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Practical Medical Oncology Textbook

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Practical Medical Oncology Textbook

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Preface

Clinical oncology is a rapidly evolving field. Within just a few years, increase in understanding of the molecular and immunological basis of cancer provided a strong base to clinical development of novel treatment options for patients across many cancer types. Several targeted therapies and immunotherapy are changing the clinical landscape and the natural history of many tumors, with an impact on patients survival. To maximize the patient benefit, prognostic and predictive biomarkers are under investigation to identify patients who will likely benefit from therapy, and multimodal diagnostic tools, such as liquid biopsy, are opening new frontiers to cancer diagnosis, screenings and therapeutic decisions.

In this textbook, many specialists in the field have covered many aspects of medical oncology. The first general section provides a comprehensive overview and background information on tumor biology and genetics, innovative technologies for clinical and translational research, and covers introductory topics on the main treatment modalities in the care of cancer patients. The following chapters are included in the clinical section on tumor presentations, diagnosis, prognosis, until the current state-of-the-art of medical treatment. It provides a systematic overview of all types of solid tumors, including epidemiology and cancer prevention, genetic aspects of hereditary cancers, differential diagnosis, typical signs and symptoms, diagnostic strategies and staging, and treatment modalities. Special attention is given to new and innovative treatments for cancer patients, such as targeted therapy and immunotherapy.

This textbook combines, therefore, essential information on clinical cancer medicine with a guide to the latest advances in molecular oncology and tumor biology. Expert commentaries at the end of each chapter highlight key points, offer hints for deeper insights, suggest further reading and discuss clinical application through the description of cases.

This textbook offers an invaluable, practice-oriented tool for medical students just beginning their clinical oncology studies, as well as medical oncology residents and young professionals.

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From Basic Research to Cancer Diagnosis

Contents

Epidemiology and Cancer Prevention

Francesco Vitale and Lucia Mangone

Contents

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1

Learning Objectives

By the end of the chapter, the reader will

- Be able to apply Public Health procedures
- Have learned the basic concepts of Public Health
- Have reached in-depth knowledge of Public Health
- Be able to put acquired knowledge into clinical practice Public Health

1.1 Introduction

The progress of cancer pathology in the world is studied by the cancer registries (CR), structures responsible for the systematic detection of cases of tumour that arise in a given population. The CRs use internationally defined standard rules for the registration of neoplasms that make data comparable on a global level. The main epidemiological indicators, which allow to describe the pathology, plan health interventions, and evaluate their impact, are incidence, mortality, survival, and prevalence.

The term incidence indicates the number of new cases diagnosed in a defined period, usually a year, in a defined population. Mortality is defined as the number of deaths for a specific disease over a defined period of time and for a specific population. Survival measures the probability of being alive after a certain time interval from diagnosis (usually 5 years from diagnosis); net survival is usually reported, i.e., the proportion of living patients net of other causes other than the tumour in question. The term prevalence indicates the number of subjects alive in a specific instant, in a given area, which in the past have faced a diagnosis of cancer: patients included in therapeutic treatments, patients in follow-up, but also subjects are included healed that have a life expectancy similar to that of the general population.

1.2 Epidemiology of Tumors

1.2.1 Incidence

There are 14 million new cancer cases per year in the world: 7,410,376 males and 6,657,518 females. Eight million (57%) occurred in the less developed regions. The overall age-standardized cancer incidence rate is almost 25% higher in men than in women, with rates of 205 and 165 per 100,000, respectively [1].

Male incidence rates vary almost fivefold across the different regions of the world, with rates ranging from 79 per 100,000 in Western Africa to 365 per 100,000 in Australia/New Zealand (Fig. 1.1). There is less variation in female incidence rates (almost threefold) with rates ranging from 103 per 100,000 in South-Central Asia to 295 per 100,000 in Northern America.

Excluding skin tumors (not melanomas), lung cancer (17% of all tumors) prevail in males followed by prostate cancer (15%), colorectal cancer (10%), stomach (9%), and liver (8%). Among females, breast cancer accounts for 25% of neoplasms, followed by colorectal cancer (9%), lung (9%), cervix uteri (8%), and corpus uteri (5%) (Table 1.1). However, the incidence is strongly influenced by the age groups: in males, leukemia is the most common cancer in both children (0–14 years) and young people (15–39 years). The liver is the most frequent neoplasm in young adults (40–44 years), while from 45 years of age, the lung tumor is the most common neoplasia with the exception of the 70–74 age group where the first neoplasm is the prostate. In females, with the exception of the 0–14 age group where the most common malignancy is leukemia, from 15 years on, the most frequent neoplasia is the breast in all age groups.

Overall, there is a strong geographical gradient between the most developed countries and the least developed countries: Australia and New Zealand, together with North America and Northern Europe and the Western European countries, have the highest incidences of tumors in the world. The countries of South Africa, Asia, and America, on the other hand, are those characterized by the lowest incidence (Fig. 1.2). This trend, however, is strongly influenced by tumor sites: in fact, while tumors such as breast and prostate, strongly related to incorrect lifestyles (nutrition, alcohol, etc.), are more frequent in developed countries, the liver and cervix are frequent neoplasms in the less developed.

With regard to time trends, in men in European countries, the incidence has increased since the first half

Fig. 1.1 Estimated cancer incidence worldwide in 2012, by sex

of the 1970s, but now some countries such as France and Denmark show a declining trend; in women, however, the incidence increases in all countries. In the Asian countries, Japan and China show a decreasing incidence, while the trend in other countries appears to be stable. Australia continues to show a growing trend; the incidence drops in the USA and New Zealand.

Table 1.1 The first five most frequently diagnosed cancers and proportion on the total of the tumors (excluding skin carcinomas) by sex

Rank	Males	Females
1°	Lung 16.8%	Breast 25.1%
2°	Prostate 14.8%	Colorectum 9.2%
3°	Colorectum 10.1%	Lung 8.8%
4°	Stomach 8.5%	Cervix uteri 7.9%
5°	Liver 7.5%	Corpus uteri 4.8%

1.2.2 Mortality

The deaths in the world for cancer are over eight million per year (about 4.5 men and 3.5 million women) with a standardized rate on the world population of 126.3 and 82.9, respectively. There is less regional variability than for incidence, the rates being 15% higher in more developed than in less developed regions in men and 8% higher in women. In men, the rates are highest in Central and Eastern Europe (173 per 100,000) and lowest in Western Africa (69) (Fig. 1.3).

In contrast, the highest rates in women are in Melanesia (119) and Eastern Africa (111) and the lowest

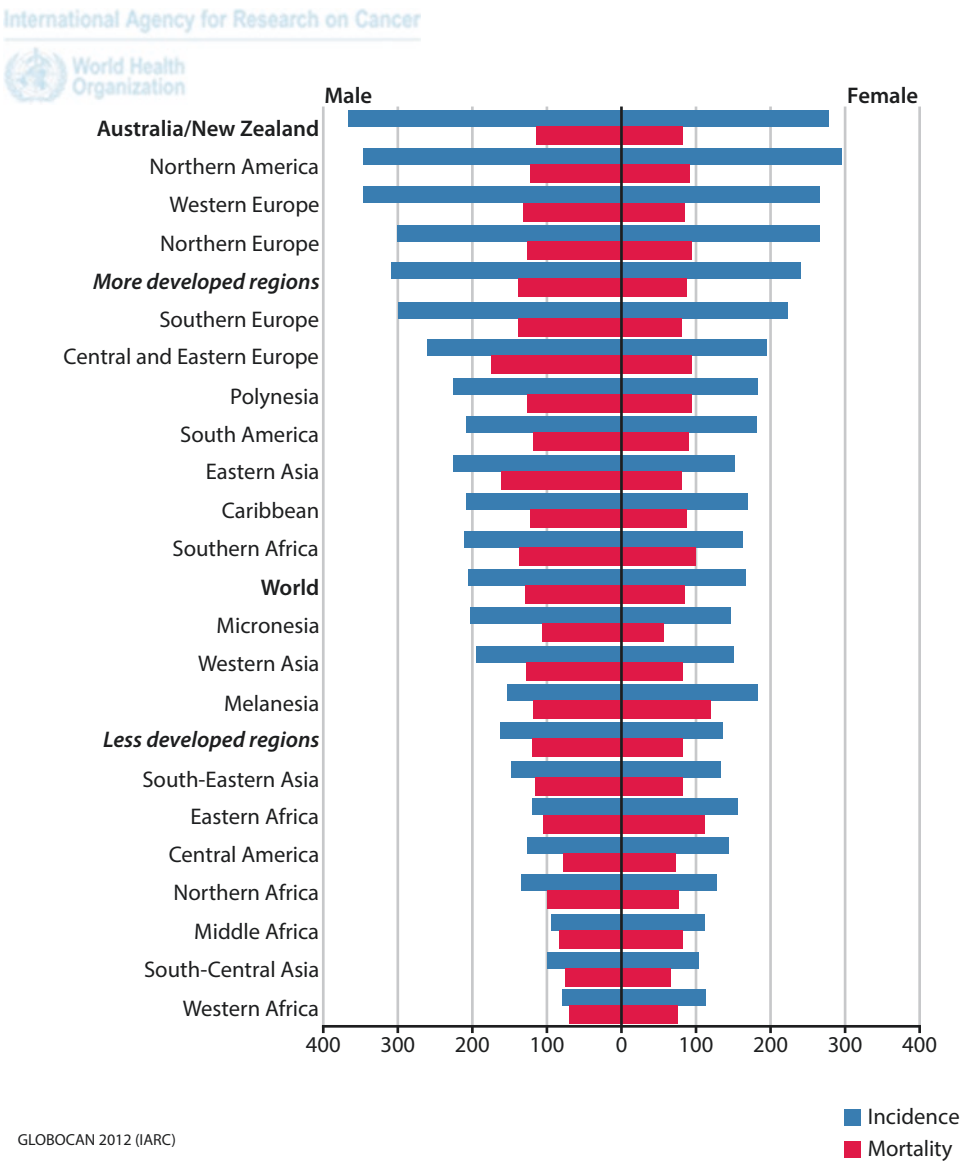

Fig. 1.2 Estimated cancer incidence and mortality by sex and region

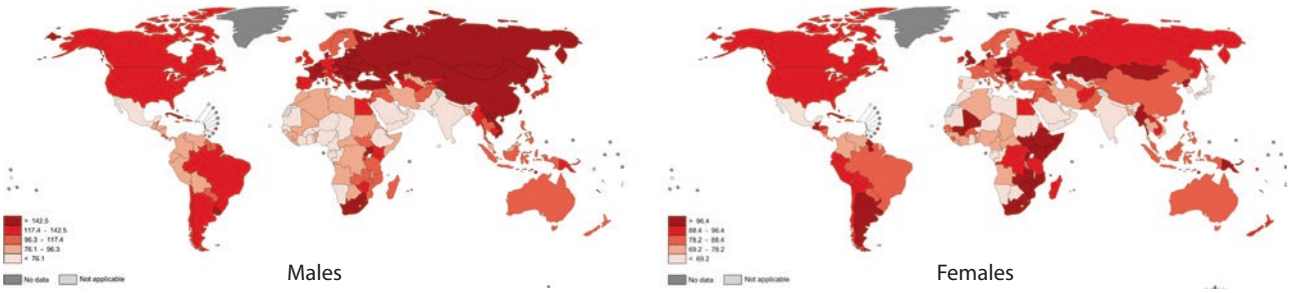

Fig. 1.3 Estimated cancer mortality worldwide in 2012: males and females

in Central America (72) and South-Central (65) Asia. The most lethal cancer are lung, liver, stomach, colon, and prostate in men and breast and lung, colon, cervix, and stomach in women.

There are also age-related differences for mortality: in children (0–14 years) and in young people (15–39 years), the highest rates of mortality are observed for leukemia. From 40 to 49 years, the highest mortality is observed for the liver, while from 50 years upwards, the lung is the leading cause of death in all age groups. In women, leukemia is the leading cause of death in children and young adults. The breast is the first cause from 40 to 64 years, while the age of 65 is the first cause of death.

With regard to mortality, there are significant differences with the highest rates in Asia. Also for mortality there is a gradient between the more developed and less developed regions with an approximately double mortality both in men (3062 vs 1592) and in women (2261 vs 1287). Fortunately, mortality rates are falling across the world, in both sexes.

1.2.3 Survival

Survival is the main outcome in the field of oncology and allows, through the measurement of time from the diagnosis, to evaluate the effectiveness of the health system as a whole against the tumor pathology. Survival, in fact, is conditioned by two aspects: the phase in which the disease is diagnosed and the effectiveness of the therapies undertaken. Therefore, both secondary prevention interventions and the availability and access to effective therapies affect survival.

CONCORD-3 updates the worldwide surveillance of cancer survival to 2014 and includes individual records for 37.5 million patients diagnosed with cancer during the 15-year period 2000–2014. For most cancers, 5-year survival remains among the highest in the world in the USA and Canada, in Australia and New Zealand, and in Finland, Iceland, Norway, and Sweden [2].

Survival trends are generally increasing, even for some of the more lethal cancers: in some countries, survival has increased by up to 5% for cancers of the liver, pancreas, and lung. For women diagnosed during 2010–2014, 5-year survival for breast cancer is now 89.5% in Australia and 90.2% in the USA, but international differences remain very wide, with levels as low as 66.1% in India. For gastrointestinal cancers, the highest levels of 5-year survival are seen in Southeast Asia. By contrast, in the same world region, survival is generally lower than elsewhere for melanoma of the skin and for both lymphoid malignancies and myeloid malignancies.

For children diagnosed during 2010–2014, 5-year survival for acute lymphoblastic leukemia ranged from 49.8% in Ecuador to 95.2% in Finland. 5-year survival from brain tumors in children is higher than for adults, but the global range is very wide (from 28.9% in Brazil to nearly 80% in Sweden and Denmark). In the poor prognosis tumors (stomach, lung, and liver), the differences were less significant, and even in more recent years, the developed countries showed very modest progress (Table 1.2).

1.2.4 Prevalence

There are 32.6 million people living with cancer (within 5 years of diagnosis) worldwide: about 15 million men and 17 million women. Prevalence is influenced by the incidence of the disease and survival, and therefore the geographical variability is very high: in men over 6 million are present in Asia (40%), 4.5 million in Europe (30%), 2.6 million in North America (17%), 1.1 million in Latin America (8%), 632,000 in Africa (4%), and 239 in Oceania (1.6%). In women over 7 million are present in Asia (42%), 4.5 million in Europe (27%), 2.6 million in North America (15%), 1.4 million in Latin America (9%), 1.1 million in Africa (7%), and 207,000 in Oceania (1.2%). There is also a strong variability linked to the site of the tumor: the breast (6 million) and prostate (4 million) are the most represented sites in women and men, respectively, followed by colorectal, lung, and cervix (Fig. 1.4).

The estimated overall trend in the present decade in Italy (+ 3.2% per year) is comparable to that estimated in the same period in the USA (+ 2.8% per year), UK (+ 3.3%), and Switzerland (+ 2.5%) [3].

Table 1.2 Age-standardized 5-year net survival (%) by continent, country and calendar period of diagnosis

Area	Good prognosis						Poor prognosis					
Site	Breast		Colon		Children LLA		Stomach		Lung		Liver	
Period	2000 2004	2010 2014	2000 2004	2010 2014	2000 2004	2010 2014	2000 2004	2010 2014	2000 2004	2010 2014	2000 2004	2010 2014
North America												
Canada	86	88	62	67	91	93	25	30	16	21	17	19
US	89	90	65	65	87	90	26	33	17	21	12	17
Europe												
Italy	84	86	59	64	83	88	32	31	14	16	16	20
Norway	85	88	60	67	88	83	22	27	12	19	8	19
Asia												
China	76	83	51	53	62	58	30	36	19	20	12	14
South America												
Brazil	69	75	45	48	68	66	19	20	11	9	15	11
Africa												
Algeria	39	77	88	74	31	–	21	42	18	34	6	14

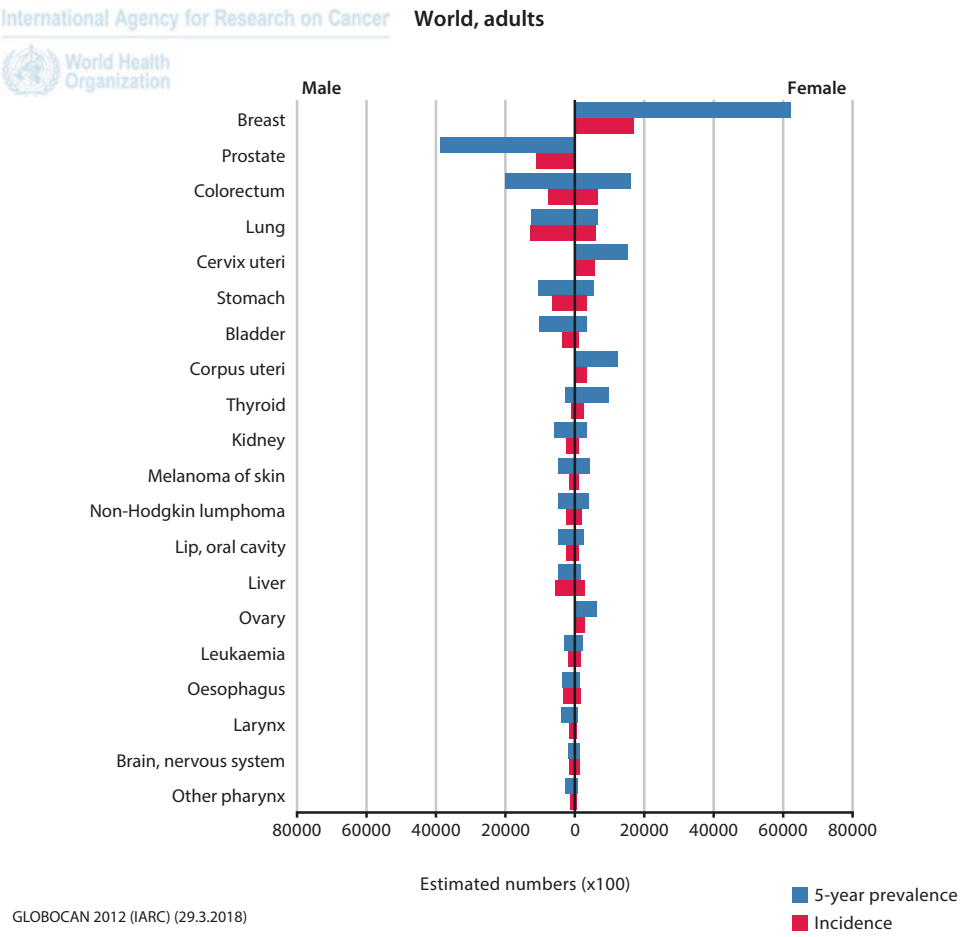

Fig. 1.4 Global estimation of cancer 5-year prevalence and annual incidence by site and sex in 2012

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1.3 Risk Factors

The known causes of DNA alterations in the genesis of cancer include environmental, genetic, infectious, lifestyle factors, and random factors. The share of tumors attributable to the various risk factors has been extensively studied: tobacco smoking is responsible for 33% of the neoplasms; the diet is responsible for 5%, but the percentage rises to 20% if one is considered overweight and obesity. Physical inactivity is associated with the development of colorectal and breast tumors. Alcohol abuse is responsible for 3% of cancers. All these risk factors depend on the habits of the individual citizen and are therefore preventable risk factors. Employment factors are responsible for 5% of cancers, infections cause about 8% of tumors, ionizing radiation and exposure to UVA are responsible for 2%, and environmental pollution contributes another 2%.

Inheritance has a very low incidence in the tumor genesis: less than 2% of the population is carrier of mutations with hereditary syndromes of neoplastic risk. BRCA 1 and 2 genes are known to increase the risk of breast and ovarian cancer, PALB 2 (partner and localization of BRCA 2), and MSH2 and MLH1 for nonpolyposis colon cancer (HNPCC).

IARC (International Agency for Research on Cancer) has published the list of human carcinogens and includes both those for which there is sufficient evidence than those with limited evidence in humans [4]. A summary is shown in Table 1.3.

Table 1.3 Agents (extract) classified as carcinogenic to humans and associated cancer sites (IARC)

	Sufficient evidence in humans	Limited evidence in humans
Chemicals and mixtures		
Formaldehyde	Leukemia, nasopharynx	Nasal cavity and paranasal sinus
Benzene	Leukemia	
Occupations		
Aluminum production	Lung, urinary bladder	
Isopropyl alcohol production	Nasal cavity and paranasal sinus	
Metals		
Chromium compounds	Lung	Nasal cavity and paranasal sinus
Nickel compounds	Lung, nasal cavity, and paranasal sinus	
Dusts and fibers		
Asbestos	Larynx, lung, mesothelioma, ovary	Colorectum, pharynx, stomach
Leather dust, wood dust	Nasal cavity and paranasal sinus	
Radiation		
Radium 226, radium 228	Bone, mastoid process, paranasal sinus	
Biological agents		
Epstein-Barr virus	Burkitt lymphoma, Hodgkin lymphoma, etc.	Lymphoepithelial-like carcinoma, stomach
Hepatitis B, C	Liver	Cholangiocarcinoma
Human papillomavirus 31, 35, 39, 45, 51, 52, 56, 58, 59	Cervix	
Helicobacter pylori	Lymphoma, stomach	

1.4 Primary Prevention

It has been known for many decades that tumors are largely preventable with individual and collective actions, a fact officially recognized for the first time in 1964 by the World Health Organization. Primary prevention includes all the procedures and interventions implemented to prevent the onset of the tumor. Since the genesis of tumors is multifactorial, it is not always possible to eliminate the causes of cancer to prevent the onset of the tumor but certainly reduces the probability that this occurs.

Between 30 and 50% of all cancer cases are preventable. Prevention offers the most cost-effective long-term strategy for the control of cancer. National policies and programs should be implemented to raise awareness, to reduce exposure to cancer risk factors, and to ensure that people are provided with the information and support they need to adopt healthy lifestyles [5].

Table 1.3 (continued)

	Sufficient evidence in humans	Limited evidence in humans
Personal habits		
Alcoholic beverages	Breast, colorectum, larynx, liver, esophagus, oral cavity, pharynx	Pancreas
Tobacco smoking	Bone marrow, cervix, colorectum, kidney, larynx, liver, lung, nasal cavity and paranasal sinus, esophagus, pancreas, pharynx, stomach, ureter, urinary bladder, in smokers' children: hepatoblastoma	Breast, in smokers' children: leukemia
Pharmaceuticals		
Cyclosporine	NHL, skin, multiple other sites	
Estrogen menopausal therapy	Endometrium, ovary	Breast
Estrogen-progestogen contraceptives	Breast, cervix, liver	
Estrogen-progestogen menopausal therapy	Breast, endometrium	

1.4.1 Tobacco

Worldwide, tobacco use is the single greatest avoidable risk factor for cancer mortality and kills approximately six million people each year, from cancer and other diseases. Tobacco smoke has more than 7000 chemicals; at least 250 are known to be harmful; and more than 50 are known to cause cancer.

Tobacco smoking causes many types of cancer (Table 1.2), including cancers of the lung, esophagus, larynx (voice box), mouth, throat, kidney, bladder, pancreas, stomach, and cervix. Second-hand smoke, also known as environmental tobacco smoke, has been proven to cause lung cancer in nonsmoking adults. Smokeless tobacco (also called oral tobacco, chewing tobacco, or snuff) causes oral, esophageal, and pancreatic cancer. Nearly 80% of the 1 billion smokers in the world live in low- and middle-income countries.

- Tobacco smoking: causes cancers of the lung, esophagus, larynx (voice box), mouth, throat, kidney, bladder, pancreas, stomach, and cervix
- Second-hand smoke (also known as environmental tobacco smoke): causes lung cancer in nonsmoking adults
- Smokeless tobacco (also called oral tobacco, chewing tobacco, or snuff): causes oral, esophageal, and pancreatic cancer

1.4.2 Physical Inactivity, Dietary Factors, Obesity, and Being Overweight

Dietary modification is another important approach to cancer control. There is a link between overweight and obesity to many types of cancer such as esophagus, colorectum, breast, endometrium, and kidney. Diets high in fruits and vegetables may have an independent protective effect against many cancers. Regular physical activity and the maintenance of a healthy body weight, along with a healthy diet, considerably reduce cancer risk. In addition, healthy eating habits that prevent the development of diet-associated cancers will also lower the risk of other noncommunicable diseases.

1.4.3 Alcohol Use

Alcohol use is a risk factor for many cancer types including cancer of the oral cavity, pharynx, larynx, esophagus, liver, colorectum, and breast. Risk of cancer increases with the amount of alcohol consumed. For several types of cancer, heavy drinking of alcohol combined with tobacco use substantially increases the risks of cancer. In 2010, alcohol-attributable cancers were estimated to be responsible for 337,400 deaths worldwide, predominantly among men.

1.4.4 Infections

In 2012, approximately 15% of all cancers were attributable to infectious agents such as *Helicobacter pylori*, human papilloma virus (HPV), hepatitis B and C, and Epstein-Barr virus. The fraction of infection-attributable cancers varied between countries and development status, from less than 5% in Australia, Canada, New Zealand, the United States, and select countries in Western and Northern Europe to more than 50% in some countries in sub-Saharan Africa. Two-thirds of infection-attributable cancers (1.4 million cases) occur in less developed countries. Vaccines are available for hepatitis B virus and some types of HPV and can reduce the risk of liver and cervical cancers, respectively.

1.4.5 Environmental Pollution

Pollution of air, water, and soil with carcinogenic chemicals contributes to the cancer burden to differing degrees depending on the geographical settings. Outdoor air pollution is classified as carcinogenic, or cancer-causing, for humans. It has been estimated that outdoor air pollution contributed to 3.2 million premature deaths worldwide in 2012 including more than 200,000 lung cancer deaths. Additionally, over four million people die prematurely from illness attributable to the household air pollution from cooking with solid fuels; 6% of these deaths are from lung cancer.

Indoor air pollution from coal fires doubles the risk of lung cancer, particularly among nonsmoking women. Exposure to carcinogens also occurs via the contamination of food, such as aflatoxins or dioxins.

1.4.6 Occupational Carcinogens

More than 40 agents, mixtures and exposure circumstances in the working environment are carcinogenic to humans and are classified as occupational carcinogens. Occupational cancers are concentrated among specific groups of the working population, for whom the risk of developing a particular form of cancer may be much higher than for the general population. It is well-documented that occupational carcinogens are causally related to lung cancer, mesothelioma, and bladder cancer. For example, mesothelioma (cancer of the outer lining of the lung or chest cavity) is to a large extent caused by work-related exposure to asbestos.

1.4.7 Radiations

Exposure to all types of ionizing radiations, from both natural and man-made sources, increases the risk of various types of malignancy including leukemia and a number of solid tumors. Risks increase when the exposure occurs at a young age and also when the exposure amount is higher. Ultraviolet (UV) radiation, and in particular solar radiation, is carcinogenic to humans, causing all major types of skin cancer, such as basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma. Avoiding excessive exposure, use of sunscreen, and protective clothing are effective preventive measures. UV-emitting tanning devices are now also classified as carcinogenic to humans based on their association with skin and ocular melanoma cancers. Radiation is used in medicine and can help save lives as well as prevent the need for more invasive procedures. However, inappropriate use may cause harm because of unnecessary and unintended radiation doses for patients. Radiologic tests and procedures should be appropriately prescribed and properly performed to reduce unnecessary radiation doses, particularly in children.

Residential exposure can also arise from radon, a naturally radioactive gas sometime present in soil, and building materials increase risk of lung cancers. Radon levels in homes can be reduced by improving the ventilation and sealing floors and walls.

1.5 Oncological Screening and Early Diagnosis

Oncological screening is a public health intervention that aims to invite an apparently healthy population to carry out a diagnostic test with the intent of discovering a possible neoplasia in a very early phase. The goal of cancer screening is to reduce mortality for that cancer and, if possible, reduce its incidence. The first objective is reached more than with the increase of the early forms, with the reduction of the advanced forms (stage IV) that bring the patient to death. On the other hand, reducing the incidence of neoplasia is only possible for those sites where the evolutionary path of the lesion is well-known: benign lesion, premalignant lesion, and cancerous lesion as in the case of the colon and the uterine cervix. To date, there are three screening programs for which a positive cost-benefit ratio has been demonstrated. Breast cancer screening for women aged 50–69 years (but many regions have widened the target population to 45–74 years); cervical screening for women aged 25 to 64; and colorectal screening involving even the male population aged 50–69 years.

The monitoring of the activity of screening programs, through appropriate indicators, is essential for the verification of the performances of the programs themselves. In fact, institutional programs are characterized not only by the offer of the test but also by the care of the person for the whole prevention path and by the presence of quality monitoring systems that are carried out through the control of the indicators in the various phases.

1.5.1 Breast Cancer

Early diagnosis strategies focus on providing timely access to cancer treatment by reducing barriers to care and/or improving access to effective diagnosis services. The goal is to increase the proportion of breast cancers identified at an early stage, allowing for more effective treatment to be used and reducing the risks of death from breast cancer.

Mammography is a radiological examination of the breast, effective for identifying breast tumors early, as it allows to identify the nodules, even small, not yet perceptible to the touch. The organized screening programs provide that the exam is performed by visualizing the breast both from the top to the bottom and from the side. A large study is published in the *Journal of Medical Screening* in September 2012, and reviewing published research on breast cancer screening programs in Europe has shown that mortality is reduced by 25% for women undergoing screening [6].

A recent Italian study shows a significant reductions among attenders for specific cancer stages; the authors observed a 39% reduction for T2 or larger (IRR = 0.61; 95% CI: 0.57–0.66), 19% for node positives (IRR = 0.81; 95% CI: 0.76–0.86), and 28% for stage II and higher (IRR = 0.72; 95% CI: 0.68–0.76) [7].

1.5.2 Cervix Cancer

WHO has reviewed the evidence regarding the possible modalities to screen for cervical cancer and has concluded that screening should be performed at least once for every woman in the target age group (30–49 years) when it is most beneficial; HPV testing, cytology, and visual inspection with acetic acid (VIA) are all recommended screening tests; cryotherapy or loop electrosurgical excision procedure (LEEP) can provide effective and appropriate treatment for the majority of women who screen positive for cervical precancer; and "screen-and-treat" and "screen, diagnose, and treat" are both valuable approaches. Regardless of the approach used, the key to an effective program is to reach the largest proportion of women at risk with quality screening and treatment. Organized screening programs designed to reach most women at risk are preferable to opportunistic screening.

A recent review report that FDA advisory panel recommended the use of HPV testing alone. This recommendation was based on data showing the long-term predictive value of a positive high-risk HPV test result. In an ideal world, in which women have regular follow-up, primary HPV screening is as effective as primary cytology screening. The duration of the protective effect of a negative HPV-negative test is twice as long as for a negative cytology test because cytologic changes are downstream of HPV acquisition. Clear algorithms for reflex cytology and for appropriate colposcopy referrals can balance the loss of specificity with HPV testing. The challenge with a new screening paradigm of primary HPV testing, which reduces the frequency of surveillance, will be to assure robust tracking and follow-up of women at risk for cervical cancer [8].

1.5.3 Colorectal Cancer (CRC)

In Europe the recommendation on cancer screening is a shared EU-level commitment to take practical steps to reduce it. Differences in cancer control strategies and survival rates among states are a further major challenge; meeting it requires a complex multidisciplinary approach. However the most important goal is to increase screening participation. Over time this will help prevent deaths due to CRC and improve the quality of life for millions of people who are at risk of developing one of the most common cancers in Europe and the world. It can no longer be accepted that a tumor that can be diagnosed by screening at an early and surgically treatable stage should continue to cause so many deaths [9].

An Italian study included 23,668 CRCs diagnosed in subjects aged 50–69 years showing a higher proportion of males, of cases in the distal colon, and a higher mean age of the patients. Compared with pre-screening cases, screen-detected CRCs showed a better distribution by stage at diagnosis (OR for stage III or IV: 0.40, 95% CI: 0.36–0.44) and grading (OR for poorly differentiated CRCs was 0.86, 95% CI: 0.75–1.00). Screen-detected CRCs have more favorable prognostic characteristics than non-screen-detected cases [10].

1

1.6 Cancer Registries

The epidemiology of cancer is monitored by the constant activity of cancer registries (CR), structures dedicated to the collection and analysis of incidence, survival, and prevalence of malignant tumors that occur in a given population. The data produced by the CR are used for descriptive epidemiology, impact assessment of cancer screening, health planning, research support, and risk assessment in environmental epidemiology. The registration activity takes place actively, using primary sources (hospital discharge records, pathological reports, death certificates, medical records, personal data, and general practitioners) and ancillary sources (exemptions, outpatient specialist services, laboratory exams, radiological examinations, palliative care, home care, and screening services). To ensure that the data collected by the CR are reliable and comparable, they adopt international standard rules (International Association of Cancer Registry) [11].

The registration activity underlies in fact mandatory rules that include:

- Completeness: elimination or minimization of the loss of incident cases
- Accuracy: minimization of the presence of incorrect, incongruent, or imprecise data
- Timeliness: guarantee of a minimum production time of the incidence and survival data
- Comparability: adoption of international standards and continuous updating
- Training: commitment to consolidate staff skills
- Respect for privacy: minimization of treatment and elimination of unnecessary use of sensitive data
- Continuity: guarantee of financial autonomy, resources, and skills
- Quality: commitment to measure, verify, and improve over time the respect of the previous principles

At an international level, the coverage of the CR is very inhomogeneous. Figure 1.5 shows the international coverage of CR: in regions with high HDI (human development index), the coverage of CR is very variable as far as 95% of North America, 78% of Oceania, and 42% of Europe. In countries with low HDI coverage, it is extremely low and does not reach 10%: 8% in Latin America, 6% in Asia, and just 2% in Africa. The same problem also afflicts the vital registration.

The availability of good quality population data would allow a continuous monitoring of the effects of the planning and prevention activities carried out in the various countries.

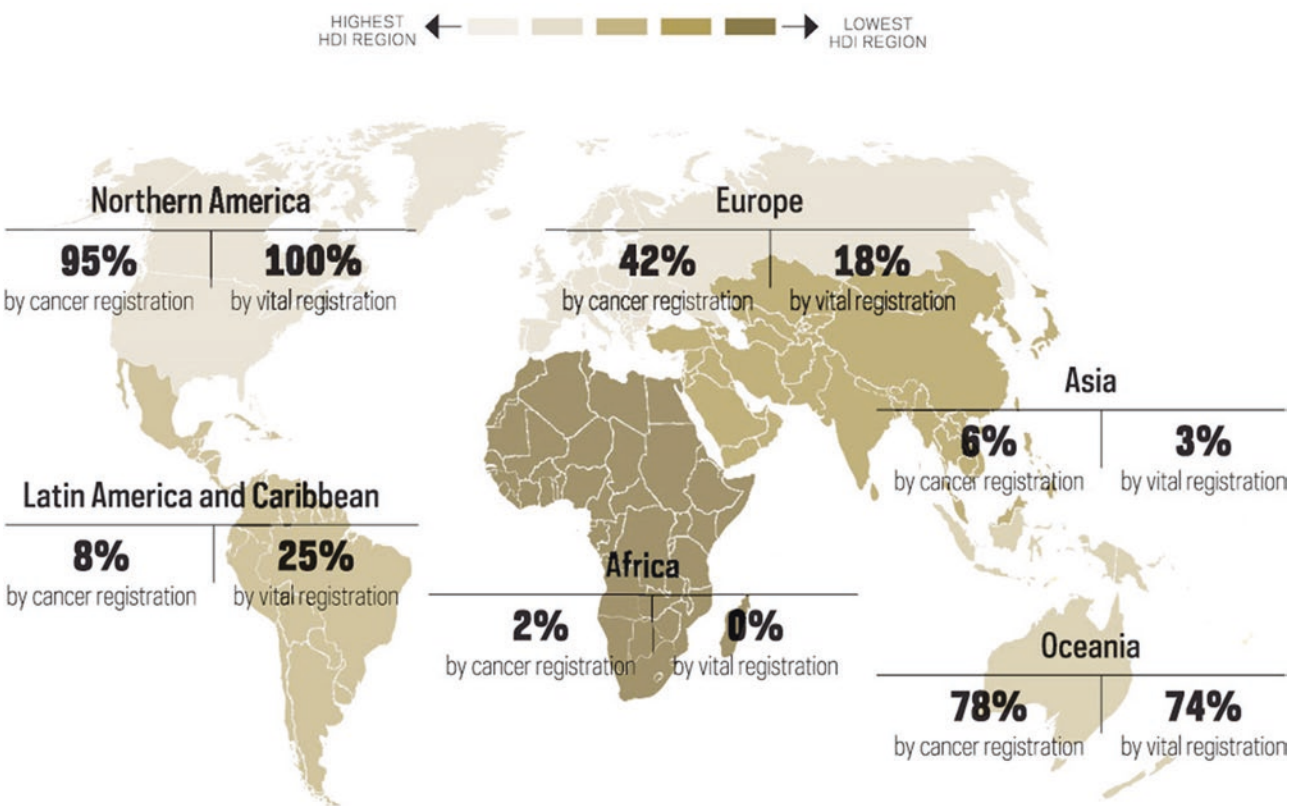

Fig. 1.5 International coverage of cancer registries by continents and human development index (HDI)

Key Points

1. There are 14 million new cancer cases per year in the world.
2. Excluding skin tumors (not melanomas), lung cancer (17% of all tumors) prevail in males followed by prostate cancer (15%), colorectal cancer (10%), stomach (9%), and liver (8%). Among females, breast cancer accounts for 25% of neoplasms, followed by colorectal cancer (9%), lung (9%), cervix uteri (8%), and corpus uteri (5%).
3. Obviously there is a consistent region variability in terms of incidence, prevalence, and types of tumors.
4. Survival trends are generally increasing, even for some of the more lethal cancers: in some countries, it has increased by up to 5% for cancers of the liver, pancreas, and lung.
5. 32.6 million people living with cancer (within 5 years of diagnosis) worldwide: about 15 million men and 17 million women. Prevalence is influenced by the incidence of the disease and survival.
6. Primary prevention includes all the procedures used to prevent the onset of the tumor.
7. Cancer registries are used to monitor constantly the number of neoplasms in a given population with the intent of studying their features and the characteristics of the patients.

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Tumor Biology and Natural History

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Contents

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Learning Objectives

By the end of the chapter, the reader will

- Have learned the basic concepts of tumor biology and intra-tumor heterogeneity
- Have acquired a good knowledge of the cancer clonal evolution process
- Have learned the basic concepts of natural history of cancer
- Have reached a good knowledge of the carcinogenesis process

2.1 Introduction

Tumors are not uniform diseases but heterogeneous entities consisting of populations of cells or cell clones, with different genetic and molecular characteristics. The growth and progression of a tumor is a heterogeneous process influenced by the surrounding tumor microenvironment. The ability of a tumor to evolve and fit to host microenvironment, by developing often resistance mechanisms to the anticancer therapies, is dependent on this biological variability [1]. In fact, the variability observed within individual tumors, known as intra-tumor heterogeneity, represents the crucial step in cancer clonal evolution process, by promoting and driving a genetic mechanism able to select the fittest cell clones [2]. A single clonal origin is usually shown by most of tumors at the early stages of the disease, whereas advanced-stage tumors may contain multiple cell populations with different characteristics which confer the ability to invade other tissues and develop distant metastases [3, 4]. The acquisition by tumor cell clones of the capability of modulating their motility or adhesion has been shown to induce variations in clinical patterns and limit therapy efficacy, by influencing treatment response. Different genetic properties, indeed, are shown by cell clones with metastatic potential compared to clones devoid of metastatic potential [5, 6]. Therefore, the identification of genetic markers of metastatic cell clones is the major purpose of many scientists [7–9]. Although little is yet known, two models have been hypothesized to explain the biological mechanisms underlying the metastasis. According to the genetic selection model, metastasis is an event that derives from a late clonal selection process involving the acquisition of a metastatic potential and an aggressive phenotype by a subgroup of cancer cells only during the late stages of the multistep process of cancerogenesis [2, 10, 11]. Another interesting model, instead, suggests the genetic background-dependent acquisition by cancer cells of a metastatic potential during relatively early stages of the cancerogenesis [12]. In this regard, Ramaswamy and collaborators [13], through a gene expression study performed on primary and metastatic tumor samples, identified a molecular signature associated with metastasis supporting this latter model.

In the last years, sequencing analysis demonstrated that genomic landscape exhibited by most of human cancers shows a small quantity of genes altered in a high number of tumors and a large amount of genes not frequently altered [14, 15]. Tumorigenesis may be induced and driven by intragenic mutations called “driver gene” mutations, whereas other mutations which confer no selective growth advantage are defined “passengers.” Driver genes can be included into 12 signaling pathways modulating 3 main molecular and cellular events, namely, cell survival, genome integrity maintenance, and cell fate [1, 16]. Understanding the genetic anomalies underlying tumor, and the specifically involved molecular pathways, has radically changed the natural history of this disease. Recent progress in understanding molecular mechanisms driving tumors led to the development of new therapeutic modalities selectively targeting specific molecular pathways, resulting in improvements for prognosis of cancer patients [17–19]. Recently, a large number of molecular alterations and genetic aberrations related to tumor cell proliferation and survival and therapy response were identified as potential biomarkers for clinical use, thanks to advances in the field of genomics, biotechnology, and molecular pathology. In addition, several evidence highlighted that treatment response can be influenced by epigenetic mechanisms able to regulate gene expression [18]. However, the simple description of genetic abnormalities is insufficient to make us understand the natural history of cancer. Therefore, an integrated view of the natural history of the tumor in the various stages of its progression is needed to identify the best treatment option, improve prognosis, and predict therapy response.

The thorough knowledge of the evolutionary history of a tumor along the space-time axis is an essential factor for developing new screening strategies able to early identify neoplasia when genetic variability is low and the disease is evolving. The implementation of more specific and sensitive clinical approaches is needed due to the correlations observed between clinical outcome and tumor diversity, in order to better characterize and evaluate tumor heterogeneity and early detect the subclonal events within tumor [20].

In this chapter, we will summarize the key concepts related to biology and natural history of tumors, describing the model of cancer clonal evolution and discussing how the understanding of biological processes may affect the natural history of the disease.

2.2 Cancer Clonal Evolution

The cancer clonal evolution model hypothesizes that tumor progression and diversity are driven by the genetic drift and natural selection, suggesting that a spontaneous or induced genetic mutation provides a selective advantage to a tumor cell, generating a dominant subpopulation able to drive tumor progression [21–23]. The theory that assumes a monoclonal origin for cancer was supported by preliminary cytogenetic analyses concerning tumor clonality that hypothesize the cancer origin from a single transformed somatic progenitor cell. This model suggests that at least one primary chromosomal alteration is shared by all cancer cells, which, afterward, undergo a clonal selection process, according to the Darwinian evolution theory, generating different cancer subclonal populations harboring secondary mutations [24]. Subsequently, other scientific studies and deeper cytogenetic analyses carried out on multiple samples from the same patient showed occasionally the appearance of several cytogenetically independent clones, raising doubts about the monoclonal theory and knowledge so far reached on tumor clonality [25–30]. Four possible different mechanisms elucidating the concept of cancer clonal evolution were postulated following the several experimental studies. The first model is based on the monoclonal theory, hypothesizing that tumor cells retain the original monoclonality during the course of the disease without acquiring further secondary mutations as those found by karyotypic analysis. In fact, some sarcomas and leukemias show only a single genetic alteration in all cancer cells (Fig. 2.1a). The second model relies on the theory of clonal divergence that confirms the monoclonality of the tumorigenesis process but hypothesizes a secondary clonal heterogeneity determined by subsequent mutations occurring over time (Fig. 2.1b). The third model involves the onset of an initial polyclonality in cancer, to which follows a clonal convergence process that causes a considerable reduction in genomic alterations and the selection of cytogenetically independent clones during tumor expansion, producing a secondary oligo- or monoclonality (Fig. 2.1c). Finally, the fourth model suggests a cancer polyclonal origin characterized by an early clonal convergence and a late clonal divergence resulting from the presence of

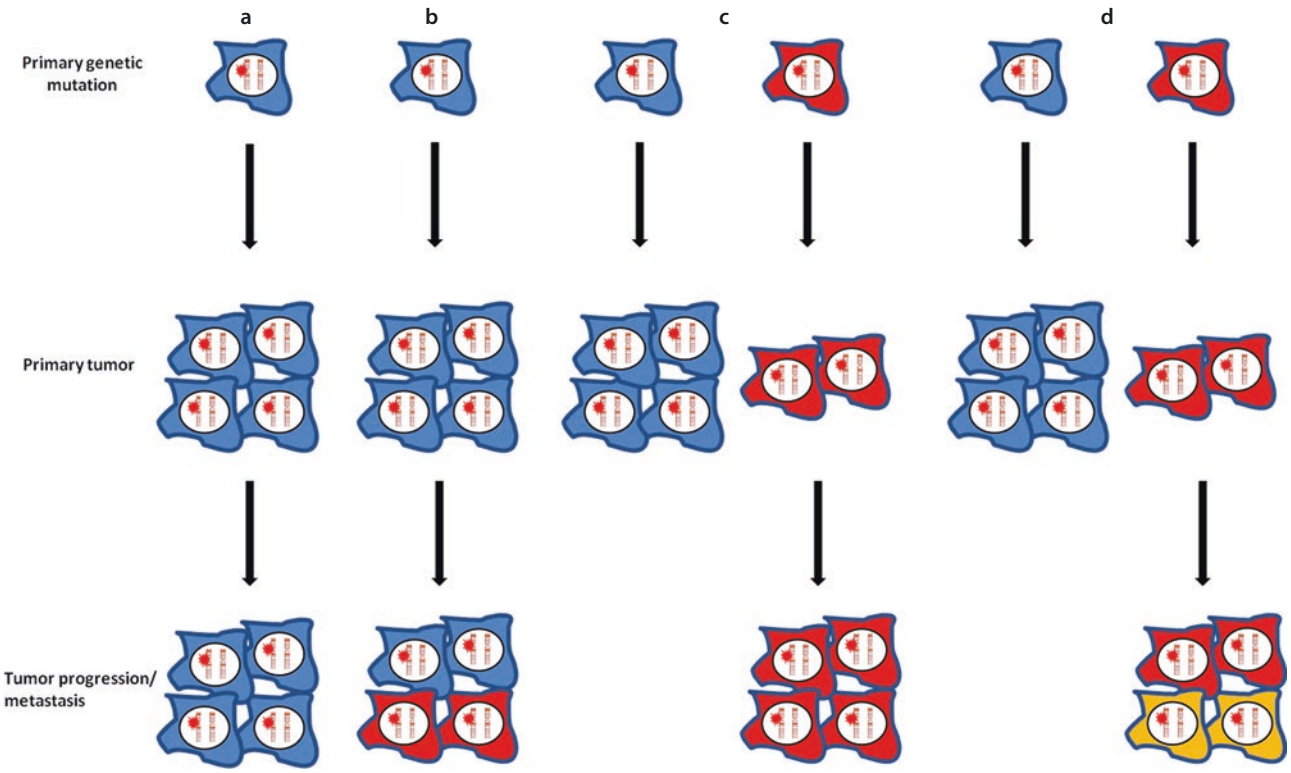

Fig. 2.1 Models of cancer clonal evolution. **a** The monoclonal hypothesis suggests that cancer cells maintain a monoclonal origin during the course of the disease without acquiring further secondary alterations. **b** The second mechanism relies on the concept of clonal divergence, confirming a monoclonal tumorigenesis process followed by a secondary clonal heterogeneity due to subsequent alterations occurring over time. **c** The third model involves an initial polyclonal tumorigenesis followed by clonal convergence resulting in a secondary mono- or oligoclonality. **d** The last model proposes a cancer polyclonal origin characterized by early clonal convergence and late clonal divergence

other cytogenetic alterations that allow specific clones to continue to exist during the intermediate stages of cancerogenesis [31–33] (Fig. 2.1d).

Experimental studies demonstrated that cancer clonal evolution is a highly heterogeneous multiple sequential process characterized by the co-existence and co-evolution of several clonal subpopulations changing along the space-time axis and acquiring selective survival advantages during tumor progression [34, 35]. It has been suggested that different tumor types may follow different evolutionary mechanisms [36, 37]. Tumor evolution can occur through four different modalities: linear evolution, clonal separation (or allopatric speciation), clonal competition (or antagonist evolution), and clonal cooperation (or symbiotic evolution). The presence of sequential alterations over time underlies the linear evolution process and may determine tumor heterogeneity when a subclone is unable to exceed its predecessors. The occurrence of subclonal populations geographically isolated within tumor and genetically distinct in different tumor districts underlies the clonal separation mechanism that is a process equivalent to the allopatric speciation [38, 39]. Recent experimental evidence revealed that distinct subclones can cooperate between them during tumor evolution (clonal cooperation) [40]. Sometimes, this cooperation can lead to a tumor collapse induced by clonal interference, when, for example, a subclone with higher proliferative capability and unable to survive alone exceeds an autonomous driver subclone (clonal competition). Therefore, innovative therapeutic approaches are needed in order to detect and target specific subclonal populations favoring survival and growth of neighboring cells in the tumor [41]. Likewise, understanding the links between phylogenetic and tumor clonality may let to genetically correlate a primary tumor with its metastases over time [42]. Two distinct pathways called microevolution and macroevolution may determine tumor evolution. While microevolution is a gradual event, conversely macroevolution is characterized by considerable, non-gradual jumps along the evolutionary lines [37].

Recent advances in the molecular biology field, such as the next-generation sequencing (NGS) analysis, and implementation of more sophisticated computational methods have allowed to better investigate the above described models of cancer clonal evolution and get a high-resolution overview of the genetic defects present in tumors, in order to deeper analyze spatial distribution of subclones and better define tumor heterogeneity [43–49]. Recently, the analysis of circulating tumor DNA (ctDNA) is allowing to predict and characterize cancer subclonal evolution during disease progression, therapy, and acquisition of drug resistance [50].

2.3 Intra-tumor Heterogeneity

A key event in cancer clonal evolution process is represented by intra-tumor heterogeneity (ITH), that is, the variability within individual tumors, able to promote and drive the genetic selection mechanism of the fittest cell clones [51, 52]. Experimental studies demonstrated that a genetic, morphological, and behavioral variability can be shown by cancer cells present in an individual tumor [53]. Using a compound microscope, the pathologist Rudolf Virchow and other researchers have detected cellular heterogeneity within single tumors, for the first time, in the 1800s [54]. Unlike the inter-tumor heterogeneity enabling to mark the differences between tumors that hamper the eradication of the disease, conversely, intra-tumor heterogeneity may affect both tumor progression and therapy efficacy [55–57]. In fact, as suggested by Heppner, in 1984, knowing the factors and events that determine the intra-tumor heterogeneity can lead to the development of new therapeutic strategies for patient cure [58]. The occurrence of a large number of genomic variations within each tumor may cause a high extent of tumor heterogeneity, despite most of these alterations, for example, chromosomal rearrangements or somatic mutations, seem to be not detected across all samples from a tumor or metastatic lesions [59]. Different genetic alterations may be found in a limited number of genes from cancer samples recruited at different stages and from different subjects [60]. Another crucial event for the intra-tumor heterogeneity seems to be the branched evolution that occurs during tumor progression, allowing to detect phylogenetic genomic changes originated during tumor clonal evolution [61, 62]. The intra-tumor heterogeneity has been shown to vary along the space-time axis, leading to the expansion of different clones which evolve independently, but not always, in a divergent way. A convergent clonal evolution may be caused by the presence of different parallel mutations in the same gene, highlighting the significant contribution of a specific molecular pathway in the tumor progression and indicating targets potentially useful for the implementation of new therapeutic options [56, 63–65]. Analyzing the molecular changes of a tumor over time in order to favor the development of tailored therapeutic strategies could be very helpful, since molecular characterization derived from tumor biopsy gives us only a picture restricted in the time and space of a given tumor, without providing information about its heterogeneity [66, 67]. Generally, there exist two theoretical models potentially complementary between them used to elucidate the origin of tumor heterogeneity: the clonal evolution model [68] and cancer stem cell (CSC) hypothesis [69]. These two theories, in the past believed mutually exclusive, seem to

have some commonalities, suggesting that tumor origin may depend on the accumulation of multiple molecular alterations, acquisition of an uncontrolled proliferation ability by single cells, and interaction with tumor microenvironment [70]. However, tumor cell organization is deemed hierarchical in CSC model and stochastic in clonal evolution model. In addition, the heterogeneity seems to be due to epigenetic and genetic alterations followed by natural selection in clonal evolution model, whereas to abnormal differentiation processes and mutations in the CSC hypothesis [71]. Furthermore, the genetic instability, proliferation rate, cell population size, and selective pressure induced by external selective thrusts, according to the Darwinian evolutionary theory, seem to drive tumor progression and therapy resistance in the clonal evolution, whereas these, instead, result depend on a small cell subgroup only in the CSC theory [72] (Fig. 2.2). In advanced-stage cancers, the intra-tumor heterogeneity found in most tumors has been shown to limit treatment response and promote drug resistance, favoring the selection of resistant subclones, sometimes identifiable prior to therapy [73] (Fig. 2.3). For that reason, the contribution that tumor heterogeneity provides to therapeutic response is essential for the success of the anticancer therapies, by studying the link between clonal heterogeneity and clinical significance of subclonal driver mutations [74–77]. Generally, the clinical choice to adopt a specific targeted therapy is dependent on the presence of target driver mutations found in the primary tumor through histological or molecular analyses. However, the main hurdle to the successful treatment is represented by the intra-tumor heterogeneity and clonal evolution within each tumor, as target mutations in the primary tumor or metastatic lesions are not harbored by all cancer cells [78]. The evolution of metastatic disease may be influenced by the microenvironment of the metastatic site, sometimes leading to the selection and enrichment of some tumor subclones and determining a genomic and phenotypic variability between primary tumor and metastases in different tumors [63]. Conversely, in other

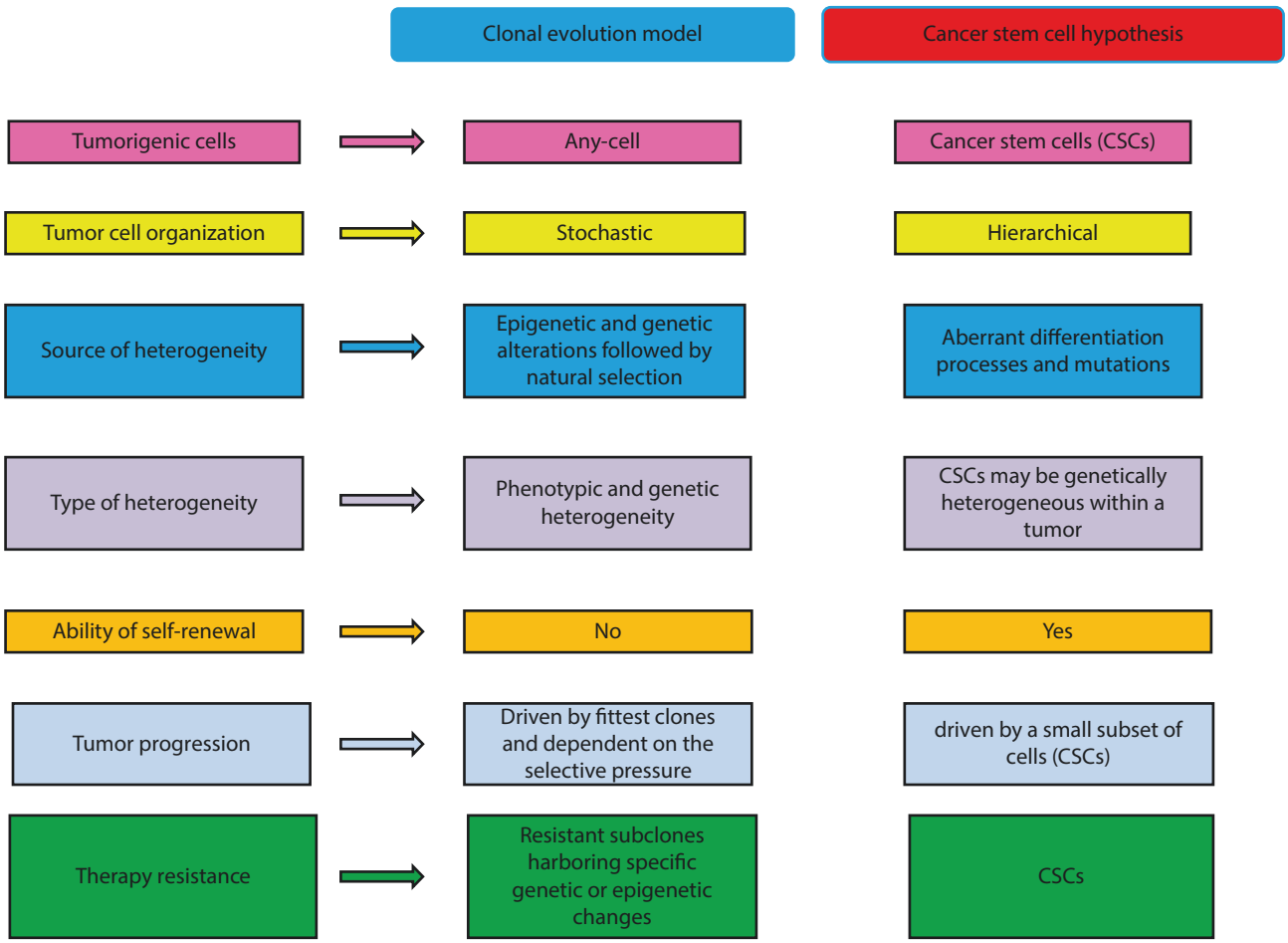

Fig. 2.2 Differences between clonal evolution model and cancer stem cell (CSC) hypothesis. The origin of tumor heterogeneity may be explained through these two theoretical models potentially complementary

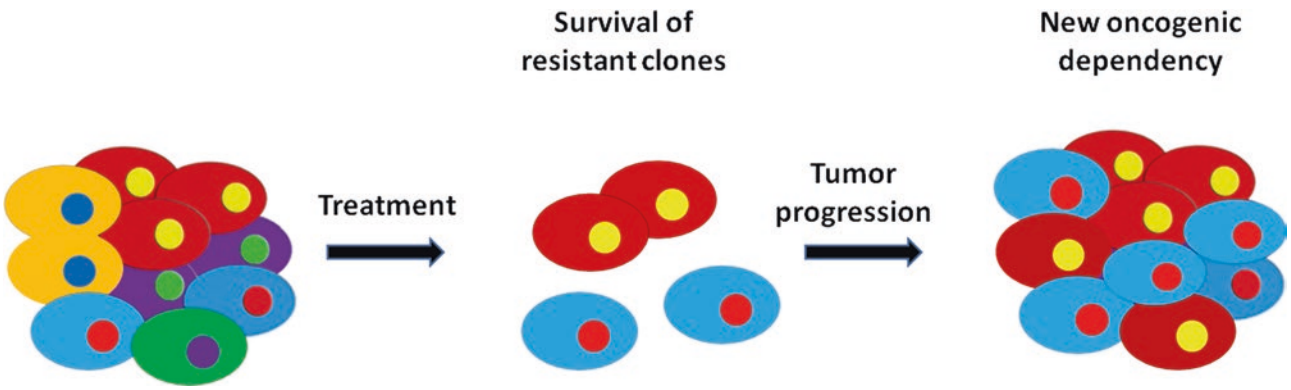

Fig. 2.3 Intra-tumor heterogeneity and resistance. Heterogeneity of tumor cells may alter the therapeutic response to specific therapies, because a small fraction of tumor clones becomes insensitive to therapy and survives, resulting in disease relapse and tumor progression

situations, the same genetic mutation has been observed both in primary tumor and metastatic lesions [79, 80].

Among tumors, an interesting example of intra-tumor heterogeneity can be represented by melanoma and non-small cell lung cancer (NSCLC). Indeed, until some time ago, mutations in *NRAS* and *BRAF* genes were considered mutually exclusive in melanoma, suggesting that tumor growth and survival do not take advantage by their simultaneous presence [81–83]. However, recent evidence showed that both two mutations may be simultaneously found in the same tumor samples [84, 85]. The intra-tumor heterogeneity has been shown to play a crucial role also in NSCLC treatment, since NSCLC patients with *EGFR* activating mutations reveal different therapeutic responses to tyrosine kinase inhibitors (TKIs) [86, 87].

The combination of different therapies may help us to overcome tumor resistance induced by intra-tumor heterogeneity, improving the efficacy of targeted agents and chemotherapy and increasing survival rates in cancer patients.

2.4 Natural History of Cancer

Cancer was described for the first time by Hippocrates which used the word "carcinoma" (from Greek "karkinos" = "crab") in order to definite it. The word "neoplasia," instead, was introduced by Galeno in the second century only and was defined as the expansion of a body region adverse to the nature. The first description of a breast tumor dates back to the Ancient Egyptians around 3500 years ago, as documented by the *Edwin Smith Surgical Papyrus*. The genetic basis of cancer concerning the hereditary chromosomal mutations able to induce uncontrolled proliferation in cells, instead, was described by Boveri in 1902 [88–90].

Tumors can be distinguished as malign or benign based on their cellular features. Cells belonging to a benign neoplasm do not alter normal tissue function and proliferate slowly [91]. Conversely, cells belonging to a malignant neoplasm exhibit numerous changes in cell biology, including ability of autonomous differentiation and proliferation, and inclination to escape from apoptosis, to invade surrounding tissues and metastasize to sites distant from the primary tumor (Fig. 2.4) [92].

A significant prognostic value is provided by degree of differentiation (or grading) of a tumor, because generally the more undifferentiated a cancer results, the greater its proliferative potential, and consequently the most unfavorable its prognosis [93].

Although the cellular features of tumors allow us to better investigate carcinogenesis, however, they are not suitable to detect molecular alterations.

The factors behind the development of cancer can be categorized into endogenous (or genetic) and exogenous (or environmental) [94, 95]. *Endogenous factors* include inherited genetic alterations, age, hormonal disorders, physiological conditions, immune system damages, and inflammatory states (e.g., pancreatitis, ulcerative colitis, etc.) [96–101]. *Exogenous factors* include lifestyle, diet, socio-economic status, chemical substances (natural and synthetic), physical agents (ionizing and non-ionizing radiations), and biological agents (bacteria, viruses, and parasites) [102–108]. Epidemiological studies on the cancer incidence revealed that cancer risk varies between population groups depending on the lifestyle-related factors and habits and defined geographical areas [109, 110]. Indeed, high incidence rates of some types of cancer in the population are determined by incorrect lifestyle and habits, such as cigarette smoke and inhalation of related products, excessive alcohol consumption, high-fat diet, ingestion of foods contemned by mycotoxins, etc. [111–115].

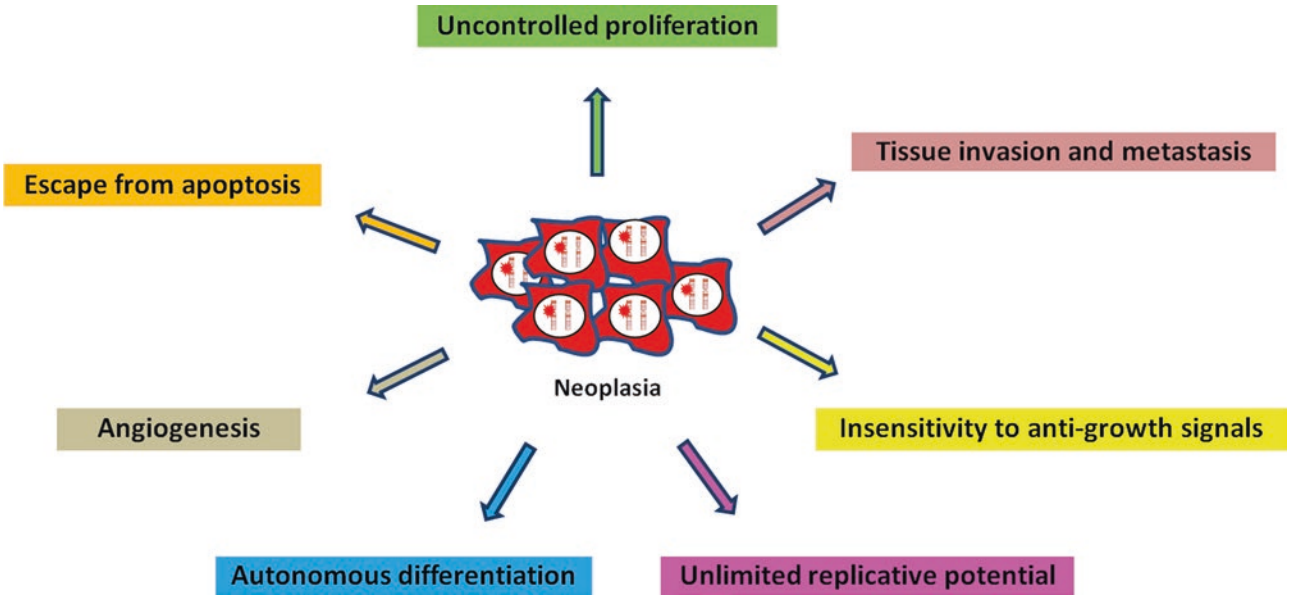

Fig. 2.4 Major biological properties of the malignant neoplasm

Cancer is a systemic disease that at first shows local events and afterward is characterized by a multistep process with several hallmarks, including rapid proliferation, apoptosis inhibition, neoangiogenesis, local invasion, and metastasis [116, 117]. Although the genetic alterations detected in human cancers have been studied and characterized by analysis of murine tumor models [118, 119], however, the natural history of tumor onset and progression and incidence and localization of primary tumors and metastases were not deeply investigated across an animal population [120]. Recent progress in the comprehension of the cancer initiation processes and the implementation of new therapeutic approaches have not been enough to definitively overcome resistance of tumor cells to different therapies, improve prognosis, and prevent tumor recurrence [121, 122]. The major goals of the oncology research are to discover the biological mechanisms underlying tumor resistance and develop therapeutic strategies able to overcome this problem, as the existing treatments not always allow to fully eliminate the disease [78, 18]. The best treatment options for cancer patients depend on several factors, including also the natural history of the disease and tumor biology.

2.4.1 Carcinogenesis

Carcinogenesis is a multistep process determined by the gradual accumulation of gene alterations and epigenetic modifications able to modulate specific molecular pathways, by producing overall a malignant phenotype [123, 124]. Starting from tumorigenesis, the cancer onset occurs through different stages over time. This process of development, in the absence of therapeutic treatment, is known as natural history of cancer and occurs over a variable period of time, even if often long. The natural history of cancer consists of a multistage process, in which the features of some most important stages are unique and well described [125, 126]. In addition, in the history of a tumor, there may be a variable period of latency, during which early onset occurs at the microscopic and cellular level. Different cancer initiation and promotion factors as well as progression history characterize every type of cancer [127]. The natural history of cancer includes three major steps described during carcinogenesis: initiation, promotion, and progression (Fig. 2.5) [128, 129]. Morphological and biochemical modifications and genetic and/or epigenetic alterations characterize each of the three stages [130–132]. Genetic changes include mutations in genes involved in the DNA repair, survival, cell proliferation control, and cell death, whereas epigenetic modifications involve the activation of several mechanisms which silence gene expression, favoring the carcinogenesis. The three major classes of genes altered in cancer are proto-oncogene, tumor-suppressor genes, and DNA repair genes. Epigenetic changes include DNA methylation and histone modifications, involved in the chromatin remodeling mechanisms [124, 133, 134, 135]. The first two stages of carcinogenesis are only known by experimental models and epidemiological studies on human tumors. Indeed, no animal model has been shown to completely mimic the complexity of these steps, because of different genomic alterations and pathological characteristics concerning cancer

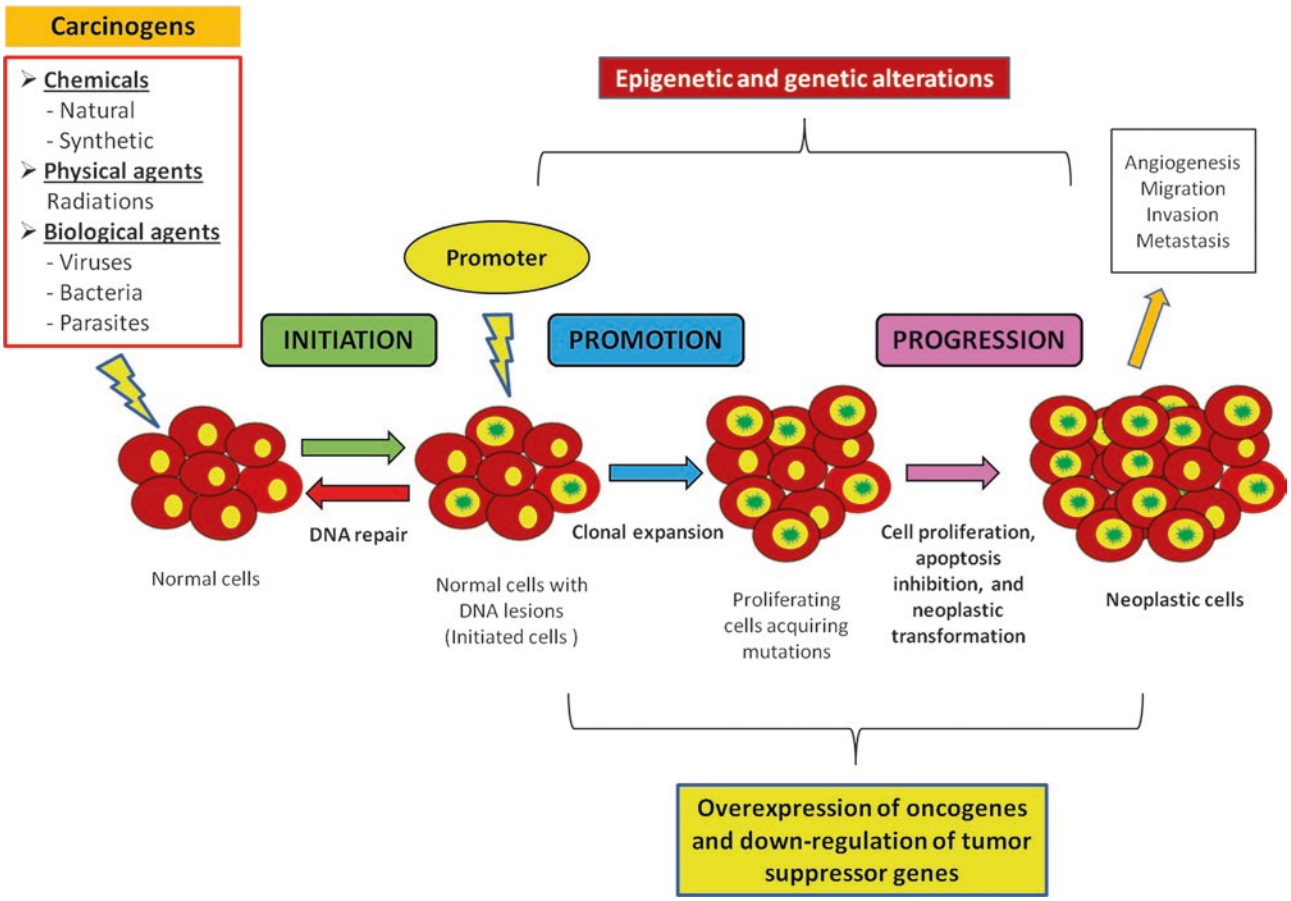

Fig. 2.5 Description of the carcinogenesis stages. The classic model of carcinogenesis consists of three major stages which describe the natural history of cancer: initiation, promotion, and progression. During these stages alterations in genome structure occur, leading, in the promotion phase, to changes in gene expression which determine the selective proliferation of initiated cells harboring DNA lesions. The initiated cells, which are phenotypically similar to other cells, are subjected to mutations which cause proliferation but not differentiation. The promoter agents do not necessarily give rise to cancer but slow the usual inhibition of the quiescent cells or in G_0, promoting the clonal expansion of initiated cells and, finally, determining malignancy. The progression is an irreversible process which describes the sequence of the consecutive transformations from pre-neoplastic or benign lesions to a malignant neoplasia

patients [136, 137]. In order to investigate the natural history of tumors, several animal cancer models, such as genetically engineered models (GEM) specifically involving the overexpression of an oncogene or loss of a tumor-suppressor gene, xenograft or allograft tumors, and chemically induced malignancies, have been developed over time. Tumor transplantation is carried out between individuals from the same species in the allografts, whereas between individuals belonging to different same species in xenografts [138, 139]. These experimental models may carefully reproduce the initiation events, evolution process, and progression of a tumor in space and time, in order to investigate the etiology, prevention, diagnosis, and treatment of cancer in all stages [137].

Usually, the different agents involved in the natural history of a tumor are classified into (1) *incomplete carcinogens* or *initiators*, which are able to trigger the first stage only; (2) *promoters*, involved in the second phase only; and (3) *complete carcinogens*, which are able to carry on the whole process from initiation to emergence of the in situ disease [129].

The *initiation* phase represents the first step in cancer development and is an irreversible and rapid process causing DNA lesions to be induced after exposure to carcinogens (chemical, physical, viral). This event predisposes susceptible normal cells to the malign evolution and immortality. At this initial stage, the cell is not yet neoplastic, but, after further genotypical and phenotypical modifications, it is addressed toward neoplastic transformation [140]. In fact, the initiated cell, which is phenotypically similar to other cells, is subjected to mutations which cause proliferation but not differentiation [141] (Fig. 2.5). The first event of the chemical carcinogenesis is represented by DNA damage which can be enzymatically fixed, if DNA repair mechanisms are activated before or during cell division. However, proliferating cells do not have much time to correct it, by

removing the covalent bonds that chemical compounds form with DNA [142]. The chemical compounds often not interact with DNA but are able to induce permanent genetic mutations which quickly propagate in the daughter cells arising from cell harboring initial mutation [143–145]. Initiated cells may stay at this phase for a while or long time (weeks, months, or years), or grow in an autonomous and clonal manner, keeping a symmetrical cellular division. An increased number of new cells and apoptosis inhibition determine the clonal expansion of initiated cells [146, 147]. There exists a correlation between the amount of carcinogen and number of produced tumor cells, since the greater its concentration and the exposure time to carcinogen, the higher the risk of carcinogenesis [148, 149]. The exposure to an initiator agent does not ensure that all cells acquiring and harboring mutations will be initiated, since, for this to happen, genes controlling the terminal differentiation process need to be mutated [150]. In some and less common cases, a spontaneous initiation event may occur, because of spontaneous mutations or replication errors in DNA [151]. Once a carcinogen has affected a cell, this is susceptible to advance to the promotion phase. Human epidemiological studies and animal experimental models showed that the initiation stage may be prevented through the protection of healthy cells to exposure to several carcinogenic agents such as tobacco, benzol, various chemical compounds, radiations, etc. [152, 153]. The discovery of the irreversibility of the process triggered by initiators has allowed to develop control measures aimed to restrict human exposure to radiations from ultraviolet light and diagnostic radiology procedures. The correlation between exposure to chemicals in the workplace and onset of specific tumors has favored the development of experimental models useful to better investigate the biopathological mechanisms underlying carcinogenesis [154, 155]. The general properties of the initiator agents are summarized in ◘ Table 2.1.

The *promotion* phase is a more prolonged event resulting from repeated or constant exposure to a compound which is not a carcinogen or initiator but a promoter agent able to maintain and stabilize the initiated lesion [146]. This stage is characterized by variations in gene expression which promote the selective proliferation of initiated cells into a large number of daughter cells carrying the mutation generated by the initiator, leading to subsequent development of pre-neoplastic cells [128, 156] (◘ Fig. 2.5). During initiation and promotion, a substantial balance between cell proliferation and apoptosis is observed, although each of these two events individually can take place at different rates. In the final phase of carcinogenesis, this equilibrium is unbalanced, triggering the onset of malignancy [157].

◘ **Table 2.1** General properties of the initiator and promoter agents

Initiators	Promoters
Promote an irreversible and additive process	Promote a reversible and non-additive process
Alone cannot lead to cancer without the subsequent presence of a promoter	Unable to promote the initiation
Carcinogens	Co-carcinogens (no carcinogens)
Mutagenic agents	Non-mutagenic agents
Administered prior to the promoter	Administered after the initiator
Affect the initiation in a dose-dependent manner	Affect the promotion in a dose-dependent manner
Undefined threshold dose	Well-defined threshold dose
Only one exposure may be sufficient	Prolonged exposure is generally required
Covalent bonds with DNA	Non-covalent bonds with DNA

The promoters do not interact directly with DNA, and they can exert their biological effects only after their metabolic activation and exposure of the cell to an initiator agent [158]. Furthermore, these substances may indirectly cause damages in DNA by oxidation processes. In the past, these events were believed to be determined by epigenetic mechanisms, whereas, today, they have been largely associated with genetic alterations [159]. There are two classes of promoters: (1) *specific promoters* which bind to receptors on the cell surface of target cells in order to modulate intracellular signaling pathways promoting cell proliferation, and (2) *non-specific promoters* which modify gene expression without the involvement of a specific receptor [160]. Promoters do not necessarily give rise to cancer but slow the usual inhibition of the quiescent cells or in G_0 by gap junctions and promote the clonal expansion of initiated cells, finally determining malignancy. Indeed, only cells undifferentiated, escaped from apoptosis and induced to divide, have been shown to have potential to give rise to tumor. Tumor development has been shown to be dependent on the exposure time and promoter dose with well-defined threshold and maximum effect, because tumor growth is not promoted at very low concentrations, and cancer risk is not greater at very high doses [161, 162]. However, studies concerning the chemical carcinogenesis showed that high concentrations of pro-

moter agents (e.g., phenobarbital, benzene, asbestos, arsenic, etc.) and prolonged exposure may induce cancer without initiation. A hypothesis aimed to explain this contradiction suggests that this phenomenon could occur because of initiated cells arisen spontaneously following an indirect effect of promoter which, by enhancing the rate of cellular divisions, promotes the occurrence of DNA replication errors [146]. A regression of cell proliferation, probably favored by apoptosis, may be observed after removal of promoter agent, making the promotion a reversible stage. Physiological factors may modulate this stage, restricting the grade of experimental carcinogenesis [163]. Like initiation stage, the promotion may be prevented through the protection of healthy cells to exposure of several substances and risk factors, such as tobacco, alcohol, high-fat diet, viruses, inflammatory states, etc. [152]. The general properties of the promoter agents are summarized in Table 2.1.

The *progression*, the most extended stage of carcinogenesis, describes the sequence of the consecutive transformations from pre-neoplastic or benign lesions, developed between initiation and promotion, to a neoplasm and to malignancy [164, 165]. This last phase, characterized by irreversibility and genetic instability, involves the acquisition by cells of autonomous and uncontrolled proliferative properties independent from the presence of stimulus and local invasion, metastasis, and loss of differentiation. Furthermore, changes in the biochemical and metabolic pathways and in morphological features of cells characterize tumor progression [166, 167]. The acquisition of a neoplastic phenotype during the progression is preceded by the occurrence of an angiogenic phenotype and is promoted by epigenetic and genetic alterations [168]. In fact, changes in karyotype, including aneuploidy, associated with increased growth rate, invasiveness, and metastasis are detected during progression, mostly in advanced tumors [169, 170]. A selective advantage for growth or survival of pre-neoplastic cells is provided not only by DNA damage but also by the down-regulation of tumor-suppressor genes, including *TP53* and *RB*, and up-regulation of oncogenes, including *myc*, *Ras*, and *Bcl-2* (Fig. 2.5). Pre-neoplastic cells show aberrant expression of growth factor receptors (e.g., epidermal growth factor receptor, EGFR), altered signal transduction pathways, deregulated cell cycle checkpoints, apoptosis resistance, neoangiogenesis, etc. [163]. Tumor progression may be prevented through well-designed and accurate screening programs of pre-cancerous lesions and small tumors or through the use of adjuvant therapies in patients who are very likely to develop metastases.

The development of tumor *metastasis* represents not only the culminating part of the natural history of cancer but is also responsible for the worse prognosis and lethal effects of many cancers. Indeed, during tumor progression, neoplastic cells lose their ability to adhere to the tissues, and acquire an invasive potential, detaching from the primary tumor and locally invading the neighboring tissues [171, 172]. The detached neoplastic cells travel through the bloodstream and lymphatic system and, after extravasation, colonize other tissues and organs distant from the primary tumor, forming at first micrometastases and then macroscopic secondary tumor lesions at these new sites. The main steps of tumor metastasis process result to be common to all cancers [173, 174]. Invasive tumors can be distinguished from in situ tumors on the basis of the infiltration or not of the basal membrane of the epithelium [175]. The body regions less resistant and most suitable to tumor invasion include nerve sheaths, organ capsules, and small vessels, whereas those more resistant are arteries, cartilage, nerves, and tendons. A typical clinical symptom revealing the tumor invasion of the vascular wall is represented by massive hemorrhagic rupture. The invasion of the bone by cancer cells destroys its structure, either by direct contact or by activation of surrounding osteoclasts, causing constant and severe pain [176, 177].

Gene alterations and angiogenesis represent the driving forces underlying tumor metastasis. Angiogenesis is an event that leads to the formation of new tumor-associated vessels able to meet its nutritional needs. Vascular endothelial growth factor (VEGF) and thrombospondin 1 (TSP-1) are significantly involved in angiogenesis induction and inhibition, respectively. Conditions such as hypoxia up-regulate the VEGF expression, promoting angiogenesis [178]. Among the main cell-to-cell adhesion molecules involved in the development of tumor metastasis, there are the cadherins, which exert a suppressive function of the cell invasion processes. In fact, for example, down-regulation of the E-cadherin expression in extracellular matrix (ECM) was detected during the onset of invasion and metastasis, whereas its up-regulation opposes to these processes, favoring the formation of adherent junctions with adjacent epithelial cells [179–181].

Metastases detected at diagnosis are classified as synchronous, whereas metastases identified during the course of the disease, months or years after treatment of the originating tumor, are defined as metachronous [182, 183]. Synchronous metastases are discovered at the same time as the originating tumor, either because they show clinical symptoms or because they are found during the systematic check-up performed before any local therapy [183, 184]. The treatment of the metastases is dependent on their chronology. A delayed metastasis that occurs long after treatment of the initial tumor may be unique (without further microscopic metastases) and be treated

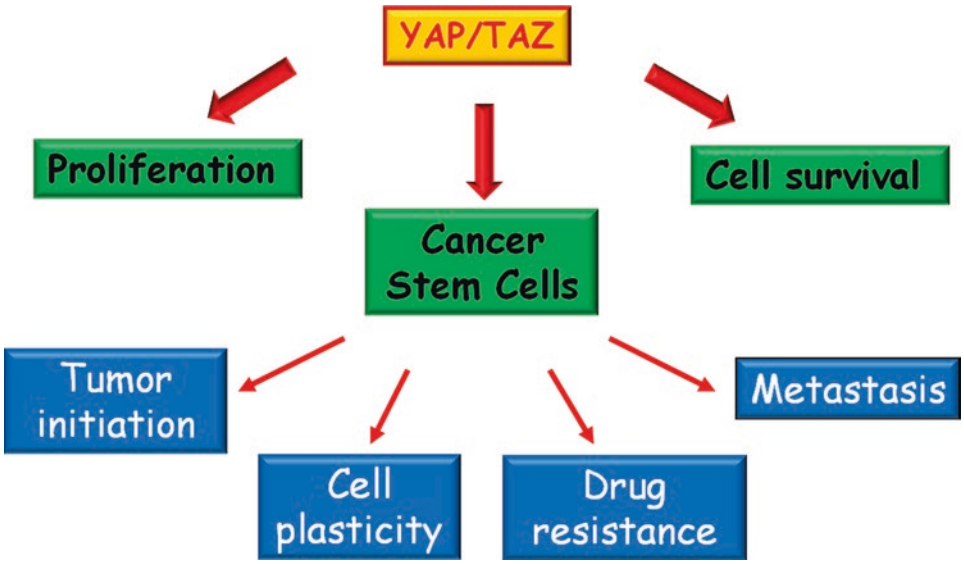

Fig. 2.6 Functions of YAP and TAZ in cancer cells and TME

locally, leading to prolonged survival. Therefore, local treatment of the metastasis sometimes may lead to the long-term complete clinical remission with good quality of life [185].

Tumors are complex entities in which cancer cells are only one of the components of a composite tumor tissue. The other component, the tumor stroma, is made up of an extracellular matrix and other cell types, including cancer-associated fibroblasts (CAFs) and immune cells, and creates multiple and bidirectional interactions with cancer cells necessary for tumorigenesis. For example, it has been found that in pancreatic cancer, the stroma, which is the connective and fibrous tissue that supports the tumor, seems to have a particularly relevant role in tumor progression and response to therapy. The molecular "players" of this tumor-stroma interaction remain partially understood. An emerging role in the tumor-stroma interplay is represented by two transcription factors called YAP ("Yes-associated protein") and TAZ ("Transcriptional co-activator with PDZ-binding motif") [186, 187]. These molecules act within cancer cells to orchestrate responses in stromal cells where they trigger signals aimed at the cancer cell growth. YAP and TAZ activation in cancer cells affects the characteristics of the tumor stroma, by modifying the composition and physical properties of the tumor extracellular matrix through the secretion of the matrix components [188]. Recognizing YAP and TAZ as key elements in the network of exchanged signals within the tumor microenvironment (TME) provides a new paradigm on the molecular principles of tumor self-organization, promising to reveal new and several interactions so far little understood [189]. The main functions played by YAP and TAZ in cancer cells and TME are summarized in the Fig. 2.6.

2.5 Conclusions

During the last years, progress in biotechnology, genomics, and molecular pathology determined improvements in understanding of tumor biology, leading to the discovery of several potential tumor biomarkers, suitable for clinical use [190]. The identification and development of molecular biomarkers in clinical oncology (e.g., KRAS in colorectal cancer, BRAF in melanoma, c-KIT and PDGFRA in gastrointestinal stromal tumor, and EGFR in lung cancer) as well as the advent of the immunotherapy have significantly modified the natural history of many tumors [191–194].

Additional molecular studies on individual cancer cells are needed to increase our knowledge about genetic variability of single cells present in several tumors and responsible for the complex question concerning cancer clonal evolution during all stages of tumorigenesis. The growing knowledge of the natural history of cancer and specific hallmarks of its phases are increasingly leading to the development of new and more specific strategies of prevention and management of tumors.

Key Points

- Tumors are not uniform diseases, but heterogeneous entities;
- The variability observed within individual tumors, known as intra-tumour heterogeneity, represents the crucial step in cancer clonal evolution process;
- Two theoretical models are used to elucidate the origin of tumor heterogeneity: clonal evolution model and cancer stem cell hypothesis;

2

- Most of tumors at the early stages of the disease usually shows a single clonal origin;
- Tumorigenesis may be induced and driven by intragenic mutations called “driver gene” mutations;
- Cancer is a systemic disease characterized by a multistep process including rapid proliferation, apoptosis inhibition, neoangiogenesis, local invasion, and metastasis;
- The cancer clonal evolution model hypothesizes that tumor progression and diversity are driven by the genetic drift and natural selection;
- Tumor evolution can occur through four different modalities: linear evolution, clonal separation, clonal competion, and clonal cooperation;
- The factors responsible for the development of cancer can be categorized into endogenous (or genetic) and exogenous (or environmental);
- The natural history of cancer includes three major step described during carcinogenesis: initiation, promotion, and progression;
- Different agents involved in the natural history of a tumor are classified into 1) incomplete carcinogens or initiators, 2) promoters, and 3) complete carcinogens;
- The identification of molecular biomarkers in clinical oncology as well as the advent of the immunotherapy have significantly modified the natural history of many tumors.

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Histopathology of the Tumors

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Contents

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Learning Objectives

By the end of the chapter, the reader will

- Be familiar with the basic concepts of histopathology of tumors
- Be able to recognize the hallmarks of malignant tumors
- Be able to integrate acquired knowledge into clinical practice

3.1 Definition

Neoplasia is an abnormal and uncontrolled cell growth; a mass of tissue that derives from this uncontrolled growth is termed *neoplasm* or *tumor* [1]. *Cancer* is the term commonly used to indicate malignant neoplasms, and the origin of the word dates back to the fourth century BC, when Hippocrates used the terms "carcinos" and "carcinoma" to describe non-ulcer-forming and ulcer-forming tumors [2]. *Cancer* comes from the Greek and Latin words referring to crab, because the swollen veins or the spreading projections from a malignant neoplasm looked like the limbs of a crab. The ability to invade adjacent tissues or spread to distant sites is, in fact, the leading feature that differentiates malignant from benign tumor. Generally, the terms *benign* and *malignant* refer to the clinical and biological behavior of a neoplasm as well as some specific morphological features. However, morphology does not always correlate with clinical course, i.e., *meningiomas*, benign tumors of meninges, may have malignant presentations and be lethal, depending on the size and location. Conversely *basal cell carcinoma*, a malignant skin tumor, is slow growing and locally aggressive but rarely metastasizes.

Benign and malignant tumor can be differentiated according to some main morphological features:

- Differentiation
- Modality of growth
- Rate of growth
- Metastasis

Differentiation describes the processes by which immature cells become mature, with specific functions [1]. As far as tumor cells, the term refers to how much the neoplastic population resembles the normal tissue: benign neoplasms are usually well-differentiated, whereas malignant neoplasm can range from well- to poorly differentiated.

3.2 Benign Neoplasms

The distinctive features of benign neoplasm are the lack of invasion of the surrounding tissues and the absence of metastases. As far as the modalities of growth are concerned, they have an expansive growth pattern in parenchymatous organs and an exophytic growth in hollow organs (Fig. 3.1). The formation of a connective tissue capsule may be observed, as a consequence of the compression atrophy of surrounding normal tissues (Fig. 3.2). Benign neoplasms are well-differentiated and closely resembling the corresponding cells of normal tissue they derive from; they have generally a slow growth rate, with a low number of mitosis (Table 3.1).

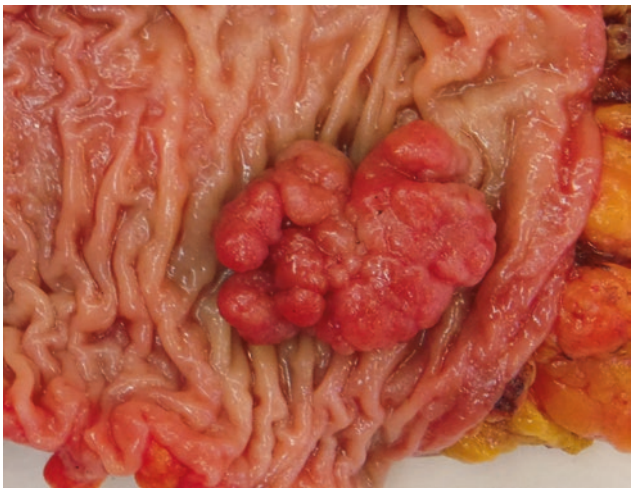

Fig. 3.1 Example of benign exophytic growth in hollow organs: adenomatous colonic polyp

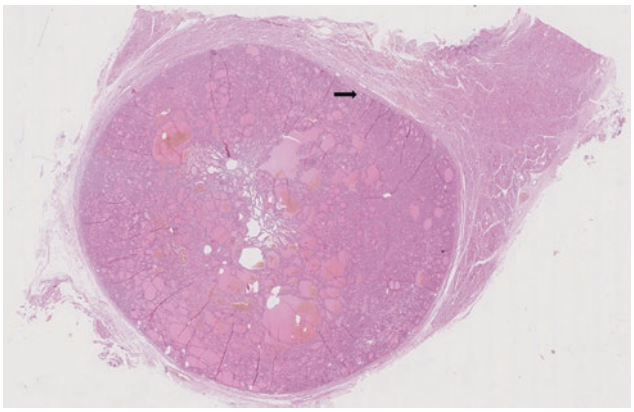

Fig. 3.2 Follicular adenoma of the thyroid: the benign neoplastic nodule is demarcated from the adjacent parenchyma by a thin, intact fibrous capsule

3.3 Malignant Neoplasms

Malignant neoplasms have the capability to invade and destroy surrounding tissue and metastasize to distant tissues [3, 4]. The diagnosis of malignancy is based on the assessment of various histopathological hallmarks (Table 3.1).

Modality of growth: The growth is usually chaotic and disorganized, with loss of polarity of tumor cells compared to the organization of the normal tissue of origin. The growth is characterized by the tendency to tissue invasion, with an infiltrative growth pattern in parenchymatous organs (Fig. 3.3); in hollow organs malignant neoplasms have the appearance of infiltrative plaques or ulcerative lesions (Fig. 3.4). Blood vessels are an essential component of neoplastic tissue, as they provide metabolic means and routes for metastatic expansion; tumor vessels tend to form tortuous networks with irregular branching patterns [5]. If neoplastic expansion is massive and fast, blood supply may be insufficient and central areas may undergo ischemic necrosis (Fig. 3.5).

Differentiation: Lack of differentiation is a distinctive feature of malignant neoplasms that can range from well- to moderately and poorly differentiated; undifferentiated tumors are defined "anaplastic" (anaplasia = loss of differentiation). *Pleomorphism* is a distinguish feature of lack of differentiation that consists in variation of shape and size of both cells and nuclei; *anisonucleosis* is the specific term to indicate the nuclei shape and size variation (Fig. 3.6).

Characteristically, nuclear size is increased but undifferentiated malignant cells may have a small appearance (i.e., malignant small round cell tumors) [6]; however, in both cases nuclear-cytoplasmic ratio (N-C ratio) is increased. Marked pleomorphism can

Table 3.1 Histopathological features of benign and malignant tumors

Feature	Benign	Malignant
Differentiation	Well-differentiated	Well- to poorly differentiated
Growth pattern	Expansive	Infiltrative
Growth rate	Slow	Rapid
Invasion	Absent	Present
Metastasis	Absent	Present
Necrosis	Absent	Present
Pleomorphism	Usually absent	Often present
Anisonucleosis	Absent	Often present
Nuclear-cytoplasmic ratio	Normal	Increased
Hyperchromasia	Absent	Often present
Nucleoli	Not prominent	Prominent
Mitosis	Rare	Increased, atypical

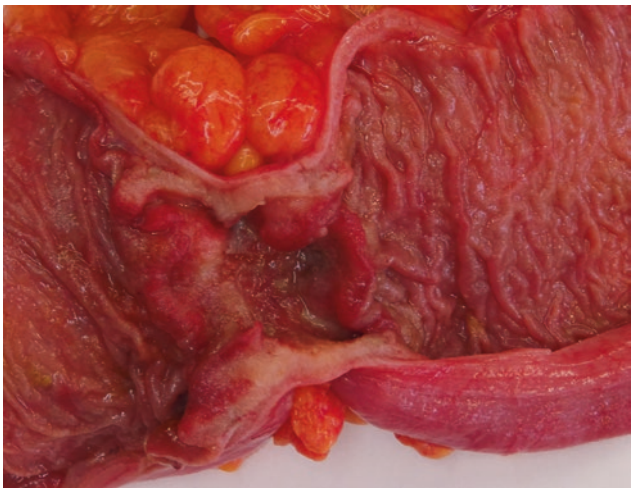

Fig. 3.4 Colorectal adenocarcinomas may have the appearance of infiltrative, ulcerated plaque

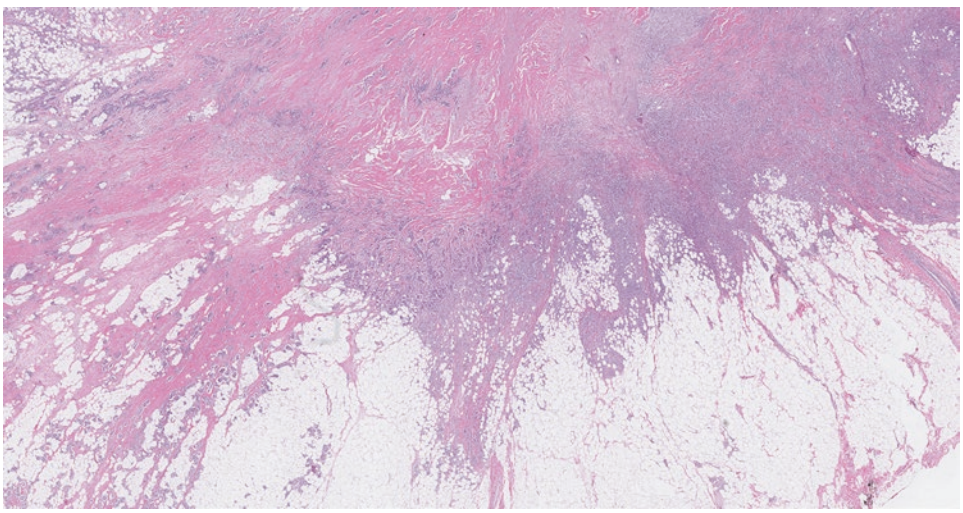

Fig. 3.3 Breast carcinoma: the growth of malignant tumor is characterized by the tendency to tissue invasion, with an infiltrative growth pattern in parenchymatous organs. Note the infiltration into breast adipose tissue

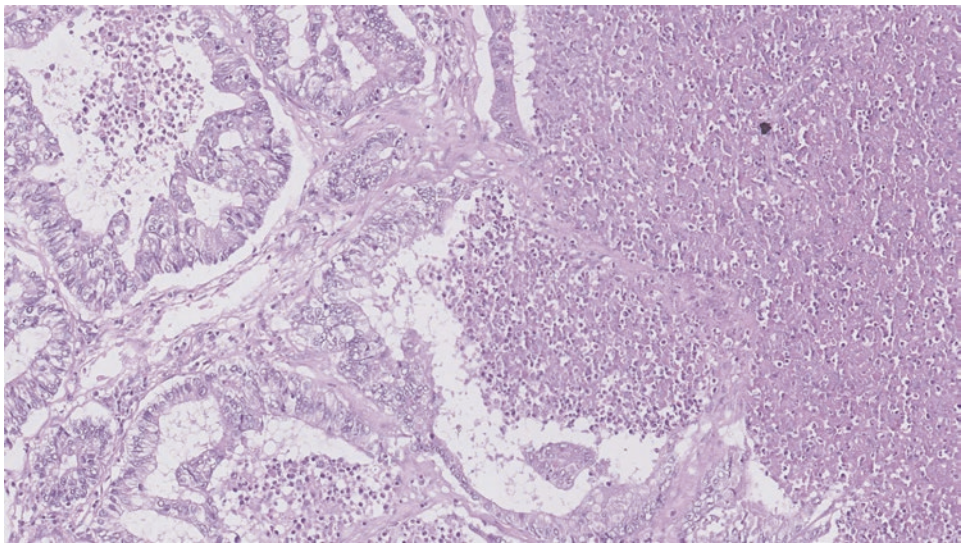

Fig. 3.5 Insufficient blood supply may cause ischemic necrosis of neoplastic central areas: a malignant neoplasm with a glandular growth pattern (on the left) and a large necrotic area (on the right)

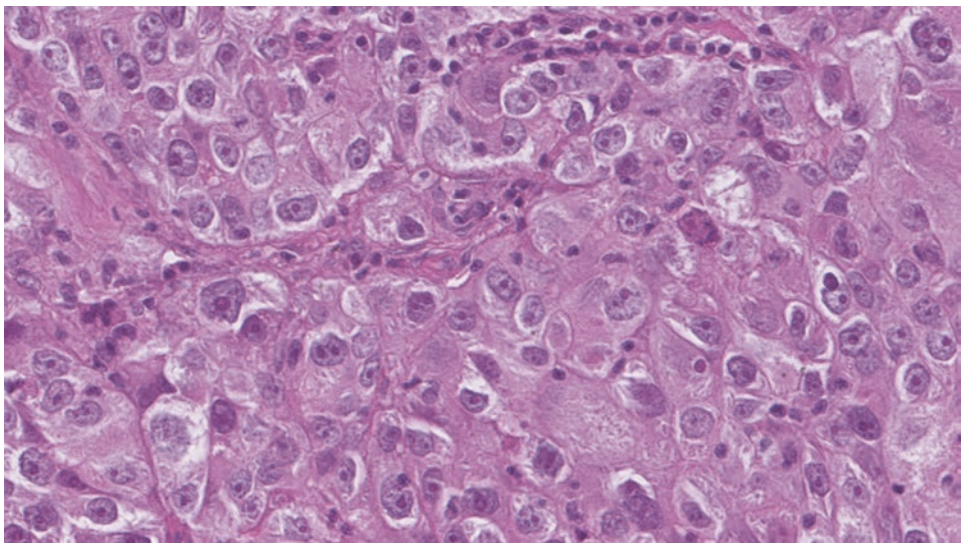

Fig. 3.6 Pleomorphism and anisonucleosis in a malignant tumor: note the variation of shape and size of cells and nuclei

lead anaplastic cells to assume the appearance of tumor giant cells, featured by the presence of a single, huge nucleus or multiple, bizarre nuclei (Fig. 3.7). Nuclear morphology is altered even with regard to nuclear chromasy: increased nuclear DNA content, resulting in a dark staining on hematoxylin and eosin (H&E) slides, is termed hyperchromasia. Otherwise chromatin may be coarse and clumped and distributed along the nuclear membrane (Fig. 3.8). Prominent, single or multiple nucleoli are usually present in malignant cells. Some types of cancer have hallmark nuclear alterations, i.e., papillary thyroid carcinoma (PTC) shows pale nuclei with powdery chromatin ("Orphan Annie" nuclei) and longitudinal nuclear grooves and intranuclear cytoplasmic inclusion, both expressions of the membrane irregularity (Fig. 3.9).

Mitotic activity: A high mitotic rate is a common feature of benign and malignant tumors, but also of hyperplasia, and reflects the higher proliferative activity of a cell population. Instead, the presence of atypical mitosis is a hallmark of malignancy. Normally, mitotic cell division occurs in a bipolar manner; however, in cancer cells, an excessive number of centrosomes may cause creation of supernumerary spindle poles [7, 8], which can result in multipolar mitosis (tripolar, quadripolar, bizarre mitotic figures) (Figs. 3.10 and 3.11). In several cancer types, the tumor mitotic rate is a significant independent prognostic factor (i.e., melanoma, neuroendocrine tumors) [9, 10].

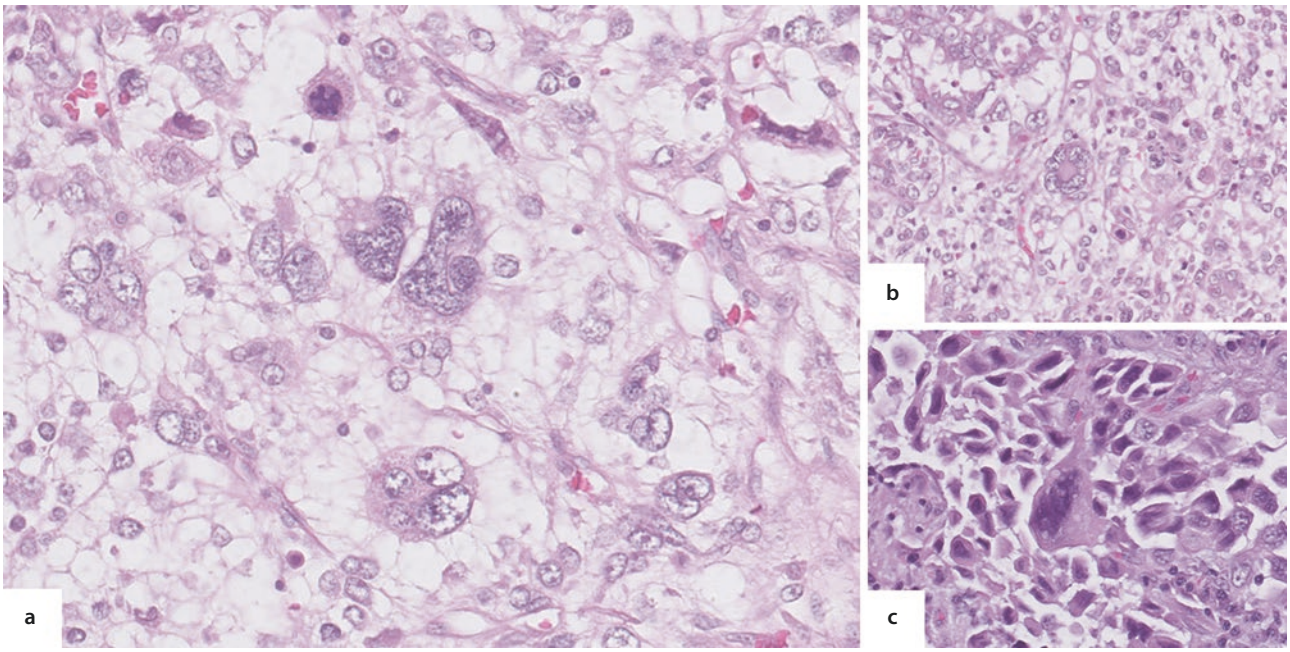

Fig. 3.7 Anaplastic tumor: malignant cells may assume the appearance of bizarre giant cells **a**. Tumor giant cells can be characterized by presence of multiple nuclei **b** or a single huge nucleus **c**

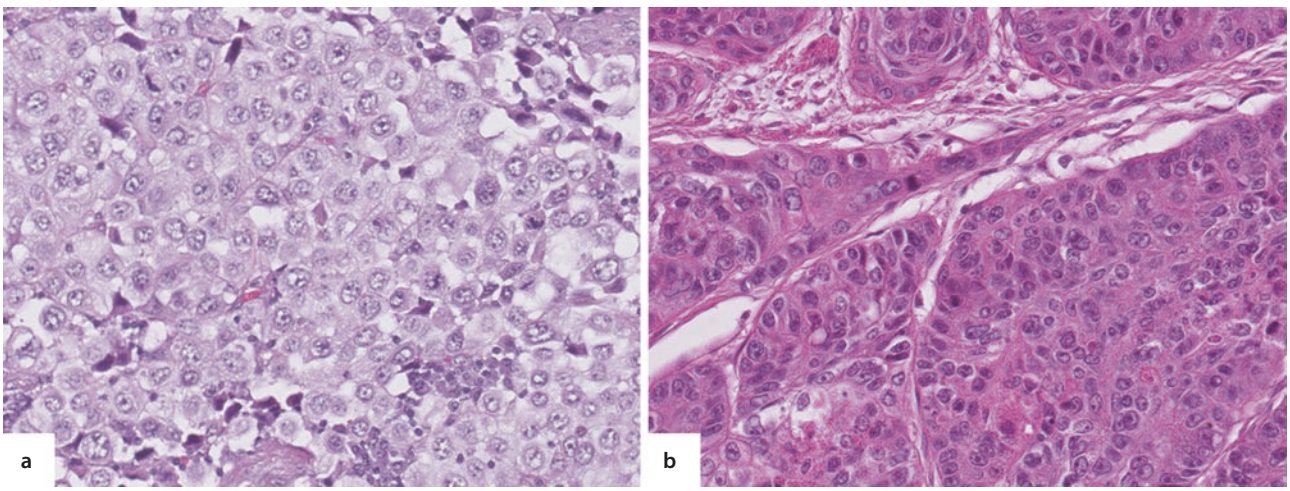

Fig. 3.8 Alteration of nuclear chromasy in malignant tumor: chromatin may be coarse, clumped, and distributed along the nuclear membrane, giving a pale appearance to the nucleus **a** or darkly stained, giving hyperchromasia to the nucleus **b**

3.4 Dysplasia

The term *dysplasia* refers to an anomaly of growth and differentiation, typically in epithelia. Dysplasia is characterized by some pathological microscopic features, namely, increase in thickness, architectural disorder, pleomorphism, nuclear enlargement with hyperchromasia, and presence of increased number of mitoses; mitoses are also present in abnormal locations and may be observed in superficial layer rather than exclusively in the basal epithelial zone [11]. These architectural and cytological atypia do not exceed basement membrane but represent a predisposition for progression to invasive neoplasia: dysplasia is a preneoplastic lesion. However, the progression to cancer is not changeless, and mild and moderate dysplasia may be reversible by removing the triggering cause [12].

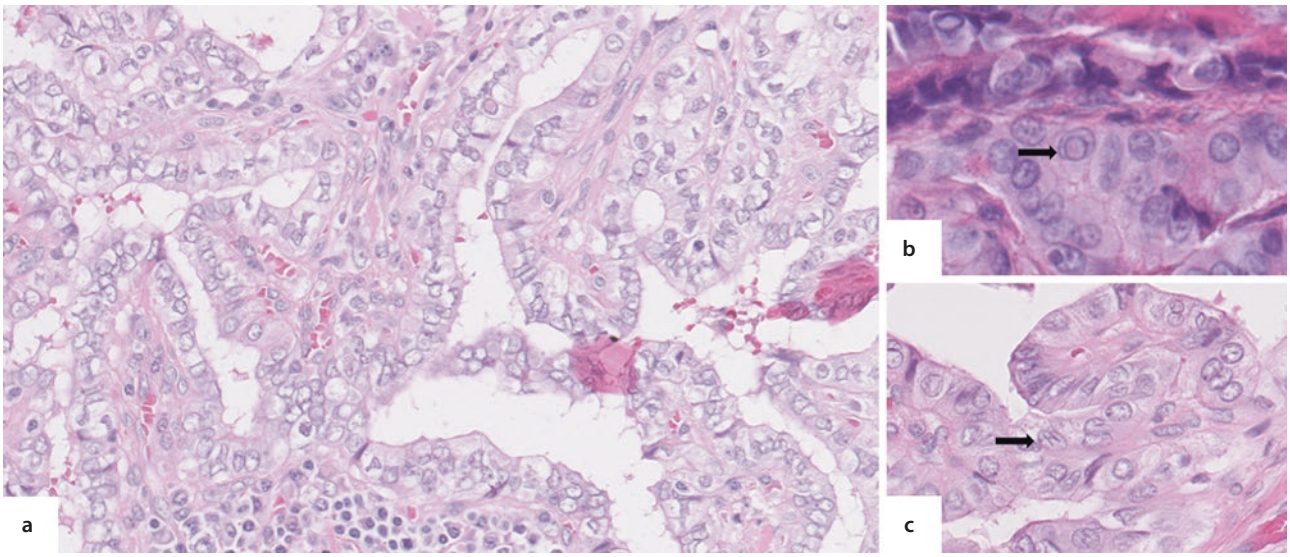

Fig. 3.9 Nuclear hallmarks of papillary thyroid carcinoma: note pale nuclei with powdery chromatin ("Orphan Annie" nuclei) **a**, intranuclear cytoplasmic inclusion (**b**, arrow), and longitudinal nuclear grooves (**c**, arrow)

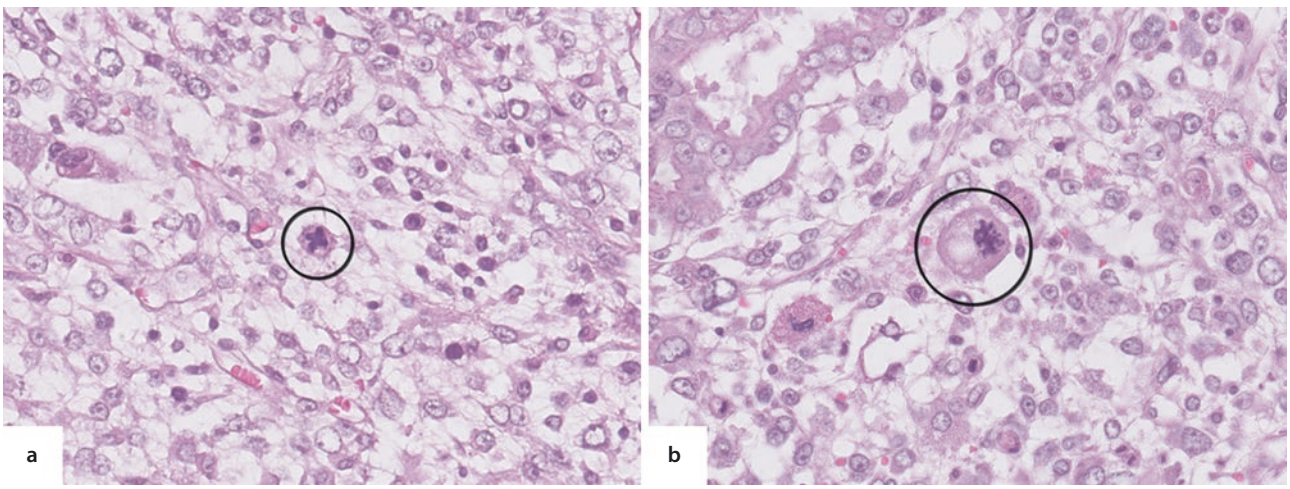

Fig. 3.10 In poorly differentiated malignant neoplasms, an increase in the number of mitoses can be observed, even with an atypical appearance, such as quadripolar (A) or bizarre (B) mitotic figures

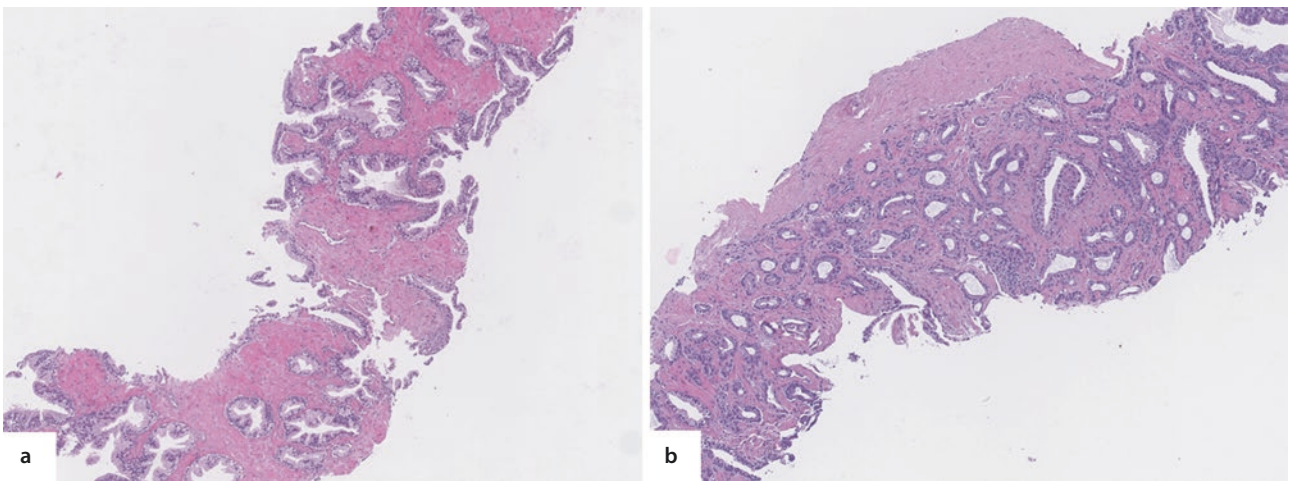

Fig. 3.11 The Gleason grading system is based on the assessment of glandular differentiation: compared to the normal prostatic tissue **a**, neoplastic glands are typically smaller and more packed **b**

Dysplasia is generally graded as "mild," "moderate," and "severe," depending on the extent and severity of morphological changes, and these criteria are generally applicable to the epithelia of all districts:

Mild dysplasia: This is characterized by proliferation of basal and parabasal cells limited to the lower third of the epithelium. Cytological and architectural atypia are minimal and mitoses are not prominent.

Moderate dysplasia: This involves the lower half of the epithelium with loss of basal polarity; stratification and maturation are preserved. The cytological changes are more prominent and increased; atypical mitoses may be present in the basal layers.

Severe dysplasia: Architectural and cytological changes can be very prominent, extending from the basal layer into the upper third of the epithelium. Suprabasal layer mitoses are usually present, even featuring atypical mitotic figures.

Carcinoma in situ is defined as severe dysplasia involving the entire thickness of the epithelium but being still confined to the normal tissue. The invasion of basement membrane defines the lesion as invasive carcinoma.

3.5 Grading

Pathological grading is a qualitative assessment that refers to the degree of differentiation of tumor cells and expresses it through a score. The most common grading system uses a four-grade score, depending on the degree of anaplasia: grade 1 tumors are well-differentiated and, although atypical, neoplastic cells resemble parent tissue. Conversely, grade 4 tumors are so anaplastic that even the recognition of their cell of origin becomes difficult; grade 2 and 3 tumors have intermediate features [13].

For many cancer types, site-specific grading systems are used, based on different pathological features.

Prostate cancer: The most widely used grading scheme worldwide is the Gleason system [14, 15]. The Gleason grading system is based on the histologic pattern of arrangement of carcinoma cells in H&E-stained prostatic tissue sections. The method assesses the glandular differentiation (neoplastic glands are typically smaller and more packed than benign glans) (Fig. 3.12) and the histologic pattern of growth of the tumor in the prostatic stroma, assigning a grade pattern from 1 to 5:

Gleason pattern 1: very well-differentiated growth of closely packed but separate, uniform, rounded to oval, medium-sized acini.

Gleason pattern 2: increase in variability in gland size and shape. The glands are not as circumscribed as pattern 1.

Gleason pattern 3: well-formed, individual glands of various sizes, including branching glands.

Gleason pattern 4: includes poorly formed, fused, and cribriform glands.

Gleason pattern 5: individual cells and cords or sheets of cells; solid nests of cells with occasional gland space formation are observed. Necrosis may be present.

The primary grade pattern (the most common seen in the tumor) and the secondary grade pattern are used to generate a histologic score, which can range from 2 to 10; each score falls into prognostically relevant Grade Groups.

Breast cancer: The most common grading system for breast cancer is the Nottingham Histologic Score system (Elston-Ellis modification of Scarff-Bloom-Richardson grading system) [16]. This method evaluates three morphological features (Fig. 3.13):

- Amount of gland formation
- Nuclear features (variation in size and shape, chromatin appearance)
- Mitotic activity

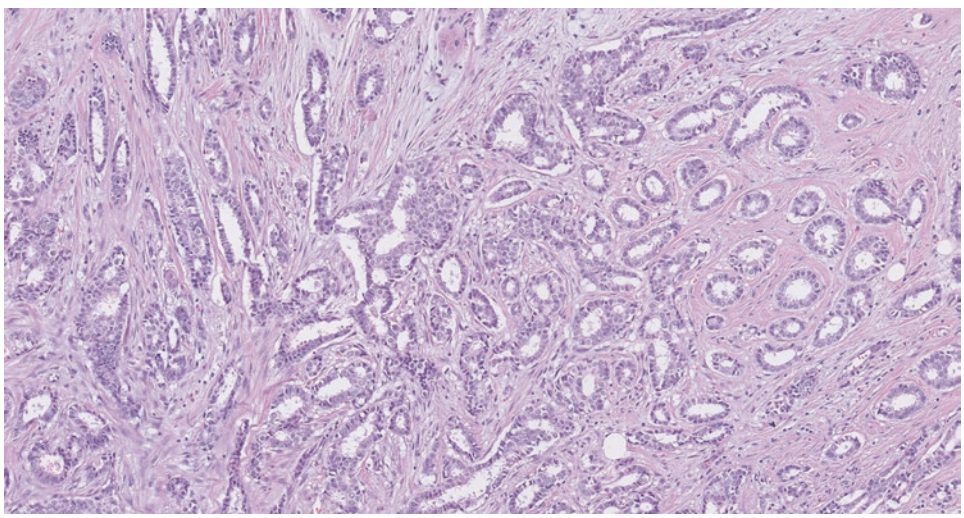

Fig. 3.12 Breast ductal carcinoma grade 1 s. Nottingham Histologic Score system: evident glandular formation, bland nuclear atypia, and low mitotic rate

Each of these features is scored from 1 to 3, and then each score is added to give a final total score ranging from 3 to 9. The final total score is used to determine the grade:

- Grade 1: score of 3–5
- Grade 2: score of 6–7
- Grade 3: score of 8–9

Malignant neoplasms are often characterized by morphological and phenotypic tumor heterogeneity, and then areas with different grade of differentiation may be present; if there is evidence of heterogeneity, the highest grade must be considered and reported.

The prognostic impact of grading is noticeable for some tumors [14, 17, 18] (i.e., sarcoma, breast and prostate carcinoma), but generally there is no direct correlation between pathological grading and clinical behavior.

3.6 Staging

Stage refers to the extent of cancer in the body and is a fundamental prognostic factor, which affects the therapeutic approach. Among the various existing cancer staging systems, the most clinically exploited is the tumor (T), node (N), and metastasis (M) staging system, developed by AJCC (American Joint Committee on Cancer) and UICC (Union for International Cancer Control) [19]. The AJCC TNM staging system provides both clinical and pathological assessment of tumor extension: the clinical stage (cTNM) is based on physical examination and imaging study information (ultrasound, computed tomography, magnetic resonance, positron emission tomography, etc.) and is integrated and/or modified by pathological evaluation of the resected specimens (pTNM). In the pTNM assessment:

- The *T* refers to the size and extent of the main tumor, measured to the nearest whole millimeter; size may be adjusted based on microscopic examination. pTis is assigned to in situ *neoplasia* identified by microscopically examination of a surgical resection .
- The *N* refers to the number of nearby involved lymph nodes. Microscopic assessment of a node may be performed by fine needle cytology (FNC), core biopsy, excisional biopsy, and regional lymph node dissection. Many cancer types have specific recommendation regarding the minimum number of lymph node to be evaluated to provide prognostic information (i.e., colon cancer).
- The *M* refers to the presence of distant metastases, spatially separated from the tumor. Direct extension of a primary tumor into a contiguous organ is classified as part of the tumor and not as metastasis.

An example of specific staging system for a single neoplasm is represented by the Ann Arbor staging system [20] for Hodgkin lymphoma (HL): the stage is mainly determined by location of the tumor (single or multiple regions, both sides of the diaphragm, extralymphatic organ involvement) and presence of constitutional symptoms. Other pathological features considered are the extension from the lymph node to adjacent tissue and presence of lesions >10 cm in diameter ("bulky" lesion).

3.7 Conclusion

The terms benign and malignant tumor refer to the clinical and biological behavior of a neoplasm as well as some specific morphological features including differentiation, modality, and rate of growth and metastatic capability. Fundamental prognostic factors are the qualitative assessment of the degree of differentiation of malignant tumor cells (grading) and the extent of cancer in the body (staging). Histopathological features of tumor should be integrated with physical examination and imaging study information for an accurate diagnosis and a proper patient management.

Summary of Clinical Recommendations

- Histopathological features of tumor should be integrated with clinical and imaging data for an accurate diagnosis and a proper patient management.
- The pathologist's decision-making process should be guided by evidence-based guidelines and consensus recommendations.
- The College of American Pathologists (CAP) provides guidelines for collecting the essential data elements for complete reporting of malignant tumors (Cancer Protocol Templates).

Key points

- Benign and malignant tumor can be differentiated according to differentiation, modality of growth, rate of growth, and metastatic capability.
- Malignant neoplasms have the capability to invade and destroy surrounding tissue and metastasize to distant sites.
- Histopathological features of tumor should be integrated with clinical and imaging data for an accurate diagnosis and a proper patient management.

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Biomarkers

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Contents

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Learning Objectives

By the end of the chapter the reader will:

- Have understood the basic concepts of using biomarkers
- Be able to discriminate between different types of biomarkers
- Have reached a good knowledge in the role of the most important biomarkers
- Be able to put into clinical practice the acquired knowledge in biomarkers

4

4.1 Introduction

In the era of personalized medicine, biomarkers represent an irreplaceable tool for cancer screening, diagnosis, and management. Even though only a minority has yet entered clinical practice, the list of potentially reliable molecular biomarkers grows longer every day.

In 1993, World Health Organizations (WHO) stated that a definition of biomarker can include "almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction," while in 1998 the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." In 2001, the International Program on Chemical Safety considered one more definition of biomarker: "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease."

In oncology, biomarkers play an important role in both the detection and management of patients affected by different types of cancers [1]. Any biological signal that can be linked to a neoplasm could be considered a biomarker, although this widely unspecific definition could refer to many different and heterogeneous classes of biological markers. In clinical practice, the term "oncological biomarker" is usually referred only to those molecules that are expressed or produced by either tumor cells or the surrounding microenvironment and play an important role in regulating disease progression.

Blood represents an extremely informative window to assess multi-organ biological responses to environmental stimulations and to detect specific markers associated with the development of the disease: it comes in contact with every organ in the body to convey information, deliver nutrients, carry waste, and survey the homeostatic status of tissues. So far, evaluation of new biomarkers from the peripheral blood has surprisingly proven to be harder than expected: this may be due to the limited presence of most of them, their high molecular complexity and instability, and the diverse origin of biomolecules in different organs and cell types.

Historically, oncological therapy has been empirically based on the histological features of the tumor, on the clinical experience of the physicians, and on the published literature.

With the diffusion of evidence-based medicine and the development of new drugs and therapeutic schedules, the clinical need of identifying outcomes to be used in large clinical trials has become compelling. However, since the 1980s the use of biomarkers as surrogate outcomes in large trials of major diseases, such as cancer and heart disease, has been widely discussed [2, 3].

Furthermore, other perspectives have been recently emerged on the usefulness of biomarkers. The oncological research has led to a very deep knowledge of cancer cells and their regulatory mechanisms, allowing to identify several pathways and driving mutations that play important roles in the cancer pathogenesis (Fig. 4.1). Thus, it is nowadays feasible to study the efficacy of a new drug not on the basis of the originating tumor tissue histology but on the basis of the molecular biomarkers and gene mutations expressed in a variety of tumor types.

The discovery of new biomarkers enabled physicians to switch from empirical therapy to a personalized medicine (so-called precision medicine), with drugs acting against specific biomolecular targets (Fig. 4.2). Biomarkers allow to identify the molecular profile of the disease, helping clinicians to select those patients that can mostly benefit from specific therapies.

The huge variety of blood tumor markers includes the wide range of biomarkers spanning from the basic changes of blood tests to the detection of plasma levels of both circulating tumor cells (CTC) and circulating tumor DNA (ctDNA) as well as the diagnostic relevance of circulating miRNAs [4].

The determination of *validity* and *relevance* is necessary to consider biomarkers useful into the clinical practice (Fig. 4.3).

- Validity can be divided in:
  - Analytical validity: the ability, inherent to the methodic, to accurately, reproducibly, and reliably measure the biomarker as an objective and quantifiable value
  - Clinical validity: the test's ability to predict or evaluate the evidence of the disease (or a clinically relevant tumor feature)
- Clinical utility: likelihood to improve a clinical outcome by using the test, based on the level of clinical evidence provided by literature and guidelines; it represents the ability to improve, i.e., overall survival or disease free survival.

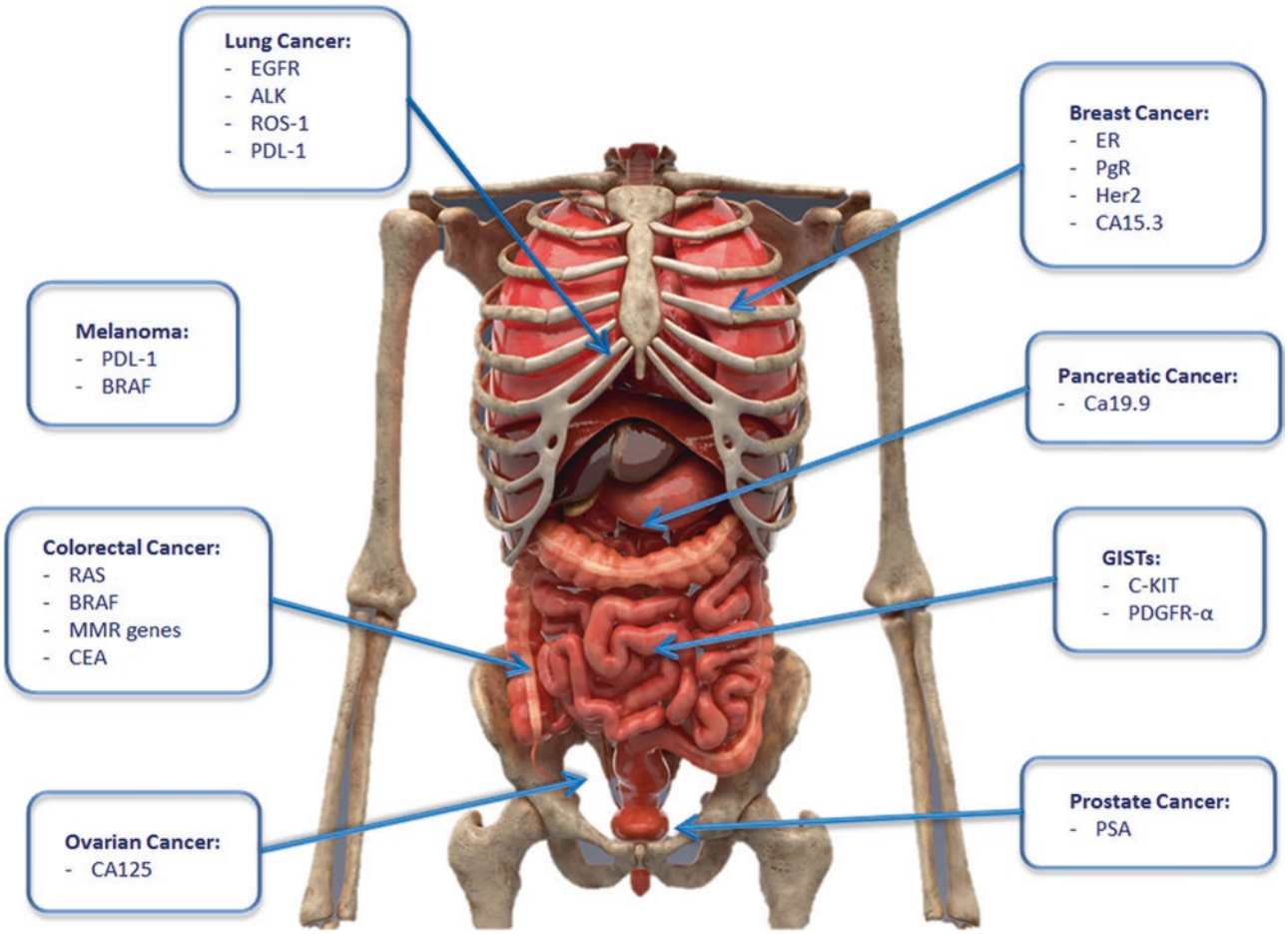


Fig. 4.1 Examples of the most relevant biomarkers used in today's clinical practice, according to the primary sites of cancers

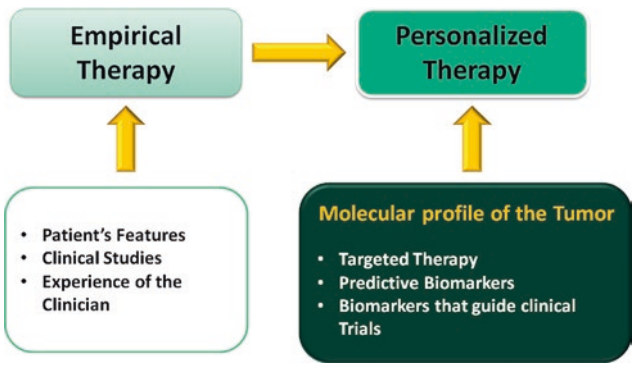


Fig. 4.2 Oncology has shifted from empirical therapy to personalized therapy

Biomarkers can be divided in five categories (Figs. 4.4, and 4.5):

- Risk markers: evaluate the risk of developing cancer in high-risk healthy subjects.
- Prognostic markers: able to stratify patients in different risk classes according to a specific outcome.
- Predictive markers: provide data on the sensibility or resistance of the tumor to a specific therapy.
- Surrogate markers: assess the activity or efficacy of the treatment.
- Diagnostic markers: usually employed in screening programs or supporting diagnostic exams.

A new group of biomarkers whose use is increasingly emerging in clinical practice are agnostic biomarkers. According to NIH, tumor-agnostic therapy can be defined as "A type of therapy that uses drugs or other substances to treat cancer based on the cancer's genetic and molecular features without regard to the cancer type or where the cancer started in the body. Tumor-agnostic therapy uses the same drug to treat all cancer types that have the genetic mutation (change) or biomarker that is targeted by the drug" (▶ https://www.cancer.gov/publications/dictionaries/cancer-terms/def/796871). Traditional oncology follows an established paradigm, utilizing a specific drug in individual tumor types that have shown to be sensitive to it during randomized clinical trials (RCT). Biomarkers are usually seen as a way to further select patients and to define subgroups more sensitive to the treatment [5]. The recent development of new technologies (i.e., high-throughput

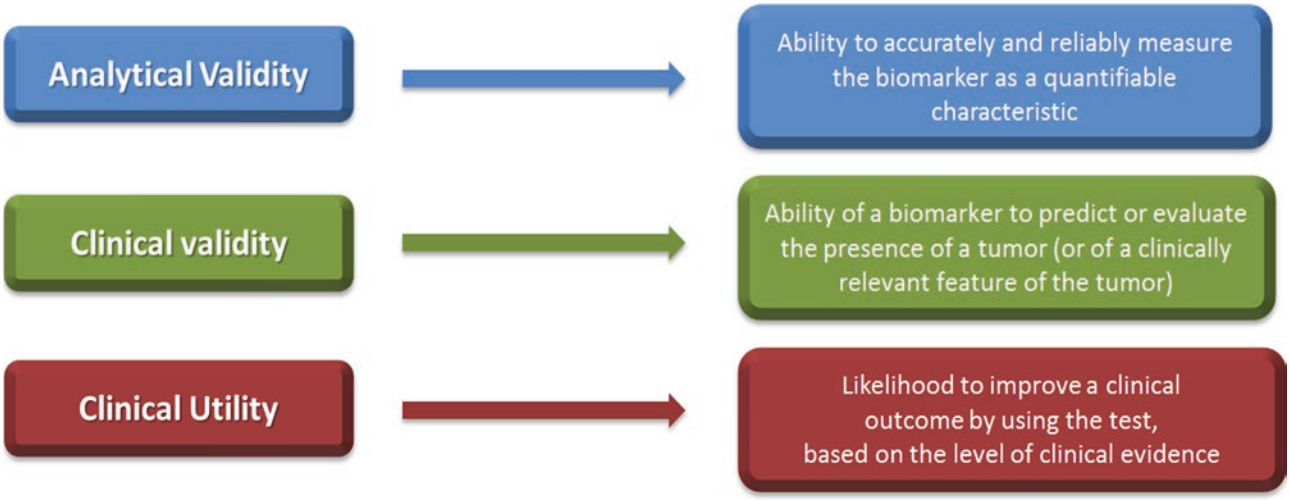


Fig. 4.3 Main features of a reliable and well-performing biomarker

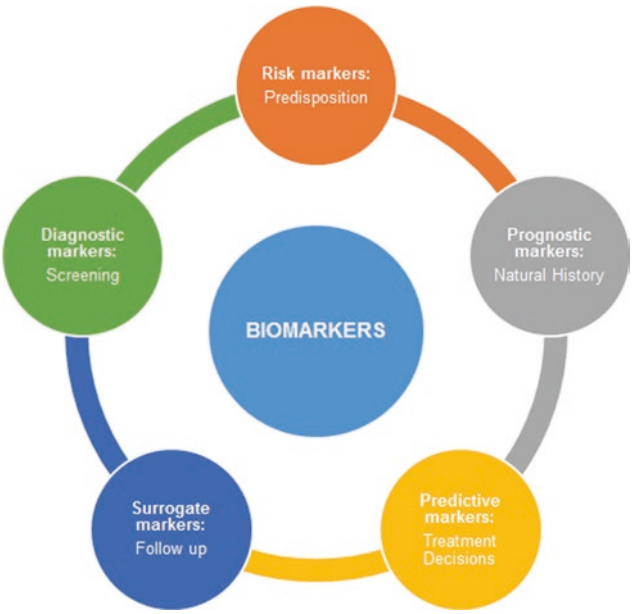


Fig. 4.4 Types of biomarkers and their use in oncology

next-generation sequencing, NGS), together with the improvements in our knowledge in genomics, has led to a rapid change in the approach to the oncological treatment and an evolution in the concept of "precision medicine." The new paradigm of mutational oncology recognizes the relevance and importance of histological and morphological characterization of the tumor but aims to guide the drug selection on the basis of genetic profiling and of actionable mutations found, independently of the tumor histology. This has led to a new kind of drug approval, defined as "agnostic approval": the drug can be administered in every patient in which the specific actionable mutation can be found, independently of tumor histology [6].

The first drug to receive tumor-agnostic approval was pembrolizumab in May 2017 when FDA granted accelerated approval for the treatment of adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or dMMR solid tumor [7]. Subsequently, larotrectinib and entrectinib, tyrosine kinase inhibitors targeting the tropomyosin receptor kinase (TRK) proteins (encoded by the neurotrophic tyrosine receptor kinase genes NTRK), become the second and third drug to receive tumor-agnostic FDA approval, respectively, in November 2018 and in 2019 [6].

These drugs are approved for patients with NTRK-positive advanced solid tumors [8–10]. Usually RCT enroll patients on the basis of tumor histology, but the evolution in tumor genomics have changed also our approach to clinical trials design too, aiming to allow the selection of treatment based on specific molecular biomarkers. With the growth of the number of known actionable mutations, we are discovering that the same genomic alterations can occur across various tumor types, albeit at low frequencies. For this reason Basket trials have been implemented: in this kind of trial eligibility is based on the presence of a specific genomic alteration, irrespectively of tumor histology [11].

4.2 Diagnostic Markers

A diagnostic biomarker is a factor that contributes, together with other tools (such as imaging techniques), to the oncological diagnosis, allowing to obtain an earlier and more precise characterization of the disease, and to better assess its aggressiveness and staging.

In recent years, the development of new technologies along with new laboratory techniques and progresses in basic, translational, and clinical research has opened

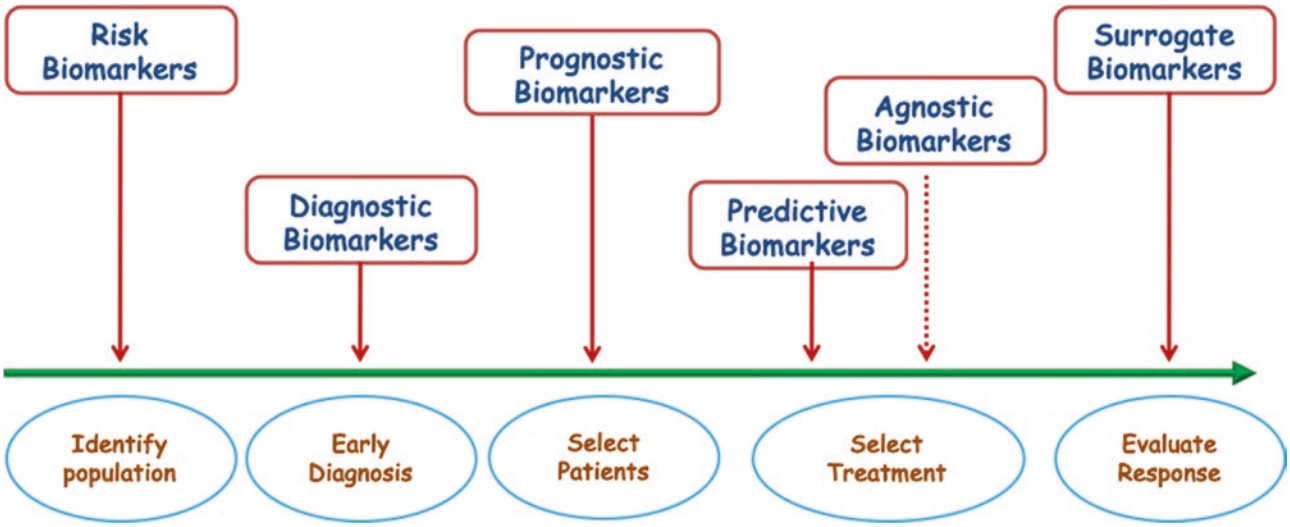
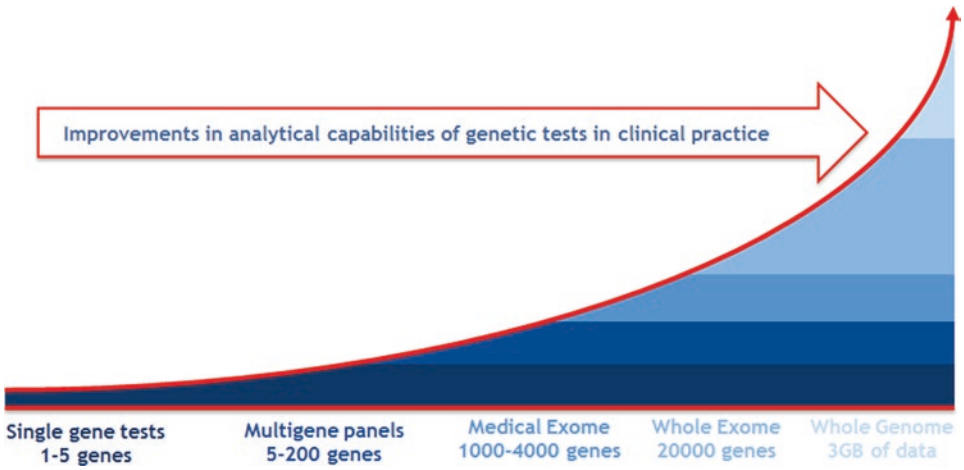


Fig. 4.5 The role of biomarkers during the natural history of the disease

Fig. 4.6 Improvements of analytical capabilities of genetic tests



new scenarios for cancer diagnosis: understanding the molecular bases of cancer pathogenesis has significantly improved and consequently been translated into clinical practice (Fig. 4.6). During the development and evolution of a cancer cell, several molecular alterations occur, including DNA, mRNA, miRNA, and proteomic alterations. These aberrations can vary not only according to the tumor origins but also to its degree of differentiation and metastatic propensity. Current methodologies for cancer diagnosis have incorporated these molecular changes into the cancer diagnostic realm, bringing to the so-called "omic" revolution: (whole) genome (WGS), exome (WES), methylome, transcriptome (including the miRnome), microbiome, metabolome, proteome, and topome, and the development of a new field called "molecular onco-diagnostics" [12, 13]. To date, a paradigm shift has occurred, and cancer diagnosis is no longer based only on morphological and histological parameters. New biomolecular platforms are now available for clinical use in cancer diagnosis, such as qualitative PCR-ARMS and RFLP, real-time PCR-TaqMan assays, nested PCR, FISH, capillary electrophoresis, sequencing/pyrosequencing, sequenom, targeted gene panel sequencing, and microarrays [14]. As a matter of fact, today molecular and biological analyses can help improving diagnostic capabilities leading to an earlier and more accurate diagnosis. Molecular alterations detected by the recently developed high-throughput technologies have become an integral part of the diagnostic armamentarium for the ultimate patients' benefit [15]. Both genetic and epigenetic alterations have been considered as good markers for the detection of ctDNA, while molecular and genetic panels can supply specific biological patterns of different cancers, providing a so-called signature for a specific tumor [16, 17].

Several circulating proteins represent potential diagnostic biomarkers in solid tumors, eventually with prognostic implications as discussed below. Nevertheless,

Table 4.1 Circulating diagnostic biomarkers

Markers	Characteristics	Tumors
AFP (alpha-feto-protein)	Glycoprotein	Hepatocarcinoma, embryonal carcinoma, yolk sac tumor, teratoma, mixed germ cell tumor
β-hCG (Human chorionic gonadotropin)	Glycoprotein	Embryonal carcinoma, choriocarcinoma, seminoma
		Hydatidiform mole
CA15-3 (Cancer antigen 15-3)	Soluble form of mucinous transmembrane glycoprotein MUC-1	Breast
CA19-9 (Carbohydrate antigen 19-9)	Soluble form of mucinous transmembrane glycoprotein	Colorectal, stomach, pancreas, biliary tract
CA125 (Carbohydrate antigen 125)	Soluble form of mucinous transmembrane glycoprotein MUC16	Epithelial ovarian, endometrial, cervical
CEA (Carcinoembryonic antigen)	Transmembrane glycoprotein	Colorectal, breast, cholangiocarcinoma, ovary, pancreas
Chromogranin A	Glycoprotein	Neuroendocrine
HE4 (Human epididymis protein 4)	Glycoprotein	Epithelial ovarian
		Lung (non-small cell, small cell)
NSE (Neuronal specific enzyme)	Glycolytic enzyme	Neuroendocrine
PSA (Prostatic specific antigen)	Glycoprotein	Prostate

their role is often controversial, and only a limited few should be used as a decision-making tool in clinical practice. Hence, we will extensively discuss only CA125 and prostate-specific antigen (PSA) for their preeminent role in ovarian and prostate cancer management, respectively. The other main circulating biomarkers and their characteristics are listed in Table 4.1.

CA125 (also known as MUC16) is a member of the mucin family, a group of proteins generally located on the surface of epithelial layers where they form a protective barrier against pathogens. In clinical practice, CA125 is useful for the management of epithelial ovarian cancer. In healthy women, CA125 serum levels are usually <35 U/ml. However, CA125 elevations may be found in nonmalignant conditions such as the follicular phase of the menstrual cycle, pelvic inflammatory disease, and liver disease (hepatitis or cirrhosis) [18]. Furthermore, increased CA125 values have also been reported in patients with non-ovarian malignancies, which include lung, breast, stomach, pancreatic, and colorectal cancers. Therefore, increasing CA125 levels may generate false positive results. CA125 displays limited sensitivity in detecting early ovarian cancer (OC) as serum levels increase in only 50% of patients with early stage disease [19]. Therefore, CA125 is not currently recommended for OC screening. However, CA125 levels may reflect the tumor burden. Hence, this biomarker may ascertain if patients receiving neoadjuvant chemotherapy (NAC) have achieved an optimal disease reduction or if they should be spared a futile surgical procedure. An initial meta-analysis evaluating the performance of preoperative CA125 in predicting adequate cytoreduction rates failed to demonstrate CA125 efficacy in advanced OC [20]. However, in a later study, Kang and colleagues showed that in patients with CA125 >2000 U/mL, the use of NAC followed by interval debulking surgery led to higher PFS than primary surgery (HR 0.5, CI 0.2–0.96; $p = 0.004$). While confirmation by additional studies is urgently needed, this result suggests that preoperative CA125 may be useful to guide physicians toward the most appropriate therapeutic approach (surgery or NAC) for OC.

Perioperative changes in CA125 have also been evaluated as a potential prognostic marker after optimal surgical debulking (<1 cm residual tumor). Chi et al. [21] demonstrated that perioperative changes in serum

CA125 may be associated with the risk of relapse in optimally resected stage IIIC patients as subjects with a "high decline" (>80%) in CA125 were at a lower risk of recurrence than those with inferior reductions or experiencing an increase in CA125 levels. As surgical removal of the primary tumor results in a rapid drop of CA125, complete biochemical remission (i.e., normalization of CA125 values) is the expected goal of any primary treatment (surgery with or without postoperative chemotherapy). Hence, the potential prognostic value of measuring the biomarker's nadir as suggested in a study by Van Altena and colleagues [22] that enrolled 331 OC patients with abnormal CA125 (>35 U/mL). The authors evaluated the CA125 nadir 1 month after surgery (in individuals receiving no chemotherapy) or 1 month after last drug infusion (in individuals subjected to chemotherapy). They found that a CA125 nadir >5 U/mL was associated with a higher risk of disease progression (HR = 1.5; 95% CI 1.0–2.3) [22]. In summary, CA125 is useful in the clinical management of epithelial OC in the diagnostic phase (but not for screening purposes) and may also be employed during therapeutic monitoring (prognostic and predictive value) and patient follow-up [23].

PSA, also known as human kallikrein 3, is an androgen-induced glycoprotein (its transcription is enhanced by the activated androgen), mainly produced by luminal prostate epithelial cells. Several conditions cause elevation of serum PSA: physiological processes like ejaculation or intense physical activity, diagnostic procedures such as digital examination or biopsy, benign diseases like hyperplasia and hypertrophy, prostatitis, or urinary retention. Conversely, 5-alpha-reductase inhibitors (finasteride, dutasteride) can lower PSA serum concentrations by approximately twofold [24]. To date, the role of PSA measurement for prostate cancer (PC) screening remains controversial. Two large studies and a Cochrane meta-analysis failed to demonstrate a survival benefit for men undergoing PSA screening showing increased overdiagnosis and overtreatment rates [25]. Hence, PSA should not be routinely used for PC screening in the overall population, as stated in the main international guidelines. Several methodological approaches have been considered in order to enhance PSA detection power. For example, in PC free PSA (fPSA) levels decrease, whereas complexed PSA increases, possibly because of impairment in PSA processing in the neoplastic tissue. Consequently the ratio between fPSA and total PSA (PSA index) is usually lower in PC displaying a higher sensibility than total PSA. Dynamic PSA measurements, such as PSA velocity and doubling time, provide information concerning marker changes over time. Nevertheless, their usefulness in early tumor detection lacks proper validation.

PSA measurement exerts a major role in PC management. Increases in PSA levels anticipate cancer relapse after surgery or definitive radiation therapy (RT), and no recurrence occurs without PSA elevation. Therefore, 6–8 weeks after radical prostatectomy PSA should be undetectable: levels ≥0.2 ng/mL, confirmed after 4 weeks, define biochemical recurrence (BR). By the same token, a PSA of 2 ng/mL above the nadir identifies BR after RT (Phoenix criteria); alternatively, three consecutive increases are needed (ASTRO definition) [26, 27].

PSA monitoring plays a major role during androgen deprivation therapy since its levels correlate with treatment responses. Thus, the deeper and faster is PSA nadir, the longer is the expected response duration. Moreover, disease progression is highly unlikely as long as PSA nadir is maintained. PSA significance in castration-resistant patients undergoing hormonal therapies (abiraterone, enzalutamide) is still under investigation, though it seems to correlate with both response and survival. Lastly, patients receiving chemotherapy for metastatic castration-resistant PC may experience PSA fluctuations, regardless of their treatment response: up to 20% present an initial PSA elevation, without clinical or radiological evidence of progression. However, a PSA drop (especially >50%) usually correlates with patient outcome [28].

4.3 Prognostic Markers

With the improvements of both surgical and clinical therapies for oncological patients, and the increasing survivals rates, it is crucial to eventually identify markers that could predict tumor's natural history and therefore select those patients who would need a more aggressive management.

The term "prognostic marker" refers to a factor (or multiple factors) that allows to stratify patients in different risk classes according to a specific clinical outcome, such as tumor progression or death. Accordingly, such a biomarker could significantly predict the natural history of the disease either in those patients who have not received any prior treatment or in those undergoing a systemic therapy. In other words, a prognostic biomarker is likely to inform about the disease outcome (e.g., disease recurrence, disease progression, death) irrespectively of treatment approach (Fig. 4.7).

In untreated patients, prognostic biomarkers reflect cancer biology, informing about disease outcomes; in pre-treated patients, while the benefit is similar in both biomarker-positive and biomarker-negative patients if the treatment resulted to be effective, the presence or absence of the biomarker may be still associated with a different outcome.

The different natural history of every single risk class could be visualized in a survival curve that shows how a

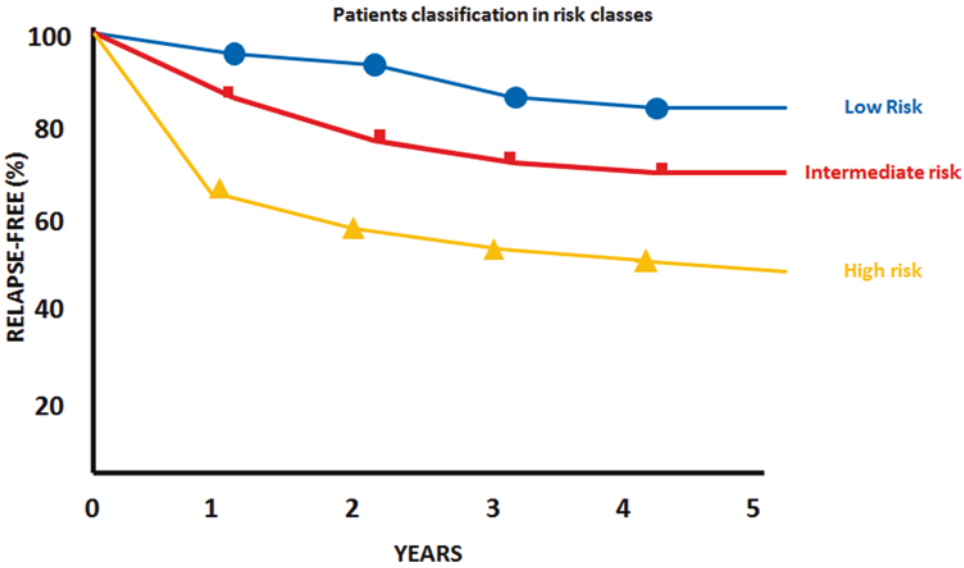


Fig. 4.7 Example of stratification in different risk classes according to a prognostic marker

prognostic biomarker might be able to stratify patients affected by an early stage of disease.

Adjuvant therapy is one of the settings that would benefit the most from the use of prognostic markers: the clinical utility of a prognostic marker relies mainly on the ability to select those patients who harbor a high risk of relapsing disease after surgery and therefore could mostly benefit from a postoperative treatment.

Examples of prognostic biomarkers are:

- CA125 and PSA, as discussed above.
- *PIK3CA* mutation status in women with HER2-positive metastatic breast cancer undergoing first-line therapy. In particular, women with tumors harboring a *PIK3CA* mutation appeared to a have worse progression-free survival when compared to *PIK3CA* wild-type patients. The *PIK3CA* mutation status is a prognostic variable since women with tumors harboring *PIK3CA* mutations used to present with worse prognosis regardless of treatment group [29].
- *BRAF* mutations (mainly V600E), acting immediately downstream of KRAS and associated with a relatively high frequency of microsatellite instability, seemed to define a molecularly specific subset (8–10%) of colorectal cancers, correlating with poor survival rates and thereby emerging as a negative prognostic marker [30]. In patients with CRC, a BRAF V600E mutation is associated with poor response and inferior survival to most systemic therapies. Furthermore, this genetic alteration is associated with a distinct pattern of metastasis, with a greater tendency to spread to the peritoneum and lymph nodes, and a lower probability of lung metastases [31].
- Amplification of the human epidermal growth factor receptor 2 (*HER-2*) gene has been regarded as a poor prognostic criterion, appearing to be associated with a more aggressive disease, poorer prognosis, and shorter overall survival in 15–20% of all breast cancers [32]. HER2 is a member of the tyrosine kinase receptor family structurally related to the EGFR that includes four members formerly known as ErbB1 (EGFR), ErbB2 (HER2), ErbB3 (HER3), and ErbB4 (HER4) [33]. HER2 is expressed in several tissues (the heart, breast, gastrointestinal tract, and kidney) where it promotes cell proliferation while suppressing apoptosis. Unlike other HER family members, HER2 lacks a direct ligand as its activation relies on heterodimerization with other HER proteins. After heterodimerization, HER2 stabilizes the ligand-receptor interaction thereby facilitating catalytic activation, tyrosine phosphorylation of selected substrates, and activation of downstream second messengers [34, 35]. Immunohistochemistry remains the "gold standard" for the evaluation of HER2 status with fluorescent in situ hybridization (FISH) employed to clarify cases showing intermediate levels (i.e., 2+) of HER2 expression. An additional method for measuring *HER2 gene* expression is quantitative real-time PCR that is characterized by high sensitivity and specificity yet is not currently in use in clinical practice [36]. Amplification or mutation of the *HER2 gene* has been reported in a number of cancers including breast carcinomas (approximately 17%), glioblastoma (7%), lung adenocarcinoma (4%), tumors of the gastro-esophageal junction and the stomach (6–29%), bladder cancer (9%), and colorectal cancer (7%).

4.4 Predictive Markers

With the advent of targeted therapies, clinical research has allowed to identify biological markers (Fig. 4.8) that could help to stratify patients according to the different benefit from a specific therapy. Drugs appeared not to show the same effectiveness nor the same adverse effects in all patients.

A predictive marker is a single factor (or a group of factors) associated with a response to a specific intervention. Additionally, predictive biomarkers can help clinicians to avoid the risk of drug-related toxicities, maximizing patient's benefit and minimizing the risk of adverse events with a more appropriate and effective use of drugs (Fig. 4.9).

Albeit not apparently correlating with nor influencing the natural history of the disease, predictive factors could stratify patients on the basis of the response to a specific therapy (more commonly a targeted therapy), assisting physicians in deciding which treatment would fit the best to every patient (Fig. 4.10).

The clinical utility of a predictive factor relies on the possibility of choosing the most appropriate therapy for each patient and, in particular, identifying those at high-risk patients sensitive to both systemic and targeted therapy, in order to (Fig. 4.11):

- Better select that fraction of patients who can benefit from targeted therapy (sensitive patients), thus achieving better outcomes and less toxicities while increasing survival rates.
- Spare from unnecessary toxicities those patients with drug-resistant micrometastases which would render a specific treatment ineffective.

The use of biomarkers at first instance aims to optimize the effectiveness of treatments, focusing the therapeutic intervention on patients with a high probability of obtaining a benefit. Secondly, the purpose of their clinical use is to avoid treatment-related toxicity in patients with a low probability of responding and to optimize economic resources.

So far, predictive biomarkers used to show not only a positive but also a negative predictive role which would relate to a low chance of response to certain therapies. The effects of such biomarkers are synergic; therefore it becomes crucial to study and exclude the presence of all negative predictive markers related to that drug before starting a new treatment approach.

In conclusion, a shift has occurred in the global approach to new drugs in oncology: while drugs have been previously administered in unselected patients evaluating the relationship among response and different

Fig. 4.8 Simplified scheme of the steps undertaken to identify the presence of a predictive marker and, subsequently, select the right treatment

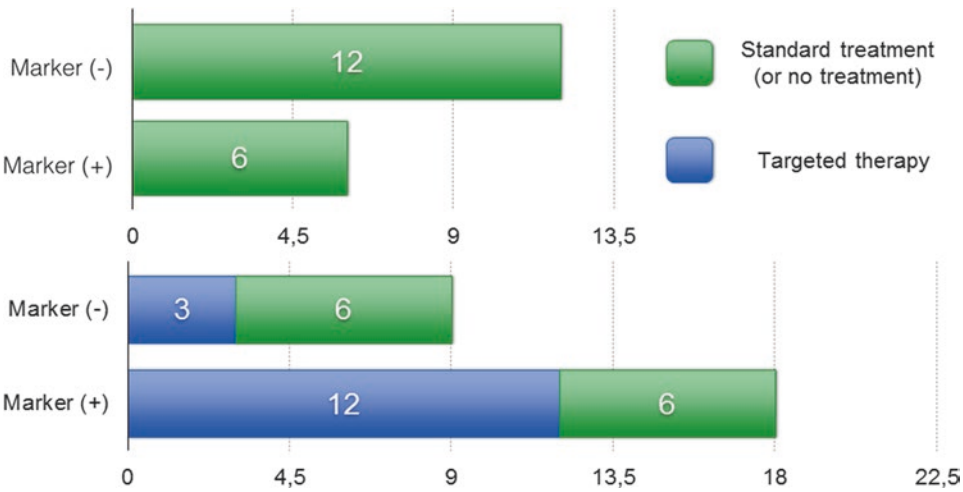


Fig. 4.9 Differences between prognostic and predictive markers: while the former assesses a disease outcome irrespectively of treatment approaches, the latter helps stratifying patients according to the different benefit from a specific therapy

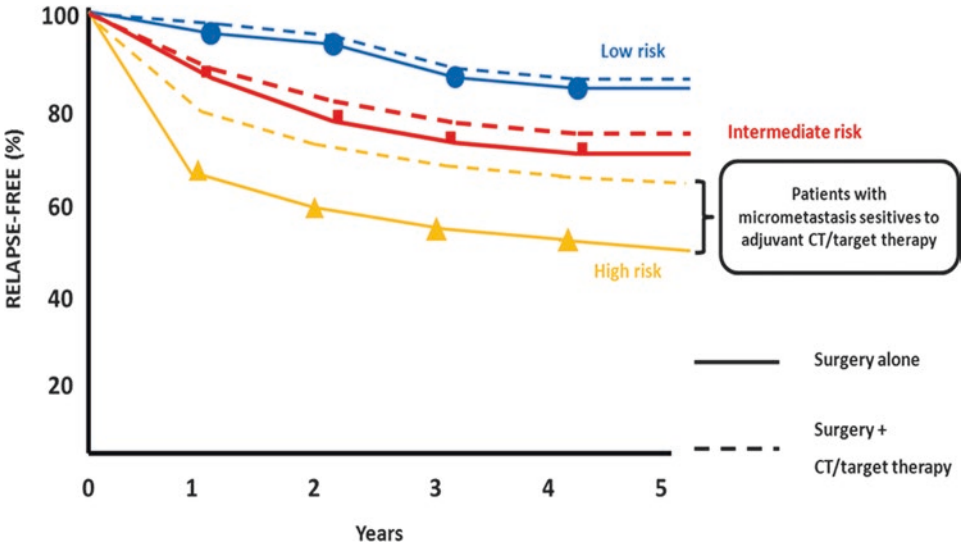


Fig. 4.10 Example of patient's stratification on the basis of the response to a specific therapy

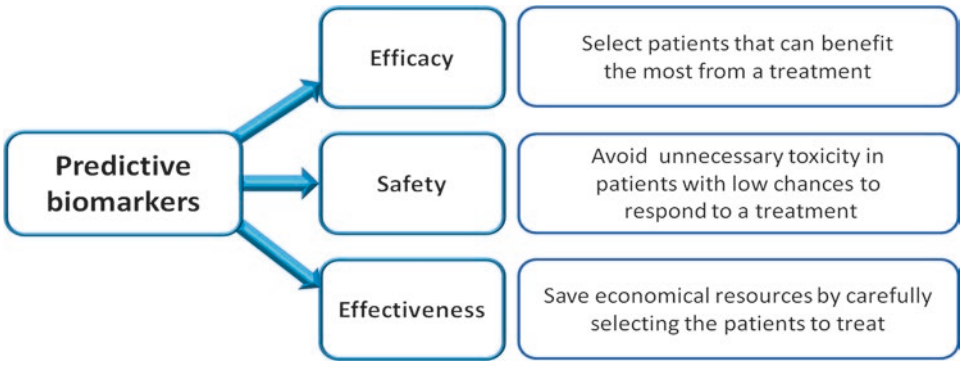


Fig. 4.11 Main features of predictive biomarkers

predictors retrospectively, predictive factors are now selected since the beginning of treatment searching for genetic and molecular patterns in order to administer specific and targeted drugs (Figs. 4.12 and 4.13).

Due to the rapid progresses in cancer biology, in the last decade, several molecular biomarkers have gained clinical relevance and currently provide essential information for the proper management of various cancer types. Examples of the most relevant predictive biomarkers in clinical practice need to be discussed:

- The anaplastic lymphoma kinase *(ALK) gene* was initially described on chromosome 2 as a fusion partner in the translocation found in anaplastic large cell lymphoma [37]. In 2007, a novel ALK fusion with the echinoderm microtubule-associated protein-like 4 (EML4) was reported as a somatic rearrangement found in 6.7% of lung adenocarcinomas [38]. The EML4-ALK fusion is generated by small inversions within chromosome 2p that fuses differing portions of the EML4 gene with part of ALK. This genetic alteration is an important therapeutic target with first-, second-, and third-generation tyrosine kinase inhibitors (crizotinib, ceritinib, alectinib, brigatinib, and lorlatinib) that have become available in clinical practice. These drugs are active both as initial treatment for ALK-addicted non-small cell lung cancer (NSCLC) and in patients failing the compound received in first line because of amplifications of the ALK locus, mutations in the ALK kinase domain (around 30%), or activation of "bypass" signaling pathways [39, 40].
- EGFR (epidermal growth factor receptor) is a tyrosine kinase receptor (TKR) that binds multiple ligands, thereby activating several downstream pathways that regulate DNA synthesis and cell proliferation. Somatic mutations in this gene, mainly targeting exons 18–21, are detected in approximately 10–12% of non-Asian patients diagnosed with lung adenocarcinoma. Mutations of the *EGFR* gene are predictive of response to anti-EGFR drugs such as erlotinib, gefitinib, osimertinib, and afatinib that represent the standard of care for the

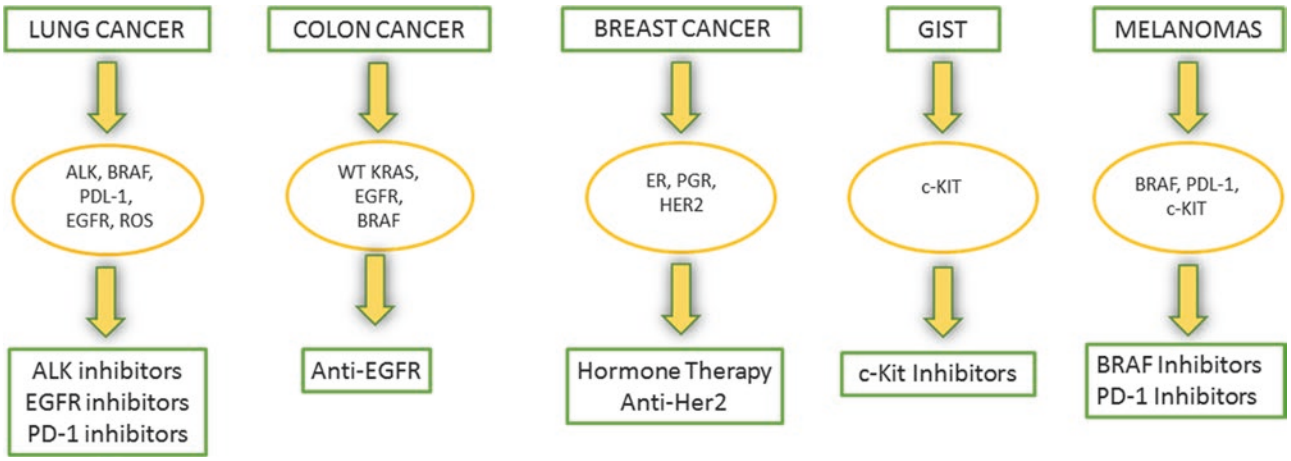


Fig. 4.12 Examples of some of the most relevant predictive biomarkers with the respective targeted treatment

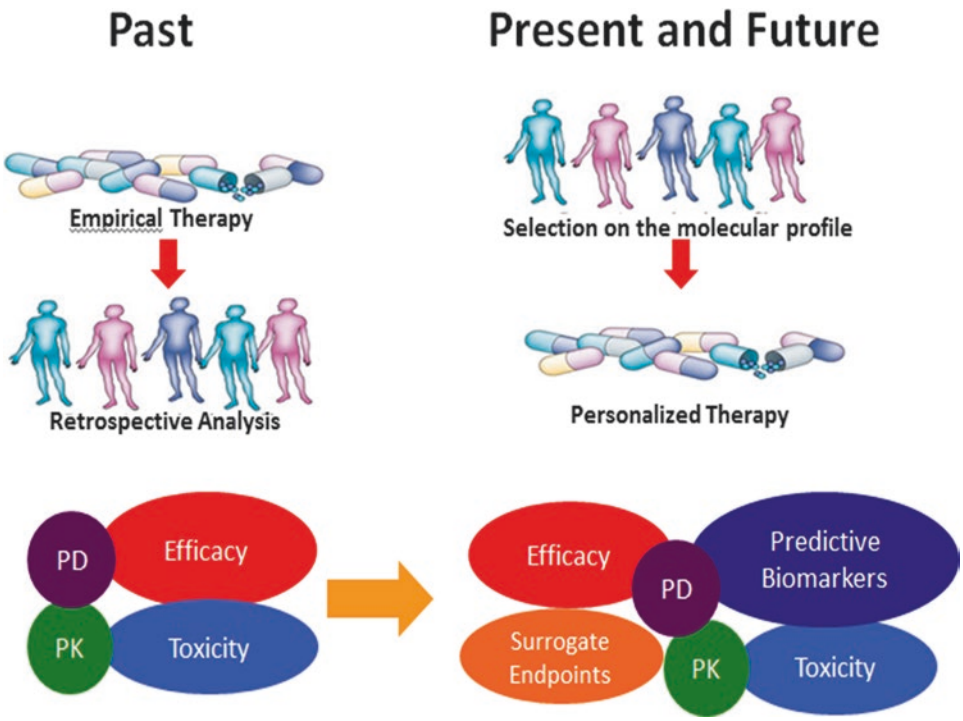


Fig. 4.13 In the past decades, drug efficacy and toxicity (based on its pharmacodynamics [PD] and pharmacokinetics [PK]) were retrospectively studied after their empirical use. Today we can select patients according to a molecular profile, administering the right drug to the right patient

first-line treatment of advanced NSCLC. The most common and best-characterized EGFR mutations are in-frame deletions involving exon 19 – which eliminate the conserved LREA motif (residues 747–750) – and the exon 21 L858R substitutions. Taken together, these two alterations constitute 80–90% of all EGFR mutations [41]. The remaining 10% of EGFR mutations appeared to harbor heterogeneous molecular alterations within exons 18–21 (so-called "uncommon" mutations) with clinically variable responses to targeted drugs and shorter survival rates when compared to classical mutations [42]. Erlotinib, gefitinib, and afatinib are competitive inhibitors of EGFR catalytic activity that currently represent the standard of care for the first-line treatment of locally advanced or metastatic NSCLC. However, after an initial response, patients loose responsiveness to these drugs often because of the development of the T790M mutation in exon 20 [43]. Recently, the third-generation tyrosine kinase inhibitor (TKI) osimertinib targeting the T790M mutation has become available in clinical practice, showing high response and improved progression-free survival [44].

- Programmed death ligand 1 (PD-L1). It is a membrane bound protein comprising 290 amino acidic residues with an extracellular region composed of an IgV domain and an IgC2 domain. The former is responsible for PD-L1 binding to the programmed death 1 (PD-1) receptor (Fig. 4.3). PD-L1 is expressed on several immune cells including T lymphocytes, dendritic cells, natural killer cells, B cells, and monocytes [45, 46]. In addition, PD-L1 can also be found on epithelial cells, vascular endothelial cells, and myeloid dendritic cells. The PD-L1/PD-1 pathway downregulates the immune response, preventing the inappropriate hyper-activation of the immune system [47]. The PD-L1/PD-1 system plays a dual role in cancer progression, as it can suppress tumor growth eliminating cancer cells but can also promote neoplastic growth eliciting immune tolerance mechanisms versus cancer cells [48]. The availability of monoclonal antibodies against PD-1 (pembrolizumab and nivolumab) or PD-L1 (atezolizumab and durvalumab) has generated unprecedented results in the treatment of NSCLC, melanoma, and renal cancer and promises to provide additional clinical benefit in the treatment of several other malignancies [49, 50].
- RAS proteins are GTPases functioning as binary switches, alternatively transducing downstream signals according to their activation state and favoring cell survival, proliferation, and migration [51]. Three different genes encode for four RAS isoforms: KRAS (Kirsten rat sarcoma viral oncogene homolog) codifies the KRAS4A and KRAS4B splicing variants, whereas NRAS (neuroblastoma rat sarcoma viral oncogene homolog) and HRAS (Harvey rat sarcoma viral oncogene homolog) encode the two homonymous proteins [52]. RAS normally switches between an active GTP-bound and an inactive GDP-bound state: the shift from one condition to the other requires additional proteins. Guanine nucleotide exchange factors (GEFs) promote RAS activation catalyzing GDP to GTP substitution. Conversely GTPase-activating proteins (GAPs) lead to RAS inactivation through GTP hydrolysis. When an upstream signal (i.e., receptor tyrosine kinase activation) activates RAS, several downstream phosphorylation cascades are initiated. At least 11 RAS effectors families are known, two of which are preeminent in mammalian cells: (i) the rapidly accelerated fibrosarcoma (RAF) family (ARAF, BRAF, CRAF) that activates the MAP kinase pathway and (ii) the phosphatidylinositol 3 kinase (PI3K) promoting AKT/mToR signaling [53]. Activating RAS mutations occur in almost 30% of human cancers as single nucleotide substitutions. Overall, KRAS mutations are most frequent (85%), especially in pancreatic ductal adenocarcinoma (PDAC), CRC, and NSCLC. Specifically, KRAS activating mutations arise in 90% of PDACs, representing a driver event in pancreatic tumorigenesis. In CRC KRAS (42%) and, less frequently, NRAS (9%) mutations are predictive of resistance to anti-EGFR monoclonal antibodies (cetuximab, panitumumab) [54]. Therefore, testing the abovementioned alterations is mandatory in metastatic CRC patients. Also, 15–25% of NSCLC harbor a KRAS oncogenic mutation in exon 2 or 3. These alterations usually occur in lung adenocarcinomas (more frequently in smokers) and, while considered mutually exclusive, can rarely coexist with EGFR mutations or ALK rearrangements. Moreover, the recent discovery of new highly selective KRAS inhibitors eliciting partial responses in NSCLC patients in phase I trials has provided a renewed opportunity to better understand the role of this mutation as an oncogenic driver [55]. In this setting, liquid biopsy proved to represent a viable option to assess KRAS mutational status on circulating tumor DNA [56], especially in the case of NSCLC tissue samples that are not always available [57]. NRAS mutations are common in melanoma and have been described in 12% of these tumors, whereas HRAS alterations are rare (3%) and are mainly found in squamous head and neck carcinomas [58]. Compared to BRAF mutant melanomas, tumors with mutant NRAS are characteristic of older patients, chronic ultraviolet exposure and tend to be located at the extremities, presenting a higher mitotic rate. Therefore, it is not surprising that NRAS-mutated patients show inferior survival rates [59].
- *BRAF*-activating mutations are clearly the most common oncogenic drivers in roughly half of all melanomas resulting in high overall response rates and frequently dramatic tumor regression in BRAF inhibitor-treated patients [60]. Interestingly, BRAF appeared to be significantly predictive of response in cutaneous melanomas, as opposed to the negative prognostic value demonstrated in colorectal cancers. The BRAF gene encodes for a serine/threonine kinase involved in the RAS/RAF/MEK/ERK signaling pathway, which governs proliferation, differentiation, and cell survival (Fig. 4.1). It is estimated that 8% of all tumors present mutations in the BRAF gene, including 50% of melanomas, 40% of papillary thyroid carcinomas, 30% of serous ovarian cancer, 10% of CRCs, and 2–3% of lung cancers [53, 61]. The most common BRAF alteration is the missense mutation V600E that leads to a conformational change resulting in constitutive activation of BRAF kinase activity [31]. BRAF mutations have important prognostic and therapeutic implications in patients

with melanoma and CRC. Indeed, BRAF-directed TKIs (BRAFi) such as vemurafenib and dabrafenib were developed for unresectable or metastatic melanoma demonstrating significant increases in both objective response rate and PFS. However, most patients eventually showed disease progression because of loss of PTEN, loss of NF1, or amplification of cyclin D1 [62, 63]. To address these issues, BRAFi have been combined with MEK inhibitors (MEKi) further improving the efficacy of the former drugs and leading to the approval of this drug combination for the treatment of metastatic melanoma displaying BRAF V600 mutations [64]. Accordingly, the combination of BRAFi dabrafenib and the MEKi trametinib has been recently approved for the treatment of advanced NSCLC patients harboring BRAF V600 mutations, based on the results of an open-label phase II trial [65].

- HER2 amplification is associated with aggressive tumor behavior, reduced responses to traditional therapies, and decreased survival [66, 67]; determining HER2 status is very important especially in breast, gastroesophageal junction, and gastric malignancies where HER2 amplification influences the therapeutic strategy. Indeed, the introduction of trastuzumab and pertuzumab (humanized HER2-targeting monoclonal antibodies) and the synthesis of trastuzumab emtansine have revolutionized the treatment of patients with HER2-positive breast cancer generating consistent improvements in overall survival [68, 69]. Likewise, patients with HER2-positive gastroesophageal and gastric cancers receiving trastuzumab plus chemotherapy show a significant increase in overall and progression-free survival compared to patient treated with chemotherapy alone [70]. HER-2-directed therapy produced unprecedented clinical improvements in both predicting responses and survival rates across all lines of treatment for advanced breast cancer. Nonetheless, HER-2 amplification or protein expression still plays a controversial role, since targeting HER-2 has been shown not to be associated with either clinical and pathological parameters in 7–34% of primary gastric tumors, in spite of survival improvement [71].
- c-KIT is a tyrosine kinase receptor (TKR) that recognizes the stem cell factor (SCF) as its ligand. In 90% of gastrointestinal stromal tumors (GIST) *c-KIT* displays point mutations that lead to constitutive c-KIT activation and therefore to tumor development [72]. Imatinib is a tyrosine kinase inhibitor (TKI) that recognizes c-KIT blocking its catalytic activity [73]. It represents the first-line treatment for high-risk operated, locally advanced, and inoperable or metastatic c-KIT-mutated GIST [74]. Therefore, c-KIT can be considered able to predict response to imatinib [75, 76].
- *ROS1* is an oncogene encoding for a tyrosine kinase receptor that is rearranged in 0.7% to 1.7% of NSCLC [77]. ROS1 rearrangements fuse the entire tyrosine kinase domain of the gene with 1 of 12 different partners generating a constitutively active chimeric kinase that drives cell transformation [78]. ROS1-positive patients are generally young, with adenocarcinoma histology and little or no history of smoking. Due to the high degree of sequence homology (>64%) between the ALK and ROS1 kinase domains and ATP binding sites (>84%), crizotinib has been evaluated in ROS1-positive patients with excellent results in terms of progression-free survival. Furthermore, several trials are already evaluating additional molecules (cabozantinib or other ALK inhibitors) for individuals displaying resistance to crizotinib [79].
- The *mesenchymal-epithelial transition (MET) gene* is a proto-oncogene located on chromosome 7 at q31.2, which encodes a tyrosine kinase receptor activated by its specific natural ligand: the hepatocyte growth factor receptor (HGFR). Activating mutation, amplification, and overexpression of this gene are also associated with multiple human tumors. Binding of HGF to MET stimulates downstream signal pathways, such as the RAS/ERK/MAPK, PI3K/AKT, Wnt/β-catenin, and STAT signaling pathways. These pathways are known to involve cell growth, migration, angiogenesis, and survival [80]. MET amplification (3–7%) and overexpression (25–75%) imply a worse prognosis for the patient. It has also been found that about 10–20% of NSCLC patients with EGFR-mutated tumor acquire resistance to EGFR-TKI through MET amplification. The evaluation of MET therefore assumes both prognostic and predictive role of response to MET TKIs (crizotinib, tepotinib, or capmatinib) [81]. Mutations of the MET gene at the level of exon 14 (METex14) are identified in about 3% of NSCLC cases. These are generally found in specific conditions: these are usually elderly patients with a history of tobacco use and lung cancer with pleomorphic (including sarcomatoid) histology or adenocarcinoma [82]. Based on overall response rate and response duration in the GEOMETRY mono-1 trial, capmatinib has only recently granted fast approval by the Food and Drug Association (FDA) for those tumors that harbor such mutations [83].
- The *NTRK1/23 proto-oncogenes* (encoding the TRKs A/B/C, respectively), influence survival and neuronal differentiation. They have recently gained considerable attention in precision oncology as they can generate fusion oncoproteins that have been identified as

oncogenic drivers in many adult and pediatric solid tumors [84]. Among the earliest to be described in cancer, translocations involving NTRK genes result from intra- or inter- chromosomal rearrangements that fuse the 3′ end of NTRK with the 5′ end [85]. The frequency of fusions of *NTRK* genes in the most frequent tumors is generally less than 5%, for example, about 0.2% in tumors of the head-neck district, 0.1–1%, in NSCLC, 0.7–1.5% in colorectal carcinomas, 0.3% in skin melanomas, and approximately 1% in soft tissue sarcomas and GIST [86].

Larotrectinib, one of the first TRK inhibitors used, has demonstrated a significant objective response rate in most patients treated with TRK fusion cancers in several clinical trials regardless of the patient's age, tumor histology, and specific involved fusion partner [87]. It was the second histology-agnostic molecularly targeted therapy approved by FDA (Food and Drug Administration) and EMA (European Medicines Agency)-approved. Entrectinib, another selective inhibitor of TRK A/B/C, ROS1, and ALK, was developed for treatment of various solid tumors, receiving FDA approval for the treatment of advanced ROS1-positive NSCLC [88]. Different approaches can be used to identify the presence of *NTRK* gene fusions in order to guide the choice of treatment. Such strategies can be direct or indirect, including immunohistochemistry (IHC), FISH, RT-PCR, and NGS techniques [89]. The implementation of these methods can be adapted to individual patients based on the histological and clinical presentation of the tumor so as to use the identification of the *NTRK gene fusions* as a biomarker for the choice of chemotherapy [86].

- Activating alterations of the rearranged during transfection (RET) kinase are therapeutically actionable oncogenic drivers across a variety of cancers. Two main activation mechanisms have been described for the oncogenic RET kinase: point mutations and genetic rearrangements. In several prospective clinical trials, the use of multi-kinase inhibitors with activity against RET has been associated with confirmed responses and long-term disease control in selected patients with RET-mutant or RET-reorganized tumors. *RET gene* alterations are more frequently implicated in the pathogenesis of lung, thyroid, and other cancers; in detail, RET fusions are observed in 10% of papillary thyroid cancers, 1–2% of NSCLC cases, and other cancer subtypes including colorectal, pancreatic, and breast cancers [90]. In lung adenocarcinomas, RET fusion occurs mainly in nonsmoking patients, and the partner most frequently associated in this context is KIF5B, histologically also present calcifications in the form of psammoma bodies. Some multiple TKIs have shown activity in NSCLC with RET fusion, as well as in other cancer types. Recently, two molecules specially designed as strong and selective inhibitors, pralsetinib and selpercatinib (previously known as BLU-667 and LOXO 292, respectively), have shown promising activity in RET-positive NSCLCs [81], demonstrating potent, durable, and extensive anticancer activity along with an acceptable toxicity profiles in advanced RET-rearranged NSCLC [91]. Accordingly, selpercatinib has just recently received FDA approval for metastatic RET fusion-positive NSCLC and advanced or metastatic RET fusion-positive thyroid cancers. The techniques used to identify RET gene alterations are NGS, PCR, and also FISH. Clinical-diagnostic insights are needed to identify new approaches to target RET-dependent tumors in order to improve the prospects for using this biomarker.
- Tumor mutation burden, also known as TMB or tumor mutation load, measures the total number of mutations within a tumor genome, sometimes defined as the total number of non-synonymous point mutations with the precise definition varying with the sequenced region size along with the localization and the nature of the included mutations [92]. Even if not without contradictory results, TMB has newly emerged as a possible independent biomarker to predict patient responses to immunotherapy in different tumor types, including lung cancer [93]. Considering the complex mechanisms causing the accumulation of somatic mutations which are likely to induce an immune response producing neoantigens, whole exome sequencing (WES) of tumor tissue initially has been the golden standard detection method technique for TMB. However, since WES was not for routine use in clinical practice due to substantial cost and turnaround time, targeted next-generation sequencing (NGS) panels have been adapted and, even if with no clear standardization among different panels, currently used to estimate TMB, presenting with a generally satisfactory correlation with TMB determined by WES. Furthermore, TMB analysis using liquid biopsy (circulating tumor DNA or ctDNA), also known as blood-based TMB (bTMB), has become an attractive method for the prediction of response to immunotherapy regimens, mostly for NSCLC patients could not always provide adequate tumor tissue for biomarker analysis [94].

  Of significance, a growing body of evidences suggested that a high TMB (either on tissue or blood) was associated with greater clinical benefit from immune checkpoint inhibitors, albeit not showing a clear survival advantage over chemotherapy alone in randomized clinical trials, especially in NSCLC patients [95]. Further ongoing trials have been assessing and validating TMB as a biomarker for response to immunotherapy.

4.5 Surrogate Markers

Understanding how activity and efficacy of oncological drugs have been evaluated so far in the context of clinical trials is crucial for introducing the concept of biomarkers as surrogate endpoints. A surrogate marker could be defined as a measure of effect of a specific treatment that may correlate with a real clinical endpoint but does not necessarily have a guaranteed relationship [96]. For instance, most targeted therapies have been recently approved for clinical practice due to their cytostatic activity that interferes with one or more pathways blocking proliferation, metastatic spread, or angiogenesis. In this setting, drug activity may not be associated with a significant radiologic shrinkage of the lesions as well as usual endpoints; such objective response according to RECIST criteria may not be suitable to evaluate their clinical efficacy. Accordingly, new surrogate outcomes whose variation can be associated to relevant clinical outcomes are needed [2].

The choice of the outcome measures is the key for designing a clinical trial: these measures can be clinical or, alternatively, indirect measures such as biomarkers. These measures (that, besides bimolecular markers, may also include physical or radiological tests) are considered as replacement endpoints or "surrogates" for clinically meaningful endpoints (Figs. 4.14, and 4.15).

Surrogate endpoints can be obtained from different modalities, such as behavioral or cognitive scores, biomarkers from electroencephalography (EEG), MRI, PET, or biochemical biomarkers. When used as outcomes in clinical trials, biomarkers are considered as surrogate endpoints, even if not all biomarkers have been validated nor regarded as such. Surrogate endpoints represent a small subset of well-characterized biomarkers with well-evaluated clinical relevance: the main difference is based on that a biomarker is an eventual "candidate" surrogate marker, whereas a surrogate marker is a test validated as a measure of the effects of a specific treatment [97] (Fig. 4.16).

A solid literature-based evidence (e.g., epidemiological, therapeutic, and/or pathophysiological) claiming that a biomarker consistently and accurately would predict a clinical outcome (either positively or negatively) needs to be considered to finally validate a surrogate endpoint. Indeed, a surrogate endpoint is a reliable biomarker that would stand in for while not replacing a clinical endpoint [98].

A biomarker may correlate with a clinical endpoint only in certain conditions. Notwithstanding, correlation with true clinical outcomes is not sufficient for a biomarker to be used as a valid surrogate endpoint, since alterations on the levels of the biomarker induced by a clinical intervention might be able to predict additional effects on the clinical outcome.

There are several advantages in using molecular biomarkers as surrogate endpoints instead of clinical and radiological outcomes:

- Enabling to design clinical trials enrolling less patients while obtaining more statistically significant results.
- Performing interim analyses, obtaining data on treatments efficacy or safety sooner than a clinical effect could be demonstrated, and eventually reducing the duration of clinical trials or allowing researchers to stop interventions potentially harmful for a subgroup of patients.
- Some events used in clinical trials as clinical endpoints may be very rare and, thus, are difficult to record, and waiting for their development may be unpractical and may also be considered unethical: surrogate endpoints allow to overcome these problems.

In clinical practice, it is essential to evaluate the levels of a certain surrogate endpoint at baseline: hence, a pro-

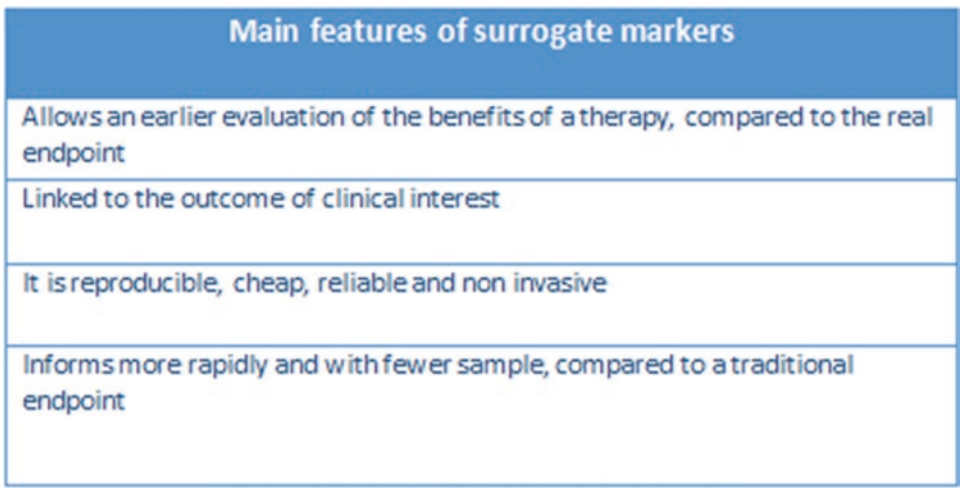


Fig. 4.14 Features of a surrogate endpoint

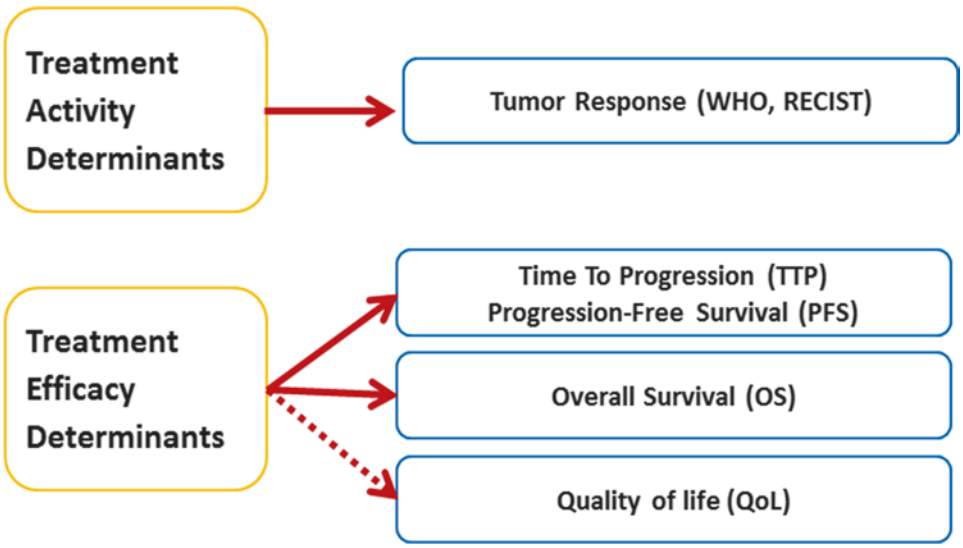


Fig. 4.15 Clinical and radiological endpoints that a surrogate biomarker may stand in for

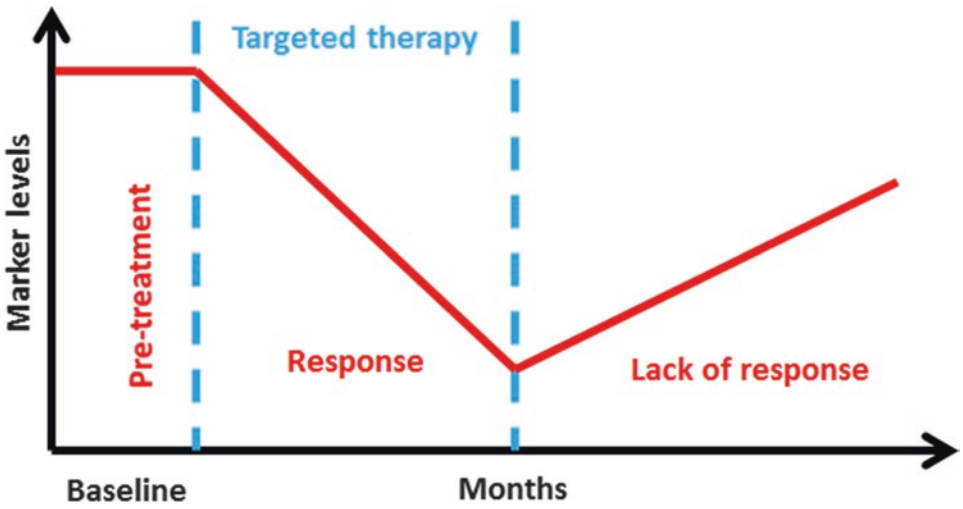


Fig. 4.16 A surrogate biomarker can be used as a measure of effect of a treatment, often allowing to evaluate its effectiveness, or the development of resistance, earlier than any imagine technique

gressive reduction of a biomarker during therapy can represent a sign of clinical efficacy, while increased values may reflect the onset of potential resistance mechanisms which could be detected before any radiologic disease progressions occurred.

4.6 Risk Markers

A risk-associated biomarker is a factor that allows to stratify general population in different risk classes, related to the cumulative risk of developing cancer, acting as a predictor of the cumulative oncological risk (Fig. 4.17). It is associated with an increased or, in some cases, decreased chance of developing a specific cancer (or a specific set of cancers) in an individual who, from a clinical standpoint, has not yet presented with that disease or medical condition. A risk marker is somehow similar to a prognostic biomarker as differences between them may not always be so clear: while the former regards healthy individuals, the latter concerns individuals who have already been diagnosed with a particular disease. Risk markers are usually represented by a mutation that may be detected many years – in some cases decades – before the onset of clinical signs and symptoms and do not describe a relationship with any specific treatment [99].

Indeed, pathological genetic variants represent the most well-established class of risk markers, characterized by a high predictive value. In recent years, a growing number of hereditary germline mutations have been studied and associated with an increased risk of developing cancer: studying these mutations is important in the context of personalized medicine in order to evaluate the oncological risk of a patient, selecting high-risk subgroups of subjects in the healthy general population, and leading to appropriate preventive strategies.

Fortunately, genetic mutations that seemed to be related with a high risk of developing hereditary or famil-

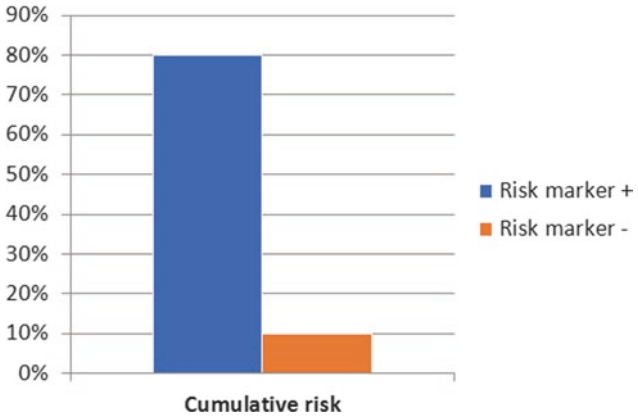


Fig. 4.17 Example of general healthy population stratification in different risk classes, related to the cumulative risk of developing cancer, according to the presence or absence of a risk biomarker

iar tumors affects only a small percentage of the general population for which careful and very specialized management solutions are needed. Such biomarkers may be used to determine whether lifestyle, nutritional, or other preventive interventions are indicated or to identify individuals who may need more aggressive surveillance and/or preventive strategies comparing to general population. However, the utility of a susceptibility/risk biomarker comprehensively depends on the availability of interventions that are able to modify the risk of disease.

Furthermore, risk markers appeared to play other roles in the set of medical research, especially in primary prevention clinical trials where it is often difficult to enroll patients such as to observe a significant number of clinical events throughout time. Risk markers allow to select patients with a higher risk of developing a disease (event), thus obtaining:

- An easier trial conduction, in a shorter amount of time and with a lower patients accrual
- A better balance between treatment benefits and side effects, reducing the number of patients that would suffer from toxicities while not taking advantage from the intervention [99]

Examples of risk markers are:

- *Germline mutations in BRCA-1* and *BRCA-2 genes,* two genes involved in homologous recombination that heavily contribute to double-stranded DNA repair, thereby acting as "genomic caretakers"(Fig. 4.18). Loss of function of *BRCA* genes confers an increased lifetime risk for multiple types of malignancies, especially breast and ovary cancer. About 5% of breast carcinomas and 10% of ovarian malignancies result from germline hereditary *BRCA1*/2 mutations. BRCA mutations are present in about 45% of families with a history of breast cancer and up to 90% of families with a history of both breast and ovarian cancer [100–102]. Moreover, sporadic (i.e., somatic) BRCA1/2 alterations are common in ovarian cancer, especially of high-grade serous histology [103, 104].
- Nowadays, BRCA mutations not only represent clinically useful risk markers but also play a predictive role, foretelling response to the new PARPi (poly-ADP-ribose-polymerase inhibitors) drugs in ovarian, breast and prostate cancers. PARP is a nuclear enzyme with dual roles in DNA repair and transcription regulation. PARP mediates single-strand break DNA repair modulating the base and nucleotide excision repair pathways. In the absence of functional BRCA genes, pharmacological inhibition of single-strand DNA repair leads to the accumulation of double-strand breaks, which can increase a cell's mutational load leading to activation of programmed death. Indeed, several PARP inhibitors (olaparib, niraparib, and rucaparib) have recently become available in clinical practice displaying unprecedented efficiency as maintenance therapy for ovarian cancer patients with platinum-sensitive and/or BRCA-mutated tumors. Accordingly, scientific societies recommended the implementation of BRCA testing into clinics with the goal of identifying both cancer patients with higher probability of benefit from specific anticancer treatments (test for response to therapy) and family carriers of pathogenic variant who have eventually inherited predisposition to cancer development (test for cancer risk) [105–107].
- Germline mismatch repair (MMR) system genes mutations which are responsible for the human nonpolyposis colon cancer (HNPCC) or Lynch syndrome. Microsatellites (MS) are short DNA sequences (1–6 bases) tandemly repeated and scattered throughout coding and noncoding regions of the genome that are highly exposed to replication errors [47]. The MMR system includes four genes *(MLH1*, *MSH2*, *MSH6*, *and PMS2)*, cooperating to detect and repair genomic aberrations. A defective MMR gathers mistakes in microsatellites, leading to genetic instability (MS instable phenotype or MSI) [48], increasing the risk of development of a wide number of tumors, especially colorectal cancers (but also including pancreatic, biliary, and urinary tract tumors). MMR alterations can be sporadic, in case of MLH1 epigenetic silencing, or inherited, in case of *MLH1*, *MSH2*, *MSH6*, *or PMS2* germline mutations (i.e., Lynch syndrome). Up to 15% of colorectal cancers (CRCs) display MSI, the majority being sporadic with Lynch syndrome the most frequent hereditary form of CRC [108]. Immunohistochemistry and PCR are currently employed to assess MSI status, and tumors are categorized as MSI-high (MSI-H) or MSI-low (MSI-L) according to the assay result [109]. MSI-H CRCs present with peculiar features, as they arise in the

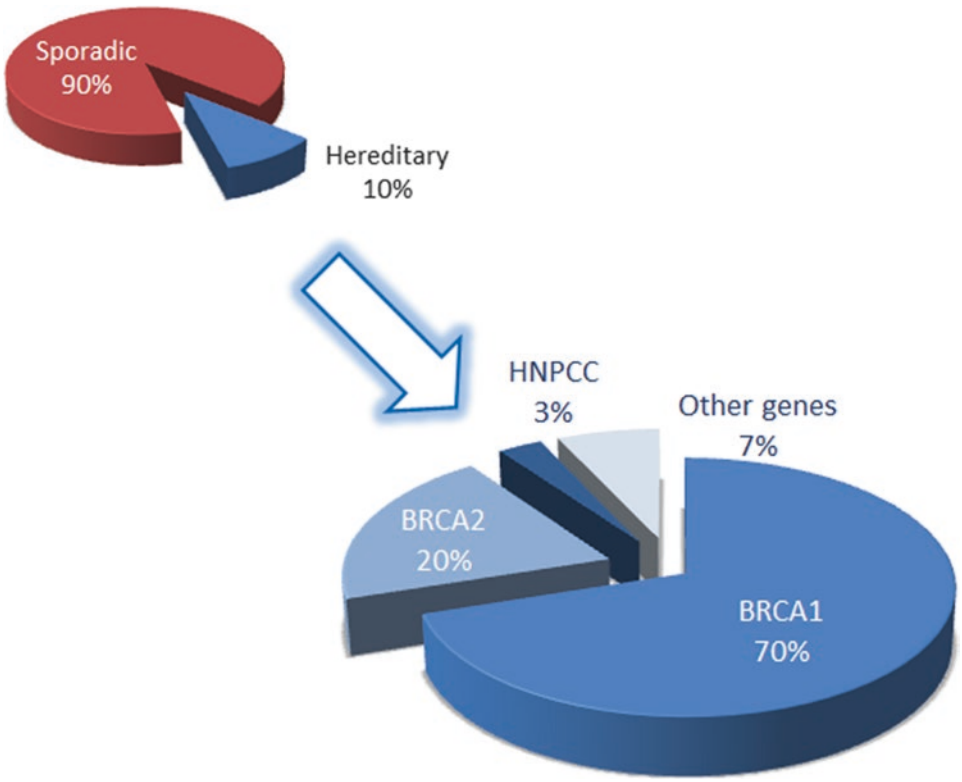


Fig. 4.18 Percentages of single genes involved in hereditary breast cancers

right colon and are diagnosed at earlier stages often exhibiting mucin production and a rich lymphocytic infiltration. Furthermore, whereas hereditary MSI-H tumors typically occur in younger patients, sporadic MSI-H CRCs tend to develop in older individuals, mainly female and smokers. The V600E BRAF mutation is often detected in sporadic MSI-H CRCs, while it is uncommon in Lynch syndrome-related tumors. The MSI status displays both a prognostic and predictive role for patients diagnosed with tumors of the colon and rectum. Indeed, according to disease extension, stage II/III MSI-H tumors have a better outcome compared with MSI-L ones, while, in stage IV CRCs, MSI-H is a negative prognostic factor. On the other hand, controversial data claim a lack of benefit of adjuvant 5-FU in MSI-H patients, whereas a defective MMR seems to enhance oxaliplatin sensitivity [110]. In addition, an emerging body of evidence demonstrates a strong correlation between MSI and immunotherapy response. As a consequence of their high mutational burden, MMR defective tumors produce numerous neoantigens favoring the efficacy of immune checkpoint modulators. Thus, several trials are testing immunotherapy in MSI-H metastatic CRC and other advanced cancer patients [111].

Key Points

- Biomarkers allow clinicians to swich from standardized medicine to a more tailored approach;
- Agnostic biomarkers allow to select patients that can benefit from specific drugs, independently from tumor site or histology;
- In basket trials, eligibility is based on the presence of a specific genomic alteration, irrespectively of tumor histology<,
- Diagnostic markers contribute, together with other tools, to oncological diagnosis, allowing for an earlier and more precise diagnosis, and a better assessment of aggressivness and stage;
- Prognostic biomarkers help predicting tumor natural history, allowing to select patients that may benefit from a more aggressive treatment. They allow to stratify patients in different risk classes according to a specifical clinical outcome, irrespectively of treatment approach;
- Predictive biomarkers allow to stratify patients on the basis of the probability of response to a specific treatment, guiding the clinician in the selection of the best treatment and helping to spare avoidable toxicities;

- Surrogate biomarkers are a surrogate (or replacement) of clinically meaningful endpoint, with wich they may correlate, and represent a measure of effect of a specific treatment.
- Risk biomarkers can help estimate the lifetime risk of developing cancer in high risk subjects: they allow to stratify general population in different classes according to the cumulative risk of cancer.

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Hereditary Cancers and Genetics

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Learning Objectives

By the end of the chapter the reader will:

- Have learned the basic concepts related to the heredofamilial and sporadic tumors
- Have reached a good knowledge of the mechanisms of action of the *gatekeeper* and *caretaker* genes
- Have acquired a good knowledge of the major hereditary tumor syndromes associated with specific susceptibility genes
- Have reached a clearer understanding of the different molecular and genetic events responsible for tumor progression and onset

5.1 Introduction

The history of hereditary tumors begins in the thirteenth century following the observation, in some families of patients with skin lesions, such as neurofibromas present in neurofibromatosis type 1 (NF1), subsequently also called von Recklinghausen's disease [1]. The insight of a generic genetic predisposition to particular types of cancer with specificity of the involved cell type dates back to the early 1900s, when Thomson emphasizes the hereditary nature of NF [2]. In 1922, Morgan first used the expression "human and experimental genetic predisposition" to cancer, referring to a Mendelian-type transmission [3, 4]. The use of murine models with genetic predisposition to the development of tumors, following the exposure to specific substances with carcinogenic effects (carcinogens), has allowed, in the course of the last century up to our days, the acquisition of new knowledge in this field [5, 6]. These models, initially adopted to understand the reason for which not all individuals exposed to tobacco smoke or asbestos developed lung cancer, have been shown to be, indeed, very useful also for the study of neoplasms arising in subjects carrying germline mutations at the level of specific genes called "susceptibility genes" [7–9]. The hypothesis proposed by Alfred Knudson, according to which a germline mutation can predispose genetically to a tumor, whose onset requires additional somatic mutations that occur secondarily in the tissue ("two-hit" hypothesis) dates back more than 30 years ago [10]. This model was identified, for the first time, in hereditary retinoblastoma, an autosomal dominant tumor at high penetrance. The affected carriers inherit a germline mutation inactivating the *RB1* gene (located on the long arm of chromosome 13) that determines a heterozygosity condition for that gene in all the cells of the organism [11]. The appearance of tumor phenotype occurs when a subsequent somatic mutation of the normal allele of the same gene occurs on a retinoblast, resulting in a homozygosity condition (loss of heterozygosity or LOH) and, consequently, the complete loss of function (loss-of-function mutation). Although *RB1* is a tumor suppressor gene and acts in a recessive manner, however, the predisposition to retinoblastoma, as previously reported, is transmitted in an autosomal dominant manner at high penetrance, since the second somatic mutation is highly probable due to chromosomal deletion events, failure of mitotic disjunction, or mitotic recombination [12, 13].

Although hereditary tumors represent only a small fraction of all the tumors which today afflict people worldwide, the knowledge of molecular genetics resulting from their study has changed not only the clinical management of affected patients and their families but provided important information on the molecular processes involved also in the corresponding, but far more numerous, sporadic tumors [14]. In addition to the hereditary tumor forms, a greater number of cancers develop with the simultaneous and synergistic contribution of multiple bland individual characters. These genetic factors at weak susceptibility, often associated with genetic polymorphisms common in the population, are known only minimally and represent the main challenge of genetic research in the years to come [15]. The identification of individuals with a hereditary risk of cancer is based on an accurate reconstruction of the personal and family clinical history and, usually, takes place in the context of an oncological genetic counseling [16]. Numerous genetic tests are currently available to confirm the clinical diagnosis and adopt the most appropriate therapies for patients but above all to early identify those subjects who exhibit an increased risk, in order to plan surveillance and prevention in the best possible way [17, 18].

In this chapter we will define the key concepts related to the heredofamilial tumors, briefly describing the major hereditary tumor syndromes associated with specific susceptibility genes (Table 5.1).

5.2 Genetic Predisposition to Cancer: Oncogenes and Tumor Suppressor Genes

Numerous studies carried out on tumor susceptibility syndromes led to a clearer understanding of the different molecular events responsible for tumor progression. Oncogenes and tumor suppressor genes are generally considered genes whose alterations, including, for example, intragenic mutations, chromosomal deletions, and variations in expression levels, are involved in the tumor onset and progression, promoting the abnormal growth of cells and their cell division [19–21]. Only a few hereditary predisposition syndromes are associated with germline mutations that determine the activation of

Table 5.1 Major hereditary tumor syndromes associated with mutations in oncogenes and tumor suppressor genes (*caretakers* and *gatekeepers*)

Syndrome	Gene (Locus)	Incidence	Penetrance
Hereditary tumor syndromes associated with mutations in caretaker genes			
Hereditary breast and/or ovarian cancer	*BRCA1* (17q21) *BRCA2* (13q12–13)	1/500–1/1000	85%
Hereditary nonpolyposis colorectal cancer (HNPCC)	*MLH1* (3p21) *MSH2* (2p22) *PMS2* (7p22) *PMS1* (2q31) *MSH6* (2p16)	1/500–1/1000	80%
Hereditary tumor syndromes associated with mutations in gatekeeper genes			
Familial adenomatous polyposis (FAP)	*APC* (5q21)	1/5000–1/10,000	~100%
Peutz-Jeghers syndrome (PJS)	*STK11* (19p13)	1/300,000	–
Hereditary diffuse gastric cancer (HDGC)	*CDH1* (16q22.1)	Rare	90%
Hereditary melanoma	*CDKN2A* (9p21)	Rare	~100%
Neurofibromatosis type 1 (NF1)	*NF1* (17q11.2)	1/3000	–
Retinoblastoma	*RB1* (13q14)	1/15,000–1/20,000	–
Cowden syndrome	*PTEN* (10q23.3)	1/200,000	90–95%
Li-Fraumeni syndrome	*TP53* (17p13)	Rare	90–95%
Hereditary tumor syndromes associated with mutations in oncogenes			
Medullary thyroid cancer (MEN 2)	*RET* (10q11.2)	1/30,000	–
Hereditary melanoma	*CDK4* (12q14)	Rare	~100%

oncogenes, leading to a gain of function (gain of function). Oncogenes induce cell proliferation by acting in a dominant manner, and, therefore, the mutation of a single allele is sufficient to promote carcinogenesis [22, 23]. It has been hypothesized that the majority of gain-of-function germline mutations are incompatible with embryonic development, highlighting the rarity of involvement of oncogenes in inherited tumors [24]. In any case, both for oncogenes and tumor suppressor genes, when cells show genetic alterations inherited via germline, subsequent somatic mutations in other genes (probably, from two to seven) are usually necessary to trigger the processes of tumor progression and metastasis [25–28].

Several crucial cellular processes, including apoptosis and cell cycle, differentiation, signal transduction, cell adhesion, maintenance of genomic integrity, and DNA damage repair (DDR) mechanisms, are regulated by tumor suppressor genes [29, 30]. It has been generally accepted that tumor suppressor genes, responsible for the heredofamilial cancer syndromes and involved in the regulation of cell proliferation and apoptosis, can be schematically divided into three main categories: gatekeepers, caretakers, and landscapers [4, 31, 32].

The "gatekeeper gene" definition was initially used to define the role of the *APC* (adenomatous polyposis coli) susceptibility gene responsible for the colon adenomatous polyposis [33]. Gatekeeper genes act by directly controlling cell growth, thus inhibiting proliferation, and leading to the apoptosis and/or promotion of terminal differentiation. Furthermore, they can promote DNA damage repair, delaying the cell cycle and thus increasing cell survival. Such genes are frequently altered in sporadic tumors at the somatic level whereas in hereditary tumors at the germline level [30, 34, 35]. Both maternal and paternal allele copies must be altered so that the tumor develops. The functional restoration of the involved gatekeeper gene will bring the tumor cell to become normal. According to Knudson's hypothesis, in the gatekeeper pathway of subjects who inherited a mutated copy of the gene, only an additional somatic mutation is needed in the other allele, in order to trigger the neoplastic process [36, 37] (Fig. 5.1).

Since gatekeeper genes have been found to be tissue-specific, therefore alterations of one of them will lead to the development of a particular form of predisposition to cancer. The major gatekeeper genes, with a descrip-

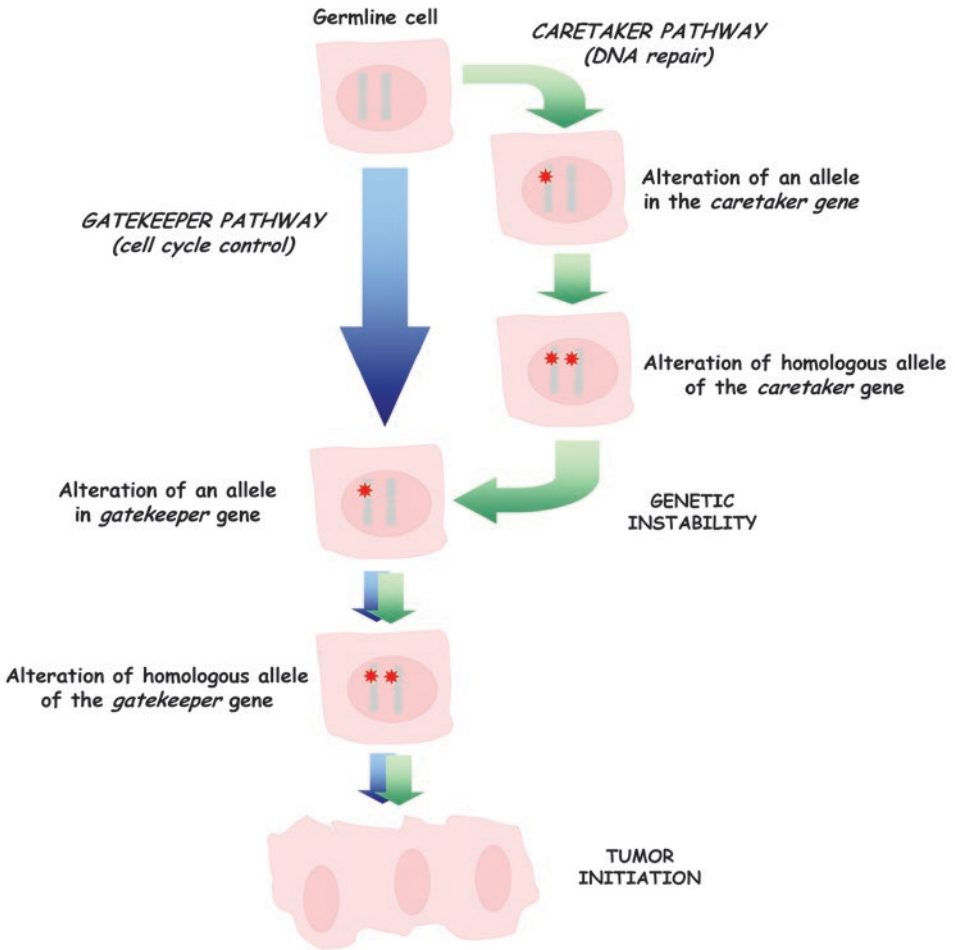

Fig. 5.1 Pathways of the caretaker and gatekeeper genes

tion of their most important functions, and the related syndromes are reported in Table 5.2.

Caretakers' genes are responsible for maintaining genomic stability and then for the genetic information integrity in each cell, by reducing the mutation rates of different genes involved in DNA repair, including gatekeepers and oncogenes [38]. These genes are considered "guardians of the genome", as they can prevent genomic instability, reducing the risk of cancer and aging. Since tumor development requires many alterations, the inactivation of such genes can lead to a significant acceleration of the tumorigenesis process. In fact, mutations of caretakers' genes determine a genetic instability that favors the appearance of further mutations in other genes important for cell cycle control [39]. This generates the so-called hypermutable phenotype, the expression of which is the instability of the microsatellite sequences (MSI) and accumulation of the mutations necessary for the neoplastic transformation. These mutations are rare in sporadic tumors but have been frequently detected in the germline [40, 41]. In the caretaker pathway of inherited syndromes, three successive somatic mutations (one mutation in the caretaker wild-type allele followed by one in each of the gatekeeper gene copies) are necessary in subjects who have already inherited an alteration in one of the two alleles, so that tumor develops [42, 43] (Fig. 5.1). Therefore, alterations of a caretaker gene are neither necessary nor sufficient for the development of a tumor. In fact, their functional restoration will not arrest the neoplastic growth, if a genetic mutation in the gatekeeper has already occurred. Overall, therefore, an altered gatekeeper gene will mainly affect the onset of a tumor, whereas a defect in the caretaker gene will accelerate tumor progression [36, 39]. The major caretakers' genes, with a description of their most important functions, and the related syndromes are shown in Table 5.3.

Tumor suppressor genes called "landscapers" encode for membrane proteins involved in intercellular communication processes, controlling the microenvironment in which cells grow. Indeed, cell growth depends on the cell-cell and cell-extracellular cell matrix (ECM) interactions [44]. The alteration of these genes can be inherited via germline and occurs mainly in the stromal cells which, by altering the microenvironment surrounding to the epithelial cells, may favor genomic instability and

Table 5.2 Major gatekeeper genes and associated sporadic and/or hereditary syndromes

Gene	Chromosome locus	Protein function	Syndrome	Associated tumors
APC	5q21	Cell adhesion, signal transduction pathway	Familial adenomatous polyposis	Colorectal cancer
WT1	11p13	Transcription factor, RNA processing	Wilms tumor	Nephroblastoma
CDKN1C	11p15.5	Cell cycle control	Beckwith-Wiedemann syndrome	Rhabdomyosarcoma, Wilms tumor, adrenocortical cancer, hepatoblastoma
NF1	17q11.2	Ras GAP activity	Neurofibromatosis type 1	Neurofibromas, sarcomas, gliomas
NF2	22q12	Cytoskeletal regulation	Neurofibromatosis type 2	Pheochromocytomas of the nervous system, myeloid leukemia
VHL	3p25.5	Transcriptional elongation regulation	Von Hippel-Lindau syndrome	Schwannomas, meningiomas, ependymomas of the nervous system, bilateral acoustic neuromas
FHIT	3p14.2	Nucleoside hydrolase	Familial clear cell renal carcinoma	Lung, kidney, stomach, cervical carcinomas
PTCH	9q22–31	Receptor for hedgehog protein	Gorlin-Goltz syndrome	Basal cell carcinomas, medulloblastomas, rhabdomyosarcoma
PTEN	10q23.3	Phosphatase	Cowden syndrome	Hamartomas, gliomas, prostate, endometrial and breast cancers
CDKN2A	9p21	Cell cycle control	Familial cutaneous melanoma	Melanoma, pancreatic cancer
MEN1	11q13.1	Unknown	Multiple endocrine neoplasia type 1	Parathyroid/pituitary adenoma, islet cell carcinoma
RET	10q11.2	Tyrosine kinase receptor for GDNF	Multiple endocrine neoplasia type 2	Medullary thyroid cancer type 2A, pheochromocytoma
RB1	13q14	Cell cycle control	Retinoblastoma	Osteosarcoma, small cell lung cancer, bladder and breast cancers
TP53	17p13	Cell cycle control, apoptosis	Li-Fraumeni syndrome	Sarcomas, leukemia, brain and breast cancers
TSC2	16p13.3	–	Tuberous sclerosis	Hamartomas, renal and brain tumors
TSC1	9q34	GTPase activation	Tuberous sclerosis	Hamartomas, renal and brain tumors
MADH4	18q21.1	Signal transduction through TGFβ/BMP	Juvenile polyposis	Hamartomas, pancreatic and colorectal cancers
NKX3A	8p21	Homeobox protein	Familial prostate carcinoma	Prostate tumors
STK11	19p13	Serine/threonine kinase	Peutz-Jeghers syndrome	Hamartomas, testicular, ovarian, breast, and colorectal cancers
CDH1	16q22.1	Epithelial cadherin	Familial gastric cancer	Breast, lung, skin, and colon cancers
CYLD	16q12–q13	Signal transduction, vesicle transport	Familial cylindromas	Cylindromas
EP300	22q13.2	E1A binding protein	Sporadic	Breast, colorectal, and pancreatic cancers

(continued)

Table 5.2 (continued)

Gene	Chromosome locus	Protein function	Syndrome	Associated tumors
EXT1	8q24.11–q24.13	Synthesis of heparan sulfate	Sporadic	Osteosarcomas, exostoses
EXT2	11p12	Synthesis of heparan sulfate	Sporadic	Osteosarcomas, exostoses
MAP2K4	17p11.2	Mitogen-activated protein kinase	Sporadic	Breast, colon, and pancreatic cancers
PRKAR1A	17q23–q24	Regulatory subunit of protein kinase A	Sporadic	Myxoma and endocrine tumors
SDHD	11q23	Subunit D of succinate dehydrogenase	Familial paragangliomas	Paragangliomas
SMARCB1	22q11.23	Actin-dependent regulator of chromatin	Rhabdoid predisposition syndrome	Rhabdoid tumors

RNA ribonucleic acid, *GAP* guanosine triphosphatase activating protein, *GDNF* glial-derived neurotrophic factor, *GTPase* guanosine triphosphatase, *TGFβ* transforming growth factor-β, *BMP* bone morphogenetic protein

consequently carcinogenesis [45]. The mutation of the landscapers' genes accelerates the process of tumor progression and metastasis by altering the surrounding microenvironment but is rarely associated with a specific hereditary syndrome of cancer predisposition (juvenile polyposis syndrome) [46].

Unlike the Knudson hypothesis, according to which heterozygotes show normal phenotype, since 50% of the product of a tumor suppressor gene is sufficient to protect a cell from neoplastic transformation, some tumor suppressor genes, such as *PTEN* (associated with Cowden syndrome), are "haploinsufficient" [47]. This means that when an inactivating germline mutation is inherited, a loss of function occurs, since the product of the remaining wild-type allele is not sufficient to adequately oppose carcinogenesis [48]. Paradoxically, the alteration of both copies of this gene involves cellular senescence, a form of permanent arrest of cell cycle that contrasts tumor progression; therefore heterozygous clones rather than cells with LOH for *PTEN* tend to be selected in the development of a neoplasm [49, 50].

Many hereditary predisposition syndromes show specific and different genotype-phenotype correlations, as usually occurs in sporadic tumors. The variability of these correlations, with reference to genetic mutations specifically associated with each of the hereditary predisposition syndromes, seems to be due to modifier genes [51, 52]. For example, in carriers of mutations in the *BRCA1* and *BRCA2* genes, which determine a genetic predisposition to breast and/or ovarian tumors, the modifier genes seem to be involved in the hormone pathway [53, 54]. The modifier genes may encode transcription factors, microRNAs, or other genomic elements not directly involved in the control of a susceptibility gene but a determinant in the molecular mechanisms of tissue-specific carcinogenesis [55, 56].

5.3 Linkage Analyses and Association Studies in Families with Genetic Predisposition to Cancer

The study of hereditary syndromes is fundamentally based on the identification of the chromosome where the genetic defect underlying the pathology is located and, therefore, on the discovery of the susceptibility gene [57]. Linkage and association studies in families with genetic predisposition to cancer allow to identify such genes and, therefore, proceed to the molecular screening of high-risk individuals. The linkage is based on the concept that neighboring loci/genes on the same chromosome are linked or associated and therefore are transmitted together, through meiosis, with a probability of recombination of less than 50% [58]. Linkage analysis, through the use of genetic markers whose localization within a chromosome is known, allows to determine the chromosomal position of a gene locus potentially associated with a susceptibility gene and so to perform an indirect diagnosis [59]. The marker linked to the susceptibility gene allows to (a) distinguish the two parental chromosomes, (b) identify the chromosome with the pathological allele, and (c) then the parental line to be analyzed in the genealogical tree. To perform a linkage analysis, specific requirements are

Table 5.3 Major caretaker genes and associated sporadic and/or hereditary syndromes

Gene	Chromosome locus	Protein function	Syndrome	Associated tumors
BRCA1	17q21	DNA repair, cell cycle checkpoint control, chromatin remodeling, estrogen responsiveness	Familial breast cancer	Breast and ovarian cancers
BRCA2	13q12–13	DNA repair, cell cycle checkpoint control, chromatin remodeling, estrogen responsiveness	Familial breast cancer	Breast and ovarian cancers
BRCA3	13q21	?	Familial breast cancer	Breast and ovarian cancers
PARP1	1q42	DNA repair, transcriptional regulation, replication, chromatin modification and apoptosis		Breast and ovarian cancer
NER system	9q22.3, 3p25, 19q13.2–13.3, 11p12–11, 16p13.3–13.13	Helicases, nucleotide excision repair	Xeroderma pigmentosum	Skin cancers
ATM	11q23.1	DNA repair	Ataxia-telangiectasia	Lymphomas
FANCA	16q23.3	DNA repair	Fanconi anemia	Acute myeloid leukemia
FANCC	9q22.3	DNA repair	Fanconi anemia	Acute myeloid leukemia
FANCD2	3p22–26	DNA repair	Fanconi anemia	Acute myeloid leukemia
MLH1	3p21	DNA mismatch repair	Hereditary nonpolyposis colorectal cancer	Lymphomas, colon and skin carcinomas, sarcomas
MSH2	2p22	DNA mismatch repair	Hereditary nonpolyposis colorectal cancer	Lymphomas, colon and skin carcinomas, sarcomas
PMS2	7p22	DNA mismatch repair	Hereditary nonpolyposis colorectal cancer	Lymphomas, colon and skin carcinomas, sarcomas
PMS1	2q31	DNA mismatch repair	Hereditary nonpolyposis colorectal cancer	Lymphomas, colon and skin carcinomas, sarcomas
MSH6	2p16	DNA mismatch repair	Hereditary nonpolyposis colorectal cancer	Lymphomas, colon and skin carcinomas, sarcomas

DNA deoxyribonucleic acid, *NER* system nucleotide excision repair system

needed, such as sample size (number of analyzed families), the presence of members of families affected by cancer, and opportunities to use genetic markers that are highly variable (polymorphic), uniformly distributed, and easily detectable with low-cost methods [60]. In addition to its localization in a contiguous position or very close to the susceptibility gene, the ideal marker must be highly variable, so as to make it stochastically impossible that all individuals with cancer inherit the same gene variant and none of the healthy subjects present it [61, 62]. For this purpose, about 300 polymorphic markers consisting of very simple sequences of DNA (2–5 base pairs) repeated in tandem and dispersed in the genome were selected. Several factors can influence the diagnostic accuracy of the linkage analyses, such as nonbiological paternity or technical errors in the execution of analyses, the acquisition of new germline mutations, or other causes of genetic heterogeneity [63]. Another factor that can negatively affect the analysis is the linkage disequilibrium (LD), that is, the nonrandom association of allelic variants in distant loci, not necessarily localized on the same chromosome [64]. In the last decades, linkage disequilibrium analyses have been performed in genome-wide association studies

(GWAS) in order to identify low-penetrance allelic variants involved in cancer susceptibility [65, 66]. GWAS are epidemiological studies, based on population genetics, aimed at identifying the associations between genetic predisposition and disease (including tumor) onset. In the cancer field, the potential of GWAS is to evaluate the association of genetic variants in different loci on different chromosomes in a wide range of cases versus control samples, simultaneously analyzing a panel of hundreds of thousands of SNP, in order to identify new alleles of cancer susceptibility [67]. These studies use a large number of single nucleotide genetic polymorphisms (SNPs) to identify the associations with disease based on linkage disequilibrium patterns in the human genome. The existence of at least two non-pathological allelic variants in a gene locus is called polymorphism. It has been estimated that in the human genome there are about seven million of common SNPs that have a minor allelic frequency (m.a.f.) of less than 5%, and, since recombination occurs in several hot spots, nascent polymorphisms are often strongly correlated [68, 69]. In 2004, Houlston and Peto [70] estimated the number of cases needed to identify low-penetrance alleles that confer a relative risk of two in both an unselected population and families with affected first-degree relatives. It has been observed that, in an unselected population, the identification of a susceptibility allele with a frequency of 5% requires more than 800 cases. In the same population, the identification of a susceptibility allele with a frequency of 1% requires more than 3700 unselected cases [70]. Therefore, the accumulation of a large amount of data in the GWAS is crucial. However, the power of the association studies can be significantly increased by using selected cases with a family history of cancer, as fewer cases are needed to demonstrate association with the disease [71]. In fact, the analyses of polymorphisms due to the variation of a single nucleotide with respect to the wild-type sequence were initially carried out on selected cases in relation, for example, to family history, in order to considerably reduce the sample size necessary to demonstrate the association with a specific tumor [72]. Subsequently, the preliminary data obtained from the selected sample were confirmed in a larger population. In general, each association study can be divided into three phases: the first phase identifies common SNPs in cases and controls; the second phase evaluates how many of these SNPs are common to a greater number of cases and controls; and, finally, the third phase aims to identify new susceptibility alleles. These studies, therefore, provide a powerful tool for identifying and mapping new genetic markers for the susceptibility and prognosis of each type of hereditary tumor, allowing a more complete chromosome localization than linkage analysis [73]. The search for alterations of these genes in patients and/or families with suspected hereditary syndromes of predisposition to cancer may allow the implementation of appropriate measures of risk reduction (primary prevention) and surveillance (secondary prevention) [74].

5.4 Sporadic and Heredofamilial Tumors

A tumor can be classified as sporadic, familial, or hereditary [75, 76] (Fig. 5.2). In sporadic tumors, spontaneous mutational events are present only in somatic cells of primary tumor, whereas in the hereditary tumors every cell of the organism harbors that specific gene alteration [77]. Although most cases of cancer are sporadic, since they occur in subjects without a significant family history for this disease, however, about 5–10% of tumors are related to hereditary factors. Sporadic tumors account for approximately 75–80% of newly diagnosed tumors and are found in subjects without evidence of inheritance [78, 79]. A tumor is considered as familial when one or more cases of cancer occur in members of the same family, in the absence of a genetic component. The trend toward family aggregation can be explained by two factors, such as the exposure to environmental conditions and family segregation of low-penetrance alleles and genes related to an increased tumor susceptibility [80]. Genetic variations in low-penetrance alleles generally involve a modest increase in cancer risk. The term "hereditary" refers, instead, to a situation in which the susceptibility of developing a certain tumor is inherited in a Mendelian way through genes of high-penetrance predisposition [81] (Fig. 5.2).

Examples of high-penetrance susceptibility genes are *BRCA1* and *BRCA2* in hereditary breast and ovarian cancers (HBOC). If one of the progenitors has a germline mutation in the genes involved in the onset of a particular tumor, the offspring has a 50% probability of inheriting that mutation [57, 82]. In the individual carriers of a germline mutation, all the cells of the organism harbor that mutation, predisposing such subject to develop a neoplasm easier and earlier with respect to the general population [23]. Therefore, carriers of mutations do not have the absolute certainty to develop a tumor during the course of their life but only have an increase in the probability of developing it as compared to the general population. Most of hereditary tumor syndromes follow an autosomal dominant inheritance pattern and are associated with germline mutations present in susceptibility genes [14]. The transmission of hereditary mutations occurs according to the Mendel's laws, depending on whether the

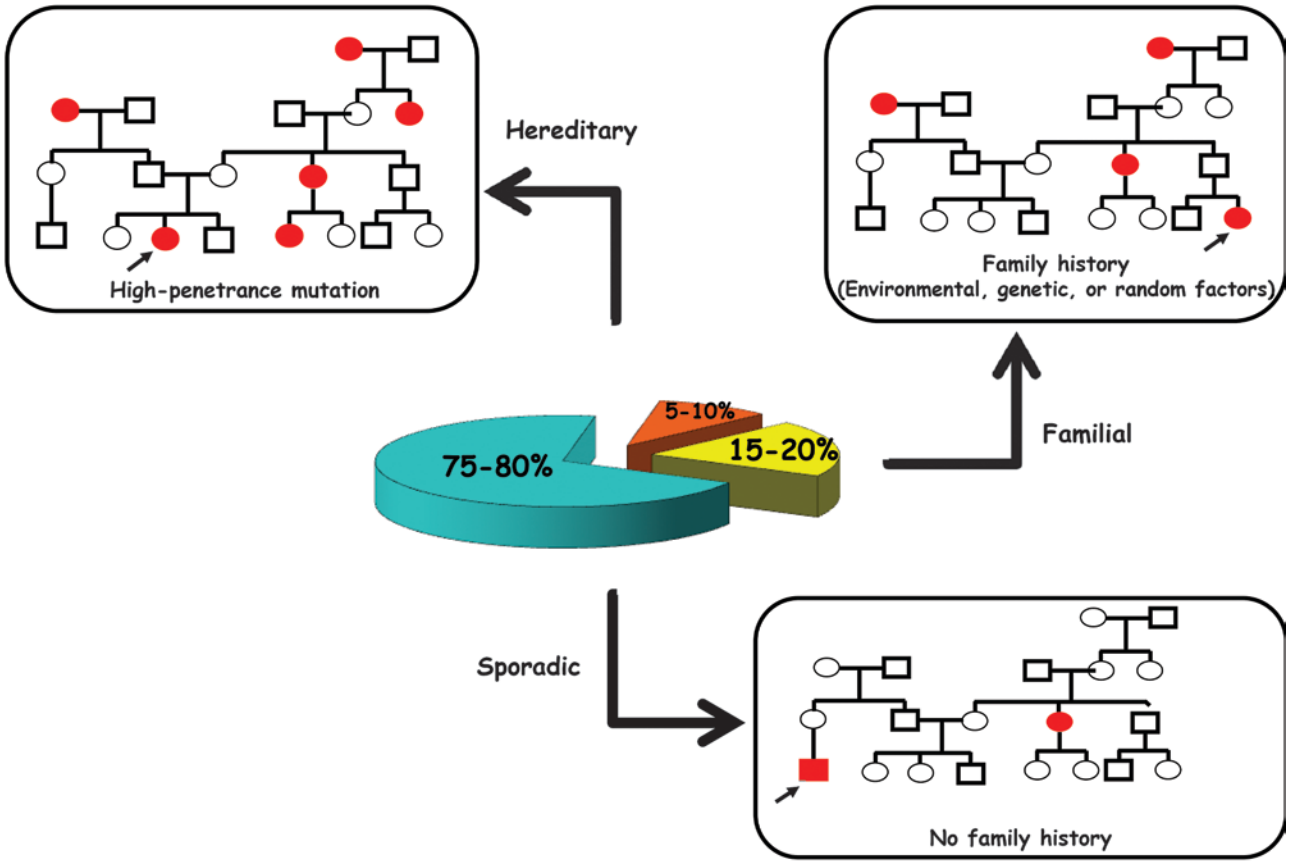

Fig. 5.2 Segregation patterns related to hereditary, familial, and sporadic tumors

mutated gene carries a dominant or recessive character [83] (Fig. 5.3).

The evidence of autosomal dominant transmission is represented by the appearance of tumors in multiple generations and association with peculiar syndromes or congenital anomalies. Furthermore, hereditary tumors often arise at a very early age and are frequently multiple (synchronous or metachronous) [83]. Additional features that suggest a hereditary syndrome of cancer predisposition include the appearance of multifocal or bilateral primary tumors, the presence of tumor cases in two or more family members (same branch of the family), and the identification of clusters of tumors associated with a specific syndrome (e.g., colorectal cancer and endometrial carcinoma in Lynch syndrome or HNPCC) [38, 84]. In a hereditary tumor, a germline mutation in a gene can be followed by the loss of heterozygosity (LOH) that hits the second allele and can result in the appearance of a point mutation (due to insertions or deletions of bases), promoter hypermethylation causing gene expression silencing, or a chromosomal deletion [85, 86]. Models of onset of sporadic and hereditary tumors are shown in Fig. 5.4.

5.5 Genetics of Hereditary Breast and/or Ovarian Cancer

Among major hereditary tumor syndromes associated with mutations in caretakers' genes, there is breast and/or ovarian cancer. Although breast (BC) and ovarian (OC) cancers are more frequently sporadic (75–80%), approximately 15–20% are familial forms, and about 5–10% of cases are hereditary [87]. Less than half of these hereditary forms are associated with germline pathogenic variants (PVs) in well-known susceptibility genes that confer a high (*BRCA1*, *BRCA2*, *TP53*) or moderate (*CHEK2*, *PTEN*, *ATM*, *etc.*) risk to develop the neoplasm over a lifetime [88–90]. Although PVs in other genes, including *CHEK2*, *ATM*, *BRIP1*, *PALB2*, *MRE11*, *NBS1*, *RAD50*, and others, have been reported in families with BC/OC recurrence, their effect on the disease risk (3–5%) was estimated as being lower than that given by *BRCA1* and *BRCA2*, and their relevance for the clinical management of mutation carriers is, to date, still debated and requires further in-depth clinical studies [91–93] (Fig. 5.5). Furthermore, these alterations are not routinely investigated due to technical (including the interpretation of such new variants) and

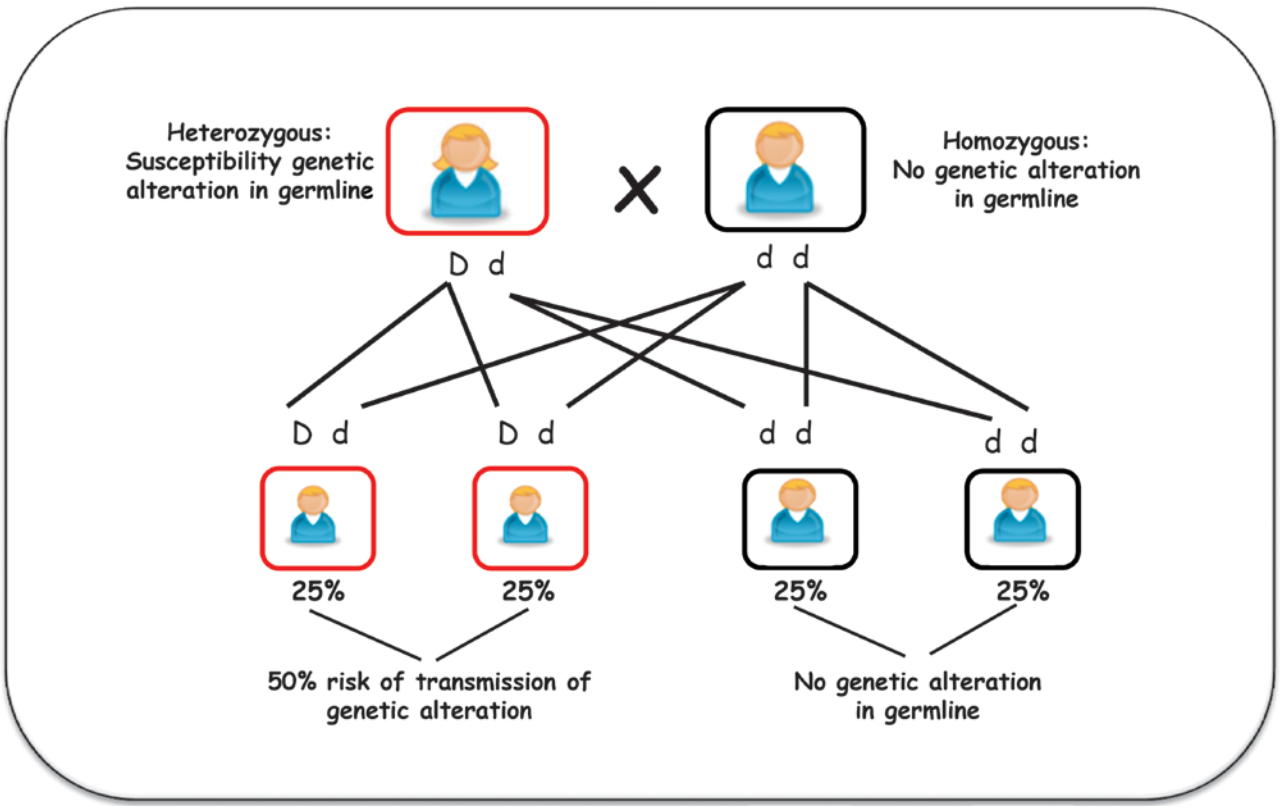

Fig. 5.3 Mendelian inheritance and autosomal dominant transmission in hereditary tumors

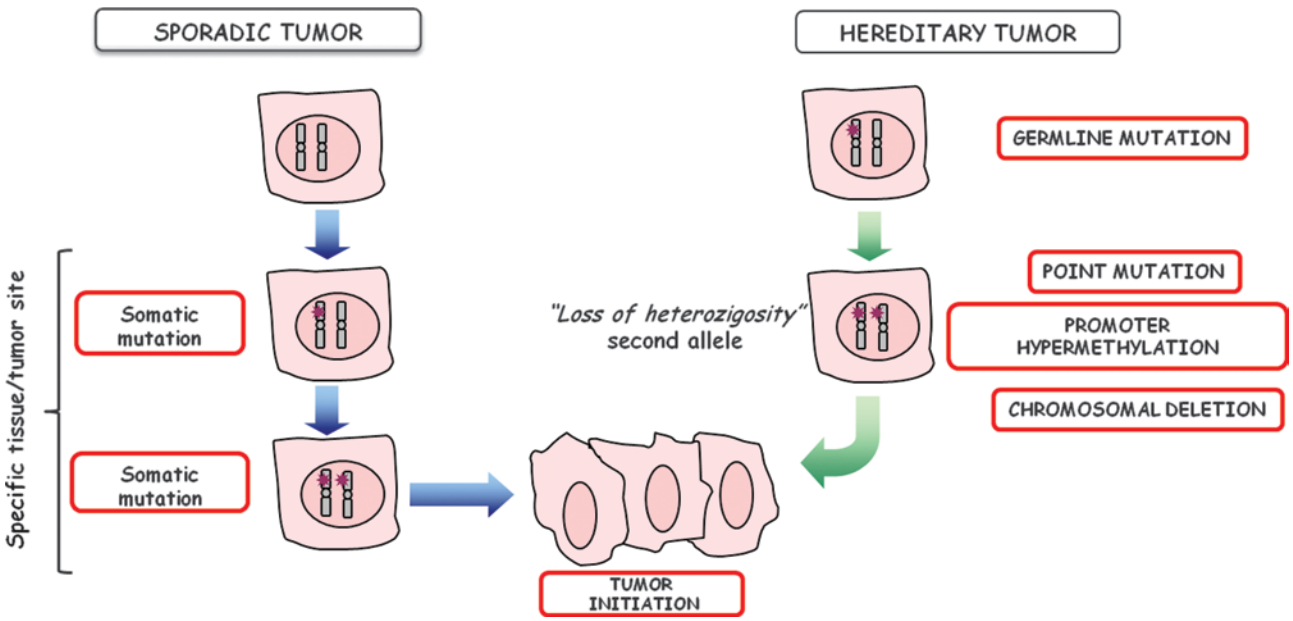

Fig. 5.4 Models of onset of sporadic and hereditary tumors

economic limitations. However, new DNA sequencing strategies (as the next-generation sequencing, NGS) have already demonstrated their capability to overcome these limitations and, soon, will allow to analyze large gene panels in addition to *BRCA1* and *BRCA2* [94].

Mutations in not yet identified high-penetrance susceptibility genes or polymorphisms in several low-penetrance loci (polygenic susceptibility) appear to be involved in more than 60% of cases of heredofamilial BC e/o OC [95, 96]. However, in most cases, hereditary

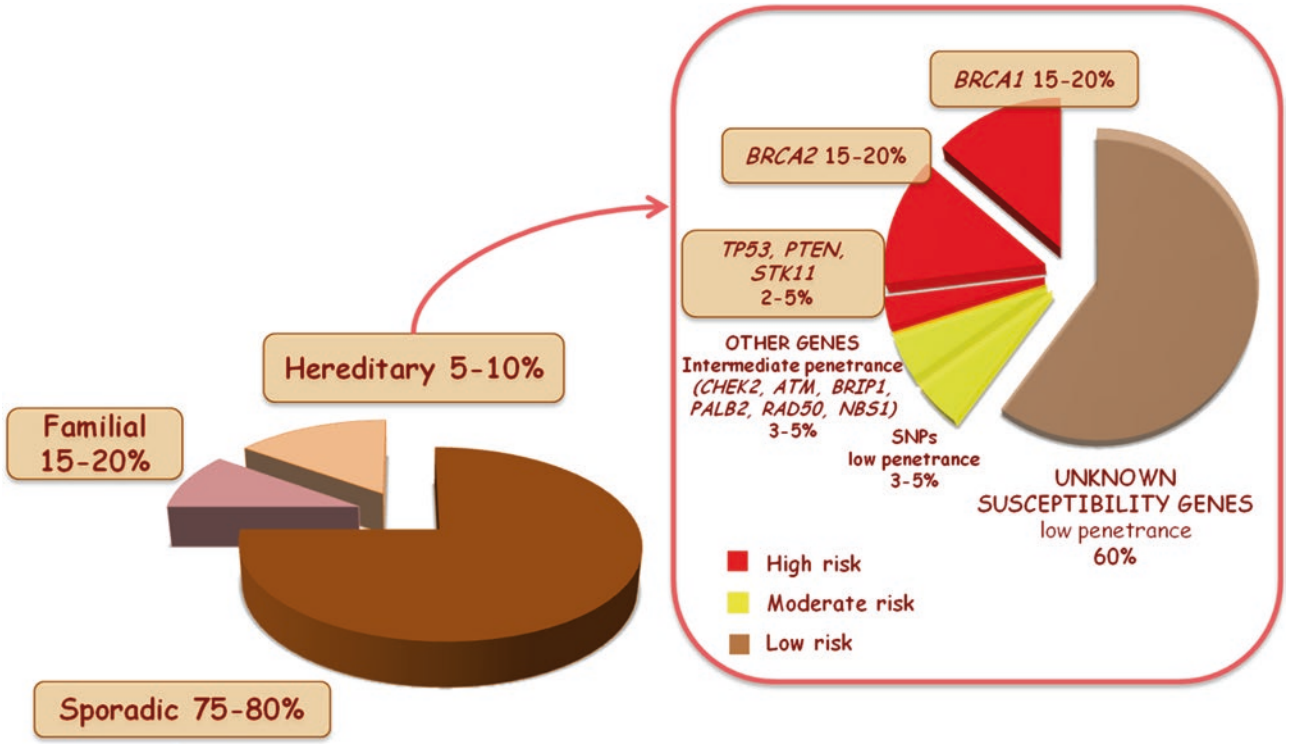

Fig. 5.5 Major susceptibility genes responsible for hereditary breast and/or ovarian tumors

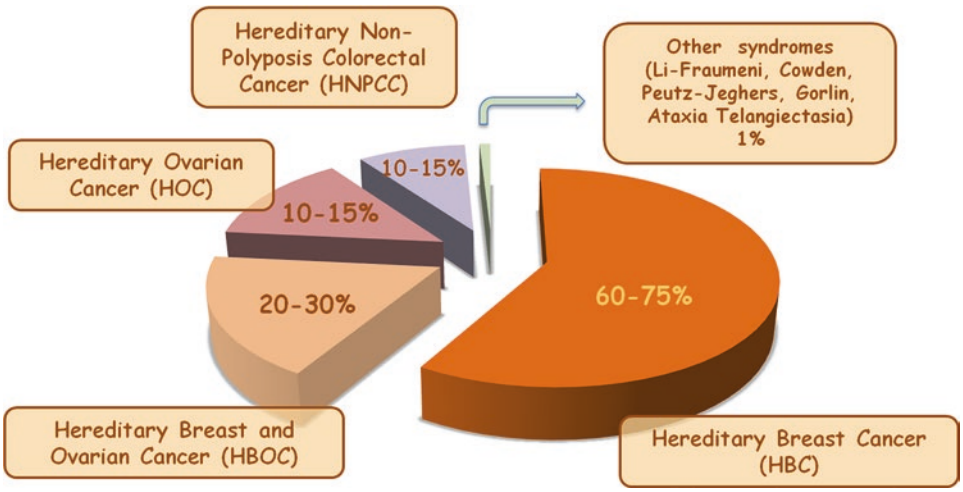

Fig. 5.6 Family profiles and syndromes related to hereditary breast and/or ovarian tumors

predisposition to BC/OC is subject to the polygenic model which involves additive or multiplicative combinations of multiple allelic variants at low-penetrance, each of which individually confers a mild-to-moderate risk of developing the tumor [71, 97, 98]. Several GWAS have allowed to identify eight allelic variants (SNPs) at low-penetrance (*FGFR2*, *TNCR9*/*Tox3*, *H19*, *MAP3K1*, *LSP1*, *8q*, *2q35*, *ECHDC*/*RNF*) responsible for only 3–5% of heredofamilial BC/OC cases [72, 92, 99] (Fig. 5.5).

Infrequently, family recurrence of BCs may be associated with PVs affecting other genes, including *PTEN* and *TP53* (responsible for Cowden syndrome and Li-Fraumeni syndrome, respectively), whereas OC recurrence may also be associated with Lynch syndrome, linked to alterations within mismatch repair (MMR) genes [38, 100] (Fig. 5.6). Patients affected by Li-Fraumeni syndrome develop various tumors, including sarcomas, leukemia, brain tumors, adrenocortical carcinomas, and also BC [101]. Instead, the Cowden syn-

drome is a condition characterized by multiple hamartomas and confers a risk of developing BC in 1% of cases [102]. Finally, the *ATM* gene, responsible for ataxia-telangiectasia syndrome, predisposes to many tumors, especially leukemia, lymphomas, pancreas adenocarcinoma, and also BC [90, 95, 103].

Patients with a BC and/or OC family history can be schematically classified into three different family profiles: hereditary breast cancer (HBC), hereditary ovarian cancer (HOC), and hereditary breast and ovarian cancer (HBOC) [104]. The HBC term is used when members of a family carrying a *BRCA1*/*BRCA2* PV only present with BC cases. On the other hand, if cases of both BC and OC segregate in their family tree, the HBOC term is used [27, 100, 105]. Finally, more rarely, the HOC term is used when there are only OC cases in a family [84]. HBC, HOC, and HBOC can be considered different phenotypic manifestations of the same genetic syndrome (Fig. 5.6).

5.5.1 *BRCA1* and *BRCA2* Genes

Two high-penetrance susceptibility *caretakers*' genes called *BRCA1* (breast cancer 1) and *BRCA2* (breast cancer 2) are considered responsible for 30 to 70% of hereditary breast and ovarian cancers. The modality of transmission of the PVs affecting these genes to the offspring is autosomal dominant [57]. Recent studies have suggested the existence of a third susceptibility gene, called *BRCA3*, probably located on chromosome 13q21 [106].

The *BRCA1*, the first of the two isolated genes, is located on chromosome 17q21 and consists of 24 exons, of which 22 encoding for a phosphoprotein of 1863 amino acids with a molecular weight of 220 kD. The *BRCA1* exon 11 (now reclassified as exon 10 with the newest nomenclature) shows a considerable size and encodes 60% of the protein [107]. The *BRCA2* gene, discovered about a year later and located on chromosome 13q21, shows larger dimensions compared to the *BRCA1* one, and it is made up of 27 exons, of which 26 encoding for a protein of 3418 amino acids [108]. Both genes show a high structural homology. BRCA proteins present several functional domains and are responsible for the DNA genomic integrity, as they are involved in the DNA repair system by homologous recombination (HR) which allows to repair DNA double-strand breaks (DSBs) [109, 110]. In addition to their implication in DNA damage response, BRCA protein is also involved in various cellular processes such as transcription regulation, cell cycle progression, apoptosis, and protein ubiquitination [111, 112]. Recent studies have also shown an epigenetic role of both BRCA proteins in chromatin remodeling and related processes such as transcription and DNA repair [53, 113]. *BRCA1* is involved in cell cycle checkpoints, genomic stability maintenance, centrosome duplication, and development of T lymphocytes [114]. This gene plays a key role in delaying cell cycle progression when DSBs occur, until they are completely repaired by the HR system. The nuclear localization and BRCA1 phosphorylation level are also regulated by DNA damage [107]. There are two different levels of monitoring for genomic integrity: the first controlled by BRCA1 and BRCA2 and the second by p53, resulting in the increase in p21 transcription and apoptosis. The p53 activation may be due to its direct interaction with BRCA1, resulting in apoptosis of tumor cells [115]. The multiple roles played by BRCA1 are due to its ability to interact with BRCA1-interacting proteins (BIPs), including RB1, p53, ATM, c-myc, BRCA2, DNA repair factors, and E2F. *BRCA1* mutations are closely related to an increase in genomic instability caused by chromosomal aberrations and aneuploidy [116]. Furthermore, *BRCA1*-mutated cells are hypersensitive to ionizing radiation (IR) and DNA lesions caused by DSBs [117].

BRCA2 has a characteristic domain containing eight BRC repeats through which it is able to bind to RAD51 protein. This suggests a potential role of BRCA2 as an assembly regulator for RAD51 on DNA double helix, during the HR system-mediated mechanism [118]. *BRCA2* also appears to be involved in Fanconi anemia, via *FANCD1* gene [119]. *BRCA2*, instead, is not associated with cell cycle checkpoints or apoptosis. Probably, the loss of function of *BRCA1/2* confers embryonal lethality in humans, since no individuals with germline mutations in the two alleles of both genes were found [120]. Pathogenic variants in *BRCA* genes do not fall within mutational "hot spots" but are uniformly distributed throughout the gene. The PVs identified in *BRCA* genes, since they were discovered, and their specific frequencies are recorded in an international database called Breast Cancer Information Core Database (BIC) which, to date, reports more than 600 pathological PVs both for *BRCA1* and *BRCA2* [121]. Nevertheless, BIC database is not still curated or updated as compared to other main databases, like ClinVar, the latter reporting more than 1500 PVs for both *BRCA1* and *BRCA2*. ClinVar database is also linked to the ENIGMA Consortium and reports the definitive classification of hundreds and hundreds variants. About 70–80% of genetic alterations are pathogenic, cause the formation of a truncated protein having smaller size, and mainly include frameshift and nonsense mutations, small insertions and deletions, and, to a lesser extent, missense mutations [122–124]. Approximately 3–5% of the pathogenic genetic alterations consist of genomic rearrangements (large duplica-

tions and deletions) and are mainly harbored by *BRCA1* [125, 126]. For the large *BRCA1* and *2* genomic rearrangements, there are no consisting data about possible founder effect, with exclusion of the Portuguese population. In addition to pathogenic alterations, there are variants of uncertain significance (VUS) and synonymous point mutations [127–129] (Fig. 5.7).

Specific PVs present only or predominantly in some populations or ethnic groups are called "*founder*" mutations, corresponding to genetic alterations that have originated in an ancestor of the observed population and were maintained in the course of evolution [130–132]. These PVs were seen, for the first time, in Ashkenazi Jews, who had the 185delAG-*BRCA1* mutation in 1% of cases, resulting in a 16–20% BC risk before the age of 50 [133]. *Founder* mutations have also been identified in various European populations [134, 135]. In Italy, the prevalence of the *BRCA1*-5083del19 and *BRCA2*-8765delAG mutations was observed [136, 137]. The *BRCA1*-5083del19 PV was identified in families of Calabrian origin and, more recently, in some families of Sicilian origin [130, 138]. In Tuscany, the *BRCA1*-1499insA mutation was identified as a hypothetical *founder* [139].

Ovarian cancers associated with *BRCA1* germline variants are four times more frequent than those arising from *BRCA2* mutations. *BRCA1* PVs confer in the *carriers* a 50–85% risk of developing BC during their lifetime, a 40–60% risk of developing also bilateral BC [139], and a 15–45% risk of developing OC or tubal carcinoma. Also germline *BRCA2* PVs confer carriers a 50% to 85% risk of developing BC and a lower risk (10–20%) of developing OC [87, 89, 140].

BRCA1-mutated BC frequently shows a poorly differentiated infiltrating ductal histotype, characterized by high proliferative activity, negativity of hormone receptors for estrogens (ERs) and progesterone (PR), and absence of *HER2/neu* amplification [141]. Therefore, this "triple-negative" phenotype results to be very aggressive and difficult to treat from the therapeutic point of view [142]. Instead, differences in *BRCA2*-mutated tumor types compared to those sporadic were not reported, except for a slight increase in the incidence of the lobular histotype [143, 144].

Recently, it has been demonstrated as the mutational status of *BRCA1/2* in OC patients which can help to determine the most suitable therapeutic treatment regimens, exploiting a new concept in oncology called "synthetic lethality" [145]. This event involves the participation of an enzyme called poly(ADP-ribose) polymerase 1 (PARP1) and its mechanism of action

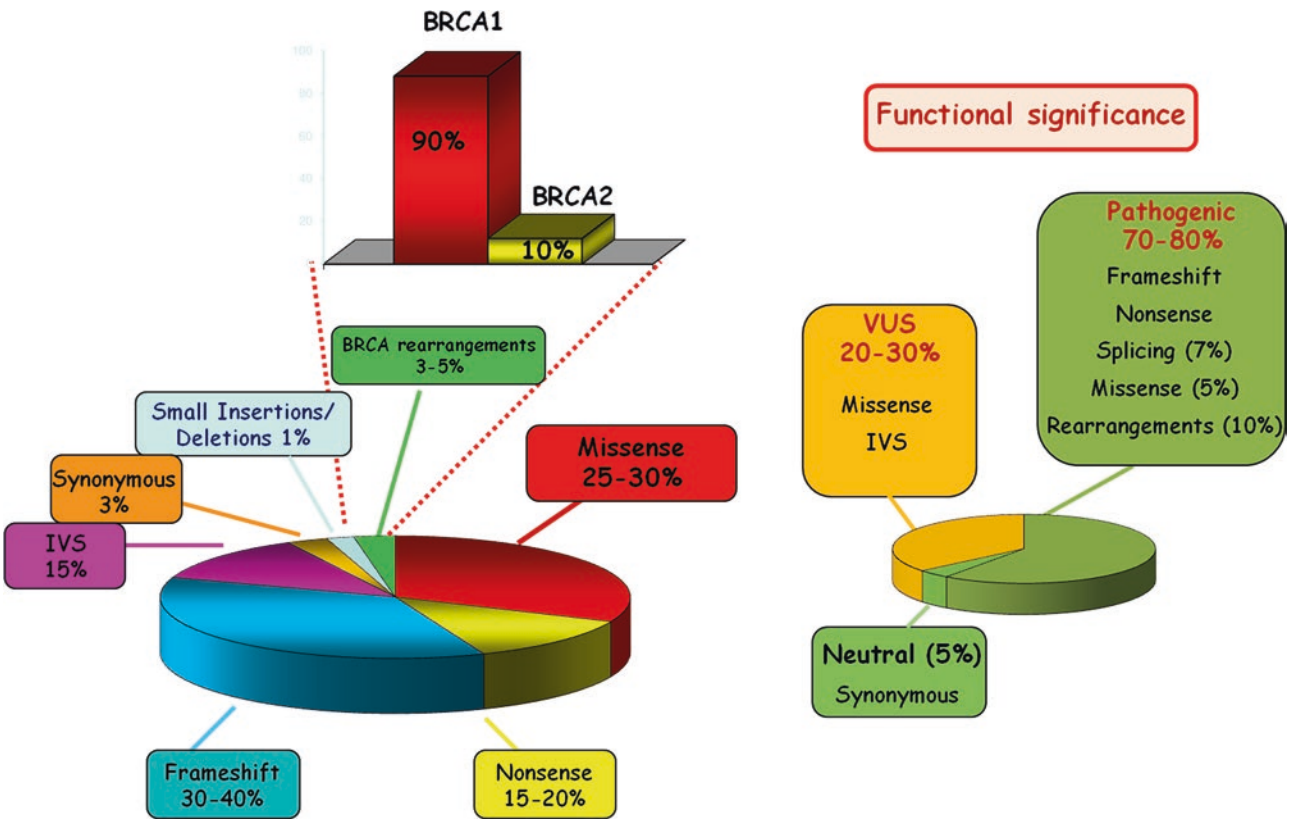

Fig. 5.7 Type and frequency of mutations in *BRCA* genes. VUS variants of uncertain significance, IVS intervening sequence (intronic)

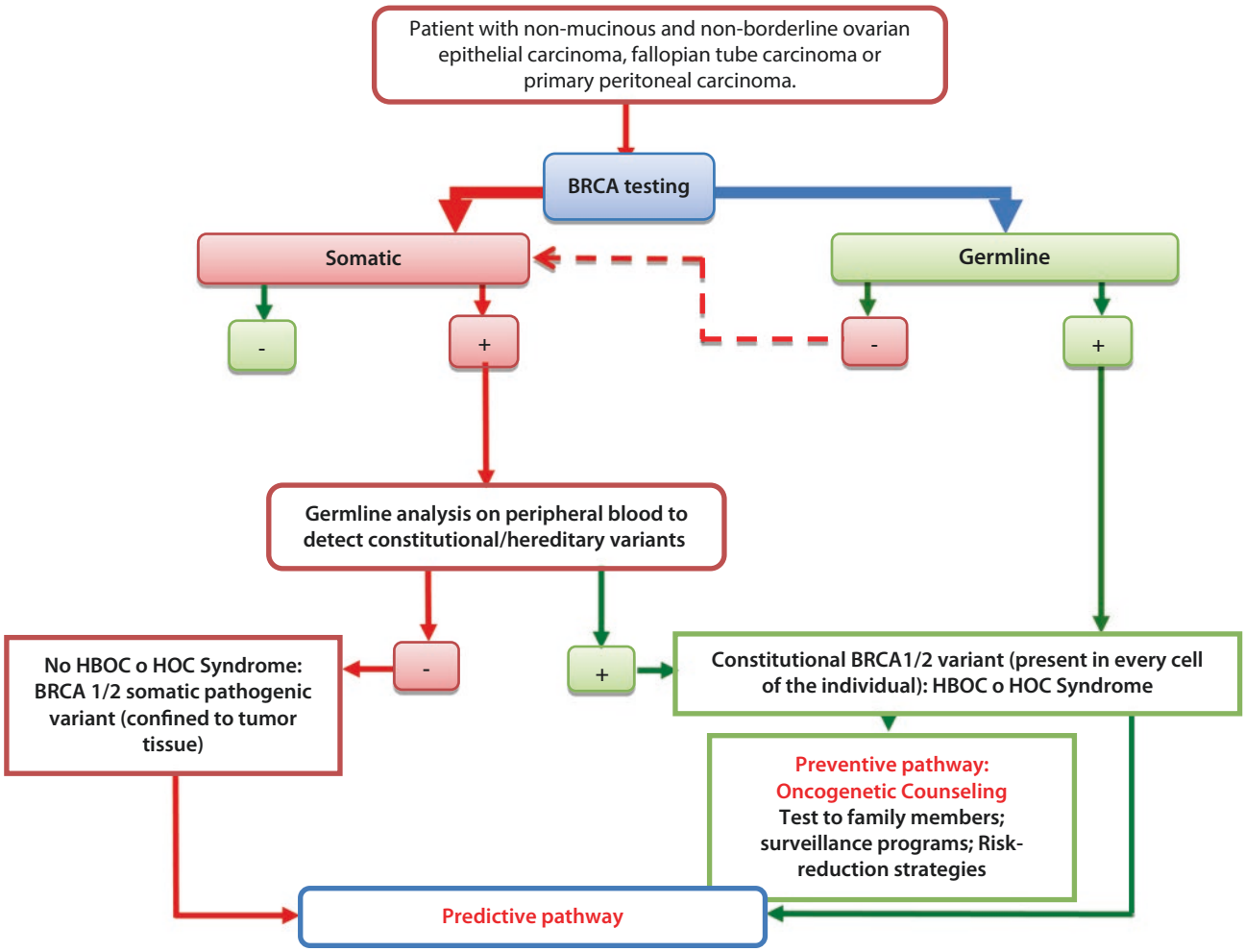

Fig. 5.8 Flow chart describing the pathway for BRCA genetic testing in OC patients (Courtesy of Associazione Italiana di Oncologia Medica (AIOM))

involved in DNA single-stranded breaks (SSBs) repair. PARP1 is the major mediator of the base excision repair (BER) system, through the regulation of several proteins, including XRCC1, involved in DNA repair and maintenance of genome integrity [146, 147]. *BRCA*-mutated OC cells have already lost their ability to repair DNA double-stranded breaks (DSBs) by the HR system; therefore, the inhibition of the PARP-mediated BER system by a PARP inhibitor prevents DNA repair and induces cell death or "synthetic lethality" [148]. Recently, the introduction of PARP inhibitors in clinical practice was shown as of significant beneficial effects in the therapeutic treatment of OC individuals with deficiency in BRCA function. PARP inhibitors represent the first example of agents targeting the loss of a tumor suppressor gene [149]. The PARP inhibition becomes synthetically lethal in tumors with inactivating mutations in *BRCA* genes, as a HR system deficiency makes them dependent on other DNA repair pathways [145, 150]. Olaparib was one of the first developed PARP inhibitors that showed therapeutic efficacy in OC patients with *BRCA* mutations [151, 152].

It is initially preferred to search the BRCA1/2 PVs on tumor tissue, because the BRCA testing on peripheral blood is able to detect only constitutional/hereditary variants. The identification of a PV, somatic or germline, allows to identify the OC patients with higher probability of response to specific PARP inhibitors. In the case of a constitutional variant, in addition to predictive information, the patient will gain the access, through the genetic counseling, to the preventive pathway (surveillance programs and risk reduction strategies). The flow chart describing the pathway for BRCA genetic testing in OC patients is shown in Fig. 5.8 [153].

5.6 Genetics of Male Breast Cancer

Male breast cancer (MBC) is a rare disease representing less than 1% of all cancers in men and less than 1% of all breast cancers in Western countries. The annual incidence of MBC is estimated at less than 1 per 100,000 men [154]; however data from the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute (NCI) indicated an increasing incidence of MBC over the last 30 years [155, 156].

Age-specific incidence rates for MBC increase linearly and steadily with age [155]. The mean age of BC presentation in males is mostly in the late 1960s [157]. Based on age-frequency distribution, age-specific incidence rate patterns, and prognostic factor profiles, MBC is considered similar to late-onset, postmenopausal estrogen/progesterone receptor-positive (ER+/PR+) female breast cancer (FBC). Compared with FBC, MBC has been reported to occur later in life, present at a higher stage, and displaying lower histologic grade, with a higher proportion of ER+ and PR+ tumors [158, 159]. MBC is recognized as an estrogen-driven disease, specifically related to hyperestrogenism. Diseases, conditions, or treatments that can increase the levels of estrogen may contribute to the development of MBC.

Approximately 15% to 20% of men with breast cancer report a family history of breast or ovarian cancer. Moreover, about 2% of patients with MBC develop a second primary breast cancer, and more than 20% of patients develop a second non-breast tumor, more frequently prostate, colon, and genitourinary cancer [160]. Overall, these associations point to a relevant genetic component in MBC.

Genetic risk factors (Table 5.4) play a key role in MBC susceptibility, and it is estimated that more than 10% of men with breast cancer have a genetic predisposition. A positive family history (FH) of BC is considered the major MBC predisposition factor. Men with a positive first-degree FH have a twofold increased risk of BC, which increases to more than fivefold with the number of affected relatives and early-onset relatives [161].

About 15% of all MBCs are hereditary forms caused by inherited germline PVs in well-identified BC susceptibility genes [162, 163]. By their mutation frequency and the magnitude of their impact in BC susceptibility, these genes can be divided into "high-penetrance," "moderate-penetrance," and "low-penetrance" genes (Table 5.5).

Table 5.4 Genetic risk factors for MBC

	Genetic risk factors
Well-established	Breast cancer family history *BRCA1*/ *BRCA2* *PALB2* Klinefelter syndrome
Possible	*CHEK2* SNPs
Suspected	Cowden syndrome Lynch syndrome

Table 5.5 MBC genetic susceptibility

	High	Moderate	Low
Genes	*BRCA1* and *BRCA2*	*CHEK2* and *PALB2*	*ESR1*, rs1314913 (*RAD51B*), rs3803662 (*TOX3*), rs1562430 and rs445114 (8q24.21), rs1011970 (*CDKN2A*/*CDKN2B*), rs614367 (*CCND1*)
Population frequency	<0.1%	MAF 1%	MAF >10%
Cancer risk (Odds ratio)	>10.0	>2.0	0.76–1.57

5.6.1 High-Penetrance Genes

BRCA1 and *BRCA2* are the most important BC susceptibility genes in high-risk families. PVs in *BRCA2* gene are estimated to be responsible for 60–76% of MBCs occurring in high-risk BC families, whereas frequency of *BRCA1* sequence alterations ranges from 10% to 16% [164]. PVs affecting both *BRCA1* and *BRCA2* genes are often found in patients with MBC who have multiple cases of breast and/or ovarian cancer in their family, but they were found in patients with MBC without FH [165].

Overall, it is reported as about 10% of all MBCs are caused by inherited germline PVs in both *BRCA1 and BRCA2* genes. The estimated lifetime risk of MBC is 5–10% in *BRCA2* and 1–5% in *BRCA1* mutation carriers [166], as compared to the risk of 0.1% of developing MBC in the general population [167]. The median age at

BC diagnosis among male *BRCA2* mutation carriers is earlier (median, 58.8 years) than that of negative cases (median, 67.9 years). Male *BRCA1* and *BRCA2* mutation carriers are also at increased risk of developing several cancer types, including prostate and pancreatic carcinomas [168]. Specific *BRCA1* and *BRCA2* PVs show high frequency in specific countries or ethnic groups, particularly, in genetically isolated populations. These variations are descended from a single founder. Founder mutations may also explain variability in BC incidence rates among countries. For example, three founder PVs, two in *BRCA1* (c.185delAG and c.5382insC) and one in *BRCA2* (c.6174delT), have been observed at higher frequency (>2% in total) in the Ashkenazi Jewish male population than in the general US population [164]. Generally, those affecting *BRCA1* are quite rare in unselected MBC cases, being more frequent in specific populations in which a founder effect is known to occur [169]. A founder effect for the *BRCA1* c.3347delAG mutation was found in Italian MBC cases [170–172].

BRCA1/2 large-scale rearrangements, including insertions, deletions, or duplications of more than 500 kb of DNA, have been also been identified in both male and female BC patients [173–176]. Interestingly, large genomic rearrangements (LRGs) in *BRCA2* are more frequent in families with MBC [175, 177], while LRGs in both *BRCA1* and *BRCA2* are infrequent in MBC cases unselected for FH [178].

Differently from both OC and women's BC, at present, there is no evidence for a correlation between the location of the mutation within *BRCA1* or *BRCA2* gene and risk of MBC [161].

It has been shown that MBC associated with *BRCA2* mutations displays specific clinicopathological features. Generally, MBC presents with lower histologic grade tumors than FBC. In contrast, MBC associated with *BRCA2* mutations presents with higher histologic grade compared both with FBC in *BRCA2* mutation carriers and with MBC in the general population from SEER [179]. In particular, higher histological grade breast tumors are more frequent among *BRCA2* mutation carriers male diagnosed at younger ages (below 50 years) than those diagnosed at older ages.

The identification of a specific *BRCA2*-associated phenotype suggestive of an aggressive behavior may define a subset of MBC patients (i.e., patients with high-grade breast tumors and with young age at diagnosis) who might particularly benefit from adjuvant chemotherapy. A similar trend is also observed for *BRCA1* mutation carriers. Overall, *BRCA1/2* MBCs display distinct pathologic characteristics compared to *BRCA1/2* FBCs. These findings should lead to the development of gender-specific risk prediction models and guide clinical strategies appropriate for MBC management.

5.6.2 Moderate-Penetrance Genes

Direct interrogation of candidate genes involved in *BRCA1/2*-associated DNA damage repair pathways led to the identification of other BC susceptibility genes, classified as moderate-penetrance genes. Variants found in this class of genes confer a smaller risk of BC than *BRCA1/2*.

CHEK2 c.1100delC was the first moderate BC risk allele identified. The *CHEK2* c.1100delC variant has been initially shown to confer approximately a tenfold increase of BC risk in men resulted as negative for *BRCA1/2* PVs: therefore, it was estimated to account for 9% of familial high-risk MBC cases [180]. On the other hand, mutations in *CHEK2* were found in 2.8% of MBC patients unselected for FH of BC and were associated with a 3.8-fold increased risk for MBC [181].

The contribution of the *CHEK2* c.1100delC mutation to MBC predisposition varies by ethnic group and from country to country [178, 180, 182–186]. A decreased frequency of the c.1100delC allele in North to South orientation has been observed in Europe.

The involvement of *BRCA1/2* in the Fanconi anemia (FA) pathway promoted mutational screening of other FA genes functionally linked to *BRCA1/2*, such as *PALB2*, *BRIP1*, and RAD51C [187].

In a recent study, Antoniou et al. [93] highlighted the relevant role played by *PALB2* in hereditary BC, suggesting that BC risk for *PALB2* mutation carriers may overlap with that for *BRCA2* mutation carriers. Therefore, *PALB2* could be considered as the third most important gene, following *BRCA1* and *BRCA2*, in BC susceptibility.

PALB2 sequence alterations were found in families with both female and male BCs [162, 188, 189]. Moreover, *PALB2* heterozygotes were fourfold more likely to have a male relative with BC [190]. To date, several studies have investigated the presence of *PALB2* PVs in MBC cases [162, 189–196]. These studies showed a variable *PALB2* mutation frequency ranging from 1% to 16% [189, 193, 194, 196]. Recent data reported a higher frequency of *PALB2* pathogenic mutations in high-risk MBC cases than that observed in high-risk FBC cases (4% vs 1%) [162].

PALB2 mutations are frequently observed in families with cases of melanoma, pancreatic, prostate, lung, and stomach cancers, in addition to BC [162, 193–195, 197, 198]. To date, the exact risk of MBC for *PALB2* mutation carriers is unknown: however, these studies suggest that *PALB2*-related families may resemble *BRCA2*-like families, in which MBC and several other cancers may be found in addition to FBC [162].

BRIP1 gene was originally suggested as a low-penetrant BC susceptibility gene [199]: nevertheless, recent studies indicated that *BRIP1* mutation carriers

have a high risk for ovarian cancer rather than BC [200, 201]. The role of *BRIP1* in MBC was investigated only in one study, and no evidences were found regarding the role of such germline *BRIP1*variants as possible factors contributing to MBC predisposition [192].

Similarly, despite a recent study reported *RAD51C* mutations in families with BC [202], mutations in this gene are mainly identified in families with either ovarian cancer only or breast and ovarian cancer [203]. Therefore, the involvement of *RAD51C* in BC is still unknown [204], and, at present, there is no evidence that *RAD51C* PVs may contribute to MBC susceptibility [205].

Rare variants in other genes, including hereditary cancer syndromes' genes (i.e., *TP53* and *PTEN*), and genes involved in DNA repair pathways (i.e., *ATM*) have been identified in a small number of pedigrees with MBC. However, the contribution of these genes to MBC risk still remains to be assessed [171, 181, 206, 207], as well as the role, in MBC, of rare mutations involved in BC susceptibility found in genes newly identified by whole exome sequencing analysis, such as *FANCM* and *RECQL* [208, 209].

5.6.3 Low-Penetrance Genes

A polygenic model, in which many genes that confer low risk individually act in combination to confer much larger risk in the population, was suggested as an explanation of the susceptibility to BC and other common cancers [210]. BCs not represented by currently known high- and moderate-penetrance BC susceptibility genes can be explained by this model. This hypothesis was confirmed by multigroup collaborations working in genome-wide association studies (GWAS) [211–216].

To date, only a few studies addressed the role of low-penetrance alleles in MBC susceptibility [217–220]. Two SNPs, rs1314913 in *RAD51B* gene and rs3803662 near *TOX3* gene, were found as being associated with MBC risk by GWAS. In particular, rs1314913 resulted specifically associated with increased BC risk in men whereas rs3803662 with an increased BC risk also in women [218]. Furthermore, by the gene candidate approach, the *ESR1* locus was found to be associated with BC risk in men and, in particular, with increased risk in ER-negative MBC cases and in male *BRCA1/2* mutation carriers [217, 219].

A significant association with MBC risk for four additional SNPs was also observed. These SNPs include rs1562430 and rs445114 both within the 8q24.21 multicancer susceptibility region, rs1011970 in *CDKN2A/CDKN2B* gene and rs614367 in *CCND1* gene. Furthermore, differences in the distribution of rs614367genotypes according to ER status and of rs1011970 genotypes according to HER2 status emerged [220]. These data suggest that the association of some SNPs with specific BC subtype seen in FBC could be also exist in MBC [221].

Overall, although the relative risk associated with SNPs is low, they are likely to be responsible for a substantial percentage of hereditary and sporadic MBCs because of their high frequency. Most SNPs that are associated with MBC risk are the same as those associated with FBC risk, but it seems that the magnitude of risk that is conferred by them is different in the two sexes [221].

Furthermore, some SNPs could act as modulators of the risk conferred by mutations in the high-penetrance BC susceptibility genes *BRCA1* and *BRCA2* [166, 222]. In the first GWAS performed in male carriers of *BRCA1/2* mutations, it was demonstrated that the combined effects of known BC susceptibility SNPs modify BC risk for male mutation carriers, with important implications on risk prediction. These results provided the first direct evidence of an overlap in the genetic susceptibility to female BC, as well as the modification of risks of BC in men with *BRCA1/2* mutations [166].

5.6.4 Oncogenetic Counseling, Screening, and Surveillance

Genetic counseling should be offered to MBC patients based on their increased risk of *BRCA* mutations, particularly in the context of a breast/ovarian cancer family history. The National Comprehensive Cancer Network (NCCN) recommendation stated that all MBC patients should be offered genetic counseling and testing based on their risk of carrying a deleterious mutation that might be relevant to their own care or the care of their family members. Risk assessment models to estimate the risk of carrying a *BRCA* mutation, such as BRCAPRO, are also considered as validated for use in male patients [223, 224].

Because incidence of MBC, adjusted by age, is only 1/100,000 individual per year, with lifetime risk of about 1/1000, there is no need of breast screening in the general male population. On the other hand, screening for BC in men at higher BC risk, including those with *BRCA1/2* mutations, strong family history of BC, such as affected mother and/or sister, Klinefelter syndrome, or transgenders, should be undertaken and should be available preferably in a clinical trial. Men at higher BC risk should be aware of the warning signs of BC and should be taught for breast self-examination. NCCN Guidelines (Version 1.2020) recommend that male *BRCA* mutation carriers have a clinical breast exam every 12 months, starting from 35 years, and have a prostate cancer screening starting at age 45 for *BRCA2* mutation carriers (Table 5.6).

Table 5.6 Management of *BRCA* mutation carriers

	BRCA mutation-positive management
Men	Breast self-exam training and education starting at age 35 yrs Clinical breast exam, every 12 months, starting at age 35 yrs Starting at age 45 yrs: Recommend prostate cancer screening for *BRCA2* carriers Consider prostate cancer screening for *BRCA1* carriers
Men and women	Education regarding signs and symptoms of cancer(s), especially those associated with *BRCA* mutations Screening may be individualized based on cancers observed in the family

Modified by NCCN Guidelines Version 1.2020

The risk of a new BC is higher in MBC survivors. MBC patients had a 30-fold increased risk of developing a contralateral BC, and this risk was greatest in men who were younger than 50 years at BC diagnosis. Thus, male survivors of early-stage BC could most benefit from breast screening. MBC survivors are also at risk of certain non-breast second malignancies, prostate being the most common [225]. Thus, MBC survivors should be offered the same screening programs for non-BC as men in the general population, unless they are found to carry deleterious genetic mutations for which specific follow-up is recommended. Overall, there is a clear need for protocols for both screening and surveillance and, more in general, for information and support to men diagnosed with BC.

5.7 Genetics of Hereditary Colorectal Cancer

Colorectal cancer (CRC) is one of the most frequently diagnosed cancers worldwide [226]. Although it often occurs sporadically, family history represents one of the strongest predictors of CRC risk, with about 30% of cases diagnosed in individuals who have one or more family members also affected with this disease [227].

CRC risk was shown to increase with the number of affected relatives and particularly in the presence of at least one early-onset CRC within the family [228]. These observations supported the hypothesis that hereditary factors influence CRC risk. Nowadays, it is estimated that approximately 5% of CRC cases are associated with highly penetrant inherited mutations related to known hereditary CRC syndromes [76].

Genetic susceptibility to CRC includes well-defined inherited syndromes such as familial adenomatous polyposis (FAP), *MUTYH*-associated polyposis (MAP), Lynch syndrome, and other less common syndromes, broadly divided into polyposis and nonpolyposis diseases, according to their different phenotypes [229] (Table 5.7 and Fig. 5.9).

5.7.1 Polyposis Syndromes

5.7.1.1 Familial Adenomatous Polyposis

Familial adenomatous polyposis (FAP) is a rare autosomal dominant condition, accounting for about 1% of CRCs. The disease is characterized by the development of 100 to thousands of adenomatous polyps in adolescence [230]. Since adenomatous polyps are the precursors to the majority of CRCs, in FAP patients the progression of polyps to CRC occurs by middle age, with a risk of CRC approaching 100% by age 50 in the absence of proctocolectomy [231]. Most of the resulting cancers occur in the left colon. Attenuated FAP (AFAP) is a variant of the disease characterized by a later onset, fewer polyps (from 10 to less than 100) often occurring in the right colon, and a CRC risk of about 70% by age 80 years [232].

Extracolonic cancers associated with FAP include adenocarcinomas of the duodenum and small intestine, desmoid tumors, papillary thyroid cancer (especially in women), medulloblastomas, and hepatoblastomas (in children <5 years of age) [233].

Both classic and attenuated phenotypes of FAP are caused by mutations in the *adenomatous polyposis coli* (*APC*) gene [234]. *APC* is a tumor suppressor gene encoding a protein involved in the *wnt* pathway, responsible to inhibit the activity of β-catenin, the transcription factor driving gut epithelial cell proliferation [228]. Loss of APC causes nuclear accumulation of β-catenin and uncontrolled proliferation due to upregulation of several oncogenes and is recognized as an early event in colorectal carcinogenesis [235]. Mutations follow the classical two-hit model of tumor suppressor inactivation. FAP patients inherit one germline PV and develop tumors from those cells in which a second hit, or loss of the wild-type allele of APC, is somatically acquired [236]. Notably, 30% of individuals with FAP do not inherit the PV but present with a de novo *APC* germline alteration; therefore they do not show a positive family history for the disease [237].

More than 1100 mutations have been identified in the *APC* gene, mostly resulting in a truncating protein product [238]. Among missense variants, the *APC* I1307K seems to moderately contribute to familial colon cancer, mostly in specific populations such as Ashkenazi Jewish [239]. Mutational hot spots are located at codons 1309 and 1061, accounting for approximately 17% and 11% of all germline APC mutations, respectively [240]. Because of the accumulation of mutations from codon 1250 to 1464, this region is termed the "mutation cluster region" [241].

Table 5.7 Hereditary CRC genes and their associated syndromes

Gene	Chromosome	Strenght of evidence	CRC risk level	Associated syndrome
APC	5q22.2	Well-established	High	Familial adenomatous polyposis (FAR) and attenuated FAP
BMPR1A	10q23.2	Well-established	High	Juvenile polyposis syndrome (JPS)
EPCAM	2p21	Well-established	High	Lynch syndrome
MLH1	3p22.2	Well-established	High	Lynch syndrome
MSH2	2p21-16	Well-established	High	Lynch syndrome
MSH6	2pl6.3	Well-established	High	lynch syndrome
MUTYH (biallelic mutations)	lp34.1	Well-established	High	*MUTYH*-associated polyposis
MUTYH (heterozygotes)	lp34.1	Not well-established	Uncertain – moderate at most	Possible increased risk for CRC
POLD1	19ql3.33	Not well-established	Uncertain – presumed high risk from limited case reports	Polymerase proofreading-associated polyposis (PPAP)
POLE	12q24.33	Not well-established	Uncertain – presumed high risk from limited case reports	Polymerase proofreading-associated polyposis (PPAP)
PMS2	7p22.1	Well-established	High	lynch syndrome
PTEN	10q23.31	Well-established	Moderate-High	Cowden/ PTEN-Hamartoma syndrome
SMAD4	18q212	Well-established	High	Juvenile polyposis syndrome (JPS)
STK11	19pl3.3	Well-established	High	Peutz-Jeghers syndrome (PJS)
TPS3	17pl3.1	Well-established	High	Li Fraumeni syndrome

Modified from NCCN Guidelines Version 3.2017 Genetic/Familial High-Risk Assessment: Colorectal

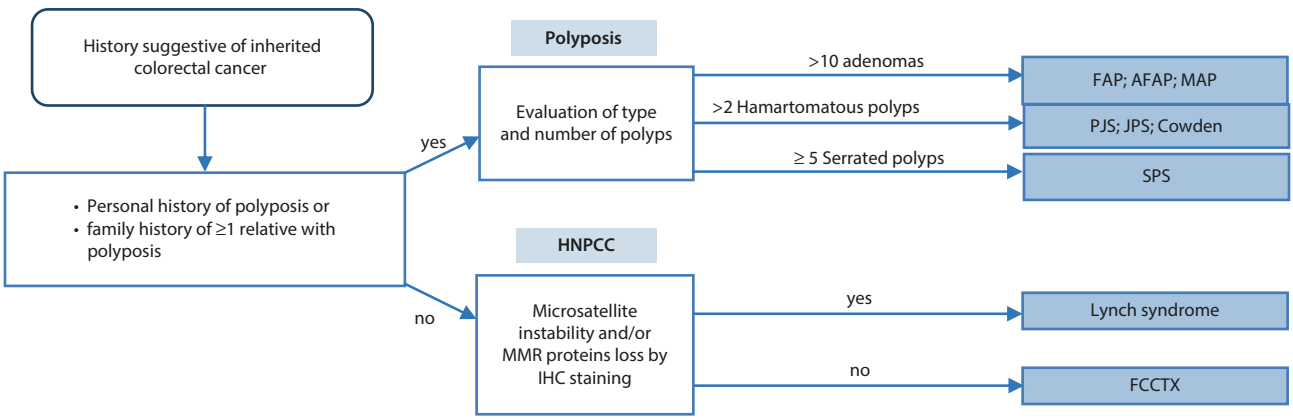

Fig. 5.9 Assessment for well-established hereditary syndromes associated with colorectal cancer. Genetic testing must follow clinical evaluation when appropriate. *HNPCC* hereditary nonpolyposis colorectal cancer, *MMR* mismatch repair, *IHC* immunohistochemistry, *FAP* familial adenomatous polyposis, *AFAP* attenuated familial adenomatous polyposis, *MAP MUTYH*-associated polyposis, *PJS* Peutz-Jeghers syndrome, *JPS* juvenile polyposis syndrome, *SPS* serrated polyposis syndrome, *FCCTX* familial colorectal cancer type X

Genotype-phenotype correlations are observed in FAP [242]. Loss-of-function PVs between codons 1250 and 1464 of the *APC* gene are associated with the most aggressive phenotype, characterized by >5000 polyps. Attenuated FAP is correlated with mutations upstream the codon 157, downstream the codon 1595, and within the alternatively spliced region of exon 9. Mutations in the remainder of the APC gene cause an intermediate

phenotype (hundreds to thousands of adenomas). In about 10% of patients with the FAP or AFAP phenotype, no germline variants in *APC* gene or its promoter, or large genomic deletions at *APC* locus, are detected, suggesting that additional genetic factors not yet identified may be associated with these phenotypes [228].

Recently, germline PVs in the proofreading domains of the DNA polymerase genes *POLE* and *POLD1* have been found in patients with attenuated FAP who resulted as negative for germline *APC* PVs [243]. These FAP variants were recognized as polymerase proofreading-associated polyposis (PPAP): nevertheless, it is a heterogeneous and still incompletely characterized disease [244].

5.7.1.2 MUTYH-Associated Polyposis

Up to one-third of individuals with suspected FAP/AFAP but negative for *APC* mutations are found carriers of biallelic germline *MUTYH* mutations [245]. *MUTYH* gene encodes for a glycosylase involved in base excision repair. MUTYH deficiency results in genetic instability of several cancer-related genes including *APC*, *KRAS*, and *TP53* [246].

MUTYH-associated polyposis (MAP) follows an autosomal-recessive inheritance pattern; therefore a family history of polyposis is rarely evident. The pathogenesis of MAP-related tumors has phenotypic similarities with FAP [247]. Patients with MAP have a lifetime risk of CRC ranging from 43% to almost 100% in the absence of timely surveillance, with a mean age at diagnosis of 50

years. They also have an increased risk for extracolonic tumors including duodenal cancer. Biallelic *MUTYH* mutations were identified in 1.7% of unselected CRC cases [248].

Among cases of European ancestry, two founder missense mutations, c.536A > G (p.Tyr179Cys) in exon 7 and c.1187G > A (p.Gly396Asp) in exon 13, account for at least 90% of all *MUTYH* pathogenic variants [249].

In population-based cohorts, monoallelic *MUTYH* mutations are found in about 1% of tested individuals. These heterozygote carriers may also be at moderate increased risk of CRC (from no to threefold increase, respectively, as compared to the general population), although study results are conflicting [250]. Recently it was shown that lifetime CRC risk in *MUTYH* heterozygotes was 7.2% for males and 5.6% for females, independent of family history. In the presence of a first-degree relative with CRC, diagnosed by age 50 years, without confirmed MAP (i.e., untested, no *MUTYH* pathogenic variant, or a heterozygous *MUTYH* pathogenic variant), the risk of CRC was 12.5% for men and 10% for women [251].

5.7.1.3 Other Rare Polyposis Syndromes

Not all CRCs develop from adenomatous polyps. Hamartomatous polyps are peculiar features of Peutz-Jeghers syndrome (PJS), juvenile polyposis syndrome (JPS), and Cowden/PTEN hamartoma tumor syndrome. All these syndromes are rare autosomal dominant conditions, associated with increased risks for gastrointestinal and other cancers, and implicated in less than 1% of all CRC cases [228].

PJS is caused by mutations in *serine threonine kinase 11* (*STK11*, also known as *LKB1*), a tumor suppressor gene involved in the mTOR pathway [252]. The lifetime cancer risk for affected individuals is estimated at 85–90% at age 70 years, including breast, pancreas, colon, small intestine, and stomach cancers [253].

JPS is mainly caused by mutations in *SMAD4* and *BMPR1A* genes, encoding proteins involved in TGF-β signaling pathway [254]. Mutations in *ENG*, another gene in the same pathway, have been reported in some patients [255]. Individuals with JPS are at increased risk for both CRC and gastric cancer, with cumulative lifetime risks approaching 40–50% [256].

Cowden syndrome is due to germline mutations in the *phosphatase and tensin homolog* (*PTEN*) gene, involved in the PI3K/AKT/mTOR signaling pathway [257]. This syndrome has been associated with a broad range of cancers, including breast, thyroid, and endometrial; although colon polyps are among the clinical features of this syndrome, the magnitude of CRC risk remains unclear [258].

Serrated polyps are premalignant lesions believed to progress to cancer via alternative pathways, different from those in adenomas, and to have unfavorable prognosis. Estimates for CRC risk associated with serrated polyposis syndrome (SPS) range from 7% to 50% [259]. The genetic basis for SPS remains elusive, probably due to the clinical heterogeneity among affected cases. Emerging evidences link mutations in *RNF43*, a regulator of *ATM*/*ATR* DNA damage response, to SPS [260].

5.7.2 Hereditary Nonpolyposis Colorectal Cancer

Most familial clusters of CRC lack the distinctive phenotypes associated with adenomatous, hamartomatous, or serrated polyposis syndromes. Hereditary nonpolyposis colorectal cancer (HNPCC) is a term traditionally used to encompass a broad spectrum of conditions, with different genetic etiologies, tumor features, and cancer risks, characterized by the presence of familial CRC cases without polyposis [228]. Patients with HNPCC are defined by clinical criteria, regardless of the results of

Table 5.8 Clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC)

Amsterdam criteria	At least three relatives with CRC and all of the following: One affected person is a first-degree relative of the other two affected persons Two successive generations affected At least one case of CRC diagnosed before age 50 years FAP excluded
Modified Amsterdam criteria	Same as the Amsterdam criteria, except that cancer must be associated with HNPCC (colon, endometrium, small bowel, ureter, renal pelvis) instead of specifically CRC
Bethesda guidelines	CRC in a patient <50 years Synchronous or metachronous CRC or the presence of other HNPCC-associated tumors[a] regardless of age Pathologic features of a microsatellite instability-high cancer (tumor infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern) in a patient <60 years CRC in one or more first-degree relatives with an HNPCC-related tumor[a] with one of the cancers diagnosed by the age of 50 y (including adenoma by 40 years) CRC in two or more first- or second-degree relatives with HNPCC-related tumors[a] regardless of age

[a]Endometrial, stomach, ovarian, pancreas, small bowel, biliary tract, ureter or renal pelvis, brain, sebaceous gland adenoma, or keratoacanthoma

genetic testing. Indeed, these criteria, referred to as Amsterdam criteria, were first established in 1991, before the genetic basis for HNPCC was known, and later modified [261, 262] (Table 5.8). The most recent and inclusive criteria, taking into consideration also molecular and pathologic features, are referred to as the Bethesda guidelines and are used to specifically diagnose Lynch syndrome [263] (Table 5.8).

5.7.2.1 Lynch Syndrome

Lynch syndrome is the most common cause of inherited CRC, accounting for about 3% of newly diagnosed cases of CRC [264]. Patients with Lynch syndrome are at increased lifetime risks not only for CRC (70–80%) but also extracolonic cancers including endometrial (50–60%), stomach (13–19%), and ovarian (9–14%) cancers. Small bowel, biliary tract, ureter or renal pelvis, brain, and pancreas cancers are also overrepresented [265]. Sebaceous adenomas and carcinomas of the skin, as well as keratoacanthomas, can be seen in the Muir-Torre variant of Lynch syndrome [229].

This hereditary cancer predisposition is caused by germline PVs in one of the DNA mismatch repair (MMR) genes, such as *mutL homolog 1* (*MLH1*), *mutS homolog 2* (*MSH2*), *mutS homolog 6* (*MSH6*), and *post-meiotic segregation increased 2* (*PMS2*) [266]. The protein products of these four MMR genes make up heterodimer complexes that have a critical role in DNA repair. The complex formed between MSH2 and MSH6 (MutS) recognizes and binds to single nucleotide base pair mismatches, after which the second heterodimer complex between MLH1 and PMS2 (MutL) binds to MutS, triggering "long-patch excision" of all the newly synthesized DNA within the vicinity of the mismatched DNA [266].

It is estimated that 80–90% of Lynch syndrome is attributable to deleterious variants in *MLH1* and *MSH2*, with the remaining 10–20% due to mutations in *MSH6* and *PMS2* [267]. Moreover, up to 3% of Lynch syndrome is due to mutations in the *epithelial cell adhesion molecule* (*EPCAM*) gene, which is directly upstream of *MSH2*. Deletions of the 3′-end of *EPCAM* result in epigenetic hypermethylation of the *MSH2* promoter, producing a phenotype very similar to Lynch syndrome [268]. Within Lynch syndrome carriers, cancer risk may vary depending on the specific type of MMR gene mutations. The cumulative incidence of CRC at 70 years of age is 40–80% for *MLH1* and *MSH2* mutation carriers but lower, about 10–22%, in *MSH6* and *PMS2* mutation carriers. In families with *MLH1* mutations, age at CRC diagnosis tends to be slightly younger when compared with families with other MMR gene mutations, *MSH6* and *PMS2* carriers developing no cancer before 40 years of age. Risk for extracolonic tumors is higher among *MSH2* and *MSH6* mutation carriers [269]. Overall, the prognosis for a Lynch syndrome colorectal tumor is significantly better compared with sporadic CRCs at the same stage [270].

Loss of DNA MMR activity results in the rapid accumulation of mutations which can occur in tumor suppressor genes or proto-oncogenes, leading to carcinogenesis. More than 90% of Lynch syndrome-associated tumors show ubiquitous mutations in specific repetitive DNA sequences, known as microsatellites, and/or lack of expression of at least one MMR protein [271, 272]. Testing of colorectal tumors for MMR deficiency is performed routinely in the clinical settings, through *microsatellite instability* (MSI) assay and/or immunohistochemical (IHC) staining. Using a panel of five microsatellite markers, tumors are classified as MSI-low or MSI-high if 1 or ≥2 markers, respectively, show instability. Immunohistochemistry can help to guide subsequent germline testing because tumor loss of MSH2, MSH6, and PMS2 expression correlates with germline PVs in the corresponding gene [271].

Clinically based criteria for identifying individuals with Lynch syndrome include Bethesda guidelines and prediction models available online (MMRpro, PREMM5, and MMRpredict): however all these criteria show suboptimal sensitivity. Therefore, a universal screening, in which all newly diagnosed CRC cases have either MSI or IHC testing, has been proposed. The better cost-effective strategy would be to limit screening to all individuals with CRC diagnosed <70 years plus those >70 years meeting Bethesda guidelines. This approach improves sensitivity compared to the Bethesda guidelines (95% vs 65%) and specificity compared to universal screening (95% vs 93%) [273].

Although nearly all Lynch-associated CRCs are MSI-high, this feature is identified in about 15% of sporadic CRCs, likely to occur through hypermethylation of *MLH1* promoter and *BRAF* somatic mutations, that are hallmarks of the serrated pathway of colorectal neoplasia. By contrast, Lynch-associated colorectal tumors are typically *BRAF* wild-type [229].

Another confounding issue is the occurrence of tumors showing MSI and/or abnormalities in the expression of MMR gene proteins on IHC testing, in the absence of germline PVs. Tumors also lack somatic *BRAF* mutation or *MLH1* promoter hypermethylation and thus resemble Lynch syndrome tumors. These cases are caused by two somatic mutations in one of the DNA MMR genes and, although this condition is not familial, can be referred to as Lynch-like syndrome [274]. The majority of patients with Lynch-like syndrome had CRC in the right colon (93%) when compared to those with Lynch syndrome (45%). In this regard, out of all patients with left-sided or rectal adenocarcinoma, 96% had germline mutations in MMR genes [275].

5.7.2.2 Familial Colorectal Cancer Type X

About 40–50% of CRC families that fulfill the HNPCC Amsterdam criteria are found to be microsatellite stable. This subgroup of HNPCC is classified as familial colorectal cancer type X (FCCTX) [276]. CRC risk in FCCTX families is only moderately increased (twofold), CRCs are diagnosed at a slightly older age compared to those with Lynch syndrome, and the risk of extracolonic cancer is equal to the average risk population [277]. The identification of the genetic etiology of FCCTX is challenging and still unknown. Several candidate genes have been proposed; however none of them appear to account for a large proportion of cases [227, 228].

5.7.2.3 Other Genes Associated with Increased Colorectal Cancer Risk

Risk for CRC might be increased in the setting of germline mutations associated with other hereditary syndromes. Li-Fraumeni syndrome, caused by germline mutations in the *TP53* tumor suppressor gene, confers increased risk for CRC and has been identified in 1.3% of individuals with early-onset disease [278]. Similarly, mutations in breast cancer susceptibility genes *BRCA1* and *BRCA2* may confer increased risk also for CRC and have been identified in 1–2% of probands referred for genetic testing for Lynch syndrome [279]. Emerging evidence showed that germline mutations in other breast cancer genes, including *ATM*, *CHEK2*, and *BLM*, may moderately increase CRC risk (NCCN Guidelines 2017) [273].

5.8 Hereditary Melanoma

Melanoma is a high-grade, poorly differentiated malignant neoplasm with unfavorable prognosis in the metastatic stage, accounting for more than 70% of the skin cancer-related deaths [280]. A familial history of melanoma is a strong predictor of melanoma onset. Up to 10% of all cases of cutaneous malignant melanoma occurs in a familial context. Familial melanoma follows an autosomal dominant transmission pattern, with incomplete penetrance and variable expressivity. Analyses of familial genetic linkage led to the identification of two high-penetrance susceptibility genes, *CDKN2A* (cyclin-dependent kinase inhibitor 2A) and *CDK4* (cyclin-dependent kinase 4), implicated in cell cycle arrest and senescence. Rare mutations or deletions in these genes confer an increased risk of developing melanoma [281, 282].

CDKN2A is considered the major high-risk melanoma susceptibility gene, since germline PVs in this gene have been described in 25% to 40% of melanoma families. *CDKN2A* maps on chromosome 9p21 and includes four exons (1α, 1β, 2 and 3) encoding for two tumor suppressor proteins called INK4A ($p16^{INK4a}$) and ARF ($p19^{ARF}$ in mice and $p14^{ARF}$ in humans) [283, 284]. Most germline mutations associated with melanoma risk are harbored by exons 1α and 2, suggesting that p16INK4a is the preferentially targeted element of *CDKN2A* [285, 286]. Infrequent deletions on the exon 1β were also identified, indicating that *p14ARF* is a susceptibility gene for melanoma not dependent on *p16INK4a* [287–289]. Even more infrequent intronic mutations of *CDKN2A* have also been detected, despite these represent only very few cases worldwide [290–293]. INK4A is a cyclin-dependent kinase inhibitor (CDKI) able to activate the pRB, negatively regulating Cdk4/6 and promoting the progression through the G1/S transition of cell cycle, while p14ARF interacts with MDM2, which usually induces the ubiquitin-dependent degradation of p53 [294, 295]. Therefore, the defect of the $p16^{INK4a}$ function favors CDK4 and CDK6 activation, inducing hyperphosphorylation and inactivation of pRB and activation of E2F1 (◘ Fig. 5.10).

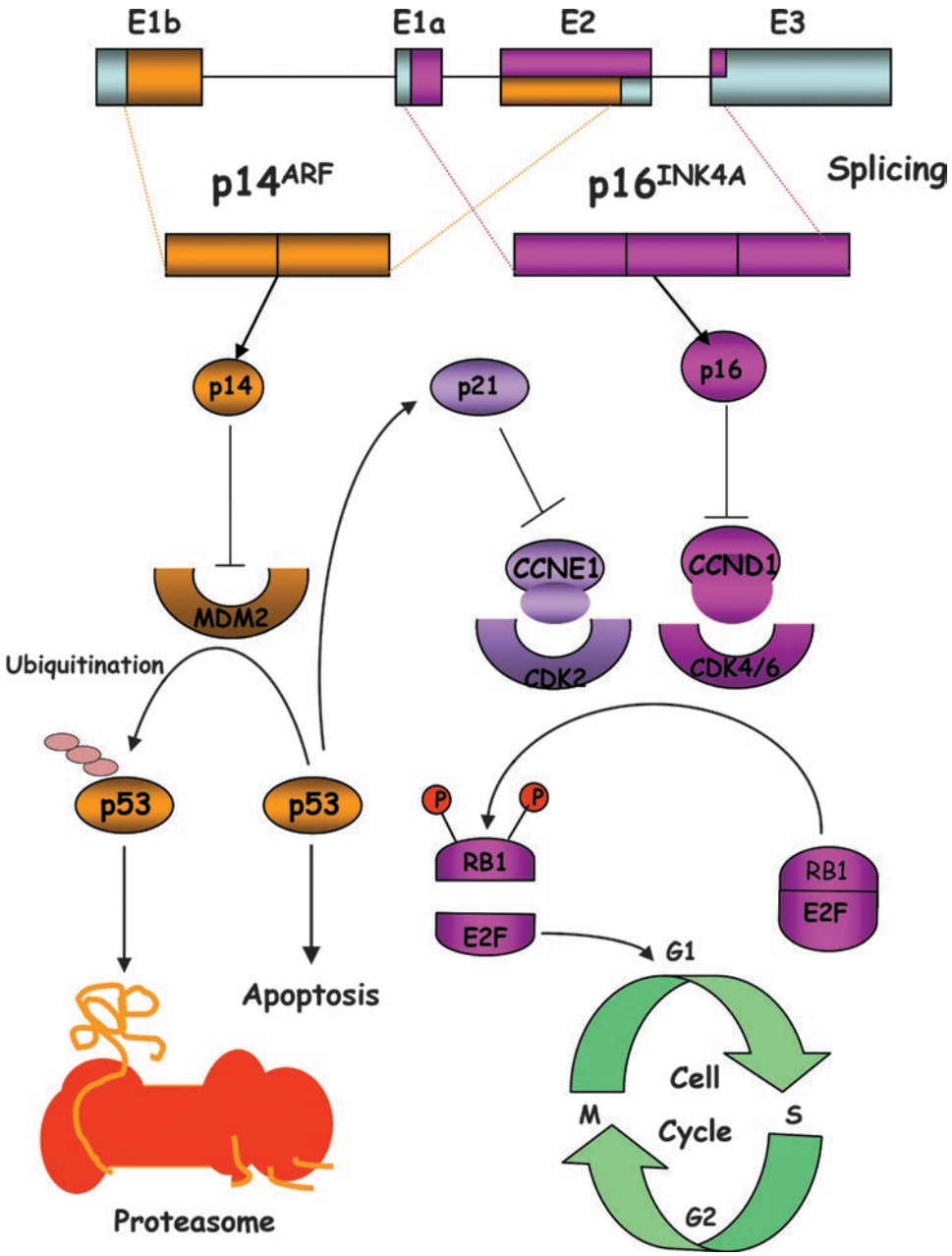

Fig. 5.10 *CDKN2A* locus structure and signaling pathways regulated by p16INK4a and p14ARF

Indeed, numerous epigenetic and genetic studies demonstrated that *INK4A* is deleted in 50% of melanoma cases, inactivated via promoter hypermethylation in approximately 10% of tumors or by point mutations in about 9% of cases [296]. Furthermore, *BRAF*-activating alterations and functional loss of $p16^{INK4a}$ and $p14^{ARF}$ were detected in the majority of melanomas [297].

An elevated number of melanoma cases in the same family, early age of onset, and appearance of multiple primary melanoma (metachronous or synchronous) have been shown to be significantly associated with *CDKN2A* mutations [298]. Approximately 3–5% of all patients affected by melanoma will develop additional primary melanomas in their lifetime. The prevalence of *CDKN2A* mutations increases with the number of diagnosis of primary melanoma. For familial melanomas related to *CDKN2A* mutations, the overall penetrance is assessed to be 30% by age 50 and 67% by age 80, despite the risk is higher in individuals residing in sunnier climates. Melanoma risk varies based on geographic area, as reported by studies performed on families with *CDKN2A* mutations from Europe, North America, and Australia. The causes of these changes are not yet well understood, but differences in the sun exposure, other genetic or individual modifications, or a combination of these components may be found [299, 300]. The frequency of *CDKN2A* mutation in familial melanoma is higher in geographical zones at low incidence of melanoma as Europe (57%) and North America (45%) than

those at high incidence as Australia (20%). In these high incidence areas, a combined effect of mutations in moderate-/low-penetrance susceptibility genes and greater sun exposure may occur [301].

Germline PVs in high-susceptibility *CDK4* gene are infrequent, as reported in the literature, and prevent the modulation of the protein by $p16^{INK4a}$ while maintaining the interaction between CDK4 and cyclin D1, causing the constitutive activation of the complex and uncontrolled cell proliferation by pRB inactivation and E2F activation. E2F, in turn, induces the transcription of S-phase genes, thus promoting cell proliferation [302]. Furthermore, as described by Rane et al., *CDK4* mutations support tumorigenesis and induce melanocytes xenografted into nude mice to escape the cellular senescence mechanisms [303].

Additionally, it was demonstrated that two low-/intermediate-penetrance susceptibility genes, called *MITF* (microphthalmia-associated transcription factor) and *MC1R* (melanocortin 1 receptor), play a significant role in the melanoma onset. *MITF* amplification was observed in 10% of primary melanomas and 20% of metastatic tumors, and it is associated with reduced 5-year overall survival [304, 305]. MITF is a member of the *MYC* supergene family of helix-loop-helix transcription factors and is implicated in survival and proliferation control [306]. It was proposed that increased MITF expression is correlated with the differentiation [307], whereas intermediate MITF levels are associated with proliferation [308] and transient low MITF levels with a melanoma-initiating cell phenotype [309]. MITF activity is modulated by posttranslational changes such as phosphorylation and degradation, through the ubiquitin-proteasome pathway in response to the activation of ERK signaling [310]. In patients with a significant family history, a germline *MITF* mutation, called p.E318K, that gives a fivefold increased melanoma risk was detected [311, 312]. This alteration confers to melanoma cells' invasive abilities, promoting tumor progression. MITF can modulate the expression of many genes implicated in cell survival (*HIF-1α*, *BCL-2*, *MET*, *APE-1*) [313–315], cytoskeleton remodeling and migration [316], and cell proliferation (*CDK2*) [317]. Moreover, MITF activity is related to the resistance to apoptosis induced by ultraviolet (UV) radiations in melanocytes.

MC1R is a transmembrane receptor localized on the cell surface of epidermal melanocytes, which, under hormonal stimulation, activates the adenylate cyclase and cAMP/PKA/CREB pathway [318]. *MC1R* allelic variants represent a significant higher risk factor for melanoma whose onset increases when a *CDKN2A* mutation occurs [319]. Differences in the pigmentation of the skin, hair, and eyes are caused by other low-risk allelic variants, which thus lead to variations in skin sensitivity to UV radiations, by raising the melanoma risk.

Sun exposure is generally considered the crucial environmental risk factor for cutaneous melanoma development, following the deleterious interactions between UV radiations and melanoma cell genome. Indeed, UV radiations may facilitate the melanoma onset by means of combined genotoxic and mitogenic effects in melanocytes [320]. As previously reported, the association of inherited intermediate-/low-penetrance variants with environmental factors, such as sun exposure, may induce the onset of melanoma [321, 322].

Key Points

- Hereditary tumors account for only a small fraction of all the tumors;
- Oncogenes and tumor suppressor genes are involved in the tumor onset and progression;
- Hereditary predisposition syndromes are associated with germline mutations;
- Tumor suppressor genes are divided into three categories: gatekeepers, caretakers and landscapers;
- Susceptibility genes may be at high, moderate, and low penetrance;
- Carriers of germline pathogenic variants in BRCA1 and BRCA2 genes confer a high risk to develop breast and/or ovarian cancer;
- BRCA genetic testing has also a predictive value in PARPi therapy response in ovarian, pancreatic, and prostate cancer patients;
- 5% of colorectal cancer cases are associated with high-penetrance mutations related to known hereditary syndromes;
- Genetic susceptibility to CRC includes well-defined inherited syndromes such as Familial Adenomatous Polyposis (FAP), MUTYH-Associated Polyposis (MAP), Lynch syndrome, and other less common syndromes;
- FAP, caused by an APC germline pathogenic variant, is a rare autosomal dominant condition which accounts for about 1% of CRC cases;
- Individuals with suspected FAP but negative for APC mutations may be carriers of biallelic germline MUTYH mutations;
- MAP follows an autosomal recessive inheritance pattern;
- Lynch syndrome (LS) is the most common cause of inherited CRC, accounting for about 3% of newly diagnosed cases of CRC;
- LS follows an autosomal dominant inheritance pattern with incomplete penetrance and includes, beyond colorectal and endometrial cancer, a broad spectrum of LS-associated cancers;
- LS is caused by germline pathogenic variants in one of the mismatch repair genes, such as MLH1, MSH2, MSH6, and PMS2, or in EPCAM gene;

- Up to 10% of all cases of cutaneous malignant melanoma occurs in a familialcontext;
- CDKN2A and CDK4 are two high-penetrance susceptibility genes conferring high risk of developing hereditary melanoma.

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Liquid Biopsy

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Learning Objectives

By the end of the chapter the reader will

- Have learned the basic concepts of potential clinical application of liquid biopsy
- Have reached knowledge about circulating tumor cells, circulating nucleic acids, and micro-vesicles
- Have learned the clinical utility of liquid biopsy in non-small cell lung cancer

6.1 Liquid Biopsy

In the last years, cancer patients' management has been completely revolutionized thanks to a better comprehension of the biological processes underlying the tumor development and progression. Indeed, we know that some tumors are "oncogene addicted" [1–3], meaning that they are strictly dependent on a hyper-activated oncogene for their own survival. Moreover, a pharmacological agent, able to specifically target specific oncogene, is efficient to selectively block cancer cells sparing normal cells from toxicity. Therefore, clinicians have changed the way to select and treat the patients, moving from one-fits all strategy to the so-called precision medicine, based on proper patient's selection [4].

Nowadays, treatment decision is strictly dependent on the tumor molecular characterization; thus, the path of cancer patients' survival is tissue dependent, but this may have several limitations [5]. A single tissue biopsy represents only a snapshot limited in time and space. Tumors generally originate from one single cell clone; nevertheless, during evolution toward advanced stage tumor cells can acquire new molecular alterations, originating a new resistant disease. Therefore, tumors are not uniform diseases but heterogeneous entities consisting of clones, with different genetic and molecular characteristics. This phenomenon is defined as tumor heterogeneity, and it is one of the main causes of treatment failure [6]. A better comprehension of tumor heterogeneity is critical to develop new therapeutic strategies.

One of the main medical needs is to develop noninvasive or minimally invasive and dynamic tools that can allow a strict patients' follow-up at different time points, and the term "liquid biopsy" encompasses this characteristic [7]. The term liquid biopsy includes several tumor components that can be detected in almost all biological fluids (plasma, serum, saliva, urine, and effusion liquids). The aim of liquid biopsy is to detect and analyze biological material originated within and from the tumor. The principal liquid biopsy components are circulating nucleic acids (circulating tumor DNA, circulating microRNA, circulating RNA), circulating tumor cells (CTC) and extracellular vesicles (exosomes and microvesicles) [8]. The information acquired through liquid biopsy can be either diagnostic, prognostic, or predictive as it can be used for the early detection of a specific malignancy, for monitoring its progression, its response to therapy, the arousal of resistant clones, or its relapse following complete remission (monitoring minimal residual disease, MRD) [9, 10] (Fig. 6.1).

6.1.1 Circulating Nucleic Acids (CNAs)

Circulating nucleic acids (CNAs), such as circulating tumor DNA (ctDNA) and circulating microRNA (miRNA), represent promising biomarkers in several diseases, including cancer, and can be isolated from many body fluids. Among these biological fluids, blood represents one of the most investigated sources for CNAs due to the very simple and minimally invasive way of sampling. A blood withdrawal can be frequently repeated at different time points and can therefore be used for a real-time monitoring of the disease. Actually, liquid biopsy is not completely new, for instance, the assessment of breast cancer genes (*BRCA1–2*) germinal mutations is carried out starting from buffy coat obtained by a blood sample. Today, the newest and most fascinating application of liquid biopsy is represented by the analysis of "somatic component" (Fig. 6.2) shared in the bloodstream directly from the primary tumor and related metastases, in active (e.g., microvescicles) or passive (apoptosis or necrosis) ways. A somatic mutation is present only in the tissue where it originates and cannot be transferred to the progeny, therefore it is an exclusive mutation of the tumor [11].

CNAs are released from both tumor and normal cells, but it has been extensively demonstrated that in cancer patients their concentration is greater [12–14]. The mechanisms of CNAs spread are not fully understood, but some hypothesis have been made. Some evidences indicate that CNAs are released through a passive mechanism; under physiologic conditions, phagocytes efficiently clear apoptotic and necrotic cells debris. This does not happen inside a tumor mass, leading to cell debris accumulation and shedding into the circulation. It has been also postulated an active mechanism driven by extracellular vesicles, such as exosomes, according to which CNAs are packed inside vesicles and actively secreted by cells. This seems to be more realistic for miRNAs, whereas for DNA, there are still conflicting data [15–18].

6.1.1.1 Circulating Tumor DNA (ctDNA)

Circulating cell-free DNA (cfDNA) is highly fragmented; it has been shown that the length of cfDNA fragments is often between 200 and 180 base pairs, suggesting that apoptosis likely produces the majority of cfDNA in circulation [19–21]. Circulating tumor DNA (ctDNA) is

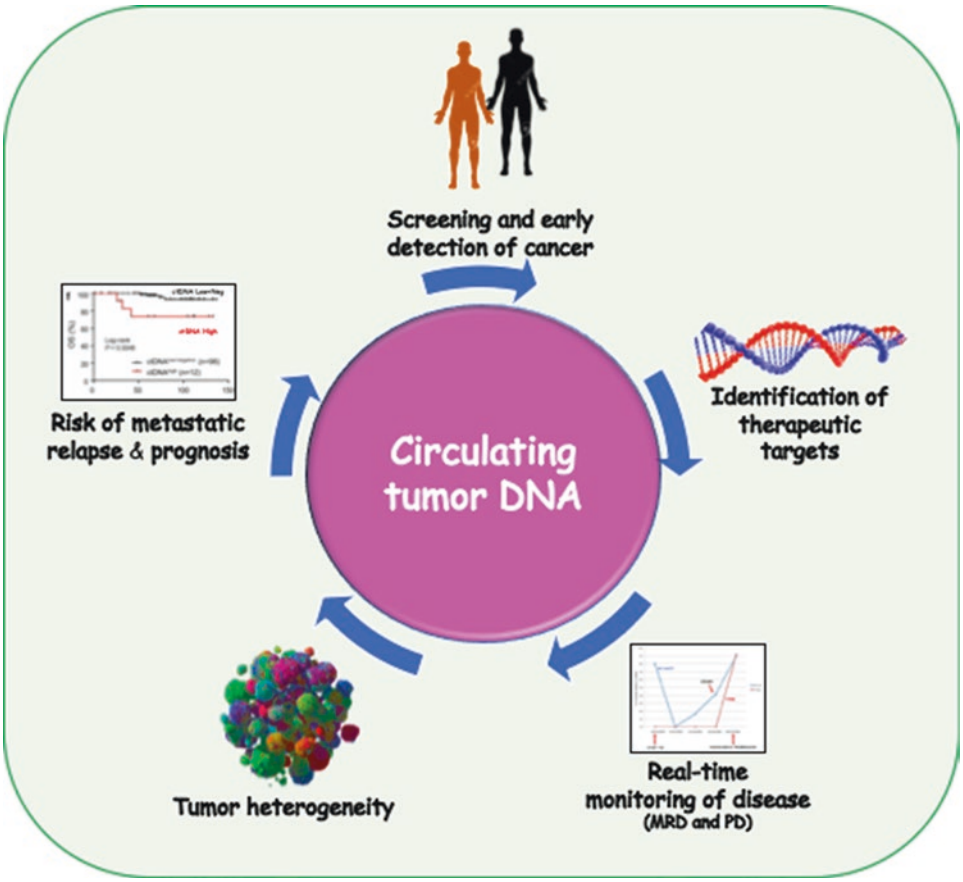

Fig. 6.1 Clinical applications of circulating tumor DNA (ctDNA)

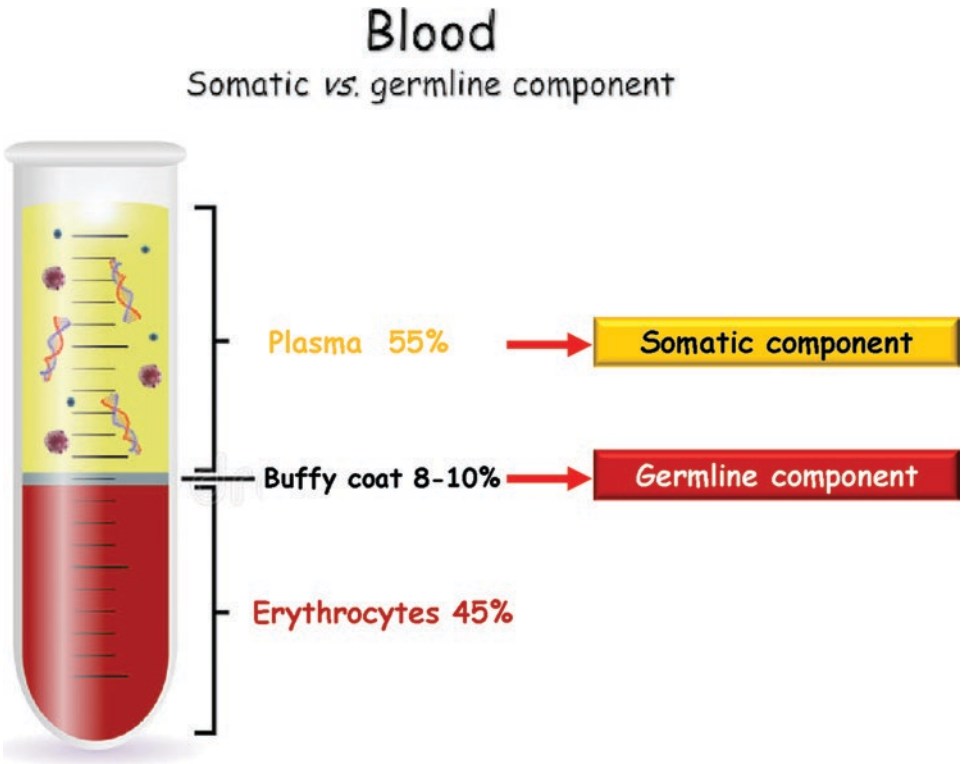

Fig. 6.2 Plasma can be considered as the somatic component of blood, and it can be used for the isolation of CNAs, CTCs, and exosome. In particular ctDNA can be used to detect somatic mutation that are exclusively present in the tumor mass and cannot be transferred to the progeny. The buffy coat contains most of the white blood cells and platelets, representing the germline component of blood, and can be used to isolate germline DNA

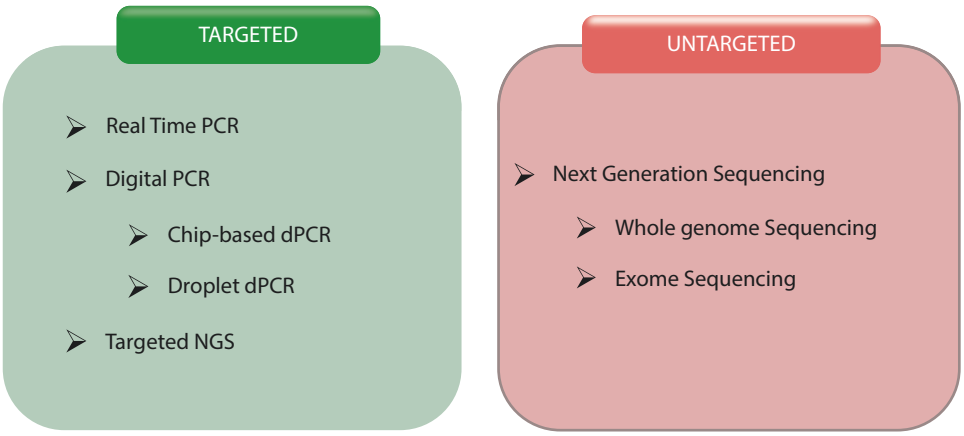

Fig. 6.3 Targeted and untargeted approaches for circulating tumor DNA evaluation

part of the cfDNA deriving from the tumor mass. The easiest way to identify ctDNA is to investigate the presence of somatic driver mutations, which can be exclusively found on tumor. Several methods have shown that the fraction of ctDNA varies greatly, between 0.01% and more than 90% [19]. Currently, it is well established that different tumor types do not release the same ctDNA amount, and, even in patients with the same disease, the ctDNA concentration may differ consistently [22, 23].

In healthy subjects, plasma cfDNA concentration is ranging from 0 to 100 ng per ml of blood, with an average of 30 ng per ml [24], whereas in cancer patients, is ranging from 0 and to over 1000 ng per ml of blood, with an average of 180 ng per ml [25]. Several pre-analytical variables, such as blood collection and handling, extraction protocols, and storage temperature, may affect the quantity and quality of ctDNA impairing its analysis [26, 27]. Even if in the majority of clinical trials plasma is the main source of ctDNA, it is still questioned whether serum could be used. Indeed, it has been reported that the amount of ctDNA in serum can be 2–24 times higher than in plasma. This can be a consequence of the clotting process that causes white blood cells breaking, finally leading to the release of wild-type DNA [28]. This phenomenon determines a further dilution of tumor-specific DNA, making it even more difficult to detect. However, it has been reported that in some cases, it might be advantageous to analyze both serum and plasma, as this increases, with respect to tissue-based analysis, the chances to detect the specific mutation [29]. To avoid ctDNA contamination with wild-type background DNA, it is important to minimize the time that elapses between blood withdrawal and plasma recovery to reduce the possibility of white blood cells lysis. Plasma can be stored for long period at −80 °C or immediately processed for ctDNA extraction [30].

Circulating tumor DNA can be analyzed using two different approaches: a targeted approach and an untargeted approach (Fig. 6.3).

The targeted approach relies on the possibility to analysis known genetic mutations that occurs in hotspot regions of specific genes with implications for therapy decisions (e.g., in Kirsten rat sarcoma viral oncogene homolog (*KRAS*), epidermal growth factor receptor (*EGFR*), and v-Raf murine sarcoma viral oncogene homolog B1 (*BRAF*) genes in colon, lung, and melanoma tumors). Among these methods, we can include real-time PCR, digital PCR (dPCR), droplet digital PCR (ddPCR), BEAMing, and targeted next-generation sequencing (NGS). In the untargeted approach, it is possible to analyze ctDNA regardless of the presence of specific mutations. This can be achieved through whole genome or whole exome sequencing using NGS platforms [31].

Liquid Biopsy in Clinical Practice: The Paradigm of Non-Small Cell Lung Cancer (NSCLC)

To better explain the utility of liquid biopsy in clinical practice, we can consider the paradigm of non-small cell lung cancer (NSCLC) patients. Indeed, in NSCLC patients, the evaluation of specific predictive biomarkers is mandatory for the choice of the most personalized targeted therapy. In particular, *EGFR*-activating mutations are predictive of response to first-, second-, and third-generation tyrosine kinase inhibitor (TKI) drugs (e.g., erlotinib, gefitinib, afatinib and osimertinib) with *EGFR* mutational status being routinely tested. Moreover, the discovery of the EML4-ALK fusion gene leads to the development of crizotinib, another TKI used in NSCLC treatment when this fusion is detected [32]. Despite these targeted therapies have profoundly improved NSCLC patients' outcome, they inevitably experience tumor progression and recurrence. As previ-

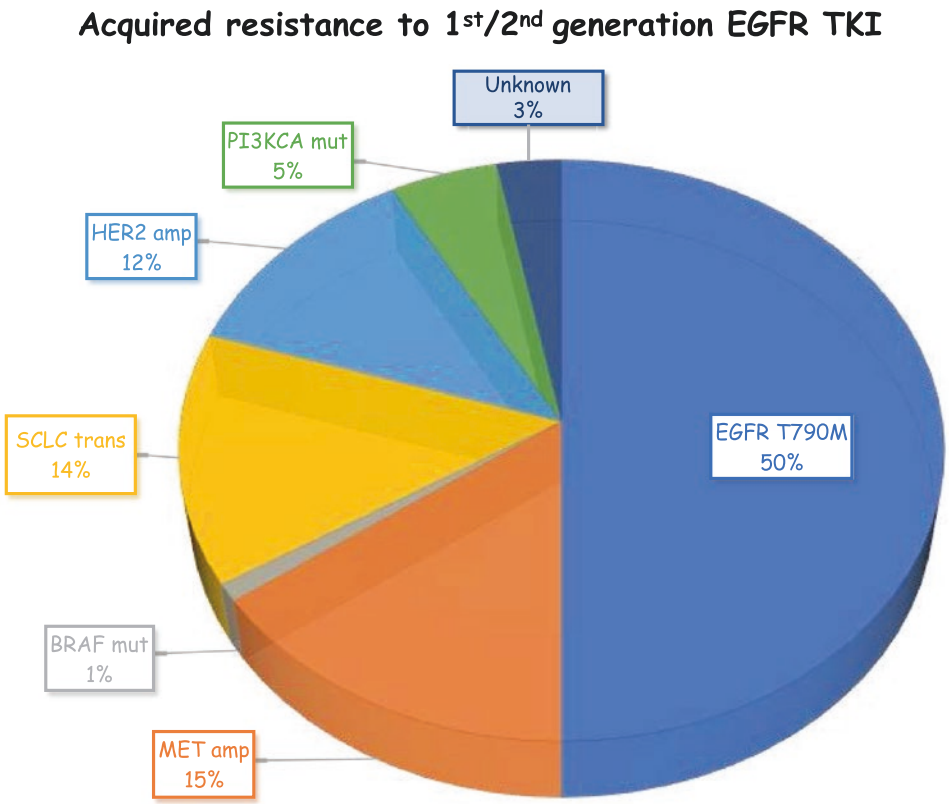

Fig. 6.4 Acquired resistance to first-line treatment with first/second-generation EGFR TKI. Mutation p.T790M occurs in exon 20 of *EGFR gene*, and it accounts for almost 50% of resistance mechanism to first- and second-generation EGFR TKI

ously mentioned, resistance onset is frequently due to the acquisition of new molecular alterations such as additional mutations or amplifications (Fig. 6.4) [33].

In some instances, these alterations have been already characterized, and pharmaceutical companies have developed new targeted agents able to overcome the resistance. Almost 50% of patients treated with erlotinib or gefitinib develop a resistance through the acquisition of the *EGFR* exon 20 p.T790M mutation.

More recently, osimertinib, a third-generation EGFR-TKI, has been developed and registered for clinical use to overcome p.T790M-associated resistance in advanced NSCLC patients [34, 35]. The phase III AURA 3 study has shown a significant survival benefit in favor of osimertinib over platinum chemotherapy in NSCLC patients who progressed to prior EGFR-TKI and were p.T790M positive [36]. Since the introduction of this new drug, the re-evaluation of *EGFR* molecular status at disease progression in TKI-treated patients is mandatory. However, in the FLAURA study, osimertinib has recently showed to improve survival rates over first-generation TKIs in the first-line treatment of *EGFR*-positive patients with common mutations in exons 19–21, thus reshaping the second-line assessment of resistant p.T790M mutation [37]. As previously reported, re-biopsy is not often achievable, and liquid biopsy may represent an alternative tool. The AURA 3 trial supported the feasibility of *EGFR* p.T790M assessment starting from derived plasma ctDNA. Indeed, progression-free survival (PFS) rates reported for the tissue and ctDNA-based p.T790M evaluation overlap [36]. Following this impressive result, the liquid biopsy (in particular ctDNA) has become part of NSCLC patients' clinical practice. In particular, it can be used in two different clinical settings in patients with advanced NSCLC (stage IIIB-C and IV) to detect *EGFR* activating and resistance mutations:

1. At the time of *diagnosis* in naive patients when tissue is not available (Fig. 6.5)
2. At the time of *disease progression* according to RECIST criteria after TKI treatment (Fig. 6.6)

In the first clinical setting, liquid biopsy represents a valid option when no tissue samples are available (Fig. 6.5) to assess *EGFR* mutational status for EGFR-TKI patients treatment selection. Conversely to *EGFR*, where robust data were reported, the assessment of other genomic alterations using ctDNA in treatment-naive patients is still limited. However, as endorsed by most international scientific societies, the detection of an actionable alteration in ctDNA, if using a validated assay, would eventually represent sufficient evidence to initiate targeted treatment, albeit not without reimbursement variations among all the different countries. Nonetheless, a negative finding of either *EGFR* or other genomic alterations using ctDNA should be considered

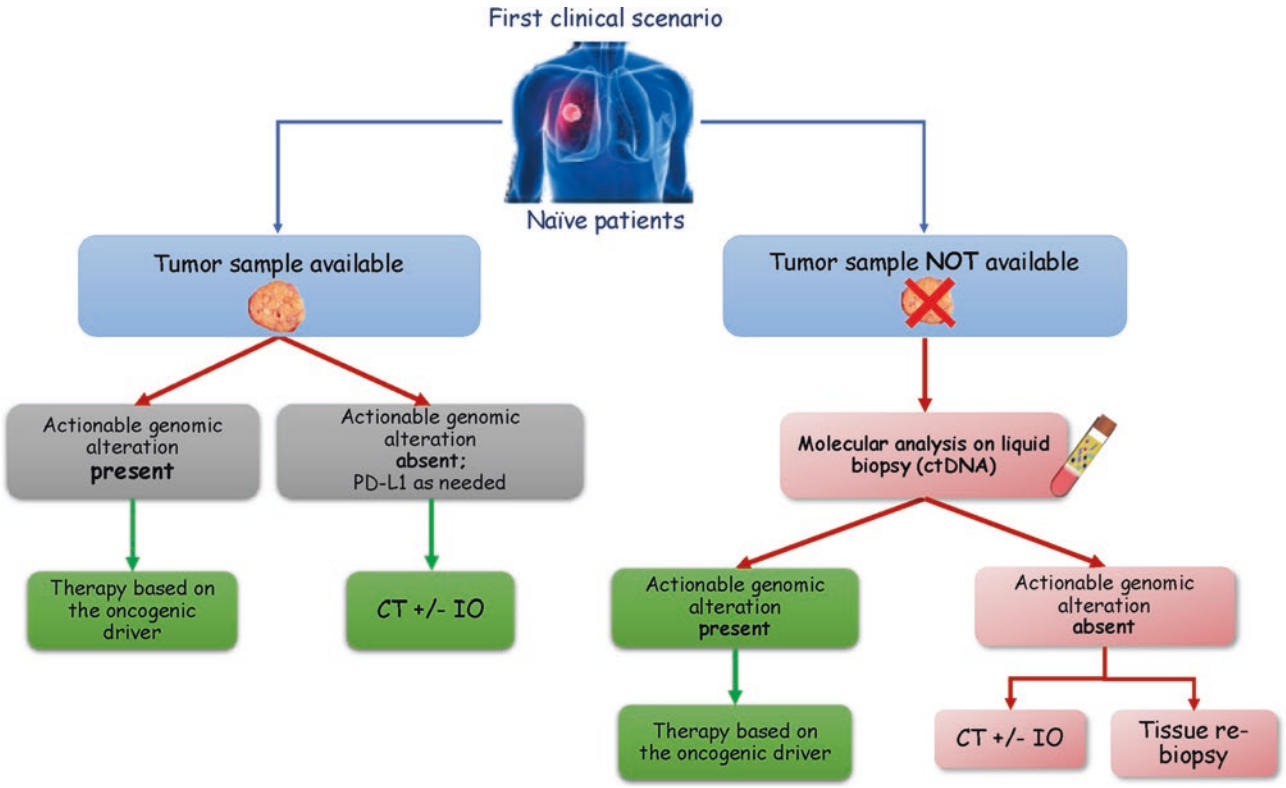

Fig. 6.5 First clinical scenario: naive patients at diagnosis. The flowchart is a simplification of the path to follow in case of available tumor sample (on the left) and no available tumor sample (on the right). When tissue sample is available, it is recommended to test at minimum for *EGFR* and *BRAF* mutations, *ALK* and *ROS1* translocations, and PDL1 expression. According to the results, patients are treated with TKI, chemotherapy (CT), or immunotherapy. When tissue sample is not available, it is possible to use liquid biopsy to detect *EGFR*-activating mutations or eventually other actionable genomic alterations; if the test is positive, the patients can be treated with a targeted treatment; if negative, the patient should undergo a tissue re-biopsy when feasible and, if the negative result is ultimately confirmed, to systemic treatment

not conclusive, and, when feasible due to patients' performance status, a tissue re-biopsy should be performed (Fig. 6.5) [38].

In the second clinical setting, ctDNA analysis could be used to detect a wide range of potentially actionable resistance alterations (Fig. 6.6).

Specifically, as concerns the *EGFR*-mutated disease, when progressing after the first-line treatment based on first- and second -generation TKIs (Fig. 6.7), liquid biopsy can be implemented as a first approach to detect p.T790M mutation. It is recommended to test both the activating mutation originally detected and the resistance mutation, since this foresight can significantly reduce the rate of false-positive results while additionally providing useful information regarding the ctDNA sharing rate of the specific analyzed patient's tumor. If the analysis on liquid biopsy is positive for the p.T790M mutation, the patients can be treated with third-generation TKI. Otherwise, when the test is negative, it is recommended to obtain a tissue biopsy and to test it for p.T790M mutation. Considering the tissue-based analysis as a gold standard, about 30% of p.T790M negative tests are false negative (FN), and this may be due to disease metastatic sites. Indeed, the location of metastatic sites significantly influences the diagnostic accuracy of ctDNA analysis in detecting *EGFR* mutations, and therefore, this parameter (intrathoracic vs. extrathoracic disease) should be considered for proper test interpretation and reporting [39].

With the increasing up-front use of osimertinib, the detection of p.T790M mutation in this setting becomes of secondary importance, since its loss has been usually associated with early resistance to osimertinib according to the drug mechanism of action [40]. Even if the mutational status of p.T790M could be readily monitored in plasma in order to precede a proven radiological progression of disease, other multiple resistance mechanisms need to be considered in this regard. Further implementation of liquid biopsy in monitoring the response to osimertinib and detecting the wide spectrum of molecular alterations responsible for treatment failure (either EGFR dependent or independent) is warranted and eagerly awaited in both ongoing and future clinical trials (Fig. 6.7). In this context, liquid biopsy using ctDNA analysis has proved to be feasible and reliable for detecting most of genomic alterations [40].

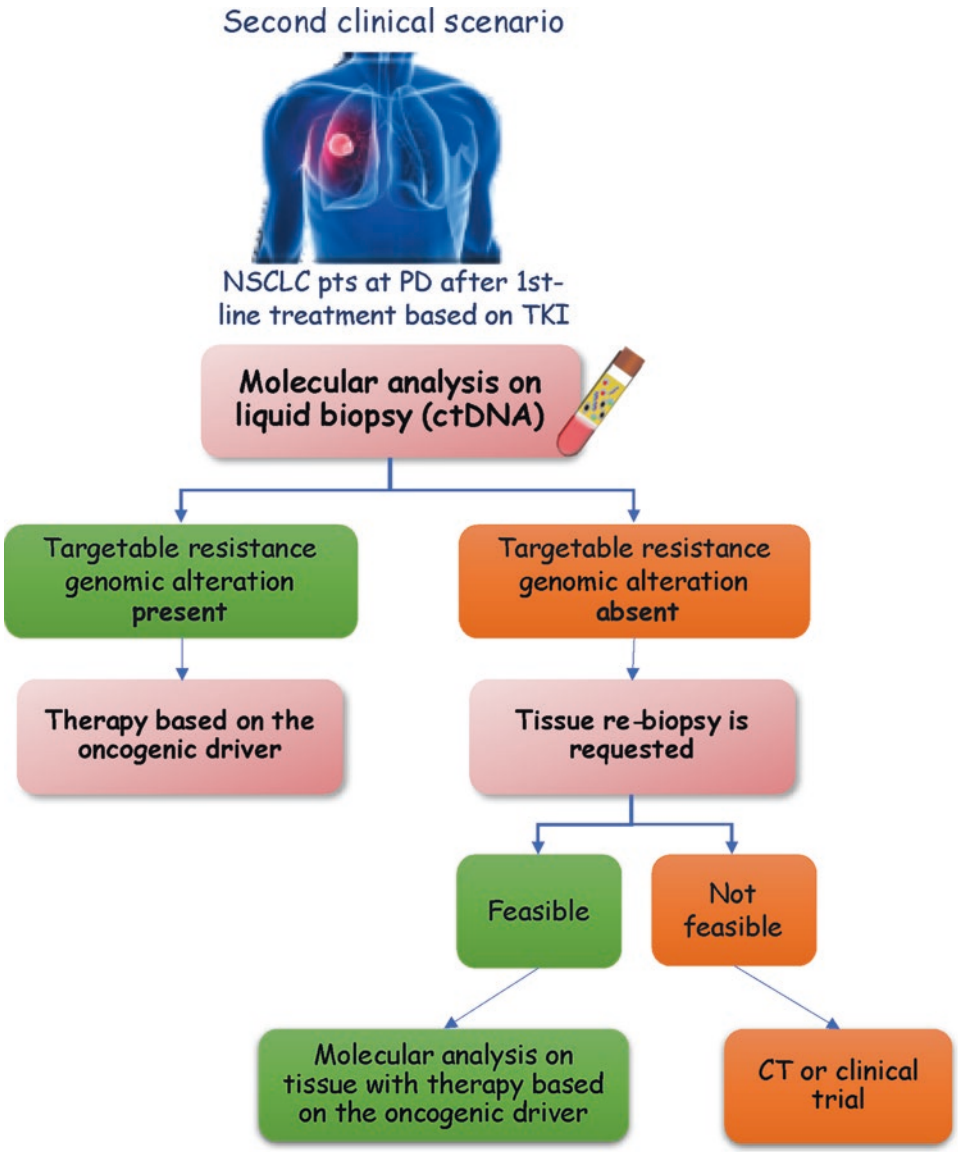

Fig. 6.6 The potential use of the liquid biopsy in patients progressing during TKI treatment

It has been reported that the appearance of the p.T790M in blood precedes disease progression by months [41] (Fig. 6.8). Seems that we are able to distinguish a molecular progression, defined by the appearance of the p.T790M in the bloodstream, and a radiological progression, following the response evaluation criteria in solid tumors (RECIST) version 1.1 (Fig. 6.7).

Therefore, the current dilemma is when it is more appropriate to switch from first/second to third- generation TKIs in order to maximize treatment response. The ongoing APPLE trial (EORTC1613) aims to evaluate the best strategy for sequencing gefitinib and osimertinib. This trial is a randomized, open-label, multicenter, three-arm, phase II study in advanced, *EGFR*-mutant and TKI-naive NSCLC patients [42]. Patients who are *EGFR*-TKI treatment naive and eligible to receive first-line treatment with *EGFR*-TKI will be randomized to:

- Arm A: osimertinib until disease progression according to RECIST v1.1
- Arm B: gefitinib until emergence of ctDNA p.T790M mutation and then switch to osimertinib until disease progression according to RECIST
- Arm C: gefitinib until disease progression according to RECIST and then switch to osimertinib until second radiologic disease progression

In all arms, plasma ctDNA p.T790M test will be performed but applied as a predictive marker for making treatment decisions only in arm B.

Although third-generation TKIs are the most advanced drugs we have, even in this case, the tumor adapts by expressing new alterations that make it resistant to these drugs (Fig. 6.9). As showed in Fig. 6.9, at resistance after osimertinib approximately 30% of

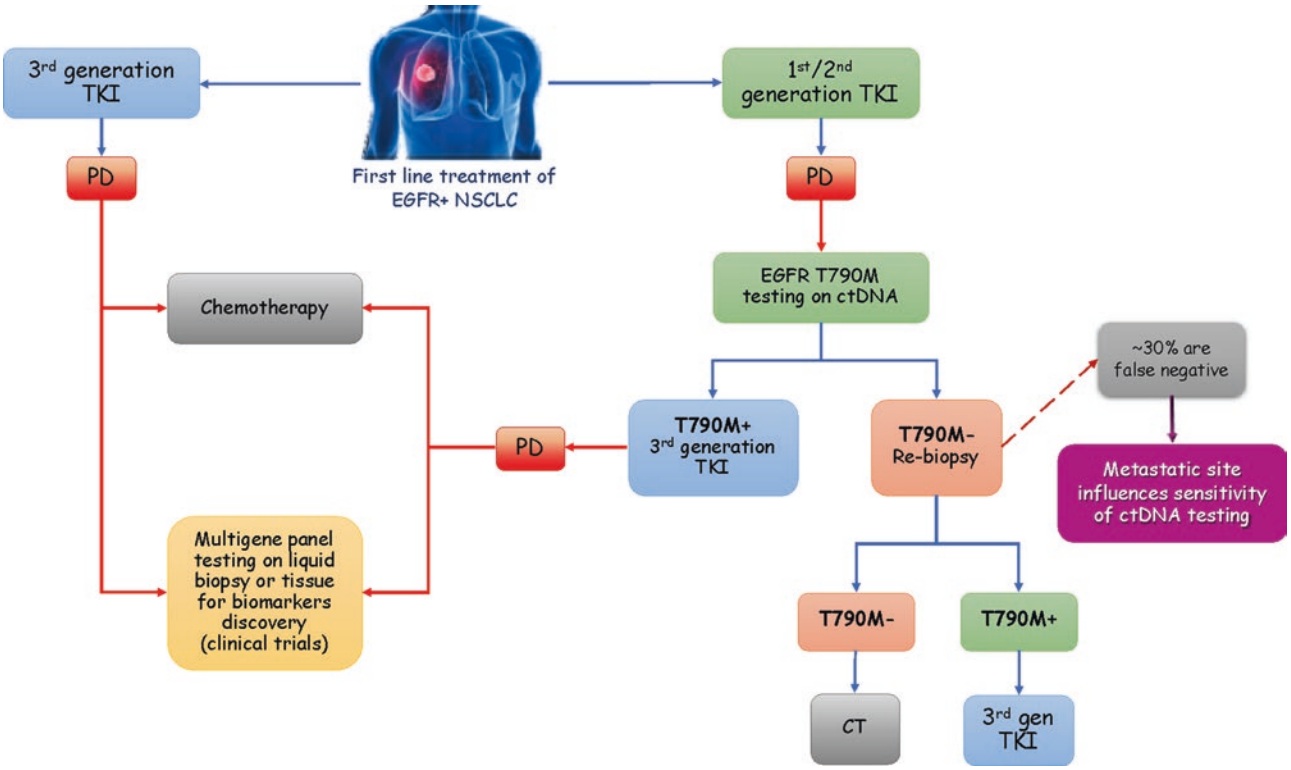

Fig. 6.7 *EGFR*-positive NSCLC patients at PD after first-line TKIs. In the case of first- or second- generation EGFR TKIs (on the right), liquid biopsy can be used as a first approach to look for p.T790M mutation. If the test is positive, the patients can be treated with third-generation TKI. When the test is negative, it is recommended to obtain a tissue biopsy and to test it for p.T790M mutation. Nevertheless 30% of p.T790M negative tests are false negative (FN). Moreover, the location of metastatic sites significantly influences the diagnostic accuracy of ctDNA analysis in detecting *EGFR* mutations, and therefore this parameter (intrathoracic vs. extra-thoracic disease) should be considered for proper test interpretation and reporting [39]. In the case of progression on third-generation TKI (on the left), patients should undergo standard chemotherapy or further mutational analysis in the context of clinical trials looking for other actionable/resistance mutations

patients maintain p.T790M, while the remaining 70% loss this mutation [43].

Among patients with maintained p.T790M mutation, the most frequent resistance mechanism is due to the development of the p.C797S mutation, resulting in an amino acid substitution at position 797 in *EGFR*, from a cysteine (C) to a serine (S) and occurs within exon 20. It has been observed that the allelic context in which p.C797S mutation is acquired may predict responsiveness to alternative treatments, and therefore, this information could have therapeutic implications for patients [44]. If the p.C797S and p.T790M mutations are in *trans* (different DNA strand, different allele), cells will be resistant to third-generation *EGFR* TKIs but will be sensitive to a combination of first- and third-generation TKIs. Whereas when the mutations are in *cis* (same DNA strand, same allele), no *EGFR* TKIs alone or in combination can suppress activity [45, 46] (Fig. 6.10).

Patients with loss of p.T790M show a more heterogeneous pattern of resistance mechanisms including small cell lung cancer (SCLC) transformation, mesenchymal to epithelial transition (*MET*) gene amplification, other rare gene fusions (involving rearranged during transfection (*RET*), fibroblast growth factor receptors (*FGFR*), or *BRAF* genes) and mutation in *KRAS*, phosphatidylInositol-3-Kinase (*PI3KCA*), and *BRAF* genes. In this context, liquid biopsy can be used to track the occurrence of such alterations and once again to provide information that can be useful for patient management.

As previously mentioned, osimertinib is active against both activating and resistance *EGFR* mutations. The FLAURA phase 3 trial has indeed compared osimertinib with standard EGFR-TKIs (first-generation TKIs, gefitinib, and erlotinib) in patients with previously untreated advanced NSCLC harboring *EGFR* activating mutation [47]. Investigators concluded that osimertinib shows superior efficacy to that of standard *EGFR*-TKIs in the first-line treatment of *EGFR* mutation-positive advanced NSCLC. Therefore, it is now questioned which are the resistance mechanisms arising after first-line treatment with osimertinib. As expected, no acquired p.T790M mutation was detected upon resistance to osimertinib [48]. The most frequent resistance mechanisms reported are *MET* amplification

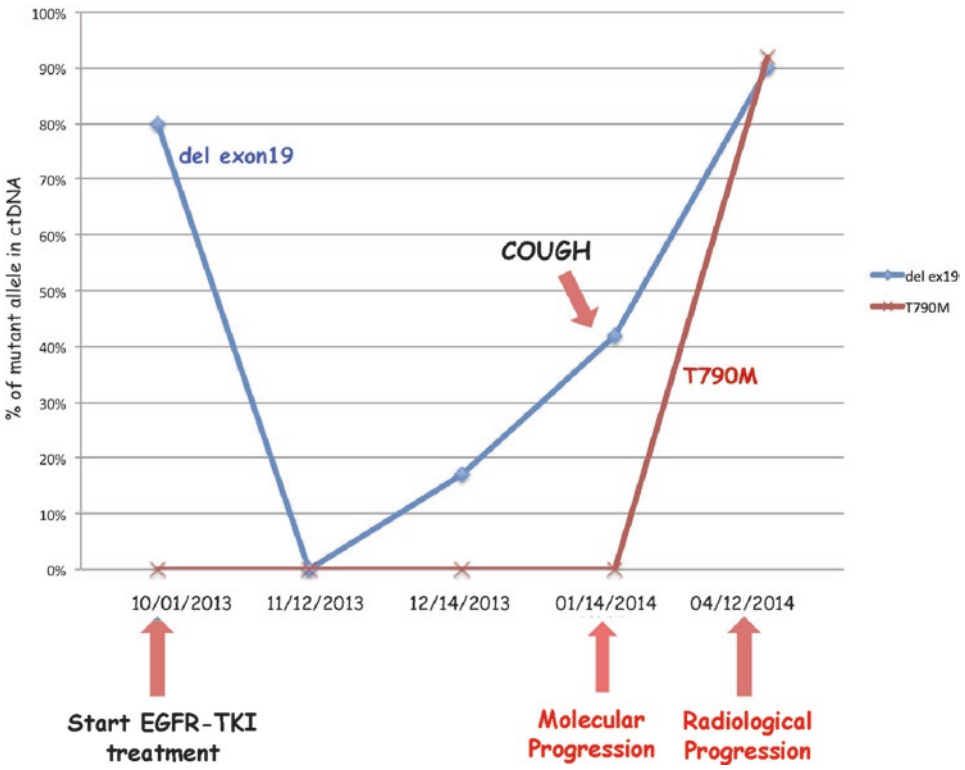

Fig. 6.8 The figure shows an example of the application of liquid biopsy in *EGFR*-mutant NSCLC patients. In 2013, the patient started a first-generation TKI treatment; at that time, the analysis of ctDNA showed the presence of the activating mutation (deletion in exon 19) with high allele fraction. After treatment initiation, the mutation significantly drops down, demonstrating the efficacy of the treatment. Four months later, a symptom appears (cough), and concomitantly, the activating mutation was again detectable in ctDNA. Notably, at this time point was also reported the appearance of the resistance mutation p.T790M in ctDNA, defining a molecular progression. Nevertheless, the patient experiences radiological progression only 3 months later, whereas liquid biopsy was able to identify it earlier

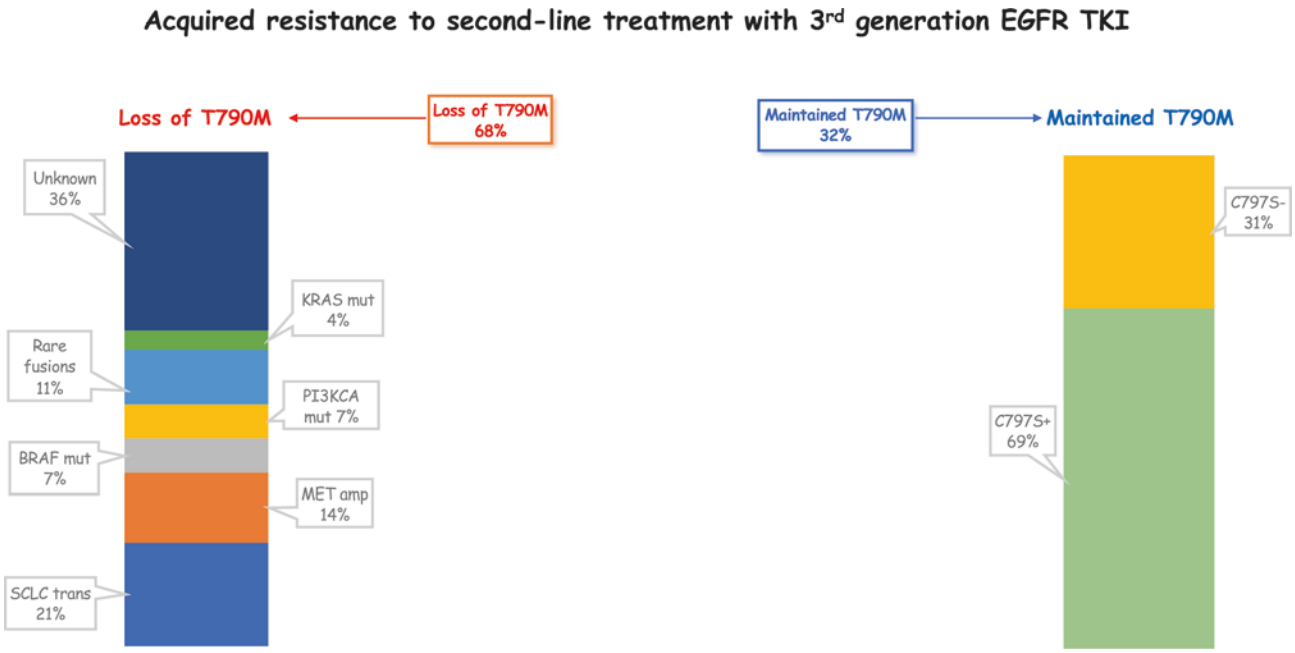

Fig. 6.9 Acquired resistance to second-line treatment with osimertinib: after treatment with osimertinib at resistance approximately 30% of patient maintain p.T790M mutation and acquire also the p.C797S mutation (on the right). Approximately 70% of patients lose p.T790M, but they acquire multiple and different alterations (small cell lung cancer transformation, gene mutations, mesenchymal to epithelial transition (*MET*) gene amplification and other rare fusion genes)

(19%), p.C797S mutation, (15%), human epidermal growth factor receptor 2 (*HER2*) amplification (5%), *PI3KCA*, and *RAS* mutations (5% and 2%, respectively) (Fig. 6.11).

Interestingly in preclinical models when p.C797S develops in cells wild type for p.T790M (when third-generation TKIs are administered in the first-line setting), the cells are resistant to third- generation TKIs but

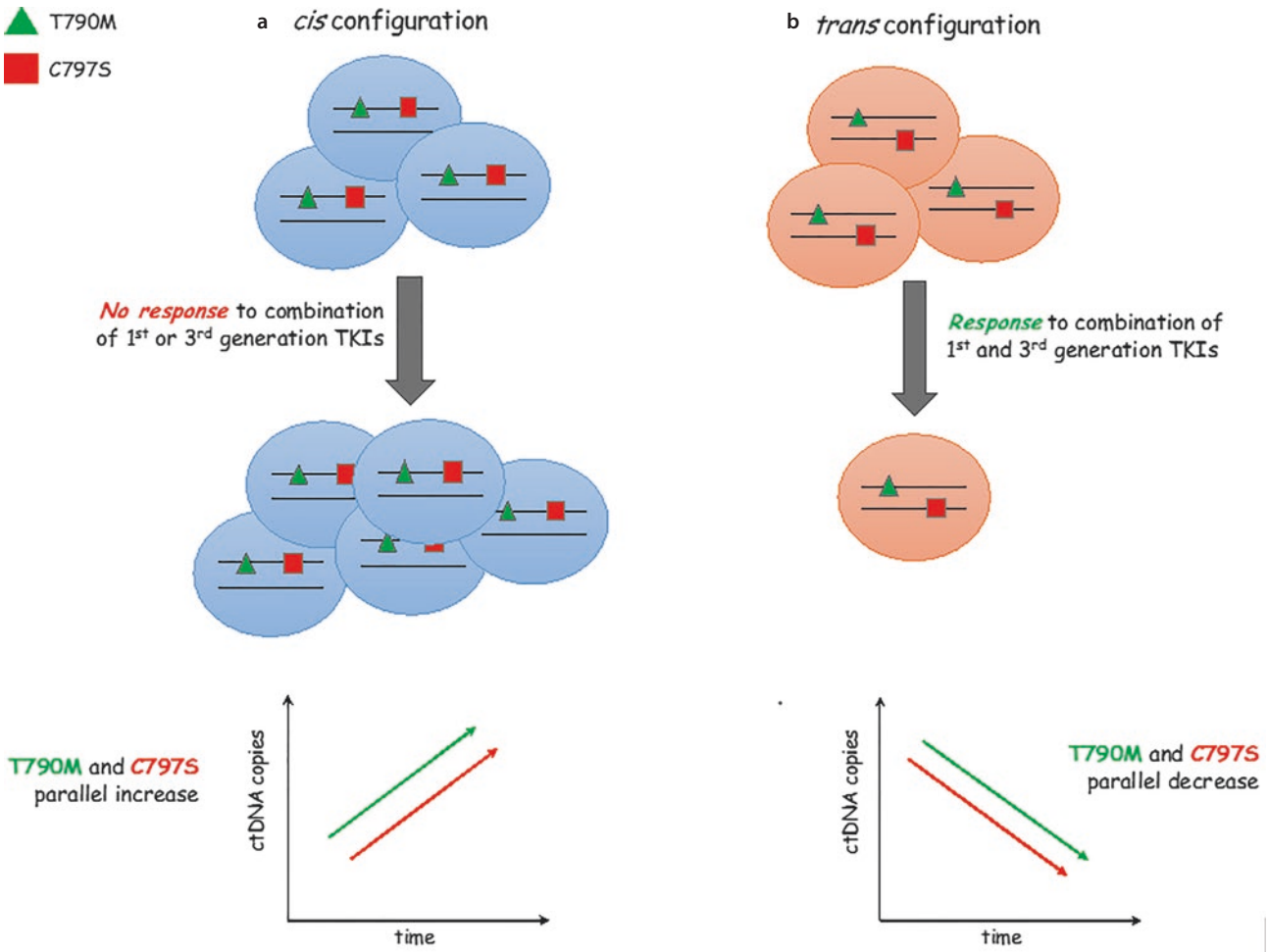

Fig. 6.10 Visual representation of p.T790M/C797S *cis* vs. *trans* configuration and the putative trend of these mutations in liquid biopsy. **a**. *cis* configuration: both mutations arise in the same DNA strand; therefore tumor cells are resistant to both first- and third-generation TKIs. If a combination of these TKIs is used, the analysis of ctDNA should reveal a parallel increase of both p.T790M and p.C797S mutations, demonstrating treatment inefficacy. **b**. *trans* configuration: mutations are present in different DNA strands. In this case, tumor cells are sensitive to both first- and third-generation TKIs when administered simultaneously. Therefore, liquid biopsy analysis should reveal a parallel decrease of the both p.T790M and p.C797S mutations, demonstrating treatment efficacy

retain sensitivity to first/second-generation TKIs [44]. This finding opens new therapeutic perspectives (Fig. 6.12). *EGFR*-mutated NSCLC (deletions in exon 19, p.L858R) patients can be either treated with first/second or third-generation TKIs. In the first case (left side of Fig. 6.12), patients experience resistance due to p.T790M and will be consequently treated with third- generation TKIs. Upon resistance development, the main acquired resistance mutation is p.C797S, with two possible configurations (*cis* and *trans*) that have different clinical implications. In case of *cis* configuration, the combination of first/second- and third-generation TKIs is ineffective, whereas in *trans* configuration, the same combination may be effective. The other treatment option in naive *EGFR*-positive patients is third-generation TKI in first-line setting (right side of Fig. 6.12); the development of p.C797S mutation is the main resistance mechanism, within this case the tumor is sensitive to treatment with first/second-generation TKIs that can therefore be used as therapeutic strategy.

In lung cancer, ctDNA testing can also have a prognostic significance. It has been demonstrated that there is a significant statistical correlation between survival and allele fraction of circulating p.T790M before and after *EGFR*-TKI administration. Indeed, the dynamic modification of circulating p.T790M mutation in ctDNA in TKI-treated patients is associated with both PFS and overall survival (OS) [49]. Interestingly, it seems that also ctDNA concentration could be a good prognostic marker; indeed the high levels of ctDNA, regardless to mutational profile, are associated with decreased survival [50]. Furthermore, OS seems to be strictly correlated with a number of variants detected in plasma. Several variants greater than three determined an OS

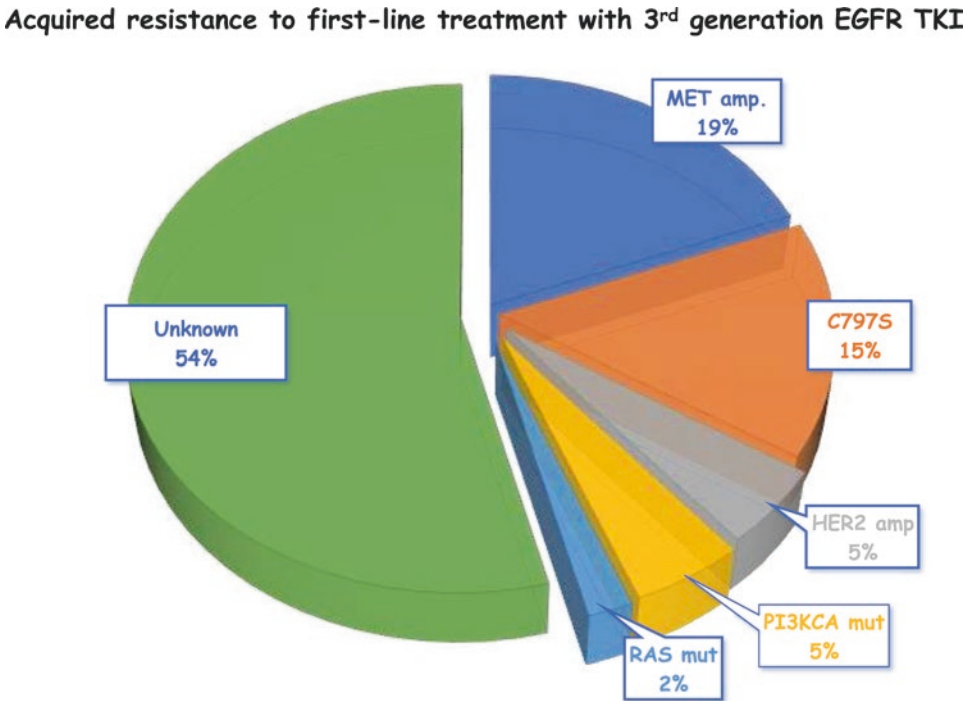

Fig. 6.11 Acquired resistance to first-line treatment with osimertinib: the most frequent resistance mechanism is *MET* amplification (19%) followed by p.C797S mutation, (15%), *HER2* amplification (5%), *PI3KCA*, and *RAS* mutations (5% and 2%, respectively)

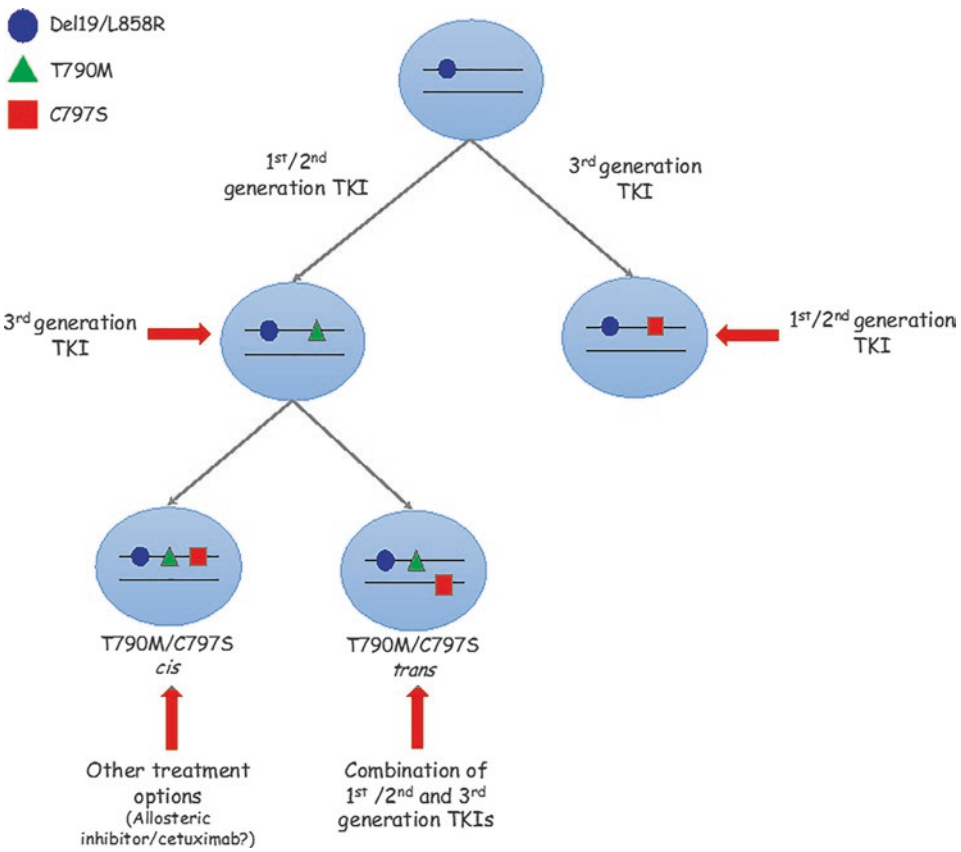

Fig. 6.12 New therapeutic perspectives in *EGFR*-positive NSCLC patients. In the first-line setting, both third- and first/second-generation TKIs are available. Upon resistance to third-generation TKIs (right side), the acquired mutation is p.C797S; in this case, cells are resistant to third-generation TKIs but retain sensitivity to first/second-generation TKIs. Resistance to first/second-generation TKIs is due to p.T790M; in this case, the tumor is sensitive to third-generation TKI. At resistance development, cells acquire p.C797S mutation in *cis* or *trans* configuration. In the first case (*cis*), tumor will not respond to a combination of first/second-generation TKIs; in the second case (trans), the same combination may be a therapeutic option

reduction, giving thus a poorer prognosis. Therefore, it seems that mutational load itself may be a good prognostic marker. In this scenario, biomarker investigations have become one of the most interesting and studied fields of translational lung cancer research with the aim to estimate patients' prognosis, to monitor treatment response and to eventually predict both treatment efficacy and tumor recurrence.

6.1.1.2 Circulating microRNA

Circulating miRNAs are promising disease biomarkers and have been deeply investigated; however, technical aspects of miRNA isolation, measurement, and quantification still represent critical steps. Sample processing, isolation, hemolysis in blood samples, and the lack of stable reference gene are among the most important critical issues [51]. The most common sources of circulating miRNAs are plasma, serum, urine, and saliva but also microvesicles and exosomes [52]. Nevertheless, the concentration of circulating miRNA in body fluids is very low, and therefore isolation and miRNA enrichment are very delicate and and important procedure that can impair downstream analysis. MicroRNAs can be analyzed through different quantitative methods, real-time PCR [53, 54], digital PCR, microarray, and also NGS. The introduction of miRNA-seq through NGS offers the opportunity to assess both known and unknown miRNAs, and this technique is very useful for miRNA discovery. Nevertheless, the major limitation of using routinely NGS is strictly correlated to its high costs as well as time-consuming. Moreover, it generally requires big amount of input RNA [55–58].

Several miRNAs have been suggested as noninvasive tool for NSCLC screening; however the numerous proposed miRNA signatures are inconsistent and still need to be properly validated in clinical setting [59]. Circulating miRNA in NSCLC seems to have a prognostic significance. MiR-34a and miR-34c in plasma are positively associated with that in tumor tissue and are also associated with disease-free survival (DFS) and OS. In particular miR-34a high expression is correlated with prolonged DFS and OS [60]. According to literature data, a recent systematic review showed that four circulating miRNAs showed high sensitivity (>80%) and AUC (>0.80) as biomarkers of stages I–II NSCLC. Additionally, four other miRNAs showed high specificity (>90%); by combining this two miRNAs panel, it is possible to reach an overall sensitivity of 92% and an overall specificity of 93% for stages I–II NSCLC [61]. Nevertheless, these and other circulating miRNAs suggested for NSCLC screening require validation in multiple independent studies before they can be proposed for clinical application. Many studies have suggested the miRNAs are also involved in sensitivity regulation to *EGFR*-TKIs. Indeed it has been shown that circulating miRNAs are differentially expressed when comparing TKI sensitive and TKI-resistant patients [62].

6.2 Circulating Tumor Cells (CTCs)

Circulating tumor cells (CTCs) have been identified almost half a century ago, when it was noticed that some cancer cells have the capacity to detach from the tumor tissue and floating in the bloodstream [63]. These cells bear the potential to seed the disease to other sites, and therefore they are responsible for metastasis development [64]. Interestingly almost 90% of all cancer deaths arise from the metastatic spread of primary tumors. The metastatic process evolves through four main steps: local invasion, intravasation, extravasation, and colonization [65, 66]. Nevertheless, we are still far from understanding how to block this crucial event.

CTC precursors have the capacity to overcome the basal membrane and the extracellular matrix through the secretion of proteases [67]. Another important feature of CTCs is the capacity to undergo epithelial-to-mesenchymal transition (EMT) by repressing expression of E-cadherin and cytokeratin as well as inducing vimentin and N-cadherin. Once CTCs intravasate onto the bloodstream, they can reach distant site where they can finally extravasate and establish a metastatic lesion (Fig. 6.13).

CTCs can be isolated either as single cells or clusters; nevertheless some evidences show that in breast cancer patients, CTC clusters have a more aggressive behavior with higher metastatic potential compared to single cells [68]. These clusters can be composed by just neoplastic cells or associated with fibroblasts, leukocytes, endothelial cells, and platelets [69]. CTCs represent a valid tumor marker in many tumor types, and they can be useful for disease progression monitoring but also for treatment response evaluation. Indeed the number of CTCs is correlated with tumor size and stage, and consequently, the dynamic modification during treatment administration may reflect therapy efficacy [70, 71].

There are different approaches for CTC isolation and detection. Isolation techniques are used for CTC enrichment from whole blood samples; indeed, the number of CTCs ranges from 1 to 10 per mL of blood. CTC selection and be achieved by exploiting both their biological and physical properties (Fig. 6.14) [72].

Immunomagnetic methods exploit some CTCs *biological characteristics* to achieve their detection. With these approaches, it is possible to couple isolation and detection phases; isolation is based on the identification of specific membrane markers, while detection can be obtained through several methods including immunofluorescence and flow cytometry.

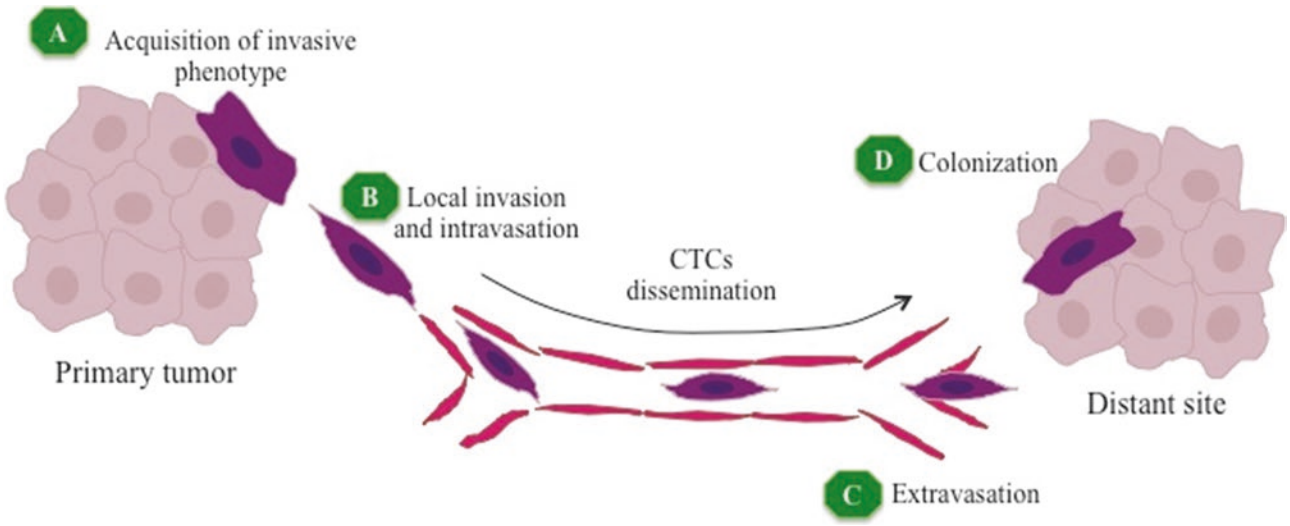

Fig. 6.13 **a** tumor cells acquire an invasive phenotype; they acquire the capacity to overcome the basal membrane and the extracellular matrix through the secretion of proteases; **b** tumor cells undergo to epithelial-to-mesenchymal transition by repressing expression of E-cadherin and cytokeratin as well as inducing vimentin and N-cadherin; this finally leads to intravasation; CTCs can now use the bloodstream to disseminate and reach distant site; **c** CTCs reach distant site, where a pre-metastatic niche has been already settled, and extravasate; **d** CTCs find fertile soil where they can grow determining the onset of a metastatic lesion

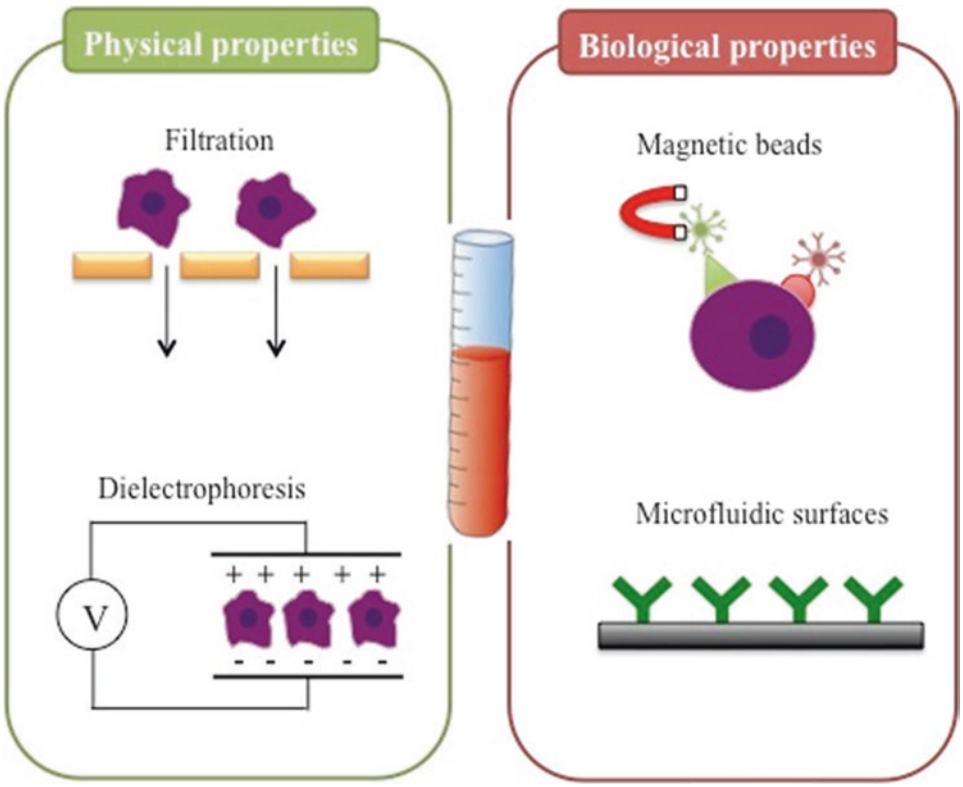

Fig. 6.14 Schematic depiction of CTC processing methods. Enrichment techniques are necessary to separate the extremely rare CTCs from peripheral blood cells; this separation can be achieved by exploiting both their physical and biological properties

Currently the election method for CTCs analysis is the CellSearch System (Veridex) that received the US Food and Drug Administration (FDA) approval in 2013. This is a simple method that evaluates the expression of both membrane epithelial cell adhesion molecule (EpCAM) and the cytoplasmic epithelial cytokeratin (CK-8, −18, and −19) markers on CTCs. Moreover $CD45^{+}$ leucocytes are negatively selected and excluded from the analysis, whereas the nuclei of CTCs are evaluated using DAPI stains [73] (Fig. 6.15).

These immunostainings are revealed through fluorescence imaging with CellTracks system; marked cells are

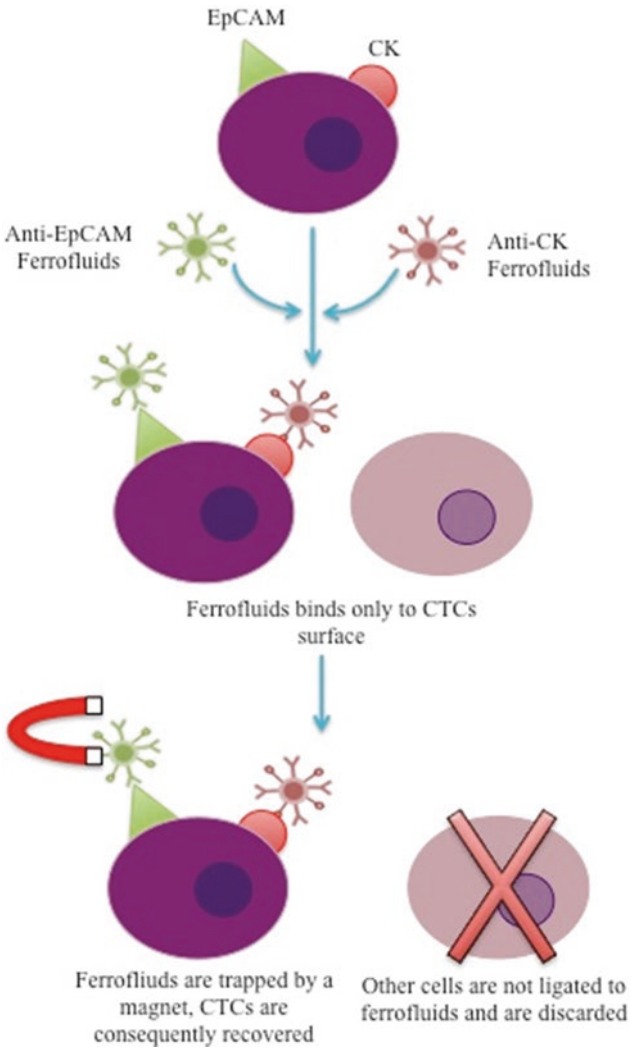

Fig. 6.15 Schematic diagram of CellSearch system technology. CTCs express EpCAM and CK on their surface. Peripheral blood (7.5 mL) is mixed with magnetic iron nanoparticles (ferrofluids) coated with anti-EpCAM and anti-CK antibody to confer CTC magnetic properties. Leukocytes are negatively selected using anti-CD45 antibody. After incubation, the mixture of CTCs and ferrofluids is exposed to a magnetic field; CTCs coated with ferrofluids are consequently isolated and further analyzed, while other cells (including leukocytes) are discarded. In addition, cell nuclei are fluorescently labeled with the DAPI nuclear dye (4′,6-diamidino-2-phenylindole) to allow microscopic identification of the relevant cell fraction

counted through flow cytometry. An interesting device recently developed is the CellCollector, GILUPI. This device could potentially have a very good application in clinic since it is intended for in vivo detection of CTCs. The device is composed by a stainless steel wire of 16 cm coated with anti-EpCAM antibodies that can be placed for 30 minutes directly in the vein (Fig. 6.16). Performances of the CellCollector® device were first tested in 12 breast cancer and 12 NSCLC cancer patients compared to 29 healthy volunteers; this study showed that CTCs could be isolated across all tumor stages,

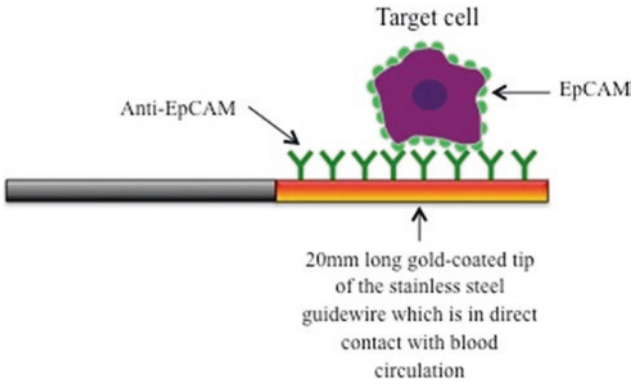

Fig. 6.16 Schematic representation of the CellCollector, GILUPI. The device is composed by a stainless steel wire of 16 cm coated with anti-EpCAM antibodies that can be placed for 30 minutes directly in the vein

including early-stage cancer, in which distant metastases were not yet diagnosed, while no CTCs could be detected in healthy volunteers [74]. Same results were obtained in prostate cancer patients [75].

Other developed methods exploit CTCs *physical characteristics*, for instance, size, membrane charge, and density. CTCs measure 7–18 μm in diameter, are larger than leukocytes, and for this reason, it is possible to separate them using specific filters, chemical materials, or through centrifugation. One of the main advantages of this approach, compared to immunomagnetic-based technology, is to yield a greater number of isolated cells. Nevertheless, this advantage can turn onto a disadvantage, as it is possible that other cell types are recovered and wrongly counted as CTCs. To avoid this inconvenient, it is fundamental to characterize CTCs after the isolation phase. Among size-based methods, the ISET (isolation by size of tumor cells) system is one of the most used. ISET allows the collection of tumor cells based on their larger size, as cells are enriched by blood filtration through filtering membranes with calibrated pores 8 μm in diameter [76] (Fig. 6.14). A direct comparison between CellSearch System and ISET showed that there is an important discrepancy between the numbers of CTC enumerated by both techniques. Indeed among 60 patients with metastatic breast, prostate, and lung carcinomas, the concordance between the two techniques was 55%, 60%, and 20%, respectively. Therefore, the discrepancies were mainly dependent by tumor type, with lung cancer showing the lowest concordance [77]. As previously mentioned after isolation through the ISET technology, it is necessary to further characterize isolated cells, both at a cellular level (microscopy, immunofluorescence, hematoxylin-eosin staining, etc.) and at molecular level. In the last case, it is necessary to extract nucleic acids (mRNA, DNA, miRNAs) from isolated cells and proceed with further analysis, for example,

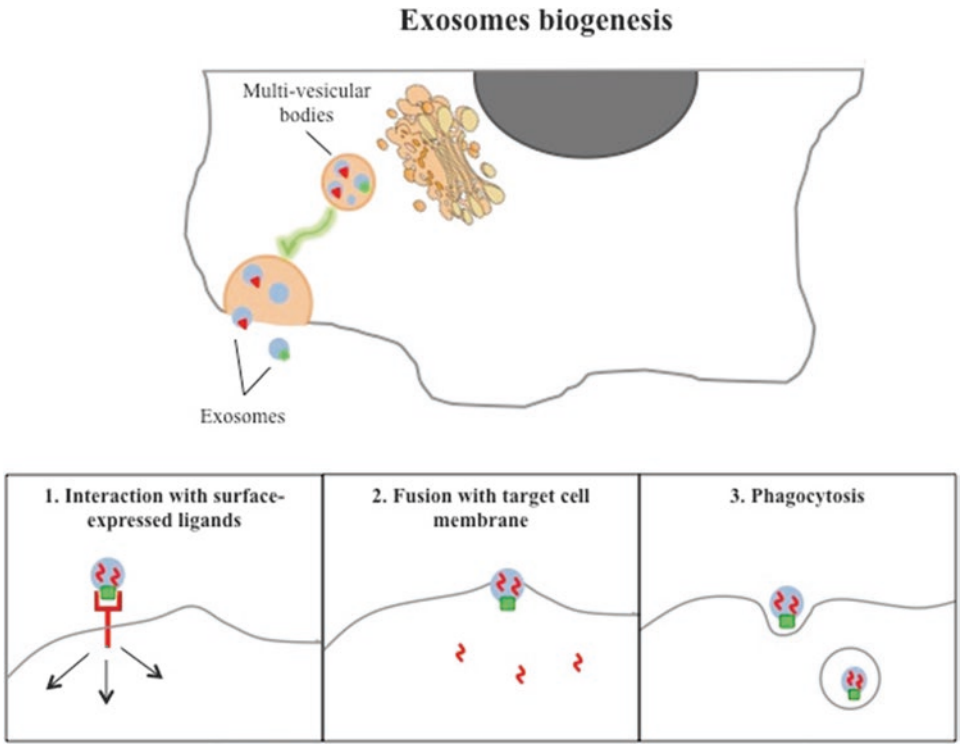

Fig. 6.17 Schematic representation of exosome biogenesis and mechanisms of action. Early endosome matures into late endosome, and finally exosomes are generated by a process of inward budding from the limiting membrane, forming the multivesicular bodies (MVB). Exosomes are released from the MVB by the fusion of the MVB with the cytoplasmic membrane. Exosome can communicate with the target cell by the interaction with surface-expressed ligands (1) by fusion with cell membrane (2) or by phocytosis (3)

through next- generation sequencing or real-time PCR [78]. Another interesting method for CTC isolation is dielectrophoresis; it is based on the evidence that cells in suspension are characterized by a specific conductivity. It is therefore possible to separate cells by applying to the suspension a specific electric field. Nowadays, the DEPArray is used to identify stem cells, leukocytes, platelets, cancer cells, and also viable CTCs [79].

Several studies have investigated the role of CTCs in NSCLC, with the aim to explore the potential of CTCs analysis for early diagnosis and outcome prediction. These studies have mainly confirmed that a greater number of CTCs are associated with poor prognosis in both early and advanced NSCLC stages [80–85]. Moreover, it has been demonstrated that the reduction of CTC number after chemotherapy administration is a surrogate biomarker of treatment response [86]. Finally the molecular profiling of single CTC in NSCLC might provide important information on tumor biology and on the mechanisms involved in tumor dissemination and in acquired resistance to targeted therapies [87, 88].

6.3 Exosomes

Exosomes are small membrane vesicles of endocytic origin and were initially isolated from the peripheral circulation of patients with cancer in 1979 [89]. These microvesicles are able to shuttle information between cells, even between distant sites, by a direct interaction with surface-expressed ligands, by fusion with target cell membrane or by phagocytosis (Fig.6.17).

Conversely to larger microvesicles, which are directly shed from the plasma membrane, exosomes derive from the intracellular endosomal compartment. Early endosome matures into late endosome, and finally exosomes are generated by a process of inward budding from the limiting membrane. Through this mechanism, several cytoplasmic components, such as miRNA, mRNA, protein, and even DNA fragments, are encapsulated into exosomes. Interestingly it has been shown that exosomal RNA determines horizontal transfer of genetic information between cells [90]. Moreover, transmembrane proteins maintain the same orientation relative to the cytoplasm and plasma membrane. In a second step, multivesicular endosomes fuse with the cellular membrane to release the exosomes into the extracellular space [91].

In recent years, exosome involvement in cancer has aroused much interest, and it seems clear that they can have different roles in cancer. On one side, exosome is able to manipulate local and systemic environment, thus favoring cancer growth and dissemination; on the other, they modulate immune system to elicit or suppress an antitumor response [92]. Another emerging utility of exosome regards the possibility to engineer them to specifically vehicle drugs inside tumor cells [93]. Since the first hypothesis of metastatic organotropism formulated in 1889 by Stephen Paget, the mechanisms underlying this process have remained poorly understood. Nevertheless, recent findings are suggesting that exosome could represent the

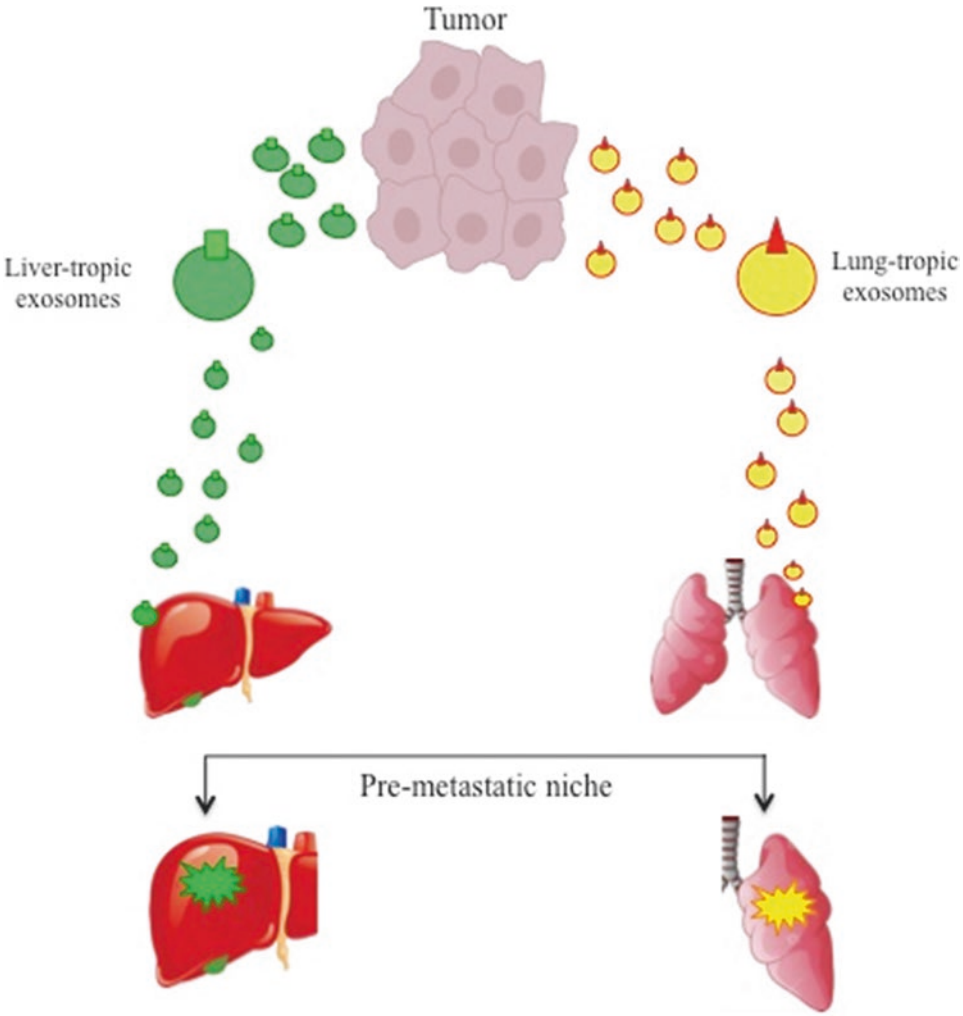

Fig. 6.18 Schematic representation of the revised "seed and soil" theory. Tumor-derived exosomes are selectively engulfed by specific organ district and are responsible for the preparation of the pre-metastatic niche. Consequently, when CTCs reach the preferential metastatic site, they find a fertile ground to take root

missing piece of the puzzle. Indeed, it has been shown that exosomes isolated from human lung, liver, and brain tropic tumor cells fuse preferentially with resident cells at their predicted destination, namely, lung fibroblasts and epithelial cells, liver Kupffer cells, and brain endothelial cells. Tumor-derived exosomes are selectively engulfed by specific organ district and are responsible for the preparation of the pre-metastatic niche. Consequently, when CTCs reach the preferential metastatic site, they find a fertile ground to take root (Fig.6.18). This peculiar exosome feature makes them an attractive and interesting target to potentially inhibit the metastasization process.

For all these reasons, exosomes are emerging as potential diagnostic, prognostic, and predictive biomarker in NSCLC. It has been recently investigated whether exosomal miRNAs content can be used as diagnostic marker for lung cancer. In the paper by Rabinowits et al., it was first compared miRNA expression profile in both tumor tissue and exosome. This approach confirmed that 12 specific miRNAs were elevated in NSCLC tissue and that was mirrored in circulating exosomes [94]. Moreover the identification of a specific exosomal miRNA profile has been shown to have a promising diagnostic performance for identifying stage I NSCLC (AUC of 0.899, sensitivity of 80.25%, specificity of 92.31%) [95].

Exosomal miRNAs have also been used to predict prognosis in NSCLC. Plasma levels of miR-21 and miR-4257 were significantly upregulated in patients with recurrence compared with those without recurrence or healthy individuals; increased level of these miRNAs was associated with shorter disease-free survival (DFS). The predictive potential of these miRNAs for recurrence was validated in a large cohort including 195 NSCLC patients and 30 healthy controls. The data indicated that increased levels of exosomal miR-21 or miR-4257 related to a worse prognosis with a shorter disease-free survival (DFS) [96].

Exosome could also be used as a source of tumor-derived DNA, and therefore, they can also have a predictive role. DNA inside exosomes can be defined as exoDNA [15, 97]. It has been reported that exoDNA can be isolated and detected from plasma and bronchoalve-

olar lavage fluid (BALF), and moreover, it can be used for *EGFR* mutation detection. In the study published by Hur JY, it was reported that liquid biopsy results using exoDNA show higher accordance with conventional tissue biopsy compared to the liquid biopsy of ctDNA alone [98]. These data are further supported by another study where the combination of exoRNA (RNA contained in exosomes) and ctDNA analysis seems to increase the sensitivity for *EGFR* mutation detection in plasma, with the largest improvement seen in the subgroup of M0/M1a disease patients known to have low levels of ctDNA.

In addition, the role of total RNA derived from exosomes has been more recently under investigation. In particular, by using an NGS-based approach, the possibility to identify the EML4-ALK translocation carried out by the exosome-derived RNA has been described for the first time in NSCLC patients [99].

Even though the potential application of exosomes analysis in clinical practice is promising, there are still some opened questions regarding the rapidity and specificity of exosome isolation and the choice of the best detection method [100]. Currently, the most commonly used approach for exosome isolation is based on ultracentrifugation [101]. However, this technique has several limitations including long processing time, lack of reproducibility, and specificity. Furthermore, the abundance of exosomes from different cellular origins makes a further enrichment of the relevant biomarkers a necessary step when considering exosomes for diagnostic or companion diagnostic purposes. NanoSight™ platform can be used for exosome concentration evaluation in fluids, but its usefulness is limited for plasma-derived exosome since they are normally too dirt for this kind of analysis [102]. Fortunately, there have been developed a series of commercial kits that enable easy microvesicle isolation [103, 104]. Recently the increased application of proteomic technologies has significantly contributed to a deeper understanding of exosome protein profiles from a wide variety of cultured cells and body fluids (such as plasma, urine, and malignant effusions) [105]. All proteomic data acquired to date demonstrate that tumor-derived exosomes (TDEs) express a discrete set of proteins specifically related to the tumor phenotype and involved in cell proliferation, antigen presentation, signal transduction, migration, invasion, and angiogenesis. Recently, it was reported the first global proteomic analysis of highly purified exosomes derived from human NSCLC malignant pleural effusion. Using nanoLC–MS/MS following 1D SDS-PAGE separation, researchers identified pathologically relevant proteins and potential diagnostic makers for NSCLC, including lung-enriched surface antigens and proteins related to *EGFR* signaling [106].

6.4 Liquid Biopsy in the Era of Immunotherapy

The introduction of immunoncology, especially immune checkpoint inhibitors, has recently become a promising frontier for the treatment of several human cancers, improving the organism's competence to direct the immune system against cancer cells, with notable success and evidence of long-term survival.

The immune checkpoint inhibitors are monoclonal antibodies that targeted specific molecules, such as PD-1 or PD-L1. Programmed death ligand 1 (PD-L1) is an inhibitory receptor expressed on the surface of tumor cells. PD-L1 interaction with its ligand PD-1 on activated T lymphocyte inhibits its cytolytic effector functions. Tumors can create an immunosuppressive microenvironment through the overexpression of PD-L1 on tumor cells, which facilitate cancer immune evasion through the downregulation of cytotoxic T-cell activity. The blockade of the PD1/PD-L1 axis with the specific antibody inhibitors prevents T-cell suppression and promotes the immune killing of the cancer cells. Although a clinically relevant median response duration is reported, only 1 out of 4 treated patient will benefit from immunotherapy. Predicting which patient will benefit from immune checkpoint inhibitors is a novel and important issue and would contribute to optimize treatment selection [107].

It has been hypothesized that expression of PD-1/PD-L1 could have a predictive and/or prognostic role. However, the value of PD-L1 expression assessment by using immunohistochemistry (IHC) staining in formalin-fixed paraffin-embedded tissue samples is currently debating and challenging [97]. The expression of immune checkpoints on immune and tumor cells is a dynamic process; therefore, evaluation at a single time point can be suboptimal for several limitations inherent to tissue sampling, IHC detection methods, and antibodies used, in addition to heterogeneous PD-L1 expression during cancer evolution and treatment. Hence, the actual effort is to identify peripheral blood biomarkers that reveal the dynamic and complex nature of the immune response and the interaction of multiple elements. Among several peripheral blood biomarkers studied, plasmatic soluble forms of PD-1 and PD-L1 (sPD-1; sPD-L1) represent the areas with more encouraging data. The prognostic and predictive role of sPD-1 and sPD-L1 seems to be dependent on the type of cancers, leading differentially to good or poor clinical outcomes [108, 109]. sPD-1 and sPD-L1 have been shown to negatively correlate with survival in NSCLC; the clinical outcome of nivolumab treatment was significantly associated with the baseline plasma sPD-L1 levels

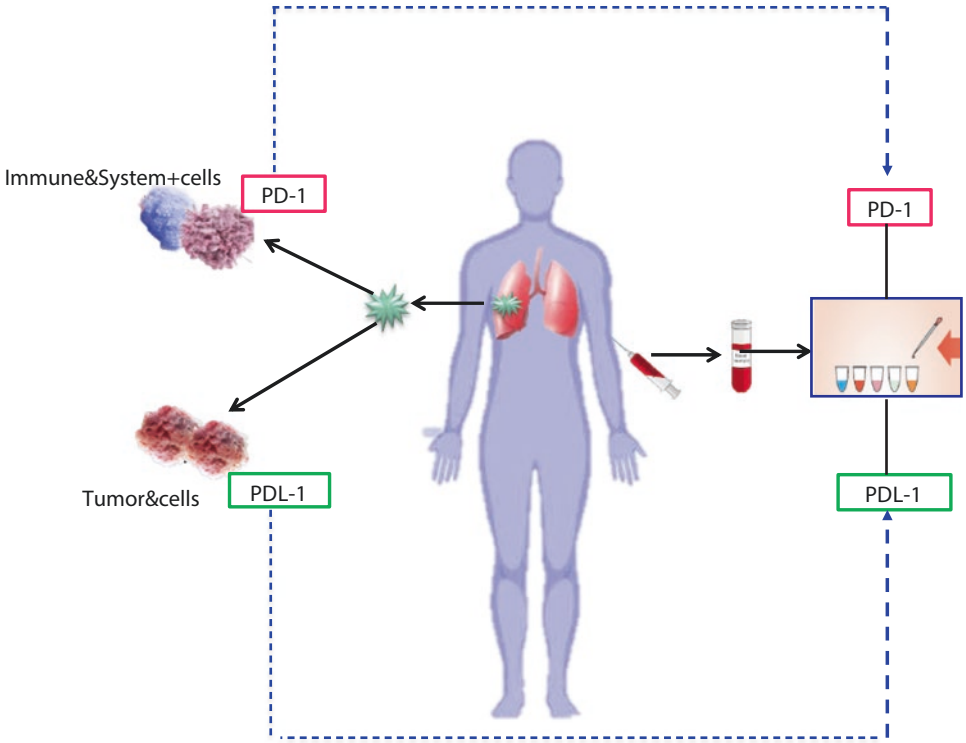

Fig. 6.19 To identify prognostic and/or predictive biomarkers requires the study of both the immune system and the tumor. Expressions of soluble forms of PD-1 and PD-L1 are associated with poor prognosis and shorter outcome in various types of solid tumors, providing the basis for a new type of liquid biopsy in the era of immunotherapy

[110]. In patients with metastatic melanoma, the level of circulating exosomal PD-L1 changes during the treatment with the anti-PD-1 pembrolizumab. A higher level before the treatment was associated with poorer clinical outcomes, and the increased levels during early stages of therapy identify the clinical responder patients [111]. It was demonstrated that high plasma levels of specific immune checkpoints correlate with dramatically poor outcome and can be used as prognostic factors in non-resectable pancreatic adenocarcinoma (PDAC) (Fig. 6.19). In this study, the researchers used specific antibodies to detect the soluble forms of PD-1 and PD-L1 and others new immune checkpoints that seem to play an important role in T-cell activation and regulation: the B7/butyrophilin-like receptors such as butyrophilin subfamily 3A/CD277 receptors (BTN3A), the butyrophilin subfamily 2 member A1 (BTN2A1), and the B and T lymphocyte attenuator (BTLA) belonging to the B7-like receptors [112]. The results show a negative correlation between plasma levels of each marker and pancreatic cancer patients' overall survival [99]. Recently, the association with clinical outcomes was showed also in head and neck squamous cell carcinoma, triple-negative breast cancer (TNBC), large B-cell lymphoma, and ovarian cancers, and several researches are ongoing to investigate this specific issue. In a new era of liquid biopsy, all these data are interesting findings that provide a rationale for the application of more precise and dynamic predictive biomarkers for checkpoint blockades.

6.5 Liquid Biopsy for Early Cancer Diagnosis

The best strategy for reducing the incidence of cancer-related death is early diagnosis; indeed cancer early detection is crucial to strongly increase the number of eligible patients for curative treatments. Screening tests nowadays available mainly focus on one tumor type (e.g., PAP test for cervical carcinoma, ultrasound and mammography for breast cancer, occult blood in the stool and colonoscopy for colorectal cancer), and yet we are still missing tests to screen aggressive diseases with very poor prognosis such as lung, pancreatic, and liver cancers. Moreover, despite the majority of screening tests are minimally invasive, some of them may not be easily accepted by patients and may have prohibitive costs. Therefore, there is the need for new noninvasive and inexpensive tests, and liquid biopsy approaches hold promise in this context.

Almost all liquid biopsy components (ctDNA, CTCs, or circulating miRNA) have been used to develop tests for early diagnosis of different tumor types. Unfortunately, none of them can be currently used in clinical practice. The road is still long and winding, but some interesting results have emerged, which still request further investigation.

In 2018 the group of J.D. Cohen et al. reported the results regarding the use of a multi-analyte blood-based test, called CancerSEEK, to detect eight common cancer types [113]. In detail, the CancerSEEK test is designed to

simultaneously evaluate levels of 8 circulating proteins and mutations in ctDNA and was applied to 1005 patients with nonmetastatic (stages I–III), clinically detected cancers of the ovary, breast, liver, stomach, lung, pancreas, esophagus, or colorectum. Considering all 8 tumor types, a median of 70% of patients were positive to the test with sensitivities ranging from 33% for breast to 98% for ovarian cancer. Interestingly a sensitivity greater than 69% was reached for five cancer types (ovary, liver, stomach, pancreas, and esophagus) for which there are no routine screening tests available. Another important aspect is the specificity which should be as highest as possible to prevent unnecessary follow-up and psychological distress associated with false-positive results. The reported specificity for CancerSEEK was greater than 99%, as only 7 out of 812 healthy controls resulted positive; therefore, the risk of false-positive is very low. The main characteristic of a screening test is the ability to intercept cancer at an early stage; CancerSEEK sensitivity was similar in stage II and III cancers (73% and 78%, respectively) but still too low in stage I cancers (43%), meaning that in early-stage disease, there is still a high risk of false-negative results. Moreover, cancer detection alone is not sufficient, and liquid biopsy alone is not able to determine the localization of a tumor in a patient that scored positive for the test.

Despite the promising results obtained in the study, there are several limitations. Control population was limited to healthy individual, whereas in a real cancer screening setting, some individuals might have inflammatory or other diseases, which could negatively impact test performance by increasing the proportion of false-positive results. Another problem is the patient cohort composed of individuals with known cancers that have already shown symptoms. Again, this is not the optimal set-up for cancer screening setting where the goal is to detect cancer before it causes symptoms. The same research group has then decided to test the CancerSEEK in a wider population, and they have recently published the results of the DETECT-A study (Detecting cancers Earlier Through Elective Mutation-Based blood Collection and Testing), an exploratory prospective, interventional study [114]. The aim of the study was to evaluate the feasibility and safety of multi-cancer blood testing coupled with PET-CT imaging to detect cancer in 10.006 women, aged from 65 to 75. DETECT-A consists in three steps: (1) a first peripheral blood sample was evaluated with CancerSEEK test; (2) individuals who tested abnormal for at least one of the biomarkers included in CancerSEEK were subjected to second blood withdrawal that was used for a second test to determine whether the same biomarker was persistently altered as well as to exclude mutations due to clonal hematopoiesis of indeterminate potential (CHIP, for more details, refer to paragraph 6); and (3) if the biomarker was reproducibly abnormal in the second test and CHIP was excluded, the blood test was considered positive, and the individual was invited to perform a full-body diagnostic positron emission tomography computed tomography (PET-CT) scan with contrast, using fluorodeoxyglugose (FDG) as the tracer. This third part of the study was used for both confirming blood test results and more importantly to localize the potential cancer in a safe and minimally invasive manner [115]. CancerSEEK test resulted positive in 490 individuals, but only 134 were confirmed in the second blood test. Of these 134 participants, 127 were evaluated by imaging, and 64 had imaging compatible with cancer. After further investigation, including cancer biopsy and other unequivocal evidence, in 26 participants, a diagnosis of cancer was confirmed. Despite this is a feasibility study with no information about clinical validity and utility of this approach, results obtained are very interesting but yet not enough robust to use this test as a routine standard-of-care screening.

One of the main limitations of detecting somatic alterations in ctDNA is that not every mutation detected is necessarily coming from a tumor. Therefore, the risk of false positive could be quite high, leading to psychological distress (for more details about false positive and false negative, refer to paragraph 6). Recently another research group, headed by M.C. Liu and G.R. Oxnard [116], used a new approach for early cancer diagnosis. Indeed, they assessed the performance of targeted methylation, instead of somatic mutations analysis, of cfDNA to detect and localize multiple cancer types across all stages at high specificity. Methylation is a process involved in gene expression regulation, and it has been demonstrated that methylation profiles are specific and can be considered as a real fingerprint of the tumor. Moreover, it has been shown that methylation profile approach outperformed both whole genome and targeted sequencing in the detection of multiple cancer types and in identifying the tissue of origin [117–119]. In the study published by M.C. Liu and G.R. Oxnard et al., methylation profile results were coupled with a machine learning system which, if properly nourished with methylation data, was able to detect and localize a large number of cancer types at sufficiently high specificity to be considered for cancer screening program [116]. The test was used in 6689 participants (2482 cancer, 4207 non-cancer), and it showed an overall specificity of 99.3% and 0.7% false-positive rate. Sensitivity for all cancer types was 44%, but it increased to 67% when considering pre-specified set of 12 cancer types which account for more than half annual cancer-related death in the United States. These new data are encouraging and justify the interest in applying the test in prospective population-level studies. Indeed, the PATHFINDER study (NCT04241796) is already ongoing, but results are expected not before 2021.

Circulating tumor cells may also be used for cancer early detection. In particular, it has been proposed that ISET, a specific CTC isolation method (refer to paragraph 2 for more details), could be used for isolation and

detection of cancer at an early stage [78]. In 2014, M. Ilie et al. used the presence of CTCs together with CT scan in chronic obstructive pulmonary disease (COPD) patients without clinically detectable lung cancer [120]. The aim was to investigate whether this approach could be useful as a first step to identify a new marker for early lung cancer diagnosis. The study included 245 people (168 with COPD, 42 smokers and 35 nonsmokers) that were tested with ISET for CTC detection. Five of the 168 COPD patients resulted positive for CTCs, and they were closely monitored by low-dose spiral CT. After 1–4 years from CTC detection, tumor became visible by CT scan, which showed nodules that were surgically removed at a very early stage. Moreover, during a follow-up period of 12 months by CT scan and ISET after surgery, none of the 5 patients experienced tumor recurrence. Therefore monitoring "sentinel" CTC-positive COPD patients may allow early diagnosis of lung cancer.

The profiling of circulating miRNA coupled with imaging has been demonstrated to be useful for detection of early-stage lung cancer. In 2008 G. Veronesi et al. demonstrated within the COSMOS study that low-dose computed tomography (LDCT) could represent a noninvasive diagnostic tool for lung cancer screening [121]. Subsequently, in 2011, the group headed by F. Bianchi and F. Nicassio developed a serum test based on the detection of 34 miRNAs that could identify patients with early-stage NSCLC. The miRNA signature was able to assign disease probability accurately either in asymptomatic or symptomatic patients and to distinguish between benign and malignant lesions [122]. Lastly, in 2014, G. Sozzi et al. showed that the combination of both miRNAs signature classifier and LDCT consistently reduces LDCT false-positive rate, thus representing a noninvasive test potentially applicable for large-scale lung cancer screening [123]. Another potential liquid biopsy approach for early cancer detection is the identification of distinct metabolomic changes occurring in cancer patients compared with healthy subjects, as recently reported in lung cancer patients with promising results [124, 125].

6.6 Remaining Challenges in Liquid Biopsy: False Positive (FP) and False-Negative (FN) Results

In the field of diagnostic tests, limit of detection or sensitivity is fundamental. The best tests should be able to efficiently distinguish between diseased and healthy individuals but also limit the possibility of false-positive results or false-negative results. The genotyping results of a liquid biopsy must faithfully reflect those obtained from the genotyping of tumor tissue, which always represents the term of comparison.

A *false-negative* results of a liquid biopsy test indicate that mutation is not detected in ctDNA, whereas in the tissue, it was. There are several reasons that lead to a false negative; one of the main aspects is the technique sensitivity. As previously mentioned, ctDNA is a portion of cfDNA, and its fraction varies greatly, between 0.01% and more than 90%. This fluctuation is strongly influenced by some tumor clinical features such as stage and metastatic spread. In early-stage disease, tumor DNA shedding is reduced, leading to a ctDNA fraction perhaps lower than 0.01%. At such low concentration, also the technique with the greatest sensitivity may not be able to intercept ctDNA mutations. Consequently, methods such as Sanger sequencing or real-time PCR are not recommended for ctDNA analysis since the false-negative rate would be too high. Plasma ctDNA analysis is frequently performed through NGS, a technique that offers the opportunity to analyze multiple genes with a high throughput without sacrificing sensitivity. NGS data analysis are performed through bioinformatic pipelines that could again determine a false-negative result. Indeed, bioinformatic filtering can lead to the exclusion of a variant considered "fake" or artifact of the technique, when it is actually present [126]. This error is greater in case of variant present at a very low allelic fraction, a typical early disease condition.

In case a liquid biopsy test indicates the occurrence of a mutation that was not detected in tumor tissue, it is called *false positive*, and it is frequently attributed to tumor heterogeneity [127]. Although heterogeneity is the main cause of plasma/tissue discordance specially in cancers that have developed drug resistance [128], many false-positive results can be attributed to other causes. The majority of cfDNA is derived from peripheral blood cells that can be subjected to clonal hematopoiesis (CH), a process that lead to the acquisition of somatic mutations within nonmalignant hematopoietic cells. Clonal hematopoiesis is challenging because it can involve relevant tumor-associated genes such as *KRAS*, Janus family Kinase 2 (*JAK2*), or tumor protein 53 (*TP53*) [129]. Therefore, mutation identification in such genes may not be indicative of the presence of a tumor and could lead to a false-positive ctDNA test. However, this problem can be easily addressed by sequencing both cfDNA and peripheral blood cell-derived DNA. In this way, it will be possible to distinguish mutations due to CH from mutations that can actually be traced back to a tumor. It has been also shown that lesions in deep infiltrating endometriosis, which are associated with virtually no risk of malignant transformation, harbor somatic cancer driver mutations [130]. In conclusion, several technical and biological aspects may influence performance of liquid biopsy-based tests. Therefore, it is needed to put effort in new investigations to fully understand the real potential of liquid biopsy as well as the limits beyond which we cannot go.

Expert Opinion

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Key Points

- An accurate profiling of the tumor molecular landscape is mandatory to tailor treatment decisions.
- The term liquid biopsy relates to the detection, quantification, and analysis of tumor components that are detected in body biological fluids (plasma, serum, saliva, urine, and effusion liquids).
- Tumor components found in body fluids are circulating nucleic acids (circulating tumor DNA, circulating microRNA, and circulating RNA), circulating tumor cells, and extracellular vesicles (exosomes and microvesicles).
- The advantage of liquid biopsy compared to traditional solid tumor biopsy is that is a minimally invasive procedure that captures tumor heterogeneity and real-time variations in tumor dynamics.
- Recent advances in biotechnology (i.e., next-generation sequencing, digital PCR) have allowed the development of liquid biopsy. With increasing novel technologies, liquid biopsy can currently be used in an easy and reproducible way in daily clinical practice.
- Liquid biopsy clinical applications include molecular diagnosis to decide treatment, determination of tumor load as a surrogate marker of early response to treatment, monitoring of mutations of resistance to targeted therapy, and detection of minimal residual disease after cancer surgery.

Hints for Deeper Insight

- A better understanding of the biology and technical aspects of liquid biopsy detection and analysis will help clinicians to maximize the potential clinical applications of liquid biopsy.
- One of the crucial clinical applications of liquid biopsy is the tracking of ctDNA through longitudinal blood extractions, which allows for a global, dynamic analysis of tumor genomic landscape. This may help in the early detection of resistance mutations that emerge during treatment preceding evidence of clinical or radiological progression. These findings can guide the clinicians to avoid continuing the administration of noneffective treatments and develop new therapeutic approaches to improve the outcome of patients. Two examples are (1) tracking of *EGFR* mutations during EGFR tyrosine kinase therapy in non-small lung cancer patients and (2) tracking of *RAS* mutations during anti-EGFR therapy in metastatic colorectal cancer patients.
- Currently, several prospective clinical trials in patients with solid tumors incorporate longitudinal ctDNA genotyping to monitor clonal dynamics and to guide treatment decisions. These studies are crucial to establish the clinical applicability of ctDNA in metastatic and localized solid malignancies, while results of ongoing studies of liquid biopsy as a tool for screening and detection of premalignant disease are highly expected.

Suggested Readings

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Diagnosis and Staging

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Learning Objectives

By the end of the chapter, the reader will be able to:

- Use a standardized approach for the diagnosis of cancer
- Order the right test in the right moment for both diagnostic and staging purposes
- Discern among established and investigational methods for cancer diagnosis
- Use appropriate systems of evaluation of response to anticancer agents

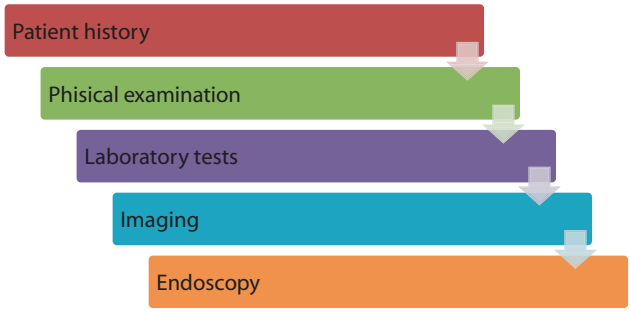

Fig. 7.1 Stepwise approach for the clinical diagnosis of cancer

7.1 Introduction

Cancer is a major public health problem worldwide and is the second leading cause of death in Western countries, exceeded only by heart disease. A sharp decline in cancer mortality has been reported over the past two decades, mostly as a result of trends in cancer-associated behaviors (smoking, excessive alcohol consumption, etc.) and advances in cancer detection and treatment [1].

Diagnosis of cancer has recently evolved, and innovative techniques currently allow early detection and characterization of tumors. In fact, while clinical and pathologic aspects, laboratory findings, and imaging keep a prominent role in the diagnosis of cancer, the advent of -omic sciences has revolutionized the approach to cancer patients, and mutatomic, transcriptomic, or metabolomic assays are being increasingly used in clinical practice for tumor diagnosis and/or prognostic stratification.

In parallel with improved diagnostic tools, a continuous refinement of staging systems has occurred in the last few decades. Staging describes the extent or spread of cancer at the time of diagnosis and subsequent evaluations and is a key factor for prognostic stratification and treatment selection. While several staging systems have been formulated, the most widely used in clinical practice is the tumor node metastasis (TNM) system, maintained cooperatively by the American Joint Committee on Cancer (AJCC) and the International Union for Cancer Control (UICC) [2]. A TNM staging has been generated for cancers of every anatomic site and histology, and aims at grouping patients based on their expected outcomes, in order to avoid under- or overtreatment and monitor the efficacy of therapeutic interventions. This chapter will summarize established and innovative concepts in cancer diagnosis and staging.

7.2 Clinical Diagnosis of Cancer

The clinical diagnosis of cancer is a multistep process (Fig. 7.1) deriving from a defined sequence of medical procedures including patient history, physical examination, laboratory tests, imaging, and endoscopic findings.

(i) *Patient history*. Given the impact of hereditary, familial, or environmental factors on cancer pathogenesis, a thorough familial and work history should be always recorded. In particular, the exact enumeration and characterization of relatives affected by specific cancer types may be extremely useful in determining whether monogenic or polygenic causes may underlie the development of a tumor. In this context, specific guidelines have been drawn for several malignancies in order to provide precise indications to the genetic consult. For example, according to major societies of medical genetics, female breast cancer patients older than 50 years should undergo genetic counseling only if at least one first-degree relative has a history of breast or ovarian cancer. Work-related factors should be also explored when interrogating the patients, and particular emphasis should be given to exposure to known cancer inducers (i.e., benzene, asbestos, cadmium, etc.). Behaviors commonly related to cancer development (i.e., cigarette smoking, excessive alcohol consumption, unprotected sun exposition, etc.) should be investigated too, since they may guide, along with current patient symptoms, in the subsequent diagnostic work-up.

(ii) *Physical examination*. A well-performed physical examination is mandatorily needed when looking for signs directly or indirectly associated with cancer. Physical examination, indeed, may provide useful information about the organs potentially involved by the primary tumor or its metastases. In several (although rare) instances, physical examination may directly lead to the diagnosis. This is the case, for example, of cutaneous melanoma, in which a precise set of morphologic changes of preexisting nevic lesions (ABCDE criteria: Asymmetry, irregular Borders, uneven Color distribution, large Diameter, temporal Evolution) may be observed by the physician during physical examination.

(iii) *Laboratory tests*. General laboratory tests including complete blood count (CBC) and comprehensive metabolic panel (CMP) may strengthen the clinical suspicion of a cancer diagnosis. Hypochromic microcytic anemia with low blood iron and high ferritin may be indicative of a chronic disease, such as cancer. High platelet number, elevated CRP, and increase of LDH or alpha2-proteins at serum electrophoresis are commonly found in patients with cancer as a result of systemic inflammation processes. Given their low sensitivity and specificity, biochemical assays for tumor-associated enzymes, hormones, or other tumor markers should never be used for cancer diagnosis. However, tumor markers may be of some utility in patients' follow-up for the therapeutic response monitoring. Nonetheless, therapeutic decisions should never be based on an isolated tumor marker rise, but should always rely on clinical or radiological findings. Table 7.1 summarizes the clinical utility of most common tumor markers.

CEA. Regular determination of carcinoembryonic antigen (CEA) throughout surveillance after curative surgery has been shown to significantly improve survival rates in patients with colorectal cancer. As a consequence, major medical oncology societies (European Society for Medical Oncology; American Society of Clinical Oncology) recommend serial postoperative CEA measurements approximately every 3 months for at least 3 years after surgery for colorectal cancer [3]. An increase higher than 30% of CEA levels in patients with colorectal cancer undergoing chemotherapy generally indicates disease progression, provided that false positives are excluded. In this context, transient spikes of CEA levels within the first weeks of treatment have been described as consequence of tumor cell apoptosis and should not be interpreted as lack of response to therapy.

CA15.3. The role of serial measurements of CA15.3 in the postoperative monitoring of breast cancer patients is controversial. In patients with advanced disease, CA15.3 may be effectively used in combination with CEA and radiology to monitor treatment response.

CA125. The role of CA125 in the surveillance of patients who underwent surgery for ovarian cancer is currently unclear. Although regular measurement of the tumor marker may detect early recurrences with a median lead time of 4 months, a recent prospective randomized study found no survival benefit from starting early treatment based on rising levels of CA125 as compared with initiating treatment at clinical/radiologic tumor progression [4]. In patients with advanced ovarian cancer, fluctuations of CA125 may be indicative of response to treatment. CA125 may be increased in patients with either benign or malignant ascites.

HCG, AFP, LDH. Measurement of human chorionic gonadotropin (HCG), alpha-fetoprotein, and lactate dehydrogenase (LDH) is mandatorily needed in determining prognosis for patients with germ cell cancer. AFP and HCG are also markers for surveillance and treatment monitoring in patients with non-seminomatous germ cell tumors. A consistent rise in AFP and HCG levels, even in the absence of clinical or radiological progression, is indicative of active disease and should lead to the initiation of treatment, provided that false positives are excluded. AFP may be increased in patients with hepatocellular carcinoma (HCC).

PSA. Data regarding the clinical utility of PSA in the screening of prostatic adenocarcinoma are controversial. In fact, increased PSA levels may be found in both benign and malignant proliferations of the prostate and may lead to prostate cancer overdiagnosis and, consequently, overtreatment. PSA may be very useful for detecting residual disease after treatment with curative intent and for monitoring response to therapies. In patients with advanced prostate cancer subjected to androgen receptor inhibition, the raise of PSA levels may be an early indicator of treatment resistance and disease progression [5].

(iv) *Innovative circulating biomarkers*. A significant evolution in the diagnostic approach to cancer has been recently represented by the so-called liquid biopsy [6]. Tumors continuously shed cells and DNA into the bloodstream, and circulating tumor cells (CTCs) as well as circulating tumor DNA (ctDNA) can be recovered, enumerated, and characterized both genomically and transcriptomically. Several different technologies including the DEPArray platform have been developed for detecting CTCs. While previous methods were based on immunomagnetic binding of CTCs to a dedicated antibody to a common epithelial protein, namely, EpCAM, the DEPArray technology enables the creation of dielectrophoretic cages around cells, so that single cell can be visualized and, if harboring the fluorescence for specific cell markers as well as typical morphology features, can be moved into a holding chamber for recovery, isolation, and subsequent molecular characterization (Fig. 7.2). CTCs have been described in a number of advanced cancers, and their number has been associated with both prognosis and response to treatment. At present, CTCs can be used as surrogate of tumor biopsy in selected clinical scenarios. For example, given the high methodological concordance between tissue and liquid biopsies, detection of mutations of resistance to anti-EGFR therapy in patients with lung adenocarcinoma may be performed by using CTCs. The applications of ctDNA are currently limited in clinical practice. However, preliminary evidence

Table 7.1 Major tumor markers in clinical oncology

Soluble biomarker	Cancers	Clinical application
AFP	Hepatocarcinoma	Diagnosis, treatment monitoring
	Germ cell tumors	Staging, prognosis, treatment monitoring
β2-Microglobulin	Multiple myeloma	Prognosis, treatment monitoring
	Chronic lymphocytic leukemia	Prognosis, treatment monitoring
	Lymphomas	Prognosis, treatment monitoring
α-hCG	Choriocarcinoma	Staging, prognosis, treatment monitoring
	Germ cell tumors	Staging, prognosis, treatment monitoring
Ca15.3	Breast cancer	Treatment monitoring
Ca19.9	Gastric cancer	Treatment monitoring
	Pancreatic cancer	Treatment monitoring
	Gallbladder cancer	Treatment monitoring
	Bile duct cancer	Treatment monitoring
Ca125	Ovarian cancer	Diagnosis, treatment monitoring
Calcitonin	Medullary thyroid cancer	Diagnosis, treatment monitoring
CEA	Colorectal cancer	Diagnosis, prognosis, treatment monitoring
	Breast cancer	Treatment monitoring
	Thyroid cancer	Treatment monitoring
	Pancreatic cancer	Treatment monitoring
Chromogranin A	Neuroendocrine tumors	Diagnosis, treatment monitoring
CYFRA21-1	Non-small cell lung cancer	Treatment monitoring
HE4	Ovarian cancer	Cancer treatment planning, treatment monitoring
Immunoglobulins	Multiple myeloma	Diagnosis, treatment monitoring
	Waldenström macroglobulinemia	Diagnosis, treatment monitoring
LDH	Multiple myeloma	Index of tumor burden, prognosis, staging
	Germ cell tumors	Prognosis, treatment monitoring
	Lymphoma	Staging, prognosis, treatment monitoring
	Melanoma	Staging, prognosis, treatment monitoring
	Neuroblastoma	Treatment monitoring
Mesothelin	Mesothelioma	Diagnosis, treatment monitoring
NSE	Small cell lung cancer	Diagnosis, treatment monitoring
	Neuroendocrine tumors	Diagnosis, treatment monitoring
PSA	Prostate cancer	Diagnosis, treatment monitoring
Thyroglobulin	Thyroid cancer	Treatment monitoring

suggests that therapeutically relevant mutations may be specifically detected in ctDNA of patients undergoing treatment with targeted agents, even months before radiological or symptomatic evidence of recurrence/progression.

(v) *Radiological imaging*. Radiological imaging plays a crucial role in both diagnosis and staging of cancer. At diagnosis, the accurate identification of all tumor lesions is essential in determining whether curative or palliative therapy should

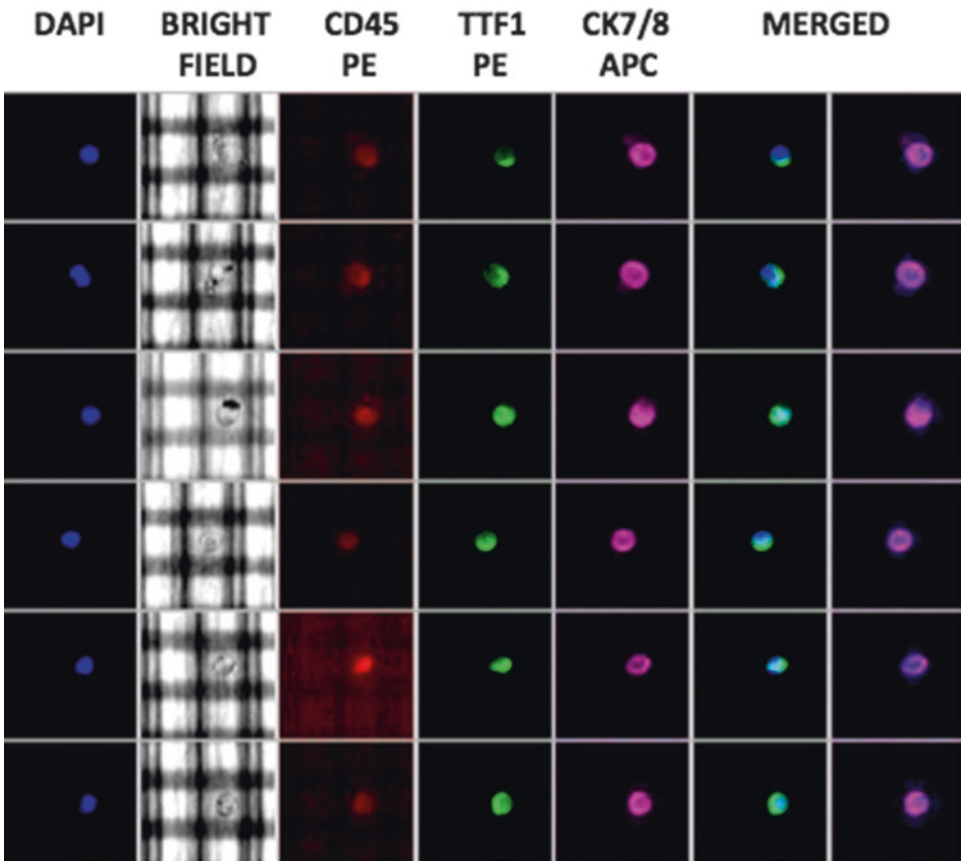

Fig. 7.2 CTC isolation and phenotypic characterization using the DEPArray technology. Nucleated circulating cells lacking hematopoietic differentiation (as expressed by DAPI positivity and CD45 negativity) are isolated and enumerated based on their expression of tumor-specific markers (in the case presented above, TTF-1 and CK7/8 are used as prototypic markers of lung neoplasms)

be addressed. By staging and restaging the patient after treatment, it is instead possible to evaluate the response to therapy. *X-rays* – X-rays are characterized by low sensitivity and specificity as compared with other radiological techniques, but may result useful at the beginning of the diagnostic work-up or in selected clinical scenarios during the follow-up. X-rays may reveal the presence of a tumor only when the diameter of the mass is larger than 10 mm.

- *Ultrasound echography (US-E)*. US-E of the abdomen is able to reveal both solid masses and cystic lesions and is usually able to distinguish between benign and malignant lesions, particularly within the liver. Primary and metastatic nodules of the peritoneum as well as neoplastic ascites may be also explored by US-E, allowing procedures like paracentesis. Other applications of US-E include transvaginal and transrectal US-E for female genitourinary or prostate cancers. On the other hand, the combination of US-E and endoscopy (US-endoscopy) allows to investigate organ layers and to perform biopsy of the pancreas, stomach, duodenum, and colon. The contrast-enhanced ultrasound (CEUS) employs microbubbles as contrast medium and may be useful to discriminate between benign regenerative nodules, hepatocellular carcinoma (HCC), and secondary lesions of the liver. The different vascularization patterns of these lesions, indeed, can be recognized by following the contrast medium during both arterial, portal, and venal phases.
- *Computed tomography (CT)*. CT is the main cross-sectional imaging technique used for the diagnosis and staging of cancer. CT scans allow the identification of both primary and secondary lesions and the evaluation of their size, the degree of infiltration of surrounding tissues, and the infiltration of vessels and adjacent organs. The use of intravenous, iodinated contrast medium may facilitate the recognition of tumor lesions and provides information on disease activity after the completion of a systemic medical treatment (Fig. 7.3). By using appropriate windows, CT scans may be used to identify lytic or blastic lesions of the bone or lesions of soft tissue.

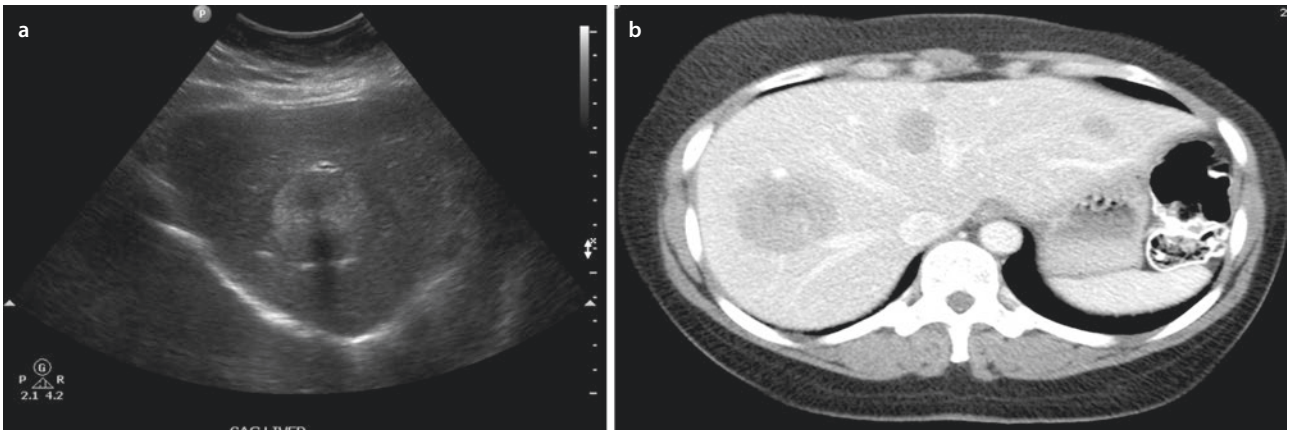

Fig. 7.3 Representative findings from US (**a**) and CT scans (**b**) in a patient with colon cancer metastatic to the liver

7

Table 7.2 Indications for nuclear medicine imaging in oncology

Radiopharmaceutical	Target	Indication
^{99m}Tc-labeled diphosphonates	Bone formation	Bone metastases
^{123}I-Metaiodobenzylguanidine	Norepinephrine transporter	Pheochromocytoma, paraganglioma, and neuroblastoma
^{111}In-DTPA-penteotride (Octreoscan)	Somatostatin receptor subtype 2, 5	Neuroendocrine tumors, medullary thyroid cancer, pheochromocytoma
^{131}I	Sodium-iodide symporter	Metastasis from thyroid cancer
^{99m}Tc-sulfur colloids	Sentinel node	Breast cancer and melanoma
^{99m}Tc-sestamibi	Mitochondrial activity	Breast cancer

Invasive procedures including fine-needle aspiration or fine-needle biopsy may be also carried out under CT scan guidance.

- *Magnetic resonance imaging (MRI)*. MRI has higher anatomical discrimination as compared with CT scan and is particularly useful to characterize the extent of tumors with respect to nearby tissues. MRI has a privileged role in the study of central nervous system tumors, head and neck tumors, and pelvic tumors including prostate, bladder, cervical, and rectal cancers and is of primary importance for the detection of primary and secondary tumors of the skeleton. Magnetic resonance spectroscopy (MRS) is a noninvasive, radiation-free analytical technique that has recently shown promise for the diagnosis of glioblastoma. MRS is able to detect different resonance frequencies from tissues with different biochemical composition and is therefore able to discriminate between tumor lesions and surrounding tissue. MRS with choline and hydrogen are used to differentiate primary from secondary lesions of the CNS.

(vi) *Functional imaging*. Functional imaging employs radiopharmaceuticals to detect biologic processes commonly associated with cancer proliferation, such as glucose overconsumption, iodine uptake, or somatostatin receptor overexpression. The most relevant applications of nuclear medicine to oncology include scintigraphy and positron emission tomography (PET).

Scintigraphy. Scintigraphy is a whole-body scanning technique where gamma radiations emitted by radiopharmaceuticals are captured by external detectors (gamma cameras) to form two-dimensional images. As summarized in Table 7.2, the most established clinical applications of scintigraphy include detection of bone metastases, identification of metastases from thyroid cancer, and diagnosis of neuroendocrine tumors (NETs). Scintigraphic techniques are not only useful for the diagnosis of cancer but can also provide prognostic and predictive information. For example, the

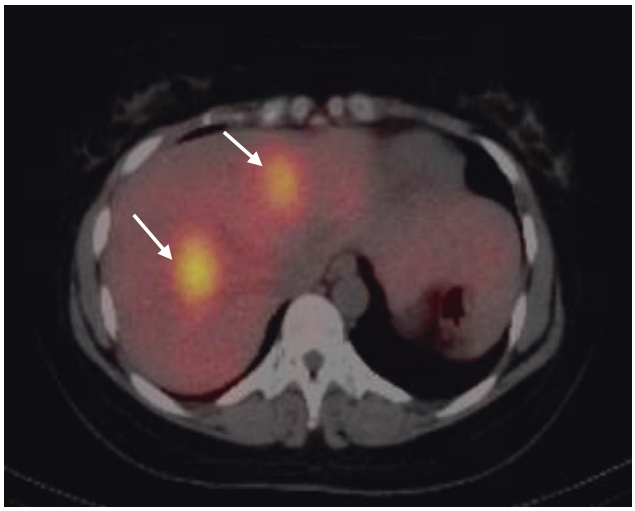

Fig. 7.4 Representative fusion image (SPECT/CT) obtained with Octreoscan in a patient with small bowel NET and liver metastases (white arrows)

^{111}In-DTPA-penteotride scintigraphy (also called Octreoscan), which is largely used for the diagnosis and staging of NETs, has demonstrated both prognostic capability (aggressive NETs are usually somatostatin receptor-negative) and predictive value (NETs overexpressing somatostatin receptors respond better to treatment with radiolabeled somatostatin analogs) (Fig. 7.4). The evolution of planar scintigraphy is represented by SPECT/CT, the combination of tomographic acquisition of single-photon emission with CT scan. SPECT/CT permits more accurate differentiation between areas of pathologic and physiologic uptake and provides better anatomical definition through the generation of hybrid images.

PET. PET is a diagnostic investigation based on the administration of a radioisotope tracer with a short half-life and can be combined with CT scan (PET/CT). The radiopharmaceutical most widely used for PET imaging is ^{18}F-fluorodeoxyglucose (^{18}F-FDG), which allows assessment of glucose transport and glycolysis within tumor cells. Given the increased glucose metabolism of cancer cells as compared with their normal counterparts, the quantification of ^{18}F-FDG uptake by PET may (i) enable the distinction between benign and malignant lesions, (ii) aid in determining the degree of tumor proliferation, and (iii) provide information on the metabolic response to treatments. Quantification of ^{18}F-FDG uptake is commonly expressed as standardized uptake value (SUV), namely, the ratio between the tumor radiopharmaceutical concentration (Bq/ml) and the injected activity (Bq) as multiplied for the body weight (g). The sensitivity of ^{18}F-FDG PET/CT varies by cancer type and histology: while certain neoplasms including lung, breast, colorectal, and head and neck tumors may be effectively imaged by PET/CT, others such as gastric or central nervous system cancers are characterized by low detection rates when using this technique. PET/CT may be especially relevant in the diagnostic work-up of patients with cancer of unknown primary. Major causes of false-positive results at PET/CT imaging are represented by infections or inflammatory processes, whereas common determinants of false negatives are small dimensions of the lesion (<1 cm) and low intratumor glucose metabolism. A number of radiotracers have been developed for PET imaging, and their clinical applications are summarized in Table 7.3.

When used for treatment response monitoring, PET/CT should be executed at least 6 weeks after surgery, radiotherapy, or chemotherapy in order to minimize the risk of false positives (as a consequence of inflammation) or false negatives (as a consequence of an early reduction in tumor metabolic activity). The Positron Emission Tomography Response Criteria in Solid Tumor (PERCIST) criteria [7] have been created (Table 7.4) to formally assess the metabolic response by PET/CT, but their use in both clinical trials and clinical practice is limited.

(vii) *Endoscopy*. Endoscopic procedures allow the direct visualization of anatomical cavities including the gastrointestinal tract and the bronchopulmonary tree. Although invasive, such procedures may be critical for the precise definition of tumor location, extent, and characteristics and allow biopsy sampling. Endoscopy may be also used therapeutically for surgical removal or ablation of superficial tumors, and has a definite role in the management of bleeding lesions, particularly in the upper or lower gastrointestinal tract. When used in combination with US, endoscopy reveals extremely accurate in staging tumors based on their diffusion across organ layers.

7.3 Pathological Diagnosis of Cancer

Clinical pathology has a crucial role in the diagnosis of cancer. First, it allows the distinction between inflammatory and neoplastic lesions; second, it is able to clearly discriminate between benign and malignant tumors; third, it provides fundamental information about tumor cell morphology, allowing the determination of the grading, the distinction between different tumor entities (i.e., small cell lung cancer versus non-small cell lung cancer), and the accurate establishment of the tumor histotype (i.e., adenocarcinoma of the lung versus squamous cell carcinoma of the lung). In addi-

Table 7.3 Established and investigational radiotracers for PET imaging

Radiotracer	Target tumor	Clinical application
^{11}C-Choline	Prostate cancer	Posttreatment evaluation of residual disease Monitoring of therapy
^{18}F-Fluoride	Bone metastases	Diagnosis and staging
^{68}Ga-DOTATOC	Neuroendocrine tumors	Differential diagnosis Diagnosis and staging Monitoring of therapy
^{11}C-Methionine	CNS cancer	Diagnosis Monitoring of therapy
^{18}F-Fluoromisonidazole	Cervical cancer, head and neck carcinoma	Investigational
^{18}F-DOPA	Carcinoids, medullary thyroid carcinoma, neuroendocrine tumors, pheochromocytoma, melanoma, neuroblastoma	Investigational
^{18}F-FDTH	Prostate cancer	Investigational
^{18}F-Estradiol	Breast cancer	Investigational
^{124}I-NaI	Thyroid cancer	Investigational
^{124}I-cG250	Renal cell carcinoma	Investigational
^{124}I-A33	Colorectal cancer	Investigational
^{68}Ga trastuzumab	Breast cancer, prostate cancer	Investigational

Table 7.4 The PERCIST criteria evaluate tumor metabolic response by PET-CT

Response by PERCIST	Description
Complete metabolic response	Disappearance of all injuries with SUL[a] peak>2.5
Partial metabolic response	Reduction of the metabolic activity of at least 30% (mean of all measurable lesions) or 0.8 SUL unit
Metabolic progression	Increase of the metabolic activity of at least 30% (mean of all measurable lesions), absolute increment of at least 0.8 SUL unit, or measurable increase of the disease or development of new lesions
Stable disease	All conditions not previously included

[a]SUL peak (SUV corrected for lean mass measured on the five most significant lesions [maximum two for organ] of at least 2 cm)

tion, by exploiting immunohistochemistry (IHC), pathology can provide clinically relevant information on the tumor immunophenotype, thus potentially driving clinical decisions (see the section "IHC" below for further details). In specific tumor types (i.e., gastrointestinal stromal tumors), molecular pathology may be also required to obtain a full set of data needed for therapeutic choices.

