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Antonio Russo
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Targeted Therapies for Solid Tumors

A Handbook for Moving Toward New Frontiers in Cancer Treatment

Humana Press

CURRENT CLINICAL PATHOLOGY

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Antonio Russo • Rafael Rosell
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Editors

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Contents

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Introduction

1

Antonio Russo, Christian Rolfo and Rafael Rosell

The landscape of cancer biology has been consistently changed since the mid of the last century. The discovery of oncogenes and tumor suppressor genes, the identification of cancer stem cells and the study of tumor immunology could be deemed the most relevant steps of an evolving scenario. Many researchers argue the opportunity yielded by a combination of different therapeutic strategies concomitantly or subsequently.

Vogelstein was one of the first researchers who identified an association between specific genomic alterations and the stages of cancer development and progression. Since the proposal of his carcinogenetic model in colon cancer, various models have been proposed in different malignancies.

Then Hanahan and Weinberg suggested the main cancer cell functions, which characterize a malignant tumor. Recently the same authors highlighted the role of targeted therapy to address the action against each hallmark of cancer.

Several years ago the idea of "magic bullets" to target oncogenes arose as a new fascinating strategy, which would have led to a definitive cure for cancer patients. This concept was based on the potential selective action on cancer cells, sparing normal cells.

Nowadays we know that targeted agents can achieve high antitumor activity, as monotherapy or as a combination with standard chemotherapy. However some side effects may develop as a consequence of the action of targeted agents on normal tissue, which express the relative target oncogenes. These adverse events are usually different from those observed when standard chemotherapy is delivered. A proper management of targeted therapy-related toxicity is needed, and international guidelines and recommendations have already included some suggestions for oncologists to manage it.

This volume about "Targeted Therapies for solid tumors" aims to help and to lead the update in this widespread field of clinical oncology.

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Oncogene Addiction in Solid Tumors

2

Stefano Caruso, Daniele Fanale and Viviana Bazan

Carcinogenesis is a multistep process resulting from the progressive accumulation of mutations and epigenetic abnormalities in expression of multiple genes that collectively give rise to a malignant phenotype [1, 2]. However, experimental evidence suggests that the suppression of an oncogene or the restoration of a tumor suppressor gene expression can be sufficient to inhibit the growth of cancer cells and even lead to improved survival rates [3].

The term "oncogene addiction" was coined by Weinstein in the early 2000s [3] to describe the phenomenon where the hyperactivity of a specific oncogene (or pathway) is required for cancer cells to survive and proliferate. Initially, some studies on hematological tumors have identified that cancer cells are often "addicted to" constitutive activation or overexpression of an oncogene for the maintenance of their malignant phenotype: It has been reported that acute inactivation of MYC in transgenic mice models of MYC-induced lymphoma and leukemia leads to the rapid induction of apoptosis and differentiation [4]. Since then some evidences that support the concept of oncogene addiction have been obtained in other tissues in murine models and using human cancer cell lines [5]. Nevertheless, the most convincing evidence for this concept comes from its application to the clinical setting. The clinical relevance of oncogene addiction paradigm is highlighted by a growing number of examples that demonstrate the efficacy of several therapeutic agents that target specific oncogenes in various cancer types. The clinical success of the multikinase inhibitor imatinib, which targets the oncogenic BCR/ABL protein in chronic myeloid leukemia (CML) [6] and also targets the product of the oncogene c-kit in gastro intestinal stromal tumors (GIST) [7], provides direct evidence for the phenomenon of oncogene addiction in the context of cancer therapy. Likewise, selective epidermal growth factor receptor (EGFR) tyrosine-kinase inhibitors (TKI), gefitinib, erlotinib, and afatinib have achieved positive outcomes in non-small cell lung cancer (NSCLC) [8, 9], pancreatic cancer [10], and glioblastoma [11]. Furthermore, similar results were obtained using the monoclonal antibody trastuzumab, which targets the receptor tyrosine kinase HER-2/NEU in patients with breast cancer [12]; the monoclonal antibody cetuximab, which targets the EGFR in patients with head and neck and colorectal cancer [13, 14]; bevacizumab, a monoclonal antibody to vascular endothelial growth factor (VEGF) in carcinomas of the breast, colon and kidney [15–17]; vemurafenib, a B-Raf enzyme inhibitor for the treatment of melanoma [18]; and crizo-

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Table 2.1 Clinical evidence of oncogene addiction[a]

Target	Therapeutic agent (monotherapy)	Cancer	Reference
BCR/ABL	Imatinib mesylate	CML	[6]
C-KIT	Imatinib mesylate	GIST	[7]
EGFR	Gefitinib/Erlotinib	NSCLC	[8]
			[9]
B-RAF	Vemurafenib	Melanoma	[18]
EML4-ALK	Crizotinib	NSCLC	[19]
Target	**Therapeutic agent (combination)**	**Cancer**	**Reference**
EGFR	Erlotinib	Pancreas	[10]
EGFR	Cetuximab	Head and neck	[13]
		Colorectum	[14]
HER-2/NEU	Trastuzumab	Breast	[12]
VEGF	Bevacizumab	Breast	[15]
		Colorectum	[16]
		Kidney	[17]

[a] Treatment regimen indicates therapeutic agent alone (monotherapy) or in combination with other chemotherapeutic agents (combination)

tinib, an ALK inhibitor, which targets the fusion protein EML4-ALK and has produced excellent results in clinical trials in NSCLC patients [19] (Table 2.1).

The principle that some cancers depend on one single oncoprotein for their continuous growth and the conclusion that this oncoprotein could represent the target for therapeutic treatment is confirmed in patients who develop acquired resistance to these therapeutic agents via de novo mutations on the same oncogene and not by mutations in other oncogenes. For example, the leukemic cells of individuals with CML can undergo a secondary mutation in the kinase domain of the BCR/ABL protein which blocks the inhibitory activity of imatinib [20]. Similarly, there may be cases of secondary resistance to gefitinib and erlotinib in patients with NSCLC due to *de novo* mutation on EGFR gene identified as T790M [21]. However, in other cases of acquired resistance, cancer cells may undertake an alternative or redundant survival pathway. For example, it has been reported that a subset of NSCLC patients with acquired resistance to EGFR TKIs exhibit amplification of the MET tyrosine kinase gene [22]. It is also known that the loss of the tumor suppressor gene PTEN is associated with treatment failure in glioblastoma patients, presumably due to the activation of pathways downstream of the EGFR [23].

The Molecular Basis of Oncogene Addiction

The molecular mechanisms underlying oncogene addiction have been extensively studied, and it has been demonstrated that these occur by processes intrinsic and exclusively dependent upon biological programs within a cancer cell. In particular, three models have been proposed to clarify the mechanisms of oncogene addiction: genetic streamlining, oncogenic shock and synthetic lethality. The genetic streamlining hypothesis is based on the concept that genetic instability in cancer cells causes the inactivation of some signaling pathways during tumor evolution, which are operational in a normal cell but not required for growth in the cancer cell. In this state, an initially nonessential oncoprotein may become essential through the genetic streamlining, and the cancer cell becomes predominantly dependent on the oncogene driven processes [24]. The blockade of the addictive receptor causes cell cycle arrest and/or apoptosis.

A second mechanism is based on the concept of "oncogene shock." According to this model, dominant oncogenes are able to sustain at the same time both prosurvival and proapoptotic signals. Normally, the prosurvival outputs dominate over the proapoptotic, but the inactivation of ad-

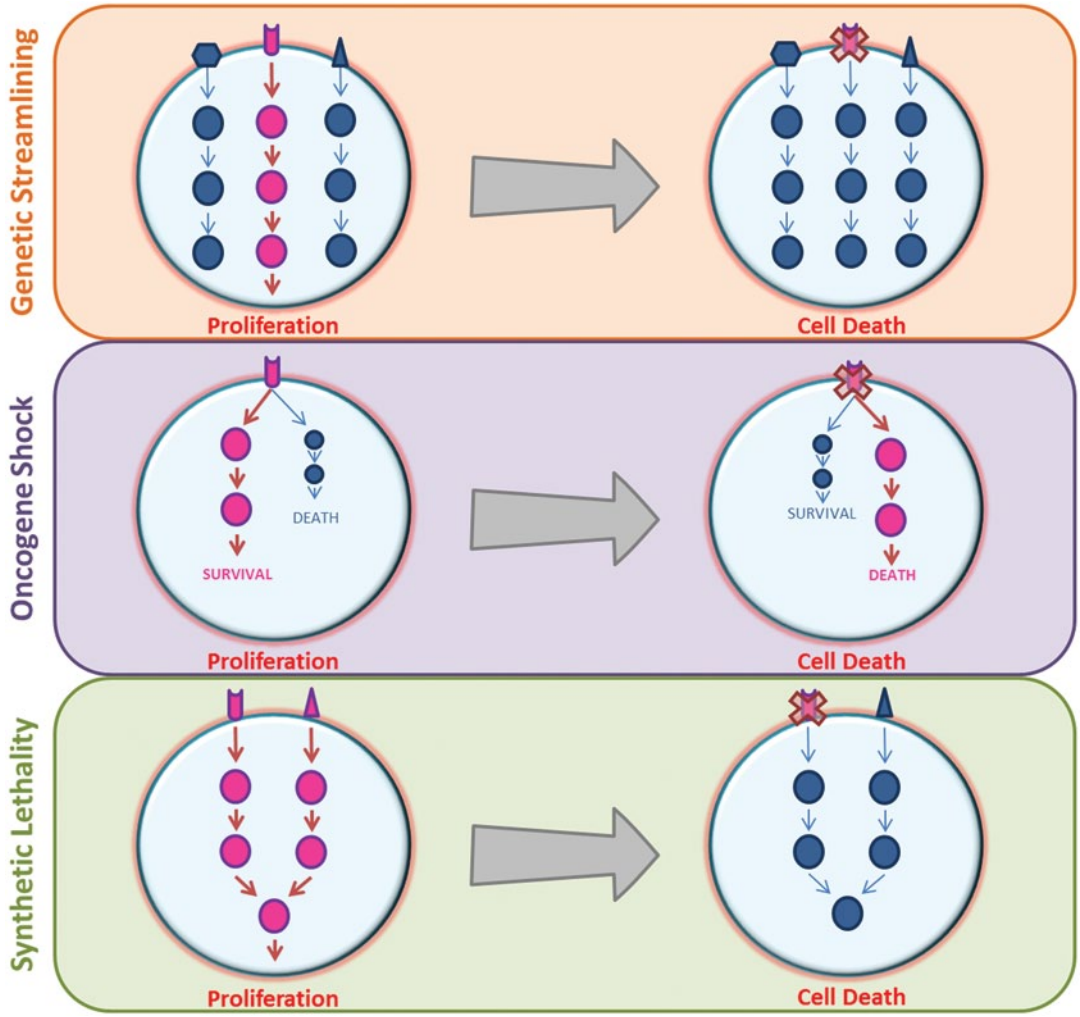

Fig 2.1 Molecular mechanisms of oncogene addiction, showing the three different hypotheses of oncogene addiction: genetic streamlining, oncogene shock and synthetic lethality

dictive receptor in cancer cells causes their death because of differential attenuation rates of pro-survival and proapoptotic signals [25].

A third hypothesis is based on the model of synthetic lethality, derived from studies in lower organisms. This theory holds that two genes are considered to be in a synthetic lethal relationship if mutation of one of the two genes is compatible with survival but mutation of both genes causes cell death [26]. This concept of synthetic lethality is rather intuitive when the two genes belong to alternative metabolic chains with a common end product, but it can also be applied to more sophisticated and integrated cellular functions, such as survival and proliferation. Furthermore, cancer cells may be more dependent on a specific oncogene with respect to normal cells as they are less adaptable because they carry several inactivated genes (Fig. 2.1).

Future Perspectives

The phenomenon of oncogene addiction has allowed novel important therapeutic opportunities through the selective elimination of tumor cells that exhibit strict dependence on a protein, providing a potential "Achilles' heel" in specific

types of human cancers. For instance, the use of small interfering ribonucleic acids (siRNAs), a class of double-stranded RNA molecules, can be useful to identify which genes are required to maintain the proliferation and survival of cancer cells and subsequently to design drugs that target the related protein [27]. Furthermore, it has been reported that a specific siRNA preparation might be administered to patients in order to knock down the expression of a critical oncogene in the tumor, thus providing a novel approach to cancer therapy [28]. In addition, oncogenes that are mutated in cancer, and not overexpressed, represent the most appropriate target for therapy because they have qualitatively different roles than oncogenes that are only overexpressed, as evidenced by the properties of mutated EGFR in NSCLC cells [29]. Today, the emerging molecular biology techniques allow us to identify different proteins and gene expression profiles between normal tissues, cancers, and subtypes of specific cancers and thus facilitate identification of specific pathways of oncogene addiction in several cancer cells. As described above, some cancers can "overcome" a given state of oncogene addiction through mutations in other genes and pathways, due to the genomic instability of cancers. Moreover, in some cases, the inactivation of the oncogene fails to cause significant tumor regression as demonstrated in a murine model of MYC-induced lung adenocarcinoma [30]. For this reason, not always the inactivation of an oncogene necessary for tumor growth and survival is sufficient to reverse tumorigenesis. In these cases, the combination therapy helps us to overcome these obstacles. It has been widely demonstrated that the efficacy of certain targeted agents can be enhanced by combining them with cytotoxic drugs, such as agents that act by inhibiting deoxyribonucleic acid (DNA) or chromosomal replication [12]. Similarly, the combination of bevacizumab or cetuximab with chemotherapy agents can improve response rates in metastatic colon and breast cancer patients, respectively [14, 15].

All these evidences support the role of oncogene addiction in the development of cancer phenotype. This phenomenon can be exploited to identify new targeted agents, which specifically target the most relevant oncogenes.

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Pharmacology and Clinical Development of New Molecularly Targeted Agents

3

Elisa Giovannetti and Elena Galvani

Introduction

Definition of Molecularly Targeted Antitumor Agents

Pharmacology can be defined as the study of substances that interact with living systems through molecular and chemical processes, especially by binding to regulatory factors and inhibiting or activating physiological body processes [1]. Such deliberate therapeutic applications may be considered the proper role of medical pharmacology, which is the science of substances used to treat human diseases.

For decades, the pharmacological treatment of cancer has used cytotoxic (i.e., cell-killing) therapy, which has been termed cancer chemotherapy [2]. Cancer chemotherapy is curative in subsets of patients who present with advanced disease, including germ cell cancer, small cell lung cancer, and ovarian cancer. Although treatment is not curative for most of the solid tumors, there has been a significant improvement in progression-free survival (PFS). These results also facilitated the study of adjuvant chemotherapy, leading to survival prolongation in a number of cancer types, and helped foster further trials in different clinical settings. Moreover, several of the most active chemotherapy regimens are being used in the neoadjuvant setting to reduce the size of the primary tumor allowing improved surgical outcome as well as preservation of vital organs, such as for anal, bladder, breast, gastroesophageal, rectal, 31 head and neck cancers, and osteogenic and soft 32 tissue sarcomas [3].

However, from its introduction, cancer chemotherapy has been encumbered by its poor selectivity because most antineoplastic drugs are toxic not only to tumor cells but also to important populations of the body's nonneoplastic cells, such as the fast-replicating cells of blood compartment, skin cells, and gastrointestinal tract lining cells. The resulting problems of unwanted side effects are compounded by difficulties in predicting the desired effectiveness of chemotherapy in individual patients. This unsatisfactory situation and the development of technology leading to the sequencing of the genome have driven intensive researches and development over the last few decades toward more specific and less toxic anticancer drugs that block the growth and spread of cancer by interfering with specific molecules involved in tumor growth and progression [4]. Because scientists refer to these molecules as "molecular targets," targeted cancer therapies are sometimes called "molecularly targeted therapies" or similar names. Several results of these efforts have reached the clinic, and

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many more are now in preclinical testing. Common to all these targeted therapies is their interaction with defined molecules present on cancer cells, which adds various degrees of increased selectivity to their toxic effects. As a consequence, detecting the target molecule on tumors before therapy holds great diagnostic potential for predicting the efficacy of the drug and personalizing therapy. Ideal anticancer drugs would indeed eradicate cancer cells without harming normal tissues. Unfortunately, no currently available agents meet this criterion, and clinical use of these drugs involves multiple challenges including the appearance of new toxicities [5], the need for biomarkers, the need of validation of genomic tests, and the evolution of cancer molecular imaging. Therefore, this chapter aims to present translational scientists and clinicians with an integrated critical view on the pharmacology (i.e., pharmacodynamics, pharmacokinetics, and pharmacogenetics), as well as on the clinical development (and related emerging problems) of the molecularly targeted antitumor agents in solid tumors.

Beyond Clinicopathological Typing: New Pharmacological Targets for Individualized Treatments

Factors such as disease stage, performance status, age and co-morbidity provide a crude discrimination of prognosis in many tumors. These clinical prognostic factors represent surrogate markers of clinical behavior and could be useful for predicting patient prognosis and guiding anticancer treatment [6]. For example, mediastinal lymph node involvement and the number of metastatic lymph nodes are important adverse prognostic factor in surgically treated stage IIIA non-small-cell lung cancer (NSCLC) [7]. Similarly, there is a significant difference in survival when the visceral pleura is involved. Indeed, visceral pleural invasion was observed more frequently in biologically aggressive tumors and, by multivariate analysis, this invasion proved to be a significant independent predictor of poor prognosis in NSCLC patients with or without lymph node involvement [8]. Therefore, in most solid tumors, the therapeutic strategy is based on the tumor type and stage as well as on the health status of the patient at diagnosis. Several data suggested that the efficacy or toxicity of anticancer treatments is also influenced by the histologic subtype. This differential therapeutic efficacy based on histologic subtype is well documented for pemetrexed in advanced or metastatic NSCLC, where a phase III trial showed that patients with nonsquamous histology had a survival benefit when treated with cisplatin/pemetrexed versus cispaltin/gemcitabine, while the reverse was observed in patients with squamous histology [9]. On the basis of these results, the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) approved pemetrexed for the use in the first-line treatment of advanced nonsquamous NSCLC.

However, the treatment of certain cancers has been revolutionized in recent years by the introduction of novel drugs designed to target specific molecular factors implicated in tumor behavior. These novel targeted therapies are based on advances in our understanding of key cellular networks and genetic nodal points around which tumors could arise and progress [10]. Genome characterization efforts have indeed highlighted the importance of "driver" somatic alterations that activate crucial oncoproteins originating tumor with a pivotal dependency. Single-agent therapeutic regimens especially designed to intercept deregulated dominant oncogenes have proven to be effective treatment in these "oncogene addicted" tumors [11]. Notable examples include imatinib, a tyrosine kinase inhibitor (TKI) in KIT-positive gastrointestinal stromal tumors, trastuzumab, a humanized monoclonal antibody (mAb) against human epidermal growth factor receptor (HER)-2 in women with HER2-positive breast cancer, sunitinib, a multitargeted TKI that inhibits both angiogenic pathways (i.e., vascular endothelial growth factor receptor and platelet-derived growth factor receptor) and direct pro-oncogenic pathways (e.g., stem-cell factor receptor and FMS-like tyrosine kinase-3),

in metastatic renal cell carcinoma (RCC). In particular, the epidermal growth factor receptor (EGFR) has been successfully targeted either by mAbs or small molecules inhibiting the tyrosine kinase domain. The mAb cetuximab blocks the extracellular domain of EGFR, thereby competing with the ligands, resulting in the inhibition of the receptor. This mAb, which is approved for the treatment of advanced colorectal cancer, has also been approved as first-line treatment combined with platinum-based chemotherapy in EGFR-positive NSCLC patients with good performance status [12, 13]. The EGFR-TKI gefitinib has been approved by the FDA and EMEA as upfront therapy replacing chemotherapy in late-stage NSCLC patients harboring activating-EGFR mutations [14]. Similarly, the manageable toxicity, along with its efficacy, makes the EGFR-TKI erlotinib an important option as maintenance therapy, and both erlotinib and gefitinib are also the only drugs of proven efficacy in the third-line setting for patients with NSCLC previously treated with chemotherapy [15]. Another example of targeted therapy is the antiangiogenic agent bevacizumab, in combination with carboplatin-paclitaxel or any platinum-based chemotherapy, which has been recently approved as first-line treatment for patients bearing tumors with nonsquamous histology [16]. More recently, the anaplastic lymphoma kinase (ALK) inhibitor crizotinib has been approved by the FDA for the treatment of locally advanced or metastatic NSCLCs with EML4-ALK translocation fusions [17]. A number of other molecular aberrations have been identified including PIK3CA mutations, IGF-1R overexpression, c-MET amplification or overexpression, or alterations in key signaling pathways, such as RAS/RAF/MEK and phosphoinositide-3 kinase (PI3K)/Akt/mTOR [18]. Several other drugs aimed to interact with these aberrant molecules are actively being investigated in the clinic, including the BRAF inhibitor sorafenib, the Src/Abl inhibitor dasatinib, and many others [11–19].

Main Targets and Pharmacodynamics of Molecularly Targeted Antitumor Agents

Pharmacodynamics is the study of the biochemical and physiological effects of drugs on the body, including the mechanisms of drug action and the relationship between drug concentration and effects. The incorporation of pharmacodynamic analyses is increasingly important in phase I clinical trials investigating whether the novel targeted agents are able to reach their targets and exert their effect in a desirable way. In contrast to the traditional nonspecific cytotoxic antiproliferative agents, which often have a small therapeutic window, steep dose–toxicity curve and an efficacy assumed to be somehow related to toxicity, molecularly targeted agents usually show less toxicity, a wider therapeutic window and an efficacy more related to growth inhibition than to tumour shrinkage. Therefore, using some representative examples of different classes of molecularly targeted agents, this chapter discusses the main pharmacological targets and mechanisms of action of such drugs, including possible suggestion for the optimization of the pharmacological studies to improve their development in the context of cancer care [20].

Agents Targeting Growth Factor Receptors

Receptor tyrosine kinases play important roles in animal development and their deregulation has been linked to several diseases, including cancer. The best example is the known role of the ERBB/HER family of receptors in the pathophysiology of breast, gastric, colorectal, lung, head, and neck tumors. There are four members of the HER family: EGFR, also termed ERBB1/HER1, HER2/Neu/ERBB2, HER3/ERBB3, and HER4/ERBB4. Activation of these receptors occurs by dimerization upon ligand binding (Fig. 3.1), and can be altered in different tumor types [21].

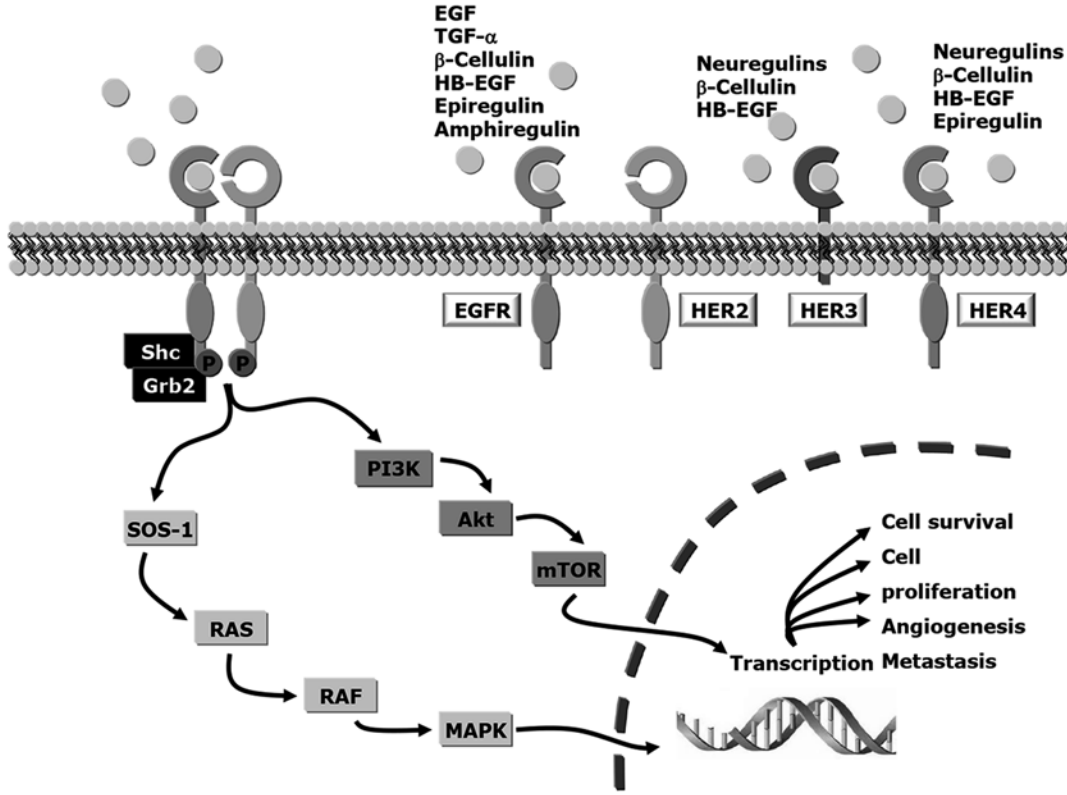

Fig. 3.1 EGFR signaling pathways: Signaling pathways and epidermal growth factor tyrosine kinase receptors involved in the tumorigenesis of NSCLC. *Akt* protein kinase B, *EGF* epidermal growth factor, *EGFR* epidermal growth factor receptor, *hb-EGF* heparin binding EGF, *MAPK* mitogen-activated protein kinase, *PI3K* phosphatidylinositol-3-kinase, *Raf* v-raf 1 murine leukemia viral oncogene homolog 1, *Ras* retrovirus-associated DNA sequences, *SOS* Son of sevenless, *TGF* transforming growth factor, *mTOR* mammalian target of rapamycin, *FGF* fibroblast growth factor, *VEGF* vascular endothelial growth factor, *Grb2* growth factor receptor-bound protein 2

Given the relevance of these receptors in cancer, multiple strategies to target HER family members have been used, but only two have successfully reached the clinic, namely antibodies (mAb) designed against the extracellular domain of the receptors, and small TKIs which interact with the intracellular domain.

Three mAbs against HER receptors are approved for the treatment of solid tumors: cetuximab and panitumumab against EGFR and trastuzumab against HER2. Cetuximab is a chimeric monoclonal anti-EGFR antibody that contains human constant domains and rodent variable domains, while panitumumab is a fully human antibody. Trastuzumab is a humanized antibody in which human sequences replace all rodent sequences except for the complementary determining regions (CDRs) which are responsible for binding to HER2 [22]. The mechanism of action of mAbs against HER receptors is thought to involve many processes, several of which depend on the region of the receptor recognized by the antibody. Stimulation of HER endocytosis and removal of HER receptors from the cell surface upon interaction with the mAbs is expected to represent a common event in the action of these treatments [23]. This reduces the total amount of the cell surface receptors and leads to reduced signaling. Another important action of the mAbs is to facilitate the attack of the tumoral cells by the immune system. The importance of the immune reaction in the mechanism of action of anti-

HERs mAbs has been demonstrated by elegant studies using mice deficient for the antibody receptor FcγRIII. Loss or blockade of the FcγRIII receptor on leucocytes severely impairs the antitumor effect of trastuzumab in vivo, indicating involvement of Fc-receptor-dependent mechanisms in the action of trastuzumab [24]. This immunological effect may also explain the clinical benefit of combining antibodies to the same molecule, but which act on different epitopes, as has been recently reported for the trastuzumab and pertuzumab combination in breast cancer [25]. Similarly, skin rash, which is one of the clinical markers of cetuximab activity, may be related to the inflammatory skin reaction mediated by this type of cytotoxic response. Cetuximab and panitumumab interact with subdomain III of the EGFR, which is a region where EGF binds to the receptor. Therefore, cetuximab is expected to impede adequate binding of EGF ligands to the cognate receptor, blocking ligand-mediated receptor activation. Trastuzumab interacts with subdomain IV of HER2. This interaction does not prevent ligand-induced HER2 oligomerization and activation. However, when the ligand is expressed as a transmembrane molecule, its ability to activate HER receptors is profoundly compromised by trastuzumab [26]. This finding is relevant since tumors fed by transmembrane growth factors of the heregulin subfamily could be targeted by trastuzumab, even in the absence of HER2 overexpression. Pertuzumab, which binds subdomain II of HER2, has been created to interfere with receptor dimerization, and, as mentioned above, has recently shown clinical efficacy [27].

Anti-HER receptor antibodies may cause an arrest of the cell cycle in G1 through induction of the cyclin-dependent kinase inhibitor p27. In addition, these agents are known to inhibit angiogenesis [28]. Trastuzumab and cetuximab also suppress DNA repair capacity through unknown pathways, contributing to the ability of the antibody to enhance the antitumor effect of DNA-damaging agents such as cisplatin [29].

In the clinical setting, trastuzumab has been approved for the treatment of metastatic and adjuvant breast cancer in combination with a taxane-based chemotherapy. In the pivotal clinical trial in metastatic breast cancer, the combination of trastuzumab with paclitaxel showed an increase in survival compared with paclitaxel alone [30]. Of note in that study the arm combining trastuzumab with anthracyclines showed an unacceptable cardiac toxicity limiting the use of trastuzumab with this type of chemotherapy. In different clinical phase II studies, trastuzumab has been combined with different chemotherapies including vinorelbine, gemcitabine, or capecitabine among others, showing different ranges of clinical activity [31, 32]. In the adjuvant setting, trastuzumab has been combined with taxanes and platinum-based regimens to avoid the concomitant administration with anthracyclines, and is also given after finishing chemotherapy to complete a total treatment of 1 year [33]. In gastric cancer, trastuzumab has recently been approved for the treatment of the metastatic disease in combination with cisplatin and a fluoropyrimidine. This randomized phase III trial, showed an increase in survival with the combination of trastuzumab and chemotherapy versus chemotherapy alone [34].

Regarding the mAbs against EGFR, such as cetuximab or panitumumab, they have been approved for the treatment of metastatic colorectal cancer, either alone or in combination with chemotherapy for patients who do not harbor mutations at the K-RAS gene [35]. Patients harbouring mutations of this molecule were resistant to EGFR inhibition by cetuximab or panitumumab; therefore, these therapies are limited to patients with wild-type K-RAS tumors. Oxaliplatin, irinotecan, and chemotherapies based on 5-fluorouracil are the most frequent drugs used when combining these antibodies [36]. Cetuximab is also approved, based on an increase in survival, for the treatment of locally advanced head and neck cancer in combination with radiotherapy and for the metastatic disease in combination with platinum-based chemotherapy. As can be seen, most of these antibodies are used in association with chemotherapies, being the platinum compounds the most used agents [37].

The second category of targeted agents in the clinical setting includes the small TKIs, which are chemical entities that neutralize the kinase

activity by binding to the enzymatic region of the receptor. These compounds are particularly attractive because of their oral availability. In addition, they are able to block receptors with molecular alterations, such as truncations of their extracellular domain, which prevent the action of anti-HER antibodies [38]. In general, TKIs act on the adenosine triphosphate (ATP)-binding domain of the kinase region, competing with ATP for the interaction with the receptor. Inhibition of the TK activity has been a successful therapeutic approach for the treatment of several tumors with pathological activation of HER receptors, and the EGFR-TKIs erlotinib and gefitinib have been incorporated into treatment paradigms for patients with advanced NSCLC, while the small EGFR-HER2 TKI lapatinib has been approved for the treatment of metastatic breast cancer in combination with capecitabine. Regarding the latter, a pivotal trial showed an increase in PFS with the combination compared with capecitabine alone [39]. Ongoing studies are currently evaluating the role of lapatinib in the adjuvant setting given in combination with chemotherapy, trastuzumab or both. In addition, lapatinib is also approved in hormone receptor positive HER2 overexpressing metastatic breast cancer in combination with letrozole [40].

Despite four large phase III trials failed to demonstrate any survival advantage from the combination of EGFR-TKIs with chemotherapy in first-line treatment, the identification of somatic EGFR mutations, followed by retrospective analyses and prospective trials with EGFR-TKIs in selected patients, explained the previous conflicting results and defined the stage for more specific use of these agents [14, 15]. Of note, erlotinib is also approved in metastatic pancreatic cancer based on a slight increase in overall survival. However, this small benefit has questioned its clinical use [41].

Two types of HER-TKIs have been described, depending on their interaction properties. Reversible inhibitors, such as erlotinib, gefitinib, or lapatinib bind to the ATP-binding pocket of the kinase region of the receptors, and can be released from this region after washing out of the drug. In contrast, inhibitors such as neratinib or canertinib irreversibly bind to the receptor, and they are thus expected to impede the function of the HER receptor even after washing out of the drug. Recovery of the HER receptors in the latter instance depends on neosynthesis by the cell machinery. The in vitro efficacy of the irreversible inhibitors is higher than the one of the reversible inhibitors. However, reversible inhibitors may result less toxic [42]. In addition to the ATP-competitive inhibitors, it is expected that future non-competitive or mutant selective inhibitors will be useful to fight resistance to the actual agents. The experience acquired with TKIs targeting EGFR in lung cancer indicates that mutations which reverse affinity of the ATP-binding pocket represent a mechanism of resistance to HER inhibitors. In particular, about 50 % of NSCLC tumors from patients that initially respond to EGFR-TKIs harbor secondary mutations that cause resistance, mainly the T790M mutation in exon 20. These mutations allow ATP to bind to the ATP-binding pocket with higher affinity than small TKIs. This would cause displacement of the inhibitors form the ATP-binding pocket by intracellular ATP [43]. To potentially overcome the issue of resistance, next-generation TKIs are being developed. Examples of irreversible TKIs include afatinib (BIBW 2992), dacomitinib (PF-00299804), or neratinib (HKI-272). Afatinib is being evaluated in a phase IIb/III trial in metastatic lung cancer patients that failed to a first line or second line of treatment including chemotherapy and gefitinib or erlotinib. A recent study showed that afatinib significantly improved PFS in a population enriched for the presence of mutations in EGFR [44].

The above-mentioned studies demonstrated that many molecularly-targeted agents are not expected to be clinically effective in common cancers. Therefore, conventional phase I/II trials may be unable to distinguish agents that modulate intended targets from those that do not. In contrast, a clinical pharmacodynamic trial can potentially identify those investigational agents that deserve full clinical development using evidence of target modulation in human malignancy as the basis for this decision. In particular, when coupled with measurement of achieved drug level in

a tumor biopsy, phase 0 pharmacodynamic trials can provide important information about investigational agents that fail to modify their candidate targets [45]. This may occur by distinguishing those agents that fail to achieve adequate intratumoral levels to affect the target, from those that do not affect a target in situ despite reaching adequate intratumoral drug levels. Because the purpose of a phase 0 pharmacodynamic clinical trial is to obtain evidence of drug action on its molecular target in a clinical setting, the results of the pharmacodynamic assessment may become the primary, and sometimes sole, objective of the phase 0 protocol. This represents an important paradigm shift from the historical practice of conducting correlative studies in oncology trials, in which clinical pharmacodynamics evaluations should be integrated in early clinical investigations using available tissue specimens for molecular evidence of drug-induced changes.

However, phase 0 trials with pharmacodynamic endpoints require reliable, validated assays to measure target modulation. Assay methodology determining target modulation should therefore be optimized in preclinical models using clinical procedures and tissue handling, processing, and storage procedures standardized prior to clinical trial initiation [46]. These will establish, for example, whether the amount of tissue obtained from an 18-gauge percutaneous needle biopsy is sufficient to reliably measure target modulation, or confirm that the sample handling procedures followed in an interventional radiology suite will not impair the evaluation of target effects. These tests require extensive resources, sophisticated and sensitive tools, and an integrated multidisciplinary team, limiting the feasibility of performing phase 0 trials only at some institutions.

Agents Targeting Key Downstream Signaling Pathways

Despite the promising results obtained with the currently used targeted therapies against growth factor receptors in extending the life expectancy of selected patients with specific solid tumors, their capability in preventing resistance is still limited. The growing knowledge about the key players in downstream pathways, including signaling cascades such as the PI3K/AKT/mTOR and the HGF-Met, makes them attractive targets for the development of new therapies that can reduce or even prevent resistance. In particular, recent preclinical data have shown that combination therapy between inhibitors of different signaling pathways might circumvent resistance against some drugs and constitute a more effective therapeutic strategy [47, 48]. Therefore, in this section, we will briefly discuss the mitogen-activated protein kinase (MAPK) cascades, which are among the most prominent pathways involved in tumor progression, and the recent advances in the development of pathway-targeting inhibitors, which might successfully be used as effective anticancer agents. In particular, The ERK1/2 MAPK pathway (usually termed as the "canonical" MAPK cascade) is composed of three MAP kinase kinase kinases (MAPKKKs) (A-Raf, B-Raf and Raf-1), two MAPKKs (MAPK ERK kinases 1/2, MEK1/2) and two terminal MAPKs (ERK1/2). The available evidence supports that this pathway—rather than being a three-tiered linear pipeline which transduces signals from the cell surface to the nucleus—involves a number of inter-players, unravelling a complex network of spatio-temporal activators and inhibitors [49]. Upon surface receptor activation, adaptor proteins (i.e., growth factor receptor-bound protein 2, Grb2) lead to the activation of GTPases belonging to the Ras family (i.e., K-Ras, N-Ras, H-Ras). Activated Ras proteins can interact with and activate members of the Raf kinase family. Regarding the canonical MAPK cascade, Ras binding is sufficient to activate B-Raf, while Raf-1 (C-Raf) and A-Raf have to go through a more complex series of activation steps. Once activated, all Raf proteins are capable of activating MEK proteins, although B-Raf is the most efficient in the task. Raf kinases bind MEK and phosphorylate two serines in the MEK activation loop during a single interaction. Two mammalian MEK isoforms have been described (i.e., MEK1/2), usually considered as a unique protein due to a large sequence identity, although

recent analyses have pointed out slight differences in their regulatory pattern [50, 51].

Moreover, the traditional view of the canonical MAPK cascade as an axis that simply transduces signaling through growth factor receptors, Ras, Raf, MEK, and ERK has been extensively reviewed in the last decades, as numerous spatio-temporal modulators of the pathway have been described. First of all, several scaffold proteins have been evidenced, each one able to modulate the final ERK1/2 activity localization. Kinase suppressor of Ras-1 (KSR1) has long been recognized as the main scaffold protein for the cascade, being capable of binding all kinase members of the pathway and thus greatly accelerating and sustaining signal transduction [52]. Other scaffold proteins such as the similar expression to Fgf genes (Sef), the IQ motif-containing GTPase-activating protein 1 (IQGAP1), and the leukocyte-specific protein-1 (LSP1), are instead able to localize the canonical MAPK cascade to different cellular compartments. Furthermore, a growing number of inhibitors/modulators of selected members of the cascade have been described, including the Raf kinase inhibitor protein (RKIP) which blocks Raf-mediated MEK phosphorylation by preventing Raf-MEK physical interaction. Interestingly, RKIP levels were found reduced in metastatic cancer cells, thereby strengthening its possible tumor suppressor role [53]. However, a recent study suggested its role in the synergistic interaction of the Raf-inhibitor sorafenib with erlotinib in NSCLC cells [54].

Several members of the canonical MAPK cascade and upstream activators are frequently altered in human tumors, and different tumor-driving alterations can lead to a constitutively activated MAPK canonical pathway. The most prominent aberrations involve constitutive activation of Ras and Raf proteins. Mutations involving these players have been extensively described. Among the three Ras human genes, KRAS is the most commonly mutated (e.g., about 85% KRAS mutations in pancreatic cancer). The large majority of somatic mutations occur on nucleotides belonging to codon 12 in exon 2. Wild-type codon 12 encodes a glycine residue that guarantees a minimal steric hindrance inside the GTP-hydrolyzing pocket. Thus, a number of missense substitutions produce residues with side chains that impair GTP-hydrolyzing capability of the protein, constitutively activating the molecule. Ras mutations involving codon 61 (exon 3) and codon 146 (exon 4) occur with a reduced frequency [55]. Among the Raf family, the BRAF gene (encoding for B-Raf) bears the largest amount of clinically relevant mutations. Up to 90% of B-Raf mutations consist in a glutamic acid substitution for valine at codon 600 (i.e., V600E). The valine residue is crucial to maintain B-Raf inactive. Thus, V600E-mutant B-Raf protein activates MEK in a Ras-independent fashion, a feature not apparent for A-Raf or C-Raf. This is due to the higher basal kinase activity of B-Raf than of C-Raf and A-Raf. In fact, B-Raf serine 445 is constitutively phosphorylated, whereas the homologous C-Raf residue needs to be phosphorylated to fully transduce a signal. B-Raf mutations are regarded as possible early tumor-initiating events in melanoma carcinogenesis [56]. Genes encoding MEK and ERK are far less subject to mutations. Exon 2 of the MAP2K1 gene (i.e., encoding the MEK1 protein) has been pointed out to harbor low-frequency mutations in melanoma, lung, and colorectal cancer [57, 58].

The aberrations of the ERK pathway frequently found in cancer cells have led to great efforts in developing compounds to strike components of the cascade. In particular, the Ras proteins were at first the most attractive targets, as their downstream activity is exerted through different survival pathways, and Ras inhibition approaches (i.e., inhibition of Ras post-translational modification), have been tested in the last decades. Additional targets in the ERK cascade are the Raf kinases. Sorafenib, the first inhibitor of B-Raf kinase activity to be approved for clinical use, is scarcely selective for B-Raf and is now regarded as a multi-kinase inhibitor, exerting its activity mainly by inhibiting pro-angiogenic receptor kinases like vascular endothelial growth factor receptor 2 and 3 (VEGFR2, 3), platelet-derived growth factor receptor beta (PDGFRB) and c-Kit [59]. Vemurafenib is a more selective B-Raf inhibitor, capable of efficiently inhibiting the V600E mutant B-Raf, and was approved in

2011 by the FDA for first-line treatment of metastatic and unresectable melanoma in patients carrying B-Raf mutations [60].

MEK inhibition seems another promising approach to target the pathway, because MEK have a unique activation loop, rendering MEK inhibitors particularly specific among kinase inhibitors [61]. Furthermore, as ERK1/2 are in close contact with MEK1/2, MEK inhibition represents a precious approach to target ERK, for which specific inhibitors have never been described. The first two described MEK inhibitors (i.e., PD98059 and U0126) displayed a great potency but a poor pharmacological profile. CI-1040 (PD184352) was then developed as an orally active drug that displayed promising activity in phase I evaluation. Anyway, due to low pharmacokinetic properties, its clinical development was stopped during phase II studies [62]. Two second-generation drugs were then developed, i.e., PD0325901 and selumetinib (AZD6244, ARRY-142886). Despite a 50-fold increased potency with respect to CI-1040, PD0325901 development was interrupted due to a high toxicity [63]. Phase II clinical trials for the use of selumetinib in melanoma, NSCLC and colorectal cancer have been completed. The drug displayed a clinical activity comparable (but not superior) to current standard therapies, although it has been suggested that its efficacy could be higher in B-Raf-mutated patients [64].

Interestingly, it was recently proposed that the clinical use of both B-Raf and MEK inhibitors may provide an additional therapeutic advantage as they may be able to control the dormancy of putative pro-metastatic disseminated tumor cells [65]. It has indeed been hypothesized that dormancy of tumor cells could be associated with ERKlow/p38 high activation pattern. In this view, the pharmacological treatment of patients with inhibitors of the ERK cascade during asymptomatic conditions may be advantageous to control the awakening of dormant cells, while inhibitors of p38 should be used cautiously, as they may accelerate the development of metastases [65]. However, strategies aiming to stimulate p38 and inhibit JNK may have benefit for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-based therapies in NSCLC [66].

Agents Inhibiting Angiogenesis

Tumor angiogenesis is the multi-step process whereby new blood vessels are formed from the existing vasculature. The new blood vessels constitute an important route for the tumor cells to exit the primary tumor site, enter the circulation, and travel to distant organs. Therefore, angiogenesis, as determined by vascular density, represents a significant prognostic indicator of tumor growth and metastatic potential in several tumor entities [67]. Being involved in tumor progression and metastasis, angiogenesis represents an attractive therapeutic target for cancer treatment. One of the major players in tumor angiogenesis is the mammalian VEGF family which is composed by five glycoproteins known as VEGFA (commonly referred to as VEGF), VEGFB, VEGFC, VEGFD (also known as FIGF), and placenta growth factor (PIGF or PGF). These ligands are able to bind and activate three tyrosine kinase receptors known as VEGFR1 (or FLT1), VEGFR2 (or KDR) and VEGFR3 (or FLT4). VEGFR2 is mainly expressed in the vasculature and is the key mediator of VEGF-induced angiogenesis. The activation of the VEGF pathway and downstream signaling network promotes tumor angiogenesis by inducing a series of cellular processes, including proliferation, survival, migration and invasion of endothelial cells, enhanced permeability of existing blood vessels, and increased chemotaxis and homing of bone marrow-derived vascular precursor cells [68]. Additionally, VEGF can act as a direct growth factor on tumors by inducing immune suppression and displaying autocrine effects on tumor cells (survival, migration, invasion). Considering the key role of the VEGF pathway on tumor angiogenesis and the relevance of this process for tumor growth and metastasis, considerable efforts have been made to develop VEGF-targeted agents that can be used in cancer therapy. These agents include neutralizing antibodies to VEGF or VEGFRs, soluble VEGF receptors or receptor hybrids, and TKIs with selectivity for VEGFRs [68].

Bevacizumab is a humanized murine mAb that binds to VEGF, leading to its functional inactivation. This antibody has been approved by

the FDA as first-line treatment for patients with metastatic colorectal cancer. This approval was mainly based on the results of a randomized phase III trial with 813 patients with previously untreated metastatic colorectal cancers. About half of the patients received a regimen of irinotecan, bolus 5-FU, and leucovorin (IFL) plus bevacizumab and the other half received IFL plus placebo. The addition of bevacizumab significantly prolonged the median overall survival (OS) of the patients by almost 5 months (20.3 months vs. 15.6 months), which corresponded to a hazard ratio (HR) for death of 0.66 (P<0.001), or a reduction of 34 % in the risk of death in the bevacizumab group. Additionally, the median duration of PFS was 10.6 months in the group given IFL plus bevacizumab, as compared with 6.2 months in the group given IFL plus placebo. Furthermore, the addition of bevacizumab to IFL was also associated with an increased response rate (44.8 % vs. 34.8 %; $P=0.004$) and an increased median duration of response (10.4 months vs. 7.1 months) [69]. More recently, the efficacy and safety of bevacizumab added to first-line oxaliplatin-based chemotherapy (either capecitabine plus oxaliplatin (XELOX) or 5-FU/folinic acid plus oxaliplatin, i.e., FOLFOX-4) was evaluated. Also in this trial, the group receiving bevacizumab experienced a statistically significant improvement in median PFS. However, the OS differences did not reach statistical significance and the response rates were similar in both arms [70]. Bevacizumab was also evaluated in combination with FOLFOX-4 as second-line treatment for metastatic colorectal cancer (mCRC) in the Eastern Cooperative Oncology Group Study E3200. In this phase III trial, 829 patients with irinotecan-refractory metastatic colorectal cancers were randomly assigned to one of three treatment groups: FOLFOX-4 plus bevacizumab, FOLFOX-4 alone, or bevacizumab alone. The combination of FOLFOX-4 with bevacizumab was superior in all efficacy parameters when compared with FOLFOX-4 or bevacizumab alone. The median OS was 12.9 months for the group treated with FOLFOX-4 plus bevacizumab, 10.8 months for the group treated with FOLFOX-4 alone (HR for death = 0.75; $P=0.0011$), and 10.2 months for the group treated with bevacizumab alone. The median PFS was 7.3 months for the group receiving the FOLFOX-4 and bevacizumab combination therapy, compared with 4.7 months for the group receiving FOLFOX-4 alone (HR for progression = 0.61; $P<0.0001$), and 2.7 months for the group treated with bevacizumab alone. Finally, the corresponding response rates were 22.7, 8.6, and 3.3 %, respectively [71]. Therefore, on June 20, 2006, the FDA granted approval for the use of bevacizumab in combination with intravenous 5-FU-based chemotherapy, as second-line treatment for metastatic colorectal cancers.

When added to standard chemotherapy, bevacizumab increased survival also in NSCLC patients [72]. In the pivotal phase III study ECOG 4599, nonsquamous NSCLC patients were randomized to receive either chemotherapy or the combination of chemotherapy with this mAb. The addition of bevacizumab prolonged the OS from 10.3 to 12.3 months, and increased the RR from 15 to 35 %. Based on this study, bevacizumab gained FDA and EMEA approval as first-line therapy for advanced nonsquamous NSCLC [73].

Several agents active against multiple tyrosine kinases including VEGFR have been investigated, and the TKIs sorafenib and sunitinib are currently used in the clinical setting. Monotherapy with sorafenib prolongs OS and delays the time to progression (TTP) in patients with advanced hepatocellular carcinoma who are not candidates for potentially curative treatment or transarterial chemoembolization. Therefore, sorafenib represents an important advance in the treatment of these tumors and is the new standard of care for this condition [74]. A phase III trial showed that, compared with placebo, treatment with sorafenib prolongs PFS also in patients with advanced clear cell RCC. The median PFS was 5.5 months in the sorafenib group and 2.8 months in the placebo group (HR for disease progression in the sorafenib group, 0.44; P < 0.01) [75]. Similarly to sorafenib, the multi-kinase inhibitor sunitinib has been tested in a number of settings/tumor types and was approved by the FDA for the treatment of RCC [76]. Sunitinib inhibits cellular signaling by targeting platelet-derived growth factor (PDGF-Rs) and VEGFRs. The simultaneous

inhibition of these targets, therefore, leads to both reduced tumor vascularization and cancer cell death, and ultimately tumor shrinkage.Sunitinib also inhibits KIT, which is a receptor TK that drives the majority of gastrointestinal stromal cell tumors (GIST). It has been recommended as a second-line therapy for patients whose tumors develop mutations in KIT that make them resistant to imatinib, or who become intolerant to the drug [76].

Pharmacokinetics

Most of the available pharmacokinetics information on new targeted agents is based on data obtained from in vitro experiments, animal studies, drug–drug interaction studies, and mass-balance studies in healthy volunteers with a single dose. In general, these TKIs are substrates of several drug transporters and metabolizing enzymes. Some of them are also capable to inhibit drug transporters and enzymes making their disposition and metabolism at steady-state pharmacokinetics rather complex and unpredictable. However, it is difficult to translate the results of these studies to the clinical oncology practice where these drugs are commonly administered on a daily basis with possible auto-inhibiting mechanisms significantly altering the pharmacokinetics outcomes as well as the relevance of claimed drug interactions. Most information is available for the TKIs that are used for the longest time in clinical practice after their approval. Therefore, in the following sections, we reported the main information on the pharmacokinetics of the EGFR-TKIs gefitinib and erlotinib [77, 78].

Bioavailability

The solubility of both erlotinib and gefitinib is pH-dependent. Agents that alter gastric pH, such as H2-receptor antagonists and proton-pump inhibitors, can substantially reduce the plasma levels of EGFR-TKIs, and their concomitant use should be avoided.

Moreover, both the bioavailability and the AUC of erlotinib increase considerably when the drug is ingested with food. Most oncologists recommend the administration of erlotinib on an empty stomach, at least 1 h before or 2 h after a meal, when it has an oral bioavailability of 60%. Conversely, when taken with food, erlotinib has a bioavailability of nearly 100%, which potentiates side effects. After 7–8 days erlotinib concentrations reach steady-state, and its elimination half-life is 31 h. Erlotinib is evenly distributed in the plasma and tumor tissue (plasma: tumor ratio = 1:1). Binding to plasma proteins is approximately 95% bound to serum albumin and alpha-1 acid glycoprotein (AAG) of the serum. For erlotinib a 30% dose reduction is allowed. This dose reduction regards 6–16% of patients because of side effects.

In contrast, food does not affect the absorption of gefitinib. The absorption after oral administration is moderately slow and peak plasma concentrations are obtained after 3–7 h from administration, with elimination half-life of 48 h, and mean bioavailability of 60%. This drug is distributed extensively in tissues, and plasma protein binding is approximately 90%.

Metabolism and Clearance

Erlotinib and gefitinib are metabolized primarily by CYP3A4 and to a lesser extent by CYP3A5 and CYP1A1 [79]. Erlotinib is metabolized primarily in the liver by different cytochrome enzymes (especially by CYP3A4), but intestinal and lung cancer cells could partly contribute to its catabolism. Moreover, cigarette smoking induces CYP1A1 and has been correlated with a reduction in erlotinib exposure after a therapeutic dose [80]. Erlotinib excretion is more than 90% by stools, while the remaining 10% is excreted through the kidney. Less than 2% of delivered dose is excreted as unchanged drug. Gefitinib is also excreted mainly as metabolites in stools, with renal elimination account for less than 4% of the administered dose.

Erlotinib is a lipophilic drug; however, its lipophilicity is about three times lower than that

of gefitinib. This could help to explain some of the differences in the pharmacokinetic and pharmacodynamic properties of the two compounds, since a greater lipophilicity also leads to a higher susceptibility to the action of catabolic mechanisms, an increase in biliary excretion and a decrease in plasma concentrations of free drug. In fact, erlotinib is less exposed to hepatic cytochrome enzyme action, resulting in a slower clearance.

Other clinical factors that affect the pharmacokinetics of erlotinib include serum total bilirubin, Alpha-1 Acid Glycoprotein (AAG) concentrations, and current smoking. Increased serum concentrations of total bilirubin and AAG concentrations were associated with a reduced erlotinib clearance. Similarly, a recent study collected interesting data on the pharmacokinetics of erlotinib and its interaction with smoke: drug exposure is reduced by 50–60 % in smokers, and the maximum tolerated dose is increased to 300 mg [80].

Pharmacogenetics

Targeted therapies should not be given to all patients irrespectively of their characteristics. Indeed, their clinical activity has been related to different clinical and biological parameters, such as the EGFR-activating mutations for gefitinib and erlotinib. However, not all clinical outcomes, including tolerability, are explained by these characteristics, and the identification of novel biomarkers is a viable area of research.

Assessing germline genetic polymorphisms as either predictive or prognostic markers is very appealing, especially in the advanced cancer setting, when diagnosis is usually done from small needle biopsy samples and tumors are either not resected or resected after chemotherapy, so that the handling of tumor material can be problematic. Polymorphisms are inherited genetic variants harbored by all the cells of the body and, although a genotype represents a static value unable to change in response to a different situation, such as exposure to chemotherapy, and may not reflect changes in tumor DNA, such as loss of heterozygosity, and previous studies showed no differences in SNPs analyzed in tumor and normal tissues [81]. Therefore, their analysis can be easily performed in blood tissue and is easier to adopt in the routine clinical setting than tumor gene expression arrays, which need core needle biopsies of patient's tumors with laser microdissection and subsequent sophisticated infrastructure.

Several germ-line DNA variations of EGFR and other genes have been associated with clinical outcome after TKIs treatment, and this section focuses on the relationship between these candidate germline polymorphisms (Fig. 3.2) and the response and toxicity to gefitinib. However, studies on polymorphisms affecting their outcome have the potential to be extended to cover TKIs of similar structure and activity such as erlotinib, sorafenib, sunitinib, imatinib, lapatinib, vandetanib, and canertinib, among a growing list of many structurally related compounds with increasing clinical application.

Several studies evaluated variants in the region which regulates the expression of the EGFR gene have been evaluated. The regulatory regions of EGFR are located within the 5'-flanking region and intron-1, and both the EGFR -191C/A and -216G/T polymorphisms lie in the transcriptional start site of the promoter region, wherein multiple nuclear regulatory affinity sites are located. The -191C/A polymorphism has been correlated with enhanced EGFR promoter gene expression and activity, while the A-G variant, which leads to the substitution of an arginine with a lysine at codon 497 (R497K), has been associated with the reduction of EGFR activity [82, 83]. Similarly the -216G/T genotype is located in the binding site for the transcription factor Sp1, and the T allele is associated with increased EGFR mRNA expression [84]. The -216G/T, -191C/A, and R497K EGFR polymorphisms were evaluated in a study conducted in 92 advanced NSCLC Caucasian patients treated with gefitinib, and the association of the -216G/T variant with longer PFS was reported. The T allele was also associated with significantly higher rates of stable disease/partial response ($P=0.01$) and a significantly higher risk of treatment-related rash/diarrhea

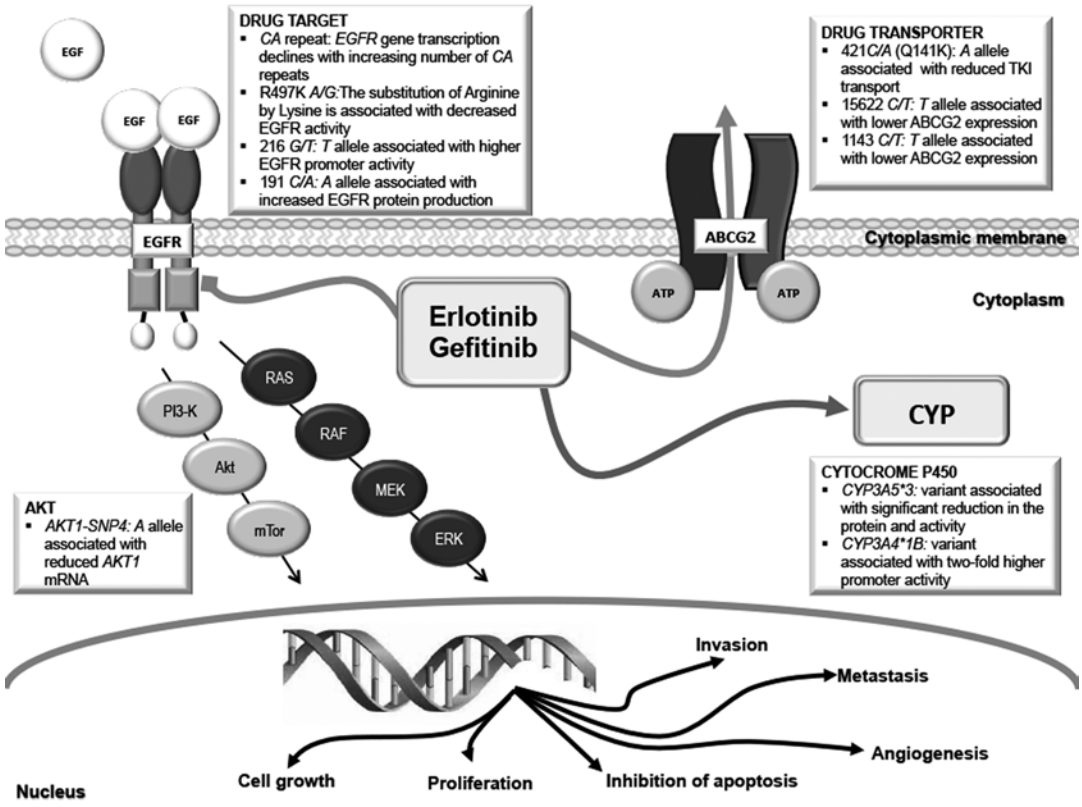

Fig. 3.2 Some of the most relevant polymorphisms in key genes involved in pharmacokinetics and pharmacodynamics of EGF receptor (*EGFR*) tyrosine kinase inhibitors (*TKI*) correlated with gefitinib and erlotinib response and toxicity in nonsmall-cell lung cancer patients. (Adapted from Galvani E, Peters GJ, and Giovannetti E. 2012 [109]

($P=0.004$) [85]. A recent study in 98 NSCLC Japanese patients treated with gefitinib screened for EGFR mutations and polymorphisms -216G/T and -191C/A reported the mutations as predictive factors of sensitivity to gefitinib, OS, and PFS, but no correlation was found between polymorphisms and clinical outcome [86]. In another study, 175 NSCLC Caucasian patients treated with gefitinib were screened for the same EGFR polymorphisms, and a significantly lower response rate was observed in patients carrying the G-C haplotype [87].

A highly polymorphic region is also located in the EGFR intron-1 as 14–21 CA-repeats [88]. Shorter alleles of a CA-dinucleotide repeat polymorphism in intron-1, of lower frequency in Asian population, are associated with an increase in transcription of EGFR [89]. In particular, the longer allele 21 has been reported to induce an 80% decrease in the gene expression compared with the shorter allele 16 [90, 91]. Most studies reported a better response to gefitinib treatment in NSCLC patients harboring the short EGFR-CA repeat genotype [92–94]. Ichihara and colleagues firstly studied in 98 NSCLC Japanese patients treated with gefitinib the relation between clinical outcome and several genetic factors, including the EGFR-CA repeat variant. In this analysis, among patients with EGFR activating mutations, individuals carrying the shorter CA alleles had a trend toward a significantly longer OS ($P=0.13$) compared with those with the long alleles (defining long CA repeats equal or greater than 19, or the sum of two alleles greater than 39, and short CA repeats as less than 19, or the sum of two alleles less than 39) [86]. Another study was fo-

cused on the correlation of clinical outcome after gefitinib treatment with EGFR mutations and CA-repeat genotype in 86 Korean patients with advanced NSCLC. In this investigation, short CA was defined as the sum of both alleles < or = 37, while long CA was defined as sum > or = 38. In agreement with the previous study, EGFR activating mutations were associated with sensitivity to gefitinib and OS, and short CA-repeat status was also correlated with better response and longer TTP [92]. In a following study performed by Nie et al. in 70 NSCLC Chinese patients treated with gefitinib, significantly higher response rate was associated with shorter CA-repeat status (defined as any allele less than or equal to 16). These patients had also higher EGFR expression and prolonged OS compared to those with long CA. No evidence of correlation was reported for clinical outcome with the R497K polymorphism or EGFR expression [95]. Shorter CA repeats (16 or less) was associated with significantly improved PFS and OS in another study in 92 Caucasians NSCLC patients treated with gefitinib [85]. However, in the largest pharmacogenetic analysis in NSCLC Caucasian patients (n = 175) treated with gefitinib, no association of the EGFR intron-1 CA-repeat status with clinical outcome was observed, grouping patients both with (1) combined CA repeat length on both alleles of ≤35 versus >35 and (2) a CA repeat length on both alleles of <18 versus all others [87].

Other potentially predictive polymorphisms include variations in the EGFR downstream signaling pathways such as AKT1, as well as the DNA repair genes and those of the genes encoding for the drug transporter ABCG2, which has been shown to be active in removing gefitinib from the cell. Different studies reported the association of the haplotype including two functional polymorphisms (AKT1-SNP3 and SNP4) with lower Akt protein levels in tissues from Caucasians, and with the lowest apoptotic response of EBV-transformed lymphoblastoids to radiation [96, 97]. Furthermore, in 96 Caucasian patients, the AKT1-SNP4 A/A genotype was correlated with shorter OS. No other poor prognostic factors were found to potentially explain the short survival of patients carrying the AKT1-SNP4-A/A variant (n = 6) since their baseline demographic and biological characteristics resulted similar to the average of the studied population. Moreover, the AKT1-SNP4 polymorphism remained an independent predictive parameter of progression and death risk at multivariate analysis [81]. A recent trial in esophageal cancer patients treated with fluoropyrimidines, platinum compounds, and taxanes, but not with EGFR-TKIs, correlated other genetic polymorphisms in AKT1 with increased recurrence and significantly shorter survival. Similarly a recent study in Korean NSCLC patients showed that other AKT1 polymorphisms could be used as prognostic markers for patients with early-stage NSCLC. These studies suggested that genetic variations in the PI3K/AKT pathway may be prognostic and/or predictive factors of response to different drugs [98, 99]. However, these results have still to be validated in a larger cohort of patients, in prospective multicenter trials, as well as additional case-control studies.

A number of common SNPs in the ABCG2 gene that might affect ABCG2 protein expression, function, and localization have been described. ABCG2 is a member of the family of ATP-binding cassette (ABC) transporters and its overexpression is commonly associated with resistance to a wide range of anticancer agents, including camptothecins, anthracyclines, and antifolates. Interactions between EGFR-TKIs and ABCG2 have been recently suggested. Of note, gefitinib is an ABCG2 substrate at clinically achievable concentrations (≤1 μM), while at higher drug concentration (>1 μM) gefitinib leads to the inhibition of the same transporter [100]. Therefore, gefitinib resistance phenotypes both in vitro and in vivo might be affected by ABCG2 expression. Furthermore, since gefitinib is an orally active compound and ABCG2 is highly expressed in the gastrointestinal tract where it participates in the uptake of several xenobiotics, one might also expect an important role for ABCG2 in the absorption and elimination of this agent. In particular, the ABCG2 421C/A polymorphism resulting in a glutamine to lysine amino acid change at position 141 (Q141K) has been correlated with the reduction of ABCG2 protein expression and/or activity and with increase accumulation of both

gefitinib and erlotinib [101]. However, no correlation between ABCG2 421C/A polymorphism and protein expression, as well as with outcome after gefitinib treatment, was observed in a tissue microarray of 50 lung cancer tissues [102].

Several other studies evaluated the correlation between selected polymorphisms and toxicity induced by gefitinib. Indeed, even if the specificity of gefitinib for its target results in a more favorable safety profile than most standard chemotherapy agents, the treatment with this agent leads to the development of rash and diarrhoea as major adverse specific effects. At present, little is known about the etiology of these effects, and there is a high interpatient variability that might be explained by the pharmacogenetic heterogeneity of patients [103]. A study in 52 NSCLC patients treated with gefitinib performed by Huang and colleagues analyzed the correlation of genetic factors with skin rash. In particular, patients were screened for the EGFR intron-1 CA repeat status, the EGFR SNPs -216G/T, -191C/A, and R521K. Among these polymorphisms, only the intron-1 CA repeat variant was correlated with grade 2–3 skin rash, observed in 21% of patients with L/L genotype (19–22 repeats), 31% S/L genotype (15–18 repeats) and 71% with S/S genotype (<15 repeats) [104]. Of note, the early grade-2/3 rash was associated with tumor response, but not the EGFR intron-1 CA-repeat genotype ($P=0.35$). No correlation was found with diarrhoea for any of these polymorphisms. Another study reported the association of the EGFR 216 G/T variant with a significantly higher risk of both rash and diarrhoea in 92 NSCLC patients treated with gefitinib [85]. Similar results were observed in our population of 96 NSCLC patients treated with gefitinib, in which grade >1 diarrhoea was significantly more frequent in patients harboring the EGFR 191C/A, A/A, EGFR 216G/G, and R497K A/A variants [81]. These results might be explained by the pathophysiology of anti-EGFR-induced diarrhoea, which is thought to result from excessive chloride secretion, inducing secretory diarrhoea. Therefore, diarrhoea might result from the higher EGFR expression in the intestinal lumen associated with the EGFR promoter polymorphism variants, as reported previously [105]. In contrast, EGFR ligand binding alterations were associated with the A allele in the R497K variant resulting in a reduced EGFR phosphorylation in colorectal cancer tissues. A strong correlation between the ABCG2 421C/A variant and diarrhoea was reported by Cusatis and colleagues in gefitinib-treated NSCLC patients [106]. In particular, they showed that only 13 (12%) of 108 patients homozygous for the wild-type genotype of ABCG2 developed diarrhoea, while 7 (44%) of 16 patients heterozygous for ABCG2 421C/A presented the adverse effect. The authors suggested that the altered ATPase activity of the polymorphic ABCG2 421C/A in the intestine, together with the reduced protein levels, might affect the oral absorption and/or elimination of gefitinib resulting in increased plasma concentrations in the steady-state and causing the diarrhoea. In contrast, no correlation between the ABCG2 421C/A variant and gefitinib-induced toxicity was found in a population of 94 Caucasian patients affected by NSCLC [102]. However, in the same population, we observed a correlation among moderate-severe diarrhoea with the ABCG2 15622C/T polymorphism and the ABCG2 (1143C/T, -15622C/T) haplotype. However, in the same population, moderate-severe diarrhoea was correlated with the ABCG2 15622C/T polymorphism and the ABCG2 (1143C/T, -15622C/T) haplotype.

Finally, both gefitinib and erlotinib are metabolized mainly by the CYP3A4, CYP3A5, and CYP1A isozymes, while CYP1A2 is involved in the metabolism of erlotinib, but not of gefitinib. Since all these CYPs are polymorphic, the distribution of specific variant CYP alleles might explain the different pharmacokinetics and activity of TKIs. However, the impact of several CYP polymorphisms to tailor in vivo treatment with TKIs remains largely to be elucidated. In the study by Rudin and collaborators [105], CYP3A4 polymorphisms were marginally associated with skin rash in erlotinib-treated patients. Individuals with lower CYP3A4 expression (A/A) were more likely to develop rash than those with higher CYP3A4 levels (A/G and G/G; $P=0.077$). Similarly, the CYP3A5*3 G polymorphism was also marginally associated with grade ≥2 rash

($P=0.094$, dominant model) and any grade diarrhoea ($P=0.062$). These marginal associations warrant further studies on the role of CYP3A4 and CYP3A5 polymorphisms in determining activity levels of EGFR-TKIs, as well as other TKIs.

In conclusion, despite the intriguing findings of several studies, the small sample size together with the interethnic differences, and the retrospective nature of most studies, make it difficult to draw any clear conclusions regarding the role of these pharmacogenetic biomarkers in determining the clinical outcome or toxicity in gefitinib treatment. Hopefully, the accurate planning of new prospective trials, the increased knowledge of key mechanisms affecting drug distribution/activity, and the use of novel technologies, including genome-wide approaches, may provide critical and essential tools to improve the prospects of pharmacogenetic research for novel molecularly targeted agents.

Conclusions

New insights into the molecular biology of cancer and tumorigenesis have recently identified key biological processes and several potential molecular targets for anticancer treatment. Novel agents targeting these aberrant processes have revolutionized the management of certain molecular subsets of cancers, and have contributed to recent improvements in survival rates, as well as in defining novel subgroups of nosological entities. For example, for EGFR mutant and EML4-ALK fusion subgroups, which are detected in approximately 15 and 4% of lung adenocarcinomas, mutation status predicts response to targeted therapy with selective inhibitors. These results led to the approval of both the EGFR-TKIs erlotinib and gefitinib and the ALK inhibitor crizotinib as first-line treatments in molecularly selected NSCLC patients [107].

However, the oncologists are still facing relevant inter-individual variability in drug activity and the occurrence of several drug resistance mechanisms. In particular, resistance to targeted agents is a general phenomenon and can be caused by several mechanisms, which are partially overlapping with the main factors involved in resistance toward chemotherapy [108]. One commonly described mechanism of resistance involves additional genetic alterations within the target oncogene itself. Additional genetic mechanisms include downstream or "bypass" activation of other components of signaling pathways, or compensatory activation of other signaling pathways. Recent studies have also shed light on nongenetic mechanisms that may have a reversible, epigenetic component, such as EMT or cancer stem cells. Taken together, these observations highlight a pressing need to further elucidate the various mechanisms that drive disease progression during drug treatment as a key step toward developing therapeutic strategies to prevent or overcome such drug resistance in individual patients, according to the specific molecular characteristics of their tumor.

Therefore, to improve the rational use of this emerging arsenal of highly selective, targeted cancer therapeutics, the conventional histopathological assessment of tumors should be associated with a refined pharmacological evaluation, including the analysis of several signaling pathways that fuel deregulated cell proliferation. Since novel genetic technologies played a pivotal role in the emergence of the so-called "personalized medicine," the integration of classical pharmacodynamics, -kinetics, and -genetics analyses with the latest generation of whole-genome analyses will be essential to further improve the customization of treatment for individual patients.

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Biomarkers as Prognostic, Predictive, and Surrogate Endpoints

4

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What Is a Biomarker?

The National Cancer Institute (NCI), defines a biomarker as: "A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease." A cancer biomarker is any biological material provided by the cancer cells that may be detected and used as indicator of tumor status or therapeutic interventions. During the last decade, advances in genomics, biotechnology, and molecular pathology led to an improved understanding of tumor biology, revealing a large number of potential tumor biomarkers, eligible for clinical use [1]. Cancer is first considered as a genetic disease; therefore, tumor-specific genetic and epigenetic alterations, such as gene mutations [2, 3], promoter-methylations [4, 5], gene copy number variations [6, 7], single nucleotide polymorphism (SNP) [8], chromosomes rearrangements [9, 10], and also alterations in the messenger ribonucleic acid (mRNA) expression or circulating microRNAs [11, 12], represent a wide group of promising genomic biomarkers. Gene mutations and SNPs can be detected either in germ-line or in tumor tissue deoxyribonucleic acid (DNA), depending on whether they are hereditary or somatic. Proteomic mass spectroscopy-based platforms have provided the ability to identify a large number of novel potential proteomic biomarkers, including cancer antigen, cell-surface receptors, or molecules produced by the host in response to the tumor [13–15]. Finally, tumor-associated metabolic alterations, such as the increase of glucose uptake by cancer cells, or other biochemical changes in the metabolic profile, may be detected by functional imaging or more innovative metabolomic technologies, and used as metabolomic biomarkers for several clinical applications [16, 17].

Potential Clinical Applications of Cancer Biomarkers

The growing number of cancer biomarkers and the advent of new testing technologies have led to the development of biomarker-guided strategies, with a greater level of individualized management of the disease in all the phases of care. Besides the well-known clinical applications, including risk assessment for disease recurrence and the early diagnosis in healthy population, a growing interest has been recently focused on the potential role of cancer biomarkers in determin-

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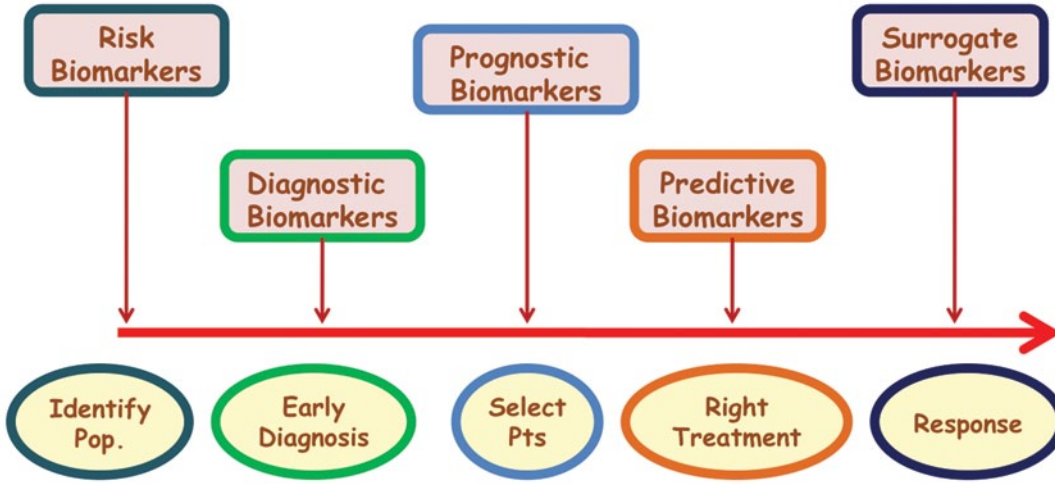

Fig. 4.1 Potential clinical applications of cancer biomarkers

ing tumor prognosis and in predicting response and/or resistance to treatments. Finally, a promising setting of biomarkers' application is as surrogate endpoint of a drug activity on the tumor and of its effects on patients' survival (Fig. 4.1). Some biomarkers may have either prognostic, predictive, and surrogate endpoint value. Because of their crucial role, it is always required a rigorous evaluation, including both analytical and clinical validation, and assessment of clinical utility prior to approving them for routinely clinical use [18].

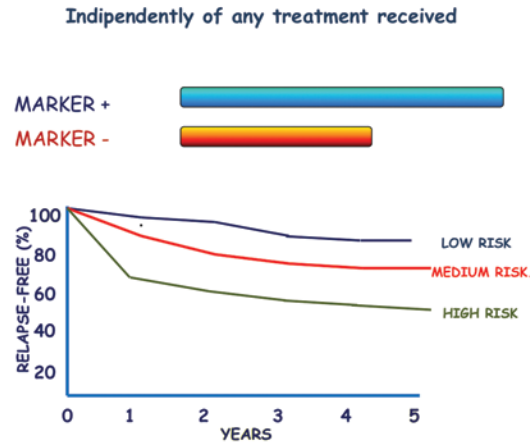

Fig. 4.2 Clinical application of prognostic biomarkers

Prognostic Biomarkers

Prognostic biomarkers refer to a single or a combination of factors related to the patient or the tumor that allow to stratify patients, at the time of diagnosis, into different classes of risk in relation to a specific outcome (such as the tumor progression or death) in the absence of any treatment or as a result of systemic, nontargeted therapies (Fig. 4.2). Therefore, they may be considered as indicators of the tumor aggressiveness and predictors of the natural history of the disease, and ultimately, of patients' outcome, independently of the treatment [19]. Prognostic biomarkers are mostly used in early stage cancer in order to select those patients at higher risk for disease recurrence or death, who may benefit more from an additional, adjuvant or neo-adjuvant, medical treatment. Besides the clinical-pathological staging system, which still remains the most important, independent, prognostic factor for all tumor types and the other tumor-tissue-related, prognostic parameters, new biological and molecular biomarkers have been recently introduced for clinical use. These biomarkers provide new tools for survival outcomes prediction and for improving the selection of patients who are candidate for adjuvant/neo-adjuvant therapies, sparing the low-risk population unnecessary treatments. In early breast cancer, for example, estrogen receptors

[20] and Her-2-neu status [21] are important, prognostic biomarkers, approved for clinical use, and always taken into account in patients' selection for a postsurgical treatment. Moreover, with the advent of genome expression profile, new, more complex tests, such as Oncotype DX or Mammaprint test, are starting to be used in a clinical setting. These platforms provide important information on the expression level of genes that are relevant to define the recurrence risk in women with early-stage hormone-positive breast cancer. These tests can also predict the potential benefit from different chemotherapies [22, 23]. In colorectal cancer, a lot of prognostic factors, including microsatellite instability (MSI), DNA mismatch repair status (MMR), single gene mutations (KRAS and BRAF) [24, 25], or genomic signature [26], have been shown to predict the risk recurrence of patients candidate for adjuvant treatment, but most of these have not been validated yet for clinical use. Furthermore, both RAS and BRAF mutations are associated with a worse prognosis, after resection of liver metastasis [27]. Finally, low ERCC1 expression and KRAS mutations are considered as negative prognostic factor in advanced non-small cell lung cancer (NSCLC) [28], while the epidermal growth factor receptor (EGFR) mutation is considered a good prognostic biomarker both in early and advanced NSCLC.

