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Therapeutic Approaches in Cancer Treatment

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Therapeutic Approaches in Cancer Treatment

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Dedications

This book is dedicated to all patients suffering from cancer and their families who are going through the pain, and to all physicians, oncologists, cancer researchers, and their families, who are working tirelessly and spending every bit of their time to save or reduce the pain of the cancer sufferers.

Foreword

Cancer treatment has come a long way in the past century, with surgery, chemotherapy, and radiotherapy being the most used modalities. While these treatments have been successful in curing many cancers and controlling tumor growth, their effectiveness decreases once the cancer metastasizes. As a result, the concept of combined therapy was introduced, resulting in significant improvements in patients' health outcomes and cancer control. However, the expected level of patient survival has not yet been achieved. Ongoing research into different tumor characteristics has led to a better understanding of cancers and the development of new treatment approaches. These include immune-mediated therapies, hormonal therapies, biological molecules, gene therapy, oncolytic virotherapy, and nanoparticle-based therapies. Despite these advances, the mortality rate of cancer has not decreased as much as hoped, and the expected level of therapy has not yet been reached to improve patient survival times. The book "Therapeutic Approaches in Cancer Treatment," edited by Dr. Asma S. Qazi and her Co-editor, provides valuable insights into the latest developments in cancer treatment and will be a valuable resource for anyone interested in this field. In this book, various treatment modalities are discussed with their respective effectiveness and potential side effects. This book is a comprehensive guide to the latest therapeutic approaches in cancer treatment. The modalities covered include radiotherapy, immunotherapy, hormonal therapy, surgeries, chemotherapy, personalized medicine approaches, nutritional therapy, gene therapy, and viral therapy.

The editor of this book, Dr. Asma S. Qazi and her Co-editor, are experts in the field of cancer research and treatment. Their vast experience and knowledge are evident throughout the pages of this book, making it an essential resource for anyone involved in cancer treatment. The chapters are written by leading experts in their respective fields, providing valuable insights into the latest research and developments. One of the most significant contributions of this book is its emphasis on personalized cancer treatment. The editors and authors highlight the importance of tailoring cancer treatment to individual patients, taking into account their unique genetic and environmental factors. I had the pleasure of working with Dr. Asma S. Qazi in the past, and their commitment to cancer research and treatment is inspiring. Their passion for helping patients and advancing cancer treatment is evident in this book, making it a valuable resource for anyone involved in cancer care. I encourage anyone interested in cancer treatment to read

this book and reflect on its contents. It is my hope that it will inspire new ideas and approaches to cancer treatment, ultimately leading to better outcomes for patients. Towards the end, I would like to congratulate Dr. Asma Qazi and her team, for their contribution to cancer research and treatment. This book is a testament to their expertise and dedication to the field.

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Preface

This book is a unique blend of structured information covering a broad area relevant to cancer therapeutics.

Cancer incidence is growing at a faster pace than the improvement in available facilities for cancer treatment. To address this holistic approach and to sensitize all the stakeholders of this cause including physicians, researchers, and patients, the information is contributed through this book, in which all the best possible treatment modalities are discussed. This book not only covers the traditional therapeutic methods but also the genomics and proteomics of the cancer, the pharmacogenetics, and the psychological elements involved in cancer treatment along with nutritional assessments for cancer patients. So, in the broad spectrum this book covers all possible elements related to cancer treatment.

This book comprises fourteen chapters covering cancer genomic approaches for cancer treatment, traditional modalities, chemotherapy, radiotherapy, hormonal therapy, immunotherapy, oncolytic virotherapy, which is another emerging field. Furthermore, it also covers targeted therapies in personalized medicine, which is again an emerging yet promising field for cancer treatment. Use of nanocarriers in the cancer treatment is another important aspect which is a developing thrive. This book is also unique about in covering psychological elements in cancer treatments and providing knowledge for nutritional assessments in cancer treatment. The book has very good text highlighting the important features with a blend of traditional and emerging treatment modalities. Furthermore, chemoresistance development and challenges it poses in cancer treatment along with the pharmacogenetics of anticancer drug, which is way important for the clinicians to understand for their individual patients, associated toxicities, and clinical responses to a particular drug.

The comprehensiveness and combination of therapeutic approaches have made this book more unique and distinctive. There are many challenges in choosing treatment modalities for cancer patients. The rapid advancements in the field have been mystifying for all those who are involved in cancer therapeutic research and treatments, as both are the curing source for this disease. However, the therapeutic approaches are advancing day by day, and there is always a room for such relevant books with the latest information to fill up the time bank.