Similar to the clinical strategy used in the diagnosis of cancer, the pathologic establishment of the nature of a tumor follows a definite stepwise approach including histopathologic examination, IHC, and molecular pathology (Fig. 7.5).

(i) *Histopathology and cytopathology*: The histopathologic examination of tissue biopsies or resection specimens is the cornerstone of cancer diagnosis. In fact, a tumor can be diagnosed exclusively on the basis of cell morphology and tissue architecture in up to 90% of cases. Although suboptimal as compared with histopathology, cytopathology may provide information relevant for cancer diagnosis. While characterized by simplicity in sampling, cytopathology is not useful for cancer staging and only seldom provides an amount of biologic material adequate for molecular investigations. One of the main applications of cytopathology is cancer screening or early cancer detection, particularly with respect to cervical cancer (Pap-test) and thyroid tumors (fine-needle aspiration of suspicious nodules). Disadvantages of cytopathology include the inability to differentiate in situ cancers from invasive tumors.

(ii) *IHC*. IHC aims at detecting tissue- or cell-specific antigens by using labeled antibodies that can be

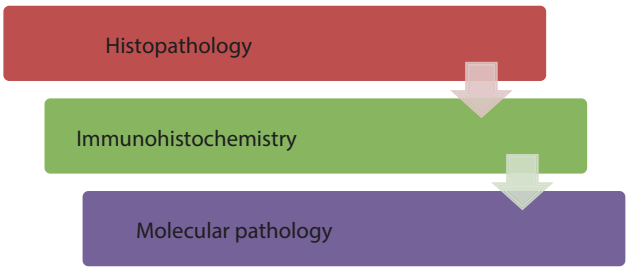

Fig. 7.5 Stepwise approach for the pathological diagnosis of cancer

visualized by light microscopy, thus allowing the recognition of the tumor cell lineage. Application of IHC is critical for the differential diagnosis among tumor entities with similar morphologic features. Among the most widely used targets employed for IHC assays, there are cytokeratins (whose positivity indicates an epithelial differentiation), CD45 (whose positivity supports a hematologic differentiation), and vimentin (whose positivity suggests a mesenchymal derivation). Further markers can be used to subclassify tumor entities; at this regard, for example, a positive staining for TTF-1 or CDX2 is indicative of bronchopulmonary or gastrointestinal tumor derivation, respectively. Apart from tumor diagnosis, IHC is also important for prognostic and/or predictive purposes. In fact, in the precision medicine era, treatment with targeted agents or with immunocheckpoint inhibitors in immunotherapy frequently relies on the expression of specific targets within the tumor. For example, detection of HER2 expression by IHC predicts response to trastuzumab or other anti-HER2 agents in both breast and gastric cancers [8]. Similarly, expression of PD-L1 in at least 50% of tumor cells predicts the therapeutic response to first-line pembrolizumab in patients with lung adenocarcinoma [9].

(iii) *Molecular pathology*. Molecular pathology examinations commonly used for the diagnosis of cancer can be subdivided into (i) assays performed on whole tissue slides (i.e., in situ hybridization) and (ii) assays performed on extracted nucleic acids (genetic sequencing, mRNA expression array). In situ hybridization is a technique based on the specific annealing of single-stranded, complementary, labeled DNA or RNA probes to localize a specific nucleotide sequence in a tumor tissue section. In fluorescence in situ hybridization (FISH), probes are labeled with a fluorochrome. This technique is widely used for the molecular characterization of several solid malignancies including lung cancer (detection of ALK translocation), breast cancer (assessment of HER2 presence in cases with indeterminate results by IHC), and Ewing sarcoma (evaluation of the *EWSR1* fusion gene). As a result of the advent of next-generation sequencing (NGS) technologies, the genetic fingerprinting of tumors has been increasingly used for clinical purposes, and specific mutations of oncogenes or tumor-suppressor genes are currently investigated to personalize cancer treatment. Tumor DNA sequencing by NGS is sensitive, reliable, and fast, requires small amounts of tissue, and provides information for both prognostic stratification and prediction to treatment response. For example, detection of KRAS mutations in colon cancer predicts lack of response to anti-EGFR mAbs, while evidence of EGFR mutations in lung cancer is predictive of response to agents targeting this receptor. RNA profiling is being increasingly used in selected clinical scenarios (i.e., MammaPrint or Oncotype DX for the molecular classification of breast cancer) and can have both prognostic and predictive relevance [10].

The pathology report must include data on both macroscopic and microscopic features of the neoplasm, detailing its diameters, intra- and extra-organ extension, as well as distance from the margins of resection. The involvement of resection margins is predictive of local relapse and has a negative impact on prognosis. The following terminologies are used to define the extent of postsurgical tumor tissue residue:

(a) R_0: absence of residual tumor
(b) R_1: presence of microscopic residual tumor (persistence of the neoplasia on a resection margin or its evidence at less than 1 mm from the margin)
(c) R_2: presence of macroscopic tumor residue

The number of excised lymph node should be always noted in the pathology report, since the ratio between metastatic lymph nodes and total lymph nodes examined can provide useful information in terms of prognosis, local relapse prediction, and overall quality of the surgery performed. The possible extension of the metastasis beyond the nodal capsule or the presence of lymph node micrometastases should be always reported.

The tumor grade represents the degree of differentiation of tumor cells and is a key factor for prognostic stratification and treatment tailoring. According to the World Health Organization (WHO) classification, tumors may be subdivided into G1 (well differentiated), G2 (moderately differentiated), and G3 (poorly differentiated). Vascular and perineural invasion, as well as the proliferative index assessed by either mitotic count or Ki-67 labeling index, is an additional prognostic factor that should be detailed in the pathology report, given their implications on therapeutic planning.

The evaluation of the so-called microsatellite instability by either IHC or molecular pathology has been recently reported to predict response to immunotherapy, and a progressive expansion of the indications to perform this test can be envisioned for the foreseeable future. Microsatellite instability is a condition of genetic hypermutability primarily resulting from defective DNA mismatch repair (MMR). Tumor cells bearing defective MMR enzymes are unable to repair DNA, thus presenting a higher mutational and neoantigen load. At present, the microsatellite status is routinely assessed in patients with colorectal cancers who may benefit from adjuvant chemotherapy. Patients with MSI-low colorectal cancer have a dismal survival as compared with those with MSI-high phenotype. Recently, the Food and Drug Administration (FDA) has approved immunotherapy in all cancers harboring a MSI-high status, thus broadening the indications to MSI testing in oncology [11, 12].

7.4 Clinical and Pathological Staging of Cancer

The clinical staging in oncology is the synthetic representation of the anatomical extension of the tumor and constitutes a system to accurately define patient prognosis. Several cancer staging systems have been developed worldwide, but the most widely used is the TNM system [2] that relies on the assumption that tumor cells from the primary site may spread to adjacent organs/structures and/or by lymphatic or blood vessels. As a result, the TNM staging system takes into account both location, extension, and size of the primary tumor (T), the involvement of the locoregional lymph nodes (N), and the presence of distant metastases (M). Numerous variants of the TNM staging system have been proposed for each tumor, and periodic modifications are proposed by the AJCC/UICC in response to newly acquired clinical data or improved understanding of cancer biology. Table 7.5 summarizes the most relevant modifications to the latest AJCC staging classifications. As a result, the majority of solid tumors can be classified by TNM, although alternative staging systems may be concurrently used for central nervous system cancers (WHO system), lymphomas (Ann Arbor classification), multiple myeloma (Durie-Salmon classification), and gynecological tumors (FIGO classification).

The TNM classification is primarily based on anatomical principles and evaluates three main parameters:

(T) - primary tumor

- T_x: tumor not evaluable.
- T_{is}: tumor in situ.
- T_0: no evidence of tumor.
- T_1-T_4: based on the size and extent of the tumor for solid organs (i.e., breast cancer or prostate cancer) and based on the degree of tumor pari-

Table 7.5 Modifications introduced in the VII–VIII AJCC staging system useful for the TNM classification of specific type of cancers

Tumor	Relevant modifications of VII and VIII AJCC classification
Esophagus	Now includes the tumors the gastroesophageal junction and the proximal 5 cm of the stomach Squamous and adenocarcinoma require separate clinical and pathological staging
Stomach	T_{2b} is reclassified as T_3 T_3 is reclassified as T_{4a} N category is revised in relation to the number of metastatic nodes in N_1 (1–2 metastatic lymph nodes) N_2 (3–6 metastatic lymph nodes) N_{3a} (7–15 metastatic lymph nodes) N_{3b} (≥16 metastatic lymph nodes) M_1 includes positive peritoneal cytology
Colorectal	N_{1a}: 1 metastatic lymph node N_{1b}: 2–3 metastatic lymph nodes N_{2a}: 4–6 metastatic lymph nodes N_{2b}: >7 metastatic lymph nodes Stage II is now classified as *a*, *b*, and *c* M_{1a}: single metastatic site M_{1b}: multiple metastatic sites
Liver	T_{3a} includes nodules ≤5 cm T_4 includes the involvement of the portal vein or intrahepatic veins
Head and neck	Separate staging for high-risk HPV-associated cancer of the oropharynx Addition of DOI (depth of invasion) in T category Addition of ENE (extra-nodal extension) to N category
Lung	T_{1-3} have been redefined and T_4 is now included
Breast	$M_{0(i+)}$ identifies cancer cells infiltrating the marrow or circulating tumor cells or cancer cells found in other organs and whose dimensions are less than 0.2 mm
Melanoma	T_{1a} includes Breslow <0.8 mm without ulceration T_{1b} includes any Breslow 0.8–1 mm or <0.8 with ulceration Mitotic count excluded by T1 category Microsatellites, satellites, or in-transit metastases are formally stratified by N category according to the number of tumor-involved lymph nodes Stage III_d is now included M_{1a-c} have been reclassified and M_{1d} added

etal invasion for hollow organs (i.e., colorectal cancer or bronchial cancer). In either cases, an appropriate definition of the "T" takes into account the involvement of surrounding organs.

(N) - Lymph nodes

- N_x: lymph nodes not evaluable.
- N_0: no evidence of metastatic lymph nodes.
- $N_{1–3}$: lymph node metastases with variable extension in terms of both location and number. For melanoma and breast cancer, the identification and removal of the sentinel lymph node is mandatorily required among the staging procedures.

(M) – Distant Metastases

- M_0: no evidence of distant metastases
- M_1: evidence of distant metastases

Based on the combination of T, N, and M, the TNM system stratifies patients in stages (stages I–IV). Stage I generally denotes cancers that are small and with negative lymph nodes. Stages II and III define cases with increasing tumor and/or nodal extent, while stage IV identifies patients with distant metastases. The TNM staging system has a prognostic validity and is key in the definition of the most appropriate treatment. For most tumors, the TNM staging can be attributed on the basis of imaging (clinical TNM, cTNM) or pathology (pathologic TNM, pTNM). However, in selected cases, the integration of other parameters (i.e., levels of soluble biomarkers for testis tumors) may be needed for stage grouping. In clinical practice, clinical and pathologic T, N, and M information are often combined to define a mixed-stage group (also named working stage). A typical example of such situation is represented by a patient who undergoes surgery only on his primary tumor and in whom the pT parameter must be combined with cN and cM status.

In patients not undergoing upfront surgical removal of the tumor (i.e., patients candidate to neoadjuvant therapy or with metastatic disease), a formal pTNM classification cannot be obtained. In these circumstances, the TNM staging will be calculated exclusively on the basis of bidimensional tumor diameters/parietal infiltration as well as presence/absence of lymph node or distant metastases, as assessed by imaging techniques. Any variations to the initial staging that occurred throughout the disease course as a result of presurgical systemic treatments should be indicated using the prefix "y" (yTNM). In patients receiving neoadjuvant therapy, the original cTNM stage should be used for surveillance or treatment definition instead of the yTNM stage. The autopsy TNM (aTNM) is used to stage cases of cancer identified only postmortem and has epidemiologic relevance.

When there are multiple simultaneous tumors of the same histology in one organ (i.e., multiple carcinoid tumors in the small bowel), the tumor with the highest T category is the one selected for classification and staging, and the multiplicity or the number of the tumors should be reported in parentheses, for example, pT2(m) or pT2(6). Metachronous primary cancers occurring in the same organ are staged as different cancer entities.

7.5 Cancer Restaging and Assessment of Response to Treatment

Historically, response to therapy for solid tumors was defined as a generic decrease in tumor size. The first international standardized criteria based on an objective tumor measurement were published by the WHO in 1979. In 2000, the initial WHO criteria were updated to produce the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines [13]. The main differences between WHO and RECIST criteria include (i) the one-dimensional (RECIST) or bidimensional (WHO) measurement of tumor lesions, (ii) the definition of progressive disease (PD), and (iii) the assessment of the tumor burden as the sum of the lesion diameters. Although the formulation of standardized criteria has allowed formal evaluation of anticancer agents in the context of clinical trials, the efficacy of the most innovative oncologic drugs cannot be accurately defined by WHO or RECIST criteria, and new systems of evaluation have been therefore proposed. Among these systems, there are the CHOI criteria that focus on tumor density modifications rather than on tumor size changes and the iRECIST criteria that have been implemented in clinical practice after the advent of immunotherapy. In fact, tumor regression is often negligible at the beginning of the treatment with immunotherapeutic agents, when a transient increase in cancer diameters may be paradoxically observed as result of tumor immune infiltration. ◘ Table 7.6 summarizes the main differences between WHO, RECIST, CHOI, and iRECIST criteria, while the PERCIST criteria are described in ◘ Table 7.4.

Traditional imaging modalities (CT, MRI) provide adequate information on the location and extension of cancer and are therefore widely used for staging and restaging purposes. Moreover, diffusion-weighted MRI and PET/CT may inform the clinician about biologic changes within the tumor (tumor necrosis, tumor cell density, tumor vascularization, and metabolism). However, cancer is a polyclonal disease subjected to branched evolution [14], and research efforts are currently undergoing to integrate both anatomical and molecular principles for the evaluation of treatment response in cancer patients. In this context, the enumer-

Table 7.6 Criteria for response evaluation in oncology

	WHO	CHOI	RECIST 1.1	iRECIST
Complete response (CR)	Disappearance of all lesions	No new lesions Disappearance of all lesions	Disappearance of all target lesions and pathologic lymph nodes (all nodal lesions have short axis <10 mm)	Disappearance of all lesions
Partial response (PR)	≥50% decrease in the sum of the area	No new lesions Decrease in size ≥10% or decrease in tumor attenuation (HU) ≥15% on CT	Decrease of ≥30% in the sum of diameters from baseline	Decrease of ≥30% in the sum of diameters from baseline
Progressive disease (PD)	>25% increase in the sum of the area	≥10% increase in tumor size or partial response criteria not met	≥20% increase in the sum of diameters, absolute increase of ≥5 mm	≥25% increase in tumor burden compared with nadir at any single time point in 2 consecutive observations at least 4 weeks apart
Stable disease (SD)	Above criteria not met	Above criteria not met	Above criteria not met	Above criteria not met

ation of CTCs or the identification of ctDNA, tumor-derived exosomes, or other soluble biomarkers before and after anticancer therapy represents a promising strategy for earlier detection of recurrence/progression or definition of molecular response [15].

7.6 Conclusions

Tumor prevention and early diagnosis of cancer represent at present the most effective way to limit the tremendous dead toll imposed by malignant diseases. Research is currently undergoing to discover novel soluble biomarkers useful for cancer diagnosis and follow-up, and innovative technologies are being exploited to improve our ability to detect tumors. In the future, a tight integration between next-generation sequencing technologies, -omic sciences, liquid biopsy, and high-resolution imaging modalities may ensure molecularly defined diagnoses of cancer that take into account not only the tumor extent but also its clonal heterogeneity.

Summary of Clinical Recommendations

- Laboratory tests and imaging procedures aimed at diagnosing cancer should be primarily oriented by patient history and physical examination.
- A pathological diagnosis of cancer is required before treatment initiation.
- Staging and restaging are fundamental processes aimed at stratifying patients based on their expected prognosis and assessing treatment response.
- Innovative diagnostic tools including the identification and characterization of CTCs, ctDNA, as well as blood multi-analyte assays carry the promise of revolutionizing the approach to cancer diagnosis and staging in the near future.

Key Points

- Tumor markers should not be used for cancer diagnosis.
- Functional imaging may provide useful information for selecting the most appropriate biopsy sites.
- Standardized criteria should be used for the staging and restaging of cancer lesions.

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Molecular Diagnostics: Innovative Technologies for Clinical and Translational Research

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Contents

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Learning Objectives

By the end of the chapter the reader will

- Have learned the basic concepts of the main molecular biology techniques
- Have reached in depth knowledge on sequencing (first generation and second generation), real-time PCR, and digital PCR

8.1 From Old to New: Where Did We Start?

8.1.1 First-Generation Sequencing

The identification of the precise order of nucleic acid residues within the DNA molecule represents the base of sequencing. Since the first natural polynucleotide sequence obtained more than 30 years ago, much work has been done, and nowadays, DNA sequencing has become a fundamental and integral part of a variety of research and clinical applications [1].

The first sequencing method was invented in 1977 by Maxam and Gilbert and was based on the selective chemical degradation of specific bases followed by the separation of the obtained fragment through electrophoresis in polyacrylamide gel (Fig. 8.1) [2]. Fragment detection was performed using radioactivity, and therefore, this method was quite laborious and very toxic. This technique should be considered the real first-generation sequencing since it was the first to be widely adopted.

Subsequently Sanger et al. experimented a new enzymatic method that takes advantage from the use of primers and chain terminators [3]. This new sequencing method quickly replaced the one of Maxam and Gilbert, because of its greater simplicity and reliability and the use of fewer toxic chemicals and lower amounts of radioactivity. As previously mentioned, Sanger sequencing is an enzymatic method that exploits the ability of a polymerase to synthesize a new strand using DNA as template starting from a primer sequence. Moreover, the sequencing reaction is supplied with modified and radiolabeled nucleotides, defined as dideoxynucleotides (ddNTPs, adenine, thymine, cytosine, and guanine) or chain terminators that by lacking the 3'OH cause termination of chain elongation (Fig. 8.2). The reactions were performed in four parallel tubes containing each individual ddNTPs, and, similarly to Maxam and Gilbert method, sequence identification was obtained by electrophoresis on polyacrylamide gel through autoradiography. Due to its increased robustness and easiness, Sanger sequencing quickly substituted the previous adopted method, and it became the most common technology used to sequence DNA for years to come. A number of improvements were made to Sanger sequencing in the following years; in the

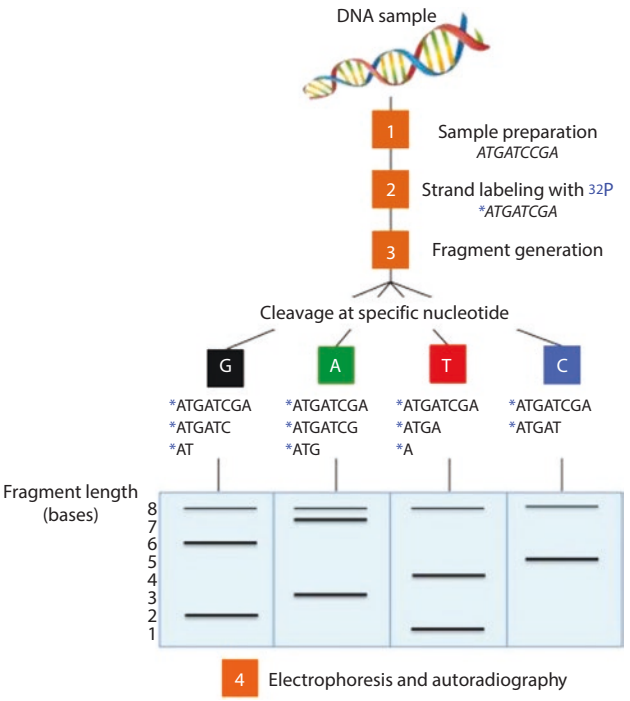

Fig. 8.1 Maxam-Gilbert sequencing method. (1) dsDNA is denatured to generate ssDNA (in italics the obtained sequence); (2) ssDNA is labeled with radioactive ^{32}P (the blue asterisk indicates the radioactive ^{32}P); (3) the labeled DNA is divided in four reactions, and it exposed to chemical substances to achieve DNA breakdown at specific nucleotide sites (G, G, and A, C and T, C; for simplicity in the figure are reported only cleavages at G, A, T, and C); (4) fragments derived from step 3 are loaded in four separated well on a polyacrylamide gel and are subjected to electrophoresis. During electrophoresis, fragments are separated according to their length; sequence detection is then performed through autoradiography. *Abbreviations*: dsDNA double-stranded DNA, ssDNA single-stranded DNA

latest chemistry, each ddNTPs is labeled with four different fluorophores that have the same or similar excitation wavelengths but different emission spectra allowing the reaction to occur in one tube instead of four.

The use of these ddNTPs eventually leads to the production of thousands DNA fragments that differ for only one nucleotide and are together labeled with a specific fluorophore (Fig. 8.3). The use of fluorescent detection technology, in combination with other advancements in chemistry and sequencing protocols, provides more accurate base calling, due to fewer "false terminations" at incorrect positions on the templates. The fragment separation and fluorescence detection are then obtained through capillary electrophoresis run on automated systems [4]. Nowadays there are several sequencers available that differ mainly in capillary number which influence sequencing throughput.

Nowadays, the latest Sanger sequencing method is widely used in clinical setting especially in the oncology field, where a personalized treatment is practically based

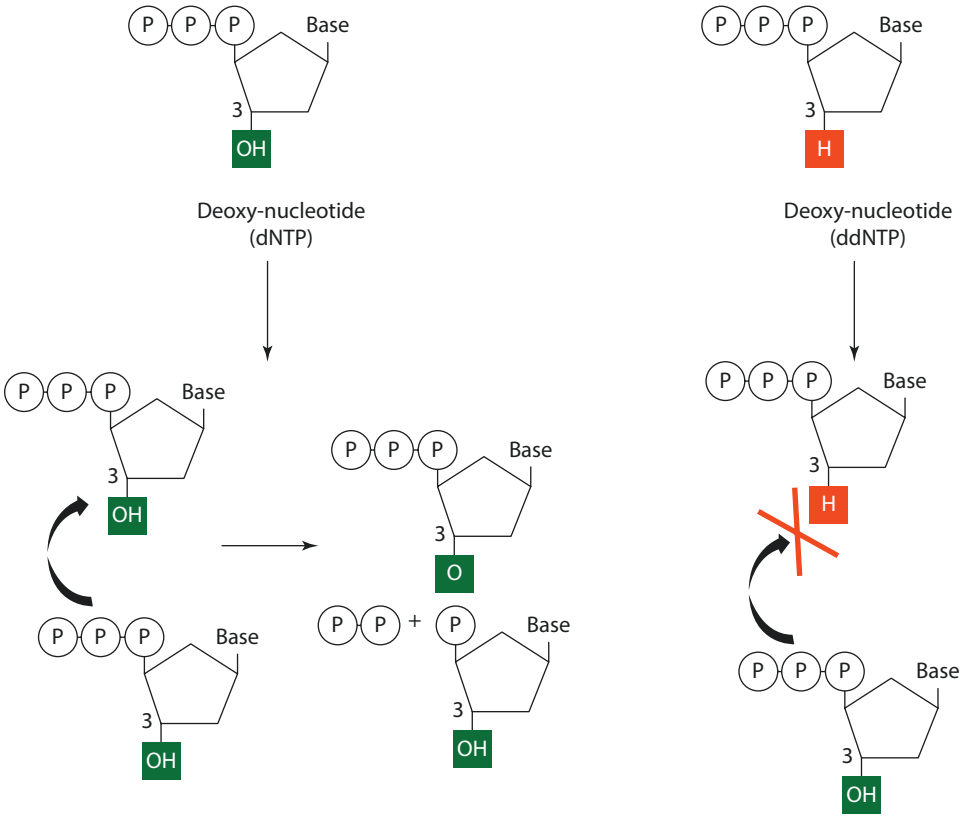

Fig. 8.2 In the upper panel are represented the structures of a dNTP (on the left) and a ddNTP or chain terminator (on the right). In the lower panel are shown two examples of polymerization. On the left is shown what happen when a dNTP is incorporated during polymerization; the 3'OH residue is available for strand elongation. On the right is shown what happens when a ddNTP is present; in this case, due to the lack of the 3'OH residue, the chain cannot be elongated. *Abbreviations*: dNTP deoxynucleotide, ddNTP dideoxy nucleotide

on the molecular characterization of the disease. Despite the so-called *first-generation sequencing* represents one of the greatest technological revolutions placed at patient's service, it has several limitations. Indeed, the sensitivity of the technique is limited (<20%) meaning that mutations present at a low allele frequency are underestimated. Moreover, even though the latest automated systems have strongly implemented processivity, it still remains a low-throughput method allowing the analysis of one sample and one gene at a time. For all these and many other reasons, in the last years, it has been developed the second-generation sequencing technology, which will be discussed in the following paragraph.

8.1.2 Real-Time PCR

Real-time quantitative PCR technique was first published by Higuchi R. and colleagues in 1993; they described a "*simple, quantitative assay for any amplifiable DNA sequence that uses a video camera to monitor multiple polymerase chain reactions (PCRs) simultaneously over the course of thermocycling*" [5]. Unlike Sanger sequencing where the detection occurs at the end of the whole process (end point), with this technique, it is possible to monitor in *real time* the number of products obtained during each PCR cycle. Therefore real-time PCR enables both quantitative and qualitative analysis, and, since its discovery, it has been used for several applications such as gene expression profiling from different biological sources (plasma, blood, cells, fresh, and paraffin embedded tissues), DNA copy number measurements, and allelic discrimination [6–8]. Real-time PCR has several advantages compared to Sanger sequencing: it is a much more sensitive technique; it is cost-effective, and it has faster turnaround time. Due to all these reasons, real-time PCR has revolutionized the field of molecular diagnostics, and the technique is now used in several clinical applications.

As previously mentioned, through real-time PCR, both quantitative analysis (absolute or relative quantification) and qualitative analysis (allelic discrimination to identify specific mutations) can be conducted. In order to perform all these functions, real-time PCR is based on two chemistries: fluorescent probes and double-stranded DNA intercalating agent SYBR® Green 1. There are different types of probe (hydrolysis probes, dual hybridization probes, molecular beacons, scorpion probes), but for simplicity, we will only describe the hydrolysis probe (e.g., TaqMan probe).

The thermostable *Thermus aquaticus* (Taq) DNA polymerase has a 5′ to 3′ exonuclease activity; it means that it can cleavage any oligonucleotide strand that it encounters during polymerization [9]. Holland and colleagues decided then to exploit this property to detect PCR product during amplification. Indeed the cleavages

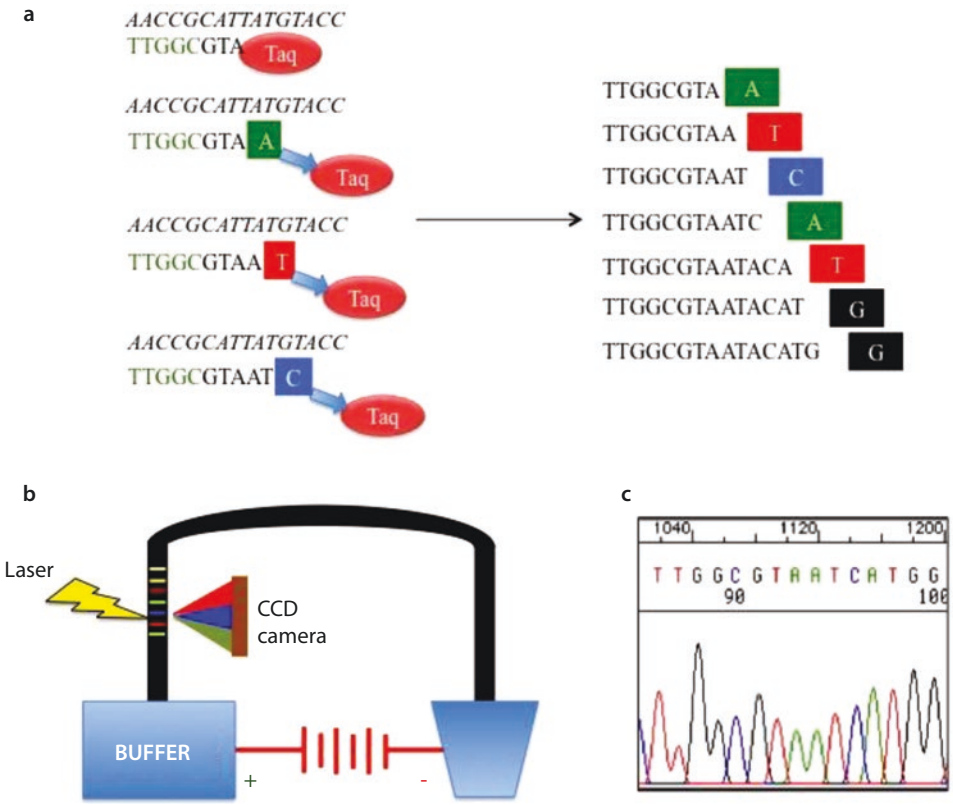

Fig. 8.3 Sanger sequencing. **a**. Sanger sequencing is an enzymatic method that exploits the ability of a polymerase to synthesize a new strand using DNA as template (in italics) starting from a primer sequence (in green). In Sanger sequencing, the reaction is supplied with chain terminators (Fig. 8.2). The incorporation of a ddNTPs eventually leads to the production of thousands DNA fragments that differ for only one nucleotide and are also labeled with a specific fluorophore (right side of panel a). **b**. Example of an automated sequencer. DNA fragments generated during sequencing reaction are subjected to capillary electrophoresis; DNA fragments are negatively charged and, when exposed to an electric field, migrate from negative to positive pole. Capillary is filled with a polymer that generates a molecular sieve enabling fragments separation. Migration speed inside the capillary is inversely correlated with fragment length; at the end of the capillary, it is settled a laser, which excites labeled ddNTPs that mark each DNA fragment. A charge-coupled device (CCD) camera collects fluorescence data; computer software then transforms the data onto an electropherogram that represent the exact nucleotide sequence **c**

of a target probe, specifically designed to hybridize within a target sequence, during PCR by the 5′-nuclease activity of Taq polymerase can be used to detect amplification of the target-specific product [9]. Similarly to the earliest sequencing chemistry, also in real-time PCR, the first probe was labeled with radioactive ^{32}P at 5′. Moreover the 3′ end of the probe was made non-extendable so that it could not function as a primer. During the course of amplification, whenever a complementary sequence is present, the probe anneals to the target region generating a substrate that is suitable for the Taq exonuclease activity. Therefore, during polymerization (when the enzyme extended from an upstream primer into the region of the probe), Taq hydrolyzes the probe; it implies that probe degradation occurs exclusively when it is annealed to the target that is being amplified during PCR. The detection was then performed using thin-layer chromatography to separate cleavage fragments from intact probe.

Subsequently the discovery of dual-labeled oligonucleotide fluorogenic probes extremely simplified the workflow, eliminating the post-PCR processing for probe degradation detection [10]. In detail the dual-labeled probes have a *reporter* fluorescent dye at the 5′ and a *quencher* dye attached at the 3′ end; when the probe is intact, no fluorescence can be detected because the 3′ dye is able to quench the fluorescence emitted by the reported dye. Consequently, only when the probe is ligated to a target sequence and the polymerase activity degrades it, the reporter emits fluorescence that can be detected. This phenomenon is called FRET (fluorescence resonance energy transfer) [11], and on this principle are based one of the most commonly used fluorogenic probes, the so-called TaqMan® probes (Fig. 8.4a).

As previously mentioned, a TaqMan® assay contain a fluorogenic non-extendable probe that has a reporter (5′ end) and a quencher (3′ end) and a primer set (forward

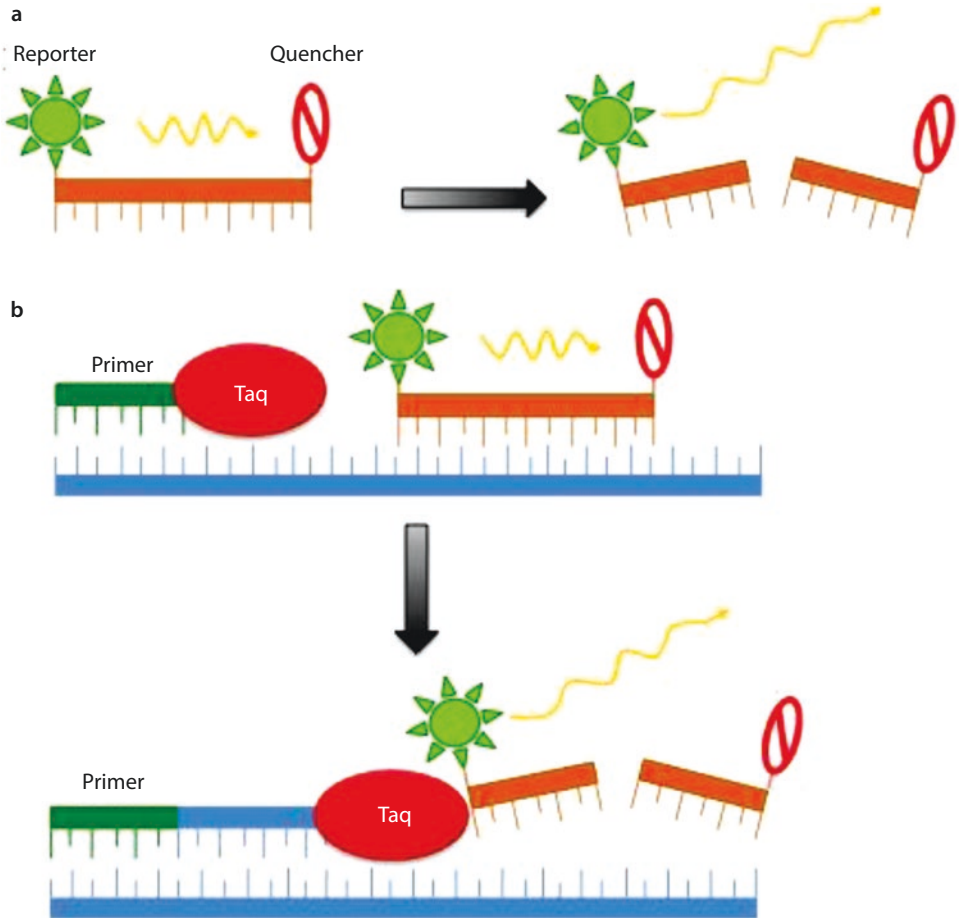

Fig. 8.4 **a**. Hydrolysis probe *(i.e., TaqMan probe)*. The hydrolysis probe has a *reporter* fluorescent dye at the 5′ and a *quencher* dye attached at the 3′ end; when the probe is intact, no fluorescence can be detected because the 3′ dye is able to quench the fluorescence emitted by the reported dye. When the probe is degraded by polymerase exonuclease activity, the quencher is removed from the reporter that can now emit fluorescence. This phenomenon is called FRET (fluorescence resonance energy transfer). **b**. TaqMan® assay contains a fluorogenic non-extendable probe and a primer set (forward and reverse primer). The probe anneals within a specific target sequence; the primer recognizes an upstream region regarding to the region where the probe binds. The TaqMan probe anneals downstream from one of the primer sites and is hydrolyzed by the exonuclease activity of the Taq polymerase. Every time the probe anneals to the target and it is cleaved, there is an increase in fluorescence, which is directly proportional to the amount of target present in the original sample

and reverse primer). The probe is designed to anneal with a specific target region, whereas the primer set recognizes an upstream and a downstream region regarding to the region where the probe binds. If the target sequence is present, the TaqMan probe anneals downstream from one of the primer sites and is hydrolyzed by the exonuclease activity of the Taq polymerase. Every time the probe anneals to the target and it is cleaved, there is an increase in fluorescence, which is directly proportional to the amount of target present (Fig. 8.4b).

Contrarily to the probe-based chemistry, SYBR Green is a nonsequence-specific fluorogenic agent that binds the minor groove of double-stranded DNA (dsDNA) but does not intercalate to single-stranded DNA. When SYBR Green is unbound in solution, it emits little fluorescence, whereas when it intercalates to dsDNA, it emits a strong fluorescent signal [12]. Consequently, during polymerization, there is an increase in fluorescence emission, but it decreases when the DNA is denatured; therefore fluorescence measurement is performed after every elongation step (Fig. 8.5a).

This allows the monitoring, at each cycle, of the precise amount of amplified DNA. The main advantage in the use of this chemistry is the low cost of the reagents and the easy workflow; nevertheless, one main drawback is related to its nonspecific nature. Indeed, any nonspecific dsDNA present in the reaction mix could generate fluorescence. Therefore, in order to increase the specificity of the reaction, it is advisable to perform a melting curve that is generated by plotting fluorescence as a function of temperature [13]. Each target amplification will be represented by a specific melting peak that allows to distinguish fluorescent emitted by real amplification from artifacts (Fig. 8.5b).

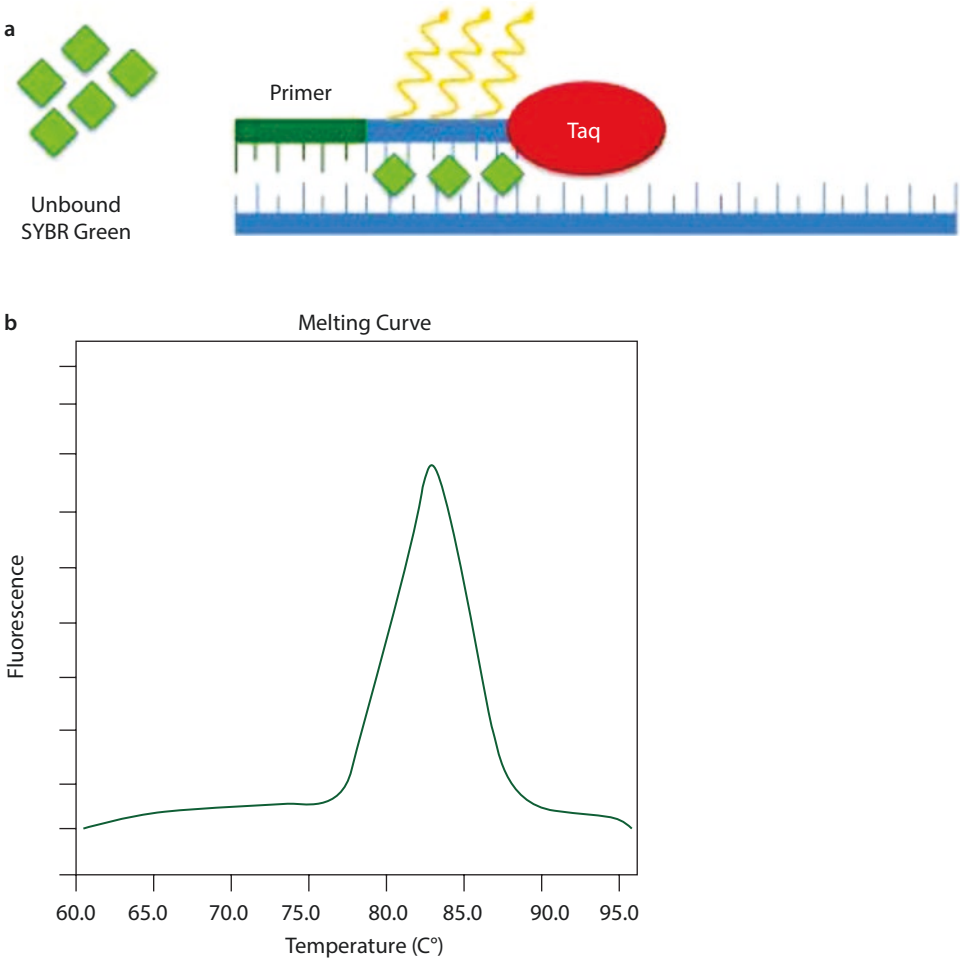

Fig. 8.5 **a**. SYBR Green chemistry. SYBR Green is a nonsequence-specific fluorogenic agent that binds the minor groove of dsDNA. When SYBR Green is unbound in solution, it emits very low fluorescence. During polymerization, SYBR Green intercalates to dsDNA and emits a strong fluorescent signal. **b**. Melting curve is generated by plotting fluorescence as a function of temperature, and it indicates the presence of nonspecific amplification. A characteristic melting peak at the melting temperature (Tm) of the desired amplicon will distinguish it from amplification artifacts that melt at lower temperatures at broader peaks

8

Independently from the use of the above-described chemistries, fluorescence is detected by a modified thermalcycler that performs amplification steps and detection at one time. After performing a real-time PCR analysis, the computer software generates an amplification plot using the fluorescence emission data that have been collected during PCR amplification (Fig. 8.6).

In Fig. 8.6 is reported a typical amplification plot with some important terms to be discussed:

- *Baseline*: the PCR cycles at which a fluorescent signal is accumulating but is beneath the limits of detection of the instrument. Normally it is set from cycles 3 to 15.
- *ΔRn*: is the difference between the fluorescence emission at each time point and the fluorescence emission of the baseline [14].
- *Threshold*: it is arbitrary chosen by the computer software, based on the variability of the baseline. Every fluorescence signal below the threshold is considered not statistically significant, whereas a signal that exceed the threshold is considered relevant, and it is used to identify the threshold cycle (C_t) for a sample.

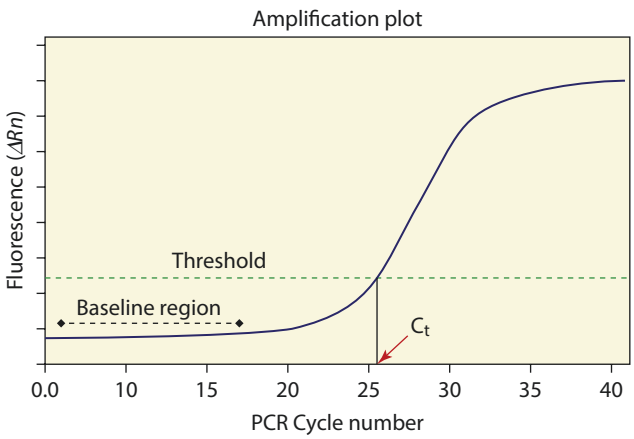

Fig. 8.6 Example of a single amplification plot showing the nomenclature commonly used in real time PCR

- C_t: it identifies the PCR cycle number at which the detected fluorescence is above the threshold [15]. The C_t is inversely correlated with the amount of the target DNA; the presence of more target in the reaction at the start of the PCR leads to a fewer number of cycles at which the fluorescence exceeds the thresh-

old. The C_t value is a basic principle of real-time PCR, and it is essential to obtain quantitative information.

Quantification in real-time PCR can be achieved using two different approaches: absolute and relative quantification. In order to obtain an absolute quantification, it is always requested to use a standard curve, by amplifying the standards (samples for which the amount of target is already known). The resulting standard curve is generated by plotting C_t as a function of the log of standard concentration. Therefore an "unknown" sample can be quantified by measuring its C_t and plotting it on the standard curve, as shown in Fig. 8.7a.

The relative quantification method does not need the construction of a standard curve, and it relies on the application of mathematical equations that are used to calculate the level of a target relative to a reference control or calibrator. The relative quantification is intended for the comparison of two conditions, for instance, it can be used when you want to investigate whether a specific mRNA is deregulated in cancer cells compared to normal cells. In this type of analysis, the difference between emitted fluorescent value of target gene and emitted fluorescent value of endogenous housekeeping gene = ΔCt(sample) is normalized in respect to a calibrator ΔCt(calibrator) to obtain target gene amount ΔΔCt = ΔCt(sample) – ΔCt(calibrator) (Fig. 8.7b).

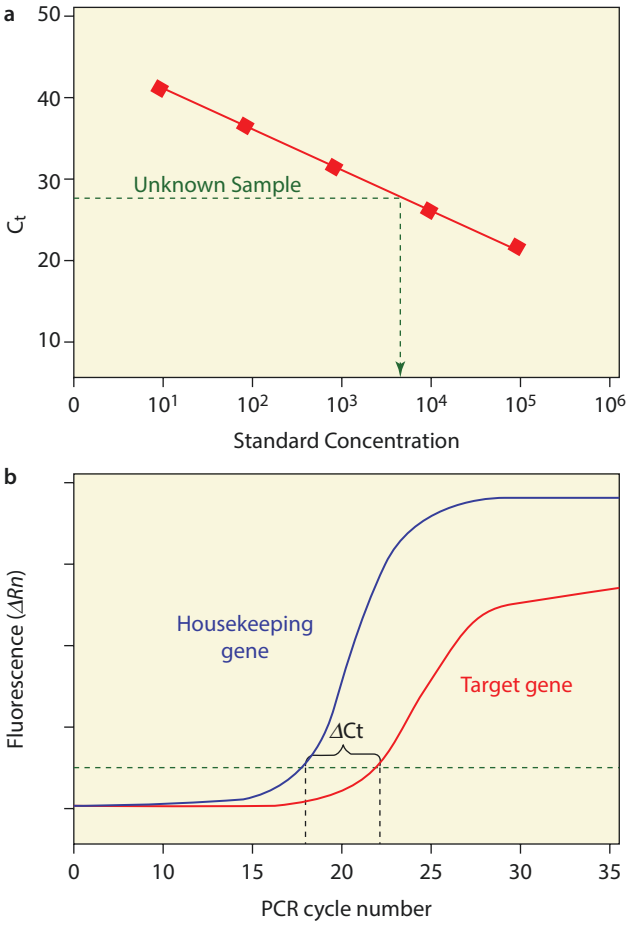

Fig. 8.7 **a**. Standard curve**.** In order to obtain an absolute quantification, it is always requested to use a standard curve, by amplifying standards (samples for which the amount of target is already known). The resulting standard curve is generated by plotting C_t as a function of standard concentration. An "unknown" sample can be quantified by measuring its C_t and plotting it on the standard curve. **b**. Example of a relative quantification. The curves (blue and red) represent housekeeping gene and target gene, ΔCt is the Ct of the target gene subtracted from the Ct of the housekeeping gene

8.1.3 In Situ Hybridization (ISH) and Fluorescent In Situ Hybridization (FISH)

In situ hybridization (ISH) represents a technique that allow the analysis of DNA structure without damaging cellular morphology or DNA integrity [16]. Initially it worked using radioactive oligonucleotide-labeled probes to examine specific genomic sequence in bacteriological studies and in leukemia. These probes were complementary to a specific genomic region, and the substitution of radioactive-labeled oligonucleotide probes with fluorescent-labeled oligonucleotide probes increased diagnostic power of this technique. Fluorescent dye can be linked to probe in two different methods: a single fluorescent dye can be directly bound to oligonucleotide backbone probe or two or more fluorescent dye can be bound to an amino linker at 5′-end of the probe, the signal was detected by fluorescence microscopy. Fluorescent in situ hybridization (FISH), a variant of ISH, was based on a hybridization process between a fluorescent marker and a complementary DNA/RNA sequence. For this reason, it was safer, increased the sensitivity of the method, and reduced the number of steps necessary to complete the analysis and currently represents an important tool adopted both in diagnostic and research field [17].

Biomarkers are molecules that play a key role for survival, growth, proliferation, or metastases of malignant cells overexpressed by several tumor types [18]. New therapeutic approaches involved different synthetic agents such as tyrosine kinase inhibitors (TKI) and antibodies able to reduce tumor growth inhibiting specifically one or few metabolic pathways [19]. Clinical benefit depends to the presence/absence of specific molecular features of gene sequence. In non-small cell lung cancer (NSCLC), epidermal grow factor receptor (EGFR) represents the primary biomarker on which

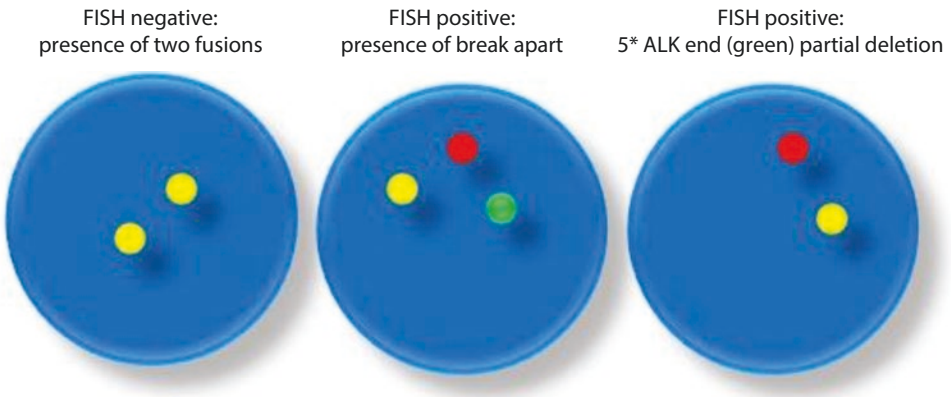

Fig. 8.8 In the figure are schematically represented an example of FISH assay for ALK translocation assessment. In particular, a not – rearranged ALK gene (FISH negative – yellow), rearranged ALK gene (FISH positive, split signal red and green probes), and a partial deletion of 5′ end of ALK gene (green probe – FISH positive)

mutational status becomes relevant for target therapy with tyrosine kinase inhibitors (TKI) [20]. In a second moment, other two biomarkers were eligible for first-line therapy with TKI in NSCLC: ALK and ROS -1 genes. These two biomarkers were affected by several translocations that made receptors constitutively active [21]. IHC and FISH are considered as standard testing modalities to detect aberrant transcriptional fusion genes, the use of FISH is also limited by the availability of the probe (Fig. 8.8). Another important element is the size of genomic aberration that can be indicated as a limiting factor [22].

8.2 Innovative Technologies

8.2.1 Next-Generation Sequencing (NGS)

The first-generation sequencing was the main method adopted worldwide; until few years ago, with the technological advancement, also sequencing witnessed an upgrade that leads to the second-generation sequencing. Moreover, in the last years, there has been a consistent increase of biomarkers discovery that are nowadays fundamental for a proper treatment choice in some tumor types. For example, in metastatic colorectal cancer, the exclusive analysis of KRAS exon 2 is no longer sufficient; indeed it has been demonstrated that also mutations in exon 3 and 4 and moreover mutations in NRAS gene (exon 2,3,4) determine primary resistance to anti-EGFR treatment [23]. The extension of KRAS testing to all RAS mutations, in addition to EGFR gene analysis to research mutation in exons 18–19 and 20 that showed a positive predictive values, favored the implementation of multi-target testing methodologies [24].

The first technique that can be counted as second generation is the pyrosequencing. As well as Sanger sequencing, this new method relies on the use of polymerase to obtain DNA fragments from a template (SBS, sequencing by synthesis), but it differs in the detection phase. Indeed, pyrosequencing does not exploit labeled dNTPs (neither radio nor fluorophores); instead it is based on a new discovered luminescent method for measuring pyrophosphate release during chain elongation. During the process, pyrophosphate is used from the ATP sulfurylase enzyme to produce ATP that will eventually activate the luciferase thus producing light (Fig. 8.9).

Moreover the amount of produced light is proportional to the amount of pyrophosphate and consequently to the number of bases incorporated during the elongation phase [25]. In this case, dNTPs are sequentially supplied to the reaction, alternating washing steps between one nucleotide and the following one. This new sequencing technique has several advantages such as the use of natural nucleotides and the possibility to observe the elongation in real time, instead of requiring lengthy electrophoreses. Nevertheless, one of the main limitations of pyrosequencing is the reading of homopolymeric regions [26].

Subsequently the pyrosequencing technology was licensed to 454 Life Sciences, a biotechnology company founded by Jonathan Rothberg, where it evolved into the first major successful commercial next-generation sequencing (NGS) platform. The first NGS platform, purchased by Roche, was the 454 pyrosequencing, which was used until a few years ago when it was discontinued from the market. The major innovation brought by this new technology is the possibility to obtain a massive parallel sequencing of thousands DNA fragments in one run [27]. Briefly the DNA is first subjected to library preparation, in which target DNA is amplified; DNA libraries are then attached to beads that subsequently undergo to water-in-oil emulsion PCR (emPCR) [28]. After this peculiar PCR, beads are coated with clonal DNA population that are subsequently distributed over a picoliter reaction plate that fits one bead per well and subjected to pyrosequencing as previously described (Fig. 8.10).

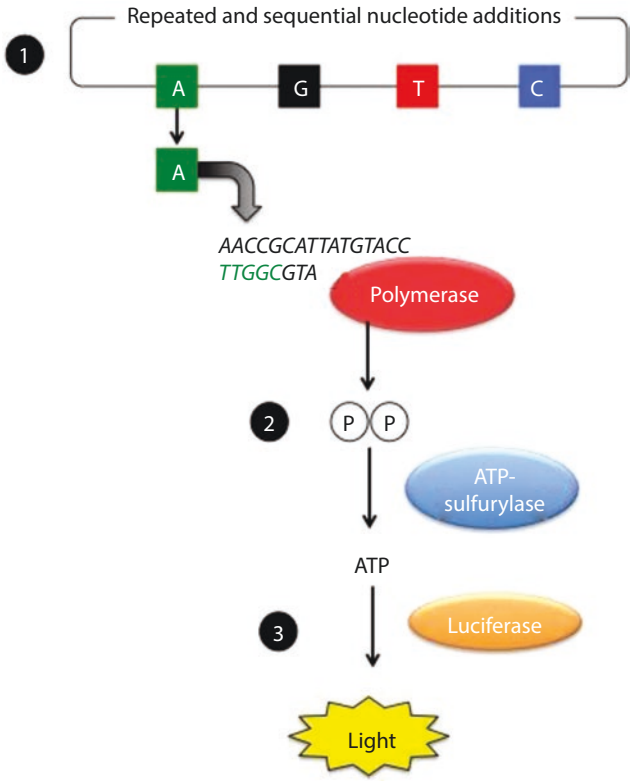

Fig. 8.9 Pyrosequencing. Natural, not fluorescent, nucleotides are sequentially added to the reaction (1), in the figure is shown the addition of ad adenine (A). When the polymerase adds a nucleotide to the elongating strand, a pyrophosphate is released (2); the pyrophosphate is used from the ATP-sulfurylase enzyme to produce ATP that will eventually activate the luciferase (3) thus producing light. The amount of produced light is proportional to the amount of pyrophosphate and consequently to the number of the same base incorporated during the elongation phase

After leaving 454 Life Sciences, Jonathan Rothberg developed another NGS platform, the so-called Ion Torrent (purchased by ThermoFisher Scientific) whose technical principle is very similar to that of pyrosequencing. Instead of measuring the release of pyrophosphate, Ion Torrent technology is based on the measurement of H^+ ion during polymerization. Moreover, unlike other NGS systems, Ion Torrent technology does not use luminescence or fluorescence for nucleotide detection ("post-light sequencing" technology) [29]. Similarly to the 454 system, DNA libraries are clonally amplified on beads through emPCR. After a washing step, beads are distributed over a chip that, through the metal-oxide-semiconductor (CMOS) technology, is able to detect the pH changes that occur during nucleotide incorporation (Fig. 8.11). As well as 454 system, also for the Ion Torrent technology, the major limitation is the homopolymer interpretation [30].

Another NGS platform that has spread worldwide due to the solidity and robustness of its chemistry is the Illumina sequencing chemistry that is different from the two ones previously described, even though there are some similarities. Indeed, as well as 454 and Ion Torrent technologies, it is an SBS method, but similarly to Sanger sequencing, it is based on the use of fluorescent "reversible-terminator" dNTPs. Instead of emPCR, libraries are bound on a flowcell that is then subjected to clonal "bridge amplification" through a solid phase PCR (Fig. 8.12) [31, 32]. Sequencing is then achieved through reversible terminators dNTPs; briefly, when a reversible terminator is incorporated in the new strand chain, elongation is temporally inhibited since the fluorophore occupies the 3'OH position. At this point, the fluorophore is excited with an appropriate laser, and the emitted fluorescence is registered thus identifying the introduced base. Subsequently, to proceed the reaction, an enzyme cleaves the fluorophore thus making the 3'OH position available again for the following cycle [33].

The most popular NGS benchtop platforms available are Ion Torrent Personal Genome Machine (PGM; ThemoFisher,) and MiSeq (Illumina) [34]. The choice of an NGS system is strongly dependent from samples' features from a quantitative and a qualitative point of view. PGM system requires only a small amount of gDNA input (10 ng), while MiSeq platform was initially optimized for 50 ng of gDNA (now it requires a smaller amount of DNA, and specific kit can be used to work with FFPE samples). PGM is a more suitable platform to adopt in routine diagnostic; this system provides different panels in which primer pools are built to cover specific gene regions. The possibility to customize primer's pool panel is an important feature of PGM system [35].

Independently from the use of one NGS system or the other, data analysis are remitted to bioinformatics tools enabled to analyze and call simultaneous detection variants in multiple genes covered by the panel. Each variant called by the software overcomes quality set up by operator, as Q30, median read length, uniformity, and coverage. Coverage plays a key role in variant calling; it indicates the number of "reads" produced during the clonal amplification, which covers a specific gene region. NGS approach allows to simultaneously analyze more genes for more patients; indeed during the step of gene selection, DNA from each patient is associated to a "barcode," a univocal molecular sequence that will mark each fragment of a single patient, and it will be identified by the instrument. Recent studies showed that cell-free-tumor DNA (cftDNA) might be indicated as a biological specimen on which analyze predictive biomarkers [36]. NGS may contribute to adopt this biologi-

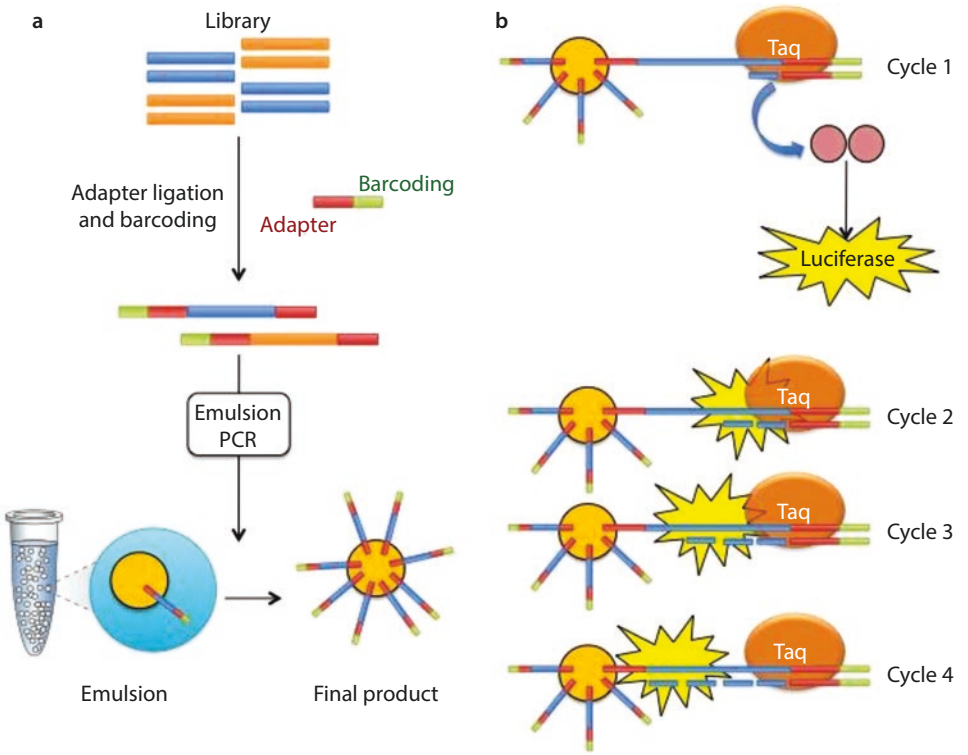

Fig. 8.10 454 pyrosequencing. **a**. Target DNA is subjected to library preparation; during this phase, DNA is amplified, and it also undergoes to adapter ligation that enable the subsequent attachment of the library to the beads. Moreover, in this step, each patient is associated with a barcode, a univocal molecular sequence that will mark each fragment of a single patient, and it will be identified by the instrument. Libraries are then attached to beads that subsequently undergo to water-in-oil emulsion PCR. After this peculiar PCR, beads are coated with clonal DNA population that are subsequently distributed over a picoliter reaction plate that fits one bead per well and subjected to pyrosequencing. **b**. During the first sequencing cycle, a single nucleotide species is added to the plate, and each complementary base is incorporated into the elongating DNA strand. The product of this reaction is the pyrophosphate that is then transformed onto ATP by the ATP sulfurylase (refer to Fig. 8.9). ATP is used by the luciferase that emits light; each burst of light is therefore associated to the incorporation of a specific nucleotide and a CCD camera detects it

cal source to avoid a slice of patients not eligible to the test for the low quantity of DNA derived from the corresponding tissue [37].

8.2.2 Nanostring

In the new era of personalized therapy, the increasing number of biomarkers to test for a single patient affected by a specific tumor process represents a problem if biological material from which DNA may be extracted is limited. In non-small cell lung cancer (NSCLC) for patients with advanced stage disease, surgical treatment is not recommended, and biomarkers analysis is often performed on the only type of biological specimen available: the cytological sample [38]. Differently from histological samples, 30–40% of this biological source for acid nucleic extraction and purification is characterized by a small amount of DNA/RNA. It is recovered without relevant differences depending from set-up preparation of the sample (smears, liquid-based cytology, thin prep) to follow a sequential diagnostic algorithm, including real-time PCR assays for EGFR mutations, fluorescent in situ hybridization (FISH) or immunohistochemistry (IHC) for ALK and ROS1 fusion detection, and IHC for programmed cell death ligand 1 (PD-L1) expression [39]. All these problems defined the necessity to substitute sequential single gene mutational assays and adopt in diagnostic routine a multiplexing assay to overcome the limits of cytological specimens in predictive biomarkers diagnostic routine [40].

The nCounter system (NanoString Technologies) is a new fascinating multiplex digital color-coded barcode technology based on hybridization system which allows to simultaneously analyzing in a single-tube multiplexed a broad-spectrum of clinical relevant biomarkers on different biological specimens, across all levels of biological expression [41]. This approach provides a method for direct analysis of targets with fluorescent molecular barcoded probes without any addictive processes of reverse transcription and/or amplification; moreover it is available for a large spectrum of sample types (FFPE,

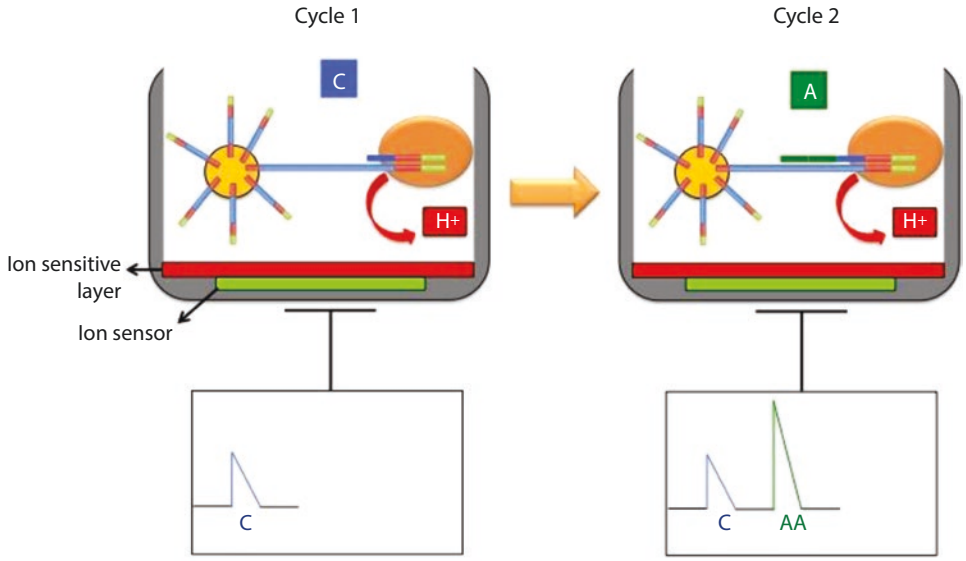

Fig. 8.11 Ion Torrent. In the Ion Torrent platform library preparation is identical to the one previously showed in Fig. 8.10a for 454 pyrosequencing. In this figure is represented a well of a typical chip, each well houses a bead coated with barcoded library. Instead of measuring the release of pyrophosphate, Ion Torrent technology is based on the measurement of H^+ ion during polymerization. As a base is incorporated, a single H^+ is released which is detected by the ion-sensitive layer and the ion sensor (represented in the figure with a red and a green bar respectively). Only one nucleotide is present during each cycle; when more than one identical nucleotide is incorporated, the sensors registered an increased H^+ release as showed on the right side of the figure. Unlike other NGS systems, Ion Torrent is a "post-light sequencing" technology since it does not use luminescence or fluorescence for nucleotide detection

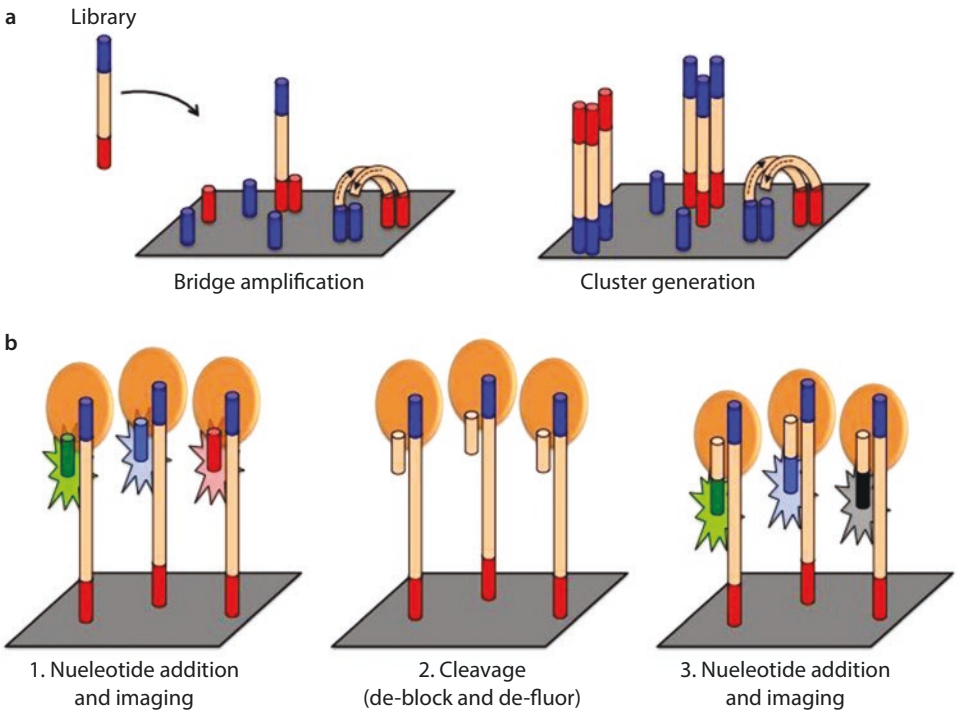

Fig. 8.12 Illumina technology. As well as 454 pyrosequencing and Ion Torrent, this is sequencing by synthesis technology, and it is based on the use of fluorescent "reversible-terminator" dNTPs. In the Illumina platform, libraries are bound to a flow cell (in gray) **a**. The flow cell undergoes to clonal bridge amplification; as showed in figure distal ends of hybridized library interact with nearby primers creating a "bridge." At the end of several rounds of amplification, millions of clusters are generated (right side of panel a.). Similarly, to Sanger sequencing, also here are used fluorescent "reversible terminators." The panel **b**. shows what happens during sequencing phase. When a reversible terminator is incorporated in the new strand, chain elongation is temporally inhibited. At this point, the fluorophore is excited with a laser, and the emitted fluorescence is registered thus identifying the introduced base (1). Subsequently, to proceed the reaction, an enzyme cleaves the fluorophore thus making the 3'OH position available again for the following cycle (2). The reaction can therefore continue with another phase of nucleotides addition and imaging (3)

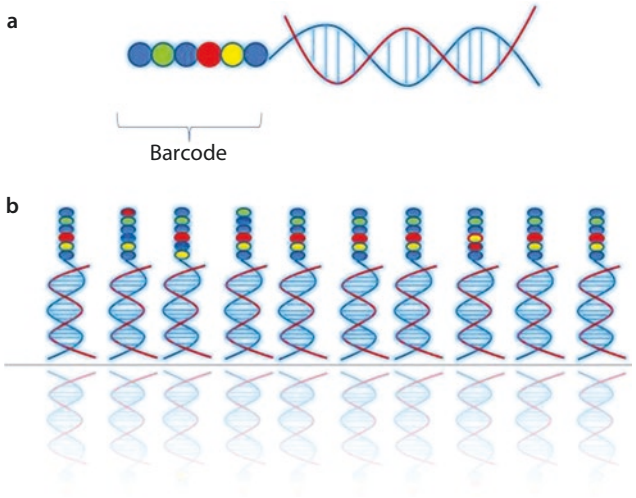

Fig. 8.13 In the figure are schematically represented a nanostring probe with specific barcode **a** and different probes with different barcode **b** in relation to specific target

cell cultures, smears) and for a large spectrum of biological sources (DNA, RNA) [42]. This platform works with few amounts of DNA/RNA concentration (15 ng/μl) and exploits different molecular barcodes and a single molecule imaging for aberrant transcripts, gene mutations, and copy number variation evaluation. In fact, each color-coded barcode is linked to a single specific target probe able to hybridize with a unique molecular target; a combination of different color coded-barcode is called multiplexed CodeSet (Fig. 8.13) [41].

The manufacturer's protocol is fast and simple; after mixed samples with capture and reporter and hybridized at 65 °C overnight, they are manually loaded in 12-plex cartridge and processed for digital data acquisition on the nCounter Sprint (NanoString). Data analysis is conducted by nSolver analysis software (V.3.00); each sample is evaluated for data containing imaging quality control (QC) metrics. Specifically, field of view (FOV) and binding density (BD) are assessed in any single case prior to gene expression data visualization. FOV indicates the discrete image units in which are divided each lane of the cartridge and eventually shows the presence of an optical issue (i.e., inability to focus). BD is a measure of the number of optical features per square micron useful for determining whether or not data collection has been compromised due to image saturation. Data interpretation is the most relevant and difficult aspect for this platform; operator should manually evaluate sample by sample for each QC to avoid the increasing number of the samples classified as "inadequate for the analysis" following default quality parameters.

8.2.3 Digital PCR

Digital PCR (dPCR) can be considered as an upgrade of the previously discussed real-time PCR technique. The term digital PCR was first used in 1999 by Vogelstein and Kinzler that described a new method able to quantify RAS mutation in a sample by partitioning the sample in hundreds microwell [43]. The development of this new technique arises from the necessity to identify mutations expected to be present at a very low frequency; this innovative approach is intended to transform the exponential, analog nature of the PCR into a linear, digital signal. In broad, real-time PCR can be used for both qualitative and quantitative purpose, either to study specific mutation of a target molecule or to determine the exact number of a target molecule. Nevertheless these results cannot be obtained at the same time, and, moreover, in real-time PCR, the absolute quantification of a target always requests a standard curve (as previously described) [44, 45]. Digital PCR overcomes this limitation; indeed in dPCR, the target is partitioned to the level of single molecules, facilitating the measurement of small percentage differences and quantification of rare variants without the aid of a standard curve. This implies that both qualitative and quantitative information can be obtained at one time for the same sample. dPCR may also be more reproducible and less susceptible to inhibition than real-time PCR, and therefore, it is a reliable tool that can be used also for diagnostic applications, as well as for research. Noteworthy dPCR is more sensible than real-time PCR, reaching a sensitivity limit of 0.1–0.01%.

The term "digital" is quite appropriate to describe the principal on which the technique is based, and we will explain why. To date there are mainly two types of dPCR: chip-based and droplet-based digital PCR. The main difference between these two technologies relies on how partitioning is achieved. In the chip-based system, the target DNA is partitioned in thousands of microwell spotted on a chip. Instead, in the droplet-based system, the DNA is subdivided in thousands of droplets obtained after an emulsion step. Therefore, there are several commercialized digital PCR platforms that mainly differ for the numbers of partitions generated, and noteworthy, this has an impact on platform sensibility. Droplet digital PCR (ddPCR) usually has approximately 20,000 partitioned droplets [46] and can have up to 10.000.000 per reaction [47], whereas the microfluidic chip-based dPCR can have up to several hundred partitions per panel. However, independently from the platform, the detection is based on fluorescence. In detail, the target DNA is first partitioned and subjected to amplification in the presence of a fluorescent probe that

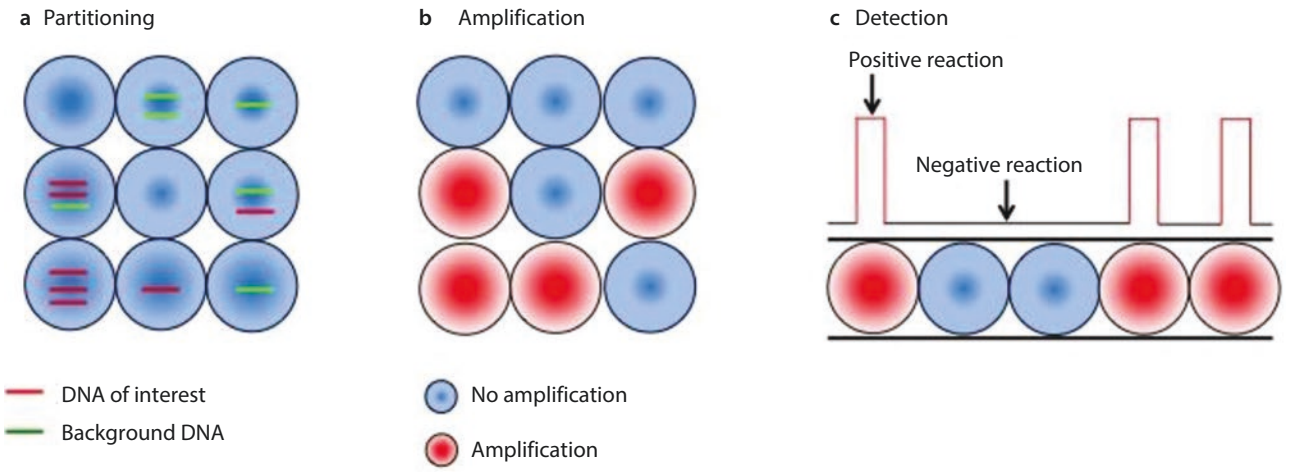

Fig. 8.14 **a**. The PCR mix reaction is partitioned in thousands of microwells or droplets. In the chip-based systems, the partition is achieved by physically distributing the reaction mix (composed by DNA, primer, probe, and polymerase) on a chip surface containing thousands of microwells; afterward the chip is subjected to several cycles of amplifications. In ddPCR system, the reaction mix is emulsified in a water-oil mixture creating the partitions; this emulsion is then amplified through an emPCR. Detection is similar for both systems; results are obtained by counting the number of *positive* (red) and *negative* (blue) reactions. A reaction is defined *positive* when the fluorescence of the specific probe is detected; accordingly a *negative* reaction is defined by the absence of the specific probe fluorescence emission. The exact number of the target molecule (expressed as DNA copy numbers per microliter) is calculated using the Poisson distribution

binds to a specific sequence. In the chip-based systems, the partition is achieved by physically distributing the reaction mix (composed by DNA, primer, probe, and polymerase) on a chip surface containing thousands of microwells; afterward the chip is subjected to several cycles of amplifications. In ddPCR system, the reaction mix is emulsified in a water-oil mixture creating the partitions; this emulsion is then amplified through an emPCR.

After the PCR amplification steps, the analysis is similar for both systems, and it relies on fluorescence detection. Indeed, results are obtained by counting the number of *positive* and *negative* partitions. A reaction (or partition) is defined *positive* when the fluorescence of the specific probe is detected; accordingly a *negative* reaction is defined by the absence of the specific probe fluorescence emission (Fig. 8.14).

Therefore, at the end, the analysis is based on a binary system where a positive reaction is "1" and a negative reaction is "0," exactly as what happens in a digital system [48]. The exact number of the target molecule (expressed as DNA copy numbers per microliter, T) is calculated using the Poisson distribution with the following equation:

$$T = \frac{-D}{Vp} \ln\left(1 - \frac{P}{N}\right)$$

where P indicates wells with positive amplified products, N represents number of total wells, Vp is the partition or droplet volume, and D is a dilution factor [49].

In clinical setting, dPCR represents a useful tool for cancer patient's management, and it is being applied for absolute allele quantification, rare mutation detection, analysis of copy number variations, DNA methylation, and gene rearrangements in different kinds of clinical samples and biological sources. Digital PCR is the answer to one main clinical need that is to have more sensitive technique to detect mutations at a very low frequency, as for the case of liquid biopsy. Indeed, to date, dPCR usefulness in cancer management is mostly focused on liquid biopsy, where sensitivity could make the difference. However, we still have to investigate whether a mutation detected at 0,01% frequency is clinically relevant and can guide medical decision.

8.3 Conclusion

In the era of precision medicine, tumor molecular profiling is central to guide a personalized treatment planning. Therefore, molecular biology techniques have become an integral and fundamental part of clinical oncology routine. Then it is important to comprehend and explore the basic molecular diagnostic technologies but also to shed light onto the most innovative technologies and the advantages that come from them. In the present chapter, we gave an overview of the main molecular biology techniques that are routinely used in clinical practice, starting from the beginning in 1977 when the first sequencing method was invented up to the most recent technological advancement such as NGS and ddPCR. Over the years, the techniques have been refined to meet the growing demand for molecular tests, trying to improve the performance and quality of the analyzes

provided. What we used to do in months or years, today can be done in weeks or even days providing a more accurate and timely response to clinical requests. Moreover, these new techniques provide an extraordinary tool for clinical and translational research in oncology. Thus, modern oncology is perfectly complemented by molecular biology; then it is important to earn a depth comprehension of the main techniques in order to understand its advantages but also its limits.

Key Points

- Given the recognized central role of tumor molecular profiling in guiding personalized treatment planning, molecular biology techniques have become an integral and fundamental part of clinical oncology routine;
- Starting from the first sequencing methods, the most recent technological advancements for application in molecular oncology are Next-Generation Sequencing and droplet digital PCR;
- Molecular biology approaches provide a feasible tool for clinical and translational research in oncology given their high sensibility and specificity;
- Nowadays, oncology cannot be anymore considered as a separate field; indeed it is perfectly complemented by molecular biology.

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Principles of Cancer Therapeutics

Contents

Chemotherapy

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Learning Objectives

By the end of the chapter the reader will:

- Be able to discuss the rational basis of cancer chemotherapy
- Have learned the basic concept of cancer cell kinetics
- Have reached knowledge on the main classes of anticancer drugs
- Have reached knowledge of mechanism of action of most anticancer drugs
- Have reached knowledge of the main toxicities of most anticancer drugs
- Have reached knowledge of the main clinical application of most anticancer drugs

9.1 Introduction

Antineoplastic medical therapy aims at the irreversible destruction of tumor cells. The term chemotherapy was initially used by Paul Ehrlich to indicate antibacterial therapy [1] and was later extended to antitumor therapy, although a substantial difference exists between the two. In fact, while antibacterial drugs only inhibit metabolic pathways specific for prokaryotic cells (i.e., bacteria), antitumor agents inhibit metabolic pathways of normal and neoplastic eukaryotic cells. It follows that antibacterial drugs are highly selective in their activity, being practically ineffective on host cells and, therefore, associated with a very low level of toxicity, while anticancer ones are less selective, being active on normal cells as well, and this may result in substantial toxicity. Selectivity and safety of a drug are conventionally expressed by the *therapeutic index* (TI), which is the ratio between the toxic and the therapeutically effective dose of a drug. Therefore, the higher the TI, the safer the drug. Antibacterial drugs are endowed with a high TI, because they are generally effective at a dose far below that responsible for toxicity. In contrast, anticancer drugs have a low TI.

Nevertheless, cancer is a life-threatening disease, and chemotherapy remains the most effective treatment for several types of neoplasms (Table 9.1). As a complement to other therapeutic strategy, chemotherapy can be used (i) as adjuvant therapy, when administered after a radical surgical resection or a definitive radiotherapy, in the absence of evident residual disease, and (ii) as neoadjuvant therapy, when used before surgery. As adjuvant treatment it is indicated in patients at high risk of relapse, e.g., breast cancer patients with axillary lymph node involvement, with the aim to eliminate potential micrometastases that may be present at the time of primary treatment. The goal of neoadjuvant chemotherapy is to reduce tumor burden to allow radical or more conservative surgery while eradicating micrometastases, as well.

Frequently, chemotherapy is administered to patients with advanced disease (metastatic cancer), when no alternative treatment is available. As shown in Table 9.1, in some advanced cancer, chemotherapy can be curative that is leading to definitive disappearance of tumor (testicular cancer, hematopoietic neoplasms); in other cases it is not able to cure, but it increases survival (breast, colon, rectum, ovary cancer).

Overall, the clinical benefits of chemotherapy overcome the possible side effects. This is the reason why oncologists use drugs with low TI to treat patients affected by cancer.

Table 9.1 Efficacy of chemotherapy in the treatment of cancer

Cancer treatment goal			
Cure			Increased survival
Neoadjuvant	Adjuvant	Metastatic	Metastatic disease
Breast cancer	Breast cancer	Acute leukemia	Breast cancer
Rectal cancer	Colorectal cancer	Lymphomas	Colorectal cancer
Anal cancer	NSCLC	Germ cell tumors	NSCLC
Esophageal cancer	Gastric cancer	Wilm's tumor	SCLC
Larynx cancer	Ovarian cancer	Ewing's tumor	Gastric cancer
Bladder cancer	Bladder cancer	Neuroblastoma	Ovarian cancer
Osteosarcoma	Osteosarcoma		Uterine cancer
Soft tissue sarcoma	Wilm's tumor		Pancreatic cancer

NSCLC non-small cell lung cancer, *SCLC* small cell lung cancer

9.2 Biological Basis of Cancer Chemotherapy

A complete knowledge of the biology of cell cycle is necessary to understand the principles of antiblastic therapy and the mechanisms of action of these drugs. The common terminology "antiproliferative drugs" used to indicate chemotherapeutic agents emphasizes the importance of the relationship between cell cycle and cytotoxic effect.

In order to replicate, cells go through a cycle composed of four distinct steps or phases of different durations (Fig. 9.1). The initial phase of the cycle, called G1, is characterized by cell growth and by the synthesis of proteins and enzymes necessary to produce new DNA, including those involved in the synthesis of purine and pyrimidine organic bases and their respective nucleotides. The next step is the synthetic phase, or S phase, in which DNA synthesis takes place with duplication of the genome. In G2 phase, cells further increase their size and the production of all cellular components required for a correct mitosis to occur. Mitosis is the last step of cell cycle, and it takes place in the so-called M phase. As showed in Fig. 9.1, after dividing, cells can follow different fates. In particular they can:

1. Turn back into G1 phase and continue the replicative process.

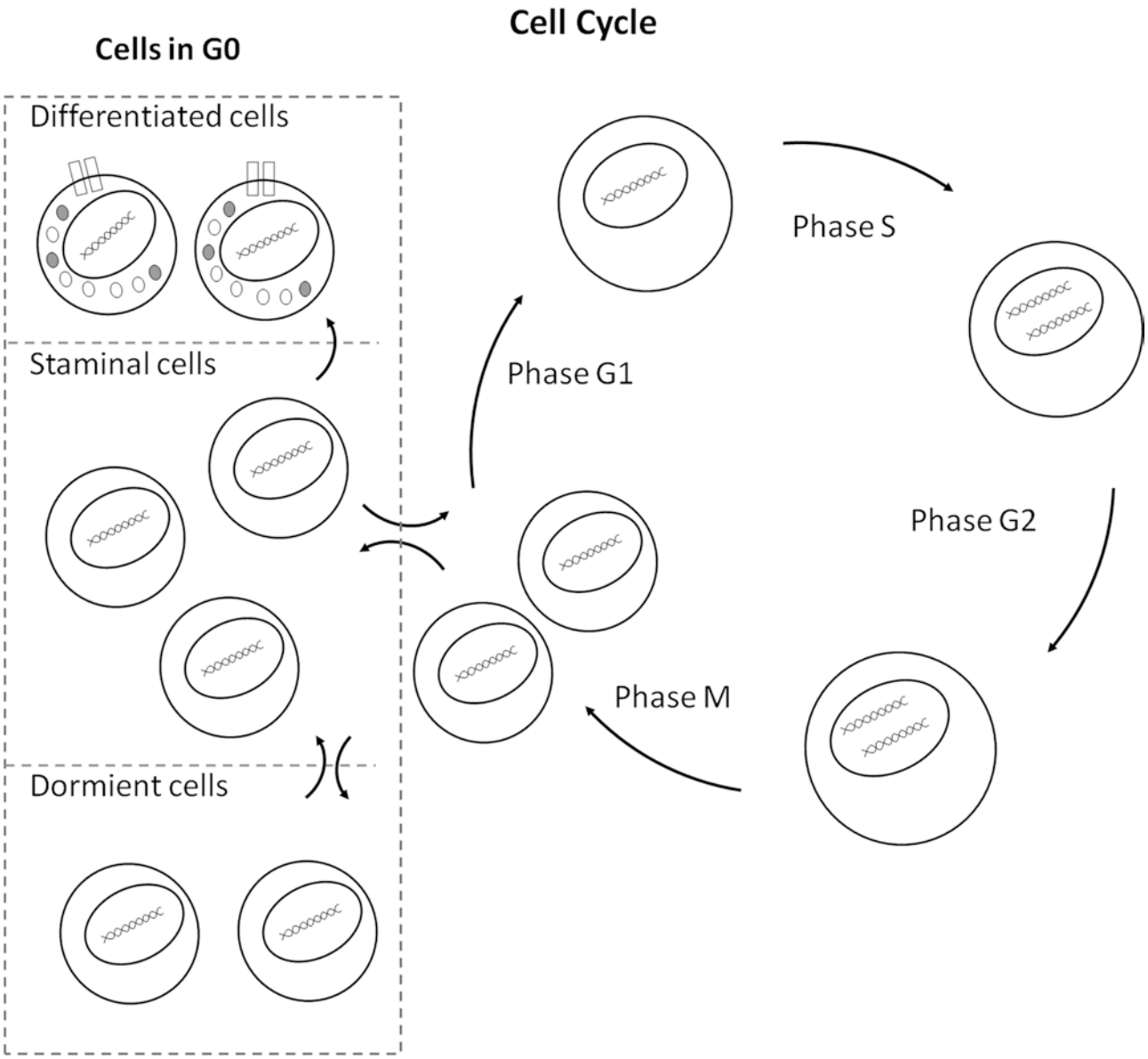

Fig. 9.1 Phases of the cell cycle

2. Get out of the loop and enter in a non-proliferative resting phase, called G0, in which they behave as staminal cells, i.e., they can (i) fully differentiate, that is, to acquire characteristics necessary to carry out the functions of the tissue they belong to, irreversibly losing their ability to replicate themselves, or (ii) stand in G0 and maintain proliferative capability as they can reenter G1 if needed; some of these cells can remain in G0 for very long time before replicating again, a phenomenon known as cell dormancy.

Therefore, based on proliferative capacity, it is possible to distinguish three cell compartments:
1. The *proliferative compartment*, or "growth fraction," which includes cells in active proliferation.
2. The *non-proliferative compartment*, represented by G0 cells that have differentiated.
3. The *staminal compartment* which is composed by G0 cells, able to reenter cell cycle thus expanding the proliferative compartments. These cells are usually resistant to the action of chemotherapy.

Classically, antiblastic drugs are divided into three main classes depending on their activity in relation to cell cycle:
(a) *Class I* or *cell cycle non-specific* drugs, i.e., agents that can kill a cell independently of the cell cycle phase. These drugs are also active on cells in G0 phase which are highly represented in most tumors at the time of diagnosis. Therefore, these drugs are extremely useful albeit, unfortunately, not numerous. Examples include some alkylating agents such as nitrosoureas.
(b) *Class II* or *cell cycle-specific drugs*, i.e., agents that are active on actively replicating cells (not in G0 phase). They can be divided in two groups:
    1. *Class IIa* or *cell cycle phase-specific* drugs, i.e., chemotherapeutic agents that kill cells at a specific phase of the cell cycle. Examples include antimetabolites and antimitotic agents that kill cells in phase S and M, respectively.
    2. *Class IIb* or *cell cycle phase-non-specific* drugs, i.e., agents active on proliferating cells, independently of the phase of cell cycle. Examples include alkylating agents such as cyclophosphamide and dacarbazine or platinum compounds such as cisplatin and carboplatin.

Some antiblastic drugs cannot be assigned exclusively to one class. For example, cisplatin, a cell cycle phase-non-specific agent, shows greater activity in S phase, although not so efficiently as an S phase-specific drug, and cyclophosphamide, another phase-non-specific agent, can kill cells even in G0 phase, but not with the efficiency it shows toward proliferating cells.

However, most antineoplastic drugs are cycle-specific and active only on proliferating cells. Therefore, the main target of chemotherapy is the proliferating compartment, but cells of the non-proliferative compartment, including differentiated cells, can also be damaged by chemotherapy.

According to what was said above, it is reasonable to expect that effectiveness of antiblastic agents is influenced by the relative abundance of the different cellular compartments.

Studies conducted in the late 1950s lend credence to the conception that a large proportion of tumor cells were included in the proliferating compartment. In the early 1960s, Skipper and Schabel, by using the murine leukemia L1210 model, showed that tumors grew exponentially [2]. On a semi-logarithmic scale chart, the kinetics of these tumors would be represented by a straight line (Fig. 9.2a). A similar kinetics implies that the tumor doubling time is constant and independent from the number of cells in the tumor. For example, if a tumor grows from 10^2 to 10^3 cells in 2 days, the same time will be required for a tumor of 10^{10} cells to reach 10^{11} cells. Obviously, this is possible only when all tumor cells are in the proliferating compartment, independently from the size of the tumor.

It was also demonstrated in animal models that when an exponentially growing tumor is treated with an effective drug at a convenient dose, the number of cell in the tumor is reduced by a constant fraction, not a constant number, regardless of the tumor burden. This phenomenon is known as the *"log-kill" hypothesis*. It follows that a dose of drug able to reduce 10^6 cells to 10 [3] will also reduce 10^9 cells to 10 [4], i.e., by 90%. Higher dose will kill a higher fraction of cells. When two or more drugs are used in combination, a multiplicative log-kill effect is achieved. Therefore, if a given dose of drug A or drug B when used as single agents is able to kill 90% of cancer cells, their combination will kill 99% of the cells (drug A kills 90% of the initial population and B 90% of the surviving 10%). Similarly, the combination of three drugs, each killing 90% of cells, will destroy 99.9% of the cells. According to this model, with repeating cycles of the same treatment, the number of cancer cells should progressively reduce to the point in which less than 1 cell survives, thus achieving the complete eradication of the tumor. As an example, let's assume that we are treating a tumor containing 10^{10} cells with a three-drug combination and that a single cycle can kill 99.9% of cells (three one-log kill) (Fig. 9.2b). However, during the interval between treatments, the tumor regrows. Assuming that this interval is short enough so that the regrowth is of only 1 log, at the moment of the second cycle, it would contain 10^8 cells, i.e., 2 log of cells less than at baseline. It follows that after 5 cycles, with a constant net loss of 2 logs per cycle, all tumor cells would be killed. An unspo-

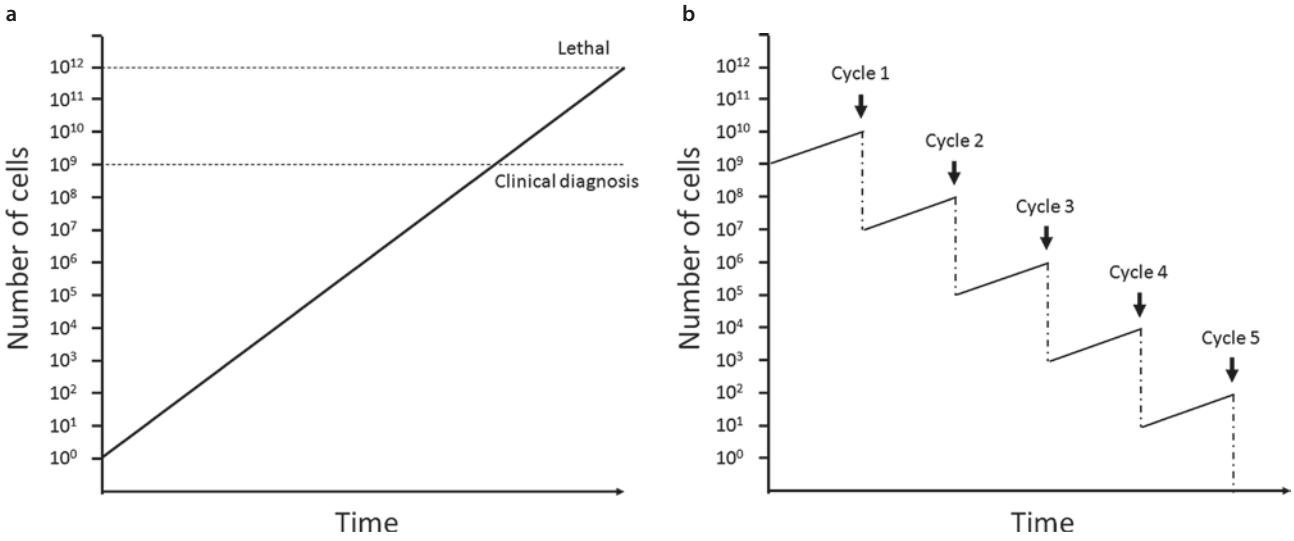

Fig. 9.2 **a** Exponential growth according to Skipper and Schabel model, **b** log-kill hypothesis

ken assumption of these model is that the tumor cell population displays a homogeneous drug sensitivity that is conserved over time, independently of tumor volume, and, therefore, that no resistant cell clone is present.

However, the model of Skipper and Schabel, assuming that tumor cells are uniformly proliferating and constantly sensitive to a certain treatment, failed when used in experimental and human solid tumors. This is due to the fact that tumors commonly do not present those characteristics: in fact, they are made of cells displaying a heterogeneous growth kinetics and with different sensitivity to treatment.

The growth of neoplasms is best described by a curve known as the *Gompertzian curve*. This is based on the mathematical model developed in the nineteenth century by the British actuary Benjamin Gompertz to describe the relationship between age and life expectancy. According to this model, tumor growth is described by a sigmoid, and tumor doubling time, which was supposed to be constant by Skipper and Schabel, actually decreases over time as tumor size increases (Fig. 9.3). The curve indicates that cells follow an exponential growth only at the beginning, when tumor size is limited. As size increases, the curve progressively deflect downward, indicating a decrease in the rate of proliferation, and soon reaches a plateau of very slow growth. Remarkably, when a tumor reaches a size that can be clinically appreciated, i.e., about 10^9 cells, it is already in the plateau phase of growth, indicating that the proliferating compartment of the tumor is extremely reduced.

Gompertzian growth has another important implication, as proposed by Norton and Simon [4]: the rate of regression under an effective treatment is directly related to the rate of tumor growth, which, according to the Gompertzian model, depends on tumor volume

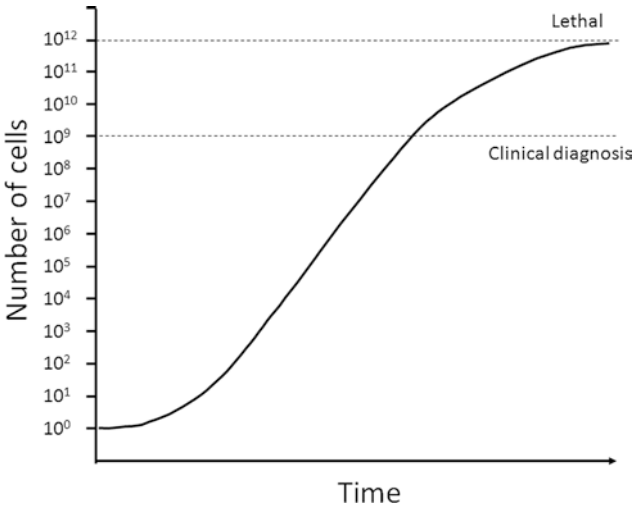

Fig. 9.3 Cell growth kinetic according to the Gompertzian model

(Norton-Simon hypothesis). In other words, keeping all other variables unchanged (dose of drug, drug sensitivity, penetration of medication), faster-growing cancer regress quicker under treatment. For example, a dose of drug able to reduce by 2 logs a tumor of 10^{11} cells would likely reduce by at least 4 logs a tumor of 10^9 cells. It follows that, as compared to larger tumors, smaller ones are more easily eradicated. This concept represents the rational basis for adjuvant therapy, i.e., the treatment applied after primary surgery to reduce the risk of recurrence that is related to the persistence of small cluster of cancer cells (micrometastasis) outside the primary site. Because of the very limited size of these clusters, the percentage of proliferating cells is expected to be very high, and, as a consequence, therapy might be more effective. However, in this setting the higher fraction of

cells killed by treatment is counterbalanced by a more rapid regrowth between treatments. It follows that relapse can be prevented only if regrowth is precluded. As postulated by Norton and Simon and demonstrated by Bonadonna and colleagues for human diseases [5], this can be achieved if chemotherapy is delivered at a higher dose rate ("dose density"), i.e., by shortening intervals between treatments.

Another obstacle to the eradication of tumors is represented by the emergence of cell clones resistant to chemotherapy. In 1979 Goldie and Coldman [3] proposed that mutations were responsible for the development of resistance to antineoplastic drugs and formulated a mathematical model to calculate the probability of occurrence of resistant clones depending on mutation rate and tumor volume. Mutation rate is estimated to be in the order of 10^{-5}: this means that at least one mutated cell is expected in a tumor composed by 10^5 cells. Mutation rate increases with the progression of cancer and with accumulation of mutated cells, so that the probability of harboring resistant clones is directly proportional to tumor size. Since a tumor becomes clinically detectable when it counts approximately 10^9 cells and given an average mutation rate of 10^{-5}, it follows that at the time of diagnosis the tumor might already contain 10^4 clones resistant to a certain drug. Obviously, the probability of having clones resistant to two different drugs will be much lower, i.e., $10^{-5} \times 10^{-5} = 10^{-10}$, that is 1 clone every 10^{10} cells. As a consequence, a tumor of 10^9 cells should not contain dual-resistant clones and could be easily eradicated by combining multiple, noncross-resistant drugs. Unfortunately, reality is quite different because of the multidrug resistance, a phenomenon in which cells are resistant to different drugs, even not structurally related.

Therefore, according to the concepts discussed above, to maximize chances of cure chemotherapy should be administered (i) when tumor size is limited, in order to maximize efficacy and reduce the chance of resistant clones; (ii) at time intervals sufficient to prevent a regrowth that overtake cell killing, i.e., at high dose density; and (iii) in the form of combination regimens.

Usually drugs are combined according to some basic principles:

(a) Efficacy as single agent.
(b) Different mechanisms of action, in order to minimize the likelihood of cross-resistance.
(c) Different toxic effects. Although this might increase the number of side effects that the patient may experience, it reduces the probability of severe toxicity.

9.3 Chemotherapeutic Drugs

The most significant advances in chemotherapy are not related to the identification of new and more effective drugs, but are represented by conceptual developments of therapy, including the use of more effective treatment with combination regimens, elucidation of the mechanisms of action of antiblastic drugs and of those underlying chemoresistance, the use of adjuvant and neoadjuvant chemotherapy, and a better understanding of the biological process involved in tumor growth.

After more than 50 years of research, chemotherapeutic drugs approved for use in humans are around 70. As discussed above, all these drugs interfere, in some way, with pathways essential for cell functions and survival and are mostly active when cells are proliferating. The relation between cell cycle progression and the point of action of a specific class of chemotherapeutic agents is illustrated in Fig. 9.3. Three main levels of activity can be recognized, resulting in:

1. Inhibition of the synthesis of nucleotides required for DNA replication and RNA transcription. These drugs are collectively called antimetabolites and include antagonists of purine nucleotides, antagonists of pyrimidine nucleotides, and antagonists of both types of nucleotides (antifolates).
2. Damage of DNA by direct binding (alkylating agents, platinum complexes, and DNA intercalators such as anthracyclines) or by inhibition of topoisomerase enzymes, (inhibitors of topoisomerase I and inhibitors of topoisomerase II).
3. Inhibition of mitosis by interference with microtubules polymerization (vinca alkaloids) or depolymerization (taxanes). These drugs are collectively called antimitotics.

A more detailed classification based on mechanism of action and molecular structure of these drugs, along with the most used agents in clinic, is reported in Table 9.2. Other classifications have been proposed that take into account drug derivation. For example, antibiotics, including anthracyclines, mitomycin, and mitoxantrone, or plant alkaloids, including vinca alkaloids, epipodophyllotoxin, taxanes, and camptothecins, are often reported as specific classes of chemotherapeutic drugs. For didactic purpose, herein we prefer to emphasize the mechanism of action of the different agents. However, each drug will be introduced by a brief description of its synthetic or biologic origin (Fig. 9.4).

Table 9.2 Classification of chemotherapeutic agents

1. *Antimetabolites*	
Antifolates:	Methotrexate, pemetrexed
Pyrimidine antagonists:	5-Fluorouracil, capecitabine, cytarabine, gemcitabine
Purine antagonists:	6-Mercaptopurine, 6-thioguanine
2. *DNA damaging agents*	
Direct damage by cross-linking of DNA or formation of DNA adducts	
Alkylants	
Nitrogen mustards:	Chlorambucil, cyclophosphamide, ifosfamide, mechlorethamine, melphalan
Alkyl sulfonates:	Busulfan
Nitrosoureas:	Carmustine, lomustine, semustine, streptozotocin
Aziridines:	Thiotepa, mitomycin
Triazenes:	Dacarbazine, temozolomide
Hydrazines:	Procarbazine
Others:	Trabectedin
Platinum analogs:	Cisplatin, carboplatin, oxaliplatin
Direct damage by DNA intercalation	
Anthracycline:	Doxorubicin, epirubicin, daunorubicin, idarubicin, liposomal doxorubicin
Others:	Mitoxantrone, actinomycin D, bleomycin
Indirect damage by topoisomerase inhibition	
Topoisomerase I inhibitors	
Camptothecins:	Irinotecan, topotecan
Topoisomerase II inhibitors	
Epipodophyllotoxins:	Etoposide, teniposide
3. *Antimitotics*	
Vinca alkaloids:	Vinblastine, vincristine, vinorelbine, vindesine, vinflunine
Taxanes:	Docetaxel, paclitaxel, cabazitaxel, Nab-paclitaxel
Others:	Eribulin, ixabepilone, emtansine (conjugated to trastuzumab,T-DM1)

9.3.1 Antimetabolites

9.3.1.1 Antifolates

The history of antifolates, and in general of antimetabolites, is an example of how crucial scientific discoveries often are the result of lucky coincidences. The identification of the antineoplastic property of antifolates in the late 1940s is paradigmatic. Farber first observed a rapid acceleration of disease in children with leukemia treated with folic acid. This observation primed the development of many folic acid antagonists. Among those, aminopterin was able to induce objective responses in a high percentage of children affected by leukemia [6].

Basically antifolates work by interfering with the synthesis of nucleic acids that occur in the late G1/S phase of the cell cycle. Therefore, they have no activity in G0 and block cell proliferation in the S phase (cell cycle phase-specific drugs). Differently from alkylating agents (see later), this group of drugs is not associated with late severe myelotoxicity (i.e., the recovery from decreased blood cell count is rapid because they do not act on stem cells), and they are not included among carcinogens. The most commonly used antifolates are the synthetic drugs methotrexate and pemetrexed.

Methotrexate (MTX) acts by inhibiting dihydrofolate reductase (DHFR) and, therefore, blocking the production of tetrahydrofolic acid (FH4), that is, the active form of folic acid. As a consequence, de novo synthesis of purine and thymidylate requiring incorporation of methyl groups transported by FH4 is interrupted. As a consequence, DNA replication and RNA transcription is abolished. The mechanism of action explains why leucovorin, also known as folinic acid, can be used to antagonize MTX-induced toxicity. In fact, leucovorin is rapidly converted in reduced folates, i.e., FH4, even in the presence of an enzymatic block of DHFR, thus restoring the synthesis of nitrogen bases.

Pemetrexed acts in part as MTX by inhibiting DHFR, but its main mechanism of action is the inhibition of thymidylate synthase (TS) and is potentiated by the intracellular glutamation of the drug. TS inhibition results in a block of the de novo synthesis of thymidylate and DNA. Moreover, pemetrexed inhibits glycinamide ribonucleotide formyltransferase (GARFT), an enzyme also involved in purine biosynthesis.

Toxicity At conventional doses, toxicity is represented by decreased cell blood count, stomatitis, diarrhea, nausea, and vomiting. To avoid severe toxicity, a supplement of folic acid (0.4 mg per os daily) and vitamin B_{12} (1000 mcg IM q9 weeks) is administered, beginning 1 week before treatment with pemetrexed.

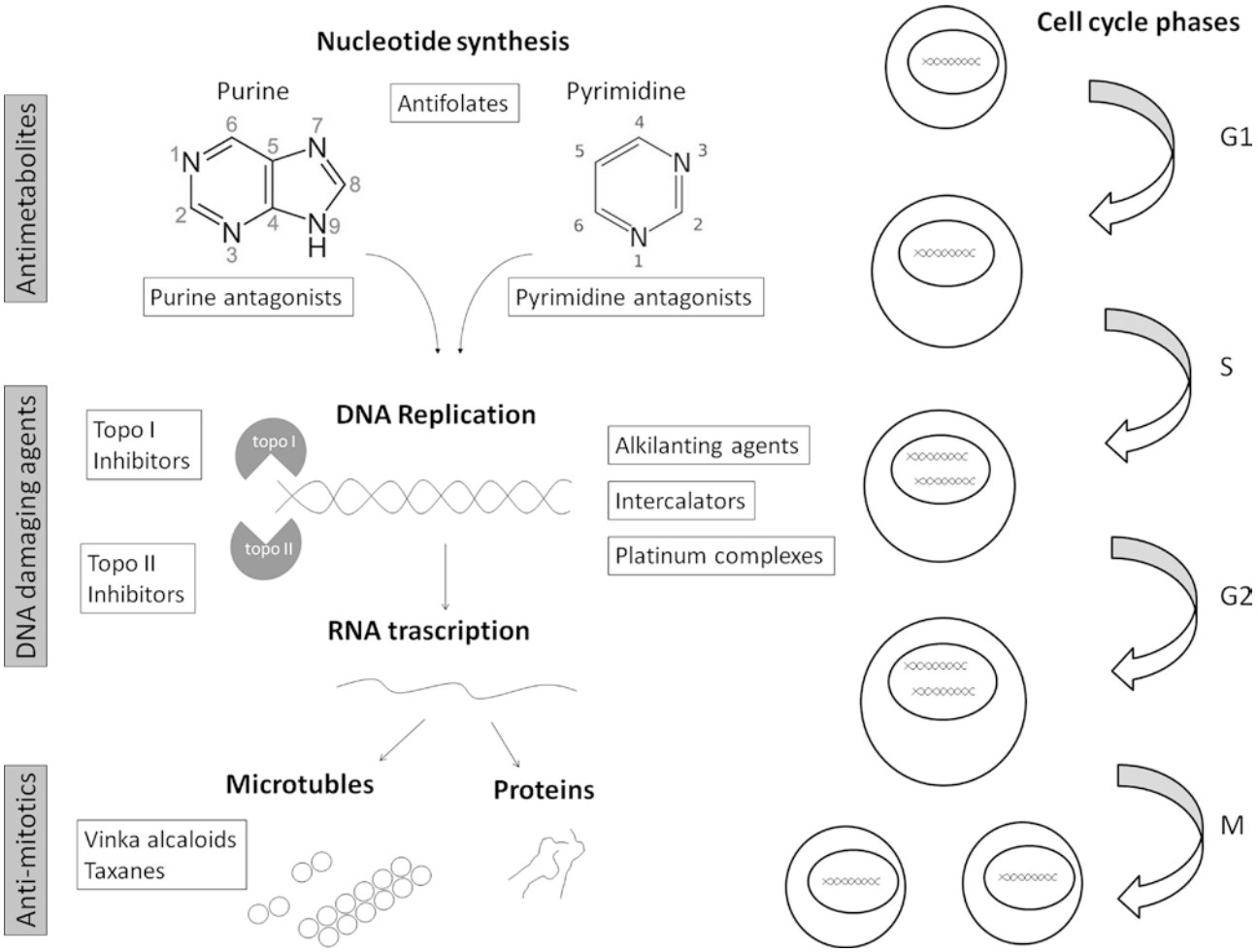

Fig. 9.4 Mechanism of action of chemotherapeutic drugs according to the different phases of cell cycle. In dark box the general classification; in white box the most important classes

Clinical Use Methotrexate is commonly used in leukemias, breast cancer, small-cell lung cancer (SCLC), bladder cancer, choriocarcinoma, and head and neck cancer. Pemetrexed is used in lung adenocarcinoma and mesothelioma.

9.3.1.2 Pyrimidine Antagonists

Agents of this class have a chemical structure mimicking, or are converted to, pyrimidine nucleotides (uridine, thymidine, cytidine). They act as "fraudulent" nucleotides and compete with the naturally occurring one, thus inhibiting enzymes involved in the synthesis of nucleic acids or being incorporated into DNA and RNA after phosphorilation. This will affect the synthesis of nucleic acids and results in a cytotoxic effect during the S phase of the cell cycle (cell cycle phase-specific drugs). The agents most commonly used are 5-fluorouracil, capecitabine, cytarabine, and gemcitabine, all synthetically produced.

5-Fluoruracil (5-FU) is one of the most used drugs in oncology. It is an analog of the nitrogen base uracil. Inside the cell 5-FU is converted into the two nucleotides responsible for its activity: 5-fluoro-2′-deoxyuridine 5′-monophosphate (5-FUMP) and 5-fluorouridine-5′-triphosphate (5-FUTP). 5-FUMP binds covalently to and inhibits thymidylate synthase, thus blocking the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) and, therefore, DNA synthesis. Binding of 5-FUMP to thymidylate synthase is potentiated by the presence of FH4. For this reason, 5-FU is often administered in association with leucovorin. 5-FUTP is incorporated into RNA altering its function.

Capecitabine is a pro-drug of 5-FU. It is administered orally and after an initial liver metabolism is converted to 5-FU in several steps, the last being catalyzed by thymidine phosphorylase. Since this enzyme is preferentially expressed in tumor cells as compared to normal ones, a selective accumulation of 5-FU in tumor cells may result. This can determine selective accumulation of 5-FU in tumor tissue.

Cytarabine, also called cytosine arabinoside or AraC, is a cytidine analogue and inside the cell is converted into the nucleotide cytarabine triphosphate (Ara-CTP) that competes with deoxycytidine triphosphate (dCTP) for incorporation in DNA, resulting in the inhibition of DNA polymerase and DNA synthesis.

Gemcitabine is structurally an analog of deoxycytidine, 2′,2′-difluoro-deoxycytidine (dFdC), similar to cytarabine but with a wider activity. In the cell it is converted into gemcitabine monophosphate (dFdCMP) and subsequently into gemcitabine diphosphate (dFdCDP) and triphosphate (dFdCTP). dFdCTP is incorporated into replicating DNA, resulting in inhibition of DNA synthesis and function. Intracellular degradation of dFdCTP and dFdCDP results in metabolites that inhibit DNA polymerase and the enzyme ribonucleotide reductase, respectively. The latter effect reduces the production of deoxyribonucleotides and inhibits DNA synthesis.

Toxicity Side effects of this class include myelosuppression, skin rush, and flu-like syndrome, while nausea and vomiting are not common. Main toxicities of 5-FU and capecitabine are stomatitis and diarrhea. Capecitabine can cause hand-foot syndrome, also known as palmar-plantar erythrodysesthesia, characterized by erythema, dryness, and swelling of the hands and feet, sometimes associated with pain or pruritus. Cytarabine can determine a usually reversible CNS toxicity with cerebellar ataxia, lethargy, and confusion.

Clinical Use 5-FU and capecitabine are used in gastrointestinal cancer, breast cancer, and head and neck cancer. Gemcitabine is mostly used in non-small cell lung cancer (NSCLC), pancreatic cancer, bladder cancer, breast cancer, ovarian cancer, and soft tissue sarcoma. Cytarabine is usually administered in hematological cancer, in particular acute leukemia.

9.3.1.3 Purine Antagonists

These drugs are structurally related to purine nucleotides (guanine, adenine) and, therefore, act with a competitive mechanism similar to that described for pyrimidine antagonists. The cytotoxic effect occurs at the S phase of the cell cycle (cell cycle phase-specific drugs). Representative drugs of this class are the synthetically manufactured 6-thioguanine and 6-mercaptopurine.

6-Thioguanine (6-TG) is phosphorylated intracellularly into thioguanine monophosphate (TGMP), which is an analogue of the guanine nucleotide, and inhibits the first step of de novo purine synthesis catalyzed by the enzyme phosphoribosylpyrophosphate amidotransferase. TGMP is subsequently converted into thioguanosine triphosphate (TGTP) that is incorporated in DNA in the place of guanosine triphosphate, causing inhibition of DNA synthesis.

6-Mercaptopurine (6-MP) acts similarly to 6-TG. It is phosphorylated intracellularly into thioinosine monophosphate (TIMP) by hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and inhibits de novo purine synthesis. TIMP is then metabolized into thioguanine monophosphate (TGMP), that further inhibits purine synthesis, and into thioguanosine triphosphate (TGTP) that is directly incorporated into DNA and blocks its replication.

Toxicity The major side effects of these drugs are myelosuppression and gastrointestinal toxicity, including nausea, vomiting, anorexia, diarrhea, and stomatitis. For their immunosuppressive effect, prolonged therapy may predispose patients to bacterial infections.

Clinical use 6-TG and 6-MP are mainly used in the treatment of acute leukemia.

9.3.2 DNA Damaging Agents

9.3.2.1 Alkylating Agents

This group of drugs has a key role in modern anticancer therapy. Some of them are among the first antiblastic drugs to be used in the clinic and were developed as by-products of military research. For its vesicant properties, sulfur mustard (mustard gas) was first used as a chemical warfare agent in 1917 by the Germans during World War I in the battle of Ypres. Thereafter the agent was also known as *yperite*. Exposure to this substance caused a severe systemic intoxication characterized by dermatologic, respiratory, gastrointestinal, and hematologic complications. In particular, a severe form of bone marrow aplasia was observed.

During World War II, nitrogen mustards, less toxic derivatives of mustard gas, were intensely studied by Gilman and Goodman for their action on lymphoid tissue. In particular, they were used for the treatment of lymphosarcomas, Hodgkin's disease, and leukemia. In the 1946 the results of these studies were published marking the official beginning of modern cancer chemotherapy [7]. Mechlorethamine was the first alkylating agent approved by FDA in March 1949. Since then several variants of nitrogen mustard chemical structure have been obtained, and today many drugs are included in this class (◘ Table 9.2).

Every member of this group is characterized by the ability to produce an alkylation reaction, i.e., the covalent binding of positively charged alkyl groups, usually methyl (CH3) or ethyl (C2H5) groups, to nucleophilic residues of proteins or nucleic acids. Most alkylating agents are bifunctional for their ability to alkylate two sites; others, such as procarbazine, dacarbazine, and temozolomide, are mono-functional.

Bifunctional agents mainly act through interaction with DNA and formation of intra-strands (in the same strand) or inter-strand (between the two strands) cross-linking. For most of these drugs, including nitrogen mustards, more frequently alkylation involves the highly nucleophilic nitrogen atom at 7-position (N-7) of guanine, although other sites can be targeted. For examples, mitomycin alkylates the extracyclic nitrogen atom on guanine, while trabectedin gives N-2 guanine alkylation. DNA cross-linking results in stable binding between the two DNA helices, the so-called welding, thus blocking DNA replication, RNA transcription, and protein synthesis.

Alkylating agents that are not bifunctional cause DNA methylation predominantly of the O-6 or N-7 guanine of DNA, resulting in point mutations and single-strand breaks.

These drugs are mainly active on proliferating cells, regardless of the cell cycle phase (cell cycle non-phase-specific). Some of them, e.g., nitrosoureas, can also act on cells in G0 (non-cell cycle-specific). With the exception of the naturally occurring substances streptozotocin (derived from *Streptomyces achromogenes*) and mitomycin (derived from *Streptomyces caespitosus* or *Streptomyces lavendulae*) and the semisynthetic trabectedin (initially extracted from the sea squirt *Ecteinascidia turbinata*), all other alkylating agents are synthetically produced.

Toxicity Side effects include myelotoxicity, particularly leukopenia and thrombocytopenia, nausea, vomiting, and stomatitis. Ifosfamide, and to a lesser extent cyclophosphamide when used at high dose, may cause hemorrhagic cystitis, due to renal elimination of their degradation products. For this reason, it is required the co-administration of MESNA, a thiol-rich substance able to "sequester" alkylating metabolites and protect urothelium.

Prolonged use of alkylating agents can cause amenorrhea in women and azoospermia in men. When administered in the first trimester of pregnancy, they can cause teratogenic effects and spontaneous abortion.

Many alkylating agents, including cyclophosphamide, chlorambucil, and phenylalanine mustard, are known carcinogens. Others, including procarbazine, cisplatin, and carmustine, are classified as probably carcinogenic. The risk of developing secondary cancers after treatment with these drugs, mainly acute leukemias, depends on dose and length of administration: after 10 years from treatment the risk is about 5–10%.

Clinical Use Cyclophosphamide is mostly used in breast cancer, SCLC, non-Hodgkin's lymphoma, chronic lymphocytic leukemia, and bone and soft tissue sarcoma. Ifosfamide is used in soft tissue sarcoma, osteogenic sarcoma, recurrent germ cell tumors, and lymphomas; carmustine and lomustine in brain tumors; dacarbazine in metastatic melanoma, Hodgkin's lymphoma, and soft tissue sarcomas; and temozolomide in metastatic melanoma and glioblastoma.

9.3.2.2 Platinum Complexes

The identification of the antitumor activity of platinum complexes arose from the observation that bacteria proliferation was inhibited in a chamber with platinum electrodes. The effect was not due to electric field, but to the formation of ammonium chloroplatinate. Further studies showed an antiproliferative effect of cis-diamminedichloroplatinum (cisplatin) on tumor cells.

Platinum complexes, including *cisplatin*, *carboplatin*, and *oxaliplatin*, are synthetic drugs that act as DNA cross-linkers, similarly to bifunctional alkylating agents, and react preferentially with N-7 guanine. Intra-strand and inter-strand cross-links block DNA replication, RNA transcription, and protein synthesis. These drugs are mainly active on proliferating cells, regardless of the cell cycle phase (cell cycle non-phase-specific).

Toxicity Cisplatin is characterized by severe kidney toxicity resulting in increase in blood creatinine and blood urea levels after 10–15 days from administration. Kidney damage is prevented by adequate hydration and mannitol infusion before treatment. Although renal toxicity is usually reversible, repeated cycles can cause permanent damage. Nausea and vomiting are very common side effects and, before the introduction of new-generation antiemetics, were among the most frequent causes of treatment refusal. Other characteristic toxicities are a dose-dependent peripheral neuropathy with sensory loss and numbness in upper and lower limbs and hearing loss. Myelotoxicity is usually moderate. Carboplatin and oxaliplatin are much less nephrotoxic and do not require pre-hydration. They also produce less nausea and vomiting, but are associated to a higher incidence of myelotoxicity, especially thrombocytopenia. Oxaliplatin produces also peripheral neurotoxicity characterized by dysesthesia and paresthesias of upper and lower limb and sometimes of the perioral region. These symptoms may be accentuated by exposure to cold. In some cases symptoms may be severe, but tend to regress after discontinuation of treatment.

Clinical Use Cisplatin and carboplatin are mainly used in gynecologic cancers, testicle cancer, lung cancer, bladder cancer, and head and neck cancers. Typically carboplatin is dosed by AUC according to the Calvert formula [8].

9.3.2.3 Intercalators

The term *intercalator* refers to molecules endowed with the capability to insert themselves between the planar bases of DNA, determining structural distortion and

inhibition of DNA and RNA synthesis. This class of drugs includes the *anthracycline antibiotics* (*doxorubicin*, *epirubicin*, *daunorubicin*, *idarubicin*) and other non-anthracycline agents such as *mitoxantrone*, *actinomycin D*, and *bleomycin*.

Anthracyclines derives from *Streptomyces peucetius* var. *caesius*. The most frequently used agents are *doxorubicin* and its 4′-epimer with a different spatial orientation of the C-4′ hydroxyl group of the amino sugar, *epirubicin*. The high toxicity profile of anthracyclines precludes their use as antibacterial agents. Their antiproliferative effect involves essentially three different mechanisms: (i) DNA intercalation, (ii) inhibition of topoisomerase II, and (iii) production of oxygen free radicals.

The planar structure of these molecules allows them to bind DNA and to act as intercalators between two consecutive base pairs of DNA, altering its steric conformation and causing a block of DNA replication and RNA transcription.

Anthracyclines also inhibit Topoisomerase II (TOP2), an enzyme that binds DNA and causes double-strand breaks thus allowing the passage of another portion of the duplex through the cut, and reseal the breaks [9]. Anthracyclines inhibit TOP2 activity through stabilization of the TOP2-DNA complexes and inhibition of DNA double-strand breaks resealing: this will trigger apoptosis.

These drugs are reduced by intracellular oxidoreductases with the formation of oxygen-free radicals responsible for single- and double-strand DNA breaks, along with peroxidation of membrane lipids and cell death for altered permeability.

The overall cytotoxic effect of anthracyclines is present throughout the cell cycle (cell cycle non-phase-specific drugs), although their activity is prevalent during cell cycle phases S and G2.

In order to limit toxicity, *liposomal formulations of doxorubicin* have been recently developed. Liposomes are nanoparticles with a diameter of 50–200 nm consisting of an aqueous core encapsulated by a phospholipid bilayer. Since capillaries of tumor vasculature have wider pores as compared to normal vessels, nanoparticles can diffuse preferentially into tumor tissues. As a consequence, reduced toxicity and enhanced efficacy are expected. Two different liposomal formulation of doxorubicin are used in clinic: non-pegylated (NPLD) and pegylated (PLD). PEGylation improves tissue distribution and prolongs half-life elimination of liposomes.

Among non-anthracycline intercalator, *mitoxantrone* is a purely synthetic compound, analogue of anthracenedione, developed to reduce anthracycline cardiotoxicity, while *actinomycin D* is an antibiotic isolated from bacteria of the genus *Streptomyces parvullus*. Both act by DNA intercalation.

Bleomycin is a mixture of glycopeptides, mainly A2 and B2 fractions, derived from *Streptomyces verticillus*, a strain of *Actinomyces*. The mechanism of action is complex and only partially elucidated. It intercalates DNA by its bithiazole rings present in the amino-terminal end of the molecule, but the cytotoxic effect results from the formation of oxygen-free radicals generated by the iron-binding region present at the carboxy-terminal end. As a consequence, single- and double-strand DNA breaks accumulate in the cells, mostly in G2 and M phases of the cycle, leading to apoptosis.

Toxicity Doxorubicin and epirubicin are associated with different and sometimes severe side effects including bone marrow suppression, alopecia, nausea, vomiting, cardiotoxicity, and tissue necrosis following extravasation. The risk of congestive heart failure is dose-dependent: it increases substantially at cumulative dose of 450 mg/m2 for doxorubicin [10, 11] and of 900 mg/m^2 for epirubicin [12]. Both liposomal formulations have a very low cardiac toxicity: NPLD in combination with cyclophosphamide can be administered safely up to a cumulative dose of 1260 mg/m^2, while PLD can be continued up to disease progression [13]. PLD is associated with palmar-plantar erythrodysesthesia.

Bleomycin is commonly associated with skin reactions such as erythema, hyperpigmentation, vesiculation, thickening, and Raynaud's phenomenon. The dose-limiting side effect is pulmonary toxicity that can progress to pulmonary fibrosis.

Clinical Use Doxorubicin and epirubicin are used to treat a large variety of neoplasms, such as breast cancer, gynecologic cancers, sarcoma, and thymic cancer.

NPLD is used in breast cancer. PLS is mostly used in Kaposi's sarcoma, ovarian, and breast cancer.

Bleomycin is indicated in lymphomas, germ cell tumors, head and neck cancer, and squamous cell carcinomas of the skin, cervix, and vulva.

9.3.2.4 Camptothecins

This class includes *irinotecan* and *topotecan*, two semisynthetic derivatives of camptothecin, an alkaloid extracted from *Camptotheca acuminata* tree, the only known natural inhibitor of topoisomerase I (TOP1). This enzyme causes single-strand breaks in DNA during replication or RNA transcription, thus allowing the passage of the intact strand through the break and relieving the torsional forces generated by the opening of the replication fork. Then it reseals the break and detaches from DNA.

Irinotecan and its active metabolite SN-38, as well as topotecan, stabilize the TOP1-DNA complex, preventing religation of DNA strand. The resulting break in DNA causes cell death during S phase (cell cycle phase-specific drugs).

Toxicity Camptothecins can cause myelosuppression, nausea, vomiting, diarrhea (especially irinotecan), arthralgias, and myalgias.

Clinical Use Irinotecan is mainly used in colorectal cancer. Topotecan is mainly used in ovarian cancer and SCLC.

9.3.2.5 Epipodophyllotoxins

These substances, including *etoposide* and *teniposide*, are semisynthetic derivatives of 4′-demethylepipodophyllotoxin, a naturally occurring compound extracted from the roots of *Podophyllum peltatum* (mayapple). They act by inhibiting topoisomerase II. As for anthracyclines, epipodophyllotoxins stabilizes the complex TOP2-DNA, thus preventing relegation of DNA at the site of double-strand break and leading to cell cycle arrest and cell death in S phase (cell cycle phase-specific drugs).

Toxicity The most important toxicities of epipodophyllotoxins are bone marrow depression, alopecia, nausea, and vomiting. When quickly administered i.v., they can cause severe hypotension. Thus, infusion time must take at least 30 minutes.

Clinical Use Etoposide is used in germ cell tumors, SCLC, and lymphomas and teniposide in neuroblastoma, non-Hodgkin's lymphoma, and acute lymphocytic leukemia.

9.3.3 Antimitotics

9.3.3.1 Vinca Alkaloids

Vinca alkaloids include naturally occurring substances extracted from the periwinkle plant *Catharanthus roseus* (*Vinca rosea*), *vincristine*, and *vinblastine* and their semisynthetic analogues *vinorelbine*, *vindesine*, and *vinflunine*. They bind tubulin and inhibit its polymerization into microtubules, a process essential for the formation of the mitotic spindle. This results in a metaphase arrest of mitosis (cell cycle phase-specific drugs). Microtubules also play a crucial role in the trafficking of neurotransmitters to the synapse.

Toxicity Vinca alkaloids are associated with myelosuppression, abdominal pain, and mucosites, but the dose-limiting toxicity is symmetrical, peripheral neuropathy, characterized by reduced deep tendon reflex, neuritic pain, paresthesia, cranial nerve palsy, and paralytic ileus.

Clinical Use Vincristine is primarily used for the treatment of lymphomas, childhood solid tumors, and testicular cancer. Vinblastine is used in lymphomas, breast cancer, and testicular cancer. Vindesine is used for the treatment of NSCLC, esophageal cancer, and breast cancer. Vinorelbine is employed in NSCLC and breast cancer. Vinflunine is used for the treatment of metastatic bladder urothelial cancer.

9.3.3.2 Taxanes

These compounds were originally extracted from plants of the genus *Taxus* (yews): *paclitaxel* from the bark of *Taxus brevifolia* (Pacific yew) and *docetaxel* from the needles of *Taxus baccata* (European yew). It has been estimated that the bark of six trees was required to obtain 500 mg of paclitaxel, almost the dose of a cycle of chemotherapy. Both paclitaxel and docetaxel are now obtained semisynthetically.

The novel taxane *cabazitaxel* is also a semisynthetic product of a natural taxoid precursor extracted from the needles of *Taxus baccata*. It is endowed with a very low affinity for the P-glycoprotein, an efflux pump associated with multidrug resistance.

Recently, a new formulation of paclitaxel, *Nab-paclitaxel*, has entered in clinic. The acronym Nab stands for nanoparticle albumin bound, to emphasize the two main characteristics of this drug: (i) its nanoparticle formulation (i.e., a compound around 100 nm in size) and (ii) its bound to albumin. In fact, Nab-paclitaxel is composed by a polymer of albumin to constitute a complex of 130 nm in diameter. An individual molecule of albumin binds 6 or 7 molecules of paclitaxel. This formulation confers to paclitaxel greater water solubility, a linear pharmacokinetic, and higher plasma concentration. More importantly, using albumin as carrier, the drug utilizes endogenous albumin transport and tissue distribution. In particular through albumin, the drug binds the gp60 receptors on the endothelial cells, diffuses in the interstitial space by transcytosis, and concentrates in the tumor interstitium by interacting with proteins such as SPARC (secreted *protein* acidic and rich in cysteine). Nab paclitaxel is not a nanoparticle, but rather a nanoformulation because albumin complexes dissolve in each single molecule once solubilized. Thus, it is not endowed with the pharmacokinetic characteristics of nanoparticles, as discussed for liposomal anthracyclines.

Taxanes preferentially bind formed microtubules, enhancing polymerization and preventing depolymerization of tubulin. It follows a cell cycle arrest in M phase (cell cycle phase-specific drugs).

Toxicity Frequent side effects include leukopenia, peripheral neuropathy, alopecia, and nausea. Paclitaxel can cause a severe allergic reaction due to hypersensitivity toward the cremophor vehicle used to solubilize the drug. Infusion-

related reactions arise after 2–3 minutes from the start of administration, and almost always within 10 minutes, and are characterized by hypotension, severe dyspnea, bronchospasm, lumbar pain, changes in heart rate, and sweating. For this reason, corticosteroids and antihistamines should be administered prior to paclitaxel infusion. Nab-paclitaxel lacks cremophor and, therefore, is not associated with hypersensitivity reaction and does not require corticosteroid pre-medication. Docetaxel can cause mucositis, water retention, skin rush, and hand-foot syndrome.

Clinical use Paclitaxel is mainly used in the treatment of ovarian cancer, breast cancer, and lung cancer. Docetaxel is used in breast cancer, lung cancer, and prostate cancer. Cabazitaxel in used in second-line prostate cancer. Nab-paclitaxel is used in breast cancer and pancreatic cancer.

9.3.3.3 Other Antimitotics

Along with vinca alkaloids and taxanes, other molecules able to bind tubulin and inhibit the dynamic disassembly and assembly of microtubules have been synthesized.

Eribulin is a fully synthetic analogue of halichondrin B, a molecule isolated from the Japanese sea sponge *Halichondria okadai*. Similarly to vinca alkaloid, it acts by binding tubulin and inhibiting its polymerization. Differently from vinca alkaloid, eribulin sequesters tubulin in nonproductive aggregates with no effect on microtubule shortening.

Ixabepilone is a semisynthetic analog of epothilone B, originally isolated from the myxobacterium *Sorangium cellulosum*. Its mechanism of action is similar to that of taxanes: binds tubulin and inhibits microtubules depolymerization.

Emtansine, also called DM1, is a derivative of myasantine, a natural product originally derived from the bark of the African shrub *Maytenus ovatus*. Emtansine is an inhibitor of tubulin polymerization more potent than vinca alkaloids, but has a very low therapeutic index because of its pronounced epatotoxicity. For this reason it never entered phase III clinical trials. However, when conjugated to trastuzumab, a monoclonal antibody directed against HER2, it distributes preferentially on tumor cell expressing the receptor, spearing normal tissues. In this formulation, called *T-DM1*, it is now available for clinical use.

Toxicity Eribulin and ixabepilone can cause myelosuppression, nausea, vomiting, mucositis, diarrhea, dyspepsia, and peripheral neuropathy. T-DM1 can cause mainly hepatotoxicity with transient elevations of transaminases and myelosuppression with thrombocytopenia.

Clinical Use Eribulin and ixabepilone are used in patients with metastatic breast cancer who have previously received anthracyclines and a taxanes. T-DM1 is used in HER2-positive metastatic breast cancer.

9.4 Conclusion

Discovery of molecular mechanisms involved in cancer growth and progression has led to the development of drugs targeting specific signaling pathways and immune checkpoints [14]. However, chemotherapy remains the most effective treatment for several types of neoplasms. Therefore, the knowledge of mechanisms of actions and side effects of these drugs represent an essential background for health professionals dealing with patients affected by cancer.

Keypoints

- Chemotherapeutic drugs are classified according to their mechanism of action and molecular structure.
- These drugs interfere with pathways essential for cell functions and survival.
- They are mostly active when cells are proliferating.
- To maximize chances of cure chemotherapy should be administered when tumor size is limited, at adequate time intervals, in the form of combination regimens.

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Endocrine Therapy

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Contents

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The development of cancer is connected to the acquisition of several specific capabilities that allow cells to grow evading apoptotic signaling, proliferate indiscriminately, and survive, independently from physiological regulatory stimuli. Several solid tumors are driven in growth by specific hormones. When tumors are driven in growth and sustained by endocrine signaling, an effective therapeutic strategy is represented by hormonal therapy with endocrine agents. The two most frequent hormone-dependent solid tumors are prostate cancer in male and breast cancer in women. The first is usually driven by androgens; the latter is driven by estrogens in approximately 60–70% of cases [1]. Other solid tumors, such as well- or moderately differentiated neuroendocrine tumors (NETs) or endometrial cancer might be treated at a certain point of their natural history with hormonal therapy, as well. Sometimes, although quite infrequently, hormone therapy might also be indicated for meningioma, kidney cancer, melanoma, and hepatocellular carcinoma [2].

Compared to chemotherapy, hormonal therapy is more selective for tumor cells and, thus less toxic, moreover is usually not burdened by myelotoxicity and alopecia, which is one of the most bothering side effects of anticancer treatments for oncologic patients.

Hormonal therapy can be administered with the purpose of inhibiting the production of specific hormones or by inhibiting their peripheral activity interfering with the hormone receptor-binding activity or downregulating hormone receptors. Hormonal therapy consists in hormones, analogues of hormones, antihormonal agents, and inhibitors of hormonal synthesis.

The main endocrine treatments available in clinical practice can be subdivided into eight main groups, as extensively reported in Table 10.1.

In the following chapter, the main "druggable" endocrine signaling pathways will be shown, in order to briefly discuss the mechanism of action, indications, metabolism, and main side effects of the related hormonal agents. With regard to side effects, only the ones that have been reported in at least 1% of patients treated in the pivotal studies will be reported.

10.1 Estrogen Receptor Signaling: A Brief Overview

Estrogen receptor is a member of the steroid nuclear receptor superfamily, which also comprises mineralocorticoid, glucocorticoid, androgen, and progesterone receptors. In humans, two different forms of ER exist, α and β. The first is expressed in the breast, endometrium, ovarian stromal cells, hypothalamus, and in the epithelium of the efferent duct; the latter is expressed in the kidney, brain, ovarian granulosa cells, bone, heart, endothelial cells, lungs, prostate, and intestinal mucosa. ER is mostly localized in the cell nucleus, even though a

Table 10.1 Main available hormonal agents for cancer therapy

Hormonal therapies			
Antiestrogens	**Antiandrogens**	**GNRH analogues**	**GNRH antagonists**
SERM	*First-generation AR antagonists*	Goserelin	Degarelix
Tamoxifen	Nilutamide	Leuprolide or Leuprorelin	
Toremifene	Flutamide	Triptorelin	
Raloxifene	Bicalutamide		
Aromatase Inhibitors	*Second-generation AR antagonists*		
Anastrozole	Enzalutamide		
Letrozole	*Androgen biosynthesis inhibitor*		
Exemestane	Abiraterone acetate		
Estrogen receptor downregulator			
Fulvestrant			
ESTROGENS	Androgens	Progestins	Somatostatin analogues
Estradiol	Fluoxymesterone	Medroxyprogesterone acetate	Octreotide
		Megestrol acetate	Octreotide LAR

SERM selective estrogen receptor modulator, *GnRH* gonadotropin releasing hormone, *AR* androgen receptor, *LAR* long-acting release

smaller amount is set in the cytosol, mitochondria, or at membrane level. ER acts via a classical nuclear/genomic and a nonnuclear/non-genomic mechanism. The classical mechanism is activated by estrogens that interact with nuclear ER, leading to their dimerization and subsequent binding to specific promoters' regions of target genes, known as estrogen response elements (EREs). ER complexes recruit several co-activators and positively or negatively modulate genomic expression of numerous growth factors and tyrosine kinase receptors (TKRs), such as EGFR, HER2, and IGFR-1, or interact with other transcriptional factors, such as AP-1 and Sp-1, leading to an indirect modulation of other genes' expression. The nonnuclear ER mechanism of action is based on an intensive cross talk between estrogen/ER nonnuclear complexes and TKRs, cytosolic tyrosine kinases, and other adaptors and/or signaling proteins that mediates the activation of PI3K/AKT/mTOR and Ras/MAPK signaling pathways. Both nuclear and nonnuclear mechanisms of action impact cell survival, proliferation, and migration. Dysregulation in ER signaling processes is on the basis of tumor development, growth, survival, and resistance to endocrine therapies, especially in breast cancer [3].

10.2 Targeting ER-Dependent Solid Tumors in Clinical Practice

ER signaling alterations are mostly involved in the development of breast cancer. Numerous hormone therapies have been developed for the treatment of hormone-dependent breast tumors, and they can be subdivided into three main categories: selective ER modulators (SERM), steroidal and nonsteroidal aromatase inhibitors (AI), and ER downregulators.

10.3 SERM [4, 5]

The selective ER modulators are a class of drugs that interact with ER and other known or less determined substrates in order to induce estrogen-like or antiestrogenic effects, depending on the tissue in which they act. The most important SERMs approved for the clinical practice are tamoxifen, toremifene, and raloxifene.

- *Tamoxifen*
  - *Mechanism of action.* Tamoxifen is a nonsteroidal compound that directly binds to ER, inducing conformational changes. Moreover, it probably interacts with several co-activators and co-repressors as well, producing both antiestrogenic and estrogenic effects. In fact, it acts as an estrogen in regulation of bone density, cholesterol metabolism, and cell proliferation in the endometrium, while in tumor cells it acts as an antiestrogen by blocking or modifying the expression of estrogen-dependent genes, directly or indirectly.
  - *Indication.* Tamoxifen is indicated for the treatment of hormone receptor-positive (HR+) breast cancer in women and men, both in the metastatic and adjuvant setting. Since several studies have shown better efficacy for AI compared to tamoxifen in postmenopausal women, tamoxifen should be preferred only in the premenopausal setting or when AI are contraindicated, suspended for toxicity, or refused by patients. It is also approved for the adjuvant treatment of HR+ ductal carcinoma in situ.
  - *Metabolism and route of elimination.* Tamoxifen is extensively metabolized in the liver, mostly by CYP2D6, CYP2C9, and CYP3A4, and is excreted with feces.
  - *Side effects.* Common side effects are represented by hot flashes, nausea, vomiting, endometriosis, vaginal bleeding, vaginal discharge, amenorrhea, altered menses, and oligomenorrhea. Among the less frequent adverse reactions (ADRs), the most peculiar that deserve more attention during the use of the drug are represented by the induction of endometrial adenocarcinoma and uterine sarcoma (with an incidence rate per 1000 women-years of 2.20 and 0.17, respectively), decreased visual acuity and retinopathy, and a slightly increased risk of thromboembolic events.
- *Toremifene*
  - *Mechanism of action.* Toremifene is a nonsteroidal compound, structurally and functionally similar to tamoxifen.
  - *Indication.* Toremifene is indicated for the treatment of metastatic HR+ breast cancer in postmenopausal women and in men.
  - *Metabolism and route of elimination.* Toremifene is extensively metabolized in the liver, mostly by CYP3A4, and is excreted with feces.
  - *Side effects.* Common side effects are represented by hot flashes, sweating, rush, pruritus, pulmonary embolism, depression, edema, pain, fatigue, dizziness, transient ischemic attack/cerebrovascular accident, hypercalcemia, ocular toxicity, vaginal discharge or bleeding, nausea, vomiting, hypertransaminasemia, hyperbilirubinemia, myocardial infarction, arrhythmia, cardiac failure, thrombophlebitis, and thrombosis. Such as tamoxifen, a very rare, yet important ADR to take into account is the development of endometrial carcinoma (less than 0.01% of cases).

- *Raloxifene*
  - *Mechanism of action.* Raloxifene is a nonsteroidal compound of the benzothiophene class with antiestrogenic effects on both breast and uterine tissues and estrogenic effects on lipid metabolism and bone tissue, reducing resorption of bone in postmenopausal women, maintaining and increasing bone mass, thus preventing osteoporosis. The molecular mechanisms of action are not fully determined, yet.
  - *Indication.* As an anticancer compound, raloxifene is indicated for the prevention of invasive breast cancer in postmenopausal women with osteoporosis or with high risk for developing breast cancer.
  - *Metabolism and route of elimination.* Raloxifene is extensively metabolized in the liver and mostly excreted with feces.
  - *Side effects.* Common side effects are represented by hot flashes, increased blood pressure, varicose vein, venous thromboembolism, flu syndrome, infections, peripheral edema, chest pain, fever, musculoskeletal disorders, sinusitis, rhinitis, other upper and lower respiratory tract inflammations, rash, sweating, breast pain/tenderness/enlargement, nausea, diarrhea, dyspepsia, vomiting, flatulence, gastrointestinal disorder, gastroenteritis, abdominal pain, genitourinary symptoms, cholelithiasis, weight gain, headache/migraine, syncope, vertigo, neuralgia, hypoesthesia, stroke, conjunctivitis, depression, and insomnia.

10.4 Aromatase Inhibitors

AI are molecules that act inhibiting the aromatase enzyme, preventing androgens (androstenedione or testosterone) to be converted in estrogens (estrone or estradiol), thus interfering with non-gonadal estrogenic synthesis. Since estrogenic synthesis in the adrenal gland, adipose, and breast and muscle tissue is the most relevant way of estrogen production in menopausal women, it is not surprising that AI are most effective in postmenopausal setting rather than in pre-menopause [6]. Alternatively, a gonadal estrogenic synthesis blockage with GnRH agonists provides an iatrogenic menopause that allows AI to be sufficiently effective also in otherwise physiologically premenopausal women [7]. At present, the most important compounds approved for clinical use are the steroidal AI exemestane and the nonsteroidal AI letrozole and anastrozole.

- *Exemestane* [4, 5]
  - *Mechanism of action.* Exemestane is an irreversible steroidal aromatase inhibitor, structurally related to androstenedione; therefore it acts as a false substrate for this enzyme, which catalyzes its conversion in an intermediate that binds it irreversibly to its active site.
  - *Indication.* Exemestane is indicated for the (neo) adjuvant treatment of postmenopausal women with HR+ breast cancer or in metastatic HR+ breast tumors. It may also be associated with mTOR inhibitor everolimus in postmenopausal women with advanced HR+ breast cancer pretreated with a nonsteroidal AI. In the premenopausal setting, it has to be used in combination with a GnRH analogue to fully preserve its efficacy, as previously explained.
  - *Metabolism and route of elimination.* Exemestane is metabolized in the liver and excreted in urine and feces.
  - *Side effects.* Common side effects are represented by arthralgia, osteoarthritis, myalgia, hypoesthesia, skeletal pain, osteoporosis, muscle cramp, hot flushes, fatigue, infections, nausea, abdominal pain, diarrhea, vomiting, constipation, dyspepsia, sweating, alopecia, dermatitis, hypertension, lymphedema, chest pain, hypertransaminasemia, hyperbilirubinemia, elevation of alkaline phosphatase, increased GGT, dyslipidemia, insomnia, depression, anxiety, confusion, headache, dizziness, paresthesia, carpal tunnel syndrome, dyspnea, coughing, bronchitis, sinusitis, upper respiratory tract infection, pharyngitis, rhinitis, visual disturbances, flu-like symptoms, increased appetite, anorexia, lymphocytopenia, and genitourinary symptoms.
- *Anastrozole* [4, 5]
  - *Mechanism of action.* Anastrozole is a reversible nonsteroidal aromatase inhibitor.
  - *Indication.* Anastrozole is indicated for the (neo) adjuvant treatment of postmenopausal women with HR+ breast cancer or in metastatic HR+ breast tumors. In advanced setting, it may also be associated with fulvestrant, preferably in first-line therapy for patients without prior exposure to adjuvant endocrine treatment or very late relapse (Rugo 2016). In the premenopausal setting, it has to be used in combination with a GnRH analogue to fully preserve its efficacy, as previously explained.
  - *Metabolism and route of elimination.* Anastrozole is metabolized in the liver and excreted with feces (around 90%) and urine (approximately 10%).
  - *Side effects.* Common side effects are represented by hypertension, edema, ischemic cardiovascular disease, arthritis/arthralgia/arthrosis/joint disorder/joint pain/joint stiffness, back pain, bone pain, osteoporosis, fracture, myalgia, anorexia, nausea vomiting, abdominal pain, diarrhea, con-

stipation, dyspepsia, dry mouth, infections, flu syndrome, fever, neck pain, malaise, mood disturbances, depression, insomnia, headache, depression, insomnia, dizziness, anxiety, paresthesia, hypertonia, confusion, nervousness, carpal tunnel syndrome, sensory disturbances, rash, sweating, alopecia/hair thinning, pruritus, dyspnea, increased cough, pharyngitis, sinusitis, bronchitis, rhinitis, genitourinary symptoms, anemia, leukopenia, increased gamma GT, hypertransaminasemia, increased alkaline phosphatase, weight gain, hypercholesterolemia, weight loss, anorexia, allergic reactions, and cataracts.

- *Letrozole* [4, 5]
  - *Mechanism of action.* Letrozole is a reversible nonsteroidal aromatase inhibitor.
  - *Indication.* Letrozole has similar indication than anastrozole. Additionally, in advanced setting, it should be preferably associated with cyclin-dependent kinase inhibitors (CDKi) palbociclib or ribociclib as first-line therapy. In the premenopausal setting, it has to be used in combination with a GnRH analogue to fully preserve its efficacy, as previously explained.
  - *Metabolism and route of elimination.* Letrozole is metabolized in the liver mostly by CYP3A4 and CYP2A6. It is primarily excreted with urine.
  - *Side effects.* Common side effects are represented by hypertension, edema, ischemic cardiovascular disease, arthritis/arthralgia/arthrosis/joint disorder/joint pain/joint stiffness, back pain, bone pain, osteoporosis, fracture, myalgia, anorexia, nausea vomiting, abdominal pain, diarrhea, constipation, dyspepsia, dry mouth, infections, flu syndrome, fever, neck pain, malaise, mood disturbances, depression, insomnia, headache, depression, insomnia, dizziness, anxiety, paresthesia, hypertonia, confusion, nervousness, carpal tunnel syndrome, sensory disturbances, renal disorders, rash, sweating, alopecia/hair thinning, pruritus, dyspnea, increased cough, pharyngitis, sinusitis, bronchitis, rhinitis, genitourinary symptoms, weight gain, hypercholesterolemia, weight loss, anorexia, allergic reactions, and cataracts.

10.5 ER Downregulators

- *Fulvestrant* [4, 5]
  - *Mechanism of action.* Fulvestrant competitively and reversibly binds to ER, preventing estrogens to bind their receptor; moreover it provides its internalization in cancer cells and leads to its subsequent degradation, as well.
  - *Indication.* Fulvestrant is indicated for the treatment of advanced HR+ breast cancer. It should be preferably combined with CDKi palbociclib in patients pretreated with endocrine therapy for advanced disease and not previously exposed to other CDK inhibitors. It may also be associated with anastrozole, preferably in first-line therapy for patients without prior exposure to adjuvant endocrine treatment or very late relapse.
  - *Metabolism and route of elimination.* Fulvestrant is metabolized in the liver and is primarily excreted with feces.
  - *Side effects.* Common side effects are represented by nausea, vomiting, constipation, diarrhea, abdominal pain, and anorexia, anemia, vasodilation, peripheral edema, bone pain, arthritis, dizziness, insomnia, paresthesia, depression, anxiety, pharyngitis, dyspnea, cough, urinary tract infection, rash, sweating, asthenia, fever, pain, headache, back pain, pain and/or reactions at injection site, pelvic pain, chest pain, and flu-like syndrome.

10.6 Androgen Receptor Signaling: A Brief Overview

Androgen receptors (AR) are primarily located in the cytosol and, such as ER, act via a nuclear and a non-nuclear mechanism of action after binding androgens, testosterone, or the more potent 5α-dihydrotestosterone (DHT). After ligands bind to AR, a subsequent interaction with several co-regulators allows the receptor to homodimerize or heterodimerize with ERα and TR4 in the nucleus and modulates gene expression via binding at DNA androgen response elements (ARE) and thus recruiting Src family members of co-activators, histone acetyltransferase (HAT), and activating the general transcription machinery. Such as ER, AR presents a non-nuclear mechanism of action, as well, that involves an intensive cross talk among AR, TKRs such as IGF-R1, KGFR, EGFR, and their downstream signaling pathways, represented by Ras/MAPK, PI3K/AKT, and Src. AR seem also to promote calcium intracellular release, formation of caveolae, and the activation of signaling cascades that regulate other nuclear receptors and transcriptional factors. Both nuclear and nonnuclear mechanisms of action may also be activated through an AR phosphorylation induced by several TKRs and membrane G-protein coupled receptors (GPCRs), independently from ligand-receptor interaction [8].

10.7 Targeting AR-Dependent Solid Tumors in Clinical Practice

Androgen signaling alterations are mostly involved in the development of almost all prostate cancers; therefore treatment with endocrine agents is usually required both in (neo)adjuvant (when required) and metastatic setting, except for rare exceptions. Numerous endocrine agents have been developed for the treatment of prostate tumors, and they can be subdivided into three main categories: first- and second-generation AR inhibitors and androgen biosynthesis inhibitors.

10.8 First-Generation AR Inhibitors

- *Nilutamide* [4, 5]
  - *Mechanism of action.* Nilutamide is a nonsteroidal agent that competitively binds to AR, preventing androgens to bind and activate their receptor.
  - *Indication.* Nilutamide is indicated for use in combination with surgical or pharmacological castration for the treatment of advanced prostate cancer.
  - *Metabolism and route of elimination.* Nilutamide is extensively metabolized in the liver and is primarily excreted with urine.
  - *Side effects.* Common side effects are represented by dyspnea, interstitial pneumonitis, lung disorder, upper respiratory infection, pneumonia, increased cough, rhinitis, hypertransaminasemia, increased alkaline phosphatase, increased BUN, increased creatinine, impaired adaptation to dark, chromatopsia, impaired adaptation to light, abnormal vision, cataract, photophobia, hypertension, angina, heart failure, anemia, hematuria, melena, increased haptoglobin, leukopenia, alcohol intolerance, hot flushes, impotence, decreased libido, gynecomastia, sweating, body hair loss, testicular atrophy, urinary tract infection, urinary tract disorders, pain, asthenia, peripheral edema, chest pain, fever, flu syndrome, malaise, syncope, headache, dizziness, hypoesthesia, paresthesia, nervousness, anorexia, weight loss, hyperglycemia, back pain, bone pain, arthritis, insomnia, depression, nausea, constipation, abdominal pain, dyspepsia, vomiting, diarrhea, gastrointestinal hemorrhage, dry mouth, dry skin, rash, and pruritus.
- *Flutamide* [4, 5]
  - *Mechanism of action.* Flutamide is a nonsteroidal agent that binds to AR, preventing androgen-AR link; it is also capable of inhibiting prostatic nuclear uptake of androgens, as well.
  - *Indication.* Flutamide is approved for the adjuvant and metastatic treatment of prostate cancer alone (with or without orchiectomy) or in combination with GnRH analogues/antagonists.
  - *Metabolism and route of elimination.* Flutamide is metabolized in the liver by CYP1A2 and is primarily excreted in urine.
  - *Side effects.* Common side effects are represented by diarrhea, nausea, vomiting, increased appetite, insomnia, weakness, hot flushes, impotence, decreased libido, gynecomastia`, altered hepatic enzymes, and transient hepatitis.
- *Bicalutamide* [4, 5]
  - *Mechanism of action.* Bicalutamide is a nonsteroidal agent that binds to AR competitively with androgens.
  - *Indication.* Bicalutamide is approved for the adjuvant and metastatic treatment of prostate cancer alone (with or without orchiectomy) or in combination with GnRH analogues/antagonists.
  - *Metabolism and route of elimination.* Bicalutamide is metabolized in the liver and equally excreted in urine and feces.
  - *Side effects.* Common side effects are represented by anemia, anorexia, diabetes, decreased libido, depression, somnolence, dizziness, myocardial infarction, cardiac failure, hot flashes, abdominal pain, constipation, nausea, diarrhea, dyspepsia, flatulence, hepatotoxicity, hypertransaminasemia, jaundice, alopecia, dry skin, rash, pruritus, hirsutism, musculoskeletal pain, hematuria, gynecomastia, impotence, asthenia, chest pain, edema, and increased body weight.

Noteworthy, most of the abovementioned common side effects of the three first-generation AR inhibitors are less frequent when these drugs are not combined with a GnRH analogue/antagonist.

10.9 Second-Generation AR Inhibitors

- *Enzalutamide* [4, 5]
  - *Mechanism of action.* Enzalutamide is a nonsteroidal agent that is capable of binding to AR, inhibiting androgens binding to their receptor; furthermore it is capable of inhibiting both AR nuclear translocation and binding to DNA.
  - *Indication.* Enzalutamide is indicated for the treatment of patients with metastatic castration-resistant prostate cancer.
  - *Metabolism and route of elimination.* Enzalutamide is hepatically metabolized, mostly by CYP2C8 and CYP3A4. Seventy-one percent of the dose

is excreted with urine; the rest is mostly excreted with feces.

- *Side effects.* Common side effects are represented by headache, dizziness, spinal cord compression and cauda equina syndrome, paresthesia, mental impairment disorders (such as amnesia, memory impairment, cognitive disorder, and disturbance in attention), hypoesthesia, dysgeusia, restless legs syndrome, seizures, hot flushes, peripheral edema, hypertension, pruritus, dry skin, gynecomastia, constipation, diarrhea, hematuria, pollakiuria, neutropenia, thrombocytopenia, hypertransaminasemia, hyperbilirubinemia, decreased appetite, decreased weight, back pain, arthralgia, musculoskeletal pain, muscular weakness, musculoskeletal stiffness, asthenia, falls, insomnia, anxiety, hallucinations, upper and lower respiratory tract infections, dyspnea, and epistaxis.

10.10 Androgen Synthesis Inhibitors

- *Abiraterone Acetate* [4, 5]
  - *Mechanism of action.* Abiraterone is a derivative of progesterone that selectively and irreversibly inhibits CYP17A1. This enzyme is involved in the androgens' synthesis by catalyzing the conversion of 17-hydroxypregnenolone to dehydroepiandrosterone (DHEA). Therefore, abiraterone effectively decreases serum levels of testosterone and other androgens by compromising its action.
  - *Indication.* Abiraterone acetate is indicated for the treatment of patients with metastatic castration-resistant prostate cancer, in combination with prednisone.
  - *Metabolism and route of elimination.* Abiraterone acetate is hydrolyzed into the active metabolite abiraterone via esterases, and then CYP3A4 and SULT2A1 are responsible for its hepatic metabolization into inactive compounds that are primarily excreted via feces and secondarily via urine (around 5%).
  - *Side effects.* Common side effects are represented by hypertransaminasemia, hyperbilirubinemia, hot flushes, hypertension, arrhythmia, chest pain/chest discomfort, angina pectoris, atrial fibrillation, tachycardia, myocardial infarction/ischemia, cardiac failure, joint swelling/discomfort, muscle discomfort, contusion, groin pain, fractures, falls, lymphopenia, anemia, hypertriglyceridemia, hypercholesterolemia, hyperglycemia, hypernatremia, hypokalemia, fluid retention/edema, hypophosphatemia, elevated alkaline phosphatase, asthenia, sepsis, fever, cough, upper respiratory tract infection, dyspnea, nasopharyngitis, urinary tract infections, hematuria, increased urinary frequency, nocturia, insomnia, constipation, diarrhea, dyspepsia, vomiting, and rash.

10.11 GnRH Agonists and Antagonists

10.11.1 The Hypothalamic-Pituitary Axis and the Clinical Use of Analogues/Antagonist of GnRH in Oncology

The hypothalamus is an endocrine organ localized in the diencephalon. It is a fundamental center of control for the endocrine system, receiving inputs from the body and other brain areas that stimulate or inhibit the production of several hypothalamic hormones responsible for the initiation of endocrine responses to environmental changes. More specifically, the hypothalamus produces a number of releasing hormones (CRH, GnRH, GHRH, TRH, and PRH) that stimulate anterior pituitary gland production and release of hormones that control a wide range of physiological functions by acting on numerous organs and tissues, such as TSH, FSH, LH, PRL, GH, and ACTH [9]. It is also responsible for the biosynthesis of ADH and oxytocin and transports them along axons to the posterior pituitary gland. It also produces and releases inhibiting hormones such as dopamine and somatostatin [8]. The production and release of these hormones are regulated by a complex mechanism of positive and negative feedbacks that ultimately involve peripheral hormones produced and released by the anterior pituitary gland, adrenal glands, thyroid, and gonads. An extensive discussion of this phenomenon is outside the scope of this chapter, but a brief explanation is important to understand the mechanism of action of GnRH agonists and antagonists. GnRH is a hypothalamic hormone responsible for the pituitary secretion of FSH and LH. These two hormones stimulate gonadal production of sexual steroidal hormones [9]. In the past years, a fundamental part of the treatment of endocrine-related breast and prostate cancer was based on the surgical castration with removal of ovaries or testicles. The advent of GnRH analogues and antagonists has dramatically reduced, if not ended, this surgical approach, improving patients' compliance to treatments and avoiding surgical and psychological side effects of these procedures. GnRH agonists' mechanisms of action are based on a continuous stimulation of the anterior pituitary gland. The initial stimulation may induce an initial increase in sex hormone levels and tumor growth, a phenomenon called tumor "flare-up," that might temporarily precipitate some symptoms, usually metastatic pain and urinary symptoms (in prostate

cancer). However, after an initial response, the continuous stimulation results in a pituitary desensitization to GnRH due to its receptor downregulation. Pituitary desensitization reduces the secretion of LH and FSH, thus inducing a state of iatrogenic menopause [10].

Differently from agonists, GnRH antagonists competitively and reversibly bind to GnRH receptors in the anterior pituitary glands, directly blocking FSH and LH secretion and quickly reducing the production and secretion of androgens and estrogens at gonadal level. Therefore, antagonists induce an iatrogenic menopausal status, as well, avoiding tumor flare-up [5].

10.12 GnRH Agonists

- *Goserelin, leuprorelin/leuprolide, and triptorelin* [4, 5]
  - *Mechanism of action.* It has been elucidated in the previous paragraph.
  - *Indication.* GnRH is indicated for the treatment of prostate cancer in both (neo)adjuvant and metastatic setting and for the treatment of pre- or perimenopausal women affected by breast cancer, in combination with other hormonal therapies.
  - *Metabolism and route of elimination.* The metabolism of triptorelin in humans is not well understood. Its elimination involves both the kidneys and liver. Goserelin has a hepatic metabolism and is mostly excreted with urine and feces. Leuprolide is mostly degraded by peptidases and excreted in urine.
  - *Side effects.* Common side effects are represented by hot flushes, impotence, decreased libido, skeletal pain, headache, hypertension, edema, chest pain, myocardial infarction, congestive heart failure, arrhythmia, peripheral vascular disorders, urinary tract infections, dysuria, decreased or loss of libido, sleep disorders, mood changes, emotional lability, depression, irritability, anemia, decreased red blood count, hypertransaminasemia, increased alkaline phosphatase, hyperglycemia, anorexia, increased BUN, flu-like syndrome, bronchitis, coughing, dyspnea, pharyngitis, pain in extremity, arthralgia, edema in legs, leg cramps, myalgia, musculoskeletal pain, muscle spasms, paresthesia in lower limbs, headache, dizziness, fatigue, asthenia, leg pain, peripheral edema, leg pain, back pain, fatigue, hyperhidrosis, rash, pruritus, hypersensitivity reaction, injection site pain, injection site reactions, injection site erythema, injection site inflammation, nausea, vomiting, constipation, dyspepsia, diarrhea, abdominal pain, abdominal discomfort, eye pain, and conjunctivitis. Moreover, in women other common side effects are represented by dyspareunia, dysmenorrhea, ovarian hyperstimulation syndrome, ovarian hypertrophy, pelvic pain, vulvovaginal dryness, bleeding/spotting, and genital hemorrhage and in men by testicular atrophy, breast pain, gynecomastia, and urinary retention. Initial administration is also associated with tumor flare-up, with consequent transient worsening of some tumor-related symptoms, in metastatic or locally advanced disease.

10.13 GnRH Antagonists

- *Degarelix* [4, 5]
  - *Mechanism of action.* It has been elucidated previously.
  - *Indication.* Degarelix is indicated for the treatment of advanced prostate cancer.
  - *Metabolism and route of elimination.* Degarelix is metabolized in the liver and excreted in urine (20–30%) and feces (70–80%).
  - *Side effects.* Common side effects are represented by hypertransaminasemia, increased γGT, injection site reactions, fever, fatigue, chills, asthenia, flu-like syndrome, anemia, hot flushes, QT/QTc interval prolongation, hypertension, abnormal BUN, abnormal creatinine level, increased or decreased weight, decreased weight, altered potassium level, antibody formation, hyperhidrosis (including night sweats), rash, nausea, constipation, diarrhea, urinary tract infections, gynecomastia, testicular atrophy, erectile dysfunction, back pain, arthralgia, musculoskeletal pain and discomfort, dizziness, headache, and insomnia.

10.14 Other Hormonal Agents

- *Octreotide* [4, 5, 11, 12]
  - *Mechanism of action.* Octreotide is a somatostatin analogue that has been administered for the treatment of carcinoid syndrome and hormonal excess syndromes associated with well-differentiated neuroendocrine tumors for a long time, due to its capability to inhibit insulin, glucagon, pancreatic polypeptide, gastric inhibitory polypeptide, and gastrin secretion. It has a much longer duration of action than somatostatin because of its greater resistance to enzymatic degradation. However, it has been recently demonstrated that the long-acting formulation (LAR), octreotide LAR, is also capable of delay time to tumor progression in patients with well- and moderately

differentiated metastatic NETs, regardless of their functional status. Its antitumor activity is relied on its action on somatostatin receptors expressed in tumor cells, which leads to a blockade of autocrine/paracrine growth-promoting hormone and growth factor production, inhibition of growth factor-mediated mitogenic signals, and induction of apoptosis. Furthermore, octreotide has also an indirect antitumor effect based on antiangiogenic action and inhibition of growth-promoting hormones and growth factor secretion. Its efficacy has been proven in different types of NETs.

- *Indication.* Octreotide is indicated for the symptomatic treatment of carcinoid syndrome and syndromes related to hormones secreted by well- or moderately differentiated NETs, especially with gastroenteropancreatic localization. The LAR formulation has also been recently indicated as an antitumoral agent for metastatic NETs, independently from their functional status.
- *Metabolism and route of elimination.* Around 30–40% of octreotide is metabolized in the liver, while 11–32% is excreted unchanged into the urine. The rest is excreted with feces.
- *Side effects.* Common side effects are represented by hypothyroidism, diabetes or hypoglycemia, anorexia, dizziness, headache, bradycardia, dyspnea, abdominal pain, dyspepsia, steatorrhea, cholecystitis, cholelithiasis, hyperbilirubinemia, pruritus, rash, alopecia, hypertransaminasemia, and pain in the site of injection.

- *Androgens, estrogens, and progestins* [2, 4, 5]
  - Androgens, estrogens and progestins in Medical Oncology have been replaced by more effective and less toxic endocrine agents that have been introduced in clinical practice during the last 40 years.
  - *Fluoxymesterone* is an androgen indicated for the treatment of metastatic HR+ breast cancer after progression on other more effective hormonal therapies. Few patients might experience antitumor responses for the last months or even years. The most common side effects are represented by hirsutism, male-pattern baldness, voice lowering, acne, enhanced libido, erythrocytosis, and elevated liver function tests.
  - High-dose estrogens, such as *estradiol*, provide an antitumor effect that was usually exploited in metastatic HR+ breast cancer before the introduction of tamoxifen and more recent, more effective, and better tolerated endocrine therapies. Although the mechanisms of such an antitumor effect remain still partially unknown, it could be addressed to the reduction in the secretion of FSH and LH by negative feedback and a sort of competitive effect on steroid receptors, as well. This is the reason why they proved to be, in a certain way, effective also in metastatic prostate cancer. In fact, their clinical indication in Medical Oncology is for the treatment of metastatic prostate cancer and HR+ breast cancer after failure of previous hormonal therapies. However, their clinical use is limited nowadays. Main side effects include nausea, vomiting, breast tenderness, darkening of the nipple-areolar complex, and thromboembolic events.
  - Progestins (*Medroxyprogesterone acetate* and *megestrol acetate*) have been classically used for the treatment of advanced HR+ breast cancer and hormonally responsive metastatic endometrial cancer. Moreover, megestrol acetate and medroxyprogesterone acetate have been occasionally used for the treatment of metastatic prostate cancer and advanced kidney cancer, respectively. However, their use as antitumor agents has been mostly replaced by more effective drugs. Progestins' antitumor effect is unclear but might be related to adrenal steroid synthesis suppression, lowering in ER levels, enhanced steroid metabolism, influence some growth factors, suppress plasma estrone sulfate formation, and direct killing of tumor cells. They are usually well tolerated, with main side effects represented by increased appetite and weight gain (therefore they are both indicated for the treatment of neoplastic cachexia). Among the less frequent ADRs, the most relevant are represented by suppression of adrenal steroid production by suppression of the pituitary-adrenal axis (although this effect appears to be asymptomatic in the majority of patients), edema, menstrual irregularities, and thromboembolic events (mostly with megestrol acetate).

The total daily dosage, route of administration, frequency of delivery, and target tumors for the main hormonal therapies are extensively reported in Table 10.2.

10.15 Endocrine Resistance and Newer Endocrine Agents

Hormonal therapies, such as almost all anticancer treatments, lose their efficacy due to the development of a broad range of molecular mechanisms of resistance. Tumors might be primarily resistant to hormonal agents, a condition called primary or de novo endocrine resis-

Table 10.2 Main available hormonal agents and standard dosages/schedules in solid tumors

Hormonal agent	Total dose	Route of delivery	Frequency of delivery	Type of cancer
Tamoxifen	20 mg	O	Daily	Breast cancer
Toremifene	60 mg	O	Daily	Breast cancer
Raloxifene	60 mg	O	Daily	Breast cancer (only for prevention)
Anastrozole	1 mg	O	Daily	Breast cancer
Letrozole	2.5 mg	O	Daily	Breast cancer
Exemestane	25 mg	O	Daily	Breast cancer
Fulvestrant	500 mg	IM	q2w for the first month, than q4w	Breast cancer
Nilutamide	Initial dose: 300 mg	O	Daily for 30 days	Prostate cancer
	Maintenance dose: 150 mg	O	Daily	Prostate cancer
Flutamide	250 mg	O	Daily	Prostate cancer
Bicalutamide	50–150 mg	O	Daily	Prostate cancer
Enzalutamide	160 mg	O	Daily	Prostate cancer
Abiraterone acetate	1000 mg	O	Daily	Prostate cancer
Goserelin	3.6 mg	SC	q4w	Prostate cancer, breast cancer
	10.8 mg	SC	q12w	Prostate cancer
Leuprolide acetate	3.75 mg	IM or SC	q4w	Breast cancer, prostate cancer
	7.5 mg	IM or SC	q4w	Prostate cancer
	11.25 mg	IM or SC	q12w	Breast cancer, prostate cancer
	22.5 mg	IM	q12w	Prostate cancer
	30 mg	IM	q16w	Prostate cancer
	45 mg	SC	q24w	Prostate cancer
	65 mg	SC implant	q48w	Prostate cancer
Triptorelin acetate	3.75 mg	IM	q4w	Breast cancer, prostate cancer
	11.25 mg	IM	q12w	Breast cancer, prostate cancer
	22.5 mg	IM	q24w	Prostate cancer
Degarelix	Initial dose: 240 mg	SC	One dose for the first month	Prostate cancer
	Maintenance dose: 80 mg	SC	q4w	Prostate cancer
Estradiol	30 mg	O	Daily	Breast cancer
	3–6 mg	O	Daily	Prostate cancer
	30 mg	IM	Qw or q2w	Prostate cancer
Fluoxymesterone	10–40 mg	O	Daily	Breast cancer

Table 10.2 (continued)

Hormonal agent	Total dose	Route of delivery	Frequency of delivery	Type of cancer
Medroxyprogesterone acetate	Initial dose: 400–1000 mg	IM	Qw	Endometrial carcinoma and kidney cancer
	Maintenance dose: 400 mg	IM	q4w	Endometrial carcinoma and kidney cancer
	200–400 mg	O	Daily	Endometrial carcinoma and kidney cancer
	≥400 mg	O	Daily	Breast cancer
Megestrol acetate	160 mg	O	Daily	Breast cancer
	40–320 mg	O	Daily	Endometrial carcinoma
Octreotide	Initial dose: 100–600 mcg	SC or IV	Daily	NETs[a]
	Maintenance dose range: 50–1500 mcg	SC or IV	Daily	NETs[a]
	Initial dose: 200–300 mcg	SC or IV	Daily	VIPoma
	Maintenance dose range: 750–150 mcg	SC or IV	Daily	VIPoma
Octreotide LAR	Initial dose: 20 mg	IM	q4w for 2 months	NETs[a]
	Maintenance: 10–30 mg	IM	q4w	NETsa

[a]excluding neuroendocrine carcinomas; *LAR* long-acting release, *NETs* neuroendocrine tumors, *O* orally, *IM* intramuscularly, *SC* subcutaneousl, *qw* every week, *q2w* every 2 weeks, *q4w* every 4 weeks, *q12w* every 3 months, *q16w* every 4 months, *q24w* every 6 months, *q48w* once every year

Attention: not all of the abovementioned formulations are worldwide available

tance, or develop a resistance at a certain point of their natural history, after a variable period of sensitiveness to endocrine treatments, a condition called secondary endocrine resistance.

The molecular mechanisms that underlie endocrine resistance are not fully understood and have been extensively investigated in the last few years, mostly in breast and prostate cancer. The most relevant are summarized as follows:

- *Breast cancer mechanisms of endocrine resistance* [13]
  - Increased activation, activity, or expression due to:
    - ER mutations
    - Altered gene regulation
    - Posttranscriptional modifications
    - Posttranslational modifications
    - Downregulation of co-repressors
    - Overexpression of co-activators
    - Increased expression of transcriptional factors
  - Cross talk between ER and TKRs
  - Overexpression of positive cell cycle regulators or reduced expression of negative regulators
  - Overexpression of antiapoptotic molecules or reduced expression of proapoptotic molecules
- *Prostate cancer mechanisms of endocrine resistance* [14]
  - Development of AR splice variants
  - Increased AR activation or expression due to:
    - Altered steroidogenesis
    - Mutation and overexpression of the receptor itself
    - Upregulation of co-activators that promote AR gene transcription
  - Altered drug efflux
  - Upregulation of glucocorticoid receptor
  - Deregulation of IL-6/STAT3 signaling
  - β-tubulin dysregulation
  - Aberrant regulation of molecules involved in cell survival and death

An in-depth discussion of these mechanisms is out of the scope of this chapter.

In order to overcome endocrine resistance, newer hormonal agents are under investigation, as well as alternative treatment strategies. These ones involve the study of specific sequences of different treatment lines, the combination of conventional endocrine agents with inhibitors of a variety of molecules involved in crucial signaling pathways (such as mTOR inhibitors or CDKi inhibitors), and sometimes the combination of endo-

crine agents with different and complementary mechanisms of action (such as the combination of AI and fulvestrant). Some of these strategies have been recently approved and have become a new standard of care, for example, the combination of letrozole and CDKi palbociclib or ribociclib in the first-line treatment of metastatic HR+ HER2 negative breast cancer. However, chapters focusing on breast and prostate cancer will provide a more thorough discussion on this topic.

Concerning breast cancer, one of the most relevant mechanisms of endocrine resistance, mostly in metastatic disease after prior hormonal therapies, is the development of ER gene activating mutations, which have been found in 14–40% of all metastatic HR+ breast tumors (in tumor specimens or in circulating tumor DNA) that developed secondary endocrine resistance [15, 16]. Thus, it is not surprising that great efforts have been made to develop newer treatment strategies or drugs to overcome this mechanism of resistance. Preclinical studies have shown that higher doses of fulvestrant or tamoxifen might be useful to overcome ER mutations-driven resistance, but also new SERMs with ER degrader function, as well, are under investigation, such as bazedoxifene. Moreover, ER degraders, elacestrant, GDC-0810 and GDC-0927, are also in early phases of clinical development [17].

In prostate cancer, a broad variety of new hormonal therapies are currently under investigation. Among newer compounds, the most promising seems to be [17]:

- AR degrader and CYP17 inhibitor galeterone (currently under investigation in phase III trials),
- AR ligand-binding domain antagonists apalutamide and darolutamide (phase III trials),
- Progesterone receptor inhibitors mifepristone and onapristone

Other AR degraders, CYP17 inhibitors, AR N-terminal domain inhibitors, and BET inhibitors are currently in early phase of development (preclinical or phase I or II clinical trials).

10

Key Points

Francesco Schettini

- Hormone therapies can be classified into three categories: SERM, AI, ER downregulators.
- Androgen signaling alterations are most involved in the development of prostate cancer.
- AR inhibitors are subdivided into three groups: first- and second-generation AR and androgen biosynthesis inhibitors.

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Targeted Therapy

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Contents

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Learning Objectives

By the end of the chapter the reader will:

- Have learned the basic concepts of targeted drugs therapeutically applicable in clinical setting;
- Have reached in depth knowledge of the most important targeted drug classes in oncology.

11.1 Introduction

The substantial increase in knowledge of many biological features of human cancer and the extraordinary biotechnological developments have been instrumental in identifying and validating a huge number of molecular targets for pharmacological approaches. Certainly, the progress in structural biology has also played a significant role in the successful design of compounds that bind and inhibit target proteins with high specificity [1]. This chapter provides an overview of the major classes of novel therapeutics and of their mechanisms of actions.

The term "targeted therapy" originally referred to inhibitors of biological targets that selectively drive malignant transformation. This would imply that the drug inhibits a cancer-specific driver that is not expressed in normal tissues.

Unfortunately, this is the case only in few instances. Many oncologists now use "targeted therapy" in a loose way, indicating compounds acting on molecular targets that are not necessarily cancer-specific, such as kinases or enzymes involved in cell growth or angiogenesis, setting them apart from the class of conventional chemotherapeutics. Actually, no clear distinction can be made between targeted therapy and chemotherapy, either regarding the higher selectivity for tumor cells versus normal tissues, or the efficacy against tumors bearing specific mutations. For example, cisplatin, that nobody classifies as a targeted agent, is particularly effective against tumors carrying specific defects in DNA repair mechanisms. It is not only a semantic issue, but it is conceptually important as it deals with the degree of selectivity of treatments.

In our opinion, it seems more adherent to reality and medically useful to classify new anticancer drugs based on different putative targets, defining their most frequent toxicities and their spectrum of activity. As emphasized at different points of this chapter, some cancers are addicted to a specific oncogene, and in this case the concept of targeted drug makes sense and is therapeutically applicable. In the majority of cases, however, we still try to use the available drugs to the best of our knowledge, knowing that their action will cause not only antitumor activity but also toxicity to normal tissues, e.g. bone marrow for drugs causing DNA damage, cardiovascular for antiangiogenic drugs, etc.

11.2 Targeting Receptor Tyrosine Kinases and Related Pathways

Kinases are one of the most important drug target classes in oncology, with over 40 drugs currently approved for a variety of solid tumors and leukemias, and many more in clinical development (Table 11.1). Tyrosine kinase activity was identified early on as a common feature of many oncogenic proteins, both at the cell membrane and intracellular levels, and targeting such activity had yielded the first undisputable proof of therapeutic concept with the development of imatinib for the treatment of chronic myeloid leukemia.

In solid tumors the major focus has been on receptor tyrosine kinases (RTKs), mainly, but not exclusively,

Table 11.1 BER = base excision repair; SSBR = single strand break repair; A-EJ = alternative-end joining; NHEJ = non-homologous end joining; HRR = homologous recombination repair

Class	Pathways	Targets	Inhibitors
Sensors and signalling proteins of DNA damage	BER, SSBR, A-EJ	PARP	Olaparib (approved), niraparib (approved), rucaparib (approved), veliparib, talazoparib
	NHEJ	DNA-PK	M3814, VX-984
	HRR	ATM	AZD0156
	HRR	ATR	VX-970, AZD6738
Cell cycle checkpoint regulators	G1/S checkpoint	CHEK1	AZD7762, PF-00477736, XL-844, LY2603618, MK-8776
	G2/M checkpoint	WEE1	AZD1775

belonging to the human epidermal growth factor receptor (HER) family. RTK activation generally requires interaction of the extracellular domain of the receptor with its cognate ligand(s), followed by receptor dimerization and transphosphorylation of the two subunits at critical tyrosine residues.

The newly phosphorylated sites enable activated receptors to recruit intracellular substrates participating in signal transduction pathways, most notably the RAS-RAF-MEK-ERK and the PI3K-AKT-mTOR pathways, involved in cell survival, proliferation, migration and invasion, as well as in tumor angiogenesis.

Constitutive RTK activation, resulting from gene mutation/amplification, autocrine ligand production or, less frequently, truncation or proteolytic cleavage of the extracellular receptor domain, has been observed to drive the growth and progression of a wide range of solid tumors. Tumor cells are known to develop a form of addiction to driver aberrant signaling pathways, a phenomenon that is commonly referred to as oncogene addiction [2], and that provides a window of opportunity for targeted therapies, as normal, non-cancer cells are much less likely to develop dependence on the same pathway. Notably, RTK-dependent pathways are characterized by a high degree of redundancy and by the presence of feedback regulatory circuits [3]. These two features play important physiological roles, by allowing collateral pathways to take over in case of temporary failure of one specific pathway, and by preventing intracellular signaling from escalating to uncontrollable levels, but they are far less prominent in cancer cells. However, when under pressure, cancer cells can also recover these properties, which may profoundly affect the long term therapeutic success of targeted agents, as will be detailed below.

The Human Epidermal growth factor Receptor (HER) family of RTKs has been shown to be frequently altered in a variety of solid tumors and has been considered as a prime target for drug development. The family includes four members: HER1, also known as the Epidermal Growth factor receptor (EGFR), HER2, HER3 and HER4. A considerable redundancy seems to characterize the receptors in the family and their respective ligands, as any given receptor can be activated by more than one ligand, and any given ligand can activate more than one receptor. A notable exception is HER2, for which no physiological ligand has been identified, qualifying it as an orphan receptor. While HER4 has been proposed to play a protective role in normal tissues, and especially in the myocardium, the other three family member have been implicated in a number of tumor types.

11.2.1 Agents Targeting EGFR

EGFR is activated in a number of tumor types, including two of the most common and deadly cancers, i.e. non-small cell lung cancer (NSCLC) and colorectal cancer (CRC). Oncogenic activation of this RTK may depend on amplification/overexpression of the wild-type gene, as observed in CRC and squamous cell head and neck cancers (SCHNC), or on point mutations that affect the intracellular domain of the receptor resulting in constitutive catalytic activity, as occurs in a subset of NSCLC. Both monoclonal antibodies and kinase inhibitors have been developed to inhibit aberrant EGFR activity. Importantly, the former appear to be particularly active against tumors overexpressing the receptor, whether in its wild type or mutated form, whereas the latter are more effective in the presence of activating mutations in the region encoding the catalytic domain (Fig.11.1).

The first EGFR-targeted monoclonal antibody to enter the clinic was *cetuximab* (Erbitux®), a chimeric IgG1 antibody that owes its anticancer effect to a dual mechanism: binding of the Fab' regions to EGFR and subsequent inhibition of ligand binding curtails downstream signal transduction and promotes receptor internalization, whereas the Fc segment recruits immune effector cells and the complement system to induce antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). As cetuximab is unable discriminate EGFR between normal and tumor cells, its therapeutic success in CRC and in SCHNC very likely relies on their oncogenic addiction to EGFR overexpression, that is not shared by normal tissues or by other tumor types. In contrast, cetuximab does not afford any major clinical benefits in patients with $EGFR^{MUT}$ NSCLC, unless the mutant receptor is also overexpressed [4]. Cetuximab was soon followed by an EGFR-targeting fully human monoclonal antibody called *panitumumab.* (Vectibix®) As compared to its predecessor, panitumumab does not contain any murine sequences, and therefore has the advantage of a lower immunogenic potential; on the other hand, it is a IgG2 and this IgG class is significantly less effective than IgG1 in recruiting immune effector cells. The last addition to the family of monoclonal antibodies directed against EGFR was *necitumumab* (Portrazza®), also fully human. In contrast to the two other members of the class, necitumumab has been approved for metastatic squamous non-small cell line cancer, based on frequent EGFR overexpression in this NSCLC subtype. Like cetuximab, necitumumab is an IgG1, so recruitment of immune effector cells is expected to make a significant contribution to its anticancer activity.

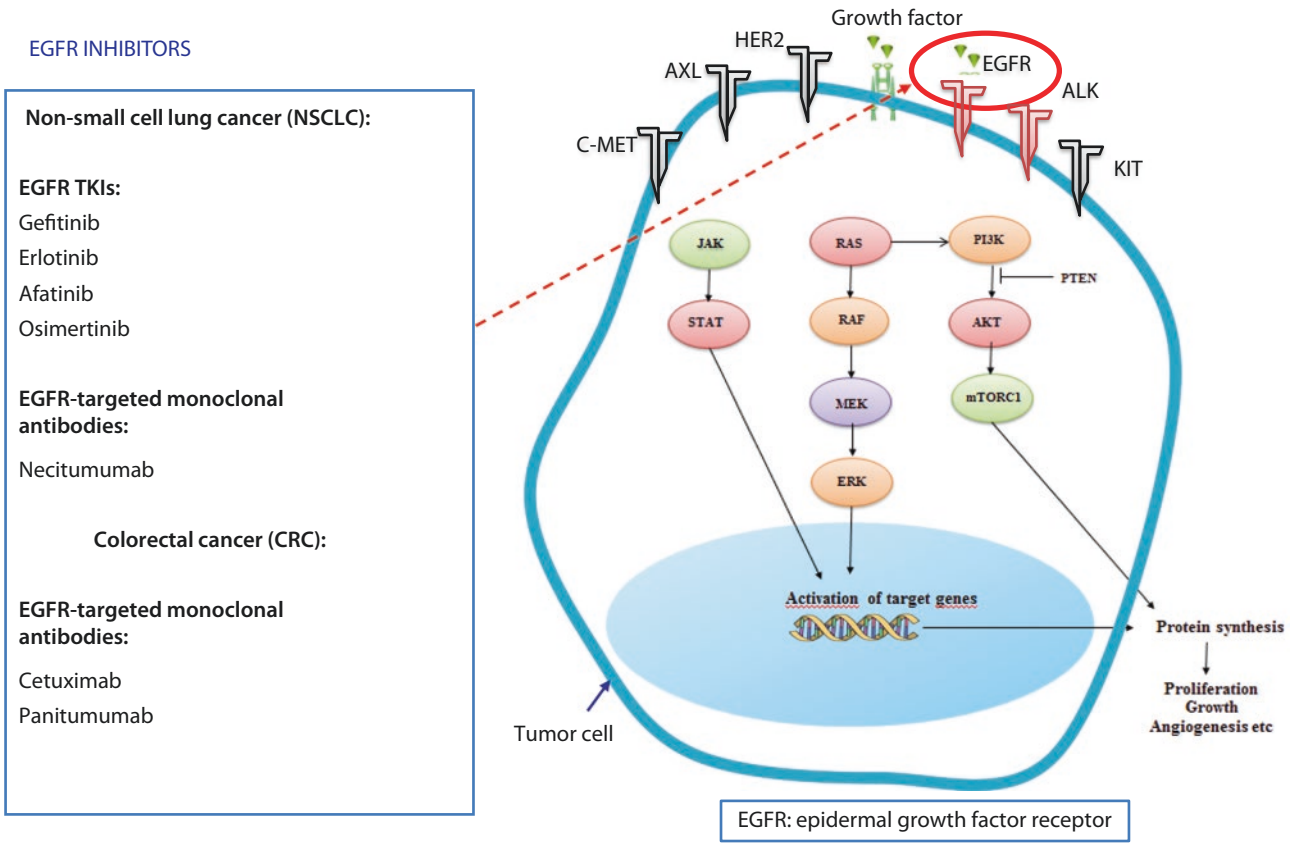

Fig. 11.1 Agents targeting EGFR in non-small cell lung cancer (NSCLC) and colorectal cancer (CRC)

Cetuximab	Monoclonal chimeric IgG1 antibody targeting EGFR, especially effective in cancers overexpressing the receptor (e.g. CRC, head and neck cancer). Recruitment of immune effector cells (ADCC) and of the complement system (CDC) contributes to its anticancer effect.
Panitumumab	Fully human monoclonal IgG2 antibody targeting EGFR. Less immunogenic than cetuximab, but less effective in recruiting ADCC and CDC
Necitumumab	Fully human monoclonal IgG1 antibody targeting EGFR. It has been approved for the treatment of metastatic squamous NSCLC

Resistance to EGFR-targeting monoclonal antibodies can occur through different mechanisms, that can be grouped into two major categories: "EGFR-dependent" mechanisms refer to the inability of the agents to block their intended target, while the target itself maintains its driver role in the tumor; in contrast, "EGFR-independent" mechanisms lead to lack of clinical response in spite of continuing target inhibition.

Ligand overexpression, EGFR amplification, acquisition of mutations in the extracellular domain are examples of EGFR-dependent mechanisms, and simply changing the specific agent can restore the receptor block. A case in point is the acquisition of the S492R mutation, which causes resistance to cetuximab, while retaining the ability to bind panitumumab [5]. "EGFR-independent" mechanisms include activation of parallel pathways that regulate the same downstream pathways (trough overexpression/activating mutations of other RTKs), as well as activation of downstream steps in the signaling cascade. RAS mutations belong to the second category; the presence of activating RAS mutations is assumed to predict innate resistance to EGFR-targeting monoclonal antibodies, which led to routine testing for KRAS mutations prior to starting cetuximab or panitumumab-based therapy in CRC patients [6].

Regarding low molecular weight kinase inhibitors, the development of agents directed at EGFR got off to a bad start: *gefitinib* (Iressa®) was first approved in 2003 for use in combination with chemotherapy in patients with advanced NSCLC, but the low response rates achieved in unselected cohorts of patients caused the FDA to retract its approval in 2005. However, a subset

of patients, predominantly never-smoking women of Asian ethnicity with adenocarcinoma histology, bearing activating mutations in the EGFR gene (exon 19 deletions or the L858R substitution in exon 21), had derived distinct benefits from treatment with gefitinib [7]. Thus, new clinical trials were designed only including $EGFR^{MUT}$-positive patients, and their results not only paved the way to the approval of gefitinib specifically for the first-line treatment of NSCLC patients with metastatic cancer bearing EGFR mutation, but also led to affirmation of an important new paradigm, i.e. that targeted therapy clinical trials should focus on patients with the specific mutation that is targeted by the drug under trial.

Another first-generation EGFR kinase inhibitor, *erlotinib* (Tarceva®), is also currently approved for first-line treatment of metastatic NSCLC patients with EGFR-activating mutations; however, this agent has a further important indication for first-line treatment of locally advanced, unresectable or metastatic pancreatic cancer, as well as orphan drug status in malignant glioma. The move from first- to second-generation EGFR inhibitors was prompted by the observation that NSCLCs relapsing following treatment with first-generation agents frequently acquired secondary mutations, most notably the T790M substitution, affecting the so called 'gatekeeper' aminoacid residue, that controls access of ATP and its competitors to the ATP-binding site. Both first-generation EGFR kinase inhibitors act by binding reversibly and with relative low affinity to ATP-binding site, and their binding is effectively precluded by the T790M mutations. Thus, a second 'wave' of EGFR inhibitors were developed, with a distinct mechanism of action from their predecessors.

Afatinib (Gilotrif®) is an orally bioavailable aniline-quinazoline derivative with a reactive acrylamide group that can covalently modify conserved cysteine residues within the catalytic domains of EGFR, HER2 and HER4, irreversibly blocking their kinase activity. Afatinib was approved for NSCLC harboring three additional EGFR mutations (L861Q, G719X and S768I) besides the two already mentioned, most common ones. In addition, binding of afatinib to C797 in the EGFR catalytic site can occur in spite of the T790M mutation; however, the drug has a greater affinity for $EGFR^{WT}$ than for $EGFR^{T790M}$, and this results in inefficient inhibition of $EGFR^{T790M}$ at clinically achievable concentrations of the drug. The most intriguing finding about afatinib is that NSCLC patients harboring $EGFR^{del19}$ derive a much greater benefit from treatment with this agent than patients with $EGFR^{L858R}$, highlighting the fact that response of EGFR mutant tumors depends on the nature of the specific activating mutation [8].

With the advent of third-generation irreversible EGFR inhibitor *osimertinib* (Tagrisso®), resistance due to the T790M mutation was finally overcome, as this inhibitor binds to EGFR bearing sensitizing and/or resistance-inducing mutations with significantly greater affinity than to the wildtype receptor, leading to a better safety profile.

Besides the acquisition of further mutations in the EGFR gene, a typical EGFR-dependent mechanism that guided the development of sequential generations of EGFR inhibitors, resistance to EGFR-targeting TKIs may occur through amplification of the mutated gene, providing a possible rationale for TKI/monoclonal antibody combinations. In addition, all approved EGFR TKIs have been shown to act as substrates for ABC transport proteins ABCB1 (P-glycoprotein) and ABCG2, that extrudes them from tumor cells and restricts their access to brain tumors and metastases.

Among EGFR-independent mechanisms, besides the already mentioned activation of parallel or downstream pathways, intrinsic resistance can also result from a deletion polymorphism in the gene encoding the proapoptotic protein BIM, which results in impaired activation of the apoptotic process following exposure to first-generation EGFR TKIs [9].

Gefitinib	First-generation kinase inhibitor targeting active mutant EGFR, used in the treatment of NSCLC
Erlotinib	First-generation EGFR kinase inhibitor used in the treatment of metastatic NSCLC and pancreatic carcinomas
Afatinib	Second-generation TKI that irreversibly blocks EGFR, HER2 and HER4 kinase activity, used in the treatment of metastatic NSCLC
Osimertinib	Third-generation irreversible EGFR inhibitor that is able to overcome the resistance due to the gatekeeper T790M mutation

11.2.2 Agents Targeting HER2

As mentioned above, HER2 differs from the other members of the family for the lack of physiological ligands. Oncogenic activation frequently depends on overexpression, that is observed in 15–20% of breast cancers (with or without overlap with hormone receptor expression), and in gastric and gastroesophageal junction carcinomas, the current clinical indications for use of HER-targeting agents; however, point mutations or loss of the extracellular domain, leading to constitutive activation, are also encountered in NSCLC and glioblastoma).

Overexpression increases the likelihood of spontaneous receptor homodimerization and ligand-independent heterodimerization with other members of the family, causing constitutive activation of downstream pathways, and it also facilitates formation of HER2-containing heterodimers following activation of other members of the family by their cognate ligands.

To date, four agents targeting HER-2 are available worldwide for the treatment of HER2+ breast cancer; their use has dramatically increased the median overall survival (now exceeding 50 months) and the 5-year survival rate of patients with this tumor type [10]. The first agent to be approved in this class was *trastuzumab* (Herceptin®), a humanized monoclonal antibody that binds to a subdomain of the extracellular portion of the receptor, that appears to be involved in ligand-independent dimer formation, curtailing downstream signal transduction and recruiting cells in the patient's immune system to activate ADCC and CDC. In addition, trastuzumab inhibits HER2 signaling by preventing cleavage of the HER2 extracellular domain, which would create a functionally active truncated isoform of HER2 (p95-HER2), contributing to tumor progression.

However, trastuzumab is less effective in inhibiting HER2 ligand-dependent heterodimerization with other members of the family (EGFR, HER3), and this led to the development and regulatory approval of a second humanized antibody, directed against a different extracellular epitope of HER-2, called *pertuzumab* (Perjeta®); nowadays a combination of the two (horizontal dual blockade), together with standard chemotherapy, is considered the best first-line approach to the treatment of HER2+ breast cancers. *Trastuzumab-emtansine* (also known as T-DM1 and Kadcyla®) is an antibody-drug conjugate (ADC) consisting of trastuzumab conjugated to a highly potent microtubule-targeting agent called mertansine (DM1) by a stable, non-reducible thioether linker. DM1 would cause unacceptable toxicity if given alone, but targeted delivery by trastuzumab to HER2-expressing breast cancer cells significantly improves its efficacy, while reducing the side effects. Upon binding of trastuzumab to HER2 on tumor cell surface, the ADC complex is internalized by receptor-mediated endocytosis and subsequent lysosomal degradation of the protein component releases DM1, which inhibits microtubule polymerization.

T-DM1 is currently used as second-line monotherapy following failure of the trastuzumab/pertuzumab combination with cytotoxic agents. Notably, it has a good safety profile, causing fewer serious adverse effects than most of other treatments regimens in HER2-positive breast cancer (Fig. 11.2).

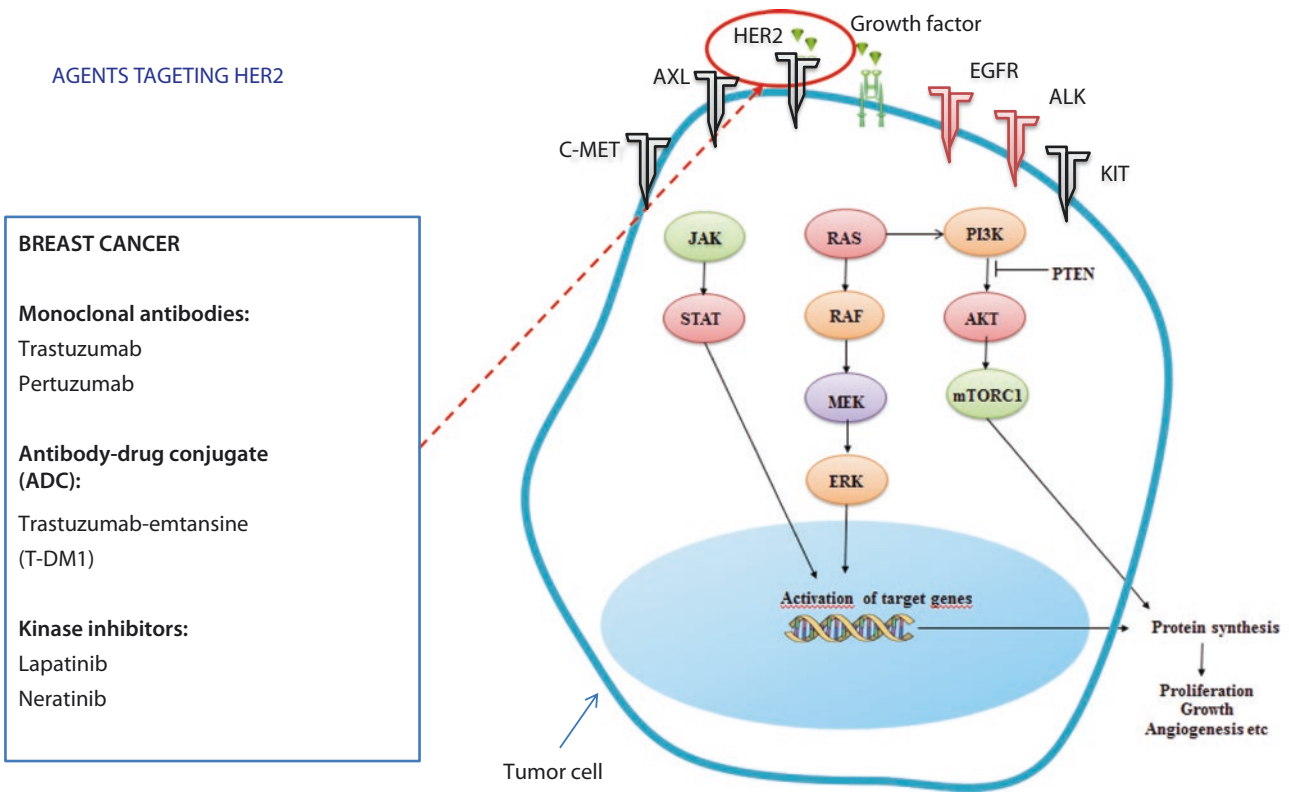

Fig. 11.2 Agents targeting HER-2 in breast cancer

Trastuzumab	Humanized monoclonal antibody targeting HER2. It inhibits ligand-independent dimer formation, thereby curtailing downstream signal transduction, and activates ADCC and CDC
Pertuzumab	Humanized monoclonal antibody targeting HER2 at an epitope different from trastuzumab. The two antibodies are frequently combined to achieve a horizontal dual blockade
Trastuzumab-emtansine (T-DM1)	Antibody-drug conjugate (ADC) obtained by conjugating trastuzumab to a highly potent microtubule-targeting agent

Resistance to trastuzumab can develop though a host of HER2-dependent and independent mechanisms [11] among the former, binding to HER2 may be reduced by epitope masking through increased expression of mucin 4 at the cell membrane and increased levels of CD44/hyaluronan complexes or by enzymatic cleavage of the extracellular domain of the receptor; in addition, mutations have also been reported to reduce trastuzumab binding and/or cause reactivation of the receptor. However, HER2-independent mechanisms, based on activation of downstream pathways and especially on upregulation of other receptors insisting on the same signaling pathways are probably more common. A receptor that is frequently involved in trastuzumab resistance is the HER3 receptor. This member of the HER family is kinase dead, and can only signal following heterodimerization with other members in the family. HER3 upregulation is a direct consequence of inhibition of RAF-MEK-ERK and PI3K-AKT-mTOR pathways downstream of HER2, because this causes the removal of negative feedback regulation of HER3 expression [12], and in cells overexpressing HER2 this results in increased HER2/HER3 dimer formation, a condition that drastically reduces the effect of trastuzumab, but could be overcome by concomitant use of pertuzumab or by HER2-directed TKIs. Antibodies targeting HER3 are also under preclinical and clinical development.

Besides antibody-based therapies, low molecular weight kinase inhibitors have also been developed, that target the catalytic domain of both HER2 and EGFR. *Lapatinib* (Tykerb®) is a reversible inhibitor that was approved in 2007 for use in combination with capecitabine in HER2 positive breast cancer, and later, in 2010, for use in combination with the aromatase inhibitor letrozole hormone receptor/HER2-positive breast cancer. However, lapatinib is more toxic and less active than trastuzumab in HER2 positive breast cancer [13]. Thus, lapatinib is currently reserved for use as an addition to trastuzumab to achieve a "vertical dual-blockade" of HER2, by targeting both extracellular and intracellular domains, in later lines of treatment in patients who cannot tolerate cytotoxic chemotherapy or with brain metastases.

Neratinib (Nerlynx®) is yet another kinase inhibitor that binds irreversibly to EGFR, HER2 and HER4. It was granted FDA approval in 2017 for the extended adjuvant treatment of adult patients with early stage HER2-positive breast cancer, following adjuvant trastuzumab-based therapy. However, a few months later, this same agent received a negative review from EMA, stating that the benefit achieved (an increase in 2-year survival rate from 92 to 94%) was not enough to outweigh the risk of side effects, including hard-to-manage diarrhea.

Lapatinib	Low molecular weight kinase inhibitor that targets the catalytic domain of HER2 and EGFR. it is currently combined with trastuzumab to achieve a "vertical dual-blockade" of HER2
Neratinib	Low molecular weight kinase inhibitor that binds irreversibly to EGFR, HER2 and HER4, only approved in the U.S.

11.2.3 ALK Inhibitors

Anaplastic lymphoma kinase (ALK) belongs to the insulin receptor superfamily and has been implicated in different cancer types. Oncogenic activation of the ALK gene can occur through translocation, as observed in lymphomas and NSCLC, whereby 22 different translocation partners have been identified; the product of the fusion gene loses its transmembrane location and becomes constitutively active. Alternatively, ALK can be activated by point mutations, that are more common in neuroblastoma and thyroid cancer. ALK rearrangements, most commonly the EML4-ALK translocation, are present in 3–5% of NSCLC, and were an unfavorable prognostic marker in the era preceding the development of ALK inhibitors [14].

Crizotinib (Xalkori ®) was the first clinically approved drug to target ALK in ALK-positive NSCLC. It is an orally active and well tolerated drug, that affords a significant improvement in overall and progression-free survival over chemotherapy in the target patient subpopulation. However, secondary mutations within the ALK kinase domain have been identified that lead to acquisition of drug resistance; most notably, a gatekeeper mutation L1196M, equivalent to the T790M substitution in

EGFR, has been observed in relapsed ALK positive NSCLC following crizotinib treatment. Second-generation inhibitors *ceritinib* (Zykadia®), *alectinib* (Alecensa®), and *brigatinib* (Alunbrig®) and the third-generation inhibitor lorlatinib (granted Priority Review in 2018) were specifically developed for sequential use following failure of previous generation therapies. Intriguingly, a recent report indicates that an ALK mutation selected by lorlatinib treatment (L1198F) may restore crizotinib sensitivity in ALK positive NSCLC [15]. All currently approved ALK inhibitors except alectinib also inhibit a further receptor kinase, ROS-1. Rearrangements of the ROS-1 gene leading to constitutive kinase activity are present in 1% of NSCLC, thus expanding the target population for crizotinib and its successors. In addition, crizotinib also inhibits the activity of MET, the membrane receptor for HGF, a property that is not shared by any other approved ALK inhibitor, whereas brigatinib is a dual ALK/EGFR inhibitor. Interestingly, while crizotinib has a very poor penetration into the CNS, second- and third-generation agents are far better at crossing the blood-brain barrier, and are accordingly far more likely to address brain metastases. Finally, recent reports suggest that ALK TKIs might be useful in the treatment of other ALK-positive cancer types besides NSCLC (Fig.11.3).

Crizotinib	Low molecular weight kinase inhibitor targeting ALK in ALK-positive NSCLC; it also inhibits the activity of ROS-1 and cMET
Ceritinib, alectinib, brigatinib	Second-generation ALK inhibitors specifically developed for sequential use following failure of previous generation therapies. Ceritinib and brigatinib also inhibit ROS-1

11.2.4 BRAF/MEK/ERK Inhibitors

The Ras-Raf-MEK-ERK pathway relays signals from cell surface receptors to the nucleus, activating a phosphorylation cascade that regulates cell growth, differentiation, and survival. RAS oncogenes (KRAS, N-RAS and H-RAS) encode a small family of cytoplasmic GTPases that under physiological conditions are transiently activated upon membrane recruitment by activated RTKs. Membrane-anchored RAS proteins promote the formation of crucial multiprotein complexes, leading to activation of downstream pathways. Aside from aberrant stimulation by upstream RTKs, constitutive activation of this pathway can depend on mutations in RAS, RAF and MEK genes, leading to unchecked ERK activity. Although RAS genes are arguably the most frequently

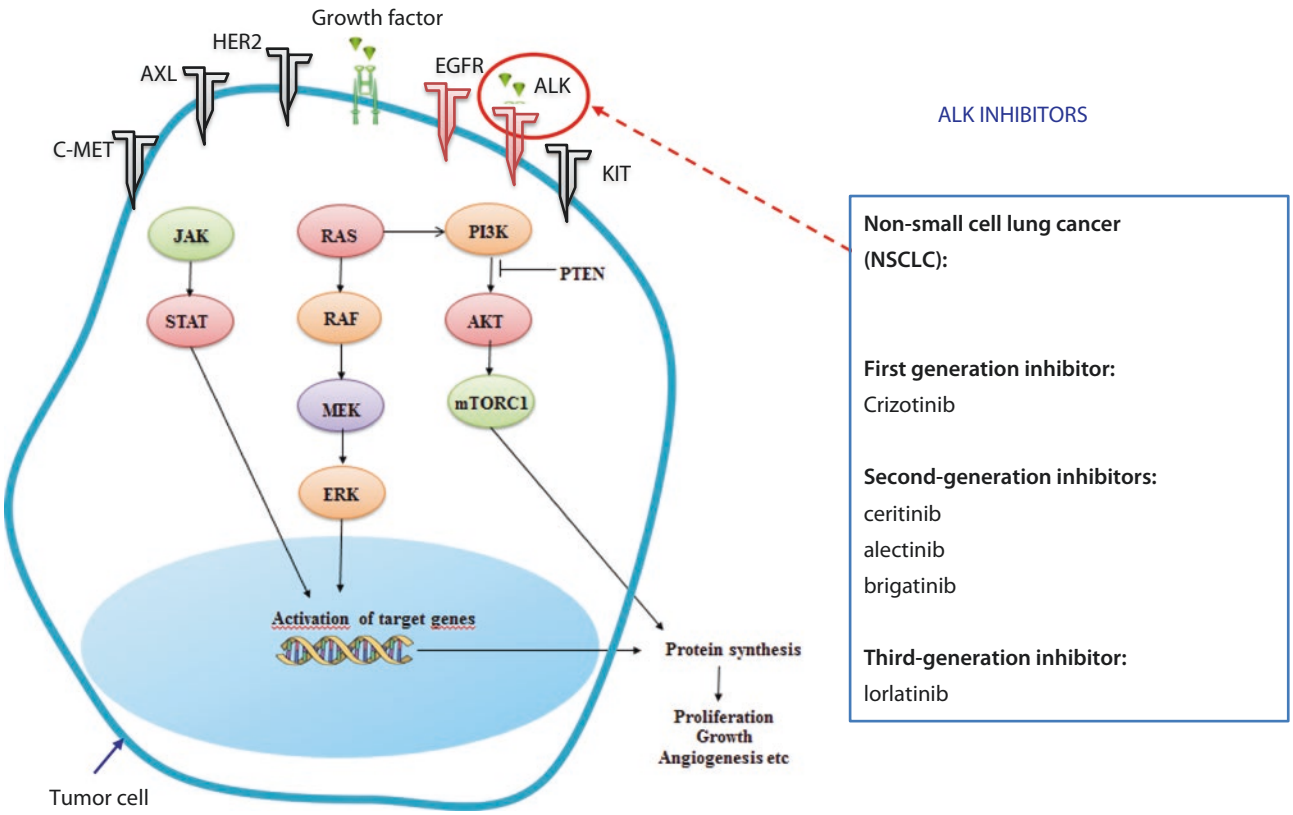

Fig. 11.3 Anaplastic lymphoma kinase (ALK) inhibitors in lung cancer

mutated oncogenes in solid tumors, RAS proteins have proven extremely elusive as drug targets, and no RAS-targeting agent has been approved for clinical use so far [16]. In contrast, downstream steps in the cascade have proven more amenable to drug modulation and currently both RAF and MEK inhibitors are used in the clinic, often in combination, whereas research on ERK inhibitors has yet to produce viable clinical candidates.

RAF proteins (ARAF, BRAF and CRAF) are serine/threonine kinases that become activated following membrane recruitment by RAS-GTP and phosphorylation. BRAF is the most frequently mutated member of the family in human cancers, including melanomas (50%), papillary thyroid cancers (45%), colorectal carcinomas (10%), non-small cell lung cancer (10%), hairy cell leukemia (~ 100%), and Langerhans cell histiocytosis (50–60%). The most common BRAF mutation is the 1799 T > A transversion, resulting in a change from valine to glutamic acid at the 600 position in the activation segment of the kinase domain and in a several-fold increase of its catalytic activity. In addition to V600E, and other V600 substitutions (V600K/D/R), a number of non-V600 missense mutations have been found, as well as fusion proteins resulting from translocations containing the catalytic domain of BRAF and in-frame deletions, causing RAS-independent dimerization and constitutive activation of the kinase domain.

The presence of $BRAF^{V600}$ mutations was identified as a potent driver for the growth and progression of melanomas, which led to the development of RAF inhibitors for the treatment of advanced disease, a condition hitherto characterized by a very poor prognosis.

First-generation RAF inhibitors were developed before the discovery of activating BRAF mutations and were basically directed at CRAF. The only first-generation inhibitor to have gained regulatory approval is *sorafenib* (Nexavar®); however, the activity of sorafenib in BRAF mutant tumors is negligible, and its clinical efficacy in hepatocellular carcinoma and renal cell carcinoma is probably due to its ability to inhibit multiple kinases [17].

In the wake of the discovery of BRAF mutations, second-generation inhibitors were developed based on their ability to inhibit BRAF V600E, which led to the approval of *vemurafenib* (Zelboraf®) and *dabrafenib* (Tafinlar®), and more recently of encorafenib (Braftovi™). All these agents induce dramatic responses, at least initially, in melanomas bearing the V600E or V600K mutations, leading to the remission of even advanced lesions. However, several important observations have emerged [18]: *a)* the response to these agents in melanoma patients is typically short-lived, generally due to reactivation of ERK signaling; *b)* both vemurafenib and dabrafenib have shown modest clinical activity when used for mutant BRAF tumors other than $BRAF^{V600}$ melanoma (e.g. CRC and thyroid cancers) bearing the same BRAF mutation. Clinical trials testing the efficacy of encorafenib on colorectal cancer are underway; *c)* while vemurafenib and dabrafenib significantly reduce ERK signaling in cells bearing $BRAF^{V600}$ mutation, paradoxical ERK activation is observed in cells harboring $BRAF^{wt}$, especially when RAS is aberrantly activated by mutations or enhanced upstream signaling. In contrast, encorafenib shows a similar IC_{50} value on mutated $BRAF^{V600}$ and wildtype BRAF, and should therefore be devoid of paradoxical effects; *d)* vemurafenib treatment is associated with increased incidence of secondary skin tumors (keratoacanthomas, squamous cell carcinomas), that disappear upon treatment discontinuation (Fig. 11.4).

A number of recent studies have begun to shed some light on the biochemical bases of the unique features of BRAFi [19]. Basically, in melanoma cells $BRAF^{V600}$ mutant proteins coexist with low RAS-GTP levels, largely due to negative feedback regulation that depends on overactive ERK signaling. Under these conditions $BRAF^{V600}$ mutants exist as active monomers that are exquisitely sensitive to BRAFi. However, several mechanisms have been observed to cause $BRAF^{V600}$ dimerization in these cells, including $BRAF^{V600E}$ amplification, expression of splice variants of $BRAF^{V600E}$, RAS mutations, upregulation of upstream RTKs (not very frequent in melanoma). Most importantly, BRFAi themselves contribute to this scenario by inhibiting ERK signaling and relieving the feedback constraint on RAS activity and RTK expression. As BRAFi are far less effective in inhibiting the activity of $BRAF^{V600}$ dimers, all these mutational or adaptive changes can account for persistent ERK activation and emergence of resistance. Along this line of reasoning, the fact that $BRAF^{V600E}$ expressing tumors other than melanoma are refractory to BRFAi might be explained by the fact that melanoma cells are less adept at upregulating membrane RTKs than CRC or thyroid cancer cells upon removal of ERK-mediated negative feedback by BRAFi [20]. On the other hand, the ability of BRAFi to discriminate between monomeric and dimeric forms of RAF proteins provides the wide therapeutic window available for these agents.

Sorafenib	First-generation RAF inhibitor; it inhibits multiple RTKs, simultaneously targeting tumor growth and angiogenesis. However, its effect in BRAF mutant tumors is negligible
Vemurafenib dabrafenib	Second-generation RAF inhibitors, specifically targeting $BRAF^{V600E}$ in malignant melanoma
Encorafenib	Second-generation RAF inhibitor with similar potency against $BRAF^{V600E}$ and $BRAF^{V600E}$, approved for malignant melanoma in 2018 and currently under clinical trials in $BRAF^{V600}$-mutated CRC

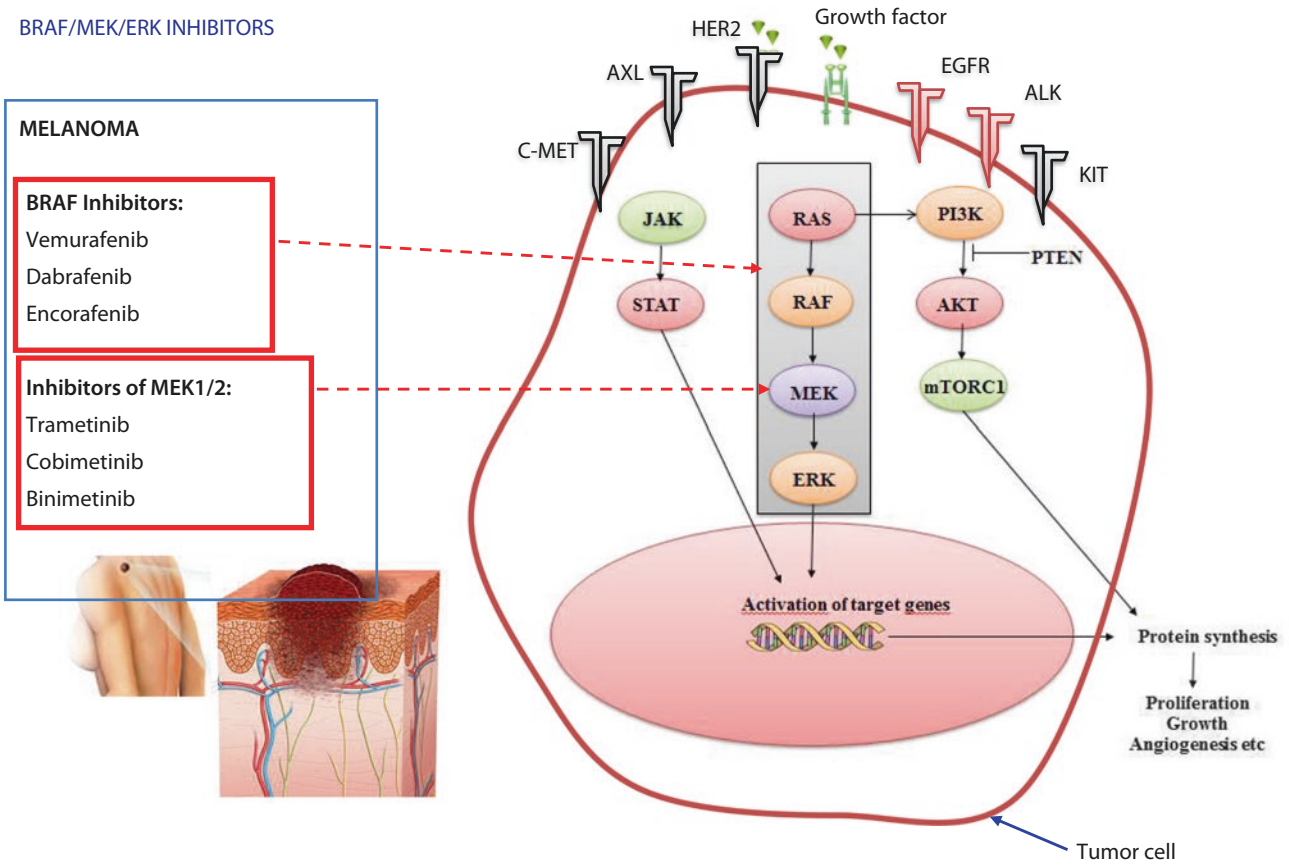

Fig. 11.4 BRAF/MEK inhibitors in malignant melanoma

Studies of the conformational changes induced by BRAFi in RAF dimers may help explain the paradoxical ERK activation induced by therapeutic concentrations of these agents in $BRAF^{WT}$ cells with active RAS [21]. The current model predicts that in such cells BRAF and CRAF will predominantly exist as dimers. Binding of the inhibitor to the first protomer in each dimer will stabilize the inactive conformation of the bound protomer, but will cause transactivation of the unbound protomer by promoting the RAF-RAS-GTP interaction, as well as the already mentioned drastic reduction in affinity for the inhibitor. This paradoxical effect is probably the cause of the observed increase in the incidence of skin lesions during treatment with vemurafenib. To avoid ERK activation by dimeric RAF, two different classes of third generation inhibitors are currently under preclinical development: the former (e.g. TAK632, MLN2480, LY3009120) are designed to equipotently inhibit both monomeric and dimeric forms of RAF (which will probably result in a significant loss in tumor selectivity), whereas the latter, named "paradox breakers" (PLX7904, PLX8394,) are more potent inhibitors of $BRAF^{V600E/K}$ than either vemurafenib or dabrafenib, and do not induce conformational changes to promote RAF/RAS-GTP interaction. Thus, paradox breakers are predicted to have fewer side effects than second-generation agents.

Since all the mechanisms of resistance observed in BRAFi refractory melanomas share the ability to reactivate ERK signaling, one obvious strategy to overcome this impasse is to directly inhibit MEK1/2, the dual specificity kinases that catalyze the next step in the signaling cascade. MEK inhibitors (MEKi) have thus been developed, and are currently routinely combined with BRAFi. *Trametinib* (Mekinist®), *cobimetinib* (Cotellic®) and *binimetinib* (Mektovi™) are allosteric inhibitors of MEK1/2, and their effects are independent of the RAS status of the cell, or the presence of RAF proteins as monomers or dimers. While these properties allow addressing both BRAFi resistance and paradoxical ERK activation in normal tissues, they also account for the poorer safety profile of these agents, as compared to BRAFi, with severe and intolerable skin rashes as the most frequent side effect when they are used as single agents. They are currently approved for the treatment of $BRAF^{V600E/K}$ melanomas in combination with dabrafenib, vemurafenib and encorafenib, respectively. Combining the two classes of agents has several advantages, including prevention of the most common mechanisms of resistance to BRAFi and decrease of the side

effects of both classes of agents, thanks to the possibility to use lower doses of both agents. However, although resistance to combined BRAFi/MEKi therapy is delayed compared to treatment with the single agents, resistance remains a significant problem to which MEK mutations or direct MEK activation contribute, along with the upstream mechanisms already reviewed. Thus, ERK inhibitors are predictably under preclinical and clinical development, with the aim of ultimately inhibiting the signaling pathways at all possible nodes. Intriguingly, a recent report suggests that melanoma cells that do not respond to BRAFi/MEKi combinations might actually become addicted to the treatment: if such observation were supported by further evidence, intermittent treatment with the combination (introducing periods of so-called 'drug holiday') might provide the best chance to control the tumor without overburdening the patients with too many different drugs.

Trametinib, cobimetinib, binimetinib	Allosteric inhibitors of MEK1/2. Since all the mechanisms of resistance observed in BRAFi refractory melanomas share the ability to reactivate ERK signaling, one strategy to overcome this impasse is to directly inhibit MEK1/2. They are currently approved for the treatment of BRAFV600E/K melanomas in combination with dabrafenib and vemurafenib, respectively

11.2.5 Inhibitors of the PI3K/AKT/mTOR Pathway

The PI3K/AKT/mTOR pathway is activated downstream of activated RTKs, either by direct or adaptor-mediated recruitment of p85, the regulatory subunit of PI3K, to the catalytic domain of RTKs, or via RAS-mediated recruitment of the catalytic subunit of PI3K, p110, to the cell membrane. The PI3K lipid kinase family is subclassified based on structure, regulation, and substrate preference, and class IA is the one most consistently involved in oncogenic pathways. Class IA isoforms are heterodimeric proteins consisting of a p110 catalytic subunit and a p85 regulatory subunit and four different catalytic paralogues (α, β, δ, and γ) have been identified. PI3K phosphorylates PIP2 in the inner leaflet of the cell membrane, creating docking sites that allow the recruitment, phosphorylation and activation of AKT; PIP3 formation by PI3K is counteracted by the PTEN phosphatase, the major negative regulator in the pathway. AKT, also known as PKB, is a serine/threonine kinase that promotes cell survival, proliferation and motility by acting on a number of diverse effectors, including transcription factors and cell cycle regulators, as well as the serine/threonine kinase mTOR (mammalian target of rapamycin). Similarly to the RAS-RAF-MEK-ERK signaling cascade, this pathway is also often dysregulated in solid tumors, due to oncogenic activation of the PI3KCA and AKT genes, or loss of the negative regulatory role played by PTEN. In contrast, aberrant mTOR activation is hardly ever caused by direct changes in the encoding gene, but generally results from disruption of the complex network of upstream regulatory signals (Fig. 11.5).

To date, more than 40 inhibitors of the PI3K–AKT–mTOR signalling pathway have reached various stages of clinical development, but relatively few have been approved for clinical use [22]. The first compounds that were developed to target this signaling pathways were mTOR inhibitors, sometimes also dubbed rapalogs because they were originally derived from rapamycin (sirolimus), a macrolide compound isolated from *Streptomyces hygroscopicus*. mTOR is the catalytic subunit of two multiprotein complexes, mTOR complex 1 (mTORC1) and mTORC2, that act as sensors for the nutritional, energetic, and redox status of the cell, and accordingly control cell metabolism and growth. Two rapalogs, *temsirolimus* (Torisel®) and *everolimus* (Afinitor®), have been approved as part of drug combinations for the treatment of renal cell cancer and breast cancer. These agents are fairly well tolerated, but on the other hand they exhibit rather limited clinical efficacy, mainly due to the different roles played by mTOR in the two signaling complexes, and to the fact that temsirolimus and everolimus, in contrast to most kinase inhibitors reviewed above, do not act by competing for the ATP-binding site of the enzyme, but by disrupting its association with accessory and regulatory subunits in the mTORC1 complex. Thus, while these agents effectively control phosphorylation of mTOR substrates that are involved in the control of protein synthesis and cell growth (e.g. the S6 kinase), mTORC2 remains unaffected and since one of the roles of mTORC2 is to provide activating phosphorylation for the upstream element of the pathway, AKT, the final outcome of the treatment is rewiring, rather than inhibition, of the pathway. Moreover, inhibiting mTORC1 also relieves a negative feedback regulatory circuit, whereby loss of S6K catalytic activity removes an upstream constraint on PI3K activation, further reducing the overall inhibitory effect of these agents. To overcome the limitations of rapamycin analogs, novel mTOR inhibitors are under pre-clinical and clinical development that revert to the more conventional, ATP-competitive mechanism of action. Such agents interfere with the catalytic site of mTOR irrespective of the molecular context, and thus inhibit both mTORC1 and mTORC2-mediated effects. Whether these newer mTORC1/2 inhibitors offer any significant clinical advantage over rapamycin analogs is

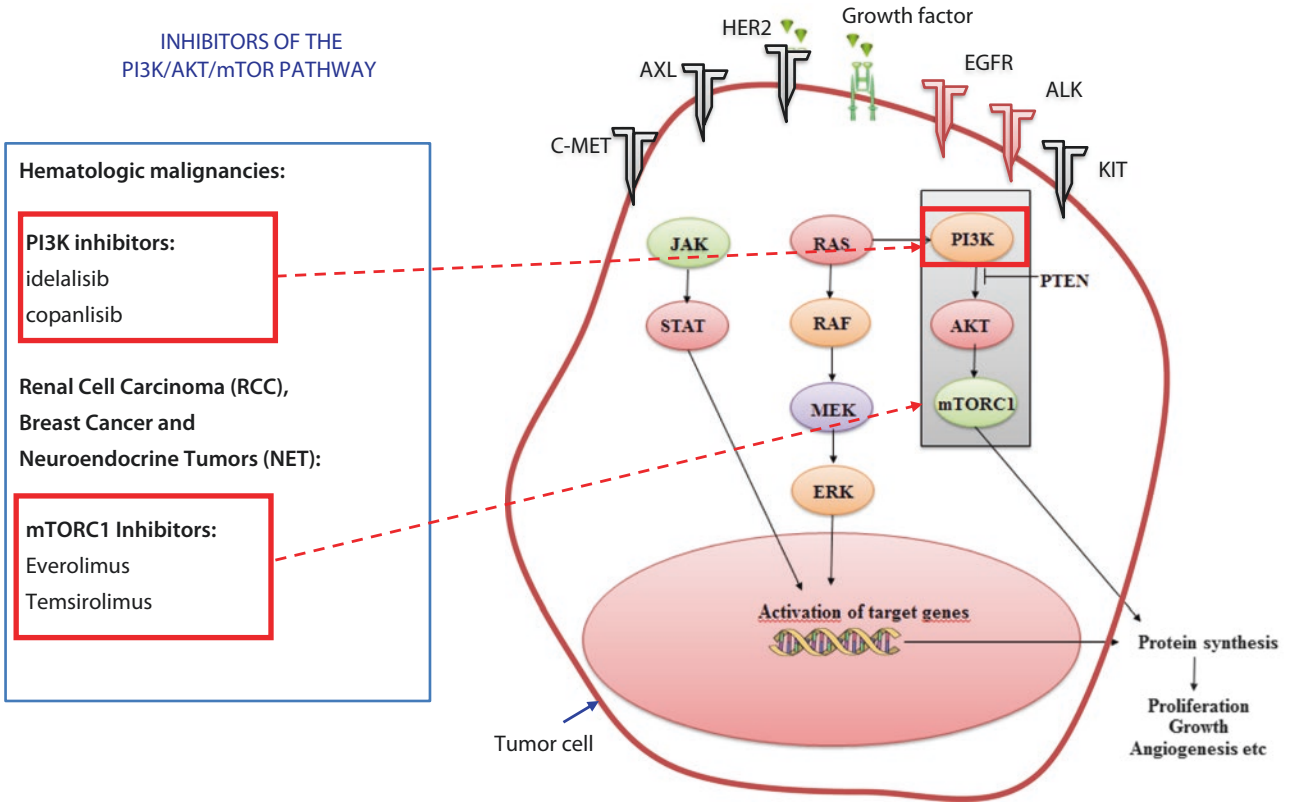

Fig. 11.5 Inhibitors of the PI3K/AKT/mTOR pathway

still unclear; perhaps their use would best be included in rationally designed combinations addressing selected patient populations.

PI3K inhibitors wortmannin and LY294002 have been used for decades as laboratory tools to inhibit PI3K activity irreversibly or reversibly, respectively, but they were developed before the complexity of the PI3K family was fully grasped and as they are *truly* pan-PI3K inhibitors (i.e. they non selectively inhibit all classes and isoforms of the enzyme) they were never approved for clinical use. A number of PI3K inhibitors are currently under clinical investigation, including (inhibitors targeting all four isoforms of class I PI3K (currently referred to as pan-PI3K inhibitors), as well as isoform-selective inhibitors. Two isoform-specific drugs have gained regulatory approval for use in hematologic malignancies: the former, *idelalisib* (Zydelig®) is a PI3K-δ-specific inhibitor used in the management of chronic lymphocytic leukemia; the latter, *copanlisib* (Aliqopa®) is directed at the α and δ isoforms and is approved for relapsed follicular lymphoma. However, in spite of the well-recognized role of class I PI3Ks in the genesis and progression of solid tumors, no drugs targeting PI3Ks have yet been approved for the treatment of solid malignancies. In addition, as of the present time the development of AKT inhibitors has failed to produce viable clinical candidates. Finally, it may be worth noting that, in contrast with the combinatorial strategy adopted for the RAS-RAF-MEK-ERK pathway (RAFi+MEKi), for this pathway a different approach has been adopted, focusing on dual PI3K/mTOR inhibitors [23], although these agents too are still awaiting approval.

Temsirolimus, everolimus	Allosteric mTOR inhibitors causing dissociation of the mTORC1 complex. They are used in the treatment of renal cell cancer and breast cancer with limited clinical effects when used as monotherapy, due to their ability to block only part of mTOR-mediated activities
Idelalisib	PI3K-δ-specific inhibitor, used in the management of chronic lymphocytic leukemia
Copanlisib	Dual PI3Kα and δ inhibitor, approved for the treatment of relapsed follicular lymphoma

Drug	Target	FDA approval	Indication
Trastuzumab (Herceptin®)	HER-2	2006	Adjuvant treatment of women with node-positive, HER2-overexpressing breast cancer in combination with doxorubicin, cyclophosphamide, and paclitaxel
		2010	HER2-overexpressing metastatic gastric or gastroesophageal junction adenocarcinoma, in combination with cisplatin and a fluoropyrimidine
Pertuzumab (Perjeta®)	HER-2	2012	HER2-positive metastatic breast cancer
		2013	Neoadjuvant treatment of early stage breast cancer
		2017	Adjuvant treatment of HER2-positive early breast cancer at high risk of recurrence, in combination with trastuzumab and chemotherapy
Trastuzumab-emtansine (T-DM1, Kadcyla®)	HER-2	2013	HER2-positive, late-stage (metastatic) breast cancer.
Lapatinib (Tykerb®)	EGFR, HER-2	2007	Advanced or metastatic breast cancer that has progressed on prior therapy, in combination with capecitabine
		2010	Hormone receptor positive, HER2-positive metastatic breast cancer, in combination with letrozole.
Neratinib (Nerlynx®)	EGFR, HER-2, HER-4	2017	Extended adjuvant treatment of early stage HER2-positive breast cancer
Cetuximab (Erbitux®)	EGFR	2004	EGFR-expressing, metastatic colorectal carcinoma refractory to irinotecan-based chemotherapy, in combination with irinotecan
		2006	Locally or regionally advanced squamous cell carcinoma of the head and neck (SCCHN) in combination with radiation therapy, or as a single agent for recurrent or metastatic SCCHN with failed prior platinum-based therapy
		2011	First-line treatment for recurrent locoregional disease and/or metastatic SCCHN, in combination with platinum-based therapy plus 5-florouracil (5-FU)
		2012	First-line treatment of *K-ras* wild-type, *EGFR*-expressing metastatic colorectal cancer (mCRC)
Panitumumab (Vectibix®)	EGFR	2006	*EGFR*-expressing mCRC after disease progression on, or following fluoropyrimidine-, oxaliplatin-, and irinotecan- containing chemotherapy
		2014	First-line treatment of *K-ras* wild-type mCRC in combination with FOLFOX
		2017	Of *K-ras* wild-type mCRC in combination with FOLFOX and as monotherapy following disease progression after prior treatment with fluoropyrimidine, oxaliplatin, and irinotecan-containing chemotherapy
Necitumumab (Portrazza®)	EGFR	2015	Advanced squamous NSCLC, in combination with chemotherapy
Gefitinib (Iressa®)	EGFR	2003	Advanced NSCLC (retracted in 2005)
		2015	First-line treatment of metastatic NSCLC with EGFR exon 19 deletions or exon 21 (L858R) substitution mutations
Erlotinib (Tarceva®)	EGFR	2004	Locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen.
		2005	Locally advanced, unresectable or metastatic pancreatic carcinoma, in combination with gemcitabine
		2010	Maintenance treatment of locally advanced or metastatic NSCLC that has not progressed after four cycles of platinum-based first-line chemotherapy.
		2013	First-line treatment of metastatic NSCLC with EGFR exon 19 deletions or exon 21 (L858R) substitution mutations

Drug	Target	FDA approval	Indication
Afatinib (Gilotrif®)	EGFR	2013	Late-stage NSCLC with specific types of epidermal growth factor receptor (EGFR) gene mutations
		2016	Squamous cell carcinoma of the lung
		2018	EGFR mutation-positive NSCLC
Osimertinib (Tagrisso®)	$EGFR^{T790M}$	2015	EGFR T790M mutation-positive non-small cell lung cancer
		2018	First-line treatment for EGFR-mutated NSCLC
Crizotinib (Xalkori®)	ALK, ROS-1	2011	Late-stage ALK positive NSCLC
		2016	ROS-1 positive NSCLC
Ceritinib (Zykadia®)	ALK	2014	Metastatic NSCLC in patients who have progressed on, or are intolerant to, crizotinib
		2017	First-line treatment for ALK-positive metastatic NSCLC
Alectinib (Alecensa®)	ALK	2015	ALK-positive NSCLC in patients who have progressed on, or are intolerant to, crizotinib
		2017	First-line treatment for ALK-positive metastatic NSCLC
Brigatinib (Alunbrig®)		2017	ALK-positive (ALK+) metastatic NSCLC in patients who have progressed on, or are intolerant to, crizotinib
Temsirolimus (Torisel®)	mTORC1	2007	Advanced RCC
Everolimus (Afinitor®, Zortress®)	mTORC1	2009	Advanced RCC after failure of either sunitinib or sorafenib
		2012	Advanced breast cancer
		2016	Progressive, nonfunctional gastrointestinal and lung neuroendocrine tumors (NET)
Copanlisib (Aliqopa®)	PI3K	2017	Relapsed follicular lymphoma in adult patients
Idelalisib (Zydelig®)	PI3Kδ	2014	Relapsed CLL, in combination rituximab
		2014	Third-line treatment of relapsed follicular B-cell NHL and relapsed small lymphocytic lymphoma
Vemurafenib (Zelboraf®)	$BRAF^{V600E}$	2011	Late-stage (metastatic) or unresectable melanoma with $BRAF^{V600E}$ mutation
		2017	Erdheim-Chester disease with $BRAF^{V600E}$ mutation
Dabrafenib (Tafinlar®)	$BRAF^{V600E}$	2013	Unresectable or metastatic in adult patients with $BRAF^{V600E}$ mutation
		2017	Metastatic NSCLC with $BRAF^{V600E}$ or $BRAF^{V600K}$ mutations, in combination with trametinib
		2018	Adjuvant treatment of melanoma with $BRAF^{V600E}$ or $BRAF^{V600K}$ mutations, in combination with trametinib
		2018	Unresectable or metastatic BRAF-positive anaplastic thyroid cancer in combination with trametinib
Encorafenib (Braftovi™)	$BRAF^{V600E/K}$	2018	BRAF-positive melanoma

Drug	Target	FDA approval	Indication
Trametinib (Mekinist®)	MEK	2013	Single-agent oral treatment for unresectable or metastatic melanoma in adult patients with BRAF ^{V600E} or BRAF ^{V600K} mutations
		2014	Unresectable or metastatic BRAF ^{V600E} or BRAF ^{V600K} mutations, in combination with dabrafenib
		2017	Metastatic NSCLC with BRAF^{V600E} or BRAF^{V600K} mutations, in combination with dabrafenib
		2018	Unresectable or metastatic BRAF-positive anaplastic thyroid cancer in combination with trametinib
Cobimetinib (Cotellic®)	MEK	2015	Advanced melanoma with BRAF ^{V600E} or BRAF ^{V600K} mutation in combination with vemurafenib
Binimetinib (Mektovi™)	MEK1/2	2018	Unresectable or metastatic BRAF ^{V600E} or V600K mutation-positive melanoma, in combination with encorafenib

11.3 Targeting DNA Damage Response

Many chemical agents present in the environment or produced by cell metabolism, e.g. oxygen free radicals, as well as physical agents like UV radiations cause DNA damage, but do not necessarily produce irreversible deleterious effects because mammalian cells have efficient and well integrated mechanisms to repair DNA damage and to neutralize their potential carcinogenic and toxic effects. The DNA damage response involves a variety of cooperating cellular pathways in which DNA repair mechanisms are closely connected with other processes, such as cell cycle checkpoints and cell death mechanisms, with the ultimate aim to maintain genomic integrity.

Growing evidence suggests that defects in DNA repair mechanisms increase the risk of neoplastic transformation after exposure to carcinogens. This has been demonstrated in many preclinical systems and is also supported by epidemiological studies in humans. Germline mutations of genes encoding proteins involved in DNA repair mechanisms, such as *BRCA1*, *BRCA2*, *BLM*, *FANCA*, *TP53*, *RAD51C* and *MSH2*, result in cancer susceptibility syndromes. In many human tumors somatic mutations of DNA repair genes have been reported, suggesting that DNA repair deficiency is a key factor in neoplastic transformation. It appears that the lack of an efficient DNA repair system is a common feature of many malignancies and explains why the therapy against many forms of human cancer is largely based on the use of drugs that cause DNA damage either by direct mechanisms, e.g. by producing DNA cross-links (like platinum drugs) or DNA breaks, or by indirect mechanisms involving DNA processing enzymes as for topoisomerase I and II poisons [24].

It has been hypothesized that the vulnerability of cancer cells to DNA damage can be further increased by compounds acting by modulating the DNA damage response and this hypothesis has led to the development of DNA repair inhibitors as potential enhancers of anticancer therapies. In this section we provide a short overview of recently developed drugs directed at DNA repair enzymes or cell cycle checkpoints involved in the response to DNA damage. Extensive reviews on this topic have been published [25–28]. In the following table are illustrate the described classes of agents targeting DNA damage response and their molecular targets.

11.3.1 Inhibitors of DNA Repair Mechanisms

DNA double strand breaks (DSBs) are crucial DNA lesions responsible for the cytotoxicity and antitumor activity of radiotherapy and of many anticancer drugs. Two sensor protein complexes are involved in the detection of DSBs, namely Ku70/Ku80 heterodimers and the MRN complex (MRN11-RAD50-NBS1). The Ku complex binds DSBs and activates the Non-Homologous End-Joining (NHEJ) pathway. The MRN complex triggers the activation of the DNA damage signaling protein ATM and a cascade of reactions ultimately activating the Homologous Recombination Repair (HRR). Other types of DNA damage repair mechanisms include Fanconi anemia, mismatch repair, base excision repair and nucleotide excision repair proteins, the latter being crucial for UV-photo-lesions and DNA-intra and -interstrand crosslinks induced by many anticancer drugs (e.g. platinum drugs).

Although several preclinical studies are currently investigating a number of sensor and signalling proteins involved in the response to DNA damage, we will only focus on PARP, DNA-PK, ATM and ATR, as they are targets of drugs that are clinically used or are under clinical development.

11.3.1.1 PARP Inhibitors

Out of 17 PARP family members with a broad range of functions, PARP1 and 2, and to a lesser extent PARP3, are involved in DNA repair processes and thus they have been targeted by drugs inhibiting DNA repair. PARP inhibitors were initially developed with the aim to potentiate the activity of DNA damaging agents, but they were unable to improve the therapeutic index these drugs, as combined administration required a significant dose reduction in order to avoid intolerable toxicity. However, an unexpected preclinical finding that was subsequently confirmed in the clinic was that PARP inhibitors were effective when used alone as single agents against some tumors, particularly those bearing specific defects in DNA repair mechanisms [25].

PARP catalytic function is activated by DNA breaks, generating extensive poly(ADP-ribose) chains on proteins in the vicinity of DNA damage. The same reaction of polyADP ribosylation occurs on PARP enzyme itself (i.e. auto-polyADP ribosylation), leading to dissociation of the enzyme from DNA. Extensive polyADP ribosylation of DNA binding proteins promotes recruitment of DNA repair proteins and modifies chromatin structure, thereby facilitating DNA repair (Fig. 11.6).

Inhibition of PARP by PARP inhibitors results in a variety of effects that are not fully elucidated and that ultimately lead to cell cycle arrest and cell death. Suppression of PARP catalytic activity hampers correct DNA repair. Moreover, PARP inhibitors cause trapping of PARP itself onto damaged DNA, causing stalling and collapse of DNA replication forks and subsequent formation of DSBs. Tumor cells deficient in HRR, e.g. those with mutations of BRCA1/2, are particularly sensitive to PARP inhibitors, highlighting the synthetic lethality mechanism that is behind the antitumor selectivity [25, 29].

So far three PARP inhibitors have been approved for the clinical use for the therapy of ovarian cancer: *olaparib*, *rucaparib* and *niraparib* [30]. Their approval has been based on an impressive improvement of Progression-free survival (PFS), even if no evidence of increased Overall Survival (OS) has been reported. Olaparib and rucaparib have been approved in Europe and US for the therapy of relapsed platinum-sensitive ovarian cancer patients with germline or somatic mutations of *BRCA1/2*. Olaparib is indicated in US also for germline *BRCA*-mutated HER2-negative metastatic breast cancer at relapse. In contrast, niraparib has been approved both in Europe and US for the maintenance therapy of platinum sensitive ovarian cancer patients, regardless of *BRCA1/2* status [31]. Indeed, also for niraparib it is evident that patients with ovarian cancer with mutations of *BRCA1/2* are more sensitive to the drug, but responses have also been observed in patients with wild type BRCA proteins. It should be remembered that several proteins other than *BRCA1/2* play a key role in HRR and thus cancer cells expressing wild type BRCA1/2 are not necessarily HRR proficient [29]. In addition, polyADP ribosylation is a post-translational modification that can change the

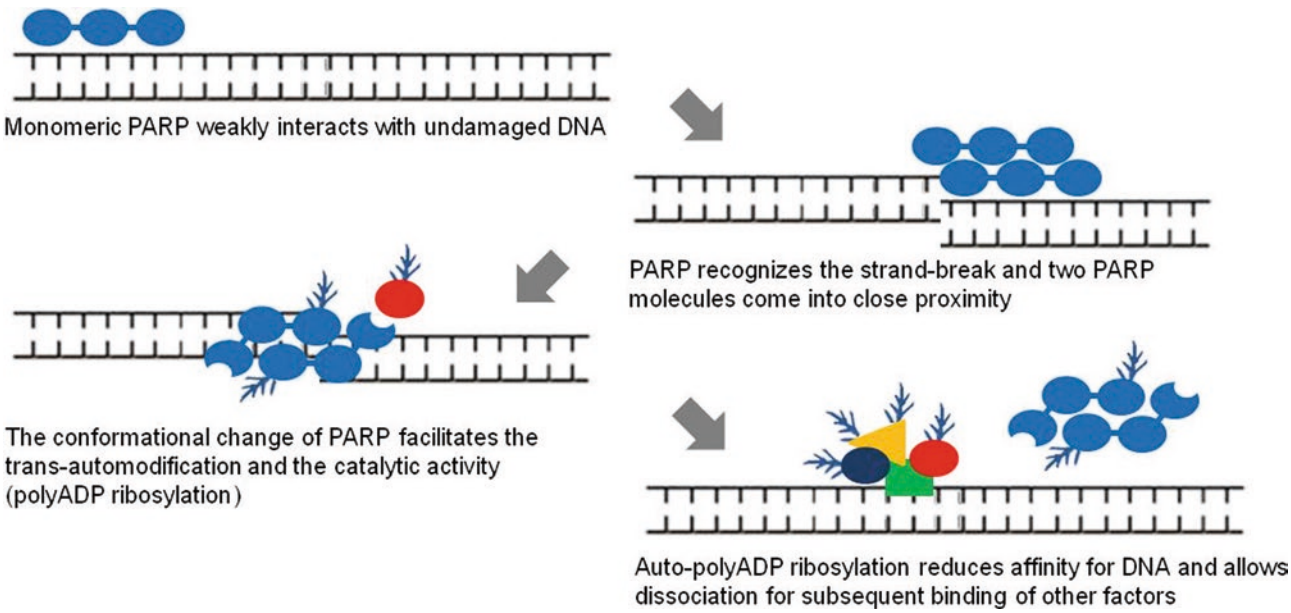

Fig. 11.6 Regulation of PARP enzymatic activity by DNA damage recognition and auto-modification

structure and the function of many proteins including transcription factors, implying dramatic changes in the transcription regulation. Evidence exists that PARP plays an important function in modulating inflammation and immune responses, thus suggesting that its inhibition could lead to profound changes in tumor biology [32]. In this respect, it may be worthwhile stressing that a small fraction (10–15%) of ovarian cancer patients receiving treatment with PARP inhibitors have very prolonged disease control that may be partially due to immunological mechanisms. This hypothesis is under investigation and in the meantime many trials are designed to evaluate whether PARP inhibitors improve the efficacy of immune checkpoint inhibitors [33].

11.3.1.2 Inhibitors of DNA-PK

DNA-PK is a key enzyme in NHEJ, a DSB repair mechanism maintained throughout the cell cycle. Its autophosphorylation induces conformational changes of NHEJ complex enabling access to Ku to DNA and other factors involved in DNA repair and in transcription regulation. Its binding to DNA is hampered by PARP itself.

Some DNA-PK inhibitors, such as *M3814* or *VX-984*, are under clinical development as single agents, although the potential impact of these inhibitors is more likely related to potentiating the activity of DNA damaging agents producing DSBs, such as topoisomerase II poisons or radiotherapy [34]. The clinical data are still too preliminary to draw any conclusions about the efficacy of these drugs.

11.3.1.3 ATM Inhibitors

ATM is the major kinase responsible for the phosphorylation of H2AX on serin 139 - known as gamma2AX - occurring as soon as a DSB is generated in a cell. ATM interacts with MRN complex involved in HRR and phosphorylates several crucial regulatory factors, including CHK2 and p53, involved in the G1/S cell cycle checkpoint. Somatic mutations of ATM are commonly found in different tumors and germline ATM mutations result in increased cancer predisposition [35].

In many preclinical systems ATM inhibitors have shown the ability to potentiate the antitumor activity of many DNA damaging agents very significantly. Clinical studies with inhibitors such as *AZD0156* used as single agent or in combination with other drugs are ongoing but any conclusions about the efficacy of this class of compound cannot be drawn so far.

11.3.1.4 ATR Inhibitors

ATR is another kinase involved in the DNA damage response, that plays a crucial role in maintaining genomic integrity. It is activated by a wide spectrum of DNA damage and replication problems, in particular by binding of RPA to single strand DNA and by DNA replication stress. In many cancers, tumor cells exhibit increased dependence on ATR signaling for survival.

A synthetic lethality mechanism has been reported between ATR and CHEK1 inhibitors in preclinical systems. Two compounds, *VX-970* and *AZD6738*, are under clinical evaluation either as single agent or in combination with DNA damaging agents [36]. Preliminary clinical data suggest that these compounds are effective in enhancing the antitumor activity of other anticancer drugs, but they also increase bone marrow toxicity. Studies are in progress to optimize dose-schedules and sequence of combination with other drugs.

11.3.2 Cell Cycle Checkpoint Inhibitors

The DNA damage response includes activation of cell cycle checkpoints that prevent premature mitosis and maintain genomic integrity, promoting cell survival or death pathways. ATM and ATR are important triggers of both DNA repair mechanisms and cell cycle checkpoints. CHEK1 (also known as CHK1) and WEE1 act to integrate signals from ATM and ATR and to induce cell cycle arrest in response to DNA damage. Defects in checkpoints allow for an accumulation of genetic alterations that can result in cancer development (◘ Fig. 11.7).

11.3.2.1 CHEK1 Inhibitors

CHEK1 is an essential regulator of both the S and the G2-M checkpoints. The first a non-selective CHEK1 inhibitor developed in the clinic was the staurosporine analogue *UCN-01;* however, its antitumor effects were hampered by its tight binding to alpha-1-acid glycoprotein present in human plasma. Other compounds able to competitively inhibit CHEK1, such as *AZD7762*, *PF-00477736*, *XL-844*, *LY2603618* and *MK-8776*, have been developed and tested for their efficacy, particularly against tumor with p53 mutations. In fact, it was proposed that tumors deficient in G1 checkpoint – due to loss of p53 – could be particularly susceptible to CHEK1 inhibitors – acting particularly in the G2 checkpoint – resulting in mitotic catastrophe and subsequent cell death [37].

Although theoretically attractive, the concept did not lead to clinically significant results and the tested CHEK1 inhibitors failed to selectively enhance the antitumor activity of other drugs. Novel inhibitors, reportedly with greater selectivity and better pharmacological properties, are being tested, particularly in combination with antimetabolites,other cell cycle checkpoint inhibitors (e.g. WEE1 inhibitors) or inhibitors of DNA repair. Several clinical studies are ongoing with the dual CHEK1/CHEK2 inhibitor *prexasertib,* eithe alone or in combination.

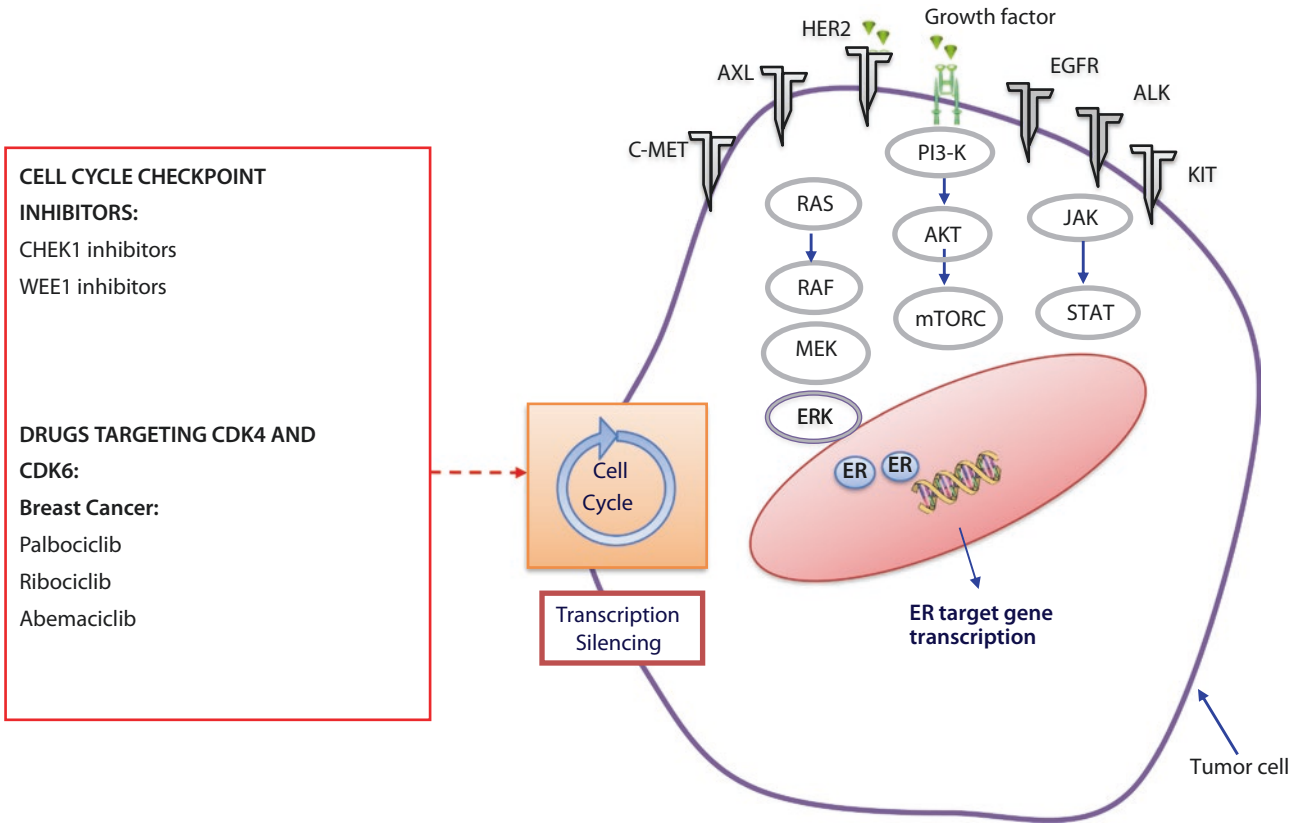

Fig. 11.7 CHEK1 inhibitors and WEE1 inhibitors interfere with the cell cycle checkpoint, a mechanism that prevent mitosis and maintain genomic integrity. CDK4/CDK6 inhibitors act on Cyclin D-dependent kinase during the G1 phase of the cell cycle. Palbociclib, ribociclib, and abemaciclib cause a cell block cells in G1, preventing entry into the S-phase

11.3.2.2 WEE1 Inhibitors

The tyrosine kinase WEE1 is a crucial component of the G2–M cell cycle checkpoint and is expressed at high levels in various cancer types. MK1775, renamed *AZD1775*, is the most potent and selective WEE1 inhibitor currently under clinical trials. Many phase I studies have been conducted combining AZD1775 with gemcitabine, cisplatin or carboplatin in patients with solid tumors. Promising results have been reported, particularly in tumors with mutated p53, with an acceptable toxicity profile. A relatively high response rate (around 40%) to AZD1775 and carboplatin was reported in ovarian cancer patients refractory or resistant to platinum-based therapies. This result is very promising and suggests that AZD1775 can counteract platinum drugresistance [38].

11.4 Drugs Targeting CDK4 and CDK6

The Cyclin D-dependent kinases CDK4 and CDK6 act during the G1 phase of the cell cycle by phosphorylating the retinoblastoma protein (Rb), thus releasing the E2F transcription factor from Rb-dependent constraints. E2F activates the transcription of genes related to DNA synthesis. Thus, inhibition of CDK4 and CDK6 causes a block of the cells in G1, preventing their entry into the S-phase (Fig.11.8).

Apparently, estrogen receptor-positive breast cancer are dependent on cyclin D1 pathway and thus it was speculated that CDK4/6 inhibitors could be more effective against these tumors. Preclinical evidence suggested a strong synergism with anti-estrogens and this observation provided the rational to investigate the combination of letrazole (an aromatase inhibitor) and *palbociclib* (CDK4/6 inhibitor) in clinical trials. The phase III study in which the combination was compared to letrazole and placebo was successful with a doubling of the PFS, which led to fast approval by FDA and EMA [39].

Similar studies with two other CDK4/6 inhibitors, *ribociclib* and, more recently, *abemaciclib*, produced a similar increase in the therapeutic effects of the associated anti-estrogen. Safety studies revealed that palbociclib and ribociclib caused a bone marrow toxicity similar to that observed with several chemotherapeutics, while abemaciclib was less myelotoxic.

Although the clinical results were consistently good in a very high fraction of patients, the drugs were not effective in all patients. However, the studies failed to

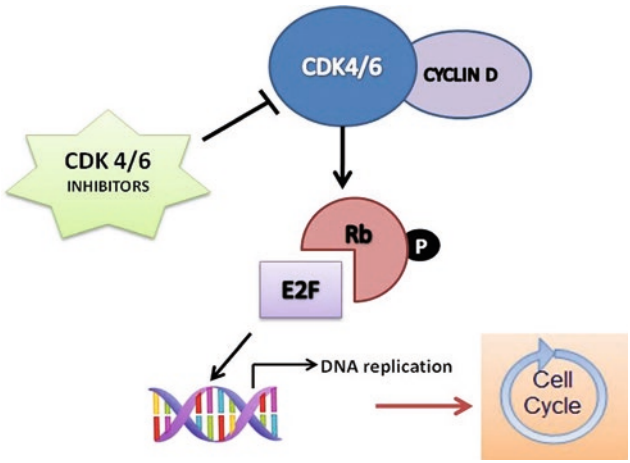

Fig. 11.8 Mechanisms of action of CDK4/CDK6 inhibitors: interference with progression to G1 phase of cell cycle

find a biomarker predictive of the response to these drugs. Apparently, no relationship was found between Cyclin D1 expression and activity of the CDK4/6 inhibitors.

Recent studies suggest that CDK4/6 inhibitors can be effective also in HER2-positive breast cancers and even in triple negative breast cancers. Clinical trial testing CDK4/6 inhibitors efficacy against other cancers, such as mantle cell lymphoma, liposarcoma and melanoma, are ongoing [40].

11.5 Drugs Targeting Epigenetic Mechanisms

Increasing evidence supports the notion that epigenetic mechanisms play an important role in cancer diseases. This suggests that "epigenetic drugs", generally considered as inhibitors developed against known epigenetic proteins, can be therapeutically effective. Howeve,r with the exception of DNA methyltransferase (DNMT) inhibitors, such as *azacytidine* and *azadeoxycytidine* (decitabine), approved for the treatment of myelodysplastic syndrome, or the histone deacetylase (HDAC) inhibitor *vorinostat* for the therapy of cutaneous T cell lymphomas, the clinical activity of epigenetic drugs has been much lower than anticipated based on biological considerations and preclinical data [41, 42]. It is possible, however, that some epigenetic therapeutic targets more recently identified will lead to effective drugs. Therefore we'll review this topic, highlighting the potential interesting mechanisms that are suitable to conceive pharmacological targets. Much research is focusing on:

1. Identifying better gene silencing agents, that are analogues of those already used in the clinic, with pharmacologically advantageous properties. In this respect, some new DNMT inhibitors, such as *SGI-110*, *RG108* or *SG1027*, have been identified and are under study.
2. Targeting transcriptional regulation, meaning inhibiting regulators of transcription that are crucial in some cancer diseases, such bromodomain and extra-terminal (BET) inhibitors, or CDK7 or CDK9 inhibitors. We do not review the many molecules under investigation as, to our knowledge, none of them is approved or disclosed to approval.
3. Targeting activating epigenetic mutations, such as mutations in EZH2, that catalyzes the methylation of histone H3 on lysine27. Various mutations of EZH2 have been observed in human neoplasms, with opposing functions in different contexts, as they can be either oncogenic or tumor suppressing, depending on other biological factors. This means that careful biological characterization of the tumors is mandatory for a therapeutic rational use of EZH2 inhibitors. A very interesting finding is that EZH2 inhibitors are effective against tumors that are deficient in SNF5 (a member of the SWI/SNF complex involved in chromatin remodeling, that is considered a bona fide tumor suppressor). Similarly, defects in the tumor suppressor gene ARID1A were found to be synthetically lethal with EZH2 inhibition, a finding of potential therapeutic interest for the fraction of ovarian cancers that do not express ARID1A.

Isocitrate dehydrogenase (IDH) inhibitors target metabolic enzymes IDH1 and IDH2, that catalyze the oxidative decarboxylation of isocitrate to alfa-KG, thus modulating alfa-KG-dependent demethylases and methylation of DNA and histones. Importantly mutant specific inhibitors have been identified (ivosidenib and *AG-121*), that specifically target mutant IDH1 and IDH2 in cancer cells. They are being investigated in clinical studies.

Finally, we cannot exclude that some used anticancer drugs that bind DNA modify the transcriptional regulation of specific genes. For example, the very high sensitivity of myxoid liposarcoma to *trabectedin* appears to be due to the ability of the drug to displace the oncogenic chimeric protein FUS-CHOP from DNA, thus modifying gene expression and reactivating adipocytic differentiation [43].

11.6 Drugs Targeting Angiogenesis

The concept of new vessel growth in tumors was described in 1971, when Folkman wrote that "*the growth of a solid neoplasm is always accompanied by neo-vascularization.*"

Angiogenesis, *the physiologic process of outgrowth of new blood vessels from pre-existing blood vessels*, is a crit-

ical step in many diseases, including cancer, and occurs in response to hypoxic microenvironment that develops within a growing tumor mass.

Hypoxia leads to the secretion of a number of pro-angiogenic growth factors, including vascular endothelial growth factor (VEGF), which are able to trigger the formation of new vessels through the process of angiogenesis. Hypoxia induces stabilization of the hypoxia-inducible factor 1α (HIF1α), which is associated with increased VEGFA production that activates the PI3K and the MAPK pathways, regulating proliferation and apoptosis and inducing angiogenesis and stromal remodeling.

Inhibition of tumor angiogenesis can thus decrease the blood flow, required for tumor development, curtailing tumor cell growth due to lack of nutrients and growth factors needed to support the formation of newly formed vessels [44].

Several angiogenesis factors regulate angiogenesis, and are potential therapeutic targets of antiangiogenic-drugs.

VEGF and its membrane receptors, VEGFR1–3, are the most extensively studied angiogenic system, and they are recognized to play an important role in regulating physiological and pathological angiogenesis. Thus, therapeutic treatments that target the VEGF systems were the first to be developed: *antibodies*, that block VEGF interaction with its receptor, and small molecule tyrosine kinase inhibitors *(TKIs),* that target VEGFR. Other angiogenic factors widely studied as potential therapeutic targets, besides VEGF and HIF-1, include platelet-derived growth factor (PDGF), fibroblast growth factors (FGF) and matrix metalloproteinases.

Therapeutic treatments that target angiogenic factors have in fact already been developed and clinically approved and angiogenesis inhibition is still considered an important strategy towards the discovery of new anticancer drugs.

The U.S Food and Drug Administration (FDA) agency has approved a number of angiogenesis inhibitors to treat cancer. Most of these agents are targeted therapies that were specifically developed to target VEGF, its receptor, or other specific molecules involved in angiogenesis, such as the serine-threonine kinase mammalian target of rapamycin (mTOR), which is a downstream effector of the PI3K signaling pathway.

The first anti-angiogenic drug approved for clinical use was *Bevacizumab* (Avastin®). It can be used alone or in combination with other drugs for the treatment of metastatic colorectal cancer, nonsquamous non-small cell lung cancer, cervical cancer, glioblastoma, ovarian epithelial, fallopian tube or primary peritoneal cancer and renal cell carcinoma [44–47]. Bevacizumab is a recombinant humanized monoclonal antibody directed against VEGF, that binds to the soluble VEGF and inhibits its ligation with the receptor, thereby preventing the growth and maintenance of tumor blood vessels [33].

Another approved monoclonal antibody is *Ramucirumab* (Cyramza®), a recombinant, fully human monoclonal antibody, directed against the vascular endothelial growth factor receptor 2 (VEGFR-2), a tyrosine kinase receptor expressed by endothelial cells. Ramucirumab specifically binds to and inhibits VEGFR-2, which may result in inhibition of tumor angiogenesis and decrease in tumor nutrient supply. This drug can be used alone or in combination for the treatment of metastatic colorectal cancer, non-small cell lung cancer and adenocarcinoma of the stomach or gastroesophageal junction [33, 47–50].

Ziv-aflibercept (Zaltrap®) is a chimeric protein composed by segments of the extracellular domains of human vascular endothelial growth factor receptors 1 (VEGFR1) and VEGFR2 fused to the constant region (Fc) of human IgG1 with antiangiogenic activity. Afilbercept, functioning as a soluble decoy receptor, binds to pro-angiogenic VEGFs, preventing its binding to cell receptors [51–53].

Bevacizumab	The first anti-angiogenic drug approved for clinical use. Is a recombinant humanized monoclonal antibody directed against soluble VEGF, inhibiting its interaction VEGFR.
Ramucirumab	A recombinant, fully human monoclonal antibody, directed against the vascular endothelial growth factor receptor 2 (VEGFR-2), a tyrosine kinase receptor expressed by endothelial cells.
Aflibercept	Aa chimeric protein composed by segments of the extracellular domains of human vascular endothelial growth factor receptors 1 (VEGFR1) and VEGFR2 fused to the constant region (Fc) of human IgG1. Functioning as a soluble decoy receptor, it binds to pro-angiogenic VEGFs, preventing their binding to cell receptors.

Other targeted agents used to inhibit angiogenesis are TKIs, and there are several clinically available TKIs that will be briefly discussed.

Sorafenib (Nexavar®) is a multikinase inhibitor of multiple growth factor receptors, including VEGFr, PDGFr, Flt-3 and c-Kit, and Raf-1, a member of RAF/MEK/ERK signaling pathway. It is approved for the treatment of hepatocellular carcinoma, renal cell carcinoma and thyroid cancer [54–56].

Sunitinib (Sutent®) blocks the tyrosine kinase activities of VEGFR2, PDGFRb, and c-KIT, thereby inhibiting angiogenesis and cell proliferation. Moreover, this agent inhibits the phosphorylation of FLT3, a receptor

expressed by some leukemia cells [57]. It is used to treat gastroinstestinal stromal tumor, pancreatic cancer and renal cell carcinoma [58–60].

Pazopanib (Votrient®) is approved for soft tissue sarcoma and renal cell carcinoma and it selectively inhibits VEGFR-1, −2 and − 3, c-KIT and PDGFR, resulting in angiogenesis inhibition [58–61].

Axitinib (Inlyta®) is a next-generation orally bioavailable TKI, that inhibits both VEGF and the platelet-derived growth factor receptors (PDGF), potent and highly selective for the VEGF receptor 1, 2 and 3; it is approved for the treatment of advanced renal cell carcinoma [62, 63].

Regorafenib (Stivarga®) is approved to treat gastrointestinal stromal tumor [64, 65], hepatocellular carcinoma and colorectal cancer [66, 67]. Regorafenib binds to and inhibits the VEGFR-2 and 3, and RET, KIT, PDGFR and RAF kinases, which may result in the inhibition of tumor angiogenesis and tumor cell proliferation.

Cabozantinib (Cabometyx®) is an orally bioavailable TKI that strongly binds and inhibits several tyrosine kinase receptors, which are often overexpressed in a variety of cancer cell types. Therefore this drugs is a pan-TKI that inhibit the activity of the hepatocyte growth factor receptor (MET), RET (rearranged during transfection), VEGFR-1, VEGFR-2, and 3 (VEGFR-3), mast/stem cell growth factor (KIT), FMS-like tyrosine kinase 3 (FLT-3), TIE-2 (TEK tyrosine kinase, endothelial), tropomyosin-related kinase B (TRKB) and AXL [68]. This may result in an inhibition of both tumor growth and angiogenesis, and eventually lead to tumor regression. It is approved for the treatment of medullary thyroid cancer and renal cell carcinoma [69–72]. *Lenvatinib mesylate* (Lenvima®) is a synthetic, orally available inhibitor, that blocks VEGFR2 activation, resulting in inhibition of the VEGF receptor signal transduction pathway, decreased vascular endothelial cell migration and proliferation, and vascular endothelial cell apoptosis. It is used in thyroid cancer and renal cell carcinoma, in association between everolimus [73, 74].

Vandetanib (Caprelsa®) selectively inhibits the tyrosine kinase activity of VEGFR2 and EGFR, thus reducing tumor vessel permeability, cell proliferation and migration. It is approved for the treatment of unrespectable and metastatic medullary thyroid cancer [75].

Sorafenib	A multikinase inhibitor of multiple growth factor receptors as VEGFr, PDGFr, Flt-3 and c-Kit and Raf-1, a member of RAF/MEK/ERK signaling pathway.
Sunitinib	Blocks the tyrosine kinase activities of VEGFR2, PDGFRb, and c-KIT, thereby inhibiting angiogenesis and cell proliferation. Moreover this agent inhibits the phosphorylation of FLT3, a receptor by some leukemia cells.
Pazopanib	It selectively inhibits VEGFR-1, −2 and −3, c-KIT and PDGFR, resulting in angiogenesis inhibition in tumors in which these receptors are up-regulated.
Axitinib	A next-generation orally bioavailable TKI, that inhibits both VEGF and the platelet-derived growth factor receptor (PDGF), potent and highly selective for the VEGF receptor 1, 2 and 3.
Regorafenib	Binds to and inhibits the VEGFR-2 and 3, and RET, KIT, PDGFR and RAF kinases, which may result in the inhibition of tumor angiogenesis and tumor cell proliferation.
Cabozantinib	Is a pan-TKI that inhibit the activity of the hepatocyte growth factor receptor (MET), RET (rearranged during transfection), VEGFR-1, VEGFR-2, and 3 (VEGFR-3), mast/stem cell growth factor (KIT), FMS-like tyrosine kinase 3 (FLT-3), TIE-2 (TEK tyrosine kinase, endothelial), tropomyosin-related kinase B (TRKB) and AXL.
Lenvatinib	A synthetic, orally available inhibitor, that blocks VEGFR2 activation, resulting in inhibition of the VEGF receptor signal transduction pathway, decreased vascular endothelial cell migration and proliferation, and vascular endothelial cell apoptosis
Vandetanib	Selectively inhibits the tyrosine kinase activity of VEGFR2 and EGFR, thus reducing tumor vessel permeability, cell proliferation and migration.

As previously mentioned, besides TKI and MoAb directed at vascular growth factors and their receptors, angiogenesis can be inhibited also by interfering with other factors such as mTOR. *Everolimus* (Afinitor®, see above) is an orally administered rapamycin analog that was initially approved for the treatment of renal cell carcinoma refractory to inhibitors of VEGF receptor signaling [76], but nowadays it can be used also for breast, lung, gastrointestinal, pancreatic cancer and subependymal giant cell astrocytoma [77, 78]. Rapalogs have been shown to suppress hypoxia-induced increases in HIF-1α, and they inhibit the response of vascular endothelial cells to stimulation by VEGF. Moreover, inhibition of mTOR activation results in the inhibition of T lymphocyte activation and proliferation associated with antigen and cytokine (IL-2, IL-4, and IL-15) stimulation and the inhibition of antibody production. Another anti-angiogenic agent is *Thalidomide* (Thalomid®), that acts primarily by inhibiting both the production of tumor necrosis factor alpha (TNFα) in stimulated peripheral monocytes and the activities of interleukins and interferons. This agent also inhibits polymorphonuclear chemotaxis and monocyte phagocytosis [79, 80]. In

addition, thalidomide inhibits VEGF and basic fibroblast growth factor (bFGF), thereby inhibiting angiogenesis. Similarly to thalidomide, *Lenalidomide* (Revlimid®) inhibits TNFαproduction, stimulates T cells, reduces serum levels VEGF and basic fibroblast growth factor bFGF and inhibits angiogenesis [81–83]. Moreover, this agent promotes G1 cell cycle arrest and apoptosis of malignant cells.

Although some anti-angiogenic drugs have shown antitumor activity, particularly in combination with other anticancer agents, the overall therapeutic results are less striking than previously anticipated. It seems likely that, as angiogenesis is a complex biological process, its inhibition by specific VEGF inhibitors can cause a compensatory upregulation of other angiogenic factors leading to resistance. The overproduction of growth factors, as well as the activation of pathways connected with hypoxia, probably may reduce the long term clinical benefits of antiangiogenic therapy and in some cases an unexpected increased biological aggressiveness of the neoplastic disease has been observed.

Even if this "rebound" effect has only been documented in a limited number of clinical reports, its existence is supported by preclinical evidence. In addition, it should be remembered that the purpose of clinical trials is to assess a therapeutic improvement and the study design is not necessarily suitable to detect increased tumor progression in non-responding patients. However, the fact that most anti-angiogenic therapies induce an increase in PFS that is not associated to an increase in survival invites to speculate that a transient delay in tumor growth is often followed by a more rapid progression of the neoplastic disease that leads patients to death.

More thorough analysis of these aspects of anti-angiogenic therapies is certainly required to critically evaluate the real benefits that these therapies produce in different neoplastic diseases.

Drug	Target	FDA approval	Indication
Bevacizumab (Avastin®)	VEGF	2004	First- or second-line treatment of metastatic colorectal cancer patients in combination with intravenous 5-fluorouracil-based chemotherapy
		2006	First-line treatment of patients with unresectable, locally advanced, recurrent or metastatic non-squamous, NSCLC in combination with carboplatin and paclitaxel chemotherapy
		2008	Treatment of patients who have not received chemotherapy for their metastatic HER2-negative breast cancer in combination with paclitaxel chemotherapy, FDA approval was revoked in 2011 because the drug is not safe and effective for that use
		2009	Glioblastoma patients that had progressed following prior therapy, fully approved in 2017
			Treatment of metastatic renal cell carcinoma patients in combination with interferon-alfa
		2014	Treatment of patients with persistent, recurrent or late-stage (metastatic) cervical cancer
			Treatment of women with platinum-resistant, recurrent ovarian cancer in combination with chemotherapy
		2016	Treatment of patients with platinum-sensitive recurrent epithelial ovarian, fallopian tube or primary peritoneal cancer either in combination with carboplatin and paclitaxel or in combination with carboplatin and gemcitabine chemotherapy, followed by Avastin alone.
		2018	Treatment of women with advanced (stage III or IV) ovarian cancer following initial surgical resection in combination with chemotherapy (carboplatin and paclitaxel), followed by Avastin as a single agent
Ramucirumab (Cyramza®)	VEGFR2	2014	Treatment of patients with advanced or metastatic gastric (stomach) or gastroesophageal junction (GEJ) adenocarcinoma whose cancer has progressed on or after prior fluoropyrimidine- or platinum-based chemotherapy
			Treatment of metastatic NSCLC patients
		2015	Indicated in combination with chemotherapy for the treatment of patients with metastatic colorectal cancer with disease progression on or after prior therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine

Drug	Target	FDA approval	Indication
Ziv-aflipercept (Zaltrap®)	VEGF	2012	Treatment of metastatic colorectal cancer resistant to or progressed after an oxaliplatin-containing chemotherapy regimen use in combination with a FOLFIRI
Sorafenib (Nexavar®)	Multi-kinase inhibitor	2005	Treatment of patients with advanced renal cell carcinoma or kidney cancer
		2013	Treatment patients with locally recurrent or metastatic, progressive differentiated thyroid cancer that no longer responds to radioactive iodine treatment
Sunitinib (Sutent®)	VEGFR2, PDGFRb, and c-KIT	2006	Indicated for the treatment of patients with gastrointestinal stromal tumors and advanced kidney cancer.
		2011	Treatment of patients with unresectable metastatic progressive pancreatic neuroendocrine tumors
		2017	Adjuvant treatment of patients with at high risk of renal cell carcinoma relapse after nephrectomy
Pazopanib (Votrient®)	VEGFR-1, −2 and −3, c-KIT and PDGFR	2009	Treatment of advanced renal cell carcinoma
		2012	Treatment of patients with advanced soft tissue sarcoma who have previously received chemotherapy
Axitinib (Inlyta®)	VEGFR and PDGFR	2012	Treatment of patients with advanced renal cell carcinoma who have not responded to other drugs
Regorafenib (Stivarga®)	Multi-kinase inhibitor	2012	Treatment of metastatic colorectal cancer progressing to prior therapy
		2013	Treatment of advanced unresectable gastrointestinal stromal tumors no longer responding to Gleevec of Sutent
		2017	Treatment of patients with hepatocellular carcinoma) who have been previously treated with sorafenib
Cabozantinib (Cabometyx®)	Multi-kinase inhibitor	2016	Treatment of patients with advanced renal cell carcinoma who have received prior anti-angiogenic therapy
		2017	Treatment of patients with advanced untreated renal cell carcinoma
Lenvatinib mesylate (Lenvima®)	VEGFR2	2015	Treatment of patients with progressive, differentiated radioactive iodine refractory thyroid cancer
		2016	Treatment of patients with advanced renal cell carcinoma, previously treated with an anti-angiogenic, in combination with everolimus
Vandetanib (Caprelsa®)	VEGFR2 and EGFR	2011	Treatment of metastatic unresectable medullary thyroid cancer
Everolimus (Afinitor®, Zortress®)	mTORC1	2009	Advanced RCC after failure of either sunitinib or sorafenib
		2011	Treatment of progressive neuroendocrine tumors of pancreatic origin in patients with unresectable, locally advanced or metastatic disease
		2012	Advanced breast cancer
		2016	Progressive, nonfunctional gastrointestinal and lung neuroendocrine tumors (NET)
Lenalidomide (Revlimid®)	Inhibitor of TNFα production	2013	Treatment of patients with mantle cell lymphoma (MCL) whose disease has relapsed or progressed after two prior therapies
		2015	Treatment of patients with multiple myeloma in combination with dexamethasone
		2017	Treatment of patients with multiple myeloma as maintenance therapy following autologous hematopoietic stem cell transplant (auto-HSCT)

11.7 Concluding Remarks

The ultimate aim of any overview is to provide an objective assessment of the available knowledge in a specific field, in this case on the novel anticancer drugs that have recently developed. We tried to filter the information of the literature with our research and clinical experience, often expressing our critical views that are not necessarily shared by all oncologists. We take the risk of criticisms as we think that the potential utility of this chapter is to stimulate discussion and highlight controversial issues requiring more knowledge. Therefore in the spirit of this chapter we conclude our overview indicating three crucial open questions for which we have only partial answers due to limited available knowledge and should be taken in account to plan research strategies in the coming years.

1. Why many compounds that are very promising in preclinical setting do not show sufficient efficacy in the clinic?

Probably the available preclinical models are still largely insufficient to adequately mimic the clinical presentation of neoplastic diseases, particularly regarding the sensitivity and resistance to antitumor agents. Cancer cell lines growing in vitro are certainly useful to investigate the mode of action of drugs, but they are not representative of the biological complexity of the tumors growing in vivo in patients. We can also rely on some molecularly characterized rodent tumors that are instrumental to investigate the mode of action and the therapeutic index of some drugs, but the quantitative prediction to their efficacy for the clinic is highly questionable. Patient derived xenografts (PDX) often maintain some specific molecular features of the original human tumor, e.g. mutations of some driver gene, nevertheless they grow in immunodeficient mice not suitable to test immunotherapies. Since the response to drugs is often dependent on, or it is influenced by, the immune system and tumor microenvironment the use of immunodeficient mice can lead to misleading results. Another challenge regards the inability to adequately translate drug dose and treatment schedule.

2. Why only a limited number of predictive biomarkers have been developed to select potentially responsive patients?

The complexity of most human tumors explains why the drug response is due to many factors and this represent an obstacle to identify reliable markers to guide the therapeutic choice, even when the drug has a good degree of specificity. In some cases, the response is not due only to changes in the levels of the specific target, but also to other factors that are crucial for the recovery or death of cancer cells. Growing evidence suggests that the tumor microenvironment plays an important role in the ultimate therapeutic response, although the contribution of different components is still only partially elucidated.

3. Why many drugs are effective in inducing an objective response but do not improve patients' survival?

Most human solid tumors are genomically unstable and very heterogeneous. The problem of heterogeneity of advanced solid tumors is the major reason for long term failure of therapies. The heterogeneity regards both the biological features of tumor cells and the drug distribution [84]. In the same neoplastic tissue, cancer cells with different biological features and drug sensitivity coexist. This means that sensitive tumors may disappear after treatment, but in most cases resistant clones will cause tumor relapse that will ultimately cause patients' death. Improving our knowledge on early molecular events causing transformation will help to identify tumors at earlier stage, when tumors are less heterogeneous and easier to be treated.

In conclusion, despite the significant improvements of the therapy of cancer and the ability to control growth of some tumors, we still need to understand how to efficiently exploit the wealth of the available therapeutic armamentarium. Rational combinations with drugs acting on different populations of cancer cells and on the tumor microenvironment should be investigated. Since most of the druggable targets identified so far are not cancer specific, we have obviously to deal with toxicity and the optimization of the sequences with the best therapeutic index requires much research. In addition, novel strategies to deliver therapeutics with high specificity to tumor cells must be elaborated.

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Immunotherapy

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Contents

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12.1 Introduction

Over the years, the treatment of cancer has seen the opening of different and new therapeutic pathways that have made it possible to overcome the many critical aspects that have always been linked to cancer and its treatment, allowing us to achieve better results in terms of toxicity.

The first validated drug for the treatment of cancer was introduced in the '70s with cisplatin, then followed by the taxanes in the 90s and then, at the beginning of our millennium, by targeted anti-HER2 and c-KIT inhibitors therapies [1].

But the real breakthrough that allowed a real step forward in cancer treatment was the discovery of cancer cells beingable to grow and develop through mechanisms of inhibition of the immune system [2–4]. In this way the cancer is able to evade the only endogenous system able to defeat it in the late nineteenth century Dr. Coley discover what was later renamed the "Coley toxin", based on the bacterium Erysipelas, pathogen of various infectious skin diseases, which cured precisely where the malignant lesion was present [5].

After Paul Ehrlich in 1909 postulated that aberrant cells, capable of triggering tumors, were constantly eliminated by the immune system thanks to the exposure of antigenic molecules against which the host could produce an antibody response sufficient to eliminate the neoplastic elements [6].

This idea was then confirmed in 1950 with Brunet and Thomas who postulated the concept of "cancer immunosurveillance" which provides that one of the physiological functions of the immune system is to recognize and destroy cellular clones transformed before they can grow and form tumor and kill cancer cells after they have formed [7] The work of Coley was then continued by the German Uwe Hobohm, a biologist chemist at the German University of Giessen, who in 2012 decided to improve Coley's idea. More specifically, he realized that, in the vitro models, bacterial immunotherapies (therapies that exploit bacteria to activate the immune system) are able to stimulate the production of cytokines, molecules that start the reaction of the immune system, documenting regression of the patient's tumor.

The immune system is able to control the disease only in the very initial phases, when the tumor is still limited and defense mechanisms still efficient; furthermore, when the cancer cells can't be destroyed, the cancer, continuing an antigenic stimulation, causing an exhaustion of the immune system. Then start a series of control mechanisms that determine immune reactions by activating cells that regulate inhibitory molecules such as PD-1 and CTLA-4.

These molecules, together with the release of immunosuppressive molecules by the tumor, reduce the activity and the proliferation of specific T lymphocytes, in order to avoid the establishment of autoimmunity phenomena, a control mechanism useful in some physiological conditions such as acute infections, which, however, in the case of tumors, turns into a powerful ally.

Cancer cells can grow and multiply in an undisturbed way because they are able to evade the immune system through various ways. They can stop the presentation of the antigen, recruit immunosuppressive cells (Treg) and recruit suppressor cells derived from myeloid cells, induce an exhaustion of specific anti-tumor response mediated by T and B lymphocytes through prolonged and ineffective stimulation as well as downregulate the molecules of the MHC I histocompatibility complex necessary for the recognition of tumor antigen by lymphocytes, deactivate the antigen processing mechanisms, release factors that suppress immune activity including adenosine, prostaglandin E2, and the indoleamin 2,3-dioxygenase enzyme (IDO) [4].

Towards the end of the 1990s, the first anticancer drugs Bacillus Calmette and Guering for bladder cancer and cytokines IFN –alpha and IL-2 for melanoma and renal cell carcinoma [8], both of which were able to stimulate immune response, were made available. These therapiees have failed to demonstrate clinical benefit in advanced carcinoma. In 2010, several immunotherapeutic drugs were introduced such as the Sipuleucel-T vaccine in the treatment of prostate cancer and the antibodies specifically designed to attack some molecules expressed on the tumor cell or produced by them (anti-HER2, anti-VEGF, anti-EGF, anti-CD20).

But the real breakthrough in the world of immunotherapy came by when the focus of th experiments shifted from directly stimulating immune response to removing the inhibition induced by the same tumor cells,thus unlocking the state of anergy in which T and B lymphocytes turned.

The antagonist of the immune checkpoint that aroused most interest was the CTLA-4 and PD-1/PDL-1 pathway, which have demonstrated a tumor response rates of 20–30% [9].

The first drug that was approved with this action mechanism was Ipilimumab, in 2011, a protein that blocks CTLA-4, a key inhibitory control molecule that counteracts the co-stimulatory signal of CD28, competitively binding to its ligands (B7.1 and B7.2) on the surface of cells presenting the antigen [10]. This link turns off the immune defense by suppressing T cell activation and proliferation as well as promoting Treg cell function. The ipilimumab, studied for the treatment of metastatic melanoma, blocking CTLA-4 restores the co-

stimulating activity of CD28, increasing the number of T cellule that can migrate and attack the tumor [11].

Promising long-term results in various cancers were made possible with the development of certain drugs such as: PD-1 inhibitors, Nivolumab and Pembrolizumab [12–15].

This is a new therapeutic approach which stimulates the patient's immune system regardless of the site of tumor, histology, the stage and the degree of differentiation [16].

12.2 The Immune System

The immune system can be described as a complex network of molecules, cells, tissues and organs. The physiological function of the immune system is the defense against infectious agents.

Non-infectious substances of foreign nature can also provoke an immune response.

The first crucial task is to eliminate all that is foreign (non self). Immune system also recognizes deadand damaged cells. In some situations, even self molecules can activate an immune response (autoimmune response). The defense against pathogens is assured by early reactions of innate immunity and the later ones of adaptive immunity.

Innate immunity: Consists of cellular and biochemical defense mechanisms pre-existing to infection and ready to react quickly.

The main components of innate immunity are:

1. Physical and chemical barriers: epithelium.
2. Cells: neutrophils and macrophages, dendritic cells and natural killer cells.
3. Blood proteins: complement system and mediators of inflammation.

The cells of innate immunity recognize pathogens through receptors (pattern recognition receptor, PRR) peculiar for the structures expressed by microbes, called PAMPs (pathogen associated molecular patterns). The most common PAMPs there: LPS lipopolysaccharide expressed on the membrane of GRAM neg, double-stranded RNA, non-methylated CPG sequences of microbial DNA, mannose-rich oligosaccharides and fucose of bacterial membranes. Some molecules released by damaged self cells, called damage associated molecular patterns (DAMP) can be recognized as danger signals from innate immunity, which is stimulated to eliminate these cells.

The main receptors that recognize PAMP and DAMP are Toll.like recptors (TLR), C leptin receptor (CLR), RIG-I-like receptors (RLR) and NOD-like receptors (NLR).

Adaptive immunity: There are two types of adaptative responses:

1. Humoral immunity: mediated by molecules present in the blood and mucosal secretions: called antibodies and products from B lymphocytes. The antibodies recognize the microbial antigens and induce their elimination through different effector mechanisms. For example they promote phagocytosis, they can trigger the release of inflammation mediators, they can be transported in the mucosal lumen of many organs and through the placenta they can protect the newborn.
2. Cellular immunity: mediated by T lymphocytes. T lymphocytes are divided in
   - Cytototxic T lymphocytes (CTL): that kill virus-infected cells or cancer cells
   - Lymphocytes T helper (Th): coordinate immune response through cytochines production
   - Lymphocytes T regulatory (Treg): with immunosoppressive functions

T cells have a membrane antigenic receptor called T cell receptor (TCR) whose repertoire variety is guaranteed by the genetic recombination of the VDJ segments. The TCR recognizes only protein fragments presented by the molecules called MHC. The activation of the T lymphocyte requires, both the TCR-MHC-peptide complex recognition and costimulatory signals. Only antigen presenting cells (APC) express both MHC and co-stimulatory molecules.

MHC: MHC molecules are distinguished in class I and class II. MHC In humans, MHC molecules are called HLA. The MHC I molecules are expressed by all the cells and present to the CTL fragments of proteins in order to control the intracellular activity. MHC II molecules are expressed by APCs (macrophages, dendritic cells) or B lymphocytes. APCs internalize circulating antigens and present them to Th lymphocytes.

12.3 Immunity and Cancer

The concept of immunosurveillance was proposed by Macfarlane Burnet in 1950 and defines that among the functions of the immune system there are the recognition and elimination of neoplastic cells. The ability of both the innate and adaptive immune system to recognize the different types of neoplasia is at the basis of the use of immunotherapy for the treatment of tumors. Today we know that tumors are able to stimulate adaptive immune responses. The presence of inflammatory infiltrates (macrophages, NK cells, T lymphocytes) has been widely demonstrated in some tumors, such as melanoma or breast cancer, and that is associated with a better prognosis.

We also know that immune responses are not always able to prevent the development of tumors because the tumors have escape mechanisms that can evade the immune system. In addition, the tumor cells are derived from the host cells and therefore they are very similar to these cells and therefore tend to to be weakly immunogenic.

Tumor Antigen:

The possibility of inducing an adaptive immune response indicates the presence of different tumor antigens compared to those of the normal cell. The antigens expressed selectively on tumor cells are called TSA-specific tumor antigens, while cancer antigen expressed also by health cells are call tumor associated antigen TAA.

Cancer antigens are divided into:

1. Products of oncogenes and tumor suppressor genes: RAS, p53, Bcr/Abl
2. Products of overexpressed but mutant oncogenes: HER2/neu
3. Mutation of genes not involved in tumorigenesis
4. Gene products that are silent in most normal tissues: tumor/testicular antigens expressed in melanomas normal
5. Non-oncogenic proteins overexpressed in tumor cells: gp100, MART
6. Oncogenic virus products: E6 protein, papillomavirus E7, EBV EBNA protein
7. Oncofetal antigens: carcinoembryonic antigen
8. Glycolipids and glycoproteins: GM2 and GD2
9. Differentiation antigens expressed normally in the tissues of origin: PSA, CD20

T Lymphocyte:

T lymphocytes are the protagonists in the response against tumors. The main mechanism of protection of the adaptive immunity against tumors is the killing of tumor cells by CD8 + CTLs. Specific CD8 + responses require cross-presentation by dendritic cells because most tumor cells do not express costimulatory molecules, typical of APC, or MHC II molecules. The cancer immunity cycle begins with the phagocytosis of tumor cells/tumor antigens by the host APCs. APCs process antigens and present them with MHC I to CD8 +. APCs express stimulatory molecules that allow the differentiation of CD8 + into CTL. CTLs are now able to recognize and kill the tumor cell.

The role of T helper CD4 + lymphocytes is less clear, could provide the cytokines necessary for the differentiation of CD8 + naïve lymphocytes in CTL effectors and memory. T helper lymphocytes release cytokines with TNF and IFN-y that increase the expression of MHC.

Antibodies: Cancer patients can produce antibodies against tumor antigens, such as in EBV-associated lymphoma. Antibodies can kill tumor cells through complement activation or antibody-dependent cellular cytotoxicity mediated by macrophages or NK cells.

NK Cells: Kill cancer cells, particularly those with a reduced expression of MHC I. Some tumors also express molecules such as MIC-A, MIC B and ULB that are ligands for the NKG2D activating receptor expressed by NK cells. Furthermore, NK cells can recognize and lyse IgG-coated tumor cells by binding to the Fc receptor (fcyRIII or CD16). The activity of NK cells is increased by some cytokines such as IFN-y, IL-15, IL-12. The IL-activated NK cells called LAK/lymhokine activated killer cells) are used in immunotherapy.

Macrophages:

Macrophages are able to both inhibit and promote growth and metastatic spread. In particular, M1 macrophages are able to kill many types of tumor cells through the recognition by the TLRs of some DAMP expressed on dying tumor cells and the activation by the IFN-Y produced by specific tumor T cells. Macrophages M1 kill cancer cells with the same mechanisms with which they kill microbes, in particular with the production of nitric oxine (NO).

M2 macrophages instead contribute to tumor progression. These cells secrete VEGF vascular endothelial Growth factors and the Transforming growth factors (TGF-b) that promote angiogenesis.

12.4 Theory of Immunoediting

Despite immunosurveillance, tumors can develop and become clinically evident. Experiments on mouse models have shown that tumors developed in immunosuppressed mice are far more immunogenic than tumors developed in immunocompetent mice. This suggests that the immune system is able to modify the neoplasm during disease progression. Immunological remodeling occurs continuously even though the major effects appear to occur in the early stage of growth when the tumor is not clinically detectable. Immunoediting is developed in three phases

1. Elimination: cells of the immune system destroy cancer cells. If at this stage all the cells are eliminated, no tumor will occur. If some cells remain active, they can enter the equilibrium phase.
2. Equilibrium: the cancer cells remain in a dormancy phase indefinitely. The selective pressure performed by the immune system can lead to the formation of tumor cells that are no longer recognized and which

become insensitive to the effector mechanisms. These cells enter the third phase.
3. Evasion: cancer cells will give rise to clinically evident tumors.

12.5 Immune Escape

Many tumors develop mechanisms that allow them to evade antitumor responses. These mechanisms can be distinguished in intrinsic to tumor cells or those mediated by other cells.

The main resistance mechanisms are:
1. Inhibition of dendritic cell maturation
2. Loss or reduction of expression of tumor antigens
3. Masking of tumor antigens
4. Reduced expression of MHC I and II
5. Lack of expression of costimulatory molecules (B7)
6. Production of immunosuppressive factors (IL-10, VEGF, TGFβ, PGE2, adenosine, IDO, IL_6)
7. Activation of immunosuppressive cells: Treg or myeloidosopressorie cells (MDSC), M2 macrophages
8. Up regulation of immune checkpoints: PD-L1/PD-L2; CTLA-4
9. Expression of pro-apoptotic molecules by tumor cells: FASL, TRAIL.

12.6 Immunotherapy

The possibility of treating cancer patients with immunological approaches dates back to the '70s even though the real revolution began only in 2011 when the first inhibitory immunocheckpoints was approved: ipilimumab for the treatment of patients with metastatic melanoma.

The introduction of inhibitory immunocheckpoints dramatically revolutionized the approach to cancer treatment by shifting the treatment target from the neoplastic cell to the T lymphocyte.

In general, immunotherapy aims to increase the patient's responses (active immunity) or to administer antibodies or tumor-specific T lymphocytes (passive immunity).

Immunization practices in vivo. Among the most promising strategies we have:
- The vaccines with tumor antigens or whole allogeneic cells
- The use of dendritic cells and the use of antibodies that target the immunitary controls of T lymphocytes

12.7 Passive Cancer Therapy

Passive immunotherapy: It develops in ex vivo activated cells or molecules that, once introduced into the body, compensate the deficiencies of the immune system. These include: specific tumor antibodies, recombinant cytokines and cell therapy.

Tumor specific monoclonal antibodies:

IgG is the most used for the stability and long half time. The binding between costant fragment and Fcy-Recptors (FcyRIIIa and FcyTIIa on NK cells, and macrophages respectively) induces the antibody dependend cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC) or antibody depended cellular phagocytosis (ADCP).

Examples of antibodies are: rituximab (anti CD20) used in non-Hodgkins lymphoma, Pertuzumab (anti-HER2) in the treatment of breast cancer, Trastuzumab (anti Her2) in the treatment of breast cancer and gastric cancer, Cetuximab (anti EGFR) in the treatment of colorectal and head-neck tumors, bevacizumab (anti-VEGFA) in the treatment of colorectal, lung, breast, kidney, glioblastoma and gynecological tumors, Panitunumab (anti-EGFR) in the treatment of colorectal tumors.

Cytokine administration: the most used are interferon-alpha (IFN-alpha), IL-2, IL12.

High dose IL 12 and IFN-A are approved for the treatment of melanoma and metatastic renal cell carcinoma.

Vaccines:

Immunization of cancer patients with tumor antigens can increase the immune response to the tumor.

The identification of the peptides recognized by the specific tumor CTL allowed to obtain different vaccines according to the type of antigen:
- Products of mutated genes: FNDC3B
- Neovax – Cellular proteins overexpressed but not mutated: Gp 100
- Testicular tumor antigens: Ny ESO-1
- Inactivated or lysed tumor cells: GVAX
- Antigens associated with HSP: HSPPC-96 - In situ tumor: OncoVax

Vaccines need different adjuvants that increase the number of dendritic cells activated on the site of vaccination: ligands TRL, CpG DNA, BCG GMCSF, IL-12.

Vaccines with dendritic cells: cells are taken from the patient, purified, incubated with cancer and then injected

again into the patient. The first dendritic cell vaccine for the treatment of prostatic carcinomas has been approved. DNA vaccines and viral vectors coding for tumor antigens are being tested. However, diary and viral cell vaccines are the best way to induce a CTL response.

12.8 Immune Checkpoint Inhibitor

The use of antibodies that inhibit the molecules that block T-lymphocytes is revolutionizing the survival of cancer patients.

Cancer cells use, in order to evade the immune response, some of the normal control circuits of the immune response called immune checkpoints that have the role in physiological conditions to degrade the activation of T lymphocytes and their effector functions.

CTLA-4 represents the first target used, it is expressed more on T lymphocytes and its function is to inhibit its activation by counteracting the activation of CD28. CTLA-4 ligands are CD80 (B7.1) and CD86 (B7.2). It has been shown on CTLA-4 knockout mice to be characterized by an overactivation of the immune response [17].

At the tumor level the activation of the immune system results in a clonal expansion of the specific tumor T lymphocytes able to counter neoplastic growth. The ipilimumab, human monoclonal IgG1 antibody against CTLA-4, represents the first FDA-approved ICI in metastatic metastatic melanoma. The ipilimumab is given at a dose of 3 mg/kg every 3 weeks for 4 times. Another anti-CTLA-4 drug is Tremelimumab currently being studied in monotherapy and in combination with anti PD-1. The mechanism of action, that is the blocking of the immunosuppressive signal, is at the base of the side effects.

PD-1 is an inhibitory receptor activated by its PD-L1 ligands (known as B7-H1 or CD274) and PD-L2 (also known as B7-DC or CD273). It is present on activated T lymphocytes, B cells, monocytes and NK.PD-L1 cells is expressed on tumor cells, on epithelial cells, lymphoid cells, myeloid and macrophages. The expression of PD-L1 is stimulated by IFN, IL-4, IL-10, can be expressed constitutively or as a mechanism of resistance. PD-L1 is expressed mainly by tumor cells and macrophages e lymphocytes. PD-1 limits the functional activation of cytotoxic T lymphocytes and can induce apoptosis and promotes the differentiation of CD4 + lymphocytes in Treg.

The monoclonal antibodies against the PD-1/PD-L1 axis are:

- Nivolumab and Pembrolizumab (anti PD-1)
- Durvalumab, Avelumab and Atezolizumab (anti PD-L1).

Drug	Target	FDA approval
NIVOLUMAB	PD-1	Patients with BRAF V600 wild-type unresectable or metastatic melanoma, as a single agent. Patients with unresectable or metastatic melanoma, in combination with ipilimumab. Patients with melanoma with lymph node involvement or metastatic disease who have undergone complete resection, in the adjuvant setting. Patients with metastatic non-small cell lung cancer and progression on or after platinum-based chemotherapy. Patients with advanced renal cell carcinoma who have received prior antiangiogenic therapy. Patients with locally advanced or metastatic urothelial carcinoma who: Have disease progression during or following platinum-containing chemotherapy Have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy. Adult and pediatric (12 years and older) patients with microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan. Patients with hepatocellular carcinoma who have been previously treated with sorafenib.

Drug	Target	FDA approval
Pembrolizumab	PD-1	For the treatment of patients with unresectable or metastatic melanoma. As a single agent for the first-line treatment of patients with metastatic NSCLC whose tumors have high PD-L1 expression [(tumor proportion score (TPS) ≥50%)] As a single agent for the treatment of patients with metastatic NSCLC whose tumors express PD-L1 (TPS ≥1%) with disease progression on or after platinum-containing chemotherapy. In combination with pemetrexed and carboplatin, as first-line treatment of patients with metastatic nonsquamous NSCLC. For the treatment of patients with recurrent or metastatic HNSCC with disease progression on or after platinum-containing chemotherapy. For the treatment of patients with locally advanced or metastatic urothelial carcinoma who are not eligible for cisplatin-containing chemotherapy. For the treatment of patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy. For the treatment of adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient o solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options, or o colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan.
Durvalumab	PD-L1	Locally advanced or metastatic urothelial carcinoma who: Have disease progression during or following platinum-containing chemotherapy. Have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy.
Atezolizumab	PD-L1	Locally advanced or metastatic urothelial carcinoma who: Are not eligible for cisplatin-containing chemotherapy, or Have disease progression during or following any platinum-containing chemotherapy, or within 12 months of neoadjuvant or adjuvant chemotherapy. Metastatic non-small cell lung cancer who have disease progression during or following platinum-containing chemotherapy.
Ipilimumab	CTLA-4	Treatment of unresectable or metastatic melanoma in adults and pediatric patients (12 years and older). (1.1) Adjuvant treatment of patients with cutaneous melanoma with pathologic involvement of regional lymph nodes of more than 1 mm who have undergone complete resection, including total lymphadenectomy. (1.2) Plus nivolumab in first line renal cell carcinoma

12.9 New Molecular Targets

In evaluation, in the advanced neoplasia, are other molecules of the immune checkpoint such as LAG3, TIM3, OX-40 and CD137, the chimeric antigen CAR that combines the properties of monoclonal antibodies to constrain antigens with the lytic capacity and the self-renewal of T cells [18] adoptive T-cell therapy (ATC) using expanded infiltrating lymphocytes of autologous tumor (TIL) that showed efficacy in metastatic melanoma [19]. Antitumor vaccines containing proteins overexpressed by tumor cells [20, 21], genetically modified virus Talimogene laherparepvec (T-VEC) which increases the secretion of immunostimulant cytokines which stimulates the colony of granulocytic macrophages (GMCSF) are also being studied [22], the latter currently being studied in the treatment of melanoma and other advanced tumors [23, 24].

12.10 Car-t

Together with the check inhibition, the adoptive cell therapy (ACT) with chimeric antigen receptor (CAR) redirected T cells is perhaps the most attractive anticancer strategy. CARs encode for transmembrane chimeric molecules with dual function: (a) immune recognition of tumor antigens expressed on the surface of tumor cells; (b) active promotion and propagation of signaling events controlling the activation of the lytic machinery. This system has several advantages: (1) to provide "reprogrammed T-cells" of an ex-novo activation mechanism; (2) to brake the tolerance acquired by tumor cells, and (3) bypass restrictions of the HLA-mediated antigen recognition, over-stepping one of the barriers to a more widespread application of cellular immunotherapy.

The use of anti-CD19 CAR T cells have demonstrated consistently high antitumor efficacy in children and adults affected by relapsed B-cell acute lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia, and B-cell nonHodgkin lymphoma, with percentage of complete remissions ranging from 70 to 94% in the different trials19. Based on these results, the FDA has approved two immunotherapies with anti-CD19 modified T cells, KYMRIAH [tisagenlecleucel (August 2017)] and YESCARTA [axicabtagene ciloleucel (October 2017).

12.11 Long Term Survival

A further advantage that allows to evaluate immunotherapy as the new weapon against cancer for the present and the future is the ability to achieve long-term survival, thanks to the memory of the immune system. In fact, the checkpoint inhibitors engage T cells with intrinsic capacity of adaptability and memory allowing lasting responses. Immunotherapy in fact tends to turn the tumour into a chronic disease, in a percentage that is around 20% as demonstrated in the meta-analysis of [25] where, among about 5000 patients with advanced melanoma treated with ipilimumab, 20% of the patients was alive at 10 years.

12.12 No Effect in Surrogate Endpoints and Atypical Patterns of Response

Ipilimumab was the first drug to show improvement in OS for over 30 years, despite its impact on the overall response rate (ORR) and progression-free survival (PFS) did not match the survival benefits achieved [20]. This lack of correlation between OS and surrogate endpoints was also demonstrated with PD-1 inhibitors in kidney tumor, in which the nivolumab compared with everolimus showed a longer OS (25 vs 19.6 months, HR 0.73, *p*: 0.0148) but not an advantage in PFS [21], non-squamous NSCLC [13] and squamous cell carcinoma of the head and neck [26], although tend to have a greater advantage in terms of ORR and PFS compared to anti-CTLA-4 therapy [27].

The first-line nivolumab has an ORR of about 43% and a PFS of 5.4 and 6.9 months [27], whereas the first or second-line pembrolizumab has an ORR of about 33% and an average PFS of 4,1 and 5.5 months, respectively [28].

This result finds a double justification: on the one hand, the mechanism of action of these drugs, having to stimulate the immune response, determines a slower response [13, 20–26]. In fact, the immuno-oncological agent does not generate visible results immediately, not directly affecting the tumor cells, in fact in some cases it may take up to 16–20 weeks for a clinical or instrumental response to occur. On the other hand there is the phenomenon of pseudo-progression in which an increase in the number of cells of the immune system, rather than of tumor cells, determines the appearance of the nodal progression that can be followed by the regression of the tumor. In particular in melanoma, a clinical response is observed in 7–12% of cases after an initial diagnosis of progressive disease according to the RECIST criteria [29].

To avoid this inconvenience, a new method of assessing response in the disease treated with immune-oncological agents has been studied, subverting what are the classical criteria for assessing the response to chemotherapy and validating new ones (IRecist) [30] that they allow not to identify a sure progression of the disease in the onset of new lesions, but to consider the trend of the tumor mass in the total.

In patients treated with nivolumab and BRAF inhibitors, about 8% had a response after initially presenting new lesions [31], as well as in the KEYNOTE 001 study, about 15% of patients treated with pembrolizumab has had an unconventional response with the development of new lesions and subsequent response to treatment beyond progression [32].

These data suggest that endpoints such as objective response rates and PFS may not be appropriate for long-term measurement of treatment benefit [33].

12.13 Persistent Responses After Cessation of Therapy

Immunotherapy has the great advantage of allowing a continuous response even after the interruption of therapy, which usually occurs due to unacceptable toxicity, as demonstrated in the CheckMate 067 study [27], in whereas 85% of patients who discontinued nivolumab due to drug-related toxicity had a complete or partial response and 70% continued to respond despite treatment interruption. Similar results occurred in the KEYNOTE 001 study with pembolizumab, where 97% of patients with a complete response maintained the response after treatment interruption [34].

The optimal duration of immunology therapy is therefore still under study.

12.14 Immunotherapy Targets a Broad Range of Tumour Types

Another important advantage, already mentioned at the beginning of the chapter, is that the efficacy of

immunotherapy is independent of histology and of cancer. Indeed, ipilimumab has shown efficacy in both cutaneous melanoma, ocular and mucosal melanoma, with 1 year OS rates of 27–34% in patients with uveal melanoma [34–36] and 35% in mucosal melanoma [37], showing no differences between the mutated BRAF subtype and NRAS in terms of OS and long-term benefits [38].

In addition, the efficacy of the therapy also extends to other types of tumors, such as non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), renal cell carcinoma, Hodgkin's lymphoma, head and neck cancer, esophageal and gastric cancers, hepatocellular cancer, bladder cancer, breast cancer and others [39, 40].

12.14.1 Special Populations

The ipilimumab allows a consistent benefit even in those patients who have a poor prognosis (e.g. elevated lactate dehydrogenase or poor performance status) [41, 42].

It also demonstrated activity in elderly patients and in those with stable and asymptomatic brain metastases [43, 44].

12.15 Therapeutic Schedule

Because of the particular mechanism of action of these immune-related drugs, their greater efficacy seems to prove at the beginning of the treatment paradigm, when the patient has a better prognosis. Furthermore, it has been shown that patients progressing after immunotherapy can use new lines of therapy due to the long survival linked to immunotherapy, not compromising the ability to respond to a subsequent therapy with BRAF inhibitors.

This advantage is also linked to the ability to act without altering the nature of tumor cells, not determining the selection of rapidly kinetic disease clones.

This discourse is not feasible to the contrary, patients who are progressing rapidly after the use of BRAF inhibitors are not always able to complete the subsequent immunotherapy, which would allow a significantly higher OS. The median OS among patients with rapid progression who did not use second-line immunotherapy was 5.7 months, compared to 18.6 months in patients who managed to complete the 4 cycles of ipilimumab.

On the contrary, in one study, with progression after previous immunotherapy with ipilimumab, all patients were able to practice therapy with BRAF inhibitors [45] without presenting a rapid progression of the disease.

So a first line of immunotherapy followed by progression from the target therapy, could be the best sequential approach to the patient with indolent disease [45–49]. In addition, previous therapy with ipilimumab does not seem to affect the efficacy of subsequent anti PD-1/PDL-1 therapy [50].

12.16 The Safety Profile

Another important advantage in favor of immunotherapy is the safety profile. These drugs, in fact, not going to act directly with cytotoxic or cytostatic effects, show a better tolerability in terms of side effects, among these the most important immunocorrelated side effects (irAE) of the anti CTLA-4 affecting the skin (pruritus and rash), gastrointestinal tract (colitis and diarrhea), liver (autoimmune hepatitis) and endocrinopathies (hypo, hyper-thyroiditis, hypophysitis), in rare cases may appear neuropathy, myositis, arthritis and uveitis.

The anti-PD-1/PD-L1 drugs have the same side effects as the anti-CTLA4, with the addition of pneumonia which, in most cases, is grade 1–2, resulting almost never due to disruption of the treatment. The incidence of grade 3–4 irAE with anti-PD-1 (nivolumab and pembrolizumab) is generally lower than ipilimumab, whereas higher incidence irAEs of grade 3–4 occurs with the combination of ipilimumab plus nivolumab, a combination that it is not associated with the appearance of new toxicities [27]. All immune-related adverse events are due to an over-stimulation of the immune system and the early recognition of these toxicities and early treatment with steroids is fundamental [51].

12.17 Combination Immuno Checkpoint Strategies

The checkpoints act at different times and locations in the tumor, the anti CTLA-4 act in an initial phase and in the periphery at the lymph node level, while the anti PD-1 act during the effector phase within the tumor microenvironment.

The ipilimumab, by inhibiting CTLA-4, promotes the proliferation of T cells, increases the number of activated T cells that can migrate to attack the tumor; while, anti-PD1 agents such nivolumab neutralize tumor defenses within the tumor microenvironment, reactivating T cell activity and inducing tumor cell death [52, 53]. The complementary roles of these two pathways in regulating adaptive immunity are supported by preclinical models in which simultaneous administration of anti-

CTLA-4 and anti-PD1 antibodies resulted in increased antitumor activity compared to single-agent treatments [54, 55].

Combining anti CTLA-4 with anti PD-1/PDL-1 results in greater efficacy in response to monotherapy [27], but accompanied by increased toxicity, 55% in the nivolumab plus ipilimumab group compared to 16.3% in the nivolumab group and 27.3% in the ipilimumab group. A strategy to maximize the benefit and reduce toxicity lies in the modification of the dosage, using ipilimumab at 1 mg/kg every 3 weeks for 4 cycles, this dose in association with a PD-1 block allows to preserve the response (ORR 57%) with an incidence of 20% of IGAs in Grade 3 and 4 [56].

With a minimum of 28 months of follow-up, the median PFS was 11.7 months with the combination (95% CI, 8.9–21.9) versus 6.9 months with nivolumab and 2.9 months with ipilimumab. The median duration of response was not included in the nivolumab/ipilimumab arm, 31.1 months in the nivolumab arm, and 18.2 months in patients who received ipilimumab. This data showed that nivolumab alone or combined with ipilimumab resulted in significantly longer progression-free survival than ipilimumab alone and led the EMA [European Medicines Agency] and FDA [U.S. BRAF V600 mutations. BRAF V600 wild-type disease and finally in BRAF V600 mutations.

In a combination of ipilimumab and nivolumab in patients with advanced melanoma, 40% of patients treated with the concomitant combination had objective responses and the 1-year OS rate was 82% [57, 58].

In the KEYNOTE 029 study in advanced melanoma the pembrolizumab used at a 2 mg/kg dose with ipilimumab at 1 mg/kg every 3 weeks for 4 cycles achieved an ORR of 57% with a slightly lower incidence of grade 3–4 AE (42%) [59].

Chemotherapy, radiotherapy and target therapy act not only through cytostatic/cytotoxic mechanisms, but also through a strengthening of the immune activity. For example, ipilimumab plus dacarbazine achieved longer-term survival longer than dacarbazine alone in metastatic melanoma [60], association with photemustine also appears to give a higher rate of disease control in metastatic melanoma [61].

Radiotherapy releases tumor antigens and promotes signals that allow the presentation of antigens from dendritic cells [62, 63], upregulate chemokines, increase the expression of MHC molecules, of ligands induced by the death receptor stress on tumor cells [64, 68], allowing a synergism of action with immunotherapy [64].

In the treatment of NSCLC the combination of chemotherapy and immunotherapy, in the KEYNOTE 189–407, 042 and the IMPOWER 150, resulted superior than the monotherapy approach.

Recentely the sequential strategy of radiochemotherapy followed by durvalumab demonstrated a significant improvement in OS in patients affected by stage III NSCLC.

Preclinical studies have in fact shown that the combination of RT with immunological agents increases the recruitment of T cells specific to fight the tumor [65–67] resulting in an affection abscopal, where the localized treatment of the tumor causes not only a narrowing of the aforesaid, but also a narrowing of neoplastic areas outside the scope of localized treatment [68, 69]. Important responses were observed in lung carcinoma [70, 71] and melanoma treated with ipilimumab in combination with hypo-fractionated RT [72]. Moreover, in a retrospective analysis performed in patients who progressed after treatment with ipilimumab and subsequently treated with radiotherapy, patients who showed an abscopal response had a better OS than those who did not (22.4 months against 8.3 months), confirming the possibility of synergy between radiation and immunotherapy [73]. This Abscopal effect was also observed in patients who had made RT before or after therapy with ipilimumab, suggesting potential synergistic effects on antitumor immunity [70, 74].

Another interesting approach is the combination of BRAF or MEK inhibitors with the anti PD-1/PDL-1 agents, a combination with lower toxicity compared to the ipilimumab combination. Early data from the combination of ipilimumab with the BRAF inhibitor (vemurafenib) were disappointing due to an increase in hepatotoxicity [75], data not confirmed by the association with another BRAF (dabrafenib) inhibitor [76].

A number of clinical trials are currently underway to provide further information on the safety and efficacy of these combinations [77].

12.18 Sequential Therapy

Given the initial disappointing results of concomitant therapy in terms of toxicity, any effects of sequential therapy were explored and the sequence with which to use the different therapeutic approaches available is currently under study.

The data now available allow us to begin to hope, in one study, in fact, the median OS among the patients treated with a BRAF inhibitor was 1.2 months after the end of BRAF inhibition for those who did not complete the treatment with ipilimumab compared to 12.7 months of those who did [78]. On the other hand, disappointing were the results for patients treated with ipilimumab after discontinuation of the BRAF inhibitor, with only half able to complete four cycles of ipilimumab and median OS of 5.0 months [79].

Luongo et al. [80] demonstrated that the presence of metastases in ≥3 three organ sites and high basal lactate dehydrogenase are negative prognostic factors in patients with mutated BRAF melanoma, and that these patients may benefit more from treatment with an inhibitor of BRAF before immunotherapy [45].

The results of two ongoing studies are awaited, the Secombit a randomized comparative three-arm study, which explores combined immunotherapy (ipilimumab plus nivolumab) followed by targeted combination therapy (encorafenib plus binimetinib) or vice-versa in patients with metastatic mutated melanoma with BRAF is a third arm with an 8-week induction with the targeted combination therapy, followed by combination immunotherapy, and subsequently by the target combo to progression (NCT02631447); the ECOG 6134 study, a randomized phase III trial comparing ipilimumab plus nivolumab followed by dabrafenib plus trametinib versus dabrafenib plus trametinib followed by ipilimumab and nivolumab in patients with advanced melanoma (NCT02224781).

12.18.1 Adjuvant Immunotherapies

Currently approved drugs for the adjuvant treatment of stage IIB-III melanoma are: high-dose IFN-a in the United States and Europe [81], at low doses for stage II in Europe [82] and IFN-a pegylated for stage III in the United States [83]. Ipilimumab was approved in October 2015 for the adjuvant treatment of patients with stage III melanoma with pathologic involvement of regional lymph nodes>1 mm who have undergone complete resection, including total lymphadenectomy. EORTC 18071 trial, in which adjuvant ipilimumab at 10 mg/kg reduced dose the risk of recurrence by 25% versus placebo (HR, 0.75; 95% CI, 0.64–0.90; $P < 0.002$), showed better RFS (HR 0.75, p: 0.0013) and at a median follow-up of 5.3 years, there was a 28% reduction in the relative risk of death (HR 0.72, p: 0.001), and the overall 5-year survival rate was 11% higher with ipilimumab adjuvant therapy compared to placebo [84].

In one study, nivolumab showed superiority to hypilimumab in the surgically resected stage III/IV patient with a high risk of relapse. The trial was stopped early by the data safety monitoring committee two to clear evidence of benefit for nivolumab. At a median follow-up of 18.5 months, relapse-free survival was 66.4% with nivolumab vs 52.7% with ipilimumab with a hazard ratio of 0.65 ($p < 0.0001$). There was a 35% reduction in the risk of recurrence with nivolumab versus ipilimumab (HR, 0.65; 95% CI, 0.53–0.80; $P < 0.0001$) [85].

The results of this study show for the first time that an anti-PD-1 drug is superior in the adjuvant setting and because of its lower toxicity nivolumab is much easier to give than ipilimumab. In December 2017, the FDA has approved the PD-1 inhibitor nivolumab (Opdivo) as an adjuvant treatment for patients with completely resected melanoma with lymph node involvement or metastatic disease, based on findings from the phase III CheckMate-238 trial.

Another anti-PD-1 drug, pembrolizumab, is being tested as adjuvant therapy against placebo in patients with resected stage III melanoma in a phase III European Organisation for Research and Treatment of Cancer (EORTC) triali, I dati preliminary dimostrano che pembrolizumab (Keytruda) reduced the risk of recurrence by 43% in patients with stage III resected high-risk melanoma, according to findings from the phase III EORTC1325/KEYNOTE-054 trial. The hazard ratio for recurrence-free survival (RFS) was 0.57 for pembrolizumab versus placebo (98.4% CI, 0.43–0.74; $P < 0.0001$) [86].

12.18.2 Efficacy in Brain Metastases

Immunotherapy has also been shown to be effective in fighting encephalic metastases [87], as shown by these two studies in which patients with melanoma and asymptomatic brain metastases, treated with nivolumab and ipilimumab, after a median follow up of 6.3 months, had an intracranial objective. Response rate of 54%, with 21% of patients showing a complete response and 33% had a partial response. The Intracranial clinical benefit rate was 60%. The six-month PFS rate was 67% and was similar for patients showing intracranial and extracranial responses [88].

Similarly, the Anti-PD1 Brain Collaboration (ABC) study assessed the activity of nivolumab, alone or in combination with ipilimumab, in melanoma patients with brain metastases.

The ABC study enrolled 3 cohorts of patients with melanoma and brain metastases. Two with asymptomatic brain metastases with no prior local brain therapy. Patients in cohort A received induction therapy with the combination of nivolumab and ipilimumab followed by nivolumab. Patients in cohort B received nivolumab only. Patients in the third cohort had brain metastases that had failed local therapy and had neurologic symptomatic brain metastases with leptomeningeal disease. The primary endpoint of intracranial response was reported for 42%, 20%, and 6% of patients respectively, with complete responses reported for 15%, 12% and 0% of patients. Among treatment-naïve patients, intracranial responses were seen in 50%, 21%, and 25% respectively. The intracranial median PFS was 4.8, 2.7, and 2.5 months for patients in cohorts respectively. The corresponding 6-month PFS rates were reported to be 46%, 28%, and 13%, respectively [89].

12.18.3 Duration of Treatment

The result from a randomized trial evaluating the impact of stopping treatment with a PD-1/PD-L1 inhibitor at 1 year vs continuing treatment in patients with advanced, previously treated NSCLC suggest the advantage of continuing the treatment with Nivolumab until rpgression of toxicities.

12.18.4 Dose and Schedule

Still on the high seas we find ourselves regarding the identification of dosage that allow the maximum benefit in terms of effectiveness with the lower incidence of irAE. The ipilimumab was compared to dosages of 3 and 10 mg/kg in advanced melanoma, obtaining an OS of 11.5 vs. 15.7 months with the respective dosages (HR 0.84, *p*: 0.04) [90], showing the latter a higher rate of irAE. The nivolumab was approved at a fixed dose of 3 mg/kg, reaching the highest number of responses [91].

The dosage of pembrolizumab is instead still being studied, the dosages of 2 mg/kg every 3 weeks, 10 mg/Kg every 2 weeks and 10 mg/kg every 3 weeks [92] were explored. Currently, the dosage of 2 mg/kg has been chosen every 3 weeks, since while showing the dosage of 10 mg/kg every 2 weeks a higher ORR, there was no difference in the OS.

12.19 Biomarkers

To date no reliable predictive factors have been identified to predict response to immune checkpoint inhibitors. Although some clinical parameters have been suggested to have a negative predictive value, i.e. high tumour burden, malignant pleural effusion, poor PS, rapidly progressive disease, brain metastases. However, these factors do not inform about neither patients' immune fitness nor tumour immunological status.

Until now the most extensively investigated biomarker is PD-L1, which is expressed both on tumour and inflammatory cells. Nonetheless, the determination of PD-L1 displays several issues: firstly PD-L1 is an extremely dynamic marker, secondly they exist different immunohistochemical antibodies and assays in clinical practice resulting in different cut-off points, and lastly biopsies may not be representative of the entire tumour. Despite its still controversial role, several studies demonstrated an association between high level of PD-L1 expression on tumour cells and increased response to anti-PD-1/PD-L1 treatment [93].

Another promising biomarker is tumour mutational load, that is well-known to reflect neoantigens burden potentially recognized by the immune system. This has been shown to correlate with better anti-PD-1 response for both pembrolizumab and nivolumab and combination of nivolumab and ipilimumab.

The same findings were demonstrated in the OAK study considering peripheral blood mutational load and response to atezolizumab. However, mutational load doesn't consider the transcriptomic and proteomic modification that depend on other mechanisms such as epigenetic and hence the rationale of the ongoing studies of association between anti PD1-PD-L1 and HDAC inhibitor (▶ ClinicalTrials.gov Identifier: NCT02437136). More interestingly, the co-occurence of a high tumor mutational burden and PD-L1 expression level of at least 50% has been suggested as predictor of response to nivolumab in NSCLC since in this subset ORR was 75% compared to 16% in that with neither factor [13].

12.20 Microbiota

Microbiota is an essential community of microorganisms that colonize from birth in different areas of the human body predominantly gut, oral and nasal mucosa, vaginal tract, etc. Is a dynamic population of over a trillion of microbes that include bacteria belonging to the different families, viruses and fungi that interact one with the other, with the local habitat and environment. The microbiome is the incredible number of genes that can be extrapolated by this complex community of cells being 100-fold larger than the whole genome.

Microbiota is strictly associated with immunity and the development of a healthy immune system, it was not surprising the finding that outcome of immunotherapeutic strategies in cancer patients can be dependent on the gut microbiome. The response to immune checkpoint inhibitors (ICI) therapy, particularly anti-CTLA-4, could be associated to microbiome composition was first suggested by the group of Zitvogel in 2015 in melanoma. Other groups have confirmed and expanded the knowledges also by means of experimental models.Very recently the observation is being extended to epithelial cancers. Using shotgun DNA analysis performed on fecal samples from 60 NSCLC and 40 RCC patients before, during and after ICI therapy, they found an overrepresentation of the Firmicutes Akkermansia muciniphila present already at diagnosis in patients that with the therapy later showed a favorable clinical outcome. Moreover, antibiotics could compromise the efficacy of PD-1 blockade. Findings were further confirmed using the "avatar mice" model where mice were recolonized by fecal microbiota transplantation from responder and non-responder ICI treated patients. Restored immunity and antitumor activity of ICI could be achieved only with transplantation of stool samples from

responder patients. Immunological mechanisms underlying these findings are still in the process of being fully elucidated. A specific Th1 response against A. muciniphila was detected associated with prolonged progression-free survival (PFS) in patients and in the antibiotic induced dysbiotic mice models the addition of Akkermansia was capable to increase T cell recirculation in lymph nodes and tumor beds. The close link with antitumor Th1 mediated immunity was already demonstrated since microbiota stimulated dendritic cells (DCs) are more efficient in activating T cell responses. In other reports in the same issue of Science melanoma patients microbiome was studied and a significant association was observed between commensal microbiome composition and clinical response of PD-1 treated patients. In the other report the amount of diversity of the microbiome repertoire appeared to be significant in defining ICI responder patients.

Key Points

- Cancer cells can proliferate thanks to different mechanism, such as the elution of immune system mechanisms of defense. This has been carefully studied and has led to the introduction of new drugs and clinical approaches.
- Immunotherapy can act in different ways or increasing the patient's responses (active immunity) or administering antibodies or tumor-specific T lymphocytes (passive immunity).
- Up regulation of immune checkpoints PD-L1/PD-L2, CTLA-4 is one the mechanism of immune evasion.
- One of the advantages of immunotherapy consists in the prolonged effect of the therapy even after the cessation of the administration of the drug for example for the unacceptable toxicity.
- Immunotherapy can be considered a better approach compared to the previous ones also for its safety profile.
- In some cases, it is possible to adopt a combination therapy using also immunotherapy.

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Integrated Treatments: The Role of Surgery

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Contents

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Learning Objectives

By the end of the chapter the reader will

- Be able to apply multidisciplinary clinical management,
- Have learned the basic concepts of multi-professional decision making,
- Have reached a depth of knowledge regardomg the role of a surgeon during tumor board meetings,
- Be able to apply to surgical oncology all the concepts learned.

13.1 Introduction

The medical and surgical treatment of primary and secondary tumors has recently received specific attention in order to increase the safety and outcomes of surgical procedures with a radical intent. In addition, perioperative mortality has progressively decreased in reference cancer centers.

An accurate multidisciplinary preoperative evaluation, specific computerized diagnostic imaging, an accurate oncological staging, an appropriate operative risk assessment, and careful post-operative management all play a key role in providing better clinical outcomes [1].

Innovative research lines, both clinical and experimental, are currently exploring the fields of pharmacology and cytology involving cancer patient support and post-operative recovery [2].

13.2 Multidisciplinary Approaches

Multidisciplinary and multi-professional management in oncology allows an appropriate approach to patient care during all phases of the disease, improves treatment response, promotes timely access to rehabilitative and supportive therapies, and allows clinicians to improve patient recovery from many different diseases [3].

The expertise and synergy of multiple health care specialists is central in managing complex cancer patients who, depending on the type of the disease and risk factors (e.g., age and comorbidities), can be indicated for different therapies, such as surgery, radiotherapy, brachytherapy, bio-therapy, chemotherapy, or those who can be monitored with active surveillance and watchful waiting.

13.3 Tumor Board Assessment

A multidisciplinary approach to the care of cancer patients is commonly incorporated in all medical institutions, and is achieved through multidisciplinary tumor board (MTB) meetings. The collaboration of surgeons, medical oncologists, radiation oncologists, pathologists, radiologists, psychologists, and other ancillary staff (e.g., family doctor, physical and respiratory therapists, palliative care, support therapist, other allied health care professionals), if well organized and structured, allows the patient to be the center of the clinical care pathway. Objective and non-contradictory information on the treatment options available for each and every disease can also be readily provided by the tumor board panel, thus avoiding multiple consultations [4].

MTBs have to be formal, regularly-scheduled meetings during which the multidisciplinary team (MDT) of above-mentioned specialists, specifically trained for the care of cancer patients, meet to review individual clinical cases. They evaluate the diagnosis, and provide clinical decision-making with an evidence-based approach. Different types of patients are discussed during MTBs, such as newly-diagnosed patients with oncologic disease, patients at high risk for tumor-progression, or patients with complex management issues [5].

MTBs can improve adherence to clinical practice guidelines, diagnostic accuracy, and clinical outcomes [6]. MTBs serve to assure that all relevant disciplines are involved in the evaluation and treatment planning-process, can reduce variation in practice settings, aid in the judicious use of health care resources, and provide educational tools for clinicians. MTBs can also support and promote the adoption of high-quality clinical practice guidelines in cancer care, potentially improving the outcomes of that cancer care. MDTs are currently the subject of many studies and trials, though a more accurate research methodology is needed in order to monitor performance, team-working, and outcomes [7]. Recently, MDTs have also been integrated into clinical trials. While subject to some limitations (e.g., lack of objective data on trial recruitment and clarity), educational initiatives such as the MDT workshops, along with whole-team involvement and commitment have been shown to improve trial recruitment and team harmony [8]. Jenkins et al. studied 22 MDT trial workshops, including an objective follow-up on a number of patients approached concerning trials 12 months after the training. They showed that team functioning was significantly improved ($p \leq 0.04$), along with participant understanding and enthusiasm toward trial recruitment, but there was no significant influence on the number of patients approached to enter trials [9].

13.4 Imaging and Diagnostic Tools

Planning for, and performing, elective surgery in patients affected with severe comorbidities is most effective when a specialist and experienced MDTs are involved. In this

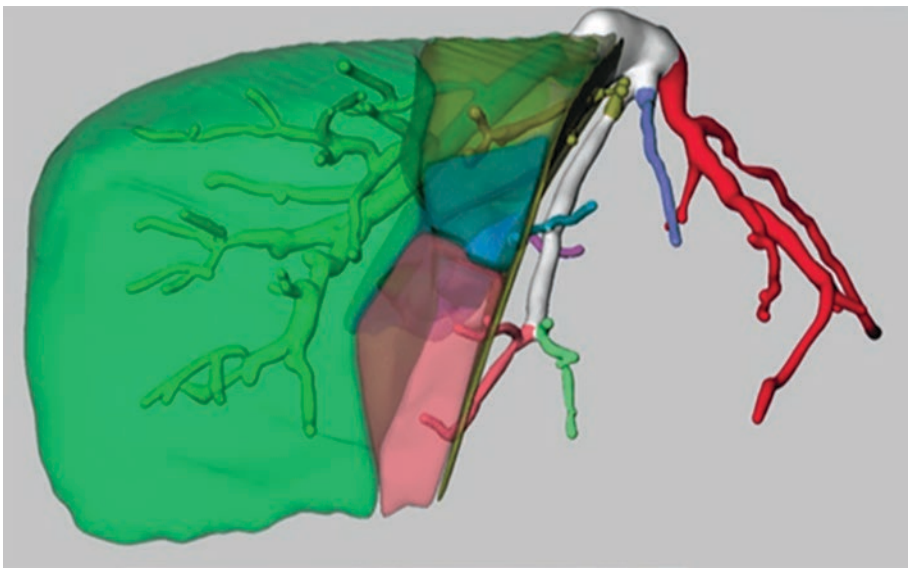
setting, notwithstanding best practice guidelines for surgery, there may be a gap between guidelines and practical application. Detailed and attentive preoperative planning is crucial for the success of elective surgery. This includes physical assessment, laboratory testing, genetic examinations, designing plans for hemostasis and pain management, and imaging. Radiological evaluation is a crucial element for surgical decision-making and for the implementation of the MTB [10, 11]. The integration of radiology into the medical, social, and political fabric of a community hospital is the thrust of the Imaging 3.0 cultural transformation, and is supported by the American College of Radiology (ACR) [12].

13.4.1 Multiparametric Evaluation

The ACR's Imaging 3.0's evidence-based criteria maps the transition of radiological practice from volume-based to value-based imaging. It is designed to aid radiologists in forging their future using tools and processes for managing practice and caring for patients (▶ https://www.acr.org/Practice-Management-Quality-Informatics/Imaging-3), and MTBs represent an extraordinary opportunity to demonstrate radiologic value in the community environment [5, 13]. The ACR strongly recommends that it become part of the basic culture in hospitals, initiating the process for the implementation of an MTB. Recently, scientific evidence has emerged that represents a primer of strategies that clinicians and radiologists can follow through practical suggestions for the implementation of MTBs, and suggests that the value of MTBs leads to the establishment of community practice [4].

The clinical uses of computed tomography (CT) and positron emission tomography (PET) imaging for assessing tumor response have several limitations in a range of anatomical sites. The routine use of interval modifications in standardized uptake values (SUVs) from sequential PET images during treatment to evaluate treatment response can be improved in terms of prognostic value and stratification of differential responders when additions of PET/CT textural features are made [14].

Multiparametric evaluation of the volume and function of parenchymal organs (e.g., liver and lungs) have become crucial elements in predicting the functional reserve in the post-operative course. In the context of hepatobiliary oncologic surgery, the methods for the measurement of the future remnant liver volume (FRLV) range from two-dimensional (2D) volume through CT, to peri-operative three-dimensional (3D) modeling (■ Fig. 13.1). Computational software allows for the definition, either manual or automatic, of the parenchymal anatomy of the liver on all the individual iconographic sections of the CT or of the magnetic resonance imaging (MRI), allowing an extremely detailed calculation of the hepatic volume [15]. Furthermore, the need to evaluate liver function in detail has led to the development of innovative methods, such as metabolic analysis performed with hepatic scintigraphy with galactosylated human serum albumin (GSA) and labeled with technetium 99 (99mTc), hepatobiliary scintigraphy with labeled mebrofenin with 99mTc with single photon emission computed tomography (SPECT). These scintigraphic investigations offer valid support in the quantification of the degree of hepatic functional impairment in relation to the diminished uptake of the radio com-



■ **Fig. 13.1** Preoperative diagnostic imaging visualization for adult-to-adult living liver donation that allows the "all- in-one" visualization of dual methods such as TC and MRI for the three-dimensional reconstruction of the biliary system. The anatomy of major and minor liver veins that drain the right lobe (displayed) is important for planning hepatic surgery and the planning of any additional back-table surgery for potential additional vascular anastomosis. (3D visualization courtesy of MeVis Medical Solutions AG, Bremen)

pound [16]. The enhancement of the function of the individual segments and sectors of the magnetic resonance liver by means of MRI with hepato-specific contrast using a gadolinium-based compound (ethoxybenzyl diethylenetriamine penta-acetic acid with Gadolinium, Gd-EOB-DTPA) provides the advantage of a radiological diagnostic evaluation simultaneous with the functional one [17].

Radiologic and endoscopic interventional procedures play a fundamental role in diagnostics, in pre-operative staging, and in the post-operative period. Symptoms related to the diagnosis of oncologic pathologies generally have a nonspecific clinical presentation, and their early recognition could allow clinicians to start a neoadjuvant treatment (e.g., for jaundice and extra-hepatic biliary oncologic disease), reduce morbidity, and increase the likelihood of obtaining a radical surgical procedure [18, 19]. For example, in patients with malignant obstructive jaundice, the typical treatment methods applied are percutaneous transhepatic biliary drainage (PTBD) and endoscopic biliary drainage (EBD). Nonetheless, there is no published consensual conclusion regarding either efficacy of the two types of drainage or the rate of complications. Based on the location of the biliary obstruction and the experience at individual treatment centers, either PTBD or EBD are specifically chosen in clinical practice as a preoperative procedure or palliative treatment to achieve prompt biliary drainage [20].

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13.4.2 Radiomics

Similar to the development of a growing integration of genomics into clinical practice to characterize the tumor phenotype with a wide range of genetic alterations (e.g., number of copies, gene expression, methylation), radiomics was recently introduced in order to characterize tumor phenotypes based on a heterogeneous aggregation of quantitative measurements derived from images (e.g., shape, morphology, intensity histogram, texture) [21]. As with genetic analysis, which provides a view of the multiple clones of tumor cells that comprise a tumor, radiomic analysis of tumor subvolumes or habitats within the tumor volume offers an imaging metric of the heterogeneous range of tumors. The analytical imaging tools provided by radiomics are based on those developed in recent years for tasks such as computer-assisted diagnosis of pulmonary nodules and breast lesions. In detail, radiomics envisions that these tools be used in very large patient datasets to derive a range of image functions. Statistical tools are then used to analyze the data. In addition to the radiological aspects related to the shape and size of the tumor, the most recent approaches of radiomics are aimed at characterizing the distribution of intensities of the gray level in the tumor area in two or three dimensions through the analytical definition of histograms or "textures." [22] Radiogenomic studies can be useful in understanding relationships between imaging characteristics of tumors, like heterogeneity, as well as their genetic characteristics, phenotype, or expected treatment outcomes [23]. In addition, they could also be helpful in finding possible high-yield targets in cases in which invasive lymph node staging becomes necessary. In order to limit the need for invasive staging procedures, introducing further surrogate parameters for detecting malignant lymph node infiltration in lung cancer patients would be helpful. Flechsig P. et al. found that density measurements in unclear mediastinal and hilar lymph nodes with equivocal 2-deoxy-2-[18F]fluoro-D-glucose ([18F]FDG) uptake in positron emission tomography/x-ray computed tomography (PET/CT) could serve as a possible surrogate parameter for N-staging in lung cancer patients, regardless of the specific lung cancer subtype [24].

13.4.3 Early Diagnosis and Screening

Ideally, early detection of cancer or pre-cancerous lesions can consistently increase the likelihood of an appropriate and successful treatment. Screening programs for colon cancer and breast cancer have led to the widespread adoption of this important approach in the general population. The benefit of a screening program must be supported by consistent data that prove a true reduction in mortality, the absence of adverse events, and affordable costs for the health care system.

Recently, lung cancer screening using low-dose spiral CT has emerged as a viable tool for effectively diagnosing early-stage lung cancer, and achieved an overall 20% reduction in mortality from lung cancer.

Screening programs are constantly evolving, from the use of invasive diagnostic tools to the use of a simple blood sample, such as circulating microRNAs to detect early lung cancer [25, 26].

13.5 Surgical Decision-Making

At present, oncologic surgery has achieved important technical breakthroughs, but there is still a lack of information in the literature regarding the outcomes of high-risk patients undergoing oncologic surgery, and only a limited number of studies have tried to address the problem. While it is difficult to produce guidelines in some clinical situations, it may not be appropriate to delay surgery for a progressive disease such as a malignant tumor to allow time for coronary artery bypass surgery

in patients affected with coronary heart disease. Some oncologic lesions that are operable at the time they are diagnosed might become inoperable if surgery is not performed promptly [27]. The amount of clinical research on the topic is increasing in order to identify ways to increase survival and meliorate outcomes in high-risk cancer patients to become candidates for oncologic surgery with different indications.

13.5.1 Patient-Related Factors

In the surgical setting, an MDT can be assembled to ensure that critical tasks are undertaken, and all available clinical evidence supports the use of surgery. At each stage, the patient and their parent/caregiver, when appropriate, should be consulted to ensure that expectations and functional goals are realistic and achievable. The degree of underlying comorbidities, e.g., liver cirrhosis, could influence therapeutic options and prognosis of primary cancer. In this specific setting, while for patients with compensated liver cirrhosis the prognosis is determined principally by the cancer staging and related surgical treatment, in patients affected with decompensated liver disease, life expectancy is influenced mainly by the underlying disease, and the therapeutic option can worsen the liver function [28].

Planning should ensure that the surgery proceeds with no adverse events. However, the surgical team should be ready to confront unexpected scenarios. Likewise, the MDT must be aware of events during surgery that may change postoperative plans. Postoperative rehabilitation should be initiated soon after surgery, with attentive management of post-operative clinical course.

13.5.1.1 The Elderly

The diagnosis of cancer currently provides the possibility of molecular target therapies, of biological drugs combined with chemotherapy, and of minimally invasive robotic surgery even in advanced disease. Innovative and personalized approaches can definitively cure or make the disease manageable over time in an increasing number of cases. But is it applicable even to elderly cancer patients? Should the clinicians follow different strategies that also take into account the patient's comorbidities (type II diabetes mellitus, arterial hypertension, cardiovascular diseases, just to mention the most common) that may be worsened by oncologic therapies?

The key point is clearly to assess the patient's general condition. The physical and psychic characteristics are crucial because in the elderly there are often cognitive deficits or dementia that can hinder compliance with and the adoption of therapies. A specific assessment by the MDT must estimate the life expectancy and, above all, the social and family living conditions; for example, an aggressive treatment plan for a patient who lives alone needs adequate health and family organization. The elderly cancer patient needs a personalized path (e.g., comprehensive geriatric assessment (CGA), based not only on the molecular profile of the tumor but also on his perceived experience and psychophysical conditions, because even a mild depression can make it difficult to undergo powerful cancer treatments. Though elective oncologic surgery in elderly patients currently has low morbidity and mortality rates, CGA improves the identification of cases at higher risk of adverse events, independent of the surgical prognostic indices [29]. Oncologic surgery in elderly patients means even more careful preparation, and should only be done by an MDT with experience in this field, and at a specialized center with a model of comprehensive care.

13.5.1.2 Obesity

Overweight and obesity affects the health and quality of life of nearly 70% of American adults age 20 years or older [30]. The cost of treating cancer patients with severe obesity and its associated comorbidities of hypertension, diabetes, cardiovascular disease, pulmonary insufficiency, and degenerative arthritis, as well as the poor long-term results of medical, drug, and behavioral therapy has increased the numbers of patients being referred for surgical treatment [31, 32]. Several multipronged initiatives have been advocated to reduce the impact of obesity on cancer. The American Society of Clinical Oncology helped accomplish this goal by (1) increasing education and knowledge of the evidence linking obesity to cancer; (2) providing resources and tools to aid oncology providers in addressing obesity with their patients; (3) building and fostering a robust research plan to better understand the pathophysiology of energy balance alterations, and evaluate the impact of behavioral change on cancer outcomes, also determining the best methods for helping cancer survivors make effective and useful changes in lifestyle behaviors; and (4) advocating for policy and system change to address societal factors that contribute to obesity and improve access to weight-management services for cancer patients [33].

Most of the evidence concerning obesity in cancer survivors comes from people who were diagnosed with breast, prostate, or colorectal cancer. Research indicates that obesity may worsen quality of life, increase cancer recurrence, progression, and prognosis (survival) [34, 35]. However, there is still no robust evidence about whether weight loss improves cancer recurrence or prognosis [36]. It has been shown that the bowel microbiomes of patients affected with obesity are less different than those of non-obese people, and this microbiota imbalance may be associated with genotoxicity, which

may in turn be related to cancer [37]. Preclinical studies show that this condition might also reduce the efficacy of oncologic therapies [38]. Researchers are beginning to think about ways to change the microbiota of cancer patients in order to improve their outcomes. Currently, gastric bypass, sleeve gastrectomy, and adjustable gastric banding are the three bariatric surgical procedures recommended for severely obese patients. The significant long-term weight control resulting from surgical therapy is associated with improvement and, often, resolution of comorbidities, including diabetes, hypertension, hyperlipidemia, and pulmonary insufficiency [39].

13.5.1.3 Chronic Lung Disease and Particularly Chronic Obstructive Pulmonary Disease

Chronic lung disease and particularly chronic obstructive pulmonary disease (COPD), including both emphysema and chronic bronchitis, can be a very frequent comorbidity of patients with cancer. Most patients with lung cancer display some degree of COPD given the fact that cigarette smoking is a common factor in the pathogenesis of the disease. Also, many patients with cancer (other than lung cancer) who require surgery may be at increased risk because of limited pulmonary function. A thorough pulmonary pre-operative assessment is mandatory in order to assess the overall surgical risk. A smoking cessation program is also imperative before surgery, and even 3 weeks of smoking cessation before surgery can reduce the likelihood of pulmonary complications after cancer surgery.

COPD patients who require lung resection for cancer are a specific subtype of individuals who may actually benefit from removal of diseased lung tissue due to the so called "LVRS (lung volume reduction)" effect. An appropriate estimate of the pulmonary pathophysiology of these patients is central for the surgeon in order to place proper indications for lung resection.

13.5.1.4 Nutritional Status

Pathological changes in nutritional status are a common framework in cancer patients, and are related to the site and the extent of the neoplasm [40]. The presence of malnutrition leads to a higher rate of hospitalization and toxicity, a lower response to chemo and radiotherapy treatments and a worsening of quality of life and prognosis [41]. If it is now established that loss of weight before surgery would increase the risk of post-operative complications, and an increase in mortality, [42, 43] even a slight weight loss that occurs during the chemo-radiotherapeutic treatments is associated a significant reduction in survival [44]. In recent years there is increasing evidence that during the treatment of more common tumors, chemo-radiotherapy toxicity and survival are influenced by muscle loss and, therefore, by the development of sarcopenia [45]. The indications for nutritional support in the oncological patient change over the course of treatment depending on whether the patient is in active cancer treatment, in remission, or in the palliative phase. It is therefore important to carry out regular nutritional monitoring, especially in patients with a tumor whose location, extension, or therapy may compromise nutritional status [46]. Though it is known that sarcopenia greatly influences the outcome in terms of surgical site infection and prolonged hospitalization after elective oncological surgery, it has recently emerged that nutritional assessment is still performed only at the request of patients in about half of the cases, and that only 16% of clinicians use nutritional screening tools [47]. The first level of intervention consists of dietary counseling by dieticians trained in the oncology field, which aims to achieve energy and protein needs even with the aid of oral nutritional supplements. If this intervention is not sufficient, artificial nutrition is indicated that must in any case privilege enteral administration. Only if this is not feasible for altered bowel function, poor tolerance to supplements or patient refusal, then nutritional support can be provided intravenously before surgery [48].

13.5.2 Tumor-Related Factors

The pathologic diagnosis of cancer relies on cytomorphology and immunohistochemistry. Microscopic evaluations are not always available and, though elective surgical procedures have low morbidity and mortality, clinical benefits have to be considered as adjuvant therapies, and must be part of an MDT approach for defining a case-by-case timeline for maximizing oncologic cares.

13.5.2.1 Prophylactic Surgery

Preventive surgery can be recommended or represent a possible therapy option for patients not yet affected with cancer, but who are carriers of genetic risk factors for the development of malignant tumoral lesions. The MDT assessment identifies the signs of preventive surgery, and entails a thorough evaluation of the clinical characteristics of complex syndromes and the risk of developing different forms of hereditary cancer. Genetic testing is one part of the genetic counseling process during the MDT evaluation. The analysis is expressly performed based on the presence of specific types of genetic mutations, and on the possibility of weighting the early detection of cancer during the monitoring. It has been shown that a bilateral prophylactic mastectomy greatly reduces the risk of breast cancer. In addition, prophylactic oophorectomy also reduces the risk of breast cancer [49]. Though BRCA status can be valuable information

for patients considering lumpectomy vs. mastectomy, studies on surgery for non-BRCA mutations are lacking. TP53 and PALB2 carry a potentially high risk of mutations for breast cancer, which may justify the indication for prophylactic surgery [50]. A comprehensive approach is mandatory in order to provide the best treatment for breast cancer patients with deleterious genetic mutations [51]. Genetic risk assessment for hereditary cancer is integral in the comprehensive care of today's patient. A tangible example is provided by patients with Lynch syndrome, in particular, those women who are considered at high risk of developing endometrial cancer. In these cases of the genetic test results, prophylactic hysterectomy can be proposed to those women in whom surgery is indicated because of the presence of uterine disorders. Prophylactic surgery can be an option for patients at risk of inherited gastrointestinal neoplasms, either on a case-by-case basis (Lynch syndrome) or, more systematically, for patients with familial adenomatous polyposis syndrome or hereditary diffuse gastric cancer. Despite its effectiveness, prophylactic surgery in a healthy individual is a procedure that requires appropriate and prompt psychological support for possible psychological, social, and physical complications [52].

13.5.2.2 Locally Advanced Tumor and Metastatic Disease

Recent evidence shows a clear advantage in the use of neo-adjuvant chemotherapy for downstaging of disease, followed by surgery in the treatment of several forms of advanced/metastatic disease [53, 54]. This surgical option is an independent factor that is associated with overall survival, and with the possibility of obtaining a surgically conservative treatment [55].

Otherwise, the high mortality rates of some types of cancer, such as pancreatic cancer, are due to the high incidence of metastases at the time of first diagnosis. In this setting, the rapid clinical course and the lack of adequate systemic therapies lead to considering oncologic surgery as the only therapeutic option of cure. Considering that 30% of patients affected with pancreatic adenocarcinoma are not amenable to resection at presentation, [56] and patients who undergo resection for localized pancreatic carcinoma have a median survival of 19.5 months [57]. Combined treatment with radiation and chemotherapy increases median survival for patients with locally advanced cancers to approximately 11 to 13 months, but rarely results in long-term survival. Clinical research efforts are currently focusing on morphologic and functional staging to provide a better patient selection, as well as the evaluation of new options for systemic and radiation therapies [58]. To increase the resection rate and achieve better prognosis, there is a need to enhance systemic effects using other, more effective antitumor drugs.

In other oncologic settings, e.g., kidney cancer, it has been reported that surgical removal of the primitive tumor improves survival in cases of locally advanced/metastatic disease. Cytoreductive nephrectomy is the standard of care for patients with metastatic clear-cell renal cancer who present with the tumor in place. The advantages are an improvement in the health status of the patient, the removal of a reservoir of neoplastic cell neoangiogenic cytokines and growth factors, and cytoreduction [59]. On the other hand, immediate CN results in a significant delay in starting systemic therapy, which fails to address the ultimately fatal metastatic disease, allowing it to progress unchecked [60, 61].

13.6 Quality of Oncologic Surgery

Data from the Italian Association of Tumor Registries indicate a constant increase in the number of Italians living after a diagnosis of cancer - about 3% a year. Prevalence increased from 2.244 million in 2006 to over 3.3 million in 2017 [62]. One in four has returned to the same life expectancy as the general population, and can be considered healed. In this scenario, the availability of new and increasingly active and expensive therapies can increase the social and economic burden [63].

Therefore, appropriate action plans and targeted investments are required in the field of cancer surgery in order to grant access for all the population to quality and curative surgery. An improved capacity of both therapeutic and palliative cancer care is needed in order to achieve better outcomes through more-appropriate allocation of surgery compared with the goal of treatment [64]. In order to obtain a high level of quality standards in terms of care and assistance, it is not only necessary to have a large pool of patients, and to easily overcome the thresholds [65].

13.6.1 Referral Center

A referral center must be able to offer a multidisciplinary team to the cancer patient composed of expert professional figures, doctors, and nurses, able to confront, manage, and solve the multiple problems presented by the cancer patient in terms of completeness and quality of care. In Italy, the definition of qualitative and quantitative, structural, and technological standards related to health care has recently demonstrated a trend towards the centralization of cancer pathologies in structures with the highest volumes of activity. The promulgation of "threshold volumes" by the Ministry of Health, beyond which a center can be considered suitable to carry out a specific surgical procedure, has allowed centers to progressively reduce the number of oncological surgical

procedures below the "threshold values," in association with an overall increase in surgical procedures, and the stability of the number of over-the-threshold structures [66]. Currently, surgical technique must be supported by an excellent knowledge in the field of tumor biology (research, epidemiology, and screening modalities), chemotherapy and radiotherapy, pain management and palliative care, a multidisciplinary approach, and diagnostic imaging. Recently, to try to make up for this need, the European Society of Oncological Surgery (ESSO) in collaboration with the Italian Society of Oncological Surgery (SICO), proposed the implementation of a "Global Curriculum," aimed at training surgeons in this multidisciplinary profile [67].

13.6.2 Minimally Invasive Surgery

A proficient oncologic surgeon should properly handle minimally invasive (MI) techniques for the surgical treatment of cancer. These techniques include endoscopy, laparoscopy, thoracoscopy, and robotics. Minimally invasive surgery allows, in selected cases, performance through small millimetric incisions of the same oncologically sound operations of surgery performed through large incisions of the chest and abdomen. The advantages of minimally invasive surgery include less postoperative pain, earlier discharge from the hospital, earlier return to normal life, a reduced immunological impact of the surgical procedure, and an easier access to, and tolerability of, adjuvant therapies. The indications and results of minimally invasive surgery must be considered selectively and specifically from organ to organ. For example, video assisted thoracoscopic (VATS) lobectomy of the lung has become the standard of care for the treatment of early stage lung cancer. The reduced impact of surgery on the patient using MI techniques allows extending the indications to patients with a reduced performance status. The adoption of rapid recovery postoperative protocols (ERAS) further enhances the benefits of minimally invasive surgery. The patient is taught to understand the fundamentals of pre- and post-operative care and does better in the postoperative rehabilitation process. [68, 69]

13.7 Conclusion

The multidisciplinary approach represents the promising and most effective way for surgical treatment with oncological intent, providing the patient with the best treatment option and the best possible care. Collaboration and interaction between clinicians is the basis of modern surgery for the treatment of cancer diseases by providing a more adequate path of therapy, both palliative and radical, to the heterogeneity and complexity of oncological diseases and comorbidities of complex patients.

Summary of Clinical Recommendations

AIOM

- # "Patients at nutritional risk should be promptly referred for comprehensive nutritional assessment and support to clinical nutrition services or medical personnel with documented skills in clinical nutrition, specifically for cancer patients. Nutritional intervention should be actively managed and targeted for each patient; it should comprise personalized dietary counseling and/or artificial nutrition according to spontaneous food intake, tolerance and effectiveness."
- Caccialanza R, Pedrazzoli P, Cereda E, Gavazzi C, Pinto C, Paccagnella A, et al. Nutritional support in cancer patients: a position paperfrom the Italian Society of Medical Oncology (AIOM) and the Italian Society of Artificial Nutrition and Metabolism (SINPE). J Cancer. 2016; 7:131–135.
- *ESMO*
  - # "A multidisciplinary team discussion is crucial for modern diagnostic and therapeutic decision making. Oncologists, surgeons, radiotherapists, molecular biologists and pathologists must give their specific recommendations for an adequate and personalized treatment strategy for each mCRC patient. The treatment quality is directly proportional to the number of treated patients: each multidisciplinary team should discuss and treat at least 50 patients per year (including early stage and advanced disease), while teams dealing with less than 50 cases per year should collaborate with referral hospitals."
  - Salvatore L, Aprile G, Arnoldi E, Aschele C, Carnaghi C, Cosimelli M, Maiello E, Normanno N, Sciallero S, Valvo F, Beretta GD. Management of metastatic colorectal cancer patients: guidelines of the Italian Medical Oncology Association (AIOM). ESMO Open. 2017; 2(1):e000147.
  - # "Liver cirrhosis may limit surgical and interventional approaches to cancer treatment, influence pharmacokinetics of anticancer drugs, increase side effects of chemotherapy, render patients susceptible for hepatotoxicity, and ultimately result in a competitive risk for morbidity and mortality."
  - Pinter M, Trauner M, Peck-Radosavljevic M, Sieghart W. Cancer and liver cirrhosis: impli-

cations on prognosis and management. ESMO Open. 2016; 1(2):e000042.

- *NCCN*
  - # "The NCCN Guidelines for Pancreatic Adenocarcinoma focus on diagnosis and treatment with systemic therapy, radiation therapy, and surgical resection."
  - Tempero MA, Malafa MP, Al-Hawary M, Asbun H, Bain A, Behrman SW, Benson AB, 3rd, Binder E, Cardin DB, Cha C, Chiorean EG, Chung V, Czito B, Dillhoff M, Dotan E, Ferrone CR, Hardacre J, Hawkins WG, Herman J, Ko AH, Komanduri S, Koong A, LoConte N, Lowy AM, Moravek C, Nakakura EK, O'Reilly EM, Obando J, Reddy S, Scaife C, Thayer S, Weekes CD, Wolff RA, Wolpin BM, Burns J. Darlow S. Pancreatic Adenocarcinoma, Version 2.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2017;15(8):1028–1061.

Key Points

- # The introduction of new imaging techniques and biochemical tests for the pre-operative MDT evaluation of the functional reserve and of body homeostasis has allowed centers to obtain a relatively low incidence of peri-operative complications after surgical treatment of cancer patients.
- # The increasing impact that surgical procedures combined with systemic therapy have in the natural history of oncological pathologies, both primary and metastatic pathologies, even in the context of chronic degenerative diseases with compromised organ function.
- *Hints for deeper insight*
  - # A better biomolecular and physiopathological understanding of the metabolic pathways and cellular mechanisms that underlie the resumption of physiological activities after surgical resection.
  - # It is particularly important to develop universal models of risk prediction of post-operative complications, increasingly advanced surgical techniques, and effective preventive measures for the codification of those risk factors that predict the lack of functional recovery with prolonged hospital stay and post-operative disability.
- *Suggested reading*
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  - # Rusby JE, Gough J, Harris PA, MacNeill FA. Oncoplastic multidisciplinary meetings: a necessity or luxury? Ann R Coll Surg Engl. 2011; 93(4):273–4.
  - # Leff DR, Ho C, Thomas H, Daniels R, Side L, Lambert F, Knight J, Griffiths M, Banwell M, Aitken J, Clayton G, Dua S, Shaw A, Smith S, Ramakrishnan V. A multidisciplinary team approach minimises prophylactic mastectomy rates. Eur J Surg Oncol. 2015;41(8):1005–12.

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Integrated Treatments: The Role of Radiotherapy

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Contents

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Learning Objectives

By the end of the chapter, the reader will:

- Be able to recognize patients who can benefit from a radiation therapy
- Have learned the importance of close collaboration with different specialists
- Know radiotherapy basic concepts

14.1 Introduction

Modern oncology makes use of the new advances in the field of new radiotherapy technologies and in new chemotherapeutic drugs.

Given that radiotherapy may have a role in almost all cancer sites, in this chapter we will discuss radiotherapy in the multimodal approach of head and neck, breast, prostate, and rectal cancer.

14.2 Effects of Radiotherapy and Chemoradiotherapy

Ionizing radiation consisting of electromagnetic radiation or photons is the type of radiation most commonly used for the treatment of patients with radiotherapy.

When cells are irradiated, they suffer *physical* and *biological* effects: the main damage is directed to DNA molecules. The damage can be *direct* if the radiation acts directly on the atoms inside the cell (typical of the high LET radiation) and *indirect* if indirectly the radiation acts producing free radicals that are responsible for the damage of the cell (e.g., breaking of chemical bonds). Radiation produces [1]:

- Delaying division of cells
- Apoptosis
- Necrosis
- Reproductive failure
- Genomic instability
- Mutation
- Bystander effect

Bystander effect is an important phenomenon: the irradiated cells send signals to adjacent nonirradiated cells inducing damage. Other possible effects produced by radiation that can be purposeless for tumor control are:

- Transformation with cancinogenesis (after many years)
- Adaptive response of the cell which then becomes more resistant to subsequent radiotherapy

In addition, radiation treatment can exert its effect by stimulating the immune system: both releasing tumor-associated antigens (TAAs) and changing tumor microenviroment. This translates into greater activation of cells presenting the antigen APC that migrate to draining lymph nodes (DLN) and exposing antigens to T cells, the latter becoming *tumor-specific* T cells (CD8 + CTL) into the tumor microenvironment. Within the tumor microenvironment, T cells perform a tumor-specific action. Moreover, multiple lines of evidence suggest that the application of ionizing radiation to a target that encompasses the tumor elicits effects that exceed cell killing per se and include specific and effective signals to the immune system of the host. The understanding of these signals and their consequences has opened a novel area of research that is based on the acknowledgment that clinical radiotherapy might impact systemic disease through the immune system (abscopal effect) [2].

The association of chemoradiotherapy can improve the therapeutic ratio. In 1979, Steel and Peckham described a theoretical framework defining the mechanisms by which these modalities interact to improve therapeutic outcome [3]:

- The spatial cooperation
- The toxicity independence
- The protection of normal tissues
- The enhancement of tumor response

The *spatial cooperation* describes the scenario whereby radiotherapy acts locoregionally, whereas drugs act against distant micrometastases. The term *toxicity independence* implies that the administration of radiochemotherapy results in improved tumor control without unacceptable toxicity. *Normal tissue protection* describes the concept that chemotherapy can allow delivering a higher dose of radiation than would be tolerated by the organs at risk when radiotherapy is used alone. Initial observations of normal tissue protection by cyclophosphamide and methotrexate when combined with radiotherapy have led to this concept. The cytotoxic *enhancement* can be defined as the enhancement of cells killing through the alteration of the induction or repair of DNA damage. To obtain a cytotoxic enhancement, the chemotherapeutic agents should be administered concomitantly to irradiation (concurrent chemoradiotherapy) with the intent to enhance the local therapeutic effect of radiotherapy.

More recently, Bentzen et al. [4] proposed a contemporary modification to this paradigm to account for the rational design of systemic agents and to account for the introduction of molecularly targeted drugs: spatial cooperation, cytotoxic enhancement, biological cooperation, temporal modulation, and normal tissue protection.

14.3 Modern Radiotherapy Techniques

External beam radiotherapy *(EBRT)* is delivered using different techniques [5]:

- Conventional 3D radiotherapy *(3D CRT)* treatment planning is manually optimized; this means that the treatment planner chooses all beams parameters, such as the number of beams, beam directions, shapes, weights, etc., and the treatment planning system (TPS) calculates the resulting dose distribution.
- Intensity-modulated radiotherapy *(IMRT)* is a relatively newer approach to 3D treatment planning and conformal therapy that optimizes delivery of irradiation to irregularly shaped volumes through complex forward or inverse treatment planning and results in modulated fluence of multiple photon beam profiles. In the inverse planning approach, dose distributions are inversely determined, meaning that the treatment planner must specify in advance the dose distribution that is desired, and the computer then calculates a set of beam intensities that will produce, as nearly as possible, the desired dose distribution.
- Volumetric arc therapy (*VMAT*) is an advanced form of IMRT that delivers dose distribution with a 360-degree rotation of the gantry in a single- or multi-arc treatment, during which the machine must rotate several times around the patient or make repeated stops and starts to treat the tumor from a number of different angles.
- Image-guided radiation therapy *(IGRT)* [6] may allow to locate and track tumors at the time of treatment and deliver more precise radiation treatment. A computer compares images taken at the time of treatment to images taken during the planning phase (simulation) and make technical adjustments when a tumor moves outside of the planned treatment range.
- Stereotactic *radiation treatment* for the body (SBRT) is a modern radiotherapy technique in which a specially designed coordinate system is used for the exact localization of the tumors in the body in order to treat it with limited but highly precise treatment fields. SBRT involves the delivery of a single high-dose radiation treatment or a few fractionated radiation treatments. A high potent biological dose of radiation is delivered to the tumor, improving the cure rates. SBRT can be used for small localized tumors (up to 6–7 cm) or a few tumors or metastasis (up to 3–5 usually) throughout the whole body. Stereotactic radiosurgery (SRS) consists in a single session of stereotactic radiotherapy delivered through a Gamma Knife system or a linear accelerator to brain tumors. These tumors can be malignant (gliomas, metastases) or benign (acoustic neurinomas, pituitary adenomas, meningiomas). SRS is also used for certain non-tumor conditions such as *vascular malformations* and *trigeminal neuralgia.*
- Particle beam radiotherapy uses beams of protons or other charged particles such as helium, carbon, or other ions instead of photons. Charged particles have different depth-dose distributions compared to photons. They deposit most of their energy in the last final millimeters of their trajectory (when their speed slows). This results in a sharp and localized peak of dose, known as the Bragg peak. The particle beam dose distribution is characterized by a lower-dose region in normal tissue proximal to the tumor, a uniform high-dose region in the tumor, and zero dose beyond the tumor, so it is useful to escalate dose and to improve local control in particular tumor sites (ocular tumors, skull base tumors, paraspinal tumors) or to reduce short- and long-term side effects limiting the dose to normal tissue in pediatric tumors.

14.4 Simulation, Contouring and Imaging Fusion, and Planning

A clinical indication to treatment is given after a physical examination and a review of medical history. Next, it will proceed with the acquisition of computed tomography (CT) images during a procedure of simulation CT to view the target and global body volumes. During the simulation, the treatment setup will be simulated by positioning the patient on the flat couch immobilized by specially designed devices (e.g., thermoplastic masks, Vac-Lock cushions, etc.). Its of great importance that the patient's position is made as comfortable as possible and that it is reproducible during all therapy sessions. For example, in pelvic treatment, the patient is instructed to follow a regimen of bladder and rectal preparation to make the anatomy reproducible.

After the CT scan, the images are then sent to the *TPS* to proceed with the contouring. The contouring consists in the delineation of the target volumes and the organs at risk neighboring to the target; *see* ◘ Fig. 14.1. In most cases, radiation oncologist uses additional diagnostic imaging procedures, including positron emission tomography (PET) and magnetic resonance imaging (MRI), procedures that allow to acquire additional anatomical and functional information. The imaging fusion, thanks to the TPS, allows to merge the images acquired at the time of the simulation with the diagnostic images for a better delineation of the volumes.

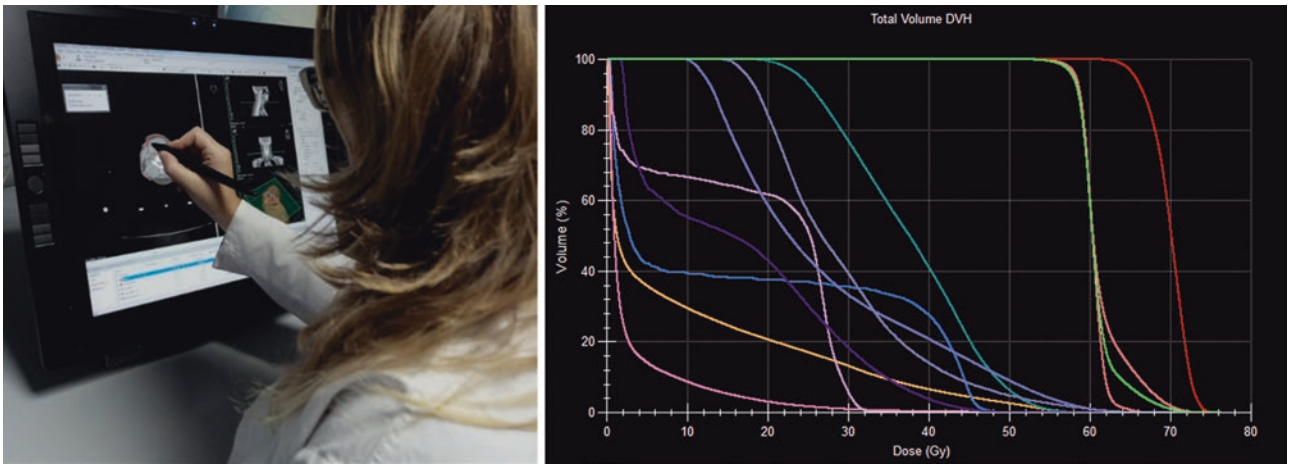


Fig. 14.1 Contouring and DVH

The recommendations for specifying gross tumor volume (GTV), clinical target volume (CTV), and planning target volume (PTV) for 3DCRT follow the International Commission on Radiation Units and Measurements (ICRU) Report Nos. 50 and 62 guidelines [7].

GTV (gross tumor volume): It is the macroscopically detectable disease, referring to the primary tumor, nodal, and or metastases.

CTV (clinical target volume): It is the tissue that includes the GTV and the subclinical disease.

PTV (planning target volume): The planning target volume (PTV) is delineated around the CTV, to allow for uncertainty related to patient positioning and systematic or internal movement.

OAR: Healthy organs to protect.

Moreover radio-oncologist provides the physical dose limits (*dose constraints*) of organs at risk to minimize side effects. The physics proceed at the planning: respecting the dose constraints and the prescription to the target. In the last step, the radio-oncologist verifies the plan, and if it respects, the specified features approve it.

Once the treatment is planned, the physician will check the dose-volume histogram (DVH), a plot in which each bin represents the volume or percentage of volume (y axis) that receives a dose equal to or greater than an indicated dose (x axis), *see* Fig. 14.1.

14.5 Role of Radiotherapy in Main Tumors

Radiation therapy has now become the most important nonsurgical modality in cancer: over 50% of all cancer patients now receive radiotherapy at some point during the illness.

Radiotherapy can be used as a *curative* treatment if there is a probability of long-term survival after adequate therapy and as a *palliative* treatment in order to palliate symptoms such as pain producing discomfort or an impending condition in patients with a little hope of survival.

In a curative setting, radiotherapy can be an *exclusive* treatment (e.g., prostate cancer) or, in association with chemotherapy (e.g., nasopharyngeal cancer), a neoadjuvant treatment or an adjuvant treatment.

Neoadjuvant treatment potentially eradicates subclinical or microscopic disease and reduces the size of the primary tumor by allowing safer or more conservative surgery (as in rectal cancer). As an *adjuvant* treatment, after surgery, radiotherapy may eliminate residual tumor in the operative field by destroying subclinical foci of cancer (including lymph node metastases) to reduce local recurrence risk (breast cancer).

Moreover, radiotherapy is frequently used in the treatment of metastatic and recurrent cancer, with cytostatic, ablative, or analgesic purposes.

14.6 Head and Neck

Radiotherapy is an important and potentially curative modality for head and neck cancers. For many primary sites within the head and neck, RT yields better functional outcomes than surgery and, thus, is often preferred for localized disease. For locoregionally advanced lesions, RT is often used in combination with chemotherapy as a definitive organ function-preserving approach or after surgery as an adjuvant.

In this paragraph we will discuss the tumor of the nasopharynx, a pathology whose surgery is hardly accessible for the localization, and radiation therapy plays a curative role with chemotherapy.

14.6.1 Indication

Sequential and/or concurrent chemotherapy is widely applied for the treatment of NPC (nasopharyngeal carcinoma) for its chemosensitivity. Concurrent chemoradiotherapy (CCRT) [8], with/without adjuvant/neoadjuvant chemotherapy, is recommended for locoregionally advanced NPC cases; radiotherapy alone is suggested only for stage I NPC patients. Currently available evidence shows trends favoring the addition of chemotherapy to concurrent chemoradiation in patients with locoregionally advanced NPC; however, it is unclear whether to administer chemotherapy to these patients before or after chemoradiation (NCCN guidelines 2018).

14.6.2 Volumes and Doses

CT simulation is performed in supine decubitus with a thermoplastic mask to immobilize head and neck and shoulders and thin slice (3–5 mm) acquisition from vertex to superior mediastinum.

Accurate delineation of the volumes requires the synthesis of clinical and imaging data.

MRI is performed within 2–3 weeks from beginning of treatment; additional information from PET/CT may be helpful to identify the primary disease and lymph node (LN) metastases.

High doses are needed to achieve optimal levels of tumor control and tumoricidal effect, but they lead also to an increased toxicity. The *gross tumor volume (GTV)* includes the nasopharyngeal tumor visible on clinical examination and imaging and involved lymph nodes. The pre-chemotherapy volumes should be considered in the contouring phase.

The *clinical target volume (CTV)* should include GTV plus natural extension pathways of nasopharyngeal carcinoma. Nasopharyngeal carcinomas tend to spread through areas of lesser resistance from the fossa of Rosenmüller, spreading muscles within the parapharyngeal space, within the pharyngobasilar fascia, neural foramina, and neural pathways. Nasopharyngeal carcinoma usually spreads to local lymph nodes, so nodal CTV systematically includes bilateral levels nodes from II to V level and retropharyngeal lymph node areas.

The *planning target volume* (PTV) is delineated by drawing a 3–5 mm margin around the CTV.

For high-risk NPC, radiation doses of 66 to 70.2 Gy given with standard fractions are necessary for control of the primary tumor and involved lymph nodes. Low-risk subclinical disease in the low neck is often treated with 44 to 54.1 Gy at 1.64 to 2.0 Gy/fraction, and for intermediate risk disease, 59.4 to 63 Gy in 1.8 to 2.0 Gy/fraction is often given with dose painting to different regions of the skull base and neck. Radiation dose-fractionation schedules may vary slightly depending on institutional preference (NCCN guidelines 2018).

Either IMRT (preferred) or 3D conformal RT is recommended for cancers of the nasopharynx to minimize dose to critical structures (Fig. 14.2).

A simultaneous integrated boost (*SIB*) allows delivering different doses per fraction within target regions in the same number of fraction: for example, in case of nasopharyngeal carcinoma, *GTV* receives 69.96 Gy in 2.12 Gy/ fraction, the positive nodes *GTV(N)* receives 66 Gy in 2 Gy/fraction, and the elective node receives from 54 to 60 Gy in 1.63–1.8 Gy/fraction, complessively 33 fractions; *see* Fig. 14.3.

The main organs at risk to delineate are:

- Brain
- Spinal cord

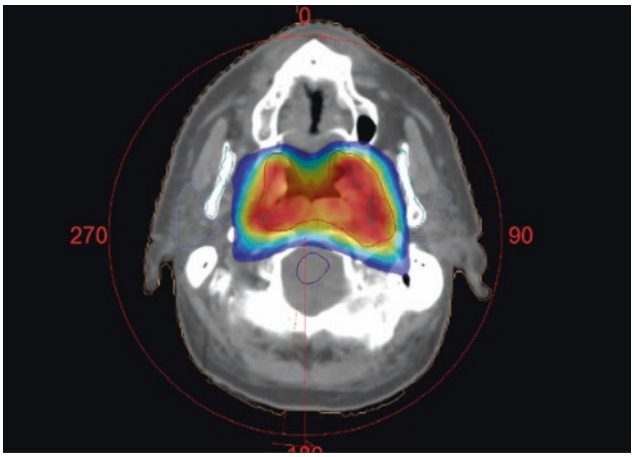


Fig. 14.2 The colorwash highlights the different delivered doses: red is the dose of 70 Gy directed to the GTV

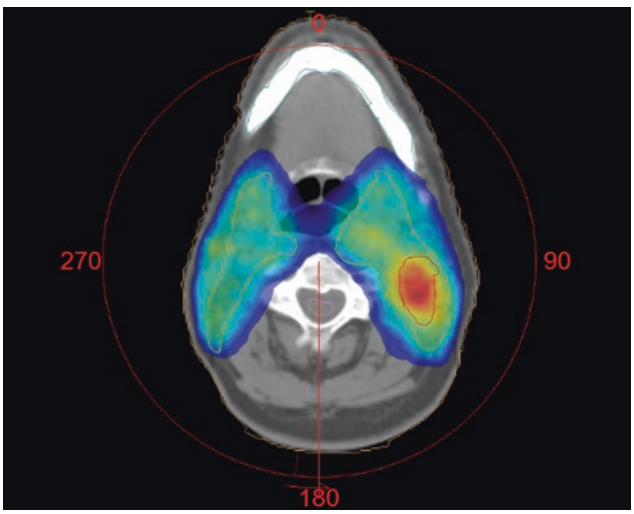


Fig. 14.3 SIB of positive node in the red isoline

- Optic chiasm, optic nerves, lenses
- Parotid glands
- Mandible
- Pharyngeal constrictor muscles
- Larynx
- Esophagus
- Thyroid
- Clavicles
- Lungs

14.6.3 Adaptive Radiation Therapy

Treated volumes can change during treatment both for changes in the patient's clinical conditions (like weight loss or as a result of steroid-based therapies) and for shrinking of tumor due to cytostatic effect of radiotherapy. Many authors [9] recommend a revaluation TAC around the 25th session to replanning the dose on new target volumes (Fig. 14.4).

14.6.4 Acute and Late Toxicities

Head and neck radiation treatment is one of the most toxic treatment for the patients. It is important to start the treatment with a good performance status, normal BMI, acceptable hepatic and renal function, and hematochemical values. After about the first week of treatment, the patient could experience fatigue, dysphagia, taste alteration, odinophagy, alteration of smell, and weight loss. Later the symptoms worsened, for which it becomes necessary medical therapy based on steroids to reduce dysphagia, antifungals to reduce mycosis and mucositis, oral mouthwashes, nutritional supplements to try to maintain adequate body weight, and analgesics for pain control.

In the last days of therapy, infusion therapy and hospitalization may be useful to maintain adequate body hydration and electrolytes in the normal range and to allow to complete the treatment.

Late toxicity varies depending on the site of the tumor and may consist of xerostomia, soft tissue or osteoradionecrosis, cataracts, radiation-induced hypopituitarism, optic pathway injury, skin/soft tissue fibrosis, swallowing dysfunction, dysgeusia, dental complication, acceleration of atherosclerosis, telangiectasias, voice alteration, hyperpigmentation, and hypothyroidism.

14.7 Breast Cancer

The main treatment available is surgery, radiotherapy and chemotherapy, hormonotherapy, or immunotherapy.

14.7.1 Indication

The absolute benefits from radiotherapy vary substantially according to the characteristics of the patient and the disease.

In the early stage:

Breast-conserving surgery (BCS) plus radiotherapy (whole breast radiation therapy (*WBRT*)) is a conservative method that provides survival rates equivalent to those of total mastectomy and axillary dissection while preserving the breast.

WBR reduces recurrence and breast cancer death rate and 10-year recurrence risk [10].

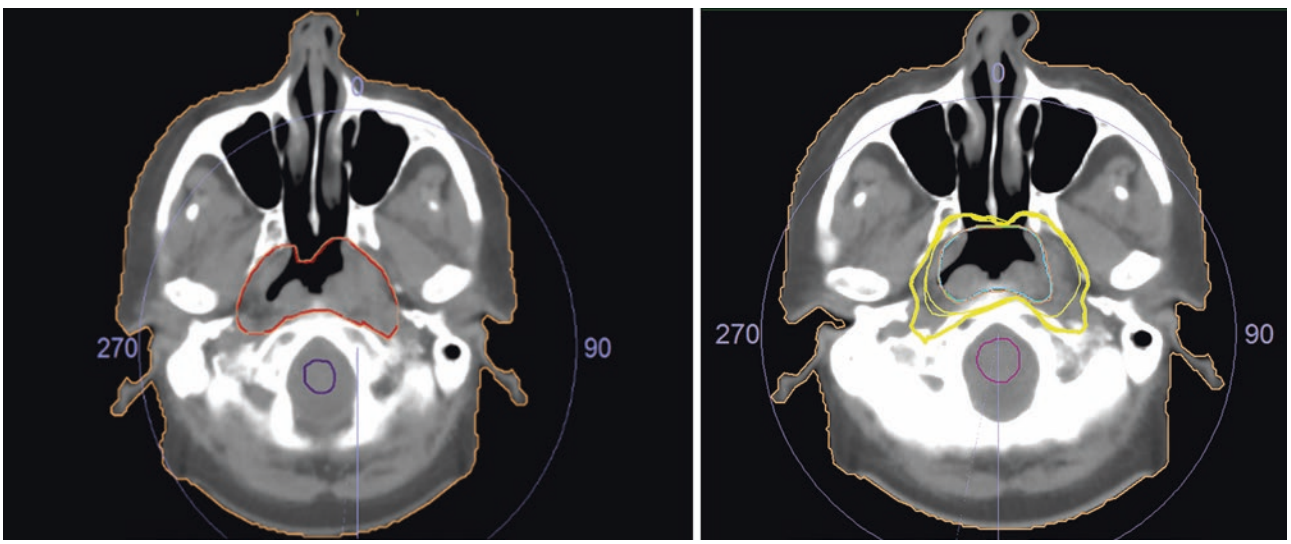


Fig. 14.4 GTV (red) before starting radiotherapy treatment; GTV (light blue) after the 20th session of radiotherapy

Radiotherapy of the infra- and supraclavicular area is recommended when four or more nodes are positive and otherwise considered individually in case of one to three nodes positive.

After *mastectomy* it is common consensus that post-mastectomy radiotherapy *(PMRT)* is mandatory for patients with risk factors as T3/T4 tumors and/or 4 or more positive axillary nodes and should be considered for patients with 1–3 involved nodes with other negative prognostic factor or when axillary dissection is omitted after a positive sentinel node biopsy. The advantage of PMRT is reducing local recurrence rates and 15-year breast cancer mortality.

A radiation boost in the *surgery bed*: can improve local control, with the largest absolute benefit in young patients, although it could increase the risk of moderate to severe fibrosis.

14.7.2 Timing to Treat After Surgery

Radiation therapy should start as soon as possible following surgery.

A delay of radiotherapy more than 8–12 weeks after surgery adversely affects local recurrence. Radiotherapy should be administered within 7 months after surgery, when chemotherapy is administered first [11].

14.7.3 Volumes and Doses

The *CTV* consists of the whole breast, up to about 0.5 cm below the skin surface. The skin is not part of *CTV* but must be included if infiltrated. The *CTV* excludes the pectoral muscle, unless there is the infiltration of the fascia; *see* Fig. 14.5. The whole breast should receive a dose of 45–50.4 Gy in 25–28 fractions or 40–42.5 Gy in 15–16 fractions in hypofractionated regimens. All dose schedules are given 5 days per week.

SPCL nodes doses amount to about 50 Gy.

Surgery bed is made up evaluating preoperative mammography, where the nodule was located. However, it is recommended to identify them on scans TC to highlight the excisional cavity and/or the possible presence of clips. A boost to the tumor bed is recommended in patients at higher risk for recurrence. Typical boost doses are 10–16 Gy in four to eight fractions.

The CTV of the *thoracic wall* consists of the cutaneous and subcutaneous tissue of the wall itself up to the costal plane and includes the entire surgical scar. Doses are 45–50.4 Gy in 25–28 fractions.

The main *organs at risk* to delineate are:

- Lung
- Heart (left breast)

14.7.4 Acute and Late Toxicities

In general, radiation for breast cancer post-lumpectomy and post-mastectomy is very well tolerated by most patients. Acute side effects of treatment are generally skin reactions, erythema, dry desquamation, edema, alteration of skin trophism, and fatigue. Rarer are ulceration, hemorrhage, and necrosis. The most common late effects on breast are persistent edema, hyperpigmentation and fibrosis; brachial plexopathy, pneumonitis, cardiac morbidity and secondary malignacy are instead uncommon.

14.8 Prostate Cancer

14.8.1 Introduction

During the last years, early diagnosis has increased, thanks to the use of PSA dosage, and management of prostate cancer has changed on the basis of the development of innovative procedures and medical therapies (new hormonal drugs, chemotherapy, bone-targeted therapies).

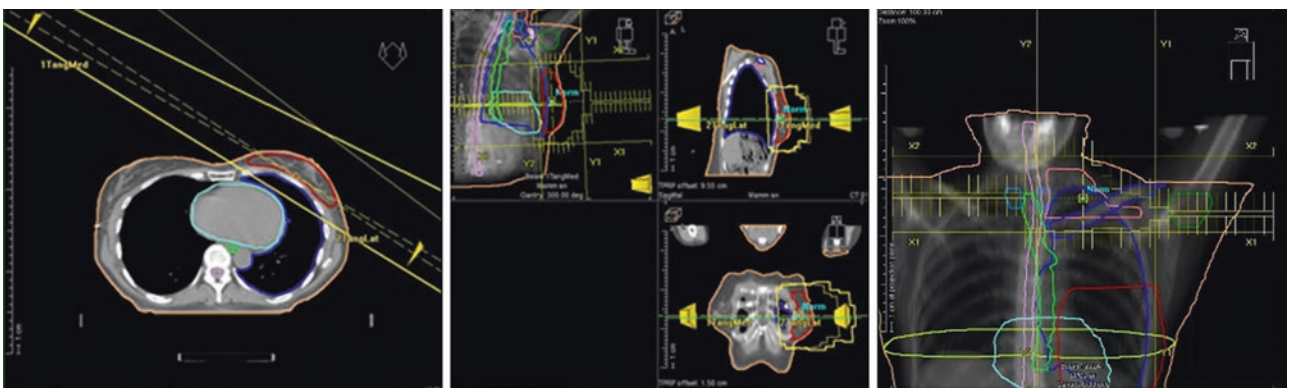


Fig. 14.5 Example of a breast planning

14.8.2 Indication

Treatment decisions should be planned based on disease staging and histological grading, by biopsy or by surgery and on life expectancy. The features considered in the classification of risk of disease are *grading* (Gleason Score), the *primary tumor stage*, and *PSA pre-biopsy*. In men with very low risk, *active surveillance* is encouraged. In men with low, intermediate, high, and very high risk, surgery or radiotherapy should be considered as *radical treatment* [12].

Advantages of radiotherapy over prostatectomy are avoiding complications associated with surgery such as bleeding. Radiotherapy has a low risk of urinary incontinence and stricture and a good chance of short-term preservation of erectile function.

The disadvantages of radiotherapy include the duration (number of fractions), the bowel, rectal, and urinary symptoms. Before starting a treatment, it is necessary understand the patient' s needs in a multidisciplinary team.

The *adjuvant* radiotherapy is indicated in case of PSA persistence (failure of PSA to fall to undetectable levels) or adverse pathologic features (i.e., positive nodes, seminal vesicle invasion, extracapsular extension, positive margins).

The salvage therapy is indicated in men who suffer biochemical recurrence after prostatectomy: (1) those whose PSA level fails to fall to undetectable levels after radical prostatectomy (persistent disease); (2) those who achieve an undetectable PSA after radical prostatectomy with a subsequent detectable PSA level that increases on two or more subsequent laboratory determinations (PSA recurrence); or (3) the occasional case with persistent but low PSA levels attributed to slow PSA metabolism or residual benign tissue (NCCN guidelines 2018).

14.8.3 Volumes and Doses

Prostate countouring is based on MRI imaging. T2-weighted MRI is currently the best modality to depict the anatomy of the prostate, as it enables a more detailed discrimination between the prostate and periprostatic tissue and the capsule infiltration (see Fig. 14.6). The use of multiparametric MRI [13] has increased in the last years helping to detect large and poorly differentiated cancers and to detect extracapsular extension: it has a good sensibility and sensitivity to detect N and M parameter.

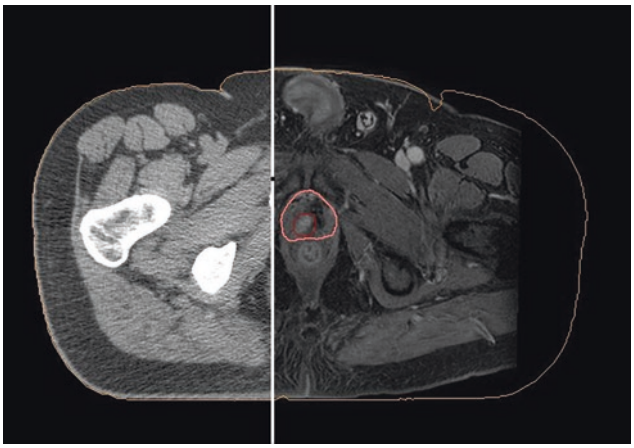


Fig. 14.6 Example of imaging fusion and *DIL*

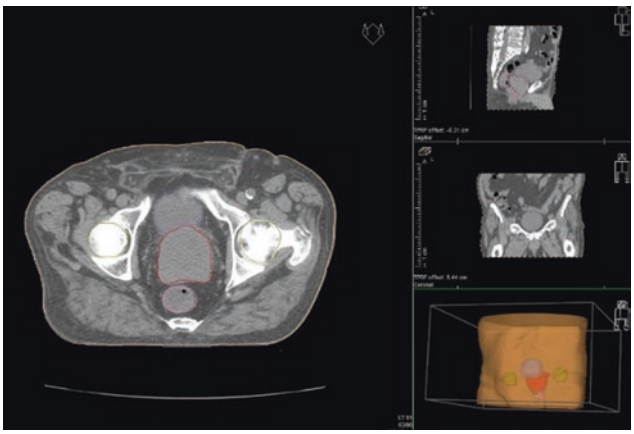


Fig. 14.7 Example of radical radiation treatment

In *radical* treatment (*see* Fig. 14.7), the dose escalation to prostate cancer is important to allow a good local control and to reduce biochemical recurrences. Doses to prostate are 78–80 Gy in 39–40 fractions. Studies have shown that local failure mainly occurs at the initial dominant intraprostatic lesion (*DIL*): in clinical trials there is growing interest in escalating the dose (till to 90–95 Gy).

Volumes are prostate and seminal vesicle in low risk; prophylactic nodal irradiation should be considered in case of aggressive tumor behavior, in high risk and very high risk disease and in case of nodal involvement (clinically positive nodes should be dose-escalated as dose-volume histogram parameters allow). In relation to the radiobiologic characteristics of prostatic cancer in the last years, many guidelines support hypofractionated regimens as an alternative to conventionally fraction-

ation both in radical and adjuvant setting: moderate hypofractionated regimens are more widely adopted (e.g., 70 Gy at 2.5 per fraction). This translates in gain for the patient and for the waiting lists of radiotherapy centers.

In *adjuvant and salvage* treatment, volumes are prostatic bed, and prophylactic nodal irradiation should be considered only in selected cases.

14.8.4 Acute and Late Toxicities

The main acute side effects consist of urethritis, cystitis, dysuria, stranguria, urgency, pollachiuria, nocturia, tenesmus, and proctitis. The prescription of drugs like cranberry supplements, anti-inflammatory and rectal suppositories could be useful in the prevention and treatment of side effects.

Possible late complications are urinary stricture, rectal bleeding, decreased volume of ejaculate, urinary incontinence, and impotence.

14.9 Rectal Cancer

14.9.1 Introduction

The multimodal approach (surgery, chemotherapy, and radiotherapy) plays a prominent role in the management of patients with carcinoma of the rectum.

14.9.2 Indication

Radiotherapy in the *preoperative* setting plays a major role in reducing the percentage of local recurrence in stages II and III [14].The advantage of preoperative radiotherapy consists in the downstaging (in some cases until complete remission) and therefore in the sphincter preservation. In the *preoperative* setting, it is feasible as *long*- and a *short*-course radiotherapy with comparable local control and OS. The *short-course* treatment should not be used if you want to get downsizing (therefore in cases where it is hoped to preserve the sphincter function) or if the tumor is close (≤1 mm) or has involved the mesorectal band. Compared to the "long-course" treatment, it allows a reduction in costs, total treatment times and acute side effects, as well as an equivalent outcome in terms of the development of local recurrence and OS.

Radiotherapy in *adjuvant setting* plays a role in patients who underwent surgery and have not received neoadjuvant chemoradiotherapy and who present risk factors that increase the likelihood of local recurrence.

Adjuvant CRT presents similar % of remote and OS recovery compared to neoadjuvant CRT treatment followed by adjuvant CT, but neoadjuvant rather than adjuvant chemoradiotherapy is preferred for patients with transmural (T3/4) or node-positive tumors, particularly if they are low-lying within the rectum since this approach includes better local control, an increased likelihood of sphincter-saving surgery, a decreased risk of posttreatment bowel dysfunction (soiling, frequent stooling), and a lower risk of chronic anastomotic stricture.

14.9.3 Volumes and Doses

The volumes to be irradiated in the *preoperative* setting consist in the rectum, mesorectum, and the pelvic lymph nodes

In the *postoperative* setting, the volumes are surgery bed with anastomosis (considering a boost), pelvic nodes.

In the long-course *preoperative setting and in the postoperative setting*, the prescribed dose is around 45–50.4 Gy in 1.8 Gy per fraction, and the radiotherapy is associated with chemotherapy with 5FU or capecitabine. In the *short-course* preoperative setting, the dose is hypofractionated and consists in five fractions of 5 Gy for a total dose of 25 Gy without chemotherapy.

14.9.4 Acute and Late Toxicities

The main toxicities observed during the radiotherapy treatment consist of proctitis, tenesmus, abdominal pain, alteration of the alve, cystitis, dysuria and pollachiuria, and weight loss.

Long-term gastrointestinal complications include change in bowel habits, rectal urgency, diarrhea, anastomotic stricture, and small bowel obstruction.

Case Study: Nasopharynx

Man: *57 years old*

- *Family history*: Negative for malignancy
- *APP:* Since 1 month appearance of laterocervical lymphadenopathy dx
- *Objective examination*: Palpatory evidence of lymphadenopathy in laterocervical dx region, at nasal fibroscopy presence of vegetant tissue at the roof of nasopharynx. Normal blood tests

Question

What action should be taken?

1. Surgery
2. Biopsy
3. Other

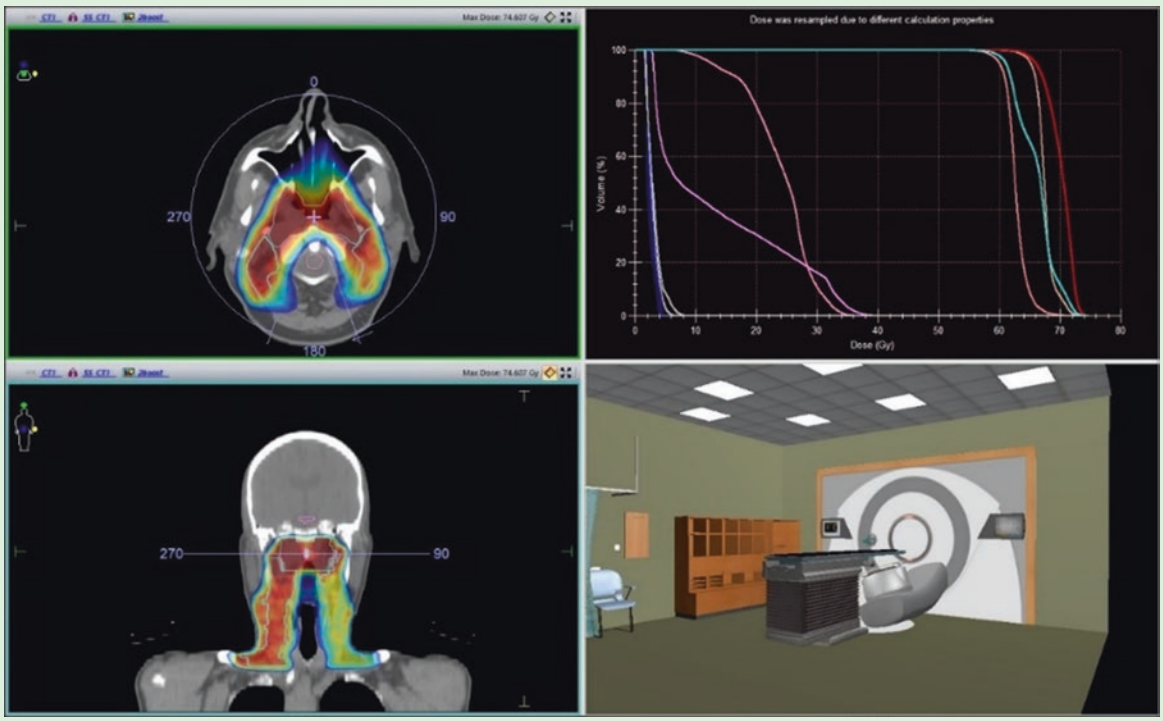
Answer

Biopsy of adenopathy and roof of nasopharynx

Question

What action should be taken?

1. Surgery
2. Medical therapy
3. Radiochemotherapy



Answer

Radiochemotherapy

Question

Which dose is needed to be delivered to the positive PET lymph nodes?

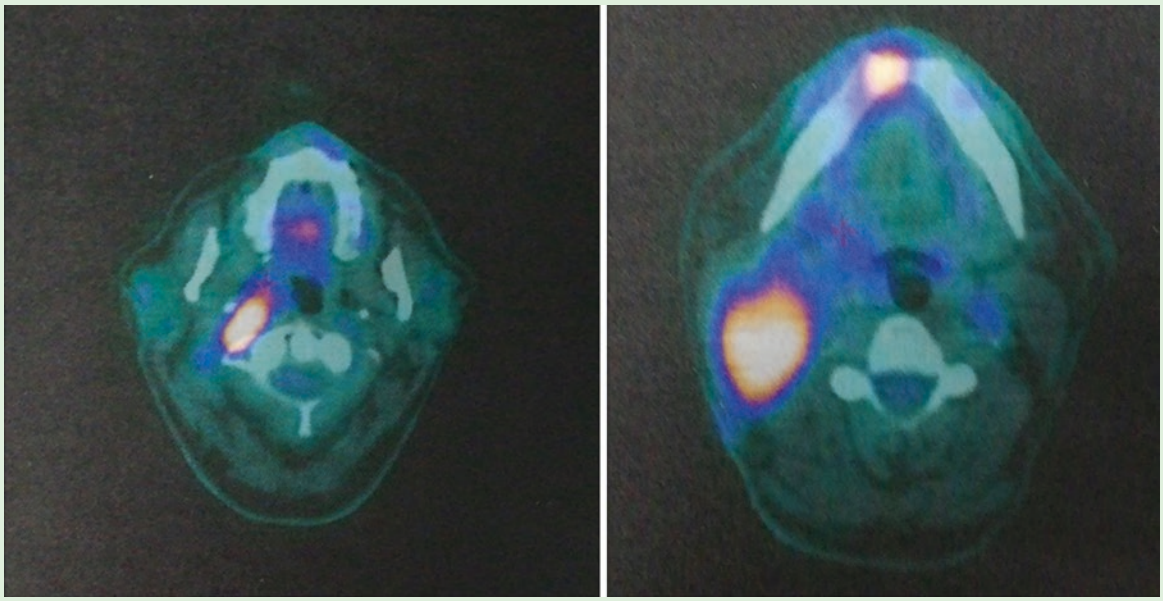
1. 66 Gy
2. 45 Gy
3. 30 Gy

Answer

66 Gy

Question

Which diagnostic method provides information about lymph node metabolic activity?



1. PET with FDG tracer
2. RX
3. TC without mdc

Answer

PET with FDG tracer

Response evaluation after 4 months after chemo and radiotherapy: complete response

Key Points

- The importance of a correct diagnosis
- The importance of full doses to the volumes to be treated
- The importance of a correct evaluation of the response to continue follow-up and the care

Key Points

Radiotherapy can be an effective part of a patient's cancer treatment regimen. It can be used as a frontline cancer treatment in almost all cancer sites.

Over the past decade, there has been a great number of technological developments in radiotherapy that have improved the planning and delivery of treatment allowing a better organ preservation, causing less toxicity, resulting in a smaller impact on quality of life.

Among the several new techniques developed, intensity-modulated radiotherapy (IMRT) and volumetric-modulated arc therapy (VMAT) can mold the shape and dose of radiation closely to the tumor cells and healthy tissue, and they are widely used for head and neck cancer, prostate cancer, etc.

Image-guided radiotherapy (IGRT) involves taking X-ray, ultrasound, or magnetic resonance imaging of the patient during treatment to assess any changes that have occurred since previous imaging and to improve the precision and accuracy of treatment delivery. IGRT is often used to treat tumors in areas of the body that move, such as the lungs.

With stereotactic radiotherapy, a high-dose, precise therapy is delivered in fewer treatments than traditional techniques. Due to the high doses, more accurate immobilization devices are used, as errors in delivery could have major consequences (such as missing the tumor). For certain conditions such as clinically inoperable stage I non-small cell lung cancer, liver and lung oligometastases, primary liver cancer, and spinal metastases, SBRT is now regarded as one of the standard therapies.

Moreover, among integrated treatments, the combination of radiotherapy and immunotherapy is becoming more and more charming for radiotherapists and oncologists. In fact the advent of immunotherapy is currently revolutionizing the field of oncology. Many preclinical data have shown that radiotherapy can synergize with immunotherapy by broadening up the immune repertoire in T cells (vaccination effect), by attracting T cells to the irradiated site (homing effect), and by rendering irradiated cells more vulnerable toward T-cell-mediated cell kill (vulnerability effect). As a consequence of this, many clinical trials are currently investigating these radiotherapy-immune interactions in patients.

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Response Assessment to Cancer Therapy

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Contents

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Learning Objectives

By the end of the chapter the reader will

- Be able to choose the best imaging technique for cancer assessment.
- Have learned the basic concepts of radiological assessment criteria for cancer.
- Be able to apply the knowledge in daily clinical practice.

15.1 Diagnostic Criteria

15.1.1 RECIST

RECIST (Response Evaluation *Criteria* in Solid Tumours) is a guide to daily clinical practice for cancer management in patients.

The first version of RECIST was published in 2000 [1] and later revised in 2009 [2].

The latest version of RECIST criteria was published in 2009 (RECIST 1.1) [2].

This version was named RECIST 1.1 rather than RECIST 2.0 because the fundamental approach to cancer assessment remains the same, based on an anatomical assessment of the disease as opposed to a functional one.

The major changes between RECIST 1.0 and 1.1 are:

- The number of lesions to be assessed.
- The evaluation of pathological lymph nodes.
- Disease progression definition is clarified.

RECIST define when tumors in cancer patients improve ("respond"), stay the same ("stabilize"), or worsen ("progress") during treatment.

The first important classification introduced by RECIST is in "measurable" and "non-measurable" lesions.

It is very important to classify as "measurable" or "non-measurable" baseline lesions.

"Measurable" lesions are defined as being at least 1 cm at CT scan (with a CT scan slice thickness no greater than 5 mm); 1 cm caliper measurement by clinical exam and 2 cm at chest X-ray.

Malignant lymph nodes are considered "pathologically enlarged" and measurable when the short axis is greater than 1.5 cm (Fig. 15.1).

Whereas all other pathological nodes (having a short axis between 10 and 15 mm) are identified as non-target lesions.

On the other hand, they do not need to be recorded or followed when the short axis is <10 mm because they are considered nonpathological.

Its important to underline that RECIST criteria consider only the lymph nodes' short axis both in the diagnosis and the follow-up phase.

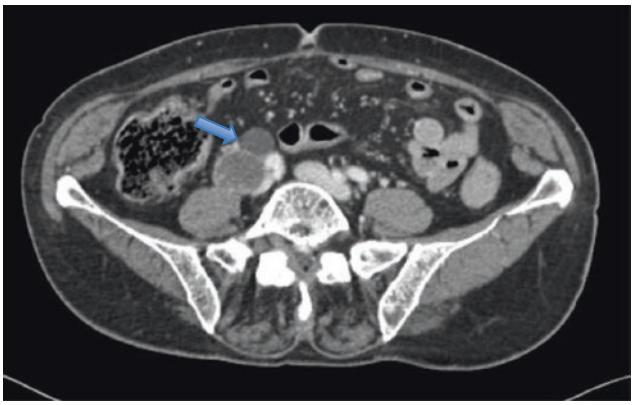

Fig. 15.1 The CT shows a pathologic iliac lymph node (arrow) in a patient with bladder cancer

By the way, we think that in radiological daily practice and not in research reporting, it could be useful for radiological and oncological follow-up to report nodes of short axis <10 mm.

On the other side "non-measurable" lesions are those where the longest diameter is inferior to 1 cm and pathological lymph nodes with a short axis between 1 cm and 1.5 cm.

Lesions which cannot be measured are always considered non-measurable.

Among "non-measurable" lesions authors list leptomeningeal disease, ascites, pleural and pericardial effusion, inflammatory breast disease, lymphangitic involvement of the skin or lung, and abdominal masses/abdominal organomegaly which can only be identified by means of a physical exam and not by reproducible imaging techniques.

Another important point is the definition of "target" and "non-target" lesions.

The radiologist tags lesions as "target" or "non-target" during the baseline examination; lesions should be representative and reproducible.

When more than one measurable lesion is present, all lesions up to a maximum of five (and a maximum of two per organ) should be chosen, recorded, and measured as "target" lesions, at baseline.

Particular consideration is reserved to the bone, cystic, and previously treated lesions. In particular:

- Blastic lesions are considered "non-measurable."
- Lesions with lytic and mixed lytic-blastic components are considered measurable only when the soft tissue component meets the criteria of a measurable lesion.
- Simple cysts are not considered malignant lesions (Fig. 15.2).
- Cystic lesions may be metastases, and when non cystic lesions are present in the same patient, cystic ones are not selected as target lesions.

Lesions located in a treated area are considered non-measurable unless a clear progression is shown.

Radiological evaluation should never be performed before 4 weeks from the beginning of treatment.

Analysis should always be performed using the same technique, and CT is acknowledged as being the best available and reproducible method to measure lesions selected for response assessment, and, as previously said, it is recommended to be applied to a slice thickness below 5 millimeters.

Another crucial point established from RECIST criteria is the definition of the four categories of response:

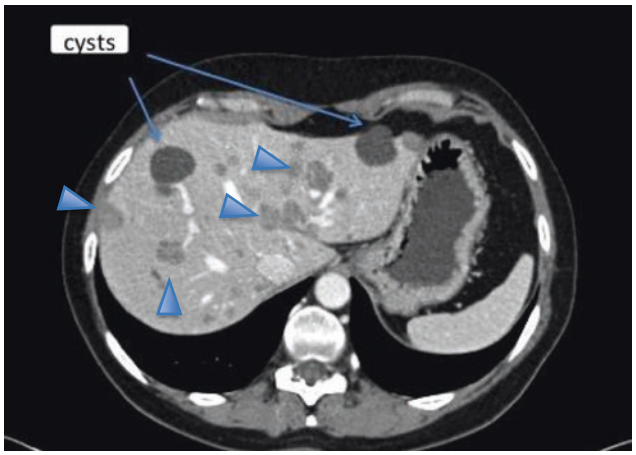

Fig. 15.2 Patient with liver metastases (arrowheads) and hepatic cysts (arrows)

- Complete response (CR): all target lesions disappear, and all pathological lymph nodes are reduced to a <10 mm short axis.
- Partial response (PR): there is at least a 30% reduction in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
- Stable disease (SD): shrinkage is not sufficient to define a partial response nor as progression because the increase is neither sufficient to define a progressive disease.
- Progressive disease (PD): the sum of diameters of target lesions shows at least a 20% increase and absolutely at least 5 mm.

There is also disease progression when one or more new lesions are found [2] (Figs. 15.3 and 15.4).

RECIST 1.1 recommends to analyze up to five lesions for lesion analysis, whereas the remaining lesions and sites of disease, including pathological lymph nodes, should be identified as "non-target lesions" [2].

FDG-PET scanning is sometimes considered reasonable in the assessment of disease progression.

There are, however, certain limitations in RECIST criteria due to differences in size measurements performed by different readers and in different moments by the same reader.

Margin irregularity may also be the cause of issue in the analysis of lesions [3] (Fig. 15.5).

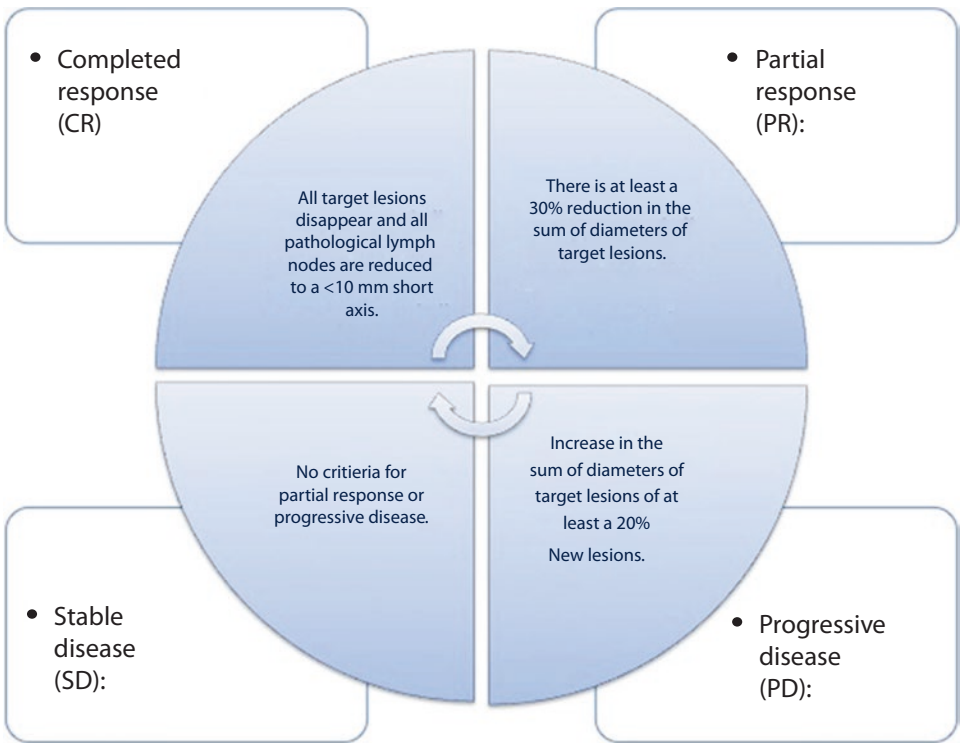

Fig. 15.3 RECIST criteria flowchart

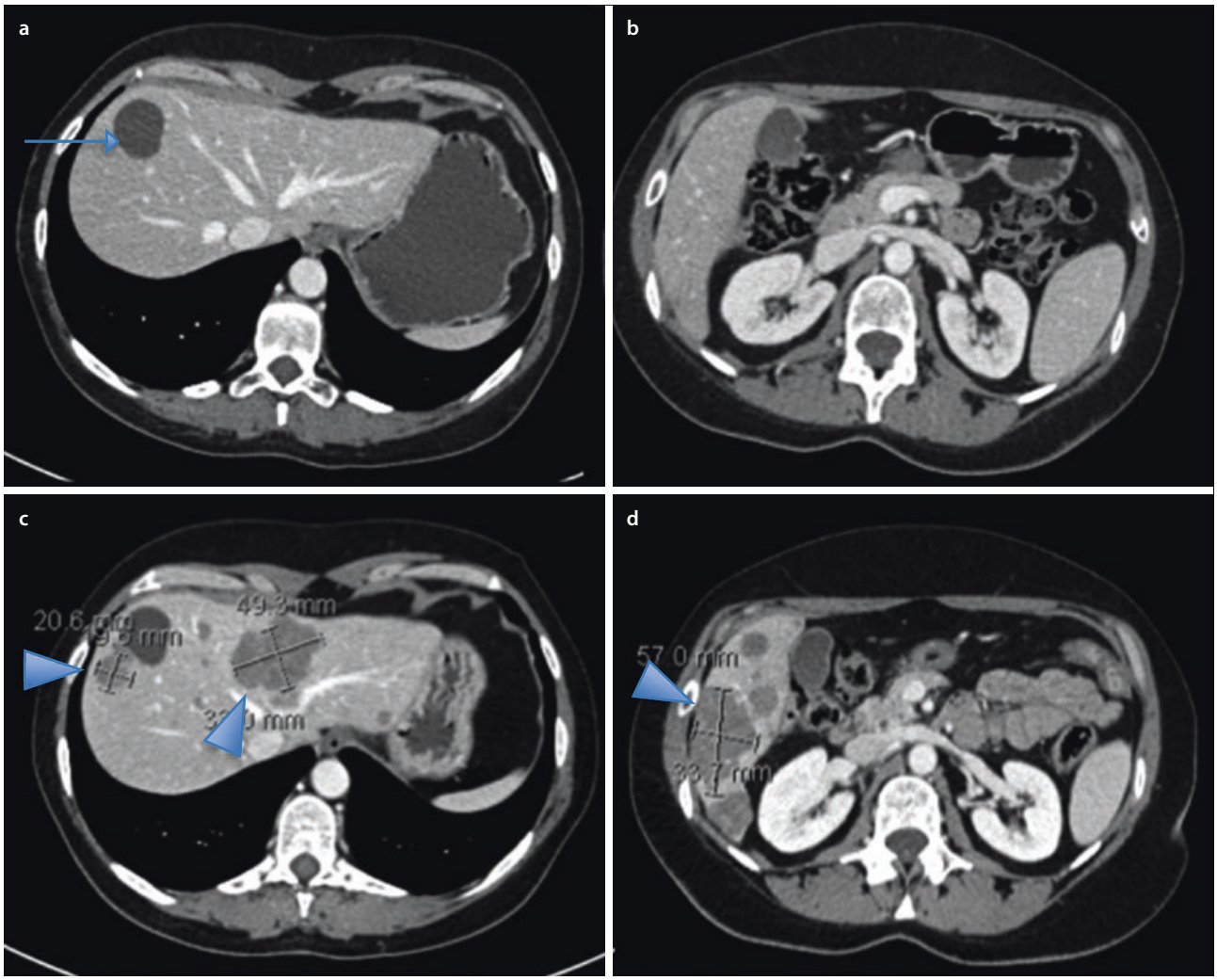

Fig. 15.4 A young woman with breast cancer and progressive disease according to RECIST criteria. The first CT scan showed only hepatic cysts (arrow in **a**). Appearance of liver metastases in the same patient after a few months (arrowheads in **c**–**d**)

15

Recent findings show that limiting the evaluation to morphological criteria may determine a limitation in cancer assessment.

Metabolic tumor responses are assessed either with the Positron Emission Tomography Response Criteria in Solid Tumors (PERCIST) or the European Organization for Research and Treatment of Cancer (EORTC) criteria.

The concordance of tumor responses between the morphologic criteria (RECIST) and metabolic criteria (EORTC and PERCIST) has been shown to be not excellent in a pooled analysis.

When adopting the metabolic criteria instead of the RECIST, overall response rates were significantly increased [4].

It is recommended to adapt frequency of tumor re-evaluation to the type and schedule of treatment.

Beyond RECIST criteria also tumor volume assessment could be useful.

Some findings have shown that volume measurement is more reproducible than size measurement in lung tumors [5, 6].

Zhao et al. demonstrated that volumetric tumor measurement is better than that of unidimensional and during gefitinib treatment it could be used to distinguish tumors with a sensitizing mutation from those without one [7].

Advances in CT technology have enabled vascular and perfusion assessment of lung lesions by using dynamic contrast-enhanced CT (DCE CT) [8].

Furthermore, tumor CT perfusion assessment in lung cancer has been shown to reflect tumor vascularity at histologic examinations [8].

In particular, several recent findings have evaluated changes in CT tumor perfusion by correlating perfusion parameters with RECIST response during treatment and survival.

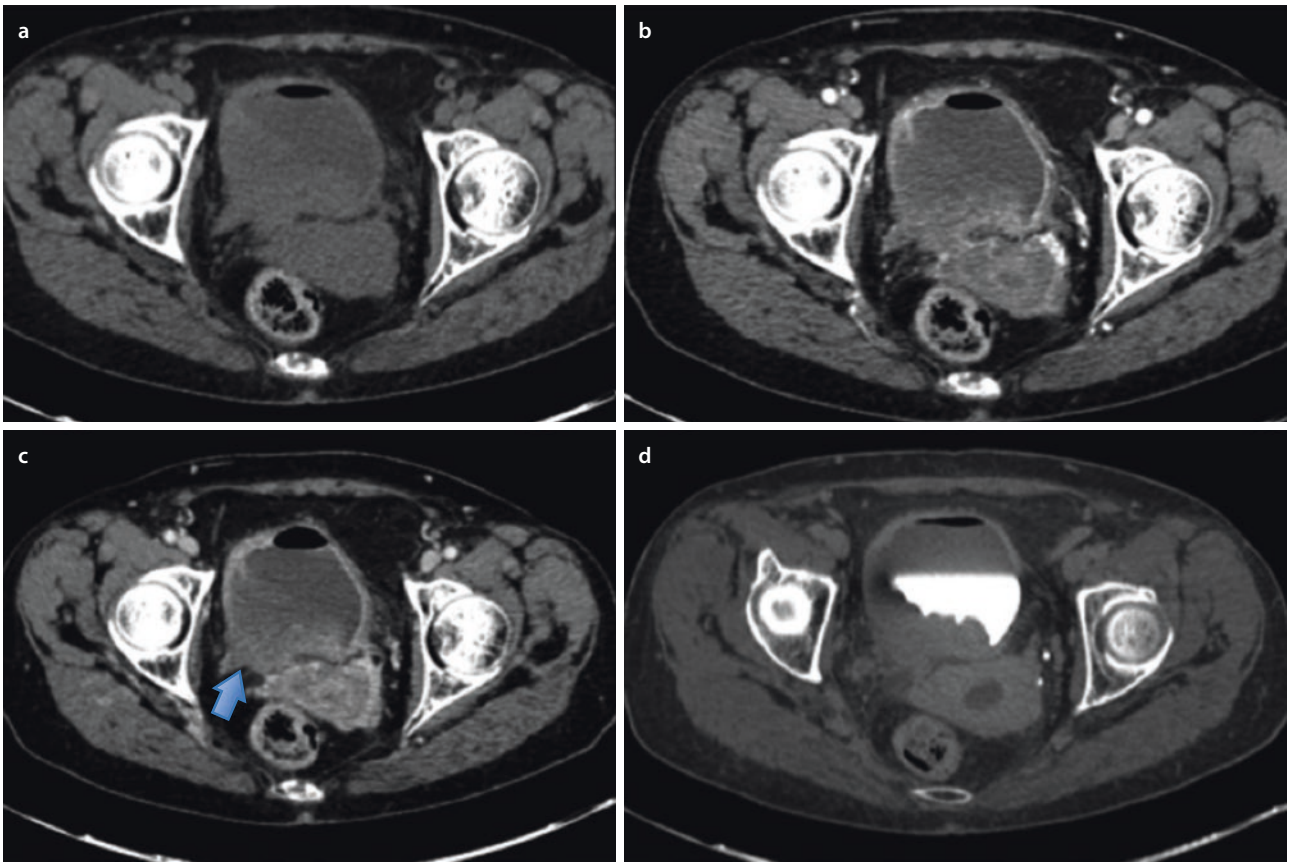

Fig. 15.5 CT examination of a patient affected by bladder cancer with irregular margins (arrow in C). **a**, pre-contrast image; **b**, arterial phase; **c**, portal venous phase; **d**, pyelographic phase

Non-small cell lung cancers with higher perfusion values are more sensitive to chemoradiation therapy than tumor with lower perfusion parameters [9].

Furthermore, authors showed that after chemoradiation therapy, findings at perfusion CT predict early tumor response and overall survival in the same cohort of patients [9].

Fraioli et al. [10] demonstrated, in a cohort of patients with unresectable lung adenocarcinoma who underwent perfusion CT before and 40 and 90 days after chemotherapy and antiangiogenetic treatment, that patients with partial response by RECIST criteria at 40-day follow-up had higher baseline blood flow and permeability compared with other patients. In conclusion perfusion CT may allow evaluation of lung cancer angiogenesis showing vascularity modifications after treatment [10].

To establish an appropriate threshold for tumor perfusion baseline and changes that may occur during the different therapies, it is necessary to introduce perfusion evaluation in daily clinical practice.

Quantitative evaluation of tumor perfusion by Dual-Energy CT could, in the near future, enter in daily clinical practice with new diagnostic criteria.

15.1.2 Targeted Therapies and CHOI Criteria

Targeted therapies arrest the growth and spread of cancer by interfering with specific molecules, the so-called molecular targets.

Usually molecular targets are involved in the growth and progression of cancer [11].

Indeed they achieve this goal by targeting specific genes or proteins found in cancer cells or in cells related to cancer growth like blood vessel cells [12].

Many of these therapies have an effect on proteins involved in cell signaling pathways,

governing basic cellular functions and activities such as the division, movement and responses of the cells to specific external stimuli, as well as cell death [3].

These therapies differ from the mechanisms of action of traditional cytotoxic chemotherapy.

Following the introduction of these news therapies, the need of new diagnostic criteria was felt owing to the growing awareness that cancer could respond to treatment and remain of the same dimension or grow but change in density.

15.1.2.1 Choi Response Criteria

Choi et al. [13] demonstrated that small changes in tumor size or density on CT are sensitive and specific procedures of response assessment of GISTs and proposed new diagnostic criteria for the evaluation of patients with GIST treated by imatinib.

Imatinib is a kinase inhibitor used to treat tumors like chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs) [14].

In particular gastrointestinal stromal tumors (GIST) are treated with imatinib [15].

GISTs are a particular kind of neoplasms that arise from special cells found in the wall of the gastrointestinal tract called the *interstitial cells of Cajal* (ICCs) [16].

Choi criteria arise from the finding that RECIST criteria, based exclusively on anatomic information only, underestimate the initial tumor response to imatinib in patients with metastatic GIST [13, 17].

At the same time, changes in tumor density were found by the authors who demonstrated that some lesions, despite clinical and PET response, increase in size.

It is believed that responding tumors decrease in density on CT because of the development of tumor necrosis, cystic, or myxoid degeneration.

Furthermore CT examination allows tumor density quantification in an objective manner, representing a valuable technique for cancer evaluation.

Measurements can be done objectively by using an optimal venous phase during the different examinations.

Furthermore, the CT triphasic imaging technique may facilitate the detection of lesions and the evaluation of tumor vascularity [17].

So, it is mandatory to use contrast medium delay automatic synchronization systems to obtain correct phases of post-contrast CT examination.

According to CHOI criteria [13], we can identify:

- Complete response (CR): when all the lesions disappear in the absence of new lesions.
- Partial response (PR): when there is a decrease in size (measured according to RECIST criteria) ≥10% or a decrease in tumor density (HU) ≥15% on CT examination, without new lesions and without progression of non-measurable lesions.
- Stable disease (SD): In the absence of criteria for CR, PR or progression of disease in the absence of symptomatic deterioration attributed to tumor progression (Fig. 15.6).
  - Progression of disease (PD): in case of an increase in tumor size ≥10% without criteria for partial response by tumor density on CT (HU), in case of new lesions or intratumoral nodules onset or dimensional growth (Fig. 15.7).

Despite its several limitations, CT is still considered the standard method for the evaluation of therapy response in patients with GIST.

The issue of intratumoral hemorrhage, which mimics disease progression, cannot indeed be solved by the Choi criteria.

Furthermore, patients with progressive GIST may present a "nodule in a mass" and not necessarily an overall increase in tumor volume because of a focal progression within a generally responsive lesion [18].

CT morphology-oriented criteria like the Choi criteria or the iodine-related attenuation measured on Dual-Energy CT have been recently developed and are more sensitive than the RECIST criteria, showing a greater correlation to the FDG changes.

An evaluation based on both changes in morphological and functional tumor data (like FDG metabolism and tumor perfusion) is required in patients with GIST [19].

15.1.3 Immunotherapeutics and iRECIST

Immunotherapy is a new a type of cancer treatment which fights cancer by strengthening the immune system.

There are several kinds of immunotherapy:

- Monoclonal antibodies
- Adoptive cell transfer
- Cytokines
- Treatment vaccines
- Bacillus Calmette-Guérin [20].

The concept of pseudoprogression was introduced by immunotherapy and described in patients with melanoma during early trials of immune-based therapeutics.

Authors noted that some patients with a RECIST diagnosis of progression showed late but deep and durable responses [21–25].

Authors proposed the modified RECIST 1.1 for immune-based therapeutics, the so-called iRECIST.

Responses related to iRECIST [26] method can be recognized by the "i" prefix (immune), as opposed to those related to RECIST 1.1.:

- —"Immune" complete response (iCR)
- —"Immune" partial response (iPR)
- —"Immune" unconfirmed progressive disease (iUPD)
- —"Immune" confirmed progressive disease (iCPD)
- —"Immune" stable disease (iSD)

The use of RECIST 1.1 is recommended to define measurable or non-measurable lesions, for the management

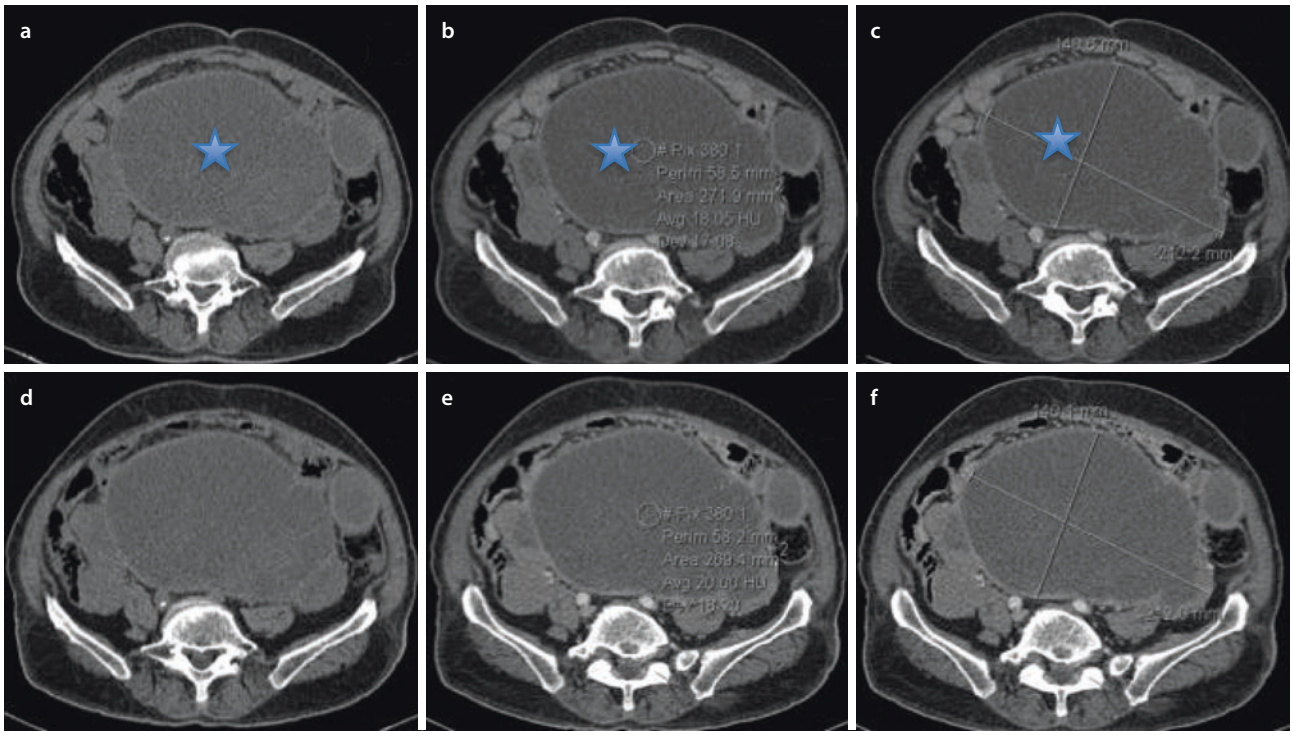

Fig. 15.6 Patient with GIST and stable disease after 1 year according to CHOI criteria. Images show stable density values and stable diameters (stars). **a**–**c** (first CT examination): **a**, non-contrast CT; **b**, **c**, post-contrast portal-venous acquisition. **d**–**f** (second CT examination): **d**, non-contrast CT; **e**, **f**, post-contrast portal-venous acquisition

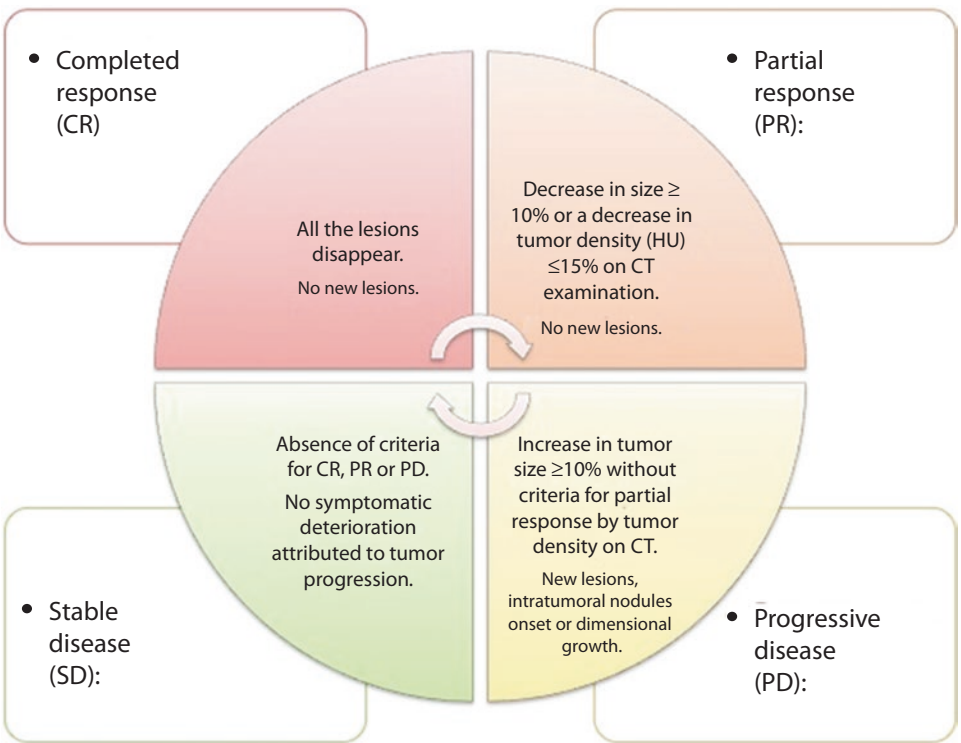

Fig. 15.7 CHOI criteria flowchart

of bone lesions, cystic lesions, and lesions with previous local treatment.

At the same time, the method of measurement was not changed by the authors.

The most important distinctive feature of iRECIST is that it resets the class of response if RECIST 1.1 progression is followed, at the next assessment, by tumor shrinkage.

In particular progression is confirmed if the next imaging assessment after unconfirmed progressive disease (4–8 weeks later) confirms a further increase in the sum of measures of target disease from iUPD of at least 5 mm [26].

15.2 Conclusion

The correct cancer assessment is crucial for the oncological patient's survival.

Radiologists must comprehend the adequate criteria for the definition of patient's response.

Development of new therapies is a challenge for radiologists.

In clinical practice, in our department, we usually make a report by using the appropriate diagnostic criteria.

At the same time, we write reports that can help clinicians in the interpretation of patient's clinical assessment.

The use of appropriate and international diagnostic criteria is important so as to share a common language between clinicians and radiologists all over the world.

Clinicians need to know the correct staging of the patient and understand changes in cancer features, also beyond the simple description of cancer dimension.

We can conclude that morphologic criteria should be used together with metabolic ones.

Key Points

- RECIST (response evaluation *criteria* in solid tumors) is a guide in daily clinical practice for cancer management; RECIST is define when tumors in cancer patients improve ("respond"), stay the same ("stabilize"), or worsen ("progress") during treatment.
- Choi response criteria arise from the finding that RECIST criteria, based exclusively on anatomic information only, underestimate the initial tumor response to imatinib in patients with metastatic GIST.
- iRECIST are a kind of modified RECIST 1.1 for immune-based therapeutics.

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Clinical Trials and Methodology of Cancer Research

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Contents

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Learning Objectives

By the end of this chapter, the reader will:

- Have learned aims and main characteristics of phase I trials
- Have learned aims and main characteristics of phase II trials
- Have learned aims and main characteristics of phase III trials
- Be able to discuss main challenges of clinical trials conducted with new anticancer drugs

16.1 Introduction

Clinical research is of paramount importance in oncology, characterized by a less than optimal outcome with the treatments currently available in clinical practice.

Clinical trials of new drugs are usually promoted by pharmaceutical companies, who will submit study results to regulatory authorities, in order to have the drug authorized for use in clinical practice. However, pivotal trials do not resolve all the clinically important questions related to the use of the drug. An important role is played by independent, academic research, which may optimize drug use in clinical practice.

All clinical trials involving human subjects must be compliant with good clinical practice (GCP), an international standard of ethics and scientific quality for the design, recording, conducting, and communication of the results of clinical trials.

Proposing a patient to participate in a clinical trial is a very delicate matter. The patient should be in the condition to make a free and conscious choice. The quality of the relationship between the investigator and the patient, that is established at this preliminary stage, will play a decisive role in the course of the entire clinical trial. Doctors should illustrate the clinical study to the patient and, according to patient's will, to the caregivers, in a personalized way, making the information about the trial clear and comprehensible, responding to all the doubts and all the requests coming from the patient, and discussing the reasons for his/her involvement and the risks and benefits associated with the inclusion in the trial. At the end of such colloquium, the informed consent sheet can be given to the patient, and it is good practice to leave a few days before meeting again for formal informed consent signature. With the signature of the informed consent, the patient who agrees to participate in the clinical trials states his/her awareness of the characteristics of the protocol and his/her rights and authorizes the investigator to the processing of personal and clinical data for the aims of the study. In any case, it should be reminded that a true and effective informed consent is, in fact, a continuous information process that takes place throughout the clinical study.

The conduction of a clinical trial is based on an essential document, the study protocol, where all the various aspects and methods of the study are reported in details. To be scientifically valid, the protocol must meet specific requirements and must clearly explicit – among the other things – the criteria for the selection of eligible patients, the definition of treatment groups, and the study endpoints. The protocol should also detail the procedures to be followed in the case of toxicity and the criteria for temporary or definitive interruption of treatment. ▶ Box 16.1 lists the main elements of a clinical trial protocol.

Traditionally, clinical trials are classified in four different phases, from phase I to phase IV. In recent years, to make faster clinical development programs, and thanks to progress of clinical trials methodology, the clear-cut distinction between clinical trials' phases is being questioned, and the separation among different phases is often not rigid. The number of "phase I-II" or "phase II-III" studies, with a seamless transition between phases with different aims within the same clinical trial, is growing. Furthermore, even the need for a complete program from phase I to phase III is being challenged by the rarity of many diseases (or the rarity of specific molecular subgroups within "common" types of cancer). For instance, several drugs have been approved by regulatory agencies after the conduction of a single-arm study, without a randomized controlled trial. In addition, the mechanism of action of many new drugs significantly differs from that of classical cytotoxic treatment. This implies the need to reconsider some important methodological concepts at various phases of clinical research, such as the optimal study design, the choice of the correct endpoint, and the proper selection of patients. Therefore, even if the ultimate aim of the clinical development of new anticancer drugs (the demonstration of benefit for the patients) has not changed over the time, the application of traditional clinical trials methodology to these new classes of drugs raises a number of relevant issues [1–3].

For educational purposes, this chapter will describe traditional study phases with some insights into how the traditional methodology is applied in recent trials, phase by phase.

Box 16.1 Main Elements of a Clinical Trial Protocol

1. Background and rationale of the trial
2. Objectives of the study (primary objective and secondary objectives)
3. Eligibility criteria (inclusion and exclusion criteria)
4. Screening procedures (tests and exams to be carried out before the patient is inserted into the study)
5. Detailed description of the methods of administration, dose, and schedule of treatment(s) in the study
6. Exams planned for the evaluation of treatment toxicity
7. Evaluation of study objectives (schedule of visits during treatment, schedule and list of exams for the evaluation of treatment activity, schedule of administration of questionnaires for the evaluation of the health-related quality of life and patient-reported outcomes, schedule of visits during follow-up, etc.)
8. Statistical hypothesis and calculation of sample size
9. Procedures of data collection
10. Statistical analysis
11. References
12. Publication rules

16.2 Phase I Trials

Phase I trials are designed to test new drugs (or new combination of drugs), in order to study their safety and to identify the dose and the schedule to be used in the subsequent phases of development.

Based on the hypothesis that antitumor agents will produce an increasing cytotoxic effect with increasing doses, phase I trials conducted with cytotoxic drugs have traditionally been based on the principle of identifying the maximum tolerated dose (MTD). Dose-limiting toxicity (DLT) is defined as the toxicity that prevents further increase in the dose of the experimental treatment. MTD is defined as the highest tested dose that is not associated with unacceptable toxicity. Once identified the MTD, dose escalation will be stopped, and usually a further group of patients ("expansion cohort") will be treated at that dose, in order to better characterize the safety profile. The MTD will be recommended as the dose to be used in the subsequent phases of clinical development.

Phase I trials are traditionally designed with dose escalation in different groups of patients, starting from low doses in the first subjects and increasing dose in subsequent subjects until this is allowed by the observed toxicity [4]. The dose escalation does not take place intra-patient (each single patient receives a fixed dose, even if the schedule of the drug provides repeated administrations) but in subsequent groups of patients. According to the classic design of phase I studies, patients are divided in groups of three (the so-called "triplets"), treated at progressively increasing doses. If the three patients treated with a dose level do not experience unacceptable toxicity during the observation period (usually a few weeks of treatment), the next three patients will be treated at a higher-dose level. If unacceptable toxicity occurs in one of the three patients, additional patients will be treated at the same dose level, to better define the actual incidence of unacceptable toxicity and rise to the next level only if the toxicity observed is not too frequent. Finally, if unacceptable adverse events occur in two of the three patients or even in all three patients, that dose level will be considered not tolerated.

Given that the main endpoint of phase I trials is the safety, they are usually conducted with a small number of patients, because the risk-benefit ratio for the patients is at the highest level of uncertainty. Recently, however, the mean sample size of phase I trials in oncology has grown, and results from phase I trials are often used to describe not only safety but also clinical activity [5].

Patients enrolled in phase I trials have a low chance of obtaining clinical benefit from the experimental treatment, because many of them will be treated at low, potentially inactive doses and also because the treatment could be completely not effective for their condition. Traditionally, cancer patients enrolled in phase I trials had a very small probability (5%) of obtaining an objective response. For these reasons, patients enrolled in phase I trials have been usually already treated with standard treatments. This probability is recently increased, considering that phase I trials of targeted agents are often conducted in molecularly selected patients. However, patients should be adequately informed about the risks associated with these trials.

Compared to the traditional methodology of phase I studies with chemotherapy, the development of new classes of anticancer drugs has determined some changes in the methodology of phase I studies. In detail, the development of target-based agents (i.e., drugs that interfere with the function of molecules that are relevant for the growth and proliferation of tumor cells) has

challenged the traditional design of phase I studies, based on dose escalation up to the identification of maximum tolerated dose. If the maximum biological effect of the drug is obtained at less than maximal doses, reaching MTD is not necessary. The identification of the minimum dose able to determine the maximum inhibition of the target (MTID, maximum target inhibiting dose) could replace the detection of the classical MTD. Phase I trials with these new drugs include the study of pharmacodynamics, i.e., the measure of the drug activity on the molecular target, measured in tumor tissue or in other tissues of the patient, that might be more easily sampled.

16.3 Phase II Trials

Phase II trials are conducted with the aim of demonstrating treatment activity against the tumor.

While phase I studies may be conducted in heterogeneous population of patients with different types of primary tumors, traditional phase II studies have the aim of describing the activity of treatment against a specific type of tumor. With molecular-target drugs, selection of patients might be based on the presence of the target at the level of the cancer cells, rather than just driven by the site of the primary tumor. The so-called umbrella trials are based on the study of different drugs in patients with the same type of tumor, choosing the drug based on molecular alterations that make the patient candidate to one or more of the experimental drugs (Fig. 16.1). The so-called basket trials imply the study of a drug (or several drugs) in patients with different histological types of solid tumors, also based on the molecular characterization (Fig. 16.2). In principle, the "umbrella" and "basket" approach can take place early in the development of drugs (also in phase I).

The objective response rate (ORR), based on tumor shrinkage that almost certainly is due to treatment activity, has been considered the standard endpoint of phase II trials in oncology. The RECIST (response evaluation criteria in solid tumors) criteria for evaluating the objective response, that today are universally used in clinical trials, were published in 2000 [6] and subsequently updated in 2009 [7]. RECIST criteria have been developed with the aim of standardizing and simplifying the measurement of the lesions, based on a one-dimensional measurement (the largest diameter of the tumor lesions or the shorter axis in the case of lymph nodes). The evaluation of the objective response assumes that the patient is subjected to a baseline instrumental evaluation (prior to the start of treatment) to identify all disease lesions and to evaluate changes subsequently induced by experimental therapy. According to RECIST criteria, the lesions which can be accurately measured in at least one dimension, with a diameter greater than at least 10 mm at the spiral CT scan, are defined as measurable lesions. Conversely, minor lesions and objectively nonmeasurable lesions (e.g., a pleural or pericardial effusion or ascites, or pulmonary lymphangitis, as well as tumor lesions previously irradiated) are defined nonmeasurable lesions. According to the updated version of the

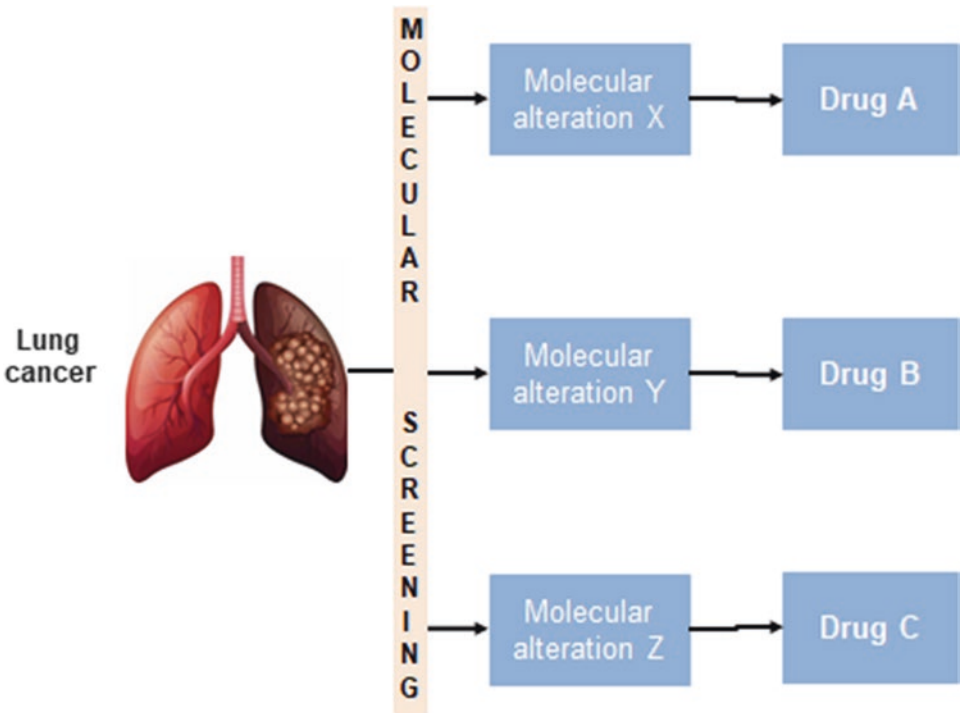

Fig. 16.1 Example of "umbrella" trial: different drugs are studied in patients with the same type of tumor, choosing the drug based on molecular alterations that make the patient candidate to one or more of the experimental drugs

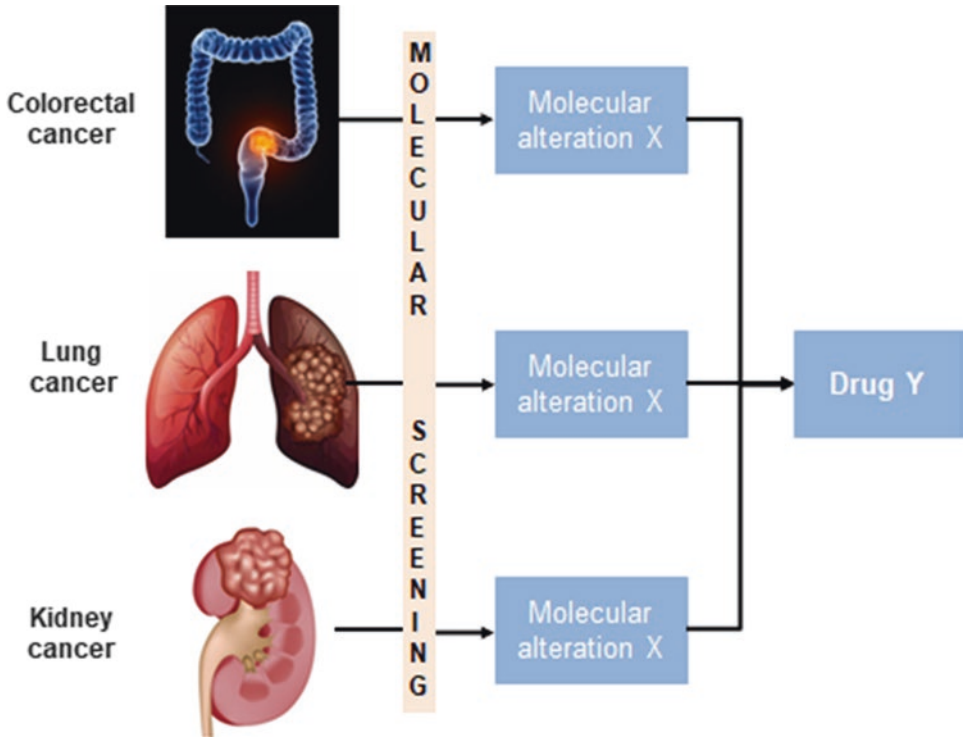

Fig. 16.2 Example of "basket" trial: one drug (or several drugs) are studied in patients with different histological types of solid tumors, based on the molecular characterization

RECIST criteria, the basal evaluation involves the identification of the so-called target lesions, that is to say all the measurable lesions, up to a maximum of two organ lesions and five lesions in total, possibly representative of all the organs involved. All other lesions are defined as "nontarget lesions," of which no measurement is required. The evaluation of the objective response is based on the repetition of the same instrumental tests carried out at the baseline evaluation, after a time defined by the study protocol. For target lesions:

- Complete response is defined as the disappearance of all lesions.
- Partial response is defined as a decrease ≥30% in the sum of the largest diameter of target lesions.
- Stable disease is neither a sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum of largest diameter of lesions since the treatment started.
- Progressive disease is defined as an increase ≥20% in the sum of the largest diameter of target lesions, taking as reference the smallest sum recorded since the treatment started or the appearance of one or more new lesions.