Predictive Biomarkers

Predictive biomarkers refer to a single or a combination of factors related to the patient or the tumor that are associated with the response or resistance to specific treatments (Fig. 4.3). Positive predictive biomarkers refer to specific oncogenic driver mutations, responsible for cancer cell proliferation and survival in different tumor types,

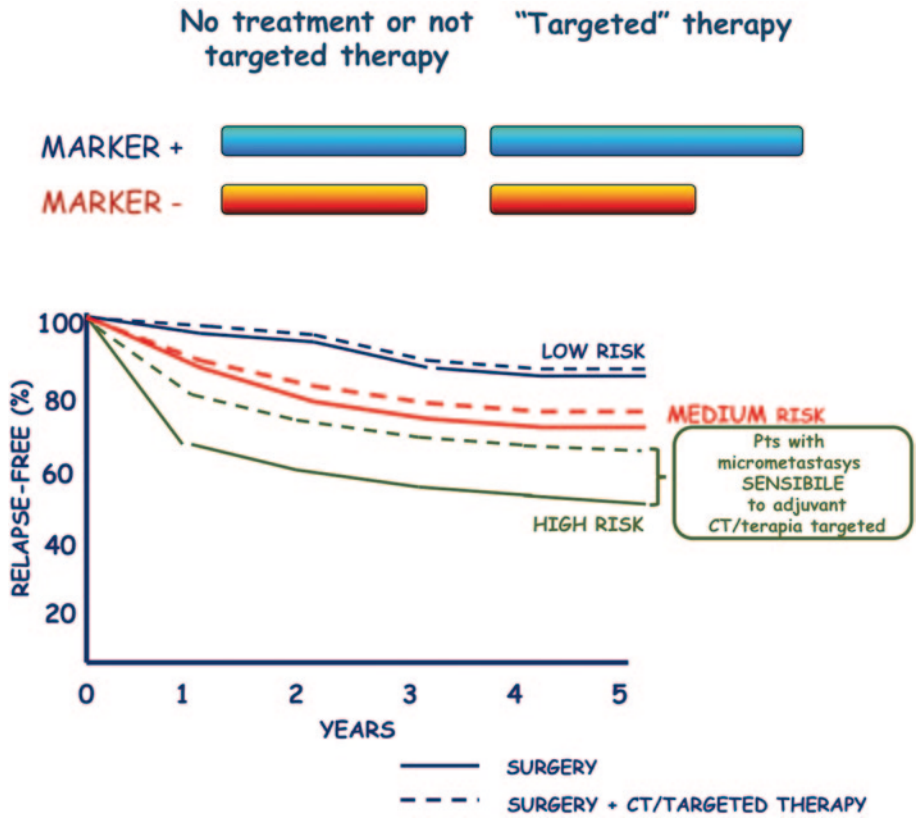

Fig. 4.3 Clinical application of predictive biomarkers. *CT* chemotherapy

that allow the selection of those patients who may experience a survival benefit from treatment with tailored agents, which target and specifically inhibit them. Otherwise, negative predictive biomarkers refer to specific cancer cell alterations which are associated with primary or acquired resistance to specific targeted therapies, allowing the exclusion of those patients who will not receive any benefit from the use of such tailored drugs. Therefore, predictive biomarkers inform about patients' outcomes exclusively in relationship with a specific target treatment received, allowing the selection of the right drug for the right patient, and promoting even more the development of personalized cancer treatments. In the last years, advances in genomics, biotechnology, and molecular pathology have generated many potential biomarkers that might help to predict response to the new targeted drugs, even if only a limited number of them have been already approved for clinical use. Her-2-neu amplification predicts response to the monoclonal antibodies (mAb) trastuzumab [29, 30] and pertuzumab [31], and the tyrosine-kinase inhibitor (TKI) lapatinib [32] has been shown to be active only in metastatic breast cancer (MBC). Hormonal receptor positivity indicates that breast cancers may respond to selective antihormone receptor antagonists [33, 34]. EGFR-activating mutations and EML-ALK/ROS1 chromosome rearrangements predict response to anti-EGFR TKIs and ALK/ROS1 inhibitors, in advanced NSCLC [35, 36]. RAS mutations exclude metastatic colorectal cancer patients from the treatment with anti-EGFR mAb [37, 38] because they are associated with poor responses and worse survival in this subgroup of molecular-selected patients. In Melanoma, BRAF-V600 mutations are predictive of response for a class of multi-target TKIs, including Vemurafenib [39]. Mutations in the exons 9–11 of c-Kit gene predict response to imatinib, while the D842V point-mutation in exon 18 of PDGFR-alfa predicts primary resistance to the same treatment in gastrointestinal stromal tumor (GIST) [40]. The approval of aforementioned predictive biomarkers in clinical setting has led to the establishment of new treatment algorithms, which always take into account the tumor molecular profile. Accordingly, biomarker-based patients' selection and targeted therapies have improved both patients' survival outcomes and quality of life, compared to the standard cytotoxic treatments. Thanks to the advances in translational research, new driver mutations have been identified in different tumor types and several ongoing trials are investigating the activity of new target agents in patients with these mutations. Therefore, the number of potential biomarkers and targeted treatment options is rapidly increasing, leading to a new insight of personalized treatment in the near future. In addition to benefiting in clinical setting, there is a growing interest in the role of predictive biomarkers in clinical trials, in order to optimize the drug-development and approval. The importance of new biomarkers' investigation is confirmed by the new trend in clinical trial design. In these biomarker-driven clinical studies, it is crucial not only to select an effective, proven, driver oncogene as biomarker but also to understand its epidemiology in cancer population and to develop a valid and reliable diagnostic test to detect it. The establishment of the predictive value of a biomarker prior to late stage, randomized, phase III trials might enhance the chances of success of such trials, reducing the time for approval of the new drugs.

Surrogate Endpoints Biomarkers

Another application of cancer biomarkers is as a surrogate endpoint. This term refers to a single or a combination of factors related to the patients or the tumors, whose changes during the treatment reflect the antitumor activity (Fig. 4.4). Surrogate

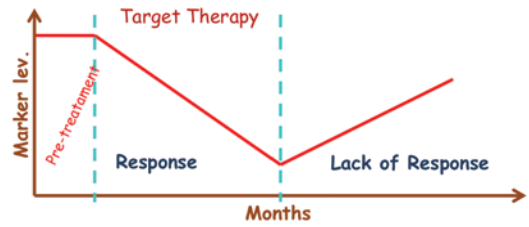

Fig. 4.4 Clinical application of surrogate endpoint biomarkers

endpoint modifications are associated with the variation of the standard clinical endpoints, such as response rates or survival. An ideal surrogate endpoint should have some features: (a) it should indicate the true benefit of a therapy earlier; (b) it should be associated with clinical outcome of interest; (c) it should be evaluated in a short or noninvasive, reproducible, reliable and cost-effective way; and (d) in clinical trials, it should be able to provide information about the efficacy of an experimental treatment more quickly and with fewer samples compared to a traditional endpoint that requires a longer follow-up time. Finally, the level of the biomarker should not change spontaneously or in response to other factors, except for cancer treatment [41]. Therefore, a validated surrogate endpoint biomarker allows the monitoring of drug activity earlier than standard clinical/instrumental evaluation by imaging or biopsy, sparing the patients of invasive medical procedures. Furthermore, these biomarkers might be used for driving early decisions concerning treatment corrections, thus saving time, effort, and money. Classical tumor biomarkers approved as surrogate endpoint include the soluble proteins CEA, CA15–3, CA125, CA19–9, and prostate-specific antigen (PSA), recommended for monitoring antitumor activity in metastatic breast, ovarian, colorectal, pancreatic, and prostate cancers, respectively [42, 43]. However, the new targeted therapies, binding and inhibiting specific molecular pathways, often produce a cytostatic effect on cancer cells, without significant decrease in tumor burden. Therefore, a lot of clinical trials have investigated the potential role of ^{18}F-fluoro-d-glucose (^{18}F-FDG) uptake variations, evaluated by positron emission tomography/computed tomography (PET/CT), as potential surrogate endpoint of early antitumor activity during the treatment with a targeted agents, in different tumor types. Interesting and positive correlation were found both in GIST [44] and NSCLC [45, 46]. Finally, the real objective of current research is the use of liquid biopsies analysis as surrogate endpoints of treatment outcome.

Circulating Biomarkers: The Concept of "Liquid Biopsy"

According to the NCI dictionary, a biopsy is defined as "the removal of cells or tissues for examination by a pathologist." In the last decades, the term "liquid biopsy" also started to be used. This definition was originally used to define circulating tumor cells (CTCs) [47] but today it can be applied also to circulating tumor DNA (ctDNA) [48]. A liquid biopsy can be defined as a liquid biomarker that can be easily isolated from any body fluids (blood, urine, saliva, ascites, pleural effusion, etc.) and that represents the tissue from which it originates, as well as a traditional biopsy (Fig. 4.5). In the field of oncology, CTCs and ctDNA are very attractive. It is now becoming increasingly clear that metastasis and tumors are extremely heterogeneous. Nowadays, the molecular profiling of the tumor is performed mainly on one tissue biopsy often performed at diagnosis. Furthermore, for the metastatic disease, it is not always feasible to obtain a biopsy for new lesions due to many reasons. Thus, the molecular profiling of one biopsy gives a spatially and temporally limited snapshot of a tumor and might fail to reflect its heterogeneity [49]. Therefore, it is very important to follow the molecular changes of a tumor in order to adapt the treatment strategies accordingly.

This is the reason why liquid biopsy is a rapidly expanding field in translational cancer research as it might be useful at different points of the diagnostic/therapeutic course of cancer patients. They may be used for early diagnosis, estimation of the risk for metastatic relapse or metastatic progression (prognostic information), stratification and real-time monitoring of therapy, identification of therapeutic targets and resistance mechanisms (predictive information), and understanding metastasis development in cancer patients [47].

In this chapter, we focus our attention on the possible role of liquid biopsy as surrogate endpoints biomarker. As explained above, the evaluation of the surrogate endpoint changing during treatment with targeted therapy is used as a measure of the drug activity.

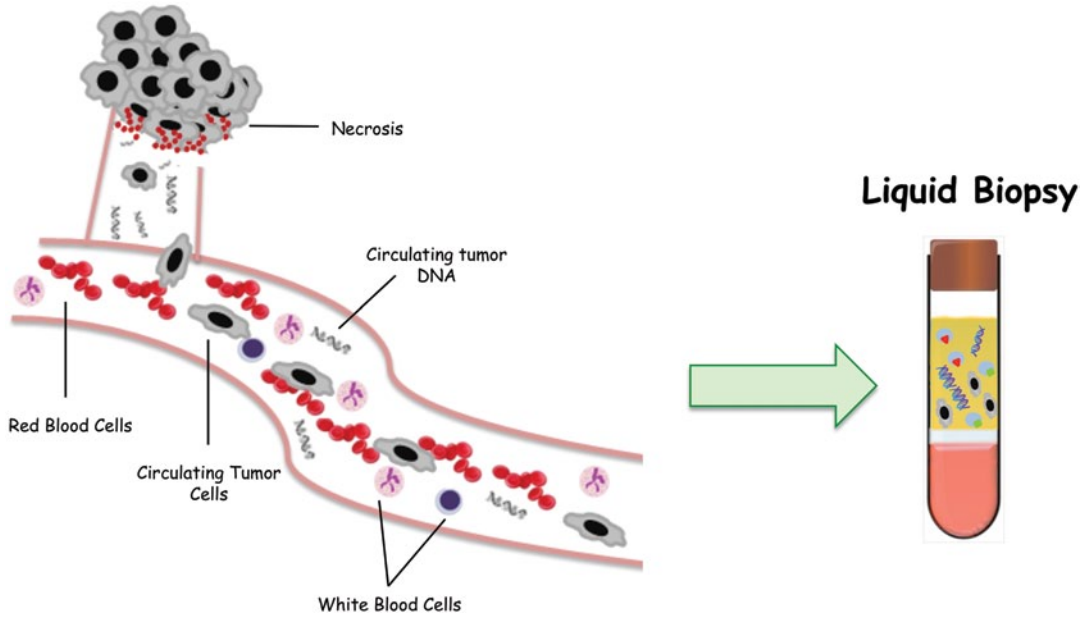

Fig. 4.5 An overview of liquid biopsy. *DNA* deoxyribonucleic acid

Circulating Tumor Cells

CTCs originate by cell detachment from the primary tumor mass, determining the migration of tumor cells to secondary sites via the lymphatic and blood system. This process is due to the epithelial-mesenchymal transition (EMT) [50] and the presence of CTCs has been demonstrated in the blood of patients with various solid tumors. Furthermore, several studies have demonstrated that the enumeration of CTCs in blood samples is a prognostic factor in several tumor types, including breast [51], prostate [52], colon cancer [53], and lung cancer [54].

The fundamental prerequisite for the introduction of the analysis of CTCs in routine clinical practice is the identification of a simple, reproducible, and reliable method for their isolation and enrichment. CTCs occur at very low concentrations of one tumor cell in the background of millions of blood cells. Their identification and characterization require, therefore, extremely sensitive and specific analytical methods, which are usually a combination of enrichment and detection procedures [55]. To date, the available techniques for CTCs isolation exploit both physical (such as size, density, electrical charges, and deformability) and biological properties (such as expression of surface proteins and the ability of invasion). Currently, there is only one technology approved by the Food and Drug Administration (FDA) for CTCs enrichment and enumeration, the CellSearch® technology (Veridex, LLC, Raritan, NJ, USA). This technique has been used in various clinical trials (breast, prostate, and colon cancers) to establish in which subset of patients' CTCs count above a known threshold might be used as a prognostic marker and a predictor of patient outcome [56–58]. All these trials have demonstrated that enumeration of CTCs, above a threshold of 5 CTCs per 7.5 ml of blood, before and after therapy is both prognostic and treatment predictive.

In metastatic castration-resistant prostate cancer patients, CTC number and patterns of metastatic spread, along with other measures of dis-

ease burden including the level of PSA and extent of disease in bone, are related [56]. Higher CTC numbers are found in patients with bone metastases and in patients who had progressed after cytotoxic therapy. Hayes et al. have analyzed the role of CTC enumeration in MBC in relation to treatment. The enumeration of CTCs was evaluated before the initiation of a new course of therapy (baseline) and at different time points after the initiation of therapy. The data reported from this study have shown that patients with <5 CTC have a longer progression-free survival (PFS) with respect to patients with ≥ 5 CTC per 7.5 ml blood. Accordingly, overall survival (OS) is significantly shorter for patients with ≥ 5 CTC compared with patients with <5 CTC. Thus, the detection of elevated CTCs at any time during therapy is an accurate indicator of subsequent rapid disease progression and mortality for MBC patients. The same trend has been shown recently in a large multicenter European study [51]. By using the same threshold of five CTCs in 7.5 ml of blood in metastatic colorectal cancer patients, it has been demonstrated that CTC analysis before and during treatment is an independent predictor of PFS and OS also in this cancer type. Furthermore, CTCs provide additional prognostic information to imaging studies [58]. For NSCLC patients, the total count of CTCs before chemotherapy initiation is associated with staging (higher detectable number of cells in stage IV patients), PFS, and OS [54]. From the same study, it is clear that the CTC enumeration, even after one chemotherapy cycle, is associated with treatment response and, therefore, they have also a predictive value. In the meantime, the evaluation of ALK rearrangements in CTCs has been shown to be feasible and reliable [59], as well as other molecular analysis in CTCs of driven oncogenic mutations such as those in EGFR, KRAS, and BRAF genes.

Circulating Tumor DNA

Cell-free DNA (cfDNA) is released from both healthy and cancer cells but patients with cancer have much higher levels of cfDNA [60–62], also defined as circulating tumor DNA (ctDNA). The levels of ctDNA seems to be related to tumor size. During tumor growth cellular turnover is faster, thus the number of apoptotic and necrotic cells increase dramatically [63]. Under normal physiologic circumstances, infiltrating phagocytes clears apoptotic and necrotic remains. This does not happen efficiently within the tumor mass, leading to the accumulation of cellular debris and its inevitable release into the circulation [64]. Based on the evidence that tumor necrosis is a frequent event in solid malignant cancers, it has been demonstrated that ctDNA is composed by a wide spectrum of DNA fragments with different strand lengths because of random and incomplete digestion of genomic DNA. Cell death in normal tissues is mainly due to apoptosis, which results in small and uniform DNA fragments of about 185–200 bp. Thus, the analysis of DNA integrity in plasma (DIA), identified as the ratio between longer fragments on the shortest, might be a useful tool to monitor cancer patients [65]. As ctDNA is directly spread from the tumor, it is a mirror of the molecular status of the tumor itself. Indeed, the exome-wide analysis of ctDNA can complement the current diagnostic/therapeutic diagram to identify mutations associated with acquired drug resistance in advanced cancers or for treatment outcome monitoring [48].

Currently, the evaluation of specific predictive biomarkers is mandatory for proper treatment selections for many tumor types (e.g., EGFR mutational status in NSCLC). As explained above, ctDNA is released from the tumor probably as a consequence of necrosis. Thus, some researches have focused the attention on the evaluation of DNA integrity in plasma of cancer patients. In this regard, Umetani et al. [66] have investigated ctDNA integrity in serum by fragment length-dependent quantitative real-time polymerase chain reaction (PCR) of ALU DNA repeats, and they found out that this test might be a promising molecular biomarker for detecting breast cancer tumor progression and regional lymph node metastases.

Even though this approach is effective, it is more interesting to analyze ctDNA for molecular alterations. The interest in ctDNA characterization has recently increased, largely because of

the development of digital genomic technologies that allow the enumeration of rare mutant variants in complex DNA mixture. Before the introduction of techniques like digital PCR (dPCR), beads-emulsion-amplification, and magnetics (BEAMing), or pyrophosphorolysis-activated polymerization (PAP), detection of ctDNA was inconsistent, suggesting that ctDNA measurement was inferior to that of other biomarkers, such as CTCs [64]. These techniques show high sensitivity especially in advanced tumors, with the mutation identified in the tumor tissue matching the mutation in the ctDNA fraction.

The whole exome sequencing of ctDNA through NGS provides relevant information about the molecular status of the tumor. Recently, Murtaza et al. have performed whole exome sequencing of plasma DNA of six patients with advanced cancers. Interestingly in NSCLC patients, the analysis of the *EGFR* gene in ctDNA has shown the occurrence of the resistance mutation T790M at progression, but not at the time of treatment initiation. This evidence demonstrates that ctDNA dynamic reflects tumor modification. Nonetheless, ctDNA turnover follows tumor growing and thus it can be used for real-time monitoring of the disease [48].

Recently, the study of Bettegowda et al. has shown the effectiveness of analyzing circulating DNA from a variety of tumors and highlights the potential investigational and clinical applications of this novel technology for early detection, monitoring resistance, and devising treatment plans to overcome resistance [67]. This study suggests that there are some intrinsic differences in ctDNA release among different tumor types.

While the studies previously presented have been focused on ctDNA analysis in advanced diseases, relatively few studies have reported ctDNA in early stage cancer. A very fascinating way to apply all these new insights about ctDNA is the possibility of selecting breast cancer patients who might benefit or not from adjuvant therapy. The recent study by Beaver et al. [68] represents a significant step toward the immediate clinical applicability of ctDNA analysis in early breast cancer patients. PIK3CA exon 9 and 20 are mutated in nearly 40 % of breast carcinomas. Thus, it might be a useful marker to be detected both in tissue and plasma samples. To this end, the group of Beaver [68] has analyzed PIK3CA mutations in early-stage breast cancer patients who have undergone to surgery. The evaluation of ctDNA before and after surgery may be used to identify patients at risk for recurrence and thus guiding chemotherapy decisions for individual patients. This approach opens different scenarios. If PIK-3CA mutation is still detectable in plasma after surgery, it might indicate residual disease. In this case, further adjuvant therapies may be recommended. Another option is that after surgery only normal circulating DNA is present. This could indicate that the procedure was curative and further adjuvant treatment is not required [69].

New Perspectives and Future Applications

The knowledge of cancer has changed dramatically over the last 30 years. We have witnessed the gradual change of cancer disease understanding; we have moved from the idea of a "chaotic disease" toward a disease that is instead determined by the acquisition of ordered and gradual mutations that characterize each growing stage. The introduction of molecular biomarkers in clinical practice has changed the natural history of many tumors (c-KIT and PDGFRA in GIST, EGFR in lung cancer, BRAF in melanoma, and KRAS in colorectal cancer).

Today, we are facing a new revolution mostly due to the introduction of novel techniques for molecular analysis in clinical practice. NGS offers the opportunity to oncologists to read and decipher the complex alterations that characterize cancer and to make the treatment decision accordingly. Due to these new opportunities, we are moving from a "one-size-fits-all" to a "personalized medicine" strategy. Despite this, we are probably not ready to manage all this information, and more researches will be needed to clarify the role and the usefulness of NGS in a clinical context.

Liquid biopsy and the use of circulating biomarker is the newest, promising, and ambitious aim that the scientific community is pursuing.

The validation of these new biomarkers is still long and winding, but in the last decades technological development of new and more sensitive technique is implementing the field quickly. CTCs and ctDNA may become fundamental parameters in the clinical management of cancer patients. Furthermore, recently an increasing number of clinical trials design take into account these variables. Thus, the clinical significance of liquid biopsy is under investigation, and probably it will be no longer a simple utopia, but it will be soon introduced in routine care.

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Evaluation of Response in Malignant Tumors Treated with Targeted Agents

5

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In the past three decades, oncology has achieved revolutionary developments. A better understanding of cancer biology and the hallmarks of human cancers has allowed the development of new treatment approaches, including the concepts of oncogenetics and target therapy [33]. Targeted therapy aims to interfere with tumor signaling pathway and thereby inhibits tumor cell growth or affects tumor angiogenesis, but does not necessarily aim for tumor cell death; this results in different radiological images. The effect of anti-angiogenic treatments, in which the blockage of vascular endothelial growth factor (VEGF) receptors inhibits the formation of new blood vessels resulting in a reduced blood supply and in tumor tissue necrosis, results on computed tomography (CT) in a reduced tumor density indirectly related to perfusion and cell density [17].

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The right assessment of tumor response to therapy is essential to state treatment success, to identify complications, and to lead decision-making for subsequent therapy. For this reason, the imaging methods have become more and more fundamental in each step throughout the assessment of cancer patients, from the screening and diagnosis of the disease, subsequent therapy, evaluation of the response to therapy, to the post-therapy follow-up of such patients. Therefore, many imaging techniques have been developed for evaluating tumor response to therapy, but measuring tumor shrinkage on CT is the current standard [22].

Various morphological approaches to assess tumor response to antitumor therapy have been introduced since the traditional methods of measuring tumor size were developed in the 1980s by the World Health Organisation (WHO), and an International Working Party was formed in the mid 1990s to standardize and simplify response criteria [14, 25, 36]. New criteria, known as response evaluation criteria in solid tumors (RECIST), were published in 2000 by a task force that comprised the European Organization for Research and Treatment in Oncology, the National Cancer Institute of the United States, and the National Cancer Institute of Canada [25].

Since the publication of the RECIST, several reports have been published regarding the low reliability of RECIST criteria in evaluating response in different types of tumors [59]. The WHO criteria and RECIST are mainly focused

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on the evaluation of anatomic tumor responses, while, as we previously said, target therapies do not necessarily cause marked tumor size reduction and thus clinically meaningful responses may be underestimated because of new targeted therapies.

Hence, RECIST was subsequently revised and in January 2009 and a revised RECIST guideline (version 1.1) was published by the RECIST Working Group based on the investigations using the database consisting of more than 6,500 patients with more than 18,000 target lesions [19, 21].

The original RECIST included definitions of minimum size of measurable lesions, instructions on how many lesions to follow, and the use of unidimensional measures for the overall evaluation of tumor burden.

Major imaging-related changes in RECIST 1.1 are a reduction in the number of lesions to be assessed, assessment of lymph node size, clarification of disease progression, and inclusion of 18-fluoro-deoxyglucose (FDG) positron emission tomography (PET) assessment exclusively in the section on detection of new lesions.

RECIST 1.1

What to Measure?

At baseline, tumor lesions/lymph nodes are categorized measurable as follows:

Tumor lesions: They must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of 10 mm by CT scan or 20 mm by chest X-ray.

Malignant lymph nodes: They have to be considered pathologically enlarged and measurable, a lymph node must be 15 mm in short axis when assessed by CT scan. At baseline and in follow-up, only the short axis will be measured and followed.

Special Considerations Regarding Lesion Measurability:

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment.

Bone lesions:

- Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, which can be evaluated by cross-sectional imaging techniques, can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.
- Blastic bone lesions are nonmeasurable.

Cystic lesions:

- "Cystic lesions" thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if noncystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with prior local treatment:

- Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion.

How to Assess Disease Progression?

All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

At baseline, a maximum of five lesions and up to two lesions in each organ are considered as target lesions. The sum of the longest diameters is calculated (long axis for nonnodal lesions, short axis for nodal lesions) for all target lesions. This baseline value is used as a reference for assessing objective tumor response at future time points. All other lesions (or sites of disease), including pathologic lymph nodes, are identified as nontarget lesions, and their presence should also be recorded at baseline.

Because treatment response is an indicator to consider in further treatment decisions and a surrogate marker for long-term survival in cancer therapy, response must be assessed as accurately and early as possible.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

Response Criteria

Evaluation of Target Lesions

Complete response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or nontarget) must have reduction in short axis to < 10 mm.

Partial response (PR): At least a 30 % decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

Progressive disease (PD): At least a 20 % increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20 %, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sumdiameterswhile on study

Evaluation of Nontarget Lesions

Complete response (CR): Disappearance of all nontarget lesions and normalization of tumor marker level. All lymph nodes must be nonpathological in size (< 10 mm short axis).

Non-CR/non-PD: Persistence of one or more nontarget lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive disease (PD): Unequivocal progression of existing nontarget lesions. (Note: the appearance of one or more new lesions is also considered progression).

When to Asses Disease?

Frequency of tumor re-evaluation while on treatment should be protocol specific and adapted to the type and schedule of treatment. However, follow-up every 6–8 weeks (timed to coincide with the end of a cycle) is reasonable. Smaller or greater time intervals than these could be justified in specific regimens or circumstances.

Imaging Considerations

RECIST 1.1 recommended maintaining standard image acquisition parameters to allow optimal comparison between studies. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.

While the precise physics of lesion size and partial volume averaging is complex, lesions smaller than 10 mm may be difficult to measure accurately and reproducibly. While this rule is applicable to baseline scans, as lesions potentially decrease in size at follow-up CT studies, they should still be measured.

Lesions which are reported as “too small to measure” should be assigned a default measurement of 5 mm if they are still visible.

CT of the chest, abdomen, and pelvis should be performed contiguously throughout the entire anatomic region of interest.

The most critical CT image acquisition parameters for optimal tumor evaluation using RECIST are anatomic coverage, contrast administration, slice thickness, and reconstruction interval. For the detection of possible new lesions, follow-up studies should cover all areas in which metastatic spread of the primary tumor in question is known to occur. Optimal visualization and measurement of metastases in solid tumors require consistent administration (dose and rate) of IV contrast as well as timing of scanning.

Positron Emission Tomography Response Criteria in Solid Tumors (PERCIST)

Limits of RECIST 1.1 in Target Therapies and Modern Criteria

Target therapies are drugs that block the growth and spread of cancer by interfering with specific molecules involved in carcinogenesis or affect tumor angiogenesis [11, 17, 23]. In the past few years, targeted agents that disrupt angiogenesis have been introduced for the treatment of various tumors. Approved agents include receptor tyrosine kinase inhibitors (TKIs), anti-VEGF antibodies, and mammalian target of rapamycin inhibitors. Collectively, these agents have allowed for a substantial improvement in the treatment of these tumors in terms of survival.

Accurate and practical methods of response assessment are critical for the optimal use of targeted therapies in clinical practice. Available evidence shows that functional imaging techniques are promising surrogate biomarkers of response of the targeted tumors and may be more appropriate than anatomic-based methods such as RECIST [20].

As previously stated, target therapies have different ways of action: some agents can induce apoptosis, some stop progression, and others block angiogenesis [11, 17, 23]. Because of differences in the mechanism of action, tumors treated with targeted therapies do not necessarily demonstrate the same radiographic findings as tumors treated with standard cytotoxic therapies. As a result, tumor response to therapy may not be observed at the same magnitude or speed on radiographic images as it used to be through traditional size-based criteria. Hence, conventional anatomy-based imaging methods for the evaluation of patient response to therapy have been found unsatisfactory because they can lead to the miscategorization for tumors like gastrointestinal stromal tumor (GIST), hepatocellular carcinoma (HCC), or melanoma when treated with targeted therapies [3, 41, 43, 44, 49, 54].

Other modern criteria proposed and recommended by professional associations for the follow-up of special tumors are summarized in Table 5.1 [2, 10, 18, 20, 29, 37, 38, 45, 47].

Imaging Modalities

Though CT is the current standard in the measurement of tumor shrinkage, other imaging modalities have also been involved in the newly developed response criteria to evaluate tumor response to targeted therapy. Continuous assessment with noninvasive imaging modalities such as ultrasound (US), magnetic resonance imaging (MRI), and PET provides data about the characteristics of the tumor concerning its vascularity, vascular permeability, blood flow, blood volume, hypoxia, metabolic activity, and cell turnover [63].

US and, in particular, its recent development, contrast-enhanced US (CEUS) using targeted microbubble contrast agents, identify necrotic and viable areas of tumors and improve the diagnostic accuracy [5]. Considering the evaluation

Table 5.1 Major proposed criteria for the evaluation of tumor response to targeted therapy

Tumor	Therapy	Criteria
Gastrointestinal stromal tumors	Imatinib	Choi criteria [37, 10, 47]
Hepatocellular carcinoma	Sorafenib	mRECIST, EASL criteria [29, 18]
Malignant melanoma	Ipilimumab	IrRC [2]
High-grade malignant glioma	All	RANO [38]
Esophageal cancer, intestinal tumors, lymphomas, nonsmall cell lung cancer and melanomas	Depending on tumor	PERCIST [20]

mRECIST modified response evaluation criteria in solid tumors, *EASL* European Association for the Study of the Liver, *IrRC* immune-related response criteria, *RANO* Response Assessment in Neuro-Oncology, *PERCIST* Positron Emission Tomography Response Criteria in Solid Tumors

of response to targeted therapy, dynamic CEUS may help in the assessment of changes in tumor perfusion and in the demonstration of therapeutic resistance to antiangiogenic treatment.

CEUS seems to be helpful in the characterization of lesions in the spleen, prostate, breast, sentinel lymph nodes in breast cancer, and pancreas. However, the role of CEUS in these organs has to be ruled out in future studies.

MRI is used in cancer detection, staging, therapy response monitoring, biopsy guidance, and minimally invasive therapy guidance [53, 62, 68]. MRI has no limitation for tissue penetration, does not use ionizing radiation, and offers higher resolution and soft tissue contrast. These advantages make MRI highly desirable for molecular imaging. MRI techniques that are used to image cancer are based on relaxivity-based imaging with and without contrast agents such as Gd-DTPA, diffusion-weighted imaging (DWI), endogenous or exogenous spectroscopic imaging determining the concentration of some metabolites, magnetic resonance elastography and blood oxygen level determination (BOLD) imaging, which has been examined as a potential means to indirectly evaluate changes in tumor oxygenation in vivo [68]. In particular, DWI measures the diffusion of water molecules (Brownian movement) providing endogenous image contrast from differences in the motion of water molecules between tissues without the need for exogenous contrast agents. Hence, it is considered a promising technique for the identification of tumors and metastases [53, 62]. As for example, DWI MRI in the liver proved to be able to see changes in hepatic metastases from neuroendocrine tumors after transarterial chemoembolization [30].

PET is a nuclear medicine imaging technique that detects gamma rays emitted indirectly by a "tracer" or positron-emitting radionuclide, such as [18F]-fluoro-2-deoxy-D-glucose, [C15O]-carbon monoxide, and [18F]- 3'-deoxy-3'-fluorothymidine [66]. Depending on the tracer that is used, images of a particular functional process of the tumor (e.g., vascularity, perfusion, hypoxia, and cell turnover) can be constructed [63]. Combining PET with CT, it is possible to a have a top-down perspective about anatomical and biological tumor information: PET/CT takes advantage of the sensitivity and functionality of PET imaging and the high spatial resolution of CT imaging [52].

One step further in multimodality imaging has been introduced combining PET and MR. PET/MRI and the resulting combination of molecular, morphological, and functional information will pave the way for a better understanding of physiological and disease mechanisms [32]. Moreover, combined PET/MR studies may provide important biomarkers to predict and monitor targeted treatment response and to document pharmacodynamic response. As for example, dynamic contrast-enhanced MR imaging allows the assessment of tumor vascularity and detects changes associated with angiogenesis-targeted therapy. However, when only dynamic contrast-enhanced MR imaging is used, the true antitumor effects of these agents cannot be completely understood, and combining PET parameters (e.g., estimates of tumor glucose metabolism, cellular proliferation, and amino-acid transport) and MR imaging methods may provide a better approach to this investigation [48]. Combined PET/MR measurements might help quantify precisely how tumor vascular properties (assessed by functional MR methods), proliferation, and antitumor effects (assessed with PET) occur and interact [32, 48].

Tumor Response Criteria in Targeted Cancer Therapies

GIST

GISTs are the most common mesenchymal tumors of the gastrointestinal tract arising from a precursor of the interstitial cells of Cajal [28, 51, 58]. These latter express the c-kit receptor tyrosin kinase. Discovery of activating mutations of the KIT and platelet-derived growth factor receptor (PDGFR)-a genes with subsequent therapeutic development of receptor TKIs has revolutionized the treatment of patients with GIST [31, 64]. In the past decade, survival of patients with GIST has improved with use of TKIs such as imatinib

[6] (Gleevec, Novartis, East Hanover, NJ) in first-line setting and sunitinib [39] (Sutent; Pfizer, New York, NY) in secondline setting. Regorafenib (Stivarga; Bayer, Berlin, Germany), an inhibitor of multiple cancer-associated kinases including KIT and PDGFR, has recently been approved by US Food and Drug Administration (FDA) in the USA as a third-line agent for TKI-resistant GIST based on data from phase II and III trials [1, 40].

In initial tumor response to imatinib in patients with malignant GISTs, dramatic changes were noted in tumor attenuation values, in the extent of enhancing intratumoral nodules, and in the extent of tumor vessels [10, 37, 47]. In some cases, size can actually increase secondary to internal hemorrhage, necrosis, or myxoid degeneration [10, 37, 47]. RECIST underestimated the effect of imatinib on metastatic GISTs especially at this early stage of treatment and was a poor predictor of clinical benefit [22]. The tumor response to treatment traditionally has been evaluated solely on the basis of tumor size, whereas Choi criteria employ both size and another quantitative parameter, tumor density. Choi response criteria, incorporating tumor density and using small changes in tumor size on CT, proved to be more sensitive and more precise than RECIST in assessing the response of GISTs [10, 37, 47].

According to the Choi criteria, a decrease in tumor size of more than 10% or a decrease in tumor attenuation of more than 15% on CT correlates well with good response by 18F-fluorodeoxyglucose (FDG) PET and shows excellent prognostic value [10, 47]. The Choi response criteria for GIST proposed that tumor attenuation could provide an additional measure of response to imatinib therapy. The response can be seen very early during treatment (Fig. 5.1).

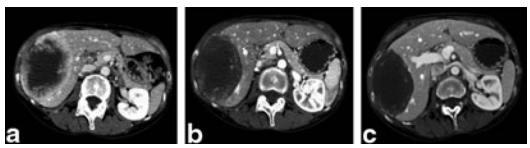

Fig. 5.1 Axial Multislice Computed Tomography images of a metastatic GIST patient before treatment (**a**), after 3 months (**b**), and after 6 months (**c**). It clearly depicts a stable disease with diameters unchanged although there is a reduction of the lesion's density

However, in a recent work Shinagare et al. [16] demonstrated that the currently used system, RECIST 1.1, is well suited for response evaluation in patients with GIST after failure of prior TKI therapy. WHO and the earlier version of RECIST are also effective. Choi criteria are far more sensitive in calling PR, and the time to best response was also shortest for Choi criteria.

Essentially, this confirms prior data that the Choi criteria serve to reclassify stable disease as "responders." The authors conclude stating that RECIST is at present well suited as the primary criteria for response evaluation in clinical trials of GIST. Choi criteria, given its high sensitivity, may be used as an adjunct or as a system to detect early proof of biological activity, but these criteria do not appear to be ideally suited as a primary tumor response criteria for definitive trials of clinical benefit.

PET has been found to be highly sensitive in detecting early response and to be useful in predicting long-term response to imatinib in patients with metastatic GIST [10]. There is good correlation between the responses based on overall tumor burden, CT attenuation, and maximum SUV (SUVmax) at FDG PET. However, as previously said, the availability of PET is still limited.

Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is recognized as one of the most chemo-resistant tumor types, and until 2007 no systemic drug was recommended for patients with advanced tumors, an unparalleled situation in oncology [27]. In 2007, FDA approved the use of sorafenib, an oral multi-TKI, which was the first and remains the only drug that has demonstrated survival benefits in patients with advanced HCC [34].

Therapy with sorafenib is indicated for patients with well-preserved liver function (Child-Pugh A class) and with advanced tumors or those tumors progressing upon loco-regional therapies. However, there are no clinical or molecular biomarkers available to identify the best responders to sorafenib [4].

Targeted therapies in HCC cause tumor necrosis. Viable tumor formation needs to be assessed using CT or MRI studies and viable tumor should

be defined as uptake of contrast agent in the arterial phase of dynamic imaging studies [57].

Growing evidence has suggested that RECIST may not be the best criteria for monitoring treatment response in HCC. Hence, the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) have proposed the development of superior methods, modified RECIST (mRECIST) and EASL criteria, respectively, to assess response to therapy in HCC [18, 29, 35, 42]. Both systems focus on changes in the viable tumor burden, which is ascertained using dynamic imaging techniques to identify the contrast-enhanced areas. EASL and AASLD guidelines adopted a modified version of a WHO criterion in which the evaluation of the treatment response accounted for the induction of intratumoral necrotic areas in estimating the decrease in tumor load, and not just a reduction in overall tumor size.

Table 5.2 summarizes the main differences in tumor response according to RECIST 1.1, mRECIST, and EASL criteria.

mRECIST and HCC

mRECIST emphasize the optimization of image acquisition protocols and consistency in the use of the same protocol throughout follow-up. Patients can be followed up with either contrast-enhanced spiral CT or contrast-enhanced MRI. The liver must be imaged using a dual-phase protocol with either modality. Delayed equilibrium phase imaging may be useful, though it is not mandatory. The viable tumor should be measured during the arterial phase. To be selected as a target lesion, the lesion should be classified as a measurable lesion according to RECIST criteria, suitable for repeat measurement and showing enhancement during the arterial phase. Ill-defined infiltrative-type HCC and malignant portal vein thrombosis are considered non target lesions.

The presence of a new lesion is considered to represent disease progression: a new lesion must have a maximum diameter of over 1 cm and show the typical vascular pattern of HCC at dynamic imaging. Otherwise, new lesions should be considered equivocal and monitored for interval growth at subsequent scans.

Table 5.2 Assessment of response to therapy in advanced HCC according to RECIST 1.1, mRECIST, and EASL criteria

	RECIST 1.1	mRECIST	EASL
CR	Disappearance of all target lesion (up to 2 measurable liver lesions)	Disappearance of any intratumoral arterial enhancement in all target lesions (up to 2 measurable liver lesions)	Disappearance of any intratumoral arterial enhancement in all measurable arterially-enhancing liver lesions
PR	>30% decrease in the sum of diameters of target lesions	>30% decrease in the sum of longest diameters of "viable" target lesion (arterial phase enhancement)	>50% decrease in the sum of the product of bidimensional diameters of "viable" target lesions
PD	>20% increase in the sum of diameters of target lesions. In addition, the sum must also demonstrate an absolute increase of at least 5 mm	>20% increase in the sum of longest diameters of "viable" target lesion (arterial phase enhancement)	>25% increase in the sum of the diameters of "viable" target lesions
SD	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD

CR complete response, *PR* partial response, *PD* progressive disease, *SD* stable disease

EASL Criteria and HCC

While RECIST criteria determine measurements based on the extent of measurable disease and the presence of arterial phase on CT, EASL criteria measure both tumor necrosis and viable tumor in order to determine extent of response.

The use of EASL criteria has been accepted in the assessment of therapy response in HCC particularly following the use of locoregional therapy, though a guideline for measurement is not currently available. Local tumor response is measured as regression of treated lesions.

The EASL criteria are applied for each target lesion as follows:

- Record the longest diameter
- Estimate the percentage of the tumor volume that appears necrotic
- Calculate the viable diameter
- Compare the viable diameter for each tumor to the baseline diameter

New lesion appearance in a previously untreated area and extrahepatic disease is considered PD.

Malignant Melanoma

Although the incidence of malignant melanoma is increasing, most cases are diagnosed at an early stage and curable through surgical excision. On the other hand, the management of patients with disseminated malignant melanoma is still a difficult problem: immunotherapy with high-dose interleukin-2, ipilimumab (a monoclonal antibody targeting CTLA-4), and monoclonal antibodies targeting the programmed death 1 protein or its ligand PD-L1 can induce durable responses or stabilization of disease in a significant proportion of patients [61].

The patterns of response to treatment with these immunotherapy drugs are different from other targeted agents, and it is mandatory to understand these different patterns of response in order to appropriately evaluate the effectiveness of this class of agents: [2]

- Patients may have a transient worsening of disease (progression of known lesions or appearance of new lesions) before disease stabilizes or tumor regresses. During antibody therapy with ipilimumab, a pseudoprogression with an increase in the size of the visible tumor occurs in some patients due to a pronounced immunological reaction and the size only begins to decrease after a period of up to 16 weeks after the start of treatment.
- Some patients who do not meet criteria for objective response can have prolonged periods of stable disease that are clinically significant.

Immune-related response criteria (irRC) were proposed in 2009 by a collaborative group of oncologists, immunotherapists, and regulatory experts [2]. For the irRC, only index and measurable new lesions are taken into account.

At the baseline tumor assessment, the sum of the products of the two largest perpendicular diameters (SPD) of all index lesions is calculated. At each subsequent tumor assessment, the SPD of the index lesions and of new, measurable lesions are added together to provide the total tumor burden:

$$\text{Tumor burden} = \text{SPD}_{\text{index lesions}} + \text{SPD}_{\text{new measurable lesions}}$$

The definitions of the irRC and guidelines on how they can be used in clinical practice are detailed below:

- *Immune-related CR*: Complete resolution of all measureable and nonmeasurable lesions, with no new lesions. After at least 4 weeks, the CR must be confirmed.
- *Immune-related PR*: >50% decrease in the total tumor burden. After at least 4 weeks the PR must be confirmed.
- *Immune-related PD*:>25% increase in tumor burden. After at least 4 weeks the PD must be confirmed.
- *Immune-related SD*: None of the above mentioned.

Immune-related altered patterns of response seen with ipilimumab are shown in Table 5.3.

The core novelty of the irRC is the incorporation of measurable new lesions into "total tumor burden" and comparison of this variable to baseline measurement.

The use of these irRC is important because the application of RECIST in patients treated with ipilimumab may lead to premature discontinua-

Table 5.3 Patterns of tumor response to ipilimumab according to irRC

Patterns of tumor response to ipilimumab	
Type A	Reduction in size of baseline lesions with no new lesions
Type B	Stable disease with no significant change in the size of the baseline lesions that may or may not be followed by a slow, steady decline in tumor size
Type C	Initial increase in tumor burden followed by response
Type D	Reduction in total tumor burden in spite of the appearance of new lesions

tion of treatment in a patient who will eventually respond to treatment or have prolonged disease stabilization.

High-Grade Malignant Glioma

Glioblastoma is the most common primary brain tumor in the United States [15]. Median overall survival for patients with newly diagnosed glioblastoma ranges from 12 to18 months when treated with the current standard of care [46, 55]. The poor prognosis underscores the need for the development and characterization of new therapeutic regimens and the need for appropriate treatment response therapy [65]. Since 1990, the primary criteria used to assess response to therapy in high-grade gliomas were those developed by Macdonald et al. [8].These criteria were based on two-dimensional tumor measurements on CT or MRI, in conjunction with clinical assessment and corticosteroid dose. Improvements in imaging technology and therapy have prompted the need to change the response assessment.

In particular, one of the challenges for response criteria is related to the use of antiangiogenic agents, especially those targeting VEGF, such as bevacizumab, and the VEGF receptor, cediranib. These agents produce high radiographic response rates, as defined by a rapid decrease in contrast enhancement on CT/MRI that occurs within days of initiation of treatment. This phenomenon, known as pseudoresponse, is partly a result of reduced vascular permeability to contrast agents rather than a true antitumor effect and can mislead to a false positive high radiological response rate [9, 38]. Moreover, a subset of patients treated with these agents develop tumor recurrence characterized by an increase in the nonenhancing component on T2-weighted/FLAIR sequences.

For these reasons, in 2010, the Response Assessment in Neuro-Oncology (RANO) Working Group published updated criteria to standardize response assessment and incorporated relevant clinical and treatment information [38]: the inclusion of contrast enhancement changes and fluid-attenuated inversion recovery (FLAIR)/T2 hyperintensity into the RANO criteria increases the sensitivity in the detection of high-grade gliomas true progression.

According to RANO, MRI is the only modality that should be used to assess response and progression of high-grade malignant glioma. The sequences required are precontrast T1, T2/FLAIR, and postcontrast T1, with two orthogonal planes (or a volume acquisition); however, additional sequence that may be helpful is DWI. Measurable disease is defined as bidimensionally contrast enhancing lesions with clearly defined margins by CT or MRI scan, with two perpendicular diameters of at least 10 mm, visible on two or more axial slices that are preferably, at most, 5 mm apart with 0-mm skip. In the event of interslice gaps, this also needs to be considered in determining the size of measurable lesions at baseline. Moreover, measurable lesions do not include cavity, cyst, or necrosis in the measurement. Among measurable lesions, the selection of target lesions considers five lesions at maximum, the largest ones and the most suitable for reproducible measurements.

At follow up, along with the measurement of previously defined target lesions, the radiologist should qualitatively assess nontarget lesions, both nonenhancing ones and those seen only on T2/FLAIR, and search for new lesions. Thereafter, oncologist has to combine lesion assessments with neurological and steroid dose information to yield an overall timepoint response, as defined in Table 5.4.

Table 5.4 RANO criteria for response assessment incorporating MRI and clinical factors

CR	Complete disappearance of all enhancing measurable and nonmeasurable disease
	CR must be confirmed after at least 4 weeks. If not confirmed, is SD
	No new lesions
	Stable or improved nonenhancing (T2/FLAIR) lesions
	Patients must be off corticosteroids and stable or improved clinically
PR	SPD of all measurable enhancing lesions decrease ≥50%
	PR must be confirmed after at least 4 weeks. If not confirmed, is SD
	No progression of nonmeasurable disease
	No new lesions
	Stable or improved nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids
	Patient is stable or improved clinically
PD	SPD of all measurable enhancing lesions increase ≥25%
	Significant increase in T2/FLAIR nonenhancing lesion on stable or increasing doses of corticosteroids, not caused by comorbidities
	Clear progression of nonmeasurable disease
	Any new lesion
	Clear clinical deterioration attributable to the tumor
	Failure to return for evaluation as a result of death or deteriorating condition
SD	None of the above

SPD sum of the product of the diameters, *CR* complete remission, *SD* stable disease, *PD* progressive disease, *RD* relapsed disease

PERCIST

Due to the increasing limits of RECIST related to the fact that they just allow a morphologic assessment, RECIST has been modified to take functional biological information into consideration. FDG-PET can provide additional anatomical information in combination with CT scan. This method is also used for the follow-up of esophageal cancer, intestinal tumors, lymphomas, and melanomas. The so-called PERCIST were proposed particularly in newer molecular treatments during response evaluation [20]. However, due to the limited availability of PET and to economic reasons, these criteria have not yet been further developed.

Because many newer cancer therapies may be more cytostatic than cytocidal, good tumor response may be associated predominantly with a decrease in metabolism, without a major reduction in tumor size [59]. Therefore, metabolic response as a leading indicator of tumor response may be even more predictive of outcome than morphologic criteria [59].

18F-FDG PET shows increased glucose uptake in metabolically active cells (and thus in a metabolically active tissue) and is most commonly used to measure glucose metabolism or tumor growth in oncology [22]. The standardized uptake value (SUV) represents a quantitative assessment of uptake in a tumor region of interest. The SUV can also represent a quantitative assessment of uptake in a tumor and is based on a ratio between tracer uptake within a tumor and homogeneous distribution of tracer within the patient body [22].

In 2009, Wahl et al. [20] proposed guidelines for the standardization of response criteria for FDG PET, the so-called PERCIST. PERCIST recommended using SUL (lean body mass–normalized SUV [SUV_{lbm}]) owing to its reduced dependence on patient weight compared with standard body weight-normalized SUV (SUV_{bw}). In PERCIST, response to therapy is evaluated as a continuous variable and expressed as a percentage change in SUL peak for the most active lesion at each time point between the pre- and post-treatment PET/CT studies, as shown in Table 5.5.

PERCIST have been integrated into RECIST 1.1.

Table 5.5 PERCIST criteria

Complete metabolic response	Visual disappearance of all metabolically active tumor
Partial metabolic response	More than a 30% and a 0.8-unit decline in SUL peak between the most intense lesion before treatment and the most intense lesion after treatment, although not necessarily the same lesion
Progressive metabolic disease	More than a 30% and 0.8-unit increase in SUL peak or new lesions Or A greater than 75% increase in total lesion glycolysis
Stable metabolic disease	None of the above

In recent years, a variety of literature has reported that 18F-FDG PET and 18F-FLT PET could predict the benefits of TKIs in NSCLC patients [12, 13, 26, 50, 56, 69]. Several studies also suggest that measuring SUVs before and after treatment is related to a prognostic value in patients with NSCLC. 18F-FDG PET has been shown to help predict response early during the course of molecular-targeted agent therapy such as EGFR-TKIs including erlotinib and gefitinib [69].

Moreover, there is a growing body of evidence suggesting that PET is becoming established as a clinical technique for assessing tumor response, also in FDG-avid lymphoma subtypes, as shown in a previous section [24].

3′-deoxy-39-(18F)fluorothymidine (FLT)-FLT PET has been more promising in measuring response to targeted therapy under some select conditions, such as in patients with a higher probability of mutations [69].

Considering esophageal cancer, it has been reported that 18F-FLT PET may discriminate tumor from esophagitis more effectively than 18F-FDG PET based on pathology evaluation [7].

Furthermore, it has been demonstrated that early SUV changes on 18F-FDG PET may help to discriminate responders from nonresponders to imatinib, a BCR-ABL and c-KIT inhibitor, that directly affects the expressions of glucose transporters [67].

In conclusion, recent preclinical studies reported that 18F-FLT PET imaging and 18F-FDG PET imaging are useful tools for early response monitoring of a novel c-Met inhibitor, BAY 853474, in a gastric cancer xenograft model [60].

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Targeted Therapies for HER2-positive Breast Cancer

6

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Introduction

The human epidermal growth factor receptor 2 (HER2) belongs to the epidermal growth factor receptor (EGFR) family. This family comprises four tyrosine-kinase transmembrane receptors (HER1/EGFR, HER2, HER3, HER4) which are involved in cell growth, survival, and differentiation. Following ligand binding to the extracellular domain (ECD), HER2 dimerizes with another receptor of the EGFR family, thus enabling autophosphorylation of the tyrosine kinase residues at the intracellular portion and triggering downstream signaling via the phosphatidylinositol 3' kinase (PI3K)/Akt and the Ras/Raf/MEK/MAPK pathways (Fig. 6.1). A higher expression of HER2 on cell surface leads to a constitutional activation of the downstream cascade. Overexpression of HER2 occurs in 15–20 % of breast cancers (BC), mainly due to HER2 gene amplification (localized on chromosome 17), and, in the absence of targeted treatment, is associated with a poorer outcome. The advent of anti-HER2-directed agents has favorably reversed the poor prognostic impact of HER2 overexpression/amplification [1]. To date, four are the approved drugs for the treatment of HER2-positive BC (trastuzumab, lapatinib, pertuzumab, and T-DM1) and many other agents are in the clinical phases of development (Fig. 6.1). Despite these advances, many patients at a certain point develop resistance to these agents and progress. In this chapter, the available data on approved anti-HER2-targeted agents, ongoing trials, and new therapeutic options will be discussed.

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Approved Anti-HER2 Agents

Trastuzumab

Metastatic Setting

Trastuzumab is an anti-HER2 fully humanized monoclonal antibody. Although its antitumor mechanism of action is not fully understood, after binding to HER2 extra cellular domain (ECD), it is supposed to exerts its function via antibody-dependent cell-mediated cytotoxicity, prevention of ECD shedding that may result in a truncated constitutively activated form, inhibition of ligand-dependent dimerization, inhibition of downstream signaling, inhibition of cell cycle progression, induction of apoptosis and inhibition of angiogenesis [1].

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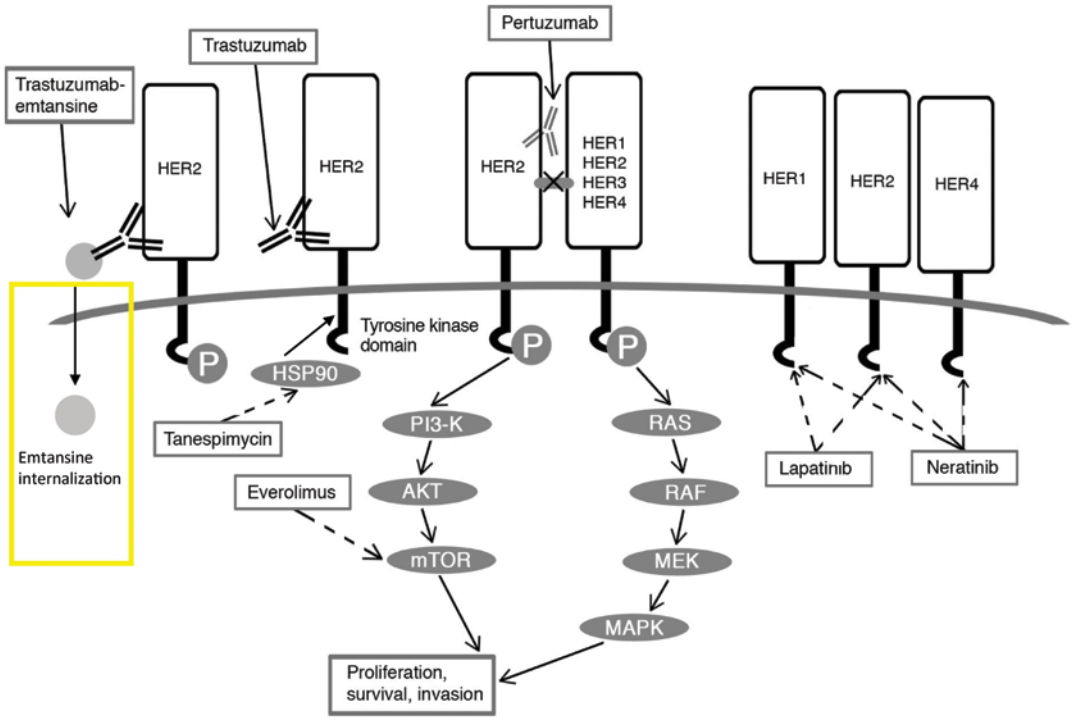

Fig. 6.1 HER family receptors and mechanisms of action of targeted agents. Trastuzumab binds to the ECD of HER2. Several mechanisms of action have been proposed, such as the inhibition of the downstreaming HER2 pathway involving PI3K/Akt and MAPK signaling. Pertuzumab is a humanized monoclonal antibody that binds to the dimerization domain on the ECD of the HER2 receptor, preventing the dimerization between HER2 and other family members. Trastuzumab emtansine (T-DM1) consists of trastuzumab bound to emtansine, a microtubule-binding chemotherapeutic agent. Following the binding of T-DM1 to HER2, the complex is internalized, emtansine is released and becomes able to interfere with microtubules in the nucleus. Everolimus is an inhibitor of mTOR, a member of the PI3K/Akt pathway. Tanespimycin is an inhibitor of heat shock protein 90 (HSP90), which is responsible for conformational stabilization of HER2. Inhibition of HSP90 impairs this stabilization, thus leading to HER2 proteasomal degradation. Lapatinib is a small molecule tyrosine kinase inhibitor of both HER1 and HER2. Neratinib is an oral irreversible small molecule tyrosine kinase inhibitor of HER1, HER2, and HER4

In early trials including HER2-positive advanced BC patients, trastuzumab monotherapy-induced response rates (RR) ranging from 11–15% in pretreated patients to 35% when used as first-line treatment for patients with 3+ score HER2 overexpression by immunohistochemistry [2–4]. Preclinical assays showed an increased efficacy of trastuzumab when it is combined with chemotherapy, and this was confirmed by clinical trials [5]. In a pivotal randomized phase III study for HER2-positive MBC, first-line chemotherapy (paclitaxel or anthracycline and cyclophosphamide) plus trastuzumab resulted in significantly higher RR (50 vs 32%, $p < 0.001$) and prolonged both progression-free survival (PFS) and overall survival (OS) as compared to chemotherapy alone (7.4 vs 4.6 months, $p < 0.001$ and 25.1 vs 20.3 months, $p = 0.46$, respectively). In this study, concurrent administration of trastuzumab and anthracyclines resulted in higher (16%) cardiac dysfunction rate (New York Heart Association class III or IV), as compared to anthracyclines alone (3%). [6] Another phase II randomized trial compared first-line docetaxel with or without trastuzumab. The combination achieved significant results in terms of RR (61 vs 34%, $p = 0.0002$), time to progression (TTP: 11.7 vs 6.1 months, $p = 0.0001$), and OS (31.2 vs 22.7 months, $p = 0.0325$). [7] Since the initial approval of trastuzumab by Food and Drug Administration (FDA) in 1998 for first-line treatment of HER2-positive metastatic breast cancer (MBC) in combination with taxanes, many other cytotoxic compounds have been safely and

effectively combined with trastuzumab in clinical trials. Overall, the RR of these chemotherapy-trastuzumab combinations ranged between 24 and 88%. Of note, all the combinations were generally associated with a tolerable toxicity profile [8]. Two phase III randomized trials deserve to be more extensively mentioned. A phase III study evaluated the association of trastuzumab and docetaxel versus trastuzumab plus vinorelbine as first-line therapy. A total of 284 patients were randomized and no differences in RR, TTP, and OS were observed between the two arms. Interestingly, the toxicity profile favored the vinorelbine-containing arm. Significantly, more treatment-related grade 3/4 febrile neutropenia and neuropathy were reported with docetaxel [9]. In a second randomized trial, 263 HER2-positive advanced BC patients were randomly assigned to trastuzumab plus docetaxel (100 mg/m^2) or trastuzumab plus carboplatin plus docetaxel (75 mg/m^2). There was no significant difference between the two regimens in terms of TTP (11.1 and 10.4 months, $p=0.57$), RR (72% for both groups), and OS (37.1 and 37.4 months, $p=0.99$). Toxicity was acceptable for both arms [10].

Noteworthy, institutional series comparing the outcome of HER2-positive MBC patients treated in the pre-trastuzumab and post-trastuzumab eras showed a significant improvement in OS after the introduction of trastuzumab, meaning that this agent has dramatically changed the natural history of this disease [11].

Early Setting

Adjuvant

In the adjuvant setting, HER2-positive BC patients outcome is remarkably improved when trastuzumab is added to chemotherapy [12]. Six phase III randomized trials have explored the benefit of adding trastuzumab to adjuvant chemotherapy for HER2-positive disease. Different schedules, timings, and durations of trastuzumab administration have been tested (Table 6.1). All experimental arms planned a 1-year treatment of trastuzumab with the exception of the Herceptin Adjuvant (HERA) trial [13], where a third arm investigates 2 years of adjuvant trastuzumab and the FinHER study [15], which planned a shorter trastuzumab treatment duration (9 weeks). Furthermore, the joint analysis of NSABP B-31 and N9831 [14], the FinHER [15] and the BCIRG006 [16] studies assessed the efficacy of trastuzumab given concurrently to adjuvant chemotherapy, while sequential schedules were investigated in the HERA [13], PACS-04 [17], and N9831 [18] trials, the last also including a sequential treatment arm.

NSABP B-31 and N9831 trials were the subjects of a planned joint analysis approved by the National cancer Institute and the FDA: data from both control groups were combined and compared to the trastuzumab concurrent arms combined together. After the release of the first interim analysis results, showing a significant improvement in disease-free survival (DFS) for the trastuzumab group, crossover from control group to trastuzumab therapy was allowed [19]. Despite a 21% crossover rate, the last analysis, at a median follow-up of 3.9 months, showed a significant outcome benefit for the trastuzumab group ($p<0.001$ for both DFS and OS) [15].

The BCIRG 006 trial randomized patients to receive four cycles of doxorubicin/cyclophosphamide and four cycles of docetaxel versus the same schedule plus 1-year trastuzumab starting concurrently to docetaxel versus six cycles of docetaxel plus carboplatin and 1-year trastuzumab starting concurrently. Data are available for the last update at a median follow-up of 65 months, for a total of 3222 patients. A significant benefit with respect to DFS and OS was seen in both groups treated with trastuzumab-containing regimens, as compared with the chemotherapy-only group. In contrast, no significant difference in DFS or OS was reported between the two trastuzumab-containing regimens. Among the two trastuzumab-containing arms, the rate of congestive heart failure (CHF) was significantly lower in the non-anthracycline group ($p<0.001$) [16].

In the FinHER study, 1010 patients were randomized to receive adjuvant docetaxel or vinorelbine followed by 5-fluorouracil, epirubicin, and cyclophosphamide (FEC). Patients with HER2 amplification ($n=232$) were further randomized to receive 9 weeks of concurrent trastuzumab or not. The last update of the study, at a median

Table 6.1 Efficacy results of trastuzumab adjuvant trials

Study	Follow-up (median)	DFS: HR (95 % CI), *p* value	OS: HR (95 % CI), *p* value
HERA [21]			
Observation vs 1 year sequential H ($n=3401$)	4 years	0·76 (NA), $p<0.0001$	0.76 (NA), $p=0.0005$
1 year sequential H vs 2 years sequential H ($n=3404$)	8 years	0.99 (0.85–1.14), $p=0.8588$	1.05 (0.86–1.28), $p=0.6333$
N9831-NSABP B31 [14]			
AC → T vs AC → T + H ($n=4045$)	3.9 years	0.52 (0.45–0.60), $p<0.001$	0.61 (0.50–0.75), $p<0.001$
N9831 [18]			
AC → T vs AC → T → H ($n=2184$)	6 years	0.69 (0.57–0.85), $p<0.001$	0.88 (0.67–1.15) $p=0.343$
AC → T → H vs AC → T + H ($n=1903$)	6 years	0.77 (0.53–1.11), $p=0.02$*	0.78 (0.58–1.05) $p=0.102$
BCIRG 006 [16]			
AC → D vs AC → D + H ($n=2147$)	5.4 years	0.64 (0.53–0.78), $p<0.001$	0.63 (0.48–0.81), $p<0.001$
AC → D vs DCaH ($n=2148$)	5.4 years	0.75 (0.63–0.90), $p=0.04$	0.77 (0.60–0.99), $p=0.04$
PACS-04 [17]			
FEC/ED vs FEC/ED → T ($n=528$)	3.9 years	0.86 (0.61–1.22), $p=0.41$	1.27 (0.68–2.38), p NR
FinHER [15]			
D → FEC vs D+H → FEC ($n=112$)	5.2 years	0.32 (0.12–0.89), $p=0.029$[a]	0.42 (0.13–1.33), $p=0.14$
PHARE [26]			
Neo/adj CHT + 6 months T vs Neo/Adj CHT + 1 year T ($n=3382$)	3.9 years	1.28[b] (1.05–1.56), $p=0.29$	1.47[b] (1.07–2.02), p NR

DFS disease-free survival, *HR* hazard ratio, *CI* confidence interval, *OS* overall survival, *HERA* Herceptin Adjuvant Trial, *NCCTG* North Central Cancer Treatment Group, *NSABP* National Surgical Adjuvant Breast and Bowel Project, *BCIRG* Breast Cancer International Research Group, *PACS* Programme d'Actions Concertées Sein, *FinHER* Finnish Herceptin, *H* trastuzumab, *AC* doxorubicin plus cyclophosphamide, *T* paclitaxel, *D* docetaxel, *Ca* carboplatin, *FEC* 5-fluorouracil plus epirubicin plus cyclophosphamide, *ED* epirubicin plus docetaxel, *CHT* chemotherapy, *NR* not reported, *Neo* neoadjuvant, *adj* adjuvant, *NA* not available

*boundary for significance preset at 0.00116

[a] distant disease-free survival

[b] noninferiority HR margin = 1.15

follow-up of 5 years, showed the benefits of adding a short course of concurrent trastuzumab to docetaxel followed by FEC versus chemotherapy alone (hazard ratio (HR) for distant disease recurrence 0.32, $p=0.029$). [14] However, due to the limited sample size, these results need to be confirmed in larger series.

The HERA trial randomized 5102 patients who completed at least four courses of adjuvant chemotherapy (anthracycline-taxane in 26 % of cases only) to receive trastuzumab for 1 year or 2 years or to observation. After the first interim analysis, patients in the control group were allowed to cross over to trastuzumab [20]. The last analysis, after a median follow-up of 8 years demonstrated a persistent significant benefit in DFS for 1 year of trastuzumab versus observation. Statistical significance was reached also for OS ($p=0.0005$) [21].

In the PACS-04 study, after a first randomization between anthracycline- and anthracycline/taxane-based chemotherapy, HER2-positive patients ($n=528$) were randomly assigned to sequential trastuzumab or observation. At a median follow-up of 4 years, the addition of sequential trastuzumab failed to detect a significant reduction in the risk of recurrence or death [17].

The only source for a direct comparison between the concurrent and the sequential strategy is the N9831 trial. In this study, patients received adjuvant doxorubicin/cyclophosphamide followed by paclitaxel alone versus paclitaxel plus 1-year trastuzumab starting sequentially versus concurrently. The sequential trastuzumab arm resulted in a 31 % reduction in the risk of recurrence ($p<0.001$), and in a 12 % reduction in the risk of death ($p=0.343$) as compared to chemotherapy alone. The 5-year DFS rate was higher, 84 versus 80 %, when trastuzumab was started concomitantly to paclitaxel instead of sequentially (HR 0.77, 99.9 % confidence interval (CI), 0.53–1.11, $p=0.0216$). Although the *p*-value did

not cross the prespecified boundary for interim analysis, a strong trend for a better outcome for concurrent trastuzumab has been defined [18].

To conclude, the inclusion of trastuzumab in the adjuvant therapy for HER2-positive BC has a clear impact on outcome and evidences suggest that this effect may be greater when the anti-HER2 agent is started during the chemotherapy treatment rather than sequentially.

Some challenges remain to be addressed. First of all, the majority of the patients enrolled in the adjuvant trastuzumab trials had tumor size more than 1 cm and nodal involvement. Recently, higher rates of recurrence for T1a-b, N0 HER2-positive tumors as compared to HER2-negative tumors have been demonstrated, thus suggesting that trastuzumab may play a role in the treatment of these patients [22]. However, since this population was not represented in the trials, we cannot draw conclusions on the optimal therapy for this subset. In a recent metanalysis of trastuzumab adjuvant pivotal trials, patients with small HER2-positive tumors (<=2 cm) seemed to derive benefit from the addition of trastuzumab to the standard chemotherapy backbone. However, the majority of patients presented stage pT1c and positive axillary nodes [23].

Some phase II, non-randomized trials have evaluated anthracycline-free taxane-based adjuvant regimens for patients with small HER2-positive BC. The APT study included 406 women with HER2-positive, node-negative, small (<3 cm) tumors. The trial was designed to evaluate a more tolerable chemotherapy regimen for patients with a limited burden of disease. Patients received paclitaxel 80 mg/m^2 plus trastuzumab 2 mg/kg for 12 weeks, followed by 9 months of trastuzumab alone at a dose of 6 mg/kg every 3 weeks. DFS at 3 years was 98.7%. By hormone receptor status, the DFS rates were 98.5% in receptor-positive patients and 99.2% in receptor-negative patients. However, this study has some limitations. It is a single-arm, nonrandomized trial, and about 20% of enrolled patients had T1a tumors that are already associated with a very favorable prognosis. Moreover, at the time of first data presentation, median follow-up was only 3.6 years [24].

Recently, a phase II study tested a combination of docetaxel and cyclophosphamide plus trastuzumab as adjuvant treatment for HER2-positive BC patients. This trial enrolled 493 patients and showed 3-years DFS, and OS rates were of 96.9 and 98.7%, respectively, with results being consistent with those obtained with longer-duration chemotherapy regimen, as reported in the major randomized trials [25].

Finally, the optimal trastuzumab duration has not been defined yet. Intriguingly, the data from the 2-year arm of HERA have been recently published, and apparently there is no difference between 1 year and 2 years of treatment in terms of efficacy. Cardiotoxicity was lower with shorter treatment duration [21]. At the same time, the results of the Phare trial, paring 6 months versus 1 year of adjuvant trastuzumab failed to show that 6 months of treatment with trastuzumab was noninferior to 12 months of trastuzumab. Despite the higher rates of cardiac events, 12 months of adjuvant trastuzmab should remain the standard of care [26]. Longer follow-up of the Phare trial and the results of other ongoing studies (the Sold trial in Finland and other North European countries, the Persephone trial in UK, and the Short-Her trial in Italy) will define the treatment duration with the most favorable therapeutic index.

Neoadjuvant

Trastuzumab has been tested in addition to neoadjuvant chemotherapy for HER2-positive stage II–IIIA patients in a phase III study. Patients were randomly assigned to paclitaxel followed by FEC or the same regimen plus weekly trastuzumab. This trial was stopped earlier by the Data Monitoring Committee after an extraordinary interim analysis showing clear superiority of the experimental arm. The addition of trastuzumab to chemotherapy induced more than doubled pathological complete responses (pCRs) as compared to chemotherapy alone (65.2 vs 26%, $p=0.016$) [27]. Another phase III trial (NOAH) evaluated the addition of trastuzumab to an anthracycline- and taxane-based chemotherapy for 334 HER2-positive patients with locally advanced breast cancer (LABC) or inflammatory BC. Event-free survival rate at 3 years was significantly better in

the chemotherapy plus trastuzumab arm (71 vs 56%; HR 0.59, $p=0.013$) as well as ORR and pCR (87 vs 74%, $p=0.009$ for ORR and 38 vs 19%, $p=0.001$ for pCR). [28] In the phase III GeparQuattro trial, 1509 patients with operable or locally advanced tumors were randomized to receive four cycles of epirubicin/cyclophosphamide followed by four cycles of docetaxel with or without capecitabine. The HER2-positive patients ($n=445$) also received trastuzumab. The pCR rate in the HER2-positive subset was 31.7%, whereas in the HER2-negative reference group it resulted in 15.7% [29].

Trastuzumab-related Cardiotoxicity

Trastuzumab regimens have been associated with an increase in both symptomatic and asymptomatic cardiac dysfunctions in the majority of large trastuzumab adjuvant trials. Although the mechanism underlying this toxicity is not completely known, HER2 is expressed on cardiac myocites and may be important for myocite repair, in particular after anthracycline exposure [30]. Symptomatic CHF occurred in 1.5–2.5% of the patients treated with sequential trastuzumab, and in 0.4–3.6% of the patients treated with concomitant chemotherapy and trastuzumab (the lowest observed BCIRG 006 arm C, not including anthracyclines). The FinHer trial is the only adjuvant trastuzumab trial without episodes of CHF [31]. As for the recently published metanalysis including eight randomized trials evaluating trastuzumab for early BC (EBC), the anti-HER2 compound significantly increases the risk of CHF (relative risk 5.11; 90% CI, 3.00 to 8.72, $p<0.00001$); and left ventricular ejection fraction decline (relative risk 1.83; 90% CI, 1.36 to 2.47, $p=0.0008$) [12]. Moreover, some data are emerging supporting the noncomplete reversibility of trastuzumab-related cardiac dysfunction [32]. In this context, data on BC populations treated outside clinical trials have also been produced. In a retrospective cohort study including 12,500 BC patients, the risk of heart failure and/or cardiomyopathy, as compared to no chemotherapy, was higher in patients treated with anthracycline alone (HR=1.40), although the increased risk was similar to other chemotherapy (HR=1.49). The risk was highly increased in patients treated with anthracycline plus trastuzumab (HR=7.19, 95% CI, 5–10.35). [33] Recently, a retrospective cohort study including 9535 patients at least 66 years old and diagnosed with stage I–III BC between 2005 and 2009, and treated with chemotherapy has been identified in the SEER-Medicare and in the Texas Cancer Registry–Medicare databases. Among trastuzumab users, the rate of cardiac heart failure was 29.4% (higher than those reported in pivotal trastuzumab adjuvant trials) compared with 18.9% in nontrastuzumab users ($p=0.001$). Other risk factors that were associated with a higher risk of developing cardiac heart failure were older age, comorbidities, hypertension, and treatment with anthracyclines [34]. Some strategies can be proposed to reduce cardiotoxicity rates. As already discussed, ongoing studies trying to define the optimal trastuzumab treatment duration are also investigating whether a shorter therapy may result in a decreased toxicity, as suggested by the results of the FinHER trial. Secondarily, chemotherapy plus trastuzumab regimens that do not contain anthracycline may be less cardiotoxic, as reported in the BCIRG 006 trial. Intriguingly, since a combined anti-HER2 and antiendocrine treatment has become an option for HER2+/HR+ advanced BC patients (discussed after), one may postulate that the same strategy might be applied even in the early setting. Finally, evidences are emerging suggesting that, in the context of trastuzumab-containing chemotherapy, by substituting standard anthracyclines with pegilated liposomal doxorubicin, a new drug formulation that may result in a more favorable drug distribution less affecting cardiac function, a less cardiotoxic effect can be achieved, without hampering treatment efficacy [35].

Lapatinib

Lapatinib is an orally available small molecule tyrosin-kinase inhibitor which acts against both HER2 and HER1, therefore suppressing their downstreaming pathways. In preclinical assays, it was shown to be active for trastuzumab-re-

sistant cancer cell lines, in particular for those expressing a truncated form of the HER2 receptor [36]. Moreover, other preclinical findings suggested that it could synergize with trastuzumab and could have a higher penetration through the blood-brain barrier [1]. Lapatinib induced only modest RR when used as a single agent for HER2-positive MBC [37, 38]. However, the large phase III registration trial was prematurely closed after an interim analysis revealed a 51% reduction in the risk of progression for capecitabine plus lapatinib as compared to capecitabine alone (HR 0.49; 95% CI, 0.34–0.71, $p<0.001$) for patients with HER2-positive disease refractory to an anthracycline, a taxane, and trastuzumab. [39] The updated efficacy analysis from 399 patients confirmed the benefit in time to progression ($p<0.001$) and in overall RR ($p=0.017$).[40]. As compared with capecitabine alone, lapatinib plus capecitabine was not associated with an increase in serious toxic effects, the most frequently reported being diarrhea, dehydration, and vomiting [41]. Asymptomatic cardiac events were identified in four women in the combination-therapy group and in one woman in the monotherapy group, whereas no symptomatic cardiac event was recorded [39] As a result, lapatinib has been approved in combination with capecitabine for patients with HER2-positive advanced disease with progression after prior anthracycline, taxane, and trastuzumab therapy. In 2011, the data of the CEREBEL study, comparing trastuzumab plus capecitabine versus lapatinib plus capecitabine as any-line therapy for HER2-positive MBC have been presented. The results, while being inconclusive with respect to the primary endpoint (central nervous system as the first site of relapse), showed a trend towards an inferior outcome in those patients treated with capecitabine/lapatinib for the intention-to-treat population. Interestingly, this difference was evident in the trastuzumab naïve group, but not in the trastuzumab-pretreated group [42]. Another trial compared paclitaxel plus lapatinib or placebo for the first-line treatment of HER2-positive MBC. The results showed a significant improvement in both OS and PFS for the experimental arm [43].

Two trials have been planned to assess the efficacy of lapatinib in the adjuvant setting. In the phase III randomized ALTTO trial, 8400 patients with HER2-positive EBC were randomized to chemotherapy plus trastuzumab (1 year), chemotherapy and lapatinib (for 1 year), chemotherapy plus trastuzumab (12 weeks), and lapatinib (34 weeks) administered either sequentially or concurrently. A pre-planned interim analysis in 2011 recommended the closure of the lapatinib alone arm as it was deemed unlikely to meet prespecified criteria to show noninferiority to trastuzumab alone [44]. Also the data from the adjuvant randomized lapatinib trial TEACH trial have been presented [45]. More than 3000 women who completed neoadjuvant or adjuvant chemotherapy without trastuzumab and were disease free were randomized to receive either placebo or lapatinib for 12 months. After a follow-up of 4 years, DFS events occurred in 13% of patients in the lapatinib arm and in 17% of patients in the placebo arm (HR = 0.83; p0.053). This modest improvement did not meet the prespecified criteria of success. Even in neoadjuvant trials, lapatinib-based chemotherapy regimens appear to be less effective than trastuzumab-containing schedules [46, 47].

Mechanisms of Resistance

Despite these advances, both de novo and acquired resistance to trastuzumab and/or lapatinib result in disease progression. Several mechanisms of trastuzumab resistance have been proposed and include: prevention of antibody-receptor binding by trastuzumab interaction with the membrane-associated glycoprotein MUC4 or by HER2 ECD shedding (leaving a truncated constitutively activated HER2 receptor form, p95), increased HER1 or HER3 expression, increased TGF-α expression (HER1 ligand). Resistance to trastuzumab may also derive from sustained downstream PI3K/Akt signaling, by means other than HER2 activation such as: *PIK3CA* or Akt mutations and/or PTEN loss and overexpression of insulin-like growth factor receptor-1 that trig-

gers the same intracellular pathway as HER2 [1]. Within these mechanisms, those involving the ECD shedding of HER2 and the upregulation of HER family members and their ligands are not supposed to affect lapatinib efficacy [1, 48]. To the opposite, those deriving from the downstream pathway members constitutive activation may confer resistance to lapatinib. However, recent evidences seem not to completely confirm this assumption [49, 50]. One possible mechanism of lapatinib resistance may derive from the upregulation of other survival pathways (such as estrogen receptor signaling) as a consequence of HER2 kinase activity inhibition [51]. Many attempts have been made to develop new drugs that may be active in trastuzumab/lapatinib resistant disease. Hereafter, the most advanced new anti-HER2 compounds will be discussed.