Overall, the book is a single compiled version of maximum possible treatment modalities with suitable breadth of coverage of the broad spectrum multisectoral audience of clinicians, researchers, policy makers, and students. There is

a hope that this book will evoke and stimulate resolutions and actions through collaborative efforts of researchers and clinicians toward the betterment of patients.

Islamabad, Pakistan Asma Saleem Qazi

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Contents

Introduction and Overview of Cancer Therapeutics

1

Asma Saleem Qazi

1.1 Introduction

Cancer has been a significant cause of mortality and morbidity both in developing and developed nations worldwide. Early identification of cancer with reliable diagnostic accuracy has still been one of the mainstay challenges of an oncologist clinic. Moreover, the routine laboratory work carried out for assessing cancer progression is equally discomforting for the patient as the disease itself. Cancer is a multifactorial disorder involving complex modifications in the genome affected by the interactions between host and the environment [16]. The major hallmarks include alteration in cell division patterns, uncontrolled replication, apoptosis evasion, sustained angiogenesis, and metastasis [23].

In the past century, cancer was effectively treated with surgery, chemotherapy, and radiotherapy in combination or either alone and thus produces cures and significant impact on tumor growth. But, once it metastasize, the treatment modalities become more complicated. With the passage of time, it was also perceived that individual treatments of surgery, chemotherapy, or radiation were not very effective [41]. The idea of combined therapy was initiated in 1960's with tremendous improvements in patients' health outcomes and cancer control. Although, the required level of expected outcome in terms of patients survival has not been achieved till date. Characteristics of different tumors are continuously under study, yet some pathways and characteristics are determined to create new revolution in understanding cancers and targeting drugs to tumors [16]. Nowadays, immune mediated therapies, hormonal therapies, biological molecules, gene therapy, and oncolytic viro-therapy. Nanoparticles-based therapies are being used to

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some extent. Although the expected therapy level has not been reached to fight cancer by decreasing the mortality rate and increasing the survival time for the cancers.

1.2 Tumor Biology and Cancer Development

When the body cells fall for uncontrolled growth and do not respond to the normal cell signaling cycle, they proliferate and undergo an abnormal condition known as cancer. If this uncontrolled behavior continues, then the cells spread to the other organs which is called as metastasis, a very fatal condition leading to majority of cancer deaths [23].

During mitosis, the normal cells grow relying to different growth factors and exhibit contact inhibition ability that is after reaching a specific threshold the cell stops dividing, but the cancerous cell grow independently of any such growth factors or signals and also lack contact inhibition ability, leading to the formation of unwanted cells [35]. Other than this, normal cells died by apoptosis and replaced by new cells with limited efficacy of DNA replication, whereas cancer cells show high activity of telomerase enzyme that keep replacing worn out ends of telomere allowing uncontrolled cell proliferation [41].

The cancer develops a large mess of cells known as hyperplasia that is formed due to uncontrolled cellular growth. The next step is dysplasia that is the cell growth accompanied with abnormalities followed by anaplasia in which these atypical cells spreads to the limited area of the tissue [15]. Till this stage the tumor is noninvasive and considered as benign. Later at the advanced stages, the tumor cells metastasize and started invading to the neighboring tissues and organs. Tumor cells require oxygen and nutrient for their growth and proliferation like other normal cells, so they develop their own blood vessels in a process called as angiogenesis. If identified earlier, the cancer can be treated [10].

1.3 Types of Cancer and Grades

Usually, they are named by the type of cells they originate.

Carcinomas originated from epithelial cells and constitute the highest ratio among all cancer types. Sarcomas are formed in bones, muscles, and connective tissues. Leukemias are specific to white blood cells. Lymphoma is the cancer of lymphatic system or cells derived from bone marrow [52]. Myelomas are specific to antibodies synthesizing white blood cells.

Cancer grades represent the abnormality in the cells. Grades are increased from 1 to 4 with the increase in the abnormality in the cells. Well differentiated cells belong to low grade tumors. Poorly differentiated cells are highly abnormal and so are included in high grade tumors [2]. To further elaborate the cells, the following are the criteria for grading:

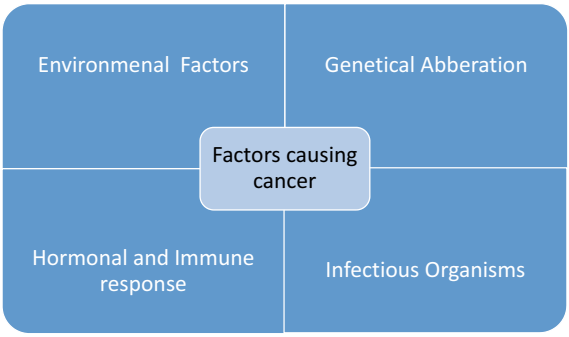

Fig. 1.1 Causative factors for cancer

Grade 1: Well differentiated cells with minor abnormalities.