In some cases, the simple measurement of the diameter of a tumor lesion may be inadequate to document the real activity of the treatment, for instance, when treatment produces necrosis without shrinkage of the lesion. Following the description of this phenomenon for several new drugs, in different types of solid tumors, modified response criteria have been proposed (e.g., the so-called modified RECIST criteria, mRECIST) that take into account, in the measurement and comparison, only the "viable" portion of the tumor lesions [8]. Recently, moreover, RECIST criteria were considered not optimal for the new immunotherapeutic drugs, for which specific criteria for evaluating the objective response were developed, including the so-called immune-related RECIST [9]. Among the peculiarities of these immune-related criteria, the appearance of new lesions does not necessarily imply disease progression. Moreover, the progression of disease should be confirmed by a subsequent instrumental evaluation, repeated after a few weeks compared to the previous one. These modifications are necessary since the enlargement of the tumor lesions in the course of immunotherapy (and therefore also the apparent appearance of lesions not previously visible) may be attributed in some cases to the lymphocytic infiltrate in the tumor sites, thus determining a so-called pseudo-progression that, if wrongly interpreted, would lead to erroneously define the failure of the therapy.

The design of a phase II study requires the definition of a proportion of objective responses below which the experimental drug will be considered inactive (P0) and the definition of a desired proportion of objective responses (P1). On the basis of these parameters, the

number of patients to be included in the study is calculated by predefining also the risk of type I error (false-positive result) and of type II error (false-negative result). The simplest design for a phase II study involves a single stage, i.e., the inclusion in the study of the total number of patients, without interim evaluations. When a trial is designed in this way, the risk is to treat too many patients with a treatment that may be inactive; this would be particularly unpleasant if there are alternative treatments that might be proposed in place of the experimental one. In order to reduce this risk, a multistage design can be applied to the phase II study: after the treatment of a first group of patients (first stage), if the number of objective responses exceeds a predetermined threshold, the study continues with the second stage; otherwise the trial is interrupted early for inactivity of the experimental treatment.

In the past decades, most phase II studies have been designed with a single treatment arm: study hypotheses and interpretation of results were based on historical controls, without prospective comparison. Ideally, a phase II trial should have both a high positive predictive value and a high negative predictive value. In other words, it should be able to discard all treatments that would be ineffective in the subsequent phase III trial, and, at the same time, it should identify as positive all effective treatments. Some authors have supported randomized design for phase II trials, with formal comparison of experimental versus standard treatment. This might lead to a better interpretation of the activity of the experimental treatment [10, 11]. Of course, the adoption of a randomized design should not transform a phase II into a phase III trial, because the latter is characterized by higher statistical power, requiring a sample size that would be too large and inappropriate for the early evaluation of an experimental treatment. Randomized phase II trials could instead be conducted according to the so-called relaxed criteria, with a power not exceeding 80% and one-tailed alpha error set to 15% or 20%, much higher than commonly accepted. Such a high risk of false-positive results, which would be of course unacceptable in a phase III trial, can be acceptable in this early context, where the aim is to quickly select promising treatments that will be subsequently tested for efficacy. According to this strategy, a low statistical power is accepted, corresponding to a small sample size, with the aim of selecting only those treatments associated with large benefits. However, empiric demonstration that randomized phase II trials are more efficient than single-arm phase II in predicting the success rate of subsequent phase III studies is not yet available, and data contrasting with such hypothesis have been reported [12].

16.4 Phase III Studies

Randomized phase III trials are universally considered the highest level of evidence to demonstrate treatment efficacy. Randomization can be difficult to accept for the patients, and somebody argues that, at least for some medical practices, randomized trials can be impractical or even unnecessary: no doubt that randomization (uncertainty about the treatment that will be assigned within the study) can be very difficult, if investigators already believe that one treatment is clearly better than the other [13, 14]. Unfortunately, in oncology like in other fields of medicine, this occurs rarely: in most cases, randomization is methodologically important to compare different strategies. In fact, randomization has the aim of obtaining treatment groups that are balanced in terms of characteristics that could affect the outcome, being different only for the treatment assigned. This will allow the comparability of treatment arms. On the contrary, if treatment would have been decided by the investigator, without randomization, this could represent a relevant bias. Within a randomized phase III trial, patients assigned to the control arm should always receive the best standard treatment. When there is no effective standard treatment, randomization to placebo (in the case of blinded studies) or to no treatment is ethically acceptable.

Given that the objective of randomized phase III trials is the demonstration of efficacy, the endpoint of the study must necessarily represent a true clinical benefit for the patient; traditionally, in the case of patients with advanced/metastatic disease, clinical benefit means "living longer or living better," which is a prolongation in survival or an improvement in health-related quality of life. Many trials in cancer patients, however, are designed with progression-free survival as the primary endpoint. This choice is often motivated by the risk that the comparison could be jeopardized by the impact of subsequent treatments on the overall life expectancy. This risk can be particularly high in some tumors (for instance, breast cancer), where the life expectancy of patients newly diagnosed with advanced disease is relatively long and the comparison between treatments could be confused by the therapy received after progression. However, this appears to be less true in many solid tumors (for instance, advanced pancreatic cancer, gastric cancer, or lung cancer), where the overall impact of subsequent treatments is (with some exceptions) less impressive than in breast cancer. Furthermore, the choice of PFS instead of overall survival as the primary endpoint can be justified if patients assigned to control arm could cross over to experimental treatment after progression, and this could mask the real difference obtained with the experimental treatment.

The design of a phase III study is based on the definition of two hypotheses: a null hypothesis that, in the case of a superiority study, means that there is no difference in efficacy between standard treatment and experimental treatment and an alternative hypothesis that means that the experimental treatment is better than the standard. The greater is the difference in efficacy assumed between the treatments, the smaller is the number of patients that will be needed. In addition to the hypothesized difference, the sample size of a phase III study is determined by the type I error (or "alpha" error), corresponding to the risk of false-positive result, and the type II error (or "beta" error) corresponding to the risk of false-negative result. The type I error is generally set at 5%, while the risk of false-negative result is generally set at 10% (thus resulting in a "power" of 90%) or 20% (resulting in a power of 80%).

16.4.1 Predictive Factors and Patients' Selection

In an ideal scenario, when complete information on predictive factors and proper selection of patients can be definitely obtained in the early phases of drug development, the conduction of subsequent phase III study could be optimized, and phase III trials should be conducted only in selected patients. Unfortunately, even with targeted agents, this ideal scenario occurs rarely. Usually, when planning a phase III trial comparing an experimental treatment with the standard, we often have contrasting or weak evidence on the exact role of predictive markers. In any case, when some evidence exists suggesting that patients with expression of the marker (M+) are benefit of the experimental treatment more than those without the marker (M-), different strategies are theoretically possible [15], namely, (a) *randomize-all* strategy, that is randomization between standard and experimental treatment without selection, possibly with stratification based on the status of the marker (in this case, *stratified trial design* or *treatment-marker interaction design*); (b) *targeted* design, that is randomization between standard and experimental treatment only in patients selected according to the status of the marker (also called *enrichment design*); and (c) *customized* strategy (also called *marker-based strategy*), that is randomization between standard arm, in which the treatment is the same for all patients, and a personalized arm, in which treatment is chosen based on the marker status of each patient.

In the *randomize-all* strategy, the marker is prospectively evaluated in all patients, allowing stratification, but no patient is excluded, and all patients are randomized, regardless of the marker status. The trial will allow comparison between experimental and standard treatment not only in M+ patients but also in the M- patients. *Randomize-all* strategy has two major drawbacks. First, if the experimental treatment is actually better than the standard in M+ patients, but not in M- patients, the reduced or absent efficacy of experimental treatment in M- patients will dilute the overall effect. This will be particularly relevant in the case M+ patients represent a small percentage of the overall number of patients. Second, subgroup analysis (in this case the separate comparison between experimental and standard treatment in M+ patients and in M- patients without a priori planned hypotheses separately for each subgroup) implies a reduced statistical power in each subgroup. If the experimental treatment is more effective than the standard one in all patients, regardless of M status, there will be a risk of falsely describing a differential effect in the two groups, because the apparent lack of effect in one of two subgroups would be actually a false-negative result.

An alternative strategy (*targeted design*) is to test the status of the marker M, excluding M- patients and randomizing only M+ patients. This strategy is acceptable only in cases where investigators have already enough evidence to completely rule out the efficacy of the experimental treatment in M- patients. Due to the absence of M- patients, compared to the *randomize-all* strategy, targeted design allows investigators to avoid potential dilution of the results. A larger difference between arms can be assumed compared to an unselected trial, determining a smaller sample size. Obviously, the sample actually enrolled in the study will correspond to a higher number of screened patients: the lower the proportion of patients M + compared to the total population, the higher the number of patients screened to reach the final sample size.

16.4.2 Non-inferiority Design

A phase III trial should answer the question: "Is the new, experimental treatment more effective than the standard?" In some cases, in oncology as well as in other medical settings, phase III trials are planned according to a so-called non-inferiority design. Because of some advantages of the experimental treatment, for example, in terms of reduced toxicity or easier administration, investigators consider that it should be preferred to the standard, if comparable efficacy is proven. However, it is virtually impossible to demonstrate that two treatments have absolutely identical efficacy. When planning a non-inferiority study, it is mandatory to determine in advance

the maximum acceptable difference (δ), i.e., the maximum reduction of efficacy considered not clinically relevant and acceptable to conclude that the experimental treatment is not worse (so-called *fixed margin method*). The results are commonly presented as confidence intervals (CI). To show non-inferiority, CI should include only values more favorable to experimental treatment than δ.

Another method adopted in non-inferiority trials is the so-called *percent retention method*. The experimental treatment is proven non-inferior if it preserves at least a specified fraction of the effect previously shown by the standard compared to placebo or supportive care.

Although non-inferiority trials have been criticized, the choice of this design for the above described studies appears to be justified when the experimental drug has clear advantages, in terms of toxicity and ease of administration, compared to the current standard.

16.5 Interpretation of Study Results and Clinical Relevance

In recent years, the concept of clinical relevance of results has gained growing importance among medical oncologists. Statistically significant difference among the arms of a clinical trial is not enough to recommend that the winning treatment should be adopted in clinical practice. Statistical significance, indeed, is attainable in trials with a very large sample size even if the absolute magnitude of benefit produced by the experimental treatment is small or very small. But a small to very small advantage might not be clinically relevant for patients (e.g., few weeks of survival prolongation without a major improvement of symptoms or quality of life or a few months prolongation of time to progression without any change in survival or quality of life). In addition, the growing cost of anticancer drugs is increasingly producing difficulties in drugs accessibility and affordability worldwide. Therefore, it becomes more and more necessary to distinguish drugs producing significant clinical improvements from those reaching the market (thanks to a formally correct development program) but not producing relevant clinical progress.

The two main world cancer societies, ASCO (American Society of Clinical Oncology) and ESMO (European Society of Medical Oncology), have produced and updated two different scoring systems (the ASCO value framework and the ESMO MCBS – magnitude of clinical benefit scale) to define the value of new anticancer drugs based on the clinical trials leading to registration in the USA by FDA or in Europe by EMA [16, 17]. Both systems try to describe and score the health benefit found in each single clinical trial, accounting for the quality of the endpoint (survival being always favored versus progression-free survival), for the size of the effect (with different thresholds), for the eventual benefit in terms of quality of life, and for the amount and type of toxicity. The ASCO framework that is projected as a tool for patient information also plans to explicit which is the price of the drug and the amount of out-of-pocket expenses that will fall directly on the patients [16]. The ESMO MCBS, on the contrary, is projected as a tool to highlight to third-party payers (e.g., governmental agencies in most European countries) which drugs are so valid that every effort should be done to make them available to citizens, and does not consider drug price at all [17]. Unfortunately, the correlation between the health benefit measured with the two scales is lower than expected, and this might create some discrepancy in the definition of clinical relevance between the USA and Europe. Nevertheless, such scales represent the first attempts to develop quantitative or semiquantitative instruments to define the value of new drugs based on clinical trial results but going somehow beyond clinical trial methodology and introducing clinical relevance and affordability as important indicators.

16.6 Conclusions

Methodology of clinical trials in oncology has been profoundly challenged by the development of target-based agents and more recently by immune checkpoint inhibitors. Sample size, study design, selection criteria, study endpoints, and even the aim and the distinction of the phases are currently very different from the traditional program. In recent years, it has become crucial to distinguish drugs producing significant clinical improvements from those positively concluding a formally correct development program, but not producing relevant clinical progress. This attention to clinical relevance is important for design, conduction, and interpretation of clinical trials.

Key Points

- Traditional methodology of clinical trials in oncology has been profoundly challenged by the development of target-based agents and more recently by immune checkpoint inhibitors.
- Sample size, study design, selection criteria, study endpoints, and even the aim and the distinction of the phases are currently very different from the traditional program.
- In recent years, the concepts of treatment value and clinical relevance of results have gained growing importance among medical oncologists.

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Basic Principles of Bioinformatics for Next-Generation Sequencing Molecular Testing in Oncology

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Contents

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Learning Objectives

By the end of the chapter, the reader will:

- Have learned the meaning of bioinformatic pipeline for next-generation sequencing and its key steps
- Have learned the differences among the most important output of a pipeline
- Have reached the knowledge of variant annotation and guidelines helpful for clinical reporting

17.1 Introduction

Cancer is a complex class of diseases affecting the genome. Thus, which is a better way to study it if not through the comprehension of DNA complexity? Revolutionary technological advances have been made since the completion of the first genome sequencing to date. High-throughput technologies pose many steps forward since the identification of tumor suppressor genes and oncogenes to the uncovering of the genomic landscape of many tumors. In particular, the advent of next-generation sequencing (NGS) platforms in the first decade of 2000 made possible to shed light in the taxonomy of cancers. Nevertheless, many questions arise from deeper knowledge deriving from these advances, starting from technical issues, e.g., depth/breadth of sequencing, to biological interpretation, e.g., how to distinguish variants with pathological significance from biological neutral ones, and ethical problems, e.g., management of incidental findings.

Precision oncology and genome-driven clinical trials [1] are the direct consequences of the introduction of NGS in the routine laboratory activity. Moreover, we are now able to exploit the tumor heterogeneity and acquired tumor resistance. However, we are still far from the real patient-tailored therapies [2]. To gain such a knowledge, the creation of consortia, e.g., The Cancer Genome Atlas, with the aim of data sharing and the creation of bioinformatic algorithms able to handle and integrate such amount of data, are mandatory.

17.2 A Brief History of Sequencing: From Sanger to Third-Generation Sequencing Platforms

A step forward in molecular biology was the development in 1983 of polymerase chain reaction (PCR) by Kary Mullis, awarded with Nobel Prize in Chemistry in 1993. Such a method, which seems to be very far from the present technologies, is still fundamental in the new sequencing platforms. Indeed, Sanger DNA sequencing, also known as chain terminator sequencing, developed in 1997, relies on PCR. It was automated through the introduction of capillary electrophoresis and was considered the gold standard until almost the first decade of 2000 [3]. In the meantime, human genome project was launched in 1990, and it requires 13 years to complete the first almost-complete sequence of human genome. However, different technological advances started to be implemented. During 1996, the first NGS platform was developed, and in 2004 it was commercialized: Roche 454. Thus, the possibility to fully sequence an individual's genome at the cost of $1000 dollars was not considered so utopistic [4]. Roche 454 was just the beginning because since then, new platforms continued to be implemented with different chemistries, lowering, by late 2015, the cost to obtain a high-quality human genome to $1500 dollars.

To date, two major companies, Illumina and Thermo Fisher, are the vendor of the most important NGS platforms. Both of them are short-read sequencer producing reads shorter than 300 bp. Both Illumina protocols and Thermo Fisher ion semiconductor sequencing (Ion Torrent) are cheap sequencing methods extensively used in clinical laboratory. The last-born sequencer from Qiagen also produces short reads: 100–150 bp length. Two new platforms are available only for research purposes, also known as third-generation sequencing platforms. They are able to produce long reads. The PacBio SMRT (single-molecule real time) technology could sequence reads longer than 2.5 Kb, while the Oxford Nanopore Technologies MinION, through the use of single-stranded pore technology, is able to sequence molecules >10 Kb.

Despite the possibility of sequencing the whole genome or the whole exome, the most used approach in molecular testing is targeted gene panels, including a discrete number of genes both as coding regions and hotspots, that are very small regions to detect a single-specific mutation. Gene panels are cost-effective and allow to obtain data with very high depth. Whole genome/exome sequencing are not routinely used in laboratories being time-consuming and with still elevated costs. Moreover, they pose ethical problems regarding incidental findings and their management.

17.3 From Wet-to-Dry Methods

17.3.1 NGS Intrinsic Errors

NGS technologies lead to the spread of bioinformatic efforts to appropriately analyze and manage data. Indeed, a clear separation between wet phase, namely the bench procedures, and data analysis exists. However, to be able to be appropriate in such a purpose, it is man-

datory to deeply know intrinsic errors related to sequencing methods. All NGS technologies primary consist of preparing a "library," which is the creation of a collection of small fragments of DNA which in turn will be sequenced. During library preparation, the fragments of DNA are linked to molecular barcodes to perform multiple sample sequencing, PCR primers and linkers which binds molecules to surface where molecules have to be sequenced. Then library have to be enriched for targeted sequencing (e.g., gene panels or whole exome sequencing). Enrichment could be performed through sequence capture which uses hybridization to complementary sequences (capture-based approach) or by PCR (amplicon-based approach). After these steps, sequencing could be performed. Illumina (e.g., HiSeq, MiSeq, NextSeq) and Ion Torrent (e.g., IonPGM, IonProton, S5) have different chemistries and thus biases. In detail, each DNA fragment is immobilized to a flow cell for Illumina and to a bead for Ion Torrent in order to clonally amplify each fragment. Sequencing by synthesis is the methods of Illumina sequencer, which uses fluorescently labelled reversible terminator-bound dNTPs. At each step, before to be washed way, the fluorophore bound to the added base is illuminated by a laser. The issue regards the similar emission spectra of fluorophores of A and C as well as G and T (red and green light, respectively, and separated by filters). Moreover, phasing (incomplete 3′ terminator removal due to erroneous enzyme kinetics) and pre-phasing (the skipping of incorporation of 3′ terminator caused by too fast synthesis) are further problems, which makes miscalls the type of error typical of Illumina platforms.

Ion Torrent chemistry is related to variation of pH due to H+ release after base incorporation, sensed by a solid-state pH sensor. When a stretch of homopolymers has to be sequenced, it was observed that AA stretch corresponds to a twofold increase in the pH with respect to single A. AAA stretch reaches only 1.5-fold increase of AA stretch and so on. Thus, reduction of increase of pH changes with the increase of the number of bases in homopolymers stretch results in incorrectly called homopolymer regions.

17.3.2 Alignment and Coverage Evaluation

Notwithstanding all the issues depicted above, sequencing run ended and data have to be correctly analyzed (Fig. 17.1).

At the end of sequencing, raw data, namely the DNA short fragment amplified, are in the format of FASTQ files, which include not only the sequences of the fragments (reads) but also quality scores of each base. They are used to check quality of FASTQ files (e.g., FASTQC),

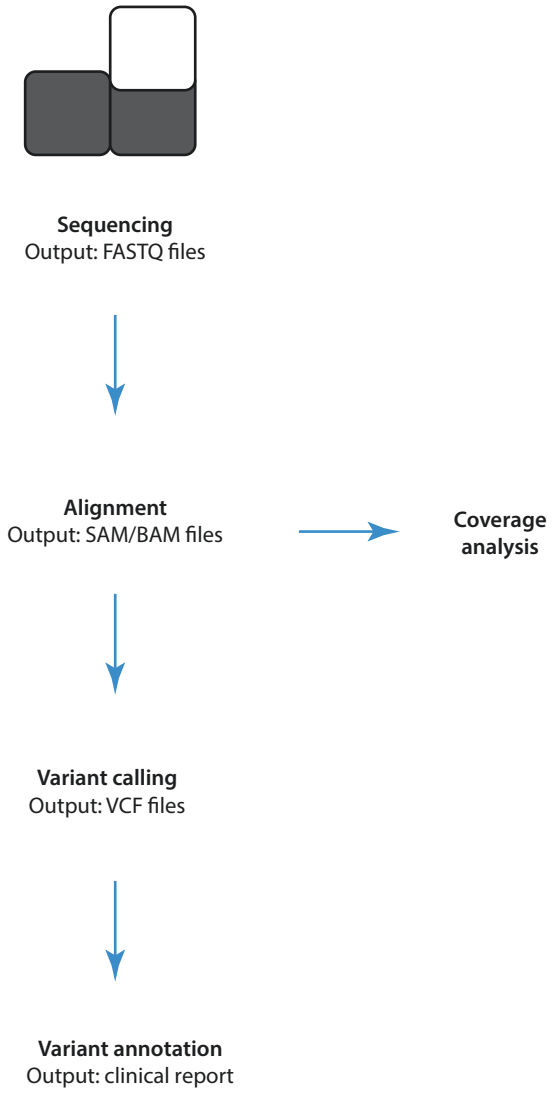

Fig. 17.1 Description of a typical bioinformatic pipeline for next-generation sequencing variant calling

which in turn could be trimmed to maintain only high-quality bases (e.g., Trimmomatic, CutAdapt). Trimming is a bioinformatic step which allows to cut low-quality bases.

A typical bioinformatic pipeline (namely, the series of steps to perform a bioinformatic analysis) includes the alignment of reads against a reference genome whose version has to be always specified to contextualize the genomic coordinate (e.g., hg19/Grch37). Different algorithms are used to perform this step. BWA [5] and Bowtie [6] are considered the best algorithms to manage short reads coming from Illumina platform. Ion Torrent has developed a "proprietary" aligner, TMAP, which is able to perform alignment for reads including also information of the flows that are the pH changes due to the incorporation of a specific base. The output is a SAM or BAM file.

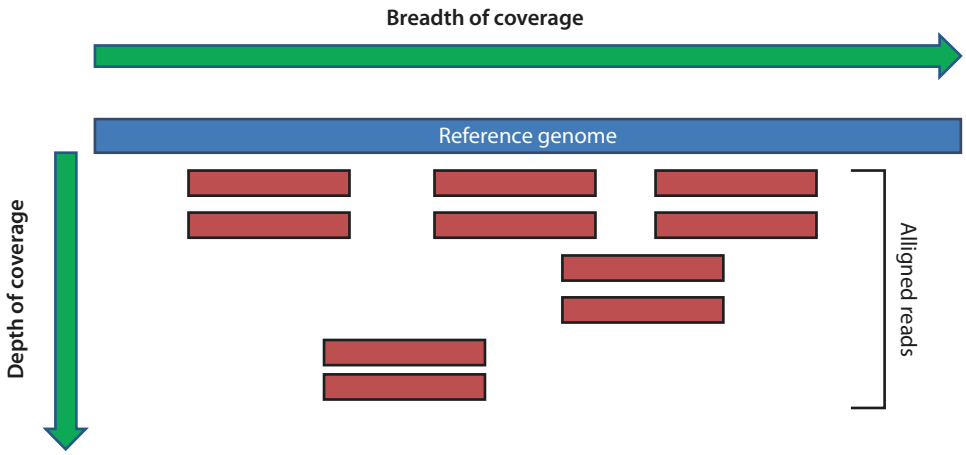

Fig. 17.2 Representation of the concept of breadth and depth of coverage

To evaluate the performance of a sequencing run to be confident on results, coverage should be checked. The term "coverage" often is misinterpreted. It is important to be able to distinguish two aspects: per-base coverage and breadth of coverage (Fig. 17.2).

Their definitions, as reported in ▶ http://www.metagenomics.wiki/, are:

"Per-base coverage is the average number of times a base of a genome is sequenced. The coverage depth of a genome is calculated as the number of bases of all short reads that match a genome divided by the length of this genome. It is often expressed as 1X, 2X, 3X,... (1, 2, or, 3 times coverage)."

"Breadth of coverage is the percentage of bases of a reference genome that are covered with a certain depth. For example: 90% of a genome is covered at 1X depth; and still 70% is covered at 5X depth."

Practically, in clinical reports coverage could be reported as average indicating percentage of targeted bases covered over the cutoff (e.g., average coverage panel of 2.5X with 99% of targeted bases covered >200X). For germline mutations, a coverage of 80X could be sufficient to confidently call variants; somatic alterations, often present at subclonal level, require higher coverage (at least 500X).

17.3.3 Variant Calling

The crucial step is variant calling, which is the identification of DNA alterations. Ion Torrent has an integrated plugin to call variants (Torrent Variant Caller). Regarding Illumina platforms, many variant caller algorithms have been implemented, as GATK HaplotypeCaller or VarScan2, each of them with different performances and with tunable options to gain confidence in variant calling process. In oncology testing, somatic variants, the so-called actionable variants, have to be reported to clinicians. Somatic alteration calling could be performed by the "tumor-normal" pipelines (e.g., MuTect, Strelka), referring to algorithms which analyze tumor samples coupled to germline control. In such a way, confounding factors related to the noise present in the germline samples are used to handle variants identified in tumor sample. Results coming from this type of approach are more reliable in particular regarding specificity.

The output is a variant call format (VCF) file, which includes not only the genomic coordinates and the type of called variants but also information about quality. In particular, variant read number, strand bias and variant allele frequency are important values to be taken into consideration when raw VCF variant filtering has to be performed to retain as much as possible true positive variants.

Variant reads are the number of reads supporting the presence of a variant. Generally, calls supported by fewer than five variant reads are typically considered to be likely false-positive calls.

Strand bias is a statistics measure of the deviation of the probability of a variant to be sequenced both on minus and plus strands. Higher values are associated with a probable sequencing artifact [7].

Variant allele frequency (VAF) is the number of reads linked to a variant divided by the overall coverage at the same locus. For germline testing, VAF is a measure of zygosity (50% VAF indicates heterozygous alterations, while 100% VAF is associated with homozygous alterations). For somatic testing, which is the most frequent in an oncology setting, VAFs are related to clonality that is the number of clones carrying a mutation. Somatic VAFs show a very high variability. For example,

in mutation related to resistance, e.g. EGFR T790M in non-small cell lung cancer patients, even very low VAF variants are reported in order to set a correct therapeutic approach.

Moreover, visual inspection of variants is allowed by the Integrative Genome Viewer [7], which works in a desktop-friendly manner (Fig. 17.3).

17.3.4 Variant Annotation

Filtered VCFs, containing as much as possible reliable variants, have to be annotated. Variant annotation, generally speaking, gives sense to the list of mutations present in VCF file in terms of biological impact in the transcription and translation of the gene. It is an impor-

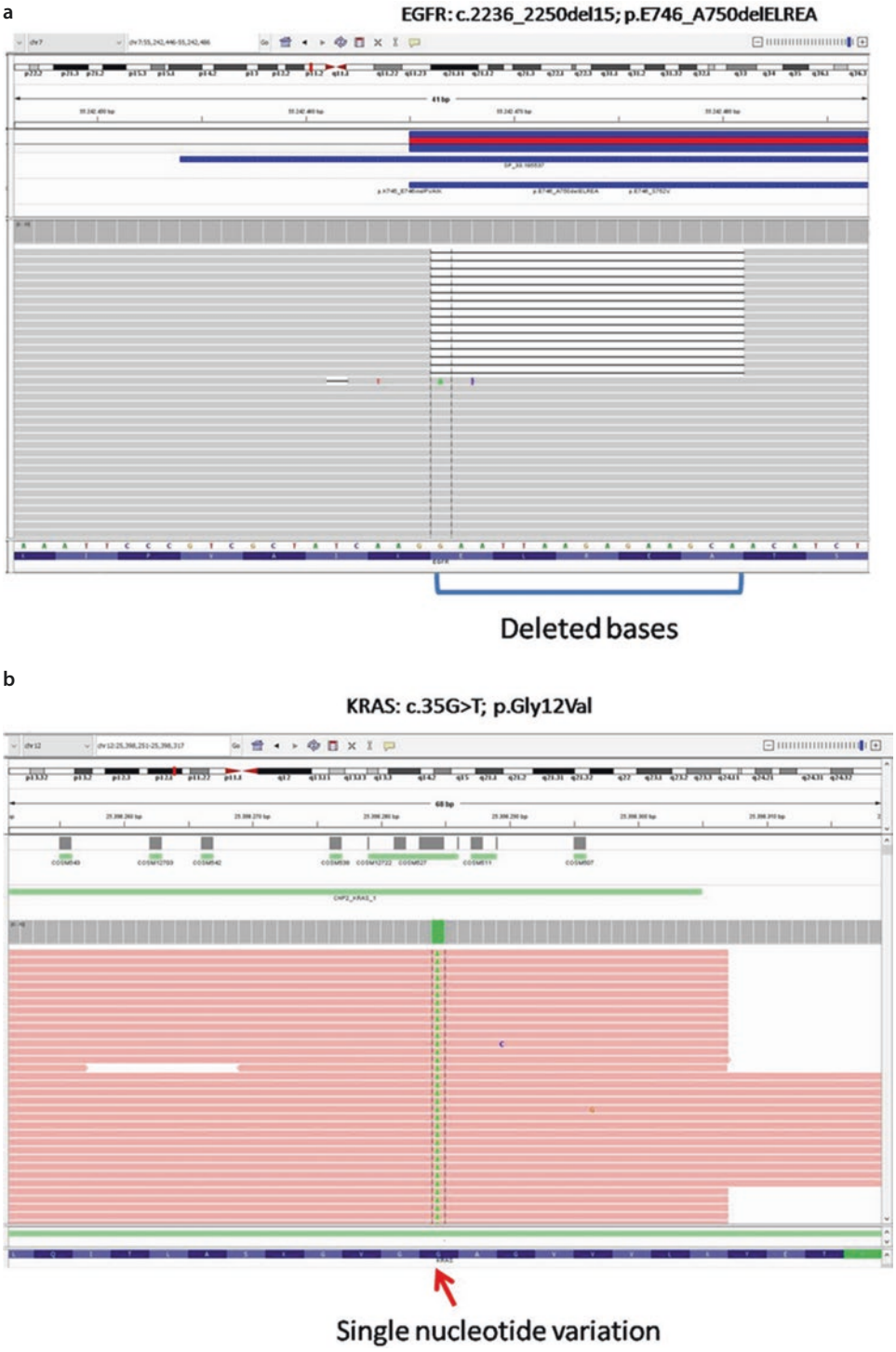

Fig. 17.3 Visual inspection of (**a**) a pathogenic deletion in EGFR gene indicating responsiveness to tyrosine kinase inhibitors in non-small cell lung cancer patients and (**b**) a pathogenic single-nucleotide variation in KRAS for patients affected by colorectal cancer which could benefit of targeted therapy

Table 17.1 Important databases used in variant annotation

Annotation databases		
Prediction databases	dbNSFP	► http://varianttools.sourceforge.net/Annotation/dbNSFP
Population databases	ExAC 1000genomes Exome sequencing project	► http://exac.broadinstitute.org/ ► http://www.internationalgenome.org/ ► http://evs.gs.washington.edu/EVS/
Oncology databases	COSMIC TCGA My cancer genome	► https://cancer.sanger.ac.uk/cosmic ► https://portal.gdc.cancer.gov/ ► https://www.mycancergenome.org/

tant step to filter germline variants and to retain only somatic ones when it is required to set a therapeutic strategy (e.g., KRAS alteration in codons 12, 13 and 61 in colon cancer; BRAF V600 alteration in melanoma, etc.) or for diagnostic or prognostic purpose. Indeed, in clinical setting tumor-normal pipelines, considering tumor and healthy genetic cell assessment in each individuous, generally could not be applied due to the lack of blood specimens. In detail, this step involves the use of several databases (Table 17.1):

- Database helpful in the prediction of deleteriousness of variants, including several in silico algorithms, e.g., SIFT and Polyphen. These tools allow to predict pathogenicity of a variants through the analysis of conserved amino acids in homologous proteins.
- Genetic population databases, reporting allele frequency of variants detected in general population or in specific-population, e.g., Caucasian. Population databases reports allele frequencies of alternative alleles in healthy individuals. In such a way, it could be possible to infer a biological impact because low frequencies could be associated to a pathology;
- Somatic databases, reporting allele frequency and, eventually, pathogenicity of cancer alterations. In such a way, it could be possible to know the penetrance of a somatic alteration in the onset of a malignancy.

17.3.5 CNV Detection

Detection of copy number variations (CNVs) is a clinical need for some malignancies (e.g., HERB2 amplification in breast cancer). NGS allows to detect CNVs, even if it is still challenging for amplicon-based panels. CNV calling requires algorithms different from tools used for variant calling. Generally laboratory confirms results through an alternative wet (e.g., MLPA) or bioinformatic method. Three main classes of method are at the basis of the algorithms.

- Depth of coverage method: bioinformatic tools detect increase or decrease of coverage in genomic region. The miscalling is due to the nonuniformity of coverage between samples or runs. The advantage is the possibility to identify large CNV (e.g., using EXCAVATOR2 tool [8]).
- Read pair analysis: this method requires paired-end sequencing and can detect only small CNV (e.g., using BreakDancer tool [9]).
- Split read: similarly, to read pair analysis, this analysis requires paired-end sequencing, but it is also able to detect breakpoint because it uses reads failing or partially failing to map (e.g., using Pindel tool [10]).

Ion Torrent platforms use a proprietary algorithm. The core of such a method is the creation of Variability Correction Informatics Baseline, including at least 48 samples. The baseline allows to perform correction on log2 ratio of amplicons. Moreover, baseline includes information about sex of samples (important for X chromosome because only a copy is present in male subjects) and tumor cellularity.

17.4 Liquid Biopsy

In 1869, the first evidence of circulating tumor cells in the blood of metastatic patients has been provided by Thomas Ashworth. Circulating tumor cells and cell-free DNA could be analyzed in all liquid compartment of the body (e.g., blood serum and plasma, urine, liquor, sputum, etc.). For diagnostic purpose, plasma is still the most used [11]. The concept underlying liquid biopsy is the monitoring of disease (e.g., minimal residual dis-

ease) and of the response to treatment (e.g., detection of resistance mutation T790M in EGFR gene to tyrosine kinase inhibitors in NSCLC patients) in a cost-effective and noninvasive fashion.

Cell-free DNA could be detected in many body fluids as a consequence of release from dying cells and circulating tumor DNA (ctDNA) is a part of the total amount, spanning from 0.01% to 90% in relation to stage of disease, tumor burden, and vascularity [12]. ctDNA could be deep sequenced with bias introduced during library preparation (e.g., 8-oxoG pairing with adenine and not citosine) and sequencing with 0.1–1% of miscalling depending of the platform used for NGS. Bioinformatic analyses are responsible in particular for false-positive calling in repetitive sequences, but the development of appropriate tools is overcoming such a problem.

The major issue is the very low allele frequency of alterations to be detected from experimental noise.

The bioinformatic pipelines are similar to those illustrated above, but some steps are performed by algorithms optimized for ctDNA (AfterQC [13], MrBam (► https://github.com/OpenGene/MrBam) and MutScan (► https://github.com/OpenGene/MutScan)).

In detail:

- AfterQC allows a better preprocessing of FASTQ files.
- MrBam improve supporting read number counting for mutations.
- MutScan is a visualization tool for interactive analysis.

Molecular barcoding sequencing [14] and CAPP-Seq [15] methods improve variant identification in ctDNA. Molecular barcodes (Unique Identifiers, UID, or Unique Molecular Identifier, UMI) are strings of complete random nucleotides, ligated to templates through ligation or through primers during PCR. Data analysis could be summarized into three steps:

1. UID extraction: the advantage of molecular tagging is the introduction of a fixed short sequence (five to seven nucleotides) between UID and DNA sequence, avoiding issues related to synthesis errors which could be responsible for alterations in the length of the barcode (FASTQ files).
2. Clustering of the reads with the same UID from BAM files.
3. Generation of a consensus read for each cluster and scoring of each position to call mutations.

CAPP-Seq is another approach for the detection of alterations in ctDNA. Basically, it is based on the definition of a "selector" from bioinformatic analyses of publicly available data to determine the most frequent mutations, ranked by their recurrence in samples. Selector is used to design biotinylated probes to reduce library to the region of interest. Then, variant calling is performed through different statistical approaches against the background of other ctDNA mutations through Bonferroni-adjusted Z-test.

17.5 Bioinformatic Pipeline Validation

Validation of an NGS process is critical because it involves both the wet methods and the subsequent bioinformatic analyses. Validation of wet procedures could be performed through the use of other laboratory techniques (e.g., Sanger sequencing, fluorescent-based method, or droplet digital PCR) or by samples with known genotype. Institutions as the National Institute of Standards and Technology (NIST) are able to certify reference standards. Their main feature is commutability, which is the "ability of a reference standard to perform comparably to treated samples" [16] in library preparation, sequencing, and analysis (e.g., FFPE samples could reduce commutability of a reference). In one established reference standards, the uncertainty could be established from the differences from the expected and the observed values. NA12878, that is the genome of a healthy European female, is one of the most used reference standard in many clinical laboratories. In detail, reference standards offer a "truth set" to evaluate NGS performance in terms of sensitivity, specificity, accuracy, and precision. Bioinformatic analyses are also a complex step to be evaluated and validated, and they require also a "ground truth." Generally, in silico datasets are generated through several available tools. FASTQ or BAM files could be easily created, providing not only datasets with known genotype but also with profile error of the used platform, and some algorithms could simulate heterogeneity in tumor samples. Of note, simulated datasets are able to validate bioinformatic step, but they could not replace the complexity of real samples and could not control wet phase of NGS testing.

17.6 Variant Interpretation and Clinical Reporting of Bioinformatic-Related Information

Clinical interpretation of variants is the most important step in the workflow of a molecular testing, even if it could be time-consuming due to the difficulty in its automation. Due to the large use of multigene targeted

sequencing panels, many variants could be detected and the reporting of results is not so simple. Variants need to be prioritized and logically interpreted in a clinical sense. For instance, it could be possible to identify variants which could be targeted by a drug not specific for the malignancy under evaluation or mutations whose consequences do not fit with the mechanism of action of a drug.

Regarding germline variants, the American College of Medical Genetics and Genomics, the Association of Molecular Pathologists, and the College of American Pathologists [16] wrote guidelines to assign clinical relevance of variants combining different approaches. Criteria include minor allele frequency reported in databases, frequency in affected individuals, prediction of the effect of the mutations, segregation, and inheritance information. Population-specific minor allele frequency (e.g., European-specific minor allele frequency) is another important factor to be taken into consideration. Minor allele frequency is the frequency observed in healthy population of the alternative allele. Population database reports data from almost 12,000 individuals, generally not affected by severe diseases; thus rare variants have great probability to have been detected and then reported. It is clear that without automation, following these recommendations could be influenced by operators. Indeed, it has been measured that the application of these guidelines to the same group of alterations reached 71% of consensus between different laboratories.

Similarly, in 2017 guidelines for somatic alterations have been drafted by American Society of Clinical Oncology, Association for Molecular Pathology, and College of American Pathologists (ASCO/AMP/CAP) [17]. They suggested to group variants into four categories based on four levels of evidence (◘ Table 17.2). Guidelines are helpful to better know a variant comparing them with knowledge-based databases, even if a deep know-how is requested to be able to manage this particular step. To date, many research groups and companies focused on the implementation of knowledge-based databases (e.g., OncoKB). Generally, they are developed by a group of specialists, from molecular biologists to clinicians, also known as curator, that "enrich" variants with information. Curators link to a variant several levels of information regarding the biology of the gene, the prediction of pathogenicity, and all detail regarding its involvement in prognosis and/or therapeutic approach.

ASCO/AMP/CAP guidelines recommend to report methodology details in the report, such as limit of detection and minimal coverage. Moreover, sequenced genomic regions (e.g., full gene or codon position) have to be clearly specified at the end of the clinical report.

More specific recommendations regarding bioinformatic analyses should be drafted, and, to date, many tools are available and under development. Thus, it would require more time to gain a consensus on bioinformatic algorithms.

17.7 Reproducibility in Bioinformatics

Given the acquired importance of bioinformatics in the last years, issues related to reproducibility regard also the analysis steps. The presence of several algorithms, each of them with different versions, to perform each part of a bioinformatic pipeline, changes in the library used to compile and install packages are responsible for such an issue. Moreover, it has been observed that only 10 papers out of 50 selected reported BWA parameters used to perform alignment, and only 11% of studies made available source code and data of simulated datasets.

Sandve [18] proposed ten rules for good practice in bioinformatic analyses (◘ Table 17.3). Many solutions are available to deal with reproducibility. For instance, Galaxy (▶ https://galaxyproject.org/) are cloud solutions that do not completely fulfill the suggested rules because pipelines are not customizable and privacy and ethical issues exist. To date the most promising approach is the container technology that is the virtualization, the so-called image of a bioinformatic pipeline. Softwares and dependencies are packed together avoiding problems related to versions and upgrading of operating system. To date, Docker (▶ http://www.docker.com) is considered the best environment to fit the rules of good practice. However, the use of such environment requires programming skills to be able to customize bioinformatic workflows. To make it easier, recently the Reproducible Bioinformatics Project (▶ http://reproducible-bioinformatics.org) has been proposed not only for the distribution of docker images but also for the implementation of a framework to build up pipelines fulfilling the ten rules.

Thus, many efforts to make reproducible bioinformatics are being made, and, at the very first instance, bioinformaticians have to be clear in the description of the workflow to allow other scientist to reproduce their results.

Table 17.2 Categories identified by ASCO/AMP/CAP guidelines and their levels of evidence useful for clinical report of variants

Categories	Evidence	Therapy	Diagnosis	Prognosis
Tier I: Variants of strong clinical significance	Level A	1. Biomarkers that predict response to FDA-approved treatments 2. Biomarkers included in professional guidelines that predict response or resistance to therapies for a specific type of tumor	Biomarkers included in professional guidelines as diagnostic for a specific type of tumor	Biomarkers included in professional guidelines as prognostic for a specific type of tumor
	Level B	Biomarkers that predict response or resistance to therapies for a specific type of tumor based on well-powered studies with consensus from experts in the field	Biomarkers of diagnostic significance for a specific type of tumor based on well-powered studies with consensus from experts in the field	Biomarkers of prognostic significance for a specific type of tumor based on well-powered studies with consensus from experts in the field
Tier II: Variants of potential clinical significance	Level C	1. Biomarkers that predict response or resistance to therapies approved by the FDA or professional societies for a different type of tumor 2. Biomarkers that serve as inclusion criteria for clinical trials 2. Biomarkers that serve as inclusion criteria for clinical trials	Biomarkers of diagnostic significance based on the results of multiple small studies	Biomarkers of prognostic significance based on the results of multiple small studies
	Level D	Biomarkers that show plausible therapeutic significance based on preclinical studies	Biomarkers that may assist disease diagnosis themselves or along with other biomarkers based on small studies or a few case reports	Biomarkers that may assist disease prognosis themselves or along with other biomarkers based on small studies or a few case reports
Tier III: Variants of unknown clinical significance	Not observed a significant allele frequency in population databases or pan-cancer/ tumor-specific databases			
Tier IV: Benign or likely benign variants	Observed at high allele frequency in population and no significant association with cancer			

Table 17.3 List of the ten rules suggested by Sandve et al. [18] for good practice in bioinformatic analyses

	Ten rules for reproducibility	
1	For every result keep track of how it was produced	Almost records of programs, version, and parameters to reproduce analyses
2	Avoid manual data manipulation steps	Manual manipulation if data is error-prone, thus it is preferable to use specific commands
3	Archive the exact versions of all external programs used	Different versions of the same program could not output the same results; thus version has to be recorded
4	Version controls all custom scripts	Workflow and modification of analysis steps have to be recorded
5	Record all intermediate results, when possible in standardized formats	Intermediate results allow to re-run an analysis step, and debugging could be simplified
6	For analyses that include randomness, note underlying random seeds	Some prediction analysis or simulations require the inclusion of a quote of causality. To be able to reproduce results, recording of the seed number is a very good practice
7	Always store raw data beyond plots	Plots summarize results, and data used to generate them have to be always stored
8	Generate hierarchical analysis output, allowing layers of increasing detail to be inspected	Plots or table is summarized results, but the presence of HTML links, for example, leading to data underlying results could be appropriate
9	Connect textual statements with underlying results	Statements result from an interpretation; thus to include link to analysis help reproducibility
10	Provide public access of scripts, runs, and results	It is a good practice to make available executables used to run an analysis workflow

17.8 Conclusions

NGS approaches posed a step forward into the deep knowledge of the human genome. The assessment of the presence of specific alterations is widely applied in the oncological clinical settings. The use of multigenic panel is both time- and cost-effective. Thus, the field of clinical bioinformatics is going to have a widespread diffusion. Data analysis is now considered the dry phase of an experimental protocol because of the importance to correctly tune parameters linked to sequencing data. A pipeline could be considered as validated not only when is the best "combination" when compared to "ground truth" but also when it is reproducible. In conclusion, there is an urgency to draw shared and unique good practices to grant "true" and reproducible results.

Key Points

- The knowledge of the intrinsic bias of the used NGS platform is the first step to perform a correct data analysis.
- Quality check of data, alignment, variant calling and variant annotation are the key steps of a variant calling pipeline.
- Pipeline has to be validated through "ground truth," which could be a simulated dataset or samples with known mutational status.
- Variant interpretation is the last step involving the use of specific databases and specific rules for clinical reporting.
- Reproducibility is an important issue in bioinformatics and there is an effort to grant it.

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New Drugs Development

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Contents

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Given the subject of medical oncology, new anticancer drug development dictates how this discipline makes its progress. However, one should always look at anticancer agents in the context of anticancer treatment as a whole. In fact, any anticancer drug, or drug regimen, will be used within a treatment strategy in a clinical presentation. Even in the advanced disease setting, but particularly if the disease is localized, cancer treatment is often multimodal. Conceptually, therefore, even the choice to treat a patient exclusively with medical therapy should always be made on a multidisciplinary basis [1]. Thus, also the development of any drug, or regimen, should be viewed under a multidisciplinary perspective. From the research viewpoint, this implies that the development of new anticancer agents should always factor the treatment strategy of the diseases they are aimed to treat, taking into account unmet clinical needs. In other words, ideally and as long as this is possible in the current drug market environment, strategies of innovation should look at the diseases, prioritizing unmet clinical needs. Drug development does not end with the regulatory approval. In this sense, if the early phases of drug development are inevitably driven by the industry, hopefully in collaboration with the academia as a proactive partner, its late stages, also beyond approval, are part of the mission of the academia to innovate cancer treatment. This should take place in partnership with patient advocacy groups, as an important component of the disease-based communities. In fact, it is important that the full disease perspective, in its clinical as well as patient-reported dimensions, is fully incorporated in clinical research on anticancer drugs. At a time of precision oncology, some anticancer drugs may be developed, and approved by regulatory bodies, in a pathologically "agnostic" fashion, i.e., looking at biomolecular markers across all neoplasms [2, 3]. However, it is important to be aware that the positioning of such drugs in the clinic will inevitably be different depending on the neoplasm. Also a pathologically agnostic approach to anticancer drug development needs to factor the disease.

As from when the first randomized clinical trial was published in 1948, clinical research in contemporary medicine is based on clinical trials [4]. In current evidence-based medicine, they are viewed as an essential tool to generate evidence about efficacy of new treatments in medicine, such as anticancer drugs [5, 6]. Clinical trials are experiments on new treatments performed in human subjects, with the aim of providing evidence of efficacy (including safety). Basically, they try to transfer the logic of the scientific experiment to the clinical world. This implies that the setting of a clinical trial is somewhat "ideal," since the conditions of the experiment will be set to optimize its chances to catch the best efficacy of a new treatment. This has to do, say, with patient selection but also with quality of care. This is sometimes recalled by contrasting "efficacy" in clinical trials and "effectiveness" in clinical practice [7]. While efficacy refers to the ideal setting of clinical trials and is thus studied by "clinical research," effectiveness refers to how new treatments are actually implemented in clinical practice and is studied by "outcome research." One should always be aware that effectiveness may correspond to worse outcomes than those observed in clinical trials. On a population basis, epidemiological research, namely, through cancer registries, looks at possible survival discrepancies among systems, which can partially depend on inequalities in quality of care. Recently, in a more clinical perspective, an area of research is exploring the value of "real-world data," i.e., data referring to real practice [8]. Of course, it is important to understand the different scopes of such different fields of research in medicine. While clinical studies tend to pursue innovation, such as the development of a new anticancer agent, or regimen, real-world evidence may explore how the real-world clinical setting is discrepant from the ideal setting in which efficacy was studied, how new technologies are transferred into clinical practice, how they perform in terms of cost/effectiveness.

It is also important to realize that "evidence" is not just about clinical trials. The whole body of biological and pathological knowledge of medicine is evidence. As a matter of fact, any clinical decision must incorporate several pieces of evidence, not only clinical trials. Then, any clinical decision should be shaped by sharing it with the patient. The output of shared clinical decision-making is obviously much more complex than a therapeutic proposal would be on which the patient is merely requested to provide an informed consent or dissent. Shared decision-making implies to go beyond informed consent: it is the clinical decision which is modelled around the single patient. If precision medicine tries to maximize the contribution of molecular biology in today's medicine, personalized medicine is all about valuing not only molecular biology but also patient's personal values in clinical decision-making [2, 9]. In the highly regulated field of anticancer drugs, one would hope that there remains room for individualizing their use at the patient's bedside. Thus, clinical development of new agents should also provide data allowing this kind of personalization of their use at the patient's bedside. Evidence-based medicine should always be viewed as the encounter between evidence of efficacy, the clinical factors pertaining to the single case, and patient's values and choices [5, 6].

Conceptually, a clinical study should be first conceived in regard to its objectives and the hypotheses that it is aimed to test. Then, the most appropriate study design should be selected, along with the study endpoints. A statistical plan should be worked out, also addressing the problem of sample size, i.e., the number

of patients to include in the study. Eventually, a clinical study protocol will be finalized. The scientific soundness, the practical feasibility, and the ethical acceptability of the study will be reviewed by independent committees, so that the patient rights are fully respected, also correcting potential biases on the researcher's side. In any case, the patient will have the right to consent to entering the study or not, based on an informed consent that needs to be independently reviewed as well. If the patient does not accept to enter a study, he/she will be followed by the institution that proposed the study to the best of available standard treatments.

As outlined in the chapter about "Clinical trials and methodology of cancer research," clinical studies developing new anticancer drugs are traditionally labelled as Phase I, II, and III. In essence, following its proper preclinical development, a new agent will be the subject of at least a Phase I study having the aim to define its dose and best regimen of administration, given its toxicity profile in human beings. Its classical end-points are thus the maximum tolerated dose (MTD) and the toxicity. In the end, MTD is a concept that in oncology is linked to the principle of a direct correlation between dose and efficacy. With cytotoxic drugs, such as typical alkylating agents, the higher the dose, the higher is the effect. This is alluded to with the expression "the more is better." MTD is thus established in Phase I studies by stepwisely increasing the dose of the drug in cohorts of study patients. When a degree of toxicity pre-specified in the protocol (the dose-limiting toxicity) is reached, the MTD is set. Once established the MTD, Phase II studies are aimed at exploring the antitumor "activity" of the new agent, generally in distinct patient populations, as defined, say, by single cancer entities, contrary to typical Phase I studies. Their classical end-point is tumor response. Traditionally, this corresponds to a degree of shrinkage in size of cancer lesions. Obviously, one will continue to study toxicity. Phase II studies are typically not randomized, though randomization may be foreseen, with a non-comparative or a comparative aim. Finally, Phase III trials are aimed to study the "efficacy" of drugs. By definition, this is described in terms of survival and/or quality of life. Classically, Phase III studies are randomized trials, testing the superiority of a drug, or a regimen, over the standard, or its non-inferiority (e.g., when the toxicity profile is expected to be better). This means that enrolled patients are allocated randomly, i.e., by chance, to one of the study arms, typically one corresponding to the experimental drug, or regimen, and the other to the standard drug, or regimen. Randomization is aimed at treating the possible systematic error in efficacy assessment that could be in place if, say, the researcher decided on his/her own which patients are due to receive the experimental or the investigational treatment, with a possible selection bias therefrom. Sometimes, studies are "blinded," if the patient is not made aware of which treatment is being received or both the patient and the physician are (double-blinded trials). This is understandable when the study end-points are such that a placebo effect cannot be ruled out, though the use of the placebo in oncology may be more questionable when it is just aimed at limiting possible clinicians' biases in assessing the treatment effect (e.g., radiologically). If the systematic errors are under control, then the random error can be addressed through statistical tests. Classically, these will assess whether, say, a difference in the end-points is statistically significant or not. Conceptually, a difference will be statistically significant if the chances of seeing it, or a more extreme one, under the hypothesis that a difference does not exist (the "null hypothesis"), were below a threshold pre-specified in the study protocol. Generally, this threshold is set to 5%. A "*P* value" <0.05 just means that, given the study results, probability was lower than 5%. Probabilistically, this will mean rejecting the null hypothesis and accepting the alternative hypothesis of some efficacy of the new treatment [10, 11]. The study sample size will have been planned at the time the study was designed to optimize chances of avoiding a false positive as well as a false negative result. This follows a statistical approach that is known as "frequentist" and may be contrasted with "Bayesian" approaches [12]. The latter could provide a probability distribution of the outcomes of interest, while frequentist approaches can just provide the statistical significance of observed outcomes and their "confidence intervals," but not probability distributions. In other words, frequentist medical statistics does not explicitly provide the physician with the probabilities of outcomes. The P value is the probability of observing the observed difference if the null hypothesis were true, not the probability that the alternative hypothesis is true. Bayesian approaches could provide such a probability, but the Bayes theorem implies that prior probability distributions should then be factored. This would mean "weighing" the study results with data external to the study, with a degree of subjectivity and thus of variability. In any case, it is important to realize that statistical significance depends on the magnitude of benefit but also on the sample size. A small difference, possibly negligible from the clinical point of view, may be statistically significant if the sample size is big, and a big difference may be non-statistically significant if the sample size is small. The clinician should always look at the magnitude of the observed clinical benefit, aside from the P value, and should always wonder whether the absence of statistical significance is just the result of an "inconclusive," rather than an actually "negative," study.

Such classical approaches to Phase I, II, and III studies are increasingly challenged by the evolving characteristics of anticancer agents. In the Phase I setting, one needs to factor that molecularly targeted drugs or immune agents do not necessarily need to be administered at the highest dose possible in order to exert their antitumor activity. This implies that reaching MTD may not necessarily be the objective and also that dose optimization will hardly be independent of assessment of activity or at least of an impact over a biomarker in terms of pharmacodynamics (e.g., measuring how the drug hits its molecular target, etc.). This may give rise to strategies to "enrich" a Phase I study with populations selected by a predictive factor of activity, possibly with designs combining Phase I with Phase II properties ("seamless" clinical studies). All this is very important under the perspective of patients entering Phase I studies. In fact, the side effects of anticancer agents are such that Phase I studies in oncology are only carried out in advanced cancer patients, definitely not healthy volunteers, as feasible in other medical areas. Given the low doses employed in the first cohorts of patients entering a classical Phase I study, a therapeutic potential may be lacking for them almost by definition. Though Phase I patients are informed about the study objectives, it is clear that therapeutic expectations are always in place. Thus, the current evolution of Phase I studies on new anticancer agents may also limit such difficulties, with their ethical and psychological implications.

In the Phase II setting, molecularly targeted drugs and immune agents, but sometimes also cytotoxic drugs, may not necessarily produce responses in terms of tumor shrinkage. Sometimes, or at least at the beginning of therapy, changes in the characteristics of lesions on medical imaging, reflecting corresponding histopathologic or functional changes of the tumor, make up the tumor response much better than shrinkage. Sometimes, tumor size may even increase, at least temporarily ("pseudoprogression"). Even more importantly, the separation of responding patients from non-respondents is artificial as long as it is based on a purely conventional threshold, and this is the case with currently employed tumor response criteria. Typically, they set such a threshold to a 30% decrease in the longest diameter of a lesion, that is to say to 50% in a corresponding regular area, which approximately means a 60–70% decrease of an ideally regular tumor volume. This does not have any biological or clinical meaning and, historically, was established essentially with a view to reproducibility concerns. Today, therefore, all degrees of variations in size are increasingly presented in study reports. Though there may be obvious reproducibility limitations, this provides a better idea of the spectrum of impacts of a drug, or regimen, in the whole patient population. Finally, it is clear that any tumor response will have a very limited clinical meaning if its duration is low. Thus, duration of response and the time to progression of responding and non-responding patients, up to progression-free survival, have become relevant endpoints of Phase II studies. Concomitantly, difficulties in accruing high numbers of patients in clinical trials, for example, in rare cancers or in small subsets of common cancer populations based on predictive biomarkers, may result in a number of clinical trials with a non-randomized design, even though they have to do more with efficacy than activity, thus with survival more than tumor response as end-points. Since classically Phase III trials have been conceived as being randomized, such non-randomized trials are often labelled as Phase II. The main difficulty is that we lack well-established methodologies for efficacy comparisons with controls that are external to a trial, not internal, as it happens when a trial is randomized.

This has to do with the general problem of "small-population" studies, i.e., clinical studies carried out in limited patient populations. They can be such in two senses. First, they may be defined, conceptually, by a biomarker, i.e., a biological predictive factor that may underlie a higher probability of efficacy of a given drug or regimen. This may well be, say, the expression of a tumor receptor liable to be hit by a molecularly targeted agent. Since the biomarker will be expressed just in a proportion of patients suffering from a given neoplasm, only a proportion of patients with that neoplasm will be eligible for clinical studies. The logic of these studies contrasts with the logic of the so-called "large and simple" clinical trials. The latter are randomized clinical trials performed in large populations of unselected patients, to provide a proof in principle of the efficacy of a treatment intervention. They reflect a notion of clinical research as trying to assess the value of new technologies in real conditions. In a sense and to some extent, they tend to offset the conceptual gap between clinical and outcome research. On the contrary, small-population trials reflect the ambitions of precision oncology, i.e., a kind of medicine revolutionized by the potential of molecular biology to individualize cancer treatment. It is clear that, if precision oncology keeps its promises, the efficacy in these small populations should be maximized, such that the sample sizes to demonstrate efficacy with a reasonable statistical precision will be lower than in trials addressing unselected populations. Thus, in a sense, while it is true that precision medicine carries the statistical difficulties of small populations, the magnitude of benefit may partially offset such difficulties.

Conversely, the other kind of small populations refers to "rare cancers." These are malignancies whose

incidence is low. They are examples of "rare diseases," often defined upon a threshold in prevalence, such as 50/100,000. Rare cancers are better defined upon incidence, and a reasonable threshold thereof may be placed around 6/100,000/year [13]. While the former threshold in prevalence would consider rare an even higher number of cancer cases, the latter threshold in incidence regards as rare a proportion of all new cancer cases as high as 10–20%. This means that rare cancers are a big problem, also quantitatively. In other words, the incidence of each rare cancer is low, but collectively rare cancers are far from being rare. They pose formidable challenges in quality of care, since the clinical expertise will not be easily accessible in the community. Conceptually, rare cancer patients should be either referred to centers of excellence specializing in their care or treated within reference networks sharing the expertise of centers of excellence. In the EU, European Reference Networks are currently trying to pursue the idea of networking in rare diseases, including rare cancers [14]. With regard to clinical research, clearly rare cancers pose all problems of small populations. Since they are defined by their pathologic partitioning, not by predictive biomarkers, it is clear that the magnitude of benefit of new agents is not necessarily high. Thus, sample size in clinical studies is undoubtedly a limiting factor in terms of statistical precision. In other words, it will be more difficult to carry out clinical studies in rare cancers as compared to common cancers. This difficulty may be partially overcome by innovative solutions [15]. Some may be organizational, enabling wide collaborations on clinical trials. However, quality of care may become an issue if large collaborations are pursued in rare diseases. Methodologically, surrogate end-points might amplify the benefit, thus reducing sample size, but their validation is problematic, all the more when numbers are low. Clinical studies may be underpowered, relaxing the type I and II errors. Bayesian approaches may be exploited to value more the whole available evidence, by incorporating prior probabilities in the evaluation of new results in a study. Given the low numbers, uncontrolled studies may be planned having efficacy, not just activity, end-points while using external comparisons (though, conceptually, the decision to randomize is independent of the planned sample size). In other words, there are ongoing efforts to work out convincing methodologies to allow to generate evidence also in rare cancers. These may well become models also for precision medicine in general. However, one should always factor that a higher degree of uncertainty needs to be accepted in rare diseases, as long as one wants not to discriminate against rare disease patients just because of the rarity of their conditions. This should also be acknowledged under the regulatory and reimbursement perspective. Under the perspective of clinical decision-making, uncertainty should always be shared with the patient. This is a way to effectively deal with the extra uncertainty implied by a rare disease, getting to a rational, shared clinical decision all the same.

Furthermore, it is hard, and unethical for patients entering it, to plan a large clinical trial without stopping rules, for superiority or inferiority, depending on interim analyses or results gathered meanwhile by other studies. Bayesian solutions are increasingly incorporated in clinical trials, though the frequentist framework is generally preserved. Surrogate end-points for survival and quality of life, such as progression-free survival, are increasingly used as evidence of efficacy, at least temporarily, to change clinical practice and to allow approval of new drugs in due time. In fact, waiting for survival differences after analyzing all patients planned by the statistical protocol of a large trial may be poorly feasible in practice, given the evolution of health technologies. To some extent, this reflects the fact that clinicians, and patients, cannot refrain from conceiving that there is a probability of efficacy of any new treatment, which depends on the whole evidence provided at any given time. Conceptually, this is a Bayesian probability that, as said, is not provided by current medical statistics.

All this relates to the currently changing landscape of clinical research on new anticancer agents, affecting both its strategic planning and its methodology. On top of that, today, artificial intelligence is increasingly employed in medicine [16]. Its most obvious use is to support clinical decision-making, by assisting, emulating, and possibly substituting, some medical skills. Expert systems may increasingly assist the physician in the diagnostic workup of patients as well as in the therapeutic decision-making process. However, there is another area of artificial intelligence that conceptually implies to gain new medical knowledge from the employment of "machine learning." This allows to process "big data," which may refer to genomics, and the like, but also to the huge amounts of clinical information that can be collected through the interoperability of electronic health records. Conceptually, there is an inherent diversity between the hypothesis-driven logic of clinical trials and the data-driven logic of machine learning [17]. There are big methodological differences between the two and big methodological challenges in their possible tension in contemporary medicine. In fact, this is an area of major changes in contemporary medicine, while, as said, the methodologies of classical clinical trials are evolving as well.

The evidence generated on new anticancer agents underlies the process of drug approval by regulatory bodies, i.e., those agencies appointed to regulate the drug market. Conceptually, regulation is based on the

assessment of a risk/benefit ratio [18]. In other words, regulatory bodies weigh the toxicities and the risks of any new agent with its efficacy. Data thereon come from clinical trials. Of course, the final judgment is based on the whole available evidence, with a degree of inevitable subjectivity. Then, the drug is made available in the health market, with discrepancies depending on the health system. In European health systems, public reimbursement choices need to be made once approval has been granted. This process is also known as "health technology assessment" (HTA). Such decisions are based on a different criterion that clearly cannot overlook costs, i.e., the cost/effectiveness ratio [19]. When these decisions are made explicitly, thresholds in cost/effectiveness may be selected, such that treatments below these thresholds are reimbursed and vice versa. A threshold in marginal cost/effectiveness of a new treatment may lie, say, in the range of 50,000 Euros per quality-adjusted life year (QALY) gained. Again, effectiveness is based on the available evidence of efficacy generated by clinical studies and possibly effectiveness explored through real-world data. In any case, in the European Union this means that a new anticancer agent approved by the European regulatory body, the *European Medicines Agency* (EMA), may be reimbursed by some national or regional health systems and not by others [20]. This gives rise to discrepancies in patient access to new drugs across countries, even across close countries.

Approval and reimbursement do not imply that a drug "should" be used in the single patient. Of course, the final decision rests with the clinician, preferably, in cancer, within a multidisciplinary cancer team, namely, a disease-based multidisciplinary tumor board. Looking at the "average patient," disease-based clinical recommendations may be conveyed through the so-called clinical practice guidelines. These are systematic guidances worked out by a given medical community to set universal standards of care [21]. Such guidances are the results of consensus processes and explicitly refer to the quality of evidence on which they are based: they are "consensus-based" and "evidence-based." Health systems, or health administrations, may translate them into "managed care patient pathways," or "diagnostic-therapeutic pathways," that take into account also available resources, in order to optimize the patient journey in the real world within a given health environment [22].

As said above, the final clinical decision should be personalized as much as possible. In the end, the doctrine of "evidence-based medicine" states that any clinical decision should be based on evidence, taking into account individual patient's characteristics, and shared with the patient on the basis of his/her values and choices [6]. Unfortunately, constraints in the access to drugs may narrow the scope of medical decision-making. If this is the case, the patient should be properly informed. As also said, the choice to use any medical therapy should always be made in a multidisciplinary manner, considering also non-medical alternative options. In any case, statistical significance should not be the only determinant of any clinical decision. In particular, the magnitude of clinical benefit should be always factored properly. As recalled in the chapter about "Clinical trials and the methodology of cancer research," scales have been devised in an attempt to categorize the magnitude of benefit provided by new anticancer agents [23]. These may be regarded as tools to prioritize the access to drugs in conditions of limited resources. Again, under the perspective of clinical decision-making, it is always important to wonder how much the "average patient" of the trial is superimposable to the patient one is treating. Prognostic and predictive factors pertaining to the single patient may well be weighed when translating the results of a trial into a personalized clinical decision. Here there is probably room for innovation through artificial intelligence tools. There is also room for methodological innovation, as it has been tried for decades in the framework of decision theory [24]. In any case, data from clinical studies are just pieces of evidence that the physician must incorporate into a personalized clinical decision. Regulatory and reimbursement constraints should just set explicit limits, within which there should be enough room to get to personalized clinical decisions, thereby optimizing the use of developed drugs in single patients.

Key Points

- Phase I, II, and III clinical studies on new anticancer agents, and regimens, are evolving, within the ongoing transformation of evidence-based medicine, at a time of precision and personalized medicine.
- Clinical research in small populations, such as in rare cancers, face special challenges, and methodological efforts are underway to provide solutions thereto.
- Clinical decision-making in cancer patients should always be viewed as multidisciplinary, and thus also the development of new anticancer drugs, and regimens, even when pathologically "agnostic," should always be conceived within a multidisciplinary approach to cancer entities.

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Management of Adverse Effects and Post Treatment Considerations

Contents

Treatment Toxicity

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Learning Objectives

- Identify the most common adverse events for approved anticancer therapies and immunotherapy.
- Review the recommended management approaches in the different organs and systems.
- Outline the signs and symptoms related to the different toxicities and the factors that may increase the risk for adverse events.

19.1 Introduction

Cancer therapy has always been a challenging focus in basic, translational and clinical research.

Over the last decades, there has been a considerable increase in the number of patients receiving anticancer chemotherapy, and novel, high-efficacy antiblastic drugs targeting various steps of cancer cell proliferation have been developed [1].

The target of cytotoxic drugs is rapidly proliferating tumour cells. Classically, cancer chemotherapy has been administered intravenously in a cyclic schedule; however, oral formulations have resulted in an important breakthrough, changing the landscape of treatment options and improving patient compliance and quality of life.

Side effects sometimes occur in rapidly proliferating tissues, such as haematopoietic, gastrointestinal mucosal, and skin tissues and their annexes. In addition, organ-specific toxicities can appear.

A significant heterogeneity in tumour response and toxicities is observed with most chemotherapeutic agents. Studies of pharmacogenetics of cancer treatment have facilitated the identification of the genetic bases for interindividual differences to better predict clinical effectiveness and drug toxicity, offering more personalised cancer treatment regimens [2].

The expanding knowledge of molecular and tumour biology and antitumour immune responses has notably impacted cancer treatment paradigms [3], with the development of novel biological and immunotherapy agents improving the outcomes of cancer patients. Important challenges are still ongoing in both fields of cancer therapy [4].

The clinical utility of anticancer drugs is limited by adverse effects.

The mechanisms involved are not yet fully understood, depending on the several biological targets, and a wide array of previously unrecognised toxicities have been increasingly observed.

In order to define and better record the adverse events reported in clinical trials, the US National Cancer Institute (NCI) published The Common Terminology Criteria for Adverse Events, also called the "Common Toxicity Criteria" (CTC or NCI-CTC). This included the description and grading of organ toxicity related to cancer therapy, generally using a range of grades from 1 (mild) to 5 (death). The current version 5.0 was released in November 2017 and became effective in April 2018.

Minimising toxicity and developing management guidelines remains one of the most challenging aspects of cancer therapy.

This chapter will summarise the pathogenesis and clinical management strategies of side effects of chemotherapy for targeted and immunotherapy agents currently approved and available for clinical use to improve patient education, define a proactive approach and recognise the peculiar symptoms and signs that prevent potentially life-threatening complications, often as a result of infections, inhibition of angiogenetic pathways, severe inflammatory syndromes, and autoimmune disorders [1].

19.2 Haematological Toxicity

The bone marrow and, secondarily, the peripheral blood cells are frequently and consistently affected by cancer treatment. Chemotherapeutic agents are effective against rapidly growing cancer cells, but simultaneously cause damage to healthy tissue with a high growth fraction, particularly all three blood cell lines.

Chemotherapy-induced neutropaenia is a common complication in cancer treatment and is one of the major dose-limiting toxicities of systemic cancer chemotherapy [5]. Neutropaenia is defined as an absolute neutrophil count (ANC) of <500 neutrophils/mcL occurring within 7–12 days following cancer chemotherapy or by an ANC of <1000 neutrophils/mcL and a predicted decline by ≤500 neutrophils/mcL over the next 48 hours [6].

Patients with neutropaenia may have an impaired ability to fight infections.

One of the most common oncologic emergencies is febrile neutropaenia (FN) associated with chemotherapy, occurring approximately in 10–50% of patients with solid tumours receiving chemotherapy [7].

FN refers to the occurrence of fever during a period of significant neutropaenia (≥38.3 °C orally or ≥38.0 °C duration over 1 hour) and can lead to delays in treatment and dose reductions of chemotherapy, which compromise treatment efficacy and increase morbidity, mortality, and treatment costs and affect the patient's quality of life [6, 8].

The risk of severe febrile neutropaenia is usually based on the treatment regimen, patient risk factors, disease characteristics and treatment intent. Age represents a major risk factor. There are other factors having a

similar role, such as advanced disease, history of prior FN, mucositis and poor performance status [8].

Risk assessment should be evaluated before the first cycle of chemotherapy. Currently, based on the different schedules of drugs and individual risk factors, patients are assigned to an overall high risk group (>20%), an intermediate risk group (10–20%) or a low risk group (<10%) of FN [6]. The standard approach for the primary prophylaxis of chemotherapy-induced and febrile neutropaenia is the use of recombinant human granulocyte colony-stimulating factor (G-CSF) filgrastim and pegylated filgrastim to prevent or reduce the incidence or duration of neutropaenia and FN and, consequently, infection-related morbidity and mortality.

Filgrastim should be administered daily at least 24 hours after cytotoxic chemotherapy until a post-nadir recovery of absolute neutrophil count has been achieved. Alternatively, due to the longer serum half-life and sustained duration of action, pegylated filgrastim requires only once per cycle administration (within 24–72 hours after chemotherapy) [6].

After EU and US patent expiration for filgrastim in 2006 and 2013, respectively, the biosimilars emerged [9], products that are designed to be 'similar' in terms of quality, safety and efficacy of an already approved original biologic drug, representing a less expensive alternative [10]. The FDA and EMA approved several biosimilar versions of filgrastim and pegfilgrastim for clinical use in patients with nonmyeloid malignancies receiving myelosuppressive anticancer drugs [9].

Most guidelines recommend prophylactic administration of G-CSF if the physician-assessed FN risk is >20%. This approach reduces the risk of FN occurrence by at least 50% in patients with solid tumours as reported by several meta-analyses [8].

The therapeutic use of myeloid growth factor for the management of patients with febrile neutropaenia is still controversial [6].

The NCCN guidelines do not recommend antibacterial prophylaxis to patients at low risk for FN [11]. However, fluoroquinolones have been used extensively in this setting, particularly levofloxacin for patients with an increased risk of *Streptococcus*-mediated oral mucositis [8].

Patients with FN should be assessed for the risk of complications to improve patient care, selecting the most appropriate antimicrobial therapy, including dosage and route of administration (oral or intravenous), drug interactions and potential side effects and predicting the need for hospitalisation.

Anaemia, defined as a decrease in haemoglobin concentration to non-physiological levels, is frequent in patients with cancers and may negatively impact survival and quality of life [12].

The incidence of anaemia increases with chemotherapy and is related to type, schedule and intensity of treatment. Chemotherapy-related anaemia can result in fatigue, impaired physical function and consequently in reduced survival rates or time to progression, representing an adverse prognostic factor.

Anaemia in cancer patients may depend on several factors, mainly related to disease extension and development and treatments used for the management of the cancer.

Cancer cells may infiltrate the bone marrow and directly affect red blood cell production and increase the release of inflammatory cytokines, inducing iron sequestration with consequent reduced production of red blood cells or impaired erythropoietin synthesis in the kidney.

Tumour growth may also result in chronic blood loss leading to progressive anaemia. In addition, chemotherapy may cause anaemia due to renal tubular damage.

Different strategies are adopted to safely manage chemotherapy-induced anaemia, including the following:

- Red blood cell transfusion in patients with Hb <7–8 g/dL and/or severe anaemia-related symptoms
- Administration of erythropoiesis-stimulating agents, such as epoetin alfa, for symptomatic anaemia and Hb level <10 g/dL or asymptomatic anaemia presenting with an Hb level <8 g/dL
- Oral or intravenous iron supplementation with or without additional anaemia therapy

Several erythropoiesis-stimulating agents have been recently developed, including biosimilar products, after patent expiration of the first-generation epoetins [13].

Chemotherapy-induced thrombocytopaenia is a common problem in cancer patients and refers to a peripheral platelet count of <100 × 109/L, increasing bleeding risk and causing treatment delays and dose reductions [14].

The incidence and severity of chemotherapy-induced thrombocytopaenia vary greatly depending on type, schedule and intensity of treatments.

In evaluating thrombocytopaenic cancer patients, it is important to exclude other potential causes of thrombocytopaenia that develop in the context of other disorders [15].

Platelet transfusions still remain the standard of care for patients with severe chemotherapy-induced thrombocytopaenia. Refractoriness may represent a relevant complication of multiple platelet transfusions. The use of thrombopoietic agents in this setting is under evaluation in several studies [14].

Haematological toxicity can occur also during treatment with anticancer-targeted therapies, particularly

tyrosine kinase inhibitors (TKIs). Grade 3 or higher haematological toxicity with oral EGFR and ALK TKIs is a rare occurrence. Neutropaenia and anaemia are the most common haematologic adverse events of these agents.

The multi-targeted TKIs, resulting in inhibition of tumour proliferation and angiogenesis, especially sunitinib, have been associated with cytopaenia.

The inhibition of FLT-3 and c-kit pathways, involved in haematopoietic process, can explain myelosuppression with these agents. Therefore, pazopanib has been associated with a limited haematological toxicity, reflecting minimal FLT-3 inhibition [16].

Myelosuppression is extremely rare in patients treated with BRAF and MEK inhibitors, although the risk of neutropaenic sepsis should be considered in patients affected by pyrexia primarily related to dabrafenib.

The most common adverse events associated with cyclin-dependent kinase 4/6 inhibitors (palbociclib and ribociclib) are haematologic, particularly neutropaenia [17], characterised by the lack of serious complications compared with cytotoxic chemotherapy, due to the cytostatic effect of CDK4/6 inhibition on the bone marrow.

Combination of chemotherapy with targeted therapies may slightly increase the risk of haematological toxicity (i.e. pertuzumab- and cetuximab-based therapies) or not result in an increased myelosuppression (i.e. combination therapy with nintedanib or lapatinib).

In addition, the administration of immune checkpoint inhibitors (ICIs) alone or in combination may seldom be associated with the occurrence of haematologic toxicity, such as anaemia, neutropaenia, thrombocytopaenia, acquired haemophilia A and cryoglobulinaemia [18, 19]. All blood cell types may potentially be involved due to autoimmunity. Symptom management is similar immune-related AEs (irAEs) and the standard approach involves the use of corticosteroid treatment or alternative immunosuppression therapy in refractory cases.

19.3 Skin Toxicity

Cutaneous adverse effects are among the most frequently observed toxicities with antineoplastic and immunomodulating drugs. Although skin toxicity is rarely life-threatening, it often impairs quality of life and compliance with therapy [20].

Characteristics of cutaneous reactions vary depending on the different classes of cancer therapies, including classical chemotherapy, targeted therapies and immunotherapy (Fig. 19.1).

Several skin conditions have been described including alopecia, papulopustular rash, dry skin, flushing, hyperpigmentation, nail changes and photosensitivity.

Chemotherapy-induced alopecia is the most emotionally difficult and distressing side effect of commonly administered chemotherapeutic agents, particularly for female patients.

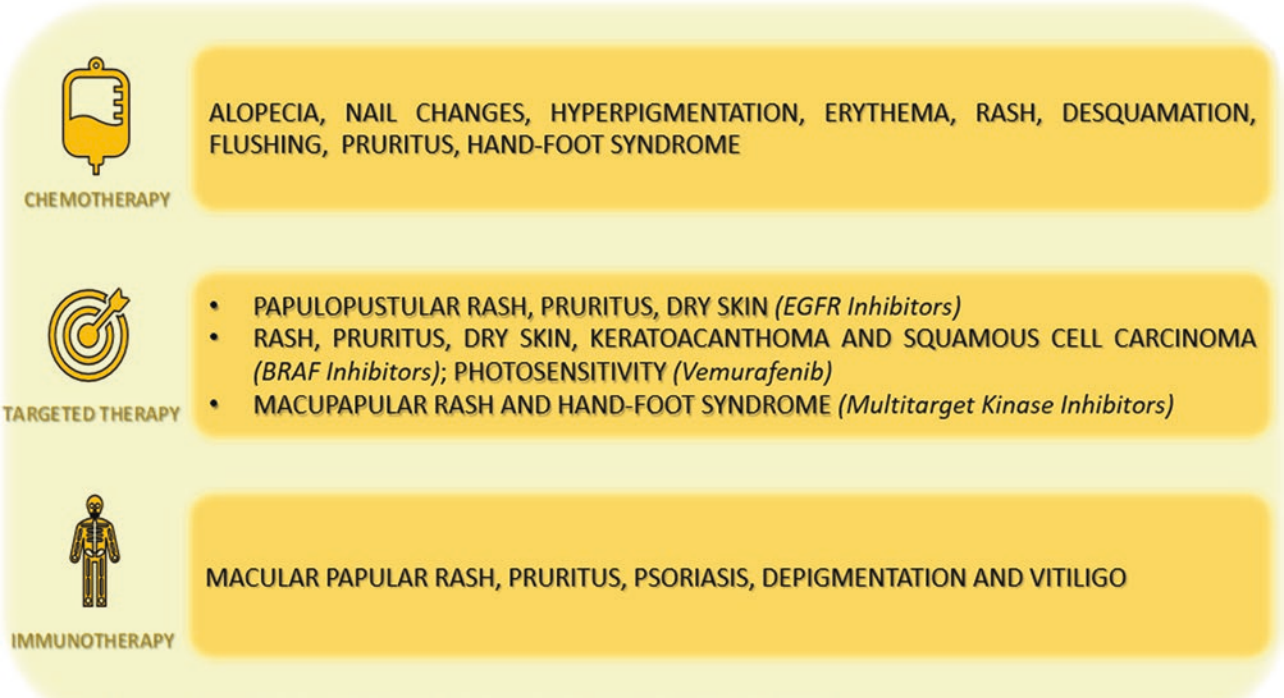

Fig. 19.1 Common skin toxicities associated with anticancer drugs and immunotherapy

Alopecia usually begins 1–3 weeks after the start of chemotherapy [21]. The timing of its appearance is influenced by the type, dose and duration of chemotherapy.

Taxanes, anthracyclines, alkylating agents and some antimetabolites are associated with higher incidence of alopecia. Scalp cooling during chemotherapy is currently the safest option to prevent chemotherapy-induced alopecia with response rates ranging from 50% to 80%. Alopecia is also a common side effect of some targeted therapies, including EGFR antibodies, BRAF inhibitors, cyclin-dependent kinase inhibitors and Hedgehog pathway inhibitors [21].

Tyrosine kinase inhibitors can cause different patterns of cutaneous lesions, often representing dose-limiting toxicities or causing discontinuation of antineoplastic therapy.

The most common dermatologic toxicity resulting from EGFR-TKI treatment is papulopustular eruption, also called acneiform rash (not appropriate for the lack of typical acne lesions, such as comedones and cystis) or folliculitis. Skin reactions are common because EGFR is expressed in keratinocytes of the basal layer of epidermis, sebaceous glands, hair follicles and endothelial cells. The rash typically begins within 1 week of treatment and has been found, in some studies, to be directly correlated to the therapeutic efficacy of EGFR-TKI and an increase in survival [22]. Several treatment strategies for skin rash have been developed including topical and systemic corticosteroid s and antibiotics, such as doxycycline or minocycline, to treat pustules or secondary bacterial infection in skin lesions.

The type of treatment is guided by the severity of the skin eruption and response to topic therapy. The EGFRI-associated rash appears to be clinically more severe with the use of monoclonal antibodies compared to EGFR-tyrosine kinase inhibitors [23]. Itching as a result of dry skin can often lead to superinfection.

Although the mechanisms underlying the development of the skin toxicity remain unclear, immunological mechanisms are considered to be involved [24].

Skin toxicities are the most common toxicities associated with BRAF inhibitors. Characteristic skin adverse events are rash, pruritus, dry skin, keratoacanthoma and cutaneous squamous cell carcinoma.

Photosensitivity is primarily associated with vemurafenib [25].

Monitoring the appearance of skin lesions associated with BRAF inhibitors is mandatory.

The development of cutaneous adverse reactions is less frequent for MEK inhibitors. Adding an MEK inhibitor to BRAF has led to the inhibition of RAS signalling in the MAPK pathway, blocking cellular proliferation, with an improvement of outcome in melanoma patients and lower incidence of skin toxicities and malignant lesions.

Multitarget kinase inhibitors can cause a dose-dependent maculopapular rash and hand-foot reaction, characterised by localised hyperkeratosis and erythema. Hand-foot syndrome, also called palmar-plantar erythrodysesthesia or acral erythema has a different clinical presentation and is a serious dose-limiting toxicity associated with many cytotoxic agents, particularly capecitabine. The pathogenetic mechanism is still unknown [22].

The clinical manifestations begin with diffuse, symmetric erythema and oedema of the palms and soles, which may progress to dryness, pain, itching and occasionally fissures and blisters.

Recommended treatment includes topical steroid and keratolytic agents for hyperkeratotic areas.

Dermatologic adverse events, mostly of immunologic origin, are common in patients treated with checkpoint inhibitors. Nonspecific macular papular rash and pruritus represent the most common manifestations, although peculiar skin adverse events have been reported, such as psoriasis, depigmentation and vitiligo [26].

Maculopapular rash can also represent the initial clinical manifestation of more severe cutaneous adverse drug reactions, although rarely described with checkpoint inhibitors, including the extensive exfoliative dermatitis, Stevens-Johnson syndrome and toxic epidermal necrolysis [26].

Cutaneous and ocular problems were the most common reported short-term and long-term sequelae.

19.4 Cardiovascular Toxicity

Patients with cancer can experience adverse cardiovascular events secondary to anticancer treatment. Cardiotoxicity due to antitumour agents is a major issue in oncology and it may have multiple and potentially clinical manifestations, including acute heart failure, ischaemia, venous thromboembolism, hypertension, QT prolongation and cardiac arrhythmias [27, 28, 29]. Treatment-related cardiotoxicity may occur during treatment or immediately after (within days or weeks) or develop after a long period of discontinuation of these therapies and can be either irreversible (Type I cardiotoxicity) or transient (Type 2 cardiotoxicity).

To date, treatment and management of cardiotoxicity is very important and a multidisciplinary approach between cardiologists and oncologists is necessary to recognise and mitigate both early and late cardiac damage [30]. Baseline cardiovascular assessment is vital before the selection of appropriate anticancer treatment

and pre-existing cardiovascular disease must be treated [31]. In particular, a baseline evaluation of cardiovascular risk (e.g. smoking, body mass index, diet and physical inactivity) [32] is needed and all potentially modifiable risk factors should be identified and treated [33]. An important role in the early identification of cardiac damage can be played by biomarkers [34]. Several different potential biomarkers have been found to detect both type I and type II cardiac damage, including troponins I and T, B-natriuretic peptide and C reactive protein [35, 36, 37]. However, due to the absence of established optimal timing and cutoff for testing these biomarkers, the routine use of any biomarker is not recommend in daily clinical practice.

Chemotherapy-related cardiotoxicity can be prevented and the late effects mitigated through primary cardioprotection and early initiation of treatment for compromised cardiac function [38].

The cardiotoxic effects of anthracycline cause myocyte injury in a dose-related and cumulative manner. Indeed, the risk of cardiac disease increases with increasing dose of anthracycline with an incidence of congestive heart failure of 5% at 400 mg/m^2 of total adriamycin dose, 26% at 550 mg/m^2, and 48% at 700 mg/m^2 [39]. For this reason, today it is recommended to not exceed the total adriamycin dose of 400–450 mg/m^2.

The prevention of cardiotoxicity from anthracycline can be performed through different levels of intervention:

- Use of chemotherapeutic schedules not containing anthracycline.
- Use of liposomal anthracyclines, less cardiotoxic.
- Identification of predictive biomarkers of ventricular dysfunction.

Asymptomatic decreases in left ventricular ejection fraction (LVEF) are common and occur in up to 20% of treated patients [40]. Left ventricular dysfunction (LVD) can be reversible and unrelated to the cumulative dose. LVD is defined, according to current guidelines, as an LVEF of <55% or a decrease in LVEF of >10% from the baseline LVEF after cancer treatment [41].

Serial measurement of LVEF is the mainstay of cardiac surveillance in patients under treatment with anti-HER2 agents, with a serial evaluation of LVEF every 3 months. The algorithm of Suter is often used for monitoring the cardiac function of patients treated with trastuzumab: if the patient has an LVEF reduction to the baseline of ≥15% or ≥10% in the presence of LVEF <50%, trastuzumab treatment should be discontinued for 3 weeks, with a re-evaluation of the LVEF thereafter [42]. If a reduction in LVEF is detected, patients should be treated in accordance with established guidelines for the management of heart failure (HF) and/or LV dysfunction [43, 44] (Fig. 19.2).

Drug therapy should include angiotensin-converting enzyme (ACE) inhibitors or β-blockers [45, 46].

Recently evidence showed that β-blockers (carvedilol and nebivolol) and ACE inhibitors (analapril) prevent LVEF reduction and decrease the incidence of heart failure during trastuzumab-based therapy. Two randomised studies evaluated the role of bisoprolol and perindopril vs. placebo (MANTICORE 101) [47] and candesartan and metoprolol (PRADA) [48] in primary prevention of ventricular dysfunction during treatment with trastuzumab, with interesting preliminary findings. However, the small patient population enrolled in both studies limits the immediate applicability of these findings in clinical practice, and further studies in larger populations are needed.

Globally, the cardiotoxicity profile of other anti-HER2 agents such as pertuzumab [49, 50], T-DM1 [51, 52, 53] and anti-HER2 TKIs (lapatinib, neratinib and afatinib) is favourable with no apparent addition of cardiotoxicity as compared to the administration of trastuzumab alone [54, 55, 56, 57, 58, 59].

Echocardiography is the technique of choice for serial measurement of LVEF [60] Due to reduced availability and higher cost, the use of cardiac magnetic resonance imaging, for evaluation LVEF, is limited to patients with unclear echocardiographic results [61, 62]. However, traditionally, echocardiography presents several limitations for the measurement of LVEF [63]. Recently, it was demonstrated that measurement of myocardial deformation (or strain imaging) provides an alternative technique, which is more sensitive and predicts cardiac damage earlier [64, 65].

Hypertension is the most common cardiovascular complication associated with Vascular Endothelial Growth Factor (VEGF) inhibitors, such as bevacizumab and multitargeted tyrosine kinase inhibitors [66]. Although hypertension induced by anti-VEGF agents can predict a good tumour response, its control does not reduce its therapeutic efficacy [67] and prevents cardiovascular complications and treatment interruption [68]. Initial assessment and close monitoring of blood pressure are recommended during therapy with these agents. Control of hypertension is particularly advisable to prevent new-onset or worsening myocardial ischaemia and heart failure in these patients [69]. ACE inhibitors and β-blockers can protect against HF development [70]. In case of severe, resistant hypertension, cancer therapy should be interrupted.

In addition, various anticancer agents can be associated with the development of cardiac arrhythmias, including bradycardia, QT interval prolongation with possible torsades de pointes and ventricular fibrillation.

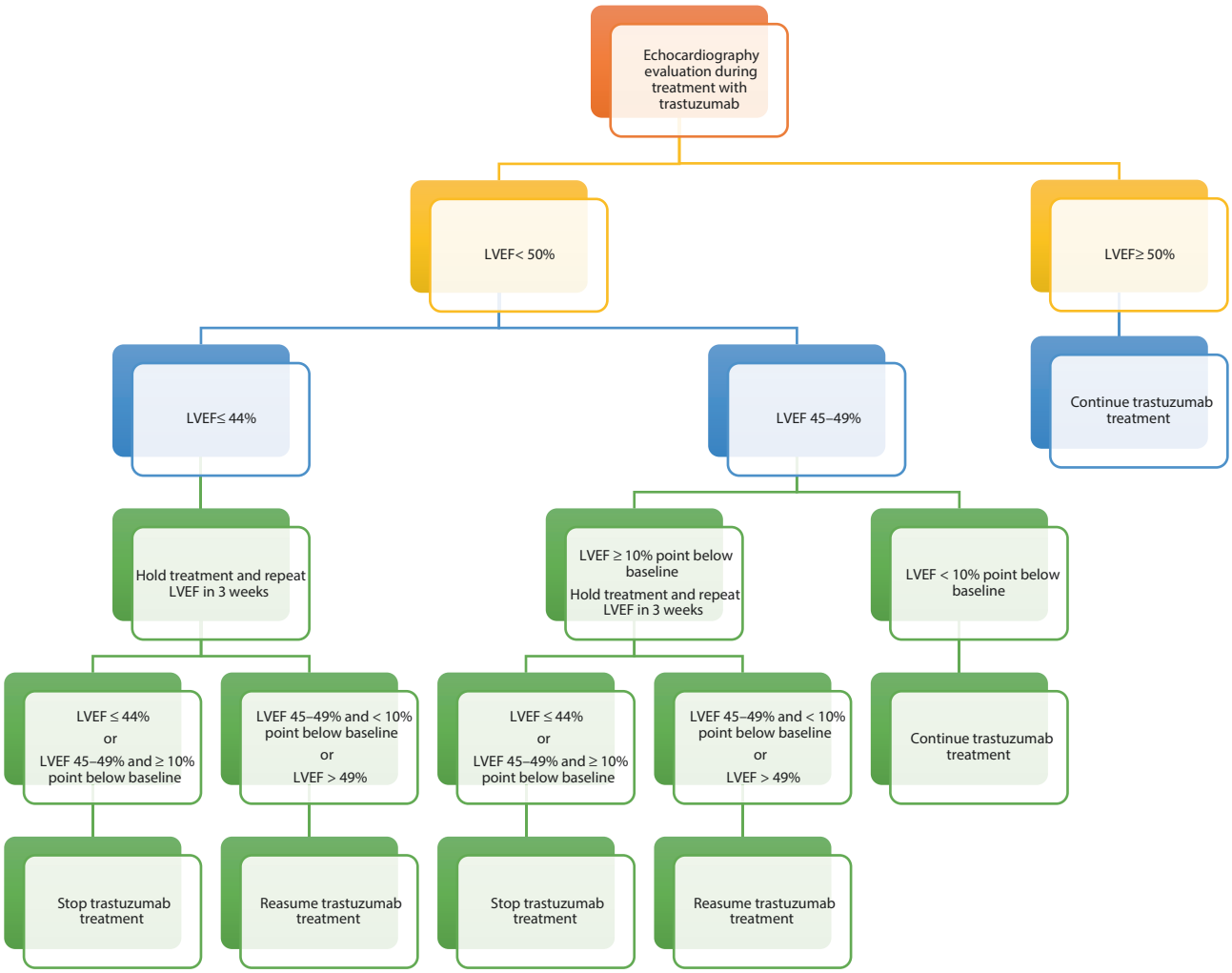

Fig. 19.2 Algorithm for continuation and discontinuation of trastuzumab based on LVEF assessments (Adapted from [31, 42])

Often rhythm disorders are transient and can be the result of multiple risk factors, such as metabolic and electrolytic disorders or drug interactions (use of antihistamines or antiemetics) [71].

Cancer patients may often have prolongation of the QT interval (from 16% to 36%), because of the concomitant presence of favourable conditions, such as renal or liver dysfunction, electrolytic disorders due to vomiting and diarrhoea and concomitant therapies with drugs that can interfere with QT interval, such as antifungal, antiemetic and antidepressive drugs [72, 73]. Monitoring the QT interval with ECG, periodic monitoring of electrolytic levels, as well as eliminating other agents known to prolong the QT interval for patients are imperative [74].

Emerging evidence suggests that treatment with checkpoint inhibitors alone or in combination may rarely result in various cardiotoxic events (myocarditis, HF, heart block, myocardial fibrosis and cardiomyopathy) [75]. Selected cases of myocardial fibrosis [76] and late-onset pericarditis were reported in melanoma patients treated with ipilimumab [77]. In a trial of ipilimumab, a case of cardiac arrest was reported [78] and a fatal case of myocardial infarction in a patient with NSCLC treated with pembrolizumab [79]. Moreover, Johnson et al. reported two cases of fatal myocarditis developed after treatment with ipilimumab and nivolumab in patients with melanoma. These events are particularly rare, but potentially fatal, with an estimated incidence in large pharmacological studies of 0.06 with nivolumab (<0.01 fatal) and 0.27 (0.17 fatal) with the combination nivolumab-ipilimumab [80]. The monitoring strategy in patients receiving checkpoint inhibitors may include baseline ECG and weekly testing of troponin levels during weeks 1–3 for patients receiving combination immunotherapy. The onset of symptoms such as chest pain, dyspnoea, palpitation and peripheral oedema should lead to further cardiac evaluation [81].

Venous thromboembolism (VTE) represents a major cause of morbidity and mortality in cancer patients [82].

Factors associated with VTE are age, genetic predisposition, tumour extent and type (more common in lymphoma, myeloma, and pancreatic, ovarian, lung, stomach, and kidney cancer) [83].

Use of antiangiogenic agents increases the risk for both arterial and venous events [84]. Baseline risk of VTE should be evaluated in patients receiving chemotherapy to decide whether to use antithrombotic prophylaxis [85]. This risk score (the Khorana score) was validated for identifying appropriate patients who need thromboprophylaxis for VTE with low-molecular weight heparin [86]. New oral anticoagulants including apixaban, rivaroxaban and edoxaban are being investigated for use in cancer patients [87], but additional studies are required to explore the safety and efficacy of these drugs in cancer patients.

19.5 Pulmonary Toxicity

Pulmonary adverse events are an important cause of respiratory failure in cancer patients and their incidence is growing with the increasing use of newer anticancer agents that may be associated with lung damage [88]. Among chemotherapeutic agents responsible for pulmonary toxicities, bleomycin is the most-well studied. This antitumour antibiotic exerts its activity, generating free radicals that disrupt cellular DNA and is degraded by an enzyme, bleomycin hydrolase, which is present at low levels in the lungs and kidneys. Therefore, it is not surprisingly that pulmonary and renal toxicities are possible adverse events with this agent. Several distinct pulmonary syndromes have been associated with the use of bleomycin, such as bronchiolitis obliterans with organising pneumonia, eosinophilic hypersensitivity, and, most commonly, interstitial pneumonitis, which ultimately may progress into fibrosis. The incidence of bleomycin-induced pneumonitis varies on diagnostic criteria utilised and may be up to approximately 40%, with 1–2% fatal events [89, 90].

Bleomycin-induced pneumonitis onset usually occurs during treatment, but may develop even after 6 months since treatment discontinuation and manifests with dyspnoea, tachypnea, and non-productive cough; it is commonly associated with pulmonary changes on restaging computed tomography scans and mostly resolve spontaneously. The decision to discontinue bleomycin administration is based on clinical signs, with no clear utility of pulmonary function tests monitoring during treatment or inflammation markers level evaluation. Moreover, bleomycin-induced pneumonitis may be associated with higher post-operative complications, including potentially fatal complications such as acute respiratory distress syndrome (ARDS) [89, 91].

The most efficient prevention measure for bleomycin-induced pneumonitis is to lower the total cumulative dose of bleomycin, since cumulative doses >400 mg increase the risk of pulmonary toxicities. The optimal management of this toxicity is not well known and bleomycin administration suspension is mandatory. In case of sudden onset of pulmonary symptoms, the administration of corticosteroids is indicated because of a high risk of bronchiolitis obliterans with organising pneumonia or eosinophilic hypersensitivity, whereas the utility of corticosteroids in patients with gradually starting symptoms is less clear [90].

Non-infectious pneumonitis has been described with use of the mTOR inhibitor everolimus, with an overall incidence of 14–17% (grade 3–4 2–4%) in clinical trials in RCC, pNET and breast cancer [92]. Symptoms include cough, dyspnoea, hypoxia and more rarely fever and haemoptysis and are associated with radiographic findings of ground-glass opacities and/or focal consolidation, primarily in the lower lobes of the lungs. Chest CT scan is the gold standard for the diagnosis, but bronchoscopy with bronchoalveolar lavage and/or biopsy may be required to definitively exclude infectious aetiologies. Treatment requires drug discontinuation in symptomatic patients with grade ≥2 AEs and steroid therapy (0.75–1 mg/kg/daily oral prednisone or, in severe cases, intravenous methylprednisolone). Everolimus may be resumed, at lower dose, in case of G2–3 toxicities after recovery to grade 1 or better, but should be permanently discontinued in case of G4 toxicities [93].

In patients treated with EGFR-tyrosine kinase inhibitors (TKIs) (geftinib, erlotinib, afatinib, osimertinib), a rare possible complication but potentially fatal is the onset of interstitial lung disease (ILD). The incidence of ILD is generally low (1.2% in a recent meta-analysis with gefitinib and erlotinib) [94], but it is more common in Asian patients and in those with previous ILD or limited residual normal lung [95, 96]. Combination therapy with EGFR-TKIs and immunotherapy may be associated with higher incidence of ILD, as recently reported, and may represent a major issue in combinatorial approaches with these drugs [97]. This complication is associated with a high mortality rate and therapeutic management includes the use of high corticosteroid (methylprednisolone 2–3 mg/kg per day) and the exclusion of infectious causes. Treatment with broad-spectrum antibiotics is usually performed in these cases, until documentation of non-infectious disease, in order to contrast the immunosuppressive effect of high-dose steroid therapy [98].

Pneumonitis may be observed during treatment with ICIs targeting the PD-1/PD-L1 pathway, with an overall incidence of 5% and a higher frequency after treatment with anti-PD1/CTLA-4 combinations com-

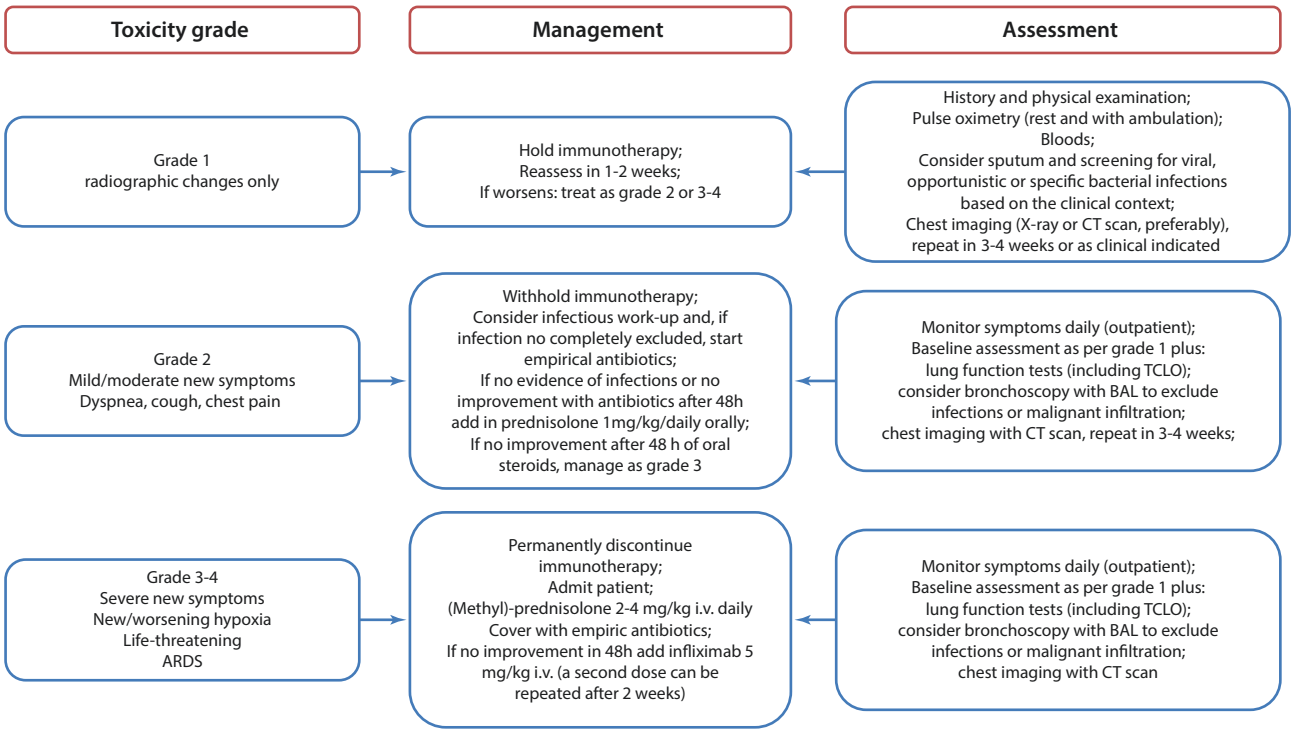

Fig. 19.3 Management of immune checkpoint inhibitors-induced pulmonary toxicity (Adapted from [108] and NCCN guidelines v1.2019)

pared with anti-PD1/PDL1 monotherapy (10% vs. 3%), irrespective of primitive tumour location [99]. Treatment with anti-PD1 agents seems to be associated with higher incidence of pneumonitis compared with anti-PDL1 agents in NSCLC, as well as in treatment-naïve patients compared with previously treated patients [100]. Interestingly, pneumonitis was not described in major trials with anti-CTLA4 agents and a recent meta-analysis reported a significant association with anti-PD1 agents [101]. As observed with other immune-related AEs (irAEs), pneumonitis is often G1–2 and improves/resolves after drug suspension and/or treatment with steroid/immunosuppressants. More rarely, this complication may evolve in a life-threatening condition, potentially fatal [99, 102].

Patients with previous chest irradiation may experience higher rates of pulmonary irAEs [103, 104], albeit the risk factors associated with development of ICIs pneumonitis should be extensively investigated further in large prospective studies [100].

The time of onset of these irAEs is variable with a very wide range (from 9 days to 19.2 months) [99]. Clinically, patients experiencing a pulmonary irAE may present with dyspnoea, cough, fatigue or respiratory failure, associated with variable radiological patterns: cryptogenic organising pneumonia (COP), ground-glass opacities, nonspecific interstitial pneumonia (NSIP), hypersensitivity pneumonia (HP), acute interstitial pneumonia (AIP)/acute respiratory distress syndrome (ARDS) and pneumonitis non-otherwise specified [99, 105, 106]. More rarely, a non-infection pleuritis may emerge [107]. Infective causes should be excluded.

According to the European Society of Medical Oncology (ESMO) guidelines, treatment of G1–2 pneumonitis requires immunotherapy interruption and prednisone start (1–2 mg/kg/daily orally). In case of G3–4 pneumonitis, treatment with ICIs should be permanently discontinued and patients should be treated with high-dose corticosteroids (methylprednisolone 2–4 mg/kg/daily i.v.), adding immunosuppressants (infliximab, MMF or cyclophosphamide) in non-responding patients. Steroid therapy should be tapered over a period of 4–6 weeks [108] (Fig. 19.3).

19.6 Gastrointestinal Toxicity

Antineoplastic drugs can damage healthy cells in the digestive tract system, resulting in adverse events such as loss of appetite, mucositis, nausea, vomiting, constipation and diarrhoea.

Immune-mediated gastrointestinal toxicity is also a common complication of immunotherapy.

Although nausea and emesis can result from several cancer treatments including targeted therapies and immunotherapy, chemotherapy-induced nausea and

vomiting (CINV) is potentially the most feared and has a detrimental effect on the quality of life of patients with cancer receiving chemotherapy, resulting in a reduction of food intake.

CINV is a highly complex condition that involves both the central and peripheral nervous systems.

Several antiemetics have been developed to target and block different pathways.

In the past decades, a high percentage of patients suffer from CINV, whereas today, about 10–20% of patients are affected [109].

The incidence and severity of CINV depend of the type of chemotherapy, doses, other drugs used in association and psychological status of the patients.

Chemotherapeutic agents or combinations are classified into four main emetic risk groups in the absence of antiemetic prophylaxis.

International guidelines for antiemetic therapy for intravenously administered chemotherapy (ASCO, NCCN AND MASCC/ESMO) have improved the management of nausea and vomiting [110].

The three categories of drugs widely used in this setting are the three 5-hydroxytryptamine (5-HT3) receptor antagonists, known as 'setrons', the neurokinin-1 receptor (NK1R) antagonists including aprepitant, fosaprepitant, a prodrug of aprepitant and the new rolapitant and netupitant, and glucocorticoids, especially dexamethasone.

Olanzapine, a second-generation antipsychotic that blocks serotonin 5-HT2 receptors and dopamine D2 receptors, added to the antiemetic regimen for high-emetic-risk chemotherapy, is a particularly useful agent for the prevention of both acute and delayed nausea and vomiting.

Anticipatory emesis, occurring before a new cycle of chemotherapy as a conditioned response, appears particularly linked to psychological processes. Behavioural therapy and/or benzodiazepines may be useful in this setting.

Many challenges remain in the prevention and treatment of CINV, particularly in the delayed phase.

Treatment-induced diarrhoea represents a constant challenge, which significantly affects morbidity and mortality of cancer patients, causing dosing delays or reductions.

Chemotherapy-induced diarrhoea (CID) can occur in 50–80% of patients and depends on the chemotherapy regimen. It is important to exclude other potential causes of diarrhoea in cancer patients [111]. Therapeutic agents commonly causing diarrhoea include fluoropyrimidines (5-fluorouracil, capecitabine and tegafur) and irinotecan.

CID is a multifactorial process involving multiple complex mechanisms that lead to damage to the intestinal mucosa and cause an altered balance between absorption and secretion in the small bowel with consequent depletion of fluids and electrolytes, dehydration, malnutrition, weight loss, fatigue, renal failure and cardiovascular alterations. Genetic factors may contribute to fluoropyrimidine toxicities. Patients with genetic deficiency of dihydropyrimidine dehydrogenase may develop early severe and potentially life-threatening toxicity, including diarrhoea. The DPYD genotyping strategies are not yet available for routine use [111].

Recent researches have investigated the effects of chemotherapeutic administration on microbiota. Anticancer therapy results in significant changes in microbiota composition.

Diarrhoea following treatment with EGFR-TKIs seems to be primarily secretory and related to the presence of EGFR on cells of the GI tract, although the mechanism remains unclear.

Diarrhoea induced by first-generation EGFR-TKI most likely occurs in the first 4 weeks after treatment initiation and within the first 7 days during afatinib treatment.

In phase I trials, diarrhoea constituted the dose-limiting toxicity of afatinib [112].

Diarrhoea is also a relatively common side effect associated with BRAF and MEK inhibitors, multikinase inhibitors and monoclonal antibodies, usually mild to moderate in severity. Loperamide is considered the mainstay pharmacologic treatment for diarrhoea. Dehydration and electrolyte replacement should be evaluated. Diarrhoea may be managed symptomatically and minimised by patient education and dose reduction in the majority of patients.

Diarrhoea is also the most common gastrointestinal immune-related adverse events followed by colitis, occurring approximately in one-third of patients, more common with anti-CTLA-4 and characterised by inflammation of the colon. The standard approach involves the use of loperamide and eventually fluid and electrolyte supplementation for non-severe diarrhoea; corticosteroid treatment in persistent grade 2 diarrhoea or severe diarrhoea or alternative infliximab in refractory cases, unless it is contraindicated [108].

In the wide spectrum of gastrointestinal toxicity due to anticancer therapies and immunomodulating drugs also stomach, the liver and pancreas represent the target organs for drug toxicity.

19.7 Nephrotoxicity

Renal dysfunction is common in patients with cancer, with a reported incidence of up to 60%. Renal failure may be caused by malignancy-associated conditions

(such as tumour lysis, disseminated intravascular coagulation, renal vein thrombosis, etc) or may be related to treatment and may widely vary depending on the antitumour agent used, type of tumour, and patient age and pre-existing renal damage [113]. Kidney injury during anticancer therapy may involve the different parts of nephrons, including renal vasculature, glomeruli, and tubulointerstitium [114].

Antiangiogenetic therapies and the antimetabolite agent gemcitabine may be responsible for renal vasculature damage, causing a thrombotic microangiopathy (TMA). TMA induced by antiogenetics targeting the VEGF/VEGFR pathway usually presents as new-onset hypertension or worsening of a pre-existing hypertension, proteinuria, acute kidney injury (AKI) or chronic kidney disease, with histopathological evidence of microvascular thrombosis [114, 115]. The incidence of TMA is rare, as suggested by the observational study MARS, evaluating the renal safety of bevacizumab in ovarian, lung, and breast cancer and has not been reported with other antiangiogenetic agents, such as axitinib and ramucirumab [116]. More frequently, bevacizumab is responsible for proteinuria, with a variable incidence (15–72.1%), mostly of grade 1. Severe proteinuria may cause renal damage and increase the risk of cardiovascular events. Therefore, close monitoring of renal function indices is recommended, including evaluation of proteinuria with urinary dipstick or on 24-h urine sample, in patients treated with bevacizumab and a prompt start of appropriate treatment in case of severe proteinuria, including therapy discontinuation and use of ACE inhibitors and angiotensin-receptor blockers [115, 116].

Acute kidney injury is thought to be a relatively uncommon AE during treatment with ICIs, with a reported incidence of ~1% in clinical trials with anti-CTLA4 and anti-PD1 agents and up to ~5% with dual checkpoint inhibitors either as combinatory or as sequential therapy [117]. However, the incidence of renal toxicities might be higher (9.9–29%), as suggested in recent studies [118], and may represent an emergent entity with the increasing use of these agents.

In contrast with other irAEs, timing of onset and resolution of AKI are variable and less informative, albeit the majority of the cases develop in the first 2–3 months. Moreover, the cumulative drug dosage does not seem to predict the risk of renal toxicity [117–119].

Clinically, renal irAEs may coexist with other immune-related toxicities and are usually asymptomatic, with the evidence of rising serum creatinine levels and pyuria in most of the cases as the sole signs of renal damage. Less frequently, AKI may be associated with haematuria, eosinophilia or nephrotic syndrome. In addition, ipilimumab-induced AKI may be associated with hyponatraemia, generally as a consequence of hypophysitis with adrenal insufficiency [117, 118, 120].

Exclusion of other causes of AKI is mandatory and a renal consultation should be sought early. A renal biopsy may be useful, with acute interstitial nephritis as the most common pathologic lesion and podocytopathy as peculiar pattern associated with ipilimumab [118]. The therapeutic management of renal irAEs depends on the severity of renal insufficiency and should include the use of corticosteroid therapy (methylprednisolone 1–2 mg/kg/daily) and may include renal biopsy [108]. The suspension of ICI therapy is recommended, but the definitive discontinuation is not always necessary and a recurrence is possible after resolution of AKI, suspension of concomitant drugs potentially associated with renal damage, and close monitoring of serum creatinine level [118].

Cisplatin is responsible for tubular injury, which occurs in the corticomedullary S3 segment of the proximal tubule, as a consequence of cisplatin uptake by renal tubular cells. Moreover, cisplatin may be associated with vascular injury, with subsequent ischaemic injury and decrease of glomerular filtration rate [113, 121]. Cisplatin nephrotoxicity is a dose-limiting toxicity and develops in approximately one-third of patients and can be associated with different clinical manifestations, including AKI (20–30% of the cases) and hypomagnesaemia (40–100%) [122]. Cisplatin-induced AKI presents ~10 days after treatment as non-oliguric renal failure, with inability to concentrate urine, due to proximal tubular dysfunction (glucosuria, aminoaciduria and, more frequently, hypomagnesaemia) and increases in serum creatinine and blood urea nitrogen. Moreover, hypomagnesaemia may potentiate cisplatin nephrotoxicity, as reported in animal models. Several nephroprotective strategies have been adopted, including the use of hydration regimens and diuretics (mannitol and furosemide) [113, 122]. Hydration limits the incidence and severity of renal injury because of reduced cisplatin half-life, urinary drug concentrations, and proximal tubule transit time. Patients should receive cisplatin in a short duration infusion (over 2–6 hours) with low volume hydration (2–4 L of normal saline hydration) and supplementation of potassium and/or magnesium. Forced diuresis with mannitol has proven effective in patients receiving high-dose cisplatin (>100 mg/m^2), whereas its utility with lower doses of cisplatin is less clear, as well as the use of furosemide [123].

Another chemotherapeutic agent associated with tubular damage is ifosfamide. This alkylating agent causes tubular cell injury through a nephrotoxic metabolite, chloracetaldehyde. Ifosfamide-induced nephrotoxicity is often reversible and in ~90% of the cases, it manifests as subclinical tubular damage with glucosuria and 2-microglobulinuria. The major preventive strategy is the concomitant use of Mesna, a uroprotective agent

that acts to neutralise the caustic metabolite acrolein [114, 124].

Hypomagnesaemia is also a possible AE seen with the anti-EGFR mAb cetuximab. The inherited mechanism responsible for magnesium depletion in patients treated with this agent is the inhibition of magnesium reabsorption in the distal tubule that is, in part, EGF-dependent [114]. Hypomagnesaemia occurrence has been correlated with outcome in mCRC [125, 126] and is usually treated with intravenous repletion of magnesium, since oral supplementation is ineffective.

Recently, treatment with the ALK inhibitor crizotinib has been associated with the development of renal simple and or complex cysts that usually regress after treatment discontinuation. The mechanisms responsible for these unusual AEs are largely unknown and may involve the inhibition of the HGF/MET pathway or the reduced levels of testosterone, usually observed during treatment [127]. Treatment of these cysts is not necessary in the vast majority of cases, unless complications. Recognition of these AEs is important since complex renal cysts may be misinterpreted as renal abscesses.

19.8 Neurotoxicity and Ocular Toxicity

Neurotoxicity is one of the most frequent AE during cancer treatment, either as a result of direct damage to neurons/glia or as a consequence of indirect injury to neuronal microenvironment and may affect both the central and peripheral nervous systems [128].

Peripheral neuropathy is the most common neurological complication of cancer treatment (38% incidence in patients treated with multiple chemotherapeutic agents) and a common reason for dose reduction or drug discontinuation, representing a well-established dose-limiting toxicity for several chemotherapeutic agents, including platinums, vinca alkaloids, taxanes and epothilones. However, targeted agents may also be responsible for peripheral neuropathy, as reported with brentuximab, bortezomib and T-DM1 [128, 129]. In cancer patients, peripheral neuropathy may be less frequently associated with other conditions, including paraneoplastic syndromes or neoplastic neuropathies. It usually presents as sensorial peripheral neuropathy and develops gradually in a dose-dependent manner. Motor and autonomic symptoms are less frequent. A genetic predisposition for chemotherapy-induced peripheral neuropathy has been hypothesised, since some single nucleotide polymorphisms have been correlated with increased susceptibility, although further studies are needed [129, 130].

To date, there are no validated strategies to prevent chemotherapy-induced peripheral neuropathy and several trials have failed to demonstrate a significant advantage with the use of different compounds. Therefore, management of this toxicity is based on dose reduction/discontinuation of causative agent and symptomatic treatment of neuropathic pain [128, 130].

Although rare, neurological AEs may observed also be with ICIs. The overall incidence of neurological disturbances in clinical trial with mAbs anti-CTLA4 is 3.8%, 6.1% with anti-PD1, and 12.0% with combinations. However, the vast majority of these AEs is of grade 1–2 and, in some cases (headache, dizziness, dysgeusia), may be associated with other clinical conditions, such as brain/leptomeningeal metastases and hypophysitys, or may be related to previous exposure to neurotoxic agents, as in the case of neuropathies. Indeed, severe neurological irAEs are relatively uncommon with an overall incidence of <1% for all types of ICIs [131]. The relative rarity of these events, as well the unspecific symptomatology, makes these irAEs difficult to diagnose. However, in some cases, these neurological AEs may be associated with life-threatening conditions and therefore a prompt diagnosis and treatment of these toxicities may prevent further morbidity for the patients. A variety of neurological disturbances have been reported with the use of both anti-CTLA4 and anti-PD1 mAbs, including aseptic meningitis, encephalitis, Guillan-Barré syndrome, myasthenia gravis, polyradiculitis and optic nerve neuritis [132]. The risk of neurological toxicities may be increased in some tumours, especially in those with higher incidence of paraneoplastic syndromes, such as SCLC [133]; therefore, the use of ICIs in these patients should be evaluated carefully.

The diagnostic algorithm for neurological irAEs should include in case of suspicious CNS disorders a brain and/or spine MRI with gadolinium in order to exclude tumour-related conditions, such as brain/leptomeningeal metastases, and the CSF analysis, which is commonly associated with abnormalities. If meningoradiculitis or Guillan-Barré syndrome is suspected, a spine MRI should be performed to exclude organic causes (i.e. spine metastases or bone metastases with spinal cord compression), as well as the CSF analysis and dosage of serum onconeural antibodies, usually seen in paraneoplastic syndromes. Finally, in case of suspicion of polyneuropathy, an electroneuromyography may be evaluated, as well as laboratory investigation (T4, TSH, B12 vitamin and serum protein immunoelectrophoresis) to rule out other possible causes [131, 134].

As for other irAEs, the majority of neurological AEs associated with ICIs can be effectively managed with corticosteroid therapy. Steroid dosage and schedules depends on the severity of neurological conditions and a multidisciplinary management is often required. In some steroid-refractory cases with an antibody-mediated

pathogenesis, the use of intravenous immunoglobulin (IVIg) and plasmapheresis may be considered [135].

A number of anticancer agents may be responsible for ocular toxicity, especially newer agents. In particular, ocular toxicities seem to be the class effect of MEK inhibitors, commonly used in melanoma, with an incidence of 5–38% in clinical trials. MEK retinopathy usually presents acutely, and it is always bilateral and often symmetrical. Clinically, it is associated with visual disturbances (blurred vision, shadows, altered colour perception, etc.) and may cause a decrease in visual acuity. Grade 1–2 toxicities may be managed with a careful monitoring, without the need for treatment suspension, which is necessary in case of grades 3 and 4 AEs. However, a rechallenge with the same agent may be proposed after recovery of symptoms and optical coherence tomography findings, with close monitoring and dosage reduction [136].

19.9 Endocrine and Metabolic Adverse Events

The endocrine system may be affected during cancer treatment and several agents have been correlated with endocrine and metabolic disturbances. The recognition and treatment of these neglected adverse events are important, since they significantly affect patients' quality of life.

Hypothyroidism is a possible treatment-related AE with multi-targeted agents sunitinib and sorafenib with a variable incidence (10–85% and 6.3–27%, respectively), based on the criteria adopted for definition of thyroid dysfunction. In particular, sunitinib is associated with a higher incidence of hypothyroidism than sorafenib, with a more frequent need of hormone replacement therapy [137]. In patients treated with the ALK inhibitor crizotinib, a significant reduction has been reported in free testosterone levels in male patients that may be associated with hypogonadism [138]. In symptomatic cases, hormone replacement therapy may be necessary.

Other agents may cause metabolic disorders. For example, the mTOR inhibitor everolimus has been associated with metabolic abnormalities, namely hyperglycaemia. In most of the cases, this AE is mild (G1–2) and does not require any drug adjustment/interruption, but anti-diabetics should be started in case of G2 toxicities. Severe hyperglycaemia (G3–4) occurs more rarely and may require, in addition to supportive measures, treatment suspension and resumption with a lower dose in case of G3 toxicity and definitive discontinuation in case of G4 hyperglycaemia [92].

More recently, a great interest for endocrine toxicities has emerged after immune checkpoint inhibitors entered into the clinics. Several different endocrine AEs may be observed after treatment with ICIs targeting CTLA4 or PD-1/PD-L1. The mechanisms underlying these irAEs are largely unknown and may involve virtually all organs and systems, although the most frequent endocrinopathies are thyroid dysfunction and hypophysitis. The relative frequency of these irAEs varies with the type of ICI used, with a higher incidence of hypo/hyperthyroidism following anti-PD1/PDL1 agents and hypophysitis after anti-CTLA4 treatment [101, 139, 140].

Retrospective studies have revealed that the median time to endocrine AEs onset with ICIs is 7–20 weeks with ipilimumab and 10–11 weeks with nivolumab and pembrolizumab [139].

The clinical presentation of endocrine irAEs may be subtle and nonspecific, mimicking other clinical conditions. Thyroiditis is usually asymptomatic and can be detected by routine thyroid hormone monitoring, although in some cases, it may be preceded by a transient hyperthyroidism state, requiring anti-thyroid and β-blocker therapy. For hypophysitis, laboratory tests and brain MRI, showing an enlargement of pituitary gland, are mandatory to exclude other possible causes, including brain metastases [102, 140]. More rarely, ICIs may cause adrenal insufficiency or diabetes mellitus type 1.

Endocrine irAEs differ from other immune toxicities because of relatively late onset time and low frequency of complete resolution, due to permanent impairment of the gland function. Moreover, cancer treatment discontinuation is not always necessary, as life-threatening endocrinopathies are rare and most of the cases can be managed with treatment delay, hormone replacement and steroid use. A multidisciplinary management is required and endocrinology consultation for monitoring diagnostic tests and modulating hormone replacement therapy is needed [139, 140, 141]. Recently, the European Society of Medical Oncology (ESMO) developed specific guidelines for diagnosis, treatment and follow-up of immunotherapy toxicities, including endocrine AEs [108]. In general, G1 toxicities do not require specific treatments, and oral steroid therapy (0.5–1.0 mg/kg/day) should be started only in case of G2 toxicities, but immunotherapy can be continued in case of thyroid dysfunctions, whereas the use of high-dose steroids (methylprednisolone 1–2 mg/kg/day oral or i.v. for 3–5 days) should be prompted in case of G3 or 4 toxicities, considering the use of immunosuppressants in case of unresolved symptoms. High-grade toxicities require cancer treatment suspension and resumption should be

considered in patients with endocrinopathies that are controlled by hormone replacement therapy (even G4) [142].

19.10 Conclusions

The last decades have seen major advances in cancer therapy with long-term remissions and increases in survival. However, a growing number of patients are exposed to different toxicity profiles.

Every organ can be involved, and severity of toxicities can vary, although most are considered mild to moderate grade, easily manageable and reversible.

Early recognition of signs and symptoms can prevent serious complications.

Accurate knowledge in recognising toxicity spectrum associated with anticancer drugs and immunotherapy warrants an appropriate diagnosis, management and support for patients.

Training to facilitate patient education, multidisciplinary approach and careful follow-up are essential.

19.11 Summary of Clinical Recommendations

Knowledge of pathogenetic mechanisms and early recognition of signs and symptoms related to different adverse events in every day practice is crucial to assure safety and optimal outcomes.

Continuous monitoring, multidisciplinary approach, effective patient-clinician communication and comprehensive education are very important in optimising proper management of treatment-related adverse events.

Key Points

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- A significant heterogeneity in tumor response and toxicities is observed with most chemotherapeutic agents
- Chemotherapy-induced neutropaenia is a common complication in cancer treatment and is one of the major dose-limiting toxicities of systemic cancer chemotherapy
- Dermatologic adverse events, mostly of immunologic origin, are common in patients treated with check-point inhibitors
- Treatment and management of cardiotoxicity is very important and a multidisciplinary approach between cardiologists and oncologists is necessary to recognize and mitigate both early and late cardiac damage
- Several different endocrine AEs may be observed after treatment with ICIs targeting CTLA4 or PD-1/PD-L1.
- Peripheral neuropathy is the most common neurological complication of cancer treatment
- Renal dysfunction is common in patients with cancer, with a reported incidence of up to 60%.
- CINV is a highly complex condition that involves both the central and peripheral nervous systems.
- Pneumonitis may be observed during treatment with ICIs targeting the PD-1/PD-L1 pathways.

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Cardio-Oncology

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Contents

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20.1 Introduction

Cardio-oncology is an emerging and rapidly developing branch of cardiology, aimed at monitoring, early diagnosis, prevention, and treatment of cardiotoxicity (CTX) related to cancer therapies through all stages of cancer and in the survival period [1–3].

Over the past decades, there has been an extraordinary improvement in the survival rates, transforming cancer from a fatal disease to a condition that in some cases becomes chronic thanks to treatment [4]. Improved survival is associated with growing evidence of treatment-related complications. These include cardiac diseases. So, the risk of death from cardiovascular disease (CVD) may go beyond that from cancer [5]. Patnaik et al. found that cardiovascular disease was the leading cause of death among older female breast cancer survivors without an initial diagnosis of CVD [6]. Cardiovascular complications are frequent and can potentially impact morbidity and mortality. For this reason, correct management of cardiovascular adverse events has become an important element in the overall care for cancer patients. Furthermore, an increasing number of patients with pre-existing CVD are now being considered for cancer therapy, which adds another level of complexity [7]. Manifestations of CTX include left ventricular dysfunction (LVD) or heart failure (HF), thromboembolism, ischemia and vasospasm, pericardial disease, hypertension, and conduction and rhythm disturbances [8–11] (Fig. 20.1).

20.2 Chemotherapy and Radiation Therapy-Induced Cardiotoxicity

Anticancer agents can be classified according to their effects on cardiovascular system: (a) drugs affecting cardiac function (e.g., anthracyclines [ANT] and trastuzumab), (b) drugs affecting vascular function (e.g., 5-fluorouracil and capecitabine), and (c) those affecting both (e.g., bevacizumab and sunitinib). Radiation therapy causes injury to the myocardium, pericardium, valvular apparatus, and coronary vasculature from the epicardial to the microvascular level, even if recently more sophisticated approaches have been developed in order to reduce cardiovascular damage [7] (Fig. 20.2).

20.2.1 Chemotherapy-Induced Cardiotoxicity

Ewer and Lippman [12] proposed a classification for CTX in two types:

- Type I CTX (e.g., anthracycline-induced cardiac damage) that is due to myocardial cells cytolysis and therefore an irreversible and dose-dependent damage;
- Type II CTX (e.g., trastuzumab-induced cardiac damage) due to dysfunction of cardiac cells, thus creating a reversible and dose-independent damage.

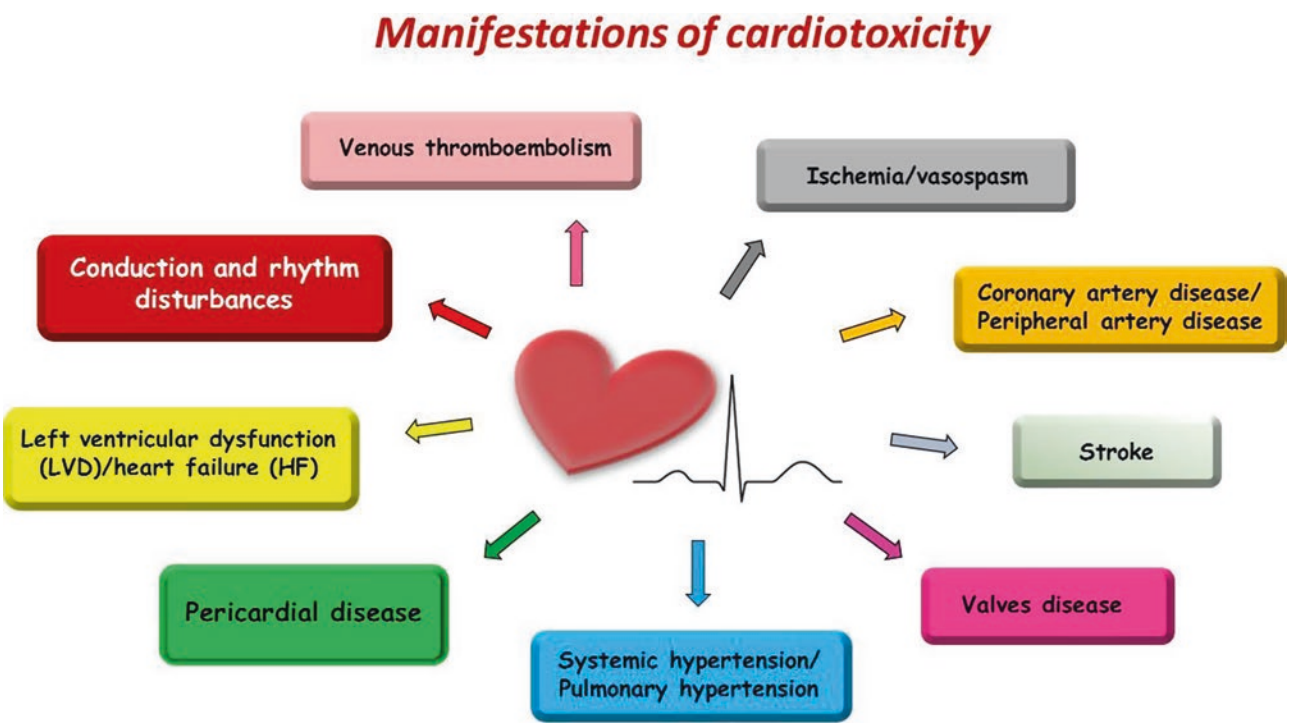

Fig. 20.1 Manifestations of cardiotoxicity

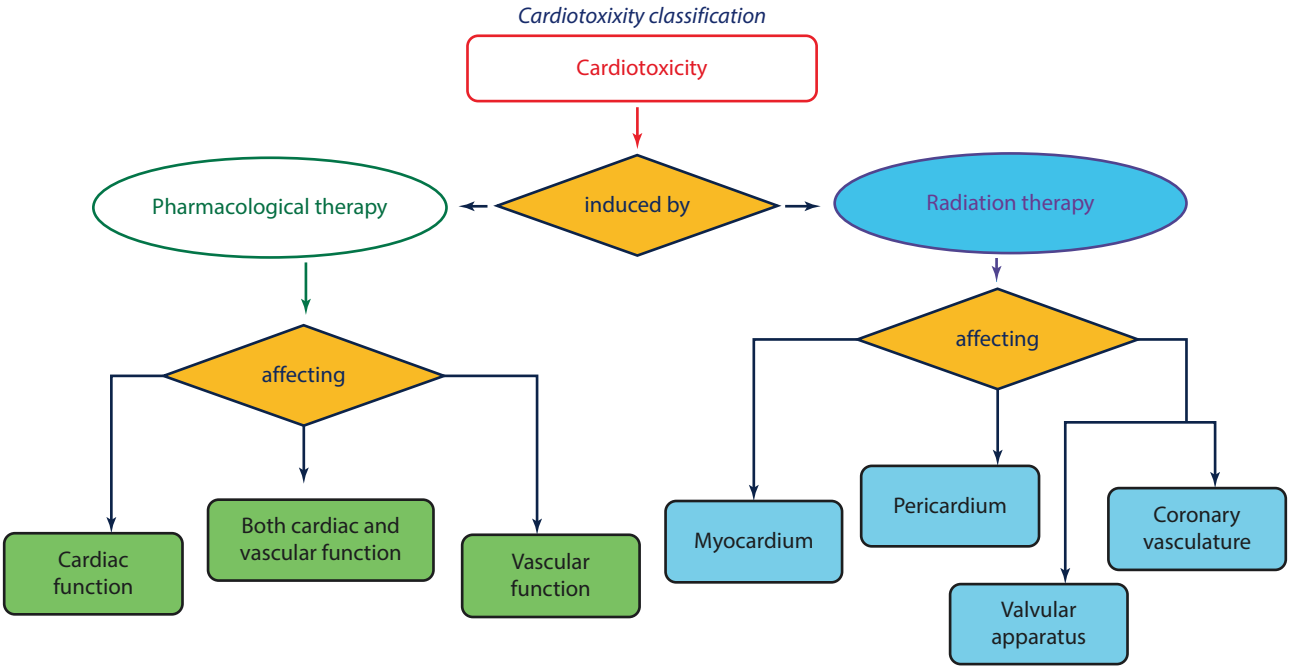

Fig. 20.2 Cardiotoxicity classification

20.2.1.1 Type I

Anthracyclines (ANT) are the prototype of drugs giving type I CTX. They are antibiotics frequently used in the treatment of many solid and hematologic tumors such as breast cancer, lymphoma, leukemia, and sarcoma. These drugs can cause cardiac damage through three mechanisms: (1) high affinity binding of ANT to DNA, with subsequent blockage of nucleic acid synthesis; (2) binding of ANT to cell membranes, altering ion transport; (3) oxidative stress leading to cardiomyocyte death [13]. These events determine structural changes, so, this type of toxicity has been widely accepted as irreversible. However, recent observation suggested that this is not completely true, because damage can be reversed if a cardioprotective therapy is started early [14].

Besides, according to the timing of occurrence of ANT-induced CTX, it could be categorized as acute or chronic. Acute toxicity is rare (<1%). It usually occurs during or shortly after chemotherapy administration. It is represented mainly by cardiac arrhythmias, transient LVD, and electrocardiography (ECG) changes. Chronic toxicity can occur early (within the first year of treatment) or late (after the first year with a median of 7 years after treatment), and it usually manifests as HF [15–17]. The risk of ANT-induced CTX increases with the increase in cumulative dose. Its incidence is low (3%) for doxorubicin dose up to 300 mg/m^2 increasing to 18% at a dose of 700 mg/m^2.

The risk factors for ANT-related CTX include lifetime cumulative dose or any other condition that increases cardiac susceptibility, including pre-existing cardiac disease, hypertension, concomitant use of other chemotherapies, mediastinal radiation therapy, or old age [18].

Alkylating agents act by causing DNA strand breaks and cross-linking [19]. Particularly, cyclophosphamide if given at high dose (140 mg/kg) causes myocarditis, pericarditis, and HF, which are irreversible in 25% of cases. Risk factors include total bolus dose, older age, combination therapy, previous treatment with ANT, and mediastinal radiation [20, 21]. Cisplatin is another alkylating agent that can cause myocardial ischemia, HF, late cardiac complications (such as hypertension), and arrhythmias (as a consequence of hypomagnesemia and hypokaliemia, due to the nephrotoxic effects of this drug) [22].

Taxanes are agents targeting microtubules that act by preventing the depolymerization of microtubules and thus the progression of cells through the M-phase of the cell cycle. Their cardiotoxic effect is moderate if used alone, while it increases if used in association with ANT or trastuzumab [23].

Paclitaxel is a prototype of pro-arrhythmogenic drug having a chronotropic effect either indirectly through histamine release or directly on the Purkinje system [24]. It interferes with the conduction of electrical stimuli, potentially causing sinus bradycardia, atrioventricular block, and ventricular tachycardia. In some cases, it can provoke hypertension and an increase in the risk of HF development, if in combination therapy with ANT and trastuzumab [25].

Docetaxel is a drug frequently used in breast cancer, in combination with or subsequent to ANT, cyclophosphamide or trastuzumab. It also appears to increase the incidence of HF [26].

20.2.1.2 Type II

Trastuzumab is a humanized monoclonal antibody. It is the prototype drug of type II CTX. It interferes with the human epidermal growth factor receptor HER2, whose inhibition on cardiac cells blocks an important protective pathway mediated by neuregulin [27]. In clinical trials, in which trastuzumab was used in the adjuvant setting, the incidence of left ventricular dysfunction was 20%, while clinically evident HF was 1.5%. So, in real life every day practice, the incidence could be higher [28]. Trastuzumab-induced cardiac damage usually disappears after treatment withdrawal [29].

Also, vascular endothelial growth factor (VEGF) inhibitors, such as bevacizumab, could cause this type of toxicity. It is a monoclonal antibody that binds to VEGF, preventing its interaction with its receptors, which reside primarily on endothelial cells, leading to inhibition of tumor angiogenesis. It can cause high blood pressure, LVD, HF, myocardial ischemia, and atherothrombotic events (ATEs). Cardiac dysfunction regression has been showed, suggesting no structural damage to cardiomyocytes, despite lacking histological confirmation. The underlying mechanisms likely include interference with endothelial function and reduced availability of nitric oxide, creating an imbalance between vasoconstriction and vasodilation, favoring vasoconstriction and capillary rarefaction [7, 30]. Antiangiogenic tyrosine kinase inhibitors (TKIs) such as pazopanib, axitinib, sorafenib, and particularly sunitinib are used to treat many metastatic tumors and show important results, but they can also cause cardiovascular complications that interfere with the VEGF pathway, particularly if they are used with or after conventional chemotherapies. The most frequent adverse effect of these drugs is arterial hypertension, even though they can also cause venous thromboembolism, coronary artery disease (CAD), and more rarely cardiac dysfunction and symptomatic HF [31–34].

20.2.2 Radiation Therapy-Related Cardiotoxicity

Radiotherapy is a well-known risk factor for CVD, even years after exposure. Radiotherapy can cause macro- and microvascular alterations along with endothelial dysfunction, atherosclerosis, valvulopathies, and pericardium fibrosis. LVD and HF manifest as a long-term effect of fibrosis that produce restrictive cardiomyopathies [35]. The presence of cardiovascular risk factors and the concomitant use of ANT could increase the probability of CTX development [36]. A recent retrospective study reported a linear association between coronary disease and dose of radiations [37]. Modifications of radiation protocols, careful radiation field planning, and techniques such as breath holding have been implemented to reduce the radiation dose to the cardiovascular structures. As outlined in recent studies and not without controversy, however, there may not be a threshold level below which radiation therapy is safe to the heart and the vascular system [38].

20.3 Cardiovascular Complications of Anticancer Treatment

Since the early identification of chemotherapy-induced cardiovascular damage is very important, the cooperation in a multidisciplinary team including oncologists, cardiologists and, of course, the patient is crucial. Before the initiation of cancer therapy, patient history and physical examination should be performed carefully to determine the baseline risk of developing cardiovascular toxicity. In particular, two kinds of risk factors should be taken into account. First, patient-related factors such as cardiovascular risk factors, age, and the concomitant presence of cardiovascular disease. Second, factors related to the antineoplastic drug such as the specific drug used and the exposure to a previous antineoplastic treatment (e.g., ANT, radiotherapy). Before starting treatment, it is advisable to manage cardiovascular risk factors and optimize treatment of concomitant cardiovascular disease. Moreover, it is recommended in most of the cases to perform a 12-lead electrocardiogram (ECG), to measure blood pressure, and to evaluate cardiac function with the best available method. Once the treatment is started, patient should be regularly followed up with a timing that depends on the baseline cardiovascular risk profile, the specific cancer treatment regimen, and the development of cardiac symptoms or events. Cancer therapies could give a wide spectrum of cardiovascular adverse events. The type of adverse events induced differs between chemotherapy, target therapies, and monoclonal antibodies (Table 20.1).

20.3.1 Heart Failure/Left Ventricular Dysfunction

HF is a clinical syndrome characterized by typical symptoms, such as shortness of breath and fatigue, and signs, which are increased jugular venous pressure, pulmonary crackles, and peripheral edema. These are caused by a

Table 20.1 Potential cardiovascular adverse events

Drug	Adverse event						
	Hypertension	Myocardial ischemia/ infarct	Thromboembolism	QT prolongation/ ECG changes	HF or related symptoms	LVD/EF reduction	Pleural or pericardial effusion
Anti-HER2							
Trastuzumab	✓		✓	✓	✓	✓	
Lapatinib				✓	✓	✓	
Pertuzumab						✓	
Trastuzumab emtansine	✓					✓	
Anti-angiogenic monoclonal antibodies							
Bevacizumab	✓		✓	✓	✓		
VEGFR-TKI							
Sorafenib	✓	✓	✓	✓	✓		
Sunitinib	✓			✓	✓	✓	
Pazopanib	✓	✓	✓	✓	✓	✓	
Vandetanib	✓		Rare	✓	✓		
Axitinib	✓	✓		✓	✓		
Vatalanib	✓				✓		
Nintedanib	✓		Rare		✓		
Regorafenib	✓	✓	✓		✓		
BRAF inhibitors							
Vemurafenib	✓			✓	✓		
Dabrafenib							
ALK inhibitors							
Crizotinib			Rare	✓			
BCR-Abl gene inhibitors							
Imatinib			Rare	Rare	Rare		Rare
Dasatinib	✓		Rare	✓			✓
Nilotinib	✓		✓	✓	✓		
Ponatinib	✓		✓	✓	✓		
Bosutinib	✓		✓	✓	✓		
Anthracyclines							
Doxorubicin				✓	✓	✓	
Epirubicin			Rare	✓	✓	✓	
Pegylated liposomal doxorubicin			Rare	Rare	✓		

structural and/or functional cardiac abnormality, resulting in reduced cardiac output and/or elevated intracardiac pressures at rest or during exertion [39]. The current definition of HF limits it to stages in which clinical symptoms are apparent. However, according to the ACC/AHA staging, symptomatic ventricular dysfunction is often an avoidable late stage of a chronic process. In fact, the onset of clinically evident cardiac HF can be delayed or prevented through the modification of risk factors and treating asymptomatic left ventricular systolic dysfunction [40]. Anticancer drugs that could determine HF/LVD are summarized in ◘ Table 20.2. In order to detect HF, the evaluation of clinical symptoms and physical examination are crucial. However, management based only on symptoms is limiting and often inefficient to prevent the occurrence of damage. In fact, symptoms occur when damage is already established. Therefore, it is advisable that patients undergo a baseline and then periodical instrumental evaluation including electrocardiography (ECG), measurement of cardiac function through cardiac imaging techniques such as echocardiography, nuclear imaging, cardiac magnetic resonance (CMR), and biomarkers such as troponin and natriuretic peptides.

◘ **Table 20.2** Anticancer drugs potentially inducing HF/left ventricular dysfunction

Drug		HF/LVD incidence (%)
Anthracyclines	*Doxorubicin*	3–48
	Idarubicin	5–18
	Epirubicin	0.9–11
	Mitoxanthone	2.6
	Liposomal anthracyclines	2
Alkylating agents	*Cyclophosphamide*	7–28
	Ifosfamide	0.5–17
Antimetabolites	*Clofarabine*	27
Antimicrotubule agents	*Docetaxel*	2.3–13
	Paclitaxel	<1
Monoclonal antibodies	*Trastuzumab*	1.7–20
	Bevacizumab	1.6–4
	Pertuzumab	0.7-1.2
TKI	*Imatinib*	0.2-2.7
	Sunitinib	2.7–19
	Pazopanib	7–11
	Sorafenib	4–8
	Nilotinib	1
	Dasatinib	2–4
	Lapatinib	0.2-1.5

20.3.1.1 Cardiovascular Evaluation of Patients Undergoing Treatment at Risk of HF/ LVD

ECG, including QTc measurement, is recommended in all patients before starting and during treatment [39]. On the other hand, echocardiography is the method of choice for the detection of systolic and diastolic dysfunction before, during, and after cancer therapy. Evaluation of left ventricular systolic function can be performed by measuring ejection fraction with biplane Simpson method, if available 3D echocardiography is preferred. In case of suboptimal acoustic windows, the use of contrast echocardiography is encouraged in order to improve profiling of the left ventricular endocardial borders. Cancer therapeutic-related cardiac dysfunction (CTRCD) is defined as a decrease of >10 percentage points in LVEF to a value below the lower limit of normal range in repeated measurements. However, the lower limit value of the normal range is still controversial because an ESC position paper considers it to be 50%, while according to the EACVI/ASE document, it is considered to be 53% [18, 41]. Besides, ejection fraction (EF) is not a sensitive tool to detect early cardiac damage since it decreases when significant toxicity has already occurred. Myocardial deformation imaging is a more sensitive tool to detect subtle cardiac damage. Particularly, a reduction of global systolic longitudinal myocardial strain (GLS) can predict a subsequent decrease in LVEF [42, 43]. According to the EACVI/ASE position paper, a decrease of GLS by >15% from baseline can be considered a marker of subclinical left ventricular dysfunction [41]. CMR could be a helpful tool for the evaluation of cardiac structure and function because it has high accuracy. It represents the gold standard for mass and volume evaluation and for ejection fraction value measurement of both ventricles. In addition, this unique technique provides additional information on tissue characterization. In fact, chemotherapeutic agents can cause edema and hyperemia, but also cellular necrosis with subsequent fibrosis [44]. This procedure is actually used to improve the accuracy of EF measurement if the quality of echo images is unsatisfactory or to confirm the EF value acquired through echocardiography, especially to determine if treatment suspension is necessary.

Cardiac biomarkers may play a complementary role to cardiac imaging. Several biomarkers have been investigated to estimate the level of CTX. The most studied are cardiac troponins (cTns). They are released in

response to myocardial injury and can be detected long before the reduction of EF. It has been shown that the increase in cTn in patients treated with high doses of ANT allows the categorization in patients with a low risk of developing chemotherapy-induced CTX and patients with a high risk. This last one requires an accurate cardiac monitoring [45]. In patients treated early with ACE inhibitors presenting a subtle damage detected through troponin elevation, the occurrence of clinically evident ejection fraction reduction can be prevented [46]. In breast cancer patients, a small study demonstrated that the combination of high sensitivity troponin with GLS might provide the greatest sensitivity (93%) and negative predictive value (91%) to predict successive CTX [42]. Based on these recent data, the EACVI/ASE expert consensus for multimodality imaging evaluation of adult patients during and after cancer treatment proposed an integrated approach using cTn measurement and echocardiography, including GLS for early detection of CTX [41].

The use of natriuretic peptides to detect HF has also been widely investigated. Increased levels can identify high-risk patients and guide therapy [47]. Currently, there is no clear evidence that allows to maintain or interrupt treatment relying only on an abnormal cardiac biomarker value or on the evidence of GLS value reduction. However, an abnormal result identifies high-risk patients that should undergo a close cardiological surveillance. Besides these patients may benefit from ACE inhibitor treatment [18].

20.3.1.2 Management

Cancer patients presenting symptoms for clinical HF during or after cancer treatment should be treated according to the current guidelines for HF. In patients in stage A, which are those at risk of HF development, receiving potentially cardiotoxic treatments, it is important to address and correct modifiable cardiovascular risk factors (e.g., smoking, hypertension, diabetes, dyslipidemia, obesity) and to optimize treatment of concomitant cardiac disease, before starting cancer therapy. Those patients who develop stage B or C, a different approach is warranted. Neuro-hormonal antagonists (ACE inhibitors, mineral-corticoid receptor antagonist, and beta-blockers [BB]) have shown improved survival. They are recommended for the treatment of every patient with HF with reduced EF (HFrEF) unless it is contraindicated or not tolerated [39]. Patients in stage C who remain symptomatic despite ACE inhibitors (ACE-I) treatment may also benefit from the combination drug sacubitril/valsartan. In stage D, treatment options are fluid restriction, inotropic agents, mechanical circulatory support, heart transplantation, and palliative or end-of-life care. Those patients that are considered at risk of developing CTRCD, the benefit/risk ratio of antineoplastic treatment should be carefully evaluated case by case by the cardio-oncology team and if there are some non-cardiotoxic drugs available, these should be preferred. If a cardiotoxic drug is started, patients should be concurrently started on cardioprotective treatment with ACE-I and/or β-blockers (BB). Besides, they should undergo a close cardiological follow-up [18]. Other potential options to reduce the risk of CTRCD include the use of preparations with a potentially less cardiotoxic profile (e.g., liposomal doxorubicin, continuous infusion), reduction of the cumulative dose, and use of dexrazoxane, an intracellular iron-chelating agent that prevents ANT-mediated damage. This last one is recommended in Europe only for adults with advanced or metastatic breast cancer who have received high cumulative doses of anthracycline and would benefit from continued anthracycline-based therapy. In patients who develop CTRCD during anticancer treatment, the opportunity to continue or withhold this treatment should be carefully evaluated by the cardio-oncology team based on the presence and severity of HF symptoms, tumor stage, expected benefits from the treatment, and availability of alternative not cardiotoxic treatment. The use of ACE-I, BB, or angiotensin receptor blockers (ARBs) for primary prevention of ANT-induced CTX is an ongoing area of active investigation, even though today evidences are not univocal. As regards BB, an observational study [48] and a randomized clinical trial [49] studying respectively carvedilol and bisoprolol found that the prophylactic use of beta-blockers decreased the risk of HF. The Prevention of Cardiac Dysfunction During Adjuvant Breast Cancer Therapy (PRADA) study demonstrated that the candesartan arm had a significant, but moderate attenuation in the decline of LVEF when compared with metoprolol or placebo [50]. However, another placebo-controlled study failed to demonstrate the cardioprotective effect of candesartan in patients with breast cancer treated with trastuzumab [51].

20.3.2 Systemic Hypertension

New-onset or worsening of systemic hypertension can be registered using several types of cancer agents. It is particularly common with drugs inhibiting the vascular endothelial growth factor (VEGF) signaling pathway. Table 20.3 reports some of the main antineoplastic drugs inducing arterial hypertension. The mechanisms through which VEGF inhibitors increase blood pressure can be directly related to the inhibition of VEGF pathway. VEGF signaling is important for proper endothelial functioning and nitric oxide synthesis; thus, its inhibition impairs vasodilation and favors an imbalance toward vasoconstriction [52] and thus causes hyperten-

Table 20.3 Anticancer drugs potentially inducing arterial hypertension

Drug		Arterial hypertension (%)
Alkylating agents	*Cysplatinum*	Dose-dependent
VEGF inhibitors-monoclonal antibodies	*Bevacizumab*	22–24
VEGFR-TKI	*Sunitinib*	15–35
	Ponatinib	67
	Pazopanib	36–46
	Sorafenib	17–29
	Regorafenib	28–48

sion. This generates a paradox, since the presence of hypertension is simultaneously an adverse cardiovascular effect and the sign of an effective oncological therapeutic response [53]. It is recommended that patients undergoing cancer treatment that has a known hypertensive risk should undergo a careful evaluation of their baseline status [54]. Hygienic and dietary measures should always be encouraged, with guidance on the practice of physical activity, low-sodium diet, and body weight control. Newly diagnosed hypertension should be treated before starting the antineoplastic treatment. Besides, treatment should be optimized if hypertension is poorly controlled. There are no specific guidelines for the treatment of hypertension in cancer patients. It could be reasonable to start antihypertensive treatment with cardioprotective drugs such as ACE-I, ARBS, and BB. Dihydropyridine calcium channel blockers could also be a therapeutic option but those that are not dihydropyridines should be avoided because they inhibit cytochrome P450 3A4, which can result in higher levels of VEGF inhibitors. Diuretics should be used with caution because they could induce an electrolyte imbalance in these patients. So, they could expose patients to the risk of developing arrhythmias [39]. In cases of severe, resistant hypertension, therapy should be interrupted. This action, usually, promptly and effectively decreases blood pressure [54].

20.3.3 Pulmonary Hypertension

A certain number of small molecule tyrosine kinase inhibitors have been associated with the development or exacerbation of pulmonary hypertension (PH) [54, 55]. These molecules may affect many molecular pathways, including those involving epidermal growth factor inhibitors or vascular endothelial growth factor receptor inhibitors. Given the relationship of several agents in this drug class with the development, exacerbation, and treatment of PAH, these pathways likely play an important role in pulmonary vascular homeostasis. Dasatinib, a second-generation Philadelphia chromosome inhibitor has been most strongly implicated as a causative agent of PAH. It appears that the development of PH increases with prolonged duration of therapy. Increases in pulmonary arterial pressure can occur in up to 11% of patients treated with dasatinib. Experimental studies suggest that this is a consequence of smooth muscle hyperplasia and endothelial dysfunction. Discontinuation of dasatinib may result in PH resolution, even though not in all cases. Response to specific PH therapies has been reported in case series and case reports. In contrast, imatinib, a first-line tyrosine kinase inhibitor used as first-line chronic myeloid leukemia therapy, appears to have a potential role in the treatment of PH in experimental studies [56]. Several chemotherapeutic agents are implicated in the development of pulmonary veno-occlusive disease (PVOD), which may be clinically indistinguishable from PH. Specifically, bleomycin and cyclophosphamide have been reported as important causes of PVOD based on registry data and several animal models [57]. The overall risk is about 10%, emerging gradually during therapy and even years later. A distinctive feature is the development of pulmonary fibrosis. Finally, interferon-α can induce pulmonary vasculitis and pulmonary hypertension for unknown reasons. All patients should undergo evaluation for signs and symptoms of underlying cardiopulmonary disease before initiation of treatment and during it with the mentioned drugs. Dyspnea, hypoxia, cough, fatigue, and abdominal and lower extremity edema should prompt an immediate evaluation, which should include an ECG, chest X-ray, and echocardiography. Contrast and high-resolution chest computed tomography is useful to address a number of potential disease processes such as pulmonary embolism and pneumonitis. Pulmonary function tests instead are useful to define the functional nature of a pulmonary disease process. Depending on the findings, these patients should be referred to a specialist. Definitely, pulmonary hypertension should be managed accordingly to published guidelines [58].

20.3.4 Coronary Artery Disease

Cancer treatment could be associated with accelerated development of ischemic heart disease [55, 59]. Cancer itself contributes to a prothrombotic state, promoting acute ischemic syndrome. Potential mechanisms of isch-

emia and arterial thrombosis through which chemotherapeutic agents cause ischemia are vasospasm (e.g., antimetabolites, anti-microtubule agents), endothelial dysfunction (e.g., angiogenesis inhibitors, alkylating agents), platelet activation and thrombosis (angiogenesis inhibitors, platinum compounds), and accelerated atherosclerosis (radiotherapy) [60]. Table 20.4 reports the main drugs that induce vascular damage. The most evident example is 5-fluorouracil (5-FU). In fact, literature data reported an incidence of angina ranging from 1% to 45%. The onset of chest pain is often abrupt during infusion of 5-FU, but can also have a delayed presentation within the first 72 h. There is a higher incidence of angina with continuous infusion compared to bolus infusions. The adverse event is not dose-dependent, besides cessation of 5-FU administration results in resolution of angina although symptoms have been reported to last up to 12 h. Re-initiation of 5-FU has shown increased incidence of angina with severe complications including acute coronary syndrome, hypotension, HF, and even death. It seems to be related to an alteration in vascular reactivity [54]. Coronary angiography usually does not reveal significant coronary artery disease (CAD) or acute plaque rupture. This leads to the suspicion that coronary vasospasm is the etiology. Capecitabine is its oral prodrug, which is converted to 5-FU. It has also been accountable for angina and acute coronary syndrome [55]. Gemcitabine, used to treat various carcinomas, has been reported to cause acute coronary syndrome. Literature data describing coronary complications from capecitabine and gemcitabine are much more limited if compared to that describing complications with 5-FU. Cisplatin, especially if combined with bleomycin or vinca alkaloids, can provoke chest pain presentations at an incidence as high as 40%. The propensity of these drugs to injure the endothelium is well-established, and endothelial dysfunction is therefore the key mechanism of altered vasoreactivity with these drugs. Another class of chemotherapeutic agents known to induce similar types of chest pain consists of taxanes, namely paclitaxel at an incidence of 0.2%–4%. Similar to 5-FU, vasospasm has been proposed to be the underlying mechanism. VEGF signaling pathway inhibitors are another class of drugs well-known to be associated with angina at an incidence of 1%–15%. Again, endothelial dysfunction likely plays an important role because inhibition of VEGF receptor signaling impairs stimulation of endothelial NO synthase activity through the Akt/PKB pathway. Moreover, endothelial NO synthase uncoupling may occur with an increase in oxidative stress, activation of the endothelin system, supporting an abnormal vascular reactivity and structure. On the other hand, bevacizumab reduced neovascularization and growth of established plaques similar to the effect determined by the other inhibitors targeting angiogenesis in experimental models. Thus, VEGF signaling pathway inhibitors may be unique in their capacity to alter vasoreactivity and the atherosclerotic disease process with potentially important differences between them [54, 55]. Radiotherapy can accelerate the atherosclerotic processes and induce coronary vasospasm. Radiotherapy-induced coronary lesions are usually ostial (left anterior descending artery is mainly affected during breast cancer irradiation of the left hemi-thorax) and potentially may induce life-threatening complications [35].

Table 20.4 Anticancer drugs potentially inducing coronary artery disease

Drug		Coronary artery disease (CAD) (%)
Alkylating agents	*Cyclophosphamide*	2
	Cysplatinum	2
Antimetabolites	*5-fluorouracil*	1–68
Antimicrotubule agents	*Docetaxel*	<1.5
	Paclitaxel	1.5–2
VEGF inhibitors-monoclonal antibodies	*Bevacizumab*	0.6–8.5
VEGFR-TKI	*Sunitinib*	1.4
	Ponatinib	12
	Sorafenib	1.7
	Nilotinib	5–9.4

20.3.4.1 Cardiovascular Evaluation of Patients Undergoing Treatment at Risk of Coronary Artery Disease

Myocardial ischemia can occur as adverse event with several anticancer therapies through different mechanisms. CAD can present with typical or atypical clinical manifestations or develop without symptoms, which is also called silent myocardial ischemia, with a prevalence that appears to be higher than that in the general population, likely because of concomitant neurotoxicity due to radio- and chemotherapy. A history of CAD is a factor that significantly increases the risk of anticancer therapy-related future events. This is why an accurate history before initiating cancer treatments is crucial. The presence of clinical symptoms suggestive of coronary artery disease (CAD) should be further investigated and in case of previous CAD, its treatment should be optimized. Moreover, a 12-lead ECG and echocardiography

should be obtained before starting the treatment and then periodically. Especially in treated mediastinal radiation, coronary artery disease can occur even many years after the exposure; therefore, a long-term surveillance program should be planned. In case of CTX, clinical scenarios can vary ranging from type I myocardial infarction, due to plaque erosion and thrombosis, to type II myocardial infarction or angina pectoris in case of significant stable atherosclerotic plaques. Since cancer itself causes a procoagulant state, in general, patients with cancer are also at risk of developing atypical mechanisms of acute coronary syndromes by thromboemboli through a patent foramen ovale or even tumor embolization, spontaneous coronary artery dissection, and extrinsic compression by a tumor mass (Fig. 20.3). Chemotherapy should be withheld if myocardial ischemia occurs. In patients undergoing antineoplastic drugs that cause vascular damage, early detection of preclinical atherosclerosis is particularly important. This is important for an accurate cardiovascular risk stratification and thus for starting protective therapies early such as aspirin and statins. For this reason, carotid ultrasound focused on the measurement of intima-media thickness (IMT) and evaluation of the presence and characteristics of plaques. These data contribute to refine cardiovascular risk stratification. An increased IMT (>0.9 mm) or the presence of plaque (focal wall thickening >1.5 mm) is associated with an increased risk of stroke and cardiac events. However, the recent guidelines of the European Society of Cardiology on cardiovascular prevention suggest only carotid artery plaque assessment, as a modifier in cardiovascular risk prediction, in patients at intermediate risk. Another early marker of vascular damage is measurement of arterial stiffness. It evaluates vascular elasticity, and thus, it is an early marker of damage. An increased arterial stiffness predicts future CVD and improves risk classification [61, 62].

Coronary artery calcium (CAC) is also a non-invasive imaging technique. It has been showed that the extent of coronary calcifications correlates with the extent of total coronary plaque burden, but it does not correlate with plaque instability [63]. This test has a particularly high negative predictive value.

In the suspicion of coronary artery disease, another test that could be performed is stress echocardiography. This technique could reveal subtle defects of kinesis and left ventricular dysfunction.

20.3.5 Peripheral Artery Disease

Peripheral artery disease (PAD) can have different clinical presentations and etiologies. The primary presentation of limb ischemia in patients with cancer has been Raynaud's and can even be ischemic fingertip necrosis [54, 64]. The incidence can be as high as 30% and can signal systemically abnormal vasoreactivity and even myocardial infarction risk. It has been reported for bleomycin, vinca alkaloids, cisplatin, carboplatin, gemcitabine, and interferon-α. A second structural form of chemotherapy-induced vascular disease, named occlusive disease, has been recognized with the emerging use of nilotinib and ponatinib. These are TKIs targeting the Bcr-Abl oncogenic fusion gene product. This entity is characterized by rapidly progressive atherosclerosis even with the institution of an appropriate treatment. Cardiovascular risk stratification and rigid correction of cardiovascular risk factors are also recommended in patients receiving these drugs, as well as surveillance of the occurrence of symptoms suggestive of claudications, pulses check, blood pressure control, and ECG. Echocardiography and ultrasound scan of carotid arteries to stratify cardiovascular risk and of the lower limbs should be considered according to clinical judgment.

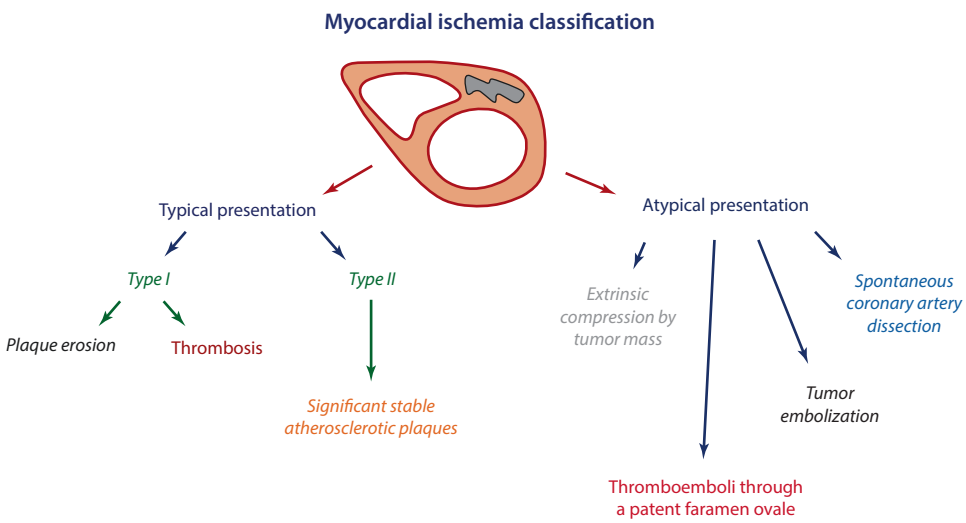

Fig. 20.3 Drug-related myocardial ischemia classification

20.3.6 Stroke

Cancer and stroke may occur independently in a certain patient. Otherwise, cancer could directly or indirectly lead to stroke through hypercoagulable, non-bacterial thrombotic endocarditis (disrupted fibrin attached to previously undamaged valves in high flow areas and developing a network into which platelets can adhere), direct tumor compression of blood vessels, or treatment-related effects that potentiate stroke [65]. Head and neck radiation cause vasculopathy of medium- and large-sized vessels that often presents years after radiation exposure. This vasculopathy is not well-characterized, but could be associated with accelerated atherosclerosis [66]. Some chemotherapeutic agents have also been associated with an increased risk of stroke, such as cisplatin, methotrexate, and L-aspariginase. The combination of bevacizumab with 5-FU or carboplatin-based therapies could double, sometimes even more, than the overall incidence of arterial thromboembolic events, especially in those patients ≥65 years of age or with a previous arterial thromboembolic event (15.7%) [67]. Consideration similar to CAD and PAD also applies to stroke prevention. Particularly, patients irradiated for head and neck cancer or lymphoma should undergo cerebrovascular ultrasound screening, especially beyond 5 years after irradiation [39].

20.3.7 Venous Thromboembolism

Venous thromboembolic events can occur in patients treated with various antineoplastic treatment such as drugs targeting the VEGF/VEGFR pathway (e.g., bevacizumab and TKIs), especially if used in combination with chemotherapy. Venous thrombosis is favored not only by treatment but also by patient-related factors such as stage, site, and histology of the tumor, comorbidity and low performance status, and presence of a central venous catheter. The suspicion is raised by clinical symptoms and signs. It can be confirmed by venous ultrasound. Venous thrombotic events are currently managed using low molecular weight heparin; however, recent evidences suggest that direct oral anticoagulants are safe and effective [68, 69].

20.3.8 Arrhythmias

Cardiac arrhythmias have been reported as an adverse effect of many chemotherapeutic drugs, including novel targeted therapies. Multiple mechanisms have been proposed to explain chemotherapy-induced cardiac arrhythmias. In general, cardiac arrhythmias can arise from direct electrophysiological effects, such as effects on cardiac sodium, calcium, and potassium ion channels and/or pumps involved in the genesis of cardiac action potentials, or cardiac indirect effects (many cardiac arrhythmias are initiated/maintained by an arrhythmogenic substrate created by the comorbidities present in cancer patients or generated by CTX induced by chemotherapy) [24].

QT prolongation (>450 ms in men and >460 ms in women) can be caused by cancer therapy, electrolyte disturbances, predisposing factors, and concomitant medications. Since it can lead to life-threatening arrhythmias such as torsade de pointes, the duration of the QT interval and risk factors for QT prolongation should be checked before, during, and after cancer treatment. The list of drugs that can induce QT prolongation includes ANT, 5-fluorouracil, taxanes, cisplatin, TKIs such as lapatinib and sunitinib, and arsenic trioxide, which is the most relevant. This last drug is used to treat some leukemias and myelomas, prolongs the QT interval in 26–93% of patients, and life-threatening ventricular tachyarrhythmias have been frequently reported [70].

As regards atrial fibrillation (AF), several studies have documented the relationship between cancer and AF. In terms of epidemiology, AF in cancer could be divided into two forms according to whether it occurs during the peri-operative period of cancer surgery (peri-operative AF) or not. This distinction is important as peri-operative AF is characterized by relatively high incidence rates, particularly in patients undergoing pulmonary resection for lung cancer. In the largest study on cancer-related peri-operative AF on nearly 14,000 patients undergoing pulmonary resection, the AF incidence rate was 12.6%. In addition, several drugs used for the treatment of cancer have been found to induce AF. These drugs include most of the cytotoxic agents such as cisplatin, 5-fluorouracil, doxorubicin, paclitaxel or docetaxel, ifosfamide, gemcitabine, and mitoxantrone; high-dose corticosteroids; antiemetic agents such as ondansetron; targeted therapies; and bisphosphonates. A single study, however, argued against bisphosphonate related-AF [71]. Inflammation could be a common denominator in both conditions. It has been suggested that AF may actually represent an inflammatory complication of cancer [72]. In a population-based study in 5806 subjects followed for a median of 7.8 years, C-reactive protein (CRP) increase was associated both with the presence of AF at baseline (odds ratio for fourth vs. first CRP quartile: 1.8) and with future AF development (HR for fourth vs. first CRP quartile: 1.3) [73]. Given the lack of evidence, there are currently no specific guidelines for AF therapy in patients with malignancies. The decision to treat patients with anticoagulant therapy could raise some concerns, especially in tumors prone to bleeding such as intracranial or gastroenterological and also for che-

motherapy-induced thrombocytopenia, and coagulation defects, even in patients at high thromboembolic risk. On the other hand, certain malignant tumors such as pancreatic, ovarian, lung, and primary hepatic cancer are associated with an increased thromboembolic risk, and the same applies to several chemotherapeutic agents such as cisplatin, gemcitabine, and 5-fluorouracil and supportive therapies like erythropoietin and granulocyte-colony stimulating factors [74, 75]. As recently proposed by Farmakis et al., an individually tailored therapy is crucial, especially for antithrombotic prophylaxis, basing clinician decision on cancer features and established thromboembolic and bleeding risk assessment tools [71]. Ventricular arrhythmias are generally related to QT prolongation, acute and chronic toxicity of chemotherapy and radiotherapy, and metabolic or hormonal alterations.

20.3.9 Pericardial Disease

Pericardial disease is also common in oncologic patients, as a consequence of cancer therapies or metastasis. It usually occurs as pericarditis and pericardial effusion and more rarely as constrictive pericarditis, especially after radiotherapy [35] or high-dose chemotherapy. The most common malignancies associated with pericardial effusions are lung cancer, breast cancer, leukemia, and lymphoma [55, 76, 77]. Acute pericarditis may occur predominantly with the use of ANT, cyclophosphamide, cytarabine, and bleomycin. Transthoracic echocardiography is the method of choice for the evaluation of patients with suspected pericardial disease due to chemotherapy, but computed tomography could be helpful to identify calcification in constrictive pericarditis, and CMR should be considered in the evaluation of primary tumors of the heart. Pericardial effusion should be quantified and graded according to standard methods. It is important to evaluate the presence of echocardiographic and Doppler signs of cardiac tamponade in this setting of patients.

20.3.10 Valvular Disease

Radiation of the mediastinum is a component of paramount importance in the treatment of a wide range of cancers including Hodgkin's lymphoma and breast cancer, both as monotherapy or also if used in combination with chemotherapy. Exposure to radiation is associated with a risk of valve damage characterized by valve fibrosis and calcification [78, 79]. The risk of developing valvular heart disease (VHD) depends on the dose of radiation received. In the study from Cutter et al., the risk increased linearly with radiation dose, especially at doses above 30 Gy [80]. There is a long latent period before the effects of radiotherapy could manifest clinically. Hull et al. reviewed 415 patients who were treated with radiation therapy for Hodgkin's lymphoma, with a delay to valvular dysfunction and disease rates of 1% at 10 years, 4% at 15 years, and 6% at 20 years [81]. Left-sided valves are affected preferentially over right-sided valves, particularly the aortic valve. There are conflicting data on the use of chemotherapy before radiotherapy. In a study by Aleman et al., the risk of developing VHD was greater in patients who were treated with radiation and chemotherapy than in patients treated with radiation therapy alone. Importantly, this study did not address the type and degree of valvular dysfunction [82]. In contrast, Cutter et al. found no association between ANT chemotherapy and the prevalence of valvular disease [80]. Echocardiography is the cornerstone for the definition of the degree of VHD. Transesophageal echocardiography is necessary when the quality of images at 2D-echocardiography is poor. Furthermore, 3D-echocardiography adds information when it is necessary to define valvular morphology. Management of VHD follows current guidelines [83]. According to an algorithm proposed by Gujral et al., in patients with abnormal valve structure but mild dysfunction, echocardiography should be repeated every 2–3 years; in patients with moderate valve dysfunction every year; in patients with severe valve dysfunction surgery should be considered, assessing the individual risk [79].

20.4 Cancer Patients Enrolled in Clinical Trials

Measuring and monitoring cardiovascular function in oncology is essential, because cancer patients treated in everyday practice have an average medium to high cardiovascular risk. This is also important since cardiovascular disease represents the first cause of death in the world. The increase of survival in oncology, especially in certain types of cancer, exposes patients to a greater cardiological risk than the only oncological one. So, correct cardiovascular management of patients enrolled in clinical trials is very important in order to define the correct management of the patient since the first stages of the development of a cancer drug (Table 20.5) (Fig. 20.4). Correct baseline evaluation and subsequent checks of patients included in clinical study protocols are not easy to define. The involvement of a cardiologist in the clinical study could certainly improve the identification of cardiovascular toxicity of anticancer drugs in clinical trials. This should be preferably performed in a dedicated ambulatory for oncological patients in profit clinical trials, that is the cardio-oncology ambulatory for clinical trials.

Table 20.5 Cardiovascular evaluation during a clinical trial

	Screening visit	First month visit	Third month visit	Sixth month visit	Ninth month visit	First year visit
General visit and medical history	✓					
Physical examination	✓	✓	✓	✓	✓	✓
Vital signs (e.g., *blood pressure*)	✓	✓	✓	✓	✓	✓
ECG (QTc, PR, QRS)	✓	✓	✓	✓	✓	✓
Echo (LVEF, GLS)	✓		✓	✓	✓	✓
Echo of the supra-aortic trunks	✓			✓		✓
CMR	To be performed in specific cases					

Note: *Make additional measurements of one or more of these parameters, if necessary*

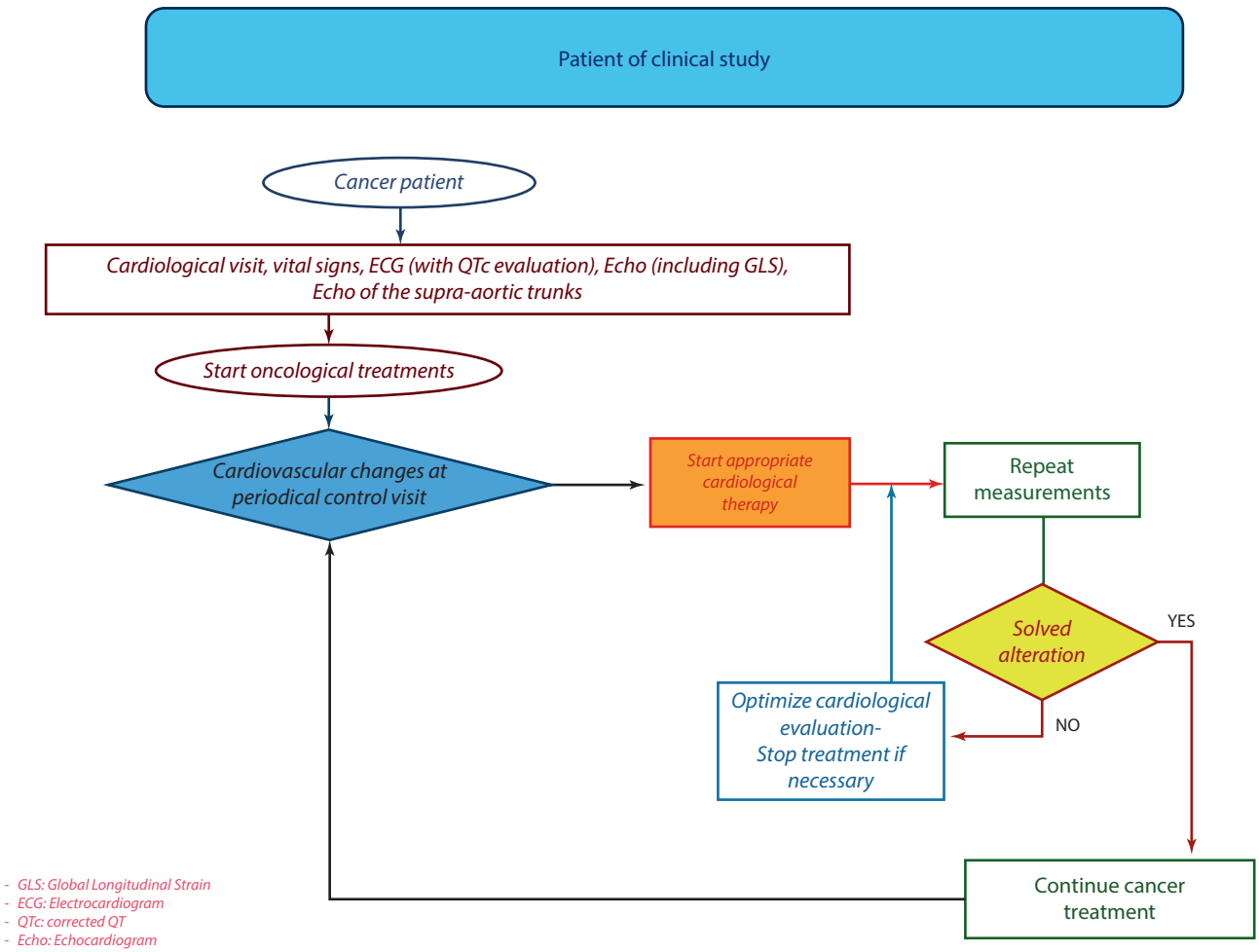

Fig. 20.4 Cardiological assessment in cancer patients enrolled in clinical trials

Management of Patients Undergoing Treatment with Potentially Cardiotoxic Antineoplastic Drugs

Before starting treatment

- Correction of cardiovascular risk factors.
- Optimization of medical treatment of current cardiac disease.
- Search for clinical symptoms underlying unknown cardiac disease.
- Complete cardiovascular examination (including pulses check especially if drugs determining vascular toxicity are used) and blood pressure measurement.
- ECG with QTc measurement.
- Measurement of cardiac function with the best available technique (usually echocardiography).
- Consider further investigation according to risk (ultrasound of carotid arteries, ABI or ultrasound of peripheral arteries, and stress test).
- Evaluation by cardio-oncology team if patient at high risk of developing cardiotoxicity.

During and after treatment

- Monitor regularly left ventricular function.
- Check vessel status if drugs causing vascular toxicity are used.
- Start readily cardioprotective drugs (ACE-I/ARB and beta-blockers) if cardiac damage is detected.
- Evaluation by cardio-oncology team if patient develops cardiotoxicity or if cardiotoxicity is not resolved.

Key Points

- Advances in cancer therapy and the increase in survival rates are associated with growing evidence of treatment-related complications, including cardiovascular disease. For this reason, this new branch of cardiology, cardio-oncology, was born.
- The aim of cardio-oncology is to facilitate cancer treatment, avoiding the occurrence of cardiovascular complications, and if they occur, to treat them promptly and efficaciously. Moreover, another target of this discipline is to promote the interaction between cardiologists, oncologists, hematologists, general practitioners, and other specialists in the interest of the health of cancer patients.
- It is important to stratify the risk of each patient to develop CTX before starting treatment through a careful cardiovascular examination.
- Besides, it is important to monitor patient cardiovascular health during treatment and to continue surveillance also after treatment interruption (see ▶ Box 20.1).
- These steps aim to minimize the burden of cardiovascular morbidity and mortality in patients with cancer who are treated with cardiotoxic agents and thus to improve their clinical outcome.

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Cancer Cachexia

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Contents

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Learning Objectives

By the end of the chapter, the reader will:

- Keep awareness off the clinical impact of cancer cachexia and the importance of early detection/interventions.
- Acquire in-depth knowledge of the pathophysiology of the syndrome.
- Get an overview of most relevant definitions and staging classifications.
- Be able to apply assessment tools to evaluate patients at risk and follow previously diagnosed patients.
- Be able to make evidence-based decisions regarding cancer cachexia treatment.
- Learn up-to-date information about recent therapeutic advances.

21.1 Introduction: Definitions and Epidemiology

Before the era of molecular medicine, the term *cachexia*, which derives from the fusion of the Greek terms *kakos* (bad) and *hexis* (habit), was extensively used to define a condition characterized by significant weight loss. Easily detectable by inspection on its advanced stages, even for the patient and for family members, it has been classically associated, whatever the underlying pathology, with an adverse clinical course and prognosis.

Cachexia is not a concept that is restricted to patients with cancer. It is a common condition associated with almost every chronic pathologic condition such as chronic kidney disease, heart failure, or long-lasting infections. It is estimated, for instance, that it might affect up to 10% of congestive heart failure (CHF) patients [1]. Nonetheless, cachexia can reach prevalence of up to 80% in patients with upper gastrointestinal tract malignant tumors [2] and is considered to be the direct cause of 20% cancer deaths [3].

In patients with advanced cancer, involuntary weight loss is often understood as a consequence that cannot be separated from neoplasm's natural history. However, the fact that not every patient with advanced cancer develops significant weight loss has led oncologists and palliative care experts to further study which pathological agents are involved in this multifactorial syndrome. The ultimate goal of these groups is developing new therapeutic approaches to specifically treat this severe cancer-associated comorbidity.

One of the most accepted definitions of cancer cachexia was published in 2011 by an international expert group, which defined cancer cachexia as a "multifactorial syndrome characterized by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support, and leads to progressive functional impairment" [4]. Its pathophysiology was defined by a negative protein and energy balance, driven by a variable combination of reduced food intake and abnormal metabolism. It was agreed to establish a body weight loss threshold on 5% over past 6 months, which was as low as 2% for patients who already presented body mass index (BMI) below 20 kg/m^2. Diagnosis of cancer cachexia could also be established by decreased muscularity determined by dual-energy X-ray absorptiometry (DEXA), bioelectrical impedance (BIA), or lumbar skeletal muscle index determined by computerized tomography (CT) imaging.

Noteworthy, cachexia prevalence is notably higher (up to 80%) among patients with gastric and pancreatic cancer [2] compared to other types of cancer. Prevalence is estimated to be above 50% for lung and advanced colorectal and prostate cancer patients and around 30% for advanced breast cancer individuals [5], although these data were recorded before consensus definition for cancer cachexia was established and, therefore, significant heterogeneity is present. Variability among specific tumor-bearing patients is influenced by the high abdominal symptom burden upper gastrointestinal cancers produce, including a significant decrease on daily nutritional intake. Therefore, healthcare professional should be cautious when labeling as cachectic every oncologic patient with significant weight loss.

Cancer cachexia should be classified as an additional paraneoplastic syndrome, since many of its causes have little to do with mass effect or invasion. It is noteworthy that, for instance, a patient with an obstructive pyloric gastric cancer who is not able to tolerate food should not be diagnosed with cachexia but with starvation (see Table 21.1).

Table 21.1 Differential diagnosis between cachexia and starvation

	Cachexia	Starvation
Caloric intake	↓	↓↓
Lean body mass	↓↓	↓
Fat mass	↓	↓↓
REE	↔ or ↑	↓
Acute-phase reactants	↔ or ↑	↔
Insulin	↑	↓
Ketogenesis	↓	↑

REE resting energy expenditure, ↓ reduced, ↓↓ markedly reduced, ↔ unchanged, ↑ increased

21.2 Clinical Impact

Cachexia is one of the most visual consequences of advanced cancer, but weight loss and its associated body image changes are just the facade of a complex metabolic syndrome that drives a myriad of highly conditioning symptoms. Cachectic patients usually refer asthenia, anorexia, and fatigue among other highly burdening symptoms. Anemia is a common associated feature, tightly related to the proinflammatory status that characterizes this syndrome.

Involuntary weight loss has been extensively related to a broad range of underlying pathologic conditions. Once a certain diagnosis is established and treatment implemented, ongoing wasting constitutes an alarm sign of an adverse clinical course and lack of therapeutic benefit. In cancer patients, data correlating pretreatment weight loss and clinical outcomes were first published in 1980 [2]. In this report, an association was made between weight loss and response to chemotherapy among breast cancer patients, even though no data were reported regarding potential differences on the amount of treatment administered to weight-losing patients.

Contemporarily, after weight loss was established as the main clinical parameter for the diagnosis of cancer cachexia [4], BMI and percentage of weight loss have been confirmed to be prognostic in a large observational study [6]. Notably, within each weight loss category, patients with lower BMI showed a shorter overall survival, suggesting a protective role of increased basal energy reserves.

All body image changes, high symptom burden, and threatened survival, which are perceived by patients and family members, lead to a significant psychological, social, and spiritual distress. Body image changes are perceived as one of the most distressing consequences of advanced cancer. Weight loss is often misunderstood by patients and relatives as a direct consequence of inadequate oral intake, which causes relevant dietary changes and even forced eating, which is often detrimental in terms of clinical outcome and quality of life [7]. Body image changes, when extreme, can lead to a significant social isolation. Particularly in cancer cachexia patients, for whom oncologic treatments and outcomes are frequently scarce and survival significantly shortened, quality-of-life issues should be correctly addressed (later in the Chapter) and must guide clinical decision-making.

21.3 Pathophysiology

Although it was initially thought that energy and substrate consumption by tumor cells significantly influenced development of cancer cachexia, the relationship between both factors remains unclear. In humans, profound cachexia often develops before tumor burden reaches 1% of body mass [8], which makes improbable that metabolism gets impaired solely by this factor.

Even with similar tumor subtypes and burden, some patients develop cachexia, while others do not. Clinical experience and research on sepsis have demonstrated that host response might be a critical factor conditioning survival in this pathology, and growing evidence shows that a similar phenomenon is probably occurring in patients with advanced cancer. For instance, a specific IL-10 allele has been consistently associated with an increased risk of developing cachexia [9]. Similarly, polymorphisms in P-selectin, a membrane glycoprotein involved in leucocyte-endothelium interactions, have been associated with weight loss in a large cancer patient cohort [10].

From an etiological point of view, there are two main causes that produce nutritional and metabolic impairment in cancer patients [11]:

- *Primary causes* are direct effects of the neoplastic disease other than those that are the consequence of anatomical invasion (i.e., biochemical factors released by tumor cells or proinflammatory cytokines produced by host cells as a consequence of the interaction with them). These mechanisms can affect both oral intake and metabolism of ingested nutrients (at an intestinal o cellular level), and they constitute the etiologic nucleus of cancer cachexia syndrome.
- *Secondary causes* are additional and partially treatable factors that contribute mainly to a decrease in oral intake. These include a great variety of nutritional-impact symptoms such as nausea and vomiting, constipation, pain, dyspnea, dysgeusia, xerostomy, and psychological disorders. Gastrointestinal obstructive symptoms must be placed here.

In terms of drawing a mechanistic scheme, there is agreement on considering cachexia a paraneoplastic and multifactorial syndrome involving changes in several metabolic pathways, tissues, and organs (Fig. 21.1). Even though the relative importance of each one of the factors can significantly vary between individuals, the overall pathologic scenario shows the following characteristics:

- Decreased oral intake due to anorexia and gastrointestinal symptoms
- Increased metabolic rate
- Muscle wasting and adipose tissue atrophy
- Proinflammatory status

21.3.1 Anorexia

Anorexia defines loss of appetite, and its presence is a constant symptom among cancer cachexia patients. As

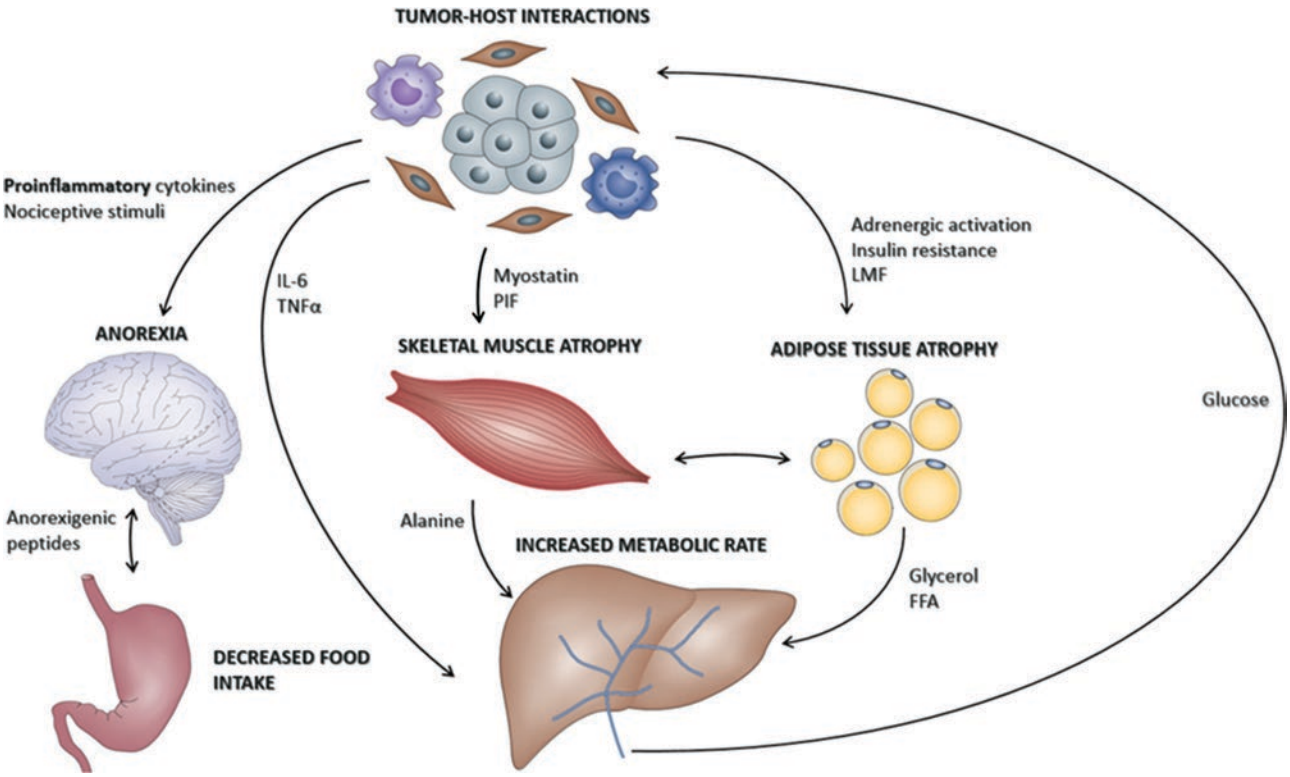

Fig. 21.1 Integrative pathophysiology of cancer cachexia. PIF proteolysis-inducing factor, LMF lipid-mobilizing factor, FFA free fatty acids

such, the term has been extensively incorporated to name the syndrome in the literature [12–14].

Central nervous system mediators are involved in food intake control, a fact that incorporates the brain to the myriad of tissues and organs involved in cachexia pathophysiology. The hypothalamus is a key region for energy balance control [15]. Its neurons secrete both orexigenic [agouti-related protein (AgRP) and neuropeptide Y (NPY)] and anorexigenic neuropeptides [cocaine- and amphetamine-regulated transcript (CART) and pro-opiomelanocortin (POMC)] under tight control. Melanocortin pathway alterations are central in oral intake regulation in cancer cachexia. POMC is a precursor of alpha melanocortin (αMSH), which is secreted by hypothalamic neurons and exerts its function via melanocortin 4 receptor (MC4R). MC4R is expressed in orexigenic neurons, which are inhibited by αMSH. NPY circulating levels have been found lower among cancer patients with weight loss and significant self-reported anorexia [16]; however, the study had a significant risk of design bias. There is a significant mechanistic link between inflammation and appetite, since the hypothalamus is a well-known target for many cytokines [15], where they increase the anorexigenic signal output.

Ghrelin, a 28-amino acid hormone produced mainly by the stomach, targets the hypothalamus to exert its orexigenic effect, where it stimulates the production of NPY by the activation of growth hormone secretagogue receptor 1a (GHSR1a) [15]. Ghrelin also increases gastric contractions and emptying, which contributes to counteract common cachexia-associated symptoms like nausea and early satiety [17]. Although ghrelin circulating levels are paradoxically high in cachectic patients [18], probably due to a compensatory response, its pathway has been extensively explored for therapeutic modulation (see *Treatment* section).

21.3.2 Decreased Food Intake

In spite of common difficulties on accurately assessing dietary intake in clinical research, there is evidence that oncologic patients get reduced food intake that is insufficient even under low physical activity conditions. For example, in a study involving newly detected lung cancer patients, dietary intake was significantly lower in weight-losing patients compared to stable-weight patients, although absolute rather than relative calorie intake data were presented [19]. However, inconsistent results have been found regarding the role of decreased nutritional intake in cancer cachexia, with cancer outpatient reported data on equivalent energy intake between weight-losing and stable-weight patients (notably, relative Kcal/Kg of body weight was higher in the former) [20].

There is, furthermore, strong indirect evidence against the fact that oral intake decrease, when present, significantly influences cancer cachexia development and progression. Refractoriness to conventional nutritional support, which reestablishes correct nutritional intake, is a key feature of cancer cachexia [4], which is supported by evidence showing that even total parenteral nutrition is of limited efficacy in this scenario [21]. Therefore, the focus was placed on a potentially increased metabolic demand in advanced cancer patients.

21.3.3 Increased Metabolic Rate

The total energy expenditure (TEE) of an individual is the result of summing resting energy expenditure (REE), diet-induced energy expenditure (DEE), and energy that is spent on physical activity (AEE). Hypermetabolism is defined by a measured REE (mREE) that is above 110% of predicted (pREE). Despite the preferred method to measure REE is indirect calorimetry [22], this is a technique that is far from being widely available in the clinic, so that remains limited to proof-of-concept trials. Predictive measurements are based on equations like Harris-Benedict's, which were developed for healthy individuals and therefore have many limitations when applied for cancer patients [23]. Hypermetabolism is estimated to affect approximately 50% of cancer patients [20], but significant variability is found in the literature [24].

Inefficient metabolic cycle activity contributes to increased energy expenditure. Tumor cells are known for their increased glucose uptake [the basis of fluorodeoxyglucose positron emission tomography (PET) imaging], which is conducted toward the inefficient glycolytic pathway. Overproduced lactate is then recycled into Cory's cycle for neoglycogenesis, which contributes to additional ATP consumption [8]. Increased gluconeogenesis in weight-losing cancer patients contributes to the significant metabolic demand to skeletal muscle and adipose tissue for appropriate substrate supply [8].

In addition, mitochondrial uncoupling proteins might contribute to increased energy consumption in cancer cachexia patients. These proteins, known for their role in shifting proton gradient between mitochondrial intermembrane space and matrix to produce heat instead of ATP, have been found overexpressed in skeletal muscle of patients with upper gastrointestinal cancer and weight loss, compared to counterparts with stable weight [25]. Other groups, however, were unable to replicate these results in patients with pancreatic cancer [24]. Similar phenomena have been replicated in adipose tissue of cancer cachexia patients, in a process that has been called adipose tissue *browning* [26]. Besides increased uncoupling, many other mitochondrial alterations have been linked to cancer cachexia syndrome, including decreased oxidative capacity [27].

Parathyroid hormone-related protein (PTHrP), a tumor-derived compound responsible for paraneoplastic hypercalcemia, has been associated to increased weight loss and per-kilogram REE increase in a cohort of non-small cell lung cancer (NSCLC) and colorectal cancer (CRC) patients [28]. Additionally, systemic inflammation probably contributes to hypermetabolism in cancer cachexia patients [29].

21.3.4 Adipose Tissue Atrophy

There is clinical evidence that increased lipolysis drives adipose tissue atrophy in cancer cachexia [30]. Adipose tissue shrinking is the consequence of a three-step triglyceride (triacylglycerol (TAG)) hydrolysis into glycerol and free fatty acids (FFA), which are released into the circulation to serve as energy substrate. The rate-limiting enzyme in this metabolic pathway is adipose triglyceride lipase (ATGL) [31] that regulates TAG conversion into diacylglycerol (DAG) and a FFA. The second step is regulated by hormone-sensitive lipase (HSL) to liberate a second FFA and monoacylglycerol (MAG). A third final enzyme, monoglyceride lipase (MGL), degrades MAG into glycerol and a third FFA. Both HSL and final products of this metabolic pathway, glycerol and FFA, are upregulated in cancer cachexia patients [30, 32]. HSL gets activated as a result of a signaling cascade initiated on β-adrenergic receptors on adipocyte cell membrane through cyclic AMP (cAMP) production and is regulated by counterpart α2 receptors [27]. Both β1-specific (atenolol) and nonspecific β-blockers (propranolol) in a small trial were shown to be able to significantly decrease REE in weight-losing cancer patients [33], but no clinically robust benefit has been published associated to these drugs (see *Treatment* section for additional insight into therapeutic adrenergic modulation). Under nonpathologic conditions, insulin acts as a well-known lipogenic factor through the activation of phosphatidyl inositol kinase (PI3K), which depletes cytoplasmic cAMP levels. Unfortunately, insulin resistance is a common feature of the cancer cachexia syndrome [27].

Furthermore, a lipid-mobilizing factor (LMF) has been identified and further characterized as a zinc α-glycoprotein (ZAG) in urine and serum of cancer cachexia patients, which activates adenylate cyclase (AC) and upregulates HSL downstream [31]. LMF is produced by both tumor cells and host tissues [34].

Fat-muscle crosstalk. Preclinical cachexia models have demonstrated unexpected effects of adipose tissue-specific changes on distant organs. Lewis lung carcinoma (LLC)- and B16 melanoma tumor-bearing mice

have been tested on loss-of-function studies. Both ATGL $^{-/-}$ and HSL $^{-/-}$ mice showed significant adipose tissue loss prevention compared to wild-type controls. Notably, muscle mass preservation and proteasomal protein breakdown downregulation were observed [32]. Although not well characterized, there is evidence of a tight interdependence between two of the most relevant target tissues in cancer cachexia, fat and muscle [32].

21.3.5 Muscle Atrophy

Muscle wasting is the dominant feature of cancer cachexia [35]. The predominant metabolic characteristic of skeletal muscle in cachectic patients is increased proteolysis, although decreased protein synthesis might contribute as well (Fig. 21.2). There are two main proteolytic pathways that regulate protein imbalance in human muscle atrophy in cachexia: autophagy and the ubiquitin-proteasome system (UPS).

Comparative genomic hybridization on a variety of muscle wasting preclinical models identified a group of genes that were upregulated in skeletal muscle, which were called *atrogenes* [36]. Among them, two muscle-specific ubiquitin ligases were identified: muscle-specific RING finger-1 (MURF1) [37] and atrogin-1 (also known as muscle atrophy F-box protein (MAFBX)) [38]. In atrophying muscle, these proteins regulate the degradation of proteins that promote structural and functional (sarcomeric) protein synthesis [36].

The IGF1-PI3K/AKT/mTOR pathway, well known for its driver role in cancer cell proliferation, is considered one of the most relevant anabolic signaling pathways in skeletal muscle physiology. In non-dividing tissues, it stimulates protein synthesis and downregulates forkhead box protein O (FOXO) transcription factors that ultimately control *atrogene* expression [36]. PI3K-AKT downregulation occurs in wasting skeletal muscle mediated by insulin resistance and increased levels of myostatin and activin A. The latter are transforming growth factor (TGF) family members that play a key role through a common membrane receptor (activin receptor type II (ActRII)) in muscle catabolism and have been found upregulated in gastric cancer patients

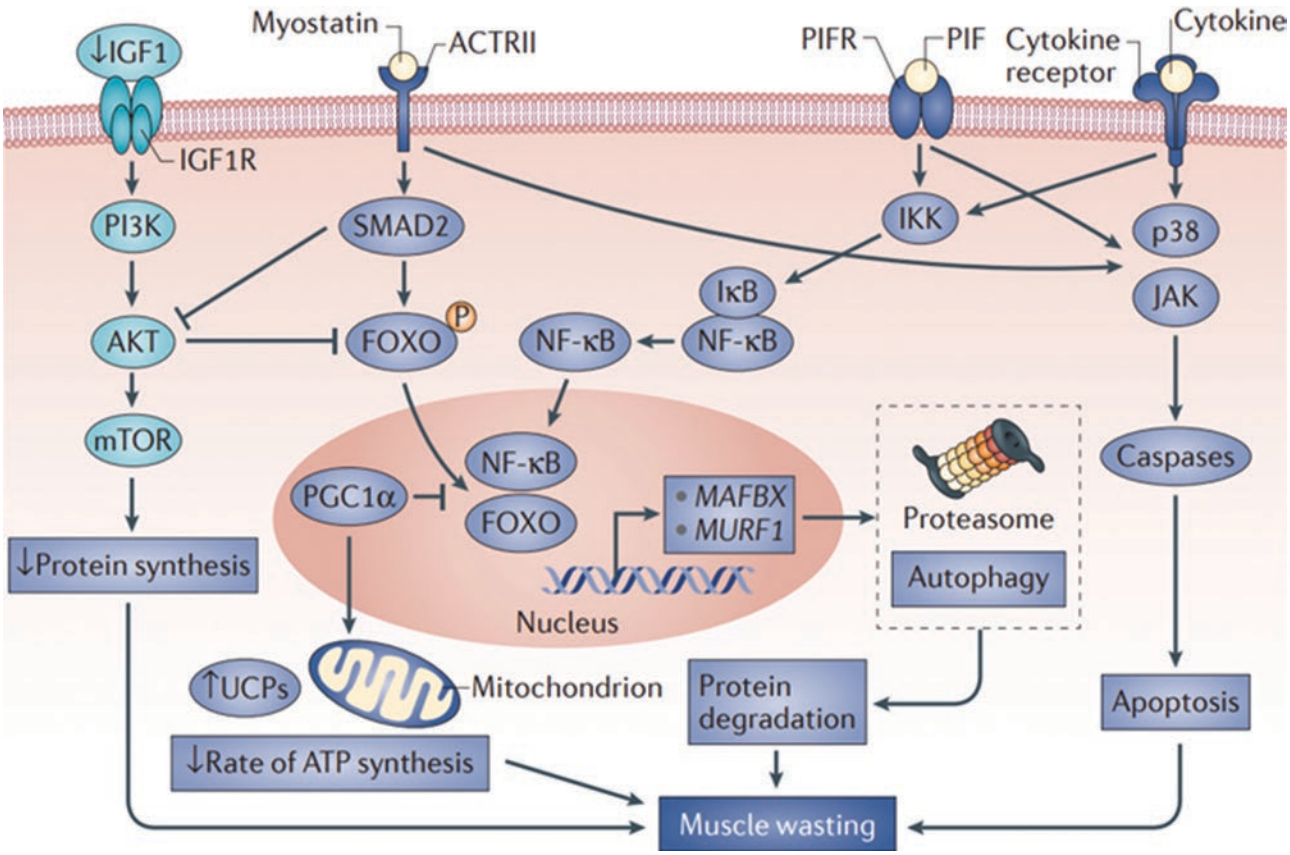

Fig. 21.2 Skeletal muscle intracellular signaling in cancer cachexia. Inflammatory mediators (i.e., cytokines like TNFα, myostatin) and tumor-derived molecules [i.e., proteolysis-inducing factor (PIF)] trigger several highly interdependent signaling pathways that ultimately produce skeletal muscle loss. ActRII activin receptor type II, IGF-1(R) insulin-like growth factor receptor 1, IκB inhibitor of NF-κB, IKK IκB kinase, NF-κB nuclear factor κB, P phosphorylation, PIF proteolysis-inducing factor, PIFR PIF receptor. [Adapted from Nature Reviews Cancer 14, 754–762 (2014) (Ref. [27])]

[39]. The peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α), which can be upregulated by physical exercise, plays a significant role in suppressing both FOXO and TNFα-mediated nuclear factor-κB (NF-κB) activity (both inhibitions lead to increased proteasomal degradation) [36]. A tumor-derived molecule, named proteolysis-inducing factor (PIF), has been found to contribute to muscle atrophy through NF-κB activation [34].

21.3.6 Inflammation

The immune system is increasingly recognized as one of the key players in cancer-host interactions [40]. Mainly acute-phase reaction and the innate immune system have been found to play a significant role in many of the metabolic pathways involved in cancer cachexia syndrome.

Initially known as *cachectin* for its role in rabbit leishmaniasis-associated wasting [8], tumor necrosis factor alpha (TNFα) was one of the first cytokines to be involved in cancer cachexia syndrome. Released mainly by activated macrophages, it exerts its influence in many of the pathophysiologic mechanisms involved in cancer cachexia. TNFα significantly contributes to central regulation of anorexia in the hypothalamus and has also been linked to adipose tissue atrophy and insulin resistance [8]. In skeletal muscle, TNFα contributes to an increased protein breakdown by the UPS [27]. Despite its wide range of ways of action, TNFα levels have not been consistently associated with cancer-related weight loss in humans [29, 41]. The absence of clinical benefit with anti-TNFα treatment in clinical trials [42] suggests that TNFα does not play a dominant role in cancer cachexia development.

Interleukin 6 (IL-6), which is secreted by activated macrophages during the first steps of innate immune activation, can also be produced by human cancer cells [8]. IL-6 exerts its main function in the liver, where it contributes to acute-phase protein synthesis activation. In contrast to TNFα, IL-6 has been consistently associated with cachexia, anemia, and shorter survival in cancer patients [43, 44].

Acute-phase reactant elevation is a common feature of cancer cachexia patients, and C-reactive protein (CRP) is one of the best characterized acute-phase proteins (APPs). Its measuring is routinely available in the clinic and has been associated with weight loss in cancer patients [29]. Synthesized in the liver in response to inflammatory cytokines, APPs are thought to contribute to an increased amino acid demand from peripheral tissues, mainly skeletal muscle [8].

21.4 Diagnosis and Staging: Initial Assessment and Follow-Up

Due to its multifactorial etiology and complexity, a consensus definition was required in order to facilitate clinical trials that were conducted in a homogenous population. The expert consensus definition published in 2011 included a definition and three diagnostic criteria [4]. Patients must meet at least one of them to establish the diagnosis of cancer cachexia:

- Weight loss of >5% over the past 6 months (in the absence of simple starvation); or
- BMI <20 Kg/m^2 AND weight loss >2%; or
- Lumbar skeletal muscle index determined by CT imaging (<55 cm^2/m^2 for men; <39 cm^2/m^2 for women) (*) AND weight loss >2%.
- (*)Fearon's consensus definition includes other possible approaches to assess lean body mass (LBM) in patients that have been omitted from the text for simplification purposes. See *Fearon K, Lancet Oncol 2011; 12: 489–95* (Ref. 5) for further details.

The clinical relevance of weight loss was confirmed in two large prospective cohorts by Martin et al. in 2015 [6]. Both BMI and percentage of body weight loss were associated with worse prognosis, and a grading system that combined both parameters was proposed. Risk-of-death increase was observed with body weight changes as limited as 2.5%. However, additional studies confirmed there was a significant interaction on the effect of elevated BMI depending on LBM: obesity was protective *only* for those patients who had maintained LBM. Sarcopenic obese patients´ mortality was significantly higher than for non-sarcopenic obese patients [45, 46]. This phenomenon had been described before for other chronic conditions as the *obesity paradox* [46]. These patients are probably detected by Fearon's third diagnostic criterium, so that appropriate and early interventions can be initiated.

BMI alone is far from being a perfect surrogate to detect patients at risk since it does not appropriately assess the predominant feature of cancer cachexia, skeletal muscle loss. Therefore, different approaches have been developed to specifically measure its loss. Although dual-energy X-ray absorptiometry (DEXA) has been historically used to determine LBM, CT and magnetic resonance imaging (MRI) are the preferred imaging techniques to specifically determine skeletal muscle mass in patients [4]. The fact that cancer patients are usually followed by CT scans for treatment outcome evaluations makes this technique especially interesting for this population, although skeletal muscle measurement is not usually included in routine-practice radiol-

ogy reports. Single-image capture is usually performed at L3-L4 vertebral level where an area within a pre-established Hounsfield unit range is quantified. This approach has shown significant correlation with whole-body measurements in healthy individuals by MRI [47], and it is potentially able to subtract muscular areas with fatty infiltration [48]. Notably, the prognostic significance of CT-measured lumbar skeletal muscle index and decreased muscle attenuation have been validated in a large Canadian cohort of lung and gastrointestinal cancer patients [49]. In addition, CT-estimated LBM has been found predictive of chemotherapy-induced toxicity [50], and some authors have suggested that LBM should guide chemotherapy dosing instead of body surface area for drugs that distribute predominantly in the LBM compartment [48]. In the case of patients not being followed by periodical CT scans, DEXA, anthropometry, and bioelectrical impedance analysis (BIA) are acceptable approaches to determine skeletal muscle mass [4].

When determining muscle mass, it is essential to assess muscle strength and functional performance. Handgrip dynamometry is a simple and fast technique to assess muscle strength that is feasible in the clinic and has been demonstrated to be prognostic and associated with lower quality of life [51]. In terms of physical function, the 6-minute walk test offers relevant information about patients' daily activities and it has been established independently prognostic in newly diagnosed NSCLC patients [52].

It is of paramount importance to assess quality of life (QOL) when evaluating advanced cancer patients in which therapeutic options remain limited. Although no gold standard questionnaires have been established, the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) is the most commonly used in this population. Additionally, a specific questionnaire and its short version have been developed for the cancer cachexia setting: the Functional Assessment of Anorexia/Cachexia Therapy (FAACT) addresses specific cachexia-related concerns and general QOL aspects [53]. The Patient-Generated Subjective Global Assessment (PG-SGA) can provide additional information [4]. The Edmonton Symptom Assessment Scale (ESAS), which has been consistently validated in palliative care settings, or other systematic symptom assessment tools, can detect and help monitor many collateral symptoms that contribute to an impaired nutritional status.

A correct nutritional assessment requires discarding inadequate nutritional intake, since this aspect of the syndrome might benefit from early diagnosis and intervention. According to the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines, inadequate oral intake is established by an energy intake below 60% of the estimated value or when a patient is not able to eat for one week or longer [22]. Oral intake can be qualitatively assessed by the PG-SGA questionnaire.

Serum albumin levels are extensively used in many different clinical scenarios to assess nutritional status of patients and can aid decision-making when cachexia patients are evaluated. Furthermore, repeated measurements provide useful information about the effectiveness of implemented therapeutic interventions. Transthyretin (also known as prealbumin) can provide similar information in the short term because of its shorter half-life in plasma (approximately 2 days compared to 18 days for albumin). Since these two proteins are known for being negative acute-phase reactants, results should be interpreted cautiously in patients with overt inflammatory conditions, which can be appropriately monitored by C-reactive protein (CRP) levels. The Glasgow prognostic scale and its modified version (GPS/mGPS), which combine high CRP (>10 mg/l) and low albumin levels (<35 g/l), have consistently demonstrated being prognostic even in early-stage cancer patients [54] and can be used to monitor patients at risk.

The consensus definition in 2011 defined three stages through which cancer cachexia usually develops. These stages are intimately associated to specific therapeutic and/or palliative interventions. A first step was defined in which before significant weight loss develops (>5%), some initial metabolic alterations can be detected (i.e., anorexia and insulin resistance) [4]. This stage was called precachexia and was established in order to stress the importance of early interventions that could contribute to restore physiologic, nutritional, and metabolic conditions before irreversible changes occur. The second stage is the proper cachexia condition that has been extensively discussed above. Finally, a third stage called refractory cachexia develops if previously initiated therapeutic interventions fail and the syndrome establishes in a patient with an advanced cancer with limited oncologic treatment options and low functional status. Under these conditions, in which survival is significantly threatened in the short term [4], therapeutic interventions have consistently been demonstrated futile, and efforts should be focused on alleviating symptoms and addressing patients and family member's psychological, social, and spiritual needs.

Fearon's group validated a slightly modified definition and a staging classification in a large cohort of advanced cancer patients [55]. In a dichotomous model, patients were labeled as cachectic if they met at least one of the two first cancer cachexia diagnostic criteria. This easy-to-apply version of the definition was able to select patients with higher CRP levels, greater appetite loss (measured by ESAS), self-reported reduced food intake (measured by PG-SGA), and lower Karnofsky

Performance Score (KPS). Notably, survival was significantly shorter for cachectic patients than for non-cachectic patients. A second model was conducted to assess a staging classification. It was based solely on BMI and weight loss percentage compared to baseline [55], but it was more accurate than the one previously given [4]. Although results were similar, no statistically significant differences were reported between precachexia group and non-cachectic patients regarding CRP levels, KPS, or survival, suggesting that additional parameters such as LBM might be required to appropriately select high-risk patients [55]. A similar four-group staging system that included weight loss, biochemical parameters, and self-reported decreased intake and functional status obtained similar results, but survival difference between precachectic and non-cachectic patients failed to reach statistical significance [56]. Another group recently validated an alternative cancer cachexia classification score (the *Ca*chexia *Sco*re – CASCO) that included total body weight and LBM loss combined with inflammation and metabolic disturbance serum markers and questionnaire-based physical performance, anorexia, and QOL assessment [57]. Even though a simplified version (MiniCASCO) appropriately correlated with the original one, the reported validation was clinically less robust. Currently, there is lack of consensus on how clinicians should detect precachectic patients, but an approach based on total body weight and LBM evaluations is considered appropriate. Complementarily, major metabolic alteration markers (hemoglobin, albumin, CRP) and symptomatic and performance status evaluations can aid the identification of patients at risk.

21.5 Treatment

Almost every previously described mechanistic pathway has been targeted by many therapeutic interventions that reach far beyond pharmacology. In the last couple of decades, an impressive effort has been made in the field, which includes nutritional and physical activity programs and targeted drugs. Despite the vast amount of literature produced, outcomes are poor, and cancer cachexia remains a challenging condition. Recently, multimodal approaches have been designed in which many of the pathogenic pathways of this multifactorial syndrome are targeted at the same time.

21.5.1 Nutritional Interventions

Many nutritional approaches have been tested for their potential role in improving patient outcomes. These include two principal modalities: (1) *nutritional counselling* (*NC*), which is based on individualized advice to patients and caregivers to improve diet in terms of quantity and/or quality, and (2) *nutritional support* (NS), which consists of a range of snacks and drinks that complement certain nutritional aspects of patient diets. This second modality can include enteral or even parenteral nutrition in cases in which decreased oral intake and a non-functional gastrointestinal tract severely compromise nutritional status and outcomes.

Nutritional interventions were recently reviewed by Lee et al. [58]. Among studies that evaluated NC without NS, no significant BMI or muscle strength improvement was found compared to control groups, although some studies reported better PG-SGA scores for patients in the intervention group. Nutritional counselling goes beyond short and unstructured advices and is intended to lead to profound and long-lasting eating habit changes [22], and its presence is warranted in multimodal cancer cachexia patient evaluations. It is of remarkable importance to systematically assess nutrition-impact symptoms like nausea and early satiety to effectively counteract their detrimental effects on oral intake.

Despite normal food is preferred to meet daily nutritional demands, oral nutritional supplements might be necessary to improve energetic and protein intake over insufficient regular diets. In some severely compromised patients, however, supplements do substitute conventional meals. Few studies have been reported comparing nutritional supplements to conventional care [58], and one of them has shown short-lasting increased nutritional intake and some long-term survival benefit for patients allocated to NS [59]. Notably, in this study survival appeared even better for patients who were offered NC. In an additional literature review on studies testing NC and NS combinations, one out of five reviewed papers reported benefit in terms of robust outcomes like LBM maintenance [60], but no survival benefit was reported [58]. Although significant heterogeneity exists between studies that limit data interpretation, outcomes for unimodal nutritional intervention were poor. Nevertheless, patients should be offered nutritional support if impaired oral intake is suspected to significantly influence patient outcomes. Artificial enteral nutrition will be established if a patient lacks functional and safe upper gastrointestinal function but maintains appropriate absorption. Since this kind of artificial nutrition is frequently associated with complications (local infection, osmotic diarrhea, tube displacement), expected survival and patient preferences should guide decision-making. Parenteral nutrition is of narrow applicability in this setting and should be offered only to patients in whom non-functional gastrointestinal tract dominates

the clinical scenario and expected survival is not limited in the short term. Clinicians must consider that removing previously initiated parenteral nutrition is often psychologically distressing for patients and relatives if clinical status worsens and priorities are modified to exclusively symptomatic care.

21.5.2 Physical Exercise

Physical activity has been analyzed for its potential benefits in terms of muscle metabolism modulation, insulin-independent glucose uptake by skeletal muscle, and immunomodulation. A Cochrane systematic review was conducted in 2014 finding no randomized clinical trials that met the established inclusion criteria [61]. However, exercise was previously explored in this context. The great majority of the studies have focused on early-stage cancer patients, mainly breast and prostate cancer, but little experience exists with advanced cancer patients, and no robust outcome improvements have been obtained [62]. A small clinical trial evaluating well-performing lung adenocarcinoma patients obtained a statistical trend showing a reduction in self-reported dyspnea compared to controls after 8 weeks of exercise training [63]. In a similar Norwegian trial in which the intervention group practiced supervised 60-minute training sessions twice a week, significant hand grip strength and walking ability improvement were reported after 8 weeks, but patients did not refer fatigue alleviation compared to the control group [64]. An additional trial focused on stage IV lung and colorectal patients in which improved mobility, fatigue, and sleep quality were reported after 8 weeks of a home-based exercise program [65].

In spite of the limited evidence for physical activity in advanced cancer patients, recommendations can be made based on available literature from cancer survival cohorts [62]. A general home-based program that includes 30–60-minute sessions of aerobic moderate-intensity exercise (50–75% of estimated maximum heart rate) three times a week [22] can fit the majority of cancer precachexia and cachexia patients' needs. Patients with higher symptomatic burden and impaired performance status should be advised low-intensity activity that will pursue psychological well-being.

21.5.3 Pharmacology

Despite the impressive amount of scientific literature produced by potentially useful drugs in cancer cachexia, in the last decade no new drugs have obtained the approval label for this condition. Patients suffering from cancer cachexia still have limited options that can alleviate their symptoms, and adverse effects are often relevant. The following sections of this chapter will provide the reader with useful information about currently used drugs, recent advances, and future trends in the pharmacologic treatment of cancer cachexia (▶ Box 21.1).

Box 21.1 Main pharmacological approaches in cancer cachexia

Anti-inflammatory drugs	
	NSAIDs
	Thalidomide
	Anti-IL-6
	Ruxolitinib
Appetite enhancers	
	Progestogens
	Corticosteroids
	Ghrelin analogs
Anabolic drugs	
	SARM
	Myostatin pathway inhibitors
	Formoterol
	Espindolol
	Proteasome inhibitors

NSAIDs nonsteroidal anti-inflammatory drugs, *SARM* selective androgen-receptor modulators

21.5.3.1 Corticosteroids

Glucocorticoids are well known for inducing notable but short-lasting appetite and oral intake increase. Although not completely understood, corticosteroids are thought to exert their action mainly through the inhibition of the proinflammatory status that characterizes cachectic patients. Clinical trials that tested their effects in advanced cancer patients reported increased appetite and well-being but no significant weight gain [12]. Great variability exists among clinicians regarding specific drugs and posology to be used for this indication. Most experience exists with dexamethasone, prednisolone, and methylprednisolone. Since well-known adverse effects are time-dependent, intermediate initial doses are recommended to reach quick clinical improvement (i.e., dexamethasone 4–8 mg/day or equivalent), followed by early progressive tapering that will establish the minimum effective dose.

21.5.3.2 Progestogens

Progesterone derivatives are the first therapeutic group that was approved for the treatment of cancer-related anorexia and cachexia syndrome and still constitute the front-line pharmacological treatment for this condition.

First tested in the oncology field for the treatment of hormone-sensitive breast and endometrial cancers, a clinically relevant appetite and weight gain was observed,

which focused research toward patients with cancer-associated weight loss. Although the mechanism of action has not been completely elucidated, both megestrol acetate (MA) and medroxyprogesterone acetate (MPA) are thought to induce their effect through neuropeptide Y release at the hypothalamus [53].

Since its approval by the US Food and Drug Administration (FDA) in 1993 for unintended weight loss in AIDS patients, MA has been extensively used for cancer patients with involuntary weight loss. In 2013, a Cochrane systematic review demonstrated MA was effective for counteracting weight and appetite loss in cancer patient when compared to placebo. However, when MA was compared to other potentially active drugs, no statistically significant difference was observed for those outcomes [13]. Moreover, the authors reported increased mortality for patients treated with MA (relative risk (RR) 1.42; 95% CI: 1.04–1.94) and warned about clinically relevant toxicity. Specifically, thromboembolic phenomena (including thrombophlebitis) that occurred in 4.3% of patients in the pooled analysis [13] might significantly limit MA administration in patients with impaired mobility or other prothrombotic comorbidities. Impotence should be considered when treating sexually active male patients. MA can significantly suppress the hypothalamic-pituitary-adrenal axis. In case a systemic infection or any other major demanding condition develops (surgery, trauma), stress doses of corticosteroids are recommended [66]. Similarly, if a clinical decision is made to suspend ongoing MA treatment, progressive tapering is preferred to abrupt discontinuation. Clinical efficacy and some forms of toxicity (i.e., edema) appear dose-dependent [13, 67], and the dose range with the best risk-benefit balance has been estimated between 320 and 800 mg/day. Dosing above that threshold was demonstrated to be of limited additional benefit compared to lower doses in a dose-response trial [67] (see ▶ Box 21.2 for additional practical information on MA prescription). Progestogens should be used with caution in castrate-resistant advanced prostate cancer, since some cases of rapid cancer progression and clinical deterioration have been reported when using MA for cancer cachexia in this population [68].

Box 21.2 *Megestrol acetate*. Practical fact sheet

Indications
- Cancer cachexia-anorexia syndrome
- Advanced breast or endometrial cancer

Posology
- Recommended initial dose: 160 mg once daily (PO)
- Increase doses, according to clinical response. Usual doses range between 320 and 800 mg/day
- Doses above 800 mg/day are not recommended
- A period between 1 and 2 months might be required to evaluate clinical efficacy

Contraindications
- Hypersensitivity
- Known or suspected pregnancy (FDA category: X)
- Gynecological bleeding of unknown origin

Precautions
- MA can produce liver and thyroid test alterations
- Cautious use in patients with previous history of thromboembolic phenomena, liver failure, or severe cardiovascular disease
- If a thromboembolic episode occurs, MA administration must be SUSPENDED
- Risk of exacerbation of previously diagnosed diabetes
- Risk of adrenal insufficiency. Consider stress doses of corticosteroids if acute illness (surgery, infection)

Interactions
- Aminoglutethimide plasmatic level reductions have been reported due to induction of its metabolism

Adverse reactions
- Skin: rash, alopecia
- Cardiovascular: thromboembolic events, palpitation, peripheral edema, cardiomyopathy
- Digestive system: gastric intolerance, nausea
- Endocrine/metabolic: hyperglycemia, hypercalcemia, cushingoid facies, adrenal crisis
- Genitourinary: breast discomfort, impotence

Some authors have highlighted that progestogen-associated weight gain is mainly dependent on extracellular water and adipose tissue, with little or no effect on LBM [12], and consistent survival benefit reports are still lacking; to date no other drug has shown superior results to progestogens in terms of appetite and weight gain in cachexia patients. Progesterone derivatives are the current mainstay appetite enhancer for cancer

cachexia in the clinic and in many multimodal approaches that are presently being tested in clinical research.

21.5.4 Nutraceuticals

Nutraceuticals or *pharmaconutrients* are food-derived natural compounds with potential pharmacodynamic properties in addition to their nutritional value. Some of these substances, which can be artificially synthesized and administered in higher concentrations in a variety of pharmaceutic forms, have been explored in cancer cachexia patients [69].

Among Ω-3 fatty acids, which are essential in human diet, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids have been extensively studied for their role counteracting acute-phase response, downregulating UPS and decreasing muscle cell apoptosis by TNF downregulation [70]. Despite promising effects on lean body mass were reported in early clinical trials, larger studies failed to confirm the initial findings [71]. In 2003 Fearon's group published results from the first large and well-controlled trial. Two hundred advanced pancreatic cancer patients were randomly assigned to receive a supplement enriched with Ω-3 fatty acids and antioxidants or an isocaloric isonitrogenous control supplement. After 8 weeks of treatment, no significant differences were found for primary outcomes (weight and LBM). A post hoc analysis revealed, however, that plasma EPA levels moderately but significantly correlated with weight and LBM gain in the experimental group, suggesting that low compliance might have influenced the results [72]. The largest trial that has tested the effect of pure EPA on weight loss included over 500 patients with advanced lung and gastrointestinal cancer. An interesting trend toward significance of 1.2 Kg (0–2.3 Kg) difference was reported for those who received 2 g/day compared to placebo. Surprisingly, patients that were assigned 4 g/day obtained no benefit [73]. Better outcomes have been reported by smaller trials. A randomized trial conducted in 40 stage III NSCLC patients reported benefit in terms of quality of life and performance status associated with oral supplements of Ω-3 fatty acids (EPA + DHA) [74]. In a similar trial that included NSCLC patients during their first-line chemotherapy, total body weight change was −2.3 ± 0.9 kg for controls, while patients receiving 2.2 g of EPA maintained stable weight (+0.5 ± 1.0 kg) [75]. Authors also reported that proportions of patients with stable muscle mass during treatment were 29% and 69%, respectively. The same group reported a relevant benefit for EPA + DHA supplementation in terms of response rate to first-line palliative chemotherapy (60.0% vs. 25.8%) and 1-year survival (60% vs. 38.7%) in stage IIIB to IV NSCLC patients [76], although treatment was not randomly assigned and results should be interpreted cautiously. Combined with MA, EPA was unable to improve patient outcomes obtained by MA alone in a large randomized trial that allocated 421 cancer patients to receive EPA, MA, or both [77]. The same group conducted a similarly designed trial that assessed dronabinol (delta-9-tetrahydrocannabinol). Appetite improvement and weight gain were greater for patients treated with MA alone compared to dronabinol alone [78], and dronabinol + MA combination did not improve MA monotherapy results. Even though significant heterogeneity exists between studies regarding supplementation composition and route of administration of Ω-3 fatty acids and clinical outcomes, which abrogates drawing robust recommendations, relevant outcomes have been reported, and the research area remains active.

Certain amino acids and their combinations have been tested in cancer cachexia for their potential role in counteracting the syndrome. A combination of β-hydroxy-β-methylbutyrate (HMB) (a leucine metabolite), glutamine (Gln), and arginine (Arg) showed promising results in terms of significant early lean body mass improvement among cancer cachexia patients. Patients who were randomly supplemented with HMB/Arg/Gln obtained a mean LBM increase of 1.12 ± 0.68 kg, whereas control group subjects lost 1.34 ± 0.78 kg [79]. Long-term results were limited because of a high dropout rate. However, a similar phase II trial was unable to confirm positive results [80].

21.5.5 Recent Advances

21.5.5.1 Appetite Enhancers

Ghrelin, an orexigenic gastrointestinal hormone that was mentioned above, and its mechanism of action have been extensively studied for pharmacological modulation in cancer cachexia syndrome. Anamorelin, an oral ghrelin analog, has been explored by two relevant phase III clinical trials. ROMANA 1 and 2 studies enrolled over 800 inoperable stage III and stage IV NSCLC cachectic patients who were well performing at enrollment (ECOG ≤2). Randomly assigned to receive anamorelin or placebo, LBM change (measured by DEXA) was reported after 12 weeks of treatment. Median LBM changes were +0.99 kg for the intervention group vs. –0.47 kg for controls and +0.65 kg vs. –0.98 kg ($p < 0.0001$) for the two studies [81]. Patient-centered outcome analysis revealed statistically significant improvement in cachexia-anorexia-related symptom burden measured by FAACT score, but no benefit in terms of a 13-item fatigue scale. A subsequently published post hoc pooled analysis revealed that LBM

improvement was greater for patients with low BMI (<20 Kg/m^2) at enrollment compared to those with normal/high basal BMI [82]. Median survival was slightly worse for the intervention group (8.9 vs. 9.2 months), a difference that did not reach statistical significance [81]. A recently published extension study revealed that additional 12-week treatment was well tolerated and could contribute to further improved outcomes [83].

21.5.5.2 Anti-inflammatory Drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) and ciclooxygenase-2 (COX-2) inhibitors have been widely studied for a possible indication in cancer cachexia syndrome. Celecoxib was administered in 24 patients with a variety of advanced cancers. After 4 months of treatment, significant LBM and GPS improvement and TNFα decrease were observed compared to baseline [84]. The absence of a control group limited the impact of these observations. Celecoxib was further compared to an active control in a small trial that randomly treated 22 lung cancer patients with celecoxib and fish oil or fish oil and placebo. After 6 weeks of treatment, intervention group patients had significantly lower CRP levels and significantly higher muscle strength and body weight [85]. Ibuprofen has also been compared with an active control in a trial that enrolled 73 patients with locally advanced or gastrointestinal cancer. Despite dropout rates above 50%, mainly due to disease progression, evaluable patient treated with ibuprofen and MA had a median weight increase of +2.3 Kg, while those who received MA and placebo lost a median of 2.8 Kg. A single trial that assigned 135 solid tumor patients to receive indomethacin, prednisolone, or placebo reported a significant survival benefit for active anti-inflammatory treatment arm patients (505 ± 65 vs. 274 ± 28 days) [86]. Currently available evidence is considered insufficient to recommend the use of nonsteroidal anti-inflammatory drugs for cancer cachexia syndrome treatment [87], but these drugs are actively being included in multidrug and multimodal approaches.

Thalidomide, a drug known for its immunomodulatory activity in multiple myeloma, has been studied in cancer cachexia patients due to its role in potentially counteracting proinflammatory cytokines [69]. Despite preliminary reports demonstrated that oral thalidomide was associated with weight and upper arm muscle mass improvement in advanced pancreatic cancer patients compared to placebo [88], more recent trials failed to obtain subjective benefit in cancer cachexia patients [14]. However, in a trial enrolling 102 cancer cachexia patients, thalidomide and MA combination obtained better results compared to controls that received MA alone in terms of body weight change, fatigue, and quality of life [89]. A Cochrane Database systematic review performed in 2012 noted that well-conducted large trials that test thalidomide in cancer cachexia patients are lacking [90].

Additional approaches have been analyzed to modulate proinflammatory pathways in cancer cachexia patients. ALD518, a humanized monoclonal anti-IL-6 antibody, showed preliminary efficacy data in weight-losing advanced NSCLC patients in terms of LBM and chronic anemia improvement [91], but data have not been validated.

Other groups have tried to modulate the effects of proinflammatory cytokines. After results with anti-TNFα drugs were discouraging [42], other approaches have been developed. Investigators have tried to counteract inflammatory cytokines through intracellular signaling inhibition on target tissues. Ruxolitinib, an oral JAK1/2 inhibitor, was found to improve weight and serum albumin and cholesterol levels in a post hoc analysis of a phase III trial in myelofibrosis patients [92]. These effects were thought to go beyond what would be expected from direct oncological improvement associated with this cytostatic drug (measured by spleen volume reduction), but results were probably influenced by a drug that counteracts a driver alteration in this chronic myeloproliferative condition. An ongoing phase II trial (NCT 02072057) that is being conducted in solid cancer patients will elucidate whether off-tumor JAK1/2 inhibition can directly improve cancer cachexia outcomes.

21.5.5.3 Anabolic Drugs

β2-agonists are commonly used for the treatment of bronchospasm. These receptors transduce such a strong anabolic signal in skeletal muscle that some β2-agonist molecules are sadly famous due to their illegal consumption as performance enhancers in elite sport. In clinical research, the only trial conducted in humans, which analyzed single-arm formoterol in combination with MA, had a relevant dropout rate of 6/13 patients, which abrogated robust conclusions in spite of some positive results for quadriceps volume and self-reported appetite [93]. Surprisingly, espindolol, which exerts opposite actions through nonspecific β1/2 antagonism, has also shown positive effects in cancer cachexia treatment, reflecting adrenergic system regulation complexity in this syndrome. In this case, benefit could be attributable to anti-catabolic effect on adipose tissue. In a trial, 87 advanced NSCLC and CRC patients were randomized to receive espindolol 10 mg, 2.5 mg, or placebo. After a follow-up of 20 weeks, intention-to-treat (ITT) analysis revealed a significant improvement in weight change rate and absolute LBM change (measured by DEXA) between high-dose espindolol group and placebo group (+0.42 vs. −0.37 Kg/4 weeks; *data not reported for absolute LBM change*) [94]. No survival benefit or quality-of-life differ-

ences were observed between the three groups. Regarding adverse events, dyspnea was referred by 19.1% of patients in high-dose espindolol group compared to 3.2% in placebo group.

Other relevant approaches have been developed to directly counteract skeletal muscle loss in cancer patients. Androgens, with known anabolic properties on skeletal muscle, are associated with dose-limiting side effects like masculinization and hepatic toxicity when administered exogenously [69]. To overcome these difficulties, nonsteroidal drugs called selective androgen receptor modulators (SARM) have been developed. These orally active molecules are designed to exert tissue-specific anabolic actions while avoiding virilizing effects [95]. Among them, enobosarm is the most advanced one in terms of clinical development. In a phase II trial, 159 well-performing (ECOG ≤1) weight-losing patients with a variety of cancers were randomized to receive enobosarm 3 mg or 1 mg daily or placebo. Among 100 evaluable patients, significant LBM improvement was reported for the 3 mg group compared to placebo at week 16 (+1.3 vs. 0.1 Kg, p 0.041) [96]. No statistically significant survival benefit was reported. Notably, neither relevant PSA plasma level changes in men nor significant hirsutism in women was observed. Enobosarm showed a safe hepatic toxicity profile. The publication of phase III trials (POWER trials) that enrolled NSCLC patient is awaited [97]. These studies were designed to include patients initiating first-line chemotherapy, and no minimum weight loss was required. Therefore, researchers will assess whether early pharmacologic intervention can improve cancer cachexia outcomes before an overt debilitating syndrome establishes.

Myostatin, a key player in skeletal muscle atrophy, and its receptor ActIIR have extensively been explored in cancer cachexia treatment. The development of myostatin pathway inhibitors was supported by a pivotal preclinical research by Zhou et al. in which colon 26 (C26) tumor-bearing mice were treated with a soluble ActIIR (sActIIR) [98]. Regardless of treatment onset time, treated mice were protected from weight and skeletal muscle mass and function loss or even recovered from previous damage. Survival was significantly prolonged for actively treated mice, which was not attributable to differences in tumor growth rate between groups. In the clinic, preliminary data of an anti-myostatin antibody in advanced cancer patients showed improved muscle strength and function, with no clear dose-response trend for thigh muscle volume [99]. Phase II trials with different myostatin pathway inhibitors are ongoing [69, 100]. Downstream on this signaling pathway, UPS is central to explain protein degradation in wasting skeletal muscle; hence proteasome inhibitors, active drugs in multiple myeloma, could potentially counteract skeletal muscle degradation in cancer cachexia. However, drugs like carfilzomib are still under preclinical development for this condition [69].

21.5.6 Multidrug and Multimodal Approaches

In spite of the impressive amount of drugs that are being analyzed, single-drug and unimodal approaches consistently failed to bring patients clinically relevant benefit. The complexity of cancer cachexia pathophysiology has been demonstrated in clinical research by the fact that no single-target approach has been able to significantly ameliorate the myriad of signs and symptoms that burden patients, family members, and healthcare providers. In recent years, multimodal strategies were developed that combine ≥2 drugs and/or behavioral interventions (nutrition +/− exercise) in an attempt to simultaneously counteract as many cachexia pathophysiological routes as possible.

Multidrug approaches often combine conventional drugs and nutraceuticals. A phase III trial allocated 332 advanced cancer patients to receive MA/MPA, EPA, L-carnitine, thalidomide, or the combination of the above. After 4 months of treatment, patients in the combination arm showed a significantly greater improvement in LBM, REE, and fatigue compared to patients in single-drug arms [101]. Contradictory results have been reported, however, when MA was combined with carnitine and celecoxib [102]. Other preliminary reports in which combined celecoxib, L-carnitine, curcumin, and lactoferrin were compared to placebo have reported LBM, appetite, and anemia improvements [12].

Evidence is scarce for interventions that combine pharmacotherapy and nutritional interventions. A Swedish group [103] conducted a large trial in which 309 patients with mainly gastrointestinal cancers were randomized to receive nutritional support: oral when food intake decreased below 90% of expected and parenteral when intake decreased to 70–80%. All patients received indomethacin and, those who had anemia, erythropoietin (EPO). Nutritionally supported patients did not show relevant benefit in the ITT analysis. In a small single-arm trial that included stage IIIB-IV NSCLC patients, a combination of MPA, celecoxib, and oral nutritional supplements for 6 weeks improved body weight loss rate, appetite, early satiety, and fatigue [104]. A clinical trial (NCT02330926) is ongoing that allocates advanced lung and pancreatic cancer and cholangiocarcinoma patients to receive combined nutritional supplementation (including EPA), home-based exercise, and ibuprofen or standard palliative care. Patients will

receive the intervention treatment during active chemotherapy, and prior weight loss will not be required for screening. The trial was designed to prevent/delay the onset of cancer cachexia.

Clinical outcomes remain poor for cancer cachexia treatment. However, disappointing results might go beyond the complexity of the syndrome, and technical factors may be playing an important role. Despite great effort was done by relevant researches in the field to unify definitions, significant heterogeneity exists between patient populations included in clinical trials. Heterogeneity often extends to control group interventions, since no consensus exists regarding the standard treatment. In terms of primary outcomes, since a clinically relevant LBM gain threshold awaits to be established, trials should be designed in an attempt to demonstrate survival benefit. It is mandatory that symptom control and QOL issues are specifically evaluated using validated scores.

Increasing consensus exists on the fact that cancer cachexia trials should enroll patients in the early phases of the metabolic disturbance, before the overt cachexia syndrome develops. In this direction, ongoing attempts will elucidate whether earlier interventions can improve previous advances ([91], NCT02330926). Recruitment rates could also be higher if patients are offered participation before the syndrome turns burdensome and in combination with active oncologic treatment. There is evidence that cancer cachexia is the consequence of cancer metabolic reprogramming that extends beyond tumor cells and neighbor microenvironment to exploit distant tissues in favor of tumor proliferation [105]. According to this hypothesis, anti-cachexia drugs could contribute to counteract tumor progression. Therefore, it is important that clinical trials on cancer cachexia directly assess tumor proliferation, which could potentially support this relevant hypothesis.

Case Study 1

A 68-year-old man was derived to the palliative care consultation for symptomatic support. Recently diagnosed with right renal pelvis urothelial cancer with retroperitoneal nodal infiltration (stage IV), he was being evaluated by medical oncology department prior to systemic first-line treatment. His medical background included arterial hypertension, type II diabetes, and grade 3 obesity.

ESAS scale-based initial symptomatic assessment detected 10/10 asthenia and 10/10 absence of well-being. Additionally, the patient complained of intense dyspepsia and early satiety. He was depressed, a status that he did not attribute to the impact of a recent advanced cancer diagnosis but to a high symptomatic burden. He referred 15 Kg weight loss in the previous three months (baseline weight was approximately 130 Kg), although obesity was still present. Additionally, relatives noted that arterial pressure was lower than usual and diabetes control had worsened despite decreased oral intake. An ECOG 2 performance status was estimated.

Physical examination revealed a BMI of 34.9 Kg/m^2 and arterial pressure of 94/69 mmHg. At inspection, central obesity and mild skin pallidity were noted, with no other relevant findings. Blood tests showed hyperglycemia (120 mg/dl) and mildly elevated Hb1AC (6.5%), grade 1 normocytic-normochromic anemia (12.3 g/dl), and leukocytosis with neutrophilia (absolute neutrophil count 11.8 × 10^3/μL). An L3-L4 vertebral level axial CT image is presented (Fig. 21.3).

Should this patient be considered at risk of developing cancer cachexia? If so, and according to Fearon's consensus definition [4]*, what cancer cachexia stage would you assign the patient to?*

What additional complementary tests would you order at this point?

What therapeutic interventions should be initiated?

After the initial assessment, since epigastric discomfort and early satiety were dominant in the clinical context, metoclopramide was initiated. However, prompt follow-up revealed absence of clinical improvement. The patient continued to complain of severe appetite decrease and progressive weight loss; hence nutritional support was started by his primary care physician.

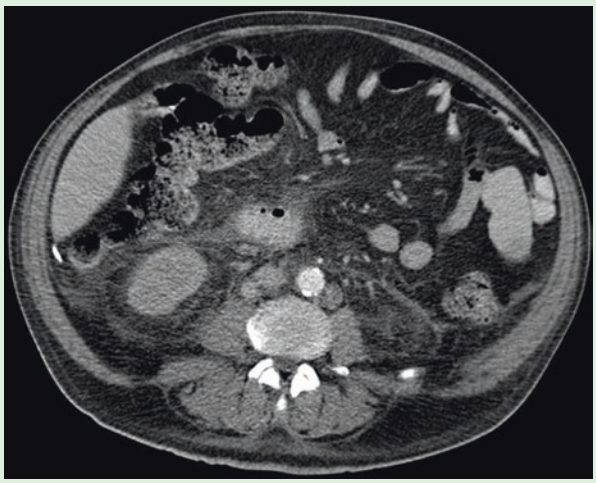

Fig. 21.3 L3-L4 vertebral level axial CT image, case study 1 patient

Patient's status continued to worsen, and hospitalization was required for a febrile urinary tract infection. A closer symptomatic support was established and MA initiated at 400 mg/day. Few days later, the patient referred improved appetite, but, despite broad-spectrum antibiotics, fever (predominantly in the evening) and elevated inflammatory parameters persisted (CRP: 170 mg/L). Urine culture was negative. Clinical suspicion of neoplastic fever augmented, and naproxen was prescribed followed by notable clinical improvement. First-line chemotherapy could finally be administered, and the patient was discharged.

This clinical case illustrates that overt cancer cachexia syndrome can occur before visually relevant weight loss establishes. Clinicians should be aware when assessing patients in which symptomatic complaints are apparently disproportionate to tumor burden. Widely available CT scans can guide accurate muscle mass determinations before clinically apparent wasting happens. The provided image is compatible with sarcopenic obesity, a condition in which predominant skeletal muscle loss is associated with less severely affected adipose tissue. High CPR levels and neutrophilia, in absence of a documented infection, further support an established proinflammatory condition.

Case Study 2

The palliative care team met a 42-year-old man while hospitalized after failed second surgical cytoreduction for a pseudomyxoma peritonei. Diagnosed 6 months before, he had received neoadjuvant chemotherapy with no clinical benefit, followed by an aggressive surgical complete cytoreduction.

Few months after the first surgery, the patient presented upper abdominal pain, and an abdominal CT scan was performed (Fig. 21.4). Images were compatible with abdominal tumor progression; hence the second salvage surgery was attempted. Surgical findings, however, revealed unresectable disease. During postoperative hospital stay, he developed malignant obstructive jaundice and postrenal kidney injury that required several stent placements. Proximal bowel obstructive symptoms were present and an enterocutaneous fistula developed, which alleviated abdominal distension but produced significant self-perception and management-related consequences.

Initial assessment revealed suboptimal pain control and emotional lability. Physical examination revealed a BMI of 20.6 Kg/m^2 (according to records, he had lost 13 Kg in the last 4 months, 17% of baseline weight). The abdomen was flat and barely depressible, and bowel movement sounds were audible. Serum albumin level was 3.2 g/dl, and CRP was mildly elevated (21 mg/L).

What etiologic factor is dominant in this patient? Should cachexia diagnosis be established in this case study?

What therapeutic interventions would you recommend?

Obstructive symptoms were symptomatically treated with a combination of metoclopramide, octreotide, and dexamethasone. Oral nutrition was progressively reintroduced and acceptably tolerated, although intermittent obstructive episodes occurred. Oral intake was highly

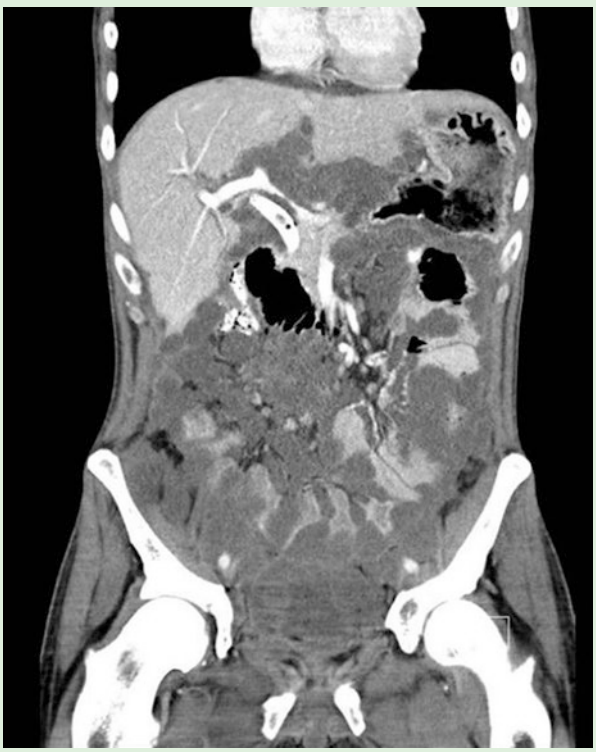

Fig. 21.4 An abdominal CT scan after cytoreduction, case study 2 patient

dependent on nausea and early satiety, but prokinetics produced only partial relief. The small intestine enterocutaneous fistula was presumably contributing to wasting due to a short intestine syndrome. Oral intake decrease, together with enterocutaneous fistula-associated losses, produced clinically relevant dehydration that required hypodermoclysis when the patient was at home. Since the patient did not tolerate high-volume ingestion, nutritional hypercaloric and hyperproteic supplements were recommended. Two chemotherapy lines were attempted that produced short-lasting benefit, but abdominal symptoms persisted and wasting progressed. Parenteral nutrition was evaluated by a multidisciplinary team, but it was finally dismissed because short survival was predicted. The patient developed obstructive cholestasis and died five months after the second surgical cytoreductive attempt.

Most extreme wasting syndromes occur in patients with high abdominal tumor burden, which is associated mainly but not exclusively with upper gastrointestinal and pancreatobiliary cancer. In these patients, nutritional-impact symptoms predominantly influence the course of the syndrome, and, in early phases, other cachexia-associated features like inflammatory markers are absent. Anatomical factors are predominant over molecular ones, and patients often develop a syndrome more similar to starvation than to cachexia. Unless tumor response is obtained, therapeutic options are of very limited efficacy in this population; hence patient preferences and expected survival should guide clinical decisions.

Key Points

- Baseline body weight must be recorded in every initial assessment of advanced cancer patients.
- Total body mass evaluations are not sensitive enough to detect all patients at risk. LBM assessment might be required for patients in which cachexia is suspected but no relevant BMI changes are present. Single-image CT-based measurements are accurate and usually available in cancer patients.
- Routinely available biochemical parameters (hemoglobin, albumin, prealbumin, CRP) can aid diagnosis, staging, and therapeutic decision-making.
- Despite evidence of efficacy is limited, cancer cachexia patients should be offered multimodal therapeutic approaches that combine nutritional and pharmacological interventions with physical activity programs.
- Participation in clinical trials is encouraged for patients with a low symptom burden.

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Supportive and Palliative Care

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Contents

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Learning Objectives

By the end of this chapter, the reader will:

- Be able to use WHO analgesic ladder
- Have learned the definition of palliative and supportive care
- Have reached in depth knowledge of breakthrough cancer pain
- Be able to put acquired knowledge into clinical practice

22.1 Introduction

The aims of palliative care are based on symptom control and amelioration of quality of life. In the recent years, there was an outstanding shift from disease-oriented medicine to patient-centered medicine. Even if the disease is incurable, it is possible to palliate the consequences. The impact of biological treatments on prolongation of survival and improvement on quality of life had determined a slight border between curative treatment and palliative care [1].

22.2 Definition

The definitions of palliative and supportive care are often overwhelming, creating some confusion in patients and professional workers. Palliative care is an approach that improves the quality of life of patients and their families facing the problem associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and assessment and treatment of pain and other problems, physical, psychosocial, and spiritual [2].

Palliative care [3]:

- Provides relief from pain and other distressing symptoms
- Affirms life and regards dying as a normal process
- Intends neither to hasten nor postpone death
- Integrates the psychological and spiritual aspects of patient care
- Offers a support system to help patients live as actively as possible until death
- Offers a support system to help the family cope during the patients illness and in their own bereavement
- Uses a team approach to address the needs of patients and their families, including bereavement counseling
- Will enhance quality of life, and may also positively influence the course of illness
- Is applicable early in the course of illness, in conjunction with other therapies that are intended to prolong life, such as chemotherapy or radiation therapy, and includes those investigations needed to better understand and manage distressing clinical complications

Oncological supportive interventions may be carried out from the earlier stages of disease to allow patients to tolerate more aggressive curative treatments. The goal of supportive care is to prevent or treat as early as possible the symptoms of a disease, side effects caused by treatment of a disease, and psychological, social, and spiritual problems related to a disease or its treatment. Basic supportive care is part of any general practitioner's medical armamentarium and consists of many subspecialties of the traditional medical and nursing care system. The driving idea lies on multidisciplinary team-working, which is outstanding in many branches of healthcare. Best supportive care for cancer patients is, as a matter of fact, the multiprofessional attention to the individual's overall physical, psychosocial, spiritual, and cultural needs, and should be available at all stages of the illness [4]. This means an attention to patient's symptoms and prevention of treatment complication with the use of symptomatic drugs, growth factors and nutritional interventions. By reducing the effects of the disease and its treatment, supportive care contributes to improved patient quality of life, to prevent therapy discontinuation for side effects, and hence to an optimization of outcomes.

Some models of supportive care [5]:

- The use of growth factors for prevention of chemotherapy induced anemia and neutropenia
- The use of antiemetics for prevention and treatment of nausea and vomiting due to chemotherapy
- The use of analgesics for treatment of cancer pain and breakthrough pain
- Prevention and treatment of symptoms and complications of neoplastic disease (i.e., thromboembolism, paraneoplastic syndromes, metastatic bone disease, etc.)
- Nutritional interventions to ameliorate patient's compliance to chemotherapy and to improve clinical conditions and quality of life
- Psychosocial care in particular for diagnosis and prognosis communication
- Rehabilitation care and prevention of secondary tumors

The old model of palliative care was restricted to the terminal phases of disease. Nowadays, the model of simultaneous care foresees an integrated approach of supportive care and palliative care from the beginning, with particular attention to quality of life [6]. Quality of life (QoL) is an important aspect of cancer patient care. The term QoL refers to the patient's subjective experience of life and it may be influenced by several parameters [7]. The World Health Organization (WHO) defines quality of life "as individual's perception of their posi-

tion in life in the context of the culture and value systems in which they live and in relation to their goals expectations standards and concern" [8]. This definition is broad and includes domains such as physical health, psychological state, level of independence, social relationships, and personal beliefs. Subjectivity and multidimensionality are the leading features of QoL. How is it possible to measure quality of life? Four modalities may be taken into account: observation, interview, daily diary of patients, and QoL questionnaires. Observation is a simple registration of patient clinical condition regarding the possibility to satisfy the normal request of daily life (to dress, to wash, to take a bag, time at rest in the house etc). There are several scales to measure performance status: Karnofsky scale or ECOG equivalent scale is the most utilized method. This assay is very useful in common clinical practice because it may select patients who may be treated or not by chemotherapy. Patients with ECOG performance status >2 may not benefit from antineoplastic treatment and present high toxicity with deterioration of clinical conditions [9].

22.3 ECOG and Karnofsky Scale (Table 22.1)

Interview and daily diary are strictly dependent on healthcare and patient judgment and this may result in confounding bias in the evaluation of quality of life. The structured questionnaire is a scientific, reproducible, feasible, and standardized assay, which explores several aspects in patient's life. There are many questionnaires utilized in common clinical practice, and some are summarized in the following table [10] (Table 22.2).

We must consider that the perception of a person's quality of life is different between individuals. At the same efficacy, one chemotherapy may be superior to another for the parameters of QoL and in the palliative setting, the oncologist should prefer the schedule of treatment with less toxicity for a particular subgroup of patients. Unfortunately, only few randomized studies have a scientific and rigorous evaluation of quality of life in cancer patients. For didactic reason, only pain and its treatment will be considered in this chapter; for other symptoms, the reader may refer to other monographies about supportive and palliative care.

Table 22.1 The ECOG scoring system versus the Karnofsky scoring system

ECOG/WHO/Zubrod score		Karnofsky score	
Fully active, no restrictions	0	Normal, no evidence of disease Able to perform normal activity with only minor symptoms	100 90
Restricted in strenuous activity Ambulatory, can carry out work	1	Normal activity with effort Able to care for self but unable to do normal activities	80 70
Ambulatory >50% of the time Capable of self-care Unable to work/ usual activities	2	Requires occasional assistance, cares for most needs Requires considerable assistance	60 50
Ambulatory ≤50% of the time Capable of limited self-care only	3	Disabled, requires special assistance Severely disabled	40 30
Disabled, no self-care Confined to bed or chair	4	Very sick, requires active support Moribund	20 10

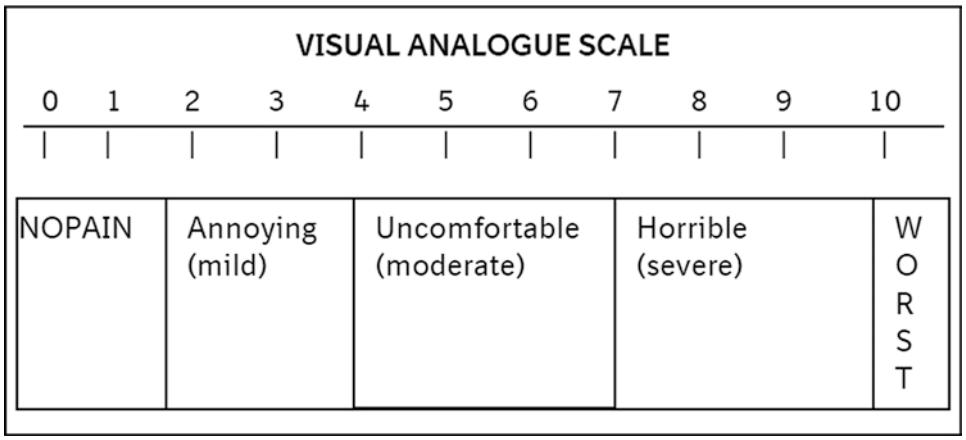
ECOG Eastern Cooperative Oncology Group, *WHO* World Health Organization

Table 22.2 Quality of life questionnaires

Generic instruments	Cancer-specific instruments
Short Form Health Survey (SF36)	EORTC quality of life questionnaire core 30 (EORTC QLQ C-30)
Ferrans and Powers QoL Index (QLI)	Functional assessment of Cancer Therapy (FACT-G)
Notthingham Health Profile (NHP)	Functional Living Index (FLI)
WHO QoL Instrument (WHOQOL-100)	Questionnaires specific for disease (Breast, Lung etc):EORTC QLQ Breast FACT-Lung Lung Cancer symptom scale sets

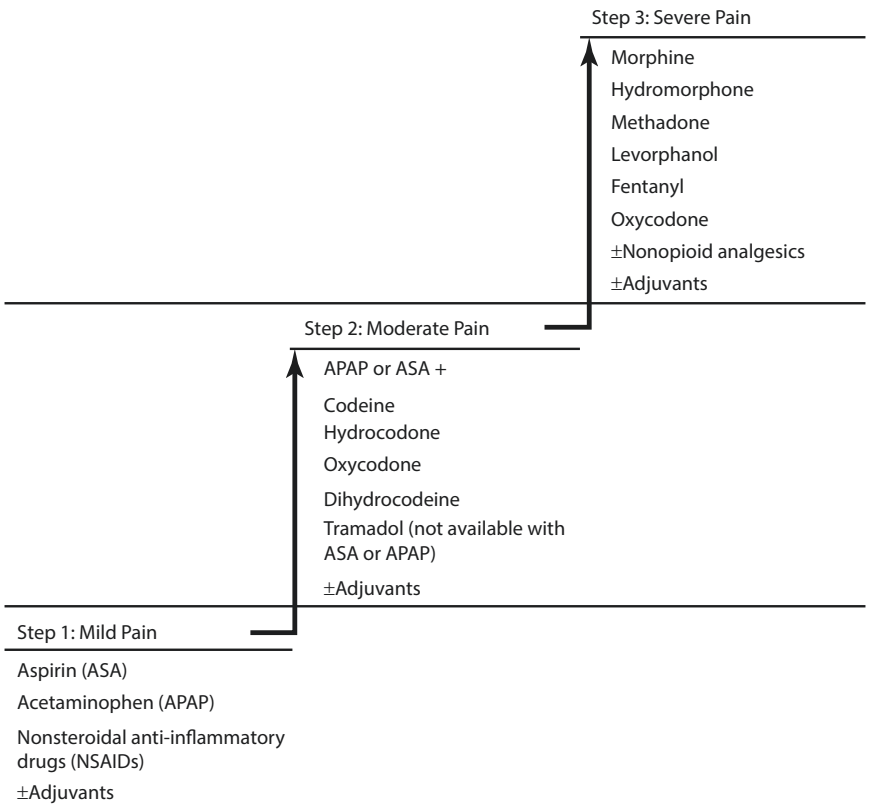
22.4 Pain and Its Treatment

Pain is a prevalent symptom experienced by at least 30% of patients undergoing an oncological treatment for metastatic disease and by more than 70% of advanced cancer patients [11]. The first step in pain treatment is correct evaluation of pain intensity. It is possible to evaluate pain with numeral scale, verbal scale, and Visual Analog Score (VAS). A VAS (or numerical score) of <4 is considered sufficient control of cancer pain due to therapy.



Pain may be caused by progression of disease, by paraneoplastic syndrome, by adverse effects of antineoplastic treatments or other clinical conditions directly or indirectly related to neoplastic disease. It is divided in nociceptive somatic pain (due to stimulation of somatic peripheral receptors), nociceptive visceral pain (due to stimulation of visceral peripheral receptors), and neuropathic pain (due to interruption or damage of neural fibers). In 1986, the World Health Organization (WHO) published a set of guidelines for cancer pain management based on the three-step analgesic ladder [12]. The main aim of WHO guidelines was to legitimize the prescription of strong opioids, arising from evidence of poor management of cancer pain, due to reluctance of health care professionals, institutions, and government to use opioids because of fear of addiction, tolerance, and illegal abuse. Its application is reported to achieve satisfactory pain relief in up to 90% of patients with cancer pain. In the last decades, studies validating the WHO analgesic ladder have been shown to have methodological limitations, including circumstances during which assessments were made, small sample size, retrospective analyses, high rate of exclusions and dropout, inadequate follow-up, and a lack of comparison with levels of analgesia before the introduction of the analgesic ladder [13].

22.5 WHO Analgesic Ladder



The first step in the WHO analgesic ladder involves the use of a non-opioid with or without an adjuvant analgesic. As regarding FANS, the development of ulcer or renal toxicity might be a concern, especially for long-term use, even if specific long-term safety profile has never been established in randomized studies. Both paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs) can be considered either alone (step 1) or in combination with opioids (steps 2 and 3) to improve analgesia and reduce opioid-related side effects. It is generally accepted that paracetamol is introduced first with an NSAID added to paracetamol or substituting paracetamol if indicated. NSAIDs are to be considered as second choice in mild pain [14, 15].

The role of so-called weak opioids in the treatment of moderate cancer pain has been questioned, and it has been speculated that this step could be by-passed. These opioids have the common characteristic to have a "ceiling effect." Some authors reported that no significant differences in pain relief were noted when the use of non-opioids alone was compared to the use of non-opioids plus opioids for moderate pain [16]. However, these results were based on single-dose studies or studies involving a small number of patients, and regular clinical use would be more effective than would be predicted on data involving single-dose administration. Some studies underlined the role of opioids for moderate pain (namely codeine, dextropropoxyphene and tramadol), in comparison to morphine in terms of efficacy and adverse effects [16]. Other studies assessed the use of strong opioids in opioid-naıve patients, skipping the second step drugs [17]. Morphine or Oxycodone used at very low doses in opioid-naıve patients may offer different advantages, including a greater tolerability while providing analgesia. Morphine is the most frequently used opioid in cancer pain management. Although morphine remains a cornerstone for the management of cancer pain, no clear data exist about the superiority of one opioid over another [12, 18] (Table 22.3).

Table 22.3 Dose of opioids commonly used for cancer pain

Drugs	Initial dose	Interval (hours)
Codeine	15–30 mg	4–6
Tramadol	50 mg	4–6
Morphine	5–10 mg	4
Methadone	5–10 mg	8–12
Hydromorphone os	8 mg	24
Oxycodone	10–20 mg	12
Fentanyl transderm	12,5–25 mg	72
Buprenorphine transderm	17,5–35 mg	72

Individualization of therapy has been emphasized to minimize the side effects and to improve the opioid response. The most important adverse effect is stipsis. For this reason, it is advisable to prescribe a laxative at the beginning of opioid therapy. It is now recognized that individual patients vary greatly in their response to different opioids. A shift from one opioid to another is called opioid rotation [19]. It is recommended when the adverse effect/analgesic equation is skewed toward the side effect component. Opioid rotation has been shown to be useful in opening the therapeutic window and in establishing a more advantageous analgesia/toxicity relationship. By substituting opioids and using lower doses than expected (according to equivalency conversion tables), it is possible in most cases not only to reduce the symptoms of opioid toxicity and to manage patients who are highly tolerant to previously used opioids but also to improve analgesia and thus the opioid responsiveness [20].

22.6 Conversion Rate

Medication	Dosage	Oral morphine equipotency
Morphine (mg/day)	1 (ev); 3 (per os)	1:1
Oxycodone (mg/day)	40 mg (per os)	2:1
Hydromorphone (mg/day)	8 mg (per os)	5:1
Fentanyl transdermal micrograms/hour	25	100:1 (25 micrograms are equivalent to 60 mg morphine per os total daily dose)
Buprenorphine transdermal micrograms/hour	35	75–100:1 (35 micrograms are equivalent to 60 mg morphine per os total daily dose)

Previously it was remembered how opioids may be associated with adverse effects, particularly opioid-induced bowel dysfunction, which may negatively influence patients' quality of life. New analgesic options may provide alternative and more effective treatments with lesser toxicity [21]. Tapentadol is a novel centrally acting analgesic with a combined mechanism of action, including μ-opioid receptor activation and a norepinephrine reuptake inhibition [22]. Tapentadol undergoes a process of glucuronization and neither significantly inhibits nor induces clinically important cytochrome enzymes. Because of its low protein binding, displacement reactions are unlikely. A recent study [23] was carried out to assess the efficacy and tolerability of tapentadol for a period of 4 weeks in 30 patients with cancer pain who

were already treated with opioids. Tapentadol was used in doses of 350–450 mg/day and was well tolerated and effective in opioid-tolerant patients with cancer pain and could be considered as a flexible drug to be used for the management of moderate to severe cancer pain. Like most studies in patients with cancer pain, it was limited by its open-label, uncontrolled design, the number of patients lost in follow-up, and discontinuation of the treatment for several reasons. Data on conversion ratios between tapentadol and other opioids are lacking. Preliminary data suggested that a conversion ratio between tapentadol and other opioids, expressed in oral morphine equivalents, could be 1:3.3 in both directions [24].

Constipation is the most common adverse effect during treatment with opioids, and is related to blockage of peripheral μ-receptors. Parenteral administration of naloxone produces a general antagonism of opioid receptors, including those producing the desired central analgesic effects. However, when naloxone is administered orally, the systemic availability of naloxone is negligible because of its extensive elimination by hepatic first-pass metabolism. Thus, the antagonist effects should presumably be limited to intestinal opioid receptors only, avoiding blockage of the desired central analgesic effects of opioids [25]. The slow-release preparation should avoid the overburdening of the hepatic enzymatic system responsible for first-pass metabolism. A tablet combining prolonged release of oxycodone and naloxone may obtain an effective analgesia while improving opioid-induced bowel dysfunction. A randomized study [26] has been performed in cancer patients; 185 patients were enrolled in a randomized, double-blind, double-dummy, parallel-group study. After 4 weeks, better intestinal function, scored by the bowel function index, was observed with prolonged-release oxycodone plus naloxone, and total laxative intake was 20% lower. Moreover, no differences between frequency and dose of laxative rescue medication were found. To demonstrate that there was no measurable loss of analgesia with higher doses of oxycodone plus naloxone, doses were extended up to 120 and 60 mg per day, respectively, with good pain control. Currently, the maximum allowed daily dose is 80 mg of oxycodone and 40 mg of naloxone per day [26].

A particular type of pain is neuropathic pain and management of neuropathic pain is a critical issue. Higher doses of opioids are warranted, and the need for adjuvant analgesics or coanalgesics [27]. The coanalgesic drug group includes gabapentinoids (gabapentin, pregabalin), antidepressants (tricyclic antidepressants, duloxetine, and venlafaxine), corticosteroids, NMDA antagonists, and cannabinoids. If metastatic bone disease with spinal cord compression is present, the use of bisphosphonates or denosumab may represent an important strategy in association with high dose of steroids. Drug toxicity can be critical for tricyclic antidepressants for the anticholinergic and antimuscarinic effect. The most common side effects are cognitive impairment, orthostatic hypotension, dry mouth, constipation, and blurred vision. Tricyclic antidepressants should be started cautiously in patients with cardiac problems, especially in the elderly. A baseline electrocardiography should be performed [28].

22.7 Adjuvant Drugs

Drugs	Indications
Non-steroidal anti-inflammatory drugs	Bone pain, Soft tissue infiltration
Corticosteroids	Raised intracranial pressure, Nerve compression, Soft tissue infiltration
Antidepressants, Anticonvulsants, Antiarrhythmics	Nerve compression or infiltration, Paraneoplastic neuropathies, Neuropathic pain
Bisphosphonates	Bone pain
Denosumab	Bone pain

22.8 Breakthrough Pain

Breakthrough cancer pain has been reported to be a relevant problem in patients with cancer pain. Breakthrough cancer pain has been defined as an acute transient worsening of pain in patients who have a controlled baseline pain [29]. It may be divided into idiopathic or incident (predictable or not predictable) pain; it may be exacerbated by movement or involuntary reflex such as bowel peristalsis and sneezing. Given the temporal characteristics of breakthrough cancer pain (rapid onset and offset), management requires drugs with rapid onset. Breakthrough cancer pain is still typically managed with a rescue dose of oral opioids usually 1/5 or 1/6 of the total daily dose. With most breakthrough cancer pain episodes peaking in intensity within a few minutes and lasting for 30–60 minutes, speed of onset is crucial for effective pain management. After oral transmucosal fentanyl was shown to be superior to oral morphine and placebo, a new generation of delivery systems was developed to improve availability through the mucosal barrier and overcome some practical problems [30].

The onset of action of an oral dose may be very slow (more than 30 minutes) and better results may be obtained with a parenteral rescue dose. Although the intravenous route is the fastest, subcutaneous administration is associated with an acceptable onset of effect and should be considered equivalent in terms of efficacy [29]. Oral transmucosal dosing is a recent non-invasive approach to the rapid onset of analgesia. Highly lipophilic agents may pass rapidly through the oral mucosa

avoiding the first-pass metabolism achieving active plasma concentrations within minutes. Fentanyl is rapidly absorbed. It has been shown to have an onset of pain relief similar to intravenous morphine, which is within 10–15 minutes. When the fentanyl matrix dissolves, approximately 25% of the total fentanyl concentration crosses the buccal mucosa and enters the blood stream. New formulations, such as effervescent preparation, intranasal or sublingual fentanyl, provide rapid analgesia and seem to be more acceptable for patients [30].

The intranasal administration of fentanyl may have some advantages (e.g., in patients with mucosal damage or salivary dysfunction). Two formulations of nasal fentanyl have been developed: an aqueous solution and a pectin-based drug delivery system in the form of a gel designed to be applied to mucosal surfaces to optimize absorption. These delivery systems provide fast and effective analgesia within 5–15 minutes, with the nasal products providing the most rapid effect. Current recommendations are for personalized titration of the dose. However, there is no proof that the use of opioid titration is more efficient than other approaches, such as the use of doses proportional to the basal opioid regimen. It is likely that appropriate dosing may enhance the advantages of such products when used at proportional doses, because this approach may produce better and rapid efficacy while maintaining safety.

22.9 Drugs for Breakthrough Pain

Opioid	Analgesic onset (minutes)	Availability (%)
Oral morphine	30–45	30
Oral oxycodone	30–45	40–50
Oral transmucosal fentanyl citrate	15	50
Fentanyl buccal tablet	15	65
Sublingual fentanyl	15	70
Intranasal fentanyl	5–10	70–90
Pectin intranasal fentanyl	5–10	70–90

Some authors suggest 10 commandments for a correct diagnosis pathway of breakthrough cancer pain: (1) assessment of background analgesia, (2) drugs used for background analgesia, (3) BTcP is a frequent phenomenon often underdiagnosed, (4) characteristics of BTcP, (5) diagnosis of BTcP, (6) continuous assessment, (7) tailored pharmacological treatment of BTcP, (8) selection of BTcP medication, (9) dosing BTcP medications, and (10) education. These steps may help clinicians to recognize and treat BTcP adequately [31].

22.10 Conclusion

Palliative and supportive care are most important in the treatment of cancer patients and may be initiated in the first phases of disease. WHO guidelines have formulated the principal aims of this simultaneous care and have suggested particular attention to physical and psychosocial aspects of patient's life. Pain treatment is a stone of supportive and palliative care, and with application of WHO guidelines, pain control may be achieved in more than 90% of patients. Neuropathic pain is a complex symptom that does not respond well to common analgesics and needs the use of adjuvants. Breakthrough pain is an exacerbation of the symptom in patients with good control of basal pain. It may be underestimated and hence undertreated. Rapid-onset analgesics such as transmucosal, sublingual, and intranasal fentanyl are used with success with amelioration of quality of life.

Key Points

- Palliative and supportive care are approaches that improves the quality of life of patients and their families facing the problem associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and assessment and treatment of pain and other problems, physical, psychosocial, and spiritual, related to a disease.
- World Health Organization (WHO), guidelines have formulated the principal aims of this simultaneous care based on multidisciplinary teamworking, which is outstanding in many branches of healthcare with particular attention to Quality of Life (QoL) and patient clinical condition (or performance status), measurable by Karnofsky scale or ECOG (Table 22.1) equivalent scale is the most utilized method in common clinical practice.
- Cancer pain treatment is a stone of supportive and palliative care. In 1986, the WHO published a set of guidelines for cancer pain management based on the three-step analgesic ladder. However, in the last decades, studies validating the ladder have been shown to have methodological limitation and the role of so-called weak opioids in the treatment of moderate cancer pain has been questioned, and it has been speculated that this second step could be by-passed.
- Opioids may be associated with adverse effects, particularly opioid induced bowel dysfunction, constipation is the most common adverse effect, which may negatively influence patients' quality of life. Opioid rotation has been shown to be useful in opening the therapeutic window and in establishing a more advantageous analgesia/toxicity rela-

tionship. By substituting opioids and using lower doses than expected (according to equivalency conversion tables in ◘ Table 22.6)

- Breakthrough cancer pain has been reported to be a relevant problem in patients with cancer pain, defined as an acute transient worsening of pain in patients who have a controlled baseline pain and is still typically managed with a rescue dose of oral Rapid-onset analgesics opioids usually 1/5 or 1/6 of the total daily dose.

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Patient–Physician Communication

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Contents

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Learning Objectives

By the end of this chapter, the reader will:

- Be able to apply communication strategy in oncological settings
- Have learned the basic concepts of communicating bad news
- Have gained in-depth knowledge of empathy and of active listening
- Be able to put acquired knowledge into clinical practice

23.1 Background and Historical Perspective

» *Of all the healers O Spitama Zarathustra, namely those who heal with the knife, with herbs, and with sacred incantations (words), the last one is the most potent as he heals from the very source of diseases.—Ardibesht Yasht (Ancient Iranian medicine)*

» *Medicine is an art whose magic and creative ability have long been recognized as residing in the inter- personal aspects of patient-physician relationship* [1].

Patient–physician communication refers to a 'patient-focused' model of medical practice rather than the more 'traditional' medical model, which is 'physician-focused' to varying degrees [2]. In the latter approach (traditional/physician-centred), the physician views his task in the medical encounter as primarily one of collecting information, which allows him to make the disease diagnosis and then to determine and communicate to the patient the treatment course.

This model does not consider the role of patient preferences and choices in determining treatment as important and employs simplistic supportive techniques in providing care. According to patient-focused approach, the physician is a 'healer' as well as an important instrument of patient support in the relationship with the patient and family. In this model, the goals of medical care include understanding the patient's concerns (agenda) and values, assessing coping and family situation, eliciting patient worries and possible barriers to implementing the treatment plan, involving patients in decision-making and in implementing the treatment plan and applying one's skills in building rapport and trust.

Effective communication is essential in developing any kind of relationship, but it is particularly true between oncologists and their patients. People diagnosed with cancer face a life-threatening event, which could be extremely challenging psychologically.

People need connection, especially in times of distress. A multitude of emotions may be precipitated during cancer diagnosis communication. Many are quite intense, often to the point of being overwhelming. Patients look to oncologists for knowledge, guidance, reassurance, hope, meaning and compassion. Unfortunately, the quality of communication in healthcare is often suboptimal. In fact, studies have shown that discussions of bad news do not meet patient needs [3, 4, 5]. Moreover, patients with cancer tend to disclose less than 50% of their concerns [6] because of the inability to communicate with their physician. The number and severity of unresolved concerns have been shown to predict high emotional distress and future anxiety and depression in patients [7, 8].

Breaking bad news is a complex communication task. In addition to the verbal component of actually giving the bad news, it also requires other skills. These include responding to patients' emotional reactions, involving the patient in decision-making, dealing with the stress created by patients' expectations for cure, involvement of multiple family members, and dilemma of how to give hope when the situation is critical.

Bad news may be defined as '*any information which adversely and seriously affects an individual's view of his or her future*' [9]. Bad news results in a cognitive, behavioural or emotional deficit in the person receiving the news that persists for some time after the news is received. Bad news is always, however, in the 'eye of the beholder', such that one cannot estimate the impact of the bad news until one has first determined the recipient's expectations or understanding. Bad news does, of course, have gradations, which to a certain extent are subjective, dependent on an individual's life experiences, personality, spiritual beliefs, philosophical standpoint, perceived social supports and emotional hardiness [10]. Bad news is the gap between how a person expected things to go and how they actually turned out.

Bad news is very isolating and the way it is delivered can extend this isolation and provoke further anger/frustration. In communicating bad news, it is therefore important to create a strong connection between the patient and the physician, so that when things go wrong the patient is able to separate the messenger from the message. With this strong relationship even though the message is bad, the messengers are perceived as part of the support system.

Patients' emotions interact with all facets of communication processes and outcomes, for instance, affecting their desire for information, their comprehension of information, the impact of information on their decisions, their willingness and ability to connect with the physician, their desire for autonomy and their perceived resilience [9].

The quality of the patient–physician communication is a key element for the psychological wellbeing of the patient but also for compliance with therapy.

Patients' concerns about illness and therapy are best addressed when oncologists and patients form a strong alliance to address psychosocial, medical and educational issues fundamental to these concerns [10]. Patient–oncologist relationship is an integral part of care processing.

Patient–physician communication plays a critical role in predicting patients' health-related attitudes and behaviours and thus ultimately their health outcomes [11]. For these reasons, communication skills are the cornerstone of comprehensive cancer care.

Objectives of communication in healthcare are as follows:

- To explain medical conditions and provide essential medical information.
- To uncover patient and/or family needs, often by engaging in therapeutic dialogue.
- To discuss goals of care.

Given the therapeutic implication of communication in health care settings, significant advances to close the communication gap have occurred over the past several decades, largely by addressing deficiencies in the various stages of an oncologist's lengthy training: undergraduate medical education, residency and fellowship and continuing medical education. Stemming from several milestones achieved by highly motivated groups of individuals, including the creation of consensus statements and guidelines by communication education experts, progress has been made to improve patient–oncologist communication [12].

Contemporary oncology practice acknowledges the importance of partnering with the patient and family in dealing with the illness [13, 14]. Patients also value their physicians as important sources of support when they provide information about the illness, encouragement and hope; discuss treatment options and address their concerns [15]. For this reason, outcomes associated with the quality of the physician-patient relationship have received increasing recognition.

For instance, Baile et al. [16] highlighted relevant studies bearing on important outcomes of communication with cancer patients and discussed the implication for training oncologists of the future.

This progress is marked by the development of evidence-based communication skill training programmes, to promote competent, communication-minded physicians necessary for effective cancer care [12]. Examples of these programmes are Oncotalk and Comskill.

OncoTalk (► https://depts.washington.edu/oncotalk/learn/, [17]) is a website designed to assist medical professionals with the difficult conversations around dying and end-of-life care. It contains a series of learning modules on critical communication topics such as fundamental communication skills; giving bad news; managing transitions to palliative care when chemotherapy is failing; talking about advance care plans and do not resuscitate orders; discussing treatment options and informed consent; conducting a family conference; handling requests for therapies that you feel are futile and cultivating personal communication skills.

ComSkill [18] is the Memorial Sloan-Kettering Cancer Center Model for Communication Skill Training, and it is based on Goals, Plans and Actions theories and sociolinguistic theory. Authors of the programme conceive of consultation communication as having five components *goals, strategies, skills, process tasks* and *cognitive appraisals* and have developed modules for each of these components.

In the recent years, with the spread of the Internet, doctors meet patients who are more informed than ever before. Social media has become a pivotal aspect in our communication with the world around us.

In health care, the rapid proliferation of health information on the Internet has resulted in more patients turning to the Internet as their first source of health information [19, 20] and acquiring knowledge on their health conditions before seeking a professional diagnosis. This may thus change the way in which patients interact with and participate in consultations with their physicians and how they feel about their relationship with their physicians. In a recent systematic review [21], results showed that Internet health information seeking can improve the patient–physician relationship depending on whether the patient discusses the information with the physician and on their prior relationship. As patients have better access to health information through the Internet and expect to be more engaged in health decision-making, traditional models of the patient–provider relationship and communication strategies must be revisited to adapt to this change.

In this chapter, we will analyse principle of patient–physician relationship outline protocols and models eliciting an empathic and effective communication.

23.2 General Outcome of Medical Communication

One useful model to represent the essential outcome of medical communication is the 'E's' [22]. Even if not all goals are pertinent to every medical visit, we could consider them as the general outcomes of a reliable patient–physician relationship.

1. *Engagement*
   To establish a therapeutic alliance between patient and physician, engagement is essential. Physicians have to help patients feel comfortable with the situation.
2. *Eliciting the patient's understanding*
   One other important issue is clearly understanding of patients (and family) knowledge and expectation about illness and treatment (see technique 'ask before telling'). Clinicians have to discover them before giving new information and to avoid misunderstandings and unrealistic expectations. Neglecting this step could lead to a disconnection between patient and physician and to inhibit a trustful relationship.
3. *Education*
   Nowadays, most people want data and prefer to have information about their illness and to have an active role in the decision-making process. Understanding patient's information needs, their comprehension ability is fundamental to better adapt the communication process onto patient's personality and values.
4. *Emotions*
   Addressing and understanding patient's emotion is central in helping relationship. It is a supportive intervention that could help reduce cancer-related distress level (see Empathy paragraph).
5. *Enlisting the patient's and family collaboration*
   To help patients and their families maintain a certain control on life-threating event, it is important to underlie their active contribution to patient care.

23.3 Principles of Patient–Physician Communication

One cannot not communicate; every communication has a content and a relationship aspect such that the latter classifies the former and is therefore a meta-communication [23].

Communication is a learned skill or a series of learned skills based on three pillars:

- Accuracy
- Efficiency
- Supportiveness

All combine to contribute to the effectiveness of communication

An expert in breaking bad news is not someone who gets it right every time—he or she is merely someone who gets it wrong less often, and who is less flustered when things do not go smoothly [9].

Communication skills are associated with important care outcomes for the oncologist, not the least of which is reduced probability of malpractice litigation. Other important outcomes of patient–physician communication include increased patient satisfaction, enhanced accrual to clinical trials, better informed consent for treatments, increased cooperation with care, decreased physician burnout and increased competence in discussing important issues such as end-of-life care.

Language plays a prominent role in all stages of the medical process, from noting symptoms, questioning patients and describing physiological functions, to history taking and noting the progress of disease, to writing a prescription (and can be extended to medical case notes and the complaint procedure). But communication, as is widely recognised by sociolinguistic, pragmatic and discourse analytic disciplines, is much more than language per se. Srikant Sarangi in his editorial 'Towards a communicative mentality in medical and healthcare practice' on first number of *Communication & Medicine* journal represented in the following mnemonic all the variables of communication [24]:

C = Code (linguistic, visual, non-verbal etc.)
O= Orderliness
M = Message
M = Mediation
U = Understanding
N = Narrative Style & Structure
I = Inferencing & Intentionality
C = Context (micro- and macro-levels)
A = Audience, Addressee
T = Tone (feeling, evaluation, key etc.)
I = Identity & Role
O= Objective/Goal
N = Norms (social, cultural, interpersonal)

Each of these variables should take into account when physicians approach patients.

ASCO Consensus Guideline on Patient–Clinician Communication [25] provides guidance to oncology clinicians on how to communicate effectively so as to optimise the patient–clinician relationship, patient care and the wellbeing of clinicians, patients and their loved ones. It also touches on key aspects of effective communication skill training. According to this guideline, the core communication skills and tasks that apply across the continuum of cancer care are as follows:

1. Before each conversation, clinicians should review the patient's medical information, establish goals for the conversation and anticipate the needs and responses of the patient and family.
2. At the beginning of conversations with patients, clinicians should explore the patient's understanding of their disease and collaboratively set an agenda with

the patient after inquiring what the patient and family wish to address and explaining what the clinician wishes to address.
3. During patient visits, clinicians should engage in behaviours that actively foster trust, confidence in the clinician and collaboration.
4. Clinicians should provide information that is timely and oriented to the patient's concerns and preferences for information. After providing information, clinicians should check for patient understanding and document important discussions in the medical record.
5. When patients display emotion through verbal or non-verbal behaviour, clinicians should respond empathically.

All of these skills could be learned through active listening and empathy.

23.3.1 Active Listening

Listening is the most fundamental component of interpersonal communication skills.

Listening is not something that just happens (that is hearing), listening is an active process in which a conscious decision is made to listen to and understand the messages of the speaker.

Active listening is a communication technique that is used in counselling, training and conflict resolution. It requires that the listener fully concentrate, understand, respond and then remember what is being said [26], not only just passively 'hearing' the message of the speaker (reflective listening)

Carl Rogers and Richard Farson coined the term 'active listening' in 1957 defining it as an important way to bring about changes in people. Despite the popular notion that listening is a passive approach, clinical and research evidence clearly shows that sensitive listening is a most effective agent for individual personality change and group development. Listening brings about changes in peoples' attitudes toward themselves and others; it also brings about changes in their basic values and personal philosophy. People who have been listened to in this new and special way become more emotionally mature, more open to their experiences, less defensive, more democratic and less authoritarian. Active listening is a communication technique used in counselling, training and conflict resolution, which requires the listener to feed back what they hear to the speaker, by way of restating or paraphrasing what they have heard in their own words, to confirm what they have heard and moreover, to confirm the understanding of both parties. Active listening does not necessarily mean long session spent listening to patients, it is a way of approaching patients, but even if it is a skill that can be acquired and developed with practice, to be effective, active listening must be firmly grounded in the basic attitude of the user.

Active listening involves listening with all senses. As well as giving full attention to the speaker, it is important that the 'active listener' is also 'seen' to be listening – otherwise the speaker may conclude that what they are talking about is uninteresting to the listener. Interest can be conveyed to the speaker by using both verbal and non-verbal messages such as maintaining eye contact, nodding your head and smiling, agreeing by saying 'Yes' or simply 'Mmm hmm' to encourage them to continue. By providing this 'feedback', the person speaking will usually feel more at ease and therefore communicate more easily, openly and honestly.

What good listeners do:

- Remain neutral and avoid judgements, this means trying not to take sides or form opinions, especially early in the conversation.
- Encourage emotional expression.
- Pay attention and observe him/herself and the other (patient).
- Be patient—pauses and short periods of silence should be accepted.
- Respect silence. Do not jump in with questions or comments every time there are a few seconds of silence.
- Give the other person time to explore their thoughts and feelings; they should, therefore, be given adequate time for that.
- Avoid distraction and therefore refrain from fidgeting, looking at a clock or watch or phone, doodling, playing with their hair or picking their fingernails.
- Use verbal sign of attentive or active listening: Positive Reinforcement with explanation, paraphrasing, remembering, Questioning, Reflection, Clarification, Summarisation.
- Actively show non-verbal signs of listening: these signs may not be appropriate in all situations and across all cultures and situations; the typical non-verbal behaviour that facilitate communication and active listening are smiling, eye contact, voice tone, posture and mirroring.

What good listener do not do:

- Interrupt
- Allow distractions
- Judge
- Criticise
- Argue

- Use cliche´d phrases such as: 'I know exactly how you feel', 'It's not that bad', or 'You'll feel better tomorrow'
- Get pulled into responding emotionally
- Change the subject or move in a new direction
- Rehearse in your head what you plan to say next
- Give advice

Active Listening Instruments

1. Using Open questions (question without yes/no answer).
2. Clarifying.
3. Paraphrasing and repeating.
4. Reflecting on feelings and emotions.
5. Verifying patient's understanding.
6. Summarising.
7. Using pause and respecting patient silence.
8. Observing non-verbal behaviour.

23.3.2 Communication Barriers

Effective communication requires paying attention to an entire process, not just the content of the message. Physicians should consider potential barriers at several stages.

Thomas Gordon, an American psychologist and the developer of 'Parent Effectiveness Training' (PET), identified twelve roadblocks to active listening (1975) that could be applicable also to patient–physician relationship.

These roadblocks are common responses that get in the way of good listening. They are not necessarily wrong, but they are not listening because they interrupt the person's own exploration, and in order to get back to his or her own process, the person must go around them (hence the term roadblock).

I. *Roadblocks that avoid patient's autonomy are as follows:*
   1. Ordering, directing or commanding.
   2. Warning or threatening.
   3. Moralising, preaching, giving 'shoulds' and 'oughts'.
   4. Advising, offering solutions or suggestions.
   5. Teaching, lecturing, giving logical arguments.
   6. Judging, criticising, directing, blaming.

II. *Roadblocks that could lead to misunderstanding between patients and physicians are as follows:*
   7. Name calling, stereotyping, labelling.
   8. Interpreting, Analysing, Diagnosing *(especially when physicians anticipate patients' feelings and thought without asking or listening)*.

III. *Roadblocks that could let patients thinks that the physician wants to deny their feelings or problems*
   9. Praising, agreeing, giving positive evaluations.
   10. Reassuring, sympathising, consoling.

IV. *Roadblocks that try to quickly solve the problem for the patient or avoid emotion.*
   11. Questioning, interrogating, cross-examining.
   12. Withdrawing, distracting, humouring, changing the subject.

These roadblocks could elicit barriers from patients that may impede effective consultation communication.

23.3.3 Emotion and Empathy

A multitude of emotions may be precipitated during and after cancer diagnosis communication. Many are quite intense, often to the point of being overwhelming. Patients' emotions interact with all facets of communicating processes and outcomes, for instance, affecting their desire for information, their comprehension of information, the impact of information on their decisions, their willingness and ability to connect with the physician, their desire for autonomy and their perceived resilience. Responding to the patient's emotions is one of the most difficult challenges of breaking bad news. Patients' emotional reactions may vary from silence to disbelief, crying, denial or anger [10].

When patients get bad news, their emotional reaction is often an expression of shock, isolation and grief.

Emotions can be addressed by three useful verbal techniques.

1. Exploring or '*clarifying' them.*
2. *Acknowledging* or 'validating' them.
3. Empathising or making statements that show the patient that the physician is in tune with how the person is feeling and why they feel that way.

A helpful mnemonic summarises what to do in responding and accepting patient emotions: N.U.R.S.E. [27].

- N—Name the emotion.
- U—Understand/normalise the emotion.
- R—Respect the patient and family for how they are coping.
- S—Support the patient so they don't feel alone.
- E—Explore the emotion.

Empathy is the cognitive process of identifying with or vicariously experiencing the feelings, thoughts or attitudes of another [28].

Empathy is not:

- Having had the same experience or problem
- Identification with the client
- Let me tell you my story

Empathy is:

- The ability to accurately understand the client's meaning

- The ability to reflect that accurate understanding back to the client

Things to consider:
1. Uncertainty about reaction
2. Fear of destroying hope
3. Fear of own inadequacy
4. Unprepared to deal with person's emotional reaction
5. Embarrassing

Understanding the patient's perspective will result in physicians discovering more about the thoughts and feelings patients are experiencing.

To promote an empathic relationship, physicians have to:
a. Accept what the patient says non-judgmentally
b. Acknowledge that patients ought to hold their own views and feelings
c. Validate the importance of the patient's contributions in a therapeutic relationship

It is important to note that acceptance is not the same as agreement. A physician could accept that a patient wishes to be cured of cancer, yet not agree that it is possible. This distinction is important is building and maintaining a relationship.

An empathic response consists of four steps [10]:
- First, observe for any emotion on the part of the patient.
- Second, identify the emotion experienced by the patient by naming it to oneself.
- Third, identify the reason for the emotion.
- Fourth, after you have given the patient a brief period of time to express his or her feelings, let the patient know that you have connected the emotion with the reason for the emotion by making a connecting statement.

23.4 Classical Communication Model and Techniques into Oncological Setting

The SPIKES protocol consists of six steps for disclosing unfavourable information—'breaking bad news'—to cancer patients about their illness [10]. The goal is to enable the clinician to fulfil the four most important objectives of the interview disclosing bad news: gathering information from the patient, transmitting the medical information, providing support to the patient, and eliciting the patient's collaboration in developing a strategy or treatment plan for the future.

The Six Steps of SPIKES
- *STEP 1: S—SETTING UP the Interview*
- Mental rehearsal is a useful way for preparing for stressful tasks. This can be accomplished by reviewing the plan for telling the patient and how one will respond to patients' emotional reactions or difficult questions. Step 1's guidelines are as follows:
  - *Arrange for some privacy*.
  - *Involve significant others*.
  - *Sit down*.
  - *Make connection with the patient*.
  - *Manage time constraints and interruptions*.
- *STEP 2: P—Assessing the Patient's PERCEPTION*
- Before discussing the medical findings, the clinician uses open-ended questions to create a reasonably accurate picture of how the patient perceives the medical situation and what are his/her expectations and feelings about illness and treatments.
- *STEP 3: I—Obtaining the Patient's INVITATION*
- While a majority of patients express a desire for full information about their diagnosis, prognosis, and details of their illness, some patients do not. When a clinician hears a patient express explicitly a desire for information, it may lessen the anxiety associated with divulging the bad news. However, shunning information is a valid psychological coping mechanism and may be more likely to be manifested as the illness becomes more severe. Discussing information disclosure at the time of ordering tests can cue the physician to plan the next discussion with the patient. If patients do not want to know details, offer to answer any questions they may have in the future or to talk to a relative or friend.
- *STEP 4: K—Giving KNOWLEDGE and Information to the Patient*
- Warning the patient that bad news is coming may lessen the shock that can follow the disclosure of bad news and may facilitate information processing.
- *STEP 5: E—Addressing the Patient's EMOTIONS with Empathic Responses*
- In this situation, the physician can offer support and solidarity to the patient by making an empathic response.
- *STEP 6: S—STRATEGY and SUMMARY*
- Patients who have a clear plan for the future are less likely to feel anxious and uncertain. Before discussing a treatment plan, it is important to ask patients if they are ready at that time for such a discussion. Checking the patient's misunderstanding of the discussion can prevent the documented tendency of patients to overestimate the efficacy or misunderstand the purpose of treatment.

Key Points

- Giving bad news becomes a task for the oncologist. Thus, a strategy for giving bad news honestly, compassionately and hopefully can be helpful to the physician and mostly for the patient.
- Communication skills are not innate abilities, they have to be learned because they are associated with important care outcomes for the oncologist, including patient satisfaction, accrual to clinical trials, increased cooperation with care, decreased physician burnout and increased competence in discussing important issues such as end-of-life care.
- Emotions play a crucial role in patient–physician relationship, so that an effective communication must take into account patient's and physician's emotion management and awareness, and to do it, oncologists must learn empathic approach and active listening.
- Recommendations when approaching patients in a clinical setting:
  1. You welcome the patient as you would like to be welcomed (and accepted).
  2. Listen to the patient the same way you would like to be listened to.
  3. Do not judge the patient as you would like not to be judged.

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Bone Health in Cancer Patients

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24

Learning Objectives

By the end of chapter, the reader will

- Have learned the basic concept of bone metastasis physiopathology;
- Have reached in-depth knowledge of bone-targeted agents;
- Be able to put acquired knowledge into clinical practice in the management of bone metastatic patients.

24.1 Bone Metastases

Bone metastases are a common complication of several types of cancers, including breast, prostate, and lung cancer. The occurrence of bone metastases led to so-called skeletal-related events (SREs), which include pathological fractures, spinal cord compression, and severe bone pain that require palliative radiotherapy and/or orthopedic surgery [1]. These complications influence patients' quality of life, reducing mobility, social functioning, and overall survival (OS). The risk of bone fractures increases in patients of both sexes above the age of 70 years, even if postmenopausal women from age 50 years onward have a major risk to develop SREs compared to men [2, 3].

Bone metastases differ depending on their tumor origin and are divided in osteolytic (breast and lung cancers), sclerotic (prostate cancer), or mixed (gastrointestinal and squamous cancers) metastases. Tumor cells secrete factors that may disrupt physiological bone remodeling processes through the deregulation of the normal osteoclast and osteoblast functions. Indeed, osteolytic bone metastases are mediated by stimulation of osteoclast activity through tumor-derived cytokines, driving to bone matrix degradation [4]. Instead, sclerotic metastases are characterized by excessive abnormal bone formation mediated by activated osteoblasts, resulting in low bone strength. Mixed metastases present both sclerotic and osteoblast features.

The abnormal activity of osteoclasts and osteoblasts, responsible for bone metastasis development, lead to the release of mitogenic factors influencing tumor growth and establishing the so-called vicious cycle of cancer. The vicious cycle, described for the first time in 1997 by Mundy [5], is a complex process based on the interaction between tumor and bone cells, where the resorption/bone formation and tumor proliferation feed off each other.

24.2 Bone Metastasis Physiopathology

Bone is a dynamic tissue that undergoes a continuous vital process of remodeling made by bone cells: osteoblasts, osteoclasts, and osteocytes [6, 7]. These cells regulate the mineralization in a coordinating network responding to different stimuli such as mechanical load, cytokines, and hormonal signals. However, bone diseases, including tumors, alter the physiological balance between bone deposition and desorption, leading to the loss of the skeleton integrity.

Bone metastases development is a consequence of several complex mechanisms that include tumor cell seeding, tumor dormancy, and the subsequent metastatic growth.

In particular, some of cells released by primary tumor reach distant organs through the circulatory system, while the majority dies. Primary tumor itself can influence and alter the environment of secondary organs promoting the formation of supportive metastatic niche [8]. Bone metastatic niche represents the ideal site for dormant tumor cells (DTCs) stabilization, where they can survive in a dormant state stopping to proliferate or proliferating at a reduced rate. DTCs are resistant to cancer therapies and can remain in quiescence for long time, even beyond 10 years and then spread and colonize other organs [9]. The switch from dormant state to proliferative one is regulated by bone metastatic niche [10]. In particular, it was known that factors including vascular endothelial growth factor (VEGF), fibronectin, and matrix metalloproteinases (MMPs) secreted from myeloid cells in the niche promote the angiogenic switch necessary to tumor cell escape from dormancy [11]. Thus, the reactivated tumor cells establish a complex interplay with bone cells leading to the *vicious cycle* of cancer that support the subsequent metastatic growth (◘ Fig. 24.1, ◘ Table 24.1). In particular, tumor cells release several soluble factors such as parathyroid hormone-related protein (PTHrP9) and interleukine-6 (IL-6) that determine a switch in receptor activator of nuclear factor kappa-B ligand/osteoprotegerin (RANKL/OPG) balance in favor of RANKL [12]. RANKL overexpression stimulates osteoclastic bone resorption and then the release of growth factors including bone morphogenetic proteins (BMPs), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), tumor necrosis factor β (TGF-β) that, in turn, promote cancer cell survival and proliferation. Recent evidences have shown that tumor cells release other factors like endothelin-1 (ET-1) and activate Wnt pathway, resulting in OPG secretion [13, 14]. OPG stimulates osteoblast differentiation and activity promoting the formation of new, but unstructured bone, prone to fracture [15]. RANKL production by activated osteoblast promotes osteoclastic activity and thus the release of bone matrix-derived factors that, in turn, stimulate cancer cells closing the cycle [12].

Several agents targeting these molecular pathways have been investigated in preclinical and clinical trials (◘ Table 24.2).

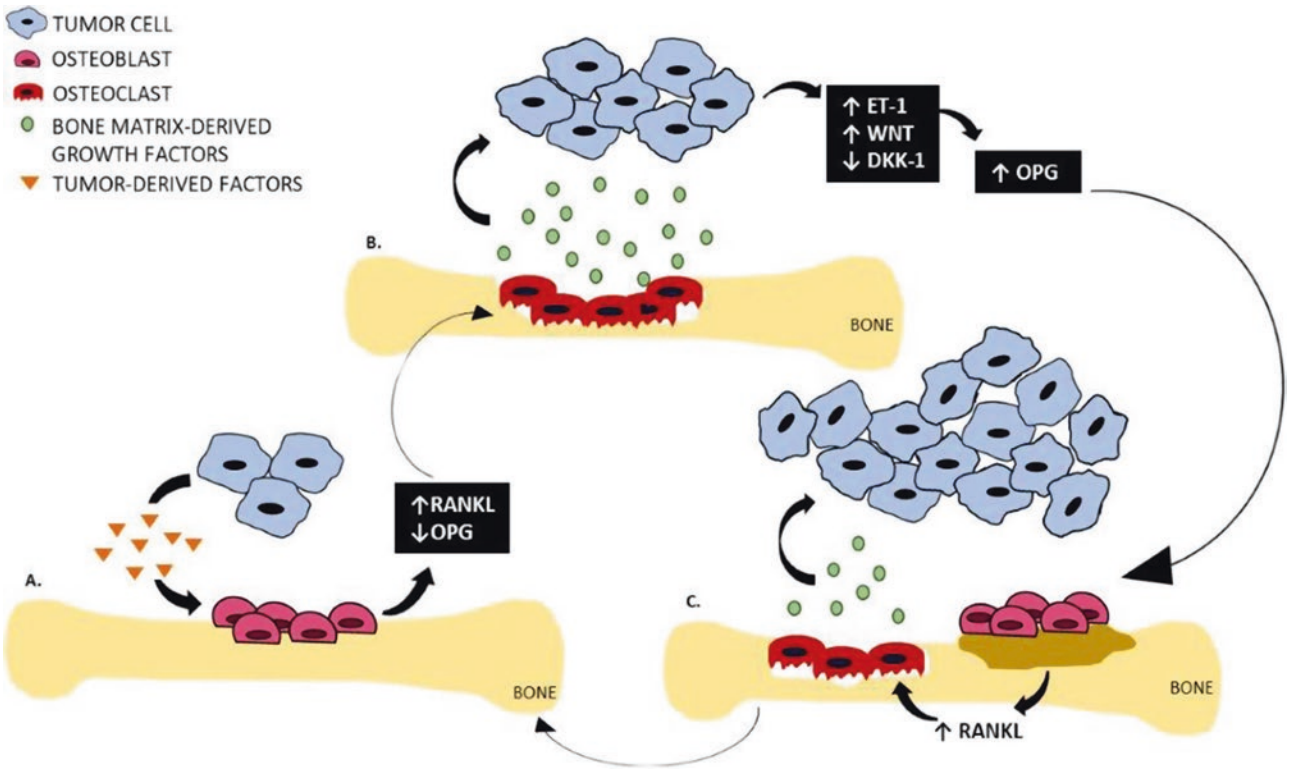

Fig. 24.1 The modern tumor vicious cycle: **a** Tumor-derived growth factors stimulate osteoblast activity inducing an increasing of RANKL that activates osteoclast bone resorption. **b** Growth factors released from bone matrix degradation promote the proliferation of tumor cells leading to OPG production through WNT pathway activation. **c** OPG stimulates osteoblast mineralization promoting RANKL secretion and thus, osteoclast activity. Osteoclastic bone resorption produces soluble factor that, in turn, stimulate cancer cells closing the cycle

Table 24.1 Principal activated pathways in the vicious cycle

Pathway	Function	References
RANK/ RANKL/ OPG axis	RANK expression on tumor cells facilities their migration into the bone Tumor cells induce a shift in RANKL/OPG balance in favor of RANKL RANKL up-regulation increases bone resorption and the release of pro-tumoral growth factors from bone matrix	[12, 15, 18–20]
Endothelin axis	ET-1 released by tumor cells stimulates osteoblast proliferation and activity	[13]
Wnt/DKK-1 axis	Canonical Wnt pathway induces OPG expression in osteoblasts promoting new bone apposition	[14]

RANK nuclear factor kappeB, *RANKL* nuclear factor kappeB ligand, *OPG* Osteoprotegerin, *ET-1* Endothelin1, *DKK-1* Dickkopf-related protein 1

24.3 Bone Metastasis Regulator Pathways

24.3.1 RANK–RANKL–OPG Axis

RANK–RANKL–OPG axis plays a crucial role in bone metastasis development. RANK expression has been founded in several tumor cell lines, including osteosarcomas and breast and prostate cancers [16, 17]. Moreover, RANK/RANKL expression has been reported in human tumor biopsies as well as breast and prostate cancers and hepatocellular carcinoma. Preclinical studies suggest that RANK expression in tumor cells facilities their migration to the bone, where RANKL is abundantly expressed. In particular, murine in vivo models showed RANKL as a potent chemoattractant in tumors and supported the pro-migratory activity of RANK-expressing breast and prostate cancer cell lines; moreover, in an in vivo melanoma model of bone metastases the inhibition of RANKL resulted in a reduction of bone lesions and tumor burden [18]. Finally, it has been demonstrated that RANK expression level in the primary tumor correlated with the occurrence of bone metastases, and RANK-expressing cancer could be found in up to 80% of bone metastases originated from solid tumor [19, 20]. Recently, evidences

Table 24.2 Principal drugs targeting molecules involved in the vicious cycle

Drug	Target	Phase study	Evidences	References
Denosumab	RANKL	Phase III randomised study Phase III randomised study Phase III randomised study Post hoc analysis of the 3 previous phase III trials	Superior to ZA in delaying or preventing SREs in bone metastatic breast cancer patients Superior to ZA in preventing SREs in mCRPC patients Not inferior to ZA in preventing or delaying first on-study SRE in patients with advanced bone metastatic cancer (excluding breast and prostate cancers) or myeloma Superior to ZA in in preventing SREs in patients with bone metastases from advanced cancer	[23, 113–115]
Zibotentan Atrasentan	ETAR	Phase III randomised study Phase III randomised trail Phase III randomised trail Phase III randomised trail	Absence of survival benefits in non-mCRPC patients Not significant improvements in OS in mCRPC patients Not improvement in OS in mCRPC patients in combination with docetaxel Not delay disease progression in men with mCRPC patients	[147–150]
BHQ880	DKK-1	Phase lb trial	Potential clinical activity in patients with relapsed MM in combination with ZA and anti-myeloma therapy	[152]

ZA Zoledronic Acid, *SREs* Skeletal-related events, *mCRPC* metastatic castration resistant prostate cancer, *ETAR* Endothelin type A receptor, *OS* Overall survival, *MM* Multiple myeloma

suggest an important role for RANKL/RANK in the immune system including in lymph node development, lymphocyte differentiation, dendritic cell survival, T-cell activation, and tolerance induction. Detailed studies in mouse models have clearly demonstrated the involvement of RANKL signaling in the functions of immune regulatory cells, such as dendritic cells, M-cells (specialized epithelial cells in mucosal tissues), and mTECs (epithelial cells localized in the thymic medulla) [21]. Notably, the functions of dendritic cells and the maintenance of M-cell numbers were impaired by the inhibition of RANKL signaling in adult mice leading to T-regs lymphocytes expansion and subsequent local and systemic immunosuppression [22]. The result of these alterations was an increase in bone resorption, tumor invasiveness, and cancer cells immune system evasion. The development and approval of denosumab, a fully monoclonal antibody against RANKL, has heralded a new era in the treatment of bone diseases by providing a potent, targeted, and reversible inhibitor of bone resorption [24].

24.3.2 Endothelin-1 (ET-1)

Endothelin-1 (ET-1) is an important factor released by tumor cells with physiological and pathological functions that promotes bone metastasis development. ET-1 is responsible to induce the release of several pro-inflammatory molecules, such as IL-6, chemokine (C–C motif) ligand 2 (CCL2), monocyte chemoattractant protein 1 (MCP-1), cyclooxygenase (COX2), and MMPs that mediated tumor invasiveness and metastasis [25–27].

ET-1 promotes osteoblast proliferation and decreases osteoclast activity, leading to the formation of typical sclerotic lesions of metastatic prostate cancer [13]. Indeed, elevated ET-1 plasma concentrations were observed in hormone refractory prostate cancer patients compared to healthy control. Moreover, immunohistochemistry of prostate cancer biopsies showed ET-1 positivity [28, 29]. In addition, ET receptor expression is associated with reduced disease-free survival time and with the major clinicopathological markers of aggressiveness and poor prognosis in breast cancer patients [30].

Atrasentan is an inhibitor of the ETA receptor that has been showed to block formation of osteoblastic metastases in mice. Nevertheless, in a placebo-controlled phase III trial in men with metastatic prostate cancer, atrasentan failed to demonstrate a reduction of overall survival, risk of disease progression, and cancer-induced bone pain [31].

Zibotentan (ZD4054) is an oral, specific ETA receptor antagonist extensively investigated in the ENTHUSE clinical development program. ENTHUSE M1 trial showed no significant improvement in OS with zibotentan monotherapy versus placebo in men with mildly symptomatic CRPC (24.5 versus 22.5 months, respectively) [32]. Moreover, the ENTHUSE M0 trial of zibotentan monotherapy in patients with non-metastatic CRPC has not demonstrated survival benefits [33]. Finally, in the ENTHUSE M1C randomized phase III trial zibotentan in combination with docetaxel has not showed improvement in OS compared to docetaxel alone in mCRPC patients [34].

24.3.3 Integrin and Cadherin

Tumor metastases require the activity of several adhesion molecules including the superfamily of integrins and cadherins.

The heterodimeric (α and β monomers) transmembrane glycoproteins *integrins* have a cell-type specificity and anchor cells to the extracellular matrix (ECM) binding their ligands. Stable adhesion to the ECM is fundamental to cell survival, indeed detached cells undergo an apoptotic process, known as anoikis [35]. Metastatic cells elude this mechanism expressing aberrant integrins [36], activating different pathways like focal adhesion kinase (FAK) [37], epidermal growth factor receptor (EGFR) [38], and Src [40] and inhibiting apoptosis [39].

Several studies correlated integrin expression (αvβ3, αvβ5, α5β1, α6β4, α4β1, and αvβ6) with the progression of breast carcinoma, prostate, pancreatic and lung cancers, and melanoma [41]. In addition, a correlation between integrins α2 e α6 – and also c-MET- expression and bone metastases development was found [42]. Integrin β1 is another fundamental integrin in prostate cancer progression that promotes bone and node metastasis formation through the activation of Akt pathway [43].

Cadherins, calcium-dependent transmembrane proteins, regulate the formation of adherence junctions to bind cells with each other. Loss of function of cadherins has linked to bone metastasis development [44]. Depending on their form (E-cadherin or N-cadherin), these proteins can act as a suppressor or promotor of cancer invasion and metastases. Indeed, the switch from E-cadherin to N-cadherin is critical for epithelial to mesenchymal transition (EMT) and thus, for metastases onset [48]. In particular, Gravdal and colleagues demonstrated that this switch is associated with decreased OS and higher skeletal recurrence in patients with prostate cancer undergone radical prostatectomy [45]. Another group showed that in human samples E-cadherin is higher express in bone metastasis compared to primary tumor [46]. The overexpression of N-cadherin in prostate cancer cells [45] probably is due to a higher aggressiveness of the tumor and not by a bone tropic behavior of the cells, but nonetheless N-cadherin expression is a good marker of further skeletal recurrence.

Among all, cadherin 11 has demonstrated to promote bone metastases. In particular, it has observed that marrow stromal cells express cadherin-11 (OB-cadherin) that facilitates the homing of breast cancer cells to the bone as well as stimulates osteoclastogenesis [47].

Similarly, in preclinical models of prostate cancer, cadherin-11 enhances migration and invasiveness of tumor regulating also the expression of pro-invasive genes [49].

24.3.4 Wnt and Dkk-1

Wnt proteins represent a secreted group of glycoproteins that bind the 7-transmembrane domain receptors regulating several cellular functions (growth, differentiation, and death). Wnt activity is also important for osteoblasts formation from their precursors, inhibiting in the same time osteoclastogenesis [50]. The activity of Wnt pathway is negatively regulated by the Dickkopf-related protein 1 (Dkk-1) that binds its receptor blocking the downstream signaling.

The role of canonical Wnt signaling has been widely demonstrated in several tumor types [54].

Wnt pathway could be also activated by fibroblast-secreted exosomes that contain active Wnt ligands or β-catenin-promoting motility and invasiveness of breast cancer cells [55].

The balance between Wnt and Dkk-1 activity determines the nature of bone metastasis in prostate cancer: Several studies have showed in preclinical settings that Wnt activation or inhibition are, respectively, linked to sclerotic and lytic bone lesions [56–58]. Indeed, prostate cancers usually express lower levels of Dkk-1 compared to normal prostate tissues, presenting mostly sclerotic metastases [53].

Higher Dkk-1 serum levels are associated with poorer OS, as demonstrated by Rachner et al. [59]. Prognostic value of Wnt–DKK1 axis was further investigated by Chen et al. who showed that high expression of miR34a in primary tumor, a negative regulator of the

Wnt downstream effector TCF7, was found to be correlated to an improved OS in a retrospective analysis of 24 patients with metastatic prostate cancer [60].

BHQ88O is a fully human anti-DKK1 neutralizing immunoglobulin G1 (IgG1) with high affinity for his target. The phase Ib trial showed that BHQ880 in combination with zoledronic acid and anti-myeloma therapy was well tolerated and demonstrated potential clinical activity in patients with relapsed/refractory multiple myeloma [61].

24.3.5 CXCR4/CXCL12

The chemokine CXCL12, called also SDF-1, is a chemoattracted cytokines that binding its receptors (CXCR4 and CXCR7) regulates cellular migration. Several studies have demonstrated the involvement of CXCL12–CXCR4–CXCR7 axis in the establishment of metastases from different tumors [62]. Indeed, in prostate, cancer high levels of CXCL12 regulates the metastatic spread in the bone marrow and the binding with its receptors activates divergent cellular responses such as cell survival, proliferation, and angiogenesis. Moreover, high levels of CXCR7 protein are associated to most aggressive tumors and promotes the release of proangiopoietic factors such as IL-8 and VEGF [63].

In breast cancer, CXCR4 and CXCL12 have a key role in the metastatic process as showed by Muller and colleagues who observed a higher expression of CXCR4 in breast tumor samples compared to normal breast tissues. Moreover, CXCR4 expression in primary tumor could predict bone metastasis occurrence over visceral metastasis onset in a case series of 40 patients with breast cancer [64]. CXCR4 down-streamed signal activated by CXCL12 causes actin polymerization and pseudopodia formation, promoting migration [65]. CXCR4–CXCL12 axis is also activated by mesenchymal stem cells and is crucial for melanoma tumor cells extravasation to the bone marrow [66].

24.3.6 TGF-β

TGF-β belongs to the TGF superfamily and has a central role in regulating cellular homeostasis. Indeed, TGF-β blocks cell cycle–inducing differentiation and apoptosis-preventing aberrant cellular proliferation [67]. Unfortunately, several tumors develop the resistance against this growth inhibition because of genetic loss of TGF-β signaling elements or downstream signaling perturbation. Moreover, TGF-β pathway is linked to bone metastasis onset in several tumor types. In particular, it has demonstrated that two TGF-β secreted proteins, bone sialoprotein and osteopontin highly expressed in prostate and breast cancer tissues, are associated with tumor grade and represent prognostic indicators for bone lesions [68, 69, 70, 71]. Although in a mouse melanoma model, TGF-β receptor 1 inhibition prevent bone metastasis development, it does not affect visceral metastases onset [73].

TGF-β exerts its protumor action, affecting directly bone microenvironment. Indeed, TGF-β secreted and activated from osteoclast bone resorption promotes the release of PTHrP from tumor cells. PTHrP promotes osteoclastogenesis, inhibiting at the same time osteoblastogenesis modulating RANKL OPG ratio [74].

24.3.7 mTOR

The mammalian target of rapamycin (mTOR) pathway is involved in cell growth and survival, thus mTOR signaling alterations are associated to several diseases such as bone metastatic cancers. Indeed, cancer cells exhibit a dysregulated growth due to genetic alterations that determine loss of function or persistent activation of common oncogenes leading to abnormal activation of mTOR. Based on these evidences, mTOR inhibitors could represent a promising treatment for bone metastases. Preclinical data demonstrated that mTOR pathway is involved in bone remodeling, decreasing osteoclast apoptosis, and promoting osteoclast survival and growth through the activation of RANK–OPG pathway. mTOR pathway influence also cathepsin K expression, in fact treatment with mTOR inhibitor (everolimus) induces a decrease of its mRNA and protein levels [72, 75–80]. Moreover, in vivo studies have showed that mTOR inhibition can also influence osteoblast differentiation [60].

24.4 Markers of Bone Metastases

Bone metastatic cancers determine changes in bone metabolism and then in bone remodeling proteins whose serum levels could predict metastasis onset [51]. These proteins represent the bone turnover markers and include markers of bone formation and markers of bone resorption [52]. Specifically, the bone formation markers include bone specific alkaline phosphatase (bALP), bone matrix proteins such as osteocalcin (OCN), and the procollagen extension peptides (P1NP and P1CP). bALP is an enzyme produced by osteoblasts that is released into circulation during the mineralization process [81].

OCN is a non-collagenous protein synthetized by osteoblasts that binds to hydroxyapatite and is involved in calcium binding [82].

P1NP and P1CP are derived from the extracellular processing of the procollagen type I molecule, which contains amino-terminal and carboxy-terminal extensions that are enzymatically cleaved upon procollagen secretion [83].

In different stages of disease of prostate cancer, several bone turnover markers could predict the presence of bone metastasis on further radiologic imaging.

Jung et al. [81] found a correlation between the levels of several cone turnover markers and the disease state (bone metastatic vs. nonmetastatic) and they found that bALP, P1NP, and CTX predict OS. Moreover, de la Piedra et al. found that high levels of these proteins can predict SREs occurrence [84].

Bone turnover markers might be a specific predictor of bone metastasis occurrence in a clinical setting since they could identify patients that are prone to bone metastasis formation due to comorbidities (i.e., osteoporosis), concomitant therapies (i.e., androgen deprivation therapy) or due to any metabolic condition that enhance bone remodeling [85].

Others bone metastasis markers are the amino-terminal-crosslinked telopeptide of type I collagen (NTX-I) and carboxy-terminal-crosslinked telopeptides of collagen type I (CTX-I and ICTP) [86]. These telopeptides are released from type I collagen degradation by proteases during bone resorption. Since serum CTX-I are influenced by food intake, urine NTX-I has been the preferred marker in the clinical setting [88].

The inhibitor of Wnt signaling Dkk-1 also represents a marker of bone metastases. With sclerostin, Dkk-1 is released into the blood and serum levels reflect inhibition of bone formation [89, 90].

Unfortunately, bone markers do not provide information about the specific lesion site and changes in serum levels are associated with only bone homeostasis alteration without identifying the specific cause [82]. Nevertheless, bone markers might be helpful in better defining the prognosis and the risk for bone complications in patients with bone metastatic disease [87, 91].

24.5 Treatment of Bone Metastases

Bone metastasis treatments depend on the features of disease and include bone-targeted agents and radiopharmaceuticals. Besides these, several molecules that are already approved, as anticancer agents (such as antiandrogens and mTOR inhibitors) are now in clinical evaluation for their potential beneficial effects on bone metabolism (Table 24.3). Bone metastatic patients commonly develop resistance to systemic treatments, thus periodic changes of therapy are required.

In order to manage patients with bone metastases a multidisciplinary team of oncologists, radiotherapists, orthopedic surgeons, and nuclear medicine physicians is necessary.

24.6 Bone-Targeted Agents

In the last two decades, the bisphosphonates and denosumab, a monoclonal antibody of receptor activator of nuclear factor kappa-B ligand (RANKL), have become established as promising therapies for bone metastasis treatment.

Bisphosphonates are analogues of pyrophosphate with a strong affinity for divalent metal ions, such as calcium ions, and thus for the skeleton. Bisphosphonates are the standard of care for the treatment of osteoporosis as well as bone metastases, thanks to their action against osteoclast bone resorption [92]. Indeed, binding hydroxyapatite crystals of bone matrix bisphosphonates form a barrier that prevents osteoclast activity and the subsequent osteoblast bone deposition. There are two classes of bisphosphonates, non-nitrogen-containing (alendronate, ibandronate, pamidronate, risedronate, and zoledronic acid) and nitrogen-containing (e.g., clodronate, etidronate, and tiludronate), that inhibit differently osteoclasts. Particularly, nitrogen-containing bisphosphonates are more active than other in blocking osteoclasts [93]. Indeed, they inhibit farnesyl pyrophosphatase, the fundamental enzyme for osteoclast function, survival, and morphology causing the accumulation of the cytotoxic nucleotide metabolite Appp1 [93–95]. Moreover, several data have demonstrated that bisphosphonates also affect immune cells (mainly macrophages and gamma delta T-cells) and tumor cells through antitumor and/or antiangiogenic effects [96].

The strong effect of bisphosphonates in bone metastatic breast cancer treatment was widely investigated. In particular, a meta-analysis which included 2806 patients showed a reduction of SREs rate after bisphosphonates treatment compared to the placebo group [97]. Although all bisphosphonates reduced SREs, the efficacy (by 20–40%) changed based on the agent [98–104]. Recently, a meta-analysis demonstrated that adjuvant bisphosphonates reduced breast cancer recurrence in bone and improved breast cancer survival in women who were postmenopausal when treatment began [105]. Starting from these evidences, the use of bisphosphonates is recommended as part of the adjuvant breast treatment in this group of women [106].

Zoledronic acid demonstrated beneficial effects also in bone metastatic prostate cancer patients. Indeed,

Table 24.3 Anticancer agents with bone effect

Drug	Subject/cells type	Bone effect	References
Everolimus	Ovariectomiced rat model metastatic ER+ breast cancer patients	Decrease of bone loss associated with estrogen deprivation Reduction of bone tumover markers and bone disease progression (BOLERO study)	[76, 127]
Abiraterone	Human primary bone cells mCRPC patients	Increase of osteoblast differentiation and activity and reduction of osteociastogenesis and bone resorption Delay of SREs development and radiologival skeletal progression (COU-AA-301 study)	[133, 128]
Enzalutamide	mCRPC patients mCRPC	Improvement of survival and skeletal responses (AFFIRM study) Reduction of radiographic progression and risk of first SRE (PREVAIL study)	[135, 161]
Cabozantinib	Human primary bone cells mCRPC patients metastatic clear-cell renal cell carcinoma	Inhibition of ostaoclast differentiation and activity Improvement of bone scan responses and reduction of SRE rates (COMET-1) Delay of SRE onset (METEOR study)	[147, 150, 151]

ER Estrogen Receptor, *SREs* Skeletal Related Events, *mCRPC* metastatic castration resistant prostate cancer

Zoledronic acid treatment increased bone density and significantly reduced bone fractures at 6, 12, 24 months in patients with nonmetastatic prostate cancer after Androgen Deprivation Therapy [107]. Zoledronic acid reduced SREs onset and pain also patients that developed hormone-therapy resistance [108, 109].

In a phase III clinical study (STAMPEDE), the addition of zoledronic acid to docetaxel showed no evidence of survival improvement or delay of SREs incidence [110]. Similar results were obtained from the CALGB/ALLIANCE 90202 study comparing early treated hormone-sensitive prostate cancer versus delayed treatment in Castration Resistant Prostate Cancer (CRPC) [111, 149].

Denosumab is a monoclonal antibody against RANKL, developed for the treatment of osteoporosis, skeletal pathologies, and bone metastasis thanks to its inhibiting activity on osteoclasts [24]. The superiority of denosumab compared to zoledronic acid in reducing SREs onset was demonstrated in a large randomized controlled trial [23]. Nevertheless, no differences in OS disease progression and rate of adverse events were observed [112]. In a castration-resistant prostate cancer patient population presenting bone metastases, the median time-to-first on-study SRE for the denosumab arm was significantly prolonged (21 months) compared to the zoledronic acid ones (17 months), with no improvements in OS or progression of disease [113]. Another trial enrolled 1776 patients with myeloma-induced osteolysis and solid tumors other than breast and prostate cancers [114]. The results showed a median time-to-first on-study SRE of 21 months in the denosumab group and 16 months in the arm receiving zoledronic acid demonstrating a non-inferiority for denosumab versus zoledronic acid, but neither a superiority after adjustment for multiple comparison nor an advantage in OS of denosumab over zoledronic acid. Nevertheless, a post hoc analysis of these three phase III trials in patients with breast cancer [23], prostate cancer [113], or other solid tumors [114] (excluding of multiple myeloma patients), showed that denosumab was superior to zoledronic acid in preventing SREs in patients with bone metastases, regardless of ECOG PS, bone metastasis number, baseline visceral metastasis presence/absence, and uNTx level [115].

On the basis of these evidences the National Comprehensive Cancer Network (NCCN), the American Society of Clinical Oncology (ASCO), and the European Society of Medical Oncology (ESMO) recommend zoledronic acid or denosumab as the standard of care in bone metastatic patients [116–118].

24.7 Radiopharmaceutical

Radiopharmaceuticals are a group of radioactive drugs that recognize reactive metastatic bone sites and emit radiations according to their nature (commonly beta emission). In patients who present metastases in different bone sites, the radiopharmaceuticals are more effec-

tive than external local beam radiation, even if the combination of both is recommended for the most painful bone lesions [119–121].

Different types of radiopharmaceuticals are currently used for bone metastasis treatment such as 131-iodine that is the approved treatment for bone metastases of follicular thyroid carcinoma. In bone metastatic breast and prostate cancers, stronzium-89 and samarium-153 represent useful palliation of bone pain. Therefore, in a randomized control trial, stronzium-89 improved progression free survival in mCRPC patients after six cycle of docetaxel [122, 123].

Most recently, FDA approved the α-particle-emitting radiopharmaceutical, radium-223 as treatment for bone metastatic prostate cancer patients. As α-emitter, radium-223 delivers a highly localized radiation to bone surface than beta-emitters, causing DNA damages and the subsequent cell death giving less irradiation to healthy bone marrow [124]. Radium-223 improved OS of bone metastatic CRPC patients previously treated with docetaxel or unfit to receive docetaxel [125]; moreover, it showed efficacy in all secondary end-points including time to the first symptomatic skeletal events (median, 15.6 months vs. 9.8 months respectively). Ongoing phase III trial are designed to evaluate the effect of a combined treatment of radium-223 and other new target therapies as abiraterone acetate in this group of patients (NCT01106352 and NCT02097303).

24.8 Anticancer Agents with Bone Effect

24.8.1 mTOR Inhibitor

The mammalian target of rapamycin (mTOR) inhibitor, everolimus, had a positive effect on bone in preclinical and clinical studies. Indeed, in vivo study in ovariectomized rat model showed that everolimus directly blocks osteoclastic bone resorption [76].

In addition, in BOLERO-2 study, the combination of the mTOR inhibitor everolimus with aromatase inhibitor showed a significant benefit in progression free survival (PFS) in postmenopausal women with estrogen receptor–positive breast cancer [126]. In particular, it has also demonstrated that this combination reduced bone disease progression, decreasing bone markers levels at 6 months and 12 months from baseline [127].

The benefit of long-term treatment with everolimus in bone metastatic breast patients who do not progress within 8 weeks of treatment has demonstrated in RADAR study showing an improvement of time to progression (37 weeks vs 12.6 weeks of placebo group).

These evidences from phase III clinical trials suggest that mTOR inhibition in combination with exemestane may have both a beneficial effect on bone health in patients with bone metastases, reducing the incidence of bone metastases morbidity and mortality.

Currently, a phase II study is ongoing in order to evaluate whether the addition to radium-223 dichloride to aromatase inhibitor and everolimus could improve skeletal response in metastatic HER2 negative hormone receptor positive breast cancer patients (NCT02258451).

24.8.2 Antiandrogen Agents

Abiraterone acetate is an androgen biosynthesis inhibitor that blocks both the hydroxylase and lyase activity of CYP17. In particular, abiraterone inhibition of CYP17A blocks glucocorticoid and adrenal androgen synthesis, leading to a virtually undetectable serum and intratumoral androgen production in the adrenals, testes, and prostate cancer cells [130, 131]. Abiraterone is co-administered with prednisone to ameliorate the secondary rise in adrenocorticotropic hormone (ACTH) that can lead to excess mineralocorticoid synthesis [132]. This agent showed not only a significant survival advantage in metastatic prostate cancer patients [128, 129], but also a strong skeletal response. Indeed chemotherapy-treated patients treated with abiraterone showed better pain relief from skeletal metastases, a delay in time to development SREs (25 months vs 20.3 of placebo group), and in radiological skeletal progression [128]. Abiraterone effects on metastatic bone disease may be not only secondary to a systemic control of the disease due to a direct antitumor effect but also due to a specific effect on bone microenvironment. Recently the effect of abiraterone both in vitro and in mCRPC patients as bone anti-resorption agents was demonstrated [133]. Our research team demonstrated that abiraterone was able to specifically modulate bone cells leading to direct anabolic and anti-reabsorptive effects, suggesting a non-canonical mechanism of action [133].

Enzalutamide is an oral AR inhibitor that targets multiple steps in the AR signaling pathway. Two large phase III trials have demonstrated the efficacy of enzalutamide in the treatment of patients with mCRPC [134, 135]. In particular, the AFFIRM study showed that mCRPC patients treated with docetaxel and then with enzalutamide had improvements in survival and skeletal responses compared to placebo group [135]. In addition, the PREVAIL study demonstrated similar results in mCRPC patients treated with enzalutamide, who had not received docetaxel compared to placebo. Indeed, it has observed improvements in primary endpoints (OS and radiographic progression) and also in the secondary endpoints, including delayed initiation of chemotherapy and reduction in risk of first SRE [134].

24.8.3 Cabozantinib

Cabozantinib is a multiple receptor tyrosine kinase inhibitor with a strong activity against c-MET and vascular endothelial growth factor receptor 2 (VEGFR2). The hepatocyte growth factor (HGF), the only known ligand for c-MET, and c-MET signaling axis, is important in the regulation of bone remodeling [136–139]. Indeed, both osteoclasts and osteoblasts express c-MET and VEGFR2, and secrete HGF [140–143]. Several preclinical studies demonstrated the involvement of cabozantinib in bone remodeling; in particular, cabozantinib inhibited tumor proliferation and bone resorption in metastatic prostate cancer animal models [144–146]. Moreover, our group showed that cabozantinib inhibited osteoclast differentiation and bone resorption activity, both directly and indirectly reducing the RANKL/OPG ratio in osteoblasts [147]. In phase II studies of CRPC patients, cabozantinib was associated with an increased resolution in bone scans, a pain relief in more than 60% of patients and a marked improvement in progression free survival (PFS) compared with placebo [111, 148, 149].

In the subsequently COMET-1 study, although cabozantinib did not increase the OS of mCRPC patients, it improved bone scan responses, progression-free survival, and reduced SRE rates, compared to prednisone [150]. In metastatic renal cell carcinoma, METEOR study demonstrated that cabozantinib reduced the risk of tumor progression and death compared to everolimus, and improved the progression-free survival and the delay of SRE onset [151–153].

24.9 Osteoimmunology in Bone Metastases

The immune system has long been known to have a central role in preventing tumor growth, but more recent evidence suggest the importance of the immune cell response in the tumor bone microenvironment as main regulator of cancer progression and metastases.

Once in the bone marrow, tumor cells can, directly or not, interact with different resident immune cells and modify the balance of immune effector and suppressor cells creating a microenvironment suitable for their growth [154, 155].

In advanced bone metastatic cancers there is a prevalence of immunosuppressive cells, mainly myeloid-derived suppressors cells (MDSCs) and regulatory T-cells. Indeed, tumor cells secrete soluble factors such as IL-4, IL-13 VEGF, granulocyte–macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and TGF-β that recruit and activate MDSCs. MDSCs stimulates osteoclast differentiation and activity and also support the polarization of macrophages into a tumor-promoting phenotype [156–158]. Recent evidences support a role of osteoblasts in osteoimmunology mediated by the release of cytokines and growth factors in the microenvironment [159]. In particular, PTHrP, produced by tumor cells, stimulates osteoblasts to produce CCL2, IL-6, and VEGF (A) that recruit and stimulate MDSCs.

Tumor-associated inflammation is not always a signal of immune system response to tumor cell growth, but sometimes creates a microenvironment that facilities neoplastic development.

Indeed, CD68+ osteal macrophages, that have a pro-tumor phenotype, establish a complex crosstalk with cancer and bone cells, leading to tumor progression in the skeleton, especially in breast and prostate cancers [160].

Finally, different immune cell types are involved in the establishment of tumor cells in the metastatic niche, mainly in bone. Indeed, some inflammatory cells express RANKL that mediates RANK+ tumor cells migration into the bone [18–20].

24.10 Conclusion

Recent advances supported the important role of bone microenvironment for metastasis adaptation and the subsequent crosstalk between tumor and bone cells that lead to cancer progression. Despite different approaches have been investigated to target this crosstalk, up to now only denosumab and bisphosphonates demonstrated to be a changing practice agent in delaying SRE. Besides these agents, others are anticancer drugs, but at the same time, have effects on bone microenvironment altering bone turnover. Anyway, currently, we are still far from fully understanding what really happens when disrupting the RANK–RANKL axis in the "real world" and we do not know which patients could benefit from these approaches. For these reasons, the goal of ongoing clinical trials is to evaluate whether combinations of different treatments could improve patient bone health.

Expert Opinion

Antonio Russo

Key points

- Bone metastases led to so-called "skeletal-related events" (SREs) that negatively affect patients' quality of life.
- Tumors alter the physiological balance between bone deposition and resorption, leading to the loss of the skeleton integrity.
- Bone metastatic niche is the ideal site for dormant tumor cells (DTCs) colonization.
- Bone metastasis onset is regulated by several pathways, including RANK–RANKL–OPG, ET-1, integrins and cadherins, WNT–DKK1, CXCR4–CXCL12, TGF-β and mTOR.
- bALP, OCN P1NP, P1CP, NTX-I, CTX-I, and ICTP are the principal markers of bone metastases.
- Bone metastasis treatments include bone-targeted agents (bisphosphonates and denosumab) and radiopharmaceuticals.
- mTOR inhibitors, antiandrogen drugs, and cabozantinib are anticancer agents with bone effects.
- In advanced bone metastatic cancers there is a prevalence of immunosuppressive cells, mainly myeloid-derived suppressors cells (MDSCs) and regulatory T-cells.
- Different immune cell types are involved in the establishment of tumor cells in the bone metastatic niche.

Hints for Deeper Insight

- Besides bone target agents, others new anticancer drugs have effects on bone microenvironment altering bone turnover. It would be interesting to deepen the direct effects of these new agents on bone cells.
- The bone marrow is a fertile soil containing a complex composition of immune cells that may actually provide an immune-privileged niche for disseminated tumor cells to colonize and proliferate. It would be interesting to investigate deeply the role of immune cells in promoting tumor cells seeding in bone niche.

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Nutrition and Cancer

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Learning Objectives

By the end of the chapter the reader will

- Have learned the most important metabolic pathways at the basis of cancer progression and development;
- Have reached a good knowledge of the main amino acids required by cancer for its growth;
- Have gained a better understanding of different dietary approaches helpful as nutritional anticancer interventions; and
- Have gained a better understanding of effects of dietary restriction on the main tumors.

25.1 Metabolic Signatures of Cancer Cells

25.1.1 Glucose

In 1924, Otto Warburg discovered that cancer cells, unlike normal cells, which heavily rely on oxidative phosphorylation to produce energy, preferentially use glycolysis for massive energy production [1].

Since then it has been demonstrated that most cancer cells, even in the presence of abundant oxygen, produce energy by high rate glycolysis followed by lactic fermentation, an effect known as "Warburg effect" (Fig. 25.1).

Glycolysis is a lesser-efficient ATP production pathway than oxidative phosphorylation, with one molecule of glucose producing two ATP molecules through glycolysis compared to the 36–38 ATPs obtained by oxidative phosphorylation. However, upregulated expression of GLUT1–3 glucose transporters can increase glucose uptake in tumor cells by over 100-fold compared to the uptake of normal cells, thus efficiently fueling energy production of tumor cells by this metabolic pathway. In addition, upregulated PFK2 overrides the ATP-based negative feedback on PFK1, a key regulator of glycolytic flux while LDH overexpression oxidizes NADH to the essential NAD^+.

This first "metabolic hallmark of cancer" has many consequences since it promotes chemotherapy resistance and excreted lactate creates an acid environment, which in turn activates immune cells helping the process of metastatization.

However, it must be noted that also normal tissues can switch to high-rate glycolysis. For example, lymphocytes upregulate glycolysis upon their activation even in the presence of oxygen and this metabolic shift may be specifically required for effector function in T-cells [2].

In normal cells hypoxia activates the transcription factors HIF-1alfa and HIF-2alfa that in turn reprogram the cellular metabolism toward high-rate aerobic glycolysis [3]. Not surprisingly, hypoxia inducible factor (HIF-1/2) is a transcription factor whose expression is increased by many key genetic alterations in cancer and by hypoxic condition [4, 5]. For example, EGFR2 and HER2 are tyrosine kinases activated in prostate and breast cancers, respectively, that increase HIF-1 synthesis as a result of PI3K and AKT signaling. HIF also activates miR-210 affecting the iron–sulfur cluster assembly that is necessary for the electron transport chain in mitochondria. HIF1-dependent transcription remodulation includes increased efflux of lactate out of cancer cells (MCT4), active pumping of H^+ ions (NHE1) and the conversion of carbon dioxide to carbonic acid (CA9). The consequences of such transcriptional reprogramming of MCT4, NHE1, and CA9 are cellular alkalization, which promotes cellular proliferation, and extracellular acidification thus helping invasion.

The increased intracellular concentration of pyruvate due to upregulated glycolysis can also be used to produce oxaloacetate and the amino acids alanine and aspartate, which can take a significant part in the anabolic processes required by cancer cells. Recent studies have also underlined the role of increased pyruvate concentration in fatty acid biosynthesis or of other nonessential amino acids. It is therefore clear that glycolysis can fulfill the energetic demand of cancer cells as well as its biosynthetic need.

In normal cells, the hypoxia-dependent transcriptional reprogramming toward increased glycolysis represents a temporary form of cellular adaptation. Cancer cells instead achieve these changes in part by mutations in oncogenes such as PI3K, RAS, and SRC which induce the expression of glucose transporter genes GLUT1,3, activate the glycolytic pathway by inducing hexokinase (HK) and phosphofructokinase (PFK) and inhibit pyruvate oxidation in mitochondria [6]. In mice, a direct involvement of RAS and SRC on GLUT-specific enhancer element has also been demonstrated [7]. In addition to its role as energy supply, increased glycolysis has been directly linked to resistance to chemotherapy at least in some cancers (e.g., cervical cancer and colon cancer).

It is increasingly evident that beside the hypoxia induction mechanism, many oncogenes (e.g., RAS, SRC, AKT, MYC) stimulate glycolysis by directly upregulating glycolytic enzymes and/or HIF1 activation which is characteristic of tumor microenvironment.

To target deregulated glucose metabolism can therefore be an effective cancer therapy.

Reducing blood glucose and/or inhibiting glycolytic enzymes are the two possible approaches toward this goal. The first approach could benefit from the characterization of the tumor avidity for glucose as determined by FDG-PET, or alternatively, tumor molecular profiling.

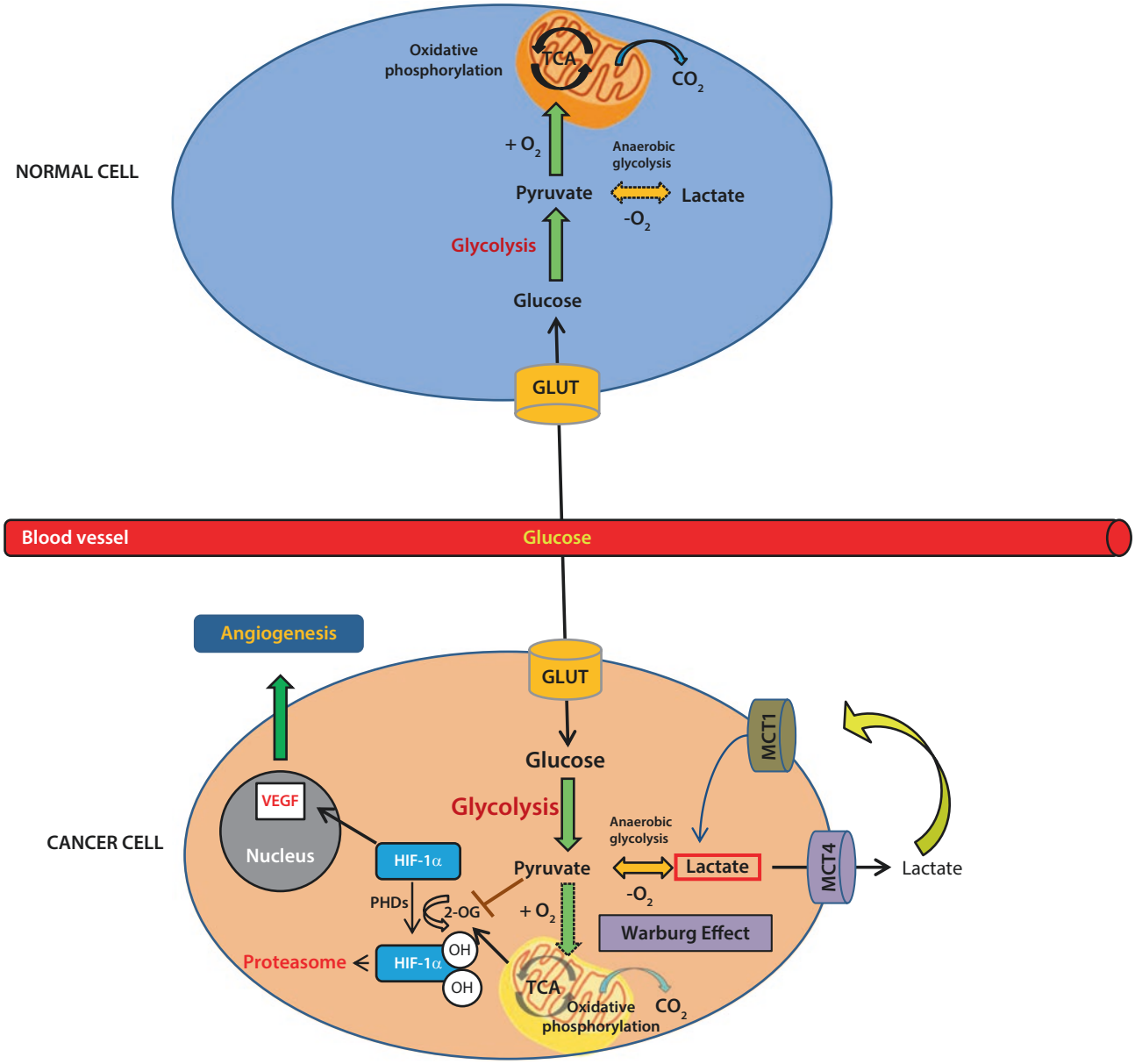

Fig. 25.1 Predominance of glycolytic metabolism in tumor cell. TCA Tricarboxylic acid cycle (or Krebs cycle), 2-OG 2-Oxoglutarate, GLUT Glucose transporter, MCT1/4 Monocarboxylate transporter 1 and 4, HIF-1a Hypoxia-inducible factor 1-alpha, PHDs Prolyl hydroxylases, VEGF Vascular endothelial growth factor

25.1.2 Amino Acids

Tumor growth requires the continuous replenishment of amino acids (AAs), which are used as building blocks for the synthesis of different macromolecules such as proteins, fatty acids, nucleotides, and the antioxidant glutathione.

However, some tumors in contrast to normal cells lose the ability to synthesize some of the nonessential amino acids or increase their need to such a high level that endogenous synthesis is no longer sufficient. In both cases cancer cells become dependent on blood supply also for these substances. This process generates auxotrophies for specific amino acids in tumor cells making tumor tissues fully dependent on the food supply of these amino acids for survival and growth.

25.1.3 Glutamine

Myc-driven cancer cells, unlike most non-transformed cells that rely on glucose, are dependent on glutamine metabolism. Myc promotes glutamine import and increases the conversion of glutamine to glutamate

which is oxidized in the TCA cycle. ^{13}C-labeled glutamine experiments demonstrated that especially under glucose or oxygen limitation, Myc overexpressing cells became dependent on glutamine for energy production and fatty acid biosynthesis. At the molecular level, the overexpression of the glutamine transporter ASCT2 is observed in Myc-transformed cells and the reliance on glutamine metabolism is more evident under stressful condition such as hypoxia and glucose limitation. Demonstrating that in these cells glutamine, eventually converted to glutamate, is an alternative to glucose metabolism.

Glutamine is the most abundant circulating amino acids representing over 20% of the amino acid blood pool and over 40% of the muscle amino acid pool. Since muscle and other organs are capable of synthesizing glutamine by scavenging the ammonia produced from the other amino acids [8], glutamine is not considered an essential amino acid. However, under circumstances such as postoperative, sepsis, and injury, the demand for glutamine of gastrointestinal tissues, kidney, and immune cells can increase, inducing organism dependence to dietary glutamine supply.

The MYC oncoprotein, whose gene is amplified in many human cancers, can alter the equilibrium between proline synthesis and degradation pushing toward proline synthesis from glutamine-derived glutamate. It must be noted that other oncogenes and oncosuppressor inhibition can alter glutamine metabolism (see Table 25.1 for a reference), and that at least in some cases different mutations within the same oncogene can produce different effects. For example, KRASG12C and G12D are much more glutamine-dependent than KRASG12V. Recent papers have also further linked this amino acid dependency to the specific mutation status. For example, colorectal cancers are glutamine-dependent only in PI3K-mutated tumors [9]. GBM and lung tumors appear to be completely independent on glutamine supply, as glucose supply seems to be sufficient for both tumor energetic and anaplerotic supply [10].

Table 25.1 Oncogenes that produce metabolic addictions

Activated oncogene	Associated metabolite addiction	References
BRAF	Glucose	[11]
KRAS	Glucose/glutamine	[12, 13]
AKT	Glucose	[14]
MYC	Glutamine	[15]
HER2	Glutamine	[16]
P53, p63, p73	Glutamine	[17–19]
Jak2-V617F	Glutamine	[20]
mTOR	Glutamine	[21]

Table 25.2 Influence of oncogene and tumor-suppressor gene loss on glutamine metabolism

Genetic change	Role in glutamine metabolism	References
MYC upregulation	Upregulates glutamine metabolism enzymes and transporters	[15, 22–25]
KRAS mutations	Drives dependence on glutamine metabolism, suppresses GLUD, and drives NADPH via malic enzyme 1 (ME1)	[13, 26–29]
HIF1α or HIF2α stabilization	Drives reductive carboxylation of glutamine to citrate for lipid production	[30–32]
HER2 upregulation	Activates glutamine metabolism through MYC and NF-κB	[16, 33]
p53, p63, or p73 activity	Activates GLS2 expression	[17–19, 34]
JAK2-V617F mutation	Activates GLS and increases glutamine metabolism	[20]
mTOR upregulation	Promotes glutamine metabolism via induction of MYC and GLUD or aminotransferases	[21, 35–37]
NRF2 activation	Promotes production of glutathione from glutamine	[38]
TGFβ-WNT upregulation	Promotes SNAIL and DLX2 activation, which upregulate GLS and activates epithelial–mesenchymal transition	[39]
PKC zeta loss	Stimulates glutamine metabolism through serine synthesis	[40]
PTEN loss	Decreased GLS ubiquitination	[41]
RB1 loss	Upregulates GLS and SLC1A5 expression	[42]

The main genetic changes affecting glutamine metabolism are listed in Table 25.2.

25.1.4 Methionine

Methionine is an essential amino acid involved in protein synthesis in methylation process of both DNA and RNA as well as in synthesis of glutathione and poly-

amines [43]. Methionine can cycle to homocysteine and excess of homocysteine, which has a clear role in cardiovascular diseases, is linked to increased methionine dietary uptake. Most cancer cells have an increased need of methionine due to the massive transmethylation process observed, the Hoffmann effect. (11C)-Methionine PET imaging confirms a very strong absorption of tumor tissues compared to normal tissue background. Interestingly, (11C)-methionine absorption seems more specific for tumor tissues than (18C) fluorodeoxyglucose suggesting that methionine avidity of tumor cells can be of greater extent than glucose avidity. Several tumor cell line, such as colon, breast, and prostate stop proliferating in the absence of methionine [44]. Dietary methionine restriction appears to be as a feasible and possible effective anticancer strategy.

25.1.5 Arginine

Arginine is a nonessential amino acid involved in the synthesis of NO, polyamine, creatine, and it could be the precursor for glutamine and proline in condition of amino acid deprivation. mTOR activation is dependent on arginine as well as the secretion of growth hormone (GH), insulin, and insulin-like growth factor 1 (IGF1).

Arginine is supplied by dietary uptake, protein degradation and endogenous synthesis.

The enzyme argininosuccinate synthetase 1 (ASS1) is essential for de novo synthesis of this amino acid. Notably, cancers like melanoma, hepatocellular carcinoma, and mesothelioma induce the methylation of the ASS1 promoter resulting in ASS1 gene inactivation [45]. It appears that ASS1 inactivation has some advantage for tumor cells such as increased pyrimidine production and glutamine independence. This suggests that molecular profiling followed by arginine deprivation or inhibition of arginine synthesis in ASS1-inhibited and other arginine-dependent tumors could provide additional strategies for tumor targeting.

25.1.6 Serine and Glycine

These nonessential amino acids can be synthetized from the 3-phosphoglycerate obtained through glycolysis, are involved in de novo synthesis of purines and glutathione, and are relevant to redox balance. Serine has been demonstrated to activate mTOR in different systems. Cancer cell during high mitotic activity, due to their higher request of these amino acids, need an exogenous continuous supply of serine and glycine [46].

Experiments in mice have demonstrated the safety and potential efficacy of serine restriction to fight cancer growth. Blood serine levels can in fact be lowered by up to 50% by dietary intervention and this reduction is associated to delayed tumor growth. In addition, the effect of serine restriction is synergic with metformin in inhibiting cancer progression [47, 48].

It must be noted that some cancers such as triple negative breast cancer and melanomas overexpress the enzymes necessary for the serine biosynthesis, becoming completely independent from serine supply for their growth. However, this gene amplification results also in depletion of glycolytic intermediates making these cancer cells particularly sensitive to glucose deprivation and to metformin treatment. These findings further stress the need for molecular profiling and the identification of "smart" multitherapy interventions.

25.1.7 Lipids and Cholesterol

Lipids are the building blocks of the cellular membrane systems with both structural and functional roles. Many cancer cells internalize lipids from the diet or obtained from surrounding tissues. However, many tumors including gastrointestinal stromal tumors, Her+ breast cancer, prostate cancer, lung cancer, head and neck, AKT-driven hepatocarcinoma, and a few others overexpress the fatty acid synthase (FAS) enzyme which can render them independent of the bloodstream fatty acid supply. FASN has therefore become an interesting drug target. It must be noted that catechins as epigallocatechin gallate (EGCG), a substance present in green tea, are capable to inhibit FASN resulting thus in anticancer activity both in vitro and in vivo. The enzyme hydroxymethylglutaryl-coA reductase is the rate-limiting step in cholesterol biosynthesis and interestingly is overexpressed in several tumors. It is therefore possible to inhibit cholesterol production through dietary or pharmacological approaches to fight these cancers [49–53].

25.2 Dietary Approach to Enhance Cancer Therapy

25.2.1 Ketogenic Diets

Ketogenic diets (KD) are high fat, low carbohydrate, and can either be high or adequate protein diets, which have been proposed as an adjuvant therapy for cancer treatment. The rationale of this approach is to reduce circulating glucose to counteract the Warburg effect and to induce ketosis. This is motivated by the fact that normal cells are capable to switch to ketone-based metabolism, while cancer cells do not. In addition, lowering the

available glucose reduces circulating insulin which is a known cancer driver for its ability to activate the (IRS)/RAS/RAF/MEK/MAPK and RAS/PI3K/AKT/mTOR signal transduction cascades. In addition, many trials have focused on the ratio of the different macronutrients and the relative proportion of medium-chain and long-chain triglycerides combined or not with caloric restriction as an important determinant [54]. Their results underline the importance of optimizing the composition of the ketogenic diet with an optimum of 75% long chain triglycerides and 25% medium triglycerides at least for neuroblastomas. Glioblastoma are also sensitive to the ketogenic diet regimen while astrocytoma and medulloblastoma are less influenced. Other studies also reported anticancer effect on prostate, colon, pancreatic, and lung cancer. Very recently was also demonstrated that ketogenic diet was capable to restore sensitivity to PI3K inhibitors [55].

In contrast to these safe applications of KD in various cancer diseases it has also been reported a rapid weight loss in a mouse model of renal carcinoma. In addition, it has been observed in a mouse model of BRAFV600E-positive melanoma that ketogenic diet increased BRAF signaling and thus determined faster cancer growth [56–63].

25.2.2 Fasting and Fasting Mimicking Diet

Chronic calorie restriction has been demonstrated to delay the growth of many cancers in mouse models. However, calorie restriction imposes weight loss and possibly also immunosuppression making it not feasible for cancer treatment.

On the contrast fasting or relatively high-calorie diets which mimic the effects of fasting (fasting mimicking diets, FMDs), for 2–7 days in a row depending on the use and organism, can rewire cellular metabolism in many species causing only a transient weight loss, which is normally reversed by the return to normal feeding. It has been demonstrated that fasting for 2–4 days, a condition which is referred to as Short-Term Fasting (STF), induces multisystemic metabolic changes which are capable to impair tumor growth in part by reduction of blood sugar, and reduction of circulating IGF1 and possibly by a transient ketone bodies increase. It has also been demonstrated that the amino acids pool is affected by fasting pointing to alanine as the most depleted amino acid while the concentration of some other amino acids an even transiently increase. This is likely due to the increased utilization of this amino acid as a precursor for glucose biosynthesis rather than to its increased urine excretion [64]. However, since some tumors directly affects the concentration of circulating amino acids pool (e.g., tryptophan) the effect of fasting on circulating amino acids in normal and cancer cells may differ and further analysis are necessary to take advantage on these differences to further ameliorate fasting cancer treatment.

Fasting and fasting-mimicking diets (FMDs) have differential effects in normal and malignant cells. These effects are partially due to IGF-1, insulin, and glucose decrease and IGFBP1 as well as ketone bodies increase. Since cancer cells have the metabolic hallmark described above, they rely more on metabolites and factors that become limited in the blood during fasting, thus resulting in cell death [65].

It has also been demonstrated that the rapid decrease of the circulating IGF-1 has a major role in the differential stress resistance observed between normal and cancer cells; in fact restoration of normal IGF1 level is sufficient to reverse the protective effect of fasting [66].

This approach is very promising, and it is receiving increasing confirmation regarding its feasibility, effectiveness, and mechanism.

A case series report demonstrated, even though in a small group of patients, that fasting started prior of chemotherapy reduced fatigue, weakness, and gastrointestinal side effects [67].

It increases the ability of commonly administered TKIs, including erlotinib, gefitinib, lapatinib, crizotinib, and regorafenib, to delay cancer cell growth, through MAPK signaling pathway and E2F-dependent transcription inhibition [68].

Oxaliplatin (OXP) treatment of colorectal tumor was combined with a 48-hour fasting showing that short-term fasting potentiated the effects of OXP on the suppression of colon carcinoma growth and glucose uptake in both in vitro and in vivo models. In vitro experiments demonstrated that STS downregulated aerobic glycolysis, and glutaminolysis, while increasing oxidative phosphorylation. Chemotherapy caused additional toxicity, which was associated with increased succinate/complex II-dependent O(2) consumption, elevated oxidative stress and apoptosis. These findings indicate that the glucose and amino acid deficiency conditions imposed by fasting promote an anti-Warburg effect characterized by increased oxygen consumption but failure to generate ATP, resulting in oxidative damage and apoptosis [69].

A combination of chemotherapy and a fasting-mimicking diet (FMD) leads to a major delay in breast cancer and melanoma progression. In breast tumors, this effect is partially mediated by the downregulation of the stress-responsive enzyme heme oxygenase-1 (HO-1). An increase of the levels of bone marrow common lymphoid progenitor cells and cytotoxic CD8(+) tumor-infiltrating lymphocytes (TILs) was observed. These

data indicate that FMD cycles combined with chemotherapy can enhance T-cell-dependent targeted killing of cancer cells both by stimulating the hematopoietic system and by enhancing CD8(+)-dependent tumor cytotoxicity [70].

Recently, Obrist et al. [71] found that periodic fasting cycles sensitize otherwise cisplatin-resistant lung adenocarcinoma, due to its dependency on glutamine, required for nucleoside biosynthesis, suggesting a further opportunity for nutritional anticancer interventions.

25.3 Conclusion

It is becoming clearer and clearer that neoplastic transformation is accompanied or even caused by cellular metabolic derangement. The altered metabolism showed by cancer cells can thus be considered as an additional target for cancer therapy to be effective.

Deregulated glucose metabolism is a common hallmark of most tumor cells and reducing blood glucose and/or inhibiting glycolytic enzymes are two possible approaches toward this goal. Dietary as well as pharmacological approaches are being developed toward this target. However, aside from few general metabolic features shared by most cancer cells, specific cancers show certain peculiarities. The molecular characterization of this specific metabolic alterations promises to be an additive tool for cancer fight. Since these metabolic alterations are dependent on specific genomic mutations, it appears that molecular profiling of tumor cells is an interesting prerequisite of dietary intervention. Specific mutations are in fact related to amino acid metabolism or to biosynthetic pathways of certain lipids. Glutamine, methionine, or arginine restriction alone or in combination are in fact a very useful strategy but only if specific genomic mutations are present. Dietary approaches, especially some extreme diets, can be a possible strategy to target these metabolic alterations of most cancer cells. Ketogenic diets have in fact been proposed as an adjuvant therapy for cancer treatment. Fasting and fasting-mimicking diets (FMDs) have demonstrated differential effects in normal and malignant cells suggesting a potential role to increase chemotherapy efficacy and to reduce the risk of recurrence.

Key Points

- Nutritional status is evaluated to identify malnutrition/cachexia;
- Some dietary strategies have been shown to be specifically suitable to fight cancer cells;
- Fasting or fasting mimicking diet are approaches used to reduce the cancer risk factors;
- The use of fasting and fasting mimicking diet during chemotherapy is an attractive opportunity to minimize the collateral effects of therapies and increase their efficacy, as shown in several mice model systems;
- Several clinical trials are ongoing to confirm the usefulness of such dietary strategies in the treatment of human cancers.

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Personalized Medicine

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Contents

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Learning Objectives

By the end of the chapter the reader will have:

- Understood the basic principles of personalized medicine and specifically of precision medical oncology;
- Have learned the basic concepts of "driver" and "passenger" mutations;
- Have reached the basic knowledge of technologies for biomarker search and validation;
- Have learned the basic current tools for biomarker use in targeted therapy and precision immunotherapy;
- Have learned the current practice of precision medical oncology.

26.1 Introduction

The emerging scenario of molecular heterogeneity of human malignancies sharing the same tissue origin and pathology features has provided novel opportunities for the treatment of human cancer by the identification and validation of biomarkers able to predict clinical response to specific therapeutics.

The antitumor activity of a particular agent is exerted against a proportion of treated individuals. The availability of validated biomarkers allows to enrich the cohort of patients achieving clinical benefit and to avoid undue toxicity in nonresponding patients (Fig. 26.1).

The basic molecular concept underlying this important idea is the finding from modern molecular profiling technologies that a tumor harbors a variety of different molecular lesions (mutations). These can be not relevant for tumor hallmarks and are in this case called "passenger mutations." In other cases, these mutations can drive tumor growth and progression and the tumor can become addicted to the mutation itself, which can in this case be called "driver mutation." The specific targeting of these molecular lesions can be of major clinical benefit. In this case, the following are needed:

(a) A validated biomarker which allows selection of tumors carrying a driver mutation
(b) A compound able to selectively target the mutated gene product

Unfortunately, not all driver mutations are "drug-gable," which means they are not amenable to selective therapeutic interventions.

This basic paradigm of personalized oncology, in the last few years, has been expanded by three basic emerging scenarios: the pharmacogenomics, the immune-genomics and the tissue-agnostic molecular-targeted therapeutics.

The therapeutic benefit can be increased or hampered by changes in the efficacy of drug metabolism in terms both of drug activation and drug detoxification. It is now becoming clear that molecular profiling technologies can allow the identification of molecular biomarkers predicting drug activity or toxicity.

The last years have witnessed the huge progress of cancer immunotherapy that has provided major survival gains in highly lethal tumors as malignant melanoma or non-small cell lung cancer (NSCLC). Even if some patients have gained major benefit, most patients appear still refractory to immunotherapy. Many studies are now pointing to the identification of biomarkers, which might allow an "a priori" selection of responsive individuals. Biomarker-directed immune therapy can be now offered to first-line locally advanced/metastatic NSCLC.

Recent studies which have taken benefit from next-generation sequencing (NGS) technologies on tumor tissue DNA or in cell free DNA (see below) have clearly demonstrated that driver mutations can be shared by tumors independently from the tissue of origin and can be successfully targeted by available agents, opening the way of multigene analysis in drug refractory tumors where there is no conventional approaches.

We will now present validated single gene biomarkers in solid and hematologic malignancies. It is noteworthy that precision medicine mainly considers "predictive" biomarkers those able to selectively predict treatment

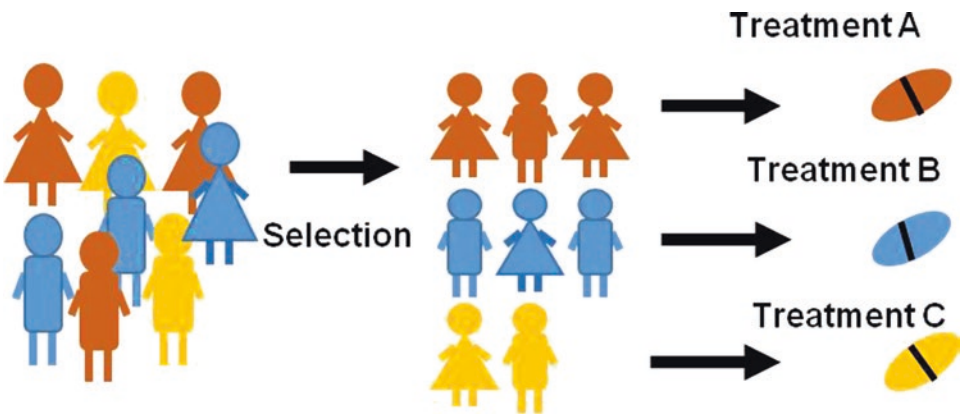


Fig. 26.1 Personalized medicine

response. Predictive factors can have a prognostic relevance. For instance, human epidermal growth factor receptor 2 (HER2) is a powerful biomarker for treatment selection in breast cancer, but it was also an adverse prognostic factor before the availability of anti-HER therapeutics.

26.2 Single Gene Biomarkers for Solid Tumors

26.2.1 Positive Predictive Molecular Biomarkers

All the following biomarkers have been validated as positive predictors of sensitivity to targeted agents:

26.2.1.1 Epidermal Growth Factor Receptor (EFGR) Mutations in NSCLC

Lung carcinomas (mostly adenocarcinomas) arising in never/lite smokers, with prevalence in female and Asian ancestry, are often carriers of tyrosine kinase mutation L858 or exon 19 deletions or T790 escape mutation. Tumor tissue profiling or liquid biopsy (see below) have been validated as predictive biomarkers for the following:

(a) First-generation noncovalent EGFR tyrosine kinase inhibitors (Gefitinib, Erlotinib)
(b) Second-generation covalent EGFR tyrosine kinase inhibitors (Afatinib)
(c) Third-generation T790-active tyrosine kinase inhibitors (Osimertinib)

26.2.1.2 ALK in NSCLC

Anaplastic lymphoma kinase (ALK) inhibitors are effective in NSLC harboring ALK–EML4 gene fusion or rearrangements. Molecular analysis by FISH or IHC has been validated as predictor of the following benefits:

(a) First-generation inhibitors (Crizotinib)
(b) Second generation inhibitors (Ceritinib)
(c) Third generation inhibitors (Alectinib)

26.2.1.3 ROS1 in NSCLC

Proto-oncogene tyrosine-protein kinase 1 (ROS1) is rearranged in 1–2% of NSCLC. ROS1 FISH is generally performed in EGFR and ALK-negative patients and predict for clinical benefit of Crizotinib.

26.2.1.4 HER2 in Breast Cancer

HER2 is a gene of the erb-b receptor (ERBB) tyrosine kinase family that includes EGFR and is overexpressed in 20% breast cancer. Molecular analysis by IHC or FISH/CISH is highly predictive for the following benefits:

(a) Trastuzumab (naked mAb)
(b) Trastuzumab–Pertuzumab (naked mAb) combination
(c) Trastuzumab emtansine (immuno-conjugated drug)
(d) Lapatinib (tyrosine kinase inhibitor)
(e) Neratinib (tyrosine kinase inhibitor)

26.2.1.5 Her2 in Gastric Cancer

Her2 is overexpressed in 17–18% gastric or gastroesophageal junction cancers and predicts for Trastuzumab clinical benefit.

26.2.1.6 Tyrosine–Protein Kinase Kit (cKIT) Mutations in Gastrointestinal Stromal Tumors (GIST)

Exon 11 and exon 9 mutations are predictive of clinical benefit in advanced GIST of the following:

(a) Imatinib mesylate
(b) Sunitinib
(c) Regorafenib

26.2.1.7 BRAF Mutations in Malignant Melanoma

Half of malignant melanomas harbor activating BRAF mutations, BRAF-v600.

These mutations are predictive of the following clinical benefit:

(a) BRAF inhibitors (Vemurafenib, Dabrafenib)
(b) MEK inhibitors (Trametinib, Cobimetinib)

26.2.1.8 BRCA1/2 Mutations in Breast, Prostate, and Ovarian Cancer

The occurrence of inherited germline or less common somatic mutations leading to inactivation of breast related cancer antigens 1 and 2 (BRCA1 and BRCA2) genes is highly predictive for Poly ADP-ribose polymerase (PARP) inhibitor activity and has led to regulatory agency approval of Olaparib in the maintenance treatment of platinum sensitive high-grade serous ovarian cancer. It also predicts for enhanced sensitivity to platinum derivatives [1]. These important genetic lesions are presently approved as a biomarker of BRCA1/2-related HER2-negative advanced breast cancer or in BRCA-mutated pancreatic and prostate cancer. This is due to the loss of homologous recombination DNA repair, which is made deficient by biallelic loss of functional BRCA genes and make tumor cells highly dependent on PARP-mediated DNA repair mechanisms. Other PARP inhibitors are Niraparib and Rucaparib. A recent meta-analysis (Staropoli et al.) has demonstrated that Olaparib, Niraparib, and Rucaparib displace a class effect in terms of activity in advanced ovarian cancer

but a different spectrum of toxicity On these base BRCA1/2 analysis can provide a crucial benefit in terms of predicting treatment efficacy and for prevention of BRACA1/2 tumors in carriers of germline mutations.

26.2.2 Negative Predictive Molecular Biomarkers

All these biomarkers have been validated as negative predictors of sensitivity to targeted agents, that means that mutations carrying tumors are insensitive to therapeutics targeted to different but related genes.

26.2.2.1 RAS Mutations in Colon Cancer

Kras is mutated in 40 % colorectal cancer in exon 2 codons 12/13 and in codons 50/61 while a lower percentage of 3–5 % harbor mutation in NR ras exon 2 codon 12/13 and exon 3 codon 61. All these mutations predict for the absence of benefit from anti-EGFR mAbs, Cetuximab and Panitumumab. Less clear is the predictive effect of BRaf mutation in colorectal cancer.

26.3 Single Gene Biomarker for Hematologic Malignancies

26.3.1 Positive Predictive Molecular Biomarkers

26.3.1.1 BCR/ABL Translocations in Chronic Myeloid Leukemia

Chronic myeloid leukemia (CML) is one of commonest forms of leukemia in the western world. The hallmark genetic alteration of most CMLs is the t(9;22) (the so-called "Philadelphia Chromosome"), which results from a chimeric protein (BCR/ABL tyrosine kinase) at stem cell level, that confers survival and proliferative advantage over normal stem cell clones. It predicts for the following clinical benefits:

(a) First-generation selective BCR/ABL inhibitor, Imatinib
(b) Second-generation Nilotinib, Dasatinib, and Bosutinib overcome resistance to Imatinib with the exception of T315I mutation
(c) Third generation TKI, Ponatinib active on T315 mutation

26.3.1.2 JAK-2 in Primary Myelofibrosis and Polycythemia Vera

Janus Kinase-2 (JAK-2) V617F activating mutation has been described in around 60 % of primary myelofibrosis (PMF) and almost all polycythemia vera (PV) patients. This genetic lesion confers a survival and proliferative advantage to the carrying BCR/ABL-negative myeloproliferative clone leading to preferential expansion of erythroid/thrombopoietic lineage or favoring the development of PMF. Apart from specific JAK-2 mutations, the pathogenic relevance of JAK–STAT pathway has been further elucidated, leading to the approval of a potent JAK-1/JAK-2 inhibitor, Ruxolitinib, in intermediate/high-risk PMF and subsequently in idroxyurea intolerant/resistant to PV patients. Indeed, efficacy of Ruxolitinib overcomes the presence of V617F in PMF as JAK–STAT pathway is activated, irrespective of constitutive activation of JAK-2. The drug exerts a thorough anti-inflammatory activity relying on the truncation of JAK–STAT-mediated signaling. Next-generation JAK inhibitors are being developed and explored in clinical trials.

26.3.1.3 BRAF Mutations in Hairy Cell Leukemia

BRAF V600E mutation has been recently described as a primary transforming event in hairy cell leukemia (HCL), accounting for virtually all cases at diagnosis. The importance of this biomarker is documented by the almost 100% ORR to BRAF inhibitor vemurafenib with around 40% CRs in relapsed/refractory setting.

26.3.2 Negative Predictive Molecular Biomarkers

26.3.2.1 B-cell Lymphomas

Several relevant prognostic biomarkers (BCL6, BCL2, MYC, TP53) have been long known in B-cell lymphomas. In the case of TP53, its mutation, despite a low frequency, is an independent prognostic marker for poor outcomes in diffuse large B-cell lymphomas (DLBCLs), follicular lymphomas, and mantle cell lymphomas. MYC IG translocations play a similar role as predictive of poorer prognosis. However, the relevance of the simultaneous presence of BCL2/MYC or BCL2/BCL6/MYC translocations (double/triple hit lymphomas, respectively) has recently led to revise the WHO classification introducing new entities for DLBCLs with distinct outcomes from non-double/triple hit like DLBCLs. This new vision will likely change the clinical approach to DLBCL as emerging evidence indicates primary refractoriness or increased probability of early relapses to standard CHOP/CHOP-like regimens, which still represent the standard of care for DLBCLs.

26.4 Liquid Biopsy

The aforementioned actionable mutations as well as the validated diagnostic analyses may have important limitations in the real-world practice scenario. A possible caveat of molecular portrait-driven therapeutic planning is the need of tissue with high percentage of tumor cells in order to avoid tumor DNA dilution by nontumor microenvironment cell-derived DNA. This representative tumor sample is often difficult to achieve. An additional relevant problem in the recent evidence of a branched evolutionary path within the tumor tissue, which can lead to different molecular portraits in different tumor sites, or in different phases of the tumor clinical history.

All of these events can be captured by a multiple biopsy strategy that can be hardly performed in the clinical setting. It is now emerging the concept that novel sources for clinical molecular diagnosis deserve to be investigated. It is becoming clear that many body fluids as blood, urine, saliva, and pleural effusions are a precious source of biological relevant molecular species. Circulating tumor cells (CTC) can be isolated from blood and human plasma contains cell free DNA (ctDNA), circulating tumor DNA (ctDNA), and other nucleic acids as microRNA, mRNA, and long noncoding RNA. Additional complexity is added to this scenario by the finding that many of these molecules can be retained within micro-vesicles or exosomes; all these fractions may have different functional relevance. The definition of a tumor portrait by the molecular analysis of these circulating species is called "liquid biopsy."

CTC can be isolated by regulatory agencies–approved methodology and offer important prognostic information, while the identification of NSCLC-associated EGFR mutation like the L858 or exon 19 deletions or T790 escape mutation by liquid biopsy has been approved for driving targeted therapy of advanced disease. An added value of liquid biopsy is the opportunity to monitor disease burden and even detect the occurrence of T790 tumor cell clones in advance of clinically overt disease progression.

Liquid biopsy is therefore leading to a paradigm shift in real-time monitoring of treatment activity and in early passage to second-line treatment in order to provide more efficient mean for giving high selective antitumor treatment.

26.5 Immuno-oncology Biomarkers

The most recent novel immune therapy approaches are mainly directed to activate a preexisting response to cancer specific neo-antigen by targeting immune checkpoint on antitumor lymphocyte such as CTLA4 and PD1 or the PDL1 (PD1 ligand1) expressed by tumor cells or by suppressive components of the tumor inflammatory microenvironment. Although the anti-CTLA4 Ipilimumab and the anti-PD1 Nivolumab and Pembrolizumab have produced a paradigm shift in cancer treatment providing long-term survival in tumors as NSCLC and malignant melanoma, many patients do not respond to this treatment. Therefore, the identification and validation of predictive biomarkers is eagerly awaited, in order to allow precision immunotherapy of human cancer [2].

The IHC search of PDL1 in the tumor tissue has been shown, by a cut-off of 50% expressing tumor cells, to identify advanced NSCLC patients in first-line treatment, where Pembrolizumab was much more effective that conventional chemotherapy, leading to approval by regulatory agency together with the companion diagnostic tools [3]. In other disease conditions, PDL1 IHC has not been validated as a predictive tool [4, 5]. Novel biomarkers as mutational burden or neo-antigen prediction on NGS data are presently under evaluation. Somatic or hereditary loss of mismatch repair genes (MSI) or polymerase epsilon genes leading to defective DNA repair or DNA proof reading are emerging as important predictive immuno-oncology biomarkers.

26.6 Tissue Agnostic Precision Oncology

It is now becoming clear that driver mutations can be a valuable target independently from the type of original tumor tissue, opening the way to the concept of tissue agnostic precision medicine. NTRAK fusion protein is a driver genetic lesion in variety of different tumors amenable to direct inhibition by different specific inhibitors as Larotrectinib and Entrectinib, amplified HER2 or mutated BRAF can be successfully targeted in a minority of different tumors, but can offer valuable options in treatment resistant tumors. Profiling platforms have been therefore developed, and tissue agnostic treatments can be offered to treatment refractory patients. These platforms are offering both profiling of tumor tissues and liquid biopsy–based approaches. Validation trials are presently ongoing. Tumor harboring MSI have been demonstrated to anti-PD1 immunotherapy and Pembrolizumab has been the first drug approved in a tissue agnostic way, considering MSI a biomarker independent from the tissue of origin [6].

26.7 Precision Medicine Clinical Research

Precision medical oncology relies on the integration of molecular analysis of tumor genomic portrait with clinical data, in order to personalize treatment of individual

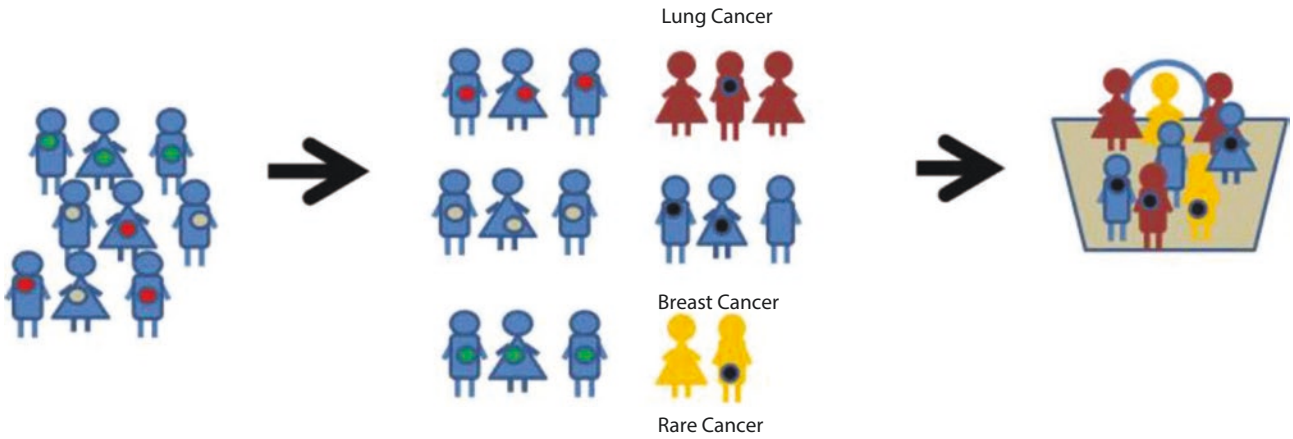


Fig. 26.2 **a** Umbrella trial. **b** Basket trial

patients and to design new clinical trials. The study of targeted therapies in different tumor types, which express low-frequency mutations (<5 %), is based on the design of *basket trials.* In these studies, a small number of patients with different kind of cancer expressing the same mutation in a hotspot site are enrolled in parallel series of phase 2 studies, which allow the study of a targeted drug in different cancer contexts. An alternative research approach relies on *umbrella trials,* which recruit patients with a single cancer type but different actionable mutations. Modern technologies for drug structure prediction on available target analysis allow a developmental path to test new drugs and biomarkers.

The limit of this trial design is that a mutation can act as driver drug-able target in a given tumor, while it can be a passenger lesion in other tumor contexts.

Another emerging issue, which can hamper the performance of basket and umbrella trials, based on mutation discovery by next-generation sequencing, relies on the emerging role of tumor stroma in conditioning therapeutic choices and future drug development (Fig. 26.2a, b).

Conclusion

Precision oncology indeed represents a paradigm shift from conventional cytotoxic drug-based treatment where the treatment was selected on the bases of cancer tissue of origin and there were nonpredictive molecular tools. Indeed, conventional chemotherapy still retains a major role in cancer treatment and its relative un-selectivity is indeed challenged by provocative findings as the induction of immunogenic cell death in tumor cells which, indeed, activate a specific immunologic tumor response and opens new promising scenarios not easy to predict.

Precision oncology, if wisely afforded, can indeed reduce toxicity and make treatment more affordable but all the stakeholders are required to redefine the regulatory approaches, to redesign clinical research, and to identify strictly validated biomarkers for novel companion diagnostics. This is the true challenge for modern oncology.

Name: **T.Z.**

Sex: F

- *Age*: 54 years
- *PS Ecog:* 0
- *Comorbidity:* NA
- *Family history of cancer:* Father's aunt diagnosed of breast cancer, cousin (maternal) with history of breast cancer diagnosed 20 years ago, maternal aunt with diagnosis of endometrial neoplasm, father died with prostate cancer, brother affected by prostate cancer. Daughter affected by breast cancer.
- *Diagnosis:* Breast cancer infiltrating ductal carcinoma in April 2017
- *Stage: IIIA*
- *Phenotype: Luminal A* (ER 100 % PgR 90 %, Mib1 il 5–10 %. Hercep test: 0)
- *Adjuvant chemotherapy:* since 28 Jun 2016 to 16 November 2016, treatment with FEC (epi-doxorubicin 75 mg/m^2+ Cyclophosphamide 500 mg/m^2+ 5FU500 mg/m^2) g1 q21 gg for 3 cycles and 12 cycles of Taxol 80 mg/m^2 weekly.
- *Hormone therapy:* Letrozole since December 2016
- *Radiotherapy treatment:* Since December 14, 2016, to February 6, 2017, treatment on breast and nodes (50 Gy + boost 16 Gy).

During follow-up monitoring, in August 2017, the patient experienced bone pain onset (VNS 10).

Which diagnostics do you suggest?

She performed bone scan of whole body that showed multiple bone metastases, especially in the lumbar region, focused on D8-D9 soma, and D5 soma.

Do you think other diagnostics are required at this point?

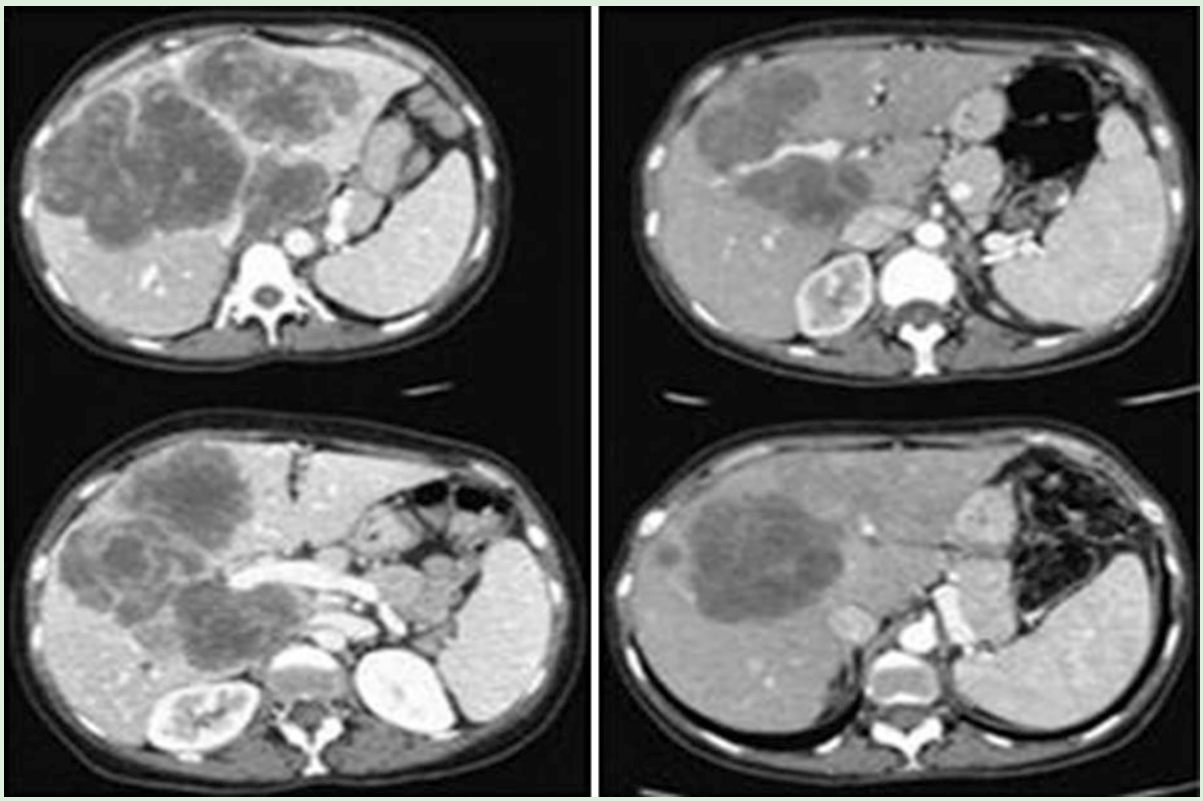
She performed CT scan of total body for restaging. Multiple liver metastases could be detected, and bone involvement was confirmed.

Do you think that the initial pathology was adequate for subsequent therapeutic strategy?

Due to the biological characteristics and the rapid disease evolution, liver biopsy was indicated. Interestingly, the re-biopsy disclosed HER2 positive (2+ at IHC with FISH amplified) breast cancer relapse. This finding allowed treatment with Taxotere and Pertuzumab and Trastuzumab since October 4, 2017.

She was also treated with palliative radiotherapy and started Denosumab therapy for bone metastases.

The patient underwent rapid clinical improvement.



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Name: **G.B.**

Sex: Male

- *Age:* 47
- *Comorbidity:* Iatrogenic diabetes
- *Family History of cancer:* None
- *Smoke History:* Heavy smoker (about 20 cigarettes till death) until 2008
- *Diagnosis:* Lung adenocarcinoma in December 2008
- *Stage: IV (brain, bones, lung and pleura effusion)*
- *First-line chemotherapy:* From 2009 February to 2009 May Cisplatin/Pemetrexed chemotherapy followed by Pemetrexed alone for three cycles
- *Second-line chemotherapy:* From 2009 March to 2009 June Taxotere chemotherapy
- *Radiotherapy:* From December 2009 to January 2010 conformational RT on mediastinum for 44 Gy; SBRT on acetabular bone lesion for 25 Gy
  - *Brain Radiotherapy:* SBRT on frontal lesion for 24 Gy in May 2010; SBRT on parietal lesion for 24 Gy in December 2014; WB RT in 2017 February 2017
- *Molecular Assessment: February 2011 EGFR mutated on EX DEL 19*
- *Target Therapy:* From February 2011 to March 2017 Erlotinib treatment

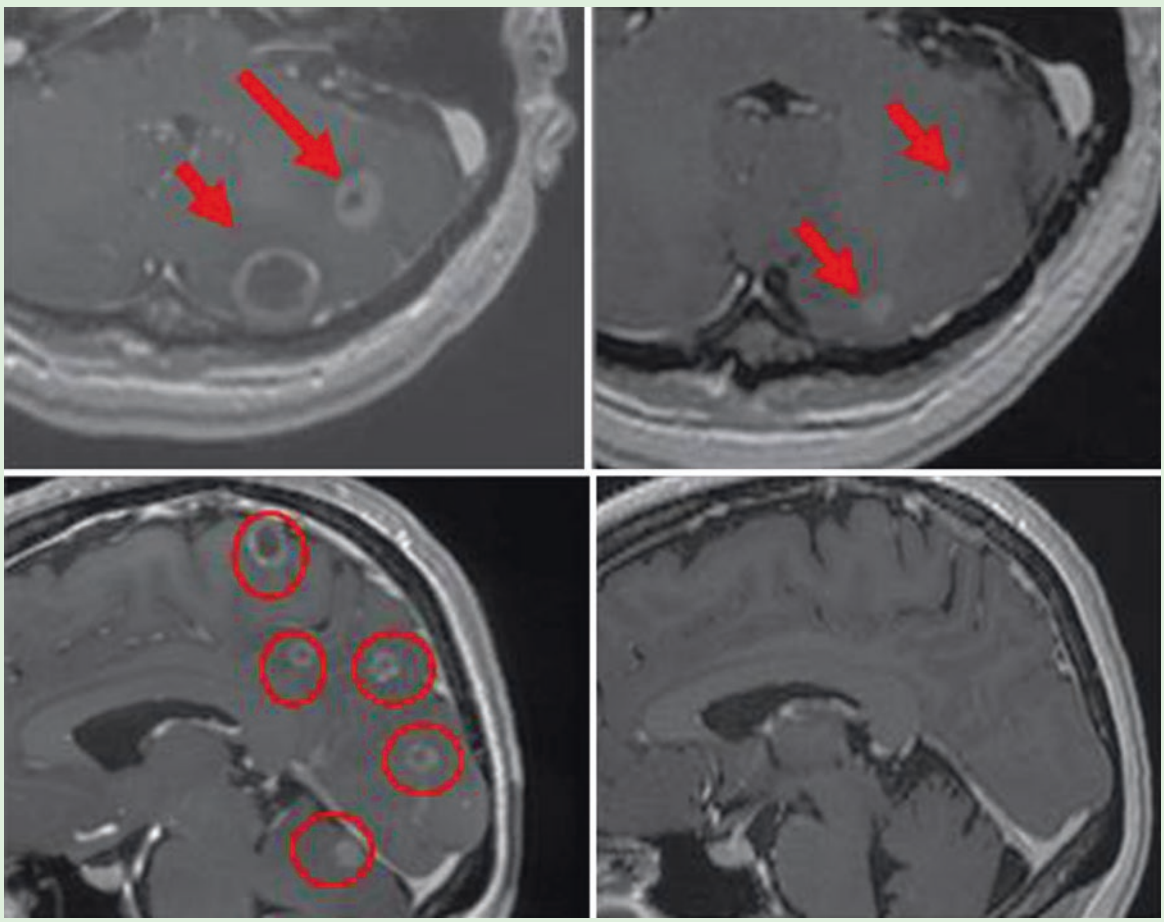
In February 2017 for volumetric increase of lung lesions and all brain lesions, patient received WB radiotherapy and lung cancer was defined as progressive disease.

Which diagnostic work-up do you suggest?

For inadequate tissue sample and difficult re-biopsy, he performed on T790M liquid biopsy; the results was positive for T790M Ex 20 mutation and in March 2017 he started Osimertinib treatment at 80 mg till death.

For bone involvement, patient underwent on July 2017 Denosumab treatment.

In June 2017, CT scan showed regression of lung and brain deposits



Key Points

- Precision medicine represents the new frontier of oncology treatments.
- The identification of molecular targets is the crucial point of precision medicine.
- Liquid biopsy grants the detection of prognostic and predictive tumor biomarkers.
- Precision medicine provides novel oncological drugs with a reduced toxicity profile compared to standard chemotherapy treatments.

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Tumor Board and Molecular Tumor Board

Lorena Incorvaia, Maria La Mantia, Giorgio Madonia, Daniele Fanale, Valerio Gristina, Viviana Bazan, Christian Rolfo, and Antonio Russo

Contents

Christian Rolfo and Antonio Russo should be considered equally co-last authors.

Lorena Incorvaia, Maria La Mantia and Giorgio Madonia should be considered equally co-first authors.

A. Russo et al. (eds.), *Practical Medical Oncology Textbook*, UNIPA Springer Series,
https://doi.org/10.1007/978-3-030-56051-5_27

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Learning Objectives

By the end of the chapter the reader will:

- Have learned what a tumor board is and what is its role in modern oncology;
- Know which figures should be part of a tumor board and which instruments are needed;
- Be aware of the rapidly evolving landscape of genetic testing in oncology and of the reasons why MTBs are becoming more and more common;
- Understand the limitations that TB and MTB may meet in specific settings and how these may be overcome;
- Be aware of the challenges still existing for the implementation and standardization of MTBs and for worldwide data-sharing;
- Have a basic knowledge of the data on the efficacy and clinical implication of TB and MTB on patient's prognosis.

27.1 Tumor Board

Cancer care management is deeply complex and often involve multiple providers of care from diagnosis to treatment to survivorship or end-of-life care. To achieve best results for the patient, an integrated approach among professionals, who share complementary skill-sets and different perspectives to the decision-making process on individualized patient treatment, is required [1].

Emerging technologies and the chance to personalize cancer treatment, including molecular therapy, target therapy, and radiation therapy, have over the last decades increasingly enhanced the need for specialized expertise and reciprocal cooperation between healthcare providers with different specialties [2]. Thus, multidisciplinary team interventions (MDTs) have been implemented across cancer care services worldwide. In literature, MDTs are referred to using a number of terms, for example, tumor boards (TB), tumor meetings, and multidisciplinary cancer team meetings. They could also be addressed as Multidisciplinary Cancer Conference (MCC), defined by Wright et al., 2007, as consultation for healthcare professionals aiming to review diagnostic and optimal treatment care of oncological patients [3, 4]. This term has been used as a synonym of TB [4, 5]; hence, we will broadly indicate this concept as TB.

TB is a group of people of different healthcare disciplines, which meets together at a given time (whether physically in one place or by video or teleconferencing) to discuss a given patient and who are each able to contribute independently to the diagnostic and treatment decisions about the recommended clinical pathway of an individual patient [3]. As an organizational instrument, TBs are becoming more common component of cancer patient care, aiming to discuss on cancer cases that are unusual and/or challenging in order to achieve a definite staging and formulate a shared treatment plan, in the light of the latest clinical evidence (Fig. 27.1). Telemedicine and telehealth have enabled the attendance of meeting, providing visualization and analysis of pathological and radiological reports from different sites [6].

Largely, the multidisciplinary approach might be a very valuable tool for clinical oncological care and integral to the patient management process [6, 7]. TBs are withal found to be part of standard cancer care management on an international level. Indeed, the American College of Surgeon's Commission on Cancer Program accreditation requires cancer programs to have a multidisciplinary cancer meeting, which prospectively analyses cases and discusses management and treatment plans [8].

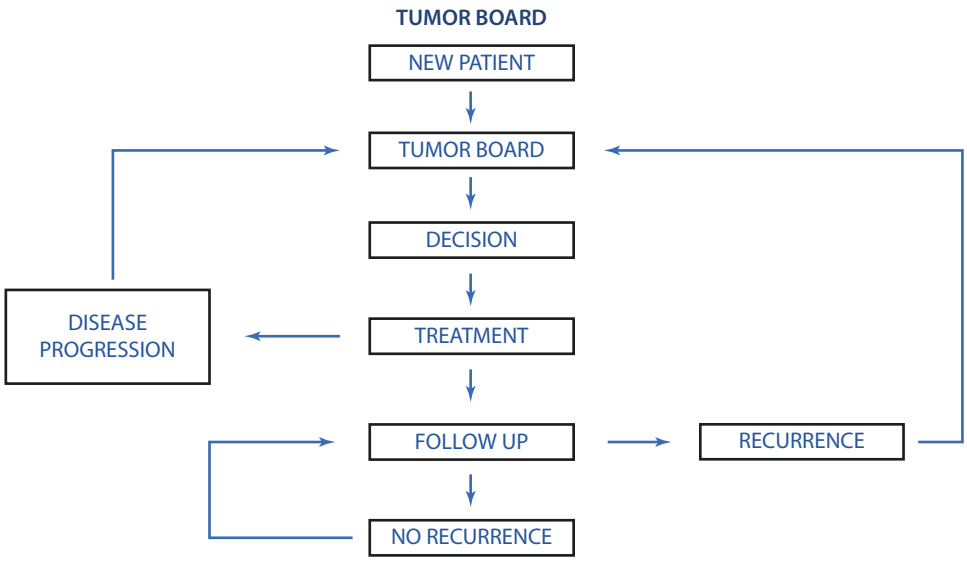

Fig. 27.1 TB workflow

Tumor board attendees might include medical oncologists, genetics counsellors, radiation oncologists, pathologists, radiologists, surgical oncologists, clinical trials and data management representatives, social workers, and oncological nurses. In addition, depending on the cases being discussed, professionals, such as gynecologists, urologists, plastic surgeons, etc., may also participate. Weekly TBs on a set day and at a set time for at least one hour seemed to be the ideal format. Meeting presentations may review cases requiring additional follow-up. By working together, the multidisciplinary team could evaluate all of the options which might improve clinical treatment's effectiveness [9]. Multidisciplinary meetings could be held either in a prospective or retrospective manner. The latter has an educational aim continuing education for healthcare providers, residents, and medical students, maintaining a tumor registry and supporting research and clinical trials [10, 11].

Owing to the significant financial cost, effort, and noticeable time used for TBs, several studies and reviews critically evaluated the real impact of multidisciplinary approach on cancer patient outcomes and management, as well as clinical practice (Table 27.1). Mostly, studies proved that a multidisciplinary approach resulted in positive oncological patient outcomes, both in terms of diagnosis and treatment planning, and in higher quality of cancer care involving consideration of multiple treatment modalities. However, strong evidence describes other functions and objectives of TBs, such as ensure the most up-to-date treatment and association with higher rates of guideline-recommended care adherence [3, 12]. Furthermore, limited evidence has demonstrated improved survival in retrospective studies for patients with breast, head and neck, and ovarian cancer. Birchall et al. [13] proved that, responding to the changes recommended by the Calman–Hine Report, more head and neck patients were evaluated in a multidisciplinary clinic (74% vs. 46%) and their 2-year survival improved (HR 0.7 $p = 0.02$).

To assess the effect of a multidisciplinary approach for patients with breast carcinoma, Chang et al. performed an evaluation for a cohort of patients examined in a multidisciplinary breast cancer center. The authors

Table 27.1 Studies which demonstrated improved outcomes with multidisciplinary management

Study	Type of study	End points assessed	Outcome
Birchall et al. [13]	Retrospective review comparing 2-year survival in H/N cancer patients in the south and west of England before and after a standards document publication (1996–2000)	2-year survival for H/N cancer patients	Patients assessed in a MD clinic (consultant oncologist, radiotherapist, and head and neck surgeon) exhibited improved 2-year survival ($p = 0.03$)
Chang et al. [14]	Retrospective study comparing treatment recommendations before and after a MD breast cancer assessment in 75 consecutive patients	Treatment recommendations made before and after a MD breast cancer assessment	Treatment change in 43% of cases (breast conservation 41% re-excision 6%, further work-up 31%, treatment change-based pathology review 9%, post-mastectomy RT9%, HT3%)
Junor et al. [15]	Retrospective population-based analysis of patients with ovarian cancer	Survival of patients with ovarian cancer based on patient factors and organizational/delivery of care factors	Referral to a MD clinic $p < 0.001$ Receipt of platinum chemotherapy
Lutterbach et al. [12]	Retrospective review of 1516 patients with a brain lesion discussed at a MDB	Assess if recommendations made at Brain TB were implemented	91% of MDB recommendations implemented
Levine et al. [23]	Prospective study of CRC patients (2008–2009), comparing patients referred to the MDC vs. patients managed outside	Comprehensiveness of the preoperative evaluation, and access to multimodal care	Complete preoperative evaluation in MDC patients was 85% vs. 23% in the CG ($p < 0.0001$) 62.5% of MDC patients vs. 41.5% of CG patients had peri-operative treatment ($p = 0.02$)76% of MDC rectal cancer patients vs. 20% of CG patients underwent neoadjuvant therapy ($p < 0.0001$)

Abbreviations: MD Multidisciplinary, *RT* Radiotherapy, *HT* Hormone Therapy, *MDB* Multidisciplinary Board, *TB* Tumor Board, *MDC* Multidisciplinary Clinic, *CG* Control Group

demonstrated that integrative program led to a change in treatment recommendation for 32 out of 75 patients (43%) evaluated [13–17].

Nevertheless, some limitations and weakness should be noted. Despite TBs representing the best approach on cancer care management, improving decision-making, the multidisciplinary aspect is still challenging forasmuch as it requires coordination, cooperation, and optimal communication, among healthcare providers from various areas of expertise [18, 19]. Moreover, considering the lack of time, the difficult in attending multidisciplinary meetings is a significant obstacle for active participation. Professionals might critically consider conference meetings as a relevant part of their work since TBs reduce waiting time for patients and improve decision-making process.

At the same time, multidisciplinary meeting should have been dedicated consistent time avoiding many clinical cases discussions in a short amount of time [20]. The lack of a financial compensation for attending a meeting and medico-legal concern assuming that TBs participants disagreed about the patient treatment plan. Similarly, strong institutional support is needed providing meeting rooms with adequate facilities, videoconferencing equipment, and computer systems for displaying radiographic images and pathology reports [21]. Additionally, notwithstanding that TBs are the cornerstone of best practice in cancer care, the lack of clear and uniform international criteria has been shown to be associated with the impact of teamwork on patient outcomes and quality of the evidence. Several efforts should be made to develop a more comprehensive and exhaustive methodology. Thus, specific guidance, team training, and investment of resources are needed. Indeed, more organizational support with education, equipment, and infrastructures might ensure higher quality standard in multidisciplinary cancer care management [22, 23].

27.2 Molecular Tumor Board

The recent developments in genomics and the advent of new technologies and sequencing platforms such as next-generation sequencing (NGS) have brought great changes and innovations in oncology. We are now able to better understand the genetic triggers of cancer, and to identify prognostic and predictive biomarkers as well as target genomic alterations against which we have developed a number of new drugs [24, 25]. Evidences indicate that cancer treatments are associated with better outcomes when based on genetical information, compared to cases where these data are lacking [26]: In different studies, significative improvements have been found in objective response rate, time to treatment failure, overall survival, and progression-free survival [27–29].

However, the mass of new data constantly pouring from in vitro studies often has a hard time being translated in everyday clinical practice [30, 31]. At the same time, the evermore complex diagnostic tools and technologies at our disposal (whole genome sequencing, WGS; whole exome sequencing, WES) and the proliferation of biomarkers and targeted therapies makes more difficult for physicians to correctly interpret the data and to select the best therapeutic path for every patients [31, 32]. This has also led to an always-more-relevant role of emerging professional figures such as bioinformaticians and biostatisticians [33].

In this context the molecular tumor board (MTB) aims to fill the gap between our knowledge on cancer genomics and everyday clinical practice, allowing confrontation among the different professional figures involved [31, 34].

MTB is a multidisciplinary board representing the interface between healthcare physicians and translational researchers, research institutions, and pharmaceutical industry (Fig. 27.2) [35]. Members periodically and regularly meet to discuss complex or rare cases where NGS may have a role in choosing the right therapeutic path [34, 36].

MTB should be composed of at least the following professionals (Fig. 27.3):

- Oncologist
- Hematologist
- Molecular pathologist
- Molecular biologist
- Clinical biologist
- Geneticist
- Surgeon
- Bioinformatician

Other professional figures that may be involved and that can participate on request to discuss specifically cases are pharmacologists, oncological nurses, radiation therapy oncologists, radiologists, biostatisticians, clinical trials investigators, patient representatives, genetic counselors, ethicists, and translational and basic scientists [37, 38].

Data have shown that molecular tumor board can help improving patient outcomes [38, 39]. Different studies (although in the absence of a control arm and biases toward fittest patients) [31] have reported that MTB can increase adherence to clinical practice guidelines [40], with improvements in different outcome measures, including progression-free survival, overall survival, and time on treatment [36, 41, 42]. In a recent publication by Shumei Kato et al, patients treated according to MTB recomendations showed significantly longer OS and PFS, with the degree of matching between patients and their genomic alterations beeing an indipendent predictor of better outcome [43]. Often these

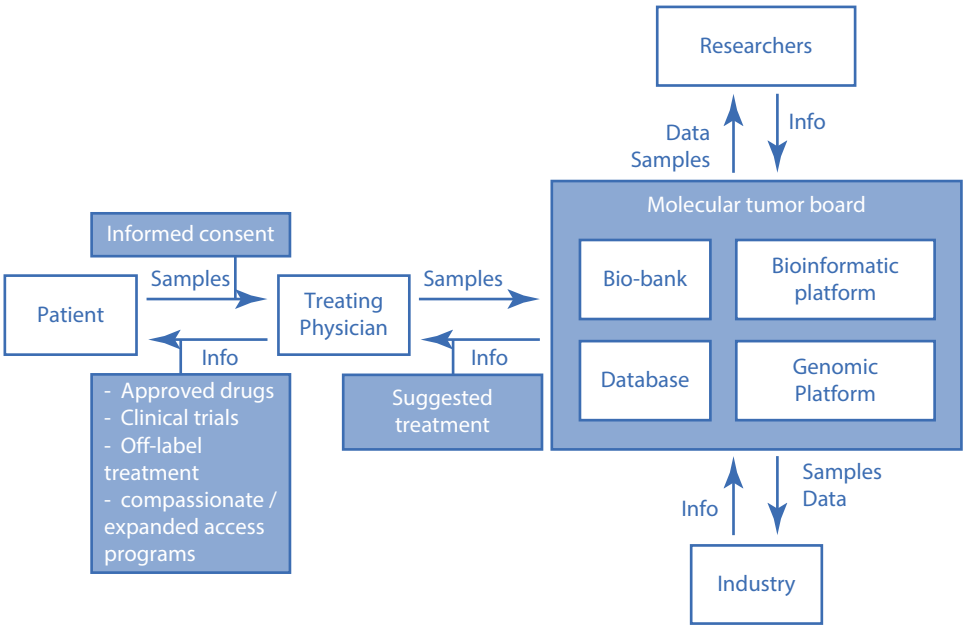

Fig. 27.2 Molecular tumor board workflow

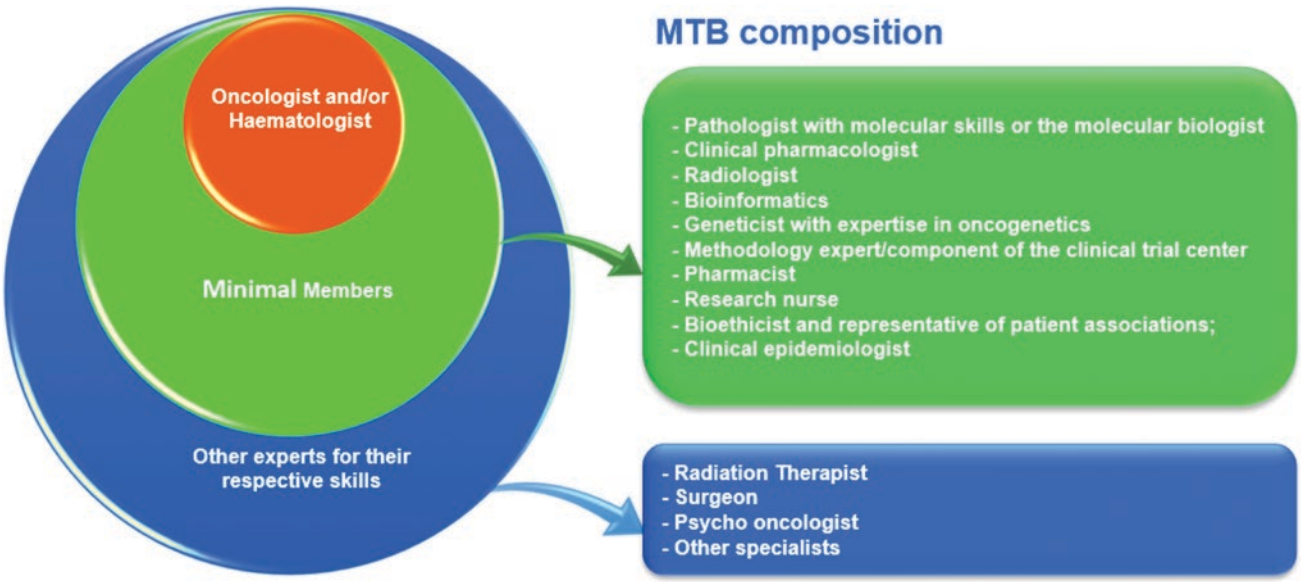

Fig. 27.3 MTB composition

new drugs can offer not only better OS or PFS, but also a significant improvement in patient's quality of life [44]. Usually MTB aims to assign approved standard-of-care agents based on high-grade evidences from randomized clinical trials. However, if this is not feasible, lower-grade evidences can be considered and experimental or not-yet-approved drugs, as well as off-label treatments, represent a valid alternative [45]. Indeed, data on the results of the application of MTB decisions have shown that patients are more often enrolled in clinical trial or in expanded or compassionate access programs [34, 36]. Moreover, genetic analyses may identify germinal mutations and thus patient with a higher genetic risk of developing cancer, allowing to implement strategies of genetic counseling and oncological screening and to extend the test to the patient's relatives, with better outcomes in terms of earlier diagnosis and treatment [26, 46]. This is, i.e., what has been happening for BRCA genes, that have recently evolved from gene predicting cancer predisposition to predictors of reponse to a specific class of drugs (PARPi). Testing has now 2 different objectives: identification of patients with higher probability of benefit from PARPi and, by testing the family members, identification of carriers of a pathogenic vari-

ant, who have inheredited predisposition to cancer development [47]. MTB also allows the sharing of knowledge and expertise among members with different fields of specialization, improving the confidence of the clinician in the use of new biotechnologies and in the interpretation of the results, and is often used as an educational tool for physicians, translational researchers trainees, and medical students [34, 38, 45].

Anyway, many gray areas remain in the implementation of a molecular tumor board [48]. It is a relatively new tool and evidences and literature are still scarce: few guidelines or quality requirements have been published. As a consequence MTBs widely vary worldwide in terms of composition, tasks, tools, and workflow, limiting the reproducibility of data and sharing of knowledge and information among different boards [31].

As an example, nowadays we dispose of a number of different sequencing techniques, platforms, and algorithms and no consensus has been achieved on which should be used within a MTB [49]. Standardization is also needed for the collection of the sample (tumor tissue, liquid biopsy), extraction of genetic material [49] and data informatization and sharing [30]. Quality of the data greatly depends on numerous factors:

- Sample used: Fine needle biopsy, core biopsy, surgical sample. Limited tumor samples may hinder the possibility to perform extensive genetic analyses and thus to identify possible targeted therapies available for the patient. In this setting, liquid biopsy may help bypass this issue by researching genetic data not in tumor tissue but in other, more accessible and abundant media (usually blood, but also urine or saliva) [9, 10, 50–52].
- Cancer genome database used to evaluate the significance of the found mutation and to compare it with available data in literature. A common problem is the interpretation of variants of almost-known significance (VAKS) that are not fully biologically characterized and for which no definitive consensus has been established. VAKS are often discussed at MTBs, because they may often offer the chance of a specific treatment. However, considering the lack of conclusive recommendations, these options must be weighed against other available options and MTB should develop standardized strategies to manage these challenging cases [51].
- Recently, evidence-based tools for interpreting actionability of genomic alterations in cancer patients have been developed. The two most compelling examples are the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) and OncoKB from the Memorial Sloan Kettering Cancer Center (MSKCC). The use of these tools within an MTB has been shown to help the choice of alteration-specific treatments through appropriate clinical trials selection [52].
- Moment in which the sample was obtained: before or after therapy (tumor can evolve during time, and treatments can modify tumor characteristics)
- Sample conservation: frozen or FFPE (formalin-fixed, paraffin-embedded)
- Time passed from the obtainment of the sample
- Analytical platform, depth, and breadth of sequence coverage: multiple panels and platforms exist, ranging from those analyzing few genes to WGS or WES platforms

The use of extensive genome sequencing techniques often also requires the sequencing of germline DNA as control. This may lead to unsolicited finding of germline mutations, with relevant implications for the patient and his relatives in terms of hereditary syndrome diagnosis and higher familiar oncological risk [31]. Appropriate informed consent, accounting for this possibility should be acquired. In addition, MTB must know how to recognize and handle these findings, knowing when and how to refer the patient and/or his relatives to genetic counseling [51], and a common policy for the management of these cases must be agreed on [37, 46]. Moreover, the identification of an actionable mutation does not always mean that the patient will be able to get access to the relative drug, because many economical (both for the genetic testing and the drug acquisition) [53] and technical limitation (local availability of clinical trials or ineligibility of the patient) may interfere [36, 38, 41]. Other reasons may limit the administration of the suggested drug, such as clinical conditions of the patient, patient's preferences, or physician's choice [26].

Most of the available data on MTB derive from single-center studies from large institutions, where most of molecular tumor boards have developed, and data on real-life applications and routine clinical practice at local level are still scarce [54]. Implementation of MTBs may not always be feasible for the lack of professional figures or of sufficient expertise of them, especially in smaller centers or local hospitals, where most of patients are treated [31, 55]. To solve this problem, clinician in smaller centers may partner with larger academic centers, with mutual benefit (increased genetic data for the academic institution, improvement in treatment selection for the local hospital) [51], or join telematic tumor boards that use computerized platforms (that must guarantee patient's privacy) to connect together various physician from distant centers [55].

Another problem may be the longer time needed to reach a clinical decision on the prosecution of patient's treatment. To avoid this, MTB meeting should guarantee a sufficient frequency of the meetings [38, 55–57].

In conclusion, with the development of ever-new techniques and the improvement of our knowledge on cancer genetics, molecular treatment is increasingly becoming a standard of care. Molecular tumor boards will become a fundamental instrument for clinical oncologists in the selection of the best therapeutic path, operating as a link between complex genetic data and a consistent, individualized treatment indication for each patient. Data sharing and standardization of procedures and workflows are therefore essential to make the most of it.

Summary of Clinical Recommendations

- Tumor boards are an irreplaceable tool to handle challenging oncological cases through confrontation and discussion between different healthcare providers and should be implemented whenever possible.
- Molecular tumor boards are increasingly needed to guide the treatment selection based on genetic information obtained thorough high-throughput platforms.
- MTB should be composed at least of oncologist, hematologist, molecular pathologist, molecular biologist, clinical biologist, geneticist, surgeon, bioinformatician. Other figures may be included depending on the specific MTB and case discussed.
- More studies and international guidelines are needed to confirm the available results on the efficacy of MTB and to guide their development worldwide.
- Data-sharing and standardization of procedures, techniques, and workflows are needed to fully take advantage of MTB.

Key Points

- Tumor boards derive from the increasing need for specialized expertise and reciprocal cooperation between cancer care providers. It consists of a group of experts of different healthcare disciplines meeting periodically to discuss challenging cases and contributing to the diagnostic and treatment decisions.
- The advances in genomics and the advent of new technologies have vastly increased our knowledge on cancer genetics; however clinicians have a hard time keeping the pace with these developments. MTB aims to fill the gap between genetic data and everyday clinical practice.
- Member can vary depending on the type of board and the specific cases discussed, but should be at least composed of oncologist, surgeon, radiologist, radiation therapy oncologist, pathologist, etc.
- TB and MTB have shown to improve patient's prognosis and outcome measures such as overall survival and progression-free-survival. Solid data on MTB are still scarce and there are not international guidelines.
- MTB aims to suggest approved drugs when possible, but clinical trials, off-label treatments, and expanded/compassionate access programs are valid alternatives.
- Difficulty in developing MTB in local centers, lack of tumor tissue, uncertain classification of the mutations founded (i.e., VAKS), handling of unsolicited findings and limitations in the effective access to the drug suggested by MTB are some opened issues, but various solutions are available (telemedicine, genetic counseling referral, liquid biopsy).
- To make the most of TB and MTB, data-sharing and standardization and validation of procedures, techniques, and workflow is necessary. Solid data on MTB are still scarce and there are not international guidelines.

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Precision Medicine in Oncology: Glossary of Relevant Scientific Terms

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Learning Objectives

By the end of the chapter the reader will

- Learn the basic concepts of precision medicine in oncology.
- Know in depth the knowledge of scientific terminology entered into the language of oncology specialists and cancer researchers.
- Know how to facilitate clear communication between clinicians about cancer research and oncology practice terminology

28

28.1 Introduction

One of the future challenges in the field of oncology is the integration of translational research and technology innovation, in order to clarify unexplored aspects of the biology and the genetics of tumors, to develop a personalized treatment based on the molecular characteristics of the patient and his disease.

"Precision Oncology" refers to the tailoring of medical treatments according to the individual characteristics and specific genomic alterations (molecular signature). "Precision" stands for an accurate selection of the patient. The selection is based on the molecular features of the tumor and the goal of "precision oncology" is to achieve a longer-lasting clinical benefit compared to mainstream treatments.

In the past, the conventional approach was defined as "one size fits all" and the disease was treated according to its localization and its histopathological features. Nowadays, all the efforts are addressed to find new therapeutic strategies according to the molecular profile of the patient [1, 2].

The human genome mapping has been completed in 2001 within the Human Genome Project. The purpose of the Human Genome Project was not only to study the genetic and epigenetic alterations, but also the genomic and proteomic along with all those variations affecting the gene expression and the signaling transduction.

New techniques such as the Next-Generation Sequencing (NGS), also called Massive Parallel Sequencing (MPS), have been recently developed. This new method allows for the sequencing of wider or full-length gene traits; its analytic sensitivity is higher than the conventional Sanger sequencing, with shorter time compared to older technologies [3]. "Druggable" mutations detected by NGS correspond to specific molecular targets (or, sometimes, definite pathways) on which targeted agents act.

In the clinical practice, the using of this new approach can be powerfully applied to the analysis of circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), corresponding to a small fraction of the total cell free DNA (cfDNA), which can be isolated from peripheral blood and other biological fluids.

Molecular profiling of circulating cellular elements (such as exosomes, CTCs, microvescicles, platelets) and circulating nucleic acids (cfDNA and ctDNA), named liquid biopsy (the blood sample is summarized by the term "liquid biopsy"), represent both a new approach helpful to identify predictive factors in patients with locally advanced disease. Nowadays the Epidermal Growth Factor Receptor (EGFR) mutation analysis in patients with Non-Small Cell Lung Cancer (NSCLC) on stage IIIB and IV [4] is validated in clinical and laboratory routine settings.

In the near future, the molecular characterization of exosomes and cell-derived vesicles might become an important tool to follow the tumorigenesis as a tool of Precision Oncology.

The efforts of the scientific community are aimed at the discovery of new potential tumor biomarkers to be targeted.

However, the identification of a genetic alteration predictive of response or resistance may be only surrogate of a possible therapeutic effectiveness. In fact, there are many "driver variants" and "passenger mutations" that can interfere with response to therapy. Importantly, the selective pressure exerted by the drugs can cause the activation of different ways of escaping for the tumor.

A further critical point is the tumor heterogeneity: The cancer genome evolves and accumulates many genetic alterations [5]. This is the reason why it could be useful to work with circulating tumor nucleic acids (ctANs), in order to be able to catch all the heterogeneous tumor landscape.

ctANs can be considered a robust marker to follow the molecular evolution of the disease. The tumor heterogeneity can be easily detected in the peripheral blood where ctANs are released by several type of tumor cells. This type of investigation, above all within the context of a standardized laboratory setting, can provide more comprehensive information compared to tissue biopsy [6].

Lastly, this new diagnostic and therapeutic approach can help to set new clinical trials: The precision medicine has changed the way of leading clinical trials. "Basket trials" and "umbrella trials" have been largely developed in the last few years: Patients can be enrolled according to the genetic and molecular characteristics of the tumor, in order to administer a treatment potentially to the most responsive patients. These new trials may reduce the toxicity and decrease the costs of future treatments [7]. In this new paradigm of "mutational oncology", the agnostic markers represent the novel frontier of precision medicine [8].

New Multidisciplinary Teams are needed to ensure the integration of different professional profiles, and the Molecular Tumor Boards are the first step to ensure the best path surrounding each personalized treatment to the patient.

Moreover, it is necessary to address the financial resources for new trials in order to allow this ambitious project called Precision Oncology [9].

In this new era of the Precision Oncology the nomenclature is of primary importance, since the correct metrics allows for a correct test prescription and a better patient management. Therefore, we report a glossary that might be useful to the clinical, basic and applied research specialists and professionals.

28.2 Glossary of Scientific Terminology

The terms included in the following glossary were identified by the Associazione Italiana di Oncologia Medica (AIOM) – Società Italiana di Anatomia Patologica e Citodiagnostica (SIAPEC) – (Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBIOC) – Società Italiana di Farmacologia (SIF) Working Group [10].

28.2.1 Pathology, Oncology and Molecular Pharmacology Section

Terminology	Definition
Copy number variations (somatic)	It refers to a change in the DNA copy number that involves tissue cells, including cancer cells. The alterations comprise loss or gain of chromosome segments or complete chromosome arms and amplifications or focal deletions.
Actionable genomic alteration	Comprises targetable alterations and genomic variations that cannot be directly targeted. They lead to a pathway dysregulation (such as the PTEN onco-suppressor gene alterations that can be targeted by PI3K/AKT inhibitors).
Targetable alterations	Genomic alteration that leads to the production of a modified protein which can be targeted by a specific drug. In this case, the rationale of the target therapy is explained by the theory of the oncogene addiction.
Gene amplification	Number of copies of a gene that is increased in certain cells and regions of chromosomes because extra copies of DNA are made in response to cell development signals or environmental stress, with subsequent increase of mRNA and its proteins. The mechanisms that generate amplification include recombination, fusion bridge breakage cycle, replication.
Liquid biopsy	Biological sample collection (blood, urine, CSF, saliva) where DNA, RNA, miRNA, proteins, exosomes, tumor cells can be isolated. These components may furnish information on the immunological and molecular characteristics of the tumor [4].
Circulating tumor cells (CTC)	Cells that detached from the tumor and go into biological fluid like blood. They can be isolated through different tools and characterized through massive parallel sequencing.
Cancer cell clone	Tumor cells originating from an ancestral cell that harbor the same somatic mutations.
Organotypic cultures	Tissue cultures (including cancer tissues) exhibiting the differentiation characteristic of the parental organ.
Primary cultures	Cell cultures obtained by cancer samples cultured in vitro through a few steps.
Circulating tumor DNA (ctDNA)	Circulating Tumor DNA (ctDNA) is a tumor-derived fragmented DNA present in the bloodstream and in many other different biological fluids (blood, urine, effusion liquids, CSF). The ctDNA can be used to obtain information on the molecular features of the disease and tumor make-up remodeling.
Exposome	The combined effects of the all environment factors/stressors on the organism, either at the genomic level or gene expression. These factors are part of the epigenetic modifications.
Intertumoral heterogeneity	Molecular hallmarks of tumor: transcriptional, genomic, epigenomic, pathological and clinical differences among tumors of different people [5, 11].
Intratumoral Hheterogeneity	Coexistence of many different subclones with different transcriptional, morphological, genomic, and epigenomic features. The temporal heterogeneity refers to the change of the subclonal structures according to the time and the treatment exposition [5, 11].
Clonal evolution	The mechanism leading to the tumor development, starting with a regular cell through the accumulation of mutations, clonal selection, and clonal expansion.
Target-specific drug	The selective interaction between the drug and its target is the main feature of this category of drugs. Target agents are highly selective (like anti-EGFR inhibitors) and they can be differentiated into "generations" (osimertinib is a third-generation of TKI drug).
Orthotopic animal models	Animal model from which tumor samples derive. These are implanted into immunodeficient mice in the same anatomical site where the tumor arose.
Humanized animal models	Animal model from which tumor samples derive. These are implanted into immunodeficient mice where the human immune system has been reestablished.

Terminology	Definition
Patients derived-xenograft (PDX) models	Animal model from which tumor samples derive. These are implanted into immunodeficient mice.
Driver mutations	Driver mutations occur in the regulatory or coding region of the tumorigenesis associated genes (driver genes). Recent evidences have shown that a tumor can harbor four different driver mutations [12].
Passenger mutations	Passenger mutations can occur in noncoding or coding gene regions, but they do not confer any advantage in terms of growth or survival. Many passenger mutations are identified in different kinds of tumor contributing to the tumor molecular make-up. Passenger mutations can also be detected in regular cells after being exposed to intrinsic and extrinsic mutational processes.
Genomic mutation/ alteration	It is defined as a stable DNA sequence alteration that can be germinal (heritable) or somatic (acquired by a cell during lifetime). Unlike the germinal variants, which can be transferred to descendants (sons), the somatic mutations are transmitted to all the cells deriving from the carrier clone during the division process (mitosis). There are many different types of genetic alterations that comprise the point mutations, structural variations, and number copy modifications.
Oncogene addiction	Tumors are characterized by the present of a large number of gene alterations: Many of them do not compromise cellular, but they are fundamental elements for the cell survival and the tumorigenesis (such as the 9–22 chromosome translocation and the subsequent establishment of the BCR–ABL fusion gene). The "oncogene addiction theory" says that the tumors growth and survival depend on a single mutated oncogene, therefore a selective inhibition of a specific oncogene negatively affect tumor growth and progression [13, 14].
Oncogenes	Oncogenes are genes able to cause the tumor transformation. Proto-oncogenes are normally present as their nonpathological variant in all the cells, while their activation is due to a chemical or environmental exposition [14].
Tumor organoids	Tumor organoids are 3D in vitro cultures of cancer cells that can be derived on an individual patient tissue.
Tumor-suppressor genes	Tumor-suppressor genes are coding genes that slow down cell division, repair DNA mistakes, or tell cells when to die (apoptosis).

Terminology	Definition
Tumor cells subclone	Mutated cells originating from a tumor clone [5].
Molecular tumor board	A multidisciplinary team that includes many different professional profiles.
Benign variant	Acquired mutation (or somatic) or hereditary variant (germline) not associated to an increased cancer risk.
Deleterious variant	The genetic alteration responsible for the inactivation of the associated protein.
Insertion/ deletion variant (in/del)	Insertion or deletion (called "indels") are characterized by gain or loss of one or more nucleotides. According to the number of bases gained or loss: short indels (1–5 bp), medium (100 bp to 30 kb) big (over 30 kb). Frameshift In/Del corresponds to a variation caused by the deletion or insertion in a DNA sequence that shifts the way the sequence is read; on the contrary, in-frame does not have any shift of the sequence, but only a change in the number of amino acid content.
Heterozygote variant	It corresponds to the alternative (altered) allele at a given locus.
Homozygous variant	This condition occurs when both the alleles coding for a protein present the alternative variant associated to the protein defect.
Variant of uncertain clinical significance	Acquired mutation (somatic) or hereditary variant (germinal) that show an unknown association with tumor or disease risk development.
Pathogenic variant	Gene variant conferring an advantage in terms of tumorigenesis or a hereditary variant which predisposes to a specific disease.
Point variant	DNA variations caused by single substitutions. If the point mutation occurs in the coding gene, two main alteration can occur: Missense variants: An amino acidic substitution is introduced within the protein sequence, where the change can sometimes alter the function or structure of both protein and its domain. Nonsense variants: The amino acid substitution leads to the development of a truncating codon (stop codon) within the protein sequence. Synonym variants: No amino acidic changes. When these changes drop close or nearby the splicing site, it is important to exclude any possible effect on mRNA maturation.
Structural variants or genomic rearrangement	It comprises all the alterations that lead to a change in the orientation, the position, and the number of copies of the genomic DNA.

Terminology	Definition
Variation in the number of copies	Germline copy number variants that contribute to the genomic variability and might predispose to hereditary diseases.
Extracellular vesicles	Small cell derived vesicles released into different biological fluids; they can be isolated to obtain information on the tumor biological features.

Comprehensive Genomics Profiling (CGP)

Comprehensive Genomics Profiling (CGP) is a next-generation sequencing (NGS) approach which evaluates by a single assay hundreds of genes including relevant driver cancer genes, in order to assess peculiar genomics signatures. CGP can detect biomarkers at nucleotide-level resolution that commonly involves all major genomic variant classes (SNVs, indels, CNVs, fusions, splice variants), as well as large genomic signatures such as Tumor Mutational Burden (TMB), blood Tumor Mutational Burden (bTMB) and Microsatellite Instability (MSI), increasing the identification of clinically actionable genomic alterations. Notably, these genomic signatures are considered as predictive and prognostic biomarkers associated with different oncological outcomes. Indeed, MSI-high is considered as negative prognostic biomarker in endometrial carcinoma, whereas it has recently emerged as an independent biomarker associated with longer survival and greater response rates following treatment with Immune-Checkpoint Inhibitors (ICIs).

Tissue-agnostic drugs

Drugs to treat tumors developed and approved regardless of tumor histology, guided by predictive and druggable genetic alterations.

28.2.2 Methodology and Clinical Trials Section

Alchemist trial	Group of trials that have the same common endpoint to define the role of the molecular target agents such as EGFR, ALK, ROS (TKI), or immunotherapeutic agents in patients affected by early stage NSCLC eligible of an adjuvant treatment.
Avatar	A real and living model that can host human tumor cells that will be treated with antineoplastic agents.
Biomarker endpoint surrogate	Tumor or patient related parameter whose modifications can give information of the antitumoral activity.
Predictive biomarker	One or more factors that can predict the response or the resistance to a specific treatment.
Prognostic biomarker	One or more factors that can be used to stratify patients at the diagnosis into different risk classes.
Circos plot	It is a graphic representation which can simplify the data on the genomic sequencing. It is commonly used to integrate a large quantity of genomic data.
Forest plot	A forest plot, also known as a blobbogram, is a graphical display of estimated results from a number of scientific studies addressing the same question, along with the overall results. It was developed for use in medical research as a means of graphically representing a meta-analysis of the results of randomized controlled trials. Although forest plots can take several forms, they are commonly presented with two columns. The left-hand column lists the names of the studies (frequently randomized controlled trials or epidemiological studies), commonly in chronological order from the top downwards. The right-hand column is a plot of the measure of effect (e.g., an odds ratio) for each of these studies (often represented by a square) incorporating confidence intervals represented by horizontal lines. A vertical line representing no effect is also plotted. If the confidence intervals for individual studies overlap with this line, it demonstrates that at the given level of confidence their effect sizes do not differ from no effect for the individual study.
Genotype driven study	Patients are enrolled according to the molecular features of the disease defined through gene/genomic sequencing.
Artificial Intelligence in the drug development	It is a discipline whose aim is to solve problems in an automatized way.
Hyperprogression	A growing number of studies have shown that immunotherapy may accelerate tumor progression in a significant subset of patients across multiple histologies. The identification of hyperprogression is done through the RECIST criteria [15].
Machine learning	It is a theory according to which a machine can fulfill a specific task thanks to the recognition of schemes without being programmed for it.
N-of-1 trials	It is a particular model of trial constitute by a single patient. The main aim is to overcome the applicability of the results coming from the classical RCT on a single individual.

Pseudo-progression	Atypical model of treatment response observed in a small subgroup of patients treated with immunotherapy [16].
Spider plot	It is a graphic representation of the dynamic percentage variation of the sum of the diameters of the tumor lesion calculated through the RECIST criteria.
Umbrella trials	This trial enrolls patients with the same tumor in order to analyze the molecular biomarkers and to address a specific target agent.
Basket trial	This kind of trials have the aim to evaluate the response to a specific drug regardless of the histology of the tumor. Patients are just enrolled on the basis of the molecular features of the tumor.
Adaptive trial	Any changes that can occur in this kind of trial should plan before and written into the protocol. Modifications are allowed and this kind of trial is also defined "flexible."
Controlled randomized trial	An experimental treatment is compared to the standard of care. The trial can be defined: double blind or single blind. In a double-blind trial both the investigator and the patients do not know the administered treatment, while in a single-blind patients do not know the administered treatment.
Swimmer plot	It is a graphic representation of all the steps of the treatment response during a trial.
Virtual clinical trials	These trials represent a new method useful to collect data of patients enrolled in many other clinical trials. Cellphones, apps, and social media can be used to enroll patients.
Waterfall plot	It is a graphic representation of the percentage variation of the sum of the diameters of the target tumoral lesions. Each patient is represented by a vertical rectangle where the percentages of grown and decrease are over or under the rectangle.
Windows of opportunity	A window of opportunity (also called a margin of opportunity or critical window) is a period of time during which some action can be taken that will achieve a desired outcome. Once this period is over, or the "window is closed", the specified outcome is no longer possible.

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Patients Categorization

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Learning Objective

Institutions and clinicians are called to respond to the needs of these patients who may live a long time with cancer disease and claim the right to recover a satisfactory quality of life (QoL). The categorization of cancer patients represents an opportunity to achieve this goal. It requires a paradigm shift in the culture of cancer survivorship care, whereby we abandon a common, general, approach to all cancer survivors in favor of the application of our epidemiologic knowledge and the developing risk assessment tools to tailor follow-up and recommendations to each individual cancer survivor.

29.1 Introduction

As a result of improved screening programs, therapeutic advances, and population aging, long-term survival is increasingly common in the path of patients with a cancer diagnosis [1]. While there has been an increase in the number of cancer diagnosis, also the number of cancer survivors has increased [2]. In recent decades, the number of patients with a history of cancer in Italy has increased from about 2 million in 2006 to over 3 million in 2016 [3]. In 2020, 4.5 million cancer patients are expected. The Italian data on cancer survivors are in accordance with the rest of the world: The number of cancer survivor in the US, for example, has increased from 3 million in 1970 to about 15 million survivors in 2012, representing 5% of the total population [2].

Oncologists must face a new scenario with new drugs that convert the metastatic disease to chronic and adjuvant treatments that have increased survival with, however, different and also unusual side effects; therefore, early detection can aid avoiding the subsequent comorbidity and allowing diagnosis of early recurrence or second cancers [4]. There is an increasing number of people of any age, health, and social status who return to an active life, despite cancer.

Institutions and clinicians are called to respond to the needs of these patients who may live a long time with a disease and claim the right to recover a satisfactory quality of life (QoL) [5].

29.2 Cancer Disease

Cancer is a highly heterogeneous disease with different types, phases, and outcomes, including forms that can be cured, according to strict statistical criteria, or may progress rapidly or through alternating periods of remission and relapses, that last for short periods or decades and that often require intermittent or continuous treatment. Living long after diagnosis can either mean being disease-free or having active chronic disease. Even the status of "*disease-free*" may be attained by some patients only after surgical resection of a small cancer or after long treatments including surgery, radiation, chemotherapy, hormones, or new anticancer agents [2]. Surviving lymphoma, breast or lung or colon cancer does not have the same meaning as regards long-term *sequelae* and quality of life [3, 6, 7]. Not only disease features and stage, but also age, gender, ethnicity and culture, and educational and social status can contribute to the experience of cancer and cancer survivorship [8].

Yet, while the menacing echo of a cancer diagnosis and its implications persist, an increasing number of patients are being cured and a growing portion of those whose cancer recurs can live for many years with good quality of life. We need to explain to all patients the variable and uncertain nature of cancer and then speak of favourable prognosis and cure rates, long-term *sequelae*, and survivorship issues to those with early stage or highly curable cancers, while speaking of relapses or progression, palliative and end-of-life care to those with advanced disease [9, 10]. Cancer patients should now be enabled to face their future with hope tempered by awareness and realistic expectations. Aware from the time of diagnosis that some cancers can be successfully treated, but also aware that lifetime follow-up and care of potential long-term treatment *sequelae* will be required, they may be better able to work with their oncologists to design an individual care plan tailored to their medical situation, life priorities, and values [11]. A young female patient's desire to have children or the importance of finetuned touch for a musician can be incorporated into the shared decision-making process [7].

Several treatments may be associated with various complications ranging from minor to serious and occasionally fatal. In addition, the increasing age of survivors is accompanied by the potential development of other disease. Consequently, the two main issues encountered in chronic cancer patients or long-term survivors are multimorbidity and long-term treatment side effects. Regarding the latter, it is possible to identify those that occur during or early after treatment and can last a long time, and those that occur long after (late) the end of treatment and can also be long-lasting. Late side effects can be classified into (a) side effects affecting specific systems (organ alterations, endocrine abnormalities, premature aging, and others); (b) functional changes (incontinence, lymphedema, ostomies, osteoporosis, arthritis, and others); and finally (c) second malignancies [6]. Late effects can be of different types and attributable to the various treatment modalities [12].

Table 29.1 Etiopathogenesis of side effects of medical treatment [12]

Side effects	Drugs ----> etiopathogenesis
Ventricular dysfunction, Heart failure, Pericarditis, Arrhythmias	Anthracyclines, trastuzumab, vinorelbine, fluoropyrimidine ---> myocardial damage sometimes dose-dependent and reversible
Photophobia, Dry eye, Keratitis, Cataract, Lacrimal disorders	Cisplatin, pemetrexed, gemcitabine, tamoxifen, TKI ----> etiopathogenetic mechanism not always known
Disorders of memory and learning, Processing difficulties, Motor disorders, Language disorders	radiotherapy, methotrexate, cytarabine, fluorouracil, cyclophosphamide ---> sclerosis cerebellar, dystrophic calcification, necrosis
Metabolic syndrome	Endocrine and chemotherapy treatments ---> increase in BMI, hyperinsulinemia, abnormal estrogen and testosterone levels
Hyper/hypothyroidismThyrotoxicosis	Use of iodinated contrast agents, radiation therapy, chemotherapy, targeted therapy ---> etiopathogenetic mechanisms not fully known, probable alteration of thyroid vascularity
Dysesthesia, paresthesia, pain, myalgia, ataxia	Taxol, vinorelbine, cisplatin
Osteoporosis	Endocrine treatments and chemotherapy, steroid therapy, alcohol, smoking, and diet low in calcium ---> OPG, RANKL, PTH, ILGF 1 interaction
Pulmonary fibrosis	Radiation therapy and chemotherapy (bleomycin, anthracyclines) sometimes dose-dependent and reversible
Genitourinary disorders	Chemotherapy (platinum and derivatives, methotrexate, nitrosoureas) ---> insult glomerular and tubular, urinary obstruction in pieces with prostate carcinoma
Gastrointestinal disorders (diarrhea)	Radiation therapy, chemotherapy, targeted therapy, liver toxicity ---> insult mucosa sometimes dose-dependent and reversible

Example of etiopathogenesis, prophylaxis, and treatment of late side effects are reported in Tables 29.1 and 29.2.

The goal of the cancer assistance must be to optimize the quality of life of these patients by controlling the side effects and comorbidity. Primary prevention interventions must be promoted by removing, where possible, the causes through the knowledge of the individual risk of each patient about the used drug [12]. The discussion and use of supportive and early palliative care in the treatment can allow advanced patients to spend their days with higher quality of life and to be cared for according to their wishes [13]. Honest continuous communication is key to caring for cancer patients: Being prepared to face life ahead can help patients improve their odds of cure and long-term survivorship, or to cope with a chronic course or death [14].

29.3 Survivorship and Cancer Survivors

The word "cancer," however, due to its persisting ominous metaphoric implications, has toxic connotations in many countries and cultures that discourage patients and their families from viewing cancer as a disease with varied outcomes, including cure [8]. Demystifying the word "cancer" will not be easy. It will require the concerted efforts of physicians, patients, and families to create a new culture in oncology. Yet the time has come for such a shift in the medical and public cancer discourse [2].

While the success is certainly true for advocacy, it is best to avoid any triumphalism regarding the medical and organizational aspects of survivorship care. Too many of our cancer patients still die soon after diagnosis, too many experience and endure great deals of physical and existential pain, and too many of those whom we proudly define as "long-term survivors" suffer from serious sequels of their primary cancer and its treatments, and remain at risk for late relapses and second primaries [9].

Debate about the definitions of "cancer survivor" and "survivorship" has intensified in the past year [1, 2, 4, 5]. According to the National Coalition for Cancer Survivorship (NCCS) in the US, any individual diagnosed with cancer is "a survivor from the time of its discovery and for the balance of life," and goes through different "seasons of survival" in a continuum [9, 11, 15].

Table 29.2 Prophylaxis and treatment of side effects of medical treatment [12]

Side effect	Prophylaxis/treatment
Cardiac toxicity	Rating FE ventricular (echocardiogram) Rating ECGDosage troponinUse of ACE inhibitors as a preventive measure
Ocular toxicity	Use of steroids and anti-inflammatory topical also prophylactic
Cognitive disorders	Rehabilitation, sleep hygiene, drugs
Metabolic syndrome	Exercise and nourishing diet to reduce insulin levels
Thyroid toxicity	Thyroid function indicators ---> replacement therapy
Neurotoxicity	Neurophysiological testsdiagnostic, identifying patients at risk----> neuroprotective
Osteoporosis	Bone mineral density; avoid smoking and alcohol; prefer a diet rich in calcium, any replacement treatment
Pulmonary fibrosis	Pulmonary rehabilitation in case of reversibility
Genitourinary abnormalities	
Gastrointestinal disorders	Antidiarrheal, intestinal disinfection

In most European and other countries, however, cancer survivors are defined as patients who have lived beyond 3–5 years from diagnosis or end of treatment with no evidence of disease [10, 16, 17].

The suffering of persons diagnosed with cancer and their experience of drastic changes in their lives, in fact, can be said to characterize them as resilient "survivors" from the day of diagnosis no matter how long they live [15]. However, in those contexts where the term "survivor" does not carry positive connotations related to "resilience," people living after a cancer diagnosis perceive it to be a negative or pessimistic label that ties them to a traumatic life event, whereas they regard the experience of cancer as contributing to their life history and identity, without defining or classifying them.

In a qualitative study, 40 persons at least 5 years post-diagnosis of breast, colorectal, or prostate cancer in the UK were asked if they considered themselves to be cancer survivors. The majority did not endorse the term: To them, "survivor" implied a high risk of death, made them feel bound to an identity that did not describe them accurately, and either suggested that a good outcome was dependent on personal characteristics or called for an advocacy role they did not wish to assume. Those who accepted it understood "survivorship" as a factual definition of having had cancer and survived, or interpreted "survivor" as implying self-empowerment or cure, despite the possibility of recurrence [17]. Some patients perceive the term as excessively heroic, as overemphasizing positive over negative feelings about cancer of equal authenticity, as not representative of those who continue to struggle with cancer, or as disrespectful to those who succumb to cancer [18]. "Living with and beyond cancer" may best describe persons who live long with cancer in a chronic form [19, 20]. A US medical oncologist, based on his personal experience, suggested "thrivorship" for those who were cancer patients as a way to "open oneself to fellowship with all in the grip of life's fragility" [1].

The NCCS definition of survivorship, now extended to "anyone touched by cancer" to reflect the profound long-lasting repercussions of cancer on patients and their families, has been useful at social and policy-making levels to promote attention to the needs of cancer patients and to request increased funding for research and standard care [7, 11]. A recent analysis of the origin, meaning and nuances of the words "survivor" and "survivorship" concluded, however, that the definition of "person who has had cancer" may be preferable, as it refers to all cancer patients and implicitly acknowledges their heterogeneity [2, 21].