Pertuzumab

Pertuzumab is a first-in-class recombinant, humanized monoclonal antibody that binds to domain II of the HER2 receptor, thus inhibiting HER2 heterodimerization with HER1, HER3, and HER4. Phase II trial data reported only a modest activity for pertuzumab monotherapy in heavily pretreated HER2-positive MBC patients and in HER2-negative patients [52, 53]. However, pertuzumab combined with trastuzumab has shown an overall RR of 24% in 66 HER2-positive MBC patients who progressed under trastuzumab [54]. The first-line randomized phase III trial CLEOPATRA, including 808 HER2-positive MBC patients, reported a significant advantage in terms of PFS for the combination of trastuzumab, pertuzumab, and docetaxel as compared to the combination of trastuzumab and docetaxel (PFS 18.5 vs 12.4 months, HR 0.62; 95% CI, 0.51–0.75, $p<0.001$). A strong trend in prolongation of OS was also shown, based on an exploratory interim analysis. No increase in cardiotoxicity with the addition of pertuzumab was observed [55]. On the basis of these results, in June 2012, the FDA approved pertuzumab used in combination with trastuzumab and docetaxel for the treatment of patients with HER2-positive MBC who have not received prior anti-HER2 therapy or chemotherapy for metastatic disease [56].

Other trials for MBC are ongoing. In the MARIANNE study (NCT01120184), 1092 patients with recurrent locally advanced or previously untreated MBC are being randomly assigned to receive T-DM1 and pertuzumab, T-DM1 and pertuzumab-placebo, or trastuzumab and docetaxel. The PHEREXA trial (NCT01026142) will evaluate—in 450 patients with HER2-positive MBC whose disease has progressed following previous trastuzumab treatment—the combination of pertuzumab, trastuzumab, and capecitabine versus trastuzumab and capecitabine.

Trastuzumab-emtansine (T-DM1)

Recently, impressive results have been reported for T-DM1, a novel antibody-drug conjugate that uses trastuzumab to specifically deliver the cytotoxic maytansinoid antimicrotubule agent to HER2-positive cells. Activation of cytotoxicity of this conjugate requires internalization into the cell after binding to HER2. As monotherapy, it induced RRs ranging from 26 to 34.5% in heavily preatreated HER2-positive MBC patients [57, 58]. T-DM1 significantly improved PFS versus docetaxel plus herceptin as first-line treatment (median 14.2 vs 9.2 months, $p=0.0353$) in a phase II trial [59]. Toxicity profile favored T-DM1, with thrombocytemia and liver enzyme elevation as the most common T-DM1-related adverse events (AEs).

In February 2013, FDA approved TDM-1 for HER2-positive MBC as a single agent, for the treatment of patients with HER2-positive, MBC who previously received trastuzumab and a taxane, separately or in combination. Patients should have either received prior therapy for metastatic disease or developed disease recurrence during or within 6 months of completing adjuvant therapy [60]. The approval was based in large part on the results of the phase III EMILIA trial. In this trial, patients with HER2-positive advanced BC who were previously treated with trastuzumab and a taxane were randomized to

receive either T-DM1 or a regimen of lapatinib and capecitabine, a standard treatment regimen for HER2-positive BC. After a median follow-up of about 20 months, the study found that patients who received T-DM1 had a 32% reduced mortality risk compared with patients who received lapatinib and capecitabine (HR = 0.68; 95% CI, 0.55–0.85, $p < 0.001$). Median OS was 30.9 months for patients who received T-DM1 compared with 25.1 months for patients who received lapatinib and capecitabine [61].

The phase III trial TH3RESA study enrolled patients whose cancer was inoperable, or had recurred after several treatments including trastuzumab and lapatinib. A total of 602 patients were randomized to receive 3.6 mg/kg intravenous infusion of T-DM1 every 3 weeks or a treatment of their physician's choice (TPC). Results showed that median PFS increased by nearly 3 months from 3.3 for the TPC patients to 6.2 for patients receiving T-DM1. Among the T-DM1 patients, 31.3% showed a response to the drug, compared with 8.6% of the TPC patients. An interim analysis of OS showed a similar positive trend, but did not reach the level at which a statistically significant benefit for T-DM1 treatment could be confirmed. Generally, there were fewer serious adverse side effects in the T-DM1 patients than in the TPC group [62].

T-DM1 is also being tested both alone and in combination with pertuzumab in patients with previously untreated HER2-positive MBC in the MARIANNE trial (NCT01120184), still ongoing.

Novel Anti-HER2 Drugs

Neratinib

Neratinib/HKI-272 is an oral, irreversible, small molecule inhibitor of EGFR/HER1, HER2, and HER4. In an open-label, phase II study, patients with advanced HER2-positive BC with and without prior trastuzumab treatment received neratinib. The 16-week PFS was 59% for patients with prior trastuzumab ($n = 63$) and 78% for those without ($n = 64$); median PFS were 22.3 and 39.6 weeks, respectively. The most frequent AEs were diarrhea, nausea, vomiting, and fatigue. Grade 3 or 4 diarrhea occurred in 30% of patients with prior trastuzumab therapy, leading to neratinib dose reduction in 29% of this cohort [63]. A large randomized, double blind, placebo- controlled adjuvant study is ongoing. In this study, patients who have completed adjuvant trastuzumab no longer than 2 years before and free from recurrence will be randomly assigned to neratinib versus placebo for 1 year. (NCT00878709) A phase III randomized study (NCT00915018) of paclitaxel with either neratinib or trastuzumab is ongoing. Other hand, the results of a randomized phase II study (NCT00777101) of neratinib alone versus the combination of capecitabine and lapatinib for MBC are inconclusive since neither inferiority nor noninferiority of treatment with neratinib versus lapatinib plus capecitabine could be demonstrated. In both treatment arms, diarrhea was the most frequently reported treatment-related adverse event of any grade (neratinib, 85% vs lapatinib plus capecitabine, 68%; $p = 0.002$) and of grade 3/4 (28% vs 10%; $p < 0.001$), [64].

Targeted Agents Combinations Including an Anti-HER2 Drug

The combination of anti-HER2 compounds together with either another anti-HER2 agent or a compound directed against a different target has been evaluated as a mean to overcome resistance and enhance therapeutic efficacy.

Dual Anti-HER2 Blockade

Recent observations have opened new opportunities for anti-HER2 treatment with less chemotherapy. In particular, recent data support the feasibility and efficacy of dual HER2 blockade with the concomitant administration of two molecularly targeted agents without chemotherapy. One of the first evidences in this context came from the metastatic setting. In a randomized study, patients who experienced disease progression on prior trastuzumab received either trastu-

zumab plus lapatinib or single-agent lapatinib. Patients in the combination arm showed a more prolonged PFS (HR 0.73; 95% CI, 0.57–0.93, $p=0.008$). [65]

A growing body of knowledge on dual HER2 blockade is deriving from the neoadjuvant setting. Chang et al. recently reported that 12 weeks of preoperative trastuzumab plus lapatinib without chemotherapy can induce pCR in 28% of HER2-positive BC patients [66]. In the phase III NeoALLTO study, the combination of trastuzumab, lapatinib, and chemotherapy resulted in the highest pCR rate (51%) as compared to the addition of either trastuzumab (29.5%, $p<0.00001$) or lapatinib (24.7%) to chemotherapy [46]. The phase II randomized CHERLOB study evaluated the activity of neoadjuvant lapatinib, trastuzumab, or both in combination with an anthracycline-taxane sequential chemotherapy [67]. A total of 121 patients were enrolled. Consistently with the NeoALLTO data, the pCR rate was increased when the dual anti-HER2 blockade, rather than each single targeted agent was added to chemotherapy (46.7, 25, and 26.3% for the combination, trastuzumab, and lapatinib arms, respectively). Diarrhea and dermatologic and hepatic toxicities were observed more frequently in patients receiving lapatinib. No episodes of CHF were observed.

In the adjuvant setting, the ALLTO trial tested the combination of lapatinb plus trastuzumab following surgery among women with early-stage HER2-positive BC. 8381 women with HER2-positive early BC joined the study and underwent random assignment to one of four treatment arms: trastuzumab alone (2097 patients), lapatinib alone (2100 patients), trastuzumab followed by lapatinib (2091 patients), or trastuzumab plus lapatinib (2093 patients). All regimens were given for 1 year and incorporated chemotherapy as a backbone, which could be delivered either prior to or concurrently with anti-HER2 therapy. In August 2011, the lapatinib alone arm was closed at the request of the Independent Data Monitoring Committee because of futility. In a comparison of concurrently administered lapatinib and trastuzumab versus trastuzumab alone, the HR for DFS reached 0.84 (97.5% CI, 0.70–1.02) with a p-value of 0.048, which failed to meet the $p\leq 0.025$ threshold for establishing statistical superiority. Similarly, sequential use of trastuzumab and lapatinib did not meet the noninferiority criteria compared with trastuzumab alone based on a HR for DFS of 0.96 (97.5% CI, 0.80–1.15) and a p-value of 0.610, which also fell below the value of 0.025 required for significance [44].

Furthermore, the higher lapatinib-related toxicities limited the optimal administration of the drug: 20–40% of patients in the lapatinib arms did not receive at least 85% of the planned lapatinib dose. In conclusion, in the adjuvant setting, the combination of lapatinib plus trastuzumab increases the toxicity without a clinical benefit.

The combination of pertuzumab plus trastuzumab was also studied. In the phase II randomized NeoSphere trial, the addition of pertuzumab to docetaxel-trastuzumab based primary chemotherapy increased the pCR from 29 to 46%. Interestingly, those patients who received trastuzumab and pertuzumab only, without chemotherapy, experienced a 16.8% pCR rate [68]. Although the highest pCR rates were still obtained when chemotherapy was added, the treatment with two anti-HER2 agents alone may represent an option for those patients not fit for chemotherapy or at lower risk of relapse.

In September 2013 in the USA, pertuzumab was granted accelerated approval for use in combination with trastuzumab and docetaxel for neoadjuvant treatment of patients with HER2-positive locally advanced, inflammatory, or early-stage BC (either >2 cm in diameter or node-positive) as part of a complete treatment regimen for early BC, based on the results of the NeoSphere trial [56].

Continued approval for this indication is contingent upon demonstration of improvement in DFS in a confirmatory trial.

The use of pertuzumab as adjuvant treatment in combination with trastuzumab is being testing by Aphinity trial (NCT01358877), still ongoing. This is a randomized, double-blind, placebo-controlled, two-arm study will assess the safety and efficacy of pertuzumab in addition to chemotherapy plus trastuzumab as adjuvant therapy in patients with operable HER2-positive primary BC.

Anti-HER2 Agents Plus Endocrine Therapies

For those patients whose tumors overexpress both HER2 and estrogen receptor (ER), the concomitant targeting of these two receptors has become a viable option in the advanced setting.

The randomized phase II TAnDEM trial included 207 patients with known HER2-positive/ER-positive MBC and reported a doubling of PFS with the addition of trastuzumab over anastrozole alone 4.8 vs 2.4 months, HR 0.63; 95% CI, 0.47–0.84, $p=0.0016$) as first-line therapy [69]. Similarly, in another phase III trial, patients with known ER+/HER2+tumors ($n=219$), derived higher benefit from the combination of lapatinib and letrozole as compared to letrozole alone (PFS 8.2 vs 3.0 months, HR 0.71; 95% CI, 0.53–0.96, $p=0.019$) [70].

The next challenge should be to evaluate whether this strategy may be translated also in the adjuvant setting. Indeed, those HER2-positive/HR-positive patients with good prognostic features such as small, node-negative tumors might not need chemotherapy and might benefit from a combined targeted treatment.

Other Combinations

Since one of the proposed mechanisms of trastuzumab resistance is the activation of the PI3K/Akt pathway, and the mammalian target of rapamycin (mTOR) is a downstream component of this cascade, the association of trastuzumab and mTOR inhibitor is under investigation. The encouraging results that have been obtained for the combination of trastuzumab and everolimus in phase I and phase II trials [71, 72], prompted the design of ongoing randomized phase III trial evaluating the addition of everolimus to a trastuzumab-based chemotherapy for MBC (NCT00876395). Furthermore, in a randomized, double-blind, placebo-controlled, phase 3 trial (Bolero-3), women with HER2-positive, trastuzumab-resistant, advanced breast carcinoma who had previously received taxane therapy were randomized to daily everolimus (5 mg/day) plus weekly trastuzumab (2 mg/kg) and vinorelbine (25 mg/mq) or to placebo plus trastuzumab plus vinorelbine, in 3-week cycles. The addition of everolimus to trastuzumab plus vinorelbine significantly prolongs PFS in patients with trastuzumab-resistant and taxane-pretreated, HER2-positive, advanced BC (7 months vs 5.18 months). However, serious adverse events were reported in 42% patients in the everolimus group and 20% in the placebo group. Because of that, the clinical benefit should be considered in the context of the adverse event profile in this population [73].

HER2 signaling has been showed to induce *VEGF* transcription and inhibition of HER2 may result in an antiangiogenic effect [1]. The inclusion of the monoclonal anti-VEGF antibody bevacizumab in a trastuzumab-containing regimen resulted in some activity in phase II trials [74, 75]. A phase III trial, the AVEREL trial, tested bevacizumab in combination with trastuzumab and docetaxel in patients with HER2-positive MBC as first-line therapy. Combining bevacizumab with docetaxel and trastuzumab did not significantly improve PFS [76]. Another phase III trial, the BETH study (treatment of HER2-positive BC with chemotherapy plus trastuzumab vs chemotherapy plus trastuzumab plus bevacizumab) included 3509 women with HER2-positive BC who were either node-positive or high-risk node-negative, with the latter group representing 41% of the population. Patients were enrolled to receive six cycles of docetaxel/carboplatin plus trastuzumab (TCH) with or without bevacizumab ($n=3231$) or three cycles of docetaxel plus trastuzumab given with or without bevacizumab followed by three cycles of 5-fluorouracil, epirubicin, and cyclophosphamide ($n=278$). In both regimens, patients continued trastuzumab with or without bevacizumab after chemotherapy to complete 1 year of targeted therapy. For the primary outcome of the study, which was DFS, there was no statistically significant difference between the patients who received bevacizumab and those who did not [77]. The results of these trials clearly defined no role for bevacizumab in HER2-positive BC. One of the main problems with antiangiogenic strategies remain the lack of tools to predict sensitivity to these compounds

and research in this area is needed. Finally, several other agents are under evaluation in combination with trastuzumab and/or lapatinib, such as heat shock protein 90 inhibitors, various multi-tyrosine kinase inhibitors, HER2 protein-derived peptide vaccines and anti-IGFR1 drugs [1].

Anti-HER2 Blockade Beyond Progression

One of the major questions in anti-HER2 treatment is whether HER2 remains a viable potential target even in subsequent lines of therapy. Most of the data on this topic derives from retrospective analysis or prospective nonrandomized trials, and they overall suggest that targeting HER2 beyond progression is an effective strategy [78]. Recently, a phase III trial has addressed this issue. Patients who progressed on trastuzumab randomly received capecitabine with or without trastuzumab. Those patients who continued trastuzumab experienced a longer PFS (8.2 vs 5.6 months, $p = 0.03$) [79]. This is consistent with findings from other trials evaluating different anti-HER2-based regimens as ≥2 line treatment, such as capecitabine and lapatinib, lapatinib plus trastuzumab, pertuzumab plus trastuzumab and T-DM1 all suggesting that continued inhibition of HER2 is important in several lines of therapy [39, 62, 65, 73, 80].

Conclusions

Given that the pool of effective anti-HER2 treatments seems to constantly grow, an essential priority for future research should be to interpret the role of each single agent and combination at the best. This goes for the definition of the optimum strategies (concurrent multi HER2 blockade, sequential approach, with or without chemotherapy, etc.) to be applied in specific settings and time points of the disease history. At the same time, further research is needed to give a deeper insight into the biology of HER2 signaling and anti-HER2 resistance, in order to prevent or reverse therapy resistance. Indeed, although many mechanisms have been proposed, no tool that could be applied into the clinics has been identified so far.

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Role of Poly ADP-Ribose Polymerase (PARP) Inhibitors in Triple-Negative Breast Cancer (TNBC)

7

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Poly ADP-ribose Polymerase Inhibitors, DNA Damage Repair Dysfunction, and the Synthetic Lethality

The treatment with poly ADP-ribose polymerase (PARP) inhibitors represents a peculiar kind of targeted therapy, an innovative way that increases the complexity of the strategy to target cancer cells, not only by counteracting a druggable biomarker or an actionable pathway, but also by specifically destroying cancer cells harboring a relevant DNA damage repair (DDR) dysfunction through the inhibition of a different other pathway of DNA repair, thus realizing the synthetic lethality.

To date, PARP inhibitors showed activity in the treatment of metastatic ovarian cancer (MOC) and metastatic breast cancer (MBC) patients carrying *BRCA1* or *BRCA2* predisposing mutations. Phase III clinical trials justified their first indication for MOC patients as maintenance treatment after reaching a clinical response with re-challenge of platinum/paclitaxel association. Ongoing randomized clinical trials would evaluate the efficacy of PARP inhibitors as first-line treatment of MBC and, specifically, of triple-negative breast cancer (TNBC) patients. Moreover, treatment with PARP inhibitors could be more widely effective in cancers with DDR dysfunction, as we would be able to more specifically detect it at the somatic level than by the surrogate *BRCA1/BRCA2* germline analysis of cancer predisposition.

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DDR Dysfunction and Clinically Relevant Biomarkers

In normal cells, a complex architecture of DNA damage checkpoints and repair pathways counteract the continuous exogenous and endogenous acquisition of DNA alterations. In cancer cells, a partial or complete dysfunction of some of these machineries is prevalent, thus increasing

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instability [1]. Dysfunctions of the DDR are common in cancer and represent potential targets for innovative therapeutic drugs [2, 3].

Cancer predisposition determining approximately 5% of the most prevalent solid tumors depends on partial (heterozygous) inactivation of genes involved in DDR. *BRCA1* and *BRCA2* genes are specifically involved in double-strand breaks repair (DSBR) through homologous recombination (HR) and their heterozygous inactivation through point mutations and chromosomal rearrangements determine breast cancer (BC) and ovarian cancer (OC) predisposition. Heterozygous inactivation of different genes involved in mismatch repair (MMR) determines colorectal cancer predisposition and hereditary nonpolyposis colorectal cancer syndromes (HNPCC).

The detection of *BRCA1/BRCA2*-related predisposition represents a surrogate biomarker of DDR dysfunction of breast or OC cells, as well as the detection of MMR genes-related predisposition of colorectal cancer cells in HNPCC syndromes, because loss of the wild-type allele probably occurs in cancer cells.

In *BRCA1/BRCA2*-related cancers, and particularly in BC and OC, the availability of assays directly detecting DDR dysfunctions of cancer cells represents an unmet need in clinical practice, while DNA microsatellite instability, occurring in approximately 15% colorectal cancers, and loss of expression of MMR proteins represent assays to directly diagnose DDR dysfunctions of colorectal cancers.

Thus, the availability of DDR assays will expand clinical evaluation of drugs targeting DDR dysfunction, from more prevalent cancers with genetic predisposition to those harboring inactivation of DDR genes, such as *BRCA1* and *BRCA2*, at the somatic level, through genetic, epigenetic, and transcriptional alterations.

DDR Dysfunction and Synthetic Lethality Through PARP Inhibition

DNA DSBs are detected and repaired by nonhomologous end-joining (NHEJ) or HR. NHEJ functions preferentially during G0 and G1, whereas HR is prevalent during late S and G2 phase. PARP-1 activity is an important sensor to signal endogenous oxidative DNA lesions and DNA single-strand breaks (SSBs), or exogenous (radiation exposure, cytotoxic chemotherapy) DNA damage.

Targeting remaining operational DDR pathways, such as base excision repair (BER) through PARP inhibitors, may selectively kill cancer cells [3, 4]. PARP inhibitors target key enzymes involved in repairing SSBs in DNA, specifically when the preferred HR mechanism for repairing DSBs is lost due to *BRCA1/BRCA2* dysfunction. In preclinical studies, cancer cells with deficient BRCA1 activity showed hypersensitivity to PARP inhibition. Exposure of cycling cells to PARP-1 inhibitors increases SSBs which may cause replication fork collapse and DNA DSBs. DNA breaks arising during replication are preferentially repaired by HR, an accurate mechanism that maintains genomic integrity [5]. HR defects such as those occurring in cancers harboring *BRCA1/BRCA2* predisposition and in BRCA-ness cancer cells are extremely sensitive to inhibitors of PARP-dependent alternative repair pathways, through synthetic lethality [6–8].

Clinical Trials with PARP Inhibitors

After the demonstration of synthetic lethality in *BRCA2*-deficient cells with the use of two different PARP inhibitors [6, 7], the inhibition of PARP was considered as a potential synthetic lethal therapeutic strategy for the treatment of cancers with specific DNA-repair defects. A first-in-men phase I study was performed with a novel, orally active PARP inhibitor Olaparib (AZD2281; KU 0059436). It identified the maximal tolerated dose (MTD), 400 mg bid and included a cohort of patients bearing *BRCA1/BRCA2* mutations and OC [9]. Antitumor activity was observed only in carriers of *BRCA1* or *BRCA2* mutation, mostly OC patients. One out of three patients with *BRCA2* BC had a complete remission lasting for more than 60 weeks. Overall response rate (RR) was 28% (13/46 patients). Most common

toxicities were G1–2 nausea and fatigue. Thus, PARP inhibition to target a specific DNA-repair pathway was selective for *BRCA*-deficient cells, supporting at the clinical level that *BRCA* mutation-associated cancers are susceptible to a synthetic lethal therapeutic approach [10, 11]. This study raises the possibility to develop anticancer drugs, as targeted therapies for patients whose tumors have the same molecular defect but different origins, such as the ovary, breast, or prostate. It may be key to accelerating the development and evaluation at a clinical level of anticancer drugs in specific adaptive trials. In the expansion studies, only *BRCA1* or *BRCA2* carriers developing ovarian, breast, and prostate cancer were enrolled, at the phase II recommended dose of 400 mg twice daily.

At the 2009 ASCO meeting, the two proof-of-concept phase II single-arm studies (ICEBERG 1 and 2), with sequential cohort design, confirmed the efficacy and tolerability of oral Olaparib in *BRCA1/BRCA2* mutation carriers with advanced, refractory OC and BC (IIIB, IIIC, IV stage), after failure of > 1 prior chemotherapy for advanced disease, with 33 and 41 % activity, respectively, at the 400 mg/m^2 bid MTD [12, 13]. Clinical trials were developed in *BRCA*-deficient and potentially BRCA-ness or homologous recombination repair (HRR)-deficient cancers, such as TNBC or basal-like BC and serous OC. Olaparib was evaluated in sporadic cancers with a presumed BRCA-ness phenotype, in heavily pretreated high-grade serous or undifferentiated ovarian carcinomas and TNBCs [14]. Both *BRCA*-mutated and wild-type ovarian carcinoma patients showed activity, while neither *BRCA*-mutated nor sporadic BC patients.

Other PARP inhibitors have also been evaluated in clinical trials [15]. Rucaparib (CO-338/AG-014699, also previously PF-01367338) was recently evaluated in phase I and II studies, and it is under evaluation as monotherapy and in combinations with cytotoxic chemotherapy [16, 17]. Niraparib (MK4827), a PARP inhibitor acting by a novel PARP trapping mechanism [18, 19], at a maximum tolerated dose of 300 mg/day [20] was evaluated in both *BRCA*-positive and sporadic tumors. BMN 673, a novel, highly potent PARP 1/2 inhibitor, that elicits DNA repair biomarkers at much lower concentrations, demonstrated high efficacy in preclinical studies, and its antitumor activity has been tested *in vitro* and in xenograft cancer models, as monotherapy and in combination [21]. Antitumor activity was seen in BRCA1, BRCA2, and PTEN deficient cells with a 20 to > 200-fold greater potency than the other PARP 1/2 inhibitors. Synergism was also seen when BMN 673 was combined with temozolamide, SN38, or platinum drugs. Thus, BMN 673 seems to be the most specific PARP inhibitor in its class. Maximum tolerated dose was 1000 mcg daily. Thrombocytopenia was the dose-limiting toxicity. Objective responses were seen in 2/6 *BRCA*-mutated BC patients. It will be studied in a phase III trial in *BRCA*-carrier metastatic or locally advanced BC patients (NCT01945775).

PARP inhibitors have also been combined with chemotherapy in *BRCA* mutation-related malignancies. Olaparib was recommended at 400 mg twice daily for 14 days with carboplatin AUC 5. Prevalent grade 3/4 adverse events were neutropenia (42 %), thrombocytopenia (20 %), and anemia (13 %). Partial responses (PR) were observed in 6/8 BC patients (duration of response 5–24 + months). More recently, the therapeutic role of PARP inhibitors was highlighted in sporadic high-grade serous OCs and TNBCs, characterized by genomic similarities as showed in the TCGA network.

Triple-Negative Breast Cancer (TNBC) and DDR Dysfunction

The molecular landscape of BCs addressing therapeutic strategies currently discriminate the following phenotypes: estrogen receptor (ER) positive, human epidermal growth factor receptor-2 (HER2)-enriched, ER/HER2-positive, and triple-negative BC (TNBC). TNBC defines a clinical subgroup without specified molecular alterations, characterized by lack of expression of ER and progesterone receptor, and lack of expression/amplification of HER2. It comprises a heterogeneous subgroup of BC patients,

accounting for approximately 15% of all BCs [22], characterized by biological aggressiveness and poorer survival, compared to the other BC subgroups. TNBC patients do not require hormone therapy, nor anti-ERBB2-targeted agents. Thus, chemotherapy represents the treatment of choice for these patients, in metastatic, adjuvant, and neoadjuvant setting.

BCs related to *BRCA1* and *BRCA2* genetic predisposition, consisting of point mutations and chromosomal rearrangements, represent approximately 5% of all BCs [23]. In high-risk families, without detected *BRCA1/BRCA2* predisposing mutation, variants of unknown significance (VUS) abrogating *BRCA1/2* function due to splicing alterations may also be clinically relevant [24]. The development of TNBC is prevalent in *BRCA1* carriers, as indicated by microarray and IHC analyses [25–29]. ER-positive BC is prevalent in *BRCA2* carriers [30].

BRCA1-related BC and TNBC share common biological features such as high histologic grade, high proliferative rate (high expression of Ki-67), a pushing border of invasiveness, and central necrotic regions [30, 31], as well as mutated *p53*, and basal-like expression profiles [32]. Conversely, more than 10% of TNBCs consists of *BRCA1* carriers with BC positive family history, and much more TNBC patients (>50%), without *BRCA1*-related BC predisposition, harbor a BRCA1/2 inactivation at the somatic level ("BRCA-ness"), by different genetic and epigenetic mechanisms specifically affecting BC cells. Thus, DDR dysfunction is prevalent in BC, and specifically in TNBC, implying the potential effectiveness of targeted treatment with PARP inhibitors more widely in BRCA1/BRCA2-ness or DDR dysfunction, TNBC, and ER-positive BC. BRCA1 inactivation via somatic mutation or epigenetic mechanisms may promote genetic instability and tumor growth [33]. However, wild-type *BRCA1* TNBCs frequently exhibit a down-regulation of BRCA1 expression or alterations in BRCA1 function, through methylation of *BRCA1* promoter or over-expression of proteins that regulate BRCA1 expression [32–35].

Gene expression profiles (GEP) showed that TNBC overlaps substantially with basal-like tumors, representing a distinct molecular subtype [22, 25–27]. More recently, the "claudin low" subtype, enriched for stem cell markers and cells capable of forming new tumors, has been identified [36, 37]. Overall, it is estimated that 65–85% of TNBCs are basal-like subtype tumors [26, 38–40]. Some patients with basal-like tumors express non-TNBC markers (i.e., ER, PR, and HER2) and have a normal breast-like phenotype. In general, overall survival (OS) in patients with TNBC patients is poor compared with other BC phenotypes. The shortest survival times were seen in patients who have the basal-like and HER2-overexpressing subtypes [26, 27, 41].

Treatment options for patients with TNBC are limited to cytotoxic chemotherapy due to the lack of a molecular target [22, 42]. Findings from small clinical studies suggest that DNA-damaging agents may be useful in TNBC and BRCA1-related tumors, due to their inherent DNA repair dysfunction [43]. Numerous targeted agents are currently under clinical evaluation.

Clinical Trials with PARP Inhibitors in BC and TNBC

Based on promising preclinical findings, several agents with PARP inhibitory activity, including olaparib (AZD2281), iniparib (BSI-201), and veliparib (ABT-888), were under clinical evaluation for the treatment of TNBC patients, either as monotherapy or in combination with chemotherapeutic agents (Table 7.1). The efficacy of PARP inhibitors has been hypothesized in TNBC and basal-like BC, as well as in serous OC due to a BRCA-ness/DDR dysfunction. The first proof-of-concept phase II single-arm study, with sequential cohort design, was developed to assess the efficacy and tolerability of oral olaparib in *BRCA1/BRCA2* mutation carriers with advanced, refractory BC (IIIB, IIIC, IV stage), after failure of >1 prior chemotherapy for advanced disease, and confirmed 41% RR (11/27 patients) at the MTD with approximately 90% disease control rate, in both *BRCA1* and *BRCA2* carriers [13]. BC patients progressing to Olaparib were all

Table 7.1 Activity and efficacy of PARP inhibitors in the treatment of metastatic BC, in patients carrying or not BRCA1/2 predisposing mutations

Reference	Drugs	Phase	BC	BRCA1/2	ORR (%)	SD	PFS (m)
Fong, NEJM'09	O	I	9	3	1	1	
Tutt, Lancet'10	O	II	27	27	11 (41)		5.7
Gelmon, Lancet Onc'11	O	II	10		0	5	
Kaufman, ASCO'13	O	II	62		8 (13)	29	
Lee, ASCO'13	O/C	I	8		7	1	
Van der Noll, ASCO'13	O/C/P	I	23		–		
Liu, EJC'13	O/Ced	I	28	3	0		
Dent, BCR'13	O/P		19		7 (37)		
Sandhu, LancOnc'13	N	I	12	4	2		
De Bono, ASCO'13	B	I	8	6	2		
Kristeleit, ASCO'13	R	I–II	17	–	1		
Huggins, ASCO'13	V	I	12		1		
Ramaswami, ASCO'13	V/C	I	38	6	2	4	
Somlo, ASCO'13	V/C	I	28		12	7	7.8
Rodler, SABCS'11	V/P/Vi	I	18	5	3	7	
Tan, ASCO'13	V/Ci/D	II	11	3	2	6	
Isakoff, ASCO'13	V/TM	II	24	24	3		

PARP-inhibitors: *O* Olaparib, *N* Niraparib (MK4827), *B* BMN673, *R* Rucaparib, *V* veliparib, *C* carboplatin, *P* paclitaxel, *Vi* Vinorelbin, *Ci* Ciclophosphamide, *D* Doxorubicin, *TM* Temozolamide, *Ced* Cediranib

P-refractory. Limiting G3 toxicities were fatigue 15% and vomiting 11%. Among TNBC patients treated with olaparib in the pivotal trial, 7/13 (54%) in the 400-mg cohort and 4/16 (25%) in the 100-mg cohort had a response; median progression-free survival was 5.7 and 3.8 months, respectively [13]. No pCR were achieved in TNBC patients. No objective responses were reported in a second study proposing olaparib 400-mg monotherapy in 10 TNBC patients [14].

A phase II randomized study tested the PARP inhibitor BSI-201 (Iniparib) in combination with Carboplatin and Gemcitabine in TNBC (0–2 prior treatment) and preliminarily showed a significant increase of median OS up to 9.2 months (HR 0.35) that was not confirmed in a subsequent phase III study [44, 45]. Recently, it was proved that iniparib at physiologic concentrations is not a PARP inhibitor, and it causes telomere-centric DNA damage [46, 47]. A phase II trial presented at the ASCO meeting 2013 evaluated this combination in the neoadjuvant setting [48].

Olaparib (200 mg daily) was recently evaluated in a phase I/II study in combination with paclitaxel 90 mg/m^2 weekly for 3 of 4 weeks cycle, in the first or second-line setting for metastatic TNBC patients: PR were 37% [49].

PARP inhibitors have also been investigated as combination therapies with other novel targeted agents [50, 51]. Cediranib, an anti-angiogenetic agent, was studied with olaparib in recurrent epithelial ovarian or TNBCs (20 OC and 8 BC patients). Patients were enrolled at four dose levels. At the dosages of cediranib 30 mg daily and olaparib 400 mg twice daily, one grade 4 neutropenia ($\geq$4 days) and one grade 4 thrombocytopenia occurred, thus the phase II recommended doses were cediranib 30 mg daily and olaparib 200 mg twice daily. Grade 3–4 toxicities occurred in 75% of patients, with grade 3 hypertension and fatigue in 25 and 18%, respectively. Overall RR was 44% in the evaluable 18 OC patients, with 61% clinical benefit (including SD). No clinical responses were observed in BC patients, but two patients had SD for >24 weeks. Moreover, in a phase II trial of MBC, veliparib in combination with temozolomide showed activity in patients with BRCA-associated disease.

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Targeted Therapies in Squamous Cell Carcinoma of the Head and Neck

8

Pol Specenier

Head and neck cancer is the sixth most common cancer worldwide [1]. More than 90% of head and neck cancers are of squamous cell histology [2]. Globally about 650,000 new cases of squamous cell carcinoma of the head and neck (SCCHN) are diagnosed each year [1].

The management of SCCHN is complex and requires a multidisciplinary approach [3]. Single modality treatment with surgery or radiotherapy (RT) is generally recommended for the approximately 40% of patients who present with stage I or II disease [3]. For patients who present with locoregionally advanced (LA) disease at diagnosis, combined modality therapy is generally recommended [3]. For patients with unresectable disease and for patients with resectable disease in whom organ preservation is desired, the current standard treatment is concurrent cisplatin-based chemoradiation (CRT) [3]. The role of induction chemotherapy remains controversial [4, 5]. A significant majority of patients with LA-SCCHN at diagnosis will develop a (loco)regional recurrence or distant metastases. Patients with recurrent or metastatic (R/M) SCCHN have a poor prognosis with a median overall survival (OS) ranging between 6 and 9 months in most studies [6, 7]. A large number of targeted agents have been tested in the treatment of SCCHN [8, 9]. However, cetuximab is still the only targeted agent which has been approved for the treatment of SCCHN [10, 11].

EGFR-Directed Monoclonal Antibodies

Cetuximab is a chimeric monoclonal antibody of the immunoglobulin G1 class, which binds with high affinity to the extracellular domain of the human epidermal growth factor receptor (EGFR) [12].

The EGFR is expressed in the vast majority of SCCHN [13]. Overexpression of EGFR is an unfavorable prognostic factor in SCCHN [14]. The affinity for the EGFR is approximately five- to tenfold higher than that of the endogenous ligands [12].

Cetuximab blocks the binding of these ligands resulting in inhibition of the receptor function. Furthermore, cetuximab induces the internalization of the EGFR, which can lead to down-regulation of the EGFR. It also targets cytotoxic immune effector cells towards EGFR expressing tumor cells (antibody dependent cell-mediated cytotoxicity) [12]. In vitro exposure of squamous cell carcinoma (SCC) cell lines derived from head and neck cancer patients to cetuximab inhibits proliferation in a time-dependent manner [15].

Cetuximab is a potent radiosensitizer and acts synergistically with cisplatin in preclinical SCC models [16–18].

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Platinum-Refractory Disease

In patients with platinum-refractory disease, response rates ranging between 10 and 13 % were observed, irrespective of whether cetuximab was administered as a single agent or in combination with a platinum compound [19–22]. The median OS was about 6 months [19–22], which is longer than commonly observed in patients with platinum-refractory disease [23]. According to the summary of product characteristics, cetuximab is administered at a loading dose of 400 mg/m^2, followed by weekly administrations of 250 mg/m^2. Fury et al. [24] observed an overall response rate of 11 % with cetuximab 500 mg/m^2 administered every 2 weeks. Median OS was 7 months. Escalating the dose to 750 mg/m^2 every 2 weeks did not result in a better outcome [24].

The modest single-agent activity of EGFR-directed monoclonal antibodies in platinum-refractory SCCHN was confirmed in a randomized trial conducted by Machiels et al., although that trial failed to meet its primary endpoint [25]. Zalutumumab is a human immunoglobulin G1 (IgG1) EGFR-directed monoclonal antibody targeting. Machiels et al. [25] randomized 286 patients in a 2:1 ratio to receive either zalutumumab plus best supportive care (zalutumumab group) or best supportive care with optional methotrexate (control group). Eligible were patients with progressive disease according to the Response Evaluation Criteria in Solid Tumors (RECIST) during or within 6 months after the failure of platinum-based chemotherapy (at least two cycles of cisplatin (≥60 mg/m^2 per cycle) or carboplatin (≥250 mg/m^2 per cycle) with an interval between the cycles of <4 weeks) and patients with platinum intolerance which was defined as discontinuation or dose reduction of platinum-based chemotherapy due to adverse or toxic effects, irrespective of response. The dose of zalutumumab was titrated according to rash. Seventy-two percent of the control patients received methotrexate from the initiation of the trial, and a further 6 % started methotrexate during the trial. Median OS (primary endpoint) was 6.7 months (95 % CI: 5.8–7.0) in the zalutumumab group and 5.2 months (95 % CI: 4.1–6.4) in the control group (Hazard Ratio (HR) for death, stratified by the World Health Organization (WHO) performance status: 0.77; 97.06 % CI: 0.57–1.05) [25]. The difference missed statistical significance by a narrow margin (unadjusted $p=0.0648$) [25]. However, there was a statistically significant difference in progression-free survival (PFS), which was a secondary endpoint of the trial (HR for progression or death, stratified by WHO performance status: 0·63; 95 % CI 0·47–0·84; $p=0.0012$) [25]. The objective response rate with zalutumumab was 6.3 %.

First Line R/M SCCHN

In the Erbitux in First-Line Treatment of Recurrent or Metastatic Head and Neck Cancer (EXTREME) trial [26], patients which previously untreated R/M SCCHN were randomized to receive cisplatin (100 mg/m^2) or carboplatin (Area under the curve (AUC 5) on day 1, followed by 5-FU 1000 mg/m^2/day as a continuous infusion for 4 days, every 3 weeks for a maximum of six cycles or the same chemotherapy plus cetuximab at a loading dose of 400 mg/m^2 followed by weekly administrations at 250 mg/m^2 [26].

Patients with at least stable disease who received chemotherapy plus cetuximab continued to receive cetuximab until disease progression or unacceptable toxic effects, whichever occurred first. OS was the primary endpoint of the EXTREME trial. The median OS was 7.4 months in the chemotherapy-alone group and 10.1 months in the group that received chemotherapy plus cetuximab (HR for death: 0.80; 95 % CI: 0.64–0.99; $p=0.04$). The median PFS was 3.3 months with chemotherapy alone and 5.6 with the addition of cetuximab (HR for progression: 0.54; 95 % CI: 0.43–0.67; $p<0.001$). The overall response rate was 20 % (95 % CI: 15–25) and 36 % (95 % CI: 29–42), respectively ($p<0.001$) [26]. Expression of EGFR or tumor EGFR copy number was not predictive for the benefit from the addition of cetuximab [27]. Adding cetuximab to the chemotherapy improved survival, irrespective of tumor

p16 or the human papillomavirus (HPV) status [28]. p16 positivity and HPV positivity were associated with a better outcome in both arms [28]. The results of the EXTREME trial are supported by a trial conducted by the Eastern Cooperative Oncology Group (ECOG), also in previously untreated R/M SCCHN [29]. In that ECOG trial patients received cisplatin 100 mg/m^2 every 4 weeks with either weekly placebo (arm A) or weekly cetuximab (arm B). The primary endpoint of the trial was a PFS [29]. Median PFS was 2.7 months for arm B and 4.2 months for arm A. The HR for progression (primary endpoint) of arm A versus arm B was 0.78 (95% CI: 0.54–1.12; $p=0.09$). The 22% reduction in risk of progression was not significant in a study powered to detect a 50% reduction in hazard rates [29]. To detect a 2-month prolongation of median PFS from 2.7 months with 90% power, approximately 173 patients would have been required, rather than the 123 enrolled in the study. Median OS was 8.0 months for arm B and 9.2 months for arm A ($p=0.21$). The objective response rate was 26% for arm A and 10% for arm B ($p=0.03$) [29]. Although the ECOG study failed to meet its primary endpoint, the results were nevertheless in line with the results of the EXTREME trial [26, 29]. Although the SPECTRUM (Study of Panitumumab Efficacy in Patients with Recurrent and/or Metastatic Head and Neck Cancer) trial also failed to meet its primary endpoint, the outcome of this trial was not contradictory to the results of the EXTREME trial as the trend in the SPECTRUM trial was strongly in favor of the arm receiving panitumumab [30]. In the SPECTRUM trial [30], 657 patients were randomized to receive either cisplatin 100 mg/m^2 on day 1 followed by 5-FU 1000 mg/m^2 on days 1–4 of each cycle or the same regimen plus panitumumab at a dose of 9 mg/kg on day 1 of each cycle. Cycles were repeated every 3 weeks. Median OS was 11.1 months (95% CI 9.8–12.2) in the panitumumab group and 9.0 months (8.1–11.2) in the control group (HR: 0.873, 95% CI 0.729–1.046; $p=0.1403$) [30]. Median PFS was 5.8 months (95% CI 5.6–6.6) in the panitumumab group and 4.6 months (4.1–5.4) in the control group (HR: 0.780, 95% CI 0.659–0.922; $p=0.0036$) [30]. Median OS in patients with p16-negative tumors was longer in the panitumumab group than in the control group (11.7 months (95% CI 9.7–13.7) vs. 8.6 months (6.9–11.1); HR: 0.73 (95% CI 0.58–0.93); $p=0.0115$), whereas there was no difference in the p16-positive patients (11.0 months (7.3–12.9) vs. 12.6 months (7.7–17.4); 1.00 (0.62–1.61); $p=0.998$) [30].

Locoregionally Advanced Disease

Cetuximab was also tested in association with irradiation in patients with LA SCCHN. Bonner et al. [31, 32] randomized 424 patients with stage III or IV, nonmetastatic SCCHN to irradiation either alone or in combination with weekly cetuximab.

The median duration of locoregional control (primary endpoint) was 24.4 months in patients treated with cetuximab plus RT and 14.9 months in patients treated with RT alone (HR for locoregional progression or death: 0.68; $p=0.005$).

Median OS was 49.0 months and 29.3 months, respectively (HR for death: 0.73; $p=0.018$) [31, 32]. However, as no results of a randomized trial comparing cetuximab plus RT with cisplatin-based CRT have been reported thus far, cisplatin-based CRT is still to be considered the standard treatment for patients with LA-SCCHN. In a meta-analysis including data on 17,346 patients, the addition of chemotherapy to RT was associated with an absolute survival benefit of 6.5% at 5 years [33]. In contrast, the benefit of cetuximab has only been demonstrated in one single randomized trial involving 424 patients.

The addition of cetuximab to cisplatin-based CRT does not further improve the outcome. In Radiation Therapy Oncology Group (RTOG) 0522 [34], 895 evaluable patients with stage III or IV nonmetastatic SCCHN were randomized to receive either CRT (72 Gy in 42 fractions over 6 weeks plus cisplatin 100 mg/m^2 on days 1 and 22) or the same regimen plus weekly cetuximab. Over 90% of the patients in both arms received the planned two doses of cisplatin. The 2-year

PFS (primary endpoint) was 64.3% with CRT and 63.4% with CRT plus cetuximab (HR: 1.05; 95% CI: 0.84–1.29; $p=0.67$). The 2-year OS rates were 79.7 and 82.6%, respectively (HR: 0.87; 95% CI: 0.66–1.15; $p=0.17$). The estimated 2-year locoregional relapse rate was 19.8 and 24.5%, respectively ($p=0.92$). The 2-year distant metastasis rate was 12.0 and 7.6%, respectively ($p=0.07$) [34]. Overall, there was no difference regarding acute grade 3 or 4 toxicities between both arms. However, grade 3 or 4 mucositis (33% in CRT vs. 43% in CRT plus cetuximab) and in-field dermatitis (15% in CRT vs. 25% in CRT plus cetuximab) was more common in the cetuximab containing arm. Grade 3 or 4 dermatitis outside the radiation field occurred in 19% of the patients treated with cetuximab [34]. In a randomized phase II trial [35] in patients with stage nonmetastatic stage III or IV oropharyngeal cancer, maintenance weekly cetuximab for 12 weeks after bioradiation with cetuximab was associated with a statistically nonsignificant higher response rate at the first evaluation at 12 weeks after the end of RT (overall response rate: 96% (complete response rate: 65%) with the addition of cetuximab vs. 85% (complete response rate 56%) without cetuximab maintenance ($p=0.073$)) and a statistically nonsignificant higher 1-year locoregional control rate (59% vs. 47%; $p=0.25$). Moreover, the difference was entirely lost after 2 and 3 years [35].

EGFR Tyrosine Kinase Inhibitors

Gefitinib and erlotinib have shown single-agent activity in nonrandomized phase II trials [36–39]. However, the addition of gefitinib to single agent docetaxel did not improve OS in a randomized phase III trial conducted by Argiris et al. [40]. In that trial, patients received docetaxel 35 mg/m^2 on day 1, 8, and 15, every 28 days, either plus placebo or plus gefitinib 250 mg/day [40]. The data monitoring a committee recommended early stopping of enrollment after inclusion of 270 patients because there was <5% chance to meet the primary endpoint (improved OS). Eligible were patients who were previously treated with chemotherapy for R/M SCCHN (73% of the patients) and patients previously untreated for R/M SCCHN either with a poor performance status (ECOG 2) or in case of relapse within 6 months after chemotherapy given as part the primary treatment with curative intent. Median OS was 6.8 months with docetaxel plus placebo versus 6.2 months with docetaxel plus gefitinib (HR: 0.99; 95% CI: 0.75–1.31; $p=0.97$). An unplanned subset analysis showed that gefitinib improved the survival in patients younger than 65 years (median 7.6 vs. 5.2 months; $p=0.04$) [40]. The time to progression was significantly longer with the addition of gefitinib (median 3.5 months vs. 2.1 months; HR: 0.69; 95% CI: 0.49–0.99; $p=0.047$). In the Iressa Versus Methotrexate (IMEX) trial [41], 486 R/M SCCHN patients were randomly assigned to oral gefitinib 250 mg/day, gefitinib 500 mg/day, or methotrexate 40 mg/m^2 intravenously weekly. Physicians and patients were blinded to the gefitinib dose. Two coprimary analyses compared OS between each gefitinib dose and methotrexate. Patients were stratified into two groups: group A ($n=256$) consisted of patients who had stable or progressive disease after at least two cycles of platinum-based chemotherapy for recurrent disease; group B ($n=230$) consisted of patients who were considered unsuitable for platinum-containing chemotherapy. Neither gefitinib 250 mg/day nor gefitinib 500 mg/day improved OS compared with methotrexate (HR: 1.22; 95% CI: 0.95–1.57; $p=0.12$; HR: 1.12; 95% CI: 0.87–1.43; $p=0.39$, respectively). Median OS was 5.6, 6.0, and 6.7 months in the gefitinib 250 mg/day, gefitinib 500 mg/day, and methotrexate groups, respectively. In group A, OS was significantly longer with methotrexate (HR for death: gefitinib 250 mg vs. methotrexate: 1.62; $p=0.01$; gefitinib 500 mg vs. methotrexate: 1.5; $p=0.02$) [41].

The addition of erlotinib to standard CRT also failed to improve the complete response rate and PFS in a randomized phase II trial conducted by Martins et al. in patients with LA-SCCHN [42].

Conclusion

The EGFR-directed monoclonal antibody cetuximab is still the only targeted agent which has shown unequivocal activity in SCCHN, both in patients with R/M disease and in patients with locoregional advanced disease. Cetuximab has modest single-agent activity in patients with platinum-refractory R/M SCCHN and improves the OS when added to platinum-based chemotherapy in patients with previously untreated R/M disease. The addition of cetuximab to irradiation improves the locoregional disease control rate and OS in patients with LA-SCCHN. However, as no results of a randomized trial comparing cetuximab plus RT with cisplatin-based CRT have been reported thus far, cisplatin-based CRT is still to be considered the standard treatment for patients with LA-SCCHN.

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Targeted Therapies for Non-Small Cell Lung Cancer

9

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Introduction

Non-small cell lung cancer (NSCLC) represents about 75–80% of lung cancer cases, mostly detected as a metastatic disease. It is considered as an aggressive and heterogeneous disease, traditionally classified on a histological basis, into adenocarcinoma, squamous-cell carcinoma, and other nonspecified subtypes, which represent about 55, 35, and 10% of all NSCLC cases, respectively. Twenty years ago, very few treatments were available for lung cancer patients, so medium survival was about 2–4 months without any [1]. Introduction of platinum-based combinations with third generation agents such as gemcitabine, vinorelbine, and docetaxel, led to a significant improvement in both response rate (RR) and survival outcomes, which reached a "plateau" of about 15–20% and 10–11 months, respectively; so it is still considered the standard treatment of the majority of NSCLC patients [2]. Subsequently, the advent of new, more specific, cytotoxic drugs, such as pemetrexed, led to a further increase of survival, which reached about 12–13 months in nonsquamous cell carcinoma subtype [3], and even 14 months if consider also the maintenance treatment [4]. Furthermore, the addiction of antiangiogenic agents to chemotherapy, such as bevacizumab, has proven to be a valid strategy for treating NSCLC, since the process of angiogenesis is essential for the growth, survival, and metastasis of solid tumors. The past decade has witnessed a radical change in the treatment of lung cancer, thanks to the discovery of key-oncogene alterations, responsible for cancer cell proliferation and survival, and the subsequent clinical development of new targeted drugs, capable to inactivate them. The detection of sensitive epidermal growth factor receptor (EGFR) mutations and subsequent correlation with clinical responses to EGFR tyrosine-kinase inhibitors (TKIs) [5, 6], led to the approval of gefitinib, erlotinib, and more recently afatinib, as first line treatment of EGFR-mutated NSCLC patients. The detection of the EML4-ALK fusion gene in another subgroup of patients with NSCLC [7], was followed by the development of the ALK and ROS1 inhibitor, crizotinib, which

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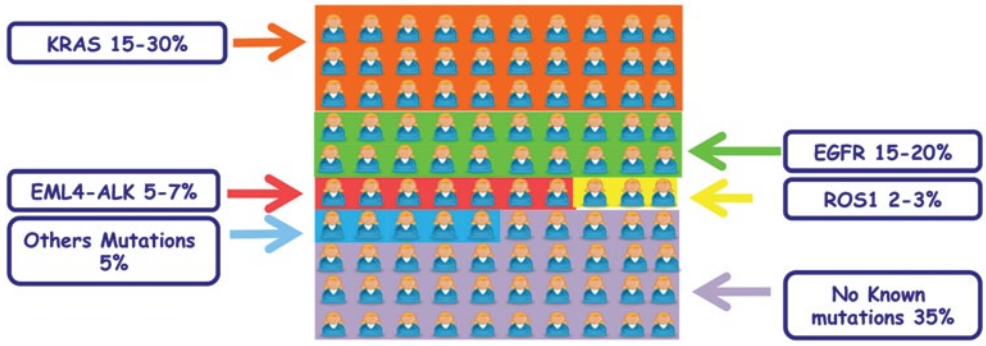

Fig. 9.1 Molecular subsets of non-squamous non-small cell lung cancer (*NSCLC*)

dramatically improved the clinical outcome of those patients. After the discovery of EGFR and ALK, and the subsequent development of the first targeted agents, several advances have been made in lung cancer translational research. The discovery of new oncogenic driver mutations, both in adenocarcinoma and squamous cell carcinoma, led to the shifting of lung cancer classification from histological to molecular basis (Fig. 9.1). Therefore, the tumor molecular profile is actually crucial in the selection of patients eligible for the new targeted therapies, leading to the establishment of new treatment algorithms, in order to provide the best treatment for each patient. Unfortunately, the percentage of patients for which a specific targeted therapy is available and whose choice is guided by the presence of the tumor molecular alterations are just about 20%, while for all the other ones, the choice among the different platinum-based combinations is still guided just by histotypes (Fig. 9.2).

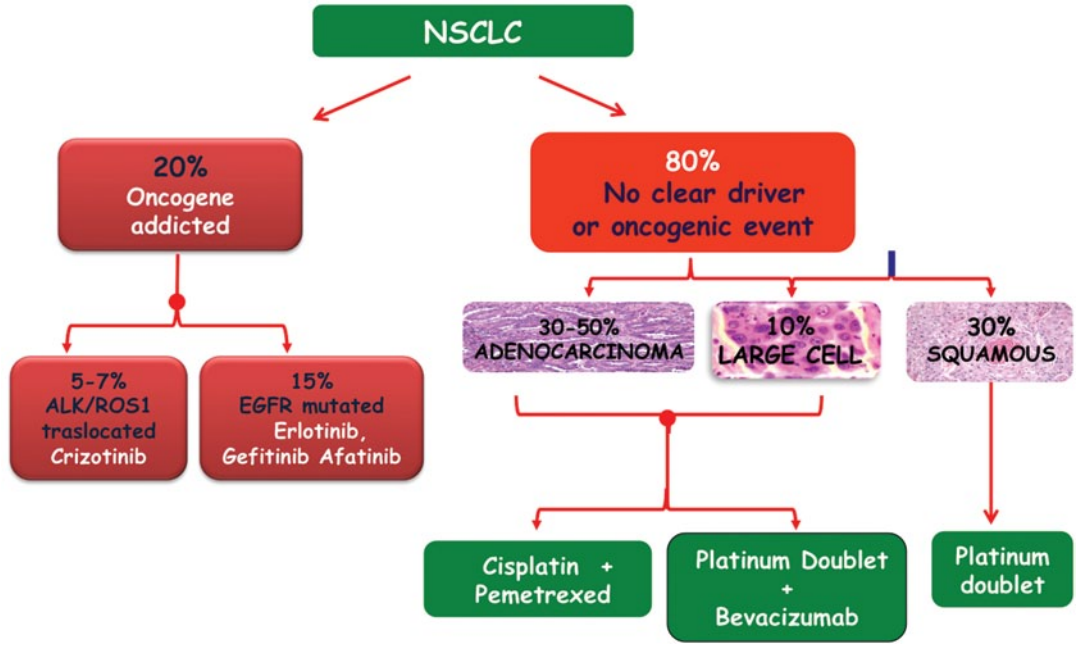

Fig. 9.2 Treatment algorithm in first-line metastatic NSCLC

EGFR-Tirosine-Kinase Inhibitors

EGFR mutations

EGFR is a trans-membrane protein with cytoplasmic kinase activity, which belongs to the HER/*erb*B family receptor tyrosine kinases, including HER1 (EGFR/*erb*B1), HER2-*neu* (*erb*B2), HER3 (*erb*B3), and HER 4 (*erb*B4). The interaction of EGFR extracellular domain with specific ligands induces a homo-dimerization (or hetero-dimerization with other HER family members) that causes the activation of the tyrosine kinase (TK) domain resulting in tyrosine autophosphorylation. Multiple signaling pathways are then activated, including RAS/RAF/ERK/MAPK and PI3K/AKT pathways. These pathways regulate several intracellular processes such as proliferation, invasion, cellular repair, protection from injury, and antiapoptosis [8, 9].

EGFR-activating mutations are the most frequent driver mutations in NSCLC, reported in about 40–60% of Asian [10–12], 15–20% of Caucasian [13, 14], and about 30% of Latin-American NSCLC patients [15], and are very important as clinical predictors of TKI sensitivity and efficacy, always taken into account in the selection of first-line treatment [16]. The most frequent mutations are exon19 deletions (over 20 variant types) and leucine-to-arginine mutation at codon 858 in exon21 (L858R), accounting for 90% of all EGFR mutations [9], but there are also some mutations with unknown biological and clinical significance (Fig. 9.3). Several cell-based studies demonstrated that these mutations increased the autophosphorylation of intracellular tyrosine residues with the subsequent activation of a subset of downstream effectors and leading to cellular proliferation, angiogenesis, tumor invasion, and metastatic potential. Following molecular

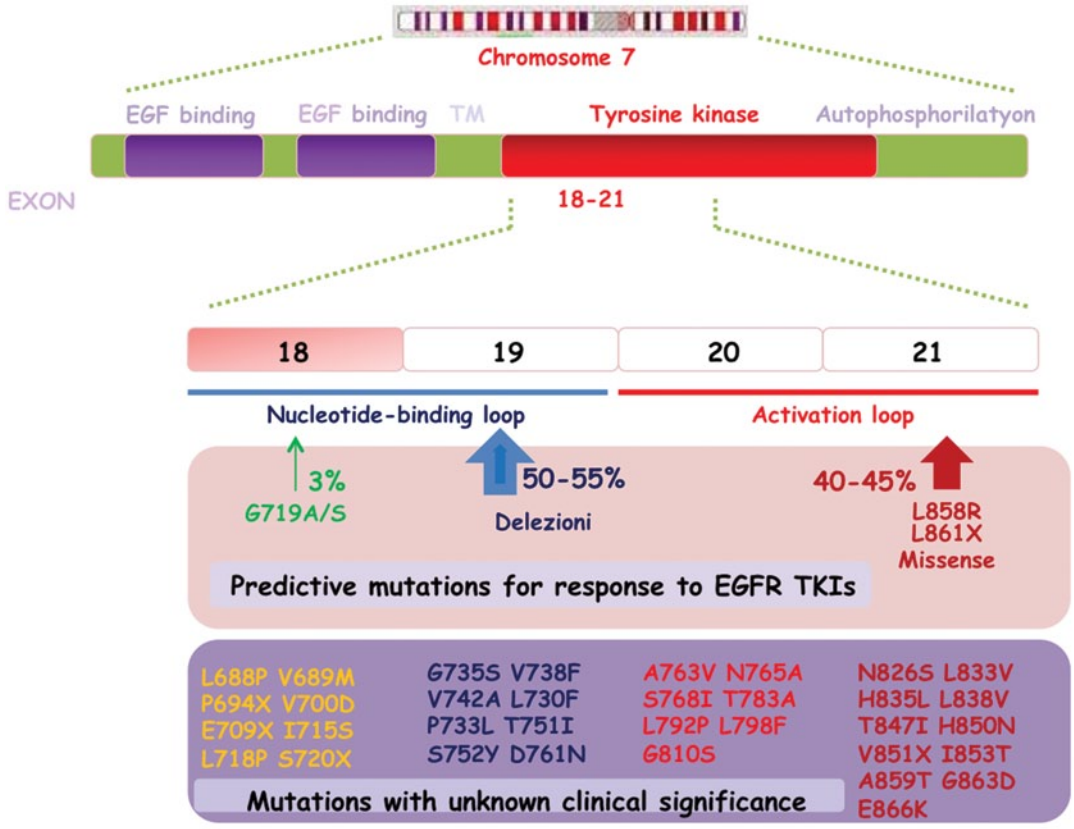

Fig. 9.3 Predictive epidermal growth factor receptor (*EGFR*)-sensitive mutations

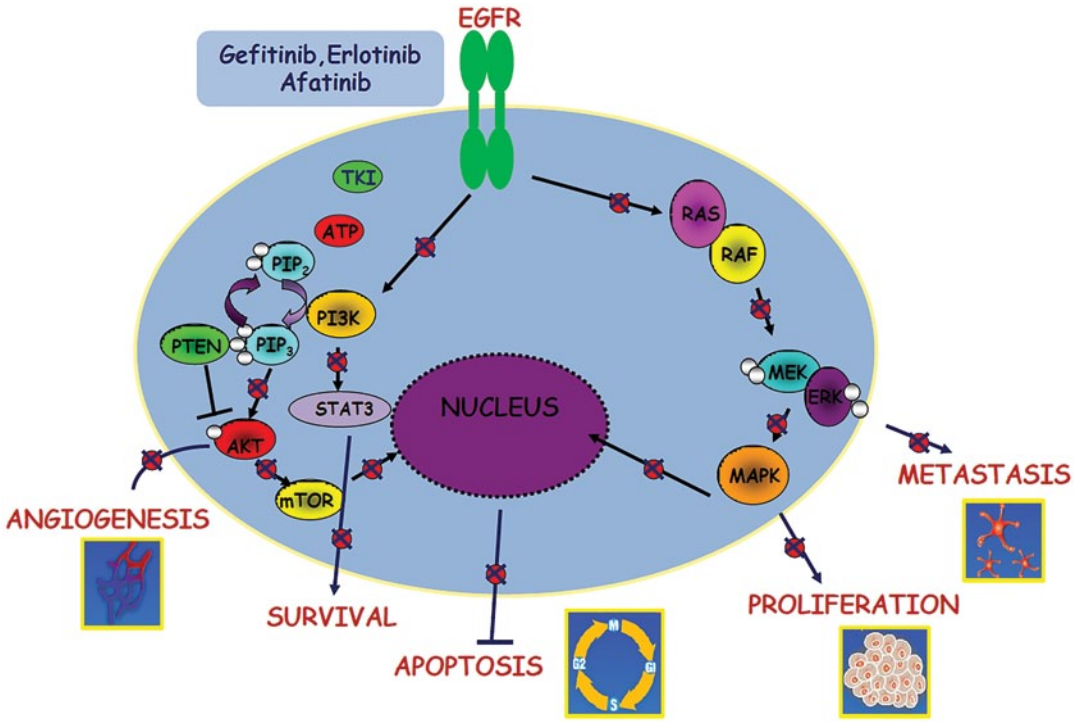

Fig. 9.4 EGFR molecular pathway and tyrosine-kinase inhibitors (*TKIs*)

interaction with the receptor, the small-molecule TKIs specifically inhibit EGFR phosphorylation and downstream signalling pathways (Fig. 9.4).

EGFR-Tirosine-Kinase Inhibitors (TKIs)

Gefitinib, erlotinib, and afatinib are orally active EGFR TKIs approved in first-line treatment of patients with advanced NSCLC whose tumor harboring EGFR-activating mutations [17]. Gefitinib has been approved only for EGFR-mutation-bearing patients, regardless the line of treatment, and is available as film-coated tablets that contain 250 mg of an active compound, taken once a day, while erlotinib is indicated both in the first-line treatment of mutated patients and in the second- or the third-line treatment of unselected patients, available in three dose-strength tablets: 25, 100, and 150 mg, whereas the recommended dose is 150 mg once a day. Finally, afatinib has been recently approved by the Food and Drug Administration (FDA) and the European Medical Agency as the first-line treatment of NSCLC patients with both activating common and uncommon EGFR-mutations, at dose of 40 mg once day. Gefitinib and erlotinib are reversible adenosine triphosphate (ATP) competitors at the ATP-binding pocket in the intracellular domain of EGFR. Afatinib is a novel irreversible, dual EGFR/HER2 inhibitor, which allows covalent modification of the ATP-binding site of the kinase domains of EGFR (Cys 773) and HER2 (Cys 805). Afatinib also shows an activity against T790M-clones, which often arise with acquired resistance to first-generation EGFR-TKIs [18]. There have now been eight separate clinical trials comparing front-line EGFR TKI treatment with standard platinum-chemotherapy in patients with EGFR-mutated NSCLC. All of these trials have shown that targeted drugs are better than standard chemotherapy in this molecular selected, patient population, leading to a significant improvement of survival, up to 24–30 months. Furthermore, the upfront treatment with EGFR-TKIs has been associated with a lower toxicity and a better quality of life in the overall patient population [12, 16, 19–24] (Table 9.1). Such

Table 9.1 Randomized studies comparing EGFR-TKI and chemotherapy in first-line treatment of NSCLC

Study	EGFR-TKI	N° $EGFR^{mut}$	RR (TKI vs. CT) (%)	Median PFS (TKI vs. CT) (months)	OS (TKI vs. CT) (months/HR)
First-signal	Gefitinib	27	84 vs. 37	8.4 vs. 6.7	30.6 vs. 26.5
IPASS	Gefitinib	132	71 vs. 47	9.8 vs. 6.4	21.6 vs. 21.9
NEJGSG002	Gefitinib	228	74 vs. 29	10.8 vs. 5.4	27.7 vs. 26.6
WJTOG3405	Gefitinib	172	62 vs. 32	9.2 vs. 6.3	NA
OPTIMAL	Erlotinib	154	83 vs. 36	13.7 vs. 4.6	NA
EURTACC	Erlotinib	173	58 vs. 15	9.7 vs. 5.2	NA
Lux-Lung 3	Afatinib	308	56 vs. 23	13.6 vs. 6.9	HR: 1.12
Lux-Lung 6	Afatinib	364	67 vs. 23	11 vs. 5.6	HR: 0.95

CT chemotherapy, *EGFR mut* epidermal growth factor receptor mutations, *HR* hazard ratio, *NA* not available, *OS* overall survival, *PFS* progression-free survival, *RR* response rate, *TKI* tirosin-kinase inhibitors

findings suggest that a molecular selection of the patients, to provide the best treatment available, may optimize their survival outcomes. On the basis of the evidence described above, the American Society of Clinical Oncology recommends EGFR mutation testing before the treatment with EGFR TKIs for the management of advanced lung cancer patients [25]. As emerging from clinical trials, EGFR-activating mutations are strongly associated with some clinical characteristics such as "women, non-smokers, Asian race and especially adenocarcinoma subtype" that could be considered as clinical predictive factors for TKI sensitivity. Nonetheless, the EGFR-activating mutations are the only, molecular predictive factors for TKI-sensitivity approved and considered in clinical practice. In summary, for front-line treatment of NSCLC, we may soon have three different drugs that are active in EGFR-mutated patients. The new question is how do we make a choice between these three different TKIs? Two randomized clinical trials are ongoing, comparing the new irreversible EGFR-TKI afatinib with both gefitinib (NCT01466660) and erlotinib (NCT01523587) in the treatment of EGFR-mutated NSCLC, but the results are not available yet.

EGFR-TKIs' Toxicity

TKIs have a good tolerability profile, with a significantly lower incidence of side effects traditionally associated with chemotherapy, such as myelosuppression, nausea, vomiting, fatigue, neurotoxicity, while they cause different kinds of toxicities, associated with the block of the EGFR pathway in healthy cells, such as skin rash, diarrhea, and asymptomatic hypertransaminasemia (mild to moderate), while severe toxicities are less frequently reported [16, 22, 23]. Skin rash is the most common adverse effect associated with TKIs, reported in more than 80% of patients treated with these drugs. It is characterized by a monomorphic papulopustular eruption often confined to seborrheic areas (mid-facial region and the upper trunk), which consists of erythematous follicular papules that evolve into pustules. As they are sometimes coalescent, they may form inflammatory plaques, which may become infected, usually with Staphylococcus aureus and form yellow crusts [26]. Although less than 20% of patients have severe symptoms [27], skin toxicity is visible and often causes physical and emotional discomfort, resulting in a significant impact on life quality [28]. However, data reported from several trials and included in a recent meta-analysis [29], showed a significant association between rash and clinical efficacy of treatment with EGFR-TKIs. Thus, rash can be considered an independent clinical predictor of effectiveness for TKI treatment, particularly for patients with EGFR unknown mutational status. Although the toxicity profile is almost comparable between the different EGFR-TKIs, the toxicity profile seems to be somewhat worse for afatinib than for erlotinib or gefitinib. For example, a higher rate of diarrhea, paronychia, and stomatitis are related to

afatinib, but the more accurate data will emerge from comparative trials. Management of low-grade diarrhea includes the use of loperamide, and sometimes of antibiotics, while rehydration, electrolyte replacement, and also hospitalization may be required for a very small proportion of patients.

ALK-Inhibitors

EML4-ALK Chromosome Rearrangement

EML4-ALK is a fusion protein between the N-terminal portion of the echinoderm microtubule-associated protein-like 4 (EML4) protein and the intracellular signaling portion of the anaplastic lymphoma kinase (ALK) tyrosine-kinase receptor. The EML4-ALK fusion gene has been recently identified by Soda et al. [7], and occurs in about 3–8 % of NSCLC patients [30]. The inversion of chromosome 2 leads to the fusion gene, and subsequently, a fusion protein that induces a constitutive activation of the intracellular domain of ALK receptor and downstream signaling pathways, such as Ras/MAPK, PI3K/Akt, and JACK/STAT3 pathways; therefore, a downstream cascade of events that lead to carcinogenesis [31]. Other fusion partners for *ALK* have been discovered in NSCLC, (such as TFG and KIF5B) [32–34], and multiple *EML4–ALK* variants have been identified [35], but their clinical significance still remains unknown. The clinical characteristics of the patients harboring these mutations and translocations are becoming apparent: They were often found in patients with adenocarcinomas, Asian ethnicity, generally never smokers or light smokers (less than 10 pack/year), but were also found, at a much lower rate, in squamous or adeno-squamous carcinomas and in smokers [30, 36]. Most of the cases do not carry other concomitant genetic abnormalities such as *EGFR or KRAS* mutations, but several simultaneously occurring *EGFR* and *KRAS* mutations in *ALK*-positive patients have been recently reported [37, 38]. These tumors have several histologic characteristics too. They tend to have a mucinous cribiform pattern in 56 % of the cases and as much as 43 % had a solid signet-ring pattern NSCLC [39]. However, there is nothing unique in the histology of these tumors that can erase the need for a fluorescent in situ hybridization (FISH) analysis for ALK translocations. FISH using break-apart probes still remains the standard and the only FDA-approved tool for testing for *EML-4/ALK* rearrangement [40]. Several studies showed a strong correlation between ALK-rearrangement positivity, as detected by FISH, and ALK protein overexpression, as detected by immunohistochemistry (IHC) [41–47]. These findings suggest that IHC could be used for screening of *ALK* rearrangements prior to FISH, leading to the development of new diagnostic algorithms, which need to be validated in large-scale concordance studies. Finally, *ALK* rearrangements define a new molecular subtype of NSCLC that is exquisitely sensitive to a new class of tailored agents, the ALK inhibitors.

EML-ALK Inhibitors: Crizotinib

Crizotinib is the first ALK-inhibitor approved for the treatment of ALK-positive NSCLC patients in progression after the first-line chemotherapy, at doses of 250 mg bid (500 mg/die) [17]. It is a potent oral ATP-competitor at the ATP-binding pocket in the intracellular tyrosine-kinase domain of ALK-receptor, with an additional anti-MET and anti-ROS1 activity. The first in human, phase I, dose-escalation trial, of crizotinib in 37, unselected patients, with advanced solid tumors, identified 250 mg twice daily as the recommended dose [48]. There were two patients with NSCLC harboring *EML4-ALK* rearrangement treated with crizotinib who showed dramatic improvement in their symptoms during the dose escalation phase. That observation led to a large prospective screening of NSCLC patients and recruitment of those with *ALK*-positive NSCLC into an expanded molecular cohort at the MTD of 250 mg twice daily. The updated results, reported by Camidge et al., including 149 previously

Table 9.2 Clinical trials with crizotinib in ALK-rearranged patients

Study	Phase	Patients included	ORR	PFS	Author
Profile 1001	Phase I	N: 149 pt (NSCLC; ALK+)	60.8% (95%CI:52.3–68.9)	9.7 months (95%CI:7.7–12.8)	Camidge 2012
Profile 1005	Phase II	N: 261 pt (NSCLC; ALK+)	60% (95%CI:53.6–65.9)	8.1 months (95% CI 6.8–9.7)	Kim 2012
Profile 1007	Phase III (CZT vs. P/D)	N: 346 pt (NSCLC; ALK+)	65% vs. 19.5% $p<0.0001$	7.7 vs. 3.0 month $p<0.0001$	Shaw 2013
Profile 1014	Phase III (CZT vs. C + P)	N: 343 pt (NSCLC; ALK+)	74% vs. 45% $p<0.0001$	10.9 vs. 7.0 month $p<0.0001$	Solomon 2014

C cisplatin or carboplatin, *CZT* crizotinib, *D* docetaxel, *NSCLC* non-small cell lung cancer, *ORR* overall response rate, *P* pemetrexed, *PFS* progression-free survival

treated and untreated, ALK+ NSCLC patients, showed an overall response rate (ORR) of 61% with a median duration of response of 49.1 weeks, and a median PFS of 9.7 months (95% CI, 7.7–12.8) [49]. The phase II trial (PROFILE1005) confirmed these striking results on 261 ALK+, pretreated, NSCLC patients. The ORR was 59.8%, with a median duration of response of 45.6 weeks, and a median PFS of 8.1 months (95% CI: 6.8, 9.7) [50]. On the basis of these impressive results, the FDA approved the use of crizotinib in October 2011, for the treatment of ALK+ advanced NSCLC. This granted approval without a phase III clinical trial is uncommon, but it was the result of the amazing data generated by this new compound in a population of patients that had a terrible prognosis. These promising results have been subsequently confirmed by two phase III, randomized trials, comparing crizotinib with standard of care both in second-line and first-line treatment, showing a great, significant improvement in RR, survival rate, and the quality of life in favor of crizotinib, for both pretreated and untreated, ALK-rearranged, NSCLC patients [51,52] (Table 9.2). Recently, another ALK-inhibitor, LDK378 (ceritinib), has been approved by FDA for the treatment of ALK-rearranged NSCLC patients who had progressed on or were intolerant to crizotinib, on the basis of the great activity showed in the recent, phase I trial by Shaw et al. [53].

ALK inhibitors Toxicitiy

Patients treated with crizotinib reported less toxicities, greater improvement in lung cancer symptoms, and greater improvement in global quality of life, when compared with chemotherapy [51]. Most treatment-related adverse events are visual effects such as visual impairment, photopsia, blurred vision, vitreous floaters, photophobia, and diplopia, often reported as flashes of light or trailing lights in the peripheral vision, overlapping shadows or after-images. Most commonly, they occur during adaptation to changes in lighting conditions, which are generally transient and diminish with the increasing number of treatment cycles, leaving patients' quality of life unaffected. Gastrointestinal events such as nausea, diarrhea, vomiting and constipation are generally mild, can be managed with a supportive care and tend to decrease in severity after the first few weeks of therapy. As the elevated liver enzymes are frequently observed (40–70%) with grade 3 in 7–15% of patients, their monitoring (along with the total bilirubin) is strongly recommended every 2 weeks of crizotinib therapy's duration. Peripheral edema, which is a common side effect, may be managed with standard medical intervention. Recent data from retrospective studies have shown that crizotinib may cause a decrease in the testosterone levels in male patients. Finally, 69% of patients experienced at least one episode of sinus bradycardia (HR ≤ 60bpm). Although as-

ymptomatic in all cases, it may sometimes cause a dizziness, hypotension or fatigue, which suggest electrocardiography (ECG) monitoring. As regards ceritinib, the most common CTCAE Grade 3–4 adverse reactions (≥5%) were diarrhea, fatigue, hyperglycemia, hypophosphatemia, increased transaminases and lipase levels, and anemia.

Overcoming Acquired Resistance: New Target Therapies

Mechanisms of EGFR-TKIs Resistance

Despite a great initial activity of first generation TKIs in molecularly defined, *EGFR*-mutated, NSCLC patients, acquired resistance frequently develops during the first year of treatment, leading to a disease progression and the subsequent discontinuation of the ongoing treatment (Fig. 9.5). Several mechanisms of resistance have been identified. Secondary mutation in the *EGFR* gene, most commonly the T790M mutation, is the leading cause of acquired resistance to first-generation EGFR-TKIs. It consists of a substitution of threonine with methionine in the point 790 of the peptidic sequence, with a subsequent steric hindrance in the ATP-binding pocket**,** so that the access of the drug is blocked [54]. It was detected in approximately 50–80% of patients who initially responded to gefitinib or erlotinib, but may also be presented at the beginning of the treatment, contributing to the primary resistance to EGFR-TKIs, characterized by shorter response duration in this subset of patients [55, 56]. In addition to the T790M mutation, there are other mechanisms involved in the development of acquired resistance, such as MET amplification (20%), HGF overexpression, Her-2 amplification (12%), PIK3CA mutation (5%), phenotypic changes in the tumor, like small-cell lung cancer (SCLC) trasformation (4%), and modifications in other signaling pathways [57]. Hence, novel agents are needed to overcome these resistance mechanisms.

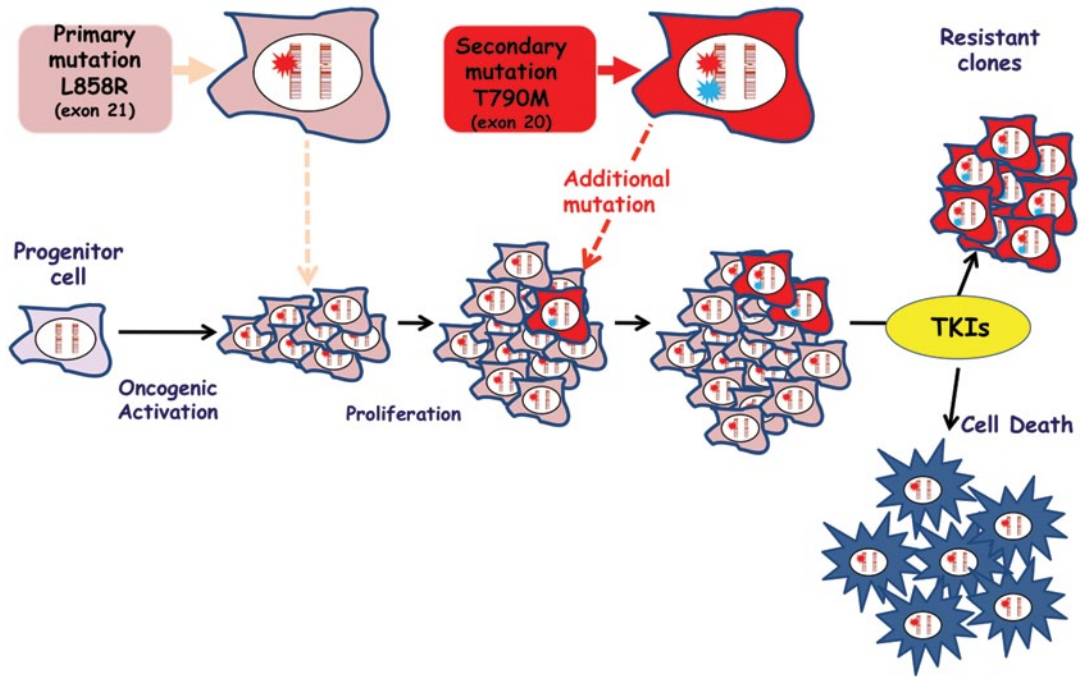

Fig. 9.5 Acquired resistance to EGFR-TKIs

New EGFR Inhibitors

Several next generation EGFR-inhibitors have been developed in order to overcome EGFR-T790M-related resistance to first-generation TKIs, and are currently under investigation in early, ongoing phase I/II clinical trials. Although afatinib was already approved for the first-line treatment of EGFR-mutated NSCLC, it has also been investigated as second- or third-line treatment, in patients previously treated with first generation EGFR TKIs, gefitinib or erlotinib, due to its peculiar activity against the T790M-clones [18]. When used as monotherapy, it has shown limited efficacy in this subset of patients (RR: 7–8%) [58]. Otherwise, the combination of afatinib with cetuximab, was associated with a disease control in all patients ($n=22$), and confirmed partial response (PR) in eight patients (36%), including four patients with T790M mutation [59]. Another EGFR, HER2, and ErbB4 inhibitor, Dacomitinib, was clinically active in a phase II trial, in patients with advanced NSCLC, who had failed one or two prior chemotherapy regimens and prior erlotinib treatment [60], but unfortunately the subsequent phase III, NCIC CTG BR.26 trial, had failed. It did not meet its primary endpoint of prolonging OS versus placebo. XL647 is an oral, small-molecule inhibitor of multiple receptor tyrosine kinases (RTKs), including EGFR, VEGFR2, HER2, and Ephrin type-B receptor 4 (EphB4). XL647, administered in an intermittent or daily-dosing schedule, exhibited antitumor activity with an overall response rate (ORR) of 3% in TKI-resistant patients selected for *EGFR*-activating mutations [61]. A novel class of nonquinazoline, oral, irreversible inhibitors of EGFR, have been recently developed, to specifically target T790M mutation. CO1686 and AZD9291, were associated with 67% and about 50% RR, respectively, in a population of NSCLC patients, progressed on first generation TKI therapy, whose tumors harbor the T790M mutation [62, 63]. Furthermore, both the new agents have shown a good tolerability profile, with a minimal incidence of grade 3/4 adverse effects, probably due to the higher selectivity of their target, compared with the other EGFR-TKIs.