29.4 Contemporary Reality of Cancer

Due to the differences in identifying and defining cancer survivors in different countries [5, 16], we must find a common language and plan appropriate models of care, rehabilitation, and communication [6, 11]. A radical shift in cancer discourse requires that oncology professionals and institutions start focusing on the contemporary reality of cancer in light of our understanding of its nature and the current treatments and outcomes, as well as the specific characteristics of each patient's cancer. This would satisfy (a) the need to align cancer patients' perceptions and expectations to those of patients with other serious illnesses; (b) the biomedical perspective of a chronic illness; (c) the patient's subjective psychosocial experiences at all stages; (d) when cured, the need for and value of participatory adaptation of each patient to tailored health-promoting programs; and (e) the role and responsibility of medical staff, nursing, and caregivers over time [9–11, 20].

29.5 Cured

The risk for death from a specific cancer is highest in the initial years after diagnosis and decreases progressively thereafter until a time at which the risk becomes negligible and surviving patients reach a life expectancy that matches that of a sex- and age-matched general population [22, 23].

Favorable long-term survival has been reached in colorectal [24, 25] and invasive cervical cancer [26], with large studies consistently showing that, in comparison with a general population, lack of excess mortality is reached in approximately 8 years. A 5-year survival is now possible for more than 95% for thyroid and testicular cancers among adult Italian cancer patients. For patients who experienced those tumor types during 2000–2004, 10-year survival reached approximately 90% [27], suggesting very good prognosis and a long-term life expectancy similar to that of the sex- and age-matched general population. In addition, the outlook for patients with differentiated thyroid cancer is very optimistic: At 30 postoperative years, the cause-specific mortality rate is only 1%, and the rate for tumor recurrence at any site is less than 15% [28]. On the other hand, even if recurrences of germ-cell tumors of the testis are rare, most relapses in patients with germ-cell tumors occur within the first 2 years of treatment [29–31], and no excess mortality has emerged in population-based studies [32].

Increasing survival is also expected for other cancer types as a result of personalized treatments based on a better understanding of the biology and potential response to therapies of each individual cancer.

Conditional relative survival—the probability of a patient surviving an additional 5 or 10 years after already surviving a given number of years—is a clinically relevant measure of long-term excess mortality in a cohort of cancer patients [24].

Use of the term "cured" for some cancer patients is being proposed in view of the increasing survival rates in some cancers [33]. As reported in the paper statement, "the word *cured* refers to complete clinical remission of a cancer, regardless of the presence or absence of late sequelae of treatments. To correctly apply the word 'cured,' the time from the cancer diagnosis must be such that the patient's risk of death does not, because of cancer, exceed that of a sex- and age-matched general population. In other words, a cancer patient can be defined as 'cured' only when his or her life expectancy is the same as that of a sex- and age-matched general population. The word 'cured' cannot be used for all cancer types, because cancer is a highly heterogeneous group of diseases with variable biologic features, clinical expressions, natural histories, responses to treatment, and outcomes."

29.6 Surveillance

Oncologists worldwide differ substantially on these issues: Some argue that long-term follow-up of cancer patients is unnecessary and yields to a consistent waste of resources, at the risk of missing some recurrences or second cancers, while others favor frequent and long-term monitoring in the hope to increase the survival odds of their patients, at the risk of repercussions on their patients' emotional wellbeing.

Usually, surveillance after cancer include detection local or distant recurrence at time in which treatment can result more effective or even curative and detecting metachronous cancer. This surveillance is defined "*cancer oriented*."

More recently, due to efficacy of treatment or early diagnosis, detecting long-term or late effects of treatment, including iatrogenic second malignancies, psychosocial sequelae, routine health maintenance, screening, counseling about modifiable risk factors, and detection of comorbidity. This surveillance could be defined "*all inclusive*."

Anyway, two conditions are necessary to achieve the objectives of surveillance: The first is to establish the coordinator of follow-up, who can be the general practitioner or the oncologist. The second, the patients categorization [34].

The benefits of categorization and a tailored surveillance appear to derive from the fact that survivors belonging to different categories cannot be treated and followed alike: High-risk patients/survivors require more frequent and intensive follow-up of lower-risk patients/survivors, while always trying to avoid as much as possible the "medicalization" of people's lives [35].

Hence, follow-up guidelines should be tailored to each patient's survival category and personal clinical history including family history and genetic mutations, environmental exposures and other risk factors, familiar, sociocultural context, and resources (▶ Box 29.1) [34].

Box 29.1 Cancer survivorship [34]

Cancer survivorship and rehabilitation

Policy recommendations for quality improvement in cancer survivorship and rehabilitation for EU Member States.

Medical follow-up: focus on late effects and tertiary prevention

1. *An early and personalized follow-up program should be systematically planned and delivered to each survivor:*
   (a) Adequately assessing the survivors' individual risk of multidimensional late effects of treatment and respective rehabilitation needs (e.g., physical, psychological, social, cognitive, sexual, nutrition)
   (b) Creating opportunities for socially disadvantaged people to fully engage in follow-up programs
2. *Adequate and updated information on medium and long-term effects of treatments should be available to:*
   (a) Survivors and their relatives
   (b) Care providers involved in the follow-up, in particular primary care professionals, for better prevention and care
3. *Identification and management of late effects of cancer treatment should be integrated in the professional training and continuous medical education of clinicians (including GPs).*
4. *In tertiary prevention, self-management should be emphasized, particularly on lifestyle recommendations and on the risks of long-term effects:*
   (a) Smoking cessation
   (b) Weight control and healthy diet including limited alcohol consumption
   (c) Sufficient sustained physical activity
   (d) Avoidance of excessive exposure to ultraviolet radiation
   (e) Stress management
5. *Physical activity should be integrated early in the care pathway for all cancer survivors.*
   It should be an important component to consider at every phase of survivorship care for all survivors in order to maintain healthy lifestyle.
6. *Evaluation of physical and psychosocial rehabilitation needs should first be screened as follows:*
   (a) Baseline screening should be performed prior to the start of any cancer-specific treatment.
   (b) Both physical and psychosocial screening should be carried out simultaneously by using simple algorithms; for physical screening, at least the following items should be screened: cardiac function, muscle strength and flexibility; for psychosocial screening, see item 8 below.
   (c) After the first screening, regular updates should be performed on individual basis.
   (d) *Needs for a person-centered approach in psychosocial rehabilitation, supportive and palliative care*
7. *Periodic screening of psychological distress and psychosocial needs should be conducted:*
   (a) During the entire cancer pathway by the health care professionals (e.g. oncologists, physicians, and nurses) and integrated in routine cancer care
   (b) Sreening should be followed by adequate provision of psychosocial care.
8. *For the diagnosis of psychological conditions, a specific assessment should be carried out by a psychological care professional:*
   (a) Using validated and simple tools and according to clinical practice guidelines for the assessment and management of psychological distress and morbidity
   (b) Anticipating the specific needs of populations at high risk, including young populations (e.g., children, adolescents, young adults) and relatives.
9. *A step-wise or tiered model of psychological care is recommended depending on the level of distress, psychological condition, and morbidity of each patient, with interventions ranging from:*
   (a) Information and psycho-education by primary oncology team to peer support
   (b) e-health platforms for psychosocial support and self-management programs
   (c) Psychological interventions by professionals trained in psycho-oncology (e.g., psychologists, social workers, psychiatrists)
   (d) Complementary spiritual support by chaplains and others
   (e) Psychotropic treatments by trained physicians (e.g., psychiatrists, oncologists).
10. *Psychosocial interventions in individual or group format should be delivered by appropriately trained professionals with specific expertise in psychosocial oncology.*
11. Increased investment in training in psycho-oncology and communication skills for primary oncology staff is highly recommended.
12. Existing clinical practice guidelines for psychosocial support of patients with cancer could be highly valuable and recommended for the provision of evidence based psychosocial care.
13. *Social and return-to-work issues should be integrated early into the cancer care pathway.*

Adaptation of working conditions for any patient returning to his/her previous work should be assessed at early stages.

Survivorship and long-term care could be provided in different clinical settings or structures according to specific categories, reducing patients' exposure to the psychological trauma that may occur, for example, when cured or long-term survivors are followed in the same clinical setting where they had received acute care. Additionally, proper categorization of survivors, as well as differentiation of dedicated facilities and modalities of survivorship care delivery, may facilitate individual patients' adherence to clinicians' proposed surveillance and follow-up, including measures to foster good general health.

Given the variety of health professionals that could play a role in survivor care, it will often be a shared responsibility, and there should be a designated individual who coordinates the care [36].

Due to the complexity of clinical cases that may arise, the surveillance program should aims not only to (a) intercept any early recurrence or metachronous cancer but also (b) evaluate in time any side effects and, in addition, (c) includes measures of health promotion and change in lifestyle.

The measures have to be implemented, however, varied depending on the characteristics of patients diagnosed with the disease and taking into account that, to date, there are now available to address specific guidelines for the follow up of these patients (► Box 29.2).

Box 29.2 Quality of assistance guidelines

Guidelines
Identifying recurrent disease
Identifying acute side effects and late iatrogenic
Identification psychosocial distress
Adherence to follow-up
Screening programs (diagnosis of second cancers)

29.7 Patients' Categorization

As always in science and medicine, what will the next decade achieve depends on the priorities that together as both survivorship care professional and cancer survivors, we clearly set for ourselves. We believe that one such priority is categorization of survivors [36]. Proper categorization will not only allow better tailored treatment, but will also address issues such as whether or not we can define an individual patient "cured" of his or her primary cancer, according to strict scientific criteria [33].

A new, more clear-cut, categorization of patients now broadly defined as "cancer survivors" may enable us to develop new clinical and organizational approaches in regard to decision-making and communication with patients, as well as to a paradigm shift in the culture of clinical oncology. Thus far, survivorship care has focused on distant medical sequels of cancer treatment, including relapses and second cancers, as well as on psychosocial repercussions of a cancer diagnosis for long-term survivors. The latter, ranging from changes in self and body image, to family dynamics and social relationships, financial issues, or more or less overt forms of discrimination, have received most attention in recent literature [37]. While the concept of "survivor," across different cultural and health-care contexts, entails both physical and psychosocial needs and concerns of patients and families that must be equally addressed by the professionals who care for them, oncologists often are reluctant to categorize their patients and survivors based on their past history of cancer and current disease status [36]. This lack of distinction among different survivors could also negatively affect communication and follow-up recommendations with and for patients (■ Table 29.3) [37].

■ Table 29.3 Category of Cancer patients [36]

	Description
Acute	Patients/survivors at first diagnosis or relapse who require acute intervention
Chronic	Chronic patients/survivors with cancer that slowly progressed or alternated between phases of remission and relapse, often accompanied by acceptable quality of life
Long-term	Patients/survivors in clinical remission for long periods of time or for their entire life who remain at risk for distant relapse or second tumors and who potentially can experience late treatment-related medical and psychosocial *sequelae*
Cured	Disease-free patients/survivors whose cancer-specific mortality and life expectancy many years after diagnosis equal that of sex- and age-matched members of the general population

29.8 Conclusions

Cancer and survivorship care should both move to personalized precision approaches, as it is done in other chronic diseases.

The categorization of cancer patients represents an opportunity to achieve this goal.

It requires a paradigm shift in the culture of cancer survivorship care, whereby we abandon a common, general, approach to all cancer survivors in favor of the

application of our epidemiologic knowledge and the developing risk assessment tools to tailor follow-up and recommendations to each individual cancer survivor.

Personalizing follow-up will improve the medical and psychosocial care for each individual cancer patients and will help reduce the stigma of disease that still persists in many cultures [38].

Key Points

- The number of cancer survivors has increased due to improved screening programs and therapeutic advances.
- New drugs convert metastatic disease into chronic.
- In the future a correct categorization will allow a more personalized treatment and will also address issues such as the possibility or not of defining a single patient as "cured" of his primary cancer, according to rigorous scientific criteria.

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Clinical Oncology: Diagnosis and Treatment of Solid Tumors

Contents

Locoregional and Locally Advanced Breast Cancer

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Learning Objectives

By the end of this chapter, the reader will:

- Have learned the basic concepts of breast cancer epidemiology
- Understand relevant prognostic and predictive factors in breast cancer
- Be aware of clinical presentation and diagnostic strategies in breast cancer
- Have learned the basic principles of breast cancer management and treatment

30.1 Epidemiology

Breast cancer (BC) is a major public health problem throughout the world. It represents the second most frequently diagnosed cancer worldwide and the most frequently diagnosed cancer among women, accounting for nearly 1.7 million cancer cases diagnosed worldwide per year. It is also the second leading cause of cancer deaths in women worldwide [1].

However, there is a significant heterogeneity in incidence rates between high incidence areas (developed Western countries, such as the United States and Western Europe) and low incidence areas (such as Africa and Asia).

In the United States, the incidence of BC has shown a consistent increase up to the first years of the twenty-first century, probably due to the increasing implementation of mammography screening programs and to the extensive use of hormonal replacement therapy in menopausal women. On the contrary, BC mortality has consistently decreased in several Western countries during the last decades, thanks to the extensive use of screening and advances in adjuvant systemic treatments [2]. Nevertheless, BC is still the leading cause of cancer-related deaths in European women. Moreover, incidence in low-income nations is increasing [3]. BC in males is rare, contributing to approximately 1% of cases.

30.2 Risk Factors for Breast Cancer

Multiple factors are associated with an increased risk of developing BC, such as age, gender, family history, genetic alterations, diet, and life style. However, most of these factors carry a small/moderate increase in risk for any individual woman. In fact, BC pathogenesis is often linked to a complex interaction among multiple factors, and it has been estimated that approximately half of BC cases do not have any identifiable risk factor beyond increasing age and female sex. Age is indeed the most relevant risk factor for BC in women with the incidence

Table 30.1 WHO classification of breast tumors. (Adapted from Lakhani et al. 2012)

Epithelial tumors
Microinvasive carcinoma
Invasive breast carcinoma
Invasive carcinoma of no special type (NST)
Invasive lobular carcinoma
Tubular carcinoma
Cribiform carcinoma
Mucinous carcinoma
Carcinoma with medullary features
Carcinoma with apocrine differentiation
Carcinoma with signet ring differentiation
Invasive micropapillary carcinoma
Metaplastic carcinoma
Rare types
Carcinoma with neuroendocrine features
Secretory carcinoma
Invasive papillary carcinoma
Acinic cell carcinoma
Mucoepidermoid carcinoma
Polymorphous carcinoma
Oncocytic carcinoma
Lipid rich carcinoma
Glycogen rich clear cell carcinoma
Sebaceous carcinoma
Salivary gland/skin adnexal type tumors
Precursor lesions
Ductal carcinoma in situ
Lobular neoplasia
Lobular carcinoma in situ
Classic lobular carcinoma in situ
Pleomorphic lobular carcinoma in situ
Atypical lobular hyperplasia
Intraductal proliferative lesions
Epithelial-myoepithelial tumors
Papillary lesions
Mesenchymal tumors
Fibroepithelial tumors
Fibroadenoma

(continued)

Table 30.1 (continued)

Phyllodes tumor
Hamartoma
Tumors of the nipple
Nipple adenoma
Syringomatous adenoma
Paget disease of the nipple
Malignant Lymphoma
Metastatic tumors
Tumors of the male breast
Gynaecomastia
Invasive carcinoma
In situ carcinoma
Clinical patterns
Inflammatory carcinoma
Bilateral breast carcinoma

of BC doubling on average every 10 years until menopause [4].

For men, major risk factors include hormonal imbalances (especially gynecomastia and cirrhosis), radiation exposure, and positive family history and genetic predisposition.

30.2.1 Hormonal and Reproductive Factors

The increase in the incidence of BC with age is at least partly explained by the role played by sex hormones in the etiology of the human BC. In fact, several evidences point out the pro-tumor role played by exposure to hormones in human BC.

BC incidence increases steeply with age until menopause and then plateaus, highlighting the role of ovarian function. Moreover, estrogen deprivation via iatrogenic premature menopause can reduce BC risk: premenopausal women undergoing oophorectomy without subsequent hormone replacement therapy have reduced risk of BC later in life, with magnitude of risk reduction increasing as the age at oophorectomy decreases [5]. The total duration of ovarian function (and thus of exposure to endogenous estrogens) seems relevant. There appears to be a relative 20% increase in BC risk for women with early menarche (<11 years of age) as compared to women with first menarche at 13 years of age [6].

The relationship with pregnancy is more complicated. Nulliparous women are at greater risk of BC than parous women (relative risk ≈ 1.4) and women whose first pregnancy occurs after age 30 have a higher BC risk as compared with women who had a pregnancy before that age [5–7]. However, BC risk increases transiently for the 10 years after a pregnancy and then declines. This appears to be related to the differentiating effect exerted by hormones on the mammary gland during pregnancy [7]. Abortion, either spontaneous or induced, does not increase the risk of BC [8].

Breastfeeding has a protective effect in lowering the risk of BC, particularly for longer durations. This effect might be linked to the inhibition of ovarian function and to structural modifications of the glandular tissue during breastfeeding.

Reproductive history and breastfeeding may at least partly part account for differences in BC risk between developed and developing nations.

Exposure to exogenous hormones also contributes to BC risk. In the Women's Health Initiative, use of combined estrogen and progestin hormone replacement therapy increased the risk of BC diagnosis (relative risk ≈ 1.5), while use of estrogen only formulations did not [9, 10]. The incidence was noted after 2 years of hormone replacement therapy and decreased after 5 years from therapy termination.

30.2.2 Dietary and Lifestyle Factors

Several studies have tried to investigate the impact of diet on BC risk. However, the results of cohort studies and meta-analyses are not always consistent, probably due to the intrinsic difficulties in accurately assessing the composition of diet over long periods of time and the effect of single components on cancer risk. The most consistent results point out that alcohol consumption is a risk factor for BC and that BC risk increases linearly with the amount of alcohol consumed (10% relative risk increase for every 10 g/day of alcohol) [11]. Risk might be enhanced by decreased intake of vitamin C, folate, and β-carotene.

In addition, existing evidence, even if not totally consistent, suggests that a diet rich in fruit, vegetables, whole grain cereals, and fiber and low in fats and refined carbohydrates might reduce BC risk.

Even if data regarding the accordant of diet composition of BC risk is not completely consistent, consistent evidence is available associating obesity with both increased risk of BC development (in postmenopausal women) and increased BC mortality. In fact, during early adult life, obesity is associated with a lower incidence of BC before menopause, but no reduction in

breast mortality. After menopause, overweight women have a 1.5-fold greater risk of developing BC than normal weight women, which increases to twofold in case of obesity [12]. In particular, weight gain after age 18 is associated with a substantial increase in postmenopausal BC [13].

On the contrary, physical exercise has been pointed out as a protective factor, and several studies have estimated that a regular physical activity reduces the relative risk of developing BC by 10–20%.

Smoking is also associated with an increase in BC risk, in addition to an increased risk of other cancers.

30.2.3 Environmental Factors

Among environmental factor, exposure to ionizing radiation is the best-known risk factor for BC risk. This has been observed in atomic bombing survivors and in patients receiving diagnostic or therapeutic irradiation. A marked increase in BC risk is observed in women who received mantle irradiation for the treatment of Hodgkin lymphoma before 15 years of age [14].

30.2.4 Family History and Inherited Predisposition to Breast Cancer

A family history of BC is a well-recognized risk factor: overall, the risk of developing BC of a woman with a first-degree relative with BC is increased 1.5–3-fold as compared to a woman with negative family history. However, the risk carried by family history depends on number of relatives affected, the exact relationship, age at diagnosis, and the number of unaffected relatives.

Only 5–10% of women diagnosed with BC have a clearly identifiable hereditary predisposition.

The most frequent and best-known genetic alterations are germline mutations in the BC susceptibility genes *BRCA1* and *BRCA2.* These mutations are inherited in an autosomal dominant manner with varying penetrance. These genes encode proteins involved in homologous recombination repair and contribute to maintain the genomic integrity of the cell. Pathogenic mutations in these genes frequently result in a loss of the functional protein produced by that allele. When the second allele of the gene is altered by a somatic event (double hit), there is a complete absence of functional protein in the cell, which leads to genomic instability. BRCA1 and BRCA2 germline mutations are relatively rare, being present in less than 1% of the general population (2% in individuals of Ashkenazi Jewish ancestry).

BRCA pathogenic mutation carriers have an estimated lifetime risk of BC ranging between 26% and 85%. *BRCA1* mutations are associated with a higher incidence of triple-negative BCs, while the phenotype of *BRCA2* associated cancers does not significantly differ from that seen in sporadic tumors [15, 16].

BRCA mutations also carry a significant lifetime risk of ovarian cancer, which ranges from 16% to 63% and 10% to 27%, respectively, for *BRCA1* and *BRCA2* [17]. *BRCA1* or *BRCA2* mutations have also been associated with male BC, fallopian tube cancer, pancreatic cancer, and prostate cancer.

However, not every BRCA1 or BRCA2 mutation is pathogenic and different mutations have been shown to carry different risk of developing cancer.

The presence of a *BRCA1* or *BRCA2* mutation may be suggested by family history, personal history of cancer, and cancer phenotype. Models are available to estimate the likelihood of mutation and evaluate referral to a genetic counselor. The implications of genetic testing are considerable for both patients and their family members and should be discussed prior to undertaking genetic testing.

If a *BRCA1/2* mutation carrier is identified, management strategies available for risk reduction include intensive surveillance, chemoprevention with selective estrogen receptor modulators (SERMs), and risk-reducing surgery (breast and salpingo-ovarian). Prospective studies demonstrated an 80–100% reduction in BC mortality in *BRCA* mutation carriers undergoing prophylactic mastectomy [18, 19]. Prophylactic bilateral salpingo-oophorectomy has the added benefit of reducing the risk of ovarian carcinoma (HR = 0.21), for which effective screening is not available, and concomitantly reduces BC risk (HR = 0.49) [20].

In families where a *BRCA* mutation is known to be present, women who test negative for the mutation are not at increased risk for BC development and do not require special surveillance.

Other genetic mutations are associated with increased BC risk: germinal mutations of *TP53* (Li-Fraumeni syndrome), *PTEN* (Cowden syndrome), and of both alleles of *ATM* (ataxia-telangiectasia syndrome) each account for <1% of cases. Mutations in CDH1 are associated with a predisposition to diffuse gastric cancer and lobular BC. Moreover, mutations in low-penetrance genes account for a significant number of non-*BRCA1* or -*BRCA2* BCs: *PALB2*, *CHEK2,* and *ATM* in heterozygosity are among these [21, 22].

Even in the absence of a known inherited predisposition, women with a family history of BC face some level of increased risk, likely from some combination of shared environmental exposures, unexplained genetic factors, or both.

30.2.5 Personal History of Breast Cancer and Benign Breast Disease

Women with a previous diagnosis of breast carcinoma have an increased risk (two- to sixfold relative risk increase) of developing a contralateral BC; however, the absolute annual risk usually remains below 1%.

Benign breast lesions are classified as proliferative or non-proliferative. Non-proliferative lesions do not increase BC risk, while proliferative lesions are a risk factor for BC. Proliferative disease without atypia usually results in a small increase in risk (relative risk ≈ 1.5–2.0), while proliferative disease with atypical hyperplasia carries a greater risk of cancer development (relative risk ≈ 4.0–5.0) [23]. A previous diagnosis of breast carcinoma in situ carries an even greater risk of developing BC (relative risk ≈ 10).

30.2.6 Mammary Density

Mammographic breast density is an index of the ratio between glandular-stromal tissue and adipose tissue in the breast. High breast density not only makes detection of BC more difficult but is also an important predictor of BC risk. Women with >75% breast density have 4.7-fold increase in BC risk than those with <10% breast density, even after adjustment for other risk factors [24].

Breast density is mainly genetically determined, although it has been shown to vary due to postmenopausal hormone replacement therapy.

30.3 Breast Cancer Prevention

30.3.1 Breast Cancer Screening for the General Population

If diagnosed based on clinical signs and symptoms, most BCs present as large nodules or with axillary node involvement. Mammography can detect smaller, clinically asymptomatic tumors or noninvasive lesions.

As disease extension is one of the principle factors determining BC prognosis, this has led to the implementation of mammographic screening programs aiming to early detection and treatment of BC, thus reducing its mortality.

Estimates of the effect of mammography screening on BC mortality vary from trial to trial: a UK review of randomized, controlled mammography trials estimated a 20% relative BC mortality reduction in women aged between 50 and 70 years old [25], while more modern case-control and cohort studies conducted in Europe and Canada have reported up to a 40% relative reduction in BC mortality for women more than 50 years of age. However, screening programs carry the risk of false-positive results, with consequent over-diagnosis and overtreatment, and risk of false-negative results, that might instill a false feeling of security among patients and doctors and delay diagnosis.

In women aged between 50 and 69 years benefits appear to outweigh risks and mammography screening, repeated every 2 years, is recommended by most countries [26].

For the age group 40–49 years, evidence of effectiveness of mammography screening is limited. Meta-analyses stratified by age have shown that reduction in BC mortality is smaller (15%) in women <50 years of age and the risk of false-positives is higher, due to lower incidence of BC in this population. Therefore, no consensus exists regarding extension of population screening to women aged between 45 and 49 years and in the age group 70–74 [9]. Moreover, no consensus exists regarding the use of ultrasound for BC screening, due to the possible increase in false-positive results [27].

Regular auto palpation of the breasts is also recommended by several countries.

30.3.2 Management of High-Risk Patients

There is no formal and unambiguous definition of which patients should be considered at *high risk* for BC. Without question, BRCA mutation carriers and women with a similar risk based on family history are considered at high risk. Other high-risk groups can include women who received mantle irradiation and those diagnosed with lobular carcinoma in situ (LCIS) or atypical hyperplasia.

The Gail model [28], which calculates a woman's risk of developing BC based on age at menarche, age at first live birth, number of previous breast biopsies, presence or absence of atypical hyperplasia, and number of first-degree female relatives with BC, has been used in several cancer prevention trials to define high-risk population. However, no clear consensus exists regarding which model should be used.

Management strategies for high-risk women include intensive surveillance, chemoprevention with endocrine agents, and, for extremely selected patients, prophylactic surgery.

30.3.2.1 BC Screening for High-Risk Patients

Annual MRI concomitantly or alternating (every 6 months) with mammography, starting 10 years younger than the youngest case in the family, is recommended for patients at high risk of BC (proven BRCA mutations or similar risk due to genetic factors or history of prior thoracic irradiation) [27] as it can detect BC at a more favorable stage than mammography screening alone. However, MRI screening is not recommended for women with atypical hyperplasia as no benefit was observed [27, 29].

30.3.2.2 Chemoprevention

Chemoprevention is an option, in addition to surveillance strategies, for patients at high risk of BC. Endocrine treatments, such as SERMs (selective estrogen receptor modulators) and aromatase inhibitors (AIs) have been evaluated in clinical trials to prevent BC occurrence.

Tamoxifen reduces the incidence of HR+ BC (a 48% reduction in a meta-analysis of 4 randomized trials), while no effect is seen in HR− cancers [30, 31]. In the largest of these studies, the NSABP P1 trial, a 49% risk reduction was seen with tamoxifen, with a reduction in both invasive and noninvasive carcinoma, and for women of all ages. A particular benefit was seen in women with atypical hyperplasia, with an 84% reduction in cancer incidence in this group. However, treatment with tamoxifen carries some well-known side effects: in these trials, tamoxifen users had an increase in thromboembolic events (RR 1.9) and endometrial cancer (RR 2.4). Therefore, despite the proven efficacy, use of tamoxifen as chemoprevention has been limited by concerns about side effects and the small absolute differences in outcomes.

Raloxifene, another SERM used for the treatment and prevention of osteoporosis, was compared to tamoxifen as chemopreventive agent in the NSABP-P2 STAR trial [32]. No difference in the incidence of invasive cancer was observed between tamoxifen and raloxifene, while more cases of noninvasive cancer were noted in the raloxifene group. Raloxifene has a more favorable side-effect profile, with less hysterectomies and endometrial cancers and significantly fewer thromboembolic events and cataracts. However, history of deep vein thrombosis, stroke, pulmonary embolism, or transient ischemic attacks is considered a contraindication to the use of both tamoxifen and raloxifene.

AIs have also been tested for BC prevention, with a reduction in invasive BC, limited to HR+ cancers. However, the side-effect profile of these drugs (arthralgia, osteoporosis) represents a limit for their use in a preventive setting [33].

30.4 Pathological Classification of Breast Cancer

Historically, classification of invasive BCs has been based on its morphologic appearance in light microscopy. The WHO (World Health Organization) classification system (Table 30.1), based on the growth pattern and cytologic features of the invasive tumor cells, recognizes invasive "ductal" and "lobular" carcinoma [34]; however, this does not imply that the former originates in the ducts and the latter in the lobules of the breast. In fact, regardless of histologic type, most invasive BCs arise from epithelial cells of the terminal duct lobular unit.

30.4.1 Invasive Ductal Carcinoma

The most common histologic type of BC is invasive ductal carcinoma, representing 65–80% of BC cases. Most classification systems use the terms *infiltrating ductal carcinoma, not otherwise specified* (NOS) or *infiltrating carcinoma of no special type* interchangeably, to emphasize that diagnosis of invasive ductal carcinoma is made by exclusion (when tumors do not present characteristics classifying them into other special categories of invasive mammary carcinoma) [34].

30.4.2 Invasive Lobular Carcinoma

Invasive lobular carcinoma is the second most common histologic type, comprising 10–15% of BC cases. It is often multifocal or multicentric, and not rarely bilateral. It is characterized by neoplastic epithelial glandular cells, which infiltrate the surrounding stroma by circling the mammary ducts. This often leads to an underestimation of tumor size by radiological techniques. It characteristically lacks expression of E-cadherin (an epithelial cell membrane molecule involved in cell-cell adhesion), a feature that distinguishes lobular from ductal disease, both in situ and invasive.

30.4.3 Special Types of Breast Carcinoma

Rarer special types comprise approximately 10% of invasive BCs and often carry a distinct prognosis as compared to ductal invasive carcinoma. BCs with pure tubular, mucinous, papillary, or cribriform features are recognized to have a more favorable outcome than ductal BC, while micropapillary tumors have a high inci-

dence of systemic recurrence [35]. Other cancers, not considered to be typical BCs, can occur in the breast, such as cystosarcoma phyllodes, angiosarcoma, and primary lymphoma.

30.4.4 Carcinoma In Situ

Carcinoma in situ is defined as the proliferation of malignant-appearing mammary epithelial cells that remain confined within the basement membrane without evidence of invasion in the stroma.

Ductal carcinoma in situ (DCIS) comprises 80–85% of in situ carcinomas and involves ductal epithelial cells. The number of ductal carcinomas in situ diagnosed has dramatically increased with the diffusion of screening mammography (15–30% of cancers detected in mammography screening programs are DCIS, especially in women aged 49–69 years), raising the problem of its management. In fact, concordance between risk factors and shared genetic alterations suggests that DCIS and invasive carcinoma might be part of the same pathologic process, and DCIS is generally considered a precursor of invasive BC. This has led to an aggressive treatment of all DCIS (see ▶ Sect. 30.11).

Lobular neoplasia is instead defined from a morphological point of view as "a proliferation of generally small and often loosely cohesive cells originating in the terminal duct lobular unit, with or without pagetoid involvement of terminal ducts" [34], a definition generally used to cover both LCIS and ALH.

Patients with LCIS treated by biopsy alone have a substantially increased risk of BC compared with women without LCIS (30–40% lifetime risk) and, although the risk for development of BC is bilateral, subsequent ipsilateral carcinoma is more likely than contralateral. This supports the view that ALH and LCIS act both as precursor lesions and as risk indicators. However, the relative risk for subsequent BC is lower in women diagnosed with ALH than in those with LCIS.

The management of lobular neoplasia must address the bilateral risk, and options include surveillance, chemoprevention, and prophylactic bilateral mastectomy. Surveillance is most commonly used, and mammography is the standard imaging technique for these patients. Prophylactic mastectomy reduces BC risk among high-risk women by approximately 90%, but there is no data indicating that the incidence of subsequent cancer is reduced by other surgical approaches, such as excision to negative margins or subsequent irradiation.

30.5 Prognostic and Predictive Pathological Factors in Breast Cancer

30.5.1 Grading

The most commonly evaluated microscopic feature is grading, which describes the grade of differentiation of a BC. Grading can be based solely on nuclear characteristics (nuclear grading) or, more commonly, on a combination of architectural and nuclear characteristics (histologic grading). In the Elston-Ellis modification of the original Scarff-Bloom-Richardson grading system, tubule formation, nuclear pleomorphism, and mitotic activity are each scored on a scale of 1–3 [36] and added. Tumors with scores of 3–5 are designated as grade 1 (well differentiated), those with sums of 6–7 as grade 2 (moderately differentiated), and those with sums of 8–9 as grade 3 (poorly differentiated). Histologic grading has prognostic significance. However, the clinical use of grade to coadjuvate clinical decision has not been implemented worldwide due to persistent challenges in standardization and inter-operator discrepancies.

30.5.2 Hormone Receptors (HR)

Estrogen (ER) and progesterone receptor (PgR) expression is evaluated using immunohistochemistry and the percentage of positive BC cells is reported (a cutoff of 1% or more positive tumor cells is generally used to define a tumor as positive). Around 80% of BCs are classified as hormone-receptor-positive.

Estrogen and progesterone receptor expression are extremely useful prognostic and predictive factors (e.g., only patients with hormone receptor-positive BC benefit from hormonal treatment) [37].

30.5.3 HER2

HER2 (human epidermal growth factor receptor 2), a transmembrane receptor of the epidermal growth factor receptor (EGFR) family with tyrosine kinase activity, is overexpressed in 15–20% of BCs. It activates pro-survival intracellular signaling pathways and is associated with clinically aggressive disease and a propensity for visceral relapses. Overexpression is generally linked to *HER2/neu* gene amplification.

In clinical practice, *HER2* status is evaluated by either immunohistochemistry or in situ hybridization. Immunohistochemical scores of 0 or 1+ are considered negative, while tumors with HER2 expression 3+ are

considered positive. Cases classified as 2+ by immunohistochemistry are evaluated using in situ hybridization: if *HER2* gene amplification is identified, the tumor is considered HER2-positive and can be treated with anti-*HER2* therapies. *HER2* status is the major predictor for benefit from *HER2*-targeted therapies [38].

30.5.4 Proliferation

Proliferation of tumor cells is usually evaluated by immunohistochemistry. Antibodies directed toward cell cycle proteins, such as Ki-67 or MIB-1, are used, thus measuring the percentage of cells in the G1 phase. Proliferation is a significant adverse prognostic factor in BC. However, due to persistent inter-operator discrepancies, this evaluation has not been implemented in clinical practice worldwide.

30.6 Molecular Classification of Breast Cancer

BC is a biologically heterogeneous disease, and it has long been appreciated that tumors with different biologic features have different clinical outcomes and responses to therapy. Clinically, BC is divided into three subgroups based on immunochemistry and in situ hybridization: HR-positive, HER2-positive, and triple-negative BC. Each of these subtypes presents specific clinical characteristics and therapeutic possibilities.

During the last 15 years, advances in molecular biology and gene expression profiling have led to the classification of BC into four intrinsic molecular subtypes (Luminal A, Luminal B, HER-2 enriched, Basal-like) and a normal breast-like group, which have been extensively characterized [39, 40].

These entities have shown significant differences in terms of incidence, risk factors, prognosis, and treatment sensitivity, giving additional information from that provided by evaluation of ER, PgR, and HER2. Moreover, intrinsic molecular subtypes only partially overlap with tumor phenotype as defined using ER, PgR, and HER2, with a discordance rate of around 30% [41].

30.6.1 Luminal A

Luminal A is the most common subtype and represents 50–60% of all BCs. It is characterized by expression of ER-activated genes, which are typically expressed in the luminal epithelium lining of mammary ducts [42]. As compared to Luminal B tumors, Luminal A tumors have lower expression of proliferation/cell cycle-related genes (e.g., MKI67 and AURKA) and higher expression of luminal-related proteins such as PgR [41]. Immunohistochemical profile is usually characterized by the expression of ER, PgR, and absence of HER2 expression. Luminal A tumors are also frequently characterized by low proliferation rate as measured by Ki67, a low degree of nuclear polymorphism and a low histological grade.

Patients with luminal A BCs have a good prognosis, the relapse rate of this subtype being significantly lower than in the other subtypes [39]. Recurrence is common in bone, whereas liver, lung, and central nervous system metastases occur less frequently than in other subtypes [42].

30.6.2 Luminal B

Luminal B represents 10–20% of all BCs. Compared to luminal A tumors, it presents a more aggressive phenotype, higher histological grade, higher proliferation rate and a worse prognosis [39]. Luminal B tumors more frequently (16.4–20.8%) present HER2-overexpression [41].

The pattern of distant relapse also differs, and although the bone is still the most common site of recurrence, this subtype more frequently presents visceral metastatic sites such as the liver. Luminal B tumors are more chemosensitive and usually benefit more from adjuvant chemotherapy [42].

30.6.3 HER2-Enriched

HER2-enriched BC is characterized at RNA and protein level by high expression of HER2, of HER2-related genes (such as other genes in the HER2 amplicon) and proliferation-related genes, intermediate expression of luminal-related genes (e.g., ESR1 and PgR), and low expression of basal-related genes. HER2-enriched BC is biologically aggressive and intrinsically carries a poor prognosis [39]. However, introduction of HER2-targeted treatment has substantially improved outcomes, both in the early and advanced settings [43].

30.6.4 Basal-Like

Approximately 15% of BCs are classified as basal-like [41]. This subtype is characterized by high expression of proliferation-related genes and keratins typically expressed by the basal layer of the skin (e.g., keratins 5, 14, and 17), and very low expression of luminal-related genes [41]. Basal-like tumors generally present high

grade, high mitotic indices and are characterized by the lack of expression of ER, PgR, and HER2. Patients with basal-like BC have poor prognosis, with higher relapse rates and short overall survival [39, 40].

30.6.5 Normal-Like Subtype

This subtype is poorly characterized, and its clinical significance remains undetermined. There are even doubts about its actual existence. In fact, some researchers believe the normal-like subtype might be a technical artifact due to contamination with normal tissue. Indeed, in a large series of samples where neoplastic cells were isolated by microdissection, no cases of normal breast-like subtype were found, supporting this hypothesis [42].

30.6.6 Gene Expression Prognostic Signatures

As previously discussed, gene expression profiles can be used to gain additional prognostic and/or predictive information to complement pathology assessment and to predict the benefit of adjuvant chemotherapy. Several of these gene signatures are commercially available, such as MammaPrint, Oncotype DX Recurrence Score, Prosigna, and Endopredict. The more clinically mature are MammaPrint and Oncotype DX.

In fact, the MammaPrint assay has been cleared by the US Food and Drug Administration for use in women younger than 61 years old with stage I or II, node-negative BC, to assess patient's risk for distant metastases. This 70-gene assay has been evaluated in the prospective MINDACT study [44], which enrolled women independently from BC grade, receptor status, and lymph node involvement. In this study, women with a low risk according to the 70-gene prognostic assay and high clinical risk (as calculated by the online tool Adjuvant! Online) were randomized to receive chemotherapy or not. Distant disease-free survival at 5 years was similar for these patients with or without chemotherapy, pointing out that MammaPrint can be used to avoid chemotherapy in a subgroup of clinically high-risk patients.

Similar evidence exists to support the use of the Oncotype DX test in node-negative HR+ BC. The OncotypeDX Recurrence Score (RS) is based on the quantitative assessment of 21 genes relevant to BC biology and is a continuous, numeric result that correlates with distant metastatic recurrence in node-negative BC patients treated with tamoxifen and with prognosis of postmenopausal women with node-positive tumors receiving endocrine treatment [45, 46]. In the prospective TAILORx (Trial Assigning Individualized Options for Treatment Rx) trial patients with HR-positive, HER2-negative, lymph node-negative BC were tested using Oncotype DX. Patients with RS <11 received endocrine therapy alone and had a 5-year distant recurrence-free survival of 99.3% without chemotherapy [47]. Patients in the intermediate-risk group (RS 11-25) were randomized to receive chemotherapy or not and the study showed non-inferiority of endocrine treatment alone in terms of disease-free survival in this group. However, subgroup analysis showed that significant benefit from adding chemotherapy exists in some subgroups of patients with intermediate risk according to OncotypeDX (such as young patients ≤50 years of age) [48]. The WSG PLAN B trial, another large randomized trial, tested the use of Oncotype DX in clinically high-risk pN0-N1 HR-positive BC patients. In this trial, patients with RS < 11 received endocrine therapy alone and had a 5-year disease-free survival of 94% without chemotherapy [49]. The RxPONDER trial is also currently testing the role of OncotypeDX in patients with HR+ HER2− BC with lymph node involvement.

For Prosigna, a 50-gene intrinsic subtype classifier that categorizes cancers into luminal A, luminal B, HER2, or basal-like subtypes [50], retrospective analyses of data from prospective trials show independent prognostic information beyond traditional pathologic markers. However, we are still waiting for data from its validation prospective randomized clinical trial, the OPTIMA trial.

For the EPclin Risk Score, a BC recurrence test integrating both gene expression data (EndoPredict) and clinicopathological features designed to accurately predict 10-year risk of distant recurrence in ER+ HER2- BC with node-negative (N0) or node-positive (N1), only retrospective analyses of data from several large prospective trials are currently available.

30.7 Clinical Presentation and Diagnosis

Most BCs are diagnosed by mammography screening in the absence of any sign or symptom.

When this is not the case, the most frequent sign of BC is the presence of a palpable, non-tender, hard breast nodule with unclear margins. Sometimes a retraction of the nipple or the skin of the breast can be present. Less frequently, BC presents with discharge of secretion or bleeding from the nipple, usually in relation with Paget disease or ductal papillomas.

In more advanced cases, the first sign of BC can be the presence of hard, fixed, non-tender lymph nodes in the axilla (or more rarely supra or infraclavicular region), eventually accompanied by lymphedema of the arm. More rarely, BC might present with mastitis or as an inflammatory carcinoma, with the diffuse involvement of the entire breast, which shows signs of inflammation (edematous, erythematous, warm, tender, enlarged breast).

30.7.1 Clinical Examination of the Breast

Breast examination includes the neck, chest wall, both breasts, and axillae and is part of a complete physical examination.

Inspection: Visual inspection of the breasts is an important component of clinical examination. The patient should be examined in both upright (arms relaxed, arms raised over the head, hands pressing on the hips) and supine positions. Asymmetry, bulging areas, skin changes (retraction, edema, dermatological lesions), and position of the nipple (inversion or retraction) should be evaluated. Spontaneous discharge should also be investigated, without squeezing the nipple.

Palpation: A first bimanual examination of the breasts should be performed with the patient in sitting position, supporting the breast with one hand and examining the breast with the other. Bimanual examination is then completed with arms raised above her head. Palpation should extend from the midaxillary line to the lateral edge of the sternum and from the clavicle to infra-mammary ridge to cover all the perimeter of the breast. Using three levels of pressure will help detect asymmetric thickenings or masses that can occur at different depths in the breast tissue. Palpation should include the careful application of pressure to the retroareolar region to check for abnormal discharge.

Regional Lymph node Examination: Clavicular and axillary lymph nodes should be examined in sitting position to allow best access to the deepest nodes. Patient should relax her shoulders and allow the examiner to support her arm while the axilla is palpated. Attention should be given to cervical, supraclavicular, infraclavicular, and axillary nodal basins. The presence of any palpable nodes and their characteristics, whether they are soft and mobile or firm, hard, tender, fixed, or matted, should be noted.

Assessment for distant metastases (bones, liver, and lungs, or neurological examination if symptoms are present) should also be conducted.

30.7.2 Imaging Techniques for Breast Cancer Diagnosis and Local Staging

The most commonly used imaging technique for BC is mammography. Mammography is used for BC screening in asymptomatic women and for differential diagnostic in patients presenting with clinical suspect of BC. Breast neoplasia usually appears at mammography as radio opaque nodule with spiculated margins. In some cases, microcalcifications are present.

Ultrasonography is often used to complement mammography and clinical examination. It is particularly useful in young women in which higher breast density might affect the sensitivity of mammography. With this exception, the sensitivity of ultrasonography is generally lower than that of mammography, even if it has a higher specificity to distinguish benign and malignant lesions. For this reason, it should be used after mammography to better characterize lesions. Ultrasonography also allows to explore regional lymph nodes and can be used to guide biopsy of suspicious lesions.

Bilateral mammography and ultrasound of the breast and regional lymph nodes are therefore usually considered standard imaging evaluation at BC diagnosis [27].

Contrast-enhanced MRI is not routinely recommended and is generally used as second-level imaging technique. It has higher sensitivity than mammography, but less specificity and its use might lead to an increase in false-positive rate. MRI is used for surveillance in women at high risk of BC or BRCA1/BRCA2 mutation carriers, in patients with breast implants, in equivocal cases at first-level imaging techniques, for staging of multifocal or bifocal lesions (particularly in lobular BC), and for evaluation of pectoral muscle infiltration. MRI may also be recommended before neoadjuvant chemotherapy, for evaluating the response to primary systemic therapy or when conventional imaging findings are inconclusive (such as a positive axillary lymph node status with an occult primary tumor in the breast) [27]. Outside these indications, MRI is generally not recommended routinely as it has a substantial false-positive rate and several studies have shown an association between MRI use and greater unwarranted use of mastectomy [51].

30.7.3 Biopsy and Diagnosis

The presence of carcinoma can only be determined by tissue biopsy. Several biopsy techniques are available: fine-needle aspiration (FNA), core needle biopsy, and excisional biopsy.

Needle biopsy techniques are usually preferred to avoid surgical scars. FNA is easily performed. However, it requires a trained cytopathologist, does not reliably distinguish invasive cancer from DCIS and often does not permit a reliable immunohistochemical characterization. For this reason, FNA use is currently only recommended to confirm involvement of axillary lymph nodes but not to evaluate primary breast lesions. Core needle biopsy, instead, provides histologic specimen suitable for interpretation by any pathologist and for ER, PR, and *HER2* testing. If preoperative systemic therapy is planned, a core needle biopsy is mandatory to ensure a complete diagnosis and assessment of biomarkers. A marker, such as a surgical clip, should be left in place into the tumor at biopsy, to ensure surgical resection of the correct site. Both core needle biopsies of breast lesions and FNA of axillary lymph nodes are usually performed using ultrasonography guiding to reduce the risk of false-negative results due to inappropriate sampling. When lesions are difficult to identify at ultrasonography (e.g., microcalcifications), mammographic guidance and vacuum-assisted biopsy can be used. False-negative rates of core biopsy are now reliably <1%.

For this reason, excisional biopsy is now a diagnostic technique reserved to patients with imaging abnormalities that cannot be targeted for core biopsy. In addition, surgical biopsy can also be indicated following core biopsy in specific cases:

- Failure to sample calcifications
- Diagnosis of atypical ductal hyperplasia
- Diagnosis of atypical lobular hyperplasia or lobular carcinoma in situ (controversial)
- Lack of concordance between imaging findings and histologic diagnosis
- Radial scar (differential diagnosis with tubular carcinoma)
- Papillary lesions (differential diagnosis with papillary carcinoma in situ)

30.7.4 Breast Cancer Staging

Once BC has been diagnosed, its extension and its prognosis should be accurately evaluated in order to define the most appropriate treatment.

Extension of the disease is usually assessed using the classical TNM staging system in which "T" refers to tumor, "N" to nodes, and "M" to metastasis. Definitions for classifying the primary tumor are the same for clinical and pathologic classification, while the clinical and pathologic classification for N staging are different (Table 30.2).

Table 30.2 AJCC Clinical and Pathological TNM staging

T category	*T criteria*
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis(DCIS)[a]	Ductal carcinoma in situ
Tis(Paget)	Paget disease of the nipple NOT associated with invasive carcinoma and/or carcinoma in situ (DCIS) in the underlying breast parenchyma. Carcinomas in the breast parenchyma associated with Paget disease are categorized based on the size and characteristics of the parenchymal disease, although the presence of Paget disease should still be noted
T1	Tumor ≤20 mm in greatest dimension
T1mi	Tumor ≤1 mm in greatest dimension
T1a	Tumor >1 mm but ≤5 mm in greatest dimension (round any measurement >1.0–1.9 mm to 2 mm).
T1b	Tumor >5 mm but ≤10 mm in greatest dimension
T1c	Tumor >10 mm but ≤20 mm in greatest dimension
T2	Tumor >20 mm but ≤50 mm in greatest dimension
T3	Tumor >50 mm in greatest dimension
T4	Tumor of any size with direct extension to the chest wall and/or to the skin (ulceration or macroscopic nodules); invasion of the dermis alone does not qualify as T4
T4a	Extension to the chest wall; invasion or adherence to pectoralis muscle in the absence of invasion of chest wall structures does not qualify as T4

Table 30.2 (continued)

T4b	Ulceration and/or ipsilateral macroscopic satellite nodules and/or edema (including peau d'orange) of the skin that does not meet the criteria for inflammatory carcinoma
T4c	Both T4a and T4b are present
T4d	Inflammatory carcinoma (see "Rules for Classification")
T suffix	Definition
(m)	Select if synchronous primary tumors are found in single organ.
N category	*N criteria*
cNX[a]	Regional lymph nodes cannot be assessed (e.g., previously removed)
cN0	No regional lymph node metastases (by imaging or clinical examination)
cN1	Metastases to movable ipsilateral Level I, II axillary lymph node(s)
cN1mi[b]	Micrometastases (approximately 200 cells, larger than 0.2 mm, but none larger than 2.0 mm)
cN2	Metastases in ipsilateral Level I, II axillary lymph nodes that are clinically fixed or matted; *or* in ipsilateral internal mammary nodes in the absence of axillary lymph node metastases
cN2a	Metastases in ipsilateral Level I, II axillary lymph nodes fixed to one another (matted) or to other structures
cN2b	Metastases only in ipsilateral internal mammary nodes in the absence of axillary lymph node metastases
cN3	Metastases in ipsilateral infraclavicular (Level III axillary) lymph node(s) with or without Level I, II axillary lymph node involvement; *or* in ipsilateral internal mammary lymph node(s) with Level I, II axillary lymph node metastases; *or* metastases in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement
cN3a	Metastases in ipsilateral infraclavicular lymph node(s)
cN3b	Metastases in ipsilateral internal mammary lymph node(s) and axillary lymph node(s)
cN3c	Metastases in ipsilateral supraclavicular lymph node(s)
[a]The cNX category is used sparingly in cases where regional lymph nodes have previously been surgically removed or where there is no documentation of physical examination of the axilla.	
[b]cN1mi is rarely used but may be appropriate in cases where sentinel node biopsy is performed before tumor resection, most likely to occur in cases treated with neoadjuvant therapy.	
N category	*N criteria*
pNX	Regional lymph nodes cannot be assessed (e.g., not removed for pathological study or previously removed)
pN0	No regional lymph node metastasis identified or ITCs only
pN0(i+)	ITCs only (malignant cell clusters no larger than 0.2 mm) in regional lymph node(s)
pN0(mol+)	Positive molecular findings by reverse transcriptase polymerase chain reaction (RT-PCR); no ITCs detected
pN1	Micrometastases; or metastases in 1–3 axillary lymph nodes; and/or clinically negative internal mammary nodes with micrometastases or macrometastases by sentinel lymph node biopsy
pN1mi	Micrometastases (approximately 200 cells, larger than 0.2 mm, but none larger than 2.0 mm)
pN1a	Metastases in 1–3 axillary lymph nodes, at least one metastasis larger than 2.0 mm
pN1b	Metastases in ipsilateral internal mammary sentinel nodes, excluding ITCs
pN1c	pN1a and pN1b combined
pN2	Metastases in 4–9 axillary lymph nodes; or positive ipsilateral internal mammary lymph nodes by imaging in the absence of axillary lymph node metastases
pN2a	Metastases in 4–9 axillary lymph nodes (at least one tumor deposit larger than 2.0 mm)
pN2b	Metastases in clinically detected internal mammary lymph nodes with or without microscopic confirmation; with pathologically negative axillary nodes

(continued)

Table 30.2 (continued)

pN3	Metastases in 10 or more axillary lymph nodes *or* in infraclavicular (Level III axillary) lymph nodes *or* positive ipsilateral internal mammary lymph nodes by imaging in the presence of one or more positive Level I, II axillary lymph nodes *or* in more than three axillary lymph nodes and micrometastases or macrometastases by sentinel lymph node biopsy in clinically negative ipsilateral internal mammary lymph nodes *or* in ipsilateral supraclavicular lymph nodes
pN3a	Metastases in 10 or more axillary lymph nodes (at least one tumor deposit larger than 2.0 mm) *or* metastases to the infraclavicular (Level III axillary lymph) nodes
pN3b	pN1a or pN2a in the presence of cN2b (positive internal mammary nodes by imaging) *or* pN2a in the presence of pN1b
pN3c	Metastases in ipsilateral supraclavicular lymph nodes
M category	*M criteria*
cM0	No clinical or radiographic evidence of distant metastases[a]
cM0(i+)	No clinical or radiographic evidence of distant metastases in the presence of tumor cells or deposits no larger than 0.2 mm detected microscopically or by molecular techniques in circulating blood, bone marrow, or other nonregional nodal tissue in a patient without symptoms or signs of metastases
cM1	Distant metastases detected by clinical and radiographic means
pM1	Any histologically proven metastases in distant organs; or if in nonregional nodes, metastases greater than 0.2 mm

Pathologic stage after neoadjuvant therapy is designated with the prefix "yp." Complete response is defined as the absence of invasive carcinoma in the breast and axillary nodes and has been clearly associated with significant improvement in disease-free survival and overall survival for the individual patient [52].

However, the principal aim of a staging system is to group patients with respect to prognosis. Despite its importance, the TNM staging has been progressively superseded by the biological characterization of the disease, which defines subgroups with different outcomes and different response to specific treatments.

This has led to a recent radical change in the staging of BC. In 2018, the Eighth edition of the American Joint Committee on Cancer (AJCC) included, in addition to the classic anatomic parameters (T, N, and M), prognostic biological parameters (grade, ER, PgR, and HER2). Moreover, the new AJCC classification also takes into account results of prognostic multigene signatures, which can be used to more accurately stratify individual patient prognosis (Table 30.3).

30.7.5 Imaging for Breast Cancer Staging and Other Pre-treatment Evaluations

In stage I–II BC without clinical suspicious of metastasis, asymptomatic distant metastases are very rare, and patients do not appear to benefit from comprehensive laboratory (including tumor markers) or radiological staging. For this reason, international guidelines do not recommend the use of imaging techniques (such as total body TC scan or bone scan) for the preoperative staging of these patients [27].

More comprehensive staging including a chest radiography or CT scan, an abdominal ultrasound or CT scan, and a bone scan can be considered for patients with clinically positive axillary nodes, large tumors (e.g., ≥5 cm), aggressive biology or clinical signs or symptoms suspicious for metastases.

Bone scan is a very sensitive technique for identifying bone metastases. However, its specificity is very low and the number of false-positives is high. Therefore, any suspicious alteration identified by bone scan should be confirmed by another imaging technique (X-ray/segmental CT scan/segmental MRI).

Functional/anatomical imaging, such as fluorodeoxyglucose positron emission tomography (FDG-PET)/CT, can be useful when conventional methods are inconclusive. It can also replace traditional imaging for staging in high-risk patient candidates to neoadjuvant chemotherapy, as well as those with locally advanced/inflammatory BC, in consideration of their high risk of metastatic disease.

Other pre-treatment assessments include: complete personal medical history, family history relating to breast/ovarian and other cancers, physical examination, a full blood count, liver and renal function tests, and alkaline phosphatase and calcium levels. Accurately assessing the menopausal status of the patient is also

Table 30.3 AJCC Pathological Prognostic Staging system. (Adapted from 8th ed). Pathological prognostic stage does not apply to patients treated with systemic or radiation prior to surgical resection

TNM	Grade	HER2 status	ER status	PR status	Pathological prognostic stage
Tis N0 M0	Any	Any	Any	Any	0
T1[a] N0 M0 T0 N1mi M0 T1[a] N1mi M0	G1	Positive	Positive	Positive	IA
				Negative	IA
			Negative	Positive	IA
				Negative	IA
		Negative	Positive	Positive	IA
				Negative	IA
			Negative	Positive	IA
				Negative	IA
	G2	Positive	Positive	Positive	IA
				Negative	IA
			Negative	Positive	IA
				Negative	IA
		Negative	Positive	Positive	IA
				Negative	IA
			Negative	Positive	IA
				Negative	IB
	G3	Positive	Positive	Positive	IA
				Negative	IA
			Negative	Positive	IA
				Negative	IA
		Negative	Positive	Positive	IA
				Negative	IA
			Negative	Positive	IA
				Negative	IB
T0 N1[b] M0 T1[a] N1[b] M0 T2 N0 M0	G1	Positive	Positive	Positive	IA
				Negative	IB
			Negative	Positive	IB
				Negative	IIA
		Negative	Positive	Positive	IA
				Negative	IB
			Negative	Positive	IB
				Negative	IIA

(continued)

Table 30.3 (continued)

TNM	Grade	HER2 status	ER status	PR status	Pathological prognostic stage
	G2	Positive	Positive	Positive	IA
				Negative	IB
			Negative	Positive	IB
				Negative	IIA
		Negative	Positive	Positive	IA
				Negative	IIA
			Negative	Positive	IIA
				Negative	IIA
	G3	Positive	Positive	Positive	IA
				Negative	IIA
			Negative	Positive	IIA
				Negative	IIA
		Negative	Positive	Positive	IB
				Negative	IIA
			Negative	Positive	IIA
				Negative	IIA
T2 N1[c] M0 T3 N0 M0	G1	Positive	Positive	Positive	IA
				Negative	IIB
			Negative	Positive	IIB
				Negative	IIB
		Negative	Positive	Positive	IA
				Negative	IIB
			Negative	Positive	IIB
				Negative	IIB
	G2	Positive	Positive	Positive	IB
				Negative	IIB
			Negative	Positive	IIB
				Negative	IIB
		Negative	Positive	Positive	IB
				Negative	IIB
			Negative	Positive	IIB
				Negative	IIB

Table 30.3 (continued)

TNM	Grade	HER2 status	ER status	PR status	Pathological prognostic stage
	G3	Positive	Positive	Positive	IB
				Negative	IIB
			Negative	Positive	IIB
				Negative	IIB
		Negative	Positive	Positive	IIA
				Negative	IIB
			Negative	Positive	IIB
				Negative	IIIA
T0 N2 M0 T1[a] N2 M0 T2 N2 M0 T3 N1[c] M0 T3 N2 M0	G1	Positive	Positive	Positive	IB
				Negative	IIIA
			Negative	Positive	IIIA
				Negative	IIIA
		Negative	Positive	Positive	IB
				Negative	IIIA
			Negative	Positive	IIIA
				Negative	IIIA
	G2	Positive	Positive	Positive	IB
				Negative	IIIA
			Negative	Positive	IIIA
				Negative	IIIA
		Negative	Positive	Positive	IB
				Negative	IIIA
			Negative	Positive	IIIA
				Negative	IIIB
	G3	Positive	Positive	Positive	IIA
				Negative	IIIA
			Negative	Positive	IIIA
				Negative	IIIA
		Negative	Positive	Positive	IIB
				Negative	IIIA
			Negative	Positive	IIIA
				Negative	IIIC

(continued)

Table 30.3 (continued)

TNM	Grade	HER2 status	ER status	PR status	Pathological prognostic stage
T4 N0 M0 T4 N1[c] M0 T4 N2 M0 Any T N3 M0	G1	Positive	Positive	Positive	IIIA
				Negative	IIIB
			Negative	Positive	IIIB
				Negative	IIIB
		Negative	Positive	Positive	IIIA
				Negative	IIIB
			Negative	Positive	IIIB
				Negative	IIIB
	G2	Positive	Positive	Positive	IIIA
				Negative	IIIB
			Negative	Positive	IIIB
				Negative	IIIB
		Negative	Positive	Positive	IIIA
				Negative	IIIB
			Negative	Positive	IIIB
				Negative	IIIC
	G3	Positive	Positive	Positive	IIIB
				Negative	IIIB
			Negative	Positive	IIIB
				Negative	IIIB
		Negative	Positive	Positive	IIIB
				Negative	IIIC
			Negative	Positive	IIIC
				Negative	IIIC
Any T Any N M1	Any	Any	Any	Any	IV

If T1N0M0 or T2 N0 M0 with ER-positive, HER2 negative and Oncotype Dx score is less than 11, then the pathological prognostic stage group is IA

[a]T1 includes T1mi

[b]N1 does not include N1mi. T1 N1mi M0 and T0 N1mi M0 cancers are included for prognostic staging with T1 N0 M0 cancers of the same prognostic factor status

[c]N1 includes N1mi. T2, T3, and T4 cancers and N1mi are included for prognostic staging with T2 N1, T3 N1, and T4 N1, respectively

imperative, and pre-treatment serum estradiol and follicle-stimulating hormone levels should be measured in case of doubt. In patients planned for (neo)adjuvant treatment, with anthracyclines and/or trastuzumab, evaluation of cardiac function with measurement of ejection fraction should also be performed [27].

30.8 Management of Nonmetastatic Breast Cancer

Treatment of BC patients should be carried out in "breast units" defined as specialized departments that treat a high volume of BC patients. Treatment should be provided by a multidisciplinary team, which includes at least one surgeon, radiation oncologist, medical oncologist, radiologist, and pathologist, who are specialized in BC. The breast team may include plastic/reconstructive surgeons, psychologists, physiotherapists, and geneticists [53]. The choice of treatment strategy, based on tumor burden, location and biological characteristics as well as on age and patient's comorbidities, should be extensively discussed with the patient and take into account her/his preferences.

Apart from metastatic disease, treatment of BC patients should take into account two components:

- Locoregional treatment (surgery and radiotherapy) aiming to the radical excision of macroscopic disease, local staging of the disease and treatment of residual tumor cells in the breast and nodes in order to limit the risk of locoregional recurrence
- Systemic treatment aiming to eradicate systemic micrometastases, which might have originated even from early-stage BC, reducing the risk of distant and locoregional recurrence at the same time

Locoregional treatment of BC is mainly guided by the evaluation of disease operability. Metastatic disease is generally considered a contraindication to surgical and local treatment, even if in some selected cases of oligometastatic disease locoregional treatments might be proposed with a potentially curative intent. Patients with T4 tumors or N2/N3 nodal disease are not candidates for surgery upfront and should be treated with neoadjuvant systemic therapy in order to shrink the tumor, allowing radical operability. However, in the N3 category, patients with N3a-b disease are considered operable and are managed as locally advanced operable BC, while patients with N3c disease (involvement of ipsilateral supraclavicular nodes) are considered inoperable and are managed as such.

Patients with lesser extent of disease are usually managed with surgery upfront. However, neoadjuvant systemic therapy can be used in these patients to allow breast conservation in a woman who would otherwise require mastectomy or to test chemosensitivity in patients with sure indication to chemotherapy (e.g., TN/HER2+ BC) (◘ Fig. 30.1).

The possibility of hereditary cancer should also be explored, allowing for appropriate genetic counseling and testing of the patient and, if needed, prophylactic procedures. In younger premenopausal patients, fertility issues should also be discussed [27].

30.8.1 Local Management of Breast Cancer: Surgery

Breast surgery represents a cornerstone in the treatment of BC. In fact, surgery is capable of eradicating macroscopic disease in breast. However, increasing knowledge of BC biology has led to the understanding that most BCs are capable of micro-metastasizing even in early stage. Over the last century, this has led to a progressive decrease in surgical aggressiveness (both on the breast and on the axilla) and a parallel increase in the use of adjuvant system treatments.

For almost one century, from its first use in 1882 to the mid-twentieth century, *Halsted's radical mastectomy* has been the classical surgical procedure for BC. This intervention involved the removal of mammary gland, nipple, skin, underlying chest muscle (including pectoralis major and pectoralis minor), and axillary lymph nodes. It was an extremely disfiguring and invalidating surgery and is nowadays used only in extremely selected cases (e.g., infiltration of the pectoralis major).

Less invasive mastectomies have been subsequently employed:

- *Patey's conservative radical mastectomy*: which preserves the pectoralis major while removing the pectoralis minor.
- *Madden's modified radical mastectomy*: which preserves both the pectoralis major and the pectoralis minor.
- *Simple mastectomy, a* term used to define a procedure in which the entire mammary gland is removed, but both pectoral muscles and axillary lymph nodes are undisturbed.

Today, mastectomy generally includes removal of the breast tissue from the clavicle to the rectus abdominous and between the sternal edge and the latissimus dorsi muscles. It also removes the nipple-areolar complex (NAC), the excess skin of the breast, and the fascia of the pectoralis major muscle. When mastectomy is

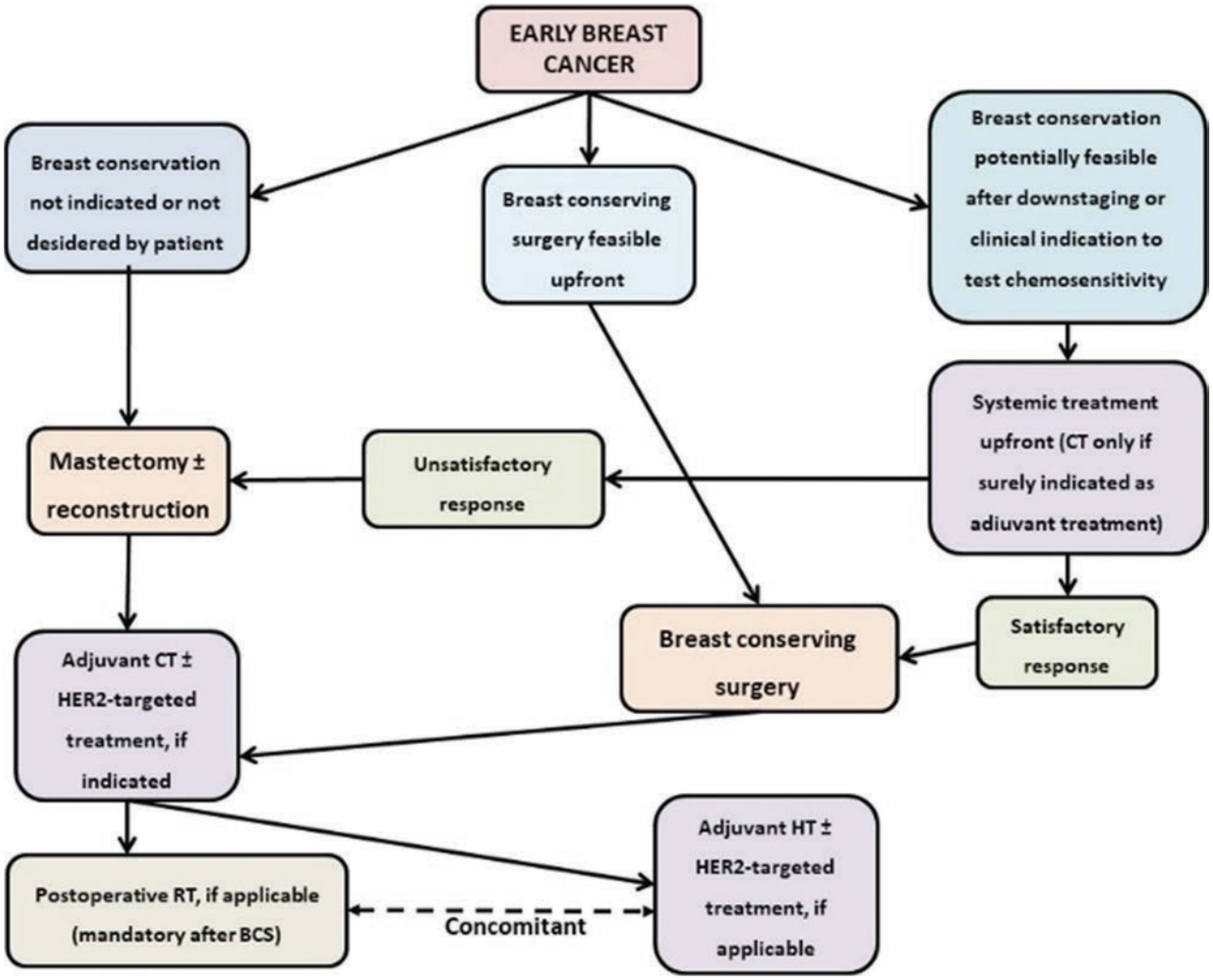


Fig. 30.1 Multidisciplinary management of early breast cancer

accompanied by axillary dissection, it is also termed modified radical mastectomy.

To ameliorate esthetic results, skin-sparing or nipple sparing mastectomy can be used. In skin-sparing mastectomy, skin excision is limited to the NAC and excisional biopsy scar, preserving the skin envelope of the breast and facilitating reconstruction. Traditionally, skin-sparing mastectomy included resection of the NAC to avoid the risk of leaving behind malignancy within the ducts of the nipple. Nipple-sparing mastectomy preserves the NAC (after residual cancer beneath the nipple is excluded by intraoperative frozen assessment) and generally guaranties excellent cosmetic results.

Over the last 50 years, the major change in the surgical treatment of primary BC has been a shift toward *breast-conserving treatment* (BCT). The goal of BCT is to use the combination of conservative surgery and radiotherapy to provide survival equivalent to mastectomy with preservation of the cosmetic appearance and a low rate of recurrence in the treated breast. Medical contraindications to the use of BCT are generally infrequent and currently, in Western Europe, 60%–80% of newly diagnosed cancers are amenable to breast conservation. Despite improvements in esthetic results, there is concern regarding the increase in rates of voluntary mastectomy (due to patient choice and not to medical reasons) in the last years. Moreover, many patients also ask for voluntary contralateral (prophylactic) breast amputation. Although patient's choice should be taken into account, physicians have a clear ethical responsibility to completely inform patients about the options and consequences.

30.8.1.1 Breast Conservation Treatment: Modalities and Risk of Local Relapse

BCT involves the combination of conservative surgery and radiotherapy for the local treatment of BC.

Conservative surgery is usually achieved through on of two main surgical procedures:

- Quadrantectomy, which implies the surgical removal of the tumor plus a large portion of surrounding healthy mammary gland, overlying skin and the pectoral fascia section beneath it.
- Lumpectomy (also known as wide local excision) only involves surgical removal of the tumor and a small portion of surrounding breast tissue.

A large number of randomized clinical trials have been conducted comparing mastectomy and BCT, and have demonstrated equivalent survival, even at long follow-up (20-year follow-up reports of the two largest studies, the NSABP B-06 and Milan I trials are available) [54, 55]. Moreover, the incidence of local relapse (LR) after BCT has declined over time, from 8–19% at 10-years in initial randomized trials to 2–7% in more recent studies. This decrease results from improved mammographic and pathologic evaluation and more frequent use of adjuvant systemic therapy.

Risk factors for locoregional recurrence after BCT can be divided into patient, tumor, and treatment factors:

- Young age (<35 or 40) is associated with increased risk of LR after BCT (even when correction is for pathologic features is applied) and is also a risk factor for LR after mastectomy
- Inherited susceptibility: BRCA1 and BRCA2 mutation carriers have a substantial risk of contralateral and late ipsilateral BC (most are second primary cancers). In these patients, the option of bilateral mastectomy should be considered (especially in young and early-stage patients) to avoid the long-term risk of a second BC in either breast.
- Margin of resection: patients with negative margins have lower rates of LR after BCT. However, there is no standard definition of a close margin and the impact of close margins on local relapse is controversial. To date, "no ink on tumor" (the absence of cancer cells at inked surfaces) remains the standard for an adequate margin in invasive cancer [56].
- Tumor biology is the most significant determinant of likelihood of LR after BCT (and mastectomy): patients with triple-negative BC are at higher risk, regardless of surgical technique.
- Supplementary irradiation to primary tumor area (boost) reduces (by 40%) the risk of ipsilateral LR, but does not impact survival [57].
- The use of adjuvant systemic therapy after BCT also significantly reduces the risk of LR

BCT requires careful patient selection and a multidisciplinary approach. A recent preoperative mammographic evaluation is necessary to determine patient eligibility (evaluate extent of the disease, presence or absence of multicentricity or microcalcifications) and should include evaluation of the contralateral breast to exclude synchronous lesions.

Some patients still undergone mastectomy due to the following:

- Tumor size (relative to breast size)
- Tumor multicentricity
- Inability to achieve negative surgical margins after multiple resections
- Patient choice
- Absolute or relative contraindications to RT, such as:
  - Ongoing pregnancy (absolute)
  - Diffuse suspicious microcalcifications (absolute)
  - Active connective tissue disease involving the skin (e.g., scleroderma and lupus) (relative)
  - Tumors >5 cm (relative)
  - Focally positive margin (relative)
  - Prophylactic bilateral mastectomy for risk reduction is under consideration (relative)

Whole-breast irradiation (WBI) is effective at eradicating subclinical multicentric foci of breast carcinoma present at the time of diagnosis but does not prevent subsequent development of new cancers in the treated breast. Therefore, patients who elect BCT require lifelong follow-up for the development of new cancers in the treated and contralateral breast.

30.8.1.2 Breast Reconstruction After Breast Surgery

For women undergoing mastectomy and wishing for breast reconstruction, a wide range of surgical options are available. The best technique should be discussed individually taking into account anatomic, treatment and patient preference.

The two major reconstructive techniques involve the following:

- Use of implants and/or tissue expanders (best suited for small/medium sized breasts with minimal ptosis)
- Use of myocutaneous tissue flaps (more flexible in the size and shape of the reconstructed breast)

For autologous tissue flaps, tissue can be taken from the latissimus dorsi muscle, from the transverse rectus abdominis muscle, from the free deep inferior epigastric perforator flap, from the superior gluteal artery-based perforator flap, or from the free gracilis-based flap. It generally tolerates postoperative RT better than implant-based reconstruction with more favorable esthetic outcomes. If postmastectomy radiotherapy is indicated, a temporary implant is usually positioned before RT.

Reconstruction may be immediate or delayed. Immediate reconstruction has the advantages of avoiding a second operative procedure and the psychological impact of breast loss. However, some patients might be advised against immediate reconstruction for oncological reasons (e.g., inflammatory BC).

30.8.1.3 Advances in Axillary Management

For many years, complete axillary dissection with removal of axillary lymph nodes has been considered standard surgical management of the axilla for patients with invasive BC and a critical component of the cure of BC. This idea was undermined by the NSABP B-04 trial, in which clinically node-negative patients were randomized to radical mastectomy (including axillary dissection), total mastectomy with RT to regional lymphatics, or total mastectomy and observation, in which delayed axillary dissection was only performed if axillary lymph nodes metastases developed. In this trial, patients treated with axillary surgery did not show a survival benefit [54].

Nevertheless, as lymph node status is one of the strongest predictors of long-term prognosis in primary BC, axillary dissection continued to be used primarily as staging procedure, while maintaining local disease control in the axilla and some therapeutic value for some patients with axillary nodal metastases.

However, axillary dissection is associated with high incidence (up to 25%) of upper limb lymphedema, which increases significantly (up to 40%) when axillary dissection is combined with RT to the axilla and that can become an invalidating sequela. On this basis, lymphatic mapping and sentinel node biopsy progressively replaced axillary dissection as staging procedure of choice in clinically node-negative patients. In the American College of Surgeons Oncology Group (ACOSOG) Z10 trial, a sentinel node could be identified in >95% of cases with the use of blue dye alone, radiocolloid alone, or the combination of the two. Complications were rare and lymphedema only occurred in 5% of patients [58]. Moreover, despite a 10% false-negative rate, patients treated by sentinel node biopsy alone showed an extremely low rate of LR in the axilla if the sentinel node did not contain metastases. Contraindications to the procedure included pregnancy and lactation (relative), and locally advanced BC (LABC). The presence of isolated tumor cells (<0.2 mm deposits) and micrometastases (>0.2 mm, <2.0 mm) in axillary nodes does not associate with any significant overall survival difference, and no difference in LR rate was observed if axillary dissection was omitted in these patients [59, 60]. Therefore, the routine use of serial sections and IHC to detect micrometastases is not warranted.

Traditionally, the presence of macrometastases in the sentinel node mandated axillary lymph node clearance. The ACOSOG Z0011 prospective randomized study tested the need for axillary dissection in clinically node-negative women with macrometastases to less than three sentinel nodes. Almost 900 patients were randomized. Five-year nodal recurrence rate was very low in both arms (0.5% in the dissection arm and 0.9% in the sentinel node biopsy alone arm) and no trend toward a survival benefit for dissection was observed. As expected, morbidity was significantly lower in the sentinel node group. However, all patients in this study underwent BCT (consequently receiving irradiation of the low axilla by breast radiotherapy tangents) and 97% of patients received systemic therapy. Therefore, these findings do not apply to women with clinically positive nodes, extensive nodal involvement, those undergoing partial breast irradiation or treatment with mastectomy [59].

Another option for the management of axilla in patients with clinically negative nodes and sentinel lymph node metastases is axillary irradiation. In fact, the AMAROS trial randomized these patients to receive irradiation of axillary and supraclavicular fields or axillary dissection and did not show a significant difference in 5-year disease-free survival (1.0% vs 0.5%) with lower incidence of lymphedema in patients treated with radiotherapy as compared to those who received surgery (14% vs 28%) [61].

At present, it is clear that axillary dissection is no longer the standard approach for all patients with positive sentinel nodes treated with BCT including whole-breast RT. However, which is the optimal approach for these patients remains a matter of debate.

Another matter of debate is axillary approach in patients receiving neoadjuvant treatment. If preoperative systemic treatment is planned, ultrasound-guided fine-needle aspiration or core biopsy of suspicious lymph nodes before treatment should be carried out as a minimum. In patients with clinically and imaging negative axilla, the best timing to carry out sentinel lymph node biopsy (if before or after preoperative therapy) remains controversial. If fact, the SENTINA and ACOSOG Z1071 trials reported lower detection rates and higher rates of false-negatives when SLNB is carried out after systemic therapy, compared with SNLB

that is carried out before neoadjuvant chemotherapy [62, 63]. However, if the axilla is negative at imaging evaluation before treatment and three or more lymph nodes are excised, a post-systemic therapy SNLB can be considered [27].

30.8.2 Local Management of Breast Cancer: Radiotherapy

30.8.2.1 Radiotherapy after Breast-Conserving Treatment

Whole-breast RT is strongly recommended after breast-conserving surgery [72], as it reduces the risk of LR and long-term BC-related mortality (3.8% reduction in BC mortality at 15 years). In elderly (>70 years old), selected patients with low risk of recurrence (small, biologically indolent tumors) receiving endocrine treatment omission of RT after breast-conserving surgery can be discussed in case of comorbidities.

Boost irradiation further reduces the risk of LR and is indicated for patients with unfavorable risk factors such as age <50 years, grade 3 tumors, extensive DCIS, vascular invasion, or focally positive margin [73, 74].

Traditionally, doses used for local and/or regional adjuvant irradiation are 45–50 Gy in 25–28 fractions followed by a 10–16 Gy boost in 2 Gy doses. Shorter fractionation schemes (e.g., 15–16 fractions with 2.5 Gy doses) have been tested showing similar efficacy without increases in side effects and are now considered a standard for whole-breast RT after breast-conserving surgery in most low-risk patients. However, young patients, patients with node-positive or high-grade tumors, and patients undergoing mastectomy and/or additional regional irradiation were under-represented in these trials and use of shorter fractionation schemes should be carefully evaluated case by case.

Another alternative schedule is accelerated partial breast irradiation (APBI), which is based on the rationale that most LR occur in the proximity of the primary tumor site. APBI can be performed using a number of different techniques, including interstitial brachytherapy, three-dimensional conformal external beam irradiation, intracavitary brachytherapy, and intraoperative radiotherapy. APBI by intraoperative radiotherapy was tested in two randomized trials, the ELIOT (single dose of electrons) and TARGIT (single intraoperative dose 50 kV X-rays) trials, which reported a significantly higher ipsilateral breast recurrence with APBI as compared to whole-breast radiotherapy [75, 76]. Despite this, APBI might be considered for the treatment of patients with an extremely low risk of LR (>60 years old, not BRCA mutated patients with unicentric, unifocal, node-negative, non-lobular BC, <2 cm, without extensive intraductal components or vascular invasion, with negative margins, which will receive adjuvant hormonal treatment) [77].

30.8.2.2 Radiotherapy After Mastectomy

The use of postmastectomy RT has been evolving over the last decade. In fact, postmastectomy RT has been always recommended for high-risk patients, including positive resection margins, cutaneous involvement or ulceration, four or more involved axillary lymph nodes, and large tumors (>5 cm). However, it has been recently shown that, in node-positive BC patients, postmastectomy RT reduces by 10% the 10-year risk of any recurrence (locoregional and distant) and by 8% the 20-year risk of BC-related mortality, independently from the number of involved axillary lymph nodes and the administration of adjuvant systemic treatment [78]. Based on these data, postmastectomy RT should also be considered for patients with 1–3 positive lymph nodes carefully evaluating on an individual patient basis (taking into account patient age and biological characteristics) [78, 79].

Older trials usually used large RT fields encompassing the chest wall and all regional lymph nodes. The European Society for Radiotherapy and Oncology guidelines advise to include only the most caudal lymph nodes surrounding the sub-clavicular arch and the base of the jugular vein, while the resected part of the axilla (after axillary lymph node dissection) should not be irradiated, except in cases of residual disease after surgery. The Danish population-based study, in which left-sided BC patients received medial supraclavicular RT, while right-sided patients also received RT to the internal mammary nodes, seems to point out the importance of including internal mammary lymph nodes in the regional target volume.

30.8.2.3 Regional Irradiation After Breast-Conserving Surgery

Whole-breast RT is considered the standard after breast-conserving surgery [72]. However, a number of findings also support the use of regional RT in intermediate/high-risk patients treated with BCT. The MA.20 trial randomized high-risk node-negative or node-positive patients treated with breast-conserving surgery to breast irradiation alone or breast irradiation plus regional RT (including internal mammary and medial supraclavicular fields). Most patients included had 1–3 involved lymph nodes. The addition of regional RT prolonged isolated locoregional disease-free survival, distant disease-free survival and disease-free survival (82% vs 77% at 10-year follow-up), but did not have a significant impact on overall survival (82.8% vs 81.8% at 10-year follow-up) [64]. However, benefits and risks should be

carefully evaluated on an individual patient basis taking into account also patient age and biological characteristics.

30.8.2.4 Radiotherapy for Unresectable Disease

Patients who present with unresectable non-metastatic disease are usually treated with primary systemic therapy. If disease is rendered resectable, it is then usually treated with surgery, followed by RT, in analogy with LABC. If the disease remains unresectable, however, RT can be considered to treat all sites of the original tumor extension with a boost to residual disease.

30.8.2.5 Toxicities of Breast Radiotherapy

Breast RT is generally well tolerated with few long-term toxicities. However, irradiation of the heart or of coronary arteries can result in premature ischemic heart disease [65]. A proportional relationship between RT dose to the heart and subsequent heart disease has been reported. Nevertheless, the absolute increase in lifetime risk is small and the risk of cardiac mortality is generally small as compared to the survival benefit from RT (both in BCT and postmastectomy). Moreover, current RT techniques spare most of the heart, reducing cardiac risk.

30.8.3 Adjuvant Systemic Treatment

The goal of adjuvant systemic therapy is to prevent BC recurrence by eradicating occult micrometastases already present at time of diagnosis. In fact, the hypothesis that, even in early stages of BC development, tumor cells are disseminated throughout the body has been validated through decades of clinical investigation. Approximately half of the decline in BC mortality observed in Westerns countries has been attributed to the use of adjuvant therapy [2].

Up to date, three systemic treatment modalities are available as adjuvant therapy for early-stage BC: endocrine treatment; chemotherapy and anti-*HER2* targeted treatment.

Selection of adjuvant treatment is based on predicted sensitivity to the specific treatment modality and on individual's risk of relapse (Table 30.4). Chemotherapy is used for HR− tumors and alongside with HER2 targeted treatment in *HER2*+ tumors. Patients with HR+ tumors are candidates for adjuvant endocrine therapy and, for high-risk patients with chemosensitive tumors, chemotherapy is added.

The estimation of recurrence risk is obtained taking into account several *prognostic factors*:

- Nodal status: Nodal status is the most important prognostic factor (risk increases progressively with number of positive lymph nodes.
- Tumor size: Risk of recurrence increases with tumor size.
- Patient age: Very young patients (≤35 years) have a poorer prognosis than older patients. Usually, BCs in these patients tend to be more often of higher grade and HR negative than in older patients. These differences probably explain in part the worse outcomes observed in very young patients [66].
- Grade: A high tumor grade (G3) is a negative prognostic factor.
- Proliferation (Ki67): High tumor proliferation is a negative prognostic factor. No clear cut-off exists, but 20% positivity for Ki67 is usually considered high proliferation.
- Histotype: Some rare special BC histotypes (e.g., tubular, mucinous, cribriform, and medullary) carry a better prognosis and a lower metastatic potential, while other rare histotypes, such as metaplastic BC have a worse prognosis.
- HR status: BCs are considered positive for HR if at least 1% of tumor cells express ER or PgR. However, tumors with an expression between 1% and 10% often present a clinical history similar to that of HR− tumors. Higher expression of HRs is associated with better prognosis.
- HER2 status: HER2 positivity is associated with more aggressive tumor biology in the absence of HER2-targeted treatment.
- Gene expression profiles: As previously discussed, several multigene tests have been recently introduced in clinical practice to select early BC patients with good prognosis which might be spared chemotherapy.

The choice of adjuvant treatment also takes into account the predicted sensitivity to the specific treatment, which is based on some *predictive factors*:

- HR status: Sensitivity to endocrine treatment is generally higher for tumors with higher levels of expression of HR as compared to tumors with lower levels. In fact, even if BCs are considered eligible for endocrine treatment if at least 1% of tumor cells express ER or PgR, it is well known that tumors with an expression between 1% and 10% are less sensitive to endocrine treatment.
- HER2 status: HER2 positivity is associated with sensitivity to HER2-targeted treatment and is used to select patients eligible for anti-HER2 treatment in clinical practice. HER2 is also a marker of benefit from adjuvant chemotherapy (HER2 overexpression being associated with a higher benefit from anthracycline-based chemotherapy) [67].
- Proliferation (Ki67): Tumors with high proliferation rates are more sensitive to chemotherapy and usually have a reduced sensitivity to endocrine treatment. In

Table 30.4 Surrogate intrinsic subtypes and recommended adjuvant therapy (Modified from Senkus et al. 2015)

Surrogate intrinsic subtype	IHC definition	Recommended adjuvant therapy
Luminal A-like	ER-positive HER2-negative Ki67 low[a] PgR high[b] low-risk molecular signature (if available)	ET alone in the majority of cases Consider CT if: high tumor burden (four or more positive LN, T3 or higher) or grade 3
Luminal B-like (HER2-negative)	ER-positive HER2-negative *and either* Ki67 high[a] *or* PgR low[b] high-risk molecular signature (if available)	ET + CT for majority of cases
Luminal B-like (HER2-positive)	ER-positive HER2-positive	CT + anti-HER2 + ET for all patients If contraindications for the use of CT, one may consider ET + anti-HER2
HER2-positive (non-luminal)	ER and PgR negative HER2-positive	CT + anti-HER2
Triple-negative (ductal)	ER and PgR negative HER2-negative	CT

ET endocrine therapy, *CT* chemotherapy, *LN* lymph node
[a]Ki-67 scores should be interpreted in the light of local laboratory values: as an example, if a laboratory has a median Ki-67 score in receptor-positive disease of 20%, values of 30% or above could be considered clearly high; those of 10% or less clearly low
[b]Suggested cut-off value is 20%

fact, Ki67 and PgR can be used as an IHC surrogate to distinguish Luminal A tumors from Luminal B tumors, which are more likely to be chemosensitive, for which chemotherapy is generally recommended in addition to endocrine treatment. However, IHC assessment of Ki67 is subjective and the St Gallen Consensus Guidelines recommend using the criteria of "clearly high" (>30%) and "clearly low" (<10%). Unclearly defined tumors according to IHC surrogates might benefit from gene expression profiling to more accurately estimate the potential benefit of adding chemotherapy (Table 30.4).

The final decision should also incorporate the predicted treatment sequelae, the patient's biological age, general health status, comorbidities, and preferences.

30.8.3.1 Endocrine Treatment

Adjuvant endocrine treatment is indicated for all patients with detectable HR expression (defined as ≥1% of invasive cancer cells) irrespective of the use of chemotherapy and/or targeted therapy. The choice of agent and its duration is primarily determined by the patient's menopausal status and risk of relapse. Differences in side-effect profiles are also present and may be taken into consideration in the decision. Tamoxifen and AIs are associated with different safety profiles. Tamoxifen is associated with an increased risk of thromboembolic complications and endometrial hyperplasia (including endometrial cancer) and should not be administered in patients using strong and moderate CYP2D6 inhibitors due to drug interaction. Patients treated with AIs are at increased risk of arthralgias and bone loss: adequate calcium and vitamin D3 intake should be administered, and they should undergo periodic assessment of their bone mineral density. All endocrine treatments can cause or worsen menopausal symptoms.

Standard duration of adjuvant endocrine treatment is at least 5 years, as shorter durations have been shown to result in inferior outcomes.

For postmenopausal women, endocrine treatment options include AIs and tamoxifen. Tamoxifen might still be a valid option for selected patients. Five years of adjuvant tamoxifen result in a 41% reduction in BC recurrence rate (HR = 0.59) and a 34% reduction in death rate (HR = 0.66) for women with HR+ BC [68]. The ATLAS trial, comparing 10 years to 5 years of adjuvant tamoxifen, have shown an improvement in overall survival and disease-free survival with the longer duration [69]. This finding is of particular relevance for women who lack the option of receiving extended adjuvant endocrine therapy with an AI, as for example premenopausal women (see the following) [27]. AIs are not appropriate for premenopausal patients not receiving ovarian suppression, as residual ovarian function can increase aromatase production overcoming the effects of AIs.

AIs can be used upfront (non-steroidal AI and exemestane), after 2–3 years of tamoxifen (non-steroidal AI and exemestane) or as extended adjuvant therapy, after 5 years of tamoxifen (letrozole and anastrozole) [27]. In the upfront setting, 5 years of AIs significantly reduce BC mortality as compared with 5 years of tamoxifen (15% more) and should be used upfront as treatment of choice in postmenopausal patients at high risk for relapse or with lobular histology [27]. For lower risk patients, the sequence can be decided on an individual basis, taking into account the

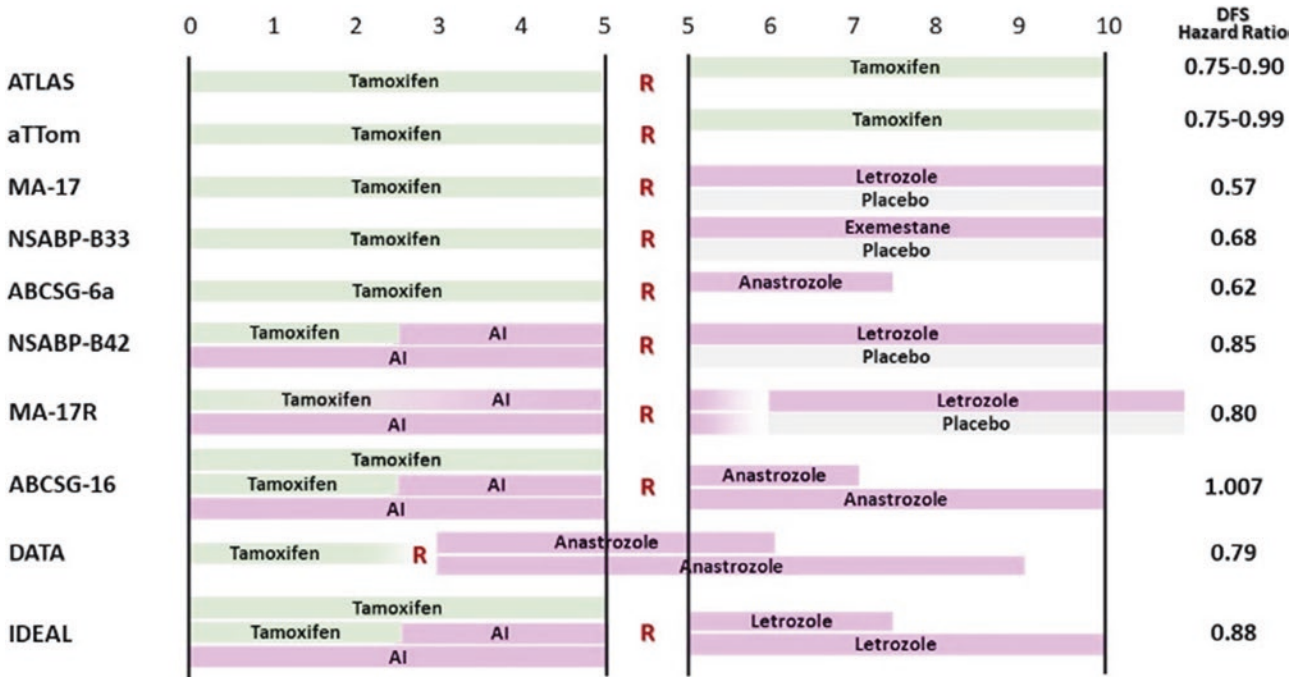


Fig. 30.2 Extended adjuvant endocrine treatment trials

different side-effect patterns. However, AIs should be considered at some point in the treatment program of postmenopausal women either as initial therapy or as sequential therapy after 2–3 years of tamoxifen [70]. The optimal duration and regimen of adjuvant ET is currently unknown. The extension of endocrine therapy beyond 5 years, underscoring the long natural history of HR+ BC, might be of benefit after initial tamoxifen, with improved outcomes seen in the ATLAS, aTTom, MA.17, and NSABP-B33 trials, but is of lesser benefit after initial AIs (IDEAL, NSABP-B42, MA-17R) (Fig. 30.2). Due to associated adverse effects, and limited absolute benefit in low-risk disease, it is more appropriate to reserve extended ET for high-risk disease.

Premenopausal women may be treated with tamoxifen alone, tamoxifen + ovarian function suppression (OFS), or an AI + OFS, according to estimated risk of relapse and patient's preference.

The combination of an AI + OFS has been shown to reduce recurrence as compared with tamoxifen + OFS, and the addition of OFS to tamoxifen has been shown to reduce recurrence as compared to tamoxifen alone. However, the addition of OFS to endocrine treatment also significantly increases adverse effects, in particular menopausal and sexual symptoms. For this reason, AI + OFS can be considered for higher risk cases (e.g., those treated with adjuvant chemotherapy), for which the absolute benefit over tamoxifen +/– OFS is greater [71]. Monitoring the bone health of these young women, especially those taking AIs or OFS, is crucial.

Tamoxifen alone can be enough for very-low-risk premenopausal patients, where outcomes are good and, in rare cases where both tamoxifen and AIs are not tolerated, a GnRH agonist alone may be considered. For premenopausal women not receiving OFS, prolongation of tamoxifen duration to 10 years, according to the ATLAS trial, might be of benefit as an improvement in overall survival and disease-free survival has been reported with the longer duration [69]. In patients becoming postmenopausal during the first 5 years of tamoxifen, switch to AIs should be considered [27].

30.8.3.2 Chemotherapy

Adjuvant chemotherapy, consisting of multiple cycles of polychemotherapy, is a well-established strategy for lowering the risk of BC recurrence and improving survival. Chemotherapy is recommended in the vast majority of triple-negative (with the possible exception of low-risk rare histological subtypes), HER2+ BCs (apart from selected cases with very low risk, such as T1aN0) and in high-risk HR+ HER2- tumors.

Over time, several chemotherapy regimens have been tested for the adjuvant treatment of BC:

- *First-generation regimens*: the combination of cyclophosphamide, methotrexate and 5-fluorouracil (CMF) was the first widely used regimen. Today it may still be used in selected patients.

- *Second-generation regimens*: anthracycline containing regimens (such as EC: epirubicin-cyclophosphamide or AC: adriamycin-cyclophosphamide) were proven to be more efficient in terms of relapse reduction (11%) and mortality reduction (16%) as compared to the same duration of first generation CMF. However, anthracyclines are associated with cardiotoxicity and might be contraindicated in case of concomitant cardiomyopathy or important cardiovascular risk factors.
- *Third-generation regimens:* The addition of taxanes to anthracyclines (in combination or sequentially) improves the efficacy of chemotherapy, at the cost of increased non-cardiac toxicity (peripheral neuropathy) [27]. Anthracyclines-taxanes based regimens reduce BC mortality by about one-third [72]. On this basis, for women who warrant chemotherapy, sequential regimens (AC/EC for 3–4 cycles followed by paclitaxel or docetaxel) are today considered the "gold standard." After four cycles of anthracyclines, weekly paclitaxel or three-weekly docetaxel (more myelotoxic) are the preferred regimens. Sequential regimens present the best efficacy combined with less toxicity as compared to combination regimens (such as TAC: docetaxel-adriamycin-cyclophosphamide) or TEC (docetaxel-epirubicin-cyclophosphamide).

The addition of 5-fluorouracil to EC (epirubicin and cyclophosphamide)-paclitaxel sequence does not improve efficacy [73]. Similarly, the addition of other drugs such as capecitabine or gemcitabine to an anthracycline-taxane regimen was not successful in phase 3 trials.

Chemotherapy is usually administered for four to eight cycles (12–24 weeks), depending on individual risk of recurrence and selected regimen. For high-risk tumors, the use of dose-dense schedules (biweekly instead of three-weekly administration with granulocyte colony-stimulating factor support) should be considered [27]. High-dose chemotherapy with stem cell support should not be used.

As an alternative to four cycles of anthracycline-based chemotherapy, taxane-based regimens, such as four cycles of docetaxel and cyclophosphamide (TC), have been developed, showing superior disease-free survival and overall survival [74]. However, these regimens are less efficacious than sequential regimens (a small 2.5% difference in invasive disease-free survival was reported when 6 cycles of TC were compared with the sequential regimen). Therefore, these regimens are not a standard for all patients, but can be used as an effective anthracycline-free option for selected patients (i.e., those with cardiac risk factors or at intermediate risk of relapse).

For patients with triple-negative BC, several neoadjuvant trials have tested the addition of platinum to a neoadjuvant anthracycline-taxane combination or sequence, improving pathological complete response. However, only some of these studies reported a consensual improvement in disease-free survival [75]. Since platinum adds toxicity and no robust, prospective randomized data exists on its use in the adjuvant setting, its addition is not routinely recommended. However, its use can be discussed with triple-negative BC patients.

In young BC patients receiving chemotherapy, the impact on subsequent fertility should be discussed. Fertility preservation techniques, such as oocyte cryopreservation or embryo cryopreservation, are available. Moreover, GnRH agonists can be used during chemotherapy to prevent chemotherapy-related ovarian failure, resulting in less premature ovarian failures and more pregnancies [76]. A decision should be taken in a case-by-case manner, after discussion with the patient regarding benefits and risks.

In general, chemotherapy should not be used concomitantly with endocrine treatment, which is usually started after chemotherapy completion, and radiotherapy, if planned, usually follows chemotherapy. Radiotherapy can be safely delivered concomitantly with trastuzumab, endocrine treatment and non-anthracycline–non-taxane-based chemotherapy [27].

30.8.3.3 Bone-Stabilizing Agents

In early BC, bisphosphonates were initially used to prevent side effects of adjuvant endocrine treatments on bone. However, several large clinical trials have shown outcome benefits for oral and intravenous adjuvant bisphosphonates. The large EBCTCG meta-analysis showed that adjuvant bisphosphonates reduced BC recurrence in bone, and improved BC survival [77]. However, the benefit appears to higher in postmenopausal patients, or in premenopausal patients receiving adjuvant ovarian suppression. Prophylactic use of bisphosphonates is not formally approved in most countries.

30.8.3.4 HER2-Targeted Treament

For HER2+ BC, the addition of trastuzumab (anti-HER2 monoclonal antibody) to adjuvant chemotherapy nearly halves recurrence risk translating into a 10% absolute improvement in long-term disease-free survival and 9% increase in 10-year overall survival. Subset analyses demonstrated comparable relative risk reduction regardless of tumor size, nodal status, or hormone receptor status.

Due to its cardiotoxicity risk, trastuzumab should not be routinely administered concomitantly with anthracyclines. However, combination with non-

anthracycline-based chemotherapy, endocrine treatment and radiotherapy is safe. Moreover, concurrent administration of trastuzumab and chemotherapy is more active than sequential therapy, and for most patients, sequential anthracycline-based followed by taxane–trastuzumab-based regimen is the preferred choice.

Trastuzumab cardiotoxicity is typically characterized by a decrease in left ventricular ejection fraction (LVEF), which is usually asymptomatic, and often resolves with drug withdrawal. In these cases, rechallenge with trastuzumab is usually feasible. Rarely, trastuzumab cardiotoxicity may evolve in symptomatic cardiac failure. Risk factors for cardiac dysfunction with adjuvant trastuzumab include anthracycline administration, preexisting cardiac disease (e.g., borderline normal LVEF or hypertension), and age >65 years. All patients being considered for adjuvant trastuzumab require baseline determination and subsequent 3-monthly monitoring of LVEF. Cardiologist input is recommended in case of cardiotoxicity.

Standard trastuzumab duration is 12 months, as in most studies trastuzumab was administered for 12 months. Several trials tested different trastuzumab durations. The HERA trial failed to demonstrate an additional benefit for 2 years vs 1 year of trastuzumab administration [78], while the PHARE and HORG trials failed to demonstrate the non-inferiority of 6 months of trastuzumab [79, 80]. The SOLD and ShortHER trial, which tested 9 weeks of trastuzumab, also failed to demonstrate non-inferiority of the shorter regimen. However, subgroup analysis suggests that in patients with stage I-II HER2+ BC the shorter regimen might have similar efficacy, with less cardiotoxicity [81, 82]. Moreover, results from the large randomized Persephone trial, enrolling more than 4000 women, have recently shown in patients treated with 6 months of trastuzumab a similar rate of disease-free survival as those treated for 12 months (4-years disease-free survival rate was 89.4% in the 6-month group and 89.8% in the 12-month group) with less cardiotoxicity (4% of patients had to stop the drug early due to cardiac toxicity vs 8%). On this basis, a shorter duration of trastuzumab might be considered in selected patients with low risk of recurrence or cardiac risk factors.

To reduce cardiac toxicity, anthracycline-free regimens such as TCH (docetaxel-carboplatin-trastuzumab) have also been proposed. However, efficacy also appeared to be lower than with the anthracycline-taxane sequence plus trastuzumab (5-year disease-free survival of 81% for TCH vs 84% for anthracycline-taxane sequence plus trastuzumab, the study was not powered for this comparison).

Moreover, in node-negative patients with tumor diameters up to 3 cm, a small prospective non-randomized trial of 12 weeks of paclitaxel plus trastuzumab, followed by 1 year of adjuvant trastuzumab, yielded a remarkably low risk of recurrence (3-year invasive disease-free survival of 98·7%) and can be considered an option for these low-risk HER2+ BCs. This regimen might also represent an option for patients with HER2+ N0 tumors 5–10 mm, which were not included in the seminal trials but that have a relatively high failure risk, particularly in HR− disease.

HR+/HER2+ early BCs are usually treated with chemotherapy followed by endocrine treatment, in combination with trastuzumab. No randomized data exist to support an endocrine therapy-trastuzumab combination without chemotherapy in this group.

Treatment escalation using a second HER2-targeting agent has been tested.

In the neoadjuvant setting, dual HER2 blockade (trastuzumab + lapatinib, trastuzumab + pertuzumab) associated with chemotherapy has led to improvements in pathological complete response (pCR) rate as compared with chemotherapy plus trastuzumab and is currently approved in several countries. However, for the combination of trastuzumab and lapatinib, the significant pathological complete response advantage observed in NeoALTTO did not translate into a significant survival advantage 29. Similarly, no significant survival advantage was observed from the addition of lapatinib in the adjuvant setting. Therefore, the combination of trastuzumab and lapatinib cannot be recommended [27]. By contrast, the combination of trastuzumab and pertuzumab combination received approval by both the US FDA and EMA for the neoadjuvant setting. In the adjuvant setting, the large randomized APHINITY trial, testing the addition of pertuzumab to standard chemotherapy plus trastuzumab, showed a small but statistically significant decrease in the risk of invasive recurrence (3-year rates of invasive-disease-free survival were 94.1% vs 93.2%). The difference was more relevant in patients with node-positive disease (3-year invasive-disease-free survival rate 92.0% vs 90.2%) and in HR− tumors. Based on these data, the combination of pertuzumab and trastuzumab has been approved in several countries. However, in consideration of its limited impact, risks and benefits for the specific patient should be carefully assessed.

The administration of neratinib for one additional year after completion of adjuvant trastuzumab has been tested in the large randomized ExteNET trial. A 5-year invasive disease-free survival benefit was reported for patients receiving neratinib (90.2% vs 87.7%), and the

benefit was more evident in the HR+ subgroup (5-year invasive disease-free survival 91.2% vs 86.8%, HR 0.60), while the HR− cohort did not show a significant benefit (88.9% vs 88.8%, HR 0.95). Based on these data, the adjuvant use of neratinib for high-risk HER2+ BC has been approved in the United States and in Europe (only in the HR+ subgroup) (Fig. 30.3).

30.8.4 Preoperative Systemic Therapy

In early BC, preoperative chemotherapy is equally effective as postoperative chemotherapy in terms of disease-free survival and overall survival. Therefore, in addition to patients with inoperable BC, neoadjuvant systemic therapy has also emerged as an option for patients in which BCT is not feasible upfront, due to tumor size, provided that the patient has a clear indication for adjuvant chemotherapy. Overtreatment for simple local tumor reduction should indeed be avoided and neoadjuvant systemic therapy should only be given if the same therapy is indicated in the adjuvant setting.

Nevertheless, neoadjuvant treatment represents an opportunity for several BC patients. Prospective, randomized trials of patients with operable BC have shown high rates of clinical response to neoadjuvant chemotherapy (50–85%, higher in HER+ and triple-negative BC), and 25–30% of patients who were not candidates for BCT upfront were able to undergo the procedure after preoperative treatment. Moreover, pCR, defined as the absence of residual invasive cancer in the breast and axilla following preoperative therapy, can be achieved in a significant number of patients (15–40%). A meta-analysis including over 13.000 BC patients has shown that patients achieving pCR have better long-term outcomes, with lower risk of cancer recurrence, as compared to women with residual cancer [52]. Since pCR is strongly correlated with patient outcome in triple-negative and HER2+ BC, neoadjuvant therapy has become a preferred option for these patients. In fact, response allows refined counseling about the expected individual prognosis.

In the neoadjuvant setting, it is generally recommended to deliver all planned chemotherapy before surgery without unnecessary breaks to maintain dose intensity and increase the probability of pCR. After delivery of 6–8 cycles of sequential anthracyclines-taxanes, additional chemotherapy in the adjuvant setting has no proven benefit, even in the absence of pCR. However, several clinical trials are now specifically available for patients with HER2+ and triple-negative BC with residual disease. Indeed, the KATHERINE trial, which randomized HER2+ BC patients with residual disease after standard neoadjuvant treatment to receive trastuzumab emtansine (TDM-1) vs trastuzumab to complete one-year of anti-HER2 therapy, reported a significant improvement in invasive disease-free survival with TDM-1. In a similar context, the CreateX trial recently reported the potential efficacy of 6 months of adjuvant capecitabine in patient with HER2- residual disease after neoadjuvant treatment.

Neoadjuvant endocrine therapy can also be used to increase BCT rates. In postmenopausal women with HR+ tumors, the preoperative use of an AI or tamoxifen significantly increases the likelihood of breast conservation (30–40% after 4 months of treatment). In clinical practice, neoadjuvant endocrine therapy (4–8 months) is typically reserved for women not considered candidates for neoadjuvant chemotherapy (i.e., comorbidities or tumor subtypes less responsive to chemotherapy such as the lobular subtype). Due to limited data, preoperative endocrine treatment is not routinely recommended in premenopausal patients outside clinical trials.

Surgery after neoadjuvant chemotherapy has dramatically changed. Initially, the rule was to excise the original tumor bed; however, currently "no ink on tumor" is considered a standard even in the post-neoadjuvant setting. Percutaneous placement of marker clips within the primary tumor prior to the initiation of neoadjuvant treatment provides a landmark for excision in case of complete response.

Radiotherapy is generally planned based on pre-treatment disease extension.

In some rare cases, a disease progression might be observed during neoadjuvant treatment. These cases usually receive non-cross resistant chemotherapy or salvage radiotherapy to achieve radical surgery.

30.9 Follow-Up for Breast Cancer Survivors

Following initial treatment for BC, patients require surveillance for LR, contralateral BC, and distant metastatic disease. Moreover, monitoring of late treatment side effects and management of endocrine treatment is needed. Even if the maximum risk of recurrence is in the first 5 years after surgery, women with HR+ BC remain at risk for many years after treatment.

The principal aim of follow-up in BC survivors is identifying potentially curable disease, in order to improve patient outcome. Locoregional recurrences and new contralateral cancers are potentially curable, so there is a clear indication for women to undergo an annual mammography and breast examination. By contrast, it is not clear if early detection of distant meta-

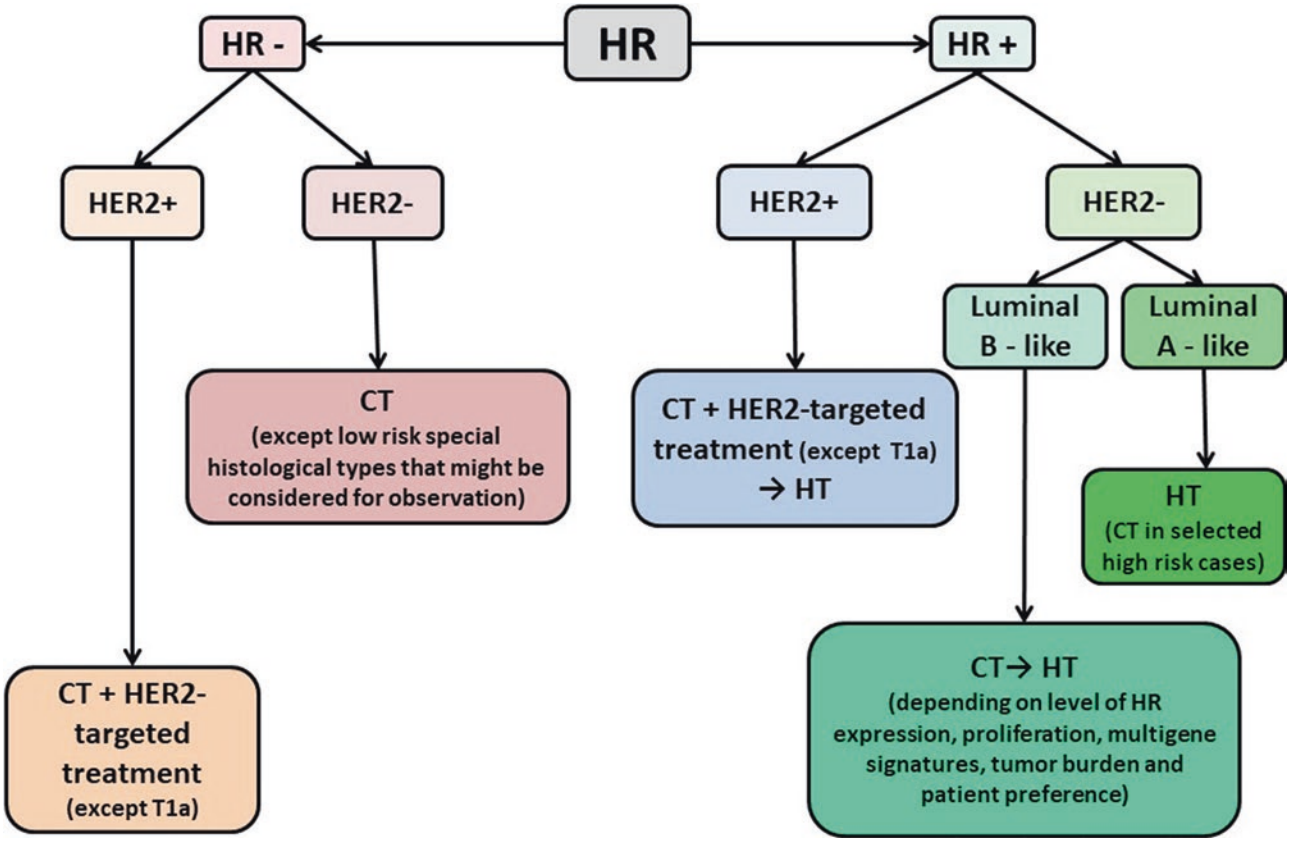


Fig. 30.3 Flowchart of adjuvant systemic treatment decision according to tumor biology

static disease can improve patient outcome. Randomized trials have compared intensive surveillance with imaging (chest X-ray, bone scan, and liver ultrasound) and blood exams (blood counts, liver function tests, and serum tumor markers) against regular physical examination and annual mammography, with additional testing performed only if clinically indicated. Intensive surveillance only achieved a modest increase in early detection of metastases in asymptomatic patients, but no impact on overall survival was observed. Based on these data, surveillance guidelines for women with early-stage BC emphasize the importance of careful history and examination to elicit symptoms or signs of recurrence and minimize the role of routine imaging and laboratory testing [27]. However, these guidelines might change in the future as efficacy of treatment in the metastatic setting increases.

30.10 Inflammatory Breast Cancer

Inflammatory BC accounts for 1–5% of all cases of BC and is characterized by diffuse erythema and edema of breast skin (peau d'orange) usually associated with a diffuse thickening of the breast. The clinical presentation results from tumor emboli in the dermal lymphatics. Inflammatory BC typically has a rapid onset and is often initially mistaken as infection. Once diagnosis is achieved, inflammatory BC is treated with neoadjuvant chemotherapy followed if possible by mastectomy. BCT is contraindicated in patients with inflammatory BC, even if a complete response is achieve, due to its diffuse nature.

30.11 Management of In Situ Malignancy

30.11.1 Surgery for In Situ Malignancy

DCIS can be treated with total mastectomy or BCT provided that adequate resection margins can be achieved. No DCIS on inked margins is considered a minimal requirement. However, no clear consensus exists regarding what should be considered an adequate margin and circumferential margins <2 mm are generally considered less than adequate. Axillary lymph node evaluation is not generally required for DCIS. Nevertheless, sentinel lymph node biopsy can be used for large and/or high-grade tumors, especially if treated with mastectomy, in case an invasive cancer is subsequently accidentally identified in the surgical specimen. Lobular neoplasia (formerly called LCIS) is only regarded as a risk factor

for future development of invasive cancer and does not require active treatment. However, its pleomorphic variant may behave similarly to DCIS and is usually treated accordingly (multidisciplinary evaluation needed).

30.11.2 Radiotherapy for In Situ Malignancy

WBRT after breast-conserving surgery for DCIS decreases the risk of LR. The decrease is evident in all subgroups; however, in some patients with low-risk DCIS (<10 mm, G1/G2 nuclear grade, adequate surgical margins), the risk of local recurrence is so low that omitting RT can be an option. After total mastectomy with clear margins for DCIS, postmastectomy RT is not recommended.

30.11.3 Systemic Adjuvant Therapy for In Situ Malignancy

In patients undergoing BCT for HR+ DCIS, tamoxifen decreases the risk of both invasive and noninvasive recurrences and reduces the incidence of second primary (contralateral) BC, without effects on overall survival. Following mastectomy, tamoxifen can decrease the risk of contralateral BC. However, the use of endocrine treatment is sometimes limited by concerns about side effects and by the absence of survival benefit.

30.12 Management of Locoregional Recurrence

Locoregional recurrence (LRR) after BC includes breast recurrences after BCT, chest wall recurrence after mastectomy, and regional nodal recurrences. It accounts for about 15% of all BC recurrences and is associated with higher stage, young age, positive margins, and intrinsic subtype (lower risk in Luminal A). Locoregional recurrence is an important predictor of metastatic disease: more than 60% of patients with LRR will eventually develop metastases (patients with short disease-free intervals, lymph node recurrence, skin lesions, and HR− tumors being at higher risk).

For this reason, patients with LRR should be accurately restaged to exclude concurrent metastatic disease.

Once metastatic disease has been ruled out, patients with LRR are treated with curative intent. Treatment is multidisciplinary and individualized based on site, prior local and systemic therapy. Usually, the first step is surgical resection. Women previously treated with BCT are treated with salvage mastectomy, patients with localized chest wall recurrences undergo surgical excision and axillary dissection is indicated for axillary nodal recurrences. Radiotherapy can also be used based on site of recurrence and previous irradiation fields.

Systemic therapy following local management is also recommended. For HR+ BC, LRR warrants the introduction or switching of endocrine therapy (e.g., for recurrences on tamoxifen, AIs should be considered). For HER2+ LRR, initiation or re-institution of anti-*HER2* therapy in an adjuvant fashion should be considered. The role of chemotherapy in this setting is more controversial, especially for patients previously treated with adjuvant chemotherapy. The CALOR trial randomized patients to "adjuvant" chemotherapy following optimal resection of LRR and a reduction in subsequent cancer recurrence, and an improvement in overall survival was observed. However, benefit was more evident in HR− tumors and no significant benefit was observed in the HR+ cohort. For patients previously treated with adjuvant chemotherapy, a non-overlapping regimen may be considered based on disease-free interval.

Case Study: Locally Advanced HER2-Positive Breast Cancer

Women, 60 years old

- *Family history* negative for malignancy
- *APR:* hypertension, hypercholesterolemia, emphysema
- *APP:* autopalpation of a mammary mass and axillary mass
- *Objective examination*: Palpable mass in the left breast (2 cm); palpable lymph nodes in the left axilla *cT1c cN2*
- *Bilateral Mammography and Ultrasonography*: in the superior external quadrant of the left breast radio opaque speculated nodule of about 1.9 cm of diameter; in the left axilla suspicious enlarged lymph nodes of 2.5 cm of maximum diameter

Question

What action should be taken?

(1) Surgery (2) Fine-needle cytology of breast mass (3) Fine-needle biopsy of the breast mass

Answer

Fine-needle biopsy of the breast mass

Histological examination:
Infiltrating ductal carcinoma, grade 3
Er 0; PgR 0, Ki-67 40%, HER2 Score 3+

+ Fine-needle biopsy of the axillary lymph node
Histological examination:
Metastasis of infiltrating ductal carcinoma

Question

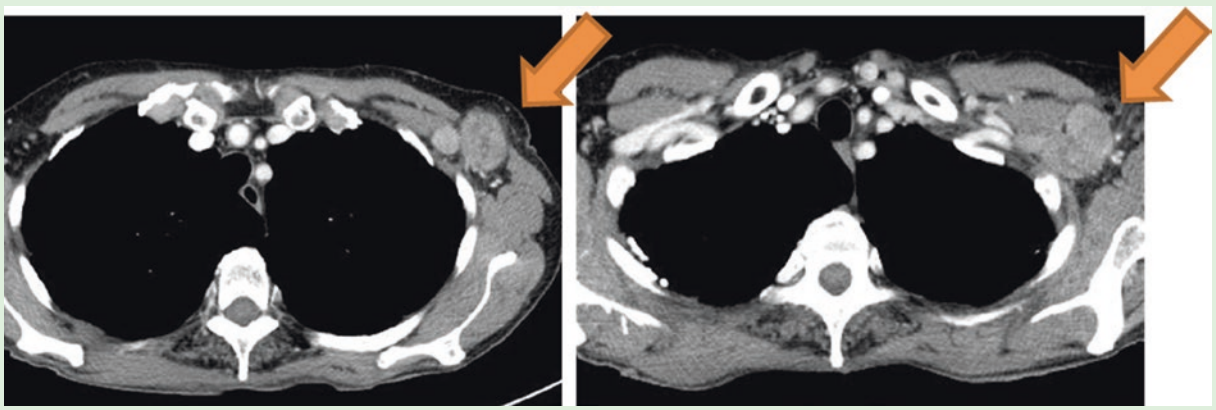
What action should be taken?

(1) Surgery (2) Start systemic treatment (3) Complete staging

Answer

Complete staging

CT scan (thorax-abdomen): 2 cm mass in the left breast, enlarged left axillary lymph nodes. No other lesions suspicious for metastases.



Bone scan: Negative

Blood tests: Normal results, CA15.3, and CEA within normality limits

Question

What action should be taken?

(1) Surgery (2) Start systemic therapy (3) Other

Answer

Start systemic therapy

Taxane + Trastuzumab (+ Pertuzumab, if available) for 3 months

⇩

Epirubicin-Cyclophosphamide for 4 cycles (3 months)

Response evaluation after neoadjuvant therapy: Complete clinical response; complete radiological response

Question

What action should be taken?

(1) Surgery (2) Continue systemic therapy (3) Start radiotherapy

Answer

Surgery: Quadrantectomy (superior external) of the left breast + axillary dissection

Histological examination:

Pathological complete response

Question

What action should be taken?

(1) Stop all treatment (2) Continue HER2-targeted treatment and start RT (3) Others

Answer

Continue HER2-targeted treatment and start RT

Trastuzumab 8 mg/kg q3w was continued for 9 months (1 year of treatment in total)

Radiation therapy delivered to the remaining breast (50 Gy + 16 Gy boost) + Regional RT

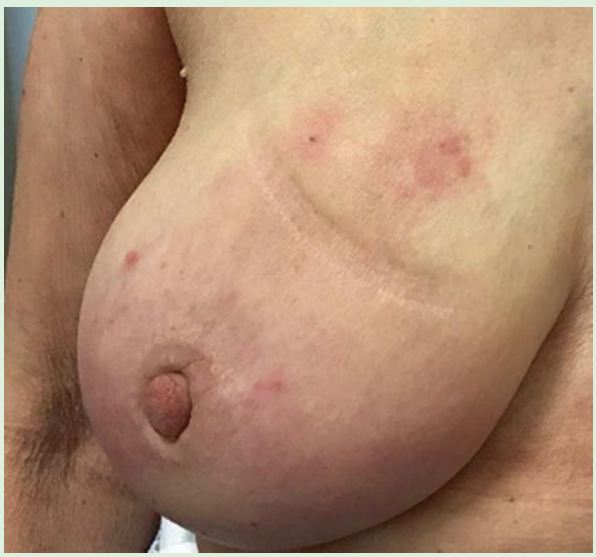
Key Points

- The importance of a correct diagnosis: histopathological diagnosis should be obtained by biopsy
- Importance of a correct staging before treatment in locally advanced breast cancer
- Use of neoadjuvant treatment in locally advanced breast cancer
- Importance of biological characteristics in treatment choice

Case Study: A Case of Triple-Negative Breast Cancer

Woman, 65 years old

- *Family history* negative for malignancy
- *APR*: negative
- *APP*: enlarged red breast since one month
- *Objective examination*: Skin edema and palpable supero-medial nodule in the right breast (4 cm)
- *Bilateral mammography and ultrasonography:* a 35 × 30 mm lesion in the upper medial quadrant of the right breast with multiple homolateral axillary lymph nodes
- *cT4b cN1*



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Question

What action should be taken?

(1) Surgery (2) Biopsy (3) Other

Answer

Fine-needle biopsy of the breast mass

Histological examination:

Infiltrating ductal carcinoma, grade 3

ER 0% PgR 0% Ki-67 80% HER2 0

CT scan (thorax-abdomen): negative for metastasis

Bone scan: negative for metastasis

Question

What action should be taken?

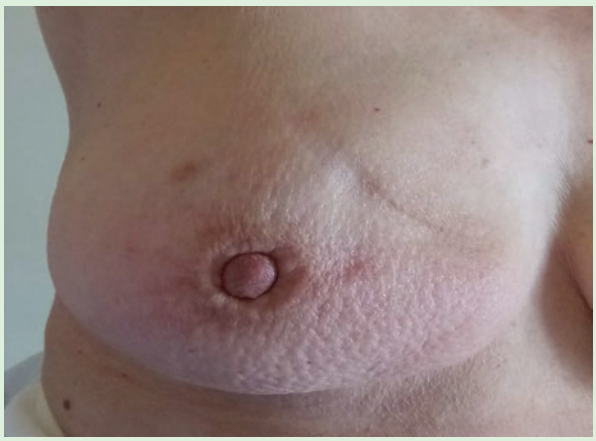
(1) Surgery (2) Start systemic therapy (3) Start Radiotherapy

Answer

Start systemic therapy

Weekly paclitaxel 80 mg/kg for 10 doses

After 10 doses, *worsening of pain, edema, and erythema in the right breast -> Clinical progression of disease*



Restaging: Chest-abdomen CT scan (negative)

*Breast ultrasound: a 40 × 35 mm l*esion in the upper medial quadrant of the right breast with multiple homolateral axillary lymph nodes

Question

What action should be taken?

(1) Surgery (2) Switch to non-cross resistant systemic therapy (3) Best Supportive Care

Answer

Switch to non-cross resistant systemic therapy

Epirubicin-Cyclophosphamide for 3 cycles (patient refuses to continue) with marginal clinical tumor response

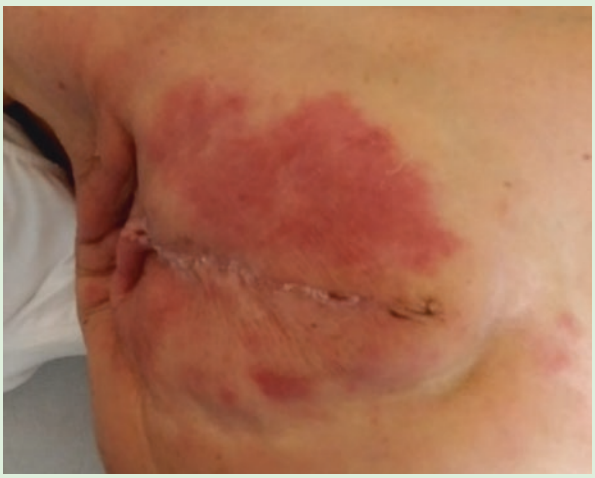
Surgery: Right mastectomy and homolateral axillary dissection

Histological examination:

Infiltrating ductal carcinoma ypT3 (6.5 cm) ypN3 (12 affected lymph nodes/13 resected lymph nodes)

ER 0%, PR 0%, Ki67 50%, HER2 0

At clinical examination: edema and erythema around the surgical scar



Skin biopsy: infiltrating ductal carcinoma G3 (triple-negative IHC) -> *locoregional recurrence*

Patient starts salvage radiotherapy + Carboplatin

Key Points

- Importance of clinical examination in breast cancer staging and re-evaluation of response to treatment
- Importance of readapting treatment based on response
- Importance of biological characteristics in treatment choice

Expert Opinion

Antonio Russo

Key Points

Breast cancer is a major public health problem throughout the world.

- Multiple factors are associated with an increased risk of developing BC, such as age, gender, family history, genetic alterations, diet, and life style.
- In women, aged between 50 and 69 years, benefits of population mammography screening appear to outwait risks, and mammography screening, repeated every 2 years, is recommended by most countries.
- Management strategies for high-risk women include intensive surveillance, chemoprevention with endocrine agents, and, for extremely selected patients, prophylactic surgery.
- Breast cancer is a biologically heterogeneous disease. Biological characteristics of the disease play a fundamental role in treatment choice.
- Histopathological diagnosis of breast cancer should be obtained by biopsy.
- Treatment of breast cancer patients should be provided by a multidisciplinary team and should be carried out in "breast units" defined as specialized departments that treat a high volume of BC patients.
- Locoregional treatment (surgery and radiotherapy) aims to the radical excision of macroscopic disease, local staging of the disease, and treatment of residual tumor cells in the breast and nodes in order to limit the risk of locoregional recurrence.
- Systemic treatment aims to eradicate systemic micrometastases which might have originated even from early stage BC, reducing the risk of distant and locoregional recurrence at the same time.

1. Breast cancer (BC) is the most frequent cancer in women (1.7 million cancer diagnosed per year), and today regarded as a major public health issue. In the last decades, there has been an increase in incidence and a reduction of mortality, probably due to the diffusion of screening campaigns.
2. BC is linked to several risk factors such as age, family history, or genetic predisposition (i.e., mutation in BRCA1, BRCA2 genes), parity, BMI, combined hormone replacement therapy (estrogen and progesterone), cigarette smoke, previous radiotherapy in thoracic region, and prior diagnosis of BC. It is almost likely that these factors interact in a multimodal manner; it is although possible to identify high-risk women who can be submitted previously to screening programs or preventive treatments (i.e., intense surveillance, chemoprevention with endocrine agents or prophylactic mastectomy).
3. Screening programs have changed the history of this neoplasm: mammography can detect small, non-palpable lesions. In women between 50 to 69 years of age, benefits of mammography appear higher than its risks; otherwise, limited evidence of effectiveness regard 40–49 year-old women.
4. According to the latest WHO classification, it is possible to recognize different types: invasive ductal carcinoma (the most frequent form), invasive lobular carcinoma (the second most frequent one), special types (i.e., pure tubular, mucinous, papillary etc.), and carcinoma in situ. It is possible to use also a molecular classification for BC that recognize five types: Luminal A, B, HER-2 enriched, basal-like, and normal-like, considering the expression of different genes. The most important prognostic and predictive factors are grading (1–3), estrogen and progesterone receptors, HER2 expression, and cellular proliferation.
5. Clinical presentation usually consists in the evidence of a palpable, non-tender, hard nodule; sometimes, the involvement of the nipple or the evidence of a cutaneous inflammation (inflammatory carcinoma), with edema, tenderness, and erythema is possible. Otherwise, it is possible to detect a nodule during the mammographic screening. Clinical examination is the first step, quite useful to understand the involvement of axillary supraclavicular, infraclavicular lymph nodes. Then a mammography is mandatory often accompanied by US, which allows to study regional lymph nodes. Biopsy ensures the presence of a BC, and it is essential to classify it; two methods can be used: FNA (usually used to assess axillary lymphnodes) or core needle biopsy; excisional biopsy nowadays is used just in particular features.
6. Treatments differ depending on stage and biology. Locoregional approaches consist in surgery (quadrantectomy, lumpectomy, or mastectomy with different techniques) and radiotherapy. Systemic therapies can be used in the neoadjuvant setting in order to shrink the tumor burden and allowing radical operability. After mastectomy, reconstructive techniques are usually implied using myocutaneous tissue flaps or implants and/or tissue expanders.

   Radiotherapy is strongly recommended after breast-conserving surgery reducing the risk of local recurrence of BC and post-mastectomy radiation and regional radiation can be used in higher-risk patients. Adjuvant therapy has the role to prevent BC recurrence, and it can consist in endocrine treatment, chemotherapy, and anti-HER2-targeted treatment (trastuzumab) considering the characteristics of the BC.
7. Follow-up is essential to early diagnose local and locoregional recurrences, contralateral BC, distant metastases and to monitor long-term treatment toxicities.

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Hints for a Deeper Insight

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Metastatic Breast Cancer

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Breast Cancer

Contents

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31.1 Introduction

Breast cancer (BC) represents the most common malignancy among women worldwide. It has been estimated that 1.67 million of new cancer cases were diagnosed in 2012, with incidence rates ranging from 27 per 100,000 in Middle Africa and Eastern Asia to 92 per 100,000 in Northern America. Despite the overall increased incidence of BC occurred worldwide in the past decades, since the late 1990s, there was a constant reduction of mortality, especially in western countries, probably due to improvement of treatment strategies, as well as early diagnosis [1]. As matter of fact, only about 6% of BC cases present at diagnosis as metastatic "de novo" disease [2]. Additionally, 20–30% of patients diagnosed at early stages are expected to develop metastatic disease [3].

Traditionally, BCs are divided into different subtypes defined by immunohistochemistry (IHC), according to the expression of estrogen receptor (ER), progesterone receptor (PgR), grading, proliferative index ki67, and overexpression/amplification of human epithelial growth factor receptor 2 (HER2).

According to the 15th St. Gallen International Breast Cancer Conference Expert Panel [4], BC could be divided in four IHC subtypes: (1) luminal A-like tumors, which are typically low-grade, strongly ER/PgR-positive, and HER2-negative and have low proliferative fraction; (2) luminal B-like tumors, which are ER-positive but may have variable degrees of ER/PgR expression, are higher grade, have higher proliferative fraction, and can be also subdivided in luminal B HER2-positive and luminal B HER2-negative [5], according to the presence of HER2 amplification/overexpression; (3) HER2-positive tumors, which are ER/PgR-negative and HER2-positive; (4) triple-negative, which are ER/PgR-negative and HER2-negative, and corresponds to the most aggressive histological subtype.

This classification still represents the mainstay for treatment choice even if gene expression profiling demonstrated to give additional prognostic information and to be more accurate in the definition of tumor cell biology than IHC. Indeed, within the same BC intrinsic subtype, a variety of biological distinct entities can be identified; as an example, within the triple-negative BC (TNBC) subgroup, several molecular subtypes have been recognized, as shown by Lehmann and colleagues, who classified TNBC into six distinct subtypes, namely, basal-like 1 and 2, mesenchymal and mesenchymal-stem-like, immunomodulatory, and luminal androgen receptor, with potential clinical implications [6].

The risk and the pattern of BC recurrence is correlated to the initial tumor stage at presentation and to tumor biology. HER2-positive and TNBCs tend to relapse within the first 5 years after initial diagnosis of early BC, whereas in hormone receptor (HR)-positive tumors, late relapses are more frequent. Tumor biology could also influence the specific sites of recurrence. In a study by Kennecke et al. [7] high rates of brain metastases were demonstrated among HER2-enriched (28.7%), basal-like (25.2%), and non-basal triple-negative (22%) tumors, whereas they were less frequent in the luminal/HER2 (15.4%) and other groups ($p = 0.001$). In contrast, bone was the predominant metastatic site for the luminal A (66.6%), luminal B (71.4%), and luminal/HER2 (65%) groups and the least a common site of metastases in the basal group (39%).

Breast cancer can metastasize anywhere in body, but the most common metastatic sites are bones, lungs, lymph nodes, liver, and brain, being the bone the most frequent initial metastatic site [8].

Metastatic BC is currently considered an incurable disease. Therefore, the main treatment objectives are improving quality of life and prolonging patient survival. In this scenario, systemic treatments represent the mainstay in the therapeutic management of metastatic BC, whereas local therapies, such as surgery and radiotherapy, are limited to peculiar situations (Fig. 31.1).

Three major therapeutic subtypes are considered for the choice of systemic treatment of metastatic BC: HR+/HER2-negative, HER2+, and triple-negative disease. Systemic treatment options for each therapeutic subtype are described separately in the next paragraphs.

31.2 Systemic Treatment

31.2.1 HR+/HER2-Negative Disease

HR+/HER2-negative (HER2−) BCs accounts for about 65% of all breast cancers. Endocrine-based therapies represent the mainstay of treatment for this BC subtype, even in presence of visceral disease [9]. Chemotherapy, instead, is the required treatment in the presence of "visceral crisis," defined by the 3rd ESO-ESMO International Consensus Guidelines for Advanced Breast Cancer (ABC) as "severe organ dysfunction as assessed by signs and symptoms, laboratory studies and rapid progression of disease. Visceral crisis is not the mere presence of visceral metastases but implies important visceral compromise leading to a clinical indication for a more rapidly efficacious therapy, particularly since another treatment option at progression will probably not be possible" [10]. Fortunately, visceral crisis is not a common clinical presentation of HR+/HER2− metastatic BC.

Another important aspect which can guide treatment choice is the presence of endocrine resistance, which is empirically classified in primary and secondary resistance. Primary (de novo) endocrine resistance is defined by the presence of a BC relapse within the first 2 years

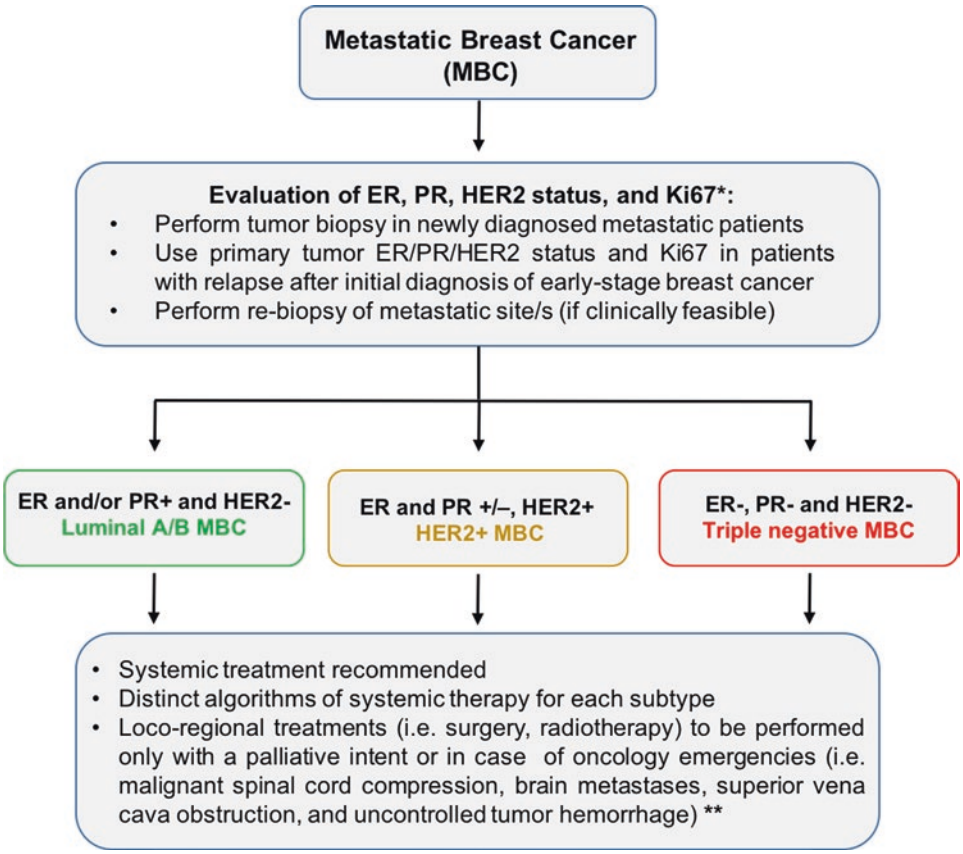

Fig. 31.1 Clinical management of metastatic breast cancer: general aspects. *Evaluation of Ki67 is relatively important to guide treatment decision in MBC. It can serve as an indicator of biologic aggressiveness and endocrine sensitivity (i.e., luminal A vs luminal B tumors). **Locoregional treatment to be performed with curative intent only in selected cases before or after systemic treatment (i.e., isolated local relapses, oligometastatic disease)

of adjuvant endocrine therapy (ET) or during the first 6 months of ET, when administered for metastatic disease. Secondary (acquired) endocrine resistance is typically defined by the presence of tumor relapse between 2 years after the beginning and 1 year after the end of adjuvant ET or by disease progression after 6 months of ET in the metastatic setting [10]. Importantly, in the presence of primary endocrine resistance, the probability of response to ET is very low; thus chemotherapy or molecularly targeted agents (see below) should be the preferred option. On the contrary, the probability of response to ET is substantially higher in the presence of acquired endocrine resistance and maximum in endocrine sensitive metastatic BC. Therefore, it is crucial to correctly predict the potential endocrine sensitivity, in order to define the best treatment option for each patient.

Our understanding about the molecular mechanisms of endocrine resistance has evolved over the past two decades. Among the different potential mechanisms identified as responsible for the development of endocrine resistance, two have been particularly studied in the last few years: genome aberrations affecting the gene encoding for ER (ESR1), considered as drivers of resistance to endocrine therapy in 15–40% of patients [11]; and activation of mammalian target of rapamycin (mTOR) signaling pathway mediated by various mechanisms, including overexpression of human epidermal growth factor receptor family members, activating mutations in PIK3CA (gene encoding for phosphatidylinositol-4, 5-bisphosphate 3-kinase – PI3K catalytic subunit alpha), in AKT1 (encoding for serine-threonine kinase 1 – AKT), and HER2, found in 30%, 4%, and 2% of patients, respectively [11]. In addition, cyclin-dependent kinase 4 (CDK4) and CDK6 have been recently identified as key drivers of tumor cell proliferation in HR+/HER2– BC [11]. Furthermore, the amplification of FGFR1 (the gene encoding fibroblast growth factor receptor 1), found in 10% of patients, was also investigated for its role in BC oncogenesis and endocrine resistance [11].

Basing on these evidences, the treatment for HR+/HER2– metastatic BC has been radically changed over the past few years by the introduction of several targeted agents administered in combination with ET, such as the selective CDK4/6 inhibitors palbociclib, ribociclib, and abemaciclib. These drugs have been studied in various lines of therapy, but their clinical benefit was demonstrated primarily in the first- and second-line setting. In first line, the randomized phase III clinical trial PALOMA 2 clearly demonstrated the advantage of adding palbociclib to the aromatase inhibitor letrozole in women with endocrine sensitive metastatic BC: median progression-free survival (PFS) was 24.8 vs. 14.5 months in palbociclib vs. placebo group ($p < 0{,}0001$) [12]. A

very similar trial, the MONALEESA 2, showed that the addition of ribociclib to letrozole significantly improved PFS compared with letrozole alone: PFS not reached vs. 14.7 months in ribociclib vs. placebo group (p = 0,00000329) [13]. More recently, also abemaciclib demonstrated its efficacy in first-line setting when added to a nonsteroidal aromatase inhibitor (NSAI – letrozole or anastrozole) in the MONARCH 3 study where the combination treatment showed similar advantages in terms of PFS over the placebo group (p = 0.000021) [14].

In second line, the PALOMA 3 study showed that the addition of palbociclib to the selective estrogen receptor downregulator (SERD) fulvestrant in patients with acquired endocrine resistance improved PFS when compared with fulvestrant alone: PFS 9.5 vs. 4.6 months (p < 0.0001) [15]. Finally, the MONARCH 2 trial assessed the benefit of adding abemaciclib to fulvestrant in endocrine-resistant patients: PFS 16.4 vs. 9.3 months (p < 0.001) [16]. Overall, the three CDK4/6 inhibitors showed a similar and good safety profile, although some differences have to be pointed out. Palbociclib and ribociclib resulted in all grade neutropenia in 66.5% and 59.3% respectively, while abemaciclib therapy was complicated by neutropenia only in 26.5% of patients. However, 13.4% of patients treated with abemaciclib experienced diarrhea, while this percentage was about 1% in patients treated with palbociclib and ribociclib.

Altogether these results suggest a remarkable benefit that can be obtained by adding CDK4/6 inhibitors to ET, and this leads to a substantial change in treatment algorithms for HR+/HER2− metastatic BC. Indeed, to date, CDK4/6 inhibitors are considered the standard for either first or second line of therapy (basing on previous treatment) associated with letrozole or fulvestrant, respectively (see Fig. 31.2).

As mentioned before, the activation of the mTOR signaling pathway is another important mechanism of treatment resistance in HR+/HER2− metastatic BC [11]. The clinical relevance of mTOR blockade has been assessed by the BOLERO 2 trial [17], in which patients, previously treated with an NSAI, were randomized to receive exemestane + everolimus vs. exemestane + placebo. The median PFS per central assessment was 11.0 vs. 4.1 months in the treatment vs. placebo group (p < 0.001). Currently, the mTOR inhibitor everolimus is indicated in the treatment of HR+/HER2− metastatic BC progressing after NSAI therapy, administered either in the adjuvant or metastatic setting (Fig. 31.2).

Recently, other trials have investigated the effect of PI3K/AKT/mTOR pathway inhibition by the use of PI3K inhibitors. The PIK3CA gene is frequently mutated in breast cancer: it is estimated that 30% of luminal HR+/HER2− BCs harbor an activating PIK3CA mutation [11], which lead to overactive downstream signaling and mediate proliferation and survival, as well as capability of migration and invasion of tumor cells [11]. Currently, there are several PI3K inhibitors in clinical development, and they could be classified in two major categories: pan-PI3K inhibitors and isoform specific inhibitors (designed to be selective to one or more of the four isoforms of the catalytic subunit of PI3K). The pan-isoform PI3K inhibitor buparlisib has been studied in the phase III randomized BELLE2 study [11], which investigated the efficacy of buparlisib plus fulvestrant versus placebo plus fulvestrant in 1147 postmenopausal women with metastatic BC progressed on an aromatase inhibitor. PFS was significantly improved from 5.0 to 6.9 months (HR 0.78, 95% CI 0·67–0·89; p < 0·001) by the addition of buparlisib. However, the treatment was complicated by several side effects: hyperglycemia, rash, fatigue, elevated transaminase, stomatitis, nausea, vomiting, and diarrhea. Mood disorders such as anxiety, irritability, and depression were also frequent, because the drug is able to cross the blood-brain barrier. BELLE2 study did not support the use of buparlisib, because of the small magnitude of benefit and induced toxicity [11].

Isoform-specific PI3K inhibitors aim to more selectively inhibit the driver oncogene and thus reduce toxicity and more potently inhibit the targeted oncogene. The ongoing phase III trial SANDPIPER and the recently published phase III trial SOLAR-1, enrolled patients with HR+/HER2-negative MBC to receive the alfa-selective PI3K inhibitors taselisib and alpelisib, respectively, in combination with fulvestrant. Alpelisib + fulvestrant showed a significant PFS improvement compared to fulvestrant alone in first-/second-line patients with tumors harboring a PIK3CA-mutation (HR: 0.65, 95% CI: 0.50–0.85, p<0.001). The study did not show any benefit for the PIK3CA-wild type cohort (HR: 0.85, 95% CI: 0.58–1.25). All patients had been pretreated with an aromatase inhibitor in the neo/adjuvant or metastatic setting. The combination was well tolerated, although G3-4 adverse events were more frequent with alpelisib and mainly represented by hyperglycemia (32.7%), diarrhea (6.7%), rash (9.9%) and fatigue (3.5%) [11, 18].

Despite the positive results achieved by ET combined with several target therapies, ET alone could still be considered a valid treatment option in the first-line setting, for some patients with endocrine sensitive disease. As support to this hypothesis, in the recent randomized phase III FALCON trial [19], fulvestrant confirmed to be a good treatment option in endocrine naïve patients, as it was superior to the aromatase inhibitor anastrozole (PFS was 16.6 vs. 13.8 months, p = 0.0486). Of note, the difference between the two endocrine agents was significant only in patients without visceral disease, where treatment with fulvestrant was associated with a particularly long median PFS (24 months).

In this complex therapeutic scenario, defining an optimal treatment algorithm is challenging, and several

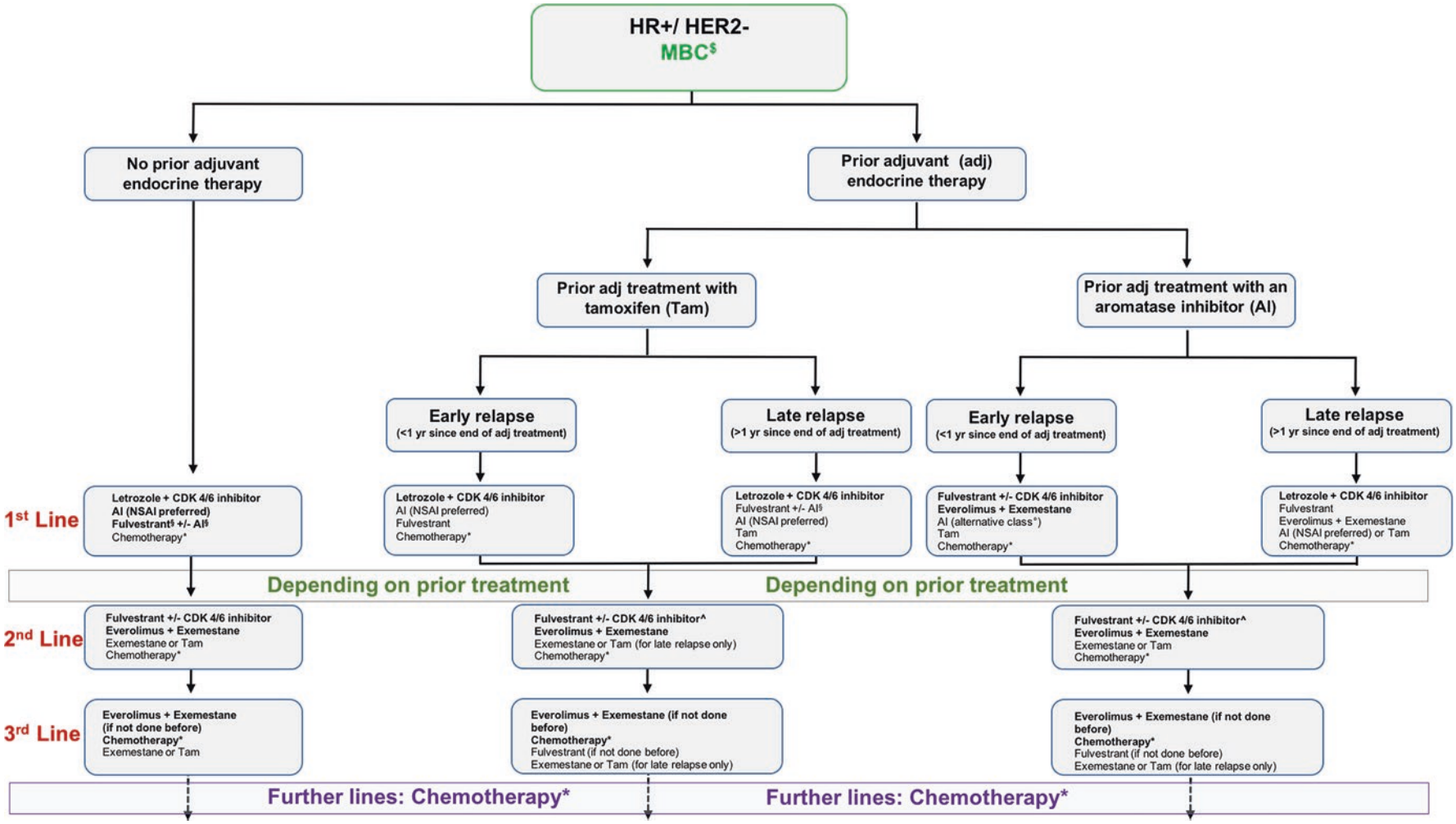

Fig. 31.2 Systemic treatment algorithm for HR+/HER2− metastatic breast cancer. *Additional abbreviations:* NSAI: Nonsteroidal aromatase inhibitor. $The present treatment algorithm applies to postmenopausal patients. For premenopausal patients consider adding LH-RH therapy (to induce pharmacological menopause) to endocrine +/− targeted agents. *Chemotherapy always preferred in case of symptomatic visceral disease. For the choice of chemotherapy scheme, please refer to treatment algorithm of triple-negative MBC. In case of objective response/stable disease achieved by chemotherapy, consider to continue with maintenance endocrine therapy or metronomic chemotherapy (in the absence of a clear standard). §Not yet authorized by AIFA. °Steroidal inhibitor if previously it was administered a nonsteroidal inhibitor, and vice versa. ^CDK 4/6 inhibitors can be administered only one time

treatment options could be considered in each case (Fig. 31.2). Therefore, the identification of predictive biomarkers of response, useful to guide treatment choice, is critical. Unfortunately, no predictive biomarkers for CDK4/6 inhibitors have been identified with certainty so far [11]. On the contrary, ESR1 mutations showed to be predictive of response in patients treated with everolimus and fulvestrant. Finally, the benefit associated with PI3K inhibitors seems to rely on the presence of PIK3CA mutations [18].

In patients with endocrine refractory disease and/or with visceral crisis, the standard indication is to perform chemotherapy with or without targeted agents. In this case the treatment algorithm is the same of that adopted in triple-negative metastatic BC with only few exceptions (see section entitled "Triple-Negative Disease" and Fig. 31.4).

31.2.2 HER2+ Disease

HER2-positive tumors accounts for about 15–20% of all BCs. Despite the aggressive biology of HER2+ BC, which is responsible for its relatively poor prognosis, the introduction of effective anti-HER2 therapies has dramatically changed the natural history of this disease. Importantly, HER2 signaling pathway represents the main driver of proliferation and survival in HER2+ cancer cell. Thus, complete inhibition of HER2 pathway represents the most effective treatment for this BC subtype [20].

HER2 status is assessed by immunohistochemistry and/or by fluorescence in situ hybridization (FISH) [21]. Considering the possibility of discordance in HER2 status between primary and metastatic tumor (discordance rate up 25%), and the critical importance of anti-HER2 therapies in this disease subtype, re-biopsy should be always taken into consideration if clinically possible, in case of relapse of HER-negative primary tumors [20].

The first anti-HER2 agent successfully introduced into clinical practice is trastuzumab, which is a monoclonal antibody directed against the extracellular domain of HER2 receptor. Trastuzumab inhibits the omo- and heterodimerization of HER2 receptors, impeding the activation of downstream signaling, determining increased endocytotic destruction of the receptor, and finally inducing immune-mediated cytotoxicity (ADCC – antibody-dependent cell-mediated cytotoxicity). For many years, the anti-HER2 monoclonal antibody trastuzumab in combination with a taxane (paclitaxel or docetaxel) has been the standard first-line treatment for HER2-positive metastatic BC basing on the pivotal trial carried out by Slamon and colleagues [20].

Recently, the phase III randomized trial CLEOPATRA [22, 23] showed that the addition of the humanized monoclonal antibody pertuzumab to the standard first-line therapy with trastuzumab and docetaxel was associated with a significant improvement of overall survival (OS) and PFS (OS 56.5 vs. 40 months for the standard and experimental treatment, respectively). Basing on these positive results, dual HER2 blockade with trastuzumab and pertuzumab in combination to a taxane has become the new standard first-line therapy for HER2-positive metastatic BC patients. The reason for the remarkable improvement of patient outcome achieved by the addition of pertuzumab to anti-HER2 therapy relies on the fact that this monoclonal antibody is designed to bind the extracellular dimerization domain of HER2 and inhibit the ability of this receptor to interact with other HER family members (HER1, HER2, HER3, and HER4), determining a complete and effective inhibition of HER signaling.

Another novel anti-HER2 drug, successfully tested in metastatic BC is the antibody-drug conjugate trastuzumab emtansine (TDM1). TDM1 is a complex molecule where the antibody trastuzumab is linked to the microtubule inhibitory agent emtansine (DM1). The molecular structure of TDM1 allows intracellular drug delivery of the potent cytotoxic drug emtansine to HER2-overexpressing cells, thereby improving the therapeutic index and minimizing exposure of normal tissue. The efficacy of TDM1 as second-line therapy for HER2+ metastatic BC was shown in the EMILIA trial, where this agent determined a significant improvement of both PFS and OS in comparison with the combination of the oral chemotherapy agent capecitabine together with the dual HER1/HER2 tyrosine kinase inhibitor lapatinib, which was the standard second-line therapy at the time of study beginning [24]. Additional trials evaluated TDM1 in subsequent lines of therapy [25, 26]. Finally, TDM1 alone or in association with pertuzumab did not demonstrate to be superior to docetaxel + trastuzumab in the first-line setting [27]. Therefore, TDM1 is currently recommended in second or subsequent line of therapy (Fig. 31.3).

An additional treatment option for HER2+ metastatic BC to be administered as second or subsequent line of therapy is lapatinib in association with capecitabine. This option could be particularly useful in patients with brain metastases, since lapatinib has been shown to penetrate the blood-brain barrier [21]. Of note brain metastases represent a major clinical challenge in HER2-positive BC since they occur in up to 50% of patients with this disease subtype [21, 28]. T-DM1 also demonstrated to be effective in case of brain metastases, in a retrospective subgroup analysis of the EMILIA trial [29].

Heavily pre-treated patients could benefit from the re-challenge of trastuzumab combined with different chemotherapy agents [21] (Fig. 31.3).

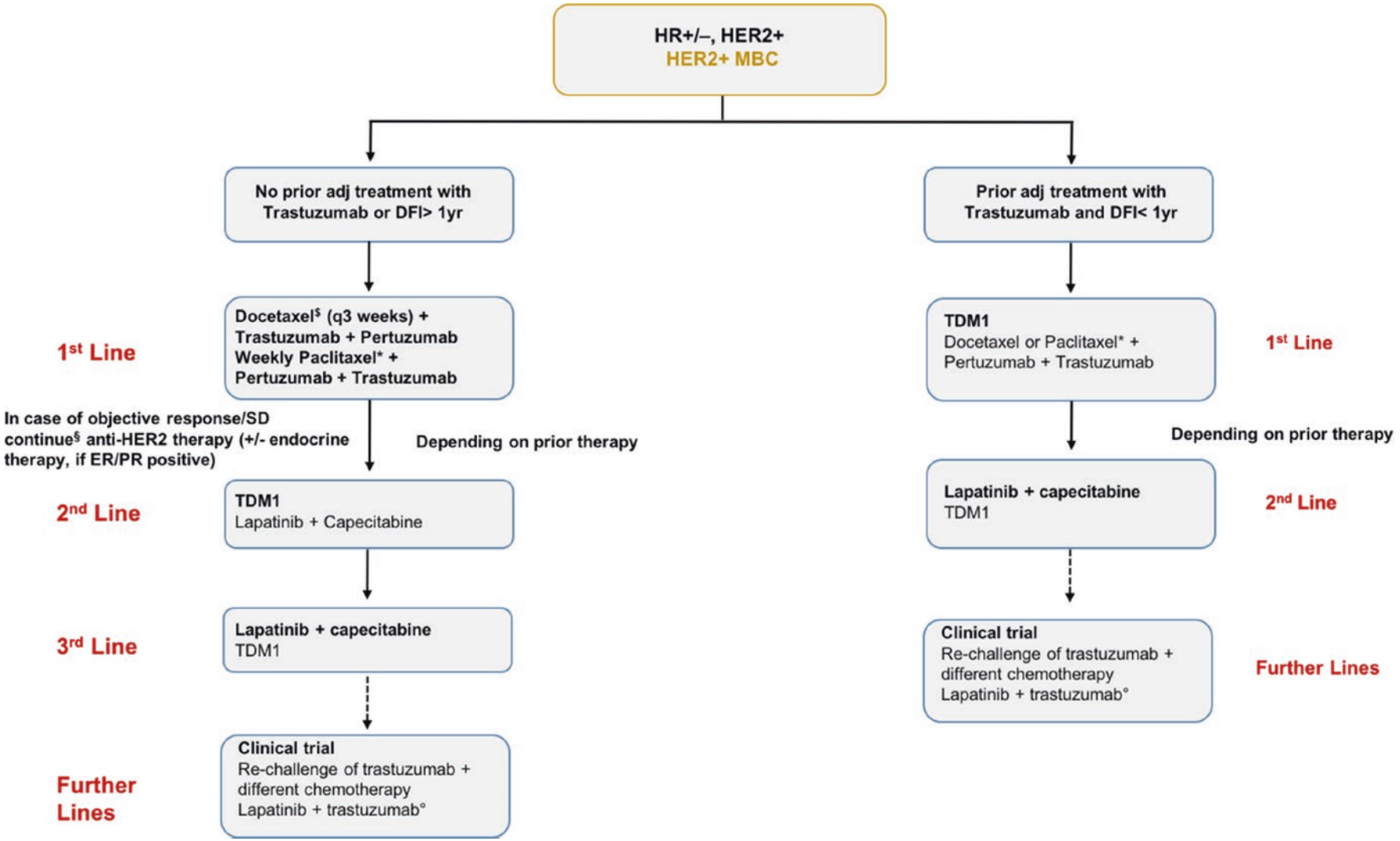

Fig. 31.3 Systemic treatment algorithm for HER2+ metastatic breast cancer. *Additional abbreviations:* DFI: Disease-free interval (from the end of adjuvant treatment). §At least 6 cycles should be administered. *Alternative to docetaxel in case of reported unacceptable toxicity or known intolerance. §Switch to maintenance therapy when maximum tolerability is reached. °Not reimbursed

Finally, for patients with HR+/HER2+ disease, anti-HER2 therapy combined with endocrine treatment is a valid option, either as maintenance therapy in case of objective response/stable disease achieved with chemotherapy + anti-HER2 therapy or as upfront treatment in patients who are not fit for chemotherapy.

31.2.3 Triple-Negative Disease

Triple-negative breast cancers (TNBC) subgroup accounts for about 15% of all BCs and is characterized by the absence of estrogen and progesterone receptor expression and the lack of overexpression/amplification of HER2 [30]. Patients affected by TNBC do not benefit from either endocrine or anti-HER2 therapies; thus standard treatment choice in this subgroup of patients is chemotherapy with or without targeted agents. The prognosis of TNBC patients remains poor (fewer than 30% of patients with metastatic TNBC survive 5 years after diagnosis) [31] due to the lack of specific "target" therapies and to the rapid onset of metastasis (probably due to the very high proliferative index), despite the high response of this BC subgroup to chemotherapeutic agents [30]. In general, international guidelines [10, 32] recommend the use of sequential single-agent chemotherapy, whereas the combination of chemotherapeutic agents (poly-chemotherapy) should be adopted for patients with symptomatic and rapidly progressive disease, which requires rapid tumor debulkying. However, only patients without impairment of multi-organ function are eligible for poly-chemotherapy, as it is associated with higher risk of toxicity. The most effective sequencing of chemotherapy agents in the treatment of metastatic TNBC has yet to be defined. In the following paragraphs, the most active chemotherapy-based therapeutic schemes for both TNBC and endocrine-refractory HR+/HER2− metastatic BC are reported.

- *Anthracyclines* [33]: these drugs are among the most active class of chemotherapy agents in breast cancer, achieving an overall response rate (ORR) in HER2-negative disease between 30% and 50%. It consists of doxorubicin, epirubicin and pegylated liposomal doxorubicin. The latter showed similar PFS and OS results comparing to the traditional form of anthracyclines, with lower rates of cardiotoxicity. Due to the frequent use of antracyclines in the neo-/adjuvant setting and considering the probability of cardiotoxicity due to the exceeding of cumulative dose levels (ranging from 450 mg/m^2 for doxorubicin to 900 mg/m^2 for epirubicin), their use in metastatic setting can be limited, although liposomal anthracyclines allow to expose the patients to much higher cumulative doses without a substantial increase of the risk of cardiotoxicity. A recent meta-analysis comparing anthracyclines and taxanes showed a modest superiority in ORR (38% vs. 33%) and PFS (7 vs. 5 months) in favor of anthracyclines group of patients. However, the strength and clinical applicability of these results were limited due to trial heterogeneity and by the cumulative toxicity in patients which were treated in adjuvant setting. As mentioned before, the combination of anthracyclines with other chemotherapy agents (i.e., taxanes, cyclophosphamide, etc.) is associated with superior ORR and PFS at the cost of higher toxicity rates.
- *Taxanes* [33]: are anti-mitotic agents widely and commonly used in BC. Taxane-based schemes are among the most effective systemic therapies in metastatic BC. This class of drugs includes docetaxel, paclitaxel, and nab-paclitaxel (paclitaxel bound to nanomolecules of albumin). The latter is a novel formulation, which requires a shorter infusion time and does not need steroid pre-medication, because of its albumin-bound formulation, and is associated with a lower risk of allergic reactions. Taxanes can be administered as single agents (for paclitaxel a weekly schedule is preferred) or in association with other chemotherapy drugs, including anthracyclines. Moreover, weekly paclitaxel associated with the antivascular endothelial growth factor (VEGF) monoclonal antibody bevacizumab is one of the standard options as first-line treatment for HER2− metastatic BC. However, the use of bevacizumab is still debated as the combination of bevacizumab + chemotherapy determined improvement of PFS but was not associated with improvement of overall survival (OS) in any of the prospective randomized trials that tested this treatment strategy.
- *Eribulin* [33]: this chemotherapeutic agent is currently approved for the treatment of HER2+ metastatic BC patients who progressed after receiving anthracyclines and taxanes. It blocks cell cycle in the M phase by inhibiting microtubule polymerization.
- *Capecitabine* [33]: is an oral chemotherapy agent, pro-drug of the anti-metabolite 5-fluorouracil (5-FU). It can used in the first-line metastatic setting (especially in patients pre-treated with antracyclines and taxanes in neo-/adjuvant setting), because of its oral administration and relatively advantageous safety profile.
- *Vinorelbine* [33]: is a commonly used chemotherapy agent in TNBC and is a semi-synthetic vinca alkaloid, with activity in heavily pretreated patients (ORR: 25–45%). This agent can be administered alone or in combination with capecitabine.
- *Gemcitabine* [33]: this antimetabolite is typically administered in combination with other drugs, such as taxanes or platinum salts, since it showed low response rates when administered as single agent.
- *Platinum salts* [33]: these drugs (i.e., carboplatin and cisplatin) cause DNA crosslink strand breaks resulting

in tumor cell apoptosis. The association of cisplatin and paclitaxel resulted in a better PFS when compared with gemcitabine and paclitaxel in unselected metastatic TNBC patients [30]. In the phase III Triple-Negative Breast Cancer Trial (TNT) [30], carboplatin monotherapy was directly compared with docetaxel in patients with metastatic TNBC. Overall, carboplatin was not superior to docetaxel. However, in patients carrying BRCA1/2 mutations carboplatin was significantly superior to docetaxel. Similar results were also found in the non-randomized TBCRC009 trial [30], where BRCA-mutated patients demonstrated increased response rates with platinum therapy. These studies are paving the way to an increasing use of platinum salts in metastatic TNBC patients, especially for those carrying mutations of BRCA genes.

To date the optimal treatment algorithm for metastatic TNBC and endocrine-refractory HER2− disease is still debated, and several options can be considered for each line of therapy (Fig. 31.4), according to previous treatments, disease burden, comorbidity, expected toxicity, and patients' preferences.

Besides chemotherapy, other biologically targeted agents (in addition to bevacizumab) have been recently tested in HER2-negative metastatic BC. In particular, the poly (ADP-ribose) polymerase (PARP) inhibitors olaparib and talazoparib showed to improve PFS in *BRCA*-mutated patients with HER2− metastatic BC, comparing with physician's choice chemotherapy [34, 35]. PARP is a constitutively expressed nuclear enzyme that modulates DNA repair and cell survival. In response to DNA single-strand and double-strand breaks, it has been reported an immediate catalytic activation [31]. In normal cells with no mutations of BRCA1 and BRCA2 genes, double-strand breaks can be repaired by homologous recombination, but in BRCA1- or BRCA2-mutated cells, homologous recombination is not functioning, and thus DNA strand breaks rely on PARP action for repair [31]. Hence, inhibition of PARP leads to severe toxicity in BRCA1- and BRCA2-mutated cells, causing the so-called synthetic lethality [31]. Importantly, sensitivity to PARP inhibition depends also on homologous recombination deficiency (HRD) which can produce a similar phenotype termed "*BRCA*ness" [31]. These results are particularly important, as they represent the first evidence of efficacy for treatments developed to inhibit selective targets in TNBC. Moreover, approximately 10–20% of TNBC patients harbor germline *BRCA* mutations, and additional cases can show "*BRCA*ness" [36].

Recently, immunotherapy showed, for the first time, to be active and effective in TNBC, as the addition of the atezolizumab, a humanized programmed death-ligand 1 (PD-L1) antibody, to nab-paclitaxel prolonged PFS in both the intention-to-treat population and the PD-L1-positive patient subgroup and OS among subjects with PD-L1-positive tumors (25.0 vs. 15.5 months, HR: 0.62) [36].

Moreover, increasing evidence suggest a potential role of anti-androgen therapy in a subset of TNBC. The expression of the androgen receptor (AR) has been described in TNBC in a range from 12 to 60%, especially in LAR subtype by Lehman and colleagues [37]. In a meta-analysis of 13 studies including 2826 patients with metastatic TNBC, it was demonstrated a rate of AR positivity of 24.4% [30]. In this context two phase II studies [37] demonstrated promising response rates with anti-androgen therapy in patients with >10% of AR expression by IHC.

Finally, many other therapeutic strategies are currently being investigated in metastatic TNBC, including among others immune checkpoint inhibitors, PI3K/AKT pathway inhibitors, and MAPK pathway inhibitors.

31.3 Local Therapies for Metastatic BC

Although systemic treatments represent the mainstay of therapy for metastatic BC, locoregional treatments performed by surgery or radiotherapy and other techniques may be useful to prevent cancer-related complications and to palliate symptoms (Fig. 31.1).

Radiation therapy has a central role in palliative care, especially in case of (1) brain metastases [radio-surgery (Gamma Knife or stereotaxic treatment) if there are few (<5 lesions) and small (<2–3 cm) metastases, or whole brain irradiation]; (2) symptomatic bone disease or risk of bone fracture; (3) medullar compression, due to vertebral fracture or endo-canalar disease; and (4) mediastinal syndrome (rare in BC), typically due to massive metastatic involvement of mediastinal nodes.

Breast surgery is indicated in case of a local relapse of BC, if there are no other metastatic sites. In metastatic de novo BCs, some evidences from retrospective and non-randomized studies seem to suggest a potential benefit deriving from the excision of primary BC in presence of metastatic disease [38]. Finally, several clinical reports suggest potential clinical benefit using locoregional treatment approaches in combination with systemic therapies for the management of oligometastatic BC, although data from prospective randomized trials are still lacking (see below) [39].

31.3.1 Management of Oligometastatic Disease

The state of "oligometastatic" breast cancer is defined by the presence of solitary or few evaluable lesions, usually in number ≤5 [40]. This particular kind of metastatic disease is estimate to represent up to 10% of patients with newly diagnosed metastatic BC [40]. Importantly

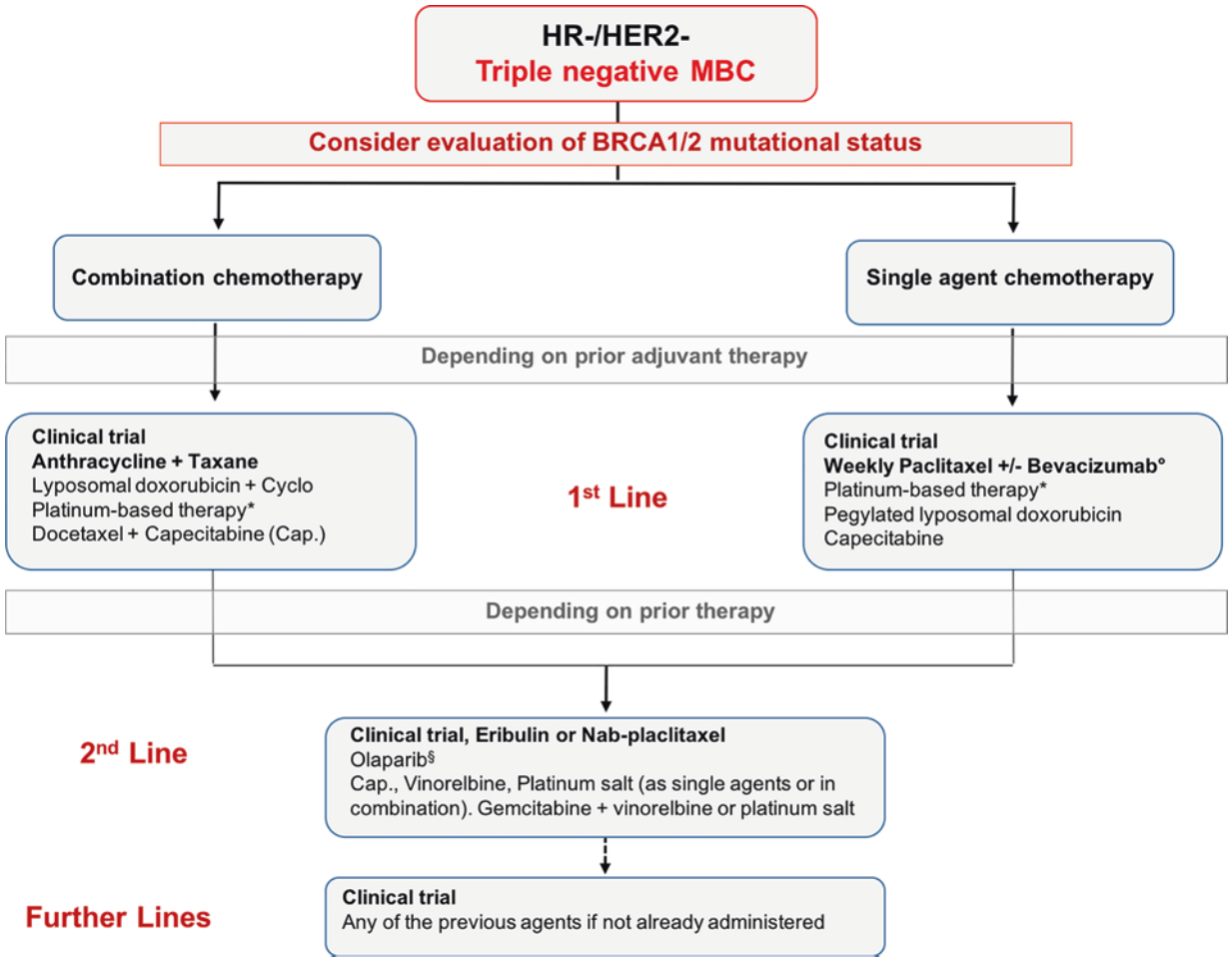

Fig. 31.4 Systemic treatment algorithm for HR-/HER2- metastatic breast cancer. *Additional abbreviations*: Cyclo: Cyclophosphamide; Cap: Capecitabine. *Platinum salt: either carboplatin or cisplatin. Platinum salts are important treatment options either in first- or second line for patients pre-treated with anthracyclines and taxanes, and in presence of BRCA1/BRCA2 mutations. °Combination of paclitaxel + bevacizumab should be considered also for patients with symptomatic visceral disease. Switch to maintenance therapy (bevacizumab +/− capecitabine) when maximum tolerability is reached. §To be approved in patients with BRCA1 or BRCA2 mutations

some of these patients could benefit from more aggressive treatment approaches administered with curative intent. Multimodal treatments are typically represented by of systemic therapy together with surgery or radiotherapy [40].

A meta-analysis by Harris et al. [40] of 28.693 MBC patients demonstrated a better 3 years OS in patients undergoing surgery of primary breast cancer, particularly in patients with smaller tumors, lower burden of metastatic disease, and fewer comorbidities, while no differences were found regarding hormone receptor status, grading, and site of metastasis. However, two randomized trials [40] failed to demonstrate a survival benefit in patients receiving surgery after systemic therapy for metastatic disease.

As mentioned before, liver represents a common metastatic site in BC, but isolated liver metastases are presents only in 4–5% of patients. Locoregional treatment approaches for liver metastases are surgical excision, transcatheter arterial chemoembolization (TACE), and radiofrequency ablation (RFA). Several lines of evidence support the use of these techniques in patients with isolated liver metastases [40].

The role of locoregional treatment of lung metastases is still unclear in BC. No studies directly compared systemic therapy alone vs. the combination with locoregional treatments.

Bone-only metastases occur in 17–37% of patients with distant relapses [40]. Radiotherapy remains the treatment approach for bone metastases, in particular to vertebral and extremities stabilization (to reduce the risk of bone fractures) and for pain relief. However, patients treated with stereotactic body radiation therapy (SBRT) demonstrated to achieve a potential survival benefit [40].

It is estimated that about 10–15% of all MBC patients develop symptomatic brain metastases and this risk is higher in triple-negative and HER2+ breast cancer. Survival of patients with central nervous system (CNS)

metastases remains poor, ranging from 2 to 16 months [40, 41]. Locoregional treatment approaches are represented by surgery, stereotactic radiosurgery (SRS), and whole brain RT (WBRT). The first two treatments are adopted in patients with limited number (1–3) and small CNS metastases, whereas WBRT is used for the remaining cases.

Overall, despite the lack of randomized trials, multimodal treatment of oligometastatic BC could represent an important strategy to improve patient outcome. However, selecting the oligometastatic patients who can benefit the most from multimodal aggressive treatment approaches remains a major challenge.

Expert Opinion

Antonio Russo

Key Points

1. Breast cancer can diffuse to other organs, and the most frequent sites of metastases are the lymph nodes, bone, liver, lung, and brain. Metastatic disease is not a curable condition, and even if with new treatments in the last years, there has been an improved survival and a better quality of life for these patients.
2. In case of hormone receptors positive and HER-2-negative breast cancer, treatment is based on endocrine therapy (ET), after having evaluated the condition of endocrine resistance which can be primary or secondary. Together with ET in first and second line of therapy, it is possible to administer new drugs such as palbociclib, ribociclib, and ademaciclib, which are CDK4/6 (involved in the resistance mechanisms) inhibitors. Palbociclib can be also added to fulvestrant in second-line treatment; another treatment is represented by everolimus, an mTOR (a factor which cause resistance) inhibitor.
3. For HER-2-positive BC, therapy consists in the administration of trastuzumab with taxane (paclitaxel or docetaxel); recently pertuzumab has been studied in this setting of patients, observing a better OS and PFS; this is the reason why the new standard of care is based on the use of trastuzumab, pertuzumab, and taxane. Another innovation is the antibody-drug conjugate trastuzumab emtansine (TDM1) which is recommended in second or subsequent lines of therapy.
4. Triple-negative BCs are characterized by a poor prognosis. Different therapeutic strategies can be used and based on chemotherapy anthracyclines, taxanes, eribulin, capecitabine, vinorelbine, gemcitabine, and platinum salts are used. It is quite important to remind that chemotherapy should be used also in HR+/HER2− BCs in case of organ crisis, also during the treatment with ET. Interesting updates come from immunotherapy: atezolizumab a PD-L1 antibody, added to nab-paclitaxel, has prolonged PFS in both the intention-to-treat population and the PD-L1-positive patient subgroup, and OS among subjects with PD-L1-positive tumors.
5. Also in the metastatic setting, locoregional treatments can be used for palliative intent and to prevent cancer-related complications. Even the absence of strong evidences, in case of oligometastatic disease, it could be useful combination of locoregional and systemic treatments. Radiotherapy (RT) is suggested when there is a bone involvement and a whole brain RT should be chosen in case of encephalic metastases.

Recommendations

- ESMO
- ► www.esmo.org/Guidelines/Breast-Cancer/4th-ESO-ESMO-International-Consensus-Guidelines-for-Advanced-Breast-Cancer-ABC-4
- ASCO
- ► https://www.asco.org/practice-guidelines/quality-guidelines/guidelines/breast-cancer#/9786
- ► https://www.asco.org/practice-guidelines/quality-guidelines/guidelines/breast-cancer#/11751
- ► https://www.asco.org/practice-guidelines/quality-guidelines/guidelines/breast-cancer#/9781

Hints for a Deeper Insight

- Atezolizumab for the treatment of triple-negative breast cancer: ► https://www.ncbi.nlm.nih.gov/pubmed/30474425
- Current state of clinical trials in breast cancer brain metastases: ► https://www.ncbi.nlm.nih.gov/pubmed/31555454
- Everolimus-based combination therapies for HR+, HER2− metastatic breast cancer: ► https://www.ncbi.nlm.nih.gov/pubmed/30092555
- Fulvestrant and palbociclib combination in heavily pretreated hormone receptor-positive, HER2-negative metastatic breast cancer patients: ► https://www.ncbi.nlm.nih.gov/pubmed/31612291

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Lung Cancer

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Thoracic Cancers

Contents

Francesco Passiglia, Valerio Gristina, and Christian Rolfo should be considered equally co-first authors.

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Learning Objectives

By the end of the chapter, the reader will

- Be able to apply diagnostic, staging, and treatment procedures of lung cancer
- Learn the basic concepts of epidemiology, pathology, and molecular biology of lung cancer
- Reach in-depth knowledge of diagnosis and treatment of lung cancer
- Be able to put acquired knowledge into clinical practice management of lung cancer patients

32.1 Introduction

Lung cancer was the most important epidemic of the twentieth century, and it's likely to remain a major public health problem also in the twenty-first century. We can ascribe different "primates" to lung cancer among all other epithelial malignant neoplasms:

- Lung cancer is the most frequent malignant tumor after non-melanocytic skin cancer.
- Lung cancer is the leading cause of cancer-related mortality worldwide.
- Lung cancer is the first malignant epithelial tumor to be successfully treated with single-agent targeted therapy.
- Lung cancer is the first malignant epithelial tumor to be successfully treated with single-agent immunotherapy.
- Lung cancer is the first malignant epithelial tumor to include liquid biopsy in the clinical management of patients.

32.2 Epidemiology

Over the last century, lung cancer switched from a rare disease to the most common malignant neoplasm in most countries and the first cause of cancer death worldwide, with about 1 of 4 cancer deaths due to lung cancer and a 5-year survival estimated to be 18%, ranging from 55% for localized disease to 4.5% for advanced disease [1] (Fig. 32.1).

The American Cancer Society's estimates for lung cancer in the United States for 2017 were [2]:

- About 222,500 new cases of lung cancer (116,990 in men and 105,510 in women).
- About 155,870 deaths from lung cancer (84,590 in men and 71,280 in women).

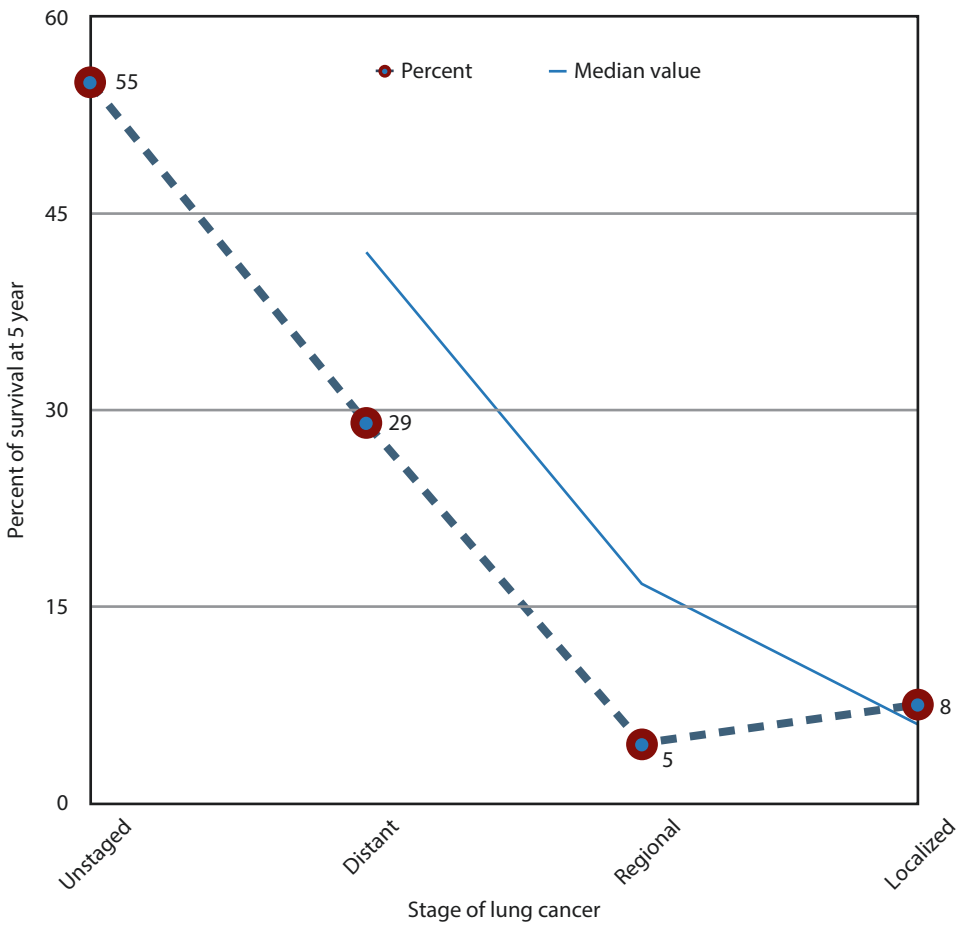


Fig. 32.1 Five-year relative survival by stage at diagnosis

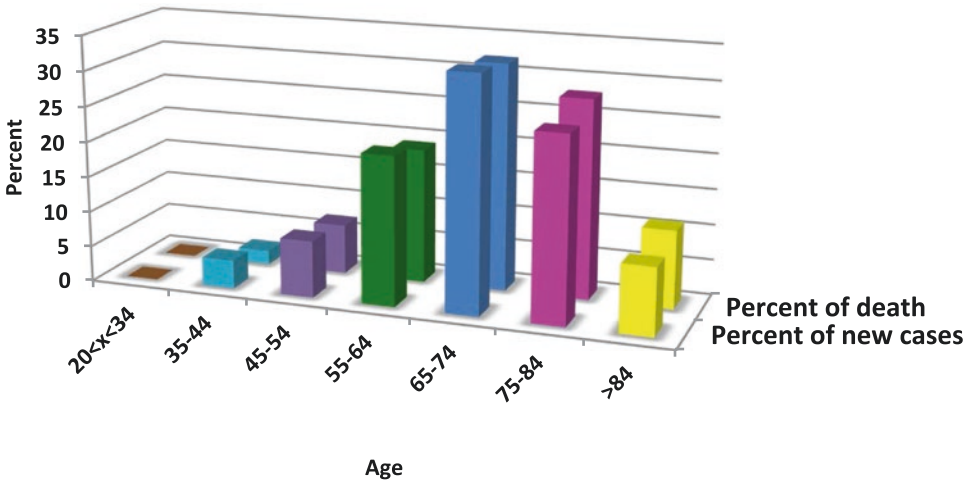


Fig. 32.2 Percent of new cases and death by age

The incidence of lung cancer increases in people who are 65 or older, and it is very rare under age 40, with an average age at the time of diagnosis of 70 years old [1, 2]. The percent of lung cancer deaths is highest among people aged 65–74 with a median age at death of 72 years old [1, 2] (Fig. 32.2).

Lung cancer has been historically most common in men; however, in the last few decades, the incidence of this disease increased among women. Since 1985 the estimated number of lung cancer cases worldwide increased by 51%, with a 44% increase in men and about 76% increase in women [2]. Interestingly women with lung cancer were usually younger at the time of diagnosis, never or former smokers, reporting adenocarcinoma as most common subtype and better survival at any stage as compared with man [3–5].

The patterns of lung cancer incidence are mainly dependent from the tobacco consumption, being tobacco smoking the main cause of lung cancer accounting for 87% of lung cancer deaths in men and for 70% in women [6], with other factors as genetic susceptibility, poor diet, asbestos, radon, and indoor air pollution less contributing to the descriptive epidemiology of this disease [7, 8] (Fig. 32.3).

A significant reduction in tobacco consumption would result in the prevention of a large fraction of lung cancers. In countries with effective tobacco control measures, the incidence of new lung cancer has begun to decline in men and is reaching a plateau for women, making lung cancer a paradigm of the superiority of prevention over treatment [9, 10].

Lung cancer in never smokers is not a rare disease especially in Asian countries and adenocarcinoma subtype, with 15% of cases in men and 53% in women, overall accounting for 25% worldwide [7]. Thus, it is emerging as a distinct disease entity with specific molecular and genetic features.

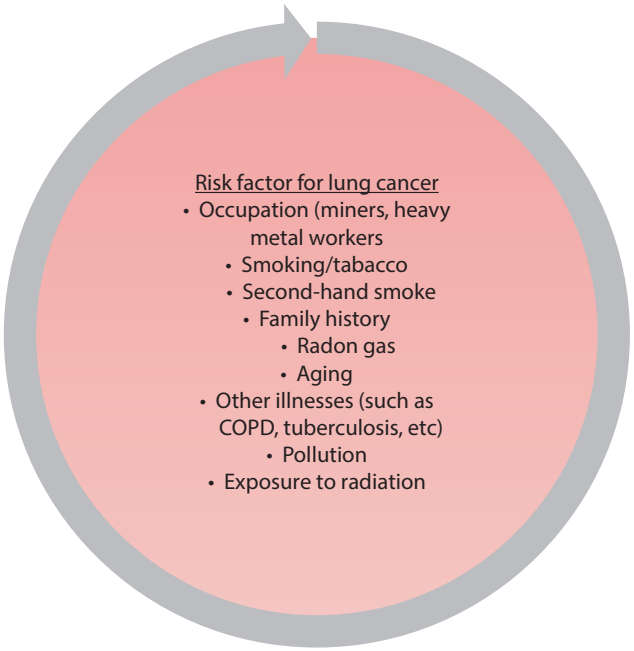


Fig. 32.3 Risk factors for lung cancer

32.3 Screening

Lung cancer diagnosis is usually performed at advanced stages with the majority of patients presenting with metastatic, not curable disease. Most early-stage lung cancers are asymptomatic, often detected by imaging procedures performed for other reasons [11–13]. Therefore, early detection by screening could be a valuable approach to detect the disease earlier, at asymptomatic and potentially curable stage [14].

Screening trials evaluating chest radiography and sputum cytology failed to demonstrate a significant decrease in lung cancer-related mortality [15, 16]. More recently the National Lung Screening Trial (NLST)

demonstrated a 20% reduction in mortality with low-dose computed tomography (LDCT) screening as compared to chest radiography in over 53,000 current or former heavy smokers [17], leading several US organizations to recommend screening for high-risk individuals in specialized centers with multidisciplinary expertise [18, 19]. Likewise, the final results of the Dutch-Belgian Randomized Lung Cancer Screening (NELSON) trial have recently shown that LDCT screening in a high-risk population reduced mortality by about 33% in women and 24% in men among more than 15,000 individuals across a 10-year follow-up period [20]. It should be noted that the interval between screens in NELSON was 2 years after the first screen and 2.5 years after the second screen, while the interval in NLST was 1 year.

However, considering the high rate of over-diagnosis of indolent cancers (20–25% of surgery performed in LDCT screening trial have been performed for benign lesions) and the fear of radiation exposure, screening with LCDT has not been endorsed in Europe yet, while an annual screening with chest LDCT in high-risk individuals (30 pack-year smoking history) from age 55 to 80 years is currently recommended in the United States.

32.4 Pathological Features

Pathological diagnosis is recommended prior to any curative treatment and should be made according to the 2015 World Health Organization (WHO) classification. In Table 32.1, we summarized the current approach for the histologic classification of surgically resected lung cancers.

The recent WHO classification, with its further subclassification of (surgically resected) adenocarcinoma (Table 32.2) by the International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (ATS), and the European Respiratory Society (ERS), showed differences in metastatic pattern, recurrence, and survival between different histological subtypes which could influence initial treatment decisions.

Non-small cell lung cancer (NSCLC) accounts for 85–90% of lung cancers including the three main histological subtypes: squamous cell carcinoma (30%), adenocarcinoma (40%), and large cell carcinoma (3–9%) [21, 22]. Immunohistochemistry (IHC), including p63, p40, and CK5/6 for squamous cell carcinoma and TTF1, napsin A, and CK7 for adenocarcinoma, is generally required to increase the specificity of diagnosis in the small sample setting and reduce the NSCLC-NOS (not otherwise specified) rate [23–25]. Large cell carcinoma is a tumor lacking morphologic or IHC evidence of clear lineage, with negative or uninformative stains for both squamous cell and adenocarcinoma (Fig. 32.4).

Table 32.1 Current classification of lung cancer

Category	Description
Adenocarcinoma	Pre-invasive lesions Minimally invasive adenocarcinoma Invasive adenocarcinoma Variants of invasive adenocarcinoma
Squamous cell carcinoma	Pre-invasive lesions Keratinizing Nonkeratinizing Basaloid carcinoma
Large cell carcinoma	
Neuroendocrine tumors	Pre-invasive lesions Carcinoid tumors (typical and atypical carcinoid) Large cell neuroendocrine carcinoma Small cell carcinoma
Adenosquamous carcinoma	
Sarcomatoid carcinoma	Pleomorphic Spindle cell Giant cell carcinoma Carcinosarcoma Pulmonary blastoma
Other unclassified carcinoma	Lymphoepithelioma-like carcinoma NUT carcinoma
Salivary gland tumors	Mucoepidermoid carcinoma Adenoid cystic carcinoma Epithelial-myoepithelial carcinoma Pleomorphic adenoma
Papillomas	Squamous cell papilloma Glandular papilloma Mixed squamous cell and glandular papilloma
Adenomas	Sclerosing pneumocytoma Alveolar adenoma Papillary adenoma Mucinous cystadenoma Pneumocytic adenomyoepithelioma Mucous gland adenoma
Mesenchymal tumors	
Lymphohistiocytic tumors	
Tumors of ectopic origin	
Metastatic tumors	

Table 32.2 Classification of lung adenocarcinomas in resection specimens

Category	Description
Pre-invasive lesions	Atypical adenomatous hyperplasia Adenocarcinoma in situ (<3 cm, formerly solitary BAC): non-mucinous, mucinous, mixed
Minimally invasive adenocarcinoma (<3 cm lepidic predominant tumor with <5 mm invasion)	Non-mucinous Mucinous Mixed
Invasive adenocarcinoma	Lepidic predominant (formerly non-mucinous BAC pattern with >5 mm invasion) Acinar predominant Papillary predominant Micropapillary predominant Solid predominant
Variants of invasive adenocarcinoma	Mucinous adenocarcinoma (including formerly mucinous BAC) Colloid Fetal (low and high grade) Enteric

BAC bronchioloalveolar carcinoma

Neuroendocrine malignant tumors account for about 15% of lung cancers, including large cell neuroendocrine carcinoma (LCNEC) (2%) and small cell lung cancer (SCLC) (13%). Immunohistochemistry to confirm the diagnosis of SCLC (synaptophysin, chromogranin A, CD56, thyroid transcription factor 1, and MIB-1) is not mandatory, but should be used in case of any doubt [26] (Fig. 32.4). SCLC originates from neuroendocrine cell precursors and is characterized by rapid growth, high response to both chemotherapy and radiotherapy, and development of treatment resistance in all patients with advanced disease [27].

Changes in composition and patterns of tobacco consumption have also led to a significant change in the distribution of lung cancer histological subtypes. Squamous cell carcinoma which was historically considered as the most common subtype in males with smoking history is now decreasing, while adenocarcinoma is increasing in both genders [28]. Smoking cessation possibly has contributed also to the decline of SCLC diagnosis [29].

The last pathologic classification of 2011 highlighted the concept that personalized medicine for patients with advanced lung cancer is determined by histology and genetics and that tissue/cell management of small biopsy/cytology samples is critical for pathologic and molecular diagnosis in order to prevent the loss of tissue in less important analysis [21, 30].

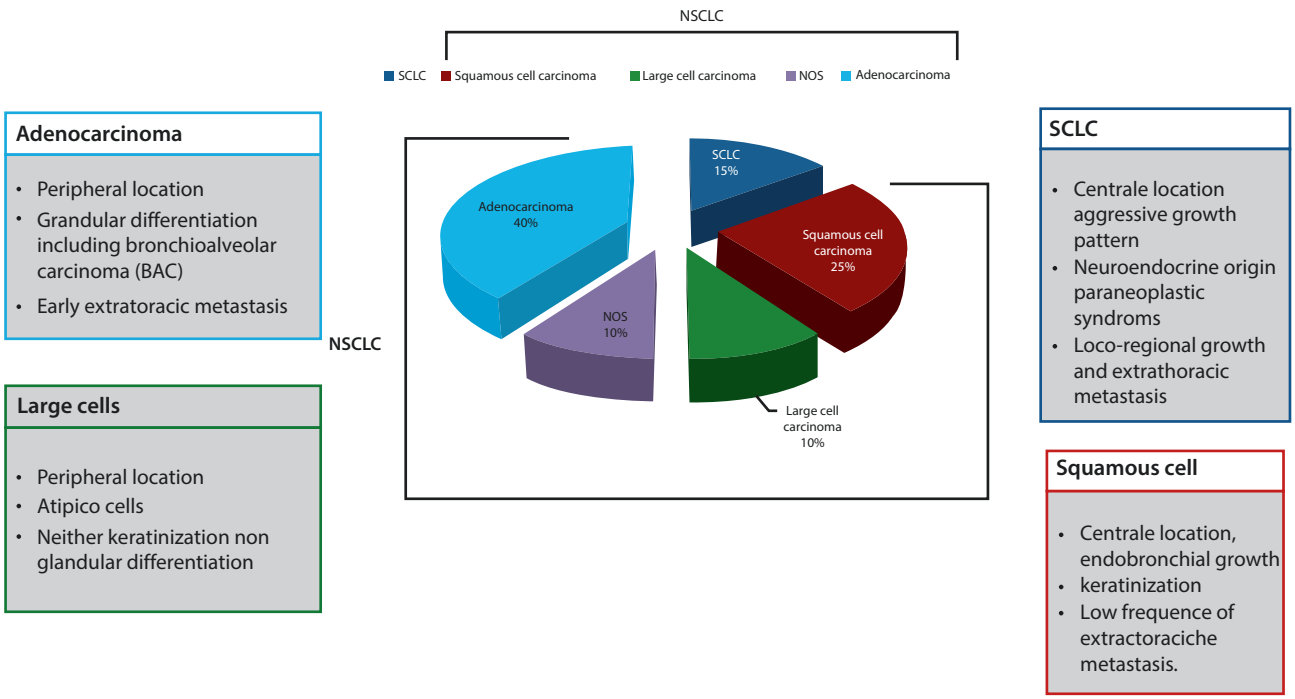


Fig. 32.4 Histological subtypes of lung cancer: Pathological features

32.5 Molecular Biology

The identification of genomic alterations as oncogene drivers in a subset of lung cancer led to a radical shift from pathological to molecular classification, establishing a new paradigm for the diagnosis and treatment of this disease known as "personalized medicine" (Fig. 32.5).

Epidermal growth factor receptor (EGFR)-activating mutations have been identified in about 40–60% of Asian [31–33], 15–20% of Caucasian [34, 35], and 30% of Latin American [36] NSCLC patients. Exon 19 deletion (Del19) and point mutation in exon 21 (L858R) account for 90% of overall EGFR-activating mutations [37], but there are also uncommon mutations in exon 18 (E709 and G719X) and in exon 21 (T854 and L861X) resulting in a constitutively activated EGFR [38]. The interaction of EGFR extracellular domain with specific ligands induced homo-dimerization or hetero-dimerization with other HER family member receptors, resulting in the activation of TK domain and tyrosine autophosphorylation. Activating mutations significantly increased autophosphorylation of intracellular tyrosine residues with the subsequent constitutive activation of downstream RAS/RAF/ERK/MAPK and PI3K/AKT pathways, ultimately favoring tumor cell proliferation, angiogenesis, and metastatic potential [37, 39] (Fig. 32.6). The EGFR mutation was the first molecular alteration in lung cancer that has been associated with clinical sensitivity to tyrosine kinase inhibitor (TKI) selectively targeting and inhibiting EGFR.

Based on currently available published data, EGFR mutations are more frequent in female, Asian, never-smoker patients with adenocarcinoma subtype. Thus, EGFR mutational testing is currently recommended in all patients with newly diagnosed advanced adenocarcinoma or large cell carcinoma and in squamous cell carcinoma patients who are never smoker and former light smokers (<15 pack-years). Based on expert consensus opinion, mutational analysis should be performed on tissue specimens, and the commonly used methods for EGFR mutation detection are reported in Table 32.3.

However, EGFR mutation analysis on circulating tumor DNA (ctDNA) demonstrated an adequate diagnostic accuracy [40, 41] as compared to tumor tissue analysis and is currently recommended as an alternative approach in a subgroup of patients with newly diagnosed metastatic disease who can't undergo biopsy or received uninformative results from tissue molecular analysis. In contrast to EGFR where strong data exists, the assessment of other genomic alterations using ctDNA in treatment-naive patients is more limited. However, as endorsed by most international scientific societies, the detection of an actionable alteration in ctDNA, if using a validated assay, would eventually represent sufficient evidence to initiate targeted treatment, albeit not without reimbursement variations among all the different countries. Nonetheless, a negative finding of either EGFR or other genomic alterations using ctDNA should be considered not conclusive, and, when feasible due to patients' performance status, a tissue re-biopsy should be performed (Fig. 32.7).

The EML4-ALK rearrangements have been detected as potent oncogene drivers in about 3–8% of NSCLC patients [42], resulting in a constitutive activation of the intracellular domain of ALK receptor and downstream RAS/MAPK, PI3K/AKT, and JAK/STAT3 signaling pathways [43], thus emerging as a predictive biomarker of clinical response to ALK inhibitors. Similarly ROS proto-oncogene 1 (ROS1) rearrangements occur in about 1–2% of NSCLC patients and were associated with a great response rate to ALK inhibitors [44, 45]. Both ALK and ROS1 rearrangements are more frequent in never smokers and younger people with adenocarcinoma, while are not associated with gender or ethnicity.

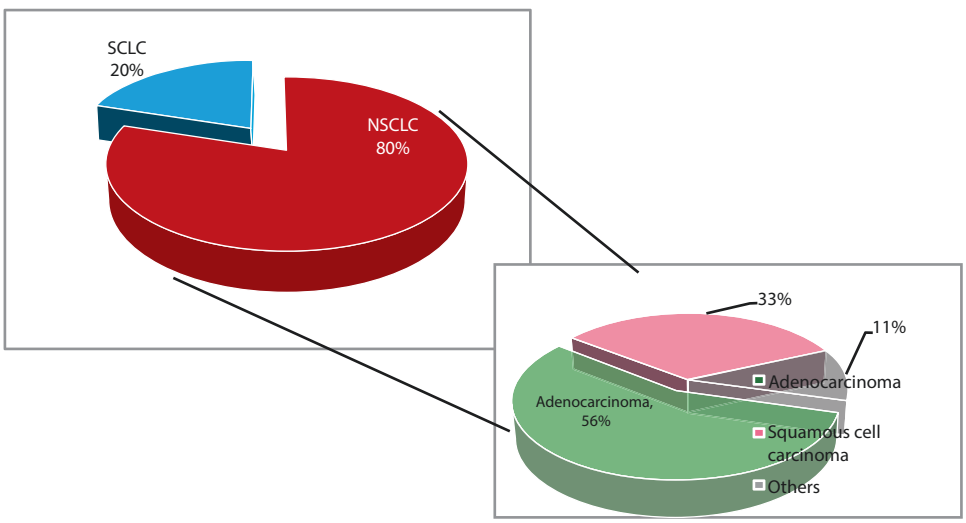


Fig. 32.5 From histological to molecular subtypes of lung cancer

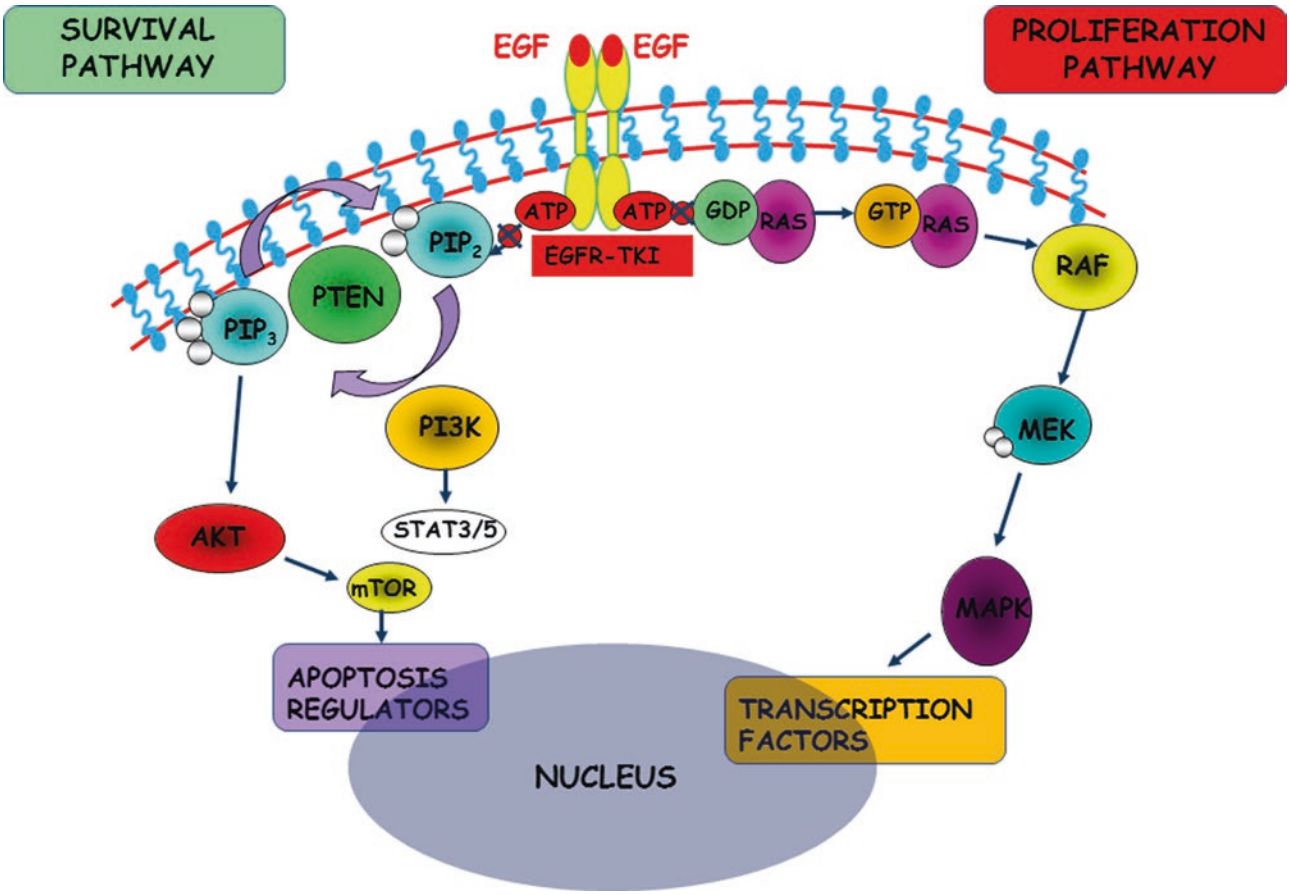


Fig. 32.6 EGFR signaling pathway and EGFR TKI

Table 32.3 Methods for EGFR mutation detection on tumor samples

Method	Tumor DNA required (%)	EGFR mutations detected	Deletions and insertions
Sanger direct sequencing	25	Known and new	Yes
Real-time/ TaqMan PCR	10	Known only	No
Cobas	5–10	Known only	Yes
Pyrosequencing	5–10	Known only	Yes
MALDI-TOF MS-based genotyping	5	Known only	No
Allelic-specific PCR/ARMS	1	Known only	No
PNA-LNA-PCR clamp	1	Known only	No
Massively parallel NGS	0.1	Known and new	Yes

The ALK/ROS1 analysis should be performed on the same patient population tested for EGFR. Fluorescence in situ hybridization (FISH) using break-apart probes on tissue specimens has been the standard Food and Drug Administration (FDA)-approved tool [46, 47] (Fig. 32.8). However, several studies showed a high concordance between FISH and immunohistochemistry (IHC) for ALK detection [48–54], suggesting IHC as a reliable screening assay for ALK rearrangements which has been adopted worldwide. Although FISH analysis on cytologic preparations is not recommended, however cell blocks may be acceptable.

Since both EGFR mutations and ALK rearrangements predicted therapeutic benefit with their respective targeted drugs in patients with adenocarcinoma, biomarker testing has been implemented and integrated into treatment decision process. The recent development of next-generation sequencing (NGS) accomplishes massive parallel gene mutation analysis and requires low amount of tissue, favoring the identification of several targetable molecular alterations, including BRAF, HER2, and MET mutations and RET and NTRK gene fusions, which may allow access to targeted treatments in the context of clinical trials (Fig. 32.8). Molecular

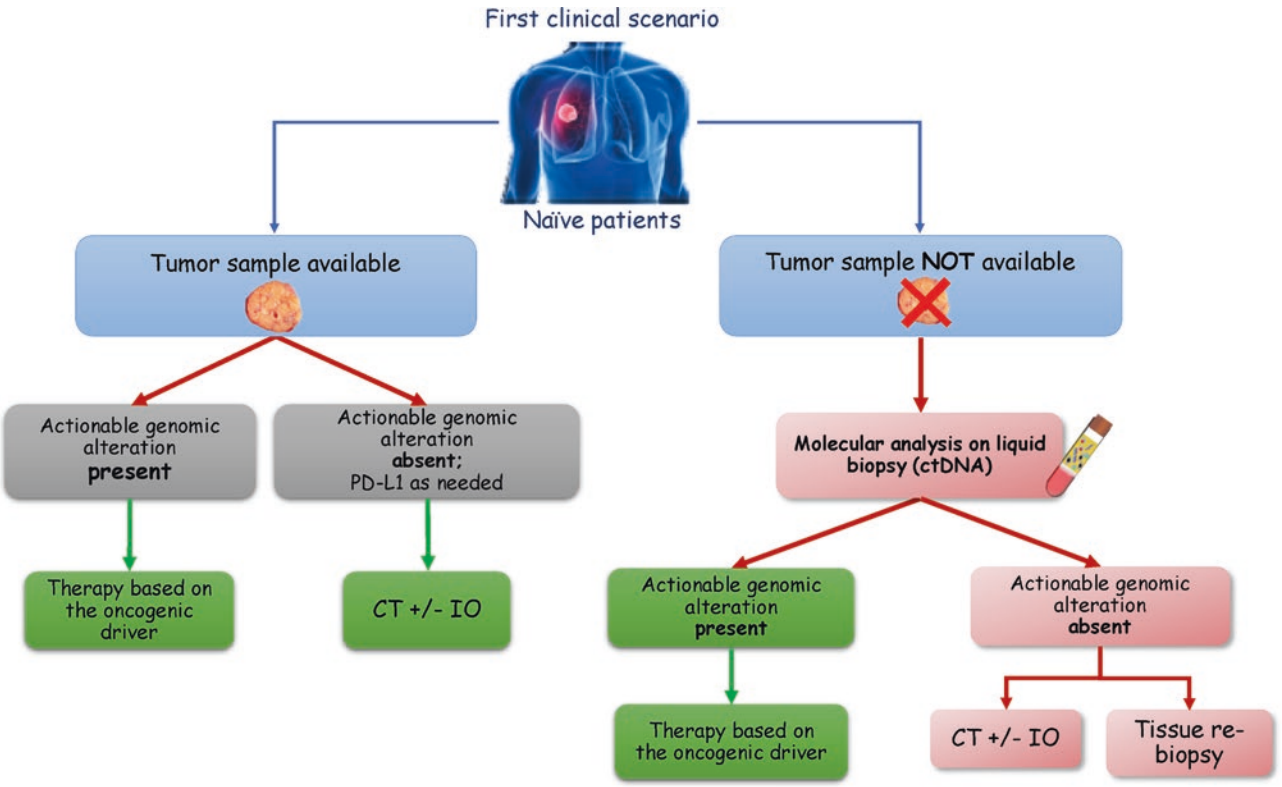


Fig. 32.7 Mutation testing at the time of advanced NSCLC diagnosis

alterations in different signaling pathways have been recently identified also in squamous cell carcinoma, including PI3KCA, PTEN, AKT, FGFR, RAS, TP53, CDKN2A, and RB1, offering new avenues of investigation for targeted treatment [55]. Despite many efforts, very few data are currently available for SCLC, revealing FGFR1, JAK2, and SOX2 gene amplifications and TP53, RB, and PTEN gene mutations [56, 57] among the most common genetic alterations, but their predictive roles need to be investigated in clinical trials.

32.6 Clinical Features

The majority of patients with lung cancer are symptomatic at the time of initial presentation, with only 5–15% of asymptomatic people in whom lung cancer is detected incidentally on a chest x-ray for other indications or in the context of screening clinical trials [11, 12]. Most symptoms may be ascribed to the loco-regional intra-thoracic tumor invasive growth and/or to the development of extra-thoracic metastasis. In a small subgroup of patients, symptoms may be related to specific paraneoplastic syndromes which require differential diagnosis with other clinical conditions [58] (Fig. 32.9).

Among the most common symptoms caused by local tumor growth, cough and dyspnea are reported in up to 60% of cases [59], and hemoptysis is reported in about one third of patients [60], while some people may refer persistent chest pain or discomfort, even if no invasion of the chest wall, mediastinum, or pleura occurred. Systemic and not specific symptoms such as fatigue and weight loss frequently occur in about 70% of patients at the time of lung cancer diagnosis. However, because these symptoms are reported also in other lung diseases associated with smoke exposure, such as COPD, special attention should be posed to any relevant symptom changing pattern in high-risk patients.

Dysphagia [61], dysphonia or hoarseness [62], and diaphragmatic paralysis may be related to the loco-regional intra-thoracic tumor growth, respectively, invading the esophagus, left recurrent laryngeal nerve, and phrenic nerve. Malignant pleural and pericardial effusions are caused by either direct tumor invasion or hematological/lymphatic spread of cancer cells, respectively, occurring in 10–20% and 5–10% of people with lung cancer [63, 64]. The "superior vena cava syndrome" occurs in less than 5% of patients with initial diagnosis of lung cancer. It is caused by obstruction or compression of vena cava by tumor and is characterized by head and neck swelling, dilatation of the veins on both neck and chest wall, cough, dizziness, headache, dyspnea, and chest pain [65, 66]. Lung cancer growing in the apex of the upper lobe, known as Pancoast tumor, may cause the

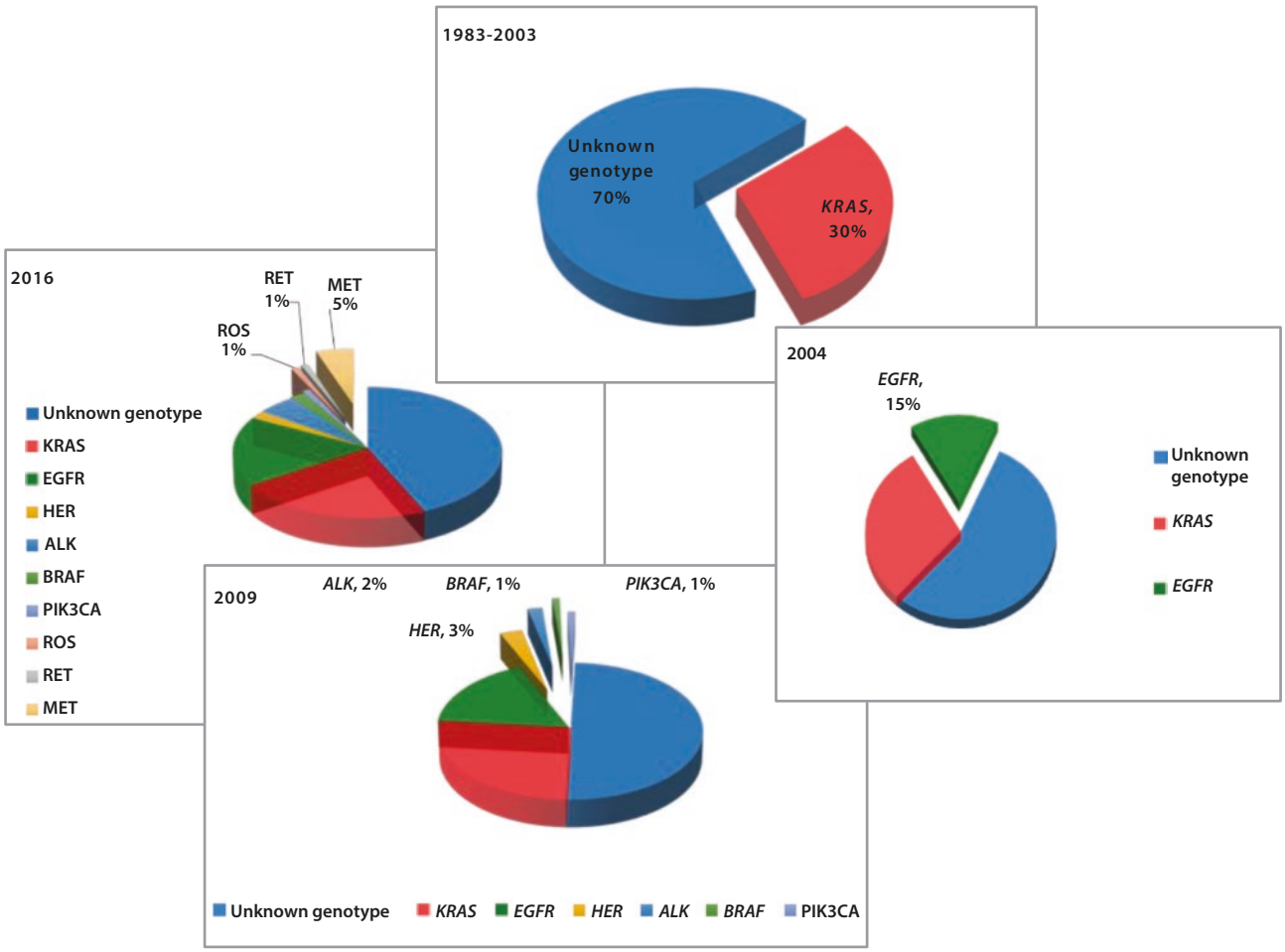
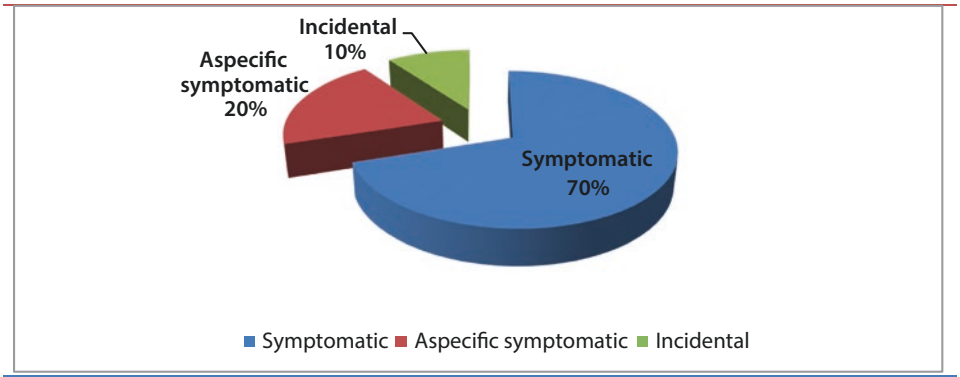


Fig. 32.8 Progress in identifying molecular alterations in lung adenocarcinoma

Fig. 32.9 Clinical presenting symptoms and signs in lung cancer



Symptomatic	
Local tumor growth	Cough, hemoptysis, dyspnea, chest pain
Loco-regional growth	Pleuro-pericardial effusion, hoarseness, superior vena cava syndrome, disphagia, Pancoast syndrome
Extra-thoracic metastasis	Bone, brain, liver, andrenal gland, others
Paraneoplastic syndromes	Muscoloskeletal, cutaneous, endocrinologic, hematologic, neurologic, miscellaneous

"Horner syndrome," overall occurring in about 4% of cases at initial presentation. It's characterized by enophthalmos, ptosis, miosis, and hemi-facial anhidrosis due to the invasion of the sympathetic chain and stellate ganglion by the tumor and is often associated with pain and muscle wasting in the arm and hand due to the invasion of the brachial plexus and chest wall pain due to the invasion of ribs and vertebrae by the tumor [67].

A significant subgroup of patients may present symptoms caused by distant tumor metastasis at the time of lung cancer diagnosis. Bone metastasis may cause pain, pathological fractures, hypercalcemia, and rarely spinal cord compression which is characterized by local pain, paralysis, sensory loss, and sphincter dysfunction and requires urgent intervention [68]. Brain metastasis is often symptomatic with variable clinical presentation including headache, focal or generalized seizures, nausea and vomiting, confusion, or visual alterations [69]. Lung cancer metastasis may occur also at other sites, including the liver, adrenal gland, and lymph nodes, with clinical presentation varying according to patient's symptoms (Fig. 32.10).

Paraneoplastic syndromes include a large spectrum of endocrine, neurologic, dermatologic, hematologic, and metabolic clinical manifestations due to the production of bioactive substances (e.g., hormone-like peptides, prostaglandins, cytokines, etc.) by tumor cells in the absence of a direct invasion/obstruction of vital organs by the tumor [70]. Paraneoplastic syndromes occur in about 10% of patients with lung cancer, mostly SCLC, and may be classified according to the clinical presentation (Table 32.4).

A careful evaluation of the aforementioned clinical manifestations and syndromes by physicians is crucial for the early detection of lung cancer, ultimately resulting in better patients' survival outcomes.

32.7 Diagnosis and Staging

The diagnostic evaluation should initially focus on careful physical examination and personal patient's history, to identify new symptoms or a significant change in the usual respiratory symptoms [58]. For all the patients with suspected lung cancer, an urgent referral for non-invasive chest imaging is recommended, including radiographs, computed tomography (CT), and positron emission tomography (PET) [71]. Chest radiography plays a crucial role in the diagnostic workup of lung cancer especially in the primary care setting [72]. However, even if it may lead to the identification of a suspected tumor, it has no sufficient diagnostic accuracy to differentiate benign from malignant lesions, often requiring additional imaging examinations, even in the case of negative result [73]. Conventional contrast-enhanced chest CT is considered the best exam to detect lung cancer, as it provides detailed information on anatomic location, margins, invasion of surrounding structures or chest wall, and mediastinal lymph node involvement [74]. PET with fluorodeoxyglucose (FDG) is very accurate for differentiating benign from malignant lesions, but plays a crucial role in the mediastinal staging, since it has shown to have both higher sensitivity and specificity than CT [75]. The overall diagnostic information which emerged from non-invasive imaging (CT, PET, or combined PET-CT), including size and location of the tumor, presence of mediastinal or distant metastasis, and the patient's clinical status, will guide the most appropriate strategy to achieve the final diagnosis and staging of lung cancer with the least risk to the patients (Fig. 32.11). Bronchoscopy with biopsy and transbronchial needle aspiration (TBNA) is the most common procedure used to obtain a pathological diagnosis

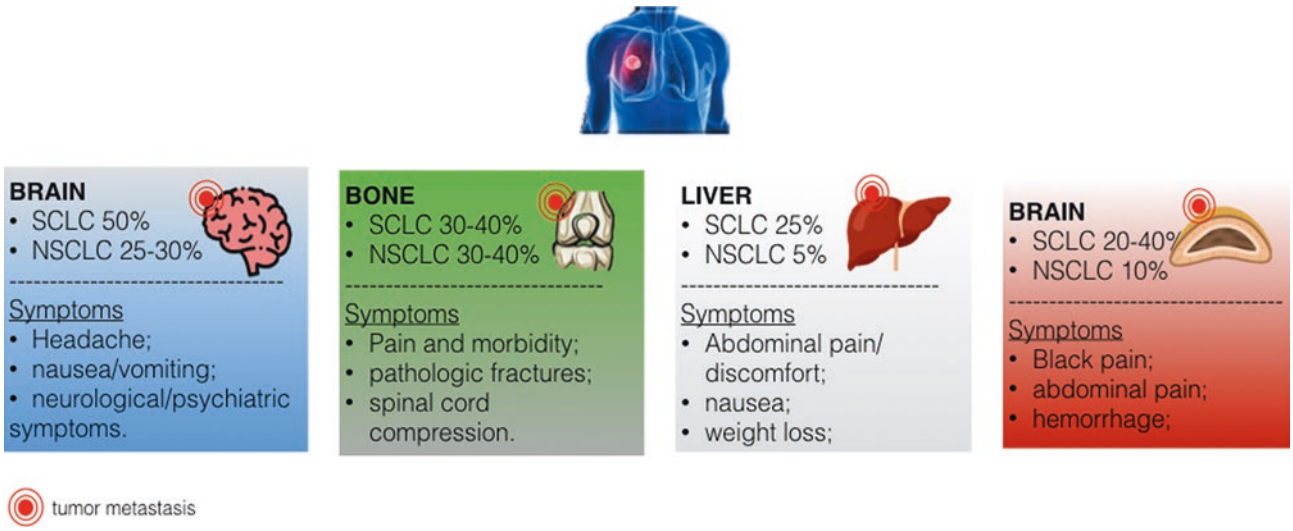


Fig. 32.10 Symptoms caused by tumor metastasis at the time of lung cancer diagnosis

Table 32.4 Classification of paraneoplastic syndromes in lung cancer

Syndrome	Lung cancer subtype	Cause
Acromegaly	Carcinoid tumors Small cell lung cancer	Growth hormone
Carcinoid syndrome	Carcinoid tumors Large cell carcinoma Small cell lung cancer	Serotonin
Ectopic adrenocorticotropic hormone (ACTH) syndrome	Carcinoid tumors Small cell lung cancer	ACTH Corticotropin-releasing hormone
Encephalomyelitis/sensory neuropathy	Small cell lung cancer	Anti-HU antibody and Hu-D antigen
Hypertrophic pulmonary osteoarthropathy	Non-small cell lung cancer Small cell lung cancer	Prostaglandin-E Inflammatory cytokines
Granulocytosis	Non-small cell lung cancer	Colony-stimulating factor (CSF) Granulocyte-CSF Granulocyte macrophage CSF Interleukin (IL)-6
Hypercalcemia	Non-small cell lung cancer	Parathormone Parathyroid hormone-related peptide
Hyponatremia	Small cell lung cancer Non-small cell lung cancer	Arginine vasopressin Atrial natriuretic peptide
Lambert-Eaton syndrome	Small cell lung cancer	Anti-P/Q channel antibody and P/Q type calcium channel (antigen)
Retinopathy	Small cell lung cancer	Antirecoverin antibody and specific antigen to photoreceptor cells
Thrombocytosis	Non-small cell lung cancer Small cell lung cancer	IL-6
Thromboembolism	Non-small cell lung cancer Small cell lung cancer	Procoagulants Inflammatory cytokines Tumor interaction with host cells

of NSCLC, especially in presence of central lesions [76]. Additional tools for biopsy include both endobronchial ultrasound (EBUS) and endoscopic ultrasound (EUS)-guided biopsy [77–79]. For peripheral pulmonary nodules not detectable by bronchoscopy, CT-guided trans-thoracic biopsy (TTB) may be considered as an alternative approach [80, 81], while in patients with pleural effusion at initial presentation, thoracentesis with cytological examination of pleural fluid should be performed [82], and, if negative, image-guided pleural biopsy or video-assisted thoracoscopy surgery (VATS) is recommended [83]. For patients in whom SCLC is suspected on the basis of both clinical and imaging findings, the easiest method among sputum cytology, bronchoscopy, trans-thoracic biopsy, and thoracentesis should be used to obtain a pathological diagnosis [76]. The diagnostic strategy should be individualized for each patient and should be decided within a multidisciplinary team.

Mediastinum staging plays a crucial role in the diagnostic workup of intra-thoracic NSCLC and significantly influences patients' prognosis and treatment strategy with the final aim of identifying patients who may benefit from surgery from those who will receive other forms of therapy. Because of the high frequency of false positive imaging tests, all patients with mediastinal lymph node involvement on CT or PET should undergo invasive tissue sampling by EBUS or EUS to confirm node disease, and if results are negative, mediastinoscopy is recommended [76, 84, 85]. For tumors without mediastinal involvement on CT or PET, invasive mediastinal staging is advised only in case of central lesion and/or a tumor size >3 cm, while peripheral tumors <3 cm should not receive additional examinations [86]. Screening for brain metastases by MRI might be useful in patients considered for curative therapy, while it's recommended in all patients reporting CNS-related symptoms. Finally, abdomen CT scan and bone scan should be performed in all patients to exclude the presence of distant metastasis [87].

During the 16th World Congress of Lung Cancer, the Union for International Cancer Control (UICC) presented the revised tumor, node, and metastasis

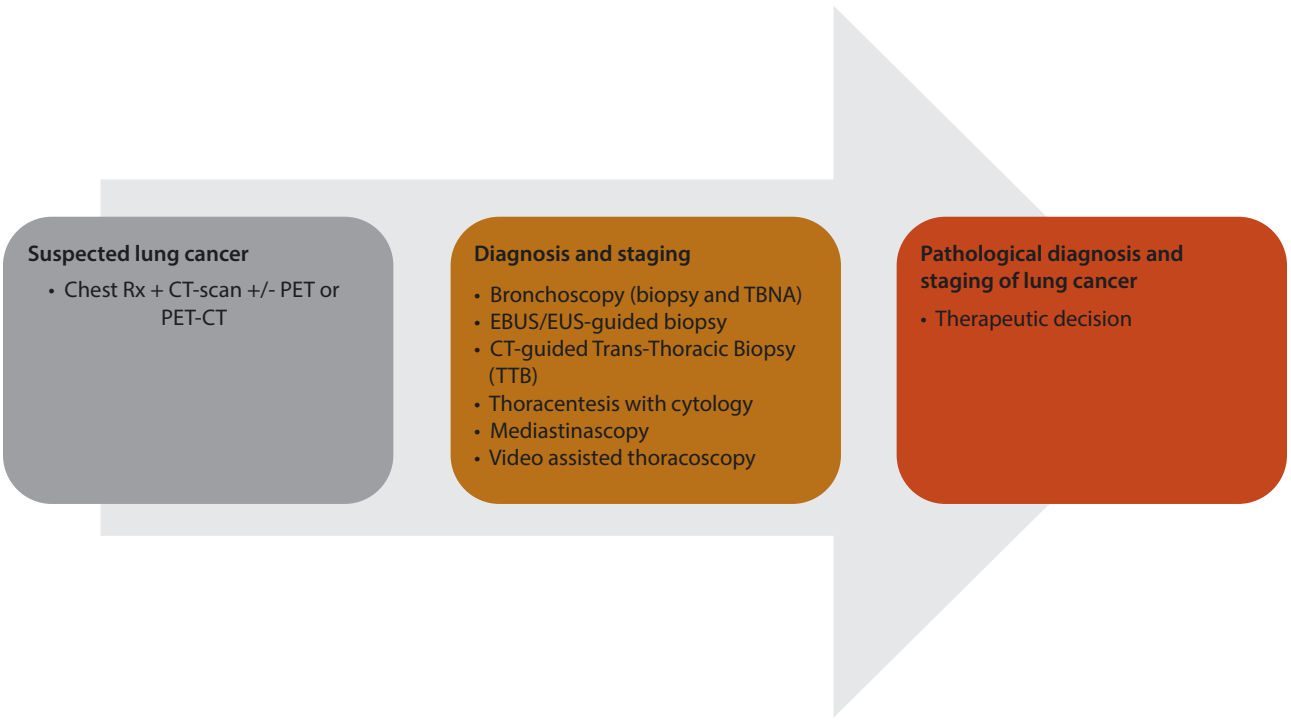


Fig. 32.11 Diagnostic algorithm in patients with suspected lung cancer

(TNM) classification of lung cancer (UICC TNM 8) [88], as shown in Table 32.5, which should be used for both NSCLC and SCLC.

The practice of classifying cancer according to anatomical extent named "stage" derives from the observation that patients' survival rates correlated with their tumor extension at the time of diagnosis [88] (Table 32.6). The TNM tumor staging remains the most important parameter informing clinicians about the prognosis for survival and guiding treatment planning and monitoring in lung cancer patient.

32.8 Treatment

32.8.1 Localized Disease

Despite recent advances in diagnostic procedures, only 20% of NSCLC patients have early-stage disease at the time of diagnosis, thus potentially operable [1] (Fig. 32.12).

The recommended treatment of patients with stage I–II NSCLC is curative-intent surgical resection [89] for all patients who are considered clinically "operable," with a 5-year survival rate reported to be 40–60% for stage I and 20–35% for stage II [1]. The current gold standard is lobectomy [90] with hilar and mediastinal lymph node sampling or dissection [91]. Either open thoracotomy or VATS is recommended as an appropriate surgical approach to the expertise of the surgeon, even if VATS should be preferred in stage I tumors [92] because it was associated with lower post-operative morbidity/mortality, resulting in improved quality of life [93]. Alternative approaches, including segmentectomy or wedge resection, could be reserved to patients with limited cardiopulmonary function [94–96]. Systematic nodal dissection of a minimum of six nodes/stations, three of which should be mediastinal including the subcarinal station, should be guaranteed to ensure "R0 resection" [91]. Curative stereotactic body radiotherapy (SABR) should be offered to patients with a peripherally located stage I NSCLC who have clinical comorbidities or are at very high surgery-related risk and those who refuse to undergo surgical procedure [97–99]. For patients with multifocal NSCLC, radical surgical resection whenever possible or alternatively SABR [100] is recommended after discussion within a multidisciplinary tumor board [83].

Adjuvant platinum doublet chemotherapy is recommended for all patients with stage II and III surgically resected disease [83]. Two meta-analyses demonstrated that post-operative platinum-based chemotherapy led to more than 10% reduction in the risk of death resulting in 5% absolute 5-year survival rate improvement [101, 102]. On the basis of the results of the JBR.10 and ANITA trials, cisplatin-vinorelbine is currently considered as the best regimen for adjuvant setting [103, 104]. The role of adjuvant therapy in stage I is still controversial, with a small survival benefit limited to patients with stage IB disease with tumor >4 cm [104, 105]. In the decision process for adjuvant therapy, several factors, including time from surgery, age, and pre- and post-operative morbidi-

Table 32.5 The eight edition of the TNM clinical classification of lung cancer

TNM clinical classification	
T	*Primary tumor*
TX	Primary tumor cannot be assessed or tumor proven by the presence of malignant cells in sputum or bronchial washing but not visualized by imaging/bronchoscopy
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor 3 cm or less in greatest dimension, surrounded by lung or visceral pleura, without bronchoscopic evidence of invasion more proximal than lobar bronchus (not in the main bronchus) T1a(mi): Minimally invasive adenocarcinoma T1a: Tumor <1 cm in greatest dimension T1b: Tumor >1 cm but <2 cm in greatest dimension T1c: Tumor >2 cm but <3 cm in greatest dimension
T2	Tumor >3 cm but <5 cm or tumor with any of the following features: involves the main bronchus regardless of distance from the carina but without involvement of the carina; invades the visceral pleura; and associated with atelectasis or obstructive pneumonitis that extends to the hilar region, involving part or all of the lung T2a: Tumor >3 cm but <4 cm in greatest dimension T2b: Tumor >4 cm but <5 cm in greatest dimension
T3	Tumor >5 cm but <7 cm in greatest dimension or associated with separate tumor nodules in the same lobe as the primary tumor or directly invades any of the following structures: chest wall (including the parietal pleura and superior sulcus tumors), phrenic nerve, and parietal pericardium
T4	Tumor >7 cm in greatest dimension or associated with separate tumor nodules in a different ipsilateral lobe than that of the primary tumor or invades any of the following structures: diaphragm, mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, esophagus, vertebral body, and carina
N	*Regional lymph nodes*
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension
N2	Metastasis in ipsilateral mediastinal and/or subcarinal lymph nodes
N3	Metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph nodes
M	*Distant metastasis*
M0	No distant metastasis
M1	Distant metastasis present M1a: Separate tumor nodules in a contralateral lobe; tumor with pleural or pericardial nodules or malignant pleural or pericardial effusion M1b: Single extra-thoracic metastasis M1c: Multiple extra-thoracic metastases in one or more organs

ties, should be taken into account and discussed within a multidisciplinary tumor board. Several studies and meta-analysis suggested that the estimated benefit from neoadjuvant chemotherapy is similar to that expected with adjuvant chemotherapy [106–108]; thus, it may be considered as a feasible and ethical approach for patients with stage II–IIIA NSCLC. However, adjuvant treatment is currently preferred because of major evidence base and clinical experience (Fig. 32.13).

Based on the efficacy interim analysis of the phase III ADAURA trial, adjuvant osimertinib has recently led to significantly improved disease-free survival (DFS) compared with placebo in NSCLC patients presenting with the complete resection of primary tumor with stage IB to IIIA and harboring EGFR common mutations. In patients with stage II to IIIA median DFS rate was not even yet reached, while the 2-year DFS was 90% with osimertinib for up to three years versus 44% with placebo with most of patients not experiencing central nervous disease relapse. No new safety signals have been observed. Even if final overall survival (OS) analyses are pending, in the EGFR-mutated radically resected NSCLC this

trial might be already considered practice-changing as opposed to the current standard of care based on adjuvant cisplatin-based chemotherapy [109]. The ongoing ITACA trial aims to identify predictive tumor molecular biomarker, such as excision repair cross-complementation group 1 (ERCC1) and thymidylate synthase (TS), useful to select patients who may derive the most clinical benefit from adjuvant chemotherapy. Post-operative radiotherapy (PORT) should be considered only after R1 resection or N2 pathological disease discovered at surgery [83]. Even if the updated results of the PORT meta-analysis showed a not clear benefit in patients with N2 pathological disease undergoing PORT after radical surgery [110], PORT is largely adopted in clinical practice. However, even if possibly retaining a role in the ablative treatment of low-volume recurrences in brain and other sites (so-called oligometastases) and as conventional chemoradiotherapy to the thorax in patients who develop isolated nodal recurrences, mediastinal post-operative radiotherapy should not be used routinely in completely resected pN2 patients with radical surgery remaining a single local modality to be followed by a watch-and-wait strategy, as recently confirmed by the LungART primary end-point analyses [111].

Only 5% of patients with SCLC have "very limited" stage I disease (T1-2, N0-1, M0) at the time of diagnosis, thus potentially benefiting from curative surgery with 5-year survival rate reported to be around 50% [112, 113]. Surgical resection with mediastinal lymph node dissection followed by four cycles of systemic platinum doublet chemotherapy is recommended as standard of care, while concurrent chemoradiotherapy may be considered as an alternative option in patients who have high perioperative risk after discussion within a multidisciplinary team [27] (Fig. 32.14).

Table 32.6 Stage grouping and 5-year OS according to the 8th TNM Clinical classification

Stage	T	N	M	5-year OS
Occult carcinoma	Tx	N0	M0	
Stage 0	Tis	N0	M0	
Stage IA1	T1a, T1a(mi)	N0	M0	**92%**
Stage IA2	T1b	N0	M0	**83%**
Stage IA3	T1c	N0	M0	**77%**
Stage IB	T2a	N0	M0	**68%**
Stage IIA	T2b	N0	M0	**60%**
Stage IIB	T1a, T1b, T1c T2a, T2b T3	N1 N1 N0	M0 M0 M0	**53%**
Stage IIIA	T1a, T1b, T1c T2a, T2b T3 T4	N2 N2 N1 N1	M0 M0 M0 M0	**36%**
Stage IIIB	T1a, T1b, T1c T2a, T2b T3 T4	N3 N3 N2 N2	M0 M0 M0 M0	**26%**
Stage IIIC	T3 T4	N3 N3	M0 M0	**13%**
Stage IVA	Any T	Any N	M1a M1b	**10%**
Stage IVB	Any T	Any N	M1c	**0%**

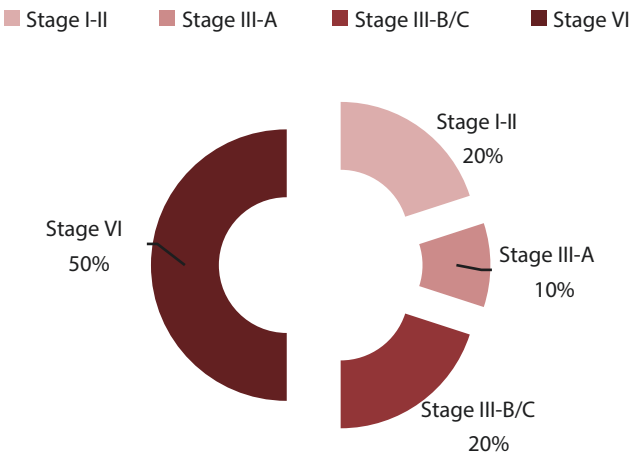


Fig. 32.12 Percentage of disease staging at the time of lung cancer diagnosis

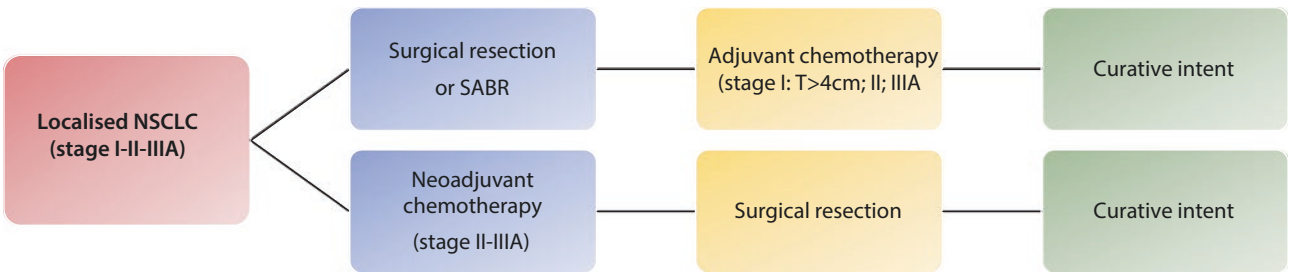


Fig. 32.13 Therapeutic algorithm for early-stage NSCLC

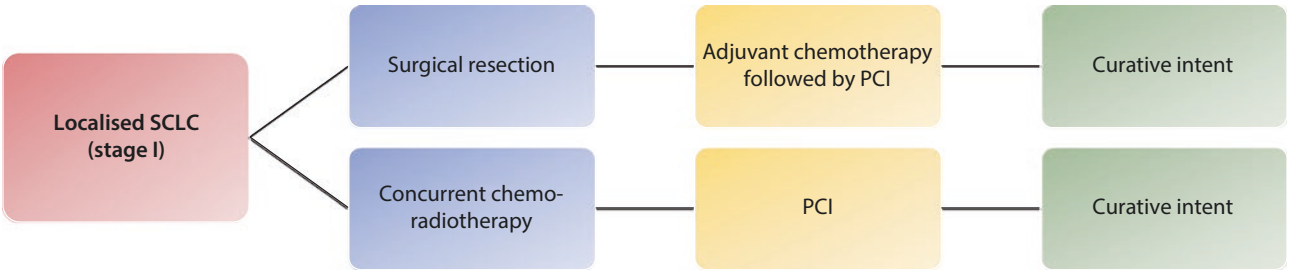


Fig. 32.14 Therapeutic algorithm for early-stage SCLC

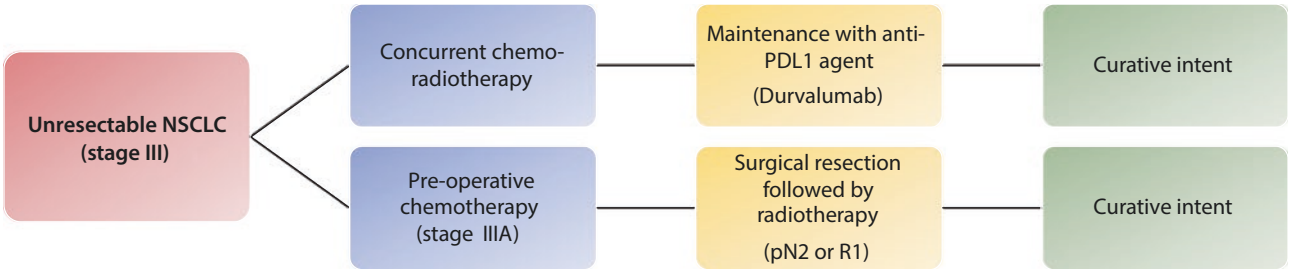
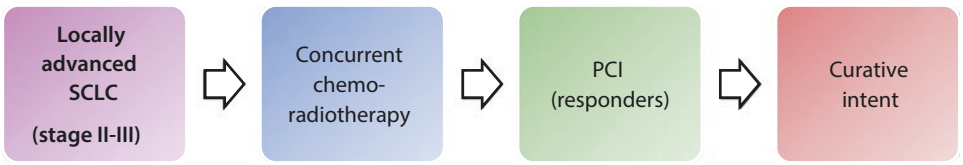


Fig. 32.15 Therapeutic algorithm for stage III NSCLC

Fig. 32.16 Therapeutic algorithm for locally advanced SCLC



32.8.2 Locally Advanced Disease

Stage III account for 25–30% of NSCLC, including a heterogeneous group of tumors with a very controversial treatment. Patients with locally advanced stage IIIA NSCLC may be candidate to surgical therapy if they have T4, N0, or single-station N2 disease with nodal staging performed by invasive methods or if they obtained nodal downstaging after pre-operative induction chemotherapy. Several studies included in the LACE meta-analysis demonstrated a 4.2% absolute 5-year survival rate improvement for patients who received adjuvant therapy, including those with stage IIIA N0-1, suggesting platinum-vinorelbine as the best regimen [102]. Eligibility for pre-operative or post-operative platinum doublets with or without radiotherapy should be evaluated in the context of an experienced multidisciplinary team. For all other patients with unresectable stage IIIA or IIIB disease, concurrent chemoradiation is currently recommended as the treatment of choice with 5-year survival rate reported to be 10% [83]. A meta-analysis including seven randomized studies demonstrated a 5.7% absolute 3-year survival rate improvement with concurrent versus sequential treatment in patients with IIIB NSCLC, even if at the cost of increased toxicity [114]. However, sequential chemotherapy followed by definitive radiotherapy is considered as an alternative valid option, especially for elderly or frail patients with clinical comorbidities. Different platinum-based combinations may be used on the clinical center experience [115–117]. Recently the randomized phase III PACIFIC study compared the anti-PDL1 monoclonal antibody durvalumab vs placebo as maintenance therapy in patients with stage III unresectable NSCLC after definitive concurrent chemoradiation, showing a significant PFS improvement in patients receiving durvalumab [118], which represents a new standard of care in this setting of patients (Fig. 32.15).

Patients with T1-4, N1-3, and M0 SCLC represent about one third of overall SCLC population. Several studies and meta-analysis demonstrated that concurrent radiochemotherapy is the best treatment option for such patients with "limited disease" and good performance status and platinum-etoposide is the most used chemo-regimen [119]. However, sequential chemotherapy followed by radiotherapy may be considered as an alternative valid option when concurrent treatment is not feasible [27]. A meta-analysis including about 1000 patients with limited disease who had complete response to chemoradiotherapy showed that prophylactic cranial irradiation (PCI) significantly improved patients' survival and reduced the risk of brain metastasis at 3 years,

without any impact on extracranial disease [120]. Because of the high incidence of brain metastasis in the natural history of the disease, all patients who responded to initial therapy should be considered for PCI within 6 months from treatment beginning [27] (Fig. 32.16).

32.8.3 Metastatic Disease

The majority of patients have distant metastasis at the time of NSCLC diagnosis, with median survival reported to be 6 months without any treatment [1]. In the last two decades, the advent of new effective drugs including targeted therapies and immunotherapies revolutionized the treatment strategies and the natural history of advanced disease, significantly improving both patients' survival and quality of life. The decision of first-line therapy should be discussed within a multidisciplinary team, taking into account both tumor histology and molecular profile, along with age, PS, comorbidities, and preference of patients [121].

Oncogene-Addicted NSCLC

For about 20% of patients whose tumors harbor oncogenic drivers, including both EGFR-activating mutations and ALK/ROS1 rearrangements, targeted agents are recommended as an upfront therapy [121] (Fig. 32.17). The recent advent of next-generation sequencing (NGS) in many centers favored molecular testing for multiple gene alterations, including BRAF, HER2, and MET alterations, as well as ROS1, RET, and NTRK fusions which may currently allow access to targeted treatment only in late lines of therapy in the context of a clinical trial [121].

Eight randomized phase III trials [33, 122–128] demonstrated that EGFR TKIs gefitinib, erlotinib, and afatinib significantly improved response rate (RR), progression-free survival (PFS), tolerability, and QoL as compared to platinum-based chemotherapy in about 12–15% of patients with metastatic NSCLC harboring EGFR (exon 19 deletion and exon 21 L858R point mutation)-activating mutations (Table 32.7). Thus, molecular EGFR testing is currently recommended in all patients with newly diagnosed, advanced, non-squamous NSCLC and in never/former and light smokers with squamous subtype before starting the first-line therapy in order to select the best treatment for each patient [121].

Recently the randomized phase III FLAURA trial [129] compared third-generation TKI osimertinib to first-generation TKI gefitinib or erlotinib in untreated EGFR-mutant NSCLC patients. The results of this study clearly demonstrated that upfront osimertinib nearly doubled median PFS and duration of response, presented with less toxicities and with intracranial activity against brain metastasis (BM) that is superior than all other TKIs, emerging as the most effective and better tolerated drug currently available for first-line treatment of EGFR-positive NSCLC patients (Table 32.8). Accordingly, the final results of the study have recently proved that osimertinib achieved a clinically meaningful improvement in median OS of almost 7 months (38.6 versus 31.8 months) in the same setting of patients [130].

Noteworthy, these EGFR mutations proved to be strong predictors of response to TKIs with the most "common" type of activating or sensitizing EGFR mutation being the in-frame deletion of exon 19 (about 45% of EGFR mutations), followed by the L858R point mutation of exon 21 (about 40% of EGFR mutations). The remaining 10% of EGFR mutations appeared to harbor heterogeneous molecular alterations within exons 18–21 (so-called "uncommon" mutations) with clinically variable responses to targeted drugs and shorter survival rates when compared to classical mutations [131].

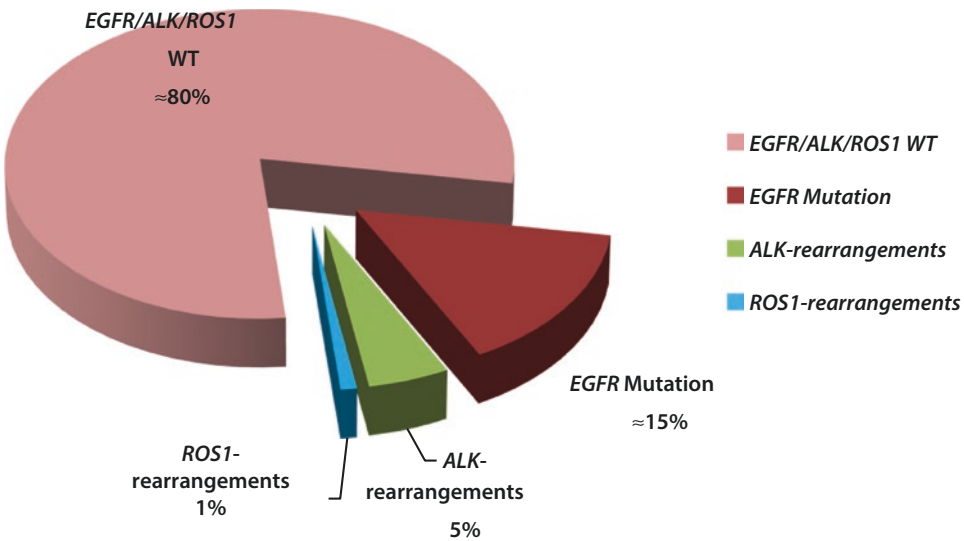


Fig. 32.17 Targetable oncogenic drivers in patients with advanced NSCLC

Table 32.7 Randomized phase III trials of EGFR TKI vs chemo as the first-line treatment in EGFR+ NSCLC

Study	Treatment	N (EGFR mut+)	PFS months (EGFR mut+)	HR for PFS (EGFR mut +)	OS months (EGFR mut+)	HR for OS (EGFR mut+)
IPASS Mok 2009	Gefitinib Chemo	132 129	9.5 6.3	0.48 (0.36–0.66)	21.6 21.9	0.91 (0.76–1.10)
First-SIGNAL Han 2012	Gefitinib Chemo	26 16	8.0 6.3	0.54 (0.26–1.10)	27.2 25.6	1.043 (0.49–2.18)
NEJ002 Maemondo 2010	Gefitinib Chemo	114 110	10.8 5.4	0.30 (0.22–0.41)	30.5 23.6	0.887 (0.63–1.24)
WJTOG3405 Mitsudomi 2010	Gefitinib Chemo	86 86	9.2 6.3	0.49 (0.34–0.71)	35.5 38.8	1.185 (0.76–1.82)
OPTIMAL Zhou 2011	Erlotinib Chemo	82 72	13.7 4.6	0.16 (0.10–0.26)	22.6 28.8	1.04 (0.69–1.58)
EURTAC Rosell 2012	Erlotinib Chemo	86 87	9.7 5.2	0.37 (0.25–0.54)	25.8 20.8	0.86 (0.54–1.38)
LUX-Lung 3 Sequist 2013	Afatinib Chemo	230 115	11.1 6.9	0.58 (0.43–0.78)	28.2 28.2	0.88 (0.66–1.17)
LUX-Lung 6 Wu 2014	Afatinib Chemo	242 122	11.0 5.6	0.28 (0.20–0.39)	23.1 23.5	0.93 (0.72–1.22)

Table 32.8 Efficacy and tolerability of EGFR TKIs as the first-line treatment in EGFR+ NSCLC

	Gefitinib	Erlotinib	Afatinib	Dacomitinib	Osimertinib
ORR	71.2%	64%	69%	75%	80%
PFS	9.5 months	9.7 months	11.1 months	14.7 months	18.9 months
Severe AEs	28.7%	32%	49%	63%	34%

Similarly, the ALK TKI crizotinib has shown to improve RR, PFS, and QoL as compared to first-line platinum chemotherapy and has been recommended as an upfront treatment in 3–8% of NSCLC harboring ALK rearrangements [132]. Thus, routine screening testing for ALK by IHC should be performed simultaneously to EGFR mutations in the same patient population, followed by the break-apart fluorescence in situ hybridization (FISH) analysis to confirm definitive positivity [121].

Recently the phase III randomized J-ALEX [133] and ALEX [134] studies compared the new-generation ALK inhibitor alectinib vs crizotinib in untreated ALK-rearranged NSCLC patients. Treatment with alectinib was associated with longer PFS and better tolerability than crizotinib, and, most importantly, it prevented the occurrence of BMs, emerging as the new standard of

Table 32.9 Efficacy and tolerability of ALK inhibitors as the first-line treatment in ALK+ NSCLC

	ORR	CNS ORR	PFS (months)	Any grade AEs	Grade 3–5 AEs
Alectinib	82.9%	81%	N.R	97%	41%
Crizotinib	75.5%	50%	11.1	97%	50%

care worldwide (Table 32.9). Likewise, according to the planned interim analysis of the CROWN trial, the third generation ALK TKI lorlatinib led to a 72% improvement in PFS along with numerical improvements in best overall response when compared with crizotinib as a first-line treatment approach, finally resulting in a higher incidence of grade 3/4 adverse events without an increased treatment discontinuation rate [135, 136].

As regards other second-generation ALK TKIs, brigatinib showed superiority to crizotinib in the phase III ALTA-1L trial as well as the third-generation ALK inhibitor lorlatinib, demonstrating a significant intracranial activity. Of significance, no randomized trials comparing alectinib with the other second-generation or third-generation ALK TKIs have been published.

Interestingly, the addition of immune checkpoint inhibitors to both first- and third-generation EGFR TKIs did not lead to any significant enhancement of clinical activity when compared to EGFR TKI alone in TKI-naïve and TKI-pre-treated NSCLC patients, however at the cost of unexpected toxicities, ultimately resulting in the limitation of further active investigation [135].

As far as other targetable alterations are concerned, BRAF mutations are identified in 2–4% of lung adenocarcinomas, half of whom presenting with a BRAF V600 mutation. Based on the results of the phase II BRF113928 trial, the combination of dabrafenib and trametinib is now approved in the first-line treatment of patients with BRAF V600 mutations, reporting excellent results that were similar in treated and untreated patients with an average ORR of 64% and a median PFS of 10 months along with a manageable safety profile, especially in the BRAF V600E population [137]. Furthermore, the discovery of new highly selective KRAS inhibitors such as AMG510 and MRTX849 eliciting partial responses in phase I trials has provided a renewed opportunity to better understand the role of KRAS mutation as an oncogenic driver, occurring in 20–30% of NSCLC patients [138]. Recently, the list of actionable targets in lung cancer has been expanded with the FDA approval of capmatinib for MET exon 14 skipping mutations, larotrectinib and entrectinib for NTRK-rearranged tumors [139, 140], and selpercatinib for RET fusion-positive NSCLCs. Other experimental molecular targets include HER-2 mutations, which recently have been shown to be therapeutically exploitable with novel potent anti-HER2 agents, such as T-DM1 and trastuzumab deruxtecan (DS-8201a) [141], and NRG1 gene fusions [142]. Moreover, the introduction of new targeted agents in clinical practice modified the lung cancer treatment strategy and was accompanied by the emergence of a new spectrum of toxicities. Skin rash, diarrhea, and asymptomatic hypertransaminasemia were the most common adverse events associated with EGFR TKIs, while visual disorders, gastrointestinal disturbances, and elevated liver enzymes were associated with ALK inhibitors [143], thus requiring an active involvement and a close collaboration between oncologists and family physicians in all phases of patients' care.

Acquired Resistance

Even if oncogene-addicted NSCLC patients benefited from TKIs, the majority of them experienced disease progression within 10–12 months of therapy, due to the development of acquired resistance by cancer cells [144] (Fig. 32.18).

The exon 20 T790M mutation is the most common finding detected in 50–60% of EGFR-mutated resistant tumors progressing to first-/second-generation EGFR TKIs. It involves the ATP-binding pocket of the receptor and results in an increased ATP affinity, causing resistance to competitive reversible EGFR TKIs [145].

The third-generation TKI osimertinib, targeting both EGFR-activating and EGFR-resistant T790M mutations, has shown to significantly improve RR, PFS, OS, and QoL as compared to standard platinum chemotherapy in patients with EGFR-mutated NSCLC who progressed to first-line EGFR TKI and were T790M-positive [146]. The results of the randomized phase III AURA trial [146] have led to the recent approval of osimertinib as new standard of care in this subset of patients emphasizing the importance of a genotype-guided approach to second-line therapy [121].

Thus, tumor re-biopsy at the time of disease progression should be considered in all patients who progressed to prior EGFR TKI [121]. Even if tissue biopsy remains the current gold standard, it is limited by several features, such as the difficult access to different tumor sites, the invasiveness of procedures, the tumor heterogeneity, and the low patients' compliance [147]. An increasing number of studies and meta-analysis [40, 41, 148, 149] evaluated the diagnostic accuracy of ctDNA for the detection of EGFR mutations in the plasma of patients with advanced NSCLC, overall suggesting a high concordance rate between these two testing methods and ultimately leading to the introduction of ctDNA in clinical practice. Molecular testing by ctDNA has been recommended as the first step of tumor genotyping for all patients who progressed to first- or second-generation EGFR TKI [121]. However, because of 30% potential false negative rate associated with this method [150], ctDNA analysis must be always followed by tissue biopsy in all cases who are T790M-negative on plasma [121]. Interestingly, the location of metastatic site appeared to significantly influence the ability to identify EGFR-activating and EGFR-resistant T790M mutations, leading to a higher diagnostic accuracy of ctDNA analysis in the event of extra-thoracic disease [151]. With the increasing upfront use of osimertinib considering the impressive results of the aforementioned FLAURA study, the detection of T790M mutation in this setting would become of secondary importance, since its loss has been usually associated with early resistance to osimertinib in the light of

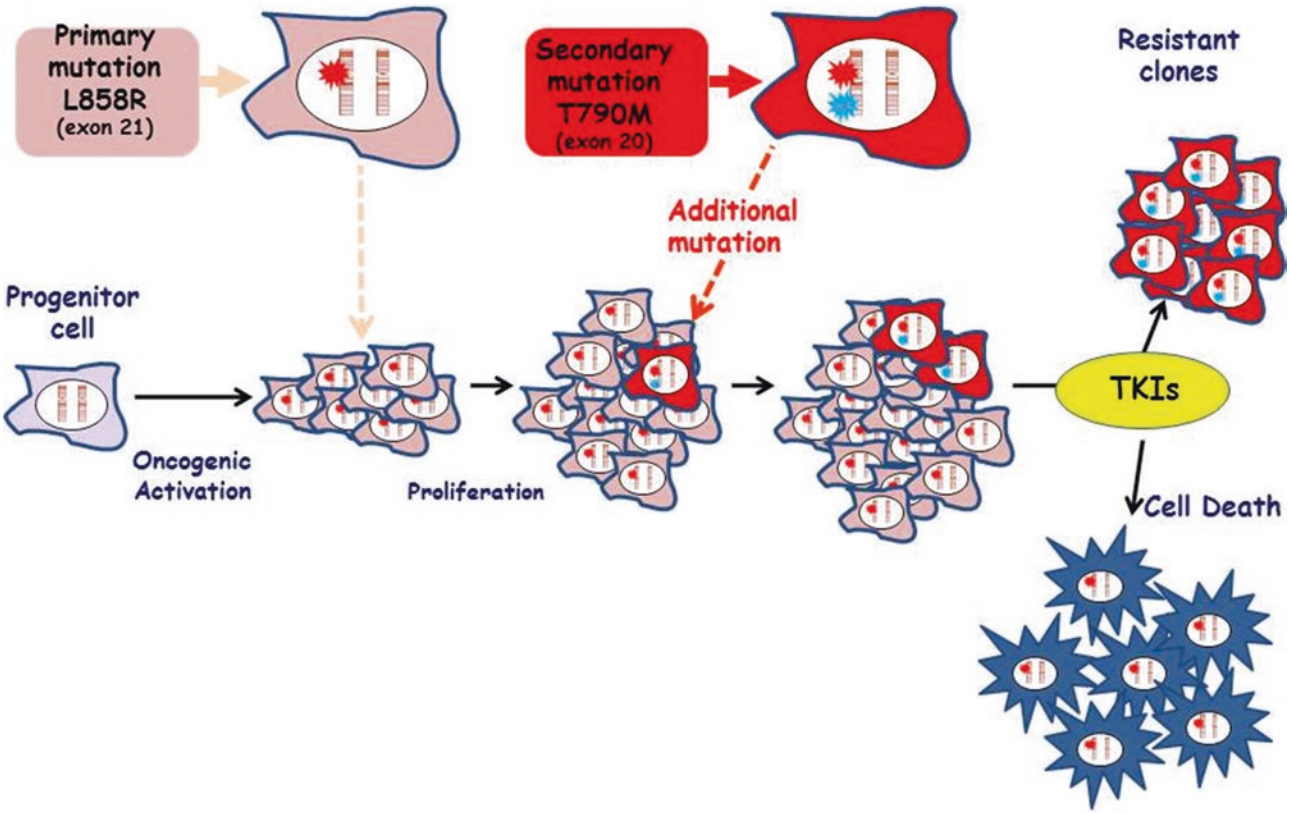


Fig. 32.18 Clonal evolution during EGFR TKI in NSCLC

the drug mechanism of action. Even if the mutational status of T790M could be readily monitored in plasma in order to precede a proven radiological progression of disease, other multiple resistance mechanisms need to be considered in this regard. Further implementation of liquid biopsy in monitoring the response to osimertinib and detecting the wide spectrum of molecular alterations responsible for treatment failure (either EGFR-dependent or EGFR-independent) is warranted and eagerly awaited in both ongoing and future clinical trials. In this context, liquid biopsy using ctDNA analysis has proved to be feasible and reliable for detecting most of genomic alterations [152] (Fig. 32.19).

Besides T790M mutation, other molecular alterations have been detected in EGFR-positive resistant tumors, including the amplification of the mesenchymal-epithelial transition (MET) factor receptor tyrosine kinase (20%), Her-2 amplification (12%), phenotypic change from NSCLC to SCLC (4%), and modifications in other parallel signaling pathways (Fig. 32.20), and new targeted agents are currently under investigation in clinical trials [144]. Waiting for the new tailored agents, platinum chemotherapy is currently recommended as the standard treatment for all patients who progressed to EGFR TKI and are T790M-negative on tumor tissue analysis.

Unfortunately, ALK-positive patients treated with crizotinib also relapse after a variable period of drug sensitivity because of the development of acquired resistance [153]. Different resistance mechanisms to crizotinib have been identified, including secondary mutations in the ATP-binding pocket of the receptor or the amplification of the ALK fusion gene or the activation of other oncogenic drivers such as increased EGFR phosphorylation and mutation, Kit amplification and mutation, and KRAS mutation, which may cause resistance independent of ALK [154, 155] (Fig. 32.21).

Second-generation ALK inhibitors ceritinib [156] and alectinib [157] have shown to significantly improve both RR, PFS, OS, and QoL as compared to platinum chemotherapy in ALK-rearranged patients who progressed to crizotinib, thanks to their ability to target the most frequent secondary mutations in the ALK domains and their higher activity against brain metastasis [158]. Although the new ALK inhibitors showed a selective spectrum of activity according to the different tumor-resistant mutations (ALK-dependent and ALK-independent), a genotype-guided approach is strongly encouraged within clinical trials but not currently recommended at the time of disease progression in clinical practice. Thus, both ceritinib and alectinib may be used in ALK-positive NSCLC patients who failed

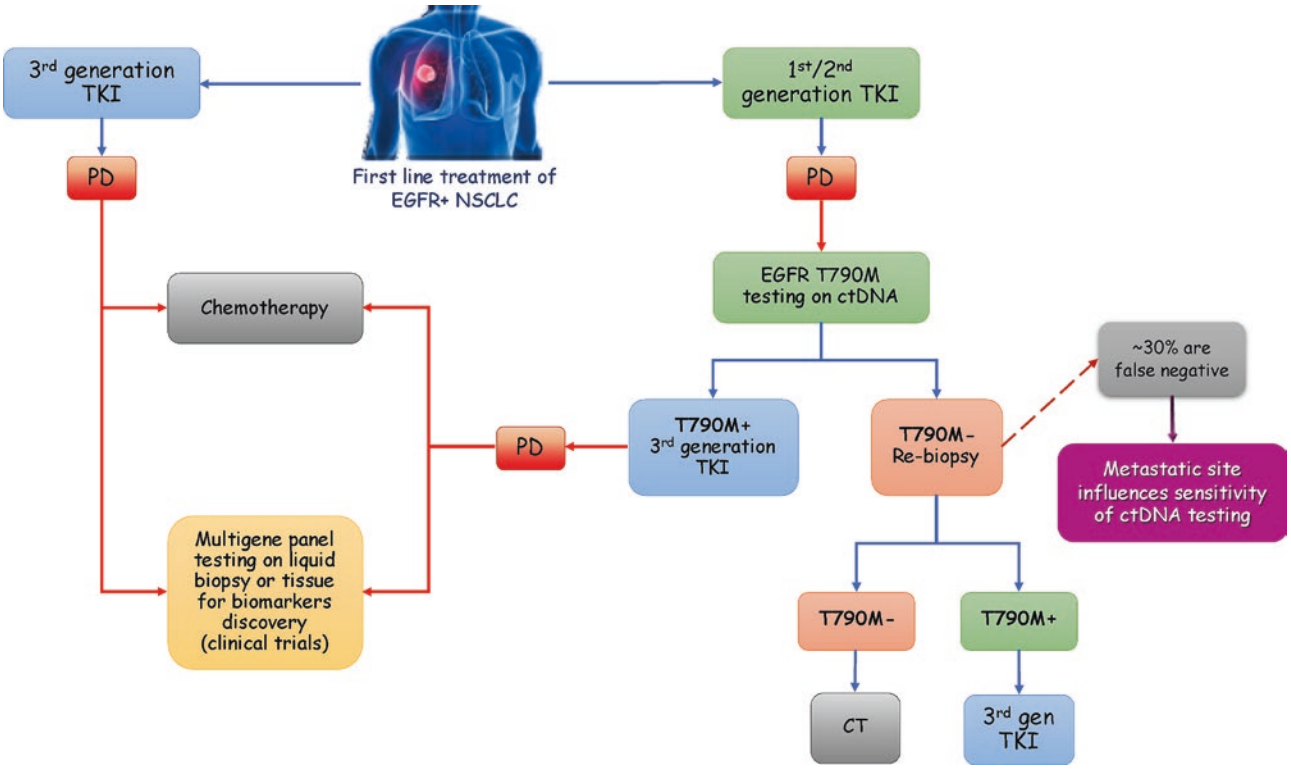


Fig. 32.19 EGFR mutation testing at the time of disease progression to EGFR TKI

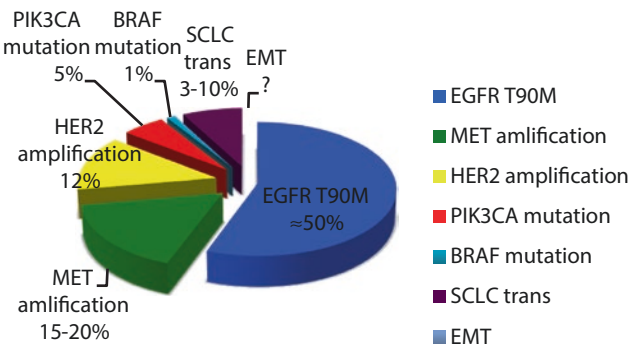


Fig. 32.20 Molecular mechanisms of acquired resistance to first-generation EGFR TKIs

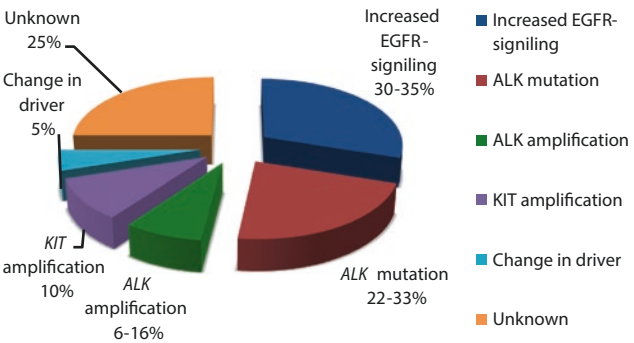


Fig. 32.21 Molecular mechanisms of acquired resistance to first-generation ALK inhibitors

crizotinib, reserving platinum combinations to further line of therapy [159]. As ctDNA and tissue NGS techniques continue to advance in this regard, a better understanding of the optimal treatment sequencing is warranted.

For all oncogene-addicted NSCLC patients who experienced limited and asymptomatic radiological progression at a single site, including the brain, bone, or adrenal gland, and were still dependent from the oncogenic signaling, continuing the TKI beyond PD in combination with local treatment, such as surgery or radiotherapy, may represent a reasonable option and could be considered on an individualized basis after discussion within a multidisciplinary team [121] (Fig. 32.22).

Non-oncogene-Addicted NSCLC

The advent of immunotherapy has recently led to a paradigm shift of first-line treatment for about 30% of patients with advanced NSCLC whose tumors overexpressed PD-L1 >50% by IHC analysis on tumor tissue samples [121, 160]. The results of the phase III KEYNOTE-024 randomized trial [161] showed that the anti-PD1 checkpoint inhibitor pembrolizumab significantly improved RR, PFS, OS, and QoL as compared to platinum chemotherapy, becoming the new standard of care in this subset of patients. Adding immunotherapy to first-line platinum regimens [162] as well as combin-

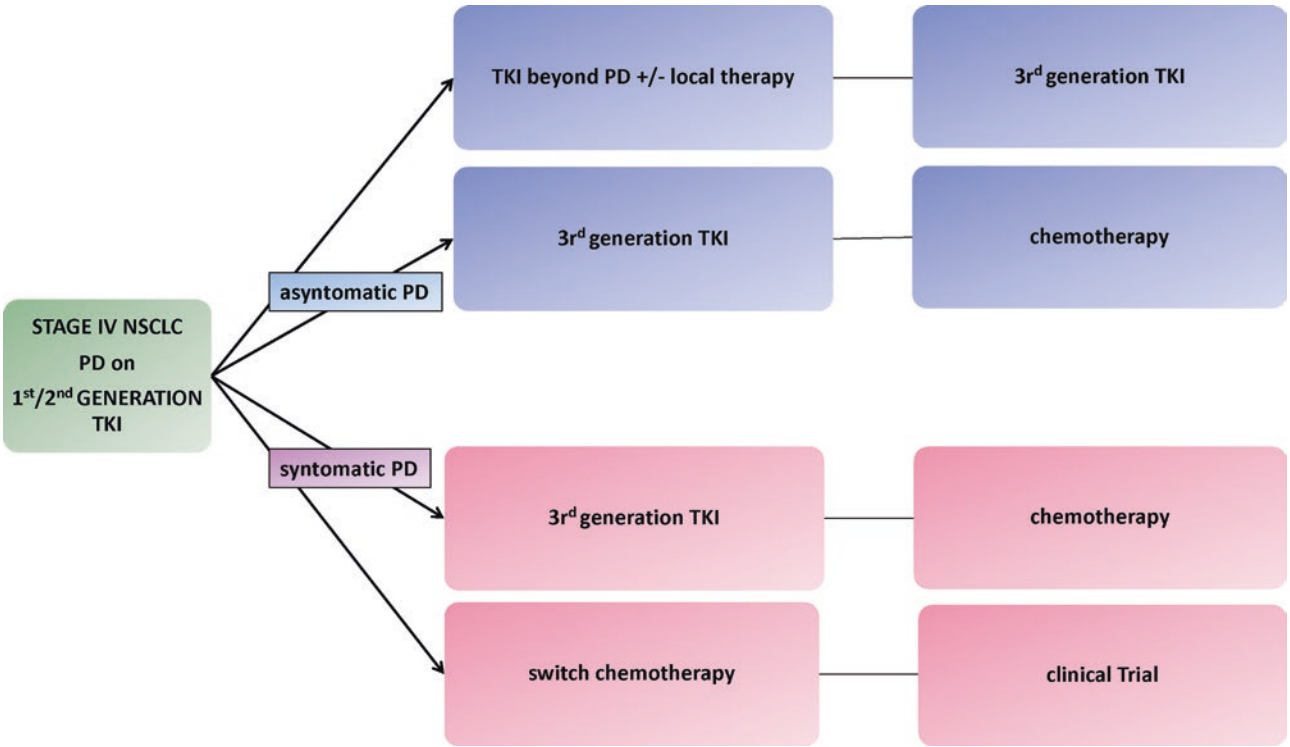


Fig. 32.22 Therapeutic options in oncogene-addicted NSCLC patients who failed first-line treatment based on TKIs

ing different anti-PD1/CTLA4 immunotherapeutic agents [163] emerged also as effective and tolerable first-line options in patients with advanced NSCLC, irrespective of PD-L1 expression. Despite some initial concerns of toxicity profiles, patients treated with the association of immune checkpoint inhibitors (pembrolizumab or atezolizumab) with platinum-based chemotherapy had improved OS, PFS, and ORR when compared to chemotherapy alone, making these regimens the current standard of care in the first-line treatment of non-oncogene-addicted NSCLC fit patients. In contrast, the results of those trials investigating the combination immunotherapy when compared to chemotherapy are difficult to interpret: the CheckMate 227 trial evaluating the association of the anti-PD1 nivolumab with the anti-CTLA4 ipilimumab showed initial improvement in PFS and later on in OS rates only in PD-L1-positive patients while being amended to use tumor mutation burden (TMB) or PD-L1 as a primary endpoint [164], whereas the MYSTIC trial evaluating the combination of the anti-PD-L1 durvalumab with the anti-CTLA4 tremelimumab did not significantly improve OS or PFS in PD-L1 selected patients [165].

For all other patients with EGFR-/ALK-/PD-L1-negative, advanced NSCLC, without major comorbidities, platinum combinations should be recommended as an upfront treatment, according to the tumor histological subtype [121]. Particularly up to six cycles of platinum combinations with a third-generation cytotoxic agent, including gemcitabine, vinorelbine, or taxanes, are recommended in patients with both squamous and non-squamous subtypes, while four cycles of platinum-pemetrexed followed by less toxic maintenance pemetrexed monotherapy until disease progression or unacceptable toxicities may be preferred in non-squamous NSCLC [166, 167]. Randomized trials and meta-analysis showed that the addition of bevacizumab to paclitaxel/carboplatin regimens improved OS in patients with non-squamous subtype and PS 0–1 and may be considered as an effective treatment option in these patients [168, 169] (Fig. 32.23). The randomized phase III SQUIRE trial [170] showed that the addition of the anti-EGFR monoclonal antibody necitumumab to platinum-gemcitabine led to a small benefit in patients with squamous EGFR-expressing tumors assessed by IHC [171], but this combination should be carefully evaluated due to the limited clinical improvement.

Different treatment options are currently available for patients with clinical or radiological progression to first-line treatment. Before the advent of chemo-immunotherapy, single-agent Immunotherapy, including the PD1 checkpoint inhibitors nivolumab and pembrolizumab and the PD-L1 inhibitor atezolizumab, represented the new standard of care in pre-treated NSCLC patients [121, 160]. Four phase III randomized studies demonstrated that PD1/PDL1 inhibitors are

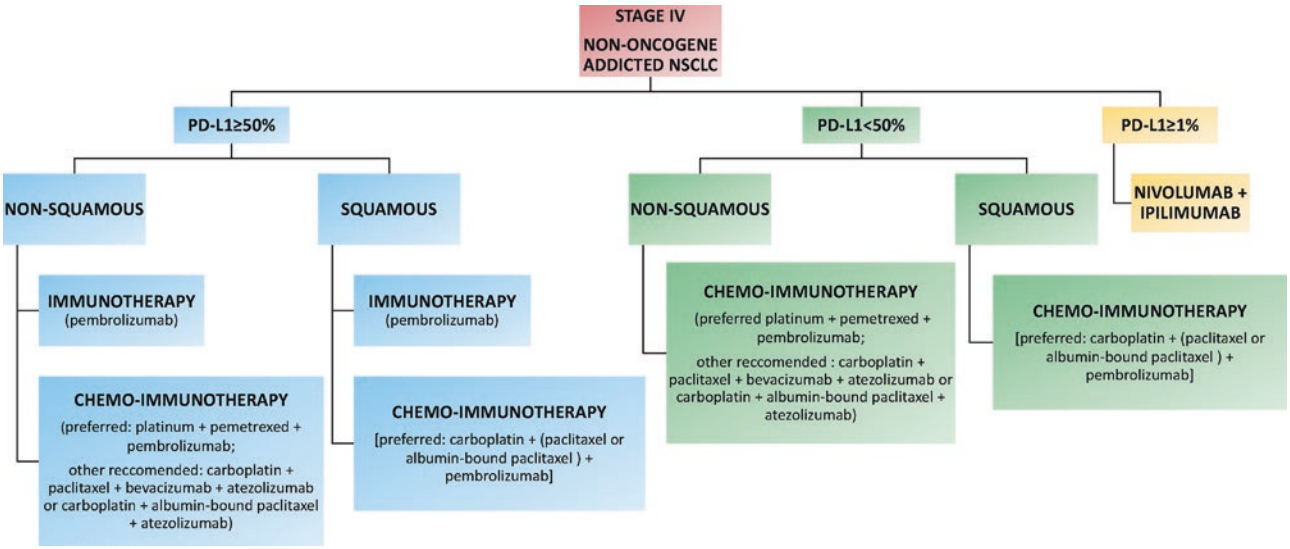


Fig. 32.23 Therapeutic upfront options in non-oncogene-addicted NSCLC patients

32

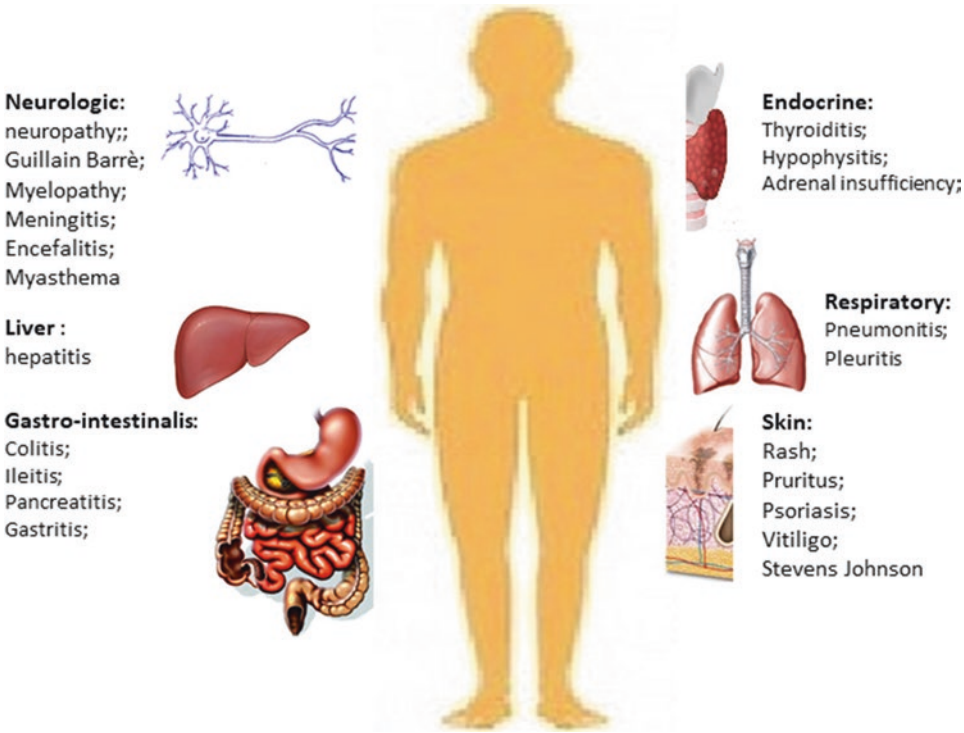


Fig. 32.24 Immune-related adverse events with checkpoint inhibitors

more effective and better tolerated than second-line single-agent docetaxel [172–175] and are currently recommended in NSCLC patients who experienced progression after platinum combinations regardless of tumor histological subtype and PD-L1 status, except for pembrolizumab, which was approved only for PD-L1-positive (IHC > 1%) tumors [121]. In this setting, an indirect comparison seemed to favor nivolumab and pembrolizumab in terms of response rates when compared to atezolizumab, whereas nivolumab appeared to be associated with a significant lower incidence of adverse events as compared to pembrolizumab and atezolizumab [176].

The use of immunotherapeutic agents in clinical practice was associated with the emergence of a new spectrum of toxicities (Fig. 32.24) derived from autoimmune response against normal tissues. Even if not frequently reported, immune-related toxicities

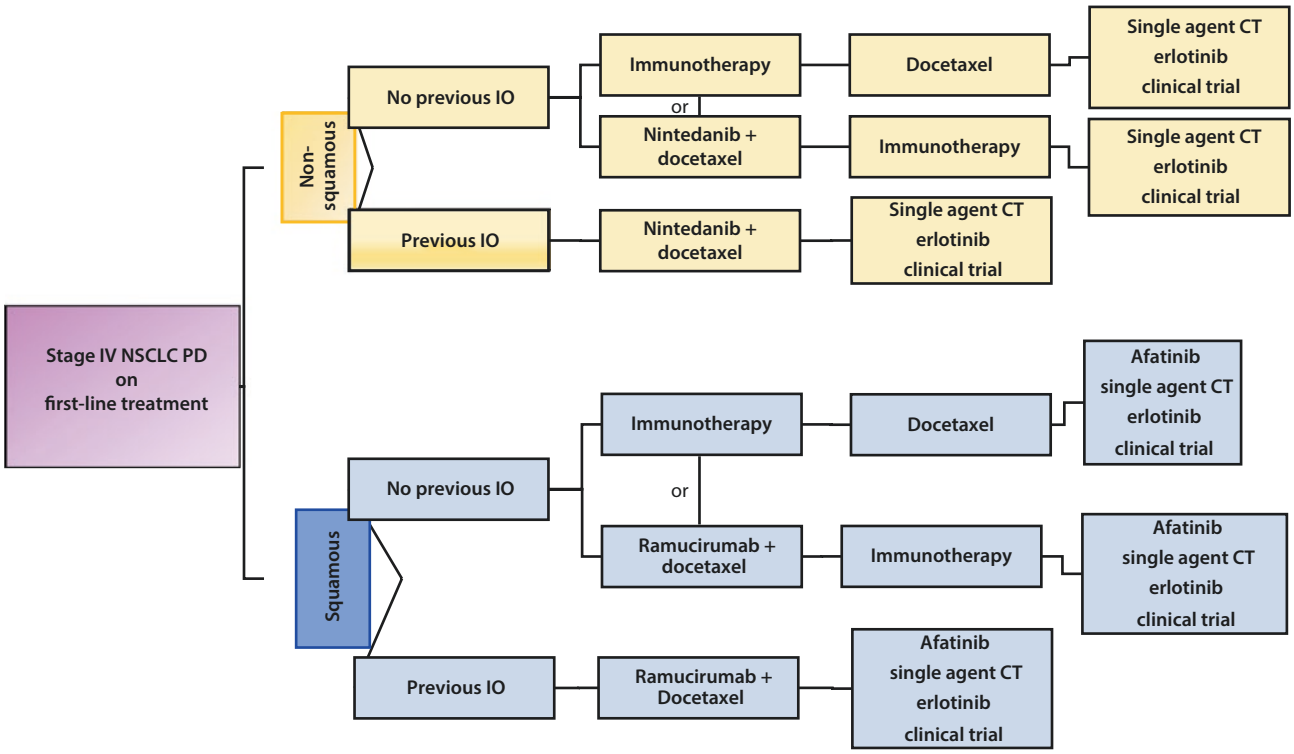


Fig. 32.25 Therapeutic options in non-oncogene-addicted NSCLC patients who failed first-line platinum chemotherapy

may be life-threatening, thus requiring an urgent and adequate pro-active management [177, 178].

Antiangiogenic agents such as the multi-kinase inhibitor nintedanib and the anti-VEGFR2 monoclonal antibody ramucirumab have been investigated in combination with docetaxel in two randomized phase III LUME-Lung1 [179] and REVEL [180] trials, showing to significantly improve OS of pre-treated NSCLC patients with adenocarcinoma and all histological subtypes, respectively. In the LUX-Lung 8 trial [181], the EGFR TKI afatinib showed significant improvements in PFS and OS as compared to erlotinib in pre-treated squamous EGFR wild-type NSCLC, emerging as an additional option in later lines of therapy. In the absence of direct comparisons among these new approved agents as well as of validated predictive biomarkers, the decision of second-line therapy should take into account several factors, including first-line treatment, tumor histology, best response and toxicities to prior treatment, and patients' comorbidities and preference in order to select the most effective and tolerable treatment for each patient (Fig. 32.25).

Brain and Bone Metastasis

The brain is the most common site of metastatic recurrence, followed by the bone, lung, liver, and adrenal glands. About 50% of patients with oncogene-addicted NSCLC will develop brain metastasis in the natural history of their disease [182]. Different from first-generation TKIs, the new EGFR TKI osimertinib [129, 183], as well as the new ALK inhibitor alectinib [134, 184], showed great activity against brain metastasis because of their ability to penetrate the blood-brain barrier (BBB) [185], emerging as new effective treatment options for patients with asymptomatic disease. For all other patients with symptomatic brain metastasis or EGFR-/ALK-negative tumors, the multimodality treatment, including surgical resection, stereotactic radiotherapy (SRS), whole brain radiotherapy, and systemic chemotherapy, should be offered or combined according to the patient's prognosis and number of metastatic sites [121].

All the patients with advanced NSCLC and bone metastasis should receive zoledronic acid or denosumab to prevent skeletal-related events (SRE), including pathological fracture, radiation or surgery to bone, or spinal cord compression. Palliative radiation should be offered to all patients with symptomatic bone lesions to achieve symptom control and is also effective in treating pain related to chest wall, soft tissue, or neural invasion [121].

Extensive-Stage SCLC

Chemotherapy with platinum-etoposide up to six cycles has been recommended as first-line treatment for all patients with metastatic SCLC and PS 0–1 for many years. Thoracic radiotherapy may be considered in patients who achieved complete extra-thoracic response

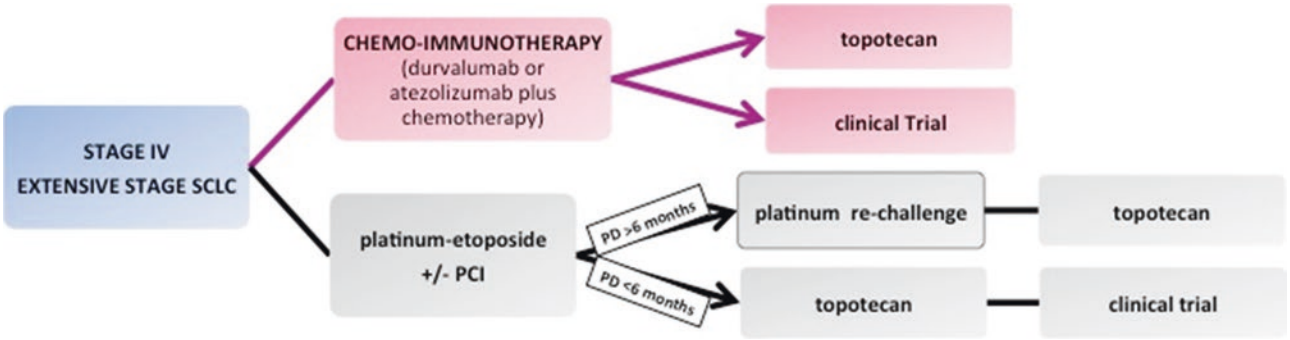


Fig. 32.26 Therapeutic algorithm in patients with extensive-stage SCLC

and partial intra-thoracic response to first-line chemotherapy, while PCI should be offered to all patients with extensive-stage SCLC who responded to first-line chemotherapy [27, 186]. However, even if the majority of patients respond to platinum chemotherapy, they inevitably will develop disease progression with median survival reported to be 3 months without any treatment [187]. Re-challenge with platinum combinations may be considered in those patients who progressed after 6 months from the end of prior chemotherapy. Second-line therapy with topotecan is recommended as standard of care in all patients who failed prior platinum chemotherapy and have PS 0–1 [27, 186].

Additionally, the combination of the anti-PD-L1 atezolizumab with platinum doublet followed by maintenance atezolizumab has been already approved and used in clinical practice in the United States, demonstrated to significantly achieve longer median OS rates (12.3 vs 10.3 months) in the IMpower133 randomized trial [188]. Likewise, the FDA approved durvalumab in combination with etoposide and either carboplatin or cisplatin as first-line treatment of such patients, since median OS was 13.0 months in the durvalumab plus chemotherapy arm compared with 10.3 months in the chemotherapy alone arm [189].

After first-line failure in small cell lung cancer, clinical trials with novel immunotherapeutic agents including anti-PD1/PD-L1 inhibitors and the DLL3-targeted antibody-drug conjugate rovalpituzumab showed encouraging activity in patients with recurrent SCLC and are currently under investigation in randomized studies [190, 191] (Fig. 32.26).

32.9 Response Evaluation

Response evaluation should be performed every 2 months for patients undergoing systemic therapy, preferably using the same radiological investigation of initial diagnosis/staging. CT scan using RECIST criteria v1.1 for measurements and response reporting is considered appropriate, while follow-up with PET is not routinely recommended. In oncogene-addicted NSCLC patients, treatment beyond RECIST disease progression is a common approach especially in patients with asymptomatic and limited PD [121]. The best criteria for response evaluation with immunomodulatory agents are still the matter of intense work and debate. Even if RECIST criteria have been adopted in the majority of clinical trials which have led to the approval of such agents, the "immune-RECIST" criteria have been recently developed by the RECIST working group to be adopted in the next cancer immunotherapy trials as well as in clinical practice [192].

32.10 Follow-Up

Due to the aggressive nature of this disease, generally close follow-up, including both clinical and radiological evaluation at least every 2–3 months after initial treatment, is advised but should also depend on individual basis [121].

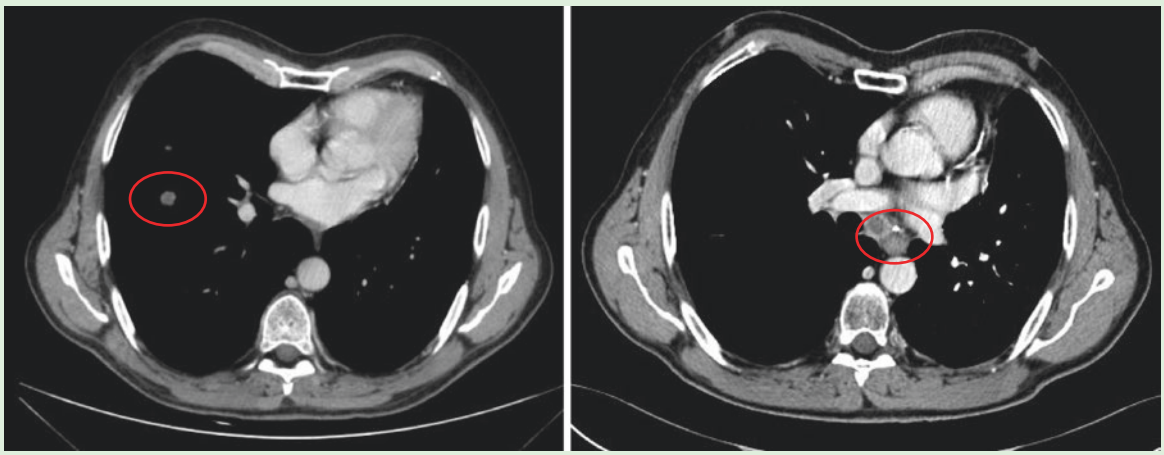
Summary of Clinical Recommendations

- *AIOM*
  - ► http://www.aiom.it/professionisti/documenti-scientifici/linee-guida/1,413,1,#TopList
- *ESMO*
  - ► http://www.esmo.org/Guidelines/Lung-and-Chest-Tumours
- *NCCN*
  - ► https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf
  - ► https://www.nccn.org/professionals/physician_gls/pdf/sclc.pdf

Case Study: Non-oncogene-Addicted Advanced NSCLC

Man: 55 years old

- *Family history*: negative for malignancy
- *APR*: hypertension
- *Smoke history*: former smoker (1 pack/day for 20 years)
- *APP*: cough
- *ECOG PS*: 0
- *CT scan total body*: multiple lung bilateral nodules, mediastinal lymph node metastasis



Bronchoscopy with biopsy: lung adenocarcinoma
Clinical stage (8th TNM staging system): stage IV (M1a)

Question

Which action should be taken?

(1) Surgery (2) Medical therapy (3) Mutational analysis

Answer

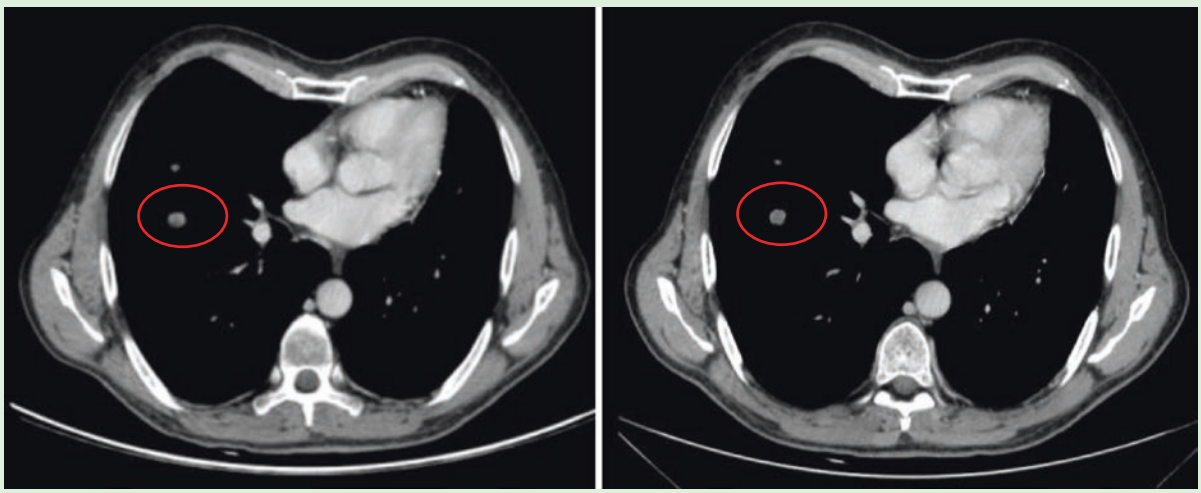
Mutational analysis: EGFR, EML/ALK, ROS1: wild-type

PD-L1 assessment: PD-L1 expression 5%

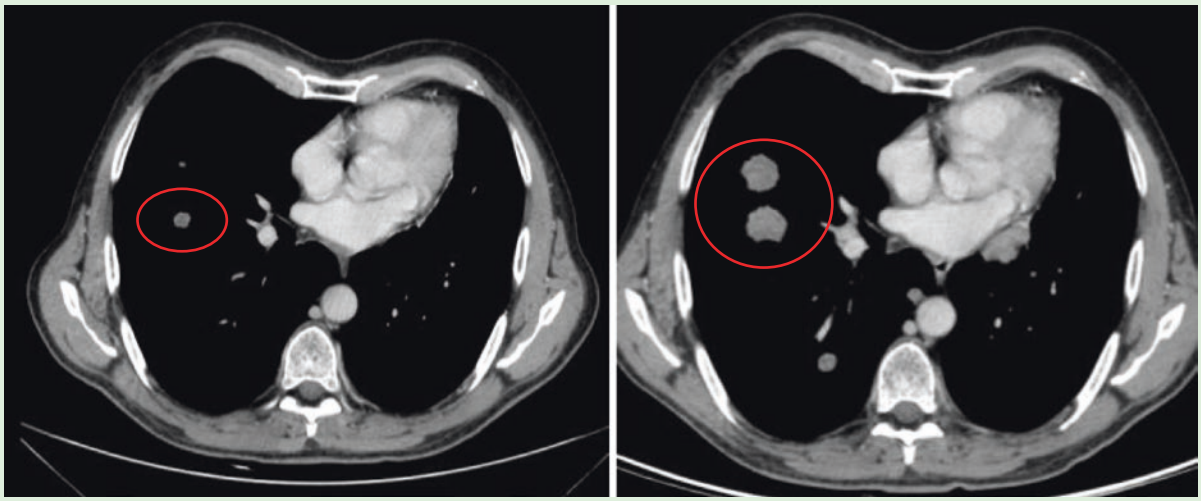
⇩

Medical therapy: cisplatin-pemetrexed × 4 cycles

- *Response evaluation after 3 months of chemotherapy*: Stable disease (SD)



- *Response evaluation after 9 months of maintenance therapy with pemetrexed*: progression disease (PD)



After 3 months | After 12 months

Question

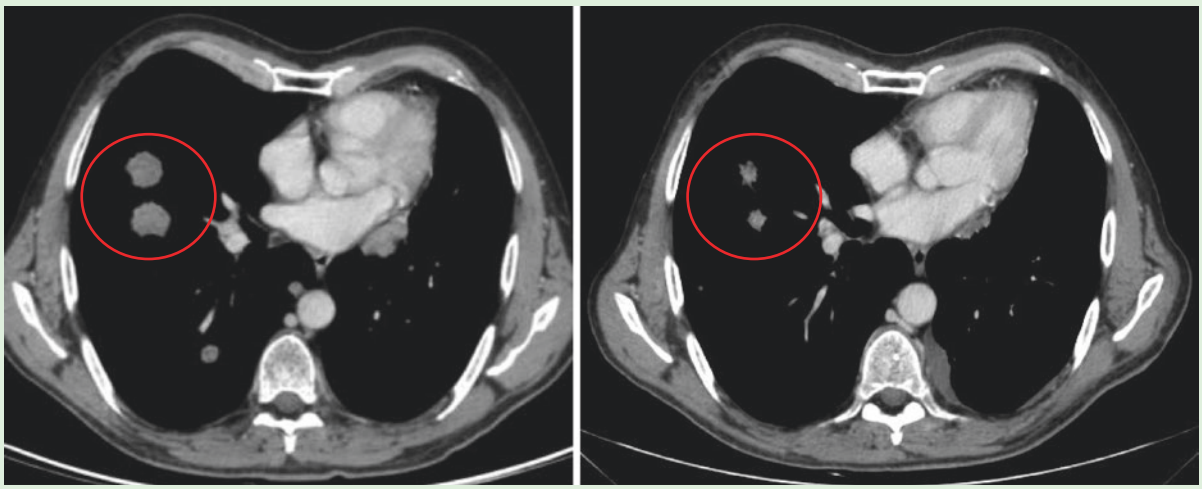
Which action should be taken?

(1) Immunotherapy (2) Chemotherapy plus nintedanib (3) Chemotherapy

Answer

Begins nivolumab 3 mg/kg q 14

- *Response evaluation after 3 months of therapy with nivolumab*: partial response (PR)



Before Nivolumab | After 3 months Nivolumab

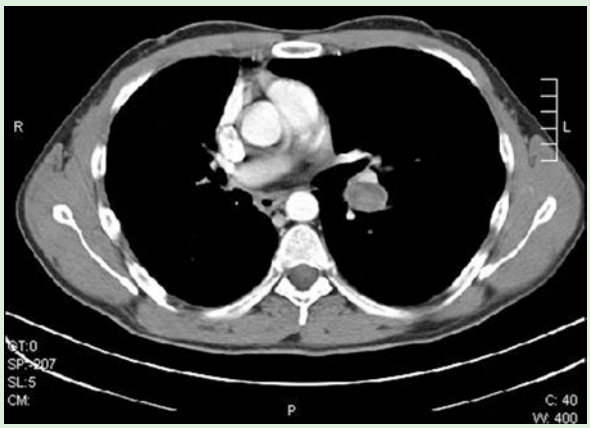
Key Points

- Importance of a correct staging and histological diagnosis
- Importance of histology and mutational analysis for first-line therapeutic choice
- Importance of PD-L1 expression for first- and second-line therapeutic choice

Case Study: Oncogene-Addicted Advanced NSCLC

Woman: 45 years old

- *Family history*: negative for malignancy
- *APR*: negative
- *Smoking history*: never smoker
- *APP*: dyspnea, cough
- *ECOG PS*: 1
- *PET-CT total body*: left lobe nodule, bilateral mediastinal lymph node metastasis *(PET 11)*



Bronchoscopy with trans-bronchial needle aspiration (TBNA): lung adenocarcinoma
Clinical stage (8th TNM staging system): stage IIIB

Question

Which action should be taken?

(1) Surgery (2) Definitive chemoradiotherapy (3) Mutational analysis

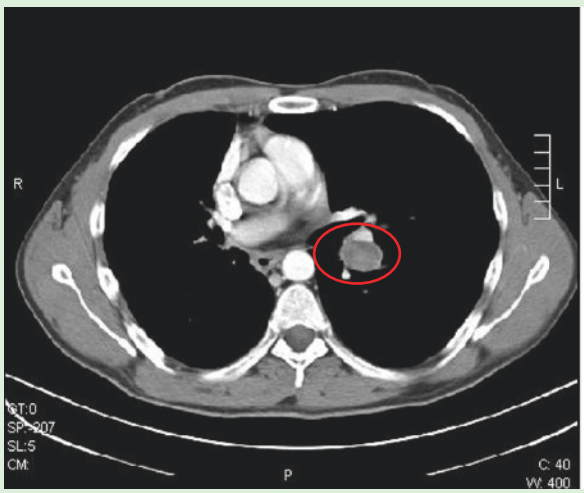
Answer

Mutational analysis: exon 19 deletion of EGFR

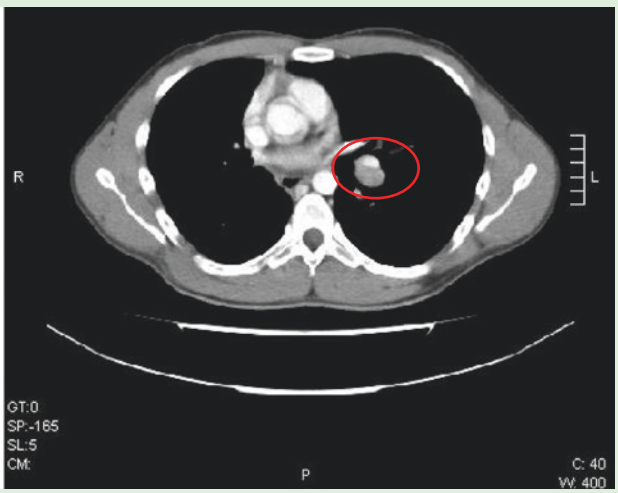
⇩

Gefitinib 250 mg os/day

- *Response evaluation after 3 months of therapy*: partial response (PR) (PET 16)

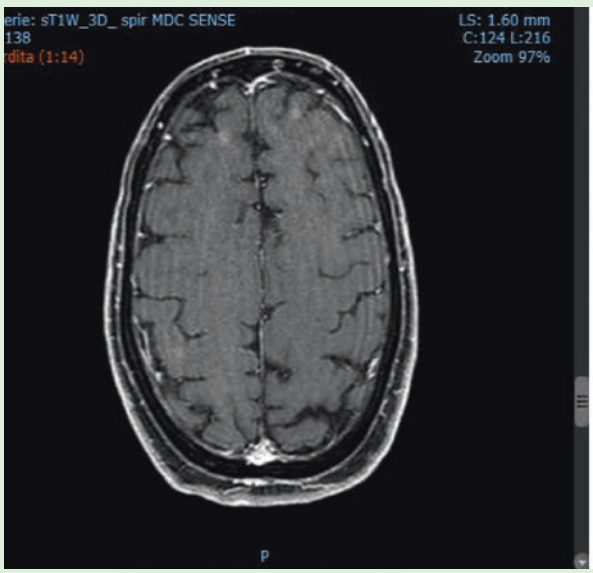


Before therapy

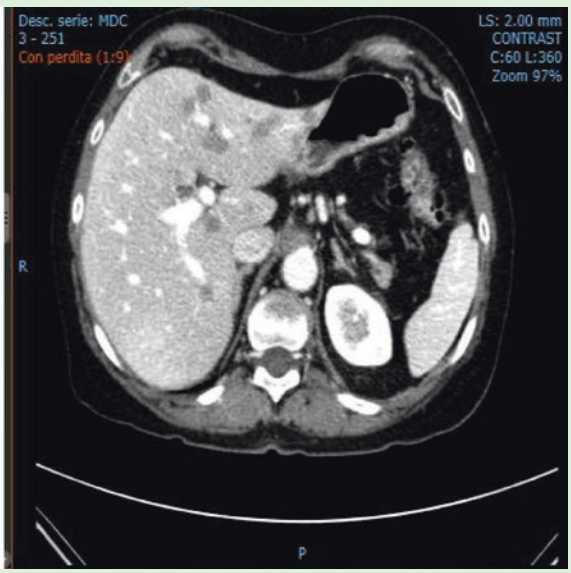


After 3 months therapy

- *Response evaluation after 12 months of therapy*: progression disease (PD) (PET e RMN 17)



Multiple asymptomatic brain lesions



Multiple liver lesions

Question

Which action should be taken?

(1) Whole brain radiotherapy (2) Chemotherapy (3) Re-biopsy

Answer

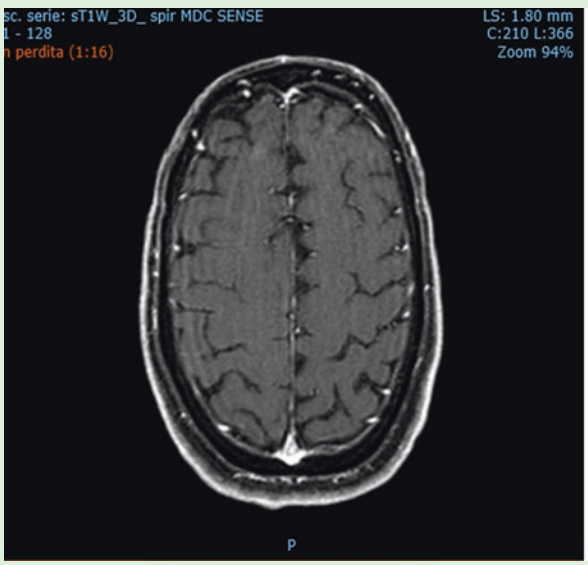
Liquid biopsy: mutational analysis on ctDNA: no evidence of EGFR-T790M mutation

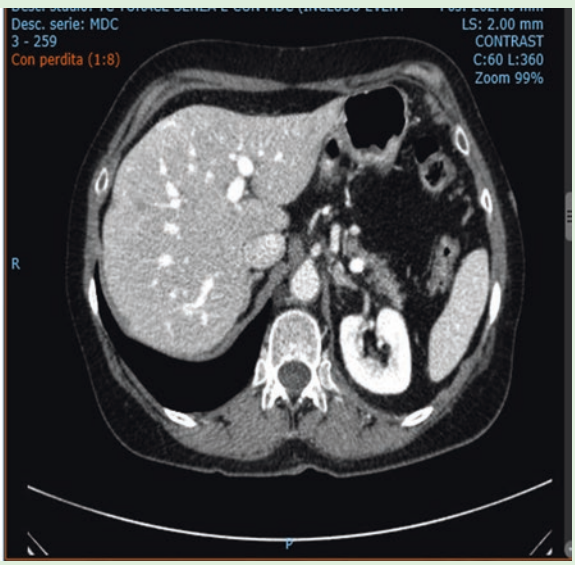
Tissue re-biopsy: positive for exon 19 deletion and exon 20 T790M mutation

⇩

Osimertinib 80 mg os/day

- *Response evaluation after 3 months of therapy with osimertinib*: partial response (PR)





Key Points

- Importance of histology and mutational analysis for first-line therapeutic choice
- Importance of re-biopsy at the time of EGFR+ NSCLC radiological progression
- Great intracranial activity of third-generation EGFR TKI

Expert Opinion

Giorgio Vittorio Scagliotti

Key Points

- Lung cancer remains the first cause of cancer death in both women and men, and, despite several therapeutic improvements over the last 15 years, 5-year survival rates remain disappointing.
- Further studies are clearly needed to implement lung cancer screening strategies to reduce significantly lung cancer mortality. Selection of high-risk participants for LDCT screening is improved by the use of multivariate risk prediction models. Additionally, a range of recruitment strategies will be required based on available health infrastructure and the distribution of the high-risk population. Simplified management algorithms for pulmonary nodules, incorporating risk prediction models and image analysis techniques, will likely lead to reduction in downstream investigations and surgery for benign disease.
- Smoking cessation is critical to the overall benefit and cost-effectiveness of a lung cancer screening program. The best strategy to optimize the intervention and integrate it into a program is not known.
- Morphological staging of lung cancer has almost reached the "efficacy" plateau, and future initiatives should integrate molecular data.
- Molecular, biological, and histological heterogeneities represent major challenges in the majority of lung cancers.
- With the completion of the human genome, we understand now that life is based on dynamic molecular networks rather than on a direct connection between genotype and phenotype.

Hints for Deeper Insight

The application of precision medicine is anticipated to improve all areas of medicine, including predicting an individual's risk of disease, disease prognosis, and risk of side effects versus positive response to disease treatment approaches.

Tissue remains the issue in the era of targeted therapies and immunotherapy because of the clear need to identify the subset of patients who benefit most from these tailored approaches. Histological and biological heterogeneities are well-known phenomena, which might significantly impact our capability to detect specific molecular targets as well as prediction of sensitivity to specific molecular targeted agents. The heterogeneity of response and outcome associated with specific molecular features is more likely a reflection of biological heterogeneity than technical issues, which might not be captured in small biopsies, fine needle aspirates, or tissue microarrays consisting of small selected tissue cores. To address all these issues, potential exploration of genomic alterations in several biological fluids, such as blood, represents a reasonable alternative with huge potential applications in the near future when test sensitivity will be optimized. The unit in precision medicine is a "biomarker ensemble" that includes a predictive biomarker, hypothesized to play a crucial role in the disease pathway; a diagnostic assay, used to determine a patient's biomarker status; and final a therapeutic agent, intended to be more effective for patients who are "biomarker-positive." If one of the three basic elements is lacking, we are lying down outside of the framework of precision medicine.

With the current emphasis on biomarker-driven drug development and the increasing inclusion of integral and integrated biomarkers in our trials, it is necessary to ensure that fit-for-purpose assays of these biomarkers are incorporated in study protocols. Briefly, markers are integral when they are essential for conducting the study as they define eligibility, stratification, and monitoring of the disease or study endpoints. Markers are considered integrated when they actually are testing a hypothesis based on preexisting data and not simply generating hypotheses.

The genomic revolution encompasses only a portion of the emerging hallmarks of cancer, which include enabling characteristics and a better understanding of the tumor microenvironment. In this context, an understanding of the immune landscape of cancers, including immune evasion strategies, has led to breakthrough therapeutic advances for patients with non-small cell lung cancer and has created a platform for future therapeutic developments.

We are at the beginning of a creative period of bottom-up research activity, organized through pilot projects of increasing scope and scale, from which best practices will progressively emerge. In particular, given the size and diversity of the healthcare enterprise, a single approach to data gathering that will populate the space is probably not appropriate for all contributors. As in any initiative of this complexity, we will need the right level of coordination and encouragement of the many players who must cooperate to create a higher level of biomedical knowledge.

In this patient-centered context, patients and their advocates are and will be more critical, each and every day, first to promote the right social pressure for the systematic implementation of the results of preclinical and clinical research and, second, to develop a work in progress and continuous discussion with the regulatory bodies and national healthcare systems in an attempt to guarantee drug accessibility to every patient, as well as help national authorities to maintain the long-term financial sustainability of the healthcare systems.

Suggested Reading

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Malignant Pleural Mesothelioma

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Thoracic Cancers

Contents

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Learning Objectives

By the end of the chapter the reader will:

- Be able to apply the right clinical and diagnostic procedures to easily recognize malignant pleural mesothelioma
- Have learned the basic concepts of epidemiology, risk factors and genetic predisposition
- Have reached in-depth knowledge of main treatment modalities
- Be able to put acquired knowledge into clinical practice

33.1 Introduction

Malignant pleural mesothelioma (MPM) is a rare and aggressive disease arising from the mesothelial cells of the pleural cavity, with a close relationship to the asbestos fibres exposure and an increasing incidence worldwide (Fig. 33.1).

Systemic chemotherapy with antifolate agents and platinum compounds still represents the standard of care for the vast majority of patients at the time of diagnosis.

The role of multimodal approaches, combining systemic treatment with radiotherapy and/or surgery, is still debated, because of the absence of adequate clinical trials designed to evaluate these strategies in patients with an early disease at diagnosis.

Despite some preliminary promising results of immunotherapy and the emerging role of biological agents for the treatment of MPM, a deeper knowledge of its pathogenesis is needed, in order to improve diagnostic tools and treatment outcomes.

33.2 Origin and Histologic Subtypes

The neoplastic transformation of the mesothelial layer leads to the onset of MPM. According to the World Health Organization (WHO) Classification of Tumours of the Pleura [1], the major MPM histologic types encounter:

- Epithelioid, with histological appearance resembling epithelial neoplasia (i.e. carcinomas)
- Sarcomatoid, with histological appearance resembling mesenchymal neoplasia (i.e. sarcomas)
- Biphasic, with the two previous variously combined together (Fig. 33.2)

Patients with sarcomatoid and biphasic tumours have significantly poorer survival compared to patients with epithelioid disease.

33.3 Epidemiology and Risk Factors

MPM is considered to be a relatively rare tumour with an estimated 2500 new cases in the United States every year and a poor survival rate (Fig. 33.3a). Incidence began to rise in 1975 and peaked around 1995, concur-

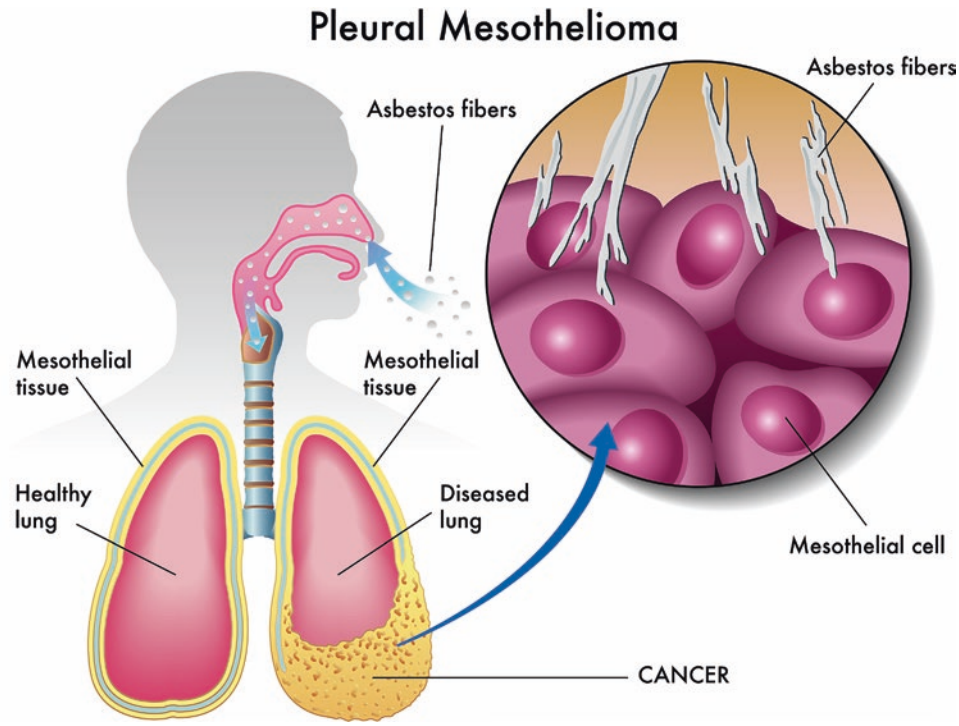

Fig. 33.1 Schematic presentation of unilateral malignant pleural mesothelioma (MPM) and its strict correlation to the inhaled asbestos fibres. (Photo credit: Dreamstime)

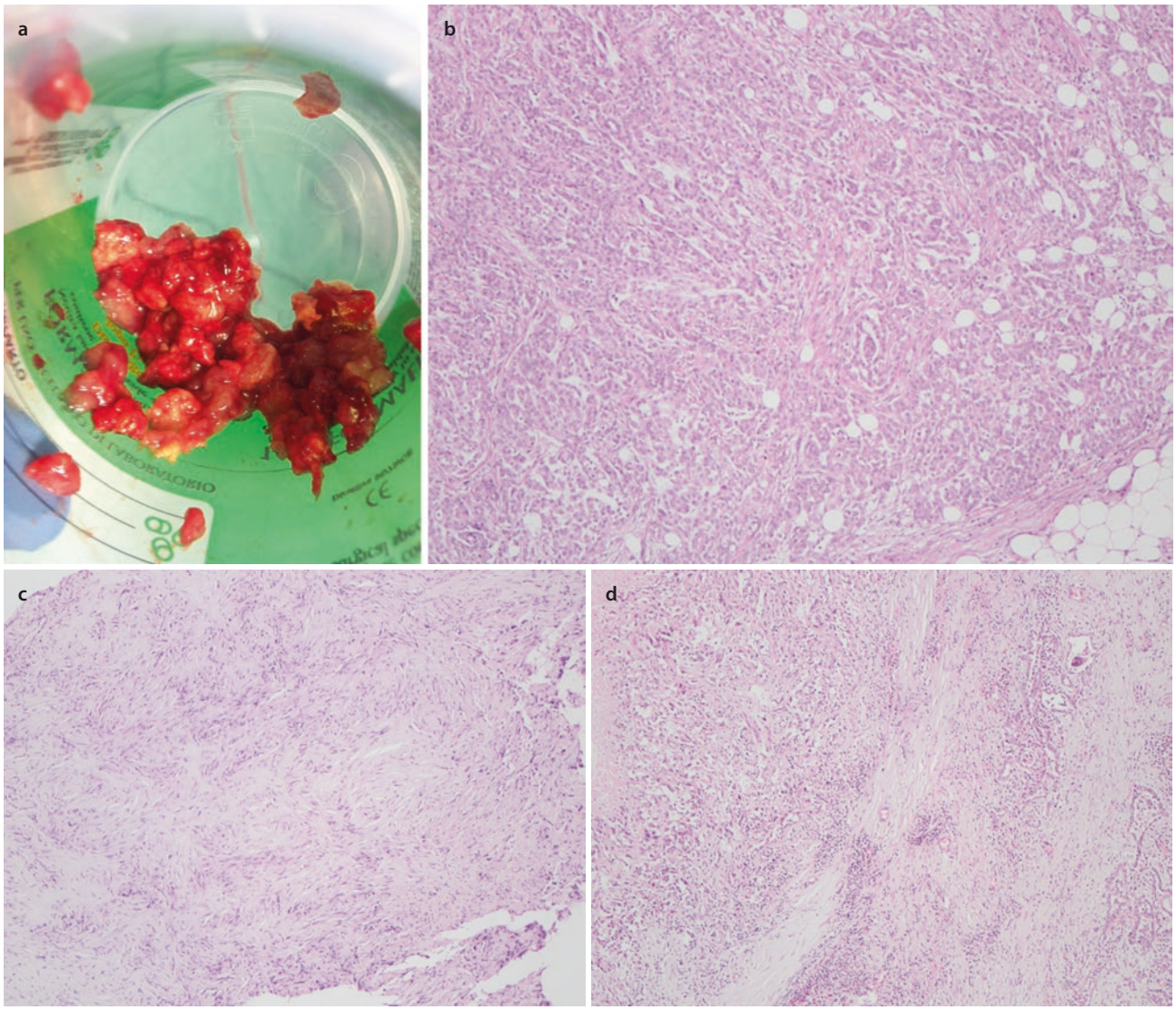

Fig. 33.2 Macroscopic aspect of a pleural tumour **a**. Microscopic aspect of epithelioid **b**, sarcomatoid **c** and biphasic **d** MPM stained with haematoxylin-eosin. (Courtesy of Dr. Luisella Righi)

rently with the diminishing of occupational and environmental exposure to asbestos (Fig. 33.3b) (SEER programme).

Professional exposure to asbestos and other mineral fibres accounts for more than 80% of the cases and makes the MPM a preventable disease. The latency time from exposure to the onset of the disease can range from 20 to 70 years and seems to be dose dependant, with a premature presentation in heavily exposed patients [3], even if there are also data suggesting a relationship between the disease and a minimal exposure [4].

In the past decades in Europe, Australia and Japan, the incidence of MPM has increased slowly, and the expected peak of incidence is between 2015 and 2025 [5]. The continued use of asbestos in the developing world and the scant regulation about the handling of many asbestos products even in some Western countries could unfortunately lead to an imminent global epidemic of MPM.

Exposure to ionizing radiations, for occupational reasons or therapeutic use for other malignancies, has also been recently described as a risk factor for MPM [6, 7].

33.4 Genetic Predisposition

Up to 20% of MPM patients do not recognize a clear exposure to asbestos, suggesting that genetic predisposition may play a central role in the pathogenesis of the disease.

A germline mutation in the BRCA1-associated protein 1 (BAP1) tumour suppressor gene, coupled with the loss of the second BAP1 allele, has been recently

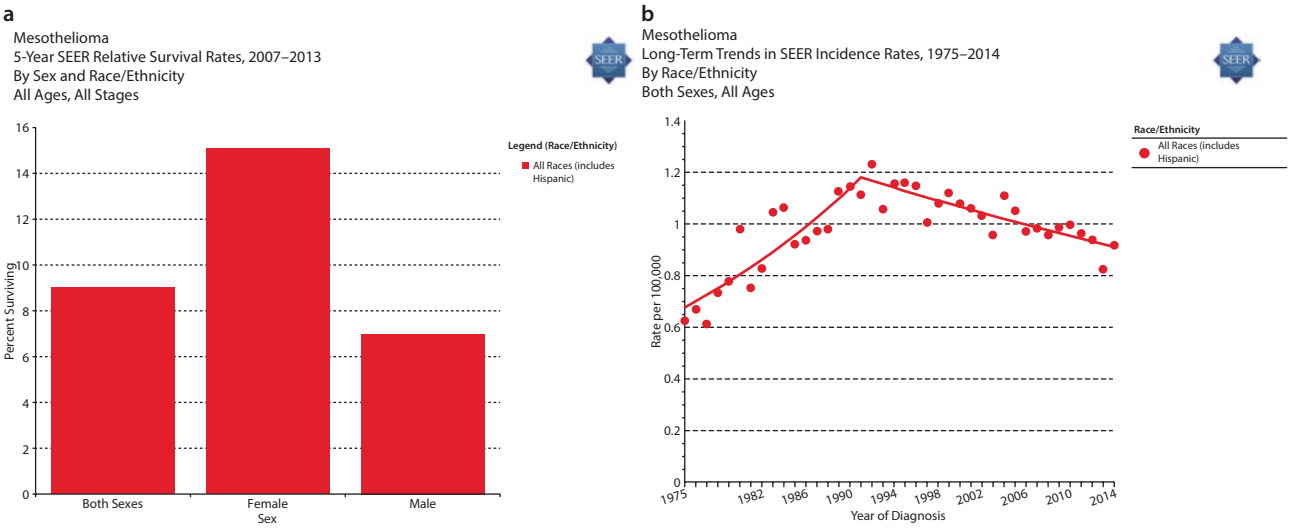

Fig. 33.3 5-year relative survival rate by sex **a** and age-adjusted incidence rates **b** for MPM. (Photo credit: SEER*Explorer: An interactive website for SEER cancer statistics [Internet]. Beta Version. Surveillance Research Program, National Cancer Institute. [Cited 2017 Apr 14]. Available from ► https://seer.cancer.gov/explorer/ [2])

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described in up to 50% of familiar cases of MPM in the presence of a modest environmental or occupational exposure to asbestos [8, 9].

The prevalence of germline BAP1 mutations is described in 6% of cases in a large asbestos-exposed cohort of patients with both mesothelioma and a family history of cancer [10].

Somatic loss of the NF2 and CDKN2A/ARF tumour suppressor genes has also been related to a higher incidence of MPM, even if with a lower frequency than BAP1 mutations [8].

33.5 Clinical Features

Median age of onset of MPM is between 65 and 70 years old, with a higher prevalence in men (80% of cases). Female incidence rate is fourfold lower than male and has remained unchanged over the past four decades in Western countries [5].

Patients typically present with shortness of breath, chest wall pain and weight loss. At physical evaluation, unilateral pleural effusion can be often detected as the main cause of respiratory symptoms and pain (Fig. 33.4). Initial symptoms are insidious and not specific, leading often to a late diagnosis, and complications due to "local invasion" are extremely common. Local complications include superior vena cava obstruction, cardiac tamponade, spinal cord compression, phrenic nerve or recurrent laryngeal nerve paralysis, dysphagia, subcutaneous involvement and direct extension through the chest wall.

MPM's spreading to the controlateral pleural cavity or to the abdomen across the diaphragm is uncommon, observed in about 10–20% of cases. Peritoneal involvement may cause ascites or bowel obstruction with high morbidity for the rapid symptoms' deteriorations [11].

Haematogenous metastasis is rare, but could potentially arise in any extrathoracic organs, strongly affecting prognosis.

33.6 Diagnosis

In the suspect of MPM, standard work-up includes:

- Chest X-ray
- Computed tomography (CT) scan of the chest and upper abdomen
- Thoracentesis (when needed, with examination of the pleural effusion)
- General laboratory blood tests

A detailed occupational history, with emphasis on asbestos exposure, is mandatory, as well as the familiar anamnesis with particular attention to other malignancy.

Chest X-ray still represents the first radiological assessment performed in the vast majority of cases of MPM. Significant volumes of pleural effusions can mask pleural or pulmonary lesions, and, conversely, small malignant pleural effusions could not be detectable with this technique. When patient's symptoms and the documented exposure to asbestos are suggestive for MPM, computed tomography (CT) scan of the chest and of the upper abdomen is necessary in order to get

Fig. 33.4 Pleural effusion with shortness of breath, chest pain and weight loss are common symptoms for MPM. (Photo credit: Shutterstock, Inc.)

more details and to proceed with more invasive procedures and/or staging (Fig. 33.5).

CT scan findings, suggesting a MPM, include nodular pleural thickening, diffuse pleural thickening with circumferential extension, pleural thickness higher than 1 cm and mediastinal pleural involvement. These radiological findings do not differentiate MPM from a metastatic pleural disease, making the differential diagnosis for MPM a challenging work [12].

Cytological features of pleural effusion may permit the diagnosis of MPM, but the sensitivity of cytology alone ranges between 32% and 76%, and tissue biopsy is generally preferred [13]. A surgical thoracoscopy is often recommended to obtain adequate tissue for the histology through pleural biopsy, to proper stage the disease and to allow pleural fluid evacuation (with or without pleurodesis) [14]. This can be performed as a pleuroscopy or as video-assisted thoracic surgery (VATS).

33.7 The Role of Biomarkers

An ideal diagnostic biomarker should be able to discriminate between MPM and normal controls, but also between malignant and benign pleural effusions or lesions, especially in healthy asbestos-exposed patients.

To date, several circulating tumour biomarkers have been investigated, but none of them is currently validated in clinical practice as MPM biomarkers due to poor specificity [15].

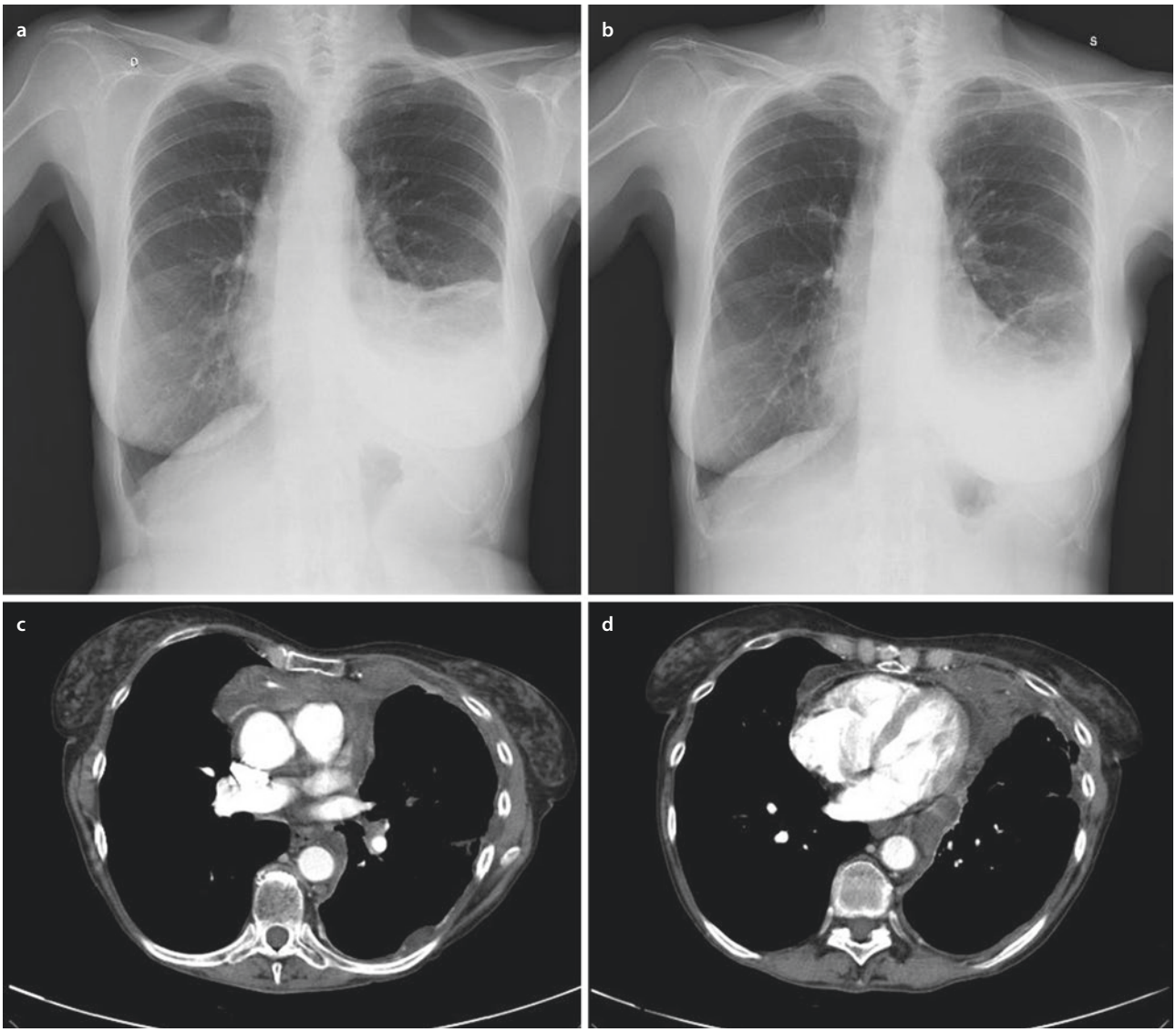

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Fig. 33.5 Chest X-ray with left pleural effusion **a** and corresponding chest X-ray **b** and CT scan **c**, **d** after thoracentesis

The osteopontin protein, a mediator of the cell-matrix interactions, can result elevated in MPM when compared with healthy asbestos-exposed patients [16]. However, pleural or serum osteopontin cannot discriminate between MPM and other pleural effusions [17], having a limited role in diagnosis.

Mesothelin is an antigen of normally differentiated mesothelial cells. The entire protein is cleaved into a C-terminal membrane-bound fragment and an N-terminal fragment, released into the blood and called megakaryocyte potentiating factor (MPF). Also part of the membrane-bound C-terminal fragment is released into the blood (soluble mesothelin). Both MPF and soluble mesothelin have been studied as potential biomarkers; the MPF seems to distinguish MPM from healthy controls with history of asbestos exposure, benign pleural diseases and pleural effusions from metastatic disease, showing high specificity and low sensitivity [18, 19]. In any case, to date the use of MPF as diagnostic biomarker for MPM is not recommended by the international guidelines for MPM, and its use in the clinical practice is extremely rare.

Circulating proteomic, fibulin-3 and high mobility group box protein 1 (HMGB1) could represent important future biomarkers, but their role in MPM differential diagnosis is still uncertain [20, 21]. Similarly, changes in microRNA signatures may differentiate MPM from lung adenocarcinoma, and MPM from benign asbestos-related pleural effusions, but data are not still mature, and confirmatory evaluation is needed [22, 23].

33.8 Pathology

Differential diagnosis of MPM may be challenging being the pleura a common site of metastasis from other sites. Furthermore, inflammatory reactive changes of the pleura could be mistaken with a malignant disease. The term "atypical mesothelial proliferation" is often adopted to describe the atypical mesothelial hyperplasia found in the pleural effusion, which could accompany a MPM, but is not sufficient for a neoplastic malignant diagnosis. For the definitive diagnosis of MPM, it is necessary to have adequate tissue biopsies and to perform an adequate immunohistochemistry (IHC) panel. The larger the tissue biopsy and the more targeted the sampling approach, the more reliable and definitive the diagnosis [24].

The latest WHO classification of tumours of the pleura has been published in 2015 (Table 33.1) with a particular attention to the prognostic value of some histological subtypes and variants of MPM [1].

Diffuse malignant mesothelioma (DMM) in the new WHO classification is well distinguished from other forms with a better prognosis, such as localized malignant mesotheliomas (LMMs) and well-differentiated papillary mesotheliomas (WDPMs).

The commonest histological subtype of DMMs is the epithelioid (70% of cases). Tumours of pure epithelioid histology can have patterns prevalently described as papillary (cells growing along exophytic fronds with vascular cores), tubulopapillary (a mixture of small tubules and papillary structures with fibrovascular cores, often with clefts and trabeculae) and solid (nests and sheets of round or polygonal cells with abundant cytoplasm and vesicular nuclei with prominent nucleoli). Sarcomatoid DMM is composed of a fascicular proliferation of spindle cells with oval nuclei, scant cytoplasm and, occasionally, prominent nucleoli, usually displaying more atypia, mitotic activity and wide foci of necrosis. Desmoplastic MPM is a rare sarcomatoid MPM variant characterized by small atypical few neoplastic cells immersed in a dense collagen stroma. Biphasic (or mixed) DMM is characterized by a combination of epithelioid and sarcomatoid patterns together.

Patients with sarcomatoid and biphasic DMMs have a significantly poorer survival when compared to the other patients [25]. Within the group of pleural epithelioid DMMs, an aggressive behaviour of epithelioid MPM with pleomorphic features (with anaplastic or prominent giant cells, often multinucleated) has been repeatedly described in multiple studies, with a similar survival to that of patients with biphasic and sarcomatoid DMMs [26, 27]. The same studies show that the combined subgroup of tubulopapillary and trabecular MPM has a more favourable prognosis than the solid subtype and the combined solid/micropapillary group [27].

WDPM represents a distinct mesothelial tumour characterized histologically by superficial spreading of papillary formations with large fibrovascular cores and myxoid stroma. It's more frequently found in the peritoneal cavity, but it shows the same features also in the thorax. WDPMs usually are indolent and clinically benign if completely resected [28]. Differential diagnosis from the papillary form of conventional DMM may be difficult, especially in small biopsy specimen.

Table 33.1 WHO classification of tumours of the pleura

Mesothelial tumours

Diffuse malignant mesothelioma (DMM)
- Epithelioid mesothelioma
- Sarcomatoid mesothelioma
- Desmoplastic mesothelioma
- Biphasic mesothelioma

Localized malignant mesotheliomas (LMMs)
- Epithelioid mesothelioma
- Sarcomatoid mesothelioma
- Biphasic mesothelioma

Well-differentiated papillary mesotheliomas (WDPMs)

Adenomatoid tumour

Lymphoproliferative disorders

Primary effusion lymphoma

Diffuse large B-cell lymphoma associated with chronic inflammation

Mesenchymal tumours

Epithelioid hemangioendothelioma

Angiosarcoma

Synovial sarcoma

Solitary fibrous tumour
- Malignant solitary fibrous tumour

Desmoid-type fibromatosis

Calcifying fibrous tumour

Desmoplastic round cell tumour

33.9 The Role of Immunohistochemistry (IHC) in the Differential Diagnosis

Site-specific carcinoma marker panels have been introduced in the 2015 WHO classification for the differential diagnosis between MPM and other malignancies affecting the pleura. Their use is largely recommended [13] in order to enhance the specificity and sensitivity of the diagnosis [29]. These markers help to discriminate a MPM from a metastatic disease from other primary sites, including:

- TTF-1 and napsin A for adenocarcinoma of the lung
- PAX-8 for renal cell and thyroid carcinoma
- Prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA) for adenocarcinoma of the prostate
- CDX2 and cytokeratin 20 for adenocarcinoma of the gastrointestinal tract
- PAX-8, PAX-2 and oestrogen receptor (ER) for serous papillary carcinoma of the ovary or peritoneum

Other useful markers in this context are CD45 and CD20 for differential diagnosis with haematogenous tumours and HMB45, melan A and SOX10 for melanoma [13].

The commonest and challenging differential diagnosis of MPM is done with primary lung adenocarcinoma. The use of at least two mesothelial markers and two carcinoma markers (including TTF-1 and CEA) is recommended to ameliorate the diagnosis [13, 29].

Immunohistochemistry has shown a limited role in the differentiation of sarcomatoid MPM from other sarcomas and sarcomatoid carcinoma of the lung with pleural involvement. Sarcomatoid MPMs often stain positive for a large spectrum of anti-cytokeratin antibodies, whereas most soft tissue sarcomas do not. Mesothelial markers used for the diagnosis of epithelioid MPM have showed limited utility for the sarcomatoid subtype, often negative for calretinin, cytokeratin 5/6 and WT-1. Sarcomatoid mesothelioma is typically positive for vimentin and may also show a positivity for S-100, actin or desmin, but these IHC markers have no diagnostic specificity [30].

Another challenging differential diagnosis for sarcomatoid mesothelioma is the differentiation from malignant solitary fibrous tumours of the pleura (SFTP), a rare mesenchymal tumour originating from the submesothelial tissue of the pleura, with a slow-growing rate and prevalently with benign histologic features. SFTPs stain positive for CD34 and bcl-2 and, usually, are keratin-negative. Recently, these tumours have been shown to be positive for STAT6 [31]. Solitary fibrous tumours of the pleura seem to have no relation to asbestos exposure, too.

Main criteria for separation of benign from malignant mesothelial proliferations are listed in Table 33.2.

Table 33.2 Reactive atypical mesothelial hyperplasia versus epithelioid malignant mesothelioma: main features

Histological features	Atypical mesothelial hyperplasia	Malignant mesothelioma
Major criteria		
Stromal invasion	Absent	Present
Cellularity	Confined to the pleural surface	Dense, with stromal reaction
Papillae	Simple, lined by single-cell layer	Complex, with cellular stratification
Growth pattern	Surface growth	Expansile nodules, disorganized pattern
Zonation	Process becomes less cellular towards chest wall	No zonation of process
Vascularity	Capillaries are perpendicular to the surface	Irregular and haphazard
Minor criteria		
Cytological atypia	Confined to areas of organizing effusion	Present in any area
Necrosis	Rare (necrosis may be within pleural exudates)	Necrosis is usually a sign of malignancy
Mitoses	Mitoses may be plentiful	Few mitoses (atypical mitoses favour malignancy)

33.10 Staging

In the oncologic field, staging always describes the anatomical extent of a tumour and plays a central role in the therapeutic decision-making process.

Historically, most staging systems for MPM have been based on single-institution databases with small retrospective surgical series. However, because of the small number of surgically resected MPMs, the main criticisms of most classifications are the discrepancy between clinical and pathological staging and the scant accuracy in describing the clinical tumour (T-) and nodal (N-) extension.

The latest developed and widely adopted staging system for MPM is the International Mesothelioma Interest Group (IMIG) classification and then approved by the Union for International Cancer Control (UICC) [32]. Details of the IMIG staging system for MPM are listed in Table 33.3.

The recent collaboration between IMIG and the International Association for the Study of Lung Cancer (IASLC) has developed an international prospective database in order to work out a data-driven revision of the current staging system: the database is geographically representative and includes thousand cases of MPM, irrespective of treatment, pathological subtype and stage.

The IASLC mesothelioma staging project is still ongoing with the aim to highlight the aspects that require further modifications in the upcoming eighth edition of the TNM classification for MPM, focusing the attention on factors, such as pleural thickness, nodal involvement and the invasion of the visceral pleura, that may influence prognosis [33, 34].

Computed tomography (CT) scan of the chest and upper abdomen still remains the only radiological assessment suggested for a proper staging of MPM non-candidate to surgical resection.

Table 33.3 International Mesothelioma Interest Group staging system for malignant pleural mesothelioma

T	*Primary tumour*
Tx	Primary tumour cannot be assessed
T0	No evidence of primary tumour
T1	Tumour limited to the ipsilateral parietal pleura with or without mediastinal pleural and with or without diaphragmatic pleural involvement
T1a	No involvement of the visceral pleural
T1b	Tumour also involving the visceral pleura
T2	Tumour involving each of the ipsilateral pleural surfaces (parietal, mediastinal, diaphragmatic and visceral pleura) with at least one of the following: Involvement of the diaphragmatic muscle Extension of tumour from visceral pleura into the underlying pulmonary parenchyma
T3	Locally advanced but potentially resectable tumour. Tumour involving all of the ipsilateral pleural surfaces (parietal, mediastinal, diaphragmatic and visceral pleura), with at least one of the following: Involvement of the endothoracic fascia Extension into the mediastinal fat Solitary, completely resectable focus of tumour extending into the soft tissue of the chest wall Non transmural involvement of the pericardium
T4	Locally advanced technically unresectable tumour. Tumour involving all the ipsilateral pleural surfaces (parietal, mediastinal, diaphragmatic and visceral pleura) with at least one of the following: Direct transdiaphragmatic extension of the tumour to the peritoneum Diffuse extension or multifocal masses of tumour in the chest wall, with or without associated rib destruction Direct extension of tumour to the contralateral pleura Direct extension of the tumour to mediastinal organs Direct extension of tumour into the spine Tumour extending through to the internal surface of the pericardium with or without a pericardial effusion or tumour involving the myocardium

N	*Regional lymph nodes*
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis to the ipsilateral bronchopulmonary or hilar lymph nodes
N2	Metastases in the subcarinal lymph node or the ipsilateral mediastinal lymph nodes including the ipsilateral internal mammary and peridiaphragmatic node
N3	Metastasis in contralateral mediastinal, contralateral internal mammary, ipsilateral or contralateral supraclavicular lymph node
M	*Distant metastasis*
M0	No distant metastasis
M1	Distant metastasis
Staging	*TNM*
Stage IA	T1aN0M0
Stage IB	T1bN0M0
Stage II	T2N0M0
Stage III	Any T3, any N1 or any N2, M0
Stage IV	Any T4, any N3 or any M1

Magnetic resonance imaging (MRI), using gadolinium, has resulted to be more accurate than CT scan in the delineation of the tumour border with regard to the surrounding tissues and in the evaluation of the diaphragmatic invasion, especially when surgical resection may be considered to be a part of the treatment plan [35].

The use of positron emission tomography (PET) scan in the staging process for MPM is still debated: false-positive findings may occur after pleurodesis, and the low spatial resolution may limit the characterization of the local growth and the nodal involvement. PET scan could be useful for the detection of distant metastases, even if rare [36].

33.11 Treatment

Treatment options for MPM potentially include surgery, radiation therapy (RT) and chemotherapy. Only selected patients with a surgically resectable disease (clinical stages I–III), good performance status and adequate respiratory function may be candidates for a multimodality treatment. For symptomatic pleural effusion in unresectable patients, pleurodesis with talc administered via tube thoracostomy represents the treatment of choice to reduce the frequency of thoracentesis and the related infective complications during chemotherapy [37].

33.11.1 Surgery

Surgical treatment with radical intent is occasionally performed in MPM patients with the aim to obtain a complete macroscopic resection.

After the establishment by the IASLC of a working group in order to recommend a uniform definition for surgical procedures [38], the main approaches to MPM are:

- Extrapleural pneumonectomy (EPP): complete en bloc removal of the involved parietal and visceral pleura including the whole ipsilateral lung. If required, the diaphragm and pericardium can also be resected.
- Extended pleurectomy/decortication (P/D): as the EPP, but with the lung left in situ.
- Pleurectomy/decortication (P/D): removal of all gross tumours, without resection of the lung, diaphragm or pericardium.

In P/D and EPP, mediastinal nodal dissection is always recommended.

The value of these procedures is extensively debated, because no definitive comparisons between EPP and P/D or between these procedures and nonsurgical treatment for MPMs are available. The only randomized clinical trial comparing the role of EPP in the context of a multimodal therapy is the Mesothelioma and Radical Surgery (MARS) trial. No difference has been observed with EPP after chemotherapy compared to chemotherapy alone. Results need to be considered with caution due to the low number of randomized patients and the mortality rate for the surgical arm, higher than expected [39].

Both surgical procedures have significant associated morbidity and mortality. P/D is resulted to be safer than EPP, with a perioperative mortality rate of about 1.5–5.4% [40]. In a large literature review including over 3700 MPM patients, perioperative mortality rates for EPP ranged from 0% to 11.8%, with major morbidities seen in 12–48% of patients (prevalently atrial arrhythmias, respiratory infections, respiratory failure, pulmonary embolus and myocardial infarction) [41].

33.11.2 Radiation Therapy (RT)

RT in MPM may have multiple indications: palliative care, preventive care or to be part of a multimodal treatment.

The purpose of RT with palliative intent is to reduce cancer pain due to chest wall invasion: this kind of treatment usually consists in short courses of RT with 10 grays (Gy) in a single fraction, or 8 Gy in three fractions [42]. However, there is no clinical evidence to support prescribing RT to reduce pain in MPM patients [43].

Preventive RT may be performed on the thoracoscopy scars and the drainage tracts to reduce the probability of seeding metastases. The efficacy of this strategy is still debated and currently is not part of the standard of care for MPM [24].

The introduction of intensity-modulated RT and 3D planning has improved the identification of the tumour border, increased the dose homogeneity and limited the normal tissue irradiation [44]. This kind of RT can be included in a multimodal strategy for the treatment of MPM, with or without chemotherapy, often after surgical approach. Data from many clinical trials suggest that RT after EPP may reduce local recurrence [45, 46]. In general, RT is not recommended as pre- or postoperative approach outside the setting of a clinical trial [24].

33.11.3 Chemotherapy

33.11.3.1 First-Line Chemotherapy and Targeted Agents

First-line chemotherapy is the only therapeutic option with proven survival benefit in patients with unresectable MPM. Chemotherapy doublets with cisplatin, in association with either pemetrexed or raltitrexed, have shown a higher median overall survival (OS) when compared to cisplatin alone (12.1 versus 9.3 months, $p = 0.02$, and 11.4 versus 8.8 months, $p = 0.04$, respectively) [47, 48]. The doublet of cisplatin/raltitrexed, however, showed a lower objective response rate compared to the other regimen, being not approved by regulatory agencies in many countries. For these reasons, to date front-line chemotherapy with cisplatin/pemetrexed represents the standard of care for unresectable MPM worldwide.

The optimum number of chemotherapy cycles is still debated. For patients with good clinical conditions, adequate organ function and radiological evidence of controlled disease, the current clinical practice is a maximum of six cycles of platinum/pemetrexed chemotherapy, even if it's not clear if four cycles would provide a similar benefit as already observed in the treatment of advanced non-small cell lung cancer patients [49]. Similarly, the use of maintenance treatment with pemetrexed as monotherapy, after induction with the combination regimen, or the switch maintenance treatment with a different agent, is uncertain and does not represent the standard of care for MPM.

The combination of carboplatin/pemetrexed is a reasonable alternative for those patients unable to receive cisplatin due to relevant clinical comorbidities. The median time to progression and the 1-year survival rate with this combination is similar to that described in patients treated with cisplatin/pemetrexed (7 versus 6.9 months and 63.1% and 64%, respectively) [50].

The use of gemcitabine, combined with a platinum agent, appears to be an active treatment in MPM; however, data coming from different studies are heterogeneous, with response rates ranging from 12% to 48% and median OS from 9.5 to 12 months [51, 52, 53].

With the attempt to improve survival for MPM patients, several targeted agents have been tested.

The role of angiogenesis is the field deeper explored: its relevance in MPM growth is demonstrated by the high levels of serum vascular endothelial growth factor (VEGF) found in MPM patients, which seem to be associated with poor prognosis [54].

A recent multicentre phase III trial has compared the addition of bevacizumab (a monoclonal antibody targeting the VEGF) to cisplatin/pemetrexed (with the possibility to be followed by the sole bevacizumab as maintenance therapy) with cisplatin/pemetrexed alone in unresectable MPM patients. This study has shown a statistically significant increase in the median OS for patients allocated in the bevacizumab-containing arm (18.8 versus 16.1 months, $p = 0.012$), reporting however a higher rate of drug-related adverse events, such as hypertension, proteinuria and arterial thrombotic events [55]. Based on this trial, the addition of bevacizumab to standard front-line chemotherapy seems to be feasible, but should be considered in selected group of patients due to the possible related adverse events.

Clinical trials are currently investigating the efficacy of small molecules, oral inhibitors of the angiogenesis, such as nintedanib, an intracellular tyrosine kinase inhibitor of the vascular endothelial growth factor receptor (VEGFR) 1-3, platelet derived growth factor receptor (PDGFR) -α and -β, and fibroblast growth factor receptor (FGFR)1-3. A recent phase II trial has reported an increased progression-free survival (PFS) for MPM patients treated with cisplatin/pemetrexed in association with nintedanib in comparison to patients treated with cisplatin/pemetrexed plus placebo (9.4 versus 5.7 months, $p = 0.0174$) [56]. A preliminary trend towards improved OS (18.3 versus 14.5 months, $p = 0.4132$) was also observed [56], but further investigations are needed and will be provided soon by the ongoing phase III trial (NCT01907100).

As previously described, the mesothelin is a potential biomarker for MPM, highly expressed in the epithelial and biphasic histology. Monoclonal antibodies, recombinant immunotoxins and antibody-drug conjugates targeting the mesothelin have been evaluated in clinical trials. The chimeric anti-mesothelin antibody amatuximab has been studied in a single-arm phase II trial in association with first-line platinum/pemetrexed chemotherapy for epithelial and epithelial-predominant biphasic MPM patients. The study did not meet its primary endpoint, the median PFS being lower than historical controls (6.1 months, 95% CI: 5.8–6.4) [57].

33.11.3.2 Second-Line Chemotherapy

To date there is no second-line standard of care for MPM patients.

In patients with good performance status, who relapse after a reasonable amount of time from front-line therapy, the retreatment with pemetrexed, used alone or in combination with a platinum salt, should be considered [58].

Many chemotherapy agents have demonstrated second-line activity in MPM, but none of them have been tested within a controlled randomized clinical trial. A phase II trial has assessed the safety and efficacy of the single-agent vinorelbine, demonstrating an objective response rate of 16% and a median OS of 9.6 months and favouring this agent as second-line treatment option in the clinical practice [59]. Similarly, on the basis of the demonstrated activity of cisplatin and gemcitabine in the front-line setting [60, 52], gemcitabine-based doublets appear to be an alternative second-line treatment as well [61].

Patients in good clinical condition should be recommended to join clinical trials in the second-line setting [24].

33.11.3.3 Immunotherapy

The immune compartment has proven to be a key component in the process of tumour initiation, progression and the response to treatment [62]. Targeting the molecular regulators of the immune function, such as cytotoxic lymphocyte antigen 4 (CTLA4) and programmed death-1/programmed death-ligand 1 (PD-1/PD-L1) signalling axis, has emerged as an effective therapeutic strategy in multiple cancers, including MPM, reported as a tumour with high infiltration of lymphocytes and macrophages and a significant T-cell inflammatory expression pattern. Preclinical studies have demonstrated that PD-1 and PD-L1 are expressed in a significant percentage of MPM and that the level of expression may characterize patients with a worse prognosis, especially in case of sarcomatoid histology [63, 64].

Tremelimumab, a fully human monoclonal antibody against CTLA-4, has been tested in a single-arm study in pretreated MPM patients, using the schedule of 10 mg/kg every 28 days. The study reported a disease control rate equal to 52%, a median OS of 11.3 months and a median immune-related PFS of 6.2 months [65]. Despite these first promising results, recent data from the phase II trial evaluating tremelimumab as second- or third-line treatment of MPM did not show a significantly longer OS compared to the placebo arm (7.7 versus 7.3 months, respectively, $p = 0.41$) [66].

A phase Ib trial that has enrolled previously treated patients with different solid tumours has evaluated the safety and efficacy of pembrolizumab, a humanized monoclonal antibody against PD-1 designed to block

the interaction between PD-1 and its ligand, the PD-L1. Eligible patients presented with more than 1% positive membranous expression of PD-L1 on tumour or stromal cells. Pembrolizumab has appeared to be safe and tolerable for patients with MPM, conferring an objective response of 20% and durable response, but further investigation is needed to validate this treatment [67]. Thirty-eight patients with advanced MPM have been evaluated in a single-centre phase II study for the use of nivolumab, a human IgG4 monoclonal antibody targeting PD-1, as second-line treatment at the dose of 3 mg/kg every 2 weeks until progression or toxicity. This limited experience has shown a disease control rate of 50% at 12 weeks, suggesting, together with the data previously reported, that targeting the PD-1/PD-L1 axis in MPM appears promising [68].

Preliminary results from a phase II randomized study evaluating the efficacy of nivolumab versus the combination of nivolumab and ipilimumab, a monoclonal antibody targeting CTLA-4, for the second- or third-line treatment of MPM, have shown a higher disease control rate at 12 weeks and a longer median OS in favour of the combination arm (51.6% versus 39.7% and median OS not reached versus 10.5, respectively) after a follow-up period of about 10 months [69]. A phase III trial comparing nivolumab/ipilimumab versus standard platinum-based chemotherapy with pemetrexed for the front-line treatment of MPM is ongoing (NCT02899299).

33.11.4 Response Evaluation and Follow-Up

Response evaluation is performed with CT scan and the examinations performed at the time of presentation (PET scan for patients undergoing multimodal treatment).

Clinical and radiological follow-up of MPM patients will depend on the local recommendations or as specified by the protocol in case of participation in a clinical trial.

33.11.5 Screening

Routine screening tests with chest X-ray or CT scan did not demonstrate to be an effective tool for the early detection of MPM. To date, there are no sufficient data to suggest that a screening programme for MPM can reduce mortality, even in patients who had a clear occupational exposure to asbestos. In a large cohort of 1045 asbestos-exposed workers, no single case of pleural mesothelioma has been detected, confirming the previous statement [70].

Summary of Clinical Recommendations

ESMO (European Society for Medical Oncology) guidelines [24]

- Occupational history with emphasis on asbestos exposure is recommended.
- Large and targeted biopsy samples facilitate definitive diagnosis. Surgical-type samples should be preferred for diagnosis.
- A major subtype diagnosis (epithelioid, biphasic, sarcomatoid) should be given in all cases of MPM.
- Antifolate/platinum doublet is the only approved standard of care for advanced MPM.

NCCN (National Comprehensive Cancer Network) Malignant Pleural Mesothelioma guidelines (version 2.2017) [71]

- Management by a multidisciplinary team with experience in MPM is recommended.
- The addition of bevacizumab to platinum/pemetrexed front-line chemotherapy should be considered in selected MPM patients.
- Immunotherapy (nivolumab +/− ipilimumab or pembrolizumab) should be considered as a treatment option for pretreated MPM patients.

Case Study: A Rare Case of Metastatic MPM

Man, 52 years old

- Family history negative for malignancy
- No documented working exposure to asbestos
- APR: no relevant anamnestic data
- APP: shortness of breath and relevant chest wall pain
- Blood tests: no relevant abnormalities. Total leukocytes 11.7 × 10^9/L. Hb 13.0 g/dL. Adequate liver and kidney function
- Urgent CT scan of the chest and upper abdomen: modest pleural effusion in the lower part of the left thorax. Widespread pleural thickening, with prevalent involvement of the mediastinal pleura. Mediastinal nodal involvement, no distant metastasis (Fig. 33.6)

Question

What action should be taken?

1. Radiological follow-up

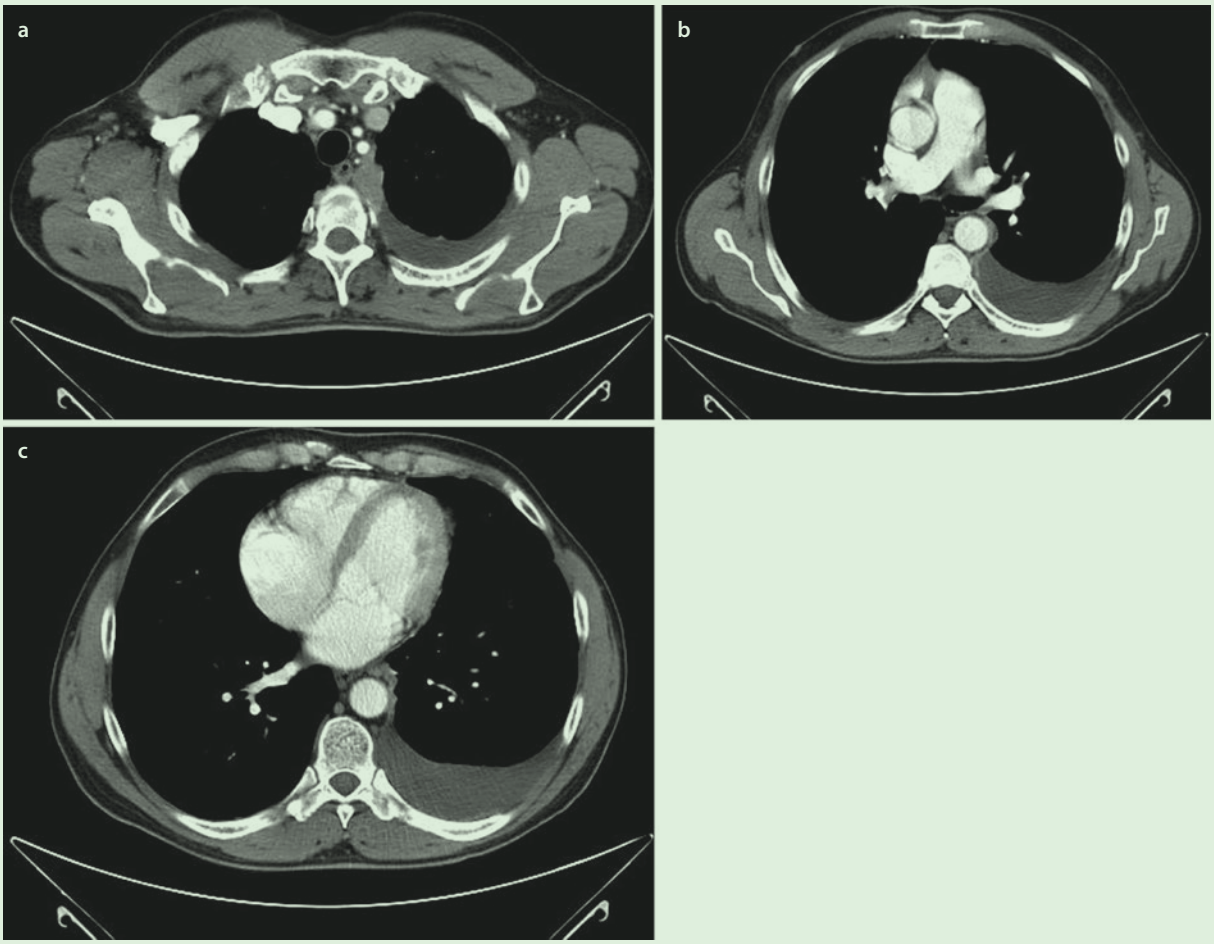

Fig. 33.6 Effusion and widespread thickening of the pleura on CT scan

2. Ultrasound-guided biopsy of the mediastinal pleura
3. Surgical thoracoscopy for multiple pleural biopsies and pleurodesis

Answer

The patient performed a surgical pleural biopsy with pleurodesis with talc.

Histological report: malignant pleural mesothelioma with epithelioid features.

Question

What action should be taken?

1. Surgery
2. Chemotherapy
3. Radiotherapy

Answer

The patient has been enrolled in a randomized clinical trial and performed four chemotherapy cycles with cisplatin/pemetrexed plus nintedanib/placebo with no relevant side effects.

He reported a fast improvement of the shortness of breath and the chest wall pain.

CT scan of the chest and upper abdomen performed after four chemotherapy cycles: great reduction of the mediastinal pleural thickening, stable mediastinal nodal involvement. No distant metastasis, no pleural effusion (Fig. 33.7). Global answer: partial response.

The widespread pleural involvement and the nodal infiltration did not indicate the possibility to perform a surgical resection. The patient started a regular clinical and radiological follow-up.

After 6 months, CT scan of the chest and upper abdomen showed a progression of the disease for the onset of multiple sub-centimetric bilateral lung nodes (Fig. 33.8a).

PET scan confirmed the progression of the disease, showing hyperactivity not only on the pleural surface and the regional lymph nodes but also on the right iliac bone. No evidence of hyperactivity on the lung nodules because of their limited size (Fig. 33.8b).

Good clinical conditions, no bone pain.

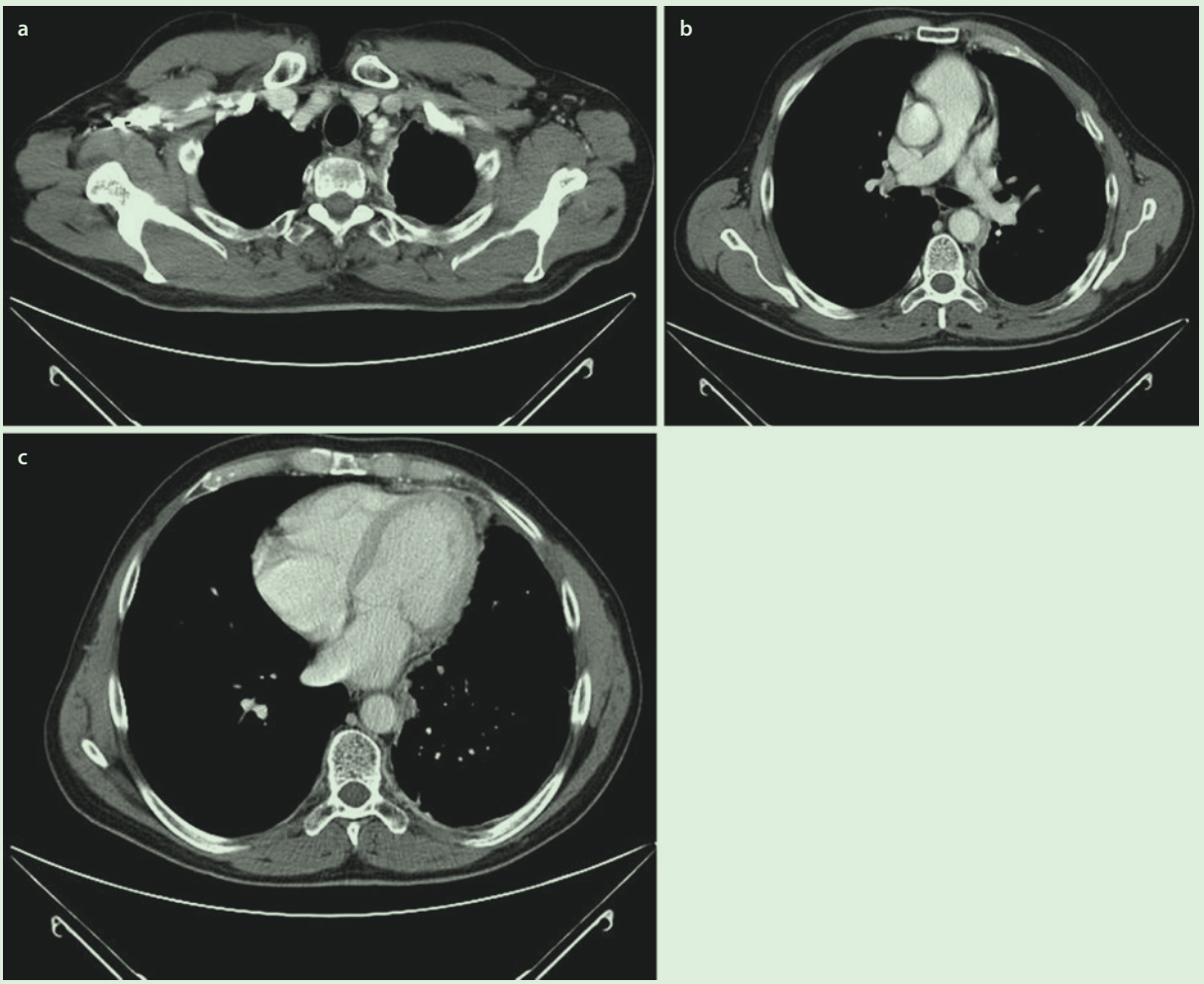

Fig. 33.7 Main radiological finding after first-line treatment

33

Question

What action should be taken?

1. Best supportive care
2. Single-agent chemotherapy
3. Enrollment in a clinical trial

Answer

No clinical trials available in our region. Patient began second-line chemotherapy with gemcitabine.

A deep genetic evaluation showed for this patient a germline mutation in the BAP1 gene, probably cause of the high predisposition to MPM in the absence of documented exposure to asbestos.

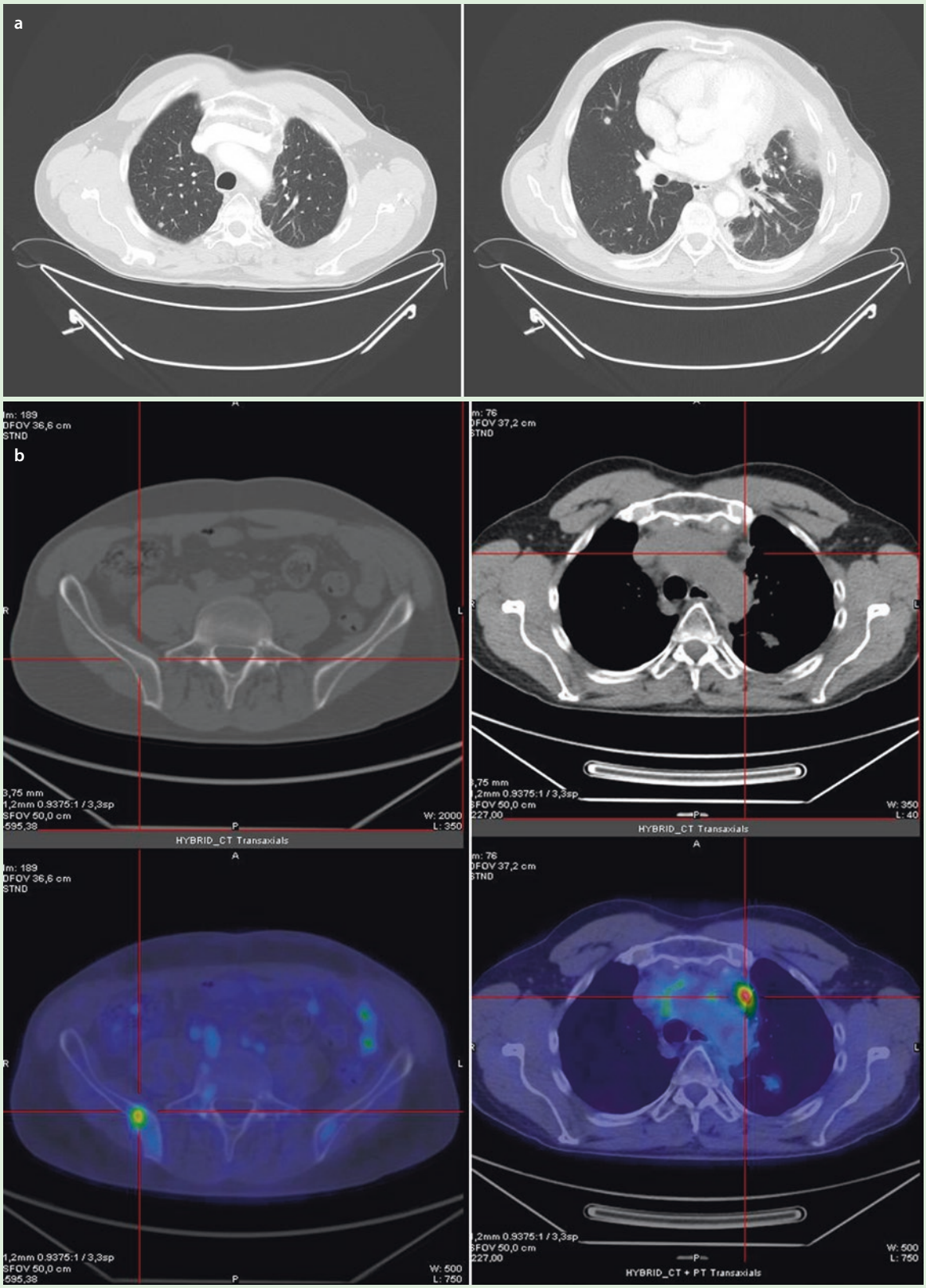

Fig. 33.8 CT scan of the chest showing multiple lung nodes, to be interpreted as of pulmonary metastases from MPM **a**. PET scan showing a bone metastasis from MPM on the right iliac bone **b**

Case Study: A Case of MPM with Aggressive Behaviour

Man, 58 years old

- Family history negative for malignancy
- Possible working exposure to asbestos (naval mechanical worker)
- Active smoker
- APR: no relevant anamnestic data
- APP: only modest chest wall pain (first assessments performed within a working prevention programme for respiratory diseases)
- Blood tests: no relevant abnormalities. Total leukocytes 7.5 × 109/L. Hb 15.4 g/dL. Adequate liver and kidney function
- Chest X-ray: right pleural effusion with apparent modest pleural thickening (Fig. 33.9)

Question

What action should be taken?

1. Medical treatment and radiological follow-up
2. Thoracentesis with cytological examination of the pleural effusion
3. Surgical thoracoscopy

Answer

The patient performed a thoracentesis with drainage of 2000 cc of pleural fluid.

Cytological examination of the pleural effusion: presence of neoplastic cells of mesothelial origin.

Question

What action should be taken?

1. Start chemotherapy.
2. Repeat thoracentesis.
3. Surgical thoracoscopy for multiple pleural biopsies and pleurodesis.

Answer

The patient performed a surgical pleural biopsy with pleurodesis with talc.

Histological report: Malignant pleural mesothelioma with sarcomatoid features.

CT scan of the chest and upper abdomen: circumferential pleural thickening with confluent mediastinal lymph nodes in the para-oesophageal region. Modest

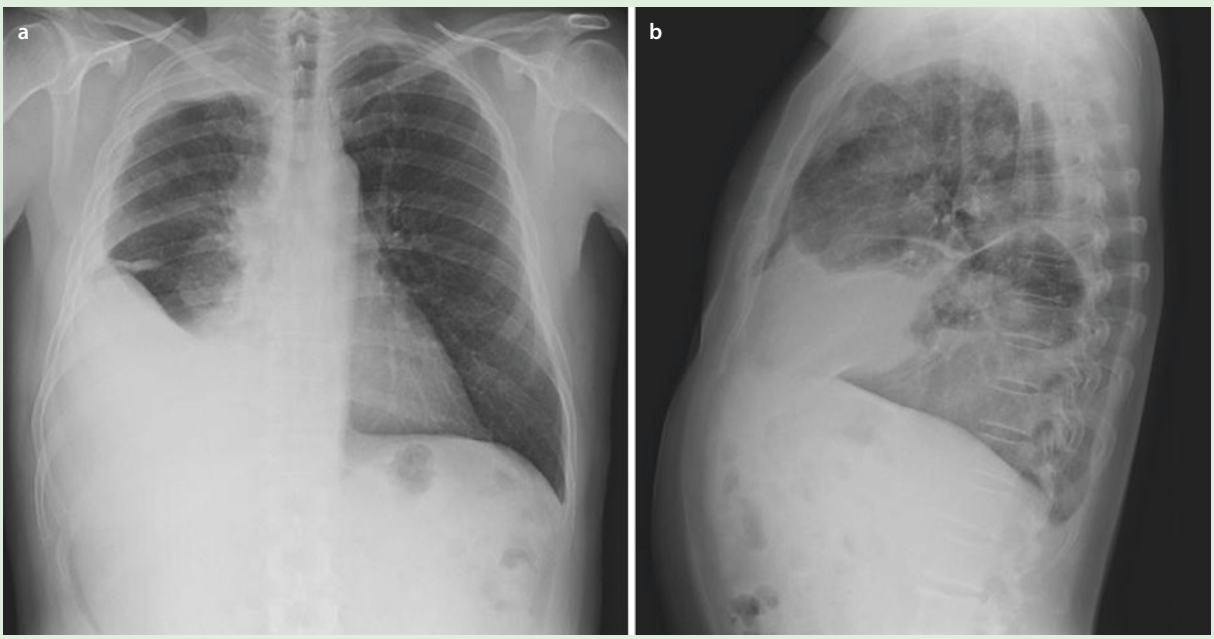

Fig. 33.9 Chest X-ray with right pleural effusion

residual right pleural effusion. No distant metastasis (Fig. 33.10).

Question

What action should be taken?

1. Surgery
2. Chemotherapy
3. Radiotherapy

Answer

The patient began chemotherapy with cisplatin/pemetrexed with rapid symptoms' deterioration in terms of chest pain, dyspnoea and weight loss.

Unscheduled CT scan of the chest and upper abdomen performed after two chemotherapy cycles: large increase of pleural thickenings with mediastinal infiltration. Stable pleural effusion. No distant metastasis (Fig. 33.11). Global answer: progression of disease.

The patient stopped chemotherapy in favour of the sole best supportive care and died after few weeks.

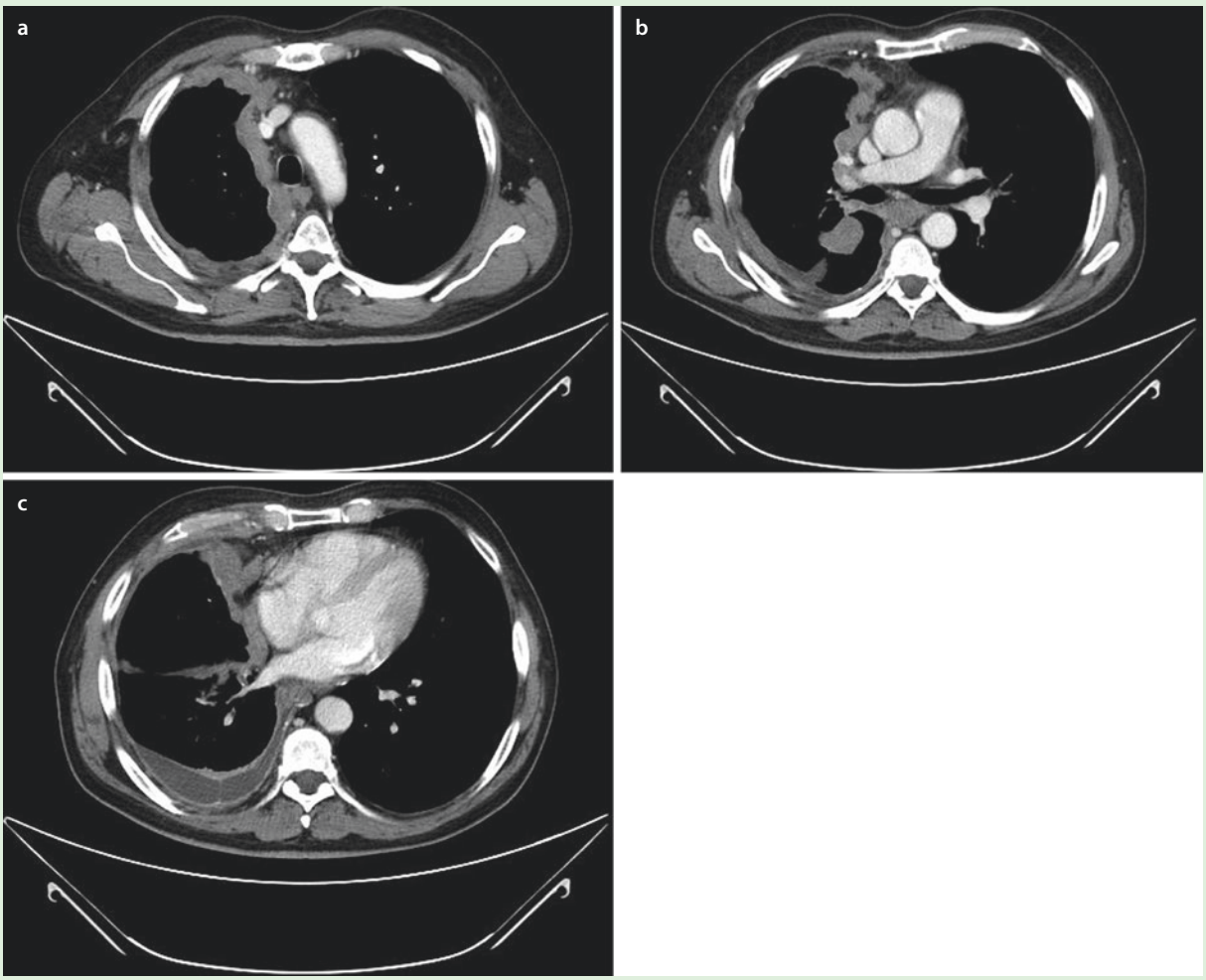

Fig. 33.10 Circumferential pleural thickening with pathological mediastinal lymph nodes on CT scan

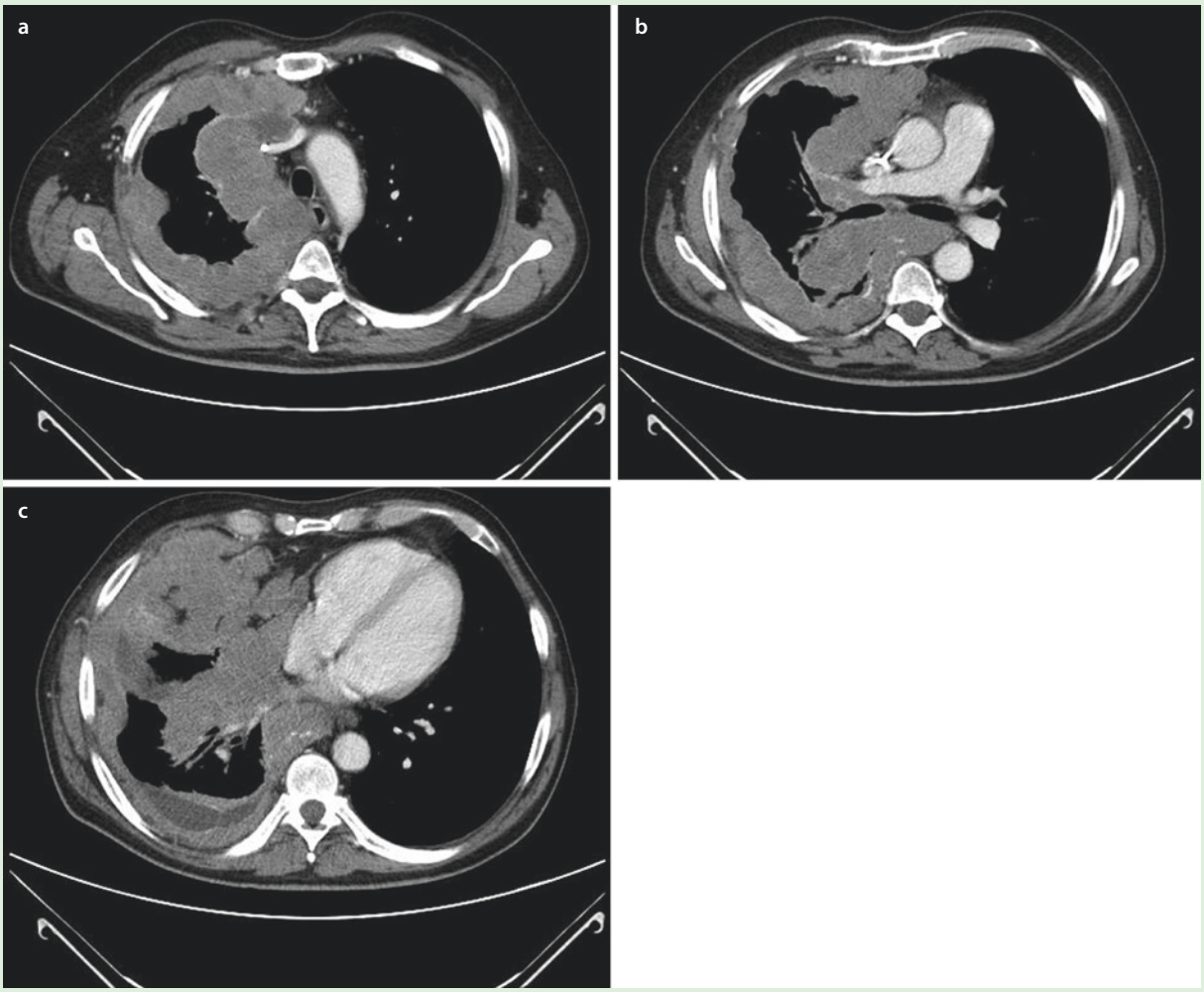

Fig. 33.11 Large circumferential pleural thickening with mediastinal infiltration

33

Expert Opinion

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Key Points

1. Malignant pleural mesothelioma is a pleural cancer raising from mesothelial cells, often linked to a previous exposure to asbestos fibres.
2. It is considered as a rare disease even if its incidence is slowly growing worldwide with an expected peak in the following years between 2015 and 2025.
3. It is more common in 65–70 year-old men and the most frequent symptoms are dyspnoea, chest pain and weight loss.
4. Chest X-ray is the first imaging diagnostic technique; CT is used for staging while MRI can be useful to study the local involvement. Thoracoscopy is the best method to collect a tissue sample; in order to make a differential diagnosis between the various pleural malignancies it is recommended (WHO 2015) to use a marker panel including TTF1, napsin A, PAX8, PSA, CDX2, CD45, CD20, HMB45, melan-A and SOX10.
5. Surgery when possible is the most successful treatment; in case of unresectable disease, chemotherapy

with cisplatin/pemetrexed is recommended with a maximum of six cycles. There are no standard approaches for a second-line therapy. New drugs have been investigated such as bevacizumab or nintedanib, but more studies are required.

6. Some trials have showed hopeful results from immunotherapy (pembrolizumab-nivolumab), and it is likely that in the future they will represent the best approach to treat these patients.

Recommendations

- ESMO
  ► www.esmo.org/Guidelines/Lung-and-Chest-Tumours/Malignant-Pleural-Mesothelioma
- ASCO
  ► www.asco.org/practice-guidelines/quality-guidelines/guidelines/thoracic-cancer#/29376
- NCCN
  ► www.nccn.org

Hints for a Deeper Insight

The incidence of MPM is increasing worldwide, due to massive asbestos exposure, and a sort of epidemic is expected in developing countries where asbestos has not been banned yet [1]. In contrast with other solid tumours, overall survival has not been increased in recent years, reflecting the scarcity of improvements in our therapeutic capabilities.

Surgery and adjuvant radiotherapy in multimodal context are associated with survival advantage in retrospective studies, but this has never been confirmed in randomized studies, and only a minority of patients are diagnosed at early stages and candidate for surgery [2]. Of the two operations proposed pleurectomy/decortication (P/D) has lower morbidity and mortality compared to extrapleural pneumonectomy (EPP) and should be preferred whenever technically feasible, especially after the failure of MARS trial which concluded that EPP in trimodality setting offers no benefit [3]. The MARS2 trial is currently randomizing patients to define the impact on survival of P/D after induction chemotherapy [4]. In the meantime, evidence is being gathered about the feasibility of hemi-thoracic intensity modulated pleural RT after P/D [5]. Recently reported randomized clinical studies showed that prophylactic irradiation of thoracic intervention sites should not be used routinely [6, 7]. Conversely, radiotherapy is an effective treatment for pain control [8].

To date the cisplatin-pemetrexed doublet is the only evidence-based treatment, associated with better quality of life and clinically significant survival improvement [9]. Carboplatin may be an alternative to cisplatin in unfit and elderly patients [10]. No predictive biomarker has a role in the everyday clinical practice. A lot of effort is ongoing to overcome the therapeutic limitations by increasing the knowledge of the molecular, biological and genetic aspects of this disease. MPM has no oncogenic driver, and future development of targeted therapies should be based on the exploration of pathways activated as a consequence of the loss of tumour suppressor genes or other targets associated with the disease phenotype [11]. Among targeted agents tested so far, only antiangiogenics in combination with standard chemotherapy showed promising results. Despite the survival benefit reported for bevacizumab plus cisplatin-pemetrexed in the phase III, randomized, open-label MAPS trial, EMA has not extended the label of the drug for this indication [12]. The phase II, randomized, placebo-controlled LUME-Meso study, testing the antiangiogenic nintedanib in combination with cisplatin-pemetrexed met its primary endpoint, and the results for the phase III part of the study are eagerly awaited [13].

Vinorelbine, rechallenge with pemetrexed and gemcitabine based chemotherapy are commonly resorted to in second-line setting, even if randomized evidence is lacking [14].

Novel immunotherapeutic approaches, including immune checkpoint inhibitors, are being explored and currently generate great expectation. Early clinical studies suggest activity in a small proportion of patients with some durable responses which constitute the proof of concept of its activity, but no clear biomarker has been found so far which is essential to exploit its full potential [15, 16].

Progresses in the MPM treatment claim appropriate designed studies, addressing the role of therapeutic/targeted agents selected on the molecular profiling of the tumour, and therefore the availability of adequate amounts of tumour tissue from each patient is critical. To warrant this, but also to satisfy other needs concerning diagnostic and staging challenges, MPM patients should be referred to centres where an expert multidisciplinary team exists. Whenever possible patients should be encouraged to enter available clinical trials in every setting of the treatment.

Suggested Reading

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Cancer of the Esophagus

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Gastrointestinal Cancers

Contents

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Learning Objectives

By the end of the chapter the reader will:

- Be able to diagnose esophageal cancer
- Have learned the basic concepts of molecular classification of esophageal cancer
- Have reached in-depth knowledge of localized, inoperable locally advanced, and metastatic esophageal cancer treatment
- Be able to put acquired knowledge into daily clinical practice

34.1 Introduction and Epidemiology

Esophageal cancer (OC) is the ninth most common incident cancer in the world and the seventh leading cause of worldwide cancer-related mortality because of the its extremely aggressive nature and the poor survival rate of affected patients. The International Agency for Research on Cancer (IARC) estimates that there were about 450,000 cases of OC in 2012 [1, 2, 3, 4, 5].

Globally, esophageal squamous cell carcinoma (SCC) is the most common histological subtype of OC, particularly in high-incidence areas of eastern Asia and in eastern and southern Africa. In the highest-risk region, the so-called Asian Esophageal Cancer Belt, which extends from northern Iran, east to China, and north to Russia, presents an estimated SCC of more than 100 cases/100,000 person-years [6, 7]. Although the incidence of SCC has decreased in many regions, a marked increase in the incidence of esophageal adenocarcinoma (ADC), which appears to be sustained, has been observed in Europe, North America, and Australia during the past four decades. So, the incidence of ADC has surpassed that of SCC in many Western countries [8, 9, 3].

Historically, while most OC were derived from stratified epithelium of the middle and lower thirds of the esophagus and therefore named SCC, ADC derived from islands of columnar glandular cells near the gastroesophageal junction. Sarcomas and small cell carcinomas generally represent less than 1–2% of all esophageal cancers. On rare occasions, other carcinomas, melanomas, leiomyosarcomas, carcinoids, and lymphomas may develop in the esophagus as well [5, 10].

SCC and ADC are the two major histological types; they differ in biological features, geographic and demographic characteristics, risk factors, pathogenesis, patients' performance status, treatment, and HRQoL; and so they are discussed separately in the two following sections [3] (Fig. 34.1).

34.2 Risk Factors

34.2.1 Squamous Cell Carcinoma

The pathophysiological pathway of SCC is typically initiated by carcinogenic compounds in direct contact with the esophageal mucosa. Mechanical injuries, such as achalasia, radiation therapy, or swallowing hot beverages or sodium hydroxide, increase susceptibility to carcinogenic compounds [3]. Transition models have described squamous epithelium undergoing inflammatory changes that progress to dysplasia and in situ carcinoma [5].

The etiology of SCC is multifactorial and strongly population dependent as a result of the following risk factors [11].

Gender

In males the SCC incidence rates are two- to threefold higher than in females [12]. A global assessment indicated an overall male-to-female ratio of 4.4 which ranged from 1.7 in sub-Saharan Africa to 8.5 in North America [13].

Smoking and Alcohol Drinking

Smoking and alcohol drinking are the main risk factors for SCC in Western countries [14], and the risk of SCC increases from threefold to sevenfold for smokers compared with non-smokers [15]. Tobacco smoke is known to contain polycyclic aromatic hydrocarbons, nitrosamines, and many other carcinogens. A commonly accepted interpretation of the synergy between ethanol and tobacco smoke is that ethanol dissolves and facilitates the transport of tobacco carcinogens to cells, making the cells more susceptible to carcinogenesis [12, 16]. Alcoholic beverage consumption has been linked causally to SCC because acetaldehyde, a class 1 carcinogen, is the first metabolite of ethanol metabolism. Microorganisms in oral cavity also produce acetaldehyde from ethanol and could contribute to the carcinogenic effects of alcohol [11].

Diet and Hot Foods and Beverages

Dietary factors have also been suggested as etiological factors of SCC for US population, especially urban African Americans, whose intake of fruit and vegetables was lower than that of other ethnic groups [12]. Higher intake of fruit and vegetables probably decreases the risk of esophageal cancer; in fact each increment of 50 g/day of raw vegetables was associated with 31% decrease in the risk of esophageal cancer, while the same increment intake of fruit was associated with 22% decrease [17, 18]. Consumption of hot foods

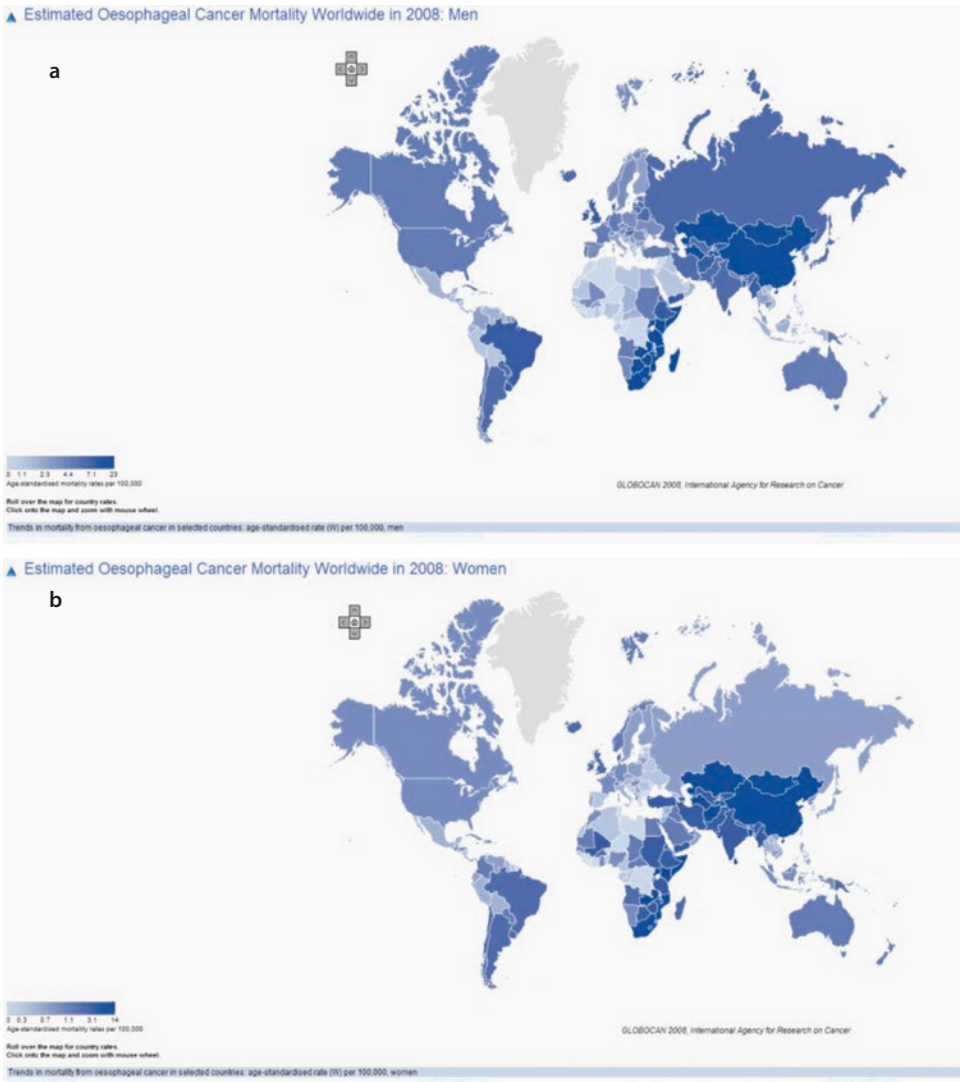

Fig. 34.1 Estimated esophageal cancer mortality worldwide in 2008. **a** Men. **b** Women. (Zhang Y. Risk factors of esophageal cancer)

and beverages has been associated with increased risk of SCC in subjects that drink the traditional herbal beverage maté, which is consumed at high temperature in large quantity (several liters per day) [11]. Other risk factors, such as micronutrients, PAHs (polycyclic aromatic hydrocarbons), BMI, medical conditions (such as Plummer-Vinson syndrome, Fanconi anemia), poor oral health, HPV infection, and mineral intakes, require confirmation with epidemiological studies in endemic areas.

Genetic Alteration

Dysplasia has been considered as a precancerous lesion of SCC with a significantly increased risk of developing into SCC. Therefore, identifying mutations occurring during SCC development could provide implications for early diagnosis and potential therapeutic strategies [16]. The genomic landscape of SCC, which frequently shows mutations in TP53, CDKN2A, and PIK3CA, has been well characterized by whole-genome sequencing (WGS) and whole-exome sequencing (WES). However, most studies of precancerous lesions of SCC were limited to hotspot genes or the allelic loss of tumor-suppressor genes. The panoramic genetic architecture of the carcinogenesis process is unknown [19].

Recent research has provided evidence that chronic inflammation is a strong risk factor in the microenvironment for the development of digestive tumors and a close association between chronic inflammation and esophageal precancerous lesions from SCC patients exists. However, evidence to prove inflammation as a pathogenic factor in SCC development hasn't been found yet; beside the last events that promote intraepithelial neoplasia (IEN) into infiltrating carcinoma haven't been identified yet [19].

34.2.2 Adenocarcinoma

Esophageal adenocarcinoma incidence rates have been steadily increasing in several Western countries, although there are differences either between countries or between regions within the same country. The upward trends are in part due to the increased prevalence of recognized risk factors such as gastroesophageal reflux disease (GORD), obesity, and male sex, while *Helicobacter pylori* infection and dietary intake of fruit and vegetables, and possibly also non-steroidal anti-inflammatory drugs, are considered protective. The increasing prevalence of reflux and obesity, combined with a decreasing prevalence of *Helicobacter pylori* infection, probably contributes to the increasing incidence of esophageal adenocarcinoma. GORD and Barrett's esophagus are among the most commonly mentioned risk factors for ADC in epidemiological studies, and the existing meta-analyses reported a gradually increased risk of ADC with the increasing frequency and duration of GORD symptoms [20].

Gender and Race

The incidence of esophageal adenocarcinoma is eightfold more common in men than in women [21] and fivefold more common in whites than in blacks in the USA. The male predominance is not readily explained by sex differences in the exposure to the established risk factors for ADC, obesity, gastroesophageal reflux disease, *Helicobacter pylori* (*H. pylori*) infection (inverse association), or tobacco smoking. On the other hand, it has been hypothesized that sex hormones and reproductive factors might play a role in the development of ADC or its precancerous lesion Barrett's esophagus, although the existing evidence is far from conclusive [22].

Barrett's Esophagus

It is a condition in which the typical squamous epithelium of the esophageal mucosa is replaced with columnar intestinal epithelium. BO is a known precursor to the development of esophageal adenocarcinoma, which has a dramatically increasing incidence over the past 40 years. The risk of ADC among patients with BO is estimated to be 30–125-fold greater than that of the general population. Endoscopically, the prevalence of BO has been estimated at 1–2% in all patients receiving endoscopy for any indication and anywhere from 5% to 15% in patients with symptoms of GORD. The incidence of endoscopically detected BO appears to have increased dramatically over the past 30 years, a finding partially attributable to the increasing frequency of endoscopy during the same period. BO on average is diagnosed in the sixth to seventh decades of life, but may develop far earlier [23].

Obesity

Measured by BMI and central adiposity, obesity has been studied extensively as a risk factor for BO. The incidences of BO and esophageal ADC have risen dramatically in the past 40–50 years in Western societies, concurrent with rapid increases in the rate of obesity. From 1976 to 1991, the prevalence of obesity at all ages rose from 25% to 33%, and it now approaches 35% in adults.

Other risk factors, such as alcohol, nutritional deficit, and tobacco use, may be considered for their effects on both GORD and BO risk. On the other hand, the use of non-steroidal anti-inflammatory drugs (NSAIDs), proton pump inhibitors (PPIs), and statins in patients with BO reduced the progression to adenocarcinoma [23].

Genetic Aspects

Very recently, it has been demonstrated using GWAS (genome-wide association study) that the risk of BO and ADC is influenced by many germline genetic variants of small effect and that shared polygenic effects contribute to the risk of these two diseases. In fact, the genetic correlation between BO and ADC was high and estimated a statistically significant polygenic overlap between BO and esophageal adenocarcinoma. This strongly suggests that shared genes underlie the development of BO and ADC. GWAS-type studies have also been conducted to elucidate susceptibility loci. The most significant results were for cancer and pre-cancer combined, suggesting that much of the genetic basis for ADC lies in the development of BO, rather than to ADC. One of the novel regions is chromosome 3p13, near FOXP1, a gene encoding a transcription factor, which regulates esophageal development. Interestingly, two of the other regions (BARX1/9q22.32 and FOXF1/16q24.1) contain risk-associated SNPs which disrupt binding of FOXP1. Further dissection of these loci is likely to lead to insights into the etiology of this rapidly fatal cancer [22].

34.3 Clinical Features

Esophageal cancers are usually asymptomatic in the early stage and after they may cause different symptoms according to progression of tumor, leading to a diagnosis in a later stage.

Weight loss and dysphagia are the most common signs and symptoms at the diagnosis. The dysphagia arises typically when there is an involvement of more than one-third of the esophageal lumen. It can be limited to the liquids or can affect also the solids, leading to a complete dysphagia. Dysphagia, thoracic pain, regur-

gitation, hiccups, drooling, and odynophagia are common symptoms in the locally advanced disease with involvement of mediastinal structures. Dysphonia and cough at the deglutition may arise in case of involvement of recurrent laryngeal nerve or presence of esophagus-tracheal fistula, respectively.

The liver, peritoneum, lungs, and bones are the most common site of distant metastasis in case of esophageal cancer, whereas the involvement of the brain is rare. Liver involvement is predominant and can lead to hepatomegaly and jaundice, while dyspnea can appear in case of diffuse lung involvement, pleural effusion, or profuse ascites. Bone pain and neurologic signs and symptoms can appear in case of bone or different areas of the brain involvement, respectively. Peritoneal metastasis may cause different entity of peritoneal carcinomatosis with ascites or secondary implants.

34.4 Pathological Features

The subsequent histopathological pictures of the SCC and the ADC were taken from and based on the description made by [24].

34.4.1 Macroscopic Aspects

34.4.1.1 Squamous Cell Carcinoma

SCC occurs most commonly in the middle third of esophagus followed by lower one-third and upper one-third, respectively. Endoscopically, it can be polypoid, flat, or ulcerated. On endoscopic ultrasound (EUS), infiltrating SCC presents as a circumscribed diffuse wall thickening with echo-poor pattern due to destruction of layers of esophageal wall. Invasion of neoplastic squamous cells into lamina propria and deeper layers defines invasive SCC.

34.4.1.2 Adenocarcinoma

The main pathophysiological pathway of ADC is likely to be chronic gastroesophageal reflux disease, causing metaplasia from the native squamous cell mucosa to a specialized columnar epithelium, known as Barrett's esophagus (BO) [3]. Barrett's esophagus is a condition in which the normal squamous epithelial lining of the distal esophagus is replaced with specialized or intestinalized columnar epithelium. BO is a complication of chronic GORD although asymptomatic subjects might also be affected [25]. For the diagnosis of BO, which is considered the premalignant condition and the main risk factor of the esophageal adenocarcinoma, the presence of intestinal metaplasia is required because currently intestinal metaplasia is the only type of columnar epithelium that clearly predisposes toward development of this highly lethal disease [26]. Gastroesophageal reflux disease or just reflux [16, 27] can damage the lining of the esophagus, which causes BO, characterized by abnormal "tongues" of salmon-colored mucosa extending proximally from the squamo-columnar junction into the normal pale esophageal mucosa. BO develops in approximately 5–8 percent of patients with reflux disease and can progress to low-grade dysplasia, high-grade dysplasia, and invasive ADC [4].

34.4.2 Microscopic Aspects

34.4.2.1 Squamous Cell Carcinoma

Chronic Esophagitis: early studies suggested that mild to moderate chronic esophagitis was associated with family history of esophageal cancer and other risk factors of SCC [7]. Subsequently, systematic studies with endoscopic surveillance, biopsy evaluation, and follow-up to the development of SCC showed that esophagitis is nonspecific and the only true precursor lesion of SCC is squamous dysplasia [1, 11].

Squamous Dysplasia: It is a histologic lesion confined to the epithelium and is characterized by cytologic and architectural abnormalities. The cytologic abnormalities include nuclear enlargement, hyperchromasia, pleomorphism, and increased and/or abnormal mitosis. The architectural changes include loss of polarity and lack of surface maturation. The abnormality starts from the basal layer, and based on the extent of involvement of thickness of epithelium by atypical cells, the dysplasia was traditionally graded as mild (up to one-third), moderate (up to two-thirds), and severe (involving upper one-third). In 2000, the WHO adopted the term "intraepithelial neoplasia" (IEN) for dysplasia and classified IEN in a two-tier system as low-grade when less than half of thickness of the epithelium is involved with atypical cells (◘ Fig. 34.2a) and high-grade when greater than half of thickness is involved. Full-thickness involvement of the epithelium is called "squamous cell carcinoma in situ" (CIS).

Histologically, the tumor can show variable differentiation. Well-differentiated SCC show presence of keratin pearls, individual cell keratinization, and intercellular bridges. Poorly differentiated SCC lack these features and are determined to be squamous in origin based on pattern of infiltration and presence of IEN or in situ lesions in the adjacent squamous mucosa or with the help of immunohistochemical markers such as CK5/6 or p63. Moderately differentiated SCC show intermediate features.

34.4.2.2 Adenocarcinoma

ADC had copy number, RNA, and methylation patterns more similar to the chromosomally unstable subtype of gastric adenocarcinoma than to esophageal SCC.

The strongest predictor of progression to high-grade dysplasia (HGD) or ADC is baseline low-grade dysplasia. Dysplasia in BO is a histologic diagnosis suggesting that epithelial cells have acquired genetic or epigenetic alterations which predispose them to the development of malignancy. Dysplasia, when identified in a patient with BO, predicts a higher risk of ADC. The annual risk of progression in BO with low-grade dysplasia (LGD) is closer to 0.5–3%. Even among experienced pathologists, the extent of interobserver agreement, when diagnosing LGD, can be less than 50%, and this is in part due to the fact that inflammation can cause cytologic atypia in the bases of crypts that mimics dysplasia. Regression of LGD, or the failure to detect dysplastic changes on subsequent endoscopies, also may occur in half or more of patients with LGD. Incidence of ADC or HGD is estimated at 1.1–6% annually, but some estimates are as high as 13.4% per year. With HGD, interobserver agreement is better but is still less than 90%.

The risk of ADC is greater in longer segments of BO compared to shorter segments. The relationship between segment length and increased risk of AC is not always linear, but the preponderance of evidence suggests that greater surface area of columnar-lined mucosa correlates with increased cancer risk [23].

Low-Grade Dysplasia: Barrett's mucosa shows loss of surface maturation and architectural distortion with glandular crowding, in the absence of active inflammation. There is a sharp contrast between neoplastic and non-neoplastic mucosa. Nuclei in the surface mucosa show hyperchromasia, nuclear enlargement, stratification, and mucin loss. Mitotic figures can be seen on the surface (Fig. 34.2).

High-Grade Dysplasia: Barrett's mucosa shows loss of surface maturation (as in LGD) and glandular crowding. The nuclei show loss of polarity and are rounded, enlarged, and hyperchromatic with inconspicuous nucleoli. Mitoses are frequent. Inflammation is less in comparison to the architectural and cytologic atypia. Presence of ulceration, active inflammation, and prominent nucleoli are features indicative of reactive/reparative changes due to a benign process or are concerning for an associated invasive carcinoma. Additional features suggestive of invasive adenocarcinoma on biopsies include cribriform glandular architecture, luminal necrotic debris, ulceration, neutrophils within dysplastic glands, and pagetoid spread of neoplastic cells in the overlying squamous mucosa.

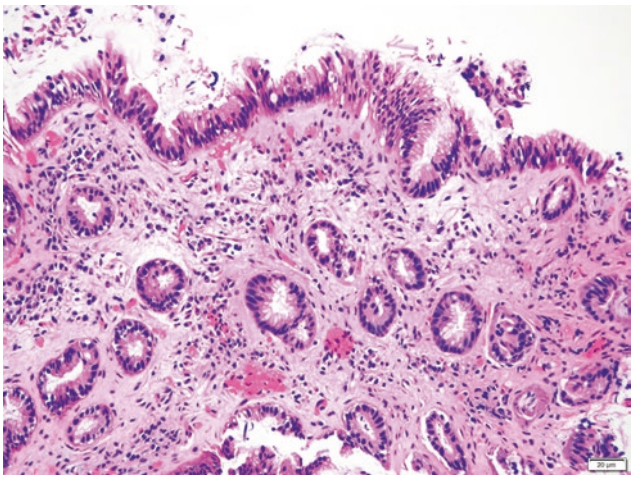

Fig. 34.2 Esophageal dysplasia: low grade; Dhingra, *MD, FACP, Associate Professor, Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX* [24])

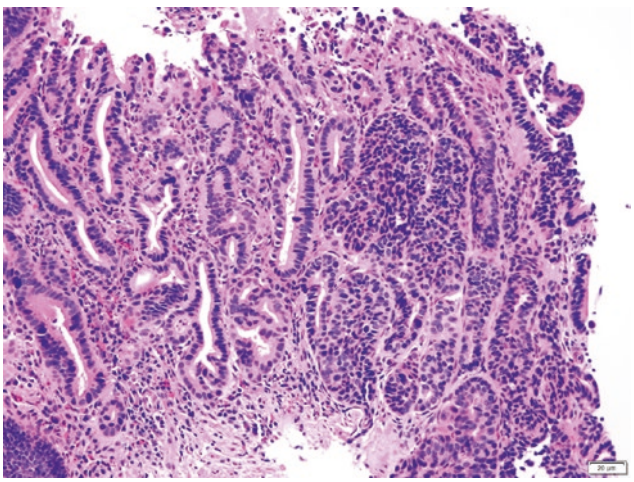

Fig. 34.3 Intramucosal adenocarcinoma. (From Dhingra, *MD, FCAP, Associate Professor, Departement of Pathology and Immunoly, Baylor College of Medicine, Houston, TX* [24])

Intramucosal Adenocarcinoma

Intramucosal adenocarcinoma is defined by invasion of carcinoma into lamina propria but not beyond muscularis mucosae. The features of intramucosal adenocarcinoma are syncytial growth pattern with back-to-back glands, presence of single cells, and small clusters within lamina propria (Fig. 34.3). Desmoplasia may not be present, but if present, it is very subtle [24].

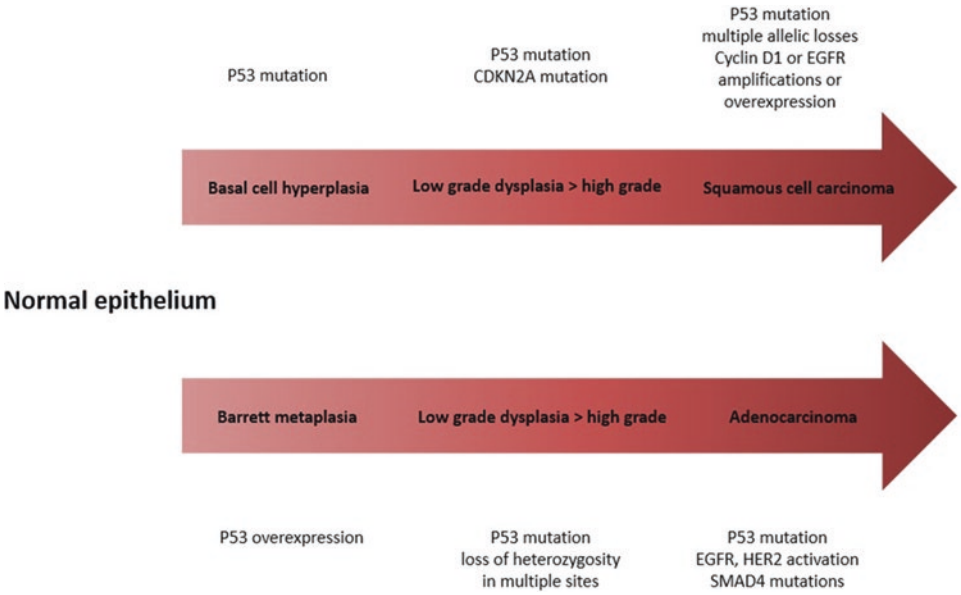

Fig. 34.4 Molecular alterations in esophageal cancers

Esophageal Adenocarcinoma

Endoscopically, if detected early, these tumors will present as mucosal irregularities. In later stages they appear as ulcerated/infiltrative or exophytic masses with obstruction. Histologically, these are gland-forming tumors with a tubular, tubulopapillary, or papillary growth pattern. A small subset of cases shows mucinous differentiation. A few cases of diffuse signet ring cell adenocarcinoma have also been reported. Foci of BO with high-grade dysplasia are commonly seen in epithelium adjacent to the tumor. The tumors show variable grades of differentiation based on the amount of gland formation, and the nuclear atypia generally follows the grade of differentiation.

Well-differentiated tumors show more than 95% gland formation, moderately differentiated tumors show 50–95% gland formation, and poorly differentiated tumors show <50% gland formation [24].

34.5 Molecular Biology

Esophageal cancer is characterized by alterations of some gene or molecules involved in different processes, such as cell proliferations, apoptosis, DNA repair, and signal transduction. In particular, loss of heterozygosis on chromosomes 1p, 3p, 5q, 9p, 9q, 13q, 17p, 17q, and 18 q, p53 mutations, Rb deletions, cyclin D1 and c-myc amplifications, NFκB hyperexpression, and bcl- 2, caspase 3, TRAIL, Fas, and Fas-L mutations are the most common alterations that can be found in these types of tumors.

RAS mutation is rare in case of esophageal tumors [28], while there is human epidermal growth factor receptor 2 (Her-2) amplification in 60% of cases of Barrett's esophagus. Her-2/neu gene, located on chromosome 17q21, encodes the Her-2 protein that belongs to the epidermal growth factor receptor (EGFR) family pathway. In case of gene amplification, there is Her-2 receptor overexpression, resulting in a prolongation of transductional signals with uncontrolled cell growth and tumorigenesis. To date, the specific ligand of this receptor has not been identified, and it is considered a ligand-independent orphan receptor.

For many years, esophageal cancers were divided into two big groups according to histopathologic and epidemiologic aspects, as already mentioned: squamous cell carcinoma (SCC) and adenocarcinoma (ADC). Furthermore, these two types of tumors are distinguished also from a molecular point of view (Fig. 34.4). SCC, in fact, showed genomic amplifications of EGFR (19%), phosphatidylinositol 3-kinase (PI3K), and p63 pathway alterations, whereas ADC showed an increased E-cadherin signaling and common amplifications of Her-2 (32%), vascular endothelial growth factor A (VEGF-A), GATA 4, and GATA 6 [29].

Nevertheless, nowadays it is known that also into these two groups it can be distinguished different kind of tumors characterized by different features. These findings led to create a molecular classifications for esophageal cancers that could became important in the future in order to develop novel target therapies directed against specific molecular targets.

34.5.1 Molecular Classifications

The Cancer Genome Atlas Research (TGCA) network [29] reported the latest molecular classification for esophageal cancer based on the evaluation of genes

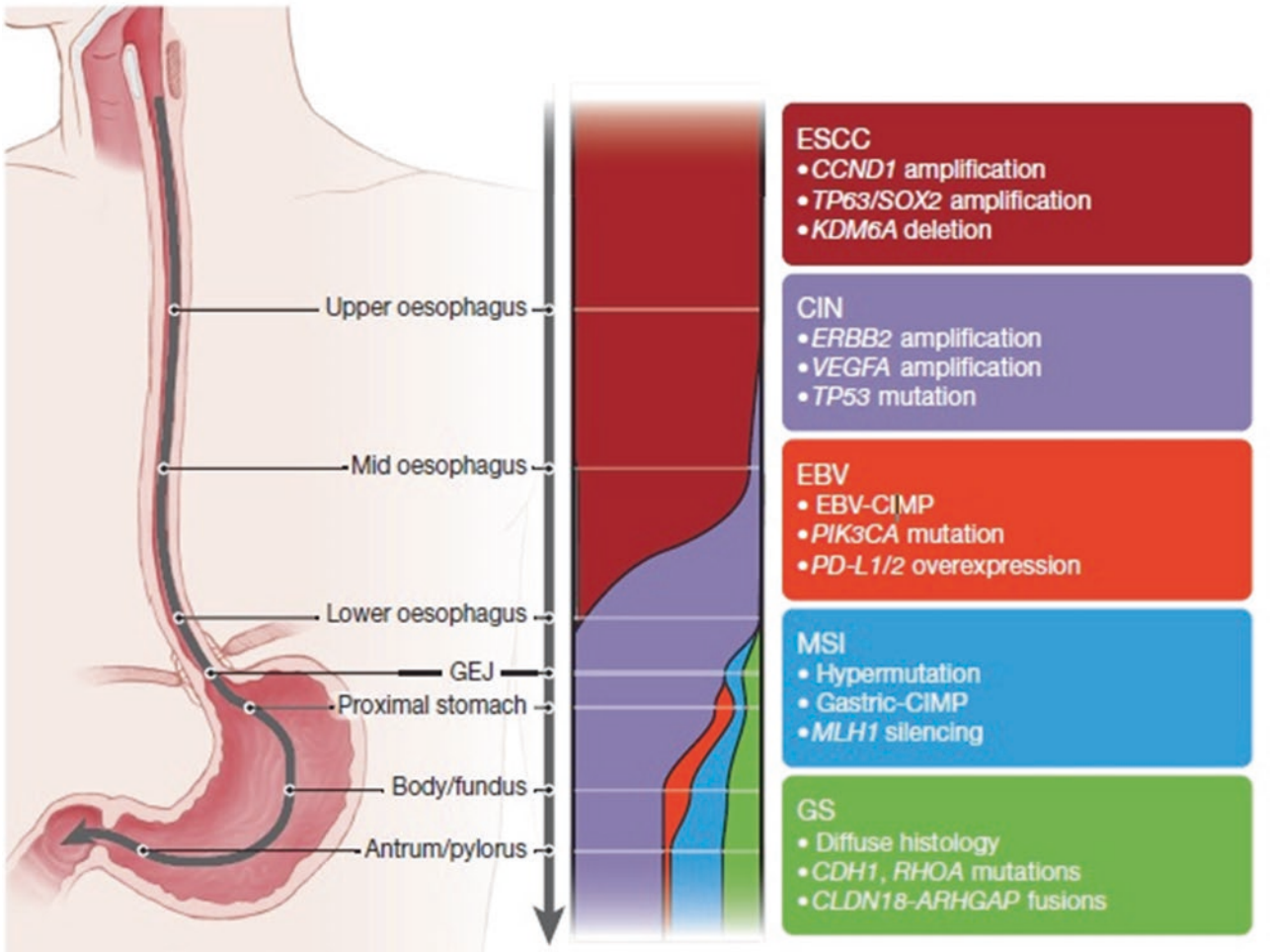

Fig. 34.5 Molecular subtypes of esophageal cancer [29]. ESCC esophageal squamous cell carcinoma, CIN chromosomal instability, EBV Epstein-Barr virus, MSI microsatellite instability, GS genomic stability

expression in 164 tumors (Fig. 34.5). According to this TGCA classification, esophageal cancer can be divided into two classical groups: SCC and ADC. Within SCC there are three molecular subtypes: SCC1, SCC2, and SCC3.

SCC1 represents the classical esophageal cancer subtype; it is predominant in Asiatic regions, and it is characterized by alterations in NRF2 pathway, involved in the regulation of response to oxidative stressors such as chemotherapy, and amplifications of SOX2 and/or p63. SCC2 is more frequent in Eastern Europe or South America; it is characterized by greater leukocyte infiltration and higher rates of PTEN inactivation and/or CDK6 amplification, whereas SCC3 is reported in the USA and Canada and shows activation of PI3K pathway. None of these subtypes was related to human papillomavirus (HPV), unlike the other types of SCC.

Esophageal ADC showed, in addition to the molecular features already mentioned, high rate of chromosomal instability (CIN) as well as gastric cancer subtype one [30], suggesting that these two types of cancer might have the same origin and be considered as a single entity. Nevertheless, other molecular characteristics, such as DNA hypermethylation, distinguish the esophageal ADC from the gastric CIN one.

34.6 Esophageal Cancer Progression

Esophageal cancer can progress by different local and contiguity diffusion, lymphatic involvement, and hematic spread of metastasis. Among all, local infiltration and lymphatic spread represent the more frequent ways of diffusion. In the first case, tumor can involve all esophageal wall leading to a visceral stenosis, whereas in case of lymphatic diffusion the neoplasm can disseminate through the lymphatic vessels in the submucosa and muscular tunics leading to also different distant synchronous lesions, known as skip lesions. This peculiarity, in addition to the lack of a serosa around the esophagus and its

close anatomical relationships with other mediastinum structures, such as the vessels, pericardium, or trachea, leads to an early diffusion of disease. Moreover, the lymphatic spread is according to the primary tumor site. In fact, there are a predominant involvement of cervical paratracheal and peribronchial lymph nodes in case of cancer of the upper third of the esophagus, a diffusion to the under-diaphragmatic stations in case of middle esophagus tumor, and involvement of the lymph nodes around the cardia in the third lower esophagus cancers.

The diffusion of disease by contiguity consists in the involvement of different organs around the primary tumor, such as the trachea and/or rachis in the upper cancers or the pericardium, diaphragm, or liver in the lower ones.

The hematic spread of tumor occurs at a later stage with frequent involvement of the liver and/or lung, whereas bone, brain, and adrenal metastases are rarer.

34.7 Diagnosis

A clinical and instrumental evaluation is mandatory in all patients with a risk condition or with new symptoms suspected for esophageal cancer. A first global clinical visit is recommended to evaluate risk factors, but specific assessment is needed to detect an esophageal tumor. Therefore, the endoscopy of the upper gastrointestinal tract is the most important exam to diagnose an esophageal cancer. The endoscopy gives a global view of esophageal mucosa and allows to obtain a biopsy on the suspected tumor lesions. In case of esophageal lesions, it is mandatory to obtain multiple biopsy on the mucosa of the lesion and around as well as a brushing of the lesion. Moreover, in case of stenosis, during the endoscopy the dilatation or the position of stents to palliate the dysphagia can be evaluated.

The radiological evaluation of the esophagus is not frequently used today. However, this study may help to define the presence, site, grade, and length of a stenosis or the presence of esophageal fistulas. The definition of length is important to plan the correct therapeutic strategy for these patients, because in case of length >5 cm, with or without distortion of the esophagus profile, there is a locally advanced disease and the upfront surgery is not recommended.

34.8 Differential Diagnosis

The most important differential diagnosis is between tumors and polyps, leiomyomas, or ulcers, because sometimes these lesions cause the same symptoms of cancer (heartburn, e.g.). In almost all cases, the endoscopy with biopsy or the morphologic characteristics at the radiological imaging of the esophagus are able to differentiate these benign lesions from the neoplastic ones.

34.9 Staging

An accurate staging is important to choose the appropriate approach to treat a patient with esophageal cancer. In order to obtain a correct staging of disease, it is important to define the site of primary tumor. In fact, it can distinguish tumors of the esophagus and tumors of the gastroesophageal junction.

The esophagus can be divided into cervical, thoracic, and abdominal, according to the following anatomical definitions [31]:

- Cervical: from the lower border of the cricoid cartilage (at the level of the sixth cervical vertebra) to the thoracic inlet (suprasternal notch); 18 cm from incisors.
- Upper thoracic: from the thoracic inlet to the level of tracheal bifurcation; 18–23 cm from incisors.
- Mid-thoracic: from the tracheal bifurcation midway to junction; 24–32 cm from incisors.
- Lower thoracic: from midway between tracheal bifurcation and gastroesophageal junction to gastroesophageal junction, including the abdominal esophagus; 32–40 cm from incisors.

The gastroesophageal junction is the point where the distal esophagus joins the proximal stomach, and it is divided from an anatomical point of view, according to Siewert classification, into [32]:

- Type I: adenocarcinoma of the distal esophagus, which usually arises from an area with specialized intestinal metaplasia of the esophagus (i.e., Barrett's esophagus) and may infiltrate the esophagogastric junction from above (center located within between 1 and 5 cm above the anatomic cardia).
- Type II: true carcinoma of the cardia arising at the esophagogastric junction (within 1 cm above and 2 cm below the cardia).
- Type III: subcardial gastric carcinoma that infiltrates the esophagogastric junction and distal esophagus from 2 to 5 cm below the cardia.

The stage is according to the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) TNM staging system (8th edition) (Tables 34.1, 34.2, and 34.3) [33]. Like the previous one, the 8th edition distinguishes between SCC and ADC and includes the grading into the stage. Nevertheless, in this last edition, there are also three separate classifications for both ADC and SCC: the pathologic stage groups (pTNM), the newly introduced

Table 34.1 Clinical TNM staging (cTNM) for esophageal cancer, 8th edition [33]

Primary tumor (cT)			
TX	Primary tumor cannot be assessed		
T0	No evidence of primary tumor		
T_{is}	Carcinoma in situ/high-grade dysplasia		
T1	Tumor invades the lamina propria, muscularis mucosae, or submucosa		
T1a	Tumor invades the mucosa or lamina propria or muscularis mucosae		
T1b	Tumor invades the submucosa		
T2	Tumor invades the muscularis propria		
T3	Tumor invades the adventitia		
T4	Tumor invades adjacent structures		
T4a	Tumor invades the pleura, pericardium, diaphragm, or adjacent peritoneum		
T4b	Tumor invades other adjacent structures such as the aorta, vertebral body, or trachea		
Regional lymph nodes (cN)			
Nx	Regional lymph nodes cannot be assessed		
N0	No regional lymph node metastasis		
N1	Metastasis in 1–2 regional lymph nodes		
N2	Metastasis in 3–6 regional lymph nodes		
N3	Metastasis in 7 or more regional lymph nodes		
Distant metastasis (cM)			
Mx	Distant metastasis cannot be assessed		
M0	No distant metastasis		
M1	Distant metastasis		
Clinical stage groups			
	Squamous cell carcinoma		
	cT	*cN*	*cM*
0	Tis	N0	M0
I	T1	N0–1	M0
II	T2 T3	N0–1 N0	M0 M0
III	T3 T1–3	N1 N2	M0 M0
IVa	T4	N0–2	M0
IVb	T1–4	N0–3	M1
	Adenocarcinoma		
0	Tis	N0	M0
I	T1	N0	M0
IIa	T1	N1	M0
IIb	T2	N0	M0
III	T2 T3–4a	N1 N0–1	M0 M0
IVa	T1–4a T4b T1–4	N2 N0–2 N3	M0 M0 M0
IVb	T1–4	N1–3	M1

postneoadjuvant pathologic stage groups (ypTNM), and clinical stage groups (cTNM). Regarding the gastroesophageal junction adenocarcinoma, Siewert I and II with involvement of the esophagus are staged according to the esophageal cancer system, whereas type III and type II tumors with distal extension to the stomach are staged according to the gastric cancer system.

Staging should include a complete clinical examination, an endoscopic ultrasound (EUS), and a computed tomography (CT) scan of the neck, chest, and abdomen with contrast.

In particular, EUS is fundamental to evaluate the local invasion (T parameter) and nodal involvement (N parameter) with high precision than CT scan (96% sensitivity for T and 81% for N). Moreover, EUS can be able to perform biopsy of primary tumor and lymph nodes and for this reason is mandatory for all patients candidate to surgery, because the involvement of regional lymph nodes represents a clear indication to neoadjuvant treatment today. The main limitation of EUS is the frequent presence of esophageal stenosis that does not allow the exam.

CT scan allows to detect locoregional involvement of continuous organs, such as aorta and trachea, and distant metastasis. On the contrary, CT scan is not recommended to distinguish between T1 and T2 invasion.

18F-Fluorodeoxyglucose-positron emission tomography (18 FDG-PET alone or in combination with CT scan: PET-CT) is considered as a second-level exam able to identify undetected distant metastases, especially in patients candidate to esophagectomy in order to prevent a surgical non-curative procedure in IV stage setting. Moreover, 18 FDG-PET may be used to evaluate the

Table 34.2 Pathological TNM staging (pTNM) for esophageal cancer, 8th edition [33]

Pathologic stage groups					
	Squamous cell carcinoma				
	pT	*pN*	*pM*	*pGRADE*	*pLOCATION*
0	Tis	N0	M0	N/A	Any
Ia	T1a	N0	M0	G1, X	Any
Ib	T1b T1 T2	N0 N0 N0	M0 M0 M0	G1, X G1–3 G1	Any Any Any
IIa	T2 T3 T3	N0 N0 N0	M0 M0 M0	G2–3, X Any G1	Any Lower Upper/middle
IIb	T3 T3 T3 T1	N0 N0 N0 N1	M0 M0 M0 M0	G2–3 Any Any Any	Upper/middle Any Any Any
IIIa	T1 T2	N2 N1	M0 M0	Any Any	Any Any
IIIb	T4a T3 T2–3	N0–1 N1 N2	M0 M0 M0	Any Any Any	Any Any Any
IVa	T4a T4b T1–4	N2 N0–3 N3	M0 M0 M0	Any Any Any	Any Any Any
IVb	T1–4	N0–3	M1	Any	Any
	Adenocarcinoma				
0	Tis	N0	M0	N/A	
Ia	T1a	N0	M0	G1, X	
Ib	T1a T1b	N0 N0	M0 M0	G2 G1–2, X	
Ic	T1 T2	N0 N0	M0 M0	G3 G1–2	
IIa	T2	N0	M0	G3,X	
IIb	T1 T3	N1 N0	M0 M0	Any Any	
IIIa	T1 T2	N2 N1	M0 M0	Any Any	
IIIb	T4a T3 T2–3	N0–1 N1 N2	M0 M0 M0	Any Any Any	
IVa	T4a T4b T1–4 T1–4	N2 N0–2 N3 N0–3	M0 M0 M0 M1	Any Any Any Any	

Table 34.3 Postneoadjuvant treatment TNM staging (ypTNM) for esophageal cancer, 8th edition [33]

	ypT	**ypN**	**ypM**
I	T0–1	N0	M0
II	T3	N0	M0
IIIA	T0–2	N1	M0
IIIB	T4a T3 T0–3	N0 N1–2 N2	M0 M0 M0
IVA	T4a T4b T1–4	N1–2, Nx N0–2 N3	M0 M0 M0
IVB	Every T	Every N	M1

response after a neoadjuvant treatment (restaging). In fact, some trials showed that an early metabolic response after the first cycle of neoadjuvant chemotherapy assessed by 18 FDG-PET could predict the pathologic response after surgery [34]. However, further confirmation is needed for this indication.

A tracheobronchoscopy should be carried out in the case of primary tumors located at thoracic esophagus in order to exclude tracheal invasion. Moreover, in the case of SCC related to chronic tobacco and alcoholism, an additional investigation of the aerodigestive tract is mandatory to exclude synchronous second cancer which is frequent in these conditions.

Ultrasonography is useful in case of cervical or upper thoracic tumor to evaluate the supraclavicular and cervical lymph nodes.

Laparoscopy can be done to detect peritoneal metastases in locally advanced ADC of the esophagus or gastroesophageal junction (15% at the diagnosis), even if this approach is not still considered mandatory.

Finally, in patients with esophageal cancer, it is important to assess the nutritional status and history of weight loss according to the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines [35], due to the primary site of tumor that leads to a difficult intake of food and subsequent weight loss. Weight loss confers an increased operative risk, worsens a patient's quality of life, and is associated with poor survival in advanced disease independent from the final body mass index. Therefore, nutritional support is mandatory at the diagnosis and during all treatment period for these patients.

34.10 Prognostic Factors

Despite the progression in knowledge and treatments for esophageal cancer, the prognosis of this type of tumor is still poor especially in case of locally advanced

or metastatic disease. The main prognostic factors for esophageal cancer are the depth of invasion, nodes, and distant metastasis that together represent the stage of disease. The median overall survival, therefore, is according to the stage, with less than 10% of patients alive at 5 years after the diagnosis.

The pathologic response to treatment represents another prognostic factor that may be considered in patients treated with a neoadjuvant approach. In fact, some trials showed that a complete pathological response (pCR) is related to better survival in these patients.

Finally, the performance status (PS) of patient can influence the prognosis by affecting the choice and the correct execution of treatments.

34.11 Treatment

Surgery represents the only curative approach in case of esophageal cancer. Unfortunately, only one-third of patients are candidate to surgery at the diagnosis, while the others showed a non-resectable locally advanced or metastatic disease. Nevertheless, the outcomes for these patients remain poor despite curative surgery, with a median overall survival from 11 to 18 months and a 5-year survival rate between 16% and 32%. In this context, an interdisciplinary planning of treatment becomes mandatory to evaluate the integration of different therapeutic approaches in addition to surgery in order to improve the prognosis [36]. The main factors for selecting primary therapy are tumor stage and location, histological type, and patient's PS, preferences, and comorbidities.

Response to systemic treatments should normally be assessed with interval imaging of the chest, abdomen, and pelvis, mostly with CT scan, although alternative imaging techniques may be used if required to monitor known sites of disease (e.g., magnetic resonance imaging for brain lesions or bone scintigraphy in case of bone lesions). The evaluation of response is according to standard radiologic criteria for solid tumor, also known as RECIST criteria, except in case of immunotherapy in which should be used the immune-modified RECIST (iRECIST). In case of neoadjuvant treatment, the restaging should comprise also a local evaluation of disease by EUS and 18FDG-PET to exclude the presence of distant metastasis before surgery.

34.11.1 Limited Disease

Surgery is the treatment of choice in limited disease (stages I and II). The goal of the surgical approach is to obtain a curative radical resection, also known as R0 resection, without macro- or microscopic residual disease. The presence of residual microscopic or macroscopic disease after surgery, known as R1 and R2 resections, respectively, represents an important bad prognostic factor for patients affected by esophageal cancer with a 5-year survival rate of 5–15% for R1 and 0% for R2.

In patients with ADC limited to the mucosa and submucosa (T1a and T1b), endoscopic therapy by endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD) is preferred. The endoscopic therapy is considered a curative approach as well as the esophagectomy in case of superficial ADC without risk factors (depth of invasion <500 μm, no ulceration, <20 mm diameter, well differentiated), whereas it is recommended a resection in case of presence of these ones.

In localized esophageal cancer beyond T1a N0, surgery is the current standard of care (Fig. 34.6). It is important to refer to specialized and dedicated surgical centers to undergo this procedure due to the high postoperative mortality and the complexity of this surgery [37]. The type of surgical approach (transhiatal or transthoracic) depends on the tumor site without differences in survival between the different types [38]. Transthoracic esophagectomy by Ivor-Lewis procedure with two- or three-field lymphadenectomy is always the approach of choice because this type of resection makes possible to explore extensively the esophagus and to obtain a good lymphadenectomy.

Recently, the role of a minimally invasive approach to the thoracic and/or abdominal cavities is increasing in clinical practice compared to the open one. In fact, a newly randomized study in patients with esophageal cancer showed that hybrid minimally invasive esophagectomy (HMIO) reduces intra- and postoperative morbidity, especially pulmonary complications, if compared to an open esophagectomy and suggests a trend in improvement of survival [39].

Regarding the tumors of the gastroesophageal junction, the partial esophagectomy with gastroresection is the first choice in case of Siewert I tumors, whereas a total gastrectomy is reserved to patients with Siewert II or III neoplasms. A two-field lymphadenectomy is mandatory in all cases in addition to the surgery of primary tumor [40].

However, despite surgery, esophageal cancer shows high rate of locoregional relapses and early distant metastasization. For these reasons, a multimodal approach was evaluated also in these types of tumors in order to improve the outcome of patients.

A recent trial involving patients with stage I and stage II esophageal cancers showed that neoadjuvant chemoradiotherapy (CRT) with cisplatin and fluorouracil did not improve R0 resection rate or survival but enhances postoperative mortality if compared with surgery alone. Based on these results, surgery alone is recommended as the pri-

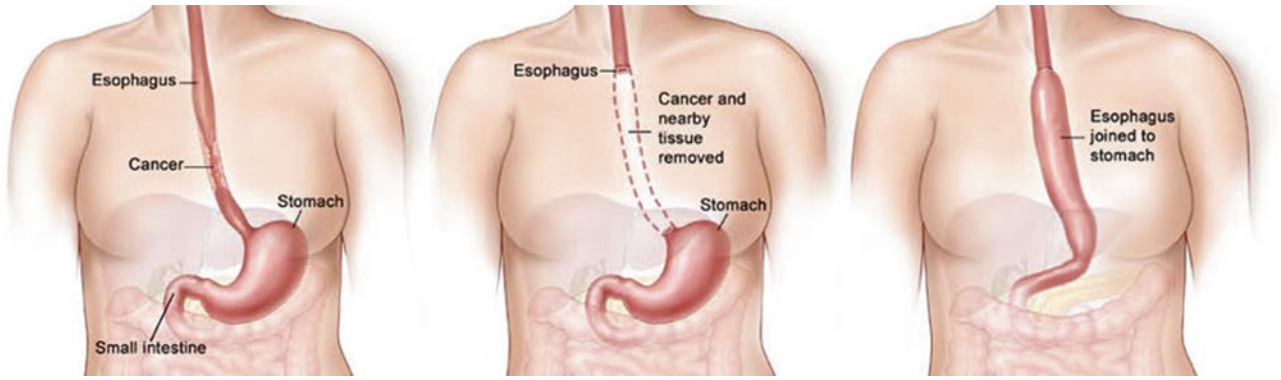

Fig. 34.6 Ivor-Lewis esophagectomy

mary treatment approach for cT2N0 esophageal cancer today [41], except in case of cervical carcinoma, in which definitive CRT represent the standard of care in localized and locally advanced disease. For these patients, in fact, the surgical approach is a total larynx-esophagectomy with high morbidity and mortality. However, his approach should be apply in cases of relapses or residual disease after multidisciplinary discussion.

In case of patients unable or unwilling to undergo surgery, combined CRT based on cisplatin/oxaliplatin and fluorouracil is superior to radiotherapy (RT) alone.

The adjuvant chemotherapy is currently limited in the esophageal disease, because the trials in this field did not show benefit with its use. All the studies, in fact, demonstrated a clear superiority for neoadjuvant approach, reserved the adjuvant RT in case of R1 or R2 resection.

34.11.2 Locally Advanced Disease

Surgery alone is not recommended as a curative approach in case of locally advanced disease (stage III), since the majority of patients cannot receive a complete R0 resection and, even after complete tumor resection, long-term survival is almost 20%. In this context, a multidisciplinary integrated neoadjuvant approach is mandatory to eradicate the micrometastatic disease and increase R0 resection and survival rates [42]. Therefore, preoperative treatment with chemotherapy or concomitant CRT is indicated in operable patients with locally advanced esophageal cancer according to the tumor's histotype. The global algorithm for treatment of non-metastatic esophageal cancer is reported in Fig. 34.6.

After a neoadjuvant treatment, all patients who did not progress during the therapy should be restaged in order to assess their response to treatment. Nowadays, pathological response is considered the only validated factor that can predict the response to treatment, according to Mandard's Tumor Regression Grade (TRG) scale (Table 34.4, [43]).

Table 34.4 Tumor Regression Grade (TRG) score according to Mandard [43]

TRG1	No viable cancer cells, complete response
TRG2	Single cells or small groups of cancer cells
TRG3	Residual cancer outgrown by fibrosis
TRG4	Significant fibrosis outgrown by cancer
TRG5	No fibrosis with extensive residual cancer

In fact, many trials demonstrated that patients who showed a pathologic complete response (pCR) had a significant survival benefit. Nevertheless, the evaluation of pathologic response is obviously obtained only on the surgical specimen of patients who underwent surgery. As already mentioned, a surrogate method that may predict the response is the evaluation of early metabolic response at 18-FDG-PET. The early metabolic response is the uptake reduction of 35% or more by PET after receiving one cycle of neoadjuvant chemotherapy [34]. Even if this represents a promising evaluation, further trials are needed to define its role as a prognostic marker, and it is not a standard in clinical practice today.

As shown in Fig. 34.7, there are two different algorithms according to histological subtypes of esophageal cancer. In case of SCC, it can be considered a neoadjuvant or a definitive approach. Regarding the neoadjuvant treatment, some trials demonstrated that patients with locally advanced disease benefit from preoperative chemo- or chemoradiotherapy with a high rate of R0 resection and better outcome [44]. The weekly administration of carboplatin and paclitaxel for 5 weeks and concurrent radiotherapy (41.4 Gy) followed by surgery represent the current standard of care

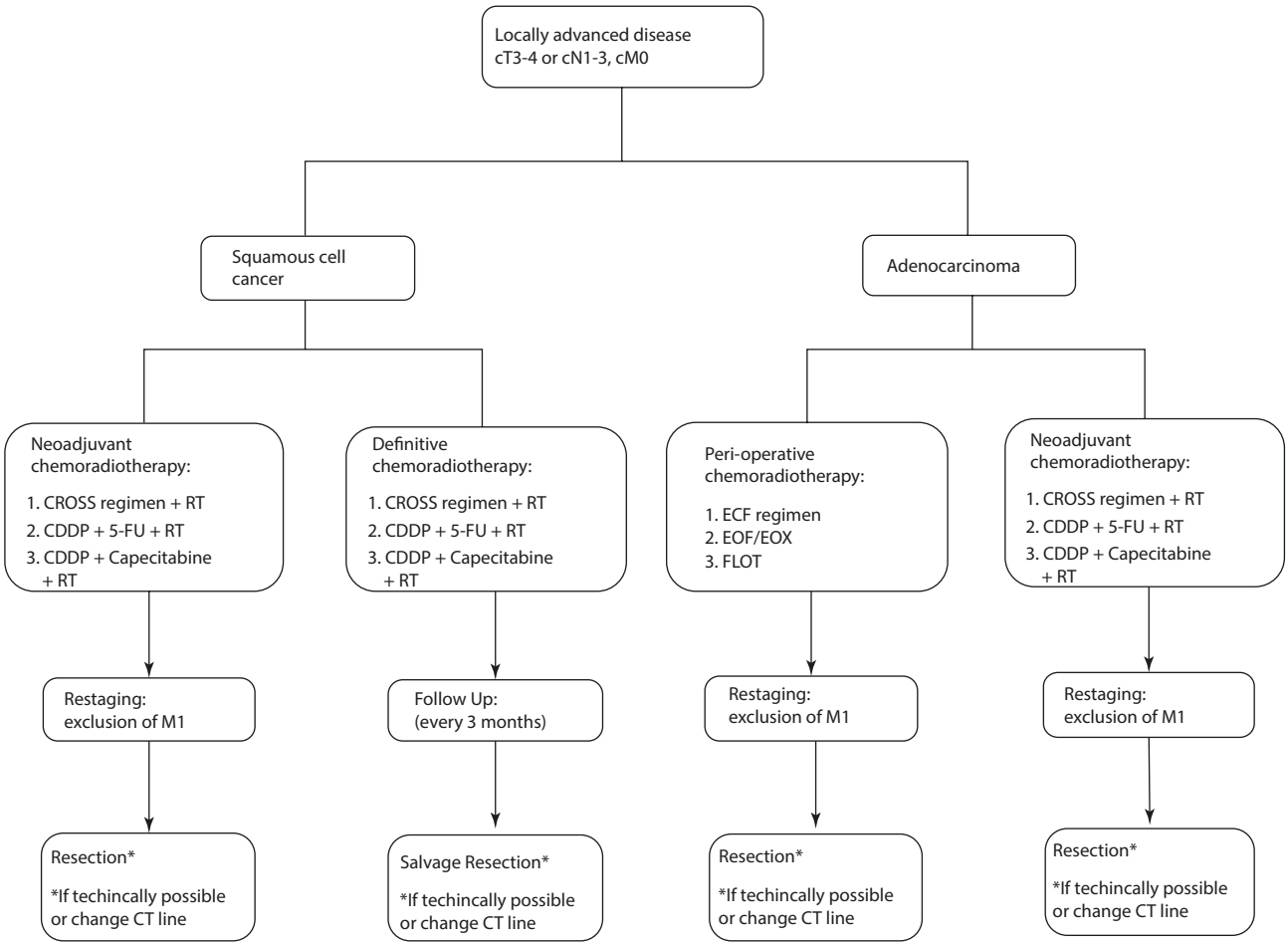

Fig. 34.7 Therapeutic algorithm for locally advanced disease

schedule for these patients [45]. However, also a cisplatin plus 5-fluorouracil schedule can be alternatively adopted. On the other hand, the comparison between definitive CRT without surgery and neoadjuvant CRT followed by surgery showed equivalent OS outcome [46, 47]. Therefore, both neoadjuvant chemoradiotherapy followed by surgery or definitive chemoradiotherapy with close surveillance and salvage surgery in case of local tumor persistence or progression can be considered for locally advanced SCC of the esophagus, except for cervical tumors in which a definitive approach is preferred. In case of response after neoadjuvant treatment, a follow-up program could be evaluated, reserved surgery at the relapse of disease.

Regarding the ADC, the standard treatment is represented by a perioperative chemotherapy with platinum- and fluoropyrimidine-based schedule or preoperative chemoradiotherapy (41.4–50.5 Gy). Moreover, in case of ADC of the gastroesophageal junction, the strongest evidences suggest the use of a perioperative approach [48, 49, 50], while chemoradiotherapy did not show a clear OS benefit if compared to chemotherapy alone [51, 52, 53]. The perioperative chemotherapy consists of a preoperative treatment period of 3–4 cycles, followed by surgery and by 3–4 postoperative cycles. The preoperative period is well tolerated, whereas only few patients are able to complete the postoperative one with an higher rate of toxicity. The most common schedules used in this setting are ECX/ECF (epirubicin, cisplatin, capecitabine, or fluorouracil) and FLOT (fluorouracil, oxaliplatin, docetaxel) for perioperative chemotherapy or cisplatin/5-FU combined with 41.4–50.4 Gy oxaliplatin/5-FU or carboplatin/paclitaxel with radiotherapy for neoadjuvant concomitant approach. Pathological response represents the principal prognostic factor after neoadjuvant treatment also for ADC. Unlike the SCC, even after complete tumor response to treatment, in ADC surgery should be done anyway.

34.11.3 Metastatic Disease

34.11.3.1 Chemotherapy

More than 50% of patients show a metastatic disease at the diagnosis. The main goals in this setting are palliation of symptoms, improvement of quality of life, and survival. Polychemotherapy is the standard first-line treatment for patients with a good performance status, while best supportive care alone is recommended in case of poor conditions. Doublet combinations of platinum (cisplatin or oxaliplatin) and fluoropyrimidines (5-fluorouracil or capecitabine) or taxanes (paclitaxel) showed a benefit in response rate if compared to monochemotherapy and are generally used in SCC. In addition to these schedules, triplet combination based on platinum, fluorouracil, taxanes, or epirubicin could be used for ADC of the esophagus or gastroesophageal junction (GEJA) like in gastric cancer. In fact, the same algorithm for gastric cancer should be applied in case of GEJA, and the determination of Her-2 status is mandatory.

Approximately 40% of patients with metastatic esophageal cancer received a second-line therapy after failure of first line. Second-line treatment is recommended in patients with a progression of disease after a first-line treatment who conserved a good performance status. In fact, an active treatment, if possible, is associated with improvement in OS and quality of life compared with best supportive care. On the other hand, in case of worsening condition, the patient is candidate to best supportive care alone.

Among different chemotherapeutic agents and schedules investigated in this setting, taxanes and irinotecan [54], alone or in association with fluorouracil (FOLFIRI), showed a survival benefit with a good toxicity profile. In case of GEJA, the same algorithm for gastric cancer should be applied (see the description in metastatic gastric cancer chapter).

There is no clear evidence for a benefit beyond the second-line treatment, but a third line with active chemotherapy may be considered in patients with a good performance status who progressed after a second-line therapy. Generally, the choice of chemotherapy schedule beyond the second line is according to previous treatments and patient's preference and performance status.

34.11.3.2 Target and Immune Therapies

Strong data with biologically targeted therapies are limited in esophageal carcinoma. Trials in this field investigated drugs against epithelial growth factor receptors (EGFRs), vascular endothelial growth factor (VEGF) pathway, and programmed cell death 1 (PD-1).

EGFR is overexpressed in 50–70% SCC and correlates with a worse prognosis in this tumor. Cetuximab, a monoclonal chimeric antibody directed against EGFR, showed to improve the response rate and PFS if used in second-line treatment for esophageal cancers, alone or in association with chemotherapy [55]. The role of cetuximab in addition to chemoradiotherapy is still under debate.

The monoclonal anti-EGFR panitumumab did not show a benefit in addition to chemotherapy in first-line setting in a phase III international trial [56]. Moreover, other studies with different EGFR inhibitors, such as erlotinib or gefitinib, did not show survival benefit [57].

Her-2 is overexpressed in 24–32% of esophageal ADC or GEJA. For treating patients with ADC, the same algorithm of gastric cancer should be applied, and the determination of Her-2 status is mandatory. In the first-line treatment of Her-2-positive gastric cancer, the phase III ToGA trial demonstrated clinically and statistically significant improvements in response rate, progression-free survival (PFS), and OS with the addition of trastuzumab to cisplatin-fluoropyrimidine doublet [58] (median OS: 13.8 versus 11.1 months), especially in the subgroup with high expression of the protein (Her-2 3+ at IHC or 2+ IHC with FISH amplification).

Trastuzumab is a humanized antibody directed against the extracellular domain of Her-2 receptor that showed in preclinical models a selected inhibition of cancer cell growth that express the receptor on their surface. Based on the ToGA results, trastuzumab was approved in many countries in addition to cisplatin-fluoropyrimidine doublet as first-line standard of care in patients with Her-2-positive disease. Trastuzumab is currently used at the same dose for Her-2-positive breast cancer (8 mg/kg in the first induction dose and then 6 mg/kg every 21 days), even if today it is clear that Her-2-positive gastric cancer is biologically different from the breast one. However, the addition of trastuzumab with different schedule to chemotherapy did not show any benefit in patients with Her-2-positive metastatic gastric cancer and performance status 2 [59]. Moreover, trastuzumab is actually investigated in adjuvant and neoadjuvant setting for Her-2-positive gastric cancer.

Despite low evidence about the use of trastuzumab in esophageal cancer, a small phase I/II trial showed that the addition of trastuzumab to cisplatin, paclitaxel, and radiotherapy is feasible in locally advanced Her-2-positive tumors [60].

Regarding target therapies against VEGF pathway, no antiangiogenetic agents are currently used in metastatic or perioperative treatment [61] for esophageal and junctional cancer (see the chapter about metastatic gastric cancer) alone or in combination with chemo- or chemoradiotherapy.

Of particular relevance, over the last few years, we have seen in oncology a big explosion of immune-oriented therapies (mainly with immune checkpoint inhibitors) that completely changed the natural history of many awful malignancies, like melanoma, lung cancer, kidney cancer, urothelial cancer, and many others. Researchers mainly focused on immunological checkpoints like programmed cell death 1 (PD-1) and its ligands (PD-L1 and PD-L2) as well as CTLA-4 pathway. More in detail, PD-1 molecule is highly expressed on T-lymphocytes, and it acts as a co-inhibitory receptor, leading to a strong suppression of immunological T-cell-mediated response in tumor microenvironment, following the engagement with its ligands, PD-L1/PD-L2, which are mainly expressed on tumor cell surface.

Preliminary data from early phase clinical trials suggested that the use of immunotherapy could improve survival also in patients with esophageal cancer. Indeed, today we have positive results about two anti-PD1 drugs, nivolumab and pembrolizumab, emerging from two pivotal phase III clinical trials, one already published [62] and the other one only presented at 2019 ASCO Meeting [63]. The Attraction-3 study [62] randomized 419 advanced SCC PD-L1 unselected patients, already refractory to one previous platinum−/fluoropyrimidine-based chemotherapy, between the anti-PD1 nivolumab and investigator's choice chemotherapy (paclitaxel or docetaxel). Overall survival (primary endpoint) was consistently improved in experimental arm versus chemotherapy group (10.9 versus 8.4 months, HR: 0.77, $p = 0.019$), with a strong reduction of grade 3–4 adverse event (18% in nivolumab arm versus 63% in chemotherapy arm). Based on these results, nivolumab could really represent a new possible standard treatment for second-line treatment of SCC patients, although this drug is not yet registered in the EU with this indication.

On the other side, second-line Keynote-181 trial [63] analyzed also advanced patients with adenocarcinoma histology (in addition to SCC). This study enrolled 628 advanced esophageal adenocarcinoma (plus Siewert I GEJ adenocarcinoma) and SCC patients, already refractory to a previous line of therapy, who were randomized between the anti-PD1 pembrolizumab and investigator's choice chemotherapy (paclitaxel, docetaxel, irinotecan). Pembrolizumab, different from nivolumab in the Attraction-3 study, did not improve OS or PFS in the overall intention-to-treat (ITT) population, but the authors clearly showed a significant benefit obtained with pembrolizumab in PD-L1-positive patients with a CPS (combined positive score) > 10%. In fact, while in the overall ITT population mOS was 7.1 months in both treatment arms, in PD-L1 CPS-positive patients a mOS of 9.3 months with pembrolizumab versus 6.7 months with conventional chemotherapy was observed. This trial strongly highlighted the necessity of PD-L1 CPS testing for metastatic patients with both adenocarcinoma and SCC refractory to a previous chemotherapy line, because for these subjects – especially for adenocarcinoma patients (not included in Attraction-3) – pembrolizumab could really make the difference, considering also the very good safety profile when compared with chemotherapy. However, to date, pembrolizumab is not registered by regulatory agencies in the EU for this indication, and we still wait for the full paper publication.

34.11.4 Supportive and Palliative Care

A multidisciplinary evaluation is important in every step of natural history of esophageal cancers due to the particular worsening of condition that these diseases could produce. In fact, a nutritional support should be evaluated after all lines of treatment as well as the palliation of dysphagia or pain. The correct choice of nutritional support (enteral, parenteral, etc.) should involve a specialist in nutrition supportive care [64].

Patients can be considered for different options of palliative treatment depending on the clinical situation. Single-dose brachytherapy may be a preferred option to treat dysphagia even after external radiotherapy with fewer complications than metal stent placement. Other possible options in case of dysphagia are the local expansion, the position of prosthesis, and the laser therapy. The local expansion is frequently used to prepare to a prosthesis placement. The use of prosthesis leads to a rapid resolution of dysphagia, but is not indicated in case of cervical or junctional tumors or in case of trachea involvement. Laser therapy is used also to obtain hemostasis in addition to treatment of dysphagia.

34.11.5 Follow-Up

There is no standardized follow-up program for esophageal cancers, because there is no evidence that regular follow-up has an impact on survival outcomes. However, follow-up should concentrate on symptoms, nutrition, and psychosocial support also by multidisciplinary evaluations. Imaging should be obtained only if clinically indicated. This program might be done every 3 months for 2 years and every 6 months for the next 3 years. In the case of complete response to definite chemoradiotherapy or after resection, a 3-month follow-up based on symptoms, endoscopy, biopsies, and CT scan may be recommended to detect early recurrence.

Case Study: An Unusual Histotype

Man, 62 years old

- *Family history*: negative for malignancy
- *APR*: no comorbidities
- *APP*: for nearly 2 months dysphagia
- *Objective examination*: negative. Performance status 0 according to ECOG
- *Blood tests*: Hb 12.1 g/dL.
- *Esophagogastroduodenoscopy*: presence of ulcerative area at the cardia (Siewert II)
- *Pathological report*: squamous cell carcinoma
- *TC chest and abdomen mdc*: lesion at the cardia with multiple perigastric lymphadenopathies. No distant metastasis
- *18-PDG-PET*: uptake at the cardia (SUV max 6.5) and perilesional lymph nodes (SUV max 7.4)

- *Diagnosis*: locally advanced SCC

Question

What action should be taken?

(1) Surgery. (2) Neoadjuvant chemotherapy. (3) Neoadjuvant chemoradiotherapy

Answer

Neoadjuvant chemoradiotherapy

The patient received treatment with carboplatin AUC 2+ weekly paclitaxel (VII courses) integrated with 45 Gy radiotherapy in 25 fractions of 1.8 Gy.

Question

After neoadjuvant treatment, what action should be taken?

(1) Surgery. (2) Restaging

Answer

Restaging

- *Esophagogastroduodenoscopy*: partial response to treatment with decrease of lesion diameter
- *TC chest and abdomen mdc*: response to treatment at the level of primary lesion. Nodular lesion at VI segment of the liver (metastatic lesion)
- *18-PDG-PET*: decrease in the uptake at the cardia (SUV max 3) and perilesional lymph nodes (SUV max 4.5). Uptake at the level of VI segment of the liver (SUV 9.4) and some bone districts (8.1, right scapula, left ribs, and right femur)
- *TC with bone window*: confirmation of the metastatic bone lesions showed at 18-FDG-PET.

- *Clinical evaluation*: arise of bone pain in the sites of metastasis.
- *Diagnosis*: progression of disease despite the partial response on the primary tumors

Question

What action should be taken?

(1) First-line chemotherapy. (2) Definition of Her-2 status. (3) Surgery

Answer

- *Revision of histotype and the definition of Her-2 status were performed.*

- Squamous cell carcinoma was confirmed. Her-2 status (IHC): 0. Microsatellite stable (MSS) and PD-L1 negative.
- *First-line chemotherapy with FOLFOX schedule after multidisciplinary evaluation*: ongoing.
- *Treatment with inhibitors of RANKL (denosumab) was evaluated.*
- *Palliative bone radiotherapy was evaluated to treat pain.*

Key Points

- The importance of a correct diagnosis even in the case of unusual histotype
- The importance of a correct choose of treatment based on the histotype
- Importance of re-staging after neoadjuvant treatment and multidisciplinary evaluation.

Case Study: A 35-Year-Old Woman with a Metastatic Esophageal Cancer

Woman, 32 years old

- *Family history:* negative for malignancy
- *APR*: negative
- *APP*: weight loss of 10 kg in the last 3 months, fatigue
- *Blood tests*: Hb 9.4 g/dL
- *Clinical evaluation*: dysphagia, weight loss, and lumbar pain
- *Esophagogastroduodenoscopy*: presence of lesion at the level of thoracic esophagus
- *Pathological report*: carcinoma with squamous aspects

Question

What action should be taken?

(1) Surgery. (2) Neoadjuvant treatment. (3) Staging

Answer

Staging

- *TC chest and abdomen mdc*: involvement of esophageal wall in the thorax part, perilesional lymph nodes. No liver or lung lesions. Osteolytic lesion at L3-L4 level, suggesting metastasis
- *Scintigraphy*: uptake at levels of L3–L4. Metastatic disease
- *Bronchoscopy*: no lesions or fistulas

Question

What action should be taken?

(1) First-line chemotherapy. (2) Multidisciplinary approach. (3) Neoadjuvant treatment

Answer

Multidisciplinary approach

- *First-line chemotherapy with FOLFOX*, ongoing
- *Palliative radiotherapy on L3–L4 to treat pain*
- *Treatment with inhibitors of RANKL*, ongoing

Key Points

- A correct and complete staging is mandatory before starting treatment.
- Importance of multidisciplinary approach.
- Importance of treatment according to primary site and histotype.

34

Summary of Clinical Recommendations and Key Points

- *AIOM*
  - All patients with new dysphagia, gastrointestinal bleeding or emesis, weight loss, and/or loss of appetite should undergo an upper intestinal endoscopy.
  - Endoscopy with ultrasonography (EUS) should be done in all patients candidate to neoadjuvant treatment.
  - 18-FDG-PET could be used to assess the response to neoadjuvant treatment in addition to CT scan and EUS, but nowadays it does not represent a standard.
- *ESMO*
  - Surgery is the treatment of choice in limited disease. In patients with T1a AC, endoscopic therapy is the preferred therapeutic approach.
  - Neoadjuvant CRT with planned surgery or definitive CRT with close surveillance and salvage surgery for local tumor persistence or progression can be considered as a recommended definitive treatment for locally advanced SCC of the esophagus. Definitive CRT is recommended for cervically localized tumors.
  - For patients with esophageal AC, perioperative chemotherapy should be considered standard in locally advanced AC of the esophagus, including esophagogastric junctional cancers.
- *NCCN*
  - All patients with R1 or R2 resection may be treated with fluorouracil-based chemoradiotherapy.
  - Chemotherapy with supportive care represents the standard treatment for metastatic patients with good PS. On the other hand, in case of poor PS, only best supportive care should be considered.
  - Trastuzumab should be used in case of Her-2-positive ADC. Other target therapies are not currently used.

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Gastric Cancer: Locoregional Disease

General Section. Locoregional Disease

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Gastrointestinal Cancers

Contents

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Learning Objectives

By the end of the chapter the reader will

- be able to apply diagnostic and therapeutic procedures
- have learned the basic concepts of gastric cancer
- have reached in depth knowledge of gastric cancer
- be able to put acquired knowledge into clinical practice.

35.1 Introduction

Gastric cancer (GC) is the fifth most common malignancy and the third most common cause of cancer-related death in both sexes with an incidence of about one million new cases worldwide [1].

Steady decline in incidence and mortality rates has been observed in most parts of the world [2], particularly limited to young patients affected by distal, sporadic, and intestinal type of GC [3]. Over the last few decades, while the incidence rate for cancers of cardia and gastroesophageal junction (GEJ) has been on a rapid upsurge and the incidence of distal GC has fallen in Western countries, the reverse occurred in Asian countries [4, 5].

By far, the large majority (about 90%) of all GCs belong to the group of adenocarcinomas, histologically classified into two major types (intestinal-type and diffuse-type) with distinct morphologic appearance, pathogenesis, and genetic profiles [6]. While diffuse-type GCs are more often determined by genetic abnormalities. Few cases (about <10%) of GCs are associated with inherited predisposition syndromes. Most of intestinal-type GCs are sporadic, mainly triggered by long-standing inflammatory conditions that result in a sequential progression from normal gastric mucosa through chronic gastritis, chronic atrophic gastritis, and intestinal metaplasia to dysplasia and carcinoma [7].

Prognosis remains dismal except in a few countries since this multifactorial tumor, with both environmental and genetic causative factors, often presents at an advanced stage of disease [8, 9] (Fig. 35.1). Despite showing similar clinical-pathological characteristics and treatments, survival rates vary from 10% to 30% in European countries [10], whereas in Asian countries much higher 5-year overall survival rates have been achieved by screening endoscopic examinations and consecutive early tumor resection [11].

Moreover, notwithstanding different epidemiology and to a certain extent different histologic features, the clinical management of GC does not take differences into account with the only potentially curative treatment approach represented by surgical resection with adequate lymphadenectomy. Current evidence seems to support perioperative therapies to improve survival in patients affected by locally advanced disease. Finally, considering that unresectable or metastatic GC could solely and regrettably benefit only from life-prolonging palliative therapy regimens; prevention and early diagnosis are the most promising strategies for GC control.

35.2 Epidemiology

Anatomically, the stomach begins at the gastroesophageal junction or GEJ (so-called Z-line, a poorly defined anatomic area separating the lower esophagus from the

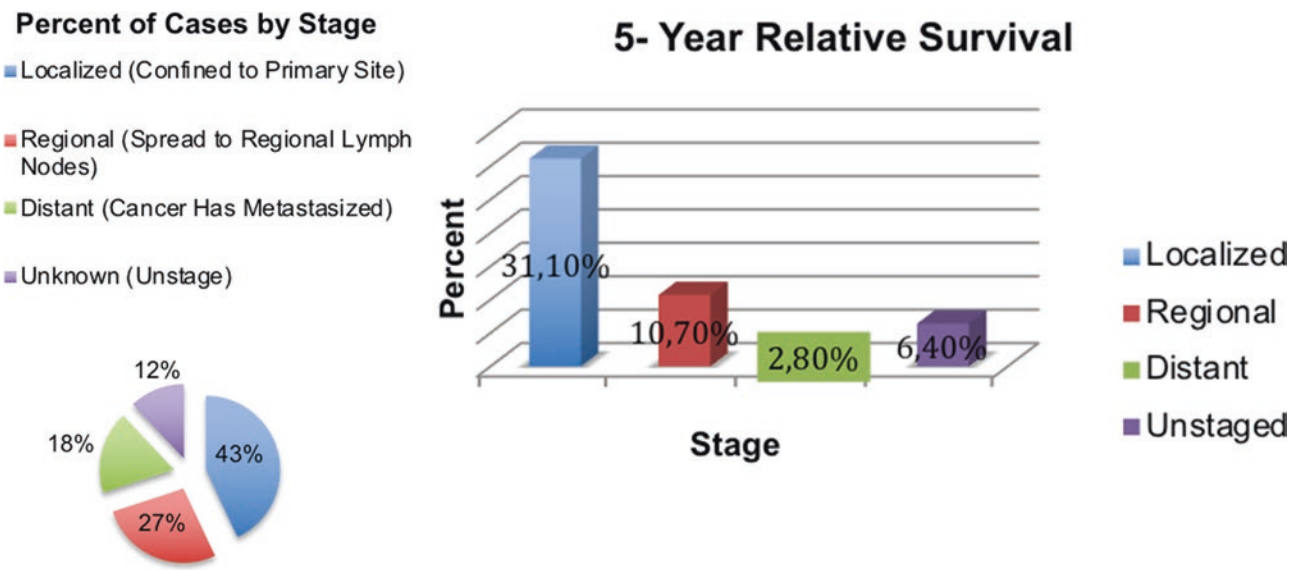


Fig. 35.1 Worldwide percentage of GC cases and 5-year survival rate by stage at diagnosis

cardia or proximal part of the stomach) and ends at the pylorus (the distal part of the stomach that connects to the duodenum).

The incidence and mortality rates for GC world wide have considerably changed over the past years, showing wide geographical variation (Fig. 35.2).

- GC used to represent the most common neoplasm and the leading cause of cancer-related death through most of the twentieth century, now ranking third to lung and liver cancer [1] and showing an overall relative 5-year survival rate of approximately 30% in most parts of the world [12].
- More than 70% of cases chiefly occur in developing countries and half the world total appears to arise in Eastern Asia (mainly in China).
- Age-standardized incidence rates are about twice as high in men as in women [1].
- The highest estimated mortality rates are present in both sexes in Eastern Asia, Central and Eastern Europe, and in Central and South America, with lower rates in Western Europe and in Northern America [1] (Fig. 35.3)
- In Japan, a mass screening program has been implemented since the 1960s, and early detection of

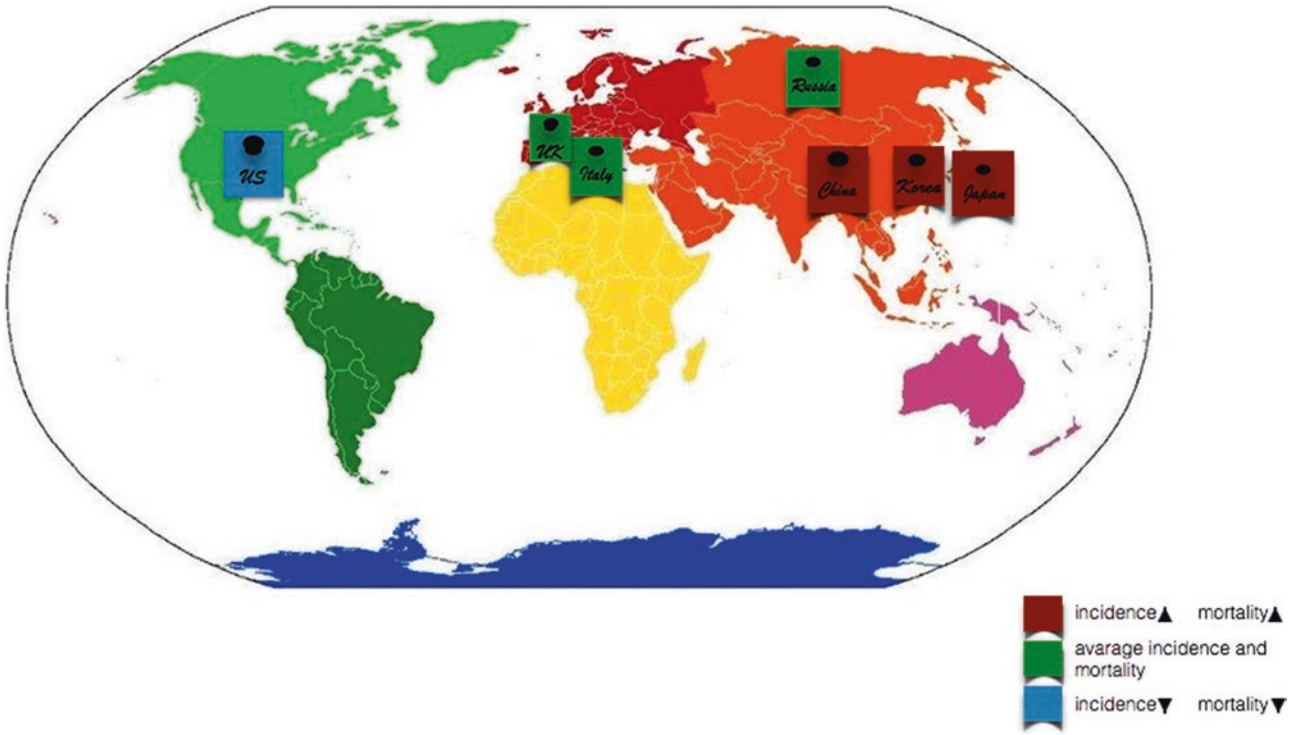
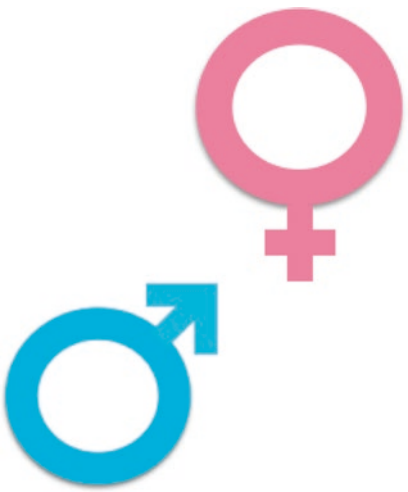


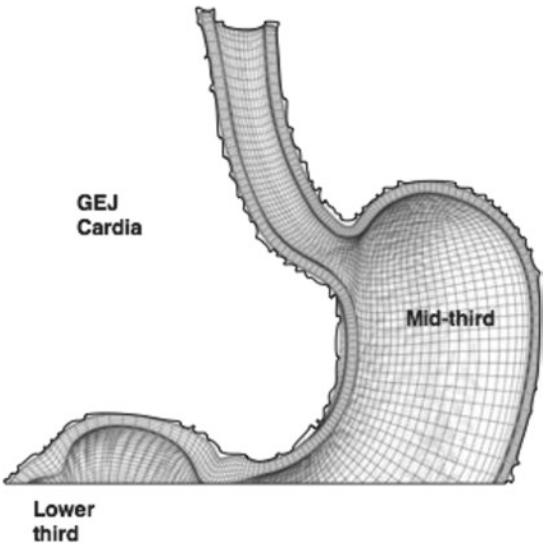
Fig. 35.2 Incidence rates and mortality for GC according to both sexes worldwide

Fig. 35.3 Cancer-related deaths according to both sexes in the European Union

LUNG	26%
PROSTATE	10%
CRC	12%
STOMACH	5,5%
PANCREAS	5%
LEUKEMIA	3%
LIVER	3%
BLADDER	4%
KIDNEY	2%
NONH. LIMPHOMA	3%
OTHER SITES	29%



LUNG	13%
BREAST	17%
CRC	13%
PANCREAS	7%
OVARY	5%
STOMACH	5%
NONH LIMPHOMA	3%
UTERINE BODY	2%
MELANOMA	1%
OTHER SITES	35%



Subtypes	Risk factor	Incidence
GEJ, Cardia:	reflux, tobacco, fat diet	↑ Western countries
Non-cardia intestinal-type:	HP	↑ Asia, Russia and part of EU ↓ Western countries
Non-cardia diffuse-type:	loss of *CDH1*	↓ Western countries

Fig. 35.4 The heterogeneity of GC epidemiology

the disease combined with improved operative techniques has led to a significant decrease in mortality.

- In Western countries, the incidence of distal GC has steadily declined potentially because of changes in diet, improved food preparation, anti-*Helicobacter pylori* therapies and earlier diagnosis of smaller lesions; on the other hand, the incidence rates of proximal GC has strikingly increased since cardia lesions are not associated with *H. pylori* infection [13] (Fig. 35.4).
- *H. pylori* accounts for at least 300,000 new cases of GC each year worldwide. Nonetheless, epidemiological studies report that only 2–3% of *H. pylori*-infected individuals eventually develop GC [14].
- Non cardia lesions seem to be more common in male, blacks, lower socioeconomic status and developing countries [15].
- Diffuse-type GC is more often seen in female and young individuals [16], while the intestinal-type adenocarcinoma is more often associated with intestinal metaplasia and Helicobacter pylori infection [17] (Fig. 35.4).
- Death rate estimates for the year 2019 from gastric cancer show the most favorable declines, with a 17.1% decrease in men and a 13.7% in women since 2014 [18].

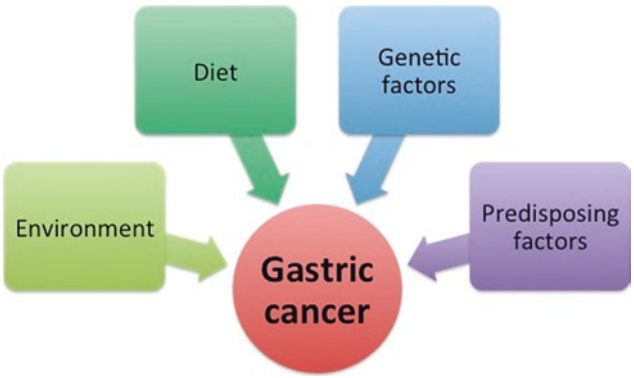


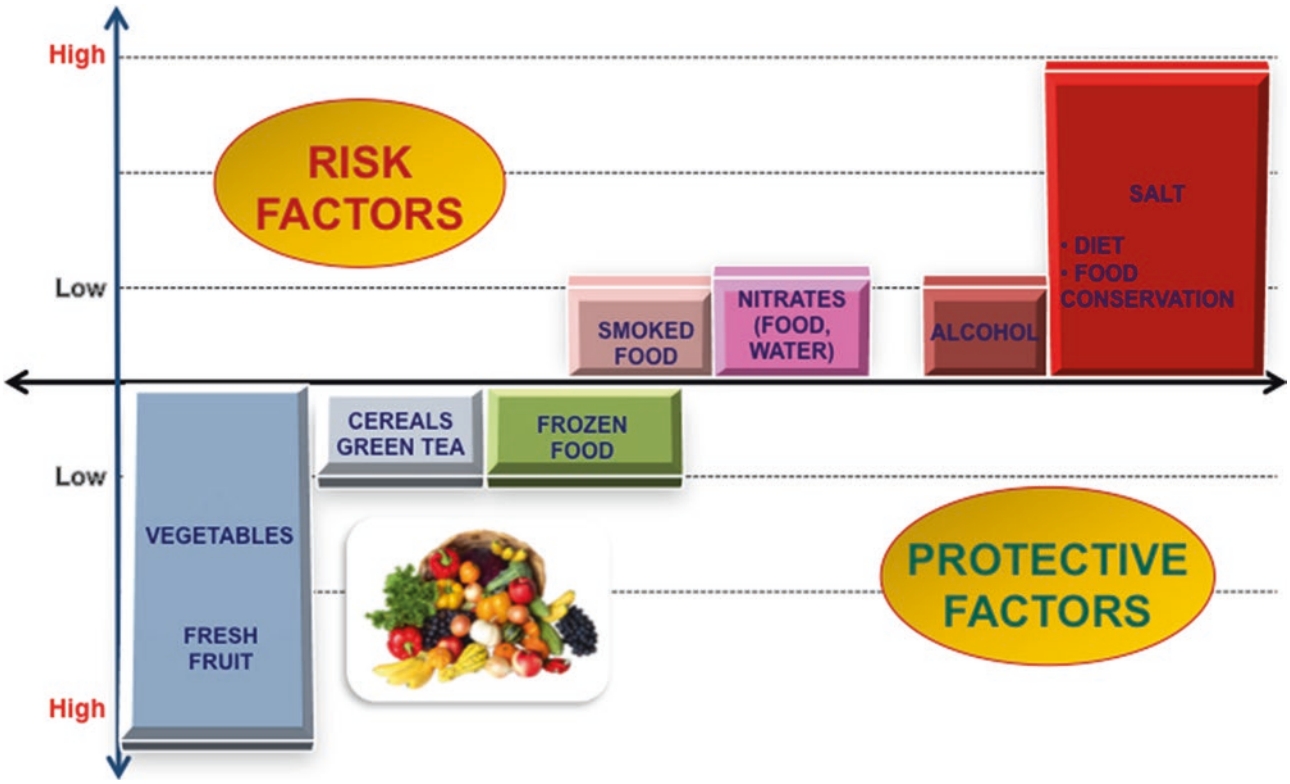
Fig. 35.5 Multifactorial etiology in GC

35.3 Etiology and Prevention

The development of GC is a multifactor process associated with a large number of risk factors (Fig. 35.5).

- Age was shown to be positively associated with a risk of gastric cancer (cardiac and non-cardiac) [19].
- Environmental exposure in early life together with the accumulation of specific genetic alterations and other cultural factors are essential in determining the risk and the predisposition to GC [20]. Several studies showed how immigrants gradually acquire the incidence rates of the country to which they move [21].

- Excessive intake of salt or salty food, low consumption of fresh fruits and vegetables likely contribute to the development of gastric cancer, while high intake of fresh fruits and vegetables, Mediterranean diet, a low-sodium diet, salt-preserved food, red and high cured meat, moderate alcohol drinking, and maintaining a proper body mass index might be significantly associated with a decreased risk of GC [22–24]. Low gastric acidity may additionally increase intraluminal formation of N-nitroso compounds (from preserved food or endogenous nitrates), which are recognized as mutagens and carcinogens [25] (◘ Fig. 35.6).
- Several studies have confirmed that tobacco smoking increases the risk of GC, both cardia and non-cardia subtypes, particularly in male [26, 27].
- Disparate data regarding occupational exposures suggested that coal and tin mining, metal processing (particularly steel and iron), and rubber manufacturing industries may lead to an increased risk of gastric cancer.
- *H. pylori* is a Gram-negative, flagellated, microaerophilic bacterium considered as a major predisposing factor for GC, increasing from three- to sixfold the risk for development of gastric carcinoma [28].
- Epstein-Barr virus (EBV) is a ubiquitous human herpes virus with oncogenic activity, which has been associated with about 10% of all gastric carcinoma cases. These EBV+ tumors are characterized by a diffuse-type histology with lymphoid infiltration (lymphoepithelioma-like carcinoma) and predominantly located in the non-antrum part of the stomach as superficial depressed (or ulcerated) lesions [29].
- Other predisposing factors, such as atrophic gastritis, gastric ulcer disease (sometimes related to *H. pylori* infection), partial gastrectomy, and Ménétrier's disease were additionally reported to increase the risk of GC [30]. In addition, adenomatous polyps of the fundic glands are considered dysplastic and most consistently associated with a cancerous transformation, mainly in the intestinal-type phenotype and in patients with familial adenomatous polyposis.
- Familiar studies have found that the risk of developing GC for relatives of cases is increased two- to threefold, suggesting a role of genetic factors [31,



◘ **Fig. 35.6** Role of lifestyle and dietary habits in determining the risk of GC

32]. Nevertheless, an inherited genetic predisposition is found in a small proportion of cases with relevant syndromes, including hereditary non-polyposis colorectal cancer, familial adenomatous polyposis colorectal cancer, hereditary diffuse gastric cancer (HDGC), gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) and Peutz Jegher's syndrome.

Prevention is a key strategy to reduce GC-related mortality. The eradication of *H. pylori* infection and a healthy lifestyle appear to be the major primary preventive interventions, whereas mass screening programs (including also serum pepsinogen evaluation and endoscopic screening) have been strikingly successful in high-risk areas, especially in Japan [33]. Moreover, serological diagnostic tests are currently available, not only informing us of the presence of *H. Pylori* but also of the microenvironment of the gastric mucosa and its possible variations: normal mucosa, gastroesophageal reflux, *H. Pylori* infection, and gastric atrophy [34].

35.4 Carcinogenesis

GC is generally viewed as the consequence of a multifactorial and multistep process, involving the host responses, bacterial virulence, diet, environmental, and other predisposing factors.

According to the general hypothesis of sporadic carcinogenesis of intestinal-type GC proposed by Correa [7], the transition from normal mucosa to non-atrophic gastritis (triggered primarily by *H. pylori* infection but also by dietary factors, chemical agents, or autoimmune diseases), initiates precancerous lesions which may then progress to atrophic gastritis and intestinal metaplasia (Fig. 35.7). Specifically, the basic components of the process are: chronic active non-atrophic gastritis, multifocal atrophy, intestinal metaplasia (first complete, then incomplete), dysplasia or advanced precancerous lesions (APLs; currently distinguished into low-grade [LG-IEN] and high-grade intraepithelial neoplasia [HG-IEN]), invasive carcinoma [35, 36].

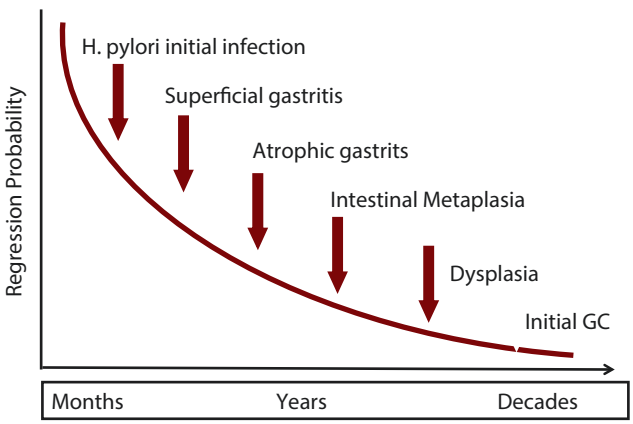


Fig. 35.7 Histologic progression of human *H. pylori* infection from the early stages of inflammation to invasive GC

H. pylori is not found in normal stomachs, but very frequently found in patients affected by chronic gastritis, even if only a minor proportion of individuals progress through the pre-neoplastic cascade [37]. *H. pylori* infection seems to play a causative role at the early phases of carcinogenesis in the intestinal-type of GC, as suggested by the significant regression of atrophic gastritis and initial precancerous lesions after antibiotic-mediated eradication of H. pylori [38]; however, no longer effective once the disease has progressed to the stage of intestinal metaplasia [39].

In the sporadic setting, the first stage of the neoplastic cascade consists of an active chronic inflammatory response to injury [40] (Fig. 35.8). *H. pylori* infection, through the action of a variety of bacterial virulence factors (such as urease, vacuolating cytotoxin A [VacA], cag pathogenicity island, cytotoxin-associated gene A [CagA], bacterial gamma-glutamyl transpeptidase [GGT]) [41, 42] and higher level of production of free radicals, recruits inflammatory cells to the host gastric mucosa, promotes gastric cell apoptosis, and reduces epithelial cell turnover, thus resulting in superficial gastritis without atrophy and gastric atrophy (defined as loss of appropriate glands, such as mucosecreting and oxyntic glands in the antrum and in the corpus of the stomach, respectively) in the majority of infected cells. Furthermore, it has been hypothesized that the initial stages of inflammation and atrophy create an abnormal microenvironment favoring engraftment of bone marrow-derived cells (BMDCs) harboring an eventual neoplastic invasive behavior [43]. Additionally, loss of glandular structures may be subsequently replaced with glands inappropriate to the location (intestinal metaplasia), reflecting a sort of an adaption to a chronic injury, but conferring a high risk for the development of dysplasia and invasive gastric cancer. So far, the distinction between dysplasia and invasive GC relies essentially in the fact that fully-developed cancer shows histological evidence of stromal invasion/infiltration by neoplastic cells into the *lamina propria*, while the different grades of dysplasia have been recently redefined as an intraepithelial/intraglandular neoplasia confined by the basal membrane of the dysplastic glands and without any apparent metastatic potency [36].

Moreover, GEJ cancers appear to arise from foci of incomplete intestinal metaplasia that may be additionally triggered by gastroesophageal reflux disease (GERD) either in the distal esophagus or in the proximal stomach. In the distal esophagus of GERD patients, the chronic reflux causes inflammation of the squamous

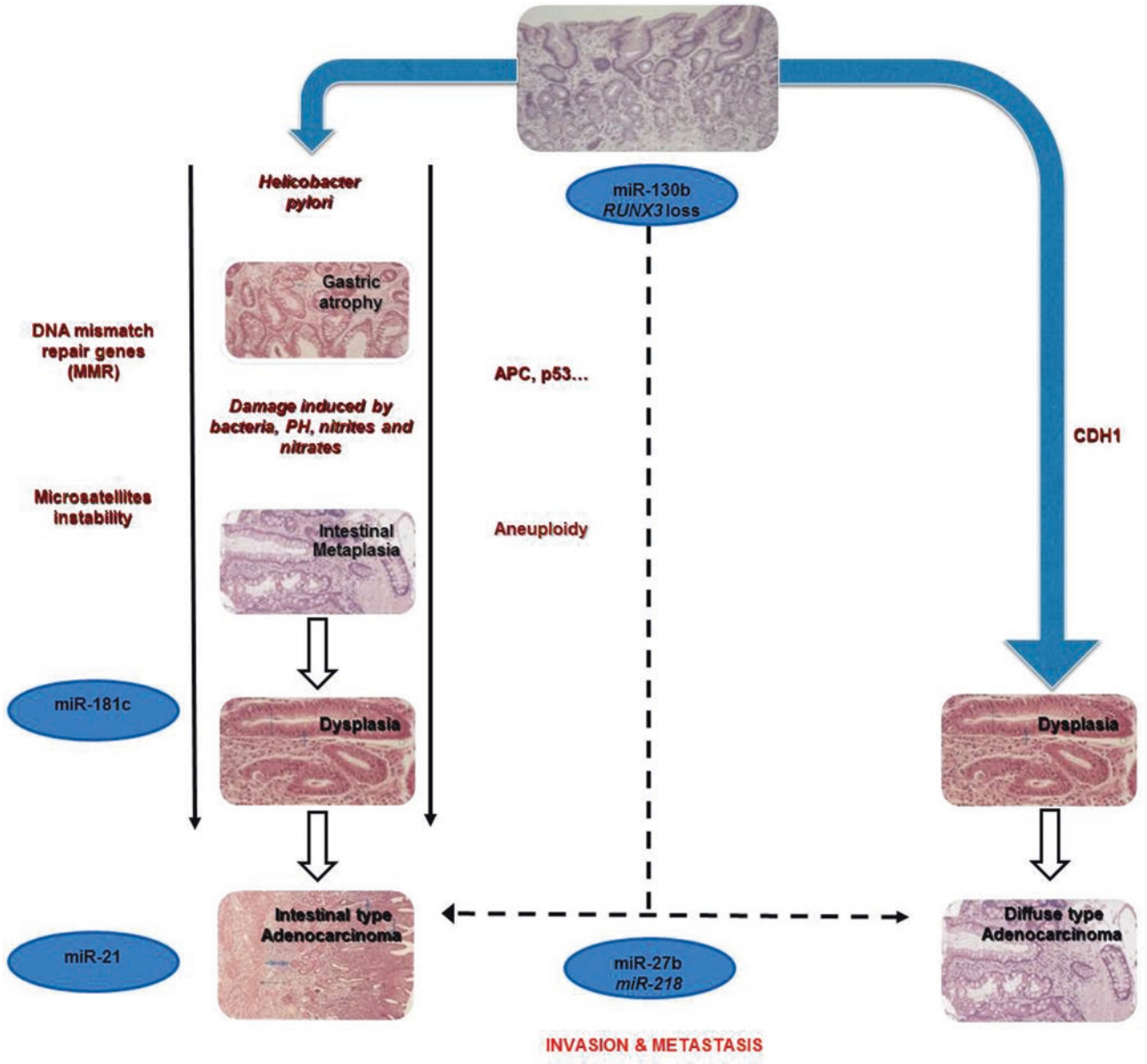


Fig. 35.8 The multi-step cascade of gastric carcinogenesis

epithelium, which is replaced by columnar epithelium as a result of metaplastic reaction, a condition known as Barrett's esophagus [44] that harbors a cumulative risk of progression to invasive cancer [45] (Fig. 35.9).

To a lesser extent, links of diffuse-type GC with atrophic gastritis or intestinal metaplasia are poor, or do not exist. Moreover, cell adhesion proteins (expressed at the adherence junctions of epithelial tissue and required for development, cell differentiation, and maintenance of epithelial architecture) seemed to act as suppressors of tumor invasion and metastasis. Indeed, a very small proportion of GCs (about 1–3%) can be caused by a specific germ-line mutation of the E-cadherin gene (*CDH1*) that harbors a 60–70% lifetime cumulative risk of advanced diffuse GC clinically resulting in a cancer syndrome, the hereditary diffuse gastric cancer (HDGC) [46] (Fig. 35.8).

The pathogenesis of GC involves multi-step genetic and epigenetic alterations, which predispose cells to neoplastic transformation. Nevertheless, molecular mechanisms underlying disease tumorigenesis and progression are still not completely understood. MicroRNAs (miRNAs), a class of small noncoding RNAs with 18–24 nucleotides, which can cause mRNA degradation or translational inhibition, seem to play a role in inflammation, cell proliferation, apoptosis regulation, and differentiation. Given the importance of miRNAs in the regulation of cell growth and viability, miRNA dysregu-

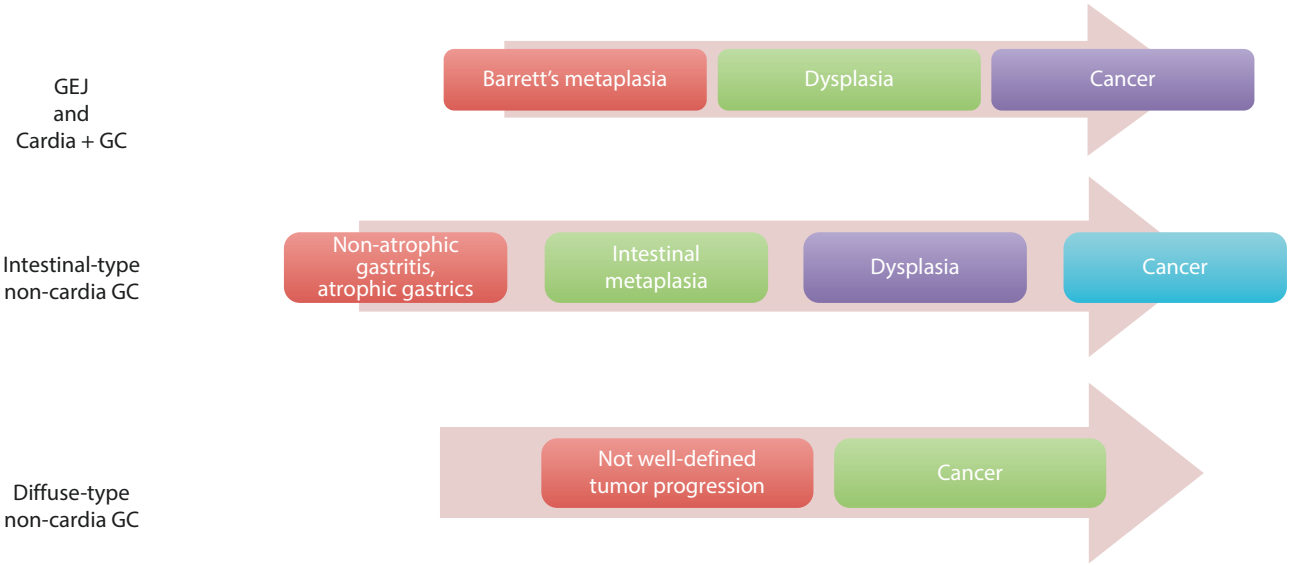


Fig. 35.9 Tumor progression models for GC

lation is believed to be closely correlated with the development and progression of gastric cancer [47]. Likewise most tumors, a growing body of evidence reveals how miRNAs influence the expression of tumor suppressor genes and oncogenes, possibly contributing to initiation and progression of GC (e.g., *miR-21* was found to be associated in the development of GC while *miR-130b* and *miR-301a* seemed to downregulate the expression of *RUNX3*, a known tumor suppressor silenced by promoter hypermethylation, leading to poor differentiated GC) [48] (Fig. 35.8)

35.5 Clinical Presentation

Because of the lack of specific symptoms that could characterize GC, most of patients are commonly diagnosed with advanced disease, presenting with a combination of signs and symptoms that are not unequivocally suggestive for GC. Nonetheless, alarm symptoms, such as dysphagia, weight loss, and palpable abdominal mass appeared to be independently associated with survival and mortality [49].

Weight loss and abdominal pain are the most common symptoms at initial presentation and should not be underestimated, since weight loss seemed to be significantly associated with shorter survival [50]. Loss of appetite, fatigue, epigastric discomfort, postprandial fullness, heart burn, indigestion, nausea, and vomiting are often related to spread of disease. Dyspeptic symptoms, such as gastroesophageal reflux, peptic ulcer disease, and functional dyspepsia can be related to GC only in a few cases.

Occasionally, symptoms may be suggestive for a lesion at a specific site in some patients. As a matter of fact, a history of dysphagia or pseudoachalasia may correlate with a cancer of the cardia extending through the GEJ. Early satiety, even if not so frequent in GC patients, could be indicative of a loss of distensibility of the gastric wall due to a diffusely infiltrative tumor, whereas later satiety and vomiting may indicate pyloric involvement. Although gastrointestinal bleeding is uncommon, hematemesis and anemia do occur in approximately 10–15% of patients.

GCs can spread by local extension to adjacent structures and can develop lymphatic, peritoneal, and distant metastases. Diffuse peritoneal spread leading to a large ovarian mass (Krukenberg's tumor) or a large peritoneal implant in the pelvis (Blumer's shelf) can produce symptoms of colorectal obstruction. Ascites, jaundice, and palpable mass are sadly related to incurable disease. Furthermore, metastatic nodules to subcutaneous tissue around the umbilicus (Sister Mary Joseph's node) or supraclavicular lymph node (Virchow's node) traditionally suggest the status of an advanced disease.

35.6 Histopathology Overview

The term *gastric cancer* usually refers to adenocarcinoma of the stomach since most of these tumors (approximately 95%) represent malignant epithelial neo-

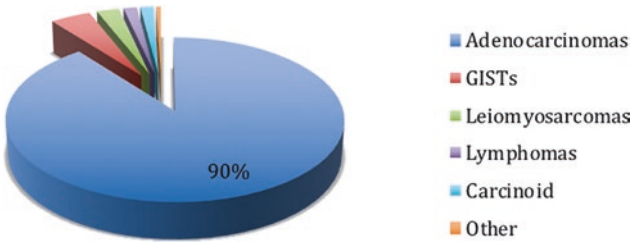


Fig. 35.10 Histologic subtypes of gastric cancer

plasms, originating from glandular epithelium of the gastric mucosa. Other malignant tumors are rare and include carcinoid tumors, leiomyosarcomas, and gastrointestinal stromal tumors. Interestingly, the stomach is the most common site for lymphomas of the gastrointestinal tract in spite of the absence of lymphoid tissue in the normal gastric mucosa (Fig. 35.10).

Several schemes have been proposed based on the morphologic (the Japanese and the Paris classification for EGCs, the Borrmann classification for advanced GC) and pathologic features (Lauren's and the World Health Organization classification) of gastric tumors. On the other hand, other classifications based on a cellular level (such as Goseki's and Ming's) have not been proven to be superior to the preexisting systems [51].

35.6.1 Macroscopic Aspects

Concerning early gastric cancer (EGC), the neoplasms are grossly classified into Type I for the tumor with protruding growth, Type II with superficial growth (further divided in elevated, flat, or depressed) and Type III with excavating growth, according to the Japanese Endoscopic Society [52] (Fig. 35.11). In addition, a more recent Paris classification, investigating also other superficial neoplastic lesions in the gastrointestinal tract, divided grossly and endoscopically the tumor as Type 0-I for polypoid growth (which is subcategorized to 0-Ip for pedunculated growth and 0-Is for sessile growth), Type 0-II for non-polypoid growth (which is subcategorized into Type 0-IIa for slightly elevated growth, Type 0-IIb for flat growth, and Type 0-IIc for slightly depressed growth) and Type 0-III for excavated growth [53].

Regarding more advanced tumors, the Borrmann classification divides GC into five types depending on macroscopic appearance and seems to be a valuable predictor for lymph node metastasis and survival [54]. Type I represents polypoid or fungating cancers (7–8%), Type II encompasses ulcerating lesions surrounded by elevated borders (30%), Type III represents ulcerated lesions infiltrating the gastric wall (30–40%), Type IV are diffusely infiltrating tumors (10–20%) and Type V are unclassifiable cancers.

35.6.2 Microscopic Aspects

Historically, the most widely used classification is by Laurén who first divided GC into either intestinal or diffuse form characterizing two varieties of tumors that distinctively present with different pathology, epidemiology, etiologies, and genetics [6] (Fig. 35.12); later, the indeterminate type was included to describe an uncommon histology. While the intestinal variety repre-

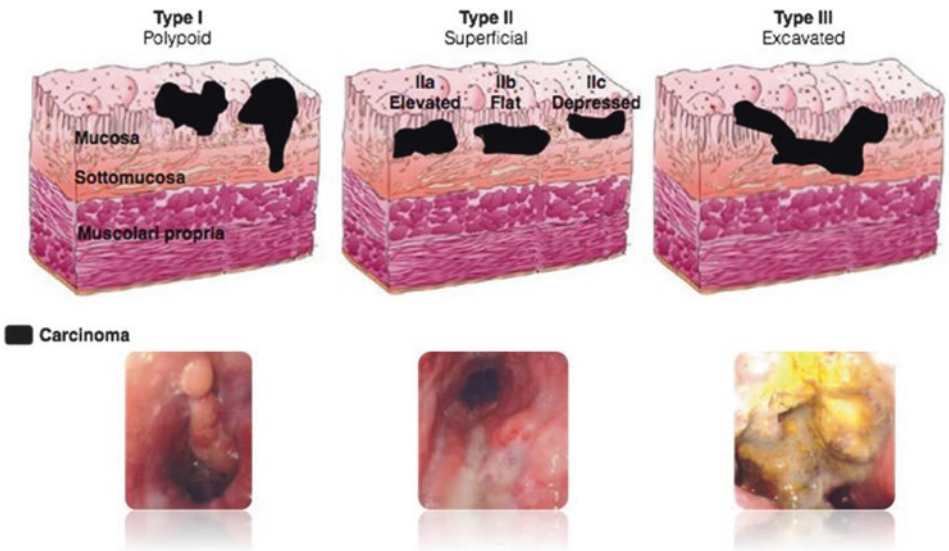


Fig. 35.11 Endoscopic classification of early gastric cancers. (Photos by courtesy of Prof. G. Genova, Surgical Oncology Unit-Department of Surgical, Oncological and Orals Sciences, University of Palermo)

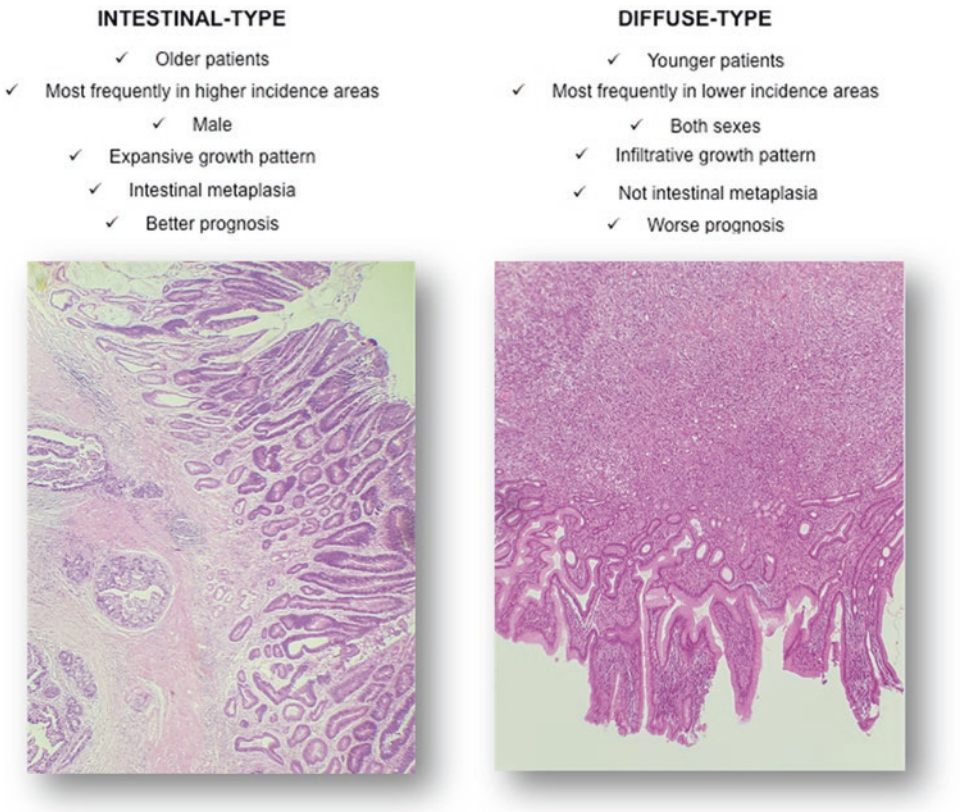


Fig. 35.12 The two histological subtypes of GC proposed by Lauren (1965). (Photos by courtesy of Prof. A. Martorana, Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, Pathologic Anatomy Unit-University of Palermo)

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sents a differentiated cancer with a tendency to form glands (similarly to colon type), the diffuse form exhibits very little cell cohesion with a predilection for extensive submucosal spread and early metastases.

Even if all other GC types of lower frequency have been included (uncommon and mixed histologic variants), four major types are currently recognized by the WHO classification: papillary, tubular, mucinous adenocarcinoma, and poorly cohesive carcinoma (with or without signet ring cells) [55].

35.7 Diagnosis and Staging

Careful clinical staging is critical to ensure that patients are appropriately selected for treatment interventions, as outlined in the most recent international guidelines. As described below, the clinical stage in the 8th edition of TNM staging is defined prior to treatment based on endoscopy imaging (Fig. 35.13a, b).

Endoscopic ultrasound (EUS) provides evidence of depth and extension of tumoral invasion (T) and presence of abnormal or enlarged lymph nodes (N), which is crucial for deciding whether to administer preoperative therapy or to undergo potential endoscopic approaches in the light of an acceptable accuracy in distinguishing T1 from T2–T4 lesions; however, the diagnostic accuracy of EUS is operator-dependent, less useful in antral tumors, and only occasionally able to highlight signs of

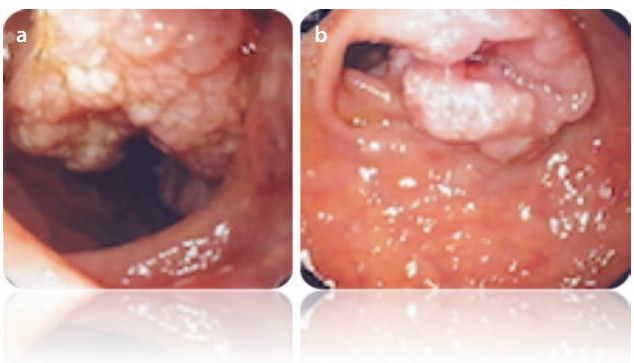

Fig. 35.13 **a**, **b** Diagnostic endoscopies are performed to determine the presence and the location of GC and biopsy for any suspicious lesions. (Photos by courtesy of Prof. G. Genova, Surgical Oncology Unit-Department of Surgical, Oncological and Oral Sciences, University of Palermo)

distant spread (M) [56]. Even if suboptimal in distant lymph nodes evaluation given the limited depth and visualization of the transducer, EUS readily identifies malignant perigastric lymph nodes (hypoechoic, round shape, smooth, distinct margin and size >1 cm). The combination of endoscopic nodal features, along with the use of fine needle-aspiration (FNA) biopsy for cytology assessment, significantly increases the accuracy of the diagnosis [57].

Contrast-enhanced computed tomography (CT) scan of thorax, abdomen, and pelvis is routinely used

for preoperative staging showing a satisfactory overall accuracy for T staging. Nonetheless, CT is less consistently accurate than EUS for the diagnosis of malignant lymph nodes showing a variable sensitivity [58], even if eventually identifying some nodal characteristics suggestive for malignancy (short-axis diameter 6–8 mm in perigastric lymph nodes round shape, central necrosis, heterogeneous or high enhancement) [59].

Combined positron emission tomography (PET) – CT imaging may improve staging by showing an improved specificity in detecting involved lymph nodes or metastatic disease. However, PET may not be informative in patients with mucinous or diffuse tumors because of the low tracer accumulation.

Laparoscopy along with peritoneal washings is recommended to exclude radiologically occult metastatic disease for clinical stage higher than T1b when chemoradiation or surgery is indicated; the benefit may be greater for patients with T3/T4 disease [60].

35.8 Staging Systems, Classification, and Prognosis

Concerning GC patients surgically treated, two pathologic systems are currently used: the Japanese system and the American joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC). While the former is more elaborate and based on anatomic involvement (particularly the lymph node stations), the latter is the system used in Western countries and more accurately estimates prognosis.

In the AJCC/UICC staging system, tumor (T) stage, which reflects the depth of tumor invasion into the gastric wall and extension into adjacent structures, is strictly related to survival rates (Fig. 35.14).

Moreover, nodal (N) stage, which is determined by the number of involved lymph nodes (a minimum of 15 examined lymph nodes is recommended for adequate staging), appeared to predict outcome more accurately than the location of affected lymph nodes (Fig. 35.15).

To date, the most important change made to the last 8th edition concerned stage III, detailing N3 staging into N3a (7–15 positive lymph nodes) and N3b (more than 15 lymph nodes) in the final pathologic stage, since they may represent diseases of differing severity (Table 35.1). For example, involvement of ≥16 lymph nodes (N3b) was associated with worse outcomes than cases involving 7–15 positive nodes (N3a) according to 5-year survival rates.

Referring to Siewert's classification of GEJ cancers [61] (Fig. 35.16), the current 8th American Joint Committee on Cancer (AJCC) classification has staged adenocarcinomas with epicenters no more than 2 cm into the gastric cardia as esophageal cancers, and those extending further as stomach cancers [62]. As opposed to the last AJCC classification system that ranks Siewert type 2 tumors with EGJ invasion as esophageal cancer whereas Siewert type 2 tumors without EGJ invasion and Siewert type 3 tumors as gastric cancer, the new stage grouping of the IGCA (The International Gastric

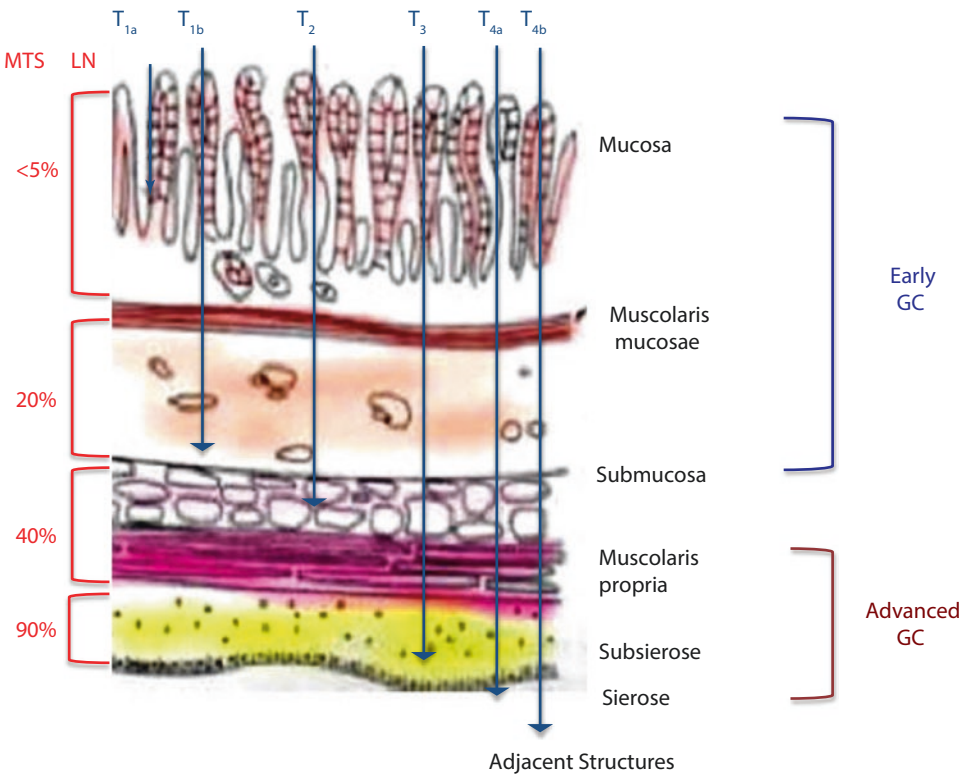

Fig. 35.14 Tumoral staging according to the 8th edition AJCC TNM system (2016)

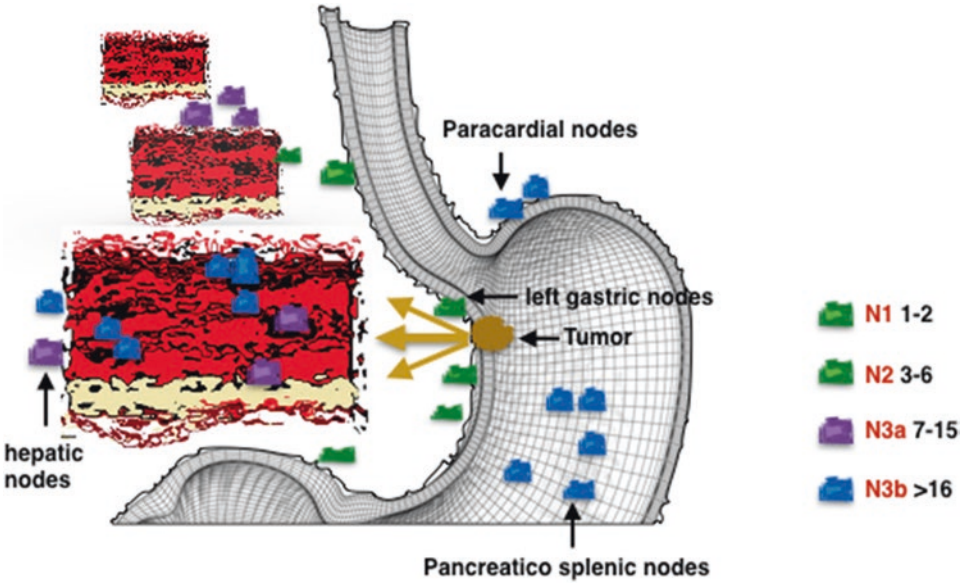

Fig. 35.15 Lymph node staging according to the 8th edition AJCC TNM system (2016)

Table 35.1 AJCC stage and TNM subgroup distributions of the patients according to the 8th edition of the TNM classification

Stage	Subgroup
STAGE IA	T1N0M0
STAGE IB	T1N1M0, T2N0M0
STAGE IIA	T1N2M0, T2N1M0, T3N0M0
STAGE IIB	T1N3aM0, T2N2M0, T3N1M0, T4aN0M0
STAGE IIIA	T2N3aM0, T3N2M0, T4aN1M0, T4aN2M0, T4bN0M0
STAGE IIIB	T1N3bM0, T2N3bM0, T3/4N3aM0, T4bN1/2M0
STADIO IIIC	T4aN3bM0, T4bN3a/bM0
STADIO IV	any T, any N, M1

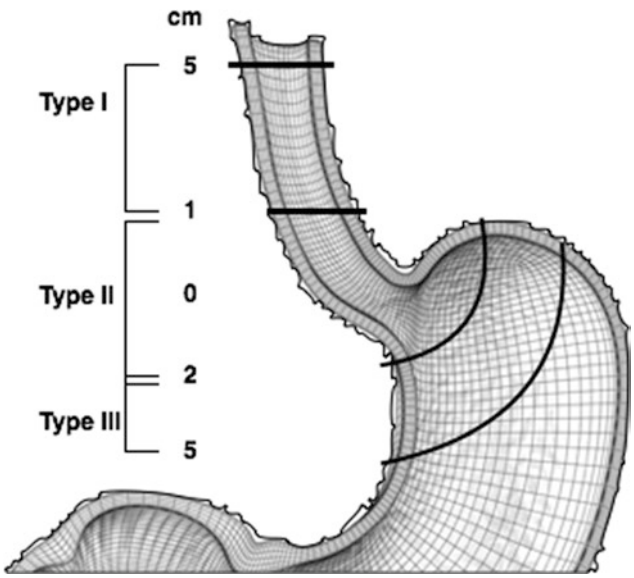

Fig. 35.16 According to Siewert's classification, cancers arising from the GEJ are anatomically classified in adenocarcinoma of the distal esophagus (type I: epicenter located within between 1–5 cm above the anatomic GEJ), true carcinoma of the cardia (type II: within 1 cm above and 2 cm below the junction) and subcardial carcinoma (type III: 2–5 cm below the junction)

Cancer Association) recommended the use of the GC staging for both Siewert type 2 and 3 tumors in the light of the not significantly different patients' overall survival and risk stratification [63]. Of note, a retrospective study suggested that cardiac carcinoma involving GEJ or distal esophagus could be more appropriately classified and staged as gastric rather than esophageal cancers, at least in the Chinese population [64]. However, more studies are warranted also in the light of the different molecular profiling and clinical follow-up data of both tumors.

35.9 Molecular Biology

GC patients can be classified according to clinical-pathological parameters together with the evaluation of serum CEA and CA-19-9 levels to predict prognosis [65] and to choose the therapeutic strategy. As regards patients with metastatic disease, the histological diagno-

sis should include the evaluation of HER-2 status from tumor tissues and plasma. Currently, the evaluation of HER-2 status in tumor tissues represents the only approved molecular biomarker taken into account by clinicians to decide the medical therapy and to predict its efficacy. Moreover, circulating tumor-derived cell-free DNA (the fraction of cell-free DNA that originates from primary tumors, metastases or from circulating tumor cells [66]) for HER2 analysis has been recently recommended in clinical practice as surrogate biomarker [67]. Nevertheless, the identification of new potential diagnostic, prognostic, and predictive molecular biomarkers represents a new challenge for current translational research. Recently, advances in next-generation sequencing technologies have enabled The Cancer Genome Atlas (TCGA) Research Network to classify GC tissue samples in four subtypes: chromosomal instability (CIN) GC (50%), microsatellite instability (MSI)+ GC (22%), genomically stable (GS) GC (20%), and Epstein-Barr Virus (EBV)+ GC (9%) [68]. These four distinct genomic subtypes appeared to differ for several genetic and epigenetic changes:

- The CIN tumors were found to be mainly located in the GEJ/cardia and show intestinal-type features. They exhibited higher prevalence of *TP53* mutations and elevated phosphorylation of epidermal growth factor receptor (*EGFR*). These tumors have a considerable number of genomic amplifications of cell cycle regulation genes, key receptor tyrosine kinases, and transcription factors [69, 70].
- The MSI+ tumors, characterized by genomic instability with high frequency of mutations due to malfunctioning in the DNA repair mechanisms. These types of GC showed to have targetable hotspot mutations in *PIK3CA*, *ERBB2*, and *EGFR*, hypermethylation in the *MLH1* promoter region, and overexpression of PD-L1 [69].
- The GS tumors showed diffuse-type features and have been associated with expression changes of molecules such as *CDH1* and *RHOA* (Ras homolog gene family, member A) gene that proved to be involved in cell adhesion and angiogenesis-related signaling pathway, respectively. These alterations might contribute to lack of cellular cohesion, uncontrolled growth, and escape to programmed cell death [69].
- The EBV+ tumors are characterized by PIK3CA mutations, extreme DNA hypermethylation, amplification of JAK2 and overexpression of both PD-L1 and PD-L2. In EBV-associated gastric carcinoma (EBVaGC), tumor cells may evade immune reactions via the PD-1/PD-L1 immune check point pathway. The cellular DNA methylation status in EBVaGC is strictly regulated by EBV infection in epithelial gastric cells. In tumor cells, EBV infection alters the mRNA expression profile, including the expression of microRNAs and long non-coding RNAs (lncRNAs) [71].

These results represented an interesting contribution to research, which aims to further personalize the management and treatment of patients with GC (Fig. 35.17).

Moreover, since the stomach harbors an abundant quantity of blood vessels, endothelial progenitor cells (EPCs), endothelial cells (ECs), vascular endothelial growth factor (VEGF), and microvessels density (MVD) may be used as candidate diagnostic and prognostic biomarkers for GC. Indeed, the evaluation of blood vessels

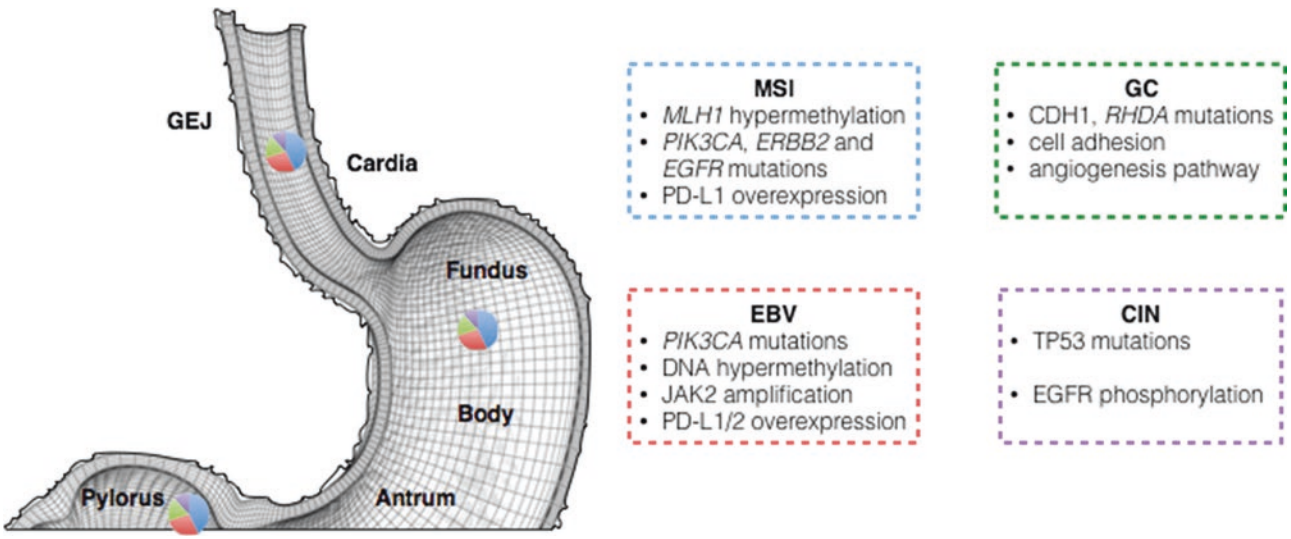

Fig. 35.17 Distribution of GC molecular subtypes according to The Cancer Genome Atlas (TCGA) Research Network

quantity and VEGF level expression along with EPCs and ECs number in patients' peripheral blood seemed to be significantly associated with TNM stage, invasion depth, and lymph node metastasis [72].

At last, the non-coding component seemed to play a key role in promoting cell growth, cell cycle progression and metastasis in GC. The TCGA analyzed miRNA expression profiles of GC tissues. Several miRNAs have shown deregulation in GC tissues and have been listed in TCGA data portal [65]. Among them, the *miR-196a*, *miR-21* (inhibiting the tumor-suppressor genes *PDCD4* that encodes a protein involved in the control of cell growth and invasion) and *miR-106a* (that positively regulates the G1-to-S transition) were revealed to be significantly overexpressed, while *miR-101* (activating *COX2* which stimulates cell proliferation) and *let-7a* appeared to be down-regulated in GC tumor samples when compared to normal tissues [73]. The expression of miRNAs seemed to be epigenetically regulated by the methylation status in gastric cancer cells [74]. A growing body of evidence has recently demonstrated that high expression levels of circulating miRNAs, evaluated in pre-operative serum and other body fluids samples, are consistent with GC tissues and turned out to be significantly reduced following surgery. Thus, miRNAs are emerging as possible noninvasive biomarkers for GC diagnosis and treatment [65]. Recent data have also shown that other emerging classes of non-coding RNA, such as lncRNA, could represent a new, valid, and largely unexplored field of investigation. LncRNAs have been arbitrarily defined according to their size, as transcribed RNA molecules greater than 200 nucleotides in length. LncRNAs regulate gene expression through mechanisms that are mostly poorly understood [75, 76]. Higher expression levels of circulating *H19*, *HOTAIR*, *MALAT1*, *HULC*, *UCA1* lncRNAs have been detected in plasma of GC patients compared to healthy controls. Overexpression of these lncRNAs was associated with proliferation, tumor metastasis, apoptosis, worse survival among GC specimens, indicating that the lncRNAs could be useful diagnostic and prognostic biomarkers [74].

In addition, immunotherapeutic agents, targeting new biological molecules, such as PD-1 and PD-L1 that would lead to immune suppression, have been recently used for treating GC patients. This strategy is showing promising results in on-going randomized clinical trials [77] (Fig. 35.18).

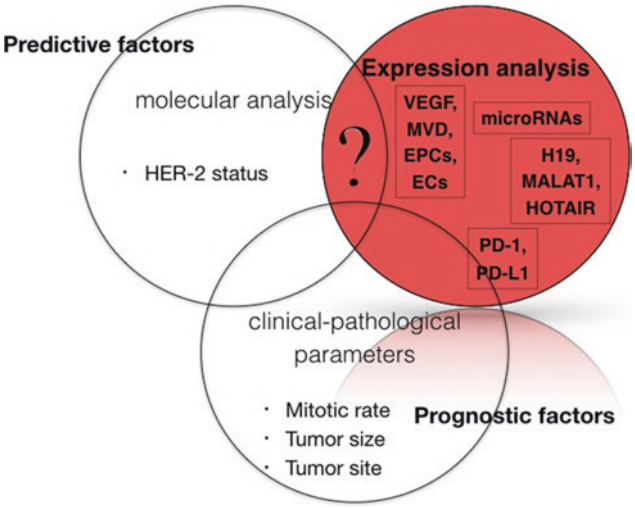

Fig. 35.18 The Diagram of Venn aims to link predictive factors with prognostic factors to assess the potential prognostic or predictive role of microRNAs, lncRNAs, or other molecular biology expression levels

35.10 Treatment

GC is clinically classified as early or advanced stage to help determine appropriate intervention. Surgical resection remains the main form of curative treatment whenever feasible. However, despite advances made in treatment strategies over past decades, the majority of patients are diagnosed at advanced stages, reflecting poor overall survival rates. In Western countries, 55–65% of patients present with locally advanced or metastatic disease. This is in contrast to Japan, where diagnosis usually occurs at an earlier stage and the majority of patients (68%) present with resectable disease. Consequently, there is an East–West division in both the surgical and medical management of gastric cancer (Fig. 35.19).

The extent of resection along with lymphadenectomy could be potentially curative and strictly depends on the assessment of the preoperative stage. Presence of comorbidities, nutritional status, and geriatric frailty should be evaluated and taken into account in the surgical risk assessment [78]. Over the past decades, not only surgical efforts have been implemented to improve patients' survival but medical oncology has also contributed a great deal with neoadjuvant and adjuvant chemotherapeutic regimens.

35.10.1 Endoscopic Therapies

EGC is defined as invasive carcinoma confined to mucosa and/or submucosa, with or without lymph node involvement and irrespective of the tumor size. Most EGCs are small (measuring 1 to 5 cm in size) and often located at lesser curvature around angularis.

There are two forms of endoscopic resection widely accepted as standard treatment for clearly confined to the mucosa, well differentiated, ≤2 cm and non-ulcerated EGCs: endoscopic mucosal resection (EMR) and

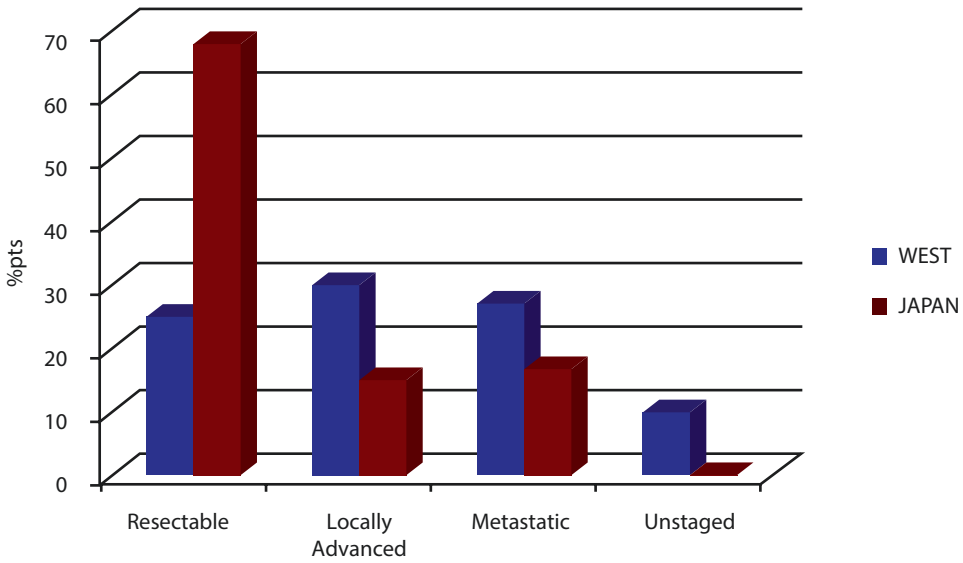

Fig. 35.19 Differences between Western and Japanese GC stage at first diagnosis

Table 35.2 Survival rates and lymph node involvement incidence in EGCs

EGC subtype	N+ incidence	5 year survival rates	
Intramucosal	3–4%	N+	90%
		N–	93%–95%~
Submucosal	19–22%	N+	80%
		N-	~90%

endoscopic submucosal dissection (ESD) have been used as valid alternatives to surgery for selected patients in medical centers with extensive experiences. According to the European Society of Gastrointestinal Endoscopy, very early gastric cancers smaller than 10–15 mm with a very low probability of advanced histology may undergo EMR since the associated lymph node metastatic risk in this group is quite low, even though ESD is strongly recommended as first-line treatment for all gastric superficial neoplastic lesions since it allows high rates of en bloc R0 curative resection with a good safety profile [79]. Thus, while EMR is minimally invasive, cost effective, and well tolerated but associated with a high local recurrence rate for incomplete resection, ESD results in higher rates of en bloc resection and histologically complete resection with low local recurrence rates but also showing higher rates of perforation and extended operation time [80]. After all, long-term survival does not appear to be compromised by the chosen technique and showed an excellent prognosis with high 5-year survival rates and very low metastatic risk (Table 35.2).

35.10.2 Surgery

Surgery is the cornerstone treatment for gastric malignancy, representing the only chance for cure in patients with localized resectable disease. Gastrectomy with complete margin resection of macro/microscopic tumor (R0) along with systematic lymphadenectomy is considered to be the only curative treatment, especially in early-stage disease with favorable prognosis (stage IB-III). As described below, perioperative therapies should be evaluated for these patients.

The extent of gastric resection depends on the site and size of the primary tumor, mainly considering that surgical morbidity was reported to be as high as about 30–40% [81] and complications after curative surgery showed a negative effect on overall and disease specific survival [82]. Therefore, subtotal gastrectomy for mid-distal third GC showed similar long-term survival results compared to total gastrectomy, with lower morbidity and mortality rates and improved postoperative quality of life as well as higher calorie intake and better nutritional status [83–85]. Hence, when the general goal of a macroscopic proximal margin of 5 cm between the tumor and the EGJ can be achieved without any microscopic (R1) or gross residual disease (R2) by a gastric-preserving approach, partial gastrectomy is preferred over total gastrecomy, especially for distal GCs (for diffuse-type cancers, a margin of 8 cm is advocated).

Nonetheless, total gastrectomy should be indicated in poorly differentiated tumors located in the angularis portion of the stomach (at high risk of microscopic invasion of the GEJ), in patients affected by multicentric disease, and/or distally located cancers with multiple lymph node metastases (in order to allow an extended lymph node dissection). Moreover, distal pancreatectomy with splenectomy for gastric cancer was found to

be related to high morbidity and poor prognosis and should not be performed, except when the primary tumor directly invades spleen and/or pancreas or definite gross lymph node metastases are present [86]. Additionally, total gastrectomy has also been advocated as a prophylactic treatment in the event of hereditary diffuse gastric cancer [87].

Concerning localized tumors of proximal stomach, the optimal surgical procedure would consist of proximal gastrectomy or total gastrectomy that seemed to be both associated with postoperative nutritional impairment.

Of interest, laparoscopic gastrectomy proved to be a safe and technically feasible procedure with a shorter hospital stay and fewer complications than open surgery. Even if associated with increased likelihood of receiving adjuvant systemic therapy when indicated and not apparently affecting lymph node staging, a higher incidence of microscopic margin positivity (above all in diffuse-type GC) was reported and long-term survival rates are yet to be determined [88, 89]. Similarly, future prospective studies and long-term results are needed to better evaluate the oncological adequacy of robotic gastric resection that was revealed not to be inferior to laparoscopic gastrectomy, except for the longer operation time and higher costs [90].

35

▪ Lymph Node Dissection

Unlike the extent of resection of the primary tumor, lymph node status and ratio are considered the most important surgical prognostic factor in advanced GC [91–93].

There has been intense debate surrounding the extent of lymphadenectomy suggesting that a more extensive dissection with the removal of an adequate number of nodes (15 or greater) may be both beneficial for staging purposes (to assign a final N pathologic stage) and associated with improved long-term survival [94, 95]. Depending on the mapped location and resection of metastatic lymph nodes, the Japanese Research Society for Gastric Cancer briefly classified the lymph node dissection at the time of gastrectomy as D1 (removal of the perigastric lymph nodes), D2 (D1 plus removal of those nodes along the left gastric, common hepatic and splenic arteries and the coeliac axis) and D0 (incomplete removal of perigastric lymph nodes) [96]. The benefits of a more extended D3 (D2 plus para-aortic nodal dissection) dissection had not been clearly demonstrated in the light of similar survival rates and higher incidence of complications when compared to D2 resection [97].

Whereas in Asian countries D2 dissection is deemed to be a standard treatment because of superior outcomes observed in randomized trials when compared to D1, none of the prospective randomized clinical trials executed in the West initially demonstrated survival advantage for more extensive lymphadenectomy. Nonetheless, fewer loco-regional recurrences and gastric cancer-related deaths were reported with D2 resection in spite of higher postoperative mortality, morbidity, and re-operation rates. However, subgroup analyses from these European trials appeared to suggest that D2 resection might be a better choice in patients affected by an advanced disease with lymph node metastases, as confirmed by long-term follow-up data [98–100]. Moreover, recent findings from an Italian systematic review and meta-analysis supported the superiority of D2 versus D1 dissection in terms of survival benefit, even if mainly limiting the advantage to the disease-specific survival and also not considering the interaction with other factors affecting patients' survival (such as complementary medical therapy) [101]. In addition, two other studies from Western countries reported longer 5-year and 10-year survival rates in the D2 group [102–104].

In summary, in the Western countries, medically fit patients affected by localized resectable GC should undergo D2 dissection that is carried out in specialized, high-volume centers with appropriate surgical expertise and postoperative care, as stated by the American and European guidelines. Notably, considering that the number of metastatic lymph nodes increases with the depth of tumor invasion through the gastric wall and EGCs showed a very low rate of lymph node involvement, a less extensive dissection could be considered in patients with T1 cancer and clinical node-negative disease (D1+, with removal of local N2 nodes according to the site of cancer).

35.10.3 Combined Modality Treatment

As stated before, many patients regrettably present with locally advanced tumors at diagnosis. In this setting, perioperative (pre- and postoperative) or neoadjuvant treatments have been considered in the last decade as attractive concepts for primary tumor downstaging, improving R0 resection rates and treating micrometastatic disease early.

Thus, considering the poor 5-year survival rate for advanced stages of GC [105] and the increasing likelihood of local recurrence or distant metastases even after macroscopic resection of the primary tumor [106], a multimodality approach, including perioperative chemotherapy or chemoradiation, has been suggested as the standard treatment for locally advanced GCs in most oncological centers today and recommended in several national guidelines (◘ Figs. 35.20 and 35.21).

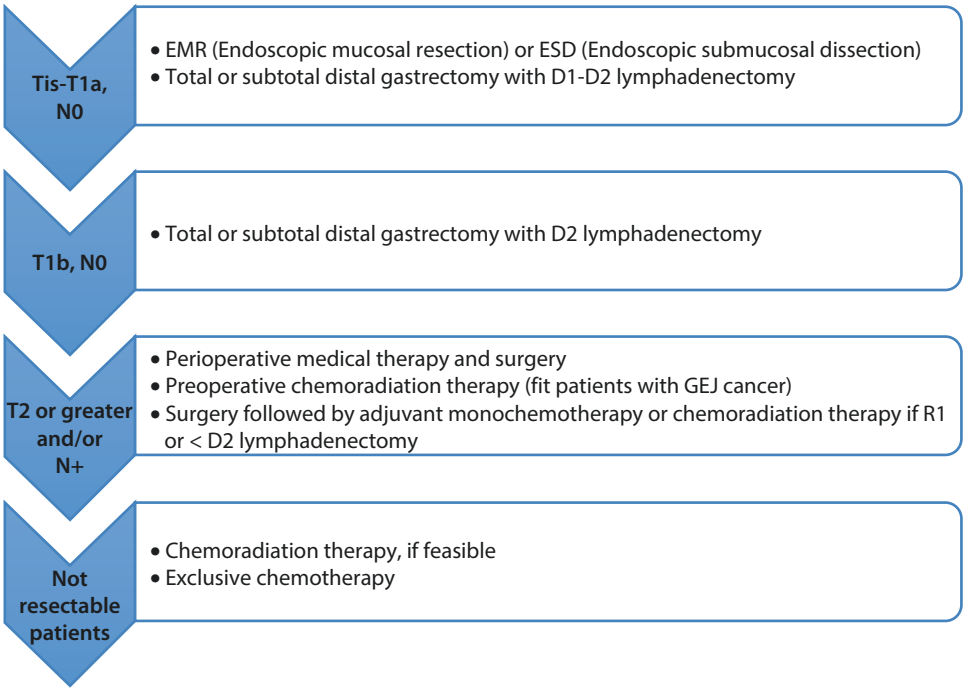

Fig. 35.20 Main clinical management of GC according to the TNM staging system

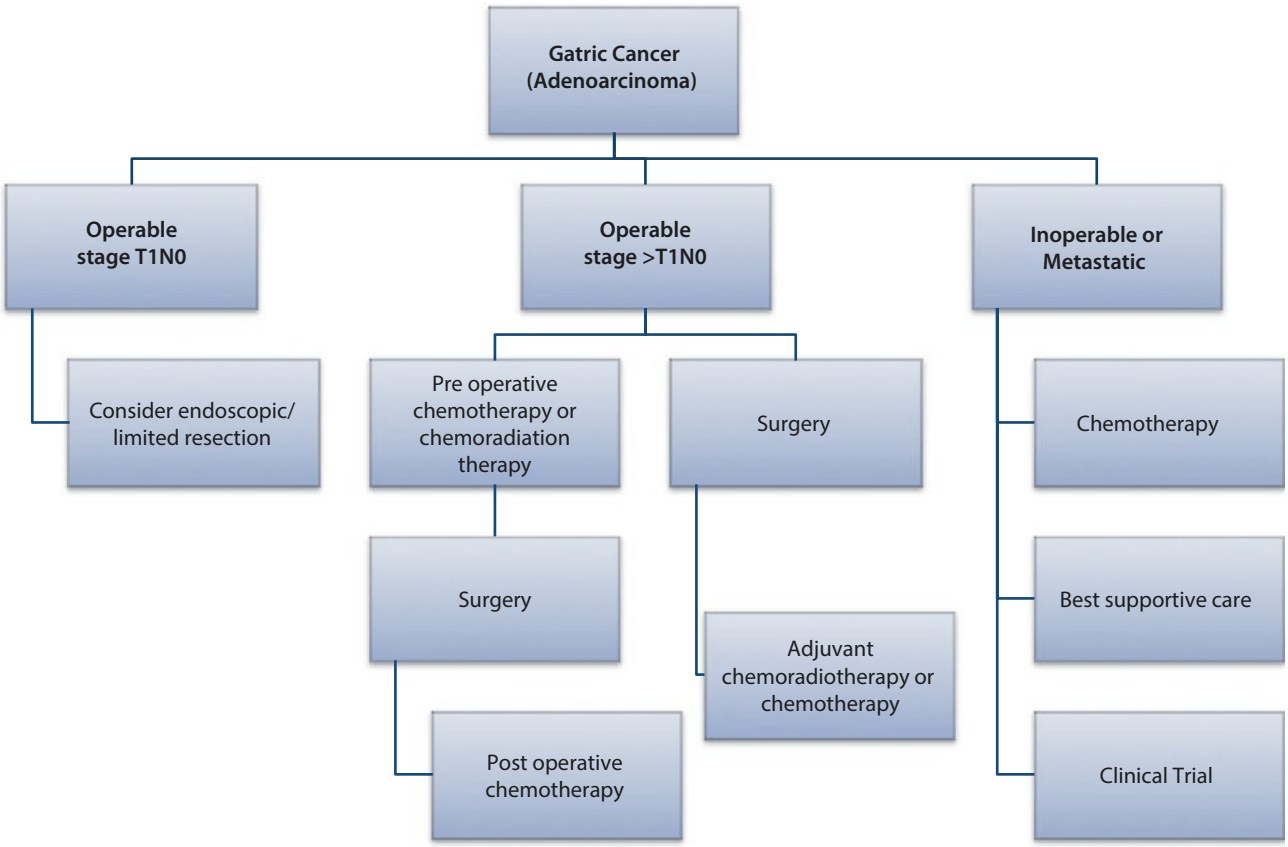

Fig. 35.21 Overview of GC treatment algorithm

Perioperative Treatment

The use of perioperative chemotherapy with a platinum/fluoropyrimidine combination has been supported by the results of the MAGIC [107] and FNCLCC-FFCD [108] randomized clinical trials that documented both an improvement in 5-year overall survival rate and a disease-free survival benefit after a median of six cycles of perioperative chemotherapy (three preoperative and three postoperative 3-week cycles of epirubicin, cisplatin, and 5-fluorouracil [5-FU] or cisplatin and 5-FU, respectively) compared to surgery alone, mostly in case of a clinically suspected lymph node involvement (cN+) or a clinical TNM stage 3 or higher (cT3+). Notwithstanding, in both trials the 5-year survival rate of chemotherapy-arms appeared to be even lower than those reported in international series after only adequate curative surgery with extended lymph node dissection. In addition, perioperative chemotherapy effects seemed to be much more evident for GEJ cancers, therefore claiming for further high-volume sample-size multicenter randomized clinical trials.

Capecitabine-containing regimens and other platinum/fluoropyrimidine doublets or triplets can also be suggested in the perioperative setting (as ECX [epirubicin, cisplatin, capecitabine] or EOX [epirubicin, oxaliplatin, capecitabine], in preference to ECF), since, in the advanced disease, capecitabine and oxaliplatin resulted not to be inferior to 5-fluorouracil and cisplatin, respectively [109]. However, dose intensification with taxanes or with prolonged ECX regimen in the perioperative setting showed some evidence of benefit in terms of progression-free survival, disease-free survival, and tumor regression at resection, but this did not translate into an overall survival improvement [110].

More recently, results from the German randomized, multicenter, open-label phase 2/3 FLOT4 trial, investigating a perioperative FLOT regimen (four preoperative and four postoperative 2-week cycles of docetaxel, oxaliplatin, and 5-FU) versus ECX/F, showed significantly higher proportion of pathological complete response, increased rate of curative surgery, and prolonged median survival rates in patients with advanced clinical stage cT2 or higher and/or nodal positive stage (cN+), intestinal- or diffuse-type GC. In locally advanced, resectable gastric or gastroesophageal junction adenocarcinoma, perioperative FLOT has revealed to improve overall survival compared with perioperative ECF/ECX (50 vs. 35 months, respectively), rates in patients with advanced clinical stage cT2 or higher and/or nodal positive stage (cN+), intestinal- or diffuse-type GC. In locally advanced, resectable gastric or gastroesophageal junction adenocarcinoma, perioperative FLOT has revealed to improve overall survival compared with perioperative ECF/ECX, reporting an acceptable drug-specific toxicity profile with no increase in surgical morbidity and mortality [111]. Accordingly, FLOT4 should be considered the new standard of care in the perioperative treatment of GC patients with a good performance status. Nonetheless, any platinum/fluoropyrimidine doublet or triplet before surgery may be reasonable with the belief that the choice of the compound should be only addressed according to the side effect profile of the cytostatic agents. In any case, the duration should be 2–3 months each for the neoadjuvant and for the adjuvant part [112].

Of interest, the novel fluoropyrimidine S-1 containing tegafur (an inactive 5-FU prodrug) and the two enzyme inhibitors, gimeracil and oteracil, proved to be effective as infusional 5-FU with an improved safety profile [113]. Data on S-1, licensed only in combination with cisplatin in advanced GC, are limited to Asian patients since this drug appeared to be curiously more toxic in western patients requiring the administration of lower doses. Finally, no evidence in the perioperative setting supported the use of those targeted therapies which significantly improved the palliative treatment of advanced GC. Furthermore, the ongoing phase III FLOT5/Renaissance and FLOT6 trials from the German AIO group will possibly answer the question whether additional surgery would confer a survival benefit over chemotherapy alone in GC patients with oligometastatic disease and if the addition of trastuzumab and pertuzumab to perioperative FLOT would affect pathological response and survival in HER2-overexpressing cancers, respectively.

Considering radiation therapy as an integral part of the treatment, the value of preoperative chemoradiation therapy for resectable GC patients has been recently assessed by the TOPGEAR study [114], an international prospective phase III randomized trial that underlined the advantage of delivering radiotherapy in the preoperative rather than postoperative setting. As a matter of fact, unlike the potential late treatment-related toxic effects showed in the North American INT0116 trial where adjuvant fluoropyrimidine-based therapy was administered in combination with conventionally fractionated RT [115], interim results of the TOPGEAR trial demonstrated that preoperative chemoradiation added to perioperative ECF resulted to be safe and feasible, not adversely affecting surgical compliance and morbidity while not increasing hematologic and non-hematologic toxicities. Further ongoing randomized trials investigating the uncertain role of preoperative chemoradiation are under evaluation in order to select the most promising strategy, especially in resectable GC (the CRITICS II trial).

Adjuvant Treatment

The use of chemoradiation in the postoperative setting is somewhat controversial. Although currently considered as standard therapy in the USA, this treatment approach has not been widely accepted in Europe due to concerns regarding toxicity. As supported by both the subgroup analyses of the INT0116 and the retrospective data from the Dutch Gastric Cancer Group trial [116], postoperative chemoradiation seemed to compensate mainly for suboptimal surgery reducing local recurrence rate after D1 resection, whereas not providing any benefit following D2 resection. So far, no strong evidence of survival benefit of chemoradiation over chemotherapy alone was demonstrated in the ARTIST trial [117], even though interim results of a phase III study (the ARTIST II trial) have recently shown that adjuvant chemotherapy (S-1 plus oxaliplatin) and/or chemoradiotherapy (S-1 plus oxaliplatin and RT) are effective in prolonging disease-free survival, when compared to S-1 monotherapy, in Asian patients with curatively resected D2, stage II/III, node-positive GC. While significantly reducing mortality and risk of tumor recurrence in terms of overall and relapse-free survival improvement when compared to surgery alone [115], the combination of radiotherapy with chemotherapeutic agents entailed a higher rate of hematologic and gastrointestinal toxicities and did not highlight a clear advantage over chemotherapy alone [118]. Hence, other alternative postoperative chemoradiation regimens have been evaluated suggesting the use of capecitabine with concurrent radiation therapy as a safe and well-tolerated option in resected GC patients. In addition, the randomized phase III CRITICS trial concluded that the addition of postoperative radiation therapy did not add any benefit in patients who have undergone preoperative chemotherapy [119].

Postoperative chemotherapy following D2 resection has not been historically associated with significant survival benefit [120–122], considering also that this approach is less well tolerated than neoadjuvant treatment. Interestingly, curative surgery alone showed very good survival rates in patients with T1 cancer [123]. However, two large randomized phase III trials conducted in Asia changed the landscape of postsurgical chemotherapy for resectable GC, reporting an improved survival benefit after curative D2 lymph node dissection in patients affected by stage II and III gastric cancer. Specifically, while the Japanese ACTS-GC trial evaluated S-1 showing the greatest survival benefit for node-negative disease [124], the Korean CLASSIC trial investigated a capecitabine-oxaliplatin doublet indicating the greatest survival benefit in N1-2 disease [125]. Moreover, results from the randomized phase III POST Trial have recently suggested that an S-1 based doublet (with cisplatin or docetaxel) could be an effective and tolerable option in Asian patients with curatively resected stage III gastric cancer [126]. In a large individual patient-level meta-analysis [127], chemotherapy based on fluorouracil regimens was associated with a 6% absolute benefit compared with surgery alone and could be consequently recommended in stage II and III GC patients who have undergone optimal surgery without the administration of preoperative treatment.

35.11 Follow-Up

Mainly considering that an improvement in survival outcomes has not been demonstrated for all types of cancers and no randomized controlled trials have been published for GC patients, the role of follow-up is still controversial and no real consensus exists. The main goal of a regular follow-up program is to diagnose local or metachronous cancer recurrence early, promptly detecting any adverse effects or treatment-related complications, while collecting data concerning cancer history and treatment outcomes.

Most relapses used to occur within the first 2–3 years, and nearly all relapses occurring by 5 years are not surgically curable. Specifically, recurrence patterns may be generally classified into locoregional recurrence (at the proximal/distal resection margin or in the adjacent tissue of the surgical bed), distant or hematogenous metastases, peritoneum implanting, and nodal recurrence (within the regional and distant lymph nodes) [128, 129] (Fig. 35.22).

Due to the lack of strong evidence, several regimens have been proposed and international guidelines slightly differ from each other. However, follow-up strategies should be always tailored to both the individual patient and the stage of the disease. The follow-up surveillance panel should be generally based on an interim history and physical examination, repeated every 3 to 6 months for the first postoperative 2 years and every 6 to 12 months for at least 5 years, thereafter annually. Complete blood chemistry lab tests along with tumor marker assays, such as CEA and Ca 19.9, are simple and inexpensive to perform, often occur earlier than imaging abnormalities, but specificity and sensitivity are quite low. Either abdominal ultrasonography or CT could be considered every 6 months, while endoscopic surveillance, especially after the endoscopic treatment of early gastric cancer, should be performed annually [130]. Likewise, endoscopic surveillance should be offered to precancerous lesions according to risk factors for progression toward gastric cancer. In case of suspected relapse/disease progression or significant clinical deterioration, physical examination along with direct blood tests and radiologic investigations should be carried out. *HER-2* testing is only recommended in metastatic or advanced disease (Fig. 35.23).

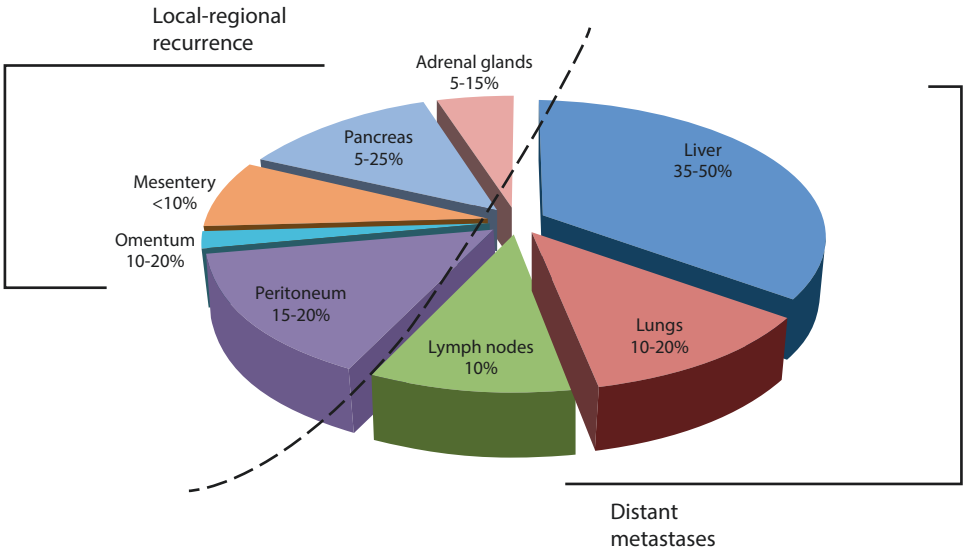

Fig. 35.22 Most frequent sites of disease recurrence in GC

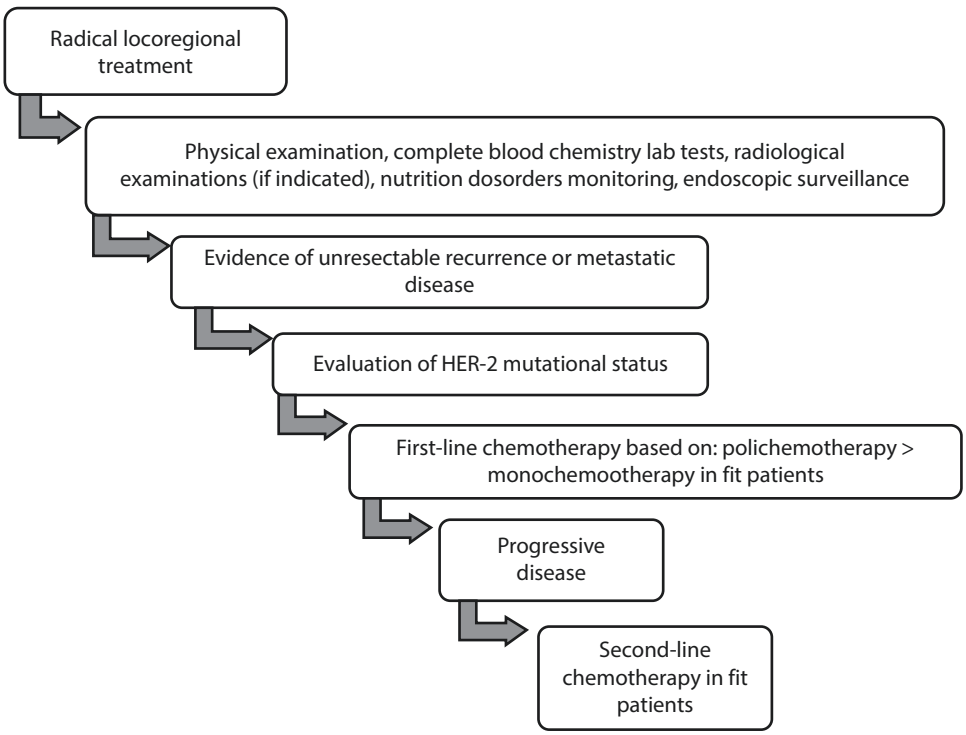

Fig. 35.23 Follow-up, long-term implications and survivorship

In addition, a proper follow-up program should allow the detection and the prompt treatment of long-term adverse effects following the primary therapy, such as digestive problems (dyspepsia, nausea, vomiting, early satiety, reflux, anorexia), post-gastrectomy syndromes (dumping syndrome, bile reflux, Roux-en-Y stasis syndrome, and afferent and efferent loop syndromes), malabsorption (iron deficiency or megaloblastic anemia in approximately 30% of patients, osteopenia, or osteoporosis) or psychological disorders. Similarly, a dietary support for patients on either a radical treatment or palliative pathway with reference to vitamin and mineral deficiencies is recommended.

Finally, further studies are warranted to better characterize the expression of vascular endothelial growth factors along with the role of several microRNAs (miRNA-328) [131] and various genes (E-cadherin [132] and cyclin E [133]) as potential biomarkers for recurrence after curative resection, allowing personalization of follow-up according to the individual risk of relapse.

Case Study: Management of a Patient Affected by Early Gastric Cancer

Male, 65 years old

- *Family history* negative for malignancies
- *PMH:* former tobacco smoker, systemic arterial hypertension
- *RMH:* Complaints of severe fatigue and nausea
- *Objective examination*: normal physical examination, except pale oral and scleral mucosa
- *Blood tests*: low hemoglobin (9.8 mg/dL) and ferritin (3.9 mg/dL) level, normal biochemical tests

Question

What action should be taken?

(1) Abdominal ultrasound. (2) Upper gastrointestinal endoscopy. (3) Abdominal computed tomography scan with contrast

Answer

Upper gastrointestinal endoscopy (for determining the etiology of the iron deficiency anemia)

An 8 mm erythematous, flat-elevated area was seen in the cardia and biopsy was obtained from the lesion. Histopathological examination confirmed well-differentiated adenocarcinoma from biopsy material, pT1Nx.

Question

What will be the next step?

(1) Endoscopic mucosal resection (EMR). (2) Subtotal gastrectomy + lymphadenectomy. (3) Endoscopic submucosal dissection (ESD)

Answer

Very early gastric cancers smaller than 10–15 mm with a very low probability of advanced histology may undergo *endoscopic mucosal resection (EMR)* since the associated lymph node metastatic risk in this group is quite low. ESD results in higher rates of en bloc resection and histologically complete resection with low local recurrence rates but also showing higher rates of perforation and extended operation time.

Question

What action should be taken after endoscopic resection?

(1) Chemotherapy. (2) Radiation therapy. (3) Endoscopic surveillance

Answer

The role of biannual or annual *endoscopic surveillance* has been well established since patients who undergo endoscopic treatment of EGC are at risk for synchronous and metachronous multiple cancers. To the contrary, however, the role of computed tomographic (CT) surveillance has not yet been well determined.

Key Points

- Start with medical history, physical examination, and diagnostic work-up.
- Endoscopic approaches remain the cornerstone of early gastric cancer initial treatment.
- Early detection and treatment contribute to decreased mortality rates.
- Follow-up strategies should be always tailored to both the individual patient and the stage of the disease.

Case Study: Management of Locally Advanced Gastric Cancer Successfully Treated by Combined Modality Treatment

Male, 54 years old.

- *Family history* positive for malignancy (mother's history of colon cancer).
- *PMH*: occupational exposure to iron processing, active tobacco smoker, no prior GI-tract disease, no drugs.
- *RMH*: a 4-month history of upper abdominal discomfort, mild nausea, anorexia, and weight loss.
- *Physical examination*: all normal findings.
- *Laboratory tests*: no abnormalities except for an increased CA 19-9 to 108 U/ml (normal range up to 39 U/ml).
- *Staging:* esophagogastroduodenoscopy revealed a 4 × 8 cm mass along the lesser curvature of the stomach, extending into the EGJ. The mass was confirmed on endoscopic ultrasound (EUS) and was staged as cT3N1 based on the presence of gastrohepatic lymphadenopathy measuring up to 1.2 cm in maximum diameter. A biopsy confirmed a poorly differentiated invasive adenocarcinoma with signet ring features. Additionally, computed tomography (CT) of the chest, abdomen, and pelvis showed no hepatic lesions and/or involvement of other organs.

Question

What action should be taken?

(1) Subtotal gastrectomy + lymphadenectomy. (2) Laparoscopy with peritoneal washings. (3) PET-FDG

Answer

Laparoscopy along with peritoneal washings is recommended to exclude radiologically occult metastatic disease for clinical stage higher than T1b when chemoradiation or surgery is indicated; the benefit may be greater for patients with T3/T4 disease since positive cytology can be present in about a third of cases and denotes M1 disease, which portends a poor prognosis, even if this is the only site of metastasis.

The preoperative peritoneal washing cytology produced a negative result.

Question

What action should be taken?

(1) Subtotal gastrectomy + lymphadenectomy. (2) Perioperative FLOTx4. (3) Perioperative ECF

Answer

In locally advanced, resectable gastric, or gastroesophageal junction adenocarcinoma, *perioperative FLOT* has revealed to improve overall survival compared with perioperative ECF/ECX (50 vs. 35 months, respectively), reporting an acceptable drug-specific toxicity profile with no increase in surgical morbidity and mortality [111]. Accordingly, FLOTx4 should be now considered the new standard of care in the perioperative treatment of GC patients with a good performance status.

Question

What will be the next step?

(1) Total gastrectomy + D2 lymphadenectomy. (2) Partial gastrectomy + lymphadenectomy. (3) Total gastrectomy + distal pancreasectomy and splenectomy

Answer

Total gastrectomy should be indicated in poorly differentiated tumors located in the angularis portion of the stomach (at high risk of microscopic invasion of the GEJ). *D2 lymphadenectomy* might be a better choice in patients affected by an advanced disease with lymph node metastases. Distal pancreatectomy with splenectomy for gastric cancer was found to be related to high morbidity and poor prognosis.

Final pathology revealed a tumor staged as yT2N0 with negative margins and 0/16 lymph nodes positive for metastatic disease, Stage IIA. The patient's postoperative staging did not show any evidence of disease and he was closely followed with adjuvant treatment.

Key Points

- The importance of proper clinical management in locally advanced GC.
- Consider imaging techniques for an appropriate preoperative staging.
- Multidisciplinary treatment is crucial for locally advanced GC.
- Understand the role of surgery along with lymphadenectomy as a part of both staging and treatment strategy.
- The choice of the most effective and tailored perioperative treatment is crucial for the patients' clinical outcome.

35

Expert Opinion

Antonio Russo

Key Points

- The global incidence of GC shows a wide geographical variation with the highest rates occurring in East Asia, while decreasing incidence and mortality in the vast majority of the developed world have been observed for non cardiac GC.
- Classification based on anatomic location and histologic subtypes has significant implications for therapy,
- New staging recommendations according to the 8th edition of the American Joint Committee on Cancer (AJCC) dramatically affects prognosis and treatment decisions.
- A multimodal approach has been implemented into the clinical practice to refine the management of locoregional disease with perioperative chemotherapy representing the standard therapy in curatively intended disease.

- Radical gastrectomy with D2 lymph node dissection has become the standard surgery in most high-volume centers

Summary of Clinical Recommendations

- Prevention and early diagnosis may be the most promising strategies for cancer control and key strategies to reduce mortality.
- Most of patients are commonly diagnosed with advanced disease, presenting with a combination of signs and symptoms that are not unequivocally suggestive for GC. Weight loss and abdominal pain are the most common symptoms at initial presentation.
- Careful clinical staging is critical to ensure that patients are appropriately selected for treatment interventions.
- Endoscopic resection is a curative option for early gastric neoplastic lesions. Surgery is the cornerstone treatment for gastric malignancy, representing the only chance for cure in patients with localized resectable disease. Lymph node status and ratio are considered the most important surgical prognostic factor in advanced GC.
- A multimodality approach including perioperative chemotherapy or chemoradiation has been suggested as the standard treatment for locally advanced GCs in most oncological centers today and recommended in several national guidelines.

Hints for Deeper Insight

Based on the exciting results reported in the metastatic setting, targeted agents and immunotherapy have been investigated in the perioperative approach in order to improve survival rates of such patients.

HER-2 status has not shown to be either predictive or prognostic in the neoadjuvant setting, even if ongoing clinical trials are currently investigating the role of trastuzumab together with pertuzumab in addition to FLOT- and XELOX-based regimens (PETRARCA and INNOVATION trials). As concerns antiangiogenic agents, bevacizumab was not associated with OS benefit when added to chemotherapy as perioperative treatment (UK MRC ST03 trial), whereas the RAMSES trial is currently evaluating ramucirumab in the perioperative treatment of Her-2 negative gastric and GEJ adenocarcinomas. Furthermore, the Phase III KEYNOTE-585 and the Phase I/II ICONIC trials are currently investigating the efficacy and the safety of pembrolizumab plus chemotherapy or FLOT and avelumab plus FLOT, respectively, in the perioperative management of gastric cancer.

To date, the most important and debated issue is the research of prognostic and predictive factors that might affect the efficacy of perioperative treatments, enabling the appropriate and prompt selection of patients for surgery and/or multimodality approach. In this field, the use of 18-fluorodeoxyglucose positron emission tomography (18-FDG-PET) scan as a predictor of early response to neoadjuvant treatment has been associated with a prognostic meaning rather that predictive, highlighting the biological aggressiveness of the tumors in non-responder patients and finally leading to a worse outcome.

The use of next-generation sequencing (NGS) has not been clearly supported by sufficient data at the time of initial diagnosis for the clinical decision-making process in the locoregional disease. The genomic and molecular characterization of gastric cancer (TCGA) has not found applications in daily clinical practice and further studies are needed to translate these findings for the management of patients. The prognostic and predictive roles of both microsatellites (MSI) and programmed cell death ligand-1 (PD-L1) are implicated in the management of the metastatic disease.

Suggested Reading

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Gastric Cancer: Advanced/ Metastatic Disease

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Gastrointestinal Cancers

Contents

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Learning Objectives

By the end of the chapter, the reader will:

- Be able to choose the correct treatment algorithm for inoperable locally advanced and metastatic gastric cancer
- Have learned the basic concepts of molecular classification of gastric cancer
- Have reached in-depth knowledge of inoperable locally advanced and metastatic stomach cancer treatment
- Be able to put acquired knowledge into daily clinical practice

36.1 Introduction

Gastric cancer (GC) is the fifth most common tumor and the second leading cause of cancer-related death worldwide. Nowadays, we know that gastric cancers can be divided into two different clinical entities, gastroesophageal junction and stomach (body/antrum) tumors, that showed different features from epidemiologic, biologic, genetic, and clinical points of view.

In this chapter, only relevant aspects for the evaluation and treatment of unresectable locally advanced and metastatic disease are reported. For a complete description of the general features of gastric cancer, see the previous chapter.

36.2 Epidemiology

Gastric cancer shows significant global differences in incidence worldwide. Indeed, the highest rates are recorded in Eastern Asia, South America, and Eastern Europe while the lowest in North America and Western Europe. In particular, in Europe, the highest rates are reported in Portugal in addition to the eastern countries, while the lower incidence is described in Denmark [1]. According to this global view, a gradual decline of the incidence of GC has been observed in Western Europe and North America in the last decades due to the improvement of life conditions and due to an epidemiologic shift that lead to the decrease of distal gastric cancer and the increase of the junctional disease [2]:

- Globally, gastric cancer had an estimated unadjusted incidence of around 18 and 9/100,000/year for men and women, respectively.
- Gastric cancer is frequently diagnosed in men with an age between 60 and 80 years.
- More than 60% of patients are older than 65 years, with an age-related increase of the risk (from 15 new diagnosis/100,000/year in under 30 years patients to 140/100,000/year in over 75 years old patients)
- 90% of gastric cancer are sporadic, while only 1–3% are hereditary.

36.3 Clinical Features

Gastric cancers are usually asymptomatic in the early stage, and they may cause specific and faded symptoms afterward, leading to a late diagnosis.

Weight loss, anorexia, dysphagia, and heartburn are the most common signs and symptoms at the diagnosis. Specific symptoms may arise in more advanced stage due to the growth of tumor that could lead to significant stenosis or hemorrhages. Dysphagia and vomit may appear in case of a stenosis located at the gastroesophageal junction or if a prominent stenosis is located at the antrum. Hematemesis, melena, or sign and symptoms of chronic anemia (malaise, fatigue, or exertional dyspnea) are the most common clinical manifestation of active bleeding.

During the natural history of these tumors, lymphonodal involvement is frequent and represents an early step in metastatic spread. The most common signs of superficial lymphonodal involvement are Troisier's sign due to the left supraclavicular lymphadenopathy (Virchow's lymph node), Sister Joseph's nodule at the navel, and Irish's sign, which is a left axillar lymphadenopathy.

The liver, peritoneum, retroperitoneal lymph nodes, and lung are the most common sites of metastasis. Bones and brain metastasis are less common but possible. Liver involvement is predominant through celiac vessels and can lead to hepatomegaly and jaundice, while dyspnea can appear in case of diffuse lung involvement, pleural effusion, or profuse ascites. Bone pain and neurologic signs and symptoms can appear in case of bone and brain involvement, respectively. Peritoneal involvement is frequent in case of GC with a signet-ring cell component or in case of undifferentiated or diffuse-type tumors (according to Lauren classification). It spreads through lymphatic vessels on the gastric wall and cause different entity of peritoneal carcinomatosis with ascites, secondary ovary involvement (Krukenberg tumor), or nodules in the pouch of Douglas, also known as a sign of Blumer's shelf.

As other tumors, also in metastatic gastric cancer, some paraneoplastic syndromes can occur, such as acanthosis nigricans, diffuse intravascular coagulation, venous thrombosis (Trousseau syndrome), and many others, due to the secretion of different active substances (cytokines, hormones, etc.) by the tumor.

36.4 Pathological Features

36.4.1 Microscopic Aspects and Immunohistochemical

In case of locally advanced, recurrent, or metastatic GC, pathological report should include not only the classical microscopic parameters, such as the histological subtypes and Lauren's classification, but also the evaluation of human epidermal growth factor receptor 2 (HER2) status.

Still today, HER2 determination represents the only validated biomarker in GC, able to influence the treatment choices. HER2 positivity is determined by quantification of the HER2 cell surface receptors by immunohistochemistry (IHC) and/or by measuring the number of HER2 gene copy numbers using fluorescence in situ hybridization (FISH). Determination of HER2 status via IHC is distinct for gastric and breast cancer, because an incomplete basolateral or lateral staining alone in gastric cancer is considered positive in addition to complete membrane staining. This difference results in tumor heterogeneity and potential inaccuracy determination of the HER2 positivity, and multiple biopsies of different sites of neoplastic lesion are recommended to overcome this risk (at least five to six biopsies are usually required).

In GC, HER2 positivity is defined by 3+ scoring on IHC or 2+ on IHC with a FISH amplification (HER2/CEP 17 ratio ≥ 2.0), according to an IHC scoring criteria specific for HER2 overexpression in gastric cancer. HER2 status is considered negative in case of results 0 or 1+ by IHC [3]. Another relevant issue in this field is that the IHC staining pattern that determines the highest level of HER2 expression by IHC (IHC 3+) depends on whether a surgical specimen or biopsy is tested. As a matter of fact, basolateral or lateral membranous reactivity in ≥10% of tumor cells represents an IHC 3+ staining pattern in a surgical specimen, while an IHC 3+ staining pattern on a tumor biopsy is determined by tumor cell clusters with a strong complete, basolateral, or lateral membranous reactivity irrespective of percentage of tumor cell stained (Fig. 36.1). Tumors with equivocal IHC scores (2+) should be tested further using FISH or other in situ methods (ISH (immunofluorescence in situ hybridization)) in order to evaluate gene amplification(Fig. 36.2).

Even if different trials have investigated the role of mesenchymal-epithelial transition factor (c-Met) in gastric cancer, the results are still controversial, and there is

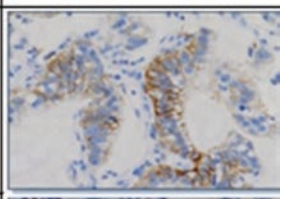
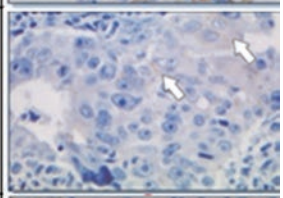
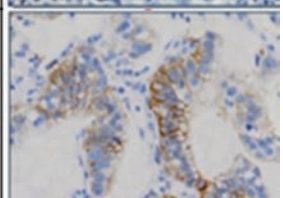
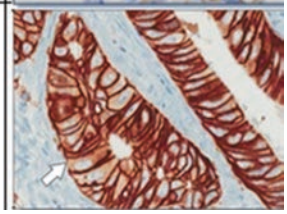
Score	Staining pattern (biopsy)	Staining pattern (resection)	Classification	IHC
0	No reactivity or no membranous reactivity in any tumor cell	No reactivity or membranous reactivity in < 10% of cells	Negative	
1+	Tumor cell cluster with a faint/barely perceptible membranous reactivity irrespective of percentage of tumor cells stained	Faint/barely perceptible membranous reactivity in >10% of cells; cells are reactive only in part of their membrane	Negative	
2+	Tumor cell cluster with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of Tumor cells stained	Weak o moderate complete or basolateral membranous reactivity in > 10% of tumor cells	Equivocal	
3+	Tumor cell cluster with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	Moderate to strong complete or basolateral membranous reactivity in > 10% of tumor cells	Positive	

Fig. 36.1 HER2 scoring system in gastric cancer

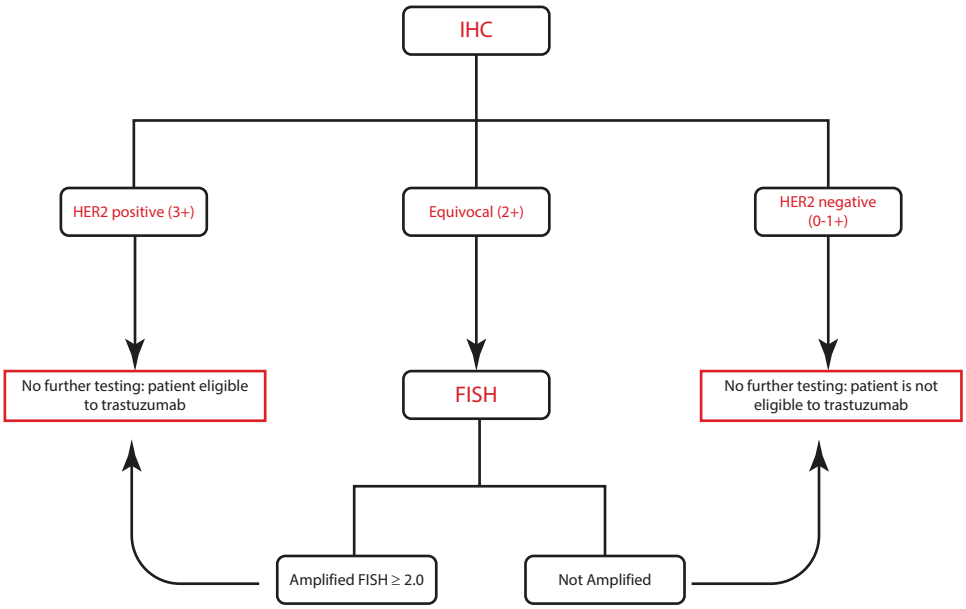


Fig. 36.2 Algorithm of HER2 status determination by IHC and FISH

not yet a validated method to assess Met amplification and overexpression. Furthermore, Met evaluation is not recommended in daily clinical practice.

With the development of immunotherapy, further biomarkers have been investigated and validated during the last years. Microsatellite instability (MSI) evaluates the genetic mutability condition. In case of impaired DNA mismatch repair (MMR), the normal function of these mechanisms leads to a genetic hypermutability and a kind of mutation accumulation that result in a high neoantigen production and a consequent sensitivity to immunotherapeutic agents. This condition is called "high microsatellite instability" (MSI-H). MMR status can also be determined by the immunohistochemical analysis of some protein expression (such as MLH1, PMS2, MSH2, MSH6).

Another possible predictive factor for immunotherapy is the programmed death-ligand 1 (PD-L1).

PD-L1 is a transmembrane protein involved in the suppressing signaling of the immune response and in the "self-tolerance," acting as an inhibition factor (coinhibitor) for T-cell activity. It is a part of those regulators that constitute the so-called immune checkpoints. The "immune checkpoint inhibitors" are the drugs mainly use as immunotherapy against cancer, thanks to their blocking action on these receptors or their ligands. A high PD-L1 expression, assessed via IHC, is considered a positive predictive factor for immunotherapy across many tumor types. Its evaluation may be carried out according to tumor proportion score (TPS) or, more effectively, according to combined positive score (CPS) analysis of not only the viable tumor cells but also the other PD-L1 staining cells in the microenvironment (lymphocytes and macrophages).

In addition to these biomarkers, also the Epstein-Barr virus (EBV) status may be a useful tool for treatment selection. Its evaluation can be done by ICH or by Epstein-Barr encoding region (EBER) in situ hybridization, even if its role is still debated and far from being already validated for GC.

36.5 Molecular Biology and Main Therapeutic Targets in Advanced Gastric Cancer

For many years, GC was considered as a single disease: however, we know that it should be considered as a collection of very different molecular entities, each characterized by different clinical and molecular features. A first attempt to define GC heterogeneity was performed by Lauren P [4], who identified two types of GC on histological bases: the first one called "intestinal," because it displayed feature characteristic of the intestinal mucosa (in fact, it arises from intestinal metaplasia), and the other one called "diffuse," because the cancer cells, often poorly cohesive, diffusely infiltrated the gastric wall. On the other side, the World Health Organization (WHO) Classification of Tumors of the Digestive System (2019) classifies GC, according to their histological appearance, in "tubular adenocarcinomas," "papillary adenocarcinomas," "mucinous adenocarcinomas," and "signet-ring cell adenocarcinomas," the latter one resembling those that are classified as "diffuse-type" in the Lauren classification. Moreover, in addition to classic histological features, we can now classify these neoplasms also by their molecular profile. In particular, many studies

have shown that gastric cancer can be driven by different genetic and/or epigenetic abnormalities: these findings led us to create robust molecular classifications that could become important especially in metastatic setting in order to develop novel target therapies.

36.5.1 Molecular Classifications

One of the first molecular GC classifications was by Patrick Tan et al. [5]: they classified GC into two distinct intrinsic subgroups – G-INT (genomic intestinal) and G-DIF (genomic diffuse). The authors used a panel of 37 GC cell lines and identified a "gene expression signature" of 171 genes that is able to distinguish between these two intrinsic subtypes, the first one called "G-INT" because more related to Lauren's intestinal subtype and the other one "G-DIF" because more related to diffuse subtype. The classification was then validated in a clinical cohort of 270 GC patients, showing that these two intrinsic classes really exist. Moreover, useful predictive information came out from in vitro experiments on 28 cell lines, with relevant implications for patient's care: G-INT cell lines were found to be more sensitive to 5-fluorouralcil and oxaliplatin, while G-DIF resulted to be more sensitive to cisplatin.

The same research group reported 2 years later [6] another GC classification based on the evaluation of gene expression in 248 tumors. According to this classification, GC can be divided into three subgroups: proliferative, metabolic, and mesenchymal. Proliferative subtypes are characterized by genomic instability, p53 mutations, and DNA hypomethylation; in the metabolic type, there is an increased activity of spasmolytic polypeptide-expressing metaplasia (SPEM metaplasia), while the mesenchymal type shows an epithelial mesenchymal transition (EMT) signature with high level of N-cadherin and low level of E-cadherin that leads to poorly differentiated tumors. Again, some interesting translational implications emerged: metabolic subtype seems more sensitive to 5-fluorouracil than the other two, while the mesenchymal subtype (probably due to "oncogenic addiction" to PI3K-AKT-mTOR pathway) seems to be more sensitive to drugs that block PI3K or mTOR, opening the way for a more precise therapy for GC.

In 2014, the Cancer Genome Atlas (TCGA) investigators published the most important and comprehensive study that we have to date on molecular GC classification. Four subtypes of gastric cancer have been described: Epstein-Barr virus (EBV)-positive, 9% of cases; microsatellite instability (MSI-H), 22% of cases; genomically stable (GS), 20% of cases; and chromosomal instability (CIN), 50% of cases ([7]; Fig. 36.3).

Each subtype shows different features and it is enriched for selected molecular abnormalities. In particular, the EBV-positive type is characterized by the posi-

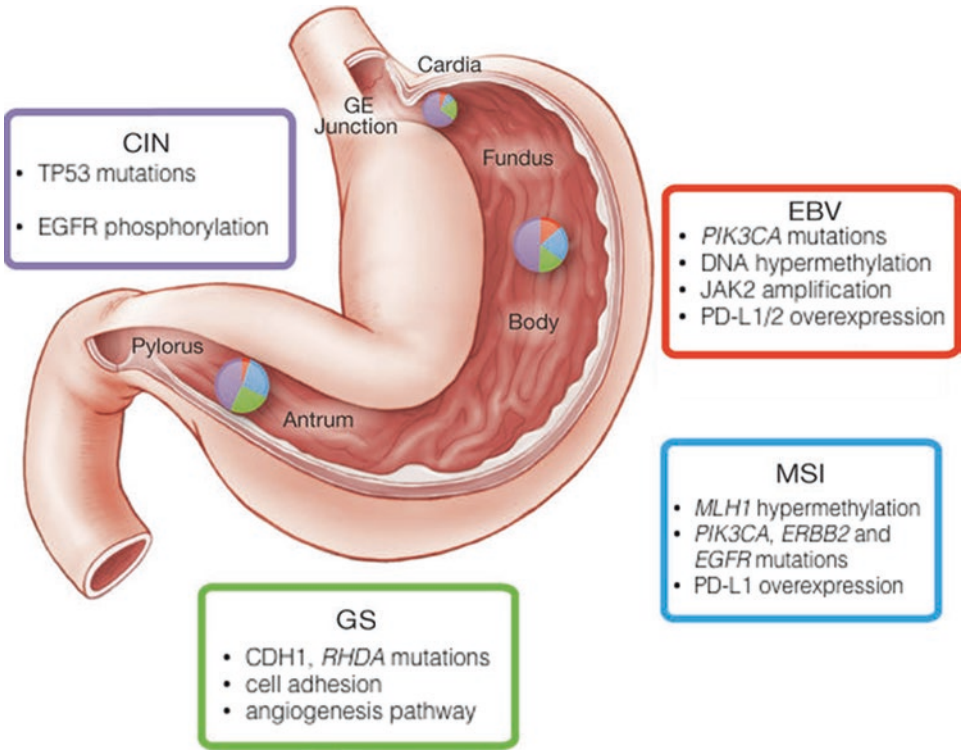


Fig. 36.3 Molecular subtypes of gastric cancer as emerged from TCGA. See the text for more information

tivity for EBV, mutations, or amplifications of PI3K, PD-L1, and JAK2; these cancers can mostly arise in the fundus or gastric body and are more frequent in men.

MSI-H tumors are more frequent in older women and comprise especially intestinal-type cancers. From a molecular point of view, this group is characterized by mutations of p53, EGFR, HER2, HER3, PTEN, or silencing of the promoter of MLH1, a gene involved in the mismatch repair process.

GS gastric cancers are frequently diffuse and arise in younger age: they lack somatic copy number aberrations and are more related to Lauren's diffuse histology than the other ones. A pathway frequently destroyed in this subtype is that related to "cell adhesion," with the most relevant genes mutated CDH1, RHOA, and chromosomal translocation involving CLDN18 and ARHGAP.

Finally, the CIN subtype is enriched for copy number changes in key receptor tyrosine kinase oncogenes such as HER2, EGFR, fibroblast growth factor receptor 2 (FGFR2), and MET. This type is composed mostly of intestinal tumors, and it involves predominantly the gastroesophageal junction. These findings have potentially important therapeutic implications in order to improve the founding of target therapies against the specific key pathways driving the tumor in each individual patient.

Recently, the Asian Cancer Research Group [8] proposed a third molecular classification based on molecular and genetic alterations in gastric cancer. According to this one, it can distinguish four groups of gastric cancer: MSI (23%), microsatellite stable with intact (MSS/TP53-, 36%), microsatellite stable with p53 mutations (MSS/TP53+, 26%), and microsatellite stable with epithelial-mesenchymal transition (MSS/EMT, 15%) [8]. Unlike the TGCA classification, the ACRG reported different outcomes for each gastric cancer's subgroup. In particular, MSI had a better prognosis, whereas MSS/EMT had a worse prognosis with high rate of recurrence and peritoneal involvement. However, further studies are needed to translate these results in clinical practice.

In the next sections, we describe the most relevant therapeutic targets in gastric cancer with notable information about pivotal clinical trials conducted in this area and some resistance mechanisms to targeted agents.

36.5.2 Human Epidermal Growth Factor Receptor 2 (HER2)-Related Pathways: Therapeutic Targeting and Resistance Mechanisms

One of the first molecular pathways studied in gastric cancer was the epidermal growth factor receptor (EGFR) family pathway, which includes EGFR/HER1, HER2/neu, HER3, and HER4 receptors. Each receptor consists of an extracellular ligand-binding domain, an intracellular domain with kinase activity, and a short, lipophilic, transmembrane domain. The binding of ligands to their own receptor leads to homodimerization or heterodimerization with other members of the EGFR family, phosphorylation of intracellular domain, and activation of downstream pathways including the Ras/Raf/mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathways. Stimulation of these pathways influences many aspects of tumor cell biology, such as proliferation, differentiation, migration, and apoptosis (Fig. 36.4). Among these receptors, HER2 plays a key role in gastric cancer.

HER2, encoded at chromosome 17q21, acts as proto-oncogene in many human cancers: its main oncogenic mechanism is represented by gene amplification (determining protein overexpression) or, less commonly, by activating mutations.

HER2 lacks of a known exogenous ligand, and it is transactivated by the interaction with other HER family members (EGFR or HER3 overall) or other tyrosine kinase receptors: its activation leads to a complex signaling cascade already described above. In GC, HER2 overexpression is mainly due to gene amplification: it occurs more frequently in proximal tumors (more than 30% of cases), than in distal cancers (less than 20%). Furthermore, Lauren intestinal subtype shows a higher expression of HER2 (up to 34%) than diffuse subtype (6%), while, concerning to TCGA classification, CIN tumors more often express HER2 as consequence of gene amplification. Different strategies to target HER2 were developed over the years: monoclonal antibodies (like trastuzumab) that bind to the extracellular domain of the receptor and TKIs (tyrosine kinase inhibitors). The pivotal phase III ToGA trial [3] showed that in HER2-positive GCs, the addition of trastuzumab to standard platinum-based first-line treatment was effective, with a median overall survival (mOS) of about 13.8 months in the experimental arm versus 11.1 in the standard one (HR: 0.74; $p = 0.0046$). This OS still represents the highest ever reached in a phase III trial recruiting GC patients. The greatest benefit was observed in high HER2-expressing patients (IHC3+ or IHC2+/FISH+), with an mOS of 16 months versus 11.8 in low HER2-expressing patients (IHC0-1+/FISH+). Therefore, this trial led to the approval of trastuzumab in HER2-positive GC, in the first-line setting for patients with IHC3+ or IHC2+/FISH+ (see Fig. 36.2). Next, it has been speculated that in GC, the addition of pertuzumab (another monoclonal antibody targeting a different HER2 domain than trastuzumab) to trastuzumab itself and platinum-based chemotherapy could improve the ToGA survival rates, leading to JACOB trial design. Unfortunately, this study [10] was negative, because mOS was 17.5 months

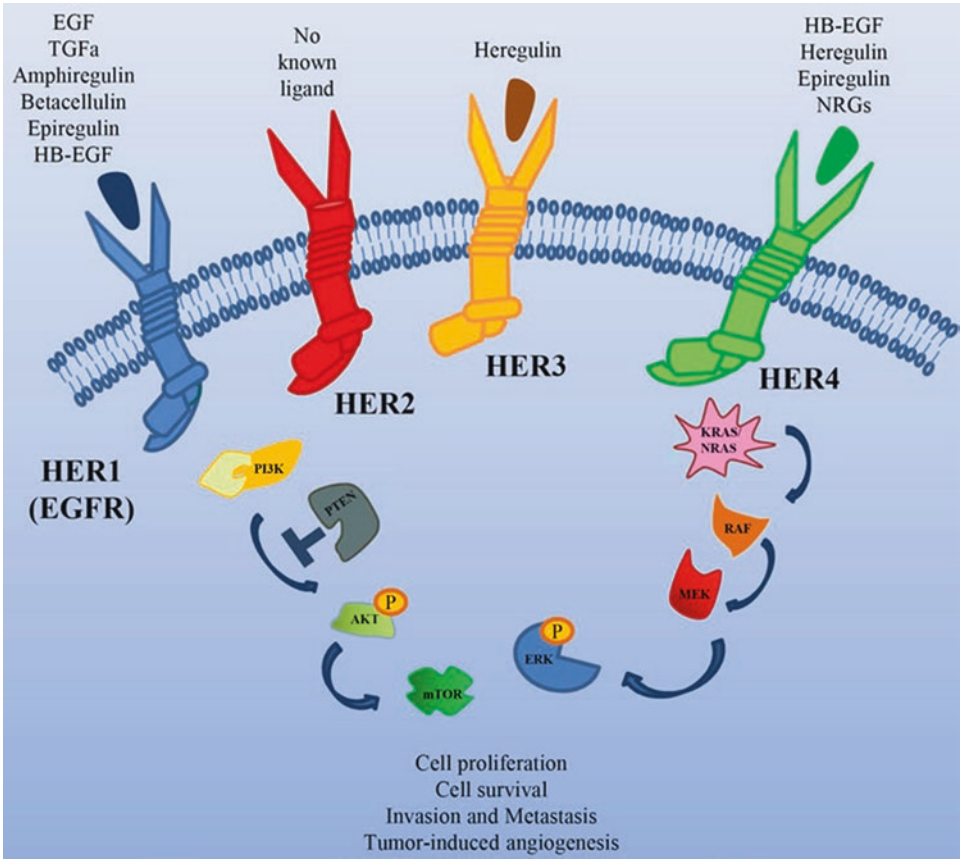


Fig. 36.4 EGFR pathways. (Used with permission from Apicella et al. [9]. See the references for the original source of this material)

in experimental arm versus 14.2 in the standard (HR: 0.84; $p = 0.0565$), a difference that did not find statistical significance. Moreover, trastuzumab emtansine (TDM-1), an antibody-drug conjugate, was studied in second-line therapy of HER2-positive GC (previously treated with trastuzumab) within the GATSBY phase III trial [11]: unfortunately, TDM-1 therapy was not superior to standard taxanes (mOS 7.9 months versus 8.6, respectively, HR: 1.15, $p = 0.86$).

Due to the disappointing results of these trials (JACOB, GATSBY), many researchers began to study mechanisms of targeted therapy resistance in GC, considering that also patients who achieved a significant response to first-line trastuzumab-based treatment can develop resistance within a few months. In fact, one main bias of the second-line trials, especially the GATSBY trial, seems to be the absence of tumor re-biopsy (e.g., at one metastatic site) at screening, taking for granted that the tumor was still HER2-positive on the basis of the "historical" diagnostic biopsy. The study by Pietrantonio et al. [12] clearly showed that a possible acquired resistance mechanism to trastuzumab-based first-line treatment could be the loss of HER2 receptor, especially for patients with dubious immunohistochemistry (IHC2+/FISH+). In that way, the negative results of the GATSBY study could be related to the fact that in a significant proportion of cases, the authors have treated with TDM-1 patients who had become HER2-negative de facto at the beginning of the second line.

More important, even primary resistance to first-line anti-HER2 drugs seems to exist: in fact, objective response rates to trastuzumab plus chemotherapy in ToGA trial was about 50% only, which implies that at least 50% of HER2-positive tumors could have coexisting molecular alterations that confer resistance. In support of this hypothesis, the group lead by Adam Bass [13] clearly showed that almost 50% of HER2-amplified gastroesophageal cancers have preexisting co-amplifications or co-mutations in key oncogenes (others than HER2), for example, cell cycle-related genes (CCNE1, CDK6, and CCND1), RTK-related genes (EGFR, HER3, MET, FGFR2), or PI3K-related genes (PIK3CA, PIK3R1, PTEN). These amplifications/mutations confer resistance to anti-HER2-targeted drugs in cell line experiments. This preliminary report was then confirmed by Pietrantonio et al. [14], who showed that mutations of EGFR, MET, KRAS, PIK3CA, and PTEN or amplifications of EGFR, MET, and KRAS can co-occur in HER2-positive GC and could explain the lack of trastuzumab efficacy and/or the appearance of primary resistance.

Among others, EGFR (or HER-1) is amplified in around 5% of gastric cancers characterized by poor prognosis.

36.5.3 Epidermal Growth Factor Receptor (EGFR)-Related Pathways: Therapeutic Targeting and Resistance Mechanisms

Epidermal growth factor receptor (EGFR) or ERBB1 is a transmembrane tyrosine kinase receptor, expressed approximately in 30% of GC, especially those with chromosomal instability.

Several studies evaluated the safety and efficacy of different anti-EGFR drugs: these therapies include – as we just discussed for HER2 – monoclonal antibodies (like cetuximab or panitumumab) and TKIs (gefitinib, erlotinib). Initial phase II trials combining these agents with cytotoxic chemotherapy in unselected patient population have encouraging results for first-line patients. Unfortunately, all of the phase III published trials investigating the role of anti-EGFR therapy in GC were negative. The EXPAND study [15] randomized first-line GC patients between cetuximab plus capecitabine/cisplatin and chemotherapy alone, showing no advantage for cetuximab arm. However, the patient recruitment was unselected for EGFR positivity, and in a post hoc analysis, the highest survival benefit was observed in a small subset of patients with high EGFR expression. The REAL-III trial [16] demonstrates that adding panitumumab to epirubicin-oxaliplatin-capecitabine was even detrimental, as the mOS for the experimental arm was 8.8 months versus 11.3 months for the standard one (HR: 1.37, $p = 0.013$).

The shocking failure of all anti-EGFR drugs in gastric cancer could be explained with the lack of a proper patient selection. In fact, a recent work by Catenacci et al. [17] showed that EGFR amplified tumors (almost 5% in this study) seem very prone to respond to cetuximab or ABT-806 (an investigational anti-EGFR drug), with an ORR of 58%, a DCR of 100%, and an mPFS of about 10 months. Thanks to the next-generation sequencing (NGS) and circulating tumor DNA (ctDNA) studies, the authors also showed the mechanisms of resistance to anti-EGFR drugs, such as the presence of EGFR-negative tumor clones, KRAS mutation/amplifications, PTEN deletion, and NRAS/HER2/MYC amplifications. This study definitively demonstrates that EGFR amplification is able to predict response to anti-EGFR therapies, despite the negative results in prior unselected phase III trials (EXPAND and REAL-III), but also showed crucial mechanisms of resistance.

36.5.4 MET Pathway: Therapeutic Targeting

MET (mesenchymal-epithelial transition) oncogene, also called hepatocyte growth factor receptor (HGF), is a receptor tyrosine kinase that appears to be deregulated in many human cancers, included in GC. The main known mechanism of MET overexpression in GC is gene amplification, which occurs in about 6% of the TCGA dataset (especially in CIN tumors). However, even tumors without gene amplification can express (or overexpress) MET, although it is not clear whether these tumors really depend on MET for survival and malignant properties. Two monoclonal antibodies, rilotumumab (an anti-HGF antibody) and onartuzumab (an anti-MET antibody), were tested in clinical trials in GC: both phase III clinical trials evaluating onartuzumab and rilotumumab were negative.

The METGastric phase III trial [18] evaluated the addition of onartuzumab to a chemotherapy backbone (mFOLFOX6) and enrolled 562 GC patients with HER2-negative/MET-positive tumors. The enrollment was early stopped due to sponsor decision, for a lack of efficacy. Unluckily, the addition of onartuzumab to mFOLFOX6 did not result in an improvement of OS (11 months in the experimental arm versus 11.3 in standard, HR: 0.82, $p = 0.24$). Negative results were obtained also with rilotumumab within the RILOMET-1 phase III trial [19], which used a different chemotherapy backbone (epirubicin plus cisplatin and capecitabine). As for the previous trial, results were clearly negative with a detrimental effect (mOS was 8.8 in experimental arm versus 10.7 in the placebo group, HR: 1.34, $p = 0.003$), and, again, study treatment was stopped early, because an independent data monitoring found a higher number of deaths in the rilotumumab group. Probably the main limit of RILOMET and METGastric trials is to have included mostly patients in whom MET was not a clear "driver" of the disease, since the highest expressing tumors (MET gene amplification) are underrepresented, which can explain the negative results described.

36.5.5 VEGF Pathway: Therapeutic Targeting

In the TCGA "CIN" subtype, vascular endothelial growth factor (VEGF), a crucial mediator of normal and pathogenic angiogenesis, is frequently amplified (up to 7% of cases). However, initial studies with bevacizumab (a monoclonal antibody targeting VEGF-A) were negative, such as the AVAGAST trial [20] and the Asiatic AVATAR trial [21], in which bevacizumab was combined with platinum-based chemotherapy

in the first-line setting. Subsequently, ramucirumab, a fully human monoclonal antibody directed against VEGFR2 (vascular endothelial growth factor receptor 2), the main receptor of the VEGF system, has been used in the second-line setting alone [22] or in combination with weekly paclitaxel [23]. Both studies were positive, with the REGARD trial showing a significant improvement in OS with ramucirumab alone versus BSC (mOS 5.2 months versus 3.8, respectively, HR: 0.776, $p = 0.047$) and the RAINBOW trial showing a significant superiority of combination arm (ramucirumab plus paclitaxel) versus paclitaxel alone (mOS 9.63 months versus 7.36 months, respectively, HR: 0.807, $p = 0.017$).

On that positive basis, ramucirumab has been tested in first-line setting in combination with cisplatin-based standard chemotherapy within the RAINFALL trial [24]: although the study formally met its primary endpoint, with an improvement in mPFS from 5.4 months (placebo arm) to 5.7 months (ramucirumab arm) (HR: 0.75, $p = 0.011$), there was no survival benefit for patients in the experimental arm, making the results negative de facto and not significant for clinical practice. Therefore, the role of antiangiogenic agents seems to be essential in second-line setting, but in the first line, like the AVAGAST and AVATAR trial, showed for bevacizumab, probably we need to better understand the patients who really benefit from this strategy.

36.5.6 Tumor Microenvironment: The Biological Basis of Immune Checkpoint Usage in Metastatic Gastric Cancer

Immunotherapy deeply changed the therapeutic landscape for several malignancies (advanced melanoma, lung, urothelial, kidney cancer, etc.) determining a completely unexpected improvement of survival by boosting the body's natural defenses to fight cancer.

As already reported, comprehensive molecular characterization performed by the TGCA group showed a relatively high mutational load (up to 10–15 mutations per megabase) in about 34% of gastric adenocarcinomas analyzed and a subset of tumors with microsatellite instability-high (MSI-H, 22%) or with an ideally favorable immune environment (the "EBV-related" subgroup that shows molecular hallmarks of sensitivity to immunotherapy, such as intratumoral or peritumoral immune cell infiltration and PD-L-1/PD-L-2 expression), suggesting that also gastric cancer could be a promising "fertile soil" for immunotherapy, especially based on immune checkpoint inhibitors [25].

36.6 Prognostic Factors

Despite the expanding knowledge about molecular mechanisms that lead to a better comprehension of GC, the prognosis of this tumor is still poor, especially in case of locally advanced or metastatic disease. In this context, the research for prognostic and predictive factors became particularly relevant.

Diffuse histotype, performance status, and number and location of distant metastasis are the principal prognostic factors in the metastatic setting. According to these and other biochemical factors, different prognostic scores have been validated over the past years. The Royal Marsden prognostic score [26, 27] divides GC patients into three risk groups on the bases of four parameters: performance status, liver metastasis, peritoneal metastasis, and serum alkaline phosphatase. Patients with peritoneal metastasis, performance status ≥2, and serum alkaline phosphatase ≥100 U/L had the worse prognosis, with a 1-year survival of 11% compared to 25.7% and 48.5% in the moderate- and low-risk groups, respectively.

In addition to these parameters, many trials showed that tumor prognosis may be influenced not only by tumor features themselves but also by tumor microenvironment. In this context, the neutrophil/lymphocyte ratio (NLR) in venous peripheral blood has been highly investigated in order to find a possible simple and quick prognostic factor. A recent research [28] showed that in a clinical cohort of 151 metastatic gastric cancer patients, NLR obtained before starting first-line chemotherapy is a strong independent predictor of poor survival, suggesting its utility for a quick and cheap patient prognostic stratification.

Regarding prognostic scores for mGC patients receiving a second-line treatment, an Italian model (Gastric life nomogram) showed to predict 12-week life expectancy for these patients [29]. However, all these promising factors need to be further validated in prospective clinical trials.

36.7 Treatment

Chemotherapy represents the standard treatment for unresectable locally advanced and metastatic gastric cancer, showing improvement of survival and quality of life compared with best supportive care [30]. ◘ Figure 36.5 summarizes the current "state of the art" for treatment selection in metastatic GC patients.

Despite of the term "advanced gastric cancer" comprising also patients with inoperable locally advanced tumors, it is important to distinguish this group of patients from the metastatic one, because in this case patients have not distant metastasis and tumor could be

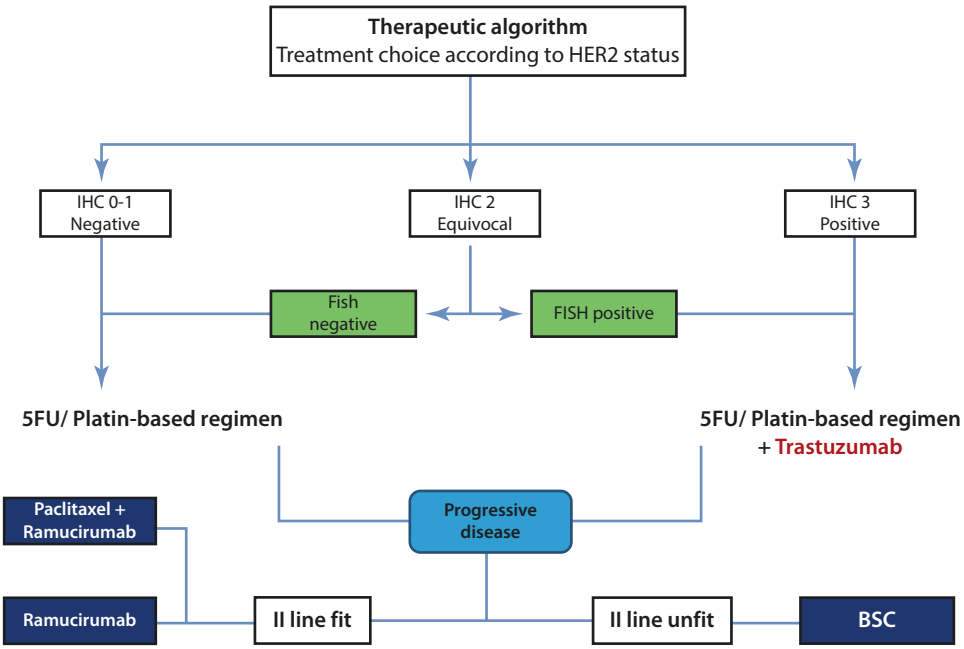


Fig. 36.5 Biomarker-driven therapy for advanced gastric cancer in 2020

converted into an operable disease after a chemotherapy response. Therefore, more aggressive and active chemotherapy schedules are recommended for these patients as a conversion therapy in order to obtain a tumor downsizing and downstaging. On the other hand, it is important to consider that the target of treatment in case of metastatic disease is the palliation, because we still do not have sufficient evidence to support the recommendation of tumor resection in this population, and surgery does not prolong survival and can even produce a detrimental effect (see below for more details). Moreover, the general clinical condition of these patients are frequently poor so a multidisciplinary evaluation of different aspects of disease, comprising a nutritional and toxicity evaluation as well as the palliation of symptoms, is fundamental to improve the efficacy of active treatments.

The nutritional assessment is crucial since the first take charge in order to prevent malnutrition and to avoid the poor compliance and tolerability caused by nutritional condition decline.

Because of tumor locations (cardia or antrum) and possible luminal obstruction, it is necessary sometimes to resort to parenteral nutrition.

Response to systemic treatments should normally be assessed with interval imaging of the chest, abdomen, and pelvis, mostly with computer tomography (CT) scan, although alternative imaging techniques may be used if required to monitor known sites of disease (e.g., magnetic resonance imaging for brain lesions). The evaluation of response is according to standard radiologic criteria for solid tumor, also known as RECIST criteria, except in case of immunotherapy in which the immune-modified RECIST (iRECIST) should be used.

36.7.1 First Line

The determination of HER2 status is essential before starting a first-line therapy in order to distinguish HER2-negative and HER2-positive gastric cancer, selecting patients for appropriate treatment with trastuzumab (an anti-HER2 monoclonal antibody). However, a more complete molecular dissection before starting a first-line chemotherapy is today highly desirable, considering the promising results of the recently presented KEYNOTE-062 trial [31], in which first-line metastatic GC patients with a MSI-H disease or a high expression of PD-L1 received greater benefit from anti-PD-1 pembrolizumab compared to standard chemotherapy arm (HR: 0.29, 95% IC 0.11–0.81). For this reason, MSI testing is absolutely recommended, although immune checkpoint inhibitors are not yet approved for this indication in EU nowadays.

No anti-HER2 agent showed a survival benefit beyond the first-line setting indeed.

36.7.1.1 Chemotherapy

In patients with HER2-negative disease, the only effective therapeutic option we have to date is chemotherapy. However, despite the use of the most modern regimens, the survival of these patients remains overall poor (median OS: 11 months), even if a correct "continuum of care" strategy and molecular selection is starting to lead to less rare longer survivals.

Polichemotherapy is still the standard first-line treatment for patients with a good performance status, while best supportive care alone is recommended in cases with poor clinical conditions considered "unfit" for active treatments.

Doublet combinations of platinum (either cisplatin or oxaliplatin) and fluoropyrimidines (5-fluorouracil or capecitabine) showed greater benefit if compared to mono-chemotherapy and are generally used in fit patients as standard regimens [30].

On the other side, the utility of triplet regimens as first-line therapy is still under debate, and their use should be evaluated, in the context of a multidisciplinary discussion, only in selected cases. For example, triplet regimen utility could be speculated in GC patients with:

1. Locally advanced disease, in which a more active regimen (like a triplet one) could lead to tumor downstaging and to a possible rescue to radical surgery on primary tumor
2. High tumor burden disease with severe symptoms, in which a rapid clinical response (such as that obtainable with triplet regimen) could be required to improve patient general clinical conditions and to achieve a more rapid symptom recovery (i.e., for severe dysphagia)
3. Oligo-metastatic diseases, in which a triplet-based "neoadjuvant" approach (e.g., with a taxane-based regimen such as "FLOT") could be followed by primary plus metastatic lesion(s) surgical resections, according to preliminary results of the phase II AIO FLOT 3 trial [32]

Triplets containing taxanes (DCF, FLOT) showed survival benefits in first-line setting, while schedules containing anthracyclines, although initially associated with better outcomes, today must not be used anymore, as we later explain.

In the phase III randomized trial TAX-325 [33], the addition of docetaxel to 5-FU/cisplatin in a three weekly regimen named DCF was associated with improved overall survival in first-line therapy (OS: 9.2 versus 8.6 months) but at the cost of significantly more toxic effects, including increased rates of febrile neutropenia. For this reason, other studies have examined the efficacy of alternative taxane-based triplets, like FLOT regimen (docetaxel, fluoropyrimidine, and oxaliplatin), with positive results both in terms of efficacy and tolerability [34].

With regard to anthracycline-based triplets, the REAL-II trial [35] demonstrated non-inferiority between ECF, ECX, EOF (epirubicin, oxaliplatin, 5-FU), and EOX (epirubicin, oxaliplatin, and capecitabine), making the substitution of 5-FU with capecitabine and cisplatin with oxaliplatin possible. However, as already anticipated, anthracycline-containing regimens should not be considered anymore for GC patient treatment: in fact, according also to a famous editorial by Jaffer Ajani, only three drugs have demonstrated an OS improvement in first-line setting – forming level I of evidence – and they are docetaxel, cisplatin, and trastuzumab, while epirubicin has never gained this "honor." As a matter of fact, a standard doublet has been demonstrated to be as effective as an anthracycline-base triplet but with significant less toxicity. For this reason and for the increased cardiac risk that is associated with these drugs, we can assert that today no GC patient should continue to receive epirubicin-based triplet.

To reinforce this concept, we refer to a fundamental study lead by Guimbaud R et al.: in this trial, the FOLFIRI regimen (irinotecan plus leucovorin and infusional 5-FU) was compared to the anthracycline-based ECX regimen in first-line setting. The authors showed a non-inferiority of doublet versus triplet regimen combination, supporting once more the necessity to avoid anthracycline from gastric cancer therapy, because it cannot add nothing to survival benefit.

Furthermore, in a different setting (neoadjuvant) the taxane-based triplet FLOT showed its superiority, in terms of responses and survival, over the epirubicin-based triplet [36].

The S1 fluoropyrimidine is an another orally choice to be evaluated in association with cisplatin in first-line setting in Asiatic population, while it is not recommended in the Caucasian due to high rate of toxicity in this population [37].

In conclusion, data are not supporting the use of triplet regimens in all patients with metastatic gastric cancer, but only in selected patients (see above), even if an increase of side effects should be considered.

36.7.1.2 Chemotherapy for HER2-Positive Disease

In the first-line treatment of HER2-positive gastric cancer, the phase III ToGA trial demonstrated clinically and statistically significant improvements in response rate, progression-free survival (PFS), and OS with the addition of trastuzumab to cisplatin/fluoropyrimidine doublet [3], especially in patients with higher expression of the protein (HER2 3+ at IHC or 2+ IHC with FISH amplification).

Based on the ToGA results, trastuzumab was approved in many countries in addition to cisplatin-fluoropyrimidine doublet as first-line standard of care in patients with HER2-positive disease. This drug is currently used at the same dose of HER2-positive breast cancer (8 mg/Kg in the first induction dose and then 6 mg/Kg every 21 days), even if today it is clear that HER2-positive gastric cancer is biologically different from the breast one. However, the addiction of trastuzumab with different schedule to chemotherapy did not show any benefit in patients with HER2-positive metastatic gastric cancer [18, 38]. Moreover, trastuzumab is actually investigated in adjuvant and neoadjuvant setting for HER2-positive gastric cancer.

Unfortunately, trastuzumab remains the only anti HER2 target therapy approved in the first-line setting.

Lapatinib, an oral inhibitor of tyrosine kinase domain of EGFR and HER2, failed to add the same efficacy as trastuzumab in addiction to capecitabine and oxaliplatin.

Similarly, negative results were achieved by Pertuzumab within the Jacob trial [10], as already reported in the previous section. For this reason, pertuzumab is not actually approved in addition to standard first-line treatment.

Anti-HER2 strategy beyond first-line setting is actually not recommended. The TDM-1 (an "antibody-drug conjugate" in which the molecule of trastuzumab is combined with a cytotoxic drug) did not show a survival benefit in the second-line treatment of patients previously treated with trastuzumab [11].

36.7.2 Second Line

Approximatively 40% of patients (and even more in high-volume centers) with metastatic gastric cancer patients receive a second-line treatment after the first-line failure. Second-line treatment is recommended in patients with a progressive disease and with a good performance status. An active treatment is associated with an improvement in OS and quality of life compared with best supportive care.

Among different chemotherapy agents and schedules investigated in this setting, taxanes, irinotecan [39], and ramucirumab (alone or in association with paclitaxel) showed a survival benefit with a good toxicity profile.

In particular, the COUGAR trial showed a benefit in OS for docetaxel if compared to best supportive care (median OS: 5.2 vs 3.6 months) [40], and the randomized phase III trial by Hironaka directly compared weekly paclitaxel with irinotecan and demonstrated similar efficacy and feasibility for both regimens [41].

In 2014, two randomized phase III clinical trials [22, 23] demonstrated the efficacy of ramucirumab (alone or in combination with weekly paclitaxel, respectively) in second-line setting. To note, until this moment, no target agents have shown a benefit in second line in association with chemotherapy with the exception of this drug. Ramucirumab is in fact a fully humanized monoclonal antibody that binds the extracellular domain of vascular endothelial growth factor receptor 2 (VEGFR2). Its mechanism of action prevents the binding with VEGF-A, VEGF-C, and VEGF-D leading to a strong antiangiogenetic property. As a single agent in the REGARD trial [22], ramucirumab was associated with a survival benefit versus best supportive care alone (median OS: 5.2 versus 3.8 months). Moreover, in addition to paclitaxel in RAINBOW trial [23], it was reported a survival benefit compared with paclitaxel alone of 2.2 months (median OS: 9.6 versus 7.4 months), with improvement also in PFS and objective response rate.

In patients with disease progression >6 months following first-line chemotherapy, the evaluation of a rechallenge with the same drug combination used in first line may be also appropriate.

Ramucirumab remains the only biological agent approved in second-line treatment for HER2-positive and HER2-negative gastric cancer today, while specific anti-HER2 drugs, such as lapatinib and TDM-1, did not improve survival in HER2-positive gastric cancer that progressed after a first-line treatment containing trastuzumab. In particular, TDM1, as already mentioned above, was studied in the GATSBY trial [11] and compared to taxanes showing no superiority in patients with previously treated, HER2-positive advanced gastric cancer. Similar results were reported for lapatinib associated with paclitaxel in the TYTAN phase III study [42], without significant difference in OS and PFS compared to paclitaxel alone.

Other targeted therapies investigated in this setting, such as sorafenib and sunitinib, did not show clinical benefit. Due to these reasons, the actual second-line treatment in HER2-positive gastric cancer is not different from HER2-negative one.

36.7.3 Third-Line Therapy and Beyond

Thanks to the novel drugs and the improvement of supportive care (especially nutritional support), a biggest amount of patient (20–25% approximately) is arriving in good clinical condition beyond a second line of treatment.

This is why a correct "continuum of care strategy" should be always supposed and tailored on the single patient features.

Current European guidelines do not recommend any specific treatment for patients with disease refractory to two or more previous regimens.

Despite this assumption, a third-line strategy with active chemotherapy should be taken into account for selected patients, if we consider the positive results of the recently published TAGS trial [43].

This was the first phase III clinical trial to evaluate GC patients who had received at least two previous chemotherapy lines: subjects were randomly assigned to receive oral trifluridine/tipiracil (TAS102) or placebo. The study met its primary endpoint, and in fact, median OS was considerably better in the experimental arm compared to placebo arm (5.7 months versus 3.6 months, HR: 0.69, $p = 0.00029$), and the treatment was well tolerated, with manageable adverse events (the most common in the TAS102 arm were neutropenia and anemia, compared to abdominal pain and deterioration of clinical condition in the placebo arm). So for the first time ever, the TAGS trial paved the way to a real "con-

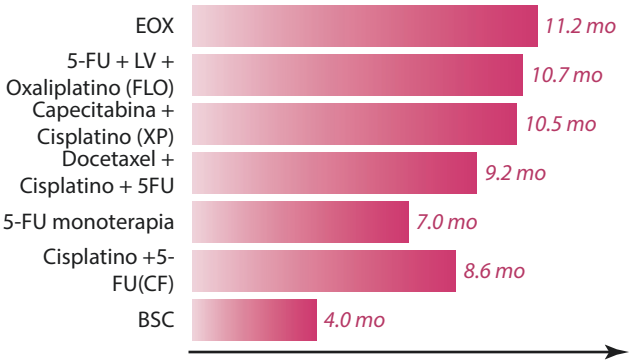


Fig. 36.6 Median OS in patients with advanced/metastatic gastric cancer. The "continuum of care" (see next in the text) has greatly improved quantity and quality of life

tinuum of care" concept even in GC, because we now have effective first-, second-, and third-line therapies, and their sequential usage could greatly expand the survival of GC patients (see Fig. 36.6) as well as their quality of life.

Moreover, a multidisciplinary evaluation is crucial in every step of natural history of gastric cancer due to the particular worsening of clinical condition that this disease produces. For example, as already reported, a nutritional support should be evaluated after all lines of treatment as well as the palliation of dysphagia or pain. After the third line, if the patient is still in good clinical conditions, the choice of new chemotherapy schedule should be done according to previous treatments, patient's preference, performance status, and clinical trials eventually available.

As reported below, in this setting of treatment, there is also a possible place for immunotherapy.

36.7.4 Immunotherapy

Emerging data from early-phase trials have suggested that the use of immunotherapy may improve survival in patients with advanced gastric cancer. In particular, the research focused on immune checkpoint of programmed cell death 1 and its ligands (PD-1/PD-L1). PD-1 is a receptor expressed on the surface of tumor cells, macrophages, activated dendritic cells, and T and B lymphocytes. As mentioned above, this receptor acts as a coinhibitor, leading to suppression of immunological T-lymphocyte-mediated response in tumor microenvironment. The TCGA molecular classification identified elevated PD-L1 expression especially in the EBV subtype.

Cancer cells use these factors and other mechanisms in order to elude the immune system reaction.

Monoclonal antibodies that target either PD-1 or PD-L1, such as pembrolizumab, nivolumab, and avelumab, can block this checkpoint inhibition and stimulate the immune response against tumor.

In a certain way, the immune system is "remodulated" in order to fight the cancer cells itself.

The phase III trial ONO-4538-12 "ATTRACTION-2" represents the current milestone for the development of immunotherapy with nivolumab (anti-PD-1 antibody) in the chemotherapy-refractory molecularly unselected population. In this entirely Asian trial, surprising survival rates of 27.3 and 10.6% at 1 year and 2 years, respectively, have been achieved in the nivolumab arm. Responders to immunotherapy had a 12-month survival rate of 86.7%, suggesting the presence of a subset of patients who greatly benefit from "checkpoint inhibition" strategy.

This trial is the only phase III positive one to date.

Immunotherapy is quickly evolving also for GC, and the correct patient selection is going to be clarified, even if the results of trials available are controversial and often negative across the different settings of treatment.

As a matter of fact, in first- and second-line setting, immunotherapy did not significantly improve survival compared to standard chemotherapy both in Asian and Western patients in two recent phase III randomized trials: KEYNOTE-062 and KEYNOTE-061.

However, although these trials have been formally negative on the whole unselected population, they have been able to recognize a subgroup of patients who benefited most from immunotherapy. Exploratory analyses identified MSI-H status and PD-L1 positivity (with CPS >1% and especially 10%) as strong positive predictor factor for immunotherapy with pembrolizumab, leading to regulatory agency approval in the USA (as previously in some Asian countries according to ATTRACTION-2 trial).

At current time, European guidelines do not recommend immunotherapy in the routine clinical practice, but the future perspectives for these drugs are promising also for GC, thanks to brilliant results in well-selected population.

36.7.5 Particular Conditions

36.7.5.1 Surgery of Primary Tumor and Metastasectomy

Surgery of primary tumor in case of metastatic disease is recommended only in the event of bleeding or luminal obstruction with a palliative intent.

Patients with metastatic cancer in fact do not benefit from addition of gastrectomy to chemotherapy as demonstrated by the randomized phase III REGATTA trial [44]. Furthermore, the surgical approach may determine a detrimental effect delaying the systemic treatment, favoring immunosuppression and aggravating the nutritional status of the patient [45].

Anyway the REGATTA trial had a number of limitations (first of all, it did not provide for the resection of the metastatic lesions, while a good surgery has always to be radical in oncology), and further trials are investigating the possible role of surgery in the "oligometastatic" population, in order to give a survival benefit in selected patients and not only a palliative meaning [46].

The most important one is currently the phase II FLOT-3 trial [32].

This trial demonstrated a possible role of surgery (both primary and metastatic lesions resection) in patients with limited metastatic disease who received neoadjuvant chemotherapy and had a good response. In patients with only retroperitoneal lymph node involvement, liver or lung involvement, and localized peritoneal involvement (all with a significant change of margin-free resection of the primary tumor and at least a macroscopic complete resection of the metastatic lesions at the posttreatment restaging), surgery showed a favorable survival (median overall survival of 31.3 months, while survival in unresected patients was 15.9 months).

This data needs a further validation and a dedicated phase III trial is ongoing at current time [47].

36.7.5.2 Peritoneal Involvement

The role of specific peritoneal treatment using hyperthermic intraperitoneal chemotherapy (HIPEC) is still controversial. Several small randomized trials in Asian patients have demonstrated a significant survival benefit for adjuvant HIPEC after cytoreductive surgery, but actually there are no solid data in non-Asian population [48–50]. For these reasons, the HIPEC is currently considered an experimental approach that should not be used in daily clinical practice, as well as the more modern PIPAC (pressurized intraperitoneal aerosol chemotherapy) [51–53].

Summary of Clinical Recommendations

- *AIOM*
  - Polichemotherapy should be considered in the first-line treatment of fit patients with advanced gastric cancer.
  - Trastuzumab in combination with platinum and fluorouracil should be considered the standard treatment for first-line HER2-positive gastric cancer patients.
  - Anti-EGFR drugs, such as cetuximab and panitumumab, are not recommended in treatment of gastric cancer.
- *ESMO*
  - Doublet or triplet platinum/fluoropyrimidine combinations are recommended for fit patients with advanced gastric cancer.
  - Trastuzumab is recommended in conjunction with platinum- and fluoropyrimidine-based chemotherapy for patients with HER2-positive advanced gastric cancer.
  - Second-line chemotherapy with a taxane (docetaxel, paclitaxel), or irinotecan, or ramucirumab as a single agent or in combination with paclitaxel is recommended for patients who are of PS 0–1.
- *NCCN*
  - Trastuzumab should be added to first-line chemotherapy for HER2 overexpressing metastatic adenocarcinoma.
  - Trastuzumab is not recommended for use with anthracyclines.
  - Two drug cytotoxic regimens are preferred because of lower toxicity, while three-drug regimens should be reserved for medically fit patients.

Case Study: An Unusual Clinical Progression

Man: 54 years old

- *Family history*: Negative for malignancy
- *APR*: Hypertension, psoriasis
- *APP*: For nearly 2 months fatigue and epigastralgia
- *Objective examination*: Negative. Performance status 0 according to ECOG
- *Blood tests*: Hb 7.1 g/dl
- *Esofagogastroduodenoscopy*: Presence of ulcerative area in the antrum of the stomach
- *Pathological report*: Gastric adenocarcinoma (diffuse type according to Lauren's classification)
- *TC chest and abdomen mdc*: Lesion at the antrum of the stomach with multiple perigastric lymphadenopathies. No distant metastasis

⇩

- *Surgery*: Partial gastrectomy with D2 lymphadenectomy
- *Pathological report*: Diffuse gastric adenocarcinoma limited to the mucosa with involvement of 4/20 lymph nodes resected. No margins or perivascular invasion
- *Pathological stage*: pT1N2
- Stage: pT1N2cM0 (stage IIA)

Question

What action should be taken?

(1) Follow-up (2) Adjuvant chemotherapy (3) Adjuvant chemoradiotherapy

Answer

Adjuvant Chemotherapy

Patient received 12 cycles of FOLFOX chemotherapy

⇩

Follow-up according to international guidelines for 5 years

- *After 3 years from the last follow-up visit*: Appearance of the right eyelid swelling with ptosis, cutaneous nodules at the neck and in the frontal region

Question

What action should be taken?

(1) Dermatologic visit (2) Cutaneous biopsy

Answer

Cutaneous biopsy

Tumor cells with an upper gastrointestinal origin. In consideration of the clinical history of patient, this record is in line with a cutaneous progression of disease.

⇩

- *Clinical evaluation*: Presence of nodules with increased consistency, no defined margins. Performance status 0 according to ECOG. No weight loss
- *CT scan*: No distant metastasis
- *Diagnosis*: Progression of disease (cutaneous non-resectable metastasis)

Question

What action should be taken?

(1) First-line chemotherapy upfront (2) Definition of HER2 status

Answer

Definition of HER2 status

HER2 status (IHC): 0

⇩

- *First-line chemotherapy with 12 cycles of Xelox schedule*: Major cutaneous response with reduction of all nodules and reduction of consistence
- *Clinical and instrumental follow-up every 3 months*: Maintenance of response
- *After PFS of 9 months*: Increase of known cutaneous lesions

Question

What action should be taken?

(1) Second-line chemotherapy upfront (2) Re-biopsy with definition of HER2 status

Answer

Re-biopsy with definition of HER2 status

Tumor cells with an upper gastrointestinal origin. HER2 status (IHC): 0

Question

What action should be taken?

(1) Rechallenge of Xelox (2) Taxolo + Ramucirumab (3) Ramucirumab (4) Irinotecan

Answer

Second-line with Taxolo + Ramucirumab. The decision was based on time of oxaliplatin exposure

Good performance status (0 according to ECOG)

Multidisciplinary evaluation

Key Points

- The importance of a correct diagnosis even in case of unusual clinical presentation
- The importance of a correct choose of treatment based on HER2 status of tumor
- Importance of re-biopsy after progression to evaluate changes in tumor characteristic

Case Study: A 32-Year-Old Man with a Metastatic Gastric Cancer

Man: 32 years old

- *Family history*: Negative for malignancy
- *APR*: Negative
- *APP*: Weight loss of 12 Kg in the last 3 months, fatigue
- *Blood tests*: Hb 10.2 g/dl
- *Esofagogastroduodenoscopy*: Presence of ulcerative area in the body of the stomach. Diffuse involvement of all stomach's wall
- *Pathological report*: Gastric adenocarcinoma (diffuse type according to Lauren's classification)
- *TC chest and abdomen mdc*: Diffuse involvement of stomach, perigastric and lombo-aorthic lymph nodes. Presence of multiple liver metastases with a maximum diameter of 12 cm

Question

What action should be taken?

(1) Surgery (2) First-line chemotherapy (3) Multidisciplinary group evaluation

Answer

Multidisciplinary group evaluation

Nutritional assessment

Pain evaluation

Oncological assessment → stage IV, performance status 1 according to ECOG

Question

What action should be taken?

(1) First-line chemotherapy upfront (2) Definition of HER2 status

Answer

Definition of HER2 status. HER2 status (IHC): 0

- *First-line chemotherapy with cisplatin/fluorouracil schedule*, ongoing
- *First instrumental assessment after three cycles*: Stable disease

Key Points

- Surgery is not recommended in case of metastatic disease at the diagnosis even in case of young patient
- Importance of multidisciplinary approach
- Importance of evaluation of performance status and HER2 status before starting treatment

Expert Opinion

Clara Montagut
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Key Points

1. The prognosis of this neoplasm is still poor above all in case of locally advanced or metastatic disease. Diffuse histotype, performance status, and number and site of distant metastasis are the principal prognostic factors in the metastatic setting. The Royal Marsden prognostic score individualizes three risk groups of patients on the base of four parameters: performance status, liver metastasis, peritoneal metastasis, and serum alkaline phosphatase.
2. In the metastatic setting, the research of prognostic and predictive factors is more than relevant in order to select patients to treat. Other important aspects are tumor microenvironment, immunological state of the patient, and molecular features of the neoplasm.
3. Polichemotherapy (doublet or triplet platinum/fluoropyrimidine) should be considered in the first-line treatment of fit patients with advanced gastric cancer.
4. Trastuzumab in combination with platinum and fluorouracil should be considered the standard treatment for first-line HER-2-positive gastric cancer patients.
5. Anti-EGFR drugs, such as cetuximab and panitumumab, are not recommended in treatment of gastric cancer.
6. Second-line chemotherapy with a taxane (docetaxel, paclitaxel), or irinotecan, or ramucirumab as a single agent or in combination with paclitaxel is recommended for patients who are of PS 0–1.
7. Trastuzumab is not recommended for use with anthracyclines.
8. Two-drug cytotoxic regimens are preferred because of lower toxicity, while three-drug regimens should be reserved for medically fit patients.

Recommendations

- *ESMO*
  ► https://www.esmo.org/Guidelines/Gastrointestinal-Cancers/Pan-Asian-adapted-ESMO-Clinical-Practice-Guidelines-for-the-management-of-patients-with-metastatic-gastric-cancer
- *ASCO*
  ► https://www.asco.org/practice-guidelines/quality-guidelines/guidelines/gastrointestinal-cancer#/14446

Hints for a Deeper Insight

- Progress in the treatment of advanced gastric cancer: ► https://www.ncbi.nlm.nih.gov/pubmed/28671042
- Expression Profile of Markers for Targeted Therapy in Gastric Cancer Patients: HER-2, Microsatellite Instability and PD-L1: ► https://www.ncbi.nlm.nih.gov/pubmed/31595457
- From Tumor Immunology to Immunotherapy in Gastric and Esophageal Cancer: ► https://www.ncbi.nlm.nih.gov/pubmed/30577521
- Prognostic value and association of Lauren classification with VEGF and VEGFR-2 expression in gastric cancer: ► https://www.ncbi.nlm.nih.gov/pubmed/31611999

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Colorectal Cancer: Locoregional Disease

Erika Martinelli, Claudia Cardone, and Giulia Martini

Gastrointestinal Cancers

Contents

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Learning Objectives

By the end of the chapter, the reader will:

- Have learned the basic concepts of CRC carcinogenesis
- Be able to apply CRC screening procedures
- Have reached in-depth knowledge of CRC clinical presentation and diagnostic work-up
- Have learned the key role of MTDs in CRC work-up
- Be able to put acquired knowledge into early CRC treatment

37.1 Introduction

Colorectal cancer (CRC) is the third most common tumour in men and the second in women, accounting for 10% of all tumour types worldwide; incidence is higher in males (ratio, 1:4). Country-specific incidence rates are available through the World Health Organization (WHO) GLOBOCAN database. Despite mortality has declined progressively due to effective screening strategies and better treatment procedures, CRC remains the fourth most common cancer-related cause of death in the world [1–3].

Risk factors for developing CRC are:

- Lifestyle or behavioural factors (smoking, high red meat consumption, obesity, low physical activity)
- Personal history (previous polyps, inflammatory bowel disease)
- Genetically determinant factors

Screening tests should be offered to average population (range according to screening programme, 50–74 years old) (Table 37.1) and high-risk subjects (Table 37.2) in order to detect precancerous conditions and early-stage disease, susceptible of a curative treatment [4–6]:

- Personal history of adenoma, previous CRC, inflammatory bowel disease (Crohn's disease and ulcerative colitis).
- Family history of CRC or polyps.

Table 37.1 Screening procedures to be offered to average risk for CRC developing population

Screening method (average-risk population)	Frequency
Faecal occult blood test (FOBT) (immunochemical test> guaiac-based test)	Yearly
Flexible sigmoidoscopy (FS)	5 years
Colonoscopy	10 years
Computed tomography colonography Circulating methylated SEPT9 DNA	Currently not recommended in guidelines

Table 37.2 Screening procedure to be offered to high risk for CRC developing population

Screening method (high-risk population)	Initiation of screening	Frequency of colonoscopy
Familial polyposis	Teen age	Every 1–2 years
Lynch syndrome	20–25 years or 5 years before the youngest case in the family	
Family history of colorectal cancer or polyps (first degree relative ≤60 years)	40 years or 10 years before the youngest case in the family	Every 5 years
Crohn's colitis/ ulcerative colitis	After 8 years of chronic disease	Every 2 years
Personal history of colorectal cancer	One year from baseline colonoscopy	
High-risk adenoma	After 3 years from baseline colonoscopy	
Low-risk adenoma	After 5 years from baseline colonoscopy	

- Genetic syndromes: familial adenomatous polyposis (FAP) coli and its variants, Lynch-associated syndromes and associated polyposis syndromes (MUTYH; Turcot; Peutz-Jeghers). In case of suspicious of genetic syndromes, it is recommended a genetic counselling.

37.2 Carcinogenesis

CRC is a heterogeneous disease, developed by complex multistep genetic and environmental influences. Three mechanisms often in overlap are implicated in CRC carcinogenesis (Fig. 37.1) [7, 8].

- Fearon and Vogelstein proposed a stepwise genetic model to explain the traditional transition from adenoma to carcinoma, initiated by mutations in *APC* (*adenomatous polyposis coli*) followed by mutations in *KRAS* and *TP53*, in the context of genomic instability, aneuploidy and loss of heterozygosity (LOH). This pathway, mainly related to chromosomal instability (CIN), constitutes most of the sporadic tumours (85%) and is associated with familial adenomatous polyposis caused by germline mutations in the *APC*.
- The microsatellite instability (MSI) pathway is caused by the loss of DNA mismatch repair (MMR) activity. MSI phenotype is detected in about 15% of CRC: in 3% is associated with familial Lynch syndrome (mutations in MMR genes, more fre-

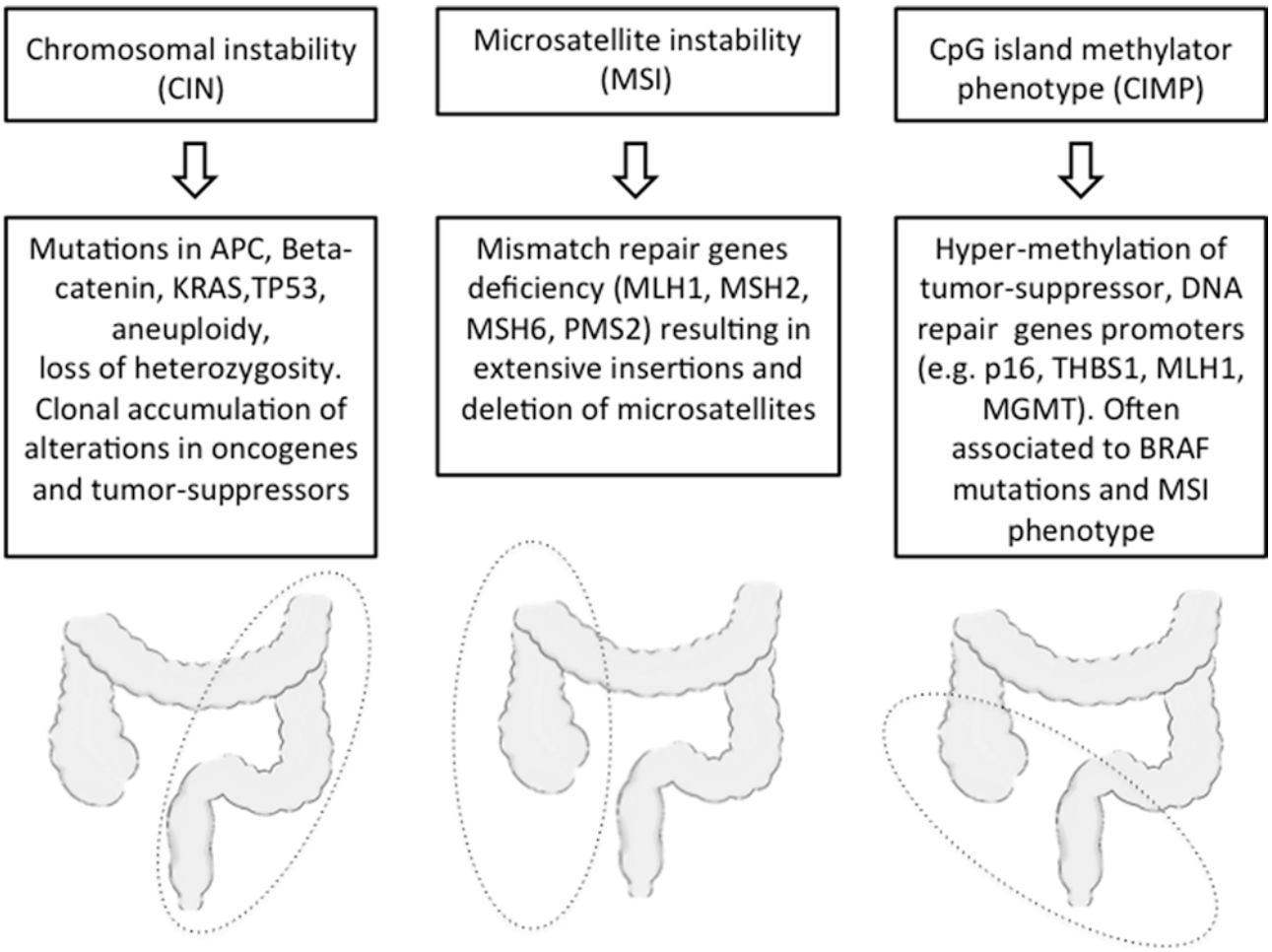


Fig. 37.1 Three pathways involved in colorectal carcinogenesis

quently in *MLH1* and *MSH2*) and in 12% with sporadic cases (mostly due to the acquired promoter hyper-methylation of *MLH1* inducing expression silencing) [9].

- The CpG island methylator phenotype (CIMP) is characterized by promoter hyper-methylation of tumour suppressor genes, such as *MGMT* and *MLH1*. It is commonly associated with serrate adenoma, BRAF mutation and microsatellite instability.

37.3 Clinical Features

Symptoms associated with CRC are usually non-specific: weakness, change in appetite, weight loss without other specific causes, general or localized abdominal pain and change in bowel habits.

In the case of right-sided tumours, clinical presentation is often insidious; iron deficiency and micro-normocytic anaemia are the most common symptoms; in left-sided tumours or rectal cancer, lower gastrointestinal bleeding and obstructive symptoms may occur.

The most frequent complications of localized CRC are acute gastrointestinal bleeding and bowel obstruction, with perforation and subsequent peritonitis and sepsis.

Synchronous CRC tumours may occur in 2.5% of cases, with identical or different histological patterns and stages of development.

As other gastrointestinal cancers, also CRC may be rarely associated with paraneoplastic syndromes (i.e. acanthosis nigricans, Leser-Trelat syndrome, dermatomyositis and thrombophlebitis migrans).

37.4 Diagnosis

Physical examinations may reveal an abdominal palpable mass in case of locally advanced disease. Digital rectal examination (DRE) is the first exam to be performed in case of suspected low rectal tumours.

Laboratory exams may reflect iron deficiency anaemia and raised inflammatory markers. The carcinoembryonic antigen (CEA), an oncofoetal antigen described

in 1965 by Gold and Freedman, is often associated with CRC, and it represents a useful tool during postoperative follow-up. An increased preoperative CEA (values >5 ng/mL indicate poor prognosis), not normalized after 4 weeks after surgery, is suspicious of persistent disease.

The main procedure for diagnosis is endoscopy, to be preferably carried out as a complete colonoscopy to the caecal pole. During endoscopic exam all suspicious lesions should undergo multiple tumour biopsies, in order to obtain definitive diagnosis. If not carried out before, a complete colonoscopy should be performed within 3–6 months after surgery.

Computed tomography (CT) colonography (virtual colonoscopy) is useful to identify tumour location and to detect synchronous lesions or polyps in case colonoscopy is contraindicated.

37.5 Staging

37.5.1 Staging Procedures

An appropriate diagnostic work-up is important to define therapeutic management. Preoperative staging of CRC should exclude metastatic disease and define the exact tumour location (right colon/left colon/rectum) and nodal involvement. A dedicated multidisciplinary team should manage patients, especially in the case of rectal neoplasia.

Tumours with distal extension to >15 cm from anal margin by using rigid sigmoidoscopy are classified as colon cancer. Tumours with distal extension ≤15 cm from anal margin by using rigid sigmoidoscopy are classified as rectal cancer and defined as low (≤5 cm), middle (>5–10 cm) and high (>10–15 cm).

Abdominal ultrasound (US) is a useful approach to exclude visceromegaly, ascites not evaluable at physical examination and, in most of cases, liver metastasis.

CT scan plays a key role in CRC staging, detecting the exact tumour site, usually defined as bowel wall thickening, tumour size, the degree of wall invasion, involvement of adjacent structures, tumour extension into the mesentery, the presence of lymph node, distant metastases and abdominal tumour-related complications. Bone scan and brain imaging should only be carried out if symptoms warrant.

However, in the case of rectal cancers, endorectal ultrasound (ERUS) and magnetic resonance imaging (MRI) are gold standard procedures. ERUS is helpful to define treatment for the earliest tumours, detecting mucosal or submucosal invasion. Pelvic MRI is useful to evaluate tumour size, tumour location, lymph node involvement and specific prognostic parameters used in rectal cancer imaging, such as the relationship between tumour and mesorectal fascia (MRF), extramural vascular invasion (EMVI) and distance to the circumferential resection margin (CRM). Moreover, MRI is indicated in the assessment of suspicious CRC liver metastasis and peritoneal implants [2, 3] (Fig. 37.2).

37.5.2 TNM Classification for Colon and Rectal Cancer

The pathological stage must be reported according to the American Joint Cancer Committee (AJCC)/Union for International Cancer Control (UICC) tumour node metastasis (TNM) staging classification system (8th edition) (Tables 37.3 and 37.4) [2, 3].

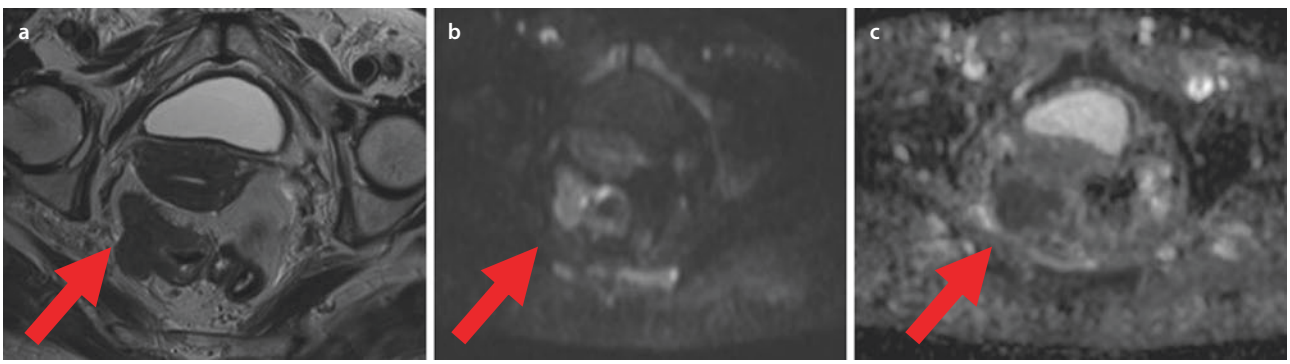


Fig. 37.2 MRI imaging for rectal tumour (lesion indicated by red arrow). **a** T2 sequence; **b** diffusion-weighted imaging sequence; **c** apparent diffusion coefficients (ADC map) sequence

Table 37.3 TNM classification for colon and rectal cancer

T	*Primary tumour*
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ: invasion of the lamina propria
T1	Tumour invades the submucosa
T2	Tumour invades the muscularis propria
T3	Tumour invades the subserosa/non-peritoneal pericolic or perirectal tissues
T4	Tumour invades other organs/structures and perforates the visceral peritoneum
T4a	Tumour perforates the visceral peritoneum
T4b	Tumour invades other organs/structures
N	*Regional nodes*
NX	Regional lymph nodes cannot be assessed
N0	No metastasis in regional lymph nodes
N1	Metastasis in 1–3 regional lymph nodes
N1a	Metastasis in 1 regional lymph node
N1b	Metastasis in 2–3 regional lymph nodes
N1c	Tumour deposit(s) in the subserosa/non-peritoneal pericolic or perirectal soft tissues without metastasis in regional lymph nodes
N2	Metastasis in 4 or more regional lymph nodes
N2a	Metastasis in 4–6 regional lymph nodes
N2b	Metastasis in 7 or more regional lymph nodes
M	*Distant metastasis*
M0	No distant metastasis
M1a	Metastasis confined to one organ (liver, lung, ovary, non-regional lymph nodes) without peritoneal metastases
M1b	Metastasis in more than one organ
M1c	Metastasis to the peritoneum with or without other organ involvement

Note: in case of rectal cancer, T3 tumours should be subclassified according to depth of invasion in the muscularis propria observed with MRI (T3a, <1 mm; T3b, 1–5 mm; T3c, 6–15 mm; T3d, >15 mm)

Table 37.4 Staging grouping of colon and rectal cancer

Stage 0	Tis N0 M0
Stage I	T1–2 N0 M0
Stage II	T3–4 N0 M0
Stage IIA	T3 N0 M0
Stage IIB	T4a N0 M0
Stage IIC	T4b N0 M0
Stage III	Any T N1–2 M0
Stage IIIA	T1–-2 N1 M0; T1 N2a M0
Stage IIIB	T1–2 N2b M0; T2–3 N2a M0; T3–T4a N1 M0
Stage IIIC	T3–4a N2b M0; T4a N2a M0; T4b any N M0
Stage IV	Any T any N M1
Stage IVA	Any T any N M1a
Stage IVB	Any T any N M1b
Stage IVC	Any T any N M1c

37.6 Pathological Features

37.6.1 Histological Features

Around 90% of CRC are adenocarcinomas originating from epithelial cells of the mucosa, characterized by glandular formation and graded according to deviation from normal glandular tissue (ranging from more differentiated Grade 1 to undifferentiated Grade 4).

Other histologic types (i.e. neuroendocrine, squamous cell, adenosquamous, spindle cell, undifferentiated carcinomas, lymphomas) are rarely observed. According to the World Health Organization (WHO) classification, there are several histologic variants of CRC, such as mucinous, signet ring cell, medullary, micropapillary, serrated, cribriform comedo-type, adenosquamous, spindle cell and undifferentiated.

In particular, mucinous adenocarcinoma, characterized by a worse prognosis, is diagnosed when mucus occurs in >50% of the tumour tissue. In case mucinous component is <50%, they are usually termed adenocarcinoma with mucinous features or mucinous differentiation.

The most used immunohistochemical markers for colorectal adenocarcinoma are cytokeratin (CK)20, CK7 and CDX2. Usually CRC adenocarcinoma

displays positivity for CK20 and negativity for CK7. CDX2 is a marker of enteric differentiation and is positive in around 90% of colorectal adenocarcinomas [10].

37.6.2 Pathological Assessment

Several information should be included in the pathological report: the morphologic description and type of surgery; presence of tumour perforation; tumour location size and invasion into adjacent structures; tumour histology and grading; status of margin; presence of tumour deposits; vascular, lymphatic, perineural invasion; tumour budding; and site and number of regional lymph nodes, considering that an adequate lymphadenectomy should include at least 12 lymph nodes.

In the case of rectal cancers, CRM distance and extranodal extension should be comprised in the pathological report.

37.7 Treatment

37.7.1 Colon Cancer

Treatment of colon cancer is based on the stage of the disease.

Stage 0 (Tis N0 M0) disease treatment options are local excision, polypectomy and segmentary en bloc resections if lesions are too large to be amenable for local excision. Colonoscopic polypectomy can be considered curative for malignant peduncolated polyps if high-risk factors as Grade 3 differentiation, level 4 Haggitt invasion (invasion of the submucosa of the bowel wall below the polyp), involved margins of excision and lymphatic or vascular invasion are excluded. Sessil polyps are graded using Kikuchi classification (involvement of submucosae, sm1, sm2 and sm3, involves the superficial, middle and deep thirds of the submucosa, respectively) and considered as a level 4 Haggitt invasion. Sm1 and sm2 lesions can be treated with polypectomy alone; otherwise, all sm3 sessile polyps and sm2 lesions with unfavourable histology should be considered for surgical resection.

For Stage I (T1–2N0M0, old staging: Dukes' A or modified Astler-Coller A and B1) surgical resection with anastomosis alone represents the standard, without adjuvant chemotherapy.

Regarding Stage II A, B and C (T3N0M0, T4 a-b N0 M0), adjuvant therapy after surgery is not recommended, but patients presenting at least one of the high-risk features (lymph nodes sampling <12, poorly differentiated tumours, vascular or lymphatic or perineural invasion, tumour presentation with obstruction or tumour perforation and pT4 stage) should receive adjuvant treatment [11]. MSI/MMR status may be useful to identify a 10–15% subset of Stage II patients who are at a very low risk of recurrence and should not receive chemotherapy [12].

Standard treatment options for Stage III (any T, N1-N2, M0) colon cancer are represented by surgical resection and anastomosis followed by chemotherapy with a doublet schedule of oxaliplatin and a fluoropyrimidine. When oxaliplatin is contraindicated, fluoropyrimidines are selected as treatment.

In the last years, several clinical trials have demonstrated the benefit of combining cytotoxic drugs. Results from the MOSAIC trial evidenced the superiority of FOLFOX4 regimen, compared with LV-5FU2, in terms of reduction in the risk of recurrence and disease-free survival (DFS) at 3 years, confirmed at 6-year follow-up 8 [13]. XELOXA phase III study assessed the safety and efficacy of adjuvant capecitabine (CPC) + oxaliplatin vs 5FU2/LV in Stage III patients, and capecitabine was defined as a well-tolerated compared with i.v. fluoropyrimidine. The X-ACT trial showed the favourable toxicity profile of capecitabine in Stage III patients and confirmed the equivalence with intravenous (i.v.) 5FU in terms of DFS.

Other agents have been studied in the adjuvant setting, but trials showed no improvement in OS when irinotecan was added to a treatment with 5-FU/LV (CALGB-89803) or LV5FU2 or AIO regimen (PETACC-3). Moreover, regarding the evaluation of targeted agents associated with chemotherapy (CT) in the adjuvant setting, all trials resulted negative, due to a different biology of early and metastatic disease.

Recently, the optimal duration of adjuvant treatment of Stage III patients has been studied by six randomized trials forming a big international collaboration called "IDEA" trial. Statistically, 3 months of treatment with FOLFOX or CAPOX was slightly inferior to 6 months in the overall study population of Stage III patients, but additional analysis of subgroups demonstrated that selected low-risk patients could be treated with CT for 3 months, given the reduction in neurotoxicity, while a 6-month treatment is reserved for those patients with a high risk of relapse (T4 or N2). Future goals are the validation of prognostic/predictive markers leading to a more personalized therapy also in the adjuvant setting [14].

37.7.2 Rectal Cancer

Rectal cancers represent approximately one third of all colorectal malignancies and have a different behaviour from colonic tumours. In fact, early and locally advanced rectal adenocarcinomas require a specific multimodal approach. Several years ago, high recurrence rates led

to an evaluation of the role of postoperative RT and adjuvant therapy with 5-fluorouracil (5FU) as the backbone in several clinical trials [15]. In the last years the role of neoadjuvant treatment with chemo-radiation (45 GY followed by surgery after 6–8 weeks) has been investigated in several clinical trials and resulted in a better local disease control, minimizing toxicity from RT and chemotherapy and eliminating local recurrence. Neoadjuvant chemo-radiation treatment has been found to determine more conservative surgery and sphincter preservation in 60–90% of cases, with a pathological complete response (pCR) in 10–25% of patients.

The choice of a preoperative treatment is based on staging and magnetic resonance imaging (MRI) or transrectal endoscopic ultrasound (EUS) that allows to a better study of pelvic structures and an involvement of nodes.

Stage I (T1, T2 N0) Early-stage tumours, defined as T1–2N0, are usually treated with surgery alone. Local excisional procedures as TEM (transanal endoscopic microsurgery) are reserved for those patients with a very early disease (cT1 N0, G1, sm1, EMVI) or for patients with a more advanced disease but with a high risk to undergo surgery. In the case of unfavourable pathological features after local excision, as the role of adjuvant CRT is not proven in preventing local recurrence, the standard treatment remains a TME (total mesorectal excision), which includes the removal of mesorectal fascia (MRF) and lymph nodes. Local RT and CRT could be used as an alternative option. TME is the chosen strategy also in those patients with tumours that are early rectal cancers but not suitable for local excision as cT1–T2 with adverse histopatologic assessment (G3, sm2–3, V1, L1) [16].

Stage II (T3–T4N0) cT3 a/b without involvement of (MRF), when located above the levators, could be treated with TME alone, only if a good-quality TME can be reached.

T3 tumours with mesorectal fat infiltration <5 mm (uT3a) should be treated with CRT followed by radical TME within 6–8 weeks. Radiation treatment consists in a traditional dose of 40–50 Gy in 25–28 fractions and concomitant treatment of infusional 5-FU or oral capecitabine. A short-course radiation treatment with high daily doses (5 × 5 Gy), followed by surgery within 10 days, could be considered as an alternative when it is not necessary to achieve a tumour-free CRM or to preserve the sphincter activity. A recent approach is represented by the use of a SCPRT not followed by immediate surgery, to avoid the risk of postoperative complications [17].

T3 tumours with mesorectal fat infiltration >5 mm (uT3b) or T4 tumours are treated with preoperative CRT followed by radical TME and adjuvant CT with infusional 5-FU or capecitabine for 4 months. SCPRT is not indicated for patients with cT4 or large bulky tumours. However, some trials are evaluating the effect of SCPRT followed by a consolidation oxaliplatin-based chemotherapy treatment prior to surgery. Results are promising, even if not yet considered as a standard of care.

Stage III (any T N+) treatment consists in preoperative CRT, followed by radical TME and adjuvant CT with oxaliplatin plus infusional 5-FU or oral capecitabine (FOLFOX or XELOX). For patients with CRM and MRF involvement, neoadjuvant treatment is necessary to shrink the cancer back away from the threatened margin, and CRT has increased the percentage of patients achieving a R0 surgery.

A different new strategy is the "watch-and-wait approach" for those patients achieving a clinical complete response (cCR) after induction treatment (10–40%). Even if several clinical trials report a similar outcome for patients not undergoing surgery, compared with operated patients, results are controversial and depend from the initial stage and unknown molecular features. Until well-designed clinical trials, longer follow-up analysis and larger numbers of patients will be able to give additional data, surgical approach remains the standard of care [18].

The role of postoperative chemotherapy in patients with locally advanced rectal cancer receiving preoperative radiation or chemoradiotherapy has been studied in clinical trials but results are not consistent, and tolerance and compliance with postoperative chemotherapy are consistently dismal [19]. Moreover, it also remains unclear which parameter choose to define the risk/benefit of an adjuvant treatment, between initial clinical (yc) and pathological (yp) stage [20].

37.8 Follow-Up

Even if surgery represents the cornerstone of early colorectal cancer treatment, a big portion of patients relapses, and, nowadays, this event is unpredictable. In the last years, four meta-analyses have showed an improvement in survival for those patients receiving a more intense follow-up, due to the possibility to detect earlier isolated locoregional recurrences. After curative resection of the cancer, patients should undergo colonoscopy 1, 3 and 5 years after the initial colonoscopy, looking for metachronous adenomas and cancers, if findings on these surveillance colonoscopies remain normal. Intervals may be shortened after the 1-year examination based on adenomatous findings or hereditary causes of colon cancer. A computer tomography of the chest and abdomen should be performed every year (abdominal CT scan could be substituted by CEUS), together with a physi-

cal examination and a evaluation of carcinoembryonic antigen (CEA) every 3–6 months for the first 3 years and every 6 months during years 4 and 5 and subsequently at the discretion of the physician. Other laboratory and radiological examinations are of unproven benefit and should be reserved for symptomatic patients.

Despite data showed a benefit of 12% in OS in patients undergoing intensive clinical and instrumental follow-up, there is still a low adherence to physicians' recommendations, giving also the heterogeneity of the trials included in the meta-analyses and the absence of an exact optimal strategy of surveillance [21–25].

Case Study: A Suspect Case of Asthenia

Man, 56 years old

- *Family history* positive for CRC
- *APR*: Essential hypertension
- *APP*: diffuse abdominal pain; change in bowel habits, asthenia
- *Objective examination*: mild tenderness on deep palpation (lower quadrants)
- *Blood tests*: Hb 9.0 g/dL; iron deficiency

Question

What action should be taken?

(1) Barium enema. (2) Sigmoidoscopy with biopsy. (3) Complete colonoscopy with biopsy

Answer

Complete colonoscopy with biopsy

An ulcerative mass is observed in the right colon. Histological examination: adenocarcinoma, moderate differentiation (G2)

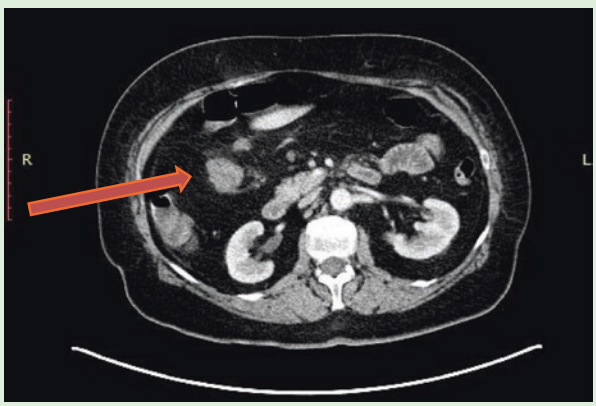
Question

What action should be taken?

(1) Thorax and abdomen CT scan. (2) Abdomen MRI. (3) Others

Answer

Thorax and abdomen CT scan. Revealed bowel wall circumferential thickening and lymph node regional metastasis. No evidence of distant metastasis



Question

What action should be taken?

(1) Surgery. (2) Medical therapy. (3) Others

Answer

Surgery (right hemicolectomy) was performed. Histological examination: adenocarcinoma, G2 stage pTa N2a cM0

Question

What action should be taken?

(1) Follow-up. (2) Medical therapy. (3) Others

Answer

Patient received medical therapy: 6 months adjuvant chemotherapy XELOX capecitabine 2000 mg/mq G1 –> G14 q21 + oxaliplatin 130 mg/mq q21.

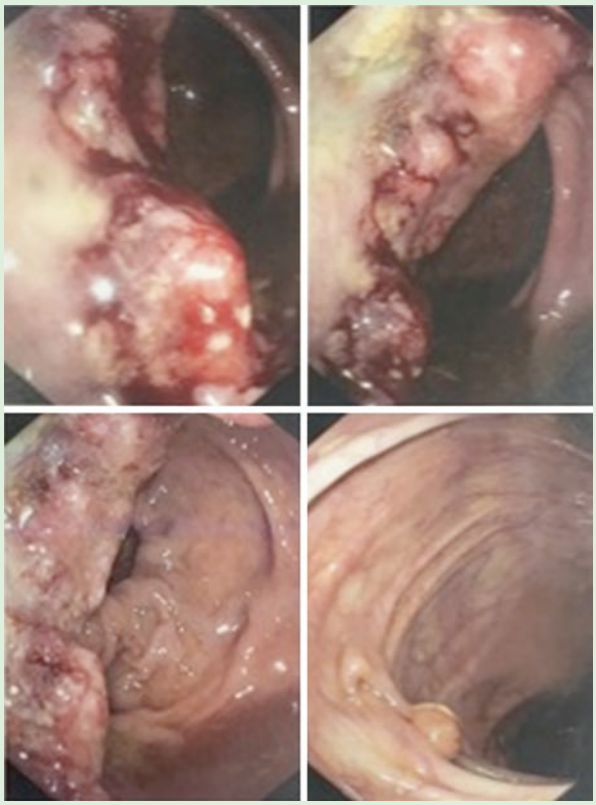
Key Points

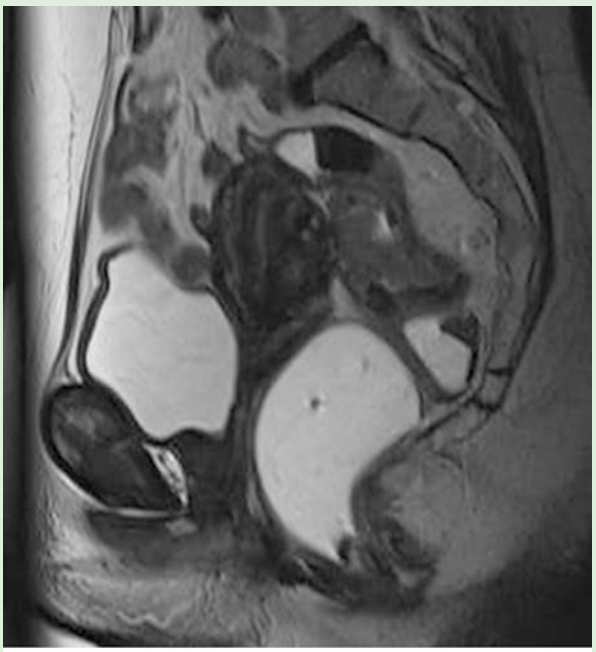
- Right colon cancer presentation is often insidious.
- Symptoms are often non-specific.
- Perform a complete colonoscopy to the caecal pole with biopsy.
- Perform adequate surgery and, if indicated, adjuvant chemotherapy.

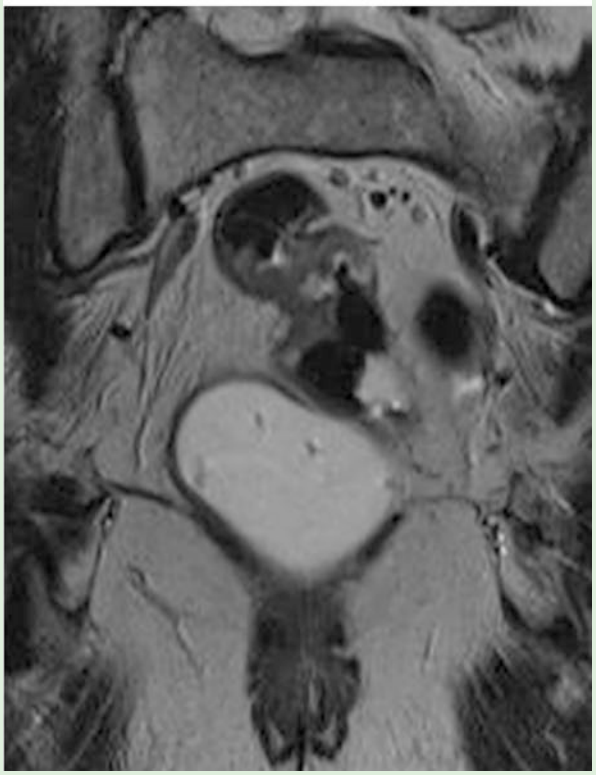
Case Study: Management of Locally Advanced Rectal Cancer

Female, 55 years old

- *Family history* negative for malignancy
- *APR*: negative, smoker 1 pack/day
- *APP*: rectorrhagia
- *Blood tests*: Hb 9.9 g/dL
- *FOBT*: positive. *DRE*: positive
- *Sigmoidoscopy with biopsy*: Ulcerative rectal mass. Histological examination: adenocarcinoma







- *TC Abdomen mdc*: lower bowel wall circumferential thickening, multiple perirectal lymph nodes. No evidence of distance metastasis.

Question

What action should be taken?

(1) Perform ERUS. (2) Perform MRI. (3) Others

Answer

Perform MRI: rectal wall circumferential thickening, thickening of the mesorectal fascia; multiple perirectal lymph nodes, EMVI present; stage cT3 N+. The case was discussed within the multidisciplinary team.

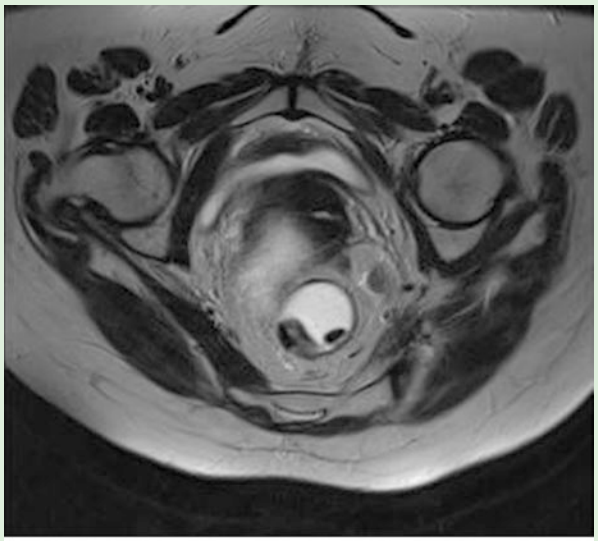
Question

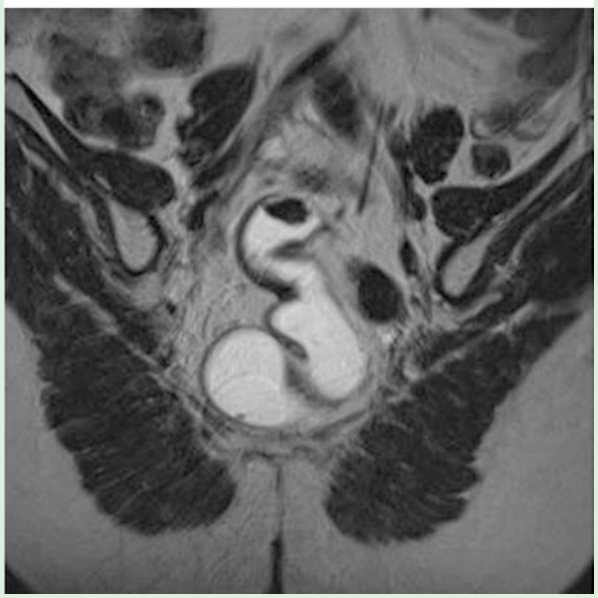
What action should be taken?

(1) Surgery. (2) Neoadjuvant CRT. (3) Others

Answer

Neoadjuvant CRT: radiotherapy (total 50 Gy) + capecitabine 825 mg/mq bis in die (bid)





MRI shows tumour and lymph node regression

Question

What action should be taken?

(1) Surgery. (2) Follow-up. (3) Others

Answer

Surgery: Total mesorectal Excision (TME) was performed. Histological examination: well-differentiated adenocarcinoma G1. stage ypT1 N0 (0/15) cM0; TRG: 2

Key Points

- Importance of proper work-up.
- Rectal cancer cases should always be discussed with the multidisciplinary team.
- ERUS and MRI are the appropriate procedures to locally stage rectal cancer.

Expert Opinion

Marc Peeters

- Colorectal cancer (CRC) is the third most common neoplasm in men and the second in women. Risk factors are represented by smoking, high red meat consumption, obesity, low physical activity, personal history such as previous polyps or IBDs and genetically determinant factors.
- Three theories are used to explain the multimodal onset of CRC: Fearon and Vogelstein model, the microsatellite instability and the cpG island methylator phenotype. These three models involve different genes such as APC, KRAS, Tp53, MLH1, MSH2 and MGMT.
- Symptoms of CRC are usually non-specific and they can vary in dependence of cancer localization in the colon: weight loss, general or localised abdominal pain, diarrhoea or constipation, weakness and lower gastro-intestinal bleeding.
- In case of suspicious of a lower rectal tumour, digital anal examination must be done. Physical examination can give useful information such as the presence of a mass in the abdomen; blood test can show iron deficiency anaemia and if investigated, increased levels of CEA (a tumour marker associated also to CRC). A colonoscopy must be performed taking tissue samples in case of suspicious masses. CT allows a correct staging with the evidence of possible metastases. For rectal cancers, MRI is the golden standard procedure.
- The most frequent histological type is adenocarcinoma; other forms are neuroendocrine, squamous cell, adenosquamous, spindle cell, undifferentiated carcinomas and lymphomas. WHO classification identifies different subtypes of CRC such as mucinous, signet ring cell, medullary, etc.
- Treatments differ from types and stage of CRC: for colon cancers, they can consist in locoregional procedures or surgery alone (0-I stages) and adjuvant chemotherapy (in some cases of II stage and in III stage) with oxaliplatin and fluoropyrimidine. For early and locally advanced rectal cancers, a neoadjuvant approach with chemo-radiation (CRT) is usually chosen; stage III tumours with mesorectal fat infiltration >5 mm or T4 tumours are treated with preoperative CRT followed by radical total mesorectal excision (TME) and adjuvant chemotherapy.
- Follow-up strategies consist in colonoscopy, CEA evaluations and CT scan in order to identify early relapses or late metastases.

Recommendations

- AIOM
  ► https://www.aiom.it/wpcontent/uploads/2020/10/2020_LG_AIOM_Colon.pdf
- ESMO
  ► www.esmo.org/Guidelines/Gastrointestinal-Cancers/Rectal-Cancer
  ► www.esmo.org/Guidelines/Gastrointestinal-Cancers/Early-Colon-Cancer
- ASCO
  ► www.asco.org/practice-guidelines/quality-guidelines/guidelines/gastrointestinal-cancer#/10251
  ► www.asco.org/practice-guidelines/quality-guidelines/guidelines/gastrointestinal-cancer#/34946
  ► www.asco.org/practice-guidelines/quality-guidelines/guidelines/gastrointestinal-cancer#/34951

Hints for a Deeper Insight

- Understanding the role of primary tumour localisation in colorectal cancer treatment and outcomes: ► https://www.ncbi.nlm.nih.gov/pubmed/28787661
- Transanal total mesorectal excision (taTME) for rectal cancer: beyond the learning curve: ► https://www.ncbi.nlm.nih.gov/pubmed/31602515
- Identification of the Risk Factors for Recurrence of Stage III Colorectal Cancer: ► https://www.ncbi.nlm.nih.gov/pubmed/31570473
- Postoperative XELOX therapy for patients with curatively resected high-risk stage II and stage III rectal cancer without preoperative chemoradiation: a prospective, multicenter, open-label, single-arm phase II study: ► https://www.ncbi.nlm.nih.gov/pubmed/31533662
- Long-term Transanal Excision Outcomes in Patients With T1 Rectal Cancer: Comparative Analysis of Radical Resection: ► https://www.ncbi.nlm.nih.gov/pubmed/31487767

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Colorectal Cancer: Metastatic Disease

Antonio Galvano, Aurelia Ada Guarini, Valerio Gristina, Nadia Barraco, Maria La Mantia, Marta Castiglia, and Antonio Russo

Gastrointestinal Cancers

Contents

Antonio Galvano and Aurelia Ada Guarini should be considered equally co-first authors.

A. Russo et al. (eds.), *Practical Medical Oncology Textbook*, UNIPA Springer Series,
https://doi.org/10.1007/978-3-030-56051-5_38

Learning Objectives

By the end of the chapter the reader will:

- Be able to apply molecular, diagnostic, and therapeutic procedures in metastatic colorectal cancer
- Have learned the basic concepts of metastatic colorectal cancer
- Have reached in-depth knowledge of colon and rectal cancer
- Be able to put newly acquired knowledge into clinical practice

38.1 Introduction

While approximately one-fourth of patients diagnosed with metastatic colorectal cancer (mCRC) most frequently present with liver metastases at the initial diagnosis (synchronous metastases) and seem to be associated with a more disseminated disease state along with a worse prognosis, the majority of them would develop metastases during the disease course after the resection of locoregional colorectal cancer (metachronous metastases). To date, the liver represents the most common site of metastatic involvement [1], with the lung also occurring almost as frequently in the specific case of rectal cancer, whereas peritoneal, bone, ovarian, and central nervous system metastatic sites are less common.

Although several different biological and clinical hallmarks exist between the colon and rectum (different embryological origin, anatomy and function) [2] along with different (neo)adjuvant treatment and surgical approaches advocated in the locoregional setting, mCRC requires similar staging procedures and systemic treatment strategies (first and subsequent lines) in terms of a multimodal approach treatment as a part of a "continuum of care."

Moreover, patients affected by oligometastatic disease confined to a single or a few organs (most frequently the liver and, secondly, the lung) should undergo an upfront evaluation by a multidisciplinary team for assessing a disease which could be initially resectable or may become completely resectable following treatment ("conversion therapy"), achieving a potentially curative approach with improved long-term survival rates.

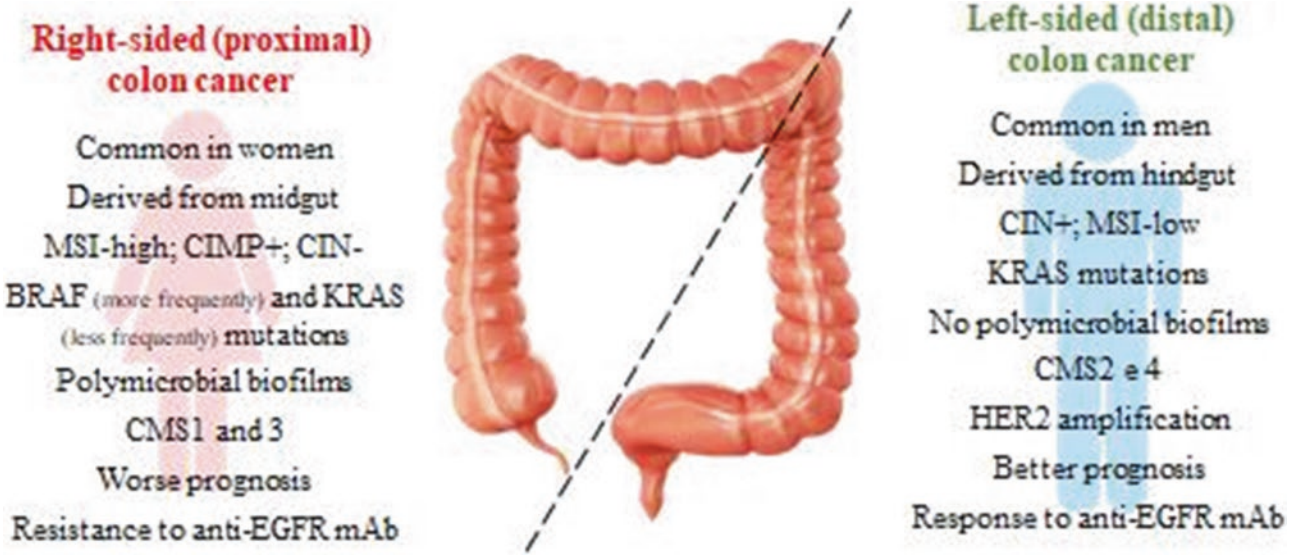
So far, the standard of care for treatment of mCRC has been built on the backbone of 5-fluorouracil (5-FU)-based chemotherapy, including first-line combination treatment with either oxaliplatin or irinotecan (FOLFOX and FOLFIRI, respectively), plus a monoclonal antibody (bevacizumab versus cetuximab or panitumumab). In the era of personalized medicine, genotyping of tumor tissue (either primary tumor or metastasis) for *RAS* and *BRAF* testing in all patients with mCRC has been associated with significant prognostic and predictive implications and therefore is strongly recommended. In addition, primary tumor location appeared to impact both prognosis and prediction of responsiveness to targeted therapy in advanced disease.

However, despite major advances in a more tailored approach to systemic treatment and a substantial rise in survival rates over the last two decades, mortality from CRC remains high with a huge number of patients who would eventually succumb to disease progression.

38.2 Molecular Biology

Colorectal cancer (CRC) exhibits differences in incidence, pathogenesis, molecular pathways, and outcome depending on tumor location. Indeed, recent epidemiological and scientific studies have showed that proximal (right-sided) and distal (left-sided) colon cancers are biologically different regarding both genetic and immunologic factors. Moreover, it has been shown that primary tumor location has a relevant prognostic value in both earlier and advanced stages of disease, and it should be acknowledged before taking important clinical decision, such as choosing between first-line or palliative treatment [3].

More than 1000 genes are differentially expressed in adult ascending versus descending colon, and biopsies of the adult colonic epithelium can be correctly classified as proximal or distal by gene expression profile, with almost 100% concordance [4]. According to their different embryological origin, the right colon and left colon have a different vascular supply; the proximal colon receives its main blood supply from the superior mesenteric artery, while the distal colon is perfused by inferior mesenteric artery. Interestingly the proximal and distal colon are exposed to different pro-carcinogenic factors that may contribute in determining the epidemiological and biological differences that characterize right and left colon cancer. The right colon and left colon differ in the expression of several antigens, but also in bile acid concentration and in the composition of the bacterial population [5–7]. Indeed, mucosal microbiota organization is a critical factor, and it is associated with a specific subset of CRC. Invasive polymicrobial bacterial biofilms have been reported in 89% of right-sided tumors but in only 12% of left-sided tumors [7].



Perhaps one of the most important discoveries in mCRC has been the recognition that mutations of the gene encoding for KRAS (a signal transduction protein which is a crucial intermediate in transmission of growth and survival signals from the EGFR to the nucleus) are early events in colorectal cancer formation, with a very tight correlation between mutation status in the primary tumor and metastases. A growing body of literature has shown that mutations in KRAS exon 2 (occurring in approximately 40% of mCRCs) are predictive of lack of response to targeted therapy, in the light of a constitutive activation of this signaling pathway which renders blocking of the EGFR binding site on the surface useless. Subsequently, update analyses from several retrospective studies have investigated the role of NRAS (exons 2, 3, and 4) and other KRAS (exons 3 and 4) mutations, resulting that targeted therapy likely has a detrimental effect in patients with *KRAS* or *NRAS* mutations [8, 9] (see ◘ Figs. 38.1 and 38.2). Moreover, approximately 8–12% of colorectal cancers have resulted to be characterized by a specific mutation in the *BRAF* gene (V600E), downstream of EGFR and mutually exclusive of *RAS* mutations, which is responsible for the constitutive activation of the MAPK pathway and postulated as a possible additional predictive biomarker of lack of response to targeted therapy [10].

BRAF-mutated (10%) mCRCs are often linked to negative prognostic implications, such as right-sided tumors. MSI results from a deficient mismatch repair system (dMMR), responsible for correcting nucleotide base mispairings which occurred during DNA replication. Immunohistochemistry (IHC) tests for MMR proteins orPCR tests for microsatellite instability (MSI) in metastatic disease setting could assist clinicians in genetic counseling and should be considered a predictive biomarker in the use of immunotherapy in mCRC, as discussed below (see ◘ Table 38.1).

Furthermore, experimental approaches have been conducted to investigate the overexpression of HER2 (human epidermal growth factor receptor 2) in mCRCs, whose prevalence is higher in *RAS*/*BRAF* wild-type tumors (5–14%). Preliminary findings demonstrate the use of HER2 as a potential predictive biomarker for refractory *HER-2* positive mCRC, yet further studies are needed. Several retrospective analyses have shown a possible prognostic or predictive role of PTEN or PIK3CA because of their relationships with EGFR pathways. Lastly, other genes like ALK, ROS1, RET, and NTRK are under investigation as predictive factors. Recent data suggested how well NTRK inhibitors work in patients carrying NTRK 1, 2, or 3 genes involving other partners.

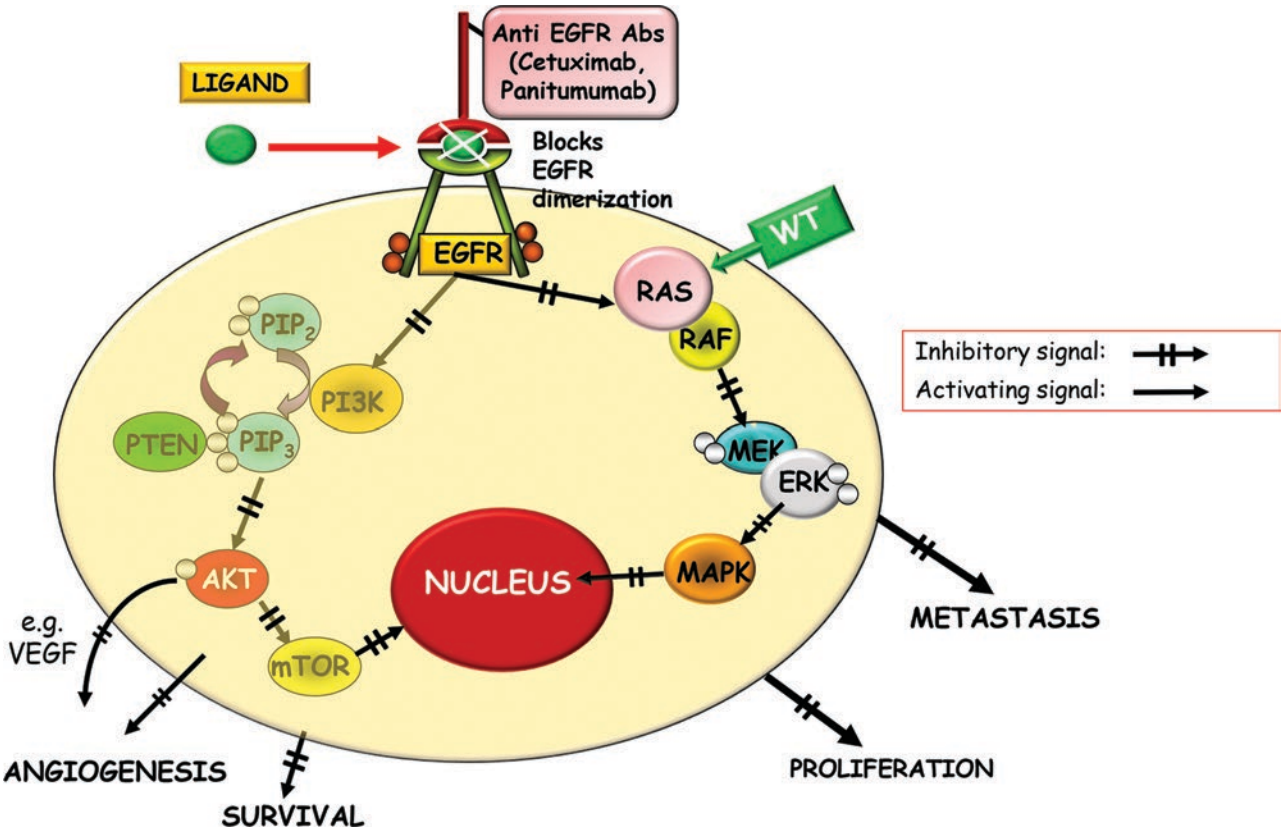
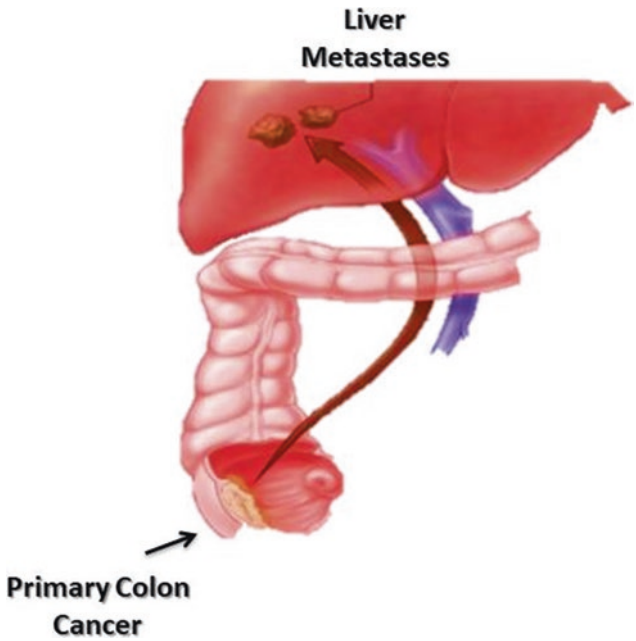


Fig. 38.1 Anti-EGFR mechanism in a wild-type KRAS scenario

38.3 Clinical Presentation and Diagnosis of Metastatic Disease



Clinical presentation of a metastatic disease can be asymptomatic and unspecific.

CRC can spread via lymphatic and hematogenous dissemination, as well as by contiguous and transperitoneal routes. The venous drainage of the intestinal tract is via the portal system; the first site of hematogenous dissemination is usually the liver, followed by the lungs, bone, and many other sites. Patients may present with signs or symptoms referable to any of these areas. It has been shown that patients with liver metastases at initial diagnosis of colon cancer have a more aggressive disease than patients with metachronous metastases. A retrospective study led by Tsai et al. showed that patients with synchronous metastases had bilobar metastases and a more aggressive disease than patients with metachronous metastases [11].

Liver metastases can be detected by CT scan, which is the first imaging technique useful for staging. Liver MRI performs better than CT scan in term of sensitivity; thus MRI is the modality preferred for liver metastases detection [12].

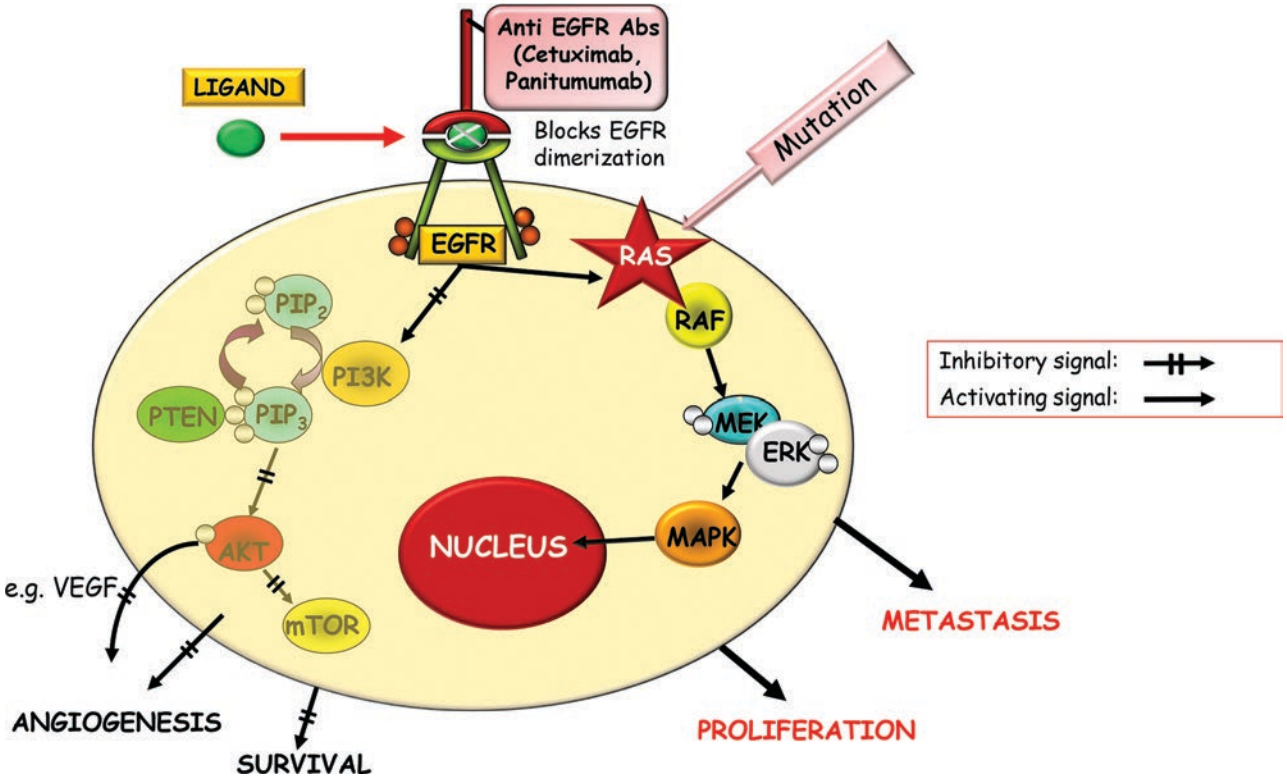


Fig. 38.2 Anti-EGFR mechanism in a KRAS-mutated scenario

38.4 Principles of Management of Metastatic Disease

As endorsed by all the international guidelines, the choice of a treatment strategy in mCRC should be evaluated as part of "continuum of care," based on consideration of the tumor- and disease-related characteristics (the mutational profile and the clinical presentation of the tumor), the goals of therapy (cytoreduction vs disease control), the type and timing of prior therapy, the patient-related factors (performance status, motivation, and expectations), and the treatment-related features (toxicity profiles of drugs).

38.4.1 Surgical Management of Colorectal Metastases

ESMO and other groups have established guidelines for the management and treatment of metastatic colorectal cancer. For selected patients, liver metastases removal and a cure are possible; this perspective should be the goal of our practice. The definition of resectable colorectal liver metastases (CLM) evolved in the past few years. There is a consensus proposing that disease should be considerable "resectable" as long as the procedure can be technically performed and the remaining liver should be at least 30%.

For technically resectable liver metastases, oncological prognostic factors should be considered since surgery upfront and perioperative chemotherapy are both feasible.

The oncological criteria provide prognostic information on DFS. These criteria are summarized by a score designed by Fung et al., and they include elements, such as number of lesions, presence of extrahepatic disease, long-term metachronous disease, and vessel relationship [13].

According to the EPOC trial, patients who have unfavorable oncological criteria but a technically resectable disease should undergo a perioperative chemotherapy: 3 months before surgery and 3 months after surgery. CAPOX and FOLFOX should be considered in this set-

Table 38.1 Summary of mCRC molecular testing recommendations

Molecular pathology and biomarkers	
Extended RAS testing	
RAS mutational status is a predictive biomarker for therapeutic choices involving EGFR antibody therapies in the metastatic disease setting RAS testing should be carried out on all patients at the time of diagnosis of mCRC RAS testing is mandatory before treatment with the EGFR-targeted monoclonal antibodies cetuximab and panitumumab Primary or metastatic colorectal tumour tissue can be used for RAS testing (see also Recommendation 3) RAS analysis should include at least KRAS exons 2, 3 and 4 (codons 12, 13, 59, 61, 117 and 146) and NRAS exons 2, 3 and 4 (codons 12, 13, 59, 61 and 117)	
BRAF testing	*MSI (MMR) testing*
Tumor BRAF mutation status (V600E) should be assessed alongside the assessment of tumor RAS mutational status for prognostic assessment	Immunohistochemistry (IHC) tests for MMR proteins or PCR tests for microsatellite instability (MSI) in the metastatic disease setting can assist clinicians in genetic counseling Tumor MMR testing has strong predictive value for the use of immune check-point inhibitors in the treatment of patients with mCRC

ting of patients, while anti-EGFR and bevacizumab are not recommended, as demonstrated in the new EPOC trial [14].

38

38.4.2 "Conversion": Strategic Treatment Goal for Unresectable Liver Metastases

Patients with limited liver/lung metastases unresectable upfront might receive a systemic therapy in order to render the disease surgically resectable. This strategy is defined as "conversion." The main limitation of trials investigating this complex therapeutic area is due to the definition of upfront unresectable liver metastases.

The CELIM trial has been one of the first trials aiming to evaluate two groups of KRAS wild-type patients, both with upfront unresectable liver metastases, who underwent two different preoperative protocols: FOLFOX6 + Cetuximab and FOLFIRI+Cetuximab. The study demonstrated that a tumor RR ranging around 60–70% was achieved in both groups and 30–40% underwent a R0 liver resection. No data on the more effective chemotherapy protocol, albeit the oxaliplatin backbone strategy, seemed to be more efficient than treatment with irinotecan [15].

Besides, the OLIVIA phase II randomized prospective trial evaluated the FOLFOX6 plus bevacizumab regimen compared to the same strategy with the addition of Irinotecan for patients with initially unresectable colorectal liver metastases. Resection rates were 49% and 61%, respectively, although it is still unknown the benefit of a third chemotherapy addition since FOLFOXIRI alone achieved high RR, as demonstrated in the phase II trial by Masi et al. [16].

Furthermore, anti-EGFR combinations were investigated to downsize colorectal liver metastases from colorectal cancer. Particularly, the PLANET-TTD [17] and the PRIME [18] trials overall efficacy results showed that panitumumab in association with FOLFOX or FOLFIRI regimens should be considered a good option in this setting.

Nevertheless, no robust data suggest a real clear advantage regarding the addition of anti-VEGF or anti-EGFR strategy, despite results from clinical trials on liver resection rate and overall response rate supposed their impact on prognosis.

Other data from the CALGB 80405 [Elez, 2015, First-Line Treatment of Metastatic Colorectal Cancer: Interpreting FIRE-3`, PEAK`, and CALGB/SWOG 80405] and FIRE-3 [19] trial showed that a doublet of chemotherapeutic agents (FOLFOX6 or FOLFIRI) plus anti-EGFR or anti-VEGF in KRAS wild-type patients was associated with higher RRs compared to bevacizumab, yet these data have not shown higher resection rates. In particular, in CALGB 80405 a subgroup of 180 pts. containing liver-only disease were converted to surgery reaching median survival times around 64 and 67 months for anti-EGFR and anti VEGF combinations.

The response after chemotherapy may be evaluated with some response parameters: early tumor shrinkage (ETS) and depth of response (DpR).

The ETS is associated with a long-term outcome, and this parameter has been first used as a response parameter in a study designed by Piesseaux et al. in patients treated with chemotherapy plus cetuximab in first line. ETS was evaluated according to cutoff values decided by the investigators (radiological tumor size at 8 weeks) [20]. However, it is still unclear the role of the antibodies post-surgery.

Local therapies like intra-arterial chemotherapy or chemoembolization might be used to shrink a large tumor as evaluated in a small study, the DEBIRI, where 296 patients among 600 patients with unresectable liver metastases had undergone hepatic arterial drug-eluting irinotecan bead (DEBIRI) therapy. Patients treated with DEBIRI achieved higher resection rates and R0 resections [21] (see Table 38.2).

Table 38.2 Conversion chemotherapy approach

Study	Chemotherapy	Liver resection rate %
Vie-LM-Bev	CAPOX + bevacizumab	93
CELIM	FOLFOX6/FOLFIRI + cetuximab	33
GONO	FOLFOXIRI + bevacizumab	40
POCHER	Chrono-IFLO + cetuximab	60
BOXER	CAPOX + bevacizumab	40
OLIVIA	FOLFOXIRI + bevacizumab versus FOLFOX + bevacizumab	49 versus 23 (RO)
Ye et al.	FOLFIRI/FOLFOX ± cetuximab	26 versus 7 (RO)

38.4.3 Local and Ablative Treatments

The goal of an ablation treatment is not curative as surgery, since the prognosis of these patients is poor due to the spread of the disease and the sites of metastases. The ablation of visible metastases could be combined with systemic therapy in order to improve the survival rate of a patient in a stage IV.

Many data for stereotactic body radiation therapy (SBRT) have been reported, assessing this technique as an optimal tool, combined with systemic therapy, in selected patients with unresectable liver metastases [22].

The CLOCC phase II trial showed radiofrequency ablation (RFA) and chemotherapy combination treatment improvement in OS [23].

38.4.3.1 Thermal Ablation

Treatments like RFA, using temperatures ranging from 55 °C to 100 °C, could be used for unresectable liver metastases with comorbidities and liver dysfunction as shown in the phase II CLOCC trial. Notably, patients underwent to RFA + FOLFOX +/– bevacizumab or FOLFOX +/– bevacizumab showing a significant improvement in overall survival in the RFA strategy.

Moreover, these thermal ablation techniques have been established to be effective in lung metastases. Petre et al. presented that RFA on the lung could improve the local tumor progression (LTP) and the survival rates by sparing the lung parenchyma. LTP-free survival rates achieved 77% at 3 years [24]. Despite these encouraging results, some limitations affected the routine use of RFA in clinical practice because of dissemination or incomplete ablation rendering this topic as controversial and limited for patients with comorbidities, unresectable lesions, or extrahepatic lesions.

38.4.3.2 Chemoembolization

Transarterial chemoembolization (TACE) is a potential option in this setting, although evidences are limited in respect to TARE. A 2013 Cochrane review has not recommended the use of TACE outside clinical trials, according to the results of a trial comparing TACE versus FOLFIRI chemotherapy regimen. A recent study by Martin et al. comparing TACE with irinotecan-loaded drug-eluting beads (DEBIRI) in addition to FOLFOX plus bevacizumab has evaluated an improvement in PFS of DEBIRI arm (15.3 vs 7.6 month)[Martin, 2015, Randomized controlled trial of irinotecan drug-eluting beads with simultaneous FOLFOX and bevacizumab for patients with unresectable colorectal liver-limited metastasis].

Notwithstanding, notably chemoembolization is still not recommended. There are few data available based on small series, like the DEBIRI [21].

Several trials based on chemotherapy-loaded particles (beads) are still ongoing.

38.4.3.3 Radioembolization

Radioembolization [selective internal radiation therapy (SIRT) or transarterial radioembolization (TARE)] is indicated in patients who have failed prior chemotherapies, and it consists in a single delivery of yttrium-90 connected to either resin or glass particles into the hepatic artery with the therapeutic effect limited to irradiation. Many trials evaluated the role of this technique as first-line or salvage approach. For chemo-refractory disease, progression-free survival and overall survival were 8.8 and 2.9, respectively [25]. In chemo-naive setting, TARE efficacy was evaluated in two randomized phase III studies in association with fluorouracil infusion (SIRFLOX and FOXFIRE), showing no clear benefit in overall survival, but only in liver-specific progression-free survival and response rates (Harpreet W, Guy V, Volker H, et al. Overall survival analysis of the FOXFIRE-SIRFLOX-FOXFIRE global prospective randomized studies of first-line selective internal radiotherapy (SIRT) in patients with liver metastases from colorectal cancer). In the same year, Garlipp B. et al. at the ASCO Annual Meeting discussed the results of the addition of TARE to chemotherapy alone in the

SIRFLOX study reaching a significant improvement in resection rate (38% vs 29%) [26]. According to these results, today this procedure should be considered as a valid option for chemotherapy-refractory liver-limited selected mCRC patients [27].

38.4.3.4 HIPEC

Hyperthermic intraperitoneal chemotherapy (HIPEC) could be considered as a valid option in patients with isolated and resectable peritoneal carcinomatosis. As shown by Elias D. et al., median survival can be prolonged in patients with resectable PC followed by HIPEC [26]. This treatment may be effective if the peritoneal dissemination is scored as "low volume," using the peritoneal cancer index (PCI). A PCI under 12 is always suggested [28].

It is still unclear whether to use oxaliplatin or mitomycin C for HIPEC; nevertheless this combination is going to become a valid standard for patients with peritoneal metastases from CRC [29].

38.4.4 Palliative Treatment

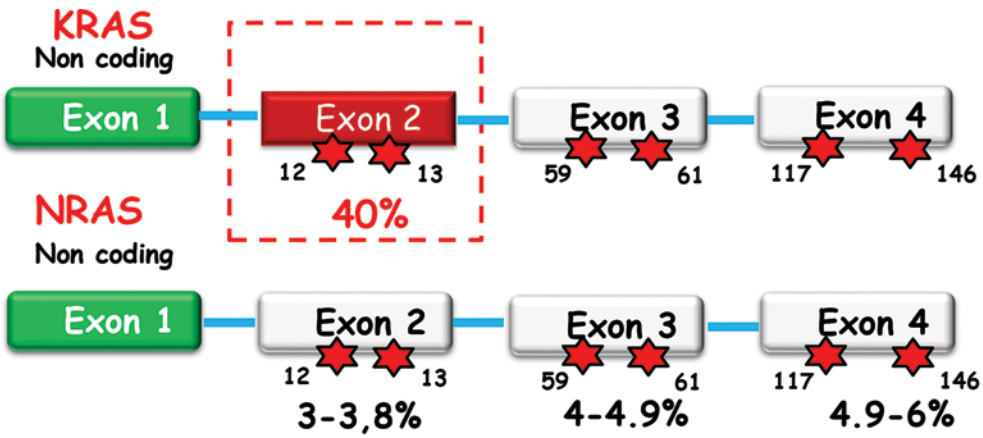
Systemic chemotherapy has been established as the main treatment approach for most patients with unresectable mCRC [30]. For decades, 5-fluorouracil (5-FU)-based chemotherapy was the only treatment option for mCRC patients, resulting in a median overall survival (mOS) of up to 12 months. Over the past 10 to 15 years, the therapeutic landscape has markedly evolved with the approval of irinotecan, oxaliplatin, capecitabine, and monoclonal antibodies [acting against vascular endothelial growth factor (bevacizumab, aflibercept, and ramucirumab) or the epidermal growth factor receptor (cetuximab and panitumumab) and are largely dependent by *RAS* and BRAF status], achieving a mOS of about 30 months. More recently, the oral multi-targeted kinase inhibitor regorafenib and TAS-102, combining trifluridine and tipiracil, have shown to be effective, reporting an improvement in OS rates in chemo-refractory setting; moreover, the use of immunotherapy checkpoint inhibitors (ICIs) is actually under evaluation within phase II or phase III trials after their investigations in preclinical and first-stage clinical trials in which their responses were associated with immunological disease status in special subgroups of mCRC patients (MSI-H).

In the specific case of advanced/metastatic rectal cancer with no surgically treated primary tumor, the treatment strategy differed since chemotherapy alone may be insufficient requiring the addition of radiotherapy for local palliation of local symptoms.

38.4.4.1 First-Line Treatment

A number of randomized clinical studies have compared two-drug regimens (FOLFOX and FOLFIRI) to the combination of 5-FU and leucovorin (5-FU/LV) as first-line therapy, showing the addition of both oxaliplatin and irinotecan offered to patients a statistically significant advantage in terms of progression-free survival (PFS), (partly) OS, and response rates (RRs) over 5-FU/LV regimens [31]. While trends appeared to favor combined chemotherapy versus serial sequential single agents, the toxicity profiles differed with oxaliplatin-based protocols leading to more neutropenia and neuropathy and irinotecan-based causing more gastrointestinal impairment and alopecia. Therefore, the choice of therapy should be considered on a patient-by-patient approach. The oral fluoropyrimidine capecitabine, less frequently used in combination with irinotecan due to early gastrointestinal toxicity concerns [32], could be used as an alternative to 5-FU/leucovorin alone [33] and in combination with oxaliplatin [34]. Moreover, the triplet combination chemotherapy regimen FOLFOXIRI has been compared with FOLFIRI as initial therapy for mCRC patients, showing to maintain long-term outcomes with statistically significant improvements in PFS (9.8 vs 6.9 months) and median OS (21.5 vs 19.5 months), albeit with some increased toxicity but no differences in the rate of toxic death [35].

Cetuximab and panitumumab, two monoclonal antibodies directedagainst EGFR inhibiting its downstream signaling pathways, have resulted to be effective either alone as salvage or in combination with FOLFIRI and FOLFOX as initial therapy options, providing a clear clinical benefit (in terms of RRs, PFS, and OS) that is limited to patients with *RAS* wild-type metastatic colorectal cancer, as demonstrated by several randomized clinical trials [36–38] and recent meta-analyses. To date, a growing body of literature has shown that expanded RAS (KRAS/NRAS) mutational analysis of tumors (either primary or metastasis) is able to predict which patients are unlikely to benefit from EGFR antibody therapy (negative predictive factor). Therefore, it should be carried out at initial diagnosis including at least detection of mutations of KRAS exons 2, 3, and 4 (codons 12, 13, 59, 61, 117, and 146) and NRAS exons 2, 3, and 4 (codons 12, 13, 59, 61, and 117) (see Fig. 38.3) in terms of a proper first-line treatment plan, as recommended by all the current guidelines. As regards BRAF mutational status, it should be assessed alongside the RAS analysis for prognostic assessment (and/or potential selection for clinical trials), in the light of a strong evidence for its use as a prognostic factor compared to its predictive value. BRAF-mutated patients have been significantly associated with more aggressive clinical features and poorer survival rates. For a long time, the addition



Trial	Sample	Technique	Evaluable for RAS (N)	New RAS mt (N)	New RAS Mutation (%)
CRYSTAL	Extracted DNA	BEAMing	430	63	15
PRIME	Macrodissection (<50% tumor cells)	Sanger Sequencing	620	108	17

Fig. 38.3 Mutation hotspot in metastatic CRC: from KRAS to Pan-RAS

of EGFR agents did not appear to increase the benefit of standard therapy in terms of PFS and OS in the BRAF-mutated group when compared to BRAF wild-type counterpart [39]. Recent data from the randomized phase III BEACON trial suggest a potential role of the binimetinib (anti-MEK), encorafenib (anti-BRAF), and cetuximab association if compared to the doublet regimen (encorafenib + cetuximab) as salvage therapy reaching a mOS of 9.0 months (vs 5.4 months) [40].

Side effects of both anti-EGFR inhibitors include severe infusion reactions (including anaphylaxis) along with the most common skin reactions which have been shown to be predictive of increased response and survival [41].

Bevacizumab, a humanized monoclonal antibody which blocks the angiogenic activity of circulating vascular endothelial growth factor (VEGF)-A, has proved to enhance activity (in terms of PFS, RR, and/or OS) in combination with FOLFIRI [42] and to be safe and effective, especially in unfit or elderly patients, when added to 5-FU/LV [43] (or capecitabine [44]). On the other hand, the addition of bevacizumab to oxaliplatin-based regimens was associated with a more modest increase of PFS with the difference in RR and OS not reaching statistical significance in a large phase III study [45]. In addition, the phase III TRIBE trial tested the possibility of adding bevacizumab to FOLFOXIRI showing significantly increased PFS and response rate when compared to FOLFIRI/bevacizumab in the first-line treatment of very selected patients with unresectable mCRC [46]. Hence, taking into account the toxicity profile of this drug (most frequently hypertension with higher risk of gastrointestinal hemorrhage, perforation, and venous thromboembolism), no validated predictive marker currently exists for bevacizumab which is therefore indicated in combination with any cytotoxic agent until disease progression or unacceptable toxicity.

As previously discussed, the location of the primary tumor (sidedness) exhibits some important prognostic and predictive implications which significantly impact on the response to targeted therapy as well as on the treatment plan strategy, especially in the first-line setting. According to recent meta-analysis results [47, 48], considering that right-sided mCRCs seem to be associated with more frequently BRAF-mutated tumors, lower response to anti-EGFR antibodies, and poorer outcomes, the initial use

of anti-EGFR agents is somewhat controversial when the treatment goal regards prolongation of survival rates along with disease control and palliation of tumor-related symptoms, even in RAS wild-type cancers; on the other hand, it is strongly recommended to initiate first-line chemotherapy in combination with an anti-EGFR antibody in RAS wild-type left-sided mCRCs, which show a markedly greater benefit from anti-EGFR therapy (see Fig. 38.4).

Although in the FIRE-3 [49] and PEAK studies (but not in the CALGB 80905 study) improved RR and OS rates have favored the addition of EGFR antibody to combination chemotherapy as first-line treatment, when compared with bevacizumab therapy that however shows similar PFS rates, no unequivocal evidence between classes superiority (bevacizumab versus the EGFR antibody therapies) in the first-line treatment of patients with RAS wild-type mCRC can be drawn. Thus, the choice of therapy should be considered depending on the individualization of the treatment approach and the therapeutic goal.

As demonstrated in the CAIRO3 [50] and AIO 0207 trials [51], fluoropyrimidine plus bevacizumab may be considered as the preferable maintenance treatment for patients receiving a first-line "induction therapy" based on the combination of fluoropyrimidine, oxaliplatin, and bevacizumab (Fig. 38.5). In the first-line setting of mCRC patients presenting with tumors deficient in DNA mismatch repair (dMMR) resulting in the phenotype of high microsatellite instability (MSI-H), the PD-1 inhibitor pembrolizumab as monotherapy has recently proved to double time to disease progression when compared to the approved treatment based on chemotherapy plus the targeted drugs bevacizumab or cetuximab. After being approved for MSI-H or dMMR solid tumors progressing on treatment and without satisfactory alternative treatment options, interim findings from the KEYNOTE-177 trial seem to offer a new standard of care as first-line therapy in such patients in the very next future, confirming a clinically meaningful and statistically significant improvement in PFS in favor of upfront pembrolizumab comparing to chemotherapy (16.5 versus 8.2 months) with fewer treatment-related adverse events [52].

38.4.4.2 Second-Line Treatment Setting

Despite the fact that few studies have addressed the sequencing of therapies in mCRC, decisions concerning therapy after progression of metastatic disease depend

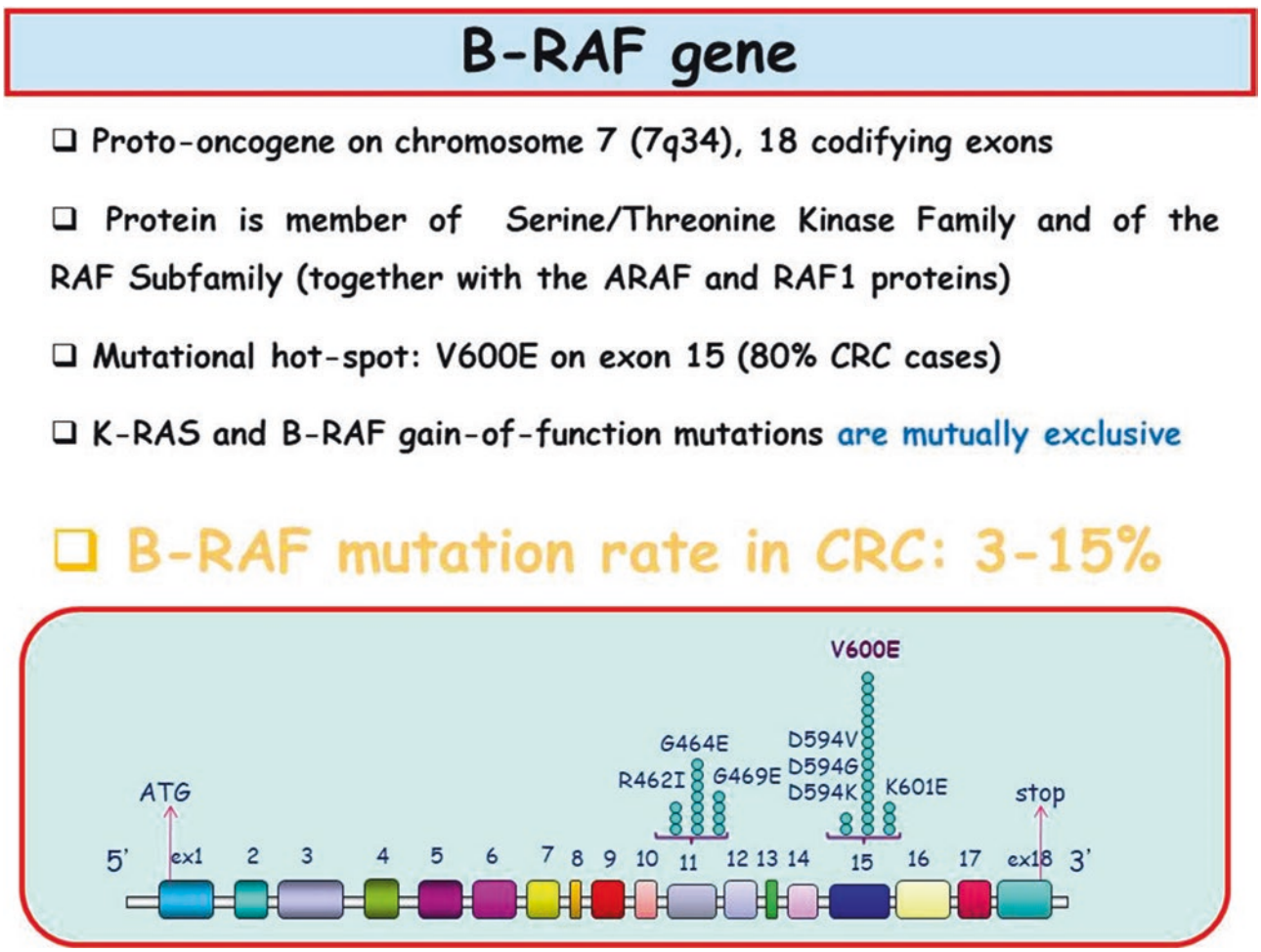


Fig. 38.4 B-RAF gene

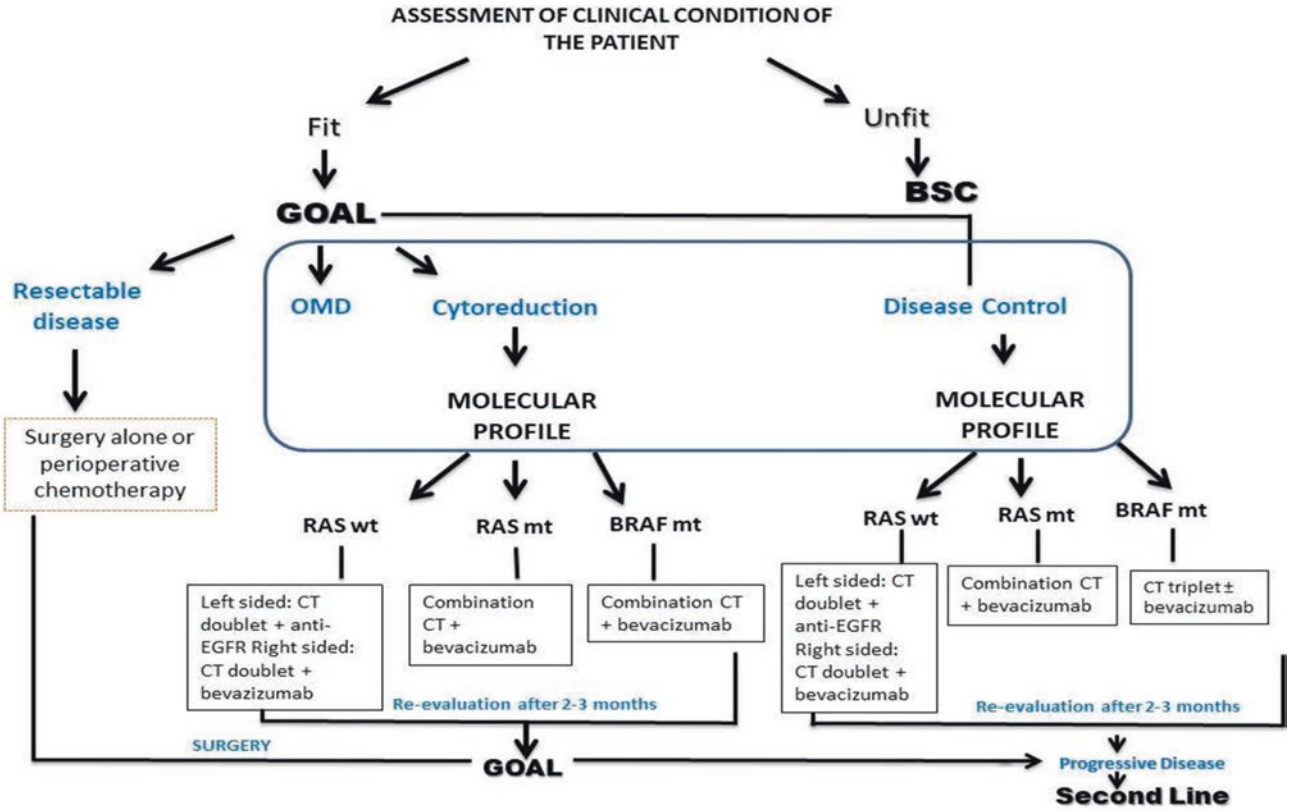


Fig. 38.5 Treatment algorithm of mCRC. *BSC* best supportive care, *CT* chemotherapy, *EGFR* epidermal growth factor receptor, *mt* mutant, *OMD* oligometastatic disease, *wt* wild-type

on upfront strategy. Second-line treatment should be offered to well-motivated patients in good performance status and adequate organ function and strictly depends on the first-line treatment choice. When considering a new treatment option, biological agents and predictive markers (e.g., tumor RAS mutation status for EGFR antibody therapy) together with a proper balance between potential treatment toxicity and efficacy should be considered in the decision-making process.

The chemotherapy backbone should be changed whenever failing in the first-line treatment. Importantly, while both EGFR antibodies have been associated with increased PFS and RR rates (but not OS) when added to irinotecan-based chemotherapy in the second-line setting [53, 54] and have shown a similar relative benefit in later lines compared with the second line of RAS wt mCRCs [55], bevacizumab has confirmed to improve OS rates in both patients who are bevacizumab naïve [56] and, albeit modestly, beyond progression in patients previously treated with bevacizumab [57], suggesting that these patients could benefit from subsequent therapies which target VEGF [58]. Hence, the anti-angiogenic fusion protein aflibercept (designed to function as a VEGF trap to prevent activation of VEGF receptors 1 and 2) has been tested in the VELOUR trial [59], resulting in survival advantage when added to FOLFIRI in patients previously progressed on a prior oxaliplatin containing regimen compared with FOLFIRI plus placebo and also in patients who are "fast progressors" on first-line bevacizumab therapy. Likewise, another anti-angiogenic agent, ramucirumab (a human monoclonal antibody that targets the extracellular domain of VEGF receptor 2), has reported a similar OS and PFS benefit, also in association with FOLFIRI, in patients whose disease progressed on first-line therapy with fluoropyrimidine/oxaliplatin/bevacizumab [60].

38.4.5 Third and Subsequent Lines

Both cetuximab and panitumumab can be used in the third line as single agents. Moreover, it has been demonstrated that cetuximab plus irinotecan is even more effective than cetuximab alone in irinotecan refractory patients [61].

Regorafenib is a multi-targeted kinase inhibitor; its activity is more effective than placebo in two trials. The CORRECT phase III trial achieved its primary endpoint OS. Patients who had progressed after all standard treatments were treated with regorafenib. The median overall survival was 6.4 months in the regorafenib group

versus 5 months in the placebo group. The main issue of this treatment remains the safety: several side effects were reported during this trial. Hypertension, diarrhea, fatigue, and hand-foot syndrome were the most common grade 3 side effects.

Another valid option in the third line is a molecule that combines trifluridine and tipiracil hydrochloride, *TAS-102*. TAS-102 reported less toxicities than regorafenib. Also, it has been shown to be effective in the refractory mCRC. A randomized trial showed an improvement in OS of the TAS group compared to the placebo, with a median OS that was, respectively, 7.1 months versus 5.3 months [62].

Even if remaining less immunogenic than other types of tumors, a small subset of mCRC patients (about 4–5%) with microsatellite instability (MSI) and deficient mismatch repair (dMMR) chemo-refractory disease seem to benefit from the novel immunotherapy checkpoint inhibitors (ICIs), nivolumab and pembrolizumab, when compared to proficient mismatch repair (pMMR) chemo-refractory patients. In recent open-label phase II studies [63, 64], these two PD-1 inhibitors have been associated with increased immune-related objective response rate in their respective dMMR arm, with pembrolizumab not still reaching median PFS and OS rates and nivolumab (also in combination with ipilimumab) showing significant PFS and OS rates at 1 year, indicating that MSI could be a predictive marker of response to ICIs independently of RAS/BRAF mutational status (see Fig. 38.6) [65].

Summary of Clinical Recommendations

- *AIOM*
  - https://www.aiom.it/wp-content/uploads/2018/11/2018_LG_AIOM_Colon.pdf
  - https://www.aiom.it/linee-guida/linee-guida-aiom-2018-neoplasie-del-retto-e-ano/
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  - https://www.nccn.org/professionals/physician_gls/pdf/colon_blocks.pdf
  - https://www.nccn.org/professionals/physician_gls/pdf/rectal_blocks.pdf

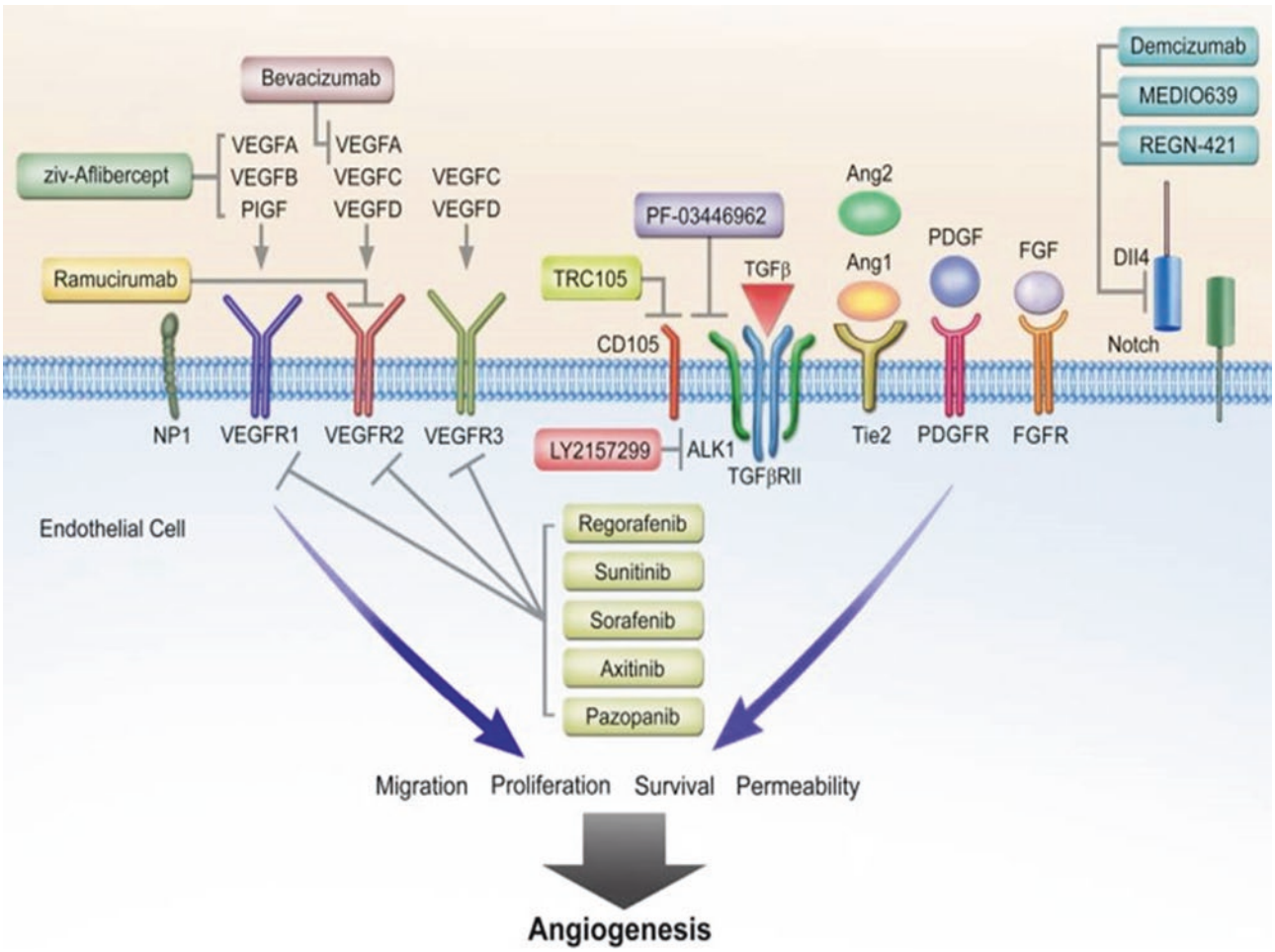


Fig. 38.6 Mechanism of selected anti-VEGF agents

Case Study: Metastatic Colon Cancer, RAS wt

Man, 70 years old

- *Family history* negative for malignancy
- *APR*: nothing to report
- *APP*: left flank pain
- *Blood tests*: Hb 12.1 g/dL; mild increased level of gamaGT, ALT, and AST

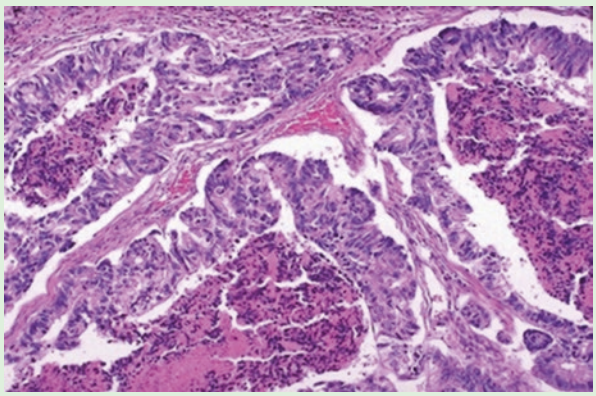
Question

What action should be taken?

(1) Surgery. (2) Colonoscopy + biopsy. (3) Others

Answer

- *Colonoscopy*: bleeding ulcerative lesion in the left colon. Biopsies were performed.
- *Histological examination*: "Colon adenocarcinoma, G2"



Question

What action should be taken?

(1) CT scan. (2) Medical therapy. (3) Others

Answer

Clinical staging → with chest-abdominal CT scan: bilobar liver metastases

Question

What would you do next?

(1) Surgery. (2) Medical therapy. (3) Others

Answer

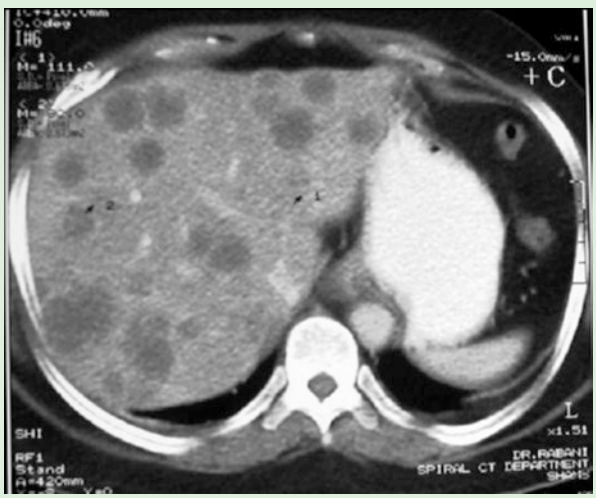
n-RAS and k-RAS are not mutated.

Medical therapy: FOLFOX bevacizumab and FOLFOX cetuximab are both options.

Case Study: Metastatic Colon Cancer, RAS Mutated

Woman, 65 years old

- *Family history* negative for malignancy
- *APR*: negative
- *APP*: asthenia, dyspepsia, change in bowel habit
- *Blood tests*: Hb 9.2 g/dL
- *Colonoscopy and biopsies*: bleeding mass at the right colon. "Colon adenocarcinoma."
- *Chest and abdominal CT scan*: multiple bilobar liver lesions
- *RAS mutated*



Question

What action should be taken?

(1) Surgery. (2) Biopsy. (3) Chemotherapy

Answer

Chemotherapy with FOLFOXIRI plus bevacizumab

Question

Is therapy with panitumumab recommended?

(1) Yes, if there is a progression disease. (2) No it is not. (3) Yes, combined with bevacizumab.

Answer

Anti-EGFR therapy is not recommended in RAS mutated colon cancer.

Key Points

- The importance of a correct diagnosis
- The importance of administering the right chemotherapy combination

Expert Opinion

Marc Peeters

- Metastatic colorectal cancer (mCRC) is the second most common cause of cancer-related death in both sexes, and, despite major advances in treatment, mortality remains high.
- A multidisciplinary team approach effort that considers patients' characteristics, tumor genomics, and treatment goals is crucial for the best treatment selection of mCRC patients.
- Treatment intensification strategies should be considered to improve response and resectability in potentially resectable mCRC.
- An upfront molecular testing (RAS/BRAF/MSI) has become of paramount importance to best determine the most effective therapeutic intervention. Likewise, the widespread use of next-generation sequencing paves the way for a more comprehensive molecular signature with potential future therapeutic implications.
- Although the optimal use and sequencing of chemotherapeutic and targeted agents across multiple lines of treatment remains unclear, the proper choice of an effective first-line therapy has resulted to be a key determinant for successful treatment outcomes.
- The impact of primary tumor location (sidedness) on the biological and clinical outcome of mCRC has recently resulted to be prognostic as well as predictive of response and survival in patients receiving biological therapies.

Hints for a Deeper Insight

Despite a substantial rise in survival over the last two decades *due* to the success of molecularly *targeted therapies,* both clinical and molecular data have shown that patients with mCRC present with heterogeneous prognosis and response to treatment.

Unfortunately, a single tissue biopsy often underestimates the dynamic molecular landscape of the disease with a limited ability to understand intra- and inter-tumoral heterogeneity, which is considered one of the major reasons for treatment failure and drug resistance. In the era of precision oncology, the noninvasive evaluation of tumor-derived biomarkers (including circulating tumor cells [CTCs], circulating tumor DNA [ctDNA], and exosomes) more frequently isolated from the peripheral blood (liquid biopsy) is a viable alternative to tissue-based genotyping, providing a comprehensive real-time picture of the tumor-associated changes in terms of a serial assessment of the clonal tumor dynamics and a meticulous characterization of drug tailoring and response in an individual cancer patient. Although the use of liquid biopsy has resulted in limited and mixed success while being still extremely limited in mCRC clinical practice, recent data demonstrate that tumor clonal evolution can be detected and longitudinally monitored in circulating ctDNA, revealing that mutant RAS clones arise in blood during EGFR blockade and exponentially decline upon withdrawal of treatment. Hence, several ongoing and future studies investigating the molecular characterization of mCRC by ctDNA detection trigger an interest in anti-EGFR retreatment and will elucidate how the dynamic clonal competition would likely impact on this therapeutic strategy (either by rechallenge strategy or by switching to alternative EGFR-targeted drugs or to new-generation agents targeting other specific subclones upon resistance), mostly considering that no phase 3 data are currently available.

BRAF mutant tumors have poorer prognosis and limited therapeutic options.

Recently, new pharmacological approaches to treat BRAF-mutated mCRC patients have shown survival benefit in a randomized phase III trial. Upon BRAF inhibition, upregulation of EGFR occurs as an escape molecular mechanism. A novel pharmacological approach that includes combination of a selective BRAF inhibitor with an anti-EFGR drug (with or without a MEK inhibitor) has shown to improve survival compared to standard arm with chemotherapy plus anti-EGFR drug. This is the first chemo-free regimen that shows efficacy in mCRC and brings hope for BRAF the very aggressive BRAF mutant tumors.

In the near future, both the emerging genome-wide gene expression analyses and the clinical proteomics will help promote personalized medicine with the identification and the refinement of new targets and signature biomarkers for combination therapy strategies.

Immunotherapy has shown striking results in the subset of mCRC tumor with microsatellite instability (MSI-H) or mismatch repair deficiency (dMMR), which accounts for approximately 5% of all mCRC. Despite these promising results and tangible advances, uncovering the molecular mechanisms responsible for primary and acquired resistance to immune checkpoint inhibitors will be crucial to develop more reliable predictive biomarkers and new potential treatment strategies, hopefully for a larger subset of mCRC patients.

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Anal Cancer

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Gastrointestinal Cancers

Contents

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Learning Objectives

By the end of the chapter the reader will:

1. Have learned the basic concepts of epidemiology, histological subtype, and clinical manifestation of anal neoplasms
2. Be able to define staging strategies, diagnostic, and therapeutic procedures
3. Be able to put acquired knowledge into clinical practice
4. Be able to realize future perspectives of anal neoplasms

39.1 Introduction

The anal canal is the terminal part of the digestive canal, between the rectum and the skin of the anal margin, approximately 3–4 cm long. The anal carcinoma is considered to be a rare type of cancer. The squamous forms of tumor of the anus consist in about 95% of the tumors of the anal canal, and only a small portion of these (about 10%) begins at an advanced stage. Conditions that increase the risk of HPV infection and/or modulate host response and persistence of infection appear to influence the epidemiology of this tumor. In particular, anal intercourse and a high number of sexual partners increase the risk of persistent HPV infection, both in men and women, with consequent development of neoplasia. Among the subtypes, HPV-16 is responsible for the infection in 73% of all HPV-related tumors and is the most commonly found variant. The importance of HPV is in its role as a potential risk factor for the development of precancerous lesions (AIN, anal intraepithelial neoplasia) and therefore neoplastic (SCCA, squamous cell carcinoma). Other important risk factors include HIV infection, immunosuppressive therapy in transplant patients, the use of immunosuppressant such as high-dose steroid therapy, a history of other HPV-related neoplasms, disadvantaged socioeconomic conditions and cigarette smoking. Smoking may also be important in modulating the persistence of HPV infection with a possible impact on treatment outcomes. Also consider the number of sexual partners, a history of anal warts, previous dysplasia or genital tract carcinomas, and smoking [1–3].

39.2 Epidemiology

The annual incidence rate is approximately 1/100,000 inhabitants per year, and its incidence is increased in developing countries: there are approximately 27,000 new cases of anal carcinoma per year.

The incidence rate is three- to sixfold for women, but men with HIV infection have a greater risk to be infected by HPV. Other risk factors are represented by a history of cervical, vulvar, or vaginal carcinoma and persistent infection with high-risk form of HPV (e.g., HPV-16, HPV-18).

Sexually transmitted diseases and certain autoimmune disorders are considered to be other important risk factors.

Most primary cancers of the anal canal are of squamous cell histology. There are other common anal canal tumors that have different histological features: adenocarcinoma, small-cell carcinoma, undifferentiated cancers, and melanomas.

The incidence rate of anal carcinoma associated with HPV infection is 88%. HPV-16 is the genotype that is most involved in the anal carcinoma HPV-related.

A 9-valent HPV vaccine is available, protecting against HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52, and HPV-58. It is predicted that this new vaccine will prevent additional 464 cases of anal cancer annually.

HPV is responsible for precancerous lesion, called anal intraepithelial neoplasia (AIN), that can be divided into low-grade and high-grade.

High-grade AIN can be a precursor of anal carcinoma, and its treatment can prevent the development of cancer. AIN can be identified by HPV testing, cytology, digital rectal examination (DRE), and high-resolution anoscopy and/or biopsy. The regression of high-grade AIN is unknown, and it is estimated to be very low in men that have sex with men [1, 2].

High-risk patients are known to be those with HIV infection. Routine screening for AIN lesions is controversial, although few guidelines recommend screening programs for HIV-positive people (Fig. 39.1) [3–6].

39.3 Anatomy, Histology, and Pathology

The anal region is made of the anal canal and the anal margin, so that we can distinguish two different types of anal cancer.

The anal canal is the most proximal part of the anal region.

Histologically, the mucosal lining of the anal canal is composed of squamous epithelium, while the mucosa of the rectum is lined with glandular epithelium.

The anal margin is lined with skin.

The most superior aspect of the anal canal is a 1–2 cm zone between the anal and rectal epithelium. The most inferior aspect of the anal canal corresponds to the area where the mucosa, lined with modified squamous epithelium, transitions to an epidermis-lined anal margin. The anatomic anal canal begins at the anorectal ring and extends to the anal verge (i.e., squamous mucocutaneous junction with the perianal skin)

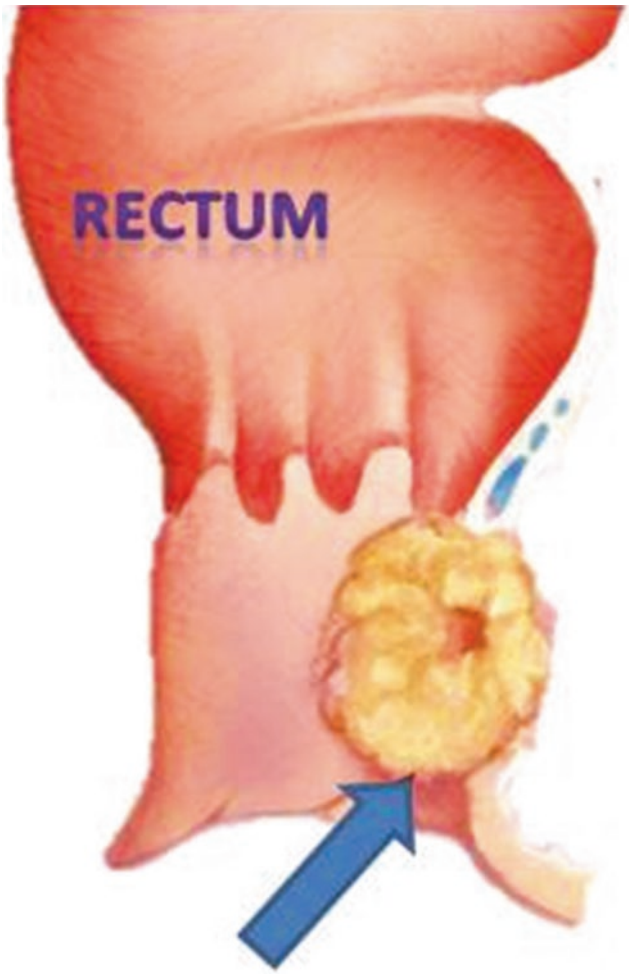


Fig. 39.1 Anal carcinoma localization

The squamous cell histology is the most common type of cancer. There are many other variants, recognized by the WHO, as large cell keratinizing, large cell non-keratinizing, and basaloid. All these subtypes are included in a single definition of squamous cell carcinoma.

Other less common anal canal cancers are adenocarcinomas, small-cell (anaplastic) carcinoma, undifferentiated carcinoma, and melanomas [7].

Anal carcinoma can be well differentiated (G1) and poorly differentiated (G4) [8, 9].

39.4 Staging and Prognostic Factors

The TNM staging for anal carcinoma is developed by the American Joint Committee on Cancer (7th edition).

Current recommendations do not involve a surgical excision, and most tumors are staged clinically by direct examination and microscopic confirmation. A biopsy is always required. Rectal ultrasound, to determine the size and the extension of the tumor, is not required during this phase.

The prognosis of anal carcinoma is related to the size of the primary tumor and the lymph node involvement.

Lymph node staging is based on location of involved lymph nodes:

1. N1, one or more perirectal nodes
2. N2, unilateral internal iliac nodes and/or inguinal nodes
3. N3, perirectal and inguinal nodes and/or bilateral internal iliac nodes and/or bilateral inguinal nodes

Surgery excision is not considered to be the initial therapy, and the lymph nodes status should be determined clinically and radiologically.

Fine needle aspiration (FNA) biopsy of inguinal nodes can be considered if node involvement is suspected. PET CT and CT scans alone are not recommended to investigate node involvement. In a series of patient that underwent abdominoperineal resection (APR), it was noted that pelvic nodal metastases were often less than 5 mm and PET and CT scans were not reliable to determinate their involvement.

Size, sex, and lymph node involvement are considered to be prognostic factors. Multivariate analysis of data from the RTOG 98-11 showed that male sex and positive nodes were independent prognostic factors for disease-free survival in patient with anal cancer treated with 5FU and radiation and either mitomycin or cisplatin. Male sex, node positivity, and tumor size greater than 5 cm were independent prognostic factors for worse overall survival.

In the EORTC 22861 trial, it has been noted that male sex, node positivity, and skin ulceration were prognostic factors for worse survival and local control (Tables 39.1 and 39.2) [13].

39.5 Clinical Presentation and Evaluation

The most common clinical presentation of anal carcinoma is represented by rectal bleeding, pain, and sensation of a rectal mass.

A clinical examination is recommended: DRE, anoscopic examination, and inguinal lymph nodes palpation.

MRI and CT scan are recommended for the evaluation of the pelvic lymph nodes. A FNA, when feasible, is always recommended to investigate lymph node involvement.

CT scan and MRI pelvis are always important to determine whether the tumor involves other abdominal/pelvic organs; however a T stage assessment is performed

Table 39.1 TNM classification for anal cancer. (American Joint Committee on Cancer, 8th edition)

Primary tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ (Bowen disease, high-grade squamous intraepithelial lesion [H-SIL], anal intraepithelial neoplasia II–III (AIN II–III)
T1	Tumor 2 cm or less in greatest dimension
T2	Tumor more than 2 cm but not more than 5 cm in greatest dimension
T3	Tumor more than 5 cm in greatest dimension
T4	Tumor of any size invades adjacent organ(s) (e.g., vagina, urethra, bladder); direct invasion of the rectal wall, perirectal skin, subcutaneous tissue, or the sphincter muscle(s) is not classified as T4
Regional lymph nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	*N1a* metastases in inguinal, mesorectal, and/or internal iliac nodes
	N1b metastases in external iliac nodes
	N1c metastases in external iliac and in inguinal, mesorectal, and/or internal iliac nodes
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis

Table 39.2 Anatomic stage/prognostic group

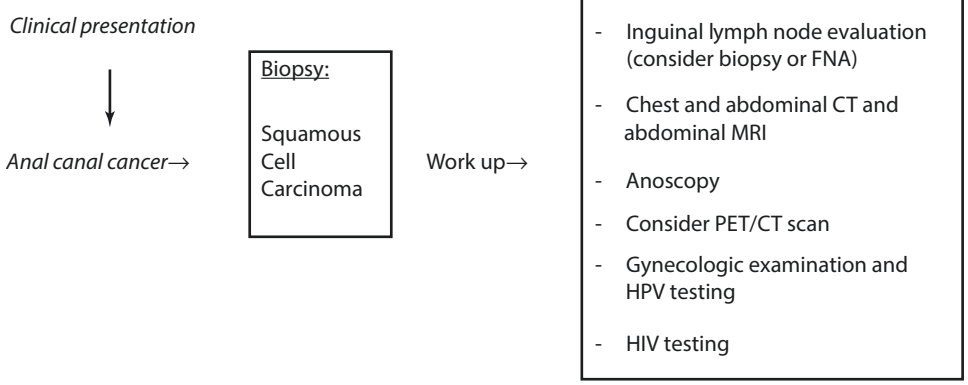
Stage		T	N	M
0		Tis	N0	M0
I		T1	N0	M0
II	*IIa*	T2	N0	M0
	IIb	T3	N0	M0
III	*IIIa*	T1	N1	M0
	IIIa	T2	N1	M0
	IIIb	T4	N0	M0
	IIIc	T3	N1	M0
I	*IIIc*	T4	N1	M0
IV		Any T	Any N	M1

through clinical examination. Chest CT scan is recommended to evaluate pulmonary metastasis.

HIV testing and measurement of CD4 levels can be performed since the anal cancer has been reported to have a higher incidence rate in HIV-positive patients.

A gynecologic examination is also suggested, including cervical cancer screening, since HPV is also associated with cervical cancer.

The staging before any treatment should be performed through PET and CT scan. PET/CT should be performed also in patients who have normal-sized lymph node at the CT scan [21–24].



39.6 Management

39.6.1 Primary Treatment of Non Metastatic Anal Carcinoma

In the past, patients with invasive carcinoma were treated with abdominoperineal resection (APR), but local recurrence rates were high, and the 5-year survival rate is about 40%.

Many non-randomized studies demonstrated that the administration of chemotherapy and radiation therapy had a higher efficacy (in terms of local recurrence) than surgery (APR).

Currently concurrent chemoRT is the recommended primary treatment for locally advanced anal canal cancer.

(a) *Chemotherapy and Radiation Therapy (chemoRT)*: A phase III study from the EORTC compared radiation therapy (RT) alone versus chemotherapy (5FU and mitomycin) and radiation therapy (chemoRT). The second option (chemoRT arm) showed more local control than RT alone.

A few studies have addressed the safety and efficacy of many chemotherapeutic agents. In a phase III intergroup study, patients receiving chemoRT with the combination of 5FU and mitomycin had a lower colostomy rate and a higher DFS compared to patients receiving chemoRT with 5FU alone, indicating that mitomycin is important in the treatment of anal carcinoma.

Capecitabine is a good alternative to 5FU in the treatment of the anal carcinoma.

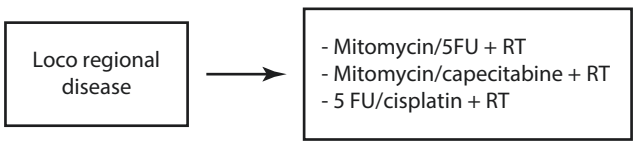
Cisplatin, as a substitute to 5FU was evaluated in a phase II trial, and results suggest that cisplatin or 5FU may be comparable for treatment of locally advanced anal cancer.

The phase III UK ACT II trial compared 5FU/mitomycin and 5FU/cisplatin, RT was also administered, and a maintenance therapy with 5FU or cisplatin was administered in one of the two arms. Results showed that mitomycin can be replaced by cisplatin because this will not affect the complete response; on the other hand, it was also demonstrated that maintenance therapy did not decrease the rate of disease recurrence.

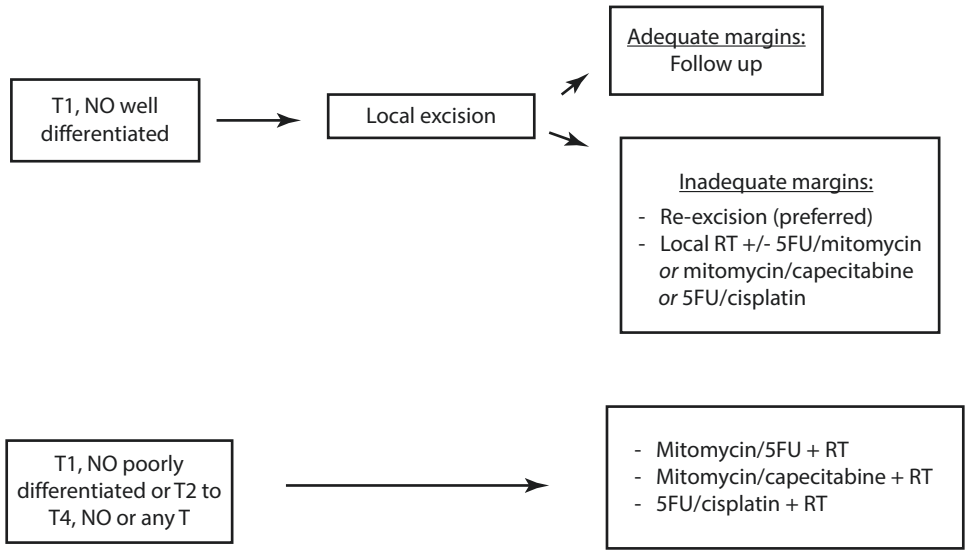
It has also been discussed the role of induction therapy, prior to a chemoRT.

The results of a recent study, the ACCORD 03, showed that there was no benefit in patients that had received induction chemotherapy prior to chemoRT. In this study patients with locally advanced anal cancer were randomized to receive induction therapy with 5FU/cisplatin or no induction therapy followed by chemoRT.

Cetuximab is an epidermal growth factor receptor (EGFR) inhibitor that works very well when RAS family genes status (KRAS and NRAS above all) is wild type. Since RAS mutations in anal cancer are very rare, cetuximab has been considered to be a promising avenue of investigation. Few studies evaluated toxicity and efficacy of chemoRT and cetuximab. The ACCORD 16 phase II trial was designed to assess response rate after chemoRT with cisplatin/5FU and cetuximab was terminated because of many adverse events. Other studies are still ongoing [17–20, 25].



1. *Surgery:* it is recommended in a few cases:
2. *Recurrence after chemoRT*: an abdominoperineal resection is recommended in patients that present a recurrence after a concurrent chemoRT.
3. *T1, N0 (well-differentiated, <1.0 cm):* a local excision is recommended for patients with a T1, N0 well-differentiated and small lesion. It is important that margins are not involved. If margins are inadequate a re-excision is a preferred treatment. If a re-excision cannot be performed, local RT with or without chemotherapy is recommended [14, 15, 16].



39.7 Metastatic Disease

It has been reported that the most common sites of metastasis outside the pelvis are the liver, lungs, and lymph nodes. Since anal carcinoma is a rare cancer, only 10–20% of patients present extra pelvic metastasis. Despite this fact, some evidences suggested that chemotherapy with fluoropyrimidine-based regimen plus cisplatin has some benefit in patients with metastatic anal carcinoma [25–28].

No evidence supports resection of metastatic disease. Recently, the INTERAACT trial results were published. This is an open-label randomized phase II trial that was aimed to compare cisplatin (CDDP) plus 5-fluorouracil (5FU) versus carboplatin (CBDCA) plus weekly paclitaxel (PTX) in patients with inoperable locally recurrent (ILR) or metastatic disease and 5FU. Patients with metastatic squamous cell carcinoma of the anus were randomly assigned to receive cisplatin and 5FU or weekly carboplatin and paclitaxel. The primary endpoint was ORR, and it was reached in the group treated with cisplatin and 5FU (59% versus 57%) [29].

39.7.1 Treatment of Recurrent Anal Carcinoma

Despite the effectiveness of chemoRT as primary treatment for locally advanced anal cancer, rates of 10–30% of local recurrence are reported. When recurrence occurs, APR is indicated. A recent retrospective analysis showed that patients who received intra-operatory RT during APR had improved local recurrence. Inguinal node dissection is reserved for recurrence in that area and can be performed without an APR in cases where recurrence is limited at inguinal nodes. Patients that presented inguinal recurrence without APR could receive chemoRT with RT to the groin, if no prior RT to the groin was given [26].

39.8 Screening

Routine screening for AIN lesions is controversial, although few guidelines recommend screening programs for HIV-positive people. Gynecologic examination, HPV testing, and cytology in high-risk patients can be performed even if it is not recommended.

High-grade AIN can be a precursor of anal carcinoma. The spontaneous regression of AIN is possible but the regression rate is unknown [9].

Anal canal lesions, according to their cytology, can be classified (Bethesda Classification 2001) into:

1. AIN: anal intraepithelial neoplasia
2. ASCUS: atypical squamous cell of undetermined significance
3. ASC-H: atypical squamous cell suspicious for H-SIL
4. L-SIL: low-grade squamous intraepithelial lesion
5. H-SIL: high-grade squamous intraepithelial lesion
6. SCC: squamous cell carcinoma

If the cytology indicates ASCUS, L-SIL, or H-SIL, the patients will perform high-resolution anoscopy and/or biopsy:

7. AIN 1 (L-SIL): a clinical assessment should be performed every 6–12 months.
8. AIN 2/3 (H-SIL): should be treated and/or clinical assessment after 6 months [9–12].

39.9 Summary: Conclusion

Anal carcinoma is a rare type of cancer but its incidence rate is increasing.

The treatment of this disease should be approached by a multidisciplinary team including physicians from GI, medical oncology, surgical oncology, and radiology.

Recommendations for the primary treatment of locally advanced anal cancer are very similar and include chemoRT.

The treatment for T1, N0 well-differentiated cancers is represented by local excision with adequate margins.

Following complete remission, patients with local recurrence should be treated with APR.

A 5FU/mitomycin or 5FU/cisplatin regimen is associated to RT. Since RAS mutations are very rare in the anal cancer, cetuximab is a promising agent, although toxicity still represents a big issue.

Case Study: Locally Advanced Anal Carcinoma

Man: 55 years old

- *Family history*: negative for malignancy
- *APR*: nothing to report
- APP: pain, bleeding, and poor bowel function unresponsive to laxative treatment of 5 months' duration.
- *Objective examination*: EDAR → depressed hard area in the anal canal with blood on the glove.
- *Blood tests*: Hb 12.1 g/dL; mild increased level of gamaGT

Question

What action should be taken?

(1) Surgery. (2) Biopsy. (3) Others

Answer

- *Anoscopy*: ulcer in the anterior anal canal and biopsies were taken.
- *Histological examination:* "Squamous cell carcinoma of the anus (SCCA)"

Question

What action should be taken?

(1) Surgery. (2) Medical therapy. (3) Clinical staging

Answer

Clinical Staging → with pelvic MRI: pelvic lymph nodes not involved. The patient was staged as *cTNM*, *T4*, *N0* → *Chest and abdominal CT scan with and without contrast*: no distant metastases

Question

What would you do next?

(1) Surgery. (2) Medical therapy. (3) Others

Answer

Medical Therapy: RT and 5FU + cisplatin

Key Points

1. The importance of a correct diagnosis: attention to rectal masses
2. Symptoms often nonspecific
3. The importance of the management of a locally advanced disease

Case Study: Metastatic SCCA

Man: 70 years old

- *Family history*: negative for malignancy
- *APR*: negative
- *APP*: asthenia, dyspepsia, change in bowel habit
- *Blood tests*: Hb 9.2 g/dL
- *Objective examination*: EDAR → hard and bleeding area in the anal canal
- *Anoscopy and biopsies*: SCCA
- *Chest and Abdominal CT scan*: multiple liver lesions

Question

What action should be taken?

(1) Surgery. (2) Biopsy. (3) Chemotherapy

Answer

Chemotherapy with 5FU and cisplatin

Question

Is metastasectomy on liver lesions recommended?

(1) Yes, after four cycles of chemotherapy. (2) Surgery is not indicated for the metastatic setting. (3) Others

Answer

No evidence supports resection of metastatic disease.

Expert Opinion

Marc Peeters

- Anal canal neoplasms are a group of diseases with a low incidence but a relative increase in the most industrialized countries. The main risk factors are HPV and HIV infection, immunosuppressive therapy in transplant patients, the use of immunosuppressant such as high-dose steroid therapy, a history of other HPV-related neoplasms, disadvantaged socioeconomic conditions, and cigarette smoking.
- The instrumental diagnostic approach in case of suspected disease is represented by clinical examination and endoscopic evaluation. More specific evaluation must be carried out using the TC and the MRI using in some circumstances PET integration.
- Local stage: currently concurrent chemoRT (using 5FU and/or mitomycin) is the recommended primary treatment for locally advanced anal canal cancer, and capecitabine is a good alternative to 5FU. Surgery could be a valid option in those superficially minimal invasive squamous cell carcinomas.
- Local recurrence/persistence: APR represents a fundamental moment.
- Advanced stage: chemotherapy with fluoropyrimidine-based regimen plus cisplatin has some benefit in patients with metastatic anal carcinoma.

Hints for a Deeper Insight

In the era of personalized medicine, the treatment of anal squamous cell carcinoma (ASCC) is changing, and several therapeutic options are going to be explored.

The development and introduction of immunotherapeutic agents have brought new possibilities in the treatment of metastatic ASCC. HPV-positive ASCC is associated with more immunogenicity, and thus immunotherapy is going to represent an intriguing alternative. Several trials indicate that immunotherapy combined with chemoradiotherapy (CRT) might be a valid option: RT can activate the immune system and promote tumor infiltration of CD8+ TILs. Immunotherapies can enhance TILs cytotoxic function and motility. Furthermore, the introduction of vaccines is bringing new hope in the treatment of ASCC; the live-attenuated listeria monocytogenes cancer vaccine ADXS11-001 targeting HPV-positive is going to be tested in combination with standard CRT in patients with ASCC treated for curative intent. For this reason, the use of immunotherapeutics need a patient stratification since the HPV infection plays an important role in predicting the treatment response.

Besides immunotherapy, efforts are being made to reduce the CRT-related toxicity CRT: the PLATO trial is testing the concept of stage-dependent RT dose adaptation. Since the dose modulations are small, the oncological outcome should not change. In this respect, data from the Danish Head and Neck Cancer Association (DAHANCA) have showed that the use of 5 fractions per week instead of 6 led to higher acute toxicity, while no differences in late toxicities were reported. Interestingly, there was an improvement in DFS.

In conclusion, although 5FU/MMC CRT still constitutes the standard of care, new approaches are currently being explored. The "dose adaptation" concept can avoid unnecessary toxicity, and the HPV status can help to stratify patients for immunotherapy.

New clinical trials are needed to test the combination between immunotherapy and CRT in the primary and metastatic setting; all these new perspectives could have an important impact on the way physicians can treat patients with ASCC in the near future.

Suggested Reading

- ► https://www.aiom.it/wp-content/uploads/2019/10/2019_LG_AIOM_Retto_ano.pdf
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Cancer of Exocrine Pancreas

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Gastrointestinal Cancers

Contents

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Learning Objectives

By the end of the chapter, the reader will

- Have reached a good knowledge of the epidemiology and risk factors of pancreatic adenocarcinoma
- Have learned the most important pathogenic mechanisms at the basis of pancreatic cancer development
- Be able to identify signs and symptoms that can raise suspect of pancreatic cancer
- Have a good knowledge of the different diagnostic tools available in diagnosis and staging of pancreatic cancer
- Be able to understand the difference between resectable, borderline resectable, unresectable, and metastatic pancreatic cancer, learning the criteria that drive the clinician in this classification
- Have gained a better understanding of well-established and innovative therapeutic algorithms

40.1 Introduction

Pancreatic cancer is one of the most lethal and aggressive human malignancies, accounting for the fourth leading cause of cancer-related death in United States of America and causing approximately 350,000 deaths world wide every year [1]. Pancreatic ductal adenocarcinoma (PDAC) is a malignant disease of the exocrine pancreas with poor prognosis and a 5-year survival rate lower than 5%. The risk of developing this tumor is equal both for men and women [2]. Many risk factors, both environmental and genetic, have been identified, the most important of which are: excessive body weight, diabetes and smoking [3]. Although, in the last few years, attempts have been made to develop early detection methods, such as spiral TC, MRCP, and EUS, and innovative therapeutic strategies in order to prolong survival and improve patient life quality, these efforts have met limited success, and surgical resection remains the only possible successful treatment option when resection margins remain negative [4, 5]. However, only a small percentage of patients (about 15%) with localized pancreatic tumors is candidate for surgical resection, since most of them, instead, exhibits a locally advanced or metastatic disease with unresectable lesions at the diagnosis. Indeed, pancreatic cancer is a silent disease, with few or no symptoms and signs until late stages [6]. Because most of surgically resected patients rapidly develop new locoregional lesions or exhibit metastatic disease progression, surgery alone appears to be inadequate and insufficient in eradicating the disease and improving prognosis [7, 8]. Therefore, along with surgery, chemo- and radiotherapy represent other treatment options, though the current therapeutic regimens provide only some small benefit for PDAC patients. Unfortunately, pancreatic cancer is inherently resistant to most currently available therapies, and, unlike other cancers, few progresses have been achieved with radio- or chemotherapy [9, 10]. Moreover, many patients with pancreatic cancer suffer from rapidly declining performance status, anorexia, and cachexia, which make it challenging to treat them. The cellular and molecular characteristics of ductal pancreatic cancer it is aggressive, with multiple levels of therapeutic resistance determined by reduced vascular density, stromal proliferation, and immune suppression. Indeed, the development and selection of pancreatic cancer cells resistant to therapies is one of the major hurdles for the clinical management of PDAC patients, leading to tumor recurrence and, consequently, a poor prognosis [11]. Therefore, adopting adequate strategies capable of overcoming the resistance which patients may develop during chemo- or radiotherapy is the main goal of clinical research. Understanding the molecular mechanisms underlying the therapy resistance and identifying new targets able to improve efficacy of therapeutic treatment may help oncologists to favor the development of personalized therapies for PDAC patients [12]. Since pancreatic cancer shows a multifactorial nature, early detection strategies or specific disease biomarkers are difficult to identify. The identification of new diagnostic, prognostic, and predictive biomarkers could represent an important tool to select patients who may benefit from a specific treatment and a crucial step toward a tailored therapy. Improvements in early screening strategies, the development of new therapies, and further progress in understanding the genetic and molecular basis of PDAC are needed in order to greatly reduce high mortality rates [13].

In this chapter, we will discuss the genetic, molecular, and clinical aspects of pancreatic cancer, by describing the alterations involved in carcinogenesis process and providing an overview of current therapeutic options and potential reasons of their failure.

40.2 Epidemiology

About 338.000 people develop pancreatic cancer every year, making it the 11th most common cancer worldwide [14]. Incidence can vary from 7.4/100.000 in the western world, where more than half of new cases are diagnosed to 2/100.000 in developing countries. These differences can be attributed to lifestyle and environment factors but, in a smaller proportion, also to differences in the accuracy of the diagnosis [15–18]. The

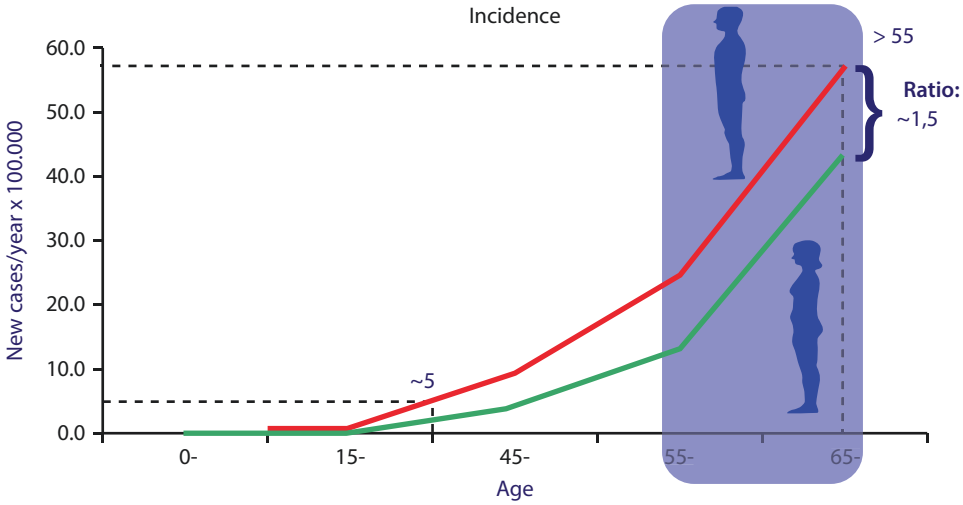


Fig. 40.1 Difference in male and female incidence of pancreatic cancer according to age

risk is higher in males, with a man/woman rate of 1.5:1, and greatly increases with aging, the highest peak being in patients older than 70 years and more than 90% of cases being diagnosed after the age of 55. On the contrary, this disease is very rare under the age of 45 (Fig. 40.1) [19–21].

Even though PDAC is not among the most frequent kinds of cancer, it certainly is one of the deadliest: PDAC is the seventh cause of death among cancers globally, both in men and women, with a total of more than 331.000 death per year, and it can be held accountable for the 6% of all cancer-related deaths [14]. In developed countries such as Europe and United States, it represents the fifth and fourth cancer-related cause of death, respectively [1, 22]. Incidence can also differ on the basis of the ethnicity of the patient, being higher in Afro-Americans, but the reasons for this are still unclear: differences in dietary habits, smoking, and obesity rates play important roles, but genetic factors are also involved [23, 24].

Notably mortality and incidence rates are very similar: due to the extremely low survival rates for this tumor, around 5% at 5 years, one of the lowest among all cancers [11, 14, 25, 26]. This data has remained stable in the last 20 years, with little progresses obtained for the prognosis of these patients [27, 28]. Incidence and mortality of pancreatic cancer are also rising globally, especially in Western world (+19% deaths in Europe from 2009 to 2014) [19, 21]: it is expected that in 2030, the number of patients affected by PDAC will have increased more than twofold over the current global rate. Considering these data, the number of deaths will probably exceed those of breast and colorectal cancer, and pancreatic cancer will be the second tumor for mortality worldwide, being surpassed only by lung cancer [17].

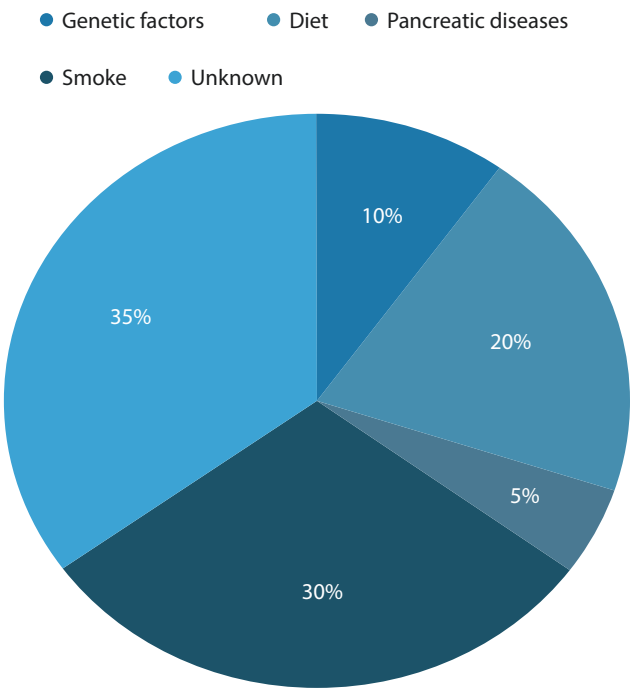


Fig. 40.2 Most important causes of pancreatic cancer

40.3 Risk factors

40.3.1 Environmental risk factors

Most of PDAC are caused by environmental causes, by lifestyle, or by other, non-oncological, reasons (80–90% of total) (Fig. 40.2) [16, 29]. Of these factors, the most important can undoubtedly be considered cigarette smoking [30–32]: chronic intake of nitro derivatives contained in tobacco can cause genetic mutations such as the activation of K-Ras and the subsequent

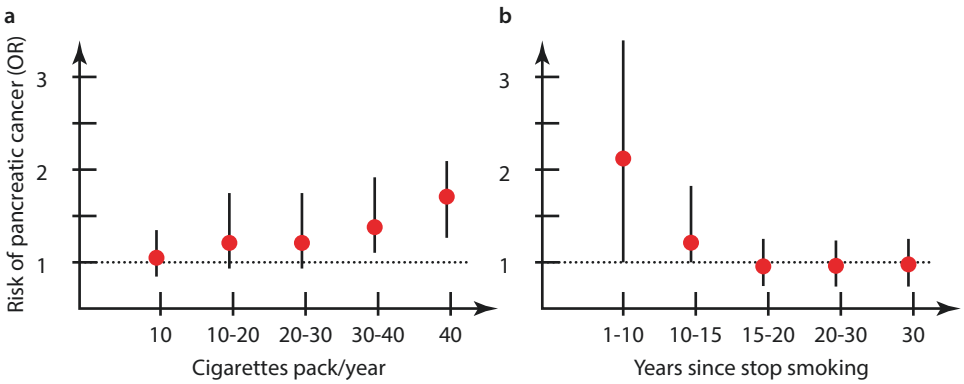


Fig. 40.3 Risk of pancreatic cancer, on the basis of the number of cigarettes smoked **a** and of the years passed since smoking has been stopped **b**

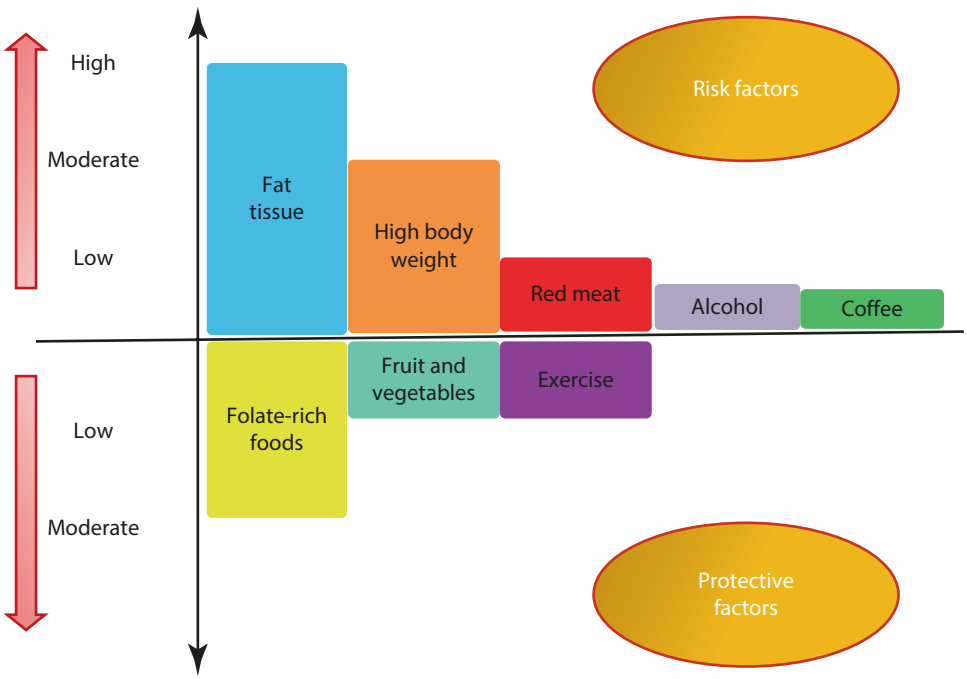


Fig. 40.4 Most important risk factors and protective factors in pancreatic cancer

development of pancreatic adenocarcinoma. Autopsies provided data that demonstrate how nitro derivatives damage pancreatic tissue. Smoking has been related up to 30% of all cases. The overall risk increases up to 2–5 times, and it keeps growing steadily with the number of cigarettes consumed, while it decreases with the number of years since smoking has been stopped, being comparable to that of nonsmokers after 15 years (Fig. 40.3) [33]. Passive smoking too has been associated with a higher risk [34, 35].

Other important environmental risk factors are, in order of importance (Fig. 40.4 and Table 40.1):

- Body fat tissue, particularly abdominal fat tissue, probably by contributing to the development of an abnormal glucose metabolism mechanism, can increase the risk of pancreatic cancer [36, 37].
- Obesity (BMI > 30): 20–40% higher risk of death by pancreatic cancer [37–39].
- Red or processed meat [40–42].
- Alcohol consumption [43].

Table 40.1 Risk factors for pancreatic

Aquired risk factors	Pathologies
Abdominal fat tissue	Diabetes mellitus
Obesity	Chronic Pancreatitis
Red or processed meat	Hereditary pancreatitis
Alcool consumption	*H. pylori*, HCV or HIV infection

Foods rich in folate (while not folate dietary supplements), fruit, vegetables, and exercise are instead considered protective factors (▶ Box 40.1) [44, 45]. It is still unclear if coffee regular consumption can increase the risk of pancreatic cancer [42, 46].

Diabetes mellitus has been linked to pancreatic cancer:

Box 40.1 Protective factors for pancreatic cancer

- Folate-rich foods
- Fruit and vegetables
- Aerobic Physical exercise

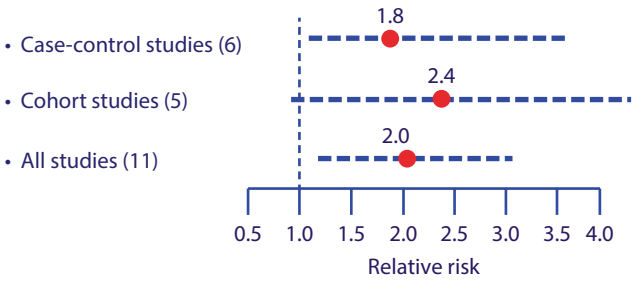
both type I and II can increase the chance of developing pancreatic cancer, with a relative risk, respectively of 2 and of 1.8 (◘ Fig. 40.5) [47–49].

The risk decreases with the duration of diabetes, and insulin and oral antidiabetic drugs have been associated with a reduction of this risk [35, 50, 51].

Chronic pancreatitis can account for 5% of all pancreatic cancer, probably because of the role that chronic inflammation can have in the genesis of cancer. Patients with chronic pancreatitis have a 26-fold increased risk, which keeps growing with the disease duration: 4% of patients affected by chronic pancreatitis for at least 20 years will develop this tumor, and the main cause of this disease, alcohol consumption, is also an independent risk factor for pancreatic cancer. Patients with hereditary pancreatitis have an extremely high risk of developing this tumor, up to 50–60 times greater than expected [17].

Infection diseases have been related to an increased risk of pancreatic cancer, even though data are not conclusive: *H. pylori* infection, human hepatitis B virus infection, and human immunodeficiency virus infection [35, 45].

Occupational risk factors are considered working in mines (especially carbon mines) or in a sawmill and being employed in the metallurgical, petrochemical, or rubber industry. All these jobs can increase the risk of pancreatic carcinoma up to fivefold [52].



◘ **Fig. 40.5** Diabetes-related risk of pancreatic cancer, according to different studies

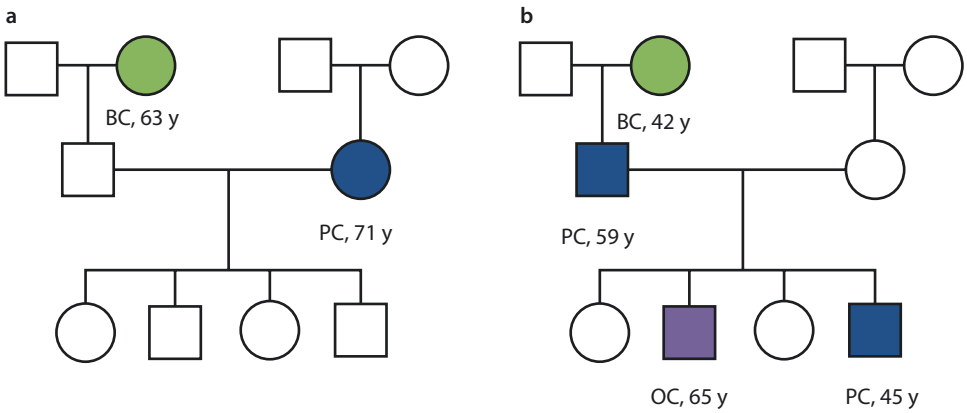
40.3.2 Genetic Risk Factors

Sporadic pancreatic cancer accounts for 70% of total, and 17% more, diagnosed before the age of 60, can be considered as early-onset sporadic pancreatic cancers.

Familial pancreatic cancers are defined by the presence of at least two first-degree relatives with pancreatic cancer (◘ Fig. 40.6 and ◘ Table 40.2) and can be held accountable for no more than 10–13% of all cases. Moreover, in less than 25–30% of familiar pancreatic cancer (3% of all pancreatic cancers), an inherited germline mutation can be found, and, therefore, a genetic syndrome can be identified (◘ Fig. 40.7) [35, 53].

Clinical features that can suggest a hereditary cancer syndrome are:

- Young age at diagnosis (<60 years)
- Multiple cases of pancreatic cancer within the same family
- Cancer clusters that can be part of a defined genetic syndrome
- Multiple tumors in the same individual



◘ **Fig. 40.6** A family tree in case of sporadic pancreatic cancer **a** and a family tree in case of familial pancreatic cancer be associated with a hereditary genetic mutation **b**

Table 40.2 Risk of pancreatic cancer based on the number of affected relatives

Affected relatives	Relative risk	Lifelong risk (%)
1 first-degree relative	4.6	6
2 first-degree relatives	6.4–9	8–12
3 or more first-degree relatives	32	40

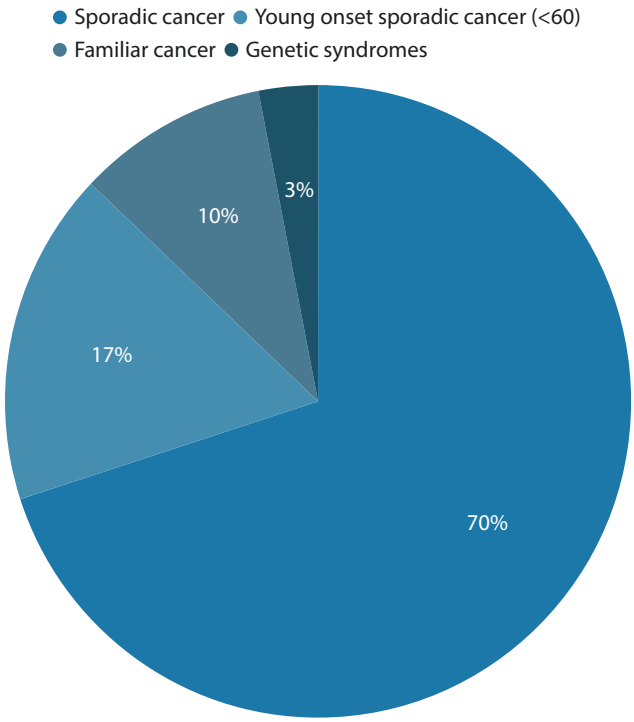


Fig. 40.7 Weight of familial and hereditary cancers on the total of pancreatic cancers

As said, specific genetic syndromes can be defined in only a third of all familial pancreatic cancers (Table 40.3). Different germline mutations are associated with varying risk of pancreatic cancer [54], and, of these, the most common are the BRCA1/2 gene mutations, cause of the hereditary breast and ovarian cancer syndrome (HBOC) [55, 56].

Other genes involved are:

- APC, whose mutation causes familial adenomatous polyposis (FAP), primarily associated with colorectal cancer
- Mismatch repair genes (MMR), whose mutations cause the Lynch syndrome, also associated with colon, endometrial, ovarian, and gastric cancer [57]
- CDKN2a and P16, associated with the hereditary melanoma syndrome
- VHL Gene

Table 40.3 Most common symptoms in pancreatic cancer

Syndrome	Genes	Relative risk
HBOC	BRCA1	3.5–10
	BRCA2	2.3
HNPCC	MSH2, MLH1, MSH6, PMS, PMS2	4.7
FAP	APC	4.5
FAMM	CDKN2A/P16	34–39
PJS	LKB1/STK11	132

- LKB1 and STK11, associated with the Peutz-Jeghers syndrome, characterized by small bowel hamartomatosis and pigmented spots on the lips

40.4 Carcinogenesis of Pancreatic Adenocarcinoma

In recent years, thanks to progress in the fields of genomics, biotechnology, and molecular pathology, a large number of molecular, genetic, and epigenetic alterations related to proliferation and survival of pancreatic tumor cell and therapy response were found, unfortunately these have not shown utility as biomarkers for clinical use [58, 59]. Also, several studies revealed that response to treatment can be affected by epigenetic mechanisms involving a gene expression regulation [60].

Other studies showed that pancreatic carcinogenesis occurs through a gradual multistep process which determinates the progression from intraepithelial neoplasia to invasive cancer, increasing the extent of cytological and morphological atypia [61, 62]. Onset of infiltrating carcinoma in patients with intraductal mucinous tumor not resected, frequent presence of ductal lesions in pancreases of infiltrating carcinoma patients and increase in the degree of atypia of lesions adjacent to infiltrating carcinoma are suggestive for the hypothesis of a multistep carcinogenesis which leads to PDAC [63]. An accurate classification of the PDAC precursor lesions was suggested only at the beginning of the 2000s, despite their discovery goes back to more than a century ago [64, 65]. Thanks to morphological analyses performed on pancreatic cancers resected from PDAC patients, three different types of histologically defined PDAC precursor lesions have been described: pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN) and mucinous cystic neoplasm (MCN) [66–68] (Fig. 40.8).

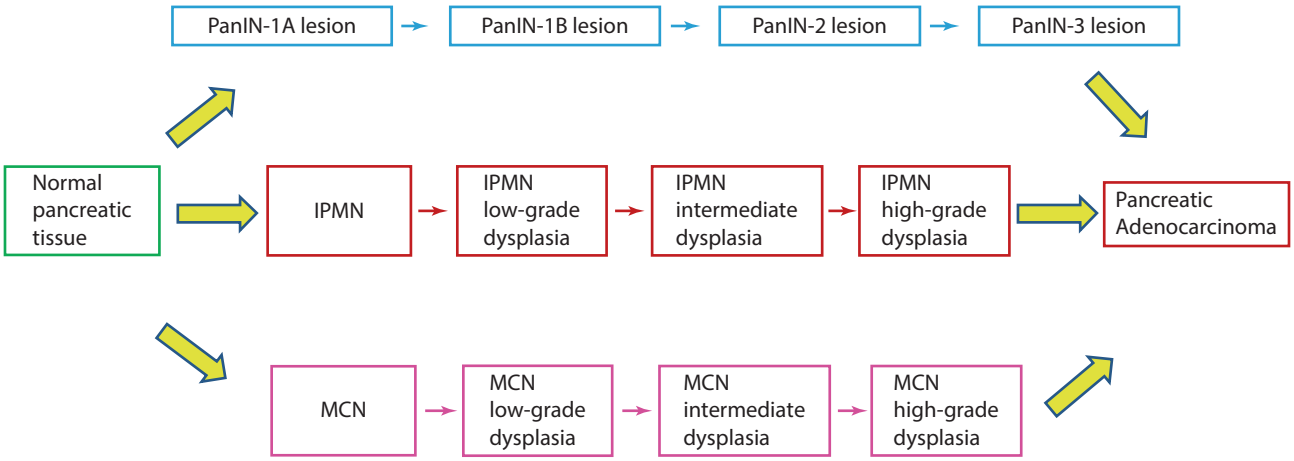


Fig. 40.8 Model of three distinct pancreatic cancer progression pathways from preneoplastic lesions to invasive pancreatic carcinoma

Nowadays, the major aim of scientific research is to early detect and genetically characterize these precancerous lesions, especially PanIN-3, before an invasive pancreatic cancer may develop. Unlike IPMNs and MCNs which are macroscopically detectable rare precancerous pancreatic lesions, PanINs are the most frequently detected microscopic lesions located in the smaller pancreatic ducts. PanIN lesions were classified in 2001 by Hruban and colleagues [65, 68] based on the degree of epithelial atypia and divided into three subtypes ranging from low-grade lesions with minimal cytological and architectural atypia (PanIN-1) to intermediate-grade lesions (PanIN-2) to in situ carcinomas (PanIN-3). These generally asymptomatic noninvasive lesions are supposed to occur before the invasion of the surrounding stroma [66, 69]. In turn, the PanIN-1 lesions have been further categorized into two different subtypes, PanIN-1A (flat) and PanIN-1B (papillary). Immunohistochemical characterization of PanINs showed that apomucin MUC1 is mainly expressed in high-grade lesions (PanIN-2/PanIN-3) and invasive PDAC as well as in the normal pancreatic ducts, whereas MUC5AC is detected in all PanIN lesions [70]. The accumulation of genetic and molecular alterations underlying these lesions has been shown to be correlated with the histological progression of PanINs. Understanding these genetic changes may allow us to early detect the transition mechanism from precancerous to malignant lesions [71–74]. One of the earliest events in pancreatic carcinogenesis is represented by overexpression of *ERBB2* oncogene encoding a tyrosine kinase growth factor receptor. The *ERBB2* activation promotes cell proliferation and was found in 82% of PanIN-1A lesions and in 100% of PanIN-3 lesions [75, 76]. Several animal models proved that the multistep progression for pancreatic cancer always involves activating mutations in *KRAS* oncogene as early event driving the carcinogenesis process [77–80]. Generally, over 90% of PDACs harbor activating point mutations mainly located in codons 12 and 13 of *KRAS* exon 2 [81, 82]. *KRAS* mutations induce cell cycle progression through activation of the MAP and AKT kinase signaling pathways [83] and were detected in 36% of PanIN-1A lesions, 44% of PanIN-1B and PanIN-2 lesions, and 87% of PanIN-3 lesions [81]. Other *HRAS* and *NRAS* mutations were not detected in PDAC patients [84].

Other early genetic events include the loss of activity of tumor suppressor gene cyclin-dependent kinase inhibitor *CDKN2A/p16*, involved in regulation of the G1/S transition of cell cycle, and telomere shortening, which determines abnormal fusion of chromosomes at the ends, resulting in the chromosome instability and induction of neoplastic progression of the cells [85, 86]. Indeed, increased cell proliferation and formation of PanIN lesions are induced by occurrence of *KRAS* mutations that alone are not sufficient for the malignant transformation process [87], which instead requires the inactivation of tumor suppressor genes, such as *CDKN2A/p16*, *SMAD4/DPC4* or others involved in the TGF-b and TP53 signaling pathways [88–93], or chronic pancreatic inflammation [94]. The loss of function of *p16/CDKN2A* can be already detected in the early PanIN stages of almost all pancreatic carcinomas with increasing frequency according to histological progression of PanINs (30% for PanIN-1A, 55% for PanIN-1B, about 90% for PanIN-2/3) [63, 95], whereas the *SMAD4/DPC4* and *TP53* inactivation occurs in the later stages of the tumorigenesis model (30% and 12% in PanIN-3, respectively) [96–98]. Since p53 modulates the cell cycle control, G2/M arrest, and apoptosis, its loss of function, detected in more than 50% of pancreatic adenocarcinomas, induces alterations in cell death and cell division processes. The mechanism that leads

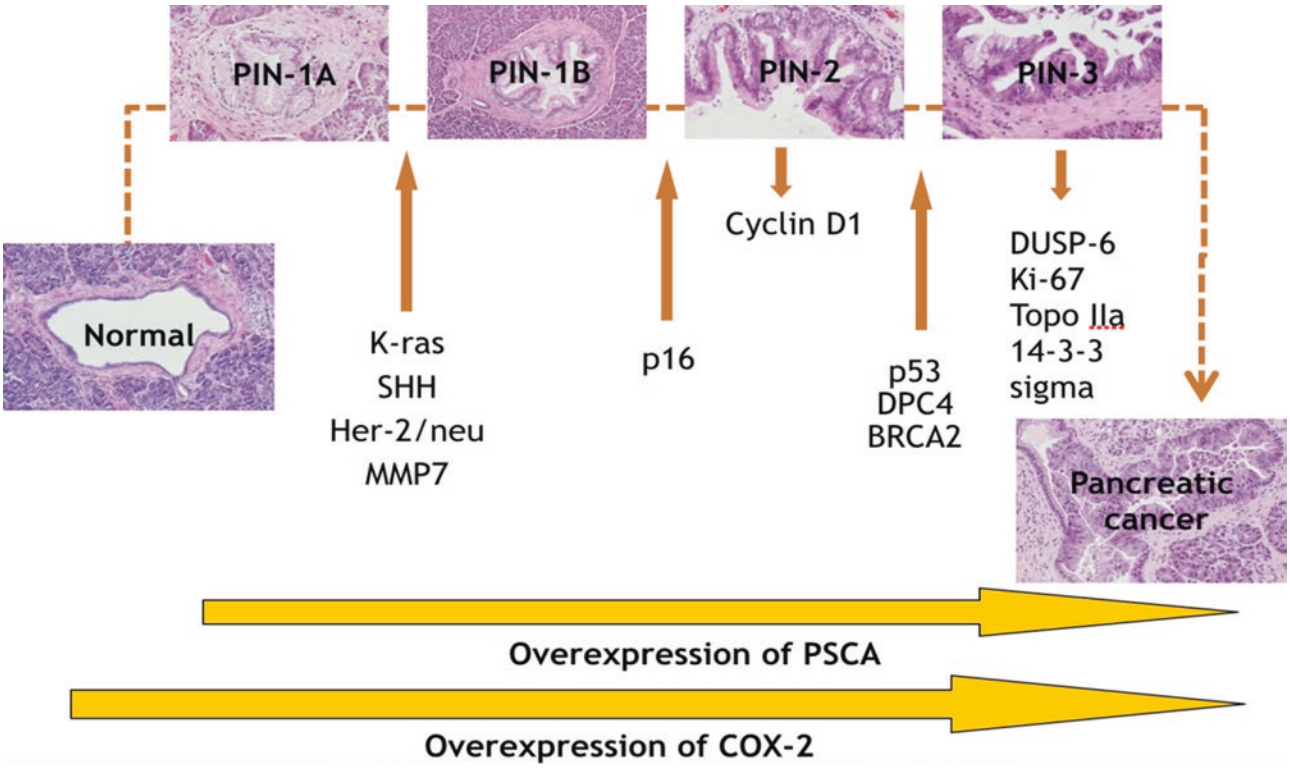


Fig. 40.9 Genetic alterations involved in the PanIN-progression model

to its inactivation involves the deletion of one allele and an inactivating mutation in the second allele [99, 100]. *SMAD4* is involved in TGF-b signaling pathway, and its inactivation promotes an abnormal cancer cell growth [83]. PanIN lesions deriving from chronic pancreatitis show *p16* inactivation with lower frequency. Three different mechanisms may cause the *p16* inactivation, such as promoter hypermethylation, homozygous deletion of the *CDKN2A*/*INK4A* locus, and intragenic mutation causing loss of the second allele [101–104]. Among the epigenetic events causing gene silencing, the hypermethylation of CpG islands at the level of the promoter of several genes is the mechanism more observed in patients with pancreatic cancer [105–107]. Using a microarray analysis, Sato and colleagues [108] demonstrated that early PanIN stages exhibit an aberrant CpG island hypermethylation which gradually enhances during neoplastic transformation. Recently, a genome-wide DNA methylation analysis has allowed to identify different molecular subtypes of pancreatic cancer [109].

In addition, the stepwise progression toward malignant transformation involves the overexpression of other molecules, such as Ki-67, topoisomerase II, and cyclin D1. The Ki-67 overexpression, correlated with cell proliferation, is more often observed in nuclei of high-grade PanIN lesions (PanIN-3) [110, 111], whereas that of cyclin D1 is detected in 30% of PanIN-2, 50% of PanIN-3, and 80% of pancreatic adenocarcinoma [112, 113]. The progressive accumulation of previously described genomic alterations determines evolution from PanIN-1A to PanIN-3 then to pancreatic adenocarcinoma (Fig. 40.9).

IPMNs are tumors of the duct epithelium characterized by ductal cystic dilatation deriving from papillary epithelial proliferation and mucin production [114, 115]. Genetic alterations identified in IPMN involves three oncogenes, such as *KRAS*, *ERBB2*, and *AKT*, and five tumor suppressor genes, such as *CDKN2A*/*p16*, *TP53*, *SMAD4*, *LKB1*, and *DUSP6*. *KRAS* mutations have been detected in about 70% of IPMN lesions both at low-grade and high-grade and seem to be responsible for the IPMN development [116]. According to the hypothesis of Yoshizawa and collaborators [117], high-grade lesions arise from low-grade lesions through a clonal pathway, whereas low-grade lesions derive by a polyclonal mechanism. Also, like PanIN lesions, IPMN lesions show the *ERBB2* overexpression as early genetic event in approximately 60% of cases [118, 119]. *AKT* activation, involved in cell growth and survival, was observed in 63% of IPMN lesions with a slightly higher frequency in high-grade than in low-grade forms [120, 121]. About 50% of all IPMN lesions shows loss of function in *CDKN2A*/*p16*, mainly caused by hypermethylation of its promoter, which increases concomitantly with the grade of dysplasia [122]. Loss of p53 function is also detected in 50% of IPMN lesions, especially in the high-grade forms, inducing defects in the genome integrity and, in turn, determining malignant transfor-

mation [123]. The *SMAD4* inactivation, instead, is considered a rare and late event in the IPMN development [124]. *LKB1* alterations were observed in 25% of IPMN lesions of patients without Peutz-Jeghers syndrome [125], while DUSP6 expression is lost or greatly reduced in some IPMN lesions [126]. A small percentage of IPMNs shows also other genetic alterations in *PIK3CA* and *BRAF* genes [127–129]. Additionally, several studies showed that almost all IPMNs harbor a mutation in *GNAS* complex gene locus (*GNAS*) or *KRAS*, and more of 50% of them is carrier of both mutations, with a higher prevalence of *GNAS* mutations in the intestinal subtype and a higher frequency of *KRAS* mutations in the pancreatobiliary subtype [130–132]. Since mutations in *GNAS*, *KRAS*, and *TP53* represent early genetic events in the IPMN onset, these alterations are not useful for identifying individuals with high-grade dysplasia or invasive disease.

MCNs are rare mucin-producing and septated cyst-forming precursor lesions of pancreatic cancer, generally asymptomatic, with favorable prognosis, and mainly observed in women [133]. Although not yet completely clear, the molecular alterations underlying MCN development and progression involve *KRAS* mutations at codon 12 observed as early event in low-grade MCNs and with increased frequency in the advanced stages, and mutations in *TP53*, *p16*, and *SMAD4*/*DPC4* genes mainly detected in high-grade MCNs and invasive disease. Since no *GNAS* mutations have been observed in MCNs, these may be used as useful genetic markers to discriminate between MCN and IPMN [134–136].

Furthermore, a familial predisposition for PDAC may be observed in about 10% of patients, some of which carry germline mutations in *BRCA2*, *P16*/*CDKN2A*, *STK11*/*LKB1*, and *PRSS1* genes, or, infrequently, also in DNA mismatch repair genes [137, 138].

40.5 Clinical Features

Pancreatic cancer is considered a silent disease, characterized by only vague and unspecific symptoms, with up to 4 months passing since their presentation to a defined diagnosis and only a third of all patients diagnosed within 2 months since the first symptoms have occurred. Moreover, often these symptoms only occur at late stages of disease. Because of this, delayed diagnosis is the most common problem in these patients.

Approximately 60–70% of pancreatic cancer occurs in the head of the pancreas, 20–25% in the body and the tail, and the remaining 10–20% diffusely involve the whole pancreatic gland [139].

Diagnosis is usually earlier for the head cancers, because of the jaundice that usually occur at early stages: in these cases, the most common symptoms are body weight loss (90%), epigastric pain (80%), and icterus (75%) (Table 40.4). Body and tail pancreatic cancers, instead, are not diagnosed until late stages, with the most common symptoms being weight loss (100%) and pain (85%), while jaundice only occurs in 5% of the cases (Table 40.5) [140, 141].

Overall, the most common symptoms and signs of pancreatic adenocarcinoma are (Table 40.6):

- Body weight loss (observed in 75% of cases), mainly caused by a combination of anorexia, subclinical malabsorption syndrome, and dyspeptic disorders.
- Epigastric pain (70%): usually severe, dull, variously radiating to the left or right ipochondria or at the back. It usually begins at early stages as a discontinu-

Table 40.4 Most common symptoms and signs at admission to hospital (pancreatic head cancer)

Signs and symptoms at admission to hospital (pancreatic head)	Frequency (%)
Body weight loss	100
Epigastric pain	85
Anorexia	35
Jaundice	5
Constipation	25
Asthenia	40
Nausea and Vomit	45

Table 40.5 Most common symptoms and signs at admission to hospital (body and tail cancer)

Signs and symptoms at admission to hospital (body and tail)	Frequency (%)
Body weight loss	90
Epigastric pain	80
Jaundice	75
Courvoisier law	25
Itching and scratching lesions	40–60

Table 40.6 Most common symptoms in pancreatic cancer

Symptom	Frequency (%)
Excessive body weight loss (8–10 kg)	75
Epigastric pain	70
Anorexia	50
Jaundice	25

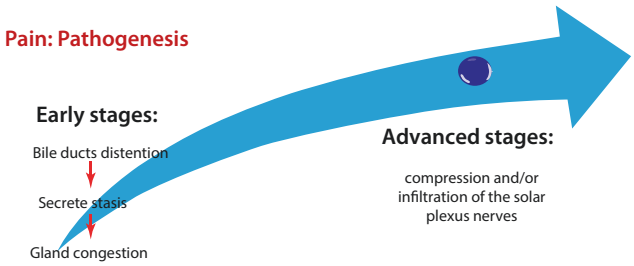


Fig. 40.10 Causes of pain in pancreatic cancer at different disease stages

ous, postprandial pain and is due to the obstruction of the pancreatic ducts, which causes ductal distention, secretes stasis, and gland congestion; at the later stages, pain can become continuous and is often caused by infiltration or compression of the celiac ganglion by the tumor (Fig. 40.10) [142].

- Anorexia (50%)
- Obstructive icterus (jaundice, 25%), usually only present in tumors occurring at the pancreatic head, is caused by compression or infiltration of the common bile duct. Signs of this event, besides jaundice, are a progressive increase of both direct and total bilirubin, together with dark urines and acholic stools.
- Courvoisier law: sometimes, at the physical examination, can be identified a palpable, enlarged and not painful cholecystitis: this is due to compression of the common bile duct.
- Trousseau syndrome: this syndrome is a cutaneous migrant thrombophlebitis, and it may be the first sign of the disease. It reflects the state of hypercoagulability often present in pancreatic cancer [143].
- Recent development of diabetes mellitus: even though this sign is not common, a rapid onset of atypical diabetes should raise suspects of pancreatic cancer [144, 145].
- Deep vein thrombosis [146].
- Nonbacterial thrombotic endocarditis, which can be easily confused with a subacute bacterial endocarditis [147].
- Ascites.
- Hepatomegaly, usually caused by hepatic metastases.
- Splenomegaly, caused by a thrombosis of the portal vein.
- Virchow sign: left over-clavicular lymph node: pancreatic cancer is the cause of 25% of metastases at cervical lymph nodes from a cancer of unknown origin.
- Sister Mary Joseph sign: palpable umbilical mass; pancreatic cancer is the cause of a metastatic umbilical lesion in 9% of cases [148].
- Blumer's shelf: presence of metastatic mass at the digital rectal examination.
- Local invasion of the duodenum can result in an upper gastro-duodenal obstruction.

Occasionally, fever, nausea, vomit, and diarrhea may occur. Itching, caused by increased bile acids in blood, may not be the most serious symptom, but can be the most distressful for the patient [141].

40.6 Diagnosis and Staging

40.6.1 Laboratory Findings

40.6.1.1 Molecular Biology

The most common laboratory tests utilized in the diagnosis of pancreatic cancer are (► Box 40.2):

- Alkaline phosphatases
- Fasting blood glucose
- Amylases and lipase

However, all these tests have proved to have low sensitivity and specificity rates, and so their usefulness is scarce [139].

Box 40.2 Laboratory testing in diagnosis of pancreatic cancer

- Alkaline phosphatase
- Fasting blood glucose
- Amylases and lipase
- CEA
- CA19.9

40.6.1.2 Biomarkers

CEA (carcinoembryonic antigen) is a glycoprotein involved in cell adhesion, expressed only in fetal gastrointestinal tissue: in adults, serum levels are usually very low but can raise in many types of cancers, especially gastrointestinal tumors, including pancreatic adenocarcinoma. However, it has low sensitivity (45%) and specificity and can increase in many non-oncological diseases or conditions such as in heavy smokers. For these reasons, it is of no use in the diagnosis of pancreatic cancer. It is instead quite useful during the follow-up in patients who had high CEA levels at diagnosis, allowing to monitor an ongoing therapy or identify a recurrent disease.

CA19.9 (also called GICA, gastrointestinal cancer antigen) has a sensitivity of 70–92% and a specificity of 68–92%, but these data can vary a lot, depending on tumor size: its levels are increased in 80% of pancreatic cancers, but it has limited sensitivity for small cancers is undetectable in patients who don't express the Lewis blood group antigen (5–10% of general population) [149]. On the other hand, it increases together with the

bilirubin levels, and high values can be found in many cholestasis-inducing conditions. Because of this, as for CEA, its usefulness in diagnosis of pancreatic cancer is limited. Ca19.9 has instead a very important prognostic value and can be used to evaluate disease burden and in follow-up, in monitoring the efficacy of a therapy or disease recurrence; level > 500 UI/ml indicates a worse prognosis after surgery [139].

40.6.2 Imaging

40.6.2.1 Transabdominal Ultrasonography

The first imaging test usually used in case of jaundice, of abdominal pain, or of clinical suspect of pancreatic cancer is transabdominal ultrasonography, because of its low cost and diffuse availability. It is performed with a low-frequency probe (2–5 MHz) and can easily study the liver and bile ducts, helping excluding other causes of jaundice. However, the pancreas is often difficult to visualize with this technique, because of constitutional factors of the patient, such as bowel gas, abdominal fat, or surgical scars: transabdominal ultrasonography has low sensitivity for pancreatic lesions (60–70%, with more than 40% of false-negative rate for tumors smaller than 3 cm), and its accuracy varies greatly depending on the operator's expertise (Table 40.7) [139].

Table 40.7 Features of the most relevant imaging techniques utilized in diagnosis and staging of pancreatic cancer

		Contrast	Biopsy	Ionizing radiations
Noninvasive imaging techniques	Ecography	No	No	No
	CT	Yes	Yes	Yes
	MRI	Yes	No	No
	MRCP	No	No	No
	PET/PET-CT	No/yes	No/yes	No/yes
Invasive imaging techniques	EUS	No	Yes	No
	ERCP	Yes	Yes	Yes

40.6.2.2 CT, MRI, and PET

Contrast CT, thanks to its diffusion and to the capability to acquire whole body images, represents the first imaging technique used in case of high suspection of pancreatic cancer. It is also the most common second level test after ultrasound, being used to confirm diagnosis or to complete staging. It can both study local vessels infiltration and perineural invasion, together with the presence of metastatic lesions. Pancreatic cancer appears as an hypodense, homogeneous lesion with indistinct margins (Fig. 40.11). Calcifications are very rare, while cystic formations can be found more frequently, especially in tumors derived from cystic lesions, and an obstruction or compression of the common bile duct (with or without dilatation) is commonly found for tumors located in the pancreatic head. Contrast CT allows to evaluate a pancreatic lesion in three different phases (Fig. 40.12):

- Before contrast: can study the presence or absence of pathological calcifications.
- Arterial phase: can study the primitive tumor and arterial involvement.
- Venous phase: can study the presence of liver metastases and venous involvement.

Maximum contrast between tumor and normal pancreatic tissue can be obtained after the enhancement peak of the arterial phase but before that of the venous phase (this is sometimes defined as "pancreatic phase") [150, 151]. Triple-phase spiral TC is capable of obtaining very thin slices (2–3 mm), increasing test sensitivity (90%), and tumor tissue samples can be obtained through percutaneous CT-guided fine needle aspiration (FNA), even though the risk of contamination has not been established yet [139].

MRI has shown no superiority to CT in diagnosis of pancreatic cancer but is useful to solve problems such as the detection of hepatic lesions that cannot be characterized by CT [152].

PET utilizes 18FDG to visualize the primitive tumor and metastatic sites and is usually used to confirm diagnosis and to evaluate nodal involvement or the presence of concealed metastases, by measuring the metabolic activity of the lesions (Fig. 40.13). It can also be used to evaluate the response to neoadjuvant therapy or to detect a relapsing disease. Anyway, this technique is particularly useful in combination with CT, enabling to correctly classify as resectable 16% of cancers considered unresectable by previous CT evaluation (Table 40.7).

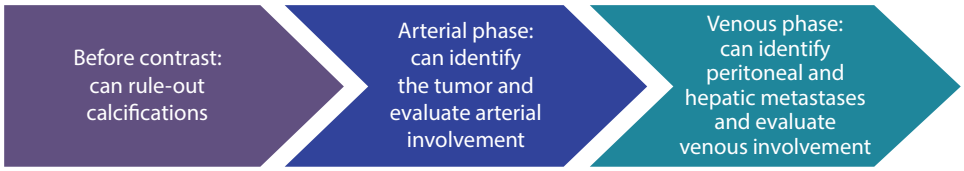


Fig. 40.11 Role of CT phases in the diagnosis and staging of pancreatic cancer

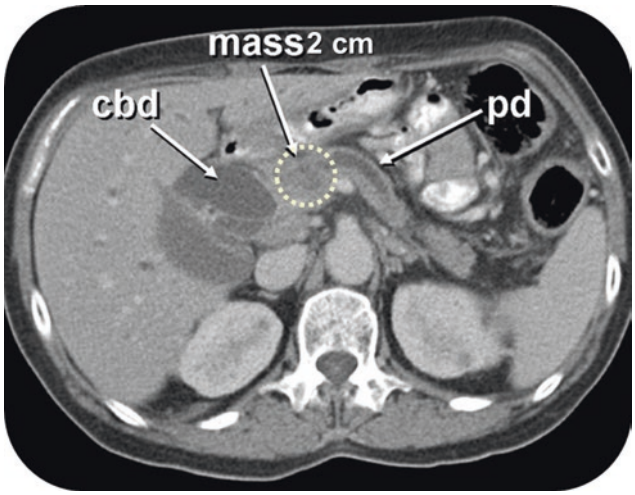


Fig. 40.12 TC scanning of pancreatic cancer. CBD common bile duct, PD pancreatic duct

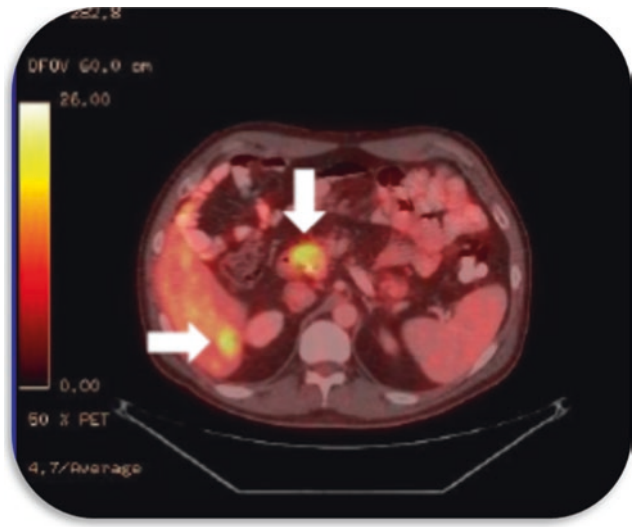


Fig. 40.13 PET imaging of a primitive pancreatic cancer and of liver metastases

40

40.6.2.3 ERCP and MRCP

Endoscopic retrograde cholangiopancreatography (ERCP) has high sensitivity (90–95%) and can help in diagnosis of uncommon forms of pancreatic cancers such as mucinous intraductal pancreatic cancers. It also allows for histological and cytological diagnosis throughout FNA or brushing, even though cytological examination has very low sensitivity (50%). However, ERCP is of little or no help in regard to disease staging and is an invasive technique, having a high risk of complications, the most common of which is acute pancreatitis (5–10%); other side effects can be infections, hemorrhages, or intestinal perforation. Because of that, today, ERCP is mostly used for therapeutic purposes, such as stenting of obstructed bile ducts with metal or plastic stents [139]. Nowadays, magnetic resonance cholangiopancreatography (MRCP), despite the lack of ability to perform biopsies, is preferred to ERCP because of the lower rate of complications and the similar sensitivity, and may also be preferred to CT for cystic neoplasms of the pancreas and to evaluate biliary anatomy. It doesn't use ionizing radiation or contrasts, but secretin can be utilized to induce pancreatic secretion: this can be used as an endogenous contrast agent to better visualize the Wirsung duct or substenoses and allows to evaluate pancreatic function by measuring the pancreatic secrete produced (◘ Table 40.7) [139].

40.6.2.4 EUS

Endoscopic ultrasonography (EUS) is today a largely used technique for pancreatic cancer staging and diagnosis: it uses a 7.5–12 MHz high-frequency probe mounted on an endoscope that can reach the stomach and the duodenum and is considered superior to CT in identifying lesion smaller than 2 cm, having a sensitivity of almost 100%, while it is able to assess the vascular and lymph node involvement with an 80% sensitivity. It is comparable to ERCP and MRCP in regard to the bile duct imaging. It also allows histological diagnosis on tumor samples through transparietal FNA, having a lower risk of contamination than percutaneous CT-guided FNA [153]. However, it still has issues with high cost, lack of operator expertise, and equipment availability. Also, different from CT and MRI, it can't evaluate distant metastases, and sedation is needed for this technique (◘ Table 40.7) [154].

40.6.2.5 Pancreatic Incidentalomas

Pancreatic incidentaloma are more and more frequently diagnosed, because of the increasing number of radiological exams performed for other reasons. When found, upfront surgery should not be the first option; instead, histological diagnosis should be obtained first, if feasible [139].

40.7 Cancer Diffusion and Resectability Evaluation

The pancreas has not a capsule and is in close proximity of other abdominal organs and of important vascular and nervous structures, such as the portal vein or the superior mesenteric artery and vein; moreover, pancreatic cancer usually shows great local aggressiveness. Because of this, at the time of diagnosis, pancreatic cancer has often already infiltrated important structures. Lymphatic diffusion occurs earlier than blood diffusion, with 40–50% of patients presenting nodal metastases at diagnosis, while instead 30–50% of patients present with hepatic metastases. Less common metastatic sites are (▶ Box 40.3):

Box 40.3 Most common metastatic sites in pancreatic cancer, ordered by frequencies:

- Nodes
- Liver
- Lung
- Bones
- Brain
- Skin

- Lung
- Skin, usually painful nodules
- Bones
- Brain, usually in the form of meningeal carcinomatosis [139]

Complete staging classification is reported in Tables 40.8 and 40.9.

Localized pancreatic cancer can be classified, on the basis of staging and vascular invasion, as (Tables 40.10 and 40.11) [155, 156]:

- Resectable: I–II stage (T1–3 Nx M0), without involvement of major blood vessels such as the celiac trunk, common hepatic artery, superior mesenteric vein, and artery and portal vein.

Table 40.8 Pancreatic cancer staging

Primary tumor (T)	*TX*	*Primary tumor not assessable*
	T0	No evidence of primary tumor
	Tis	In situ carcinoma
	T1	Tumor limited to the pancreas, <2 cm in maximum diameter
	T2	Tumor limited to the pancreas, >2 cm in maximum diameter
	T3	Tumor extended beyond the pancreas but without involvement of the celiac axis or of the superior mesenteric artery
	T4	Involvement of the celiac axis or of the superior mesenteric artery
Regional lymph nodes (N)	NX	Regional lymph nodes are unassessable
	N0	No regional lymph nodes involvement
	N1	Regional limph nodes involvement
Distant metastasis (M)	M0	No distant metastasis
	M1	Presence of distant metastasis

Table 40.9 Pancreatic cancer staging (2)

0	Tis, N0, M0
IA	T1, N0, M0
IB	T2, N0, M0
IIA	T3, N0, M0
IIB	T1, N1, M0 T2, N1, M0 T3, N1, M0
III	T4, any N, M0
IV	Any T, any N, M0

Table 40.10 Resectability criteria for localized pancreatic cancer according to staging and vascular invasion

	Stage	Arterial invasion	Venous invasion
Resectable	I–II (T1–3)	No	No
Borderline resectable	II–III (T3–4)	<50%	Reconstructable
Unresectable	III (T4)	>50%	Unreconstructable

Table 40.11 Therapeutic options based on cancer resectability and on the presence/absence of metastatic lesions

	Surgery	Chemotherapy	Radiotherapy
Resectable	+	+	+
Borderline resectable	+	+	+
Locally advanced	–	+	?
Metastatic	–	+	–

- Borderline resectable: II–III stages (T3–4 Nx M0), with marginal arterial involvement (<50% of circumference) or reconstructable invasion of the superior mesenteric vein and portal vein.
- Locally advanced or unresectable: III stage (T4 Nx M0), with major arterial involvement (>50% of circumference) or not-reconstructable vein invasion; mesenteric or para-aortic node invasion is considered an absolute unresectability criteria.

Extrapancreatic disease precludes curative resection, and surgery may have only palliative purposes this case.

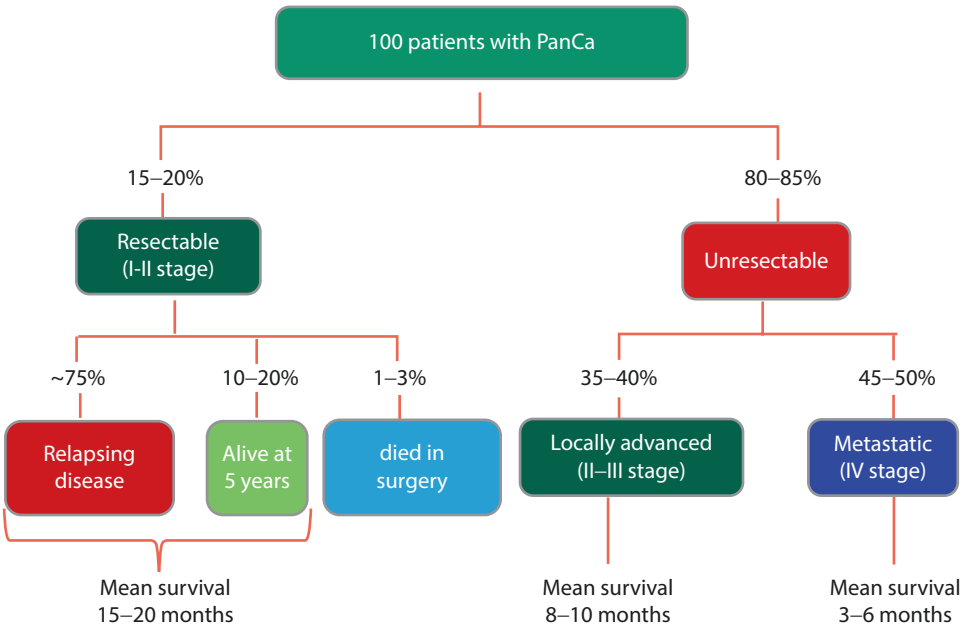


Fig. 40.14 Patient distribution according to tumor stage at diagnosis and their relative mean survival

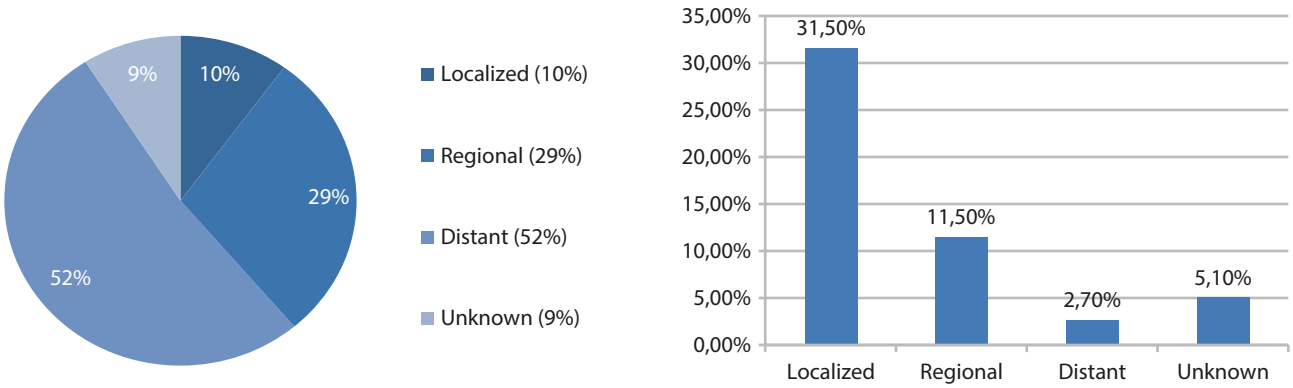


Fig. 40.15 Percentage of cases and 5-year-survival rates by stage

Historically, vascular involvement has been considered a contraindication to resective cure, but nowadays, the invasion of the superior mesenteric or portal vein is not an absolute contraindication. These veins can be partially resected, and, also, complete reconstruction is possible, using native veins as replacement. Nonetheless, invasion of the superior mesenteric, celiac, and hepatic arteries still presents a barrier to resection.

Inclusion in the borderline resectable category also depends on surgeon's expertise, on the clinical status, and personal choice of the patient [139].

Only 15–20% of pancreatic carcinomas are considered resectable at diagnosis, and, moreover, while CT or MRI can assess non-resectability with a positive predictive value of more than 90%, the positive predictive value for resectability is lower than 50% [157]. The remaining 80–85% of cancers are unresectable (35–40%) or metastatic (45–50%) and will not undergo curative surgery but only palliative chemotherapy and/or radiotherapy (Figs. 40.14 and 40.15). Medical comorbidities, performance, and nutritional status must be considered before evaluating any of these treatment modalities, whereas age alone must never be considered as an absolute contraindication [139].

40.8 Treatment

40.8.1 Resectable cancer

40.8.1.1 Surgery

Surgery is the only curative treatment for pancreatic adenocarcinoma: to this date, open surgery remains the gold standard, and data on laparoscopic surgery are still scarce [158]. The main goal is to obtain microscopically

negative margins (R0); R1 is defined by the presence of microscopically positive margins, while R2 corresponds to macroscopically positive margins or unresected positive nodes [159].

After complete preoperative evaluation, surgical approach must be chosen on the basis of tumor's size, localization, and aggressiveness; the most common kind of resection are:

- Head: Whipple pancreatoduodenectomy (preserving the body and tail, with or without conservation of the pylorus) [160]
- Body/tale: Pancreatectomy (preserving the head) and splenectomy [161].

Standard node dissection, with at least 15 lymph nodes removed, should always be performed to allow proper staging, but extended lymphadenectomy is not recommended [162]. Considering the higher complication risk, preoperatory bile drainage should not be performed routinely but only in patients with active cholangitis or bilirubin serum levels higher than 250 micromoloes/L [163]. An open question remains whether or not radical pancreatectomy can improve prognosis, especially in patients with macroscopically positive margins (R2).

R1 or R2 margins are also considered independent negative surgical prognostic factors, together with surgeon's expertise and the entity of blood loss. Pathological or molecular prognostic factors are (◘ Table 40.12):

- Staging (tumor size, node involvement)
- Vascular and perineurial invasion
- Location
- Proliferation indexes
- Chromosomal abnormalities

40.8.1.2 Adjuvant and Neoadjuvant Therapies

Chemotherapy, with or without radiotherapy, is essential to improve outcomes in patients with pancreatic cancer eligible for surgical treatment [164, 165]. Adjuvant regimens achieve this result by eliminating possible micrometastasis, thus reducing the risk of relapsing disease and increasing survival rates. Therapy should ideally be initiated within 8 weeks after surgery.

◘ **Table 40.12** Main surgical, pathological and molecular prognostic factors in pancreatic cancer

Surgical	Anatomical/ pathological	Biomolecular
Surgeon and surgical equipe	Primitive tumor size	Proliferation index
Resection margins	Tumor site (worse at body tail)	Chromosomal abnormalities
Blood loss during surgery	LN involvement or metastasis	
	Neural or vascular invasion	

Standard regimens are considered single-drug chemotherapy with up to 6 cycles of gemcitabine or of 5-fluorouracil plus leucovorin [166]. Other options may include 5-FU in continuous infusion (CI 5-FU) or capecitabine monotherapy, if other options are not feasible. Of note, recent evidence have showed that combination regimen could represent innovative strategies to achieve significant improvements. In particular, gemcitabine plus capecitabine [167] or modified FOLFIRINOX (ASCO Annual meeting 2018) represent the new standard regimens in this setting of patients with an acceptable toxicity profile. The gemcitabine + nab-paclitaxel combination treatment has been studied against standard gemcitabine monotherapy in the adjuvant setting in the APACT study, presented at the 2019 annual ASCO meeting [168]. In this study, even though overall survival and investigator-assessed disease-free survival showed an advantage for gemcitabine + nab-paclitaxel, the primary endpoint, independent reviewer disease-free survival, was not reached.

Radiotherapy can be added for patients at a high risk for local recurrence (i.e., positive resection

margins and/or lymph nodes) but has shown no improvement in disease-free survival rates outside of these subsets of patients and therefore is not routinely utilized in clinical practice for all of pancreatic cancer cases.

Sadly, only 25% of patients who could possibly undergo surgery, and 50% of those who obtain complete macroscopic resection (R0 or R1), can initiate adjuvant chemotherapy (◘ Fig. 40.16). This is because of various reasons:

- The poor performance status of many patients with pancreatic cancer (after surgery)
- An inadequate recover from surgery
- Because previously unnoticed metastases are found at the postoperative restaging

These problems could be overcome by using a neoadjuvant chemotherapy regimen: in this case, therapy is administered before surgery and allows for an earlier treatment of micrometastases and a higher chance to obtain complete resection. It also increases the number of patients that can receive chemotherapy or radiations, and, moreover, surgery appears to be safe, with a possible reduction of the risk of tumor spread during surgery. Finally, it allows to stratify patients on the basis of their response to chemotherapy to better select those who may benefit from surgery (◘ Fig. 40.17) (NCCN guidelines,Version 2.2019). The downside is the risk of a progression of the disease in patients who will not

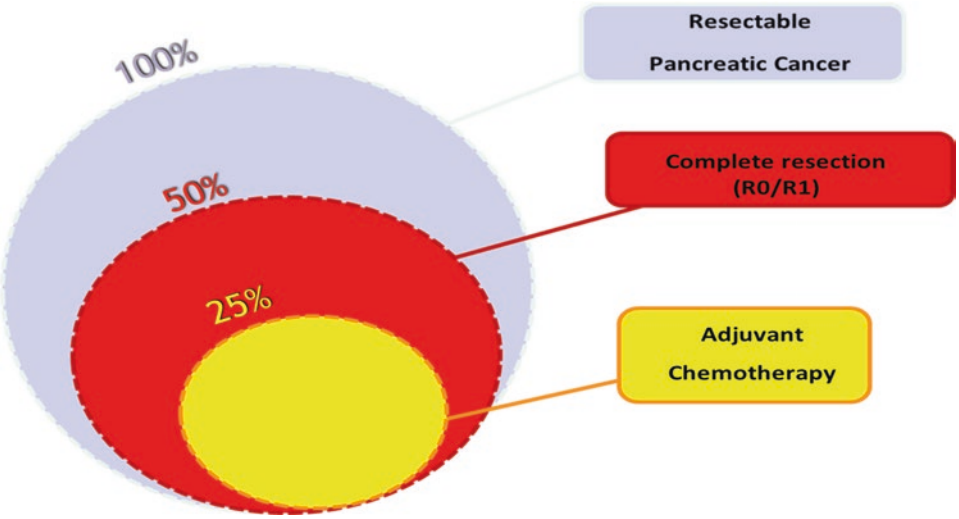


Fig. 40.16 Patients with cancer that will undergo adjuvant chemotherapy

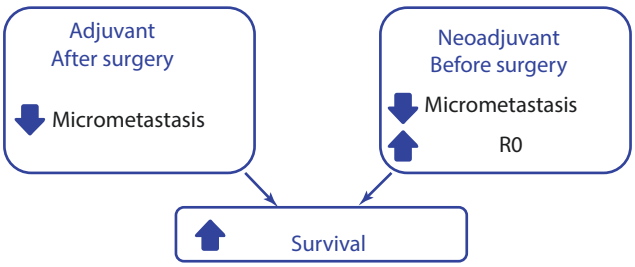
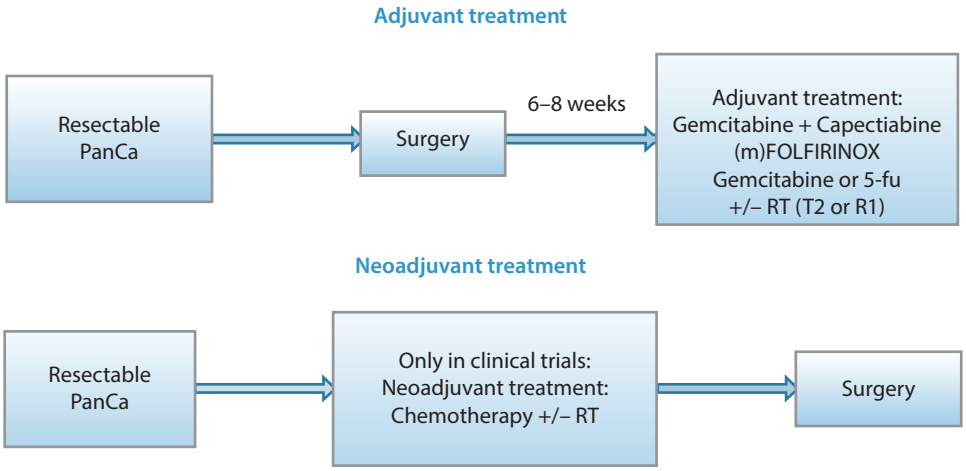


Fig. 40.17 Advantages of both adjuvant and neoadjuvant chemotherapy

Fig. 40.18 Therapeutic algorithm in resectable cancer



respond to neoadjuvant treatment: this can result in a poorer surgical outcome for these subjects or even in the progression to a stage in which surgery is no longer an option. However, at the present day, in the setting of resectable disease, this regimen, as well as neoadjuvant chemoradiation therapy with standard 50Gy fractionation, is not part of clinical practice and is not recommended outside of clinical trials (Fig. 40.18) [159].

40.8.2 Borderline Resectable and Unresectable Cancer

Borderline resectable disease represents up to 50% of all pancreatic cancers. Nowadays, surgery is no more considered the upfront treatment for this disease. However, no standard chemotherapy/chemoradiation treatment has been identified for these patients, and many options

are available, with reported resectability ranging around 30–90% rates. Probably the major limitation is due to the absence of randomized phase III trials comparing sequences and combinations and from the high heterogeneity of studies.

Acceptable treatments involve FOLFIRINOX (or modified FOLFIRINOX) and gemcitabine-based multiagent chemotherapy (i.e., gemcitabine + albumin-bound paclitaxel).

The patient may also be scheduled to receive a multimodal induction therapy [169], usually using 3 or 4 cycles of a gemcitabine-based multi-agent chemotherapy or FOLFIRINOX regimen (if good performance status) as a first step. This is usually followed by a low-dose 5-fluorouracil monotherapy infusion at a dose of 200–250 mg/mq or oral capecitabine treatment, together with radiotherapy at a dose of at least 50Gy [170]. Gemcitabine monotherapy can be associated to radiations instead of fluoropyrimidine-based chemotherapy [171].

Despite no differences in long-term survival rates have been demonstrated, multimodal regimens have been associated with better response rates than chemotherapy alone. In particular, gemcitabine-based drug combinations reached up to about 90% of response rates if compared to fluoropyrimidine-based (25–70%), accounting for an increased rate in toxicities and worse quality of life [172].

Upfront chemoradiation is not usual in this setting, but it may represent an option for patients presenting with poorly controlled pain or local invasion with bleeding (NCCN guidelines, Version 2.2019).

Independently from the kind of treatment that has been used, the subsequent steps depend on the results of the neoadjuvant treatment: if downstaging has been obtained and the tumor can now be considered resectable, the patient will undergo potentially curative surgical treatment. If, otherwise, the disease is progressed to locally advanced or metastatic disease, palliative chemotherapy will be initiated (Fig. 40.19) [156, 159]. In the latter case, the choice of the subsequent treatment will depend on the patient's PS and on the kind of drugs previously administered. Anyway, retrospective studies [173, 174] suggest that radiographic response doesn't always correlate with pathological response: if no apparent tumor shrinkage is observed after neoadjuvant treatment and no extrapancreatic progressive disease is evident, surgery could still be attempted.

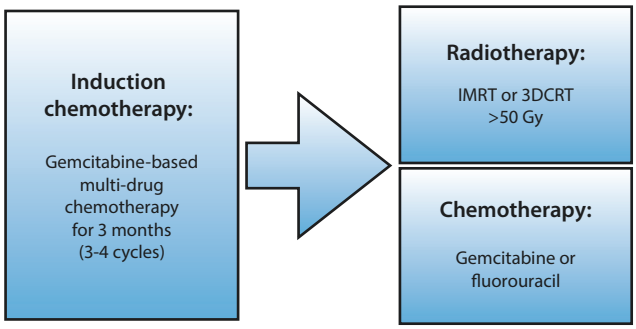


Fig. 40.19 Therapeutic algorithm in borderline resectable pancreatic cancer

According to guidelines, locally advanced cancers classified as unresectable will never undergo curative surgery. Standard treatment nowadays is represented by 6-month gemcitabine-based chemotherapy (i.e., gemcitabine + albumin-bound paclitaxel) or FOLFIRINOX / modified FOLFIRINOX. Gemcitabine monotherapy, 5-fluorouracil plus leucovorin, 5-FU in continuous infusion (CI 5-FU) or capecitabine monotherapy may also be used (NCCN guidelines, Version 2.2019).

A new, common approach to this disease is a multistep combination of chemotherapy and radiotherapy. After 2 or 3 months of chemotherapy alone (any of the aforementioned regimens may be used), the patient will be restaged to evaluate if objective response or, at least, stable disease have been achieved, and progression has not occurred [175–177]. If so, and if the patient's performance status is good enough, chemoradiation therapy can be started; this usually consists in a 5-fluorouracil, capecitabine or, alternatively, gemcitabine monotherapy, associated with radiotherapy [169, 170]. If disease has progressed, the patient will undergo palliative chemotherapy without radiation, using a different drug than the one that has been used previously. In any case, standard durations and drugs for this regimen have not been defined yet, and recent evidence are questioning the effectiveness of this approach (Fig. 40.20) [178].

40.8.3 Metastatic Disease

Patients with metastatic disease at diagnosis have a mean survival of only about 6 months, and standard therapy have not achieved satisfying results in improving survival. On the other hand, quality of life is of

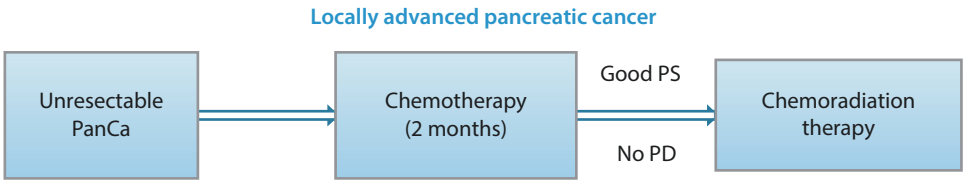


Fig. 40.20 Therapeutic algorithm in unresectable pancreatic cancer

great relevance for these subjects, and the most common symptoms such as pain, weight loss, nausea, or anorexia should be properly handled [179]. Therefore, in pancreatic cancer, the role of palliative treatment is not only to increase survival but also:

- To improve quality of life
- To obtain an adequate control of symptoms

These factors are difficult to evaluate through objective measurements but can be measured through the analysis of clinical benefit. This is an efficacy criteria created to evaluate response when therapy achieves minimal or no results in terms of standard criteria (such as overall survival or progression-free survival). It can be achieved by satisfying at least one of the following four main goals:

- 50% or more reduction of pain, measured daily with visual analogue scale (VAS), for at least 4 weeks
- At least 50% reduction of opioid drugs administration, expressed in mg morphine equivalent, for at least 4 weeks
- Karnofsky PS score improvement of 20% or more for at least 4 weeks
- At least 7% of body weight gain

Clinical benefit evaluation has received both great praise and great criticism. The first because it allows to keep in count factors other than life expectancy, which were previously dismissed but can be very important in the everyday life of an oncological patient; the latter for the lack of reproducibility of the results: even though body weight and opioid consumption can be objectively measured, clinical benefit still is a criteria prone to subjective evaluation from the patient (pain evaluation) and from the physician (PS analysis) [139].

Until recent times, gemcitabine monotherapy has been considered the standard of care in metastatic pancreatic cancer, having shown better results than 5-fluorouracil, both in clinical benefit, and overall survival improvements [180]. However, new data have shown the benefits of combination regimens, at least in selected patients [181]:

- FOLFIRINOX is a multi-agent chemotherapy combining 5-fluorouracil, irinotecan, and oxaliplatin. It is more effective than gemcitabine, improving overall survival of about 4 months in mean, but at the cost of higher toxicity (i.e., higher risk of febrile neutropenia): its use is limited to patients with good performance status (0–1 ECOG performance status and normal or subnormal levels of serum bilirubin), who can tolerate these side effects [182].
- Nab-paclitaxel is a drug that combines a taxane with a molecule of albumin, which is usually eagerly absorbed by tumors, allowing to obtain higher doses of drug inside the cancer cells associated with lower toxicity. It has been used in pancreatic cancer together with gemcitabine, proving itself superior to gemcitabine alone (2 months of median improvement in overall survival) with slightly lower activity but also a more favorable toxicity profile than FOLFIRINOX [183].
- As regards to target therapy, the efficacy of erlotinib, an EGFR inhibitor, in addition to gemcitabine has been evaluated too. However, although statistically significative, improvements in terms of overall survival have been very limited (median overall survival benefit of only 12 days), and so its role in advanced pancreatic cancer management is arguable [178, 184].
- 5-fluorouracil plus leucovorin, 5-FU in continuous infusion (CI 5-FU) or capecitabine monotherapy may also be considered (NCCN guidelines,Version 2.2019).

The current standard of care can be summarized as follows (Tables 40.13 and 40.14) [139]:

- Patients with 0–1 ECOG performance status, no comorbidities and bilirubin levels lower than 1.5× ULN: FOLFIRINOX should be considered.
- Patients with ECOG performance status 2 or minor comorbidities: nab-paclitaxel plus gemcitabine regimen can be used.

Table 40.13 Main factors involved in the decision of the right therapeutic regimen in patients affected by metastatic pancreatic cancer

Patient	Disease	Treatment
Age	Stenting	Toxicity
Performance status	Bilirubin serum levels	Quality of life
Comorbidities	Aggressiveness of the disease	Cost
Patient's choice		Clinical experience of the oncologist

Table 40.14 Choice of chemotherapy regimen based on PS, comorbidities, and bilirubin level

PS	Comorbidities	Bilirubin levels	Regimen
0–1	None/minor	<1.5 × ULN	FOLFIRINOX
2	Minor	<1.5 × ULN	Nab-paclitaxel+ gemcitabine
2	Yes	>1.5 × ULN	Gemcitabine alone
3–4	Severe	>1.5 × ULN	Only BSC

- Patients with performance status 2, comorbidities, or bilirubin serum levels higher than 1.5× ULN: gemcitabine monotherapy remains the standard.
- Patients with poor performance status, many comorbidities, or high levels of serum bilirubin: only best supportive cares (BSC) should be administered.

Second-line treatments are currently undefined, and their suitability must be evaluated case by case. However, in progressive, gemcitabine refractory, metastatic pancreatic cancer, the most commonly used regimens are 5-fluorouracil and leucovorin alone or in combination with oxaliplatin or irinotecan (FOLFOX and FOLFIRI regimens) [185] (NCCN guidelines,Version 2.2019). On the basis of the recently published NAPOLI-1 phase III trial, the NALIRI regimen (nanoliposomal Irinotecan with 5-FU and leucovorin) is now considered a new II line treatment option for these patients [186].

Gemcitabine monotherapy can be considered in patients previously treated with fluoropyrimidine-based first-line therapy.

Pembrolizumab has been approved in the United States as a second-line treatment option in patients with MSI-H or dMMR tumor without other satisfactory treatment options [187].

40.8.4 BRCA-Mutated Pancreatic Cancer

BRCA-mutated pancreatic cancer has been associated to better response to platinum-based treatments and could be good candidate to FOLFIRINOX or 5-fluorouracil and cisplatin regimens [183].

The POLO trial, presented at the 2019 annual ASCO meeting, evaluated the role of Olaparib, a PARP-inhibitor, vs placebo as maintenance therapy in BRCA1/2 germline-mutated patients with metastatic pancreatic adenocarcinoma who did not progressed after at least 16 weeks of first-line platinum-based chemotherapy. The results have shown a doubling of the median progression-free survival (7.4 months in the olaparib group vs 3.8 months in the placebo group), with a statistically significant hazard ratio of 0.53 [188].

40.8.5 Palliative Treatments

Palliative surgery plays an important role in the management of patients with pancreatic adenocarcinoma. Approximately 65–75% of patients with pancreatic cancer develop symptomatic biliary obstruction [189]. In this case, the best palliative option consists is the endoscopical insertion of a biliary stent. Metallic covered stents should be preferred to plastic or uncovered stents, having a lower biliary obstruction recurrence rate. If endoscopic management is not feasible, it is possible to surgically perform a biliopancreatic or gastric derivation [190]. Symptomatic gastric outlet obstruction occurs in 10–25% of patients with pancreatic cancer [189]. Similar to biliary obstruction management, duodenal obstruction can be handled endoscopically, positioning an expandable metallic stent or, as a second choice, surgically, positioning a percutaneous endoscopic gastrostomy (PEG) tube or performing a gastrojejunostomy [190].

Endoscopic surgery can also be used to reduce pain in pancreatic cancer, by blocking the coeliac plexus by performing a celiac plexus neurolysis: this method is safer than percutaneous insertion and equally effective [190]. Palliative radiotherapy can be used to relieve pain, bleeding, and/or local obstructive symptoms (NCCN guidelines,Version 2.2019).

Oral pancreatic exocrine enzyme replacement therapy can be administered to patients with pancreatic cancer with symptoms of pancreatic enzyme deficiency. This therapy may be initiated without diagnostic tests, considering the high frequency of this deficiency (94%) [191, 192] (NCCN guidelines,Version 2.2019).

40.9 Surveillance

NCCN guidelines recommend history and physical examination every 3–6 months with 2 years and then every 6–12 months in patients for resected pancreatic adenocarcinoma without evidence of active disease. CA19.9 measurement and chest-abdomen-pelvis CT scans every 3–6 months for 2 years can be performed, even though no significant survival benefit for patients who received regular CT scans surveillance has been shown [193].

Case Study: Pancreatic Cancer Diagnosis and Treatment

Man, 47 years old

- *Family history* positive for malignancy: maternal grandmother with gastric cancer.
- *PMH*: Diabetes mellitus type II, smoker of 1 pack/day for 20 years.
- *RMH*: development of jaundice in the last 2 months, with *ECOG PS*: 0.
- *Blood tests*: high blood levels of bilirubin, mostly direct (5 mg/dL).

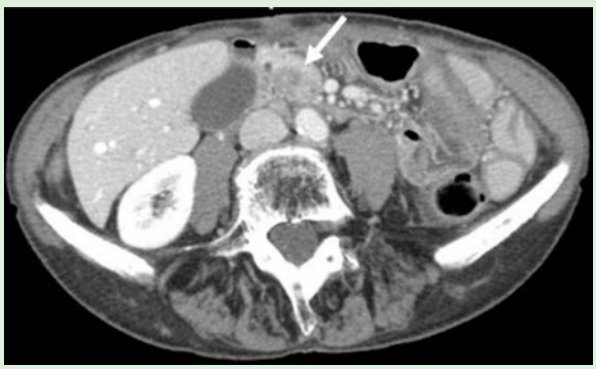
Question

What imaging technique should be chosen?

(1) PET. (2) Abdominal contrast CT. (3) ERCP

Answer

Contrast CT



- *Abdominal contrast CT*: hyperdense lesion at the head of the pancreas (maximum diameter 2.5 cm) in the context of a modest increase in the dimensions of the pancreatic head (3 cm). No lymphadenopathies.

Question

No thoracic lesions at the CT evaluation. What action should be taken?

(1) Surgery. (2) CT-guided FNA biopsy. (3) Chemotherapy

Answer

CT-guided FNA biopsy

- *Cytological examination*: compatible with pancreatic adenocarcinoma.

Question

Which one is the right treatment?

(1) Surgery. (2) Radiotherapy. (3) Chemotherapy

Answer

Surgery

- *Whipple pancreatoduodenectomy without preservation of the pylorus.*
- *Histology*: Pancreatic adenocarcinoma, pT3N0M0, G2, R0.

Question

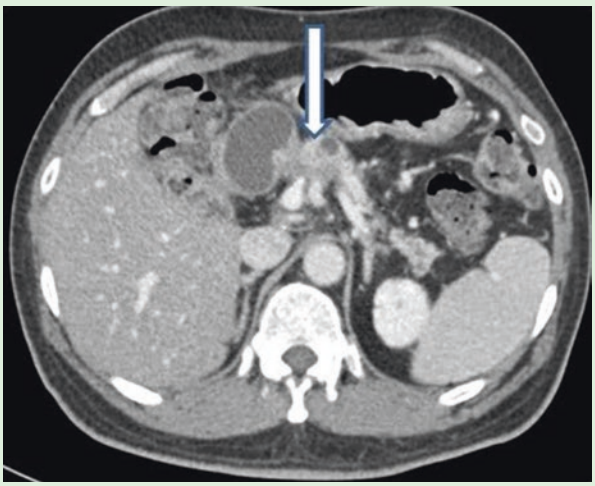
What to do now?

(1) Palliative chemotherapy. (2) Adjuvant radiotherapy. (3) Adjuvant chemotherapy

Answer

Adjuvant chemotherapy

- *Patient underwent adjuvant chemotherapy*: single-drug gemcitabine schedule for 6 months (7 cycles).
- *CT follow-up*: 2 years after surgery, detection of local pancreatic recurrence (maximum diameter 2.1 cm), infiltrating the splenic and mesenteric vein, together with their confluence and part of the portal vein.



- *EUS FNA biopsy*: diagnosis confirmed through cytological examination

Question

How to treat this local recurrence?

(1) New surgery. (2) Radiotherapy. (3) Palliative chemotherapy

Answer

- *Palliative chemotherapy*: the entity of vascular invasion contraindicates both surgery and radiotherapy.

- *Clinical evaluation*: The patient has very good performance status (0) and no relevant comorbidities.

Question

Which therapy?

(1) FOLFIRINOX. (2) Gemcitabine. (3) Gemcitabine + nab-paclitaxel

Answer

FOLFIRINOX: Considering the very good PS of the patient, he underwent six cycles of FOLFIRINOX, with partial response, and relevant, but incomplete regression of venous involvement.

- *New evaluation of the case*, considering the high compliance of the patient and his will to undergo surgery or radiotherapy.

Question

What action should be taken?

(1) New surgery. (2) Radiotherapy. (3) Follow-up

Answer

Palliative radiotherapy: Vascular invasion still forbids surgery, but considering patients' will and good PS radiotherapy is, though risky, the selected option.

Key Points

- Consider with suspect a gradually developing, direct bilirubin jaundice
- Importance of appropriate diagnosis and staging
- Carefully choose the right therapeutic option
- How to treat local relapse
- Importance of patient's will in clinical decisions

Case Study: Pancreatic Cancer Diagnosis and Treatment

Woman, 70 years old

- *Family history*: negative for malignancies
- *PMH*: HCV-positive hepatitis (treated in 2017 with new oral antiviral therapy), recurrent lung and bladder infections, ascending aortic aneurysm, and moderate mitral regurgitation
- *RMH*: occurrence of recurrent abdominal pain in the last 3 months, investigate with abdominal echotomography that showed nothing relevant.
- *ECOG PS*: 1/2.

Question

How would you proceed?

(1) Diagnostic laparoscopy. (2) Abdominal contrast CT. (3) New echography

Answer

Abdominal contrast CT: hyperdense lesion at the body of the pancreas (maximum diameter 1.5 cm), apparently infiltrating the peripancreatic fat tissue.

Question

What action should be taken?

(1) Surgery. (2) EUS with FNA biopsy. (3) Chemotherapy

Answer

EUS with FNA biopsy

- *Cytological examination*: compatible with pancreatic adenocarcinoma.

Question

How would you complete staging?

(1) PET. (2) Chest contrast CT. (3) EUS

Answer

Chest contrast CT: no signs of metastatic lesions.

Question

Which one is the right treatment?

(1) Whipple pancreatoduodenectomy. (2) Radiotherapy. (3) Pancreatectomy and splenectomy

Answer

Pancreatectomy and splenectomy (with preservation of pancreatic head)

- *Histology*: Pancreatic adenocarcinoma, pT1N1M0, (5/22 nodes positive for metastases) G2, R0, presence of vascular and neural infiltration, no infiltration of the adipose tissue.

Question

What to do now?

(1) Palliative chemotherapy. (2) Adjuvant radiotherapy. (3) Adjuvant chemotherapy

Answer

Adjuvant chemotherapy

- *Patient underwent adjuvant chemotherapy*: single-drug gemcitabine schedule (6 cycles), with dose reduction

because of the scarce PS and many comorbidities of the patient.

- *MRI follow-up*: 3 years after surgery, detection of local pancreatic recurrence (maximum diameter 2.1 cm), not infiltrating any major arterial or venous vessel, and local nodal metastases, confirmed with PET-CT.

Question

How to treat this local recurrence?

(1) New surgery. (2) Radiotherapy. (3) Palliative chemotherapy

Answer

Radiotherapy: the PS of the patient doesn't allow for a new surgical procedure, but, considering the absence of vascular involvement, RT is a possible option.

- *Stereotaxic helical RT* is performed in five sessions, for a total of 50 Gy. The treatment is well tolerated by the patient, and partial response is obtained.
- After 6 months, at follow-up MRI, detection of new *peritoneal and hepatic metastatic lesions*, confirmed at PET-CT.

Question

What action should be taken?

(1) New surgery. (2) I line palliative chemotherapy. (3) Best supportive cares

Answer

I line palliative chemotherapy

Question

Which therapy?

(1) FOLFIRINOX. (2) Gemcitabine. (3) Gemcitabine + nab-paclitaxel

Answer

Gemcitabine: considering the low PS of the patient and the many comorbidities, gemcitabine alone is the most suitable regimen.

- After four cycles of gemcitabine, RMI detection of peritoneal and hepatic *progressive disease*.

Question

What action should be taken?

(1) Best supportive cares. (2) FOLFIRINOX. (3) Capecitabine

Answer

II line palliative chemotherapy with capecitabine

Key Points

- Intermittent abdominal pain, in the absence of jaundice, can be a sign of body-tail pancreatic cancer.
- Always consider PS and comorbidities when selecting a treatment.
- Role of II line chemotherapy.

Expert Opinion

Marc Peeters

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Key Points

Pancreatic cancer represents one of the deadliest among all cancers, with extremely low survival rates. Because of the lack of early symptoms and the not-so-high sensitivity of first-line diagnostic techniques such as abdominal US, diagnosis is often retarded, being reached when it is already too late and there are no more chances of curative treatments.

In case of local disease, proper staging and arterial and venous involvement evaluation are essential to appropriately evaluate the best therapeutic path and to select between immediate surgery, neoadjuvant chemo- or chemoradioterapy or definitive chemo- or chemoradiotherapy. Modern imaging techniques such as MRCP and EUS are thus fundamental for proper staging, and all patients should be referred to hub centers which dispose of them.

In contrast to other cancers, where targeted therapies and immunotherapy have revolutionized treatments, offering a significant improvement in life expectancy, the backbone of pancreatic cancer treatment is still based on chemotherapy, with few or no improvements in survival until recently. The introduction of new drugs (nab-paclitaxel, nanoliposomal irinotecan) or combination schedules (FOLFIRINOX, adjuvant gemcitabine and capecitabine combination) have shown significant results, but the overall prognosis of the patient have not changed much. Interesting results have been shown by the recently published POLO trial in patients harboring germline BRCA 1 or 2 mutations, demonstrating the efficacy of adding olaparib as maintenance therapy after

first-line platinum-based chemotherapy in this subgroup of patients. This trial showed a statistically and clinically significant progression risk reduction opening the way to the use of PARPi as a new class of targeted therapies in pancreatic adenocarcinoma.

However, more efforts must be put in place to better understand the genetic and biological bases of this pathology, to develop and select drugs that can be active against it, and to find those subgroups of patients that could benefit the most from these treatments.

- Main risk factors are tobacco and excessive body weight; pancreatic cancer is usually asymptomatic in early stages, and the most frequent symptoms are jaundice (pancreatic head), abdominal pain, and weight loss.
- In case of high suspect of pancreatic cancer, contrast CT is the first imaging technique to consider. PET, MRI, EUS, ERCP, and MRCP could be needed to complete staging; CA19.9 is the most utilized biomarker in pancreatic cancer, even though its usefulness for diagnosis is limited;
- Resectable stage: pancreatoduodenectomy and pancreatectomy+splenectomy with preservation of the head are the two main surgical procedures performed; adjuvant chemotherapy with 5-FU or gemcitabine should be carried out.
- Borderline stage: tumors should be treated with a multimodal chemoradiation therapy, followed by surgery if feasible; the actual standard of care for unresectable cancer is 6 month of gemcitabine, but chemo-radiotherapy schedules should be considered.
- Advanced cancer: in selected patients, with very good performance status FOLFIRINOX is an option, while in patients with good performance status nab-paclitaxel should be considered; other patients should be treated with gemcitabine alone or only BSC.

Recommendations

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Hints for a Deeper Insight

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Biliary Cancer

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41.1 Epidemiology and Risk Factors

Biliary tract cancers (BTCs) comprise a heterogeneous group of neoplasms including cholangiocarcinoma (CCA), classified as intrahepatic (iCCA), perihilar or extrahepatic (eCCA) and gallbladder cancer. CCA is the second most common primary hepatic malignancy accounting for 10–20% of primary liver cancers [1]. The epidemiology of CCA and its subtypes display enormous geographic differences reflecting the distribution of different risk factors, both environmental and genetic alike [2, 3, 4]. eCCA represents the most common form of CCA; whereas in East Asian countries, iCCA is more the common form [5]. Globally, CCA is exceptionally common in Chile, Bolivia, South Korea and North Thailand, while it is a rare cancer (incidence less than 6 cases per 100,000) in Western countries [6].

Several conditions have been linked to CCA carcinogenesis. Some are considered established risk factors such as primary sclerosing cholangitis (PSC), while some have a weak association and are therefore considered possible risk factors. Several studies have demonstrated that individuals with PSC have a high risk of developing CCA [7, 8, 9, 10]. Liver fluke infestation is also strongly associated with CCA [11]. Indeed, prevalence rates of CCA are maximum in regions with higher prevalence rates of liver fluke infestations [5, 12] caused by *Opisthorchis viverrini* and *Clonorchis sinensis* species acquired by oral ingestion of undercooked fish [13, 14]. Choledochal cysts [15] especially type I (solitary, extrahepatic) and type IV (extrahepatic and intrahepatic) cysts are also associated with a high risk for cholangiocarcinogenesis. In addition, Caroli's disease, a rare congenital disorder characterized by nonobstructive dilatation of segmental intrahepatic bile duct, has been linked with intrahepatic CCA [16]. Other common risk factors is the presence of gallstones in the intrahepatic biliary tree, known as hepatolithiasis [17] and toxic agents like thorotrast and dioxin [18, 19]. Recent evidences have demonstrated a correlation between hepatitis B virus (HBV) and HCV infections with cholangiocarcinogenesis [20, 21]. Finally, other pathological conditions as cirrhosis [22], obesity [23, 24] and diabetes [25] have been associated to CCA incidence even if they need to be further validated.

41.2 Classification and Histological Types

Cholangiocarcinomas (CCA) can develop anywhere along the biliary tree and are anatomically classified as intrahepatic (10%), perihilar (50–60%) and distal CCA (20–30%) [6]. Lesions can be defined as mass-like, periductal, intraductal or mixed based on clinical presentation and site of origin. Most cholangiocarcinomas are well or moderately differentiated tumours with a locally aggressive behaviour. They present mainly with infiltration of contiguous structures (liver, hepatic artery and portal vein), nodal involvement (up to 30% at diagnosis), invasive spread with neural, perineural and lymphatic involvement and subepithelial extension [26]. In particular, iCCAs arise from the small bile ducts in the liver and can be divided into mass-forming, periductal infiltrating and intraductal growth types [27]; pCCAs originate in the main hepatic ducts or at the bifurcation of the common biliary tract, and they can have exophytic (forming-mass) or intraductal macroscopic growth patterns; dCCAs arise from the extrahepatic tract comprehending the cystic duct up to the ampulla of Vater. Macroscopic subtypes are: sclerosing tumours, nodular (often both) and papillary.

41.3 Screening and Diagnosis

The prevention of underlying liver disease and the identification of high risk patients is the best choice to improve clinical outcome. It is important pay attention to the potential symptoms of the disease, even if clinical presentation of CCA is unspecific. Patients with CCA could present several symptoms such as malaise, abdominal pain, loss of weight and appetite as well as obstructive jaundice, common also with other pathologies (i.e. hepatocellular cancer, pancreatic cancer, biliary stones) [28, 29, 30]. CCA screening cannot be performed with any degree of reliability in individuals who are not experiencing symptoms. Moreover, there are not effective lab tests that can identify biliary cancers at early stage and the disease is usually diagnosed late.

Since CCA recommended screening methods are not available in clinical practice, the surveillance of risk patients is crucial for early diagnosis at resectable stage.

Patients with hepatolithiasis, PSC, cholangitis, hepatobiliary flukes and choledochal cysts could be the best candidates for the screening [31, 32]. Nevertheless, patients with hepatolithiasis usually develop several complications that make the diagnosis of CCA more difficult; thus patients' prognosis is significantly worst.

It is known that PSC patients have an increased risk to develop CCA, but given the low annual incidence, it is difficult to identify a high-risk group within those patients that could benefit from a screening program. In particular, the current technologies used for CCA screening and diagnosis (ultrasound (US), magnetic resonance imaging (MRI/MRCP), computed tomography (CT) scan, cholangiography and endoscopic retrograde cholangiopancreatography (ERCP), as well as serum and bile markers for cancer) lack of efficacy, cost-effectiveness and reliability.

Carbohydrate antigen 19-9 (CA 19-9), a glycolipid expressed by cancer cells, is the most common circulated marker associated with CCA. Nevertheless, it cannot be considered a screening marker, given its variability of sensitivity and specificity. Indeed its serum levels increased after several inflammatory processes common in different types of cancer and in infectious conditions (i.e. cholangitis). At contrary, patients who are negative for Lewis antigen do not produce CA 19-9 neither in presence of CCA [33]. Recently, novel molecular markers have been introduced to differentiate CCA from other biliary diseases like angiopoietin-2 secreted by tumour cells [34, 35].

Imaging studies allow a noninvasive examination of the biliary tree, but they are inadequate when used alone. For example, US is not recommended for screening or diagnosis of CCA given its limited resolution [36].

CT and MRI are useful for the distinction of CCA from hepatocellular carcinoma for tumours >2 cm. In particular, CT has a high accuracy for evaluating portal vein and arterial involvement, even if it has low sensitivity in detecting lymph node metastases.

Another imaging technique used for CCA detection is MRI with magnetic resonance cholangiopancreatography (MRCP), especially to evaluate pCCAs with an accuracy up to 95% [37].

When these imaging systems are inadequate to diagnose CCA, positron emission tomography (PET) can be used. PET-CT has high sensitivity and specificity to detect primary tumours and metastasis of iCCA, even if its sensitivity and specificity decrease in pCCA evaluation [38].

Another imaging system used for the evaluation of CCAs is cholangiography performed by percutaneous transhepatic cholangiography (PTC), MRCP or endoscopically using endoscopic retrograde cholangiopancreatography (ERCP). ERCP is useful to diagnose perihilar and distal extrahepatic CCA and, with PTC, allows biliary stent placement when biliary obstruction occurs. Moreover, ERCP with brush cytology is used to sample tissue biopsies thanks to the advantage of wire guidance with high specificity of CCA diagnosis. Nevertheless, meta-analysis study showed that the sensitivity of ERCP is around 40–50%, and thus it cannot be used as diagnostic method for early diagnosis [38, 39]. To increase the sensitivity of cytology, fluorescent in situ hybridization can be used [40].

41.4 Staging and Prognosis

The previous edition of the American Joint Committee on Cancer (AJCC) staging system staged intrahepatic tumours as hepatocarcinoma and extrahepatic CCAs (perihilar and distal) considered as unique entity. The new classification for iCCAs focuses on tumour extension, vascular invasion and extrahepatic structures infiltration, representing a useful prognostic factor (Table 41.1). The classification for eCCAs includes separate TNM for perihilar and distal carcinomas [41] (Tables 41.2 and 41.3).

Table 41.1 The AJCC/UICC staging of intrahepatic cholangiocarcinoma

Primary Tumor (T)	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma *in situ*
T1	Tumor confined to the bile duct, with extension up to the muscle layer or fibrous tissue
T2A	Tumor invades beyond the wall of the bile duct to the surrounding adipose tissue
T2B	Tumor invades the adjacent hepatic parenchyma Tumor invades unilateral branches of the portal vein or the hepatic artery
T3	Tumor invades the main portal vein or its branches bilaterally; or the common hepatic artery; or the second-order biliary radicals bilaterally; or unilateral second-order biliary radicals with contralateral portal vein or hepatic artery involvement
T4	
Regional lymph nodes (N)	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis (including nodes along the cystic duct, common bile duct, hepatic artery and portal vein) Metastasis to periaortic, pericaval, superior mesenteric artery and/or coeliac artery lymph nodes
N2	
Distant Metastasis (M)	
M0	No distant metastases
M1	Distant metastases present

Surgical treatment represents the only curative treatment option. If radical surgery is performed, prognosis is not influenced by cancer primary site and extension of resection. Patients with unresectable iCCA have a life expectancy <5% at 5 years that increases to 20–44% for patients with early stage disease (T1–T2). Survival is related to the presence of multiple tumours, vascular invasion, regional nodal involvement and large tumour size [42], and it is also linked to the macroscopic subtype (better for papillary) and tumour differentiation grading (better if well-differentiated). In particular, vascular invasion and the presence of multiple tumour sites are poor prognostic factors only in N0 stage disease, while regional nodal involvement has an important prognostic value just in localized disease (M0). For extrahepatic neoplasms, the depth of tumour invasion has been identified as an independent predictor of outcome. Recently

Table 41.2 The AJCC/UICC staging of perihilar cholangiocarcinoma

Primary Tumor (T)	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma *in situ* (intraductal tumor)
T1a	Solitary tumor without vascular invasion (maximum diameter < 5 cm)
T1b	
T2a	Solitary tumor without vascular invasion (maximum diameter > 5 cm)
T2b	
T3	Solitary tumor with vascular invasion
T4	Multiple tumors, with or without vascular invasion Tumor perforating the visceral peritoneum or involving the local extrahepatic structures by direct invasion Tumor with periductal invasion
Regional lymph nodes (N)	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis present
Distant Metastasis (M)	
M0	No distant metastases
M1	Distant metastases present

Table 41.3 The AJCC/UICC staging of distal cholangiocarcinoma

Primary Tumor (T)	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma *in situ*
T1	Tumor depth of invasion <5 mm
T2	Tumor depth of invasion between 5 and 12 mm
T3	Tumor depth of invasion >12 mm
T4	Tumor involves the coeliac axis, or the superior mesenteric artery
Regional lymph nodes (N)	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
Distant Metastasis (M)	
M0	Non distant metastasis
M1	Distant metastasis present

other prognostic factors have been identified in advanced unresectable BTC including poor performance status (ECOG ≥2), high neutrophils and bilirubin serum levels, low haemoglobin and disease stage (metastatic vs locally advanced) associated with worse outcome [43]. Finally, high levels of neutrophil/lymphocyte ratio (NLR) are correlated with poor outcome [44, 45].

41.5 Treatment

The therapeutic strategies include surgery, adjuvant and neoadjuvant treatments and palliative therapies. A detailed treatment algorithm is shown in Fig. 41.1.

41.6 Surgery

Surgical resection of CCA remains the only potentially curative treatment associated with long-term survival [46]. Unfortunately, the surgical approach is feasible in only about 20–40% of patients because of they often present advanced unresectable tumours. For this reason, it is important to select patients who can really benefit from surgery, considering the tumour's anatomical site and the extent of local and metastatic spread [47].

Radical resection with clear pathological margins is crucial to achieve a long survival as well as the absence of vascular invasion, lymph node metastasis and adequate functional liver [42, 48]. Hepatic resection is usually performed as standard treatment of CCA. In particular, resection of intrahepatic and extrahepatic bile ducts and affected segments or lobe is recommended for iCCA, pancreatoduodenectomy for dCCA and resection of the involved intrahepatic and extrahepatic bile ducts, the gallbladder and regional lymph nodes for pCCA [42]. The cholecystectomy is the treatment for gallbladder cancer at early stage, while reoperation is indicated in advance stages (liver resection and nodal dissection) [49].

CCA has a median overall survival at 5 years of 20–36 months, while in patients undergone to complete pathological resection, overall survival increases to 65 months [50, 51]. After surgery, some complications occur including hepatic failure, cholangitis, wound infection and sepsis which lead to a significant decrease in survival rates. Several co-morbidities could affect patients' survival such as hypoalbuminemia and jaundice in the preoperative period, even if mortality is similar to those of extended resection (with or without vascular resection) patients [50–53].

Although the recent advances in surgical treatment, about 60% of patients have a recurrent disease after resection within 2 years. Nevertheless, the mortality after recurrence is worst in patients treated with standard therapies compared to those undergone to pathological resection (3-year overall survival 32% vs 3%; $p < 0.0001$) [54].

Liver transplantation is not recommended as treatment for unresectable CCA, because it is associated with rapid tumour recurrence and low survival. A best novel approach for CCA is preoperative chemoradiotherapy followed by liver transplantation [55].

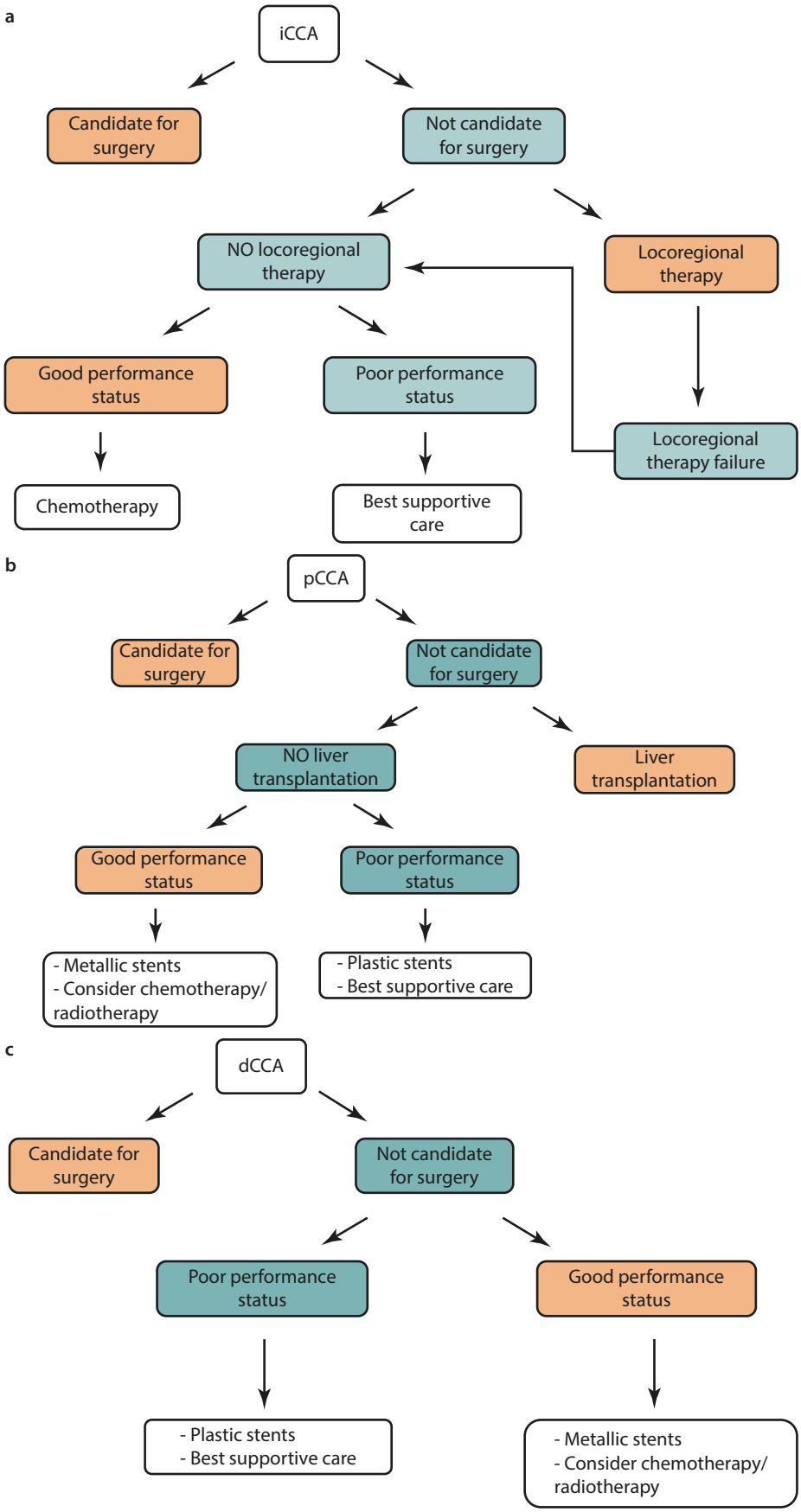

Fig. 41.1 Clinical management algorithms for adult patients with **a** iCCA; **b** pCCA; **c** dCCA

Data supporting the benefit of this treatment in iCCA are controversial, due to the small number of sample size and the heterogeneity of perioperative treatment. Moreover, some factors contraindicate this procedure including multiple tumour, perineural, vascular and liver infiltration and lack of adjuvant and/or neoadjuvant therapy [56, 57] (Fu et al. 2011). A recent multicentre study reported that 73% of patients with cirrhosis and early iCCA have a high actuarial survival at 5 years [58].

In early stage of pCCA, the rate of recurrence-free survival at 5 years is about 65–68% in patients undergone liver transplantation previously treated with neoadjuvant therapy [59].

Based on promising results of liver transplantation, this approach needs to further investigations and could be applied only in specific groups of patients in specialized centres.

41.7 Adjuvant and Neoadjuvant Treatment

Both radiation and chemotherapy have been analysed in the adjuvant setting (either apart or concomitantly). In particular, a large meta-analysis performed by Horgan et al. [60] evaluating 6712 patients, among which 1792 received adjuvant treatment (both radiotherapy and chemotherapy) demonstrated no survival benefit of the overall population. Anyway, authors observed that adjuvant chemotherapy is associated with improved survival among patients with R1 resection and N1 stage [60]. Therefore, adjuvant chemotherapy should not be considered a standard in BTC but could be discussed in patients with high risk of tumour recurrence.

Similarly, retrospective analysis suggested that adjuvant radiation therapy can achieve survival advantage just in selected patients' subgroups, especially in R1 resection [61, 62], but no randomized trials assessing are available.

The main data assessing the role of adjuvant chemoradiotherapy derive only from retrospective analysis. The most commonly used concomitant chemotherapeutic agents were 5-fluorouracil and gemcitabine. Although several evidences suggest that adjuvant concomitant chemoradiotherapy could achieve survival advantage mainly in R1–R2 and N1 stage disease [63, 64, 65], it cannot be considered a standard of treatment.

Regarding neoadjuvant setting, a novel optional treatment for CCA is preoperative chemoradiotherapy followed by liver transplantation [66]. Promising results were obtained treating patients affected by hilar CCA with stereotactic radiotherapy followed by chemotherapy and liver transplantation [55]. Further studies are needed to confirm the efficacy of this therapy, which, at the moment, should be proposed only in selected cases.

41.8 Palliative Therapy

41.8.1 Chemoradiotherapy in Locally Advanced Disease

Concomitant chemoradiotherapy has been largely investigated in locally advanced CCA showing survival benefit. A retrospective analysis evaluated the activity of concomitant treatment in patients receiving capecitabine and cisplatin alone or in combination with radiotherapy. Data showed that overall survival and progression free survival were significantly longer in the chemoradiotherapy group and concurrent treatment achieved a higher (but no statistically significant) disease control rate [67]. These results should be confirmed in prospective randomized trials in order to define a standard of treatment.

41.8.2 Chemotherapy in Advanced Disease

For patients with unresectable or metastatic CCA, systemic chemotherapy remains the mainstay palliative treatment modality. Unfortunately, advanced CCA is often associated with liver function impairment, jaundice, weight loss, pain and poor performance status which contraindicate chemotherapy. The most active cytotoxic chemotherapy agents in the management of BTCs are gemcitabine and platinum agents [68, 69].

The role of chemotherapy in improving patient outcome and quality of life was reported in 1996, and gemcitabine was established as reference treatment in advanced disease [70]. Many following trials have been performed [68], but they were negative or lacked the statistical power to change clinical practice.

In 2010, ABC-02 study phase III trial defined gemcitabine and cisplatin (GEMCIS) combination as standard treatment for advanced CCA [71]. This combination showed an improvement in overall survival compared to gemcitabine alone (11.7 months versus 8.1 months). The following studies confirmed these findings [72, 73]. A large meta-analysis comparing oxaliplatin (GEMOX) and cisplatin (GEMCIS) combination with gemcitabine found a longer survival in patients treated with GEMCIS (11.7 vs 9.7 months), although a higher toxicity rate [74]. Other potentially active regimens, such as gemcitabine and capecitabine combination [75], or triplets comprehending fluoropyrimidines, gemcitabine and platinum compound [76, 77] need to further investigations.

41.8.3 Second-Line Chemotherapy

Failure of first-line treatment is often associated with rapidly worsening performance status, and only a small number of patients may be suitable for further treatments. In addition, patients often have the inherent problems of biliary obstruction and sepsis associated with BTC, which may contraindicate further chemotherapy. Considering the low number of patients able to undergo to second-line, very limited literature data are available in this setting.

Small prospective and retrospective studies have shown potential benefit in selected patients (good performance status, long PFS after first-line chemotherapy (>6 months), resected primary tumour and low CA 19.9 value) [78, 79]. A British trial (ABC-06), comparing second-line chemotherapy with oxaliplatin and 5-fluorouracil versus best supportive care is currently ongoing (NCT01926236).

41.8.4 Locoregional Treatment

Locoregional treatment is indicated in the patients where surgical resection is not to be performed. This approach includes transarterial chemoembolization (TACE), intraarterial chemotherapy and radiofrequency ablation (RFA). Retrospective and prospective analyses demonstrated that TACE [80, 81] and intraarterial chemotherapy [82, 83] are associated with a good disease control rate.

Moreover, photodynamic therapy is another treatment option in locally advanced hilar and perihilar CCA that gives a good locoregional tumour control. Recent data demonstrated that photodynamic therapy with concomitant biliary stenting is associated with improved survival and quality of life compared to biliary stenting alone [84].

41.9 Key Genomic Alterations and Emerging Therapies

Advances in molecular medicine allowed the identification of a wide range of mutations, amplifications and deletions in BTC, many of which have targetable options. Several studies have documented important driver mutations reported in other tumours including the epidermal growth factor (EGF) pathway with EGF receptor (EGFR), k-ras and b-raf mutations or overexpression and alteration in the mitogen-activated protein kinase and PI3K/mammalian target of rapamycin pathways. More recently, recurrent translocation events involving fibroblast growth factor receptor (FGFR) loci and mutations in the metabolic pathway involving isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2) have been reported. Finally, mutations in chromatin-remodelling genes BAP1 (encoding a nuclear deubiquitinase), ARID1A (encoding a subunit of the SWI/SNF chromatin-remodelling complexes) and PBRM1 (encoding a subunit of the ATP-dependent SWI/SNF chromatin remodelling complexes) have been described [85].

41.9.1 EGFR Pathway

The EGFR family comprises four tyrosine kinase receptors (ERBB1–4) that regulate cell proliferation, survival, angiogenesis and invasion through ligand binding and subsequent activation of signal transduction cascades involving the MAPK pathway (Ras-Raf-MEK-ERK) and the PI3K/AKT pathway [86].

EGFR amplifications are seen in 8% of BTC cases, while mutations in 13–15% [87, 88, 89].

Despite encouraging results from early studies [90, 91], randomized trials of EGFR antagonists erlotinib and cetuximab, each added to gemcitabine and oxaliplatin, showed no improvement in survival outcomes in advanced BTC [92, 93].

HER2 (v-ERB-B2, erythroblastic leukaemia viral oncogene homolog-2) overexpression and gene amplification are also described in BTCs with a higher incidence in gallbladder cancer (19%) [94]. Similarly to EGFR inhibitors, HER2 antagonists, trastuzumab, lapatinib and afatinib have demonstrated no clinical benefit in advanced BTC [95, 96].

41.9.2 MAPK Pathway

Aberrations in cell-surface receptors and their ligands (e.g. EGFR, VEGF) can lead to constitutive activation of downstream cascades, including the MAPK signalling (RAS-RAF-MEK-ERK). KRAS mutations are very common in BTCs, with highest rates seen in eCCA, followed by iHCC, and lowest in GBC (Li et al. 2014; [85, 97]). KRAS mutations have been associated with perineural invasion, advanced stage and poor prognosis [98].

Principal BRAF mutation (V600E) was reported also in CCA with varying frequency [99]. Despite the recognized frequency of KRAS and BRAF mutations, targeting this pathway remains challenging. A phase II study of selumetinib, a MEK inhibitor, showed a disease control rate of 80% and median overall survival of

9.8 months in patients with advanced biliary tract cancer [100]. Conversely, clinical trials of sorafenib, a multikinase inhibitor targeting the MAPK axis, failed to show clinical benefit in BTC [101, 102].

41.9.3 PI3K Pathway

The phosphoinositide-3-kinase (PI3K), signalling pathway involved in cell proliferation, is upregulated in CCA, and its activation was associated both with poor and good prognosis in BTC patients [103]. Several studies have reported PIK3CA hotspot mutations in BTC [104], mostly in GBC (Jeffrey et al. 2015; [105, 106]). Clinical trials are lacking except for an early study of everolimus, a mTOR inhibitor acting downstream of the PI3K signal. This study showed evidence of antitumour activity with a median overall survival of 9.5 months in 27 advanced BTC patients [107]. Moreover, a phase II trial using a PI3K inhibitor, copanlisib, in combination with gemcitabine and cisplatin as first-line therapy is ongoing (NCT02631590).

41.9.4 FGF Pathway

The fibroblast growth factor (FGF) ligands and receptors (FGFR1-4) are involved in cancer development and progression via activation of mitogenic and mesenchymal signals [108]. Genome-wide structural analyses in BTCs showed recurrent translocation events that involved the FGFR2 locus [109]. In particular, chromosomal fusions occur between FGFR2 and various genomic partners (e.g. AHCYL1, BICC1, PARK2, KCTD1, MGEA5, TACC3, TXLNA) ([110, 111]; Nakamura et al. 2014).

FGFR translocations were reported in 13% of iCCA with improved survival in these cases [110]. The recent discovery of recurrent FGFR2 fusions has opened a promising therapeutic avenue. Multitargeted tyrosine kinase inhibitor (TKIs) that also inhibits FGFR (such as ponatinib, nintedanib, dovitinib and brivanib) and FGFR antibodies and FGFR trap molecules have been developed. These FGF inhibitors are currently in their early phases, and few trials focusing on BTC are underway to investigate their potential clinical utility.

41.9.5 IDH Pathway

The IDH family of enzymes comprises the proteins IDH1, IDH2 and IDH3 that are involved in different cellular processes, including mitochondrial oxidative phosphorylation, glutamine metabolism, lipogenesis, glucose sensing and regulation of cellular redox status [112]. Mutations in IDH1 and IDH2 genes lead to accumulation of metabolites that result in altered intracellular processes including DNA methylation and hypoxia responses, ultimately leading to oncogenesis [113].

Mutations in IDH1 and IDH2 have been identified in BTC, especially in iCC, but the prognostic significance remains conflicting. Indeed, several studies have demonstrated that IDH mutations were correlated with decreased overall survival [114], while other studies reported that IDH alterations were not associated with survival [115] or associated with longer time to disease recurrence [116].

A large recent genomic profiling analysis identified IDH mutations in 20% of iCCA cases but none in eCCA or GBC. Pharmacologic IDH inhibitors have been developed and some clinical trials are underway. Early results from studies of oral IDH1 (AG-120) and IDH2 (AG-221) inhibitors have shown encouraging results in acute myeloid leukaemia setting [117, 118]. Trials with these agents are being expanded to solid tumours including BTC patients (NCT02073994, NCT02273739).

41.9.6 Chromatin Modifiers

Chromatin remodelling allows genomic DNA to access regulatory transcriptional proteins and thereby controls gene expression. Genetic alterations in ARID, BAP1 and PBRM, responsible for chromatin remodelling, have been implicated in BTC. In particular, ARID1A, encoding a subunit of the SWI/SNF chromatin-remodelling complex, seems to act as a tumour suppressor gene, and its inactivation is linked to multiple malignancies [119]. Exome sequencing analysis identified ARID1A mutations in 19% of iCCA [114].

BAP1 and PBRM1 are also involved in chromatin remodelling, and their alterations are described in 7–25% of cases of BTC and associated to worse survival in iCCA [120]. Histone deacetylase inhibitors such as vorinostat and panobinostat may offer new therapeutic chances in this setting [121].

41.9.7 Other Molecular Pathways

The WNT/β-CATENIN pathway is involved in the regulation of cell invasion and migration. Preclinical evidences demonstrated that WNT pathway activation is associated with chemo-resistance and metastatic

spread [122, 123]. High levels of activated β-CATENIN into the nucleus has been described in iCCA (15%) [124]. Although multiple WNT pathway inhibitors are currently under development in solid tumours [125], only few clinical trial have been reported for BTC as yet.

The Hedgehog pathway may also be involved in the development of BTC [126, 127, 128, 129]. Indeed, suppression of Hedgehog pathway reduced tumour volume in preclinical BTC models [130, 131]; in addition, BTC patients with activated Hedgehog pathway had a more aggressive behaviour and worse outcome [132, 133].

c-MET tyrosine kinase plays a key role in carcinogenesis by promoting angiogenesis, tumour invasion and metastasis. c-MET overexpression has been reported in CCA patients associated with a poor prognosis [89, 134, 135], while c-MET amplification is very rare. HGF/c-MET pathway promotes the invasive progression of gallbladder carcinoma cell lines [136] and data from human tissue confirmed the higher c-MET expression in cancer cells compared to normal gallbladder tissue [137].

41.10 Conclusion

BTCs are a group of devastating heterogeneous tumours difficult to diagnose and associated with poor outcome. The incidence of iCCA is increasing worldwide due to a complex interplay between predisposing genetic factors and environmental triggers. Surgery with complete resection is the only chance for cure, but this approach is applicable in few patients and, often, is associated with a high percentage of disease recurrence. Recent evidences suggest that liver transplantation combined with neoadjuvant chemoradiotherapy could offer long-term benefit in selected patients. Unfortunately, the most part of BTCs are diagnosed at advanced unresectable stage, and the chemotherapy remains the only treatment available to improve symptoms palliation but with a poor impact on patients survival. To achieve improved outcomes, better understanding of tumour biology, combined with the development of novel diagnostic and treatment strategies, is crucial. Several genomic alterations have been identified in BTCs, and newer targeted therapies acting on these pathways are being tested in clinical trials.

Expert Opinion

Marc Peeters

1. Biliary tract cancers are a group of heterogeneous neoplasms that comprise gallbladder cancer and cholangiocarcinoma (CCA) which can be classified in an intrahepatic, perihilar and extrahepatic form, actually the most frequent type (50–60%).
2. Different risk factors (such as primary sclerosing cholangitis and liver fluke infestation) and a genetic predisposition account for the various worldwide diffusion of this neoplasm, more common in the southwest of Asia and South America than in Western countries. Although the awareness about the risk factors for the low incidence of this neoplasm and for the lack of efficacy, cost-effectiveness and reliability no screening programs can be adopted.
3. No specific symptoms are present at the diagnosis, and frequently they consist in jaundice, abdominal pain and weight loss.
4. Just a multimodal diagnostic approach can give the necessary information about the neoplasm: CT scan is quite useful for masses >2 cm in order to study the portal vein and arterial involvement; magnetic resonance cholangiopancreatography (MRCP), has an important role in evaluating perihilar CCAs with an accuracy up to 95%. Moreover, ERCP with brush cytology can be used to sample tissue biopsies thanks to the advantage of wire guidance.
5. Surgery is the only curative treatment (20–40% of patients); furthermore, in selected patients with high risk of recurrence after surgery, adjuvant chemoradiotherapy can be evaluated. New studies have shown that liver transplantation combined with neoadjuvant chemoradiotherapy could offer long-term benefit in selected patients.
6. In case of unresectable cancer, chemotherapy with platinum agents and gemcitabine is the most recommended treatment even if it is linked to a poor prognosis (<5% at 5 years). There is not a standard recommended second-line chemotherapy, so more studies are needed; locoregional treatments (TACE, RFA) must be considered in this setting of patients.
7. Innovative drugs (such as MEK or mTOR inhibitors), have shown interesting results and more trials are ongoing in order to verify the efficacy of these drugs. More evidences will be obtained continuing the study of biological features of these neoplasms in order to obtain new therapies.

Recommendations

- AIOM
  ► https://www.aiom.it/linee-guida-aiom-tumori-delle-vie-biliari/
- ESMO
  ► https://www.esmo.org/Guidelines/Gastrointestinal-Cancers/Biliary-Cancer
- ASCO
  ► https://ascopubs.org/doi/abs/10.1200/JCO.18.02178

Hints for a Deeper Insight

- Randomized clinical trial of adjuvant gemcitabine chemotherapy versus observation in resected bile duct cancer: ► https://www.ncbi.nlm.nih.gov/pubmed/29405274
- Salvage radiotherapy for locoregionally recurrent extrahepatic bile duct cancer after radical surgery: ► https://www.ncbi.nlm.nih.gov/pubmed/28937265
- Karnofsky Performance Score Is Predictive of Survival After Palliative Irradiation of Metastatic Bile Duct Cancer: ► https://www.ncbi.nlm.nih.gov/pubmed/28179357
- Race, ethnicity, and socioeconomic factors in cholangiocarcinoma: What is driving disparities in receipt of treatment? ► https://www.ncbi.nlm.nih.gov/pubmed/31301148
- Smoking, Alcohol, and Biliary Tract Cancer Risk: A Pooling Project of 26 Prospective Studies. ► https://www.ncbi.nlm.nih.gov/pubmed/31127946

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Hepatocellular Cancer

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Gastrointestinal Cancers

Contents

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42.1 Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer in adults. Even if improvements in prevention and diagnosis have been done in recent years, HCC still remains the third leading cause of cancer death [1].

It occurs in the setting of chronic liver inflammation, mostly linked to chronic viral hepatitis B or C. Exposure to toxins such as alcohol or aflatoxin could conceivably be causes of HCC. Also metabolic syndrome and nonalcoholic steatohepatitis (NASH) are increasingly recognized as risk factors for HCC. Hemochromatosis and α1-antitrypsin deficiency could increase the risk of developing HCC.

Often, but not always, HCC develops through a fibrotic degenerative process with the formation of nodules called cirrhosis. So far, HCC is the most common cause of death in people affected by cirrhosis [2].

Most patients affected by HCC have signs and symptoms of chronic liver disease (jaundice, ascites, abnormalities of blood coagulation, hyporexia, weight loss, abdominal pain, nausea, and vomiting). Sometimes they do not show any symptoms. In some cases, HCC patients could present worsening of the symptoms.

42.2 Epidemiology

In the US surveillance, epidemiology, and outcome (SEER) database program, HCC accounts for 65 % of all cases of liver cancer [3, 4]. The incidence rate of HCC increased from 1.4/100,000 cases/year in the 1980s to 6.2/100,000 cases in 2011 [3, 5]. HCC is more frequent in men than in women, with a ratio of about 2.4:1 [6]. It is generally diagnosed between 50 and 70 years of age [7], is predominant in Asian and African countries, and is not very common in Northern Europe and North America [4]. The main risk factors are hepatotropic viruses infection, such as HBV and HCV, and alcohol abuse. About 80–90 % of HCCs occur within the context of cirrhosis [8]. In recent years an increase in the number of cases associated with metabolic syndrome has been observed.

42.3 Physiopathology

Hepatitis B virus is the principal cause of hepatocellular carcinoma. There are clear evidences of such an association, accumulated from biological studies in patients with chronic liver disease degenerated into neoplastic disease and from prospective and retrospective epidemiological studies conducted on populations from Africa, Malaysia, Japan [9, 10], China [11], Europe [12], and the USA [13]. Hepatitis C is also strongly associated with the risk of primitive HCC [14, 15], with a relative risk estimated up to more than a 20%, which is a figure similar to the one of hepatitis B.

Alcohol abuse is another risk factor for the development of this tumor type.

In recent years it has been shown as in developed countries there is a correlation between the metabolic syndrome (NASH and NAFLD) and HCC. However, the above form is still poorly studied.

In the world, the principal liver carcinogen aflatoxin content in food is a product of the metabolism of the fungus *Aspergillus flavus* that contaminates foods (usually the produce of grain stored in hot and humid environment) in many tropical countries, particularly in Southern Africa and Southeast Asia. Experimentally, it is among the most potent liver carcinogen known for certain animal species, and it is likely that it is a potential carcinogen also for men. In addition, the incidence of primitive HCC in some areas of Southern Africa (where this cancer is particularly prevalent) is positively correlated with the content of aflatoxin in the diet [16]. In developed countries, food is less contaminated by *Aspergillus flavus*, and this fungus is not involved in the carcinogenesis of HCC.

There is also a difference in the incidence of hepatitis B infection between developed and developing countries. In developing countries infection with hepatitis B, it is more common, while in developed countries hepatitis C infection is more frequent. The hepatitis B virus is a direct carcinogenic, while the hepatitis C virus is an indirect carcinogen: hepatitis C exerts its carcinogenic action through the inflammatory process and the resulting cirrhosis that develops in the liver. These etiological differences are reflected in a different biological behavior of HCC: the majority of Caucasian patients have a slow-growing and expansive cancer [17], whereas South African patients have a rapid-growing cancer [18]. As a consequence, there are significant different etiologies between primary HCC in Africans and Europeans and North Americans.

In turn, even among Europeans there are pathway and genetic differences between patients with HCC related to hepatitis and HCC patients related to metabolic syndrome.

Being a major player in the inflammation in carcinogenesis of this tumor, the expression of hepatitis virus-related proteins very likely reflects the differences between the various types of HCC.