Mechanisms of Crizotinib Resistance

Unfortunately, PFS in patients on crizotinib are short-lived despite of a great clinical and radiographic responses. Ultimately, these NSCLC harboring the EML4-ALK translocation become resistant to crizotinib. Mechanisms of acquired resistance to crizotinib may be divided into two groups. The first one includes additional genetic alterations in the target, such as secondary mutations of the ALK kinase domain or amplification of the *ALK* fusion gene [64, 65], responsible for about 30% of acquired resistance to crizotinib. The most common and well-characterized mutation is the L1196M mutation [66], consisting of a substitution of methionine for leucine in the "gatekeeper" residue, promoting the active conformation of the protein and leading to an increased protein kinase activity [67]. Other resistance mutations include: G1269A, C1156Y, L1152R, G1202R, S1206Y, and 1151Tins. The second group includes the activation of other oncogenic drivers, such as, *KRAS* mutations, *KIT* amplification and increased EGFR autophosphorylation and mutations, which may cause resistance through reactivation of downstream signaling pathways via bypass tracts, independently of ALK genetic alterations [64, 65], suggesting the need of combination therapies. Finally, the specific mechanism of acquired resistance development during crizotinib treatment remains still unknown in about 20% of patients.

New ALK Inhibitors

A new generation of ALK inhibitors has been developed, showing promising results in early clinical studies. LDK378 (ceritinib) is the novel, potent, and selective ALK-inhibitor, recently approved by the FDA for the treatment of ALK-rearranged NSCLC patients who had progressed on or were intolerant to crizotinib. A phase I study was conducted in 163 patients with metastatic, ALK-positive, NSCLC who had progressed on or were intolerant to crizotinib, showing about 60% of responses, with a median progression-free survival of 7.0 months (95% CI, 5.6–9.5)

[53]. Several phase II and III studies are currently investigating the activity of this new compound ALK-rearranged NSCLC. AP26113 is a novel, synthetic, orally-active TKI which potently inhibits mutant-activated forms of ALK and EGFR, including the gatekeeper mutation *L1196M* and T790M [60–61]. Preliminary data available from a phase I/II ongoing trial of AP26113 in *ALK*-positive patients, have shown a great activity and a good tolerability profile of this compound, both in crizotinib naïve (RR: 50%) and in crizotinib-resistant patients (RR: 76%) [68]. The planned phase II expansion will include five cohorts including *ALK*-positive NSCLC patients who are naïve or resistant to prior ALK-targeted therapy, also *EGFR*-mutated NSCLC patients who are resistant to EGFR-targeted therapy, other cancers with abnormalities in the ALK gene or other AP26113 targets, and finally an ALK+, brain metastasis, dedicated cohort. This study is currently recruiting patients [63]. CH5424802 is a potent, selective oral ALK inhibitor with preferential antitumor activity against NSCLC cells expressing *EML4/ALK* fusion, including also the L1196M gatekeeper mutation and C1156Y mutation [69]. CH5424802 has shown a great activity and a good tolerability profile in two early clinical trials. The reported ORR was 73.3% and 82%, in crizotinib naïve and in crizotinib pretreated, ALK+, NSCLC patients, respectively [70, 71]. No treatment-related adverse events (AEs) led to dose reductions. Main treatment-related AEs were liver function test abnormalities, dysgeusia, rash, nausea, and myalgia, most of them grade 1 except for neutropenia.

Conclusions

The clinical development of target therapy has been an amazing success story in lung cancer translational research, leading to a radical change in the treatment of NSCLC. Both EGFR-TKIs gefitinib, erlotinib, and afatinib, and ALK-inhibitors crizotinib and ceritinib, have shown to be more effective and better tolerated than cytotoxic drugs in a subgroup of molecular selected, NSCLC patients, leading a significant improvement of their RR, median survival and QoL, and marking the beginning of a new era in NSCLC treatment, characterized by new ethical and scientific considerations (Fig. 9.6). Thanks to the advances made in translational research, the number of biomarkers in NSCLC is rapidly increasing, and a lot of new molecules are currently undergoing investigation in early clinical trials. The future is very promising: a growing number of target agents will be available for clinical use, and new genetic-technologies like the "next-generation-sequencing," will make possible to create a molecular-genomic profile of every patient's tumor, based on the analysis of either a single tissue sample, or circulating tumor cells, or circulating tumor DNA, leading to new fascinating chances for a personalized treatment in overall NSCLC population. Unfortunately, despite the promising activity showed in clinical setting, acquired resistance to new targeted agents inevitably develops during treatment, leading to a clinical progression of the disease and the discontinuation of the ongoing treatment. As acquired resistance appears to be pleomorphic,

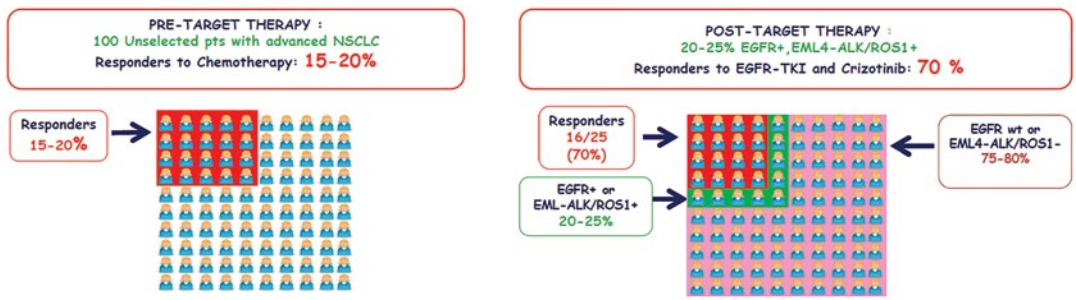

Fig. 9.6 Evolution of response rate in advanced NSCLC

a deeper understanding of the specific genetic alterations of tumor cells occurring at the time of disease progression is crucial in order to lead the decision making of subsequent treatments. Elucidating acquired resistance mechanisms and developing adequate therapeutic strategies such as the optimal sequence of treatment and the best combination regimens are crucial questions to be answered by dedicated translational research studies. Only a close collaboration between oncologists, pathologists, and molecular biologists may help to find the right answers in an efficient and timely fashion.

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Targeted Therapies for Gastric Cancer

10

Nishi Kothari and Khaldoun Almhanna

Introduction

Gastric cancer (GC) is the 14th most common cancer in the USA. In 2014, an estimated 22,220 new cases will be diagnosed and 10,990 patients will die from the disease in the USA alone [1, 2]. Patients in the USA often present with advanced stage, partially due to the nonspecific early symptoms of the disease as well as the absence of screening guidelines. Survival of patients with GC has improved only modestly over the last 50 years despite considerable improvement in diagnosis, surgical techniques, and multidisciplinary approaches to care. The 5-year overall survival (OS) rate for advanced GC remains between 5 and 15%. Surgical resection is the only potentially curative treatment. However, 5-year survival after R0 resection remains very low as between 40 and 60% of patients develop recurrent disease.

Chemotherapy persists as the cornerstone of treatment for patients with metastatic disease. Many chemotherapeutic agents are active in this disease including platinums, irinotecan, fluorouracil, taxans, and epirubicin. Despite the variety of agents, median survival for metastatic disease remains between 8 and 10 months. Treatment with a combination of three agents has been shown to lead to modest improvements in survival compared to two agents, but at the expense of significant toxicity [3].

During recent years, the underlying molecular heterogeneity, underlying GC carcinogenesis, and progression have been described. Our improved understanding of disease biology has stimulated the search for novel therapeutic approaches. The development of new agents to be combined with cytotoxic treatment is an urgent priority.

Targeted therapy has emerged as a new strategy to improve outcomes in several malignancies including colon, lung, and breast cancer among others. Molecules related to cell proliferation, invasion, and tumor metastasis have been studied in GC. Agents targeting these molecules have been evaluated in the preclinical setting and are rapidly moving to clinical trials. The vascular endothelial growth factor (VEGF) receptor, epidermal growth factor receptor (EGFR), human epidermal growth factor type 2 (HER2), insulin-like growth factor receptor (IGF-R), P13k/Akt/mTor pathway, c-Met, and fibroblast growth factor receptor (FGFR) have all been investigated as potential targets.

In this chapter, we will discuss these molecular targets and the novel drugs currently in development for patients with GC from bench to clinical practice.

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Molecular Targets in Gastric Cancer

Pathogenesis of GC involves multiple genetic and epigenetic alterations, chromosomal aberrations, gene mutations, and altered molecular pathways. Some of these molecular abnormalities and signaling pathways are amenable to pharmacological interventions (Fig. 10.1). Multiple agents targeting these pathways are now in clinical development and are being tested in patients with GC (Table 10.1).

Cell Surface Receptor Inhibitors

Human Epidermal Growth Factor Type 2 (HER2) Inhibition

HER2 is a member of the EGFR/HER family, which is composed of HER1, HER2, HER3, and HER4. The HER2 gene is a proto-oncogene located at the long arm of human chromosome 17 [4], which encodes for a 185-kd transmembrane glycoprotein receptor with intracellular tyrosine kinase activity [5].

The HER2 receptor is involved in signal transduction, which leads to cell growth and differentiation. None of the epidermal growth factor (EGF) family of ligands is known to directly activate HER2, however, HER2 is the preferenw-

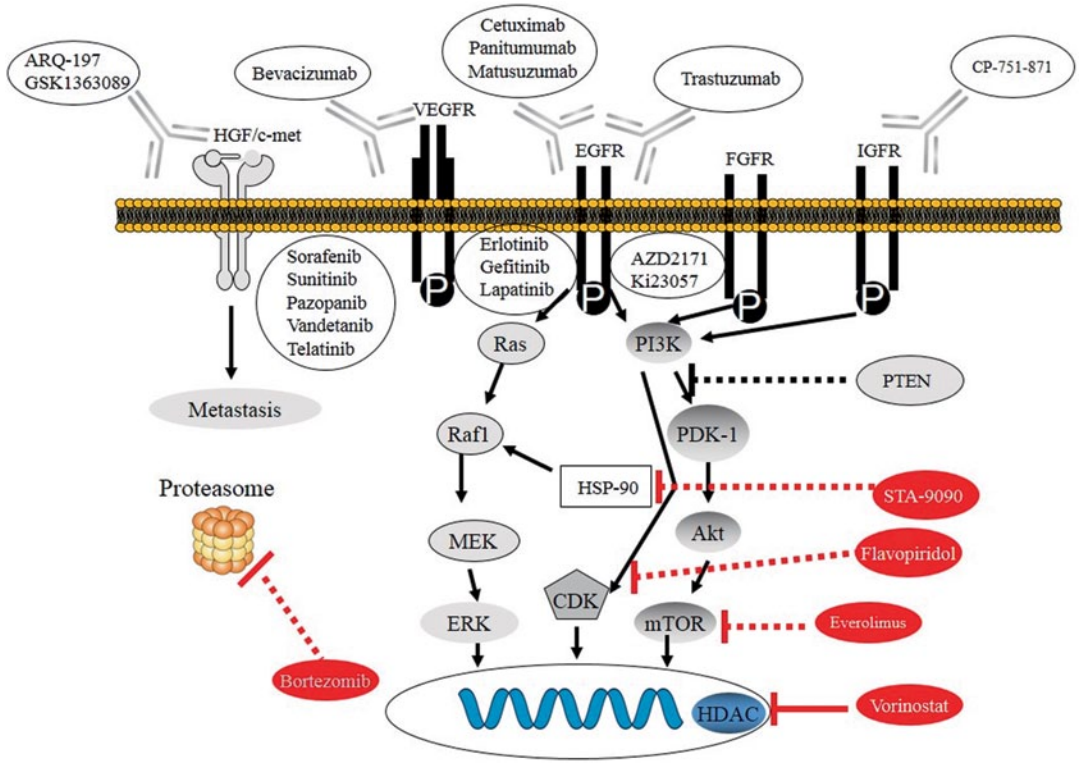

Fig. 10.1 Targeted therapy in gastric cancer and sites of action. Akt protein kinase B, *CDK* cyclin-dependent kinases, *EGFR* epidermal growth factor receptor, *ERK* extracellular signal-regulated kinase, *FGFR* fiborblast growth factor receptor, *HDAC* histone deacetylases, *HGF* hepatocyte growth factor, HSP-90 heat shock protein-90, *IGFR* insulin growth factor receptor, *MAPK* mitogen-activated protein kinase, *MEK MAP* kinase kinase, *mTOR* mammalian target of rapamycin, *PDK-1* pyruvate dehydrogenase lipoamide kinase isozyme 1, *PI3K* phosphatadylinositol 3-kinase, *PTEN* phosphatase and tensin homolog, *VEGFR* vascular endothelial cell growth factor receptor

Table 10.1 Targeted agents and clinical trials for gastric and gastroesophageal cancer

Drugs and their targets	Agents	Clinical trials
VGFR inhibitors		
Monoclonal antibody	Bevacizumab	Phase III
Receptor tyrosine kinase	Sunitinib	Phase II
	Sorafenib	Phase I/II
	Pazopanib	Phase II
	Vandetanib	Phase I/II
	Telatinib	Phase II
EGFR inhibitors		
Monoclonal antibody	Cetuximab	Phase III
	Panitumumab	Phase III
	Matuzumab	Phase I/II
Receptor tyrosine kinase	Gefitinib	Phase II
	Erlotinib	Phase II
HER2 inhibitors		
Monoclonal antibody	Trastuzumab	Phase III
Receptor tyrosine kinase	Lapatinib	Phase II
IGF-1R inhibitors		
Monoclonal antibody	CP-751–871	Phase I
c-Met inhibitors		
Receptor tyrosine kinase	GSK1363089	Phase II
	ARQ197	Phase I/II
FGFR inhibitors		
Receptor tyrosine kinase	Ki23057	Preclinical
	AZD2171	Phase I
Aurora kinase inhibitors		
	SNS-314	Phase I
	AT9283	Phase I
Polo-like kinase inhibitor		
	GSK461364	Phase I
Cyclin-dependent kinase inhibitor		
	Flavopiridol	Phase I
PI3Kinase inhibitors		
	Everolimus	Phase I, II
Heat shock protein 90 inhibitor		
	STA-9090	Phase I
Ubiquitin–proteasome pathway inhibitor		
	Bortezomib	Phase II
Matrix metalloproteinases (*MMPs*)		
	Marimastat	Phase III
Histone deacetylase inhibitor		
	Vorinostat	Phase I
Protein kinase C inhibitor		
	Bryostatin	Phase II
PARP inhibitors	Olaparib	Phase II/III
	Veliparib	Phase I

VEGFR vascular endothelial growth factor receptor, *EGFR* epidermal growth factor receptor, *HER2* human epidermal growth factor receptor type 2, *IGF* insulin-like growth factor, *FGFR* fibroblast growth factor, *PI3K* phosphatidylinositol 3-kinases, *HGF* hepatocyte growth factor

tial dimerization partner of other members of the ErbB family [6].

In general, HER2 overexpression and amplification in GC ranges from 7 to 34% of patients, depending on the population studied. HER2 overexpression correlates with poor prognosis in ovarian and breast cancer [7]. Higher amplification was originally shown to be associated with worse survival in Japanese GC patients [8]. However, these results have not been reproduced in follow-up studies. In addition, the primary tumor site appears to have higher concordance of HER2 amplification by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) than regional lymph node or distant metastases [9–11]. This should be considered when biopsy specimens are obtained.

Preclinical studies have shown that anti-HER2 therapies have significant antitumor activity for both in vitro and in vivo GC models [12, 13]. The most common approaches to target HER2 are by inhibition by monoclonal antibodies (trastuzumab and pertuzumab) or tyrosine kinase inhibitors (lapatinib). Both types of blockade have been examined in clinical trials of GC patients and are discussed below.

Trastuzumab

Trastuzumab is a humanized monoclonal antibody that has been approved by the US Food and Drug Administration (FDA) since 1998 for the treatment of breast cancer. Trastuzumab targets the extracellular-binding domain of the HER2 receptor and has been combined with cytotoxic chemotherapy in patients with gastric and gastroesophageal junction (GEJ) tumors in several trials.

Most notably, the ToGA study [14] was an open-label, international phase III randomized controlled trial performed across 24 countries. Patients with treatment-naive metastatic or locally advanced unresectable gastric or GEJ adenocarcinoma with overexpressed HER2 protein were eligible. HER2 overexpression was defined as staining 3 + by IHC or by FISH positivity (HER2:CEP17 ratio $\geq$2). HER2 positivity was reported in 22.1% of screened patients. HER2 expression varied according to GC subtype: proximal tumors overexpressed HER2 most frequently (20–30%) and diffuse tumors less often (6%). Distal intestinal type tumors were intermediate.

Patients were randomized to receive cisplatin plus fluoropyrimidine every 3 weeks for six cycles, with or without intravenous trastuzumab at 6 mg/kg after a one-time loading dose of 8 mg/kg. Patient who completed six cycles of treatment in the trastuzumab arm were allowed to continue on trastuzumab maintenance until progression.

There was a 2.7-month improvement in median OS for patients who received trastuzumab (median OS 13.8 months compared with 11.1 months with a hazard ratio of 0.74). Response rate, time to progression, and duration of response were significantly higher in the trastuzumab plus chemotherapy group as well. Of note, the median survival in the chemotherapy only arm was higher than expected in this study and could be related to the high proportion of Asian patients in the study (55%). A treatment benefit was found in all the predefined subgroups, including GEJ tumors. The combination therapy was generally well tolerated with only a slightly increased risk of asymptomatic left ventricular dysfunction and transfusion reaction. This study led to the first FDA approval for targeted therapy for gastric and gastroesophageal (GE) junction adenocarcinoma in 2010 [8].

Based on these encouraging results, the HELOISE study was formulated to evaluate the optimal dose of trastuzumab in advanced gastric and GE junction tumors. Patients are randomized to the currently approved dose of 6 mg/kg versus 10 mg/kg. This study is currently recruiting patients [15].

Trastuzumab is also being evaluated in the nonmetastatic setting. An ongoing phase II study, NCT01130337, treats patients with trastuzumab, capecitabine, and oxaliplatin for three cycles prior to surgery. If an R0 or R1 resection is achieved, patients are given an additional three cycles of treatment. Trastuzumab will be continued for a total of 1 year [16]. Similarly, the TOXAG study is evaluating the role of adjuvant trastuzumab with chemotherapy (oxaliplatin and capecitabine) with concurrent radiation after surgical resection

[17]. The HerFLOT study gives trastuzumab with FLOT (5FU, leucovorin, docetaxol, and oxaliplatin) for four cycles prior to surgical intervention. Patients then receive an additional four cycles of chemotherapy with trastuzumab and nine additional cycles of trastuzumab alone [18]. For locally advanced esophageal or GE junction adenocarcinoma, RTOG 1010 is a phase III trial which randomizes patients to weekly paclitaxel, carboplatin, and radiation with or without trastuzumab prior to surgery [19]. The results of these eagerly anticipated studies could change the treatment paradigm for GC.

As resistance to HER2 therapy has begun to arise, there has been increased interest in the second generation HER2 targeting agent pertuzumab, which binds to a different site on the HER2 (and potentially HER3) receptor and then leads to the disruption of dimerization and blockade of downstream signaling. Based on preclinical work in GC, as well as the efficacy of the combination of trastuzumab and pertuzumab in breast cancer [20], the JACOB first-line phase III study was developed. Patients with metastatic or locally advanced unresectable gastric or GE junction adenocarcinoma are randomized to cisplatin, fluoropyrimidine, and trastuzumab with or without pertuzumab [21].

Another agent active in breast cancer is also being tested in GC. TDM-1 (trastuzumab emtansine) is an antibody-drug conjugate which utilizes HER2 overexpression to deliver a cytotoxic agent directly to cancer cells. This combination has had favorable responses in preclinical GC. A second-line phase II/III trial of T-DM1 in advanced GC is currently recruiting. The study has three arms; TDM-1 at 3.6 mg/kg every 3 weeks, TDM-1 at 2.4 mg/kg every week, or physician's choice of single-agent paclitaxel or docetaxol [22].

Of the monoclonal antibodies, at present only trastuzumab is approved for locally advanced unresectable and metastatic GE junction and GCs. However, with the results of these adjuvant trastuzumab trials as well as the pertuzumab and TDM-1 studies, the role for monoclonal antibodies in GC will likely expand significantly.

Lapatinib

Lapatinib is an oral small molecule dual tyrosine kinase inhibitor of EGFR and HER2. It has been approved for the treatment of HER2 positive advanced breast cancer previously treated with trastuzumab and in conjunction with hormonal therapy for triple positive metastatic breast cancer [23–25]. Lapatinib monotherapy in advanced GC was evaluated in a phase II study and showed limited single-agent activity with a 12 % response rate [26]. However, it is difficult to draw conclusions from this work as patients were not selected based on HER2 overexpression.

Lapatinib has since been evaluated in combination with standard chemotherapy. In the phase III LOGIC study, patients with HER2 overexpressed advanced gastric and GE junction adenocarcinomas were randomized to chemotherapy (capecitabine and oxaliplatin) plus lapatinib versus placebo [27]. This study did not meet its primary endpoint of improvement in OS, though certain subgroups (the Asian population and patients under age 60 years) were shown to have a benefit.

The second-line phase III TYTAN trial compared weekly paclitaxel with or without lapatanib. Again, there was no OS or progression-free survival (PFS) benefit for the lapatinib group, though there was a statistically significant increased response rate [28]. At present, lapatinib does not appear to be ready for widespread implementation in GC but ongoing studies might change its role, likely in combination with other targeted agents.

Epidermal Growth Factor Receptor (EGFR) Inhibition

The EGFR is a transmembrane glycoprotein receptor for the EGF family of extracellular protein ligands [29] and is overexpressed in several gastrointestinal (GI) malignancies. Ligand binding to the extracellular domain leads to EGFR activation and phosphorylation of the intracellular tyrosine kinase, which then directs activation of Ras/Raf/mitogen-activated protein kinase (MAPK) or the Akt/mTOR pathway [30]. EGFR overexpression occurs in 30–50 % of all gastric and GEJ cancers. It is associated with older age, more

aggressive histology, and more advanced stage [31–33].The EGFR gene copy number has also been hypothesized to be a predictive biomarker.

The most common approaches to inhibit the EGFR are by inhibition of the EGFR via monoclonal antibodies (i.e., cetuximab, matuzumab and panitumumab) or tyrosine kinase inhibitors (i.e., gefitinib, erlotinib). Both methods have been studied in patients with GC.

Monoclonal Antibodies Targeting EGFR

Cetuximab

Cetuximab is an IgG_1 type chimeric monoclonal antibody that binds to the extracellular domain of the human EGFR and competitively inhibits the binding of EGF and other ligands, as well as ligand-induced tyrosine kinase autophosphorylation. This antibody–receptor interaction prevents receptor dimerization and thereby blocks ligand-induced EGFR tyrosine kinase activation. Cetuximab also induces EGFR internalization, downregulation, and degradation [34] and is currently approved for the treatment of advanced colorectal cancer as well as squamous cell head and neck cancer [35, 36].

Cetuximab has been evaluated extensively in phase II studies of patients with advanced GC as monotherapy or in combination with chemotherapy (Table 10.2). In patients with untreated or recurrent advanced gastric and GEJ cancer, cetuximab was combined with several chemotherapy regimens in different clinical setting with varying results. When it was combined with FOLFIRI (5-fluorouracil, irinotecan, folinic acid) in 38 patients, overall response rate (ORR) was 44% and OS was 16 months [37]. In combination with FUFOX/FOLFOX (5-fluorouracil, oxaliplatin, folinic acid), cetuximab produced ORR of 65% and OS of 9.5 months [38]. Other combinations with cetuximab have been evaluated in metastatic disease as well, including carboplatin/paclitaxel, cisplatin/docetaxel, capecitabine/cisplatin, and XELOX (capecitabine and oxaliplatin). Response rates ranged between 6 and 69% with an OS between 4.0 and 16.6 months [39–50].

Cetuximab-related adverse events were common in all of these trials. Infusion-related reactions, skin toxicity, and diarrhea were the most prevalent. Based on promising efficacy in several phase II studies, the phase III trial EXPAND (Erbitux in combination with Xeloda and cisplatin in advanced esophagogastric cancer) was performed. 904 patients were randomized to cisplatin with capecitabine with or without cetuximab. Results showed no PFS or OS benefit for the cetuximab group [51].

Unlike in colorectal cancer, the *KRAS* mutation has not been shown to be an accurate negative predictive biomarker for response to cetuximab in GC [52]. However, EGFR expression, copy number, and phosphorylation have also been evaluated as potential biomarkers. In a phase II study of FOLFOX plus cetuximab, correlative analyses on 75% of the 52 patients treated showed that increased EGFR gene copy number ($\geq$4.0) was significantly associated with better OS (HR 0.2, 95% CI: 0–0.8; $P=0.022$)[53]. In another phase II study, higher levels of EGFR expression were associated with increased response rates, but not with time to progression or OS [42]. In the same study, expression of phosphatase and tensin homolog (PTEN) was significantly associated with improvements in response rate, progression-free, and OS. However, these results were not confirmed in the DOCETUX study, in which negative/low EGFR expression and high extracellular signal-regulated protein kinase (ERK) expression were associated with response to therapy [37]. In another work, response rate and time to progression were significantly better in patients without evidence of EGFR phosphorylation [38]. The small sample sizes and retrospective nature of these analyses impede our ability to draw meaningful conclusions regarding the prognostic or predictive value biomarkers for anti-EGFR therapy in GC at this time.

Panitumumab

Panitumumab is the first fully human immunoglobulin G_2 monoclonal antibody targeting EGFR. The clinical benefit of panitumumab was demonstrated in patients with advanced colorectal cancer without the KRAS mutation [54]. In GC, a randomized trial of epirubicin, oxaliplatin, and capecitabine (EOX) with or without

Table 10.2 Clinical trials of the EGFR pathway in gastric and esophageal cancer

Study	Phase	Agent(s)	*n*	ORR	TTP	OS
Pinto et al.	II	Cetuximab + FOLFIRI	38	44%	8	16
Lordick et al.	II	Cetuximab + FUFOX	52	65%	7.6	9.5
Safran et al.	II	Cetuximab + Carbo/paclitaxel/RT	60	27%	NA	NA
Tebbutt et al.	II	Cetuximab + docetaxel	38	6%	2.1	5.2
Ma et al.	II	Cetuximab + CDDP/CPT-11/surgery	20	0%	NA	NA
Kanzler et al.	II	Cetuximab + IF	49	42%	8.5	16.6
Han et al.	II	Cetuximab + FOLFOX	40	50%	5.5	9.9
Pinto et al.	II	Cetuximab + CDDP/docetaxel	48	41%	NA	NA
Woell et al.	II	Cetuximab + oxaliplatin/CPT-11	51	63%	6.2	9.5
Zhang et al.	II	Cetuximab + CDDP/capecitabine	49	48%	5.2	NA
Yeh et al.	II	Cetuximab + CIV 5-FU/LV/CDDP	35	69%	11	14.5
Bjerregaard et al.	II	Cetuximab + CPT-11	31	6%	3;2	NA
Kim et al.	II	Cetuximab + XELOX	44	52%	6.5	11.8
Moehler et al.	II	Cetuximab + FOLFIRI	49	46%	9	16.5
Lordick et al.	II	Cetuximab + FOLFOX	52	65%	7.6	9.5
Chan et al.	II	Cetuximab	35	3%	1.6	3.1
Rao et al.	II	Matuzumab + ECX	21	65%	5.2	NA
Rojo et al.	II	Gefitinib	75	NA	NA	NA
Dragovich et al.	II	Erlotinib	70	9	2	6.7
Wainberg et al.	II	Erlotinib + FOLFOX	34	50	NA	11

ORR objective response rate, *TTP* time to progression, *OS* overall survival, *5-FU* 5-fluorouracil, *NA* not applicable, *FOLFIRI* biweekly bolus 5-FU/leucovorin, irinotecan, infusional 5-FU, *FUFOX* weekly oxaliplatin/leucovorin, infusional 5-FU, *CDDP* cisplatin, *CPT-11* irinotecan, *IF* weekly irinotecan, infusional folinic acid/5-FU, *FOLFOX* biweekly bolus 5-FU/leucovorin/oxaliplatin and infusional 5-FU, *LV* leucovorin, *XELOX* capecitabine plus oxaliplatin, *ECX* epirubicin/cisplatin/capecitabine

panitumumab (REAL-3) did not show any benefit at preplanned interim analysis and was stopped early [55]. However, these negative results may have been partly due to decreased doses of chemotherapy in the combination arm [56].

Matuzumab

Matuzumab is another fully humanized IgG_1 monoclonal antibody against EGFR. In a phase I study of matuzumab in combination with ECX (epirubicin/cisplatin/capecitabine) as first-line therapy for patients with EGFR-positive gastric and GEJ cancer, treatment was well tolerated without major dose-limiting toxicities other than grade 3 fatigue [57]. This combination went on to a phase II study of 72 patients who were randomized to ECX with or without matuzumab. There was no improvement in ORR, progression-free survival, or OS, and a phase III study was not recommended [58].

Though some studies with the monoclonal antibodies are ongoing, these agents are not currently recommended in an unselected GC population.

Tyrosine Kinase Inhibitors Targeting EGFR

Clinical trials using tyrosine kinase inhibitors in GC have shown modest efficacy when used as a single agent or in combination with cytotoxic therapy.

Gefitinib

Gefitinib is an oral EGFR tyrosine kinase inhibitor with promising activity against several types of malignancy in early phase trials. In gastric and GEJ cancer, a phase II study of single-agent gefitinib enrolled 75 patients with previously treated gastric and GEJ cancer. They received gefitinib at 250 mg or 500 mg daily. Gefitinib was shown to reach the tumors at sufficient concentrations to inhibit EGFR activation, but this did

not translate into clinical benefit. Disease control was achieved only in 18% of patients [59]. A complete phase III trial (NCT01243398) randomized patients with advanced esophageal and GE junction tumors to gefitinib versus placebo after progression on chemotherapy. The study is complete and the pending results will help better delineate the activity of gefitinib in esophageal and GCs [60].

Erlotinib

Erlotinib is another oral EGFR tyrosine kinase inhibitor. Erlotinib has been approved in the USA for the treatment of lung and pancreatic cancer. In gastric and GEJ cancer, erlotinib was found to be active only in patients with GEJ cancer. A phase II trial in 70 patients with advanced gastric and GEJ cancer showed a response in 9% of patients with GEJ cancer but no responses in the GC group [61].

Vascular Endothelial Growth Factors Inhibition (Anti-Angiogenesis)

Angiogenesis is an important aspect of tumorigenesis and is critical for tumor growth and survival. The vascular endothelial growth factor (VEGF) plays a pivotal role in the control of angiogenesis, tumor growth, and metastasis in many human cancers [62] including GC, which makes it an attractive target for treatment. VEGF-A is an essential mediator of physiologic and pathologic angiogenesis [63], and its activities are mediated by two tyrosine kinase receptors, vascular endothelial growth factor receptor (VEGFR)-1 and VEGFR-2. Serum VEGF concentration has been related to metastasis and worse outcome in GC and GEJ tumors [64, 65]. Multiple agents have been developed to target the VEGF pathway, including monoclonal antibodies and tyrosine kinase inhibitors.

Monoclonal Antibodies Targeting VEGF

Bevacizumab

Bevacizumab is a recombinant humanized IgG_1 monoclonal antibody against VEGF. Bevacizumab has been extensively evaluated alone and in combination with chemotherapy in many solid tumors. Bevacizumab significantly enhances the antitumor efficacy in colorectal, lung, ovarian, renal cell, and breast cancer [66–70]. However, it does have side effects including thromboembolic events, gastrointestinal perforation, and hypertension.

Several phase II trials have evaluated bevacizumab in the treatment of GC as well as GEJ tumors. One study combined bevacizumab with irinotecan and cisplatin in 47 patients with metastatic gastric and GEJ cancer and resulted in response rate of 65% in the 34 patients with measurable disease. Median survival was 12.3 months. However, 25% of patients had thromboembolic events [71].

Another study of oxaliplatin, docetaxel, and bevacizumab was conducted in 38 previously untreated patients with locally advanced or metastatic GC and GEJ tumors and showed median PFS of 6.6 months and OS of 11.1 months. Gastrointestinal perforation occurred in three patients [72].

The combination of modified docetaxel, cisplatin, and 5-fluorouracil (DCF) and bevacizumab in 44 patients with metastatic GC and GEJ tumors resulted in response rate of 67% and median OS of 16.8 months. Venous thromboembolism was seen in 39% of patients [73].

A phase II trial combining bevacizumab with 5-FU, leucovorin, and oxaliplatin (FOLFOX) was conducted. Out of 16 patients enrolled, ten patients (63%) achieved a pulmonary rehabilitation (PR) and six patients (37%) achieved minor response or disease stabilization. The median time to progression (TTP) and OS were 7 and 8.9 months, respectively. There were no observed bevacizumab-related toxic events, such as perforation or thrombosis [74]. These trials are summarized in Table 10.3.

The promising results of phase II trials led to the Avastin in Gastric Cancer (AVAGAST) study [75]. This was a phase III multinational, randomized, placebo-controlled trial to evaluate the efficacy of adding bevacizumab to cisplatin-based chemotherapy in the first-line treatment of advanced GC. Seven hundred and seventy-four patients from 93 centers in 17 countries were enrolled. Approximately 50% of patients were from

Table 10.3 Clinical trials targeting VEGFR in gastric and GEJ tumors

Study	Phase	Agent(s)	*n*	ORR	TTP	OS
Shah et al.	II	Bevacizumab + CDDP/CPT-11	47	65	8.3	12.3
El-Rayes et al.	II	Bevacizumab + docetaxel/oxaliplatin	8	50	NA	NA
Enzinger et al.	II	Bevacizumab + docetaxel/CDDP/CPT-11	32	63	NA	NA
Kelsen et al.	II	Bevacizumab + docetaxel/CDDP/5-FU	44	67	12	16.2
Jhawer et al.	II	Bevacizumab + docetaxel/CDDP/5-FU	42	64	NA	NA
Ohtsu et al.[a]	III	Bevacizumab + Cisplatin + 5-FU	774	29/38[a]	5.3/6.7[a]	10/12[a]
Bang et al.	II	Sunitinib (second-line)	42	5	4.3	12.7
Moehler et al.	II	Sunitinib (second-line)	38	5	1.5	6.3
Kim et al.	I	Sorafenib + capecitabine/CDDP	21	63	10	14.7
Sun et al.	II	Sorafenib + docetaxel/CDDP	44	39	5.8	13.6

ORR objective response rate, *n* sample size, *TTP* time to progression, *OS* overall survival, *CDDP* cisplatin, *CPT-11* irinotecan, *NA* not applicable, *5fu* 5-flurouracil

[a] This was a randomized phase III trial. OR, TTP, and OS for patients treated without and with bevacizumab, respectively

Asian countries. Median OS was 12.1 months in the bevacizumab plus chemotherapy arm compared to 10.1 months with placebo plus chemotherapy arm (hazard ratio 0.87; 95% CI, 0.73 to 1.03; $p = .1002$). However, though the trial did not meet its primary objective of OS, both median PFS and ORR were significantly improved in the bevacizumab group. No bevacizumab-related safety signals were identified. The heterogeneity of GC might explain the discordant results between phase II and III trials. In addition, the patients with GEJ tumors on the AVAGAST study treated with bevacizumab arm had an exceptionally high response rate of 85% and improved OS. Asian patients showed better OS and PFS regardless of the treatment received when compared to European and Americans. Selection bias, sample size, and study design might have limited the conclusions of single-arm phase II studies.

In order to better select patients who might benefit from anti-VEGF therapy, a panel of tumor angiogenic factors was evaluated in the AVAGAST study, including EGFR, VEGF-A, VEGFR-1, VEGFR-2, and neuropilin (NRP) [76]. Low-tumor neuropilin expression was associated with shorter OS in the placebo group. Adding bevacizumab seems to correct this effect as patients with low-tumor neuropilin, a co-receptor for VEGF-A, had an OS treatment hazard ratio numerically better than those with high neuropilin (low NRP HR 0.75; 95% CI 0.59–0.97; high NRP HR 1.07; 95% CI 0.81–1.40) in the bevacizumab group. Neuropilin thus appeared to be a promising prognostic biomarker candidate, with potential predictive properties for bevacizumab as well. In addition, lower baseline plasma VEGF-A correlated with longer OS. Further evaluation of these potential biomarkers is ongoing.

Another approach to targeting the VEGF pathway is through so-called dirty kinase inhibitors, which inhibit the VEGF receptor as well as FLT-3, c-kit, and RET.

Several tyrosine kinase inhibitors are currently being evaluated in GC.

Tyrosine Kinase Inhibitors Targeting VGFR

Sunitinib

Sunitinib is an oral multitargeted tyrosine kinase inhibitor of VEGFR, platelet-derived growth factor receptors (PDGFRs), c-kit, RET, and FLT-3 that has been approved for the treatment of advanced renal cell carcinoma (RCC) and imatinib resistant or intolerant gastrointestinal stromal tumors (GIST).

Several trials have evaluated single-agent sunitinib in the treatment of GC. A phase II second-line trial of single-agent sunitinib in 78 patients with advanced gastric and GEJ cancer showed promising results: two patients had partial response and 25 patients had stable disease for ≥6 weeks. Median PFS was 2.3 months and median OS was 6.8 months (95% CI, 4.4–9.6 months). Grade ≥3 thrombocytopenia and neutropenia were reported in 34.6% and 29.4% of

patients, respectively, and the most common nonhematologic adverse events were fatigue, anorexia, nausea, diarrhea, and stomatitis [77]. Another phase II study in 52 pretreated patients with advanced GC reported that sunitinib (50 mg/day for 4 weeks, followed by 2 weeks' rest) was well tolerated [78]. In the intention to treat population, the ORR was 3.9%, median PFS and OS were 1.28 months and 5.81 months, respectively. In a subgroup analysis, VEGF-C expression in the tumor was associated with significantly shorter median PFS, but there was no difference in tumor control rate.

Sunitinib has also been evaluated in combination with chemotherapy. A second-line phase II trial randomized 107 patients to docetaxel with or without sunitinib. The TTP was not significantly different (3.9 months in the sunitinib arm vs. 2.6 months), but there was an increased response rate of 41.4% compared to 14.3% [79].

Similar to other tyrosine kinase inhibitors (TKIs), sunitinib has multiple drug interactions and can lead to QTc prolongation and changes in the metabolism of CYP3A4 substrates. Common toxicities include hypertension, hand–foot syndrome, and liver dysfunction.

Sorafenib

Sorafenib is a potent inhibitor of Raf tyrosine kinase and several other receptor tyrosine kinases, including VEGFR-2, VEGFR-3, and PDGFR-β. Sorafenib has been approved for the treatment of both RCC and hepatocellular carcinoma based on the results of phase III trials [80, 81]. In tumor xenografts models, sorafenib effectively inhibited tumor growth and angiogenesis in gastric tumors [82].

Sorafenib has been evaluated for the treatment of advanced GC in several studies. It was combined with capecitabine and cisplatin in a phase I trial [83] as first-line therapy and the objective response rate was 62.5%. The median PFS and OS were 10.0 and 14.7 months, respectively. A phase II study of 44 patients combined sorafenib with docetaxel and cisplatin and showed a median PFS of 5.8 months and median OS of 13.6 months, which warranted further study [84]. However, another phase II study of 40 patients combined sorafenib with second-line docetaxel and oxaliplatin and showed a disappointing PFS of 3 months and OS of 6.5 months [85]. A phase II trial of sorafenib monotherapy in metastatic GC was terminated early because of the low response rate [86].

Pazopanib

Pazopanib is an oral agent which inhibits angiogenesis through multiple pathways, including the VEGF receptor, the platelet-derived growth factor (PDGF) receptor, as well as c-kit. It has been approved by the FDA for use in the treatment of metastatic RCC as well as metastatic soft tissue sarcoma based on the results of phase III trials [87, 88]. Pazopanib has also been shown to have activity in metastatic thyroid cancer [89].

Pazopanib is currently being evaluated with chemotherapy in two GC trials. The phase II PaFLO trial randomized first-line advanced GC patients to 5-fluorouracil, leucovorin, and oxaliplatin with or without pazopanib and is currently accruing patients [90]. Another first-line phase II trial adds pazopanib to capecitabine and oxaliplatin in advanced GC patients and is also recruiting [91]. The results of these studies will help determine if pazopanib has a role in the treatment of advanced GC.

Vandetanib (ZD6474)

Vandetanib is a dual VEGFR and EGFR tyrosine kinase inhibitor. It also inhibits RET-tyrosine kinase activity, an important growth driver in certain types of thyroid cancer. In 2011, vandetanib became the first drug to be approved by the FDA for the treatment of metastatic medullary thyroid cancer. In an orthotopic GC model, vandetanib inhibited tumor growth, decreased microvessel density, and slowed tumor cell proliferation [92].

A recently reported phase I trial evaluating vandetanib plus paclitaxel, carboplatin, 5-fluorouracil, and XRT induction therapy followed by surgery for previously untreated locally advanced cancer of the esophagus and GE junction found that the combination was well tolerated and warranted further evaluation [93]. However, when vandetanib was evaluated with docetaxel in a second-line, randomized GC study, the study was

terminated early because of insufficient power to show results [94].

Telatinib

Telatinib is a potent small molecule oral tyrosine kinase inhibitor that selectively targets the VEGF and PDGF receptor families. Telatinib has showed activity in GC in an early phase I trial, which led to a phase II study in combination with capecitabine and cisplatin as first-line treatment in patients with advanced cancer of the stomach or GE junction [95]. In the 39 patients eligible for analysis, the ORR was 67%, and an additional 28% of patients had stable disease [96]. Median OS results are still pending.

Several studies are investigating the VEGF pathway inhibition in GC despite the negative results of the AVAGAST trial. There is potentially a subset of GC patients who would benefit from targeting this pathway. To this end, several prognostic and predictive markers to predict clinical outcome in patients treated with VEGF inhibition are being actively investigated. Trials of VEGF pathway inhibitors in the neoadjuvant setting are also ongoing. In the UK, the Medical Research Council Adjuvant Gastric Infusional Chemotherapy trial (MAGIC)-B is evaluating the role of bevacizumab for peri-operative chemotherapy in operable adenocarcinoma of the stomach and GEJ.

Ramucirumab

Ramucirumab is a new fully human IgG1 monoclonal antibody that specifically and potently inhibits VEGFR-2. Ramucirumab has demonstrated efficacy and tolerability in several studies. The phase III REGARD (ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma) study randomized second-line gastric or GE junction adenocarcinoma patients to single-agent ramucirumab or best supportive care. They found a median OS of 5.2 months in the treatment arm compared to 3.8 months, with a p value of 0.042 [97]. Based on the known activity of the agent, the phase III RAINBOW (a global, phase III, randomized, double-blind study of ramucirumab plus paclitaxel versus placebo plus paclitaxel in the treatment of metastatic gastroesophageal junction (GEJ) and gastric adenocarcinoma following disease progression on first-line platinum- and fluoropyrimidine-containing combination therapy rainbow) study randomized 665 s line advanced gastric or GE junction cancer patients to paclitaxel with or without ramucirumab. Median OS was 9.63 months in the combination arm versus 7.36 months for paclitaxel alone. Patients in the combination arm had more neutropenia and hypertension [98].

Based on the REGARD study, the FDA approved ramucirumab in 2014 for use as a single agent in gastric and GE junction cancer after progression on a platinum or fluropyrimidine-containing regimen [99]. This is the first targeted agent approved in the treatment of GC since trastuzumab.

Insulin-like Growth Factor-1 (IGF-1) Inhibition

The IGF-1 receptor belongs to the insulin receptor family (IGF-1 and IGF-2). IGF-1R is expressed on the cell surface and phosphorylation of intracellular substrates leads to activation of the MAPK and PI3K/Akt pathways which promotes tumor growth, progression, and invasion in several cancers, including GC [100]. The IGF-1R and its associated signaling system have gained significant interest in the treatment of several malignancies. The IGF-1R pathway is targeted through monoclonal antibodies, IGF-1R antisense/siRNA, and receptor tyrosine kinases. IGF-1R signaling has been associated with resistance to cytotoxic therapy. Inhibition of IGF-1R enhances tumor cell apoptosis in numerous models [101]. IGF-1R signaling has also been causally linked to de novo or acquired resistance to EGFR-targeting agents in several malignancies [102]. In GC, IGF-1R expression in resected tumors correlates with poorer clinical outcomes [103]. In a study of 86 patients with resected gastric tumors, patients with low expression of both IGF-1R and EGFR had significantly longer OS compared to those who lack the low coexpression [103]. A phase I trial of docetaxel combined with CP-751,871, an IGF-1R antibody, has demonstrated promising results [104]. However, the

data on IGF-1R inhibition in GC is still premature.

Fibroblast Growth Factor Tyrosine Kinase Inhibitors

Fibroblast growth factor (FGF) and its signaling receptors have multiple biological properties including cell proliferation, differentiation, motility, and transformation [105, 106]. Fibroblast growth factor receptor 2 (FGFR2) is amplified in poorly differentiated GC (scirrhous cancer) with malignant phenotypes [107], which makes it a promising molecular target for treatment.

In preclinical models, AZD2171, a highly potent oral VEGF, FGFR1, PDGFRB, and VEFGR2 tyrosine kinases inhibitor, led to tumor inhibition in GC xenografts in a dose-dependent fashion. The most potent antitumor activity was seen in xenografts over-expressing FGFR2. These results suggest that AZD2171 might be clinically beneficial in patients with FGFR2 expressing gastric tumors [108].

Ki23057, a broad-range tyrosine kinase inhibitor of FGFR2, also inhibits FGFR1, FGFR2, and VEGF2 tyrosine kinases. It inhibits the proliferation of gastric scirrhous cancer cells with FGFR2 gene amplification only. Oral administration of Ki23057 inhibits the growth and peritoneal dissemination of GC cells through FGFR2-RAS/ERK inhibition, rather than through FGFR2–PI3k–AKT signaling inhibition [109]. To our knowledge, no clinical trials are currently available for this compound in GC.

c-Met Tyrosine Kinase Inhibitors

Met is a membrane receptor that is essential for embryonic development and wound healing. C-Met is a receptor tyrosine kinase that is expressed in epithelial and endothelial cells. Hepatocyte growth factor (HGF), its ligand, is expressed by cells of the mesenchymal lineage. Overexpression of c-Met and activating c-Met mutations have been widely documented in many tumor types including GC [110], and c-Met deregulation correlates with poor outcomes. In a study of 121 patients with advanced GC, HGF, and c-Met were significantly overexpressed in patients with liver metastases [111]. Coexpression of c-Met and HER2 proteins in patients with GC has been associated with poorer survival [112].

c-Met inhibition has been evaluated in early phase trials with promising results. Two phase I trials of ARQ197 (a nonadenosine triphosphate (ATP) competitive small-molecule inhibitor of c-Met) in patients with solid tumors showed disease stabilization in 7 out of 11 patients, with prolonged stabilization for >32 weeks in five tumor types, including GC [113]. Another trial of 36 patients reported that 5.5% of patients achieved a PR, and 53% had SD [114].

A phase II study examined the safety and efficacy of two dosing schedules of foretonib (GSK1363089), an oral small-molecule inhibitor of c-Met and VEGFR-2, as a single agent in patients with metastatic GC. Foretonib was well tolerated in both dosing schedules. However, c-Met amplification in metastatic GC was found to be less common than anticipated, and occurred in only 3 out of 43 patients. Amplification of the met oncogene was not associated with a higher response rate. However, the lack of a well-validated method to assess c-Met makes any conclusive interpretations difficult. Single-agent foretonib demonstrated minimal antitumor activity in a c-Met unselected GC population. Mandatory pre- and on-treatment biopsies to better define c-Met pathway and target inhibition were added to the protocol [115]. Other clinical trials of various c-MET inhibitors (both TKIs and monoclonal antibodies) are ongoing.

Cell Cycle Inhibition

Polo-like Kinase Inhibitors

Polo-like kinases (PLKs) are a family of conserved serine/threonine kinases, which are involved in signal transduction pathways leading to the formation and changes in the mitotic spindle. As such, they are involved in the regulation of cell-cycle progression through G_2 and mitosis. These enzymes also activate cyclin-dependent kinase/cyclin complexes during the M-phase of the cell cycle. PLK-1 overexpression is seen in various malignancies including GC [116], and is associated with the accumulation of

proliferation-related genes and oncogenes. Inhibiting PLK-1 leads to cell growth inhibition and apoptosis. Moreover, PLK-1 is a prognostic marker for GC [117]. Patients with PLK1-positive tumors have more lymph node metastasis and diffuse growth pattern and thus worse outcome when compared to those with PLK-1-negative tumors [118].

The inhibition of PLK-1 via small interfering RNA (siRNA) resulted in increased cdc2 activity, increased cyclin B expression, and accumulation of GC cells at the G2/M phase. This led to improper mitotic spindle formation, delayed chromosome separation, attenuated pro-caspase three levels, and increased apoptosis [119]. A phase I trial investigating the role of PLK inhibitors in various solid tumors, including GC, found that some patients had stable disease but with a high VTE rate of 20 %[120].

Aurora Kinase Inhibitors

Aurora kinases (A, B, and C) are serine/threonine kinases that have been recognized as important regulators of cell proliferation from mitotic entry to cytokinesis [121]. In normal cells, aurora kinase protein levels increase from the G2 to the M phase. Overexpression of aurora kinase A results in chromosomal instability in a variety of tumors including GC. In addition, aurora kinase A inhibits drug-induced apoptosis leading to drug resistance [122]. Aurora kinase A overexpression in upper gastrointestinal cancers indirectly activates HDM2 leading to p53 suppression and cancer cell survival [123], which translates into poorer clinical outcomes [124].

Various aurora TKIs are currently under investigation in phase I trials. In a phase I trial of SNS-314 in patients with solid tumors, a novel selective inhibitor of aurora kinases A, B, and C showed no objective responses [125]. In another phase I trial of AT9283, a multitargeted kinase inhibitor including aurora kinases A and B, 33 patients were treated. The best response was a PR in one patient and two patients with SD [126].

Cyclin-Dependent Kinase Inhibitors

Cyclin-dependent kinases (CDKs) comprise a group of protein kinases (cdk1–cdk9) that participate in cell-cycle regulation via the retinoblastoma product (Rb). The inactivation of the Rb pathway results from overexpression or amplification of CDKs, downregulation of negative factors such as endogenous CDK inhibitors, or from mutations in the Rb gene or its product. This pathway is deregulated in different malignancies, resulting in a disturbed $G_1$to S phase of the cell cycle [127].

Flavopiridol is a synthetic flavone that inhibits in vitro tumor cell growth at nanomolar concentrations by blocking cell-cycle progression at G_1 or G_2 [128, 129]. Flavopiridol is a potent inhibitor of CDKs with respect to the ATP-binding site including cdk-1, cdk-2, cdk-4, and cdk-7, and hypophosphorylation of Rb [130]. Flavopiridol has also been shown to induce apoptosis, inhibit angiogenesis, and potentiate the effects of chemotherapy by arresting the cell in the G1 or G2/M phase [131, 132]. In a phase I study of 38 patients with advanced cancer, flavopiridol was administered as a continuous infusion. One patient with GC had a complete response (CR) lasting more than 48 months [133]. A phase I trial of FOLFIRI in combination with flavopiridol in patients with GC and other solid tumors showed clinical benefits in 39 % of patients[134]. However, a phase II study of flavopiridol as a single agent in 16 patients with advanced GC showed no activity [135].

Other Novel Targets

Ubiquitin–Proteasome Pathway Inhibitors

The ubiquitin–proteasome pathway is essential for protein quality control through degradation. It plays an important role in cell-cycle regulation, transcription, signaling, protein transport, DNA repair, and stress responses. Disturbance in proteasome activity leads to the accumulation of polyubiquitinylated proteins, endoplasmic reticulum stress, and even cell death [136].

Bortezomib

Bortezomib is a potent inhibitor of the proteasome and has prominent effects in vitro and in vivo against several solid tumors. It has been

approved for the treatment of hematological malignancies but its role in solid tumors is not well established. In preclinical models, bortezomib-induced apoptosis in three GC cell lines: SNU638, MUGC-3, and MKN-28. When combined with cisplatin and docetaxel, bortezomib dramatically decreased tumor cell growth compared with chemotherapy alone [137] .

This preclinical efficacy led to multiple phase II studies being developed. One phase II study of bortezomib in 16 patients with advanced gastric adenocarcinoma was performed. No patients had an objective response but one patient achieved SD [138]. In another phase II trial of 44 patients with advanced gastric and GEJ cancer, 28 chemo-naïve patients (arm A) received irinotecan in combination with bortezomib, and 12 patients who were previously treated received bortezomib alone (arm B). Response rates of 44% in arm A and 9% in arm B were seen. The PFS and OS were 1.9 and 5.4 months in arm A and 1.4 and 4.1 months in arm B, respectively [139]. In another phase II trial of bortezomib combined with paclitaxel and carboplatin in first-line treatment of 35 patients with metastatic gastric and GEJ cancer, tumor response rates were lower than anticipated at only 23% and the OS was 8.9 months [140].

PI3 Kinase Pathway Inhibition

The PI3K enzymes are involved in the phosphorylation of membrane inositol lipids [141]. The activation of PI3K generates the second messenger phosphatidylinositol (3,4,5)-trisphosphate (PIP3) from phosphatidylinositol 4,5-bisphosphate (PIP2). This recruits proteins to the cell membrane, including the Akt/PKB kinases, resulting in their phosphorylation by phosphoinositide-dependent kinase 1 (PDK1) [142], and by PDK2 [143].

Dysregulation of the PIP3/Akt/mTOR pathway can occur secondary to oncogenic mutations of PIK3CA [144], loss of PTEN function [145, 146], mutation of Akt/PKB isoforms [147], or upstream activation through other pathways like IGF-1R. Abnormal expression of the PTEN protein in GC is found in 11% of tumors and is related to the tumor differentiation, advanced staging, and chemoresistance [148]. Upregulation of the PI3k/Akt/mTOR downstream pathway correlates with a worse prognosis and may contribute to the resistance to chemotherapy [149].

Everolimus

Everolimus (RAD001) is an oral mammalian target of rapamycin (mTOR) inhibitor that has shown anticancer activity both in preclinical GC models [150], as well as in a phase I study in Japanese GC patients [151]. Based on these promising results, a multicenter phase II study was performed in pretreated patients with metastatic GC [152]. Fifty-three patients were eligible for analysis. At a median follow-up of 9.6 months, median PFS was 2.7 months and median OS was 10.1 months. Common grade 3 or 4 adverse events included anemia, hyponatremia, increased gamma-glutamyltransferase, and lymphopenia. The short PFS compared to the relatively long OS is puzzling and requires further evaluation. On the basis of these results, the phase III GRANITE-1 trial was performed. 656 s or third line advanced GC patients were randomized to everolimus as monotherapy or placebo with best supportive care. The median OS was not significantly different, at 5.39 months in the everolimus group compared to 4.34 months [153].

Heat Shock Protein 90 Inhibitors

Heat shock protein 90 (HSP90) is a molecular chaperone and is one of the most abundantly expressed proteins in the cell. Multiple cell-specific oncogenic processes are tightly regulated by binding of HSP90 [154, 155]. In GC, HSP90 expression correlates with tumorigenesis and lymph node metastasis [156]. The downregulation of HSP90 can increase drug sensitivity of tumor cells. In preclinical studies, HSP90 inhibition reduced the constitutive and inducible activation of extracellular signal-regulated kinase 1/2, Akt, and signal transducer and activator of transcription (STAT3), and decreased the protein expression of the nuclear hypoxia-inducible factor-1α (HIF-1α) [157]. There are several ongoing studies evaluating HSP90 inhibitors in various malignancies.

STA-9090 is a potent, next-generation HSP90 inhibitor. STA-9090 has shown superior activity

and improved safety profile relative to other agents in preclinical models. Two phase I dose-escalation studies of STA-9090 in patients with solid tumors, including GC, have shown STA-9090 to be well tolerated at dose levels up to 216 mg/m^2 once weekly [158] or 25 mg/m^2 twice weekly [159]. These studies warrant further evaluation of STA-9090 in solid tumors, including GC.

Matrix Metalloproteinases (MMPs)

The matrix metalloproteinases (MMPs) are a family of highly homologous protein-degrading zinc-dependent endopeptidases that degrade components of the extracellular matrix. This family currently includes more than 25 members that play an important role in normal cellular growth and repair. They are aberrantly expressed in several solid tumors and are thought to contribute to the invasive potential of these malignancies [160]. Based on promising phase I results, a phase III study of marimastat, an MMP inhibitor, was undertaken [161]. Altogether 396 patients with inoperable/metastatic gastric or GE junction adenocarcinoma who had received no more than first-line 5-FU-based chemotherapy were randomized to receive either placebo or marimastat. At 2-year follow-up, there was a small but statistically significant difference ($p=0.02$) in median OS (160 vs. 138 days) and 2-year survival (9% vs. 3%) favoring the marimastat group. Despite these promising results, further development of this drug has been halted secondary to poor tolerability because of musculoskeletal toxicity.

Histone Deacetylase Inhibitors

Epigenetic modulation of gene expression has an important role in regulating cell biology [162]. Epigenetic silencing of tumor suppressor genes, induced by the overexpression of histone deacetylase (HDAC), plays a crucial role in carcinogenesis. Further understanding of the cancer cell cycle and the role of HDAC inhibition has led to the development of several new anticancer agents [163].

18 HDAC enzymes have been identified and categorized in three classes in humans. HDAC is thought to be independent prognostic marker in GC. Moderate to strong expression of HDAC2 was found in 44 (62%) out of 71 gastric tumors and was associated with tumor aggressiveness and nodal spread [164, 165].

HDAC inhibitors act by binding to a critical zinc ion required for catalytic function of the HDAC enzyme [166]. These compounds have varying potency and specificity, with variable effects on the acetylation of nonhistone substrates [167], leading to distinct efficacy, toxicities, and therapeutic effects [168].

More than 15 HDAC inhibitors have been tested in preclinical and early clinical studies, but the only HDAC inhibitor approved by the FDA is vorinostat for hematological malignancies. In a phase I trial of vorinostat monotherapy in 16 Japanese patients with gastrointestinal cancer, including 10 with GC, 8 patients had SD as the best response [169]. Another phase I trial of vorinostat combined with FOLFIRI in patients with upper gastrointestinal tumors has been reported. Among eight patients in whom the response was assessable, two had a PR and five had SD [170]. A phase I/II study of vorinostat plus capecitabine and cisplatin for first-line treatment of metastatic or recurrent GC is currently accruing [171].

Protein Kinase C Inhibition

Protein kinase C (PKC) is part of a family of enzymes that are involved in controlling the function of other proteins. These enzymes work through the phosphorylation of hydroxyl groups of serine and threonine amino acid residues. PKC inhibitors are currently being investigated in both malignant and nonmalignant conditions.

Bryostatin-1, an inhibitor of protein kinase C, has been evaluated in combination with paclitaxel sequentially in esophagogastric tumors [172]; despite the initially promising results, the drug has been discontinued secondary to unexpected grade 3/4 myalgia in approximately half of all patients.

Poly ADP-ribose Polymerase Inhibitors

The function of poly (adenosine diphosphate (ADP)-ribose) polymerase (PARP) is to repair single-stranded breaks (SSBs). If these SSBs are not repaired, they become double-stranded

breaks (DSBs) at the next fork replication, which leads to cell death. The PARP inhibitors function by preventing the SSB repair and thus ultimately allow cancer cell death to occur [173]. These agents have shown activity in ovarian and breast cancer, particularly in patients with *BRCA* mutation.

Second-line therapy for metastatic or recurrent GC, randomized to paclitaxel with or without olaparib [174]. Because preclinical data has shown that there is more olaparib sensitivity in patients with low ataxia telangiectasia mutated (ATM) protein [175], this study enriched for low ATM protein levels.

Based on these results, there is an ongoing phase III study of second-line GC patients randomized to paclitaxel with or without olaparib [176].

A phase I study of another PARP inhibitor veliparib with FOLFIRI is ongoing [177] .

Conclusion

GC is one of the most common malignancies worldwide, with approximately 990,000 new cases and 738,000 deaths per year, accounting for about 8% of new cancers [178]. At diagnosis, approximately 50% of patients have disease that extends beyond locoregional confines. Only half of eligible patients will ultimately undergo curative resection. Screening is not widely performed outside of high prevalence areas. Cytotoxic agents have been the mainstay of systemic treatment for decades but carry significant toxicity. The need for novel agents is urgent.

During recent years, several molecular abnormalities underlying gastric carcinogenesis and progression have been identified. This has stimulated the search for novel therapeutic approaches, and many studies are now incorporating these targeted agents with chemotherapy. However, given the highly complex nature of the underlying molecular abnormalities and concurrent aberrations in multiple signaling pathways, targeted agents used as monotherapy or even added to a chemotherapy backbone are unlikely to result in significant efficacy. The inherent redundancies in these molecular pathways also preclude effective blockade of proliferation and survival if only one receptor is targeted. Pursuing multiple targets simultaneously should be considered. However, this method is severely hampered by our current limited understanding of how to combine targeted agents, the logistical issue of designing multi-sponsor trials, as well as the potential for additional toxicities. Molecular profiling will be important to identify the specific patient who might benefit from targeted therapy, validate whether the drug inhibits the target, and determine if the tumor having the target is even of functional importance.

Biomarkers are increasingly utilized in cancer treatment to predict the efficacy and toxicity of anticancer agents. Increased use of biomarkers is expected to lead to treatments suited for individual patients, such as HER2 inhibition in GC. However, at the present time, few biomarkers are used clinically, as most have not gone beyond the investigational phase. In clinical trials, selecting patients based on predictive factors is ideal, but this is difficult with the lack of validated biomarkers in GC and the diversity of molecular alterations acquired during malignant transformation, recurrence, or metastasis.

Many of the agents discussed in this article have poorly defined targets in individual patients, which hampers their optimal development. Measuring the efficacy of these agents on the targeted pathway is crucial to further define their role. To help refine the use of these agents, they could be first tested in the neoadjuvant setting with multiple biopsies specimens collected. The tissue could then be correlated with the patient's outcome, with regard to whether the target is of functional importance. The target could be evaluated to assess if it was actually inhibited by the agent. However, this schema has several limitations. The response rate in the neoadjuvant setting might not translate into survival for metastatic disease. There is also significant morbidity and inconvenience associated with serial biopsies. Similarly, evaluating targeted agents in a refractory population might not be the optimal way to identify clinical benefits of novel agents. In the future, combining targeted therapy with

cytotoxic agents and or radiation should be based on sound scientific evidence and rational design.

The failure of phase III trials to demonstrate survival benefit despite what may sometimes be considered promising results from phaseII studies indicates the need to change the current drug evaluation system. Targeted agents often result in stable disease rather than disease response, which makes assessment more challenging. The increased emphasis on randomized phase II screening trials can minimize the likelihood of erroneous conclusions regarding efficacy. The results of such trials must be confirmed in phase III trials or even in additional phase II studies. OS must remain the primary end point of clinical trials because of the short survival and the lack of surrogate clinical endpoints to predict survival in GC. The magnitude of benefit in survival seen in pilot studies to generate a phase III trial must be considered on a case-by-case basis.

Apart from the molecular targeted therapies described in this article, many other agents are currently being evaluated in GC. Further studies are needed to determine the optimal use of targeted therapy. The success of trastuzumab in HER2 overexpressed tumors should serve as a model for identifying the appropriate GC patients with various biological subsets of the disease. Adequately powered, randomized trials are necessary to define the role of targeted therapies in advanced GC. Biomarker-driven studies to correlate with treatment outcomes will be critical to identify patients who will benefit most from targeted therapy.

Conflict of Interest The authors have no conflicts of interest to declare

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Targeted Therapies for Pancreatic Cancer

11

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Introduction

The majority of patients with pancreatic cancer are diagnosed with locally advanced or metastatic disease. The organs that are more likely to receive distant metastasis from pancreatic tumors are the liver and peritoneal cavity. Treatment of patients with advanced disease remains palliative with a median overall survival (OS) that only ranges from 9 to 10 months with current systemic chemotherapeutics. By contrast, local management of early-stage pancreatic cancer is based on radical surgery and adjuvant chemoradiation [14, 28].

Despite increasing research activities in the field of pancreatic tumors, no outstanding new target therapies have changed the landscape in this malignancy over the past decades. Gemcitabine has become the first-line chemotherapeutic agent in pancreatic cancer for years, utilized in locally advanced or metastasized disease and in all patients that cannot undergo surgery. The combination of irinotecan, 5-fluorouracil, and oxaliplatin, in selected patients, has also demonstrated a survival benefit [6]. The combination of erlotinib and gemcitabine resulted in a statistical but non-clinical benefit in overall survival [25].

Overall, however, with a 5-year survival rate of 1–4 % and a median survival period of 4–6 months, the prognosis of patients with pancreatic cancer has remained extremely poor in the last decades [33]. The development of advanced therapeutic strategies is a prerequisite for eventually achieving a better outcome, as sufficiently early detection of pancreatic cancer is unlikely to occur in the near future.

Many novel agents for advanced pancreatic cancer have been or are being evaluated in phase 2 and phase 3 clinical trials. The majority of studies have not demonstrated a significant treatment or survival advantage to these therapeutic agents, and many investigators have observed potent toxicities that may affect quality of life [40]. An exception to this, is the recently communicated results from the metastatic pancreatic adenocarcinoma clinical trial (MPACT) study, in which nab-paclitaxel plus gemcitabine demonstrated a statistically significant improvement in overall survival compared to patients receiving gemcitabine alone (median of 8.5 vs. 6.7 months; hazard ratio 0.72: $p = 0.000015$). In addition, the combination of nab-paclitaxel and gemcitabine

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showed a 59% increase in 1-year survival (35 vs. 22%, $p=0.0002$) and demonstrated double the rate of survival at 2 years (9 vs. 4%, $p=0.02$) as compared to gemcitabine alone [46]. Newer approaches to drug development should focus on agents that target the physiologic effects of the mutated cellular signaling pathways.

Tumor Stroma

The stroma contains the extracellular matrix and many cell types including fibroblasts, endothelial cells, and immune cells; the complex interactions between the tumor and the stroma constitute a microenvironment favorable for its growth [12]. Therefore, understanding the interaction of pancreatic tumor cells with stromal components is critical for developing improved therapeutic options for patients.

Pancreatic ductal adenocarcinoma is characterized by a strong desmoplastic reaction. Interactions between cancer cells and the surrounding stromal fibroblast play a critical role in tumor invasion and metastasis. To date, very little is known about this tumor–stroma interaction and whether the desmoplastic reaction is an obstacle for tumor cure. A number of reports suggest that desmoplastic reaction in pancreatic carcinomas promotes the malignant phenotype of cancer cells to detriment to the host. It is suggested that the tumor–stroma interactions that induce the hallmark desmoplastic reaction may also provide a microenvironment that promotes the highly malignant pancreatic cancer phenotype.

Pancreatic stellate cells (PSC) have recently been described as a stromal component in the pancreas and were identified to be responsible for the development of pancreatic fibrosis after various kinds of pancreatic insults. Activated PSC synthetize extracellular matrix proteins and are involved in repair of pancreatic injury, however, prolonged PSC activation as seen in chronic pancreatitis and in pancreatic cancer is implicated in the generation of pancreatic fibrosis and tumor desmoplasia [2]. Some reports suggest that the effect of fibrosis could restrict tumor growth while other authors have related fibrosis with bad blood supply, making difficult the chemotherapy delivery to the tumor [42]. Nevertheless, we have to better understand the role that PSC are playing in pancreatic tumors since a subpopulation of them named $CD271^+$ is significantly correlated with a better prognosis in patients with advanced pancreatic carcinoma [9].

The expression of osteonectin by fibroblastic cells in the stromal compartment in pancreatic adenocarcinoma is strongly associated with poor patient outcome [16]. Osteonectin, also known as SPARC (secreted protein, acidic and rich in cysteine) is a highly conserved multifunctional glycoprotein that belongs to the matricellular class of proteins, and plays a critical role for collagen deposition and fibrilogenesis (Table 11.1). Expression of osteonectin in the stroma likely facilitates the development of the dense collagenous stroma associated with pancreatic cancer.

Consistent with its function as a mediator of tissue remodeling, SPARC regulates the expression of proteins involved in cell-extracellular matrix turnover and formation including collagens and matrix metalloproteinases (MMPs). SPARC can also directly affect endothelial cell behavior by regulating proliferation, cell shape, fibroblast growth factor (FGF), vascular endothelial growth factor (VEFG) and platelet-derived growth factor (PDGF) [5]. SPARC may exert divergent actions in tumors reflecting the complexity of this protein [37].

Osteonectin is known to bind albumin, and interaction of osteonectin with nab-paclitaxel may concentrate the drug in the tumor vicinity and increase efficacy. Therefore, nab-paclitaxel can increase intratumoral concentration of the paclitaxel by a receptor-mediated transport process across the endothelial cell wall, thereby breaching the blood/tumor interface. Nab-paclitaxel offers the additional advantages of delivery of a relatively high dose of paclitaxel, the avoidance of the Cremophor EL medium, and ease of administration. In addition, it has shown promising activity in a recent phase I/II trial with pancreatic cancer patients, testing three different doses in combination with gemcitabine [45]. SPARC

Table 11.1 Effects of matricellular proteins osteopontin and osteonectin in pancreatic ductal adenocarcinoma (PDA)

Matricellular protein	Source	Regulation	Effect in PDA	Relevance
Osteopontin/ osteopontin-c	Tumor-associated macrophages	Paracrine regulator	Prometastatic promotes PDA growth	Target for therapeutic intervention
	PDA cells	Autocrine regulator induced by nicotine alternative splicing		
Osteonectin	PDA cells	Autocrine regulator epigenetic silencing (aberrant methylation)	Inhibits PD growth	Possible tumor suppressor
	Fibroblastic cells in tumor stroma	Paracrine regulator	Prometastatic	Associated with poor prognosis
		Contributes to formation of dense desmoplastic stroma	Profibrotic	Dense stroma may hamper penetration of chemotherapeutic agents
			Interacts with albumin	Promote accumulation of albumin-paclitaxel nanoparticles

expression in the stroma but not in the tumor was correlated with improved survival. In another small phase II trial, 19 patients were treated with nab-paclitaxel after progression to first-line gemcitabine, achieving a progression-free survival (PFS) of 1.7 months and an overall survival of 7.3 months [15]. Results from the subgroups according to the different expression of SPARC in the tissue samples of patients treated in the MPACT trial are awaited shortly.

Transforming growth factor β (TGFβ) has been closely correlated with both radiation-induced fibrosis and the desmoplastic reaction in pancreatic carcinoma. Moreover, the TGFβ pathway is frequently altered in pancreatic carcinoma through mutations or methylation of DPC4 (deleted in pancreatic cancer 4, also known as SMAD 4) or overexpression of the TGFβ ligand [8]. Many drugs have been developed to target TGFβ signaling. The antisense oligodeoxynucleotide trabedersen (AP 12009) specifically inhibits TGFβ2 expression, and it has demonstrated a good toxicity profile and encouraging survival results in patients with refractory solid tumors including pancreatic cancer [29].

Several compounds targeting the TGFβ pathway are under development. Perhaps, one of the most promising is LY2157299, a small molecule that inhibit TGFβ type I receptor selectively, that is being tested in combination with gemcitabine for patients with advanced or metastatic pancreatic cancer in a phase I trial at this time.

Pancreatic Cancer Stem Cells

A growing body of evidence now supports the concept that cancers are diseases driven by subpopulation of self-renewing cancer stem cells (CSCs). In 2007, two groups of investigators found the presence of CSCs in human pancreatic cancer [13, 22]. These cells would have the ability to self-renew and generate the diverse cell population and through asymmetric division, they give rise to more differentiated cells. One important recent observation is that the putative CSCs are very plastic and can transition between different states, such as epithelial and mesenchymal states. Therefore, the pancreatic CSCs may be involved in the metastatic spread of pancreatic ductal adenocarcinoma (PDA) [39].

Pancreatic CSCs are resistant to chemotherapy and radiation therapy, which may explain why these treatments do not cure the disease and why there is much interest in targeting these specific cells.

There are three main nonexclusive scenarios for depleting CSC populations. Firstly, developing therapeutic agents that selectively kill CSCs by targeting their self-renewal machinery with-

out affecting normal stem cells. Secondly, identifying cues to force CSCs into differentiation, since this process may be reversible due to the enhanced plasticity of cancer cells; these treatment modalities most certainly need to be accompanied by cytotoxic or other targeted therapies. Thirdly, inhibiting the specific machinery of CSCs enable them with enhanced DNA damage response and/or antiapoptotic response.

Successful targeting of CSC may require the inhibition of multiple stemness pathways as a consequence of their redundancy and/or non-exclusiveness. One of the most promising approaches to target CSCs is certainly the inhibition of developmental pathways (e.g., sonic hedgehog, mammalian target of rapamycin (mTOR), bone-morphogenic protein (BMP), Notch, or Wnt). Interestingly, Mueller et al. have recently shown that neither SHH inhibition alone nor SHH inhibition as a supplement to chemotherapy was capable of effectively diminishing the CSC pool [26]. Inhibition of the mTOR pathway by rapamycin was not sufficient to eliminate CSCs completely, but the combined inhibition of SHH and mTOR, together with chemotherapy, resulted in the desired complete targeting of the CSCs. This triple therapy resulted in the virtually complete depletion of the pancreatic CSC pool.

Oncofetal Signaling Pathways: Notch and Hedehog

The Notch signaling pathway has been known to play critical mechanistic roles in the development of organs, tissue proliferation, differentiation, and apoptosis. It is believed that Notch interacts in the early developmental stages by maintaining pancreatic epithelial cells in a progenitor state and, thus, delaying their differentiation until it becomes appropriate. In the adult pancreas, little or no expression of Notch signaling has been found [27].

Notch activity is required for TGF-induced acinar-to-ductal transition. It was shown that both Notch activation and activated K-Ras signaling act cooperatively to initiate pancreatic carcinogenesis [7]. The molecular mechanisms by which Notch contributes to pancreatic cancer are poorly understood. Recently, Notch signaling was also found to be involved in pancreatic CSCs, which may be related to pancreatic cancer aggressiveness [48].

The molecular knowledge of the Notch signaling pathway with respect to pancreatic cancer is considered important for discovering new drugs and the design of novel therapeutic strategies for the treatment of pancreatic cancer.

γ-secretase inhibitors (GSI)

Notch signaling is activated *via* the activity of γ-secretase. Therefore, γ-secretase becomes a target for cancer therapy. Several forms of GSIs have been found to have antitumor effects [36]. Prevention of Notch activation by γ-secretase inhibitors prevents acinar-to-ductal metaplasia in TGF-α-treated cells. Downregulation of Notch-1 using GSI has been found to be correlated with decreased proliferative rates, increased apoptosis, reduced cell migration, and decreased invasive properties of pancreatic cancer cells [47].

Sulforaphane

Sulforaphane is a natural compound with anticancer activity in many human cancers. Sulforaphane was shown to target the pancreatic tumor-initiating cells [17]. Rausch et al. have described the synergistic activity of sulforaphane and sorafenib in eliminating CSCs from pancreatic cancer cells [38]. Moreover, sulforaphane increased the sensitivity of cells to several chemotherapeutic agents (cisplatin, gemcitabine, doxorubicin, and 5-flurouracil) especially by targeting CSCs, which was, in part, due to targeted inactivation of Notch-1 in pancreatic cancer.

Diferuloylmethane

This flavoring agent in food (curcumin) inhibits the cell growth and induced apoptosis in pancreatic cancer through inactivation of the Notch

pathway. Furthermore, it has been reported that curcumin downregulates miR-21 and upregulated miR-200 in pancreatic cancer, leading to increased sensitivity to gemcitabine [1].

Sonic hedgehog (SHH) and other proteins downstream of the hedgehog pathway were recently detected in precursor lesions and samples of primary tumors from patients with pancreatic adenocarcinoma [18]. Several reports have implicated the misregulation of the hedgehog signaling pathway in the initiation and progression of pancreatic cancer. Expression of SHH contributes to the formation of desmoplasia in pancreatic cancer.

Agents like sulforaphane or cyclopamine are been investigated in transgenic mouse models of islet cell tumors. Cyclopamine has shown to increase apoptosis, decrease tumor cell proliferation, and reduce tumor volume. Furthermore, hedgehog inhibition with cyclopamine significantly prolonged median survival in this model. But in another study, cyclopamine decresased chemosensitivity to 5-fluorouracil and gemcitabine under hipoxic conditions in pancreatic carcinoma [31]. Vismodegib (GDC-0449), a small-molecule inhibitor of smoothened (SMO), a key component of Hh signaling, have been tested in a phase I trial in 68 patients, eight of them diagnosed of pancreatic cancer [23]. Tumor responses were observed in 20 patients (19 with basal cell carcinoma and 1 unconfirmed response in medulloblastoma), but no response was seen in the pancreatic cancer patients.

Another inhibitor of smoothened is IPI-926 [30]. Although this compound has been shown an interesting activity in a phase I trial, a phase II study has been halted, after early results indicated a median survival rate less than the 6-month median of gemcitabine alone.