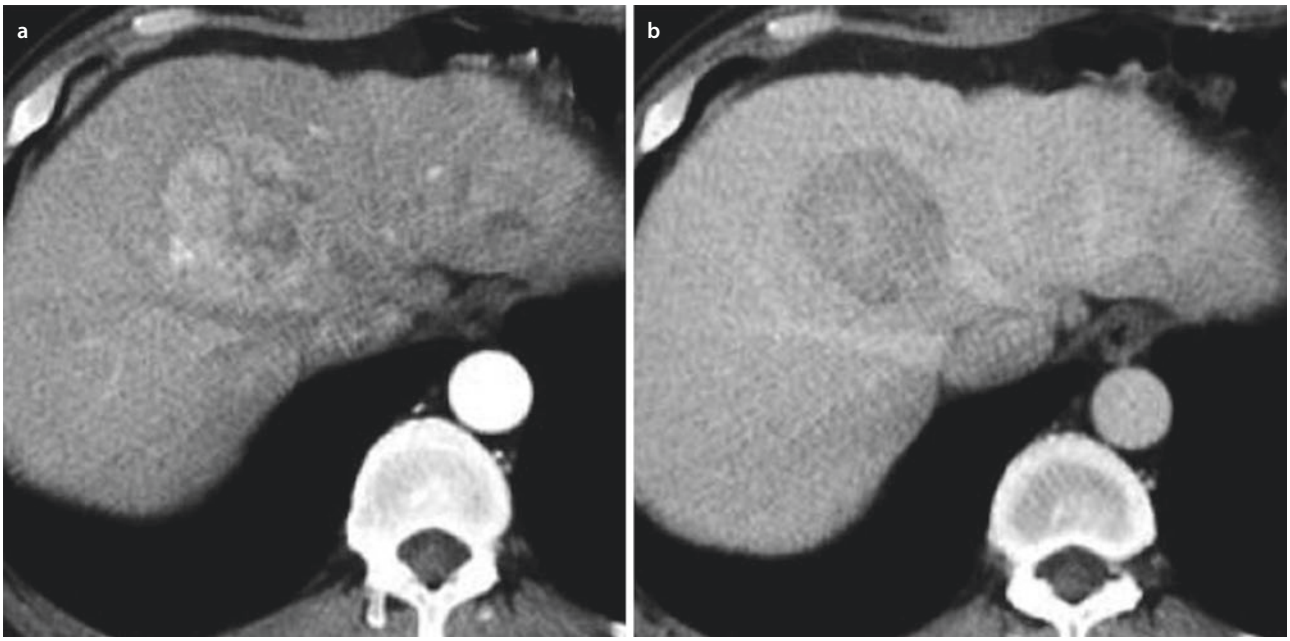


Fig. 42.1 HCC CT-scan. **a** Arterial phase sequence with wash in and **b** washout

42.4 Diagnosis

42.4.1 Radiological Criteria

The presence of small nodules in a cirrhotic liver is normal, making the differential diagnosis between regeneration nodules and neoplastic nodules often difficult. A "focal lesion," i.e., a lesion measuring at least 5 mm detected by ultrasound or another method is first identified [19]. Hepatic carcinogenesis occurs in stages in 90% of cases: the lesion progresses from regenerative micronodule to regenerative macronodule, with histological changes that lead from mild to severe dysplasia to carcinoma, extending to the entire nodule and beyond.

From a histological point of view, the transformations that occur during carcinogenesis are generally accompanied by a progressive formation of anomalous arterial vessels (tumor neoangiogenesis) and loss of the portal component [20]. The imbalance between the components of the vascular support gives HCC a unique behavior in the different contrast phases that enables imaging techniques to identify the tumor, i.e., an increase in the arterial phase signal in the lesion compared to the surrounding parenchyma (commonly called arterial hypervascularization or wash-in), followed by a reduction in the venous phase that makes the lesion appear moderately less contrast-enhanced than the parenchyma (appearance defined as premature washing or washout). In the presence of wash-in followed by washout, a 10-mm lesion in a cirrhotic liver can be fairly confidently diagnosed as HCC.

Suspicious nodules should be evaluated with contrast-enhanced MRI and/or CT scan to identify a diagnostic pattern typical of HCC (hypervascularization in the arterial phase and washout in the venous/late phase) and to carry out staging in order to define prognosis and the most suitable therapy if malignancy is confirmed (Fig. 42.1). The role of contrast-enhanced ultrasound (CEUS) in the diagnosis of HCC has been questioned due to its poor ability to differentiate intrahepatic cholangiocarcinoma from HCC [21].

In the case of a typical MRI and/or CT (with wash-in and washout) appearance of lesions exceeding 10 mm, a diagnosis of HCC can be considered confirmed. Conversely, for lesions with an atypical appearance (lack of arterial hypervascularization and/or washout), further evaluation with an alternative contrastographic technique (MRI or CT) or CEUS is performed, or it may be decided to proceed directly to biopsy, if technically feasible [21] (Fig. 42.2).

42.4.2 Role of Alpha-Fetoprotein

Alpha-fetoprotein is the most commonly used serum marker for HCC. Alpha-fetoprotein is no longer recommended as a diagnostic test because of the low sensitivity of its threshold value (about 20%), especially in small nodules, and also because of its lack of specificity when lower limits are used, e.g., >20 ng/dL). Thus, diagnosis of HCC is based on the results from typical imaging of malignancy in a cirrhotic liver or histological confirmation. High values of alpha-fetoprotein have a clear negative prognostic significance [21].

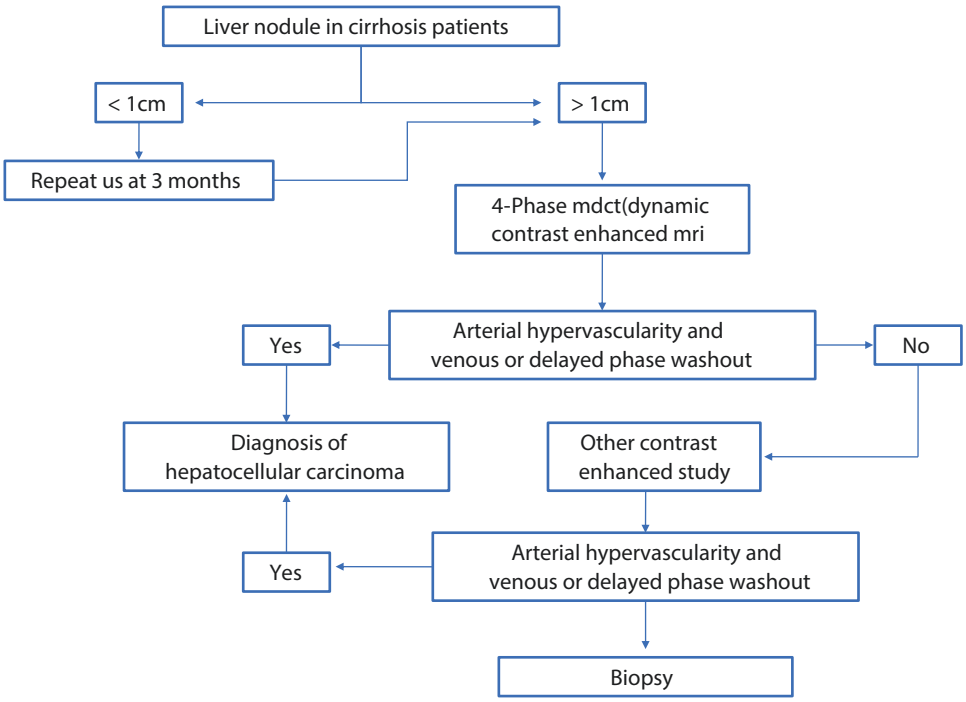


Fig. 42.2 Diagnostic flow chart

42.4.3 Histological Criteria and Classification

42.4.3.1 Liver Biopsy

Even if instrumental investigations could be able to achieve a diagnosis, sometimes HCC should be investigated by the histological examination of the lesion through ultrasound- or CT-guided percutaneous biopsy usually when radiological examinations lead to diagnostic doubts.

42.4.3.2 Pathology

Macroscopic Features

Macroscopic characteristics of HCC are related to both the size of the tumor and the presence or absence of liver cirrhosis. In fact, HCCs associated with liver cirrhosis show fibrous capsule and intratumoral septa, while the ones without cirrhosis tend to be massive and nonencapsulated (Fig. 42.3). HCC could occasionally present itself as a pedunculated lesion. Surrounding intrahepatic metastases are frequent in advanced phases.

Due to its significant angiogenesis features (Longo et al.), macrovascular invasion of portal vein could be present in more than 70% of advanced HCC. Furthermore, intrahepatic metastases are caused mostly by tumor spread in the portal vein branches. Less frequently, tumor invades the major bile ducts. Extrahepatic metastases are mostly hematogenous (i.e., liver, lung and less frequently bone). Regional lymph node metastases are frequent.

Microscopic Features

Neoplastic cells resemble polygonals with distinct cell membranes and abundant granular eosinophilic cytoplasm with a nucleus/cytoplasm ratio which is higher than normal. Moreover, the nucleus is round with coarse chromatin and a thickened nuclear membrane. The presence of sinusoidal vessels surrounding tumor cells is an important diagnostic feature. Common characteristics are portal vein thrombosis and microvascular invasion with presence of mitotic figures. The presence of abundant fat or bile canaliculi, copper, intracellular hyaline bodies, and intranuclear pseudoinclusions could be less frequent (Fig. 42.4). HCC is immunohistochemically positive for HepPar-1 and AFP, even if these markers may be negative in high-grade tumors. Also glypican-3 may be positive in both cytoplasm and membrane. Unlike the sinusoidal endothelial cells in normal liver tissue, those in HCC are immunohistochemically positive for CD34 and factor-VIII-related antigen.

A variable number of macrophages with similar features of well-differentiated tumors Kupffer cells are present in the sinusoidal blood spaces. They bear an immunohistochemical positivity for CD68 and antilysozyme [22].

Different Histological Patterns

The trabecular (plate-like) pattern is the most common in well- and moderately differentiated HCCs. Neoplastic cells are grouped in cords of variable thickness which are separated by sinusoid-like blood spaces. Sinusoid-like blood spaces often show varying degrees of dilata-

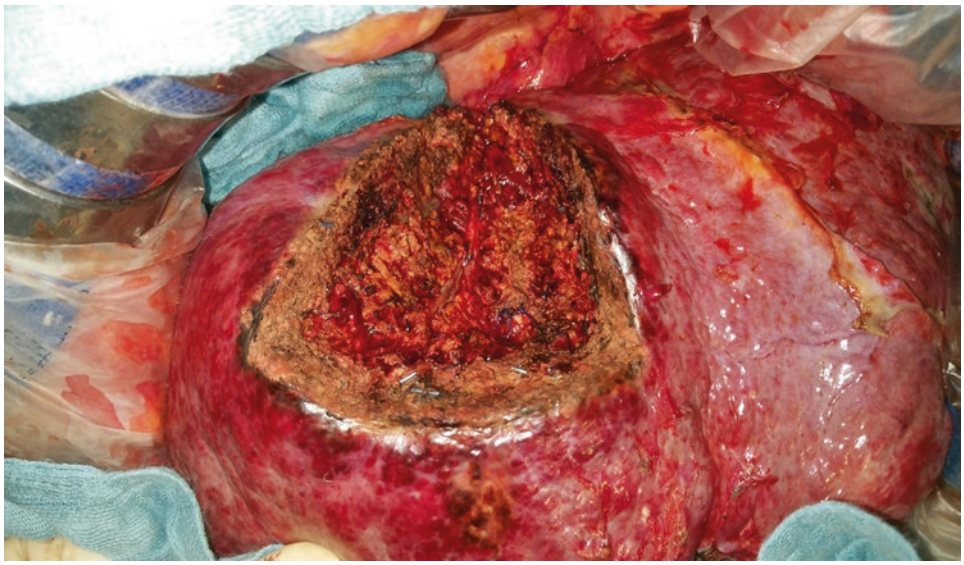


Fig. 42.3 Macroscopic aspect of hepatocellular carcinoma on cirrhotic liver

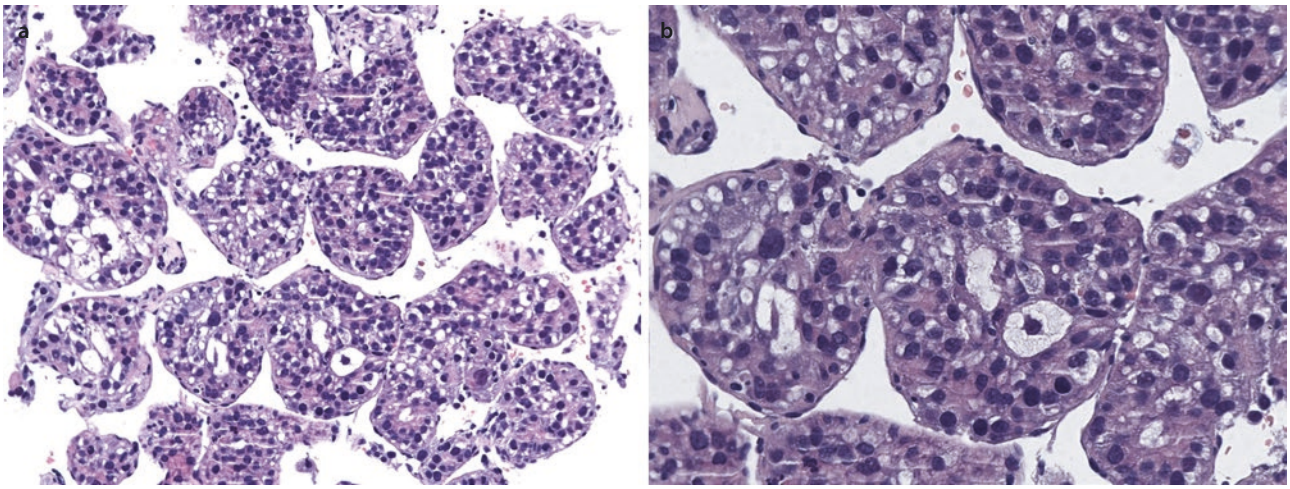


Fig. 42.4 **a** Well-differentiated HCC. Typical roll-off appearance due to the capillaryization of sinusoids. 20× (H/E). **b** Greater magnification (40×). Endothelins continuously delimit the aggregates of atypical hepatocytes (H/E)

tion, and peliosis hepatis-like changes are occasionally observed in advanced HCCs (Fig. 42.5).

Pseudoglandular and acinar variants of HCC frequently show a glandular pattern, usually admixed with the trabecular pattern.

An uncommon HCC subtype is scirrhous. It is characterized by marked fibrosis along the sinusoid-like blood spaces with varying degrees of atrophy of tumor trabeculae. The scirrhous type must not be confused with cholangiocarcinoma or fibrolamellar carcinoma.

The term "sclerosing hepatic carcinoma" has been used to designate a variety of tumors arising in non-cirrhotic livers. This variant is often associated with hypercalcemia, but it doesn't constitute a distinct histopathological entity [23].

Cell Variants

*Pleomorphic HCC*s show marked variation in cellular and nuclear size, shape, and staining. Multinucleated or mononuclear giant cells are often present, appearing as osteoclast-like giant cells. They are frequently observed as common in poorly differentiated tumors. In clear cell HCC, cancer cells present clear cytoplasm due to the presence of abundant glycogen. Those features make the differential diagnosis from metastatic clear cell type renal carcinoma challenging.

Sarcomatoid HCC is a subtype with sarcomatous change which is characterized by the proliferation of spindle cells or bizarre giant cells. It is more frequent in patients who have undergone TACE. Most of them are positive for vimentin or desmin.

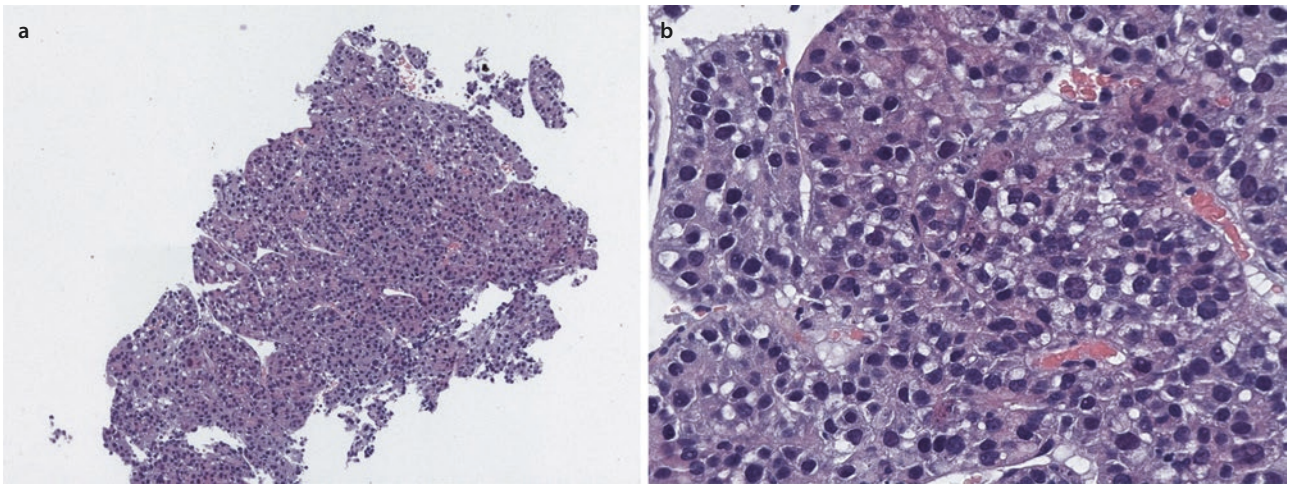


Fig. 42.5 **a** Moderately differentiated trabecular hepatocarcinoma. 20× hematoxylin/eosin. **b** Greater magnification (40×). Evident nuclear dysmetries with hypercromasia (H/E)

Fatty change HCC is most frequent in early-stage tumors with a diameter lower than 2 cm. Its frequency declines as tumor size increases, with rather infrequent fatty changes in advanced tumors. It could be associated with metabolic disorders related to hepatocarcinogenesis and insufficient blood supply in the early neoplastic stages.

Bile production HCC is occasionally observed, usually as plugs in dilated biliar ducts, with a prominent bile production. It is interesting to see that cancer cells turn green after formalin fixation. Mallory hyaline bodies are intracytoplasmic, irregular in shape, eosinophilic, and PAS-negative.

Fibrolamellar HCC is usually observed in noncirrhotic livers with a higher incidence in adolescents or young adults. Cancer cells are grouped in sheets or small trabeculae which are divided by hyalinized collagen bundles with a characteristic lamellar pattern. These cells contain deeply eosinophilic and coarsely granular cytoplasm and distinct nucleoli. Pale bodies are present, and stainable copper, usually in association with bile, can occasionally be shown.

Undifferentiated carcinoma represents about 2% of epithelial liver tumors. Its characteristics resemble those of all the undifferentiated cancers, with poorly differentiated small cells and a high mitotic cell rate. Its prognosis is worst compared to other HCC variants [23].

Grading

According to the histological grade of differentiation, HCC can be divided into well-differentiated, moderately differentiated, and poorly differentiated.

Well-differentiated HCC cells present minimal atypia and increased nuclear/cytoplasmic ratio. They are organized in trabecular patterns: pseudoglandular or acinar structures are frequently observed.

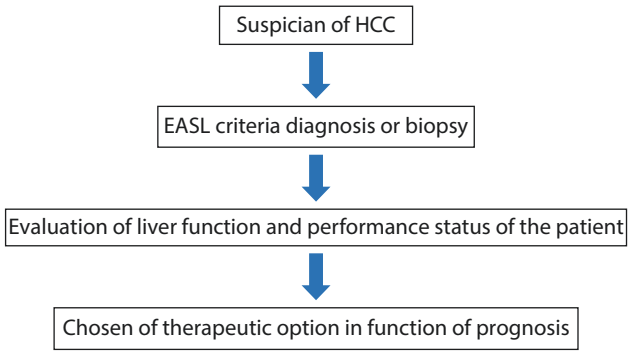
Moderately differentiated HCC is the most common in tumors which are larger than 3 cm in diameter. Cells show abundant eosinophilic cytoplasm and round nuclei. A pseudoglandular pattern is also frequent with bile or proteinaceous fluid. Cancer cells are organized in trabeculae.

In *poorly differentiated HCC*, cancer cells show an increased nuclear/cytoplasmic ratio, frequent pleomorphism, and high proliferation rate. Poorly differentiated HCC is frequent in late stages of the disease [23].

42.5 Staging

One of the most important moments in the onset of an HCC is the possibility to achieve a correct staging of the cancer to choose the best therapeutic option. Currently, the most common staging system for HCC is the Barcelona Clinic Liver Cancer (BCLC) system, which determines cancer stage and patient's prognosis based on tumor burden, severity of the diseases, and patient's performance status [24].

We identify very early and early stage (BCLC 0 and BCLC A) in patients with solitary lesion or up to three nodules ≤3 cm (no macrovascular invasion or extrahepatic disease). In this case patients can benefit from potentially curative treatment (resection, transplant, or ablation). In case of intermediate stage HCC (BCLC B), in asymptomatic patients with multifocal HCC, without vascular invasion or extrahepatic disease, patients could be candidate for transarterial chemoembolization (TACE). In case of multifocal HCC with vascular invasion or extrahepatic disease, systemic treatment with tyrosine kinase inhibitor (sorafenib) currently offers the best therapeutic option. Patients with end-stage liver disease (BCLC D) have a very poor prognosis and require supportive care alone.



Ablation	Preserved liver function, 1 nodule <2cm
Resection	Preserved liver function, 1 nodule
TACE	Internmediate liver fuction, multiple nodules
Transplant	Any liver function, 1 nodule <5 cm or 3 nodules >3cm
Systemic therapy	Preserved liver function, and in rarecase intermediate liver function, advanced and metastatic stages
Bestsupportive care	Terminal stages

Fig. 42.6 Therapeutic algorithm

42.6 Treatment

Considering the multifactorial evaluation of cirrhotic patient with HCC, different therapeutical options are available to treat cancer (Fig. 42.6).

42.6.1 Surgery

In order to achieve a correct diagnosis of HCC in cirrhotic patients, the EASL panel of experts and the American Association for the Study of Liver Disease (AASLD) [1] adopted the definition of HCC radiological hallmark, considering radiological criteria for diagnosis, based on typical contrast uptake of the nodule in arterial phase and washout in the late phase. In case of >1 cm nodule, one radiological technique (CT, MRI, US-contrast) could be sufficient for diagnosis. If the diagnosis is uncertain, a second radiological exam could integrate the result. In case of further doubts, a specimen biopsy is necessary. The AFP value might be useful for diagnosis but in practice it will not affect the treatment strategy.

42.6.1.1 Liver Resection

With a 50% 5-year overall survival (OS), liver resection is considered the only therapy which seems to cure the disease while maintaining liver function. Liver resection remains the most accessible treatment for liver malignancies, because a limited availability of graft limits transplantation in selected cases. There has been some progress recently which has aimed at improving the results of liver resection. Better patient selection and preoperative studies, associated with the improvement of surgical tools and techniques including laparoscopic [25] and robotic surgery [26], have enhanced postoperative outcome. Unfortunately, only 20–30% of patients have resectable disease at diagnosis. The ideal resection candidate is a patient with a single nodule, Child-Pugh A, without satellite nodules or vascular invasion, and the possibility to perform an anatomical resection to reduce the risk of untreated satellite nodules. Bilobar pathology is usually a surgery contraindication, and more conservative strategies are preferred in order to control the pathology.

42.6.1.2 Preoperative Assessment of the Patient Plays a Key Role

The main risks related to liver resection are hepatic insufficiency and failure [27]. This risk is heightened in case of an excessively large amount of hepatic parenchyma liver resection [28]. For that reason, the preoperative risk assessment is a fundamental process before liver resection. In case of liver resection, we should consider two fundamental evaluations: a quantitative evaluation based on the percentage of hepatic parenchyma [29] that could be resected and a qualitative evaluation [30] involving functional reserve of the whole liver. For liver resection in cirrhotic patients, a minimal amount of 40% of liver should be preserved to avoid liver failure. For qualitative measurement, the main test is the evaluation of the indocyanine green at 15 min retention rate. Another feature evaluated before liver resection is portal hypertension [31], which should be absent in order to achieve better postoperative course and Child-Pugh classification, which allows the calculation of a score based on biological tests and clinical evidence to estimate the cirrhosis severity [32]. This classification is used to assess the prognosis of chronic liver disease, mainly in cirrhotic patients. It is based on the analysis of five items and divides patients in three classes in function according to the cumulative score. Analyzed items are total bilirubin, serum albumin, prothrombin time or INR, ascites and hepatic encephalopathy. The combination of these factors could minimize the risk of liver failure.

The ECOG (Eastern Cooperative Oncology Group) [33] (Table 42.1) scale of performance status is a scale which helps to understand how the disease can impact the patient's daily life. It measures the patients' level of functioning in terms of their ability to take care of themselves in terms of daily activity and physical ability. Grade 0 and 1 describe patients who are able to perform the same activity before disease or patients, who, although with restrictions in performing physical activ-

Table 42.1 ECOG performance status

ECOG	ECOG performance status
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but able to move and to carry out tasks of a light or sedentary nature, e.g., light house work, office work
2	Able to move and capable of any personal tasks but unable to carry out any work activities; up and about for more than 50% of waking hours
3	Capable of only limited self-care; confined to bed or chair for more than 50% of waking hours
4	Completely disabled; unable to carry on any self-care; totally confined to bed or chair
5	Dead

ity, could nonetheless perform simple tasks. These categories are the ideal categories of patients who could undergo treatment, with a low risk of posttreatment complications.

Firstly, a CT scan of abdomen and thorax is mandatory to exclude major parenchymal involvement or distal metastases. The role of the CT can facilitate both the definition of a correct diagnosis and the evaluation of the relationship between nodules and both vascular and biliary structures. In case of major resection, it is mandatory to calculate the amount of theoretical future remnant liver (FRL) through a CT 3D reconstruction [34]. FRL corresponds to the quantity of liver which should be preserved after surgery in order to be sufficient to guarantee a normal liver function. In case of insufficient FRL, portal vein embolization [35] (selective occlusion of monolateral portal flow to obtain contralateral hypertrophy of the liver) could be useful for its increase. In case of major resection, at least 40 % of FRL should be preserved in cirrhotic patients.

The most important aspect related to liver resection is the identification of appropriate candidates who could stand liver resection. A correct assessment of the patient's general status and liver function must be performed to reduce the risk of an uneventful postoperative course to a minimum. One of the main concepts in liver resection is the necessity to preserve a quantity of functional liver parenchyma after surgery to avoid postoperative liver failure. This quantity of functional liver is called FRL, and it is calculated before surgery with an appropriate software. According to Couinaud's classification and the division of the anatomy of the liver in eight segments [36], minor liver resection is the definition used when ≤3 segments are resected, or there is a major resection involving >3 segments. According to these classifications, patients that can be considered for minor resection should be Child A with bilirubin levels ≤2 mg/dL and an absence of ascites and with more than 100.000/mm^3 platelets. If major resection indicated, criteria for minor resection should be respected with the addition of bilirubin levels ≤1 mg/dL, the absence of portal hypertension, and portal vein embolization for future remnant liver of <40 %.

Surgical Technique

The aim of liver resection is to offer the best treatment with adequate resection margins [37]. A tumor-free margin of at least 1 cm should be guaranteed, with better results when there are more than 2 cm of margins. This is due to the necessity to remove the zone in which satellite nodule could be present and therefore inducing an early pathology recurrence. For the same reason, anatomical resection is preferred to nonanatomical resection [38] due to intrahepatic diffusion following portal vein pedicle, which could be ideal in patients with inadequate liver function, in order to reduce the liver failure risk.

Liver resection needs an initial intraoperative ultrasound, in order to identify liver lesions and anatomical relation among liver lesion and vascular and biliary structure. Once assessed the resection feasibility and identified a surgical plan, liver resection could be performed using different techniques and devices, to reduce blood loss and perform an easier hepatectomy [39]. In the majority of liver resections, a tape is passed around the round ligament in order to clamp the inflow (Pringle maneuver) of the liver and to control a possible intraoperative bleeding, even if the duration of pedicle clamping is limited in time. More measures could be adopted to achieve a better control of bleeding, including vascular exclusion of the liver with pedicle clamping associated to caval and hepatic vein clamping, along with an important hemodynamic impact.

42.6.1.3 Laparoscopic Liver Surgery

In last 20 years, the improved accuracy and diffusion of laparoscopic liver surgery in combination with the development of new surgical tools have made liver resection easier and increasingly less invasive. Apart from the advantage of minimally invasive access on postoperative pain, laparoscopic liver surgery has been demonstrated to reduce intraoperative bleeding, leading to faster recovery and with the same short- and long-term oncological results [40]. It is possible to associate liver resection and radiofrequency ablation. Recently, robotic surgery has increased the number and reproducibility of liver resection. In terms of percentage with robotic surgery a 5-year disease-free survival is almost 45%, compared with a 25% disease-free survival due to the high rate of recurrence and the presence of vascular invasion or microsatellite nodules, most of the time with the presence of liver cirrhosis.

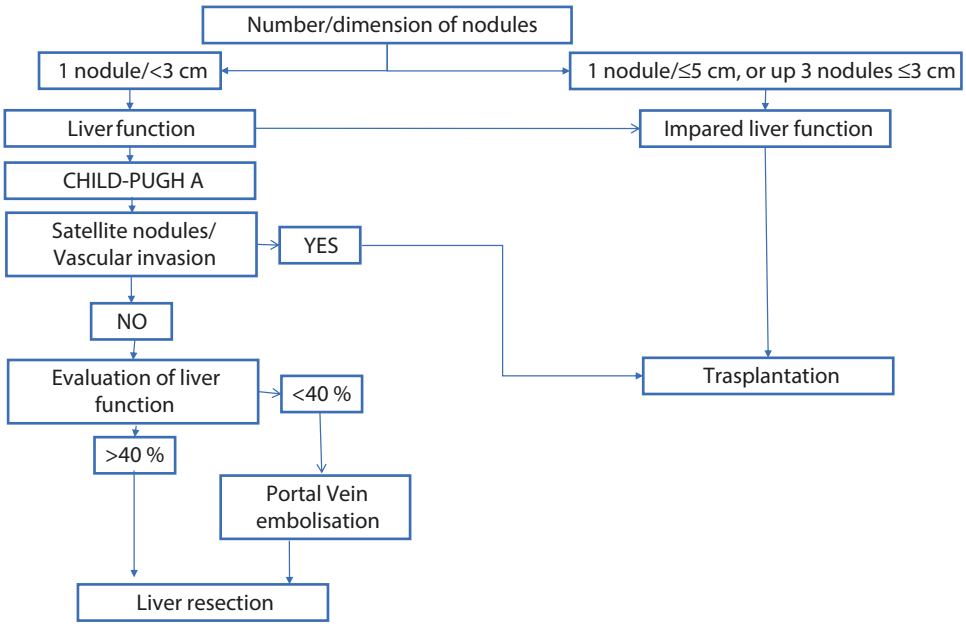


Fig. 42.7 Surgery: flow chart

42.6.1.4 Liver Transplant

Liver transplant offers a better (OS) (70 % at 5 years); it is limited by strict selection criteria and organ shortage. It's indicated especially for HCC patients with impaired liver function.

HCC often onsets on a pathological liver condition. Even if viral hepatitis reduced its frequency after the development of antiviral therapies, other causes including fatty liver disease and alcohol still represent a fertile ground on which HCC can easily develop, compared to a non-pathological liver [41]. Transplant offers the possibility to treat both the cancer and the underlying disease. Unfortunately, not all patients with liver disease and HCC could benefit from liver transplant, due to organ shortage and to limited benefit of treatment for patients with advanced liver disease. For this reason, to optimize transplant benefits, some criteria have been established. The most common criteria are "Milan criteria" [42], which consider the presence of any solitary HCC ≤5 cm, or up to three lesions ≤3 cm each, without vascular invasion or metastasis as the ideal candidate for liver transplant.

In order to treat patients who are beyond transplant criteria, it is possible to treat liver nodules in order to reduce tumor load, for example, with liver resection [43], or locoregional therapies, allowing the patients to fill translatability criteria. This strategy allows the HCC downstaging within Milan criteria in 40 % of patients outside criteria; however, posttransplant HCC recurrence rates are high at 16 % [44].

In order to allow more patients to be transplanted, some strategies have been considered to expand donor pools [45]: partial graft, deriving from living donor, or donor after cardiac death and recently, some tools as perfusion machine are used to improve the quality of grafts and to prolong their viability before being transplanted to recipient patients.

Even if transplant centers are trying to expand the donor pool, one of the main problems of liver transplant remains the dropout [46] of those patients waiting for liver transplant, in whom liver disease progresses.

Nowadays, surgery represents the only change of long-term survival in these patients. Figure 42.7 is a summary of the characteristics of HCC patients able to underwent to surgery (Fig. 42.7).

42.6.2 Locoregional Procedures

42.6.2.1 Ablation

HCC locoregional treatment [47] is gaining increasing treatment interest. Even if surgical resection guarantees the possibility to ablate the tumor and eventually satellite nodules, recent studies demonstrate that locoregional treatment leads to equivalent results. It could also be considered as a palliative treatment for patients who can't undergo other treatments for HCC.

The most common ablation treatments are percutaneous ethanol injection (PEI), radiofrequency ablation (RFA), and microwave ablation (MWA). All these approaches are image-guided procedures, in most cases performed through ultrasound.

42.6.2.2 PEI

This procedure [48] needs to monitor the distribution of alcohol in the nodule to achieve the best results. The particularity of this procedure is the low cost of the material. It is feasible and safe, especially for lesions

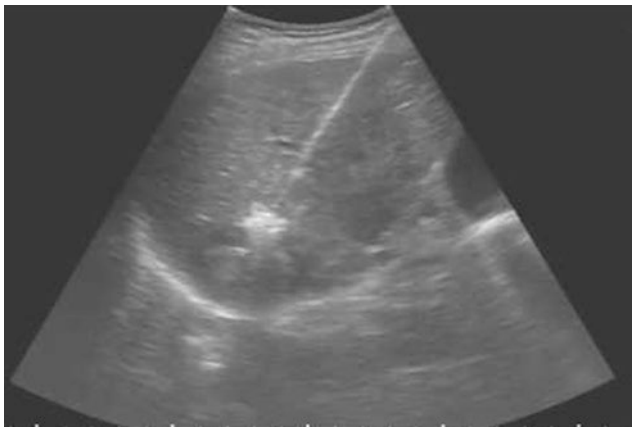


Fig. 42.8 Ultrasound guided ablation of liver lesion

close to the bile duct or to the bowel, due to the non-transmission of energy during the procedure. In fact, alcohol is easily diffused in hyper vascularized HCC. Furthermore, it can be performed in patients with portal thrombosis.

42.6.2.3 RFA and MWA

RF [49, 50, 51] is considered the gold-standard ablation technique. Even if transplantation and liver resection represents the best chance for patients concerning long-term survival, RF represents a valid alternative, and it could be used in association with resection or could be part of a downstaging treatment before liver transplant. Based on constant radiofrequency, energy-generated heat, it transmits the energy to the lesion and to surrounding tissue. It can be performed in sedation or general anesthesia. Under ultrasound control (Fig. 42.8), the needle is placed in the middle of the lesion, to transmit energy uniformly in and around the lesion. In case of more than one lesion, simultaneous treatment could be performed.

In literature, the best results are described for HCC Child A patients with lesions <3 cm, with long-term 5-year OS (50–60 %) comparable to surgical resection and liver transplantation. Small solitary HCC can achieve 5-year OS of 85 %. It is associated with a shorter postoperative stay and lower mortality rate compared to resection [50].

MWA [52, 53] is a recent technique which proposes faster and more extensive ablation areas, allowing the treatment of larger lesions closer to large vessels and biliary structures.

42.6.3 TACE

TACE is a radiological technique which combines inflow occlusion of feeding artery tumor inflow with

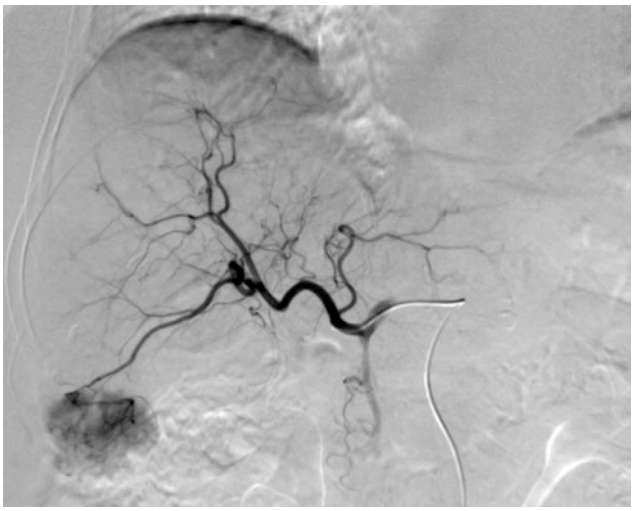


Fig. 42.9 TACE of HCC of right liver

the locoregional therapy directly in the tumor area [35] (Fig. 42.9). This treatment induces the local necrosis of the tumor associated with high intratumor concentration of chemotherapy.

TACE could allow the treatment either of multiple nodules or a selective treatment of a single nodule. Moreover, when during the radiological evaluation of tumor response, the treatment results incomplete, it can be repeated, since it is well tolerated by liver function, due to the low impact on liver function. It is indicated for patients with liver disease associated with impaired liver function.

Herein (Fig. 42.10), it is represented the summary of HCC patients features able to underwent to locoregional approaches.

42.6.4 Systemic Treatments

Even if for the last 10 years, sorafenib was the only therapeutic strategy, nowadays new tyrosine kinase inhibitors [54] and immune checkpoint inhibitors [55] improved the survival of HCC patients.

42.6.4.1 Sorafenib

The efficacy of sorafenib, a small-molecule multitarget kinase inhibitor, in the treatment of advanced HCC has been demonstrated in two randomized phase III trials, the SHARP [56] study and the Asia-Pacific study [57]. Both studies enrolled patients not eligible for locoregional treatment (at diagnosis or after failure of any previous treatment) but with good hepatic function (Child-Pugh A). In both trials, sorafenib treatment (400 mg twice daily up to instrumental and clinical progression or unacceptable toxicity) resulted in a significant prolongation of OS and time to progression (TTP).

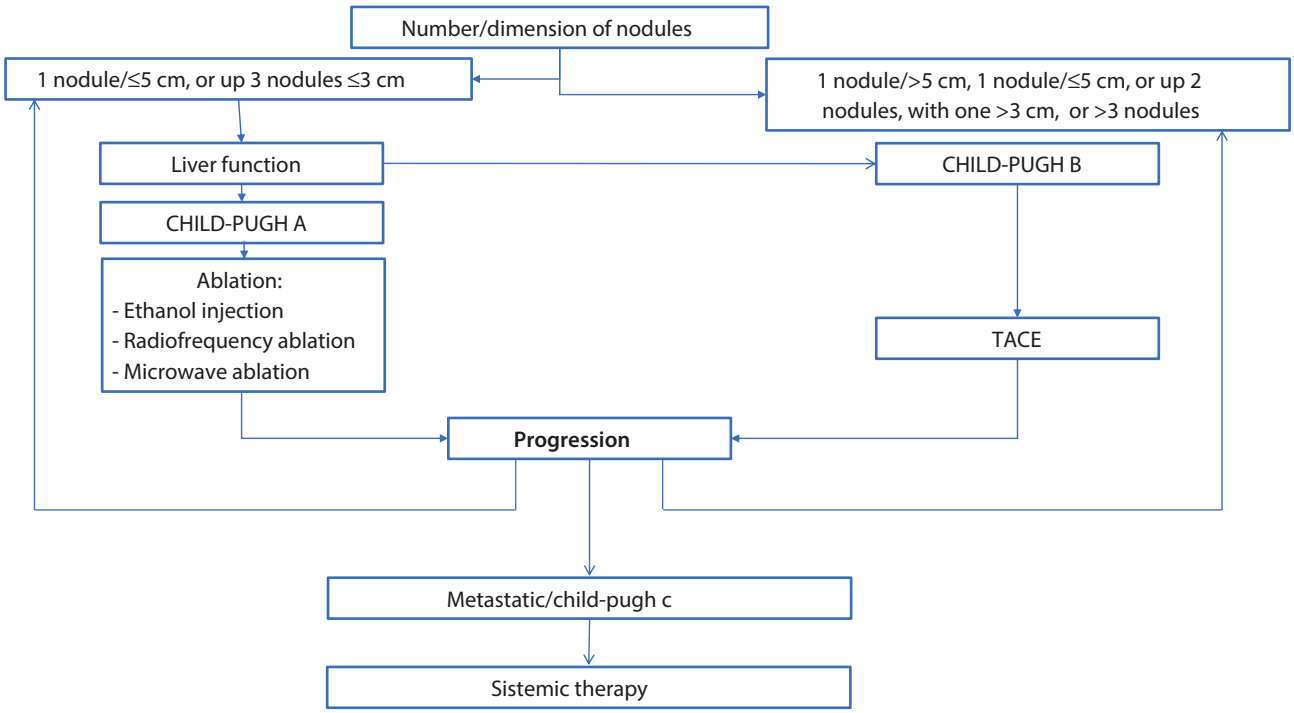


Fig. 42.10 Locoregional procedures: flow chart

In absolute terms, the median survival prolongation was approximately 3 months in the SHARP study and approximately 2 months in the Asian study, but findings are only comparable in relative terms (hazard ratio 0.69 and 0.68, 95 % CI 0.55–0.87, and 0.50–0.93, respectively). On the basis of these results, sorafenib was approved by the EMA for the treatment of HCC in October 2007 (Table 42.2).

The main adverse events of sorafenib are hand-foot skin reaction, hypertension, and diarrhea. Numerous studies have focused on the role of factors and biomarkers predictive and/or prognostic to response to sorafenib, but currently no marker is used in current clinical trials. The most interesting factors studied are the correlation between toxicity and response [58, 59], immune inflammation indicators, and level of lactate dehydrogenase [60, 61, 62].

42.6.4.2 Lenvatinib

Recently, the results from a multicenter randomized non-inferiority phase 3 study comparing lenvatinib and sorafenib were published [63]. Patients with advanced HCC or HCC not recommendable for locoregional treatment and who had never received systemic treatment were recruited and randomized to receive lenvatinib (12 mg/day (body weight ≥ 60 kg) or 8 mg/day (body weight < 60 kg) or sorafenib (400 mg twice daily for 28-day cycles). The primary endpoint was OS,

Table 42.2 Main TKI in use for HCC, lines of indication, survival, side effects

Drug	Lines of indication	Overall survival in phase 3 trial [Months (95 % CI)]	Adverse events
Sorafenib	First	10.7 (9.4–13.3)	Hand-foot skin reaction, hypertension, and diarrhea
Lenvatinib	First	13.6 (12.1–14.9)	Hypertension, fatigue, diarrhea, joint and muscle pain
Regorafenib	Second	10.6 (9.1–12.1)	Breathlessness and looking pale, bruising, bleeding gums or nosebleeds, fatigue, hand-foot skin reaction
Cabozantinib	Second	10.2 (9.1–12.0)	Severe bleeding (hemorrhage), emesis, blood red or black tarry stool

measured from the date of randomization to the date of death from any cause. Median survival time for lenvatinib was 13.6 months (95 % CI 12.1–14.9), therefore not lower than sorafenib (12.3 months, 10.4–13.9; HR 092, 95 % CI 0.79–1.06). Among secondary endpoints (progression-free survival [PFS] and TTP), although lenvatinib was superior to sorafenib, in the study design, the evaluation of the radiological response according to mRECIST was not centralized. Among adverse events of any grade, hypertension occurred more frequently in lenvatinib-arm patients (42 % vs. 30 %), while palmar-plantar erythrodysesthesia syndrome was more frequent in those treated with sorafenib, as expected. In conclusion, lenvatinib did not result inferior to sorafenib in terms of OS in untreated advanced HCC. The safety and tolerability profiles of lenvatinib were consistent with those previously observed (Table 42.2).

42.6.4.3 Atezolizumab Plus Bevacizumab

IMbrave150 trial [64], a randomized double-blind phase III trial, evaluated the efficacy of atezolizumab plus bevacizumab versus sorafenib in first-line chemotherapy. Study meets the co-primary endpoint for OS and PFS. Atezolizumab plus bevacizumab improved OS (hazard ratio [HR] 0.58; 95 % CI 0.42–0.79, $p = 0.0006$) and PFS (hazard ratio [HR] 0.59; 95 % CI 0.47–0.76, $p < 0.0001$) with respect to sorafenib. mOS was not reach in atezolizumab plus bevacizumab arm compared to 13.2 months for sorafenib arm; PFS was 6.8 months in atezolizumab plus bevacizumab arm compared to 4.3 months for sorafenib arm.

42.6.4.4 Regorafenib

In the RESORCE study [65], a randomized double-blind phase III study, Child-Pugh A patients with advanced or intermediate HCC (the latter was not eligible for locoregional treatment) who had tolerated first-line sorafenib at a dose of at least 400 mg/day for at least 20 of the 28 days prior to discontinuation but had progressed during treatment were randomized to receive the best supportive therapy (BSC) in combination with oral regorafenib (160 mg once a day for 21 days of each 4-week cycle) vs. BSC and placebo. The primary endpoint was OS (defined as the time from randomization to death from any cause). Regorafenib improved OS (HR 0.63; 95 % CI 0.50–0.79, $p < 0.0001$). Median OS was 10.6 months (95 % CI 9.1–12.1) for regorafenib compared to 7.8 months (6.3–8.8) for placebo. Adverse events (AEs) were reported in all patients treated with regorafenib. In particular, the AEs with the highest grade (3 or 4) were hypertension (15 % in the regorafenib group vs. 5 % in the placebo group), hemorrhagic fever with renal syndrome (HFRS) (13 % vs.1 %), fatigue (9 % vs. 5 %), and diarrhea (3 % vs. no patient in the placebo group). In all additional efficacy endpoints (PFS, TTP, response rate [RR] and disease control rate [DCR]), regorafenib was statistically superior to placebo (Table 42.2).

42.6.4.5 Cabozantinib

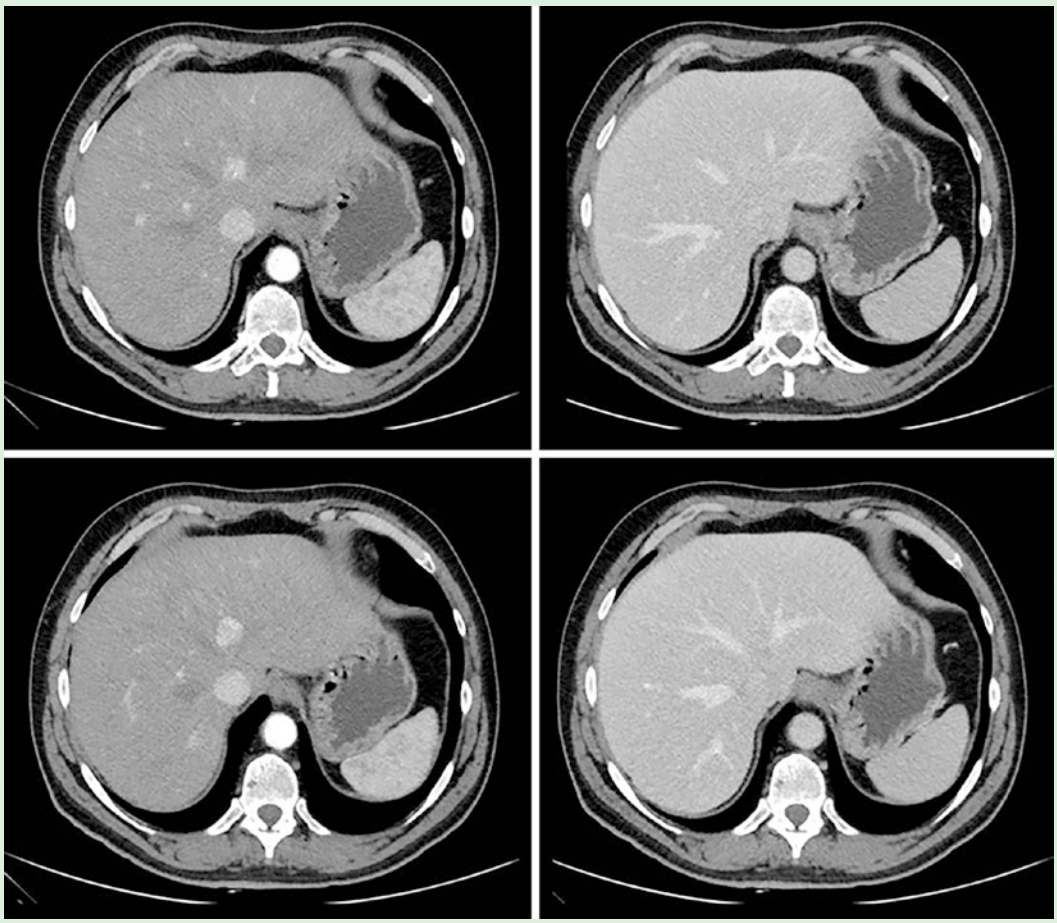
The CELESTIAL study [66], a randomized double-blind phase III trial, evaluated the efficacy of cabozantinib in patients progressing on sorafenib. Cabozantinib improved OS (hazard ratio [HR] 0.76; 95 % CI 0.63–0.92, $p = 0.0049$). mOS was 10.2 months (95 % CI 9.1–12.0) for cabozantinib compared to 8 months (95 % CI 6.8–9.4) for placebo. In addition to being statistically superior to placebo in terms of PFS, TTP, RR, and DCR, cabozantinib was also superior in terms of PFS and ORR (Table 42.2).

42.6.4.6 Ramucirumab

REACH-2 trial [67], a randomized double-blind phase III trial, evaluated the efficacy of ramucirumab versus placebo sorafenib in patients progressing on sorafenib with α-fetoprotein concentrations of 400 ng/mL or higher. Study meets the primary endpoint for OS. Ramucirumab improved OS (hazard ratio [HR] 0.71; 95% CI 0.53–0.95, $p = 0.0199$). mOS was 8.5 months (95% CI 7.0–10.6) for ramucirumab compared to 7.3 months (95% CI 5.4–9.1) for placebo. In addition, to confirm the better results compared to placebo in terms of PFS, no difference was found in terms of DCR.

Case Study

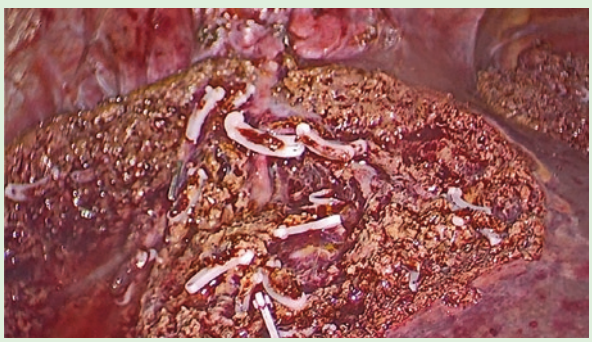
Man: 55 years old
- Family history: negative for malignancies
- APR: treated HCV infection, cirrhosis
- Blood test: normal liver function test, Child A, Meld 8, Afp 200 ng/mL
- TC abdomen and MRI: lesion of 24 × 20 × 22 mm in segment 4, confirmed for HCC



Question

What action should be taken?

1. Surgery
2. RFA
3. Others



Answer

A. Liver resection, if possible laparoscopy

Question

Which is the best follow-up?

1. CT scan every 3 months
2. Nexavar
3. Others

Answer

1. CT scan

Question

Which is the best treatment in case of recurrence?

1. Liver resection
2. Liver transplant
3. Others

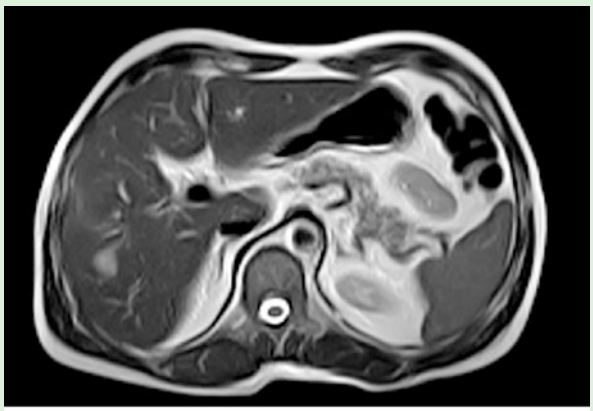
Answer

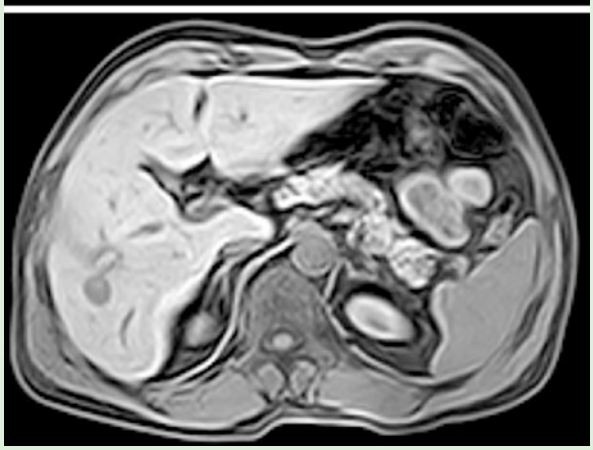
2. In case of recurrence, treatment of choice should be liver transplant, which guarantees best overall and disease-free survival.

Case Study

Man: 75 years old

- Family history: negative for malignancies
- APR: treated HCV infection, cirrhosis, PS 2
- Blood test: normal liver function test, Child A, Meld 8, Afp 500 ng/mL
- TC abdomen and MRI: lesion of 15 × 10 × 12 mm in segment 6, confirmed for HCC





Question

Which is the best treatment of choice?

1. Resection
2. RFA
3. Others

Answer

1. RFA in consideration of performance status of patient and small size of the lesion. Results are comparable to liver resection, with better postoperative outcome in such a fragile patient.

Question

Which is the best treatment in case of recurrence?

1. Liver resection
2. RFA
3. Others

Answer

2. In case of recurrence, treatment of choice should be radiofrequency ablation or TACE in case of multinodular lesions

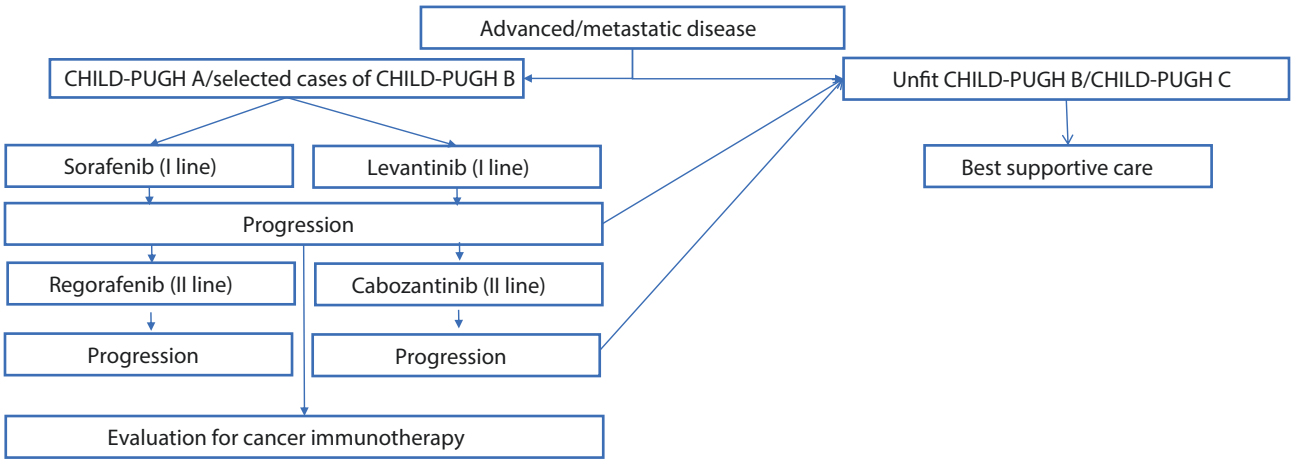


Fig. 42.11 Systemic therapy: flow chart

42.7 Future Perspectives

Even if new molecular approaches have been experimented, only slightly significant improvements have been achieved in survival. Therefore, clinicians need to both identify new therapeutic approaches and select patients suitable for these treatments.

Moreover, it must be pointed out that cancer immunotherapy is the new open option for solid treatments. Different clinical trials evaluating the role of immunotherapy in treating HCC have been conducted. Initial promising results have been obtained among cytokine-induced killer cells and immune checkpoint inhibitors in the adjuvant setting and advanced stages, respectively. Anyway, there are several ongoing trials, the results of which appear intriguing. Conclusively, since the liver immune system the plays an important role in immune tolerance, the possibility of unmasking these mechanisms can be a winning weapon in HCC, so immunotherapy [68] will represent the future therapy in this cancer (Fig. 42.11).

42.8 Highlights

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer in adults.

It occurs in the setting of chronic liver inflammation, mostly linked to chronic viral hepatitis B or C.

Hepatic carcinogenesis occurs in stages in 90% of cases: the lesion progresses from regenerative micronodule to regenerative macronodule.

Suspicious nodules should be evaluated with contrast-enhanced MRI and/or CT scan to identify a diagnostic pattern typical of HCC

One of the most important moments in the onset of an HCC is the possibility to achieve a correct staging of the cancer to choose the best therapeutic option. Currently, the most common staging system for HCC is the Barcelona Clinic Liver Cancer (BCLC) system, which determines cancer stage and patient's prognosis based on tumor burden, severity of the diseases, and patient's performance status

Very early and early stage (BCLC 0 and BCLC A) in patients with solitary lesion or up to three nodules ≤3 cm (no macrovascular invasion or extrahepatic disease). In this case patients can benefit from potentially curative treatment (resection, transplant, or ablation).

In case of intermediate stage HCC (BCLC B), in asymptomatic patients with multifocal HCC, without vascular invasion or extrahepatic disease, patients could be candidate for transarterial chemoembolization (TACE).

In case of multifocal HCC with vascular invasion or extrahepatic disease, systemic treatment with tyrosine kinase inhibitor (sorafenib/lenvatinib) or in the next future with atezolizumab plus bevacizumab it could be suggested.

Different clinical trials evaluating the role of immunotherapy, antiangiogenic, and TKI or their combinations in treating HCC have been conducted.

Expert Opinion

Vito Di Marco

1. Hepatocellular carcinoma is one of the leading causes of cancer on cirrhotic patients.
2. Many different approaches are available, depending on tumor diffusion and status of the patient.
3. To date, sorafenib and regorafenib are the approved therapies in advanced HCC. Levantinib and cabozantinib could represent other therapies that have shown efficacy in advanced HCC. Even if new molecular approaches have been experimented, only slightly significant improvements have been achieved in survival. Therefore, clinicians need to both identify new therapeutic approaches and select patients suitable for these treatments.
4. Moreover, it must be pointed out that cancer immunotherapy is the new open option for solid treatments. Different clinical trials evaluating the role of immunotherapy in treating HCC have been conducted. Initial promising results have been obtained among cytokine-induced killer cells and immune checkpoint inhibitors in the adjuvant setting and advanced stages, respectively. However, there are several ongoing trials, the results of which appear intriguing. Conclusively, since the liver immune system plays an important role in immune tolerance, the possibility of unmasking these mechanisms can be a winning weapon in HCC, so immunotherapy will represent the future therapy in this cancer.

Recommendations

- ESMO
  ► https://www.esmo.org/Guidelines/Gastrointestinal-Cancers/Hepatocellular-Carcinoma

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Head and Neck Cancers

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Learning Objectives

By the end of this chapter, the reader will:

- Be able to correctly apply diagnostic and staging procedures
- Have learned the basic concepts of treatments and supportive care
- Have reached the basic knowledge for the management of HNC patients
- Be able to apply the knowledge in clinical practice

43.1 Introduction

Head and neck cancers (HNCs) represent a heterogeneous group of tumors arising from the epithelial tissue of the upper aerodigestive track. Oral cavity, oropharynx, larynx, and hypopharynx carcinoma are classically included in the defintion of HNCs. Although these cancers share some risk factors (e.g., smoking and alcohol exposure, human papilloma virus infection, premalignant lesions) and histotype (squamocellular carcinoma in at least 90% of the cases), the disease management (e.g., surgery, external beam radiation, and systemic therapy alone or combined) and natural history differ quite significantly in the curative setting. On the other hand, squamocellular carcinomas of the head and neck area are generally considered altogether in the recurrent/metastatic setting.

Epithelial malignant tumors originating from other sites, such as nasopharynx, paranasal sinuses, and salivary glands (majors and minors) have different risk factors (e.g., Epstein-Barr virus infection; exposure to leather and wood dust), histologies (e.g., in the last WHO classification, more than 20 different histotypes have been described for salivary gland carcinomas), and treatment approaches (e.g., surgery; external beam radiotherapy with photons or particles). This chapter focuses only on classical squamous cell carcinoma (SCC) of the HN (SCCHN).

43.2 Epidemiology and Risk Factors

43.2.1 Epidemiology

SCCHNs are rare malignancies, according to RARECARE definition (incidence <6/100,000 year) [1]. Worldwide, the incidence rates of SCCHNs exhibit a wide geographical heterogeneity, reflecting variability in the prevalence of risk factors [2]. In the United States, it is estimated that about 64,690 new cases of SCCHN will occur in 2018 (33,950 oral cavity, 17,590 pharyngeal, and 13,150 laryngeal), which will account for about 3.7% of new cancer cases [3]. In Europe, new cases are expected to affect approximately 151,000 patients in 2020 [2].

Over the last decades, in economically developed countries, a trend toward increased incidence of human papillomavirus (HPV)-related oropharyngeal cancers has been detected, especially and among men. Conversely, the incidence of HPV negative cancers has been decreasing [4, 5].

European data showed improvement in survival for most HNCs, with the highest 5-year relative survival detected for larynx (59%) and the poorest for hypopharynx (25%). Results for other subsites were intermediate (oropharynx, 39%; tongue, 43%; oral cavity, 45%) [6].

43.2.2 Risk Factors

Tobacco consumption and alcohol intake are the main recognized risk factors for SCCHNs. Smoke from tobacco combustion contains several harmful chemicals able to cause DNA damage leading to mutations. Alcohol has an intrinsic transforming action via its metabolite, acetaldehyde, and heavy consumption of alcohol is recognized as an independent risk factor for SCCHN, especially for hypopharynx. Furthermore, alcohol has the ability to magnify the effect of tobacco smoke in a synergistic manner: the risk of cancer development among heavy smokers and drinkers is much higher than expected based on the additive effects of the individual risks [7]. The ability of alcohol to potentiate the effects of smoking more likely resides in its nature as a chemical solvent, enhancing and prolonging mucosal exposure to the carcinogens present in tobacco smoke. The entire aerodigestive track epithelium and other organs are continuously exposed to these carcinogens, thus their transforming effects act synergically on the whole mucosal complex. Therefore, patients diagnosed and treated for SCCHN are at risk of developing second primary tumors, both in the same region and elsewhere (i.e., lung, bladder, esophagus). The estimated risk is 12%, but it is thought to be lower for HPV-related disease [8].

These agents act altering the normal activity of immune system, inducing a state of immunosuppression through different mechanisms. The host defense impairment as well as the inflammation environment caused by smoking and alcohol consumption increase the risk of cancer development.

High-risk HPV oral infection is an established risk factor for oropharyngeal SCC, with no or limited effect on other subsites of the region [9]. More than 200 HPV genotypes have been identified and categorized by their risk of inducing malignancies; among these 12 HPV

types are considered oncogenic by the International Agency for research on cancer (IARC). HPV16 is the most frequent "high-risk" virus involved in head and neck carcinogenesis, followed by HPV18. Whereas other genotypes are much less frequent (HPV 33, HPV 35, HPV 58) [10].

HPV-positive patients tend to be younger compared to HPV-negative patients; they also have less exposure to tobacco and alcohol. HPV infections are mainly transmitted by oral sex, although other factors have been implicated, such as marijuana consumption, dietary factors, and genetic polymorphisms. It should be noted that, even if HPV infection represents a causal factor for tumor occurrence, it is also a positive prognostic factor [11]. In fact, HPV-associated oropharyngeal carcinomas (OPCs) are more responding to both chemotherapy (CT) and radiotherapy (RT), therefore higher survival rates are recorded after curative treatments of patients with HPV-associated OPC compared to those with a HPV-negative OPC. A longer survival is also observed in HPV-related OPCs in the recurrent/metastatic setting, which again illustrates a different natural history of this disease entity. Even though a different metastatic pattern has been suggested, this data is controversial and it might reflect the longer survival time observed in this subset of patients [12].

Other factors may also contribute to the development of HNC in selected patients, such as poor oral hygiene, oral cavity infections, as well as betel nut chewing, a widespread habit in certain regions of Asia [13]. Some dietary measures may have a role in protecting individuals from HNC, such as using a diet high in fruit and vegetable and low in red meat intake [14].

43.3 Clinical Features

Specific signs and symptoms, related to the anatomy, the local lymphatic system and the innervations of primary involved sites, characterized SCCHN. Dysphonia, pharyngodynia, dysphagia, lump in the neck, etc., are frequently reported. Although unspecific, the persistence of these symptoms for a long period (>3 weeks), requires a prompt clinical evaluation with an otolaryngologist or ear-nose-throat (ENT) surgeon. Common signs and symptoms usually present at diagnosis of SCCHNC are listed in Table 43.1.

Clinical history (e.g., smoking history; alcohol exposure; HPV infection; comorbidities) and physical examination play an essential role in both treatment planning and follow-up. The primary purpose is to define the locoregional tumor extension (T and N categories according to tumor staging [15]), to exclude second primaries, evaluate airway patency or quantify alterations related to previous treatments. Inspection of the entire upper aerodigestive tract (UADT) and bilateral neck is mandatory. Digital palpation may add further information, for example, in the evaluation of the deep extension of lesions involving the oral cavity and/or oropharynx or in assessing the nature of neck nodes. Palpable nodes with increased consistency, poorly defined margins, reduced mobility on superficial or deep planes should be always considered as suspicious. The clinical evidence of a "fixed lymph node" should be remarked and taken into account in the treatment plan. All suspicious neck lymph nodes have to be evaluated by

Table 43.1 Signs and symptoms at presentation and presumptive correlated with primary site

Signs and symptoms	Primary tumor subsite
Lingual pain, persistent ulceration, leukoplakia erythroplakia; bleeding lesions	Oral cavity; oropharynx
Odynophagia	Oral cavity, oropharynx, hypopharynx
Pharyngodynia	Oropharynx, hypopharynx, larynx
Dysphonia	Larynx, hypopharynx
Swollen neck lymph nodes	Each subsite, depending on the nodes levels (see Fig. 43.1)

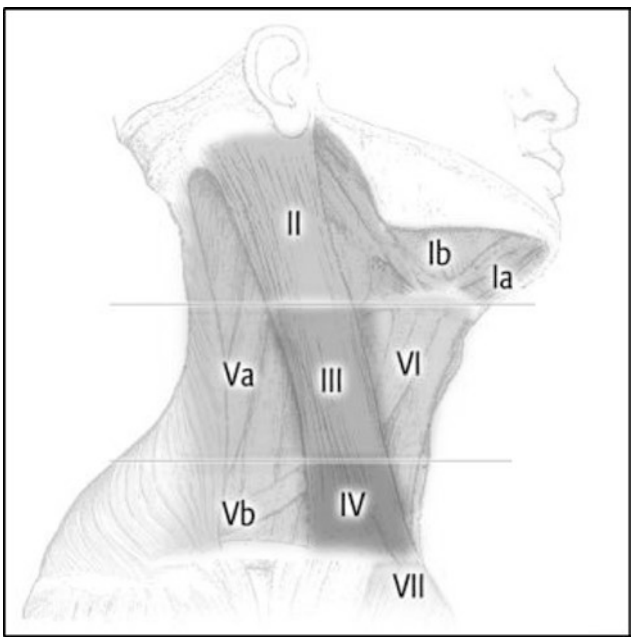

Fig. 43.1 Neck nodal structures divided into levels according to Robbins

an imaging technique, such as ultrasonography, contrast-enhanced computer tomography scan (CECT) or magnetic resonance imaging (MRI).

43.3.1 Clinical Issues According to the Anatomic Subsite

Evaluation of SCCHNs is still primarily based on simple mucosal inspection [16], even though several adjunctive visual aids have been introduced in the past decades to provide deeper insight into the specific biological behavior of such lesions [17]. As a consequence, the concept of "optical biopsy" has been gradually developed to designate a non-invasive, real-time diagnostic approach aimed at a more accurate early diagnosis of (pre)malignant lesions, and to avoid unnecessary biopsies or incomplete surgical removal. Narrow Band Imaging (NBI) has been already proven to be a useful diagnostic tool in identifying early-stage mucosal SCCHNs [18]. It applies optical filters to enhance visualization of the mucosal and submucosal microvascular pattern. These filters enhance blue and green light (wavelengths of 415 and 540 nm, respectively), corresponding to the peaks of hemoglobin absorption, thus penetrating superficial mucosal layers and highlighting the underlying capillary network without scattering in the deeper layers. Therefore, it is thus possible to identify specific neoangiogenic patterns suggestive of premalignant and neoplastic transformation (see also Fig. 43.2). Apart from NBI, a number of other biologic endoscopy tools have been widely described and adopted in the head and neck clinical examination (e.g., supravital stainings by toluidine blue, autofluorescence, confocal microendoscopy, optical coherence tomography, and others). However, clinical examination of each head and neck region needs to consider its specific characteristics and is therefore presented separately.

43.3.2 Oral Cavity

The oral cavity is the entrance of the digestive tube. It spans between the oral fissure (anteriorly), and the oropharyngeal isthmus (posteriorly) and includes the following subsites: buccal mucosa, upper and lower alveolar ridge, anterior two-thirds of the tongue, retromolar trigone, floor of the mouth, and hard palate [19].

Inspection of the oral cavity can be performed by direct visualization or employing video-endoscopes that allow image magnification and application of filters such as NBI.

Superficial premalignant/malignant lesions of oral cavity may appear as leukoplakia (white patch), erythroplakia (red patch), or erythroleukoplakia (mixed or speckled, white, and red patch). These are merely descriptive terms, just referring to the whitish or erythematous appearance of the lesion at the mucosal level that may be the presentation of a number of different benign and malignant diseases. In fact, oral leukopla-

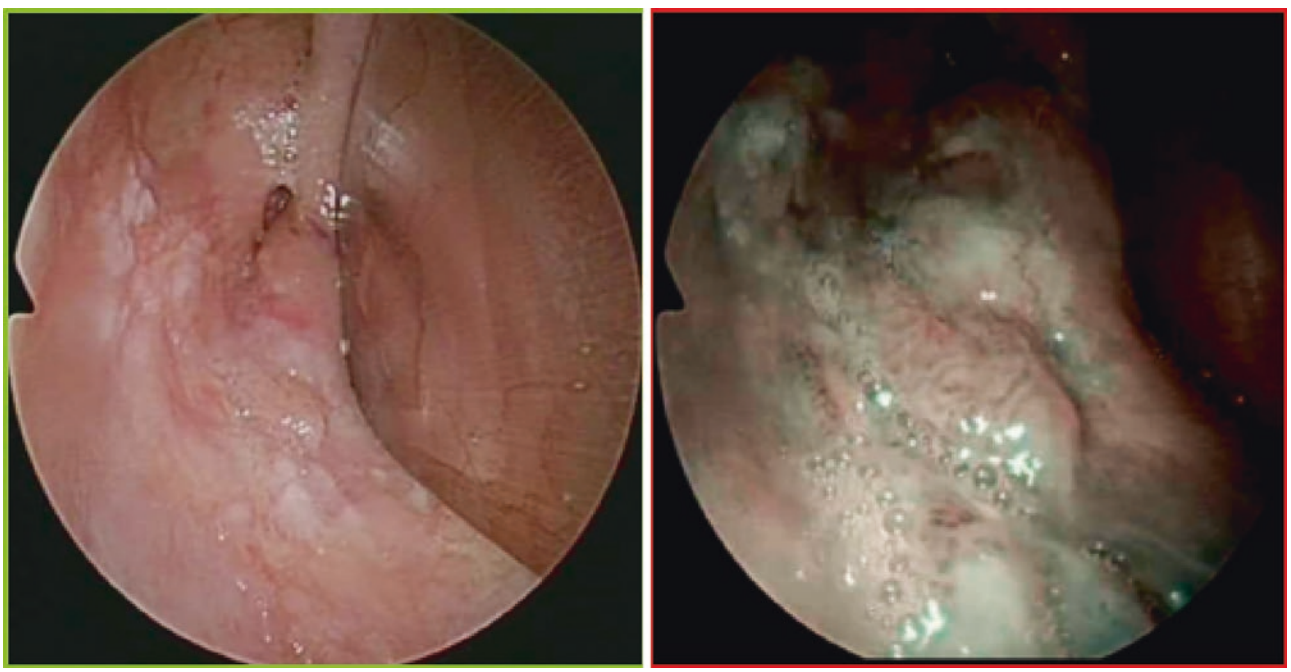

Fig. 43.2 Buccal mucosa aspect upon inspection with normal light (left) and NBI (right), highlighting the underlying capillary network with neoangiogenic patterns, indicating a neoplastic lesion

kias may present a malignant transformation rate from 0.13% to 17.5%, while erythroplakias, although rarer, may harbor severe dysplasia or invasive carcinoma. According to different experiences, the transformation rate of oral dysplasia is approximately 8% [20], while for erythroplakia it might range from 14% to 60% [21–23]. The indication for a biopsy is dependent on the characteristics of the lesion and the patient's clinical history (previous SCCHNs or inflammatory diseases of the UADT). Irregular margins, prominent vascularity, increased consistency, and signs of deep infiltration are typical factors that increase the risk of malignancy, requiring a biopsy or a closer follow-up.

All subsites should be carefully examined, taking care of those where direct visualization is naturally impaired, such as the oral vestibule, floor of the mouth, and alveolar ridge. The presence and state of dentition should be always evaluated given its influence on the risk of mandibular invasion. In tumors reaching the alveolar crest, the likelihood of bone infiltration is significantly increased in edentulous patients. In fact, in the presence of teeth, tumors invade the mandible by extending through the dental sockets and advance into the cancellous part of the bone after overcoming the natural barrier represented by the dental ligaments. Conversely, in the edentulous subjects, tumors extend up to the alveolar process and infiltrate the dental pores to extend to the cancellous part of the mandible. Moreover, in this situation, the mandible and maxilla are usually much more limited in their vertical height and, therefore, the distance between the bony cancellous portion and the mucosal surface of the gum is greatly reduced. Bone infiltration has to be assessed by bimanual palpation of the lesion that appears to be fixed to the alveolus. However, local evaluation by imaging techniques is always needed.

In tongue tumors, critical points are represented by the depth of infiltration and the extension beyond the median line. It is difficult to obtain a fine evaluation of the deep extension by palpation alone. However, it is often possible to assess if the tumor reaches the median septum of the tongue or if it deeply infiltrates the floor of the mouth. These are fundamental anatomic boundaries that help in giving a better definition of tumor extension inside the hemi-tongue (and hemi-floor of the mouth) compartment. This is a key concept that results in marked differences in the surgical approach.

43.3.3 Oropharynx

The oropharynx extends from the plane of the hard palate superiorly to the plane of the hyoid bone inferiorly. It communicates with the nasopharynx above, the hypopharynx inferiorly, and the oral cavity anteriorly. Oropharyngeal subsites are the base of the tongue, the tonsils and the tonsillar pillars, the soft palate, and the posterior pharyngeal wall [19].

Most of the oropharynx can be visualized transorally, while the base of tongue usually requires a transnasal flexible endoscope or a transoral rigid 70°/90° endoscope to be examined. While the concepts of superficial examination are similar to those described for the oral cavity, palpation and examination of the deep extent of the tumor are focused on different critical sites. In particular, in tonsillar tumors, trismus (inability to completely open the mouth) and pain during mastication are suggestive of infiltration of the medial pterygoid muscle. This can be verified by palpation of the tonsillar lesion and the surrounding structures. When the tumor appears to be fixed to the deep plane, an infiltration of the medial pterygoid muscle or the mandible is more likely.

In tumors of the base of tongue, a critical sign suggesting deep infiltration is hypoglossal nerve palsy. At rest, if the nerve is injured, a tongue may have the appearance of a "bag of worms" (fasciculations) or wasting (atrophy). The nerve is then tested by extruding the tongue that will deviate toward the palsy side, in case of nerve palsy.

Finally, a superficial or deep extension to nearby regions should also be considered, since it is a significant factor influencing the treatment choice.

43.3.4 Larynx

The larynx (voice box) is a component of the respiratory tract located in the anterior neck. It is a complex organ whose primary function is to protect the lower airway from the entry of foreign matter, but it has also other important functions, such as phonation and swallowing. The larynx is divided into three regions: supraglottis, glottis, and sublottis [19].

Clinical examination of the larynx is mainly dependent on endoscopic instruments (fibro- or videolaryngoscopes) that allow to completely visualize all laryngeal subsites (i.e., supraglottic, glottic, and subglottic regions). The vocal folds are the primary site of origin of laryngeal cancer, followed by the supraglottis. Tumors rarely originate from the subglottis that is generally involved in case of secondary extension of glottic tumors.

In glottic tumors, the superficial extension over the glottic plane (up to the anterior commissure and contralateral vocal fold, T1b) to different subsites (supraglottis or subglottis, T2) and the motility of vocal folds are critical factors to be ascertained before making any ther-

apeutic decision. Furthermore, high-definition videoendoscopes may help in better defining the tumor's superficial spread, also thanks to adjunctive techniques, such as NBI. Concerning the tumor's depth of invasion, the clinical examination is capable of giving precise information, especially in early and intermediate tumors. This is especially important when a transoral laser cordectomy is planned. In fact, in such a conservative approach, type of cordectomy is dependent on the deep tumor extension (i.e., mucosa, vocal ligament, vocal muscle, paraglottic space, cartilage). A first step is to evaluate the mucosal wave by laryngostroboscopy. This exam allows visualizing the vocal fold's mucosal wave during phonation. In case of invasion through the epithelium into the vocal ligament, the mucosal wave progression is altered. It is also possible to confirm this evidence by an intraoperative evaluation thanks to the saline injection into the Reinke space: when a complete "ballooning" of the mucosa is not possible due to tumor adhesions to the vocal ligament, this should be considered an indirect sign of infiltration. Finally, vocal muscle and/or cricoarytenoid joint involvement should be assessed considering the vocal fold and arytenoid motility. In case of deep muscle infiltration, the vocal cord motility is impaired, while the arytenoid motility is normal ("impaired vocal cord motility", T2). Conversely, in case of arytenoid and cricoarytenoid joint infiltration, both vocal cord and arytenoid motility are impaired ("fixed vocal fold", T3). Concerning supraglottic carcinomas, early-stage lesions are significantly less frequent in view of their non-specific symptom profile. The deep extension is mainly represented by upper paraglottic and pre-epiglottic spaces involvement. Spread to neck lymph nodes is more common than in other larynx subsites. However, the primary role of clinical examination is visualizing the superficial tumor spread, while imaging techniques give a better view of deep laryngeal compartments.

43.3.5 Hypopharynx

Hypopharynx is the anatomical region that connects the oropharynx superiorly with both the larynx and esophagus inferiorly. It extends from the superior border of the hyoid bone to the lower border of the cricoid cartilage and comprises posterolateral pharyngeal wall, pyriform sinuses, and pharyngo-oesophageal junction (postcricoid area) [19].

As in supraglottic tumors, early diagnosis is infrequent in hypopharyngeal cancers due to a lack of notable symptoms in the initial phases of disease. Transnasal laryngoscopy is the main clinical examination for such tumors and should be focused on determining their superficial extension and quantifying laryngeal involvement. The Valsalva maneuver and phonation may help in distending the piriform sinus mucosa, further improving visualization. When considering the inferior extension, the Betz fold (created by the superior border of the cricopharyngeus muscle), located at the apex of the piriform sinus, delineates the junction with the esophageal inlet. Tumor spreading below this anatomical boundary should dictate a better evaluation of the upper esophagus to adequately delineate its inferior margin of extension. Similarly, each hypopharyngeal subsite harbors different challenges and critical issues.

Tumors frequently arise from the piriform sinus and can superficially spread to the entire hypopharyngeal mucosa. The medial wall is in direct connection with the larynx; thus, deep infiltration may present as impairment or fixation of the ipsilateral hemilarynx. As previously mentioned, this may be related to vocal muscle (and paraglottic space) infiltration or extension to the arytenoid and cricoarytenoid joint. This is possible even without radiologic signs of cartilage infiltration. Conversely, deep infiltration from the lateral wall of the piriform sinus may lead to involvement of lateral neck structures. In particular, relationships with the carotid artery should be thoroughly clarified before the treatment planning. Finally, in case of bulky or deeply infiltrating disease at the level of the posterior wall of the hypopharynx, it is mandatory to clarify its relationship with the prevertebral fascia and prevertebral muscles. The infiltration of these structures evaluated by CT or MRI (better) contraindicates a surgical approach.

Ultimately, postcricoid carcinomas are significantly less frequent in the general population. In these cases, a precise evaluation of laryngeal subsites and motility gives important information on their superficial and deep extension. However, the inferior spread should also be considered in view of their aggressive biologic behavior.

Hypopharynx tumor has the worse prognosis within the SCCHN subsites (10-year survival 10% in locally advanced cases). Distant metastasis (lung) can be present also at diagnosis. A complete disease staging (locoregional plus distant) is recommended at diagnosis.

43.3.6 Occult Primary Head and Neck Cancer

A tumor is defined as occult or unknown primary cancer (CUP) when it presents in metastatic stage without an identifiable primary site after appropriate investigation. This category includes tumors with different histologies (SCC, adenocarcinoma, melanoma, anaplastic tumors). SCCs account for 5% of all CUPs and are most

frequently detected in cervical lymph nodes [24]. The neck level site of metastases is indicative of the possible origin of the neoplasm: tumor involving upper or mid-level cervical nodes (levels I–III, VA) likely originates from the head and neck district. Conversely, a primary site beneath the clavicles (tracheal-bronchial, lung, esophagus) or skin cancer should be suspected in those cases with lower cervical lymph nodes (supraclavicular area, levels IV and Vb), although a head and neck primary is still possible. Thyroid neoplasms can metastasize to all nodal levels.

Patients presenting with a neck mass should have a complete H&N examination using fiber-optic endoscopy, as well as a careful examination of the skin and skin appendages of the entire cervico-cephalic region. A detailed anamnesis of risk factors, previous history of malignancy or resection of cutaneous lesions should be collected.

In the absence of a suspected primary lesion, fine needle aspiration biopsy (FNAB) is the preferred pathological assessment. Core or open biopsy should be avoided, because they may alter the physiological cervical lymphatic drainage and expose the patient to tumor cell seeding with consequent negative therapeutic and prognostic implications. HPV and Epstein Barr Virus (EBV) testing are suggested for SCC or undifferentiated histology. An HPV-positive test strongly suggests an oropharyngeal occult primary located in the homolateral tonsil or base of tongue. Positive EBV testing hints at a nasopharyngeal tumor.

Computed tomography (CT) and/or magnetic resonance imaging (MRI) with contrast are usually the first line of imaging. In case of negative results, a total body PET-CT scan (preferably before the biopsy) should be performed. Examination under anesthesia with NBI inspection of the entire mucosal sites is a recommended diagnostic step, together with clinical/radiological guided biopsies of primary site.

Transoral diagnostic surgery [lingual or palatine tonsillectomy with Transoral Laser Microsurgery (TLM) and transoral Robotic Surgery (TORS)] have emerged as effective modalities to increase the detection of occult primary [25]. However, the therapeutic benefit of these surgical procedures over radiation treatment is still uncertain.

43.4 Natural History

The vast majority of HNCs arise from the surface epithelium of the aerodigestive track; therefore, their early presentation is usually one of a superficial lesion. Invasion of the underling muscular layer is frequent, allowing for tumor spread along muscular fibers and fascias even further away from the primary site. Advanced lesions usually present bony structure erosions, but sometimes periosteal invasion can be found also in smaller lesion arising from the gum, nasal, and paranasal mucosa. Usually, bony and cartilage structures represent a barrier for tumor spread. On the other hand, cancer cells can grow and migrate along nerves fibers, with histotypes such as SCC or salivary gland cancers (especially adenoid cystic carcinoma) able to recur at distant sites from their origin, such as the skull base. Also, vascular space invasion is associated with a higher metastatic rate.

HNCs, especially SCCs, usually do not cause discomfort and symptoms at early stages, thus leading to frequent diagnosis (about 80% of the cases) in a locoregionally advanced disease stage [6]. Neck lymph nodes enlargement is one of the most common signs that prompts diagnostic workup. Indeed, nodal involvement at diagnosis is quite common, with variable probability according to T stage and relative richness of lymphatic vessels of the district. Metastases are rarely detected at baseline; metastatic spread is more common in cases with higher nodal stage or pathologic lymph nodes below the level of the thyroid notch.

Early diagnosis and timely start of treatment are crucial to improve HNC outcome. Oral cancer occurs in site easily accessible by physical examination. Therefore, prevention in high-risk individuals could be carried out through routine oral mucosa examinations. Screening initiatives have been undertaken worldwide, demonstrating that a primary care strategy reduces the mortality rate of oral cancer in high-risk individuals and increases the proportion of tumors detected in early stages. Whether these strategies are cost-effective is not known and further randomized controlled trials are necessary to assess benefit of a visual examination as part of a population-based screening program [26].

Educational campaigns have been organized worldwide with the aim of raising awareness on HNCs symptoms and subsequently drive earlier presentation, diagnosis, and referral (e.g., *Oral, Head and Neck Cancer Awareness Week* in the US; *Make Sense* campaign in Europe).

43.5 Pathological Features

43.5.1 Histological Type

Almost 90% of epithelial HNCs arise from the surface epithelium and are SCC or one of its several described variants, such as lymphoepithelioma, spindle cell carcinoma, verrucous carcinoma, and undifferentiated carcinoma. Spindle cell carcinoma, usually located in the

larynx, consists of a high grade carcinoma with a component of mesenchyimal-like cell [27]. Verrucous carcinoma is more often found in the oral cavity (gum) and is usually a low-grade carcinoma frequently associated with chronic chewing of tobacco. Neuroendocrine neoplasms of the larynx, although rare, are the most common nonsquamous tumors of this organ. Tumor classification is based on its grade of differentiation, with aggressiveness being inversely correlated to differentiation.

43.6 Diagnostic Work-Up

43.6.1 Assessment of HPV Infection

Assessment of HPV infection is indicated in all cases of SCC arising from the oropharynx and from cervical nodes metastasis of unknown primary. No single analysis is considered the *gold standard* for HPV identification. Due to its cheap and reproducible methodology, p16 immunohistochemistry (IHC) is the optimal surrogate for detection of HPV infection and this is the diagnostic test approved for OPC in the latest TNM classification (VIII edition). p16 has high sensitivity, close to 100%, although up to 25% of analyzed cases have discordant results between p16 IHC and HPV in situ *hybridization* (HPV ISH) – which is a more specific but less sensitive test. In those cases, a more sophisticated test, such as mRNA and or DNA qPCR for viral protein E6, is indicated.

Commonly, diagnostic algorithm recommends upfront p16 IHC, given its ability to spare further testing. Human papilloma virus ISH's high specificity allows its use either simultaneously to IHC or as a confirmation test for p16 positive cases. In case of discordant results, it is possible to employ ISH for less common HPV types and perform qPCR (Table 43.2).

Table 43.2 Methods for HPV detection

Methods	Cost	Sensitivity	Specificity
p16 immunohistochemestry (p16 IHC)	+	+++	+
In situ hybridization for high-risk HPV (HPV ISH)	++	++	+++
Viral E6 mRNA/DNA quantitative polymerase chain reaction (E6 mRNA-DNA qPCR)	+++	++	+++

43.6.2 Imaging

Imaging plays a crucial role in HNC detection, particularly in those tumors not assessable by direct clinical examination or endoscopy. Furthermore, imaging helps to stage the tumor according to the TNM system: delineating lesion size and extension, its invasion of adjacent structures, local lymph nodes involvement, and the presence of distant metastases [28].

Imaging is essential for initial evaluation and guiding biopsies for pathological diagnosis in order to plan proper oncologic treatment; it is also crucial in evaluating tumor response during follow up and therefore plays a pivotal tool to detect early recurrences, which may allow for salvage therapy.

Appropriate imaging modality selection is crucial. Cross-sectional imaging modalities available include: ultrasound (US), CECT, MRI, and positron emission tomography-CT with fluorine-18-deoxy-D-glucose (FDG-PET-CT) [29].

Several factors influence the selection of the imaging techniques, such as the availability of the technology, the primary tumor site and histology, and the presence of contraindications. For example, MRI is contraindicated in patients with pacemakers, metal foreign body, or implants, while CT examination is contraindicated in patients with allergy to iodinated contrast media or renal failure.

Ultrasound is an easy and cheap imaging modality. It has the advantage of sparing ionizing radiation to patients and is ideal for guiding biopsy of superficial lesions. US has the limitation of being operator-dependent and not being able to visualize deeper structures. In fact, US waves are not transmitted through bones and air. In the head and neck area, it is usually reserved for evaluation of *major salivary glands, thyroid gland, and cervical lymph nodes*.

Contrast Enhanced Computer Tomography is a widely available modality with limited execution time. CECT is the best imaging technique to assess bone structures. It has the limitations of using ionizing radiations and showing poor tissue contrast resolution compared to MRI. However, in some cases, its short time of examination resulting in less motion artifacts may make it preferable to MRI. CECT is the preferred technique for the evaluation of *primary laryngeal* or *hypopharyngeal* malignancies and for the detection of adjacent cartilaginous and bony structures involvement. Computed tomography is also crucial in staging malignancies as it allows for detection of distant metastases. It may be used as an alternative to US in guiding biopsies, especially in deeper lesions adjacent to vascular and nervous structures (e.g., parapharyngeal space lesions) [29, 30].

Magnetic Resonance Imaging is the gold standard technique for the assessment of most HNCs based on its superb soft tissue contrast resolution. MRI has the advantage of not using ionizing radiations and is the best modality to assess perineural spread, evident as irregular thickening and post-contrast abnormal enhancement of the affected nerves [30]. MRI also allows for evaluation of blood vessels without injection of contrast media, by means of specific sequences.

More recently, MRI functional techniques, such as diffusion weighted imaging (DWI) or dynamic contrast enhanced (DCE) imaging has been developed. These techniques may help to evaluate response to treatment, to detect early recurrences, to distinguish between residual/recurrence disease and post-treatment changes. The main disadvantages of MRI include its high costs and long acquisition time, with low image's quality due to motion artifacts (e.g., swallowing). Therefore, adequate patient compliance is required.

Flourine-18-Deoxy-Glucose-Positron Emission Tomography/Computer Tomography permits whole body evaluation with a single exam, including the site of primary disease; for this reason, it is used as an alternative modality to computed tomography for staging malignancies. In addition to computed tomography, it has the advantage of evaluating metabolic activity of the tumor, measured by the uptake of a radioactive tracer (FDG, fluoro-deoxy-glucose). Thanks to this peculiarity, FDG-PET is essential during follow up of patient with head and neck malignancies as it helps to detect disease persistence/recurrence [31]. However, since infection and inflammation may also result in FDG uptake, this exam is usually performed at least 12 weeks after the end of treatments to minimize false-positive findings. Compared to CECT, it has superior accuracy for detecting nodal metastases, but may produce false-negative results in cases of nodal disease measuring less than 1 cm. FDG-PET/CT does not adequately assess deep, soft-tissue extension or bone involvement and, therefore, does not provide satisfying anatomical road map for treatment. It has the limitation of using ionizing radiation and requires long acquisition time (total investigation time: 2–3 hours).

43.7 Staging

In order to standardize communication between health professionals, the *American Joint Committee on Cancer Staging System* has been adopted. We invite to refer to the latest edition in order to properly stage your patients. Recently, the VIII edition has been released, introducing major changes [15, 32]. Among them, we want to underline:

A. A distinct staging system adopted for HPV-positive and negative OPC.
B. The introduction of different T-stages in oral cavity carcinomas depending on the depth of tumor invasion. This change acknowledges the different biological behavior of invasive tumors, where deeply invasive cancers (>10 mm) are associated with worse prognosis and are classified with higher T stage (T3).
C. The emphasis on extranodal extension (ENE) for HPV-negative neoplasm. ENE is defined as the presence of carcinoma extension through the fibrous capsule of the lymph node into the surrounding connective tissue. It negatively affects prognosis and it has been classified as N3b.

Neck nodal structures are commonly divided into levels [33], as shown in Fig. 43.1. Positive nodal involvement is predictive of the site of origin. Indeed, nasal cavity, lip and oral cavity malignancies initially spread to level I-II, while oropharyngeal neoplasm are associated to level II-III. Laryngeal and hypopharyngeal carcinomas spread to level II-III-IV. Level V involvement is typical of nasopharyngeal malignancies.

43.8 General Principles of Curative Treatment

HNCs affect organs vital to a patient's social life (e.g., larynx, oral cavity). Treatments needed to eradicate the malignancy can lead to several physical and functional sequelae with a serious impact on quality of life. Since therapy of locoregional malignancies has a curative intent, therapeutic efforts must not only be focused on cure of the patient but also should aim for minimizing disfigurement outcomes and late side effects. Given the complexity of multimodality treatment, patient's management should be handled from the initial diagnosis by a multidisciplinary team of health care providers with relevant expertise (surgeons, radiation oncologists, medical oncologists, dentists, speech pathologists, physical/occupational therapists, nutritionists, and skilled nurses) [34, 35].

Furthermore, SCCHN patients usually bear several comorbidities, especially metabolic (e.g., diabetes) and cardiovascular (e.g., arterial vasculopathies; hypertension; renal failure). A thorough inquiry on patient's medical history and social environment (i.e., presence of accountable caregiver) is of paramount importance for determining the actual ability to sustain the treatments.

Surgery and RT, alone or combined, are the curative treatments for HNC patients. Although chemotherapy by itself is not considered a curative treatment, it

enhances the effects of RT and it is routinely used as part of combined modality treatment (i.e., concomitant chemoradiation), particularly in patients with locally advanced disease. The optimal combination of these treatment modalities depends on the anatomic site of the cancer as well as the disease stage.

43.8.1 Surgical Principles

Surgical treatment is strictly dependent on tumor site, size, and involved structures. Accurate preoperative clinical and radiological evaluations allow to adequately plan surgery according to such specific characteristics. The basic principle of HN oncologic surgery is the achievement of complete tumor resection (with free surgical margins), while maintaining as low as possible the occurrence of postoperative sequelae (attended and unavoidable negative consequences of a given therapeutic act) and complications (unattended and avoidable negative consequences). In this view, whenever feasible, minimally-invasive endoscopic/endoluminal approaches are often favored. These are represented by a number of continuously evolving techniques and approaches, such as TLM, TORS [36], and transnasal endoscopic surgery (TES) [37]. These therapeutic modalities accomplish tumor resection through natural orifices (mouth and nostrils), thus limiting the morbidity due to external scars and traumas to uninvolved surrounding tissues. However, locally advanced tumors may require an extensive resection with more conventional "open" procedures, in which cervicotomy, facial bone osteotomy and/or craniotomy must be applied in order to access the lesion itself and allow its safe removal. In these scenarios, reconstructive surgical techniques play an essential role in granting an effective esthetic and functional restoration, leading to acceptable results even in case of wide composite resections [38]. In association with surgery of the primary lesion, it is often necessary to remove cervical lymph nodes that may be involved by metastatic (clinically overt or occult) neoplastic localization. This is obtained through different types of lateral and/or central compartment neck dissections. This is a major surgical procedure that has to be well distinguished from the neck node biopsy that is usually used in hematologic diseases for a diagnostic purpose, but not recommended in HNCs. During neck dissections, all involved or potentially involved lymph nodes with the surrounding fat tissue within the neck fascial compartments are removed for prophylactic or therapeutic reasons. While in the first clinical scenario, elective neck dissection will remove only the neck levels at higher risk of harboring metastatic cells according to the site of the primary tumor, in the latter one, therapeutic neck dissection will accomplish removal of all the six neck levels (uni- or bilaterally, according to the tumor relationships with the midline).

43.8.2 Radiotherapy Principles

RT plays a pivotal role in the curative treatment of early and locally advanced SCCHN [39]. Intensity Modulated RT (IMRT) is the standard of care in HNCs. IMRT with or without CT has been established as a radical and effective treatment approach, scoring better than 3D-conformal RT in terms of toxicity and quality of life. However, late radiation-related effects, such as xerostomia or dysgeusia, are important issues that need to be addressed. The advantages of irradiation over surgery may include the following: (1) the avoidance of major postoperative complications, (2) reduction of functional or cosmetic defects since no tissues are removed, (3) elective irradiation of the neck lymph nodes, and (4) irradiation failures could be surgically salvaged.

The treatment intent could be either curative or palliative. Curative IMRT is used for the purpose of permanently eradicating the tumor; in this context, RT could be radical or adjuvant (i.e., postoperative). Palliative RT is designed to ameliorate a specific symptom (e.g., pain, bleeding, etc.) within incurable malignancy.

For early-stage cancer, surgery or RT alone are both effective. RT can be delivered via external beam or interstitial brachytherapy (where radiation sources are inserted into needles placed through the tumor). For intermediate- and advanced-stage cancers, possible alternative strategies are surgery followed by radiation or definitive RT, with or without chemotherapy. Unresectable cancers can be cured by RT alone or chemo-radiation. Nasopharyngeal cancers are treated only by RT or chemoradiation, reserving surgery as salvage treatment in case of failure.

Adjuvant RT is indicated when factors predicting local recurrence after surgery are present: positive resection margins when no further surgery is possible, locally advanced tumors (stage III-IV, nodal extension to level IV or V, extracapsular spread), close resection margins (<5 mm), high-grade tumors and perineural or vascular invasion [40]. In those cases, the addition of concomitant cisplatin 100 mg/m^2 each 3 weeks significantly improves the PFS at least in case of close/positive margins and/or extracapsular spread of disease.

The best evidence for estimating the risk of recurrence in the clinically negative neck comes from historical series of neck node dissections or observational follow-up. If the risk of nodal recurrence is 20% or higher, prophylactic neck treatment is recommended. Selecting the appropriate nodal levels to be treated

depends on a thorough knowledge of lymph node drainage pathways of the head and neck areas as well as data from previous series of patients found to have nodal metastases when clinically N0. The choice between surgery or RT to treat the N0 neck usually depends on the treatment of the primary tumor.

When staging exams indicate lymph node involvement, the neck is treated with a neck dissection, RT, or a combination of the two. RT with or without chemotherapy can control neck disease, particularly when involved nodes are smaller than 3 cm at diagnosis. For N2 and N3 disease, particularly where nodes are 3 cm in diameter or larger, a combination of surgery and RT is recommended. Surgery followed by RT presents the advantage of obtaining fast local control of disease, useful for rapidly growing mass or in cases of skin involvement; this approach also provides definitive staging information. Anyway, postoperative RT volumes are more difficult to define with certainty. A selective neck dissection 3 months after radiation should be performed in cases of residual nodal disease. For HPV-positive OPCs, a longer follow up is required to obtain the clearance of the neck nodes after RT or chemoradiotherapy.

After neck dissection, adjuvant RT is recommended in the presence of macroscopic residual disease (e.g., nodes dissected off the carotid artery) or if two or more nodes contain tumor. Adjuvant RT should also be considered if a single involved node exceeds 3 cm in diameter. Chemotherapy can be avoided in the lack of negative pathological prognostic factors listed in Table 43.3.

Side Effects

Side effects depend on site, extent, and dose of irradiation to the head and neck areas. They may be divided into acute, when they develop during or soon after treatment, and late, if they appear months (at least >6 months) or years after RT completion. Generally, acute side effects become apparent about 2 weeks after RT start, when dysphagia, dysgeusia, xerostomia, and skin reactions may occur. Dysphagia is the main side effect that makes the course of RT difficult. In fact, when patient's nutritional and fluids oral intake becomes insufficient, causing severe weight loss, feeding tube placement is required. In cases with adequate baseline nutritional status, a reactive approach with temporary naso-gastric tube placement is preferred. On the contrary, when baseline malnutrition or dysphagia are present, or in case of extended field of RT for locally advanced disease (where dysphagia is predictable), a more stable solution, such as gastrostomy, might provide durable support.

Other RT toxicities are changes in voice caused by swelling and scarring, loss of appetite, edema, bone pain, nausea, fatigue, mouth sores. Late side effects may include xerostomia, skin fibrosis, hearing loss, hypothyroidism.

Because RT can cause tooth decay, damaged teeth may need to be removed. Therefore, prior to any RT for HNCs, patients should be examined by a dentist or oral oncologist.

Table 43.3 High-risk pathologic features and relative indications for adjuvant treatment (SCC from oral cavity, oro-hypopharynx, and larynx)

Pathologic features	Indications for adjuvant treatment
AJCC Disease stage III-IV	Postoperative RT (PORT)
Level IV-V nodal extension	PORT
Perineural invasion, vascular embolism	PORT
Microscopic marginal resection (R1)	PORT + concurrent CT
Extra nodal extension	PORT + concurrent CT

Technical Tips

Before starting RT, the patient undergoes a computed tomography-simulation. It consists of acquiring a computed tomography scan while the patient is positioned and immobilized in the same setting as future treatment. Therefore, the patient must be in a proper position suitable for acquiring CT images and treatment delivery, but at the same time, the patient should also be in a comfortable and reproducible position. The patient is positioned supine on the treatment couch with the head and neck supported by a neck rest, which can be customized as required to find the best position for treatment. A thermoplastic mask constructed from a cast of the patient's head has been regarded as the most accurate method for immobilizing the patient.

Conformal volume-based RT of HNCs requires knowledge of anatomy and patterns of disease spread, which are often specific to each primary tumor site and histology. Cancers in the head and neck region spread in four main ways: (1) direct extension from the primary site to adjacent structures; (2) spread through the lymphatic vessels to lymph nodes; (3) diffusion along nerves (perineural spread) to other HN areas; (4) enter blood vessels and disseminate to distant sites. In SCCHN, a spread to the lymph nodes in the neck is relatively common. When planning treatment volumes, all these dissemination pathways should be taken into account. Furthermore, surrounding critical normal structures and their own radiation sensitivity should also be delineated and may significantly influence treatment planning and/or prescribed dose.

A radiation therapy regimen (schedule) usually consists of a specific number of treatments given over a set time period. The standard of care in curative RT for SCCHN consists in 2-Gy daily fractions delivered 5 days a week. The standard radical dose to the primary tumor and involved nodes is 70 Gy. In the adjuvant setting, the standard dose is 60 Gy, with 66 Gy delivered on sites of positive resection margins or extranodal spread. 50 Gy prophylactic dose to uninvolved nodal levels is recommended. Improved local control and cure rates can be achieved by using altered fractionation regimens, by combining systemic agents with radiation or possibly by a combination of these approaches.

Altered fractionation regimens comprise accelerated RT, hyperfractionation, and hypofractionation. Accelerated radiation schedules shorten the overall treatment time to reduce tumor repopulation during a course of RT and theoretically increase local control and cure. Hyperfractionation schedules reduce the dose per fraction and use two fractions per day in order to reduce the risk of late effects and allow dose escalation with an improved therapeutic ratio. Hypofractionated regimens give larger doses per fraction in a shorter overall treatment time with the aim of reducing the risk of tumor repopulation at the cost of a theoretical increase in late effects.

- *Brachytherapy*

Brachytherapy is a form of RT where a sealed radiation source is placed inside or next to the neoplastic lesion, thereby concentrating the radiation dose, with a rapid dose fall-off. When expertise is available, it can be used as definitive treatment for small tumors of the lip, oral tongue, floor of mouth, buccal mucosa, and nasal vestibule. It can also be used as retreatment modality in selected cases.

- *Radiotherapic Salvage Treatment*

Despite the advances made in the primary disease setting, still up to 30–50% of all curatively treated SCCHN patients will develop a locoregional recurrence. In addition, the development of a second primary tumor in the HN region represents a constant threat for those who survive. Salvage surgery remains the standard of care for these patients, although it is feasible in only 20% of the cases, with 25% to 45% of patients experiencing long-term disease control.

Potentially curative re-irradiation, with or without concurrent chemotherapy, could be considered whenever the disease is unresectable or the patient is ineligible for surgery. In case of adverse prognostic factors, immediate postoperative (chemo-)re-irradiation after salvage surgery can be administered safely and significantly improves locoregional control. Re-irradiation for locoregional failure or second primary tumors poses a daunting problem for radiation oncologists. Traditionally, the administration of a second course of RT to tissues within a previous radiation field has been considered unsafe and avoided due to concerns regarding toxicity. Nevertheless, when RT can be safely administered, it provides a reasonable chance of long-term survival (approximately 15–20%). Therefore, patient selection is of utmost importance. Suitable patients should have excellent performance status, no significant medical comorbidities, no severe sequelae from the previous course of radiation. Furthermore, disease-free interval from previous malignancy should be at least 1 year and the target volume as small as possible. Patient should be fully aware of the increased risk of potentially serious acute and late effects. Intensity Modulated RT (IMRT) techniques, Stereotactic Body RT (SBRT), and heavy particle therapy (proton or carbon ion-therapy) can be useful to conform more closely the dose to target volumes, to minimize the high-dose treated volume and the dose to surrounding critical structures.

43.8.3 Systemic Agents as Part of Curative Treatments

Systemic agents might be employed in the curative setting as part of: (1) induction chemotherapy (CT); (2) concomitant CT during radiation treatment; (3) adjuvant CT.

43.8.3.1 Induction Chemotherapy

The role of induction CT is still under discussion. Since the ability of induction CT to increase overall survival in SCCHN patient is not clear, its use is currently reserved only for organ preservation strategy of hypopharyngeal and laryngeal tumors [41–44]. The presently preferred induction chemotherapy regimen (TPF) comprises a combination of three drugs, docetaxel, cisplatin, and 5-fluorouracil (5FU). In previously untreated SCCHN, this combination yields major response rates approximating 70% to 90%, depending on the type of patients treated (resectable vs. unresectable) with clinical complete response rates in the 15–30% range. Induction chemotherapy could be used as part of multimodality treatment in unresectable disease and in organ preservation strategy. It may be employed also in cases of rapid tumor evolution in symptomatic patients – bleeding, reduced airway patency – when other techniques are not promptly available or whenever a tumor shrinkage could avoid a tracheostomy or a gastrostomy.

43.8.3.2 Concomitant Chemotherapy During Radiation Treatment

Several evidences support the use of concurrent CT during RT (CTRT). The meta-analysis of CT in HNC (MACH-NC) showed a 6.5% of 5-year survival benefit and better locoregional control in favor of the combined treatment over RT alone [45]. However, based on calendar age (not biological age) this advantage was shown to be less in older patients and in particular seemingly negligible in those over 70 years of age. Concurrent CTRT treatment could be used as single treatment strategy with a curative aim or as part of the postsurgical, adjuvant treatment. When employed in combination with adjuvant RT, CT indications are restricted to patient at high risk of recurrence, identified through specific pathologic features, reported in ◘ Table 43.3.

Although several drugs have been tested, either as single agent or in combination, concomitant treatments with platinum regimens represent the standard of care being more effective than other types of mono-chemotherapy [45]. Cisplatin 100 mg/m^2 q3weeks is considered the standard of care in association to RT. The 3-weekly cisplatin schedule seems preferable to weekly cisplatin, since it resulted in a significantly better locoregional control (but without overall survival benefit) in a randomized controlled trial comparing the 3-weekly high-dose with a weekly rather low dose of 30 mg/m^2, albeit at the cost of increased toxicitiy [46]. The total cumulative dose of the drug has a significant positive correlation with survival; efforts should be aimed to reach at least a dose of 200 mg/m^2 throughout the course of radiotherapy [47].

In case of absolute contraindications to cisplatin (e.g., impaired renal function, inability to sustain high infusion volume due to cardiac pathology, severe hearing loss, and neuropathy), alternatives are available [48]. Carboplatin is generally tolerated better than cisplatin, but it is also less effective. Cetuximab, a monoclonal antibody targeting EGFR, has proved to increase RT performance (cetuximab+RT OS 49 months vs. RT 29.3 months) [49], although cetuximab added to platinum-based RT was not superior to standard platinum-based RT. [50]

43.8.3.3 Adjuvant Chemotherapy

There is no clear evidence of a benefit from adding adjuvant chemotherapy to locoregional treatment [45]; hence, it should not be used in clinical practice.

43.8.4 Supportive Care During Radiation Treatment

HNC treatment can cause a wide spectrum of disabling toxicities. The addition of cisplatin to RT in CTRT enhances both the therapeutic effect and the toxicities. In the curative setting, it is of paramount importance to avoid RT breaks and treatment suspensions as they could negatively affect outcomes. For this reason, a supportive care program should be planned before treatment start, helping patient to overcome side effects.

First, patients should be encouraged to quit smoking and to reduce alcohol consumption. In fact, these habits may decrease treatment efficacy and increase treatment-related side effects [51]. Behavioral counseling combined with medications to support smoking cessation could be helpful.

Before treatment start, all patients should perform a thorough assessment of nutritional and dental status. Any dental condition at risk of complications must be managed so as not to create complications that could interrupt RT and to reduce the risk of long-term complications. Dental care after RT requires special attention. Fluoride treatments can help to decrease caries incidence. Long-term maintenance of oral hygiene should be initiated in conjunction with cancer treatment [52].

While most HNC patients are already nutritionally compromised because of their disease and/or their unhealthy life-style habits, RT toxicity, such as mucositis, may cause further difficulties in eating. Thus, patients should receive dietary counseling and be evaluated for nutritional risks before treatment start to select those requiring prophylactic gastric tube placement and those to monitor closely during treatment for the need of temporary nasogastric tube feeding interventions. Prophylactic feeding tube placement should be considered in cases of severe weight loss prior to treatment (i.e., 5% weight loss over prior month; 10% weight loss over 6 months), in patients with ongoing dysphagia, those with severe aspiration, or patients in whom long-term swallowing disorders are expected [53, 54].

Baseline assessment by a speech or language therapist should be undertaken and appropriate interventions organized to maintain functions before treatment starts. This may be beneficial in case of aspiration or risk that the treatment itself induces dysphagia problems or complications, such as aspiration pneumonia. Severe swallowing impairment is hardly rehabilitated. In those patients, gastrostomy should be considered.

Here, we will briefly focus on frequent acute side effects occurring during initial treatment.

Xerostomia is usually a late RT side effect but changes in saliva quantity and composition can occur shortly after RT start (1–2 weeks). The best way to minimize salivary gland toxicity is to use highly conformal RT techniques, avoiding unnecessary RT dose to sensitive targets, such as parotid glands. Patients should drink adequate amounts of fluids, rinse and gargle with a weak salt solution or baking soda several times daily during treatment.

Mucositis occurs in nearly all patients receiving head and neck RT. Multinational Association of Supportive Care (MASCC, ► http://www.mascc.org) guidelines suggest the use of oral zinc supplements to prevent mucositis in patients receiving radiation or chemoradiation for oral cancer, while the use of benzydamine mouthwash is recommended only in patients receiving moderate radiation doses (up to 50 Gy) without concomitant chemotherapy. Although there is evidence that low-level laser therapy might be beneficial for the prevention of oral mucositis during RT, this expensive technology requires daily treatment and its use is scarce [55]. Mucositis is managed symptomatically with scrupulous oral hygiene (excluding alcohol-containing mouthwashes), dietary modifications, and pain control. Acidic and spicy foods, sharp foods, caffeine, and alcohol consumption should be avoided. Mucosal superinfections by bacterial, fungal, and viral agents should be treated with appropriate therapy. Pain may be significant during and shortly after the course of RT and therefore it should be treated adequately. 0.2% morphine mouthwash may be effective to treat pain due to oral mucositis during CTRT [55], while transdermal fentanyl is an option for dysphagic patients. In some cases, a nasogastric tube placement is necessary to prevent weight loss.

Radiation dermatitis in the treatment field is common during RT and it occurs within the first 4 weeks of treatment. Patients should be instructed about the harm of exposure to potential chemical irritants or to direct sunlight without adequate protection. Hygienic routine should be carried out with water and mild soap/shampoo gentle washing [56]. Beyond that, there is little evidence to support the use of one topical approach over another [57]. Topical products should not be applied shortly before radiation because they can cause a bolus effect, thereby artificially increasing the radiation dose to the epidermis. Prophylactic topical steroids could be used to reduce discomfort or burning and itching [56].

Dysgeusia is an abnormal or impaired sense of taste and it may contribute to nutritional difficulties and weight loss. Pharmacologic intervention using zinc supplementation or amifostine have not shown consistent benefit; however, dietary counseling may be of value [58].

Cisplatin toxicity. As previously mentioned, cisplatin is the chemotherapeutic drug most frequently used in combination to RT. High cisplatin doses can cause neuropathy, nephrotoxicity, and ototoxicity. Conventional audiometry may be used to detect and monitor hearing impairment. Before using cisplatin, it is important to assess patient's baseline renal function by calculating or measuring urinary creatinine clearance and consider all the potential nephrotoxic comorbidities (e.g., uncontrolled hypertension, diabetes, etc.) and drugs in use. Electrolytes should be frequently monitored, as hypomagnesemia or other urinary wasting syndromes can occur requiring prompt correction. The administration of intravenous saline with magnesium supplementation is the primary approach for preventing cisplatin-induced nephrotoxicity and must be adopted in all patients treated with cisplatin [59].

Pain is experienced frequently and it could be related to the cancer itself and also to treatment toxicities, such as mucositis, inflammation, superinfection and scarring from surgery, or other treatments. Adequate pain management is mandatory and often requires opioid drugs.

Anxiety and depression are common in patients treated for head and neck cancer, and these symptoms can have a significant negative impact on quality of life. Psychological support and antidepressant drugs should be employed as needed.

43.9 Principle for Curative Treatment of Specific Subsite Tumor

43.9.1 Oral Cavity

Surgery is the first-line treatment for oral SCC. Each surgical procedure, together with adjuvant therapies, should be tailored according to the specific risk profile of every single patient. This is especially true considering tumors involving the mobile tongue/floor of the mouth, with the aim to precisely balance surgical invasiveness, functional results, and oncologic outcomes.

Early tumors, with minimal depth of invasion (less than 4 mm) and no clinical and radiologic evidence of lateral neck metastasis, can be considered as low-risk diseases and should be managed by a unimodal treatment (i.e., surgery alone). Transoral resection can adequately remove the entire disease with wide free surgical margins of at least 1 cm from the visible border of the lesion itself, leaving only minimal or null functional impairment with no impacting on speech and swallowing. Complex reconstructive techniques, such as pedicled or free flaps, are rarely required in these cases even if, in some instances, local mucosal flaps may speed up and help the healing process, thereby improving functional outcomes. In situations where the depth of infiltration is greater than 4 mm, most studies agree on the indication for a prophylactic selective (levels I to III) lateral neck dissection due to the high risk of occult (subclinical) nodal metastases.

Locally advanced tumors characterized by a higher depth of invasion (greater than 1 cm) usually require more aggressive surgical approaches, followed by adjuvant radiotherapy, alone or in combination with chemotherapy. The recently proposed concept of tongue

compartmental surgery [60] aims at removing the entire tumor-containing anatomic compartment (i.e., hemi-tongue and ipsilateral floor of the mouth) via a pull-through transoral-transcervical approach, with or without mandibulectomy, in continuity with the T-N tract and the draining lymph nodes (levels I to V). After resection, the separation between oral cavity and the neck is restored using microvascular free flaps or pedicled flaps as a second-line option. This approach effectively closes the surgical defect, while granting a sufficient volume to restore function of the resected hemi-tongue. High-risk histopathological features as an advanced local extension or microscopically positive – inadequate surgical margins are the primary indications for adjuvant treatment. Similarly, a significant nodal burden and extranodal extension are associated with an increased risk of regional recurrence that can be adequately managed by postoperative (chemo)-radiotherapy.

In summary, while the low-risk disease may be adequately managed with a unimodal treatment, high-risk tumors should be handled in a multimodal manner (i.e., surgery + (chemo)-radiotherapy).

43.9.2 Larynx

TLM (or, in some cases, TORS) is the treatment of choice in case of early-intermediate neoplasms, shifting to open partial laryngectomies and total laryngectomy in more advanced tumors. Elective neck dissection is frequently considered for supraglottic and advanced glottic carcinoma.

For early laryngeal cancer (T1 and T2), TLM and radiotherapy showed comparable results in terms of survival outcomes. Concerning vocal outcome, while no consistent data are available, radiotherapy seems to induce better results in selected subgroups of patients (T1b). However, TLM has the advantage to be a quick, cost-effective, minimally invasive, and repeatable treatment, with a high success rate especially in early neoplasms (T1 and superficially spreading T2). This is particularly true when it is compared with the lengthy treatment course and the significantly lower chances of organ preservation in case of disease relapse when radiotherapy is the first treatment delivered.

Concerning intermediate tumors (bulky T2 and T3), the choice between possible treatment options is even more extensive. TLM offers good results in terms of functional and oncologic outcomes in T2 and selected T3 tumors when performed in experienced centers. However, open partial laryngectomies represent the most frequently applied surgical approach for these diseases. Supraglottic, supracricoid, and supratracheal laryngectomies (type I, II, and III according to the European Laryngological Society classification) can effectively address endolaryngeal tumors (T3) with at least one uninvolved cricoarytenoid unit, and even early T4a with a limited anterior extralaryngeal extension. In these procedures, patient selection is crucial: pulmonary and cardiac functions should be carefully assessed, since unfit patients may not tolerate the resulting degree of chronic subclinical aspiration. Furthermore, non-surgical organ preservation strategies (i.e., induction chemo plus radiation in responding patients) play a pivotal role in selected T3 (glottic site; mobile vocal cords) and should always be considered before resorting to a total laryngectomy. On the other hand, their use in T4 lesions has been demonstrated to carry to an unfavorable locoregional control with dismal organ preservation rates [61, 62].

Total laryngectomy followed by adjuvant therapy proved to be the most effective treatment for laryngeal cancers with extensive cartilage infiltration or diffuse extralaryngeal invasion. This procedure consists of the complete removal of the larynx with associated prelaryngeal strap muscles and results in a permanent tracheostomy. While swallowing frequently returns to normality after the end of the healing process (10–15 days), speech ability is impaired and voice can be restored with a variety of technical and surgical approaches (e.g., external devices, tracheoesophageal puncture with placement of a voice prosthesis) [63].

43.9.3 Oropharynx

Oropharyngeal cancer has been classically considered as a primarily non-surgical neoplasm due to its remarkable radio- and chemosensitivity and to the invasiveness of conventional (transmandibular) surgical approaches. Therefore, open surgical resection and reconstruction are usually reserved for persistent/recurrent tumors after (chemo)-radiotherapy or locally advanced tumors that need a multimodal treatment to achieve a reasonable chance of cure [i.e., surgery + (chemo)-radiotherapy]. However, recent technical developments such as TLM and TORS led to an expansion of minimally invasive procedures for early-stage neoplasms. This is also related to the recent epidemics of HPV-related tumors, resulting in an increased incidence among young (40- and 50-year-old), healthy subjects with less or no alcoholic/tobacco abuse history if compared to the "classic" 60 and 70-year-old head and neck cancer population. The higher curative rates and longer life-span after treatment of this new type of HNC patients shifted the balance between oncologic outcomes and post-treatment sequelae toward the latter, opening a discussion regarding the role of TORS (and transoral surgery in general)

as an alternative to (chemo)-radiotherapy, at least in selected cases. While randomized trials comparing minimally invasive surgery with (chemo)-radiotherapy are still ongoing in HPV-positive OPC, there is some limited evidence that surgery may grant better functional outcomes for early disease, still maintaining high chances of cure. However, it is crucial to weight each approach according to a careful pretreatment staging to avoid overtreatment (surgery + chemoradiotherapy) in patients that could have been successfully treated by a less aggressive strategy (chemo-radiotherapy or surgery alone). In this view, evaluation of the primary lesion should take into account its pattern of infiltration in order to avoid unexpected postoperative margins positivity or involvement of functionally essential structures (bilateral lingual vessels or hypoglossal nerves). Lateral neck metastasis should also be carefully assessed to exclude bilateral involvement or extranodal extension before embarking on a primary surgical approach.

43.9.4 Hypopharynx

The surgical principles for treatment of hypopharyngeal cancer are strictly related to its aggressive biologic behavior with frequent laryngeal involvement. Most of these tumors are diagnosed in late stages (III-IV). A diffuse field of cancerization with multiple neoplastic foci and a significant submucosal spread, associated to multiple lymph nodes metastases, represent the typical clinical scenario of hypopharyngeal carcinoma, resulting in the need for extensive resections even in presence of relatively small primary tumors. Therefore, conservative surgical approaches, such as TLM, TORS, or open partial hypopharyngectomies, are only exceptionally employed. Considering early and intermediate tumors, (chemo)-radiotherapy often offers the more favorable balance between adverse effects and oncologic results [64]. In these cases, surgery is generally a salvage option, with minimal chances of organ preservation.

On the other hand, hypopharyngolaryngectomy is a viable option for advanced tumors and those not responding to induction chemotherapy. This is always associated with an elective or therapeutic lateral neck dissection in consideration of the advanced stage of the disease and the high frequency of nodal metastasis. Functional results are comparable with those obtained after total laryngectomy alone, but resection of the hypopharyngeal mucosa results more frequently than not in the need for complex reconstructive techniques (pedicled or free flaps) that significantly increase the operative times and the risk for postoperative complications.

43.10 General Principles for HNC Palliative Treatment

Approximately one third of SCCHN patients are diagnosed with early stage disease (T1-2, N0) with excellent prognosis. On the contrary, overall survival for late stage patients is poor, with 40–50% of them alive after 5 years. Distant metastases usually involve the lung. Recurrent/metastatic SCCHN prognosis is dismal; median OS from standard first-line therapy is about 10 months, with only 20% of patients alive after 2 years [65].

The presence of a locoregional recurrence poses significant challenges that require the ability to anticipate as much as possible the issues correlated with the natural disease evolution. Therefore, foreseeing patient's needs and tailoring therapeutic intervention accordingly is crucial for maintaining adequate quality of life. An example of such process is represented by the regular assessment of airway patency and nutritional status. These areas are of paramount importance and frequent evaluations are mandatory. Risk of bleeding by recurrent lesions close or involving the blood vessels of the neck is another relevant medical issue. Hemorrhage might be lethal and rarely anticipated, although a prior hemorrhagic episode is an important "red flag" signaling the potential risk of major complication. Discussion with the patient over invasive procedures, such as gastrostomy and tracheostomy, are recommended, especially considering residual prognosis.

43.10.1 Radiotherapy: Palliative Treatment

Palliative RT may provide control of local symptoms, such as pain. It is indicated in cases when a curative approach is not feasible due to disease characteristics or patients' comorbidities. Palliative RT to the primary tumor could also be useful when metastases are present at diagnosis, or in locally recurrent disease to ameliorate fungating tumor or reduce bleeding.

43.10.2 Systemic Treatment

First-line treatment for recurrent/metastatic SCCHN is an evolving field. For almost a decade, standard first-line treatment for recurrent/metastatic SCCHN has been a combination of a cisplatin, 5FU, and cetuximab [65]. In clinical trial setting, this regimen registered an overall response rate of 36% with a median OS of 10 months. Nevertheless, suboptimal performance status, frequent cardiovascular conditions and anticipated toxicities reduce the proportion of patients susceptible

to such combination. Therefore, alternatives, such as two-drug combination without 5FU or three drug regimens with the introduction of paclitaxel (response rate >50%) in spite of 5FU, could be used [66].

Very recently, data from a phase III trial with pembrolizumab in first-line treatment changed the scenario in this setting. The study evaluated pembrolizumab alone or in combination with platinum and 5FU against the SoC (cetuximab-platinum - 5FU combination). Pembrolizumab combined with chemotherapy showed a benefit over SoC in OS in patients with a programmed cell death ligand 1 (PD-L1) combined positive score (CPS) ≥ 20 (median 14.7 months vs. 11.0 months, HR 0.60, 95% CI, 0.45 to 0.82, $P = 0.0004$), CPS ≥ 1 (median 13.6 months vs. 10.4 months, HR 0.65, 95% CI, 0.53 to 0.80, $P < 0.0001$), and in total population. Furthermore, pembrolizumab alone proved to be superior to SoC in the CPS ≥ 20 and CPS ≥ 1 population [67]. Interestingly, the overall response rate was lower for pembrolizumab alone in comparison to the combination of pembrolizumab and chemotherapy. Overall, these data support the use of pembrolizumab, alone or in combination with chemotherapy, as a new SoC in first-line setting for CPS ≥ 1 recurrent/metastatic SCCHN.

The choice of which first-line treatment to deliver to which patient should be informed by the following factors: (1) CPS score; (2) the urgency of a clinical response. For example, an asymptomatic patient with high CPS could be proposed with a chemo-free scheme. On the other hand, a symptomatic, CPS low patient in need for a rapid clinical benefit should be proposed with a treatment that includes chemotherapy in order to obtain higher chance of response.

Platinum-resistant disease, defined as persistent or recurrent tumor within the 6 months after curative therapy with platinum-based agents, is an aggressive disease with a worse prognosis.

Second-line treatment armamentarium for recurrent/metastatic SCCHN includes immunotherapy as well [68]. A randomized trial compared nivolumab to systemic chemotherapy, showed for the first time a significantly prolonged survival of immunotherapy in this subset of patients (7.5 months vs. 5.1 months, HR 0.70; 97.73% CI, 0.51 to 0.96; $P = 0.01$) [69]. Also pembrolizumab demonstrated a benefit in OS compared to standard chemotherapy (8.4 months vs. 6.9 months; HR: 0.80; 95% CI, 0.65 to 0.98; $P = 0.016$) [70].

However, more than 50% of patients do not receive a second-line treatment, and the expected response rate is low (less than 10% with chemotherapy, 13% immunotherapy). Performance status is the main factor that drives treatment choices. However, more than 50% of patients do not receive a second line treatment and in fact half of them cannot receive this because of a poor general condition [71]. Therefore, a multiprofessional balanced care is mandatory in this disease setting with symptomatic progression.

Case Study Oral Lesion

Man, 68 years old.

- *Family history* negative for malignancy.
- *Comorbidities:* Diabetes mellitus type II, chronic atrial fibrillation, active smoker (20 pack/year), and alcohol consumption (3 drinks per day).
- *Recent history:* Long history of oral leukoplakia, occasional surgical removal with negative histological examination. For nearly 2 months, pain located under dental prosthesis.
- *Objective examination*: painful ulcerated area on the mucosal surface of left mandibular gum. Infracentimetric palpable node at level IIa.
- *Blood tests*: Hb 12,1 g/dl; regular biochemistry.

Question

What action should be taken?

(1) Surgery (2) Biopsy (3) Other

Answer

Transoral biopsy under local anesthesia

Histological examination:

SCC G1 in a field of high-grade dysplasia.

Question

What radiological exams should be performed?

(1) FDG-PET. (2) Head and neck MRI. (3) EGDS

Answer

FDG-PET and MRI.

Findings: local lesion at left inferior mandibular mucosa with contrast enhancement and FDG uptake diffuse to left mouth floor, no clear signs of bone invasion. Borderline lymph node at left level IIa.

Question

What action should be taken?

(1) Surgery + adjuvant therapy. (2) Induction chemotherapy + RT. (3) Brachytherapy

Answer

→ Compartmental local surgery with bilateral neck dissection. Tumor pathological stage: pT4a (floor of the mouth invasion) pN3b (1/22 ECS positive lymph node) according to AJCC VIII edition.

→ Adjuvant therapy with IMRT plus three high-dose of cisplatin

Key Points

- The importance of a correct follow-up of premalignant lesion: attention to symptoms
- Adequate presurgical staging and postsurgical staging with latest TNM
- The importance of adjuvant therapy
- Importance of multidisciplinary management

Case Study Neck Mass

Man, 56 years old

- *Family history* negative for malignancy
- *Comorbidities*: Negative, smoking history (10 packs/year)
- *Recent history*: Asymptomatic slowly growing left neck mass
- *Blood tests*: No abnormalities
- *Objective examination*: No signs of malignancy in the ENT region, both in transoral and fibroscopic NBI/with light examination. Neck mass at the III level.

Question

What action should be taken?

(1) Surgery. (2) Biopsy. 3) Staging

Answer

Node biopsy, preferable under US: SCC G3, research of HPV and EBV is mandatory. In this case, p16 IHC/HPV DNA was positive.

Local MRI and whole body FDG PET: confirm left neck cystic adenopathy, 3.5 cm, without clear primitive lesion. No distant lesion.

General anesthesia with oropharyngeal (bilateral tonsils and base of the tongue) biopsy: negative for malignancy.

AJCC TNM VIII edition: TxN1M0, stage I

Question

What action should be taken?

(1) Surgery. (2) Induction chemotherapy. (3) Chemoradiotherapy

Answer:

Chemoradiation with IMRT delivered with radical intent on oropharynx and bilateral neck combined with 3-weekly cisplatin.

Key Points

- HPV and EBV assessment to guide diagnosis and therapy.
- Supportive care in order to complete curative treatment.

Expert Opinion

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A synthetic and schematic description of head and neck cancers (HNCs), due to their heterogeneity is not simple: primitive sites or origin, stage and presence of comorbidities influence the diagnostic process and the therapeutic course. Otherwise, it has been seen that HNCs share lots of risk factors such as smoking, alcohol consumption, HPV infection, or genetic predisposition. Primary prevention is essential to avoid the onset of HNCs, and as a result smoking should be avoided and so the alcohol consumption which has a well-known synergic action with smoke. As other tumours, early diagnosis is essential to provide a radical and non-invasive treatment. Prognosis is very poor in case of advanced stage or metastases: in this setting of patients palliative cares are crucial in order to guarantee a good quality of life; furthermore is relevant to remember the importance of toxicities during the various treatments which can be used: in these situations a multimodal approach is the best option. Nowadays, hopes rely on immunotherapy as it can determine a better OS and PFS in this setting of patients in case of the need of a second-line therapy, so enrolment in clinical trails is encouraged.

1. Head and neck cancers (HNCs) are a group of heterogeneous neoplasms arising from epithelial tissue of the upper aerodigestive regions. They share almost

the same risk factors such as smoking, alcohol consumption, HPV infection, and previous HNCs.
2. Symptoms can be quite characteristic in dependence of the primary site of origin: it is possible to observe dysphagia, pharyngodynia, dysphagia, dysphonia, enlarged lymph nodes, persistent ulcerations, leukoplakia, or erithroplakia.
3. Clinical history and physical examination are useful approaches in order to understand site and features of HNCs. Obviously lots of imaging investigations can be employed such as US, CT, MRI, PET-FDG/CT or techniques such fibro or video laryngoscopes with the eventual use of the narrow band imaging (NBI). In case of occult neoplasm and lymph nodes enlargement, a FNA can be evaluated to determine the origin site of HNC.
4. The most frequent histologic subtype is the classic squamous carcinoma and its variants; other types are the neuroendocrine neoplasms of the larynx, which are the most common nonsquamous tumors in this region.
5. New therapeutic techniques have changed the clinical approach to HNCs: a multimodal approach must be considered in order to guarantee the best therapeutic options; so surgery and radiotherapy (RT), alone or combined, are regarded as curative treatments; chemotherapy has today the role to improve radiation effects. Obviously, the different approaches depend on the site of origin and the stage of each patient; chemotherapy can be used in three different scenarios: induction, concomitant with RT, or adjuvant approach. Otherwise, an assessment of patient's conditions before and after treatments is essential to avoid or control complications such as swallowing disorders.
6. After curative treatments, follow-up strategies must be adopted; in case of incurable disease, periodic evaluations are generally used to understand the effect of therapy or cancer progression. In this peculiar setting, palliative care should be considered.

Recommendations

ESMO

- ► www.esmo.org/Guidelines/Head-and-Neck-Cancers/Squamous-Cell-Carcinoma-of-the-Head-and-Neck

ASCO

- ► www.asco.org/practice-guidelines/quality-guidelines/guidelines/head-and-neck-cancer#/34961
- ► www.asco.org/practice-guidelines/quality-guidelines/guidelines/head-and-neck-cancer#/32806
- ► www.asco.org/practice-guidelines/quality-guidelines/guidelines/head-and-neck-cancer#/28176

AIOM

- ► https://www.aiom.it/linee-guida-aiom-tumori-della-testa-e-del-collo/

Hints for a Deeper Insight

- The potential for liquid biopsies in head and neck cancer: ► https://www.ncbi.nlm.nih.gov/pubmed/29906408
- Experiences of psychological flow as described by people diagnosed with and treated for head and neck cancer: ► https://www.ncbi.nlm.nih.gov/pubmed/31622871
- The emerging use of immune checkpoint blockade in the adjuvant setting for solid tumors: a review: ► https://www.ncbi.nlm.nih.gov/pubmed/31621445
- Immunotherapy for head and neck cancer : Highlights of the 2019 ASCO Annual Meeting: ► https://www.ncbi.nlm.nih.gov/pubmed/31612261

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Central Nervous System Malignancies

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Central Nervous System Malignancies

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Learning Objectives

By the end of this chapter, the reader will:

- Have learned the key facts of epidemiology and pathophysiology of CNS malignancies and BMs
- Recognize the clinical presentation of CNS malignancies and BMs
- Be able to plan the appropriate diagnostic work-up
- Be able to manage symptoms
- Have learned the basic concepts of treatment

44.1 Introduction

The most frequent malignancies of the central nervous system (CNS) are not primitive neoplasms, but metastasis originating from other sites, such as lung (small cell lung cancer), breast, and skin (melanoma) are the most frequent intracranial lesions [1]. The primitive neoplasms of the CNS are a rare and heterogeneous group of malignancies with different biological behavior and consequently with different prognosis (Fig. 44.1).

In some cases, CNS malignancies can be part of manifestations of a genetic syndrome, such as von Hippel-Lindau (VHL) or neurofibromatosis. In these rare circumstances, the patients show other neoplasms in different sites (kidney, skin, etc.) with facial abnormalities or peculiar manifestations (café-au-lait macules) [2]. In the following sections, a synthetic view of the most important and frequent CNS neoplasms will be provided with a particular insight to the 2016 WHO classification and, subsequently, the mainly pathophysiologic features of brain metastasis (BMs).

44.2 Primary Brain Tumors

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44.2.1 Classification

The WHO classification provides a good system to categorize this heterogeneous group of CNS neoplasms. The 2007 version was replaced in 2016 with the introduction of new categories and groups of tumors together with a fundamental role of the molecular biology. The integration of genetic and phenotypic aspects is the milestone of the new classification [3] (Fig. 44.1).

44.2.2 Diagnosis

Frequently, in case of asymptomatic lesions like meningiomas, diagnosis could be accidental. However, in presence of signs and symptoms of CNS involvement, a correct diagnostic process must be established. First of all, personal and family history of the patient should be collected in order to focus on the symptoms' onset or to investigate the exposure to risk factors. Then physical and neurological examinations should be performed: focal signs and symptoms can conduct to the correct imaging investigation. Indeed, if the clinical suspect is a neoplasm, the patient should be addressed to a contrast MRI exam. Obviously, other tests, such as CT scan,

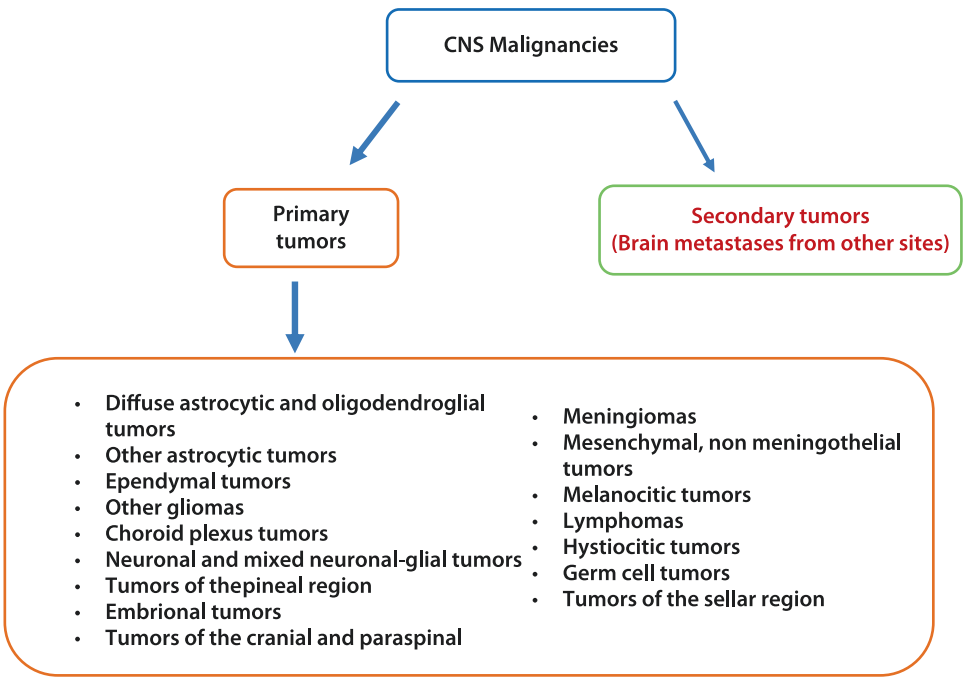

Fig. 44.1 2016 World Health Organization (WHO) Classification of tumors of the Central Nervous System: A summary

angiography, or PET can be used also as pre-operative investigations [4].

44.2.3 Gliomas

The new 2016 WHO classification has redrawn this group of tumors, which include astrocytomas (II and III grade), oligondendrogliomas (II and III grade), and glioblastomas (IV) together with diffuse gliomas typical of childhood.

The evaluation of the IDH1 status is the first step in the classification system. We can thus identify "IDH mutant gliomas" and "IDH wild type gliomas."

In the context of IDH mutant gliomas, other features must be evaluated to distinguish oligodendrogliomas from astrocitomas, as the co-deletion of 1p/19q and the mutation of ATRX and tp53.

Co-deletion of 1p/19q: diagnosis of oligodendrogliomas (II and III grade).

Mutation of ATRX and tp53: astrocitomas (II and III grade).

Moreover, it is possible to identify different types with different prognosis and molecular features:

- *Diffuse low-grade gliomas*: These include WHO grade II diffuse astrocytomas and oligodendrogliomas, which can be divided into two groups considering the mutation of IDH-1. Early and maximal safe resection is the initial treatment for those patients. Post-operative treatment decisions are based on risk stratification, although the delineation between low-risk and highrisk glioma is highly variable. The wild-type forms are typically diagnosed in elderly patients and the prognosis is poor. Patients with a mutation of IDH-1 are usually younger (≤40 years) and they have best prognosis. In these patients, a watch and wait policy with MRI every 3–6 months is accepted. Patients with highrisk lowgrade gliomas are clinically defined as older than 40 years, have neurological symptoms, large tumor (>5 cm), or subtotal resection. In these cases, current postsurgical standard of care is focal radiotherapy to 50–54Gy followed by six cycles of adjuvant treatment with procarbazine, lomustine, and vincristine (PCV) [5].
- *Diffuse high-grade gliomas*: These include WHO grade III anaplastic astrocytomas and anaplastic oligodendrogliomas. The standard of care for patients with highgrade gliomas is maximal safe surgical resection followed by chemoradiation. Chemoradiation consists of radiotherapy to 60 Gy with either six cycles of adjuvant PCV or concurrent and adjuvant temozolomide. It is important to notice the role of temozolomide in newly diagnosed anaplastic astrocytomas without 1p/19q co-deletion: in this case temozolomide in the adjuvant setting is linked to a longer overall survival after radiotherapy [6]. Bevacizumab is an option in case of relapse.

44.2.4 Glioblastomas

Glioblastoma is the most lethal of the primary brain tumors in adults.

With the new classification, three different groups of this neoplasm were recognized:

- *IDH1 wild-type glioblastoma*: it is the most frequent form and in general arises in patients older than 55 years old. It can be described as a "the novo" type to easily distinguish it from the other form of glioblastoma, which usually develops after previous gliomas. The milestone of treatment consists in the association of radiotherapy and temozolomide (concurrent and adjuvant), which have improved survival of patients with glioblastoma [7].

Given the important role of temozolomide, the analyses of MGMT promoter methylation in the first steps of the diagnostic process has a prognostic relevance and may inform the treatment, especially in older patients [8]. Another possible treatment is the tumor-treating field, but it is not used today as a first line therapy. In case of progression, nitrosureas, bevacizumab, or temozolomide rechallenge should be considered.

- *IDH1 mutant glioblastoma*: This considers younger patients with a prior diagnosis of lower grade diffuse glioma. The treatment is quite similar to the group of anaplastic astrocytomas [9].
- *NOS glioblastoma*: In this group, all the forms in which a correct evaluation of IDH was not possible are categorized [10].

Recently regorafenib, an oral multikinase inhibitor of angiogenic, stromal, and oncogenic receptor tyrosine kinases, showed an encouraging overall survival benefit in recurrent glioblastoma [11].

44.2.5 Gliomatosis Cerebri

A diffuse clinical involvement defines a clinical condition actually called gliomatosis cerebri, whose treatment differs from other types of malignant forms. In fact, surgery

is not the standard of care and some studies have shown an advantage of temozolomide as first line therapy [12].

44.2.6 Ependymomas

Ependymomas are not frequent CNS malignancies. These tumors account for 3.5% of all cases [13] and they usually develop in the IV ventricle, also in children.

Other sites are III ventricle and the vertebral canal. Obviously, signs and symptoms vary according to the site of the tumor, but often they are linked to cranial hypertension. There is also the possibility of spread into the vertebral canal and it depends on the site and grade of the neoplasm.

The risk factors, which can modify the prognosis, are age, the site of the neoplasm, histological grade, and the possibility to perform a curative surgery, although some of them appear controversial even today and there is no solid consensus [14].

In the last 2016 classification, a new variant was introduced: ependymoma RELA fusion-positive, which is actually typical among children. Surgery is the main option in trying to preserve the normal neurologic functions. Post-operative radiotherapy should be considered in case of grade III tumor [15] as some studies have shown an advantage in terms of overall survival. In case of relapse, a medical treatment with cisplatinum or temozolomide should be used [16].

44.2.7 Medulloblastoma

Medulloblastoma is a neoplasm which arises from precursor neuronal cells in the posterior cranial fossa; meanwhile, it is the most frequent tumor of the CNS among children; in adults, it is quite rare. Signs and symptoms are mostly caused by its position and they can be considered a direct consequence of cranial hypertension. The new classification has introduced new molecular entities and actually, they should be integrated with the well-known histologic types, which are as follows:

- Classic
- Desmoplastic/nodular
- Extensive nodularity
- Large cells/anaplastic

The molecular groups are as follows:

- WNT-activated
- SHH-activated
- Group 3
- Group 4

The standard treatment in adults with a standard risk is post-surgery radiotherapy. New studies have shown a better prognosis for patients treated with the combination of both radiotherapy and chemotherapy (cisplatin, etoposide +/− cyclophosphamide) [17].

In case of high risk patients, a combination of radio therapy and chemotherapy is recommended.

During the follow-up, a multidisciplinary approach should be considered for each patient for a correct evaluation of the endocrine, neurologic, and cognitive aspects [18].

44.2.8 Meningiomas

Meningiomas are a group of neoplasms arising from the meninges, which represents the second most frequent tumor in the CNS.

Some well-defined risk factors are: genetic syndromes, such as Neurofibromatosis 2 (NF2), and radiation exposure. Meningiomas are much more frequent in women and a hormonal role in the development of these tumors has been hypothesized [19].

Most frequently, they are diagnosed accidentally, but otherwise, in some cases, they can cause symptoms, such as headache, seizures, nausea, and vomiting as part of a cranial hypertension syndrome.

There are different strategies in case of a diagnosis of meningioma: if it is asymptomatic and characterized by a slow-growth rate, a watchful-wait can be a good approach, while if the neoplasm is associated to symptoms or has a high-growth rate, surgery can be the first approach.

In case of recurrence or unresectable masses, radiotherapy is usually used; chemotherapy is addressed to those patients with a relapse disease who cannot be treated again with surgery or radiotherapy even if solid evidences are lacking [20].

44.2.9 Other Tumors

Other forms of neoplasms which concern the CNS system are as follows:

- Neuromas: This tumor has its origin from the VIII cranial nerve and, even rarely, can be part of a genetic syndrome, such as Neurofibromatosis 2. In most cases, it is localized in the pontocerebellar angle and the most important symptom is tinnitus.
- Pituitary gland neoplasms: Usually they are adenomas and can adopt both secretory and non-secretory forms. Symptoms can depend on both the local compression and the hormones which can be secreted. These neoplasms can be divided into macro and micro adenomas and the standard imaging exam is MRI. In most cases, surgery is the main treatment.

Just in case of a prolactinoma, a medical treatment can be used as Dopamine agonists show an inhibitory effect; another option can be gamma-knife treatment.
- Pineal gland tumors: Mainly are germinomas and can be characterized for focal symptoms due to local compression.

44.2.10 Genetic Syndromes

Some genetic syndromes can be characterized for the presence of benign or malign neoplasms in the CNS. Even if they account just for a minority of cases, they should be always taken into consideration in young patients with different lesions also in other sites, such as kidney or skin.

Neurofibromatosis 1 is an autosomal dominant syndrome caused by a mutation in NF1 gene (chromosome 17). It is easy to recognize thanks to peculiar skin lesions called café-au-lait macules, together with neurofibromas and axillary freckling. Regarding the CNS, astrocytomas, meningiomas, gliomas, and ependymomas can appear. Other clinical manifestations can be seizures, mental retardation, and hydrocephalus. *Neurofibromatosis 2* is characterized by a mutation in NF2 gene (chromosome 22). Differently to NF1, café-au-lait macules are less frequent; typically it is possible to find bilateral neuromas which involve the VIII cranial nerve. In addition, in this case, other possible manifestations are meningiomas and gliomas.

In the *von Hippel-Lindau syndrome*, the involvement of CNS is limited to the cerebellum, where the growth of hemangioblastomas is possible; these patients can also suffer from kidney cancer, pheochromocytoma, or liver and pancreatic cysts [21].

Key Points
- Personal and family histories of the patient, together with a correct physical and neurological examination, are fundamental steps to choose the best imaging test in order to confirm or exclude the diagnosis of a CNS neoplasm.
- In some rare cases, CNS neoplasms can be part of a genetic syndrome, above all in young patients.
- The new WHO classification has improved the role of biological features of CNS malignancies, such as IDH mutation or tp53/ATRX. Biological features are very important for diagnosis and prognosis.
- In most cases, surgery represents the most important treatment. Maximal safe resection improves functional status and reduces mortality in both lowgrade and highgrade glioma. Other options are radiotherapy and chemotherapy as for glioblastomas (temozolomide with radiotherapy).
- Medulloblastoma is a frequent neoplasm in children, but much less frequent in adults; it is located in the posterior cranial fossa and it can be treated with different approaches.
- Meningiomas are frequent neoplasms; they are usually treated in case of symptomatic conditions.

Recommendations
- ASCO
  ► https://www.asco.org/research-guidelines/quality-guidelines/guidelines/Neurooncology
- Hints for a deeper insight
- The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary
  ► https://link.springer.com/article/10.1007/s00401-016-1545-1
- PD-1/PD-L1 immune-checkpoint inhibitors in glioblastoma: A concise review
  ► https://www.sciencedirect.com/science/article/abs/pii/S1040842818303172?via%3Dihub
- AIOM
  ► https://www.aiom.it/linee-guida-aiom-neoplasie-cerebrali/

44.3 Brain Metastases

Alessandro Inno and Stefania Gori

Brain metastases (BMs) occur when cancer cells originating in tissues outside the central nervous system (CNS) spread secondarily to the brain. They represent a common complication of many cancers, mainly lung cancer, breast cancer, and melanoma.

The incidence of BMs is thought to be increasing over time due to a combination of factors, including: (1) the improvement in the quality of neuroimaging together with a more frequent use of routine imaging studies of the brain, leading to early detection of clinically silent lesions; (2) effective systemic therapies for the primary cancer resulting into extended survival of cancer patients, thus leading to a larger population of cancer patients at risk for BMs [22].

The occurrence of BMs is generally associated with an adverse impact on survival and quality of life. In fact, despite recent advances in the diagnosis and management of this condition, BMs still carry a dismal prognosis and, therefore, represent an unmet clinical need.

44.3.1 Epidemiology and Risk Factors

BMs are the most frequent intracranial tumor, occurring up to ten times more frequently than primary brain tumors, although their exact incidence is not known. In population studies, the incidence of BMs among cancer patients ranged from 8.5% to 9.6% [23–27]. However, population studies may underestimate the true incidence of BMs. Data from old autopsy studies, in fact, suggest higher frequencies and it is now believed that 20–40% of patients with metastatic cancer will develop BMs during the course of the disease [28, 29].

Virtually, any primary cancer may spread to the brain. The majority of BMs originate from lung cancer (40–50%), breast cancer (15–30%), melanoma (5–20%), kidney cancer (3–10%), colorectal cancer (3–10%) and unknown primary (3–15%) [30, 31]. Therefore, lung cancer, breast cancer, and melanoma account for approximately 80% of BMs overall. However, in more recent cohorts, an increasing prevalence of BMs from colorectal and kidney cancers was reported, possibly due to general improvement in detection, treatment, and prognosis of these two types of cancer [27].

According to the number of brain lesions and the extent of systemic disease, the metastatic involvement of the brain may be defined as follows: solitary BM, in presence of only one brain lesion with a controlled primary tumor and no other metastases; single BM, in presence of only one brain lesion with an active primary tumor and/or systemic metastases; oligo BMs in presence of 2–3 brain lesions; multiple BMs in presence of more than 3 brain lesions [30].

Surgical series and cohort studies reported that among patients with BMs, approximately 40–45% present with one brain lesion, 25–30% with 2–3 lesions, and 20–30% with more than 3 lesions [31, 32]. Breast, colorectal, and kidney cancers have a slightly higher likelihood to be associated with a single BM, whereas lung cancer and melanoma are more likely to develop multiple BMs [30]. The number of patients with a single BM has decreased over time, whereas the proportion of patients with three or more BMs has increased, and this is likely due to the more frequent use of contrast-enhancement brain magnetic resonance imaging (MRI) in the diagnostic work-up.

According to the timing of diagnosis of BMs, they can be classified as: synchronous, if BMs are diagnosed within 2 months from the diagnosis of the primary tumor; metachronous, if BMs are diagnosed more than 2 months after the diagnosis of the primary tumor.

Synchronous BMs are most frequent in lung cancer, while for breast cancer, BMs often represent a late event, with a median time interval of more than 3 years between the diagnosis of primary breast cancer and detection of BMs [31].

The simultaneous diagnosis of BMs and primary cancer has become more common over time, likely because of a more frequent use of neuroimaging in the initial staging assessment [27]. In a recently published descriptive analysis of 2419 patients with BMs, in fact, approximately a quarter of patients presented with synchronous diagnosis of primary tumor and BMs, and 20% of patients received the diagnosis of BMs through routinely performed radiological staging procedures [31].

Several potential risk factors for the development of BMs have been investigated, particularly in breast cancer. Initial studies identified lung metastases as first site of relapse and a negative hormone receptors status as risk factors for the occurrence of BMs in patients with non-brain metastatic breast cancer [33]. Refined knowledge of the molecular classification of breast cancer led to the observation that different intrinsic molecular subtypes are associated with distinctive patterns of metastatic spread, with HER2-positive and triple negative breast cancer having higher the risk of developing BMs, as compared with luminal A subtype [34].

In 2010, Graesslin and colleagues identified age, tumor grade, negative status of hormone receptors and HER2, number of metastatic sites, and short disease-free survival as independent risk factors for subsequent BMs in patients with non-brain metastatic breast cancer. Based on this data, a prediction nomogram for BMs in metastatic breast cancer was developed [35] and, more recently, its validity and exportability were further confirmed by an external validation study [36].

Similarly, nomograms for the prediction of BMs were developed also for patients with non-small-cell lung cancer (NSCLC). Tumor histology, smoking status, pT stage, and the interaction between adenocarcinoma and pN stage were used in a Korean study to build a nomogram for the prediction of BMs as first site of relapse [37], whereas, in a Chinese study, neuron-specific enolase, histology, number of metastatic lymph nodes, and tumor grade were included into a nomogram for predicting BMs in patients with curatively resected NSCLC [38].

Nomograms to predict BMs may represent a helpful tool for identifying high-risk patients in order to personalize follow-up or select candidates for trials specifically designed to evaluate preventive interventions.

44.3.2 Pathophysiology

The propensity to generate BMs differs among different tumor types and also among different cellular clones of the same tumor [39]. Not all cells of a given tumor are able to reach the brain and lead to macroscopic BMs, therefore primary tumors and corresponding BMs may be biologically different [40]. According to the "seed" and "soil" hypothesis, the development of BMs is possibly related to a series of unique characteristics of some tumor cells that allow them to find the brain microenvironment a favorable place for their growth, and that are not necessarily required for successful growth at other organs [41]. Recent investigations are beginning to shed some light into cellular and molecular mechanisms of BMs development. For instance, in metastatic breast cancer, cyclooxygenase-2 (COX2), the epidermal growth factor receptor (EGFR) ligand HB-EGF and a2,6-sialyltransferase ST6GALNAC5 have been identified as mediators of cell passage through the blood–brain barrier (BBB) [42], and the upregulation of SOX2 and OLIG2 genes seems to play a role for the growth of BMs [43].

The metastatic process is a complex series of sequential events governed by a cascade of molecular changes [44]. Metastatic cells that successfully colonize the brain must complete the following steps:

1. Invasion
   Tumor cells dissociate from the primary tumor mass by the loss of the cell-cell adhesion capacity and invade the surrounding stroma through the upregulation of matrix-degrading enzymes and dysregulation of proteins involved in cell motility and migration. In this phase, tumor initiates angiogenesis, which is necessary for tumor growth and also provides a route for detached cells to enter the circulatory system.
2. Intravasation
   Tumor cells interact with more permeable tumor-induced endothelial cells, produce enzymes that degrade the vessel basement membrane, and enter the lumen of capillaries or lymph channels, thus spreading through venous circulation.
3. Transportation
   Once in the bloodstream, tumor cells must avoid detachment-induced apoptosis and escape destruction by the immune system and the mechanical forces to survive.
4. Extravasation
   Through the bloodstream, circulating tumor cells reach the arterial vessels that supply the brain. Access of tumor cells to the brain is governed by the BBB, a physiologic and anatomic structure composed by a monolayer of specialized endothelial cells connected by tight junctions and surrounded by a thick basement membrane without fenestration, and underlying astrocytes that regulate the flow of nutrients, ions, and cells into the brain. The arrest of tumor cells into brain microvessels is favored by specific adhesion molecules to brain endothelials cells, and the process of extravasation and invasion of BBB requires the expression of various cell surface receptors and degradative enzymes.
5. Growth:
   Once in the brain, tumor cells may die or remain quiescent in a dormant state for months or even for years, if the soil is not propitious for tumor growth. Alternatively, the brain may provide a hospitable microenvironment and several growth factors, such as nerve growth factor (NGF) or vascular endothelial growth factor (VEGF), may facilitate the proliferation of tumor cells and the development of macroscopic BMs.

Each step of the metastatic cascade is relatively inefficient and only a small number of primary tumor cells that reach the bloodstream is able to form viable BMs [45].

Although occasionally BMs may occur by direct extension into the CNS from the primary tumor (for instance in case of outer ear, mastoid, rhinopharyngeal, paranasal sinus, or orbital cancer) or from skull metastases, the vast majority of BMs result from hematogenous spread. Therefore, the pattern of distribution of BMs reflects the proportional blood flow to the different anatomical regions of the brain [46], with nearly 80% of BMs involving the cerebral hemispheres, followed by cerebellum (15%) and brainstem (5%).

Within the brain vasculature, single malignant cells or tumor emboli may be entrapped in small size terminal arteries. This might explain the propensity of BMs to develop at the gray/white matter junction and in wathershed zones of the cerebral circulation [47].

44.3.3 Clinical Manifestations

Approximately two-thirds of patients with BMs develop neurologic symptoms. Symptoms are extremely variable depending on the location of BMs [48, 49].

Clinical presentation is similar to that of other brain tumors and includes the following:

- Headache
- Seizures
- Nausea and/or vomiting
- Focal neurological dysfunction
- Cognitive dysfunction
- Gait disorders

- Nuchal rigidity
- Photophobia

Headache is the most frequent symptom, occurring in 40–50% of patients with BMs [50]. It may be located on the same side of the tumor mass but can be also diffuse. Patients with multiple BMs or with lesions located in the posterior fossa are at higher risk of headache. Metastasis-related headache is generally a manifestation of intracranial hypertension and it can mainly occur in early morning hours, can be associated with nausea, vomiting, and transient visual impairment, and can be also exacerbated by cough or straining. These typical features, however, are present only in a minority of patients. In most cases, metastasis-related headache is indistinguishable from tension headache or migraine. Therefore, headache characteristics, other than recent worsening, usually fail to predict reliably the presence of BMs, unless focal deficits or papill-edema coexist [51].

Seizures occur as the first manifestation of BMs in 20% of patients and a similar percentage of patients may develop symptomatic epilepsy at some point in the course of their disease [52]. Multiple BMs or metastatic melanoma are associated with an increased risk of seizures. Metastasis-related seizures are generally focal with or without secondary generalization.

Focal neurological dysfunction, such as weakness of one limb or hemiparesis, with or without sensory changes, language disorders or visual deficits, is the presenting sign of BMs in 20–40% of patients. Gait disorders characterized by unsteadiness, short steps and widening of lower limbs may develop even in the absence of focal motor deficit and they are typically caused by multiple, bilateral, small size BMs [49].

Patients with BMs can also have cognitive dysfunction, including memory problems and mood or personality changes, especially in case of multiple BMs [53]. Nuchal rigidity or photophobia represent signs of meningeal involvement.

In the majority of cases, the onset of symptoms is subacute due to the gradual growth of the tumor mass and surrounding edema, although some patients can present acutely with seizures or with neurological signs and symptoms resembling stroke or transitory ischemic attack. An acute onset may be due to metastatic hemorrhage, embolization of tumor cells, invasion or compression of cerebral artery by the tumor mass. Melanoma and renal carcinoma are more often associated with hemorrhagic brain metastases [54].

44.3.4 Diagnosis

Neurologic symptoms that are suggestive for BMs require always appropriate investigation, both in patients with and those without a known history of cancer. In fact, neurologic symptoms may represent the first presentation of cancer in about 15% of patients, thus appearing before systemic cancer is diagnosed. On the other hand, up to 10% of brain lesions in cancer patients can be non-metastatic. Differential diagnosis includes primary brain tumors, abscesses, demyelinating diseases, cerebral infarctions or hemorrhages, intracranial hematomas, progressive multifocal leukoencephalopathy, intravascular thrombosis and radiation necrosis [49].

Brain imaging plays a key role for the diagnosis of BMs in cancer patients who develop new neurologic symptoms, but also for the screening of BMs in asymptomatic cancer patients at high-risk of CNS involvement. Computed tomography (CT) scan and MRI represent the key imaging modalities for the diagnosis of BMs (Fig. 44.2) [55].

At imaging, BMs are usually spherical, solid, or cystic, and well-circumscribed lesions of various sizes

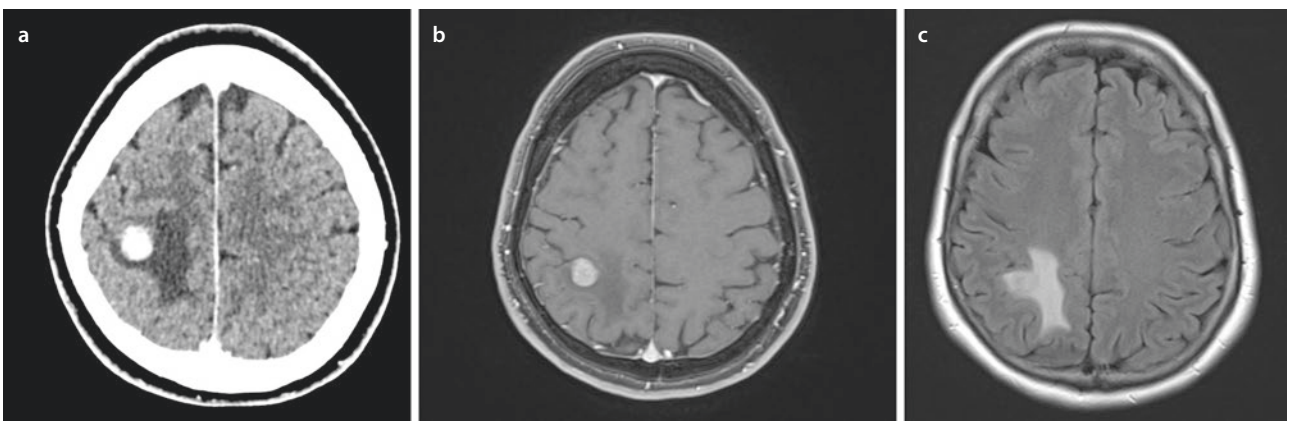

Fig. 44.2 Right parietal BM from lung adenocarcinoma, with surrounding edema, as showed by: **a** contrast-enhanced CT scan; **b** T1-WI MRI with gadolinium; **c** T2-WI MRI

located at the cortico-medullary junction or in watershed areas, with varying amounts of surrounding edema. On non-enhanced CT scan, BMs usually are hypo- or isodense lesions, although hemorrhagic metastases or metastases from melanoma may appear as hyperdense lesions. After iodinate contrast injection, BMs demonstrate enhancement, with surrounding vasogenic edema appearing as hypodense area [56].

Although CT scan is able to detect BMs, MRI represents the gold standard for the diagnosis because of its higher resolution, superior tissue contrast, and no bone artifacts. Standard MRI sequences include T1-weighted imaging (T1-WI) with or without contrast medium, T2-weighted imaging (T2-WI), and fluid-attenuated inversion recovery (FLAIR). On T1-W1, BMs usually generate a low-intermediate intensity signal surrounded by a decreased signal in case of peritumoral edema. When BMs show increased intensity, it can be a sign of intralesional hemorrhage or melanin deposits. After injection of paramagnetic contrast, BMs are often enhanced and may present peripheral ring enhancement with a non-enhancing core corresponding to central necrosis. On T2-WI and FLAIR sequences, both BMs and surrounding edema appear as an area of increased intensity. Metastases from mucinous gastrointestinal adenocarcinomas may appear hypointense in T2-WI, because of high protein content within the lesions. Advanced MRI techniques, such as diffusion-weighted MRI, perfusion MRI, and MRI spectroscopy, represent additional tools for distinguishing BMs from other entities, such as high-grade primary glial tumors, CNS lymphomas, or cerebral abscesses [49, 57].

Fluorodeoxyglucose (FDG) - positron emission tomography (PET)/CT scan is an increasingly used tool in the staging of cancer, particularly lung cancer. However, it is not as sensitive as MRI in the evaluation of BMs [58]. In fact, some BMs may manifest as focal hypermetabolic areas, therefore difficult to detect within the normal cerebral cortex which is FDG avid, or may appear as focal hypometabolic areas indistinguishable from other non-neoplastic conditions, such as brain infarction. Therefore, PET/CT is not routinely indicated for the assessment of BMs. In selected cases, however, FDG-PET/CT or PET/CT with aminoacidic tracers, such as 18F-Tirosine may be helpful to distinguish hypermetabolic local recurrent BMs from hypometabolic post-radiation necrotic lesions [59, 60].

In patients with history of cancer, imaging is generally sufficient to provide diagnosis of BMs, and in most cases, histological confirmation is not required. Stereotactic or open biopsy, or surgical resection of a cerebral lesion should be considered only if at imaging there is some concern regarding diagnosis of BMs, and in selected patients with BMs as the only site of relapse after successful treatment of the primary tumor and no evidence of extracranial disease.

In patients without known history of cancer, the identification of primary tumor is part of the diagnostic procedure. The diagnostic work-up should consist at least in complete clinical examination, including skin scrutiny, chest and abdomen contrast-enhanced CT and, if CT scan does not show any evidence of primary or systemic cancer, a whole-body FDG-PET/CT. When these examinations are inconclusive, then stereotactic or open biopsy or surgical resection should be performed to establish histological diagnosis and orient to the location of primary tumor [61].

44.3.5 Treatment

(a) *Prognostic Factors*

Despite active treatments, prognosis of patients with BMs remains poor, with a wide heterogeneity of outcomes depending on several prognostic variables. In this regard, a sound prognostic classification is important for both clinical decision-making and design of clinical trials.

In 1997, the Radiation Therapy Oncology Group (RTOG) developed a prognostic index for patients with BMs performing a recursive partitioning analysis (RPA) from a database of 1200 patients treated with whole brain radiotherapy (WBRT) from three RTOG trials conducted between 1979 and 1993 [62]. Based on Karnofsky performance score (KPS), age, control of primary tumor, and extent of extracranial disease, three prognostic classes with different median survival times were identified: class I (patients with KPS $\geq$ 70, age < 65, controlled primary tumor and no extracranial disease), with a median survival of 7.1 months; class II (KPS $\geq$ 70 and one of the following: age $\geq$ 65, uncontrolled primary or extracranial disease), with a median survival of 4.2 months; class III (KPS < 70), with a median survival of 3.4 months.

Since then, several other models have been developed, with the aim to further assess prognostic factors and better predict survival of patients with BMs. Among them, the grading prognostic assessment (GPA), developed in 2008 by Sperduto et al., is considered the least subjective, most quantitative, and based on the most current data from randomized trials [63]. Compared with RPA, GPA excluded the estimation of control of primary tumor, which is subjective and often difficult to evaluate, whereas included the number of BMs, which had proven to represent a relevant prognostic factor.

Table 44.1 Graded prognostic assessment (GPA)

	Score		
	0	0.5	1
Age, years	>60	50–59	<50
KPS	<70	70–80	>80
No. of CNS metastases	>3	2–3	1
Extracranial metastases	Present	–	Absent

CNS central nervous system, *KPS* Karnofsky performance score

According to GPA, a score was assigned for each considered prognostic factor (age, KPS, number of BMs, presence or absence of extracranial metastases), as shown in Table 44.1.

On the basis of GPA score, four prognostic groups were identified with significantly different median survival times:

- GPA 0–1, 2.6 months
- GPA 1.5–2.5, 3.8 months
- GPA 3, 6.9 months
- GPA 3.5–4, 11.0 months

The original GPA was subsequently refined with diagnosis specific indexes (DS-GPA) which considered different significant prognostic factors for each cancer type (lung, breast, GI, kidney, and melanoma), as summarized in Table 44.2 [64, 65]. Of note, besides clinical prognostic factors, a relevant prognostic value of molecular characteristics was recognized for breast cancer and NSCLC. Particularly, the status of hormone receptors and HER2 is an integral part of the breast-GPA [66, 67] and, more recently, *EGFR* mutational status and rearrangements of *ALK* have been included into the updated version of DS-GPA for NSCLC, namely, the Lung-molGPA [68].

(b) *Management*

The management of BMs consists of a multimodal approach, including symptomatic treatment, local therapy, such as surgical resection and/or radiation therapy, and systemic therapy [69]. The therapeutic strategy for the individual patient with BMs depends on several factors including the prognosis of the patient, the status of systemic cancer, and the number, size and location of BMs. The appropriate treatment should be discussed within a skilled multidisciplinary team.

For most patients with newly diagnosed BMs, local treatment is the primary approach:

- For patients with newly diagnosed single BM or oligo-BMs, with good performance status (KPS ≥ 70), life expectancy >3 months, controlled systemic disease and/or available active drugs for systemic disease (RPA class I), upfront treatment options are generally represented by surgical resection or SRS. Surgical resection may be preferred in case of large BMs, BMs surrounded by extensive edema or when histologic diagnosis is needed. The addition of post-operative radiotherapy (WBRT or SRS) or adjuvant WBRT after SRS reduces the risk of intracranial recurrence, without a proven survival benefit.
- For patients with multiple BMs and good prognosis (RPA class I-II), WBRT or SRS represent the primary treatment.
- For patients with BMs and poor performance status (RPA class III) BSC alone is a reasonable option, and alternative options are represented by WBRT or SRS.

Upfront systemic therapy for patients with newly diagnosed BMs can be considered in case of asymptomatic or paucisymptomatic BMs from a chemo-sensitive primary tumor (i.e., germ cell tumor, small-cell lung cancer) or a primary tumor harboring a druggable target (i.e., NSCLC with *EGFR* mutation, *ALK* or *ROS-1* rearrangements, *BRAF*-mutated melanoma or selected cases of *HER2*-positive breast cancer).

A proposal of clinical decision making is summarized in Table 44.3 [70].

For relapsed or progressive BMs, the treatment should be established considering the local and/or systemic therapies previously done.

Symptomatic treatment should be generally offered to all patients with symptoms related to BMs.

(i) *Symptomatic Treatment*

Symptomatic treatment is often used to reduce the symptoms of BMs. It includes medical decompressive therapy for symptoms associated with increased intracranial pressure, antiepileptic drugs for seizures and analgesic medications for headache.

Medical Decompressive Therapy

Vasogenic edema associated with BMs plays a major role in the development of neurologic symptoms. By causing an additional mass effect, often exceeding the volume of the BM itself, edema determines an increased intracranial pressure and also leads to neurological disturbances by reducing local blood flow [71].

Corticosteroids are typically used to control cerebral edema in patients with newly diagnosed BMs [72]. The antiedema effects of corticosteroids is attributed to a reduction in the permeability of abnormal tumor capillaries and a stabilization of the disrupted BBB. Dexamethasone is generally considered the drug of choice because of its minimal mineralocorticoid effect and long half-life, although probably any other

Table 44.2 Disease-specific graded prognostic assessment (DS-GPA)

Primary tumor	Prognostic factor	GPA scoring criteria					Score[a]
Lung cancer							
		0	**0.5**		**1**		
	Age, years	≥70	<70		NA		–
	KPS	<70	80		90–100		–
	ECM	Present	–		Absent		–
	BMs, no	>4	1–4		NA		–
	Gene status	*EGFR* neg/unk and *ALK* neg/unk	NA		*EGFR* pos or *ALK* pos		–
	Total						–
	Adenocarcinoma MS by GPA: 0–1.0, 6.9; 1.5–2.0, 13.7; 2.5–3.0,26.5; 3.5–4.0, 46.8; non-adenocarcinoma MS by GPA: 0–1.0, 5.3; 1.5–2.0, 9.8; 2.5–3.0, 12.8.						
Breast cancer							
		0	**0.5**	**1.0**	**1.5**	**2.0**	
	KPS	≤50	60	70–80	90–100	–	–
	Age, y	≥60	<60	–	–	–	–
	Subtype	Basal like	–	Luminal A	HER2	Luminal B	–
	Total						–
	MS by GPA: 0–1.0 = 3.4; 1.5–2.0 = 7.7; 2.5–3.0 = 15.1; 3.5–4.0 = 25.3						
Melanoma/ RCC							
		0	**1**		**2**		
	KPS	<70	70–80		90–100		–
	BMs, no	>3	2–3		1		–
	Total						–
	Melanoma MS by GPA: 0–1.0 = 3.4; 1.5–2.0 = 4.7; 2.5–3.0 = 8.8; 3.5–4.0 = 13.2: RCC MS by GPA: 0–1.0 = 3.3; 1.5–2.0 = 7.3; 2.5–3.0 = 11.3; 3.5–4.0 = 14.8						
GI cancers							
		0	**1**	**2**	**3**	**4**	
	KPS	<70	70	80	90	100	–
	MS by GPA: 0–1.0 = 3.1; 2.0 = 4.4; 3.0 = 6.9; 4.0 = 13.5						

BMs brain metastases, *ECM* extracranial metastases, *GI* gastrointestinal, *MS* median survival in months, *KPS* Karnofsky Performance Status, *RCC* renal cell carcinoma

[a]Evaluating clinician completes this column

corticosteroid can be effective if given in equipotent doses. Starting doses of 4–8 mg/day of dexamethasone may be considered, unless patients exhibit severe symptoms due to increased intracranial pressure. In these patients, higher doses, such as 16 mg/day or more, should be considered [73]. In view of the definite increase in toxicity with daily doses more than 24 mg and inconclusive dose-response data, daily doses beyond 24 mg are not recommended. The long biological half-life of dexamethasone suggests that the daily dose may be given in two doses, rather than three or four doses. The reported response rates (RRs) in terms of symptom improvements with steroids ranged from 33% to 80% in different studies [74].

Corticosteroids' toxicity includes gastrointestinal adverse events (peptic ulceration, upper gastrointestinal

Table 44.3 Clinical decision-making for BMs

1. Consider systemic therapy when:	BM from highly chemotherapy-sensitive primary tumor BM found on screening MRI with planned systemic treatment BM from primary tumor with identified molecular alteration amenable to targeted therapy Other therapeutic options have been exhausted and there is a reasonable drug available
2. Consider WBRT when:	CNS and systemic progressive disease, with few systemic treatment options and poor PS Multiple (>3) BMs, especially if primary tumor is known to be radiotherapy sensitive Large (>4 cm) BM, not amenable to SRS Postsurgical resection of a dominant BM with multiple (>3) remaining BMs Salvage therapy for recurrent BM after SRS or WBRT failure
3. Consider SRS when:	Oligo-BMs or multiple BMs, especially if primary tumor is known to be radiotherapy resistant Postsurgical resection of a single BM, especially if >3 cm and in the posterior fossa Local relapse after surgical resection of a single BM Salvage therapy for recurrent oligo-BMs after WBRT
4. Consider surgery when:	Uncertain diagnosis of CNS lesion(s) Oligo-BMs, especially when associated with extensive cerebral edema Dominant BM in a critical location
5. BSC alone is reasonable when:	Systemic progressive disease, with few treatment options and poor PS

Modified from Lin and DeAngelis (2015)

BM brain metastasis, *BSC* best supportive care, *CNS* central nervous system, *MRI* magnetic resonance imaging, *PS* performance status, *SRS* stereotactic radiosurgery, *WBRT* whole brain radiotherapy

bleeding or perforation), myopathy, opportunistic infections, cushingoid features, hyperglycemia, behavioral changes (irritability, insomnia, anxiety, depression, and, rarely, florid psychosis), and osteoporosis. Incidence and severity of toxicity are related to higher doses and prolonged treatment duration. Since the majority of responding patients achieve symptoms relief within 48–72 hours, continued use of high starting doses may be neither necessary nor safe. For responding patients, steroid dose has been reduced by 25–50% every fifth day in most studies, although a more rapid tapering every third day may be considered when starting from 16 mg/day, in order to avoid the increased toxicity of steroid use beyond 3 weeks. In patients who worsen on dose reduction, prolonged steroid use may be required. For patients receiving corticosteroids for more than 1 month, prophylaxis of opportunistic infections with trimethoprim/sulfamethoxazole should be considered.

Steroids may be combined with non-steroideal antiedema agents, such as osmotic cerebral decongestants. Of these agents, only mannitol is currently used in clinical practice. Mannitol is generally reserved for severe neurological manifestations or when a rapid reduction in the intracranial pressure is desirable, such as in impending cerebral herniation [75]. The usual dose is 0.75–1 g/kg given intravenously (usually 125 ml of 18% solution is appropriate for adult patients) every 6 hours. Treatment should be continued for up to 48 hours. Animal experiments suggest that a rapid rate of infusion reduces intracranial pressure more effectively than a slow infusion [76]. Serum electrolytes should be monitored with mannitol use and corrected when required.

Antiepileptic Drugs

Patients with BMs presenting with seizures or those who develop seizures during the course of their disease should be started on antiepileptic drugs.

Among antiepileptic drugs, those inducing P450 cytochromes should be avoided in order to prevent interactions with systemic therapies, as well as those with potential neurotoxic effects in order to not aggravate the neurologic state of the patients with new symptoms that could be wrongly interpreted as progressive disease. For these reasons, phenytoin, phenobarbital, carbamazepine, and oxcarbazepine are not routinely used. Levetiracetam (1000 mg/day–3000 mg/day) has emerged as the preferred treatment because it does not induce the P450 system and does not exhibit any relevant drug interactions. Moreover, levetiracetam is generally well-tolerated, although behavioral irritability has been reported. If necessary, the addition of valproate (20 mg/kg/day) may be considered.

In the absence of seizures, prophylactic antiepileptic drugs should not be routinely started [77]. This recommendation is mostly based on the results of a randomized clinical trial of antiepileptic versus non-antiepileptic prophylaxis in 100 patients with primary brain tumors (n = 40) or BMs (n = 60). In the subgroup of patients with BMs there was no significant difference in terms of seizure incidence between the two arms [78]. Given the lack of benefit and the potential risk of adverse events, published guidelines have recommended against the prophylactic use of antiepileptic drugs for patients with BMs [79, 80]. However, these conclusions are based on

data derived from studies with old antiepileptic drugs, including phenytoin and phenobarbital, which are no longer first-choice drugs, whereas newer agents such as levetiracetam, topiramate, lamotrigine, or pregabalin have been not yet systematically investigated in this setting. Therefore, the issue of antiepileptic prophylactic therapy in patients with BMs remains controversial, especially for patients with lesions in highly epileptogenic areas or patients with metastatic melanoma that frequently involves the cerebral cortex.

For patients with BMs undergoing brain surgery, prophylactic antiepileptic drugs may be considered since they reduce the incidence of seizures of 40–50% in the first week after surgery [81]. In patients who do not experience seizures, antiepileptic drugs should be tapered and discontinued after the first post-operative week [80].

(c) *Surgery*

Surgical resection plays a critical role in the treatment of newly diagnosed single BM.

In the 1990s, three randomized clinical trials compared surgery plus WBRT versus WBRT alone for single BM [82–84]. In the first two studies [82, 83], a significant OS benefit for surgery followed by WBRT compared with WBRT alone was reported (approximately 9–10 months vs. 4–6 months) and, in one of these trials [83], the greatest survival advantage was obtained in patients with controlled extracranial disease (12 vs. 7 months; $p = 0.02$). The third trial, which included more patients with an active systemic disease (80% vs. 30–40%) and a lower KPS compared with the first two trials, did not show any survival benefit with the addition of surgery to WBRT [84]. Overall, these data suggest that the survival benefit of surgery is limited to patients with good performance status and controlled systemic disease. There is also some evidence suggesting that in selected patients with 2–3 BMs, complete surgical resection may be beneficial, yielding results that are comparable to those obtained in patients with a single lesion [85].

The goal of surgery is the complete removal of BMs, while protecting functional cortex, subcortical structures, and vascular structures [86]. Although surgical resection is an invasive approach, it is generally well-tolerated in patients with BMs. In fact, a large retrospective review of 208 patients undergoing resection for BMs (191 with single lesions) reported an overall operative mortality of 1.9% [87]. Gross total resection of BMs can be achieved with low morbidity using contemporary image-guided systems, such as preoperative functional MRI, intraoperative neuronavigation, and cortical mapping [88].

Surgical resection allows an immediate relief of symptoms caused by increased intracranial pressure, a reduction of focal neurological deficits, and a rapid steroid tapering in the majority of patients. Furthermore, surgery helps to establish the histological diagnosis in case of unknown primary or multiple primary tumors, or when imaging is not conclusive.

(i) *SRS*

In the 1980s, SRS was introduced as a minimally invasive option as opposed to surgery for the treatment of oligo-BMs [89].

SRS is a type of external radiation therapy delivered in a single dose to a small target volume (3–4 cm) with high precision. SRS requires precise location of the tumor and head immobilization systems. It can be delivered using either gamma-knife, consisting of multiple collimated cobalt-60 sources, or linear accelerator (Linac). There is no difference in outcome between gamma-knife and Linac. Compared to gamma-knife, however, Linac allows treatment of larger, non-spherical lesions and can deliver treatment both in a single dose and in multiple fractions. In the latter case, the technique is called stereotactic fractionated stereosurgery (SFRT), it represents an alternative to single-dose SRS and may be used for patients with larger lesions or lesions located near critical structures [90]. Studies comparing surgery and SRS suggest similar outcomes, although most of them are not randomized trials [91–93]. SRS for newly diagnosed oligo-BMs achieves symptomatic improvement, a local control of 80–90% at 1 year and median OS of 6–12 months [94]. Patients with single BMs, good performance status (KPS > 70) and controlled extracranial disease have longer survival [95]. Age seems to not affect the outcome, since elderly patients achieve the same benefit as younger patients [96]. BMs from radioresistant primary tumors, such as melanoma or kidney cancer, respond to SRS as well as BMs from radiosensitive tumors [97].

RTOG9508, a randomized phase 3 study in patients with 1–3 BMs, investigated the role of SRS + WBRT compared to WBRT alone, reporting better local control and performance status at six months in the combined therapy group [96]. However, a survival advantage was observed only for patients with a single BM and, in a secondary analysis, for patients with good GPA score (3.5–4.0) regardless of the number of BMs [98], and these observations highlight the need for an appropriate selection of patients for SRS.

In the past 5–10 years, SRS has been increasingly used for patients with higher number of brain metastases, due to improved technology that allows the delivery of SRS with increasing speed while maintaining precision and accuracy [95, 99]. A prospective multicenter Japanese study investigated the use of SRS alone in 1194 patients with 1, 2–4 or 5–10 BMs, and found similar OS and treatment-related toxicity rates between the groups with 2 to 4 and 5 to 10 metastases. Cumulative volume

of BMs, rather than the number, was reported as a significant prognostic factor [95].

In recent years, SRS has been used also to treat postsurgical cavities. Several retrospective and one prospective phase 2 trial reported 1-year local control rates ranging from 70% to 90% and a median OS of 10–17 months, suggesting that postoperative SRS may be as effective as WBRT in achieving local control [100]. However, the balance between benefit and risk is currently unknown with unsolved issues (optimal dose and fractionation, impact on survival, quality of life and cognitive function, incidence of complications), therefore randomized trials are needed to clarify the role of postoperative SRS.

Complications of SRS are reported in 10–30% of patients, but severe adverse events are rare. Early complications occur within 2 weeks from treatment and are represented by symptoms related to transient increased intracranial pressure (headache, nausea and vomiting, worsening of preexistent neurological deficits, and seizures) that are generally reversible with steroids. Late adverse events occur months to years after the treatment and include hemorrhage and radionecrosis. The risk of adverse events increases with the increase of lesion size [101].

(ii) *WBRT*

WBRT was historically considered a mainstay in the treatment of BMs and, in the modern era, still plays multiple roles. WBRT is indicated in case of multiple BMs, BMs larger than 4 cm, BMs with poorly controlled systemic disease or BMs in patients with poor performance status. It may be also used as adjuvant therapy with the aim of reducing recurrence after surgery, as salvage therapy after surgery or SRS, or for reirradiation after late WBRT. Standard fractionations are 30 Gy in 10 fractions or 20 Gy in 5 fractions. The addition of radiotherapy sensitizers does not translate into a survival benefit [69, 102].

Different studies in the past reported symptomatic response in up to 60% of patients treated with WBRT, although neurological improvement could be partially attributable to steroids. Median OS reported with WBRT (3–6 months) is longer than that observed in patients not receiving treatment (1–3 months). However, a phase 3 non-inferiority trial on NSCLC patients with BMs that were not candidates to surgery or SRS did not show any survival difference in OS and quality of life between WBRT and BSC [103].

It is still controversial whether, after complete surgical resection or SRS, WBRT should be offered with the aim of destroying microscopic metastatic foci at the original tumor site or at distant intracranial locations. In fact, three large phase 3 trials [104–106] and a meta-analysis [107] demonstrated that omitting WBRT in patients with a limited number of BMs after either complete surgery or SRS results in significantly worse local and distant control in the brain, however, without a significant impact on OS. A recent individual patient data meta-analysis of three randomized studies comparing SRS alone with SRS + WBRT in patients with 1 to 4 BMs suggested a survival advantage for SRS alone and no risk reduction for new BMs with the addition of WBRT in patients aged <50 years, whereas in patients aged ≥50 years the addition of WBRT reduced the risk of recurrence, without improving survival. The reason of these results is not completely clear [108]. WBRT may cause early adverse effects (fatigue, alopecia) and late neurotoxicity. Several studies assessed the impact of adjuvant WBRT on cognitive functions and quality of life, reporting more frequent decline of cognitive functions and more fatigue for SRS + WBRT compared with SRS alone [109–112]. Based on the lack of survival benefit and the increased risk of neurotoxicity, the American Society for Radiation Oncology (ASTRO) has recommended against the routine use of adjuvant WBRT after SRS. The issue of adjuvant WBRT after surgical resection is less well-defined. In a randomized trial on 95 patients with completely resected single BM, the addition of WBRT to surgery compared with surgery alone significantly prevented brain recurrence at site of the original BM (10% vs. 46%, $p < 0.001$) and at other sites in the brain (14% vs. 37%), but, again, without significant difference in survival, that was a secondary endpoint of the study [104].

For patients who do not receive WBRT after surgery or after SRS, close follow up with a brain MRI repeated every 3 months should be performed for early detection and treatment of local or distant brain recurrence. However, it remains unclear whether an active surveillance with salvage local therapy is as effective as immediate adjuvant WBRT. There are no randomized trials in this setting, but case series reported symptom relief in 30–70% patients receiving salvage WBRT [113, 114].

In order to reduce WBRT-associated neurotoxicity, new approaches, including neuroprotective agents and new radiation techniques, have been investigated. In a randomized phase 3 trial, the addition of metamine to WBRT delayed cognitive impairment, but with only 149 patients enrolled, the study was underpowered to achieve significant results [115]. Hippocampus avoidance-WBRT, a novel technique used to reduce the radiation dose to critical hippocampal areas, may be associated with preservation of memory and quality of life without increasing risk of recurrence in the low dose region, as suggested by a phase 2 study [116].

(iii) *Systemic Therapy*

Half of patients with BMs die from progressive systemic cancer, therefore systemic therapy often represents an integral part of the overall treatment strategy. Indeed, the use of systemic therapy as upfront treatment of BMs

has been neglected for years, mainly because of the prevailing belief that antitumor drugs do not cross the BBB and also because patients with symptomatic or uncontrolled BMs have been generally excluded from clinical trials of systemic therapies. However, growing evidence suggests that the presence of macroscopic BMs may disrupt the BBB, thus allowing the penetration of therapeutics into the tumor tissue and providing a rationale for clinical investigations of systemic therapy for BMs [117].

For patients with chemo-sensitive primary tumors or tumors harboring a druggable target, systemic therapy may be a reasonable option for upfront treatment, thus delaying the need for local therapy, especially when tumor burden in extracranial sites is prominent and the control of systemic cancer is an urgent issue. Instead, when BMs are symptomatic, large or located in critical areas, or when primary tumor is low chemo-sensitive or not harboring a druggable target, systemic therapy can be postponed after the local treatment of BMs [70].

The choice of systemic therapy in the individual patients depends on multiple factors, including the performance status of the patient, the tumor type and molecular characteristics, and the previous lines of systemic therapy already administered. The following paragraphs are focused on NSCLC and breast cancer, the two cancers that most often metastasize to the brain.

NSCLC

NSCLC is a heterogeneous disease composed of several molecular subtypes, some of them associated with specific oncogenic drivers amenable to target therapy.

For patients with metastatic NSCLC, harboring an activating mutation in the *EGFR* gene (10–15% of Caucasian patients and up to 50% of Asian patients), standard first-line systemic therapy is an EGFR tyrosine kinase inhibitor (TKI). EGFR-TKIs are active both against systemic disease and BMs. First-generation (gefitinib, erlotinib, icotinib) and second-generation (afatinib, dacomitinib) agents achieve intracranial RRs of 60–80%, with median PFS in the brain of approximately 7–12 months and median OS of 15–20 months [118]. However, the upfront treatment with EGFR-TKIs may be questionable, since retrospective series with first-generation EGFR-TKIs suggest better OS for patients treated with upfront radiotherapy, especially SRS, compared with patients receiving EGFR-TKIs alone [119]. Moreover, after the initial intracranial response, 26–33% patients eventually experience intracranial progression [120].

About 50% of progressive NSCLC acquire the EGFR T790M mutation, that is a well-known mechanism of resistance to first- and second-generation TKIs [121]. Recently osimertinib, a third-generation EGFR-TKI targeting not only the classic EGFR activating mutations but also T790M mutation, has been approved for metastatic NSCLC. Osimertinib has demonstrated promising activity against BMs. In fact, a pooled analysis of two phase 2 studies on CNS response to osimertinib in patients with T790M-positive metastatic NSCLC reported an encouraging intracranial RR and disease-control rate of 50% and 92%, respectively [122]. These data have been corroborated by the results of a phase 3 randomized trial comparing osimertinib versus platinum-pemetrexed as second-line treatment for patients with T790M-mutated metastatic NSCLC who had progressed during receipt of first-line EGFR-TKI [123]. Among the subgroup of patients with BMs, osimertinib achieved a median PFS significantly longer than chemotherapy (8.5 vs. 4.2 months). Osimertinib seems to be even more effective when administered as first-line treatment as demonstrated by the FLAURA study, a phase 3 study on patients with EGFR-mutant NSCLC randomized to receive osimertinib or a first-generation EGFR-TKI (gefitinib or erlotinib) as first line treatment [124]. Among patients with BMs at trial entry (about 20% of the entire trial population), osimertinib achieved higher intracranial RR compared with standard EGFR-TKI (66% vs. 43%), with longer CNS PFS (not reached vs. 13.9 months), longer duration of response (13.8 vs. 8.5 months) and lower frequency of CNS progression [125].

Although *ALK* rearrangements involve a minority of NSCLC (4–6%), up to 50% of patients with ALK-positive NSCLC eventually develop BMs [126]. Crizotinib was the first ALK inhibitor approved. Pooled analysis of a phase 3 randomized trial (PROFILE 1007) and a single-arm phase 2 trial (PROFILE 1005) with crizotinib reported a 56% of intracranial disease control rate at 3 months, with a RR of 18% in patients with previously untreated BMs and 33% in patients who had previously received brain radiotherapy [127]. Second-generation ALK-TKIs, alectinib, lorlatinib, and brigatinib, have demonstrated efficacy in crizotinib-resistant patients. In a pooled analysis of two studies evaluating CNS response of alectinib in pretreated patients, intracranial RR was 64% [127]. When compared head-to-head as first line treatment in randomized phase 3 trials, alectinib demonstrated an intracranial RR higher than crizotinib (59% vs. 26%) [128, 129].

Based on the high intracranial RRs observed with new-generation TKIs in EGFR-mutant and ALK-positive NSCLC patients, upfront systemic treatment with these agents may be a reasonable option, in order to delay the need for local treatment thus preserving neurocognitive functions [130]. However, as discussed before, some evidence suggests that TKIs given after radiotherapy, particularly SRS, may be a more effective approach than target therapy given upfront, but it should be emphasized that such data derive from non-

randomized, retrospective studies with old-generation TKIs [119, 131, 132].

The combination of EGFR-TKIs with radiotherapy is still a controversial approach. In fact, some phase 2 studies investigating the combination of erlotinib plus WBRT or icotinib plus WBRT suggested prolonged survivals [133, 134], but randomized phase 2 and 3 studies on patients unselected for EGFR mutations failed to demonstrate a superiority of the combination of erlotinib with either SRS or WBRT over radiotherapy alone, with higher risk of toxicity [135, 136]. There are only limited data about the safety and tolerability of concomitant ALK-TKIs and radiation therapy [137]. Therefore, a prudential temporary discontinuation of TKIs during radiation therapy may represent an acceptable option. In this regard, SRS has the advantage of few days of temporary systemic therapy discontinuation, as compared to WBRT.

In recent years, immune checkpoint inhibitors have prolonged survival of a subgroup of patients with metastatic NSCLC, and the use of immunotherapy is expected to increase in the near future. Pembrolizumab is approved as first-line treatment for patients with EGFR wild-type, ALK-negative NSCLC with PD-L1 expression ≥50%, and as second-line treatment for patients with PD-L1 expression ≥1%, whereas nivolumab and atezolizumab are approved as second-line treatment, regardless of PD-L1 expression. Although data on immune checkpoint in patients with NSCLC and BMs are limited since most studies excluded untreated BMs and patients requiring a steroid dose ≥10 mg/day of prednisone or equivalent, available evidence suggest activity against BMs. In fact, in a non-randomized, phase 2 study, patients with untreated BMs (18 patients with melanoma and 18 patients with NSCLC) received pembrolizumab, and 33% of patients with NSCLC achieved an intracranial response [138]. Case reports suggest intracranial activity also for nivolumab [139]. Results from larger randomized studies investigating immunotherapy for NSCLC-derived BMs are awaited.

Chemotherapy still represents the mainstay of treatment for many patients with advanced NSCLC who are not candidates to target therapy or immunotherapy. Platinum-based regimens have clinical activity against BMs from NSCLC, with intracranial RR ranging from 23% to 50%, comparable to that expected for the systemic disease. However, the best regimen for BMs has not been identified. In fact, in a randomized phase 3 trial comparing three different chemotherapy regimens (carboplatin plus gemcitabine, paclitaxel plus gemcitabine, or paclitaxel plus carboplatin), in the subgroup of 194 patients with clinically stable BMs, no chemotherapy regimen was proven to be superior to the others in terms of RR, PFS, or OS. In non-squamous histology, the combination of platinum compounds plus pemetrexed has demonstrated interesting activity, with RR of 50% and median OS up to 9 months [140].

Breast Cancer

HER2-positive breast cancer and TNBC have high propensity to metastasize to the brain [141].

The introduction of HER2-targeted agents has dramatically improved the outcome of patients with HER2-positive breast cancer, both in early and in metastatic disease. Despite these improvements, however, approximately 40% of patients with advanced HER2-positive breast cancer relapse in the CNS [142]. Until recently, trastuzumab-based chemotherapy has been the mainstay of treatment for HER2-positive metastatic breast cancer. A survival benefit with trastuzumab was reported also for patients with BMs, but this seems due to the control of systemic disease rather than to an intracranial activity [143]. The addition of pertuzumab to trastuzumab and docetaxel has demonstrated a survival advantage over trastuzumab plus docetaxel, thus becoming the current standard of care as first-line treatment [144]. Unfortunately, patients with BMs were excluded from pivotal trials investigating this combination; therefore, its possible role as upfront treatment of BMs is still unclear. Of note, a secondary analysis of the CLEOPATRA trial reported a longer median time to development of BMs as first site of disease progression for patients in the pertuzumab-trastuzumab-docetaxel arm compared with those in the trastuzumab-docetaxel arm (15.0 versus 11.9 months), suggesting a protective role of the triplet combination against BMs [145]. It is therefore conceivable that for naïve patients, systemic therapy with pertuzumab, trastuzumab, and a taxane following the local treatment of BMs would be a reasonable option to delay disease progression, both in the brain and in extracranial sites.

The dual EGFR/HER2 TKI lapatinib has been extensively investigated in patients with HER2-positive BMs, both as single agent and in combination. As a single agent in pretreated patients, lapatinib has negligible activity with an intracranial RR of less than 3% [146]. Greater activity has been observed in combination with capecitabine, leading to an intracranial RR of 38% in pretreated patients [147], and an intracranial RR of 66% with 1-year survival of 70% in the newly diagnosed setting, as demonstrated by the single-arm phase 2 LANDSCAPE study [148].

Historically, anti-HER2 monoclonal antibodies were thought to be too large to cross the BBB, but there is now evidence from studies utilizing 89Zr-labeled trastuzumab as a PET tracer that there is some penetration of antibodies through BBB disrupted by BMs. This is further supported by accumulating evidence of intracranial activity of T-DM1, with RRs similar to those observed for extracranial disease [149]. Furthermore, an exploratory retrospective analysis of the EMILIA trial showed a survival benefit for patients with brain metastases treated with T-DM1 compared with patients treated with lapatinib and capecitabine (26.8 months versus 12.9 months) [150].

Therefore, for patients with progressive BMs after trastuzumab-based therapy, possible systemic options are T-DM1 or lapatinib plus capecitabine.

Up to 40% of patients with TNBC develop BMs. Unfortunately, no target therapy is available for this subtype, and the only option of systemic therapy is chemotherapy. When treating BMs from breast cancer with chemotherapy, drugs with proven antitumor activity in extracranial sites should be preferred to agents like temozolomide, with known penetration of BBB but limited systemic activity. In fact, temozolomide showed no activity in patients with breast cancer and BMs [151]. Conversely, cisplatin or carboplatin-based combinations achieved objective responses in patients with BMs from breast cancer, particularly TNBC. For instance, a complete brain response of 13% and a partial response of 25% were described with the combination of cisplatin and etoposide in patients with BMs [152]. Data from phase 2 studies also suggest that, in naïve patients with BMs, conventional combination therapies, such as cyclophosphamide/methotrexate/5-fluorouracil (CMF) or 5-fluorouracil/doxorubicin/cyclophosphamide (FAC), may have clinical activity. There is growing investigation of immune checkpoint inhibitors and PARP inhibitors for the treatment of TNBC, but the role of these agents in the treatment of BMs has yet to be elucidated.

(d) *Assessment of Response*

The assessment of response for BMs is still an open issue and there are no standard criteria. Across clinical trials in oncology, dimensional criteria, such as WHO, RECIST, and RECIST 1.1, have been often used to assess the response [153–155]. However, these criteria have many limitations. Particularly, they consider the intracranial and extracranial sites together for the assessment of response, and do not take into account the control of neurologic symptoms, that for patients with BMs represents a crucial goal, as it has an important impact on quality of life. For this reason, Macdonald and colleagues developed response criteria specifically for CNS malignancies [156]. These criteria consider dimensional changes together with neurologic symptoms and need for steroids (Table 44.4).

An important limitation of Macdonald's criteria is that they are derived by WHO criteria which are based on bi-dimensional measurement. Bi-dimensional criteria are more time-consuming and increase the risk of measurement errors compared with uni-dimensional criteria. In order to standardize the response assessment for BMs in clinical trials, the Response Assessment in Neuro-Oncology (RANO) working group has recently proposed new response criteria based on uni-dimensional measurement of lesions, corticosteroids use, and clinical status, but their use in clinical trials is not yet widespread [157].

44.3.6 Conclusion

BMs represent a frequent complication of several solid tumors and their incidence has been rising in the last decades, possibly due to the more widespread use of neuroimaging in asymptomatic patients, but also due to the improved survival of cancer patients obtained by novel anticancer drugs that effectively control systemic disease. Treatment of BMs should be personalized, often requiring a multimodal approach, including both local and systemic treatment.

Although survival of patients with BMs has improved over the last years mainly due to the progress of radiotherapy techniques and the availability of more effective systemic treatments, BMs still have an adverse impact on prognosis and quality of life. The development of more effective treatment for BMs still represents an urgent clinical need.

Table 44.4 Comparison of response criteria

	Image modality	Target lesion	Maximum number of CNS target lesions	Measurement technique	Shrinkage required for partial response	Confirmatory scans	Steroids	Neurological symptoms	Extracranial disease
WHO	Not specified	Minimum size not specified	Not specified	Bi-dimensional	≥50%	Required at least 4 weeks Apart	Not included	Not included	Included
RECIST	CT or MRI	Longest diameter ≥10 mm	5	Uni-dimensional	≥30%	Required in non-randomized trials where response is the primary endpoint	Not included	Not included	Included
RECIST 1.1	CT or MRI	Longest diameter ≥10 mm	2	Uni-dimensional	≥30%	Required in non-randomized trials where response is the primary endpoint	Not included	Not included	Included
Macdonald	CT or MRI	Minimum size not Specified	Not specified	Bi-dimensional	≥50%	Required at least one month Apart	Stable or decreased	Stable to improved	Not applicable

Modified from Lin et al. (2015)
CNS central nervous system, *CT* computed tomography, *MRI* magnetic resonance imaging, *RECIST* Response Evaluation Criteria in Solid Tumors, *WHO* World Health Organization

Expert Opinion

Christian Rolfo

- Although any cancer may virtually spread to the brain, the majority of BMs originate from lung cancer, breast cancer, melanoma, kidney cancer, colorectal cancer, and unknown primary.
- In case of neurologic symptoms suggestive for BMs, brain CT scan and/or MRI represent the mainstay of the diagnostic work-up.
- An accurate prognostic assessment through validated prognostic indexes may inform the decision-making process.
- Clinical management is based on a multidisciplinary approach including symptomatic treatment, surgery, radiotherapy (SRS or WBRT), and systemic therapy.
- The goal of symptomatic treatment is to reduce symptoms associated with BMs, particularly symptoms related to cerebral edema through the administration of steroids or mannitol, and seizures through the administration of antiepileptic drugs.
- Surgery represents an option for patients with good performance status and single or oligo-BMs, especially when associated with extensive edema or in case of uncertain diagnosis.
- SRS is a non-invasive approach for patients with good performance status and oligo-BMs, and for selected patients with multiple BMs it can be also delivered as adjuvant treatment after surgical resection of a single BM, or as salvage treatment for recurrence after surgery or WBRT.
- WBRT may be considered for patients with multiple BMs, or as salvage treatment for recurrence after surgery or SRS.
- Systemic treatment is often an integral part of the overall treatment strategy, mainly for the control of systemic disease; some anticancer agents such as *EGFR* or *ALK* inhibitors for oncogene-addicted NSCLC or anti-HER2 drugs for HER2-positive breast cancer have also activity against BMs.

Recommendations

- NICE
  ► https://www.nice.org.uk/guidance/ng99/chapter/Recommendations

Hints for a Deeper Insight

- Recent advances in managing brain metastasis**:** ► https://www.ncbi.nlm.nih.gov/pubmed/30473769
- Brain metastases: radiosurgery**:** ► https://www.ncbi.nlm.nih.gov/pubmed/29307350
- Surgery for brain metastases: An analysis of outcomes and factors affecting survival: ► https://www.ncbi.nlm.nih.gov/pubmed/29554624
- Management of breast cancer brain metastases: A practical review: ► https://www.ncbi.nlm.nih.gov/pubmed/27829201

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Renal Cancer

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Genitourinary Cancers

Contents

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Learning Objectives

By the end of this chapter, the reader will:

- Have learned the basic concepts of epidemiology, risk factors, histological subtype, and molecular profile of Renal cell carcinoma (RCC)
- Have reached in depth knowledge of diagnosis, staging, and clinical management of RCC
- Be able to put acquired knowledge on RCC into clinical practice

45.1 Introduction

Over the past 10 years, the advances in identification of the molecular mechanisms related to renal cancer tumorigenesis and the understanding of the central role of angiogenesis in cell growth and proliferation allowed in identifying several targets of clinical interest. The development of new drugs, such as tyrosine kinase inhibitors, revolutionized the medical treatment of renal cancer by making possible to target the signaling pathway and the molecular events that are key events for pathogenesis of this malignancy.

Recently, immunoncology, has become a promising frontier for the treatment of renal cancer, improving the organism's competence to direct the immune system against cancer cells. All these findings have resulted in significant improvements in median overall survival (OS) for patients and in a greater number of therapeutic opportunities (Fig. 45.1).

45.2 Epidemiology

- Renal cell carcinoma (RCC), derived from renal tubular epithelial cells, accounts for ~2% of all adult malignancies; it is the seventh most common cancer in men and the tenth most common cancer in women, with a median age of diagnosis of around 60–65 years [1, 2].
- Occurrence in younger ages could be indicative of *hereditary kidney cancer syndrome* (3–5% of all RCCs); the most common is the *Von Hippel Lindau (VHL) disease* [2].
- Over the past two decades, a divergent pattern of increasing incidence and decreasing mortality was observed, especially in the western, industrialized, world [3].
- This is due, probably, to the combined effect of the increasingly incidental detection with abdominal imaging (Fig. 45.2) and the effectiveness of several systemic therapies developed.

45.3 Risk Factors

- Established and well-known risk factors for RCC are *cigarette smoking, hypertension*, and *obesity* [4]. Further clinical conditions that are common in patients with RCC are chronic kidney disease, dialysis, and kidney transplantation [5].
- Furthermore, *genetic factors* also contribute to RCC risk. The *Von Hippel Lindau (VHL) disease* is an autosomal dominant syndrome characterized by mutations affecting the *VHL* tumor suppressor gene [2]. Inactivation of the *VHL gene* leads to accumulation of *HIF (hypoxia inducible factor)*: under normal conditions, HIF is constitutively degraded. HIF promotes transcription of gene involved in the angiogenesis-pathway and tumor progression, including vascular endothelial growth factor (VEGF), PDGF, fibroblast growth factor (FGF), and hepatocyte growth factor (HGF). The aberrant accumulation of HIF results in uncontrolled

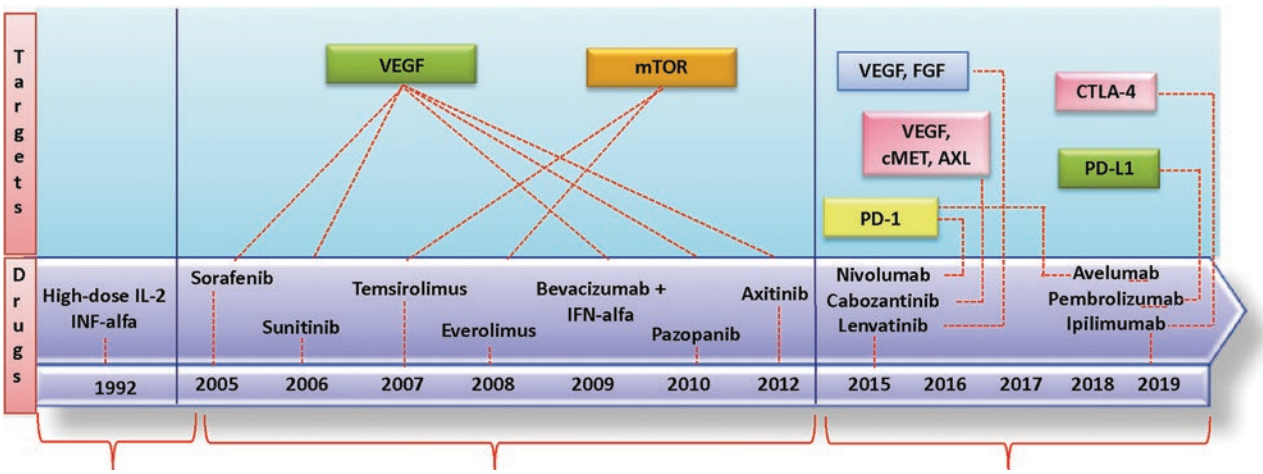


Fig. 45.1 Parallel development of new targets and new drugs for metastatic renal cell carcinoma (RCC)

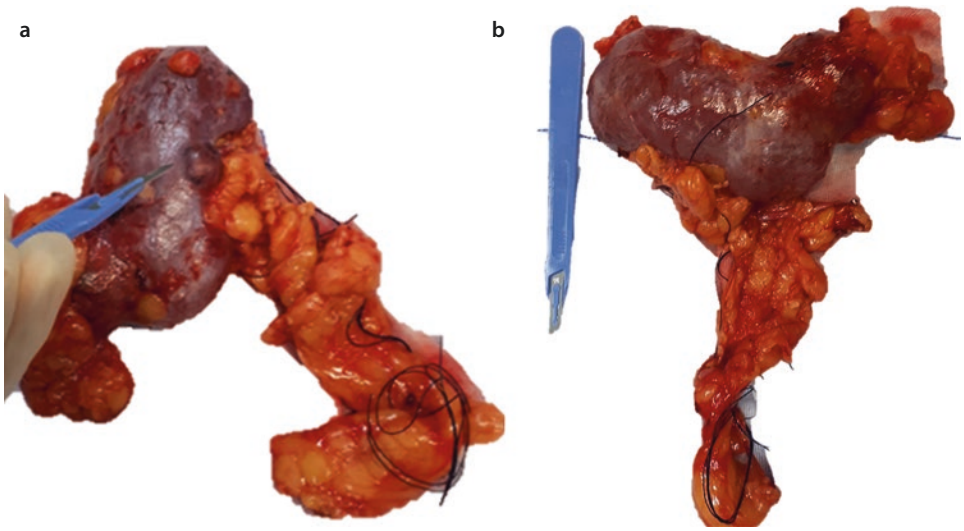


Fig. 45.2 Von Hippel Lindau (VHL) disease. **a** Numerous renal cysts. **b** Renal cell carcinoma, clear cell. (Courtesy of Prof. A. Simonato)

activation of transcription factors and several target genes, predominantly mediators of angiogenesis, that enhance cell survival [6].

- Clinically, suggestive for VHL disease is RCC in young patient, with personal or family history of any other tumor typical of VHL. The most frequent are as follows:
  - Retinal angioma
  - Spinal or cerebellar hemangioblastoma
  - Adrenal or extra-adrenal pheochromocytoma
  - Multiple renal and pancreatic cysts
  - Neuroendocrine tumors of the pancreas. PMID 20301636 (Fig. 45.3)

45.4 Histological Subtype and Molecular Profile

Approximately 75% of RCC are *clear cell carcinoma (ccRCC)* (Fig. 45.3). The other RCC subtypes are a heterogeneous group of cancer with different morphology, genetic and molecular pathogenesis, and clinical behavior, as a whole known as *non-clear cell RCC (nccRCC)*. In terms of prognosis, the survival of the vast majority of nccRCC patients is significantly inferior compared to ccRCC patients [7].

Today we know that between the histological subtype there are not only histologic difference but also cytogenetic alterations with specific genes mutated (Fig. 45.4):

- The *clear cell carcinoma* is a "disease of a chromosome 3p": the *VHL* gene is the most frequently inactivated. This gene resides in the short arm of chromosome 3 (3p), at 3p25, and results mutated, deleted, or hypermethylated. Interestingly, mutations in others tumor suppressor genes located in the 3p, (at 3p21), have also been reported, including Polybromo 1 (*PBRM1*), BRCA associated protein-1 (*BAP1*), and SET Domain Containing1 (*SETD2*). These genes encode chromatin-regulating and histone-regulating proteins. Together with the abnormal expression of kinase of mTOR pathway, these genetic alterations are present in more than 50% of ccRCC patients [8]. According to some authors, ccRCC patients harboring mutations in the mTOR gene should be considered as having a "metabolic" neoplasm, due to the key role of mTOR in the regulation of cell metabolism.
- The *papillary renal cell carcinoma* are ~15% of all renal cancers and are divided into two main subtypes, type 1 and type 2. The type 1 is associated with mutation of gene MET, and type 2 with mutations of CDKN2A, SETD2, and NRF2.
- The *chromophobe renal cell carcinoma* makes up ~5% of kidney tumors. Frequent are mutations of TP53 or PTEN.
- The *oncocytoma*, ~5% of all RCC, is a benign tumor associated with mitochondrial genes alterations (COX1, COX2, MTND4, and MTCYB). Hybrid tumors have been described that present overlapping features of chromophobe RCC and oncocytomas, often observed in Birt–Hogg–Dubé syndrome.

Other minor subtypes include the following:

- *MiT family translocation renal cell carcinoma*, with recurrent translocations, involving Xp11.23 (TFE3), 6p21 (TFEB) and others, that occur typically in young patients.

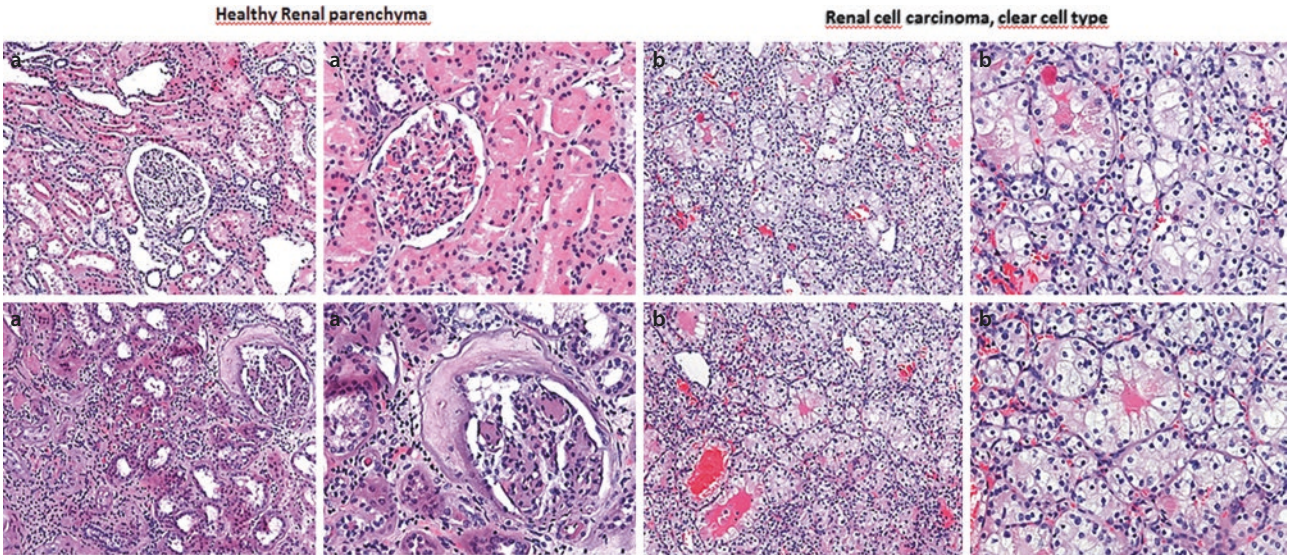


Fig. 45.3 Microscopic picture (H&E 10×, 20× magnification) showing healthy renal parenchima **a** and renal cell carcinoma, clear cell type **b**

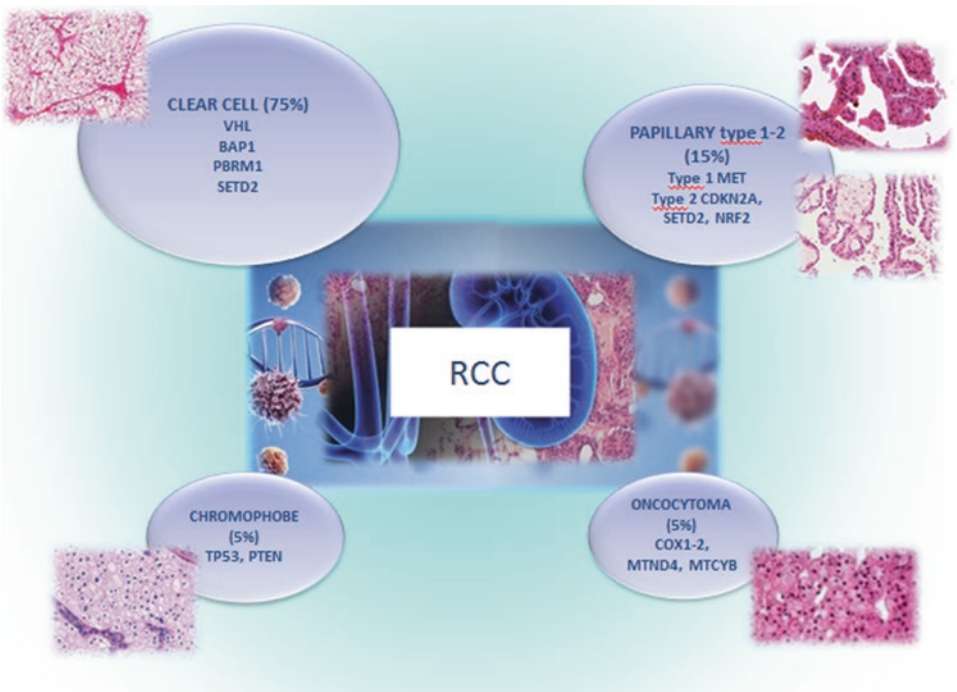


Fig. 45.4 Histological subtype and molecular profile of RCC

- *Collecting duct carcinoma*, rare and highly aggressive, with unknown gene alterations. Should be considered and treated like urothelial carcinomas of the upper urinary tract, though their prognosis is even worse.

Additional minor subtypes include renal medullary carcinoma, clear cell papillary RCC, hereditary leiomyomatosis, tubulocystic RCC, acquired cystic disease-associated RCC, mucinous tubular and spindle RCC, succinate dehydrogenase-deficient RCC, and RCC-associated RCC [9].

45.5 RCC Pathogenesis and Tumor Evolution

45.5.1 Role of Genes

The genetic and molecular pathogenesis of RCC appears to be much more complex than originally thought. Only 2–3% of ccRCC are accounted for hereditary diseases characterized by a germline mutation of the VHL gene. Conversely, mutations or silencing of the same VHL gene are associated with >80% of sporadic ccRCC.

VHL inactivation is the founding event; *BAP1, PBRM1, SETD2, KDM5C* mutations seem to be involved in disease progression and to have effect on clinical outcome [10]. For example, BAP1 is related to larger tumor size, higher Fuhrman nuclear grade, and worse cancer-specific survival [11].

At the same time, VHL loss is early events evident in all ccRCC cell of tumor sampled; driver mutations of BAP1, PBRM1, SET2, MTOR, KDM5C are present heterogeneously (branched mutations) and are mutually exclusive [12]. This leads to hypothesis of molecular subclassification of ccRCC in the future.

Therefore, the *genomic heterogeneity* adds further complexity to RCC pathogenesis: sequential and parallel accumulation of mutations is responsible of subclonal evolution with hypothetical effect on the clinical outcome. How individual genetic alterations and their interactions contribute to the pathogenesis in RCC are largely unknown and no prognostic and predictive biomarkers have been validated to date. But recent report on BAP1, PBRM1, and SETD2 as potential prognostic and predictive biomarkers may foster the possibility of impacting risk profiling.

Genomic heterogeneity translates into *clinical tumor heterogeneity* that has important therapeutic implications. RCC variability, beyond between patients (inter-patient), exists within the same patient (intra-patient) and within a given tumor sample (intra-tumoral). This *spatial* and *temporal biological diversity* changes over time and in response to treatment, and contributes to the development of compensatory mechanisms that result in resistance. The selective pressure first-line antiangiogenic treatments induced on the tumor has been demonstrated to be able to induce further mutations; notably enough, the rate of VHL mutations seems to increase moving from first- to second-line treatment.

45.5.2 Role of Angiogenesis and Tumor Microenvironment

45.5.2.1 Angiogenesis

Due to the above seminal genetic alterations (i.e., those affecting the *VHL* gene), RCCs are *highly vascular*, and *angiogenesis*, the process of new blood vessel formation, is a crucial step in their pathogenesis [13].

The frequent loss of the *VHL* tumor suppressor gene, results in HIF up-regulation. This aberrant accumulation of HIF proteins, translocating into the nucleus, leads to the transcription of several HIF target genes. These genes include angiogenic factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and transforming growth factor-α (TGF-α), following the stimulation of angiogenesis (Fig. 45.5).

Furthermore, renal tumor angiogenesis is also stimulated by growth factors through the phosphatidylinositol-3 kinase PI3K-AKT-mTOR signal transduction pathway.

Given the highly vascular nature and the central role of angiogenesis in RCCs, several agents targeting the vascular endothelial growth factor (VEGF) pathway have explored this feature. Before 2005, only two drugs were available to treat RCC: High-dose IL-2 (HD IL-2) and interferon α (INF-α), with substantial toxicity and a median survival of ~15 months.

Since 2005, *antiangiogenic drugs*, such as the monoclonal antibody anti-VEGF *bevacizumab*, but especially tyrosine kinase inhibitors (TKI) targeting (mainly, but not exclusively) the VEGF/VEGFRs signaling axis (*sunitinib*, *pazopanib*, *axitinib*, *sorafenib*, and *tivozanib*) and inhibitors of mammalian target of rapamycin (mTOR) pathway (*everolimus*, *temsirolimus*) revolutionized the treatment of advanced or metastatic RCC, reaching the median overall survival (OS) to ~30 months in 2014.

Sorafenib	The first TKI approved for mRCC treatment. It is a multikinase inhibitor of multiple growth factor receptors as VEGFr, PDGFr, Flt-3 and c-Kit and Raf-1, a member of RAF/MEK/ERK signaling pathway.
Sunitinib, pazopanib	Multitarget oral TKI, with inhibitory activity against VEGF and PDGF receptors.
Axitinib	The next-generation TKI, potent and highly selective for the VEGF receptor 1, 2, and 3.
Everolimus, temsirolimus	The kinase inhibitors of mTOR complex 1 (mTORC1).
Bevacizumab	Unique monoclonal antibody anti-VEGF approved, in combination with immunomodulator interferon α.
Tivozanib	An oral, highly potent, and selective tyrosine kinase inhibitor of VEGF receptors 1, 2, and 3. It has recently been approved by the European Medicines Agency (EMA) for first-line treatment and is at various stages of development in EU countries [14].

However, anti-angiogenic agents have typically *transitory efficacy* because the inhibition of tumor angiogenesis by VEGFR-TKI is reversible. Indeed, clinically durable responses are rare and after an initial period of response, most patients will experience disease progression for the development of treatment resistance.

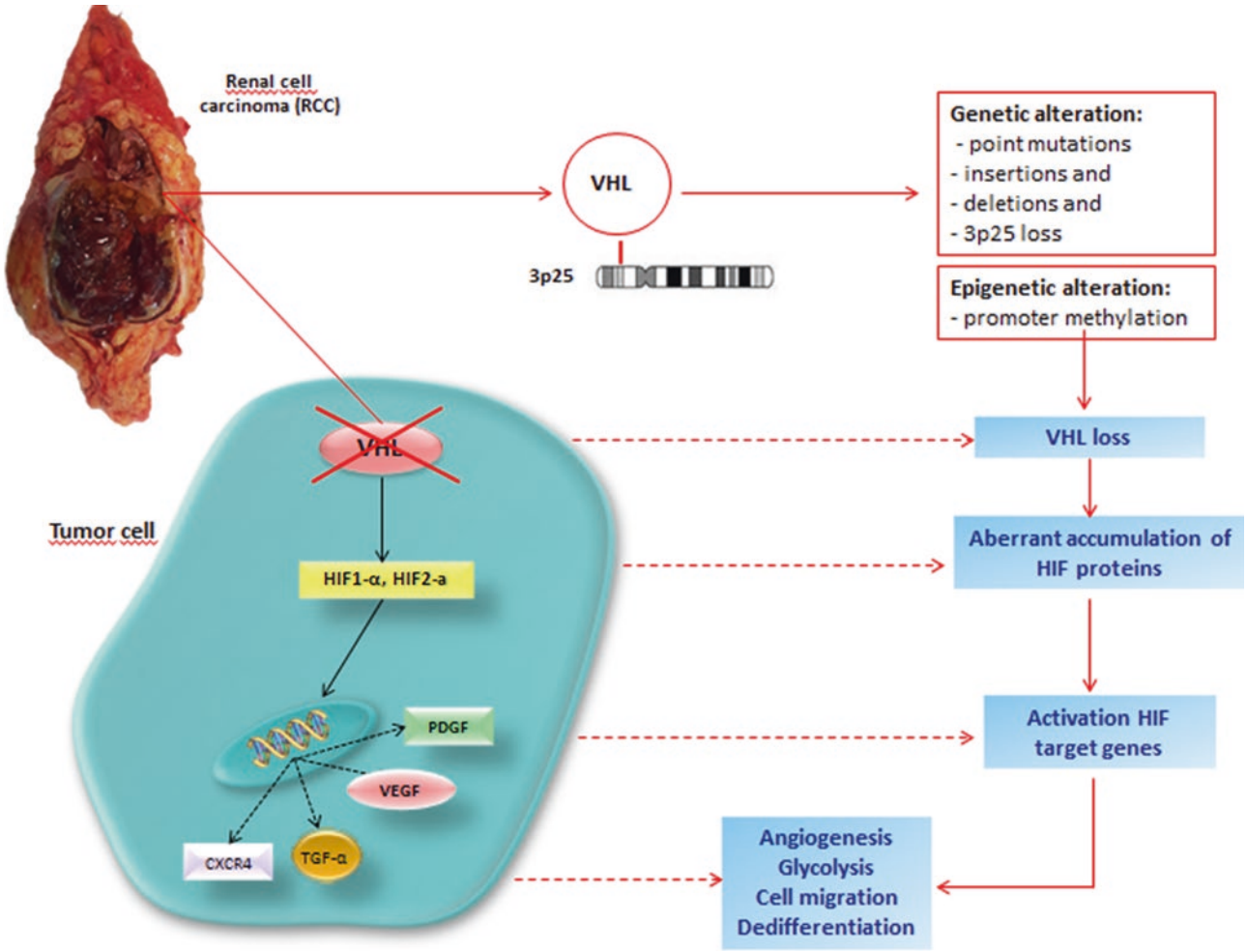


Fig. 45.5 VHL inactivation in clear cell renal cell carcinoma (ccRCC), with accumulation of HIF proteins, activation of HIF target genes and increased angiogenesis

Resistance is mainly caused by adaptive mechanisms of cancer cells with the activation of angiogenesis-related pathways independent of VEGFR and PDGFR.

Furthermore, anti-VEGFR therapies are not definitely precise and "targeted", because VEGF is ubiquitously expressed in solid tumors, although much more in RCC, and anti-VEGFR therapies target endothelial, and not cancer cells.

A strategy to *overcome the resistance* to VEGFR inhibitors is the development of new-generation antiangiogenic drugs targeting multiple distinct pathways, such as *cabozantinib* and *lenvatinib*, characterized by additional targeted mechanism of action.

Inhibiting both VEGFR and accessory pathways simultaneously might avoid the development of resistance to treatment.

Cabozantinib	A receptor tyrosine kinase inhibitor whose targets include MET (hepatocyte growth factor receptor), VEGFR2, and AXL receptor tyrosine kinase (AXL).
Lenvatinib	Third-generation of VEGFR inhibitors. It is a multi-TKI of VEGFR1-3, with inhibitory activity against fibroblast growth factor receptors (FGFR1-4), PDGFRα, glial-cell-line-derived neurotrophic factor receptor (RET) and KIT.

The Inhibition of MET and AXL with *cabozantinib* has a strong rationale:

- cMET is overexpressed in many ccRCC
- cMET and AXL are induced by VEGF inhibition
- Targeting MET and AXL could to overcome resistance to anti-VEGF [15].

Also the combination of *lenvatinib* + *everolimus* have an attractive biological rationale of synergistic activity of VEGFR and mTOR pathways inhibition and a randomized phase II trial that show a PFS benefit for this combination, was published recently [16] (Fig. 45.6).

45.5.2.2 Tumor Microenvironment

The observations of high levels of immune infiltrate in the RCC microenvironment and the parallel occurrence of some spontaneous tumor regression of metastases after radical nephrectomy suggested a natural antitumor immunity for metastatic RCC [17].

RCC lesions are often infiltrated by tumor-infiltrating lymphocytes; analyzing T cell infiltration score (TIS) and the corresponding mutation load in 19 cancer types by The Cancer Genome Atlas research program, ccRCC shows the highest TIS. Tumor immune microenvironment characterization in ccRCC identifies prognostic and immunotherapeutically relevant messenger RNA signatures [18].

Despite numerous evidences in solid tumors suggest that increased TILs are associated with good prognosis, several studies showed that high density of CD8+ TILs is associated with poor clinical outcome in RCC: studying the tumor-specific survival in immune infiltration classes, the T cell enriched class has the poorest survival, whereas the non-infiltrated class is associated with better outcomes. Furthermore, the increase in TILs was found to be associated with higher tumor grade and stage [19].

Since the 1990s, before the introduction of TKI for the mRCC treatment, cytokines, such as high-dose IL-2 and interferon-α (IFN-α), were used, alone or in combination, to enhance antitumor immunity in RCC and represented the standards of care, however limited by poor efficacy and severe dose-limiting toxicities. The new generation of *immunotherapy agents*, the immune checkpoint-blocking therapies, are of increasing interest in this disease today.

Immuno-oncology is a promising frontier for RCC and the recent new information relative to the complex role of tumor microenvironment (TME) are resulting in a greater number of therapeutic opportunities [20].

Tumors, indeed, can create an immunosuppressive microenvironment by upregulating inhibitory molecules,

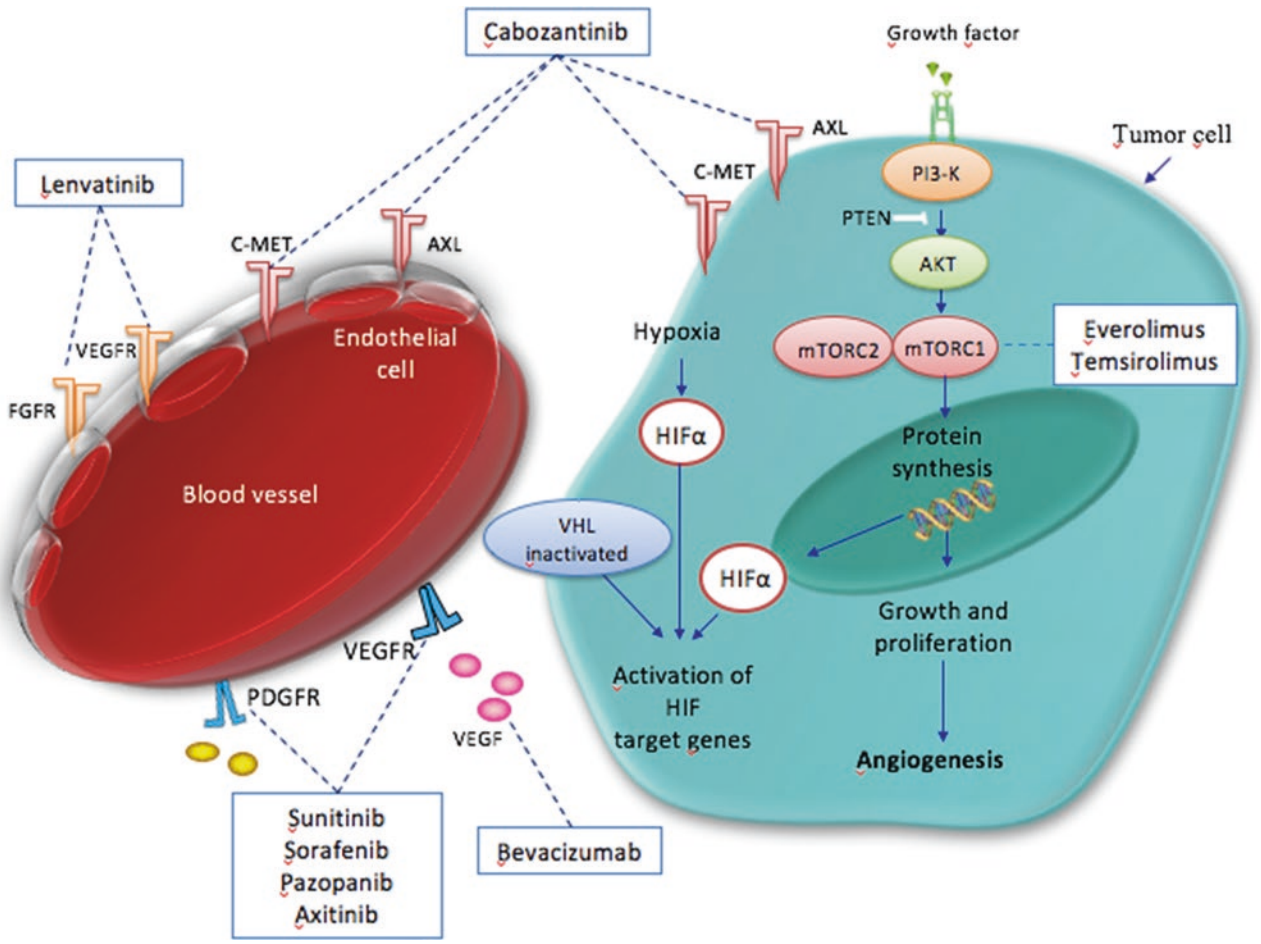


Fig. 45.6 Signaling pathways inhibition by targeted agents in mRCC. (Adapted from Ref. [6])

such as *programmed cell death protein (PD-1)* on tumor-infiltrating T cells, or its ligand *PD-L1*, on tumors cells [21].

The PD1- PD-L1 pathway downregulates cytotoxic T-cell activity [22].

The blockade of the PD1-PD-L1 interactions with specific antibodies inhibitors, may prevent T-cell suppression: the T-cells remain active and promote the immune killing of the tumor cells.

The *immune checkpoints inhibitors* already developed, or under exploration (alone or within different combinations) in RCC clinical trials are as follows:

- Nivolumab and pembrolizumab, monoclonal antibodies that target PD-1 receptor
- Avelumab and atezolizumab, monoclonal antibodies that target PD-L1-receptor
- Ipilimumab, monoclonal antibody against the cytotoxic T-lymphocyte antigen-4 (CTLA-4), another immune-inhibitory molecule expressed in activated T cells and in suppressor T regulatory cells studied in clinical trials, alone or in combination (Fig. 45.7).

The inhibition of immune checkpoint PD-1 with *nivolumab*, has been demonstrated to be clinically effective on metastatic disease, with significant improvements in median OS for kidney cancer patients treated after the failure of an anti-VEGFR agent [23, 24]. The introduction of these immunotherapy drugs contributed to the revolution in the treatment of mRCC and promise to be translated to a significant number of patients, achieving durable remissions in the near future [25].

45.6 Diagnosis and Staging

The clinical presentation of RCC was, historically, characterized by *flank pain, gross hematuria*, and *a palpable abdominal mass*, depending on the localization and the large size of the tumor.

Currently, the majority of diagnoses (>50%) results from *incidental findings*, suggested by non-invasive radiological techniques, ultrasonography (US), or computed tomography (CT), are often performed for another clinical reason. These RCCs detected incidentally are often early and small tumors, making the classical clinical triad mentioned above less frequent than in the past. Some patients show symptoms related to *paraneoplastic syndromes*, caused by cytokines and hormones production by cancer cells and characterized by hypercalcemia, fever, erythrocytosis, and Stauffer's syn-

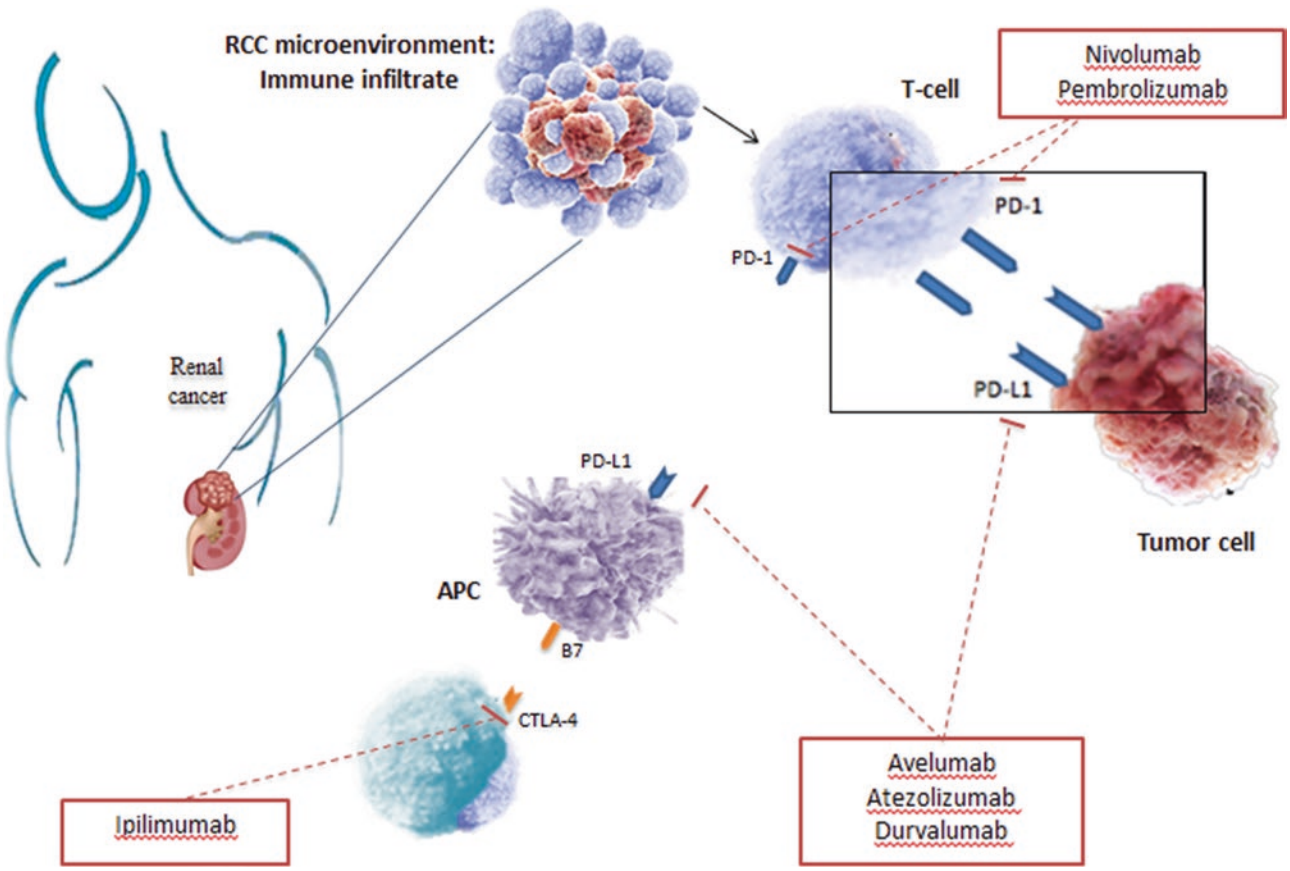


Fig. 45.7 Immune checkpoints inhibitors approved or available in RCC clinical trials

drome (signs of cholestasis unrelated to tumor infiltration of the liver or intrinsic liver disease).

Laboratory examinations could show alterations of several parameters, such as serum creatinine, hemoglobin, leukocyte and platelet counts, lymphocyte to neutrophil ratio, serum calcium and lactate dehydrogenase levels. Some of these tests are used for risk assessment within different prognostic score systems.

Most RCCs are strongly suspected by *imaging studies*, because they have typical radiological features, including intra-tumoral heterogeneity due to necrosis or hemorrhage and exophytic growth, and high uptake of contrast- enhancement agents.

Ultrasonography (US) is usually the first radiological technique that allows the detection of RCC.

Further exams to investigate local invasiveness, lymph node involvement, and distant metastases are contrast-enhanced *chest, abdominal, and pelvic CT scan*, for the study of lung, liver, and lymph nodes metastasis (Fig. 45.8).

The use of either bone scan or CT (or MRI) of the brain are usually performed only in symptomatic subject, i.e., when a clinical suspicion of bone or cerebral involvement is present, while 18FDG-PET is not a standard investigation in the diagnosis and staging of ccRCC and should not be used routinely. Abdominal magnetic resonance imaging (MRI) may provide additional information, especially to investigate the venous involvement from the tumors, which frequently causes the vena cava tumor thrombus. *TNM staging* of RCC is based on size, position, and lymph node involvement. The staging system used is the AJCC/UICC TNM classification (American Joint Committee on Cancer -AJCC/Union for International Cancer Control – UICC/tumor–node–metastasis – TNM; 7th edition-2010) (Fig. 45.9).

45.7 Management

Approximately 65% of patients with RCC have *localized* tumors, which are treated with *surgery* and can be cured by total nephrectomy or nephron-sparing surgery (e.g., partial nephrectomy). The remaining ~35% of patients present with *metastatic* RCC. Finally, about 20–40% of patients with confined primary tumor at diagnosis will develop metastatic disease after local therapy [26].

45.7.1 Localized Disease

Surgery

The standard treatment of localized RCCs is complete surgical excision of the lesion by *partial or radical nephrectomy*, with a curative intent.

The goal of modern surgery is to completely remove the primary tumor, while preserving the largest possible amount of healthy renal parenchyma, limiting invasiveness, iatrogenic renal function impairment, and overtreatment that can increase patients' morbidity.

Radical and partial nephrectomies show similar OS on the basis of a randomized trial (EORTC) and a meta-analysis that included 107 studies with over 180,000 patients [27].

The choice between partial or radical nephrectomy is related to the clinical stage of the disease, predominantly diameter, location, depth, proximity to hilar vessels and the urinary collecting system, and the type of surgical

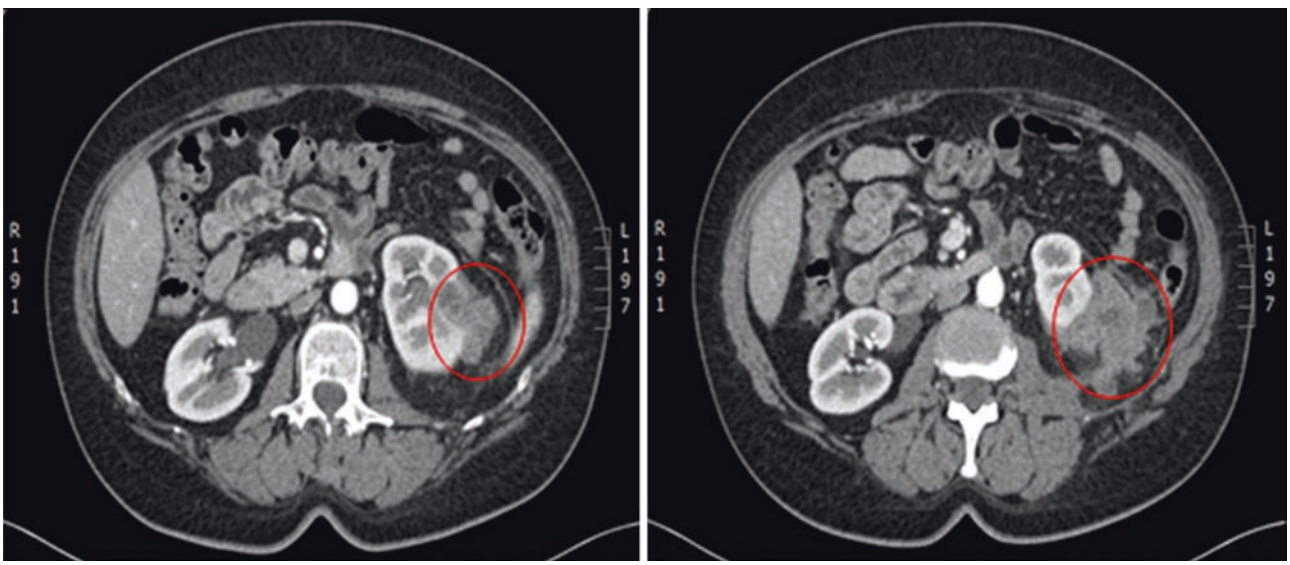


Fig. 45.8 Contrast enhanced CT demonstrates a heterogeneously enhancing mass arising from the lower pole of the left kidney

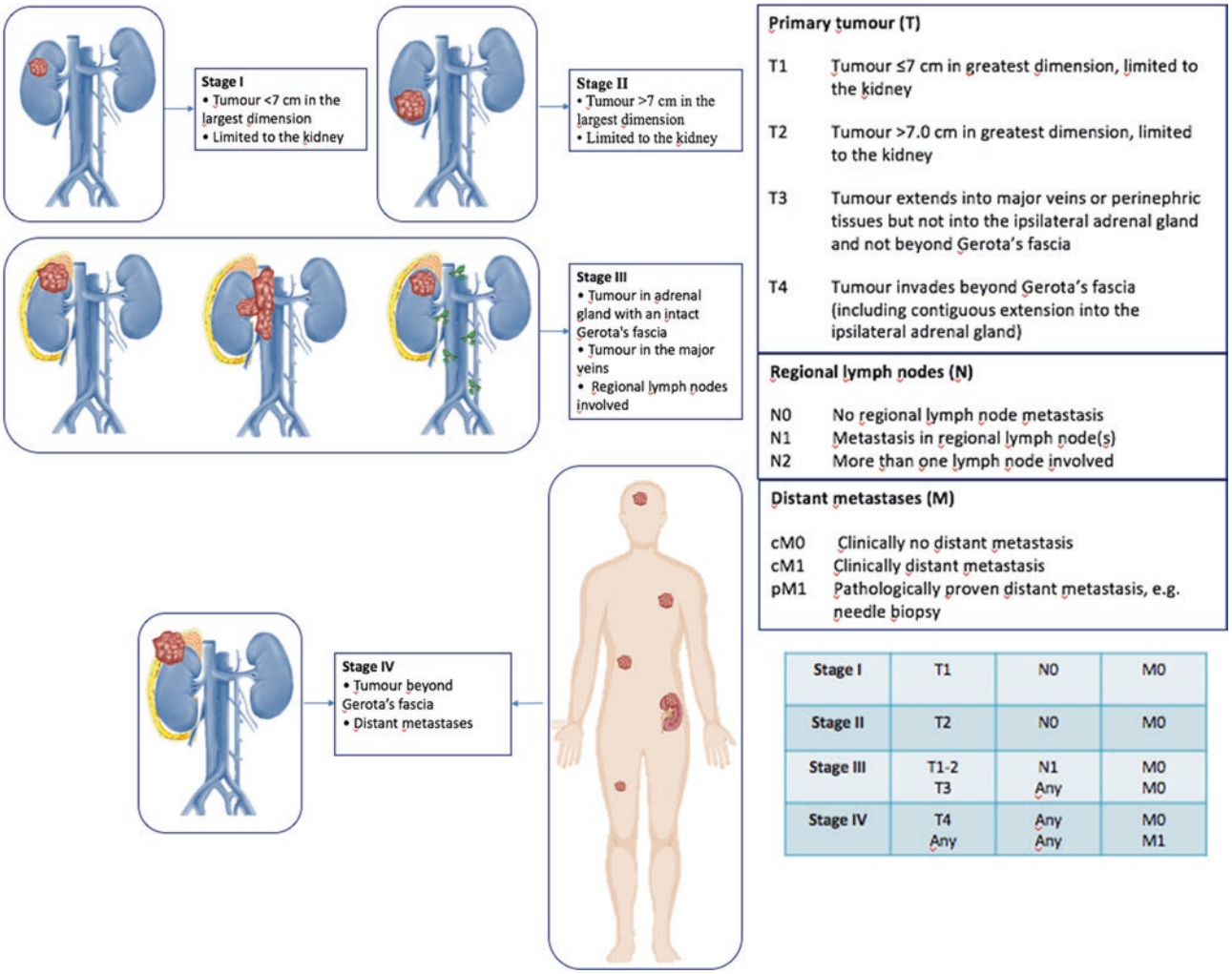


Fig. 45.9 AJCC/UICC TNM classification of RCC, 7th edition-2010

approach (open, laparoscopic, or robotic), and depends, as well as tumor features, also on the surgeon's expertise. Together, these surgical trends highlight the importance of preserved renal function [28] (Fig. 45.10).

Active Surveillance

Although surgery represents the standard of care for localized RCC, there exists a rationale for active surveillance in well-selected patients. This strategy seems reasonable because of the following:

- Small renal masses often harbor benign final pathology
- Some renal tumors have a slow median growth rate with low risk of metastatic progression (2–3 mm/year)
- A significant proportion of RCC patients with severe comorbidity, particularly the elderly patients, are unfit for surgery, with high risk of surgical complications including death.

A definite protocol for active surveillance, with specifics indications for tumor size and growth rate cut-off, is not yet defined. For these patients, it is commonly suggested imaging every 3 months in the first year, every 6 months during the next 2–3 years, and annually thereafter. Intervention should be proposed for growth >3–4 cm or by >0.4–0.5 cm/year [10].

45.7.1.1 Risk Assessment in Localized Disease

The patient's individual risk of disease recurrence after surgery varies significantly: clinical and pathological variables, such as histology, grading, extent of tumor, have prognostic value in RCC and may be used for the risk assessment. All these features are not perfectly accurate when used alone, but, when combined into integrated systems, have shown to be valuable tools to predict RCC prognosis [29].

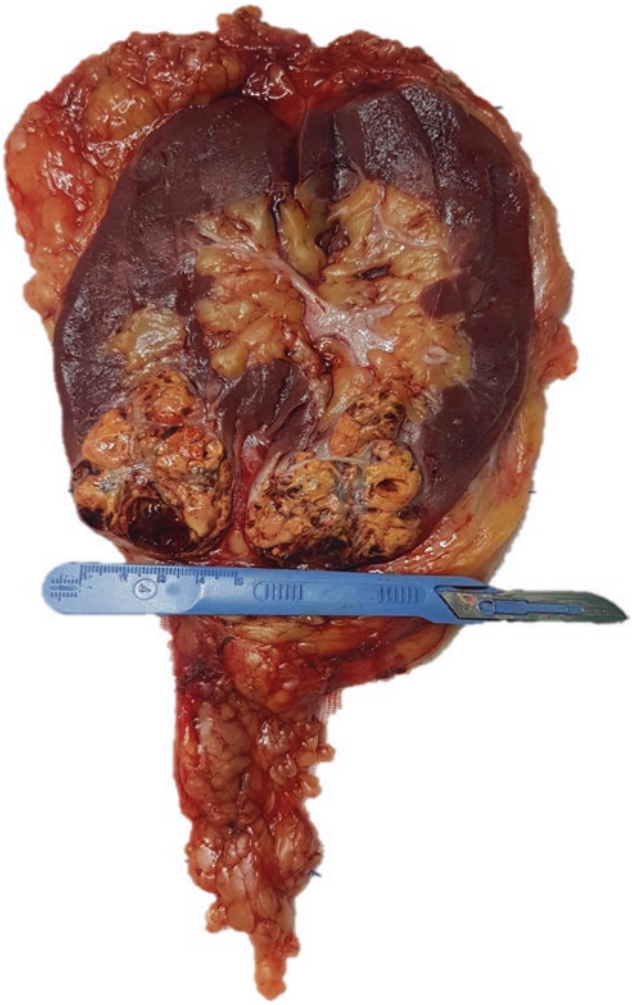


Fig. 45.10 Radical nephrectomy for RCC in the lower pole of the kidney. (Courtesy of Prof. A. Simonato)

The most used and validated systems are as follows:

- The *UCLA Integrated Staging System (UISS)*, that combines TNM stage, Fuhrman grade, and Eastern Cooperative Oncology Group (ECOG) performance status (PS) [30].
- The *SSIGN system (Stage, Size, Grade and Necrosis)*, developed from the Mayo Clinic. It does not consider the performance status or other clinical parameters, and includes tumor necrosis; a limitation of this scoring system is that it is only useful for clear cell renal carcinomas [30].
- The *Karakiewicz nomogram*, similar to the UISS, but tumor size is used as a continuous variable and the ECOG performance status is replaced by a symptom classification that distinguishes asymptomatic, local, and systemic symptoms [31].
- The most recent scoring algorithm developed by *Leibovich et al.* that is a modification of the SSIGN score. It differs from the SSIGN score and from many others because the endpoint is progression to metastatic RCC rather than survival [32].

These risk assessment tools have the potential to allow better risk stratification of patients into low-intermediate- and high-risk groups. To date, no clear preference for a specific prognostic score may be given [33].

Adjuvant Therapy

Currently, almost no adjuvant treatment tested, have proved able to improve either disease-free survival (DFS) or OS, within a randomized controlled, phase III trial. A recent study (S-TRAC adjuvant study) reported a DFS benefit of 1 year of sunitinib therapy in comparison with placebo in 615 patients with resected, non-metastatic, high-risk RCC. However, the lack of any OS benefit, together with the suboptimal trade-off of 1 year of toxic therapy in exchange for 1.2 years of DFS benefit, are among the major criticisms regarding this study [34]. Several other trials of adjuvant targeted therapies and immunotherapy are ongoing and the results will be reported in the near future.

45.7.2 Metastatic Disease

One-third of patients with RCC present with distant metastases at diagnosis, and approximately a quarter of patients with localized disease treated with nephrectomy have relapses in distant sites [35]. Distant metastases occur most often in the lymph nodes, lungs, bone, liver, and brain [36] (Fig. 45.11).

45.7.2.1 Risk Assessment in Advanced Disease

Different prognostic models to stratify patients with metastatic RCC for systemic treatment were developed. Key prognostic factors identified include performance status (PS), time from diagnosis to systemic treatment, hemoglobin, calcium, neutrophil and platelet counts in the blood.

The most recent "*International Metastatic RCC Database Consortium (IMDC) score*" is based on six factors. The patients are stratified into three *categories of risk* based on the number of prognostic factors: *favorable* (0 risk factor); *intermediate* (1–2 risk factors); and *poor risk* (3–6 risk factors) (Fig. 45.12).

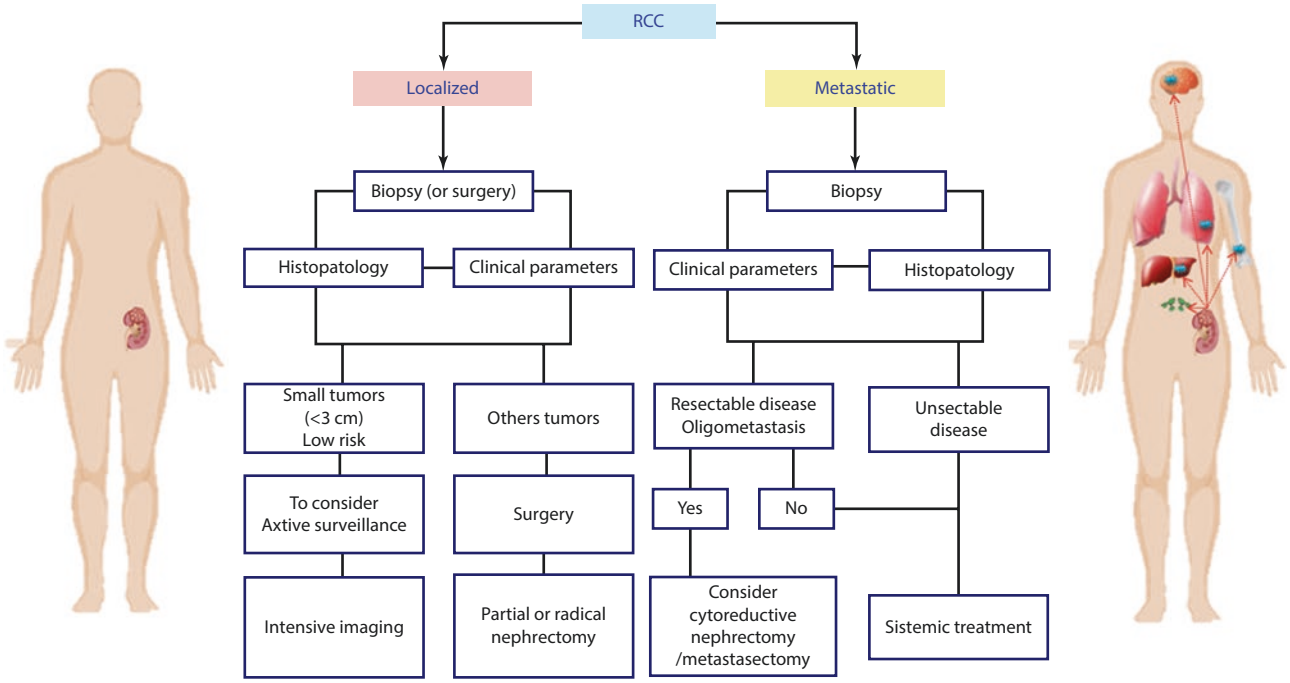


Fig. 45.11 Treatment strategy for renal cell carcinoma; **a** localized disease; **b** metastatic disease and distant sites most frequently involved

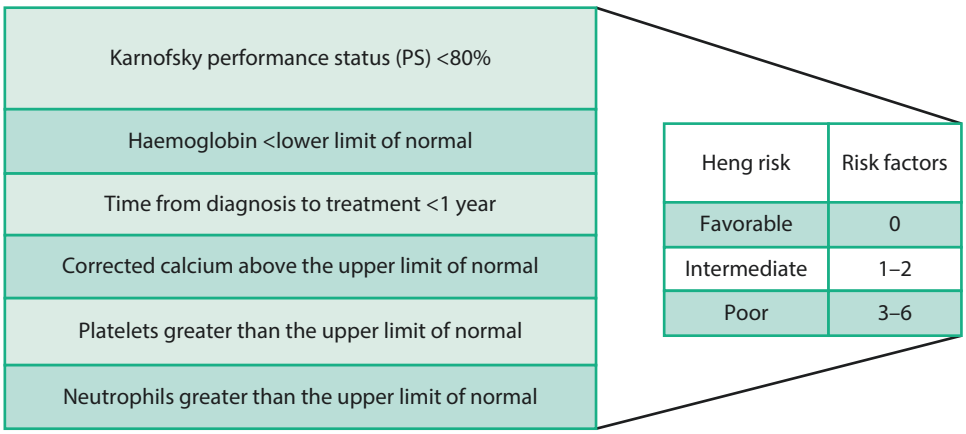


Fig. 45.12 Heng criteria (IMDC) and IMDC score. (Heng et al. J Clin Oncol. 2009)

Medical Treatment

Systemic treatment is indicated for patients with *unresectable* or *metastatic* RCC.

Expanding knowledge of RCC biology and a better understanding of pathways involved in RCC pathophysiology produced in the past 10 years the approval of several novel therapeutic agents tailored to specific molecular drivers described before.

The highly vascular nature of RCCs and the role of "functional hypoxia" and angiogenesis made this tumor an ideal target to exploit this feature with anti-angiogenic drugs.

Tyrosine kinase inhibitors (TKI) targeting *VEGF signaling pathways* have been, in fact, the first drugs that have improved patient outcomes compared with the previous cytokines-based standard of care.

After the approval of Sorafenib in 2005, several targeted agents have been specifically designed and approved, quickly changing the landscape of RCC treatment, targeting VEGF signaling axis (sunitinib, pazopanib, bevacizumab, axitinib) or mTOR pathway (everolimus).

The next paradigm shift occurred in 2015, when three novel agents showed improved outcomes in the post-first-line setting: 2 new-generation antiangiogenic drugs targeting *multiple distinct pathways* (cabozantinib and lenvatinib) and nivolumab, targeting the immune system/tumor microenvironment (Fig. 45.13).

Currently, several drugs are available for the treatment of mRCC:

1. Three agents were the first approved for previously untreated mRCC, in *first-line* setting: *sunitinib*, *pazo-*

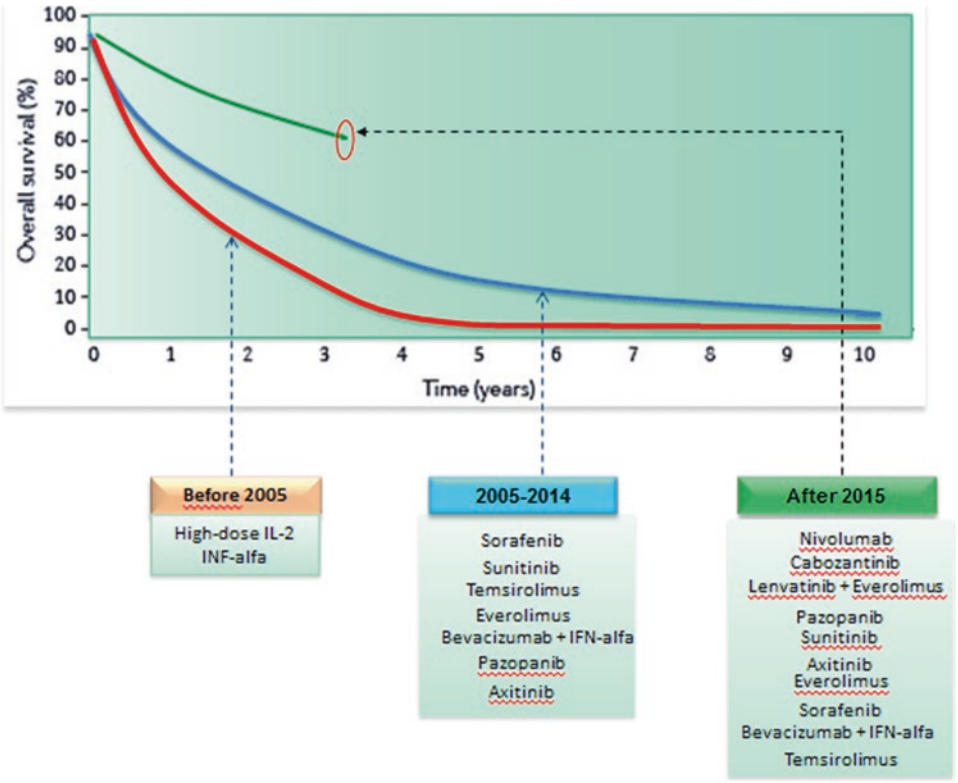


Fig. 45.13 Survival outcome and introduction of new therapeutic options for mRCC treatment

panib and the combination of *bevacizumab* and the immunomodulator *interferon-α*.

Sunitinib and pazopanib demonstrated similar median PFS in a randomized trial in first-line setting (COMPARZ), with different safety profile.

Bevacizumab is a monoclonal antibody anti-VEGF approved in 2009, but the TKI sunitinib and pazopanib, showed comparable efficacy, but a distinct safety profile, with the advantage of oral administration compared to the bevacizumab plus interferon regimen.

Afterward, the U.S. Food and Drug Administration (FDA) approved cabozantinib for treatment in the first-line setting, based on data from the CABOSUN trial. This is a randomized phase II study in patients with intermediate and poor-risk previously untreated RCC. Patients received cabozantinib or sunitinib and cabozantinib treatment significantly prolonged PFS compared with sunitinib [37].

Furthermore, in April 2018, the FDA granted approvals to *nivolumab and ipilimumab* in combination for the treatment of intermediate or poor risk, previously untreated advanced renal cell carcinoma. The approvals were based on the phase 3 CheckMate 214 trial of nivolumab plus ipilimumab versus sunitinib in previously untreated advanced RCC. In this trial, overall survival and objective response rates were significantly higher with nivolumab plus ipilimumab than with sunitinib among intermediate- and poor-risk patients [38].

Tivozanib is another option of care when available.

For patients with poor risk, *temsirolimus* is approved, but is associated with modest survival benefits, and requires weekly intravenous administration; thus, sunitinib or pazopanib are usually the preferred options in this setting, not to take into account those unfortunate patients who can receive just best supportive care.

Anti-angiogenic drugs typically have transitory efficacy: they produce more or less durable responses (usually in the range of 8–12 months), followed by disease progression due to the development of resistance to anti-VEGF therapy [39, 40].

2. Current *second-line* therapeutic options for mRCC include *nivolumab*, *cabozantinib*, *lenvatinib* + *everolimus*, and *axitinib*.

Nivolumab has been compared to everolimus in patients who had failed prior after either one or two TKIs within the CheckMate 025 trial, demonstrating an OS benefit, which led to regulatory approval in both the EU and the USA. However, predicting which patient will benefit from nivolumab still remains an issue, as well as an unmet need. PDL1 is dynamic and the expression in paraffin-embedded tumor from the primary site could not be representative of all metastatic disease.

The subsequent introduction of cabozantinib created more therapeutic options for mRCC patients, but also increased the difficulty in choosing a second-line treatment.

Cabozantinib increased progression-free survival (PFS), overall survival (OS), and objective response rate (ORR) in patients with advanced RCC after previous anti-VEGF targeted therapy, in the phase III METEOR trial [41].

To date, there are no data about direct comparison between nivolumab and cabozantinib and both are effective options after first-line VEGFr-TKI failure. The absence of head-to-head comparisons does not solve the controversy for the choice of treatment at present [42].

Axitinib, everolimus, and lenvatinib + everolimus are considered as alternative options.

Given the increased survival of patients with advanced disease, an increasing number of patients are able to undergo three lines of therapy. Treatment in *third-line* setting depends largely on the choices made previously.

The optimal sequence of agents for the treatment of mRCC is not well defined. Ongoing trials and others recently concluded continue to research new therapies and to elucidate the optimal sequence of the known ones, making the *RCC treatment, a continuously changing scenario.*

Especially, combination therapy with *avelumab + axitinib*, *pembrolizumab + axitinib*, and *bevacizumab + atezolizumab* are showing promising efficacy in first-line setting.

Given the availability of several promising new drugs with novel mechanisms of action, the immediate challenge is to choose the most effective and specific combination or sequential therapy to prevent resistance in individual patients. The biomarkers can help in the future to formulate personalized treatment, driving the choice of treatment and preventing or overcoming drug resistance.

45.7.2.2 Integrated Management Strategy

Although typically reserved for localized tumors, surgical debulking can also be used with cytoreductive intent in patients with metastatic disease.

Candidates for *cytoreductive nephrectomy* are those patients with good performance status and low systemic disease burden [43]. Although a positive impact of cytoreductive nephrectomy in metastatic ccRCC patients has been demonstrated within two randomized controlled, phase III trials in the era of cytokines, retrospective data suggest that this paradigm is still valid in the present era of targeted agents.

In addition, surgical resection of metastatic sites remains a treatment option in patients with a solitary metastasis or oligometastatic disease, especially for lung-confined lesions [44].

A subset of patients with advanced tumor, especially having limited sites of metastasis and few adverse prognostic factors, has indolent progression of disease. For these patients, active surveillance can be an initial strategy, that is, observation for a period of time before the start of systemic therapy.

The safety of observation before starting treatment has also been suggested by retrospective and prospective studies, and should be considered in patients with limited tumor burden and in the absence of symptoms [45].

Local treatment strategies of metastases should be discussed in a multidisciplinary team: conventional radiotherapy, whole brain radiotherapy (WBRT), and other type of local radiotherapy, including stereotactic radiosurgery (SRS), stereotactic body radiotherapy (SBRT), CyberKnife radiotherapy, and hypofractionated radiotherapy, can be considered for selected patients after multidisciplinary review [33].

45.8 Emerging Treatment

Recently, immunoncology, represented a new and promising frontier for RCC, with the opportunity of long term survival.

Immune checkpoint inhibitors account for the majority of immunotherapies in use today, but there is a great potential to future developments, including a new generation of immunotherapy agents (Fig. 45.14).

Furthermore, there is a scientific rationale for immunotherapy in combination with VEGF-Inhibitors: angiogenesis, hypoxia, and immunosuppression seem to be strongly related.

The hypothesis of synergistic effect is supported by the observation that antiangiogenic drugs are capable of decreasing immunosuppressive cells and cytokines, such as regulatory T cells, TGF b and IL-10, and inhibitory molecules on T cells, such as immune checkpoint PD-1, enhancing eventually the antitumor immunity. Therefore, targeting the VEGF/VEGFR pathway may attenuate RCC-induced immunosuppression, achieving improved response rates and theoretically can prevent the emergence of escape mechanisms from either agent.

Fig. 45.14 Immune checkpoint inhibitors and new generation of immunotherapy agents

Novel immunotherapy			
Checkpoint inhibitors	Vaccines	Adoptive T-cell therapy	T-cell agonists
PD-1 inhibitors - Nivolumab - Pembrolizumab PD-L1 inhibitors - Atezolizumab - Avelumab - Durvalumab CTLA-4 inhibitors - Ipilomumab - Tremelimumab	- Dendritic cell - Single peptide - Multipeptide	- CAR T cells - CIK cells	- Agonist antibodies - Cytokines

As mentioned before, several trials with immune checkpoint in combination with VEGF-targeted therapy, or new TKIs, are ongoing to investigate if the bidirectional link between VEGFR- and checkpoint inhibitors is translated into potential clinical benefit for the patients without high toxicity [46–49].

45.9 Follow-Up

No standard recommendation can be given for the follow-up in RCC.

For localized RCC, the time interval of follow-up depends on risk factors. CT scans of thorax and abdomen are routinely carried out; it is recommended to perform CT scans every 3–6 months in high-risk patients for the first 2 years, while yearly on low-risk patients.

During systemic therapy in RCC patients with advanced disease, 2- to 4-month follow-up schemes with CT scan should be advised to determine response and resistance [33].

45.10 Conclusion

Advanced RCC, characterized by a continuously changing treatment scenario.

The introduction of immunotherapy and new generation of oral TKI resulted in a paradigm shift for mRCC, introducing the concept of a possibility of cure.

Sequencing remains, at the moment, the option of choice for treating mRCC, but new available data may change this condition adding an innovative concept of "best patient selection": *predictive biomarkers* are needed to identify patient subgroups for appropriate treatment and to prevent and overcome drug resistance. *Patient selection* will be the key for personalization in the future.

New drugs and new combinations continue to be explored.

Advances in diagnosis, local management, and systemic therapy will help to develop more effective therapeutic strategies and new algorithms for precision therapy (Fig. 45.15).

Summary of Clinical Recommendations

- Linee Guida dell'Associazione Italiana di Oncologia Medica (AIOM)
- Tumori del rene. Edizione 2020.
- Renal cell carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology 30: 706–720, 2019. ▶ https://doi.org/10.1093/annonc/mdz056. Published online 21 February 2019.
- NCCN (National Comprehensive Cancer Network) GUIDELINES FOR TREATMENT OF CANCER BY SITE: Kidney cancer.

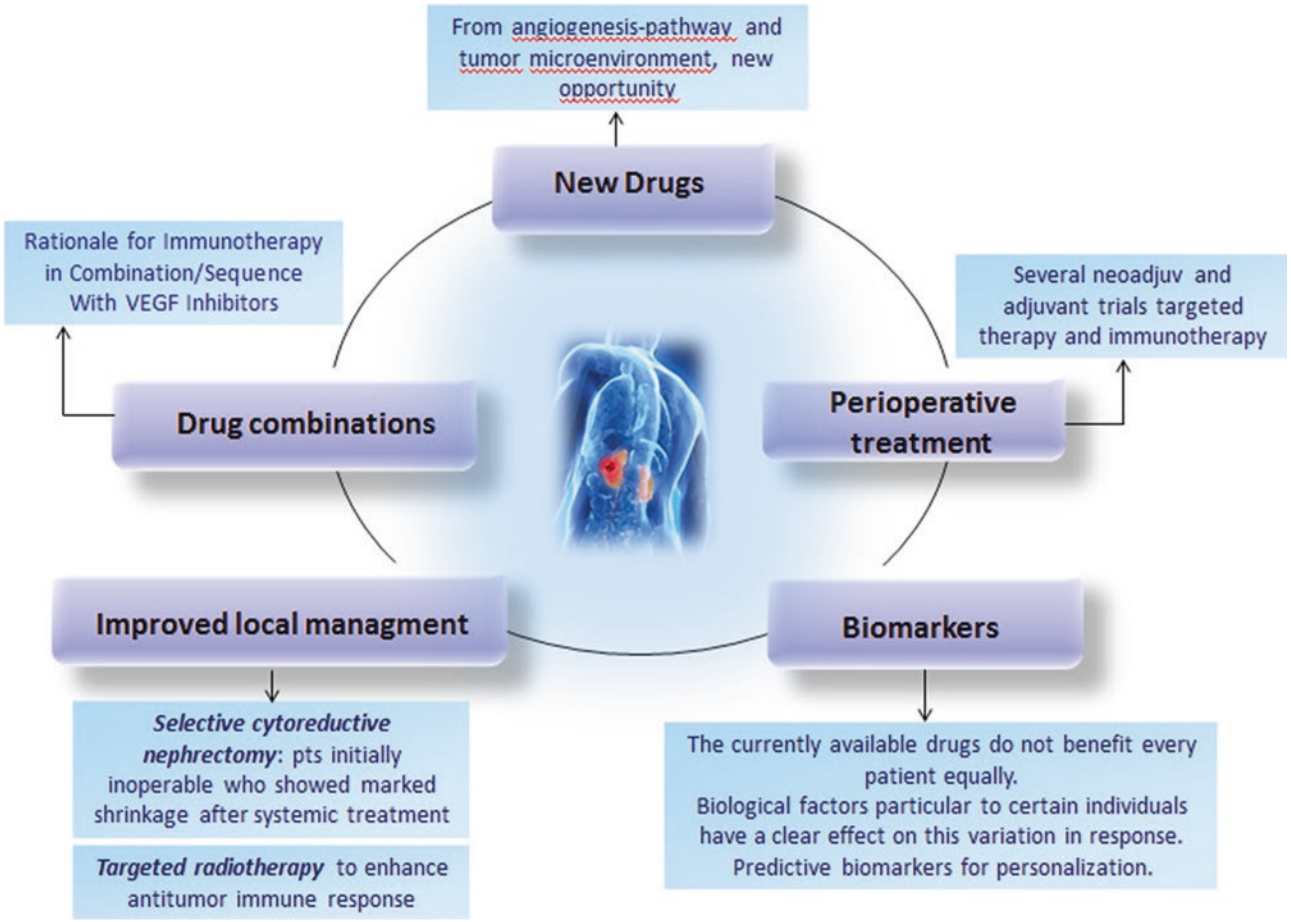
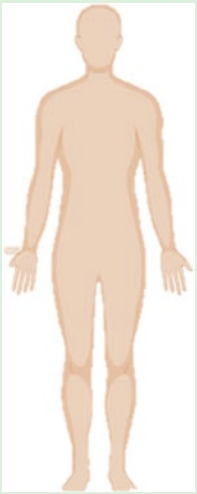
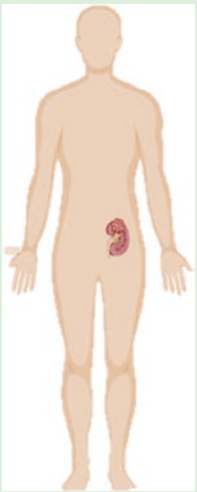


Fig. 45.15 The near future for the RCC management

Case Study





Man, 65 years old

- *Family history* negative for malignancy
- *APR*: hypertension; cigarette smoking; history of renal lithiasis
- *APP*: in the last 2 months flank pain, then an episode of gross hematuria
- *Objective examination*: Globose abdomen; mild tenderness on deep palpation (quadrant inf.sx); No palpable mass.
- *Blood tests*: Hb 9.2 g/dl; hypercalcemia; platelets and neutrophils greater than the upper limit of normal.
- *Contrast enhanced CT abdomen*: heterogeneously enhancing mass arising from the lower pole of the left kidney.
- *Staging* negative for metastasis.

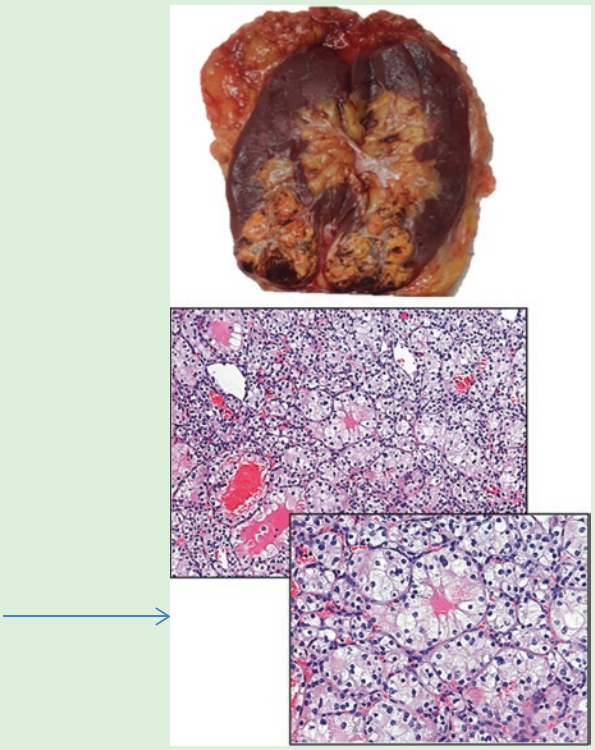
Question

What action should be taken?

1. Surgery
2. neoadjuvant chemotherapy treatment
3. Medical treatment with TKI

Answer

Radical nephrectomy, with a curative intent



Histological examination: *clear cell carcinoma (ccRCC)*

Question

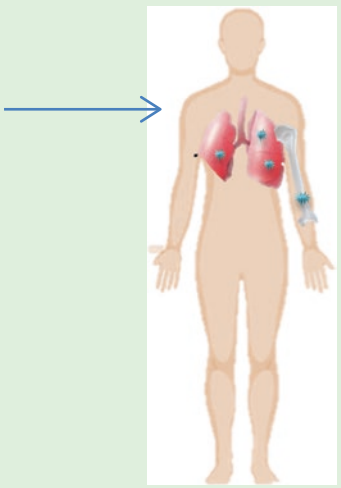
After surgery?

1. Adjuvant treatment
2. Observation
3. Follow-up

Answer

Follow-up

After 2 years, multiple pulmonary and bone metastases



Question

1. Surgery of metastases?
2. Start medical treatment?
3. Follow-up?

Answer

Starts medical treatment

Expert Opinion

(Genitourinary Cancers: Kidney, Antonio Russo, Department of Surgical, Oncological and Oral Sciences, University of Palermo, Palermo, Italy; Marc Peeters, Oncology Department, University of Antwerp, Edegem, Belgium; Lorena Incorvaia, Department of Biomedicine, Neuroscience and Advanced Diagnostics, University of Palermo, Palermo, Italy; Christian Rolfo, Thoracic Medical Oncology, University of Maryl and Greenebaum Comprehensive Cancer Center, Baltimore, MD, USA)

Key Points

- Renal cell carcinoma (RCC), derived from renal tubular epithelial cells, accounts for ~2% of all adult malignancies; it is the seventh most common cancer in men and the tenth most common cancer in women.
- Approximately 75% of RCC are clear cell carcinoma (ccRCC). The other RCC subtypes are a heterogeneous group of cancer with different morphology, genetic and molecular pathogenesis, and clinical behavior, as a whole known as non-clear cell RCC (nccRCC).
- Today we know that between the histological subtypes there are not only histologic difference, but also cytogenetic alterations with specific genes mutated.
- RCC is, indeed, a tumor where new biological knowledge has changed the landscape: antiangiogenic agents and immunotherapy are changing the natural history of the disease.
- Given the availability of several promising new drugs with novel mechanisms of action, the immediate challenge is to choose the most effective and specific combination or sequential therapy to prevent resistance in individual patients. The biomarkers can help in the future to formulate personalized treatment, driving the choice of treatment and preventing or overcoming drug resistance.

Recommendations

- AIOM:
- ► https://www.aiom.it/linee-guida-aiom-tumori-del-rene-2019/
- ESMO:
- ► https://www.esmo.org/guidelines/genitourinary-cancers/renal-cell-carcinoma

Hints for a Deeper Insight/Suggested Reading

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Bladder Cancer

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Genitourinary Cancers

Contents

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https://doi.org/10.1007/978-3-030-56051-5_46

Learning Objectives

- By the end of this chapter, the reader will:
- Be able to apply staging and diagnostic procedures for bladder cancer
- Have learned the basic concepts of bladder cancer
- Have reached in-depth knowledge of epidemiology, staging, molecular biology, and treatment of bladder cancer
- Be able to put acquired knowledge into clinical practice of bladder cancer

46.1 Introduction

Bladder cancer is one of the most common urologic cancers with the highest recurrence rate of any malignant disease. In North and South America, Europe, and Asia, the most common histologic subtype is transitional cell carcinoma. Other histotypes include squamous cell carcinoma and adenocarcinomas.

46.2 Epidemiology

Bladder cancer represents the ninth tumor by incidence in the World with an incidence rate of 9 per 100,000 in men and 2.2 per 100,000 in women, turning out to be the fourth tumor by incidence in men (9% of all diagnoses of cancer), and the 11th tumor in women (2.7% of all diagnoses of cancer). In terms of mortality, it represents 4.5% of total cancer deaths in males and 1.7% in women. These data indicate that survival of bladder cancer, considering all the stages, is averagely long, with a statistically declining trend of mortality rate in men (−1.5% per year) and a stable mortality trend in women (+0.3% per year):

- Incidence of bladder cancer increases with age, with a median of diagnosis around 72 years.
- Bladder cancer is rarely diagnosed before age 40 years.
- Bladder cancer is about three times more common in men than in women.
- In the past two decades, the incidence of bladder cancer has been stable in men, but has increased in women (+0.2% per year).

46.3 Etiology

Factors that may increase bladder cancer risk include the following:

- Cigarette Smoke: it is the main risk factor; it causes 50–60% of bladder cancers in men and 20–30% in women. The correlation between bladder cancer's incidence, number of cigarettes and years of smoking is statistically relevant.
- Occupational exposure: exposure to paint components, PAHs, aromatic amines, and diesel exhaust are the second risk factor.
- Diet: consumption of fruits and vegetables reduces bladder cancer incidence.
- Chronic infections: according to numerous scientific works a correlation was found between chronic urinary infections and infiltrating tumor forms and squamous histological subtype (e.g., Schistosoma, Haematobium, and Bilharzia).
- Previous chemotherapy exposures: cyclophosphamide is associated with an increased risk of approximately nine times; radiotherapy of the abdomen and/or pelvis increases the risk of bladder cancer even many years later.
- Genetics: Although some polymorphisms seem to increase susceptibility to bladder cancer in persons with work exposure to substances associated with increased risk, no strong evidence have been observed for hereditary factors in the development of bladder cancer even if familial clusters of bladder cancer have been described.

There is currently no evidence that non-invasive screening investigations could reduce risk of mortality of bladder cancer.

46.4 Clinical Features

Bladder cancer could be asymptomatic for a long time. Symptoms may also vary depending on the location and extension of the tumor. Bladder cancer located at the urethral level could cause acute urinary retention, while superficial forms may be asymptomatic or give irritative symptoms (e.g., increased frequency of urination, increased urgency of urination, urge incontinence, excessive passage of urine at night). Bladder tumors at the ureteral openings could cause hydroureteronephrosis. In advanced stages, the symptomatology may be characterized by asthenia, loss of appetite, weight loss, pain in the sites of metastasis, or organ failure.

46.5 Pathological Features

Ninety percent of bladder cancer derives from the urothelial epithelium so it's called urothelial cell carcinoma. Other uncommon forms are squamous cell carcinoma, mixed forms, sarcomatoid tumors, and small cell carcinomas. Bladder cancer is distinct into invasive and non-invasive carcinoma based on the infiltration of the basal membrane.

46.5.1 Non-Invasive Bladder Cancer

It includes 70% of urothelial cell carcinomas, it is characterized by papillary architecture and subdivided on the basis of cytological nucleus differentiation grade (WHO 2016) in three forms:

- Non-invasive urothelial cell carcinoma of low malignant potential (50% of recurrence risk)
- Non-invasive urothelial cell carcinoma of low grade: >50% of recurrence risk and 10% of metastatic risk
- Non-invasive urothelial cell carcinoma of high grade: >60% of recurrence risk and 30% of metastatic risk.

46.5.2 Invasive Bladder Cancer

It includes all bladder cancers which infiltrate the basal membrane, which are usually high-grade neoplasia with a high recurrence and metastatic risk.

46.6 Molecular Biology

Somatic mutations in fibroblast growth receptor 3 (FGFR-3) and tumor protein p53 (TP53) in tumor cells seem to be crucial molecular events in the non-invasive and invasive pathways, respectively.

FGFR-3, Ras, and PIK3CA mutations occur more frequently in non-invasive bladder cancer, upregulating the AKT and mitogen-activated protein kinase (MAPK) pathway. Loss of heterozygosity (LOH) on chromosome 9 is among the most frequent genetic alterations in bladder cancers and is considered an initial event.

The TP53 gene is found to be altered in approximately 60–65% of invasive bladder cancers and its mutation is significantly related with a short Progression-Free Survival (PFS). The TP53 gene mutation is also considered an independent predictive factor of death in patients with muscle-invasive bladder cancer.

Usually, high-grade invasive cancers also present alterations in PTEN and retinoblastoma (Rb) genes.

In addition to specific cancer cell genetic alterations, the tumor microenvironment may influence the tumor growth and cell proliferation by production of vascular endothelial growth factor (VEGF) and abnormal E-cadherin expression.

46.7 Diagnosis

In case of clinical suspicion of bladder cancer, it is mandatory to proceed with clinical examination, blood tests, urinalysis (to evaluate the presence of anemia from hematuria, acute renal failure from hydronephrosis, and exclude the presence of urinary tract infections). If a bladder cancer is suspected, it is convenient to perform cytological analysis of urinary sediment to eventually identify tumor cells. Eco-ultrasound has a diagnostic sensibility of 80–95% and high diagnostic specificity, with some limitations, such as the inability to diagnose flat bladder neoplasms and to study the upper urinary tract and the fact that it is an operator-dependent exam. Due to these limitations, in case of neoplasm found with the echography or strong clinical suspicion, it is mandatory to perform an endoscopic evaluation. The local staging could be performed both with computerized tomography (CT) and with magnetic resonance imaging (MRI); however, the MRI is more detailed to define the local staging. Once a radiological description of the lesion is obtained it is necessary to complete the diagnostic work-flow with an endoscopic evaluation with flexible cystoscopy in order to evaluate both the number and morphological features (papillary or solid) of the lesions and to describe mucosal anomalies. The transurethral resection of bladder tumor (TURBT) is a diagnostic-staging procedure, also, in the case of small lesions (smaller than a centimeter), allows to perform the radical removal of its implant base and the surrounding margins, to identify a possible involvement of the muscular tunic. These data are essential for the subsequent therapeutic strategy.

46.8 Staging and Prognosis

Bladder tumors could be subdivided into non-muscle invasive (T1, Ta, Tis) and muscle-invasive (T2–T4 – Table 46.1). The prognosis of non-invasive muscle neoplasms is significantly influenced by the grading of differentiation, by the size, by the number of diagnosed neoplasms, by the number of recurrences, by submucosal invasion, and by the presence of in situ carcinoma, a high-grade flat neoplasm with high risk of invasion. Related to these characteristics, 5 years progression risk ranges from 1% to 45%. In muscle-invasive cancers, recurrence risk and 5-year survival are, respectively, 68% and 66%.

46.9 Treatment

The treatment of bladder cancer is multidisciplinary and should involve urologist, oncologist, and radiotherapist. Treatment algorithm for management of bladder cancer includes non-muscle-invasive bladder cancer, localized muscle-invasive bladder cancer, and metastatic disease (Fig. 46.1).

Table 46.1 TNM staging system

Primary tumor (T)		*Stage*	*T*	*N*	*M*
TX	Primary tumor cannot be assessed	Stage 0a	Ta	N0	M0
T0	No evidence of primary tumor	Stage 0is	Tis	N0	M0
Ta	Noninvasive papillary carcinoma	Stage I	T1	N0	M0
Tis	Carcinoma in situ: "flat tumor"	Stage II	T2a	N0	M0
T1	Tumor invades lamina propria (subepithelial connective tissue)		T2b	N0	M0
T2	Tumor invades muscularis propria	Stage IIIA	T3a	N0	M0
pT2a	Tumor invades superficial muscularis propria (inner half)		T3b	N0	M0
pT2b	Tumor invades deep muscularis propria (outer half)		T4a	N0	M0
T3	Tumor invades perivesical tissue		T1–T4a	N1	M0
pT3a	Microscopically	Stage IIIB	T1–T4a	N2, N3	M0
pT3b	Macroscopically (extravesical mass)	Stage IVA	T4b	Any N	M0
T4	Tumor invades any of the following: prostatic stroma, seminal vesicles, uterus, vagina, pelvic wall, abdominal wall		Any T	Any N	M1a
T4a	Tumor invades prostatic stroma, uterus, vagina	Stage IVB	Any T	Any N	M1b
T4b	Tumor invades pelvic wall, abdominal wall				
Regional lymph nodes (N)					
Regional lymph nodes include both primary and secondary drainage regions. All other nodes above the aortic bifurcation are considered distant lymph nodes.					
NX	Lymph nodes cannot be assessed				
N0	No lymph node metastasis				
N1	Single regional lymph node metastasis in the true pelvis (perivesical, obturator, internal and external iliac, or sacral lymph node)				
N2	Multiple regional lymph node metastasis in the true pelvis (perivesical, obturator, internal and external iliac, or sacral lymph node metastasis)				
N3	Lymph node metastasis to the common iliac lymph nodes				
Distant metastasis (M)					
MO	No distant metastasis				
M1	Distant metastasis				
M1a	Distant metastasis limited to lymph nodes beyond the common iliacs				
M1b	Non–lymph node distant metastases				

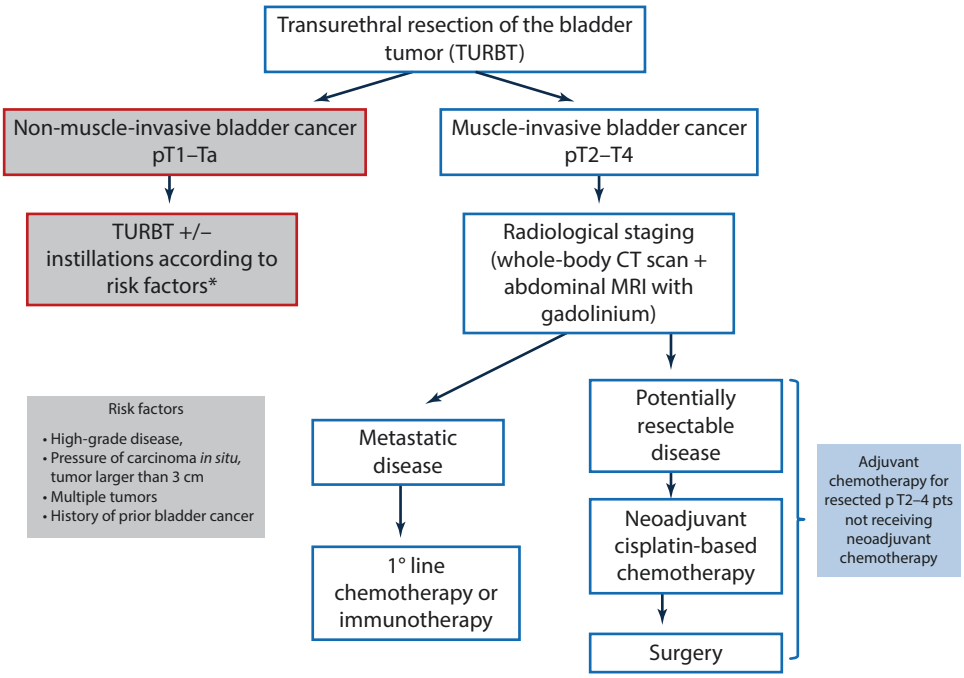


Fig. 46.1 Treatment algorithm for management of blabber cancer

46.9.1 Treatment of Non-Muscle-Invasive Bladder Cancer

Complete TURBT is the treatment of choice for any initial bladder tumor, followed by instillations according to risk. Risk factors for recurrence and progression include high-grade disease, presence of carcinoma in situ, tumor larger than 3 cm, multiple tumors, and history of prior bladder cancer. Intravesical BCG is the treatment of choice for reducing the risk of cancer progression and is mainly used for cancers with an intermediate or high risk of progressing. It is associated with a risk of significant toxicity, including rare deaths from BCG sepsis, local toxicity, and systemic side effects. Because of concerns about side effects, BCG is not generally used for patients with a low risk of progression. Intravesical therapy with thiotepa, mitomycin C, or doxorubicin is also often used for treatment of patients with multiple tumors or recurrent tumors or as a prophylactic measure. Segmental cystectomy or radical cystectomy is used in highly selected patients at high risk of progression with extensive or refractory superficial high-grade tumors based on reports that up to 20% of patients are at risk of death.

46.9.2 Treatment of Muscle-Invasive Bladder Cancer

Radical cystectomy is the standard treatment option of muscle-invasive bladder cancer and its effectiveness at prolonging survival increases if it is preceded by cisplatin-based multiagent chemotherapy. Radical cystectomy is a major operation with a perioperative mortality rate of 2–3% that is accompanied by pelvic lymph node dissection and includes removal of the bladder, perivesical tissues, prostate, and seminal vesicles in men and removal of the uterus, fallopian tubes, ovaries, anterior vaginal wall, and urethra in women. Postoperative complications may include erectile dysfunction in men and sexual dysfunction in women. After radical cystectomy, approximate 30–40% risk of recurrence still exists for patients with muscle-invasive disease and overall survival has generally been reported to be in the range of 50–60%. Combined treatments with the highest level of evidence supporting their effectiveness are radical cystectomy preceded by multiagent cisplatin-based chemotherapy and radiation therapy with concomitant chemotherapy.

46.9.2.1 Neoadjuvant and Adjuvant Chemotherapy

Because bladder cancer commonly recurs with distant metastases, systemic chemotherapy administered before cystectomy may be preferable to postoperative treatment in order to enhance tumor resectability and treat occult metastatic disease. Additionally, neoadjuvant chemotherapy is better tolerated. The use of cisplatin-based neoadjuvant chemotherapy for bladder cancer is supported by a 5% absolute increase in 5-year OS and a 9% absolute increase in 5-year disease-free survival compared with radical cystectomy alone. This demonstrated survival benefit encourages the use of this approach in patients with good performance status (ECOG 0–1) and renal function (GFR > 60 ml/min) with clinical stage T2–T4, N0. While there is still insufficient evidence for

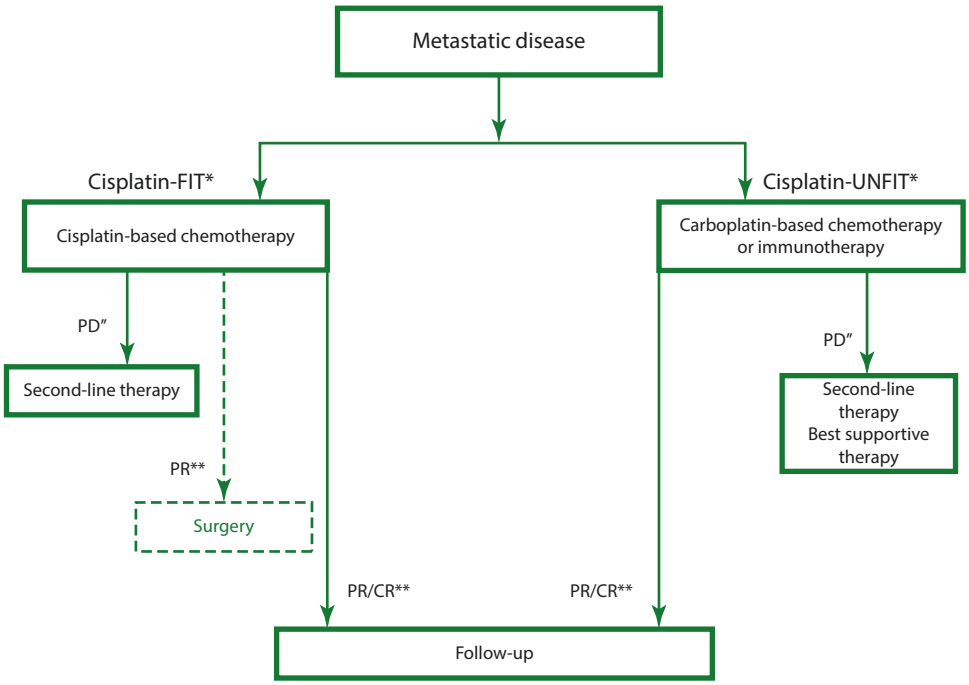


Fig. 46.2 Treatment algorithm for metastatic disease

*Cisplatin-FIT: ECOG P5 O-1, Creatinine clearance >60 ml/min, no serious comorbid conditions
** PD: Progressive disease, PR: Partial response, CR: Complete response

the routine use of adjuvant chemotherapy in clinical practice, it is likely that high-risk patients that have not received neoadjuvant chemotherapy, such as those with pT2–T4 and/or node-positive disease, will benefit most from adjuvant cisplatin-based chemotherapy.

46.9.2.2 Radiotherapy With or Without Concomitant Chemotherapy

The approach of organ preservation therapy is a reasonable option for patients seeking an alternative to cystectomy and a palliative option for those who are medically unfit for surgery. In these cases, definitive radiation therapy is a treatment option that yields a 5-year survival of approximately 30–40%, and best results are seen in patients with solitary lesions and without carcinoma in situ or hydronephrosis. The addition of chemotherapy to radiation therapy has been shown to reduce local relapse rates, although it has not been shown to result in increased survival or improved quality of life.

46.9.3 Metastatic Disease

Metastatic disease can arise at the time of diagnosis (about 10% of cases) or, more often, occur after primary surgical treatment in about half of all patients. Distant metastases are more common than local recurrences and they can involve lymph nodes, bones, lungs, or other organs. Patients with metastatic disease are treated with systemic therapy, even if metastasectomy could be taken into consideration in selected cases with oligometastatic disease, especially located in lung or lymph nodes, and good response to pharmacological treatments (Fig. 46.2).

46.9.3.1 First-Line Therapy

Since the 1980s, cisplatin-containing chemotherapy is the gold standard treatment for advanced bladder cancer. In particular, GC (Gemcitabine plus Cisplatin) and HD-MVAC (High Dose of Methotrexate, Vinblastine, Doxorubicin and Cisplatin with growth factor support) regimens represent the recommended first-line treatment options for cisplatin eligible patients thanks to the results of two large phase III trials (Von Der Maase et al., 2000; Sternberg et al., 2001). Cisplatin-based chemotherapy is associated with a median overall survival of 14 months with better efficacy in patients with only lymph node disease and good performance status. For cisplatin ineligible patients (due to compromised renal or liver status, poor performance status, or serious comorbid conditions), representing about one-third of all patients, regimens with lower toxicity profiles are recommended. For example, the use of carboplatin may be a substitute for cisplatin and non-platin-containing regimens, including taxanes and gemcitabine, may be considered in some cases. For selected patients with only lymph node subdiaphragmatic disease who present a partial response after first-line chemotherapy, a surgical approach on residual disease can be considered as part of a multidisciplinary choice. Results of ongoing phase III clinical

Table 46.2 Clinical trials of immune-checkpoint inhibitors plus chemotherapy for first-line urothelial cancer

Study	Agent	Phase and type	Primary endpoint
MK347536/ KEYNOTE-361	Pembrolizumab ± chemotherapy vs chemotherapy	Randomised. controlled	PFS, OS
IMvigor130	Alezolizumab ± chemotherapy vs chemotherapy	Randomised, controlled	PFS, OS, % with AEs
DANUBE	Durvalumab ± tremelmimab vs SOC chemotherapy	Randomised, open label	PFS, OS
CheckMate901	Nivolumab ± ipilimmab vs chemotherapy	Randomised, open label	PFS, OS

trials investigating immune-checkpoint inhibitors in combination with chemotherapy for the treatment of first-line urothelial cancer are awaited (Table 46.2). Early data from KEYNOTE-361 (pembrolizumab ± chemotherapy versus chemotherapy) and IMvigor130 (atezolizumab ± chemotherapy versus chemotherapy) showed a detrimental effect in terms of overall survival of immunotherapy alone compared to chemotherapy for those patients with a negative PD-L1 expression. For this reason, on May 2018, FDA restricted first-line use of atezolizumab and pembrolizumab for patients who are not eligible for cisplatin and whose tumors express PD-L1 (PD-L1 expression ≥5% and ≥10%, respectively) or in patients who are not eligible for any platinum-containing chemotherapy regardless of PD-L1 expression (NCCN Guidelines Bladder Cancer Version 05.2018).

46.9.3.2 Second-Line Therapy

In patients progressing after first-line therapy, prognosis is very poor with a median overall survival of 5–7 months. Treatment options for subsequent line of therapies include several chemotherapeutic agents (docetaxel, paclitaxel, gemcitabine, pemetrexed), many of which tested in phase II trials with a modest antitumor activity (overall response rate – ORR from 0% to 30%). Vinflunine, a third-generation member of the vinca alkaloid family, is the only drug that showed a survival benefit in this setting. Based on a 2.6-month median survival gain compared with best supportive care in a randomized phase III clinical trial (Bellmunt et al., 2009), it was approved as second-line treatment option in metastatic transitional cell carcinoma of the urothelium by the European Medicines Agency (EMA), but not by the US Food and Drug Administration (FDA).

46.9.3.3 New Agents

Given the high rate of somatic mutations reported in bladder cancer, immunotherapy has shown a significant impact as a treatment option for this pathology. Table 46.3 summarizes recent data of immunotherapy in metastatic urothelial cancer both in the first and second lines. Based on these results, the PD-1 inhibitors nivolumab and pembrolizumab as well as the PD-L1 inhibitor atezolizumab are approved by FDA and EMA for the treatment of locally advanced or metastatic urothelial cell carcinoma that has progressed during or after platinum-based chemotherapy or within 12 months of neoadjuvant or adjuvant platinum-containing chemotherapy. In addition, durvalumab and avelumab (PD-L1 inhibitors) are approved for the same indication only by FDA.

Although immune checkpoint inhibitors have improved outcomes in some patients with platinum-resistant metastatic or unresectable urothelial carcinoma, many others may not benefit from this kind of approach. A part of immune-refractory patients are carriers of FGFR (fibroblast growth factor receptor) alterations, which are found in 10–20% of metastatic urothelial carcinoma and are typical of the immunologically "cold" luminal 1 molecular subtype. Erdafitinib, a pan-FGFR inhibitor, demonstrated promising activity among patients with FGFR alterations in the recently presented open-label phase 2 study BLC2001: 42% confirmed ORR (3% CR, 39% PR) and 80% disease control rate (CR + PR + SD), data through which FDA granted Breakthrough Therapy Designation for erdafitinib in the treatment of urothelial cancer (Siefker-Radkte et al., ASCO-GU abstract #411, ASCO Annual Meeting 2018 abstract 4503). A phase III trial is ongoing.

46.10 Follow-Up

Timing, plan, and follow-up duration may vary based on risk categories. In general, for non-muscle invasive disease, the first cystoscopy performed after 3 months since the diagnosis represents an important prognostic factor. In the following tables, useful follow-up algorithms are shown.

Table 46.3 Trials with immunotherapy in metastatic urothelial cancer both in the first and second lines (Jiang et al., 2018)

Setting	ICI	Phase	*n*	ORR (%)	PFS (mo)	OS (mo)	PD-L1 biomarker analysis			SAEs	HrQOL
							Assay	Cells	Cut-off		
Metastatic second line therapy	Alezolizumab vs. chemotherapy[a]	III	931	22	2.1	11.1 vs 10.6	VentanaSP142	1C	5%	20	Improved fatigue and physical function
	Prembroliziamab vs. chemotherapy[a]	III	542	210	2.1	10.3 vs 7.4	Dako IIIC22C3	TC and IC	10%	15	–
	Nivolumab	II	270	20	2.0	8.74	Ventana SP142	TC and IC	1%, 5%	18	Stable or improved
	Avelumab	I	249	17	1.58	6.5	Dako IHC73-10	TC	5%	8	–
	Durvalumab	I/II	191	17.8	1.5	18.2	Ventana SP263	TC or IC	25%	4.9	–
Metastatic firstline[b]	Atezolizumab	II	119	23	2.7	15.9	Ventana SP142	TC	<1%, 1–4%, ≥5%	16	–
	Pembrolizumab	III	370	29	2.0	11.0	Dako IHC22C3	TC and IC	<1%, 1–9%, ≥10%	16	–

ICI immune checkpoint inhibitor, *ORR* objective response rate, *PFS* progression free survival, *OS* overall survival, *SAEs* severe adverse events, *HrQOL* health-related quality of life, *IC* immune cells, *TC* tumor cells

[a]Chemotherapy was physician's choice of docetauel (74 mg/m^2), paclitaxel (175 mg/m^2), or vinflunine (320 mg/m^2) every 3 weeks

[b]Cisplatin-ineligible patient

46.10.1 Non-Muscle Invasive Bladder Cancer

Low risk	*Cystoscopy*: after 3 and 12 months; then annually up to 5 years *Imaging*: upper tract and abdominal-pelvic imaging baseline
Intermediate risk	*Cystoscopy and urine cytology*: after 3 and 6 months, then every 6 months up to 2 years, then annually up to 5 years *Imaging*: upper tract and abdominal-pelvic imaging baseline
High risk	*Cystoscopy and urine cytology*: every 3 months up to 2 years, every 6 months up to 5 years, then annually up to 10 years *Imaging*: upper tract and abdominal-pelvic imaging baseline, upper tract imaging every 1–2 years up to 5 years

46.10.2 Muscle Invasive Bladder Cancer

Post-bladder sparing (partial cystectomy or chemoradiation)	*Cystoscopy*: every 3 months up to 2 years; every 6 months up to 4 years, then annually up to 10 years *Urine cytology*: every 6–12 months up to 2 years *Blood tests*: every 3–6 months up to 2 years *Imaging*: chest, upper tract, and abdominal-pelvic imaging every 3–6 months up to 2 years, then annually up to 5 years
Post-cystectomy pT2 N0	*Urine cytology*: every 6–12 months up to 2 years *Blood tests*: every 3–6 months up to 2 years, then every 9–12 months up to 5 years *Imaging*: chest and abdominal-pelvic imaging (CT scan) every 6 months up to 2 years, then annually up to 5 years
Post-cystectomy pT3–4 and/or pN+	*Urine cytology*: every 6–12 months up to 2 years *Blood tests*: every 3–6 months up to 2 years, then every 9–12 months up to 5 years *Imaging*: chest and abdominal-pelvic imaging (CT scan) every 4 months up to 2 years, then every 6 months up to 5 years *Blood tests*: CBC (cell blood count), renal, and liver function testing

Case Study: Cisplatin-Fit Patient

Man, 67 years old. Smoker of 20 cigarettes die

- *Family history* negative for malignancy
- *APR*: Hypertension
- *APP*: Hematuria and dysuria for nearly 4 months
- *Objective examination*: Globose abdomen; mild pain during deep palpation on right flank with palpable mass
- *Blood tests*: Anemia (Hb 9.1 g/dl)
- *Urine cytology*: Positive for malignant tumor cells
- *CT abdomen mdc*: Lesion in the right renal pelvis
  - No lymphadenopathies
  - No distant metastases

Question

What action should be taken?

(1) Surgery. (2) Biopsy. (3) Other

Answer

Surgery: right nefro-ureterectomy + TURBT

Histological examination:

(a) Kidney, paracaval lymph node, and ureter: high-grade papillary and solid urothelial carcinoma infiltrating the pelvis wall at full thickness and the peripielic fat. Free the renal parenchyma. Multiple foci of high-grade urothelial neoplasia along the ureter mucosa. pT3N0

(b) Bladder: high-grade solid urothelial carcinoma infiltrating the muscularis propria. pT2

Conclusion:

Resected renal pelvis urothelial carcinoma pT3N0 + endoscopically resected bladder urothelial carcinoma pT2

Question

What action should be taken?

(1) Surgery. (2) Medical therapy. (3) Radiotherapy

Answer

Surgery: radical cystectomy + bilateral pelvic lymphadenectomy

Histological examination: high-grade urothelial carcinoma of the bladder infiltrating the bladder wall and the perivesical fat. Metastasis in 1/6 right iliac lymph nodes. pT3a N1

Staging of disease with CT thorax-abdomen and PET-FDG: right lung metastasis in the medium lobe + right external iliac lymphadenopathies and metastasis on the abdominal wall in hypogastrium

Question

What action should be taken?

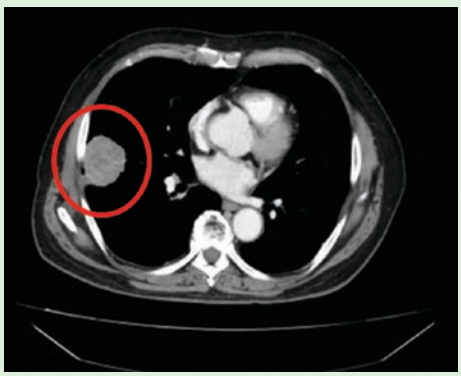
(1) Surgery. (2) Medical therapy. (3) Radiotherapy

Answer

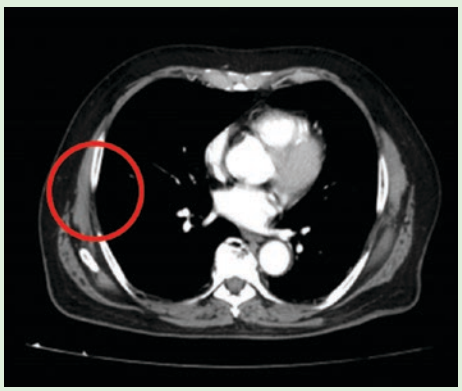
Medical therapy: the patient begins first-line chemotherapy with Cisplatin + Gemcitabine

Response evaluation after 6 months of therapy with Cisplatin + Gemcitabine: Partial response to CT; partial metabolic response to PET-FDG (reduction in extension and intensity of metastatic lesions)

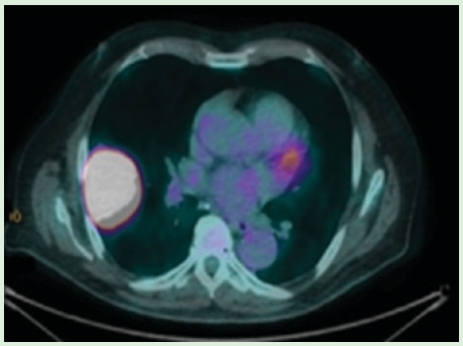
Lung Metastasis
Basal

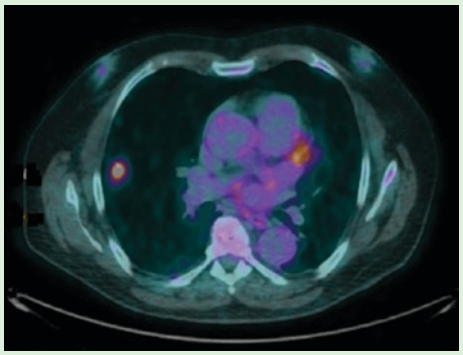


After 6 months of chemotherapy



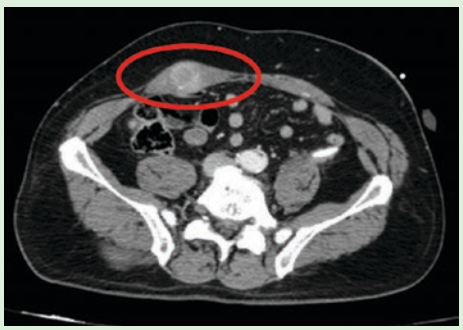
CT



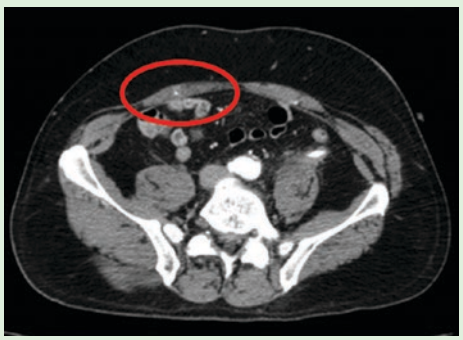


PET-FDG

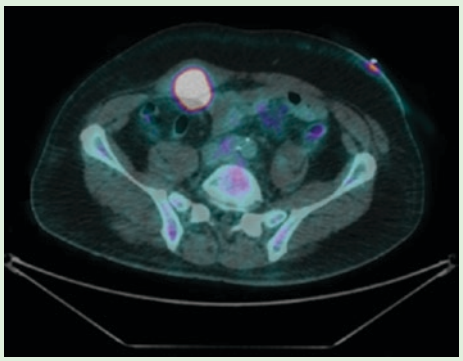
Hypogastric Abdominal Wall Metastasis
Basal

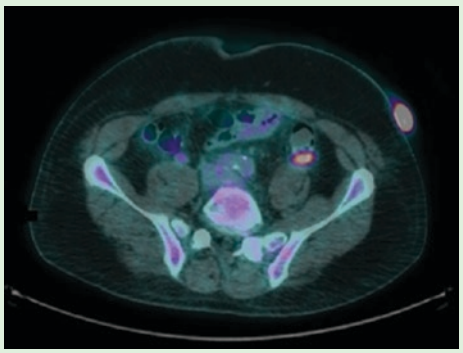


After 6 months of chemotherapy



CT





PET-FDG

Question

What action should be taken?

(1) Metastasectomy. (2) Follow-up. (3) Continues chemotherapy

Answer

Surgical evaluation: metastasectomy not indicated due to initial disease extension and tumor aggressiveness → the patient begins *follow-up*

After 6 months of follow-up, progression disease (lung right lesion 40 versus 14 mm and appearance of new nodules + increase of the right external iliac lymphadenopathy)

Question

What action should be taken?

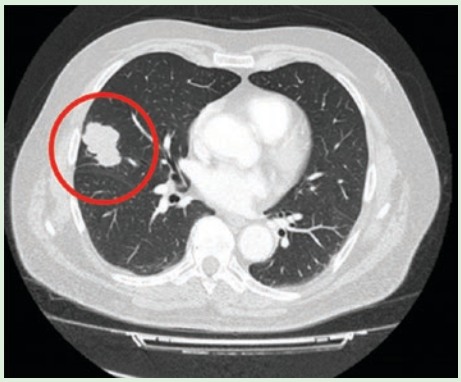
(1) Second-line chemotherapy. (2) Cisplatin-gemcitabine re-challenge. (3) Immunotherapy

Answer

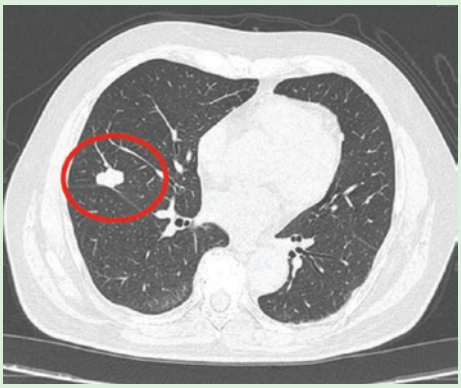
The patient begins second-line chemotherapy with Vinflunine (immunotherapy not available)

- *Response evaluation after 3 months of therapy with Vinflunine*: Partial pulmonary response, lymph nodal stable disease to CT, he continues chemotherapy until progression or unacceptable toxicity

Before treatment



After 3 months of Vinflunine



Key Points

- The importance of a correct disease staging to avoid unnecessary surgery
- The importance of a multidisciplinary approach
- Correct management of medical treatment side effects
- Choice of best therapeutic option for the patient

Case Study: Platin-Unfit Patient

Man, 77 years old

- *Family history* negative for malignancy
- *APR*: severe ischemic cardiopathy, hypertension, renal failure
- *APP*: after the appearance of hematuria TURBT with diagnosis of high-grade urothelial papillary carcinoma infiltrating the prostate (pT4)
- *CT Thorax-Abdomen mdc*: thickening of the bladder wall that involves the ureteral meatus causing hydroureteronephrosis, iliac lymphadenopathies, and lung metastasis (metastatic disease)
- *Blood tests*: serum creatinine 1.7 mg/dl (renal failure due to hydroureteronephrosis)

Question

What action should be taken?

(1) Surgery. (2) Nephrostomy. (3) Immediate first-line chemotherapy

Answer

Nephrostomy: the patient underwent bilateral nephrostomy placement with only partial renal function recovery

Question

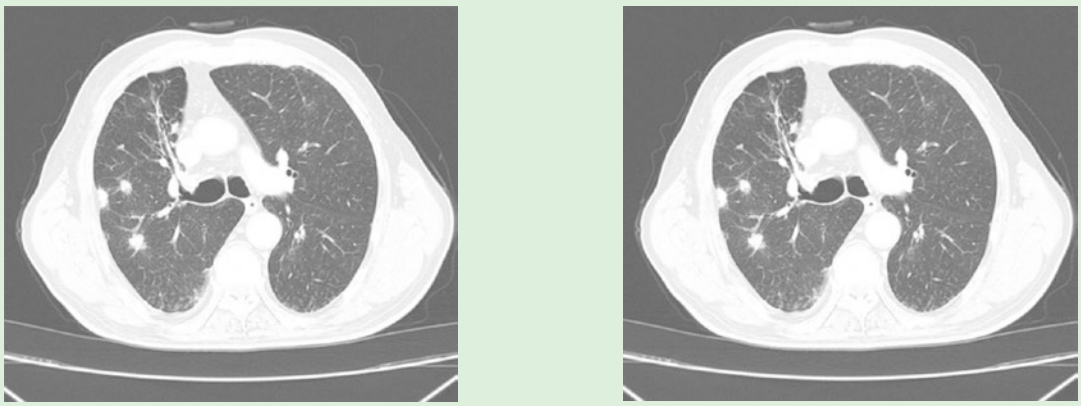
What action should be taken?

(1) Platin-based poli-chemotherapy. (2) Mono-chemotherapy. (3) Immunotherapy

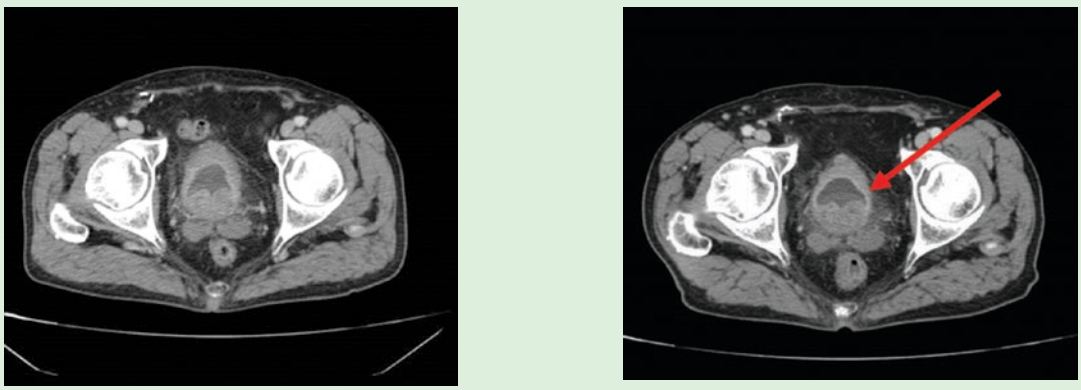
Answer

Mono-chemotherapy: patient begins first-line mono-chemotherapy with gemcitabine due to persistent moderate renal failure and concomitant cardiopathy (immunotherapy not available)

After 3 months of Gemcitabine: SD, after 6 months: local progression of the bladder lesion with urinary symptoms, pulmonary stability



Pulmonary stability after 3 and 6 months of gemcitabine



Local PD after 6 months

Question

What action should be taken?

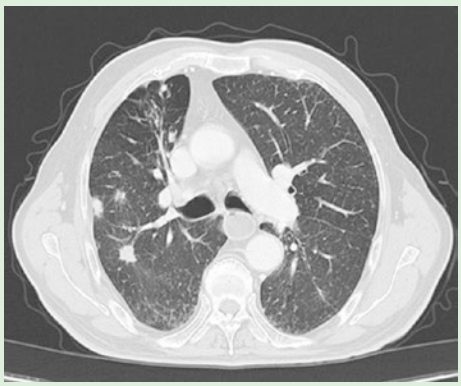
(1) Second-line chemotherapy. (2) Immunotherapy. (3) Best supportive care

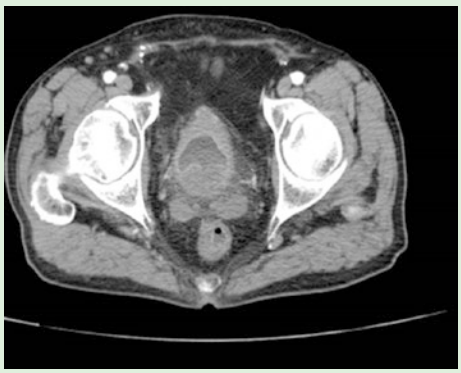
Answer

Second-line chemotherapy: the patient begins second-line chemotherapy with weekly paclitaxel

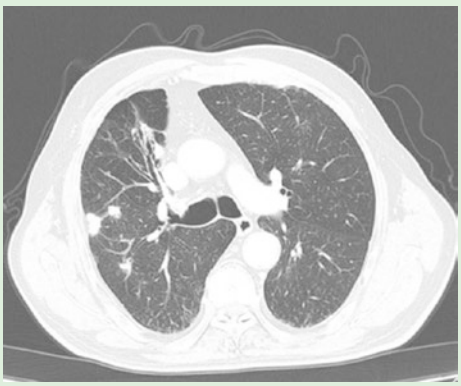
After 6 months of paclitaxel: stable disease, he continues with the same therapy until progression or unacceptable toxicity

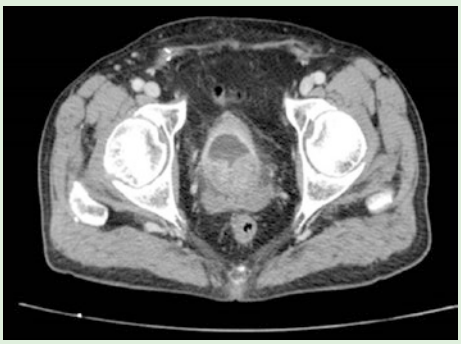
Before paclitaxel





After 6 months of paclitaxel





Key Points

- Importance of comorbidity evaluation in order to decide the best medical treatment
- It could be useful to consider immunotherapy for platin-unfit patients when possible
- The duration of therapy is based on tolerability and efficacy
- Importance of symptoms management in the context of simultaneous care

Expert Opinion

Giuseppe Procopio

Key Points

1. Bladder cancer is a common neoplasm that affects frequently men with a median age at the diagnosis of 72 years old. The most important risk factors are cigarette smoke, professional exposure to paint components or aromatic amines, chronic infections (i.e., Schistosoma Haemotobium, and Bilharzia), previous chemotherapies, and gene predisposition.
2. Symptoms vary from hematuria, increased frequency or urgency of urination, hydroureteronephrosis, and acute urinary retention, and they actually depend on the primitive site of cancer. Systemic symptoms are asthenia and weight loss, while others can depend on the sites of metastases.
3. There are different histological types, but the most frequent is the transitional cell carcinoma. Other types are squamous cell carcinoma, mixed forms, sarcomatoid tumors, and small cell carcinomas. Moreover, they can be divided in non-invasive and invasive subtypes considering the basal membrane infiltration. It is crucial to understand this aspect in order to administer the correct treatment.
4. Clinical examination, blood test, and urine analyses are the first steps in the diagnostic approach. US is quite useful but flexible cystoscopy is essential to study both the number and morphological features of the lesions. It is possible to investigate the local involvement thanks to MRI or CT which can be implied also for a complete staging. The transurethral resection of bladder tumor (TURBT) is the most important step, which can have also a therapeutic intent, and it allows to study the basal membrane invasion.
5. Treatments are different considering the infiltration of the basal membrane: in non-invasive cancers, TURBT is the treatment of choice followed by instillations according to the risk. In invasive forms, radical cystectomy is recommended preferable if preceded by cisplatin-based multitarget chemotherapy.
6. Radiation therapy can be used to prevent cystectomy or for those who are unfit for surgery. In case of metastatic disease, a platinum-based chemotherapy is recommended together with gemcitabine or high dose of Methotrexate, Vinblastine, Doxorubicin with growth factor support. In case of second-line therapy, Vinflunine is the only drug that showed a survival benefit.
7. PD-1 inhibitors Nivolumab and Pembrolizumab and the PD-L1 inhibitor Atezolizumab are approved for the treatment of locally advanced or metastatic urothelial cell carcinoma that has progressed during or after platinum-based chemotherapy or within 12 months of neoadjuvant or adjuvant platinum-containing chemotherapy.
8. Timing, plan, and follow-up duration may vary based on risk categories and can consist in cystoscopy, urine cytology, blood test, or imaging such as CT scan.

Recommendations

- ESMO
- ► https://www.esmo.org/Guidelines/Genitourinary-Cancers/Bladder-Cancer
- ASCO
- ► www.asco.org/practice-guidelines/quality-guidelines/guidelines/genitourinary-cancer#/25246
- ► www.asco.org/practice-guidelines/quality-guidelines/guidelines/genitourinary-cancer#/10691
- AIOM
- ► www.aiom.it/linee-guida-aiom-2020-tumori-urotelio/

Hints for a Deeper Insight

- Update on the Guideline of Guidelines: Non-Muscle Invasive Bladder Cancer: ► https://www.ncbi.nlm.nih.gov/pubmed/31597003
- Epidemiology of Bladder Cancer: A Systematic Review and Contemporary Update of Risk Factors in 2018: ► https://www.ncbi.nlm.nih.gov/pubmed/30268659
- Bladder cancer: Present and future: ► https://www.ncbi.nlm.nih.gov/pubmed/28736063

Suggested Reading

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Prostate Cancer: Locoregional Disease

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Genitourinary Cancers

Contents

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Learning Objectives

By the end of this chapter, the reader will:

- Be aware of the current recommendation statement of prostate cancer screening program
- Have learned the basic notions of localized prostate cancer diagnosis and staging
- Have reached in depth knowledge of all treatment options for localized prostate cancer, their efficacy, and side effects

47.1 Epidemiologic Evidence and Risk Factors of Prostate Cancer

47.1.1 Epidemiology

Prostate cancer (PCa) is the most frequently diagnosed non-skin cancer among Western men aged >50 years with an estimated incidence of 161,360 new cases in 2017 in the United States. More than 80% of PCa diagnoses are represented by localized disease [1].

Incidence rates in the United States fluctuated during the last decade with a peak of 240,000 new cases in the 1993. The great increase in incidence between the late 1980s and the mid-1990s were due to the large number of cases detected once PSA became available and widely utilized. Since 1992, incidence rates declined as a result of changing of PCa screening [2].

With regard to mortality rates in Western countries, PCa is the third leading cause of cancer death behind lung and colorectal cancer. In 2017, in the United States, 26,730 people died of PCa. A steady decline in mortality has been noted during the last decade due to the screening and numerous new treatments. Currently, the 5-year survival is approximately 91% [1].

47.1.2 Risk Factors

PCa is a multifactorial disease. The different role of constitutional and environmental risk factors in tumor carcinogenesis has yet to be elucidated [2].

The established constitutional risk factors are advancing age, positive family history, and the race. PCa develops mainly in older men; about six cases in ten are diagnosed in men aged 65 or older. A study of age-specific incidence reveals that PCa risk begins to rise after age 55 years and peaks at age 70–74, declining slightly thereafter [2].

Heredity plays a significant role in PCa. Men who have a family history of PCa are more likely to develop it themselves. Pathogenic variants in genes of high and moderate penetrance (e.g., BRCA1, BRCA2, the mismatch repair genes, and HOXB13) confer lifetime risk of PCa. However, these alterations probably explain no more than 10% of all cases [3].

Regarding race, PCa is more common among men of African descent than in Caucasian and Asiatic ethnicity. These men seem to develop a more aggressive disease and at a younger age than others racial groups [4]. The exact reasons for these differences are not known and may involve socioeconomic causes or other factors. Observation of Asian migrants provides the most compelling argument for environmental influences linked to Western lifestyle as causal factors [5].

The environmental risk factors potentially associated with PCa development include obesity, physical activity, sexual activity, smoking, and occupational exposures [5, 6].

47.2 Initial Prostate Cancer Diagnosis and Disease Staging

47.2.1 Prostate Cancer Screening

Secondary prevention is the most appropriate instrument to influence the natural history of a disease and to reduce its lethality. For a long time, the periodic dosage of prostate specific antigen (PSA) was considered the more useful screening test for early detection of PCa with a consequent dramatic increase in the incidence rate between the 1980s and the 1990s [2].

PSA evaluation and digital rectal examination (DRE) are the two components of the modern PCa screening program. The ERSPC (European Randomized Study of Screening for Prostate Cancer) and PLCO (Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial) trials were designed to define the effects of screening on prostate cancer-related mortality [7, 8]. Both trials showed that PSA screening, with or without the support of DRE, was associated with an increased of diagnosis rate. However, only the European study showed a reduction in PCa mortality in the screening arm compared to the control arm (RR 0.8; 95% CI, 0.65–0.98) after a median follow-up of 9 years [7–9]. In other words, to prevent one death from prostate cancer, 1410 (95% CI, 1132–1721) men need to be screened and 48 men treated [7].

Therefore, there are convincing evidence that the screening is associated with over-diagnosis and over-treatment [9]. Consequently, the decision to undergo PSA testing should be discussed between the patient and his physician, balancing advantages and disadvantages (opportunistic screening). Currently, the principal international guidelines do not recommend population-based PSA screening for prostate cancer annually. Individual screening may be considered for high-risk

populations: from the age of 50 years (or from the age of 45 years for African American men and family history of PCa) for men who have at least a 10-year life expectancy, with positive family history [10, 11].

47.2.2 Laboratory Test and Imaging for Diagnosis of Prostate Cancer

The main diagnostic tools to identify a prostate tumor include serum concentration of PSA, DRE, and prostate imaging. However, the only test that can confirm the diagnosis of PCa is tumor biopsy.

The PSA is a protein produced within the prostate gland and secreted into seminal fluid. Circulating PSA level can be elevated not only in the presence of cancer, but also in physiological conditions (recent ejaculation or intense physical activity), in case of benign pathologies (prostatic hypertrophy, prostatitis, prostatic infarction, urinary retention) and after diagnostic investigations (digital rectal examination, transrectal ultrasound, endorectal coil prostate magnetic resonance) [12]. There are two types of PSA: free PSA that moves freely in the blood, and complex PSA attached to other proteins. PCa cells produce more complex PSA than other physiological and pathological situations. Consequently, the higher the amount of free PSA, the less likely prostate cancer will be diagnosed. Therefore, several controversies exist regarding PSA level cutoffs and reference ranges [13].

DRE is a simple procedure to examine the peripheral zone of prostate gland where most often PCa is found. Any lumps, hard or irregular areas encountered during this procedure may indicate the presence of cancer. A prospective clinical trial, conducted by Catalona et al., compared DRE and serum PSA in the early detection of PCa. The study enrolled 6630 males, 50 years or older, who underwent PSA determination and/or DRE. The biopsies were performed if the PSA level was greater than 4 mcg/l and/or the DRE was suspicious. Of 1167 biopsies performed, cancer was detected in 264 cases. PSA identified significantly more tumors (82%, 216 of 264 cancers) than DRE (55%, 146 of 264 cancers, $p = 0.001$). The cancer detection rate was 3.2% for DRE, 4.6% for PSA and 5.8% for the two methods combined. The author concluded that the use of PSA in conjunction with DRE enhanced early PCa diagnosis; prostatic biopsy should be considered if either the PSA level was greater than 4 mcg/l or DRE was suspicious for cancer [14].

Transrectal ultrasound (TRUS) and multiparametric magnetic resonance imaging (mpMRI) are the two main imaging methods used for localized PCa detection [15]. TRUS could identify hypoechoic areas that are commonly associated with cancer. Currently, this technique plays an essential role in guiding prostate biopsy. mpMRI has better soft tissue resolution than TRUS. It uses more sequence in addition to the anatomic T2-weighted images, such as diffusion-weighted MRI, derived apparent-diffusion coefficient from diffusion-weighted MRI, and dynamic contrast-enhanced MRI [16]. mpMRI can be helpful in the characterization of suspicious lesions and in the staging of PCa to accurately define the capsular infiltration and assist the physician for surgical planning. Moreover, it is suggested for low-risk localized PCa in the active surveillance program [17, 18]. The use of mpMRI prior to starting active surveillance could identify missed lesions or, conversely, support this option for patients with minimal disease. Preliminary results speculate on the role of mpMRI in selecting patients for active surveillance [17, 18]. Less certain is the role of mpMRI in monitoring patients on active surveillance because larger validation studies are still necessary.

On the basis of the PSA level and/or a suspicious DRE, the TRUS-guided transperineal prostate core biopsy has become the standard way to obtain material for an accurate histopathologic diagnosis [19].

47.2.3 Histology and Grading Score

Prostatic adenocarcinoma is the most commonly diagnosed form of PCa (more than 90% of cases). One of the major characteristics of prostate adenocarcinoma is its heterogenic structure, with variably differentiated glandular structures formed by tumor cells that express PSA and androgen receptors [20].

Prostatic adenocarcinoma is subject to Gleason scoring to give an overall evaluation of the tumor differentiation and heterogeneity. The grading system consists of five different histologic patterns (from 1 to 5) based on the differentiation of tumor growth pattern compared to normal glandular structure. Two different scores are assigned at each prostate tumor, the first one refers to the most common pattern (which represents more than 50% of the tumor tissue) and the second one is the non-dominant cell pattern with the highest grade; the final Gleason score (GS) is the sum of these two scores [21]. The Gleason grading system remains one of the most powerful prognostic predictors in prostate cancer. However, this system has undergone significant revisions. The last revision was made during the 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma [22]. The new grading system includes five distinct Grade Groups based on the Gleason score groups (Grade group 1 = GS 6, Grade group 2 = GS 7 3 + 4, Grade group 3 = GS 7 4 + 3, Grade group 4 = GS 8, Grade group 5 = GS 9–10). Gleason score groups, initially described in 2013 in a study from Johns Hopkins

Hospital and then validated in a multi-institutional study on 20,845 radical prostatectomies, were shown to be more accurate in predicting tumor progression than the Gleason risk stratification groups (GS ≤ 6, GS 7, GS 8–10) [23, 24]. Moreover, even though the GS can range from 2 to 10, the last ISUP revision identified GS 6 as the lowest score that can be assigned.

47.2.4 Staging of Localized Prostate Cancer

The decision to proceed with further staging workup is guided by clinical and pathological features that define the risk of systemic spread: the number and the site of positive biopsy cores (Table 47.1), the tumor grade, and the level of serum PSA [25].

The most common sites of PCa spread are bone and lymph nodes. Abdominal-pelvic CT scan and bone scan are useful for defining the cancer dissemination [26].

Patients with clinical stage T2 or less, PSA < 10 ng/ml, Gleason score ≤7% and <50% positive biopsy cores have <10% likelihood of having node metastases and can be spared nodal evaluation with CT scan [23]. In addition, the bone scan may be avoided in asymptomatic cT1 patients, if the serum PSA level is <20 ng/ml and in asymptomatic cT2 patients with PSA level <10 ng/ml, but only for well-differentiated or moderately differentiated tumors (Gleason score ≤7) [27].

The international guidelines recommend the use of thoraco-abdominal computed tomography (CT) scan, abdominal Magnetic Resonance Imaging (MRI), and bone scan to complete PCa staging in intermediate- and

Table 47.1 TNM staging for prostate cancer

Clinical (c) primary tumor (T)	*Pathologic (p) primary tumor (T)*
cTx Primary tumor cannot be assessed	
cT0 No evidence of primary tumor	
cT1 Clinically inapparent tumor neither palpable nor visible by imaging *cT1a* Tumor incidental histologic finding in 5% or less of tissue resected *cT1b* Tumor incidental histologic finding in more than 5% of tissue resected *cT1c* Tumor identified by needle biopsy (e.g., because of elevated PSA)	
cT2 Tumor confined within prostate *cT2a* Tumor involves one-half of one lobe or less *cT2b* Tumor involves more than one-half of one lobe but not both lobes *cT2c* Tumor involves both lobes	*pT2* Organ confined *pT2a* Unilateral, involving one-half of one side or less *pT2b* Unilateral, involving more than one-half of one side but not both sides *pT2c* Bilateral disease
cT3 Tumor extends through the prostatic capsule *cT3a* Extracapsular extension *cT3b* Tumor invades the seminal vesicle	*pT3* Extraprostatic extension *pT3a* Extraprostatic extension or microscopic invasion of the bladder neck *pT3b* Seminal vesicle invasion
cT4 Tumor is fixed or invades adjacent structures other than seminal vesicles: bladder, levator muscles, and/or pelvic wall	*pT4* Invasion of bladder, rectum
Clinical (c) regional lymph nodes (N)	*Pathological (p) regional lymph nodes (N)*
cNx Regional lymph nodes were not assessed	*pNx* Regional nodes not sampled
cN0 No regional lymph node metastasis	*pN0* No positive regional nodes
cN1 Metastasis in regional lymph node(s)	*pN1* Metastasis in regional nodes(s)
Clinical (c) distant metastasis (M)	
cM0 No distant metastasis	
cM1 Distant metastasis *cM1a* Non-regional lymph node(s) *cM1b* Bone(s) *cM1c* Other site(s) with or without bone disease	

high-risk disease or in symptomatic patients [28]. Choline positron emission tomography/CT (PET/CT) is not considered standard for PCa staging.

47.3 Treatment Options for Localized Prostate Cancer

According to clinical risk groups for recurrence of localized and locally advanced PCa (Table 47.2), several treatments can be proposed [25]. The international guidelines consider radiotherapy (RT) and radical prostatectomy (RP) the standard treatments with curative intent for localized PCa. However, which of these approaches offers survival benefit over the other remains controversial. Therefore, choosing between RP and RT is based on the different toxicity profile. In addition, in particular conditions observational strategies can be adopted.

47.3.1 Observational Strategies: Active Surveillance and Watchful-Waiting

The active surveillance (AS) is an observational strategy that consists in a periodic monitoring of disease course, reserving surgery or radiation therapy in case of disease progression. The AS offers to selected men with PCa the chance to delay or avoid an invasive treatment and its associated side effects. Currently, this approach includes the PSA blood test and the DRE every 6 months, and prostate biopsies every year.

The ProtecT is the only prospective randomized clinical trial comparing the AS with an immediate curative treatment. Of the 82,429 patients who were screened, 1643 men were randomized in three arms: AS (n = 545), RP (n = 533), and 74 Gy of 3D conformal external beam RT (EBRT) (n = 545). In the AS group, the trigger for intervention included PSA kinetics (PSA doubling time less than 12 months), appearance of symptoms, changes in DRE, and patient anxiety. The patients had a median age of 62 years, median PSA of 4.6 ng/ml (90% of patients have PSA < 10 ng/ml) and not palpable disease (T1c). Moreover, 75% of the entire population had a Gleason Score 6. The primary aim was 10-year disease-specific survival. The secondary endpoints were all-cause mortality, the incidence of clinical progression, and the incidence of metastasis. Approximately, 80% of patients assigned to the surveillance arm did not demonstrate any clinical progression. However, almost 50% of men enrolled in this arm opted for treatment intervention. There was no statistical difference in OS among the three study arms (98.8% in the AS group; 99% in the surgery group; 99.8% in the radiant therapy group at 10 years; p = 0.48). However, the prostatectomy and the radiant therapy were associated with lower rates of disease progression, including metastasis, than active monitoring (112 patients in the AS group; 46 patients in the surgery group; 46 patients in the radiotherapy group; p < 0.001 for the overall comparison) [29].

According to the international guidelines, the AS strategy can be offered to patients with a life expectancy of 10 years or less with low-risk disease (T1–2a, Gleason score ≤6 and the PSA level <10 ng/ml), but also to patients with low-risk disease and a life expectancy greater than 10 years to avoid side effects related to surgery or radiant treatments.

The watchful-waiting is another option for men with early-stage prostate cancer. It consists in a less intensive follow-up with specific attention to the cancer-related symptoms to decide if a treatment is needed.

The Scandinavian Prostate Cancer Group Study number 4 (SPCG-4) is a prospective, randomized trial to compare the surgical treatment with watchful-waiting in men with localized PCa. After randomization, 348 patients were assigned to watchful-waiting arm and 347 men to RP. The study enrolled patients with localized PCa (cT1–2), age <75 years and PSA values <50 ng/ml. Moreover, approximately 50% of patients were classified as intermediate or high risk. The endpoints of this trial were all-cause mortality, specific survival and the incidence of metastasis. After a follow-up of 18 years, the data revealed that the risk of death from PCa was significantly lower in the RP arm than in the watchful-waiting group (17.7% vs. 28.7%, HR: 0.56; p = 0.001). Similarly, the risk of death from all causes was 56.1% in the RP arm and 68.9% in the watchful-waiting group (HR: 0.71; p < 0.001). Moreover, the risk of spreading was significantly lower for patients treated with RP (26.1% vs. 38.3%; RR 0.56; p < 0.001). Moreover, the

Table 47.2 Clinical risk groups for recurrence of localized and locally advanced prostate cancer

Risk group	Low	Intermediate	High
Localized PCa	PSA < 10 ng/ml and GS < 7 (ISUP grade 1) and cT1–2a	PSA 10–20 ng/ml or GS7 (ISUP grade 2/3) or cT2b	PSA > 20 ng/ml or GS > 7 (ISUP grade 4/5) or cT2c
Locally advanced PCa			Any PSA; any GS; cT3–4 or cN+; any ISUP grade

PCa prostate cancer, *PSA* prostatic specific antigen, *GS* gleason score, *ISUP* International Society of Urological Pathology

subgroup analysis showed a greater survival benefit of immediate treatment for younger patients (<65 years) and for high-risk disease [30].

By contrast, the Prostate Cancer Intervention versus Observation Trial (PIVOT) showed that RP did not significantly reduce PCa specific and overall mortality after a follow-up of 12 years. The PIVOT study enrolled 731 patients with a localized PCa (cT1–2) and PSA values <50 ng/ml who were younger than 75 years and had a life expectancy <10 years [31]. Although the inclusion criteria were similar, the two studies did not enroll a homogeneous population (cT1c: 12% in the SPCG-4 trial and 50% in the PIVOT; intermediate and high risk: 50% in the SPCG-4 trial vs. 35% in the PIVOT). The differences between study populations and the historic period (before or during the era of PSA testing in the SPCG-4 and PIVOT, respectively) make the results of these trials not fully comparable. In conclusion, watchful-waiting could be recommended for patients with cT1c prostate tumor and a life expectancy <10 years.

47.3.2 Surgical Approaches

RP represents the most common and effective treatment for localized PCa. RP can be performed by open, laparoscopic, or robot-assisted (RARP) approaches. The goal of each approach must be complete eradication of disease, while preserving continence and, whenever possible, potency [32]. In a randomized phase III trial, RARP showed to reduce admission times and blood loss but not early (12 weeks) functional or oncological outcomes [33, 34].

Actually many men with localized PCa will not benefit from definitive treatment, and 45% of men with PSA-detected PCa may be candidates for deferred management. In men with co-morbidity and a limited life expectancy, treatment of localized PCa may be deferred to avoid loss of quality of life (QoL).

47.3.2.1 Radical Prostatectomy and Prostate Cancer Risk Groups

RP has to be offered to patients with low- and intermediate-risk PCa and a life expectancy >10 years.

In low-risk PCa, a lymph node dissection (LND) is not recommended. In intermediate-risk PCa, a nerve-sparing surgery is recommended in patients with a low-risk of extracapsular disease (referring to specific nomograms). In order to select patients for nerve sparing procedures it is possible to use mpMRI.

An extended LND (eLND) has to be performed if the estimated risk for positive lymph nodes exceeds 5%. A limited LND is not recommended.

RP can be offered in patients with high-risk localized PCa and a life expectancy of >10 years only as a part of multimodal strategy. In high-risk PCa, a neoadjuvant hormonal therapy before RP is not recommended. In high-risk PCa an eLND is mandatory.

It is possible to offer RP also in selected patients with locally advanced (cT3a) disease and a life expectancy >10 years, but only as a part of multi-modal therapy. A nerve-sparing surgery can be offered to patients with a low risk of extracapsular disease (referring to specific nomograms). In high-risk disease the use of mpMRI is recommended as a decision-making tool to select patients for nerve-paring procedures. In highly selected patients with high-risk locally advanced PCa (cT3b-T4 N0 or any T N1), an RP can be offered only as a part of multimodal therapy.

47.3.2.2 Radical Prostatectomy in Senior Adult Patients (>70 Years of Age)

In senior adults with PCa it is recommended to perform a systematic health status evaluation using a geriatric screening with G8 and mini-COGTM.

Treatment options for senior adults according to their health status are as follows:

- Standard treatment to fit or healthy older men
- Standard treatment to frail patients with reversible impairment after the resolution of geriatric problems
- Adapted treatment to disabled patients
- Only symptomatic palliative treatment to patients who are too sick with terminal illness

47.3.2.3 After Radical Prostatectomy for Curative Intent

After RP, PSA should be undetectable (<0.1 ng/ml). A PSA of >0.1 ng/ml after RP is a signal of residual prostate tissue. Palpable nodules and increasing serum PSA are often signs of local recurrence. After an undetectable PSA is obtained following RP, a PSA >0.2 ng/ml and rising is associated with recurrent disease. In patients with pT3N0M0 PCa and an undetectable PSA, it is recommended to discuss with the patients about the possibility of adjuvant external beam radiotherapy (EBRT) because it at least improves biochemical-free survival. It is recommended to inform patients with pT3N0M0 PCa and an undetectable PSA the possibility to use a salvage irradiation as an alternative to adjuvant EBRT when PSA increases. An adjuvant hormonal therapy for pN0 disease is not recommended.

The role of adjuvant ADT after RP is controversial. The only prospective randomized trial, designed to evaluate the efficacy of immediate ADT compared to ADT deferred at disease progression in node-positive PCa

patients after radical prostatectomy and pelvic lymphadenectomy, showed significant OS and PFS improvements with immediate ADT [35]. However, the positive results might be affected by the gross lymph node disease involvement and the high percentage of positive margins and seminal vesicle invasion (more than 60%). Therefore, in PCa patients with microscopic lymph node metastases, adjuvant ADT cannot be recommended.

A routine follow-up of asymptomatic patients should be obtained by a disease-specific history and PSA measurement supplemented by DRE. This kind of follow-up should be performed at 3, 6, and 12 months after treatment, then every 6 months until 3 years, and then annually. Imaging to detect local recurrence is only recommended if it affects treatment planning. Biopsy is usually not necessary before second-line therapy. Bone scans and other imaging modalities are not routinely recommended for asymptomatic patients if there are no signs of biochemical relapse. In case of bone pain or other symptoms of progression, a re-staging should be considered irrespective of serum PSA level.

47.3.2.4 Oncological Outcomes

It is very difficult to compare open RP with the laparoscopic (LRP) and RARP approaches because the available clinical studies have several limitations. Almost all of the available data derive from prospective non-randomized trials, or retrospective studies, which provide a low level of evidence [36].

Positive surgical margins (PSMs) are the most used and collected data for oncological RP analysis. This is mainly because of the lack of long-term biochemical recurrence and disease-free survival rate data. Analysing the overall PSM rates and pT2 PSM rates among comparative studies, similar PSM rates have been found for RRP and LRP (22.45% and 22.04%, respectively, $p = 0.000$), whereas RARP was only slightly better compared with the other techniques (21.14%). These differences become significant considering only the pT2 stage with similar rates for the RRP and LRP series (16.64% and 17.44% pT2 PSM rates, respectively, $p = 0.045$) and lower rates for RARP (10.53% pT2 PSM rates). Randomized trials are necessary, however, to draw definitive conclusions.

47.3.2.5 Functional Outcomes

A critical point in the evaluation of the RP outcomes is whether patients who obtain good cancer control also obtain a good functional result. This is a relevant issue considering that urinary incontinence (UI) and erectile dysfunction can have a significant negative impact on patients' health-related quality of life.

Thus, in the last decade, the desire to reduce the invasiveness of traditional open and laparoscopic surgery and, above all, the attempt of achieving better functional results, produced the increased interest in and the popularity of robotic techniques both in Europe and the USA.

Although recent systematic reviews and meta-analysis found that RARP had higher postoperative continence rates than retropubic or laparoscopic radical prostatectomy, UI and sexual dysfunction remain the most bothersome postoperative complications even after RARP [36, 37].

The evaluation of UI rates between different studies published in the literature is difficult. This is due mainly to the lack of standard data collection methods (the use of non-validated questionnaires or simple interviews) and the use of different definitions. Furthermore, follow-up is often insufficient or only partial.

The weighted mean continence rates at 6 months for the RRP, LRP, and RARP series are 73.71%, 63.82%, and 89.12%, respectively ($p = 0.000$). After a 12-month follow-up, the continence rates for the RRP, LRP, and RARP series are 83.22%, 70.77%, and 92.78%, respectively ($p = 0.001$). Evaluation at 24 months of follow-up is not possible because few papers conducted follow-up using this interval. These data support the statement that the continence rates after RRP and LRP are similar, with RRP performing slightly better than LRP. Randomized prospective studies are necessary, however, to accurately compare the continence rates between the three surgical approaches.

Regarding erectile dysfunction rates, the data are too limited for definitive conclusions. Data from the available comparative studies suggest an advantage in terms of urinary continence and erectile function for patients who underwent RARP compared with those patients subjected to the RRP and LRP techniques, but future studies are needed to confirm this trend.

47.3.2.6 RP as Second-Line Treatment

RP as salvage treatment can be offered to treat highly selected patients with localized PCa and a histologically proven local recurrence. Due to the increased rate of side effects, a salvage RP should be performed only in experienced centers.

47.3.3 Radiotherapy

RT for PCa was first introduced during the second decade of the twentieth century, by positioning radium applicators in hollow organs adjacent to the prostate, like urethra, bladder, or rectum. Unfortunately, this type of treatment, a form of endocavitary brachytherapy, was associated with high-dose to the organ's mucosa that caused significant morbidity. During the 1920s–1940s,

EBRT was introduced, but due to the availability of machines generating low-energy x-ray beams, it had palliative intent with significant side effects. The role of EBRT in the management of prostate carcinoma became clearer with the introduction of technological advancements that allowed the use of megavoltage radiation (energy >1000 kV) that penetrated more deeply in the body, and that were associated with less skin and subcutaneous morbidity. During the 1950s and 1960s, megavoltage radiation was more commonly available from the decay of radioactive isotopes (Cobalt-60 units, 1.25 MeV), while in the following decades, high-energy X-rays, produced by linear accelerators, became increasingly popular and are now the most common form of EBRT [38]. Furthermore, linear accelerators provide beams with more sharply delineated borders, thus allowing to escalate tumor dose and to minimize acute and late toxicity to normal tissues. Improved technology, including 3-dimensional conformal radiotherapy (3D-CRT), intensity-modulated radiotherapy (IMRT), and image-guided radiotherapy (IGRT), associated with improvement in treatment planning and dosimetry, made RT, together with RP, one of the primary treatment options for patients with both localized and locally advanced non-metastatic PCa, and a treatment option for patients with persistent disease or who relapse after surgery. RT may be administered using EBRT alone, EBRT combined with a brachytherapy (BT) boost, or BT alone.

47.3.3.1 External Beam Radiation Therapy

A large body of medical literature on radiotherapy in the management of PCa regards the use of conventional EBRT, which was typically delivered using a 4-field technique (anteroposterior, posteroanterior, left lateral, right lateral) usually designed to include the prostate, seminal vesicles, and regional lymphatics, for a cumulative dose of 45–50 Gy delivered over 5–5.5 weeks. Subsequently, an additional dose of approximately 20 Gy to a boost field was administered to the prostate and periprostatic tissues to a total dose of 66.6–70 Gy. The results of this treatment in patients with T1–2 disease were similar to those achieved after RP, with 10-year survival rates for both treatments in excess of 60% [39, 40]. More modern techniques, 3D-CRT and IMRT, allow delivering of higher doses of at least 72–80 Gy with an improvement of local and regional control [41, 42].

Clinical trials involving 3D-CRT, demonstrated relevant advantages of this technique over conventional RT: less morbidity associated to 3D-CRT, make possible dose escalation to the target organs with improvement of biochemical outcome, as well documented in a trial by MD Anderson CC, in which the outcomes following 70 and 78 Gy were compared, and by MGH with dose comparisons of 70 and 79 Gy [41–43]. 3D-CRT techniques are now considered the minimum standard EBRT approach in patients with prostate cancer.

During the last decades, IMRT has rapidly become a highly precise method of delivering increasing doses of radiotherapy to the prostate and immediate periprostatic tissues, by achieving tightly conformal dose distributions with the use of non-uniform radiation beams. Complex treatment-planning software algorithms allow exceedingly high doses of radiation to be delivered to the target, while significantly smaller doses are delivered to the adjacent normal tissue. Data from the Memorial Sloan Kettering Cancer Center have demonstrated the safe delivery of doses of more than 80 Gy using IMRT [44].

The treatment of prostate cancer with IMRT techniques need the reproducible identification of the target and surrounding organs on daily treatments. IGRT, which refers to the use of verification tools in an attempt to ensure proper target localization during the course of radiotherapy, is of paramount importance to assure daily reproducibility if high conformal techniques are used [45, 46]. IGRT thus further minimizes the margin of normal tissue that would otherwise need to be irradiated. The term IGRT has been used to identify wide range imaging techniques as simple as daily port films, to those as complex as computer-assisted patient repositioning devices. Intraprostatic implantable fiducial markers, and daily three-dimensional imaging IGRT, are frequently utilized for accurate target localization if IMRT is the modality of choice; without such specificity, the logic of using IMRT is questionable.

Furthermore, many efforts have been recently attempted to address the importance of organ movement during the daily treatment fraction [47]. This issue is particularly important because many intensity-modulated radiation treatments can require 20–30 minutes or longer to be performed. In the attempt to manage intrafraction movement, new forms of target tracking have become clinically available, such as the use of small radio transponder devices implanted into the prostate [48].

Conventional EBRT is usually delivered using photon beams. Charged particle therapy, i.e., proton beam therapy, has been successfully used in the management of PCa. Early work from the cyclotron center at Harvard formed an important basis for current clinical trials. A unique feature of proton beam therapy is the way in which it deposits its most concentrated radiation dose. Proton beams have a characteristic Bragg peak; beyond this point, where energy deposit in target tissues is at a maximum, radiation rapidly falls off, which is important in the management of normal tissue toxicity.

Data from Loma Linda and Harvard suggest that prostate cancer can be effectively managed with conformal proton beam therapy [49, 50]. Although proton beam therapy is being more widely used in men with

prostate cancer, as new treatment facilities become available, there is currently no evidence that this approach offers any advantages over IMRT.

Radiation therapy is also widely used in an adjuvant setting after radical prostatectomy. Selection of candidates for this approach is difficult. Multi-institutional data from the American Society of Therapeutic Radiation Oncology (ASTRO) consensus conference suggest that in patients treated for rising PSA levels, postoperative radiotherapy (dose range of 60–65 Gy) offers a PSA remission rate of 70% with a durability of the response ranging from 25 to 67 months.

47.3.3.2 Brachytherapy

In brachytherapy (BT), radioactive sources are directly implanted within the prostate, thus providing the highest of radiation over a very limited distance, allowing to maximize irradiation to the tumor, while minimizing the dose to normal structures [51]. BT can be used as a single modality, or as a boost in association to EBRT. BT implants may be permanent or temporary; in both cases radiation sources are inserted into the prostate using a transperineal approach, under transrectal ultrasound guidance. BT requires only one or limited number of treatments, rather than the daily therapy required by EBRT.

Permanent implants are characteristic of low dose-rate BT (LDR), which is delivered by permanently implanting numerous radioactive seed, typically either Iodine-125 or Palladium-103. The recommended prescribed doses for LDR monotherapy are 145 Gy for Iodine-125 and 125 Gy for Palladium-103. The corresponding boost dose after 40–50 Gy EBRT are 110 Gy and 90–100 Gy, respectively. LDR BT is usually completed in a single outpatient procedure [52].

Temporary implants are used in High Dose-Rate BT (HDR). This type of BT uses a single radiation source (Iridium-192) which is inserted into the prostate, by a computer-driven after-loading machine, through hollow catheters or needles, that have been previously positioned under transrectal ultrasound, and then removed at the end of the treatment. HDR BT can be used alone or in combination with EBRT (40–50 Gy). A commonly used regimen for HDR treatment alone includes 13.5 Gy × 2 fractions, while commonly used boost regimens include 9.5–11.5 Gy × 2 fractions, 5.5–7.5 Gy × 3 fractions, and 4.0–6.0 Gy × 4 fractions. HDR BT typically requires a 48-hour hospitalization for each session, and can be completed in one or few procedures [53].

Indication of BT for individual patients is based upon technical feasibility, the absence of coexistent urinary conditions and the ability to adequately irradiate all disease. A large prostate gland (usually more than 60 g) is associated with a higher rate of treatment-related complications and represents a relative contraindication to BT. In these cases, a course of androgen deprivation therapy prior to BT may help to reduce the organ volume. BT alone is considered an appropriate option in men with low or intermediate-risk disease, but its interest for high-risk patients, particularly as a boost in association with EBRT, is increasing [54].

47.3.3.3 Complications of RT

The morbidity of both EBRT and BT when performed with advanced techniques and in high experience Centers are very low. Acute radiation proctitis of moderate severity is reported in less than 20% of patients treated with EBRT, depending on radiation dose and treatment volume [55]. If pelvic lymph nodes are included in the target volume, radiation enteritis may also be observed. After RT completion, acute symptoms usually disappeared within 1–2 months. A small percentage of patients require a procedure, such as colonoscopy, following EBRT due to the persistence of diarrhea, rectal urgency, or hematochezia [56]. Less than 50% of patients experience urinary symptoms, including urinary frequency, dysuria, or urgency due to cystitis or urethritis during RT. Symptoms completely disappear after the completion of therapy. With modern techniques, late side effects are extremely uncommon.

After RT, erectile dysfunction increases over time and its frequency is associated to other factors, including older age, concurrent comorbidities, such as hypertension, cardiovascular disease, and diabetes [55]. The use of anti-androgen deprivation therapy is also an important factor.

47.3.3.4 Results of RT

For man with low-risk clinically localized prostate cancer, EBRT, BT, and radical prostatectomy all provide an extremely high degree of freedom from local or distal recurrence in series with long follow-up. For selected patients with a low or very low-risk of recurrence, active surveillance with delayed definitive treatment if necessary, represent an appropriate option [57, 58]. If disease control is similar, different treatment approaches show important differences in the pattern of associated toxicity that may address patients' choice. For man with regionally localized intermediate, high, and very high-risk prostate cancer, RT administered using EBRT alone or combined with a BT boost and associated with ADT, or radical prostatectomy with pelvic lymph node dissection, in patients without tumor fixation to adjacent structure, are both treatment options. In patients managed with radical prostatectomy and with more extensive local disease, positive surgical margins, or lymph nodes involvement at histologic examination, adjuvant RT should be recommended. The choice of treatment,

surgery versus RT, depends upon a detailed, informed, patient decision, taking into consideration the potential advantages and disadvantages associated with each approach, balanced with the specific side effects associated with each different treatment technique. For men treated with RT, therapy should be administered using high conformal techniques, such as image guided IMRT, with the aim to ensure the delivery of high curative doses to the target, while minimizing the dose to surrounding normal tissues [44]. The association of EBRT plus BT may be helpful in attempting dose escalation [59–63].

47.3.4 Neoadjuvant and Adjuvant ADT After RT

The role of neoadjuvant ADT has been evaluated in several randomized trials. The Trans-Tasman Radiation Oncology Group (TROG) 96-01 trial demonstrated a significant OS advantage [HR 0.63 (0.48–0.83)] with RT plus 6 months neoadjuvant and concurrent combined androgen blockade (CAB) compared to RT alone in 818 locally advanced PCa patients [64]. Similarly, the Radiation Therapy Oncology Group (RTOG) trial 8610 showed an improvement in 10-year prostate cancer-specific mortality (23% vs. 36%; $p = 0.01$) with the addition of 4 months neoadjuvant and concurrent ADT to RT in 456 PCa patients with T2–4 disease [65].

Therefore, neoadjuvant and concurrent ADT for 4–6 months are recommended for high-risk PCa patients receiving radical RT, and can be considered for men with intermediate-risk disease.

Adjuvant ADT has been investigated in several trials, showing an OS improvement among patients with locally advanced PCa treated with EBRT combined with androgen suppression as compared with the use of EBRT alone and deferral of hormonal treatment until relapse. An EORTC randomized phase III trial comparing EBRT alone and EBRT combined with an ADT for 3 years for T1–2 PCa tumors of WHO grade 3 or T3–4 N0–1 M0 tumors, revealed a significant improvement in disease-free (5-year DFS 40% vs. 74%; $p = 0.0001$) and overall survival (5-year OS 62% vs. 78%; $p = 0.0002$) in favor of the combined therapy [66].

As concerns the optimal duration of adjuvant ADT, two randomized trials support the role of long-term ADT. In particular, the RTOG 92-02 trial demonstrated significant improvement with long-term ADT (28 months) compared to short-term ADT (4 months) in addition to RT in term of disease-free survival, disease-specific survival, local progression, distant metastasis, and biochemical failure. An OS advantage was limited to the subgroup of patients with a Gleason score of 8–10 (81.0% vs. 70.7%, $p = 0.044$) [67]. Analogously, the EORTC-22961 trial showed a 4.7% advantage in 5-year OS in favor of long-term adjuvant ADT (36 months, 6 months concurrent to RT and 2.5 years of further treatment) compared to short-term hormonal therapy (6 months, concurrent to RT) in locally advanced PCa patients treated with external-beam radiotherapy [68].

Therefore, concomitant (with or without neoadjuvant) and adjuvant ADT, for 2–3 years, is recommended for high-risk locally advanced PCa patients treated with radical EBRT.

Updates:

- Prostate-specific membrane antigen (PSMA) positron-emission tomography (PET)/computed tomography (CT) scanning could be more sensitive than conventional imaging to detect occult lesions in prostate cancer patients.
- A novel magnetic resonance imaging (MRI)-guided ultrasound procedure for localized prostate cancer (TULSA) is able to spare healthy nerve tissue enveloping prostate gland. The TACT trial results showed 80% of patients without clinically relevant prostate cancer and 65% with negative biopsy at 12 months.
- Data from the RADICALS-RT trials suggested the role of radiotherapy as salvage strategy rather than as adjuvant treatment of men with prostate cancer.

Expert Opinion
Giuseppe Procopio

Key Points

- Prostate cancer is the most frequent solid tumor diagnosed in male people and due to its high incidence and prevalence, screening programs have been adopted among population such as the valuation of PSA; otherwise, for the frequent over-diagnosis and over-treatments, nowadays, the screening program should be carefully discussed with the patient.
- After diagnosis of PCa, the decision to proceed with systemic staging workup is guided by the risk of disease systemic spread. Curative treatments or observational strategies may be proposed according to the risk of recurrence, life expectancy, and patients' preferences.
- RP or radiotherapy (external beam or brachytherapy) are two options for low- or intermediate-risk disease.
- RP plus pelvic lymphadenectomy or external beam RT plus hormone treatment are two alternative options for high-risk or locally advanced PCa.

- Long-term adjuvant ADT is recommended for high-risk PCa patients treated with radical EBRT.
- ADT represents the cornerstone of treatment for metastatic prostate cancer.
- The early addiction of docetaxel or abiraterone acetate to ADT improves the overall survival of mHSPC, mainly in the subpopulation of high-volume and in high-risk patients.
- Several therapeutic options have demonstrated to improve patients' outcomes in the mCRPC setting, including docetaxel, cabazitaxel, abiraterone and enzalutamide, and Radium-223.

Summary of Clinical Recommendations

- The annual PSA-based screening program should be discussed with the patient for the risk of over-diagnosis and over-treatment.
- After diagnosis of PCa, the decision to proceed with systemic staging workup is guided by the risk of disease systemic spread.
- Curative treatments or observational strategies may be proposed according to the risk of recurrence, life expectancy, and patients' preferences.
- RP or radiotherapy (external beam or brachytherapy) are two options for low- or intermediate-risk disease.
- RP plus pelvic lymphadenectomy or external beam RT plus hormone treatment are two alternative options for high-risk or locally advanced PCa.
- Long-term adjuvant ADT is recommended for high-risk PCa patients treated with radical EBRT.

Recommendations

- ESMO
  ► www.esmo.org/Guidelines/Genitourinary-Cancers/ESMO-Consensus-Guidelines-Prostate-cancer
- NCCN
  ► jnccn.org/view/journals/jnccn/17/5/article-p479.xml
- ASCO
  ► www.asco.org/practice-guidelines/quality-guidelines/guidelines/genitourinary-cancer#/32796
  ► www.asco.org/practice-guidelines/quality-guidelines/guidelines/genitourinary-cancer#/33301
  ► www.asco.org/practice-guidelines/quality-guidelines/guidelines/genitourinary-cancer#/25251
  ► www.asco.org/practice-guidelines/quality-guidelines/guidelines/genitourinary-cancer#/24836

Hints for a Deeper Insight

- Phase II study of pembrolizumab (MK-3475) in patients with metastatic castration-resistant prostate cancer (KEYNOTE-199)-study AP 93/16 of the AUO: ► https://www.ncbi.nlm.nih.gov/pubmed/28980011
- Prostate cancer between prognosis and adequate/proper therapy: ► https://www.ncbi.nlm.nih.gov/pubmed/28255369
- Prostate Cancer Genetics: Variation by Race, Ethnicity, and Geography: ► https://www.ncbi.nlm.nih.gov/pubmed/27986209

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Prostate Cancer: Advanced and Metastatic Disease

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Genitourinary Cancers

Contents

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Learning Objectives

By the end of the chapter, the reader will:

- Be able to distinguish between hormone-sensitive and castration-resistant prostate cancer
- Have learned the basic concepts of metastatic prostate cancer therapeutic algorithm
- Have reached in-depth knowledge of prostate cancer hormonal therapy, chemotherapy, and particle-emitting radionuclides (efficacy and safety profile)
- Be able to put acquired knowledge into clinical practice for the evaluation of metastatic prostate cancer patients' prognosis and the management of different treatment options

48.1 Introduction

Advanced or metastatic prostate cancer includes two main clinical patterns: patients that develop metachronous locoregional or distant metastases (after a variable interval from the diagnosis, and the treatment, of the primary tumor) and patients that present with metastasis at the time of diagnosis.

Although the spread of prostate cancer screening has resulted in an apparent migration of the diagnosis in earlier stages, with consequent improvement in long-term survival and decrease in the rate of patients with metastatic cancer, however, it is estimated that currently up to 3–6% of patients have metastatic prostate cancer at the time of diagnosis in the United States [1] and in Europe [2], with an incidence of 6.7 per 100,000 cases per year [3]. De novo metastatic prostate cancer usually affects younger subjects, with a mean age at diagnosis of 62 years, and a proportion of diagnoses in men aged 35–50, 51–55, and 56–60 of 4.4%, 8.5%, and 12.5%, respectively [3].

According to tumor sensibility to hormonal therapy, which constitutes the fundamental treatment of advanced prostate cancer as will be clarified later, it is possible to distinguish two different metastatic prostate cancer stages: hormone-sensitive disease (metastatic hormone-sensitive prostate cancer (mHSPC)) and castration-resistant disease (metastatic castration-resistant prostate cancer (mCRPC)).

48.2 Metastatic Hormone-Sensitive Prostate Cancer

48.2.1 Prognosis

Compared to patients that develop metachronous metastases, the worst prognosis of patients with newly diagnosed metastatic prostate cancer reflects the peculiar aggressiveness of this condition [4]. The median survival of patients with de novo metastatic prostate cancer is about 42 months [5, 6]. However, it must be underlined that this population is extremely heterogeneous and differs in terms of clinical presentation, tumor biology, and prognosis. In fact, alongside very aggressive forms (symptomatic patients, undifferentiated tumors, visceral metastases, large bone involvement), there are considerably more indolent clinical patterns (asymptomatic, oligo-metastatic patients).

The heterogeneity of prostate carcinoma makes it difficult to correctly predict its clinical evolution since the diagnosis; therefore, the debate on the prognostic evaluation of the single men affected by this pathology is still open.

In contrast to CRPC, there is little evidence regarding the main factors influencing prognosis in the hormone-sensitive disease setting. Several clinical features have been recognized as potential prognostic factors, including the number and the site of bone metastases, the presence of visceral metastases, the Gleason score of the primary tumor, the performance status and the patient's age, and the initial values of PSA and alkaline phosphatase [7]. Of note, no prognostic factor has been validated prospectively.

- *Gleason score*: As reported by Crawford, well-differentiated tumors (Gleason 2–4) at the time of diagnosis are associated with lower rates of progression and mortality and, vice versa, prostate cancer presenting a low degree of differentiation (Gleason 7–10) progresses faster toward metastatic disease [8].
- *PSA*: The prognostic role of PSA at the time of the diagnosis is still a matter of debate, as it is not always correlated with the biological behavior of the tumor. For example, tumors with high Gleason score and low PSA levels at the diagnosis have been associated with worse prognosis than those with higher PSA [9]. However, it should be noted that high-grade tumors could produce less PSA per gram of tumor, reducing the reliability of this marker. Therefore PSA is more reliable in the diagnostic phase and as a parameter to monitor responses to treatments.

  The potential prognostic role of the absolute value of PSA was prospectively evaluated after 7 months from the beginning of androgen deprivation therapy in the cohort of patients enrolled in the SWOG-9346 study. Three different prognostic groups were distinguished: PSA <0.2 ng/mL with median overall survival (mOS) of 75 months, PSA <4 ng/mL with mOS of 44 months, and PSA > 4 ng/mL with mOS of only 13 months [10]. However, this stratification requires further confirmation.
- *Alkaline phosphatase*: A recent sub-analysis of the GETUG-AFU-15 study reported the role of alkaline phosphatase (ALP) – an indicator of disease bone involvement – as a potentially useful factor for

assessing patients with high bone metastases who might benefit from more aggressive treatments. ALP was in fact the strongest prognostic factor in the discrimination of patients with good or poor prognosis, with mOS of 69.1 months in patients with normal ALP compared to 33.6 months of patients with high ALP values and 5-year survival rates of 62.1% and 23.2%, respectively [11].

- *Site of metastases and tumor burden*: Bone and lymph nodes are the most common sites of distant metastases. It has been estimated, through autopsy studies, that the prevalence of skeletal involvement in patients who die of prostate cancer is more than 90%. The presence of clinically evident bone metastases and the appearance of skeletal events represent a negative prognostic factor [12]. Up to 15% of patients with metastatic prostate cancer at diagnosis have visceral metastases [13, 14]. The visceral metastatic involvement and the presence of multiple sites affected represent two unfavorable prognostic factors, so that any new metastatic site involved is associated with an increase of about 20% of the mortality risk. However, we should note that the negative impact of visceral metastases on mortality is maintained even when focusing exclusively on patients with only one metastatic site involved [13].
  Although there is a consensus in considering the disease volume as one of the main factors influencing the prognosis of mHSPC patients and therefore in guiding the therapeutic choices, the correct definition of high volume (or low volume) still remains to be stated unequivocally. Several classifications have in fact been used over the years in many studies, many of which agree to consider the visceral metastatic involvement and/or the involvement of the appendicular skeleton as the main factors correlated to poor prognosis.

Of interest, Glass defined a prognostic model based on the outcome of patients enrolled in the prospective SWOG-8894 study, identifying three different prognostic groups on the basis of four risk factors: bone metastases (appendicular or axial skeleton involvement), performance status (PS) of the patient according to ECOG classification, PSA value, and tumor Gleason score [7]. Patients at good prognosis include those without appendicular disease and without visceral metastases or with appendicular and/or visceral involvement but with ECOG-PS of 0 and Gleason <8; patients at intermediate prognosis are those with appendicular and/or visceral involvement and ECOG-PS of 0 with Gleason ≥8, or with ECOG-PS ≥1 and PSA <65 ng/ml; and finally poor prognosis patients are those with appendicular skeletal and/or visceral metastases and PS-ECOG ≥1 and PSA ≥65 ng/ml.

Good, intermediate, and poor prognosis risk groups were associated with 5-year survival rates of 42%, 21%, and 9%, respectively [7]. However, this model was based on data from patients treated more than 20 years ago (in the period between 1989 and 1994). To limit this bias, Gravis et al. re-tested Glass's prognostic system in a more updated patient cohort, the GETUG-15 study population, highlighting the persistence of a significant difference in OS between good and intermediate and between good and poor prognostic groups, while the difference was not confirmed among patients belonging to the intermediate group and to poor prognosis. However, the small sample size of the poor prognosis group (83 patients) may have affected these results [11].

48.2.2 Therapy

Prostate cancer treatment has different objectives, depending on the extent and aggressiveness of the disease but also on the patient's life expectancy and the presence of comorbidities that may represent a risk of death higher than that represented by the same prostatic cancer. In contrast to the treatment of localized and locally advanced disease, in which surgery and radiotherapy play a central role, the therapeutic approach for metastatic prostate cancer is systemic. Radiotherapy is a palliative option for controlling cancer-related pain.

48.2.2.1 Androgen Deprivation Therapy for mHSPC

Prostate cancer is an androgen-dependent tumor; therefore, androgen deprivation therapy (ADT) represents the fundamental treatment of mHSPC since the 1940s when Huggins and Hodges demonstrated for the first time the responsiveness of prostate cancer to androgen deprivation [15].

Survival and proliferation of tumor cells, as the normal ones, depend on the binding of androgens (testosterone and dihydrotestosterone) to the androgen receptor (AR). AR is a member of the superfamily of nuclear steroid receptors that, in the absence of its ligand, is kept confined in the cytoplasm by the chaperone protein HSP90. The binding of androgenic hormones with AR, instead, causes the dissociation of AR from HSP90 and its migration into the nucleus, where it dimers and subsequently binds the promoter regions of target genes, inducing their transcription. If prostate cells (or neoplastic cells) are deprived of androgen stimulation, resulting from the reduction of testosterone to castration levels, they undergo apoptosis. Any treatment aimed at suppressing androgen activity (reduction of circulating testosterone levels) is called ADT. The purpose of ADT is therefore to lower serum testosterone to castration levels (<50 ng/dl, or more recently <20 ng/dl),

thus limiting the survival of tumor cells and inducing tumor regression. DT can be achieved by surgery (orchiectomy) or with medical castration drugs.

Surgical Castration

Surgical castration, which permanently reduces circulating testosterone levels to less than 50 ng/dl, is still the most rapid and economical method to achieve this goal. Bilateral orchiectomy is a simple and low-cost surgical procedure; however, it has fallen into disuse due to the negative psychological impact on patients. Since orchiectomy induces a rapid fall of testosterone levels (95% within 3 hours), it can still be reserved for patients with bone metastases at high risk of bone marrow compression.

Medical Castration

The medical approach remains the most used therapeutic modality for castration. Several classes of drugs, with different mechanisms of action, are able to induce a reduction of serum testosterone up to castration levels. Medical castration is, at least in part, reversible.

- LHRH analogs: Medical therapy with analogs of hypothalamic LHRH has provided results, in the short and long term, comparable to those of bilateral orchiectomy [16]. LHRH is normally secreted by the hypothalamus in a pulsatile way and stimulates the pituitary gland to secrete LH and FSH, which in turn promote testicular testosterone synthesis. Exposure to stable concentrations of LHRH inhibits the production of pituitary hormones. The chemical castration is nowadays mostly carried out by LHRH analog molecules that lead to a saturation of the pituitary receptors for Gn-RH and therefore inhibition of the increase in LH. This inhibition is however preceded by a transient phase of stimulation of the pituitary LHRH receptors and consequently of increased testosterone levels. This phenomenon is called flare-up and starts about 2–3 days after the first injection of LHRH analogs and persists for at least a week. Chronic exposure to LHRH analogs results in a downregulation of LHRH receptors; this suppresses the pituitary secretion of FSH and LH and therefore the production of testosterone, whose levels fall to castration values generally within 2–4 weeks.

Flare-up may be responsible for a worsening of symptoms due to an initial transient increase in testosterone levels that, by stimulating tumor growth, may precipitate bone marrow compression or urinary tract obstruction, or lead to a worsening of the bone-metastases pain. Flare-up can be avoided by the concomitant use of anti-androgens, which antagonize the action of androgens at the peripheral (receptor) level, thus neutralizing the proliferative effects of testosterone on the target tissues, including the primary prostate tumor.

- LHRH antagonists: These drugs compete with the LHRH for binding to the pituitary receptors, therefore blocking the secretion of LH and FSH as well as of testosterone. The LHRH antagonists, by definition, have no agonist effect and therefore are responsible for a more rapid reduction of testosterone with an optimal safety profile, avoiding the flare-up phenomenon [17]. The efficacy of this class of drugs is comparable to LHRH analogs in reducing testosterone to castration levels [18].
- Anti-androgens: Anti-androgens compete with testosterone and DHT for binding to the prostatic nuclear receptor, promoting apoptosis and inhibiting tumor growth.

According to their chemical structure, anti-androgens are classified into steroidal (synthetic hydroxyprogesterone derivatives) and non-steroidal anti-androgens. Both classes compete with androgens at the receptor level. This is the only non-steroidal anti-androgen action. In addition steroidal anti-androgens have progestogenic properties due to central pituitary inhibition (they inhibit the release of gonadotropins, LH, and FSH). As a consequence, non-steroidal anti-androgens, not suppressing testosterone secretion, are associated with preservation of libido, physical potency, and bone mass.

The use of anti-androgens as monotherapy can only be considered in M0 patients, but not in metastatic disease. In fact, a meta-analysis assessing the efficacy of different steroidal (cyproterone acetate) and non-steroidal (flutamide, nilutamide, bicalutamide) anti-androgens showed the inferiority of anti-androgen monotherapy compared to other methods of surgical or pharmacological castration in metastatic patients [19].

- Total androgenic blockade (BAT): One of the strategies still under debate is the total androgenic blockade (BAT), obtained by associating to the medical or surgical castration the anti-androgens not only for the short period of time necessary to counteract the flare effect. Numerous studies have been conducted to confirm the superiority of BAT compared to monotherapy with LHRH analogs, with conflicting results [20–22].

A meta-analysis of the Prostate Cancer Trialists' Collaborative Group, published in 2000 on *The Lancet*, examined the results of 27 randomized trials (8275 patients) comparing LHRH analog monotherapy to BAT. The 5-year survival rate was 25.4% in patients undergoing BAT compared to 23.6% in patients treated with ADT alone ($p = 0.11$). However, the subgroup analysis showed that BAT induced a 3% increase in

5-year survival in patients treated with non-steroidal anti-androgens (27.6% BAT vs. 24.7% with LHRH analogs, $p = 0.005$); on the contrary, in patients treated with cyproterone acetate, the combination therapy reduced survival compared to LHRH analog monotherapy (15.4% BAT vs. 18.1% LHRH analogs, $p = 0.04$). This difference is due to the increase in non-cancer-related mortality in patients treated with cyproterone acetate in combination with LHRH analogs [23]. A Cochrane review, which excluded studies with cyproterone acetate, confirmed a statistically significant benefit in terms of 5-year survival in favor of BAT (risk difference, 0.048; 95% CI, 0.02–0.077) [24].

Therefore, BAT determines a small but statistically significant advantage in terms of survival compared to monotherapy, accompanied however by a further deterioration of the quality of life in several areas: sexuality, cognitive functions, and thermoregulation. For this reason, BAT is an option to be considered only in selected patients.

ADT is generally well tolerated, but this therapy is not free from side effects such as hot flashes, loss of power and libido, fatigue, muscle mass reduction, osteoporosis, dysmetabolic syndrome, and increased cardiovascular risk.

In order to overcome ADT side effects, intermittent androgen deprivation (IAD) has been evaluated as a potential therapeutic strategy. IAD consists in alternating periods of treatment with LHRH analogs to periods of therapy interruption. The rationale of the IAD is based on the fact that an intermittent therapy would allow a cyclic recovery of the gonadal function with consequent reduction of the collateral side effects and improvement of the quality of life; moreover the reestablishment of the testosterone blood concentration would delay the selection of androgen-independent cellular clones, procrastinating disease progression and increasing overall survival.

A non-inferiority phase 3 study that compared IAD to continuous ADT treatment enrolled 3040 patients with metastatic disease and PSA >5 ng/ml. Patients treated with goserelin and bicalutamide for 7 months whose PSA values reached <4 ng/ml were randomized to continue the current therapy or to interrupt it with the reserve or take it back in case of clinical or biochemical disease progression. Although some indicators of quality of life have improved among patients undergoing IAD, overall survival was not non-inferior compared to continuous therapy (5.1 years vs. 5.8 years, HR 1.10, 90% CI 0.99–1.23) [25]. Therefore, IAD should not be considered an alternative to continuous ADT in metastatic prostate cancer patients, out of highly personalized strategies.

48.2.2.2 Chemotherapy for mHSPC

Historically, the role of chemotherapy in prostate cancer was reserved to castration-resistant disease. Recently the research has focused on HSPC, significantly modifying the whole therapeutic paradigm. In recent years, in fact, several studies have been carried out to evaluate the possibility of associating to ADT other therapeutic agents in order to enhance the antitumor activity, delay the development of resistance, and improve the patients' prognosis. A relevant question is whether the administration of chemotherapy to mHSPC patients may improve the efficacy and tolerability of docetaxel. Docetaxel, indeed, exerts its cytotoxic activity through androgen-mediated effects that target androgen-dependent cells before they can adapt to become androgen-independent. Taxanes have a direct effect on the androgen signaling pathway. In fact, docetaxel stabilizes microtubules and maintains the AR in the cytoplasm, inhibiting its translocation into the nucleus in response to androgens or via ligand-independent pathways. In addition, taxanes act through the FOXO1 transcriptional repressor to prevent gene expression responsive to androgens. The inhibition of AR signal, rather than antimitotic activity, may indeed be the reason that explains the antitumor activity of taxanes in prostate cancer [26, 27]. Therefore, the early combination of docetaxel with ADT could delay the development of resistance to ADT (delay the CRPC phase) and maximize the efficacy of docetaxel.

The efficacy of first-line therapy with the association of ADT and docetaxel in mHSPC patients has been shown in three randomized phase 3 trials:

- GETUG-AFU15 study: It is a small phase 3 study conducted in France and published in November 2015 [28]. It randomized a total of 385 patients to receive ADT monotherapy (orchiectomy or LHRH analogs, with or without non-steroidal anti-androgens) or the association of ADT with docetaxel for nine cycles. The majority of patients had metastatic disease at the time of diagnosis (71%, 272/385), while only a minority of them became metastatic after treatment for localized disease. The study failed to demonstrate a survival advantage with chemotherapy in the overall population: median survival was 58.9 months (95% CI 50.8–69.1) in the docetaxel plus ADT arm compared to 54.2 months (42.2–not achieved) in the ADT arm (HR 1.01, 95% CI 0.75–1.36). Combination therapy had been shown to significantly prolong the biochemical PFS (22.9 vs. 12.9 months, HR 0.72, 0.57–0.91, $p = 0.005$) and the radiological PFS (23.5 vs. 15.4 months, HR 0.75, 0.59–0.94, $p = 0.015$) [28]. One of the major limits of this study was the enrollment of patients mainly with low disease burden. A subsequent retrospective analysis, in fact, has reclassi-

fied patients according to the disease volume using the CHAARTED study criteria. In the high-volume subgroup of patients (47% of total), there was a trend in favor of the combination therapy of ADT plus docetaxel, with a 22% reduction in the risk of death and an improvement of 4.7 months in overall survival (mOS 39.8 vs. 35.1 months, HR 0.78, 95% CI 0.56–1.09, $p = 0.14$) [29].

- CHAARTED study: It is a randomized phase 3 study, published in August 2015 in the *New England Journal of Medicine*, involving a total of 790 patients with mHSPC randomized to receive ADT alone or the association of ADT with docetaxel for six cycles within 4 months from the beginning of the ADT. The study included mainly metastatic patients at diagnosis (75%), compared to patients who developed metachronous metastases. At a median follow-up of 28.9 months, there was a statistically significant and clinically relevant overall survival advantage (about 13.6 months) for patients treated with docetaxel plus ADT (mOS 57.6 vs. 44 months, HR 0.61, 95% CI 0.47–0.80, $p < 0.001$), which translates into a reduction in the risk of death by 39%. The survival benefit was more evident in patients with high-volume disease (65% of cases), defined by the presence of visceral metastases or four or more bone lesions, of which at least one is located outside the axial skeleton and pelvis (mOS 49.2 vs. 32.2 months, HR 0.60, 95% CI 0.45–0.81, $p < 0.001$) [30].
- The STAMPEDE study: It is a multi-factorial study that enrolled 2962 patients, with metastatic disease at diagnosis (M1, 1817 subjects), with localized high-risk disease (N0, 697), or with lymph node involvement (N+, 448), but all candidates received long-term hormone therapy. Patients were randomized to ADT alone ($n = 1184$), ADT in association with docetaxel for six cycles ($n = 592$), the combination of ADT and zoledronic acid for 2 years ($n = 593$), or ADT in combination with docetaxel and zoledronic acid ($n = 592$). The main objective of the study was to evaluate the efficacy of combined treatment with ADT and docetaxel compared to ADT monotherapy and to evaluate the possible benefit of the addition of zoledronic acid in the population of hormone-sensitive patients. At a median follow-up of 43 months, patients who received docetaxel (with or without zoledronic acid) associated with ADT showed a significant advantage in overall survival of about 10 months (mOS 81 vs. 71 months, HR 0.78, $p = 0.006$). This advantage was even more significant in the subgroup of metastatic patients (mOS 60 vs. 45 months, $p = 0.0005$) compared to those with only biochemical disease recurrence. However, the limited number of patients with non-metastatic disease, along with the small number of deaths in this subgroup, underestimated the power of all survival analyses. Early docetaxel was also associated with a PFS advantage, while no benefit in terms of DFS and OS was observed by the addition of zoledronic acid [31].

The results suggest a paradigmatic shift in the therapeutic algorithm of mHSPC, providing a solid rationale about the possibility of improving patients' survival by starting docetaxel in a hormone-sensitive stage, rather than procrastinating chemotherapy in the castration-resistant phase.

The results of GETUG-AFU15, CHAARTED, and STAMPEDE trials were included in two meta-analyses, which analyzed the role of docetaxel addition to ADT in the treatment of hormone-naïve metastatic patients, confirming the statistically significant advantage in favor of early combination therapy [32, 33]. In particular, the meta-analysis of Vale and co-authors showed an increase in overall survival with the early use of docetaxel together with ADT in patients with metastatic disease at diagnosis estimated at around 9% at 4 years (from 40% to 49%), with a hazard ratio of 0.77 (95% CI 0.68–0.87, $p < 0.0001$). Furthermore, chemotherapy in combination with ADT improved the failure-free survival, with an HR of 0.64 (0.58–0.70, $p < 0.0001$), which translated into an absolute reduction in the rate of therapeutic failure of 16% at 4 years (95% CI 12–19) [32]. For this reason, the combination of ADT and docetaxel is a viable option for those hormone-naïve patients who have metastases at diagnosis, are with "high-volume" disease, and are in good clinical condition.

The adequate selection of mHSPC patients destined to achieve a significant benefit from this therapeutic strategy still represents a matter of debate. It should also be emphasized that chemotherapy with docetaxel is a treatment associated with remarkable toxicity, especially hematologic; in the CHAARTED study, the grade 3–4 neutropenia rate was about 12%, with febrile neutropenia in about 6% and severe infections associated with neutropenia in 2% of cases. Obviously it is important to remember that in patients with multiple bone metastases, of advanced age, and with comorbidities, the expected toxicity is even higher. The results of the CHAARTED study showed a major advantage of docetaxel restricted to the subgroup of "high-volume" disease compared to patients with "low-volume" disease. Robust data are not available to recommend the routine use of the early combination of docetaxel and ADT in patients with low-volume, oligo-metastatic, and slowly evolving hormone-sensitive disease. In this subgroup ADT remains the therapeutic standard of care.

48.2.2.3 Second-Generation Hormonal Therapy for mHSPC

Similarly to what has been described for chemotherapy, the efficacy of early use of the combination of ADT with second-generation anti-androgen in mHSPC disease has recently been investigated. Abiraterone acetate, pro-drug of the corresponding active form abiraterone, is a selective and irreversible inhibitor of cytochrome P-450c17 (17α-hydroxylase/C17,20-lyase), a crucial enzyme in the biosensitization of androgen hormones in testicular and adrenal tissues and in neoplastic prostate tissues. Blocking CYP17 inhibits the testicular, adrenal, and neoplastic biosynthesis of androgens.

The LATITUDE trial evaluated the use of abiraterone acetate and prednisone in association with ADT in 1199 patients with mHSPC. All patients included in the study had a high-risk disease, defined by the presence of at least two of the following criteria: Gleason score equal to or greater than 8, a minimum number of bone lesions equal to 3, and evidence of measurable visceral metastases. The main objective of the study was to demonstrate an advantage in terms of overall survival and radiological PFS resulting from the early addition of abiraterone to standard hormone therapy. At a median follow-up of 30.4 months, the addition of abiraterone to ADT resulted in a statistically significant prolongation of survival (mOS not reached vs. 34.7 months, HR 0.62, 95% CI 0.51–0.76, $p < 0.001$), with a 38% reduction in the risk of death compared to the placebo group. Abiraterone also prolonged PFS (33.0 vs. 14.8 months, HR 0.47, 95% CI 0.39–0.55, $p < 0.001$), time to pain worsening, time to the beginning of subsequent therapies, and time to biochemical progression [34]. It is important to underline that, given the significant benefit in OS observed at the interim analysis, crossover to abiraterone was allowed for patients in the placebo treatment arm. Grade 3 and 4 toxicities were reported in 63% of patients in the abiraterone treatment arm (mainly mineralocorticoid toxicity, with hypertension and hypokalemia) and in 48% of those in the placebo group [34]. Therefore, the data support the hypothesis that a more effective inhibition of the AR-mediated signal pathway as initial systemic therapy in mHSPC patients at higher risk, albeit with a greater incidence of side effects related to the use of abiraterone compared to ADT alone, leads to better results than ADT alone.

The role of abiraterone plus ADT for mHSPC was confirmed in the STAMPEDE study. The *New England Journal of Medicine* published the results of the STAMPEDE study relative to the comparison between abiraterone acetate and prednisolone in addition to ADT compared to ADT alone in a cohort of 1917 mHSPC patients (52% with newly diagnosed metastatic prostate cancer, 20% with lymph node metastasis, and 28% with a locally advanced disease or a disease previously treated with surgery or radiotherapy and relapse with high-risk characteristics). At a median follow-up of 40 months, the combination of abiraterone/prednisone and ADT showed significantly longer survival compared to ADT alone, with a 3-year survival rate of 83% versus 76% (HR 0.63, 95% CI 0.52–0.76, $p < 0.001$). The survival advantage in favor of abiraterone was even more significant in the subgroup of patients with metastatic disease (HR 0.61, 95% CI 0.49–0.75). Grade 3–5 adverse events occurred in 47% of patients in the abiraterone treatment arm and in 33% of patients treated with ADT and were primarily hypertension, transaminase increase, and respiratory disorders [35]. It is important to underline that a randomized phase 3 study comparing the combination of ADT and enzalutamide to ADT plus placebo in patients with mHSPC is currently ongoing (NCT02677896).

Of interest, at the 2017 ESMO Congress, the results of an analysis about the direct comparison between the two treatment cohorts evaluated in the STAMPEDE trial (the combination of abiraterone acetate and ADT vs. the combination of docetaxel and ADT) were presented. There was no difference in survival between the two different combinations (HR for the OS of 1.16), while a statistically significant advantage in terms of biochemical relapse and disease progression in favor of abiraterone compared to docetaxel was noticed [36]. However, these preliminary data do not allow to draw definitive recommendations.

48.3 Metastatic Castration-Resistant Prostate Cancer

48.3.1 Introduction

All men with metastatic prostate cancer will progress to castration-resistant disease with a mortality rate of over 50% [37]. Castrate-resistant prostate cancer (CRPC) is defined by disease progression despite androgen deprivation therapy (ADT) and may present as one or any combination of castrate serum testosterone <50 ng/dL or 1.7 nmol/L plus either biochemical progression (defined as three consecutive rises in PSA 1 week apart resulting in two 50% increases over the nadir and a PSA > 2 ng/mL) or radiological progression (defined by the appearance of two or more new bone lesions on bone scan or a soft tissue lesion using RECIST (Response Evaluation Criteria in Solid Tumours)) [38]. Symptomatic progression alone is not sufficient to diagnose CRPC.

There are currently six systemic therapies approved by the Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medicinal

Table 48.1 Phase 3 trials of single agents leading to regulatory approval in castration-resistant prostate cancer

Trial: therapy (approved date)	N	Disease state	Comparator	HR	OS (months)	P value
TAX327: docetaxel (2004) [11]	1.006	First-line	Mitoxantrone Prednisone	0.76	18.9 vs. 16.5	0.009
TROPIC: cabazitaxel (2010) [13]	755	Post-chemotherapy	Mitoxantrone Prednisone	0.70	15.1 vs. 12.7	<0.0001
COU-AA-301: abiraterone acetate (2011) [24]	1195	Post-docetaxel	Placebo Prednisone	0.74	15.8 vs. 11.2	<0.0001
COU-AA-302: abiraterone acetate (2013) [26]	1.088	Pre-chemotherapy	Placebo Prednisone	0.81	34.7 vs. 30.3	0.0033
AFFIRM: enzalutamide (2012) [34]	1199	Post-docetaxel	Placebo	0.63	18.4 vs. 13.6	<0.0001
PREVAIL: enzalutamide (2014) [35]	1717	Pre-chemotherapy	Placebo	0.71	32.4 vs. 30.2	p < 0.001
ALSYMPCA: radium-223 (2013) [22]	922	Pre- and post-docetaxel Symptomatic	Placebo	0.695	14.0 vs. 11.2	0.00085

Products (EMEA) that offer a survival benefit for the treatment of metastatic castration-resistant prostate cancer (mCRPC). These include docetaxel, cabazitaxel, enzalutamide, abiraterone acetate, sipuleucel-T (approved only in the United States), and radium-223 (Table 48.1).

48.3.2 Therapy

48.3.2.1 Chemotherapy for mCRPC

The first available chemotherapeutic options for patients with mCRPC, mitoxantrone and estramustine, had a limited clinical benefit because these agents did not show to prolong overall survival (OS) [39–41]. Estramustine was approved for the treatment of mCRPC in 1981 on the basis of small non-randomized studies, which showed improved rates of disease control over comparators [42, 43]. Estramustine was associated with a high rate of toxicity when given in combination, and while it may improve PSA response, it did not consistently improve OS [44, 45]. A meta-analysis of 742 patients demonstrated better PSA response and OS with the addition of estramustine to chemotherapy, but at the cost of significant adverse events (AEs) [46]. Mitoxantrone was associated with significant palliative benefits and improved PSA response rates, which led to its approval in 1996 and subsequent establishment as standard of care [40–43].

- *Docetaxel*

In 2004 the taxane chemotherapy, docetaxel, replaced mitoxantrone as the standard of care following two phase 3 studies (TAX327 and SWOG-9916) in which docetaxel prolonged OS in patients with mCRPC [47, 48].

TAX327 was a randomized, non-blinded, phase 3 study in which 1006 patients with mCRPC received 5 mg of prednisone twice daily and were randomly assigned to receive 12 mg of mitoxantrone per square meter of body-surface area every 3 weeks, 75 mg of docetaxel per square meter every 3 weeks, or 30 mg of docetaxel per square meter weekly for 5 of every 6 weeks.

The primary end point of the study was overall survival; secondary end points were predefined reductions in pain, an improvement in the quality of life, a reduction in serum PSA levels of at least 50%, and objective tumor responses.

Patients treated with docetaxel every 3 weeks had a significantly higher survival rate compared with the mitoxantrone group ($p = 0.009$); on the contrary, patients treated with weekly docetaxel did not show any survival superiority ($p = 0.36$). The median duration of survival was 18.9 months (95% confidence interval [CI], 17.0–21.2) in the group given docetaxel every 3 weeks, 17.4 months (95% CI, 15.7–19.0) in the group given weekly docetaxel, and 16.5 months (95% CI, 14.4–18.6) in the mitoxantrone group. The hazard ratio for death in the group treated with docetaxel every 3 weeks, as compared with the mitoxantrone group, was 0.76. Visceral involvement, high baseline alkaline phosphatase level, and low hemoglobin level were negative prognostic factors in the multivariate models, whereas rising serum PSA as the sole indicator of progression was a favorable factor. Post hoc analysis indicated that high Gleason score (8, 9, or 10) was an adverse prognostic factor for survival.

A reduction in pain was more frequent among patients receiving docetaxel every 3 weeks than among those treated with mitoxantrone (35% vs. 22%, $p = 0.01$),

but the percentage of patients with reduced pain in the weekly docetaxel group (31%) did not differ significantly from that of the mitoxantrone group.

Rates of PSA response were significantly higher in the docetaxel groups (45% in the group treated with docetaxel every 3 weeks and 48% in the group of weekly docetaxel, $p < 0.001$ for both comparisons) than in the mitoxantrone group (32%). Patients with measurable soft-tissue lesions who received docetaxel every 3 weeks had a higher rate of tumor response than patients who received mitoxantrone every 3 weeks (12% vs. 7%, $p = 0.11$), but this difference was not significant.

As to the AEs, the incidence of grade 3 and 4 neutropenia was relatively low, and febrile neutropenia was rare. There was a higher incidence of cardiac events among patients who received mitoxantrone. Most other types of AEs were more frequent among patients treated with docetaxel, and there was no trend toward a lower frequency with weekly docetaxel than with docetaxel given every 3 weeks. Low-grade AEs that occurred in at least 15% of patients in one of the groups included fatigue, nausea or vomiting or both, alopecia, diarrhea, nail changes, sensory neuropathy, anorexia, changes in taste, stomatitis, dyspnea, tearing, peripheral edema, and epistaxis. More patients in the docetaxel groups than in the mitoxantrone group had at least one serious adverse event, with rates of 26% among those in the group given docetaxel every 3 weeks, 29% among those given weekly docetaxel, and 20% among those given mitoxantrone. AEs leading to discontinuation of treatment included fatigue, musculoskeletal or nail changes, sensory neuropathy, and infection in the docetaxel groups and cardiac dysfunction in the mitoxantrone group.

The percentage of patients who had an improvement in the quality of life was similar in the two docetaxel groups (22% in the group given docetaxel every 3 weeks and 23% in the group given weekly docetaxel) and significantly higher than that in the mitoxantrone group (13%, $p = 0.009$ and $p = 0.005$, respectively).

SWOG-9916 was also a randomized, phase 3 trial in which 770 men were randomly assigned to one of two treatments, each given in 21-day cycles: 280 mg of estramustine three times daily on days 1 through 5, 60 mg of docetaxel per square meter of body-surface area on day 2, and 60 mg of dexamethasone in three divided doses before docetaxel, or 12 mg of mitoxantrone per square meter on day 1 plus 5 mg of prednisone twice daily. The primary end point was overall survival; secondary end points were progression-free survival, objective response rates, and post-treatment declines of at least 50 percent in PSA levels.

In an intention-to-treat analysis, the median overall survival was longer in the group of patients treated with docetaxel and estramustine than in the group who received mitoxantrone and prednisone (17.5 months vs. 15.6 months, $p = 0.02$), and the corresponding hazard ratio for death was 0.80 (95% CI, 0.67–0.97). The median time to progression was 6.3 months in the group given docetaxel and estramustine and 3.2 months in the group given mitoxantrone and prednisone ($p < 0.001$). PSA declines of at least 50 percent occurred in 50% and 27% of patients, respectively ($p < 0.001$), and objective tumor responses were observed in 17% and 11% of patients with bidimensionally measurable disease, respectively ($p = 0.30$). Grade 3 or 4 neutropenic fevers ($p = 0.01$), nausea and vomiting ($p < 0.001$), and cardiovascular events ($p = 0.001$) were more common among patients receiving docetaxel and estramustine than among those receiving mitoxantrone and prednisone.

The TAX327 and the SWOG-9916 trials have provided support for the treatment with docetaxel in men with mCRPC.

Cabazitaxel

Cabazitaxel, a second-generation taxane, was developed to overcome resistance to docetaxel. Its efficacy was evaluated in the TROPIC phase 3 trial [49]. This was a randomized trial in which 755 mCRPC patients received oral prednisone 10 mg daily and were randomly assigned to receive cabazitaxel 25 mg per square meter intravenously or mitoxantrone 12 mg per square meter intravenously on day 1 of each 21-day cycle and were stratified for disease measurability (measurable vs. non-measurable) and ECOG performance status (0–1 vs. 2). 50% of patients had measurable soft-tissue disease and 25% had visceral (poor prognosis) disease. One dose reduction (cabazitaxel 20 mg per square meter or mitoxantrone 10 mg per square meter) per patient was allowed in this study.

Median overall survival was 15.1 months for patients in the cabazitaxel arm (95% CI, 14.1–16.3) versus 12.7 months for the mitoxantrone arm (95% CI, 11.6–13.7). This result corresponds to a 30% reduction in relative risk of death (HR 0.70; 95% CI, 0.59–0.83; $p < 0.0001$). Median progression-free survival was 2.8 months (95% CI, 2.4–3.0) in the cabazitaxel group and 1.4 months (95% CI, 1.4–1.7) in the mitoxantrone group (HR 0.74; 95% CI, 0.64–0.86; $p < 0.0001$). Patients treated with cabazitaxel had significantly higher rates of tumor response and PSA response than did those who received mitoxantrone, as well as significant improvements in time to tumor progression and time to PSA progression.

The most common toxic effects of cabazitaxel were hematological; the most frequent hematological grade 3 or higher AEs were neutropenia, leukopenia, and anemia. The most common non-hematological grade 3 or higher adverse event was diarrhea. Grade 3 peripheral neuropathy was uncommon.

On the basis of these results, cabazitaxel was approved in 2010 for the treatment of patients with mCRPC who have previously received docetaxel-based regimens [42].

The PROSELICA study, which compared the two allowed doses of cabazitaxel (20 and 25 mg per square meter) as second-line therapy in patients with mCRPC, concluded that the 20 mg per square meter dose maintains at least 50% of the survival benefit observed in the TROPIC study [49, 50]. This study reported lower toxicity for 20 mg per square meter than for 25 mg per square meter cabazitaxel dose with similar OS, suggesting that the dose may be reduced in patients who require the reduction [50].

More recently, cabazitaxel 25 and 20 mg per square meter (every 3 weeks) were compared with docetaxel in terms of OS in patients with chemotherapy-naïve mCRPC (FIRSTANA) [51]. No statistically significant differences between the three treatment groups were observed for OS or PFS; the study did not demonstrate the superiority of cabazitaxel over docetaxel. Treatment with cabazitaxel at the lower dose resulted in a similar OS and less hematological toxicity than the higher dose.

48.3.2.2 Radiopharmaceutical

Radium-223

Radium-223, a bone-seeking calcium mimetic, forms hydroxyapatite complexes during bone mineralization in areas of high osteoblast activity and increased bone turnover around prostate cancer metastatic lesions [52–54]. Radium-223 decays to emit predominantly high-energy alpha particles over a short range (<1 mm), leading to cytotoxicity through the production of predominantly unrepairable DNA double-strand breaks in nearby tumor and cells forming the cancer microenvironment. The short path of the alpha particles also means that toxic effects on adjacent healthy tissue and particularly the bone marrow may be minimized.

In phase 1 and 2 clinical trials of patients with bone metastases, radium-223 was associated with a favorable safety profile, with minimal myelotoxicity [55, 56]. Phase 2 studies have shown that radium-223 reduces pain and improves disease-related biomarkers (e.g., bone alkaline phosphatase and PSA) [57, 58], suggesting a survival benefit in patients with CRPC and bone metastases. To evaluate the effect of radium-223 on survival, a phase 3, randomized, double-blind, multinational study has been conducted (the Alpharadin in Symptomatic Prostate Cancer Patients (ALSYMPCA) study), which has compared the efficacy and safety of radium-223 versus placebo in patients with CRPC and bone metastases [59]. A total of 921 patients have been enrolled in the ALSYMPCA study (614 in the radium-223 group and 307 in the placebo group). The median number of injections was six in the radium-223 group and five in the placebo group.

At the interim analysis, the median overall survival was 14.0 months in the radium-223 group and 11.2 months in the placebo group; radium-223 was associated with a 30% reduction in the risk of death (HR 0.70; 95% CI, 0.55–0.88; two-sided $p = 0.002$). The advantage of radium-223 over placebo was confirmed also in the updated analysis, where median overall survival was 14.9 months in the radium-223 group and 11.3 months in the placebo group; the updated analysis confirmed the 30% reduction in the risk of death among patients in the radium-223 group as compared with the placebo group (HR 0.70; 95% CI, 0.58–0.83; $p < 0.001$). Radium-223, as compared with placebo, significantly prolonged the time to the first symptomatic skeletal event (median, 15.6 months vs. 9.8 months; HR 0.66; 95% CI, 0.52–0.83; p < 0.001), the time to an increase in the total alkaline phosphatase level (HR 0.17; 95% CI, 0.13–0.22; $p < 0.001$), and the time to an increase in the PSA level (HR, 0.64; 95% CI, 0.54–0.77; $p < 0.001$).

The associated toxicity was mild and, apart from slightly more hematologic toxicity and diarrhea with radium-223, this did not differ significantly from that in the placebo arm. Grade 3 febrile neutropenia was reported in one patient (<1%) in each group. A significantly higher percentage of patients who received radium-223, as compared with those who received placebo, had a meaningful improvement in the quality of life during the period of study-drug administration (25% vs. 16%, $p = 0.02$).

Therefore, although radium-223 is most often used as a second- or third-line therapy for mCRPC, it is reasonable to use it in bone-predominant, symptomatic disease even in the pre-docetaxel setting.

48.3.2.3 Novel Androgen-Directed Agents

Abiraterone Acetate

Abiraterone acetate is a selective inhibitor of androgen biosynthesis that potently blocks cytochrome P450 c17 (CYP17), a critical enzyme in testosterone synthesis, thereby blocking androgen synthesis by the adrenal glands and testes and within the prostate tumor. The efficacy of abiraterone acetate, in combination with prednisone, has been evaluated in two pivotal phase 3 studies in both men with mCRPC after chemotherapy with docetaxel (COU-AA-301 trial) [60, 61] and in men who were chemotherapy-naïve (COU-AA-302 trial) [62–64].

In the two phase 3 trials, patients received oral abiraterone acetate 1000 mg or placebo once daily in combination with oral prednisone 5 mg twice daily.

In the COU-AA-301 trial, 1195 patients were randomly assigned to receive abiraterone acetate plus prednisone (797 patients) or placebo plus prednisone (398 patients) [60]. At the time of the preplanned interim analysis, treatment with abiraterone acetate plus prednisone resulted in a 35.4% reduction in the risk of death as compared with placebo plus prednisone (HR 0.65; 95% CI, 0.54–0.77; $p < 0.001$). The mOS was

14.8 months in the abiraterone acetate group and 10.9 months in the placebo group. The effect of abiraterone acetate and prednisone on OS was consistent across all subgroups, and the significance of the treatment effect on OS was robust after adjustment for stratification factors in a multivariate analysis (HR for death, 0.66; 95% CI, 0.55–0.78; $p < 0.001$). Abiraterone acetate demonstrated its superiority over placebo for all the secondary end points analyzed, including the confirmed PSA response rate (29% vs. 6%, $p < 0.001$), the objective response rate on the basis of RECIST among patients with measurable disease at baseline (14% vs. 3%, $p < 0.001$), time to PSA progression (10.2 months vs. 6.6 months), and median PFS on the basis of radiographic evidence (5.6 vs. 3.6 months). At a median follow-up of 20.2 months, median OS was 15.8 months (95% CI, 14.8–17.0) in the abiraterone group compared with 11.2 months (10.4–13.1) in the placebo group (HR 0.74, 95% CI 0.64–0.86, $p < 0.0001$) [61]. The most common adverse event was fatigue, which occurred at a similar frequency in the two treatment groups [60]. Other common AEs in both groups were back pain (30% in the abiraterone acetate group and 33% in the placebo group), nausea (30% and 32%, respectively), constipation (26% and 31%), bone pain (25% and 28%), and arthralgia (27% and 23%). Most of these events were grade 1 or 2. AEs associated with elevated mineralocorticoid levels due to CYP17 blockade (fluid retention and edema, hypokalemia, and hypertension), as well as cardiac disorders and liver-function test abnormalities, were more common in the abiraterone acetate group than in the placebo group (55% vs. 43%, $p < 0.001$). The incidence of fluid retention and edema was higher in the abiraterone acetate group (31%, vs. 22% in the placebo group, $p = 0.04$). Grade 1 or 2 peripheral edema accounted for most of these events. Hypokalemia also occurred in a higher proportion of patients in the abiraterone acetate group (17%, vs. 8% in the placebo group, $p < 0.001$). Cardiac events (primarily grade 1 or 2) occurred at a higher rate in the abiraterone acetate group than in the placebo group (13% vs. 11%, $p = 0.14$), but the difference was not significant. The most frequently reported cardiac events were tachycardia (3% in the abiraterone acetate group and 2% in the placebo group, $p = 0.22$) and atrial fibrillation (2% and 1%, respectively, $p = 0.29$). Abiraterone acetate treatment has been associated with an elevation in aminotransferase levels. A grade 4 elevation in an aminotransferase level early in the study led to a protocol amendment specifying more frequent monitoring with liver-function tests during the first 12 weeks of treatment. Overall, however, abnormalities in liver-function tests occurred at a similar frequency in the abiraterone acetate and placebo groups, including changes of any grade in liver-function tests (10% and 8%, respectively), grade 3 or 4 changes in liver-function tests (3.5% and 3.0%), grade 3 or 4 elevations in aspartate aminotransferase levels (1.4% and 1.6%), grade 3 or 4 elevations in alanine aminotransferase levels (1.0% and 1.1%), and grade 4 elevations in aminotransferase levels (0.3% and 0.5%).

In the COU-AA-302 trial, co-primary end points were radiographic progression-free survival (rPFS) and OS defined as the time from randomization to death from any cause [62–64]. The median follow-up duration for all patients was 22.2 months. At the time of the first interim analysis, treatment with abiraterone plus prednisone, as compared with placebo plus prednisone, resulted in a 57% reduction in the risk of radiographic progression or death (median not reached vs. median of 8.3 months; HR for abiraterone-prednisone vs. prednisone alone, 0.43; 95% CI, 0.35–0.52; $p < 0.001$). At the time of the second interim analysis, the median time to rPFS was 16.5 months in the abiraterone-prednisone group and 8.3 months in the prednisone-alone group (HR 0.53; 95% CI, 0.45–0.62; $p < 0.001$).

The planned interim analysis of overall survival was performed after 333 deaths (43% of 773 events) were observed. Median OS was not reached for the abiraterone-prednisone group and was 27.2 months (95% CI, 26.0 to not reached) in the prednisone alone group. A 25% decrease in the risk of death in the abiraterone-prednisone group was observed (HR, 0.75; 95% CI, 0.61–0.93; $p = 0.01$), indicating a strong trend toward improved survival with abiraterone-prednisone. The effect of abiraterone on OS was consistently favorable across all prespecified subgroups.

The final analysis showed that there was a significant decrease in the risk of death in the abiraterone acetate group compared with the placebo group (HR 0.81, 95% CI 0.70–0.93, $p = 0.0033$) [63]. At a median follow-up of 49.2 months, mOS was significantly longer in the abiraterone acetate group than in the placebo group (34.7 months [95% CI 32.7–36.8] vs. 30.3 months [28.7–33.3], HR 0.81 [95% CI 0.70–0.93], $p = 0.0033$) [63].

In a multivariate analysis baseline PSA, lactate dehydrogenase, alkaline phosphatase, hemoglobin, bone metastases, and age were all significant prognostic factors for overall survival but ECOG performance status score was not. Abiraterone acetate plus prednisone decreased the risk of time to opiate use for prostate cancer-related pain compared with placebo plus prednisone at this final analysis (HR 0.72, 95% CI 0.61–0.85, $p < 0.0001$). Median time to opiate use for prostate cancer-related pain was 33.4 months (95% CI 30.2–39.8) in the abiraterone acetate group versus 23.4 months (95% CI 20.3–27.5) in the placebo group [63].

As to the safety profile, AEs of special interest, including events related to mineralocorticoid excess,

were more common in the abiraterone acetate group than in the placebo group. Most of them were of grade 1 or grade 2 in severity. The most common AEs in the final analysis resulting in death in the abiraterone acetate group were disease progression and general physical health deterioration as a sign of clinical progression in three (1%) and three (1%) patients, respectively. No treatment-related deaths occurred. Abiraterone acetate therapy was also associated with significant ($p < 0.05$) improvements in health-related quality of life (HR-QOL) compared with placebo plus prednisone in terms of patient-reported fatigue (assessed by Brief Fatigue Inventory questionnaire) [65] and functional status (assessed by Functional Assessment of Cancer Therapy-Prostate total score (FACT-P)) [66].

On the basis of the results of the COU-AA-301 and COU-AA-302 trials, abiraterone acetate has been approved by the national agencies for drug regulation and is now part of clinical practice in mCRPC treatment algorithm.

Enzalutamide

Enzalutamide is an androgen-receptor-signaling inhibitor chosen for clinical development on the basis of activity in prostate-cancer models with overexpression of the androgen receptor. Enzalutamide inhibits nuclear translocation of the androgen receptor, DNA binding, and coactivator recruitment. It also has a greater affinity for the receptor, induces tumor shrinkage in xenograft models (in which conventional anti-androgen agents only retard growth), and has no known agonistic effects [67, 68].

In a phase 1–2 trial enrolling men with CRPC (some of whom had undergone previous chemotherapy) conducted by the Prostate Cancer Clinical Trials Consortium [69], enzalutamide had shown significant antitumor activity regardless of previous chemotherapy status. On the basis of these findings, a dose of enzalutamide was identified for further study [70].

The efficacy of enzalutamide has been evaluated in two pivotal phase 3 studies in both men with mCRPC after chemotherapy with docetaxel (AFFIRM trial) [71] and in men who were chemotherapy-naïve (PREVAIL trial) [72].

In the AFFIRM trial, 1199 patients were randomly assigned to receive either enzalutamide (800 patients) or placebo (399 patients) [71]. The primary end point was OS, defined as the time from randomization to death from any cause. Secondary end points included measures of response (in the PSA level, in soft tissue, and in the quality-of-life score) and measures of progression (time to PSA progression, radiographic PFS, and time to the first skeletal-related event).

The mOS was 18.4 months (95% CI, 17.3 to not yet reached) for patients receiving enzalutamide and 13.6 months (95% CI, 11.3–15.8) among patients who received placebo. The use of enzalutamide resulted in a 37% reduction in the risk of death, as compared with placebo (HR for death 0.63; 95% CI, 0.53–0.75; $p < 0.001$). The survival benefit was consistent across all subgroups, including age, baseline pain intensity, geographic region, and type of disease progression at entry. The superiority of enzalutamide over placebo was shown for all secondary end points, including PSA-level response rate (54% vs. 2%, $p < 0.001$), soft-tissue response rate (29% vs. 4%, $p < 0.001$), FACT-P quality-of-life response (43% vs. 18%, $p < 0.001$), the time to PSA progression (8.3 vs. 3.0 months; HR, 0.25; $p < 0.001$), radiographic PFS (8.3 vs. 2.9 months; HR, 0.40; $p < 0.001$), and the time to the first skeletal-related event (16.7 vs. 13.3 months, HR 0.69, $p < 0.001$).

In terms of safety, the enzalutamide group had a lower incidence of AEs of grade 3 or above (45.3%, vs. 53.1% in the placebo group). The median time to the first adverse event was 12.6 months in the enzalutamide group, as compared with 4.2 months in the placebo group. A higher incidence of all grades of fatigue, diarrhea, hot flashes, musculoskeletal pain, and headache was observed in the enzalutamide group than in the placebo group. Cardiac disorders were noted in 6% of patients receiving enzalutamide and in 8% of patients receiving placebo (with cardiac disorders of grade 3 in 1% and 2%, respectively). Hypertension or increased blood pressure was observed in 6.6% of patients in the enzalutamide group and 3.3% of those in the placebo group. Liver-function abnormalities were reported as AEs in 1% of patients receiving enzalutamide and in 2% of those receiving placebo. Five of the 800 patients in the enzalutamide group (0.6%) were reported by the investigators to have had a seizure; no seizures were reported in the placebo group. One case of status epilepticus (confusion associated with partial complex status epilepticus) required medical intervention; the four other seizures were self-limited and did not recur after study-drug discontinuation. However, potentially predisposing factors were present in several patients. Caution should be used in administering enzalutamide to patients with a history of seizure or who have other predisposing factors, including underlying brain injury, stroke, brain metastases, or alcoholism, or to patients receiving concomitant medication that may lower the seizure threshold.

In the PREVAIL trial, a total of 1717 patients were enrolled randomly assigned to enzalutamide ($n = 872$) and placebo ($n = 845$) [72]. At 12 months of follow-up, the rate of radiographic PFS was 65% in the enzalutamide group and 14% in the placebo group. Treatment

with enzalutamide, as compared with placebo, resulted in an 81% reduction in the risk of radiographic progression or death (HR in the enzalutamide group, 0.19; 95% CI, 0.15–0.23; $p < 0.001$). Fewer patients in the enzalutamide group than in the placebo group had radiographic progression or died (118 of 832 patients [14%] vs. 321 of 801 patients [40%]). The median radiographic PFS was not reached in the enzalutamide group, as compared with 3.9 months in the placebo group. The treatment effect of enzalutamide on radiographic PFS was consistent across all prespecified subgroups.

As to the OS, at the planned interim analysis, the median duration of follow-up for survival was approximately 22 months. Fewer deaths occurred in the enzalutamide group than in the placebo group (241 of 872 patients [28%] vs. 299 of 845 patients [35%]). Treatment with enzalutamide, as compared with placebo, resulted in a 29% decrease in the risk of death (HR, 0.71; 95% CI, 0.60–0.84; $p < 0.001$). The mOS was estimated at 32.4 months in the enzalutamide group and 30.2 months in the placebo group. The treatment effect of enzalutamide on overall survival was consistent across all prespecified subgroups.

Enzalutamide has showed superiority over placebo with respect to all secondary end points. The median time to the initiation of cytotoxic chemotherapy was 28.0 months in the enzalutamide group, as compared with 10.8 months in the placebo group (HR, 0.35; $p < 0.001$). Treatment with enzalutamide also resulted in a reduction in the risk of a first skeletal-related event, which occurred in 278 patients (32%) in the enzalutamide group and 309 patients (37%) in the placebo group (HR, 0.72; $p < 0.001$) at a median of approximately 31 months in each of the two groups. Among patients with measurable soft-tissue disease at baseline, 59% of the patients in the enzalutamide group, as compared with 5% in the placebo group, had an objective response ($p < 0.001$): complete and partial responses were observed in 20% and 39% of the patients, respectively, in the enzalutamide group, as compared with 1% and 4%, respectively, in the placebo group. Enzalutamide was also superior to placebo with respect to reductions of at least 50% and 90% in the PSA level, the time until PSA progression, and the time until a decline in the quality of life. The median time until a quality-of-life deterioration, as measured on the FACT-P scale, was 11.3 months in the enzalutamide group and 5.6 months in the placebo group (HR, 0.63; $p < 0.001$).

As to the safety profile, a grade 3 or higher adverse event was reported in 43% of the patients in the enzalutamide group, as compared with 37% in the placebo group; however, the median time until the first event of grade 3 or higher was 22.3 months in the enzalutamide group and 13.3 months in the placebo group. The most common adverse events leading to death were disease progression and a general deterioration in physical health, with similar incidences in the two groups. Adverse events that occurred in 20% or more of patients receiving enzalutamide at a rate that was at least 2 percentage points higher than that in the placebo group were fatigue, back pain, constipation, and arthralgia. The most common event of grade 3 or higher in the enzalutamide group was hypertension, which was reported in 7% of the patients. The most common cardiac event was atrial fibrillation, which was reported in 2% of the patients in the enzalutamide group and in 1% of those in the placebo group. One patient in each study group had a seizure. No evidence of hepatotoxicity was observed in the enzalutamide group.

On the basis of the results of the AFFIRM and PREVAIL trials, enzalutamide has been approved by the national agencies for drug regulation and is now part of clinical practice in mCRPC treatment algorithm.

Updates:

- The phase 3 PROfound trial results suggested a role for olaparib, an inhibitor of PARP enzyme, for castration-resistant prostate cancer patients who carry genetic -alteration in BRCA1, BRCA2, or ATM genes and other genes involved in the homologous recombination mechanism.
- TITAN phase 3 randomized trial results showed a significant delay in second progression-free survival for castration-sensitive metastatic prostate cancer patients who received apalutamide in association with standard androgen deprivation therapy (ADT) versus standard ADT.
- Immunotherapy could have a significant role in advanced prostate cancer management. In particular, preliminary results from the phase 2 KEYNOTE-199 underlined a role for the addition of anti-PD1 pembrolizumab to standard enzalutamide in a cohort of chemo-naïve metastatic castration-resistant prostate cancer patients.

Case Study Metastatic Prostate Cancer

48

Man, 50 years old

- *Family history:* Negative for malignancy
- *APR*: Negative
- *APP*: April 2018, lower back pain on the right, VAS 9. Prostate adenocarcinoma Gleason score 9 (4 + 5)
- Right femoral metastasis and treated since 2016 with ADT
- *Blood tests*: PSA 6 ng/dl (vs. 2.5 ng/dl vs. 11.8 ng/dl), testosterone <0.04 ng/dl
- *PET with choline:* Hypermetabolic areas in the right femur (SUV max 6 vs. 4) + right iliac wing (SUV max 5 vs. 3.8) + left iliac wing (SUV max 6 vs. 3.4) + L3-L4-L5 (SUV max 3.2). Evidence of hypermetabolic area at L1 (SUV max 5.0) and left femur (SUV max 6)

Questions

What is the disease setting?

1. mCRPC
2. mCSPC
3. Locally advanced prostate cancer

Answer

mCRPC

Question

What is the preferred therapy option in this setting?

1. Abiraterone acetate
2. Enzalutamide
3. Chemotherapy

Answer

Abiraterone acetate + corticosteroid if there are no important cardiovascular comorbidities, otherwise enzalutamide. Chemotherapy could be preferred if high disease volume or visceral metastatic sites are involved.

Key Points

- The importance of the correct disease setting
- The importance of new-generation hormonal therapies

Expert Opinion

Giuseppe Procopio

Key Points

- Prostate cancer is the most frequent solid tumor diagnosed in male people and due to its high incidence and prevalence, screening programs have been adopted among population such as the valuation of PSA; otherwise, for the frequent over-diagnosis and over-treatments, nowadays, the screening program should be carefully discussed with the patient.
- After diagnosis of PCa, the decision to proceed with systemic staging workup is guided by the risk of disease systemic spread. Curative treatments or observational strategies may be proposed according to the risk of recurrence, life expectancy, and patients' preferences.
- RP or radiotherapy (external beam or brachytherapy) are two options for low- or intermediate-risk disease.
- RP plus pelvic lymphadenectomy or external beam RT plus hormone treatment are two alternative options for high-risk or locally advanced PCa.
- Long-term adjuvant ADT is recommended for high-risk PCa patients treated with radical EBRT.
- ADT represents the cornerstone of treatment for metastatic prostate cancer.
- The early addiction of docetaxel or abiraterone acetate to ADT improves the overall survival of mHSPC, mainly in the subpopulation of high-volume and in high-risk patients.
- Several therapeutic options have demonstrated to improve patients' outcomes in the mCRPC setting, including docetaxel, cabazitaxel, abiraterone and enzalutamide, and Radium-223.

Recommendations

- ESMO
  ► www.esmo.org/Guidelines/Genitourinary-Cancers/ESMO-Consensus-Guidelines-Prostate-cancer
- NCCN
  ► jnccn.org/view/journals/jnccn/17/5/article-p479.xml
- ASCO
  ► www.asco.org/practice-guidelines/quality-guidelines/guidelines/genitourinary-cancer#/32796
  ► www.asco.org/practice-guidelines/quality-guidelines/guidelines/genitourinary-cancer#/33301

► www.asco.org/practice-guidelines/quality-guidelines/guidelines/genitourinary-cancer#/25251
► www.asco.org/practice-guidelines/quality-guidelines/guidelines/genitourinary-cancer#/24836

Hints for a Deeper Insight

- Phase II study of pembrolizumab (MK-3475) in patients with metastatic castration-resistant prostate cancer (KEYNOTE-199)-study AP 93/16 of the AUO: ► https://www.ncbi.nlm.nih.gov/pubmed/28980011
- Prostate cancer between prognosis and adequate/proper therapy: ► https://www.ncbi.nlm.nih.gov/pubmed/28255369
- Prostate Cancer Genetics: Variation by Race, Ethnicity, and Geography: ► https://www.ncbi.nlm.nih.gov/pubmed/27986209

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Testicular Cancer

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Genitourinary Cancers

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Learning Objectives

By the end of the chapter, the reader will:

- Be able to detect a possible patient with testicular cancer
- Have learned how to manage the work-up and diagnosis of testicular cancer
- Have reached in-depth knowledge of treatment of this pathology
- Be able to put acquired knowledge into clinical practice
- Be able to follow-up these patients both to detect relapse and late toxicity

49.1 Introduction

Testicular cancer is the most common malignant solid tumor in young men between the second and fourth decade of life, and it accounts for approximately 1% of all cancers in men [1].

The classification of testicular cancer includes several types of testicular cancers but the germ-cell tumor (GCT) is the most frequent (about 95%). Approximately 50% are pure seminoma and the other 50% are non-seminoma [2].

The 5-year survival for localized testicular cancer is 99.2%, while for metastatic testicular cancer it is 73.2% [3]; therefore, a careful staging at diagnosis, adequate early treatment based on a multidisciplinary approach, and strict follow-up and salvage therapies are very important approaches for the delivery of the best treatment.

49.2 Epidemiology

Nearly 8.850 men are diagnosed with testicular cancer yearly in the United States, but only around 410 will die of their disease [1]. In Europe the rate of incidence is 5.8% (21.532/100.000) and the mortality rate is 0.4% (1612/100.000) [4].

There are some known risk factors such as:

- Cryptorchidism [5]
- Personal or family history of testicular cancer [6–8]
- Infertility or subfertility [9]

49.3 Clinical Features

Testicular cancer usually presents as a nodule or a painless swelling in one testicle.

When there are metastases, symptoms can vary from neck mass (supraclavicular adenopathy), cough or dyspnea (lung metastases), abdominal or lumbar back pain (retroperitoneal disease), bone pain (bone metastases), central nervous system (CNS) symptoms (CNS metastases), or lower extremities swelling (obstruction or thrombosis).

In about 5% of the GCT patients, they can be presented with gynecomastia, which is a systemic endocrine manifestation associated with production of human chorionic gonadotropin (hCG) by foci of choriocarcinoma or trophoblastic cells in the tumor [10].

49.4 Diagnosis

49.4.1 Clinical Examination

In the case of a suspected testicular nodule or swelling, the physical examination should include scrotum palpation to evaluate the nodule or swelling. A complete physical examination should be performed to search for any other findings such as gynecomastia, abdominal palpable mass, or supraclavicular mass.

49.4.2 Imaging

Testicular ultrasound is useful to confirm the presence of a testicular mass and explore the contralateral testis [2]. It is a very sensitive diagnostic method and it is important to evaluate whether the mass is intra- or extra-testicular.

If a patient is diagnosed with a retroperitoneal mass or has elevated serum tumor marker suggesting extragonadal GCT, a testicular ultrasound should be performed even in the absence of palpable testicular mass [11] (EAU guidelines).

The imaging studies should also include a chest radiography.

49.4.3 Serum Tumor Markers

The serum tumor markers assume a crucial role in testicular cancer. Alpha-fetoprotein (AFP), lactate dehydrogenase (LDH), and beta-hCG are essential in the diagnosis, staging, prognosis, and assessment of treatment outcome. They should be measured before and after treatment and throughout the follow-up period [11].

AFP is produced by non-seminomatous cells and it has a half-life of 5–7 days; therefore, a non-seminoma is associated with elevated AFP. If a pure seminoma has an elevated AFP, then an undetected focus of non-seminoma is present [12]. Beta-hCG can be elevated in both seminoma and non-seminoma tumors and it has a half-life of about 1–3 days.

49.4.4 Screening

There are no recommendations for screening for testicular cancer. However, individuals with risk factors and especially in patients with a family history of testicular cancer, family members and the patient should be informed about the importance of physical self-examination [11].

49.5 Differential Diagnosis

The differential diagnoses are:
- Epididymitis
- Orchitis
- Hydrocele
- Abdominal hernias
- Varicocele
- Lymphoma
- Trauma
- Metastases from other tumors
- Testicular torsion

49.5.1 Pathology

The natural evolution of the disease depends of the histological subtype [13, 14] (▶ Box 49.1):

Box 49.1 Classification of testicular cancer according to the World Health Organization Classification of Tumors 2016

Germ-cell tumors

Seminoma

Non-seminoma
- Embryonal carcinoma
- Choriocarcinoma
- Yolk sac tumor
- Teratoma
- Teratoma with malignant/somatic transformation
- Mixed germ-cell tumor

Spermatocytic tumor

Sex cord-stromal tumors
- Sertoli cell tumor
- Leydig cell tumor
- Granulosa cell tumor
- Mixed types
- Unclassified

Mixed germ-cell and stromal tumors
- Gonadoblastoma

Adnexal and paratesticular tumors
- Adenocarcinoma of rete testis
- Adenocarcinoma of the epididymis
- Mesothelioma
  - Malignant mesothelioma
  - Adenomatoid tumor

Miscellaneous tumors
- Carcinoid
- Lymphoma
- Metastatic tumors

- Seminoma: It represents approximately 45% of testicular tumors. At diagnosis 25% of the patients presented lymphatic and up to 5% visceral metastases (lung and bone mainly).
- Spermatocytic seminoma represents 4% of seminomas and usually appears in older patients with germ-cell tumor and more frequent in patients older than 70 years. They are most often bilateral and its metastatic potential is minimal.
- Pure choriocarcinoma: It is rare (0.3%). It is the most aggressive and metastasizes quickly through hematogenous spread. It has elevated HCG and normal alpha-fetoprotein concentrations.
- Yolk sac tumor: It produces AFP; it has worse prognosis in adults compared to children.
- Embryonal carcinoma: In pure form it represents 3% of the cases and in the mixed form it is present in more than 40% of adult testicular tumors. It is a tumor consisting of undifferentiated cells. 33% of elevation of AFP is associated.
- Teratoma: You can see the three germ layers' (ectoderm, mesoderm, and endoderm) fabrics; it may undergo a malignant transformation and this produces metastasis. The most common is the mesodermal differentiation.

Other tumors with less constraints:
- Leydig cell tumor, Sertoli cell tumor, and granulosa cell tumor: They do not present serious elevations of AFP or hCG. They can produce metastases. Sertoli cell tumors are chemo-resistant. Granulosa cell tumors have juvenile and adult forms and usually have a benign behavior [15–17].
- Rhabdomyosarcoma: It is more frequent in those younger than 20 years old. Metastatic potential is fundamentally to lymph nodes and lungs.

49.6 Staging

Physical examination; history; determination of serum level of AFP, beta-hCG, and LDH; pathology; and imaging studies define the extension of disease and appropriate treatment [13].

The recommended staging system is based on the classification of the International Union Against Cancer (UICC), with the TNMS system (tumor, node, metastasis, and serum markers) including the anatomical extension (T), the invasion of regional nodes (N), and the presence of metastasis (M) with local characterization (Tables 49.1 and 49.2). Serum concentrations of tumor markers, AFP, beta-hCG, and LDH and the nadir value post-orchiectomy are incorporated into the S category [18].

49.6.1 Imaging Studies

Computed tomography (CT): It is used to identify metastatic involvement above and below the diaphragm. Oral and intravenous contrast is the best for identifying retroperitoneal lymphadenopathy [19].

Positron emission tomography (PET): It yields no improvement in clinical staging and no value in post-chemotherapy management [20].

Magnetic resonance imaging (MRI): It occasionally provides valuable information regarding vascular anatomy or liver disease [13].

49.6.2 Risk Classification for Advanced Disease

For the advanced disease, the International Germ Cell Cancer Collaborative Group (IGCCCG) defined a prognostic classification system based on the extent of disease and levels of serum tumor markers post-orchiectomy and divides seminomas and non-seminomas in good-, intermediate-, and poor-risk groups (Table 49.3) [2].

49.7 Treatment

49.7.1 Fertility Issues

Patients with testicular cancer frequently present sperm alterations, and the chemotherapy and radiotherapy contribute to fertility impairment. It is important to assess their fertility pretreatment and they should be informed of their options, e.g., cryopreservation [11].

Table 49.1 Staging system of testicular cancer according to the TNMS system

TNM category	Description
Primary tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Intratubular germ-cell neoplasia
T1	Tumor limited to the testis and epididymis or tumor invasion into the tunica albuginea only
T2	Tumor extending through the tunica albuginea with involvement of the tunica vaginalis
T3	Tumor invades the spermatic cord
T4	Tumor invades the scrotum
Regional lymph nodes – Clinical (N) or pathologic (pN) staging	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastases to single or multiple lymph nodes, each <2 cm in size
N2	Metastases to single or multiple lymph nodes, >2 cm but <5 cm in size
N3	Metastases to lymph node, >5 cm in greatest dimension
Distant metastasis (M)	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis
M1a	Nonregional nodal or pulmonary metastasis
M1b	Distant metastasis other than to nonregional lymph nodes and lungs
Serum tumor markers	
SX	Unavailable or not performed
S0	Within normal limits
S1	Lactate dehydrogenase (LDH) level <1.5 times normal, human chorionic gonadotropin (HCG) level <5000 IU/L, alpha-fetoprotein (AFP) level <1000 ng/mL
S2	LDH 1.5–10 times normal; HCG level, 5000–50,000 IU/L; AFP level, 1000–10,000 ng/mL
S3	LDH >10 times normal; HCG level >50,000 IU/L; AFP level >10,000 ng/mL

Table 49.2 Anatomical staging and prognostic groups

Stage	T	N	M	S
0	pTis	N0	M0	S0, Sx
I	pT1 – pT4	N0	M0	Sx
IA	pT1	N0	M0	S0
IB	pT2-pT4	N0	M0	S0
IS	Any pT	N0	M0	S1 – 3
II	Any pT	N1 – N3	M0	Sx
IIA	Any pT	N1 N1	M0 M0	S0 S1
IIB	Any pT Any pT	N2 N2	M0 M0	S0 S1
IIC	Any pT Any pT	N3 N3	M0 M0	S0 S1
III	Any pT	Any N	M1	Sx
IIIA	Any pT Any pT	Any N Any N	M1a M1a	S0 S1
IIIB	Any pT Any pT	N1-N3 Any N	M0 M1a	S2 S2
IIIC	Any pT Any pT Any pT	N1-N3 Any N Any N	M0 M1a M1b	S3 S3 Any S

49.7.2 Management of Testicular Cancer

The treatment of seminoma and NSGCT involves surgery, radiotherapy, and chemotherapy and depends on the disease stage [2, 11, 18].

49.7.2.1 Primary Treatment

The primary treatment for the majority of testis tumors is radical inguinal orchiectomy. A testicular prosthesis should be offered to every patient.

Seminoma germ-cell tumor first-line treatment (Algorithm 49.1)

49.7.2.2 Stage I Seminoma

In this stage, most of the patients are cured after surgery and the rate of relapse is small, so the toxicity should be minimized. Surveillance is the preferred option for this stage.

In alternative, one course of adjuvant carboplatin therapy AUC 7 can be used or adjuvant radiotherapy as seminoma cells are extremely radiosensitive.

The risk factors that divide seminoma stage I into low- and high-risk groups for occult metastatic disease are tumor size >4 cm and rete testis invasion.

Table 49.3 Risk classification for advanced disease

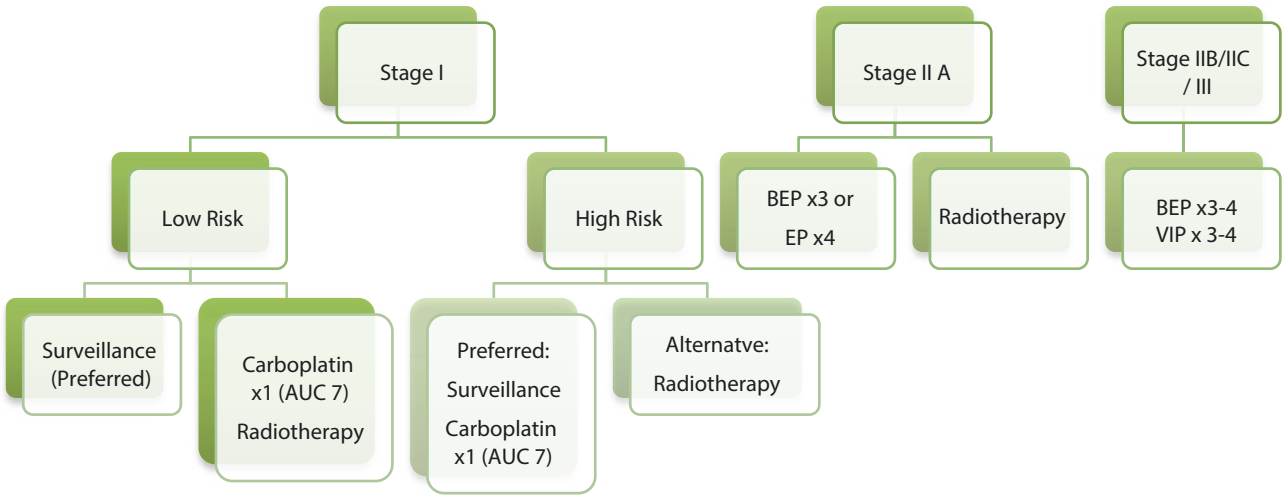
Risk status	Non-seminoma	Seminoma
Good risk	Testicular or retroperitoneal primary tumor	Any primary site
	No nonpulmonary visceral metastases	No nonpulmonary visceral metastases
	AFP <1000 ng/mL hCG <5000 IU/L LDH <1.5 × upper limit of normal	Normal AFP Any hCG Any LDH
Intermediate risk	Testicular or retroperitoneal primary tumor	Any primary site
	No nonpulmonary visceral metastases	Nonpulmonary visceral metastases
	Post-orchiectomy markers – Any of the following: hCG 5000–50,000 IU/L LDH 1.5–10 × upper limit of normal	Normal AFP Any hCG Any LDH
Poor risk	Mediastinal primary tumor	No patients classified as poor Prognosis
	Nonpulmonary visceral metastases	
	Post-orchiectomy markers – any of the following: AFP >10,000 ng/mL hCG >50,000 IU/L LDH >10 × upper limit of normal	

49.7.2.3 Stage IS Seminoma

Stage IS is a very rare type of seminoma with persistent elevation of serum tumor markers after surgery, which can be evidence of metastatic disease. The extent of disease should be determined by imaging studies. The chemotherapy is similar to the non-seminoma tumors.

49.7.2.4 Stage IIA Seminoma

In this stage, adjuvant radiotherapy of the para-aortic region and ipsilateral iliac nodes reaches an overall survival of almost 100%. In case of multiple node involvement, chemotherapy with EP (etoposide and cisplatin) × 4 or BEP (bleomycin, etoposide, and cisplatin) × 3 is an option.



Algorithm 49.1 Seminoma germ-cell tumor first-line treatment

49.7.2.5 Stage IIB Seminoma

Adjuvant radiotherapy can be an option for stage IIB seminoma (non-bulky disease). For cases with adenopathy greater than 3 cm, adjuvant chemotherapy with EP × 4 or BEP × 3 is an option.

49.7.2.6 Stage IIC Seminoma

Adjuvant chemotherapy with BEP × 3 or EP × 4 is recommended.

49.7.2.7 Stage III Seminoma

Stage III patients are divided into good or intermediate risk (nonpulmonary visceral metastases).

In the good-risk group, adjuvant chemotherapy with BEP × 3 or EP × 4 is recommended.

In the intermediate group, chemotherapy with BEP × 4 or VIP (etoposide, mesna, ifosfamide, and cisplatin) × 4 is recommended.

49.7.2.8 Post-chemotherapy Management of Seminoma Stages II–III

Serum tumor markers and CT scan are used to evaluate the presence of residual mass. In case of normal serum tumor markers and no residual mass or mass less than 3 cm, no more treatment is needed and the patient should be on surveillance.

In case of residual tumor, a PET scan should be performed 6 weeks after chemotherapy. If the PET scan is negative, the patient should go under follow-up. If the PET scan is positive, biopsy of the mass or resection should be considered, and if the results show seminoma, chemotherapy with EP × 2 or TIP (paclitaxel, ifosfamide, and cisplatin) × 2 is recommended. In case of incomplete resection, TIP × 4 or VeIP (vinblastine, ifosfamide, and cisplatin) × 4 is recommended.

Non-seminoma germ-cell tumor first-line treatment

49.7.2.9 Stage I Non-seminoma

This stage has high survival rates. It can be divided into low or high risk based on absence or presence of vascular invasion, respectively.

In the low-risk group, surveillance is standard, but if it is not possible, adjuvant chemotherapy with one or two cycles of BEP is recommended. If the patient is not fit for chemotherapy, open nerve-sparing retroperitoneal lymph node dissection (RPLND) is an option.

In the high-risk group, surveillance and chemotherapy (one or two cycles of BEP) are options. Open nerve-sparing RPLND can be an option.

49.7.2.10 Stage IS Non-seminoma

Chemotherapy with EP ×4 or BEP ×3 is recommended. Hepatobiliary disease, use of marijuana, and hypogonadism may be the reason for elevated serum tumor markers post-orchiectomy, so results should be interpreted with caution.