Survival Pathways

EGFR

Blocking epidermal growth factor receptor (EGFR) signaling decreases growth and metastasis of human pancreatic tumor in animal models and enhance the effects of gemcitabine. Both small-molecule tyrosine kinase inhibitors of the EGFR (erlotinib) as well as monoclonal antibodies directed against this molecule (cetuximab or panitumumab) have been studied in patients with pancreatic adenocarcinoma.

A phase III trial from the National Cancer Institute of Canada compared gemcitabine with and without erlotinib in 569 patients with locally advanced or metastatic pancreatic cancer [25]. Combined therapy was associated with few objective responses, and although overall survival was significantly better compared to gemcitabine alone (hazard ratio 0.81, $p=0.038$, median 6.2 vs. 5.9 months), this difference is rather considered clinically relevant. In a recent systematic review including sixteen studies containing 1308 advanced pancreatic cancer patients treated with gemcitabine plus erlotinib [50], the weighted 1-year survival rate, objective response rate and disease control rate based on studies reporting robust results were 27.9, 9.1, and 57.0 %, respectively.

In another phase III randomized trial, the addition of cetuximab to gemcitabine failed to demonstrate a clinically significant advantage over gemcitabine alone [34]. Median survival time was similar between the two arms of the study (6.3 months for the combination arm vs. 5.9 months for the gemcitabine arm; hazard ratio = 1.06; 95 % confidence interval (CI), 0.91–1.23; $p=0.23$, one-sided).

Other EGFR tyrosine kinase inhibitors including gefitinb and lapatinib have been tested in advanced pancreatic carcinoma, but pilot studies did not show sufficient activity to warrant further development.

PI3K/AKT/mTOR

The mTOR signaling network contains a number of tumor suppressor genes including PTEN, LKB1, TSC1, and TSC2, and number of proto-oncogenes including phosphoinositide 3-kinase (PI3K), Akt, and mTOR that are constitutively activated in many tumor types. mTOR plays a pivotal role in integrating a variety of cellular

signals such as the presence of growth factors or nutrient levels to control various cellular processes including cell proliferation, cell survival, and angiogenesis.

The PI3 K/Akt pathway is implicated in increased resistance to radiation and poor overall survival in many human malignancies. In preclinical models, inhibition of PI3K, Akt, and mTOR have demonstrated antitumor activity in pancreatic cancer cells when used alone or in combination with other agents [4]. Multiple PI3K/AKT/mTOR inhibitors are currently being tested in early phase trials in solid tumors including pancreatic cancer. RX-0201 is a novel antisense oligonucleotide that antagonizes Akt signaling. This agent is being assessed in phase II studies in renal cell carcinoma and pancreatic cancer. Other inhibitors, like PBI-05204, MK-2206, and GSK2141795 are currently being examined in phase I studies [32].

Wolpin et al. studied the activity of everolimus in 33 patients with gemcitabine-refractory, metastatic pancreatic cancer [49]. The results were disappointing, with a PFS and OS of 1.8 and 4.5 months, respectively. No objective responses were seen, and only 21% of patients achieved stabilization disease. Nevertheless, other phase II trials with others mTOR inhibitors as metformin, a commonly used antidiabetic drug, are underway. Other groups are testing multitarget drugs, like the dual PI3K and mTOR inhibitor, BEZ235 [44].

IGF1-R

Insulin-like growth factor-1 (IGF-1) leads via its receptor IGF-1R to the activation of the PI3K/Akt pathway, providing antiapoptotic signals to malignant cells. In pancreatic cancer, IGF-1 and its receptor are constitutively overexpressed, and represent a promising survival target, which might be functionally relevant even in K-Ras-mutated tumors. The IGF-1R inhibitor cixutumumab (IMC-A12) has combined with erlotinib and gemcitabine in a phase II trial. Unfortunately, cixutumumab has no shown benefit on PFS or OS [35]. In another phase II trial, patients with a previously untreated metastatic pancreatic adenocarcinoma were randomized to gemcitabine combined with open-label ganitumab, double-blind conatumumab, or double-blind placebo [21]. In total, 125 patients were randomized. The 6-month survival rates were 57% (95% CI 41–70) in the ganitumab arm, 59% (42–73) in the conatumumab arm, and 50% (33–64) in the placebo arm.

Approaches Targeting Angiogenesis

Pancreatic cancers frequently overexpress vascular endothelial growth factor and its receptor. Encouraging data from phase I and II trials justified the development of a phase III trial conducted by the Cancer and Leukemia Group B comparing the addition of bevacizumab to gemcitabine alone in 602 patients with advanced pancreatic cancer [19]. Unfortunately, no differences in median overall survival (5.8 months for gemcitabine/bevacizumab and 5.9 months for gemcitabine/placebo, $p=0.95$) neither in median PFS (3.8 and 2.9 months, respectively, $p=0.07$) were seen.

In another phase III trial, the addition of bevacizumab to gemcitabine plus erlotinib in patients with previously untreated metastatic pancreatic cancer did not improve overall survival (median 7.1 vs. 6 months, $p=0.21$), with a marginally improvement in PFS (median 4.6 vs. 3.6 months) [43].

Another two phase III trials have failed to demonstrate a benefit for the combination of sorafenib or axitinib with gemcitabine [10, 20]. The hypovascularity of the stroma in pancreatic cancers may have been responsible for lack of benefit for this class of agents.

Inmunotherapy

Immunotherapy is well tolerated with less toxicity than chemotherapy, and its rational is to stimulate a host immune response that results in long-term tumor destruction. Since it has been postulated that benefit of immunotherapy could be greater in early steps of the disease, with less

tumor burden, many studies have been performed in the adjuvant setting.

There are two major strategies against the tumor cell and surrounding stroma: (1) Vaccine therapy and (2) antibodies against immune checkpoints controlling self-tolerance and modulating the immune response.

1. *Vaccines* GV1001 vaccine therapy has shown good tolerability in phase I/II trials. Other vaccine therapies that are been tested in phase I or II trials are based on targeting survivin or heat shock protein (HSP). In a single-center phase II study, GM-CSF vaccine showed promising results when combined to surgery followed by chemoradiation [24]. Algenpantucel-L is composed of irradiated, live, allogeneic human pancreatic cancer cells expressing the enzyme alpha-1,3 galactosyl transferase (alpha-GT). Recently Hardacre et al. have published the results of a multicenter phase II trial of algenpantucel-L with gemcitabine and 5-FU/radiation after R0/R1 resection [11]. After a median follow-up of 21 months, the 12-month disease-free survival was 62 %, and the 12-month overall survival was 86 %. Nevertheless these data need to be confirmed in phase III trials.
2. *Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4)* is one immune checkpoint that plays a critical role in the immune response driven by T cells. Ipilimumab, a monoclonal antibody against CTLA-4 have shown benefit in melanoma or lung cancer patients, and also is being evaluated in pancreatic cancer. A phase II study of ipilimumab in 27 patients with locally advanced or metastatic pancreatic cancer showed no survival advantage, but one of these patients had delayed clinical response [41]. CD40 is a tumor necrosis factor receptor superfamily member that has been shown to be a key regulatory step in the development of T-cell-dependent antitumor immunity. In a phase I trial a CD40 agonist CP 870,893 has been combined with gemcitabine in patients with advanced pancreatic cancer [3]. After initial promising results phase II studies have been started.

Conclusions

In the last couple of decades, no relevant advances in the treatment of patients with metastatic pancreatic cancer were seen. However, in the last months, we have noticed that the advances of the molecular understanding of pancreatic cancer have translated into active clinical drugs. Another important point is the deeper knowledge of the role that stroma has for both cancer development and progression and as a barrier to the optimal delivery of chemotherapy. We are also aware of the existence of a different subset of cells like CSCs and PSCs that seem to be involved in tumor development and tumor resistance to chemotherapy and radiotherapy and even in the resistance to novel targeted agents.

Some of these recent advances in the molecular biology of pancreatic cancers are translating in new therapeutic targets and treatment strategies. These advances have recently made that a SPARC-binding nab-paclitaxel have been shown to improve overall survival when given in combination with gemcitabine. More effort should be placed in understanding the molecular effects of new drugs. In this sense, the design of new clinical trials should be driven to those patient populations with high expression or susceptibility to the targets we are acting against. There is still a long way to go.

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Targeted Therapies in Hepatocellular Carcinoma

12

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Introduction

Hepatocellular carcinoma (HCC) is a multistep process which starts from liver cirrhosis (LC). The most common causes of LC include Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) chronic liver viral infections, metabolic diseases such as nonalcoholic fatty liver disease (NAFLD), and less commonly, genetic diseases such as alpha-1 antitrypsine deficit and hemochromatosis; other minor causes are autoimmune disease and B1 aflatoxin intoxication [1]. The geographical distribution of HCC reflects the distribution of these causes. It could be classified in areas with very high incidence, as Africa and Asia, and areas with lower incidence, as North America, North Europe, and Australia [2]. In the last decade, many biomolecular mechanisms have been discovered allowing the development of numerous molecules active against HCC defined as "targeted therapies." Actually the only therapy approved for HCC is sorafenib, but there are many drugs approved in other tumors that are effective in HCC, but are still under investigation in preclinical and clinical studies. These are effective as monotherapies or as combination therapy with chemotherapeutic agents and other targeted agents.

Target Therapy Related to Biological Pathways

Target therapies for HCC could be classified according to the biological pathways involved in HCC. The regimens under investigation are distinguished in monotherapy or combination therapy. The efficacy in terms of progression-free survival (PFS), time to progression (TTP), and overall survival (OS) is the main goal of these regimens. Molecular mechanisms involved in hepatocarcinogenesis are: mitogen-activated protein kinases (MAPK), phosphoinositide-3-kinase, AKT/mTOR, c-MET, IGF, Wnt-β-catenin, hedgehog, VEGFR, platelet-derived growth factor receptors (PDGFR); and many targeted therapies act through these mechanisms.

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Monotherapy

MAP-Kinase-Mediated Pathway

One of the most important pathways is that related to the MAPK cascade. Many transmembrane receptors including EGFR, VEGFR, and PDGFR exploit this pathway. It can be blocked by two different mechanisms: through the inhibition of the kinase or through a binding of monoclonal antibodies with the receptor extracellular domain; and they transduce the signal to the nucleus. The first step is Ras phosphorylation by guanosine-diphosphate (GDP) release and substitution with guanosine-triphosphate (GTP), which makes it activated. This activation allows the formation of the binding site for Raf, which can be activated in turn. A direct inhibitor of Ras is sorafenib that blocks tyrosine-kinase activity in membrane receptors, such as VEGFR and PDGFR, and determines the inhibition of tumor vascularization and proliferation. This drug was approved for the systemic treatment of patients with advanced HCC, class C according to Barcelona clinic liver cancer (BCLC), not susceptible for surgery. SHARP [3] and Asian-Pacific studies [4] showed a statistically significant increase of median survival by about 3 months compared to placebo in the SHARP trial and by about 2 months in the Asian-Pacific trial (SHARP: 10.7 vs. 7.9 months, HR = 0.69, 95% CI 0.55–0.87, $p < 0.001$; Asian-Pacific: 6.5 vs. 4.2 months, HR = 0.68, 95% CI 0.50–0.93, $p = 0.014$) [3, 4]. Similar results were observed in both these studies for median time to progression (TTP), which was doubled in sorafenib arm (SHARP: 5.5 vs. 2.8 months, HR = 0.58 95% CI 0.45–0.74, $p < 0.001$; Asian-Pacific: 2.8 vs. 1.4 months, HR = 0.57, 95% CI 0.42–0.79, $p = 0.0005$) (Table 12.1). Therefore, sorafenib globally achieved a good efficacy over placebo in terms of TTP and OS and kept an acceptable tolerability profile. These results could be intended as an increase by about 11% for 1-year survival rate and by about 31% of death risk reduction. This benefit remains valid where these data are adjusted for some prognostic factors by multivariate analysis, including eastern cooperative oncology group (ECOG) performance status, vascular invasion, extrahepatic extension, Child-Pugh status, α-fetoprotein, serum albumin, alkaline phosphatase, and bilirubin [3, 4]. The results relative to response are disappointing. Indeed, partial responses are just 7 out of 299 (2%) in the SHARP trial and just 5 out of 150 patients (3.3%) in the Asian-Pacific trial. In the meantime, no complete responses were observed. Though the sorafenib blocks the Ras protein, other biological pathways are involved in the oncogenetic process through which the signal transduction continues to the nucleus. In fact, the activation of Ras induces activation of Raf which induces MAP-MEK-ERK cascade activation. A drug active on MAP Kinase (MEK) protein is selumetinib, which showed poor efficacy, with very low PFS, TTP, and OS (1.4, 1.4 and 4.2 months, respectively) [5–7] (Table 12.1). Later, the activated MAP-MEK-ERK complex translocates to the nucleus where activates transcriptional factors and other nuclear proteins prompting cell proliferation by cell cycle regulation [8–11].

Many other drugs are active on this cascade. For example, gefitinib is an EGFR-inhibitor, which in vitro decreases cell growth and supports apoptosis through Bcl-2 blockade [12]. Few clinical data on HCC treatment with gefitinib are available. A phase II clinical trial with gefitinib as first-line treatment of advanced HCC in patients with Child-Pugh C class showed no complete responses (CR), one partial response (PR) and seven stable diseases but only 31 patients are enrolled. Such poor efficacy is added to a high toxicity, frequently G3 adverse reactions, in most cases neutropenia, particularly important in patients already showing cirrhotic neutropenia [13] (Table 12.1).

Erlotinib, another tyrosine-kinase EGFR-inhibitor, has proved to be effective against HCC in different phase II clinical trials. Recent clinical trials [14], conducted on patients with different etiology (alcohol, HCV, and HBV), showed that the first-line treatment with erlotinib induces a 4–9 months PFS and a 10.8–15 median OS. Similar results are showed by Melanie et al. with 9 months PFS and 15,7 median OS (Table 12.1). Despite the good efficacy demonstrated by erlotinib and similar results in all studies, this drug is

Table 12.1 Targeted therapies according to biological pathways and clinical benefit

Drugs	Pathway	*n*. patients	PFS (months)	TTP (months)	OS (months)
Sorafenib Llovet et al. [3]	*MAP-kinase*	299	–	5.5	10.7
Cheng et al. [4]		150	–	2.8	6.7
Selumetinib O'Neil et al. [5]	*MEK-ERK*	17	1.4	1.4	4.2
Gefitinib O'Dwyer et al. [13]	*EGFR inhibitor*	–	–	–	–
Erlotinib Philip et al. [14]	*EGFR inhibitor*	40	6.5	–	10.75
Melanie B. Thomas [15]		40	9	–	15.7
Lipatinib Bekaii-Saab et al. [16]	*HER2-/ EGFR-inhibitor*	–	1.9	–	–
Ramanathan et al. [17]		–	2.3	–	–
Brivanib Park et al. [18]	*VEGFR/FGFR inhibitor*	55	4.7	5	10
Llovet et al. [19]		–	–	4.2	9.4
Finn et al. [20]		46	–	6.9	9.79
Raoul et al. [21]		96		2.8	10
Linifanib Toh et al. [22]	*VEGF/PDGF/c-kit inhibitor*	44	1.4	5.4	9.7
Sunitinib Zhu et al. [23]	*VEGF inhibitor*	34	3.9	–	9.8
Faivre et al. [24]		37	–	5.3	8
Koeberle et al. [25]		45	12	–	–
Worns et al. [26]		11		3.5	8.4
Cedranib Alberts et al. [27]	*VEGF inhibitor*	28	–	2.8	5.8
Cetuximab Zhu et al. [28]	*Anti-EGFR monoclonal ab*	30	1.4	–	–
Bevacizumab Siegel et al. [29]	*Anti-VEGF monoclonal ab*	46	6.9	–	–
Everolimus Zhu et al. [34]	*PI3K/Akt inhibitor*	28	3.8	–	8.4
Shiah et al. [35]		39	3.4	–	8
Sirolimus Rizell et al. [2008]	*mTOR inhibitor*	21	6.5	–	–
Decaens et al. [2012]		25	–	15.3	6.6
Sorafenib + TACE Sansonno et al. [6]		62	–	9.2	–
Erlotinib + Bevacizumab Kaseb et al. [51]		59	7.2	–	13.7
GEMOX-B Zhu et al. [7]		33	5.3	–	9.6
Bevacizumab + Capecitabina Hsu et al. [53]		45	2.7	–	5.9
Bevacizumab + Capecitabine + Oxaliplatino Sun et al. [55]		40	6.8	–	9.8
Bevacizumab + Sirolimus Choo et al. [57]		24	5.5	–	9.4

burdened by severe toxicity with G3–4 toxicity [15].

Also lapatinib, another tyrosine-kinase HER2- and EGFR-inhibitor, has shown poor efficacy on HCC, obtaining a PFS of 1.9 and 2.3 months in the studies by Bakaii-Sab et al. [16] and Ramanathan et al. [17], respectively.

Brivanib is a tyrosine-kinase VEGFR- and FGFR-inhibitor. The first study that demonstrated its efficacy was a phase II study, which tested its antitumor effect in first-line treatment of HCC. This study demonstrated a PFS, a TTP, and an OS of 4.7, 5.4, and 10 months, respectively [18]. These data are confirmed in a recent phase III study on brivanib, reported in EASL 2012 proceedings by Llovet et al. showed a greater efficacy (OS and TTP up to 9.4 and 4.2 months, respectively) than placebo arm [19]. Brivanib is also effective in second-line treatment in patients treated with Sorafenib and Talidomide, achieving TTP (according to mRECIST criteria) and OS by 6.9 and 9.79 months. Besides, it has a good safety profile, with few side effects. Its effect on IV collagen, which could be a good response predictor factor, has been demonstrated [20]. Raoul et al. [21] conducted a phase II study of brivanib in patients with advanced or metastatic HCC who had no prior systemic therapy or one prior regimen of an angiogenesis inhibitor. 96 patients were enrolled, 55 in cohorts with no prior systemic therapy, median OS was 10 months and median TTP was 2.8 months. Therefore, Brivanib appears to have activity as both first-line and second-line postsorafenib systemic treatment in HCC. Linifanib is a tyrosine-kinase inhibitor active on VEGF, PDGF, and c-kit. To date, a phase II study has enrolled 44 patients and has shown a PFS of 16 weeks and a TTP and an OS of 5.4 and 9.7 months, respectively 31% [22].

Sunitinib, a multi-targeted receptor tyrosine-kinase inhibitor active on VEGF, has shown its efficacy in phase II studies with a PFS and an OS of 3.9 months (95% CI, 2.6–6.9 months) and 9.8 months (95% CI, 7.4 months). Moreover, IL-6, SDF-1-alfa, scKIT, and circulating progenitor cells (CPC) high levels correlate with a worse prognosis [23].

Better effects have been shown by Faivre et al. with a 5.3 months TTP (95% CI 2.7–7.9 months) and an 8.0 months OS (95% CI 4.4–13 months) [24]. A cooperative Swiss study on 45 patients has shown a 33% PFS 12 of Sunitinib (95% CI 20–47%) [25]. Worns et al. evidenced a 3.5 months TTP and an 8.4 months OS [26]. Even this inhibitor shows significant side effects, such as thrombocytopenia and intestinal bleeding.

Cedranib, a tyrosine-kinase inhibitor active on VEGF, showed a 5.8 months OS (95% CI 3.4–7.3 months) as well as a 2.8 months TTP (95% CI 2.3–4.4 months) [27].

Conversely, there are other molecules directed against the extracellular domain.

The first drug is cetuximab, an anti-EGFR monoclonal antibody, in phase II studies, has demonstrated poor activity against HCC, obtaining a PFS of 1.4 months [28].

The second one, bevacizumab, an anti-VEGF monoclonal antibody, has demonstrated efficacy in HCC patients, with an improvement up to 6.9 months of PFS. However, this efficacy is limited by high toxicity such as bleeding and deep venous thrombosis, which may worsen the liver function in already cirrhotic patients [29].

It has been demonstrated that bevacizumab significantly reduces VEGF serum levels and increases circulating endothelial cells (CEC), showing a high OR in about 14% patients (6 of 43 patients). Moreover, patients with low IL-6 and IL-8 levels at baseline had a better disease control (DCR), so high levels of these interleukins at baseline would be predictor factors of low PFS [30].

PI3K/Akt/mTOR-Mediated Pathway

PI3K/Akt/mTOR-mediated pathway starts its signal transduction through the activation of phosphatidylinositol 4,5 phosphate (PIP2) by PI3K-mediated phosphorylation. PIP2 becomes phosphatidylinositole 3,4,5 triphosphate (PIP3), which binds and activates serine/threonine kinase Akt. Phosphatase and tensin homolog (PTEN) blocks PIP3K, because is a lipidic phosphatase which dephosphorylates PIP3. So PTEN inactiva-

tion for gene deletion increases PIP3 levels, with subsequent higher levels of active Akt, which inhibits apoptosis and prompts cell proliferation [31]. Since the RAS/RAF/MAPK pathway is the main target of sorafenib, a subsequent activation of PI3K/AKT/mTOR pathway could explain resistance development. A higher activation of Akt and a lower expression of PTEN were found in 40–60% of HCC [32]. Even mTOR overexpression has been reported in 15–40% of HCC [33]. To date, some targeted drugs for this pathway have been identified, such as RG7321, a PI3K inhibitor and perifosins, Akt inhibitor, but these are still under preclinical investigation. mTOR inhibitor including everolimus (RAD001), sirolimus, temsirolimus, reached clinical investigation for HCC [34–36]. It seems really interesting to test the synergistic effects of rapidly accelerated fibrosarcoma (RAF) and mTOR inhibitors on the control of HCC progression. Some preclinical studies started to test this relevant topic [37]. The mTOR everolimus, at a 10 mg/day dose, showed a 3.8 months PFS (95% CI 2.1–4.6 months) and an 8.4 months OS (CI 3.9–21.1 months) [34]; similar results were obtained by another study showing a 16 weeks PFS (CI.11–21 weeks) and a 33.4 weeks OS (CI 9.2–57.6 weeks) [35]. Sirolimus, a mTOR inhibitor, has proven to be efficacious either on cholangiocarcinoma or on HCC, with an OS by 6.5 months (range 0.2–36 months) in one study [38], as well as a 15.3 weeks TTP and a 6.6 months OS in another one [39].

Wnt/β-catenin-Mediated Pathway

Wnt-mediated pathway is mediated by transmembrane receptors, which belong to Frizzled family. These receptors need a signal protein called dishwelled, which regulates the multifunction β-catenin protein. This one influences both cell–cell adhesion and gene regulation. This kind of Frizzled receptor bounds contemporarily a coreceptor which is related to LDL-LRP receptor. The complex Frizzled-LRP activates β-catenins, which binds to cadherins for cell–cell adhesion. If β-catenin is not activated, it is degraded by GSK3β-APC-AXIN1 complex. The binding between Wnt and Frizzled-LRP complex leads to inhibition of β-catenin phosphorylation and degradation with subsequent storage in cytoplasm and nucleus. The excess of β-catenin in the nucleus binds to LEF-1/TC complex, a gene regulator, and shifts Groucho, a corepressor, resulting in coactivation of target genes, such as c-myc, cyclin D1, and survivin, involved in mRNA translation, cell cycle and dedifferentiation, so that cell proliferation and survival is favored. The aberrant activation of the Wnt/β-catenin pathway is induced by various molecular alterations in HCC. These include gain-of-function mutations of CTNNBI gene encoding for β-catenin, loss-of-function mutations of negative regulators of Wnt/β-catenin pathway, such as AXIN1, AXIN2, and APC genes, epigenetic events that change the expression profiles of pathway components, as a consequence of a deregulated interaction between tumor cells and their microenvironment. Several strategies have been proposed to target the Wnt/β-catenin pathway in HCC. These include targeting the interaction between the Wnt ligand and the Frizzled receptor; targeting the destruction complex; targeting the catenin/Lef-Tcf transcriptional complex [40, 41]. Alterations in various genes could activate this cascade, including mutations of APC promoter [42], mutation of β-catenin (more frequent in HCV-related infection) [43], deletions and point mutations of axin-1 [44], and dishwelled overexpression. There are not any drugs developed that act against this biological pathway.

Hedgehog-Mediated Pathway

Even though this oncogenic pathway is well known, specific targeted therapies were not found. In liver carcinogenesis, hedgehog proteins are included in a family of signal molecules, which are normally bound to cholesterol, limiting the spread of those proteins. When these proteins get free, they bind the transmembrane receptor Patched, which activates another membrane protein, Smoothened, triggering its signal to nucleus. Through this way, some genes involved in protein synthesis and cell cycle regulation, induce dedif-

ferentiation and promote cell proliferation and survival [45]. Recently a drug, NVP-LDE225, has been developed in preclinical studies to target this pathway [46].

Combination Therapy

For this reason, some researchers tried to design new therapeutic protocols including both monotherapy regimens and combinations with cytotoxic drugs, such as 5-fluorouracil, octreotide, and doxorubicin. Recent clinical studies evaluating these regimens achieved a limited benefit in terms of TTP. Anyway, by these treatments, higher liver toxicity was observed, including increase of transaminases, bilirubin, and hemorrhagic events, all factors related to LC prognosis [47–49]. For this reason, it is mandatory to accurately evaluate liver function and the stage of cirrhosis when sorafenib is combined with a cytotoxic agent. There are few studies about the association therapy. In a study by Thomas et al., PFS is 3.3 months [50], while Kaseb et al. report that the erlotinib-bevacizumab association increases PFS to 7.2 months [51]. However, this association is not effective in second-line treatment in sorafenib refractory patients, showing a 1.5 months PFS [52]. Due to its high efficacy profile, the effects of adding bevacizumab to gemcitabine-oxaliplatin treatment (GEMOX-B) or to capecitabine have been recently evaluated. Compared to bevacizumab monotherapy, GEMOX-B does not define a better PFS (5.3 months); bevacizumab added to capecitabine does not either improve PFS and OS, which are 2.7 and 5.9 months, respectively, [53] versus 6.9 months PFS with bevacizumab in monotherapy [54]. The bevacizumab association with capecitabine-oxaliplatin shows the same efficacy of bevacizumab in monotherapy in terms of PFS and OS, which are 6.8 and 9.8 months, respectively [55]. The comparison between TACE + sorafenib and TACE + bevacizumab efficacy shows the same good results with a PFS of 6 months in about 65% and 1 year in about 25% of patients; however, these data have low statistical power because the study was conducted in a small population ($n=25$) [56]. To date, a unique phase I study exists on bevacizumab + sirolimus association which does not evidence an improved efficacy, showing a PFS and an OS of 5.5 and 9.4 months, respectively [57]. These studies about bevacizumab in HCC suggest that in this setting it has better efficacy in monotherapy than in combination with chemotherapy. These results are opposed to those obtained for other cancers and need to be explained by specific studies about biological bases.

There are many ongoing trials (phase I–II) that are evaluating the combination of sorafenib with other target agents, such as anti-EGFR TKIs, monoclonal antibodies, and mTOR inhibitors.

Conclusions

Since the discovery of sorafenib and the demonstration of its efficacy against HCC many strides have been made in the systemic therapy of this malignancy. In fact, the discovery of new biological pathways has allowed the development of new drugs active against HCC with selective mechanisms that reduce the progression of this cancer. This allowed leading the way toward personalized therapy to the patient. Although many of these drugs have demonstrated clinical efficacy in terms of PFS, TTP, and OS, most of them have a limited clinical application due to the high percentage of side effects. Also the stage of the underlying liver disease may limit the use of targeted therapy in HCC patients. Most of these patients are cirrhotic, and this disease can reduce the function of the liver parenchyma thus reducing the clinical applicability of these drugs. Another weapon available for clinicians is the opportunity to choose whether to apply targeted therapy as monotherapy or combination therapy. The latter could be a great chance since in HCC many pathogenic pathways are involved. Currently, sorafenib is the only targeted therapy on label and active against HCC. Indeed, it has demonstrated high profiles of OS, TTP, and PFS compared to other therapies. Conversely, the combination therapy achieving the best outcomes includes erlotinib and bevacizumab. However, until now this combination has shown low clini-

cal safety profiles being burdened with numerous side effects. So nowadays an optimal staging is important to allow the patient to decide the best personalization of therapy. Much work remains to be done to assess the role of targeted therapy in the adjuvant, neoadjuvant, and metastatic setting, to determine the optimal combination of treatments, either tandem-targeted agents or with conventional cytotoxins, and evaluate the role of sequential versus concurrent therapy.

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Targeted Therapies for Colorectal Cancer

13

Antonio Russo, Antonio Galvano, Giuseppe Bronte and Marc Peeters

Salvage Targeted Therapy in mCRC

A great portion of colorectal cancer (CRC) patients develop distant metastases, which are not resectable. These patients are suitable for first-line systemic chemotherapy, with the aim to prolong survival and even improve the quality of life in most cases.

Nowadays, there are many regimens approved for the treatment of metastatic CRC (mCRC). For this reason, the main challenge for oncologists is represented by the choice of the best combination of drugs for each patient, taking into account, especially the relationship between the costs (in terms of toxicity and economic resources) and the benefits expected from the chosen treatment. This choice is difficult because the major international organizations of oncologists (National Comprehensive Cancer Network (NCCN), European Society for Medical Oncology (ESMO), and American Society of Clinical Oncology (ASCO)) use the same schedules of treatment, but propose different algorithms according to the results of some questionable studies. For these reasons, there is an absolute need to identify quickly a sequence of treatments as much as possible uniquely, able to ensure the best possible outcomes for our patients.

Recently, the treatment of unresectable mCRC includes several drugs used in combination regimen or as monotherapy. The first important drug developed was 5-fluorouracil. The introduction of new cytotoxic agents into clinical practice, such as irinotecan and oxaliplatin has improved the response rate (RR), progression-free survival (PFS), and overall survival (OS) from 15–20 %, 5–6, and 10–12 months to 30–40 %, 8–10, and 20–24 months, respectively [1, 2].

The addition of targeted agents to such chemotherapy regimens has helped to substantially improve survival in mCRC patients.

The targeted drugs approved for mCRC include three groups: monoclonal antibodies (mAbs) against VEGF (bevacizumab) and epidermal growth factor receptor (EGFR) (cetuximab and panitumumab), recombinant fusion proteins against angiogenic factors (aflibercept), molecules that inhibit the tyrosine kinase receptors located on the cancer cell membrane (TKIs, such as regorafenib) (Table 13.1).

As regards antiangiogenic agents, the process of angiogenesis is one of the fundamental steps

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Table 13.1 Summary of trials comparing the combination of standard chemotherapy (CT) + targeted drug with standard chemotherapy alone

Trials	Targeted drugs vs. CT comparison	Number of patients	Biomarkers	Outcomes (months)	
				PFS	OS
Crystal	Folfiri Cetuximab vs. folfiri	1198	KRAS WT	8.9 vs. 8.0	19.9 vs. 18.6
		267	All-RAS WT	11.4 vs. 8.4	28.4 vs. 20.2
Prime	Folfox4 Panitumumab vs. folfox4	1096	KRAS WT	10.0 vs. 8.6	23.9 vs. 19.7
		512	All-RAS WT	10.1 vs. 7.9	26.0 vs. 20.2
E3200	Folfox4 Bevacizumab vs. folfox4	829	ND	7.3 vs. 4.7	12.9 vs. 10.8
Velour	Folfiri Aflibercept vs. folfiri	1226	ND	6.9 vs. 4.6	13.5 vs. 12.0
TML (ML 18147)	CT Bevacizumab vs. CT	820	ND	11.2 vs. 9.8	5.7 vs. 4.1

necessary for growth and tumor development. In particular, it is the process by which new blood vessels are formed from preexisting vessels, a key step in the spread of tumors. In addition, it is precisely for these reasons that the inhibition of angiogenesis is one of the most interesting and possible anticancer strategies, through the inhibition of VEGF and its signaling pathway. The family of VEGF consists of at least five known ligands, VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PGF) and three receptors, VEGFR-1, 2, and 3 [3].

The first anti-VEGF mAb has been developed against VEGF-A, which is the main promoter of angiogenesis. The first of these drugs to be approved in combination with chemotherapy was bevacizumab, a humanized mAb. It is the mechanism of action consists of the sequestration of VEGF from the circulation thus preventing binding to its receptor [4].

This drug induces the regression of newly formed malignant vessels, the normalization of vascular architecture, the inhibition of neovascularization, and tumor growth. Bevacizumab has shown its efficacy in combination with standard chemotherapy compared to chemotherapy alone. However, it does not have efficacy as a single agent [5]. This phenomenon is probably due to its action on tumor vessels, and it would seem to be reflected in a better diffusion of the other chemotherapeutic agents into the tumor structure [6].

As regards toxicity, for all the antiangiogenic drugs, there are common side effects, which include hypertension, arterial thromboembolism, bleeding, proteinuria, wound healing complications, voice changes, and rarely, intestinal perforations and reversible posterior leukoencephalopathy. All these effects are treatable and reversible with appropriate treatment or drug discontinuation.

Another group of targeted agents include anti-EGFR mAbs. EGFR regulates the signaling pathways involved in cell differentiation, cell proliferation, and angiogenesis, and it is overexpressed in about 60–75 % of CRCs [7].

EGFR pathway is mediated by the binding of ligand to the receptor. This receptor activates a cascade of critical signaling pathways, including the RAS-RAF-MEK-ERK and PI3K-Akt-mTOR pathways, which involve several cellular functions and cancer cell survival ([8, 9]; Fig. 13.1).

Currently, there are anti-EGFR two molecules approved for the treatment in various lines of chemotherapy for mCRC patients: cetuximab, a chimeric immunoglobulin G (IgG)1 and panitumumab, a fully human IgG2 antibody.

The main help in the difficult choice of these regimens comes from the research of prognostic and predictive factors. Among the predictive factors for the treatment of mCRC patients, the most studied and validated include the genes belonging to the RAS family (H-RAS, NRAS, and KRAS). In addition, BRAF seems to have a predictive role, although more scientific evidences are available to support it as prognostic factors.

Recent findings have shown that the mutations in KRAS and NRAS codons 12, 13, and 61 result in constitutive activation of the RAS-RAF-ERK pathway, which then results in resistance to anti-EGFR therapy in approximately 35–40 % of CRC tumors. Thus, KRAS status became the

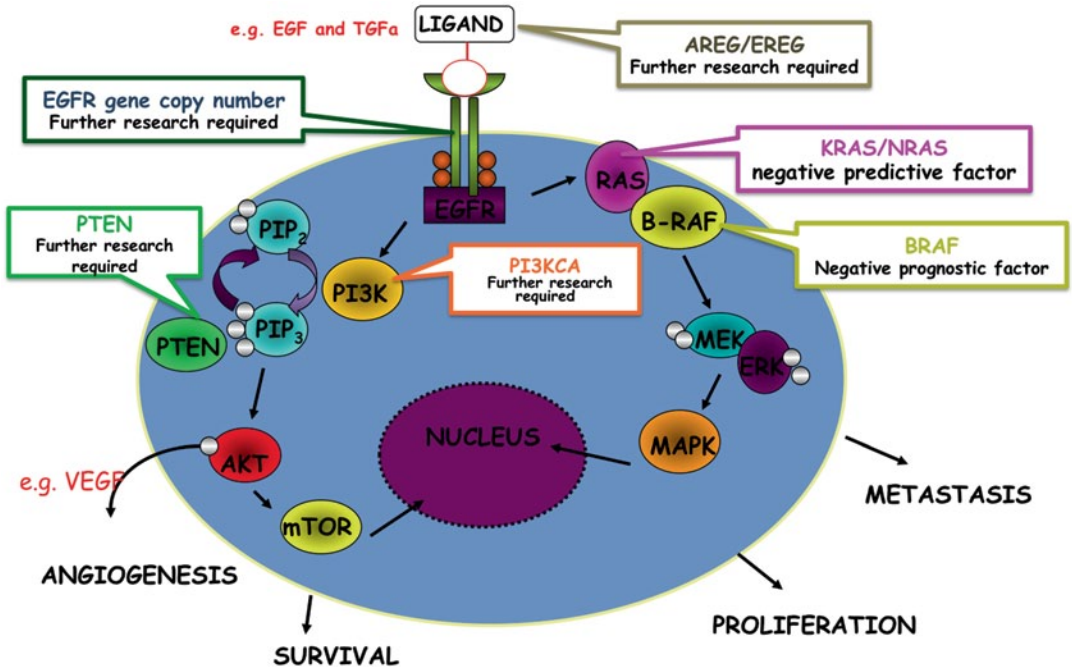

Fig. 13.1 EGFR pathway and corresponding alterations in the molecules involved in transduction signaling

most important predictive biomarker for mCRC. In the meantime, some evidence suggest a role of KRAS mutations to identify those patients with poor prognosis [10–12].

However, a pooled analysis of refractory cetuximab-treated mCRC suggested that not all KRAS mutations are equal in their ability to confer resistance to anti-EGFR therapy, showing a clinical benefit by cetuximab in patients with a Gly13Asp KRAS mutation in codon 13 [13, 14].

This charming biological hypothesis, however, seems finally waned in the light of recent phase II study result presented at the 2014 ASCO Annual Meeting in which no benefit was demonstrated in a cohort of patients with this particular mutation [15].

NRAS is a gene closely related to KRAS and its mutations are present in approximately 3–5 % of patients with CRC. Even the state of mutant NRAS is shown to be related to a lower response to an anti-EGFR mAb. In particular, it seems that when patients with additional mutations in exons 2, 3, and 4 of NRAS and in exons 3 and 4 of KRAS are excluded from the population of patients without exon 2 of KRAS mutations (wild-type), the results of effectiveness are better, assuming that mutations of RAS may be assumed as a biological negative predictive marker of efficacy for the treatment with anti-EGFR agents in mCRC patients [16–20].

BRAF is another gene involved in the genesis of resistance to anti-EGFR drugs and its mutant status (V600E) is present in approximately 10 % of CRC cases. Both NRAS and BRAF mutations appear to be mutually exclusive in respect of KRAS status. BRAF mutational status is considered important both as a prognostic factor and as a predictor for anti-EGFR response, although the first one appears predominant.

PIK3CA mutations have been reported in approximately 15–20 % of CRC and in 20 % are coexpressed in KRAS mutant CRC. The mutations were found in exons 9 and 20, although it is the latter that was associated with increased resistance to anti- EGFR (Fig. 13.2).

Because the number of biomarkers of resistance to anti-EGFR-based treatment is increasing, in the next few years a proper way has to be found to identify those patients who can really benefit by an anti-EGFR strategy.

Skin alterations, in all their forms (acneiform eruptions, xerosis, nail changes, hairy alterations,

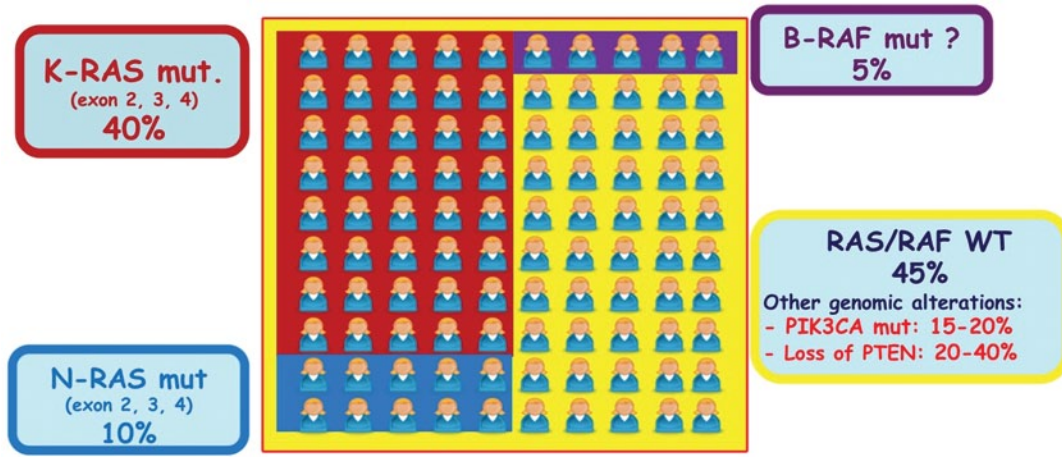

Fig. 13.2 Distribution of gene mutations playing prognostic and predictive roles for treatment with anti-EGFR agents

hyperpigmentation, and telangiectasia) are the most common examples of skin toxicity, present in about 80% of the patients enrolled in the main registration studies, demonstrating that its grading was positively correlated with the effectiveness of treatment [21–24]. Among other class-specific toxicity hypomagnesemia, infusion-related reactions and hepatic abnormalities could also be mentioned for a potential predictive role of drug response [25, 26].

All the findings from clinical trials on targeted therapy allow the proposal of an algorithm to select the best treatment sequence. On the basis of the results in terms of PFS and OS from the major clinical studies, molecular profile seems the main tool to guide the assignment of each mCRC patient to the right treatment.

The main conditioning factor in the first-line treatment choice is clearly the mutational status of KRAS and NRAS genes. The absence of both KRAS and NRAS mutations (H-RAS results wild type in more than 99% of cases) allows to define a new subset of mCRC patients defined as "All-RAS wild-type" particularly sensitive to the action of anti-EGFR mAbs in addition to a standard chemotherapy doublet.

This benefit results from the analysis of the CRYSTAL study (FOLFIRI plus cetuximab) and PRIME study (FOLFOX plus panitumumab). CRYSTAL study results were favorable to FOLFIRI plus cetuximab when compared to FOLFIRI alone improving significantly OS (median survival, 23.5 vs. 20 months; hazard ratio (HR), 0.796; $p=0.0093$) in the KRAS wild-type subgroup but not in the KRAS and BRAF wild-type subgroup, confirming the probable only poor prognostic role of the BRAF mutant status [23, 27]. At the ASCO 2014 Annual Meeting, the retrospective efficacy data of the All-RAS wild-type subgroup were presented. New RAS mutations resulted in about 15% of patients, based on the data coming from the same analysis conducted on the PRIME study (see below), confirming the advantage in OS (28.4 vs. 20.2 months) for this subgroup of patients [28].

Moreover, panitumumab has been evaluated in first-line setting by the PRIME study, in which a standard FOLFOX regimen was tested with or without panitumumab in patients with mCRC no previously treated, showing a modest advantage in PFS only for the KRAS wild-type subgroup (9.6 vs. 8 months HR 0.80, $p=0.02$) but not in terms of OS (23.9 vs. 19.7 months), suggesting that panitumumab should have a lower efficacy compared to cetuximab [29]. Furthermore, a detrimental effect was observed in KRAS mutant patients, who received panitumumab in combination with FOLFOX-4 compared to FOLFOX-4 alone arm, like in the OPUS trial, with no significant difference in PFS between patients receiving

panitumumab plus irinotecan-based chemotherapy (FOLFIRI) and those receiving FOLFIRI alone in the second-line treatment trial [30]. The negative effect of panitumumab in addition to FOLFOX was highlighted in a population of patients without mutation in exon 2 of the KRAS gene, but those patients could have mutations in the other RAS genes. A test of interaction was performed within a recent retrospective analysis of the PRIME study that analyzed the results based on the new discoveries on the role of mutations in the RAS genes, and it showed that there was a difference in OS between patients mutant for RAS and those who were All-RAS wild-type, suggesting that this subset of mutations could contribute to a worse outcome [31]. Indeed, All-RAS wild-type patients had a higher OS of approximately 5.8 months compared to mutant RAS and also the severity of adverse events associated with panitumumab–FOLFOX4 in the wild-type group and mutant RAS subgroups were similar to the previously reported safety findings for KRAS in the PRIME trial [29].

In conclusion, deeming retrospective analyses as exploratory because of some limitations (alpha error calculation, ITT population), the authors conclude that probably RAS mutations in addition to KRAS exon 2 mutations could predict a lack of response to anti-EGFR therapy in mCRC patients and that the pooled trials or meta-analyses of anti-EGFR therapy are needed to confirm these findings. From the results above, there is a widespread idea that the priority for first-line treatment in mCRC patients must be a combination of a doublet of standard chemotherapy (FOLFOX or FOLFIRI) plus an anti-EGFR mAb (panitumumab or cetuximab, respectively) as standard treatment suggested. Indeed, as regards the use of bevacizumab in first-line treatment, it was evaluated in addition to irinotecan or oxaliplatin-containing regimens, but for all phase III trials available, bevacizumab has never granted a clear advantage in terms of OS but only in PFS [32–34]. As a consequence, for KRAS or NRAS (RAS) mutant mCRC patients who cannot receive an anti-EGFR antibody, an oxaliplatin-based doublet with bevacizumab (not FOLFIRI because of the lack of phase III studies comparing FOLFIRI + bevacizumab versus FOLFIRI alone) represents a good option for delaying disease progression and thus reducing the risk of death.

After a first-line progression, the choice of a second-line treatment depends on the clinical condition of the patient and on the drugs that have been used as a first-line regimen. On the basis of these preliminary considerations, for All-RAS wild-type patients progressing after a first-line FOLFIRI plus cetuximab, the best option available seems to be an oxaliplatin-based doublet plus bevacizumab (capecitabine plus oxaliplatin (CAPOX) or FOLFOX + bevacizumab). This indication is based on the results from the phase III E3200 study, in which an advantage in terms of OS (12.9 vs. 10.8 months) was reached in the experimental arm (FOLFOX 4+bevacizumab), if compared to a standard control arm (FOLFOX4 alone) [35]. Whereas for those patients progressing after a first-line FOLFOX + panitumumab regimen, the main option seems to be the combination of the standard FOLFIRI regimen with aflibercept, as evidenced by the results of the VELOUR trial. Aflibercept is a soluble fusion protein of the human extracellular domains of VEGFR-1 and VEGFR-2 and the Fc portion of human IgG. Aflibercept binds to both VEGF-A and PlGF with a higher affinity than mAbs and essentially renders the VEGF-A and PlGF ligands unable to bind and activate cell receptors. Aflibercept was engineered to optimize pharmacokinetic properties while still maintaining the potent VEGF blocking activity compared with that demonstrated by other anti-VEGF antibodies [36]. Aflibercept was tested in combination with a FOLFIRI standard regimen versus a FOLFIRI alone regimen (VELOUR trial) [37], adding a significant increase in terms of OS (13.5 vs. 12.1 months; HR 0.81). For these reasons aflibercept can be considered a new treatment option in combination with FOLFIRI, above all for those RAS mutant patients in progression after oxaliplatin-based chemotherapy (the subgroup analysis revealed that this advantage was not statistically significant in patients undergone first-line bevacizumab-containing regimen) or with symptomatic disease [38]. In the view of its

antiangiogenic mechanism of action aflibercept is also an example of rechallenge after a progression from a prior antiangiogenic containing chemotherapy. It is interesting to note that the results are very similar to those reported by the study TML as regards OS and PFS, with an increase in toxicity of chemotherapy-associated adverse events: diarrhea, stomatitis, fatigue, neutropenia, neutropenia, and complicated [39].

An important clinical question is whether or not it is appropriate to deliver bevacizumab with other chemotherapeutic agents in patients with mCRC progressing from a first-line treatment containing bevacizumab. The feeling that the topic would be of great interest to be investigated was given by the initial data of the BRiTE study, a large prospective observational study, whose results suggested that continuing VEGF inhibition by bevacizumab beyond an initial progression could play an important role by improving the global success of therapy [40]. Bevacizumab beyond progression was mainly explored by the TML trial, in which 820 patients who were progressing up to 3 months after discontinuing first-line bevacizumab plus chemotherapy were assigned to a different schedule of treatment with or without bevacizumab, showing a benefit of the continuation of bevacizumab in both PFS (5.7 vs. 4.1 months) and OS (11.2 vs. 9.8 months, HR 0.81). A subgroup analysis in this study also showed that these advantages were particularly evident for those patients with a first-line PFS>9 months (HR 0.73; IC 95% 0.58−0.92) [41]. This benefit was further confirmed by a small randomized phase II Italian trial (BEBYP trial—PFS, HR: 0.65, $p=0.0062$) whose results in OS are currently immature [42]. Therefore, these data support the idea that continuing bevacizumab beyond progression by changing only the chemotherapy regimen leads to better outcomes. This fact could be suggested, also considering that it would represent a further line of targeted therapy into the global strategy of continuum of care giving a profound rationale to suggest this approach even for first-line therapy in RAS mutant progressing patients. As regards the cetuximab-based rechallenge, no confirmatory data are available after the positive report of small experiences [43].

As a result of all these available treatment options, the choice of second-line treatment in patients with mutant RAS must take into account the duration of first-line containing bevacizumab treatment. Indeed, if it is quite short, a better therapeutic option could be to use aflibercept + FOLFIRI instead of continuing bevacizumab.

By the addition of these new agents, patients with mCRC have now earned numerous therapeutic opportunities for the treatment of their disease. In particular, the achieved improvement in clinical benefit that is directly responsible for an improvement of the performance status too. As a logical consequence, those patients in progression after a second-line chemotherapy, who are still fit, could benefit from a further chemotherapeutic treatment. In the field of biological therapies, a new molecule has been provided. It has recently been approved in this setting of patients, which encloses about 40% of patients with mCRC. Regorafenib is a tyrosine kinase inhibitor that inhibits VEGFR-1, VEGFR-2, and VEGFR-3, as well as PDGFRβ, Tie-2, c-KIT, FGFR-1, RET, and BRAF [44]. Because of its strong antiproliferative and antiangiogenic activity, regorafenib has been indicated in the treatment of mCRC patients. It is based on the data coming from the CORRECT study, a randomized controlled phase III study, in which patients were randomized to regorafenib or placebo in chemorefractory patients [45]. However, the results of this study showed in the experimental arm only a modest but significant benefit in OS and a low RR (1%) compatible with the action of delaying tumor growth rather than the tumor response itself. The drug was weighed down by heavy toxicity attributable to the more frequent hand-foot skin reaction, fatigue, hypertension, diarrhea, and skin rash. This discussion has been provided an overview about the most valid therapeutic alternatives available today in the treatment of mCRC. If an oncologist has to treat particular situations it would be a reasonable option to resort to suboptimal and/or underpowered chemotherapy. An important consideration we have to do about the performance status role. For elderly or unfit patients, bevacizumab has demonstrated its efficacy when added to fluoropyrimidines, in

particular with the oral analogous capecitabine. The findings about this regimen strengthen the theory that bevacizumab improves the action of classical chemotherapeutics into the tumor structures. It is not clear whether the small benefits justify the high cost of the drugs. Anyway, in respect of these new available data about the high safety of this combination with few recorded side effects, it would be considered a reasonable alternative especially for elderly patients (AVEX and MAX trials) [46–48].

On the other hand, in an effort to ensure the highest response in younger patients with an optimal performance status, the results of the randomized phase III TRIBE study were recently disclosed. This study evaluated the role of bevacizumab in first-line treatment for mCRC patients by a triplet chemotherapy compared with the standard schedule for this setting as FOLFIRI plus bevacizumab. The results of this study showed a significant advantage for the triplet chemotherapy in terms of PFS (12.1 vs. 9.7 months) with a significant incremental toxicity, such as diarrhea, stomatitis, and neutropenia. At the 2014 ASCO Annual Meeting, the updated analysis was presented according to the RAS mutational status. All-RAS wild-type patients treated with FOLFOXIRI plus bevacizumab as first-line treatment achieved a PFS and median OS of 13.3 and 41.7 months, respectively. Whereas the patients with mutant RAS showed no difference in PFS but had a significantly reduced OS. Of note, B-RAF mutant patients (about 8% mCRC patients), who commonly have a life expectancy by no more than 1 year, achieved an OS by about 19.1 months through the combination of bevacizumab and FOLFOXIRI. This finding suggested a possible role for this combination in this particular setting of patients [49].

A question that could easily arise from this discussion is what drug represents the best option for mCRC patients among anti-VEGF and anti-EGFR agents nowadays. By a careful analysis, we could suppose that some useful considerations could be derived from indirect comparisons more than from studies with direct comparison. This is currently a very active and important line of research, which led in the recent past to the publication of several articles on this topic. Bevacizumab was tested in first-line treatment against cetuximab with a head-to-head randomized phase III study (FIRE-3) in patients with KRAS wild-type both in combination with FOLFIRI regimen, aiming to establish the best therapeutic choice between anti-VEGF and anti-EGFR agents. The recently published data, however, do not clarify definitively this question by demonstrating a questionable significant benefit for cetuximab in OS (28.7 vs. 25 months) in the absence of a benefit in PFS, both secondary endpoints of the study. The primary endpoint was the RR, that resulted in no significant modifications in favor of cetuximab (62 vs. 58%, $p=0.183$) [50]. A recent update of data on the basis of the mutational status of the genes of EGFR pathway was presented at the 2014 Gastrointestinal Cancers Symposium. The researchers observed that ORR and OS within the RAS wild-type patients' group was higher in the FOLFIRI plus cetuximab arm, suggesting that the exclusion of patients with RAS mutations identifies a new population which seemed more likely to benefit from cetuximab [51]. This topic was also discussed by other experiences recently published like the PEAK and CALGB trials. Especially from the latter, which is a large trial with more than 1100 patients, a possible solution for this important question is awaited, since PFS was chosen as the primary endpoint. However, even in this case the data presented only partially at the 2014 ASCO Annual Meeting revealed no difference with regard to the primary endpoint between the use of an anti-EGFR or anti-VEGF in a population of selected patients with wild-type KRAS. The publication of the definitive study's results with the retrospective RAS analysis is awaited to reach any definitive conclusions ([52, 53]; Table 13.2).

Systemic Therapies for Patients with Initially Resectable Liver Metastases

Approximately, 50% of CRC patients are diagnosed with metastases confined to the liver. Surgical approach, whenever possible, does not just guarantee a cure since about 70% of these

Table 13.2 Summary of trials comparing different targeted drugs + standard chemotherapy

Trials	Targeted drugs comparison[a]	Number of patients	Biomarkers	Outcomes (months)	
				PFS	OS
Fire-3	Cetuximab vs. bevacizumab	592	KRAS WT	10.3 vs. 10.4	28.8 vs. 25
CALGB 80405	Cetuximab vs. bevacizumab	1142	KRAS WT	10.4 vs. 10.8	29.9 vs. 29
Peak	Panitumumab vs. bevacizumab	285	KRAS WT	10.9 vs. 10.1	34.2 vs. 24.3
		170	All-RAS WT	13 vs. 9.5	41.3 vs. 28.9

[a] Each treatment arm includes chemotherapy plus target drug

patients will a recur [54] and 5 years OS is around 30–40% [55, 56].

For these reasons, the combination of surgery and chemotherapy should be considered the main multidisciplinary approach to reduce recurrence risk and to increase OS. This aim was tested for different chemotherapy options both in pre-, peri-, and postoperative settings.

Pre- and Perioperative Chemotherapy

As regards the first approach, its advantages are partially similar to those of other neoadjuvant chemotherapy regimens, such as to facilitate the removal of large masses in case of good response or to test the sensitivity to chemotherapy used or also to determine a pathological response that is considered a strong predictor of survival outcome after the combination of surgery and chemotherapy [57, 58]. Following the advantage in terms of delay of disease recurrence demonstrated by the European Organisation for Research and Treatment of Cancer (EORTC) 40983 intergroup trial—the EPOC trial, the next step has been to validate whether the addition of targeted drugs could provide an advantage only in patients with liver disease who are candidates for surgery [59]. This was the aim of the new EPOC trial [60], in which cetuximab was added to chemotherapy regimens (FOLFOX, FOLFIRI, XELOX, XELIRI) to improve PFS in KRAS wild-type patients. In the latter study, the inclusion criteria did not provide limitation for the number of liver metastases (EPOC trial <=4), resulting in a PFS in favor of chemotherapy alone (HR 1.94; $p=0.030$). No significant difference was observed in OS, whereas a subgroup analysis confirmed the trend of distrust in using cetuximab plus oxaliplatin-based chemotherapy raising the concern for a probably different interaction with irinotecan and oxaliplatin. These data confirm that at this time anti-EGFR mAbs do not haveto be used in patients with resectable liver metastases. The use of an anti-VEGF in pre perioperative liver limited disease is evaluated first with the phase II BOXER trial, in which a cohort of patients with liver-limited disease were treated with XELOX plus bevacizumab and resulted in a 78% RR, with no grade 4 toxicities related to the surgery, even though the use of antiangiogenic drugs is historically linked to a risk of bleeding/thromboembolism/delay in wound healing that do not recommend surgery until 4–6 weeks the end of treatment [61–65].

Moreover, as demonstrated in the first BEAT trial, bevacizumab seemed to have an important role before surgery to improve R0 liver metastasis resections rate safely (173 out of 225 patients, 76.9%), in patients originally deemed unresectable. This finding provides a rationale to make prospective randomized trials evaluating the use of bevacizumab before resection of liver metastases. Grade 3/4 bleeding and wound-healing events were reported in 0.4 and 1.8%, respectively [63].

Other two important prospective randomized trials study have investigated the use of bevacizumab in this context. In the TRIBE study (triplet plus bevacizumab), authors analyzed secondary R0-resection rate as secondary endpoint and did not demonstrate a trend for R0-resection rate (15 vs. 12%; $p=0.327$) despite of a significant improvement in RR (65 vs. 53% $p=0.006$) [49]. Recently published data of the OLIVIA trial confirmed significant differences in ORR considering only the subgroup R0 without a significant increase in toxicity of grade G3–4 including neutropenia and diarrhea [66].

Postoperative or Adjuvant Chemotherapy

A pooled analysis of two prospective randomized trials [67] resulted in a marginal statistical benefit in PFS for systemic 5-fluorouracil (5-FU)-based chemotherapy after resection of colorectal liver confined metastases (HR 1.32; CI 95%: 0.95 1.82; $p=0.0095$) [68,69]. Even standard chemotherapy regimens were analyzed in this setting. Irinotecan-based regimens did not show any clinically significant advantage if compared with 5-fluorouracil [70]. No randomized studies evaluated oxaliplatin. Nevertheless, the combination containing oxaliplatin, 5 fluorouracil, and folinic acid (FOLFOX) is now widely used in the United States [71]. Other options like adjuvant hepatic artery infusion therapy with 5-FU or floxuridine have been proposed as standard therapy for this subset of patients, but because of lacking results in long-term OS, this approach has not been accepted yet as a valid therapeutic option [72].

The role of biological therapies in this setting has been evaluated in the Dutch study HEPATICA closed prematurely, in which patients undergoing resection of liver metastases were randomized to 6 months of chemotherapy plus 12 months of bevacizumab or 6 months of CAPOX chemotherapy alone. The results had shown no statistically significant advantage in terms of disease-free survival (DFS) at 2 years (70 vs. 52%) in the intervention group, placing high hopes for a longer follow-up [73].

Combining Anti-VEGF and Anti-EGFR

The interesting results of targeted agents in the treatment of patients with mCRC, in terms of OS, PFS, resection rate of synchronous and metachronous metastases, and symptom control rate, prompted the design of studies on the combination of multiple targeted drugs. Most of these studies have just been completed, a lot of them are ongoing. Despite a strong rationale, there is insufficient evidence to support a greater clinical impact of targeted combination regimens compared to the standard regimen. Among the most important experiences, panitumumab advanced colorectal cancer evaluation (PACCE) trial did not show any improvement in RR when panitumumab was added to FOLFOX plus bevacizumab in first-line setting. This study was terminated early because those patients randomized to the panitumumab arm experienced a higher toxicity rate. As a consequence of this increased toxicity, clinical benefit in terms of PFS was impaired compared to the control arm [74].

The CAIRO-2 trial evaluated the efficacy of XELOX plus bevacizumab with or without the addition of cetuximab, and the results confirmed the data of PACCE trial showing a lower PFS for the combination of the targeted drugs but pointing out a trend toward a reduced incidence of grade 3 oxaliplatin-related neurotoxicity in patients receiving the XELOX regimen along with the dual biologic combination [75].

Because of these preliminary results, the third arm of the study head-to-head CALGB/SWOG 80405 which included the combination of bevacizumab and cetuximab with FOLFOX or FOLFIRI was closed [76].

Based on the positive results from studies in which these drugs were used alone, many expectations were placed in the trials that evaluated the use of these molecules in combination. Despite the strong scientific rationale, the results showed no clinical benefit of the combination of an anti VEGF and and anti-EGFR in this setting of patients [77].

In conclusion, the results of these studies show that currently the dual-targeted therapy should not be used in combination with cytotoxic chemotherapy if not within a clinical trial. It has been available with the results of the DREAM trial/OPTIMOX 3 in which the use of bevacizumab + erlotinib (an anti-EGFR tyrosine kinase inhibitor) has been compared with bevacizumab alone in 700 patients as maintenance therapy after a first-line standard regimen (FOLFIRI + bevacizumab, XELOX2 + bevacizumab, mFOLFOX7 + bevacizumab) delivered for 6–12 cycles [78]. Although the study was weighed down by more than 40% of drop-outers, encouraging results in terms of PFS would show that the combination of EGFR and VEGF-targeted agents has not been dead yet but it has to be evaluated in more clinical trials.

The Development of New Targeted Agents

In spite of the huge amount of resources invested in cancer research, nowadays only a few new molecules have been approved for the treatment of metastatic CRC. Furthermore, there are only few agents used in randomized phase III trials. These include ramucirumab, a specific VEGF-directed therapy, brivanib, and perifosine, which represent further new strategies for intracellular signal blockade.

Ramucirumab is a human mAb directed against VEGFR-2, which is considered to be the main vascular endothelial growth factor (VEGFR) isoform, which mediates the process of tumor angiogenesis, blocks the binding of VEGF to its receptor [79]. As with other VEGF-directed therapies, the main side effects associated with ramucirumab include hypertension, thrombotic events, proteinuria, and bleeding [80]. The current planned phase III study of ramucirumab in mCRC, which has not started yet the patients' enrollment, will investigate its role as second-line treatment in combination with FOLFIRI [81]. Recently, the results of a phase II study of ramucirumab in the first-line setting in combination with FOLFOX6 therapy were published. The study tested ramucirumab for PFS, objective RR, OS, and safety. The authors found that ramucirumab may enhance the efficacy of modified FOLFOX-6 chemotherapy with an acceptable safety profile in mCRC [82].

Despite the negative results reported to date for dual biologic therapy with bevacizumab and cetuximab in mCRC, a phase II study evaluating the combination of cetuximab and ramucirumab is under development [83].

Brivanib is a novel oral receptor TKI that inhibits in particular the VEGF and fibroblast growth factor (FGF) signaling pathways [84, 85].

The importance of the study of these molecules in this tumor arose from new discoveries concerning the mechanisms of resistance to anti-VEGF agents. Namely, this resistance seems to be related to the increased expression of FGF and that brivanib would be able to restore the sensitivity to anti- VEGF agents in resistant patients by now and also facilitate its interactions with anti-EGFR antibody therapies, as demonstrated in preliminary studies in in vivo tumor xenograft models [86–88].

The activity of brivanib has been investigated in various phase I/II and especially in a phase III study that evaluated cetuximab plus brivanib alaninate versus cetuximab plus placebo in patients with metastatic, chemotherapy-refractory, wild-type KRAS CRC patients, with an increase of G3–4 toxicity in the experimental arm (78 vs. 53 %). The only advantages were showed up in modest benefit in PFS and ORR. Despite the positive effects on PFS and objective response, the use of brivanib was not to recommend in clinical practice because of its poor tolerability profile [89].

In addition to combination studies with cetuximab, future studies will evaluate brivanib in combination with bevacizumab in the metastatic setting of CRC patients.

Perifosine is an oral alkylphospholipid, that inhibits several key signal transduction pathways, including Akt and NF-kB. This signal transduction pathway was deemed crucial in the genesis of many malignancies. The inhibition of the NF-kB pathway could be able to restore the cell sensitivity to 5-FU as demonstrated in experiments in vivo and in vitro [90, 91].

As with brivanib, perifosine may have efficacy in combination with currently approved targeted agents in mCRC, as well as potential for single-agent activity. Its efficacy has been investigated in several phase II and phase III studies. In particular, the preliminary results of X-PECT study are now available. It is a randomized phase III study comparing capecitabine alone versus capecitabine plus perifosine in terms of OS in those patients who have undergone an average of at least four prior lines of chemotherapy. The endpoint of this study was not met, even considering the stratification by KRAS status [92].

Targeted Therapies in Adjuvant Treatment

The high antitumor activity by targeted agents in the metastatic setting, as highlighted by the improvement of RRs, prompted the design of numerous clinical trials to evaluate their possible role in the adjuvant setting. Two studies has investigated the usefulness of bevacizumab in combination or as monotherapy in patients with stage II and III colon cancer: the National Surgical Adjuvant Breast and Bowel Project (NSABP) C-08 trial and the international multicenter AVANT trial [93–95]. Although these studies have produced promising early results, neither of them showed a significant benefit for the experimental arm containing the antiangiogenic agent [96].

Ongoing studies are evaluating the role of bevacizumab in this setting, as for example, the QUASAR-2 trial. As consequence, bevacizumab should not be used in the adjuvant setting if outside of clinical trials.

In addition, the role of anti-EGFR agents in the adjuvant setting has been studied in several trials. Even for these drugs, the results were disappointing. Cetuximab was evaluated in the United States National Cancer Institute Intergroup Study N1047 trial [97], and after a median follow up of 28 months, this study has stopped for a lack of clinical benefit at the interim analysis, without considerations about KRAS and BRAF status. Of note, when this study started, there was no sufficient information about the importance of the mutational status of these genes and as a consequence either the KRAS/BRAF wild-type and mutant patients were included.

Also the Pan-European Trials in Alimentary Tract Cancer (PETACC8 tested cetuximab in the adjuvant setting without any promising result, probably due to a shorter period of cetuximab treatment because of its toxicity or because of the detrimental association of oxaliplatin-based chemotherapy plus an anti-EGFR antibody [31, 98]. Sub group analysis and long-term interim analysis are planned in the next few years. Studies containing the combination of FOLFIRI plus cetuximab were interrupted as soon as the results of PETACC 3 trial were available [99]. These data showed that irinotecan was not an active drug compared to a combination of folinic acid and 5-fluorouracyl in the adjuvant setting [100].

According to these findings, no targeted therapies are actually indicated for use in the adjuvant setting. The inclusion of patients in clinical trials for adjuvant treatment is encouraged to explore the real role of these drugs in this setting.

Conclusions

The mCRC is still a condition with a large prevalence in the general population. In recent years, numerous molecular targeted agents were added to standard chemotherapy contributing to an increase of OS and to an improvement of the palliation of symptoms with a consequent increase in quality of life. The assessment of the mutational status of the key genes involved in the signal transmission of proliferation pathway and the identification of prognostic and predictive factors of response to these new drugs represent a milestone in the treatment of this disease. It is possible to design a strategy to tailor every treatment based on the molecular profile of each patient, providing for each of them the best sequence of treatment available. These drugs are useful not only in the rescue strategy but also in the conversion therapy for those patients with potentially resectable liver metastases. For these drugs, a role in the adjuvant setting has not been shown yet. Finally, new important weapons are nowadays available for the oncologists, despite further efforts should still be made to validate the prognostic and predictive role of genes potentially involved in the pathogenesis of this disease.

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Targeted Therapy in Gastrointestinal Stromal Tumors

14

Piotr Rutkowski, Joanna Przybył, Agnieszka Wozniak and Giuseppe Badalamenti

Introduction

Gastrointestinal stromal tumors (GISTs) comprise a heterogeneous group of the most common mesenchymal neoplasms of the gastrointestinal (GI) tract. GISTs ranged from small benign lesions to aggressive malignant tumors which may originate anywhere in the GI tract, but the most frequently in stomach (~70% of cases) and small intestine (~20% of cases; [115, 116, 161]). Approximately 20–25% of gastric and 40–50% of small intestinal GISTs are clinically malignant [116]. Metastases develop mainly in the liver and may occur even after more than 10 years after surgery of the primary lesion which brings the necessity of a long-term follow-up of GIST patients [116]. Epidemiological studies indicate mean annual GIST incidence of 10–15 cases per million people, affecting mainly older individuals at the median age of 55–65 years [22, 111, 126, 132]. GISTs are believed to arise from a progenitor related to the interstitial cells of Cajal (ICC) which are the pacemakers for peristaltic contractions [76, 94, 123]. Approximately 85–95% of GISTs are immunopositive for KIT (also known as CD117) which is currently used for routinely diagnosis [162]. However, recent studies showed that DOG1 (discovered on GIST, also known as TMEM16A, ANO1), is more sensitive and specific marker than KIT [90, 107, 122]. Other well-established immunohistochemical markers used for differential diagnosis are CD34 (hematopoietic progenitor stem cell antigen), smooth muscle actin (SMA), S100 protein, desmin (muscle cell marker), and vimentin (mesenchymal cell marker; [117, 121, 165]) or recently described carbonic anhydrase II [138]. Cytogenetically, both benign and malignant GISTs are characterized mainly by chromosomal losses of 14q, 22q, and 1p. Additional genomic alterations present in metastatic GISTs involve losses of chromosomes 13q, 15q, 18, and a partial deletions of 11p and 9p (including tumor suppressor genes *CDKN2A* and *CDKN2B*), as well as gains of 5p, 8q, and 17q [7, 53, 54, 68, 93, 114, 200].

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Oncogenic Mutations in GISTs

The first report linking GISTs with the activating mutations in receptor tyrosine kinase (RTK) *KIT* gene was published by Hirota et al. [76], proving that mutated KIT protein was constitutively activated without binding the KIT ligand, stem cell factor (SCF). Since then, the mutational status of *KIT* and other members of type III transmembrane RTK family has been extensively investigated in GISTs. Activating mutations in the gene encoding platelet-derived growth factor receptor A (*PDGFRA*), a kinase sharing a high level of homology with KIT have been also found in GISTs [71]. Both genes are located at 4q12 probably having evolved as a duplication of an ancestral gene [172]. Advances in the understanding of molecular events underlying GIST tumorigenesis made KIT and PDGFRA oncoproteins the essential diagnostic and therapeutic targets and lead to the paradigm for genotype-driven targeted therapy (see below; [4]).

It is well documented that 75–80% of sporadic GISTs harbor *KIT*-activating mutations and 5–8% of sporadic GISTs carry *PDGFRA*-activating mutations [95]. The most frequent mutation site in *KIT* is the 5′ end of exon 11 (about 67% of all mutations in GISTs) where in-frame deletions or point mutations may occur. Less common primary mutation sites in *KIT* include the 3′ end of exon 11 typically exhibiting internal tandem duplications (ITDs) and exon 9, where an insertion of two amino acids (p.A502_Y503dup) is often reported. The most common mutation in *PDGFRA* is the p.D842V substitution in exon 18. *KIT* and *PDGFRA* mutations in GISTs are mutually exclusive and they are early oncogenic events in GISTs development [5, 23, 26, 27, 37, 55, 71, 95, 151, 167, 174, 201]. Interestingly *KIT* exon 9 duplication is most frequently observed in intestinal GISTs while *PDGFRA* exon 18 mutations are most often found in tumors localized in stomach [95, 97, 99, 200]. Figure 14.1 shows a schematic diagram of the most common *KIT* and

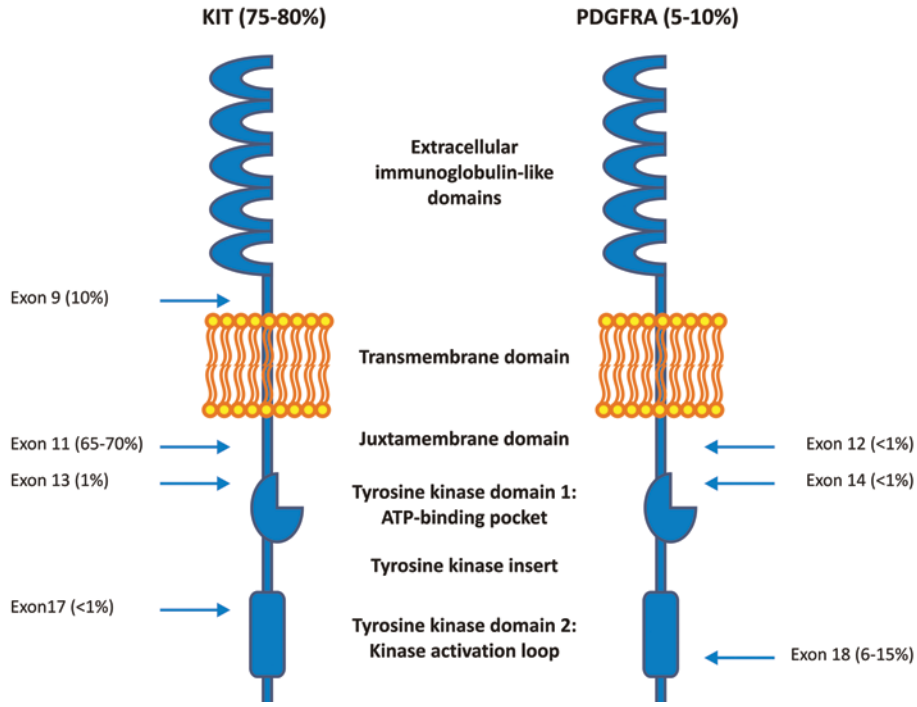

Fig. 14.1 Activating mutations of KIT and PDGFRA described in human primary GISTs

Table 14.1 Molecular classification of RTK-naïve GISTs

KIT mutations (75–80% of sporadic GISTs)	
Exon 11 (deletions, substitutions, duplications/insertions)	Most common mutation in sporadic GISTs (65–70%); Present in tumors localized in all GI sites; Best response to imatinib; Reported also in familial GISTs
Exon 9 (A502_Y503dup)	More common in GISTs originating from small bowel/colon; Intermediate/dose-dependent response to imatinib; Good response to sunitinib
Exon 13 (K642E)	Present in tumors localized in all GI sites; Observed clinical responses to imatinib; Reported in familial GISTs; More often as secondary mutations in imatinib-resistant tumors
Exon 17 (D816V, D820Y, N822K, Y823D)	Present in tumors localized in all GI sites; Observed clinical responses to imatinib (except p.D816V); Reported in familial GISTs; More often as secondary mutations in ima/sun-resistant tumors
PDGFRA mutations (5–10% of sporadic GISTs)	
Exon 12 (V561D)	Present in tumors localized in all GI sites; Observed clinical responses to imatinib
Exon 14 (N659K)	Only few cases described in literature; More common in GISTs originating from stomach
Exon 18 (substitutions, deletions)	More common in GISTs originating from small bowel/colon; Often related to indolent clinical behavior; p.D842V is the most common—resistant to ima/sun; Other exon 18 mutations are sensitive to imatinib
KIT/PDGFRA wild-type	
	Frequent in pediatric GISTs; Poor response to imatinib, better to sunitinib; Typical for GISTs related to neurofibromatosis type 1 or Carney's triad (gastric GIST + pulmonary chondromas ± paraganglioma); In some cases associated with: *IGF1R* overexpression *BRAF* mutation (p.V600E) *SDHs* mutations

Ima imatinib, *sun* sunitinib

PDGFRA mutation sites. Table 14.1 summarizes the most important molecular features of GISTs according to the *KIT* and *PDGFRA* mutational status.

Approximately 10–15% of GISTs do not present detectable mutations neither in *KIT* nor *PDGFRA*—these tumors are often called "wild-type" (WT) GISTs. They are indistinguishable from mutant ones in terms of morphology, KIT expression and tumor localization. They are also characterized by uncontrolled KIT activation *via* yet unknown mechanisms [49]. Notably, in 7–20% of WT GISTs, the *BRAF* p.V600E mutation is detected [1, 33, 77, 125]. In addition, defects in the succinate dehydrogenase (SDH) complex have been recently revealed in WT GISTs [79, 123].

Activating mutations of *KIT* and *PDGFRA* lead to the kinase conformational changes, dimerization and subsequent autophosphorylation of tyrosine residues causing constitutive activation of the downstream effectors in the phosphatidylinositol 3′-kinase (PI3K)/AKT, mitogen-activated protein kinase (MAPK), Janus kinase/signal transducers and activators of transcription (JAK/STAT) and RAS pathways, leading to increased cell proliferation and inhibition of apoptosis [33, 50, 150].

Markers of Progression in the GIST Management

The treatment of choice in primary, resectable, localized GISTs is radical surgery with negative margins. However, approximately 40–50% of the patients develop recurrent or metastatic disease after potentially curative operations [115, 154]. Identification of the risk factors for recurrence after primary surgery is crucial for reliable prognosis, follow-up schedule and recognition of patients who may potentially benefit from the adjuvant therapy. The main criteria of aggressive behavior of GIST are based on the presence of invasion of surrounding structures and/or metastases (overtly malignant cases), as well as on primary tumor site, size and mitotic index [116]. Several risk stratification systems have been proposed in the recent years. A Consensus Conference held at the National Institutes of Health (NIH) in 2001 provided the first evidence-based definition and a practical scheme for the risk assessment in the clinical course of this disease. The risk categorization was based on evaluation of the tumor size and mitotic rate (evaluated per 50 high-powered fields; HPF) as the most reliable prognostic factors [56]. Additional analysis in patients with primary tumor after complete macroscopic resection confirmed the significance of tumor anatomic location as the independent prognostic factor. Miettinen and Lasota created the classification for risk assessment in gastric, duodenal, and intestinal GISTs (NCCN-AFPI; National Comprehensive Cancer Network-American Forced Institute of Pathology; [116, 118–121]) which constituted the basis for new staging system of American Joint Committee on Cancer (AJCC; [51]). It combines the principal features (i.e. size, site and mitotic index) and reflects the fact that gastric GISTs show a much lower rate of aggressive behavior than jejunal and ileal GISTs of comparable size and/or mitotic rate [116, 120, 121]. Recently, it was established that tumor rupture (spontaneous or iatrogenic) is an additional important risk factor strongly associated with the increased recurrence rates [153, 155]. Therefore, in 2008, Joensuu proposed another simplified classification system based on four prognostic factors (tumor size, site, mitotic count, and the presence of tumor rupture; [81, 87, 156]). In addition to the clinicopathological factors, *KIT* and *PDGFRA* mutational status may have a prognostic significance in primary GISTs; however, at present, insufficient data exist to incorporate the kinase mutation status into stratification of the risk of primary tumors. Several studies indicated more favorable prognosis for patients carrying exon 11 point mutations or insertions as well as *PDGFRA* exon 18 mutations, whereas tumors harboring *KIT* exon 11 deletions especially involving codons 557 and/or 558 or in homozygous state, as well as *KIT* exon 9 duplications were associated with more aggressive behavior of GISTs [2, 96, 98–101, 110, 192, 201].