49.7.2.11 Stage IIA Non-seminoma

The treatment for these patients depends on the serum tumor marker levels:

- In case of normal serum tumor markers post-orchiectomy, RPLND or chemotherapy with four cycles of EP or three cycles of BEP is recommended.
  - If the disease is multifocal, chemotherapy is the best option.
- In case of persistent elevation of serum tumor markers, the risk of relapse is elevated, so induction chemotherapy is recommended.

49.7.2.12 Management of Non-seminoma Stage IIA After Primary Treatment

After primary chemotherapy, AFP and beta-hCG levels should be assessed and an abdominal and pelvic CT with contrast should be done and a chest CT or X-rays may be considered.

In case of negative serum tumor markers or residual mass <1 cm, surveillance is an option. In case of residual mass >1 cm, RPLND must be considered. This procedure must be done in high-volume centers.

After primary RPLND:

- Surveillance for pN0 and pN1
- Chemotherapy for selected pN1, pN2, and pN3

For pN1 and pN2, the regimen is BEP or EP for two cycles. For pN3 disease, four cycles of EP or three cycles of BEP are recommended.

49.7.2.13 Stage IIB Non-seminoma

The patient's treatment also depends on both post-orchiectomy tumor marker levels and radiographic findings:

- If normal tumor markers and imagological findings of retroperitoneum disease:
  - Nerve-sparing RPLND followed for adjuvant treatment
  - Primary chemotherapy and nerve-sparing RPLND or surveillance
- In presence of imagological findings of metastatic disease:
  - Chemotherapy, followed by RPLND or surveillance
- In case of persistent elevation of tumor markers, the primary treatment should be chemotherapy and RPLND is not recommended.

49.7.2.14 Advanced Metastatic Non-seminoma

The choice of the chemotherapy regimen depends on the risk classification:

Good-risk group:

- There are two regimens recommended for this group: BEP ×3 or EP ×4.

Intermediate-risk group:

- There are two regimens recommended for this group: BEP ×4 or VIP ×4 (patients with bleomycin intolerance).

Poor-risk group:

- The regimen recommended is BEP ×4 and VIP ×4 (patients with bleomycin intolerance) (◘ Algorithm 49.2).

Post-chemotherapy management:

- In the end of chemotherapy, the patient should undergo a CT scan and evaluation of serum tumor markers.

In case of negative tumor markers and imagological complete response, the following are recommended:

- Surveillance in case of initial stage IS
- Surveillance or RPLND in case of IIA, S1, IIB, S1, IIC, or IIIA

In case of residual mass, the recommended treatment is surgery followed by chemotherapy.

Second-Line Therapy for Metastatic Germ-Cell Tumors

Patients who present recurrence or do not have a durable complete response to first-line therapy can be divided in two groups: favorable or unfavorable prognosis based on prognostic factors.

In the favorable prognosis group (complete response to first-line therapy, low levels of post-orchiectomy serum tumor markers, and low-volume disease), the use of conventional chemotherapy or high-dose chemotherapy is recommended. Participation in clinical trials is encouraged.

In the unfavorable prognosis group (incomplete response to first-line treatment, high levels of serum markers, high-volume disease, and presence of extragonadal primary tumor), participation in a clinical trial is the preferred option, or conventional chemotherapy or high-dose chemotherapy.

49.8 Follow-Up

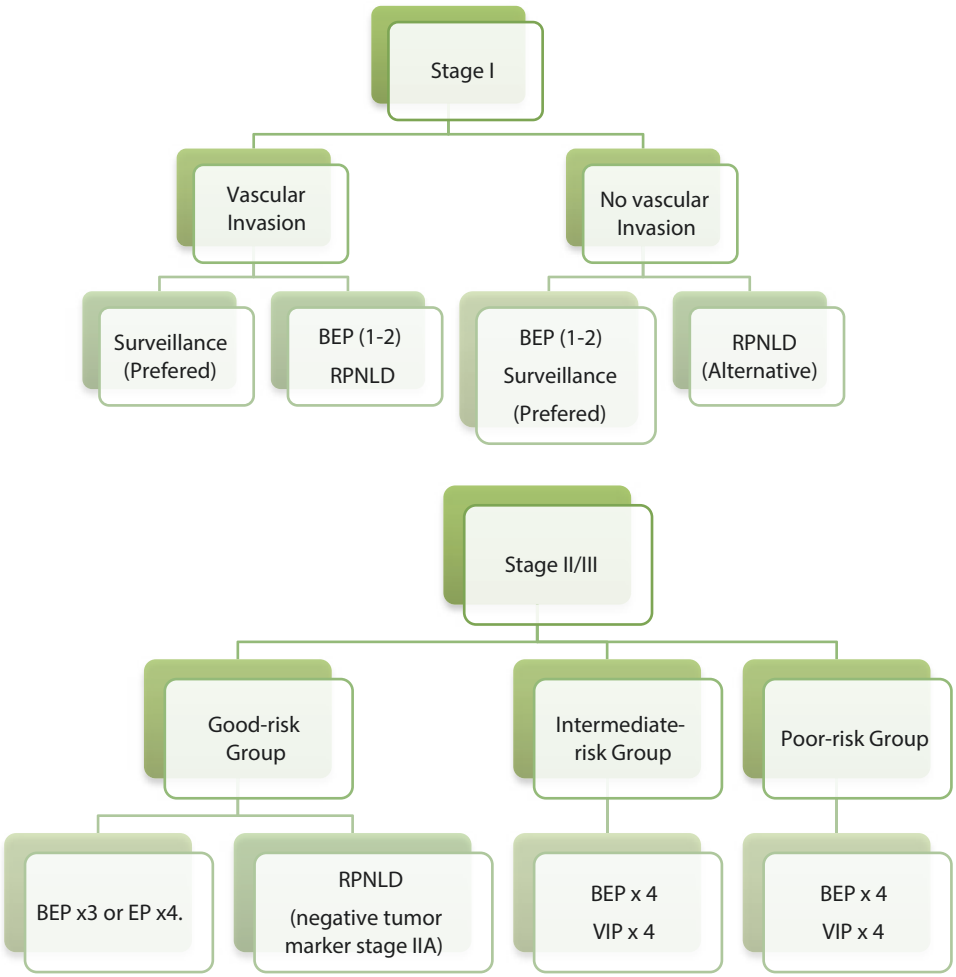
The main objective of follow-up visits is to allow an early detection and treatment of relapse. The follow-up plan must be adapted to the individual patients and the schedules published should only provide a general guidance.

Late relapses after 5 years are a rare event occurring in nearly 0.5% of patients. Therefore, beyond 5 years of follow-up, its aim shifts toward detection of late side effects of treatment [11].

49.9 Survivorship

Although testicular cancer represents the most curable solid tumor, there is considerable long-term morbidity related to the treatment and extensive follow-up. Neurotoxicity, nephrotoxicity, cardiovascular disease,

Algorithm 49.2
Non-seminoma germ-cell tumor first-line treatment

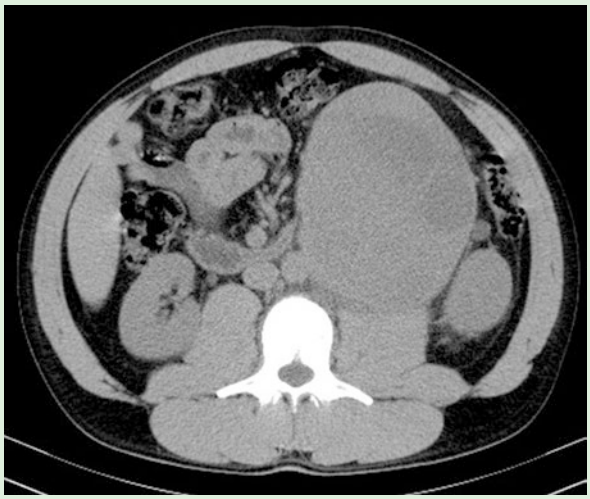


pulmonary toxicity, hypogonadism, decreased fertility, psychosocial problems, and even the development of second malignant neoplasms are all possible outcomes late in life for testicular cancer patients. In this regard, the institution of lifelong follow-up of testicular cancer survivors should be considered [21].

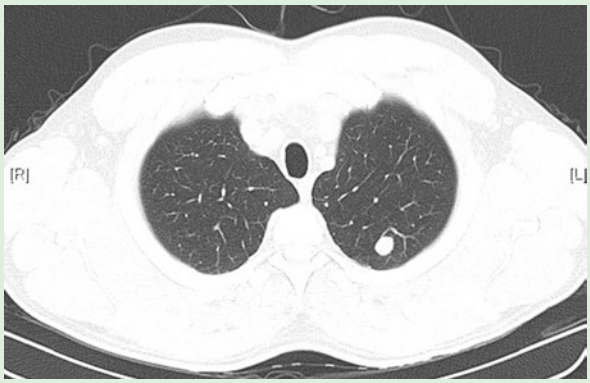
Case study

Male, 36 years old, healthy

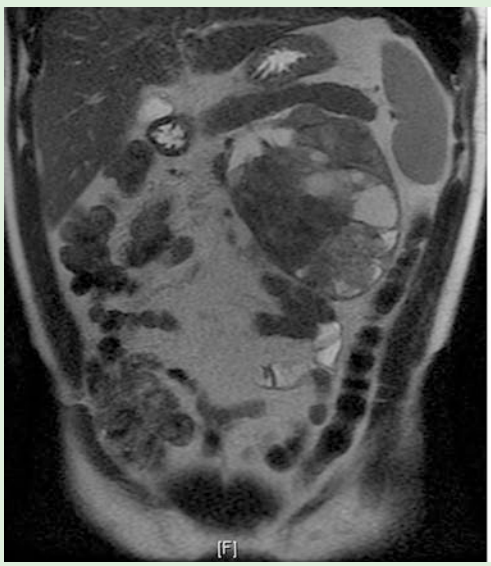
- *Family history:* Negative for malignancy
- *APP:* For nearly 4 months, left lower back pain. Recently with irradiation to the abdomen and palpable abdominal mass
- *Objective examination*: Palpable mass of stony consistency in the upper left abdominal quadrant with 60 × 100 mm
- *Blood tests*: Elevated LDH (1668 U/L)
- *Abdominal ultrasound:* Well-defined and lobulated bulky mass (retroperitoneal?) of 108 × 134 mm, solid, heterogeneous, with cystic areas
- *Abdominal and pelvic CT:* Bulky left retroperitoneal mass (139 × 109, 7 mm) as described by the ultrasound. Suggesting MRI

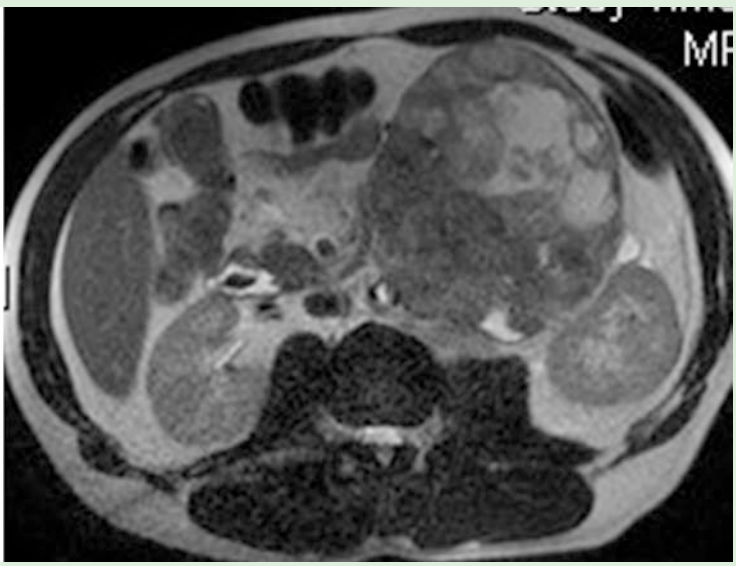


- *Thoracic CT:* Multiple lung metastasis



- *Abdominal and pelvic* MRI: Bulky expansive left retroperitoneal mass, with 11/12 cm, heterogeneous, cystic areas and hemorrhagic areas. Probably a sarcoma or extragonadal germ-cell tumor





Question

What should we do?

1. Serum tumor markers
2. Surgery
3. Biopsy

Answer

Serum tumor markers and biopsy

Beta-hCG was elevated (1804 mlU/mL)
Histology: Carcinoma extensively necrotic

Question

What should we do next?

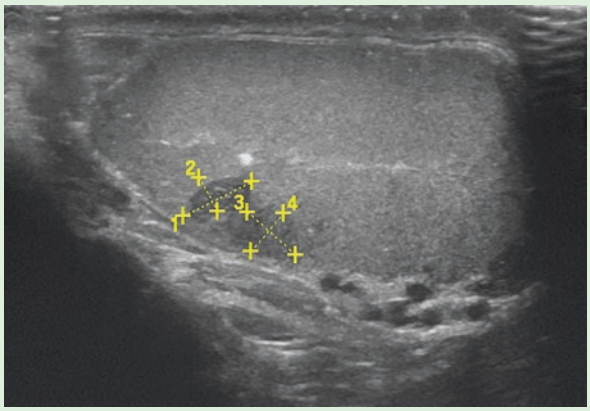
1. Surgery
2. Chemotherapy
3. Testicular ultrasound

Answer

Testicular ultrasound

Multifocal tumor in the left testis

49



Question

What should we do next?

1. Biopsy
2. Chemotherapy
3. Other

Answer

Chemotherapy

Four cycles of BEP (bleomycin, etoposide, and cisplatin)

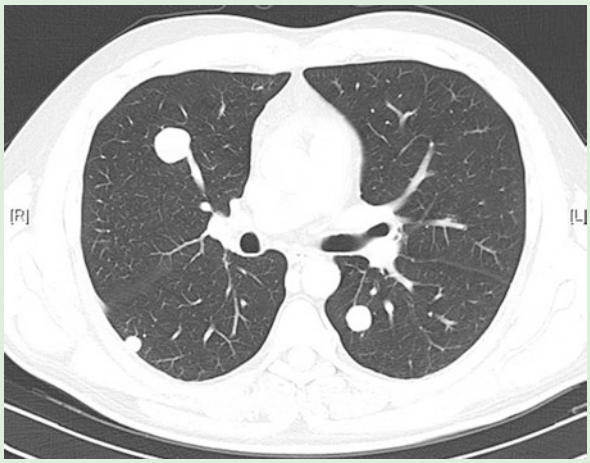
Response Assessment

Thoracic, abdominal, and pelvic CT: Significant reduction of retroperitoneal mass dimension as well as lung metastasis

The patient was sent to the IPO (Portuguese Oncology Institute) of Lisbon where they performed surgery: radical left orchiectomy + appendectomy + excision of the retroperitoneal residual mass.

- Histology: Germ-cell intratubular tumor with 2 mm in the left testis and a metastasis of a non-seminomatous germ-cell tumor, with tumor in the surgical margins
- Serum tumor markers: AFP and beta-hCG normal

Two months later, the patient had an increase of AFP and the CT shows disease progression (lung and retroperitoneal).



Question

What should we do next?

1. Chemotherapy
2. Surgery
3. Others

Answer

Chemotherapy

The patient started chemotherapy with TIP (paclitaxel, ifosfamide, and cisplatin) followed by autologous bone marrow transplantation.

Response Assessment

Thoracic, abdominal, and pelvic CT: No evidence of oncological disease

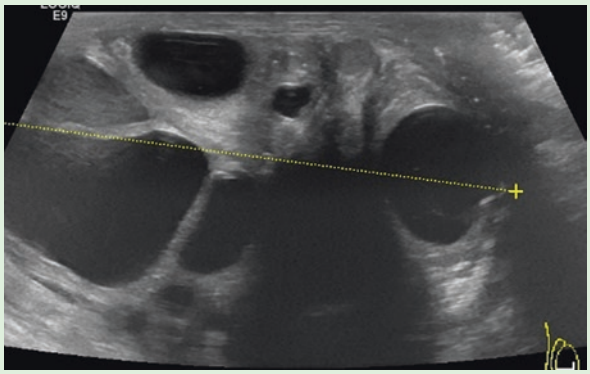
Key Points

- Importance of serum tumor markers and biopsy: Differential diagnosis with other neoplasia.
- Do not forget to look for a testicular mass even in the extragonadal germ-cell tumors.
- When appropriately treated, testicular cancer can have high survival rates even in the metastatic setting.

Case study

Man, 33 years old, healthy

- *Family history:* Negative for malignancy
- *APP:* Palpable nodule in the right testis after a trauma that increased its dimensions
- *Objective examination*: Palpable suspicious mass in the right testis
- *Blood tests*: AFP and LDH normal, beta-hCG elevated (5.7 U/L)
- *Scrotum ultrasound:* Increased volume of the right testis with cystic suspicious testicular cancer



- *Thoracic, abdominal, and pelvic CT:* Increased volume of right testis with multiple cystic formations, no evidence of other disease sites

Question

What should we do next?

1. Biopsy
2. Surgery
3. Others

Answer

Surgery

The patient underwent a right radical orchiectomy with prosthetic implantation.

Histology: Cystic teratoma with foci of embryonal carcinoma and foci of seminoma.

Question

What should we do next?

1. Chemotherapy
2. Serum tumor markers
3. Radiotherapy

Answer

Serum tumor markers

Post-orchiectomy tumor marker levels are used for risk stratification and are incorporated into the American Joint Committee on Cancer TNM Staging System for Testis Cancer.

- LDH, AFP, and beta-hCG normal
- Stage IA, low risk

Question

What should we do next?

1. Chemotherapy
2. Active surveillance
3. Radiotherapy

Answer

Active surveillance

Key Points

- Importance of serum tumor markers before and after the surgery.
- Early diagnosis leads to high rates of survival.
- Active surveillance is the preferred treatment option in low-risk patients.

Expert Opinion

Antonio Russo

Key Points

1. Testicular cancer is the most common cancer in 20-40 years old men; its incidence in Europe is around 5.8%, with a mortality rate of 0.4%.
2. Clinically it usually appears as a nodule or a painless swelling in one testicle; sometimes symptoms are linked to the metastatic diffusion (i.e., dyspnea or cough can appear in case of lung metastases). Some testicular cancers can produce human chorionic gonadotropin (hCG) which causes gynecomastia.
3. It is possible to identify different histological subtypes according to the latest WHO classification: seminoma (the most frequent form), spermatocytic seminoma, choriocarcinoma, yolk sac tumor, embryonal carcinoma, teratoma, Leydig cell tumor, Sertoli tumor, granulosa cell tumor, and rhabdomyosarcoma.
4. When a testicular cancer is suspected, a physical examination should be performed followed by US and blood test with the evaluation of beta-hCG, alpha-fetoprotein (AFP), and lactate dehydrogenase (LDH). They can give useful information about the type of testicular cancer and they are also implied during the follow-up. For a correct and complete staging, CT is recommended.
5. Treatment differs from each patient; the primary one in most cases consist in a radical inguinal orchiectomy. The subsequent approaches depend on the his-

tological subtype and stage; they can comprise just follow-up (stage I seminoma), radiotherapy, or even chemotherapy with different schedules.

6. At the end of the treatment, the patient should undergo follow-up periodic evaluations which must be adapted to the single patient.

Reccomendetions

- ESMO
- ► www.esmo.org/Guidelines/Genitourinary-Cancers/Testicular-germ-cell-cancer
- ► www.esmo.org/Guidelines/Genitourinary-Cancers/Testicular-Seminoma-and-Non-Seminoma
  American Urological Association
- ► www.auanet.org/guidelines/testicular-cancer-guideline

Hints for a Deeper Insight

- Epidemiology and Diagnosis of Testis Cancer: ► www.ncbi.nlm.nih.gov/pubmed/26216814
- Clinical presentation, management and follow-up of 83 patients with Leydig cell tumors of the testis: a prospective case-cohort study: ► https://www.ncbi.nlm.nih.gov/pubmed/31532522
- Relapse surveillance of patients with testicular germ cell tumor: ► https://www.ncbi.nlm.nih.gov/pubmed/31495441
- Cancer-testis antigens and immunotherapy in the light of cancer complexity: ► https://www.ncbi.nlm.nih.gov/pubmed/25901859
- Testicular Cancer Biomarkers: A Role for Precision Medicine in Testicular Cancer: ► https://www.ncbi.nlm.nih.gov/pubmed/30497810

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Cancer of the Penis

Alchiede Simonato, Cristina Scalici Gesolfo, and Alberto Abrate

Genitourinary Cancers

Contents

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Learning Objectives

By the end of the chapter, the reader will:

- Be able to identify patients/lesions at risk for penile cancer
- Have learned the basic investigation method for a good staging of penile cancer
- Be able to apply the acquired knowledge in clinical practice in order to make an early diagnosis and choose the best treatment for the patient

50

50.1 Epidemiology and Cancer Prevention

Penile carcinoma, though a rare neoplasm in developed countries, is an aggressive disease with devastating effect in affected patients. In developing countries such as South America, Africa, and Asia, where its incidence is higher, early detection is a goal that urologists try to achieve, in order to limit the damage and the mortality related to the progression of this tumor.

Squamous cell carcinoma (SCC) represents the commonest histological type followed by basaloid carcinoma, warty carcinoma, and papillary carcinoma as shown in Table 50.1. It arises from the prepuce or glans and its natural history and pathology are similar to other locations of SCC such as the oropharynx, female genitalia, and anus. The incidence of penile SCC is related to age, with a peak in the sixth decade, and changes dramatically from the Western countries to the Third World.

In fact, while in Europe and the USA it is a rare disease, with an incidence <1/100,000 males, in some parts of Africa, South America, and Asia, it can represent the 1–2% of malignant disease in men [1].

Furthermore, distribution around the world in terms of incidence is related to the prevalence of HPV. The higher the prevalence of HPV in a certain country, the higher the incidence of penile carcinoma [1]. According to this evidence, HPV infection (especially sustained by subtypes HPV-16 and HPV-18) is one of the major risk factors, as it probably acts as a cofactor in the carcinogenesis through an interaction with oncogenes and oncosuppressor genes such as p53 and Rb [2]. Supporting this hypothesis, HPV DNA is present in the histological samples of the 70–100% intraepithelial neoplasms and in the 30–40% of invasive penile cancers.

However, HPV infection is not the only cause of penile carcinoma. It is probable that chronic infection/inflammation in general could promote this oncogenesis. This fact could explain the association between phimosis and penile carcinoma [3]. The mechanical micro-trauma and the poor hygienic conditions linked to phimosis could promote infections and inflammations and sustain their chronicity. In this sense, it is not surprising that the lowest incidence of penile carcinoma is recorded in those cultures or countries where neonatal circumcision is routinely performed, as this not only improves hygiene and reduces the risk of chronic infection/inflammation but also removes the majority of the tissue that could develop a penile carcinoma.

Table 50.1 Prevalence and prognosis according to histological type of penile carcinoma

Prevalence and prognosis according to histological type of penile carcinoma			
Histological type	**% of cases**	**Prognosis**	**Metastasis**
Common squamous cell carcinoma (SCC)	48–65	Depends on location, stage, and grade	Early inguinal nodal metastasis could be present
Basaloid carcinoma	4–10	Poor prognosis	Early inguinal nodal metastasis
Warty carcinoma	7–10	Good prognosis	Rare
Verrucous carcinoma	3–8	Good prognosis	None
Papillary carcinoma	5–15	Good prognosis	Rare
Sarcomatoid carcinoma	1–3	Very poor prognosis	Early vascular metastasis
Mixed carcinoma	9–10	Heterogeneous group	Depending on histological types
Pseudohyperplastic carcinoma	<1	Good prognosis	Not reported
Carcinoma cuniculatum	<1	Good prognosis	Not reported
Pseudoglandular carcinoma	<1	Poor prognosis	Early metastasis
Warty-basaloid carcinoma	9–14	Poor prognosis	High metastatic potential
Adenosquamous carcinoma	<1	Low mortality	High metastatic potential
Mucoepidermoid carcinoma	<1	Poor prognosis	Not reported
Clear cell variant of penile carcinoma	1–2	Poor prognosis	Early metastasis, frequent lymphatic metastasis

Despite this encouraging data, the incidence of carcinoma in situ (CIS) seems not to be affected by neonatal circumcision [3]; furthermore, no significant changes in incidence have been recorded in adults having undergone circumcision. Another important risk factor for penile carcinoma is cigarette smoking, which increases three- to fivefold the risk of penile carcinoma, which in its turn has been found to be dose dependent [3].

50.2 Genetic Aspects of Hereditary Cancer

Currently, cancer of the penis has not been correlated to a hereditary disorder or a hereditary genetic mutation.

50.3 Differential Diagnosis

Squamous cell carcinoma represents the commonest histological type of penile carcinoma (up to 95%), with smaller percentages also of melanoma, basal cell carcinoma, and Paget disease. Furthermore, the incidence of penile Kaposi's disease increased following the incidence of HIV.

SCC is often preceded by a premalignant lesion [4]. Recognizing and treating the premalignant lesion is important to prevent the evolution to penile cancer. Table 50.2 summarizes the most common premalignant lesions and their characteristics.

Table 50.2 Types of premalignant lesions

Types of premalignant lesions		
Premalignant lesion	**Risk factor**	**Appearance**
Leukoplakia	Diabetes	White, hard, may ulcerate
Balanitis xerotica obliterans (BXO)	Chronic phimosis, chronic infections, poor hygiene, vigorous sexual activity, lichen sclerosus, paraphimosis	Penile skin fusion to the head of the penis, indurated and narrowed
Giant condyloma acuminata	HPV infection	Bulky exophytic growth and tumor size that often exceeds 10 cm in greatest diameter
Bowen disease		Sharply defined plaques of scaly erythema, may ulcerate and crusted

50.4 Typical Signs and Symptoms

The primitive tumor is localized on the glans in the 48% of cases, on the prepuce in the 21% of cases, on both in the 9% of cases, and on the coronal line and on the penile rod in the 6% and 2% of cases, respectively [5]. At physical examination, penile carcinoma presents as a small, hard, and erythematous area, sometimes ulcerated, or as a small endophytic or exophytic node. The commonest symptoms are pain, discomfort, and burning sensations.

50.5 Diagnostic Strategies and Staging

Physical examination is the first important step for the diagnosis of penile carcinoma. Lesions could be hidden by a phimosis; in this case circumcision should be performed before choosing local treatment of the lesion in order to avoid under- or over-treatment.

During physical examination, attention must be paid to the inguinal lymph nodes. The physical examination should be reported as complete as possible with indication of side, number, and mobility of enlarged nodes. The absence of palpable lymph nodes in the presence of penile cancer deposes for an early lymphadenectomy without need for further imaging investigation; in fact 20% of patients with absence of palpable lymph nodes have nodal micrometastases [6]. At diagnosis enlarged palpable inguinal lymph nodes are present in about 58% of patients, of which 17–45% are positive for metastasis [7], while in the other cases the enlargement is due to inflammation. In order to distinguish the inflammatory enlarged nodes from the metastatic ones, patient should be reexamined after at least a week of antibiotics. Bilateral involvement of lymph nodes is possible due to the presence of a high number of lymphatic vessels that cross in the subcutaneous tissue of the penis. Patients with positive lymph nodes should be assessed for distant metastasis through a CT scan of the abdomen and pelvis and chest X-rays [8].

Histological examination is crucial for the diagnosis and treatment of penile carcinoma.

Based on the clinical presentation of the primitive lesion, a total excision or a biopsy should be considered. When the lesion appears deep and invasiveness is suspected, a penile US or MRI must be performed in order to exclude involvement of the corpora cavernosa [9].

In any case a biopsy should be performed.

Aggressiveness criteria are used to choose the timing for demolitive treatment. One of these criteria is the differentiation grading that varies from 0 to 4, from more differentiated to more undifferentiated and aggressive disease. The staging of penile carcinoma follows the

Jackson classification and the TNM classification as reported in Tables 50.3 and 50.4. Negative prognostic factors for metastatic spread are tumors with vertical growths and with vascular and lymphatic invasion.

Table 50.3 Jackson classification of penile carcinoma

Jackson classification	
Stage	**Description**
I	Confined to the glans or prepuce
II	Invasion into shaft or corpora
III	Operable inguinal lymph node metastasis
IV	Tumor invades adjacent structures, inoperable inguinal lymph node metastasis

50.6 Treatment Options

The treatment of penile carcinoma tries to achieve two ideal goals:

- Complete eradication of the tumor
- Organ preservation

For small, superficial, and localized lesions, organ preservation is generally an achievable goal as complete eradication can be performed with excisional surgery, laser ablation, brachytherapy, or external beam radiotherapy.

First-line treatment of carcinoma in situ (CIS) can consist of topical chemotherapy with imiquimod or 5-FU, though a strict follow-up is required in consideration of the high risk of failure of the treatment or recurrence both in the short and long term. Total or partial glans resurfacing can be performed both in the first or second line of treatment.

Table 50.4 2016 TNM clinical and pathological classification of penile carcinoma

2016 TNM clinical classification of penile carcinoma	
T – Primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
Ta	Non-invasive verrucous carcinoma
T1	Tumor invades subepithelial connective tissue
	T1a without lymphovascular invasion and is not poorly differentiated
	T1b with lymphovascular invasion or is poorly differentiated
T2	Tumor invades corpus spongiosum with or without invasion of the urethra
T3	Tumor invades corpus cavernosum with or without invasion of the urethra
T4	Tumor invades other adjacent structures
N – Regional lymph nodes	
NX	Regional lymph nodes cannot be assessed
N0	No palpable or visibly enlarged inguinal lymph node
N1	Palpable mobile unilateral inguinal lymph node
N2	Palpable multiple unilateral or bilateral inguinal lymph nodes
N3	Fixed inguinal nodal mass or pelvic lymphadenopathy, unilateral or bilateral

Table 50.4 (continued)

M – Distant metastasis	
M0	No distant metastasis
M1	Distant metastasis
2016 TNM pathological classification of penile carcinoma	
pT – Categories that correspond to the clinical T categories	
pN – Regional lymph nodes (from biopsy or surgical excision)	
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Metastasis in one or two inguinal lymph nodes
pN2	Metastasis in more than two unilateral inguinal nodes or bilateral inguinal lymph nodes
pN3	Metastasis in pelvic lymph node(s), unilateral or bilateral extranodal or extension of regional lymph node metastasis
pM – Distant metastasis	
pM1	Distant metastasis microscopically confirmed
G – Histopathological grading	
GX	Grade of differentiation cannot be assessed
G1	Well differentiated
G2	Moderately differentiated
G3	Poorly differentiated
G4	Undifferentiated

In patients with small, localized, invasive lesions, a conservative approach is recommended with an extemporary analysis of the margins. To consider the reliability of the negativity of a margin, it should be at least 5 mm from the lesion.

Possible conservative treatments for T1/T2 diseases are:

- Laser therapy
- Mohs micrographic surgery
- Glans resurfacing
- Glansectomy
- Partial penectomy

There is not enough evidence to prefer one organ-conserving strategy over another in terms of outcome and conservative surgery could improve the patient's quality of life.

In patients with T1 and T2 disease with a diameter <4 cm, radiotherapy could be a valid conservative treatment with local control rate ranging from 70% to 90%; however, recurrence rates after radiotherapy are higher than after partial penectomy. Common complications of radiant treatments are urethral stenosis, meatal stenosis, glans necrosis, and late fibrosis of corpora cavernosa.

Treatment of T2/T3 disease consists of partial amputation with at least 5 mm of free margin. Surgery must be followed by a strict follow-up. Radiotherapy could be considered as treatment.

In patients with locally advanced disease (T3/T4), a total penectomy with perineal urethrostomy must be performed. In patients with T4 penile cancer, neoadjuvant chemotherapy should be performed and followed by surgery in responders. In non-responders, adjuvant chemotherapy and palliative radiotherapy are options.

50.6.1 Nodal Anatomy, Drainage, and Treatment

It is important to devote a paragraph to the treatment of the inguinal nodes. In fact, nodal involvement could be considered the major prognostic factor for survival in patients affected by penile SCC. As discussed above, survival is related to the absence or presence of nodal metastases.

The lymphatic drainage of the penis is entrusted to superficial and deep inguinal nodes and is characterized by a well-known anatomy crossover between those two groups, both ipsilateral and bilateral. The sentinel node of the prepuce is located on the upper-medial zone and drains from this to the superficial inguinal nodes (8–25 nodes), while glans and corpora cavernosa could drain into superficial inguinal node or directly into the deep inguinal nodes and into the external iliac nodes. For this reason, in patients undergoing lymphadenectomy, both the superficial and deep inguinal nodes are removed according to the ilioinguinal lymph node dissection (IILND). In fact, contralateral metastases could be found in more than 50% of patients treated with a bilateral inguinal lymphadenectomy, despite the absence of palpable lymph nodes.

Several studies demonstrated the importance of an early lymphadenectomy, considering that micrometastases were found in the 25% of patients with non-palpable lymph nodes who underwent surgery. An improvement of survival has been found in patients undergoing early nodal dissection while delayed nodal dissection could only rarely save recurring patients.

Surveillance in case of non-palpable lymph nodes should be offered only to Ta, T1, and CIS patients with high compliance and after a complete information about the risk of worst survival in case of lymphadenectomy of lateral regional recurrence. In this case survival decreases from 90% to 40% at 5 years comparing early lymphadenectomy with lymphadenectomy for later regional recurrence. Whenever surveillance is indicated, it is important to schedule a strict follow-up schedule in order to intervene immediately, should there appear to be a change in nodal stage.

The choice of timing and extension of lymphadenectomy should follow the algorithm shown in Fig. 50.1. Approaching inguinal nodal dissection, it is important to establish the correct balance between therapeutic goals and minimal morbidity for the patient. In fact the IILND often causes important complications such as severe lymphedema and necrosis of the skin flap (30–50%), wound infection, phlebitis, and pulmonary embolism. In order to decrease this rate of complications in patients with clinically negative inguinal lymph nodes, different procedures have been tested:

- Fine-needle aspiration cytology (FNAC): In both ultrasonography and lymphangiography guidance, the FNAC did not show sufficient sensitivity to be considered as a staging procedure [10].
- Sentinel lymph node biopsy: This procedure is no longer recommended due to unreliability in identifying microscopic metastasis.
- Dynamic sentinel node biopsy: This procedure uses the injection of radiant and colored substances that produce gamma emissions near the lesion. These substances are absorbed from the lymphatic system and collected to regional lymph nodes that can be detected, identified, and dissected during surgery. However, this technique currently shows good results in terms of sensitivity only in high-volume centers with trained surgeons and nuclear medicine specialists [11].
- Superficial node dissection: This consists of the removal of those nodes which are superficial to the

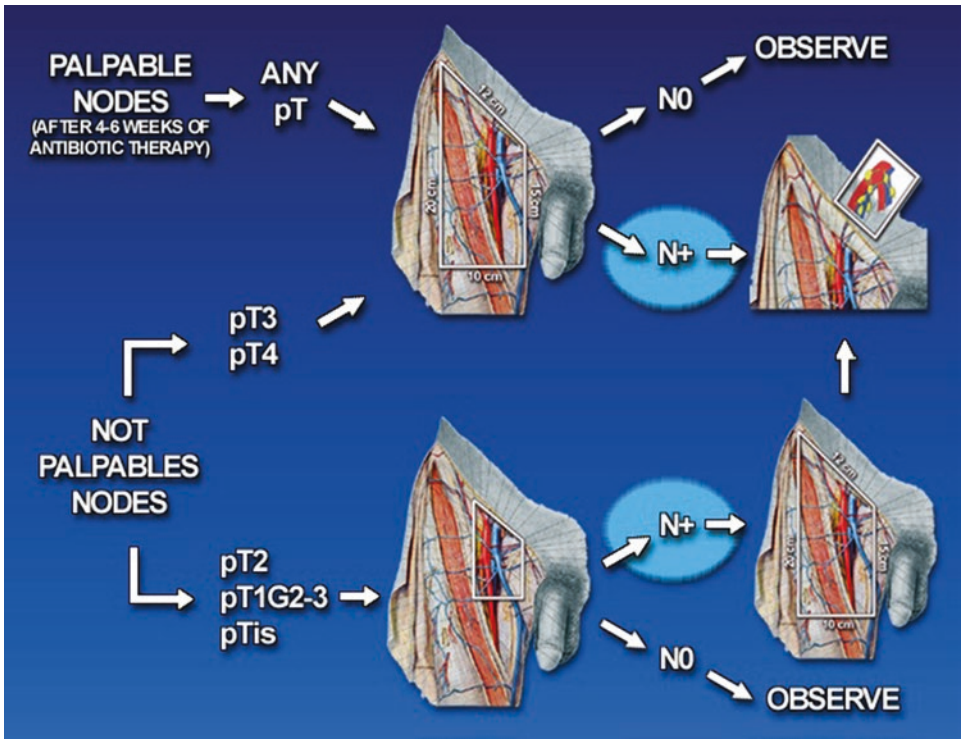

Fig. 50.1 Algorithm of timing and extension of lymphadenectomy

fascia lata. If no metastatic nodes are found, a complete IILND is not performed due to evidence of absence of recurrence in up to 3 years of follow-up in cohort studies [12].

- Complete modified inguinal dissection: This technique was proposed by Catalona in 1988 [13] and allows the performance of a small cutaneous incision, preserving the saphenous vein. No muscle transposition is needed in order to protect the femoral vessel, and furthermore this technique allows the dissection of both superficial and deep nodes. The Catalona modified inguinal dissection is shown in Fig. 50.2. With the modified IILND by Catalona, though still present, comorbidities are less frequent and less severe as demonstrated by different studies [14–16].
- Laparoscopic and robotic minimally invasive inguinal lymphadenectomy: At present, the results obtained with minimally invasive approaches are comparable to open surgery [17].
- Pelvic lymphadenectomy: It should be performed in case of positivity of the inguinal node due to the uncommon presence of pelvic lymph node metastasis with negative inguinal nodes. Suspicion of nodal pelvic involvement in absence of inguinal node metastasis should be evaluated through pelvic CT scan.

50.6.2 Non-surgical Treatments

Neoadjuvant chemotherapy (four cycles of cisplatin- and taxane-based regimen) should precede radical surgery in patients with non-resectable or recurrent lymph nodes (LE 2a GR B) [18].

Adjuvant chemotherapy should be offered to patients with pN2/pN3 or systemic disease and a limited metastatic load (LE 2b-3 GR C). Second-line therapy with anti-EGFR monoclonal antibodies and tyrosine-kinase inhibitor has been investigated but further studies are necessary (LE 4) [19, 20].

50.7 Conclusions

Penile carcinoma is a malignant disease which benefits from early diagnosis and treatment. After a complete staging, a multidisciplinary approach is mandatory to ensure the best therapy for the patients. However, timing plays a crucial role; whatever the chosen treatment, it must be performed as early as possible to increase the chances of success.

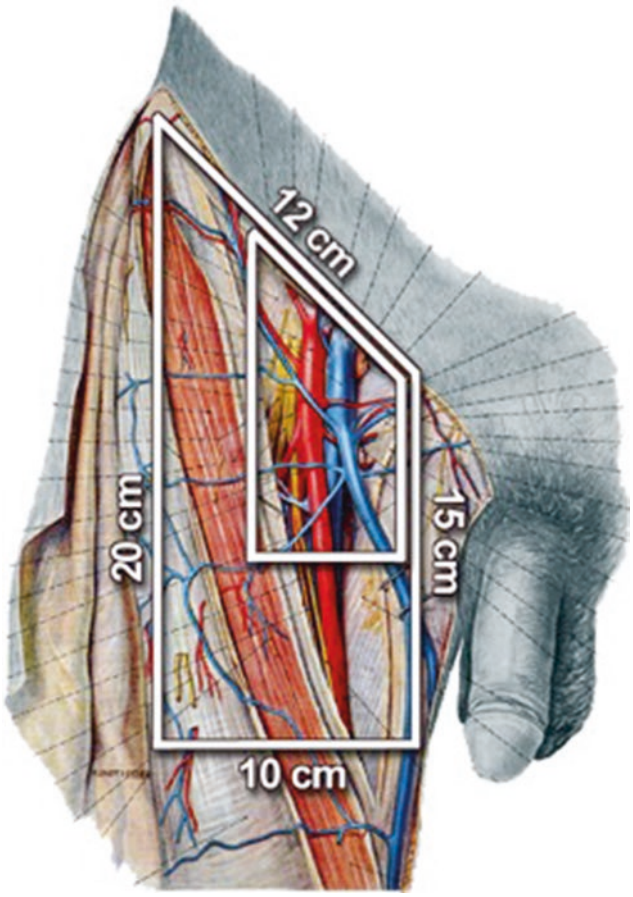

Fig. 50.2 IILND resection area: **a** Classic IILND involves lymph node both superficial and deep to the fascia lata contained within the femoral triangle. **b** Completed modified inguinal dissection according to Catalona that excludes the area lateral to the femoral artery and caudal to the fossa ovalis and saphenous vein preservation, with no need for sartorius muscle transposition

Summary of Clinical Recommendation

Diagnosis and Staging:

- Perform a physical examination, and record morphology, extent, and invasion of penile structures. Perform a physical examination of both groins, and record the number, laterality, and characteristics of inguinal lymph nodes and:
  - Non-palpable nodes → offer invasive lymph node staging in high-risk patients (≥T1b).
  - Palpable nodes → abdominopelvic computed tomography (CT) or positron emission tomography (PET)/CT and chest X-ray for staging.
- In patients with systemic disease or with relevant symptoms, obtain a bone scan.

Treatment:

- For localized penile cancer (from Tis to T2 confined to the glans) → offer local treatment like laser, glans resurfacing, radiotherapy, and glansectomy.
- For T2 with invasion of the corpora cavernosa → offer partial amputation and reconstruction/radiotherapy/brachytherapy.
- For T3 with invasion of the urethra → offer partial/total penectomy with perineal urethrostomy.
- T4 → offer neoadjuvant chemotherapy followed by surgery in responders or palliative external beam radiation.

Management of Nodal Metastases:

- Non-palpable inguinal nodes (cN0):
  - Tis, Ta G1, T1a → surveillance
  - ≥ T1b → invasive staging by bilateral modified inguinal lymphadenectomy/dynamic sentinel node biopsy
- Palpable inguinal nodes (cN1/cN2) → radical inguinal lymphadenectomy
- Fixed inguinal lymph nodes(cN3) → neoadjuvant chemotherapy followed by radical inguinal lymphadenectomy in responders
- Pelvic lymphadenopathy → ipsilateral pelvic lymphadenectomy if two or more inguinal nodes are involved on one side (pN2) and if extracapsular nodal metastasis (pN3) is confirmed
- pN2/pN3 patients after radical lymphadenectomy → adjuvant chemotherapy

Follow-Up:

- Minimum length of 5 years, with an interval of 3 months for the first 2 years for any categories of patients.
- Penile-preserving treatment: Interval of 6 months after the first 2 years with regular physician/self-examination. If positive, repeat biopsy after topical or laser treatment for carcinoma in situ.
- Amputation: Interval of 1 year after the first 2 years with regular physician/self-examination.
- Inguinal lymph nodes under surveillance: Interval of 6 months after the first 2 years with regular physician/self-examination.
- Inguinal lymph nodes pN0 at initial treatment: Interval of 1 year after the second year of follow-up with regular physician/self-examination. Ultrasound with fine-needle aspiration biopsy optional.
- Inguinal lymph nodes pN+ at initial treatment: Interval of 6 months after 2 years of follow-up with regular physician/self-examination. Ultrasound with fine-needle aspiration cytology optional, CT/MRI optional.

[a]According to the most recent guidelines available [e.g., ASCO, EAU, AUA]

Expert Opinion
Lorena Incorvaia

Key Points

- In case of suspected penile cancer, early diagnosis and treatment are mandatory.
- Treatment of penile carcinoma aims to completely eradicate the tumor, while preserving the organ integrity when possible.
- Nodal involvement is the major prognostic factor.

Hints for Deeper Insight

- Pathological subtype, perineural invasion, lymphovascular invasion, depth of invasion, and grade in the primary tumor are strong predictors of poor prognosis and high cancer-specific mortality.
- In doubtful cases, before definitive surgical treatment, confirmatory frozen section excisional biopsy can be done.
- In case of clinically normal inguinal regions (cN0), imaging studies are not helpful (except in obese patients) for N-staging.

Suggested Reading

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Ovarian Cancer, Early Primary Disease

Domenica Lorusso, Giuseppa Maltese, Ilaria Sabatucci, and Elisa Tripodi

Gynecological Cancers

Contents

A. Russo et al. (eds.), *Practical Medical Oncology Textbook*, UNIPA Springer Series,
https://doi.org/10.1007/978-3-030-56051-5_51

Learning Objectives

By the end of the chapter, the reader will:

- Be able to apply diagnostic and staging procedures in the management of early ovarian cancer
- Have learned the basic concepts of surgical management of disease
- Have reached in-depth knowledge of indications to adjuvant treatments
- Be able to put acquired knowledge into clinical practice for the management of early stage particularly by referring patients to tertiary centers where multidisciplinary management can be performed

51

51.1 Introduction

Ovarian cancer represents the seventh most common cause of cancer among women worldwide, and the vast majority of malignant ovarian cancers (about 90%) are epithelial tumors (EOC) [1, 2].

Ovarian cancer is staged according to FIGO staging system (International Federation of Gynecology and Obstetrics) and, less commonly, to AJCC-TNM staging system.

FIGO stage I disease describes a neoplasm exclusively limited to ovaries, while in FIGO stage II the tumor is confined to the pelvis, as shown in ◘ Fig. 51.1. Both conditions are defined as "early-stage ovarian cancers"[3]. Early-stage ovarian cancers are usually asymptomatic and have a relatively good prognosis with a 5-year survival rate of about 90%. Unfortunately, only 20–30% of all ovarian cancer cases are diagnosed at early stage [4].

51.2 Epidemiology

The incidence of ovarian cancer differs among geographic areas, with the higher rates in industrialized countries, especially Europe and North America, with approximately 22,200 new cases diagnosed during 2016 in the USA resulting in 14,240 deaths [5].

The incidence increases with age and is prevalent in postmenopause, with a median age at presentation of 60 years. The age at diagnosis is earlier in patients with genetic or familial predisposition, generally in the fifth decade of life [6].

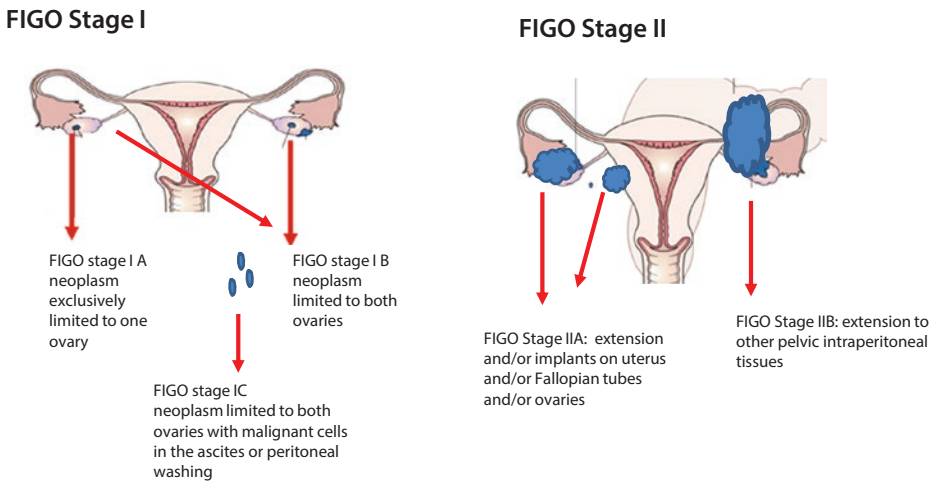
Over the past 30 years, the 5-year survival rate of women with ovarian cancer has increased to about 10%, ranging from 30% to 40%, depending primarily on the stage of disease at diagnosis. The improvement of surgical techniques [6], surgical expertise, and the decrease of the use of postmenopausal hormonal therapy [7] have possibly contributed to the survival amelioration.

Approximately 25% of women with ovarian cancer are diagnosed at FIGO stages I and II, generally due to an accidental finding, for example, during sonography, computerized tomography (CT) scanning, or laparoscopy performed for other reasons.

51.3 Ovarian Cancer Staging

All the most important international scientific guidelines underline the importance of staging on treatment and prognosis of ovarian cancer.

Ovarian cancer is classified according to size, extent, and localization of the disease, using two different staging systems: the FIGO (International Federation of



◘ **Fig. 51.1** FIGO staging of early stage ovarian cancer (2014) (IIC has recently been eliminated in 2014 FIGO Staging)

Table 51.1 FIGO and AJCC-TNM staging classification of ovarian, fallopian tube, and peritoneal cancer

Stage FIGO	Classification	TNM	Incidence (%)	Year survival (%)
I	*Tumor limited to ovaries or fallopian tube(s)* *Stage IA*: Tumor is limited to one ovary or fallopian tube; the capsule is intact, no tumor on ovarian surface. No malignant cells on the ovarian surface or in peritoneal washings or ascites. *Stage IB*: Tumor is limited to both ovaries or fallopian tube; the capsule is intact, no tumor on ovarian surface. No malignant cells on the ovarian surface or in peritoneal washings or ascites *Stage IC:* Tumor is limited to one or both ovaries or fallopian tubes, with any the following: surgical spill (*IC1*), capsule rupture before surgery or tumor on surface (*IC2*), or malignant cell in the ascites or peritoneal washings (*IC3*) *Capsule rupture and positive cytological washings are considered as independent predictors of poor disease-free survival [9]	T1 T1a T1b T1c1 T1c2 T1c3	20%	92
II	*Tumor involves one or both ovaries with pelvic extension* *IIA:* Extension and/or implants on uterus and/or fallopian tubes and/or ovaries *IIB:* Extension to other pelvic intraperitoneal tissues *IIC is recently eliminated in 2014 FIGO stage	T2 T2a T2b	5%	73–78

Gynecology and Obstetrics) [3] and the AJCC-TNM staging systems [8]. Both staging systems are also applied to fallopian tube carcinoma and primary peritoneal adenocarcinoma. In Table 51.1 incidence and survival rates by stage are shown.

Objectives of staging are:

- To describe prognosis
- To plan appropriate treatment

The FIGO staging system is exclusively pathological and based on the findings at surgical intervention. A preoperative instrumental evaluation is necessary to evaluate neoplasm extension and to exclude the presence of extraperitoneal metastases. Surgical exploration and adequate staging are necessary to determine postoperative treatment.

Table 51.2 Risk factors for ovarian cancer

Patient characteristics	Increasing age Personal history of breast cancer Familial history of breast and ovarian cancer
Reproductive factors	Nulliparity Early menarche and late menopause Infertility Hormonal replacement therapy
Environmental factors	Obesity Talc exposure
Genetic factors	BRCA1/2 mutations Lynch syndrome Other genetic syndromes

51.4 Risk Factors

51.4.1 Non-genetic

Most part of epithelial ovarian tumors is sporadic. Nulliparity, older age (>40 years), obesity, long-term postmenopausal estrogen therapy use, and infertility increase the risk for ovarian cancer. On the contrary, multiparity, oral contraceptive use, younger age at first pregnancy, and breastfeeding are protective factors (Table 51.2) [10].

A recent meta-analysis demonstrated that a long-term oral contraceptive use reduces the risk of ovarian cancer in general population, especially in patients with BRCA mutation [11].

The role of smoking, talc exposure, diet, and non-steroidal anti-inflammatory drugs is still controversial [12, 13].

51.4.2 Genetic Syndromes

Familial genetic syndromes are diagnosed in approximately 10–12% of women with EOC [14, 15]. Hereditary breast-ovarian cancer syndrome and hereditary non-polyposis colorectal cancer (Lynch syndrome) are the most frequent; other syndromes, although less frequent, have been associated with an increased risk of ovarian cancer (Table 51.3).

BRCA-associated ovarian cancer

An important risk factor for ovarian cancer is the BRCA1 or BRCA2 gene mutation, which is the cause of hereditary breast-ovarian cancer syndrome. In families with a history of breast or ovarian cancer, BRCA germline mutations are responsible for approximately 90% of cases of ovarian cancer [16].

Both BRCA genes are tumor suppressor genes that produce proteins involved in DNA damage repair; BRCA mutation carriers are unable to repair double-strand DNA damage, which ultimately leads to the accumulation of genetic alterations and to cancer development (Fig. 51.2).

Characteristics of BRCA-mutated ovarian cancer patients:

- BRCA mutations are prevalent in the Jewish population [17].
- BRCA1 mutations are more common than BRCA2 mutations (incidence: 20–40% vs. 10–20%, respectively) [18].
- The predicted lifetime risk of ovarian cancer is greater in patient with BRCA1 than BRCA2 mutation (40–60% vs. 10–30%, respectively).
- BRCA-mutated tumors are associated with improved progression-free survival (PFS) and overall survival (OS), with a better prognosis compared to sporadic ovarian cancers [19, 20].
- BRCA mutations are generally associated with high-grade serous ovarian cancer and less frequently with other histological subtypes (e.g., high-grade endometrioid tumors and clear cells).
- BRCA1/2-mutated tumors are particularly sensitive to PARP inhibitors.
- BRCA status is associated with greater chemosensitivity, mainly to platinum [18, 20] but also to other chemotherapies, for example, pegylated liposomal doxorubicin [21, 22] or trabectedin [23].

Table 51.3 Genetic syndromes associated with an increased risk of ovaria cancer

Syndrome	Gene mutation	Pathologies
Hereditary breast and ovarian cancer	BRCA1 and BRCA2 mutation, HRD positive	Breast, ovarian, fallopian tube, peritoneal, and pancreatic cancer
Hereditary non-polyposis colorectal cancer (Lynch syndrome)	MLH1, MLH3, MSH2, MSH6, tgfbr2, pms1, pms2	Colorectal, endometrial, and ovarian cancer
Peutz-Jeghers syndrome	STH11	Colorectal, stomach, esophageal, small intestine, and ovarian cancer
PTEN hamartoma tumor syndrome (Cowden syndrome)	PTEN	Thyroid, breast, and ovarian cancer
MUTYH-associated polyposis	MUTYH	Colorectal, small intestine, bladder, and ovarian cancer

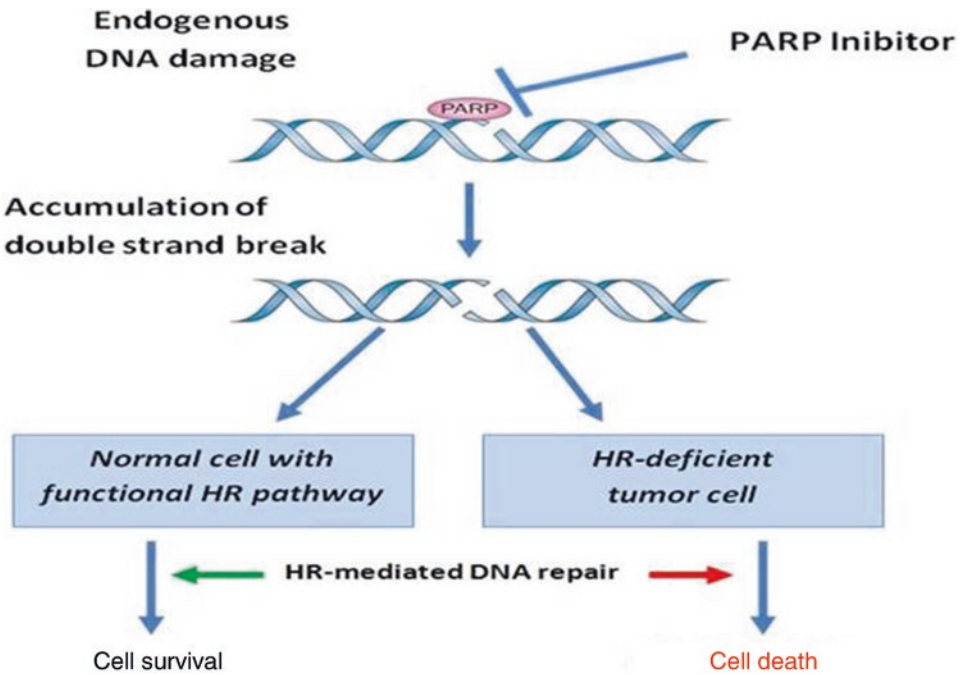


Fig. 51.2 Role of BRCA 1–2 genes

Other genetic syndromes:

Hereditary non-polyposis colorectal cancer (Lynch syndrome) is an autosomal-dominant inherited disorder, associated with several cancers mainly with colorectal and less frequently with gastric, small intestine, endometrial, ovarian and hepatobiliary malignancies [24].

The typical germline mutations are in DNA mismatch repair (MMR) genes MLH1, MLH2, MSH2, MSH6, and PMS2 [25] and only 2–3% of ovarian cancers are attributable to this syndrome [24, 26].

The lifetime risk of ovarian cancer is approximately 10% in patients with MMR gene mutations [25].

A small proportion of ovarian cancer, generally early stage at diagnosis, is diagnosed in women with Li-Fraumeni syndrome, a cancer predisposition characterized by germline mutations in p53 gene.

51.5 Screening

Several studies have been conducted to verify the feasibility of ovarian cancer screening, evaluating both the benefits and the costs of a needless surgery. Systematic pelvic examination, transvaginal ultrasonography, and biomarker levels have been evaluated, but at present, there is no valid screening program for ovarian cancer, because simply there is no test able to anticipate the diagnosis at an earlier stage [27, 28]. The most important screening studies are as follows:

1. PLCO study (USA) compared annual transvaginal ultrasound and 4-month CA125 blood tests for 4 years versus no screening. After a median follow-up of 12 years, no decrease in mortality was reported; in addition, false-positive results led to serious complications after surgery in 15% of women.
2. UKCTOCS study (UK) compared transvaginal ultrasound plus CA-125 (annual multimodality screening) versus ultrasound alone versus no screening. Preliminary results suggested that the multimodality screening is more effective in detecting early-stage tumors; however, after a median follow-up of 11 years, a significant mortality reduction was not observed.
3. Birmingham School of Medicine (USA) randomized 32,000 women to receive transvaginal ultrasound or CA125 evaluation annually versus no screening, without benefit in cancer-related mortality.

Ultrasound and biochemical monitoring are however suggested for patients with familiarity and with BRCA mutations, even though, also in these cases, no benefit in survival was reported.

51.6 Histological Subtypes

Epithelial ovarian tumors are classified according to the WHO histological classification (Fig. 51.3). Recent classification of these tumors considers cell type, degree of malignancy, and infiltration, distinguishing benign lesions from low malignant potential lesions and from malignant invasive carcinomas. Borderline tumors (low malignant potential) do not exhibit stromal invasion and therefore have a good prognosis, while invasive carcinomas, which have a worse prognosis, have a papillary structure, stromal invasion, and high mitotic activity [29].

Epithelial ovarian cancer comprises five main histological subtypes, including high-grade serous carcinoma (HGSC), low-grade serous carcinoma (LGSC), endometrioid carcinoma (EC), clear-cell carcinoma (CCC),

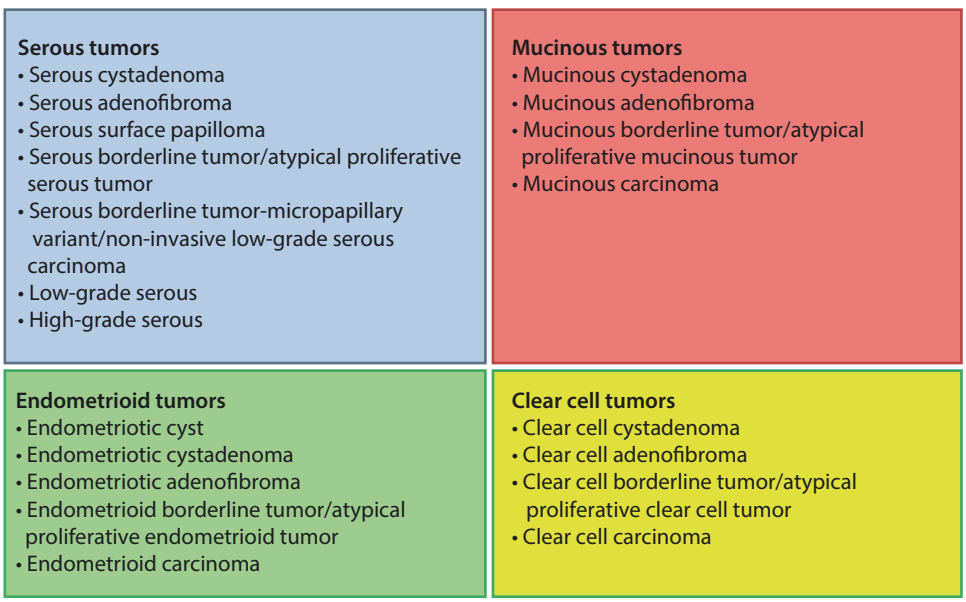
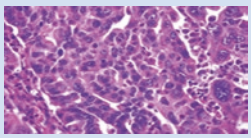
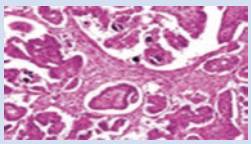
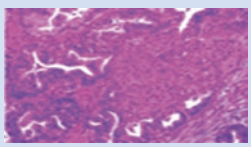
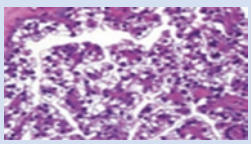
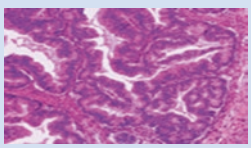


Fig. 51.3 WHO histological classification (2014)

Table 51.4 Tumor histotypes, incidence, and involved genetic pathway

Subtypes	Incidence	Genetic pathway correlated	Microscopic features
High-grade serous carcinoma (HGSC)	70%	TP53: Encodes a protein that regulates the cell cycle BRCA1/2: Encodes proteins that are involved in DNA repair mechanism	
Low-grade serous carcinoma (LGSC)	5%	BRAF KRAS	
Endometrioid carcinoma (EC)	15%	PTEN TP53/BRCA1/2	
Clear-cell carcinoma (CCC)	5%	PTEN ARID1A PIK3CA	
Mucinous carcinoma (MC)	2%	KRAS	

and mucinous carcinoma (MC) [30]. These tumor types really represent different diseases because they are associated with different risk factors (epidemiologic and genetic factors); different incidences, prognosis, and outcomes; different types of response to chemotherapy; and finally different abnormal biomolecular pathways. About 70% of patients have high-grade serous histology, while mucinous and clear-cell carcinomas are extremely rare [31, 32]. Recent histological review distinguished low-grade (grade 1) from high-grade (grade 2 or 3) serous carcinomas recognizing them as different tumor entities (Table 51.4) [33, 34].

51.7 Patterns of Spread of Epithelial Ovarian Cancer

- Lymphatic dissemination to pelvic and para-aortic lymph nodes is common in epithelial ovarian cancer. Retroperitoneal lymphatic dissemination in stage I and II tumors has been reported in 5–20% of cases depending on grade and histology [35]. Spread through the retroperitoneal and diaphragmatic lymphatics can result in metastasis to the supraclavicular lymph nodes; in rare cases, retrograde invasion of inguinal/femoral lymph nodes is reported.
- Direct extension to adjoining organs, like adhesions to the intestine, is frequent, while the involvement of the lumen of the intestine is uncommon.
- Exfoliation of clonogenic cells that directly implant on peritoneal surfaces (pelvis, paracolic gutters, intestinal mesenteries, right hemidiaphragm) is the principal pattern of spread; tumor cells tend to follow the path of circulation of peritoneal fluid from the right pericolic gutter cephalic to the right hemidiaphragm.
- Hematogenous spread is uncommon, with involvement of the liver, lung, CNS, and bone.

Patterns of spread and dissemination of epithelial ovarian cancer are shown in Fig. 51.4.

Various genetic and molecular factors responsible for ovarian carcinoma cell dissemination, able to impact either on peritoneal dissemination or vascular metastasization, have been identified.

The passive dissemination, which impacts on peritoneal involvement, interests numerous enzymes with proteolytic activity (integrin, spheroids, transglutaminases, various interleukins, vascular endothelial growth factor)

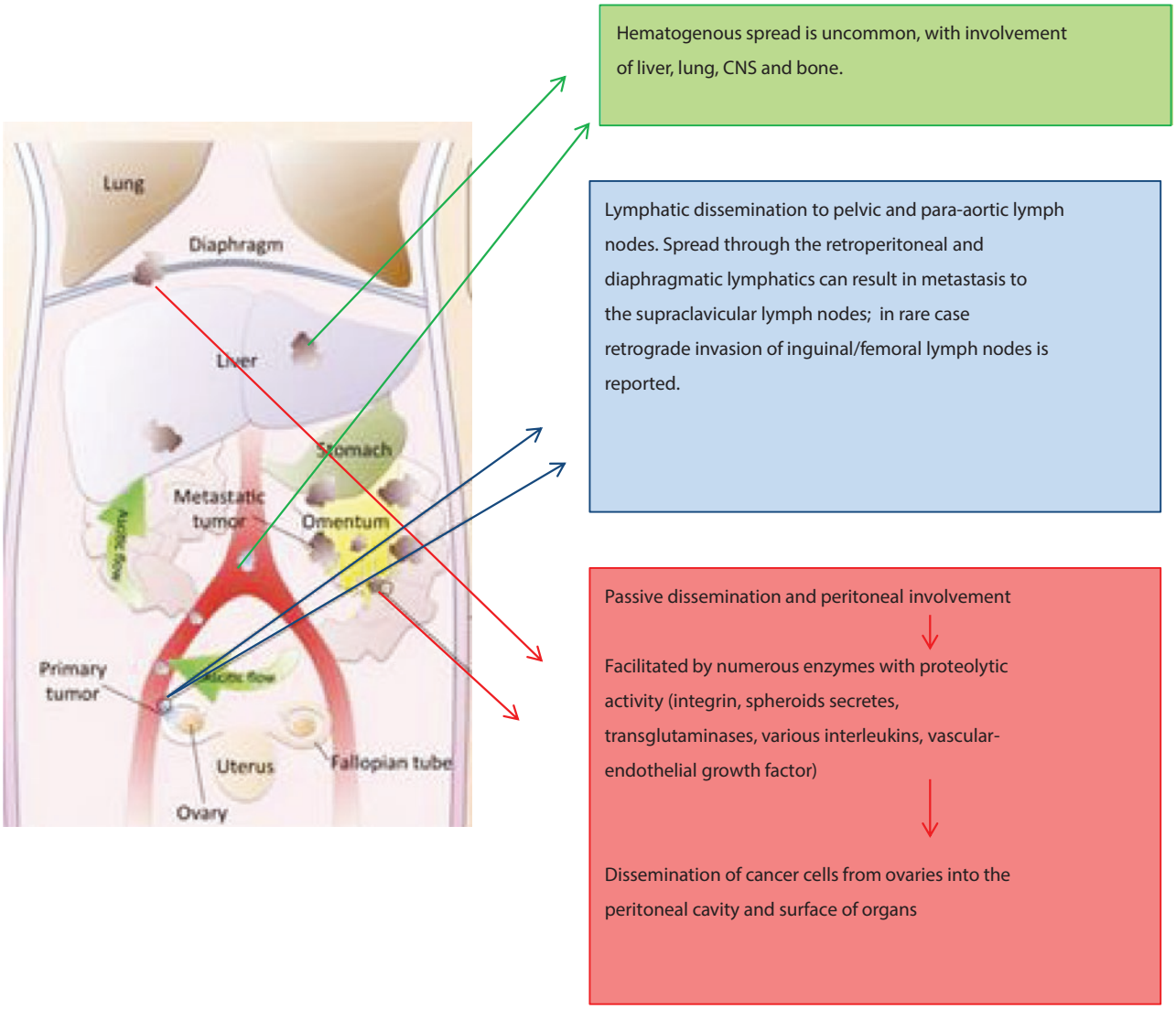


Fig. 51.4 Patterns of spread and dissemination of epithelial ovarian cancer

responsible for dissemination and adhesion of cancer cells into the peritoneal surface [36, 37].

Thereafter, peritoneal implants produce enzymes necessary for new vessel creation. A group of vascular endothelial growth factors (VEGFs) activate vascular and lymphatic endothelium receptors to form new blood and lymphatic vessels with high permeability.

51.8 Diagnosis

Approximately 70% of women with ovarian cancers are diagnosed with advanced disease.

The most common symptoms associated with ovarian cancer are vague and non-specific and include pelvic or abdominal pain, abdominal discomfort, bloating, change in bowel habits, increased abdominal size, dyspepsia and nausea, difficulty eating, early satiety, weight loss, vomiting, and acute abdomen [38].

Rarely ovarian cancer may appear with paraneoplastic syndromes, such as hypercalcemia, thrombophlebitis, Cushing syndrome, and neurologic syndrome with cerebellar ataxia and peripheral neuropathy [39].

The diagnostic evaluation of ovarian cancer is based on:

- Pelvic examination: Gynecologic evaluation with rectovaginal examination is indicated to assess suspicious pelvic or abdominal masses.
- Physical examination: It is indicated, in advanced stage, to assess ascites, superficial lymphadenopathy (generally in supraclavicular and inguinal areas), and pleural effusion.
- Laboratory testing: Cancer biomarkers suggestive of ovarian cancer are CA125 (cancer antigen 125) and HE4 (human epididymis protein 4). CA125 level is elevated in approximately 80% of advanced epithelial ovarian tumors and in 50–60% of patients with early-stage disease [40]. The sensitivity of CA125 in ovarian cancer correlates to tumor stage; specificity

is low because the marker is increased in other benign and malignant disorders, as shown in Table 51.5 [41]. Better positive predictive values of CA125 are reported in postmenopausal women, because of the higher probability of cancer and the lower prevalence of benign lesions after menopause.

Serum HE4 has a better specificity than CA125 because its levels are rarely increased in benign disorders [42] and in premenopausal women [40].

Moore et al. [43] in 2009 developed the Risk of Ovarian Malignancy Algorithm (ROMA) that utilized HE4, CA125, and menopausal status for the prediction of ovarian cancer in patients with pelvic mass. High ROMA score is associated with a greater risk of ovarian cancer and, since from 2012, it is used to differentiate benign and malignant ovarian masses.

Imaging techniques: Transvaginal ultrasound (TVU) is an important diagnostic tool in the evaluation of patients with a pelvic mass. The typical sonographic finding of malignancy is a "complex" cyst, defined as containing cystic and solid components; presence of septa and papillae can also be observed (Fig. 51.5 and Table 51.6). Although, TVU is able to evaluate ovarian architecture and mass vascularization and to detect ascites, the sensitivity and specificity in distinguishing benign from malignant adnexal lesions varies from 86% to 94% and 94% to 96%, respectively [44].

CT scan and MRI are generally used to evaluate the peritoneal and lymph node extension in women with suspected ovarian cancer. Moreover they are useful for differential diagnosis with other abdominal neoplasms and are important for planning the type of surgery. Chest study, with X-ray or CT scan, is essential for identifying a pleural effusion.

Table 51.5 Disorders associated to elevated CA125 levels

Benign disorders	Malignant disorders
Pelvic mass correlated Ovarian hyperstimulation syndrome Meigs syndrome	Primary pelvic tumor Ovarian cancer Uterine cancer (advanced stage) Fallopian-tube cancer (advanced stage) Rectal or bladder cancer (advanced stage)
Non-pelvic mass associated Pancreatitis Nephrotic syndrome Liver failure Peritonitis	Secondary pelvic association Peritoneal metastasis in breast cancer Pancreatic carcinoma Peritoneal metastasis in gastric cancer (Krukenberg) Lymphoma

Table 51.6 Typical sonographic finding of benign and malignant masses

Benign mass	Malignant mass
Unilocular cysts	Irregular solid tumor
Presence of solid components <7 mm	Presence of ascites
Presence of acoustic shadowing	At least four papillary structures
Smooth multilocular mass with largest diameter <100 mm	Irregular multilocular solid tumor with largest diameter >100 mm
No blood flow (color score 1)	Very strong blood flow (color score 4)

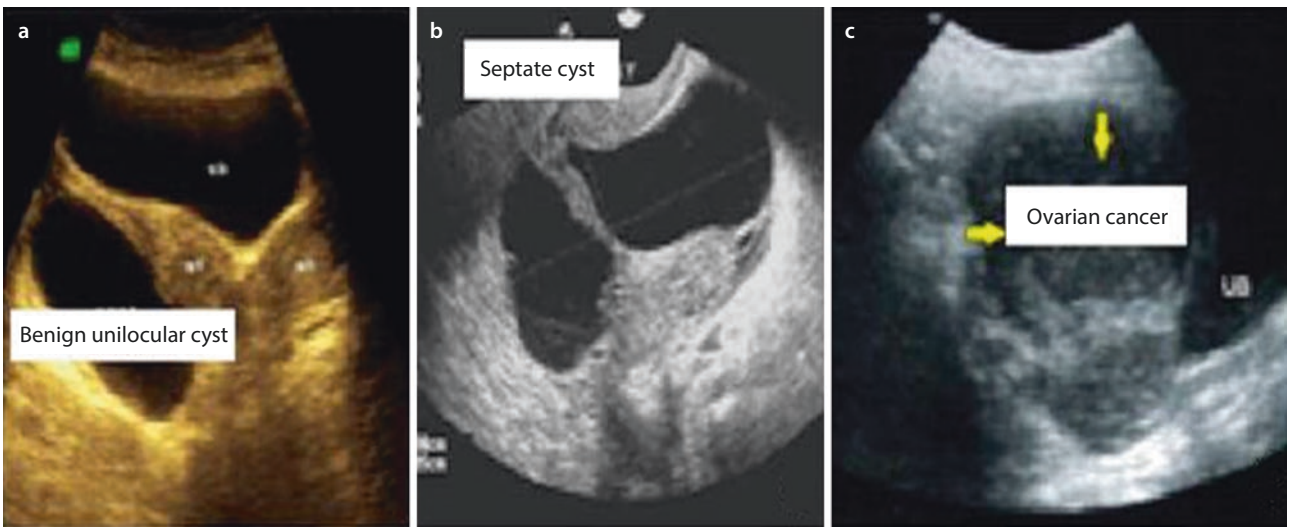


Fig. 51.5 **a** Benign unilocular cyst. **b** Septate cyst. **c** Ovarian cancer

The value of positron emission tomography (PET) has been recently studied. Hypermetabolic lesions are often associated with adnexal malignancies, but several false-positives (follicular cysts or benign cystadenomas) have been identified in pre-menopausal women. PET positivity in postmenopausal patients is always suspicious and must be investigated. However, several studies have documented a sensitivity and specificity inferior to other techniques such as CT scan or MRI (58% and 78%, respectively) and in consequence PET scan is not routinely used [45].

More recently, the role of 18-F-FDG-PET-CT (positron emission tomography CT scan) has been investigated as a more accurate method to characterize adnexal masses. The studies appear very encouraging, demonstrating a superior sensitivity with respect to other techniques (93% and 77% vs. 96% and 38% for PET-CT scan and CT scan alone, respectively) [46]. Unfortunately, a significant percentage of false-negative (borderline tumors, low-grade serous carcinomas, mucinous and clear-cell carcinomas) and false-positive (myomas or corpus luteum) does not advice a routine use of this method.

51.9 Prognostic Factors

Prognostic factors in ovarian cancer are:

- *Ethnicity and race*: At the same stage of diagnosis, Afro-American patients have a 30% greater risk to die when compared to Caucasian women [47].
- *Age:* Younger patents have a survival rate higher than older population across all stages (75% vs. 40%, respectively) [48].
- *Performance status* is an independent prognostic factor: Patients with good PS have a better tolerance to treatments (both surgery and chemotherapy) [49, 50].
- *FIGO stage:* Most powerful predictor of prognosis and most important factor influencing survival (as shown in Table 51.7) [51]. Careful surgical staging is crucial to address appropriate treatment and assure better survival.
- *In early stage* the most important factors correlating with poor prognosis are histopathological subtype (serous vs. other histotypes), grade of differentiation (G1-G2-G3), and cyst rupture (spontaneous before surgery or during surgery) [52, 53].
- *Grading* is particularly important for stage I disease and distinguishes three subcategories [54]:
  1. Low risk (good prognosis) → FIGO stage IA, grade 1 with 5-year overall survival >90%
  2. Intermediate risk(FIGO stage IA G2, or IB and IC G1)
  3. High risk (poor prognosis) → (FIGO stage IA grade 3, IB or IC grade 2–3, FIGO stage II, any clear-cell carcinoma) with 5-year overall survival of 50–60%
- *Genetic factors:* Genetic predisposition linked to BRCA1/2 mutation genes is associated with a better prognosis [55]. In several studies, BRCA mutations have been correlated with younger age and improved response to antitumoral treatment, mainly PARP inhibitor [56] and platinum-based chemotherapy [18, 20] but also pegylated liposomal doxorubicin (PLD) [21, 57] or trabectedin [23, 58].
- *Immunologic factors*: Presence of tumor-infiltrating lymphocytes and higher expression of immune signature are considered as good prognostic factors. Immunohistochemical studies demonstrated that the elevated expression of PD-1 and PD-L1 and the elevated concentration of CD3+ and CD8+ TILs, mostly in HR-deficient tumors, were independent positive prognostic factors [59].

Table 51.7 Correlation between FIGO stage and 5-year overall survival

Stage FIGO	5-year overall survival
I	70–90%
II	50–60%
III	20–40%
IV	10%

51.10 Treatment

The treatment of ovarian cancer is based on the stage of disease which is the reflection of the extent and spread of the cancer. There are generally three approaches for the treatment of ovarian cancer: surgery, chemotherapy, and, only in selected cases, radiation treatment.

51.10.1 Surgery

Surgery is the primary treatment for ovarian cancer. It is used for diagnosis, staging (according to FIGO system), and treatment with the intent of maximal cytoreduction [60]. A small rate of patients with early-stage disease is treated with surgery alone. In all other cases, systemic chemotherapy is added. The standard surgical management of early-stage invasive ovarian cancer consists of:

- Peritoneal washings
- Intact tumor removal
- Total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH/BSO), only unilateral salpingo-oophorectomy (USO) in selected cases
- Infracolic omentectomy
- Random peritoneal biopsies

- Biopsy of all adhesions and suspicious lesions
- Bilateral pelvic and para-aortic lymph node sampling

A midline vertical incision is critical for an adequate exploration of abdomen. The pelvis and upper abdomen and specifically all peritoneal surfaces (liver, stomach, spleen, large and small bowel, and diaphragms) are carefully explored to identify metastatic implants.

Any ascites is collected for cytology. If no ascites, peritoneal washings should be obtained.

Laparoscopy Several studies have investigated the safety of minimally invasive laparoscopic approach (with the same intra-abdominal procedures) for staging and treatment of early-stage ovarian cancer, mainly to reduce hospital stay and postoperative complications (reduced blood loss, fewer infections) [61, 62]. A systematic review [63] demonstrated that laparoscopy is associated with several disadvantages such as a higher rate of intraoperative cyst rupture and port-site metastasis [64, 65, 66] so it is only considered in experienced centers.

Conservative management of patients desiring to preserve fertility (fertility-sparing surgery) This approach can be considered for young and nulliparous woman with unilateral, low-risk tumors (stage IA or stage IC with grade 1 or 2 and favorable histology [67]. Fertility- sparing surgery includes unilateral salpingo-oophorectomy (preserving the uterus and contralateral ovary) after careful exploration and biopsies of abdominal cavity to exclude metastatic disease, lymphadenectomy, and curettage of the uterine cavity to exclude a synchronous endometrial tumor [60].

The rate of microscopic disease (including positive node, cytology, peritoneal and omental metastases), in apparently EOC, is up to 25% [68]. A recent GOG review on early- stage high-risk ovarian cancer established a 5-year recurrence and overall survival of 75.5% and 81.7%, respectively [12]. Thus surgical restaging in apparent stage I tumors may help in identifying patients requiring adjuvant treatment and is an independent prognostic factor.

51.10.2 Adjuvant Chemotherapy

Systemic adjuvant chemotherapy for early-stage disease is recommended in all patients with high-risk tumors stage IA and IB grade 3 or IC any grade serous, endometrioid, and mucinous and for all stages of clear-cell carcinomas.

The optimal adjuvant therapy for intermediate-risk group (FIGO stage IA G2, IB and IC G1) and high-risk group (FIGO stage IAG3, IB G2-G3, IC G2-G3, and clear cell) had not yet been established until 2003 when solid scientific proof of the clinical effectiveness of adjuvant chemotherapy was provided.

Adjuvant Trials

In 2003 two large prospective randomized trials (ICON1 and ACTION) [69, 70, 71] and two relevant meta-analyses [72] demonstrated that women who received adjuvant platinum-based chemotherapy had better overall survival and progression-free survival than those who did not.

ICON1: In this trial patients with FIGO stage I–II ovarian cancers requiring adjuvant chemotherapy were randomized to receive platinum-based chemotherapy or observation. Carboplatin (AUC5) for six cycles was administered in most patients (87%). The trial reported a significant benefit in OS (72% vs. 64%) and PFS (70% vs. 60%) for chemotherapy-treated patients versus the observation arm [73]. Subgroup post hoc analysis suggested that high-risk patients (IA G3, IB or IC G2 or G3, clear cell) benefit more from adjuvant chemotherapy.

ACTION: Patients with stage IA and IB G2-G3, all stage IC, and stage IIA, after intensive surgical staging, were randomized to receive platinum-based chemotherapy (47% cisplatin plus cyclophosphamide and 33% single-agent carboplatin) for at least four cycles versus no treatment. The final results reported a significant benefit for chemotherapy-treated patients in terms of PFS (HR 0.63) and OS (HR 0.69). In a subgroup analysis, significant advantages in terms of OS and PFS were identified in sub-optimally staged patients, whereas, among optimally staged patients, there was no significant difference in survival outcomes.

The analysis of the combined trials [70, 71], a Cochrane review [54], and a relevant meta-analysis [74] confirmed the amelioration of survival and PFS for patients receiving adjuvant chemotherapy despite the non-uniformity of data on the type of surgery, the number of cycles, and the type of chemotherapy.

Type of Chemotherapy

The standard treatment for early-stage disease is carboplatin-based chemotherapy. Single agent or combination is a controversial issue. Literature data present several limitations (different chemotherapy regimens, retrospective data, small number of patients, different surgical approaches, and different postoperative residuals). Specifically, three retrospective trials have compared platinum monotherapy versus platinum-paclitaxel combination in early-stage ovarian cancer [75, 76] suggesting no significant advantage in terms of recurrence and deaths for the combined treatment at the price of a higher toxicity. Despite controversial data, according to published guidelines of the 4th Ovarian Cancer Consensus Conference [2], carboplatin-paclitaxel remains the standard treatment for early-stage disease. Carboplatin alone is a reasonable alternative for patients with poor performance status and comorbidity and, probably, for intermediate-risk disease.

Duration of Treatment

The optimal number of cycles of adjuvant chemotherapy in early-stage ovarian cancer is not defined. ACTION and ICON trials have demonstrated identical benefit when using four or six courses of platinum-based chemotherapy. The GOG 157 trial [77], comparing three versus six cycles of platinum-paclitaxel chemotherapy, has demonstrated no significant difference in recurrence rate (25% and 20%, respectively) with a higher risk of toxicity in the six-cycle arm. The authors concluded that three cycles of carboplatin-paclitaxel chemotherapy could be considered a sufficient number of cycles in early stage. A subgroup analysis of the same study [78] showed a significant reduction of the risk of recurrence with six cycles of chemotherapy for serous histotypes, while no benefit was reported for other histotypes with a longer treatment.

Viewpoint

The objective of future research should be to identify possible prognostic and predictive factors able to identify which patients with EOC can benefit from adjuvant chemotherapy. Several studies [79, 80, 81] have shown that DNA ploidy is an independent prognostic factor in early-stage disease distinguishing poor from good prognosis patients and able to separate patients who do not require adjuvant chemotherapy. Other ongoing studies attempt to identify molecular markers, serum protein patterns, gene expression, and microarray profiles with prognostic and predictive roles [82, 83, 84].

51.10.3 Radiotherapy

Radiotherapy is not generally used in the management of patients with early-stage ovarian cancer. Two randomized phase II studies [85, 86] have investigated the role of pelvic radiotherapy in stage I epithelial ovarian cancer, comparing radiotherapy with no postoperative treatment. The trials suggested that pelvic irradiation presents severe toxicity and could reduce the rate of pelvic relapses but does increase OS, because relapses occurred generally in the peritoneal cavity. Otherwise, for clear-cell carcinomas, a mono-institutional study [87] reported a significantly higher 5-year OS and PFS in women with stage I to III OCCC when treated with adjuvant whole abdominal radiation (WAR) probably because the majority of cases are confined to the pelvis and because the disease is generally chemoresistant. On the contrary a recent study [88] has not reported a survival benefit for patients with stage I and II ovarian clear-cell carcinoma treated with adjuvant RT.

Summary of Clinical Recommendations

Chemotherapy recommendations based on risk group:

- Low-risk group: FIGO stage IA and IB grade 1 → chemotherapy is not recommended; patients in this stage have an excellent prognosis without adjuvant treatment.
- Intermediate-risk group: FIGO IA G2, IB G1–G2, IC G1 → the advantage of chemotherapy (carboplatin AUC5-7.5 g1 q 21 single agent or carboplatin plus paclitaxel 175 mg/mq g1 q 21 for three to six cycles) is minimal and this option is to be discussed with patients.
- High-risk group: FIGO stage IA G3, IB G3, IC G2–G3, FIGO stage II, any clear-cell carcinoma: Adjuvant chemotherapy is recommended with carboplatin AUC5-7.5 g1 q 21 plus paclitaxel 175 mg/mq g1 q 21 for three to six cycles; single-agent carboplatin AUC5 is considered for patients with contraindication for doublets.

Case Study 1: Management of Solid Ovarian Masses

36-year-old female, G2P2.

- Family history: Mother (deceased) for endometrial cancer.
- *APR:* No prior history of pelvic infections or abnormal Pap tests.
- *APP:* In the last 3 months, pelvic pain, worsening with defecation.
- Pelvic examination revealed a solid pelvic mass.

Question

What action should be taken?

(1) Surgery. (2) Pelvis transvaginal ultrasound. (3) PET-FDG

Answer

Pelvic ultrasound showed an enlarged right ovary containing a 2.8 × 2.8 cm heterogeneous mass (hypoechoic cystic with a solid component). The image demonstrates increased vascularity within the solid component of the right ovarian mass (suspicion for malignancy).

Question

What action should be taken?

(1) MRI. (2) Surgery. (3) Follow-up

Answer

MRI showed an enlarged right ovary plus peripheral follicles with heterogeneous enhancement. Pelvic free fluid is also apparent.

- CT scan: No evidence of distant metastasis.
- CA125 level was normal.

Question

What action should be taken?

(1) Surgery. (2) Chemotherapy. (3) Radiotherapy

Answer

Monolateral right oophorectomy was performed with frozen section: The histologic report was a serous high-grade ovarian cancer. Immediately contralateral salpingo-oophorectomy, total abdominal hysterectomy, omentectomy, peritoneal washings, random peritoneal biopsies, and bilateral pelvic and para-aortic lymph node sampling were performed → The final pathologic report: tumor is limited to one ovary; the capsule is intact, no tumor on ovarian surface. No malignant cells are present in peritoneal washings in the peritoneum or lymph nodes (FIGO stage IA grade 1).

Question

What action should be taken?

(1) Follow-up. (2) Chemotherapy. (3) Radiotherapy

Answer

Follow-up

Low-risk group: FIGO stage IA and IB grade 1 → Chemotherapy is not recommended; patients in this stage have an excellent prognosis without other treatments.

Key Points

- Multidisciplinary consultation in the primary management of EOC
- Role of surgery for adequate diagnosis and staging

Case Study 2: Management of Solid Ovarian Masses

46-year-old female

- *APR and APP:* No prior history of gynecologic disorders
- During routine gynecological examination, the gynecologist revealed a pelvic mass with complex characteristics by transvaginal ultrasound

Question

What action should be taken?

(1) Surgery. (2) Follow up. (3) Consultation at a reference center

Answer

The patient was referred to a gynecological center where she received accurate work-up by:

- Transvaginal ultrasound (TVU) → Irregular solid mass with papillary structures and high-density vascularization
- Laboratory testing → CA125 and HE4 at normal levels
- CT scan: Negative for secondary lesions
- Pelvic MRI: Suspicious implants on the uterus and homolateral fallopian tube

Question

What action should be taken?

(1) Surgery. (2) Chemotherapy. (3) Radiotherapy

- The patient underwent surgery → Mass removal with frozen section intraoperative examination was performed. The histology report was suggestive for an epithelial ovarian tumor. The patient underwent complete surgical staging with bilateral salpingo-oophorectomy, total abdominal hysterectomy, omentectomy, peritoneal washings, random peritoneal biopsies, and homolateral pelvic and para-aortic lymphadenectomy → The final pathologic report was ovarian endometrioid carcinoma G3 with implants on the uterus (FIGO stage IIB).

Question

What action should be taken?

(1) Chemotherapy. (2) Follow-up. (3) Radiotherapy

Answer

- In patients with FIGO stage II, classified as high risk, adjuvant chemotherapy is recommended with carboplatin AUC5-7.5 g1 q 21 plus paclitaxel 175 mg/mq g1 q 21 for six cycles.

Key Points

- Surgery has a key role in early-stage tumors for diagnosis and adequate staging.
- High-risk patients should receive adjuvant chemotherapy.
- Suspicious mass should be referred to referral centers for ovarian cancer treatment where adequate preoperative work-up and surgical procedures can be performed.

Expert Opinion

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Key Points

1. Ovarian cancer is a neoplasm affecting female people with a median age of 60 years old; incidence has increased in the last years, but on the contrary mortality has decreased. The main risk factors are age, genetic predisposition, obesity, and long-term postmenopausal oestrogen therapy. Some genetic syndromes have been associated with the onset of ovarian cancer such as Lynch syndrome and hereditary breast and ovarian syndrome (BRCA1/2).
2. Almost 70% of women are diagnosed with an advanced disease; symptoms are vague and nonspecific, such as abdominal pain, bloating, weight loss, nausea, vomiting, or acute abdomen. The diagnostic assessment is based on pelvic and physical examination, evaluation of Ca125 and HE4 (ROMA Index), US, CT, MRI, and 18F-FDG-PET. Up to date, no screening programs are useful for an early diagnosis, even if ultrasound (US) and biochemical monitoring is suggested for patients with familiarity and BRCA mutations.
3. The main classification schemes are the FIGO and the AJCC staging systems. There are several histological subtypes, such as high-grade serous carcinoma (70% of types), low-grade serous carcinoma, endometroid carcinoma, clear-cell carcinoma, and mucinous carcinoma. Each subtype has different features, and prognosis varies according to the histological group.
4. Ovarian cancer can spread to other organs thanks to different mechanisms: lymphatic dissemination, direct extension to adjoining organs, exfoliation of clonogenic cells that directly implant on peritoneal surfaces and hematogenous spread, which is actually uncommon.
5. Treatment is based on the stage of the neoplasm and it can consist in surgery, chemotherapy, and in selected cases, radiotherapy. Surgery has a diagnostic, staging, and therapeutic intent. A small percentage of patients with early stage are treated with surgery alone; otherwise, chemotherapy is usually added. Carboplatin-paclitaxel remains the standard for early stage disease. Carboplatin alone can be used in patients with comorbidity, poor performance status, and maybe intermediate risk disease.

Recommendations

- ESMO
  ► https://www.esmo.org/Guidelines/Gynaecological-Cancers/ESMO-ESGO-Consensus-Conference-Recommendations-on-Ovarian-Cancer
- AIOM

Hints for a Deeper Insight

- Laparoscopy versus laparotomy for FIGO stage I ovarian cancer: ► https://www.ncbi.nlm.nih.gov/pubmed/27737492
- Newly Diagnosed and Relapsed Epithelial Ovarian Carcinoma: ESMO Clinical Practice Guidelines: ► https://www.esmo.org/Guidelines/Gynaecological-Cancers/Newly-Diagnosed-and-Relapsed-Epithelial-Ovarian-Carcinoma
- Staging classification for cancer of the ovary, fallopian tube, and peritoneum: ► https://www.ncbi.nlm.nih.gov/pubmed/24219974
- 2010 Gynecologic Cancer InterGroup (GCIG) consensus statement on clinical trials in ovarian cancer: report from the Fourth Ovarian Cancer Consensus Conference: ► https://www.ncbi.nlm.nih.gov/pubmed/21543936
- Adjuvant (post-surgery) chemotherapy for early stage epithelial ovarian cancer: ► https://www.ncbi.nlm.nih.gov/pubmed/22419298

Surgery for Ovarian Cancer

- Surgery is the corner stone of treatment of ovarian cancer. It enables the clinician to confirm the diagnosis histologically, to assess the extent and spread of the disease, and to attempt resect all visible tumor if possible. The concept of cytoreduction was introduced by Griffiths in 1975 and has been validated in several subsequent studies. In patients with advanced epithelial ovarian cancer, surgery is used in conjunction with chemotherapy consisting of a taxane and platinum compound. In most cases, six cycles of paclitaxel and carboplatin are given. Patients with early ovarian cancer often do not require adjuvant chemotherapy. The optimal type of surgical access to the abdomen depends upon the clinical presentation. In young patients who want to preserve their fertility with a tumor of less than 10 cm diameter, which seems to be limited to the ovary(ies), a laparoscopic approach can be considered. In patients with larger tumors and disseminated disease, a (midline) laparotomy is preferred. For patients with no apparent extra-ovarian

disease, adequate surgical staging is mandatory as microscopic metastasis can be found in up to 25% of cases, which is an indication for additional chemotherapy. The staging procedure should include inspection of the entire peritoneal cavity, multiple peritoneal biopsies, an (infracolic) omentectomy, a pelvic and paraaortic-lymph node sampling. In patients who do not want to become pregnant anymore and in patients with advanced disease the ovaries, fallopian tubes and uterus are removed. An appendectomy is performed in case of a mucinous tumor. If extra-ovarian disease is visualized, the surgeon should aim to remove all macroscopic tumor. This may require extensive surgery including resection of large- and/or small bowel, excision of implants of peritoneal or liver surface, a splenectomy, etc. Organ resections are performed in about 30% of patients in order to remove all macroscopic disease. Complete surgical resection rates of epithelial ovarian cancer are higher if the surgery is performed by a specialized gynecological oncologist or in high-volume hospitals, but also depend on the biology of the disease (e.g., higher for endometrioid carcinoma). Survival of patients is clearly better after a complete (optimal) debulking. In expert centers, this can be achieved in 70–85% of patients with advanced disease.

- If peri-operative assessment shows that not all macroscopic disease can be removed surgically, interval debulking surgery should be considered. This may be particularly the case in patients with a high upper abdominal tumor load and extensive peritoneal carcinomatosis with a lot of ascites. These patients should receive three to four cycles of chemotherapy after their initial diagnostic and/or staging procedure. Approximately 60% of these patients can have a successful optimal interval cytoreductive operation. Postoperatively these patients are treated with three cycles of additional chemotherapy. A prospective randomized trial showed that the survival of these patients was significantly improved by the second attempt to remove disease. A meta-analysis found no survival benefit of interval debulking surgery compared to primary surgery, but noted a better survival in patients whose primary surgery was not performed by gynecologic oncologists or who had primary suboptimal surgery. The current consensus is that patients should have at least one surgical attempt to remove all visible disease by an experienced team. If possible this should be performed as primary procedure but if not feasible an interval debulking operation should be performed.
- The role of hyperthermic intraperitoneal chemotherapy (HIPEC) in conjunction with cytoreductive surgery remains controversial for patients with ovarian cancer. HIPEC is a highly concentrated, heated chemotherapy treatment that is delivered directly to the abdomen during surgery. A recent Dutch and Korean randomized study gave contradictory results, so the final word remains to be said about this indication. Secondary cytoreductive surgery in patients with recurrent ovarian cancer is safe and effective in selected cases. Preferentially the patients should have platinum-sensitive disease, a disease-free interval of more than 24 months after primary treatment and optimal cytoreduction.

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Ovarian Cancer: Primary Advanced and Recurrent Disease

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Learning Objectives

By the end of the chapter, the reader will:

- Be able to apply medical and surgical procedures in the management of primary advanced and recurrent EOC
- Have learned the basic concepts and the clinical indications to primary and secondary cytoreductive surgery
- Have reached in-depth knowledge of recurrence treatment strategy in order to build an algorithm that allow patients to receive all the available treatment options, possibly in the more appropriate temporary order
- Be able to apply acquired knowledge about molecular and genetic characteristics of ovarian cancer to ameliorate treatment options and targeted therapy approaches

52.1 Introduction

About 70% of patients with epithelial ovarian cancer (EOC) are diagnosed at advanced International Federation of Gynecology and Obstetrics (FIGO) stage III or IV disease, with a 5-year survival that ranges from 39% to 17% [1].

Generally, more than 70% of women with advanced disease obtain a complete clinical and instrumental remission at the completion of primary treatment, but unfortunately 50–70% of them will develop a recurrence after a median PFS of approximately 18 months [2].

In the last years, the survival rate of patients with advanced and recurrent ovarian cancer has increased thanks to the improvement of surgery and the utilization of novel antitumoral agents. Moreover, a significant increase in the knowledge of molecular and genetic characteristics of ovarian cancer has led to the improvement of treatment options including targeted therapies.

52.2 Epidemiology, Diagnosis, Pathogenesis, and Prognosis

Ovarian cancer typically spreads to peritoneal surfaces and the omentum and can diffuse by local extension, lymphatic invasion, intraperitoneal implantation, hematogenous dissemination, and transdiaphragmatic passage. Intraperitoneal dissemination is the most common: malignant cells can spread anywhere in the peritoneal cavity but are more likely to implant in sites of stasis along the peritoneal fluid circulation (e.g., pelvis, paracolic gutters, intestinal mesenteries, right hemidiaphragm).

When disease spreads beyond the ovaries, determining an advanced stage, patients experience persistent but not specific symptoms such as abdominal bloating, constipation, digestive difficulties, nausea, loss of appetite, sense of pelvic weight, or lower back pain. Patients with advanced disease are instrumentally evaluated with CT scan, PET-FDG, or MRI in order to assess site, size, and distribution of metastases particularly for preoperative appraisal of resectability.

Preoperative serum Ca125 level is elevated in 75% of cases; it frequently reflects the burden of disease and does not appear to be predictive of survival. The postoperative and during chemotherapy reduction of Ca125 value is associated with a more favorable outcome.

Recently, Zeng et al. reported that normalization of Ca125 levels after three cycles of neoadjuvant chemotherapy is associated with more favorable outcomes, as well as achievement of a Ca125 nadir equal or less than 10 U/L after completion of treatment [3].

Other prognostic and predictive factors are [4]:

- FIGO tumor stage
- Age
- Histology (mucinous and clear cell histotypes are associated with poor prognosis than other histotypes)
- Grade of differentiation
- Performance status
- BRCA mutational status
- Residual tumor after cytoreductive surgery
- Markers of proliferation and growth factors receptors (Bcl-2, EGFR, GST, LRP, p16, p21, P-pg, and TNF-α)
- Expression of genes associated with metastasization

52.3 Staging of Ovarian Cancer

Ovarian cancer is staged according to the International Federation of Gynecology and Obstetrics (FIGO) surgical staging system (Table 52.1) [5].

52.4 Primary Treatment of Advanced Disease

Management of primary advanced disease includes:

- Primary cytoreductive surgery or neoadjuvant chemotherapy (NACT) followed by interval debulking surgery (IDS)
- Chemotherapy (intravenous chemotherapy or intraperitoneal chemotherapy)
- Maintenance therapy

Table 52.1 FIGO staging of advanced ovarian cancer (2014)

Stage III	**Tumor involves one or both ovaries with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lymph nodes**
IIIA	Positive retroperitoneal lymph nodes and/or microscopic metastasis beyond the pelvis IIIA1 positive retroperitoneal lymph nodes only IIIA1(i) metastasis ≤10 mm IIIA1(ii) metastasis >10 mm
IIIA2	Microscopic, extrapelvic (above the brim) Peritoneal involvement ± positive Retroperitoneal lymph nodes
IIIB	Macroscopic, extrapelvic, peritoneal Metastasis ≤2 cm ± positive Retroperitoneal lymph nodes. Includes Extension to capsule of the liver/spleen
IIIC	Macroscopic, extrapelvic, peritoneal Metastasis >2 cm ± positive Retroperitoneal lymph nodes. Includes Extension to capsule of the liver/spleen
Stage IV	**Distant metastasis excluding peritoneal metastasis**
IVA	IVA pleural effusion with positive cytology
IVB	Hepatic and/or splenic parenchymal Metastasis, metastasis to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside of the abdominal cavity)

52.4.1 Primary Debulking

The standard treatment of patients with advanced EOC is radical cytoreductive surgery followed by platinum-based chemotherapy for six to eight cycles [6]. The role of primary surgery is to provide histological diagnosis, stage the disease, and provide, when possible, a complete tumor debulking [7, 8].

The primary debulking surgery (PDS) involves:

- Total abdominal hysterectomy (TAH)
- Bilateral salpingo-oophorectomy (BSO)
- Peritoneal washing
- Omentectomy
- Biopsies of peritoneal surfaces
- Pelvic and para-aortic lymphadenectomy of bulky nodes
- Removal of all visible lesions and biopsies of any suspected areas

Furthermore, other more invasive procedures, such as splenectomy, bowel resection, partial hepatectomy/gastrectomy, or cystectomy, are frequently required to adequately and radically debulk all visible disease [9, 10].

Primary optimal cytoreductive surgery is considered an essential step in the management of advanced ovarian cancer because it confers better outcome and prognosis to patients [11]. Another important role of primary surgery is to remove large necrotic lesions promoting drug failure and chemoresistance; furthermore, removing bulky intra-abdominal lesions ameliorates patient symptoms decreasing the risk of bowel obstruction or perforation [12].

The success of debulking surgery depends on various aspects, such as patient performance status, extension of disease, and, probably the most important one, surgeon expertise [13].

The objective of debulking surgery is to achieve maximal cytoreduction by removing all visible disease; in general, cytoreductive surgery is defined as optimal when all macroscopic disease is resected and is defined optimal when the largest residual tumor after procedure is less than 1 cm in maximum diameter [14]. Residual disease at primary surgery and outcome are strictly related; in this context, all studies report a statistical survival advantage in patients with ≤1 cm residual disease compared to patients with residual disease >1 cm [11, 15].

Systematic Lymphadenectomy

Actually, the standard management of stage IIIC–IV ovarian cancer involves removal of pelvic and para-aortic lymph nodes only if clinically suspicious. Systematic lymphadenectomy of non-suspicious nodes during primary surgery has recently been discouraged because of the lack of evidence for therapeutic role [16].

An Italian perspective randomized trial evaluated FIGO stage IIIB–IIIC and IV EOC patients to receive systematic pelvic and para-aortic lymphadenectomy at primary debulking surgery versus removal of only bulky nodes. The final results showed an improvement in progression-free but not in overall survival in patients who had undergone systematic lymphadenectomy [17].

The recently published results of a prospective randomized AGO trial (LION), which investigated the role of systematic pelvic and para-aortic lymphadenectomy versus no lymphadenectomy in FIGO stage IIB–IV epithelial ovarian cancer patients achieving complete intraperitoneal debulking during primary surgery, showed no improvement in overall and progression-free survival in patients subjected to systematic lymphadenectomy.

Recent international guidelines do not recommend systematic lymphadenectomy other than the removal of suspicious and/or enlarged nodes in patients with advanced EOC [18, 19].

Ultra-radical Surgery

The criteria of "extension of debulking" evolved in the last years since postoperative residual tumor is considered the most important survival prognostic factor.

Unanimously recent international guidelines recommend the maximum surgical effort at primary cytoreduction, with the goal of no macroscopic residual disease [20].

Ultra-radical surgery is defined as an aggressive approach aiming to obtain the complete macroscopic resection of all visible disease. The ultra-radical debulking is comprehensive of the already described surgical procedures with eventually bowel resection, splenectomy, cholecystectomy, caudal pancreatectomy, large stripping of the peritoneum, and diaphragm or hepatic resection, if involvement is demonstrated. Retrospective data suggest that patients receiving aggressive surgical debulking have a significant improved survival when compared to those with suboptimal cytoreduction at the price of increased complications [9, 10, 21].

Neoadjuvant Chemotherapy (NACT)

Neoadjuvant chemotherapy is defined as a treatment given before surgery (defined as interval debulking surgery or IDS) to reduce tumor dimensions and increasing surgical radicality. The objective of NACT is to increase the complete resection rate at interval debulking surgery and decrease perioperative morbidity and mortality. This approach is typically considered an option for patients with stage IIIC and IV EOC who are not good candidates to upfront surgery for several reasons (comorbidities, poor performance status, high perioperative risk, low possibility of optimal cytoreduction, or non-removable sites of metastasis) [22]. Generally patients receive three cycles of carboplatin-paclitaxel chemotherapy, and subsequently, if there is evidence of response, they undergo interval debulking surgery followed by additional three cycles of the same chemotherapy.

Actually, information about the therapeutic role of NACT followed by interval debulking surgery is controversial. The approach is supported by two phase III international randomized trials, EORTC 55971 and CHORUS studies, which compared NACT followed by interval debulking surgery to primary debulking surgery followed by adjuvant chemotherapy in stage IIIC–IV patients with potentially resectable disease. The final results showed no difference in PFS and OS in both arms with less morbidity in patients receiving NACT [23, 24]. Both studies have been criticized because of the scanty median OS, the slow mean operative time, and the rates of optimal cytoreduction in the primary surgery arm (Fig. 52.1).

A recently published meta-analysis showed that neoadjuvant chemotherapy helps the gynecologic surgeons to achieve an increased rate of optimal cytoreduction, while a meta-analysis by Bristow et al. showed a negative survival effect in patients undergoing interval debulking surgery compared with those receiving primary surgery [25].

Therefore, the choice between primary cytoreductive surgery and NACT remains unclear. A position

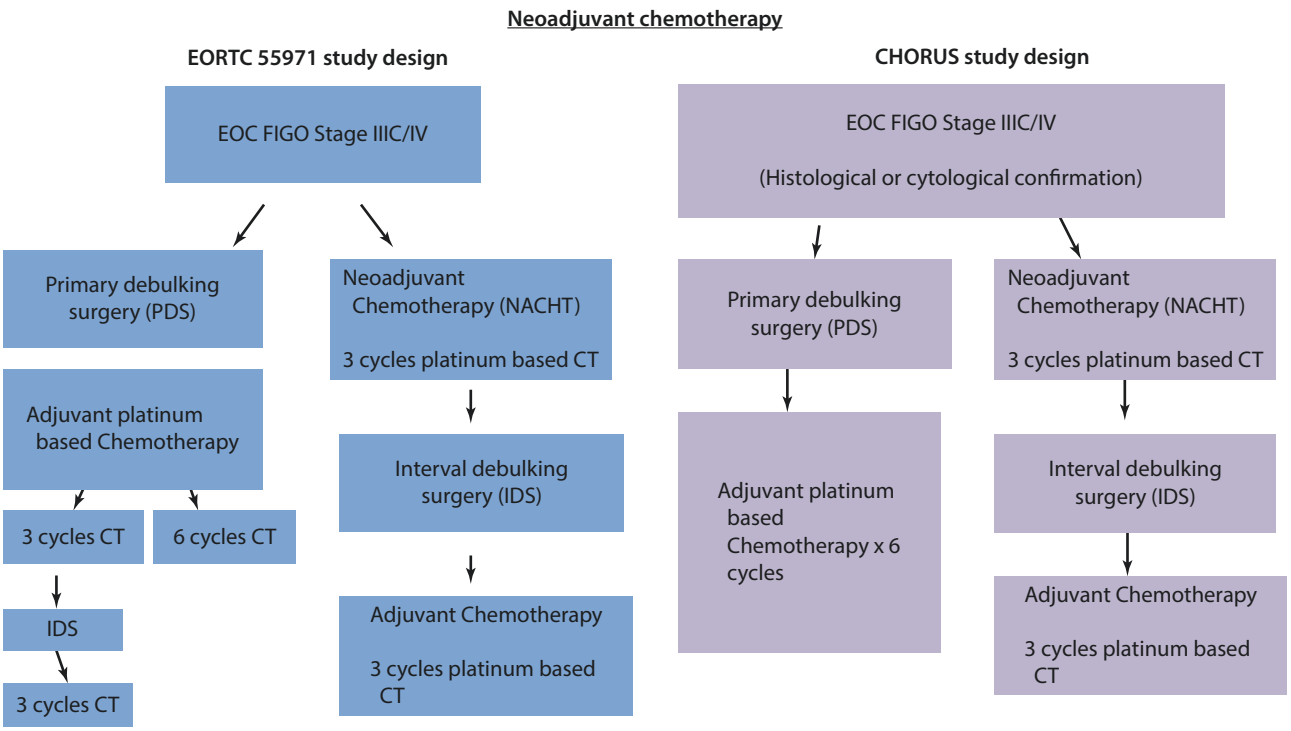


Fig. 52.1 EORTC 55971 and CHORUS study design

paper concerning the appropriate use of NACT in advanced ovarian cancer has recently been published by the American Society of Clinical Oncology and the Society of Gynecologic Oncology [26]. The consensus panel concluded that PDS is recommended in patients fit for surgery and with a good chance of achieving optimal cytoreduction while NACT followed by IDS is the preferred approach for patients with advanced-stage disease less fit for surgery and with low possibility of achieving upfront radical cytoreduction. Both conditions have to be assessed by a gynecologic oncologist.

The number of NACT cycles (ranging from three to six) and consequently the optimal surgery timing are still controversial. Colombo et al. retrospectively evaluated patients with stages IIIC–IV EOC according to the number of neoadjuvant chemotherapy cycles (<4 = group B1; >4 = group B2) and compared them with patients receiving PDS (group A) [27]. Final results showed an inverse relationship between prognosis and number of NACT cycles; patients receiving late IDS had a worse survival compared to patients treated with early IDS or PDS. Similar results are reported in the Bristow meta-analysis, which demonstrated an inverse relationship between survival and the number of NACT cycles with each additional chemotherapy cycle beyond the third associated with a 4-month decrease in overall survival. In conclusion, IDS should be attempted as soon as possible, preferably no later than three or four cycles of NACT.

52.5 Systemic Treatment

Intravenous platinum-paclitaxel chemotherapy is the standard of care in patients with stage III and IV ovarian cancer. A complete clinical remission is achieved in approximately 70% of treated patients, but up to 80% of them will experience disease recurrence after a median PFS of approximately 18 months [28]. At current time, platinum-taxane combination is the gold standard of treatment, showing improved survival compared to platinum single agent or to platinum-non-taxane combinations [29].

As reported by several studies, the addition of a third non-cross-resistant agent (e.g., liposomal doxorubicin, gemcitabine, or topotecan) does not improve survival compared to platinum-paclitaxel doublet [30, 31, 32, 33, 34, 35].

There is no evidence that continuing first-line platinum-paclitaxel chemotherapy beyond six cycles confers additional benefit to patients with advanced ovarian cancer; similarly there is no evidence that maintenance treatment with a different chemotherapy agent increases OS in advanced disease.

Schedules of Intravenous Chemotherapy

The established doses of chemotherapy are carboplatin AUC 5–6 and paclitaxel 175 mg/m^2 every 3 weeks. The JGOG 3016 study compared platinum-taxane doublet every 3 weeks with the "dose-dense" paclitaxel schedule (carboplatin AUC 6 administered every 3 weeks plus weekly paclitaxel 80 mg/m^2) for six cycles, in women with stages III–IV EOC. The final results showed that dose-dense schedule was associated with improved PFS (median 28 vs. 17.5 months) and OS (100.5 vs. 62 months) and a better toxicity profile with respect to the standard approach [36, 37].

In the Western populations, the same advantages have not been documented. The GOG 262 trial [38], the MITO 7 trial [39], and the ICON8 trial [40] did not report any difference in PFS or OS between the schedules at the price of notable differences in the toxicity profile and quality of life (QOL).

Bevacizumab in First-Line Treatment

Bevacizumab, a potent inhibitor of vascular endothelial growth factor receptor (VEGFR), blocks the growth of new tumor blood vessels, starving the cancer of the nutrition and oxygen it needs to survive; moreover it increases the effects of chemotherapy by improving drug delivery to the tumor. In ovarian cancer, bevacizumab has been explored as a single agent and in combination and maintenance after chemotherapy. GOG 218 and ICON7 are the two phase III trials investigating bevacizumab in combination with carboplatin-paclitaxel in the adjuvant setting.

GOG 218

Patients with FIGO stage IIIB–C and IV epithelial ovarian cancer who had undergone optimal debulking surgery were randomized to receive three different treatments, as shown in Fig. 52.2:

A. Standard chemotherapy with 3-weekly intravenous paclitaxel plus carboplatin for 6 cycles plus 3-weekly placebo for 22 cycles
B. Standard chemotherapy with 3-weekly intravenous paclitaxel plus carboplatin for 6 cycles plus bevacizumab 15 mg/kg during chemotherapy followed by 3-weekly placebo for 22 cycles
C. Standard chemotherapy with 3-weekly intravenous paclitaxel plus carboplatin for 6 cycles plus bevacizumab 15 mg/kg during chemotherapy followed by bevacizumab for a total of 15 months

Final results showed a prolongation of PFS in arm C with respect to control arm A (10.3 vs. 14.1 months, respectively). Progression was assessed by biochemical progression based on Ca125 levels (GCIG criteria) and radiological progression with imaging RECIST criteria. When an analysis of treatment efficacy was done only

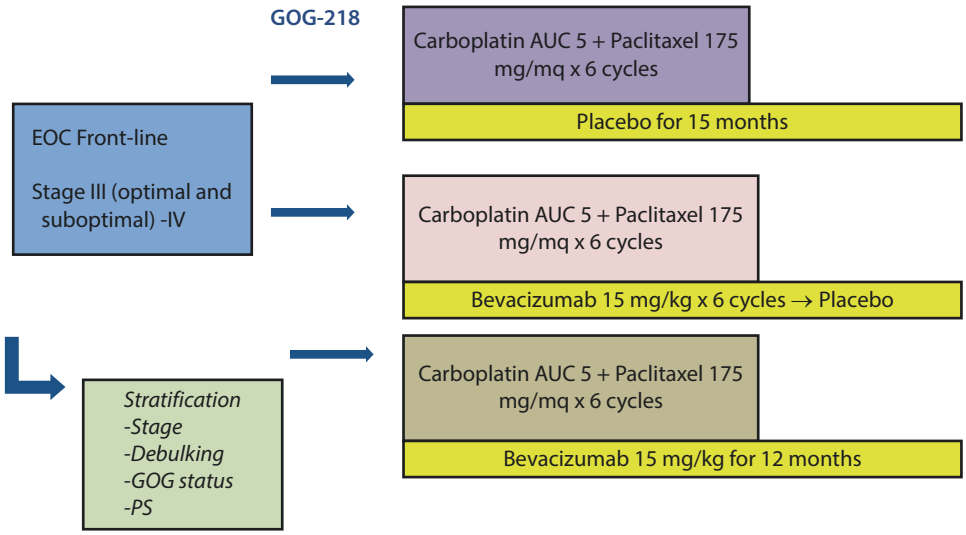


Fig. 52.2 GOG-218 study design

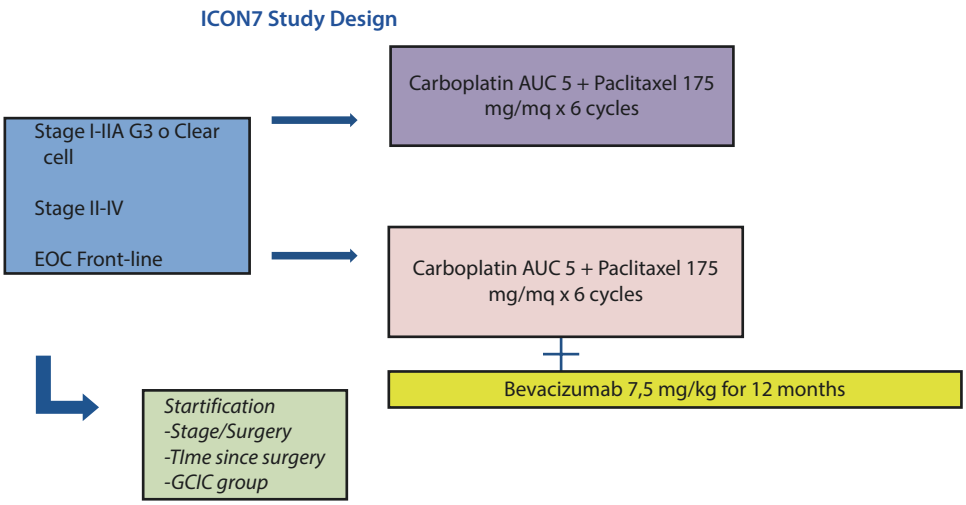


Fig. 52.3 ICON-7 study design

with radiological method, patients in arm C had a 6.2-month improvement in PFS compared to the control group and a 36% reduction in the risk of cancer progression or death [41].

ICON 7

Patients with high-risk stage I or II and stage III and IV epithelial ovarian cancer that had undergone debulking surgery (optimal and suboptimal) were randomized to receive:

A. Standard chemotherapy with 3-weekly intravenous paclitaxel plus carboplatin for six cycles
B. Six cycles of three weekly carboplatin-paclitaxel plus bevacizumab (7.5 mg/kg) for six cycles followed by bevacizumab in maintenance for 12 months (Fig. 52.3)

In the intention-to-treat population, a 1.7-month improvement in PFS was reported in the bevacizumab arm. In high-risk patients (stage III with >1.0 cm residual disease or stage IV), a median PFS improvement from 10.5 to 15.9 months in the bevacizumab arm was registered [42]. A recently published final analysis of survival data showed a statistically significant advantage in OS in patients with high-risk disease (39.3 vs. 34.9 months, in bevacizumab- and non-bevacizumab-treated patients, respectively).

Results from these two studies supported the European Commission (EMA) approval of bevacizumab in combination with carboplatin and paclitaxel in front-line treatment of patients with advanced FIGO stage IIIB–C and IV ovarian cancer (Table 52.2).

Intraperitoneal Chemotherapy

This procedure provides direct delivery of chemotherapy (cisplatin and/or paclitaxel) into the peritoneal cavity through a catheter, in addition to administering intravenous chemotherapy (Fig. 52.4) [43].

The rationale of this approach is based on the following:

- The most common route of ovarian cancer spread is within the peritoneal cavity.
- The ability to reduce tumor volume with debulking is essential to favor drug penetration.
- The residual peritoneal tumor is exposed to increased concentration of drug for a prolonged time period compared to intravenous (IV) treatment.

Patients eligible for this treatment should present:

- Good performance status
- Stage III–IV EOC with optimally cytoreduced disease (residual <1 cm after surgery) because penetration of IP chemotherapy into tumors is limited to 1–2 mm

Table 52.2 PFS and OS results in ICON7 and GOG 218 studies

Trial	Arms	PFS	OS (HR, *p* value)
ICON7 *N* = 1528 Beva: 7.5 mg/kg	A: CP B: CP + Beva → Beva 12 cycles	17.4 months 19.8 months	44.6 months 44.5 months
GOG 218 *N* = 1873 Beva: 15 mg/kg	CP CP + Beva CP + Beva → Beva 22 cycles	10.3 months 11.2 months 14.1 months	39.3 months 38.7 months 39.7 months

Abbreviations: *CP* carboplatin-paclitaxel, *Beva* bevacizumab

Side effects: Abdominal pain, nausea, and vomiting.

Complications: Bowel obstructions, infections (peritonitis, abdominal wall or catheter infections), and intestinal perforations.

A Cochrane meta-analysis [44] showed that IV/IP therapy improved median progression-free survival and overall survival and decreased the risk of recurrence and death, compared to IV therapy; unfortunately a relevant rate of patients is unable to complete IP cycles for related treatment-related toxicities (neurotoxicity and abdominal discomfort impacting on self-reported QOL) or catheter-related complications [45, 46]. In the GOG 172 trial, only 42% of patients completed treatment, 8% never started, and 34% received only one or two cycles.

For these reasons, despite the interesting data, this approach is difficult to apply in clinical practice to the majority of patients.

Treatment of Recurrent Disease

In advanced-stage EOC, the relapse rate is approximately 70–80%, even after complete response to systemic first-line treatment [47].

At recurrence the treatment options are:

1. Systemic therapy (standard and novel chemotherapeutic agents and biological agents)
2. Surgery: Secondary cytoreductive surgery (SCS) followed by chemotherapy

The choice depends on different factors such as previous treatments, the BRCA mutational status, the performance status, the number and sites of metastases, and finally the interval time between the last cycle of first-line chemotherapy and recurrence (platinum-free interval: PFI) (Figs. 52.5 and 52.6).

Platinum Agents

Platinum compounds remain the most active agents currently used in the treatment of recurrent platinum-sensitive epithelial ovarian cancer (PFI >6 months). As

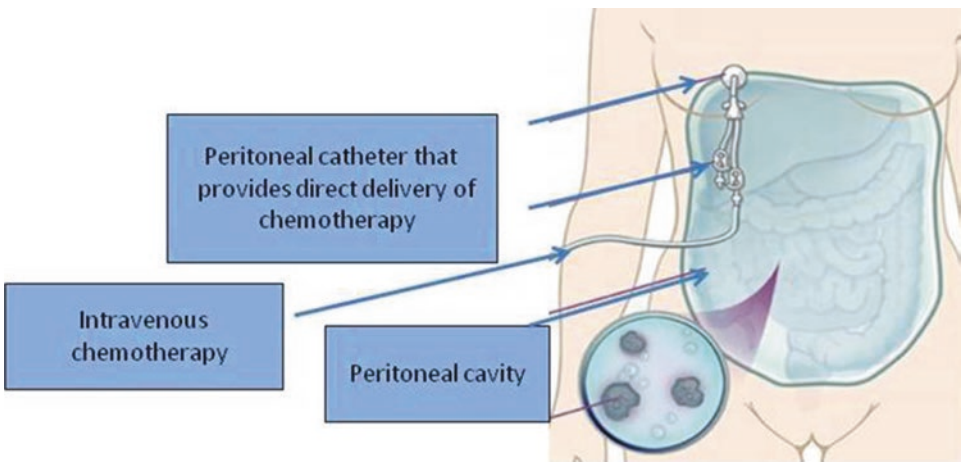


Fig. 52.4 Intraperitoneal chemotherapy procedures and mechanism of action

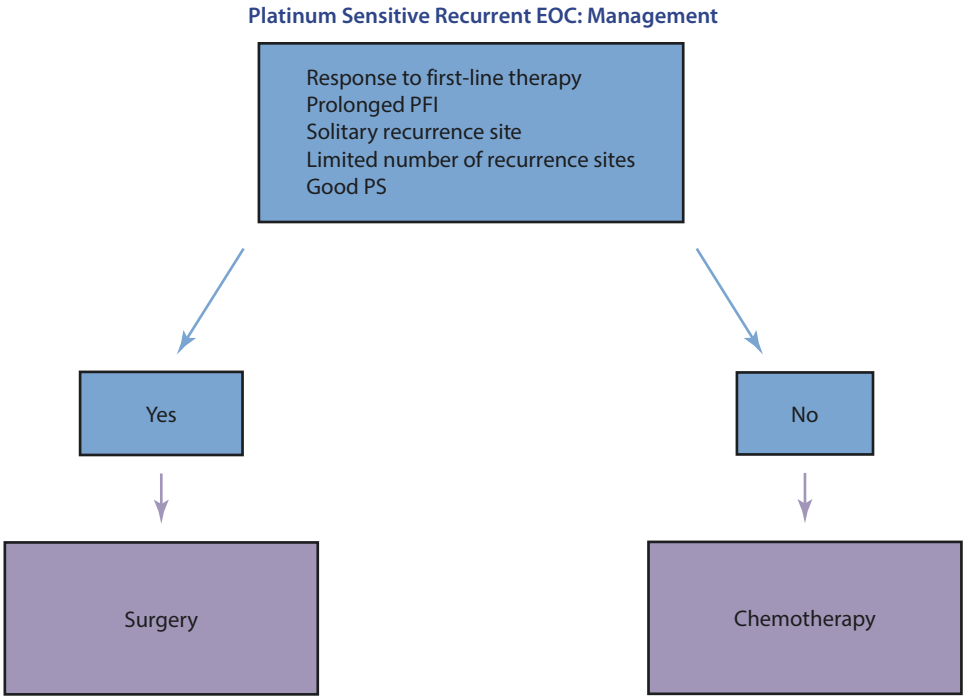


Fig. 52.5 Treatment algorithm of recurrent ovarian cancer

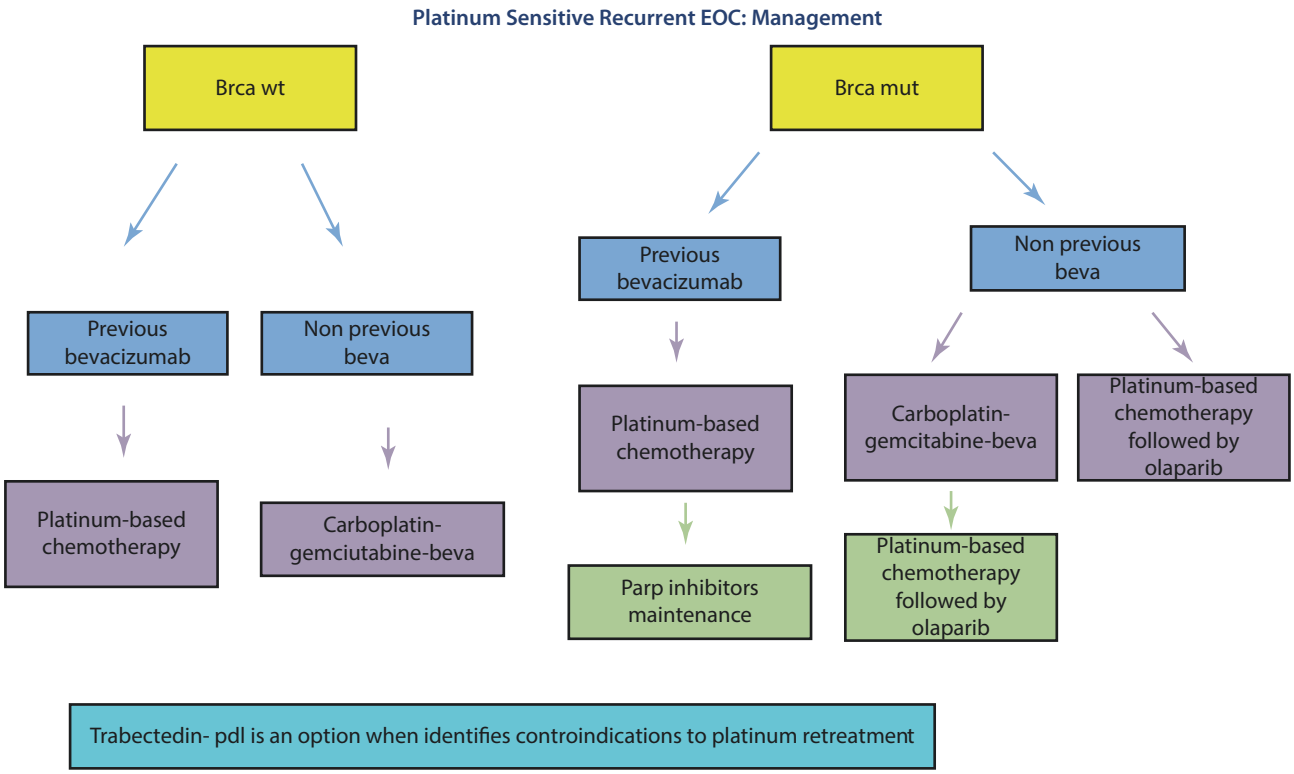


Fig. 52.6 Platinum sensitive recurrent EOC management

for the adjuvant setting, extending administration beyond six cycles has not improved long-term outcomes but increased the risk of hematologic and non-hematologic cumulative toxicity [48]. In patients with platinum-sensitive recurrence, platinum-based treatment, generally given as a doublet, is recommended. Usually carboplatin (AUC 4–5) is given in association with pegylated liposomal doxorubicin (PLD, 30 mg/m^2) every 4 weeks or with paclitaxel (175 mg/m^2) every 3 weeks or with gemcitabine (1000 mg/m^2 days 1 and 8) every 3 weeks. Overlapping oncologic outcome with different hematologic and non-hematologic toxicities has been reported with the different regimens, so that the choice is mainly based on previous toxicity and patient's preference.

Cisplatin has a comparable efficacy to carboplatin and could be associated with the same drugs (gemcitabine, paclitaxel, and PLD) but is usually considered a second choice because of the worse toxicity profile. Moreover it is generally used in case of hypersensitivity reactions to carboplatin [49].

Carboplatin-PLD combination: The efficacy of this association was established by the CALYPSO study which compared, in platinum-sensitive recurrent EOC, carboplatin-paclitaxel to carboplatin-PLD. The final results showed an improvement in median PFS in the PLD regimen compared to the paclitaxel regimen (11.3 vs. 9.4 months, respectively) and equivalent OS. As for the toxicity profile, carboplatin-PLD was characterized by less neuropathy (5% vs. 27%), myalgia (4% vs. 19%), and carboplatin-hypersensitivity reactions (16% vs. 33%), but higher percentages of mucositis (14% vs. 7%) and hand-foot syndrome (HFS) (12% vs. 2%) compared to paclitaxel regimen were reported [50, 51].

Carboplatin-paclitaxel combination: The efficacy of this association was evaluated in ICON4 and AGO-OVAR-2.2 trials [52, 53] which compared single-agent platinum to platinum-paclitaxel combination in platinum-sensitive recurrent ovarian cancer. Final data showed a significant OS benefit (29 vs. 24 months) in the experimental arm with a higher percentage of neurotoxicity and alopecia, compared to carboplatin alone.

Carboplatin-gemcitabine combination: An AGO-GCIG study, comparing carboplatin-gemcitabine to carboplatin alone, has reported an advantage in response rate (47% vs. 31%) and PFS (8.6 vs. 5.8 months) for the combination arm without a significant OS advantage. The doublet is associated with a significant myelosuppression, mainly thrombocytopenia [54].

In platinum-sensitive patients not able to receive platinum for residual neurotoxicity or for anaphylactic reaction, a non-platinum doublet is available. The combination of trabectedin-PLD has reported increased PFS (9.2 vs. 7.5, respectively) and increased OS (23.0 vs. 17.1, respectively) with respect to PLD single agent in partially platinum-sensitive recurrent EOC [55, 56].

Non-platinum agents

Generally, recurrence in platinum-resistant patients (platinum-free interval <6 months) is treated with single-agent non-platinum chemotherapy. Among available agents, the most used are:

Paclitaxel: Generally the weekly schedule (60–80 mg/m^2 continuously or the 3 weeks on and 1 week off schedule) is a well-tolerated regimen and it is recommended in patients without residual neuropathy. The response rate is approximately 13–25%. Neuropathy is the most frequent toxicity [57].

PLD: Single-agent PLD is administered once every 4 weeks at the dose of 40 mg/m^2. This schedule is well tolerated and hand-foot syndrome and stomatitis are the main toxicities. Approximately, the ORR is about 12–15%, the time to progression 9–12 weeks, and the median OS 35–40 weeks [58].

Gemcitabine: Single agent is administered at the dose of 1000 mg/m^{2}d1 and d8 every 21 days. The objective response rate is 9–11% with 55% of stabilizations of disease. Median PFS and OS are 3 and 13 months, respectively. Gemcitabine is associated with considerable myelosuppression, mostly thrombocytopenia [59].

Topotecan: Single-agent administration considers two different schedules: daily (1.5 mg/mq/day on days 1–5 of a 21-day cycle) or weekly (4 mg/mq on days 1, 8, and 15 of a 28-day cycle). High-grade hematological toxicity was reported in 27.9% and 4.8% of patients in the 3-weekly and weekly schedule, respectively, without difference in objective response rate (9–19%), PFS, and OS [60].

Other drugs used as single agent in this setting are etoposide (50 mg/m^2 daily for 21 days every 4 weeks) and pemetrexed (900 mg/m^2 every 21 days) with response rate of 27% [61] and 10–20%, respectively [62].

Antiangiogenic Agents:

Bevacizumab: The OCEANS study is a phase III randomized trial evaluating the role of bevacizumab in combination with chemotherapy in platinum-sensitive recurrent ovarian cancer. Patients received gemcitabine and carboplatin for six cycles plus placebo or bevacizumab in combination and maintenance until disease progression. The final results reported a median 4-month PFS advantage in patients receiving bevacizumab compared with placebo (12.4 months vs. 8.4 months, respectively), without difference in overall survival [63]. Moreover the bevacizumab arm registered an increased response rate compared to placebo arm (78.5% vs. 57.4%, respectively).

The phase III randomized AURELIA trial [64] evaluated the efficacy of bevacizumab in combination with standard chemotherapy versus chemotherapy alone in patients with platinum-resistant recurrent ovarian cancer. Enrolled patients received either single-agent

chemotherapy (at investigator's choice between weekly paclitaxel, pegylated liposomal doxorubicin (PLD), or topotecan) or chemotherapy in combination and maintenance with bevacizumab until disease progression or unacceptable toxicity. Patients enrolled in the bevacizumab arm experienced a 3.3-month improvement in median PFS and a significant amelioration in quality of life [65] with respect to chemotherapy alone-treated patients. Final results did not show a significant improvement in OS, probably due to crossover of 40% of patients receiving bevacizumab therapy after progression.

Actually, the prescription of bevacizumab has different indications across the world. In fact, despite evidence of activity in different treatment settings, there is no international consensus about the most appropriate setting of disease in which to use the antiangiogenic agent [66].

Multitargeted TKIs: Various agents are being investigated in several phase II–III studies demonstrating single-agent activity in EOC:

(a) *Pazopanib*: It is an oral tyrosine kinase inhibitor targeting VEGF receptor 1, 2, and 3, platelet-derived growth factor receptor α and β, and c-kit and inhibiting angiogenesis and tumor proliferation. When used as maintenance treatment after first-line carboplatin-paclitaxel chemotherapy in advanced ovarian cancer (AGO-OVAR 16), the drug demonstrated a 6-month PFS benefit [67]). The MITO 11 trial [68] is a phase II trial, evaluating the efficacy of pazopanib in combination with weekly paclitaxel in patients with platinum-resistant recurrent ovarian cancer versus chemotherapy alone. The study showed a significant 2.9-month improvement in PFS for the pazopanib arm (median 6.3 vs. 3.4 months for experimental arm vs. standard arm, respectively).

(b) *Cediranib:* It is a VEGFR 1, 2, and 3 oral tyrosine kinase inhibitor demonstrating a particular activity in recurrent ovarian cancer [69]. In ICON6 trial [70, 71], a randomized controlled phase III trial, the efficacy of cediranib given concurrently with platinum-based chemotherapy and as maintenance in women with platinum-sensitive relapsed ovarian cancer was assessed. Final results showed a significant improvement in terms of PFS and OS for the experimental arm. Recently, data on the association to olaparib plus cediranib versus olaparib alone in patients with relapsed platinum-sensitive ovarian cancer were reported and documented. Improvements in objective response rate (80% vs. 48%), disease stabilization (17.7 vs. 9 months), and PFS for the combination arm were reported regardless of BRCA status [72].

(c) *Nintedanib* is an oral triple angiokinase inhibitor that blocks VEGFR 1, 2, and 3, platelet-derived growth factor receptors (PDGFR), and fibroblast growth factor receptors (FGFR) 1, 2, and 3. When used as maintenance treatment in first-line setting after chemotherapy (AGO-OVAR 12), nintedanib showed a 1.2-month increase in PFS versus placebo. This advantage was considered insufficient for promoting further development of the drug [73].

PARP Inhibitors

Approximately 50% of patients with high-grade EOC are deficient in the DNA homologous recombination repair pathway.

In about 25% of cases, this defect is related to mutations (germline or somatic) of BRCA 1/2 genes or epigenetic inactivation of the same genes. In the remaining 25% of cases, patients present mutations in a series of minor genes, involved in the homologous recombination deficiency (HRD) [74], as shown in Fig. 52.7.

Poly(ADP-ribose) polymerase (PARP) plays an integral role in single-strand DNA break repair via the base excision pathway. Normal cells can repair DNA damage using alternative pathways, for example, homologous recombination pathway (HR), sufficient to maintain genomic integrity; in cells with deficient homologous recombination (as are BRCA-mutated cells), DNA damage accumulates and consequently leads to cell death (apoptosis).

Based on this mechanism, PARP inhibitors selectively kill tumor cells compared with normal cells, a concept recognized as "synthetic lethality." HRD increases sensitivity also to platinum-based chemotherapy because the deficiency impairs the ability of cancer cells to repair the direct platinum-induced double-strand DNA breaks. For these reasons, platinum sensitivity is often associated with an HRD tumor phenotype (Fig. 52.8).

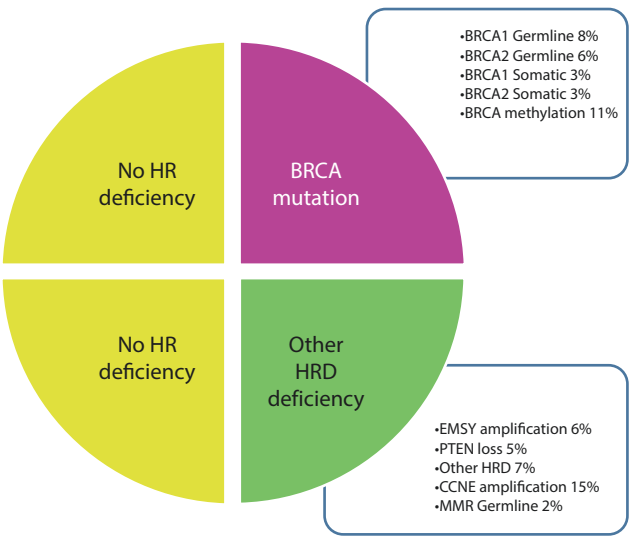


Fig. 52.7 Genes and intracellular proteins involved in homologous recombination deficiency

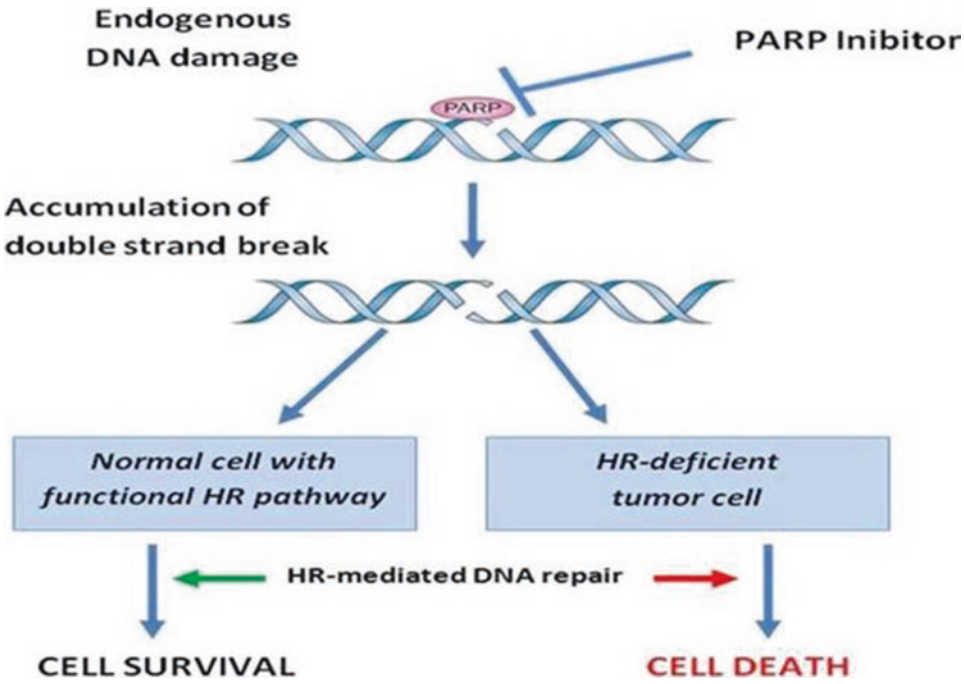


Fig. 52.8 PARP inhibition and tumor-selective synthetic lethality

PARP inhibition is a novel approach to target tumors with deficiencies in DNA repair mechanisms. BRCA mutation is the first and currently the unique predictive biomarker for targeted therapy in ovarian cancer. The availability of PARP inhibitors as a treatment option in EOC opened the door for routine testing of BRCA mutations in blood (germline test) and in the tumor specimen (somatic test) [75]. Information about BRCA status, according to the most recent guidelines, should be obtained at the time of diagnosis, in order to create a suitable therapeutic algorithm. Robust data support the role of PARP inhibitors in the treatment of patients with *BRCA*-associated ovarian cancer. Moreover responses are also described in non-*BRCA*-mutated patients (particularly in platinum-sensitive) suggesting that the clinical utility of PARP inhibitors can be extended to a larger patient population [76].

Common features of PARP inhibitors:

- Inhibition of PARP-associated DNA repair pathway.
- Particularly effective in presence of BRCA mutation.
- Oral drug.
- Well tolerated.
- Common side effects are nausea, fatigue, vomiting, diarrhea, and bone marrow suppression (increased risk infection, bleeding, anemia).
- Rare serious toxicity such as leukemia and lung inflammation (interstitial pneumonia).

Olaparib:

Olaparib (AZD2281, KU-0059436) is a potent PARP inhibitor (PARP 1, 2, and 3) that is being developed as an oral therapy, both as single agent (including maintenance) and in combination with chemotherapy and other antineoplastic agents. Actually, olaparib indications are different in the United States and European Union.

In the United States olaparib is approved in monotherapy for patients with BRCA-mutated ovarian cancer who received three or more previous chemotherapy treatments and as maintenance in platinum-sensitive, platinum-responding ovarian cancer, regardless of BRCA mutational status. In Europe olaparib is approved for the maintenance treatment of patients with relapsed platinum-sensitive, BRCA-mutated (germline or somatic), high-grade serous epithelial ovarian cancer who are responding (partial or complete) to platinum-based chemotherapy. In both cases, the recommended dose is 400 mg twice daily, until disease progression or unacceptable toxicity.

Study 19 is an international, double-blind, pivotal, randomized, phase II trial showing antitumor activity of olaparib in maintenance treatment of patients with platinum-sensitive high-grade serous relapsed EOC (Fig. 52.9).

The study reported a significant 3.6-month increase in median PFS for olaparib maintenance therapy compared with placebo (8.4 months vs. 4.8 months, respectively) in the overall population; moreover, in a subgroup analysis, the benefit was greater in patients with germline or somatic BRCA mutation, with a significant 6.9-month increase in median PFS and a 82% risk reduction of disease progression or death in olaparib arm without detrimental impact on global health-related quality of life (HRQoL).

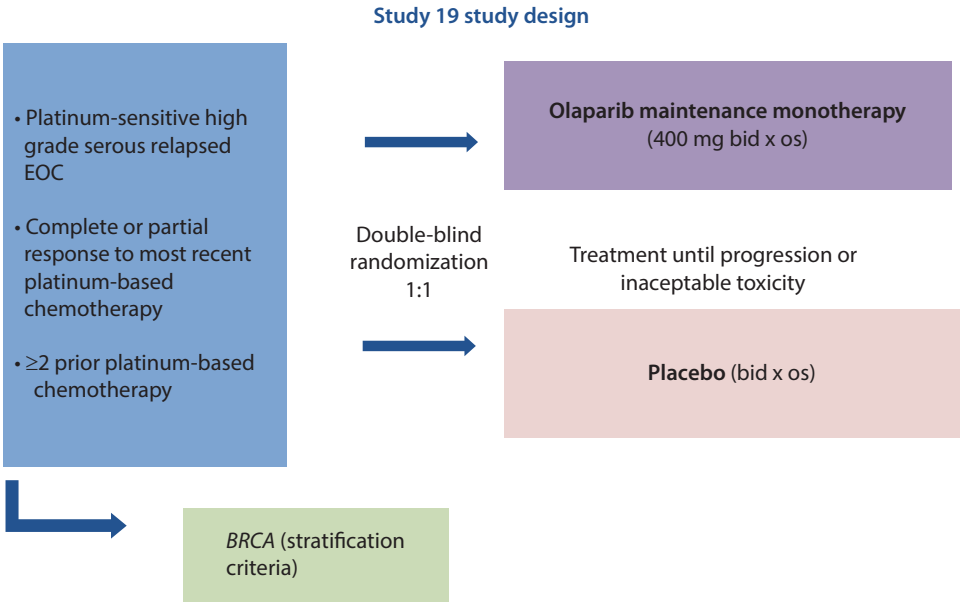


Fig. 52.9 STUDY-19 study design

On November 2016, the Lancet Oncology reported overall survival data of Study 19 demonstrating that patients with platinum-sensitive recurrent EOC receiving olaparib maintenance treatment have longer overall survival with respect to patients receiving placebo (median survival was 29.8 months vs. 27.8 months in olaparib and placebo arm, respectively). Moreover, in BRCA-mutated patients, the median survival was 34.9 months versus 30.2 months for olaparib and placebo, respectively [70, 71].

Rucaparib

Rucaparib is a small-molecule PARP 1 and PARP 2 inhibitor which is administered at the dose of 600 mg twice daily. This drug is being developed as maintenance treatment for recurrent platinum-sensitive EOC in ARIEL3 study, in which rucaparib was administered to prespecified groups of patients, categorized according to homologous recombination deficiency (HRD) status (BRCA-mutated, BRCA-like/high loss of heterozygosity (LOH), and the intention-to-treat population), as maintenance treatment after platinum-based chemotherapy in comparison to placebo.

Final results showed a statistically significant improvement in progression-free survival (PFS) in each of the three populations: median progression-free survival in patients with BRCA mutation was 16.6 months in the rucaparib arm versus 5.4 months in the placebo arm. In patients with a homologous recombination deficiency, median PFS was 13.6 months versus 5.4 months in the rucaparib and placebo arm, respectively. In the intention-to-treat population, median PFS was 10.8 months versus 5.4 months for rucaparib and placebo, respectively.

Rucaparib is already approved by the FDA as single agent for the treatment of BRCA-mutated (either germline or somatic) recurrent EOC patients who had received at least two previous CHT lines based on a pooled analysis of two phase II trials (ARIEL2 and Study 10) reporting 54% response rate and 10-month median PFS when rucaparib was used a single agent for the treatment of active disease [77].

The ARIEL4 trial is a phase III ongoing multicenter randomized study evaluating rucaparib versus clinician choice chemotherapy in relapsed ovarian cancer patients with BRCA mutations who failed two prior lines of therapy.

Niraparib

Niraparib is a small-molecule PARP 1 and PARP 2 inhibitor administered at the dose of 300 mg daily. In the phase III NOVA trial, niraparib was given as maintenance treatment in patients with recurrent epithelial ovarian cancer who are in complete or partial response to platinum-based chemotherapy in comparison to placebo. Two parallel and independent cohorts of patients were enrolled: germline BRCA-mutated patients and platinum-sensitive patients without germline mutation. Final results reported a decrease in risk of progression or death compared with placebo for the BRCA-mutated patients.

Median PFS was 21 months in patients with germline *BRCA* mutations, 12.9 months in germline BRCA wild-type patients who carry a homologous recombination deficiency (HRD), and 9.3 months in BRCA wild-type patients without HRD deficiency. The corresponding figures for the placebo arm were 5.5 months, 3.7 months, and 3.8 months, respectively. Based on these data, the FDA has approved niraparib as maintenance treatment for patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer in complete or partial response to platinum-based chemotherapy, regardless of BRCA mutational status (Fig. 52.10).

	Germline BRCA mut	Somatic BRCA mut HRD +	BRCAwt HRD +	BRCAwt HRD neg
Niraparib	21.0 months	20.9 months	9.3 months	6.9 months
Placebo	5.5 months	11.0 months	3.7 months	3.8 months

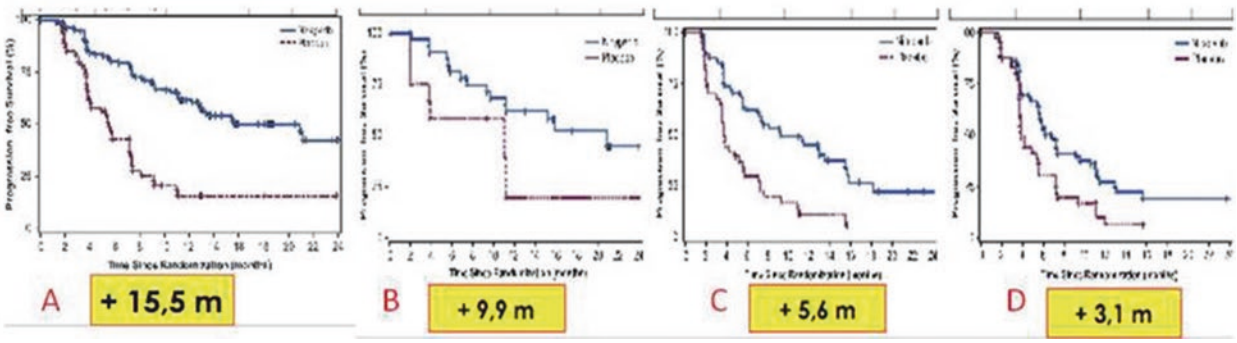


Fig. 52.10 The Kaplan-Meier curves of PFS for the 2 treatments arms in the different population: A: gBRCA mutated cohort; B: sBRCA mutated cohort; C: HRD positive-BRCA WT patients; D: HRD Negative patients

Alpha-Folate Receptor

The folate receptors (FR) constitute a group of proteins that mediate accumulation of folate into cells and regulate folate homeostasis and consequently synthesis, methylation, and DNA repair [78].

Alpha-folate receptor (aFR), an isoform of this family, can be over-expressed by several epithelial-derived tumors, including ovarian cancer, where it is present in approximately 75% of cases [79]. The over-expression of aFR is considered a negative prognostic factor and associated with poorer overall survival (OS) [80].

Recently, aFR is receiving more interest in gynecologic cancers as an excellent target for new targeted therapies [81]. aFR is expressed on cancer cell surface and has the ability to connect through folic acid several ligand molecules (e.g., antineoplastic agents) that can selectively penetrate into cancer cells minimizing systemic toxic side effects. This family of molecules, also called immunoconjugates, includes:

- Farletuzumab (MORAb-003), a humanized monoclonal antibody that targets glycoprotein 3 (GP-3) and triggers a host immune response against GP-3-expressing cells resulting in apoptosis [82]. This drug has shown activity against advanced epithelial ovarian cancer, mainly in platinum-sensitive disease (MORAB study).
- Mirvetuximab soravtansine (IMGN853), an alpha-folate receptor-targeting antibody-drug conjugate that combines an alpha-folate receptor-binding antibody and a novel antitumoral agent (tubulin-disrupting maytansinoid DM4). This drug has shown activity against advanced epithelial ovarian cancer, mainly in platinum-resistant disease (FORWARD 1 study) [83].
- Vintafolide (EC145), an alpha-folate receptor ligand conjugated with vinca alkaloid-derived drug that targets FR-expressing cells, explored in a randomized phase II trial in "platinum-resistant" ovarian cancer versus PLD with deluding results (PRECEDENT study) [84].

52.6 Secondary Cytoreductive Surgery

Chemotherapy is the standard treatment of recurrent epithelial ovarian cancer, but surgery can also be performed in selected patients.

The role of secondary cytoreductive surgery (SCS) in the standard management of recurrence remains poorly defined. Generally, the eligibility criteria for secondary cytoreduction are:

1. Response to first-line therapy and prolonged platinum-free interval (PFI) [12, 85, 86]
2. Solitary recurrence site or limited number of recurrence sites [86, 87]
3. Good PS

The DESKTOP OVAR I retrospective trial and the DESKTOP OVAR II prospective trial have identified and validated a panel of selection criteria for SCS and a predictive score to identify patients who could have a complete resection during secondary cytoreductive surgery (AGO score: ECOG PS 0, no residual tumor after first surgery, and ascites less than 500 ml) [88].

DESKTOP IIII and GOG 213 are two prospective randomized controlled phase III trials investigating the role of secondary cytoreductive surgery for recurrent EOC.

Final results of DESKTOP III trial showed that secondary cytoreductive surgery translates in 6-month improvement in PFS with respect to chemotherapy alone; the benefit was exclusively seen in patients with complete resection (CR) indicating the importance of selecting patients and centers in which the surgical procedure is performed.

Treatment Algorithms for Recurrent Ovarian Cancer

The option of second-line chemotherapy for recurrent disease is based on platinum-free interval (PFI), defined as the time interval between the last dose of platinum to the progression of disease [89].

Patients are generally divided into four groups:

A. Platinum-refractory: Patients who progressed during platinum-based chemotherapy or within 4 weeks after last dose
B. Platinum-resistant: Patients with a disease progressing within 6 months from last platinum dose
C. Partially platinum-sensitive: Patients who progressed between 6 and 12 months from last platinum dose
D. Fully platinum-sensitive: Patients who progressed with an interval of more than 12 months from last platinum dose

PFI is considered the most important criterion for predicting the response to chemotherapy in recurrent ovarian cancer and is also the main factor leading the choice of therapeutic strategy. PFI is not only expression of the biology of the disease but is highly influenced by several factors, such as the type of surgery (primary debulking surgery or neoadjuvant chemotherapy) and the type of chemotherapy (with or without bevacizumab in first line). Moreover, the PFI value has recently been criticized for arbitrary definition and categorizations of recurrences particularly because the date of disease progression is somewhat variable according to the method used to evaluate progression (either radiological assessment, Ca125 level increase, or clinical progression) [90].

Recently, the Fifth Word Consensus Conference on Ovarian Cancer reported that the PFI should not be considered more as the only parameter to take into account in choosing treatment at recurrence of disease particularly in the era of targeted therapies and biological characterization of ovarian tumors.

Other important tools for the decision-making are platinum-free-interval, BRCA mutational status, previous treatments, and toxicity. The ultimate goal of treatment strategy is to build an algorithm which allows patients to receive all the available treatment options, possibly in the more appropriate temporary order.

When Platinum Is an Option

In patients with platinum-sensitive recurrent epithelial ovarian cancer who do not present contraindication to platinum retreatment, platinum-based combinations with paclitaxel, gemcitabine, or pegylated liposomal doxorubicin are recommended.

Some other considerations are mandatory before starting with second-line chemotherapy such as ECOG performance status, mutational status of BRCA 1 and 2 genes, and previous received treatments.

A. BRCA 1/2 wild-type patients never exposed to bevacizumab

Recommended choice is represented by the combination of carboplatin AUC 4 on day 1 plus gemcitabine 1000 mg/m^2 on days 1 and 8 associated with bevacizumab 15 mg/kg on day 1 every 21 days until progression of disease or unacceptable toxicity.

B. BRCA 1/2 wild-type patients previously exposed to bevacizumab

Recommended choice, according to physician's judgment, is platinum-based combinations with paclitaxel, gemcitabine, or pegylated liposomal doxorubicin chosen according to the toxicity profile and patient's preference. The recommended schedules are:

- Carboplatin AUC 4 on day 1 plus gemcitabine 1000 mg/m^2 on days 1 and 8 every 21 days [54]
- Carboplatin AUC 5 plus pegylated liposomal doxorubicin (PLD) 30 mg/m^2 on day 1 every 28 days [50, 51]
- Carboplatin AUC 5 plus paclitaxel 175 mg/m^2 on day 1 every 21 days [53]

C. BRCA 1/2 mutation carrier patients previously exposed to bevacizumab

Recommended choice is a platinum-based chemotherapy (for four to six cycles) followed by maintenance treatment with the licensed PARP inhibitor until progression of disease or unacceptable toxicity.

D. BRCA 1/2 mutation carriers never exposed to bevacizumab

Available choices are:

Combination of carboplatin AUC 4 on day 1 plus gemcitabine 1000 mg/m^2 on days 1 and 8 associated with bevacizumab 15 mg/kg on day 1 every 21 days until progression of disease or unacceptable toxicity

Platinum-based chemotherapy (for four to six cycles) followed by maintenance treatment with the licensed

PARP inhibitor until progression of disease or unacceptable toxicity

The choice between two regimens should be based on patient's preference and disease characteristics and discussed with patients.

When Platinum Is Not an Option

When the physician identifies contraindications to platinum retreatment, despite the patient being platinum-sensitive, such as in the case of previous anaphylactic reactions to platinum [91] which occurs in up to 40% of cases, residual neurotoxicity, or intermediate sensitivity to platinum (patients who progressed between 6 and 12 months from last platinum dose), a platinum-free strategy with the combination of trabectedin 1.1 mg/m^2 plus PLD 30 mg/m^2 on day 1 every 21 days can be offered [55].

OVA-301 trial is a randomized phase III trial comparing the efficacy and the safety of trabectedin 1.1 mg/m^2 associated with PLD 30 mg/m^2 on day 1 every 21 days versus PLD 50 mg/m^2 alone on day 1 every 28 days. The study reported a benefit in PFS in the combination arm, especially in the platinum-partially sensitive cohort (median PFS was 9.2 months vs. 7.5 months and median OS was 23.0 months vs. 17.1 months, respectively) [92].

Moreover a post hoc analysis suggests that this combination is particularly active in terms of response rate, PFS, and OS in BRCA-mutated patients [93]. Preclinical and clinical data suggest a benefit in the trabectedin → platinum sequence suggesting that trabectedin administered before carboplatin is able to select cellular clones more sensitive to subsequent carboplatin treatment. This hypothesis will be tested in the ongoing prospective randomized INOVATYON trial.

In patients with platinum-resistant disease, the objective of treatment is symptoms palliation and maintenance of QoL. Sequential single-agent non-platinum therapies are recommended and, as reported in a recent Cochrane systematic review, paclitaxel, PLD, and topotecan have similar efficacy (ORR 10–15% and median PFS 3–4 months) but different toxicity profile, which should be discussed with the patient [94].

Summary of Clinical Recommendations

Management of primary advanced disease includes:

- Primary cytoreductive surgery or NACT followed by interval cytoreductive surgery
- Intravenous chemotherapy
- Intraperitoneal chemotherapy
- Dose-dense chemotherapy
- Maintenance treatment

Intravenous chemotherapy → carboplatin AUC 5–6 + paclitaxel 175 mg/m^2 every 3 weeks plus bevacizumab in combination with chemotherapy and in maintenance for 15 months

Management of recurrent disease includes:

1. Systemic therapy (standard and novel chemotherapeutic agents and biological agents)
2. Surgery: Secondary cytoreductive surgery (SCS)

The choice depends on many factors such as the previous received treatments, the BRCA mutational status, the performance status, the site and number of recurrences, and finally the time interval between the last cycle of first-line chemotherapy and recurrence.

When Platinum Is an Option

BRCA 1/2 wild-type patients never exposed to bevacizumab → carboplatin AUC 4 on day 1 plus gemcitabine 1000 mg/m^2 on days 1 and 8 associated with bevacizumab 15 mg/kg on day 1 every 21 days until progression of disease or unacceptable toxicity

BRCA 1/2 wild-type previously exposed to bevacizumab → platinum-based combinations with paclitaxel, gemcitabine, or pegylated liposomal doxorubicin

BRCA 1/2 mutation carriers previously exposed to bevacizumab → platinum-based chemotherapy (for four to six cycles) followed by maintenance treatment with the licensed PARP inhibitor until progression of disease or unacceptable toxicity.

BRCA 1/2 mutation carriers never exposed to bevacizumab → (a) Combination of carboplatin AUC 4 on day 1 plus gemcitabine 1000 mg/m^2 on days 1 and 8 associated with bevacizumab 15 mg/kg on day 1 every 21 days until progression of disease or unacceptable toxicity. (b) Platinum-based chemotherapy (for four to six cycles) followed by maintenance treatment with the licensed PARP inhibitor until progression of disease or unacceptable toxicity.

When Platinum Is Not an Option

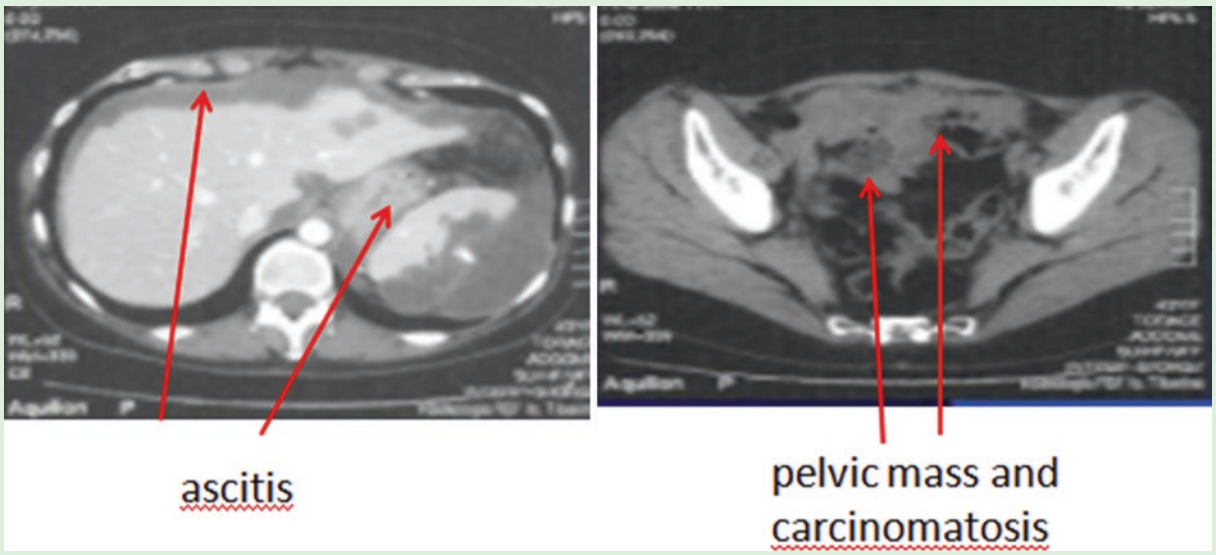
In patients with platinum-partially sensitive disease → trabectedin 1.1 mg/m^2 plus PLD 30 mg/m^2 on day 1 every 21 days

In patients with platinum-resistant disease → sequential single non-platinum agents (paclitaxel, PLD, and topotecan)

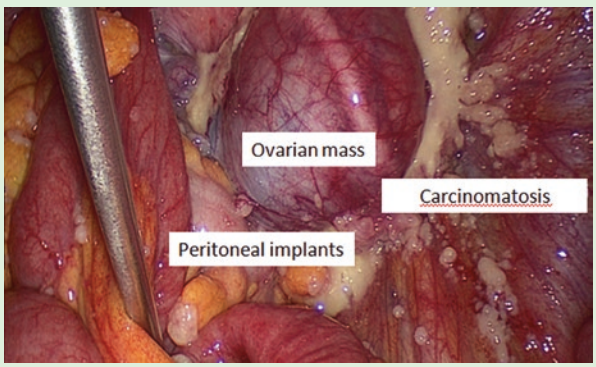
Case study 1: Management of patient with BRCA germline mutation and recurrent ovarian cancer

Woman, 64 years old, ECOG PS0

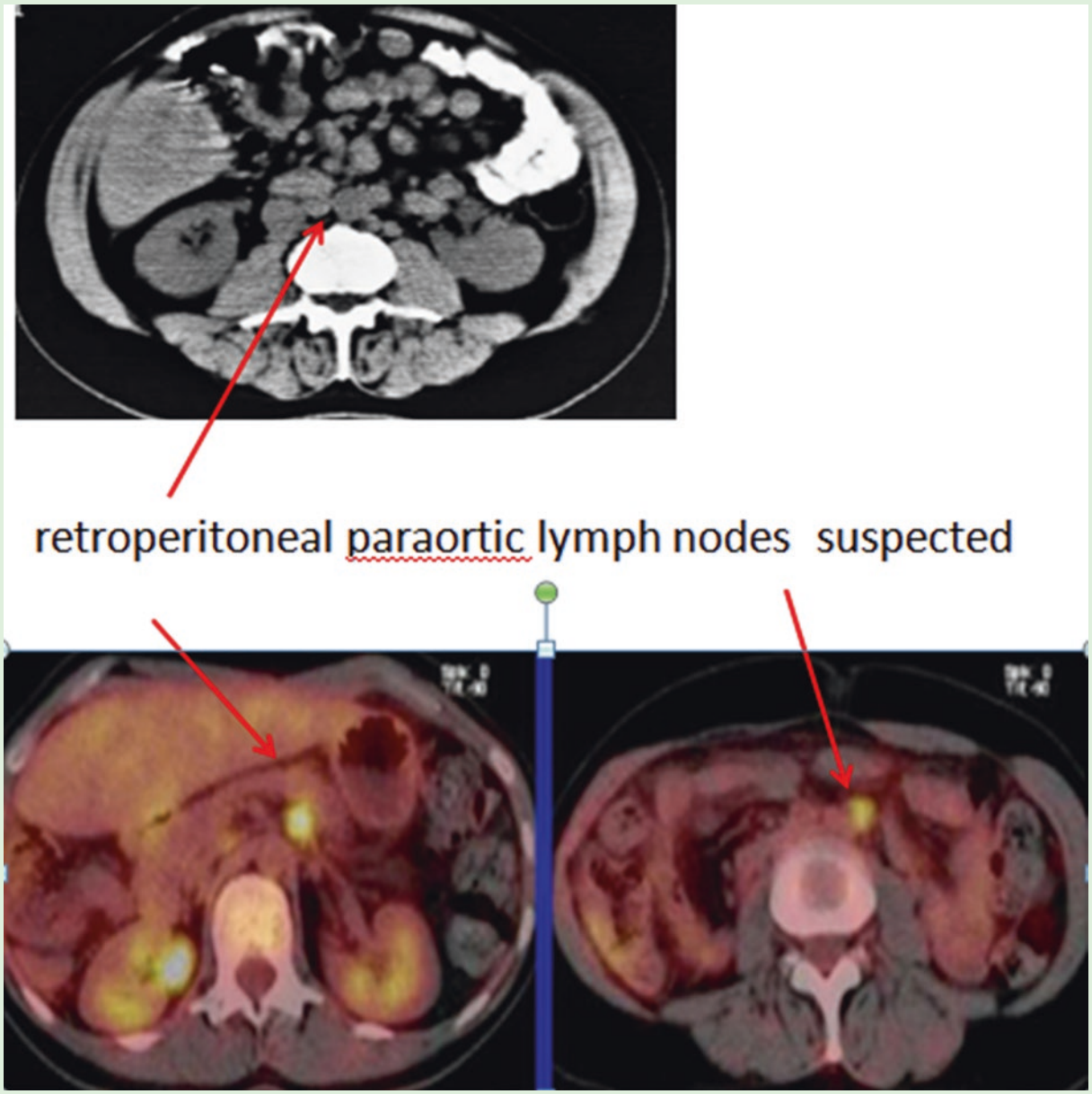
- *Family history:* Negative for malignancy
- *APR:* Hypercholesterolemia treated with statin, well-controlled hypertension treated with spironolactone
- *APP:* Nausea, asthenia, and diffuse abdominal pain
- *CT scan chest-abdomen* (20/09/2002): Presence of pelvic mass, ascites, carcinomatosis, and clinically suspected pelvic lymph nodes, Ca125 level of 622 U/ml



- *Surgery* (02/10/2002): Laparotomy with total abdominal hysterectomy, bilateral salpingo-oophorectomy (BSO), peritoneal washing, total peritonectomy and omentectomy, removal of bulky pelvic and para-aortic lymph nodes, and removal of peritoneal nodules. Postoperative residual tumor = absent (RT0)



- *Pathologic assessment* showed stage IIIC high-grade serous ovarian cancer.
- From 22/11/2002 to 4/04/2003, the patient received six cycles of chemotherapy treatment with carboplatin AUC 5 plus paclitaxel 175 mg/mq g1 q 21.
- Follow-up labs showed normalization of Ca125 to less than 10 U/ml and CT scan was NED (non-evidential disease upon completion of chemotherapy).
- In 2016 the patients developed abdominal pain. Physical examination was normal but laboratory analyses showed a Ca125 level of 190 U/ml.
- *CT scan* (30/06/2016) revealed enlarged retroperitoneal para-aortic lymph nodes suspected to be metastatic. This data were confirmed by PET-FGD performed on 11/07/2016.



Question

What action should be taken?

1. Secondary cytoreductive surgery
2. Systemic chemotherapy
3. Wait and see strategy

Answer

Considering the good PS, the prolonged PFI, and the limited number of recurrent sites of disease, the choice was for secondary cytoreduction surgery.

22/07/2016: Patient underwent secondary cytoreductive surgery (removal of para-aortic lymph nodes through laparotomy).

20/06/2016: Although she had no family history of ovarian and breast malignancies, she was offered BRCA testing for hereditary risk assessment. The test showed a pathogenic BRCA 2 mutation.

What action should be taken?

1. Carboplatin-gemcitabine followed by licensed PARP inhibitor maintenance
2. Carboplatin -gemcitabine-bevacizumab followed by bevacizumab as maintenance
3. Non-platinum combination

Answer

Considering the platinum-free interval (>12 months) and the prior received treatments (only platinum-based chemotherapy without bevacizumab), despite the BRCA mutation, given the limitation in bevacizumab reimbursement which is labelled only in the first platinum-sensitive

recurrence, after discussing with the patient the treatment strategy, our choice was carboplatin-gemcitabine in combination and maintenance with bevacizumab.

- From 22/08/2016 to 14/12/2016, patient received six cycles of carboplatin AUC 4 g1 plus gemcitabine 1000 mg/mq g1, 8 q21 plus bevacizumab 15 mg/kg.
- Since 8/01/2017, maintenance therapy with bevacizumab 15 mg/kg is ongoing.

Key Points

- The role of surgery at diagnosis and at recurrence
- Implications of a positive BRCA mutation test in the treatment decision-making
- The choice of a drug based on prescriptive limits (i.e., bevacizumab at first platinum-sensitive recurrence)

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Case study 2: Management of patient with stage IV ovarian cancer and BRCA germline mutation

Woman, 60 years old, ECOG PS1

- *APR:* Severe obesity, well-controlled hypertension and diabetes mellitus treated with oral hypoglycemic drug
- *BRCA 1 germline mutation carrier*
- *APP:* In December2016, she referred abdominal pain, increased abdominal size, and anorexia. Patient has undergone CT scan and PET-FDG showing multiple liver metastasis, peritoneal nodules, and multiple para-aortic bulky lymph nodes.

Question

What action should be taken?

A. Cytoreductive surgery and subsequently adjuvant platinum-based chemotherapy
B. Laparoscopic-TC-guided biopsy and subsequently neoadjuvant platinum-based chemotherapy
C. Platinum-based chemotherapy alone

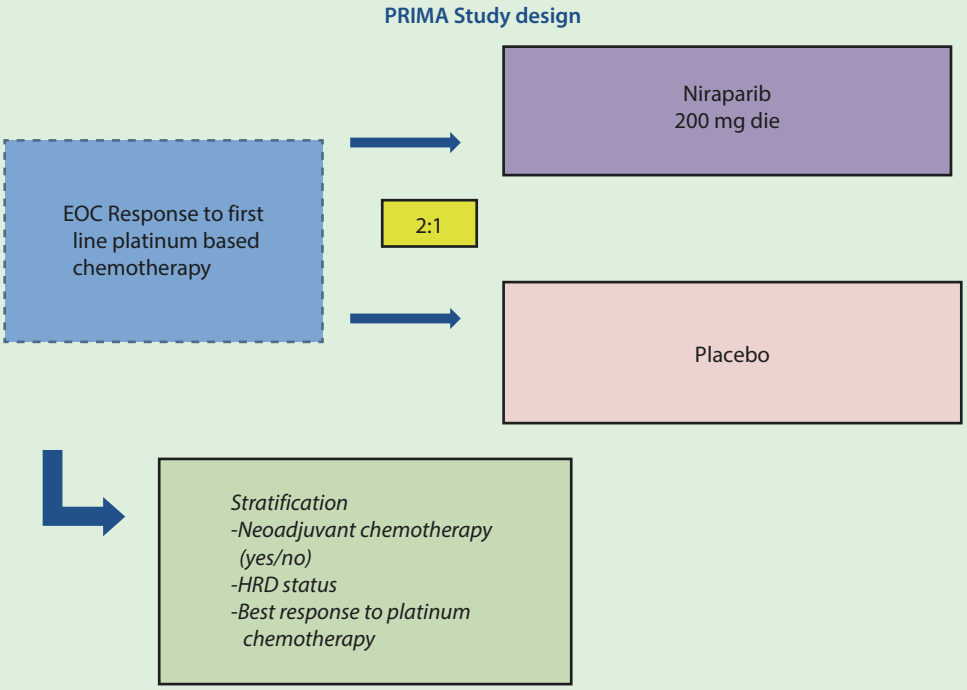
Answer

- Considered the comorbidities, the poor performance status, the low possibility of optimal cytoreduction, and the non-resectable sites of metastasis, the choice was option B.
- On 09/01/2017, during laparoscopy, unilateral salpingo-oophorectomy and biopsy of liver metastasis were performed. The histologic examination showed a high-grade serous carcinoma FIGO stage IV.
- From 20/01/2017 to 10/03/2017, the patient received three cycles of chemotherapy treatment with carboplatin AUC 5 plus paclitaxel 175 mg/mq g1 q 21.
- After the third CHT cycle, the Ca125 level was within the range of normality; radiologic imaging showed a partial response in lymph nodes and a complete response in liver metastasis (with only a residual small subglissonian metastases).
- On 10/04/2017, the patient underwent interval debulking surgery (total abdominal hysterectomy, unilateral salpingo-oophorectomy, peritoneal washing, total omentectomy, biopsy of suspected peritoneal surfaces, and removal of pelvic and para-aortic bulky lymph nodes). Residual disease at the end of the procedure was absent.

What action should be taken?

(a) Other three cycles of carboplatin-paclitaxel
(b) Other three cycles of carboplatin-paclitaxel plus bevacizumab followed by bevacizumab maintenance
(c) Other three cycles of carboplatin-paclitaxel followed by PARP inhibitor maintenance

Considering the excellent response to chemotherapy, the known BRCA mutational status, and the possibility of subsequently administering bevacizumab (at first recurrence), our choice was option C. In fact, the patient was enrolled in PRIMA trial, a phase III randomized, double-blind multicenter study, evaluating the efficacy of niraparib versus placebo as maintenance treatment in patients with stage III or IV ovarian cancer responding to front-line platinum-based chemotherapy.



Key Points

- Multidisciplinary consultation in the primary management of EOC.
- Patient selection for neoadjuvant systemic treatment.
- Personalization of treatment according to BRCA mutation is important (also considering patients as possible candidates for clinical trials).

Expert Opinion

Domenica Lorusso

Key Points

1. Management of primary advanced disease includes the following:
   - Primary cytoreductive surgery or NACT followed by interval cytoreductive surgery
   - Intravenous chemotherapy
   - Intraperitoneal chemotherapy
   - Dose-dense chemotherapy
   - Maintenance treatment
2. Management of recurrent disease includes the following:
   - Systemic therapy (standard and novel chemotherapeutic agents and biological agents)
   - Surgery: secondary cytoreductive surgery (SCS)

   The choice depends on many factors such as the previous received treatments, the BRCA mutational status, the performance status, the site and numbers of recurrences, and finally the time interval between the last cycle of first-line chemotherapy and recurrence.

Recommendations

ESMO

- ► https://www.esmo.org/Guidelines/Gynaecological-Cancers/ESMO-ESGO-Consensus-Conference-Recommendations-on-Ovarian-Cancer

AIOM

Hints for a Deeper Insight

- Adherence to treatment guidelines for ovarian cancer as a measure of quality care: ► https://www.ncbi.nlm.nih.gov/pubmed/23812456
- 2004 consensus statements on the management of ovarian cancer: final document of the 3rd International Gynecologic Cancer Intergroup Ovarian Cancer Consensus Conference (GCIG OCCC 2004): ► https://www.ncbi.nlm.nih.gov/pubmed/16239238
- Optimal primary surgical treatment for advanced epithelial ovarian cancer: ► https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD007565.pub2/full
- Fifth Ovarian Cancer Consensus Conference of the Gynecologic Cancer InterGroup: first-line interventions: ► https://www.ncbi.nlm.nih.gov/pubmed/28327917
- Survival benefits with diverse chemotherapy regimens for ovarian cancer: meta-analysis of multiple treatments: ► https://www.ncbi.nlm.nih.gov/pubmed/17105988
- Recurrent ovarian cancer: ► https://academic.oup.com/annonc/article/27/suppl_1/i63/1785593

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Endometrial and Cervical Cancers

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Gynecological Cancers

Contents

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Learning Objectives

By the end of the chapter, the reader will:

- Have learned the most important knowledge of uterine and cervical cancers
- Be able to define the diagnostic and therapeutic procedures relative to uterine cancers and apply them into clinical practice
- Have understood the most innovative therapeutic strategies of uterine cancers
- Have known the potential future therapeutic perspectives of both uterine and cervical cancers

53.1 Introduction

The uterus can be essentially divided into two distinct anatomic regions (Fig. 53.1):

- Corpus
- Cervix

Uterine corpus and cervical cancers represent two malignancies very different from each other in terms of epidemiology, risk and etiological factors, histopathology/molecular biology, and therapeutic approaches. Thus, we will deal with these two arguments as separated topics in this chapter.

53.2 Uterine Corpus Cancers

Uterine corpus cancers are the fourth most common malignancy and the sixth leading cause of cancer-related death in female sex in the United States, with estimated incidence and mortality rates of 7% (61,880 new cases) and 4% (12,160 deaths), respectively, in 2019 [1] (Figs. 53.2 and 53.3).

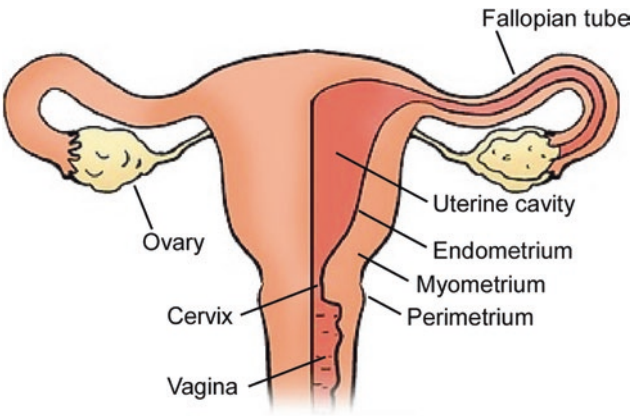


Fig. 53.1 In the cranio-caudal direction, we can distinguish the *fundus* (the uppermost rounded part), *uterine corpus* and *uterine cavity*, *internal uterine orifice*, *cervix* (known also as "uterine neck" and protruding into the vagina) and *cervical canal*, and *external uterine orifice*. The uterine wall is composed of three layers: *endometrium* (the innermost), *myometrium*, and *perimetrium* (the outermost)

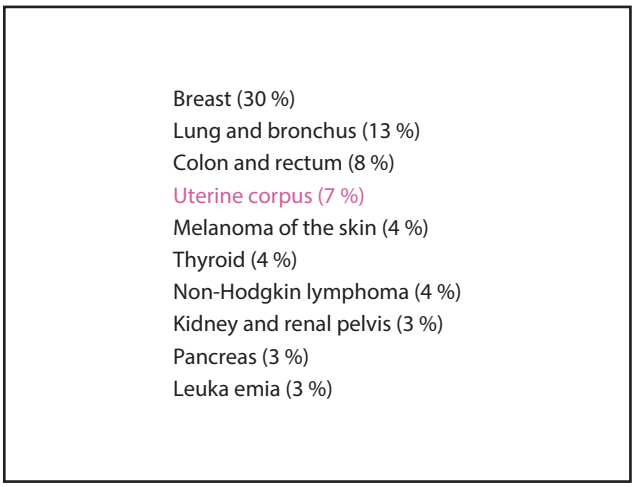


Fig. 53.2 Estimated incidence rate by cancer types in female sex, United States, 2019

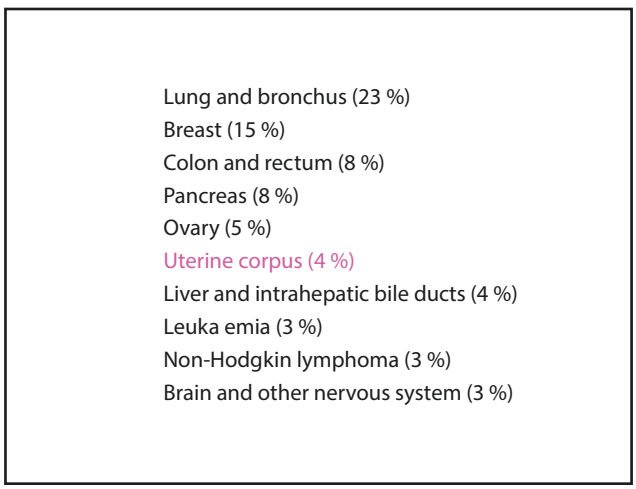


Fig. 53.3 Estimated mortality rate by cancer types in female sex, United States, 2019

These numbers are in line with epidemiological data emerged from Europe in 2018: incidence rate 6.6% (121,600 new cases) and mortality rate 3.5% (29,600 deaths). Particularly, corpus cancers were found to be at the fourth and seventh places, respectively, in terms of incidence and mortality [2] (Figs. 53.4 and 53.5).

53.3 Endometrial Cancer

53.3.1 Epidemiology

Endometrial cancer (EC) is the sixth most common cancer in women with over 382,000 new cases and 90,000

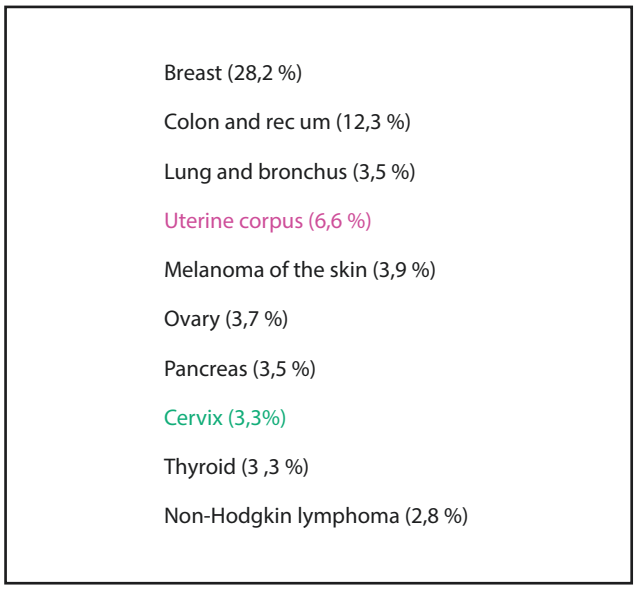


Fig. 53.4 Incidence rate by cancer types in female sex, Europe, 2018

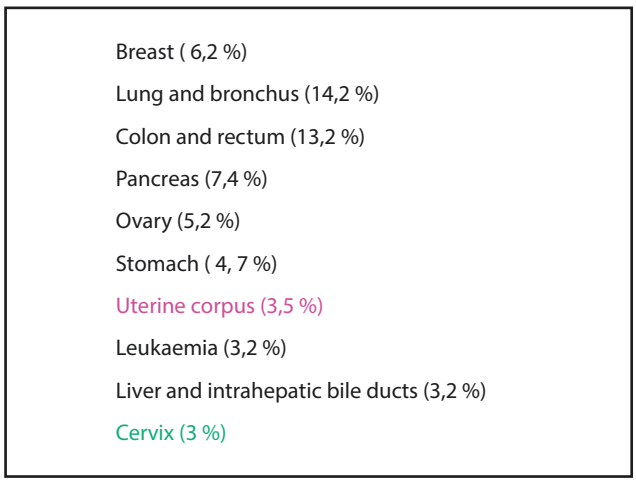


Fig. 53.5 Mortality rate by cancer types in female sex, Europe, 2018

deaths found in 2018 worldwide. The countries with the highest incidence rate in 2018 are represented in the Fig. 53.6 [3]. EC is at the first place in terms of frequency among uterine corpus cancers and the most representative gynecological tumor in developed countries (likely due to environmental and dietetic factors); there, over the last decades, a gradual increase both in the incidence and mortality rate has been registered, related to prolonged life expectancy, bad lifestyle habits, rise of advanced-stage cases, and poor-risk histologies [4]. EC is typically diagnosed in post-menopausal women (>90% in the 50–70 age group, median age 63 years) and at an early stage (≃67% at stage I) [5] (Fig. 53.7). Five-year survival rate varies according to the stage at the diagnosis [6] (Fig. 53.8).

53.3.2 Etiological, Risk, and Protective Factors

Increased estrogen levels, especially not enough to be counterbalanced by adequate progesterone levels, are the most important predisposing factor to the endometrial cancer onset. We can divide the *risk factors* as shown in Table 53.1.

Contrariwise, we can recognize as *protective factors*:

- Active lifestyle, maintaining of a normal weight, consumption of dietary fibers, and coffee consumption [24]
- Use of combined estrogen-progestin contraceptives (CCs) [25–27]
- Use of combined estrogen-progestin HRT in menopause [28]

53.3.3 Prevention and Screening

Any validated screening test for EC is designed for the general population; certainly, it is recommended to adopt primary and/or secondary prevention measures for those women who present with increased or high risk to develop an endometrial cancer.

Primary prevention may carry out correcting some risk factors: adoption of healthy dietary habits and active lifestyle.

Women who have an *increased risk* of EC onset (for the presence of concomitant risk factors) should undergo a careful surveillance if any endometrial thickness (≥3 mm) is detected on ultrasonography or if any unexpected vaginal bleeding has recently appeared, especially when in treatment with unopposed estrogen or tamoxifen. Likewise, the same can be even more so applied to *high-risk* groups: women who underwent a fertility-sparing treatment for adult granulosa cell tumor (AGCT), epithelial estrogen-secreting ovarian tumor, endometrioid EC (EEC), and well-differentiated (G1), premalignant lesions (atypical endometrial hyperplasia (AEH), endometrial intraepithelial neoplasia (EIN)).

Lynch syndrome (LS) type II deserves a separate discussion. Mutations relative to genes involved in the mismatch repair (MMR) system (MLH1, MSH2, MSH6, PMS2) can occur up to 5% of EC cases, thus typically presenting with microsatellite instability (MSI) on immunohistochemistry (IHC) [29, 30].

Genetic counselling and testing should be proposed to all those patients who had an EC and colorectal cancer, especially when younger than 50 years and/or with a significant related family history [31–35].

Patients with known germline LS mutations should respect at least a close surveillance program from the age of 35 years that includes annual clinical gynecological examination, trans-vaginal ultrasonography (TVUS), and endometrial biopsy [33–36].

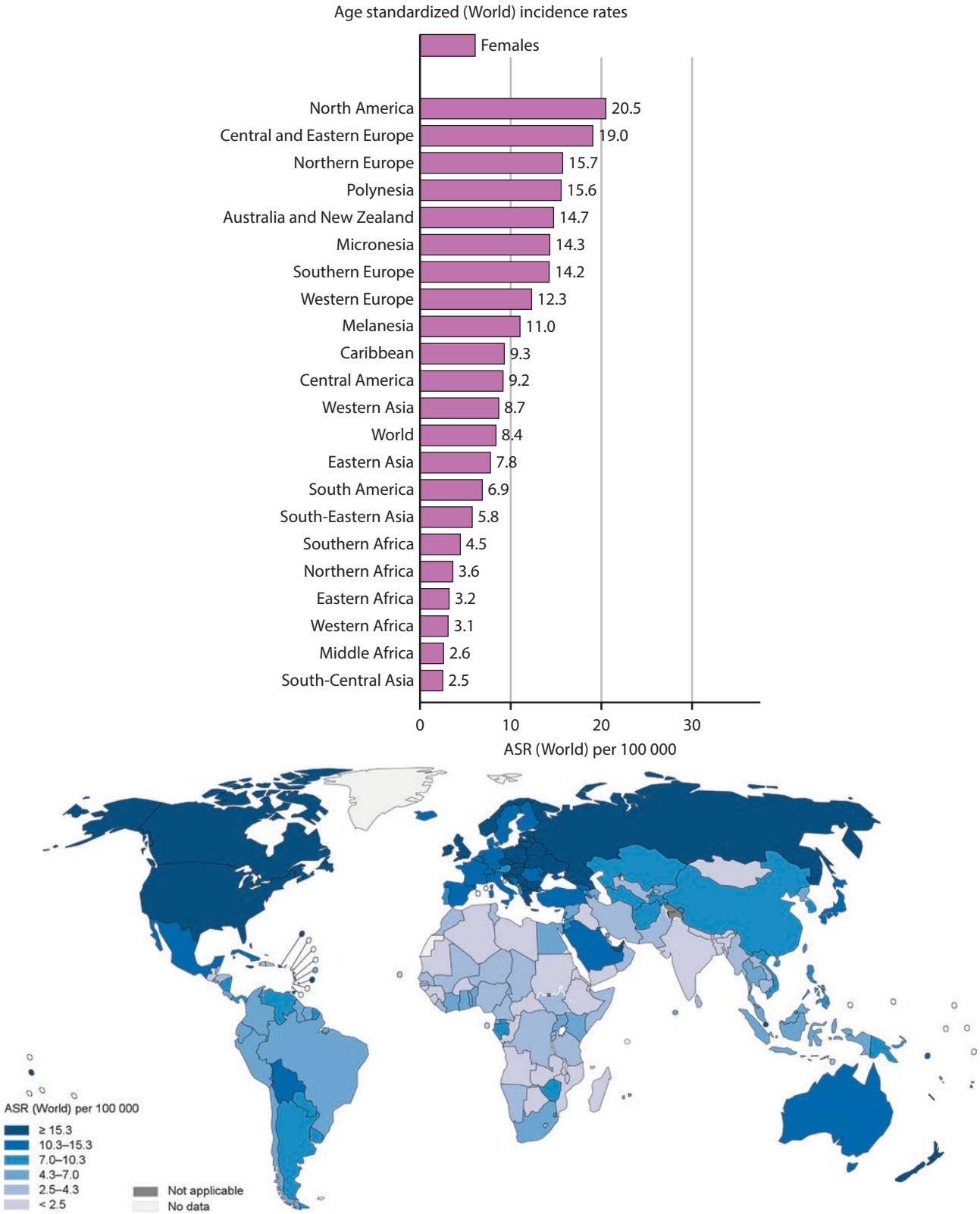


Fig. 53.6 Age-standardized incidence rates of EC in the world

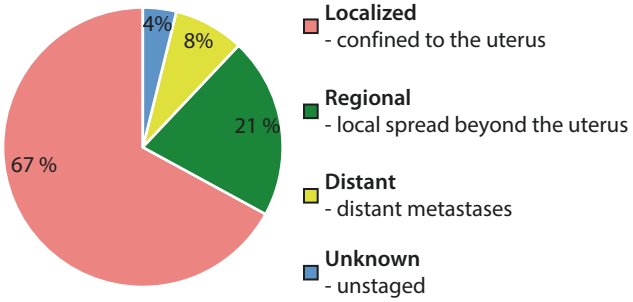


Fig. 53.7 Percent of cases by stage at the diagnosis

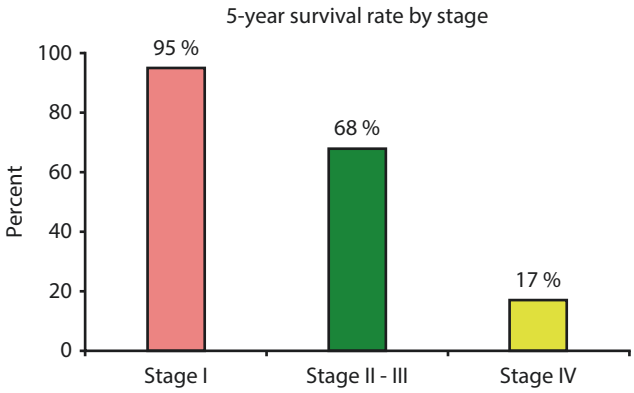


Fig. 53.8 5-year survival rate according to the stage

53

Table 53.1 Principal *risk factors* for endometrial cancer

Enviromental factors	Hormonal factors	Hereditary/familial factors
Overweight (BMI 25–29,9 kg/m²)/obesity (BMI ≥ 30 kg/m²): *Higher BMI* associated with higher relative risk (RR) [7–10] *Chronic hyperinsulinemia* leads to: (a) Higher levels of free insulin-like growth factor (IGF) with mitogenic/anti-apoptotic effect (b) Higher estrogen levels secondary to sex hormone-binding globulin (SHBG) lowering [11] *Medical history of metabolic syndrome, type II diabetes mellitus (T2DM), hypertension, chronic hepatopathy, and sedentary habits* may be observed [12] Correlation with good prognostic factors: low grade, endometrioid histology, and presentation at early stage	*Early menarche (<12 years)* *Late menopause (>55 years)* [15]	*Lynch syndrome (LS)/hereditary Non-polyposis colorectal cancer Syndrome (HNPCC):* Lifetime risk for EC and colorectal cancer 40–60% [23] Lifetime risk for ovarian cancer 9–12% Correlation with poor prognosis
Eating habits: *High consumption of red meat* (100 g/die) [13] *High consumption of saturated fat* [14]	*Nulliparity/infertility*	
	Polycistic ovarian syndrome (PCOS) [16]	
	Estrogen-producing tumors *Ovarian granulosa tumors* *Theca cell tumors* [17]	
	Use of uncombined menopausal hormone replacement therapy (HRT) [18]	
	Use of tamoxifen for breast cancer treatment: Estrogenic/proliferative activity on endometrium [19–21] Increased risk in post-menopausal women [22] Increased risk correlated to dose and time of treatment	

The risk-reducing surgical strategies (hysterectomy and bilateral salpingo-oophorectomy), should be taken into consideration and evaluated within a multidisciplinary context, at the age of 40 years or after the offspring desire is satisfied [37, 38].

53.3.4 Histopathology and Molecular Biology

An old and outdated dualistic model divided ECs into two pathogenic entities, as shown in ◘ Table 53.2 [39]. Recently, The Cancer Genome Atlas (TCAG) Research Network, innovatively, identified four different EC subgroups on the basis of molecular profiles [40] (◘ Table 53.3).

POLE ultramutated and MSI hypermutated subgroups appear to be related to a better prognosis [41]; thus, the preliminary molecular characterization of an endometrial carcinoma could help clinicians to make more tailored therapeutic decisions, mostly in those apparent high-risk cases which could benefit or not from such a treatment on the basis of their molecular profiles.

◘ Table 53.4 shows the main EC histological subtypes and their relative frequencies. The ECC is typically composed of glands, recalling those of normal endometrium, well/moderately differentiated with respective solid component <5% (G1) and 6–50% (G2). Atypical endometrial hyperplasia (AEH) and endometrial intraepithelial neoplasia (EIN) are considered precursor lesions. Likewise, the serous endometrial intraepithelial carcinoma (SEIC) would seem the premalignant lesion of serous ECs.

All the other special histotypes represent high-grade and more aggressive epithelial variants of ECs. Serous-papillary and clear cell ECs usually involve more elderly women [42]. The first is frequently related to pelvic irradiation and tamoxifen-based hormone treatment; the second, indeed, embraces a heterogeneous group of typical and less typical (serous-like) clear cell carcinoma. Mucinous and squamous ECs must be carefully distinguished from respective cervical cancer histotypes. The carcinosarcoma, also known as malignant-mixed Müllerian tumor (MMMT), is considered a metaplastic epithelial tumor [43, 44]. The undifferentiated ECs are very rare clinical entities, microscopically composed of undifferentiated cells organized in solid mass. They included both small cell neuroendocrine (chromogranin, synaptophysin-positive) and de-differentiated carcinomas [45]. The latter is commonly found in LS and is characterized by the concomitant presence of G1/G2 adeno- and undifferentiated components.

◘ **Table 53.2** *Dualistic model*

Principal characteristics	Type I	Type II
Histology	Endometrioid	Serous, clear cell
Relation to estrogen	Yes	No
Differentiation grade	G1/G2	G3
Prognosis	Good	Poor
Molecular alterations	PI3K, PTEN silencing, defects on repair system genes, MSI, KRAS, CTNNB1	Serous → TP53, p16 inactivation, E-cadherin lowering, HER-2 overexpression Clear cell → ARID1A

G1 well differentiated, *G2* moderately differentiated, G3 poorly differentiated, PI3K phosphatidylinositol 3-kinase, *PTEN* phosphatase and tensin homolog, *HER-2* human epidermal growth factor receptor 2, *CTNNB1* catenin-β 1 gene, *ARID1A* AT-rich interactive domain-containing protein 1A

53.3.5 Clinical Presentation and Diagnosis

Almost all patients with EC present with abnormal (post-menopausal or intermenstrual) vaginal bleeding. Leukorrhea, pelvic and low back pain, leg edema consequent to intra-abdominal lymph node involvement, bowel obstruction, bone pain related to the presence of metastases, and dyspnea could be other possible symptoms complained by patients usually with advanced EC.

When an EC is suspected, the common clinical practice provides, first of all, the execution of a TVUS (+/− color Doppler) to detect any eventual focal or diffuse endometrial thickening [46, 47]. A post-menopausal endometrial thickness >3 mm or an inappropriate pre-menopausal endometrial thickening, associated with a vaginal bleeding, must be seen as a "wake-up call," and further investigations are necessary. Particularly, endometrial sampling through US-/hysteroscopy-guided biopsy or dilatation and curettage (D & C) is usually the next step. Conventional D & C is associated with lower accuracy and discomfort for patients, whereas highly sensitive devices, like Pipelle, Vabra aspirator or Tao brush, and SAP-1, are increasingly established and better-tolerated endometrial samplers]. Obviously, the visually direct endometrial biopsy by hysteroscopy guide implies a higher accuracy [48]. Besides, saline infusion sonography enables to differentiate focal from diffuse endometrial involvement. Histological examination is based on morphological features, supported by IHC stains and, sometimes, by the research for specific molecular alterations. The differential diagnosis between benign and malignant lesions is allowed by

Table 53.3 *New model* by TCGA

POLE ultramutated	MSI hypermutated	Copy number (CN) low	Copy number (CN) high
High mutagenicity	High mutagenicity	Low mutagenicity	Low mutagenicity
Mutations on POLE 58 exonucleasic domain	MSI consequent to dysfunction of MMR proteins (above all, MLH1 promoter hypermethylation)	Microsatellite stability (MSS)	MSS
Infrequent copy number aberration	Infrequent copy number aberration	Low copy number aberration	High copy number aberration
Mutations on PI3KCA, PI3KR1, PTEN, KRAS, and FBXW7	Mutations on PTEN, KRAS, and RPL22	Mutations on CTNNB1	Mutations on TP53, FBXW7, and PPP2R1A
Mostly high-grade (G3) EECs	Mostly EECs	Mostly EECs with positive estrogen and progesterone receptors (ER/PR)	Mostly serous ECs
Good prognosis (5-yr RFS = 93%)	Good prognosis (5-yr RFS = 95%)	Poor prognosis (5-yr RFS = 52%)	Poor prognosis (5-yr RFS = 42%)

POLE 58 DNA polymerase subunit ε, *RFS* relapse-free survival, *PI3KCA* PI3K catalytic subunit α, *PI3KR1* PI3K regulatory subunit α, *FBXW7* F-box/WD repeat-containing protein 7, *yr* year, *RPL22* ribosomal protein L22, *PPP2R1A* protein phosphatase 2 scaffold subunit α

Table 53.4 *EC histotypes*

Histotype	Frequency (%)
Endometrioid	≃80%
Serous-papillary	<10%
Clear cell	≃4%
Mucinous	≃1%
Squamous	<1%
Mixed[a]	<1%
Carcinosarcoma	<1%
Undifferentiated	<1%

[a]All tumors whose non-predominant component exceeds 10%

all these analyses. For example, AEH/EIN is typically characterized by loss of PTEN and PAX-2 (paired box gene-2) expression compared to benign lesions; or the loss of p53 commonly identifies a SEIC, in contrast to its benign mimics. Wilms tumor gene (WT-1) is searched for serous EC. Moreover, to distinguish an endocervical EC from a cervical cancer, searching for ER/PR, vimentin, carcinoembryonic antigen (CEA), and p16 is recommended. Histotype and grade differentiation are the most important features to be taken into account when a malignant endometrial sample is analyzed, as these will influence the therapeutic choice.

53.3.6 Pre-operative Work-Up, Staging, and Risk Groups

The pre-operative work-up may be schematized as shown in Fig. 53.9.

The current staging system for EC is based on the last FIGO (*Fédération Internationale de Gynécologie et d'Obstétrique*) classification, revised and published in the year 2009 [49] (Table 53.5).

Unfavorable prognostic factors associated with high risk of recurrence not reported by the FIGO staging but to be taken into consideration are myometrial invasion ≥50%; special histotypes; high-grade differentiation; lymphovascular space invasion (LVSI); tumor size >2 cm; nodal, lower uterine segment and extrauterine involvement; young age; and molecular LS profile [50–52]. Indeed, based on these clinico-pathological factors, a risk-group classification can help clinicians to make therapeutic decisions in adjuvant setting (Table 53.6).

53.3.7 Surgical Treatment, Lymphadenectomy, and SLND

In the figure below (Fig. 53.10), we report the surgical management algorithm of EC.

As regards stage I, the gold standard for surgery consists of extrafascial simple total hysterectomy without vaginal cuff [55]. Minimally (laparoscopic, robotic) invasive surgery would not seem to negatively affect the overall survival (OS) and progression-free survival

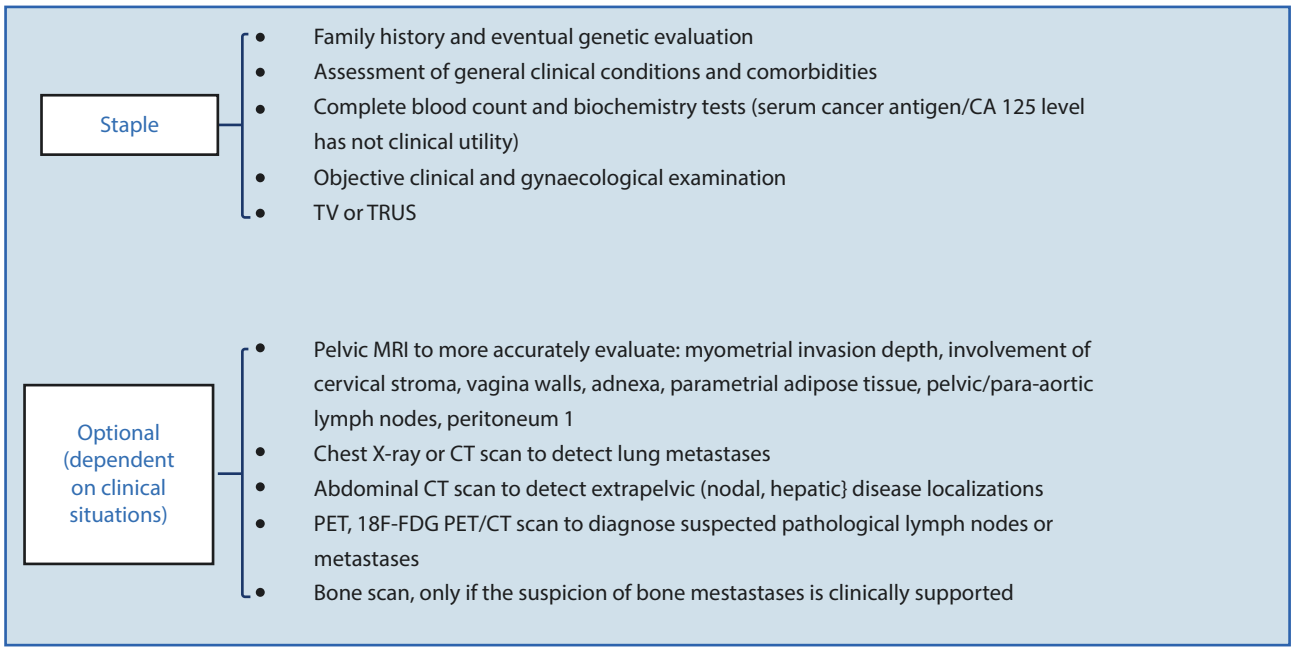


Fig. 53.9 Pre-operative work-up. TRUS trans-rectal ultrasonography, MRI magnetic resonance imaging, CT computed tomography, PET positron emission tomography, FDG fluorodeoxyglucose

Table 53.5 EC staging according to the FIGO classification (2009)

Stage I	*Tumor confined to the uterine corpus*
IA	<50% myometrial invasion
IB	≥50% myometrial invasion
Stage II	*Tumor confined to the uterus, but with cervical stromal involvement*
Stage III	*Local and/or regional extension of the tumor*
IIIA	Uterine corpus serosa and/or adnexa
IIIB	Vagina and/or parametrium
IIIC	Pelvic or para-aortic lymph nodes
IIIC1	Pelvic lymph nodes
IIIC2	Para-aortic lymph nodes (+/− pelvic lymph nodes)
Stage IV	*Tumor involves bladder and/or bowel mucosa and/or distant metastases*
IVA	Bladder and/or bowel mucosa
IVB	Distant metastases[a] (including also intra-abdominal and inguinal lymph nodes)

[a]Most frequent metastasizing sites are the lymph nodes, liver, lung, brain, and bone (vertebrae)

Table 53.6 Risk groups

Risk group	Prognostic factors
Low	Stage IA, endometrioid, G1/2, LVSI negative
Low/ intermediate	Stage IB, endometrioid, G1/2, LVSI negative
High/ intermediate	Stage IA, endometrioid, G3, LVSI negative/ positive
	Stage IA/IB, endometrioid, G1/2, LVSI positive
High	Stage IB, endometrioid, G3, LVSI negative/ positive
	Stage II
	Stage III without residual disease (R0)
	All stage special histotypes (serous-papillary, clear cell, mucinous, squamous, carcinosarcoma, small cell neuroendocrine carcinoma, de-differentiated carcinoma)

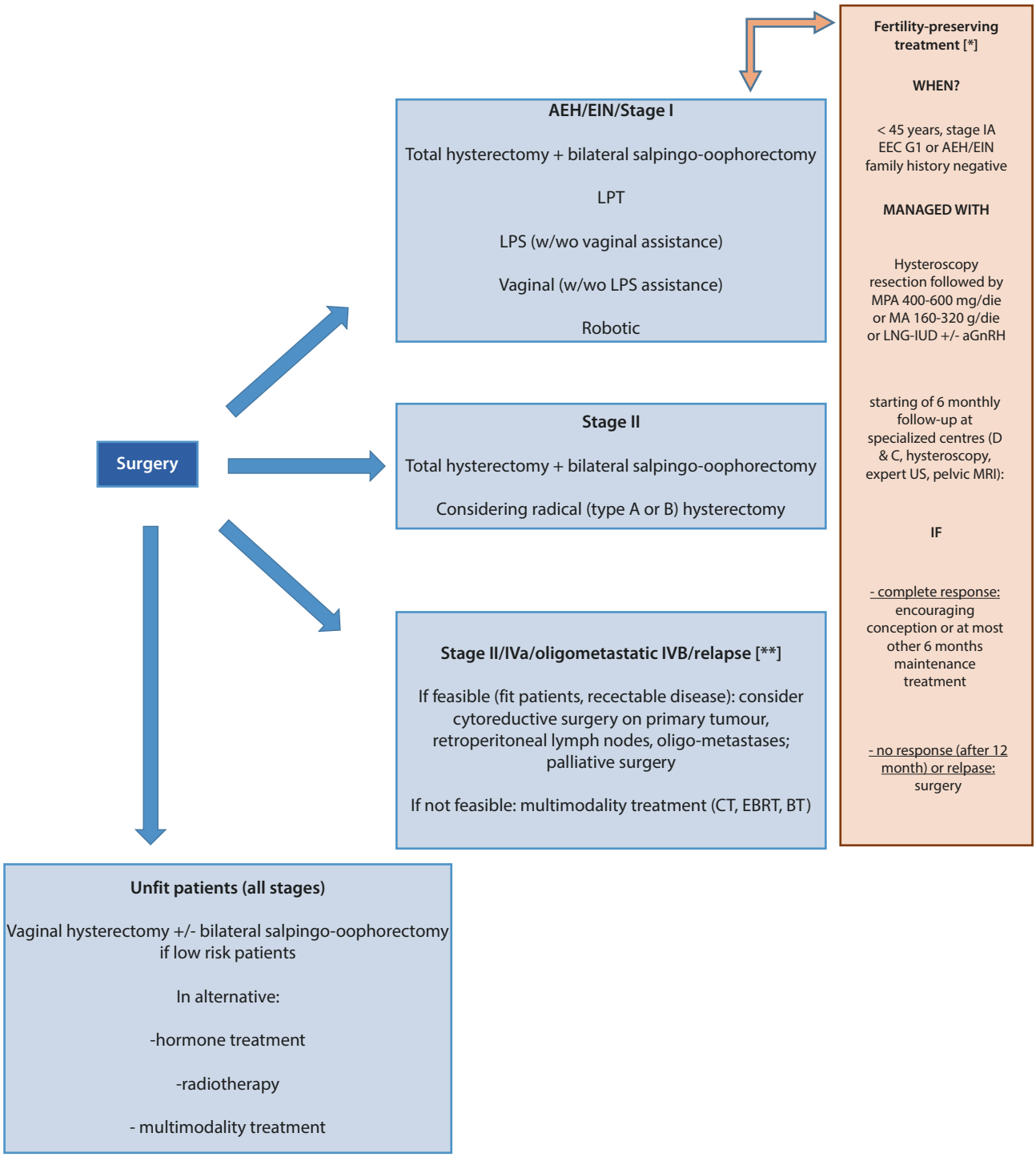


Fig. 53.10 Surgical management algorithm. LPT laparotomy, LPS laparoscopy, w/wo with or without, MPA medroxyprogesterone acetate, MA megestrol acetate, LNG-IUD levonorgestrel intrauterine device, aGnRH gonadotropin-releasing hormone analogue, CT chemotherapy, EBRT external beam radiotherapy, BT intracavitary brachytherapy, * [53], ** [54]

(PFS), as demonstrated by Gynecologic Oncology Group/GOG-LAP2 and LACE studies; besides, it is associated, particularly the robotic approach, with shorter hospitalization, less intra- and post-operative complications, and better quality of life versus laparotomy [56–59]. Thus, it may be recommended in low- and intermediate-risk patients and could be considered for high-risk ones. The vaginal approach is contemplated for low-risk or unfit patients [60].

A radical hysterectomy, with the lateral extension of resection on parametrium (type A or modified-type B depending on the lateral level of resection), is the surgical procedure carried out from stage II with clear peritoneum involvement, to ensure the highest possibility of free margins. Exenteration (removal of the uterus, bladder, and rectum and permanent uro- and colostomy) is an option in extensively locally advanced stage III/IV cases or in central recurrence (after RT), when the possibility to obtain no residual macroscopic disease is high.

The standard surgical approach remains the same for special histotypes, but in addition a staging omentectomy should be executed for serous-papillary histotype.

The role of systematic lymphadenectomy (pelvic and para-aortic up to the level of renal veins) is crucial to reduce the lymphatic spread, for staging purposes, and to guide adjuvant therapies (Fig. 53.11). As demonstrated by the SEPAL study results, the removal of para-aortic lymph nodes increases the OS in high-risk population, and the number of excised lymph nodes has an important impact [61].

Intermediate-risk patients would not seem to benefit from systematic lymphadenectomy in terms of OS and PFS, but it should be considered for staging intent and, therefore, to choose the proper adjuvant treatment [62, 63]. Also stage III/IV cases do not gain advantages in OS and PFS from a systematic lymphadenectomy, but it represents an integral part of the comprehensive staging.

The technique of SLND (sentinel lymph node dissection) has been studied over the last few years for the uterine cancers and to date is to be considered experimental. The cervical tracer injection, like fluorescent indocyanine green, enables to individuate bilaterally sentinel lymph nodes (SLNs) with high sensitivity, identifying even micrometastases or isolated tumor cells (ITC). This could allow to avoid an improper and not free from morbidity lymphadenectomy in those clinico-pathological high-risk cases with SLN negative or, conversely, to ensure a more radical surgery in low-risk patients with SLN positive, for which initially there was no planning for a lymph node systematic removal [64, 65].

53.3.8 Adjuvant Treatment

In the figures below (Figs. 53.12, 53.13, 53.14, and 53.15), we report different flowcharts relative to adjuvant treatment algorithms by disease stage and risk groups, based on more recent clinical trial results.

- **Stage I – *low, intermediate, and high risk***

BT has the role to reduce vaginal recurrence, while EBRT has been associated with a lower risk of pelvic recurrence and major local toxic effects versus BT. BT does not seem to increase neither the local control of disease nor the OS in low-risk patients. As demonstrated from several studies, the intermediate-risk patients do not gain advantages in terms of OS from EBRT, though this increases the local control of disease, mostly in the presence of high-risk factors. Therefore, BT is consid-

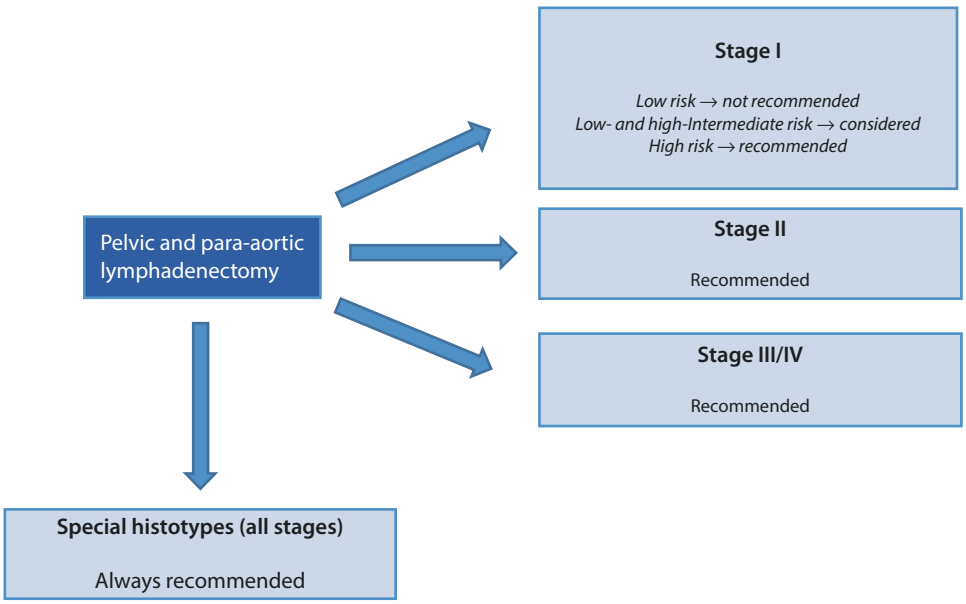


Fig. 53.11 Role of systematic lymphadenectomy. SLND sentinel lymph node dissection

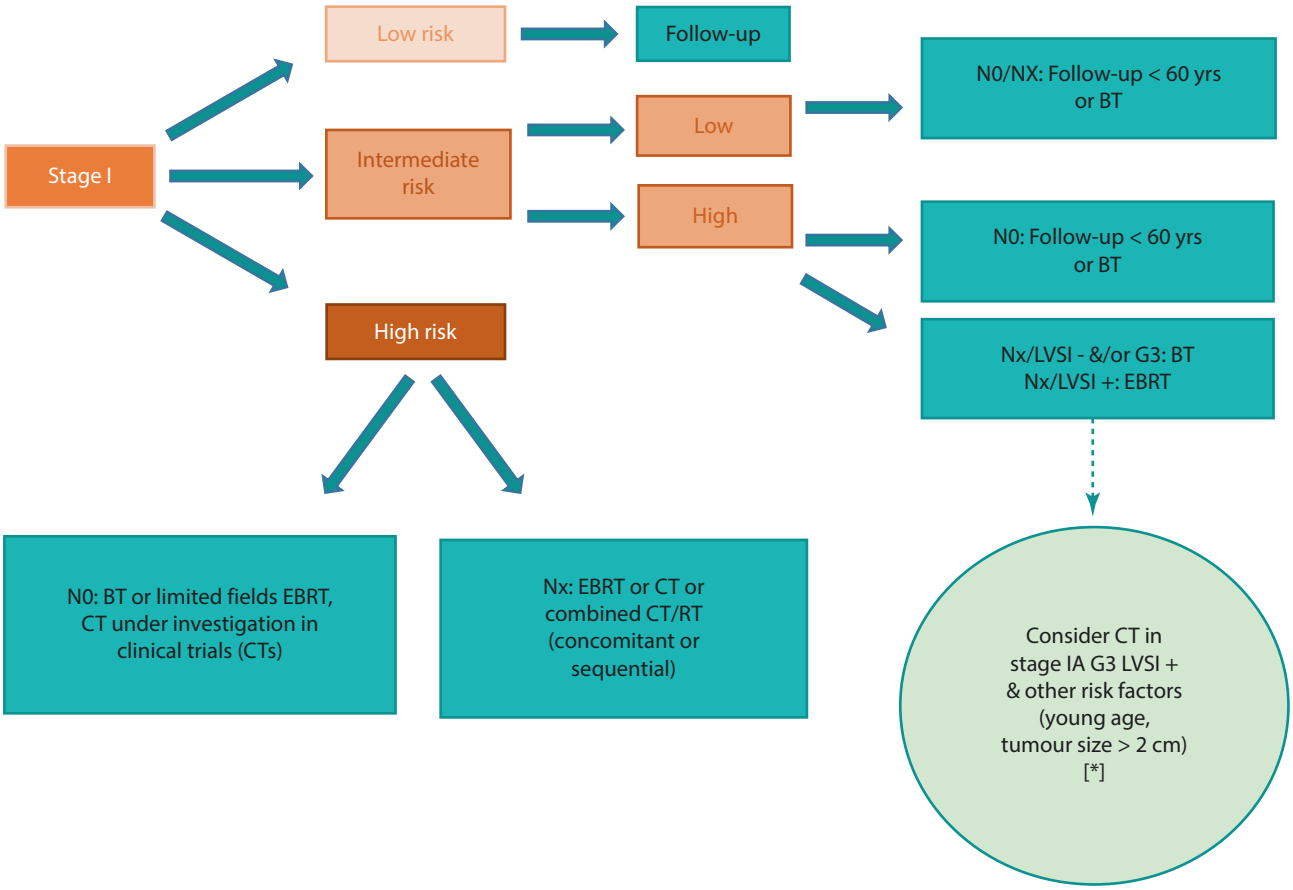


Fig. 53.12 Adjuvant treatment in stage I. N0 no regional lymph nodes involved, Nx regional lymph nodes not assessable, * [66]

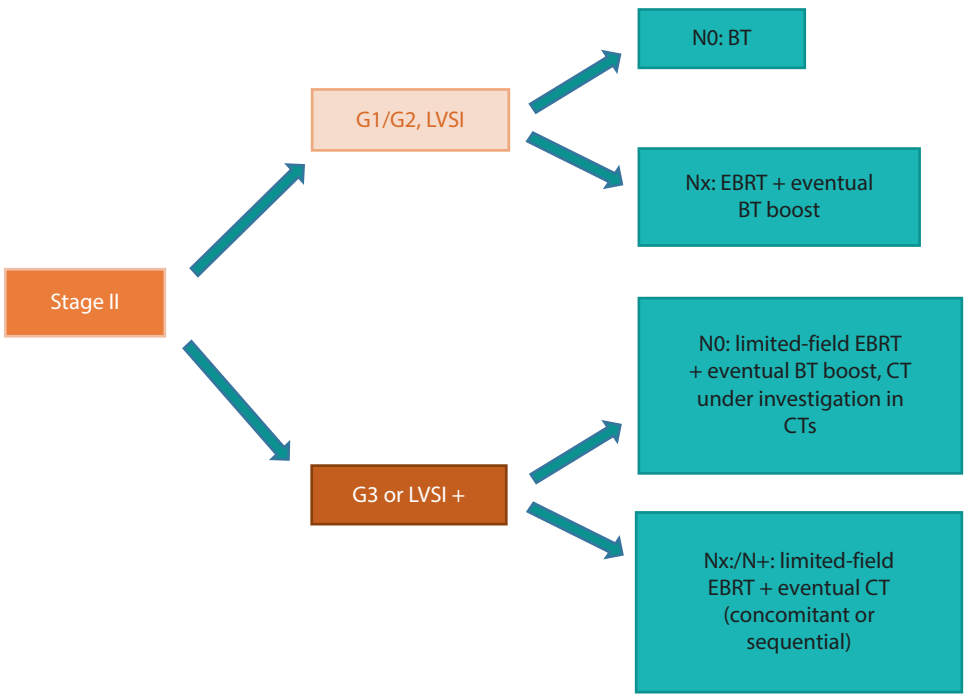


Fig. 53.13 Adjuvant treatment in stage II. N+ regional lymph nodes involved

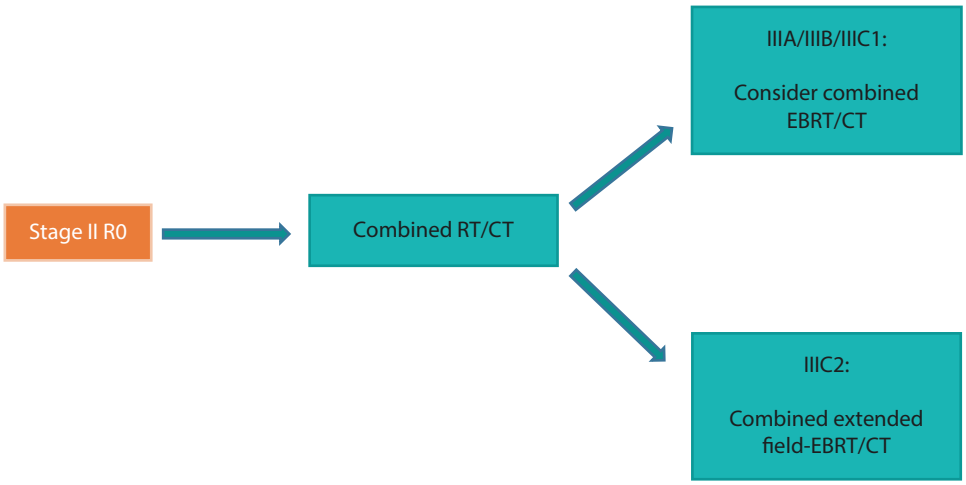


Fig. 53.14 Adjuvant treatment in stage III R0

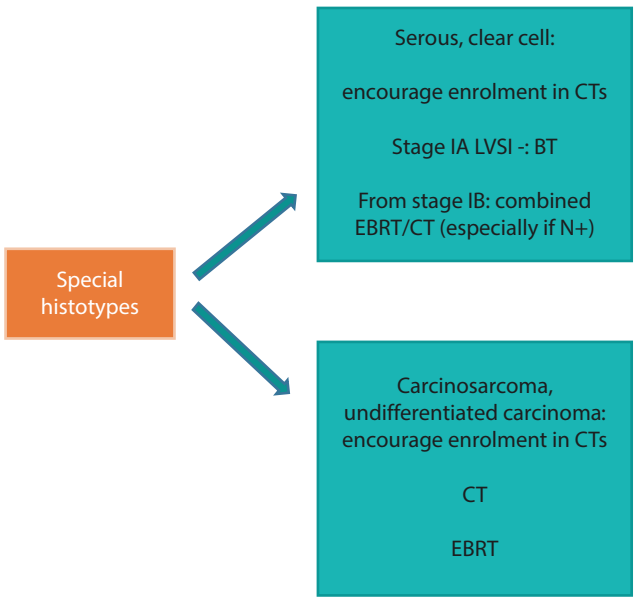


Fig. 53.15 Adjuvant treatment for special histotypes

ered sufficient to reduce vaginal recurrence in this group, except in those cases with several high-risk factors which likely deserve an EBRT [67].

- **Stage II – *high risk***
- **Stage III without Residual Disease – *high risk***
- **Special Histotypes – *high risk***

The PORTEC-3 trial, after a median follow-up of 6 years, has recently revealed that combined platinum-based CT/RT (concomitant cisplatin/RT followed by four carboplatin-paclitaxel cycles) increases both relapse-free survival (RFS) and OS in all high-risk groups (from stage I-high risk, without nodal status assessment, to stage III and special histotypes) [68, 69].

Interestingly, the impact of adjuvant CT was also investigated for each of the four molecular subgroups (see Table 53.3), using tissue samples from PORTEC-3 trial patients, and the results of this research were presented at the European Society for Medical Oncology (ESMO) 2019 meeting. The aim was to assign to specific molecular alterations the proper predictive value in terms of response to adjuvant CT/RT, to better identify patients who could benefit more from concomitant therapies. It was found that CN-high and POLE ultramutated subgroups report improved RFS if treated with the combination, unlike the MSI hypermutated population, who seems to not benefit from CT [70].

53.3.9 Advanced and Recurrent Disease

Locally advanced disease (stage IIIA/IIIB/IIIC with residual disease, IVA) typically benefits from multimodality treatments.

The role of surgery, as discussed above, has to be considered when the maximum cytoreductive effort (on primary tumor, pelvic and para-aortic and/or other enlarged lymph nodes, oligo-metastases in stage IVB) could likely ensure the absence of post-operative residual disease, though there are few evidence about its efficacy in such cases as distant metastatic diseases. Alternatively, the surgical approach may have a palliative purpose.

When surgery is not feasible (unfit patients, medical contraindications, unresectable tumors), radical frontline RT (EBRT and BT) plays an incisive role. Likewise, as surgery, also radiotherapy may exercise a palliative role, both on regional complications (bleeding, local pain, etc.) and distant lesions (painful bone metastases).

Sometimes, a multimodality approach is associated with a better outcome, such as reported for bulky diseases, in which carrying out systemic therapy (chemo- or

hormone) or surgery before RT could provide a more radical result.

A doublet chemotherapy, generally 3-weekly carboplatin-paclitaxel administered for six cycles, represents the standard of first-line medical care in unresectable patients [71]. Recently, as revealed by a phase II single-arm study (KCOGG1303), a dose-dense paclitaxel (days 1, 8, 15) plus carboplatin (day 1 every 3 weeks) regimen, in advanced or recurrent uterine corpus cancers, was assessed alike as safe and effective [72]. The triplet cisplatin-paclitaxel-adriamycin has been demonstrated to increase the response rate (RR), PFS, and OS versus the doublet cisplatin-adriamycin, at the price of increased toxic effects especially in fragile patients [73]. Interestingly, mono-platinum, anthracyclines, and taxane-based therapy have been associated with objective response rate (ORR) > 20%.

To date, there is not a standard second-line chemotherapy validated for patients who progressed on first-line platinum-based treatment. A recent meta-analysis has reported ifosfamide, oxaliplatin, pegylated liposomal doxorubicin (PLD), topotecan, and docetaxel as the most active chemotherapeutics in this setting [74, 75].

In selected cases, including either ER/PR-positive G1/G2 not rapidly progressive EEC or unfit patients, a progestin-based front-line treatment (MPA 200 mg/die, MA 160 mg/die), resulting in ORR 15–30% and OS 7–11 months, has to be taken into consideration [76]. After disease progression, tamoxifen, fulvestrant, and aromatase inhibitors (AIs) w/wo aGnRH could be considered as second-line hormone therapy [77]. The results of a phase II trial, based on the use of ribociclib (400 mg/die) and letrozole (2.5 mg/die) in patients with relapsed ER-positive EC, were recently presented at the American Society of Clinical Oncology (ASCO) 2019 meeting. After a median of two previous chemo-regimens, a PFS12 (PFS at 12 weeks) rate of 55% was achieved, thus encouraging efforts in revisiting old standard hormone treatment in specific subsets of EC patients [78].

Local relapse disease, similarly to locally advanced tumors, could take advantages from combined treatments. Patients who previously received RT and present with pelvic recurrence could undergo where feasible to prompt surgery (even exenteration) or chemo-/hormone therapy with neoadjuvant intent followed by surgery. Conversely, when never received, RT could be curative in a high percent of central-vaginal recurrence thanks to combined EBRT/BT [79]; besides, in regional or high-risk relapsed disease, patients could benefit from the RT/CT combination. The ongoing trial GOG-0238 will evaluate if the concomitant RT/cisplatin-based CT is also valid for vaginal relapse versus the only RT treatment [80].

53.3.10 New and Potential Future Therapeutic Perspectives

The treatment of metastatic endometrial cancer still represents an unmet clinical need; in fact, the median OS (mOS) is no longer than 12–15 months in advanced and recurrent disease. That is why a lot of efforts are moving toward the search and development of innovative tailored therapeutic opportunities, mainly considering patients' genetic and molecular characteristics and incorporating them as eligibility and stratification factors into CTs.

Several targeted therapies, relying on molecular pathways typically altered in EC, are being studied into phase II/III clinical trials; however, to date, none has been extended to clinical practice.

- *Mammalian Target of Rapamycin (mTOR) Inhibitors*
  Discouraging results come from phase II CTs, evaluating the use of mTOR inhibitors (temsirolimus, ridaforolimus) in chemo-naïve or pretreated patients and reporting alterations on PTEN-PIK3CA-AKT-mTOR signaling proliferative pathway [81]. A more recent phase II study reported an increased OS for ridaforolimus versus hormone and chemotherapy [82]. Other altered pathways as object of study include RAS-RAF-MEK-ERK-MAPK and FGFR-2 [83].
- *Anti-angiogenic Agents*
  It has been hypothesized that patients overexpressing vascular endothelial growth factor (VEGF), which is known to play an immunosuppressive action, could benefit from the use of anti-angiogenic agents, as resulted from preliminary clinical data [84–86]. Unfortunately, some randomized CTs (GOG-86P, MITO (Multicenter Italian Trials in Ovarian Cancer and Gynecologic Malignancies), END-2) have recently reported that the addition of the anti-angiogenic bevacizumab to standard CT does not significantly improve PFS nor in never-treated patients [87, 88].

  The ongoing NICCC study is recruiting patients with ovarian or endometrial recurrent clear cell carcinoma, randomizing them to receive standard CT or the multi-kinase anti-angiogenic inhibitor nintedanib, to evaluate if the experimental arm is associated or not to a longer PFS as primary endpoint [89].
- *Immune Checkpoint Inhibitors (iCKPi)*
  Encouraging results are emerging from the possible use of iCKPi particularly in those endometrial cancers associated with high genomic instability and mutational and neoantigen load, like POLE ultra-mutated and MSI hypermutated subgroups, usually presenting increase in tumor-infiltrating lymphocytes (TILs) and PD-1/PD-L1 protein expression

[90]. Preliminary positive clinical data moved the research toward the design of prospective randomized CTs, to compare immunotherapy (alone or combined with CT) to standard of care.

Recently, the Food and Drug Administration (FDA) approved the use of the anti-PD-1 monoclonal antibody pembrolizumab for PD-L1-positive pretreated EC patients, after the phase II KEYNOTE-158 study results, showing durable disease control rate (DCR) (73%) in heavily pretreated MSI-H (high) advanced EC [91].

The phase II PHAEDRA trial, discussed at the ASCO 2019 meeting, showed the activity of the anti-PD-L1 monoclonal antibody durvalumab in patients with advanced EC who received ≤3 prior CT. In detail, the d-MMR (deficient mismatch repair) cohort obtained higher objective tumor response rate (OTRR) and DCR compared to pMMR (proficient mismatch repair) cohort [92].

The phase I/II GARNET trial reported the efficacy of dostarlimab/TSR-042 (anti-PD-1 monoclonal antibody) in treating advanced/recurrent EC, obtaining significant RR regardless of MMR status [93].

The ongoing phase II randomized MITO END-3 trial will confront the experimental combination of carboplatin-paclitaxel with the anti-PD-L1 monoclonal antibody avelumab versus carboplatin-paclitaxel in first or subsequent lines of therapy. Analogously, the phase III randomized AtTEnd/ENGOT-en 7 (European Network for Gynaecological Oncological Trial groups) study is recruiting patients with advanced or recurrent EC to evaluate if the addition of the anti-PD-L1 monoclonal antibody atezolizumab to carboplatin-paclitaxel would improve PFS and OS compared to CT alone. An ongoing phase II trial by Oaknin et al. is investigating the role of the combination pembrolizumab-doxorubicin in advanced EC patients, treated with at least one previous platinum-based CT [94].

- *Combined Anti-angiogenic-iCKPi Therapy*

At the ESMO 2019 meeting, Mekker et al. presented the results of a phase Ib/II trial comprising a cohort of patients with metastatic EC, pretreated with no more than two CT lines and enrolled to receive the combination of the multi-kinase anti-angiogenic inhibitor lenvatinib with pembrolizumab. The synergistic combination showed a promising antitumor activity with an ORR at 24 weeks of 40% in overall population and significant efficacy also in not MSI-H/d-MMR subgroup. This leads to the combination approval by FDA as second-line treatment or for patients not candidate to definitively curative surgery or RT or, finally, in not MSI-H/d-MMR subgroups [95]. However, a high percent of severe adverse events (AEs) was recorded (grade 3–4 AEs almost in 70% of population, discontinuation rate 20%). The respective prospective randomized phase III trial (lenvatinib-pembrolizumab versus CT) is still recruiting [96].

- *Poly(ADP-Ribose) Polymerase (PARP) Inhibitors*

Also poly(ADP-ribose) polymerase (PARP) inhibitors – alone or combined with anti-angiogenic agents – could have a role in the treatment of advanced EC showing mutations in homologous recombination repair (HRR) genes (PTEN loss, ARID1A, etc.), as already shown from preclinical data. The rationale of a PARPi-anti-angiogenic combination therapy arises from the observation, into preclinical studies, of an HRR gene suppression and a major sensitivity to PARPi, in hypoxic states, with consequent synergistic effect [97, 98]. The randomized phase II three-arm NRG GY012 study is investigating if single-agent olaparib, single-agent cediranib, or the combination would prolong the PFS in recurrent, persistent, or metastatic EC [99]. Also the synergistic association of PARPi and iCKPi in more immunogenic EC subtypes (POLE and MSI positive) would seem promising, leading physicians to test this combination into CTs [100]; the phase II DOMEC study was designed to evaluate the efficacy of olaparib + durvalumab in advanced, persistent, or metastatic EC [101].

53.3.11 Follow-Up

The surveillance program after a radical and curative treatment for EC may be schematized as in Table 53.7.

Besides the clinical and physical examination, further investigation would not seem to impact on OS and should be performed only when the clinical suspicion of relapsed disease is high [103]. Certainly, these decisions must be entrusted to the clinical judgment depending on the individual and primary tumor risk group. The ongoing Italian multicenter randomized TOTEM trial could provide more indications regarding the best surveillance attitude, as it is evaluating different follow-up strategies, depending on relative patient risk [104].

Table 53.7 Follow-up for EC after curative therapy

Clinical and physical (gynecological/pelvic) examination	Every 3–4 months for the first 2 years and then 6-monthly until the 5th year from primary treatment [102]
Not ordinarily recommended exams	Tumor markers: CEA, CA 125, CA 19.9, and AFP, chest X-ray, abdominal US, CT scan, pelvic MRI, 18F-FDG PET/CT scan, whole-body bone scan

53.4 Cervical Cancer

53.4.1 Epidemiology

Cervical cancer (CC) is the most common gynecological tumor in developing countries, where it is even reported as a leading cause of cancer-related death, as consequence of a higher human papillomavirus (HPV) infection prevalence and a less availability of effective screening tools. Contrariwise, industrialized countries are experiencing a progressive decrease in the incidence and mortality rate, thanks to the implementation of efficacious primary and secondary prevention measures against HPV infection, and this is contributing also to the steady decline of global CC incidence and mortality. It has been calculated that the global prevalence of HPV infection exceeds 80%.

Worldwide, CC represents the fourth most frequent tumor in female sex with 570,000 new cases and 310,000 deaths registered in 2018, predominantly concentrated in Africa, Latin America, and Asia (these countries contributing to almost 90% of global deaths) [105] (Fig. 53.16). The estimated new cases and deaths in the United States in 2019 are 13,170 and 4250, respectively, whereas the not insignificant numbers in Europe in 2018 were 61,100 and 25,800, respectively, with an incidence rate of 3.3% and a mortality rate of 3% [1, 2].

Most cases of cervical carcinoma in situ (CIS) are diagnosed in younger age (25–35 years), whereas the peak incidence of invasive CC concerns the 40–65 age group, with a 5-year survival rate variable according to the stage (90%, 66%, and 40% for early, locally advanced, and metastatic disease, respectively) [106].

53.4.2 Pathogenesis and Molecular Biology

The primary cause of CC is represented by a persistent HPV infection, most commonly involving the basal cells of the transformation zone (TZ), which is a transitional area between the endocervical columnar epithelium and the squamous epithelium of the vagina; indeed, the HPV DNA is detected in almost all cases of cervical cancer (99.7%) [107]. Most (≃ 80%) carriers naturally eliminate HPV within 1–2 years, but the contemporary intervention of other risk factors predisposes to a chronic infection, spreading locally without systemic viremic phase, tough to overcome, initially leading to the development of precancerous lesions and secondarily of cervical cancer [108, 109] (Fig. 53.17).

Precancerous lesions refer to dysplastic conditions characterized by abnormal cervical cell growth and are known as cervical intraepithelial neoplasia (CIN) 1, 2, or 3, on the basis of dysplasia grade (see ▶ Sect. 53.3.4).

The human papillomavirus is a double-stranded DNA virus, with a capsid consisting of 72 capsomeres (Fig. 53.18). Its genome codifies for six early proteins (E1, E2, E4, E5, E6, and E7), which are responsible for the viral replication, and for two late structural proteins (L1 and L2), then assembled into capsomeres in different percentages (80 and 20%, respectively). When HPV integrates its DNA with that of the host cell, E2 stops inhibiting E6/E7 with consequent p53-retinoblastoma protein (pRb) suppression and morphological/functional cellular alterations. When persistently repeated, this process leads to a neoplastic transformation and progression within about 15 years or less.

Two-thirds of invasive CC cases are related to HPV 16 and 18 oncogenic genotypes, which predominantly affect the 30–39 age group [112] (Table 53.8). Less frequent oncogenic subtypes are responsible for the other 30% of cervical carcinomas, with HPV 31, 33, 35, 45, 52, and 58 being the most common after HPV 16 and 18.

As we can observe from Table 53.8, the prevalence of HPV 16 and 18 varies according to the histotype, with HPV 16 more related to squamous histology and HPV 18 to adenocarcinomas [113]. Other subtypes (6, 11) are frequently related to benign conditions, being responsible for 90% of genital warts [114].

As for ECs, the efforts of physicians are moving toward the attempt of characterizing CCs on the basis of their molecular profiles. At last ESMO 2019, some authors present the molecular characterization of 37 patients with advanced cervical carcinoma, finding 34 different pathogenic mutations (PI3KCA and KRAS being the most frequent) in about the 70% of the population. They individuated a correlation between KRAS mutations and adeno-/adenosquamous histologies, associated with a worse prognosis. Instead, PIK3CA mutations seem to be related to a better prognosis of mixed histology tumors. Knowing the biological profile could help clinicians to direct patients to increasing tailored therapies (see ▶ Sect. 53.3.12).

53.4.3 Primary and Secondary Prevention

For several decades, the Papanicolaou test (PAP test/smear) has represented the only validated screening tool to anticipate the diagnosis and the treatment of cervical precancerous/cancerous lesions. It works in identifying cytological alterations on a small scratched TZ cell sample, resulting in a low-sensitivity (50%) and strongly operator-dependent procedure (both on the execution and the interpretation of results). Lately, it has been outdone by the more sensitive and effective HPV test, which works in detecting the higher-risk genotype DNA on cervical cells [115, 116]. However, the PAP test maintains its usefulness in the 21–29 age group, whereas the HPV test alone or combined with the PAP test (co-test) is recommended from 30 years [117] (Fig. 53.19).

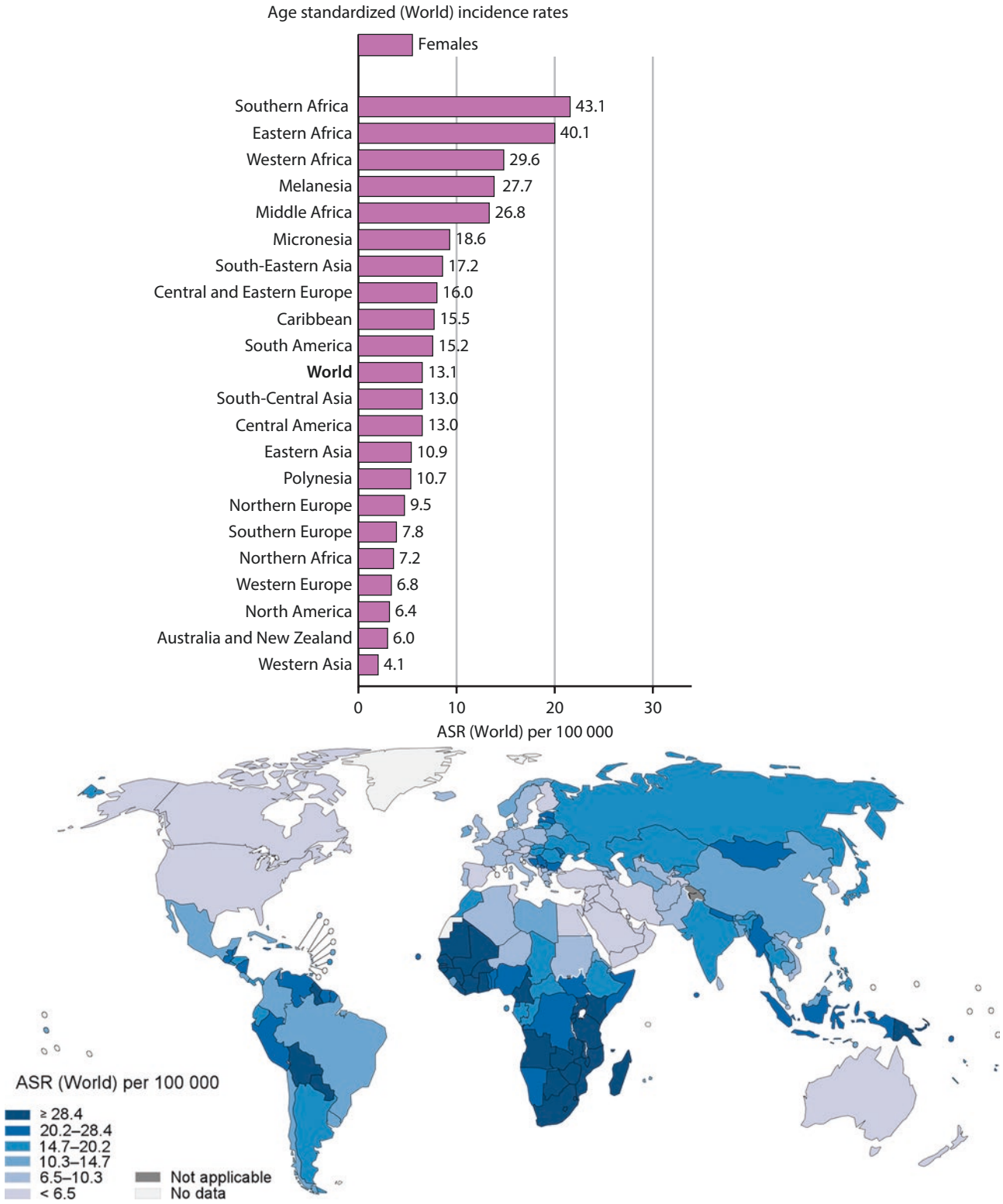


Fig. 53.16 Age-standardized incidence rates of cervical cancer in the world

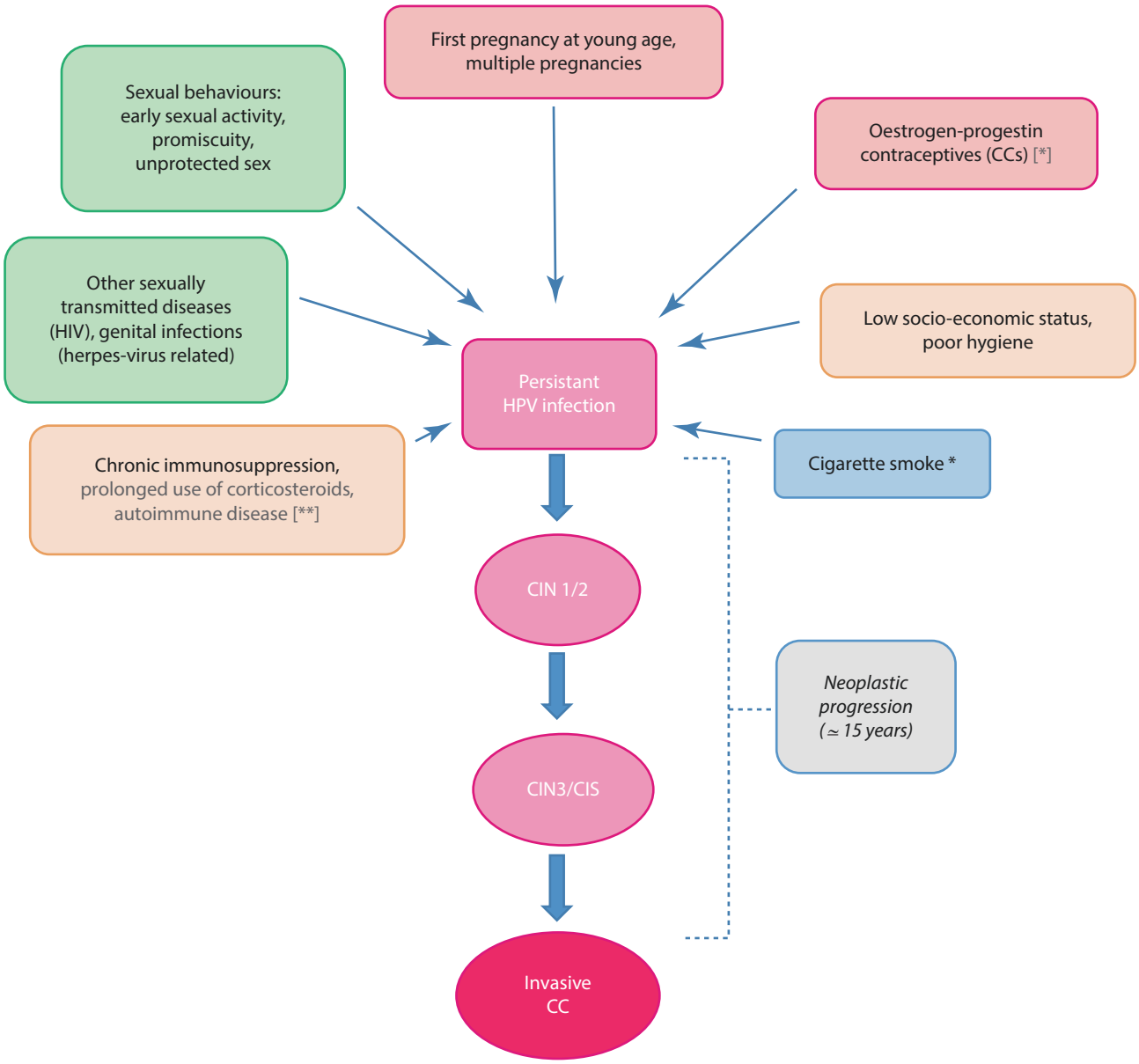


Fig. 53.17 Risk factors intervening on chronic HPV infection. HIV human immunodeficiency virus. *The correlation smoke-CC is strong for squamous histotype and, depending on lower immune local defense, consequent to a smoke-induced reduction of cervical Langerhans cells, * [110],** [111]

- Vaccines

Almost 90% of the general population comes, at least once in life, into contact with HPV and the peak incidence of the infection regards the 16–25 age group. Accordingly, the pharmaceutical industry has developed, in recent years, efficacious vaccines directed against the higher-risk HPV genotypes and to be administered at an early age. Currently, three vaccines are available for the primary prevention of HPV infection and its related pathologies (Table 53.9).

These three vaccines, in addition to offering a type-specific protection, would seem to have some cross-protective activity against other oncogenic viruses.

The duration of vaccine-induced protection, differentiated according to the number of doses received, will be better defined by longer follow-up of CTs. From observational studies till now conducted, it has emerged that vaccines reach almost 100% of protection efficacy against persistent infection and precancerous lesions up to 9 years (Cervarix®) [119, 120]. Furthermore, early findings from some clinical trials would support the

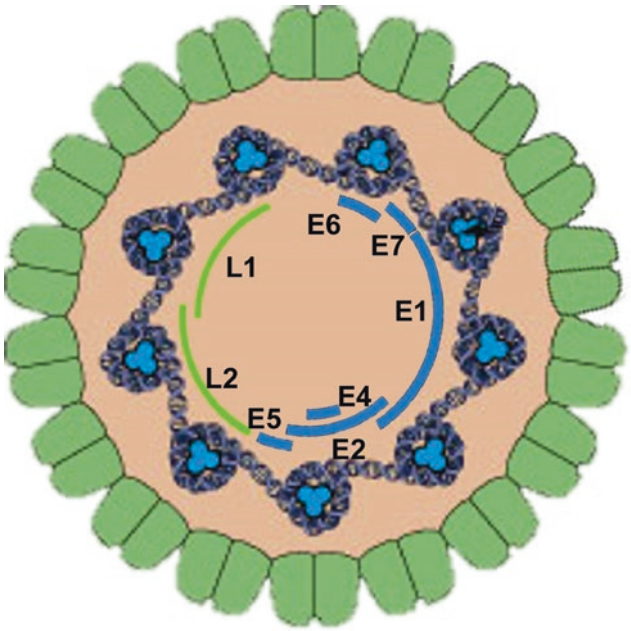


Fig. 53.18 Human papillomavirus (HPV)

Table 53.8 Prevalence of HPV genotypes by most frequent histotypes (squamous and adenocarcinoma)

Genotypes	Squamous carcinoma	Adenocarcinoma
16	59%	36%
18	13%	37%
31, 33,35, 39, 45, 52, 58, 59, 67, 68, 70, 85	≃ 28%	≃ 27%

comparable effect in long-term protection of one single vaccine dose versus two or three dose schedules. This could lead, in the future, to the introduction of one single dose schedule, facilitating the adhesion to screening program from low-income countries [121, 122].

A large randomized trial revealed that the nine-valent vaccine immunizes almost 100% of the population against all nine HPV genotypes, preventing effectively precancerous lesions, carcinomas in situ, and invasive cancers and showing 96% of efficacy against 6-month persistent infection, sustained by HPV 31, 33, 45, 52, and 58 genotypes. Thus, Gardasil 9® adds a cover against the genotypes responsible for 15% of cervical cancer and 4% of HPV-related pathologies in men (penile, anal, oropharyngeal cancers, genital warts) [123–125].

The immunization of young population will produce a drastic reduction in the prevalence of HPV infection and relative benign/malignant pathologies. Some CTs confirmed the efficacy, although lower, of vaccines even when administered to adult population (24–45 years) [126, 127]. Consequently, current screening programs need to be extended to older women and men. In women aged 20–29 with 80% vaccine coverage, a reduction in the invasive CC incidence rate of 63% within 2025 is expected.

Obviously, proper lifestyle (quitting smoking) and sexual habits (avoiding promiscuity and unprotected sex) have to be considered as useful primary prevention tools in reducing the risk of CC onset.

53.4.4 Histopathology

The World Health Organization (WHO) recognizes three categories of cervical epithelial tumors (Table 53.10).

- *Squamous Tumors*
  All squamous tumors and their precursors are related to HPV infections, mostly sustained by HPV 16 genotype, which is also associated with poorer prognosis (see Table 53.8).
  The *squamous cell carcinoma*, based on the growth pattern and morphological features, could microscopically present as one of the following variants: *keratinizing*, characterized by rare mitosis and the presence of keratin pearls, *non-keratinizing*, and *special histotypes* (*basaloid, verrucous, warty, papillary, lymphoepithelioma-like, squamo-transitional*).
- *Squamous intraepithelial neoplasia* refers to *CIN3*/*CIS*. Cervical intraepithelial neoplasia is generally considered as a precancerous lesion limited to the cervical epithelium (usually the TZ epithelium), which may present with various grades of dysplasia extension:
  - CIN1, mild dysplasia, involves the lower third of the epithelial thickness.
  - CIN2, moderate dysplasia, involves from one-third to two-thirds of the epithelial thickness.
  - CIN3, severe dysplasia, involves ≥ two-thirds of the epithelial thickness and practically coinciding with CIS, without going beyond the basement membrane.
- *Glandular Tumors*
  Most *adenocarcinomas* (80%) are endocervical and microscopically presenting architecturally well-differentiated (cytologically G2/G3) and eosinophilic cytoplasm. Variants include *mucinous,* the most common, with mucin-rich cells, usually G1 and associated with good prognosis and including, in turn, *endocervical*, *intestinal*, *signet-ring cell*, *minimal deviation*, and *villoglandular* subtypes; *endometrioid, clear cell, serous,* and *mesonephric* are all other rare variants.
  Glandular tumors and their precursors present a heterogeneous correlation with HPV, with the usual-type endocervical adenocarcinomas and AIS being

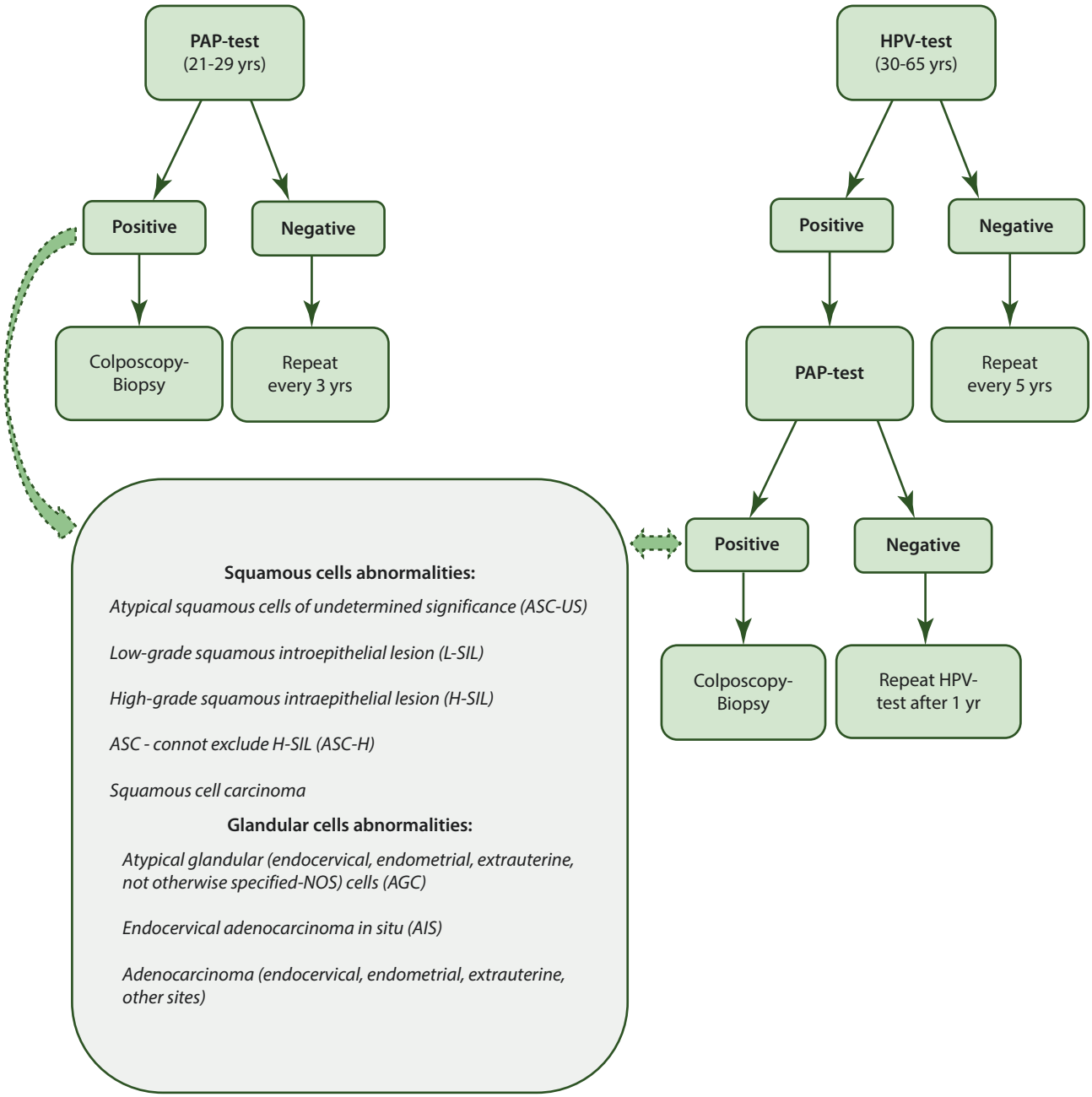


Fig. 53.19 PAP and HPV tests execution flowchart. The cytological alterations detected on PAP test are reported according to the *Bethesda system* (2001) [118]. L-SIL corresponds to mild dysplasia/CIN1 and H-SIL to moderate-severe dysplasia/CIN2 and CIN3/CIS. Women with known risk factors (HIV positivity, immunosuppression, previous cervical precancerous/cancerous lesions) and beyond 65 years old should undergo more frequent screening tests

highly related to HPV (90 and 100%, respectively), especially with HPV 18 genotype; conversely rarer variants appear unrelated to viral etiology. Although AIS occurs rarely, its incidence rate seems to be rising up; it is typically difficult to detect at colposcopy and to manage, being multifocal and extending inside the cervical canal [128].

- *Other Epithelial Tumors*

  Also *adenosquamous carcinomas* show a correlation with HPV 18. *Neuroendocrine tumors* (carcinoid, atypical carcinoid, small cell carcinoma, large cell carcinoma) are diagnosed on histology and usually present with higher neuroendocrine marker distant spread.

Table 53.9 Principal characteristics of the three licensed HPV vaccines

Characteristics	Bivalent (Cervarix®)	Quadrivalent (Gardasil®)	Nine-valent (Gardasil 9®)
Genotype protection	16, 18	6, 11 16, 18	6, 11 16, 18 31, 33, 45, 52, 58
Indications	*Prevention of:*	*Prevention of:*	*Prevention of:*
	Cervical, vaginal, vulvar precancerous lesions	Cervical, vaginal, vulvar precancerous lesions	Cervical, vaginal, vulvar precancerous lesions
	Cervical cancer	Cervical cancer	Cervical cancer
		Anal precancerous lesions, anal cancer	Anal precancerous lesions, anal cancer
		Genital warts	Genital warts
Indications by age, sex, and relative schedule	Females	Females	Females
		Males	Males
	9–14 years old: Two doses (0–6 months)	9–13 years old: Two doses (0-6 months)	9–14 years old: Two doses (0–6 months)
	≥15 years old: Three doses (0–1–6 months)	≥14 years old: Three doses (0–2–6 months)	≥15 years old: Three doses (0–2–6 months)

53.4.5 Clinical Presentation and Diagnosis

CC is often asymptomatic, especially at an early stage; instead, when locally advanced, patients could complain spontaneous or after coitus abnormal vaginal bleeding and discharge, dyspareunia, and pelvic pain. Patients with metastatic small cell neuroendocrine cervical carcinoma may clearly present with paraneoplastic syndromes and relative symptoms: syndrome of inappropriate antidiuretic hormone secretion (SIADH), Cushing syndrome, hypercalcemia, neurological disorders, and weight loss.

Ordinarily, the suspicion of a cervical carcinoma arises from an abnormal PAP test or a positive HPV test. As shown in Fig. 53.19, the second-level procedure to further investigate the presence of a CC is represented by the colposcopy w/wo biopsy. Particularly, this exam allows to obtain a magnified view of the cervix, thanks to the use of a binocular microscope equipped with a light source, called colposcope. During the observation, the cervix will be first cleansed with saline solution to detect eventual abnormal vascularization; then, an acetic acid 3–5% wash will show up as whitish areas possible dysplastic lesions; and, finally, the *Schiller test*, which consists of the application of an iodine solution (*Lugol's solution*) on the cervical surface, will individuate as negative-iodine (clearer) eventual pathological areas. Therefore, colposcopy enhances the possibility to individuate suspected lesions and to achieve more addressed biopsies and histological characterization.

Macroscopically, a cervical cancer could appear as exophytic with outward-growing or endophytic with predominant stromal infiltration.

53.4.6 Pre-operative Work-Up, Staging, and Risk Assessment

After histological diagnosis, a pre-operative work-up to better define the cervical carcinoma extension is mandatory; it should include clinical examination and radiological imaging as schematized in Fig. 53.20.

Until recently, the staging system for CC was based on the 8th FIGO and Union for International Cancer Control (UICC)-Tumor-Node-Metastasis (TNM) classification [131] (Table 53.11).

A new revisited FIGO classification, published in 2018, reported some changes in cervical cancer staging, shown in green in Table 53.11 [132].The tumor risk assessment is based on the evaluation of some clinico-pathological factors: tumor size, stromal invasion depth, and LVSI, which help physicians to define the relative risk class (low, intermediate, high) and to choose the proper adjuvant treatment (Table 53.12). Greater tumor size (>2 cm), deeper stromal invasion, and the presence of LVSI (correlating with a higher risk of lymph node metastasis) are associated with a worse prognosis. Other prognostic factors include lymph node status/number of lymph nodes involved and stage, which appear directly related to each other, differentiation

Table 53.10 WHO histological classification of cervical tumors

Histotype		Frequency (%)
Epithelial		*95%*
Squamous tumors and precursors	Squamous cell carcinoma, NOS: Keratinizing Non-keratinizing Special histotypes	85%
	Early invasive/microinvasive squamous cell carcinoma	
	Squamous intraepithelial neoplasia (CIN3/CIS)	
	Benign squamous cell lesions (condyloma acuminatum, squamous papilloma, fibroepithelial polyp)	
Glandular tumors and precursors	Adenocarcinoma: Mucinous Endometrioid Clear cell Serous Mesonephric	10–12%
	Early invasive adenocarcinoma	
	Adenocarcinoma in situ (AIS)	
	Glandular dysplasia	
	Benign glandular lesions (Müllerian papilloma, endocervical polyp)	
Other epithelial tumors	Adenosquamous carcinoma (glassy cell carcinoma variant)	3–5%
	Neuroendocrine tumors	
	Undifferentiated carcinoma	
	Adenoid cystic carcinoma	
	Adenoid basal carcinoma	
Not epithelial		*<5%*
Mesenchymal tumors		
Mixed epithelial and mesenchymal tumors		
Melanocytic tumors		
Miscellaneous tumors		
Lymphoid and hematopoietic tumors		
Secondary tumors		

grade, histological subtype (adenocarcinoma – worse than squamous carcinoma), margin status, parametria and vaginal cuff status, and levels of squamous cell carcinoma antigen (SCC) and hemoglobin at the moment of diagnosis [133, 134].

53.4.7 Treatment of Pre-invasive Tumors

Usually, CIN1 lesions spontaneously regress; hence, no excisional treatment is routinely recommended in these cases [135]. Patients presenting with a CIN1 at colposcopy will have to repeat, after 1 year, co-test (≥30 years) or only PAP smear (<30 years) and eventually a new colposcopy. If CIN1 persists, an excision will be preferred, primarily when H-SIL or ASC-H has been cytologically detected.

Conversely, CIN2 and CIN3/CIS should always deserve an excisional treatment, even if CIN2 could regress without intervening, certainly more easily than CIN3. Thus, young patients presenting with a CIN2 could be alternatively addressed to a surveillance strategy, repeating PAP test and colposcopy every 6 months for 1 year and undergoing an excisional procedure if CIN2 persists.

The excisional treatment consists of the removal of a cervical cone (conization), trying to obtain clear margins and to allow the re-establishment of a new TZ. Possible procedures include LEEP (loop electrosurgical excision procedure), cold knife conization, and laser conization. Besides, ablative techniques, such as cryosurgery or laser ablation (CO2 laser), are admitted when the entire borders of the lesion are visible, the endocervical sampling is negative, and there are no glandular abnormalities at cytological test; generally, ablation seems to be associated with higher recurrence rate than excision [136, 137].

Total hysterectomy represents the gold standard for women who satisfied the offspring desire and presenting with an AIS, with the risk of post-conization persistent disease being high (multifocal, endocervical growth). Alternatively, a conservative fertility-sparing excisional treatment may be preferred for fertile women.

53.4.8 Treatment of Early Invasive Tumors *(FIGO 2018 - IA1/2, IB1/2, IIA1)*

In the figure below (Fig. 53.21), we report the treatment algorithms for early cervical carcinomas.

The standard primary treatment for early invasive CC is represented by simple or radical hysterectomy, depending on the substage and class risk, and bilateral

Fig. 53.20 Pre-operative work-up, * [129],** [130]

Gynaecological examination (if difficult or unclear vaginal/parametrial involvement, examination under anaesthesia w/wo cervical and vaginal mapping has to prefer)

Cystoscopy/rectoscopy +/- biopsies (if infiltration is suspected)

Abdominal/pelvic MRI w/wo contrast to evaluate with high sensitivity and specifity: tumour size, distance between tumour and internal uterine orifice, cervical length, involvement of cervical stroma/parametrial tissue and infiltration depth, involvement of corpus uteri, vagina, bladder, rectum, pelvic/para-aortic lymph nodes, peritoneum, presence of hydronephrosis [*]

Abdominal CT scan w/wo contrast to study eventual pathological lymph nodes and abdominal metastases; to evaluate the response to neoadjuvant treatments

CT scan w/wo contrast to detect possible lung metastases

18F-FDG PET/CT scan to diagnose with high sensitivity and specificity suspected pathological lymph nodes or metastases, mostly in advanced disease rather than early tumours [**]

Table 53.11 CC staging according to the 8th edition of FIGO (2014) and UICC-TNM classification

TNM	FIGO	Definition
T – primary tumor		
Tx		**Primary tumor cannot be assessed**
T0		**No evidence of primary tumor**
Tis		**Carcinoma in situ**
T1	**I**	**Tumor confined to the cervix**
T1a	**IA**	Microinvasive carcinoma (diagnosed only by microscopy) Stromal invasion[a]<5 mm and horizontal spread ≤7 mm
T1a1	IA1	Stromal invasion <3 mm, horizontal spread ≤7 mm
T1a2	IA2	Stromal invasion ≥3 mm and <5 mm, horizontal spread ≤7 mm
T1b	**IB**	Clinically visible carcinoma or microscopic carcinoma greater than IA
T1b1	IB1	≤4 cm
	IB1 *(FIGO 2018)*	Stromal invasion ≥5 mm and tumor size <2 cm
T1b2	IB2	>4 cm
	IB2 *(FIGO 2018)*	Tumor size ≥2 cm and <4 cm
	IB3 *(FIGO 2018)*	Tumor size ≥4 cm
T2	**II**	**Tumor extends beyond the uterus but not to the lower third vagina/pelvic wall**
T2a	IIA	No parametrial invasion
T2a1	IIA1	<4 cm
T2a2	IIA2	≥4 cm
T2b	IIB	Parametrial invasion

Table 53.11 (continued)

TNM	FIGO	Definition
T3	**III**	**Tumor extends to the lower third vagina/pelvic wall or causes hydronephrosis/ non-functioning kidney**
T3a	IIIA	Tumor extends to the lower third vagina
T3b	IIIB	Tumor extends to the pelvic wall or causes hydronephrosis/non-functioning kidney
	IIIC1r/p (*FIGO 2018*)	Pelvic lymph node metastasis
	IIIC2r/p (*FIGO 2018*)	Para-aortic lymph node metastasis
T4	**IVA**	**Tumor involves bladder/rectum mucosa or extends beyond true pelvis**
N – regional lymph nodes		
Nx		**Regional lymph nodes cannot be assessed**
N0		**No regional nodal metastasis**
N1		**Regional nodal metastasis**
M – distant metastasis		
M0		**No distant metastasis**
M1	**IVB**	**Distant metastasis**

[a]Stromal invasion is calculated from the base of epithelium to the deepest point of infiltration. In green, changes in staging system reported by FIGO 2018 classification. r radiological, p pathological. M1 includes inguinal lymph nodes and peritoneal disease; the extension of tumor to the vagina, adnexa, and pelvic serosa is not to be defined as M1

Table 53.12 Risk groups

Risk group	Prognostic factors		
	Tumor size	Stromal invasion depth	LVSI
Low	<2 cm	Superficial 1/3	Negative
Intermediate	<2 cm	Any	Positive
	≥2 cm	Any	Negative
High	≥2 cm	Any	Positive

PLND (except for squamous IA1 LVSI-negative carcinomas), w/wo PALND [138]. Some ongoing randomized CTs will better establish which is more appropriate between a simple and a radical procedure [139]. Bilateral annessiectomy is also usually executed, in addition to hysterectomy, especially in post-menopause or patients who satisfied the offspring desire.

The sentinel lymph node mapping, through the intraoperative injection on the cervix of a tracer (fluorescent indocyanine green, the most favorite), would allow to avoid an inappropriate and not completely free from morbidity PLND or, conversely, to support a lymphadenectomy (if SLNs are negative or positive, respectively), above all in stages from IA1 with LVSI positivity to IB1, for which high detection rate and sensitivity have been reported in literature [140, 141].

However, considering the not widely standardized procedure, often physicians prefer to remove PLNs, regardless of SLN mapping results [142].

The PALN involvement appears to be more related to the presence of pelvic lymph node metastases and tumors larger than 2 cm; hence, PALND should ensure a better prognosis from stage IB1.

Minimally invasive (laparoscopic, robotic) surgery, in the past years, had been thought to offer similar outcomes than laparotomy in early invasive CC and to be advantageous in terms of less intra- and post-operative complications. Nevertheless, some recent CTs have dem-

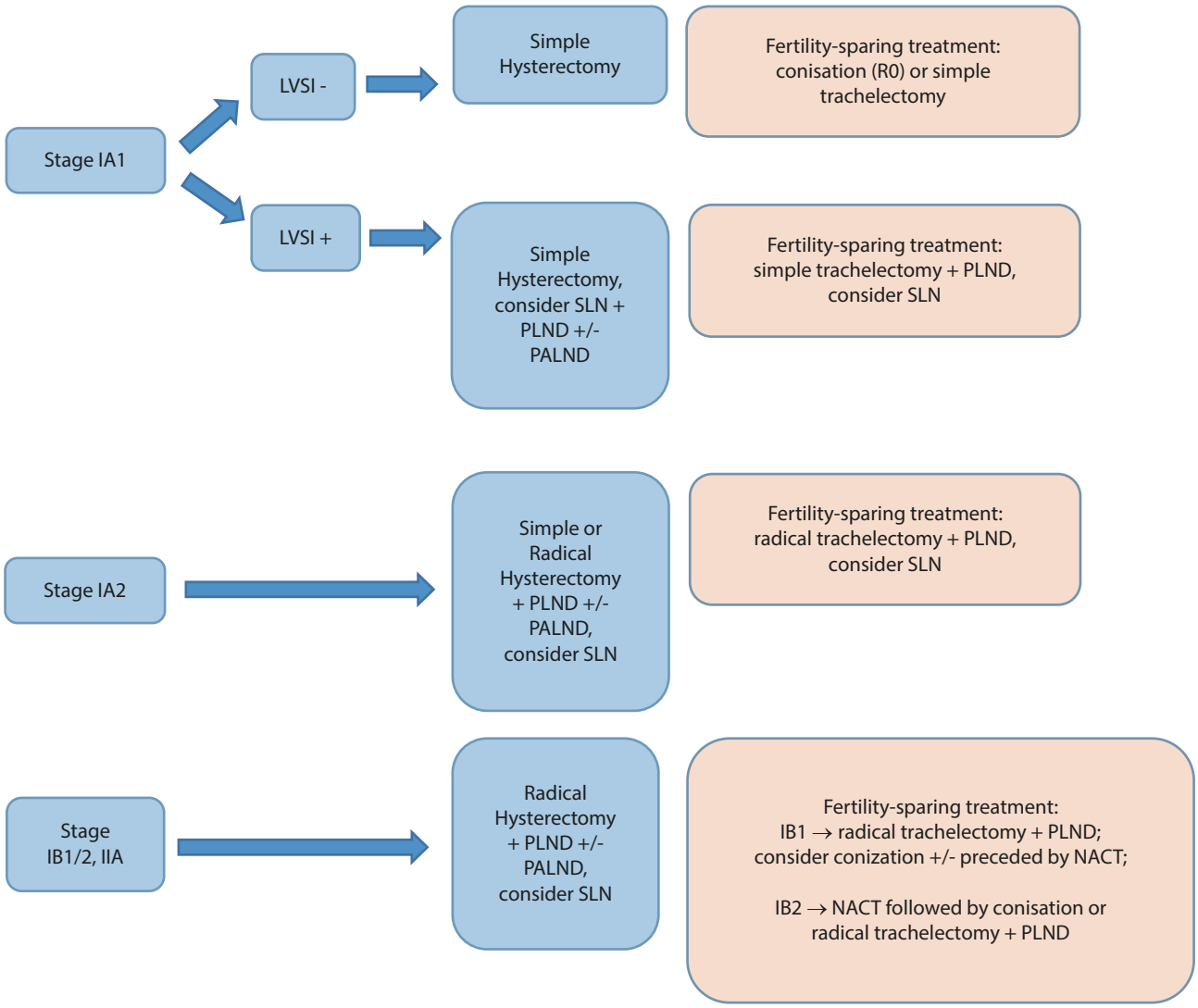


Fig. 53.21 Surgical algorithms for early invasive cervical tumors. PLND pelvic lymph nodes dissection, PALND para-aortic lymph node dissection, NACT neoadjuvant chemotherapy

onstrated that laparoscopic and robotic approaches failed in improving PFS and OS and reducing recurrence rate, compared to open surgery, moreover for tumors >2 cm.

Almost half of early tumors concern women of childbearing age; thus, it is fundamental to define fertility-sparing approaches. Trachelectomy consists of the removal of the cervix (via the abdomen or vagina) and nearby tissue (paracervix/parametrial tissue), upper part of the vagina, and pelvic lymph nodes [143]. Depending on the lateral extension level of resection, trachelectomy may be defined as simple (resection at cervical border) or radical (resection at ureter bed), with the latter preferred for tumors >2 cm as they are associated with higher risk of parametrial involvement, LVSI, lymph node metastases, and recurrence [144].

NACT can be exploited to downstage IB1/2 disease, before fertility-sparing treatments (both conization and trachelectomy), and, although under experimental validation, also IB3 stage (FIGO 2018) [145].

- Exclusive Radiotherapy

  Exclusive RT approach, consisting of simultaneous EBRT and intravaginal-cervical BT (80–85 Gy overall dose), could be also applied, as valid primary treatment and alternatively to surgery, in IB1–IIA1 stages [146]. In fact, RT seems to ensure comparable outcomes in terms of local control of disease, PFS and OS, and safety profile [147]. Surgery could be preferred in adeno-histologies, younger age, and low-risk groups who would not necessitate further adjuvant treatments (CT or RT), in relation to favorable prognostic factors and considering the increased toxicity arising from combined approaches. Otherwise, RT should be considered as the treatment of choice.

53.4.9 Adjuvant Treatment

Patients presenting with concomitant pathological risk factors (see ▶ Sect. 53.3.6 and ◘ Table 53.12) are candidates to receive further adjuvant therapies, after surgery (◘ Fig. 53.22).

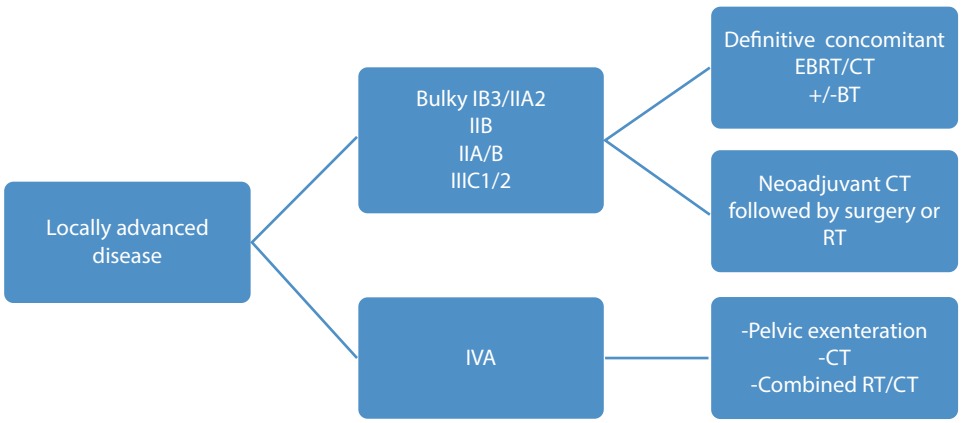
Particularly, it seems that intermediate-risk group could benefit from adjuvant pelvic EBRT alone in terms of PFS, without improving of overall survival; therefore, the option of no further treatment after surgery is equally valid for these patients [148]. Conversely, high-risk patients should undergo concomitant adjuvant RT (45–50, 4 Gy total dose) and at least three to four cycles of cisplatin-based CT (weekly radio-sensitizing dose of 40 mg/mq), resulting in increased PFS and OS when the combined strategy is used [149]. In addition, there is also a role for BT (10 Gy) when surgical vaginal margin resulted positive or close at pathology. Finally, large-field EBRT on eventual positive common iliac and para-aortic lymph nodes is recommended.

53.4.10 Treatment of Locally Advanced Disease

The treatment algorithm for locally advanced disease may be schematized as in the flowchart below (◘ Fig. 53.23).

The standard of care for locally advanced cervical carcinomas (w/wo positive pelvic/para-aortic lymph nodes) is represented by definitive concomitant EBRT/platinum-based CT, with the addition of endocavitary/interstitial vaginal-cervical brachytherapy. In fact, this multimodality approach allows to gain better both local and distant control of disease and also absolute improvement in OS and PFS, compared to any monotherapy; however, these advantages mostly concern I/II stages than III/IVA ones [150]. Normally, EBRT is directed on the uterus, upper third vagina, parametria, and obturator/pre-sacral lymph nodes (45–50 Gy total dose), but an extended-field RT could be necessary in case of positive iliac/para-aortic lymph nodes (until

◘ **Fig. 53.22** Algorithm for adjuvant treatment according to Sedlis (on the top) and Peters (on the bottom) criteria



◘ **Fig. 53.23** Flowchart on locally advanced CC treatment

55–60 Gy of overall dose for macroscopic metastases) and of inguinal lymph nodes in stage IIIA (extension to lower third of vagina). In IIIB stage, protective endoureteral stent or nephrostomy placement is required to ensure both kidney function and nephrotoxic cisplatin administration.

The addition of BT (25–30 Gy) is crucial to increase the local disease-free survival (DFS) in IIB/IIIB stages, although its effect on OS is unknown; besides, the interstitial approach could be associated with the traditional endocavitary BT for high paracervical tumoral residue, after EBRT/CT, with probable benefits on survival [151]. Therefore, an optimal RT treatment has to reach elevated overall dose (90–95 Gy) and, furthermore, should be administered within 7–8 weeks [152].

The concomitant CT consists of the administration of weekly 40 mg/mq cisplatin for the whole time of RT, generally for six cycles. Alternatively, a concomitant doublet cisplatin-gemcitabine, followed by two further adjuvant cycles of the same CT regimen, has shown to improve OS and PFS. Noteworthy, the worse safety profile of this combination makes difficult the routine application in clinical practice [153].

Some authors are trying to establish the potential role on survival of salvage hysterectomy after definitive radiotherapy/concurrent chemoradiotherapy in case of residual cervical disease [154].

As alternative to concomitant RT/CT, high-dose neoadjuvant CT followed by radical surgery (type C hysterectomy) may be also carried out. Generally, doublet cisplatin-paclitaxel is the most preferred regimen, considering the higher hematological toxicity occurring with use of triplets, such as cisplatin-paclitaxel-ifosfamide.

Recently, the phase III randomized EORTC trial results have been presented at ASCO 2019. This study has compared outcomes arising from platinum-based NACT followed within 6 weeks by radical surgery versus concomitant RT/CT in IB2 (FIGO 2014) and IIA/B stages. It was found that sequential NACT-radical surgery approach does not improve DFS and OS compared to concomitant treatment [155]. A similar phase III clinical trial found that concomitant RT/CT produces advantages in terms of PFS but not OS versus sequential NACT-radical surgery, with a mildly worse toxicity profile for concomitant treatment [156]. Thus, the choice of the most appropriate therapy should individually rely on safety and quality of life.

The role of sequential RT or concomitant CT/RT following NACT is still controversial. An ongoing phase III trial (INTERLACE) will provide insights on the better treatment between induction dose-dense CT (carboplatin AUC2-paclitaxel for six cycles) followed by concomitant RT/CT and standard RT/CT alone [157]. Besides, a phase III randomized study (OUTBACK) is testing the role of additional four cycles of adjuvant carboplatin-paclitaxel-based CT, after definitive concurrent RT/CT in locally advanced disease [158].

Finally, efforts are moving toward the definition of a molecular prediction model of response to chemoradiation, so as to improve clinical outcome in cervical cancer patients [151].

53.4.11 Recurrent and Metastatic Disease

Generally, most relapses occur within 2 years from first diagnosis of CC and may present as locoregional, extrapelvic, and metastatic distant disease (lungs, bones, etc.).

CC local relapses may occur at vaginal cuff (central relapse) or at pelvic wall (lateral relapse). Managing of recurrence depends on previous received treatment. Particularly, pelvic recurrences after surgery or outside the previous field of radiation may be treated with radical concurrent RT/platinum-based mono-CT (or combined with 5-fluorouracil) w/wo BT. Alternatively, post-radiated patients may undergo pelvic exenteration w/wo intraoperative RT (IORT) and subsequent reconstructive surgery. Besides, patients with small (<2 cm) isolated central pelvic relapse could be treated with a more conservative surgery (radical hysterectomy) or endocavitary/interstitial BT. Lateral relapses could benefit too from both concurrent EBRT/CT and surgery w/wo IORT [159].

The standard first-line chemotherapy regimen for patients presenting with distant metastases (stage IVB) is represented by 3-weekly cisplatin-paclitaxel doublet associated with bevacizumab, since the GOG-240 trial reported improvement of response rate, PFS, and OS (gain of 4 months) with the addition of the anti-angiogenic agent [160]. Alternatively, for patients not candidate to receive cisplatin (renal dysfunction, early relapse after previous cisplatin-based treatment), carboplatin-based doublet CT could be applied [161]. Other active platinum-based doublets include agents such as topotecan, gemcitabine, vinorelbine, ifosfamide, and 5-fluorouracil which result in higher response rate compared to monotherapies [162].

Any standardized regimen is expected for patients who progress following a first-line CT, but, usually, one of the most active abovementioned agents is used, if not previously administered. Other possible chemotherapeutics, which are in any way not associated with impressive improving in OS, are pegylated liposomal doxorubicin, docetaxel, and irinotecan.

Also in metastatic setting, there is a role for high-dose RT, especially in controlling oligo-metastatic disease and lymph node metastases (pelvic, para-aortic, mediastinal, supraclavicular), whereas short-course RT could be applied for treating symptomatic distant metastases (e.g., painful bone metastases) [163].

53.4.12 New and Potential Future Perspectives

As for ECs, the treatment of recurrent and metastatic cervical cancers is still considered an unmet clinical need, considering the short survival related to this disease setting (<17 months after first-line therapy) [164]. Therefore, it is not surprising that clinical efforts are moving toward the identification of innovative and targeted therapies, which could affect selected altered molecular pathways.

- *Anti-angiogenic Agents*
  The viral oncogenic E5 and E7 proteins are known to act inducing VEGF overexpression; in fact, in addition to bevacizumab, some CTs have tested multikinase inhibitors with anti-angiogenic activity (sunitinib, pazopanib, apatinib), finding a mild prolongation in terms of PFS associated with the use of pazopanib [165, 166].
- *Anti-EGFR Agents*
  Although the EGFR (epidermal growth factor receptor) overexpression is reported in more than 50% of CCs, studies on the administration of anti-EGFR molecules (erlotinib, gefitinib, cetuximab) have reported uncertain and discouraging results [167].
- *iCKPi*
  Promising results are coming from CTs testing iCKPi, such as nivolumab (monoclonal anti-PD-1 antibody), ipilimumab (monoclonal anti-CTLA4), and pembrolizumab, which have shown to increase ORR in recurrent or metastatic CCs [168].

  Particularly, the phase II KEYNOTE-158 trial resulted in the FDA approval of pembrolizumab for PD-L1 positive patients, pretreated with chemotherapy (from second-line) [169]. An ongoing phase III randomized trial (GOG-3016) is testing the activity of a new anti-PD-1 antibody (cemiplimab) versus standard of care in advanced setting [170]. Recently, at ESMO 2019, the interim analysis results of the CheckMate 358 trial have been presented, showing the safety of combination nivolumab + ipilimumab in recurrent/metastatic CC pretreated or not with CT, regardless of PD-L1 expression.

  Probably, the most satisfying results will come from the synergistic effect of combined iCKPi-based treatment with chemotherapy and anti-angiogenic agents. The phase III randomized KEYNOTE-826 trial is enrolling patients with persistent, recurrent, or metastatic cervical cancer to receive pembrolizumab plus CT versus CT alone. Interestingly, BEATcc is an ongoing randomized phase III study on the use of standard cisplatin-paclitaxel-bevacizumab with or without atezolizumab as first-line treatment for advanced disease. Besides, iCKPi-CT is being evaluated also in locally advanced setting, such as in the phase III CALLA trial; particularly it is randomizing patients to receive durvalumab with and following concurrent RT/CT versus RT/CT alone.
- *Therapeutic Vaccines*
  Encouraging activities are emerging from the use of therapeutic vaccines, consisting of the infusion of E6/E7-activated T lymphocytes or autologous tumor-infiltrating lymphocytes (LN-145) after in vitro clonal expansion, in combination with interleukin 2 (IL-2) and/or CT.

53.4.13 Follow-Up

The surveillance program after a definitive treatment for CC may be schematized as in Table 53.13 [171, 172]. Effect on survival of follow-up strategies should be defined within prospective studies.

53.5 Conclusions

Endometrial and cervical cancers are showing, over the last decades, increasing incidence and mortality in developed and low-income countries, respectively. Regarding EC, this is essentially related to prolonged life expectancy and bad lifestyle habits, whereas, for CC, it depends on low availability of effective screening programs and tests in poor countries. On the other hand, innovative therapeutic strategies are emerging for the management of advanced gynecological malignancies. In fact, efforts of clinicians are increasingly moving toward the experimentation of targeted therapies, trying to ensure to patients more proper and tailored solutions, on the basis of predictive molecular profiles of response to specific and selected agents or combination of them. Thus, in the next years, this could lead to prolonging

Table 53.13 Follow-up for CC after radical treatment

Clinical and physical (gynecological/pelvic/rectal) examination	Every 3–6 months for the first 2 years and then 6-monthly until the 5th year from primary treatment
PAP test if feasible +/– HPV test	Annually
Not ordinarily recommended exams *Admitted for symptomatic or high-risk patients when physical examination appears difficult*	Tumor markers: CEA, CA 125, CA 19.9, and AFP, chest X-ray, abdominal US, CT scan, pelvic MRI, 18F-FDG PET/CT scan, whole-body bone scan

overall survival and progression-free survival of more advanced settings, whose management is still considered an unmet clinical need. Furthermore, thanks to the wide implementation of clinical trials on the definition of risk factors and predisposing conditions, prevention, through lifestyle correction and/or validated screening tests, represents an efficacious tool to diagnose earlier and reduce mortality of uterine cancers.

Case Study: Management of a Patient Affected by Locally Advanced Endometrial Cancer

63 years old

- *Family history:* negative for malignancies
- *PMH:* Obese (BMI = 32 kg/m^2), Systemic Arterial Hypertension
- *RMH:* Complain of abnormal vaginal bleeding and pelvic pain
- *Objective examination*: painful lower abdominal regions
- *Blood tests*: lower hemoglobin (10.1 mg/dL)

Question

What action should be taken in the first instance?

1. Abdominal Ultrasound
2. Gynaecological examination and Trans-Vaginal Ultrasound
3. Pelvic MRI w/wo contrast

Answer

Gynaecological examination and Trans-Vaginal Ultrasound (for searching eventual origin of bleeding from uterus)

Question

A diffuse (9 mm) endometrial thickness is detected on TVUS. What should be the next step?

1. Hysteroscopy-guided biopsy
2. Pelvic MRI w/wo contrast
3. Planning of hysterectomy

Answer

Hysteroscopy-guided biopsy

Although the presence of an abnormal vaginal bleeding associated to pelvic pain and endometrial thickness at US is highly suggestive for an endometrial cancer, a histological characterization should be recommended before considering other staging exams and/or surgery.

Question

Pathology concludes for a serous-papillary EC. *What should be the next step?*

1. Combined RT/CT
2. Complete staging (Pelvic MRI, Abdominal CT scan, Chest CT scan) and planning of surgery
3. Hysteroscopy surveillance

Answer

Complete staging (Pelvic MRI, Abdominal CT scan, Chest CT scan) and planning of surgery

The patient is affected by a high-risk histology EC. A pre-operative work-up should be recommended in all patients with pathological confirmed endometrial tumours, considering as optional some radiological exams. In this case, because of the more aggressive and prognostically unfavorable histology, a depth pre-operative evaluation should be highly taken into account to better establish the disease loco-regional and distant spread.

Question

The pre-operative work-up has detected a suspected pelvic lymph nodes involvement (stage IIIC), without distant localizations. Which surgical strategy should be preferred?

1. Extrafascial simple total hysterectomy without vaginal cuff + bilateral salpingo-oophorectomy
2. Radical hysterectomy + bilateral salpingo-oophorectomy + pelvic and para-aortic lymphadenectomy + staging omentectomy
3. Radical hysterectomy + pelvic lymphadenectomy

Answer

Radical hysterectomy + bilateral salpingo-oophorectomy + pelvic and para-aortic lymphadenectomy + staging omentectomy

Simple hysterectomy is considered the gold standard for stage I EC. Instead, starting from stage II a radical hysterectomy associated to systematic lymphadenectomy is recommended, independently from the macroscopic lymph nodes involvement. Our patient was clinically staged as a IIIC, thus even more so deserving a systematic lymph nodes removal to reduce lymphatic spread risk, but also for staging purposes and better guiding adjuvant treatment. Moreover, a staging omentectomy is strongly recommended, because of the high peritoneal spread risk by serous EC.

Question

Pathology confirms a IIIC1 stage for pelvic and no para-aortic lymph nodes involvement. Should the patient undergo to further treatments?

1. No, but she must follow a surveillance program
2. Yes: 3-weekly carboplatin-paclitaxel administered for 6 cycles
3. Yes: combined platinum-based CT/RT

Answer

Yes: combined platinum-based CT/RT

A multimodality approach is strongly suggested for special histotypes, especially in presence of a N-positive disease, as several clinical studies have found longer PFS and/or OS linked to combined treatments.

Key Points

- Start with medical history, physical examination and histological definition
- A proper pre-operative work-up should be always proposed, especially in presence of high-risk disease
- A radical hysterectomy should be accompanied by an optimal surgical staging (omentectomy), when a serous-papillary histology is diagnosed
- Adjuvant combined RT/CT is basically the preferred approach after EC surgery, especially in high-risk clinico-pathological conditions.

53 Case Study: Management of a Patient Affected by Metastatic Cervical Cancer

49 years old

- *Family history:* negative for malignancies
- *PMH:* tobacco smoker for 30 years; 3 years ago → positive HPV-test for 16 genotype; positive PAP-TEST for L-SIL; colposcopy-guided biopsy conclusive for CIN1
- *RMH:* 6-months history of vaginal discharge, dyspareunia, pelvic discomfort, lumbar pain, legs lymphedema
- *Physical examination*: painful hypogastric region, pain on pressing lumbar region, vaginal discharge on gynaecological examination
- *Laboratory tests*: no abnormalities
- *New HPV and PAP-tests:* positivity for 16 genotype HPV and the presence of squamous carcinoma cells, respectively.

Question

Which investigations should be performed?

1. 18F-FDG PET/CT scan
2. Colposcopy-guided biopsy and a complete radiological staging
3. Hysteroscopy

Answer

Colposcopy-guided biopsy and a complete radiological staging

Considering the presence of squamous carcinoma cells on PAP-TEST, past and recent medical history and objective findings on physical examination, the suspicion for an advanced cervical cancer is very high. Thus, a colposcopy-guided biopsy and a complete staging of the disease are mandatory.

Pathology confirms the presence of a squamous cell carcinoma and the radiological staging the extension to pelvic lymph nodes with right hydronephrosis, two suspected left lung and one L3 metastases.

Question

What action should be taken?

1. First-line CT
2. Radical hysterectomy + bilateral salpingo-oophorectomy + systematic lymphadenectomy
3. First-line CT and consider high-dose and/or short-course RT

Answer

First-line CT and consider high-dose and/or short-course RT

Although the patient is affected by a metastatic cervical cancer and a first-line chemotherapy is tightly required, a multimodality approach should be considered. Indeed, the role of high-dose RT in controlling oligometastatic disease and lymph nodes metastases is quite recognized. Moreover, a short-course RT could be applied for treating symptomatic distant metastases, for example painful bone metastases like in this case (L3 lesion).

Question

Which CT regimen should be preferred?

1. 3 weekly cisplatin-paclitaxel doublet associated to Bevacizumab
2. 3 weekly cisplatin-paclitaxel doublet
3. 3 weekly carboplatin-paclitaxel doublet +/- Bevacizumab

Answer

3 weekly carboplatin-paclitaxel doublet associated +/- Bevacizumab

The standard first-line CT regimen for metastatic patients consists in the combination of the 3 weekly cisplatin-paclitaxel doublet and the anti-angiogenic Bevacizumab. But, considering the right hydronephrosis detected on radiological exams, our patient should be candidate

to receive carboplatin, instead of cisplatin, to reduce the renal dysfunction risk. In fact, in similar cases, a protection nephrostomy placement should be recommended, primarily in those patients could benefit from a high-dose RT on abdominal lymph nodes.

Question

After 12-months treatment, the radiological revaluation shows a disease progression on lungs and mediastinal lymph nodes. What should be the next step?

1. Pegylated liposomal doxorubicin administration
2. Gemcitabine administration
3. Refer the patient to a specialized center for the eventual enrolling into clinical trials

Answer

All the options are valid therapeutic strategies.

To date, any standardized regimen is recommended for patients who progress to a first-line platinum-based doublet; in fact, a series of several chemotherapy agents could be used in this setting: pegylated liposomal doxorubicin, gemcitabine, topotecan, vinorelbine, ifosfamide, 5-fluorouracil docetaxel, irinotecan. Besides that, the phase II KEYNOTE 158 trial resulted in the FDA approval of pembrolizumab for PD-L1 positive patients, pretreated with chemotherapy (from second-line); thus, also in this case, the possibility to evaluate the PD-L1 expression by tumoural cells and, consequently, to administer iCKPi should be taken into account. Certainly, referring the patient to a center that handles ongoing clinical trials could represent an optimal therapeutic strategy and opportunity.

Key Points

- Start with medical history, physical examination and histological definition
- Proper pre-operative work-up and imagine techniques are crucial to establish the effective extension of the disease
- Multimodality treatment could find its usefulness even in metastatic setting
- The choice of the most effective and tailored treatment should be discussed or realized in high volume gynaecologic oncology centers handling clinical trials on targeted therapies

Expert Opinion

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Key Points

Endometrial cancer is the most common gynecological cancer affecting about 320000 women worldwide. As most cases are diagnosed in an early stage due to the occurrence of vaginal bleeding, the overall survival rate is high. However, nearly one-fifth of women have aggressive endometrial cancer with a survival of about 1 year. There are two different types of endometrial carcinoma, named as type I and type II. Type I endometrioid endometrial cancer represents most sporadic cases, is driven by estrogens, often is well differentiated, and has a good prognosis. Type II endometrial cancer consists of clear cell, serous, mucinous, adenosquamous, and mixed carcinomas and typically presents with advanced stage disease and is associated with a high mortality. About 10% of endometrial carcinomas are triggered by germline alterations in DNA mismatch repair genes (MMR). Those patients often develop endometrial cancer at a young age. Standard management of endometrial cancer at diagnosis involves surgery consisting of a hysterectomy, bilateral salpingo-oophorectomy, and in high-risk cases pelvic- and paraaortic lymphadenectomy and staging. A 2012 review found that for early-stage primary endometrioid adenocarcinoma of the endometrium, laparoscopy and laparotomy are associated with similar rates of disease-free and overall survival and that laparoscopy is associated with reduced operative morbidity and shorter hospital stays. In patients with advanced disease, adjuvant chemotherapy and/or radiotherapy should be considered. The Cancer Genome Atlas (TCGA) group used whole genome sequencing to characterize genetic aberrations in endometrial cancers. Recurrent translocations of gene were found in important cancer pathways, such as WNT-EGFR-MAPK-RAS, PI3K, and RB1. Frequent translocations were discovered in the BCL family and novel POLE hotspot mutations were identified. PTEN, PIK3R1, PIK3CA, FBXW7, and KRAS were found to be frequently mutated. These genomic alterations are crucial for the development of genome-driven precision care, pharmacogenomics, and the development of targeted drugs. Next-generation sequencing assays looking at MMR genes are currently already actively used for the identification of individuals at risk for developing endometrial cancer.

Cancer of the uterine cervix (CC) reflects the disparities in access to healthcare across the world. Although

being highly preventable, this disease is still a major public health problem in less developed regions. Globally it is the second most prevalent cancer in women, with most cases diagnosed in an advanced stage. Inoperable CC will continue to be highly prevalent during the next decades as screening programs and vaccination campaigns are still unavailable in most countries while not being entirely effective. Currently, locally advanced disease is treated with (chemo)radiotherapy and metastatic disease with platinum-based chemotherapy (+/- bevacizumab). First- and second-line systemic treatments are not very effective, and early clinical trials with targeted therapy have not yet identified new targeted drugs with superior response rates. Up to now, the scientific society and pharmaceutical industry have shown little interest in developing new (targeted) treatment modalities to improve the outcome of patients with advanced and recurrent CC. In fact, CC is often not included in phase I-II basket trials assessing experimental drugs. Traditionally, testing novel systemic treatments in advanced CC requires large and expensive randomized clinical trials involving hundreds of patients, with follow-up extending several years before results emerge. Neoadjuvant experimental therapy (NET) provides a unique opportunity for faster and cheaper studies assessing the responsiveness to targeted drugs and/or immunotherapy. CC is easily and safely accessible for repeated tumor biopsies allowing intra-patient comparisons. NET can be performed in a concept of proof setting as short (2-4 weeks) treatment courses before standard treatment, using biomarkers as endpoints. It can also be tested with randomized trials comparing standard chemotherapy versus a combination of experimental treatment using operability and/or pathological response as endpoints, jointly with biomarkers for response, thereby gaining insight into molecular changes associated with tumor response. While immunotherapy is emerging as a potential treatment modality of CC, NET may offer a unique opportunity to assess the immune response in vivo. Patients with recurrent metastatic disease are often immunocompromised, and it is therefore important to assess immunotherapy in earlier stage patients. We therefore advocate the use of NET as a tool to accelerate translational and clinical research into better treatment of CC. Performing this research in parts of the world with a high incidence of the disease is mandatory to achieve this goal. Such an approach is only ethically defendable if strategies are developed to reduce costs and access to new active drugs in these countries.

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Vulvar and Vaginal Cancers

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Gynecological Cancers

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Learning Objectives

By the end of the chapter, the reader will:

- Have learned the basic concepts of epidemiology, histological subtype, and clinical manifestation of vulvar and vaginal cancer
- Be able to define staging strategies and diagnostic and therapeutic procedures
- Be able to put acquired knowledge into clinical practice
- Be able to realize future perspectives of vulvar and vaginal cancer

54.1 Vulvar Cancer

54.1.1 Overview

Vulvar cancer is considered as a rare tumor and it accounts for 4 % of gynecologic malignancies; the median age of diagnosis is 68 years. The 5-year survival rates range from 86 % of localized disease (stage I and II) to 53 % of locally advanced disease (stage III) and 19 % for patients with metastatic disease (stage IV). The most common histologic path is the squamous cell carcinoma (SCC). There are also many other rarer histologies: melanoma, Bartholin gland adenocarcinoma, verrucous carcinoma, extramammary Paget disease, and sarcoma.

Risk factors are represented by human papillomavirus (HPV) infection, cigarette smoking, inflammatory conditions of the vulva, aging, and immunodeficiency.

Ninety percent of vulvar cancer is of SCC histology. The noninvasive vulvar intraepithelial neoplasias (VINs) are correlated in approximately 52–100 % of cases to human papillomavirus (HPV). VINs can be divided into "usual" VIN (uVIN), normally caused by HPV infection, and "differentiated" VIN (dVIN), not caused by infection but correlated to inflammatory lesions, like lichen sclerosus.

The International Society for the Study of Vulvovaginal Disease defined a new classification in low-grade squamous intraepithelial lesion (LSIL), including condyloma and HPV effect, and high-grade squamous intraepithelial lesion (HSIL), which corresponds to uVIN of the previous classification and dVIN. There is another variant called Bowenoid papulosis (BP) that can appear similar to uVIN or HSIL but it disappears spontaneously (World Health Organization Classification of Tumours of Female Reproductive Organs, 4th edn., IARC).

There are many benign conditions that may develop into vulvar carcinoma; *lichen sclerosus* is the most common inflammatory, noninfectious disorder of the vulva, and it can be associated with VIN and vulvar carcinoma in 15% to 40% of cases. Lichen planus is a dermatosis but, as well as lichen sclerosis, it can evolve into erosive vulvar disease, which has been associated with invasive vulvar squamous cell carcinoma.

Extramammary Paget disease of the vulva is an eczematous lesion that appears on the vulva and, rarely, it may be associated with underlying cutaneous adenocarcinoma [1].

54.1.2 Clinical Presentation, Diagnosis, and Work-Up

Currently the guidelines used are the International Federation of Gynecology and Obstetrics (FIGO) and American Joint Committee on Cancer (AJCC) TNM staging systems. There are a few updates on these available guidelines that include the revision of stage III that now includes the positivity of the groin lymph nodes, while patients with positive pelvic lymph nodes are considered stage IVB.

The clinical presentation of vulvar cancer can be varied. The most common presentation is within the labia majora; other possible sites are the clitoris, mons, or perineum. Patients with HPV-positive tumors can have multifocal lesions and concurrent cervical neoplasia can be present. Many cases may be asymptomatic while itchiness, bleeding, pain, and irritation can occur as the most common symptoms.

Diagnosis is made through biopsy of the suspicious areas. Once the diagnosis of vulvar cancer is confirmed, the work-up includes history and physical examination, while the imaging techniques used are CT, PET-CT, and MRI that may be helpful to stage the disease and to delineate the extent of the tumor. CT scan is a useful tool to do a clinical staging of the disease and to detect distant metastases. MRI is performed to understand the real extension of the disease; in the TNM system staging, the parameters T and N can be studied through this abovementioned technique. PET-CT scan is performed when the disease is locally advanced to better understand if the first approach should be surgical or medical. HPV testing is always recommended while HIV testing is suggested in younger patients.

54.1.3 Prognostic Factors and Surgical Staging

AJCC and FIGO TNM staging systems are both used to delineate the disease. The clinical staging alone is not useful to define the lymph node involvement; the node (N) parameter is a fundamental prognostic factor to establish the vulvar cancer survival [2].

A complete lymph node staging requires a full inguinofemoral lymphadenectomy. However, common

practice has included the use of the sentinel lymph node (SLN) biopsy in order to obtain a proper disease staging in the early disease, where lymphadenectomy could be avoided because of its morbidity.

Other prognostic factors in vulvar cancer represent the tumor site, tumor size, number of tumor foci, histologic type and grade, depth of stromal invasion, surgical margin status, and presence of lymphovascular invasion. Additionally, tumor involvement of tissues/organs such as vagina, urethra, anus, and rectal mucosa is an important prognostic factor [3, 4].

Primary Tumor Resection

The surgical technique used can be local excision or vulvectomy: it depends on tumor extent. However, the surgical techniques mentioned involve resection of approximately 1- to 2-cm radial margin of grossly normal tissue and a minimum of 1-cm-deep margin of deep fascia.

Vulvar cancer has a high recurrence rate and the goal of primary resection is the complete removal with 1- to 2-cm margins; moreover, in a recent study of Arvas et al., tumor-free margins of at least 2 mm have been associated to a decreased local recurrence risk [5].

Lymph Node Evaluation

Lymph node dissection in patients can be omitted in patients with stage IA, since the lymph node involvement at this stage is less than 1%. The SLN and inguinofemoral lymphadenectomy is recommended starting from the stage IB, because the risk of lymph node involvement is greater than 8% and it grows for stages beyond the IB [6].

The *SLN assessment* has the role to avoid a unilateral or bilateral inguinofemoral lymphadenectomy that can have many side effects like lymphedema. The safety and accuracy of SLN assessment has been examined in a multicenter observational study (GROINSS-VI). 403 women with vulvar tumors<4 cm did not undergo lymphadenectomy if SLN was negative. The median follow-up period was 35 months and recurrence was observed in only 6 of 259 patients with a unifocal primary tumor and negative SLN. The study demonstrated that in early-stage vulvar cancer, the groin recurrence was low when the SLN assessment was performed [7].

54.1.4 Management

Early-Stage Disease

In the early-stage disease, the better treatment is represented by radical local incision or vulvectomy; the right approach is still debated and remains a surgical decision.

Stehman et al. have compared groin dissection versus groin irradiation and they noted that the surgical removal of lymph nodes had a better outcome and disease control and lower recurrence rates than radiation therapy.

T1 tumors should undergo local resection or radical local resection and the SLN is recommended. Surgery of T1b or smaller T2 is led by tumor location. Lateralized lesions at >2 cm from the vulvar midline should undergo radical resection or modified radical vulvectomy and ipsilateral groin node evaluation.

Patients with midline vulvar lesions should undergo radical local resection or modified radical vulvectomy [8].

Adjuvant Treatment

There are limited prospective randomized trials on the adjuvant treatment of vulvar cancer due to the rarity of the disease.

Node involvement is an important prognostic factor and adjuvant treatment should be addressed to these patients.

The GOG 37 enrolled 114 patients with groin node-positive vulvar cancer after radical vulvectomy and bilateral inguinofemoral lymphadenectomy. Patients were randomly assigned to receive pelvic node dissection or adjuvant radiotherapy to the groin/pelvis. A long-term follow-up demonstrated that the higher rates of disease-related death were registered in the group that received pelvic node dissection compared with pelvic/groin RT [9].

A more recent study showed that, among 444 elderly patients (median age of 78) with node-positive vulvar cancer, the better outcomes were reached from the patients that underwent adjuvant radiotherapy.

External beam irradiation should be performed as adjuvant treatment in patients with close margins or with positive sentinel lymph node or with one or more lymph nodes positive for metastases at inguinofemoral lymphadenectomy (see Table 54.1).

Locally Advanced Disease

In the past, the locally advanced disease was primarily treated with radical surgery such as en bloc radical vulvectomy with bilateral inguinofemoral lymphadenectomy; however, these surgeries had significant postoperative complications.

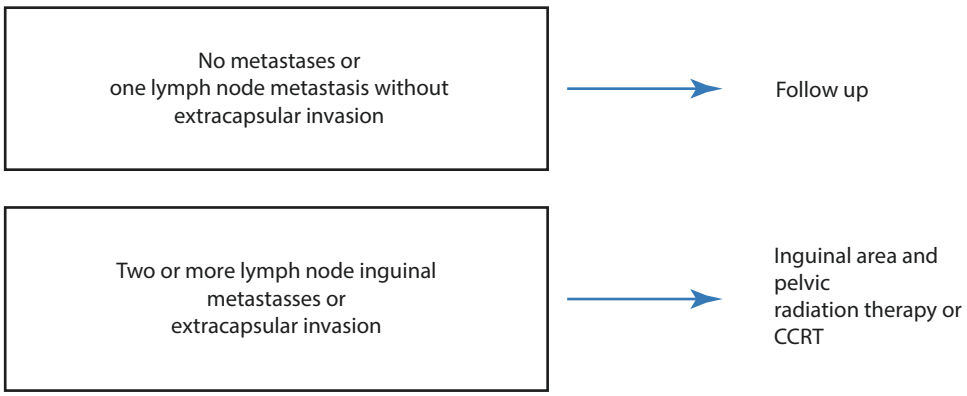
Nowadays, a multimodality treatment has been explored and implemented. Preoperative radiotherapy is demonstrated to have a debulking role and to reduce the surgical treatment morbidity. Additionally, chemotherapy is demonstrated to sensitize the disease to radiations [10].

Chemoradiation

Patients with stage III/IV disease may benefit from a concurrent chemo- and radiation therapy treatment: this choice results in a longer survival rate and recurrence rate [11].

Table 54.1 Management of inguinal lymph nodes of vulvar cancer

Management after inguinal node dissection



In the GOG 101 study, 73 patients underwent chemoradiation before surgery and a residual disease was just detected in 3% of patients treated [12].

An analysis of NCDB data (2004–2012) compared the outcomes of 2046 women with locally advanced vulvar carcinoma who underwent chemotherapy or chemoradiation treatment before surgery. Patients who underwent surgery after the combination treatment had a higher OS compared to patients that received chemotherapy alone [13].

Many other studies assessed the efficacy and safety of a preoperative surgery in a locally advanced disease, and the preferred chemotherapy regimens used were cisplatin and 5-fluorouracil and mitomycin and 5-fluorouracil.

Recurrent and Metastatic Disease

According to NCCN guidelines, the preferred chemotherapy regimens used in the recurrent/metastatic setting are represented by cisplatin and carboplatin as single agents, as well as the combination of cisplatin or carboplatin with paclitaxel, with or without bevacizumab.

Cisplatin is used as a radiosensitizing agent and it has been demonstrated that short- and long-term complication rates were acceptable, with a promising OS and DFS. Additionally, due to the lack of tissue toxicity, this strategy allowed physicians to surgically treat regional lymph node recurrence safely [14].

Carboplatin can be a valid alternative to cisplatin. The JCOG0505 randomized phase III trial assessed that carboplatin-based regimen was non-inferior to cisplatin-based regimen [15].

Prospective trials confirmed that the prognosis for inguinofemoral recurrence is poor. If this event occurs, radiotherapy or concomitant chemotherapy and radiation therapy can be considered. Re-excision for inguinal node recurrence is not a standard of care but can be performed in selected patients [16].

Paclitaxel can also be used as single agent in patients not eligible for locoregional treatment. A phase II study (EORTC-GCG, European Organisation for Research and Treatment of Cancer – Gynaecological Cancer Group) demonstrated that taxol administered once every 3 weeks in patients with metastatic/recurrent vulvar cancer, not amenable for locoregional treatment, had moderate activity for local control. The study registered an ORR of 13.8% and a median PFS of 2.6 months (median follow-up was 24 months).

Immunotherapy has been recently introduced in trial enrolling patients with vulvar cancer. In fact, pembrolizumab has been studied and approved for a second-line therapy for PDL-1-positive or MSI (microsatellite instability) cervical cancers [17]. KEYNOTE-158 is an ongoing trial that is enrolling patients with advanced vulvar cancer to receive pembrolizumab as second-line treatment (NCT02628067).

54.1.5 Follow-Up

Most recurrences of vulvar cancer occur within the first 1 or 2 years. A retrospective analysis of 330 patients with vulvar cancer at Mayo Clinic was conducted and showed that the higher rates of treatment failure were registered in patients with inguinofemoral node involvement, within a 2-year follow up, suggesting that the node involvement is one of the most important prognostic factors. In 35% of patients, disease occurred 5 years or more after diagnosis; this last information suggests the importance of a long-term follow-up [18].

The recommended surveillance is based on the disease stage. History and physical examination should be performed for all patients every 2–3 months for the first 2 years and every 6 months for another 3–5 years. Patients with high risk of recurrence (stage III) can be

assessed more frequently. Annual cervical/vaginal cytology can be indicated in order to detect lower tract dysplasia. Imaging techniques such as CT, PET-CT, and MRI are indicated for suspicious examination findings or symptoms.

54.2 Vaginal Cancer

54.2.1 Overview

Vaginal cancer is a rare disease (1% of the gynecological cancers). The commonest histology is the squamous cell carcinoma, while only 5–10% is adenocarcinoma. The risk factors are similar to the cervical cancer ones; in particular HPV infection and age are involved (PMID: 26411952).

The most common sites for vaginal cancer are the upper third of the vagina (56%) followed by the lower third (31%) and the middle third (13%) [19].

The upper two-thirds of the vagina are drained into pelvic nodes while the lower third drains into the inguinal nodes so that the metastatic routes depend on the site of the primary tumor.

The surgical approach is chosen based on the site of the primary tumor and the surgeon should consider the removal of both the primary tumor and the regional lymph nodes.

Recurrences are usually treated with chemotherapy; however, due to the rarity of this disease, there are few studies in this setting [19].

54.2.2 Histopathological Approaches

The most common histology of vaginal cancer is the squamous cell carcinoma (SCC).

SCC can be histologically divided into five different types: keratinizing, non-keratinizing, basaloid, verrucous, and warty. HPV infection is detected in 80% of cases of vaginal cancer, mostly in the non-keratinizing variant [20].

As for cervical intraepithelial neoplasia (CIN), there is a vaginal intraepithelial neoplasia that is defined as the presence of atypical squamous cells within the vagina epithelium that is not accompanied by interstitial infiltrate. Vaginal intraepithelial neoplasia (VAIN) is classified into three grades: VAIN 1, VAIN 2, and VAIN 3. The largest part of VAIN is caused by HPV infection. VAIN 1 is also called LSIL while VAIN 2 and 3 correspond to HSIL [21].

A less frequent histology is the adenocarcinoma of the vulva that is frequently diagnosed in women who had been exposed in utero to synthetic non-steroid estrogens such as diethylstilbestrol (DES). It was typically used in pregnant women in the 1950s and many cases of vaginal cancers were diagnosed in their young children in the 1970s [22, 23].

The staging of vaginal cancer is performed according to the FIGO classification. In stage I and II the carcinoma is limited at vaginal and subvaginal tissue, in stage III it is extended to the pelvic wall, and in stage IV it is extended beyond the true pelvis and invades bladder and/or rectal mucosa (IVa) or is spread to distant organs (IVb) [23].

54.2.3 Management

- **Principles of Surgical and Radiation Therapy**

Surgery remains the gold standard for resectable vaginal cancer. The type of surgery depends on the site of the disease and on its extension. If the tumor occurs in the upper third of the vagina, surgery consists of hysterectomy extended to the vagina. If vaginal cancer also presents VAIN, a total vaginectomy is recommended. Moreover, pelvic exenteration could represent an option in selected cases [24]. In particular, surgery is recommended for stage I disease and tumor localized in the upper third of the vagina [23]. If the tumor has a large extension and it is mainly localized in the lower part of the vagina, radiation therapy is preferred. If the tumor is small and is localized in the lower third of the vagina, surgery remains highly recommended [24].

Extended surgery such as pelvic exenteration may be considered if the patient has the invasive tumor to the rectum or urinary bladder, a rectovaginal or vesicovaginal fistula, or local recurrent tumors after radiation therapy.

A retrospective study performed by the Magee Hospital of Pittsburgh reported a better prognosis for patients that underwent surgery than irradiation therapy alone in stage I and II disease with an upper third vagina localization [25].

Additionally, the histological features of the disease can determine the treatment strategy; the majority of vaginal cancer has a squamous histopathology while a minority is adenocarcinoma. It has been reported that the adenocarcinoma is poorly sensitive to radiation therapy; thus, surgical therapy is recommended.

Due to the rarity of the disease, there is a lack of randomized trials and only retrospective studies are reported. According to these reports, relevant prognostic factors are tumor size and lymph node involvement.

Radiation therapy is recommended to preserve the function of adjacent organs, when the disease is locally advanced. Methods of irradiation therapy include brachytherapy and more recently image-guided brachy-

therapy (IMBT). External beam irradiation based on 3D treatment planning using CT and MRI has become a standard [23, 26].

More specifically, stage I disease may benefit from brachytherapy alone or in combination with external beam irradiation (with tumor thickness <5 mm, while for tumor thickness greater than 5 mm or at stages II to IVA, external beam irradiation is recommended). Also, concurrent chemotherapy with carboplatin or cisplatin may be considered [27].

Concurrent chemo- and radiation therapy is performed using sensitizing agents such as cisplatin and 5-fluorouracil. Because of the rarity of this disease, it is hard to find out the real efficacy of combining treatments. Given that, it is reasonable to apply results of clinical trials regarding cervical cancer, based on similarities of organ sites, risk factors, and histopathology. Physicians must consider concurrent use of chemotherapy in combination with radiation therapy if the tumor is stage III or IVA, >4 cm in diameter, or positive for lymph node metastasis [23, 27, 28] (see Tables 54.2 and 54.3).

54

54.2.4 Follow-Up

History and physical examination, cytology, chest X-ray examination, tumor markers, and CT should be performed every 2–3 months for the first 2 years, every 6 months through the fifth year, and once a year for the sixth and subsequent years.

The higher risk of recurrence has been registered in the first 2 years after the diagnosis. The recurrence rates decrease after 5 years [29].

Recurrence can be confirmed using cytology and biopsy and CT, MRI, and PET are indicated for suspicious findings.

The first choice for locoregional relapse is radiation therapy; when distant metastases occur, chemotherapy is the treatment selected [23].

54.3 Summary: Conclusion

Vulvar and Vaginal cancer are considered rare diseases with a low incidence but a relative increase in the most industrialized countries. surgery is the recommended primary treatment for localized vulvar and vaginal cancer (stage I), while locally advanced diseases may benefit of concurrent chemo and radiation therapy. The preferred chemotherapy regimens are cisplatin or carboplatin and 5FU. Immunotherapy (Pembrolizumab) is still under investigation in the second line treatment of metastatic vulvar cancer.

Table 54.2 Primary treatment for early-stage vaginal cancer

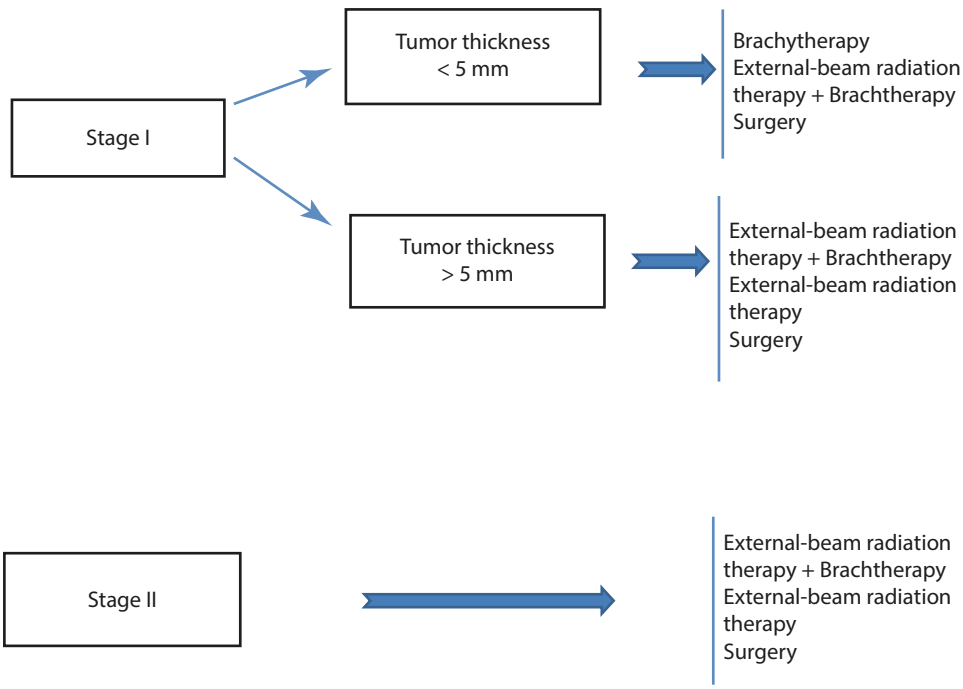
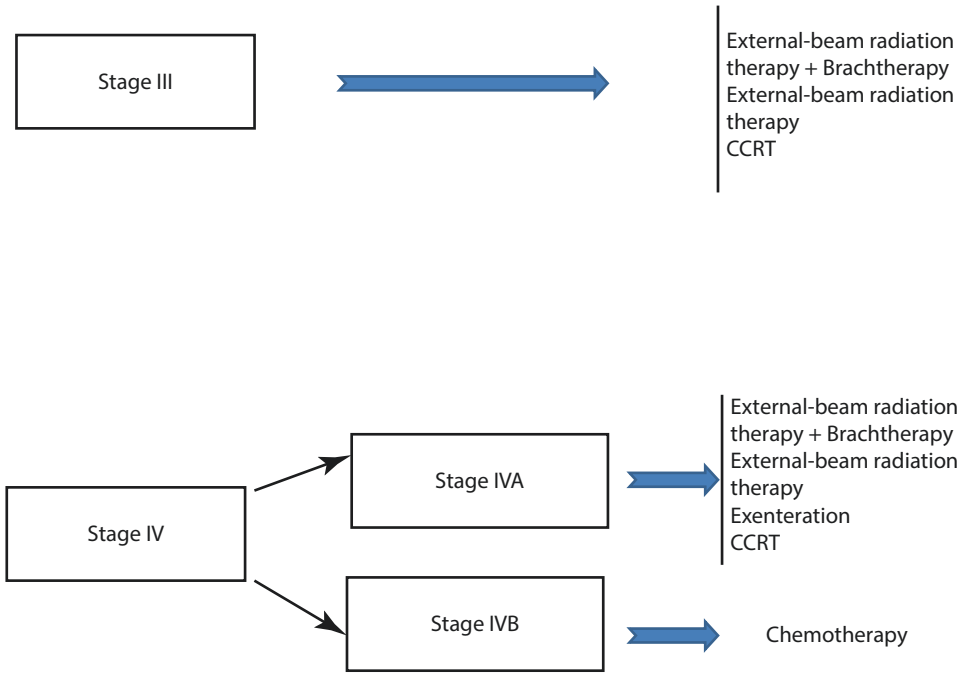


Table 54.3 Primary treatment for locally advanced and metastatic vaginal cancer



Key Points

- Vulvar and vaginal cancer are considered rare diseases with a low incidence but a relative increase in the most industrialized countries. The main risk factors are HPV infection, age, disadvantaged socio-economic conditions, and cigarette smoking.
- The instrumental diagnostic approach in case of suspected disease is represented by clinical examination, cytology, and biopsy. A more specific evaluation must be carried out using the TC and the MRI; in some circumstances, PET integration can be helpful.
- Currently surgery is the recommended primary treatment for localized vulvar and vaginal cancer (stage I), while locally advanced diseases may benefit from concurrent chemo- and radiation therapy. The preferred chemotherapy regimens are cisplatin or carboplatin and 5-FU. The addition of bevacizumab can be considered in the treatment of metastatic vulvar cancer.
- Local recurrence/persistence: Radiation therapy should be considered for local recurrence. Re-excision of inguinofemoral lymph nodes is discouraged because of postoperative complications.
- Immunotherapy: Pembrolizumab is still under investigation in the second-line treatment of metastatic vulvar cancer.

Case Study: Vulvar Cancer In Situ

Woman, 55 years old

- *Family history:* Negative for malignancy
- *APR:* Nothing to report
- *APP:* Bleeding and itchiness on her vulva
- *Objective examination*: Papules, plaques, and ulcerated lesions on the vulva

Question

What action should be taken?

(1) Surgery. (2) Biopsy and/or cytology. (3) Others

Answer

Biopsy and path: Usual-type VIN, warty subtype, with HPV changes

Question

What action should be taken?

(1) Surgery. (2) Medical therapy. (3) Clinical staging

Answer

Surgery: Wide local excision of the lesions

Path: VIN 2 (HSIL)

Question

What would you do next?

(1) Surgery. (2) Medical therapy. (3) Follow-up

Answer

Follow-up: Clinical examination every 6 months for the first 2 years

Key Points

- The importance of a correct diagnosis: Attention to rectal masses
- Symptoms often nonspecific
- The importance of the management of a locally advanced disease

Case Study: Metastatic Vaginal Cancer

Woman, 70 years old

- *Family history:* Negative for malignancy
- *APR*: Negative
- *APP*: Asthenia, bleeding on the vagina
- *Blood tests*: Hb 9,2 g/dl
- *Objective examination*: Wide ulcerative and bleeding lesion on her vagina
- *Chest and abdominal CT scan*: Multiple lung lesions
- *Biopsy*: Squamous cell carcinoma of the vagina

Question

What action should be taken?

(1) Surgery. (2) Metastasectomy. 3) Chemotherapy

Answer

Chemotherapy with 5-FU and cisplatin

Question

Is metastasectomy on lung lesions recommended?

(1) Yes, after four cycles of chemotherapy. (2) Surgery is not indicated for the metastatic setting. (3) Others

Answer:

No evidence supports resection of metastatic disease.

Key Points

- The importance of a correct diagnosis: Attention to rectal masses
- Importance of the right management for metastatic disease

Expert Opinion

Domenica Lorusso

Key Points

It is estimated that approximately 27.000 and 15000 women worldwide are diagnosed with vulvar cancer and vaginal cancer, respectively, each year thus meaning that both diseases represent rare conditions for which solid literature evidences about treatment are hard to be produced.

Both conditions are, at some extent, related to HPV infections and, in this case, are associated to a better prognosis than non-HPV-related tumors. As such, HPV vaccines represent the best form of primary prevention for both tumors, and last-generation 9-valent HPV vaccine has been calculated to be able to eradicate 90% of HPV-driven cases. Both tumors are preceded by pre-invasive conditions (VIN and VAIN) whose treatment represents a tool to reduce the risk of developing invasive cancer (secondary prevention).

Stage of disease represents the most important prognostic factor with 5-year survival ranging from 80% to 15% for stage I and IV, respectively, for vulvar cancer and from 60 to less than 10% for vaginal cancer.

For vulvar cancers, the mainstay of treatment is represented by the surgical excision of the primary tumor providing free radical 1 cm margins and the surgical assessment of the inguinofemoral nodes through the excision of sentinel lymph node (SLN) in less than 4 cm lateral tumors and by bilateral linguino-femoral lymphadenectomy in all other cases. The role of SLN in tumors larger than 4 cm need to be addressed in future clinical trials.

Radiotherapy and chemoradiation represent the standard of care for more advanced disease when free radical margins are impossible to obtain without extremely demolitive surgical procedures and the adjuvant treatment for node positive patients after radical surgery.

In vaginal cancers, surgery (radical local excision and pelvic lymphadenectomy) has a role limited to stage I disease involving the upper posterior vagina, while radiation or chemoradiotherapy represents the standard of care for most patients.

Chemotherapy has a palliative role in recurrent disease. Most used drugs are platinum, paclitaxel, gemcitabine, and 5-fluorouacil with response rate of about 10–15% and median PFS of less than 3 months. No biological agent has been approved for the treatment of both diseases, but a strong scientific rationale and preliminary clinical data suggest that antiangiogenic agents, tyrosine kinase inhibitors, and EGFR receptor inhibitors may play a role in the treatment of the disease. New agents and new treatment strategies are needed to improve outcome in such setting, and immunotherapy may represent a potent tool particularly for HPV–related tumors as recently reported during the last ASCO meeting.

In most cases, vulvar and vaginal tumors are squamous: to further complicate the scenario, the presence of several rarest histotypes in both the anatomical locations (adenocarcinomas, melanomas, sarcomas) make treatment evidences even more scanty.

The major difficulties in producing strong scientific evidences in these diseases leading to new drug approval is represented by the infrequency of the tumors. I strongly believe this is an area in which international cooperation of groups involved in clinical research may play a fundamental role in ameliorating treatment outcome. Moreover, as for all rare cancers, different study designs and simplified drug approval procedures are mandatory.

In conclusion, vulvar and vaginal cancers are rare malignancies that need to be treated in tertiary referral centers where these diseases can be managed through a multidisciplinary approach.

Key Message

- Vaginal and vulvar cancer are rare diseases that need to be managed in tertiary referral centers in a multidisciplinary approach.
- Most part of these tumors are HPV related and can be prevented by HPV vaccines (primary prevention).
- Both the tumors are anticipated by premalignant lesions that can be cured in order to reduce the incidence of malignant disease (secondary prevention).
- Radical surgery with clear margins and inguinofemoral lymph node evaluation is the mainstay of treatment in early stage vulvar cancers
- Radiotherapy/chemoradiation is the treatment of choice in most part of vaginal cancer and in advanced stage vulvar cancer.
- Chemotherapy has a palliative role in recurrent disease; no biological agents have been approved for the treatment of these rare tumors.
- International collaboration is mandatory in producing evidences for the management of rare tumors.

Discussion Points

- The role of sentinel lymph node in larger than 4 cm tumors need to be addressed.
- The role of isolated tumor cells and micrometastasis on prognosis need to be better clarified.
- New biological agents (antiangiogenic agents, tyrosine kinase inhibitors, and EGFR receptor inhibitors) need to be studied and possibly approved for the management of advanced disease where prognosis remains dismal.
- Different study designs and simplified drug approval procedures are mandatory in rare disease.

Summary of Clinical Recommendations

- *NCCN*
  ► https://www.nccn.org/professionals/physician_gls/pdf/vulvar_blocks.pdf
- *ESGO*
  ► https://guidelines.esgo.org/media/2016/08/ESGO-Vulvar-cancer-Complete-report-fxd2.pdf
- *AIOM*

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Cancer of the Adrenal Gland

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Endocrine Cancers

Contents

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Learning Objectives

By the end of the chapter the reader will

- have reached in-depth knowledge of biology and clinical presentation of paraganglioma/pheochromocytoma and adrenocortical carcinoma
- have learned the basic concepts of diagnosis and natural history of these rare diseases be able to put acquired knowledge to select the best treatment strategies for every patient bearing these rare diseases.

55.1 Introduction

The adrenal gland is composed of two embryological and functional distinct organs (Fig. 55.1). The inner adrenal medulla is derived from neural ectoderm, and in the adult, it is a mediator of the acute stress response through secretion of catecholamines. The adrenal cortex is derived from intermediate mesoderm, and it is organized into three distinct concentric zones with three distinct functions [1]. The outer zona glomerulosa synthesizes and secretes mineralocorticoids that function to maintain sodium balance and intravascular volume, the zona fasciculate synthesizes glucocorticoids that function to regulate energy storage, and the zona reticularis synthesizes sex-steroid precursors.

Tumors may arise both from medulla and cortex. The majority of them are benign. Pheochromocytomas are tumors derived from the chromaffin cells of the embryonic neural crest [2]. Chromaffin cells are postganglionic parasympathetic and sympathetic neurons which are located in the adrenal medulla or along the paravertebral and para-aortic axes (Fig. 55.2). Sympathetic paraganglia have a neck-to-pelvis distribution and produce catecholamines, while parasympathetic paraganglia, which do not produce catecholamines, are found almost exclusively in the neck and skull base, along the branches of glossopharyngeal and vagus nerve. Tumors arising from extra-adrenal chromaffin cells are termed paragangliomas.

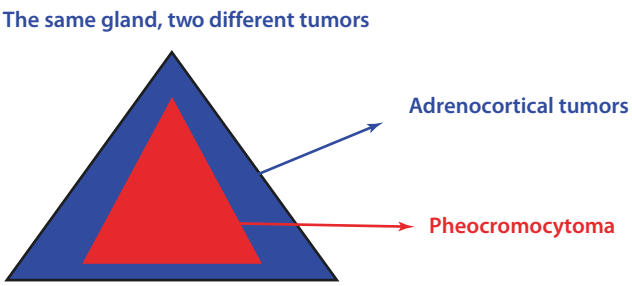


Fig. 55.1 Adrenal gland is composed of two different organs. Pheochromocytomas derived from the inner medulla, instead adrenocortical tumors from the outer zones

Tumors deriving from malignant transformation of adrenal cortex are either adenoma or adrenocortical carcinoma.

55.2 Epidemiology

Pheochromocytomas (PCCs) and paragangliomas (PGLs) are rare diseases with an estimated incidence in Western countries between 2 and 8 new cases per million population per year [3]. Many cases are discovered incidentally by computed tomography or magnetic resonance imaging. The peak age of occurrence is in the third to fifth decade of life with almost equal distribution among male and female patients. About 10–20 % occur in pediatric patients. Between 5 % and 20 % of PCCs and 15 % and 35 % of sympathetic PGLs is malignant with the occurrence of metastatic disease either at diagnosis or during the natural history of the disease.

Adrenocortical neoplasms are relatively frequent, with an estimated incidence in the general population ranging from 3 % to 10 % [4]. A large Italian study showed that of 380 operated adrenal incidetalomas, the 52 % were adenomas [5].

The incidence of adrenocortical carcinoma (ACC) in Western countries is between 0.5 and 2 new cases per

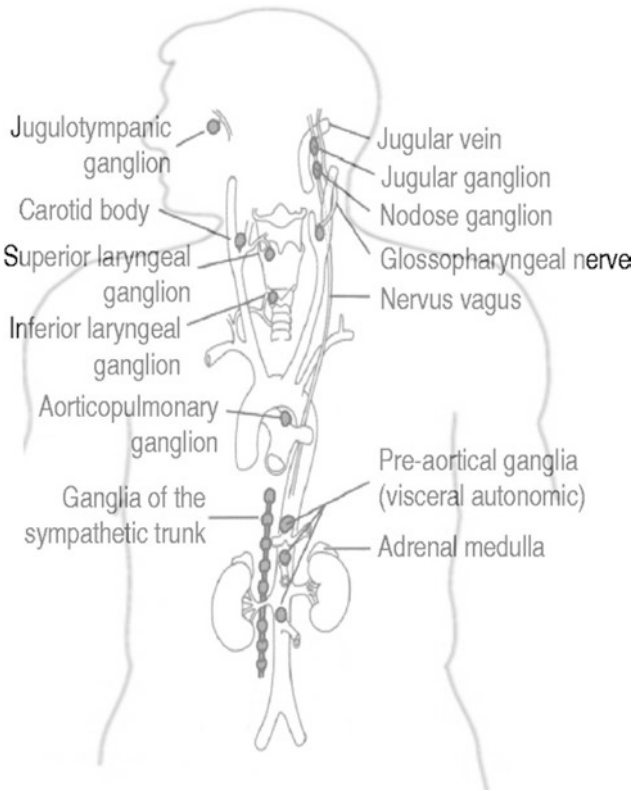


Fig. 55.2 Potential sites of paragangliomas and pheochromocytomas

million population per year. The male/female ratio is 1/1.5, and according to age, there is a bimodal distribution with 2 peaks in childhood and young adults between 4th and 5th decade [6].

55.3 Heritability

PCCs and PGLs are mainly sporadic, but they may also be associated with specific familiar disorders. More than 30% of PCCs/PGLs present with germline mutations being associated with hereditary syndromes [7], that is, multiple endocrine neoplasia type 2 (MEN2), neurofibromatosis type 1 (NF), von Hippel–Lindau syndrome (VHL), and pheochromocytoma-paraganglioma syndrome (PGL 1, 3, and 4) (Table 55.1).

Since this group of tumors is the most strongly inherited among all human tumors, genetic screening is recommended, particularly in case of young age of tumor appearance, positive family history, bilaterally multifocal tumors, and recurrence or malignancy.

Genetic disorders associated with ACC are Li-Fraumeni and Beckwith-Wiedemann syndromes. Less clear is the association of ACC with familial adenomatous polyposis, multiple endocrine neoplasia 1, and neurofibromatosis [8] (Table 55.1). Genetic tests for ACC are not routinely recommended but may be performed in selected young patients.

55.4 Adrenal Incidentaloma

Adrenal incidentaloma (AI) is an asymptomatic adrenal mass detected on imaging not performed for suspected adrenal disease. With modern imaging techniques, AIs are increasingly detected, and based on published literature, the frequencies of the different underlying tumor types are adrenocortical adenomas in 80 % (75 % of them are nonfunctioning, and the majority of functioning are cortisol secreting), adrenocortical carcinoma in 8 %, pheochromocytomas in 7 %, and metastatic tumors in 5 %. In surgical series, the distribution is as follows: adenoma 55 % (nonfunctioning 69 %, cortisol-secreting 10 %, aldosterone-secreting 6 %), pheochromocytoma 10 %, adrenocortical carcinoma 11 %, myelolipoma 8%, cyst 5%, ganglioneuroma 4 %, and metastasis 7 % [11].

The prevalence of adrenal adenomas decreases with the tumor size in favor of ACC that represents a minor part of AI if they are less than 4 cm (2 % of cases) or 4–6 cm (6 % of cases) in size. However, their prevalence increases substantially, and among adrenal tumors >6 cm, it can be up to 25 %.

According to the guidelines of the European Society of Endocrinology, surgical treatment of AI should be considered in an individualized approach; the appropriateness of surgical intervention should be guided by the likelihood of malignancy, the presence and degree of hormone excess, age, general health, and patient preference.

55.5 Clinical Features

The vast majority of symptoms and signs of PCCs are due to the associate excess of catecholamines released by tumors either continuously or paroxysmally [12]. The most frequent symptoms and sign is hypertension typically sustained, paroxysmal, or sustained with paroxysms. The paroxysmal release of catecholamines constitutes the characteristic classic triad of episodic headache, sweating, and palpitations. This may be triggered by anesthesia and tumor manipulation; positional change, exercise, and various medications (e.g., tricyclic antidepressants, opiates, metoclopramide, and radiographic contrast agents) are other possible precipitating factors. Frequently, the episodes occur in a random pattern with no clearly defined precipitating event. Other symptoms associated to PCCs are anxiety, dyspnea, chest, abdominal or flank pain, nausea and vomiting, tremor, flushing, dizziness, visual symptoms such as blurred vision, and paresthesia. Persistent vasoconstriction in patients with pheochromocytoma declines the blood volume leading to orthostatic hypotension. Chronic exposure to catecholamine may lead to irreversible myocardial fibrosis [13].

The majority of ACC are functioning at presentation; according to the Orbassano and Brescia database, 52 % of ACC at diagnosis are hormone secreting (Fig. 55.3). Cortisol either alone or in association with androgens is the hormone most frequently secreted, so Cushing syndrome is the most frequent clinical manifestation. Less frequently, the tumors may produce androgens or other hormones such as estrogens or mineral corticoid hormones, and consequently, the symptoms and signs can be amenorrhea and virilization or hypertension with hypokalemia, respectively (Table 55.2).

Both malignant PCCs and ACC patients may suffer from symptoms and signs related to malignancy such as weight loss and fatigue and symptoms related to primary tumor mass and/or relevant metastases.

55.6 Pathological Features

The pathological differential diagnosis of adrenal neoplasias is still largely based on morphological features requiring an experienced pathologist.

There is no histological system that is currently endorsed for the biological aggressiveness of PCCs/

Table 55.1 Hereditary syndromes in PCC, PGL, and ACC

Adrenal Tumor	Syndrome	Mutation	Prevalence in general population	Clinical features
PCC	Von Hippel-Lindau	VHL	1:36.000	Hemangioblastomas of the brain, spinal cord, and retina, renal cysts and clear cell renal cell carcinoma, pheochromocytoma, pancreatic cysts, and neuroendocrine tumors, endolymphatic sac tumors, and epididymal and broad ligament cysts
PCC	MEN2A MEN2B	RET	1–9:100.000	MEN2A: Medullary thyroid carcinoma, pheochromocytoma, parathyroid adenoma or hyperplasia. MEN2B: Medullary thyroid carcinoma, pheochromocytoma, mucosal neuromas of the lips and tongue, distinctive facies with enlarged lips, ganglioneuromatosis of the gastrointestinal tract, and a "marfanoid" habitus
PCC	Pheochromocytoma-paraganglioma syndrome	SDHA SDHB SDHC SDHD SDHAF2	1:30.000–100.000	Leigh syndrome, late-onset optic atrophy, ataxia and myopathy, PGLs High malignant potential extra-adrenal PGLs, adrenal PCCs and HNPGLs HNPGLs, rare cases of adrenal PCCs and extra-adrenal PGLs Multifocal HNPGLs, adrenal PCCs and extra-adrenal PGLs (usually benign) Young age onset multifocal HNPGLs
PCC and ACC	Von Recklinghausen	NF1	1:3.000	Malignant peripheral nerve sheet tumor, pheochromocytoma, café au lait spots, neurofibroma, optic glioma, Lisch nodule, skeletal abnormalities
ACC	Li-Fraumeni syndrome	TP53	1:20.000–1.000.000	Sarcoma, choroid plexus tumor, brain cancer, early breast cancer, leukemia, lymphoma
ACC	Lynch syndrome	MSH2, MSH6, MLH1, PMS2	1:440	Colorectal cancer, endometrial cancer, sebaceous neoplasms, ovarian cancer, pancreatic cancer, brain cancer
ACC	MEN1	MENIN	1:30.000	Foregut neuroendocrine tumors, pituitary tumors, parathyroid hyperplasia, collagenoma, angiofibroma, adrenal adenoma/hyperplasia
ACC	Beckwith-Wiedemann syndrome	IGF2, CDKN1C, H19 locus changes on 11p15	1:13.000	Wilms' tumor, hepatoblastoma, macrosomia, adrenocortical cytomegaly, adrenal adenoma, adrenal cyst, hemihypertrophy, macroglossia, omphalocele, ear pits
ACC	FAP	APC	1:30.000	Intestinal polyps, colon cancer, duodenal carcinoma, thyroid cancer, desmoid tumor, adrenal adenoma, supernumerary teeth, congenital hypertrophy of the retina, osteoma, epidermoid cysts
ACC	Carney complex	PRKAR1A	700 cases worldwide	Primary pigmented nodular adrenal disease, large-cell calcifying Sertoli cell tumors, thyroid adenoma, myxoma, somatotroph pituitary adenoma, lentigines

VHL von Hippel-Lindau, *MEN1* Multiple endocrine neoplasia type 1, *MEN2* Multiple endocrine neoplasia type 2, *RET* rearranged during transfection proto-oncogene, *SDH* succinate dehydrogenase, *HNPGLs* head and neck region paragangliomas, *FAP* familial adenomatous polyposis, *NF1* neurofibromatosis type 1
Modified from [9, 10]

PGLs. The certainty of malignant behavior is done by the presence of metastases. According to the last WHO classification [14], all pheochromocytomas could have metastatic potential. Several histologic features such as invasion (vascular, capsular, and/or periadrenal adipose tissue), large nests or diffuse growth, focal or confluent necrosis, high cellularity, tumor cell spindling, cellular monotony, increased and/or atypical mitotic figures, profound nuclear pleomorphism, and hyperchromasia included in the Pheochromocytoma Adrenal gland Scaled Score (PASS) have been associated with malignancy. However, the validity of this scoring system is a matter of controversy.

Several markers have been introduced to establish the adrenocortical origin of adrenal masses, with steroidogenesis factor-1 immunohistochemistry and Melan-A being particularly useful. The differential diagnosis between adrenocortical adenoma versus carcinoma may be challenging.

The most widely used diagnostic score has been introduced by Weiss et al. [15] and includes the following parameters: mitosis, atypical mitosis, necrosis, venous invasion, sinusal invasion, capsular invasion, nuclear atypia, diffuse architecture, and clear cell. A score of ≥3 suggests malignancy. Ki67 as a marker of proliferative activity is useful particularly as independent prognostic factor.

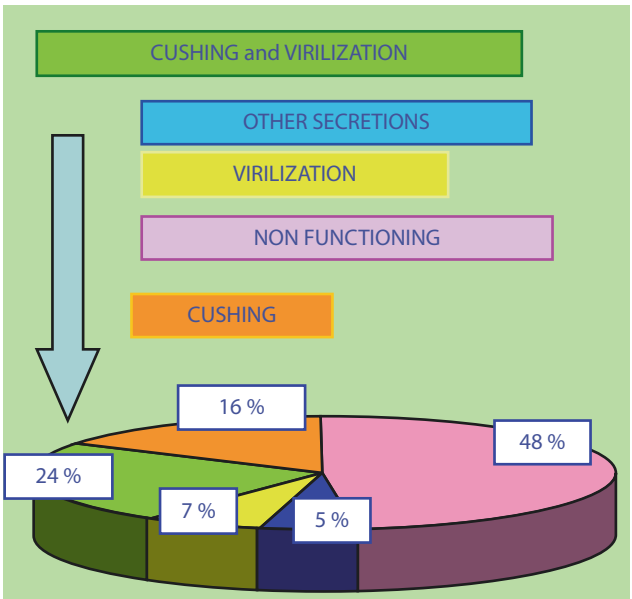


Fig. 55.3 Adrenocortical cancer. Clinical presentation

55.7 Molecular Biology

The inherited basis of PCCs/PGLs has been well characterized since many years. About 12 % to 16 % of them have SDHx or FH mutations. The gene encoding subunit B of the SDH complex is by far the most important contributor to a hereditary malignant disease. Between 1 % and 13 % of PCCs/PGLs have germline VHL mutations, whereas the frequency of RET, NF1, TMEM, and MAX considered together is between 1 % and 11 %. Sporadic PCCs/PGLs may retain the same driver genes as seen in inherited tumors; however, the number of driver genes has grown to more than 20 over the past decade suggesting great complexity of these diseases. A comprehensive molecular analysis, recently published, revealed that PCCs/PGLs have a low genome alteration rate with a remarkable diversity of driver alterations including germline and somatic mutations and somatic fusion genes. New driver genes were discovered including a Wnt-altered subtype driven by a MAML3 fusion gene and CSDE1 somatic mutation [16]. Put these new data in the context of the established literature five molecular subtypes of both inherited and sporadic PCCs/PGLs have been identified: (1) pseudohypoxic

Table 55.2 Hormone syndromes related to secreting ACC and PCC

Syndrome	Incidence	Hormone profile	Signs and symptoms
Cushing's syndrome	50–80 % of cases	Hypercortisolism, suppressed ACTH levels	Plethora, dorsal fat hump, diabetes mellitus, muscle weakness/atrophy, osteoporosis, hypokalemia, hypertension, mood alterations, insomnia, skin atrophy, higher susceptibility to infectious diseases
Hyperandrogenism	40–60 % of cases	Excess of dehydroepiandrostenedione sulfate, 17-hydroxyprogesterone, testosterone, androstenedione	In women: hirsutism, virilization, menstrual irregularities, temporal balding, acne.
Pheochromocytoma	All cases	Paroxysmal and chronic release of catecholamines	Classic triad: episodic headache, sweating, and palpitations; anxiety, dyspnea, chest, abdominal or flank pain, nausea and vomiting, tremor, flushing, dizziness, blurred vision, and paresthesia, orthostatic hypotension, irreversible myocardial fibrosis.

Table 55.3 Clusters and driven alterations

Cluster	Driven alteration	Degree hereditary	Altered pathways
Pseudohypoxia	TCA cycle-related	100 %	Mitochondrial dysfunction *(SDHA, SDHB, SDHC, SDHD, SDHAF2, FH)* and pseudohypoxia
Pseudohypoxia	VHL/EPAS1-related	25 %	Pseudohypoxia *(VHL, EPAS1)*
Wnt signaling	CSDE1, MAML3-fusion	0 %	Wnt-signaling *(CSDE1, MAML3)*
Kinase signaling	NF1> HRAS> RET> TMEM127 > MAX	20 %	MYC *(MAX)*, MAPK *(RET, NF1, HRAS)*, mTOR *(TMEM127)*

PCCs/PGLs, (2) pseudohypoxic PCCs/PGLs, TCA cycle-related, (3) pseudohypoxic PCCs/PGLs, VHL, and EPAS1-related, (4) Wnt signaling PCCs/PGLs, and (5) Kinase signaling PCCs/PGLs (Table 55.3). This molecular classification provides opportunities for prognostic stratifications and future targeted therapies [17].

Numerous genetic and molecular studies have recently been performed on adrenocortical tumors, including carcinoma. These studies have detected nine driver genes CTNNB1, ZNRF3, TP53, RB1, CDKN2A, MEN1, DAXX, TERT, and MED12 and the involvement of three major pathways, including the p53, Wnt/b-catenin, and IGFII pathways. Alteration in microRNA (miRNA) profiling or hypermethylation of the CpG island methylator phenotype in up to 50 % of cases of ACC was also described [8].

55.8 Diagnosis

55.8.1 Hormone and Biochemical Assessment

Since both PCCs/PGLs and ACC are hormone secreting, a comprehensive hormonal analysis is recommended when an adrenal mass is diagnosed. The ACC working group of the European Network for the Study of Adrenal Tumors [18] suggests a preoperative hormonal work-up including basal cortisol, ACTH, dehydroepiandrostenedione sulfate, 17-hydroxyprogesterone, testosterone, androstenedione, estradiol, urinary free cortisol, and dexamethasone suppression test [19, 20]. The endocrine assessment is essential in order to confirm a suspected hormonal excess and to establish the origin (cortex or medulla) and nature (malignant or benign) of the adrenal lesion, i.e., the coexistence of cortisol and androgen hypersecretion is a sign of malignancy [11, 14, 19] (Table 55.4). The best screening test for initial assessment of PCCs/PGLs is measurement of free plasma and urinary fractionated metanephrines [21]. Although elevation of plasma or urinary normetanephrines slightly above the upper reference range only marginally increases the probability of PCs/PGLs, a more than fourfold elevation is associated with a 100 % probability [22].

Table 55.4 Homonal work-up for adrenal cancers

Glucocorticoid excess (minimum of 3 of 4 tests)
Dexamethasone suppression test (1 mg, 23:00 h)
Excretion of free urinary cortisol (24 h urine)
Basal cortisol (serum)
Basal ACTH (plasma)
Sexual steroids and steroid precursor
DHEA-S (serum)
17-OH-progesterone (serum)
Androstenedione (serum)
Testosterone (serum)
17-beta-estradiol (serum, only in men and postmenopausal women)
24-h urine steroid metabolite examination
Mineralocorticoid excess
Potassium (serum)
Aldosterone/renin ratio (only in patients with arterial hypertension and /or hypokalemia)
Catecholamine excess
Normetanephrine, metanephrine, and methoxytyramine (plasma)
Alternatively: fractionated metanephrine excretion (24 h urine)

In metastatic PCs/PGLs, any increment of normetanephrines indicates disease relapse and activity often before the onset of symptoms [23]. Plasma methoxytyramine is another useful marker. Elevated levels (more than fourfold higher the normal range) are associated

with SDHB mutations and extra-adrenal disease and can be predictive of malignancy [22]. Plasma chromogranin (CgA) levels may be elevated in both benign and metastatic PCCs/PGLs although it is significantly higher in metastatic tumors and associated with poor prognosis. CgA is a valuable complementary in malignant PCCs/PGLs since supranormal levels are frequently found in patients with metastatic disease and normal normetanephrine levels [24].

55.8.2 Imaging

The role of imaging procedures is of paramount importance to differentiate benign and malignant adrenal lesions and to correctly stage the disease. Computed tomography (CT) scan is the first choice imaging technique [19]. Tumor size, lipid content of the mass, and the velocity of the washout of contrast medium are the best criteria for diagnosing ACC [25].

As previously mentioned, the risk for malignancy increases for lesions >4 cm (sensitivity, 97 %; specificity, 52 %) and >6 cm (sensitivity, 91 %; specificity, 80 %) [26], while a density mass ≤10 Hounsfield Unit (HU) in unenhanced CT scan is significant for a lipid-rich content and, thus, for the benign nature of the lesion [11, 20]. In case of basal density >10 HU, a rapid contrast-medium washout (>50 %) is diagnostic for the benignity of the tumor [19, 20]. ACCs are usually irregular large masses, with heterogeneous enhancement for the presence of necrotic, calcific, and hemorrhagic areas in the solid component [20, 25]. Local invasion and tumor extension into the inferior vena cava are indicative of malignant behavior [19]. A chest CT scan must be performed to exclude the presence of lung metastases before surgery [19, 20].

Magnetic resonance imaging (MRI) of the abdomen is considered as effective as CT scan in detecting ACCs [19]. Adrenal carcinomas appear isointense to hypointense on T1-weighted images and hyperintense on T2-weighted images and show a heterogeneous signal drop on chemical shift [27, 28]. In radiologically indeterminate adrenal lesions, functional imaging can be a helpful integrative diagnostic tool, as a high uptake at the ^{18}F-fluorodeoxyglucose-positron emission tomography (FDG-PET) is suggestive for ACC [29, 30]. To prove the adrenocortical origin of a lesion, a new tracer can be used: metomidate ([11C]MTO). It specifically binds to adrenocortical CYP11B, key step enzymes in steroid synthesis. ACCs show a higher uptake at [11C]MTO-PET compared to normal gland [31].

CT scanning of the abdomen and pelvis is the recommended initial imaging modality also for pheochromocytoma [5]. CT provides high tomographic resolution with a localization sensitivity between 88 % and 100 %. On CT imaging, PCCs can be homogeneous or heterogeneous, solid or cystic, and with or without calcification. MRI is another useful tool in localizing PCCs. The most common MR imaging appearance of a PCC is of low signal intensity on T1 imaging and high signal intensity on T2-weighted imaging. Although MRI lacks the superior spatial resolution of CT, it is useful to detect skull base and neck paragangliomas. Contrast-enhanced ultrasound has attracted interest, but there are insufficient data to recommend it for PPGL screening [32].

Functional imaging is another widely used imaging modality for pheochromocytomas. Metaiodobenzylguanidine (MIBG) is a radiopharmaceutical agent that accumulates preferentially in catecholamine-producing cells. ^{123}I-labelled MIBG has a sensitivity between 85 % and 88 % for PCCs and between 56 % and 75 % for PGLs. Its specificity ranges from 70–100 % to 84–100 %, respectively [33, 34, 35, 36]. ^{123}I-MIBG is the recommended agent for functional imaging in patients with PCC. Its major diagnostic uses are confirmation that an adrenal lesion is a PCC, the identification of metastases, and assessing suitability for ^{131}I-MIBG therapy. Prior to ^{123}I-MIBG imaging, thyroid uptake of radioactive iodine must be blocked with potassium iodide.

In addition to MIBG, several other functional imaging modalities have been identified including PET scanning using 18Ffluorodopamine, 18F-fluorodihydroxy-phenylalanine (^{18}F-DOPA), or ^{18}F-fluoro-deoxy-glucose (FDG). FDG-PET is especially used in paragangliomas or metastatic; it is a highly sensitive disease in tumors showing SDH mutations [37]. ^{68}Ga-DOTATATE PET/CT was recently found to be superior to ^{123}I-MIBG and SRS and is considered as the first-line investigation in high-risk patients of metastatic PCCs/PGLs and familial PGLs harboring SHDB mutations [38].

55.9 Differential Diagnosis

Tumors which should be considered in the differential diagnosis of adrenal pheochromocytomas and adrenal adenoma/carcinoma include myelolipoma, cyst, ganglioneuroma, and metastasis.

55.10 Prognostic Factors

The progression of PCCs/PGLs is strongly influenced by genetics. Currently, the only reliable predictor of malignancy is the SDHB gene germline mutation as it is found in more than 40 % of metastatic PCCs/PGLs (especially extra-adrenal PGLs) [39]. There is no staging system for malignant PCCs/PGLs. The survival rate depends mainly on the tumor size and primary tumor

location (extra-adrenal location is associated with poor prognosis) [19]. Short-term survivors (<5 years) are patients with metastases to the liver and lungs, whereas long-term survivors have bone metastases [40].

The most important prognostic factors in early ACC are the disease stage, margin-free resection, age, the proliferation marker Ki67, and the glucocorticoid excess [19]. In patients with metastatic disease, the prognosis is generally poor, but it is more heterogeneous than previously believed, and long-term survivors are rarely seen. The number of tumor organs (Table 55.5) has a major prognostic role together with four other parameters grouped together under the label GRAS, defined by grade (Weiss score <6 or >6 or Ki67 <20 % or >20 %), resection status of the primary, age younger than or older than 50 years, and the absence or presence of tumor-related or hormone-related symptoms at diagnosis. The GRAS parameters are defined favorable if Ki67 < 20 %, primary R0 resection is performed, age <50 years, and there is the absence of symptoms at diagnosis (either related to cortisol hypersecretion or tumor mass). The GRAS parameters are classified as pejorative in case of grading as defined by Ki67 >20 % and/or primary R1-2 resection status [41].

55

Table 55.5 mENSAT + GRAS classification of ACC

Stage	mENSAT + GRAS
I	T1–2, favorable GRAS[a]
II	II-A: T1–2, unfavorable GRAS
	II-B: T1–2, pejorative GRAS
III	III-A: T3, or T4, N0, M0, and favorable GRAS
	III-B: T3, or T4, N0, M0, and unfavorable GRAS
	III-C: T3, or T4, N0, M0, and pejorative GRAS
IV	IV-A: 2 or 3 tumor organs[b] and favorable GRAS
	IV-B: 2 or 3 tumor organs and unfavorable GRAS
	IV-C: 2 or ≥3 tumor organs and pejorative GRAS

[a]GRAS parameters are considered favorable if grading defined by Ki67 is <20 %, primary R0 resection status performed, age <50 y, and there is the absence of symptoms at diagnosis. GRAS parameters are classified unfavorable in case of age >50 y, or the presence of symptoms at diagnosis. GRAS parameters are classified as pejorative in case of grading as defined by Ki67 >20 % and/or primary R1-2 resection status.
[b]Tumor organ counts include the primary and lymph nodes if not resected (Baudin E. et al., 2015)

55.11 Treatment

Surgery is the mainstay of therapy in the management of both ACC and pheochromocytoma with local regional disease. It is advisable that adrenal surgery should be performed in referenced centers with a documented number for adrenal cancer per year (>10 adrenalectomies) [19]. Open surgery is the standard treatment of ACC patients when complete resection can be achieved. Laparoscopic adrenalectomy is the standard procedure for pheochromocytoma and for a selected group of patients with small ACCs without preoperative evidence for invasiveness and adrenal masses (e.g., incidentalomas) that are judged as only potentially malignant.

The major principles of the management of metastatic PCCs/PGLs include control of symptoms related to catecholamine overproduction and of tumor growth, but no curative treatment is achievable. Treatment choices include a wait-and-see policy, locoregional therapies, systemic chemotherapy, and radiopharmaceutical agents. The decision on the best treatment for each individual patient is often complex and requires a multidisciplinary approach.

Phenoxybenzamine, a long-acting nonselective (alpha1 and alpha-2), noncompetitive alpha-adrenergic blocker, and doxazosin, a selective alpha-1-adrenergic blocker, are the most frequently used drugs to obtain symptom control and prepare patients for surgery. In patients with PCC and secreting PGL, in fact, exposure to high levels of circulating catecholamines during surgery could cause hypertensive crises and arrhythmias. Therefore, a preoperative preparation with an alpha-adrenergic blocker at least 10–14 days before surgery is required [42].

As regards the antineoplastic therapy, the wait-and-see strategy could be an option for selected patients with slowly progressive tumors, while active therapeutic intervention is generally required in the presence of uncontrolled hormone- or tumor-related symptoms, high tumor burden, or significant radiographic progression [43].

Cytoreductive (R2) resection in malignant PCC may sometimes improve the quality of life and survival by reducing the tumor burden and controlling hormonal hypersecretion [40].

^{131}I-MIBG therapy should be considered as a first-line approach in patients with significant tumor burden, slowly progressive disease, and adequate ^{131}I-MIBG uptake on diagnostic imaging. With ^{131}I-MIBG therapy, a disease stabilization and partial hormonal responses can be achieved in 50 % and 40 % of patients. Although objective responses are common, complete response

rates are low [44]. The use of ^{131}I-MIBG therapy may be limited by hematologic toxicity [45] and by the need of a prolonged inpatient admission for radiation safety purposes.

Because a significant number of metastatic sites express SSRTs, peptide receptor radionuclide therapy (PRRT) using ^{90}Y-DOTATOC and ^{177}Lu-DOTATOC can be potentially used.

The results of a retrospective study on 20 consecutive advanced PCCs/PGLs patients, in which PRRT was administered, showed disease regression in 36 % of patients (29 % partial and 7 % minor response), while 50 % had stable disease. Eight of 14 patients treated for uncontrolled secondary hypertension obtained the reduction of medication doses [46].

Combination chemotherapy with cyclophosphamide, vincristine, and dacarbazine (CVD) administered to malignant PCCs/PGLs can obtain 37 % tumor response and 40 % hormonal response; complete remissions are rare [47]. Temozolomide is a 3-methyl analogue of mitozolomide developed as an oral alternative to intravenous dacarbazine. A retrospective study on 15 consecutive patients with metastatic PCs/PGLs showed five partial responses (33 %), seven stable (47 %), and three progressive diseases (20 %). Interestingly, disease responses were confined to the 10 patients carrying a mutation in SDHB [48].

In cases of unresectable liver metastases, transarterial-(chemo)-embolization (TACE) has been shown to reduce metastatic deposits and catecholamine and CgA levels [49]. Other options include radiofrequency ablation and alcohol injection to unresectable lesions.

Approximately 70 % of patients with metastatic PCCs/PGLs develop bone metastases that are mainly lytic. These patients require a combination of therapeutic modalities including antiresorptive medications such as bisphosphonates or RANKL inhibitors, external-beam irradiation and radiofrequency ablation of bone metastases or surgical stabilization, and cementation [49]. Table 55.6 summarizes the treatment options for PCCs/PGLs.

Table 55.6 Therapeutic algorithm for the primary treatment of metastatic PCCs and PGLs

Disease status	Medical Treatment	Therapeutic Options
If resectable tumor	Alpha blockade ± alpha-methyltyrosine ± beta blockade (pre-operatively)	Resection (laparoscopic preferred when safe and feasible)
If unresectable locally	Alpha blockade ± alpha-methyltyrosine ± beta blockade (pre-operatively)	If possible cytoreductive (R2) resection and/or
		Local radiotherapy
If distant metastasis	Alpha blockade ± alpha-methyltyrosine ± beta blockade (pre-operatively)	If possible cytoreductive (R2) resection and/or
		^{131}I-MIBG (if positive MIBG scan with dosimetry) or SSR analogs if positive receptors or
		Systemic chemotherapy (CVD) or TMZ or
		Clinical Trial
If asymptomatic tumor without significant radiographic progression (RECIST)		Wait and see strategy Active radiological surveillance (at 3, 6 months, 1 year)

Surgical series have shown that up to 80 % of ACC patients are destined to develop locoregional recurrence or distant metastases after an apparent complete surgical excision [50, 51]. On these bases, there is a strong rationale for the use of adjuvant therapy in ACC patients. The evidence in favor of this therapeutic option, however, is still limited since the results of prospective randomized clinical trials are lacking.

Mitotane is the only drug approved by international pharmaceutical agencies for treatment of advanced ACC. A large retrospective case-control study reported that patients treated with adjuvant mitotane had a significantly longer recurrence-free survival (RFS) and overall survival (OS), compared with two independent groups of patients untreated after surgery [52]. Recently, the same group has updated the follow-up of these cohorts of patients with almost 10 years of additional observation, confirming that adjuvant mitotane treatment is associated with a significant benefit in terms of RFS regardless of the hormone secretory status [53]. Advantage on OS is less evident, but this may be explained by different treatment of ACC recurrence between groups and the introduction of a landmark analysis. Despite its retrospective nature, this study remains the most informative piece of evidence on the topic, and it represents a reference for decision making in ACC patients. On the basis of the results of this study, adjuvant mitotane therapy is currently recommended by international guidelines [19].

The management of patients under long-term mitotane therapy is not easy and requires experienced endocrinologists or medical oncologists. The most common side effects are gastrointestinal (nausea, vomiting, diarrhea, anorexia, and mucositis) and neurological (lethargy, somnolence, vertigo, ataxia, confusion, depression, dizziness, decreased memory, and polyneuropathy) (Table 55.7). The management of them is complicated by the long half-life of drug plasma levels (40 days). The maintenance of mitotane serum levels within the so-called therapeutic range (14–20 mg/L) allows the attainment of the best benefit from the drug and the prevention of side effects (neurological, in particular) in most cases [54].

About 50 % of newly diagnosed ACC patients present with metastatic or unresectable disease [19]. Moreover, despite initial complete resection of ACC, up to 70–80 % of patients are destined to develop recurrent or metastatic disease [6]. The management of these patients is mainly centered on systemic therapy that since many years include mitotane alone or mitotane in combination with chemotherapy. The standard chemotherapy regimen for advanced ACC is EDP (etoposide, doxorubicin, and cisplatin) plus mitotane (EDP-M) [55]. The efficacy of the EDP-M regimen was demonstrated by the results of a prospective randomized clinical trial in which 304 patients were prospectively enrolled in about 6 years and randomized to receive either EDP-M or streptozotocyn plus mitotane (Sz-M). Patients with disease progression to the first-line treatment received the alternate regimen. EDP-M was supe-

Table 55.7 Side effects of mitotane therapy

System organ class	Very common	Common	Rare	Very rare
Nervous system disorders	Dizziness, somnolence, vertigo, depression, decreased memory	Lethargy, ataxia, confusion, polyneuropathy		
Gastrointestinal disorders	Nausea, vomiting, diarrhea, mucositis			
Blood and lymphatic system disorders		Leucopenia	Thrombocytopenia, anemia	
Endocrine disorders	Adrenal insufficiency	Primary hypogonadism in men		
Skin and subcutaneous tissue disorders		Rash, gynecomastia		
Metabolism and nutrition disorders	Anorexia; hypercholesterolemia, hypertriglyceridemia			
Cardiac disorders				Hypertension
Hepatobiliary disorders	Increase in hepatic enzymes (mostly GGT); hepatic microsomal enzyme induction			
Immune-related adverse reaction			Autoimmune hepatitis	
Eye disorders				Blurred vision, double vision, toxic retinopathy, macular edema, cataract
Renal and urinary disorders				Hemorrhagic cystitis, hematuria, albuminuria
Investigations	Increase in hormone binding globulins (CBG, SHBG, TBG, vitamin D binding protein); reduction of fT4;			

rior to Sz-M both in terms of disease response rate and progression-free survival (PFS). Analysis of OS also favored patients initially randomized to receive EDP-M, but due to the attenuating effect of the crossover to EDP-M of patients randomized to the Sz-M at disease progression, the difference just failed to attain statistical significance [56].

In addition to systemic therapy, also local regional therapies, i.e., radiofrequency ablation (RFA) [57] and chemoembolization [58, 59], can be taken into consideration in a selected patient population.

Finally, the morbidity caused by ACC and the prognosis derives not only from the spread of malignant cells into other organs, but also from the consequences of hormone excess. Consequently, the goals of treatment in ACC include both control of tumor growth and mitigation of the effects derived from hormone excess in patients with clinical and biochemical finding of hormone hyperscretion. Patients with metastatic ACC that exhibits autonomous steroid secretion should be treated with steroidogenic inhibitors to ameliorate the effects of excessive mineralocorticoids (hypertension and hypokalemia) and glucocorticoids (hypertension, hyperglycemia, hypokalemia, and muscle atrophy). The management of hormone excess in patients with metastatic ACC is often challenging. The presence of Cushing syndrome may consistently increase the toxicity of chemotherapy since it is associated by immune depression that favors infections particularly in the neutropenia phase. Therefore, a rapid control of hormone hypersecretion is mandatory. Mitotane has both antisecretive and antiproliferative activities; however, the slow onset of its activity is a main limitation for the management of Cushing's syndrome [6]. Faster drug in lowering the serum cortisol levels is needed. Ketoconazole is more rapid than mitotane in controlling Cushing syndrome [60], but it requires several weeks, and its clinical employment is hampered by the hepatic toxicity. Metyrapone (Cormeto) is an adrenolytic molecule targeting the 11-beta-hydroxylase. In a recently published experience by our group, metyrapone was associated upfront to the EDP-M regimen, and this combination was very well tolerated and led to a rapid control of Cushing's syndrome induced by cortisol secreting ACC [61]. In patients with advanced ACC with severe Cushing syndrome, the EDP-M plus metyrapone regimen (EDP-MM) is the best treatment strategy.

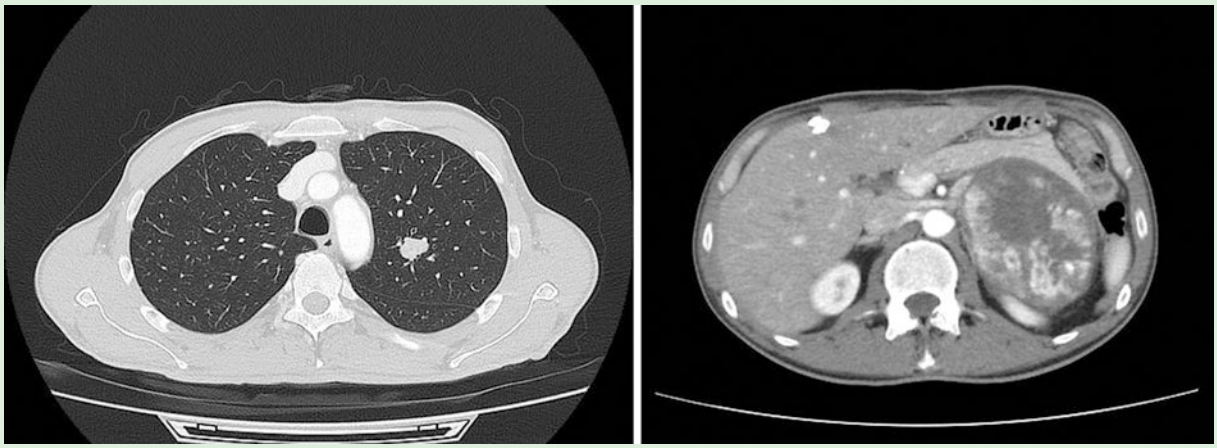
55.12 Follow-up

Patients who underwent successful surgery for nonmetastatic PCC/PGL are at risk of malignant recurrence and require long-term clinical (adrenergic symptoms and blood pressure levels) and biochemical follow-up [19]. The follow-up is especially important for patients with extra-adrenal primary disease, tumor size >5 cm, or SDHB mutations. Biochemical testing (plasma or urinary metanephrine, normetanephrine, chromogranin A, and methoxythyramine) should be repeated ~14 days following surgery to check for remaining disease and thereafter every 3–4 months for 2–3 years. This should subsequently be repeated every 6 months. Patients with new events (high blood pressure, adrenergic symptoms, or pain) and/or elevated circulating or urinary biochemical tests should undergo imaging that includes thorax and abdomen CT and best functioning imaging (PET FDG in most cases).

For patients with ACC after complete resection, a regular follow-up every 3 months including abdominal CT (or MRI), thoracic CT, and monitoring of initially elevated steroids is recommended. After 2 years, intervals may be gradually increased. In case of long-term persistence of the disease-free status, follow-up should be continued for at least 10 years [19].

Adrenocortical Carcinoma: A Case Report

1. *Man, 57 years old*
2. *Family history:* Negative for malignancies
3. *APR:* Negative
4. *APP:* Insomnia, palpitation
5. *Objective examination*: Moon face, central obesity, buffalo hump, hypertension
6. *Blood tests*: Hyperglycemia, hypokalemia;
7. *TC abdomen mdc*: Adrenal lesion of 10 × 9 × 9 cm. few lung lesions (maximum diameter of 3 cm)



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Question

What action should be taken?

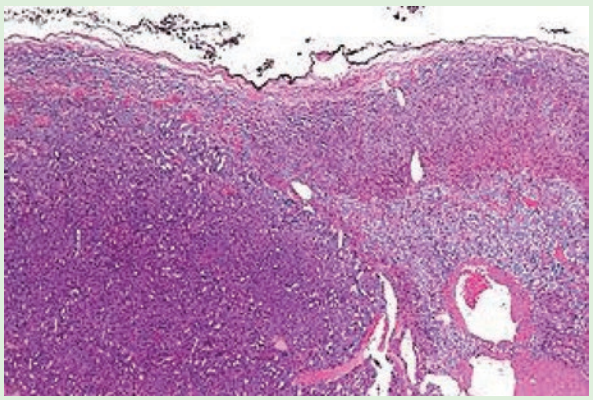
(1) Surgery. (2) Hormonal assessment and biopsy. (3) Biopsy alone

Answer

Hormonal assessment and biopsy

8. *Baseline hormonal assessment*: hypercortisoluria, hypercortisolemia, ACTH suppression, negative metanephrine and normetanephrine.
9. *Lung biopsy*
   Histological examination: Adrenocortical carcinoma. MART-1 +, MELAN-A +inhibin +, Ki67 30 %.

Question



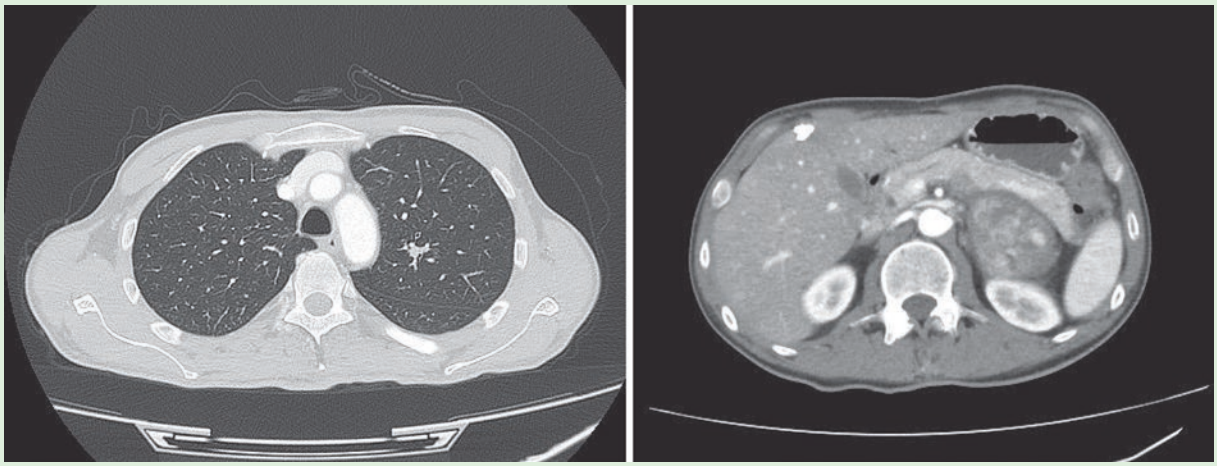
What action should be taken?

(1) Surgery. (2) Chemotherapy plus Mitotane. (3) Chemotherapy plus Mitotane plus Metyrapone

Answer

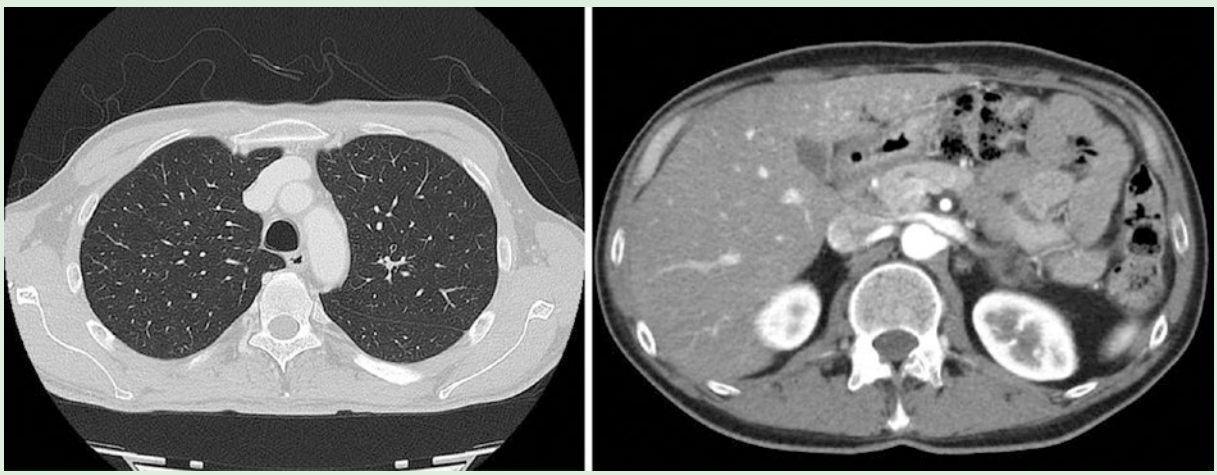
Chemotherapy plus Mitotane plus Metyrapone

10. *Chemotherapy* with Etoposide, Doxorubicin and Cisplatin (EDP scheme) plus Mitotane and Metyrapone.
11. *Hormonal assessment after one month*: normalization of cortisoluria, cortisolemia, and ACTH
12. *Response evaluation after 5 cycles of chemotherapy (EDP scheme) plus Mitotane:* Partial response



13. *Response evaluation after 7 cycles of chemotherapy (EDP scheme) plus mitotane:*

Partial response



Question

What action should be taken?

(1) Continue chemotherapy. (2) Continue only Mitotane. (3) Surgery

Answer

Surgery

14. Surgery: Left surrenectomy and lymphadenectomy of the renal hilum.
    Histological examination: Adrenocortical carcinoma. Negative lymph nodes. R0.

Question

What action should be taken?

(1) Continue chemotherapy plus mitotane. (2) Continue only mitotane. (3) Follow-up

Answer

Continue only mitotane and perform an instrumental follow-up

15. The patient is actually treated with mitotane. A periodic follow-up with a CT scan is performed every 3–4 months.

Key Points

- The importance of a correct approach of metastatic ACC with Cushing syndrome:
  - Complete hormonal assessment to evaluate the concomitant secretion of other hormones in addition to cortisol
  - Biopsy of one lesion to confirm the diagnosis
- The importance to obtain the rapid control of Cushing syndrome by adding metyrapone to the EDP-M scheme
- The importance of a correct monitoring to evaluate the response
- The potential positive impact of resection of primary adrenal disease in a patient with oligo metastatic ACC
- The importance to individualize the treatment length in order to obtain the maximum cytoreductive effect

Advanced Pheochromocytoma: A Clinical Case

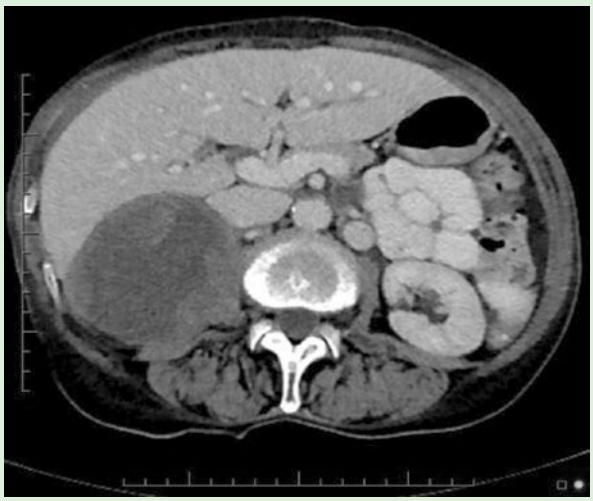
Man, 56 years old

1. *Family history:* father and mother deceased for a not specified abdominal malignancy
2. *Comorbidities:* arterial hypertension since 15 years
3. *Recent history:* recurrent hypertensive crisis and episodes of hypotension requiring access to the emergency response service
4. *CT scan:* right adrenal mass, diameter max 8.5 cm, inhomogeneous. No evidence of metastases.

Question

What should be done first?

- Biopsy
- Surgery
- Antihypertensive therapy

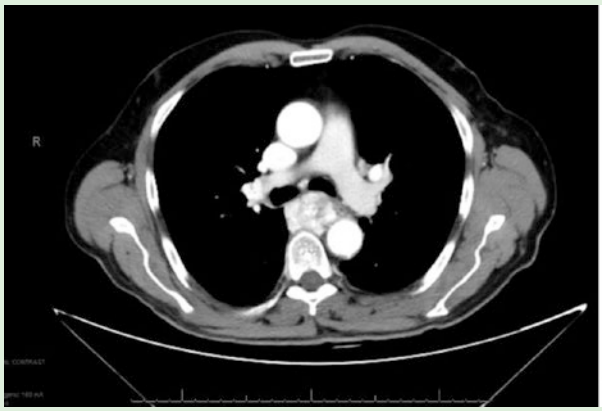
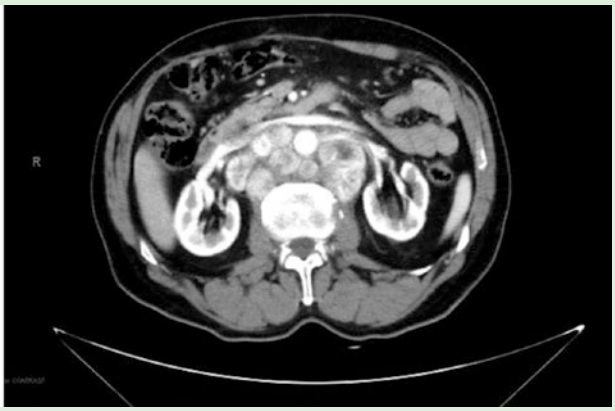


Answer

Anti-hypertensive therapy

2 weeks before surgery, the patient was treated with noncompetitive alpha-adrenoreceptor antagonist. In suspected pheochromocytomas, fine needle biopsy is contraindicated.

- *Surgery:* right adrenalectomy
- *Pathology:* IHC positive for CgA, NSE, synaptophysin, and CD56 and negative for CEA, S100, Melan A, cytokeratins, alpha-inhibin, and vimentin
- *Follow-up:* periodic clinical, abdominal US sonography and tumor markers (CgA and NSE) evaluations, with no evidence of disease recurrence for 6 years
- *Disease recurrence after 6 years. Laboratory analysis:* NSE 34.8 ng/ml (nv < 16); CgA 1066 ng/mL (nv), metanephrine 0.660 mg/24 h (nv), normetanephrine 39,390 mg/24 h (nv), 3-metossithyramine 1.865 mg/24 h (nv)
- *Abdominal US sonography:* in the retroperitoneum; in para-aortic; in the presence of multiple, voluminous, and confluent formations; in hypoechoic; and in compatible with adenopathy
- *Total body CT scan:* multiple confluent retroperitoneal adenopathy, 10 cm in diameter, and bulky thoracic mass, with necrotic areas

Question

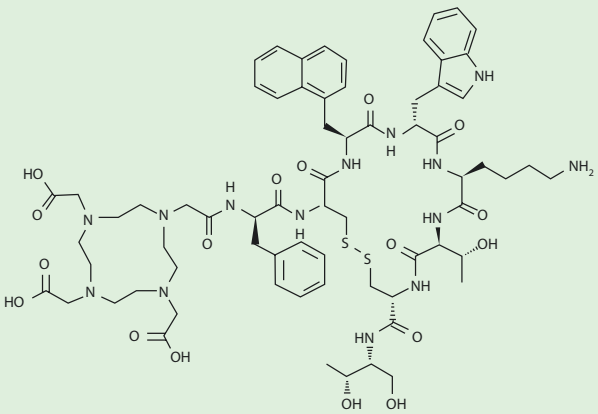
What should be done now?

- Surgery
- Medical therapy
- Metabolic imaging

Answer

Metabolic Imaging:

- *68Ga-DOTA-NOC PET/TC:* Multiple high intensity uptakes of radionuclide in mediastinum, bilateral lung ilus, Barety lymph nodes, carenal and paraesophageal adenopathy, precardiac area, and common iliac lymph nodes. The presence of intense uptake also in inferior left and right lung lobes



- *MIBG-Scintigraphy:* Evidence of bulky abdominal and thoracic disease with high intensity uptake of MIBG

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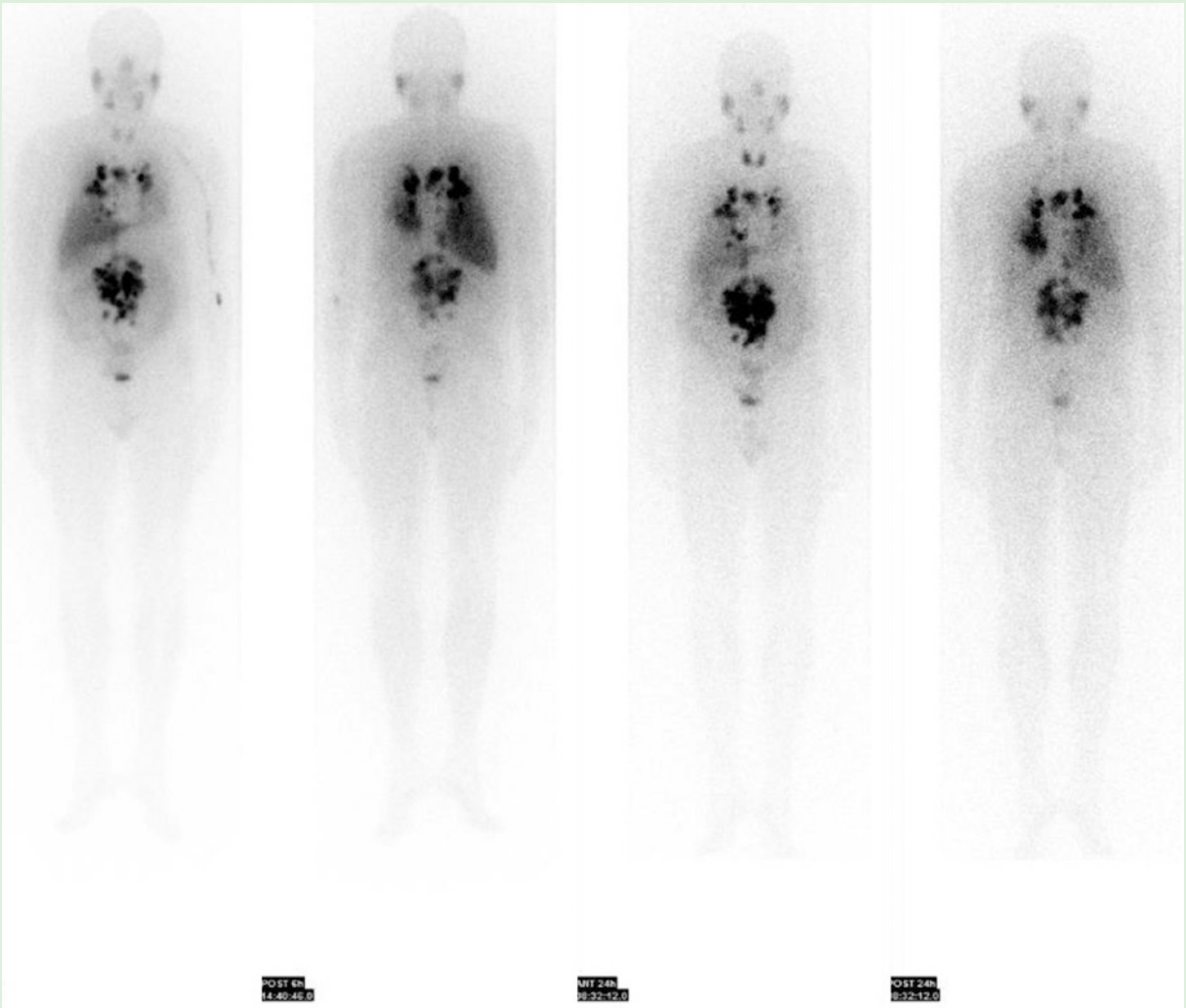


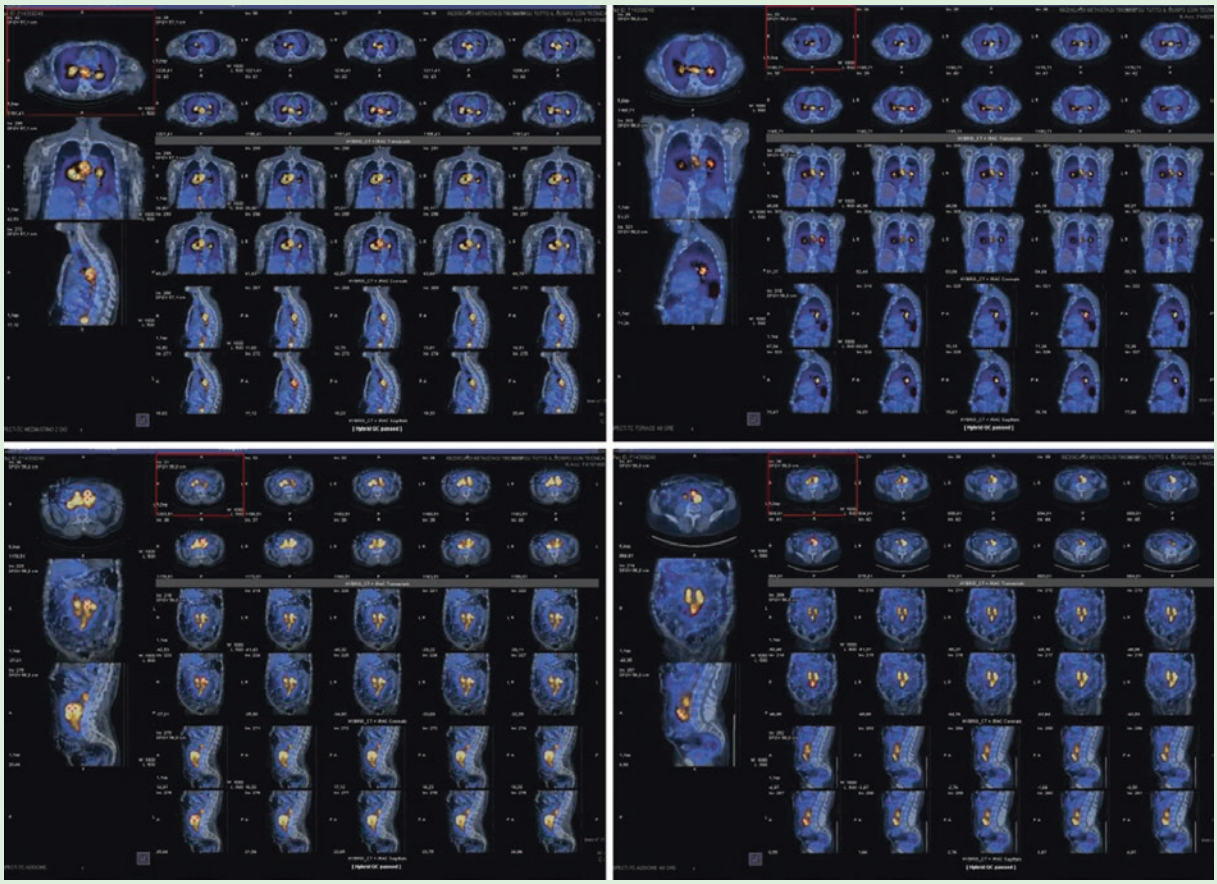
Question

Which therapy for this patient?

- Radionuclide therapy
- Chemotherapy
- Clinical Trial

Answer

Radionuclide therapy with MIBG: The patient was treated with 1850 MBq MIBG I-131. The MIBG-scintigraphy evaluation 4 months after the PRRT showed the reduction of the metabolic activity of all the metastatic sites.



Key Points

1. The importance of a correct study of an incidental adrenal mass.
2. The importance of long-term follow-up and the diagnosis of malignancy of pheochromocytoma are extremely difficult at diagnosis and are done in case of metastatic disease.
3. Multidisciplinary management of patients affected with pheochromocytoma.
4. The role of radionuclides for diagnosis and treatment.

Expert Opinion

Alfredo Berruti

Key Points

- The adrenal gland is composed of two embryological and functional distinct organs: medulla and cortex. The majority of adrenal tumors are benign. Malignant transformation is rare. Tumors arising from adrenal chromaffin cells of the medulla are called pheochromocytomas (PCCs), whereas those arising from extra-adrenal chromaffin cells are termed paragangliomas (PGLs). Tumors deriving from the transformation of adrenal cortex are either adenoma or adrenocortical carcinoma (ACC).
- PCCs and PGLs are rare diseases with an estimated incidence in Western countries between 2 and 8 new cases per million population per year. Benign adrenocortical neoplasms (adenomas) are frequent, whereas ACC is extremely disease with an estimated incidence between 0.5 and 2 new cases per million population per year.
- PCCs and PGLs are mainly sporadic. Thirty percent of them, however, are associated with specific familiar disorders. Therefore, genetic counseling is recommended in all PCCs and PGLs patients. This is not the case of ACC patients that are rarely associated to genetic disorders. In these latter patients, genetic tests are not routinely recommended.
- Adrenal incidentaloma is an asymptomatic adrenal mass detected on imaging not performed for suspected adrenal disease. A surgical treatment should be considered individually, on the basis of the likelihood of malignancy (i.e., tumor size >4 cm), the presence and degree of hormone excess, age, general health, and patient preference.
- The most frequent symptoms and sign of PCCs is hypertension that is associated to an excess of catecholamines released by tumors either continuously or paroxysmally. Forty to sixty percent of ACC are functioning at presentation, being cortisol hypersecretion (Cushing syndrome) the most frequent clinical manifestation.
- Since both PCCs/PGLs and ACC are hormone secreting, a comprehensive hormonal analysis is recommended when an adrenal mass is diagnosed in order to establish the origin (cortex or medulla) and nature (malignant or benign) of the lesion. Hormone monitoring is essential also during follow-up as hormone increase may indicate early disease relapse. Moreover, plasma chromogranin levels could be additionally evaluated in PCCs/PGLs.
- No histological system currently available can predict the biological aggressiveness of PCCs/PGLs. The certainty of malignant behavior is done by the evidence of metastases. The Weiss score is widely used to discriminate benign versus malignant ACC.
- Imaging procedures are fundamental to both differentiate benign and malignant adrenal lesions and to correctly stage the disease. Computed tomography scan is the first choice imaging technique for PCCs/PGLs and ACC. FDG PET scan could be of help in the staging of both PCCs and PGLs. Functional imaging techniques, such as ^{123}I MIBG and ^{68}Ga-DOTATATE PET/CT, offer both diagnostic and theranostic information and are widely used for PCCs/PGLs.
- In PCCs/PGLs, the only reliable predictor of malignancy is the SDHB gene germline mutation. There is no staging system for malignant PCCs/PGLs. The most important prognostic factors in early ACC are the disease stage, margin-free resection, age, the proliferation marker Ki67, and the glucocorticoid excess. Additional prognostic factors in metastatic patients may be mENSAT stage and GRAS parameters, but they are not validated yet.
- Surgery is the mainstay of therapy in the management of both ACC and PCCs/PGLs with local regional disease. It is advisable that adrenal surgery should be performed in referenced centers.
- Currently available guidelines recommend adjuvant therapy with mitotane in radically resected ACC patients with high risk of recurrence and death. This recommendation, however, is based on a weak evidence, since it is not supported by the results of prospective randomized clinical trials.
- The major principles in the management of metastatic PCCs/PGLs include control of symptoms related to catecholamine overproduction and tumor growth, but no curative treatment is achievable. Treatment choices include locoregional therapies, systemic chemotherapy, and radiopharmaceutical agents, and a wait-and-see policy in case of indolent disease with low tumor burden. The decision on the best treatment for each individual patient is often complex and requires a multidisciplinary approach.
- In metastatic ACC patients, the goals of treatment include both tumor growth control and mitigation of the effects derived from hormone excess when there is a biochemical and clinical evidence of hormone hypersecretion. Mitotane and metirapone play a major role in the control of Cushing syndrome. The standard first-line approach in ACC patients is mitotane alone or mitotane in combination with EDP chemotherapy scheme (etoposide, doxorubicin, and cisplatin). In

addition to systemic therapy also local regional therapies, that is, radiofrequency ablation and chemoembolization can be taken into consideration in a selected patient population.
- A hormonal, clinical, and imaging follow-up is suggested after surgery in both PCCs/PGLs and ACC patients.

Summary of Clinical Recommendation

ESMO
- ACC is defined by a Weiss score of 3 or more. Malignant pheochromocytomas/paragangliomas are defined by the presence of metastasis.
- Patients suspected to harbor primary adrenal tumors should undergo a standardized diagnostic work-up consisting of endocrine assessment for excess hormone production and modern imaging (CT/MRI of abdomen, chest CT, and in selected cases supplemented by isotope functional imaging mainly FDG-PET). The diagnostic work-up differs between ACC and pheochromocytoma.
- Guided biopsies of potentially resectable primary adrenal tumors are not informative in most cases, but these are potentially harmful and should be avoided.
- The ENSAT TNM staging system should be used for ACC staging.
- Histological diagnosis should be done by an experienced pathologist and should rely on morphological, mitotic, and immunohistochemical parameters.
- Complete surgical extirpation of localized and locally advanced ACC or pheochromocytoma (R0 resection) is the mainstay of potentially curative approaches. Additionally, a locoregional lymphadenectomy is suggested for ACC.
- In pheochromocytoma, cytoreductive surgery might be considered. In advanced ACC, this approach is only reasonable for patients with severe hormone excess.
- Meticulous perioperative management of hormonal, glucose, electrolytes, cardiac, and fluid/blood pressure abnormalities is a critical component of patient care.
- Despite the limited literature evidence, adjuvant systemic mitotane is recommended for patients with ACC and incomplete resection (R1, Rx stage III) or in the presence of high-risk features (Ki67>10 %). R1 and Rx ACC resections may be followed by additional adjuvant radiotherapy to the tumor bed.
- Fit patients with inoperable ACC, high tumor volume, and rapid disease progression should be treated with combination cytotoxic chemotherapy plus mitotane (EDP-M). Less fit patients and/or patients with low tumor burden and slow progression can (first) be managed with mitotane monotherapy combined or not with locoregional options.
- Disease and symptom control is the main treatment goal for patients with inoperable pheochromocytoma and can be attempted by radiopharmaceuticals (^{131}I-MIBG), locoregional ablative procedures, and/or combination chemotherapy (CVD) in selected cases.
- Wait-and-see policy is recommended in low tumor burden and asymptomatic malignant pheochromocytoma and paraganglioma.
- Patients with resected ACC or pheochromocytoma should be followed at regular intervals with clinical, imaging, and biochemical screens for at least 10 years. Lifelong surveillance with an increased interval of time is favored in malignant pheochromocytoma/paraganglioma.
- The follow-up of patients with inoperable disease should be performed every 2–4 months for ACC and every 3–6 months for pheochromocytoma/paraganglioma during the first year of follow-up and then adjusted.

NCCN

1. PCC
    1. For the correct diagnosis and tumor staging, it is recommended to measure plasma-free or 24-hour urine fractionated metanephrines and to perform a chest CT with or without contrast and abdominal/pelvic multiphasic CT or MRI. The genetic counseling recommended too.
    2. For metastatic disease, tumor staging should include MIBG scan, somatostatin receptor-based imaging (i. e., see Primary Treatment (PHEO-2) somatostatin receptor scintigraphy or gallium-68 dotatate PET/Ctg), FDG-PET/CT (skull base to mid-thigh), and bone scan (if bone symptoms).
    3. Medical therapy should include alpha blockade with volume repletion and high salt diet for 7–14 days or until stable.
    4. Resectable disease should undergo surgery, preferring the laparoscopic approach when feasible
    5. Locally unresectable disease should continue medical therapy and should be referred to multidisciplinary center and then evaluated for radiotherapy with or without cytoreductive resection (R2) when possible; if positivity to MIBG scan, 131I-MIBG should be considered
    6. Metastatic disease should continue medical therapy and should receive one of the following therapies:
        - Cytoreductive resection (R2) when possible.
        - 131I-MIBG (if positivity to MIBG scan).
        - Clinical trial.
        - Systemic chemotherapy.
        - Palliative RT for bone metastases.

- Surveillance program should be offered both to resected and metastatic patients and comprises every 3–12 months H&P, blood pressure, markers, chest CT ± contrast, and abdominal/pelvic CT or MRI with contrast or FDG-PET/CT.

2. ACC
   - The basal evaluation of an adrenal mass should include the following:
     - Adrenal protocol for morphologic evaluation: CT with contrast or MRI with/without contrast to determine size, heterogeneity, lipid content (MRI), contrast washout (CT), and margin characteristics.
     - A functional evaluation, in order to identify functioning or nonfunctioning tumors. The hormonal work-up is specific for hyperaldosterism, Cushing's syndrome, and pheochromocytoma.
   - When a carcinoma is suspected (greater dimension >4 cm or inhomogeneous, irregular margins, local invasion or other malignant imaging characteristics), it is necessary to complete the staging with chest CT with or without contrast and abdominal/pelvic CT or MRI with/without contrast to evaluate for metastases and local invasion.
   - Localized disease should undergo surgery. Open adrenalectomy is recommended.
   - If high risk of recurrence, consider adjuvant mitotane therapy and external-beam RT to tumor bed.
   - In metastatic disease, consider observation with chest CT with or without contrast and abdominal/pelvic CT or MRI with contrast for clinically indolent disease every 3 months and biomarkers (if tumor initially functional).
   - If primary tumor and >90 % of metastases are removable, the surgical resection should be considered, particularly if functional
   - In metastatic disease, systemic therapy should be considered, preferably in clinical trial:
     - Cisplatin/carboplatin + etoposide ± doxorubicin ± mitotane.
     - Streptozocin ± mitotane.
     - Mitotane monotherapy.
   - After disease, resection consider chest CT with or without contrast and abdominal/pelvic CT or MRI with contrast and biomarkers (if tumor initially functional) every 3–12 months up to 5 years.

Hints for Deeper Insight

Pheochromocytomas and Paragangliomas

- *International guidelines*
  Berruti A, Baudin E, Gelderblom H, Haak HR, Porpiglia F, Fassnacht M, Pentheroudakis G; ESMO Guidelines Working Group. Adrenal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2012 Oct;23 Suppl 7:vii131–8. PMID: 22997446.
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- *Prognostic stratifications and future targeted therapies.*
  Crona J, Taïeb D, Pacak K. New Perspectives on Pheochromocytoma and Paraganglioma: Toward a Molecular Classification. Endocr Rev. 2017 Dec 1;38(6):489–515. PMID: 28938417.
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- *Novel targeted therapy in PCCs and PGLs*
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Adrenocortical carcinoma:

- *International guidelines*
  Fassnacht M, Dekkers OM, Else T, Baudin E, Berruti A, de Krijger R, Haak HR, Mihai R, Assie G, Terzolo M. European Society of Endocrinology Clinical Practice Guidelines on the management of adrenocortical carcinoma in adults, in collaboration with the European Network for the Study of Adrenal Tumors. Eur J Endocrinol. 2018 Oct 1;179(4):G1-G46. PMID: 30299884.
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- *Therapeutic range in mitotane treatment.*
  Hermsen IG, Fassnacht M, Terzolo M, et al. Plasma concentrations of o,p'DDD, o,p'DDA, and o,p'DDE as predictors of tumor response to mitotane in adrenocortical carcinoma: results of a retrospective ENS@T multicenter study. J. Clin. Endocrinol. Metab. 2011;96:1844–1851. PMID: 21470991.
- *Targeted therapies and immunotherapy in ACC.*
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Cosentini D, Grisanti S, Dalla Volta A, et al Immunotherapy failure in adrenocortical cancer: where next? Endocr Connect. 2018 Nov 1. pii: EC-18–0398. R1. PMID: 30400026.

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Pheochromocytomas and Paragangliomas

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Cancer of the Thyroid

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Endocrine Cancers

Contents

Authors Valerio Gristina, Nadia Barraco, and Silvio Buscemi should be considered equally co-first authors.

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Learning Objectives

By the end of the chapter, the reader will:

- Be able to apply diagnostic and therapeutic procedures in thyroid cancer
- Have learned the basic concepts of thyroid cancer
- Have reached in depth knowledge of thyroid cancer management
- Be able to put acquired knowledge into clinical practice

56.1 Introduction

Thyroid cancers are the most common endocrine neoplasms, accounting for more than 90% of the total newly diagnosed endocrine cancers [1] while representing <1% of all human tumors and about 3% of visceral malignancies.

Thyroid cancers are basically derived from either follicular cells (papillary, follicular, anaplastic and poorly differentiated carcinoma) or parafollicular cells (medullary carcinoma) and share a common classification based on differentiation (well, intermediate, and poor differentiated). Nevertheless, thyroid carcinoma can be additionally categorized by increasing clinical aggressiveness reflecting the wide range of clinical behavior from low mortality and long-term survival in most cases of well and intermediately differentiated tumors to frequently incurable poorly differentiated cancers.

Both papillary and follicular cancers, grouped together under the header of "well-differentiated thyroid cancer" (WDTC), account for 95% of cases and are effectively treated with surgery, radioactive iodine (RAI), and thyroid-stimulating hormone suppressive therapy unless patients present with advanced disease [2]. Medullary thyroid cancer (MTC) is less common constituting between 2 and 5% of all thyroid malignancies but much more clinically aggressive whenever surgery is not feasible. Anaplastic carcinoma (ATC) is one of the most aggressive cancers in humans and fortunately appears to be declining over time. Poorly differentiated thyroid carcinoma (PDTC) was introduced as a separate entity in 2004 in the WHO Classification of Tumors [3] showing an intermediate prognosis between differentiated and undifferentiated neoplasms (Table 56.1).

In recent years, the development of targeted therapy has led to the approval of different multikinase inhibitors for iodine refractory-DTC (sorafenib and lenvatinib) and for progressive or metastatic MTC (cabozantinib and vandetanib).

Table 56.1 Frequency and mortality rates in thyroid cancers

Thyroid cancer	Frequency	Mortality
Papillary (PTC)	85–90%	1–2% at 20 years
Follicular (FTC)	10–15%	10–20% at 10 years
Midollary (MTC)	2–5%	25–50% at 10 years
Poorly differentiated (PDTC)	1–3%	60% at 5 years
Anaplastic (ATC)	1%	90% at 5 years

56.2 Epidemiology and Etiology

The incidence of thyroid cancers has tripled over the past 30 years varying considerably by geographic area, age, and sex.

- The incidence of thyroid cancers is increasing worldwide probably due to two coexisting processes: increased detection of (apparent increase) and increased number of cases (true increase) due to unrecognized thyroid-specific carcinogens [4, 5].
- Nearly 60–80% of thyroid carcinomas detected nowadays are micropapillary thyroid carcinomas (<1 cm in size).
- WDTCs have a greater incidence in whites than in blacks of both genders.
- Both papillary and follicular thyroid carcinomas are approximately 2.5 times more common in females with an earlier median age at diagnosis that tends to be even earlier for papillary cancer as compared to follicular cancer in either gender.
- Older patients are more likely to have higher risk PTC variants, PDTC or ATC.
- The incidence rates of MTC and ATC do not show any substantial differences by race/ethnicity. Up to 75% of MTC cases occur sporadically with other distinct familial syndromes accounting for the remainder. Occasionally, ATC may arise via dedifferentiation of prior WDTC.
- Radiation exposure, age, gender, family history, and low iodine intake are known risk factors for WDTC and probably for ATC while autoimmune thyroiditis and obesity remain controversial. MTC is not associated with radiation exposure but significantly related to hereditary conditions.

56.3 Histopathology Overview

The cellular consistency of the normal thyroid gland is made up of two main parenchymal cell types: follicular (Fig. 56.1) and parafollicular cells. While the former line colloid follicles, concentrate on iodine and produce thyroid hormones giving rise to both DTC and ATC, the latter produce the hormone calcitonin and are the cells of origin for MTC. Immune cells and stromal cells are responsible for extremely rare lymphoma [5] and sarcoma [6] of the thyroid, respectively.

56.4 Clinical Features

While thyroid nodules are common in the general population, the risk of malignancy is rare (approximately 5–10%) and easily assessed by obtaining information from the history and physical exam.

The vast majority of thyroid cancers presents as a palpable neck mass which may represent a primary tumor or metastatic lymphadenopathy, detected either by the patient or by clinician's physical examination. Conversely, patients first present with a non-palpable mass diagnosed incidentally with neck imaging.

On physical exam, particular attention to the firmness, mobility, irregularities, and size of the nodules, their adherence to the surrounding structures, and the presence of lymphadenopathy are significant clues to the presence of carcinoma.

The presence of a solitary nodule and evolution of symptoms such as rapid growth of the mass, worsening of dysphagia and breathing, hoarseness, fatigue, and weight loss should be queried albeit these features do lack specificity for malignancy. Vocal cord paralysis is generally associated with advanced disease (Fig. 56.2).

Most differentiated thyroid cancers are clinically indolent and have a favorable outcome with two-thirds of patients exhibiting gross disease localized to the thyroid at presentation. Conversely, approximately 10% of patients have recurrent or persistent disease with follicular carcinoma (FTC) showing more of a propensity to spread to distant sites (such as bone and lung) than papillary carcinoma (PTC), which tends to metastasize to lymph nodes [7]. However, prognosis seems to be similar in age-matched and disease stage-matched patients [8].

Alternatively, MTC may present either as an asymptomatic mass or as a bulky disease with high levels of serum calcitonin and severe secretory diarrhea. If not metastatic or relapsed, the natural history of localized and regional MTC is generally indolent with many patients having excellent long-term outcomes. Moreover, an increasing number of patients have been identified in one of the familial settings (Table 56.2).

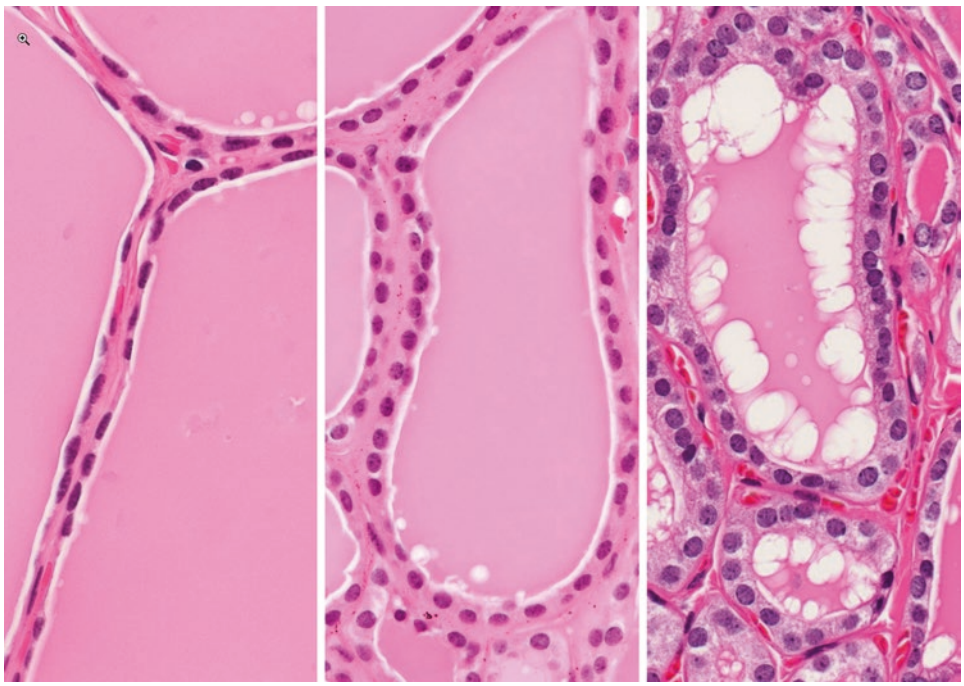

Fig. 56.1 Normal thyroid follicular cells and parafollicular cells. (Photos by courtesy of Prof. A. Martorana, Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, Pathologic Anatomy Unit-University of Palermo, University of Palermo)

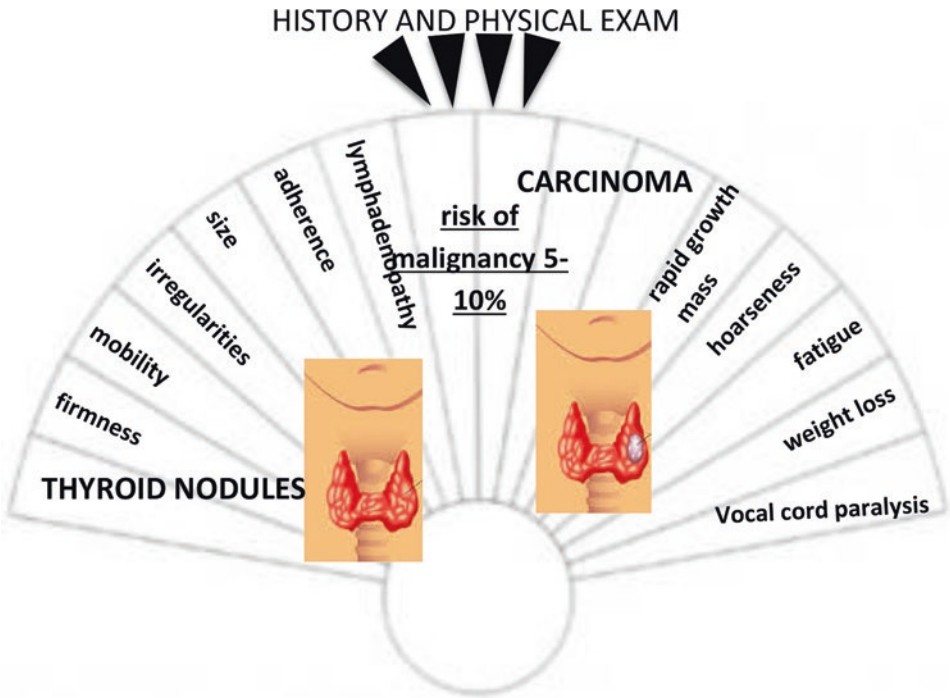

Fig. 56.2 Main clinical features suggestive for thyroid malignancies

Table 56.2 Clinical and genetic characteristics of familial medullary thyroid cancer syndromes

Syndrome	Characteristics Features
FMTC	MTC
MEN-2A	MTC Adrenal medulla (pheochromocytoma) Parathyroid hyperplasia
MEN-2A with cutaneous lichen amyloidosis	MEN-2A and a priuritic cutaneous lesion located over the upper back
MEN-2A or FMTC with Hirschsprung disease	MEN-2A or FMTC with Hirschsprung disease
MEN-2B	MTC Adrenal medulla (pheochromocytoma) Intestinal and mucosal ganglioneuromatosis Characteristic Marfanoid habitus

FMTC familial medullary thyroid cancer, *MEN* multiple endocrine neoplasia, *MTC* medullary thyroid carcinoma

On the contrary, ATC and PDTC uniformly present with a large and hard palpable mass invading the neck and often causing rapid compressive symptoms. To date, the majority of patients affected by ATC primarily die from upper airway respiratory failure. Regardless of treatment strategy, survival after diagnosis is unfortunately very poor.

56.5 Pathological Features

56.5.1 Macroscopic Aspect

PTCs show a variable appearance from minute subcapsular white scars to large tumors greater than 5 to 6 cm that may present with cystic change, calcification, or even ossification grossly invading surrounding structures.

FTC usually presents as unifocal and thickly encapsulated showing invasion of the capsule or vessels. Grossly, MTC may be circumscribed or infiltrative and is usually encapsulated and white-yellow. PDTC is a follicular-derived neoplasm that usually presents with a large infiltrative mass and a solid growth pattern, grossly showing intraglandular lymphatic and vascular spread. However, certain examples are encapsulated, at least partially. ATCs are large, extrathyroidal, and fleshy with obvious hemorrhage, necrosis, and aggressive growth pattern that may replace all previous evidence of WDTC.

56.5.2 Microscopic Aspects and Immunohistochemical

Microscopically, PTCs are characterized by the presence of papillae with ground glass nuclei and necrotic changes ("psammoma bodies"), but some variants are totally follicular in pattern and are identified as a follicular variant. Further subtypes are tall cell variant (TCV),

columnar cell variant (CCV), diffuse scleroting variant (DSV), solid variant (SV), and hobnail variant [9].

FTCs show trabecular or solid pattern of follicles with nuclear atypia, focal splinded areas, mitotic figures, and no necrosis. Oncocytic carcinoma (OTC or Hurtle cell carcinoma) is considered a variant of follicular neoplasms (Fig. 56.3).

MTC cells are monomorphic with round, oval, or spindle shape and a low nuclear/cytoplasmatic ratio often containing a characteristic amyloid substance (deposit from calcitonin). PDTCs usually present with a solid, trabecular, or insular pattern with at least one of the following: convoluted nuclei, >3 mitotic figures/10 HPF, and tumor necrosis [10]. ATC displays three patterns often mixed with better differentiated cells: large pleomorphic, spindle, or squamoid cells rarely showing rhabdoid inclusions (Fig. 56.4).

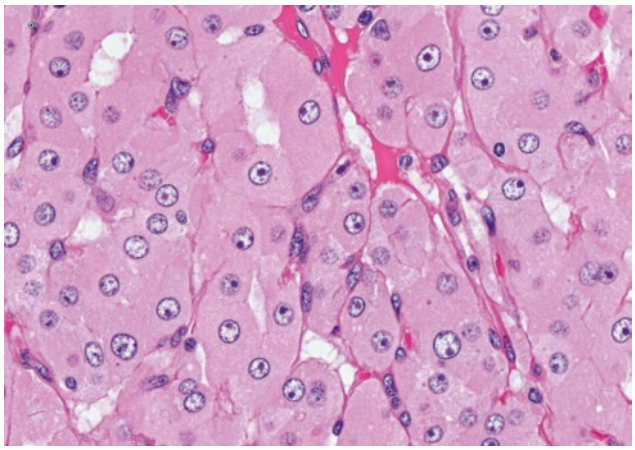

Fig. 56.3 Hurtle cell carcinoma microscopic aspect. (Photos by courtesy of Prof. A. Martorana, Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, Pathologic Anatomy Unit-University of Palermo, University of Palermo)

56.6 Diagnosis, Classification, and Staging Systems

Based on cancer statistics, incidence of thyroid tumors has been largely and globally increasing during the last decades. The diagnostic evaluation of thyroid cancer is mainly based on neck ultrasonography (US) encompassing the thyroid as well as the central and lateral neck compartments (Fig. 56.5a, b).

Specifically, some US parameters are traditionally associated with high risk of malignancy but poorly predictive when evaluated singly (Table 56.3). Moreover, US determination of tissue stiffness (elastography) has been recently suggested to detect malignancy in thyroid nodules with high sensitivity and specificity [11]. Nevertheless, larger prospective studies are needed for routine clinical use. Other imaging modalities (such as CT, MRI and PET) are less sensitive in diagnosing thyroid malignancies but important for eventually staging the extrathyroidal spread of the disease.

Fine needle aspiration (FNA) along with US is proved to be the most sensitive, reliable, and cost-effective technique in the evaluation of the thyroid nodules. FNA cytology (FNAC) plays an important role in the diagnostic work-up by estimating the risk of malignancy of the nodule in order to prevent unnecessary surgeries for benign conditions and avoid missing malignant nodules (Fig. 56.6). In particular, any patients affected by thyroid nodule >1 cm or <1 cm if there is any clinical or ultrasonographic suspicion of malignancy should undergo FNAC [12].

However, several factors can affect the diagnostic value of FNA including sampling error, heterogeneity of the nodule, physician's experience, and follicular neoplasia [13]. Furthermore, considering also the confusion related to diagnostic terminology between cytopa-

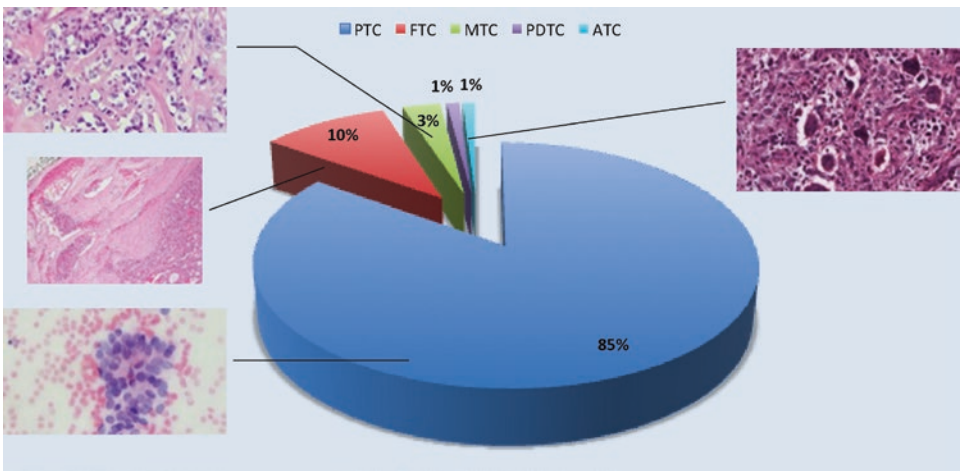

Fig. 56.4 Histologic subtypes of thyroid cancers. (Photos by courtesy of Prof. A. Martorana, University of Palermo)

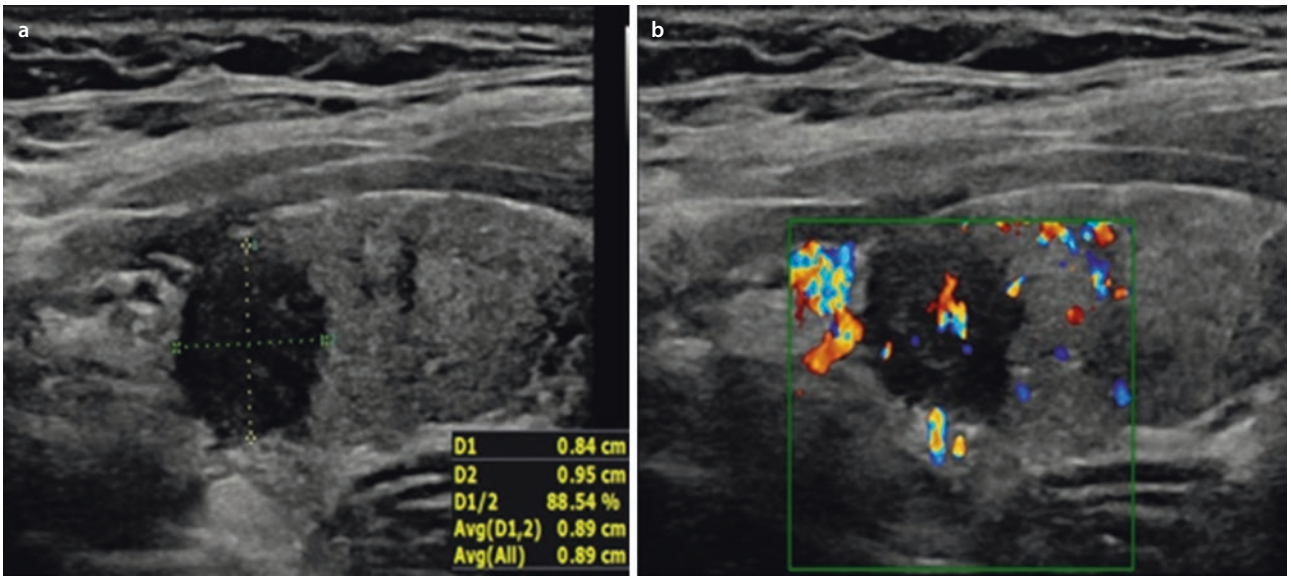

Fig. 56.5 *Thyroid US*: **a** Ultrasound scan shows a well-defined, homogeneous, solid hypoechoic oval-shaped nodule with irregular margins in upper left thyroid lobe suggestive for PTC; **b** Color-Doppler mode scan shows peripheral and intranodular vascularity. (Photos by courtesy of Department of Radiology, University of Palermo)

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Table 56.3 US features suggestive of malignancy in thyroid cancers

Thyroid nodule features	Lymphnode features
Microcalcifications	Microcalcifications
Hypoechogenicity, absence of halo	Hyperechogenicity
Irregular margins (infiltrative, microlobulated or spiculated)	Peripheral vascularity
Shape "taller than wide" on transverse view	Rounded shape
Solid aspect	Cystic aspect

thologists despite the wide application of FNA, many researchers suggested the unification of FNA reports in order to improve the clinical management and reduce the number of indeterminate cases (Table 56.4).

Moreover, during the initial evaluation of a patient with a thyroid nodule, serum thyrotropin (TSH) level should be measured while routine measurement of serum thyroglobulin (Tg) is not recommended and the use of routine serum calcitonin (CT), even if crucial for early detection and screening in MTC, is still debated. If the serum TSH is subnormal, a radionuclide (preferably ^{123}I) thyroid scan should be obtained to document whether the nodule is hyperfunctioning ("hot" appearance) or not ("cold" appearance) since hyperfunctioning nodules rarely harbor malignancy and do not need any further cytologic evaluation. Conversely, a higher serum TSH level is associated with increased risk of malignancy as well as more advanced stage thyroid cancer [14].

Although several systems have been proposed and validated for staging differentiated thyroid cancers without any clear superiority (Table 56.5), TNM (tumor-node-metastasis) staging system is internationally adopted providing a good risk stratification while failing to predict the risk of recurrence and the individual response to treatment in DTC [15].

In order to adequately predict the risk of disease recurrence/permanence in DTC, first initial risk evaluation should be carried out postoperatively according to American Thyroid Association (ATA) guidelines (Fig. 56.7). Since the risk of recurrence and disease-specific mortality can change over time as a function of the clinical course of the disease and the response to therapy, a dynamic risk stratification (DRS) should be continually assessed according to treatment response (excellent, incomplete biochemical, incomplete structural, or indeterminate) during the whole follow-up in order to avoid overtreatment in low-risk patients and on the other hand undertreatment in high-risk subjects [12, 16].

Finally, all patients with suspicious MTC should undergo a staging work-up before surgery including basal serum CT, CEA, calcium, and plasma metanephrines and normetanephrines, or 24-h urine collection for metanephrines and normetanephrines. The goal is to define the extent of disease and to identify the comorbid conditions of hyperparathyroidism and/or pheochromocytoma in the case of hereditary forms.

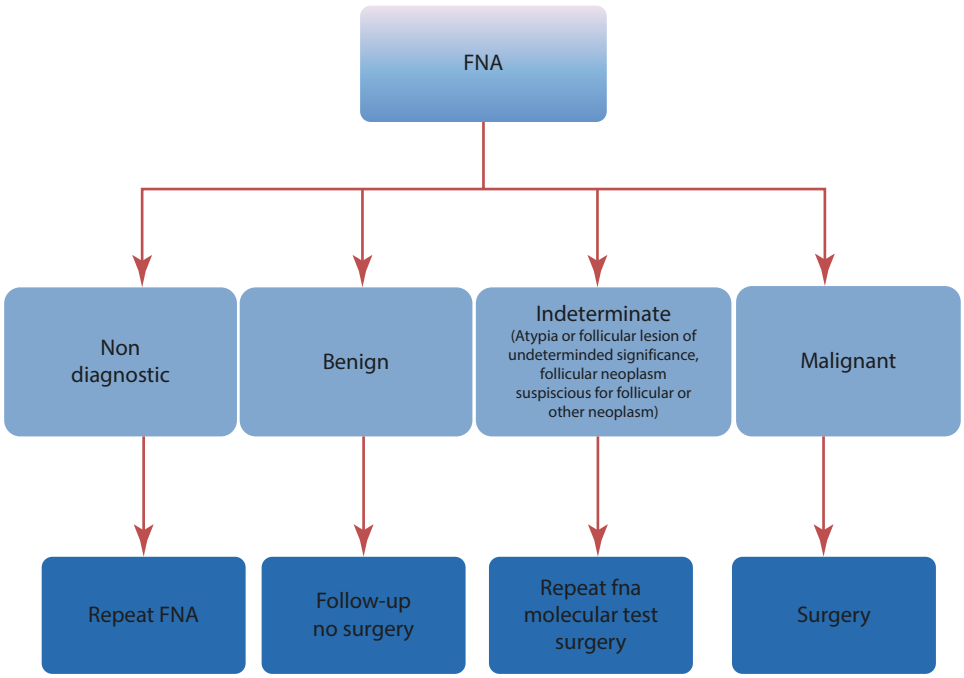

Fig. 56.6 Algorithm for evaluation and management of patients with thyroid nodules based on FNAC

Table 56.4 Recommended diagnostic categories according to Italian (SIAPEC-IAP, AIT, AME, SIE), American (The Bethesda System for Reporting Thyroid Cytopathology), and UK (UKRCP) cytopathology classification

Diagnostic Category	Risk of malignancy	Clinical management
Not diagnostic, cystic	–	Repeat FNA
Not malignant, Benign	0–3	Clinical follow-up
Low risk undetermined lesion, atypia or follicular lesion of undetermined significance	5–15	Repeat FNA
High risk undetermined lesion, follicular neoplasia or suspicious	15–30	Surgical lobectomy
Suspicious for malignancy	60–75	Near-total thyroidectomy or lobectomy
Malignant	97–99	Near-total thyroidectomy

Table 56.5 Prognostic classification systems in DTC

System	Criteria
AGES	Age, Grade of tumor, Extent, Size
AMES	Age, Metastasis, Extent, Size
MACIS	Mestasis, Age, Completeness of resection. Invasion, Size
Ohio State	Size, Cervical metastasis. Multiplicity, Invasion, Size
Sloan-Kettering	Age, Histology, Size, Extension, Metastasis
NCTTS	Size, Multifocality, invasion. Differentiation, Metastasis
TNM	Size, Extension, Nodal metastasis. Distant metastasis

56.7 Molecular Biology

Several studies demonstrated that traditional histopathological features of WDTCs, ATCs, and PDTCs are associated with genetic changes indicating how molecular alterations in thyroid cancer could closely correlate with specific stages in a multistep tumorigenic process. MTC, whether sporadic or inherited, has a detectable association with mutations of the rearranged during transfection (RET) proto-oncogene; mutations in RET are associated with autosomal dominant syndromes including MEN2A, MEN2B, and familial MTC and are found in approximately 50% of sporadic cases (Fig. 56.8).

Although ultrasound and ultrasound-guided FNA remain the first-line diagnostic tools for detecting and characterizing thyroid tumors, cytology alone fails to define thyroid nodules in 15–30% of cases [17] probably due to the high heterogeneous nature of the lesions and

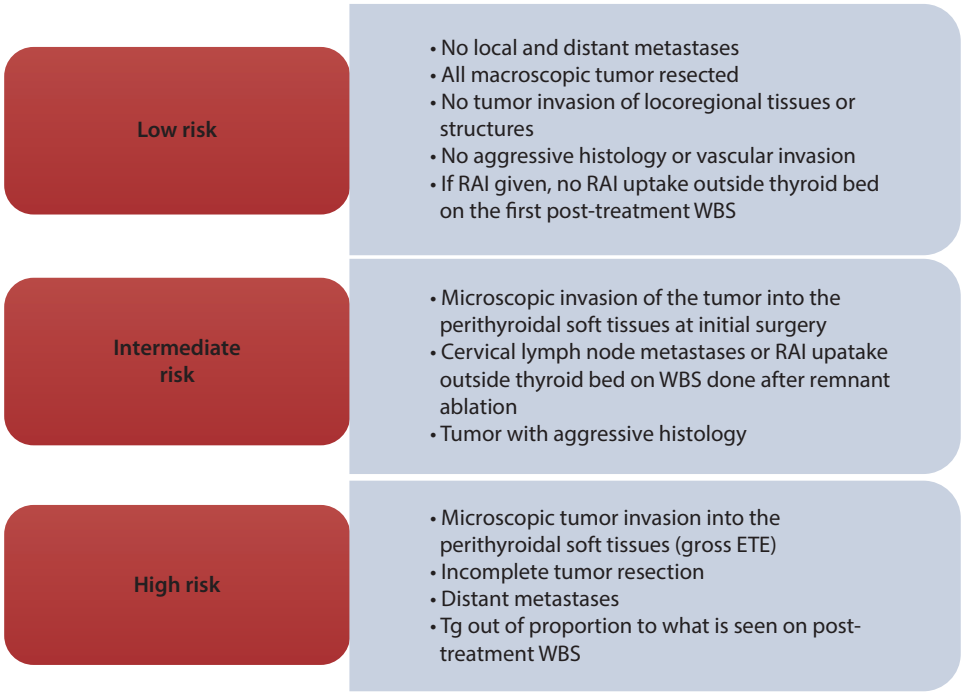

Fig. 56.7 Initial risk evaluation in DTC according to 2015 American Thyroid Association guidelines

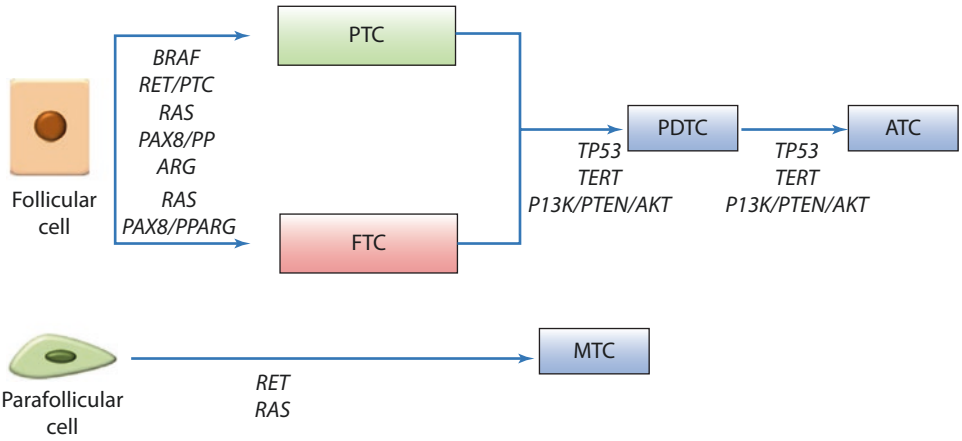

Fig. 56.8 Multistep tumorigenesis in thyroid cancer

the lack of specific markers. Taking into account the need to reduce unnecessary diagnostic thyroid surgery for indeterminate thyroid nodules, molecular testing has been also studied to help define prognosis and improve precise personalized treatments.

Specifically, two main pathways (RAS/RAF/MEK/ERK and PI3K/AKT/mTOR) seem to be involved in the propagation of signals from the cell membrane tyrosine kinase receptors (RET, EGF, VEGF, PDGF) into the nucleus. Gene alteration in the RAF/ RAS/ MEK pathway leads to promotion of cell proliferation, cell growth, and angiogenesis and loss of differentiation, while mutation in the PI3K/AKT/mTOR pathway results in tumor progression [17].

Recently, the application of the next-generation sequencing (NGS) technique has detected common molecular alterations, such as BRAF p.V600E, RAS point mutations, fusion oncogenes (RET/PTC, PAX8/PPARγ), and other aberrations of the MAPK and PI3K–PTEN–AKT signaling (Table 56.6), as strong indicators of malignancy because ~97% of mutation-positive nodules had confirmed malignant diagnosis at histology [18, 19].

Moreover, two update gene panels using NGS have been developed to rule-in PTC and FTC with high specificity and positive predictive value (PPV) in the case of cytologically indeterminate nodules and rule-out malignancies with high negative predictive value (NPV) in benign nodules [20]. Nevertheless, considering the relative high percentage of false negatives, the lack of long-term outcomes, and the standardization of these molecular tests, further research into the implications on

Table 56.6 Principal genes detected by NGS in thyroid tumors

Gene	Expression	Main Alteration	Tumor
AKT1	Ubiquitous	Activating mutation	PDTC
BRAF	Ubiquitous	Activating mutation in exon 15 (95% p.V600E)	PTC (40–80%) PDTC (5–35%) ATC (10–50%)
NTRK1	Nervous system, not in normal follicular cell	Rearrangement with TMP53 and TGF	PTC (0–10%)
PIK3CA	Ubiquitous	Activating mutations, copy number gain	FTC (0–10%) PDT (0–15%) ATC (5–25%)
PPARG	High levels in adipose tissue, low levels in follicular cells	Rearrangement with PAX8	FTC (20–50%)
RAS	Ubiquitous	Activating mutation	FTC (30–50%) PTC (0–10%) PDTC (20–50%) ATC (10–50%)
RET	*Normally expressed in C-cells, not in follicular cells*	Activating mutation, rearrangement with PTC	PTC (5–25%) MTC

treatment decisions, disease prognosis, and risk stratification is warranted.

Additionally, more clinical studies are needed in order to validate miRNAs as effective molecular markers in the diagnosis and prognosis of thyroid cancer in serum samples [21].

56.8 Prognostic Factors

As previously described, different staging and scoring systems exist and appear to be essential for accurate prognostic evaluation and treatment algorithms. Despite the variability between these systems, both patient and tumor characteristics were found to be independently associated with survival and therefore considered "widely accepted" prognostic factors.

While tumor size, distant metastasis, lymph node involvement, and clinical stage were all found to have definite prognostic value, both the result of histologic grade and the number of clinically positive lymph nodes seemed to show a controversial effect on survival. Moreover, gender and age should be reconsidered as prognostic factors since several studies have shown contradictory results regarding male gender as a negative prognostic factor and a different cutoff point than 45 years may be more accurate for prognosis [15].

Concerning DTC, a number of new factors with potential prognostic implications have recently emerged, including clinical factors (postoperative radiation, LN ratio, postoperative Tg levels, and positive PET-CT findings) and molecular markers (BRAF, Ki67, P53, PAX8-PPARγ), but yet to be included in new predicting systems.

56.9 Treatment

Whereas in WDTC combination of surgery, adjuvant radioactive iodine (RAI) ablation and TSH-suppressive therapy enable high rates of cure even in cases of extrathyroidal tumor manifestation [15], in PDTC and in MTC surgical resection may represent the only definitive therapy. Unfortunately, in ATC there is not yet a standardized and efficient treatment that could improve survival [22].

In WDTCs, the treatment for thyroid cancer is predominantly surgical considering also that the completeness of resection has been associated with less recurrence and improved survival [23]. Total thyroidectomy, removing both lobes and the isthmus (plus the pyramidal lobe, if present), is considered the mainstay of curative-intent therapy. The key decisions in the surgical management of differentiated tumors basically are whom to operate and how extensive a resection to perform.

Surgery is usually followed by the administration of ^{131}I, a selective and targeted approach for delivering tumoricidal doses of radiation to thyroid tumors, which showed reductions in both recurrence and cause-specific mortality in several large retrospective studies [24, 25]. The aim of RAI is to destroy any residual thyroid tissue preventing locoregional recurrence especially in high-

risk patients and to facilitate long-term surveillance with whole-body iodine scans or stimulated thyroglobulin measurements.

Postoperative thyroid hormone therapy should be immediately initiated with the aim to replace the thyroid hormone deficiency (replacement therapy) or suppress the potential growth stimulus of TSH on tumor cells (suppressive therapy).

Although about one-third of advanced DTC have metastatic lesions with low avidity for iodine at the time of diagnosis, relapsed or metastatic DTC is frequently ^{131}I-enriched and responds well to RAI. External beam radiation therapy (EBRT) should be only considered for critical metastasis and when complete surgical excision is not possible or when there is no significant radioiodine uptake in the tumor. In iodine-refractory WDTC, the role of chemotherapy has been limited, while two new drugs, sorafenib and lenvatinib, recently demonstrated prolongation of PFS compared with placebo in the phase III trials, DECISION and SELECT [26, 27].

Likewise WDTC, surgery is the primary treatment of MTC and can result in cure in locoregional recurrences whenever feasible. Postoperative thyroid hormone replacing therapy should be given to maintain serum TSH concentration within the normal range while no indications exist for RAI therapy. The results of EBRT and chemotherapy in patients affected by metastatic MTC are disappointing. Two drugs, vandetanib and cabozantinib, have been approved for use in progressive or metastatic MTC.

PDTC and ATC generally do not take up RAI and may not secrete Tg, and their proliferative activity may not be influenced by TSH. In this setting, surgery is indicated to improve the local control. A comprehensive and aggressive multimodal approach including high-dose EBRT and chemotherapy is the current treatment of choice in highly selected patients.

The role of neoadjuvant chemotherapy or targeted therapy in thyroid cancer is not well established [28], although this approach can be of benefit in selected cases.

56.9.1 Localized Disease

In the management of localized thyroid cancer, a long-standing controversy exists among international guidelines regarding the extent of surgical resection, the use of RAI therapy, the intensity and length of follow-up, and the degree of TSH suppression, particularly in DTCs.

There is a clear trend in the evolution of guidelines addressing surgical management of WDTC de-escalation, with recommendations recognizing the role of active surveillance of low-risk disease, higher thresholds for surgery, and acceptance of less than total thyroidectomy when surgery is recommended [29]. Indeed, active surveillance can be a safe and effective option for small subcentimetric PTCs [30], while three single observational cohort studies suggested that for WDTC between >1 cm and <4 cm, without extrathyroidal extension, and without clinical evidence of lymph node metastases (cN0), thyroid lobectomy alone may be sufficient as initial treatment [31–33]. As a matter of fact, these studies comparing lobectomy with total thyroidectomy did not show any substantial differences in overall survival and disease-specific survival rates contradicting a previous study by Bilimoria et al. in whom total thyroidectomy for PTC >1 cm was found to provide an overall survival advantage. Despite offering several advantages over thyroidectomy such as lower rate of both permanent hypoparathyroidism and hypothyroidism with subsequent lifelong levothyroxine (LT4) replacement therapy and bilateral recurrent laryngeal nerve palsy, it is worth noting that lobectomy was associated with higher risk of disease recurrence [23, 34]. Furthermore, there is international consensus that lobectomy (instead of total thyroidectomy) could be offered to low-risk small tumors, while a therapeutic lymph node dissection is necessary with clinically positive nodal (N1) disease in the central or lateral neck compartment. However, prophylactic lymph node dissection is still controversial. No guidelines actively recommend routine prophylactic lateral neck dissection, though some previous retrospective studies have considered it [35, 36].

Following surgical resection, radioiodine (RAI) ablation is recommended for selected patients with primary tumors measuring 1–4 cm and clinical-histologic features predicting intermediate to high risk of tumor recurrence (Fig. 56.9). RAI treatment is performed 1–6 months following thyroidectomy, while patients are significantly hypothyroid (low iodine diet of approximately 1–2 weeks suggested) or iatrogenically stimulated (with recombinant human TSH, rhTSH), in order to deliver a targeted ablative dose to any remnant thyroid tissue within the thyroid bed and/or elsewhere (e.g., thyroglossal duct tract and/or metastatic foci). Additionally, the dosing of ^{131}I is somewhat controversial even if in the recent years it has become increasingly apparent that successful thyroid ablation may be achieved using low radioiodine activities [37, 38]. Acute adverse effects of RAI are represented by nausea, neck pain, lacrimal gland dysfunction, salivary gland dysfunction, and altered taste, while the long-term toxicities include secondary primary malignancy, sialadenitis, nasolacrimal duct obstruction, and infertility [39].

Adjuvant thyroid hormone suppression therapy is indicated in high-risk patients (initial TSH suppression to below 0.1 mU/L is recommended) in whom it may decrease progression of metastatic disease, thus reducing cancer-related mortality (Fig. 56.10).

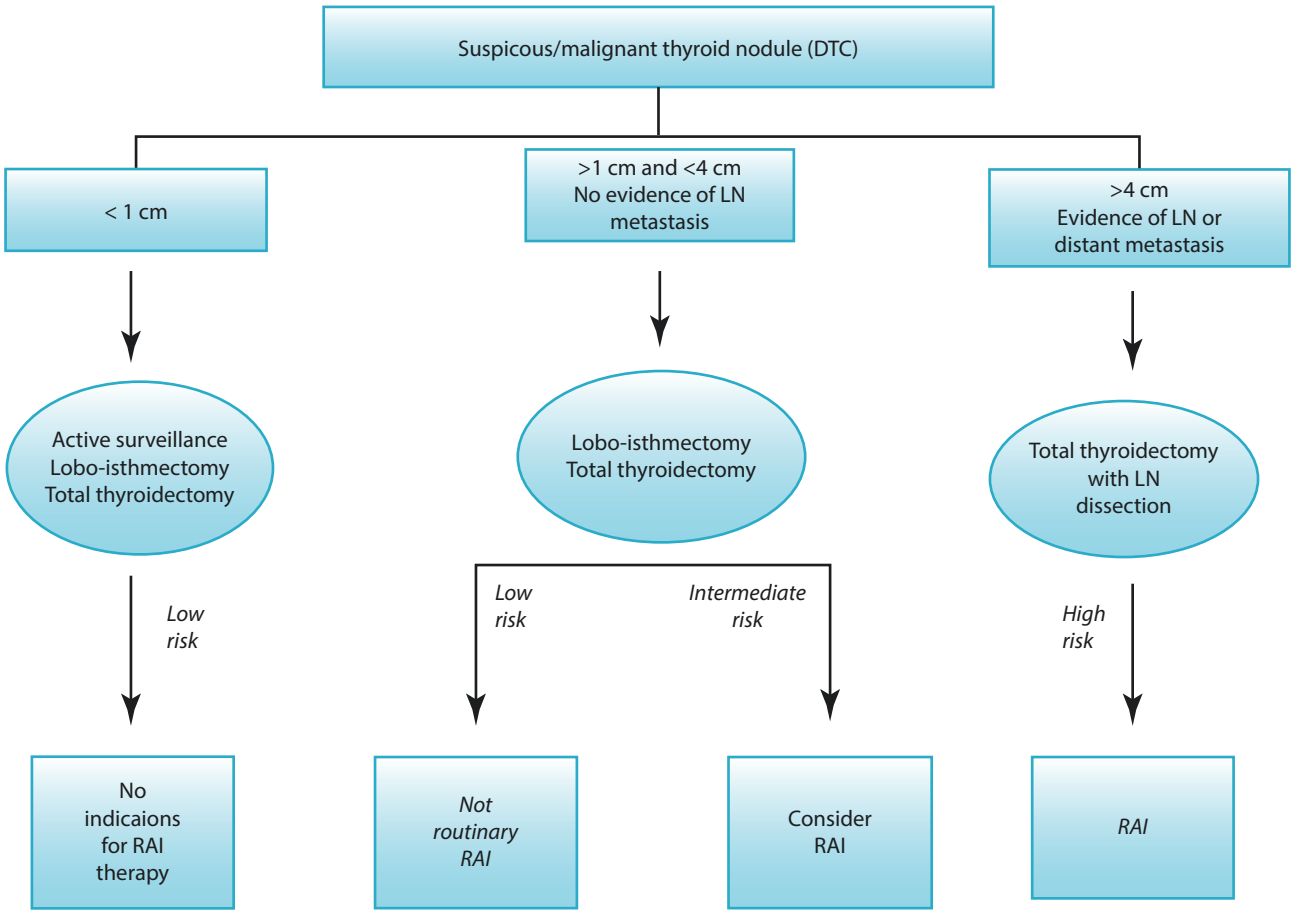

Fig. 56.9 Surgical and RAI treatment in differentiated thyroid cancers

Low risk	TSH 0.5-2 MU/L, if not evidence of disease
Intermediate risk	TSH 0.1-0.5 MU/L
High risk	TSH < 0,1 MU/L

Fig. 56.10 Risk-stratified management of thyroid hormone therapy

The drug of choice is levothyroxine (LT4). While for intermediate-risk thyroid cancer patients initial TSH suppression to 0.1–0.5 mU/L is recommended, no substantial benefits are demonstrated in low-risk patients [40]. Adverse effects of TSH suppression may include the known consequences of subclinical thyrotoxicosis, including exacerbation of angina in patients with ischemic heart disease, increased risk for atrial fibrillation in older patients, and increased risk of osteoporosis in postmenopausal women.

Likewise WDTC, the cornerstone of local treatment of MTC is surgical resection consisting of total thyroidectomy with dissection of central lymph node compartment and resection of the involved lateral compartment. Total thyroidectomy is also indicated in the sporadic setting because a small portion of lesions may be bilateral and because at the time of diagnosis it may not be clear whether the patient is affected by a familial disease or a true sporadic case. Unlike differentiated tumors, where the iodine avidity of follicular cells makes even metastatic DTC amenable to treatment, parafollicular cells do not concentrate iodine. Little randomized control data support the use of adjuvant radiotherapy for microscopic or macroscopic residual disease, extrathyroidal extension or extensive lymph node metastases, and in cases where there is a concern for airway obstruction [41]. Preoperative serum calcitonin levels and neck imaging findings should guide the initial surgical approach, since some retrospective cohort studies demonstrated the clinical benefit of elective neck dissection with serum calcitonin levels.

Unfortunately, in ATC complete resection does not significantly correlate with longer overall survival and cannot be even performed in most cases because of extensive disease.

Assessment and Management After Initial Treatment

Serum Tg determination, neck US, and ^{131}I whole-body scan (WBS) specifically detect recurrent or residual disease in most patients affected by DTCs who have undergone total thyroid ablation with thyroidectomy and remnant ablation (Fig. 56.11)

Serum Tg levels should be assessed periodically, but the test results more sensitive when the thyroxine is stopped or when recombinant human TSH (rhTSH) is given to increase the serum TSH [41]. However, patients dislike periodic hormone therapy withdrawal because of symptomatic hypothyroidism; intramuscular administration of rhTSH represents a safe and well-tolerated alternative with significantly fewer adverse events [42].

Even if showing higher false-negative rate than serum Tg evaluation, ^{131}I WBS should be performed several days after RAI therapy is given to assess iodine uptake by the tumor. Posttreatment imaging appeared to be much more sensitive in patients younger than 45 years old who previously had received RAI-therapy [42].

Concerning MTC, calcitonin (CT) is the only hormone produced by parafollicular cell, thus resulting crucial for postoperative surveillance. Measurements of both serum CEA and calcitonin are established prognostic markers in MTC and represent the cornerstone of postoperative assessment for residual disease (Fig. 56.15). In addition, it is recommended to maintain serum TSH and calcium levels within the normal range 4–6 weeks after surgery.

56.9.2 Recurrent or Metastatic Disease

The treatment of choice for localized thyroid cancer recurrences remains surgery, performed according to the type of thyroid cancer, the stage, and the patient's age. In some DTCs at high risk of recurrence, surgery may be supplemented by RAI treatment.

Tumor relapses are not uncommon (~20%) with the most frequent sites of distant metastases for WDTC occurring in the lung (50%), bone (25%), brain, liver, and skin. Liver is a major site of distant metastasis for MTC.

Treatment for metastatic WDTC includes TSH suppression and RAI therapy since the disease remains iodine avid and sensitive in the two-thirds of patients. Moreover, one-third of these tumors considered to be radiosensitive eventually become resistant due to a mutation of the sodium-iodide symporter (NIS) gene [43]. Specifically, tumors considered to be iodine refractory are those having some of the following characteristics: persistent neoplastic tissue that does not take up RAI, disease characterized by heterogeneous RAI uptake, or disease that progresses after RAI treatment despite RAI uptake (Fig. 56.12).

RAI-refractory patients should benefit from other locally ablative treatments such as radiofrequency ablation (RFA) or stereotactic radiation therapy (SBRT) that appeared to be as effective as surgery in selected patients, improving local tumor control and delaying the initiation of systemic treatment. To a lesser extent,

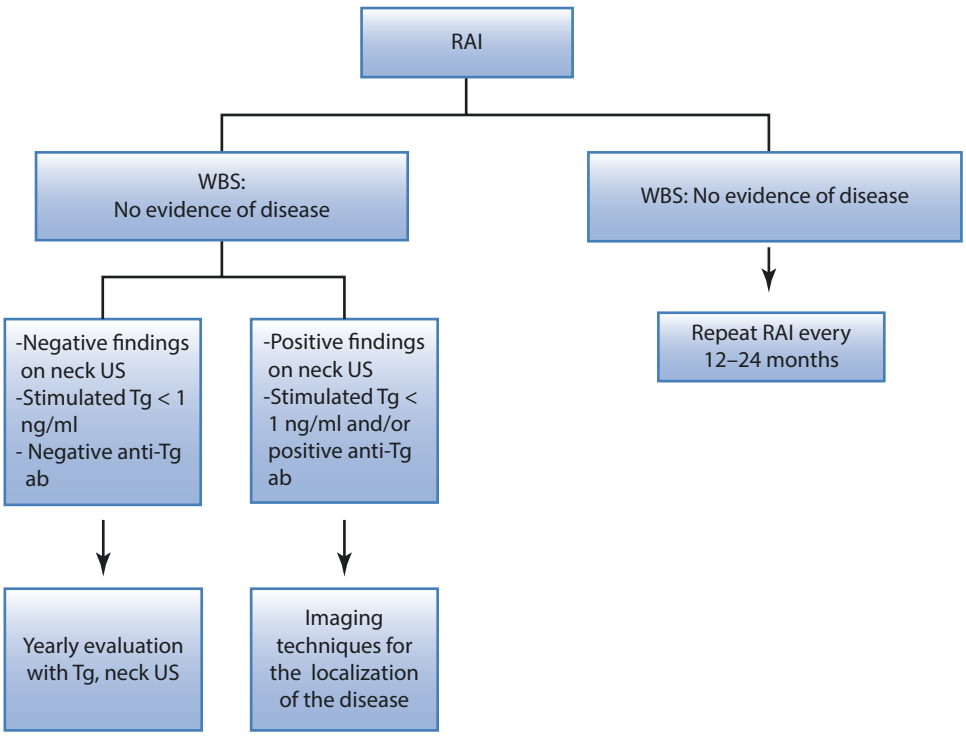

Fig. 56.11 Assessment algorithm at the time of the first control post-initial treatment in RAI-treated patients with differentiated thyroid carcinoma (DTC)

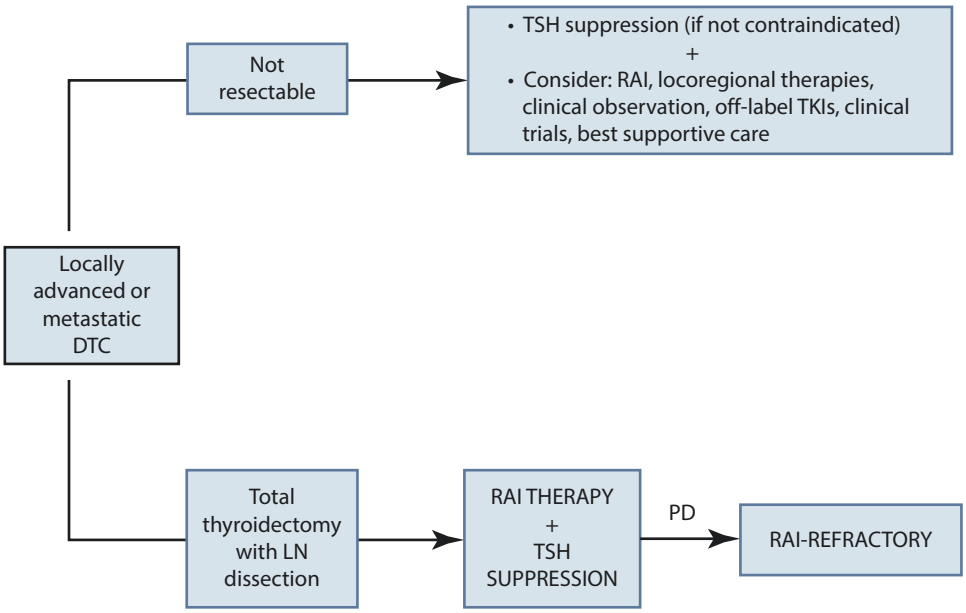

Fig. 56.12 Treatment algorithm for locally advanced or metastatic DTC

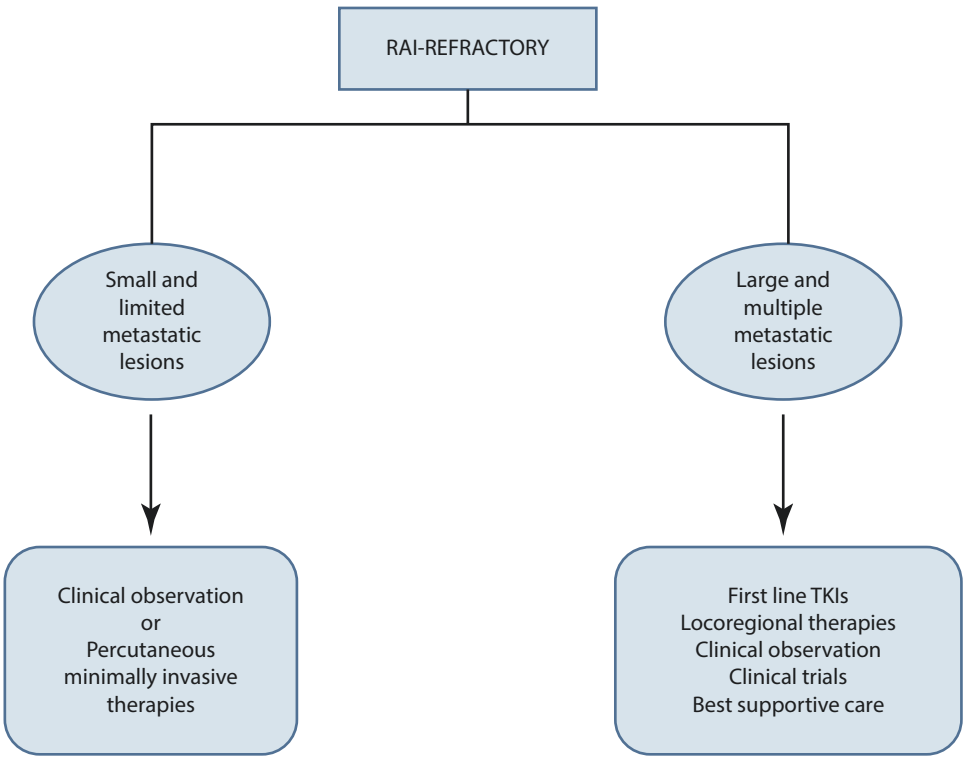

Fig. 56.13 Treatment options for progressive RAI-refractory DTC

these local treatments are indicated for symptomatic or imminently symptomatic disease, even in the presence of RAI uptake. EBRT may be indicated when complete surgical excision is not feasible or when there is no significant radioiodine uptake in the tumor, as also in the case of ATC.

When disease progression occurs at multiple sites where other better tolerated and more accessible local treatments have been exhausted and when target lesions appear to be radiologically measurable and in progression over the previous 12–14 months (as defined by RECIST criteria), treatment with tyrosine kinase inhibitors (TKIs) should be considered (Fig. 56.13).

For several decades, chemotherapy was the only option for treating patients with metastatic thyroid cancer. Several chemotherapeutic agents, including platinum compounds, bleomycin, doxorubicin, and paclitaxel, either administered alone or in combination,

have been considered to be potentially effective in treating RAI-refractory and poorly differentiated thyroid cancers. In addition, dacarbazine-containing regimens have been used for MTC [44]. These drugs showed universally poor response rates with significant toxicities and had short, if any, duration of effect [45].

Serine (BRAF) and tyrosine kinases (RAS, RET) act in tumors as intermediaries in cell signaling stimulating tumor proliferation and angiogenesis and favoring the capacity for invasion and metastasis. Therefore, the discovery of the antiangiogenic and antiproliferative effect of multitarget TKIs has led to the approval of four molecules that block these inappropriately activated pathways within the cancer cells. Four phase III studies have been conducted up to now showing a benefit for sorafenib, lenvatinib, vandetanib, and cabozantinib. According to drug regulatory agencies (Food and Drug Administration and European Medicines Agency), sorafenib (DECISION trial) and lenvatinib (SELECT trial) are approved for the use in progressive radioiodine-refractory PTC and FTC, while vandetanib (ZETA trial) and cabozantinib (EXAM trial) are approved for the treatment of metastatic MTC.

Sorafenib is a multitargeted kinase inhibitor targeting vascular endothelial growth factor receptor (VEGFR)-2 and (VEGFR)-3, platelet-derived growth factor receptor (PDGFR)-β, fibroblast growth factor receptors (FGFRs) 1–4, c-KIT, BRAF, and RET. In the DECISION trial, sorafenib showed a mean PFS of 10.8 months compared to 5.8 months in the placebo group in patients with locally advanced or metastatic radiation-resistant DTCs. 98.6% of the patients experienced adverse events (fatigue, hand–foot syndrome, and diarrhea) with 37.2% of these being grade 3 or higher [26].

Lenvatinib is an oral molecule capable of inhibiting receptors involved in the modulation of angiogenesis and lymphangiogenesis such as VEGFRs 1–3, FGFRs 1–4, PDGFR-α, RET, and KIT. In 2015, Schlumberger and colleagues conducted a phase III, double-blind, placebo-controlled study in patients affected by a pretreated or non-pretreated RAI-resistant DTC, showing a marked and statistically significant increase in median PFS for the lenvatinib group (18.3 months versus 3.6 months) while achieving a high clinical benefit. In the SELECT trial, lenvatinib showed a higher PFS (18.3 vs 10.8 months) and higher percentage of partial response (63.2% vs 12.2%) than sorafenib with some cases of complete response. However, 97.3% of patients mostly presented grade 3 or higher adverse events (hypertension, proteinuria, thromboembolism or renal failure), and a higher number of deaths were described for lenvatinib. Notwithstanding this, the shorter PFS observed in the placebo arm with respect to the PFS in the placebo arm of DECISION study (3.6 vs 5.8 months) suggested that patients enrolled in the SELECT study could have been affected by a more severe disease. Recently, lenvatinib activity has been also evaluated in ATC preclinical studies [46].

Vandetanib is also an oral multikinase inhibitor molecule mainly targeting the VEGFR, the epidermal growth factor receptor (EGFR), and RET-tyrosine kinase. The efficacy and safety of this drug was compared with placebo in patients with locally advanced or metastatic MTC in the ZETA trial [47]. The median PFS in the vandetanib group was not reached, but it was estimated at 30.5 months; it was 19.3 months in the placebo group. Frequent adverse events were diarrhea, rash, nausea, hypertension, dry skin, dry mouth, and headache.

Cabozantinib is an oral, small-molecule, multitargeted TKI with potent activity against VEGFRs, MET, RET, and the c-KIT, TIE2, and FLT3 genes. In the EXAM trial [48], a statistically significant prolongation in median progression-free survival was observed in patients with documented radiographic progression of MTC treated with cabozantinib (11.2 months vs 4.0 months in the placebo group). Most frequent adverse events include stomatitis, hypertension and diarrhea, fatigue, weight loss, and palmar-plantar erythrodysesthesia syndrome.

All TKI phase III studies have demonstrated significant improvement in progression-free survival, but not in overall survival (Table 56.7). This can be explained by the possibility of crossover in most of the studies. However, in a subgroup analysis, a statistically significant difference in overall survival (44.3 months vs. 18.9 months) was observed in patients with MTC and somatic RET M918T mutations who received cabozantinib compared with placebo [27]; similarly, patients with sporadic MTCs harboring a somatic *M918T* mutation had a higher response rate to vandetanib in the ZETA trial. In another subgroup, analysis of older patients (>65 years) from the SELECT trial who were treated with lenvatinib had an improved overall survival compared with placebo [49].

56.9.3 TKI Resistance: The "Escape Phenomenon"

The main limitation of targeted therapy is the development of an escape mechanism, called "escape phenomenon," that allows cancer cells to grow after a variable period of time from the beginning of the treatment. Resistance to TKI-based treatment is almost always present, regardless of TKI efficacy or tumor type [50–52].

This is likely due to the method of action of TKIs, which are cytostatic and not cytotoxic molecules, implying that tumoral cells are not killed but made quiescent

Table 56.7 Phase III studies in locally advanced or metastatic thyroid cancers resistant to radioiodine

Trial	Treatment	Phase study	Cell type	Prior treatments	Crossover	Number of patients	Response rate	PFS
DECISION	Sorafenib Placebo	III	DTC	No	Yes	207 210		10.8 5.8
SELECT	Lenvatinib Placebo	III	DTC	Yes	Yes	261 131	64% 1.5%	18.3 3.6
ZETA	Vandetanib Placebo	III	MTC	Yes	Yes	231 100	45% 13%	30.5 19.3
EXAM	Cabozantinib Placebo	III	MTC	Yes	No	219 111	283% 0%	11.2 4.0

DTC differentiated thyroid carcinoma, *HR* hazard ratio, *MTC* medullary thyroid carcinoma, *OS* overall survival, *PFS* progression-free survival

and not proliferative. Therefore, surviving cells can develop a mechanism of drug resistance determined by both the activation or upregulation of alternative pro-angiogenic pathways and the selective pressures of the microenvironment during malignant progression.

Since TKIs are cytostatic drugs, they should be indefinitely continued until there is clear evidence of disease progression or severe side effects occur; however, if the progression appears to be somehow limited, it is clinically reasonable to continue the administration of the drug until the possibility of substitution with another drug. There is also some evidence that once the TKI is stopped, the disease progresses even rapidly.

There are two main types of resistance: a "primary" or intrinsic resistance that is already present before targeted treatment is begun and a "secondary" resistance that develops after a variable period of definite response. An example of primary resistance is represented by *RET V804M* and *V804L* gatekeeper mutations that appeared to confer resistance to vandetanib in MTC by preventing the binding of the drug to the receptor in vitro studies [53]. Examples of secondary resistance in thyroid cancer treated with TKIs are still unknown but possibly due to secondary site mutations that are usually located downstream from the TKI target or in parallel pathways, as observed in some in vitro models [54].

Strategies to Overcome Resistance

A genotype-directed therapy using a TKI that acts via more than one pathway is one way of countering resistance. This can be explained by the sustained therapeutic effect of cabozantinib that effectively blocks the onset of MET-driven evasive resistance by inhibiting both MET and VEGFR2, unlike agents targeting the VEGF pathway alone. Similarly, the administration of another TKI with other mechanisms of action seemed to be able to revert the trend of growth after the escape mainly considering that in the SELECT trial lenvatinib showed efficacy in prolonging PFS also in patients previously treated with other TKIs (no data available on the use of Sorafenib as second line in patients who develop resistance to other TKIs).

Furthermore, addition of a synergistic agent is another way of evading resistance since dual inhibition of the MAPK and mTOR pathways or the MEK and mTOR pathways showed an interesting and effective inhibitory synergism in thyroid cancer cell lines, including ATC [55, 56]. Since early mutation of BRAF and RAS has been reported in almost one-third of ATC cases, a phase II open-label basket trial was conducted in patients with BRAF V600E-positive malignancies (including 16 with ATC), showing an acceptable safety profile and an objective response rate of 69% (11/16; 95% CI 41–89%) when administering the BRAF inhibitor dabrafenib plus the MEK inhibitor trametinib.

Another way of overcoming drug resistance mechanism and/or enhancing drug efficacy is the combination of targeted therapy with chemotherapy or radiotherapy that resulted to be safe and tolerable in ATC and PTC patients in phase I trials [57, 58].

Alternative TKIs

Pazopanib, a multikinase inhibitor mainly acting against VEGF 1–3, PGDF, FGFR, and RET, found to have activity against MTCs in preclinical studies. Likewise, sunitinib showed efficacy in phase II trials though not without adverse effects (asthenia, mucosal and cutaneous toxicities, hand–foot syndrome, and cardiac events).

No phase III clinical trials of axitinib (a second-generation VEGFR1–3 inhibitor) are available, but in a phase II trial, a progression-free survival of 15 months was observed with a 93% rate of adverse events, the most common being diarrhea, nausea, and hypertension [59]. In 2008, this molecule was first evaluated in a phase II

trial showing partial responses and stable diseases in 60 subjects affected by advanced thyroid cancer including 11 MTC patients [60].

Furthermore, current experimental strategies aim to target oncogenic signaling pathways that diminish iodide avidity in thyroid cancer. As previously described, oncogenic activation of the mitogen-activated protein kinase (MAPK) signaling pathway (RET/RAS/RAF/MEK/ERK), suppressing the expression of follicular cell-specific genes that are responsible for iodide uptake (e.g., the sodium-iodine symporter, or NIS) and metabolism, is a central event for the development of the majority of thyroid malignancies. Hence, MAPK pathway inhibition can enhance RAI incorporation and efficacy in a subset of RAI-refractory patients: selumetinib, a MEK 1/2 inhibitor, was found to reverse refractoriness to RAI in patients with metastatic DTC in combination with therapeutic radioiodine [61].

Moreover, the understanding of this biology provides the molecular basis of the well-established clinical observation that BRAF mutant tumors present with more aggressive clinical behavior and are more often RAI-refractory. For BRAF mutant patients, treatment with dabrafenib, a BRAF inhibitor, increased iodide incorporation in 6 of 10 BRAF mutant patients, resulting in tumor shrinkage after subsequent treatment with I-131 in 5 of the 6 patients [62]. In patients with ATC and BRAF V600F mutation, a study with the selective BRAF inhibitor vemurafenib was also done [63].

Immunotherapy and Other Agents

In the presence of nondruggable mutations, immunotherapy could represent an alternative approach, even if inclusion of targeted therapy, immunotherapy, chemotherapy, and/or radiotherapy, administered in combination or sequentially, should be tested within the context of a clinical trial. Few data are available with antibodies targeting programmed cell death 1 (PD-1) receptor or programmed cell death ligand-1 (PD-L1). The anti-PD-1 monoclonal antibody spartalizumab was tested in 41 heavily pretreated patients with advanced ATC, and responses were observed in 19.5%.

Patients who undergo PET/CT scan with tumor lesions having strong avidity for Ga-DOTATATE may benefit from a new type of radionuclide-based therapy, a technique called peptide receptor radionuclide therapy (PRRT). This is a molecule of lutetium-177 or yttrium-90 linked to a somatostatin analog that has high affinity for the overexpressed somatostatin type 2 receptors (SSTR) in MTC. Several series reported a good response rate and a beneficial effect on QOL for both DTCs and MTCs [64].

Histone deacetylase inhibitors (HDACIs), such as valproic acid or romidepsin, cause selective cell death of tumor cells since HDAC exert a pro-oncogenic effect by keeping genes involved in apoptosis in a transcriptionally quiescent state [65]. Though several HDACIs have not yet shown clinical benefit, these small molecule inhibitors are good potential therapeutic agents and under trial for all advanced thyroid cancers, including MTC.

The rise of immunotherapy may further alter the fate of patients with thyroid carcinoma, whether differentiated, medullary, or anaplastic. Chowdhury and colleagues showed that overexpression of PD-L1, involved in controlling the immune response of T cells, is associated with an increased risk of relapse and, thus, is a marker of poor prognosis in PTCs. In addition, Zwaenepoel and colleagues described this overexpression in about one-third of ATCs. The first two checkpoint inhibitors developed targeting PD-1 are pembrolizumab and nivolumab whose responses observed are encouraging in terms of continuing research on the subject.

56.10 Follow-up

The aim of follow-up is to define the absence of persistent tumor 6–12 months after the primary treatment, ascertaining whether or not the patient is free of disease and eventually leading to the early discovery and treatment of persistent or recurrent locoregional or distant disease.

No evidence of disease (NED) is defined by the absence of both clinical and imaging evidence of tumor, undetectable Tg levels (during either THS suppression or TSH stimulation) and the absence of anti-Tg antibodies.

Regarding DTC, patients treated with RAI therapy may be followed with unstimulated Tg annually and periodic neck US if they have negative clinical and ultrasound findings, stimulated Tg less that 1 ng/ml with negative anti-Tg antibodies, and negative whole body scan (Fig. 56.11). The trend in Tg levels over time, which should be assessed with the same laboratory and same assay, can help indicate those patients with a clinically significant residual disease that should be studied using further imaging techniques. However, when basal serum Tg is ≤0.1 ng/ml and neck US is unremarkable, patients may be clinically considered free of disease and are able to avoid an rhTSH stimulation; conversely, rhTSH stimulation testing may still be informative on the absence or presence of disease when basal serum Tg is >0.1 ng/ml but <1.0 ng/ml. If thyroid function tests (FT3, FT4, TSH) additionally confirm the adequacy of hormone suppressive or substitutive therapy after 3–6 months from the initial treatment, patients may be considered in complete remission with a very low subsequent recurrence rate (<1.0% at 10 years). RAI scans may be used to characterize the functional status of structural disease and are recommended every 12–24 months until no

clinically significant response to RAI is seen. Non-RAI imaging (such as CT and or FDG-PET/TC) might be considered if negative RAI imaging and rising stimulated Tg levels.

On the contrary, diagnostic total body ^{131}I imaging is less often used for low-risk patients and absolutely not specific for thyroid cancer in patients who have not undergone remnant ablation. These patients may be followed with periodic neck US and Tg level measurements (Fig. 56.14).

Regarding MTC, if serum CT after a provocative (pentagastrin or calcium) test results undetectable from 1 to 3 month after surgery, no other diagnostic test is warranted, and serum CT should be repeated every 6 months for the first 2–3 years and annually thereafter. In patients with serum CT concentration <150 pg/ml, localization of disease should be limited to a careful examination by neck US. Patients with basal CT >150 pg/ml should be screened for distant metastases (Fig. 56.15)

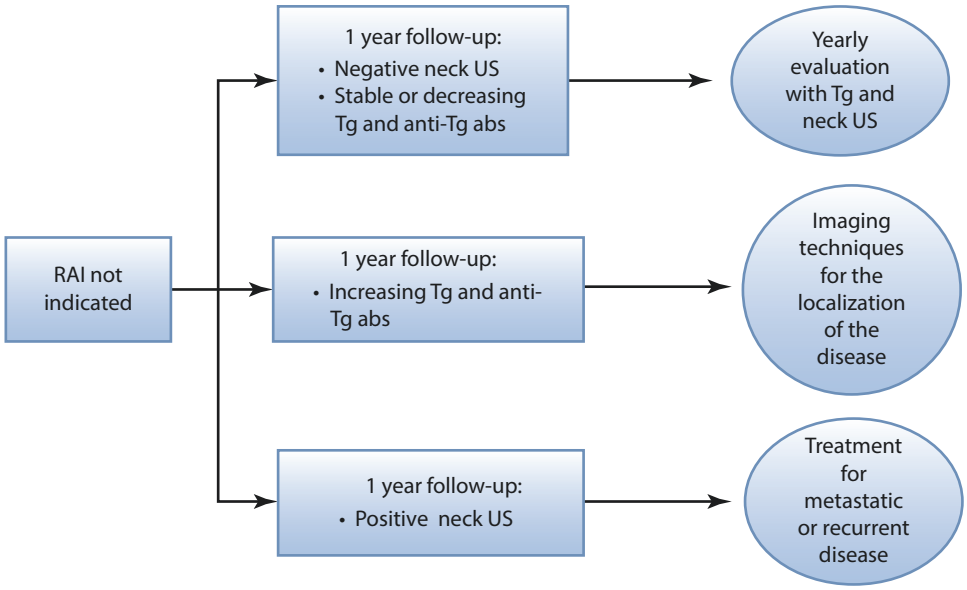

Fig. 56.14 Follow-up algorithm in DTC patients not previously treated with RAI therapy

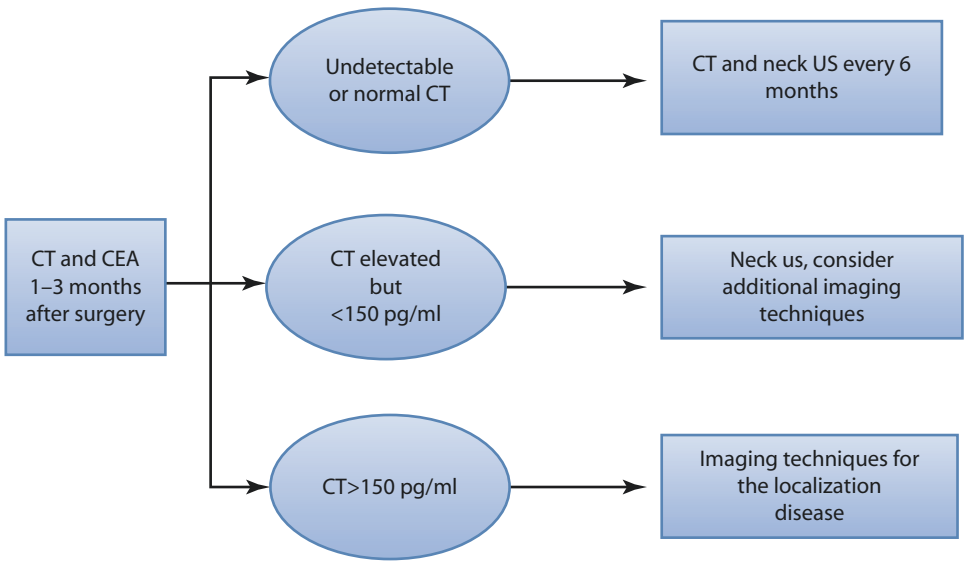

Fig. 56.15 Diagnostic algorithm based on calcitonin levels obtained 1–3 months after initial surgery in patients with medullary thyroid carcinoma

Case Study: Management of a Patient with a Progressive Metastatic Papillary Thyroid Cancer

Female, 45 years old

- *Family history* negative for malignancies
- *APR:* prior exposure to ionizing radiation for Hodgkin's lymphoma
- *APP:* Incidental US finding of an asymptomatic and painless mass located on the thyroid left lobe, unintentional weight loss, no skin changes on the overlying skin, no obstructive symptoms, no swellings
- *Objective examination*: solitary and palpable nodule with firm consistency, fixed in respect to surrounding tissues and mobile with the trachea at swallowing; absence of hoarseness, cervical lymphadenopathies and tracheal or esophageal compression
- *Blood tests*: normal thyroid function tests (fT4, fT3, TSH)
- *Ultrasonography*: well-defined, homogeneous, solid hypoechoic oval-shaped nodule measuring up to 4 cm in diameter with irregular margins in upper left thyroid lobe
- *FNAC*: cellular smears with papillary fronds composed of cells with enlarged oval nuclei with intranuclear grooves, inclusions with nuclear crowding and overlapping, and background "chewing-gum" colloid suggestive for classical PTC

Question

What action should be taken?

(1) Lobectomy. (2) Total thyroidectomy. (3) Total thyroidectomy with LN dissection

Answer

Total thyroidectomy (in light of the radiation history, the size of the tumor and the absence of involved lymph nodes)

Histological examination confirmed classical PTC with lymphovascular invasion, with a high mitotic index (5 mitosis × 10 high power fields) and high Ki67 expression (15%) (Foto papillare Martorana)

WBS ⇨ residual disease in the thyroid bed

Question

What action should be taken postoperatively?

(1) Substitutive hormone therapy. (2) RAI therapy + suppressive hormone therapy. (3) Active surveillance

Answer

RAI therapy + suppressive hormone therapy

⇩

RAI therapy until radioiodine uptake is completely absent + initial TSH suppression to 0.1– 0.5 mU/L (intermediate risk patient)

Follow-up at 1 year: positive findings on neck US and rising TG serum levels. Total body CT and PET scans revealed the new appearance of pulmonary and vertebral osteolytic lesions

Question

What action should be taken?

(1) Axitinib. (2) RAI therapy. (3) Lenvatinib

Answer

Begins Lenvatinib 24 mg cp daily with significant reduction of bone lesion volume

Key Points

- Start with medical history, physical examination and diagnostic work-up
- Surgery remains the cornerstone of thyroid cancer initial treatment
- The importance of the postoperative risk-stratified management in DTC
- Consider TKIs in the progressive life-threatening metastatic disease

Case Study Management of MTC in a Patient Affected by MEN2A

Female, 30 years old

- *Family history* positive for malignancy (mother's history of pheochromocytoma)
- *APR*: negative
- *APP*: Lump at the base of the neck becoming more prominent during swallowing, episodic sweating, hypertensive crisis in the last weeks, no intestinal disorders
- *Objective examination*: dominant thyroid nodule at the base of the neck; nontender palpable left lateral cervical lymph node; elevated blood pressure and heart rate; no mucosal neuromas in the tongue, lips or eyelids; no musculoskeletal abnormalities.
- *Laboratory tests*: serum calcium, parathyroid hormone, TSH and cathecolamines within reference range; rising levels of both serum calcitonin (420 pg/ml) and CEA; 24 h urinary cathecolamine was 1100 nmol/24 h (normal range < 230 nmol/24 h); 24 h urinary vanillylmandelic acid weakly positive.
- *US*: hypoechogenic mass with diameter of 2.3 cm x 1.5 cm on the left lobe of the thyroid

Question

What action should be taken?

(1) Surgery. (2) CT scan. (3) FNAB

Answer

Total body *CT scan* did not detect metastatic disease and confirmed the presence of a right adrenal pheochromocytoma. Avoid FNAC since the diagnosis could be consistent with MEN2A and biopsy could increase the possibility of tumor spread.

Question

What action should be taken?

(1) Surgery. (2) RAI therapy. (3) Other

Answer

Surgery: right total adrenalectomy followed by total thyroidectomy with lymph nodes dissection to both central and latero-cervical compartments

Question

What action should be taken?

(1) Hormone substitutive therapy + Genetic screening for RET. (2) Other. (3) Follow-up

Answer

Administer *hormone replacement therapy* and perform *genetic screening for RET* mutation to the patient and the family members (parents, siblings, and children)

Key Points

- The importance of both family history assessment and proper clinical management in MTC
- Consider lab tests and imaging techniques for differential diagnosis
- Understand the use of RET testing in families with MEN2 as a part of strategy to prevent MTC

Expert Opinion

Alfredo Berruti

Key Points

- The cellular origin of the thyroid tumor has important implications for prognosis and planning the therapeutic and follow-up strategies.
- Differentiated tumor cells, both papillary and follicular, are able to take up iodine and to secrete thyroglobulin (Tg) under stimulus from thyrotropin-stimulating hormone (TSH).
- Medullary thyroid cancer (MTC) derived from parafollicular C cells, which are not involved in iodine metabolism and are not TSH dependent. MTC is not able to concentrate 131I and does not produce Tg. It instead pruduces Calcitonin
- Both differentiated thyroid cancer and MTC can be cured by surgery, that is the mainstay of therapy.
- Postoperative radioactive iodine (131I) therapy can improve the cure rate of differentiated thyroid cancers.
- Tg is an important and useful marker in the follow-up to early detect recurrent or residual disease. Calcitonin and carcinoembryonic antigen (CEA) are the reference tumor markers for MTC.
- The prognosis of patients affected by thyroid cancer is highly variable and depend on the histotype and the degree of differentiation. The survival perspective of non metastatic patients with differentiated thyroid cancer is very good, while it is poor in patients with poorly differentiated and anaplastic tumors. The survival perspective of MTC is generally poorer than that of differentiated tumors.
- Recurrences of differentiated thyroid cancer can be identified early and tcured by measuring the basal and/or TSH-stimulated serum Tg levels and via neck ultrasound.
- Serum calcitonin is a sensitive marker in the follow-up of MTC patients. Diagnostic imaging could be negative in patients with calcitonin levels <150 pg/ml, whereas the probability of detecting metastases increases with growing of calcitonin and CEA levels.
- In about one-third of advanced differentiated thyroid cancers, the metastatic lesions have a very low avidity for iodine, and 131I therapy has no effects. Anaplastic and poorly differentiated thyroid cancers are no longer able to take up iodine, secrete Tg, or respond to TSH stimulus.
- The increasing knowledge about the molecular alterations underlying thyroid cancer has provided the rationale for the use of targeted cancer therapies that represent newer options for patients with advanced thyroid cancer.
- Randomized prospective clinical trials have shown that sorafenib and lenvatinib are efficacious in the management of metastatic differentiated thyroid cancer that are radioiodine refractory, while vandetanib and cabo-

zantinib are efficacious in metastatic MTC. All these molecular target agents improved progression-free survival but not overall survival, and their administration is associated with relevant (although manageable) toxicity. The prescription of these drugs on individual patients should be done taking carefully into account the cost/benefit balance.

- Since both differentiated thyroid cancers and MTC may follow and indolent disease course, it is not always clear which patient needs a systemic therapy. Discussion within a multidisciplinary team is of paramount importance to guarantee the best therapeutic approach to these patients.
- The therapeutic strategies actually available have had only a limited impact on survival of metastatic patients with both differentiated thyroid cancer and MTC, and the management of anaplastic and poorly differentiated malignancies still remains challenging. Moreover, risk stratification is currently based mainly on clinic pathologic risk factors. New drugs and new prognostic parameters are therefore needed.

Summary of Clinical Recommendations

- Lobectomy (instead of total thyroidectomy) could be offered to low-risk small DTCs, while a therapeutic lymph node dissection is necessary with clinically positive nodal (N1) disease in the central or lateral neck compartment. Preoperative serum calcitonin levels and neck imaging findings should guide the initial surgical approach of MTC. In ATC, complete resection cannot be even performed in most cases because of extensive disease.
- RAI administration is not recommended for small (≤1 cm) intrathyroidal DTC with no evidence of locoregional metastases (classified as low-risk cases), may be considered in intermediate-risk patients, and is recommended for patients at high risks of recurrence.
- In DTCs, high-sensitivity assays of basal Tg can be used in testing to verify the absence of disease (excellent response), whereas serial measurements of basal Tg should be obtained in patients with residual thyroid tissue and following lobectomy; neck US is the most effective tool for detecting structural disease in the neck, and other Imaging studies are indicated if locoregional and/or distant metastases are clinically suspected or in patients with known metastases; TSH should be suppressed when structural disease persists or when high-risk patients have a less-than-excellent response to therapy. As regards MTC, CT and CEA monitoring along with multimodality imaging should be included in the early and long-term postoperative staging work-ups
- DTC patients with distant metastases should receive radioactive iodine after TSH stimulation; non-RAI-avid lesions and those that lose their ability to concentrate RAI or progress despite RAI avidity should be considered RAI-refractory; lenvatinib and sorafenib should be considered the first-line systemic therapy for RAI-refractory DTC. Cabozantinib and vandetanib should be considered the first-line systemic therapy for MTC patients; in patients with RETM918T or RAS-mutant MTCs, cabozantinib offers significant PFS and OS advantages over wild-type cancers. Patients with BRAF V600E-positive ATCs should be treated with the BRAF inhibitor dabrafenib plus the MEK inhibitor trametinib; clinical trial enrolment should be encouraged for patients with good clinical PS.

Hints for Deeper Insight and Suggested Reading

1. S Filetti, C Durante, D Hartl, S Leboulleux, LD Locati, K Newbold, MG Papotti, A Berruti, ESMO Guidelines Committee, Thyroid cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Annals of Oncology, mdz400, ► https://doi.org/10.1093/annonc/mdz400
2. AIOM Guidelines (Associazione Italiana Oncologia Medica)- Tumori della Tiroide 2017/2018. ► https://www.aiom.it/tumori-della-tiroide-2017/
3. NCCN Clinical Practice Guidelines in Oncology-Thyroid Carcinoma

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Cutaneous Melanoma and Other Skin Cancers

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Skin Cancers

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Learning Objectives

By the end of the chapter, the reader will:

- Have notions of incidence, mortality, and the main risk factors for cutaneous melanoma
- Have learned the basic concepts of pathogenesis of melanoma
- Be able to identify a suspected skin lesion for melanoma
- Know the staging of melanoma
- Have learned basic concepts on melanoma therapy
- Be able to put acquired knowledge into clinical practice.

57.1 Cutaneous Melanoma

Paola Queirolo and Andrea Boutros

57.1.1 Introduction

The majority of melanomas arise from the skin, but other types, with substantially different pathogenesis and biological behavior, include mucosal or uveal melanoma too. This chapter will focus essentially on cutaneous melanoma.

Cutaneous melanoma is the deadliest form of skin cancer, despite being relatively rare, representing less than 5% of all skin cancers.

According to the American Cancer Society, cutaneous melanoma is the fifth leading type of cancer in the United States, with about 96 000 new estimated cases and about 7 000 expected deaths in 2019 [1].

Before the introduction of immunotherapy and targeted therapy, the median survival of patients with stage IV disease was less than 1 year [2–5]. For more than 30 years, the standard of care for advanced melanoma was chemotherapy, but in the last decade, significant therapeutic achievements were obtained in patients with advanced melanoma thanks to targeted therapy and immunotherapy.

In this chapter, data from the most relevant clinical trials in early-stage and advanced melanoma will be discussed with some insight on the treatment of real-world patients.

57.1.2 Epidemiology and Risk factors

According to the Centers for Disease Control and Prevention (CDC), in 2015 in the United States, a total of 80 442 new cases of cutaneous melanoma were observed. The CDC reported a total of 22 new cases and 2 deaths for every 100 000 people [6].

Melanoma tends to be more frequent in fair-skinned people who tends to develop sunburns more easily [7]. Indeed, sunburns, especially early in life, are considered a crucial risk factor in the tumor genesis of melanoma [8]. In particular, ultraviolet lights in the UVB spectrum (290–320 nm) are the principal ones to be associated to sunburns, leading to DNA damage, inflammation, and local immunosuppression.

Other risk factors include the total number of melanocytic nevi, family history of melanoma or any other skin cancer, immunosuppression, male sex, and age. Even if the average age at diagnosis is around 60 years old, cutaneous melanoma is not uncommon among young adults [9].

57.1.3 Pathogenesis

The two most important predisposing factors to the development of cutaneous melanoma are sun exposure and genetic susceptibility. Indeed, cutaneous melanoma occurs more commonly on sun-exposed areas of the back in men, and of the extremities in women, with an overall increased risk for fair-skinned subjects versus dark-skinned subjects. However, the relationship between melanoma and sun exposure is not as evident as it is in other non-melanoma skin tumors, since melanoma can also occur on dark-skinned people and in non-photo-exposed areas (acral melanoma).

57.1.3.1 Familial Melanoma

Familiarity is another very important risk factor, since it is estimated that about 10–15% of all melanomas occur on a genetic basis.

The main mutations observed are involved in the decreased activity of the retinoblastoma (RB) tumor suppressor proteins and may also be involved in the pathogenesis of sporadic melanoma, such as CDKN2A and cyclin-dependent kinase 4 (CDK4).

In particular, the CDKN2A gene encodes three different oncosuppressors (p15/INK4b, p16/INK4a, and p14/ARF), and it is involved in an autosomal dominant inherited mutation. In particular, p16/INK4a increases the activity of the tumor suppressor proteins of the RB family by inhibiting CDK4, while p14/ARF increases the activity of the p53 oncosuppressor by inhibiting the activity of the MDM2 oncoprotein. These mutations lead to an increase in uncontrolled melanocyte proliferation [9].

The CDKN2A germline mutation has been associated with the multiple mole/melanoma/pancreatic cancer syndrome [10].

Other rare familial melanomas have been associated with mutations of the MITF susceptibility gene, involved in the development of melanoma and renal cell carcinoma [11].

Other predisposing conditions are xeroderma pigmentosum, dysplastic nevus syndrome, and family history of melanoma (even without the evidence of pathogenetic mutations).

57.1.3.2 Sporadic Melanoma

Melanoma may occur sporadically on somatic mutations that inhibit the activity of tumor suppressor proteins (e.g., CDK4 and CDKN2A), or on mutations that increase the activity of signal-transduction oncoproteins (RAS and PI-3K/AKT), leading to increased cell growth and survival.

About half of melanoma patients bear the BRAF mutation, a gene encoding a serine/threonine kinase downstream of RAS, and about 10% carry the NRAS mutation. Melanomas that arise in non-sun-exposed areas, on the other hand, can lead to activating mutations of the C-KIT receptor tyrosine kinase. These somatic mutations have today become a fundamental therapeutic target.

Note that these mutations are probably necessary, but not sufficient for the development of melanoma, since even the melanocytic nevi carry the same activating mutations as NRAS and BRAF, without having an evolution in malignancy [9].

57.1.4 Clinical Presentation

Melanoma occurs classically according to the acronym ABCDE:

- Asymmetry.
- Border irregularity.
- Color: variegated and uneven with streaks of black, red-brown, gray, blue.
- Dimensions more than 6 mm in diameter.
- Evolution. The early recognition of suspicious skin lesions is important, as melanoma can assume a rapidly progressive behavior and give distant metastasis [12].

However, not all melanomas occur according to the ABCDE rule. In many cases, it may present as a nodular symmetrical lesion; in other cases, it may not be pigmented; and in others, it may have small dimensions. On the other hand, in subjects with dysplastic nevus syndrome, most nevi fall within the characteristics of ABCDE, without being melanoma. For this reason, it is important to integrate the ABCDE rule with the sign of the "ugly duckling," where lesions presenting uneven characteristics that deviate from all the other patient's nevi pattern must be considered as suspect [12].

Usually, melanoma is an asymptomatic lesion, but sometimes it can cause itching, local pain, or bleeding [9].

57.1.5 Diagnosis

Biopsy is the only valid way to diagnose melanoma. Furthermore, biopsy is essential for proper staging.

The excisional biopsy must include all the lesion in all its thickness (without necessarily including the subcutis) and a narrow margin of about 1 mm.

When an excisional biopsy is not possible (large lesions, lesions of the face or genital mucosa), an incisional biopsy is taken from a representative site.

In ungual lesions, a punch biopsy can be the first diagnostic step [12].

57.1.5.1 Histopathology

In its early stage, melanoma may present a horizontal diffusion within the epidermis and superficial dermis (radial growth phase). After a variable period of time, melanoma can begin to grow vertically (vertical growth phase). Having acquired the ability to metastasize, melanoma can invade the deeper dermal layers in this phase.

The Breslow thickness has a strong prognostic value, and it represents the distance from the granular layer of the epidermis to the deeper intradermal tumor cells. Other factors are the presence of ulceration and the number of mitoses.

Melanoma cells are larger than normal melanocytes and contain large nuclei, condensed chromatin, and prominent nucleoli [9].

The different histological variants of melanoma are described in Table 57.1 [12].

Table 57.1 Melanoma histologic subtypes

Superficial spreading melanoma	The most frequent type of melanoma. It occurs mainly on trunk and extremities (except on acral sites). This variant is the mainly associated with sun exposure.
Nodular melanoma	The second histotype in frequency, associated with a worse prognosis due to its thickness at time of diagnosis.
Acral melanoma	Relatively rare variant, despite being the most common in dark-skinned subjects. It arises in the acral sites (palms, plants, subungual).
Lentigo maligna melanoma	Lesion that typically occurs on the face of elderly subjects, characterized by a very long phase of radial growth.
Desmoplastic melanoma	Uncommon variant. Histologically it presents melanocytes in the dermis with intense stromal infiltrate. Often clinically unpigmented.

[12]

57.1.6 Staging

A correct staging is a fundamental step in the planning of the appropriate diagnostic-therapeutic strategy. The approved classification is the TNM by the American Joint Committee on Cancer (AJCC), 8th edition, reported in Table 57.2 [13].

Table 57.2 Melanoma staging system, AJCC 8th edition

T	*Breslow thickness*	*Ulceration*	
Tx	Not applicable because the primary tumor thickness cannot be assessed	Not applicable	Not applicable
T0	Not applicable	Not applicable	Unknown primary or complete regression
Tis	Not applicable Melanoma in situ	Not applicable	Stage 0
T1	T1a: <0.8 mm	Absent	Stage I
	T1b: <0.8	Present	
	T1b: 0.8–1.0 mm	Absent	
T2	T2a: >1.0–2.0 mm	Absent	
	T2b: >1.0–2.0 mm	Present	Stage II
T3	T3a: >2.0–4.0 mm	Absent	
	T3b: >2.0–4.0 mm	Present	
T4	T4a: >4.0 mm	Absent	
	T4b: >4.0 mm	Present	
N	*No. of involved regional lymph nodes*	*In transit metastasis, satellite, and/or microsatellitosis*	
Nx	Not assessed	No	
N0	Not detected	No	
N1	N1a: one clinically occult	No	Stage III
	N1b: one clinically detected	No	
	N1c: not detected	Yes	
N2	N2a: 2–3 clinically occult	No	
	N2b: 2–3, at least one clinically detected	No	
	N2c: one clinically occult or clinically detected	Yes	
N3	N3a: ≥4 clinically occult	No	
	N3b: ≥4, at least one clinically detected or any number of matted nodes	No	
	N3c: ≥2 clinically occult or clinically detected and/or any number of matted nodes	Yes	
M	*Anatomic site of distant metastasis*	*LDH level*	
M0	Nonevident distant metastasis	Not applicable	
M1	M1a: skin, soft tissue, and/or nonregional lymph nodes	(0) Not elevated (1) Elevated	Stage IV
	M1b: lung	(0) Not elevated (1) Elevated	
	M1c: non-central nervous system (CNS) visceral sites	(0) Not elevated (1) Elevated	
	M1d: CNS	(0) Not elevated (1) Elevated	

The most significant prognostic factors are the Breslow thickness and the presence of ulceration.

In fact, the 5-year survival goes from 99% for melanomas <1 mm to 90% for those >4 mm [13]. Ulceration has a similar impact, especially in thicker melanomas, ranging from a 5-year survival of 94% and 90% in pT3a and pT4a melanomas to 86% and 82%, respectively, in pT3b and pT4b [13].

The number of mitoses per mm^2 has a real impact on survival for values greater than 4 [13].

Stage III 5-year survival ranges from 82% in N1 melanoma, to 76% in N2, and 57% in N3 [13].

In stage IV disease, serum LDH level is a clinically significant factor with a predictive and prognostic value [13].

57.1.7 Treatment of Early-Stage Disease

57.1.7.1 Surgery

Early-stage melanoma has an excellent long-term prognosis [13]: surgical excision is the standard treatment, with a 5-year survival rates at 98% for stage I and ranging from 96% to 82% for stage II disease [13]. Wide local excision is the definitive approach. Several meta-analyses [14] summarized the evidence regarding width of excision margins for primary cutaneous melanoma. In particular, recommended excision margins are as follows:

- 5 mm in melanoma in situ
- 10 mm if Breslow's thickness <2 mm
- 20 mm if Breslow's thickness ≥2 mm

When Breslow's thickness is ≥0.8 mm, a sentinel lymph node biopsy (SLNB) is indicated. In fact, the risk of lymph node metastatic involvement is proportional to depth. The SLNB is a minimally invasive procedure that confers prognostic information regarding risk stratification.

Risk stratification, as shown in a recent meta-analysis, should be based on ulceration (present/absent) of the primary tumor and SN tumor burden (high > 1 mm/low < 1 mm) as shown by Rotterdam criteria for Sentinel Node Tumor Load [15].

This risk stratification is in line with the recent evidence that the complete lymph node dissection (CLND) does not have a significant impact on survival [16], with an important and unnecessary exposure to morbidity over time.

57.1.7.2 Adjuvant Setting

Patients with thick and/or ulcerated-primary melanoma and/or regional lymph node involvement at diagnosis (stage III) have higher risk of recurrence after surgical resection. So, in this category of patients, there is strong indication to start an adjuvant treatment, in order to improve the risk of relapse. The main adjuvant clinical studies are summarized in Table 57.3.

Figure 57.1 shows a therapeutic algorithm for melanoma at high risk of relapse.

57.1.7.3 Interferon Alpha

During the last two decades [17], the only approved treatment in the adjuvant setting was interferon alpha. Different doses and schedules were studied in different nations.

In terms of event-free survival (EFS) an improvement has been observed, leading to absolute increases in 5- and 10-year EFS of 3.5% and 2.7%, respectively,

Table 57.3 A summary of the main clinical studies in the adjuvant setting in melanoma

Study	Treatment	Stage	HR_{RFS}	HR_{OS}
Interferon alpha meta-analyses [17]	Interferon – different regimens	IIB-IIIC	**0.82** *vs.* placebo	**0.89**
EORTC 18071/ CA184-029 [18]	Ipilimumab (10 mg/kg; q3w × 4 → q3m) vs. placebo	IIIA(>1mm)/IIIB/IIIC	**0.76** *vs.* placebo	**0.72**
CheckMate-238 [19]	Nivolumab (3 mg/kg; q2w) vs. ipilimumab (10 mg/kg; q3w × 4 → q12w)	IIIB/IIIC/resected IV	**0.65** *vs.* ipilimumab	Not available
KEYNOTE 054 [20]	Pembrolizumab (200 mg/kg; q3w) vs. placebo	IIIA(>1mm)/IIIB/IIIC	**0.57** *vs.* placebo	Not available
BRIM-8 [22]	Vemurafenib (960 mg bid) vs. placebo	IIC/IIIA(>1mm)/IIIB/IIIC	**0.65** *vs.* placebo **0.80** IIIC **0.54** IIC-IIIB	Not available
Combi-AD [21]	Dabrafenib + trametinib (150 mg bid + 2 mg qd) vs. placebo	IIIA(>1mm)/IIIB/IIIC	**0.47** *vs.* placebo	**0.57**

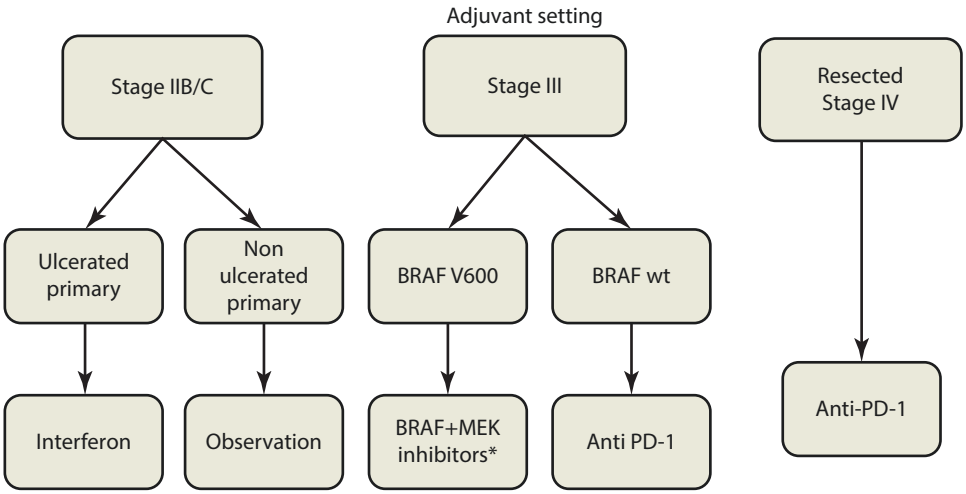

Fig. 57.1 A therapeutic algorithm for melanoma at high risk of relapse

with a significantly greater benefit in ulcerated primary tumor patients. Note that disease stages I and II were also included in those clinical trials.

In the recent years, great progress has been made in the immunologic treatment of metastatic melanoma. Now we see the same progress in the adjuvant setting.

Available drugs are anti-CTLA4 ipilimumab, and anti-PD-1 nivolumab and pembrolizumab.

57.1.7.4 Ipilimumab

EORTC 18071/CA184-029 [18] is a phase 3, randomized trial where ipilimumab – administered at 10 mg/kg dose every 21 days, for 4 cycles, followed by maintenance doses every 3 months up to 3 years – was compared to placebo. Ipilimumab showed greater efficacy than placebo in terms of relapse-free survival with a 5-year RFS of 41% for the ipilimumab arm and 30% for the placebo and in terms of distant metastases-free survival (DMFS), where, at 5 years, ipilimumab showed superior data compared to placebo: 48% were alive metastases-free in the ipilimumab arm versus 39% in the placebo arm.

In particular, the superiority of ipilimumab was more evident in the ulcerated-primary and >3 positive lymph nodes subgroups.

However, the treatment arm showed a higher toxicity profile (high incidence of grade >3 adverse events), which occurred in more than a half of the ipilimumab arm patients. In order to overcome this issue, another study was designed to compare ipilimumab 10 mg/kg and ipilimumab 3 mg/kg, with no significant difference in terms of efficacy and with an improvement of the severe adverse event (SAE) rate.

57.1.7.5 Nivolumab

CheckMate 238 [19] is a phase 3, double-blind study, which enrolled completely resected stage IIIB, IIIC, and IV patients to receive nivolumab 3 mg/kg every 2 weeks for 1 year or ipilimumab 10 mg/kg every 3 weeks for four cycles. This trial compared an anti-PD-1 to an anti-CTLA4 (which efficacy was already demonstrated versus placebo).

RFS at 24 months was 63% and 50%, respectively, in the nivolumab and the ipilimumab arm, with a more favorable toxicity profile in the nivolumab group.

57.1.7.6 Pembrolizumab

Pembrolizumab is another anti-PD-1 agent, approved in the advanced disease, that was also experimented in the adjuvant setting KEYNOTE-054 [20] clinical study.

Patients were randomized to receive pembrolizumab 200 every 3 weeks or placebo up to 1 year or placebo. RFS and toxicity profile were as manageable and tolerable as shown in the metastatic setting and substantially the same of nivolumab.

57.1.7.7 Dabrafenib Plus Trametinib

As for the immune checkpoint inhibitors, BRAF and MEK inhibitors have been tested as well in the adjuvant setting, both in monotherapy and in combination.

In particular, the COMBI-AD [21] study, presented in 2017, investigated the efficacy of BRAF and MEK inhibitors in combination. Patients with completely resected, high risk stage III (lymph node metastasis > 1 mm), BRAF V600-mutated cutaneous melanoma were enrolled and randomized in two arms: placebo or dabrafenib 150 mg bid plus trametinib 2 mg qd, for

maximum 1 year. RFS showed a highly significant superiority of the experimental arm. After 36 months of follow-up, DMFS was 71% in the treatment group versus 57% in the placebo arm.

57.1.8 Treatment of Advanced Stage Disease

1. *Targeted Therapy*

Molecular Basis of Targeted Therapy

The MAPK (RAS-RAF-MEK-ERK) signaling pathway is a critical regulator of cellular growth, tissue invasion, and survival.

Nearly half of patients with metastatic melanoma harbor a BRAF V600-mutation [23] (most commonly V600E or V600K). The analysis of anatomic subtypes showed that BRAF mutations are common in cutaneous melanoma (43%) and less commonly observed in patients whose tumors arise from sun-damaged skin (e.g., lentigo maligna melanoma), from mucosal (6%) and uveal (<2%) sites. Among cutaneous melanomas, BRAF mutations are more frequently found in superficial spreading melanomas (53%) and less frequently present in acral melanoma (18%) and lentigo maligna melanoma (9%).

This somatic mutation activates the MAPK signaling pathway. In particular, mutated BRAF signals as a monomer, becoming independent of upstream growth stimuli and constitutively activated as well as insensitive to negative feedback signals.

BRAF V600-mutation has been implicated in different mechanisms of melanoma progression [23]:

- Activation of the downstream MEK/ERK signaling pathway and unchecked tumor replication
- Evasion from apoptosis and cellular senescence mechanisms
- Increased MEK-dependent angiogenesis
- Evasion from the immunologic surveillance
- Metastatic potential through upregulation of proteins involved in cellular migration.

The BRAF inhibitors vemurafenib and dabrafenib were both approved as single agents by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) in 2011 and 2013, for the treatment of unresectable or metastatic BRAF V600-mutated melanoma. These BRAF inhibitors are able to reduce MAPK pathway activation and prevent melanoma cell growth.

However, chronic BRAF inhibition can lead to acquired resistance through various mechanisms, such as the following:

1. Reactivation of MAPK pathway signaling
2. Activation of PI3K-Akt-mTOR pathway signaling
3. NRAS mutation

In addition, rapid responses are often short-lived, and recurrent tumors are often more aggressive.

These issues led to preclinical studies and clinical investigations of combination therapy with MEK inhibitors. In Table 57.4, those clinical studies are summarized.

In 2014, the randomized, phase 3 coBRIM [4] study evaluated the combination of the BRAF inhibitor vemurafenib and the MEK inhibitor cobimetinib versus vemurafenib single-agent first-line treatment, in patients with unresectable, BRAF V600-mutated, melanoma. The combination treatment showed a significant advantage (2016 update [24]) in terms of median progression-free survival (PFS), the primary endpoint of the study 12.3 months for cobimetinib and vemurafenib versus 7.2 months for placebo and vemurafenib (HR = 0.58, CI 0.46–0.72, $p < 0.0001$), and in terms of overall survival (HR = 0.70, CI 0.55–0.90), with a median OS of 22.3 and 17.4 months, respectively, in the combination and single-agent arms.

Table 57.4 A summary of the main clinical studies of targeted therapy in melanoma

Study	Treatment	ORR (%)	mPFS (months)	3-year OS (%)	Grade 3/4 AEs (%)
coBRIM [24]	Vemurafenib (960 mg; bid) + cobimetinib (60 mg; qd) vs. vemurafenib + placebo	68 45	12.3 7.3	38.5 31	62 52
COMBI-v [5]	Dabrafenib (150 mg; bid) + trametinib (2 mg; qd) vs. vemurafenib (960 mg; bid)	64 51	11.4 7.3	45 32	52 63
COMBI-d [25]	Dabrafenib (150 mg; bid) + trametinib (2 mg; qd) vs. dabrafenib (150 mg; bid) + placebo	67 51	9.3 8.8	44 32	48 50
COLUMBUS [26, 27]	Encorafenib + binimetinib (450 mg + 45 mg; qd) vs. vemurafenib (960 mg; qd) or encorafenib (300 mg; qd)	64 41 52	14.9 7.3 9.6	47 – –	58 63 66

In the same year, the randomized, open-label, phase 3, COMBI-v [5] trial showed the efficacy of the combination of dabrafenib and trametinib, compared with vemurafenib alone, in patients with previously untreated unresectable melanoma with BRAF V600E or V600K mutations. The combination significantly improved the OS (HR = 0.69, CI 0.53–0.89, p = 0.005) and the PFS (HR = 0.56, CI 0.46–0.69, p<0.001).

COMBI-d [25] is another phase 3, randomized, double-blind, clinical trial showing the superiority of dabrafenib and trametinib versus dabrafenib single agent. The 2017 update showed significant advantage for the combination arm in terms of 3-year PFS (22% vs. 12%), HR = 0.71 (CI 0.57–0.88).

COLUMBUS [26, 27] is a two-part, randomized, open-label, phase 3, clinical trial investigating efficacy and safety of the combination of encorafenib, a BRAF inhibitor and binimetinib, a MEK inhibitor, in unresectable stage III or IV, BRAF V600 mutated melanoma.

In Part 1, encorafenib (450 mg) plus binimetinib combination was compared to encorafenib (300 mg) alone and vemurafenib alone. The primary endpoint was PFS of encorafenib plus binimetinib versus vemurafenib. The combination arm showed median PFS of 14.9 months versus 7.3 of the vemurafenib single-agent arm (HR = 0.54. CI 0.41–0.71, p < 0.0001). In addition, the combination arm showed an advantage in terms of median OS: 33.6 months versus 16.9 months (HR = 0.61, 0.47–0.79). The 3-year OS was 47% in the encorafenib + binimetinib arm.

In Part 2, encorafenib (300 mg) plus binimetinib was compared to encorafenib (300 mg) alone. The combination treatment showed superiority in terms of PFS (HR = 0.77. CI 0.61–0.97, p = 0.029).

Note that in the COLUMBUS trial, a lesser number of patients had baseline LDH > ULN.

Targeted therapy is well tolerated as confirmed by all clinical trials. Some toxicities, such as skin toxicity, arthralgia, fatigue, and gastrointestinal adverse events, are common to all BRAF and MEK inhibitors.

In particular, photosensitivity and diarrhea are more common with vemurafenib plus cobimetinib, while fever and chills are more common with dabrafenib plus trametinib.

Type and severity of these toxicities vary considerably and may influence the choice of drug.

C-KIT

Mutations in the KIT proto-oncogene – arising on mucosal, acral, and chronically sun-damaged (CSD) skin melanomas – could be responsive to the tyrosine kinase inhibitor imatinib mesylate [28]. In fact, three phase 2 studies demonstrated a clinical efficacy of imatinib targeting KIT, with a median time to progression (TTP) of 4 months in the KIT-mutated (exons 9, 11, and 13) group of patients.

2. *Immunotherapy*

Melanoma has always been described as an immunogenic tumor. In fact, before the immune checkpoint inhibitors (ICIs), many immunomodulatory drugs have been tested, such as high-dose interleukin-2 (IL-2) [29].

ICIs showed survival benefits with several randomized clinical trials, leading to the approval by the FDA (Food and Drug Administration) and the EMA (European Medicines Agency) of the anti-CTLA-4 (cytotoxic T-lymphocyte antigen 4) antibody ipilimumab and the anti-PD-1 (programmed cell death protein 1) antibodies nivolumab and pembrolizumab. Table 57.5 shows the main clinical studies on immunotherapy in melanoma.

Table 57.5 A summary of the main clinical studies of immunotherapy in melanoma

Study	Treatment	ORR (%)	mPFS (months)	3-year OS (%)	Grade 3/4 AEs (%)
CA184-024 [3]	Ipilimumab (10 mg/kg; q3w) + dacarbazine (DTIC) vs. DTIC (850 mg/m^2; q3w)	15.2 10.3	3 3	20.8 12.2	56.3 27.5
KEYNOTE-002 [35]	Pembrolizumab (2 mg/kg or 10 mg/kg; q3w) vs. ChT	21–25 4	2.9 2.7	– 	11–14 26
KEYNOTE-006 [32]	Pembrolizumab (10 mg/kg; q2w or q3w) vs. ipilimumab (3 mg/kg; q3w)	36–37 13	5.6–4.1 2.8	48.1 37.8	17 20
CheckMate 037 [30]	Nivolumab (3 mg/kg; q2w) vs. ChT	31.7 10	3.1 3.7	– 	14 34
CheckMate 066 [31]	Nivolumab (3 mg/kg; q2w) vs. DTIC (1000 mg/m^2; q3w)	40 14	5.1 2.2	51.2 21.6	11.7 17.6
CheckMate 067 [33]	Nivolumab + ipilimumab (1 mg/kg + 3 mg/kg; q3w × 4 → nivolumab 3 mg/kg; q2w) vs. nivolumab (3 mg/kg; q2w) or ipilimumab (3 mg/kg; q3w)	58 44 19	11.5 6.9 2.9	58 52 34	59 21 28

Ipilimumab showed in two main phase 3 clinical trials (CA 184-002 [2], comparing ipilimumab 3 mg/kg vs. gp100 vaccine therapy in pre-treated patients and CA 184-024 [3], ipilimumab 10 mg/kg plus dacarbazine vs. dacarbazine alone, in treatment-naïve patients) an improvement in terms of long-term benefit, with a 3-year OS of 21% and with a plateau on the OS curve, representing a long-term responders subgroup.

However, the CTLA-4 blockage is associated with an increased risk of developing autoimmune disorders, known as immune-related adverse events (irAEs). In fact, 10–26% presented grade ≥3 irAEs, principally enterocolitis, hepatitis, dermatitis, and endocrinopathies.

Currently, ipilimumab as single agent has an uncertain role in the treatment of melanoma due to the superiority, in terms of both clinical activity and safety, showed in clinical trials by the anti-PD-1 agents.

In particular, the phase 3 CheckMate 037 [30] trial compared the ORR of nivolumab 3 mg/kg every 2 weeks with investigator's choice chemotherapy in pre-treated patients who progressed on BRAF inhibitors or ipilimumab. The ORR was higher in the nivolumab arm: 31.7% versus 10.6%. The CheckMate 066 [31] showed its superiority in the first-line setting as well. Compared with dacarbazine in BRAF wild-type melanoma patients, nivolumab obtained a 3-year OS of 51% versus 22%, with an ORR of 40% versus 14%.

KEYNOTE-006 [32] is a phase 3 study designed to compare pembrolizumab 10 mg/kg (every 2 weeks or every 3 weeks) with ipilimumab 3 mg/kg every 3 weeks. Results showed a median PFS of 5.6 and 4.1 months for each schedule of pembrolizumab versus 2.8 months in the ipilimumab arm, with an ORR of 36% and 37% versus 13%, also showing an advantage in the pembrolizumab arms; 4-year OS was 42% in the pooled pembrolizumab arms and 34% in the ipilimumab arm.

As in the case of ipilimumab, the anti-PD-1 treated patients show a prolonged clinical response, as seen mainly in the KEYNOTE-006 study. This potential is leading to take in consideration the question of how long to continue treatment in these patients.

In addition, pembrolizumab and nivolumab showed a more favorable safety profile as well when compared with ipilimumab. In fact, the observed incidence of grade ≥3 irAEs with an anti-PD-1 agent was about 10–16% versus 19–27% with ipilimumab. The most common anti-PD-1 adverse events are fatigue, rash and pruritus, diarrhea, and endocrinopathies.

Combination of Immune Checkpoint Inhibitors

In order to improve the ORR, several clinical trials were designed to investigate the combination of different ICI. In particular, the CheckMate 067 [33] is a phase 3 clinical trial that randomly assigned, in a 1:1:1 ratio, treatment-naïve patients to receive nivolumab 1 mg/kg plus ipilimumab 3 mg/kg every 3 weeks for four cycles, followed by nivolumab 3 mg/kg every 2 weeks; nivolumab 3 mg/kg every 2 weeks plus placebo; or ipilimumab 3 mg/kg every 3 weeks for four cycles plus placebo.

ORR and PFS were significantly higher for the combination arm: the observed ORR were 58% versus 44% and 19% in the nivolumab and ipilimumab arms, respectively; with a median PFS of 11.5 months in the combination arm versus 7 and 3 months for nivolumab and ipilimumab alone, respectively. In addition, a recent 3-year OS update showed that 58% of patients who received nivolumab plus ipilimumab were alive at 3 years, versus 52% of the nivolumab single-agent group and 34% of the ipilimumab group.

However, these encouraging results were obtained with a high toxicity profile: grade 3/4 adverse events have been observed in 58% of patients in the combination arm.

Despite the poor safety profile, the combination regimen of ipilimumab plus nivolumab was approved by the regulatory authorities.

In order to decrease the incidence of grade ≥3 irAEs, a combination of pembrolizumab and reduced-dose ipilimumab (1 mg/kg) was studied in the phase 1 study KEYNOTE-029 [34], with an incidence of grade 3–4 AEs of 42%, with no difference in term of ORR (57%).

3. *Radiation Therapy*

Melanoma has a variable spectrum of radiosensitivity; therefore, it should be considered as a radioresistant neoplasm and requires high doses of radiation to show efficacy [37].

Its application is mainly in the treatment of encephalic lesions through stereotaxis or whole brain radiation [38, 39], and of bone lesions through an 8 Gy single fraction radiation therapy [40, 41]. However, potentially any metastatic localization can be irradiated in a palliative setting or to increase local disease control (Fig. 57.2).

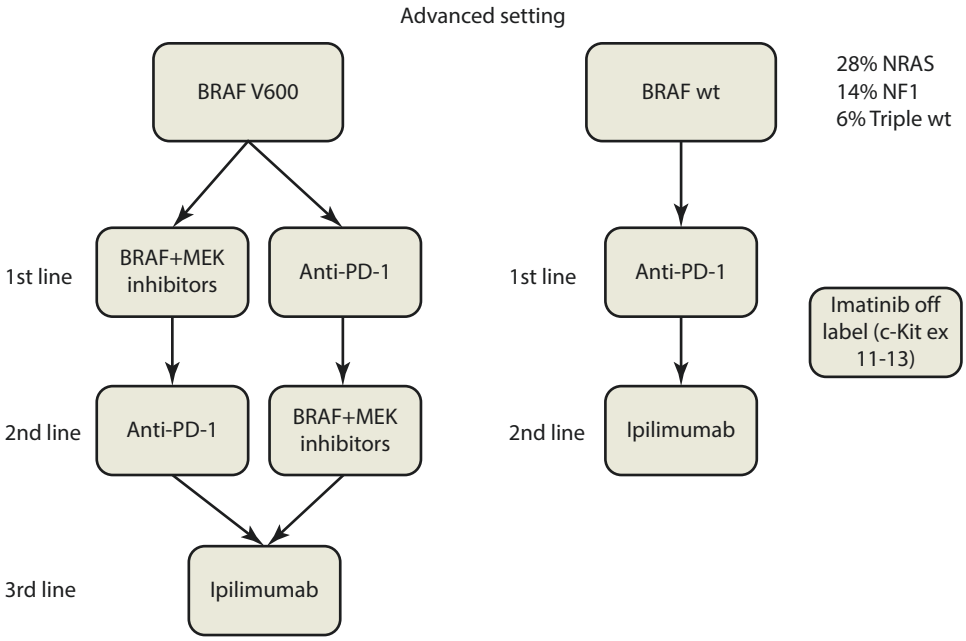

Fig. 57.2 A therapeutic algorithm for advanced stage disease setting melanoma

Case Study

57

A 43-year-old man comes to your attention after having surgically removed a suspect pigmented skin lesion of the back with the following histological report:

Histological report 1

Sample of skin of 23 mm × 9 mm
Superficial spreading melanoma
Breslow thickness: 1.2 mm
Ulceration: present
Mitoses/mm^2: 2

Question 1

What action should be taken?

(a) Start an adjuvant treatment with nivolumab + ipilimumab
(b) Wide excision with 10-mm margins
(c) Wide excision with 5-mm margins
(d) Wide excision with 10-mm margins + sentinel lymph node biopsy

Answer

Wide excision with 10-mm margins + sentinel lymph node biopsy.

The patient also performs a CT that shows no evidence of distant metastases. He shows you the report of the wide excision and sentinel lymph node biopsy:

Histological report 2

Margins: free
Left axillary sentinel lymph node: presence of 1 micrometastasis.
Molecular biology
Primitive melanoma BRAF V600E positive

Question 2

What is the TNM stage?

(a) pT1b pN0 M0
(b) pT2b pN1a M0
(c) pT2a pN1a M0
(d) pT1a pN1a M0

Answer 2

pT2b pN1a M0

Question 3

What action should be taken?

(a) Start a 1-year adjuvant treatment with vemurafenib.
(b) Start a 2-year adjuvant treatment with pembrolizumab.
(c) Start a 1-year adjuvant treatment with dabrafenib + tramentinib.
(d) Perform a left axillary complete lymph node dissection and start an 18-months adjuvant treatment with interferon.

Answer 3

Start a 1-year adjuvant treatment with dabrafenib + tramentinib.

After 1 year, the patient has completed the adjuvant therapy without any particular adverse event, with the exception of some febrile peaks. At the follow-up CT, there is no evidence of distant metastasis.

Expert Opinion

Paola Queirolo

Key Points

1. Cutaneous melanoma represents less than 5% of all skin cancers but it is regarded as the deadliest. The main risk factors are fair skin, male sex, age, sunburns in early life, total number of melanocytic nevi, family history of melanoma, and immunosuppression. Mutations in CDKN2A and MITF genes are associated to the predisposition of melanoma's onset, together with other conditions such as xeroderma pigmentosum and dysplastic nevus syndrome. Other important mutations regard BRAF and c-KIT genes.
2. Melanoma usually appears as an asymmetric nodule with border irregularity, variegated and uneven with streaks of black, red-brown, gray or blue coloration, more than 6 mm in diameter and with a rapidly progressive behavior (ABCDE). Local pain or bleeding can be present.
3. Excisional biopsy (with a narrow margin of about 1 mm), is the golden standard; in case of big nodules or particular sites, an incisional biopsy can be done. In order to understand the main features and risk of melanoma evolution, the Breslow's thickness (BT) must be evaluated. There are different histologic subtypes: superficial spreading, nodular (linked to the worst prognosis), acral, lentigo maligna, and desmoplastic melanoma.
4. In case of early-stage melanoma, wide local excision is the definitive approach, according to the BT: 5 mm in melanoma in situ, 10 mm (when BT is <2 mm), and 20 mm (when >2 mm). A sentinel lymph node is indicated when BT is >0.8 mm.
5. Adjuvant therapy consists in interferon (stage IIB/C for ulcerated primary lesions), BRAF and MEK inhibitors (stage III and mutation in BRAF V600), nivolumab, pembrolizumab (stage III and BRAF wild type and stage IV). Nivolumab and pemrbolizumab are anti-PD-1 antibodies (immune checkpoint inhibitors) which have shown good responses in this setting of patients, alone or in combination. Vemurafenib and dafrafenib are a group of tyrosine kinase inhibitors, which can be used in case of BRAF mutations for unresectable or metastatic melanoma. MEK inhibitors such as trametinib and cobimetinib are also used in this setting of patients. Some trials have shown better responses in case of combination treatments, that is dabrafenib + trametinib or encorafenib + binimetinib. C-KIT inhibitors can be otherwise used in case of KIT mutations.

Suggested Reading

Haanen JBaG. et al. Management of toxicities from immunotherapy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol Off J Eur Soc Med Oncol. 2017;28, iv119–iv142.

Immunotoxicity is a new field of Medicine, born with the increasingly use of immune checkpoint inhibitors in clinical practice in oncology. It is therefore important to know the main manifestations, the diagnostic and therapeutic approach, and the natural course of these conditions [36].

Recommendations

- AIOM
- ► www.aiom.it/wp-content/uploads/2020/10/2020_LG_AIOM_Melanoma.pdf
- ESMO
- ► www.esmo.org/Guidelines/Melanoma/Cutaneous-Melanoma

The Word to the Expert

The awareness about cutaneous melanoma and its risks has increased in the last years thanks to prevention campaigns among the population. Although more and more must be done above all among young people that tend to ignore the major risk factors linked to this neoplasm such as unprotected sun exposure. In case of suspected lesions, a dermatology expert should be consulted, in order to exclude the presence of cutaneous melanoma. The most interesting results concerning the therapeutic successes, regard the use of tyrosine kinase inhibitors (TKIs) and immunotherapy, which have modified the natural history of this cancer. Nowadays more attention is given to the possible relapses after the end of immunotherapy and in those patients who do not response to this category of drugs. In conclusion, it is essential that in case of advanced disease the patient is addressed to a reference center, which has a good experience in managing TKIs, immunotherapy, and its possible side effects.

Hints for a Deeper Insight

- Cutaneous melanoma: From pathogenesis to therapy (Review): ► https://www.ncbi.nlm.nih.gov/pubmed/29532857
- Advances in Immunotherapy for Melanoma: A Comprehensive Review: ► https://www.ncbi.nlm.nih.gov/pubmed/28848246
- Acral lentiginous melanoma: differences in survival compared to other subtypes: ► https://www.ncbi.nlm.nih.gov/pubmed/31628856
- Pericardial effusion under nivolumab: case-reports and review of the literature: ► https://www.ncbi.nlm.nih.gov/pubmed/31627742

57.2 Nonmelanoma Skin Cancer

Paola Queirolo Andrea Boutros, and Enrica Teresa Tanda

57.2.1 Introduction

Nonmelanoma skin cancer (NMSC) is a huge group of skin tumors that constitutes the most common form of human cancer, with an estimated incidence >3 million new cases each year in the US, with about 2000 estimated deaths each year [42, 43].

The main risk factor for NMSC is ultraviolet (UV A, UV B) rays exposure, and its incidence increases with age [44].

Many NMSC present as an erythematous lesion or a nodule. Definitive diagnosis can be obtained by shave, punch, or excisional biopsy.

57.2.2 Actinic Keratosis

AKs are common precancerous lesions that mainly occur in fair-skinned individuals as a result of cumulative sun exposure. AKs may potentially undergo spontaneous regression or progress into in situ or invasive SCC [45, 46].

Pathogenesis of AK includes UVB light exposure, DNA instability (e.g., xeroderma pigmentosum), albinism, age, and history of immunosuppression.

AKs present as a reddish-pink hyperkeratotic surface on a sun-exposed area (mainly head and neck, forearms).

There are many histologic subtypes: hyperplastic, atrophic, Bowenoid, acantholytic, and pigmented, with common features, such as atypical keratinocytes and nuclear atypia [47].

57.2.2.1 Treatment

Firstly, preventive measures consist of avoidance of excessive sun exposure and sun protection. AKs have low but actual SCC transformation potential; this is the reason why therapy is recommended. The approach can be lesion-directed or field-directed.

Lesion-directed therapy is suitable in case of few clinically visible AKs and consists mainly in cryotherapy. A multicenter study evaluated the efficacy of cryotherapy for AKs of the face and scalp, with an average response rate of 67% per patient [48].

Field-directed therapy offers an advantage [45] when a huge amount of clinically evident and subclinical lesions are present. In this case, treatment may also have a role in terms of prevention.

- *PDT*. AK is currently the only FDA-approved indication for PDT. When topically applicated, 5-aminolevulinic acid (ALA) and methylaminolevulinic acid (MAL) accumulate in malignant and premalignant cells and are metabolized to protoporphyrin IX, a photoactive agent that promotes the generation of free radicals when irradiated with an appropriate light source, leading to irreversible cell damage, and precancerous cell death [49, 50].
- *Cryotherapy*. This technique exposes a precancerous lesion to temperatures reaching −60 °C, provoking tissue damage, and a thermal shock leading to premalignant cell death. Cryotherapy does not allow a precise margin control [48].
- *5-FU*. A systematic review of randomized clinical trials showed that 5-FU 0.5% resulted in an average reduction of lesions of 86%, with a higher compliance profile from lower side effect rate [51].
- *Imiquimod*. The FDA-approved protocol is twice weekly for 16 weeks. When compared to cryotherapy and 5-FU [52], imiquimod showed similar efficacy but higher sustained response at 1 year [45].

Another possible option is diclofenac 3% gel, with similar outcomes [53].

57.2.3 Basal Cell Carcinoma

BCC is the most common human cancer, accounting for 25% of all cancers in the US. It typically develops from sun-exposed areas, but it has been reported in non-exposed regions too [54]. BCC is a slow-growing tumor originating from the basal layer of epidermis, with a poor metastasizing potential, but a locally invasive behavior. Extensive sun exposure, age, and immunosuppression are the most important risk factors [44].

57.2.3.1 Clinical Presentation

Many hereditary syndromes can manifest through multiple BCCs. Among these, the nevoid BCC syndrome (NBCC, also known as Gorlin syndrome, autosomal dominant mutation of PTCH1 gene, with broad nasal root, odontogenic keratocysts, palmo-plantar pits, calcification of the falx cerebri, medulloblastomas, multiple skeletal abnormalities, and multiple BCCs) [55–57], Bazex syndrome (X-linked, follicular atrophoderma, hypotrichosis, hypohidrosis, milia, epidermoid cysts, facial BCCs), Rombo syndrome (similar to Bazex), xeroderma pigmentosum (autosomal recessive, DNA repair defect, multiple NMSC, and melanomas), Rasmussen, Darier, and albinism.

BCC is clinically variable and includes many subtypes: superficial, infiltrative, nodular (the most common subtype, accounting approximately 50% of all variants), morpheaform, pigmented, and fibroepithelioma of Pinkus [58, 59].

Superficial BCC is a well-defined erythematous-pink macule. It is difficult to differentiate from AK. Nodular BCC is a pearly papule with telangiectasias. History of central ulceration or easy bleeding is not rare. *Morpheaform* (also known as sclerosing) BCC presents as an indurated, firm, not well-demarcable, scar-like lesion. The actual extension of the tumor is often greater than its clinical appearance. Fibroepithelioma of Pinkus is a rare variant of BCC, presenting as a pink dome-shaped or pedunculated papule or nodule [60]. It may be difficult to distinguish from amelanotic melanoma.

57.2.3.2 Treatment

Radical surgical excision – when applicable – is the treatment of choice for BCC [61]. In fact, surgery gives advantage in terms of histologic evaluation of the excised specimen. In case of anatomic sites requiring maximal tissue conservation (i.e., face), Mohs micrographic surgery (MMS) allows an optimal margin control.

Imiquimod, 5-FU, and PDT are FDA-approved local treatments for superficial BCC. A clinical study randomized 601 patients with superficial BCC to receive MAL PDT, imiquimod, or 5-FU. Complete clinical response at 1 year was 73% in the PDT arm, 83% in the imiquimod arm, and 80% for 5-FU. However, imiquimod and 5-FU showed higher rates of local side effects [62].

Radiation therapy has a role when surgery is contraindicated (such as relapse or extensive, unresectable BCC).

Systemic therapy is indicated in case of unresectable or metastatic BCC, when both radiation therapy and surgery are not anymore suitable. The only available systemic therapy in the past for metastatic disease was chemotherapy, but no randomized clinical trial has been conducted.

A clinical study showed the efficacy of systemic retinoids, but with unacceptable toxicity rates.

Currently, new treatment options targeting the "Hedgehog" pathway are available: vismodegib is a smoothened (SMO) inhibitor (approved in 2012 by the FDA) showing, in a clinical trial, an objective response rate of 67% in locally advanced disease patients and of 38% in metastatic disease patients [63]. Most common adverse events are muscle spasms, alopecia, and dysgeusia (leading to weight loss and malnutrition) [64–67].

Case Study

A 53-year-old woman comes to your attention presenting the lesion shown in Fig. 57.3. She tells you that the lesion evolved very slowly over a decade.

Question 1

What action should be taken?

(a) Try to perform a radical surgery
(b) Start a radiation therapy
(c) Obtain an incisional biopsy
(d) Put on antibiotics for 14 days

Answer 1

Obtain an incisional biopsy.

The patient returns with the following histological report:

Histological report

Infiltrative basal cell carcinoma
Infiltrating the fascia
Perineural invasion: present
Lymphovascular invasion: present

Question 2

What action should be taken?

(a) Try to perform a radical surgery
(b) Start a radiation therapy
(c) Start a systemic treatment with vismodegib
(d) Mohs micrographic surgery

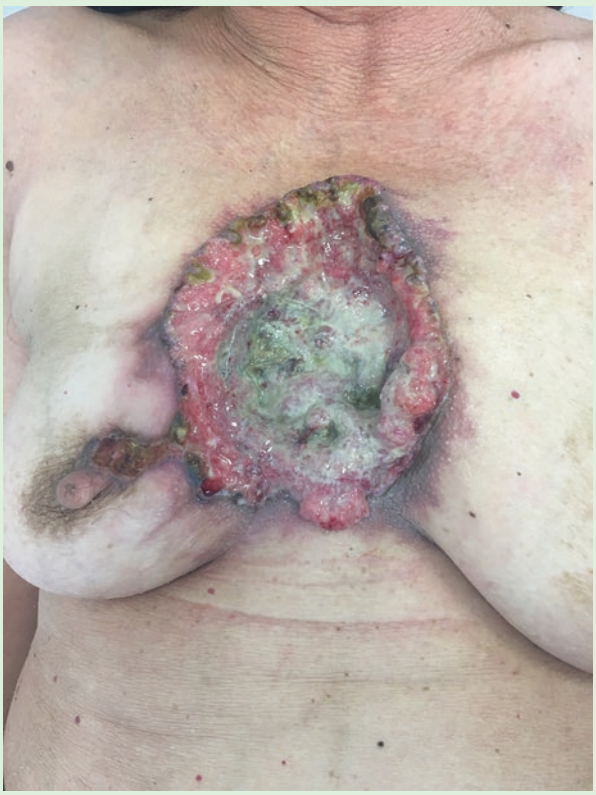

Fig. 57.3 Huge carcinomatous lesion of the chest in a 53-year-old woman

Answer 2

Start a systemic treatment with vismodegib.

The patient returns for an 8-week check. She has always taken her medications without adverse events. The lesion is shown in Fig. 57.4.

Question 3

What action should be taken?

(a) Try to perform a radical surgery
(b) Start an adjuvant radiation therapy
(c) Continue with the current treatment with vismodegib
(d) Switch to chemotherapy

Answer 3

Continue with the current treatment with vismodegib

The patient returns again after other 8 weeks of treatment. She continued her treatment with no adverse events. The lesion is shown in Fig. 57.5.

Patient's locally advanced basal cell carcinoma is responding successfully to treatment. You decide to continue until disease progression or unacceptable toxicity.

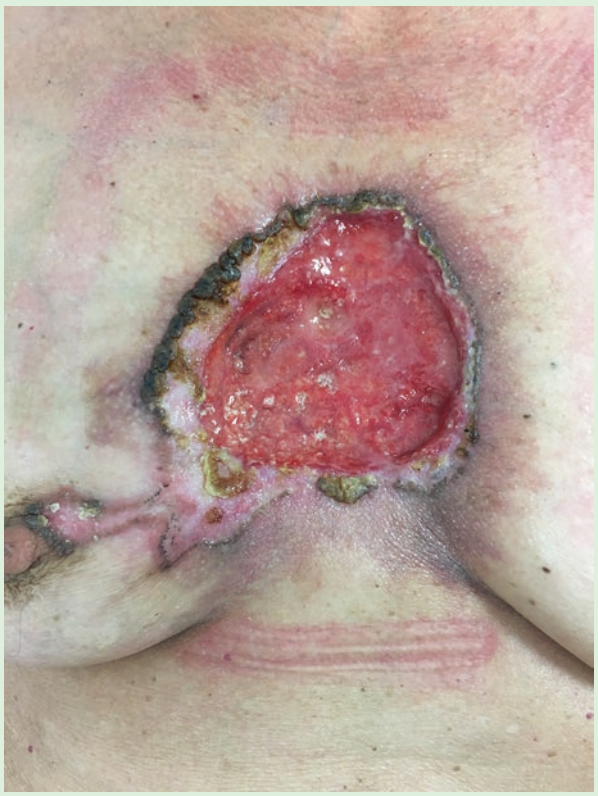

Fig. 57.4 Partial response after 8 weeks of treatment with vismodegib

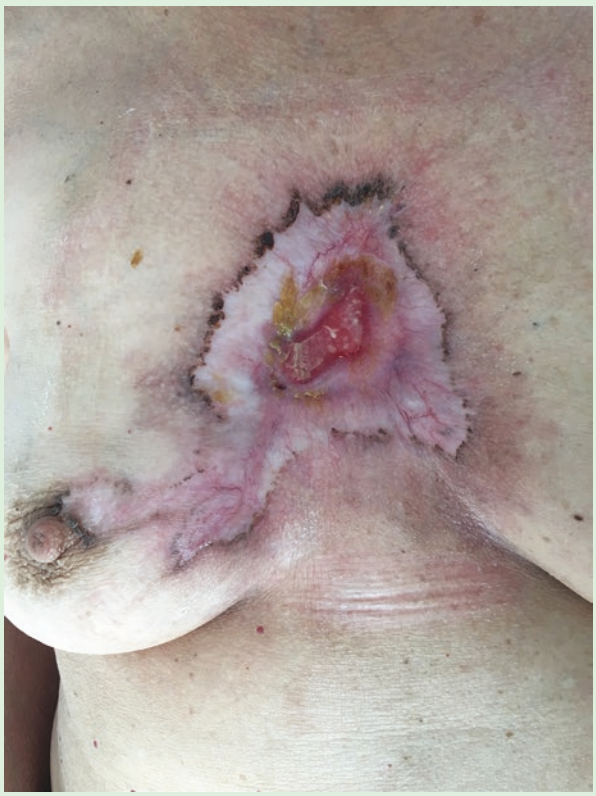

Fig. 57.5 Further response after 16 weeks of treatment with vismodegib

Sonidegib is another FDA-approved oral SMO antagonist that showed efficacy in the multicenter phase 2 BOLT trial for locally advanced or metastatic BCC patients who are not suitable for surgery or RT, or for recurrent locally advanced BCC following surgery or RT. The primary endpoint was an objective tumor response rate (ORR) point estimate of ≥30%. Sonidegib showed an ORR of 56% [68], with an acceptable risk profile and comparable adverse events to that observed in vismodegib [69].

57.2.4 Squamous Cell Carcinoma

Cutaneous SCC (cSCC) is one of the most common NMSCs [70], originating from epidermal keratinizing cells [43].

In the USA, 2.1 million new cases have been registered in 2017, making it the second most common human cancer after BCC. Anyway, it is difficult to estimate the exact incidence and mortality due to the absence of cSCC in the US tumor registers. European data show that inci-

dence of this tumor ranges from 9 to 96 per 100.000 ab. in male, and 5 to 68 per 100.000 in female. In Australia [71], the incidence in 2002 was 499 per 100.000 in male and 291 per 100.000 in female. In 2011, the cSCC mortality incidence in Australia was 2 per 100.000. Higher data coming from a Danish [72] study showed that disease-specific mortality was 2–4% in 1984.

Principal risk factors are cumulative sun exposure over life (especially in fair-skinned subjects), age, immunosuppression, HPV chronic infection, and male sex (3:1 ratio). However, among black subjects, cSCC arises more often on sites of preexisting inflammatory conditions, with a high mortality rate, because of delayed diagnosis [73]. Other risk factors are chemical (petroleum, coal, arsenic) and physical (ionizing radiations) agents and smoking.

57.2.4.1 Histology

CSCC originates from epidermis (or follicles) as atypical single cells or cellular nests [74]. In situ cSCC is an intraepidermal carcinoma (isolated to the epidermis) that seldom progresses to an invasive disease. Dermal invasion differentiates invasive SCC from in situ SCC. Invasive SCC is characterized by large cellular size, nuclear hyperchromatism, and the presence of mitotic figures. In well-differentiated SCC, the presence of keratin pearls is a sign of cytoplasmic keratinization.

Generally, the prognosis of cSCC is excellent, but it has been estimated that almost 3% of patients develop a metastatic disease. In 2012, a study suggested that from 5.604 to 12.572 people with cSCC developed nodal metastasis in the USA [70]. In particular, tumors arising from chronically inflamed skin, at mucocutaneous junction, presenting perineural invasion, diameter >40 mm, depth >4 mm, or a locally recurrent lesion, have a high risk (10–30%) of progression to metastatic disease.

Many distinct histologic subtypes of cSCC already exist; most of them are well-known, but their malignant potential is still not adequately well-recognized.

Previous classifications were not based on malignant potential. Ackerman defined cSCCs as "one entity with many faces" in 1978, when he described cSCC arising from actinic keratoses and carcinoma in situ, as well as keratotic, pseudoglandular (adenoid), pale-cell (clear cell), necrotizing, verrucous, spindle cell, and keratoacanthoma (KA)-like variants of SCC [75]. This histopathologic classification was not based on the malignant potential.

Cassarino divided in 2006 invasive cSCC into low (<3%), intermediate (3–10%), and high risk (>10%), based on risk rated of recurrence and metastasis [76, 77].

57.2.4.2 Low-Risk Invasive cSCC

Most of cSCC have an indolent behavior. The majority arise from AKs, sun-damaged skin of elderly people. Low-grade variants include the following:

- *Arising on AK*. About 95% of invasive cSCC arise from AKs, but the estimated rate of AKs leading to invasive cSCC is 0.2–10% per year. This variety of cSCC is often superficially invasive and well differentiated, with a low risk of metastasis, and it could be considered cured by surgical excision.
- *Verrucous carcinoma and other HPV-related cSCC*. A group of cSCC variants including verrucous carcinoma, oral (florid papillomatosis), anogenital (giant condyloma of Buschke-Lowenstein), plantar (epithelioma cuniculatum), epidermodysplasia verruciformis (genetic autosomal recessive disorder), sporadic, and HIV-related forms. It is a low-grade group of tumors, with an eso-/endophytic growth, associated to chronic HPV-6 and HPV-11 (epithelioma cuniculatum) and HPV-16 and HPV-18 (oral florid papillomatosis and giant condyloma of Buschke-Lowenstein) infection. Radiation therapy is not recommended, as it has been reported to lead the tumor to dedifferentiation and higher-grade SCC.
- *Spindle cell/sarcomatoid SCC (nonradiation associated)*. Uncommon variant, usually arising on sun-damaged skin, head, and neck. Cases related to radiation or arising on scars have a more aggressive behavior, and these will be discussed under the section on radiation-induced SCC. This tumor is usually composed of poorly differentiated dermal spindle cells.
- *Trichilemmal carcinoma (TLC)*. Rare subtype of cSCC arising upon sun-damaged skin of elderly people. Excellent prognosis.
- *Keratoacanthoma (KA)*. KA is a low malignant potential variant of cSCC characterized by its rapid growth and – often – a clinical spontaneous regression. KA presents clinically as a dome-shaped papule with a crateriform architecture. It should be treated as a variant of cSCC because it is impossible to predict which lesions regress and which progress.

57.2.4.3 Intermediate Risk Invasive cSCC

The following are less common group of tumors with a controversial prognosis:

- *Acantholytic/adenoid*. Arising on sun-exposed skin, mainly of elderly males. Its malignant potential ranges between 3% and 19% of distant metastasis rate.
- *Lymphoepithelioma-like carcinoma of the skin (LELCS)*. Rare tumor arising on sun-exposed skin in elderly people, not EBV-related as the nasopha-

ryngeal LELC. Its malignant potential is still not totally known.

- *Intraepidermal epithelioma (IEE)/Borst-Jadassohn tumor with invasion*. This tumor has been described as "the most controversial entity in dermatopathology." However, its malignant potential, ranging 6–10% of distant metastasis, should not be underestimated.

57.2.4.4 High-Risk Invasive cSCC

Many of these skin cancers are rare tumors with few large studies able to determine their real malignant potential.

- *Invasive Bowen's disease*. Rapidly growing ulcerated tumor occurring in a scaly or erythematous patch. About 5% of Bowen's disease may become invasive and 13–20% of those develop distant metastasis.
- *Desmoplastic*. Aggressive variant arising principally on sun-damaged skin of elderly males, characterized by high rates of recurrence and distant metastasis (22–77%). Histologically composed by cords of spindled cells in a desmoplastic stroma, with frequent perineural invasion.
- *Malignant proliferating pilar tumor (PPT)/cyst*. Rare tumor arising on the scalp of elderly men, presenting as a cystic mass that may be ulcerate. PTTs are benign tumors but with a high recurrence potential, while malignant PTT/SCC arising in PTT is highly aggressive and metastatic in about 30%.
- *De novo SCC*. Uncommon variant arising on both sun-exposed and non-exposed skin, presenting as an erythematous nodule or induration with crusting or ulceration. 8–15% rate of local or distant metastasis.
- *Adenosquamous cell carcinoma*. Highly aggressive, rare tumor arising in the head and neck or genitalia of elderly patients, characterized by frequent recurrences and distant metastasis (up to 50%) associated with high tumor-related death. Histologically characterized by mucin-producing cuboid-columnar cells.
- *Arising in association with radiation, burn scars, chronic conditions, and immunosuppression*. Generally, cSCC arises more often in chronically injured skin affected by chronic inflammatory disorders, or immunosuppressed skin, including ulcers, burns, organ-transplanted patients, discoid lupus, lichen sclerosus, lichen planus, dystrophic epidermolysis bullosa, and lupus vulgaris. These tumors bear an aggressive behavior with high rates of invasion, recurrence, and metastatic potential.
  1. Burn scar SCC (Marjolin's ulcer). Arising on a scar with a latency period ranging from 4 months to 35 years. Characterized by a high metastatic rate (35–50%)
  2. SCC arising in discoid lupus. Common among African-Americans. High metastatic rate (30%)
  3. Radiation-induced. Radiations lead to a 3 times increased risk. Any histologic subtype is possible, but spindle cell is the most common. Tumors are aggressive and frequently metastatic
  4. Immunosuppression-related. Related to degree and duration of immunosuppression

Based on these data, the National Comprehensive Cancer Network (NCCN) [78] identified key risk factors for recurrence (summarized in Table 57.6 [79]).

Some sites are considered high-risk factors independently of size, such as area H, where optimal tumor clearance is not always possible.

These risk criteria have been revised by Schmults in a multivariate analysis of 256 high-risk cSCC, in another four risk factors:

1. Tumor diameter 2 cm or greater
2. Depth of invasion beyond subcutaneous fat
3. Poor differentiation
4. Perineural invasion [80]

In conclusion, cSCC includes distinct subtypes of varying malignant potential. This is the reason why it is recommended that the pathology report includes the following:

1. Histologic subtype
2. Degree of differentiation [81]. Poorly differentiated cSCC (Broder's grades III–IV) has a higher risk of developing distant metastasis than well and moderately differentiated (33% vs 9%).
3. Approximate depth of invasion [82]. The metastatic potential is related to depth of invasion: <2 mm (about 0% of metastasis rate), 2–6 mm (4.5% of metastasis rate), >6 mm (15% of metastasis rate).
4. Perineural invasion [81]. It is associated with about 50% of recurrence and metastasis rate.
5. Hematolymphatic invasion.

57.2.4.5 Treatment

There are four general approaches to treat cSCC:

- *Surgical excision.* Surgical excision using margins of 4 mm and 6 mm for low-risk and high-risk tumors, respectively, is the treatment of choice for cSCC. Traditional surgery showed lower recurrence rates than Mohs micrographic surgery [83].
- *Cautery or electrodesiccation.* This technique is suitable only in case of low-risk cSCC due to the impossibility to have a complete histopathology review of the tumor and the risk of residual foci of invasive tumor [84].
- *Radiation therapy.* RT is the treatment of choice in case of special sites (such as lip) or advanced SCC. In addition, advanced cutaneous carcinomas may be treated with surgery and adjuvant RT when

Table 57.6 A summary of the key risk factors of cSCC recurrence identified by the NCCN [79]

		Low risk	High risk
History and physical examination	Location/size	Area L (trunk and extremities) <20 mm Area M (cheeks, forehead, scalp, neck, pretibial) <10 mm	Area L ≥ 20 mm Area M ≥ 10 mm Area H (face, genitalia, hands, feet)
	Borders	Well-defined	Poorly-defined
	Primary vs. recurrent	Primary	Recurrent
	Immunosuppression	No	Yes
	Site of prior RT or chronic inflammatory process	No	Yes
	Rapidly growing tumor	No	Yes
	Neurologic symptoms	No	Yes
Pathology	Degree of differentiation	G1 – G2	G3
	Acantholytic (adenoid), adenosquamous (mucin production), desmoplastic, or metaplastic (carcinosarcomatous)	No	Yes
	Depth/level of invasion	≤6 mm	>6 mm
	Perineural, lymphatic, vascular invasion	No	Yes

the possibility of residual disease is high. Indications for postsurgical RT are as follows:
- Positive margins
- Perineural invasion
- Multiple recurrences
- Underlying tissue invasion

In case of regional lymph node involvement, treatment may include local RT, lymphadenectomy, or both.

Unresectable cSCC is treated with RT alone [85].

Electrochemotherapy (ECT) This recent therapeutic technique is used in primary and metastatic skin tumors. The procedure exploits high intensity electric pulses, applicated on the tumoral mass, in order to increase the permeability of cell membrane to a systemically infused chemotherapeutic agent. In particular, bleomycin and cisplatin local cytotoxicity is significantly augmented by the electroporation [86].

Medical treatment Treatment of metastatic disease may include chemotherapy, treatment with targeted therapy, or – from few months – immunotherapy.

In particular, *platinum*-based chemotherapy showed to have a radio-sensitizing effect in this setting, as showed in two retrospective studies [87, 88].

Cetuximab is a chimeric monoclonal antibody that inhibits the EGFR signaling pathway and used off-label for treatment of unresectable or metastatic cSCC. A phase 2 clinical study showed that cetuximab monotherapy obtained an overall response rate of 22% [89]. Other reports showed that, when combined with adjuvant RT, cetuximab reached 50% of complete responses [90].

Cemiplimab is an FDA-approved anti-PD-1 immunotherapeutic agent that has shown interesting results in two clinical trials in a group of patients with locally advanced disease without surgical indications or with metastatic disease. The overall responses were 50% and 48% in both cases, respectively. The toxicity profile was not different from that of immune-checkpoint inhibitors when used in monotherapy, in both trials.

Among 108 patients with advanced cSCC, including metastatic ($N = 75$) or locally advanced ($N = 33$) disease, the overall response rate (ORR) was 47% (95% CI: 38, 57). 61% of responses were 6 months durable or longer.

Observed severe adverse events were principally immune-related in both trials (pneumonitis, hepatitis, colitis, adrenal insufficiency, hypo- and hyperthyroidism, diabetes mellitus, and nephritis).

Among patients with advanced cSCC, cemiplimab induced a response in approximately half the patients and was associated with adverse events that usually occur with immune checkpoint inhibitors [91].

57.2.5 Merkel Cell Carcinoma

Merkel cell carcinoma (MCC) is a rare, primary, and highly aggressive neuroendocrine skin tumor, described for the first time by Toker in 1972 as a "trabecular carcinoma of the skin" [92]. Some cytological characteristics like the presence of neurosecretory granules led to

identify the Merkel cells, cutaneous mechanoreceptors, as the cells of origin of this tumor. For this reason, the name of this malignancy was redefined as MCC in the early 1980s.

57.2.5.1 Epidemiology and Risk Factors

The incidence of MCC is increasing more rapidly than other skin tumors, such as malignant melanoma, probably due to longevity, improved detection, and increased reporting. Indeed, the incidence rate passed from 0.22 to 0.79 cases per 100.000/year in the USA [93], from 0.13 to 0.35 in Europe, and reaching the highest rate in Australia [94]. In a recent study by Paulson and colleagues, the total number of cases reported annually showed a 95.2% increase (from 334 cases in 2000 to 652 cases in 2013) [95].

Several demographic factors are associated to this phenomenon. First of all, the incidence of MCC increases dramatically with age, by approximately 10-fold between 40–44 and 60–64 years and 10-fold again between 60–64 and 85 years. Indeed, data about the incidence rate (from 2011 to 2013) are consistent: 0.1 cases/100,000 person-years among subjects of 40–44 years and 9.8/100,000 person-years for people older than 85.

Incidence is higher among men, with a men/women ratio of 2–3:1, and this effect is most pronounced among the oldest age groups.

Most frequently, MCC presents with local disease, but regional lymph node and distant metastases may be present in up to 30% of new cases. In a minority of cases, MCC is diagnosed as a lymph node metastasis without an identifiable primary lesion, which may have spontaneously regressed or be occult [96]. MCC is highly aggressive, with a disease-related mortality rate of 46%, higher than the one seen among melanoma patients, but the stage at diagnosis strongly influences this parameter [97].

The main risk factor for the development of MCC includes infection with the Merkel cell polyomavirus (MCPyV), ultraviolet radiation exposure (UVB irradiation), and immunosuppression [98, 99].

Approximately 80% of MCCs are caused by a ubiquitous virus called Merkel cell polyomavirus (MCPyV) [100]. In these cases, carcinogenesis is caused by the clonal integration of the MCPyV into the host genome: we will talk more extensively about the role of MCPyV in the pathogenesis of MCC in the dedicated section.

UV exposure is a significant risk factor for MCC and may contribute by causing immunosuppression and mutagenesis [101]. Several observations support this data. First of all, there is a great difference in incidence between non-Hispanic white individuals and other ethnical groups, with a white–black ratio of 20:1. Secondly, MCC commonly arise on chronically sun-exposed skin and/or in individuals treated with UVA photochemotherapy. Moreover, usually MCC patients have a history of other skin cancers associated with sun exposure like melanoma or cutaneous SCC. Finally, a molecular UV signature (DNA mutations that are typically caused by UV damage) has been demonstrated in MCPyV-negative MCCs [102].

A separate category is represented by immunocompromised people, such as organ transplant recipients, HIV-infected subjects, people using immunosuppressant medications, and those with lymphoproliferative disorders or other malignancies. In this group, a younger age and higher mortality are observed. This emphasizes the crucial role of the efficient immune surveillance [103]. On the other hand, chronic inflammatory disorders such as rheumatoid arthritis are also associated with higher incidence of MCC [104].

57.2.5.2 Histopathology

Even though MCCs share some morphologic and histologic features with normal Merkel cells (MCs), emerging data suggest that MCs are not the cells of origin.

One of the most accredited hypotheses affirm that MCC could originate from an immature totipotential stem cell with neuroendocrine features acquired during malignant transformation [105]. Other fascinating hypothesis sees the pre-pro B cell or fibroblasts as the origin cell.

The pre-pro B-cell origin is based on the expression of some elements that are normally restricted to early B cells, like Paired Box 5 (PAX-5), Terminal deoxynucleotidyl Transferase (TdT), and immunoglobulins rearrangement, and are expressed in MCCs [106].Finally, the discovery that human dermal fibroblasts support productive MCPyV infection has generated the hypothesis that fibroblast could be the origin cell [107].

Even if no clinically significant differences have been described, we recognize three histologic form of MCC:

- Trabecular, rare, and less aggressive
- Intermediate, more common, and with a high number of mitotic figures
- Small cell MCC, indistinguishable from small cell carcinoma of other origin (e.g., lung)

The histopathologic differential includes basal cell carcinoma, melanoma, Ewing sarcoma, neuroblastoma, leukemia cutis, and poorly differentiated carcinoma (e.g., metastatic small cell lung cancer).

The definitive diagnosis of MCC is based on immunohistochemistry: MCC is positive for EMA, CK20 with a distinctive pattern, neurofilament, and neuroendocrine markers including synaptophysin and chromogranin.

Several histological parameters can be used as independent prognostic factors: first of all, the tumor thickness that reflects the deep invasion of MCCs is measured from the granular layer to the deepest extent

of the tumor. This data is associated with decreased survival, like higher mast cell counts, and vascular density in the tumor and surrounding stroma. Infiltrative tumor growth pattern ($p = 0.001$) and lymphovascular invasion ($p = 0.007$) are also features associated with more aggressive tumor behavior. Moreover, nodular growth pattern, shallow invasion, and the absence of lymphovascular invasion are associated with longer survival [108].

Also, immunohistochemical features such as p53 and p63 immunopositivity have been shown to negatively predict survival, with p63 expression showing the greatest prognostic value [109].

Finally, several small studies since 2010 have shown that MCV-positive MCC confers a better prognosis than its MCV-negative counterpart [110].

57.2.5.3 Clinical Presentation

Clinically, MCC usually presents as an asymptomatic erythematous or violaceous nodule.

The surface can be ulcerated or crusted (especially among "old" lesions, ◘ Fig. 57.3), lucid or opaque, and dome-shaped with multiple peripheric telangiectasia.

To better summarize the clinical presentation of MCC, in 2007 Heath et al. [101] analyzed 106 patients and identified some clinical characteristics which appeared more frequent. On the basis of this observation, it has been created an acronym (AEIOU) that resumes the most significative characters. As we said, MCC often presents as follows:

(A) Asymptomatic nodule with a rapid
(E) Evolution; more frequently affects
(I) Immunosuppressed patients
(O) Older people over 70 years old and strongly exposed to
(U) UV radiation

At presentation, 65% of patients have skin-limited disease, 26% have nodal involvement, and 8% have distant metastases [111]. The most common sites of metastases are regional nodal basins (inguinal, axillary, or head and neck nodes), distant skin, lung, bone, and brain.

Spontaneous regression of the primary has occasionally been seen on re-excision specimens with a dense lymphocytic infiltrate of T cells around the site of the prior biopsy [112]. Metastatic MCC with no known primary has also been reported and represents 4% of all MCC cases.

57.2.5.4 Staging

Once the diagnosis of MCC has been established on clinical and histopathologic grounds, appropriate staging should be performed.

First of all, sentinel lymph node biopsy (SLNB) is recommended for all MCC patients because approximately one-third of patients without clinical nodal involvement have microscopic involvement detected by SLNB [97].

The impact of SLNB on survival has been mixed in the literature: patients with a negative SLNB had about 85% 5-year MCC-specific survival rate compared with about 55% of patients with positive nodes [113].

57.2.5.5 Treatment

The rarity of MCC has made clinical studies of treatment difficult to perform. The absence of univocal information generated a lack of consensus around the most effective treatment algorithm.

The treatment depends on the stage of the disease, the tumor site, and any comorbid conditions.

57.2.5.6 Local Disease

Complete surgical excision of the primary site with 1–2 cm negative margins with sentinel lymph node biopsy is the first step in treating localized MCC [114]. The rate of local recurrence ranges from 25% to 40% [115–118]. Retrospective studies showed that also Mohs micrographic surgery could be an effective surgical option, even if prospective clinical trials comparing MMS to wide local excision have not been performed [117, 119–121].

Radiation monotherapy could be an alternative to surgery for patients who are poor surgical candidates or for those in whom surgery would result in significant functional compromise [122, 123]. However, the outcomes of radiation monotherapy may be inferior compared to complete surgical resection. Higher doses of radiation are typically recommended for radiation monotherapy as compared to doses used for adjuvant therapy. The NCCN guidelines recommend doses of 60 to 66 Gy for curative-intent radiation, with a wide treatment margin (5 cm) around the primary site [114].

In patients with *negative SLNBs* (stage II), if the primary tumor is less than 1 cm, widely excised with negative resection margins, and contains no high-risk features (lymphovascular invasion, location on the head and neck, immunosuppression), data suggest that no adjuvant therapy is needed [114]. On the other hand, patients with high-risk tumors should undergo 50 Gy to 66 Gy of adjuvant radiation to the primary site [122, 124]. Data from a retrospective analysis of 6908 cases from the National Cancer Database demonstrate that a combination between adjuvant radiation and surgery could reduce local recurrence and improve survival compared to surgery alone [125, 126].

Patients with *positive SLNBs* (stage III) should be discussed in a multidisciplinary tumor board because optimal management has not been established. Standard treatment options include CLND, definitive nodal radiation, or a combination of the two. Actually, most studies have not sufficient power to draw meaningful conclu-

sions. In this landscape, two independent studies found no difference in regional recurrence or overall survival between groups treated with CLND, definitive radiation, or combination therapy [127]. However, NCCN recommends adjuvant radiation to the draining nodal basin after CLND in the presence of multiple involved nodes or extracapsular extension of tumor [114].

57.2.5.7 Advanced Disease

Patients with distant metastatic disease should be referred expediently to a multidisciplinary tumor board. They may benefit from a combination of surgical excision for local debulking, radiation for palliation of symptoms or nodal disease, and/or systemic therapy, often through clinical trials.

Chemotherapy regimens were based on small cell lung cancer protocols, due to the similar neuroendocrine properties to MCC. The most common regimens are carboplatin (or cisplatin) and etoposide or a combination of cyclophosphamide, doxorubicin (or epirubicin), and vincristine. All these chemotherapic regimens are associated with considerable toxicity, especially in patients older than 65, and can worse immunosuppression.

MCC is chemosensitive, with initial response rates that range from 53% to 76%, but these responses tend not to be durable, with a usually short median progression-free survival (3–8 months) and progressive disease developing in 90% of patients at 10 months [128–130]. The few real-world, retrospective studies which assessed second-line or later chemotherapy showed low objective response rates (ORRs; from 8.8 to 23.0% with no complete responses) and very limited durability (1.3–3.3 months) [131].

57.2.5.8 Immune Checkpoint Inhibitors

Nowadays, considerable evidence suggests that immunosuppression contributes significantly to develop of MCC, and this consideration implies that therapeutic agents might be beneficial in this neoplasm.

Advancements in immunotherapy have greatly extended survival for patients with metastatic disease, particularly with the use of checkpoint immunotherapy involving the PD-1 (programmed death) and PD-L1 (programmed death-ligand) pathways. Both avelumab (MSB0010718C; anti-PD-L1) and pembrolizumab (anti-PD-1) have shown great results in clinical trials performed on patients with metastatic MCC. Results of these trials led to the addition of ICIs in the most recent update of the National Comprehensive Cancer Network (NCCN) Clinical Practice guidelines as a treatment option for stages III–IV MCC, and these agents are now the standard, first-line agents for metastatic MCC [114].

Avelumab, a monoclonal antibody that specifically inhibits PD-L1, is the first systemic immunotherapy for use in metastatic MCC. In the international multicenter phase II JAVELIN Merkel 200 trial, 88 patients with stage IV chemotherapy refractory MCC were treated in a single arm with avelumab 10 mg/kg every 2 weeks until confirmed disease progression or unacceptable toxicity [132]. After 1 year of follow-up, avelumab demonstrated an overall response rate (primary endpoint) of 33% with 11.4% of complete response rate and a disease control rate of 43.2%. 1-year progression-free survival (PFS) rate was 29%, and an overall survival (OS) was 52% [133]. Updated analysis, published during ASCO 2018, showed 2-year PFS rate of 26% and 2-year OS of 36%. This trial gave a lot of information regarding also the great safety profile of avelumab, with just 9.1% grade 3 adverse events and 4.5% grade 3 immunorelated adverse events, no grade 4 adverse events, or treatment-related deaths. The most common adverse events were fatigue (24%), infusion-related reactions (IRRs) (17%), diarrhea, and nausea (9% each). However, five patients developed serious adverse events leading to permanent discontinuation: elevated aminotransferases, enterocolitis, IRRs, chondrocalcinosis, synovitis, and interstitial nephritis. Based on these results and on the safety profile, the FDA accelerated approval to avelumab for the treatment of patients with metastatic MCC, in the first- and second-line settings.

Data from an interim analysis of avelumab as first-line treatment in patients with metastatic MCC have been recently published [134]. First-line avelumab treatment was associated with early and durable responses and a manageable safety profile.

Pembrolizumab, an anti-PD-1 monoclonal antibody, has been tested in a multicenter, phase II non-controlled study of 25 systemic therapy-naive patients. Preliminary results of this study, after 33 weeks of median follow-up, showed 6-month PFS of 67% and median PFS of 9 months [135].

Updated results after a median follow-up of 6.8 months, published during ASCO 2018, demonstrated a median PFS of 16.8 months and an 18-month OS of 68%. The disease control rate was 66% with an ORR of 56% and 24% of complete response. Among 21 confirmed responders, median response duration was not reached (range 3.9–25.6 months). The safety analysis confirmed the good safety profile of pembrolizumab with a rate of grade 3–4 adverse events of 30%.

The largest study of *nivolumab* to date consisted of a single-arm, open-label trial with nivolumab 240 mg every 2 weeks, for 25 systemic therapy-naive and previously treated patients [136]. After a median follow-up of 26 weeks, 22 patients responded, with a higher percentage occurring in treatment-naive patients and a PFS of 82%. ORR was 73% among treatment-naive population and 50% in pretreated population. Survival analysis showed a 3-month OS of 92%. Regarding safety, the rate of grade 3–4 adverse events was 24%.

In all these studies, response was seen in both virus-positive and virus-negative tumors, although studies have suggested that PD-L1 expression is higher on virus positive tumors [137].

Other immune checkpoint inhibitors investigated by clinical trials are ipilimumab, atezolizumab, durvalumab, tremelimumab, and daratumumab.

Finally, combinations of immunotherapeutic options are being evaluated: a lot of attention has been generated around *talimogene laherparepvec* (TVEC), a genetically altered herpes simplex type I virus that selectively replicates in tumor cells and express human granulocyte-macrophage colony-stimulating factor, which activates dendritic cells to present tumor antigens and encourage an innate cell-mediated host response. There have been a few reported cases regarding the success of TVEC in treating advanced locoregional MCC in elderly patients who were not good surgical or chemotherapy candidates [138]. Primary nodules regressed and did not recur for 7 months to 11 months after the last dose. A multicenter phase II trial is under way to further investigate its success in treating MCC and other cutaneous tumors.

57.2.5.9 Target Therapies

Finally, targeted therapies remain an area of research for MCCs dominated by specific mutations. Several different pathways have been identified as potential targets of therapy. One case of complete response to idelalisib in a PI3K/AKT-mutated MCC and one in a stage IV MCC patient has been reported since now [139]. MLN0128 is a target of the mTOR pathway currently in phase II trial for advanced MCC. Pazopanib was reported to induce partial remission in a case report [140].

57.2.6 Dermatofibrosarcoma Protuberans

DFSP is a rare (incidence: 3 per million) soft tissue sarcoma of histiocytic origins [141] and arising from the dermis, with a locally aggressive potential to deeper soft tissues. DFSP represents 1% of all sarcomas, and it principally affects young subjects in their mid-30s. Blacks have slightly higher incidence than whites; men and women are equally affected [142].

57.2.6.1 Histopathology

Histologically, DFSP is composed of monomorphous, dense, spindle cells, arranged in a storiform pattern that takes over the dermis. This tumor is characterized by tentacle-like projections, and often, no defined border can be recognized between the tumor and normal tissue. This may be the reason why the incidence of local recurrence is so high.

A pigmented variant of DFSP – also known as Bednar tumor – presents melanin-containing dendritic cells. The juvenile form – called giant cell fibroblastoma – is characterized by loose hypocellular areas that resemble mature DFSP.

Immunohistochemistry helps to differentiate DFSP from dermatofibroma (DF) through the exclusive expression of the human progenitor cell antigen CD34 in DFSP.

More than 90% of DFSP bear a particular translocation between chromosomes 17 and 22, provoking the fusion of the collagen type I-alfa1 gene (COL1A1) to the platelet-derived growth factor (PDGF) beta-chain gene (PDGFB). This fusion results in the deletion of exon 1 of PDGFB, leading to the constitutive activation of PDGF receptor (PDGFR) protein tyrosine kinase, providing signals for the cells to proliferate.

57.2.6.2 Clinical Presentation

DFSP is a slow-growing tumor (most commonly occurring on the trunk and proximal extremities), starting as a small asymptomatic firm, indurated papule, or patch, and it may gradually evolve into a nodule or a sclerotic plaque; ulceration may be present in case of accelerated growth.

Possible differential diagnoses may include cutaneous melanoma, dermatofibroma, keloid, and morphea.

57.2.6.3 Treatment

Surgery Wide excision without elective lymph node dissection is the standard of care for DFSP. A study suggested a 5 cm margin of excision in order to prevent local recurrences, as the likelihood of local recurrence is directly proportional to the adequacy of surgical margins [143].

Radiation therapy is an alternative treatment option to surgery, as this can lead the neoplasm to have a more aggressive behavior. RT is used, in particular, if surgical resection was not possible, or would result in major cosmetic or functional loss, with good local response. In addition, RT may be recommended in case of positivity of the margins of resection or in an adjuvant setting.

The complete RT dose ranges from 50 to 70 Gy [144].

A medical treatment option in case of advanced or metastatic disease may include *imatinib mesylate*, a BCR/ABL (the fusion product responsible of chronic myelogenous leukemia), and a specific tyrosine kinase (including c-kit and PDGF receptors) inhibitor. Imatinib has been used in DFSP based on the central role of the constitutively activated PDGFB-PDGFBR signaling pathway in the proliferation of DFSP cells, showing a clinical success.

In 2006, the US Food and Drug Administration approved imatinib mesylate for treatment of unresectable, recurrent, and/or metastatic DFSP. Note that a small group of DFSP patients lacking the t (17;22) translocation have no response to imatinib.

Recent studies showed a decreased tumor load using imatinib in a neoadjuvant setting.

Conventional chemotherapy is rarely used to treat DFSP [145, 146].

57.2.7 Kaposi Sarcoma

Kaposi sarcoma (KS) was described first in 1872 by the Hungarian dermatologist Moritz Kaposi. KS is a spindle-cell tumor deriving from endothelial cell lineage. KS can be categorized into four types:

1. *Epidemic*. The epidemic type is the most commonly observed in the USA. This form tends to have an aggressive behavior, and it is considered to be typically AIDS related. Positivity to human herpesvirus 8 (HHV-8) has been associated to this form, and the infection can predate the epidemic KS by about 10 years [147].
2. *Iatrogenic*. Principally related to immunosuppressive treatments, especially in transplant patients [148]. The observed time to development of KS following transplantation ranges 15–30 months. In these cases, the disease shows an aggressive behavior with visceral involvement, but withdrawal of immunosuppression may cause regression of the disease [149].
3. *Classic, sporadic*. This form, typical of the Mediterranean and Eastern European elderly men, has a more indolent course, with rare lymph nodes or visceral involvement. Its development may be due to aging immune dysregulation (subsequent immune suppression and reactivation), history of other neoplasm, and possible concomitant infections, as malaria. Cigarette smoking has been noted to have a protective effect [150].
4. *Endemic, African*. This entity occurs in African HIV-seronegative people. It represents the first form of cancer observed in men in the African countries of Malawi, Swaziland, Uganda, Zambia, and Zimbabwe (9% of all cancers in Ugandan males) and the second cancer in women. The high prevalence of shoeless people in these areas has been associated with an increase of endemic KS, possibly related to chronic lymphatic obstruction in the lower limbs from fine soil particles. The endemic form has a more common lymph node involvement than the classic variant [151].

The involvement of HHV-8 (identified by polymerase chain reaction in more than 90% of all subtypes of KS lesion), HIV infection, immunologic dysregulation, and environmental factors requires further investigation to understand the complex pathogenesis of KS.

KS cutaneous lesions are typically brown-violaceous nodules and typically concentrated on the lower extremities and the head and neck. KS nodules may be single or symmetrically distributed, following Langer lines.

Mucous membrane involvement is not uncommon (oral cavity, conjunctiva). The bulky tumor mass may interfere with speech or mastication.

57.2.7.1 Treatment

Epidemic KS In the AIDS-associated form, the treatment of choice is always centered on the highly active antiretroviral therapy (HAART). In some high-risk patients, a combination of HAART and chemotherapy is still needed. However, no data are still available to show that treatment improves overall survival [152].

Classic KS In this indolent form, surgical excision may be enough especially for patients with small lesions. However, local recurrence is very common.

Local treatment by RT may be effective in a palliative setting, against bleeding and pain.

Other topical treatment options include intralesional therapy with vinca alkaloids, cryotherapy, laser therapy, and topical retinoids [153].

In case of visceral involvement, symptomatic disease, or rapidly progressive mucocutaneous disease, chemotherapy can be used with a palliative intent. The chemotherapy protocol of choice is doxorubicin, bleomycin, and vincristine (ABV). Single drug (in liposomal preparation) regimens have also been approved in AIDS-related KS [152].

A recent study with imatinib mesylate has shown response in 4 of 5 patients [154]. In another small trial of nine patients with AIDS-related KS, immune-checkpoint inhibition with nivolumab or pembrolizumab leads to partial responses in six patients and complete response in one patient, with a low toxicity profile [155].

Finally, study of the complex multiple pathways of pathogenesis may lead to develop inhibitors of the principal tumor growth-stimulating factors. Recent ongoing studies are now involving the VEGF, the basic fibroblast growth factor (bFGF) pathways, as the matrix metalloproteinases and oligonucleotides, showing good preliminary results [156].

Topical Treatment Insights

The general approach to management of skin cancer depends on the biologic aggressiveness of the tumor. Surgical options are generally considered the gold standard treatment, including excision and Mohs surgery. In case of superficial tumors or precancerous lesions, many other options can include curettage, cautery/electrodessication, cryosurgery, photodynamic therapy (PDT), and laser surgery, with limited efficacy. Other options are topical therapy (such as imiquimod, diclofenac, or 5-fluorouracil) and radiation therapy (RT).

Imiquimod

Imiquimod is an imidazoquinoline-binding toll-like receptors (TLR) 7 and 8 acting as an immunomodulator. This effect promotes tumor regression by the cell-mediated immune response (CD4 T-helper 1 and CD8-T cytotoxic lymphocytes) through the upregulation of interferon (IFN)-α, IFN-γ, and proinflammatory interleukins (IL)-8, 6, 12, by the innate immunity cells.

Imiquimod has been approved by the US Food and Drug Administration (FDA) for treatment of low-risk skin cancers, such as actinic keratosis (AK) and superficial basocellular carcinoma (BCC). Variable results were obtained in case of nodular BCC, in situ squamous cell carcinoma (SCC), or melanoma in situ.

Imiquimod 5% cream should be administered for 6–12 weeks, and it may show several adverse events including application site reactions, but also systemic flu-like syndromes [157, 158].

5-Fluorouracil

5-FU is a chemotherapeutic agent that inhibits the DNA synthesis, blocking the thymidylate synthetase. It is approved by the FDA for AKs and superficial BCC [159]. The main adverse event is application site reaction.

Diclofenac

Diclofenac is a nonsteroidal anti-inflammatory agent, characterized by a high affinity for cyclooxygenase-2 (COX-2), a prostaglandin-producing enzyme, frequently elevated in AK, NMSC, and melanoma, involved in the UV-induced skin damage [160]. Diclofenac inhibits the prostaglandin-mediated UV-induced mutagenic effect also by the reduction of proinflammatory cytokines such as IL-1 and TNF-α [161].

Photodynamic Therapy

PDT is effective in the treatment of certain NMSCs. 5-aminolevulinic acid (ALA) and methyl-aminolevulinic acid (MAL), when topically applicated, accumulate in malignant and premalignant cells and metabolized to protoporphyrin IX, a photoactive agent, generating reactive oxygen species when exposed to a specific wavelength of light (from 400 nm to infrared). This leads to irreversible damage and cancerous cell death [162].

PDT is currently approved by the FDA for AKs, but many off-label uses in other dermatologic conditions are currently under investigation [49].

Postprocedural scarring are depigmentation are rare adverse events [162].

Radiation Therapy

RT is a treatment option in many NMSCs, such as Merkel cell carcinoma, cutaneous lymphomas, BCC, and cutaneous SCC, especially when surgery is precluded due to poor patients' performance status, or in case of unresectable tumors.

Another important indication of RT is in the adjuvant setting, in case of the following:

- Positive surgical margins
- Perineural invasion
- Locoregional nodal metastasis

RT can be delivered as electrons or superficially penetrating photons (X-rays) [163, 164].

Postprocedural adverse events may be acute and chronic radiation dermatitis or necrosis, epidermal atrophy, telangiectasias, altered pigmentation, alopecia, and secondary NMSCs [165].

Expert Opinion

Paola Queirolo

Key Points

1. Nonmelanoma skin cancer are heterogeneous group of neoplasms which affect primarily the skin. They can have a different biological behavior and risk factors. Most frequently, they are associated to sun exposure, prior skin neoplasm, previous treatments, and genetic conditions.
2. Actinic keratosis (AK) are cutaneous precancerous lesions as a result of sun exposure, and they can evolve into in situ or invasive SCC. Usually they appear as reddish-pink hyperkeratotic surface on a sun-exposed area. Treatment can consist in photodynamic therapy, cryotherapy, and the use of 5-FU or imiquimod. Obviously, it is recommended to avoid further sun exposure.
3. Basal cell carcinoma is the most common human cancer, and it is characterized by a locally invasive

behavior. It is associated to some genetic syndromes such as the nevoid BCC (Gorlin syndrome) or to the Bazex syndrome. It usually appears as a well-defined erythematous-pink macule, quite difficult to differentiate from AK. There are different subtypes such as superficial, infiltrative, nodular (the most frequent), morpheaform, fibroepithelioma of Pinkus, and the pigmented one. Treatment is based on radical surgery excision, imiquimod, 5-FU, and PDT. In case of inoperable or metastatic cancers, a new drug is represented by vismodegib, approved in 2012 by FDA *and sonidegib*.

4. Cutaneous squamous cell carcinoma (cSCC) arises from epidermal keratinizing cells. The most important risk factors are sun exposure, age, immunosuppression, HPV chronic infection, and male sex. Prognosis of cSCC is excellent, but sometimes a metastatic diffusion of this neoplasm is possible. Thanks to dermal invasion, it is possible to differentiate an in situ cSCC from an invasive one, which can be divided according to the risk of recurrence and metastases in three categories: low (<3%), intermediate (3–10%), and high risk (>10%). As the cSCC includes different subtypes of varying malignant potential, the pathology should report the following: histologic subtype, degree of differentiation, depth of invasion, perineural invasion, and hematolymphatic invasion. Treatment is based on surgical excision, cautery or electrodesiccation, radiation therapy, electrochemotherapy, and chemotherapy in case of metastatic disease (platinum-based regimens, cetuximab, cemiplimab).
5. Merkel cell carcinoma is a rare neuroendocrine cancer of the skin. Its incidence has increased in the last years, probably for a better knowledge of this neoplasm. It is associated to advanced age, previous UV exposure, immunodeficiency, and Merkel cell polyomavirus (MCPyV). Clinical presentation consists in cutaneous erythematous or violaceous nodules with possible ulcerations. Metastases can occur, most frequently in locoregional lymph nodes; distant sites are bone, distant skin, lung, and brain. After the excisional surgery, when possible, adjuvant radiotherapy (RT), complete local nodal dissection or both are recommended in case of positive sentinel lymph-node biopsy or in high-risk patients (in this setting just RT can be sufficient). In advanced stages, platinum agents and etoposide chemotherapy were the main treatment options before the use of avelumab, a PD-L1 inhibitor, which has shown successful results in controlling neoplastic proliferation. New drugs under investigation are other immunotherapy drugs and some targeted therapies (idelasib and pazopanib).
6. Dermatofibrosarcoma protuberans is a soft tissue sarcoma arising from histiocytic cells. It tends to recur locally, and the treatment is a surgical excision with almost 5 cm of margin. In case of positivity of the margins or when surgery is not practicable, RT is recommended. For unresectable, recurrent or metastatic disease, imatinib is a possible treatment.
7. Kaposi sarcoma is characterized by brown-violaceous nodules typically localized in lower extremities. There are four types with a different population distribution: epidemic (HIV-correlated), iatrogenic (caused by iatrogenic immunodeficiency as in case of organ transplants), sporadic (in elderly men of Mediterranean and Eastern European areas), and endemic (in African regions). Classic form can be surgically treated; radiotherapy can have an effective palliative intent and other possible topic approaches are cryotherapy, intralesional therapy with vinca alkaloids, or laser therapy. In case of visceral involvement, symptomatic disease or rapidly progressive mucocutaneous disease, chemotherapy with doxorubicin, bleomycin, and vincristine is recommended. Targeted therapies and immunotherapy are under study, and some trials are on-going.

Recommendations

- American Academy of Dermatology
- ► https://www.aad.org/news/guidelines-to-treat-nonmelanoma-skin-cancer

Hints for a Deeper Insight

- Understanding the Molecular Genetics of Basal Cell Carcinoma: ► https://www.ncbi.nlm.nih.gov/pubmed/29165358
- Patient-centered management of actinic keratosis. Results of a multi-center clinical consensus analyzing non-melanoma skin cancer patient profiles and field-treatment strategies: ► https://www.ncbi.nlm.nih.gov/pubmed/31625770
- Kaposi sarcoma herpesvirus pathogenesis: ► https://www.ncbi.nlm.nih.gov/pubmed/28893942
- Efficacy and Safety of First-line Avelumab Treatment in Patients With Stage IV Metastatic Merkel Cell Carcinoma: A Preplanned Interim Analysis of a Clinical Trial: ► https://www.ncbi.nlm.nih.gov/pubmed/29566106

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Soft Tissue Sarcomas (STS)

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Soft Tissue Sarcoma, GIST and Neuroendocrine Neoplasms

Contents

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Learning Objectives

By the end of the chapter, the reader will:

- Have learned the basic concepts of epidemiology, histological subtype, and molecular profile of STS.
- Have reached in-depth knowledge of diagnosis, staging, and clinical management of STS.
- Be able to put acquired knowledge on STS into clinical practice

58.1 Introduction

Soft tissue sarcomas (STSs) represent a rare and heterogeneous group of solid tumors derived from mesenchymal progenitors and account for 1% of all adult malignancies [1]. Approximately 80% of sarcomas arise from soft tissue and viscera, whereas the remaining 20% originate from bone. STSs potentially may occur at all body anatomic sites, even though the majority arise from the extremities.

As classified by the World Health Organization (WHO), the group of STSs comprise more than 100 different histologies according to the presumptive tissue in origin [2]. Histological diagnosis is crucial in order to define staging and prognosis and to deliver appropriate therapy. Unfortunately, sometimes it causes a diagnostic challenge for pathologist, particularly when the diagnostic material is a small biopsy and when clinical information is incomplete. After the development of distant metastasis, the median overall survival (OS) is 12–19 months, and almost 20% of patients are still alive at 3 years [3].

58.1.1 Diagnosis and Pathology

There is agreement on the recommendation that the pathological diagnosis of STM should contain the following information:

- Macroscopic description
- Status of margins, so as to allow the attribution of surgical intervention to the categories "radical," "broad," "marginal," and "intralesional"
- Histotype according to WHO 2013

The malignancy grade is described by the classification of the French Federation of Cancer Centers:

- Grade 1: Low grade
- Grade 2: Intermediate grade
- Grade 3: High grade

The WHO 2013 classification of mesenchymal tumors distinguishes (1) benign lesion, (2) lesion with intermediate biological behavior, and (3) lesion with malignant biological behavior.

Intermediate lesions are defined as follows:

- Locally aggressive but not metastasizing tumors (e.g., aggressive fibromatosis)
- Tumors with a metastasis rate of less than 2% (e.g., plexiform fibrohistiocytic tumor)

58.1.2 Staging and Risk Assessment

Available staging classifications have limited relevance and should be improved. The Union for International Cancer Control (UICC) stage classification system, eighth edition, stresses the importance of the malignancy grade in sarcoma [4]. In general, in addition to grading, other prognostic factors are tumor size and tumor depth for limb sarcomas. Of course, site, tumor resectability, and the presence of metastases are also important. Nomograms are available, which can help personalize risk assessment and thus clinical decision-making, especially on adjuvant/neoadjuvant treatments [5, 6].

58.2 STS Management

58.2.1 Essential Elements Prior to the Initiation of Therapy

According to major national and international guidelines, the optimal therapeutic strategy of all soft tissue sarcomas (STS) patients should be discussed within multidisciplinary teams. Disease histology, stage, anatomical localization, and patient preferences are the most important elements for a correct decisional process [7, 9]. Notably, compliance to guidelines and relapse-free survival of sarcoma patients are significantly better when the initial treatment is guided by a pretherapeutic specialized multidisciplinary tumor board [10].

Adequate imaging of primary tumor, i.e., MRI with and without contrast +/− CT with contrast, is necessary to provide details about the size of the tumor and its contiguity to nearby visceral and neurovascular structures. A chest spiral CT scan without contrast is recommended in the US guidelines [1] and mandatory in the European ones [2]. In selected circumstances, other imaging studies might be required.

Histological diagnosis prior to therapy should be acquired whenever possible. Core needle biopsy or incisional biopsy usually provides sufficient tissue to perform a correct pathological and molecular diagnosis e must always be carried out in the case of lesions over 5 cm in diameter (Fig. 58.1).

The STS clinical presentation can be very different in relation to the place of origin. In the case of a limbs or trunk localization, the sarcoma is presented as a clini-

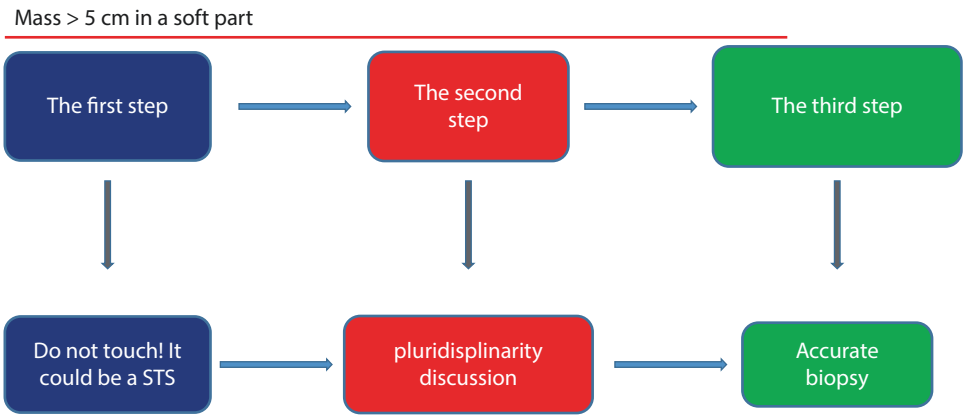


Fig. 58.1 The role of biopsy for all lesions greater than 5 cm. (Diagnosis: flow chart)

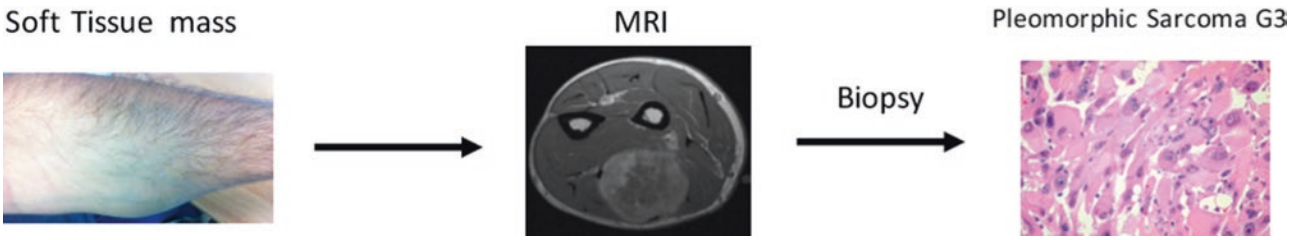


Fig. 58.2 Soft tissue mass of the forearm compatible with sarcoma; magnetic resonance imaging confirms the suspicion and the biopsy confirm pleomorphic sarcoma G3

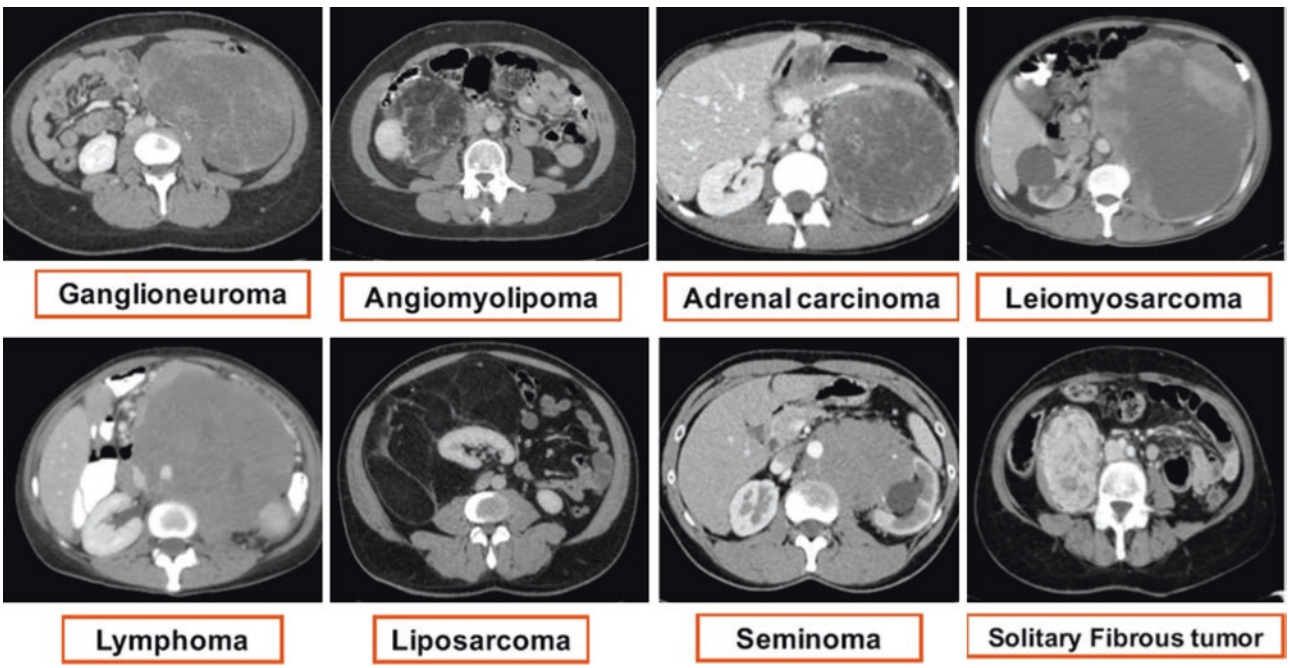


Fig. 58.3 The retroperitoneum may be the site of different type of cancers and imaging does not allow a differential diagnosis

cally evident swelling, with stretched-elastic consistency and rapid growth. In this case, only biopsy can confirm the diagnosis and define the histotype (Fig. 58.2).

Retroperitoneal sarcomas, on the other hand, can reach considerable size because they are very often asymptomatic. In this case, as in the case of the sarcomas of the limbs and of the trunk, the biopsy is mandatory. The retroperitoneum may also be the site of different type of cancers, and imaging does not allow a differential diagnosis (Fig. 58.3).

Finally, visceral sarcomas, which are much rarer and are clinically similar to the most frequent carcinomas.

Pathological review by national and international STS experts should be obtained in all cases where the histological, immunohistochemical, and molecular data do not allow a straightforward diagnosis. In fact, selected histologic subtypes characteristically display unusual biological behaviors. For example, epithelioid hemangioendothelioma is often indolent, whereas visceral Ewing(−like) sarcomas tend to be particularly aggressive. These histologic subtypes do not usually follow the principles of therapy hereby discussed.

58.2.2 Principles of Multidisciplinary Therapeutic Approach

58.2.2.1 Surgery

Surgical resection with appropriately negative margins is the standard treatment for most patients with STS. Dissection should be through grossly normal tissue planes uncontaminated by tumor and should be performed by a surgeon specifically trained in the treatment of STS. In fact, the volume and expertise of the center where the surgery is conducted does significantly impact overall and progression-free survival [4]. The biopsy site should be excised en bloc with the definitive surgical specimen, to minimize the risk of seeding. Currently, there is no universal agreement on the dimensions of the margins, ideally >2 cm. Closer margins might be necessary to preserve bones, joints, major vessels, or nerves, especially in extremity STS. Surgical clips might be placed to mark the periphery of the surgical field to help guide potential future radiotherapy, particularly for retroperitoneal and abdominal sarcomas.

In extremity STS, limb-sparing surgery should be performed, whenever possible. Stage I disease of the extremities should be treated with radical surgery and oncologically appropriate margins. In case of appropriate margins, patients should be evaluated for rehabilitation and start clinical and radiological follow-up. In case of positive surgical margins, surgical re-resection is strongly advised; if the reintervention does not significantly affect organ function [5], adjuvant RT should be considered. Patients with stage II, III resectable disease might follow several therapeutic strategies according to size, histologic subtype, and localization.

Appropriate multimodal strategies include the following:

1. Surgery followed by adjuvant RT +/− chemotherapy
2. Preoperative (chemo)RT followed by surgery +/− adjuvant chemotherapy
3. Preoperative chemotherapy followed by surgery + adjuvant RT +/− chemotherapy.

Preoperative RT and/or chemotherapy should be considered to reduce the likelihood of a local relapse and to improve the outcomes of surgery [6]. In selected cases, either resectable with adverse functional outcome or unresectable, regional limb therapy (perfusion and infusion) with chemotherapy +/− TNF-alpha can be considered in institutions with experience [7]. Amputation should be performed for patient preference or if the gross total resection of the tumor is expected to render the limb nonfunctional [8].

For STS of the retroperitoneum, the standard surgical treatment is multi-visceral en bloc resection, often including nephrectomy, partial colectomy, and resection of vascular and muscular structures. This type of surgery is considered safe when carried out at a specialist sarcoma center. High-risk resections should be carefully considered on an individual basis and weighed against anticipated disease biology [9].

Notably, patients with limited metastasis confined to a single organ and limited tumor bulk that are amenable to local therapy should receive primary tumor management as described for stage II or III tumors and consider metastasectomy +/− chemotherapy +/− RT [10] (Fig. 58.4).

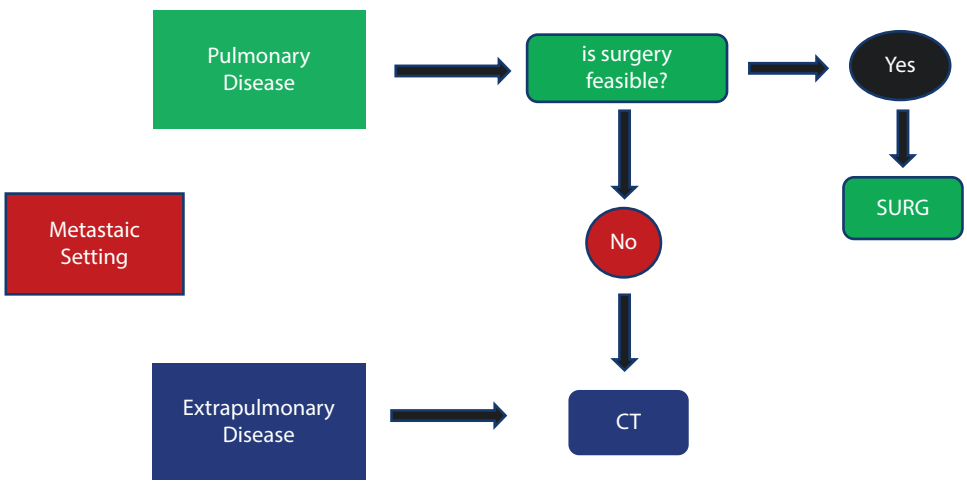


Fig. 58.4 Therapeutic approach in advanced disease

58.2.2.2 Radiotherapy

Radiotherapy is widely used in the treatment of STS patients. Adjuvant (i.e., postoperative) external beam RT (50 Gy + a variable boost dose based on margin status) should be considered for a close soft tissue margin (10–16 Gy boost) or a microscopically positive margin on bone, major blood vessels, or a major nerve (16–18 Gy boost). Randomized clinical trial data support the use of adjuvant RT to reduce local relapse, although there is no clear improvement in overall survival rates [11]. Preoperative RT is believed to reduce the risk of seeding due to surgical manipulation of the tumor. It is usually administered at a dosage of 50 Gy in 1.8–2 Gy fractions. Preoperative and adjuvant RT does not differ in terms of local or global disease control. Compared to adjuvant RT, preoperative RT is associated to greater risk of wound complications [12], but usually targets smaller radiation fields, reducing side effects, such as fibrosis, joint stiffness, and oedema [13]. A recent meta-analysis combining 16 studies also supports the use of external beam RT (both pre- and postoperative) for local tumor control in patients with resectable STS, both in the extremities and in the retroperitoneum [14]. Brachytherapy can also be considered in selected patients as an alternative to external beam RT [15].

58.3 Medical Therapy

58.3.1 Neoadjuvant Chemotherapy

The cornerstone of the medical therapy for most STS patients in all settings is represented by anthracyclines (doxorubicin and epirubicin), alone or in association to other drugs.

In the last few years, the efficacy of neoadjuvant treatment has been evaluated in different trials. The advantages of a neoadjuvant treatment are different: tumor shrinkage with the possibility of a conservative surgery, early control of micrometastases, and in vivo evaluation of treatment activity (Fig. 58.5).

In this setting, the data are conflicting and the benefit of chemotherapy seems to be limited to patients with high-grade large tumours [16]. Importantly, in patients with high-risk localized STS, three cycles of full-dose pre-operative CT are not inferior to five cycles [17]. Recently, it was reported that neoadjuvant full-dose epirubicin + ifosfamide was superior to histotype-tailored chemotherapy for most histological STS subtypes [18]. Among the histology-driven regimens, the use of trabectedin in high-grade myxoid liposarcoma has shown particularly interesting results, with response rates comparable to the standard epirubicin regimen [18]. Neoadjuvant therapy is proposed in experienced centers high risk to patients where primary surgical treatment would not be feasible or would be only feasible with adverse functional outcome.

In specific histologies, neoadjuvant chemoradiotherapy treatment may be particularly active and must be considered before surgery (Fig. 58.6).

Neoadjuvant treatment: Theoretical advantages

- Tumor cytoreduction
- Immediate treatment of micrometastases
- Early indication as to the effectiveness of chemotherapy/radiotherapy

Fig. 58.5 Theoretical advantages of neoadjuvant treatment

58.3.2 Adjuvant Chemotherapy

The finality of adjuvant treatment in STS is to improve overall survival (OS) and relapse-free survival (RFS) (Fig. 58.7).

The role of adjuvant chemotherapy in STS therapy is debatable [19]. Large meta-analysis including several trials conducted up to the year 2000 showed a statistically significant 6–10% increase in recurrence-free survival at 10 years, associated to a non significant 4% increase in overall survival [20]. In a 2001 Italian trial, restricted selection criteria for high-risk cases and high-dose intensities of doxorubicin and ifosfamide resulted in a positive impact on the disease-free survival and overall survival [21]. A second, updated meta-analysis published in 2008 confirmed a significant, although marginal, efficacy of chemotherapy in localized resectable soft-tissue sarcoma with respect to local recurrence, distant recurrence, overall recurrence and overall survival. These benefits are further improved with the addition of ifosfamide to doxorubicin-based regimens, but must be weighed against associated toxicities [22]. Notably, in 2012, the randomized clinical trial EORTC 62931 showed no significant benefit deriving from an adjuvant chemotherapy with doxorubicin, ifosfamide, and granulocyte colony-stimulating factor [23]. This study, however, was limited by a long period of accrual, a large number of ineligible patients, inadequate dosing of ifosfamide, and inclusion of patients with leiomyosarcoma, an histology known to be poorly responsive to ifosfamide. Currently, adjuvant chemotherapy is generally considered for young fit patients with high-grade disease after discussion of risk-benefit ratio [24].

The Italian AIOM guidelines and European ESMO guidelines suggest an adjuvant treatment in the case of lesions greater than 5 centimeters in diameter, G3, and with deep localization.

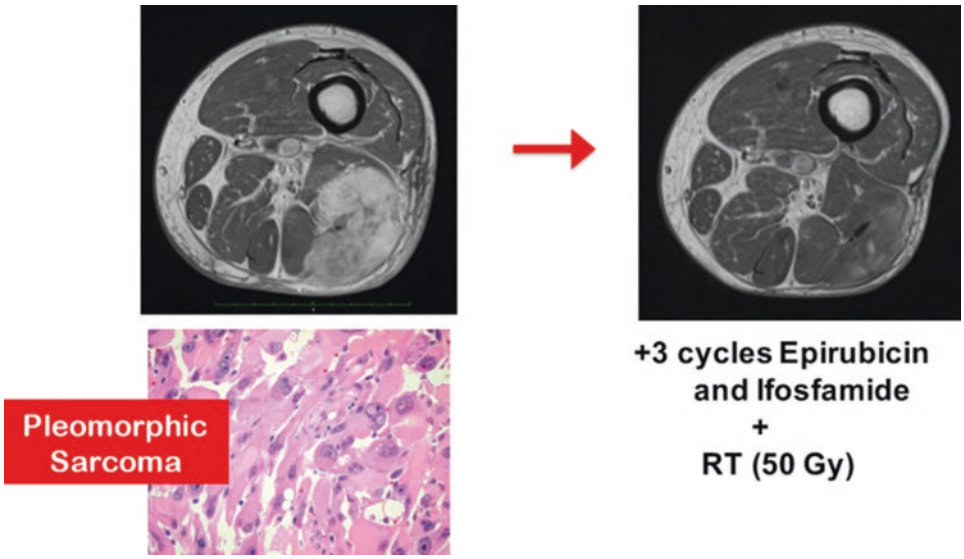


Fig. 58.6 Pleomorphic Sarcoma: good response after neoadjuvant chemoradiotherapy treatment

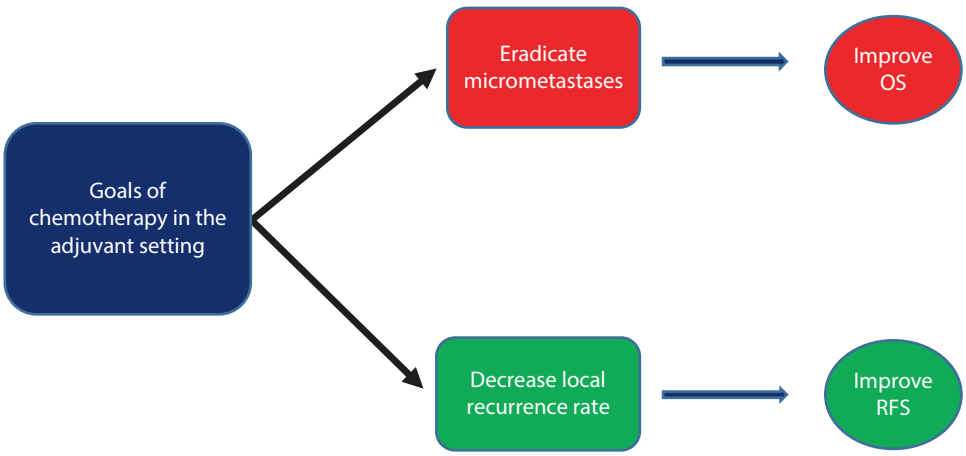


Fig. 58.7 Benefits of adjuvant chemotherapy

Age, performance status, and sensitivity to chemotherapy are further parameters to be evaluated (Fig. 58.8).

58.3.3 Palliative Chemotherapy

The benefit of doxorubicin in metastatic STS patients was first reported by Benjamin et al. in 1975 [25]. Median survival for patients with metastatic STS treated with doxorubicin-containing regimens is however only 12–16 months, and the 2-year survival rate is ~30% [26, 27]. It must be noted that the addition of ifosfamide to doxorubicin does not significantly increase overall survival, but is associated to higher response rates and longer progression-free survival, with usually manageable increases in toxicity [26].

Two other chemotherapeutic regimens, i.e., doxorubicin + evofosfamide, a hypoxia-activated prodrug similar to ifosfamide [28], and gemcitabine + docetaxel [29], have been recently studied as potential first-line therapies in randomized controlled phase III trials, both with no benefit in survival compared to doxorubicin alone. Alternative regimens should be proposed if anthracyclines are contraindicated (e.g., in case of reached cumulative dose due to previous chemotherapy for other cancers, in presence of known cardiologic morbidity) or based on patient preference [30].

In second line, based on the specific histologic subtypes, other drugs and regimens can be chosen (see), for example, gemcitabine+/−docetaxel or dacarbazine in leiomyosarcomas [31, 32], trabectedin in liposarcoma and leiomyosarcoma [33], and the multi-tyrosine kinase inhibitor pazopanib for non-adipocytic sarcomas [35]. Among these agents, eribuline showed impressive results with improved overall survival, particularly in liposarcomas [34].

Moreover, in selected histologies, targeted therapies should be considered based on their molecular specificity [36], e.g., in dermatofibrosarcoma protuber-

ans (a subtype driven by PDGF-β/PDGFR signaling), the multi-tyrosine kinase inhibitor imatinib has strong activity [37, 38]; and in myofibroblastic inflammatory tumor, a subtype often driven by ALK translocation, ALK inhibitors can be used [39, 40] (Table 58.1).

Immunotherapy in STS is not approved yet, although recently promising results have been observed with pembrolizumab in a limited number of histologies. [41]

Figure 58.9 shows the treatment flow chart in the case of metastatic disease (Fig. 58.10).

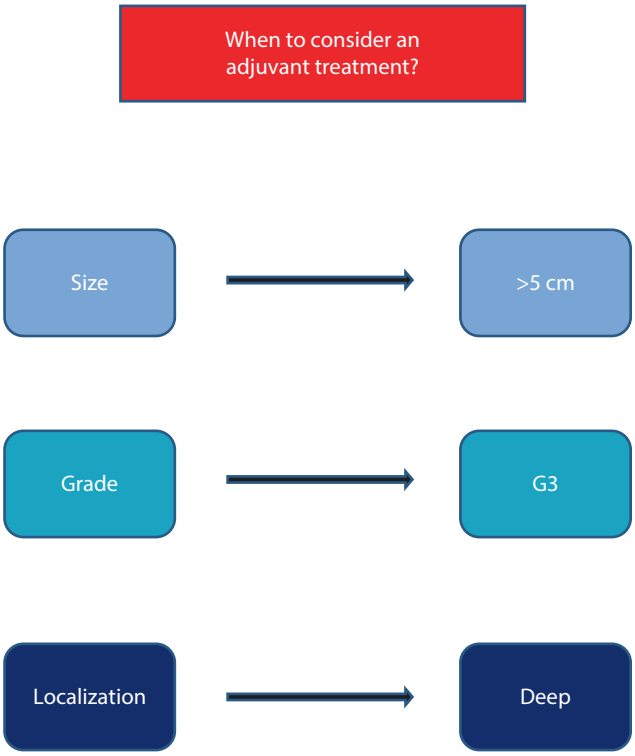
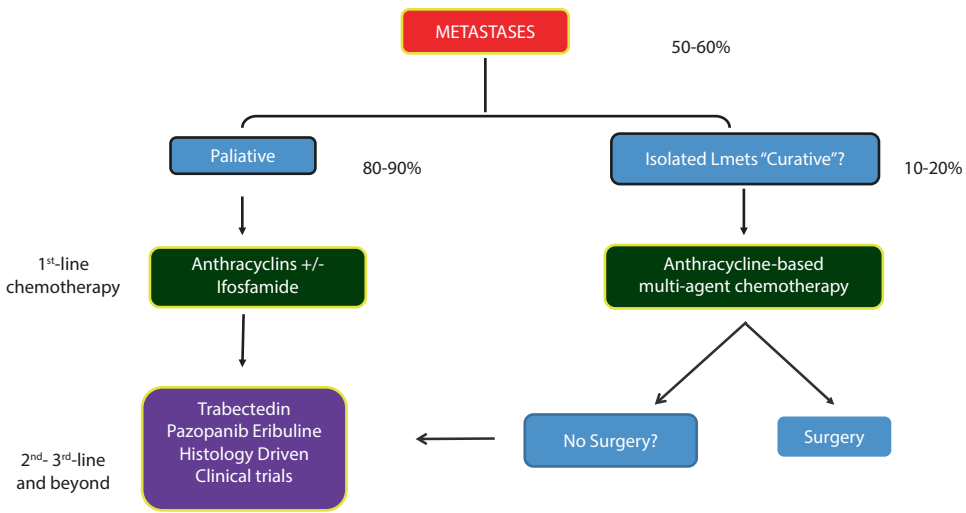


Fig. 58.8 Indications for adjuvant treatment

Table 58.1 Histology-driven treatment

Histotypes	specific treatments
Non myxoid liposarcoma	Doxorubicin +/– ifosfamide
Myxoid liposarcoma	Trabectedin
Leiomyosarcoma	Doxorubicin + DTIC, Gem-TAX, Gem-DTIC
Synovialosarcoma	High-dose Ifosfamide
UPS	Ifosfamide. Gem-TAX
Angiosarcoma	Taxol, gemcitabine
MPNST	Etoposide-HD ifosfamide
GIST, dermatofibrosarcoma	Imatinib
Pecomas	mTOR inhibitors
Alveolar soft tissue sarcoma	Anti-VEGFR agents
Endometrial Stromal sarcoma	Hormonal treatment (aromatase inhibitor)

Fig. 58.9 Flow chart treatment in metastatic setting



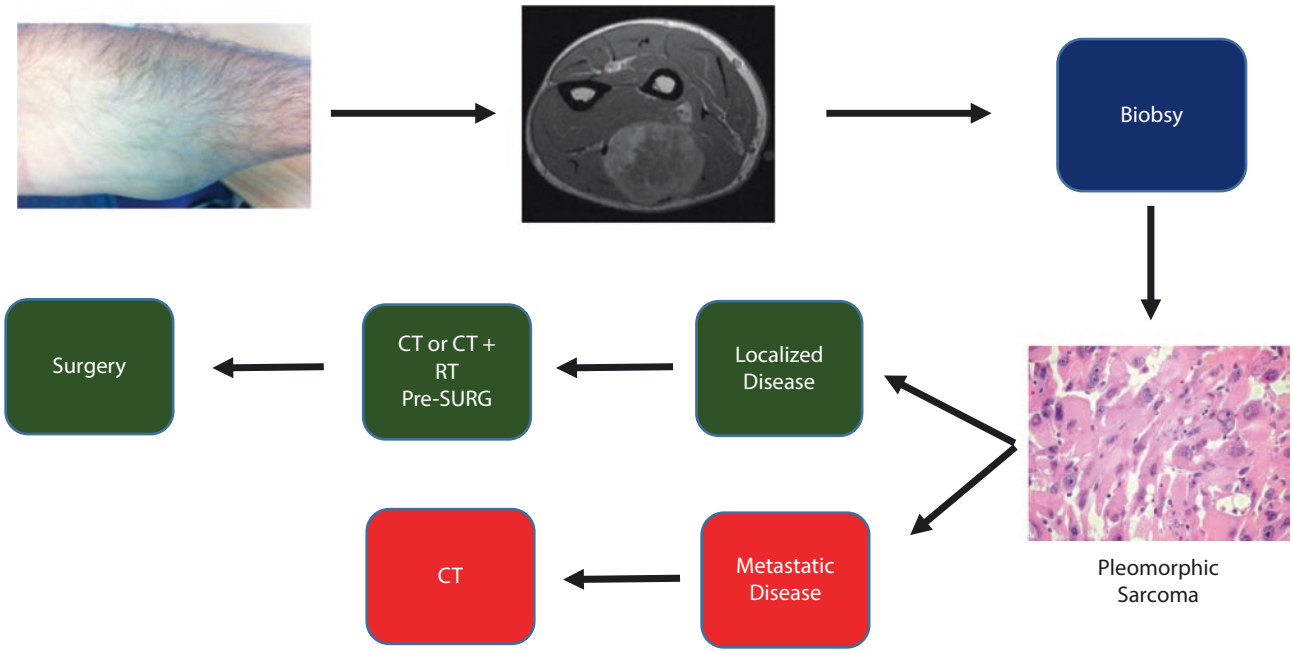


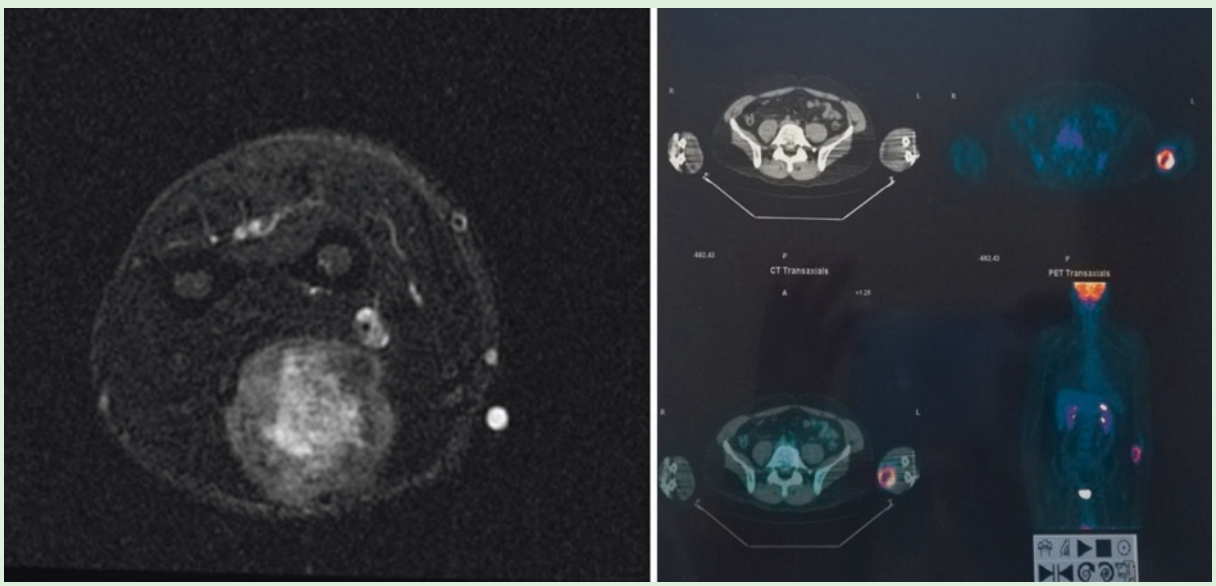
Fig. 58.10 Global therapeutic approach in a patient with a mass of soft tissues. First, biopsy. Second, staging. If localized disease, consider neoadjuvant treatment and then surgery. If metastatic disease, palliative chemotherapy

58

Case Study

Man, 50 years old

- *Family history* negative for malignancy
- *APR:* hypertension
- *APP:* contusive trauma on the left forearm with the appearance of a rapidly growing lesion
- *Objective examination*: stretch-elastic swelling of soft parts
- *Blood tests*: normal blood tests



- *RMI mdc*: In correspondence of the proximal third of the fly side of the forearm, round formation with sharp margins. DT max 3.8 cm × 5.6 cm
- *FDG-PET:* metabolic radiocomposed localized in correspondence of the left forearm, with a diameter of 38 mm and with an SUV = 12.1
- *CT-scan: negative for distant metastases*

Question

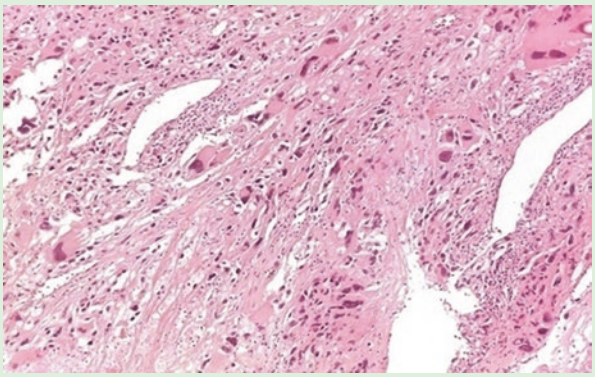
What action should be taken?

1. Surgery
2. Biopsy
3. Other

Answer

Biopsy

Pleomorphic saroma G3



Question

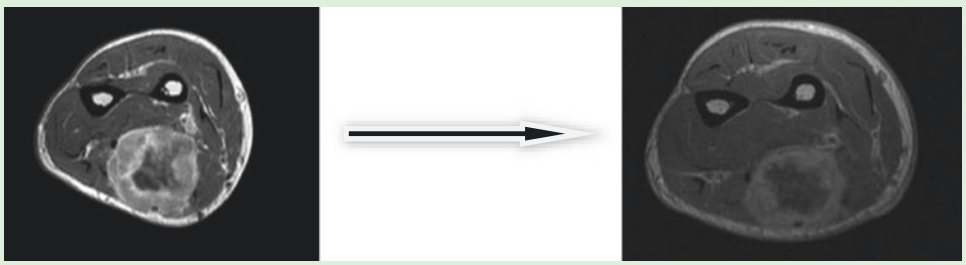
What action should be taken?

1. Surgery
2. Neoadjuvant treatment

Answer

Neoadjuvant treatment

- *Response evaluation after three cycles with epirubicin and ifosfamide:* partial response (Choi criteria)



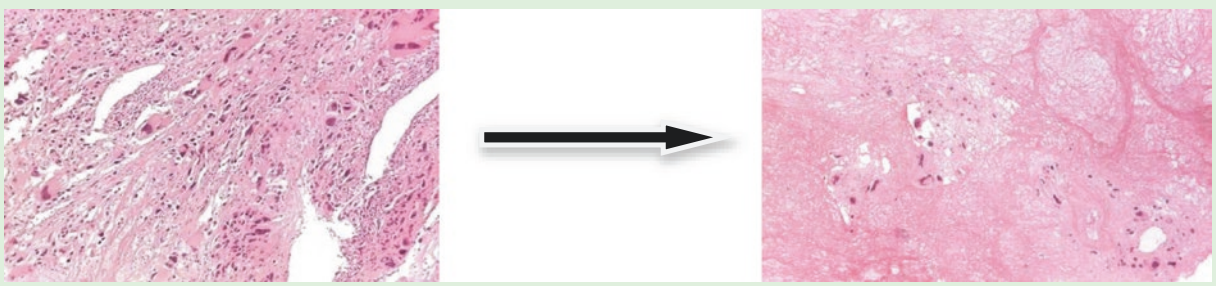
Question

What action should be taken?

1. Surgery
2. Radiotherapy
3. Continue chemotherapy

Answer

Surgery: Undifferentiated pleomorphic sarcoma with a high degree of malignancy, largely necrotic, with residual groups of vital cellular elements. Necrosis 95%. HWOS grade 3.



Pre-chemotherapy

Post-chemotherapy

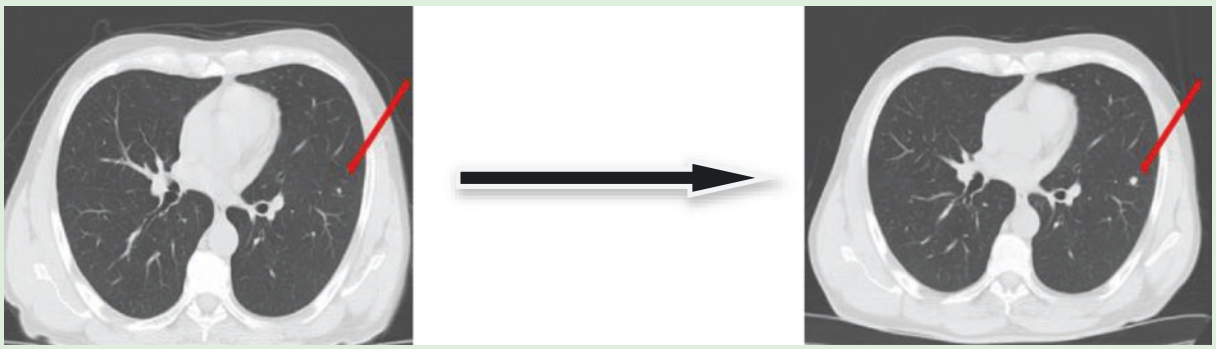
Key Points

- The importance of a correct diagnosis: biopsy is essential
- Considers a neoadjuvant treatment in the case of high grade sarcomas over 5 cm in diameter

Case Study

Man, 45 years old

- *Family history* negative for malignancy
- *APR:* 2 years ago, surgery for a leiomyosarcoma of the right arm followed by adjuvant chemotherapy
- *APP:* in the course of the follow up finding of a single growing pulmonary nodule



Question

What action should be taken?

1. Surgery
2. Radiotherapy
3. Chemotherapy
4. Biopsy

Answer

Surgery: Thoracotomy and transsegmental resection of the left lower lobe with diagnosis of metastases from leiomyosarcoma G2

Question

What action should be taken?

1. Follow up
2. Radiotherapy
3. Chemotherapy

Answer

Follow up

Key Points

- In case of single pulmonary metastases, consider surgery
- After pulmonary metastasectomy, chemotherapy is not a standard

58

Expert Opinion

Giuseppe Badalamenti

Key Points

- Soft tissue sarcomas (STSs) include over 80 histological rare entities, with even more molecular subsets, characterized by a low to very low incidence in all populations.
- A multidisciplinary approach is mandatory in all cases, involving pathologists, radiologists, surgeons, radiation therapists, medical oncologists, and pediatric oncologists, as well as nuclear medicine specialists and organ-based specialists.
- Surgery is the standard treatment of all patients with an adult type, localized STS. The standard surgical procedure is a wide excision with negative margins (no tumor at the margin, R0).
- Surgery (wide excision) can be completed with adjuvant RT in case of STS >5 cm diameter, G3, and deep localization.
- There is no consensus on the current role of adjuvant chemotherapy. Study results are conflicting, though some data available from smaller studies suggesting that adjuvant ChT might improve, or at least delay, distant, and local recurrence in high-risk patients. The choice of an adjuvant treatment must therefore be individualized especially in the case of chemosensitive histology.
- In the advanced/metastatic disease, the goal is palliative, and the decision-making is complex, depending on diverse presentations and histologies and should always be multidisciplinary. Monotherapy with anthracyclin remains the gold standard. The histology-driven treatment is an option in particular cases.

- (Ref. ESMO Clinical Practice Guidelines – Soft Tissue and Visceral Sarcomas)

Hints for Deeper Insight and Suggested Reading

- Soft Tissue and Visceral Sarcomas: ESMO-EURACAN Clinical Practice Guidelines for Diagnosis, Treatment and Follow-up. P.G. Casali, N. Abecassis et al., on behalf of the ESMO Guidelines Committee and EURACAN. Annals of Oncology 29 (Supplement

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Gastrointestinal Stromal Tumors (GISTs)

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Soft Tissue Sarcoma, GIST and Neuroendocrine Neoplasms

Contents

Lorena Incorvaia and Giuseppe Badalamenti should be considered equally co-first authors.

A. Russo et al. (eds.), *Practical Medical Oncology Textbook*, UNIPA Springer Series,
https://doi.org/10.1007/978-3-030-56051-5_59

Learning Objectives

By the end of this chapter, the reader will:

- Have learned the basic concepts of epidemiology, histological subtype, and molecular profile of gastrointestinal stromal tumors (GISTs).
- Have reached in-depth knowledge of diagnosis, staging, and clinical management of gist.
- Be able to put acquired knowledge on GIST into clinical practice

59.1 The Role of Medical Treatment in the Management of GIST

Lorena Incorvaia, Giuseppe Badalamenti, Sergio RizzoViviana Bazan and Antonio Russo

59.1.1 Introduction

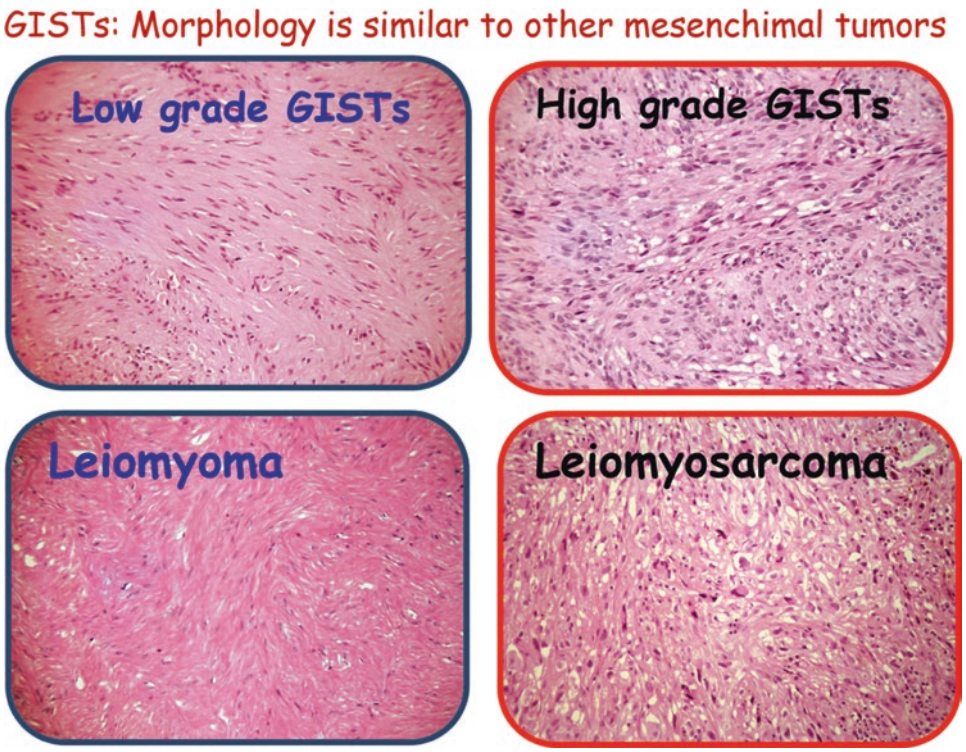
GISTs, while *relatively rare*, are the most common primary mesenchymal neoplasms of the gastrointestinal tract.

GISTs are typically *highly resistant to conventional chemotherapy*; the discovery of activating mutations in the *proto-oncogene KIT* and the development of *tyrosine kinase inhibitors* (*TKI*), such as imatinib, first introduced in 2002, revolutionized the treatment strategy for GISTs, by making possible to target the specific molecular events that are key events for pathogenesis of the disease.

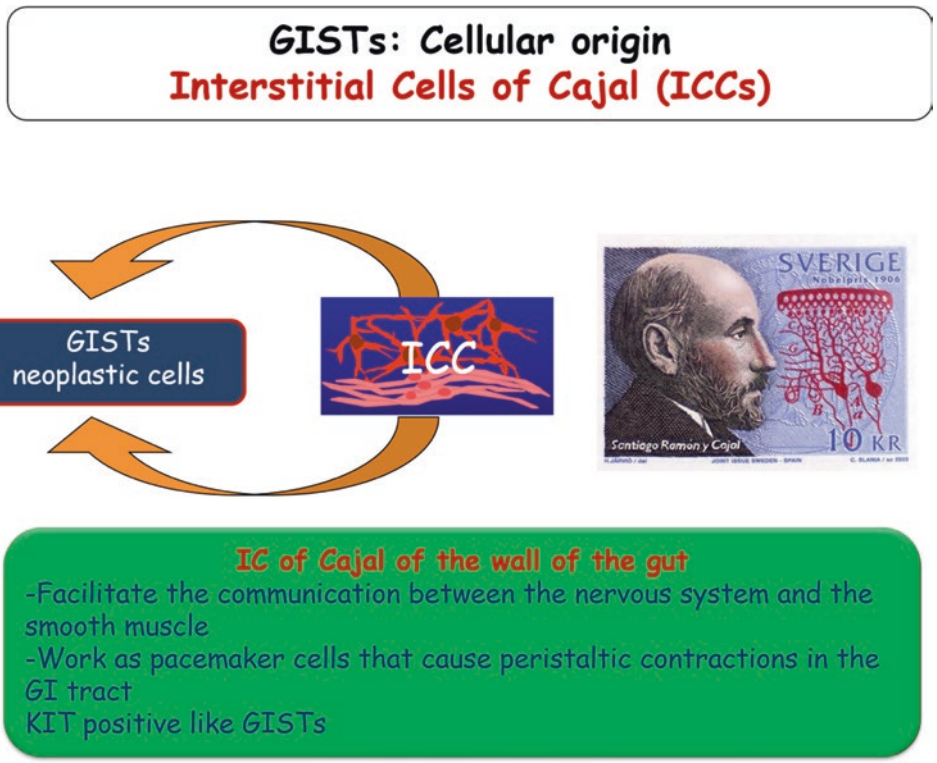
- GISTs can arise at *any age*, with a median of diagnosis *around 60–65 years.*
- More than 80% of the patients are older than 50 years.
- Occurrence in children is rare, and pediatric GIST represents a distinct subset, with the absence of KIT/platelet-derived growth factor alpha (PDGFRA) mutations, female predominance, and multifocal pattern of gastric GISTs [1, 2].
- GISTs can be found *anywhere in the gastrointestinal tract*, but the most frequent location is stomach (55%), followed by small intestine (30%). Less frequent are colon/ rectum (5%) and esophagus (<1%).
- Exceptionally rarely, GISTs can occur outside the gastrointestinal tract, such as in the omentum, mesentery, or retroperitoneal (<5%) (Fig. 59.1).

59.1.2 Origin

For many years, GISTs were initially classified as smooth muscle sarcomas, such as leiomyoma, leiomyoblastoma, or leiomyosarcomas.



Further studies identified similarity to a cell population in the gastrointestinal tract called *interstitial cells of Cajal* (*ICCs*), present in the wall of the gut. These cells facilitate the communication between the nervous system and the smooth muscle and work as pacemaker cells that cause peristaltic contractions in the GI tract. Data proving this relationship are based on similar histological findings and above all on the common expression of certain antigens such as CD117, the product of the oncogene c-KIT, and myoid antigens [3].



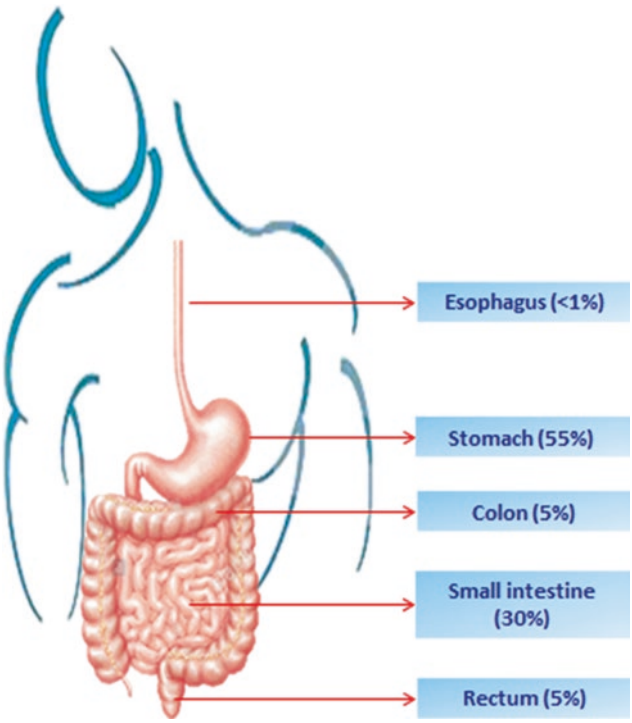


Fig. 59.1 GISTs distribution on the gastrointestinal tract

59.1.3 Pathological Features

Pathologically, the diagnosis of GIST relies on morphology and immunohistochemistry.

59.1.3.1 Macroscopic Aspects (Fig. 59.2)

59.1.3.2 Microscopic Aspects and Immunohistochemistry (IHC)

A. Microscopic evaluation reveals *three principal subtypes of GIST* depending on the cytomorphology: spindle cell, epithelioid cell, and the less frequent GISTs with mixed morphology, both spindle and epithelioid cells (Fig. 59.3).
B. Approximately, the *95%* GISTs are immunohistochemically *positive for the tyrosine kinase receptor KIT (CD117)*. About 5% of GISTs are, instead, negative for detectable KIT expression [4].
C. In the diagnosis of c-kit-negative cases, DOG1 expression is a new immunohistochemical marker with unknown functions selectively expressed in GISTs.

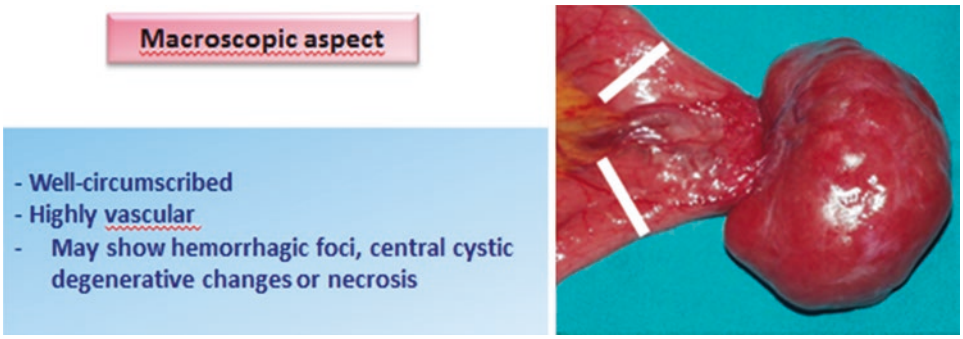


Fig. 59.2 Macroscopic appearance of a small bowel GIST. (Courtesy of Dr. A. Gronchi)

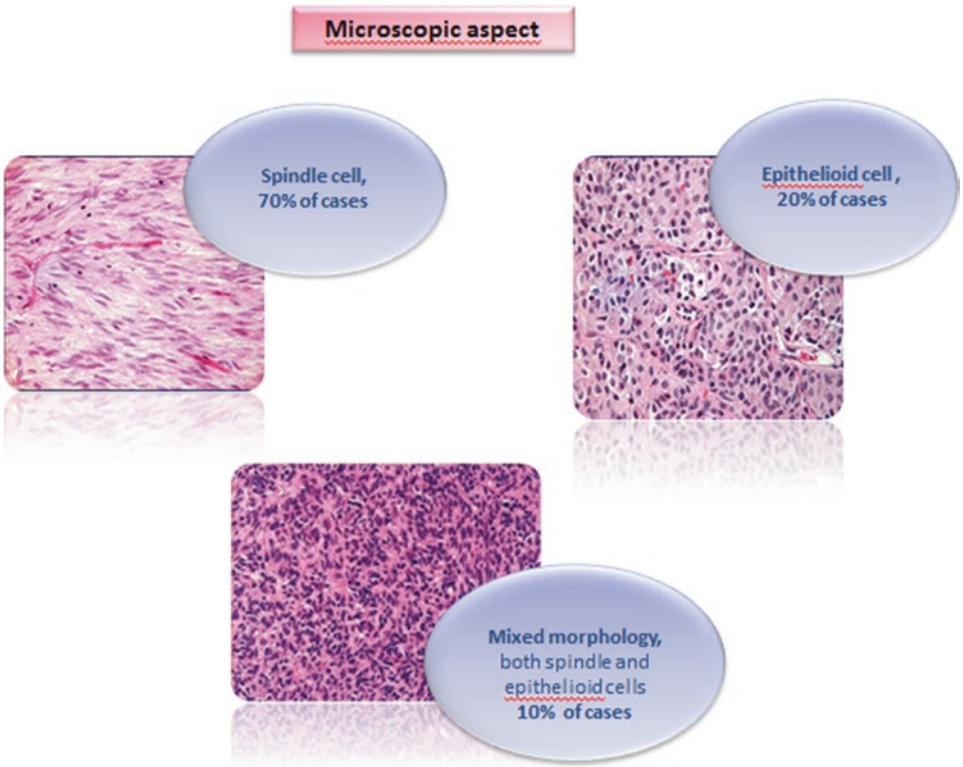
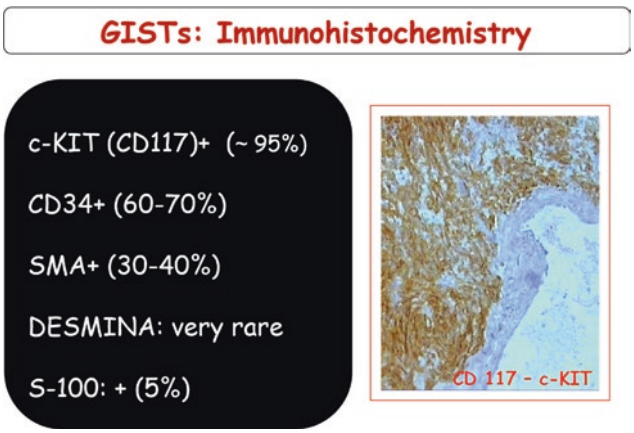


Fig. 59.3 Histological subtype of GISTs

Because the receptor KIT (CD117) is commonly expressed on GIST cells, it represents an important feature for a correct histological diagnosis [5]. Other antigens to be studied are CD34, an antigen common in hematopoietic stem cells, endotheliocytes, and fibroblasts, positive in 70–80% of GISTs, smooth muscle actin (SMA) positive in around 30% of GISTs, and usually reciprocal to CD34 and vimentin, while S100 and desmin expression is usually rare [2, 3].



59.1.4 Molecular Biology

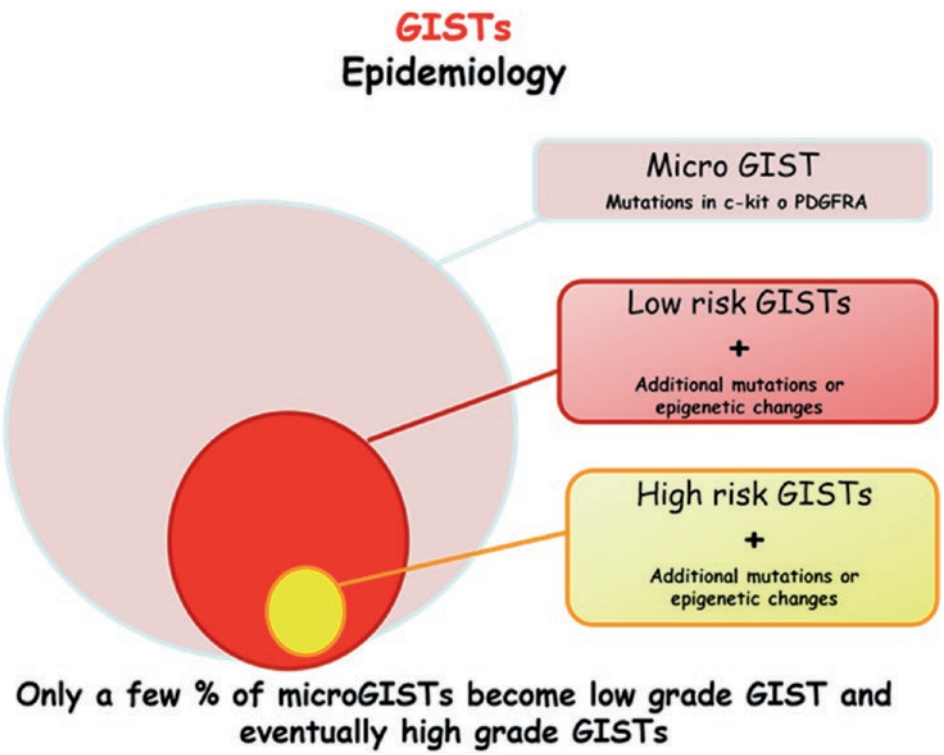
The identification of *activating mutations in the proto-oncogene KIT* in 1998 triggered a sea change in our understanding of the GIST pathogenesis and has resulted in a new paradigm for the use of molecular genetic diagnostics to guide targeted therapies.

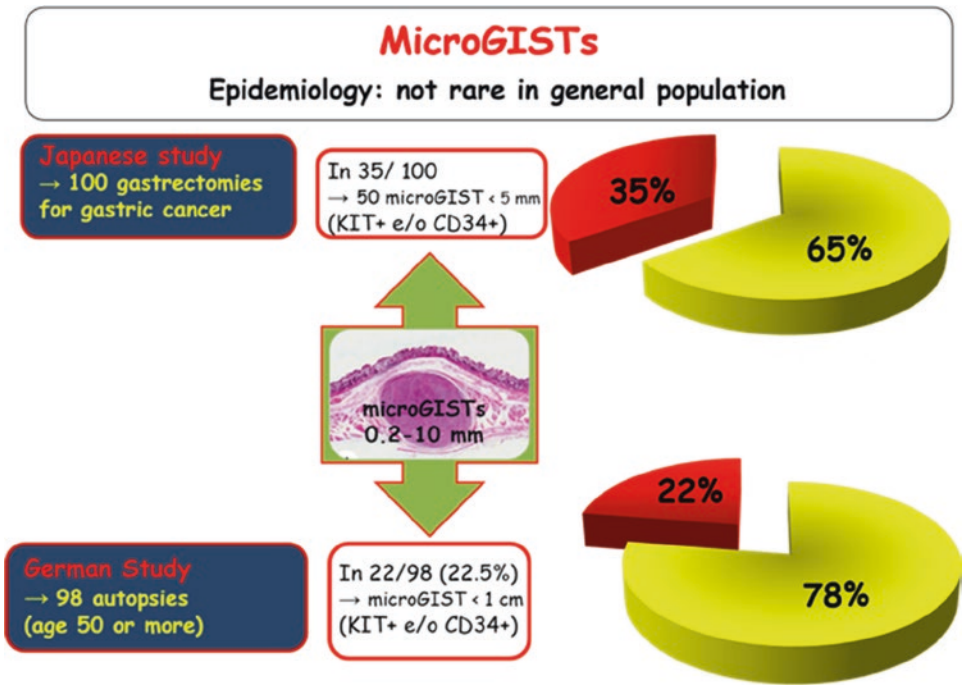
KIT gain-of-function mutations, together with those in *platelet-derived growth factor receptor A* (*PDGFRA*), are now well established as the *driver mutations* in the majority of GISTs [3, 6].

While pediatric and Mendelian inheritance-based GISTs are often wild type for PDGFRα and C-KIT and may be mutated in other genes such as *SDH*, sporadic GISTs often need a mutation of these genes as a fundamental step in their pathogenesis [7].

However, KIT and PDGFRα mutations are not sufficient for the development of a high-risk GIST since it seems other mutations or chromosomal aberrations are required. In fact, similar to the carcinogenetic model hypothesized for colon cancer by Vogelstein, a model of tumor evolution has also been proposed for GIST, that is, the high-risk GIST commonly seen in clinical practice would be the result of the evolution of a micro-GIST, usually characterized by the mutation of C-KIT or PDGFRα, to a low-risk GIST by acquiring new mutations such as secondary point mutations or epigenetic alterations and then to a clinically evident disease by new KIT or PDGFRα activating mutations, telomerase activation, or chromosomal aberrations [8].

Furthermore, the so-called micro-GISTs are probably extremely common in the population – about 30% in different studies – though only a very small number of these will progress to low- and then high-risk GISTs [9, 10].





The *frequency* of mutations in KIT and PDGFRA is different, and the mutations are mutually exclusive [11]:

- *Approximately 70%* of GISTs are driven by mutations in the *oncogene KIT.*
- Of those GISTs without KIT mutations, the majority harbor mutations in the gene encoding (*PDGFRA*) (15%).
- The remaining 15% of GISTs initially were genetically unclassified and described as KIT/PDGFRA "wild-type" GISTs. Today, with the expansion of our knowledge about molecular profile, further different and less frequent genetic mutations in other genes, such as *BRAF* and *KRAS,* have been recognized.

Therefore, at the state of current knowledge of molecular spectrum of mutations, GISTs can be divided into two distinct clusters: *succinate dehydrogenase (SDH)-competent* and *SDH-deficient subgroups*, each with distinct clinical and genetic characteristics (◘ Fig. 59.4) [3, 12].

1. *SDH-Competent GISTs*
   Heterogeneous group of tumors that primarily comprises KIT/PDGFRA/BRAF/NF1-mutated GISTs with normal genomic methylation patterns, in most cases presenting as sporadic tumors.
2. *SDH-Deficient GISTs*
   Characterized by a pattern of global, genome-wide DNA hypermethylation and are diagnosed primarily in pediatric patients or young adults. SDH-deficient GISTs almost always arise in the stomach, show prevalent epithelioid histology, and undergo early metastasis to liver and lymph nodes, with a relatively indolent long-term course [13].

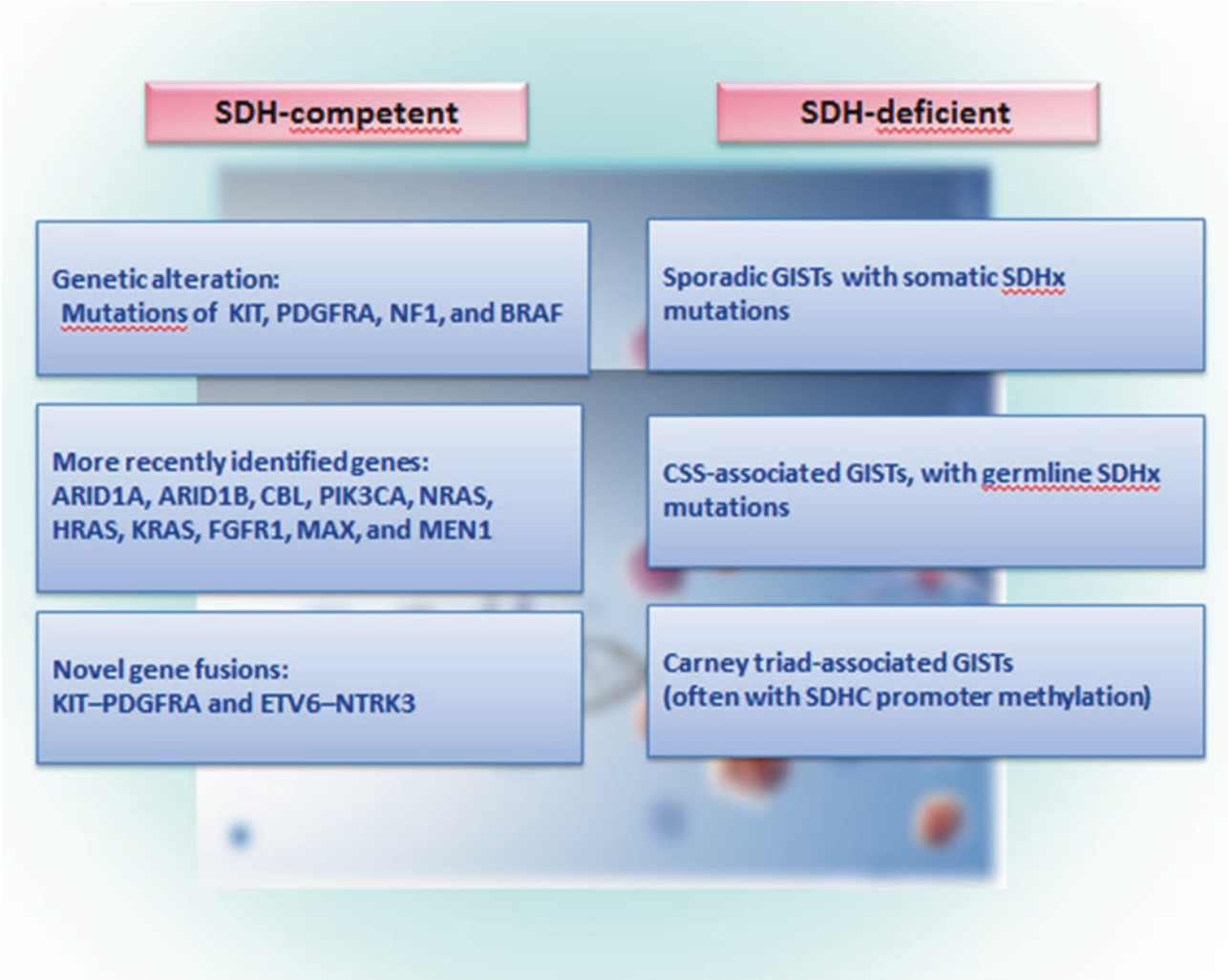
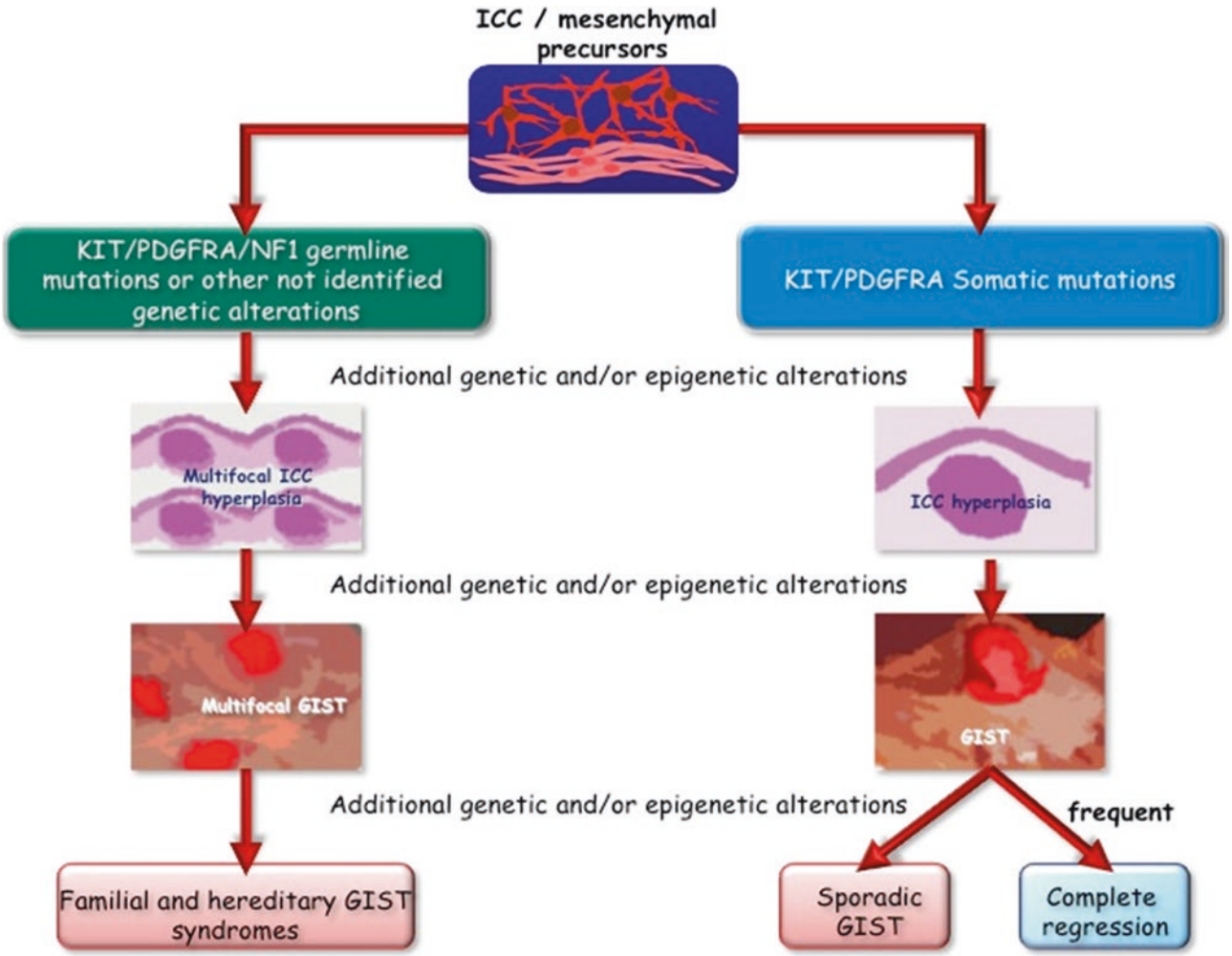


Fig. 59.4 Succinate dehydrogenase (SDH)-competent and SDH-deficient subgroups of GISTs

Features	Corney's triad	Corney's-Stratakis diad
GISTs	yes	yes
Paraganglioma	yes	yes
Pulmonary chondroma	yes	No
Hereditary	No	yes
Gender	> F	F = M
c-Kit or PDGFRA mutations	yes	No
Mutations on the SDH subunits SDHD, SDHC and SDHB	yes	In 9/11 families
SDH gene loss	In some cases	In some cases

Biology of Familial GISTs The initial role of mutations leading to the acquisition of function by the genes KIT or PDGFRA in the oncogenesis of GISTs is suggested by their transmission through the germinal line in different familial cases. Germinal mutations in these genes have been observed in 14 families. The mean age at diagnosis in patients with familial GISTs is 46 years. This familial form is not so common in children. Nevertheless, it is important to evaluate patients according to the effects and symptoms associated with germinal mutations in the genes KIT and PDGFRA, which include melanomas, freckles, urticaria pigmentosa, perioral and perianal hyperpigmentation, and achalasia. The various clinical manifestations in patients with germinal mutations in KIT are closely dependent on the specific domain of the KIT involved in the mutation. Aberrant mutations affecting the juxtamembrane domain (exon 11) are associated with mastocytosis and hyperpigmentation, apart from the generalized hyperplasia of the progenitor intestinal Cajal cells (ICC). Nevertheless, such symptoms do not seem to be present when the mutation involves the kinasic activity domain.

The initial phases of familial GISTs appear biologically similar to those of sporadic GISTs, with similar cytogenetic progression mechanisms and genic expression profiles.

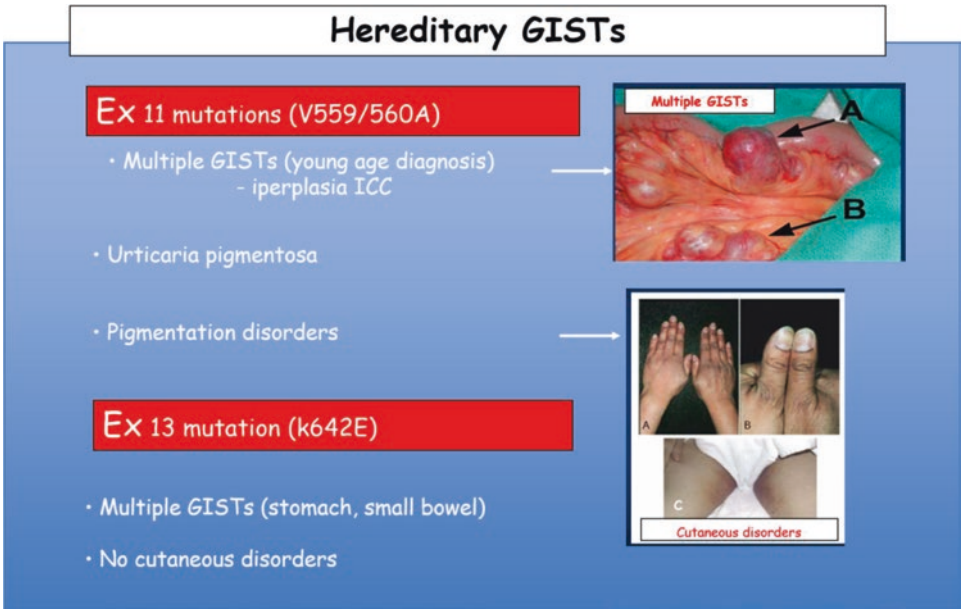


In familial GISTs, germinal mutations in KIT and PDGFRA are mostly similar to those found in sporadic forms. Two mutations which have never been found in sporadic GISTs, Asp419del in KIT and Tyr555Cys in PDGFRA, have, however, been identified in two families presenting hereditary GISTs. Furthermore, a recent study reports the case of a patient who developed lipomas and GISTs and who showed the germinal mutation Asp561Val in PDGFRA.

Two very similar models of transgenic mice have been developed in an attempt to identify the germinal mutations of KIT found in familial GIST syndromes

63. Such mutations are exactly the same as those found in patients with sporadic GISTs. Transgenic mice with these mutations maintain both their vitality and fertility and develop GISTs with a penetrance of about 100% 64.

The first case of familial GIST observed involved a Japanese family where the deletion of one of the two consecutive residues of valine (codon 559 or 560, GTTGTT) in exon 11 of KIT was identified throughout three generations. The subjects affected presented perianal hyperpigmentation and developed both malignant and benign multiple GISTs 65. A germinal mutation in the kinasic domain I of KIT has been identified in France in a 67-year-old woman and her 40-year-old son. Both these patients presented a dozen duodenal and jejunal GISTs and presented a constitutive substitution (K642E) in exon 13 of KIT 66 [10].



GISTs are not often diagnosed in children. Up till now, pediatric forms make up only 1% of all the identified cases. The current know-how regarding adult GISTs and correlated tumors, for example, paragangliomas, together with the development of new methods, such as microarray techniques, have led to remarkable progress in the comprehension of the rare pediatric forms. These may, however, show a different pathogenesis from that of adult GISTs, since apparently no mutations of *KIT* and *PDGFRA* are present (*wild-type* GIST). This might indicate that there exist other activation mechanisms of *KIT* or oncogenic *pathways* which are not linked to the gene and which are active within the cells. In the majority of pediatric GISTs examined, no other cytogenetic anomaly or alterations of exons 9, 11, or 13 of *KIT* have been identified. Of the 64 pediatric GISTs undergoing mutational analysis reported in literature, only 7 (11%) show a mutation in the genes *KIT* and *PDGFRA*. These mutations were equally distributed between exons 11 and 9 of *KIT* and were relatively common in *PDGFRA*. A homozygous punctiform mutation in exon 9 of *KIT* (C>T): Pro456Ser and a nonsense mutation in exon 18 of *PDGFRA* were found in two different cases of pediatric GISTs. This is a different model from that observed in adult sporadic GISTs, where *KIT* mutations are ten times as common as *PDGFRA* mutations.

59.1.4.1 KIT and PDGFRA

As mentioned before, the main initial event in GIST tumorigenesis are often gain-of-function mutations in *KIT* or *PDGFRA* genes, located on the long arm of chromosome 4 (4q12) (◘ Figs. 59.5 and 59.6).

- In GIST, the most common mutations are found in *KIT exon 11* (60–70%) that affects the juxtamembrane domain (Corless et al., 2011). The most frequent types of mutation are in-frame deletions, followed by single nucleotide substitution, resulting in constitutive activity of the kit receptor. Approximately 80% of exon 11-mutated tumors are located in the stomach and typically show more spindled than epithelioid histology.
- Mutations in *KIT exon 9* are the second most common following the exon 11 mutations. Account for 8–10% of GISTs, affecting the extracellular domain

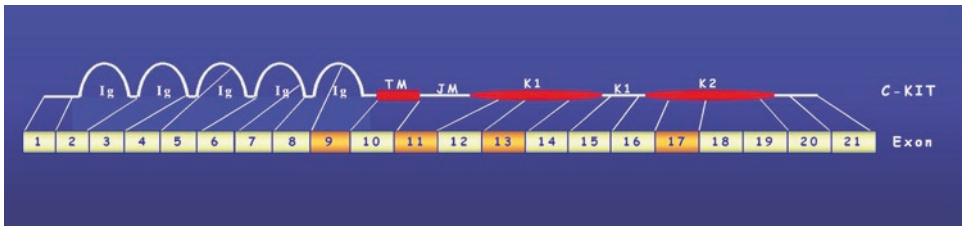


Fig. 59.5 C-KIT oncogene gene structure. The members of type III tyrosine kinase receptor family consist of a ligand-binding extracellular domain of 5 immunoglobulin (Ig) regions, an autoinhibitory intracellular juxtamembrane domain, and a kinase domain of an amino terminal ATP-binding region (activation loop)

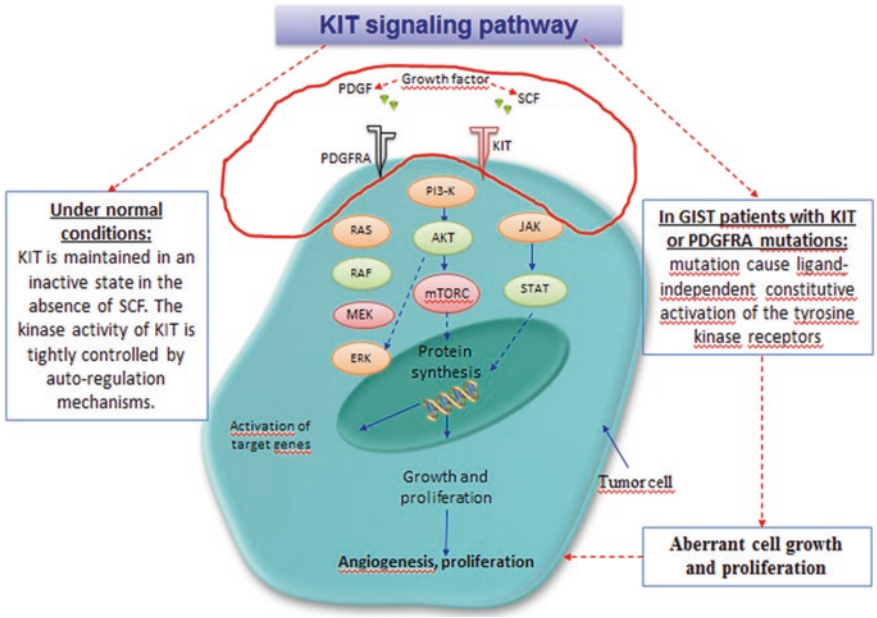
Fig. 59.6 KIT and PFGFRA signaling pathways

KIT and PDGFRA genes encode transmembrane glycoprotein receptor tyrosine kinase **(RTK).**

The **ligands** of KIT and PDGFRA are stem cell factor **(SCF)** and platelet-derived growth factor **(PDGF),** respectively.

Binding to the receptors results in **kinase activation**, initiating downstream signaling pathways, mainly through phosphoinositide 3-kinase (PI3K)/AKT, RAS/RAF/mitogen-activated protein kinase (MAPK) and signal transducer and activator of transcription 3 (STAT3) pathways, that promote cell **proliferation** and **survival**. PMID: 24267995

Mutations in the **KIT or PDGFRA genes** may result in expression of a protein with constitutive RTK activity and **aberrant cell growth and proliferation.** (Fig 1.6)



59

and 95% are duplications of codons 502 and 503 (Lux et al., 2000). These tumors have a higher prevalence in the small or large bowel.

Generally uncommon are the mutations is in *exons 13 and 17* of KIT (Corless et al., 2011).

- About 10% of GISTs harbor *PDGFRA mutations* (Heinrich et al., 2003b; Hirota et al., 2003). PDGFRA and KIT mutations are mutually exclusive. The majority of PDGFRA-mutated GISTs occur in the stomach, usually with epithelioid or mixed epithelioid and spindle cell histology. Although the activated pathways downstream are identical to KIT mutations, PDGFRA-mutated GISTs tend to have a lower risk of recurrence, and among metastatic GISTs, only 2.1% showed PDGFRA mutation compared with 82.8% in those with KIT mutations.

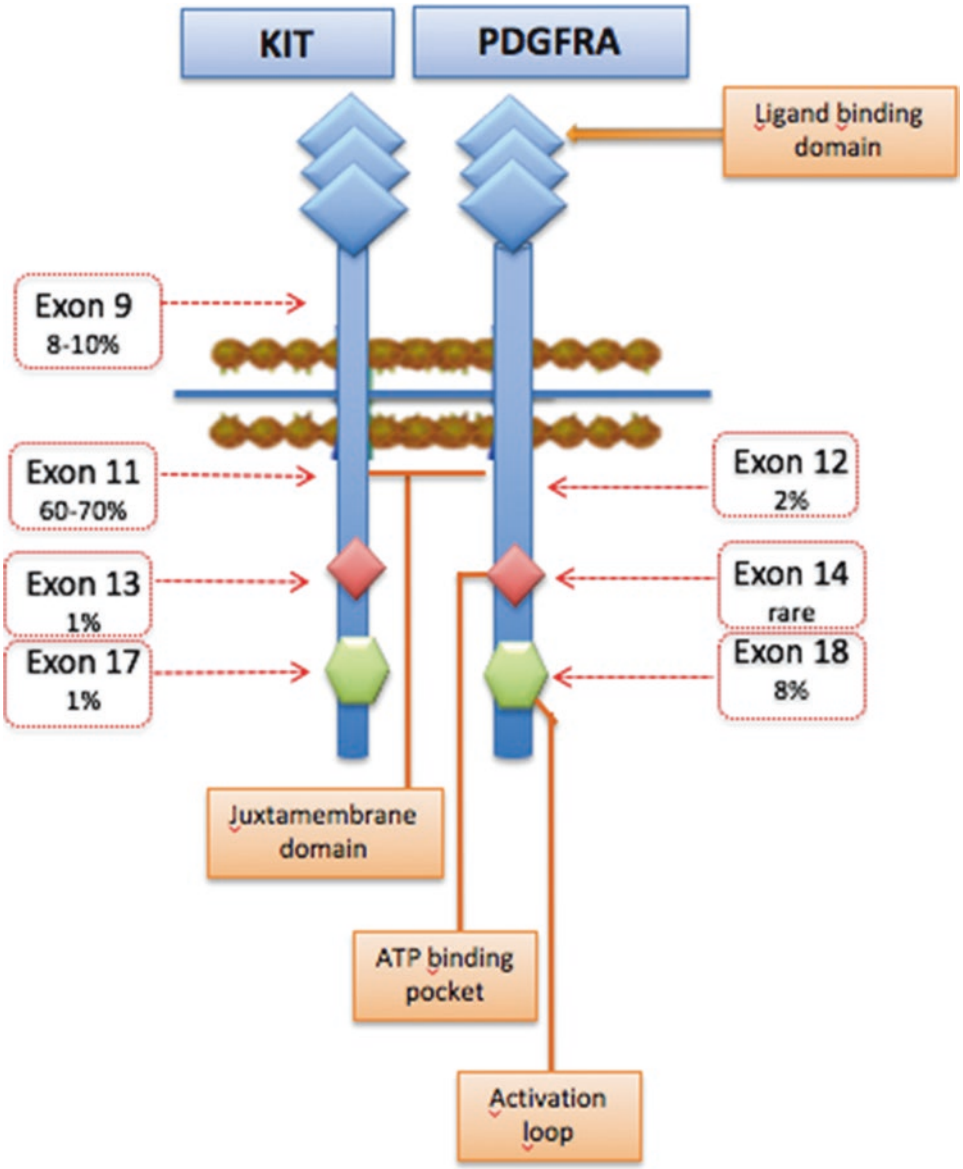


Fig. 59.7 Location and frequency of KIT and PDGFRA mutations

PDGFRA-mutated GISTs showed a variability of response to medical treatment. Most PDGFRA mutations in GISTs have been identified in *exon 18*: the most frequent mutation, *D842V*, represents 70% of PDGFRA mutations and 5% of metastatic GISTs and is the most common cause of primary resistance to therapy. The second most frequent mutation of exon 18, instead, the *deletion of codons 842 to 845*, confers imatinib sensitivity [14, 15] (Fig. 59.7).

59.1.5 Clinical Features

Unlike gastrointestinal carcinoma that has epithelial origin, GISTs are tumors of ► connective tissue, and therefore, most commonly grow extrinsically from the wall of GI tract. For this submucosal location, the GISTs achieve usually a large size without causing gastrointestinal obstruction or other symptoms typical of epithelial cancers (Fig. 59.8).

The *clinical presentation* of GIST is not characteristic and depends on the localization and the size of the tumor.

In contrast with epithelial carcinoma of the GI tract, which has an irregular mucosal or polypoidal growth with or without intestinal obstruction, GIST has a predominant *exophytic component* and displaces rather than invades the surrounding structures.

The GISTs *tumor size* at the time of diagnosis varies widely, from small nodules <2 cm to large masses, up to 30 cm in size (Corless et al., 2002).

The small tumors are, frequently, *asymptomatic* or associated with *nonspecific symptoms* and often diag-

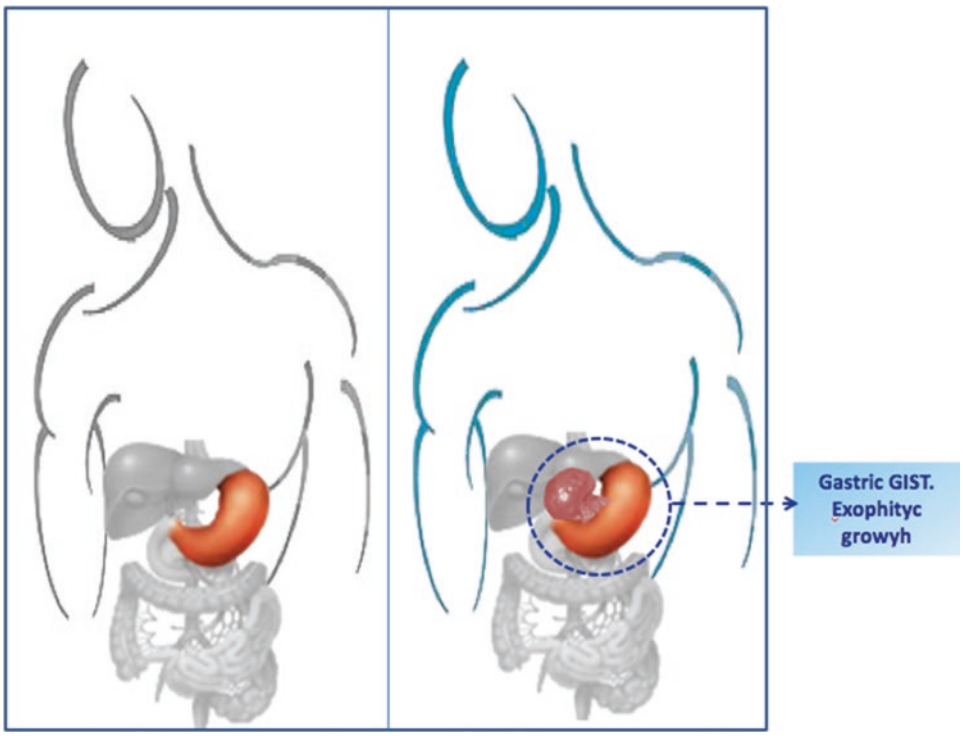


Fig. 59.8 Pattern of growth

nosed incidentally during endoscopic/surgical procedures or during radiologic studies performed to investigate manifestations of gastrointestinal tract disease.

Also for the voluminous tumors, the *symptoms* associated with GISTs are nonspecific and can include the following:

- Abdominal pain
- Nausea and early satiety
- Vomiting
- Anorexia and Weight loss
- Epigastric fullness

Localization of the tumor	Several clinical symptoms depending on localization of the tumor: for example, the esophageal tumors are present with dysphagia, odynophagia, retrosternal pain, and hematemesis; gastric tumors may cause epigastric pain, anorexia, nausea, vomiting, and weight loss
Obstruction	GISTs may also produce site-specific symptoms secondary to obstruction, for intraluminal growth of the tumors or for exophytic luminal compression (e.g., constipation in colorectal GIST or obstructive jaundice in duodenal GISTs)
GI bleeding	It can be produced by pressure and ulceration of the overlying mucosa with resultant blood loss and fatigue

In loss frequent cases, especially for large GISTs, the *GIST rupture* can occur into the abdominal cavity with life-threatening intraperitoneal hemorrhage [17].

59.1.6 Diagnosis

The diagnostic evaluation of gastrointestinal stromal tumors is based on imaging techniques, but the most important diagnostic tools remain the histology with the immunohistochemical examinations.

Small, asymptomatic lesions are usually discovered accidentally during endoscopy, ultrasonography, or computer tomography performed for other indications.

Endoscopy	Usually describes GIST as submucosal changes, in the majority of cases as oval protrusion, observed through the gastrointestinal lumen, with a covering mucosa often intact
Computed tomography	Shows these lesions as a solid mass with exophytic growth from the muscularis propria that displays contrast enhancement and may contain areas of necrosis (Fig. 59.9)
Endoscopic ultrasonography (EUS)	Besides endoscopy and computer tomography, it plays an important role in the diagnostic work-up of GISTs. Frequently, EUS shows GIST as hypoechogenic mass originating from different layers of the gastrointestinal tract wall, usually from the muscularis propria and muscularis mucosa, with an irregular outer margin and nonhomogeneous echo pattern

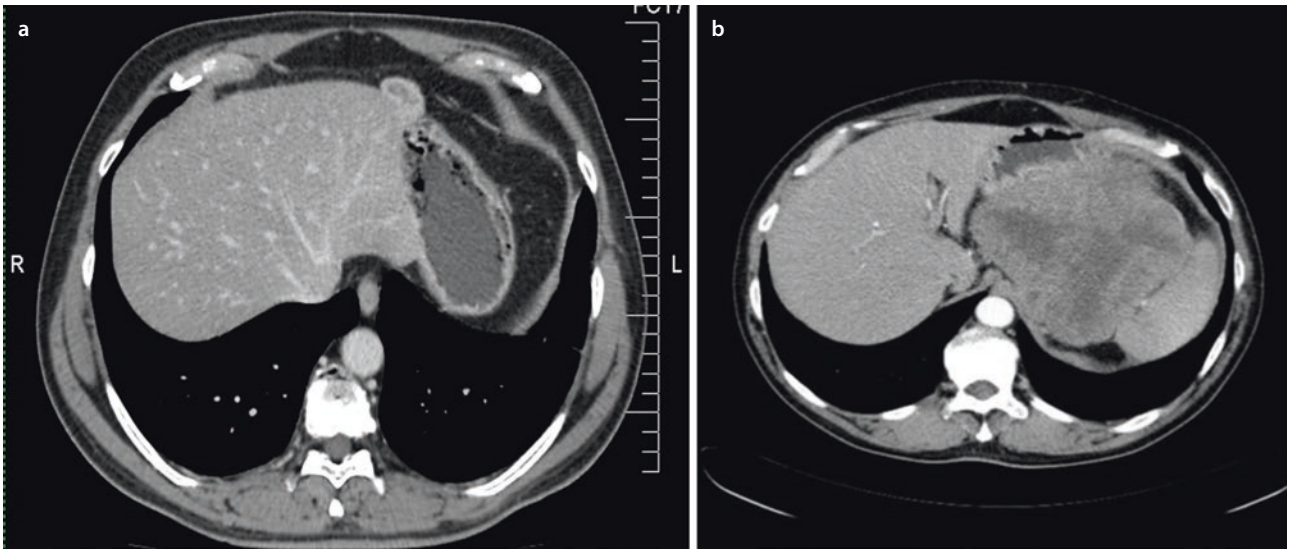
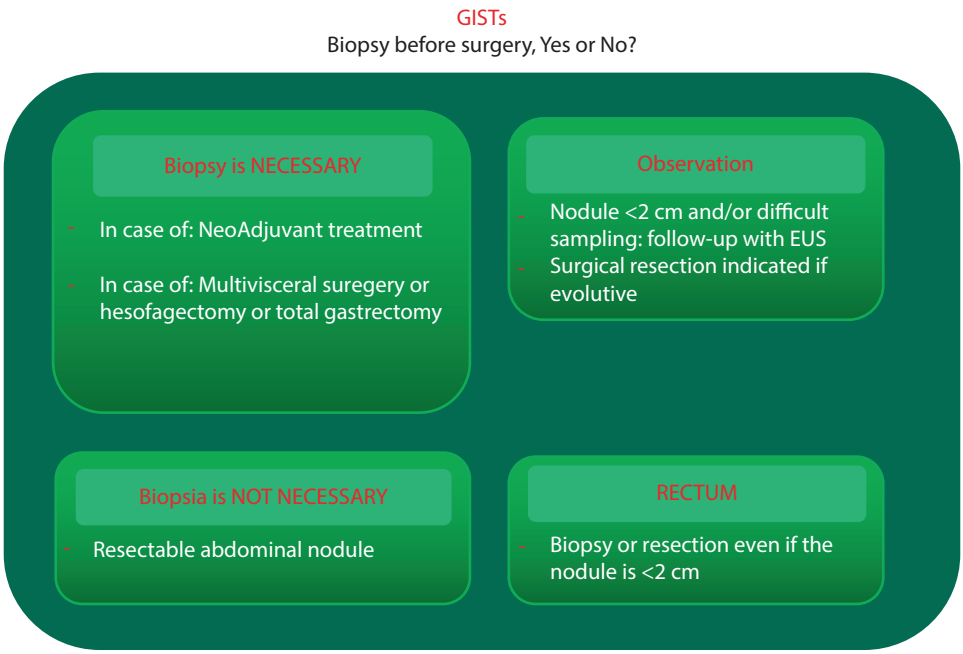


Fig. 59.9 **a** Small Gastric GIST. **b** Heterogeneously enhancing mass in the stomach, with necrosis

Magnetic resonance imaging (MRI)	May be an alternative to abdominal and pelvic CT scan. For rectal GISTs, MRI provides better preoperative staging information.

The final diagnosis is established on the basis of *histological examination of biopsy* with *immunohistochemical investigations.*



The evaluation of fluorodeoxyglucose (FDG) uptake using an FDG-positron emission tomography *(PET) scan*, or FDG-PET–CT, is useful mainly for early detection of the tumor response to molecular-targeted therapy [3, 17] (Fig. 59.10).

59.1.7 Prognostic Factors

Current ESMO guidelines do not recommend the use of TNM system for the classification and staging of GIST, due to the limitations of this system.

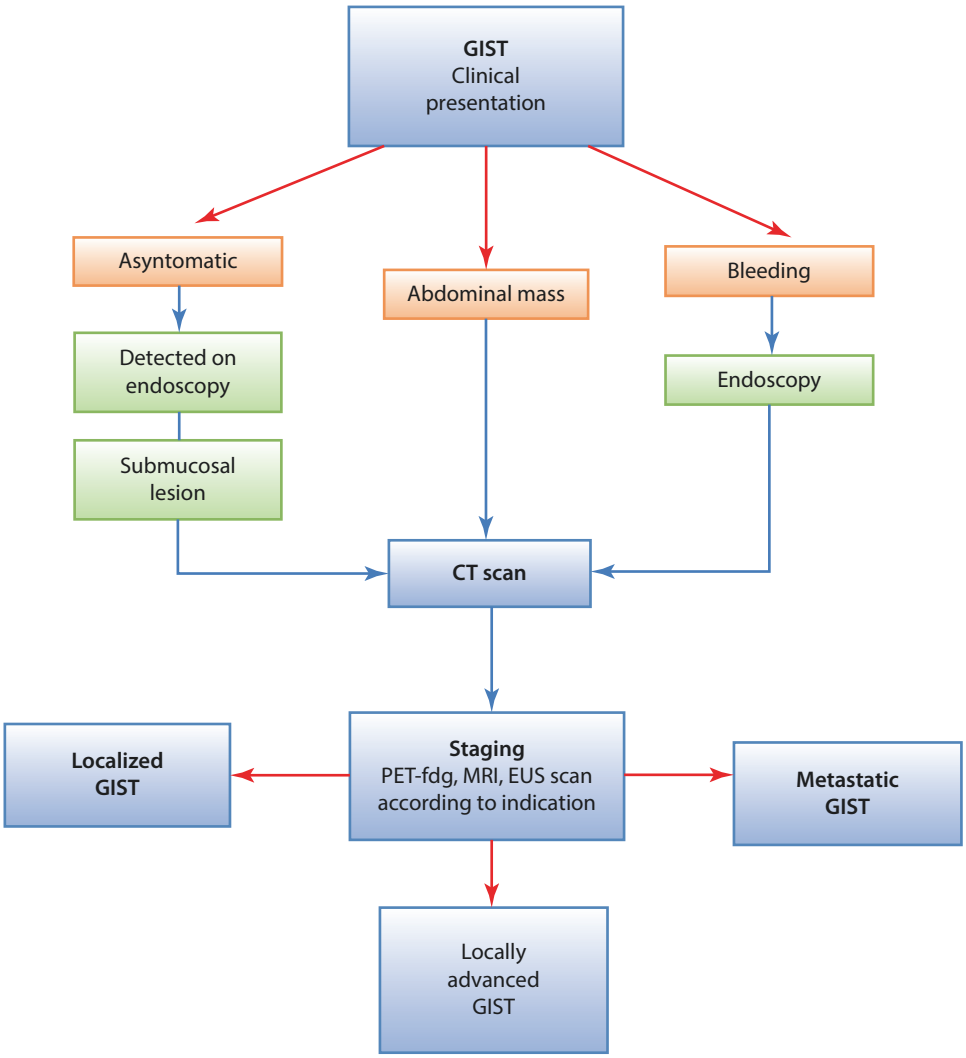


Fig. 59.10 Diagnostic evaluation of GIST. CT: computerized tomography; PET: positron emission tomography; MRI: magnetic resonance imaging; EUS: endoscopic ultrasound

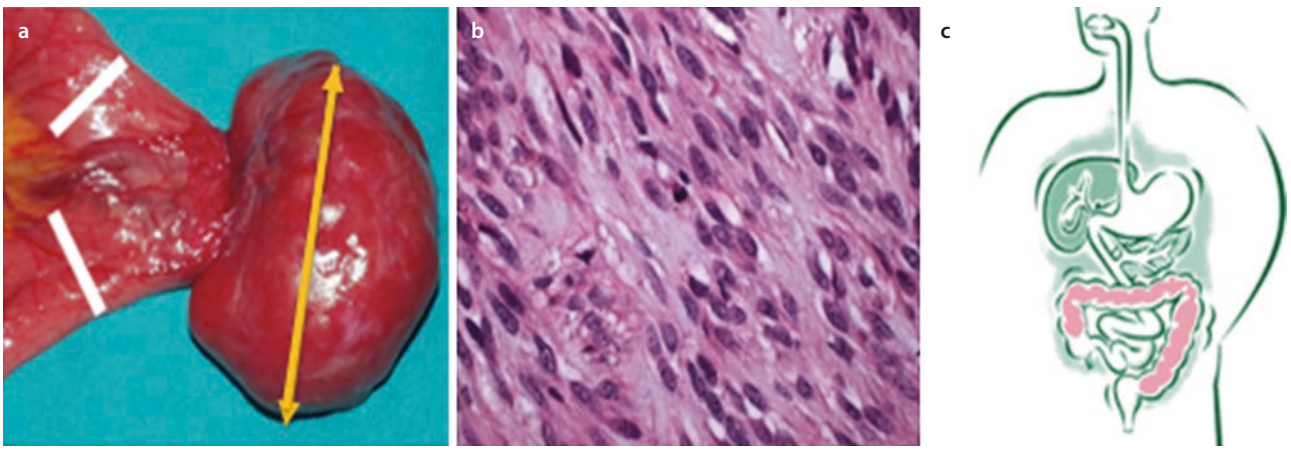


Fig. 59.11 Prognostic factors **a** tumor size; **b** mitotic activity; **c** anatomic site

Prognostic factors used for risk assessment affect the primary tumor site (Fig. 59.11):

- *Mitotic index*
- *Tumor size*
- *Tumor site: gastric* GISTs have a better prognosis than *small bowel or rectal* GISTs.
- *Tumor rupture* is an additional adverse prognostic factor.

This version of the risk assessment scheme is based on several large series published by Mietinnen and colleagues (2006) (Fig. 59.12), after integrated by Joensuu (Fig. 59.13).

More recently, prognostic heat and contour maps have been developed which should address issues associated with the nonlinear continuous variables of tumor size, mitotic index, and tumor rupture (Fig. 59.12).

Fig. 59.12 Joensuu's risk stratification for gastrointestinal stromal tumors

Risk category	Tumor size (cm)	Mitotic index (5 HPF)	Primary tumor site
Very low-risk	>2	<5	Any
Low-risk	2.1-5	<5	Any
Intermediate-risk	<5 5-10	6-10 <5	Gastric Gastric
High-risk	Any >10 Any >5 5.1-10	Any Any >10 >5 >5 >5	Tumor rupure Any Any Any Non-gastric Non-gastric

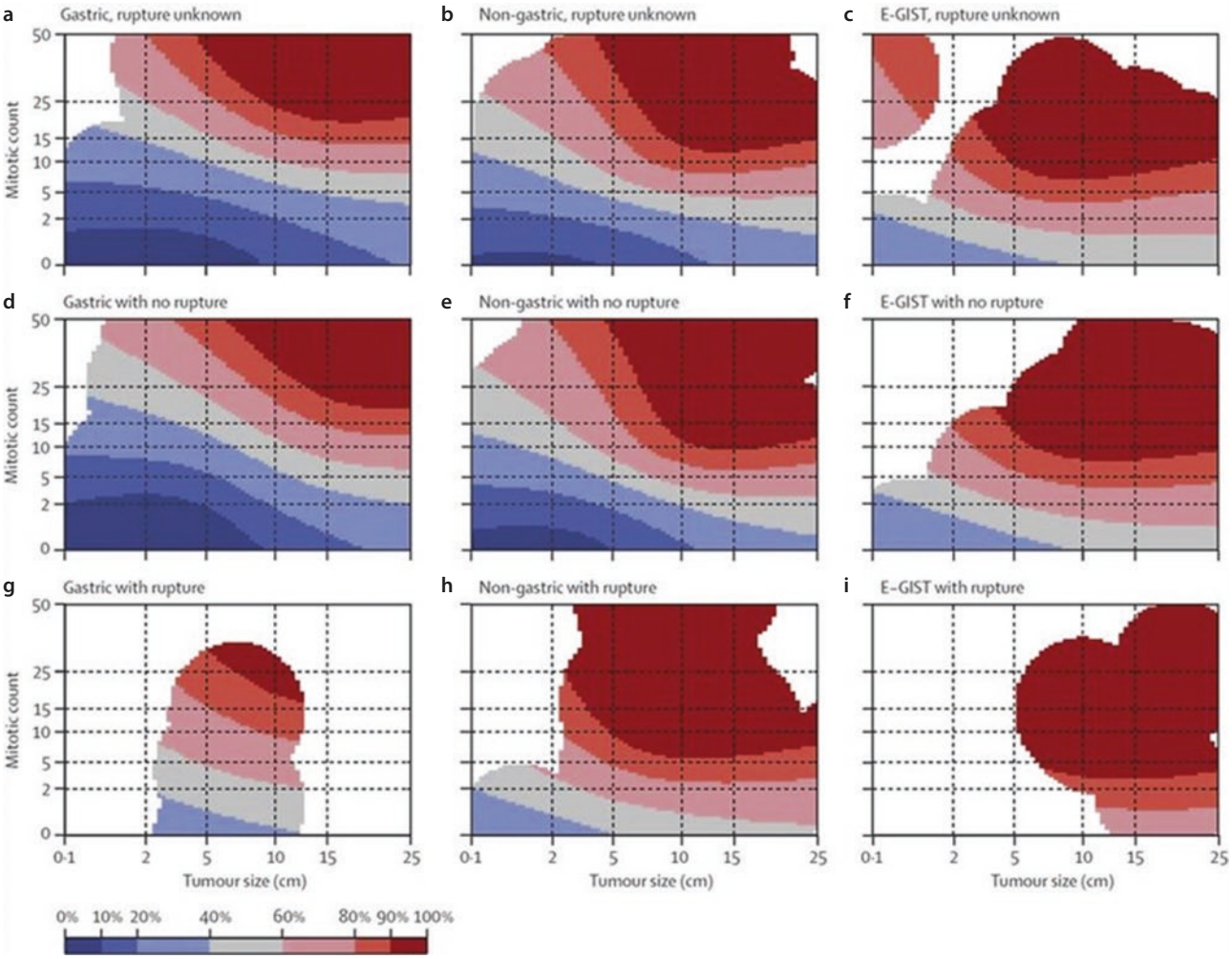


Fig. 59.13 Prognostic heat map for the risk assessment

In the future, also the *molecular profiling* of GISTs should be considered in risk classification systems. For example, GIST with exon 11 mutation has a higher risk of relapse than GIST WT.

Tumor mutational status is particularly important in GIST because it is predictive of response to TKI treatment, but has also a prognostic value: the type of mutation affects prognosis in metastatic disease. Patients with advanced GISTs and *KIT exon 11 mutation* have the superior prognosis and the longest progression-free survival (PFS) compared with *exon 9 mutations* or patients lacking both KIT and PDGFRA, who have less favorable PFS [15].

59.1.8 GIST Management

Prior to the advent of the tyrosine kinase inhibitors (TKIs), there were few treatment options available to patients with advanced GIST; the response rate to conventional chemotherapy agents was extremely low and the survival generally measured in few months [16].

Advances in understanding the molecular background of GIST allow the identification of abnormal receptor tyrosine kinase (RTK) signaling and the development of specific TKI, such as the first approved imatinib, that has become a paradigm for molecularly targeted therapies in solid tumors [17].

59.1.8.1 Focus on Imatinib (◘ Fig. 59.14)

59

59.1.9 The Medical Treatment

59.1.9.1 Advanced and Metastatic GIST

In locally advanced inoperable and metastatic GIST patients, imatinib is the standard first-line treatment. The standard dose of imatinib is 400 mg daily. A higher dosage (800 mg/day) demonstrated a PFS advantage for KIT exon 9-mutated GISTs, despite no difference in overall survival (OS) and is endorsed by the NCCN, ESMO, and AIOM guidelines.

Treatment should be continued indefinitely, since treatment interruption is generally followed by relatively rapid tumor progression.
Imatinib achieved disease control in 70–85% of patients, but, despite the high response rate, the median time to progression (TTP) is approximately 24 to 30 months.
Median OS is approximately 57–60 months
5–23% of patients show a durable response lasting for more than 10 years.
10–15% of patients show progressive disease to imatinib within 3/6 months of starting therapy (primary resistance) and show stronger correlation with certain genotypes. These tumors most commonly are those with mutations in PDGFRA, particularly the D842V mutation in exon 18, or those lacking mutations in either KIT or PDGFRA.

- Despite the high efficacy of imatinib, virtually all metastatic GISTs will become resistant due to additional acquired mutation in KIT.

Secondary or acquired resistance to imatinib, it develops in the large proportion of patients who demonstrate disease control and ultimately develop progressive disease, usually within 2–3 years [18].

59.1.9.2 Molecular Profile of Primary and Secondary Resistance

The **primary resistance** arises in GSTs with no identifiable KIT or PDGFRA mutations is likely due to different mechanisms causing the disease development and activation of alternative signaling pathways. Therefore, treatment of these GISTs with the targeted agents other than imatinib, such as VEGFR, BRAF or MEK inhibitors, might be a better clinical alternative (Janeway et al, 2009)

In 1996, Druker and colleagues published their identification of a small molecule TKI, now known as imatinib, that can *selectively block the ABL kinase activity and induce cell death of BCR-ABL positive chronic myeloid lymphoma (CML) cells* (Druker et al, 1996).

Concurrently imatinib was shown **not only specific to BCR-ABL,** but *also blocks the enzymatic activity of the trasnmembrane receptor tyrosine kinases KIT and PDGFRA.* (Buchdunger, 2000; Heinrich, 2000a)

Imatinib binds to the ATP-binding site located in the amino-terminal lobe of the kinase domain that **competitively blocks ATP binding and consequent phosphorylation of KIT** (fig. 1.11).

Inhibition of mutant receptor KIT by imatnib led to GIST cell growth arrest and apoptosis (Tuveson, 2001).

Therafter, clinical development of imatinib for GIST therapy repidly progressed and has been considered the **standard first-line therapy** *for inoperable or metastatic GISTs since its approval in 2002.*

IN 2008, FDA approved ***adjuvant use of imatinib*** for patients with high risk of recurrence.

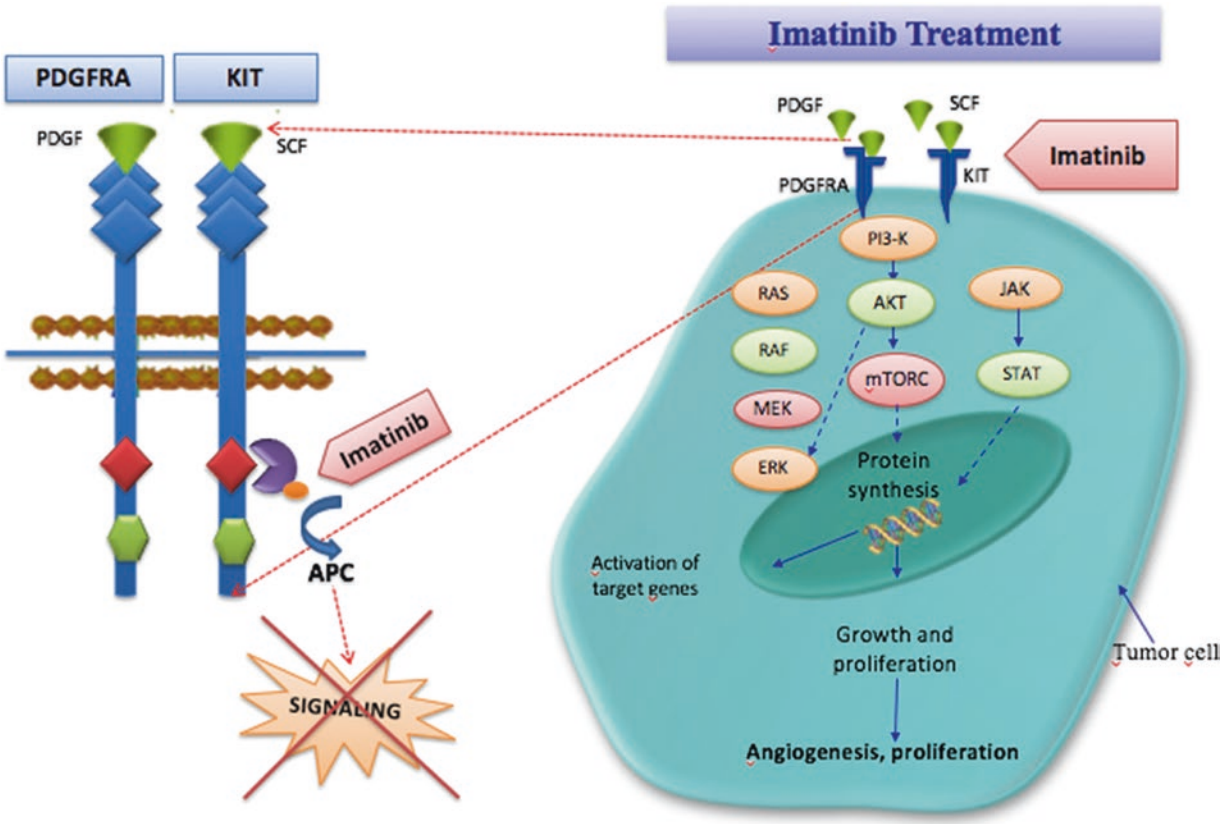
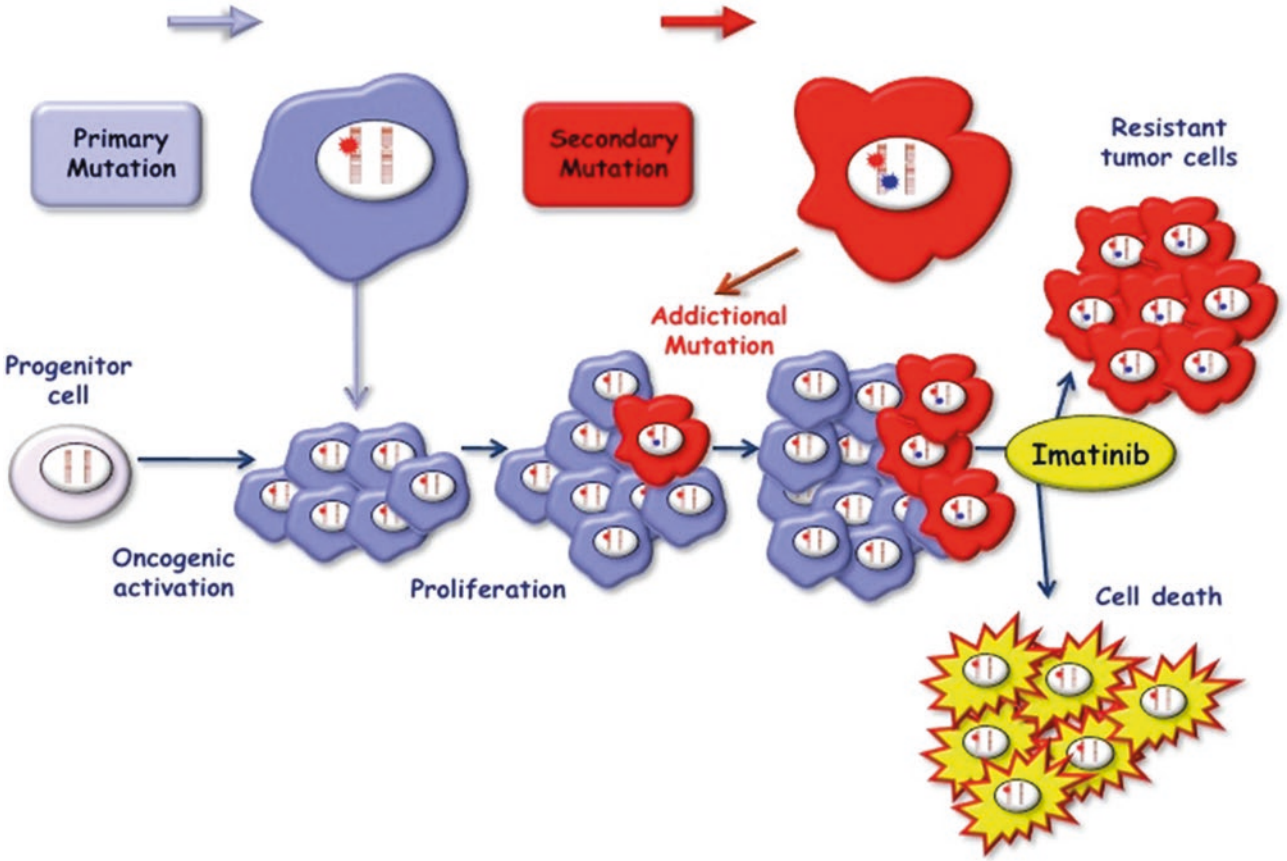


Fig. 59.14 Mechanism of action of imatinib

- Mutations in *exon 9* affect the extracellular KIT domain, mimicking the conformation change when SCF binds to the receptor, which induces higher degree of dimerization (Yuzawa et al., 2007). Since this mutation does not interfere with the kinase domain, exon 9 mutated KIT has the kinase domain same as the wild-type KIT, in which decreased sensitivity to imatinib was observed in vitro compared to exon 11 mutant KIT (Corless et al., 2011). Dose escalation is suggested for treatment of GISTs harboring these mutations (MetaGIST, 2010).
- Both clinical and in vitro studies have reported that *PDGFRA D842V mutation* is strongly resistant to imatinib (Corless et al., 2005; Heinrich et al., 2008a; Weisberg et al., 2006). This mutation results in a change in the kinase activation loop that strongly favors the active conformation of the kinase domain, which consequently disfavors imatinib binding (Gajiwala et al., 2009; Heinrich et al., 2003a). Patients with D842V-mutant GISTs show low response rates and short progression-free and overall survival during imatinib treatment (Biron et al., 2010).
- In addition to mutations, *gene amplification of KIT or PDGFRA* was shown as a potential mechanism leading to either primary or secondary resistance (Debiec-Rychter et al., 2005; Liegl et al., 2008; Miselli et al., 2007).



Secondary mutations is the main known mechanism for deveoping imatinib acquired resistance (Antonescu et al, 2005; Grimpen et al, 2005; Heinrich et al. 2006).
The most common mechanism is the occurrence of secondary mutations in the same gane that was originally activated and that render these clones resistant to imatinib (clonal evolution).
The most common secondary mutations occur in the ATP-binding pocket (encoded by exon 13 and 14) and in the kinase activation loop (encoded by exons 17 and 18).

59.1.9.3 Type of Progression

Most of the imatinib-resistant tumors exhibit inter- and intratumor heterogeneity (Liegl et al., 2008; Loughrey et al., 2006; Wardelmann et al., 2006): different types of secondary mutations across the multiple nodules of the same patient, and in different areas of the same tumor, cause the onset of resistant subclones.

This heterogeneity has important implications onto the efficacy of second-line TKI therapy after the first-line imatinib treatment.

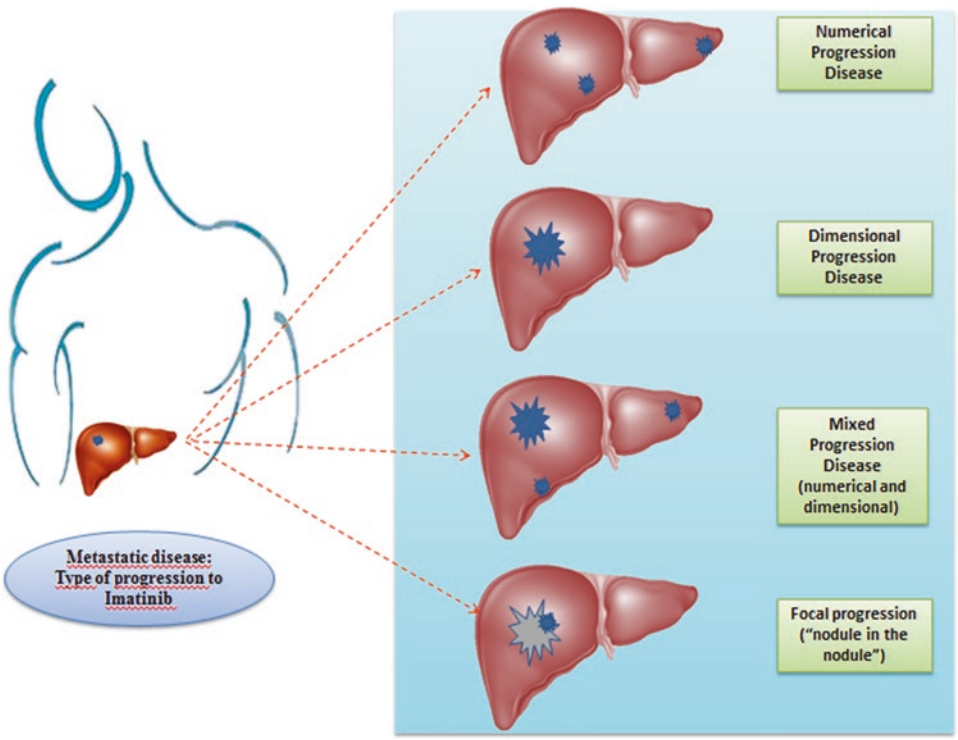
The type of progression disease (PD) evaluated with CT scan can be distinguished into different groups:

- *Dimensional PD*: characterized exclusively by dimensional growth of pre-existing lesions
- *Numerical PD*: characterized by the occurrence of new lesions
- *Mixed PD*: characterized by both dimensional and numerical PD
- Exists also a "focal progression" into a lesion in previous response to the treatment, the so-called nodule in the nodule (◘ Fig. 59.15)

59.1.9.4 Strategies to Overcome the Resistance

- *Second-line treatment*
  For GIST patients who progress on the standard dose of imatinib (400 mg daily), both imatinib dose escalation (800 mg daly) and sunitinib are feasible options.
- *Imatinib 800* mg daily should be considered for patients who was started on first-line imatinib 400 mg daily and experienced disease progression, on the basis of two large dose finding randomized phase III trials 14–15.
- *Sunitinib*, an oral multitarget tyrosine kinase inhibitor with high selectivity for KIT and PDGFRα, is an alternative strategy to overcome resistance for imatinib-refractory patients. In a randomized phase III trial, sunitinib 50 mg 4 weeks on and 2 off improved significantly PFS over placebo in second-line setting for those patients who had progression to first-line imatinib 17. However, sunitinib 37.5 mg continuously seems to be similarly effective and safe to sunitinib standard dose.
  The degree of disease control, including length of PFS and median OS, is significantly higher in patients whose GIST is with primary exon 9 mutation in KIT or those with no mutations in either KIT or PDGFRA.
- *Third-line treatment*
  Regorafenib is a recent third-line standard of care for metastatic GISTs resistant to both imatinib and sunitinib [19].

  Besides KIT and PDGFRA, this TKI also inhibits VEGFR1–3, TEK, RET, RAF1, BRAF, and BRAFV600E and FGFR (Wilhelm et al., 2011). Similar to sunitinib, regorafenib delayed the progression of patients for only 3.9 months compared to the placebo treatment (Demetri et al., 2013).



◘ **Fig. 59.15** Type of progression to imatinib in metastatic disease

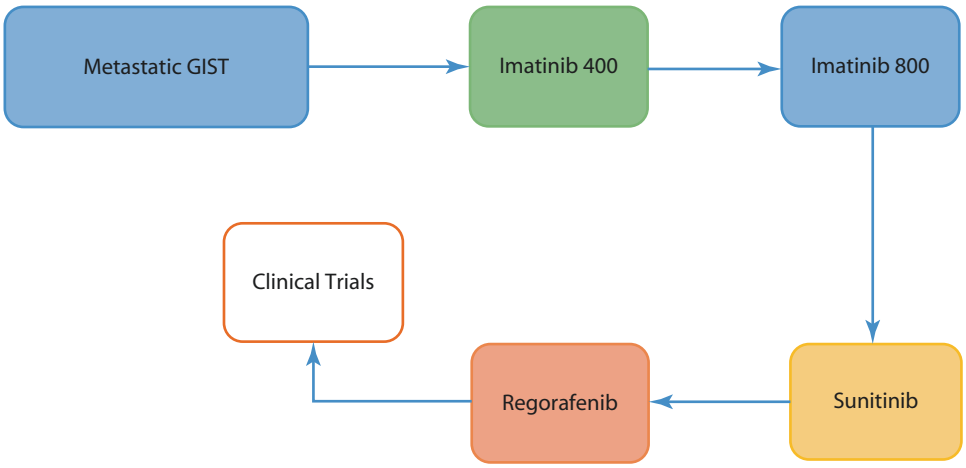


Fig. 59.16 Therapeutic algorithm for Metastatic GIST

For patients progressing to regorafenib, inclusion in clinical trials is indicated. In the absence of clinical trials, an option may be the treatment rechallenge with imatinib [20, 21] (Fig. 59.16).

59.1.9.5 New Therapeutic Targets and Treatments to Overcome Resistance to TKI

Several alternative TKI targeting KIT/PDGFRA (nilotinib, masatinib, sorafenib, dovitinib, pazopanib), multiple RTK (crizotinib, cabozantinib), or downstream signaling pathways (buparlisib, alpelisib, binimetinib) were studied in GIST patients with resistance to approved TKI.

Many clinical trials testing the compounds alone and in combination are ongoing, but unfortunately, none of these drugs has been registered for GIST treatment.

Novel agents, with an enhanced activity against specific secondary KIT/PDGFRA mutations, are currently being evaluated in preclinical and clinical settings [22].

Ponatinib	Multitarget inhibitor (PDGFRA, VEGFR2, FGFR1, and Src) approved for TKI-refractory leukemia. Potently inhibits KIT exon 11 primary mutants and a range of secondary mutants and has been shown to induce regression in engineered and GIST-derived tumor models containing these secondary mutations. Demonstrated a clinical benefit rate (CR, PR, or SD ≥16 weeks) of 55% in patients with primary KIT exon 11 mutation
Crenolanib	Inhibits the imatinib-resistant PDGFRA p.D842V-mutated kinase and also reduced the expression of KIT/PDGFRA by inhibiting MAPK and stabilizing ETS translocation variant 1 (ETV1) in mutated GIST. A phase III study is currently ongoing
BLU-285 (a vapritinib)	Highly selective inhibitor of KIT exon 17 mutations was also found to inhibit PDGFRA p.D842V mutant activity. Preliminary data from clinical trial showed a tumor reduction in all PDGFRA p.D842V-mutated patients
PLX9486 (Plexxikon)	Had an inhibitory effect on proliferation in a TKI-resistant PDX model (KIT exon 11 + 17), where its activity was more pronounced than imatinib. Currently, is evaluated alone and also in combination with pexidartinib
DCC-2618 (ripretibib)	Switch-control tyrosine kinase inhibitor active against a broad spectrum of KIT and PDGFRA mutations, under evaluation in clinical trials

59.1.9.6 Role of Medical Treatment in Localized Disease

Given the efficacy of imatinib in the metastatic setting, the use of imatinib has been extended to the *adjuvant setting* for the treatment of adult patients following GIST resection.

Risk stratification is essential to identify and better define the patients with GIST who are most likely to benefit from adjuvant imatinib therapy.

Three randomized phase III clinical trials have examined the use of imatinib 400 mg daily as an adjuvant for 1, 2, and 3 years; all three showed that it extends recurrence-free survival (RFS) in comparison with placebo or surveillance.

Additionally, the initial and long-term results provided by the AIO study demonstrated that 3 years of imatinib significantly improves RFS and OS compared with 1 year of therapy.

According to survival findings in the AIO trial, 3 years of adjuvant imatinib therapy are recommended for patients with GIST with high-risk features.

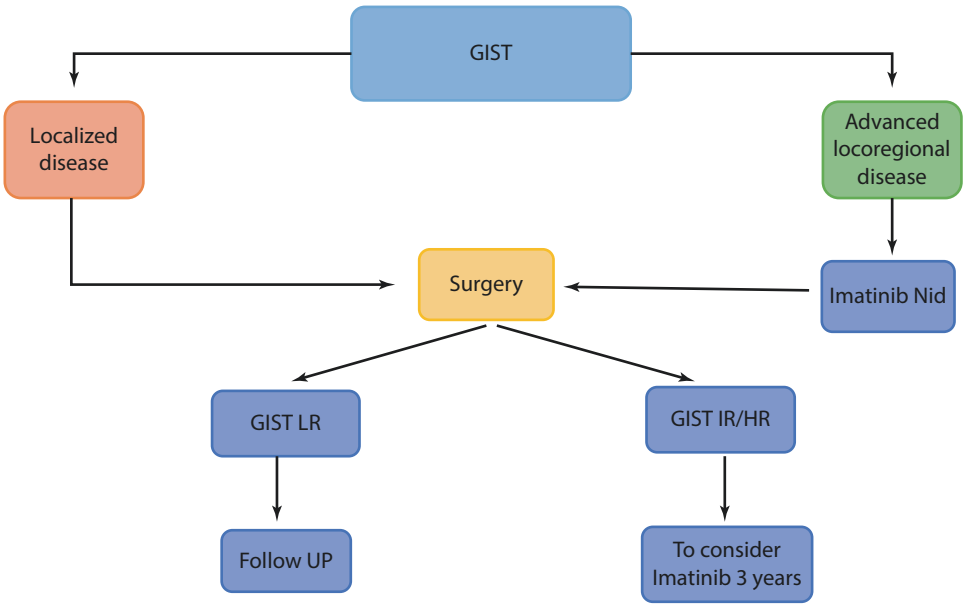


Fig. 59.17 Treatment strategy for GIST; LR: Low risk; IR: intermediate risk; HR: high risk; Njd:

Moreover, two randomized trials are ongoing in high-risk GIST patients: a Scandinavian study comparing 5 years with 3 years and a French study comparing 6 years to 3 years of imatinib.

The use of adjuvant imatinib is not recommended for low risk and very low risk, but there is no consensus for intermediate risk. In this situation, the risks and benefits of treatment should be shared with the patient.

In the *neoadjuvant setting,* its preoperative use is proposed in tumor bulk reduction in order to ease complete surgical resection or make organ preservation more likely in initially unresectable or borderline resectable disease. Imatinib should be continued for 6–9 months but not extended beyond 12 months because of the risk of imatinib resistance and of usually minor additional tumor shrinkage

If an adjuvant or neoadjuvant treatment is indicated, the mutational analysis is required to predict the response to treatment with imatinib [23, 24] (Fig. 59.17).

neoadjuvant

59.1.10 Response Evaluation

Response evaluation is complex, and early progression should be confirmed by an experienced team. Antitumor activity translates into tumor shrinkage in the majority of patients, but some patients may show changes only in tumor density on CT scan, or these changes may precede delayed tumor shrinkage. These changes in tumor radiological appearance should be considered as the tumor response. Even increase in the tumor size, in particular, may be indicative of the tumor response if the tumor density on CT scan is decreased. Even the "appearance" of new lesions may be due to their being more evident when becoming less dense [25].

Therefore, both tumor size and tumor density on CT scan, or consistent changes in MRI or contrast-enhanced ultrasound, should be considered as criteria for tumor response. An FDG-PET scan has proved to be highly sensitive in early assessment of tumor response and may be useful in cases where there is doubt, or when early prediction of the response is particularly useful (e.g., preoperative cytoreductive treatments) (Fig. 59.18).

A small proportion of GISTs have no FDG uptake, however. The absence of tumor progression at 6 months after months of treatment also amounts to a tumor response. On the other hand, tumor progression may not be accompanied by changes in the tumor size. In fact, some increase in the tumor density within tumor lesions may be indicative of tumor progression. A typical progression pattern is the "nodule within the mass," by which a portion of a responding lesion becomes hyperdense [24, 26].

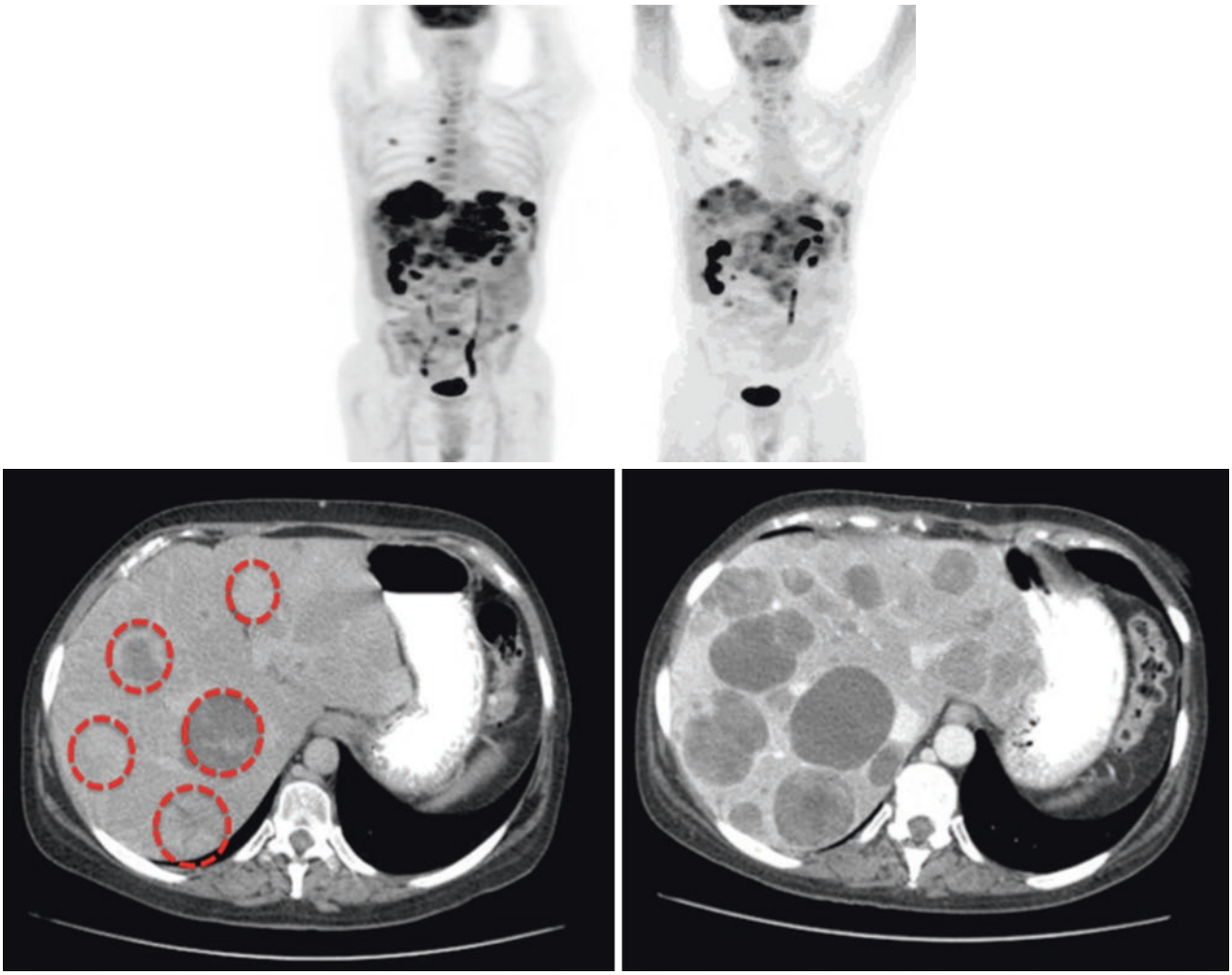
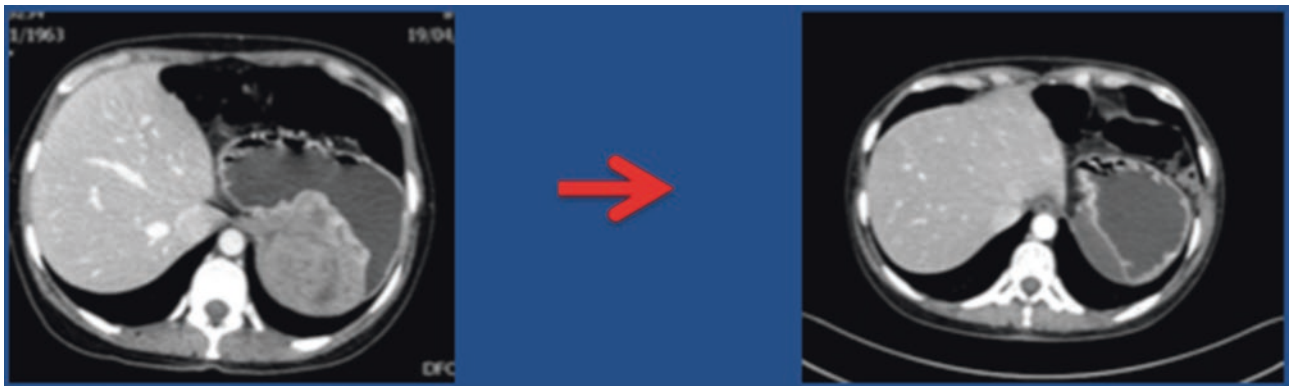
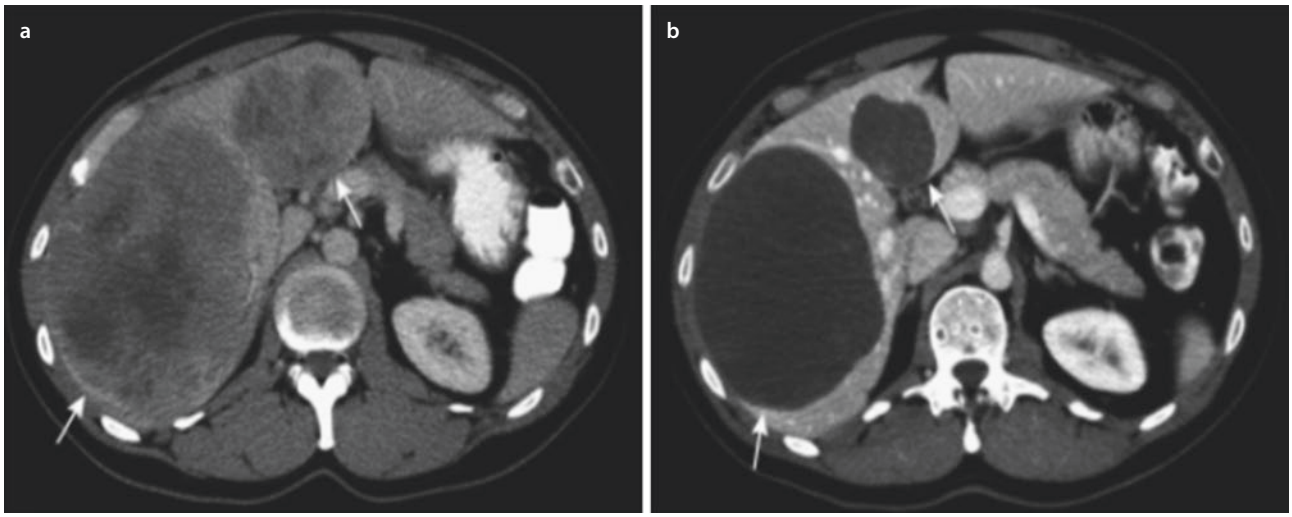


Fig. 59.18 Effect of imatinib therapy using positron emission tomography on fluorodeoxyglucose (FDG) levels: tumors that had a robust response to imatinib present a significant decrease in FDG signal, even within 24 hours of the first dose (Van den Abbeele & Badawi, 2002)

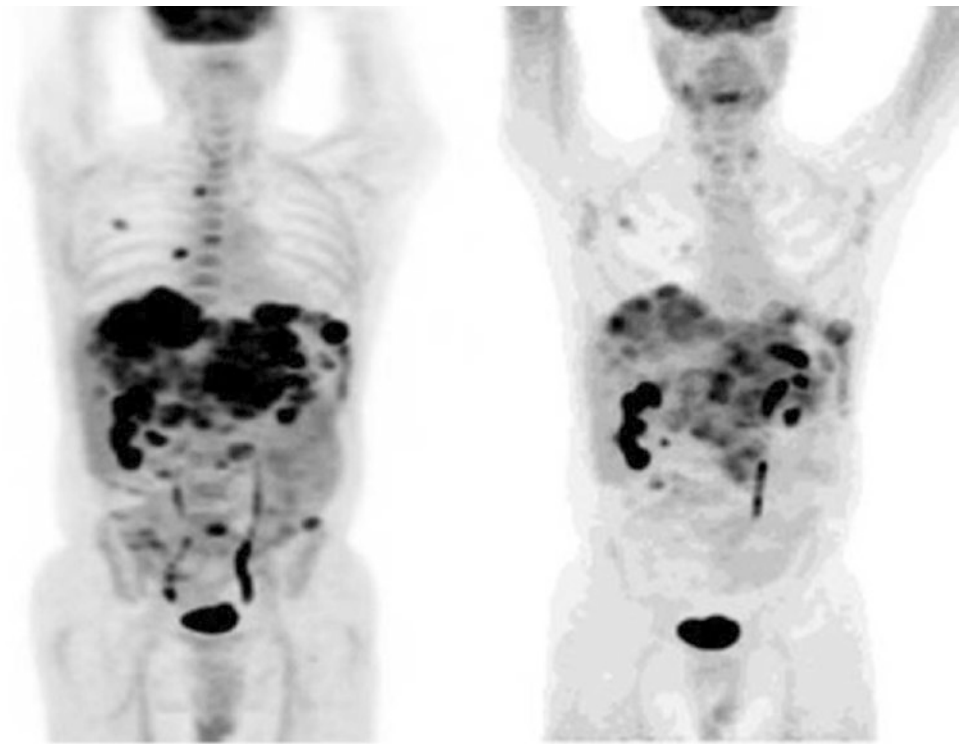


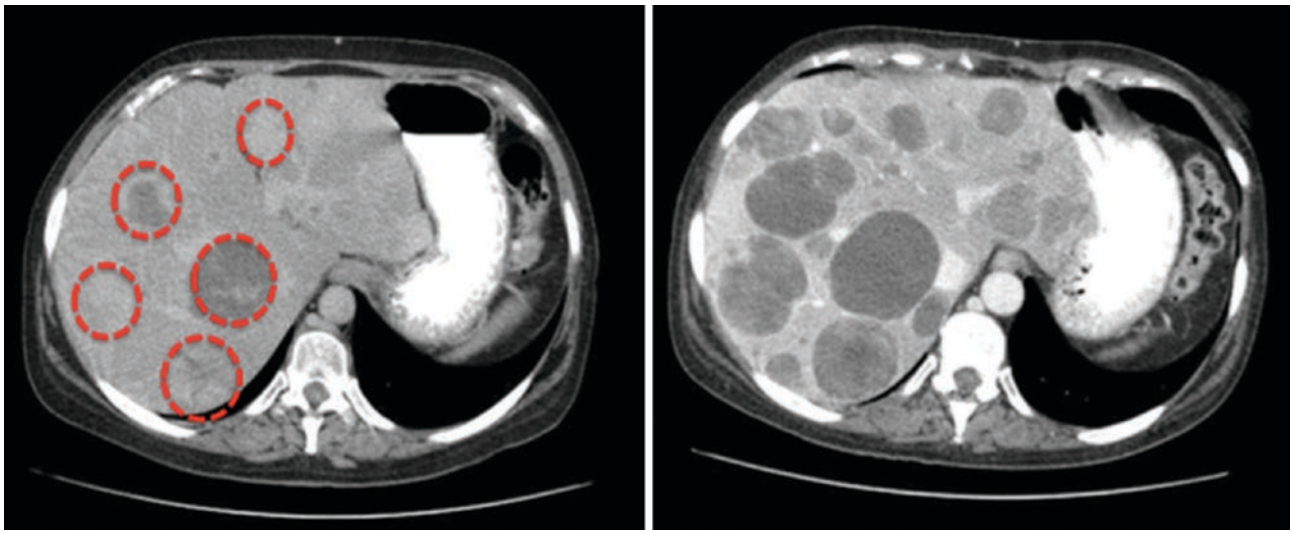
RECIST Response



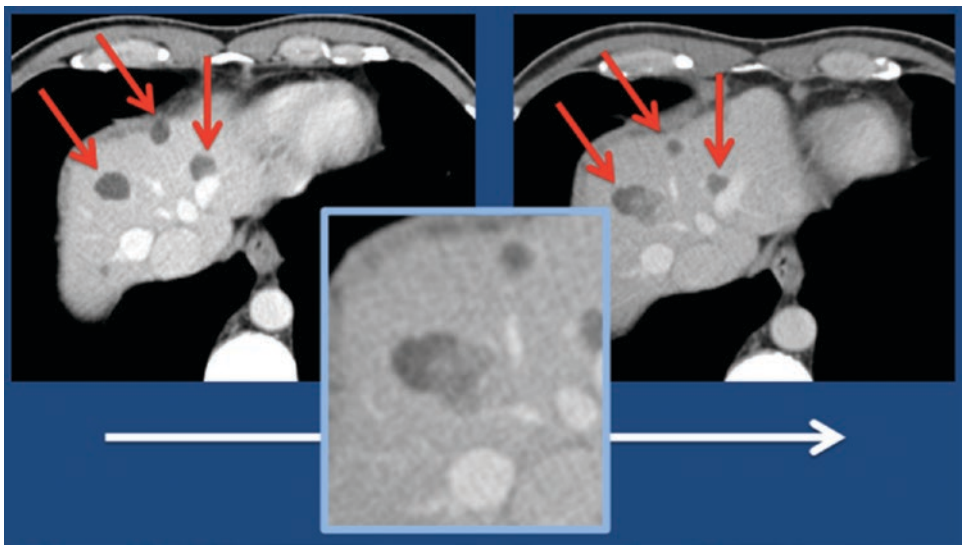
CHOI Response

Fig. 59.18 (continued)

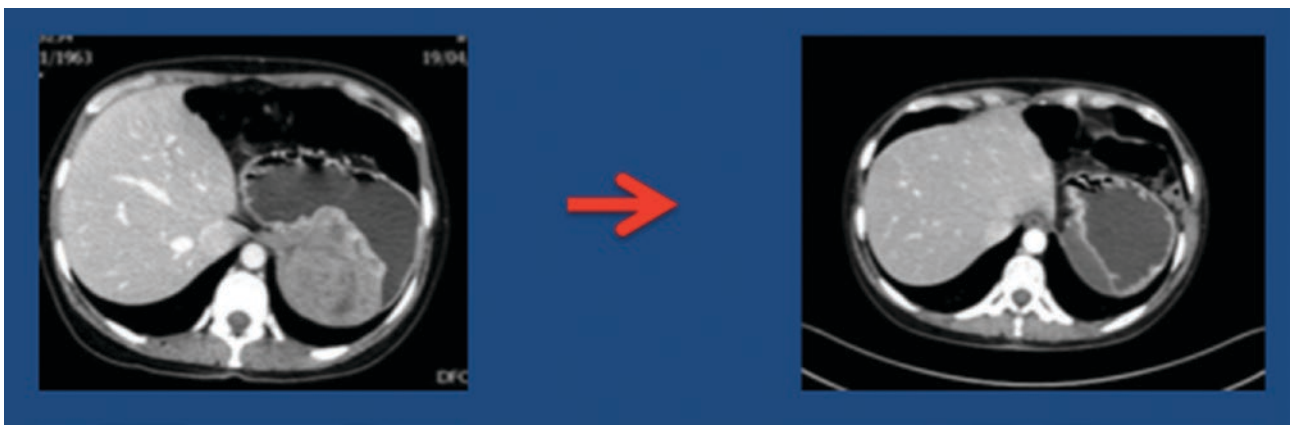




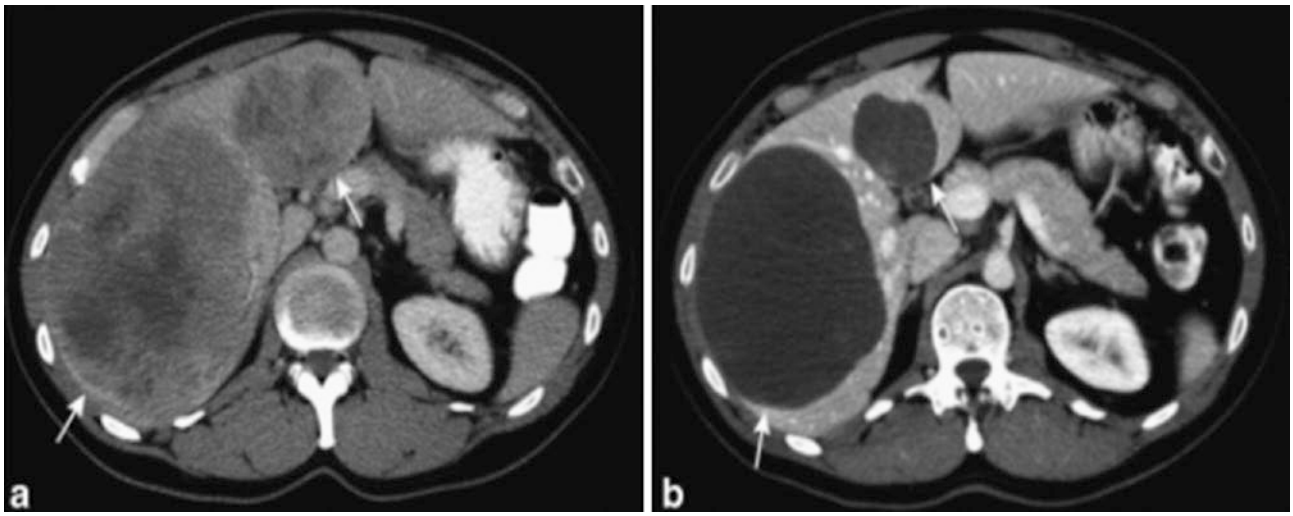
Pseudoprogression



Nodular Progression



RECIST Response



CHOI Response

59.2 The Role of Surgery in the Management of GIST

Sinziana Dumitra and Alessandro Gronchi

59.2.1 Introduction

While the management and ultimately survival of GIST was revolutionized at the dawn of the twenty-first century with the discovery of the *c-kit* tyrosine kinase mutations [27, 28] and that of targeted therapy [29], allowing disease control in a historically difficult to manage disease [30–36], surgical management remains the cornerstone of GISTs management and is based on the phase of disease at presentation. While a well-established and valid staging system is not currently in use for GIST, however a practical way to conceptualize this disease and its surgical management is to think of it as localized, locally advanced, or metastatic disease.

59.2.2 Localized GIST

Much of the surgical management of GIST truly depends on the primary site of disease occurrence; the most common site being stomach (50%), followed by small bowel (25%), and colon and rectum (10%) [37–41]. There are some reports of less common locations of GIST, namely, omentum, mesentery, and retroperitoneum [42]. The overall disease prognosis depends on size, location, mitotic count, and tumor rupture [43]. While the surgical options might differ based on location, the principles of an oncologic surgical resection remain the same. First of all, it is important to thoroughly inspect the abdomen to ensure absence of peritoneal metastases. Secondly, achieving negative resection margins over the organ of origin is recommended, even if a clear association between quality of surgical margins and disease free and overall survival has not been demonstrated [37], save for rectal GIST. This is mainly due to the variety of presentations, with the majority of GIST having an intra-abdominal growth. When the tumor is confined to the GI wall, quality of surgical margins is likely to be more critical. A main advantage in GIST is that compared to other sarcomas and adenocarcinomas margins need be less wide, allowing for less extensive and morbid surgery. Thirdly, surgeons should manipulate GISTS with great care as not to rupture these friable tumors. Lastly, given that these stromal tumors rarely metastasize to lymph nodes, a lymphadenectomy is not performed routinely unless the presence of suspicious nodes is detected preoperatively.

59.2.3 Gastric GIST

Adequate preoperative assessment includes imaging as well as upper endoscopy. In the stomach, GIST often presents as an exophytic mass that can be easily resected or wedged out with the aid of a stapler (Fig. 59.19, panel a). While the authors believe that all GISTs should be resected given the fact, some have argued for potential observation at smaller sizes (<2 cm) after discussion with the patient [44]. It is important to highlight that symptomatic GIST (e.g., bleeding, perforation, obstruction) should undergo resection. Emerging endoscopic techniques have also been successful in adequately

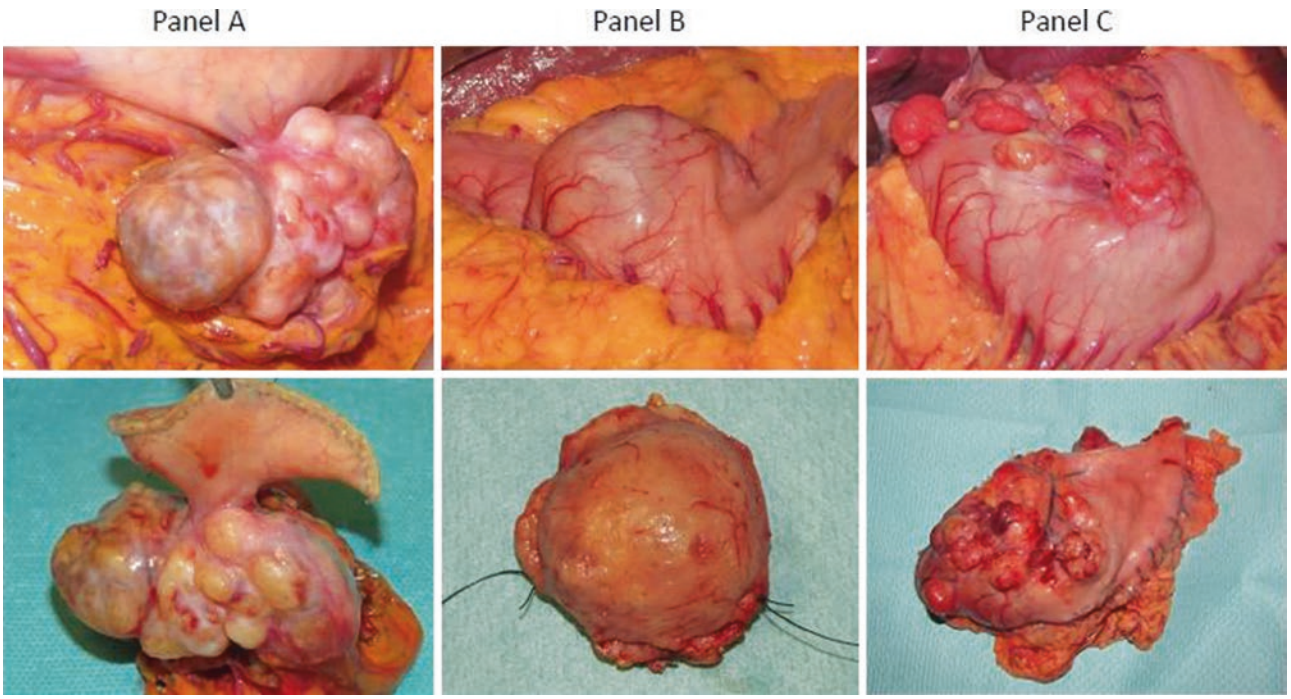


Fig. 59.19 Macroscopic appearance of gastric GIST and its implication for surgical management: extraluminal growth, which can be resected with a wedge mechanical suture in panel **a**; intraluminal growth, which can be resected conservatively with a wedge manual suture in panel **b**; multinodular growth, which can only be resected with a conventional subtotal/total gastrectomy in panel **c**

removing gastric small (i.e., <2 cm) GIST [45, 46]. These are particularly useful in patients with multiple comorbidities who could not undergo a surgical procedure or as an alternative to active surveillance.

If larger non-exophytic GISTs are encountered often times, a large resection can be avoided by simply incising the gastric wall and resecting the tumor with an adequate margin, under direct vision, and subsequently closing the gastric wall by approximating the edges (Fig. 59.19, panel b). This allows for a controlled gastric wedge, while avoiding resecting a large portion of the stomach. A particular case where great care needs to be taken when resecting a gastric GIST is one at or close to the gastroesophageal (GE) junction; a 32 French bougie should be utilized to ensure that the GE junction remains patent and sufficiently wide after wedge resection. These patients need to be carefully assessed in the preoperative setting and if a gastoesophageal resection would be necessary in order to obtain negative margins; then neoadjuvant targeted therapy should be considered in order to spare such an extensive resection and anastomosis. Very rarely is a subtotal or total gastrectomy required for GIST. Likewise multivisceral resections, including pancreas, spleen, and liver, are rarely required, as the tumor can often be separated from surrounding organs. However, when this is anticipated not to be the case, a preoperative therapy with imatinib should be considered, unless the tumor harbors an insensitive mutation, such as PDGFRA D842V, or belongs to the SDH-deficient subgroup, both insensitive to imatinib and all other approved TKIs. Finally, SDH-deficient GIST is predominantly located to the stomach and is multifocal (Fig. 59.19, panel c). As a result of this specific subgroup, subtotal/total gastrectomy is more often required, along with regional lymphadenectomy, as lymph node metastases are more common.

An important surgical modality to discuss is the utilization of laparoscopic surgery in GIST that allows for a faster recovery, shorter hospital stay, and decreased overall costs of care. Recent studies and meta-analyses did not identify oncologic outcome differences when using laparoscopic surgery when compared to open [47–49]. Even in studies assessing larger GIST >5 cm, oncologic results were similar [50]. The authors do caution in case of large tumors to ensure the extraction site is large enough and suggest the tumor be extracted in a specimen bag as to avoid rupture and spillage. Of note, imatinib therapy can be used to downsize the tumor and allow a laparoscopic procedure.

59.2.4 Duodenal GIST

The surgical management of duodenal GISTs can be more challenging and greatly depends on size as well as the portion of the duodenum affected. The most com-

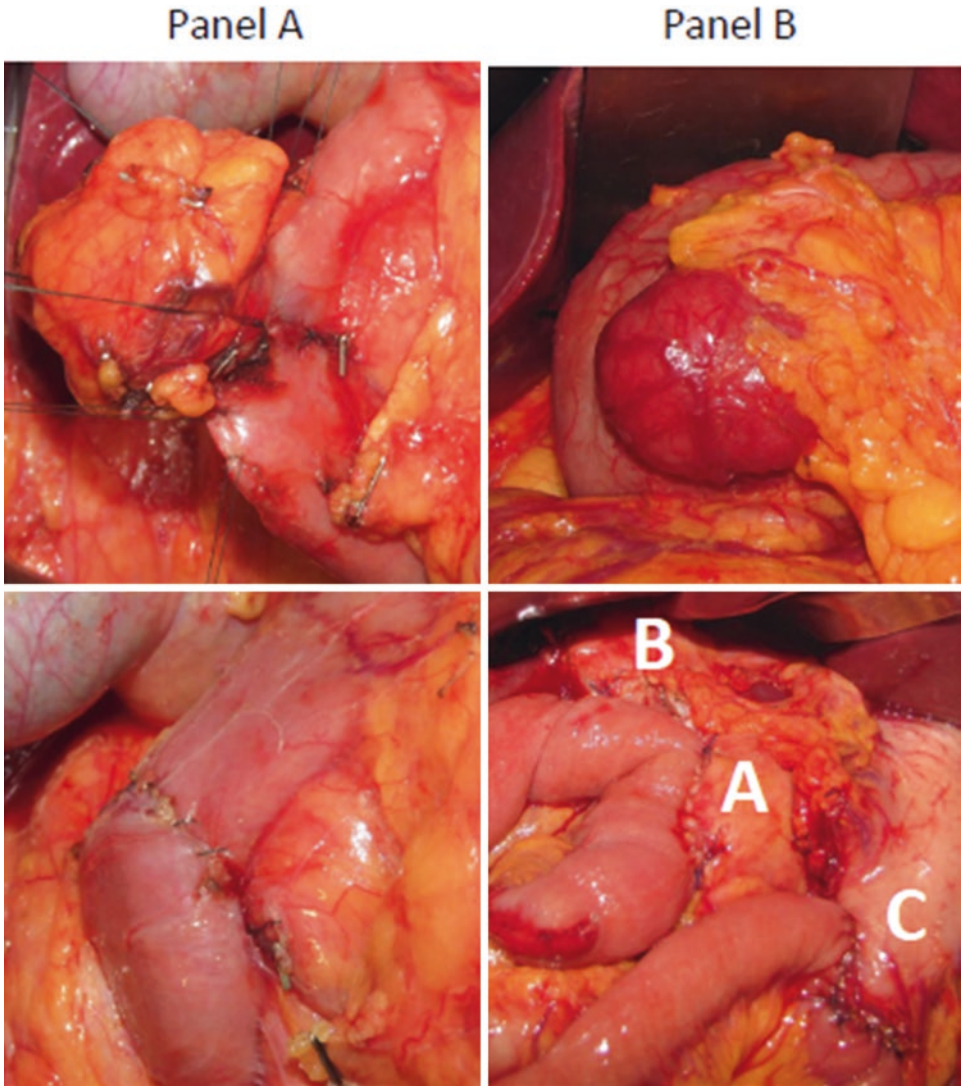


Fig. 59.20 Macroscopic appearance of a duodenal GIST occurring on the 2nd portion of the duodenum and its implication for surgical management: antimesenteric growth, which can be resected conservatively with a wedge manual primary suture (or at times with a jejunal loop interposition) in panel **a**; mesenteric growth, which can be resected only with a pancreaticoduodenectomy and a Whipple reconstruction (pancreatic [A], biliary [B] and gastrointestinal [C] anastomoses) in panel **b**

mon site of duodenal GIST occurrence is the second portion followed by the third, fourth, and finally the first portion of the duodenum [51, 52].

Another important limitation that might not allow for a local excision is whether the tumor occurs on the mesenteric or non-mesenteric side [52]. Given the risk of duodenal stricture, after an extensive Kocher maneuver, we suggest a wedge excision under direct vision and primary closure (Fig. 59.20, panel a). If necessary, the common bile duct can be identified by using a pediatric feeding tube. More specific reconstructions are mandated by the size of the defect and the location (Fig. 59.20, panel b).

As with GE junction tumors if they occur at the insertion of the bile duct in the D2 or D2-D3 area, for which a pancreaticoduodenectomy might be required to obtain an adequate negative margin excision, then neoadjuvant therapy is suggested in order to downsize the tumor and allow for a less morbid resection. In our experience, the extent of surgery does not confer a disease-free or survival advantage [53].

59.2.5 Small Bowel GIST

Small bowel GISTs can have widely varying presentations such as palpable masses, obstruction, bleeding, and rupture, and more increasingly often they present incidentally based on imaging or endoscopy. Their prognosis varies widely based on size and mitotic count [54]. Surgery upfront should be offered upfront when disease is limited. Often time small bowel GIST is easily amenable to resection and can even be considered for laparoscopic resection [55]. Often times it is much easier to proceed to a segmental resection and primary anastomosis rather than perform a wedge resection (Fig. 59.21, panel a). GIST associated to neurofibromatosis type 1 is predominantly located to the small bowel and is virtually always multifocal (Fig. 59.21, panel b). Their risk does not depend in the number of lesions, while it depends on the features of the most aggressive one. Surgical resection may be directed only to remove the one or the ones at high risk, as removing

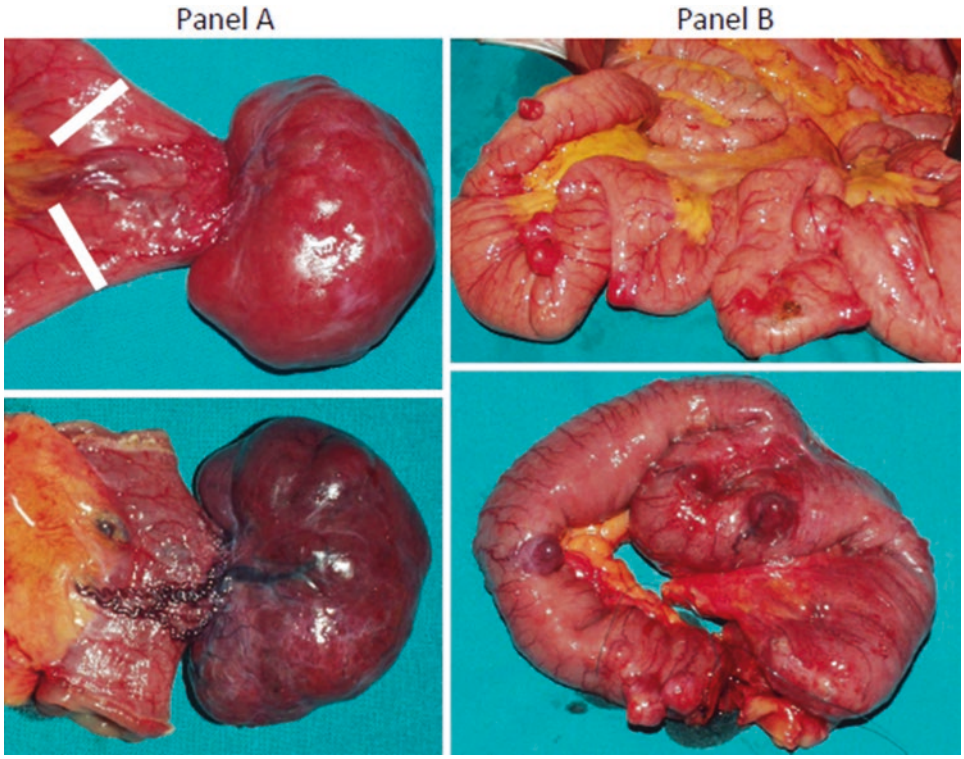


Fig. 59.21 Macroscopic appearance of a small bowel GIST and its implication for surgical management: single nodule, which can be resected with a simple small bowel segmental resection in panel **a**; multiple nodules (typical scenario in Neurofibromatosis type 1 patients), which can be resected with a more extended small bowel resection

all lesions may at times require an extended procedure followed by short bowel syndrome.

59.2.6 Rectal GIST

The most common site of presentation of colonic GIST is the lower rectum. Rectal GIST, while rare, often displays a more aggressive behavior than GISTs occurring in other locations [56]; indeed even small GISTs <2 cm with mitotic activity can recur and even metastasize [57]. Indeed, local recurrence rates are much higher than at other locations even after correcting for number of mitoses. Studies have shown that obtaining R0 margins of resection is paramount in rectal cancer for disease-free survival and overall survival. Neoadjuvant treatment with imatinib is associated with improved survival [58]. Depending on the size of the tumor, local approach to resection can be performed via transanal, transarcral, or perineal approaches (Fig. 59.22, panel a). It is important when performing a resection to achieve a complete removal of the tumor-bearing rectal wall and the tumor-covering tissue layer as GISTs originate from the muscularis propria and not the mucosa [58]. Often times if rectal GISTs are large and not amenable to local resection, abdominal resection or abdominoperineal resections should be undertaken (Fig. 59.22, panel b). There have been some case reports of laparoscopic techniques being used in rectal tumor resection, but the authors believe it should be undertaken only in small tumors when rupture-free and negative resection can be achieved.

59.2.7 Locally Advanced GIST

Perhaps, one of the important indications for neoadjuvant treatment is in the case of locally advanced borderline resectable GIST. Indeed, the conceptual advantage of therapy in these patients is twofold: first, the potential to avoid a multiorgan resection and organ preservation, thanks to tumor downstaging and the increased ability to obtain R0-negative resection margins (Fig. 59.23, panel a, b). Another advantage is the fact that treatment can render the tumor less vascular and friable allowing for easier manipulation and decreased risk of rupture which is key especially in larger or difficult to access tumors (Fig. 59.23, panel c, d) [59]. The use of imatinib prior to surgical intervention was based initially on institutional series demonstrating good radiologic responses of 60–70% with disease-free survival rates of 70% at 3 years [59]. In the radiation therapy oncology group, RTOG 0132 trial assesses the use of neoadjuvant imatinib in patients with locally advanced disease among others. Despite the short duration of neoadjuvant treatment in this cohort, the rate of R0 resection was 77%, quiet high in this fairly high-risk group [60]. A much larger 10 center retrospective study

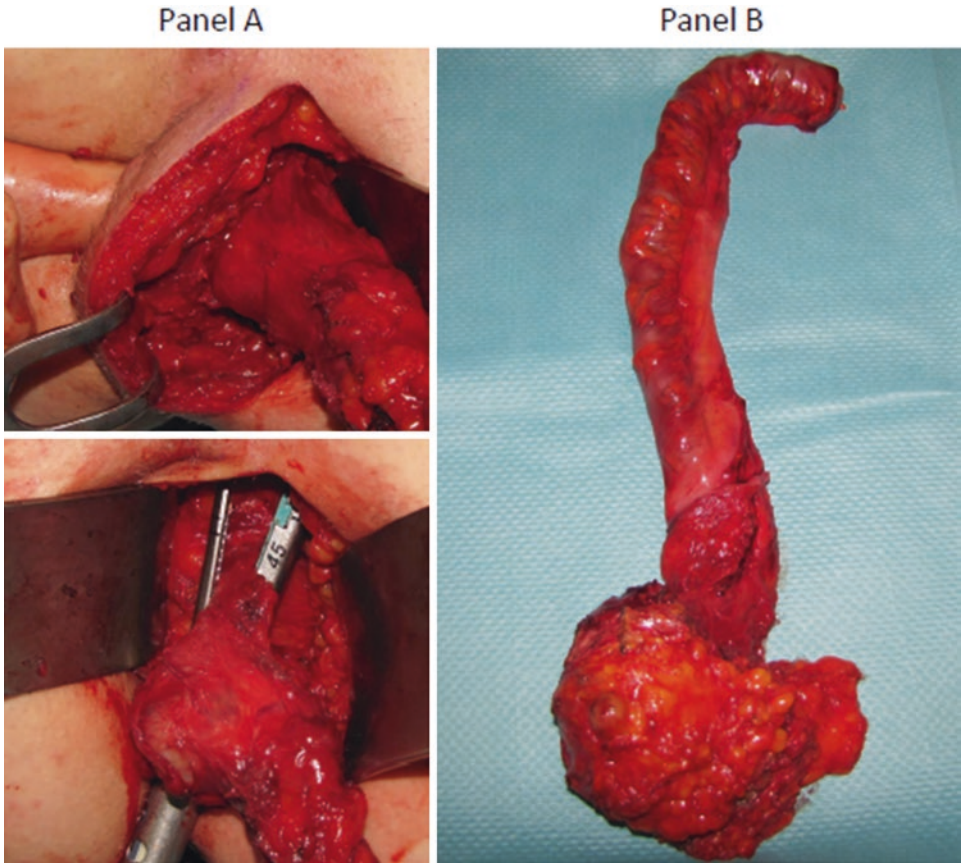


Fig. 59.22 Macroscopic appearance of a rectal GIST and its implication for surgical management: small nodule, which can be resected with a local approach in panel **a**; big tumor, occupying the whole pelvis, which can only be removed with an abdominoperineal resection in panel **b**

of neoadjuvant treatment with imatinib until maximal response was achieved or the lesion was no longer borderline. While the rate of R0 resection was of 80%, the rate of recurrence was 23% at 46 months [61].

There are particular clinical scenarios where neoadjuvant treatment is particularly important as tumor location might require an extensive, morbid resection with more complex long-term effects. In GISTs of the gastroesophageal junction, a two-cavity approach may be avoided by downstaging the tumor as it would be the case for duodenal GISTs where three patient might be spared a pancreaticoduodenectomy with all the possible morbidity it entails. Another important scenario is that of rectal GISTs where sphincter might be preserved and continence maintained, improving the patient's quality of life.

As impressive as the results obtained with neoadjuvant therapy, it is paramount for the surgeons to regularly assess the response to treatment. Indeed while the duration of preoperative treatment varies widely in the literature between 12 and 40 weeks and it does seem that optimal time for intervention is situated somewhere between 6 and 12 months. It is critical to assess response to treatment at the initiation of therapy and to continue this assessment regularly as not to miss the window of resectabilty. Moreover, the resection should occur ideally before the development of clonal resistance to the drug given.

59.2.8 Metastatic GIST

The main goal in the treatment of metastatic GIST at presentation is disease control, and the only way to do so is via systemic treatment as can be demonstrated by historical series where debulkings were attempted with dismal results with 25% median survival at 5 years [62]. While there remains a fervent debate on the utility of surgery in the setting of metastatic disease, there are some clinical scenarios in which patients might benefit from metastasectomy. The main rationale behind cytoreductive surgery in the era of third and even fourth line systemic therapy for GISTs is the concept of clonal resistance and the delay of subsequent lines of therapy [59]. It is important to recall that imatinib and other targeted therapies are not cytotoxic; rather, they produce cell senescence; they thus do not provide a cure for GIST.

Multiple institutional series have described promising results in disease control [63, 64]; however, patient

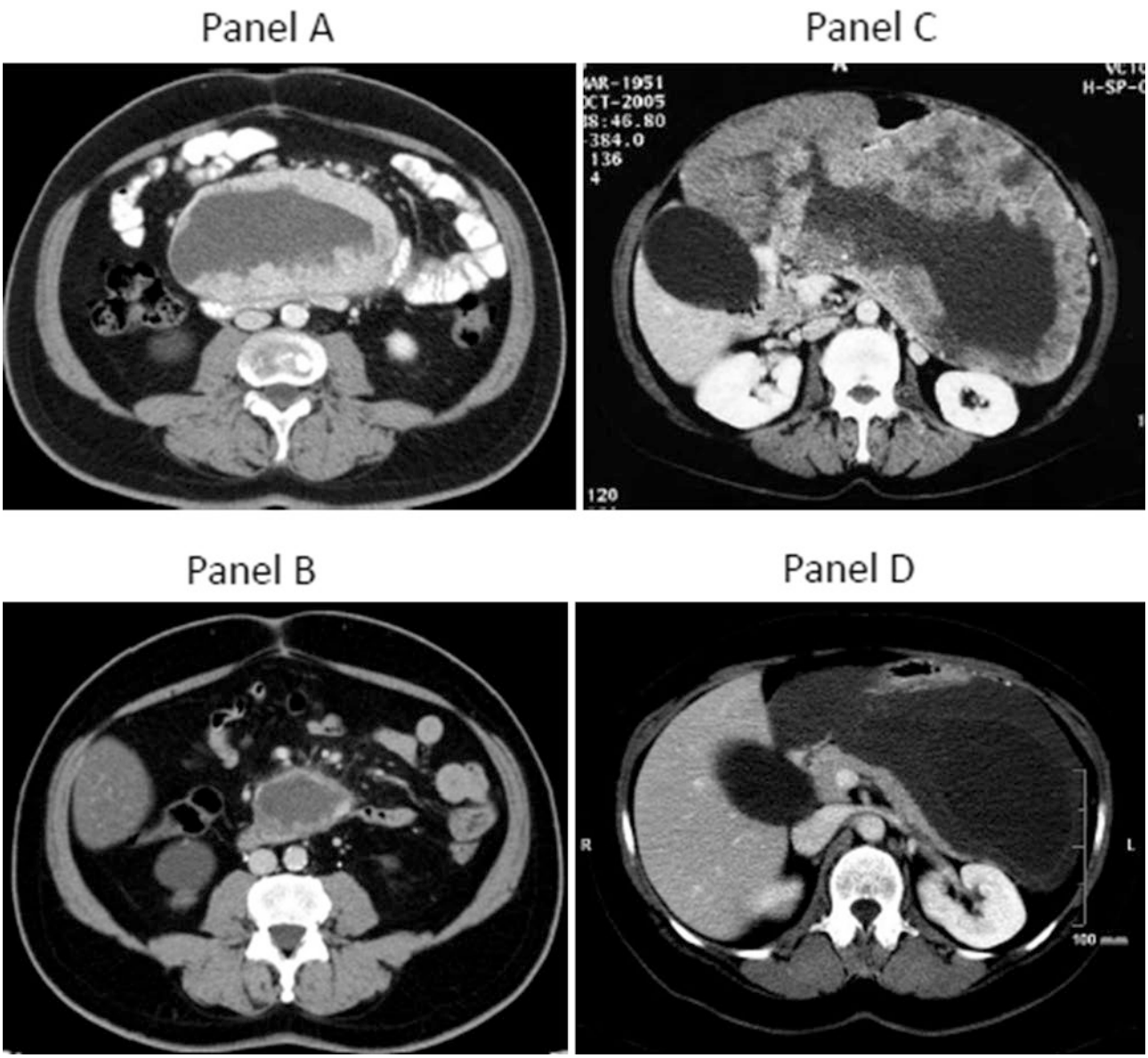


Fig. 59.23 Contrast enhanced CT scan, venous phase, of a primary large duodenal GIST abutting superior mesenteric vessels before (panel **a**) and after (panel **b**) 12 months of medical therapy with Imatinib: a major shrinkage has occurred, improving quality of surgical margins. Contrast enhanced CT scan, venous phase, of a primary large necrotic and highly vascularized gastric GIST before (panel **c**) and after (panel **d**) 12 months of medical therapy with Imatinib: no shrinkage has occurred, but an important change in tumor density has taken place with a significant reduction of vascularization, which makes tumor resection much less at risk of tumor rupture and safer

selection and optimal intervention timing are key when performing metastasectomies [65]. Indeed, patients with localized, persistent, and slow-growing metastases seem to benefit from surgical intervention much more than those with multifocal progression [59]. This might be secondary to the limited ability to obtain a complete debulkings in patients with multifocal disease. In a the large multicenter study by Bauer et al., an important prognosis factor in the patients selected for resection is site of disease with disease limited to the liver surviving significantly longer than those with peritoneal disease [63]. As with localized disease, the widow of opportunity after initiation of treatment with imatinib seems to be 6–12 months (Fig. 59.24, panel a, b) [63].

Some groups have suggested that cytoreductive surgery should be offered at the outset in order to clear all macroscopic disease. However, retrospective series did not find a survival advantage of initiating the treatment sequence with surgery. Moreover, surgery at the outset did not delay the initiation of second-line treatment [66]. Indeed starting the treatment sequence with imatinib allows for disease biology to declare itself and enables

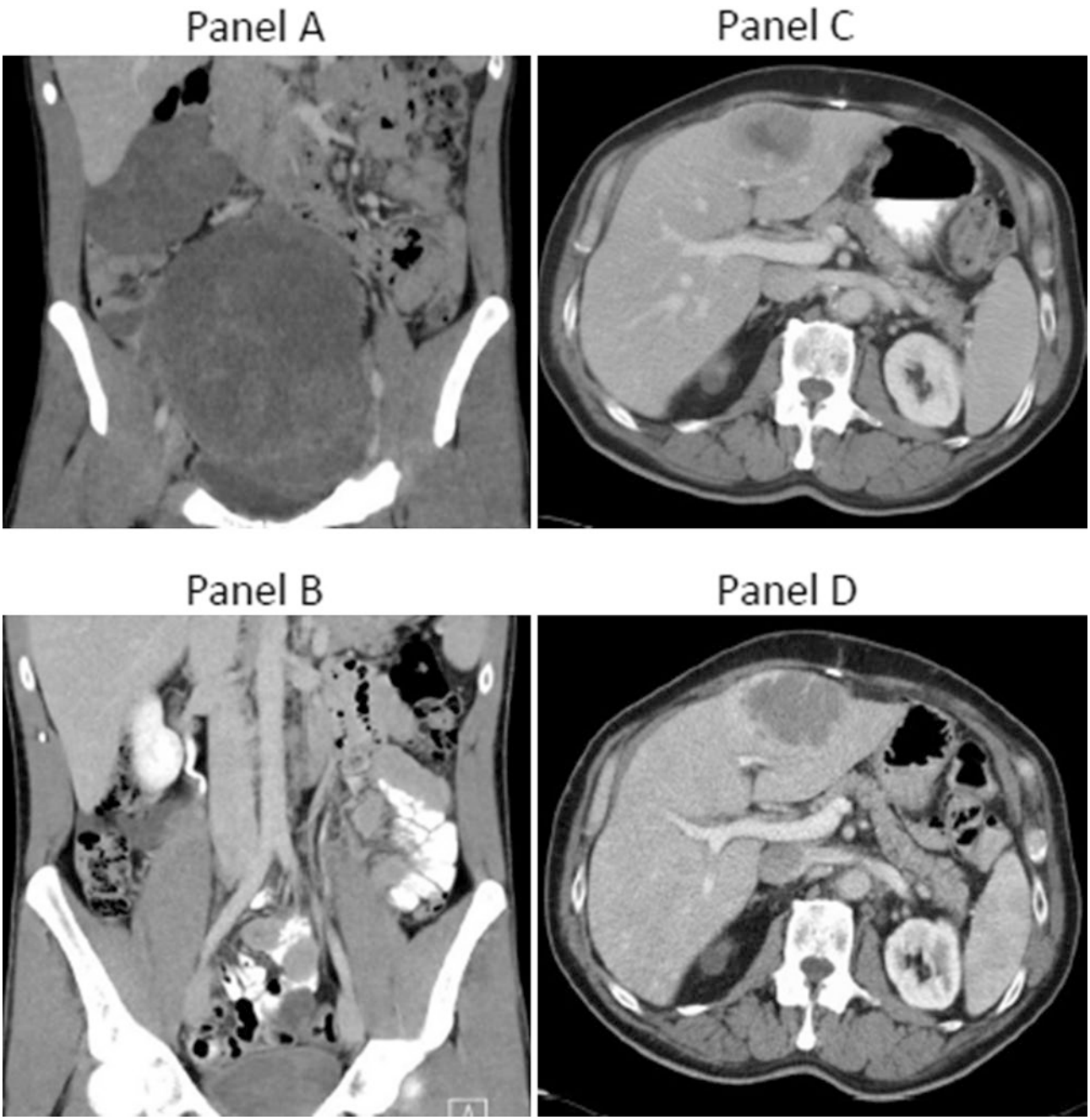


Fig. 59.24 Contrast enhanced CT scan, venous phase, of a large small bowel GIST metastatic to the peritoneum before (panel **a**) and after (panel **b**) 12 months of medical therapy with Imatinib: a major shrinkage has occurred of both primary and metastatic sites. Contrast enhanced CT scan, venous phase, of a single GIST liver metastasis before (panel **c**) and after (panel **d**) progressing on Imatinib: surgical resection of the single liver nodule is an option to consider

selecting patients that will have a favorable response to intervention. Another important factor in the choice of timing of intervention is disease progression. Indeed, patients undergoing interventions at the time of progression have shorter disease-free intervals postoperatively than those in remission at the time of intervention [64]. However, the use of surgery in limited progression may be of help to postpone the switch to a further line therapy, as this may maximize the time a patient stay on the given drug and therefore the control of the disease (Fig. 59.24, panel c, d) [63–65].

Another juncture when surgery could be considered for metastatic GIST is at the time of second-line therapy. In a study assessing survival in patients undergoing surgery for metastatic disease while on sunitinib, surgery was much less successful when compared to results

described in patients on first-line therapy, with lower macroscopically negative excision rates, higher complication rates, and lower survival [67].

Finally, surgery may play a role in the subgroup of TKI-insensitive GIST (PDGFRA D842V-mutated GIST or SDH-deficient GIST), as the natural history is usually more indolent and patients may survive several years with metastatic disease. The same does not apply to metastatic NF1-associated GIST, the prognosis of which is generally very poor.

59.2.9 Conclusion

Surgery remains the cornerstone treatment modality in GIST and the only one to provide a cure. Surgical techniques and their roles in the continuum of care are dictated by disease location and stage. With the advent of targeted therapies has been an increased utilization of neo-adjuvant imatinib in the treatment of localized disease leading to increased rates of complete resection and an associated disease free survival benefit. While surgery for metastatic GIST does remain controversial, there are certain patients that may benefit from resection especially when the disease is stable on systemic treatment and limited or an isolated progression has occurred.

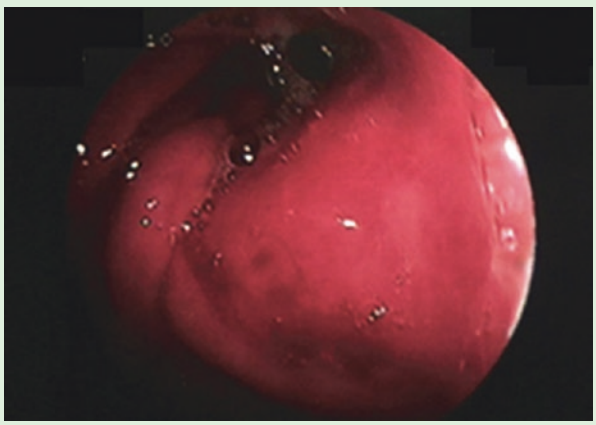
Summary of Clinical Recommendations

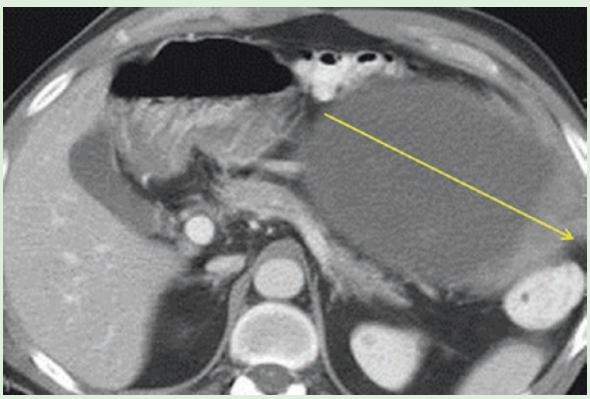
- *Linee Guida dell'Associazione Italiana di Oncologia Medica (AIOM)*
- *Sarcomi dei Tessuti molli e GIST. Edizione 2019.*
- *ESMO Clinical Practice Guidelines. Sarcoma and GIST.*
- *Annals of Oncology 2018*
- *NCCN (National Comprehensive Cancer Network) GUIDELINES FOR TREATMENT OF CANCER BY SITE-2018: Soft Tissue Sarcoma and GIST.*

Case Study Author: Please Indicate the Clinical Case TITLE Here

Man, 56 years old

- *Family history* negative for malignancy
- *APR:* Diabetes Mellitus type II
- *APP:* For nearly 2 months nausea and asthenia; diffuse abdominal pain
- *Objective examination*: Globose abdomen; mild tenderness on deep palpation (quadrant sup.sx); Palpable mass in the left hip
- *Blood tests*: Hb 9,1 g/dl; mildly impaired liver function tests (GOT; GPT)
- *Esofagogastroduodenoscopy*: Normal mucosa; compression of the gastric wall





- *TC abdomen mdc*: Lesion of 34 × 23 × 10 cm in continuity with small curvature, no cleavage plane from the gastric wall
- No lymphadenopathies
- Peritoneal implants and multiple liver metastases

Question

What action should be taken?

(1) Surgery (2) Biopsy (3) Other

Answer

Ecoendoscopy with biopsy

Histological examination:
GIST spindle cell; gastric origin
CD 117+; 2 mitosis/50 hpf

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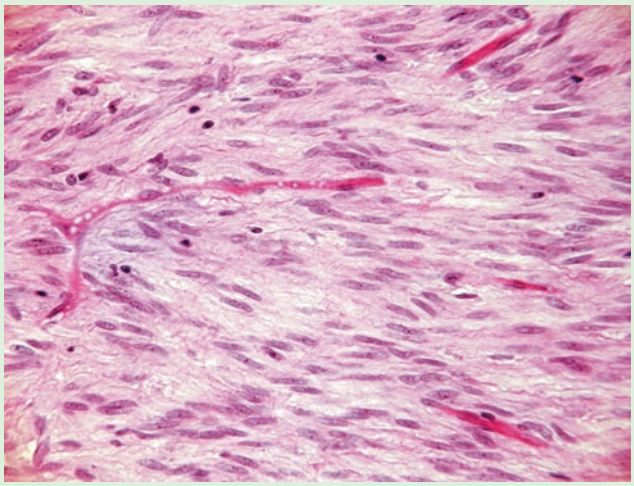
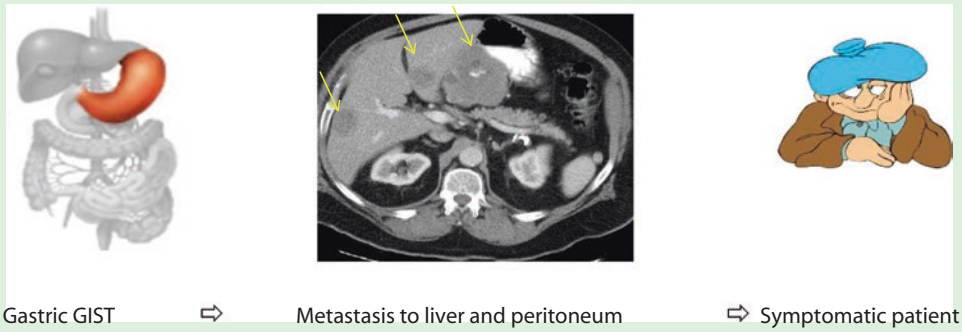




Gastric GIST ⇨ Metastasis to liver and peritoneum ⇨ Symptomatic patient

Question

What action should be taken?

(1) Surgery (2) Medical therapy (3) Mutational analysis

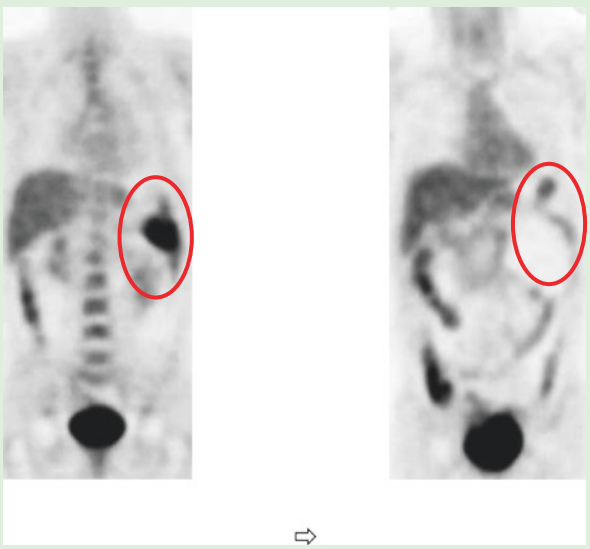
Answer

Mutational analysis: Exon 9 KIT mutation

⇩

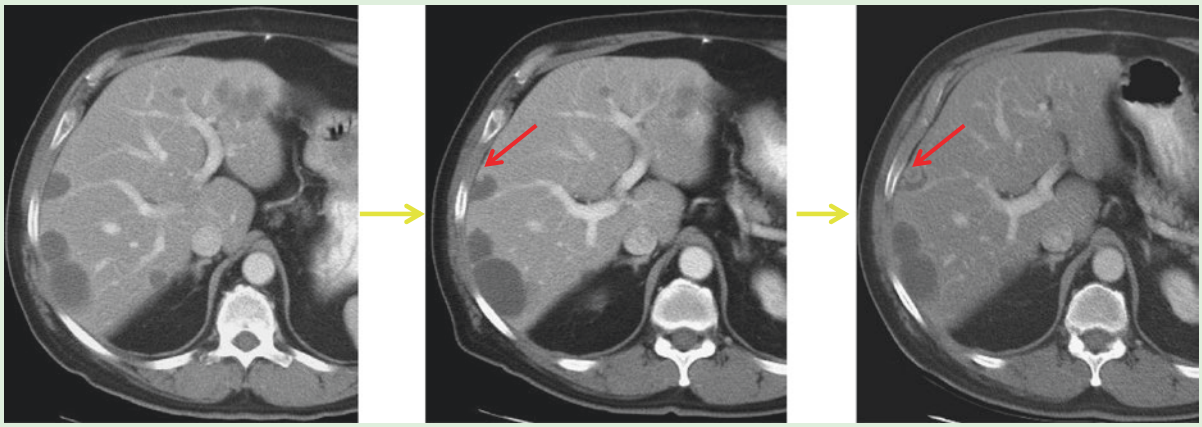
Medical therapy: Imatinib 800 mg/die

Response evaluation after 3 months of therapy with Imatinib 800 mg/die: Complete metabolic response to PET-FDG



Before Imatinib ⇨ After 3 months of Imatinib

Response evaluation after 12 months of therapy with Imatinib 800 mg/die: Appears "nodule in nodule" that increases in size after a further 2 months (14 months of therapy with Imatinib 800 mg/die)



After 9 months | After 12 months | After 14 months

Question

What action should be taken?

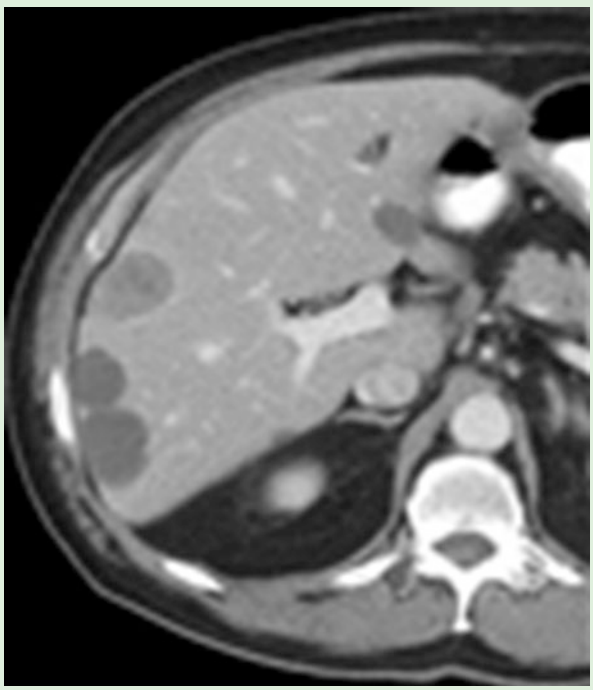
(1) Sunitinib (2) Regorafenib (3) Continues Imatinib 800

Answer

Begins Sunitinib 37.5 mg/die

Response evaluation after 3 months of therapy with Sunitinib 37.5 mg/die:

Tissue response to TC



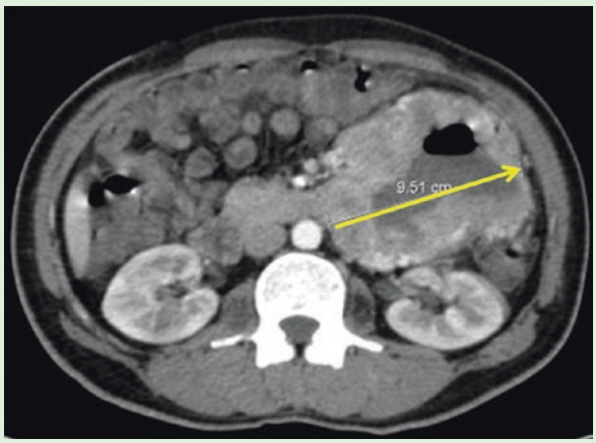
Key Points

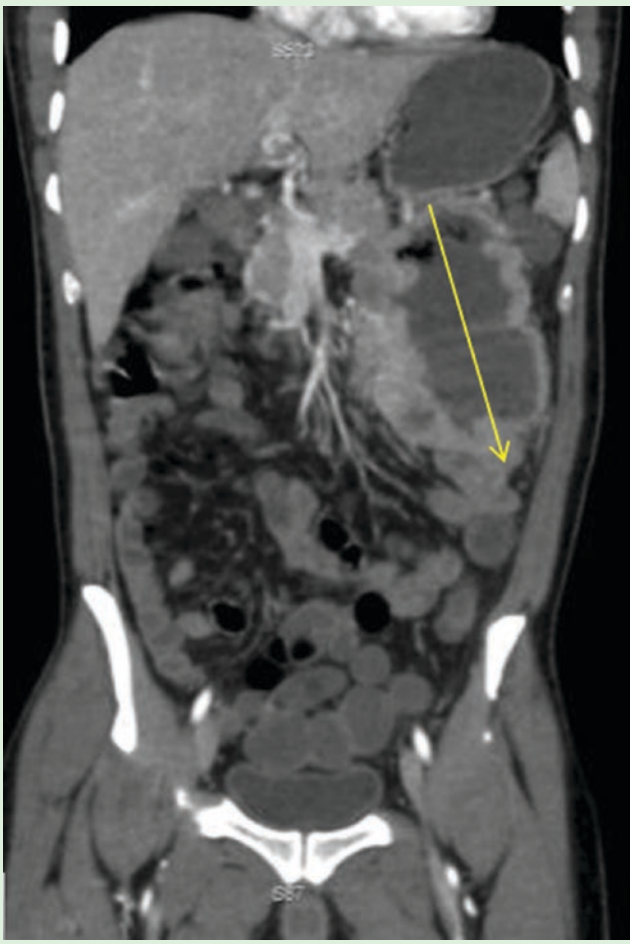
- The importance of a correct diagnosis: attention to the large bowel masses
- Symptoms often nonspecific; mucosa normally not involved
- The importance of a correct evaluation of the response
- Importance of mutational analysis in the therapeutic choice

Case Study Author: Please Indicate the Clinical Case TITLE Here

Man, 56 years old

- *Family history* negative for malignancy
- *APR*: negative
- *APP*: asthenia, dyspepsia, change in bowel habit
- *Blood tests*: Hb 9,2 g/dl
- *TC Abdomen mdc*: Voluminous abdominal lesion of 10 × 9.5 × 8 cm. located between stomach, spleen, pancreas, transverse colon and the first ileal loops. (localized disease)





Question

What action should be taken?

(1) Surgery (2) Biopsy (3) Other

Answer

Biopsy: GIST spindle cell, CD 117 + Mutational analysis: Exon 11 Kit mutation

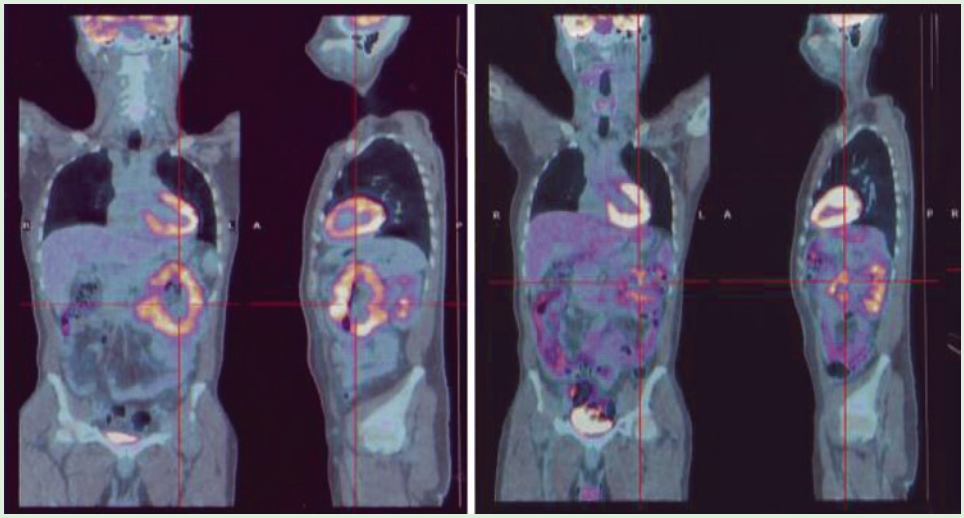
Question

What action should be taken?

(1) Surgery (2) Medical therapy (3) Other

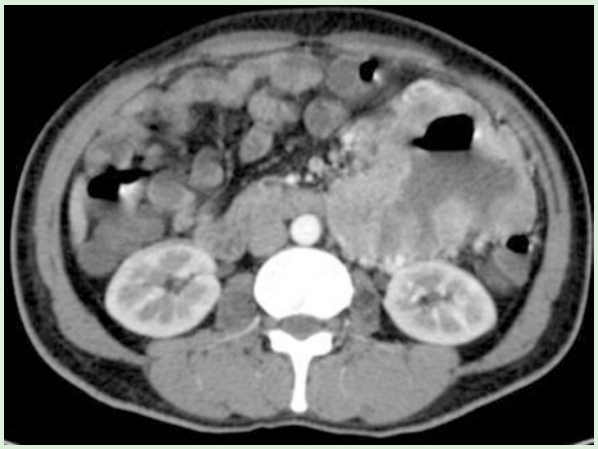
Answer

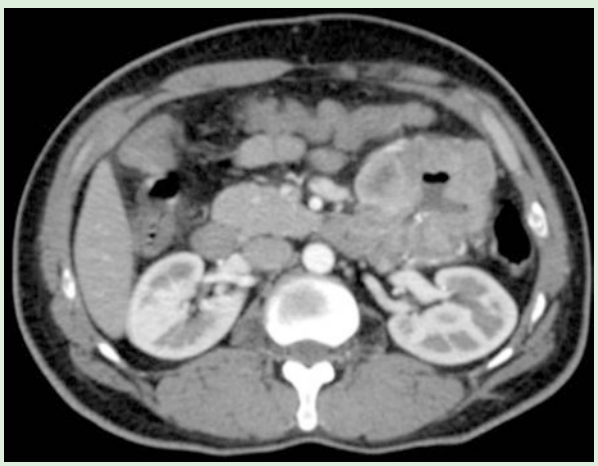
Preoperative treatment: Imatinib 400 mg/die



Before treatment

After 1 month of Imatinib





After 6 months of Imatinib: SD

Question

What action should be taken?

(1) Surgery (2) Continues Imatinib (3) Other

Answer

Surgery: R0

Key Points

- Importance of preoperative biopsy:
- Differential diagnosis with other neoplasia: Other sarcomas, germ cell tumors and lymphomas not need the same surgery!
- Possibility of medical treatment preoperative: it would be desirable to know the mutated exon before deciding whether or not to initiate a preoperative treatment
- Is appropriate to assess early response by PET
- The maximum response is obtained after 6–12 month

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Expert Opinion

Giuseppe Badalamenti

Key Points

GISTs are rare cancer that originate from the gastrointestinal tract; the most frequent location is stomach (55%), followed by small intestine (30%). Less frequent are colon/rectum (5%) and esophagus (<1%).

- Approximately 70% of GISTs are driven by mutations in the oncogene KIT; of those GISTs without KIT mutations, the majority harbor mutations in the gene encoding (PDGFRA) (15%). The remaining 15% of GISTs were described as KIT/PDGFRA "wild-type" GISTs.
- The mutational analysis is essential to predict the response to treatment with imatinib
- Surgery is the standard treatment in operable localized disease; locally advanced borderline resectable GIST or avoid multi-organ resection are the important indications for neoadjuvant treatment with imatinib. Surgery should be proposed between 6 and 12 months after starting a neoadjuvant treatment.
- In the case of high-risk GIST, an adjuvant treatment with imatinib for 3 years is the standard; in this case, the mutational analysis is mandatory to identify GISTs sensitive to imatinib.
- In metastatic setting, imatinib 400 mg is the standard treatment; in the case of GIST, exon 9 mutated, the treatment with imatinib high doses might be preferred. In the case of mutations resistant to imatinib, a clinical trial should be proposed.
- For GIST resistant to imatinib, sunitinib is indicated in the second line and regorafenib in the third line.
- Given the rarity of the pathology and the opportunity to participate in clinical trials, the patient's reference to highly experienced centers is always recommended.

Hints for Deeper Insight and Suggested Reading

- Recommendations for the implementation of mutational analysis and management of gastrointestinal stromal tumor (GIST) patients. Raccomandazioni 2019 per l'implementazione dell'analisi mutazionale e la gestione del paziente con Tumore Stromale Gastrointestinale (GIST). October 2019.
- Position paper of Italian Scientific Societies (AIOM – Fondazione AIOM – ISG – SIAPEC-IAP – SIBIOC – SICO – SIF). ► www.aiom.it
- Tailored management of primary gastrointestinal stromal tumors. Etherington MS, DeMatteo RP. Cancer. 2019. doi: 10.1002/cncr.32067.

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Neuroendocrine Neoplasms (NENs)

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Soft Tissue Sarcoma, GIST and Neuroendocrine Neoplasms

Contents

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Learning Objectives

- Have learned the basic concepts of neuroendocrine neoplasms (NENs).
- Have reached in-depth knowledge about terminology, classification, diagnostic, and therapeutic features of gastroenteropancreatic and lung NENs.

60.1 Terminology/Classification

Neuroendocrine neoplasms (NENs) represent a rare and heterogeneous group of malignancies which can develop in many different sites of our body. They originate from the cells of the diffuse neuroendocrine system.

The main classification of NENs is based on their pathology features.

60.1.1 GEP NENs

Gastroenteropancreatic (GEP) NENs are classified according to the grade of differentiation and proliferation index.

Particularly, they are named neuroendocrine tumors (NETs) when they are well differentiated (WD) whereas neuroendocrine carcinomas (NECs) when they are poorly differentiated (PD).

Gastroenteropancreatic NENs were classified in four categories, including NETs G1 (WD with <3% Ki-67), NETs G2 (WD with 3–20% Ki-67), NETs G3 (WD with >20% Ki-67), and NECs (PD with >20% Ki-67) in accordance with the 2019 WHO classification [1].

Gastroenteropancreatic NENs can be differently named on the basis of their biological and clinical features as low/intermediate grade of malignancy (comprising NET G1,G2) and high grade of malignancy (NET G3 and NEC) (Table 60.1).

Table 60.1 GEP NENs WHO/IARC classification

Type	Ki-67 (%)	Mitosis	Grade of malignancy
NET G1	<3	<2	Low
NET G2	3–20	2–20	Intermediate
NET G3	>20	>20	High
NEC	>20	>20	High

60.1.2 Lung NENs

Lung NENs were classified on the basis of some pathological parameters, such as mitosis and necrosis. In accordance with the latest WHO classification, 2015 edition, they are distinguished in small cell lung cancer (SCLC), large cell neuroendocrine carcinoma (LCNEC), atypical carcinoid (AC), and typical carcinoid (TC) (Table 60.2) [2].

The two forms of carcinoids, such as TC and AC, are also called lung NETs, and they have low/intermediate grade of malignancy. Large cell NEC and SCLC are both called lung NECs, and they have a high grade of malignancy.

Table 60.2 Lung NENs WHO classification

Type	Mitosis	Necrosis	Grade of malignancy
SCLC	>10	Present	High
LCNEC	>10	Present	High
AC	2–10	Focal, if any	Intermediate
TC	<2	None	Low

60.1.3 Clinical Classification of NENs

From a clinical perspective, it is critical to distinguish GEP NENs in functioning and nonfunctioning [3]. The former regards the presence of a clinical syndrome related to the production of one or more substances or hormones by the tumor, whereas the latter indicates the absence of a clinical syndrome related to the tumor although the patient can be symptomatic due to mass-effect symptoms related to the tumor and/or the tumor can secrete some substances without any clinical implication. The majority of GEP NETs are nonfunctioning.

The most common NEN-related clinical syndrome is the carcinoid syndrome. This is associated mainly with WD, small bowel origin, and metastatic stage NETs. A carcinoid syndrome has been reported in around 20% of all NETs, ranging from 8% of lung to 32% of small intestine NETs [4].

A further manner to classify GEP NENs is the distinction into sporadic and inherited. The most common inherited syndromes which can be associated with GEP NENs are multiple endocrine neoplasia type 1 (MEN-1) and von Hippel-Lindau (VHL) syndrome. Much rarely, GEP NENs can be associated with neurofibromatosis

Table 60.3 Genetic syndromes associated with GEP NENs

Syndrome	NET
MEN-1 (Wermer's syndrome)	Pituitary adenoma PanNET Thymic NET Lung NET Gastric, type 2, NET (ZES related)
Von Hippel-Lindau (VHL)	PanNET Pheocromocytoma
Neurofibromatosis (NF-1)	Periampullary NET Pheocromocytoma
Tuberous sclerosis complex (TSC)	PanNET

type 1 (NF-1) and tuberous sclerosis complex (TSC) syndromes (Table 60.3).

60.2 Epidemiology

60.2.1 GEP NENs

Epidemiologic data about NENs are fragmented and derive from different sources all over the world. One of the richest registry database is the surveillance, epidemiology, and end results (SEER). This is a comprehensive source of population-based information initiated in 1973 and updated annually. The current (SEER 18) registry grouping now includes approximately 30% of the US population. Based on the latest updated publication [5], incidence of GEP NENs was 3.56 per 100,000 persons per year, with small intestine and rectum representing the most common sites (1.05 and 1.04 per 100,000 persons, respectively) and pancreas much rarer (0.48 per 100,000 persons).

Interestingly, prevalence of all NENs is clearly increasing due to the good prognosis of most of them. This is particularly important if it is considered that GEP NENs were reported as the second type of malignancy of the digestive system in terms of prevalence just after colorectal cancer.

Low-grade (G1) and early-stage (localized) GEP NENs showed the most increasing incidence. This can be referred to the <2 cm pancreatic incidentalomas and small GI polyps, especially in the rectum, probably related to increasing use of imaging procedure in clinical practice.

Gastroenteropancreatic NECs are extremely rare. They represent around 3% of extrapulmonary NEC that are about 9% of all NECs. The vast majority of NEC are represented by small cell lung cancer (SCLC) [6].

With regard to survival rectum and appendix, NETs showed a median survival (24.6 and >30 years, respectively) much better than panNETs (3.6 years). Of course survival resulted related to the stage and grade. Metastatic small intestine NETs had the best survival (5.8 years) and metastatic colon NET the worst (4 months).

Globally, the updated SEER database data showed that incidence of NENs increased 6.4 folds from 1973 to 2012, with stomach and rectum representing the highest rate (fifteenfold and ninefold, respectively). Also survival improved over time, especially for metastatic panNET, probably due to improvements of therapy.

60.2.2 Lung NENs

Lung NENs represent around 25% of all lung cancers. Unlike GEP NENs that are for the vast majority WD, lung NENs are dominated by the PD forms. Small cell lung cancer (SCLC) represents roughly 20% of all lung cancers, LCNEC 3%, AC 0.3%, and TC 2%.

Epidemiology of lung NETs can be different if considered from two different points of view. Indeed while in clinical practice of a lung cancer medical oncologist lung NET represents a very rare entity (<3% of all lung cancers), they are quite frequent in the clinical practice of a NET-dedicated medical oncologist, representing around one third of all low-/intermediate-grade NEN (Fig. 60.1).

Lung NET, particularly TC, can be associated to a clinical syndrome. Carcinoid syndrome is the most frequently associated syndrome, and it regards around 10% of lung NET; ectopic ACTH and acromegaly are the two other possible syndromes [7].

Lung NET can be associated in very rare cases to an inherited syndrome, mainly MEN-1.

60.3 Diagnostic Features of the Functioning and Nonfunctioning GEP NETs

The diagnosis of GEP NENs is based on multiple features including clinical presentation, biochemical markers, imaging, and endoscopy. However, in any case of suspected GEP NEN, a histological confirmation is required to define the diagnosis and to plan a proper multidisciplinary management.

60.3.1 Clinical Presentation

The clinical manifestations of GEP NENs are heterogeneous as these malignancies may be asymptomatic or

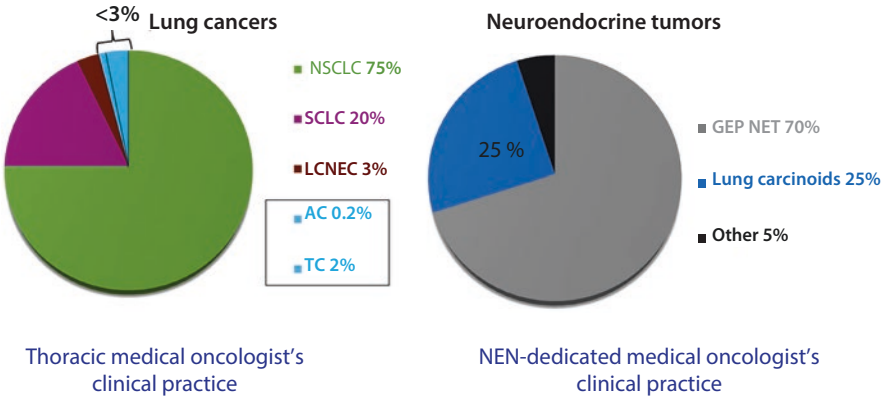


Fig. 60.1 Lung NET represent <3% of all lung cancers and 25% of all NET. (References Rekhtman N et al. Arch Pathol Lab Med 2010; 134:1628-38; Modlin IM et al. Cancer 2003; 97:934-959; Halperin, D.M.; Shen, C.; Dasari, A.; et al. Lancet Oncol. 2017, 18, 525–534; and Ejaz, S.; Vassilopoulou-Sellin, R.; Busaidy, N.L. et al. Cancer 2011, 117, 4381–4389)

may cause nonspecific or obstructive symptoms, particularly in those cases where metastases are already present at the first diagnosis. However, functioning tumors show typical syndromes which are the consequence of hormonal hypersecretion.

60.3.2 Gastrointestinal Neuroendocrine Neoplasms (GI NENs)

Patients with GI NENs are often asymptomatic, although these neoplasms might be responsible for nonspecific symptoms, which are often confused with irritable bowel syndrome (abdominal pain/discomfort, change in bowel movements). Moreover, intestinal NENs can cause obstructive symptoms due to a local fibrotic reaction; thus their prompt diagnosis with consequent surgical resection of the primary tumor is needed. Intestinal tumors with liver metastases can be responsible for the typical carcinoid syndrome, present in 18% of patients with jejunal-ileal carcinoids [3], and characterized by flushing, diarrhea, abdominal pain, and more rarely from tearing, profuse sweating, telangiectasia, cardiac fibrosis, and cutaneous manifestations. It depends on the release of serotonin, which is not any more metabolized in the liver, together with other molecules (tachykinins, prostaglandins, bradykinin) [4].

Appendicular NETs are usually small, well differentiated, and often incidentally found during appendectomy, with a frequency of 3–9/1000 appendectomy [8].

Gastric NETs, which are rare malignancies of the stomach that develop from enterochromaffin-like (ECL) cells in the gastric wall, represent 0.5–1.7% of all gastric cancers and 7.1% of all GI NETs [9]. Three distinct tumor types have been proposed: type 1, which develops as a consequence of hypergastrinemia secondary to achlorhydria in type A chronic atrophic gastritis (CAG), usually not metastatizing; type 2, which is associated with Zollinger–Ellison syndrome in multiple endocrine neoplasia (MEN) type 1, potentially metastatizing; and type 3, which is not associated with hypergastrinemia (sporadic gastric NETs) and is often malignant with frequent metastases to regional nodes (55%) and liver (24%) [6–8]. These tumors are generally asymptomatic, and they are often incidentally discovered during gastroscopy; however, they can rarely give an atypical carcinoid syndrome with prolonged flushing, sialorrhea, sweating, tearing, hypotension, and widespread itching.

Neuroendocrine tumors of the colon (8.6% of all carcinoids) are often voluminous [10]. Finally, rectal NENs (1: 1000–2500 endoscopies) are usually small, nonfunctional, and rarely metastatic.

60.3.3 Pancreatic Neuroendocrine Neoplasms (PanNENs)

Neuroendocrine neoplasms of the pancreas (PanNENs) are a heterogenous, malignant disease with varying tumor biology and clinical presentation. The annual incidence of all PanNENs is 0.8/100,000, which includes both functioning and nonfunctioning PanNENs [11].

Nonfunctioning tumors contribute 60% of all PanNENs. Functioning PanNENs with specific clinical syndromes include [12] the following:

1. Insulinoma, which is characterized by hypoglycemia-related symptoms.
2. Zollinger–Ellison syndrome which includes diarrhea, recurrent peptic ulcers, gastroesophageal reflux symptoms and pain.
3. Verner–Morrison syndrome (VIPoma syndrome), characterized by diarrhea, hypokalemia, and hypochloridia.
4. Glucagonoma which is characterized by the so-called 4D syndrome consisting of diabetes, dermatitis, deep vein thrombosis, and depression.
5. Somatostatinoma which includes diarrhea, diabetes mellitus, and cholelithiasis.
6. ACTH-producing PanNENs which is characterized by the Cushing syndrome.

60.3.4 Biochemical Markers

There are generic and specific biochemical markers for GEP NENs.

Chromogranin A (CgA), which is an acidic glycoprotein of 439 amino acids and a molecular mass of 48 kDa, is found throughout the diffuse neuroendocrine system and shows a sensitivity of 96% and 75% in functioning and nonfunctioning NENs, respectively, and a specificity of 68–100% [13–17]. However, CgA is not highly specific to GEP NENs since it can be found in other malignancies and other non-tumor-related conditions [18–20] and during proton pump inhibitor (PPI) therapy. In addition, blood CgA is also elevated in other neoplasms of non-endocrine origin [18–20] and is not increased in all patients with NENs. Even if CgA does not seem to be particularly accurate as a biomarker in the diagnosis of NENs, it may be useful in the follow-up of patients with NENs [17, 21–27].

Nevertheless, over the last decades new biomarkers have been developed, and they may overcome CgA, NETest being the most studied one, that is, an RNA transcript panel of peripheral blood [28, 29].

In the cases of carcinoid syndrome, the specific marker is the urinary 5-hydroxyindolacetic acid (5-HIAA), serotonin metabolite, which is characterized by a sensitivity of 65–75%, with a specificity of 90–100% [30]. The 5-HIAA dosage can be influenced by some foods or drugs that should be avoided in 3–5 days prior to urine collection.

Regarding functioning NENs, the diagnosis should be based on the following serological tests, summarized in Table 60.4.

Table 60.4 Diagnostic and clinical features of functioning PanNENs

Functioning PanNENs	Diagnosis	Main manifestations
Insulinoma	Plasma glucose <55 mg/dl, insulin ≥3.0 μU/ml, C-peptide ≥0.6 ng/ml and proinsulin ≥5.0 pmol/l	Hypoglycemia-related symptoms
Gastrinoma	Gastrin levels >1000 ng/l with a gastric pH <2. Positive secretin test	Zollinger–Ellison syndrome: diarrhea, recurrent peptic ulcers, gastroesophageal reflux symptoms, pain
VIPoma	Increased VIP	Verner-Morrison syndrome: diarrhea, hypokalemia, hypochloridia
Glucagonoma	Increased glucagon	4D syndrome: diabetes, dermatitis, deep vein thrombosis, depression
Somatostatinoma	Increased somatostatin	Diarrhea, diabetes mellitus, cholelithiasis
ACTH-producing PanNENs	Increased ACTH	Cushing syndrome

60.3.5 Radiological Techniques and Nuclear Medicine Tests

Conventional radiological techniques including abdomen ultrasound (US), computed tomography (CT) scan, and magnetic resonance imaging (MRI) are useful to localize both the primary tumor and possible metastases. However, the identification of a small bowel primary NEN on CT and MRI either via the standard technique or in combination with enteroclysis is challenging [31]; thus in this specific subgroup of patients, endoscopy plays a pivotal role [32]. Computed tomography or MRI scans should be also repeated during the follow-up to assess tumor recurrence/progression after therapy.

In recent years, PET/CT with ^{68}Ga-labeled somatostatin analogues *(SSAs)* has shown the highest sensitivity for localizing NENs and also a high specificity. According to several studies, the sensitivity varied from 86 to 100% and the specificity from 79% to 100% [11], except insulinomas, in which case the sensitivity was only 25% [33]. PET/CT with ^{68}Ga-labeled SSAs is therefore the method of choice to fully stage and localize the extent of disease in patients with NENs, except for insulinoma [34, 35].

60.3.6 Endoscopy

Digestive tract endoscopy allows to identify and to diagnose, by targeted biopsy, mucosal and submucosal NENs located in all the sites of the digestive tract reachable from the endoscope. The diagnosis of small bowel NENs may be challenging with upper and lower GI endoscopy, and their diagnosis has improved with the advent of capsule endoscopy (CE) and double balloon enteroscopy (DBE), which allow for direct visualization of the entire small bowel. CE and DBE may be complementary and show a similar diagnostic yield even if their role in routine staging needs further clarification, also considering the lack of data on potential procedural risks of these methods in NENs [35]. Endoscopic ultrasound (EUS) is

the modality of choice for diagnosing PanNENs and for the locoregional staging of gastric, duodenal, pancreatic, and rectal NETs. In the setting of PanNENs, it has demonstrated higher accuracy in tumor detection than other imaging modalities with sensitivity ranging up to 94%. The sampling adequacy rate of EUS-fine needle aspiration has been reported to be of 83–93%, with an overall complication rate of about 1–2% [36–38].

The diagnostic yield of combined EUS imaging and cytology is significantly better than EUS imaging alone. Moreover, the preoperative availability of the Ki-67 index of a pancreatic lesion may help to decide between typical and atypical resection, and EUS may also have a potential role in the surveillance of multiple endocrine neoplasia type 1 (MEN-1) patients [39, 40].

As regards gastric NENs, the ENETS guidelines suggest to perform EUS in case of lesions >1 cm [7]. A staging EUS is frequently performed to confirm the appropriateness of endoscopic resection, usually endoscopic mucosal resection (EMR). EUS is important also for the staging of duodenal NENs as the exclusion of any locoregional lymph node metastases by EUS is required prior to EMR [41]. Finally, EUS plays a key role in the staging of rectal tumors, especially if >20 mm, with muscularis propria invasion or aggressive histological features, as EUS allows to accurately assess the depth of invasion and the possible presence of locoregional lymph node metastases.

The role of endoscopic technique in the diagnosis of GEP NENs is summarized in Table 60.5.

60.3.7 Histology

The definitive diagnosis of GEP NENs is based on histopathological examination, which is also essential for NEN classification and allocation to therapy [1].

However, obtaining adequate tissues by endoscopic forceps biopsy is often difficult due to the location of gastrointestinal NENs in the deep mucosa and submucosa. Moreover, even if biopsy is successful, the diagnosis may be difficult due to small specimen size or "crush" artifacts, which can lead to misdiagnosis [42].

A complete histopathological examination, which should be performed by histopathologists with a proper expertise in this specific field, must consider the size of the tumor, the number of mitosis, the presence of cellular atypia, the proliferative index, angioinvasiveness, and local invasiveness.

The histological diagnosis of NENs is generally confirmed by immunohistochemical demonstration of neuroendocrine markers [43]. Several general neuroendocrine markers are known: chromogranin-A (CgA), synaptophysin, protein cell product 9.5, neural cell adhesion molecule (NCAM/CD56), neuron-specific enolase (NSE), and Leu 7. However, CgA and synaptophysin are the most common markers to confirm the endocrine nature of the neoplastic cells.

60.4 Diagnostic Features of Lung NETs

In accordance with the 2015 World Health Classification (WHO), lung NENs include four morphological entities: typical carcinoid (TC), atypical carcinoid (AC), large cell neuroendocrine carcinoma (LCNEC), and small cell lung carcinoma (SCLC) [2]. They have specific pathological and clinical features as shown in Table 60.6.

The distinction between TC and AC requires a surgical sample and cannot be reliably assigned to a cytological or biopsy sample [2]. From a clinical point of view, it is essential to not confuse a lung carcinoid, either typical or atypical, with a poorly differentiated neuroendocrine carcinoma (NECs), be it small or large cells

Table 60.5 The role of endoscopic technique in the diagnosis of GEP NENs

Neoplasm	Gastroscopy	Colonoscopy	DBE/CE	EUS
Gastric neoplasms	*Yes*	/	/	For the staging of lesions >1 cm
Duodenal neoplasms	*Yes*	/	/	For the locoregional staging of all duodenal neoplasms
Small bowel neoplasms	Yes	Yes	*Yes*	/
Colorectal neoplasms	/	*Yes*	/	For the staging of rectal tumors, if >20 mm, with muscularis propria invasion or aggressive histological features
PanNENs	/	/	/	*Yes*

Table 60.6 Clinical and pathological features of lung NENs

		TC	AT	LCNEC	SCLC
Age	Mean	45 ys	55 ys	65	65
	Decade	IV–V	V–VI	VI–VII	VI–VII
Sex		F > M	M > F	M > F	M > F
Prevalence		1–2%	0.2%	3%	15%
Smoke		No	Yes (or past)	Yes	Yes
MEN-1		5%	Rare	No	No
Metastases		10–15%	45–50%	50–70%	>80%
Grade		Low	Intermediate	High	High
Morphology		Well–diff.	Well–diff.	Poorly–diff.	Poorly–diff
Mitosis (x 2 mm^2)		<2	2–10	>10 (median 70)	>10 (median 80)
Necrosis		No	Yes (focal)	Yes (extended)	Yes (extended)

60.4.1 Minimum Requirements of an Anatomopathological Report of Lung Neuroendocrine Neoplasms (NENs)

Mitotic counts, the presence of necrosis, and Ki-67 labeling index (LI) should be indicated in the pathological diagnosis of a surgical or biopsy (noncytological) sample for at least two reasons: (a) mitosis and necrosis are integral parts of the current diagnostic-classification criteria, they allow comparative crossed studies and, for mitotic counts, identify CA with different prognosis; (b) although Ki-67 LI has no recognized diagnostic role in lung NEN, many studies have suggested a prognostic role in the carcinoid category (TC and AC), even in the individual subcategories, in addition to orienting the clinician to one or the other extreme of the clinical-pathological spectrum of pulmonary NETs.

60.4.2 Role of Ki67 in Pulmonary NEN

The role of Ki-67 LI is not yet well codified in the lung NEN [44]. However, its scopes of use can be exemplified as follows:

(a) Utility in distinguishing CT and CA from poorly differentiated NE carcinomas, in particular, SCLC, in limited diagnostic material (cytology and biopsies) [45].
(b) Unreliability as the sole diagnostic criterion in individual cases, although there are significant differences in mean or median distribution values between the different subtypes of pulmonary NET.
(c) Possibility of using Ki-67 LI as a prognostic criterion (various "cut-offs" have been proposed in the literature) within carcinoids, even with independent value in multivariate analysis, while there are no data in poorly differentiated NECs.
(d) Nonuniformity of literature data on the methodologies to be followed to calculate Ki-67 [44].

60.4.3 Immunohistochemistry (IHC) in Lung NENs

Immunohistochemical characterization of neuroendocrine differentiation markers (chromogranin A, synaptophysin, and CD56/NCAM and, in some cases, hormones) may be useful to confirm the neuroendocrine nature of neoplastic proliferation or the origin of the tumour, especially in poorly differentiated NENs or when the diagnostic material (biopsy or cytology) is limited where the neuroendocrine nature may not be immediately evident [2]. In case of metastatic sample, the positivity for TTF1 may suggest the lung origin of a TC or AC, while there is often an unfaithful expression of nuclear transcription factors (TTF1, CDX-2, Isl-1, PAX-8, WT1) in NECs independently by the original anatomical site.

60.4.4 Endoscopic Diagnosis

Endoscopic procedures (flexible bronchoscopy) represent the first choice to get a cytohistological diagnosis of patients with suspected airway tumor [46]. Neuroendocrine neoplasms may present as typical carci-

noids with endobronchial lesions, endoscopically visible, and well-delimited, regular surface, sometimes polypoid and easily bleeding upon contact with the instrument. *Large cell neuroendocrine carcinomas* often present as invasive lesions of the airways, necrotic, with evident infiltration of the bronchial wall and, sometimes, of adjacent mediastinal structures. *Atypical carcinoids* have intermediate endoscopic characteristics between TC and LCNECs with variable degree of invasiveness of the bronchial wall and adjacent structures. Currently, EBUS-TBNA is the most used method for the diagnosis and staging of pulmonary neoplasms; it presents high diagnostic performance and guarantees a quality of sampling sufficient for different immunohistochemical analyses and differentiation between different types of neuroendocrine tumors [47, 48]. Invasive staging methods of pulmonary neoplasms (mediastinoscopy, video thoracoscopy, mediastinotomy) are only indicated in cases of highly suspicious lymph nodes if EBUS-TBNA is negative for malignancy [49].

60.4.5 Radiological Imaging

The radiological diagnosis is based on two main procedures: multislices computed tomography (MSCT) and magnetic resonance (MR).

Pulmonary carcinoids in MSCT occur as well-circumscribed nodular alterations, usually <5 cm in size, often associated with the presence of a perihilar mass.

In most cases, the carcinoid has a central location, while less commonly it is located in the peripheral pulmonary site [50]. With MSCT, it is possible to identify the location of the disease to undergo biopsy. Percutaneous CT-guided biopsy is the best technique for histological diagnosis of both medial and pulmonary solid lesions. Indeed, in addition to providing adequate material for a reliable histological diagnosis [51], the needle gauge used (18 Gauge) to perform the sampling does not significantly affect the percentage of expected complications as for other body districts [52]. The LCNEC shows radiological characteristics very similar to those of the NSCLC, so it is difficult to distinguish them on the only morphological basis. The LCNEC [53] develops peripherally in the vast majority of cases, while, in a minority of cases, it is found in the central lung, with concomitant atelectasis. The margins are usually well defined often with lobulations, but there are also presentations with nodules with spiculated margins, with cavitations, aerial bronchogram in their context, and central necrosis [52, 53]. A characteristic contrast enhancement for this type of injury is not appreciated. The SCLC develops centrally, and the diagnosis is almost always made when the disease is in an advanced stage.

Patients who cannot undergo CT (i.e., allergy to iodine m.d.c.) can be studied with MR for the abdominopelvic evaluation. In this case, it is recommended to use standard weighted T1 and T2 sequences for the study of the abdomen and multiphase dynamic sequences during and after the injection of hepato-specific m.d.c. (Gd -EOB -DTPA) [54–56]. Moreover, MR is more sensitive than MSCT in recognizing very small lesions in the liver [57, 58].

60.5 Molecular Biology Features

60.5.1 Gastroenteropancreatic Neuroendocrine Neoplasms (GEP NENs)

In the last years, the development of new technologies has allowed the study of innovative aspects about tumors and their mechanisms, e.g., their genesis, growth, and strategies of resistance to chemotherapy. This new awareness has taken importance on the landscape of the biological features of tumours and allowed the research and the use of new key strategies. The classical therapies are now joined by new drugs known as "targeted therapies" which actually are more effective and characterized by a different and lower toxicity. This is the reason why it is important not just studying but also understanding which are the genetic and epigenetic features of a tumor; the knowledge about the molecular aspects of GEP NENs is not so deep unlike other neoplasms, above all for their low frequency and their heterogeneous behavior; what we know is that the majority of mutations which lead the tumorigenesis are expressed in those genes which usually regulate in a negative sense the growth and the proliferation of cells; they are known as "suppressor genes." This is an atypical aspect as in other tumors, the uncontrolled cell proliferation starts from activating mutations which involve other types of genes (proto-oncogene).

Classically, it is possible to divide the mutations in two main categories: the germline and the somatic ones.

60.5.2 Germline Mutations

They account for about 5% of NENs and above all pancreatic ones, even if they can be involved also in the midgut. There are different genes implicated, in particular, MEN1, VHL, NF1, TSC1, and TSC2 [59]. Mutations in these genes are present not only in GEP NENs' tumorigenesis but also in more complex and multiple organ diseases in which GEP NENs are just a part of the syndrome.

60.5.2.1 MEN1 and Menin

MEN-1 (11q) by the expression of its protein interacts with several transcription factors such as JUN-D (resulting in a negative control of cell-proliferation), c-Myb and c-Myc, and NF-kB; furthermore menin controls TGF-beta, Wnt and Hedgehog, and PI3K/AKT signals; its role implies also the regulation of RNA and, in particular, of miRNA [60]. Considering these pathways is possible to understand the role of menin as negative controller of cell cycle. The synthetic view of Fig. 60.2 shows how complicated is the role of menin and how many pathways can be modified by its mutation.

Clinically, MEN-1 mutations lead to a condition characterized by at least two of these three tumors: pancreatic NENs, parathyroid adenomas, and anterior pituitary adenomas, even if there are lots of other manifestations like adrenal cortical tumor or skin alterations (facial angiofibroma) [62].

60.5.2.2 VHL (Von Hippel Lindau)

VHL gene codifies for a protein, VHL, which contributes to regulate the cell proliferation: in particular, it interacts with elongin C, forming a complex which allows the degradation (via ubiquitinylation) of HIF-alpha (hypoxia-induced factor), a transcript factor of genes like VEGF, EPO, TGF-alpha, and PDGF-beta [63]. Von Hippel Lindau disease can be distinguished into two types, and both of them are associated to the development of NENs of pancreas (type 1 and subtype 2B) [64].

60.5.2.3 NF1 (Neurofibromatosis-1)

Neurofibromin 1 (17q11) has an important role in inhibiting RAS protein, thanks to its GTPase activity. Germline mutation of NF1 causes a particular disease characterized by skin alteration like "café au lait" spots, neurofibromas, malignant peripheral nerve sheath tumor, and rarely neuroendocrine tumors [65].

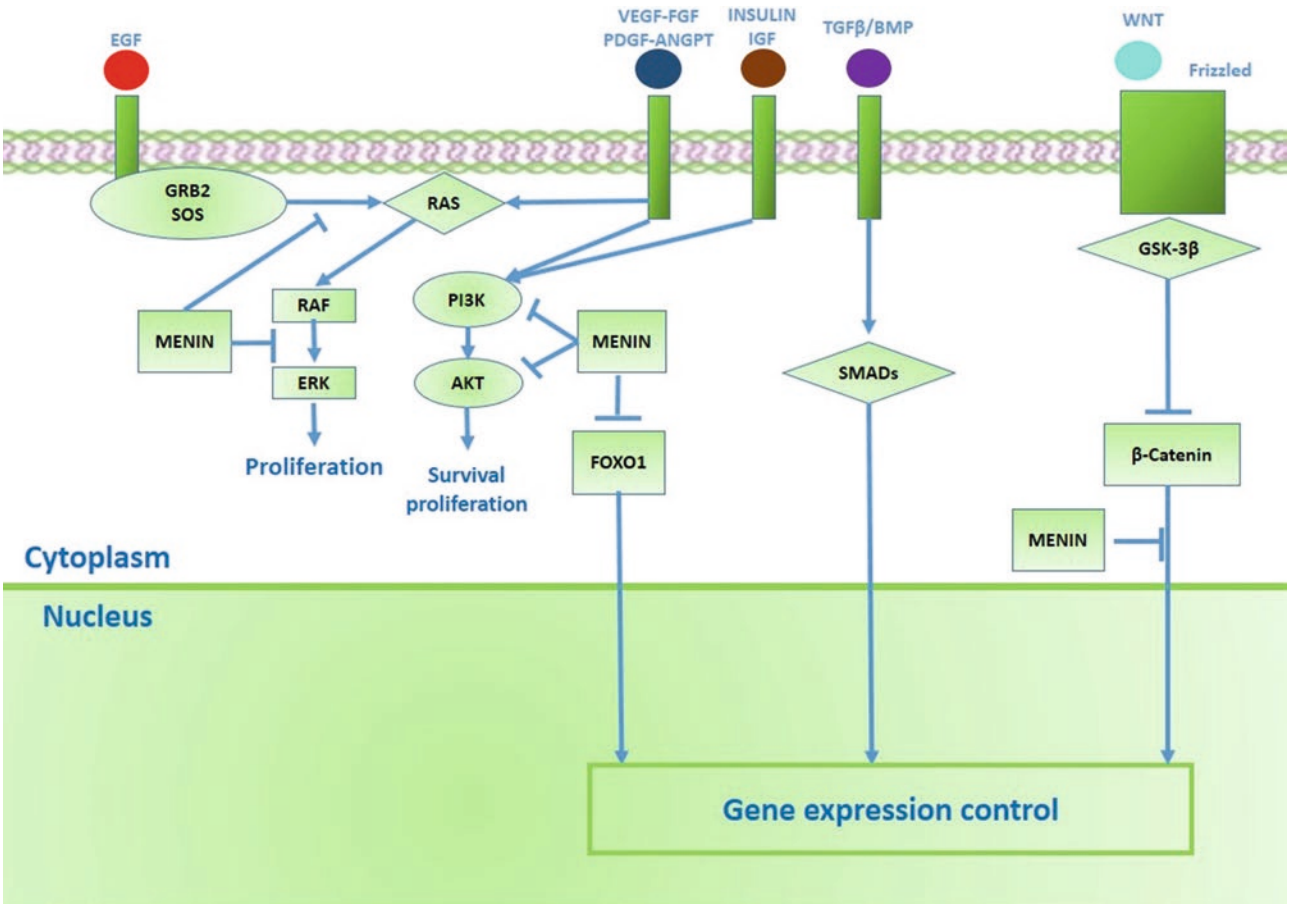


Fig. 60.2 Role of Menin: modified from "Towards a new classification of gastroenteropancreatic neuroendocrine neoplasms" [61]

60.5.2.4 TSC (Tuberous Sclerosis)1 and 2

Tuberous sclerosis complex 1 and 2 codes for hamartin and tuberin; their role involves the regulation of cell adhesion, thanks to the interaction with PI3K/AKT/mTOR pathways [66]. Some clinical manifestations consist in skin lesions (hypomelanotic macules), ungueal fibromas, renal angiolipomas, and hamartomas [59].

For a synthetic view, see ◘ Table 60.7.

60.5.3 Somatic Mutations

Between PanNENs and small-intestinal NENs, there are some differences regarding the types and the frequency of somatic mutations.

The most frequent genetic alteration in PanNENs consists in the loss of heterozygosis (LOH) of MEN1; other alterations include YY1 (ying-yang 1) which is a transcriptional repressor involved in the control of some factors of the mTOR pathway; it is correlated to a more advanced age at the diagnosis, and it accounts for about 30% of patients with sporadic insulinomas [67].

Death domain-associated protein (DAXX) and ATRX are other two factors implied in the tumorigenesis of NETs. ATRX (involved also in an inherited X-linked disease characterized by alpha-thalassemia and mental retardation), with DAXX, forming a histone H3.3 chaperone, contributes to regulate most aspects of cell regulation-like apoptosis and transcription.

In particular, it seems that they are linked to the chromosome instability (CIN) when their levels are lower than normal, and it is also correlated with tumor stage and the presence of metastasis [68].

Some tumors are characterized by the presence of specific alteration, like MEN1 for gastrinomas or YY1 for insulinomas [67, 69].

Usually, NENs of small intestine are genetically stable, and it was not possible to find somatic alterations in MEN1, ATRX, or DAXX. Although the biological landscape presents some modifications like the mutation of CDKN1B, it encodes for cyclin-dependent kinase inhibitor 1B (p27KIP1), and its inactivation leads to a worse prognosis.

Other mutations which have been discovered in small intestine NENs regard SDHD gene (involved in a hypoxic response when mutated and already studied for the onset of paraganglioma); it has been proved a loss of heterozygosis of this gene, and this correlates with a possible role of hypoxia in small intestine tumor [70].

60.5.4 Role of Chromosomes

Understanding the chromosomal alterations is another strategy to study the biological aspect of tumors to find and at the same time new criteria useful to classify GEP NENs.

It is possible to identify two kinds of events: a chromosome or a microsatellite instability. The latter is actually not well-known, and it seems to be correlated to a better prognosis; differently, CIN is a well-known mechanism in both types of NENs (pancreatic and small intestinal): loss of chromosomes is more frequent, and probably during the development of the tumor, there is an accumulation of chromosomal alteration [35]. Some alterations in PanNENs regard [61] the following:

- Deletion of chromosome 9p: It leads to the loss of CDKN2A, which expresses p16 and p14, two tumor-suppressor proteins.
- Deletion of chromosome 16p: In this case, there is the loss of expression of TSC2, involved in the regulation of PI3K/AKT/mTOR pathway.
- Deletion of chromosome 10p: Found more in malignant lesions, it leads to a deregulation of AKT/mTOR pathway.

◘ **Table 60.7** Genetic syndromes

Syndrome	Gene	Localization	Main manifestations
Multi-endocrine neoplasia 1	MEN	11q13	Pancreatic NENs, parathyroid adenomas and anterior pituitary adenomas, adrenal cortical tumor or skin alterations (facial angiofibroma), lipomas, collagenomas, meningiomas
Von Hippel Lindau disease	VHL	3p25–26	Angiomiomatosis, pheochromocytoma, renal cell carcinoma, NENs, hemangioblastomas
Neurofibromatosis (Von Recklinghausen's disease)	NF1	17q11	Café au lait spots, neurofibromas, malignant peripheral nerve sheath tumor, neuroendocrine tumors
Tuberous sclerosis complex	TSC1 TSC2	9q34 16p13.3	Skin lesions (hypomelanotic macules), ungueal fibromas, renal angiolipomas, hamartomas

60.5.5 Mechanism of Methylation

Methylation of CpG islands is a known mechanism, not just for colorectal carcinoma but also for GEP NENs; in particular, this process involves different loci like MGMT, RASSF1A, MLH, and CDKN2A [22]. The most remarkable aspect regards the methylation status of MGMT: it correlates with a better response to alkylating agents like dacarbazine or temozolomide [38]. Methylation mechanism is more common among midgut NENs than pancreatic ones [61].

60.5.6 Gene Expression Patterns

As a result of what is reported until now, it could be useful to remind the necessity of classifying NENs considering the biological aspects of the tumors. Some studies have already done this: Duerr et al. have identified, using DNA microarray analyses, two categories of PanNENs: a benign and a malignant form; the latter is characterized by the overexpression of the genes ADCY2, FEV, GADD45beta, and NR4A2 and is compared to the well-differentiated NENs. Other genes like PDGFR are expressed in the two subtypes [71].

60.6 Molecular Pathways and Biological Drugs

See related paragraph.

60.6.1 Bases of Treatment

60.6.1.1 Gastroenteropancreatic Neuroendocrine Neoplasms (GEP NENs)

Each patient with a GEP NEN or suspicious for that should be referred as soon as possible to a referral center for NENs.

First of all, it is extremely important that pathological diagnosis of GEP NEN is reliable, that means that the neoplasm should be a *pure* NEN. Indeed, a *non-pure* GEP NEN might be an adenocarcinoma with *neuroendocrine differentiation* or a mixed neuroendocrine/non-neuroendocrine neoplasm (MiNEN) that are two entities distinct from NENs and therefore requiring a different clinical management [1].

Tumor staging and characterization Fig. 52.5 - See below "Lung neuroendocrine neoplasm staging" are two critical steps. Clinical staging should be performed by means of morphological (radiological) tools, such as contrast-medium computed tomography (CT) scan of the chest and abdomen or abdominal magnetic resonance imaging (MRI) + chest CT scan. Pathological staging is related to the TNM eighth edition *(AJCC Cancer Staging Manual, eighth ed, Amin MB (Ed), Springer, Chicago 2017)*. Somatostatin receptor subtype 2 (SSTR-2)-related imaging should be performed in patients with low-/intermediate-grade GEP NET. Metabolic imaging with 18-fluorodeoxyglucose (FDG)-positron emission tomography (PET) should be considered for high- and intermediate-grade GEP NEN.

60.6.1.2 Local/Locally Advanced Stage

For patients presenting a pure G1–G2 GEP NET at a local or locally advanced radically resectable stage, an upfront surgical approach should be discussed within the MDT.

For patients presenting a pure G3 GEP NEC at a local or locally advanced radically resectable stage, a chemotherapy +/– radiotherapy should be discussed integrated with a possible surgical approach and its timing.

For patients presenting a pure G3 GEP NET at a local or locally advanced radically resectable stage, an upfront surgical approach versus an upfront medical treatment should be discussed within the MDT.

60.6.1.3 Advanced Stage

In patients with advanced GEP NETs, no specific sequence or integration of therapies has been validated so far. Therapeutic decision about the single-line therapy depends on a number of factors, including level of evidence, regulatory aspects, guidelines, local expertise/experience, logistics, and clinical trials availability. Furthermore, it should be linked to a number of tumor-related factors, such as the presence of a clinical syndrome, inherited condition, tumor grade, and SSTR-2 functional expression. The metastatic tumor burden, tumor primary site, resectability, patient symptomaticity (tumor's mass effect), and rate and pattern of tumor progression are also important factors to be considered for the therapeutic choice (◘ Tables 60.8 and 60.9).

It is therefore clear that ideally each clinical case should be discussed within a NEN-dedicated multispecialist team and that early and late therapeutic goals should be shared. Different goals and strategies can induce distinct therapeutic choices for therapies with different level of evidence in the same clinical settings (◘ Fig. 60.3).

Locoregional treatments, mainly in the liver, can be discussed in selected cases, such as monofocal or oligofocal liver progression, minimal residual disease after tumor response on systemic therapies, or within a global debulking strategy. However, the level of evidence is quite low, coming mostly from retrospective analyses [72].

Table 60.8 Main criteria for therapeutic choice

Clinical syndrome	Functioning vs. nonfunctioning
Tumor grade	Histology (morphology + Ki-67)
Tumor stage	Clinical (morphological and functional imaging) or pathologic (TNM)
Tumor primary site	Midgut, pancreas, other GI, unknown primary
SSTR-2 imaging	^{68}Ga-SSA-PET/CT
Genetic syndrome	Sporadic vs. inherited

Table 60.9 Further criteria for therapeutic choice

Tumor related symptoms (mass-effect)	Symptomatic vs. asymptomatic
Performance status	0 vs >0 (ECOG)
Comorbidity	
Tumor status	Stable vs. slowly progressing vs. rapidly progressing
Tumor burden	Radiological imaging
Goal of the single therapy	Syndrome control vs. symptoms control vs. tumor growth control Cytoreduction vs. stabilization
Goal of the therapeutic strategy	Debulking (partial or absolute) vs. tumor growth control over time (QoL)

Bases of therapeutic approach to GEP NET patients

- Involvement of a NET referral center
- Multidisciplinary discussion (1° step of diagnostic-therapeutic management)
- The MDTshould be composed by NEN-dedicated specialists
- The MDT should share a therapeutic strategy rather than the single therapy

Fig. 60.3 Key-points of clinical management of patients with GEP NET

In advanced *nonfunctioning* pNET, an SSA can be considered as first-line therapy.

For the so-called GEP *NET G3*, there is no absolute evidence about a specific first-line therapy and sequencing. As

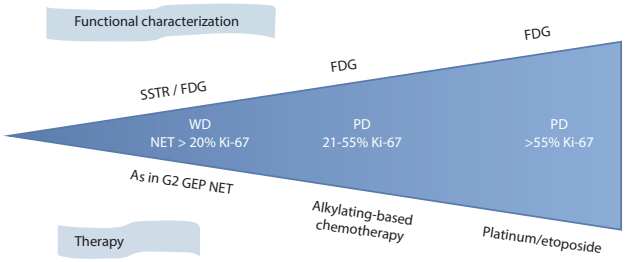


Fig. 60.4 Functional characterization of high-grade GEP NENs

they are *high-grade* neoplasms, a chemotherapy is usually considered, with alkylating-based regimens or fluoropyrimidines/oxaliplatin combinations preferred to platinum/etoposide [73]. However, EVE and SUN can be evaluated in advanced pancreatic NETs G3 considering that tumor morphology rather than proliferation index was the inclusion criterion for the regulatory trials. Furthermore, some recent reports indicated specific activity of EVE and SUN in panNET G3 [74, 75], and recommendations [76] suggest that clinicians should manage NET G3 in a different manner than the NEC G3 even considering therapeutic options usually discussed in the G2.

In a patient with an advanced GEP NEC, the SSTR-2 imaging does not have a role, whereas ^{18}FDG-PET/CT should be considered to stage and characterize the disease.

In this latter context chemotherapy represents the universally shared option. Historically, a combination of cisplatin or, less commonly, carboplatin plus etoposide was proposed (Fig. 60.4).

60.6.1.4 Lung Neuroendocrine Neoplasms (GEP NENs)

Staging and Characterization (Fig. 60.5)

Local or Locally Advanced Stage

A patient with a resectable locally advanced lung NET should be considered for resective surgery.

A patient with a locally advanced high-grade lung LCNEC should be considered for upfront or delayed resection, including neo-/adjuvant chemotherapy +/– radiotherapy.

A patient with locally advanced SCLC should be considered for chemotherapy +/– radiotherapy.

Advanced Stage

Patientis with a metastatic lung NET can receive two general types of therapies, comprising locoregional and systemic therapies. Among the former, there are palliative surgical resection of the primary site or metastatic disease, palliative external beam radiotherapy, palliative interventional radiology procedures including liver transarterial embolization (TAE), thermoablation radiofrequency (TARF), and liver transarterial radioem-

Fig. 60.5 Staging and characterization of lung neuroendocrine neoplasms

Lung neuroendocrine neoplasm staging

Type of lung NEN	Morphological imaging	Functional imaging	Circulating markers
Well differentiated Very low Ki-67 Low grade TC	Total-body CT scan	^{68}GaPET-CT-DOTA-peptide CgA	
Well/moderately differentiated Intermediate Ki-67 (e.g. 3–20) AC	Total-body CT scan	^{68}GaPET-CT-DOTA-peptide + ^{18}FDGPET-CT	CgA + NSE
Poorly differentiated, High Ki-67 (e.g. > 20%) LCNEC/SCLC	Total-body CT scan	^{18}FDGPET-CT	N SE

Caplin ME, et al. Ann Oncol. 2015; 26:1604-20. Gasparri R, et al. Q J Nucl Med Mol Imaging. 2015;59:446-54. Wolin ME. Oncologist. 2015;20:1123-31.

bolization (TARE) with ^{90}Yttrium. The latter category comprises somatostatin receptor 2 (SSTR-2)-directed therapies, including somatostatin analogs (SSAs) and peptide receptor radionuclide therapy (PRRT), molecular targeted agents (MTAs) like Everolimus, chemotherapy (several regimens), and interferon (IFN).

Criteria for choosing therapy and therapeutic strategy in lung NET are similar to those of GEP NET.

60.7 Theragnostic Role of Nuclear Medicine

Nuclear medicine has acquired a central role for the management of NEN, mainly as a consequence of several factors including a high diagnostic accuracy and clinical availability of different radiopharmaceuticals (which may prove more valuable in specific clinical settings) and for the possibility to employ the same compounds for target therapy. In fact, being very heterogeneous both at presentation and during the disease natural course, NEN still represents a challenge for the clinicians.

PET/CT presents several advantages including a higher spatial resolution [77, 78], the possibility to semi-quantify the tracer uptake in the region of interest (SUVmax) [77, 78], lower costs [79], and shorter image acquisition protocol (2 hours vs acquisitions at 4-24 hours). Moreover, several β+ emitting radiopharmaceuticals are currently available for PET/CT imaging to study either somatostatin receptor (SSTR) expression (68Ga-DOTA-peptides, the most frequently employed tracers in well differentiated NEN) or metabolism (18F-DOPA, 18F-FDG).

60.7.1 β+ Emitting Radiopharmaceuticals Employed for PET/CT Imaging

68Ga-DOTA-peptides (DOTA-TATE, DOTA-NOC, DOTA-TOC): are somatostatin receptor analogues, internalized after binding. The currently available compounds differ for the affinity to SSTR subtypes (DOTA-TATE shows higher affinity for SSTR-2; DOTA-NOC shows the wider SSTR subtype affinity, binding to SSTR-2,3,5) [80]. Sensitivity and specificity for the detection of well-differentiated NEN lesions is very high (90–98% and 92–98, respectively) [80–82], and a very high interobserver agreement has been reported [83].

Indications include evaluation of disease extension (staging/restaging) (Fig. 60.6), detection of relapse, selection for targeted therapy (with either cold or hot SSAs), and identification of the unknown primary tumour site in pts. with proven NEN metastatic lesions [80].

Potential utility of SSA therapy withdrawal has been suggested (1 day is suggested for short-lived molecules and at least 3–4 weeks for long-acting SSAs I.V.); however, there is no consensus;

18F-DOPA [80]: at present, the clinical setting in which 18F-DOPA is most frequently employed is the detection of NEN presenting with low/variable SR-expression (neuroblastoma, pheochromocytoma, paraganglioma-abdominal, medullary thyroid cancer).

18F-FDG [84]: is the most frequently employed radiopharmaceutical in oncology; its uptake reflects cell glucose metabolism and is therefore an indirect measure of dedifferentiation and aggressiveness of tumour cells. Most solid tumors are FDG-avid.

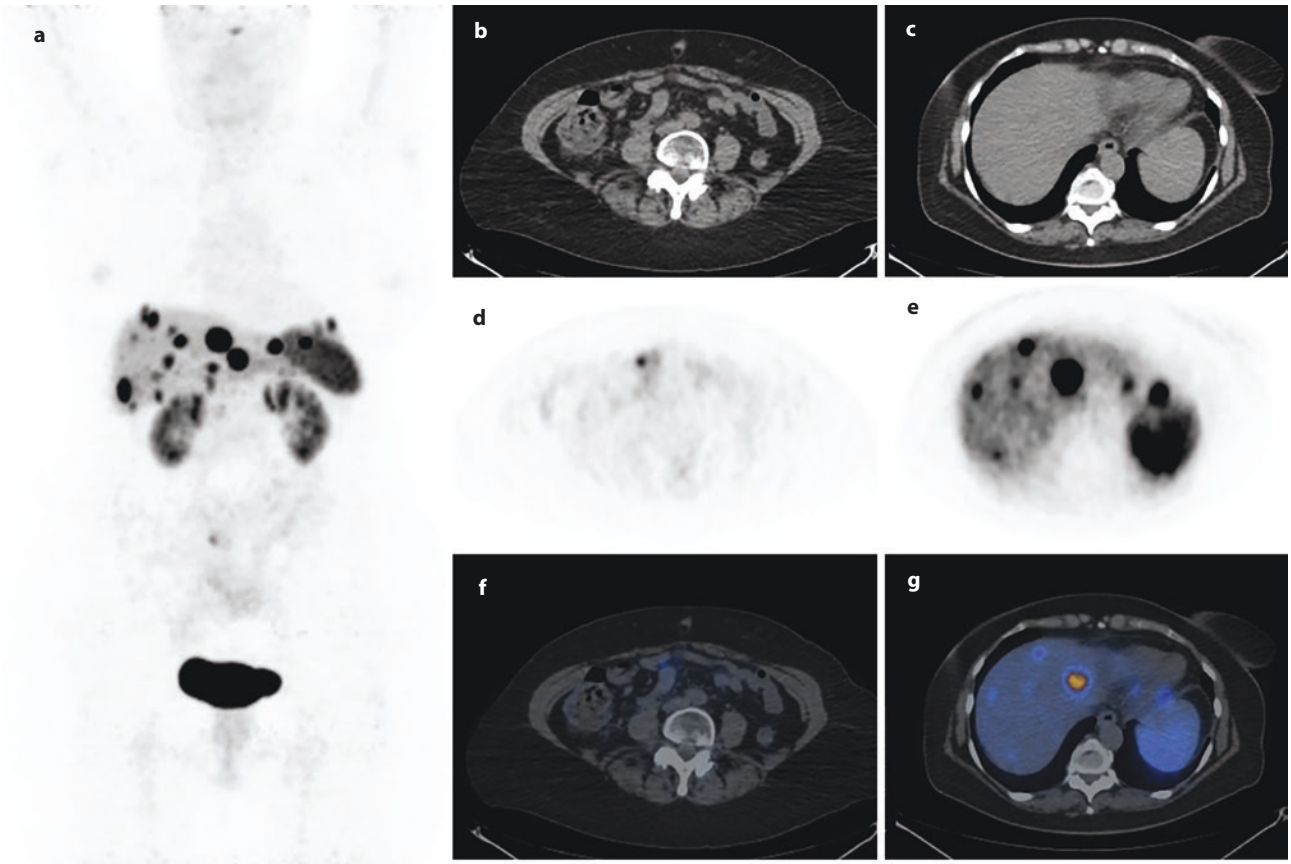


Fig. 60.6 PET/CT MIP **a**, low dose CT **b**, **c**, PET **d**, **e** and fused PET/CT **f**, **g** transaxial images of a patient with multiple metastatic liver NEN lesions **e**, **g** and ileal primary **d**, **f**. All lesions show high SR expression. Of note: ileal NEN may present with very small lesions that may be better appreciated in PET only images

60.7.2 Choice of the Radiopharmaceutical

The choice of the radiopharmaceutical to employ first is guided by evaluation of several factors: tracer availability in the nuclear medicine center, SSTR expression (some histotypes are known to show low/variable SSTR expression), and differentiation grade (SSTR expression is higher in well-differentiated forms).

The 2017 EANM (European Association of Nuclear Medicine) guidelines [80] indicated in the primary tumor site a fundamental criterion choice (► https://www.eanm.org/publications/guidelines/): in particular, 68Ga-DOTA-peptides should be considered as first choice for the assessment of NEN of the foregut and midgut and for paragangliomas of the head and neck. On the contrary, 18F-DOPA is to be considered first choice for abdominal paragangliomas, while 18F-FDG should be employed before 68Ga-DOTA-peptides to study NEN of the hindgut.

Additional factors to be considered when assessing the potential additional role of FDG are tumor grade and whether the detection of the presence of a more aggressive (dedifferentiated and therefore FDG-avid) clone is mandatory. In particular, much attention has been devoted to the potentially complimentary role of 68Ga-DOTA-peptides and 18F-FDG [80, 85]. On one side, obtaining images with both tracers could provide a complete biological characterization of the whole tumor burden: confirming both a significant SR expression (which would drive somatostatin target therapy) and detecting if aggressive clones are also present (providing prognostic patients stratification). On the other side, there is no international consensus on whether FDG should be performed regardless of tumor grading (therefore in all NEN grades) [80, 85] and how often it should be repeated during the disease natural history (taking into account that the generally better prognosis of NEN as compared with other solid tumors corresponds to a longer life expectancy). International experts agree however that, if positive, FDG is prognostic, allowing the idnetification of patients with more aggressive disease tumors. Although the current approach to FDG/DOTA-peptides combined imaging is different across centers and countries, based on current knowledge [80, 85], experts agree that FDG should be the first choice for NEC and it may provide addi-

tional clinically useful information in G3 (ki67 values 21–55%) and G2 (ki67 values 3–20%) NEN. The most accurate approach seems to plan combined imaging based on accurate evaluation on the clinical case and on multidisciplinary discussion.

60.7.3 Radionuclide Target Therapy (PRRT)

PRRT consists of the systemic administration of radiopharmaceuticals that bind to SR (overexpressed on NEN lesions) and that are labeled with isotopes that can deliver a cytotoxic radiation (β-emission) to target cells, resulting in target radiotherapy. The short pathway of emitted radiation ensures a target cytotoxic effect. DOTA-TATE and DOTA-TOC can therefore be used for both diagnosis (when labeled with the β+ emitting 68Gallium) and therapy (when labeled with 90Y or 177Lu), acquiring a theranostic role in NEN management.

Critical organs are represented by the kidneys (infusion of positively charged amino acids can reduce radiation to kidneys of approximately 60%) and the bone marrow (including late hematologic toxicity).

However, mainly due to regulatory issues, PRRT has been employed up to now only as an experimental treatment. In 2013, the EANM published the first procedural guidelines for PRRT in NEN (detailed schemes and doses can be found at ► https://www.eanm.org/publications/guidelines/). In 2017, the first international phase 3 multicenter trail was published [86]. This latter study evaluated the efficacy and safety of 177Lu-DOTA-TATE as compared with high-dose octreotide long acting in patients with advanced, progressive, SR–positive mid-gut NEN. The study reported how PRRT with 177Lu-DOTA-TATE resulted in markedly longer progression-free survival and a significantly higher response rate as compared to the arm treated with high-dose octreotide. Severe adverse effects were minor.

Current ENETS guidelines [85] consider PRRT for treatment of patients with positive expression of SR-2, or metastatic or inoperable, after failure of other treatment or at progression. There is an open debate on when to position PRRT in the NEN management flow chart and to what extent is the impact of potential late complications (e.g., hematologic toxicity) on subsequent treatment options.

60.7.3.1 Contraindications to PRRT

Absolute: pregnancy, severe acute concomitant illnesses/unmanageable psychiatric disorders.

Relative: breast feeding (if not discontinued), severely compromised renal function (especially when 90Y-labeled compounds are employed, while for 177Lu-labeled radiopharmaceuticals, a mild-/moderate-grade renal impairment can be tolerated, e.g., creatinine ≤1.7 mg/dl); severely compromised bone marrow (EANM suggested reference values are WBC <3000/μl, with absolute neutrophil count <1000/μl, PLT <75,000/μl for 177Lu-DOTATATE, <90,000/μl for 90Y-DOTATOC, RBC <3,000,000/μl).

Figure legend: PET/CT MIP **a**, low-dose CT **b**, **c**, PET **d**, **e**, and fused PET/CT **f**, **g** transaxial images of a patient with multiple metastatic liver NEN lesions **e**, **g** and ileal primary **d**, **f**. All lesions show high SR expression. Of note: ileal NEN may present with very small lesions that may be better appreciated in PET only images

60.8 Chemotherapy

60.8.1 Gastroenteropancreatic Neuroendocrine Neoplasms (GEP NENs)

Chemotherapy has been used for several decades in NEN patients, although no clear evidence about its survival impact was demonstrated. Since no validated and universally shared predictors of response and efficacy has been found so far, clinical and tumor features are the only drivers for chemotherapeutic regimens and schedules choice.

60.8.1.1 Chemotherapy in Neuroendocrine Carcinomas (NECs)

Chemotherapy is the most common option proposed in advanced NECs. Although these neoplasms appear relatively chemosensitive, their prognosis remains dismal. Cisplatin (CDDP)/etoposide (VP-16) is the regimen of choice based on the assumption that the clinical behavior of NECs is similar to that of SCLC. The literature, however, is quite scant and limited to studies rather dated and not specifically designed to clarify this topic [87–90].

Carboplatin (CBDCA) has been reported as a valid alternative to CDDP and irinotecan to VP16 for lung NECs [91, 92].

According to the latter point, it has been reported that patients with <55% Ki67 GEP NECs had a low response rate but lived longer than those with >55% Ki67 [93].

Oral etoposide has been reported safe and efficient in treating G3 GEP NEN patients scheduled for cisplatin/carboplatin + etoposide therapy [1, 94].

Over the latest years, the heterogeneity of high-grade category has been deeply explored [93, 95, 96].

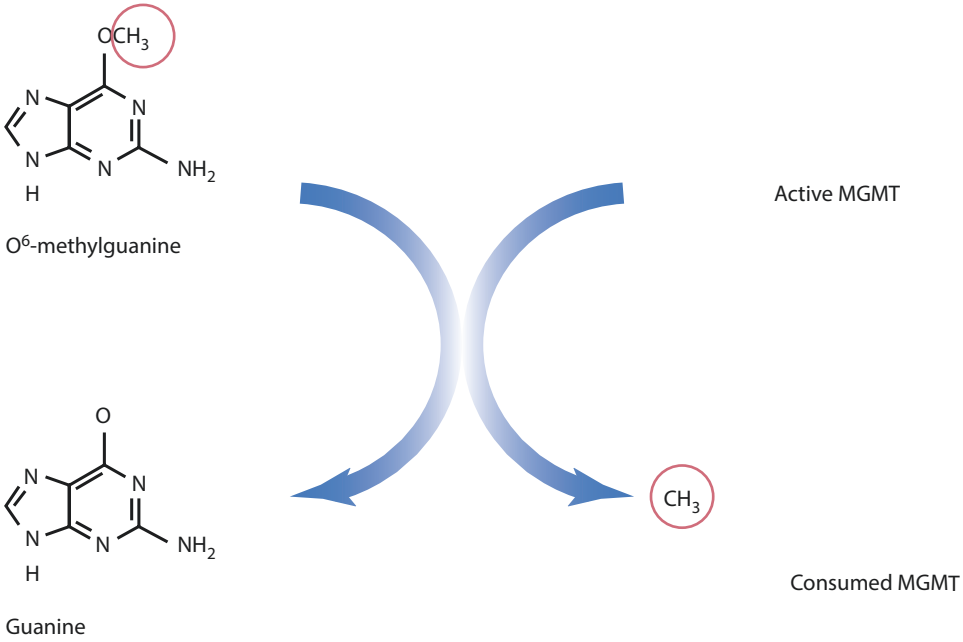


Fig. 60.7 MGMT mechanism of action. (From Ref. [100])

Among GEP NENs G3 (WHO 2010), a particular subgroup is represented by morphologically well-differentiated and Ki67 > 20% and/or mitosis >20 HPF NENs. Recent reports suggest that these neoplasms have a better prognosis than the other GEP NECs and respond less to conventional chemotherapies [93, 95, 97].

On these bases in patients with Ki67 < 55% NEC, it is possible to consider chemotherapeutic regimens alternative to those containing platinum.

60.8.1.2 Chemotherapy in Neuroendocrine Tumors (NETs)

In NETs, chemotherapy has been widely used as single-agent or combination regimens.

Evidence came from retrospective and phase II studies, mostly using alkylating agents [streptozotocin (STZ), dacarbazine (DTIC), temozolomide (TMZ)], antimetabolites [5-fluorouracil (5-FU), capecitabine)], and, more recently, oxaliplatin.

Dacarbazine has been reported active as single agent and combination [98, 99].

Temozolomide is the latest compound from this category; it is an oral agent, usually well tolerated. A number of retrospective and prospective studies with TMZ were published as single agent and combination; although a specific combination regimen was not defined, TMZ + capecitabine is one of the most proposed. The methylguanine-methyltransferase (MGMT) enzyme can methylate the oxygen in 8-guanine position allowing the repair of the DNA damage induced by alkylating agents as TMZ. Therefore, it is supposed that the expression of MGMT is inversely proportional to the response to the TMZ itself (Fig. 60.7). However, so far no absolute validation of this concept in clinical practice was done.

Among the other type of chemotherapy, oxaliplatin has been largely used all around the world [100–106].

Lung Neuroendocrine Neoplasms (NENs)

There is no standard chemotherapy regimen for lung NETs [107].

Five-FU, CDDP, carboplatin, irinotecan, TMZ, gemcitabine, VP-16, doxorubicin, STZ, dacarbazine, paclitaxel, docetaxel, and pemetrexed were the mostly drugs used as single agent. Polychemotherapy was able to produce a radiological PR in only 5–10% of patients, but with symptomatic responses in 40–60% of cases.

A retrospective study on just lung NETs [108] reported activity of TMZ as monotherapy in 31 patients (66% ORR) and good tolerability. Oxaliplatin has been reported active and potentially effective in retrospective analyses of patients with metastatic lung NETs alone or lung NETs mixed with other primary sites, treated with GEMOX, CAPOX, or FOLFOX regimens [101, 105, 109].

60.9 Systemic Biological Therapies

60.9.1 Gastroenteropancreatic Neuroendocrine Neoplasms (GEP NENs)

Biological systemic therapies investigation was limited to GEP NETs. Some molecular pathways represented the targets, including somatostatin receptor (*SSR*), phosphoinositide 3-kinase (*PI3K*)/protein kinase B also known as *AKT*/mammalian target of rapamycin (*mTOR*), insulin-like growth factor receptor (*IGFR*)/epidermal growth factor receptor (*EGFR*), and vascular endothelial growth factor and vascular endothelial growth factor receptor (*VEGF*/*VEGFR*). Understanding these pathways is a key strategy to a correct use of new therapeutic approaches.

60.9.2 Somatostatin Receptors (SSTRs)

The PROMID trial was a placebo-controlled phase III study, demonstrating the efficacy of using octreotide 30 mg every 4 weeks in metastatic functionally active or inactive neuroendocrine midgut NETs. In particular, median time to tumor progression (TTP) in octreotide LAR and placebo groups was 14.3 and 6 months, respectively; after 6 months of treatment, stable disease was observed in 66.7% of patients treated with octreotide LAR and 32.7% of placebo groups [110].

The randomized double-blind CLARINET study compared lanreotide 120 mg every 4 weeks with placebo in patients with nonfunctioning enteropancreatic advanced NENs with a Ki-67 < 10%. After 24 months, estimated rates of progression-free survival (PFS) were 65.1% in the Lanreotide group and 33.0% in the placebo group: concluding that treatment with somatostatin analogue (SSA) was associated with prolonged PFS [111].

60.9.3 mTOR Pathway

The serine/threonine kinase mTOR and its complexes (mTORC 1 and 2) contribute to the regulation of cell growth, protein synthesis, and autophagy, thanks to the interaction with lots of stimuli such as nutrition availability which involves AMPK or insulin and IGF1/IGF2 [61] that can activate PI3K and AKT signals; both pathways lead to the activation of mTORC 1–2 [61]. Some studies have shown that the expression and the activation of this pathway is higher in PanNENs than in small intestine NETs. According to this, in PanNENs there is also a lower expression of tuberin (TSC2), an inhibitor of mTOR signaling, and this leads to a more aggressive behavior of the tumor with a worse prognosis. The PI3K/AKT/mTOR pathway is regulated also by the phosphate PTEN which acts as inhibitor; its levels are lower in NECs and in the most aggressive forms, and the loss of its expression might correlate with sensibility to mTOR inhibitors [112–114].

For a general view of mTOR pathway, see the image below.

The RADIANT-4 trial has studied the activity of a rapalog inhibitor of mTORC1, Everolimus, in progressive nonfunctioning NENs of lung and GI tract. The results of the trial have demonstrated the significant improvement in PFS in the group of patients treated with Everolimus compared to the placebo group [115].

Sunitinib, which is a multitargeted tyrosin-kinase inhibitor (VEGFR-PDGFR), is a possible strategy since a randomized phase III trial in patients with progressive pancreatic NENs has proven its efficacy (PFS 11.4 months against 5.5 in the placebo group) [116]. Thanks to that, sunitinib is recommended in progressive PanNENs with a high grade of recommendation. Predictors of efficacy to sunitinib can be IL-8 and VEGFR3 levels [117].

For a short view on the biological mechanisms of NENs, see ◘ Table 60.10 and ◘ Fig. 60.8.

◘ **Table 60.10** An easy view on the biological pathway of NENs

Molecular target	Role	Drugs	Trials	Notes
SSR	Inhibitory effects on cell-growth and proliferation and on protein synthesis.	SSA: lanreotide or octreotide	PROMID/ CLARINET	–
mTOR	Regulation of cell growth, protein synthesis, and autophagy	Everolimus	RADIANT	–
VEGFR/ EGFR	Proliferation of new vessels, PI3K/AKT/mTOR signal, TGF-beta and the connective tissue growth factor	Sunitinib	NCT00428597	–

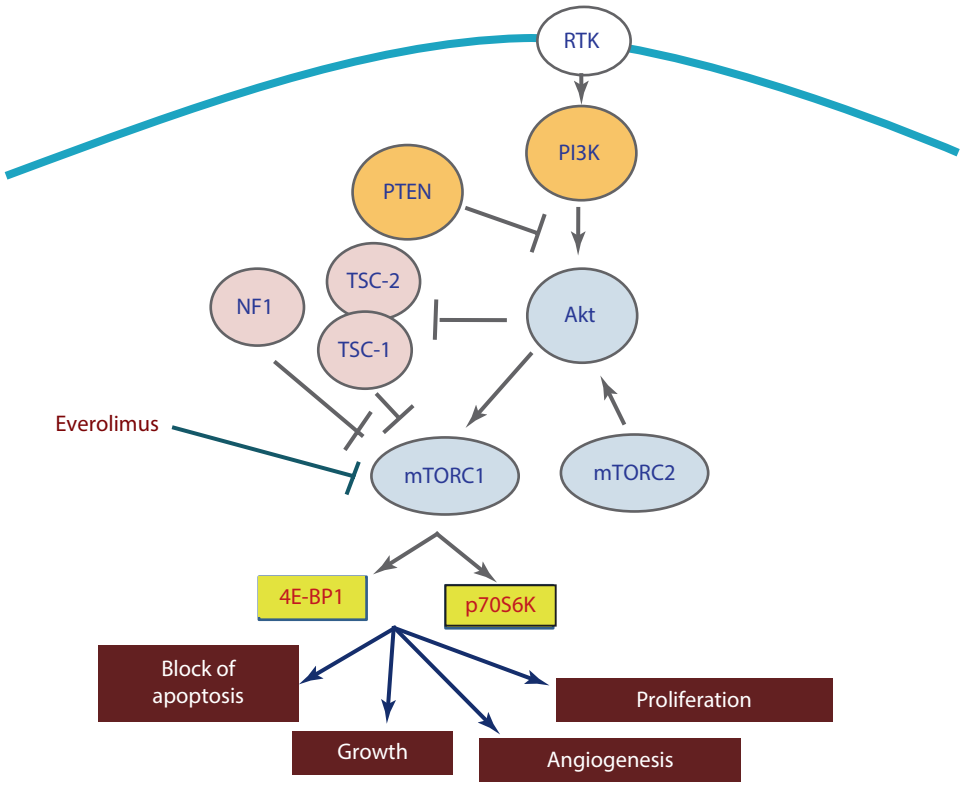


Fig. 60.8 mTOR pathway 1

60.9.4 Lung NETs

Somatostatin analogues (SSAs) such as octreotide or lanreotide were compared to placebo and showed antiproliferative effects in midgut carcinoids, including patients with lung tumors, with a progression-free survival (PFS) increase from 6 months to 14 months [110]. These agents are also recommended to control symptoms caused by secretion of biologically active peptides or amine, occurring in 60% of patients with lung carcinoids. Recently, the subgroup analysis of the phase III randomized RADIANT-4 trial demonstrated that everolimus led to a median PFS improvement of 5.6 months preserving overall health-related quality of life as compared to placebo in patients with advanced, nonfunctional, lung carcinoids, emerging as new standard of care in this subgroup of patients [115, 118, 119]. The phase II randomized LUNA trial compared the long-acting SRA pasireotide versus everolimus versus pasireotide plus everolimus in patients with advanced lung carcinoids, showing that the proportion of patients progression-free at month 9 were 39%, 33.3%, 58.5%, respectively, with tolerability consistent with the known safety profiles of these agents [120]. The results of this study indicate that combination therapy of an SSA with everolimus would need further clinical investigation in this rare subset of patients.

60.10 Liver-Directed Treatments

Liver-directed treatments (LDTs) are mostly represented by interventional radiology procedures. They include ablative and vascular treatments.

60.10.1 Ablative Treatments

Local ablative techniques play an important role in the treatment of liver metastases when there is no surgical indication as for location and/or number and/or size of lesions.

Although more ablative treatments are available such as cryotherapy, microwave ablation, laser, or electroporation, the most common technique is the *radiofrequency thermal ablation (RFA)*. In well-selected patients, this method allows to reach results quite overlapping to surgery [121].

This type of treatment should be proposed to patients with localized and limited or residual disease after other therapies. Radiofrequency thermal ablation acts by converting the energy of radiofrequency waves into heat: a high-frequency alternating current, approximately 460 kHz, passes through the tip of a needle-electrode, spreading into the surrounding tissue and causing an ionic vibration. The vibration in turn determines the

progressive heating of the cell walls of the tumor tissue surrounding the electrode and resulting in cell death at temperatures of 60–90 °.

In patients with liver metastases from GEP-NETs or lung NETs, RFA is effective both in symptom control and tumor growth control; it can be performed through both a percutaneous and surgical approach and in this latter case by both an open and laparoscopic technique [122]. A published large series of NEN patients with liver metastases treated with RFA showed that this therapy could provide effective local control with prompt symptomatic improvement [123].

One of the RFA limits is the treatment of liver lesions close to vital organs, or superficial metastases in contiguity with the stomach, colon, and diaphragm.

In case of liver lesions close to large vessels (portal venous branches or hepatic veins), there is a high risk of disease recurrence after thermal ablation due to a "cooling" effect induced by the blood flow which dissipates the heat induced by electrode.

Although surgical resection represents the treatment of choice in patients with low liver tumor burden, RFA could replace the surgery itself, particularly in patients in whom the metastases are unresectable or when the surgical access is particularly difficult. The combination of the surgical resection with the RFA may give the opportunity to completely treat the metastatic liver metastases in case of lesions less than 3 cm diameter and when the number of them is limited.

Microwave ablation (MWA) over the last years has been spreading a lot due to a better efficiency of the equipment available. Microwave generators use electromagnetic energy at a minimum of 900 MHz to cause thermal ablation of tumor cells, reaching a temperature of 160 °C. Compared to RFA, MWA acts with energy release in the active tissue, determining the dehydration and carbonization of the tissue and generally completing the treatment in a much shorter time than RFA. Moreover, due to the differences in energy release, MWA is involved in the "cooling" effect of the tissue caused by the surrounding vasculature less than RFA.

The most important limit for both RFA and MWA is liver lesion size, even though it has been described the possibility of performing the thermoablation also of large lesions by the multi-positioning of the needle in the context of the same metastasis and during the same session. However, it is difficult to radically treat a liver lesion greater than 3 cm, obtaining an adequate thermo-induced necrosis margin in the surrounding healthy hepatic parenchyma (comparable to the surgical resection margin).

60.10.2 Vascular Treatments

The hepatic transarterial embolization (TAE) is performed under radiological control, and it is based on the Seldinger percutaneous technique and on the principle that liver metastases and primary tumors in NENs are vascularized by the hepatic arterial circulation, whereas the nonpathological hepatocytes are mainly supplied by the portal vein [124–127].

The procedure requires the hepatic artery or its anatomical variants catheterization for an angiographic study of the hepatic arterial circle and to evaluate which arterial branches are involved in the pathological circle of the lesion. Then with a superselective technique, a slow infusion of embolizing microspheres (tens to hundreds microns in size) carries on through the arterial branches belonging to the lesion.

The main purpose of this procedure is to embolize the pathological arterial path as distally as possible with the embolizing material to induce ischemia and tissue necrosis (Fig. 60.9).

The treatment can be repeated after 1–3 months for several sessions.

From a clinical point of view, TAE could reduce tumor-related symptoms and induce a tumor debulking in order to improve the efficacy of systemic treatments or surgery inside a multidisciplinary strategy.

The chemoembolization (TACE) differs from TAE only for the type of material infused which is a chemotherapy (adriamycin, streptozotocin) mixed to an embolizing agent like an oil (Lipiodol) or microparticles with different composition based on the chosen material. The utility of this procedure is increasing tissue damage induced by ischemia through a chemotherapy [128].

These treatments are often associated with a post-embolization syndrome, characterized by transient liver failure which may be caused by treatment-induced necrosis. The clinical symptoms are fever, nausea, vomiting, and, in particular, after TACE, abdominal pain. It is generally lasting for 24–48 hours, and the therapy is based on hydration, antibiotics, and antipyretics; in case of patients with functioning tumor, an infusion treatment with somatostatin analogs (SSAs) should be considered.

The selective internal radiation therapy (SIRT) is another treatment with a vascular approach [25, 26], and it can be performed by intra-arterial infusion of microspheres preloaded with Ittrio90 (90Y), in patients with unresectable liver metastases, who already underwent TAE and/or TACE.

Its efficacy seems independent by the neoplastic tissue already treated, while it is dependent by the doses administered which, in turn, depends on the relationship between liver disease and liver healthy. Higher this ratio is, higher should be the dose delivered to the lesion compared to the healthy liver parenchyma. Contraindications to SIRT are the same for TAE and TACE.

Based on the observed results (PR or CR in 63% of patients and median OS of 36–70 months), long-term clinical results should be performed and encouraged.

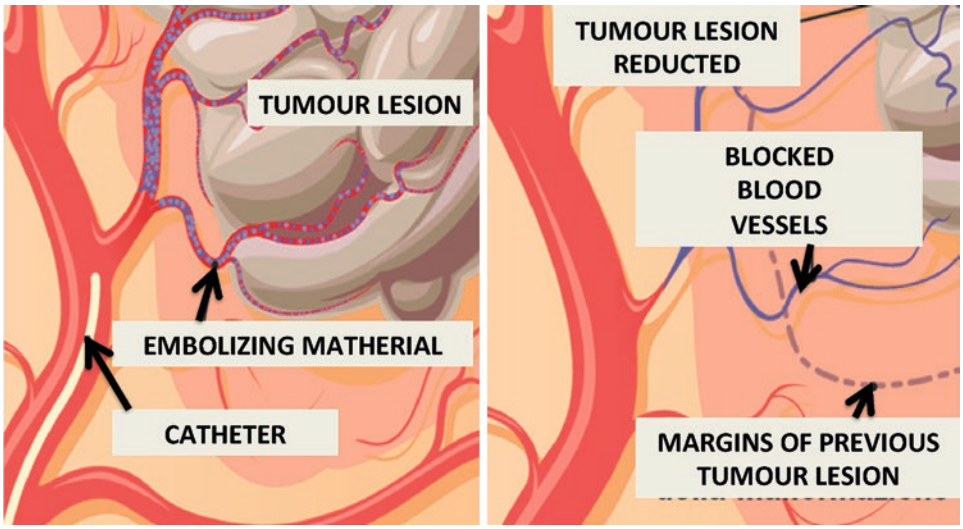


Fig. 60.9 TAE: The embolizing materials block the blood flow and the lesion reduction

Expert Opinion

Nicola Fazio

Key Points

1. Neuroendocrine neoplasms (NENs) are a group of heterogeneous malignancies raising from cells of the diffuse neuroendocrine system. Neuroendocrine neoplasms are rare cancers as their incidence is <6 new cases/100.000 × year.
2. Gastroenteropancreatic (GEP), which are the most common among NENs, are classified according to tumor morphology + ki-67 and/or number of mitosis into four categories: neuroendocrine tumors (NET) G1, NET G2, NET G3, and neuroendocrine carcinomas (NEC). Neuroendocrine tumors are the well differentiated, whereas NEC represent the poorly differentiated. In case of mixed forms, they will be indicated as mixed neuroendocrine non ndocrine neoplasm (MiNEN).

   Lung NENs are the second most frequent subgroup of NENs; they are classified into typical and atypical carcinoids, which are the well differentiated, and small cell and large cell neuroendocrine carcinomas, which are the poorly differentiated.
3. Clinical presentation of NENs is heterogeneous. Particularly, NENs comprise functioning and nonfunctioning types, depending on the presence or absence of a clinical syndrome due to hormones or other substances produced by the tumor. The most frequent syndrome is the carcinoid syndrome, more often characterized by diarrhea and facial flushing.
4. Morphological (cross-sectional) and functional (somatostatin receptor, metabolic) imaging are indicated for diagnosis, staging, and characterization of a NEN. Upper and lower digestive endoscopy +/− endoscopic ultrasound (EUS) are tools to diagnose, to stage, and to treat NENs from the stomach, duodenum, and rectum. Pancreatic EUS is useful for

diagnosis of pancreatic NEN. However, a pathologic diagnosis, preferably histologic, should always be obtained.

5. Therapeutic approach to NENs is various. Localized low-grade NETs can be removed endoscopically or surgically. Radical surgery should be performed in locally advanced radically resectable NETs. In the advanced setting, systemic medical therapies, surgical or interventional radiology debulking, and primary tumor removal should all be discussed within an NEN-dedicated multidisciplinary team (MDT).
   Somatostatin analogs (SSA), sunitinib, everolimus, peptide receptor radionuclide therapy (PRRT), and various chemotherapeutic regimens can be proposed in clinical practice to patients with advanced NENs.
6. Liver-directed treatments, including surgical and nonsurgical approaches, are usually discussed within the NEN-dedicated MDT for patients with metastatic GEP or lung NETs.

Recommendations

- ESMO
- ► https://www.esmo.org/Guidelines/Endocrine-and-Neuroendocrine-Cancers/Neuroendocrine-Bronchial-and-Thymic-Tumours
- ► https://www.esmo.org/Guidelines/Endocrine-and-Neuroendocrine-Cancers/Neuroendocrine-Gastroenteropancreatic-Tumours

The knowledge about NENs has been hugely increasing over the last three decades. Although this led to a wider awareness about this disease, improved diagnostic work-up and characterization, and a higher number of therapeutic options, unfortunately currently there is no validated predictive factor of efficacy to some therapy or specific sequence/integration of the different treatments. This may be due on the one hand to the limitations of our research approach and on the other hand to the biological and clinical heterogeneity of NENs. On this basis, it looks clear that the key of a successful therapy for a patient with a NEN should be his/her management within a NEN-dedicated MDT. Luckily, several centers all over the world constituted the own internal NEN MDT, many of them certified by the European Society of Neuroendocrine Tumors (ENETS) as Centers of Excellence for GEP NEN. The NEN-dedicated MDT involvement is critical to individualize the therapeutic strategy in line with the tumor/patient characteristics and goals of treatment.

Guidelines from the main scientific societies may give some help in terms of algorithm of clinical thinking, but it should be always kept in mind that the level of evidence is often very low and therefore exposed to bias. The NEN-dedicated MDT makes less difficult to contextualize the evidence, experience, approvals, and investigations into the specific clinical context of the patient who should be treated.

Although the aforementioned limitations basic and clinical research on NEN have been clearly improved over the last decades by leading to important new insights and laying solid foundations for practice-changing future investigations.

Hints for a Deeper Insight

- Anti-tumour effects of lanreotide for pancreatic and intestinal neuroendocrine tumours: the CLARINET open-label extension study: ► https://www.ncbi.nlm.nih.gov/pubmed/26743120
- Everolimus for the treatment of advanced, non-functional neuroendocrine tumours of the lung or gastrointestinal tract (RADIANT-4): a randomised, placebo-controlled, phase 3 study: ► https://www.ncbi.nlm.nih.gov/pubmed/26703889
- Non-conventional doses of somatostatin analogs in patients with progressing well differentiated neuroendocrine tumor: ► https://www.ncbi.nlm.nih.gov/pubmed/31545377
- Therapeutic schemes in 177Lu and 90Y-PRRT: radiobiological considerations: ► https://www.ncbi.nlm.nih.gov/pubmed/26576734

Case Study: A Huge Abdominal Mass

Woman, 35 years old

- *Family history* negative for malignancy
- *APR:* Rheumatic heart disease (mitral valve)
- *APP:* Frequent episodes of nausea and vomiting with abdominal pain
- *Objective examination*: Mild tenderness on deep palpation of the abdomen and a palpable mass in the mesogastric area
- *Blood tests*: Normal blood test
- *CT abdomen mdc*: Evidence of a retroperitoneal mass (DT max: 14 × 13.7 × 6.7 cm) originating from pancreatic tissue with a consistent compressive effect on the surrounding structures. Negative for distant metastases

Question

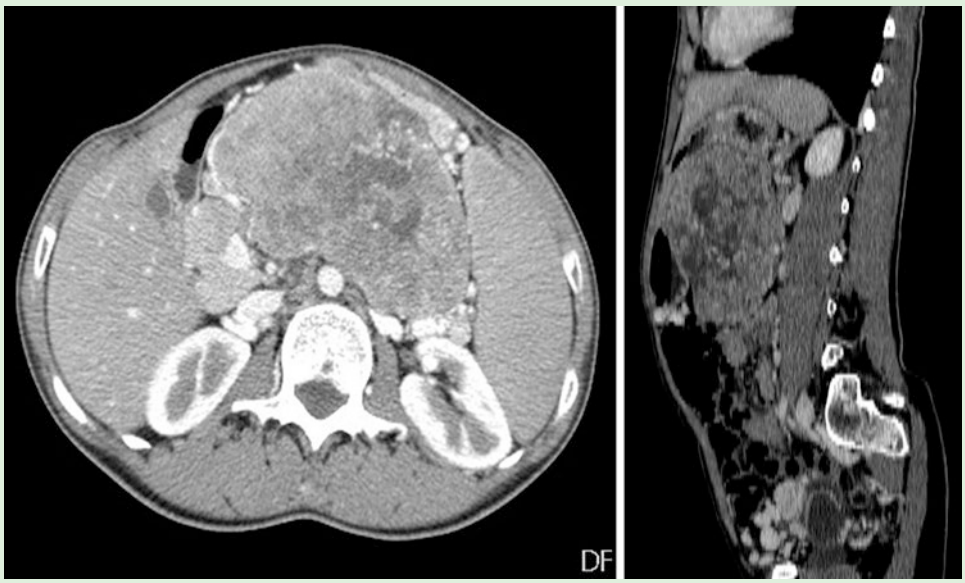
What action should be taken?

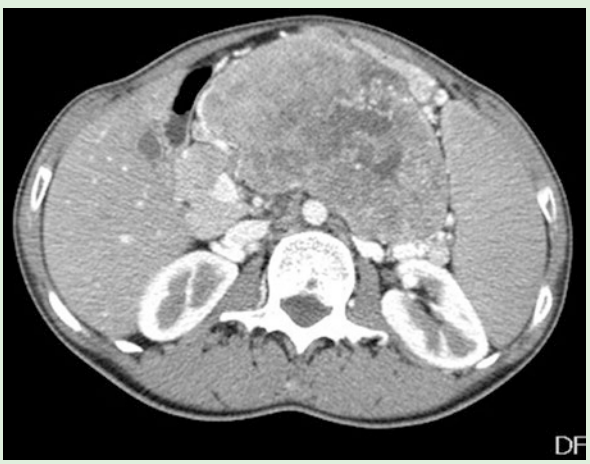
(1) Surgery. (2) Biopsy. (3) Other

Answer

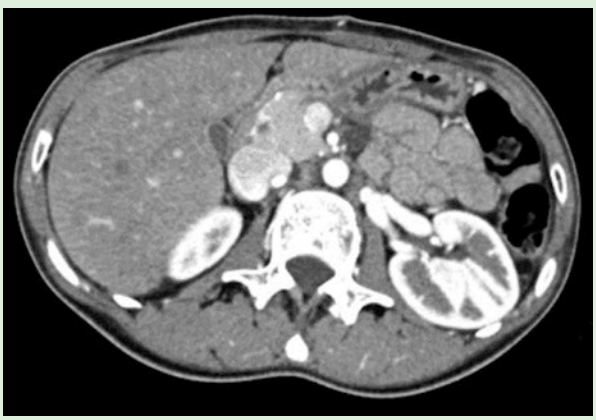
Biopsy

Neuroendocrine tumor (NET) G2, chromogranin A+, ki67: 10%, 7/10 mitosis HPF.

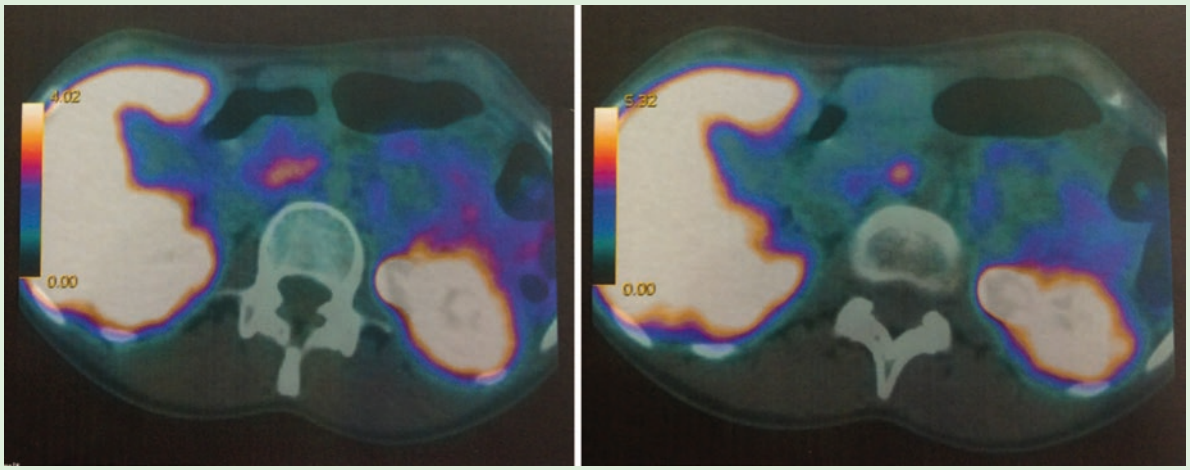




Before surgery



After surgery



Question

What action should be taken?

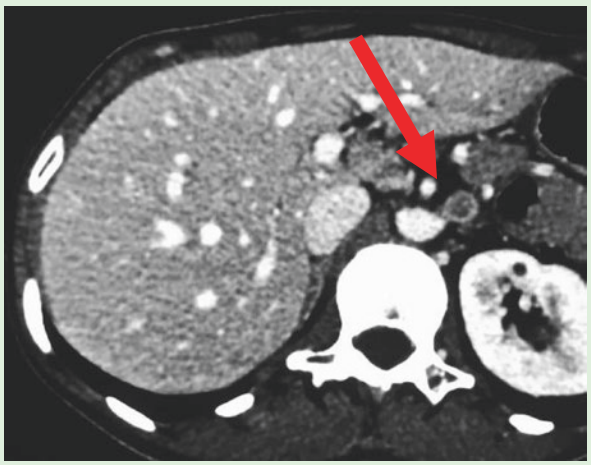
(1) Surgery. (2) Medical therapy. (3) Other

Answer

Surgery.

Distal splenopancreatectomy. Histopathological examination: neuroendocrine tumor, G2, ki67: 12%

Nine months later, at the CT scan, evidence of a new nodule on the left suprarenal region. (DT max 6 mm).



68Ga-PET: Positive uptake in the celiac region.

Question

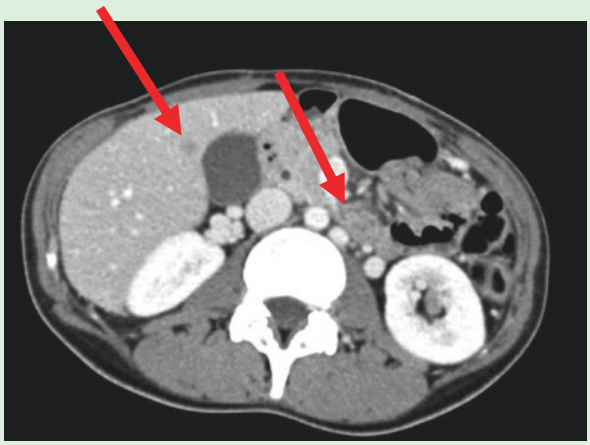
What action should be taken?

(1) Somatostain analogues. (2) Sunitinib. (3) Other

Answer

The patient begins somatostatin analogues (SSA) at standard dose.

At the next CT scan evaluation, evidence of progression disease of the previous nodule (DT max 13 mm), and arising of a new hepatic lesion (DT max 8 mm).



Question

What action should be taken?

(1) Everolimus. (2) Sunitinib. (3) Other

Answer

Everolimus 10 mg.

Key Points

- The importance of a correct surgical approach
- Symptoms often nonspecific
- The main role of the targeted therapies (i.e., everolimus).

Case Study: The Importance of PRRT

Woman, 72 years old

- *Family history* negative for malignancy
- *APR:* Hysterectomy, for leiomyoma, appendectomy, major depressive disorder
- *APP:* Intense acute abdominal pain
- *Objective examination*: Tenderness on palpation of the abdomen
- *Blood tests*: Normal blood test
- *CT abdomen MDC*: Evidence of enlarged mesenteric lymph nodes
- *Colonoscopy:* Negative
- *Esophagogastroduodenoscopy:* Negative

An exploratory laparotomy is performed in the critical surgery unit: resection of a small tract of the bowel. Histopathological examination: neuroendocrine tumor, ki67: 7%, 3/10 mitosis HPF, G2.

- *Octreoscan*: Negative
- One year later, after a suspicious CT scan and because of the presence of abdominal pain, a ^{68}Ga-PET is performed with the positive uptake of the tracer at some mesenteric lymph nodes and small tracts of bowel.

Question

What action should be taken?

(1) Surgery. (2) Biopsy. (3) Other

Answer

Surgery.

Resection of lymph nodes and the involved tracts of bowel.

Histopathological examination: Neuroendocrine tumor (NET) G1, ki67: <2%

- As the presence of symptoms like nausea, vomiting, abdominal pain, the patient is treated with somatostatin analogs at standard dose with a relief of the symptoms.
- After six years, at a follow-up CT scan, multiple mesenteric lymph nodes appear bigger and suspicious.

Question

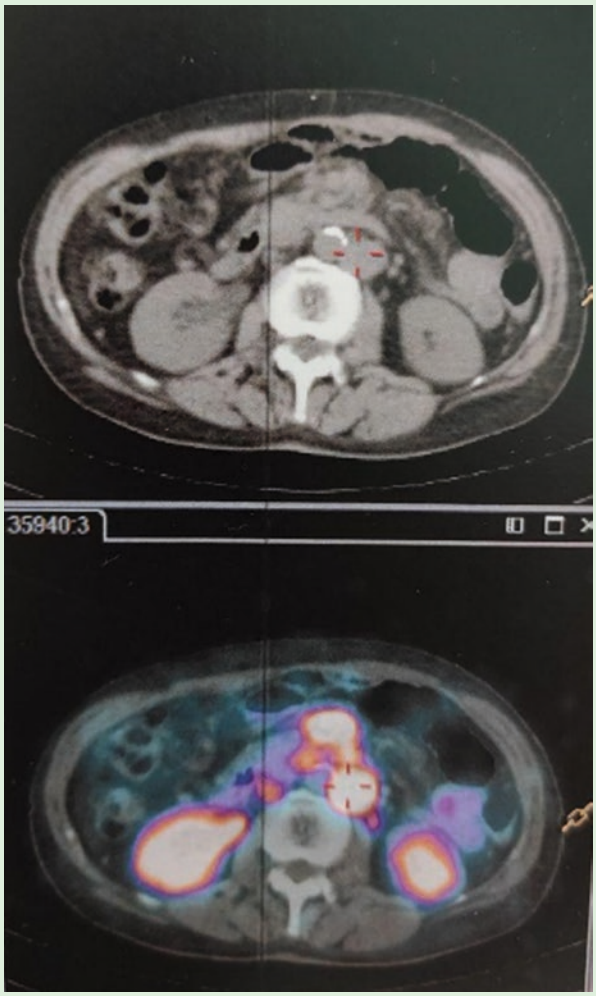
What action should be taken?

(1) Watchful waiting. (2) ^{68}Ga-PET. (3) Other

Answer

^{68}Ga-PET.

Uptake of the tracer in multiple mesenteric and supradiaphragmatic lymph nodes. Evidence of doubtful uptake in the liver.



Question

What action should be taken?

(1) Surgery. (2) Medical therapy. (3) Other

Answer

Other: Peptide receptor radionuclide therapy (PRRT)

Key Points

- The possibility of acute presentation of neuroendocrine neoplasms (bowel obstruction)
- The importance of a correct follow-up
- The role of peptide receptor radionuclide therapy in a multidisciplinary approach

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