Introduction of Imatinib Mesylate in the Therapy of GISTs

Classic cytotoxic chemotherapy is an ineffective method of treatment in advanced GISTs. Also, radiotherapy has a limited value in the management of GISTs, mainly due to the tumor location surrounded by dose-limiting vital organs. Recurrent and/or metastatic and/or unresectable cases had a very poor prognosis until the beginning of the twenty-first century, when advances in the understanding of the molecular background of GIST pathogenesis have resulted in the development of a treatment approach which has become a model of targeted therapy in oncology. The introduction of imatinib mesylate [Gleevec™, Glivec®; Novartis], a small-molecule selective inhibitor of RTK, has revolutionized the therapy of advanced (inoperable and/or metastatic) GISTs. Imatinib mesylate, previously known as STI571 (signal transduction inhibitor), is a derivative of 2-phenylaminopyrimidine which competitively binds to the ATP binding site and inhibits phosphorylation of kinase substrates which leads to the growth inhibition in affected cells [32, 67, 130] (Fig. 14.2). Initially, it was applied in the treatment of chronic myelogenous leukemia (CML), to specifically inhibit the tyrosine kinase activity of BCR-ABL fusion oncoprotein [47]. Moreover, in pre-clinical studies, imatinib has been demon-

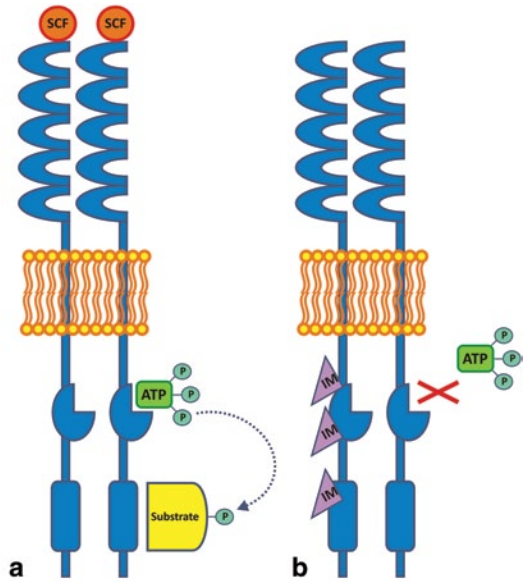

Fig. 14.2 Mechanisms of KIT activation and its inhibition by imatinib. **a** KIT is activated by the ligand SCF (stem cell factor) in normal conditions or becomes constantly activated by a gain-of-function mutation which causes KIT dimerization, binding of adenosine triphosphate (ATP) and autophosphorylation of selected tyrosine residues which subsequently leads to the substrate phosphorylation and activation of downstream signaling pathways. **b** Imatinib (IM) binds to the inactive conformation of tyrosine kinase domain 1 or 2 and prevents binding of ATP. There are three imatinib contact points—two in tyrosine kinase domain 1 and one in tyrosine kinase domain 2 (marked by the *triangles*)

strated to inhibit the activity of KIT, PDGFRA/B, ABL, and ARG (ABL-related gene) tyrosine kinases [134, 163]. Imatinib is administered orally with 98% bioavailability and metabolized by hepatic cytochrom P450 isoforms including CYP3A4; therefore, other drugs administered concomitantly may cause changes in its pharmacokinetics and *vice versa* [181]. Imatinib was first applied to a GIST patient with multiple metastatic lesions in 2001 resulting in an impressive response [83].

Imatinib Mesylate in the Targeted First-line Therapy of Advanced GISTs

Imatinib mesylate at initial dose of 400 mg daily has quickly become the first line standard treatment of patients with metastatic, recurrent and/or inoperable GISTs [32]. Results of several clinical trials (Phase I/II EORTC 62001, US/Finland Phase II trial, phase III EORTC 62005, and US S0033) confirmed the high efficacy of imatinib in the treatment of GISTs in the majority of patients with inoperable/metastatic disease [12, 13, 39, 102, 185, 187] (Table 14.2). As compared to the historical data with estimated 10–19 months of median survival in patients with advanced disease, currently the survival dramatically improved with median overall survival (OS) reaching approximately 5–6 years (with approximately 40% surviving currently 8 years) and median progression-free survival (PFS) in the range of 2–3 years. About two-thirds of GIST patients achieve an objective response during imatinib treatment with a standard dose of 400 mg daily, and further 20% of patients show durable disease stabilization [39, 153, 158, 188]. Yet, the complete remissions are rare. Two large, parallel, very similar international studies comparing a standard imatinib dose of 400 mg daily with a high-dose of 800 mg daily have demonstrated similar response rates and overall survival for both imatinib doses, but better PFS in the high-dose arm [143, 188]. This trend for the improvement of PFS in the high-dose arm was confirmed in the meta-analyses of these trials (hazard ratio [HR] 0.89, log rank $p=0.04$; [184]). Response to imatinib does not always result in the immediate decrease of the tumor size but rather in the inhibition of growth and apoptosis of tumor cells [182]. It may be visualized with functional imaging, either as the disappearance of metabolically active regions using positron emission tomography (PET) or as hypoattenuation on computed tomography (CT; Fig. 14.3)—methods recommended in the Choi's criteria of assessing treatment response [24]. Finally, a randomized trial conducted by the French Sarcoma Group has demonstrated that imatinib therapy should be continued as long as clinical benefits of therapy are observed, even after ra-

Table 14.2 Summary of the results of clinical trials in advanced GISTs treated with imatinib

Study name; phase and patients number, (reference)	Objective response		Stable disease (%)	Disease progression	Overall survival	Progression-free survival
	Partial response (%)	Complete response (%)				
EORTC 62001; I ($n=36$) [185]	54	0	37	8%	n/a	n/a
US B2222; II ($n=147$) [39]	67	1	16	12%	Median 57 months	Median time to progression 24 months
EORTC 62002; II ($n=27$) [187]	67	4	18	11%	n/a	73% (1-year)
Intergroup S0033; III ($n=746$) [13]						
400 mg daily	45	3	27	25	78% (2-year)	50% (2-year)
800 mg daily	45	3	26	26	73% (2-year)	53% (2-year)
EORTC 62005; III ($n=946$) [188]						Median 19 months
400 mg daily	45	5	32	13%	69% (2-year)	44% (2-year)
800 mg daily	48%	6%	32	9%	74% (2-year)	52% (2-year)

n/a not available

diologically assessed complete response, as termination of treatment results in a rapid disease progression [14].

Completed trials demonstrated that imatinib is generally well tolerated up to a dose of 800 mg/day; however, the majority of patients reported at least mild or moderate treatment-related adverse events (grade 1 or 2). The most common adverse events include edema (especially periorbital), fatigue, nausea, diarrhea, musculoskeletal pain, rash, anemia, and granulocytopenia [25, 183] [67]; Table 14.3.

Recently emerging issue is the surgical removal of disease remnants during imatinib therapy, which may lead to complete remission in selected GIST patients after the achievement of partial response. This policy appears attractive especially in the fact that it can theoretically

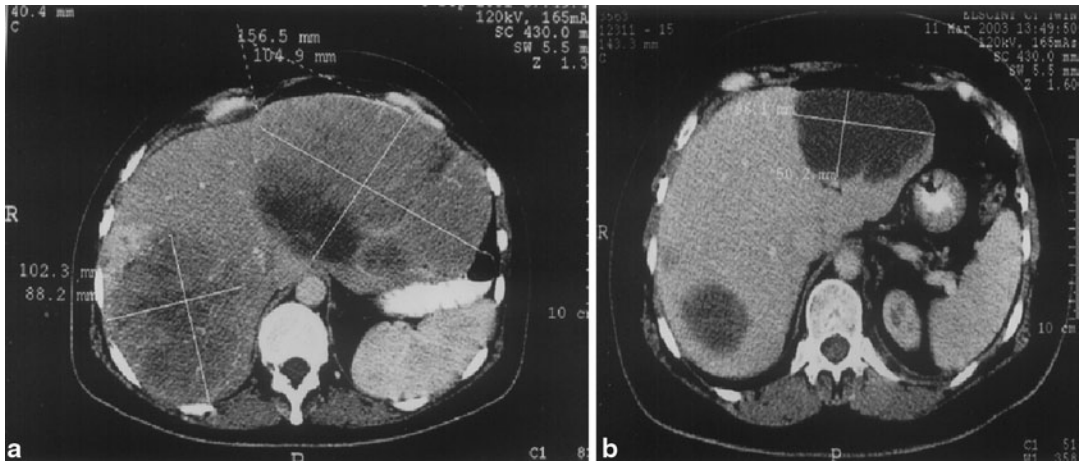

Fig. 14.3 Computed tomography images demonstrating response to imatinib therapy in advanced GIST with liver metastases; characteristic decrease of lesion density is observed (**a**—before imatinib therapy, **b**—after maximal response to imatinib therapy). **a** 4/09/2001; **b** 11/03/2003

Table 14.3 The most common adverse events during imatinib therapy in advanced GISTs (based on phase I–III clinical trials)

Adverse event	All grades (%)	Grade 3 or 4 (%)
Fluid retention or edema	55–74	0.9–2.9
Eyelids	40–48	0
Extremities	20–37.5	0
Nausea	44–52	0.7–1.4
Diarrhoea	12.5–45	2.0
Muscle-skeletal pain, muscle cramps	39	0-1.3
Fatigue	30–67.8	0–5.9
Skin rash	31	2.7–4.3
Headache	19–31	0
Abdominal pain	25	0
Meteorism	21	0
Vomiting	12.9–26.4	0.7
Loss of apetite	26	1.9
Bleeding	10.8–12.9	2.7–4.8
Dyspepsia	10.9	0
Anemia	9–89	2.0–7.0
Neutropenia	6.8	4.8
Leucopenia	4.8–34.0	1.4–2.7
Thrombocytopenia	5.9	1.5

prolong durable remission, as the excision of the tumor is performed before the development of imatinib resistance, and thus the risk of resistant clone selection is reduced. The optimal time for the implementation of surgical treatment is probably the moment of disease stabilization without further response to imatinib after observing radiological observation of the maximal initial remission—usually it corresponds to the interval between 6th and the 18th month from the onset of imatinib therapy [152]. Several series of patients treated surgically during imatinib therapy were published; however, according to the European Society of Medical Oncology (ESMO) consensus guidelines the surgical therapy during imatinib treatment remains individualized option, without formally confirmed survival benefit in randomized trial [176].

KIT and *PDGFRA* mutational status strongly correlates with the response and PFS in GIST patients treated with imatinib. Heinrich et al. examined 127 patients with advanced GISTs enrolled into phase II trial and found that patients with tumors carrying the most common *KIT* exon 11 mutation showed the highest response rate (83.5%) and the longest PFS. Patients with tumors harboring *KIT* exon 9 mutations demonstrated response rate at approximately 45% and shorter PFS, whereas WT GISTs showed the lowest rates of response to imatinib and the shortest PFS [72]. Other studies have confirmed that mutational status predicts the clinical response to imatinib [35]. In general, patients with tumors harboring *KIT* exon 11 mutations show the best clinical response to imatinib with the highest rate of objective responses (70–85% of patients) and the longest OS and PFS (Table 14.4). However, it has been demonstrated that not all *KIT* exon 11 mutants response to imatinib therapy equally well. Debiec-Rychter et al. [37] suggested that the involvement of codons in the distal part of *KIT* exon 11 translates into worse response to the therapy in comparison with tumors bearing mutations in the proximal part of the exon. It is possible that mutations inducing conformational changes, such as large deletions or insertions may reduce the affinity of KIT for imatinib and moderate drug efficacy [37, 72]. As far as tumors carrying *KIT* exon 9 mutations are concerned, approximately 15–30% of these cases

Table 14.4 The correlation of tumor genotype (*KIT* mutations) and response to imatinib therapy in GIST patients in clinical trials

	B2222 Phase II ($n=127$) (%) [72]	EORTC-AustralAsian Phase III ($n=363$) (%) [37]	North America SWOG S0033 Phase III ($n=428$) [75]
Objective response[a]			
KIT exon 11	83[b]	70[b]	64%[b]
KIT exon 9	48	35	38%
Wild-type	0	25	37%
Disease progression			
KIT exon 11	4.7	3.2	n/a
KIT exon 9	17.4	17.2	n/a
Wild type	55.6	19.2	n/a

n/a data not available, *RECIST* Response Evaluation Criteria in Solid Tumors

[a] Defined as complete or partial radiological response according to RECIST (Response Evaluation Criteria in Solid Tumors; [177])

[b] Statistically significant difference between *KIT* exon 11 versus *KIT* exon 9 and patients without *KIT* mutations

demonstrate primary resistance to imatinib therapy [155]. However, the results of EORTC-ISG-AGITG 62005 trial and combined meta-analysis of S0033 and EORTC-ISG-AGITG 62005 trials demonstrated that GIST patients carrying *KIT* exon 9 mutations benefit from the higher dose of imatinib (median PFS 18 vs 6 months for doses of 800 mg and 400 mg daily, respectively; [37, 113, 137, 165, 184]. It is assumed that *KIT* exon 9 p.A502_Y503dup disrupts the antidimerization motif in the extracellular KIT domain, leading to spontaneous receptor homodimerization and activation of related kinase receptors, the activity of which might be more effectively modulated by the higher dose of imatinib. The influence of the higher dose may be also indirect and associated with the inherent biological differences of GISTs harboring *KIT* exon 9 and exon 11 mutations. Based on these observations, the NCCN and ESMO recommended that the dose of 800 mg/day should be used as a standard treatment in the subgroup of patients with advanced GISTs carrying *KIT* exon 9 mutations [20, 43]. Both clinical and laboratory studies demonstrated that tumors with *PDGFRA* exon 18 p.D842V mutation are resistant to imatinib therapy, similarly to approximately 50% of WT GISTs. Other *PDGFRA*-mutant GISTs show variable response [27, 37, 75].

Adjuvant Therapy with Imatinib

Post-operative recurrence of moderate and high-risk GISTs is commonly observed; therefore, the idea of adjuvant therapy with imatinib after primary surgery has been evoked to prevent or delay the recurrence and to prolong patient's survival. In 2008, imatinib has been registered for use in adjuvant therapy, in patients after resection of primary GIST at significant risk of relapse based on the published results of clinical trials demonstrating substantial reduction of the risk of recurrence but without definite guidance as to optimal duration of treatment [38].

The role of imatinib therapy in the adjuvant setting has been evaluated in several phase II and III clinical trials, i.e., ACOSOG Z9000 and Z9001 (conducted by the American College of Surgeons Oncology Group); SSGXVIII/AIO (conducted by the Scandinavian Sarcoma Group and the Sarcoma Group of the Arbeitsgemeinschaft Internistische Onkologie XVIII); RTOG S0132 (conducted by the Radiation Therapy Oncology Group) and EORTC 62024 (conducted by the European Organization for Research and Treatment of Cancer) (Table 14.5). Data from the ACOSOG Z9001 phase III study, comparing 1-year adjuvant therapy with imatinib 400 mg/

Table 14.5 The most important clinical trials of adjuvant therapy with imatinib in GISTs

Study	ACOSOG Z9000	Kang et al. [89]	Li et al. [104]	Jiang et al. [80]	ACOSOG Z9001	SSGXVIII/AIO	EORTC 62024
	Phase II				Phase III		
Study design	One-arm, open, multicenter;	One-arm, open, multicenter, prospective;	Open, non-randomized, prospective, one-center;	Non-randomized, one-center, prospective;	Double-blinded, placebo-controlled, randomized, multicenter;	Two-arms, open, randomized, multicenter, prospective;	Two-arms, open, randomized, multicenter, prospective;
	Imatinib 400 mg daily for 1 year	Imatinib 400 mg daily for 2 years	Imatinib 400 mg daily for 3 years vs observation	Imatinib 400 mg daily for 5 years vs observation	Imatinib 400 mg daily vs placebo for 1 year	Imatinib 400 mg daily for 1 year vs 3 years	Imatinib 400 mg daily for 2 years vs observation
Number of patients	$n=107$	$n=47$	$n=56$ (imatinib) $n=49$ (observation)	$n=35$ (imatinib) $n=55$ (observation)	$n=359$ (imatinib) $n=354$ (placebo)	$n=200$ (1 year); $n=200$ (3 years)	$n=906$
Major eligibility criteria	Primary GIST CD117(+) after radical resection High risk of relapse: Tumor size ≥10 cm OR Tumor rupture OR Intraperitoneal metastases 5	Primary GIST with exon 11*KIT* mutation after radical resection High risk of relapse: Tumor size ≥10 cm OR Mitotic index ≥10/50 HPFs OR Tumor size ≥5 cm and mitotic index ≥5/50 HPFs	Primary GIST CD 117(+) after resection Intermediate or high risk of recurrence (NIH classification): - Tumor size 5 cm AND/OR - Mitotic index 5/50 HPFs	Primary GIST CD 117(+) after resection R0 High risk of relapse (modified NIH classification)	Primary GIST CD117(+) after radical resection Tumor size ≥3 cm Low, intermediate and high risk of relapse	Primary GIST CD 117(+) after radical resection High risk of relapse (modified NIH classification): Tumor size 10 cm OR Mitotic rate 10/50 HPFs OR Mitotic rate 5/50 and tumor size 5 cm OR Tumor rupture	Primary GIST CD 117(+) after radical resection Intermediate Or high risk of relapse (NIH classification): - Tumor size 5 cm AND/OR - Mitotic index 5/50 HPF

Table 14.5 (continued)

Study	ACOSOG Z9000	Kang et al. [89]	Li et al. [104]	Jiang et al. [80]	ACOSOG Z9001	SSGXVIII/AIO	EORTC 62024
	Phase II				Phase III		
Results—primary end-point:							
	OS at median follow-up 4 years: 1-year: 99% 2-years: 97% 3-years: 97%	RFS at median follow-up 26.9 months: 1-year: 97.7% 2-years: 92.7%	Significantly better RFS in imatinib arm as compared to observation at median follow-up 45 months (HR 0.188, 95% CI 0.085–0.417; p 0.001): 1-year: 100% vs 90% 2-years: 96% vs 57% 3-years: 89% vs 48%	Significantly better RFS with imatinib as compared to observation only at median follow-up of 44.0 months (HR 0.122; 95% CI 0.041−0.363; p 0.001): 1-year: 100% vs 70.9% 2-year: 88.0% vs 37.8% 3-year: 88.0% vs 27.5%	Significant improvement of 1-year RFS in imatinib arm (98%) as compared with placebo (83%); median follow-up time 19.7 months HR 0.35, p 0.0001	Significant improvement of RFS with 3-year imatinib therapy as compared with 1-year therapy at median follow-up 54 months (HR 0.46, 95% CI 0.32–0.65; p 0.0001): 5-letnie: 65.6 vs 47.9%	Time to imatinib failure at relapse (changed from OS)
Results—secondary end-point							
	RFS at median follow-up 4 years: 1-year: 94% 2-years: 73% 3-years: 61%		Significantly decreased risk of death due to GIST with imatinib adjuvant therapy in comparison to observation at median follow-up of 45 months (HR 0.254, 95% CI 0.070–0.931; $p=0.025$)		Lack of statistically significant differences in 1-year OS between study arms - HR 0.66, $p=0.47$	Significant improvement of OS with 3-year imatinib therapy as compared with 1-year therapy at median follow-up 54 months (HR 0.45,95% CI 0.22–0.89; $p=0.019$): 5-year OS: 92.0% vs 81.7%	RFS significantly improved with 2-year adjuvant imatinib therapy, 5-year imatinib failure-free survival rate was 87% in imatinib-treated group vs 84% in the control arm; OS data need longer follow-up

ACOSOG American College of Surgeons Oncology Group, *GIST* gastrointestinal stromal tumors, *HPF* high-power microscope field, *HR* hazard ratio, *OS* overall survival, *RFS* recurrence-free survival, *AE* adverse event, *AIG* Arbeitsgemeinschaft Interistisch Onkologie, *CI* confidence interval, *EORTC* European Organisation for Research and Treatment of Cancer, *Gr* Grade, *NIH* National Institutes of Health, *RTOG* Radiation Therapy Oncology Group, *SSG* Scandinavian Sarcoma Group

day to placebo in patients after R0 resection of GISTs at least 3 cm in diameter, have shown a significant reduction in the risk of recurrence from 17 to 2% at 1 year (20 months of follow-up; $p=0.0001$), with a hazard ratio of 0.35. The treatment was well tolerated; however, no significant impact on overall survival has been observed, thus implying that adjuvant imatinib rather delays than prevents the relapse. The major clinical benefit of adjuvant therapy was limited to the group of patients at high-risk of relapse according to NCCN-AFIP criteria [11, 38].

Data from the SSGXVIII/AIO trial, comparing 12 versus 36 months of adjuvant imatinib treatment after resection of GIST with a high risk of recurrence, were presented in 2011 at the 47th Annual Meeting of the American Society of Clinical Oncology (ASCO). The results showed significant improvement in the 36-month arm compared to the 12-month arm, both in recurrence-free survival (5-year RFS: 65.6% vs 47.9%; $p<0.0001$) and overall survival (5-year OS: 92.0% vs 81.7%; $p=0.01$). The best results were obtained for patients harboring *KIT* exon 11 mutations. Imatinib was generally well tolerated with anemia, periorbital edema, fatigue, nausea, diarrhea, leucopenia, and muscle cramps as the most common adverse events. More patients discontinued imatinib therapy in the 3-year arm as compared to the 1-year arm, without features of GIST recurrence (26% vs 12%; $p<0.001$; [82, 85, 86]). Based on these data, FDA and EMA recommended 36 months of treatment with imatinib after surgery for adult patients with CD117-positive GISTs with the high risk of relapse.

Ongoing EORTC 62024 trial, which aims to compare 2-year imatinib adjuvant treatment with observation only, will provide data on imatinib resistance upon rechallenge after disease relapse in the intermediate and high-risk patients who have undergone the resection of primary tumor.

Mutational status also has a predictive value for clinical response to imatinib adjuvant therapy and may help to tailor the treatment to patients with more sensitive mutations, such as *KIT* exon 11 mutants, or to exclude patients with imatinib-resistant mutations, such as *PDGFRA* p.D842V mutation. Although controversial in the adjuvant setting, patients with metastatic GISTs harboring mutations in *KIT* exon 9 may benefit from imatinib dose increase up to 800 mg/day. Thus, *KIT* and *PDGFRA* genotyping in GISTs should be mandatory also in the adjuvant setting [21, 29].

Neoadjuvant Therapy with Imatinib

In selected cases of locally advanced GISTs, the strategy of neoadjuvant imatinib therapy has become a common approach recommended both by the European and US guidelines [20, 43]. Preoperative imatinib treatment gives the opportunity to increase resectability of locally advanced GIST and to avoid mutilating surgery by decreasing tumor volume. Devitalized tumor facilitates its resection, which results in less post-operative morbidity, less blood transfusions, and lower risk of tumor rupture. The duration of neoadjuvant treatment should be limited to the maximal response to therapy (usually 6–12 months after start of imatinib when no further response is observed in two consecutive imaging examinations—careful response assessment should be undertaken not to miss the best timing for surgery; [3, 15]).

Only few formal phase II trials in locally advanced GISTs with pre-operative imatinib treatment were performed (Table 14.6). The results of Radiation Therapy Oncology Group (RTOG) 0132/American College of Radiology Imaging Network (ACRIN) 6665 phase II trial confirmed safety of this approach and high rate of relapse-free survival in the long-term observation after surgery. The results of this study may have also lead to the discovery of gene expression signatures associated with response to imatinib as predictor factors [147]. In a group of 53 patients with locally advanced GISTs and difficult tumor localization, pre-operative imatinib allowed for organ-sparing resection in the majority of cases [52, 191]. In a series of 161 patients analyzed by EORTC Soft Tissue and Bone Sarcoma Group (STBSG) study, only two patients demonstrated disease progression during the neoadjuvant therapy. The 5-year disease specific survival rate was 95%, which is significantly better than in the case of unresectable localized GISTs treated with

Table 14.6 Clinical trials of neoadjuvant treatment with imatinib in GISTs

Study	Eligibility criteria	Number of patients	Study design	End points and results
Phase II RTOG-S0132/ACRIN 6665	Locally advanced ≥5 cm (group A) Or metastatic/recurrent (group B) potentially resectable GIST KIT+	Group A = 30 Group B = 22 *n* = 52	Pre-operative imatinib 600 mg daily for 8–12 weeks, then resection and post-operative imatinib for 2 years; non-randomized, open study	2-year PFS: 80.5 %; objective response rate: 6 %; 2-year OS: 92.3 %; 5-year RFS: 57 %; 5-year OS: 77 %; R0 resection in 65 % patients; post-operative morbidity 29 % grade 3 and 16 % grade 4, 4 % grade 5
Phase II APOLLON CST1571-BDE43	Locally advanced histologically confirmed GIST KIT+	*n* = 40	Pre-operative imatinib 400 mg daily for 6 months; non-randomized, open study	Primary end-point: ORR; secondary end-points: resectability R0 and organ-sparing resection, TTP, OS, safety
Phase II in MD Anderson Cancer Center	GIST ≥1 cm histologically confirmed KIT+	*n* = 19	Pre-operative imatinib 600 mg for 3, 5 or 7 days and post-operatively for 2 years; randomized study	1-year DFS = 94 % 2-year DFS = 87 %

DFS disease free survival, *ORR* objective response rate, *OS* overall survival, *RFS* recurrence-free survival, *TTP* time to progression

imatinib only [158]. Goh et al. analyzed response rates and the outcomes of 37 patients with advanced GISTs treated with neoadjuvant imatinib followed by surgical resection. They observed that the R0 resection after neoadjuvant therapy was possible in 33 (89 %) of patients, with significant post-operative complications reported in only four patients [65].

Pre-operative therapy with imatinib is a safe option, which should be considered in the case of problems with margin-free resection or technically difficult, localized primary tumors (e.g., in rectum, duodenum, and gastroesophageal junction) with a high risk of post-operative complications or in unavoidable necessity of mutilation. Pre-operative biopsy and *KIT*/*PDGFRA* genotyping is required when planning the neoadjuvant therapy to exclude potentially primary resistant tumors (e.g., WT GISTs and *PDGFRA* p.D842V mutants). Importantly, imatinib therapy in locally advanced potentially resectable tumors does not replace the surgical resection of GIST as patients treated with imatinib alone (without surgery) have worse outcomes similar to the metastatic cases [15]. According to the current guidelines, after neoadjuvant therapy, the adjuvant therapy with imatinib should be implemented.

Present indications for pre-operative imatinib treatment in GISTs include locally advanced tumor, amenable only to mutilating surgery (e.g., abdomino-perineal resection, pelvic evisceration); when negative resection margins over the organ of origin are difficult to obtain; and when function-sparing resection and minimizing the extent of surgery can be possible after tumor shrinkage (e.g., wedge resection instead of total gastrectomy with splenectomy, local excision instead of pancreatoduodenectomy; [159]).

Imatinib Resistant GISTs

There are different clinical and biological factors predicting initial and late resistance to imatinib. From the molecular point of view, primary resistance to imatinib has its origins in the *KIT*/*PDGFRA* mutational status. Although early resistance may be observed in association with all mutation types, it is the most often seen in *KIT* exon 9 mutants, *PDGFRA* exon 18 p.D842V mutants [37, 113, 165, 188, 203]. Early disease progression within the first 6 months of imatinib treatment may be also caused by the lack of mutations in both kinase genes [73]. It has been also

proposed that the individual imatinib metabolism resulting in certain imatinib plasma level may correlate with the early resistance [42]. Distinct mechanisms are involved in the late resistance to imatinib therapy. Secondary resistance in patients with limited or multifocal disease progression, who initially responded to the therapy, is related to the acquisition of additional *KIT* or *PDGFRA* mutations in approximately 60% of cases. Secondary mutations are found the most often in exons encoding the ATP binding site of the kinase domain (exons 13 and 14) or the kinase activation loop (exons 17 and 18). These mutations result in the conformational changes in the kinase domains which prevents drug molecule binding to the enzymatic pocket of the receptor or favors the kinase activated state [6, 72, 73, 106, 165, 166, 182]. For instance, a common secondary *KIT* exon 13 p.V654A mutation decreases the binding affinity between imatinib and the receptor. Val654 forms hydrophobic bonds with imatinib while Ala654 in the mutant oncoprotein does not form these bonds [148, 149]. Another frequent secondary mutation is *KIT* exon 14 T670I, often called the "gatekeeper" mutation. Thr670 forms a hydrogen bond with imatinib which stabilizes drug molecule and thus allows the molecule to enter a hydrophobic pocket but Ile670 cannot form such a bond [66, 149]. In addition, secondary mutation in *KIT* exon 17 encoding activation loop domain, such as p.D816V, changes the kinase domain conformation making the receptor unable to bind imatinib molecule [18]. Cases of imatinib-resistant GISTs associated with the acquisition of *PDGFRA* p.D842V mutation in tumors harboring primary *PDGFRA* exon 12 mutations were also reported [27, 36, 73]. Secondary mutations more frequently develop in tumors harboring primary mutations in *KIT* exon 11 rather than exon 9 (73% vs 19%; $p=0.0003$), probably because patients with initially imatinib-sensitive tumors have been treated for longer periods, providing both the selection pressure and time for the emergence of imatinib resistant clones [75, 88, 194]. The problem of secondary mutations is additionally complicated by the fact that multiple resistant clones can develop in the course of therapy. In some cases, multiple secondary *KIT* mutations may be detected with a distinct mutation in each separate anatomical site in a patient with progressive disease [73, 106, 193, 194]. Besides the acquisition of secondary *KIT*/*PDGFRA* mutations, the other mechanisms of late resistance to imatinib include: (1) *KIT* genomic amplification and overexpression outweighing inhibitory capacity of imatinib; (2) activation of alternative receptor tyrosine kinases, loss of *KIT* expression in previously KIT-positive tumors or development of multidrug resistance; (3) functional resistance in kinase-expressing tumors; (4) pharmacokinetics disturbances (e.g., overexpression of drug-efflux pump leading to decreased intratumoral imatinib levels; high blood level of alpha1-acid glycoprotein binding and inactivating imatinib; increased clearance of imatinib over time causing decreased systemic concentrations of imatinib; [66, 106, 194]).

Treatment Options for Imatinib Resistant GISTs

The spectacular response to imatinib therapy is time-limited and followed by the development of secondary resistance (after initial stabilization or response) in the majority of patients. In the pivotal US-Finland B2222 phase II study, 5% of patients demonstrated primary resistance to imatinib and in another 14% of patients early resistance occurred, and within 2–3 years of imatinib treatment approximately 40–50% of patients progressed showing secondary resistance to imatinib [39]. Initial or primary resistance is considered as disease progression during the first 3–6 months of imatinib therapy. Further progressions are considered as late or secondary resistance. There are several therapeutic strategies in patients showing progression during imatinib treatment, such as escalation of the dose of imatinib to 800 mg/day, surgical removal of focus progression-lesions, and therapy with registered second-line drug sunitinib malate (multi-targeted tyrosine kinase inhibitor with antiangiogenic properties; Fig. 14.4; [3, 40]).

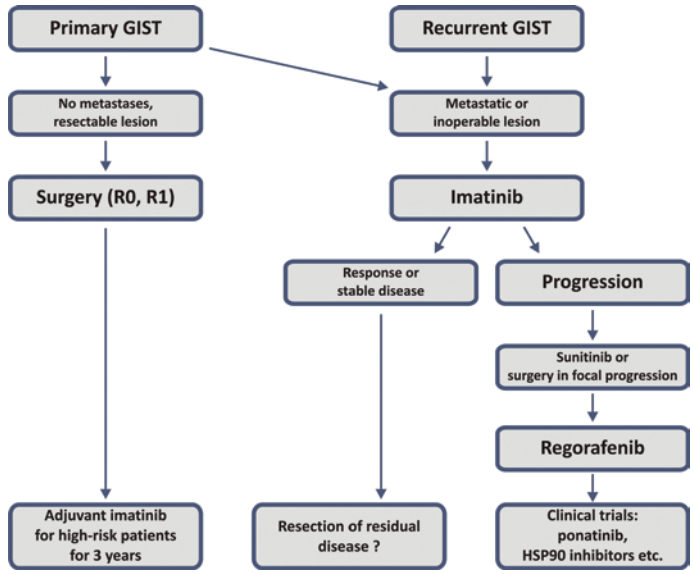

Fig. 14.4 Algorithm for GISTs patients' management

In the case of GIST progression during standard-dose imatinib therapy, the commonly used strategy is to increase of the imatinib dose to 800 mg/day. Approximately one-third of the patients whose tumor progressed on 400 mg initial dose demonstrate partial response or stable disease after a dose increase to 800 mg/day [139]. The results of this approach may depend on the primary mutational status, because patients with WT GISTs and tumors carrying primary *KIT* exon 9 mutations seem to be more likely to benefit from such strategy. In addition, the determination of plasma imatinib concentration may serve as a valuable parameter for optimal dose selection. It has been demonstrated that in patients with advanced GISTs imatinib trough level is associated with the clinical benefit [42]. In the case of limited progression (such as "node within a mass" pattern), if other metastatic lesions are still sensitive to imatinib, surgical resection, or ablation of the resistant tumor may be performed.

With further/generalized progression or intolerance to imatinib, second-line standard treatment using monotherapy with alternative RTK-targeting inhibitors is recommended. Sunitinib malate (Sutent®, SU11248; Pfizer) is an oral multitargeted tyrosine kinase inhibitor, which is active against a broad spectrum of tyrosine kinases: KIT and PDGFRA/B, vascular endothelial growth factor receptors (VEGFRs)-1, -2, and -3, FMS-like tyrosine kinase-3 (FLT3), colony stimulating factor 1 receptor (CSF-1R), glial cell-line derived neurotrophic factor receptor (Rearranged during Transfection [RET]; [92]) and to a lesser extent fibroblast growth factor receptor-1 (FGFR-1; [112]). Sunitinib was approved multinationally for the treatment of imatinib-resistant or -intolerant GISTs, since it demonstrated a significant antitumor and antiangiogenic activity in the clinical setting. Two phase II, one phase III and one "treatment-use" trials have investigated the activity of sunitinib in GIST patients after the failure of prior imatinib treatment, and all these trials have shown the significant activity of sunitinib in this population of patients (Table 14.7). The objective clinical benefit was achieved in approximately 60 % of GIST patients who received sunitinib after failure of imatinib therapy. Median PFS time on sunitinib is 6–8 months [40, 63, 109, 144]. In a phase I/II study sunitinib was administered to 97 patients who demonstrated progression or intolerance when treated with imatinib [73, 128]. Patients received 25, 50, or 75 mg of sunitinib per day in different schedules and 8 %

Table 14.7 Summary of the results of clinical trials evaluating second-line therapy of advanced GISTs with sunitinib

Phase; patients number Regiment (reference)	Partial response (%)	Stable disease	Disease progression	Median time to progression (months)
I/II; $n=97$ 50 mg/day 4 weeks on—2 weeks off (continuation study); [109]	8	70	22	7.8
II; $n=60$ 37.5 mg/day continuous dosing [63]	12	62	8	Median progression-free survival 35.1 weeks
III; $n=312$ 50 mg/day 4 weeks on—2 weeks off; [40]	19	58	7	6.8
$n=1,091$ 50 mg/day 4 weeks on—2 weeks off "treatment-use" [128]	14	63	22	37 weeks

of them experienced partial responses and 70% of patients showed stabilization of disease. In 37% of patients, stabilization lasted for more than 6 months. The median survival was 19.8 months (95% confidence interval [CI] 13.6–25.8) with 58% of patients surviving more than 1 year. Placebo-controlled phase III trial showed the median time to tumor progression for patients treated with sunitinib more than four times longer than that for patients receiving placebo (27.3 vs 6.4 weeks; $p<0.0001$; [40]). Patients were randomized (2:1) to receive either sunitinib or placebo orally, on a regimen of 4 weeks on treatment followed by 4 weeks off (4/2 schedule) 50 mg once per day, which is considered to be the highest tolerated dose. Partial response was observed in 6.8% of sunitinib-treated patients and in none of placebo-treated patients [40]. It has been also demonstrated that many advanced GIST patients benefit from sunitinib therapy (mainly due to stabilization of disease according to RECIST, not Choi criteria) with OS exceeding 1.5 years [157]. However, as with imatinib, complete radiological responses are extremely rare and responses are time-limited. The biological and pharmacological results (such as rebound phenomenon documented by PET scan) have stimulated the exploration of a continuous dosing schedule, which theoretically could prevent the decrease of tumor suppression during rest periods, perhaps resulting also in a better antitumor efficacy. The results of multicenter phase II trial have indicated that the continuous regimen with a lower daily dose (37.5 mg) may be equally effective and possibly better tolerated [63]. The most common treatment-related adverse events during sunitinib therapy include fatigue, diarrhea, skin discoloration, nausea, mucositis, arterial hypertension, hand and foot syndrome (palmar–plantar erythrodysesthesia), impairment of left ventricular ejection fraction, and hypothyroidism [40, 144] (Table 14.8). Importantly, arterial hypertension is not only the common side effect observed during sunitinib treatment but it also serves as a predictive factor of sunitinib antitumor efficacy in renal-cell carcinoma (RCC) and GIST patients, as it influences both the PFS and OS [146, 157]. *In vitro* studies have determined that sunitinib metabolism is mediated primarily by the cytochrome P450 CYP3A4 isoenzyme; therefore, an extreme caution should be undertaken when it is necessary to administer sunitinib in combination with known inhibitors or inducers of CYP3A4 [108].

KIT mutational status appears to serve as a predictor of tumor response to sunitinib as well. Unlike imatinib, tumors initially (prior to imatinib treatment) harboring *KIT* exon 9 mutation or wild-type GISTs have a higher chance to respond to sunitinib. Moreover, GISTs carrying *KIT* exon 9 mutations appear to be more sensitive to sunitinib than those with primary *KIT* exon 11 mutations. Clinical benefit of sunitinib in the WT cases (e.g., in pediatric GISTs) is also clear. However, patients with *PDGFRA* mutations (mainly p.D842V) do not respond to sunitinib treatment [142, 157]. In a phase I/II trial of sunitinib conducted in patients with imatinib-resistant tu-

Table 14.8 The most common adverse events during sunitinib therapy in advanced GISTs (based on phase I–III clinical trials and post-registration)

Adverse event	All grades (%)	Grade 3 or 4 (%)
Hand and foot syndrome	13.0–21.4	0–5.4
Skin rash	13.0–15.2	0–0.8
Yellowish skin discoloration	25.3	0
Arterial hypertension	13.0–43	0–7.0
Hypothyroidism	5.8–62.0	0.4
Loss of apetite	7.1–33.0	0.4–5.0
Headache	10.5	0.8
Diarrhoea	29.0–40.0	0–5.1
Nausea	24.0–31.0	0–2.0
Stomatitis	16.0–19.1	0.8
Vomitings	16.0–24.0	0.4–2.0
Dyspepsia	12.5	0.8
Abdominal pain	11.7–33.0	2.3–11.0
Meteorism	5.8	0
Gastro-esophageal reflux	5.8	0
Pain of extremities	8.2	0.4
Joint-muscle pain	5.8–12.0	0.8–1.0
Anemia	12.8–58.0	4.0–5.5
Neutropenia	9.3–43.0	6.2–10.0
Thrombocytopenia	8.9–36.0	2.7–5.0
Fatigue/malaise	34–52.5	0–9.7
Mucositis	11.7	0
Decrease of left ventricle ejection fraction	5.1–10.0	0.4–1.0
Bleedings (all) including: epistaxis	3.0–18.0 2.0–7.0	0–7.0 0
Xerostomia	6.0	0
Dysgeusia	11.0–21.0	0

mors, the partial response rate was significantly higher in patients with GISTs carrying primary *KIT* exon 9 mutations than those with exon 11 mutations (37 vs 5%). In addition, patients with *KIT* exon 9 mutations or WT genotype had four times longer PFS and doubled OS compared to patients with *KIT* exon 11 mutations [75]. Biochemical profiling of secondary kinase mutations revealed *in vitro* sensitivity of *KIT* exon 13 and 14 mutations to sunitinib, while secondary *KIT* exon 17 and 18 mutations remained resistant to sunitinib [70, 142]. The observation was confirmed by the clinical studies that sunitinib is not active against most imatinib-resistant secondary mutations affecting KIT activation loop [74, 75]. On the other hand, the utility of secondary mutation analysis is very challenging because imatinib-resistant GISTs are very heterogeneous with multiple clones having different secondary mutations within the same or different nodule [6, 62, 106, 194].

Regorafenib (BAY 73-4506; Bayer) is an oral multikinase inhibitor of angiogenic (VEGFR-1, VEGFR-3 and TIE2), stromal (PDGFRB and FGFR) and oncogenic (KIT, RET and BRAF) receptor tyrosine kinases [64, 165, 196], which has been recently approved in the third-line therapy after imatinib and sunitinib failure. Regorafenib demonstrated encouraging KIT inhibitory activity *in vitro* on imatinib-resistant KIT double mutants, which carried exon 11 mutation and secondary mutations, including p.T670I and p.V654A in ATP-binding pocket or p.D816G, p.N882K, and p.Y832D in the activation loop [196, 197]. The results of a phase II trial showed clinical benefit in 73% of GIST patients following failure

of imatinib and sunitinib with median PFS of 10 months [64]. The phase III study of regorafenib in metastatic or unresectable GIST patients whose disease had progressed despite prior treatment with at least imatinib and sunitinib showed significant improvement of PFS for patients who received regorafenib as compared to patients who received placebo (4.8 months vs 0.9 months, respectively; hazard ratio 0.27; [44]). The most common regorafenib-related adverse events at grade 3/4 were arterial hypertension, hand-foot skin reaction and diarrhea.

Experimental Therapy of Imatinib- and Sunitinib-resistant GISTs

Alternative Kinase Inhibitors

If dose escalation with imatinib and sunitinib/regorafenib therapy fails, clinical trials with novel agents alone or in combinations may be considered. The future management of GISTs is likely to be altered not only by the availability of novel drugs, but also by better understanding of biological mechanisms underlying response to the certain type of therapy. The first strategy in the treatment of advanced GISTs refractory to the standard therapy is the application of agents that inhibit a broad spectrum of kinases (including KIT) and that exhibit inhibitory effects against imatinib-resistant mutants. Numerous new generation RTK inhibitors, other than imatinib and sunitinib, are currently available (Table 14.9) and most of them affect a broad range of tyrosine kinases, thus they may overcome the resistance to the standard targeted therapy. RTK inhibitors which have demonstrated antitumor activity in imatinib-naïve and imatinib-resistant GISTs include nilotinib (AMN107), masitinib (AB1010), sorafenib (BAY43-9006), vatalanib (PTK787/ZK222584), dasatinib (BMS-354825), motesanib (AMG706), cediranib (AZD2171; [46]), ponatinib (AP24534), and midostaurin (PKC412).

Pre-clinical studies on cell lines carrying mutations sensitive and resistant to imatinib have shown that nilotinib (AMN107; Tasigna; Novartis) inhibits growth of these cells more potently than imatinib. Moreover, nilotinib intracellular concentrations are 7–10-fold higher than those of imatinib in the analyzed cell lines [142]. Weisberg et al. [195] demonstrated that nilotinib inhibits growth of *KIT* exon 11 p.V560del and *KIT* exon 13 p.K642E mutant Ba/F3 cells as effectively as imatinib. It has been also shown that nilotinib is a potent inhibitor of *KIT* p.V560G mutant cells [195], but is not effective against the gatekeeper *KIT* exon 14 single (*KIT* p.T670I) or double (*KIT* p.W557_K5588del/T670I) mutants [70]. Moreover, nilotinib induced cell growth inhibition in imatinib-resistant *KIT* p.V560del/V654A and *KIT* p.V559D/D820Y mutant cells at lower concentrations than dasatinib and sorafenib (see below; [70]). The results of a phase I study with nilotinib alone or in combination with imatinib have shown its relevant activity in imatinib-resistant GISTs with more than two-thirds of patients exhibiting disease stabilization and with the median time to progression in the third-line therapy of about 6 months [189]. However, the advanced phase III studies of the third and first-line treatment with nilotinib have been stopped recently as the results did not show any significant benefit from the use of nilotinib as compared to the standard therapeutic options.

Another potent KIT inhibitor masitinib mesylate (AB1010; AB Science) has shown greater *in vitro* activity and selectivity than imatinib by the means of inhibition of recombinant human WT KIT, human, and murine KIT with activating mutations in the juxtamembrane domain and recombinant PDGFRA [48]. Masitinib was evaluated in the first-line setting in patients with advanced GISTs in a small phase II trial [17, 102]. Preliminary data suggested that the progression-free survival with masitinib (PFS rates of 59.7% [95% CI: 37.9; 76.0] and 55.4% [95% CI: 33.9; 72.5] at 2 and 3 years, respectively) in treatment-naïve GISTs may be longer than with imatinib. The OS at 2 and 3 years was stable at 89.9% (95% CI: 71.8; 96.6) [102]). Furthermore, masitinib's selective inhibition of KIT and PDGFRA but not ABL kinase was hypothesized to cause less cardiotoxicity than imatinib and, at least in this small study, there were no reports of cardiotoxicity with masitinib [92, 102]. Preliminary results

Table 14.9 Novel agents with promising activity in advanced GISTs

Compound name	Company	Target	Examples of known sensitive KIT/PDGFRA mutations	Examples of known resistant *KIT/PDGFRA* mutations	Phase of development (Clinical-Trial.gov ID)
Receptor tyrosine kinase inhibitors					
Nilotinib (AMN107)	Novartis	KIT, PDGFRA/B, ABL	KIT: V560G/V654A; V559D/D820Y;K642E	KIT: W557_K558del/T670I PDGFRA:D842V	Phase III (NCT00785785)
Sorafenib (BAY43-9006)	Bayer	VEGFR2/3, PDGFRB, KIT, BRAF, FLT-3, RET	KIT: W557_K558del/T670I; V560del/V654A; V559D/D820Y	KIT: 670I; V654A; D816G; N882K; Y832D	Phase II (NCT01091207)
Regorafenib (BAY73-4506)	Bayer	VEGFR1,2,3, DGFRB, KIT, FGFR1, BRAF, RET	KIT: T670I; D816G; N822K A829P	KIT: V654A	Phase III, recently approved in third line (NCT01271712)
Masitinib (AB1010)	AB Science	KIT, PDGFR, FGFR3			Phase III (NCT00812240)
Motesanib (AMG706)	Amgen	KIT, PDGFRA/B, VEGFR1-3, RET			Phase II (NCT00254267)
Vatalanib (PTK787/ ZK222584)	Bayer/Novartis	VEGFR -3, PDGFRA/B, KIT			Phase II (NCT00117299)
Midostaurin (PKC412)	Novartis	KIT, PDGFRA/B, VEGFR2, PKC	KIT: D816V; T670I; V654A; PDGFRA: V561D; D842V		

Table 14.9 (continued)

Compound name	Company	Target	Examples of known sensitive KIT/PDGFRA mutations	Examples of known resistant *KIT/PDGFRA* mutations	Phase of development (Clinical-Trial.gov ID)
Dasatinib (BMS-354825)	BMS	SRC, ABL, KIT, PDGFRA	KIT: D816Y; V559D; V560G; W557_K558del D816Y	KIT: W557_K558del/T670I PDGFRA: D842V	Phase II (NCT00568750)
Vandetanib (ZD6474)	AstraZeneca	VEGFR-2, EGFR, RET			
Pazopanib (GW786034)	GSK	VEGFR-1,-2,-3, PDGFRA/B, KIT			Phase II (NCT01323400)
Crenolanib	Arog Pharmaceuticals	PDGFRA		PDGFRA: D842V	Phase II (NCT01243346)
Ponatinib (AP24534)	ARIAD	KIT, PDGFRA	KIT: D816A/G/H/V D820A/E/G/Y N822H/K Y823D A829P T670I	KIT: V654A	Phase II (NCT01874665)
Cediranib (AZD2171)	AstraZeneca	KIT, PDGFRA/B, VEGFR1-3, Flt-3			Phase II (NCT00385203)
Inhibitors of PI3K/mTOR pathway					
GDC0941	Genentech	PI3K			Phase I
BYL719	Novartis	PI3K			Phase I (NCT01219699)
BEZ235	Novartis	PI3K/mTOR			Phase I/II
BKM120	Novartis	PI3K			Phase I (NCT01468688)
Everolimus (RAD001)	Novartis	mTOR			Phase II (NCT00510354)
Perifosine (KRX-0401)	Keryx Biopharmaceuticals	AKT			Phase II (NCT00455559)

Table 14.9 (continued)

Compound name	Company	Target	Examples of known sensitive KIT/PDGFRA mutations	Examples of known resistant *KIT/PDGFRA* mutations	Phase of development (Clinical-Trial.gov ID)
Monoclonal antibodies					
Olaratumab (IMC-3G3)	ImClone	PDGFRA			Phase II (NCT01316263)
Bevacizumab (NSC-704865)	Genentech	VEGFR			Phase III (NCT00324987)
HSP-90 inhibitors:					
Retaspimycin (IPI-504)	Infinity Pharm.	HSP-90			Phase I (NCT00276302)
IPI-493	Infinity Pharm.	HSP-90			
STA-9090	Synta Pharmaceuticals	HSP-90			Phase II (NCT01039519)
AUY922	Novartis	HSP-90			Phase II ((NCT01404650 and NCT01389583)
AT-13387	Astex Pharm	HSP-90			Phase II (NCT01294202)
BIIB021	Biogen Idec	HSP-90			Phase II NCT00618319
HDAC inhibitors					
Panobinostat (LBH589)	Novartis	HDAC			
Inhibitors of IGF1R					
Linsitinib (OSI-906)	OSI Pharmaceuticals	IGF1R			Phase II (NCT01560260)

VEGFR Vascular Endothelial Cell Growth Factor Receptor, *EGFR* Endothelial Cell Growth Factor Receptor, *PDGFRA* Platelet-Derived Growth Factor Receptor, Alpha/Beta polypeptide, *PKC* Protein Kinase C, *FLT3* (FMS-like tyrosine kinase 3), *HSP-90* Heat shock protein 90, *mTOR* mammalian target of rapamycin, *HDAC* Histone deacetylase, *IGF1R* Insulin-like growth factor 1 receptor

of another phase II study evaluating masitinib in imatinib-resistant GISTs patients showed the promising 2-year OS rate of 53% with a good safety profile of the drug.

Sorafenib (BAY 43-9006, Nexavar; Bayer) is a multitargeted inhibitor of selected RTKs, including VEGFR-2, VEGFR-3, PDGFRB, BRAF, and KIT. The results of a study evaluating *in vitro* activity of sorafenib in KIT mutant cells demonstrated the inhibition of imatinib-resistant *KIT* p.T670I gatekeeper mutation in the GIST model, thus suggesting its clinical activity in cases with acquired resistance [69]. The results of a phase II trial examining sorafenib in 38 patients with imatinib- and sunitinib-resistant GISTs showed that five patients (one imatinib-resistant and four imatinib- and sunitinib-resistant) demonstrated partial response and further 21 patients (three imatinib-resistant and 18 imatinib- and sunitinib-resistant) demonstrated stabilization of disease. Grade 3/4 toxicities included hand-foot syndrome, hypertension, diarrhea, hypophosphatemia, GI bleed, rash, thrombosis, GI perforation, fatigue, and anemia. Median PFS was 3.4 months in the group resistant to imatinib only and 5.2 months in the group resistant to both agents. Median OS was 13.6 and 10.5 months, respectively. These data demonstrated that sorafenib has definite activity in imatinib- and sunitinib-resistant GISTs and gives a prolonged disease control in selected patients [19, 127]. Based on these results, several studies evaluating regorafenib (a sorafenib-derived compound) in GISTs were undertaken.

Vatalanib (PTK787/ZK222584; Bayer and Novartis) is another orally bioavailable small molecule multitargeted RTK inhibitor active against VEGFR-1, -2, and -3, KIT, and PDGFRB [199]. This agent was tested in a phase II trial in the treatment of imatinib-resistant metastatic GISTs and demonstrated a clinical benefit (partial response or stable disease) in 10 of 15 patients with a median time to progression of 8.5 months [84].

Also, dasatinib (BMS-354825; Sprycel; BMS) is a novel oral small molecule ATP-competitive tyrosine kinase inhibitor exhibiting activity against BCR-ABL, the Src family of kinases, KIT and PDGFRA [45, 164, 178]. Interestingly, it has been estimated that dasatinib is 325-fold more potent than imatinib against cells expressing WT BCR-ABL [132]. *In vitro* studies showed that dasatinib inhibits activity of WT KIT, as well as juxtamembrane and activation loop imatinib-resistant mutant KIT isoforms including KIT p.V559D, p.V560G, p.W557_K558del, and p.D816Y [45, 164]. Furthermore, it has been demonstrated that dasatinib may successfully inhibit the imatinib-resistant PDGFRA p.D842V mutant isoform [45]. However, a phase II study in imatinib- and sunitib-refractory advanced GIST patients did not confirm significant activity of dasatinib [179]. Dasatinib was also tested as the first-line treatment of GISTs but the recruitment was stopped early due to the high toxicity of the drug.

Motesanib (AMG 706; Amgen) is another small molecule orally bioavaliable that potently inhibits VEGFR-1, -2, -3, KIT, and to a lesser extent RET and PDGFR. Similarly to sorafenib and vatalanib, motesanib has the potential to enhance the clinical benefit of KIT or PDGFR inhibition by affecting angiogenesis, regarding its ability to target VEGF receptors. The antiangiogenic activity of motesanib was demonstrated to contribute to the reduction of tumor growth in a pre-clinical xenograft model of breast cancer through decrease in neovascularization [30, 141]. In a phase II study on motesanib, the clinical benefit was achieved in 62% of 102 patients with a median PFS estimated at 4 months [10].

Staurosporine derivative and protein kinase C inhibitor additionally affecting VEGFR-2, PDGFRA/B, KIT and FLT-3, called midostaurin (PKC412, Novartis), was also tested against imatinib-resistant GISTs both in *in vitro* and *in vivo* studies. This agent was demonstrated to be active in GISTs harboring imatinib-resistant *PDGFRA* p.D842V, gate-keeper *KIT* p.T670I, *KIT* p.V654A, and *KIT* p.D816V mutations [36].

Since the insulin-like growth factor 1 receptor (IGF1R) is overexpressed in the majority of wild-type GISTs (including pediatric GISTs; [16, 28, 78, 134]), it was proposed as a novel therapeutic target. Recent *in vitro* studies confirmed that GIST cells are sensitive to the IGF1R in-

hibitor NVP-AEW541, alone and in combination with imatinib [136, 175].

Oncogenic signaling in GISTs is sustained mainly *via* phosphoinositide 3-kinase/mammalian target of rapamycin (PI3K/AKT) rather than RAS/MAPK, which are both activated by mutated forms of KIT and PDGFRA [9]. Phosphorylation/activation of the downstream proteins these signaling pathways leading to uncontrolled cell proliferation and survival as well as inhibition of proapoptotic signaling [140]. Several PI3K inhibitors such as GDC0941 (Genentech), BYL719 (Novartis), BKM120 (Novartis), or PI3K/mTOR dual inhibitors BEZ235 (Novartis) were sucesfully tested *in vitro* and *in vivo* using different GIST models [58, 105]. Floris et al. showed that combination of GDC-0941 and imatinib has extensive antitumor efficacy in GIST xenograft, inducing more substantial apoptosis and durable effects compared to imatinib alone. This effect was sustained even after treatment withdrawal [58].

Moreover, agents listed above are tested in combinations to target different pathways involved in GIST pathogenesis. Usually such combinations include one RTK inhibitor affecting KIT with: (1) other RTK inhibitors targeting a broader range of *KIT* mutations and acting on other tyrosine kinases, (2) inhibitors of VEGFR decreasing the interstitial fluid pressure in tumors resulting in enhanced drug uptake and synergistic effect of therapy [198], (3) agents targeting *KIT* and *PDGFRA* downstream effector pathways (especially, PI3K/AKT/mTOR pathway), (4) conventional chemotherapeutics since the compounds inhibiting KIT and VEGFR may potentially sensitize tumor cells to the cytotoxic drugs. It is obvious that resistant tumors have more than one target; therefore, the systemic treatment in such cases should be multitargeted. Several drug combinations have been the subject of early clinical testing: (1) imatinib with oblimersen (inhibitor of antiapoptotic protein BCL-2), (2) imatinib or sunitinib with perifosine (KRX-0401, a synthetic alkylphospholipid targeting cell membrane signal transduction pathways and AKT activation), (3) imatinib with everolimus (RAD001—an oral inhibitor of the mammalian target of rapamycin (mTOR) which is a downstream effector in the PI3K/AKT pathway; [186]), and (4) sunitinib with vandetanib (ZD6474—an antiangiogenic compound and inhibitor of VEGFR-2, EGFR, and RET; [202]). Successful induction of growth arrest and apoptosis of GIST-T1 cells in association with inhibition of KIT and its downstream effectors in PI3K/AKT/mTOR pathway caused by the synergistic activity of sunitinib and vandetanib provide a strong rationale for further investigation of such combinations [202]. However, Sambol et al. [160] have demonstrated that the efficacy of another novel agent called flavopiridol (a cyclin-dependent kinase inhibitor) in a GIST882 cell line carrying *KIT* exon 13 p.K642E mutation, which induced apoptosis and downregulation of KIT more potently than imatinib, but the combination of flavopiridol and imatinib was essentially equivalent to flavopiridol alone.

Recent development of monoclonal antibodies against PDGFRA and VEGFR contributed to the introduction of a new direction in the GIST therapy. These antibodies are currently being tested in two clinical studies in patients with metastatic or unresectable GISTs: a phase II trial evaluating human anti-PDGFRA monoclonal antibody called olaratumab (IMC-3G3) in previously treated patients and a phase III study of imatinib with or without anti-VEGFR monoclonal antibody bevacizumab (NSC-704865).

Alternative Targets and Their Antagonists

A novel promising strategy for treating GISTs resistant to tyrosine kinase inhibitors (TKIs) may be the application of drugs leading to the oncoprotein degradation. Both KIT and PDGFRA are stabilized and protected from proteasome-mediated degradation by the molecular chaperone heat shock protein 90 (HSP-90; [61]). There are several compounds with HSP-90 inhibitor activity which are currently tested in the clinical trials involving TKI-resistant GIST patients, such as IPI-504, IPI-493, STA-9090, AUY922, AT-13387, and BIIB021 (CNF2024). It has been demonstrated that the inhibition of HSP-

90 causes loss of KIT expression on cell surface and loss of KIT phosphorylation, especially in cells with mutated KIT [8, 103, 131]. However, HSP-90 inhibition induces KIT degradation regardless of specific activating mutations, as all of KIT isoforms need HSP-90 for their stabilization. Bauer et al. [8] have shown that HSP-90 inhibitor 17-allylamino-18-demethoxy-geldanamycin (17-AAG) affected both imatinib-sensitive and imatinib-resistant KIT oncoproteins with their downstream effectors and repressed proliferation in several different GIST cell lines. *In vivo* experiments proved that retaspimycin (17-allylamino-17-demethoxygeldanamycin hydroquinone hydrochloride, IPI-504, Infinity Pharmaceuticals, Inc.) alone resulted in tumor regression, proliferation arrest, and induction of tumor necrosis. The treatment effects were enhanced by combining IPI-504 with imatinib or sunitinib [59]. Demetri et al. presented early data from a phase I trial evaluating retaspimycin an intravenous compound inhibiting HSP-90 which is a highly soluble hydroquinone hydrochloride derivative of 17-AAG with favorable pharmaceutical properties. Retaspimicyn administration resulted in disease remission on PET imaging, CT and in pathological examination of patients with metastatic TKI-resistant GISTs [41, 173, 180, 190]. IPI-504 was also demonstrated to suppress the proliferation of GIST cells carrying PDGFRA p.D842V mutation [45]. Despite the fact that recently a phase III trial evaluating the efficacy of IPI-504 in advanced GIST patients, resistant to imatinib and sunitinib treatment, was terminated prematurely due to the unexpected liver toxicity, which was also shown in preclinical experiments [59], other compounds with HSP-90 inhibition activity are still under study. IPI-493 (Infinity) has been studied in mouse xenograft model by Floris et al. [60] showing that as a single agent it has consistent antitumor activity and induces KIT down-regulation in GISTs with heterogeneous KIT mutations. In addition, IPI-493 synergizes with TKIs that are commonly used for the treatment of advanced or imatinib resistant GISTs. The antitumor response of IPI-493 is particularly enhanced in combination with sunitinib [60]. Another HPS90 inhibitor, a non-geldanamycin derivative, AT13387 shows an effectiveness against both imatinib-sensitive and imatinib-resistant GISTs. AT13387 inhibits cell proliferation and KIT signalling ablation in both *in vitro* and *in vivo* models. The drug was well tolerated *in vivo*, also in combination with imatinib [169, 170]. AT13387 is currently being evaluated in the clinic in a phase II GIST trial in combination with imatinib.

Promising preclinical data were demonstrated regarding the activity of histone deacetylase (HDAC) inhibitor panobinostat (LBH589) in human GIST mouse xenograft model [57]. Human HDAC family is a well-recognized anticancer target which plays a key role in the control of transcriptional regulation [31, 34, 91]. Panobinostat affects HDAC, modulates expression of cell cycle proteins, induces cell cycle arrest, apoptosis, and influences the angiogenesis-related genes [57, 129]. It was shown that the combination of panobinostat with imatinib enhanced the therapeutic effect in GIST xenograft model, as well as the proapoptotic effect in GIST cell lines, what may constitute promising strategy for overcoming the resistance to imatinib during treatment of advanced GISTs [57, 129].

GIST cells are often characterized by overexpression of prosurvival Bcl-2 family members such as Bcl-2, Bcl-xL, and Mcl-1 [171]. *In vitro* studies proved that ABT737, a BCL-2 family inhibitor, showed antiproliferative and apoptotic effects alone and in combination with imatinib in GIST cell lines—direct engagement of apoptotic cell death may be an effective approach to circumvent imatinib-resistance in GISTs [145].

One of the major challenges in the therapy of advanced GISTs is the individualization of treatment which seems to become possible in the nearest future. The first and most crucial parameter, serving as a predictive factor for the choice of therapeutic agent, is the mutational status of the primary tumor. In general, patients bearing *KIT* exon 11 mutations are more likely to benefit from imatinib treatment than patients with *KIT* exon 9 mutations, *PDGDRA* p.D842V mutations or WT GISTs. Moreover, it is now well known that patients harboring *KIT* exon 9 mutations should receive higher than standard dose of imatinib to increase their chances for prolonged

PFS. Similarly, imatinib-treated patients carrying a point mutation/deletion at *KIT* codons 565 or 579 have worse outcomes as compared to the patients harboring exon 11 mutation at a different site. Personally tailored therapy in these imatinib-refractory GISTs should be based on the understanding of individual molecular mechanisms of resistance, such as the secondary mutations and their sensitivity to specific agents. For instance, the presence of a *KIT* p.T670I gatekeeper mutation in resistant GISTs may indicate sorafenib or sunitinib as the next therapeutic option, whereas other secondary mutations may be more susceptible to nilotinib or PKC412. On the other hand, sunitinib is less active against tumors with secondary *KIT* exon 17 and 18 mutations as compared to exon 13 or 14 mutations. Of note, although novel small molecule kinase inhibitors are potent against different *KIT*/*PDGFRA* mutants, essentially they are imatinib alternatives and they do not effectively inhibit all imatinib-resistant molecular alterations. Therefore, novel approaches targeting pathways downstream of KIT and PDGFRA regardless of the specific mutational activation mechanisms, or affecting the oncoprotein stability, are worth further exploration.

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Targeted Therapies in Kidney Cancer

15

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Introduction

Around 65,000 patients are diagnosed every year with kidney cancer in the United States and this disease, representing 4–5 % of all new cancer diagnosis, causes nearly 12,000 deaths [1]. Approximately 25–30 % of patients with kidney cancer are diagnosed with locally advanced or metastatic disease and one-third of patients with local disease who undergo surgery will recur. Renal cell carcinoma (RCC) arises from the renal epithelium and represents the most common form of kidney malignancy. Although historically considered as a single entity, we have recently learned that RCC includes a number of different tumor subtypes that occur in the kidney (clear cell, papillary, chromophobe, etc.). Each one has different genetics and molecular basis, a particular clinical course, and distinctive response to the available treatments [2]. Traditionally, therapeutic options for advanced RCC have been limited to cytokines that provide responses in the range of 10–15 % with a median overall survival (OS) of 10–12 months and notable toxicity [3]. However, in the last decade, parallel to substantial advances in the understanding of the molecular biology of clear cell RCC (ccRCC), several targeted agents have been developed for this disease leading to a relevant improvement in treatment outcomes. Currently, patients with advanced RCC can achieve median survivals exceeding the 2-year mark [4].

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Molecular Biology of Kidney Cancer

Clear Cell Renal Cell Carcinoma

ccRCC can be sporadic (95 %) or familial (4–5 %) [5]. Mutations in the Von Hippel–Lindau (VHL) gene are associated with the homonymous syndrome, characterized by an increased risk of several medical disorders with a characteristic hypervascularization, such as retinal angiomas, hemangioblastomas, and also ccRCC. Patients with VHL syndrome have 40–60 % risk of developing ccRCC in their lifetime. Abnormal function of the VHL gene (either by mutation or by methylation) has been also found in approximately 90 % of the sporadic forms of ccRCC [6], pointing out the relevance of this gene in the tumorogenesis of this particular histological subtype. Moreover, understanding the VHL gene pathway has provided the foundation for the development of targeted therapies in ccRCC.

The VHL gene encodes a protein (pVHL) that regulates the levels of a transcription factor named hypoxia-inducible factor (HIF) that regulates cellular response to oxygen availability.

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Under normoxic conditions, HIF is eliminated by ubiquitinization when interacts with pVHL. Under low oxygen conditions or in the case of defective pVHL function, there is an accumulation of HIF, which activates the transcription of genes involved in adaptation to hypoxia and closely related to tumorogenesis. These include angiogenesis factors, such as vascular endothelial growth factor (VEGF), cell growth factors such as transforming growth factor alpha and beta (TGF alpha–beta), platelet derived growth factor (PDGF), and genes that promote glycolysis [7]. Many of the current targeted therapies for ccRCC act on the products of these genes and their cognate receptors.

Papillary Type I Renal Cell Carcinoma

Hereditary papillary renal cell carcinoma (HPRC) is an inherited disease with a high risk of developing bilateral type I papillary RCC. It is associated with an activating mutation in MET gene on chromosome 7. MET seems to be less frequently mutated than VHL in sporadic RCC, with an estimate of around 10 % of the cases [8]. MET encodes a protein that is a cell surface receptor of hepatocyte growth factor (HGF). The activation of this receptor by binding HGF promotes binding of second messenger molecules as GRB2, GAB1, or PI3K and triggers cell proliferation pathways. The MET mutation causes permanent activation of the tyrosine-kinase domain of the receptor, without ligand HGF stimulation. MET overexpression confers a growth advantage but additional steps might be necessary for the development of papillary RCC tumors [9]. Based on this, MET and their regulators could represent appropriate treatment targets in RCC, especially in papillary type I tumors.

Papillary Type II RCC

The familial form of papillary type II RCC is part of a syndrome, namely, Hereditary Leiomyomatosis RCC (HLRCC) that confers an increased risk of this unusual aggressive kidney cancer and uterine and cutaneous leiomyomas [10]. The inactivating mutation involved in this syndrome affects the fumarate hydratase (FH) gene, which encodes this Krebs cycle enzyme. When FH is inactivated, levels of fumarate increase and prevent HIF degradation by hydroxylation. HIF accumulation, as already noted above, promotes transcription of genes related to tumorogenesis. This is the basis of the therapeutic approaches targeting the HIF pathway, in both ccRCC and in papillary type II tumors.

Chromophobe Renal Cell Carcinoma

Inherited inactivating mutation of the Birt–Hogg–Dube (BHD) gene on the short arm of chromosome 17 leads to a syndrome that bears this name, characterized by increased risk for different types of renal tumors, such as chromophobe RCC (33 %), hybrid oncocytic neoplasms (50 %), ccRCC (10 %), or oncocytoma (7 %) [11]. This syndrome has characteristically the presence of fibrofolliculomas (85 %), which are benign hair follicle tumors, and pulmonary cysts (> 85 %) associated with spontaneous pneumothorax. This germ line BHD gene mutation is found in around 90 % of BHD-affected family members [12]; however, it seems to be rare in sporadic chromophobe RCC [13]. BHD gene encodes a protein, namely, folliculin, which interacts with two proteins (FNIP1 and FNIP2) and binds AMPK. This complex down-regulates mTOR activity. Therefore, when folliculin is defective, it exist an increased activity of mTOR pathway, resulting in HIF up-regulation [14]. Therefore, from a mechanistic perspective, both mTOR and HIF pathways are considered therapeutics targets in this type of renal tumor, and has been shown in animal models.

Clinical Development of VEGF-Targeted Therapy in RCC

Once the molecular bases of RCC have been reviewed, we will present the current targeted agents for the treatment of this disease.

Targeting the Ligand: Anti-VEGF Antibody

VEGF-mediated activity in RCC can be targeted through different strategies. Those include blocking the ligand itself through the administration of monoclonal antibodies and inhibiting the tyrosine-kinase activity of the cognate receptor (vascular endothelial growth factor receptor [VEGFR]) with small molecules. Recently, drugs with a combined effect have also been developed and will be shortly described.

Bevacizumab

Bevacizumab is an intravenously (i.v.) administered recombinant human monoclonal antibody directed against VEGF. This compound binds and neutralizes all biologically active isoforms of VEGF [15]. Bevacizumab was the first of its class to demonstrate activity in the treatment of advanced RCC. A randomized phase II trial, in which 116 patients with treatment-refractory metastatic ccRCC were randomized to receive placebo, low-dose (3 mg/kg), or high-dose bevacizumab (10 mg/kg) i.v. every 2 weeks, demonstrated that the superiority of this antibody and opened the field of anti-angiogenics for the treatment of metastatic RCC [16]. Later on, two multicenter international phase III studies, one in North America (CALGB 90206 trial) and one in Europe (AVOREN study), investigated bevacizumab in combination with interferon alfa-2a (IFN) versus IFN, in the first line setting [17, 18] (Table 15.1).

Both evaluated the same doses of bevacizumab (10 mg/kg every 2 weeks i.v.) and IFN alfa-2a (9 MU three times weekly subcutaneous) but differed slightly on the populations that were included and the use of placebo. The CALGB 90206 allowed inclusion of patients without cytoreductive nephrectomy and did not use placebo in the IFN alone arm. In both studies, progression free survival (PFS) was significantly longer with bevacizumab plus IFN than with IFN monotherapy. In addition, the experimental arm showed trends towards a longer overall survival (OS) but not reaching statistical significance [19, 20].

These OS results could be explained by the fact that the European trial patients in the placebo–IFN arm were allowed to cross over to the bevacizumab arm and more than 50 % of the patients in each arm who discontinued received subsequent therapy. In the CALGB 90206 study, crossover was not permitted but the patients who received IFN received subsequent therapy. In terms of response rate (RR), both studies showed a significant increase in objective RR in the experimental arm (25.5 vs. 13.1 %, $p<0.0001$ and 31 vs. 13 %, $p<0.0001$ in the American and European studies, respectively). Main toxicities of Bevacizumab plus IFN included fatigue, asthenia, anorexia, hypertension and proteinuria.

Based on these results, Bevacizumab plus interferon alfa-2a was approved by the European Medicines Agency (EMA) in November 2007 and by the US Food and Drug Administration (FDA) in August 2009 for the first-line treatment of metastatic renal cell carcinoma (mRCC) patients with good or intermediate risk according to Memorial Sloan Kettering Cancer Center (MSKCC) classification.

Recently, bevacizumab has been investigated in combination with mTOR inhibitors (Temsirolimus and everolimus) in mRCC with additive toxicity and no clear benefit [21–24].

Targeting the Receptor: Small-Molecules Blocking VEGFR Activity

An alternative approach to direct inhibition of VEGF is blocking the activity of its cognate

Table 15.1 Randomized clinical trials testing the combination of IFN and bevacizumab in mRCC

Bevacizumab-IFN vs IFN	AVOREN ($n=649$)	CALGB 90206 ($n=732$)
PFS	10.2 vs 5.5 months, HR 0.63, $p>0.001$	8.5 vs 5.2 months, HR 0.71, $p<0.0001$
ORR	31 vs 13 %	25.5 vs 13.1 %
OS	23.3 vs 21.3 months, HR 0.86, $p=0.129$	18.3 vs 17.4 months, HR 0.86, $p=0.07$

PFS progression free survival, *ORR* overall response rate, *OS* overall survival, *HR* hazard-ratio

receptors. Several tyrosine-kinase inhibitors (TKIs) have been developed in this setting to treat advanced RCC and are here presented.

Sorafenib (BAY 43-9006)

Sorafenib is an oral multikinase inhibitor that inhibits VEGFR 1–3, platelet-derived growth factor receptor (PDGFR), stem cell factor receptor, c-Kit, and the serine–threonine kinase Raf-1.

The activity of sorafenib in advanced RCC was demonstrated in the phase III Treatment Approaches in Renal Cancer Global Evaluation Trial (TARGET) study. An international phase III randomized, double blind, placebo-controlled, trial of single-agent sorafenib in 905 cytokine-refractory mRCC patients with favorable or intermediate MSKCC risk score for survival. Patients received continuous oral sorafenib, 400 mg twice daily or placebo. Few objective responses were observed (10 vs 2%, $p<0.001$) but a PFS advantage (median 5.5 vs 2.8 months, hazard ratio (HR) 0.44, 95% CI 0.35–0.55, $p<0.01$) was obtained [25]. Improvement in the primary endpoint of OS did not reach significance in the intent-to-treat analysis. However, after censoring the placebo patients who crossed over to the sorafenib arm, there was an increased overall survival with sorafenib (17.8 vs 14.3 months, HR 0.78, $p=0.029$) [26]. On the other hand, a small randomized phase II study of sorafenib versus IFN alfa-2b ($n=189$) in the first-line setting failed to demonstrate a PFS advantage over IFN (5.6 vs 5.7 months, respectively) [27]. The most common grade 3 or 4 treatment related adverse events (AEs) associated with Sorafenib were hand-foot-syndrome (86%), fatigue (5%), dyspnea (4%), and hypertension (4%).

Sorafenib was approved by the FDA in December 2005 and by the EMA in July 2006 for the treatment of patients with cytokine-refractory advanced RCC.

Sorafenib has also been recently investigated in second-line after progression to another TKI (Sunitinib) in a multicenter phase III trial (INTORSECT) in which 512 mRCC patients with progressive disease after sunitinib were randomized to receive the mTOR inhibitor temsirolimus ($n=259$) or sorafenib ($n=253$). There was no statistically significant difference in the primary endpoint (PFS 4.2 vs 3.9 months, respectively) but a benefit in OS in favor of sorafenib was observed (12.27 vs 16.6 months, HR 1.31, CI 95% 1.05–1.63) [28]. Sorafenib is also currently being evaluated in sequential and adjuvant setting in other trials (ASSURE trial: Adjuvant Sorafenib or Sunitinib for Unfavorable Renal Carcinoma; NCT00326898).

Sunitinib (SU11248)

Sunitinib is an oral multitargeted receptor TKI that inhibits VEGFR 1–3, PDGFR, c-Kit, and FMS-like tyrosine kinase-3 (Flt3). Two initial phase II trials of sunitinib in mRCC patients who had failed previous immunotherapy obtained an objective RR of 45%, a PFS of 8.4 months and an OS of 22.3 months in 168 evaluable patients [29]. These encouraging results led to a randomized phase III study that compared single-agent sunitinib versus IFN-2a in 750 systemically untreated patients with mRCC. Patients received oral sunitinib, 50 mg once daily in 6-week cycles (4 weeks on treatment followed by a 2-week rest period) or subcutaneous IFN-2a thrice weekly, escalated in weekly increments from 3 to 6 to 9 MU). This trial demonstrated a statistically significant advantage in objective RR (39 vs 8%, $p<0.001$) and PFS (11 vs 5 months, HR 0.54, $p<0.001$) for sunitinib treated patients and was consistent across patient subgroups, although only 7% were poor prognostic as per MSKCC risk classification. In addition, quality of life (QoL) was superior with sunitinib than with IFN-2a and scores indicated clinically significant differences ($p<0.001$) [30]. A final survival analysis of these patients was reported suggesting a trend for improved median OS with sunitinib therapy (26.4 vs 21.8 months; HR 0.82, 95% CI 0.67–1.00; $p=0.051$) [31]. The survival benefit may have been diluted by crossover of more than 50% of placebo-assigned patients to sunitinib and/or other VEGFR inhibitor therapy. In addition, a separate exploratory analysis of patients who did not receive post-study cancer treatment showed that the median OS time with sunitinib was double than with IFN-2a (28.1 months vs 14.1 months, respectively; $p=0.003$). The OS

seen with sunitinib in this trial was more than double the OS reported in previous trials involving cytokines, increasing from 13 to 26 months. The most common grade 3 treatment related AEs in this trial were hypertension (12%), fatigue (11%), diarrhea (9%), hand and foot syndrome (9%), and hypothyroidism (14%).

Sunitinib was approved by the FDA in January 2006 based on responses in patients with mRCC who had failed cytokine therapy and received full approval in February 2007 from the FDA and EMA based on results obtained in the first-line treatment of patients with locally advanced or mRCC.

Sunitinib has emerged as a standard of care for patients with untreated mRCC and is currently being investigated in a number of ongoing clinical trials to assess efficacy of sequential treatment and its efficacy in the adjuvant setting after surgery (ASSURE trial: Adjuvant Sorafenib or Sunitinib for Unfavorable Renal Carcinoma [NCT00326898]; STRAC Trial: Sunitinib Treatment of Renal Adjuvant Cancer (S-TRAC): a randomized double blind phase III study of adjuvant sunitinib vs placebo in subjects at high risk of recurrent RCC [NCT00375674]).

Pazopanib (GW786034)

Pazopanib is a second-generation orally administered multitargeted TKI that inhibits VEGFR1-3, PDGFR and c-kit and is the third oral VEGFR inhibitor to achieve regulatory approval status in the United States and Europe based on results from a randomized, double-blind, placebo controlled phase III study of single-agent pazopanib in 435 patients, including cytokine-pre-treated and treatment-naïve, with locally advanced and/or mRCC. The majority of patients were of good or intermediate risk according to MSKCC classification. Patients received continuous oral pazopanib, 800 mg daily ($n=290$), or placebo ($n=145$) once daily. Patients who progressed on placebo were eligible to receive open-label pazopanib. There was a significant increase in PFS with pazopanib compared with placebo in the entire population (median PFS 9.2 vs 4.2 months, respectively, HR 0.46, $p<0.0001$), and in the cytokine-pretreated (7.4 months vs 4.2 months, HR 0.54, $p<0.001$) and treatment-naïve (11.1 months vs 2.8 months, HR 0.40, $p<0.0001$) subpopulations as well as a significantly higher ORR (30% vs 3%, $p<0.0001$) [32]. Pazopanib led to a significantly longer PFS time regardless of MSKCC risk score, age, sex, or performance status and QoL with pazopanib did not differ significantly from that with placebo. In a recent survival data update, there was no difference in OS for pazopanib-assigned and placebo-assigned patients (median OS 22.9 vs 20.5 months, HR 0.91, $p=0.22$) but 54% of the latter received pazopanib after progression [33]. Pazopanib was approved by the FDA in October 2009 for the first-line treatment of patients with advanced RCC.

Pazopanib has been compared with sunitinib as first-line therapy for advanced RCC in the phase III COMPARZ study. This is the first "face-to-face" randomized comparison of two approved VEGF-targeted therapy in this setting. Over 1000 patients with mRCC were randomized to receive either pazopanib or sunitinib as first-line therapy. The study had a non-inferiority design and aimed to exclude a difference >25% in the HR, being the primary endpoint PFS. The results showed a median PFS of 9.5 and 8.4 months for sunitinib and pazopanib (HR 1.47 CI 95% 0.89–1.21), respectively, suggesting that pazopanib is non-inferior to sunitinib. Interestingly, a different safety profile between both drugs was observed. While fatigue and hand and foot syndrome were more frequent with sunitinib, diarrhea, and hepatotoxicity were more frequent with pazopanib [34].

A complementary phase II randomized study (PISCES) evaluated 169 treatment naïve patients with a novel primary endpoint: patient preference. Patients received blinded treatment with pazopanib for 10 weeks with a 2-week washout period before 50 mg of sunitinib for 10 weeks, or vice versa. Following the double blind phase (total of 22 weeks), patients were allowed to continue on treatment based on which agent they preferred. About 70% of patients reported preferring treatment with pazopanib, 22% preferred sunitinib, and 8% had no preference. This study was not designed to assess efficacy [35].

Based on these data, pazopanib represents another valid option along with sunitinib and bevacizumab-INF in the first-line setting in mRCC. Pazopanib is also being evaluated as second-line therapy in mRCC patients previously treated with VEGF-targeted therapy in a single arm phase II study (NCT00731211) and also in the adjuvant setting (PROTECT trial).

Axitinib (AG013736)

Axitinib is a potent, selective, second-generation inhibitor of VEGFR1-3 that inhibits the receptor activity at sub-nanomolar drug concentrations. Axitinib has a greater relative potency when compared with first line VEGFR inhibitors and a less "promiscuous" mechanism of action [36]. In a phase II open-label, single-arm study, axitinib monotherapy was evaluated in 52 patients with cytokine-refractory mRCC. The ORR was 44%. The median time to progression was 15.7 months and the median OS time was 29.9 months [37]. A subsequent phase II, open-label, single-arm trial assessed the activity of axitinib in 62 patients who had received prior sorafenib. MSKCC risk status was not determined in this population. Treatment with axitinib achieved 22.6% ORR with a median PFS time of 7.4 months and median OS time was 13.6 months [38]. After demonstrating clinical activity in these two phase II studies, axitinib was evaluated in a second-line open-label, randomized phase III trial (the AXIS study). In this trial, 723 patients with mRCC who progressed despite initial systemic therapy including sunitinib (54%), cytokines (35%) and bevacizumab-IFN or temsirolimus (11%) were randomized to receive either axitinib 5 mg twice daily or Sorafenib at standard dose. Axitinib dose escalation up to 10 mg twice daily was allowed in the absence of hypertension or other grade 3 toxicities. The primary endpoint, PFS, was significantly better for axitinib than sorafenib regardless of prior treatment (median PFS for all patients was 6.7 vs 4.7 months, HR 0.67, $p<0.001$; HR 0.74 after prior sunitinib, HR 0.46 after cytokine). Responses were seen more often after axitinib than sorafenib (19.4 vs 9.4%, $p<0.001$) [39]. The most common toxicities associated with axitinib included hypertension, diarrhea and fatigue. Based on these results, the FDA approved axitinib for the treatment of mRCC patients in the second-line setting. In a recent survival data update of the AXIS study, there was no difference in overall survival for the whole group: 20.1 to 19.2 months for axitinib and sorafenib ($p=0.3744$) [40].

Axitinib has also being evaluated in the first-line setting versus sorafenib, and results have been recently communicated. The study had 90% power to detect a 78% PFS improvement from 5.5 months with sorafenib to 9.8 months with axitinib, corresponding to a HR of 0.561 (overall one-sided $\alpha=0.025$). Although numerically axitinib provided better PFS, this study failed to achieve statistically the primary endpoint. In the overall study population median, PFS was 10.1 versus 6.5 months with axitinib versus sorafenib (HR adjusted for PS, 0.767; 95% CI 0.559–1.053; one-sided $p=0.0377$). Objective RR with axitinib versus sorafenib was 32.3% versus 14.6% (one-sided $p=0.0006$ adjusted for PS) [41].

Tivozanib (AV-951)

Tivozanib is another second-generation oral pan-VEGFR-TKI that was investigated in a phase II randomized, placebo-controlled, discontinuation trial of 272 patient's naïve to VEGF-targeted therapy. Patients received Tivozanib 1.5 mg daily in 4-week cycles (3 weeks on treatment and 1 week off). The ORR was 25.4% and the median PFS 11.8 months; a subgroup analysis of these data suggested that patients with ccRCC and prior nephrectomy appear to respond better to tivozanib, with an ORR of 29.6% and a median PFS time that was not reached [42]. These results led to a bigger trial named TIVO-1. The TIVO-1 is a phase III randomized, open-label multicenter trial that compared tivozanib versus sorafenib in 517 mRCC patients. Approximately, one-third of them had received citokines previously. Median PFS was 11.9 months versus 9.1 months for tivozanib and sorafenib, respectively (HR = 0.79, 95% CI 0.639–0.993; $p=0.042$). A more pronounced difference in median PFS was seen with treatment-naive patients (12.7 vs 9.1 months, respectively; HR 0.76; 95% CI 0.580–0.985; $p=0.037$) and the response rate was 33 versus

23%, $p=0.014$, respectively. Patients who progressed on sorafenib were subsequently treated with tivozanib, which could have confounded the overall survival measurement. Hypertension and dysphonia were more common with tivozanib while diarrhea and hand and foot syndrome were more frequent with sorafenib. The incidence of fatigue was low with both drugs. Tivozanib showed a favorable toxicity profile with <10% of patients developing common terminology criteria for adverse events (CTCAE) grade 3 or 4 adverse events [43]. In conclusion, a favorable risk-benefit profile was demonstrated for Tivozanib by a clinically meaningful and statistically significant prolongation of PFS and improvement in ORR over sorafenib. However, the trial showed a non-significant trend toward worse overall survival among patients assigned to Tivozanib after all patients had been followed for at least 2 years raising some concern and leading to a negative evaluation by FDA that declined approval of this compound.

Mechanistic Target of Rapamycin (mTOR) Inhibitors

The mechanistic target of rapamycin (mTOR) is a serine–threonine kinase that exists in two functionally distinct multiprotein complexes, TORC1 and TORC2. The TORC1 complex includes mTOR and regulatory-associated protein of mTOR (RAPTOR), regulates cell cycle progression, protein translation, and several aspects of metabolism. On the other hand, the TORC2 complex includes mTOR and rapamycin insensitive companion of mTOR (RICTOR) and regulates the activity of the kinase AKT. Different drugs targeting this pathway have been developed in renal cell cancer therapeutics. Two TORC1 inhibitors (Temsirolimus and Everolimus) have received regulatory approval for use in advanced RCC in first and second line, respectively, based on positive results from two randomized phase III trials [44, 45]. Other compounds targeting possible mechanisms of resistance/escape to TORC1 inhibition, such as PI3K and TORC2 activation, have been recently developed and are in different stages. We will summarize the data available in RCC for this family of drugs as follows.

Temsirolimus (CCI-779)

Temsirolimus (sirolimus 42-ester 2,2-bis hydroxymethyl propionic-acid) is an mTOR inhibitor, administered IV and rapidly converted into its major metabolite sirolimus. Sirolimus binds the protein FKBP12 and exerts its anticancer effect through inhibition of the TORC1 complex. Early in the clinical development of temsirolimus activity in RCC patients was observed leading to posterior phase II disease oriented trials that confirmed activity and defined 25 mg in a weekly basis as the recommended dose for further testing [46, 47]. A pivotal phase III, randomized, open-label study compared IFN, temsirolimus, and temsirolimus in combination with IFN in patients with previously untreated advanced RCC who had at least three of six protocol-specified risk factors for short survival. The primary endpoint was OS. This study was positive favoring the arm of temsirolimus alone with a median OS of 10.9 months. Temsirolimus was overall well tolerated. Main toxicities were metabolic and/or hematological. Differently from other big trials in advanced RCC, this study included about 20% of patients with non-clear cell histology and temsirolimus also demonstrated benefit in this population [44]. Currently, temsirolimus was approved in 2007 by the FDA and EMA for first-line treatment of mRCC with poor prognosis criteria. It is also considered a valid option for the treatment of patients with non-clear cell RCC.

Everolimus (RAD001)

Everolimus is an orally administered inhibitor of mTOR. Different phase I studies defined a daily oral dosing schedule of 10 mg continuously and revealed remarkable activity in various solid malignancies with a favorable toxicity profile [48]. Later on, everolimus was tested in an uncontrolled phase II trial in patients with advanced RCC who had been previously treated

with cytokines, and showed a high proportion of durable disease stabilization or tumor shrinkage [49]. Those results prompted an international, multicentre, double-blind, randomized phase III trial (RECORD1), where everolimus was compared with placebo for the treatment of metastatic RCC patients whose disease had progressed on treatment with VEGF receptor TKIs (sunitinib, sorafenib or both). Patients were randomly assigned in a 2:1 fashion to receive everolimus 10 mg once daily ($n=272$) or placebo ($n=138$). The study primary endpoint was PFS assessed by independent central reviewer, and secondary endpoints included OS and safety. The results of the second interim analysis revealed a significant difference in PFS (4.0 vs 1.9 months) favoring the everolimus arm and the trial was halted. A recent update of this study has been published confirming the benefit of everolimus in this population [median PFS of 49 vs 1.9 months; HR, 033; $p<0.001$]. Stomatitis, rash, and fatigue were the most commonly reported adverse events, but were mostly mild or moderate in severity. Pneumonitis (any grade) was detected in 22 (8%) patients in the everolimus group, of whom eight had pneumonitis of grade 3 severity. No statistically significant differences in OS were observed [14.8 months (everolimus) vs 14.4 months (placebo); HR, 0.87; $p=0.162$].

Everolimus was approved in 2009 by the FDA and EMA for the treatment of mRCC after progression to VEGF targeted therapies.

New Generation PI3K-AKT-mTOR Agents

Double PI3K-mTOR Inhibition: NVPBEZ235

New drugs targeting PI3K and mTOR are currently in clinical development. NVPBEZ235 is a new, orally bioavailable imidazoquinoline that exert its anticancer effect through blocking of the ATP-binding domain of PI3K and mTOR thereby inhibiting both TORC1 and TORC2. Pre-clinical studies have demonstrated that this double inhibition is superior to only TORC1 blocking leading to clinical studies where BEZ235 is being tested either as a single agent or in combination (Studies: NCT01453595; NCT01482156). Results are eagerly awaited [50].

AKT Inhibition: Perifosine

Two phase II trials (Perifosine-228 and 231) have been conducted testing the efficacy and safety of a novel AKT inhibitor, perifosine, in patients with advanced RCC who had failed on previous VEGF targeted therapy and/or mTOR inhibitor. Efficacy data reported is similar to that of other already tested second line agents. The median PFS was around 14 weeks in both studies and response rate ranged from 4 to 10% and 30 to 46% of stabilizations. Perifosine was well tolerated with scarce grade 3 and 4 events. Most common toxicities included nausea, diarrhea, musculoskeletal pain, and fatigue. Given its mechanism of action and toxicity profile, it could be worthy a further evaluation of perifosine in combination with other currently available drugs for advanced RCC [51].

Upcoming Agents in Renal Cell Cancer

There are a number of agents in development in advanced RCC therapeutics. These molecules could be classified based on their mechanism of action:

1. *Last generation anti-angiogenics*: Cediranib, Dovitinib, AMG-386 (Trebananib), Aflibercept, and Regorafenib.
2. *Met inhibitors*: Foretinib, AMG102, and Tivantinib.

Last Generation Anti-Angiogenics

Cediranib

Cediranib is an orally available, high-potent VEGFR inhibitor with activity against VEGF receptors 1–3. Two studies (one European and one North-American) have reported activity of this compound in mRCC. First, a phase II,

randomized, double-blind, parallel-group study compared the efficacy of cediranib versus placebo in patients with metastatic ccRCC who had not been treated with VEGFR inhibitors. Patients were randomized (3:1) to cediranib 45 mg/day or placebo. The primary objective was comparison of tumor size change from baseline to 12 weeks of therapy. Secondary objectives included response rate and duration, progression-free survival (PFS) and safety, and tolerability. Crossover was permitted. Seventy-one patients were randomized (53 to cediranib/18 to placebo). After 12 weeks of therapy, there was a significant difference in mean percentage change from baseline in tumor size between the cediranib (−20%) and placebo (+20%) arms ($p<0.0001$). Eighteen patients (34%) achieved a partial response on cediranib and almost half [25 (47%)] experienced stable disease. Cediranib treatment was also effective at prolonging PFS compared with placebo [median PFS 1.1 vs 2.8 months). The toxicity profile was quite consistent with a VEGFR inhibitor with diarrhea (74%), hypertension (64%), fatigue (58%), and dysphonia (58%) as the most frequent adverse event [52]. Another single-arm phase II trial conducted by a Canadian consortium tested cediranib at the same dose and in a similar population in 44 patients. The primary endpoint was objective response and secondary objectives included clinical benefit rate, duration of response, PFS, OS, and safety. Thirty-eight percentage of the patients in this trial achieved a partial response and the clinical benefit rate reached 85%. Median PFS was 8.9 months (95% CI: 5.1–12.9); and median OS was 28.6 months (95% CI: 18.2–37.3 months). Consistent with the previous study the most frequent grade 3 or higher AEs included hypertension, fatigue, hand-foot syndrome, and diarrhea [53]. These very encouraging results open a window of launching a phase III trial in this setting, although probably using lower doses such as 30 mg would make adhesion to treatment easier based on long-term toxicity profile.

Dovitinib

Dovitinib is an oral agent able to inhibit the tyrosine kinase domain of VEGFR and FGFR receptors. In a phase II trial of pre-treated patients with advanced RCC, the median PFS and OS were 6.1 and 10.2 months, respectively. This led to the launch of an open-label, randomized, multi-center, phase III study to compare the safety and efficacy of dovitinib versus sorafenib in patients with metastatic RCC after failure to VEGF-targeted and mTOR inhibitor therapies (the GOLD trial; TKI258-A2302). Recently, data from the first interpretable results of this trial have been published, confirming that the GOLD study did not meet the primary endpoint. Median PFS based on central review was not statistically different between the two treatment arms (log-rank test stratified by MSKCC risk group; $p=0.063$; one-sided with alpha-level $=0.0248$) with an estimated 14% risk reduction in the dovitinib arm compared to the sorafenib arm. Median PFS was 3.7 (3.5–3.9 months) and 3.6 (3.5–3.7 months) for dovitnib and sorafenib, respectively. There were no differences in other secondary end-points such as OS or PFS based on investigator's radiology review. Therefore, the third line setting in mRCC remains an unmet need in genitourinary oncology [54]. Other ongoing studies with this compound are testing the role of this molecule in first line mRCC and in combination with other drugs (NCT01791387 and NCT01714765).

AMG-386 (Trebananib)

The Tie/angiopoietin via is an alternative mechanism of angiogenesis parallel to the VEGF/VEGFR pathway. AMG-386 is a recombinant peptide-Fc fusion protein that binds angiopoietin 1 and angiopoietin 2, blocking their interaction with the Tie 2 receptor. In a tumor xenograft mouse model, treatment with AMG-386 inhibited tumor growth [55] and, in clinical studies, AMG-386 showed antitumor activity and good safety profile [56]. There is data of this molecule in combination with sorafenib and other molecules. AMG-386 plus sorafenib induced tumor response in 29% of the patients with RCC previously treated [57]. Currently, there are a number of phase II studies ongoing, testing AMG-386 in combination with sunitinib and sorafenib in patients with advanced RCC in the first- and

second-line setting (ClinicalTrials.gov identifier NCT 00853372 and NCT 00467025).

Aflibercept

Aflibercept (also known as VEGF-Trap), is a recombinant protein composed of two VEFGR domains fused with the Fc protein of human IgG1. Thus, aflibercept binds circulating VEGF and other pro-angiogenic factors such as PlGF, preventing its action on their cognate receptors [58]. Phase I studies confirmed that aflibercept was safe and well tolerated, and the recommended phase II dose was established at 4 mg/kg in a weekly fashion. Preliminary antitumor activity was seen in various tumor types including RCC [59]. These data has led to the implementation of a phase II study to evaluate aflibercept in metastatic or unresectable RCC previously treated with TKIs which is currently recruiting patients (NCT 00357760).

Regorafenib

Regorafenib is an oral multitargeted TKI. This compound inhibits VEGF receptors that are also blocked by first- and second- generation TKIs, such as VEGFR1-3, PDGFR-beta, and KIT. In addition, it has inhibitory activity on other receptors that are considered as "escape mechanisms" and that could be involved in anti-angiogenic resistance, such as TIE2, FGFR, and other. Phase I studies defined its toxicity profile and determined the recommended phase II dose as 160 mg per 24 h for 3 weeks in a 4-week cycle. Moreover, promising preliminary antitumor activity was observed, including patients with RCC [60]. The results of a phase II study in patients with advanced RCC and no previous treatment have been recently communicated revealing remarkable activity but also significant toxicity. Around 40% of the patients achieved a partial response; nevertheless, drug-related serious adverse events occurred in up to 35% of the patients. Grade 3 treatment related toxicities were common; most frequently, hand and foot skin reaction (33%), diarrhea (10%), renal failure (10%), fatigue (8%), and hypertension (6%). Two patients had grade 4 treatment-related adverse events: two cardiac ischemia, one hypomagnesaemia, and one pain in the chest. Four patients died during study treatment or within 30 days of last dose, of which two were deemed likely to be related to the study drug [61]. This toxicity profile will determine future development of this compound.

MET Inhibitors

Foretinib

Foretinib is an small-molecule TKI which acts blocking *in vitro* several receptors involved in angiogenesis, such as MET, VEGFR-2, PDGFR-beta, Tie-2, RON, kit, and FLT 3; in the case of the first two receptors, such antiangiogenic activity has also been demonstrated *in vivo*, resulting in stopping tumor growth in xenograft models [62]. Up-regulation of MET has been described in patients after VEGF-inhibitory therapy, as a mechanism of resistance to this treatment [63]. In the phase I study of foretinib in humans with advanced solid tumors, four patients with sporadic papillary RCC were included and two of them achieved a maintained partial response. The recommended dose of foretinib was 240 mg, given on the first 5 days of a 14-day cycle. Reversible elevations in serum aspartate aminotransferase and lipase were the dose-limiting toxicities [64]. A phase II study in patients with hereditary or sporadic papillary RCC has been recently published, demonstrating some antitumor activity (ORR 13.5% and median PFS of 9.3 months) and also a high predictive value of the presence of the germline MET mutation [65].

AMG 102

AMG102 is a mononuclear antibody IgG2 that neutralizes fully human hepatocyte growth factor (HGF). This growth factor is the ligand of the MET receptor and is involved in multiple cellular functions including proliferation and survival. In pre-clinical studies, HGF antagonist inhibited tumor xenograft growth [66] showing antitumor activity. Forty patients were treated in a phase I study with this compound and five of them had RCC. Overall, remarkable activity and a favorable safety profile were reported. One patient with RCC achieved a tumor stabilization.

Suitable treatment schedule is to be determined [67]. Due to the high selectivity of AMG 102 for the MET ligand HGF, the toxicity profile is better than other MET inhibitors. This makes AMG a good candidate for combination studies with cytotoxics [68] and with other targeted agents, such as bevacizumab [69]. Several phase II studies have been completed to determinate the clinical activity of AMG 102 in several tumor types. An RCC trial with 61 patients revealed 44% disease control rate [70].

ARQ 197 (Tivantinib)

ARQ 197 is an oral non-ATP competitive selective MET inhibitor. Pre-clinical studies showed *in vitro* and *in vivo* growth inhibition [71]. The clinical studies conducted included patients with RCC. Tivantinib was well tolerated and exhibited antitumor effect. The recommended dose of tivantinib for evaluation in phase II trial is 360 mg twice a day [72]. There is a phase II study that is recruiting patients with mRCC to be treated with tivantinib plus erlotinib or tivantinib alone (NCT01688973). The primary endpoint is overall response rate.

Cabozantinib

Cabozantinib is a potent oral TKI of MET, VEGFR2, and RET which in pre-clinical studies has showed a decrease of tumor invasiveness and metastasis compared with other drugs targeting VEGF pathway without MET inhibition [63]. In a phase I trial, 85 patients were included, two of them with renal cancer [73]. The results of a phase II trial with 25 patients with refractory mRCC treated with cabozantinib was presented at the 2012 ASCO annual meeting, showing 28% of partial responses and a median PFS of 15 months. The recommended dose of cabozantinib was 140 mg daily. Toxicities included hypophosphatemia, hiponatremia, fatigue, diarrhea, and hypertension [74]. Currently, it has been designed a phase II trial, not yet recruiting, which will compare cabozantinib versus sunitinib in previously untreated locally advanced or metastatic kidney cancer, whose primary endpoints are progression free survival and overall survival (NCT identifier 01835158).

Conclusions

1. Parallel to remarkable advances in the knowledge of the molecular biology or renal cell cancer in the last decade, multiple targeted agents have been developed, and others are entering clinical development.
2. Elements related to angiogenesis and their regulatory mechanisms remain the most validated targets.
3. Several drugs have reached the clinical with remarkable success, doubling the historical survival times of patients with mRCC.
4. Emergent treatment strategies include the dual blocking of elements of the PI3K-AKT-mTOR pathway and MET inhibition.

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Targeted Therapies in Melanoma

16

Daniele Fanale, Giuseppe Bronte and Antonio Russo

Introduction

Melanoma is the most serious and aggressive form of skin cancer and the sixth most common cancer in North America. The incidence of melanoma has been continuously increasing in the last decades, and faster than any other cancers. It is estimated that 76,100 Americans will be diagnosed with melanoma and 9710 will die from the disease in 2014 [1].

Melanoma is a high-grade, poorly differentiated malignant tumor of melanin pigment-producing cells (melanocytes) with poor prognosis in the metastatic stage, accounting for more than 70% of the skin cancer related deaths. Melanomas may arise from the mucosal epithelium covering the respiratory, alimentary, and genitourinary tracts (55, 24, and 18% of cases, respectively), all of which contain melanocytes, as well as from the skin. Mucosal melanomas are rare, account for approximately 1% of all melanomas and generally carry a worse prognosis than those arising from cutaneous sites. Rare sites of origin include the urinary tract, gall bladder, and small intestine. However, due to the rarity of mucosal melanoma, the understanding of these malignancies and their optimal clinical management remains limited [2]. Instead, there are four major subtypes of invasive cutaneous melanoma: superficial spreading, nodular melanoma, lentigo maligna, and acral lentiginous. For patients with cutaneous melanoma, the prognosis is related to the location and depth of the primary tumor, and the presence or absence of locoregional and distant metastatic disease [3].

Malignant melanoma arises from the neoplastic transformation of epidermal melanocytes resulting from complex interaction between genetic and environmental factors [4, 5]. Sun exposure is widely considered as the critical environmental risk factor for cutaneous malignant melanoma, which originates as a consequence of deleterious interactions between ultraviolet (UV) radiations and the melanocyte genome [6]. In fact, UV radiations may contribute to melanoma development through combined genotoxic and mitogenic effects in melanocytes.

Melanoma is the most dangerous form of skin cancer in the white population, being largely resistant to conventional therapies at advanced stages. The management of patients with advanced melanoma represents a significant challenge considering that, historically, chemotherapy and immunologic therapies have produced only modest results in the treatment of metastatic melanoma. Patients with metastatic melanomas have a median survival rate that typically ranges from 6 to 10 months [7]. Although new lines of targeted therapy and immunotherapy were

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introduced recently, clinical responses are still either too transient or limited to restricted subsets of patients as it is hard to target the elusive metastatic phenotype. Currently, prevention and early detection represent the only effective strategies to reduce the incidence of this tumor. Despite improvements in early melanoma diagnosis, the 5-year survival rate remains low in advanced disease [8]. Understanding the molecular mechanisms underlying this disease might be the key factor for the development of novel therapeutic strategies.

Molecular Biology of Melanoma

Tumor growth is the result of genetic and/or epigenetic alterations in key genes, regulating processes such as apoptosis, proliferation, cell cycle, survival, senescence, and DNA damage repair. These changes lead to the synthesis of biologically modified proteins by promoting an increase of the tumor progression. At the initial stage, the genetic modifications can be germline and the detection of cancer susceptibility genes plays a key role to identify and monitor patients at risk of developing melanoma. For this reason, the prognosis is closely associated with the early diagnosis.

An increasing understanding of melanocyte biology and melanoma pathogenesis is leading to the development of targeted therapies and the potential for major improvements in the care of patients with advanced melanoma. This section provides an overview of the key genes and associated pathways involved in the acquisition of the malignant melanoma phenotype.

Genetic Risk Factors

The melanomas are genetically and phenotypically heterogeneous tumors harboring various genetic alterations, as revealed by recent clinical, epidemiological, and genetic studies. In 2005, Curtin et al. [9] proposed a molecular classification based on the sites where the melanoma occurs, the genetic alterations and the sun exposure history. *BRAF*, *NRAS*, and *KIT* are three well-known oncogenes involved in melanoma pathogenesis. A high frequency of activating *BRAF* mutations (80%) was detected in nevi, indicating that these alterations occur early during melanoma progression, leading to the activation of the cell proliferation followed by induction of senescence [10]. Recent evidence showed that the BRAF V600E mutation was found in the majority of melanomas [11]. Targeting of mutated BRAF kinase has recently been shown to significantly improve overall survival of patients with metastatic melanoma, highlighting the important role of this oncogene in melanoma biology [12]. Mutations in *BRAF* were significantly more common in melanomas located in areas without chronic sun-induced damage. Melanomas arising in chronically sun-damaged skin, mucosal surfaces, and acral skin were characterized by wild-type *BRAF* and wild-type *NRAS*, but exhibited alterations in *KIT* and, frequently, increased copy number of the genes encoding for cyclin-dependent kinase 4 (CDK4) and cyclin D1 (CCND1), downstream components of the RAS–BRAF pathway [9].

The initial mediator of senescence seems to be $p16^{INK4a}$, which blocks the CCND1/CDK4 complexes and inhibits cell proliferation. Moreover, although KIT mediates the cell cycle activity, its effect seems to be limited to a subset of melanomas. PTEN phosphatase loss activates the PI3K/AKT signaling pathway by overcoming the $BRAF^{V600E}$-mediated senescence. Therefore, PTEN loss could evade senescence mediated by $p16^{INK4}$ loss, promoting melanoma progression via the PI3K/AKT cascade. Indeed, the deregulation of PI3K/AKT pathway is considered a late event in melanoma progression [13]. AKT activation was detected in about 60% of sporadic melanomas thereafter to gene amplification or to inactivation of PTEN, which negatively regulates the PI3K/AKT pathway [14, 15]. The presence of both PTEN and BRAF mutations has been reported in 17% of melanomas ([16]; Fig. 16.1).

Also, several studies identified less frequent mutations in other genes, such as *PREX2* (phosphatidylinositol-3,4,5-trisphosphatedependent Rac exchange factor 2), encoding for a nega-

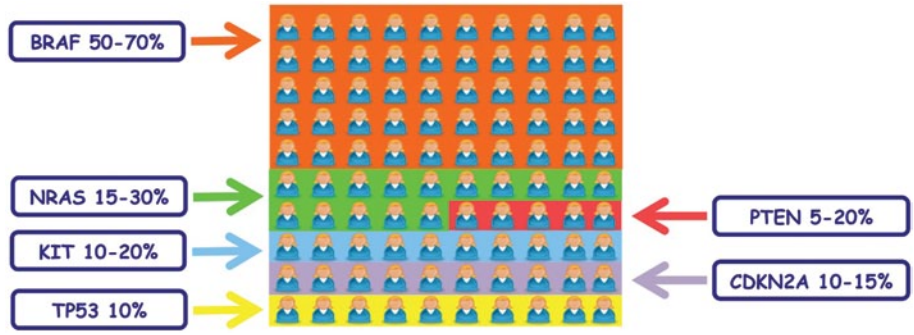

Fig. 16.1 Distribution of somatic gene mutations in melanoma patients

tive regulator of PTEN [17], *PPP6C*, encoding for a serine/threonine phosphatase, and *RAC1*, encoding for a GTPase of the RAS superfamily. Noteworthy, melanomas that were mutated for both *BRAF* and *NRAS* exhibited more frequent mutations in *PPP6C*, while melanomas that were wild-type for both *BRAF* and *NRAS* showed more frequent mutations in *RAC1* [18, 19]. Furthermore, germline mutations in the genes encoding for CDK4 and CDKN2A (cyclin-dependent kinase inhibitor 2A), involved in regulation of the cell cycle, have been shown to confer a high malignant melanoma risk [20, 21]. In addition, the identification of genetic variants with low/intermediate allele frequency conferring a moderate risk of cancer represents an important scientific approach to discover novel melanoma-predisposing genes [22]. Therefore, frequent germline allelic variants in the *Casp8*, *MTAP*, *MATP*, *MC1R* (melanocortin 1 receptor) and *ASIP* genes have been identified as low-risk susceptibility genes or as modifiers of high-risk susceptibility genes [23, 24]. Recently, an increase of the risk of developing melanoma was associated with a germline mutation in the *MITF* (microphthalmia-associated transcription factor) gene, involved in control of melanocyte homeostasis [25–28]. Functional genomic studies showed that MITF regulates the transcription of several genes involved in DNA replication and repair, though the molecular mechanisms have remained to be elucidated yet [29]. These genes are involved in melanoma progression by conferring metastatic genome stabilization during the metastatic process [30]. Recently, in addition to the commonly mutated genes *BRAF*, *NRAS*, *PTEN*, *TP53* and *p16*, new oncogene candidates such as *MAPK1/2*, *ERBB4*, *GRIN2A*, *MMP8* and *GRM3* were identified [31–33]. Their particular role in melanoma biology is currently under investigation through *in vitro* and *in vivo* experiments, but requires further validation in clinical studies. In the future, these new gene candidates could provide more individualized treatment approaches for metastatic melanoma patients [34].

The RAS/RAF/MEK/ERK Signaling Pathway

In recent years, the most important advance has been the discovery that the mitogen activated protein kinase (MAPK) cascade is the pivotal signaling pathway in melanoma progression and development. In fact, the novel therapeutic approaches rely on the inhibition of some members of this cascade. BRAF and MEK molecular pathways appear to be key players in this field. The RAS/RAF/MEK/ERK cascade is activated by various receptors, including c-KIT, FGF receptor, and c-MET. Dysregulation of signaling can occur at various levels, from alterations at the receptor level to changes in the intracellular signaling cascade, resulting in aberrant cell proliferation and/or apoptosis [35]. The RAS family is made up of small G proteins divided into three different isoforms: NRAS, HRAS, and KRAS. The members consist of a catalytic domain that mediates the guanine nucleotide binding and hydrolysis and of an hypervariable region con-

taining the membrane targeting domain required for its activation. Mutations in *NRAS*, the most common in melanoma, were detected in 33% of primary and 26% of metastatic tumors, and are correlated with sun exposure and nodular lesions [36, 37]. The most frequent *NRAS* mutations are substitutions of glutamine at position 61 by a lysine or an arginine (Q61K, Q61R) [38]. *HRAS* point mutations have only been found in benign lesions that does not progress to melanoma [39]. No mutations of *KRAS* have been described in melanoma.

The family of serine/threonine kinases RAF consists of three isoforms, ARAF, BRAF, and CRAF (RAF-1), activated by the small GTPases RAS. Activating mutations in BRAF are present in approximately 40–60% of advanced melanomas [40, 41]. In 80–90% of cases, this activating mutation consists of the substitution of glutamic acid for valine at amino acid 600 (V600E mutation) with most of the remainder consisting of an alternate substitution (lysine for valine) at the V600 locus (V–K) that accounts about 16% of mutations in melanoma [42–44]. The latter and other less common mutations were found at slightly higher rates in melanomas arising in older patients. Advanced melanomas with a mutation in *BRAF* appear to have some clinical differences that are associated with a more aggressive clinical course. Patients with BRAF mutations are younger and have greater number of nevi. Current results from melanoma cohorts showed that *NRAS* and *BRAF* mutations are almost always mutually exclusive [45–47], indicating that the occurrence of each mutation may be specific to certain subtypes of melanoma [46]. The V600E mutation creates a constitutively active status for BRAF, independent of a previous activation by RAS and upstream extracellular stimulus, determining an increased proliferation and promoting a checkpoint for malignant transformation. However, BRAF requires the cooperation of other determinants to drive melanoma progression. BRAF can regulate various aspects of the cell survival. Activated BRAF promotes IκB degradation, while inhibition of BRAF sensitizes cells to apoptosis[48]. BRAF can also control cell growth by regulating $p27^{kip1}$ levels [49]. Recently, NRAS/BRAF signaling activation was shown to mediate the epithelial-to-mesenchymal transition (EMT) in advanced melanoma [50].

BRAF together with other two isoforms activates via phosphorylation a second protein known as mitogen-activated protein kinase (MEK), which in turn activates downstream extracellular signal-regulated kinase (ERK). The ERK signaling pathway can regulate various molecules important for tumorigenesis, survival, and senescence. Conversely, the inhibition of RAS, BRAF, or MEK blocks ERK activity and inhibits the growth of melanoma cells both *in vitro* and *in vivo* [51]. In wild-type BRAF or NRAS cells, ERK activation is low in comparison to mutant cells and can control proteins involved in extracellular adherence, cell motility, and angiogenesis [52]. In melanoma cells, ERK can inhibit the cell cycle regulator $p27^{kip1}$ and also alter *in vitro* invasion capability by regulating the production of matrix metalloproteinase-1 (MMP-1) [53, 54].

Current and Emerging Approaches in Melanoma Treatment

After melanoma diagnosis, the next step is to determine the tumor stage, the extent of its spread and its aggressiveness. Staging is important to plan the most appropriate treatment. Surgical excision is the treatment of choice for early localized cutaneous melanoma and is curative in most cases. Therefore, an appropriate excision is important to lessen the risk of a local recurrence. Although patients with localized disease can be treated successfully with surgical resection in the majority of cases, some individuals develop disseminated disease [55]. The recurrence rates remain high for stage III disease, with relapse-free survival rates of 63, 32, and 11% for stages IIIA, IIIB, and IIIC, respectively. The prognosis for melanoma patients with distant metastases is poor, and the vast majority of those with stage IV melanoma will die from disease [56].The identification of specific oncogenic-driving mutations and the evolving knowledge of the molecular biology of melanoma have led to notable advances in the treatment of metastatic melanoma. It aims

Table 16.1 Summary of the clinical development for melanoma patients

Class	Drug	Target	Clinical research advancement	Positive Outcomes	FDA approval
Targeted therapy	Vemurafenib	BRAF	Phase III	RR, PFS, OS	Yes
	Dabrafenib	BRAF	Phase III	RR, PFS	Yes
	Trametinib	MEK	Phase III	RR, PFS, OS	Yes
	Selumetinib	MEK	Phase II	–	No
	Imatinib	KIT	Phase II	RR	No
Immunotherapy	Ipilimumab	CTLA-4	Phase III	OS	Yes
	Nivolumab	PD-1	Phase I	RR	No
	Lambrolizumab	PD-1	Phase I	RR, PFS	No

to prolong survival, to block the spread of metastases and to prevent the development of new sites of disease. Approaches that can provide clinically important benefits for appropriately chosen subsets of patients with metastatic melanoma can include surgical excision, immunotherapy, targeted inhibition of the MAP kinase pathway, and radiation therapy to sites of metastases, depending upon the localization and the extent of metastases. Although cytotoxic chemotherapy was widely used prior to the development of targeted therapies, it does not have an established role for patients with metastatic melanoma [57]. Instead, the radiation therapy may be used to reduce tumor mass, to prevent recurrence, and to treat those sites of metastases, such as brain, which are difficult to be treated by surgery [58]. There are three main categories of drug treatment: chemotherapy, targeted therapy, and immunotherapy. This section provides an overview of current and emerging treatment options for melanoma patients (Table 16.1).

Chemotherapy

Conventional chemotherapy is based on the use of alkylating agents such as fotemustine, dacarbazine, and temozolomide which trigger cytotoxic effects able to inhibit or slow the cancer cell growth by blocking cell replication. However, these drugs showed objective response rates of approximately 10–15%, with no improvement of overall survival [59, 60]. Other cytotoxic agents, including taxanes, have been tested in melanoma with response rates similar to that of dacarbazine. Trials of polychemotherapy and combinations with cytokines, in the 1980s and 1990s, yielded better response rates for multi-agent regimens, but no improvement in overall survival. Therefore, cytotoxic chemotherapy generally is not used as the initial treatment for patients with advanced disease [60]. More recent researches have led to the development of immunotherapy, using an anti-CTLA4 monoclonal antibody, and to targeted therapies (BRAF or MEK inhibition), which prolong progression free and overall survival compared with chemotherapy. Thus, these cytotoxic drugs are actually used for patients harboring non-BRAF mutated melanomas or for patients who developed resistance to previous treatments.

New Targeted Therapies in Melanoma

Targeted therapy is a form of treatment in which drugs (or other substances) are developed with the aim of destroying cancer cells by leaving normal cells intact. These drugs are designed to interfere with the specific molecules that drive the growth and spread of the tumor, and are associated with fewer side effects compared to chemotherapy and radiation therapy.

The recent characterization of the molecular alterations in melanoma led to the development of personalized targeted therapies, which have revolutionized the treatment for advanced melanoma. These treatment options are designed to target tumors according to their molecular diversity and activated intracellular signaling pathways [61]. The BRAF/MEK/ERK signal-

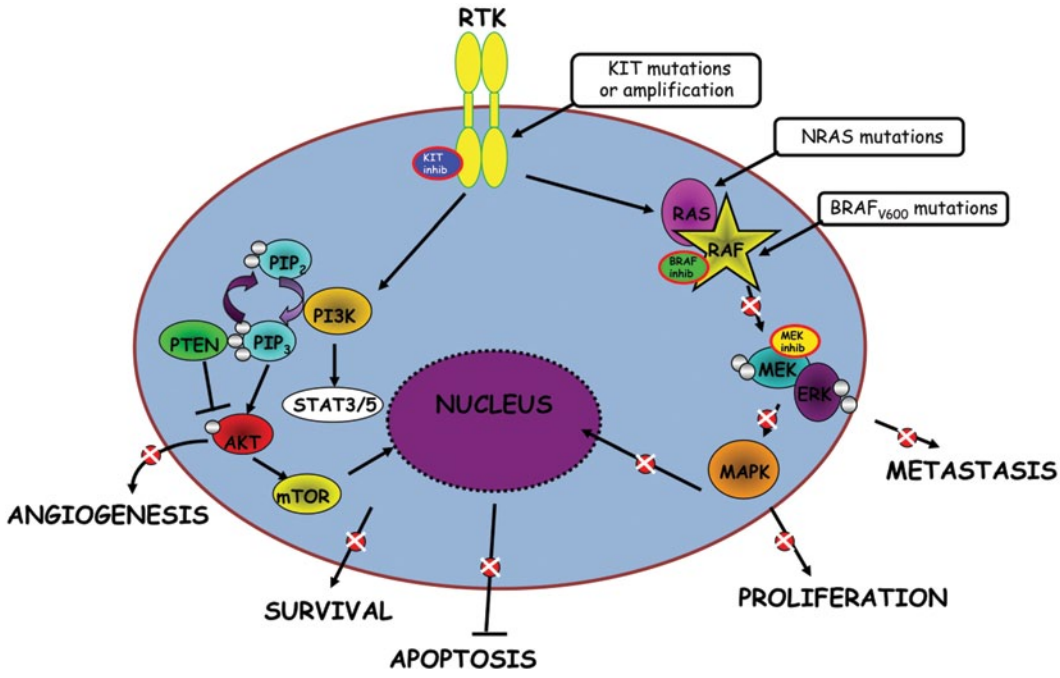

Fig. 16.2 Biological effects of targeted therapy, BRAF, MEK, and KIT inhibitors, in the intracellular pathways

ing pathway has attracted considerable attention as a target for anticancer therapy, due to its high frequency of mutations and its important role in melanoma [57]. Furthermore, less frequent activating *KIT* mutations were detected in a small portion of patients (15–20%) with acral lentiginous or mucosal melanomas [62, 63] and with melanoma arising in areas of chronic skin damage [64]. For this reason, there are two main classes of agents used in targeted therapies for melanoma: (1) drugs targeting melanoma cells with alterations in the BRAF/MEK signaling pathway (BRAF and MEK inhibitors) and (2) drugs targeting melanoma cells with alterations in the *c-KIT* gene. Three agents have showed significant clinical benefit and have been approved for use in patients with BRAF mutations: the BRAF inhibitors, vemurafenib and dabrafenib, and the MEK inhibitor trametinib ([65, 66]; Fig. 16.2).

Other pathways have also been investigated in melanoma to identify new potential targets for therapy.

Some researchers argued that the PI3K–AKT–mTOR pathway could be involved in melanoma genesis. Indeed, AKT3 could be deregulated and PTEN decreased in melanomas, as reported above. Pre-clinical studies showed that rapamycin, an mTOR inhibitor, decreases the proliferation of melanoma cells [67]. These molecular alterations seem to play a role in the resistance to BRAF and MEK inhibitors. However, the use of the mTOR inhibitors, temsirolimus and everolimus, in melanoma patients has not shown a significant activity [68, 69]. These controversial results from pre-clinical and clinical studies may be attributed to a hyper-activation of AKT as a compensatory mechanism.

Tumor angiogenesis has also been involved in melanoma proliferation and progression. Bevacizumab, the anti-VEGF monoclonal antibody, was studied both alone and in combination with chemotherapy or interferon-alpha-2b. Even though some responses were observed, it has not been demonstrated yet whether VEGF-targeted therapy plays a role in improving clinical outcomes [70–74].

BRAF Inhibitors

About half of all melanomas harbor activating mutations in the *BRAF* gene. As mentioned

before, the two most commonly observed *BRAF* mutations are V600E and V600K, which account for 95% of these mutations. These changes produce an altered BRAF protein that drives melanoma cells to grow and divide quickly [43]. The presence of a V600 mutation predicts responsiveness to BRAF and MEK inhibitors as they are not likely to act in melanomas harboring the wild-type *BRAF* gene [75].

The first agent developed to target oncogenic BRAF in melanomas was sorafenib (BAY 43-9006), a multikinase inhibitor that inhibits BRAF (wild-type or V600E), but also PDGFR, VEGFR, and c-KIT [76, 77]. As a monotherapy, sorafenib showed limited clinical activity and proved to be inefficient in the treatment of unresectable or metastatic melanoma. This lack of activity is likely explained by lack of specificity for BRAF [78].

During the last decade, many BRAF inhibitors have been discovered and most of them exhibited potent antitumor activity, especially on tumors that harbor V600E mutations, with little cross-reactivity for wild-type BRAF and CRAF [79]. Some of these compounds have been entered clinical trials and displayed encouraging results. The best validated drugs that appear to have the highest affinity for the catalytic domain of the BRAF kinase, exhibiting unprecedented survival benefits in advanced melanoma, are vemurafenib and dabrafenib. Clinical trials have demonstrated that the first potent and effective drug targeting mutated BRAF in melanoma was vemurafenib [80, 81]. **Vemurafenib** is a potent inhibitor that selectively binds to mutant BRAF proteins containing V600E amino acid substitutions, preventing constitutive activation of the MAPK pathway, and resulting in antitumor effects of cell proliferation inhibition and apoptosis induction [82, 83]. This drug was approved by the US Food and Drug Administration (FDA) in 2011 for the treatment of $BRAF^{V600E}$ mutant melanomas that cannot be removed by surgery and only for those patients who have tested positive for the *BRAF* mutation [84]. In phase 1 and 2 clinical trials, vemurafenib showed an objective response rate >50% in patients suffering from melanoma. These results were confirmed in a phase 3 clinical trial, which compared vemurafenib to dacarbazine. It showed an improvement of both response rate (RR) and survival outcomes, progression-free survival (PFS) and overall survival (OS) [85]. Based on these results, vemurafenib was approved by FDA in 2011 in those patients with BRAF V600E mutation. The most common side effects are joint pain, fatigue, hair loss, rash, itching, sensitivity to the sun, and nausea. Less common but serious side effects can occur, such as heart rhythm problems, liver function test impairment, severe allergic reactions, and severe skin or eye side effects [86]. Some people may develop new skin cancers called squamous cell carcinomas. These cancers are usually less serious than melanoma and they can be definitively treated by surgery [87].

Despite these excellent results, a subset of $BRAF^{V600E}$-mutant patients was found initially resistant to vemurafenib (intrinsic resistance) and most of the others developed secondary resistance. Almost all tumors showed reactivation of the MAP kinase pathway or upregulation of parallel signaling pathways with increased ERK phosphorylation at the time of resistance and restored cell survival [88]. The MAPK pathway may also be activated when $BRAF^{V600E}$ splice variants lacking the RAS-binding domain develop. These variants dimerize in the absence of RAS activation by reactivating the pathway [89]. Both PTEN and cyclin D1 are involved in mechanisms of intrinsic resistance. Patients whose tumors exhibit both *BRAF* mutations and PTEN dysfunction showed a lower response rate than dabrafenib. In addition, cell lines with both cyclin D1 amplification as well as *BRAF* mutation do not undergo apoptosis when exposed to BRAF inhibitors [90, 91]. Different mechanisms involved in acquired and secondary resistance have been reported [92]. Multiple genetic changes may contribute to this event, and research is currently ongoing to further clarify patterns of resistance to improve the clinical outcome of the patients [93]. Insights into mechanisms of resistance aim to potential drug combinations to overcome this important clinical problem, by promoting the concept of dual inhibition of the MAPK pathway. In this perspective, other BRAF inhibitor agents are developing [94].

Dabrafenib is another new generation BRAF inhibitor showing significant activity in patients with advanced melanoma compared with dacarbazine chemotherapy both in terms of RR and PFS. The difference in OS was not statistically significant. Dabrafenib was approved by the FDA in 2013 for the treatment of patients with advanced melanoma containing the $BRAF^{V600E}$ mutation. This drug is not indicated for the treatment of patients harboring wild-type *BRAF*, but only for those patients who have tested positive for the *BRAF* mutation [93]. Dabrafenib belongs to the same class of vemurafenib, working with a similar efficiency, but it seems to be more efficient in melanomas with brain metastasis [95, 96]. Like vemurafenib, dabrafenib decreases phosphorylated ERK and causes cell cycle arrest. In pre-clinical studies, dabrafenib has demonstrated to be almost 20 times more selective at inhibiting $BRAF^{V600E}$-mutants than wild-type *BRAF* in several cancer cell lines. In addition, dabrafenib shows inhibitory effects in cell lines containing other activating *BRAF* mutations, including V600K and V600D [97].

Since dabrafenib and vemurafenib appear to have similar clinical activity, the choice between two agents likely relies on other factors including their toxicity profiles. Common side effects include thickening of the skin (hyperkeratosis), headache, fever, joint pain, non-cancerous skin tumors, hair loss, and hand-foot syndrome (redness, pain, and irritation of the hands and feet). Although it also can cause squamous cell carcinomas of the skin, these may occur less often than with vemurafenib. Some other more serious side effects that can occur with dabrafenib include severe fevers, dehydration, kidney failure, eye problems, and increased blood glycemic levels. However, unlike the vemurafenib, dabrafenib-does not induce photosensitivity [87].

MEK Inhibitors

As mentioned before, downstream of RAF in the MAPK cascade, there are the MEK and ERK kinases. Since RAF moves from the cytoplasm to the cell membrane during cellular signaling, the new activated complex triggers the signal cascade via consecutive phosphorylations through MEK1 and MEK2. This, in turn, activates ERK 1 and 2 which are able to enter the nucleus and interact with several transcription factors to promote cellular growth and differentiation [98]. Multiple *in vitro* studies demonstrated that mutated BRAF signaling is mediated via MEK and ERK [99]. Therefore, inhibition of MEK is another option for targeting the MAPK pathway and several studies are currently evaluating the role of MEK inhibitors in patients with BRAF-mutant metastatic melanoma [100]. MEK inhibition is associated with improved response rate, progression-free survival, and overall survival in patients with BRAF-mutated metastatic melanoma.

Pre-clinical studies of the MEK inhibitor, PD0325901, and its precursor, CI-1040, showed direct inhibition of ERK in cell lines and reduced tumor growth in animal models, but they were not brought forward due to their toxicity in early phase trials [101, 102].

Selumetinib was the first allosteric selective MEK inhibitor to be evaluated in a phase II clinical trial in patients with metastatic melanoma. This agent determined a 12% objective response rate in patients with BRAF mutant tumors, whereas no response was observed in wild-type tumors, enhancing the importance of selecting a specific patient population [103].

Trametinib and MEK162 are potent, highly specific inhibitors of MEK1/MEK2 that provide responses in 20% of the melanomas harboring a BRAF mutation [104, 105]. **MEK162** showed activity in patients with advanced melanoma and a *NRAS* mutation. MEK inhibition showed efficacy in NRAS-mutated patients, for whom there is no specific targeted therapy [104].

Trametinib was recently approved by the FDA for the treatment of patients with unresectable or metastatic melanoma harboring *BRAF* V600E or V600K mutations, because in the phase III METRIC trial it achieved a significant improvement of RR, PFS, and OS when compared to dacarbazine or paclitaxel. It is not indicated for the treatment of patients who have received previously a BRAF inhibitor therapy [106]. Common side effects include rash, diarrhea, and swelling. Rare but serious side effects can include heart damage, loss of vision, lung

side effects, and skin infections. Combination therapy with a BRAF inhibitor may improve the efficacy and reduce BRAF inhibition-associated side effects, including skin toxicity [107]. There are no clinical trials comparing vemurafenib, dabrafenib, and trametinib with each other, however, data suggest that the BRAF inhibitors, vemurafenib and dabrafenib, are more active than the MEK inhibitor trametinib. The combination of dabrafenib and trametinib appears to have a superior response rate and progression free survival than dabrafenib alone with less skin toxicity, however, comparison of the efficacy of the combination with dabrafenib alone awaits the completion of ongoing phase III trials [108]. Moreover, there are no randomized trials that compare targeted therapy with immunotherapy.

c-KIT Inhibitors

A small portion of melanomas exhibit activating *c-KIT* mutations that help them develop and grow. These changes are more common in melanomas that arise in certain parts of the body (acral or mucosal melanomas). Some drugs used for the treatment of other cancers, such as imatinib mesylate and nilotinib, are known to target cells with changes in *c*-KIT. The KIT receptor tyrosine kinase is a transmembrane protein consisting of extracellular and intracellular domains. Most *KIT* mutations are located in exon 11, which encodes for the juxtamembrane domain, and in exon 13, which encodes for a kinase domain [64]. In the subgroups of patients with melanoma on chronic sun damaged skin, acral lentiginous or mucosal melanoma the incidence of *KIT* mutations or amplification is up to 25 % [109]. It has been demonstrated *in vitro* that imatinib mesylate inhibits proliferation and induces apoptosis in melanoma cells with hyperactivation of c-KIT. These biological effects go through the increase of $p27^{KIP}$ and inhibition of the ERK, PI3K/AKT, and STAT signaling pathways [110]. For patients without a *BRAF* V600 mutation but with a *KIT* mutation, the use of a KIT inhibitor may provide an important treatment option. Phase II studies using imatinib in unselected groups of patients with advanced melanoma showed no clinical efficacy [111, 112]. However, phase II clinical trials performed on patients with c-KIT mutations showed objective response rates in 33 % of cases [113]. Furthermore, results from a phase II trial showed that imatinib could be effective when tumors harbor *KIT* mutations, but not if KIT only is amplified [114].

Immunotherapy

Several evidences reported that melanoma is an immunogenic tumor but metastatic melanoma cells have developed mechanisms to escape from immunosurveillance and to survive. Immunological strategies based on the use of drugs with effects on immune system to stop or slow the growth of cancer cells could improve the prognosis of metastatic melanoma [115]. The approaches that have allowed to provide clinically important benefit for patients with disseminated melanoma in appropriately selected patients include immunotherapy with high-dose interleukin-2 (IL-2), immunotherapy with ipilimumab, a monoclonal antibody targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4), and immunotherapy with monoclonal anti-PD-1 antibody.

In 1998, the FDA approved the use of the immune molecule IL-2 as a treatment option for advanced melanoma. IL-2 was first identified as a T cell growth factor in 1976. Subsequently, recombinant IL-2 was shown to have potent, dose-dependent immunomodulatory and antitumor activity in a number of murine tumor models [116]. These observations led to the development of high-dose IL-2 regimens for clinical use. IL-2 is a form of immunotherapy that has allowed to help some people with metastatic melanoma when administered in high doses, leading to complete disappearance of the disease or tumor growth arrest for a prolonged period. However, high dose IL-2 can cause serious side effects, including low blood pressure, irregular heart rhythms, accumulation of fluid in the lungs, fever, and rarely death. For this reason, treatment with high dose IL-2 is generally reserved for younger patients who have good heart and lung function [117].

Conversely, the immune molecule interferon alpha (IFN-α) was used only after surgery as a

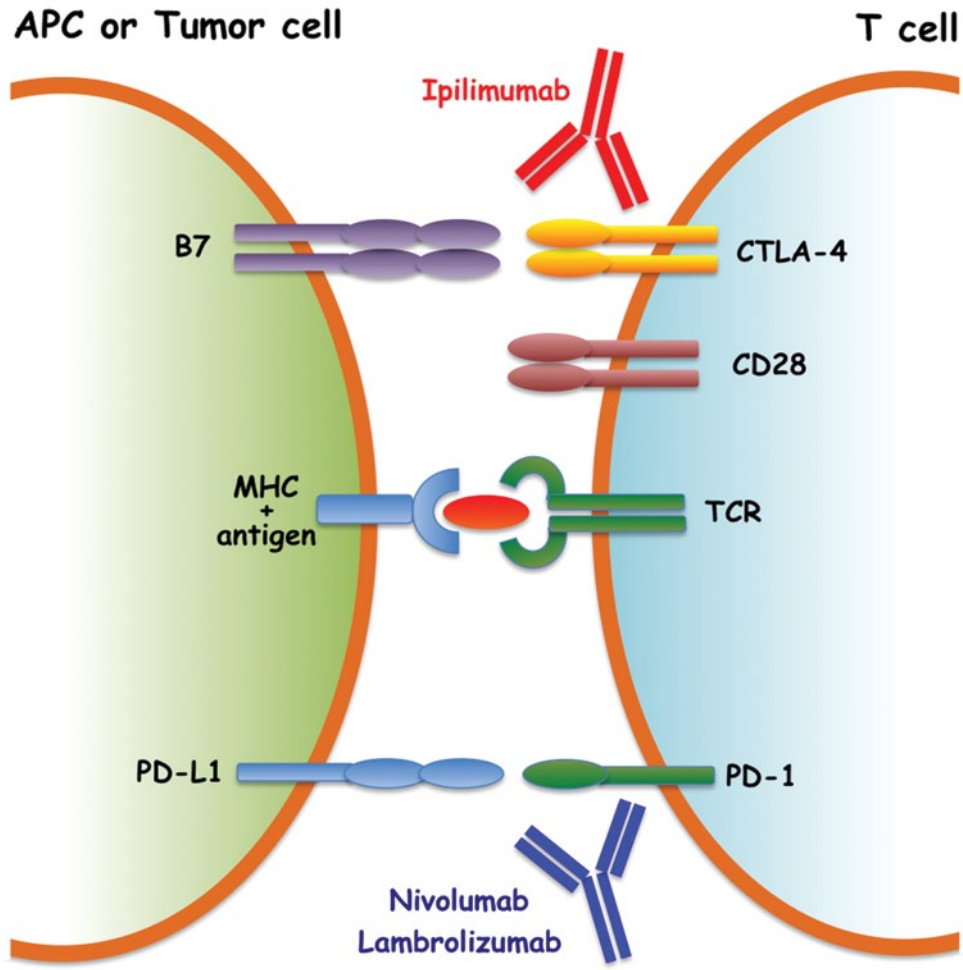

Fig. 16.3 Mechanism of action of immunotherapy, anti-CTLA-4, and anti-PD-1 monoclonal antibodies, in melanoma

adjuvant immunotherapy, or in combination with other agents used for treatment of advanced melanoma. However, most of clinical trials based on this immune system activation did not translate into clinically significant objective response rates and any improvement in overall survival [118]. This led to the targeting of T-cell signaling pathways, initially CTLA-4 and more recently programmed death 1 (PD-1) and its ligand PD-L1 (Fig. 16.3).

Immunotherapy Targeting CTLA-4

CTLA-4 is expressed on the surface of activated CD4+and CD8+T-cells and binds B7 molecules on antigen-presenting cells (APC), repressing T-cell activation. After T-cell activation, CTLA-4 is recruited to the plasma membrane where it plays an autoregulatory role, attenuating T-cell activation and proliferation, thereby maintaining effective antitumor immunity [119].

Ipilimumab, a novel antibody blocking CTLA-4, is a fully human immunoglobulin that inhibits this negative feedback, potentiating the T-cell-mediated immune response. No drug or combination of drugs showed an impact on overall survival until 2011, when ipilimumab was approved for clinical use by the FDA following the publication of results of a pivotal phase III trial [120]. Major weaknesses of this treatment were the low rate of objective response (10%), a

small percentage of patients achieving long-term disease control, and the serious side effects. It was first compared to the gp100 peptide vaccine with an improvement of OS by 3.6 months. Then it was added to dacarbazine and this combination was compared to dacarbazine alone with a subsequent improvement of OS by 2.1 months. These findings suggest that ipilimumab exerts its function against melanoma regardless a peptide-mediated vaccination. However, the effects of ipilimumab on OS seem to not only be mediated by tumor responses, but also perhaps by prolonged stable diseases, regression after an initial progression and regression of target lesions in the presence of new lesions. Ipilimumab toxicity differs from that of other antibody-based therapies [121]. These are mainly immune-related adverse effects. The most common of them include effects in the gastrointestinal tract, skin and liver. The initial observation of toxic deaths was not reported in the most recent phase III trial with dacarbazine, since the toxicity management protocols were standardized for this drug [122, 123]. Tremelimumab, the other anti-CTLA-4 antibody in clinical development, did not show a statistically significant survival rate in its pivotal trials, although this result may have been affected by the availability of ipilimumab in the United States at the time of that trial [124].

Immunotherapy Targeting PD-1 and PD-L1

The programmed death 1 (PD-1) receptor, expressed at the surface of activated T cells, is a negative regulator of T cells [125]. It was first isolated in 1992 by Ishida and Honjo and initially cloned as a molecule overexpressed in apoptotic cells [126]. Its role as a negative regulator of the immune response was demonstrated and studied in PD-$1^{-/-}$ knockout mice that showed a variety of autoimmune diseases [127]. Unlike CTLA-4, PD-1 receptor ligand (PD-L1) is directly expressed on tumor cells. When PD-L1 binds to its receptor, the T cell ability to target the tumor cell is inhibited. The difference between the CTLA-4/B7 and PD-1/PD-L1 interactions is linked to the phase of T-cell response: the priming phase for the first one and the effector phase for the latter. According to this difference, it was argued that PD-1 blockade could prevent tissue damage. Anti-PD-1 and anti-PD-L1 antibodies directly activate cancer-specific T cells [128]. Nivolumab (also known as BMS-936558) is a fully human anti-PD-1 antibody being explored in lung, melanoma, and renal cancers, demonstrating an approximately 30% objective response rate in melanoma. Interestingly, tumor PD-L1 expression might provide a basis for selecting patients for the treatment, as none of patients with tumors negative for PD-L1 showed a response. Interstitial pneumonitis is the most serious immune complication of this agent, with deaths resulting from this complication. Immune toxicities were seen with these agents but at a lesser rate and reduced severity compared to other immunomodulating molecules such as ipilimumab [129].

The anti-PD-L1 antibody MDX-1105 (also known as BMS-936559) exhibited objective responses (17%) in melanoma. Thus far, both the response and toxicity rates were lower than those reported with anti-PD-1 antibodies [130]. Recently, monoclonal anti-PD-1 antibody lambrolizumab (MK-3475) was evaluated in metastatic or unresectable melanomas. Objective response rate was obtained in 38% of patients and the responses were durable in the majority of patients [131].

Conclusions

Recent advances in the molecular biology field have allowed for the development of treatments able to improve, for the first time, the overall disease-free survival of metastatic melanoma patients. Advances in the use of immunotherapy and targeted therapy have been shown to potentially improve survival and have become the preferred approaches for most patients with metastatic melanoma. However, clinical responses are still either too transient or limited to restricted patient subsets. The complete cure of metastatic melanoma therefore remains a challenge in the clinic. For instance, new molecular targets need to be identified to help the subset of patients who do not harbor BRAF mutations and overcome the limitations of the current therapeutic agents.

Moreover, combinations of targeted therapies are required and are being studied to prevent or delay the resistance mechanisms.

In the last few years, the treatment strategy for patients with metastatic malignant melanoma has been changed by the results of clinical trials on BRAF inhibitors and immunomodulators. Therefore, actually oncologists can treat a half of melanoma patients with BRAF inhibitors instead of chemotherapy as they harbor a BRAF activating mutation. For the other ones who are BRAF wild-type, ipilimumab represents the best option, so that the use of chemotherapy is limited to those patients refractory to targeted therapy.

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Targeted Therapies for Prostate Cancer

17

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Introduction

Historically, the treatment of advanced or metastatic prostate cancer patients was based on hormonal treatment. Unfortunately, virtually all patients will progress to hormones, and in this scenario, the combination of docetaxel and prednisone is considered as the standard first-line chemotherapy. Recently published results support the use of other different drugs such as cabazitaxel (CBZ), abiraterone (AA), enzalutamide (ENZ), and radium 223 in these patients. Moreover, in recent years, there are a large number of new therapies with different mechanisms of action for patients with metastatic prostate cancer.

In this chapter, we review new alternatives of treatment for patients with metastatic prostate cancer, including new androgen-directed strategies, non-AR-mediated therapies, immunotherapy, and bone targeted treatments.

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Targeting Androgen Receptor (AR)-Associated Signaling in the Treatment of Prostate Cancer

Although androgen-deprivation therapies typically result in a rapid response in metastatic patients, almost all of them finally develop a progressive disease, resistant to hormonal deprivation. Most of the secondary hormone manipulation techniques despite achieving castrate levels of testosterone only provide temporary disease control, and on average, after 12–18 months, the malignant cells become resistant to treatment, with a reported median overall survival of approximately 30 months [41].

The Prostate Cancer Clinical Trials Working Group 2 (PCWG2) defines castration-resistant prostate cancer (CRPC) as patients with serum castration levels of testosterone (testosterone <50 ng/dl or <1.7 nmol/l), PSA and/or clinical progression to castration, and progression despite anti-androgen withdrawal for at least 4–6 weeks [72].

Several treatment options have been described for these patients with metastatic (m) CPRC. Up-regulated androgen receptor (AR) expression and autonomous synthesis of androgens by neoplastic prostate epithelium (either de novo from cholesterol or through metabolism of adrenal

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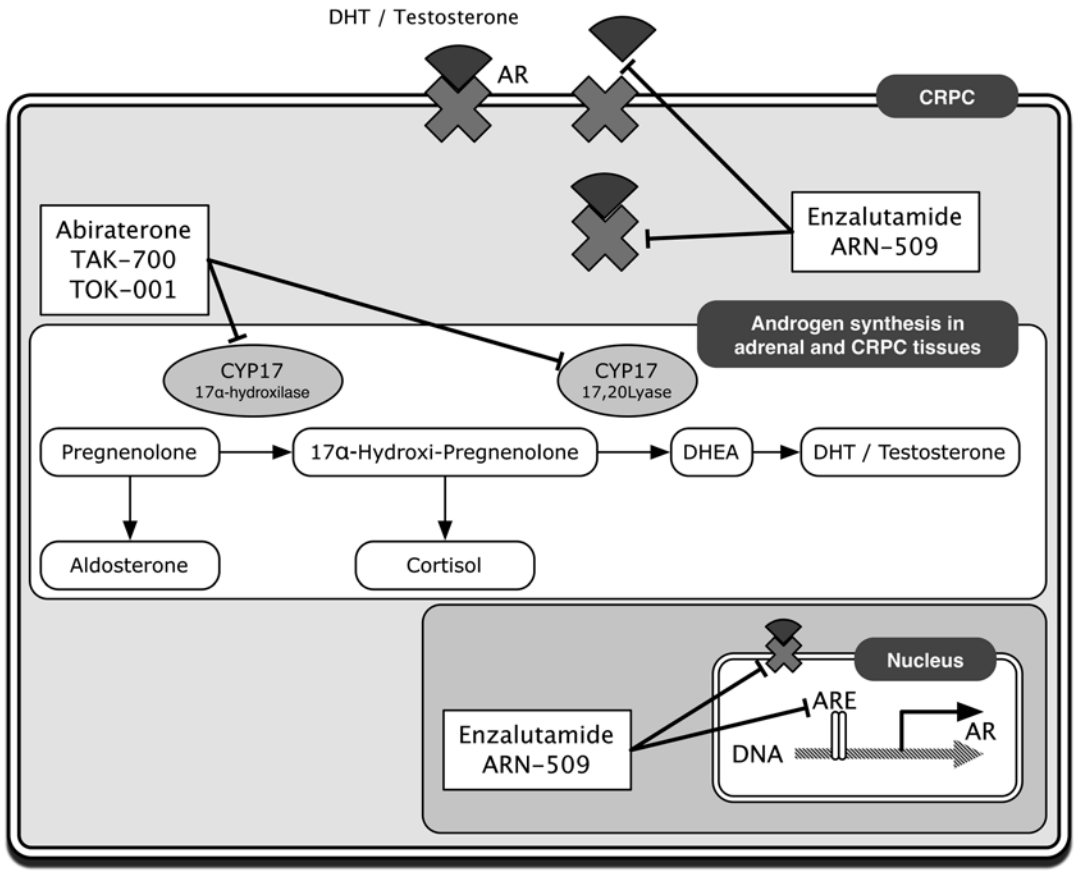

Fig. 17.1 Interaction between testosterone and its receptor in the prostate cell

precursors) are important contributors to CRPC growth (Fig. 17.1) [12, 51]. Different mechanism of resistance to treatment have been described: (1) increased expression of androgen synthesis-related genes, including *CYP17A1*, *AKR1C3*, and *HSD17B3*; (2) up-regulation of AR transcription and the development of splice variants; (3) androgen receptor mutation; and (4) other alterations.

Here, we review some of the drugs with activity in patients with castration-resistant prostate cancer (Table 17.1).

Abiraterone acetate (AA) is a novel, selective, irreversible, and potent inhibitor of 17-[alpha]-hydroxylase/17,20-lyase (CYP17), a critical enzyme in testosterone synthesis, thereby blocking androgen synthesis by the adrenal glands and testes and within the prostate tumor. This enzymatic activity has recently been demonstrated to further reduce testosterone levels in the blood to undetectable range (<1 ng/dl) and it is suggested to reduce de novo intratumor androgen synthesis. Recently, abiraterone has demonstrated activity in castration resistant prostate cancer patients before and after docetaxel administration. In the COU-AA-301 phase III study, 1,195 patients who failed to first or second lines of chemotherapy (at least one docetaxel-based regimen) were randomized to receive abiraterone 1,000 mg daily plus prednisone 5 mg bid versus placebo plus prednisone [17, 23]. Primary objective was overall survival (OS), and secondary objectives were time to PSA progression, radiographic progression-free survival (rPFS), and PSA response.

Median survival in patients treated with abiraterone was 15.8 months in comparison with 11.2 months in placebo treated patients

Table 17.1 Novel hormonal treatments in castration resistant prostate cancer patients

Drugs	Study	Setting	Treatment arms	Results
Abiraterone [65]	Phase III COU-AA-302	Pre-docetaxel	Abiraterone + Prednisone vs Prednisone	OS: NR vs 27.2 HR 0.75 (95% CI 0.61–0.93)
Enzalutamide [5]	Phase III PREVAIL	Pre-docetaxel	Enzalutamide vs Placebo	OS: 32.4 vs 30.2 HR 0.70 (95% CI: 0.59-0.83)
Abiraterone [23]	Phase III COU-AA-301	Post-docetaxel	Abiraterone + Prednisone vs Prednisone	OS: 15.8 vs 11.2 HR 0.74 (95% CI 0.64–0.86)
Enzalutamide [73]	Phase III AFFIRM	Post-docetaxel	Enzalutamide vs Placebo	OS: 18.4 vs 13.6 HR 0.63 (95% CI 0.53–0.75)
Orteronel (TAK-700) [20]	Phase III	Post-docetaxel	Orteronel	OS: 17 vs 15.2 HR 0.886 (95% CI: 0.73–1.06)
Galeterone (TOK-001) [91]	Phase II	CRPC (M0 or M1)	Galeterone	PSA response in 43–71% patients
ARN-509 [61]	Phase I/II	CRPC (M1)	ARN-509	PSA response in 46% patients
ODM-201 [25]	Phase I/II	CRPC(M1)	ODM-201	SD in 60% post-chemotherapy patients

CI confidence interval, *CRPC* castration resistant prostate cancer, *HR* hazard ratio, *M0* no metastatic disease, *M1* metastatic disease, *NR* not reached, *OS* overall survival, *Pts* patients, *SD* stable disease

($p<0.0001$). Patients treated with abiraterone also obtained higher PSA response rate (29.5% vs 5.5%; $p<0.0001$).

In the pre-docetaxel setting, COU-AA-302 phase III study has evaluated the clinical benefit of AA versus prednisone in mildly symptomatic or asymptomatic chemo-naïve patients with progressive metastatic CRPC [65]. About 1,088 patients were randomized 1:1 to AA 1,000 mg plus prednisone 5 mg bid versus placebo plus prednisone. In an interim analysis with 55% of the required events, overall survival, radiographic PFS and secondary endpoints all favored the AA arm. Overall survival was not reached for AA compared to 27.2 for placebo, respectively (HR 0.75; 95% CI 0.61–0.93), although the p value (0.01) did not reach the pre-specified alpha level for the interim analysis (0.0035). Radiographic PFS was 16.5 and 8.3 months for AA and placebo, respectively (HR 0.53; 95% CI 0.45–0.62; $p<0.0001$).

In both studies, abiraterone presented an excellent tolerance profile although it needs administration of prednisone to prevent the toxicity derived from the excess of mineralocorticoids due to the CYP17 blockade. Most frequent grade 3–4 toxicity was edema and fluid retention (<3% severe), hypokalemia (<4%), hypertension (4%) and hypertransaminemia (3–5%)[17, 65].

Orteronel (TAK-700), a non-steroidal selective inhibitor of 17,20-lyase, suppresses androgen production and appears to have less effect on cortisol synthesis, allowing steroid-free dosing. According to phase II study results, orteronel produced PSA responses in 52% of patients, with manageable toxic effects, making it attractive for long-term use [26]. Based on these results, two phase III studies are exploring the efficacy of orteronel plus prednisone versus prednisone alone in men with either chemotherapy-naive or docetaxel-treated metastatic CRPC.

In the ELM-PC5 trial, 1,099 mCRPC patients who failed to docetaxel-based therapy were randomized 2:1 to continuous 28-day cycles of oral orteronel 400 mg BID + prednisone 5 mg BID, or placebo [20]. Primary endpoint was overall survival, and secondary endpoints were radiographical progression-free survival, 50% or more PSA decrease at 12 weeks, pain response at 12 weeks

and safety. The study was terminated for failing to meet its primary endpoint. Median OS was 17.0 months (95% CI 15.2, 19.9) in patients receiving orteronel versus 15.2 months (95% CI 13.5, 16.9) in those receiving placebo (HR: 0.886 [95% CI: 0.739, 1.062]; $p=0.1898$). Median rPFS was significantly improved in the orteronel arm versus the placebo arm: 8.3 months versus 5.7 months (HR: 0.76 [95% CI: 0.653, 0.885]; $p=0.00038$). Main drug-related adverse events included nausea (30%), vomiting (23%), fatigue (17/11%), and diarrhea (16/9%).

Galeterone (TOK-001) is another inhibitor of CYP17. In addition to its activity against CYP17, it also competitively blocks androgen binding at the AR and downregulates AR expression in cell lines [95]. In the phase I ARMOR-1 study, 49 patients with chemotherapy naive non-metastatic CRPC received galeterone (650–2600 mg) plus prednisone; PSA reductions of 30% or greater were seen in 49% of patients, with 22% of them having a 50% or greater decline in PSA. ARMOR-2 is an open label, two-part phase II trial that evaluates safety and efficacy of SDD galeterone in four populations of CRPC patients. Twenty-eight patients were enrolled in part 1. There were four grade 3 adverse events; PSA response was seen in 43–71% of patients [91].

Enzalutamide (MDV3100) is an androgen-receptor and signaling inhibitor chosen for clinical development based on the activity in prostate cancer models with overexpression of the androgen receptor [37, 93]. Enzalutamide inhibits nuclear translocation of the androgen receptor, DNA binding, and coactivator recruitment. Compared to the currently available antiandrogen agents enzalutamide has a greater affinity for the receptor, induces tumor shrinkage in xenograft models (in which conventional agents only retard growth), and has no known agonistic effects.

In the double-blind, placebo-controlled AFFIRM trial, 1,199 men with castration resistant prostate cancer after chemotherapy were randomly assigned in a 2:1 ratio, to receive oral enzalutamide at a dose of 160 mg per day (800 patients) or placebo (399 patients). Corticoesteroids administration was optional in both arms. Enzalutamide was superior to placebo in the primary end point, overall survival (HR = 0.63 [0.53, 0.75], $p<0.001$)[72]. Enzalutamide was also superior over placebo with respect to all secondary end points: reduction in PSA level by 50% or more (54% vs 2%, $p<0.001$), the soft-tissue response rate (29% vs 4%, $p<0.001$), the quality of life response rate (43% vs 18%, $p<0.001$), the time to PSA progression (8.3 vs 3.0 months; HR 0.25; $p<0.001$), radiographic progression-free survival (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; $p<0.001$).

Enzalutamide has a good toxicity profile, with fatigue (6% grade 3), hypertension (6.6%), and hot flushes (20 and 0% grade 3) as the main side effects; the cardiac events were observed in 6% of patients with enzalutamide versus 8% in the placebo arm. Seizures were reported in five patients (0.6%) receiving enzalutamide [73].

In the double-blind, placebo-controlled, PREVAIL phase III study, chemotherapy-naive patients with mCRPC were stratified by site and randomized 1:1 to enzalutamide 160 mg/day or placebo [5]. OS and rPFS were co-primary endpoints and analyzed for the intent-to-treat population. A total of 1,717 men were randomized. Median OS was 32.4 months (95% CI 31.5—upper limit not yet reached [NYR]) in the enzalutamide arm versus 30.2 months (95% CI, 28—upper limit NYR) in the placebo arm, with a 30% reduction in risk of death (OS: HR 0.70; 95% CI: 0.59–0.83; $p<0.0001$). Median rPFS was NYR (95% CI: 13.8—upper limit NYR) in the enzalutamide arm versus 3.9 months (95% CI: 3.7–5.4) in the placebo arm, with an 8% reduction in risk of rPFS (HR 0.19; 95% CI: 0.15–0.23; $p<0.0001$).

ARN-509 is a structural analog of enzalutamide, which has been shown to have similar in vitro but greater in vivo activity [15]. ARN-509 is currently being evaluated in phase I–III trials in different scenarios, including in men with non-metastatic CRPC, as well as in men previously treated with chemotherapy and/or abiraterone acetate. In a phase I/II trial, 46% of patients showed a >50% PSA response at 12 weeks, with a good tolerance to the drug [61].

The phase III SPARTAN trial evaluates the efficacy and safety of ARN-509 versus placebo in adult men with high-risk non-metastatic castration-resistant prostate cancer. Approximately 1,200 participants will be randomly assigned in a 2:1 ratio to receive either ARN-509 or placebo. This study is currently open to the inclusion of patients.

ODM-201 is a novel full androgen receptor inhibitor for CRPC with no AR agonist activity. ODM-201 has a high-PSA response rate in CYP17 inhibitor (CYP17i)-naïve CRPC patients in the phase I/II ARADES study. Patients were enrolled into three dose levels 100, 200, and 700 mg bid. All 124 patients had progressive mCRPC; 37 patients were pre-chemo/CYP17i-naïve, 32 post-chemo/CYP17i-naïve, and 55 post-CYP17i [25]. In 54 post-chemo patients with tissue evaluable disease, the response rate was 6–10%, with stabilization disease in 60% of patients.

Targeting Non-AR-Mediated Signaling

Besides AR-mediated pathways, several alternative signaling pathways may also be involved in the disease progression of prostate cancer. Like other solid tumors, prostate cancers are often characterized by abnormalities in a variety of growth factor signaling pathways that control cell cycle and apoptosis. As these pathways are being understood, new therapeutic targets are driving the development of new agents with activity against advanced disease.

Different pathways that are a current focus for research on specific agents are discussed below (Tables 17.2 and 17.3).

Endothelins

Endothelins are peptides produced by both the tumor and the microenvironment. **Endothelin-1 (ET-1)** binds to the **endothelin-A receptor (ET-A)** and modulates vasomotor tone, nociception, and cellular proliferation in a variety of tissues [42]. Endothelins facilitate formation of blastic metastases in pre-clinical models producing growth factors and enhancing tumor cells proliferation. Serum ET-1 concentration is higher in patients with bone metastases than in patients with localized disease. On the other hand, ET-A is overexpressed in castration-resistant prostate tumors. For this reason, ET-1 and its receptors could be a therapeutic target in this disease.

Two orally active selective inhibitors of the ET-A receptor, **atrasentan and zibotentan**, have been extensively studied in mCRPC to target the supporting environment for metastatic growth. Based on the phase II results with promising data in terms of PFS and even OS, multiple phase III trials (single-agent and combination with docetaxel) were conducted with both **atrasentan** [10, 54, 60] and **zibotentan** [55, 24]. Unfortunately, none of these trials showed significant benefit compared with placebo, and for that reason these agents are no longer being actively developed.

Antiangiogenic agents

Antivascular agents have been also tested in prostate cancer targeting vascular endothelial growth factor (VEGF), related tyrosine kinase pathways, and other novel targets. VEGF expression has been found in localized and metastatic prostate cancer and higher plasma VEGF levels have been correlated with disease severity.

Bevacizumab

Bevacizumab, a humanized monoclonal antibody directed against VEGF has been tested also in prostate cancer. In early phase II studies, bevacizumab was evaluated in chemotherapy-naïve patients with CRPC, but no objective responses were seen, although there was a PSA reduction in 27% of patients [62]. As first-line therapy, bevacizumab in combination with docetaxel/prednisone provided no OS benefit, though there was an improvement in PFS [40].

Aflibercept

Another way of targeting VEGF is by binding its receptors. VEGF-trap is a fusion protein of

Table 17.2 Ongoing clinical trials of targeted therapy for patients with metastatic castration-resistant prostate cancer

Agent	Phase	*n*	Setting	Target antigen	Comparison treatment	Primary endpoint	ClinicalTrials.gov identifier
Chaperone protein							
Custirsen	III	1023	Chemotherapy naive mCRPC	Clusterin	Custirsen + Docetaxel/Prednisone Docetaxel/Prednisone	Overall Survival	NCT01188187 SINERGY
Custirsen	III	630	mCRPC after docetaxel failure	Clusterin	Custirsen + Cabazitaxel/Prednisone Cabazitaxel/Prednisone	Overall survival	NCT01578655 AFFINITY (recruiting)
OGX-427	II Rand	74	mCRPC PSA progression with Abiraterone	Hsp27	OGX-427 + Abiraterone/Prednisone Abiraterone/Prednisone	PFS	NCT 01681433 (recruiting)
OGX-427	II Rand	72	mCRPC in progression without prior chemotherapy	Hsp27	OGX-427 + Prednisone Prednisone 5 mg BID	Disease progression 12 weeks	NCT01120470
PI3K/PTEN/mTOR							
BEZ235	Ib/II	74	Asymptomatic or minimally symptomatic mCRPC	PI3K	BEZ235 + Abiraterone/Prednisone	DLT/PSA response	NCT01717898 (finish)
GDC-0068/ GDC-0980	Ib/II	262	mCRPC previously treated with docetaxel	PTEN	GDC-0068 + Abiraterone/Prednisone Abiraterone/Prednisone	DLT/rPFS	NCT01485861 (recruiting)
Everolimus	I/II	60	mCRPC	mTOR	Everolimus + docetaxel/Prednisone	Safety, Tm response	NCT00459186 (completed)
Temsirolimus	II	24	Chemotherapy-naive CRPC	MTOR	Single arm	Tm response	NCT00919035 (completed)
IGFR-1							
Cixutumumab (IMC-A12)	II	41	Asymptomatic mCPRC Chemo naive	IGFR	Cixutumumab (single arm)	TTP	NCT 00520481 (completed)
Ramucirumab (IMC-1121B)	II Rand	133	mCRPC after docetaxel failure	IGFR	Cixutumumab + Mitoxantrone / Prednisone Ramucirumab + Mitoxantrone/ Prednisone	PFS	NCT00683475 (completed)
Figitumumab (CP-751,871)	II	120	mCRPC chemotherapy-naive or docetaxel-refractory	IGFR	Figitumumab + Docetaxel/Prednisone (single arm)	PSA, TUMOR RESPONSE	NCT00313781 (completed)
PARP inhibitor							
Olaparib (AZD2281)	II	89	mCPRC postdocetaxel	PARP	Single arm	RR	NCT01682772 TOPARP(recruiting)

Table 17.2 (continued)

Agent	Phase	*n*	Setting	Target antigen	Comparison treatment	Primary endpoint	ClinicalTrials.gov identifier
Veliparib (ABT-888)	II Rand	148	mCRPC	PARP	Veliparib + Abiraterone/Prednisone Abiraterone/Prednisone	PSA response	NCT01576172 (recruiting)
Agent	*Phase*	*n*	*Setting*	*Target*	*Comparison treatment*	*Primary endpoint*	*ClinicalTrials.gov identifier*
MET inhibitor							
Cabozantinib (XL 184)	III	960	mCRPC treated with Docetaxel and Abiraterone or Enzalutamide	MET/VEGFR2	Cabozantinib Prednisone	Overall survival	NCT1605227 COMET-1
Cabozantinib (XL 184)	III	246	CPRC treated with Docetaxel and Abiraterone	MET/VEGFR2	Cabozantinib Mitoxantrone/Prednisone	Pain response	NCT1522443 COMET-2
Immunomodulator							
Tasquinimob	III	1200	Asymptomatic or mildly symptomatic mCRPC		Tasquinimob Placebo	PFS	NCT 01234311
Tasquinimob	I	32	Heavily pre-treated mCRPC		Tasquinimob + Cabazitaxel/P	MTD	NCT01513733
Src inhibitor							
Saracatininb	ii	132	Recurrent/progressive PC with bone metastases	Src	Saracatinib Zoledronic acid	Bone markers	NCT00558272 (Completed)

DLT dose limiting toxicities, *mCRPC* metastatic castration-resistant prostate cancer, *n* patient number, *PSA* prostate-specific antigen, *rPFS* radiographic progression-free survival, *TTP* time to progression

Table 17.3 Negative phase III trials with targeted therapies and docetaxel

Trials	Treatment	Results
VITAL II	GVAX vaccine	Poorer survival in experimental group
SWOG SO421	Atrasentan	No difference in progression-free survival or survival
ENTHUSE	Zibotentan	No significant difference in survival
MAINSAIL	Lenalidomide	No survival difference (more toxicity)
CALGB 90401	Bevacizumab	No survival difference (better progression-free survival)
READY	Dasatinib	No difference in survival
VENICE	Aflibercet	No survival difference (more toxicity)

VEGF receptor that binds VEGF-A, and blocks all its isoforms and also placental growth factor (PIGF). A phase III study with aflibercept recently has been reported [90] and did not show benefit in overall survival when added to docetaxel/prednisone in mCRPC in the phase III VENICE trial. Although the aflibercept combination had evidence of biologic activity compared with docetaxel/prednisone (median decline in PSA: 68.6% vs 63.5%; objective response rate: 38.4% vs 28.1%), aflibercept increased severe toxicity, which substantially reduced the overall treatment duration.

Results from the VENICE and CALGB 90401 trials have showed similarities between an improvement in survival and an increase in fatal treatment-related adverse events with the experimental combinations. And, in both trials, the fatal events were mainly infectious, rather than vascular (Table 17.3).

Tasquinimod

Tasquinimod is a quinoline-3-carboxamide derivative and novel immunomodulator that promotes upregulation in prostate cancer of thrombspondin-1, an inhibitor of angiogenesis and cell migration, and blocks S100A9, a protein that has been implicated in regulating myeloid-derived suppressor cells in the tumor microenvironment [56, 97].

A recent, randomized, phase II study of tasquinimod compared with placebo in more than 200 men with mCRPC and minimal symptoms demonstrated a significant clinical benefit with a 6-month PFS of 69% versus 37% ($p=0.001$), and median PFS was 7.6 versus 3.3 months ($p=0.0042$) [58]. Based on the favorable phase II trial results, a large phase III randomized, double-blind placebo-controlled study was conducted in asymptomatic or mildly symptomatic mCRPC patients with recruitment recently completed (NCT 01234311), the results are pending.

Additional trials are currently ongoing with tasquinimod, including CATCH trial in which tasquinimod is being combined with cabazitaxel (NCT01513733).

Lenalinomide

Lenalinomide is a thalidomide analog with improved tolerability and has a dual activity; its antiangiogenic activity, due to inhibition of VEGF and fibroblast growth factor secretion from tumor and stroma cells, but it also has an immunomodulatory effect by stimulating T-cells inhibiting T-regulatory cells and increasing Natural Killer cells activity.

The first results of a phase II trial combining bevacizumab, lenalidomide, docetaxel, and prednisone in CRPC patients were presented at the 2011 ASCO Genitourinary Cancer Symposium, the combination was associated with a high-response rate, showing manageable toxicity [35]. Unfortunately, the phase III trial comparing different doses of lenalidomide combined with docetaxel–prednisone versus placebo were stopped and did not meet the primary endpoint of survival benefit (MAINSAIL study, NCT00988208).

Tirosin Kinases

Tyrosine kinases (TK) are key enzymes that modulate several intracellular pathways of growth and proliferation of tumor cells. The most studied in prostate cancer are **sorafenib and sunitinib**.

Sorafenib

Sorafenib targets RAF kinase, VEGFR-2 and platelet-derived growth factor receptor (PDGFR-β). Clinical experience in prostate cancer is limited to phase II studies. One of them with mCRPC patients in first-line therapy obtained biochemical response and stable disease, with no partial or complete response and no severe (grade 4) adverse effects reported [88]. Another study showed an important difference between PSA evolution and radiologic response. Median PFS and median OS were 3.7 and 28.3 months, respectively. These findings show sorafenib may be an active drug in the CRPC setting, although PSA evaluation does not seem to be the ideal way to test its efficacy [16].

Sunitinib

Sunitinib is an oral tyrosine kinase inhibitor with activity against VEGFR-2, PDGFR β, and KIT. Some phase I -II studies explored sunitinib activity (37.5 mg/d on days 1–14) in combination with docetaxel and prednisone in first-line metastatic CRPC patients. Objective biochemical RR was 56%, and time to progression (TTP) was 42 weeks. This regimen was well tolerated [105]. In another phase II trial with a standard schedule of 50 mg/day, 4-weeks-on/2-weeks-off, patients with CRPC, who had progressed after one or two chemotherapy regimens including docetaxel, were included; 12.1% had a >50% decrease in PSA levels and 21.2% had a >30% PSA decline, with a median PFS of 19.4 weeks [48]. However, the phase III trial comparing sunitinib and prednisone versus placebo was early stopped due to futility in an interim analysis [86].

Chaperone Proteins

Chaperone (heat-shock proteins) plays central roles in stress responses by maintaining protein homeostasis. They facilitate intracellular transport, nuclear translocation, and antiapoptotic properties [102] and they are an established target for anticancer therapy in different solid tumors.

Two cytoprotective chaperones, **clusterin and Hsp27** are targets in current clinical trials of CRPC.

Clusterin is a stress-induced, cell survival protein overexpressed in several solid tumors including CRPC and associated with disease progression and treatment resistance [104]. Clusterin expression is upregulated in patients with prostate cancer who have received androgen-deprivation therapy (ADT) [36].

Custirsen (OGX-011) is an antisense oligonucleotide that inhibits translation initiation of clusterin mRNA. In vitro, custirsen was found to resensitize docetaxel-refractory prostate cancer cell lines to docetaxel [87]. Custirsen downregulated clusterin expression in primary prostate cancers in a phase I study [13]. Later, in a randomized phase 2 trial of first-line CRPC ($n=82$), Custirsen in combination with docetaxel and prednisone compared with standard chemotherapy alone showed an improvement in OS (23.8 vs 16.9 months, HR 0.61, $p=0.06$) although there was no statistically significant improvement in the rate of PSA decline, objective response rate, and PFS. However, on multivariate analysis, patients treated with docetaxel plus custirsen had 51% lower death rate than patients treated with docetaxel alone (HR: 0.49; $p=0.012$)[14, 68].

Based on these results, custirsen is being further evaluated in two large randomized phase III trials. These two trials focus on survival. In the first one, custirsen or placebo is being combined with the docetaxel plus prednisone regimen to assess the effect on overall survival in men with castrate resistant prostate cancer (SYNERGY NCT01188187). Results of this trial are expected in 2014. The second trial is a comparison of cabazitaxel/prednisone alone or in combination with custirsen for second-line chemotherapy in mCRPC (AFFINITY NCT01578655). This study is currently recruiting participants.

Heat shock protein-27 (Hsp27) is another stress-induced chaperone protein involved with the AR and treatment resistance [103]. **OGX-427** is a second generation antisense oligonucleotide against Hsp27 in phase II trials in combination with abiraterone in mCRPC and in combination with prednisone as second-line mCRPC treatment.

Phosphoinositide-3-Kinase–Akt–Mammalian Target of Rapamycin Pathway

Upregulation of the PI3K (phosphoinositide-3-kinase)–Akt–mTOR (mammalian target of rapamycin) pathway has been detected in various tumors, including prostate cancer [50]. PI3K activation is regulated by tumor suppressor phosphatase and tensin homolog (PTEN), and loss of PTEN function has been involved in androgen-independent prostate cancer growth [74]. Deletion of PTEN has been associated with earlier disease progression in patients with prostate cancer, greater AR expression, and poor clinical outcome [76]. Activated PI3K induces Akt to phosphorylate and activate mTOR, which promotes cell division.

Several mTOR1 inhibitors in monotherapy, such as **rapamycin, temsirolimus, or everolimus** have not shown significant clinical activity, suggesting the need for combination studies. Everolimus is being investigated in combination with bicalutamide, docetaxel, and bevacizumab in phase II trials [28]. Given the pre-clinical data for AR pathway reciprocal cross-talk, several early phase studies are evaluating combination AR and PI3K/Akt/mTOR blockade [70].

IGF-1R Pathway

The insulin-like growth factor I receptor (IGF-IR) is a receptor tyrosine kinase with antiapoptotic and transforming activities, and IGF-1R-mediated signaling can be identified during several stages of metastasis, including, migration, and invasion [96]. Expression of ligands IGF-I and IGF-II was higher in high-grade than in low-grade tumors [43]. Moreover, in a meta-analysis of clinical studies, elevated circulating concentrations of IGF-1 were associated with a greater risk for prostate cancer [63].

Three monoclonal antibodies against IGF-1R **cixutumumab (IMC-A12), ramucirumab (IMC-1121B), and figitumumab (CP-751,871)** are being assessed in mCRPC patients.

Poly (ADP-ribose) polymerase

Poly (ADP-ribose) polymerase (PARP) is an enzyme with strong affinity for DNA single-strand breaks and allows DNA repair. Inhibition of this enzyme leads to alterations in the ability of DNA replication to occur, causing cell death [52]. Recent pre-clinical evidence demonstrates that the TMPRSS:ERG gene product interacts with PARP, induces DNA damage, and is required for ERG transcription and cell invasion [7]. Identification of those patients harboring a TMPRSS:ERG fusion may increased benefit from PARP inhibition with either agent.

Several PARP inhibitors are in development in CPRC and currently there are two phase II trials ongoing in metastatic CRPC utilizing **Olaparib (AZD 2281)** as monotherapy and **Veliparib (ABT-888)** with abiraterone/prednisone.

MET

MET is a receptor tyrosine kinase (RTK) that binds to hepatocyte growth factor (HGF) and subsequently activates multiple signaling cascades, including PI3K and MAPK. MET has roles in oncogenic signaling, angiogenesis, and metastasis and it is dysregulated in multiple malignancies, including prostate cancer [11, 101]. In prostate cancer cells, androgen deprivation activates MET signaling. Activated MET is particularly highly expressed in bone. Pre-clinical studies have suggested that MET signaling may promote survival of prostate cancer cells

Cabozantinib (XL184) is a small molecule receptor tyrosine kinase (RTK) inhibitor of hepatocyte growth factor receptor (MET), vascular endothelial growth factor receptor 2 (VEGFR2), and RET [98].

Cabozantinib has shown preliminary evidence of activity against bone metastases in patients with castration-resistant prostate cancer. In a phase II randomized discontinuation trial, 171 docetaxel-pre-treated and docetaxel-naive patients with measurable, progressive mCRPC cancer received 12 weeks of treatment with cabozantinib. After the initial treatment, patients

with a partial or complete response were allowed to continue treatment in an open label extension. Those with stable disease were randomized to cabozantinib or placebo, while those who received placebo were given cabozantinib. Patients with progressive disease after the initial 12 weeks of treatment and those who progressed on cabozantinib following randomization came off protocol.

Although only 5% of patients met RECIST criteria for a partial response at 12 weeks, 72% had regression of soft tissue lesions, and 68% of evaluable patients had improvement on bone scan. Interestingly, PSA changes were inconsistent and independent of clinical or radiographic activity. Among the 31 patients who were randomized after the initial 12 weeks of cabozantinib treatment, progression-free survival following randomization was significantly longer with cabozantinib compared with placebo (24 versus 6 weeks, hazard ratio 0.12). Bone turnover markers (alkaline phosphatase, C-terminal telopeptide) decreased 57% in evaluable patients. Subjective improvement in bone pain was seen in 67% of cases, and 56% decreased or stopped narcotic usage. The toxicities observed in more than 40% of cases included fatigue (63%), decreased appetite (54%), diarrhea (51%), and nausea (49%) [85].

Two randomized, phase III trials of cabozantinib (60 mg/day) have been initiated in men who have progressed on docetaxel and either abiraterone or enzalutamide as treatment for bone metastases from castrate resistant prostate cancer. These trials are being conducted in unselected men without regard for c-met activity.

- The COMET-1 trial compares cabozantinib with/to prednisone. The primary endpoint of this trial is overall survival.
- In the COMET-2 trial, patients are being randomly assigned to either cabozantinib or to a combination of mitoxantrone plus prednisone. The primary endpoint is confirmed durable pain response.

Src

Src is a membrane associated protein and non-receptor tyrosine kinase that modulates signal transduction through several pathways, including the PI3K, focal adhesion kinase (FAK), and MAPK resulting in regulation of cell survival, proliferation, and angiogenesis [47]. Src-family kinases play a role in invasion, tumor spread, chemomodulation, and malignant bone disease, which are all important for prostate cancer control, both of the tumors itself and the preferential site of metastasis [22]. There is evidence that Src-family kinases play an important role in the transition from androgen-sensitive to castration resistant disease in the preclinical setting [92]. And, there is rationale for inhibiting the Src and Src-family kinases due to their activity that is increased in prostate cancer, and the role they play in regulating osteoclast and osteoblast function. Thus, adequate inhibition of Src and Src-family kinases should lead to tumor growth reductions, reductions in tumor metastases, reductions in areas of bone absorption and related complications, as well as tumor angiogenesis.

There are several Src and Src-family kinases inhibitors that are being investigated at different levels as follows.

Saracatinib In a phase 2 study, 5 of 28 patients had a PSA decline. However, no patient achieved and 30% decline. The median PFS was about 8 weeks.

Dasatinib is a small-molecule multityrosine kinase inhibitor of several signaling proteins, including receptor tyrosine kinases, Src family kinases, Bcr-Abl, c-Kit, PDGFR, and ephrins [27]. Dasatinib has shown preclinical activity in prostate cancer [53]. In phase II monotherapy trials in 48 men with mCRPC before chemotherapy, no responses were seen with dasatinib 100 mg daily, but there was a lack of progression in 43% of patients at 12 weeks [99, 100]. Common adverse events of Dasatinib include fatigue, nausea, diarrhea, headache, and anorexia.

In phase I/II trials, evaluating dasatinib in combination with docetaxel, 30% of patients had disappearance of lesions on bone scan and 57% of patients had a durable PSA response [1].

On this basis, a phase III trial was conducted, in which 1,522 men with metastatic castration resistant prostate cancer (mCRPC) were randomly assigned to either dasatinib with docetaxel plus prednisone or docetaxel plus prednisone alone. Preliminary results presented at the 2013 ASCO Genitourinary Symposium found no improvement in overall survival, the primary endpoint of the trial, with a median follow-up of 19 months (median 21.5 vs 21.2 months, HR: 0.99) [2].

Immunotherapy

Prostate cancer disease provides a test system to determine the efficacy of vaccines for different reasons. This cancer is a tumor that grows relatively slowly, recurrence is often diagnosed early, there is a biological marker that can early detect relapse (PSA doubling time), various specific antigens have been identified and characterized, and vaccines can be used with a good safety profile in combination with anti-androgen therapy, chemotherapy, or radiotherapy (Table 17.4).

Antibodies

Ipilimumab (Anti-CTLA4, MDX-010)

Ipilimumab is a human anti-CTLA-4 monoclonal antibody that demonstrated a PSA decline ≥50% in 2/14 CRPC patients in a phase I trial [82]. Most common adverse events (AEs) were arthralgia, malaise, bone pain, pallor, back pain, constipation, fatigue, and decreased appetite.

In a phase II study [79], the combination of ipilimumab with docetaxel was tested: ipilimumab (23 patients) versus ipilimumab and a single dose of docetaxel 75 mg/m^2 (20 patients). PSA responses were observed in 6 patients (3 in each group) and 52 serious adverse events (SAEs) were observed. Five of these 52 SAEs, were considered to be related to ipilimumab treatment: adrenal insufficiency (one patient), diarrhea, colitis and melena (all in one patient), and colitis (one patient).

A phase I dose-escalation trial [94] evaluated ipilimumab-GVAX in patients with metastatic CRPC. PSA responses >50% were seen in 7/28(25%) patients. The most common adverse events were injection-site reactions, fatigue, and pyrexia. Two patients had grade 3 hypophysitis and one patient developed grade 4 sarcoid alveolitis.

Another phase I trial [46] tested PROSTVAC-Ipilimumab and this combination did not seem to exacerbate the immune-related adverse events associated with ipilimumab.

The addition of radiotherapy as a potential immune enhancer to improve clinical responses to ipilimumab has also been reported, in phase I and II trials, to be well tolerated [4, 77]

Prostate Specific Membrane Antigens and Antibodies (J591)

J591 is a monoclonal antibody that binds to the external domain of PSMA, and has been used in combination with radionuclei for therapeutic purposes. After a phase I trial [3] of ^{177}Lutetium-labeled J591 (^{177}Lu-J591) that reported biological activity, the same group presented a phase II study (Tagawa et al. 2008) with 30 patients, where 18 (60%) patients had progressed to docetaxel chemotherapy. A decline >50% and 30% in PSA was observed in 10 and 30% of patients, respectively. The most common adverse event was thrombocytopenia.

In a phase I trial of Yttrium-90-labeled J591 (^{90}Y-J591) [49], anti-tumor activity was observed in two patients, who experienced 85 and 70% PSA decline. Thrombocytopenia was the dose-limiting toxicity.

Vaccines

Sipuleucel-T (APC8015, Provenge)

Sipuleucel-T contains mature, autologous antigen-presenting cells (APCs). APCs are obtained from the patient via a standard leukapheresis procedure approximately 2 days before each scheduled infusion. The patient's APCs are co-cultured with a recombinant fusion protein (PA2024)

Table 17.4 Ongoing clinical trials of immunotherapy for patients with metastatic castration-resistant prostate cancer

Agent	Phase	*n*	Setting	Target antigen	Comparison treatment	Primary endpoint	ClinicalTrials.gov identifier
Ipilimumab							
Ipilimumab	III	600	Asymptomatic or minimally symptomatic metastatic CRPC	CTLA-4	Placebo	Overall survival	NCT01057810
Ipilimumab	III	800	Metastatic CRPC prior treatment with docetaxel	CTLA-4	Placebo	Overall survival	NCT00861614
Ipilimumab plus leuprolide acetate	II	20	Neoadjuvant treatment	CTLA-4	–	Safety	NCT01194271
Ipilimumab plus androgen deprivation therapy	II	48	Metastatic non-CRPC	CTLA-4	–	Safety	NCT01377389
ProstVac							
ProstVac plus Ipilimumab	I	30	Asymptomatic metastatic CRPC	CTLA4 PSA		Safety	NCT00124670
ProstVac plus docetaxel/prednisone	II	144	Metastatic CRPC	CTLA4 PSA	Docetaxel/Prednisone	Overall survival	NCT01145508
ProstVac plus flutamide	II	65	Metastatic CRPC	CTLA4 PSA	Flutamide	Efficacy	NCT00450463
Sipuleucel T							
Sipuleucel T plus abiraterone	II	60	Metastatic CRPC	APC8015	–	Safety/Efficacy	NCT01487863
Sipuleucel T plus androgen deprivation therapy	II	60	Non-metastatic prostate cancer and rising PSA after androgen deprivation therapy	APC8015	–	Safety/Efficacy	NCT01431391
^{177}Lu-J591							
^{177}Lu-J591	I	30	Metastatic CRPC	PSMA	–	Safety	NCT00538668
^{177}Lu-J591 plus docetaxel/prednisone	I	30	Metastatic CRPC	PSMA	–	Safety	NCT00916123
^{177}Lu-J591 plus ketoconazole	II	140	Nonmetastatic CRPC	PSMA	Ketoconazole	Time to radiologic progression	NCT00859781

CRPC castration-resistant prostate cancer, *CTL-4* cytotoxic T lymphocyte antigen, *n* patient number, *PSA* prostate-specific antigen, *PSMA* prostate-specific membrane antigen

containing prostatic acid phosphatase (PAP) and granulocyte macrophage colony stimulating factor (GM-CSF). The activated, antigen loaded APCs are then re-infused into the patient, where they stimulate a T-cell response against prostate cancer cells [75]. Results from phase I and II trials revealed an increase in T-cell mediated immune responses against PAP and a decrease in the serum PSA level with low toxicity [6, 8, 9, 78].

Three phase III trials have been conducted with this vaccine in prostate cancer. In the first study (D9901; [79, 80]), 127 men with asymptomatic CRPC were randomized (2:1) to receive either Sipuleucel-T ($n=82$) or placebo ($n=45$). The primary endpoint was the time to progression, which was 11.7 weeks in the vaccine group compared with 10 weeks in the placebo group ($p=0.052$).

A similar phase III study (D9902A) was initiated, but enrollment was stopped after 98 patients, based on initial results in D9901, and an integrated analysis of D9901 and D9902A was reported [34]. A total of 225 patients were randomized to Sipuleucel-T ($n=147$) or placebo ($n=78$). The results showed a median benefit in overall survival of 4.3 months for Sipuleucel-T (23.2 vs 18.9 months), translating to a 33% reduction in the risk of death [hazard ratio (HR)=1.50, $p=0.011$].

Finally, the third phase III trial (IMPACT or D9902B) [38] randomly assigned 512 patients to receive either Sipuleucel-T (341 patients) or placebo (171 patients) in a 2:1 patient ratio. Unlike previous studies, overall survival was the primary end point. A relative reduction of 22% in the risk of death was observed as compared with the placebo group (HR=0.78, $p=0.03$), which represents an increase of 4.1 months in the overall survival for the Sipuleucel-T group (25.8 vs 21.7 months). The 36-month survival probability was 31.7 and 23.0% in the Sipuleucel-T and placebo groups, respectively. Paradoxically, no benefit in the time to disease progression was observed. Common adverse events reported in the Sipuleucel-T group included chills, fever, and headache. On April 29, 2010, Sipuleucel-T was approved by the FDA for the treatment of patients with asymptomatic or minimally symptomatic CRPC.

GVAX

GVAX is a vaccine obtained from prostate cancer cells genetically modified to secrete GM-CSF. Unlike Sipuleucel-T, where prostatic acid phosphatase is the antigen source, GVAX uses whole tumor cells as antigens.

GVAX was shown in phase II studies to be immunogenic, clinically active, and generally well tolerated. In an initial trial [81, 82], 55 chemotherapy-naïve CRPC patients with radiologic metastases ($n=34$) or elevated PSA levels only ($n=21$), were treated with two different doses (low-dose and high-dose). Actual median survival was compared with estimated median survival obtained using the Halabi nomogram [31], a pre-treatment prognostic model. The metastatic group showed an increased median survival, when compared to predicted values of 26.2 vs 19.5 months, respectively. The median survival for high-dose and low-dose treatment was 34.9 ($n=10$) and 24 months ($n=24$), respectively. Immunogenicity and clinical activity were dose-dependent as reported in another multicenter phase I/II trial [32] were the median overall survival was 35.0, 20.0 and 23.1 months for the high, medium, and low-dose groups, respectively.

These results led to two phase III studies. The first (VITAL-1; [33, 34]) compared GVAX to docetaxel plus prednisone (D + P) in asymptomatic CRPC chemotherapy-naïve patients. The study was prematurely terminated based on the results of a previously unplanned futility analysis which determined that the study had less than a 30% chance of meeting its predefined primary endpoint of improvement in overall survival. The median survival was 20.7 months in the GVAX group and 21.7 months in the D + P group, but these differences were not statistically significant.

The second phase III trial (VITAL-2) [83] compared D + P to docetaxel-GVAX (D + G) in CRPC taxane-naïve symptomatic patients. The study was designed to enroll 600 patients, but was prematurely terminated after performing a futility analysis after accrual of 408 patients, that

showed a higher survival in the standard treatment group due to an excess of deaths in the GVAX group, 76 deaths in the D + P group vs 85 in the D + G group (HR = 1.4, $p = 0.02$).

PROSTVAC-VF Vaccine (PSA-TRICOM)

PROSTVAC-VF [45] is a recombinant vaccine based on the vaccinia virus and a fowl pox virus encoding human PSA and three co-stimulatory agents, lymphocyte function-associated antigen 3 (LFA-3), ICAM-1, and B7.1. Both vaccines infect antigen-presenting cells (APCs) resulting in expression of proteins on the surface of APCS. The interaction of transduced APCs with T-cells promotes a targeted immune response and T-cell-mediated tumor cell destruction. This vaccine successfully demonstrated immunologic activity with a low-toxicity profile in several phase I trials [21, 29, 69]. These positive results encouraged further assessment of this product in several phase II studies.

The Eastern Cooperative Oncology Group (ECOG) published a phase II study evaluating the response to vaccinations in 64 patients with biochemical progression after local therapy and different schedules [30]. At 19 months, 45.3% of the patients were free of PSA progression and overall time to PSA progression was 13.6 months. A phase II study [18], based on the same population, included 50 patients. At that time, the results were presented only 29 patients were evaluated. Sixty-six percentage of patients were free of PSA progression at 6 months (primary end point).

A phase II trial analyzed the effect of PSA-TRICOM with or without GM-CSF in 32 patients with metastatic chemotherapy-naïve CRPC [44]. Twelve of 32 patients had a PSA decline (37.5%) and 22 out of 32 patients had a longer survival compared to those predicted based on the Halabi score.

Finally, a randomized double-blind phase II trial was conducted in 125 men with asymptomatic CRPC [39]. Similar to the Sipuleucel-T phase III study, the primary end point, progression-free survival, was not met (3.8 months in the PROSTVAC group and 3.7 months in the control group), but the treatment was associated with a 44% reduction in the risk of death and a 8.5 month improvement in median OS (25.1 vs 16.6 months).

TG4010

MVA-MUC1-IL-2 (TG4010) is a recombinant modified Vaccinia Ankara (MVA) viral vector encoding MUC1 and interleukin-2 (IL-2), which has shown in phase I trials to be safe [57, 64]. It has not been investigated in CRPC, but was assessed in a randomized phase II study in patients with PSA progression after local treatment [19]. None of the patients showed a 50% decline in PSA (primary endpoint); however, 10 patients had stabilized PSA for over 8 months.

Bone-targeted therapy

Prostate cancer is a bone-predominant disease, and there are two classes of agents in development and those affect bone resorption and radiopharmaceuticals [71].

Bisphosphonates

The first class of osteoclast-targeted agent is the bisphosphonates. Their activity is derived from structural similarity to pyrophosphate, a normal component of bone. When administered orally or intravenously, bisphosphonates are incorporated into bone matrix by binding to exposed hydroxyapatite crystals. This binding provides a barrier to osteoclast-mediated bone resorption and has direct inhibitory effects on osteoblasts. **Zoledronic acid** is the most potent available bisphosphonate, 1,000 times more potent in vitro than clodronate [66, 67].

Denosumab

RANKL-induced signaling plays an important role in osteoclast regulation, making it a logical target for therapeutic intervention. Denosumab is a subcutaneously administered monoclonal antibody with a high-binding affinity for RANKL. It has a half-life of more than 30 days at its highest

doses and can produce sustained inhibition of bone turnover markers (e.g., NTx) for more than 6 months in certain clinical settings.

We have phase 3 results of the two key agents in this setting: zoledronic acid and denosumab. Zoledronic acid showed a significant improvement in skeletal-related event outcomes when compared to placebo (median time to first SER 16 vs 10.5 HR 0.64). It became standard of care. Recently, denosumab showed an improvement over zoledronic acid (20.7 vs 17.1 RR 0.82. These agents have activity as measured by improvement in skeletal-related events (Fizazi et al. 2010). Both zoledronic acid and denosumab have been shown to significantly reduce the incidence of skeletal events, such as pathologic fractures and spinal cord compression, in prostate cancer patients with advanced disease. Intensive osteoclast inhibition with monthly denosumab has been shown to modestly prolong metastasis-free survival by about 4 months in men who have progressed on first-line androgen-deprivation therapy [84].

Recently, results from the Zometa European Study (ZEUS), presented at the EAU Congress, indicate that zoledronic acid is not better for preventing bone metastasis in high-risk prostate cancer patients than standard treatment.

Radiopharmaceuticals

The last agent is **radium-223** which is an alpha-emitting radiopharmaceutical that delivers high-energy irradiation with a short range, and therefore lower penetration into surrounding tissue than beta-emitting radiopharmaceuticals such as samarium-153 and strontium-89. This agents historically has been used for pain relief; radium-223 not only does it appear to offer pain relief but also improve survival. These are data from a study of symptomatic, heavily pre-treated metastatic CRPC patients treated with radium-223 or with placebo, with an improvement in survival that is similar (considering hazard ratio) to chemotherapy, and even approaching some of the newer AR signaling inhibitors. The effects are not only on survival but also on skeletal-related events, with a hazard ratio of 0.61. Recently Radium-223 has been approved by the FDA for prostate cancer with bone metastases [58].

Conclusions

CRPC is a heterogeneous disease with different signaling pathways involved in disease progression. Several potential molecular targets for treating CRPC, including those that inhibit AR-mediated and non-AR-mediated signaling have been identified. In recent years, novel agents have shown promise in clinical trials, including agents targeting the androgen axis (inhibitors of androgen production and novel AR antagonists) and agents with other targets (Src, IGF-1R, PI3K, PTEN, mTOR, MET, and clusterin).

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Targeted Therapies for Bone Metastases

18

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Abbreviations

AP-1	Activator protein 1
bALP	Bone-specific alkaline phosphatase
BM	Bone microenvironment
BMSCs	Bone marrow stromal cells
BPs	Bisphosphonates
CCR1	Chemokine receptor for MIP1α-1
CXCR-4	Chemokine receptor type 4
DKK1	Dickkopf-related protein 1
DLX5	Distal-less homeobox 5
HGF	Hepatocyte growth factor
IGFBP5,	Insulin-like growth factor 5
LRP	Low-density lipoprotein receptor-related protein 5/6
MIP	Macrophage inflammatory protein-1α
MMP-1	Matrix metalloproteinase-1
MM	Multiple myeloma
MSCs	Mesenchymal stem cells
NFATC1	Nuclear factor of activated T cells
OPG	Osteoprotegerin
PAM	Pamidronate
PCs	Plasma cells
PTHrP	Parathyroid hormone-related protein
Runx2	Run-related transcription factor 2
SDF-1	Stromal-derived factor
SREs	Skeletal-related events
Src	Sarcoma
TK	Tyrosine kinase
TGF-β	transforming growth factor beta
TBRI	TGF-β type I receptor
uNTX	Urinary N-telopeptide
VCAM-1	Vascular cell adhesion molecule-1
VLA-4	Cell surface molecule very late antigen-4
ZA	Zoledronic acid

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Introduction

Bone, particularly trabecular bone, is one of the most preferential metastatic target sites for malignancies such as breast, prostate, and lung cancers. Skeletal metastasis frequently leads to pain,

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fractures and other complications. Crosstalk between tumor cells and bone cells, both through direct cell–cell contact and through soluble factors, is considered critical for the development and progression of bone metastases [24, 55, 67, 81, 131].

Depending on their radiographic appearance, bone metastases can be predominantly osteolytic, involving bone destruction, or osteoblastic characterized by increased deposition of new bone. The lesion phenotype reflects the local interaction between tumor cells and the bone remodeling system [119]. Prostate cancer metastases are typically osteoblastic [67], whereas breast cancer metastases are usually osteolytic [60]. More precisely, recent observations suggest that bone metastases represent a spectrum. At one end, osteolytic lesions are associated with increased bone resorption and reduced osteoblast (OB) activity, but an attempt at bone repair is often also present, whereas bone metastases that are predominantly osteoblastic also show an enhanced bone resorption. Dysregulated bone resorption by osteoclasts (OCs) is necessary for the establishment of metastases, as it releases growth factors from the bone matrix, fueling tumor growth [18, 41, 54, 55, 60].

Tumor cells produce chemokine receptors, cell adhesion molecules and cell surface receptors that enable them to home to the bone and attach to the endosteal surfaces [131]. Although tumor cells secrete proteolytic enzymes and can directly destroy the bone matrix in vitro, the main mediators of bone destruction within a metastatic lesion are the OCs [60]. The bone matrix is a rich deposit of growth factors that are released into the tumor microenvironment as a result of osteolysis. These factors stimulate the growth of tumor cells and alter their phenotype, thus promoting a vicious cycle of metastasis and bone pathology. Physical factors within the bone microenvironment, including low oxygen levels, acidic pH, and high extracellular calcium concentrations, may also enhance tumor growth [57, 119].

Tumor-produced parathyroid hormone-related protein (PTHrP) is one of the important mediators of the osteolytic process occurring in metastatic breast carcinoma. Transforming growth factor-β (TGFβ), which is abundant in the bone matrix and is released as a result of osteoclastic bone resorption, promotes osteolysis by stimulating PTHrP production by tumor cells. PTHrP then stimulates osteoclastic bone resorption by increasing OB production of receptor activator of nuclear factor-kappaB ligand (RANKL) and by decreasing their production of osteoprotegerin (OPG) [40, 60]. Cancer cells can also secrete multiple other cytokines that stimulate osteoclastogenesis [119].

Furthermore, there is evidence that osteolytic lesions are linked with impaired OB differentiation and activity [13, 44, 45, 74] and elevated OB apoptosis [72].

In contrast, in the case of osteoblastic metastasis, OB proliferation and matrix deposition have increased [44, 45, 67, 130]. Prostate cancer cells alter bone homeostasis by secreting factors such as BMPs, WNTs and endothelin 1 (ET1) that directly affect OB function, as well as prostate-specific antigen (PSA) and other proteases that influence bone formation indirectly, e.g., by releasing and activating growth factors present in the bone microenvironment [67, 90, 131]. The net result is increased OB proliferation and differentiation, leading to increased deposition of abnormal, woven bone. OB secreted factors, in turn, promote tumor cell survival and growth, enforcing the cycle [19, 67, 80, 131]. Prostate cancer cells also produce factors that stimulate OC activity [54]. Indeed different prostate cancer cell lines are capable of causing either osteolytic or osteoblastic bone metastases in immunocompromised mouse models [30, 83, 115]. The degree of osteolytic or osteoblastic capability can be linked to differences in the cancer cell secretome, more specifically— to the secreted factors that affect the bone microenvironment. In particular, recent studies show that cancer cell-secreted DKK1 and NOG, which suppress bone formation by inhibiting WNT and BMP signaling, respectively, in OBs, are crucial determinants of osteolytic metastasis [22, 44, 46, 90, 100].

Targeted Therapies for Bone Metastatic Solid Cancers

The treatment of bone metastases aims to prevent further progression and skeletal-related events (SREs), relieve the bone pain and improve the patient's quality of life (QOL). A multimodal approach, including collaboration among radiologists, pathologists, hemato-oncologists, radiation oncologists, nuclear medicine, and orthopedic oncologists, is a prerequisite for the effective management of patients with bone metastases [62]. Current therapeutic options include surgical intervention, targeted radiotherapy, radioisotope treatment, and targeted medical treatments.

Surgical management relieves bone pain, improves neurological functions, and inhibits the local tumor growth [62]. Radiotherapy is used to relieve bone pain, but it can also induce tumor regression and bone healing [59].

Major improvements have been recently made in medical treatments. Thanks to acquisitions on the biology of bone metastases, new therapies blocking key targets for the establishment and progression of secondary bone lesions have been developed.

Targeted treatments for bone metastases can be divided into tumor-targeted therapies and bone-targeted therapies. Owing to the "vicious circle" in the bone microenviroment, by inhibiting cancer cell growth we can block the tumor stimulation on OC differentiation and bone resorption. Several tumor-targeted therapies (chemo-, hormone-, target-, and immuno-therapies) were found to induce a bone scan objective response or improvement in bone pain in patients with bone metastatic cancer. The most representative tumor-targeted therapies with proven efficacy also in bone localization of disease will be discussed below. Bisphosphonates and the RANKL antibody denosumab are the only bone-targeted therapies approved, but many emerging bone molecular targets are under investigation. Systemic radionuclide therapies could be considered as bone-targeted treatments too.

Tumor-Targeted Therapies: The Example of Advanced Prostate Cancer

Hormonotherapy in Bone Metastatic Prostate Cancer

The critical role of androgens for prostate cancer growth was established in 1941 by Charles Huggins, and these findings established androgen deprivation therapy (ADT) as the primary treatment for patients with advanced prostate cancer [50, 103].

Standard approaches include orchiectomy, a gonadotropin-releasing hormone (GnRH) agonist, or a combination of a GnRH agonist plus an antiandrogen (complete androgen blockade) [27].

Although ADT is palliative and not curative [121], it can normalize serum levels of PSA in over 90% of patients and can produce objective tumor responses in 80–90% of patients. This antitumor activity can improve the QOL by reducing bone pain as well as the rate of complications, such as pathologic fracture, spinal cord compression, and ureteral obstruction.

Patients who have progressed while on ADT are said to have castration-resistant disease. Secondary hormone manipulations often lead to clinical benefit after progression on ADT, although the duration of such effects is usually limited.

A number of options are available for secondary hormone manipulation following failure of initial ADT for advanced disease: withdrawal of antiandrogens or other hormones, administration of antiandrogens, estrogen, and progestins, P450 enzyme inhibitors, and glucocorticoids. Recent advances have demonstrated that androgen-based pathways continue to have a clinically significant role in the progression of castrate-resistant prostate cancer. In addition to androgen production by the adrenal gland and the testis, several enzymes involved in the synthesis of testosterone and dihydrotestosterone, including cytochrome P450 17 alpha-hydroxysteroid dehydrogenase (CYP17), are highly expressed in tumor tissue [78].

Abiraterone irreversibly inhibits the products of the CYP17 gene. Abiraterone plus prednisone in men who had previously been treated with a docetaxel-containing chemotherapy significantly improved overall survival (OS) compared with placebo plus prednisone in the randomized phase III trial. There was also a statistically significant improvement in palliation of pain due to bone metastases and a statistically significant increase in the time to first SRE [68]. In another phase III trial that included chemotherapy-naive patients with metastatic castration-resistant prostate cancer, the clinical benefit of abiraterone in terms of radiographic progression-free survival (rPFS) and OS [96] was shown.

Some metastatic and primary prostate tumors retain activation of the androgen receptor in processes that are entirely independent of the androgen ligand. Several mechanisms, including upregulation of androgen receptor expression through amplification of the androgen receptor gene [25] increased sensitivity of androgen receptor via overexpression of nuclear coactivators [39], and splice variant mutations of the receptor [42, 122], have been proposed and may coexist. Small molecule antagonists of the androgen receptor, such as MDV3100, are being developed and have demonstrated promising clinical activity.

On the basis of phase I/II study results, two phase III trials were drawn to MDV 3100 with placebo. In the AFFIRM trial, 1199 men with castrate-resistant prostate cancer who had received prior docetaxel-based chemotherapy were randomly assigned to either MDV3100 (160 mg per day) or placebo [101]. The results of the trial were recently published. OS, the primary endpoint of the trial, was significantly increased in patients assigned to MDV3100 (median 18.4 versus 13.6 months; hazard ratio (HR), 0.63). The superiority of enzalutamide over placebo was shown with respect to all secondary endpoints: the proportion of patients with a reduction in the PSA level by 50% or more (54 versus 2%, $p < 0.001$), the soft tissue response rate (29 versus 4%, $p < 0.001$), the QOL response rate (43 versus 18%, $p < 0.001$), the time to PSA progression (8.3 versus 3.0 months; HR, 0.25; $p < 0.001$), rPFS (8.3 versus 2.9 months; HR, 0.40; $p < 0.001$), and the time to the first SRE (16.7 versus 13.3 months; HR, 0.69; $p < 0.001$).

In the PREVAIL trial, asymptomatic or minimally symptomatic men with metastatic prostate cancer who are chemotherapy naive are being randomly assigned to MDV3100 or placebo (Clinical Trial NCT01212991).

Biologic Therapy in Bone Metastatic Prostate Cancer

In castration-resistant disease, the overall impact of cytotoxic chemotherapy is limited, and this has led to the development of newer approaches, as biologic therapy, for the treatment of advanced prostate cancer.

Cabozantinib (XL184) is an inhibitor of c-MET, VEGFR2 and RET, receptor tyrosine kinases (TKs) that are frequently activated in metastatic processes. In particular, XL184 has shown preliminary evidence of activity against bone metastases in patients with castration-resistant prostate cancer.

In a phase II randomized discontinuation trial, patients with measurable progressive metastatic prostate cancer treated with XL184 obtained an objective response in bone metastases and subjective improvement in bone pain [51].

At the 2012 American Society of Clinical Oncology (ASCO) meeting, the results from the subsequent non-randomized expansion cohort in docetaxel-pretreated patients were presented. XL184 demonstrated to improve bone scan response (BSR), bone pain reduction with a decrease of markers of bone resorption (C-telopeptide (CTX) N-telopeptide (NTx) and bone-specific alkaline phosphatase (bALP)). The mechanism explaining the activity of XL184 on bone metastases is largely unknown and all these promising results are awaiting confirmation in controlled phase III trials.

Bone-Targeted Therapies

Bisphosphonates, denosumab, emerging targeted treatments (bone anabolic and/or anticatabolic agents) and systemic radionuclide therapies belong to the group of bone-targeted therapies.

Bisphosphonates

Bisphosphonates have a strong affinity to bone hydroxyapatite and surfaces undergoing active remodeling. Bone-resorbing OCs internalize bisphosphonates, leading to multiple consequences in OCs. Nitrogen-containing bisphosphonates (alendronate, risedronate and zoledronic acid (ZA)) inhibit farnesyl pyrophosphatase, an enzyme responsible for the prenylation of GTPases that are essential for OC function, structural integrity and prevention of apoptosis [70, 92, 118].

Inhibition of farnesyl pyrophosphatase also results in the accumulation of isopentenyl diphosphate that is incorporated into a cytotoxic nucleotide metabolite, ApppI [77]. Besides the effects on OCs, there are indications for direct effects of bisphosphonates on cancer cells. Bisphosphonates have also been suggested to activate γδ-T cells, modulate tumor-associated macrophages and inhibit angiogenesis [61, 73, 126].

Clinical trial data, individually and in meta-analysis, have established that in women with metastatic breast cancer and clinically evident bone metastases, the use of bisphosphonates reduces the frequency SREs by approximately one-third when compared with placebo or no bisphosphonate therapy. This was associated with significant delays in median time to SRE, improvements in bone pain and improvements in global QOL [125].

Both oral (clodronate and ibandronate) and intravenous (ibandronate, pamidronate (PAM) and ZA) formulations have become integral components of treatment in patients with breast cancer and bone metastases. In the USA, only two bisphosphonates, PAM and ZA, are FDA approved for the management of metastatic bone disease in patients with breast cancer.

ZA is also approved for use in men with bone metastases that is progressing on initial hormone therapy in patients with bone metastases from prostate cancer. The benefit of ZA in this setting of patients was demonstrated in a phase III trial showing a significant reduction in the frequency of SREs, a longer median time to develop a SREs and lower pain and analgesic scores [97].

In a placebo-controlled trial of 773 patients with skeletal metastases from cancers other than breast and prostate (including non-small cell and small cell lung, renal cell, thyroid, and head and neck cancers), comparing ZA versus placebo, patients who were randomly assigned to ZA had a significant reduction in the number of SREs (38 versus 47%) and a significantly longer time to the first event (230 versus 163 days) [94].

Finally, bisphosphonates significantly decrease bone turnover and increase bone mineral density in men receiving ADT for prostate cancer [21, 47].

Denosumab

Given the fundamental role of the RANK–RANKL system in the maturation and function of OCs and thereby in the development of bone metastasis, inhibition of this system has been extensively investigated as a therapeutic tool for the treatment of osteolytic metastasis. Denosumab is a non-cytotoxic IgG2 monoclonal antibody with an extremely high affinity for human RANKL and thereby prevents the interaction between RANK, which is expressed by OCs, and RANKL produced by OBs. Clinical trials in breast cancer patients with bone metastases have demonstrated that denosumab reduces SREs and bone resorption, as shown by the reduced levels of bone turnover markers N-telopeptide of type I collagene (uNTX) [7, 66]. Furthermore, denosumab suppresses bone resorption marker in a manner independent of prior treatment with biphosphonate, and also in patients who had responded poorly to biphosphonate treatment [8]. A phase III clinical study that compared denosumab and ZOL in the treatment of breast cancer patients with bone metastases, revealed that denosumab is more effective in delaying or preventing first and subsequent SREs. The overall incidence of SREs, the adverse effects of renal toxicity and osteonecrosis of the jaw as well as the OS of patients were similar between the two treatments [111].

In a 2012 meta-analysis, compared to bisphosphonates, denosumab was significantly more effective and was associated with reduction in the SRE rate, delays in time to SREs, and prolongation in the time to developing bone pain (in breast

cancer patients without pain at baseline). There was no difference in OS [125].

Moreover, denosumab has demonstrated to significantly delay the time to first-on-study SRE compared with ZOL bone metastases, also in patients with castration-resistant prostate cancer [31].

A phase III trial compared denosumab and ZA in 1776 patients with multiple myeloma (MM) or bone metastases from a solid tumor other than breast or prostate cancer (40% non-small cell lung cancer, 10% MM, 9% renal cell carcinoma, 6% small cell lung cancer, and 5% other tumor types) [48]. Denosumab was not significantly inferior to ZA in delaying time to first-on-study SRE (20.6 versus 16.3 months; HR, 0.84; 0.95% CI, 0.71 to 0.98; $p=0.03$). However, when adjusted for multiple comparisons to test for superiority, the difference was no longer statistically significant ($p=0.06$). OS and disease progression were similar between the two groups. In a subgroup analysis, mortality appeared to be higher with denosumab compared with ZA in patients with MM (HR for death, 2.26; 95% CI, 1.13–4.50), but the number of patients in this group was limited.

On the basis of these and other data, denosumab has been approved for the treatment of patients with bone metastases from solid tumors, but not MM.

Importantly, denosumab induced an 86% tumor response rate in patients with giant cell tumor of bone; this type of tumor consists of RANK expressing OC-like giant cells and mononuclear (stromal) cells that express RANKL [116].

Recently, the ability of denosumab to prevent the development of bone metastasis in high-risk prostate cancer patients has been demonstrated. Indeed some have hypothesized that, by limiting bone turnover and resorption, denosumab may make bone an environment that is less amenable to circulating tumor cells remaining and clonally expanding.

In a phase III trial, 1432 men with nonmetastatic castration-resistant prostate cancer were randomly assigned to denosumab or placebo. Denosumab increased the time to development of first bone metastasis by a median of 4.2 months compared with placebo, in a population of men deemed to be at high risk for the development of metastatic disease (baseline PSA value $\geq$ 8.0 ng/mL and/or PSA doubling time (PSADT) $\leq$ 10.0 months). No difference in OS was noted, however (median 44 versus 45 months; HR, 1.01) [109].

To determine the efficacy of denosumab in men at greatest risk for bone metastases, the researchers evaluated bone-metastasis free survival (BMFS) in a subset of men with PSADT $\leq$6 months. Results were showed at the 2012 ASCO meeting. Median BMFS in the placebo group of men with PSADT $\leq$6 months was 6.5 months shorter than for the placebo group in the full population (18.7 months versus 25.2 months), indicating that these men are at particularly high risk. In this group of men with PSADT $\leq$6 months, denosumab prolonged BMFS by a median of 7.2 months, and with a 23% reduction in risk compared with placebo. Patients with shortened PSADT are at higher risk of developing bone metastasis and denosumab is markedly effective at prolonging BMFS in this subset of patients [98].

Finally, denosumab is approved drug to prevent bone loss in men at high risk for fracture receiving ADT for nonmetastatic prostate cancer [107, 108], and to increase bone mass in women at high risk for fracture receiving adjuvant aromatase inhibitor therapy for breast cancer [28].

Bone Anabolic and Anticatabolic Agents

Breast and other osteotropic cancers affect the skeleton not only through increased OC activity, but also by concurrently defective OB function that appears strictly related to both growth and treatment of tumors. Therefore, new drugs targeting both OB and OC deregulated pathways may be essential in these diseases to concomitantly exert bone anabolic and anti-catabolic therapeutic effects. In this context, a number of compounds are currently under intensive investigation in both preclinical and clinical studies.

c-Src Inhibitors

The Src sarcoma (src) oncogene encodes for a non-receptor-TK involved in several molecular pathways including those signaling for cell adhe-

sion, proliferation and chemotaxis. Activation of the c-Src pathway has been reported to drive the tumor progression of prostate, breast and other solid cancers [10], while it regulates both OC and OB activities in the physiology of bone turn-over. In fact, in OCs, src signaling is essential for both differentiation and the cytoskeleton rearrangement, necessary for bone resorption, whereas it acts as a negative regulator of OB maturation through the inhibition of run-related transcription factor 2 (*Runx2*) gene [132]. Recently, it has been demonstrated that the pathways activated by *src* and IL-6 engage a functional loop inducing the expression of insulin-like growth factor 5 (IGFBP5), a c-Src activating factor that maintains the OBs in a persistent immature condition [87]. Dasatinib, a functional double inhibitor affecting both *src* and Abl families, down-regulates OC formation and its bone resorptive functions by reducing both c-Fos and nuclear factor of activated T cells (NFATC1) levels as well as the expression of cathepsin K, αVβ3 and chemokine receptor for macrophage inflammatory protein (MIP)-1α (CCR1). Moreover, it regulates OB differentiation by priming the canonical WNT/β-catenin pathway [34]. A combinatory treatment of dasatinib with docetaxel in patients with prostate cancer showed a significant reduction of urinary N-telopeptide (uNTX) and bALP [3], whereas in a parallel study enrolling patients with MM, dasatinib was ineffective on OB function while modifying the expression of phenotypic OC markers [124]. In a phase II trial, bosutinib, a Src/Abl inhibitor, demonstrated promising efficacy in extending the time to progression in patients with breast cancer, although no effects were reported on bone health [14]. However, several clinical trials are presently evaluating the role of *src* inhibitors as single agents, as well as in combination with hormone therapy or chemotherapeutic drugs in bone metastasis.

Activin A

Activin A, a cytokine belonging to TGF-β family, is primarily secreted by cells of the OB lineage including not only bone marrow stromal cells (BMSCs) but also secreted by OCs. This cytokine stimulates OC formation by synergic effect with RANKL while inhibiting OB differentiation by down-regulation of distal-less homeobox 5 (DLX5) gene expression [33]. Its serum levels have been described to be increased in both breast and prostate cancer patients with bone metastases in comparison to patients with no skeletal involvement [64].

Moreover, *RAP-011*, a murine IgG-Fc fusion protein resembling the soluble activin receptor type IIA, apparently stimulates bone remodeling and reduces the formation of osteolytic lesions in animal models of MM and breast cancer [17]. The RAP-011 human analog, ACE-011, is currently under clinical investigation for similar effects in patients with MM. Preliminary results provide evidence that this drug significantly increases the levels of bone regeneration biomarkers with a concurrent decrease of bone pain in the absence of evaluable toxicity [1]. ACE-011 also increases hemoglobin levels, and its utilization to treat chemotherapy-induced anemia is presently being evaluated in patients with either breast or non-small lung cell cancer.

TGF-β Receptor Inhibitors

TGF-β is segregated at high levels in the bone matrix and plays a critical role in bone remodeling and cancer progression. It regulates the expression of several factors involved in the pathogenesis of bone metastases, such as integrin αvβ3, IL-6, IL-8, IL-11, matrix metalloproteinase-1 (MMP-1), chemokine receptor type 4 (CXCR-4), and others [91]. Moreover, TGF-β, which is largely released during bone resorption, stimulates breast cancer cells to produce osteolytic factors, including IL-11 and PTHrP [26]. It has also been reported that the inhibition of TGF-β type I receptor (TBRI) kinase accelerates OB differentiation by priming both Runx2 and ephrin(Eph)B4 expression, while downregulating OC maturation through EphB2 expression as an OC inhibitor [76]. Additional studies have also demonstrated that the blocking of the TGF-β pathway attenuates the development of bone metastasis from breast cancer cells [26], while Takeuchi and co-workers proved that its inhibition resulted in the restraining of bone lytic lesions in a model of MM bearing SCID-rab mice [113].

Polyphenols

Polyphenols have been reported to exert antitumor properties and their effects have been exhaustively evaluated on a bone metastatic variant of a breast cancer cell line, namely MDA-MB-435. In fact, their inhibitory activity has been proven on both the growth of the primary breast tumor and the progression of metastatic cancer in the skeleton. Their antitumor activity seems to be supported by pharmacodynamic mechanisms related to modifications of the tumor microenviroment within the bone rather than to their direct suppression of the tumor [16]. Furthermore, it has also been shown that resveratrol inhibits, at least partly, OC differentiation by impairing RANKL signaling and concurrently promoting OB differentiation by upregulating the nuclear receptor of 1,25(OH)2D3 [9]. Taken together, these findings strongly suggest that the polyphenols can be useful in treating bone metastases as well as bone disease in MM.

Cytotherapy

Transplantation of mesenchymal stem cells (MSCs) in patients with osteogenesis imperfect leads to an increase in bone mineral density with a reduction of bone fractures [49]. In view of these results, an analogous model of cytotherapy has also been proposed for myeloma bone disease. Intrabone injections of MSCs in a SCID-rab model of MM promotes bone formation through activation of endogenous OBs and suppression of OC activity. Moreover, it has been reported that by restoring OB maturation, MSCs also delay myeloma cell growth in bone [65]. Further studies are required to improve our understanding of MSCs/tumor interactions [58].

Integrins Inhibitors

Integrins are a family of cell surface receptors that primarily mediate interactions of normal cells with components of the extracellular matrix. They form heterodimeric transmembrane receptors consisting of noncovalently associated α and β subunits. Several tumor cell types express an abnormal integrin profile compared to nontumor cells [20, 23] providing an opportunity for specific targeting. Targeting integrins on both tumor and/or host cells has proven to be effective not only in blocking local cancer progression, but also in reducing tumor cell detachment from their primary site in preclinical models [56, 89]. Although OCs express various integrins, it is now well accepted that integrin avb3 is a central molecule for OC function [29]. For the treatment of skeletal metastasis, the αvβ3 integrin has become an attractive target because of its expression in tumor and angiogenic cells, its role in OC differentiation and function and its role in tumor cell homing to bone [86, 106, 112]. There is preclinical evidence that αvβ3 integrin-targeting drugs, including peptides (S247, ATN-161, cilengitide) and non-peptidic small molecules (PSK1404), successfully block osteolysis and tumor growth in animal models of bone metastasis [4, 20]. There are several ongoing clinical trials evaluating the anticancer effect of integrin antagonists in advanced refractory and metastatic cancers but only a few integrin antagonists are being evaluated in patients with bone metastatic cancer. Clinical trials of function-blocking antibodies are also ongoing, including Vitaxin (LM609), a humanized monoclonal IgG1 antibody against the extracellular domain of the αvβ3 integrin heterodimer. Vitaxin had substantial anti-angiogenic effects in preclinical models and has shown direct antitumor effects as well as impaired bone resorption by inhibiting OC attachment to the bone surface [38].

Another integrin involved in bone tropism is a5b1, a specific receptor of fibronectin, often up-regulated in tumor cells that undergo epithelial–mesenchymal transition (EMT) mainly stimulated by tumor growth factor β (TGF-β) [71]. a5b1 expression on leukemia, prostate and breast cancer cells facilitates their interaction with bone marrow stroma. Thus, therapeutic targeting avb1 would be particularly promising for the treatment of advanced cancers associated with skeletal lesions as potential inhibitor of bone colonization. Volociximab is a chimeric monoclonal antibody that blocks the a5b1 receptor and it has been, currently, evaluated in two phase II clinical trials in the treatment of metastatic renal cell carcinoma, in advanced epithelial ovarian cancer, melanoma, NSCLC and pancreatic cancer (NIH clinical trials database: http://clinicaltrials.gov/).

Cathepsin K inhibitors

Cathepsin K (cathK) represents the key enzyme responsible for osteoclastic bone resorption actively participating in the process of bone turnover. This cysteine protease plays a key role in bone matrix degradation and appears to be a limiting step in osteoclastic bone resorption [35].

Until now, a role for cathepsin K in bone metastasis has been mainly attributed to its ability to effectively degrade native collagen I, a process necessary for the expansion of tumour within the bone. These evidence suggest that inhibition of cathepsin K may disrupt two processes essential to the development of bone metastases: cancer cell invasion and OC-mediated bone resorption. Hence the role of cathK in bone resorption makes it an attractive therapeutic target in the treatment of those disorders involving bone loss, such as osteoporosis and bone metastasis.

Nowadays, several small molecule inhibitors of cathepsin K have been developed; due to its selectivity, Odanacatib is the only cathepsin k inhibitor in clinical development [36]. A Phase II controlled study on women with breast cancer metastatic to bone randomized to receive daily administration of odanacatib or zoledronic acid showed bone remodeling markers reduction (urinary NTx) after 4 weeks treatment [53]. Despite these promising results, two phase III trials of odanacatib that were initiated in breast and prostate cancer patients with MBD were closed before their completion and no further evaluation is ongoing in the oncology setting. However, odanacatib is still under investigation in phase II–III trials for the treatment of osteoporosis.

WNT Signaling Pathway Modulators

Wnt proteins bind Frizzled receptor family members and, in association with low-density lipoprotein receptor-related protein (LRP)5/6, trigger downstream signaling via β-catenin, which induces activation of different genes involved in osteoblastogenesis [5, 43]. DKK-1 binds to LRP5/6 and blocks the interaction with Wnt-1, resulting in b-catenin degradation and inhibition of OB differentiation. Wnt signaling in OBs upregulates OPG expression and downregulates RANKL expression [44], suggesting a mechanism by which Wnt signaling in OBs indirectly regulates osteoclastogenesis.

Data from several tumor types suggest that DKK1 promotes osteolytic metastases, and may facilitate the conversion of osteoblastic metastases to an osteolytic phenotype [45].

Elevated levels of DKK-1 were first described in the serum and bone marrow of patients with multiple myeloma. The blockade of DKK-1 using neutralizing antibodies resulted in a decrease of both osteolysis and skeletal tumor growth in a severe combined immunodeficiency (SCID)-hu murine model of multiple myeloma. Moreover, DKK-1 antibody treatment led to a significant increase in OB number, serum human osteocalcin level, and trabecular bone, indicating that this antibody had bone anabolic effects [129]. A clinical trial combining the DKK1-neutralizing antibody BHQ880 and zoledronate in relapsed/refractory myeloma patients is currently ongoing (NCT00741377).

Further studies are therefore required to examine the importance of DKK-1 as a therapeutic target for cancer bone metastasis.

Endothelin Receptor Antagonists

Endothelins (ET-1, ET-2, ET-3) are a group of 21-amino acid peptides that are produced in a variety of tissues, where they act as modulators of vasomotor tone, nociception, cell proliferation, and hormone production [81]. Although ET-1 can act alone as a mitogen for a number of cancers, its effects are greatest as a comitogen with a variety of growth factors. Two endothelin receptors, ETA, and ETB, are found in humans. ETA primarily binds with ET-1 and ET-2, while ETB binds equally with all three endothelins [52].

Prostate cancer cell lines are characterized by loss of ETB and increased ET-1 levels, which inhibit apoptosis through an interaction with ETA [37]. The ETA receptor is expressed to a greater degree in high-grade as compared to low-grade prostate cancer and in men with bone metastases [37]. ET-1 is thought to alter the balance of osteoblasts and osteoclasts to favor the new bone formation that is characteristic of prostate metastases [81], and also to mediate metastasis-related bone pain [63].

The orally-active ETA receptor antagonists atrasentan and zibotentan have been extensively studied in advanced prostate cancer.

However, phase III trials did not demonstrate a statistically significant benefit from atrasentan, also when in combination with docetaxel [15, 82, 88].

Similar resuls were obtained phase III trials that were drawn to investigate the efficacy of Zibotentan in men with castrate-resistant prostate cancer and bone metastases. Phase III trials in patients with non-metastatic castrate-resistant prostate cancer (NCT00626548) and in combination with docetaxel (NCT00617669) are ongoing [84]. The studies cited have been published, moreover these trials have recently been evaluated by a meta-analysis, thus the bibliographic voice should be referred to this meta-analysis.

Systemic Radionuclide Therapies

Men with extensive multifocal painful bone metastases and those with persistent or recurrent pain despite receiving external beam RT to maximal normal tissue tolerance may achieve palliation of their symptoms by treatment with bone-targeted radioisotopes.

A prerequisite for treatment with these radioisotopes is the presence of uptake on bone scan due to metastatic disease at the sites that correlate with pain. Although these radioisotopes are appropriate for other histologic tumor types with osteoblastic bone metastases, they are mainly used in men with advanced prostate cancer and women with breast cancer since these cancers are often characterized by a high ratio of bone to soft tissue metastases.

Two classes of bone targeted radioisotopes have been used: beta emitters and alpha emitters.

The beta emitters samarium-153 and strontium-89 are approved for use in men with metastatic prostate cancer. Retreatment with radioisotopes such as samarium-153 is feasible if clinically indicated, once bone marrow toxicity has resolved. The alpha-emitting radioisotope radium-223 (Alpharadin**)** may offer significant advantages because of its more localized deposition of radiation. Radium-223 has been demonstrated to improve survival and decrease skeletal-related events but is not yet approved for general use [99].

In a randomized phase III trial it was evaluated radiopharmaceuticals and zoledronic acid in the palliation of osteoblastic metastases from lung, breast, and prostate cancer. The result were presented at the last ASCO meeting: the addition of Sr89 or Sm153 did not result in a difference in SREs, OS, or QOL [102].

Myeloma Bone Disease (MBD): Approaches of Target Therapy

MM is part of the trio, with prostate and breast cancers, that promotes severe skeletal devastation. MBD is characterized by osteolytic lesions that rarely heal while usually producing severe SREs such as pathologic fractures, spinal cord compression and chronic bone pain, resulting not only in severe morbidity but also in a dramatic increase in mortality. In contrast with other osteotropic cancers where bone destruction is accompanied by local new bone formation to tentatively repair the skeleton lesions, MBD represents a typical osteolytic disease with concurrent reduction of OB function in terms of both OB numbers and repairing activity. In this context, the bone microenvironment (BM) plays a major role in the deregulation of the OB–OC axis, including the interactions between stromal, endothelial, immune, and bone cells and the extracellular matrix components such as osteopontin and fibronectin. All these components constitute a neoplastic unit which supports both tumor progression and the related bone destruction [127] (Fig. 18.1).

MM plasma cells (PCs) act by increasing the expression of osteoclastogenic factors such as RANKL, MIP-1α, IL-3 and stromal-derived factor (SDF)-1. Moreover, MMPCs disrupt Wnt-regulated OPG expression by OBs while, by the expression of the heparan sulfate proteoglycan, syndecan-1 (CD138), they disactivate OPG through its internalization and subsequent lysosomal destruction, resulting in an imbalance of the RANKL/OPG ratio [110].

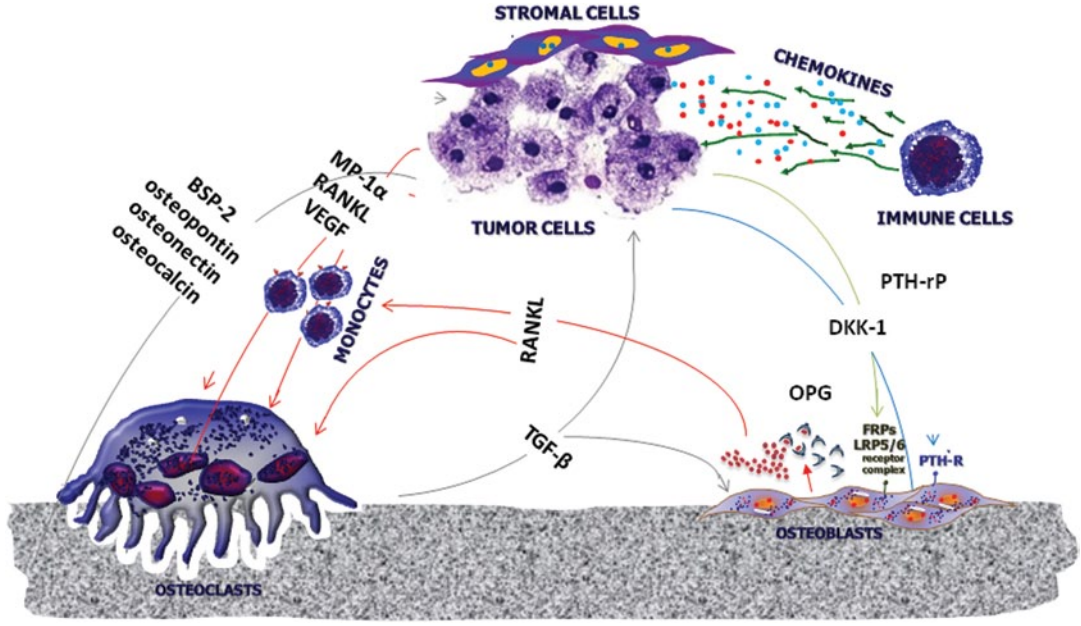

Fig. 18.1 The drawing illustrates the pathogenesis of osteolytic metastasis. The interplays between tumor cells and bone marrow microenvironment, such as stromal cells and immune cells, play a major role in deregulation of OB–OC axis. Moreover, the resorption of mineralized matrix by osteoclast releases chemotatic and adhesive factor for cancer cells. At the same time, tumor cells directly stimulate osteoclastogenesis while inhibiting OB maturation with a reduction of their anabolic activity and a concurrent increase of receptor activator of nuclear factor-kappaB ligand (RANKL) secretion

MIP-1α is a chemokine secreted by OCs and MMPCs that stimulates osteoclastogenesis independently from the OPG/RANKL/RANK pathway and promotes chemotaxis and survival of MMPCs [46]. Both bone marrow expression and serum levels of MIP-1α correlate with bone disease extension and survival in MM patients.

Interestingly, MMPCs, besides stimulating OC pathways, may also directly contribute to bone resorption. Several authors have demonstrated that bone-resorbing osteoclasts from MM patients include nuclei with translocated chromosomes of myeloma cell derivation, in addition to nuclei without these translocations [2], whereas studies from our group showed that, under certain conditions, MMPCs may acquire the OC-like phenotype as multinucleated cells (Fig. 18.2a) expressing TRAcP that produce erosive pits (Fig. 18.2b) on experimental bone substrate [104, 105].

MMPCs suppress osteoblastogenesis by direct cell–cell contact as well as by the production of soluble factors. The contact between MMPCs and mesenchymal stromal cells is mediated by both the cell surface molecule very late antigen-4 (VLA-4) and the vascular cell adhesion molecule-1 (VCAM-1) resulting in down-regulation of the OB transcription factor RUNX2 [93]. Furthermore, soluble OB inhibitors are secreted by MMPCs and by cells of the MM marrow microenvironment. They include: Dickkopf-related protein 1 (DKK1); secreted frizzled-related protein 2, IL-7, hepatocyte growth factor (HGF) and IL-3 (Yaccoby 2010). In particular, DKK1 is a soluble inhibitor of WNT signaling that binds to low-density lipoprotein receptor-related protein (LRP) 5/6 and prevents interaction with Wnt-1, thus inhibiting OB differentiation. Elevated levels of DKK1 have been described in the serum and bone marrow of MM patients, particularly in those with diffuse osteolytic lesions, thus suggesting the inefficacy of OB function [32]. Although mesenchymal stromal cells have been reported to interact with MM tumor growth, ma-

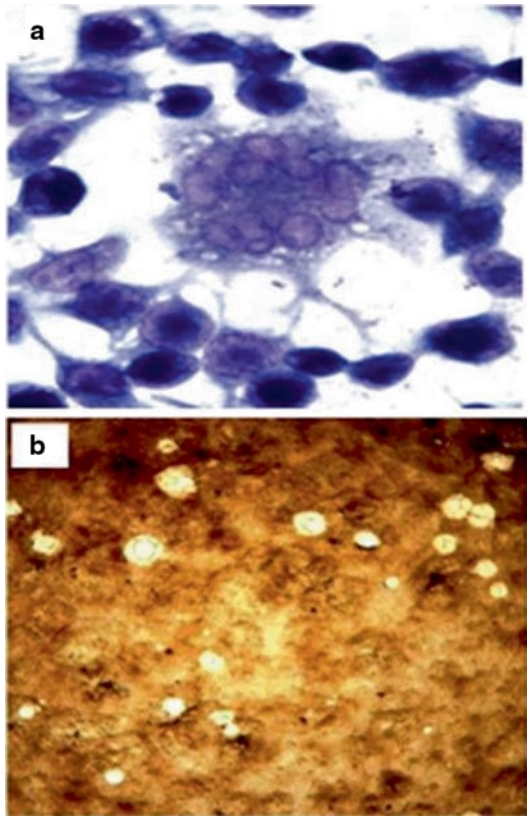

Fig. 18.2 **a** Myeloma cells polykarions generated from cultures of the U-266 cell line. The spontaneous formation of polykarions was observed after 3–4 weeks of cultures with a 10% FCS supplemented RPMI. **b** Experimental bone resorption by U-266 cells. The cells were removed after 9 days of incubation and the substrate of calcium phosphate was inspected by light microscopy after von Kossa. The erosive lacunaes on calcium phosphate depict the bone erosive capability of these cells

ture OBs produce smaller amounts of IL-6 and apparently prime MMPCs to undergo apoptosis by cell-cycle arrest in the G1 stage, at least partly through the expression of small leucine-rich proteoglycans such as decorin and lumican [128]. Therefore, the restoration of OB function in MM patients may result in a double effect, namely the impairment of the anabolic activity of bone and the concomitant inhibition of tumor growth.

Like for other osteotropic cancers, bisphosphonates (BPs) represent the standard of care for MBD. Since their advent, BPs have dramatically changed the evolution of MM. Before the "BP era" SREs occurred in approximately 75% of MM patients whereas with second generation BPs such as PAM and ZA their occurrence has dropped to 25% [6, 95]. These compounds have been also shown to display anti-MM pleiotropic effects including the activation of apoptosis in MMPCs, inhibition of tumor-associated angiogenesis and improvement of anticancer response by Vγ9Vδ2 T cells [75]. In agreement with these data, a study enrolling 1960 MM patients showed that ZA, in association with anti-MM drugs, not only reduced the frequency of SREs, but also increased OS [79]. However, BPs also induce adverse effects such as kidney failure and osteonecrosis of the jaw (ONJ) [12, 123] and since MM patients are constitutively at risk for kidney failure, alternative therapeutic approaches are needed for patients ineligible to receive BPs [69].

In a recent double-blind study, Denosumab was evaluated in comparison with ZA in patients with MM or advanced cancers and bone metastases, excluding breast and prostate cancers. Denosumab resulted non-inferior to ZA in preventing or delaying SREs with ONJ occurring at similar rates in both groups. However, treatment with Denosumab resulted in lower rates of renal failure and acute-phase reactions than ZA [48].

MIP-1α targeting by neutralizing antibodies or antisense strategies reduces the tumor burden and inhibits the development of osteolytic lesions in the murine 5TGM1 model of MBD [85], whereas MLN3897, a small molecule antagonist of the CCR1 which has been developed in clinical trials investigating certain immunological diseases, inhibits OC differentiation by down-regulating cell-fusion and c-fos expression, and abrogates the proliferative advantage of MMPCs mediated by OCs [117]. Future trials targeting the MIP-1a pathway are necessary to assess whether these results are capable of clinical application.

The blockade of DKK-1 by neutralizing antibodies showed both restoration of bone mineral density and reduction of tumor burden in a (SCID)-hu murine model of MM [129]. A phase I–II clinical trial combining the human IgG1 anti-DKK1 BHQ880 with conventional chemotherapy and ZA in relapsed/refractory myeloma patients is ongoing.

The important contribution of BM to MM progression may account for the efficacy of treatments targeting both the bone microenvironment and the tumor, such as the use of bortezomib and the immunomodulatory drugs. Bortezomib, a 26S proteasome inhibitor, downregulates the Nuclear Factor-KappaB (NF–KB) pathway, and reduces MM/BMM interplays by restraining the tumor burden and the progression of MBD. It disrupts accelerated OC differentiation by inhibiting *p*38 protein kinase, NF-KB and activator protein 1 (AP-1) signaling, while promoting OB differentiation by the stabilization of β-catenin in osteogenic cells, increasing bone morphogenetic protein 2 level in the bone marrow microenvironment and blocking the degradation of RUNX-2 [120]. These effects have been clinically confirmed with the reduction of bone resorption markers and the increase of bALP and osteocalcin serum levels in MM patients responsive to Bortezomib [114]. Immunomodulatory drugs, such as Thalidomide, Lenalidomide and Pomalidomide, in addition to their antitumor effect, restrain MBD progression by targeting BMM. It has been demonstrated that Lenalidomide downregulates PU.1, an early transcription factor implicated in OC differentiation, and inhibits RANKL secretion by marrow stromal cells while downregulating Cathepsin-K [11].

Conclusion

The coupling of bone resorption and bone formation ensures that the removal of the mineralized matrix is replaced by an equivalent quantity of new bone. Skeletal metastases alter this equilibrium, which, during disease, can shift from an excess of bone resorption to an excess of bone formation. Bone metastases can have phenotype heterogeneity in lesions from the same patients or even within a single lesion. Therefore, agents that are able to reset the balance between deregulated bone resorption and formation may represent the "magic bullet" for bone metastases. At present, BPs still remain the standard of care for the treatment of skeletal metastases because of their ability in reducing the number of SREs and delaying time to the first SRE. Nevertheless, there are limited data on the optimal duration of treatment and BPs are necessarily discontinued in patients who develop ONJ or kidney failure. Alternative therapeutic approaches are thus needed for patients ineligible to receive BPs in the presence of novel SREs.

Several drugs which target the OC and the OB pathways such as the RANK/RANKL/OPG axis, or molecules such as cathepsin K, SRC, WNT, TGF-β, Activin-A, and others, have been tested with encouraging preliminary data from preclinical and clinical studies. In particular, denosumab has been recently approved for breast and prostate cancers. Interestingly, cytotherapy approaches, previously utilized in regenerative bone treatments of several metabolic and traumatic bone diseases, appear efficient in repairing bone loss in a mouse model of MBD and are a fascinating prospect for bone osteotropic cancers. New clinical trials should thus be aimed at evaluating novel combinatory approaches involving conventional BPs as well as new OC inhibitors and other drugs capable of restoring normal OB function.

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Index

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