Grade 2: Bit higher level of abnormality and moderately differentiated cells.

Grade 3: Improperly differentiated cells with highly mutated chromosomes effecting nearby cells and producing harmful chemicals.

Grade 4: Undifferentiated, immature, and primitive cells.

1.4 Factors Involved and Cancer Causes

The initiation and cancer advancement in the body depends on factors like immune conditions, environmental factors, life style, mutations, hormonal imbalance, infectious organism, exposure to radiations and chemicals [23]. Any of these factors may cause abnormal cell growth and uncontrolled proliferation which ultimately lead to tumor and then the spread of these cells to other tissues and organs causing metastasis. Mutations may be in the master genes or tumor suppressor gene that leads to cancer [7]. Abnormal chromosomes replication resulting in the deletion or duplication of entire or partial set of chromosome, defective DNA repair are also considered as one of the cause of cancer spread. Genetic aberration leads to abnormal protein synthesis or modification that may halt the normal protein functioning and thus leading to cancer [44]. At times these changes take months to years to be detectable, also there are several mechanism that involves in cancer development, these factors thus creating it difficult to get cancer diagnosed at earlier stages (Fig. 1.1). However, it is not entirely impossible to detect the cancer at earlier stage.

1.5 Cancer Therapeutic Modalities

Knowing the severity of cancer, extensive research is required to understand the disease diagnosis and therapeutics completely. All the treatment methods which are used to treat cancers still require continuous research to better cope with the

heterogeneity of disease [12]. Along with the basic or bench side research, quality treatment trials are also required. Currently, more than 60% of all ongoing medical treatment trials are concentrating on cancers [12]. Commonly used treatments are surgery, chemotherapy, and radiotherapy which are decided on the type, stage, and locality of cancers [6]. Some of the modern treatment modalities includes hormonal and immunotherapy, antiangiogenic modalities and stem cell therapies, genomic therapy, and oncolytic viro-therapy [15]. Along with these the genetic control, nutritional assessments for cancer patients and psychological treatments all add to the success of the treatment strategy for cancer patients [7, 10]. Further, in this book almost all treatment strategies will be discussed that are helpful in cancer treatment.

1.6 Surgeries

One of the most commonly used modalities is surgery for many benign and malignant tumors. It also has lesser side effects on other associated or neighboring tissues as compared to chemotherapy and radiotherapy. Surgeries can be less invasive or open depending upon localization of tumor, size of tumor removed, patient immune condition, and reason for surgery [50].

During the surgery, entire tumor can be removed or certain specific part can also be removed. Tumor mass can also be debulk and ease the tumor pressure and pain on the certain area. Surgery also improves the chances of successful chemotherapy [9, 24].

During open surgery, a cut is made to remove the tumor mass along with some healthy tissues and lymph nodes to ensure complete removal of tumor mass. While in invasive surgery, a small incision is made, and a thin tube with camera is inserted in the body to see the tumor in detail. With the help of images from the camera, the surgeon decided about the removal of tumor mass using specialized surgical tools [50].

1.7 Chemotherapy

The cancer treatment with chemotherapy dates back to 1930s and was introduced by Paul Ehrlich, a German scientist, while working with alkylating agents. During the World War I and II, the soldiers, exposed to mustard gas, experienced decreased level of leukocytes count which was treated by Gilman in 1943 by nitrogen mustard as a first chemotherapeutic agent to treat lymphomas [1]. These were highly electrophilic in nature and can react with cellular nucleophiles, adding alkyl groups to DNA bases, resulting in cancer cell death. At the same time, another class of antibiotics called antimetabolites (e.g., aminopterin and amethopterin) that interfere with folate synthesis or mimic DNA precursors, halting DNA replication leading to death of cancer cells [20]. After 1948 these antimetabolites were used to treat leukemia in children. Later, cyclophosphamide and chlorambucil were

synthesized to treat cancers. In 1951, 6-thioquanine and 6-mercaptopurine were developed by Elion and Hitchings for treating leukemia [18]. Similarly, a new drug was developed by Heidelberger for solid tumors, 5-fluorouracil (5-FU), which is used as chemotherapy agent against colorectal, head, and neck cancer until now [13]. Prior to chemotherapy, the surgical removal was the only choice to treat tumors.

The national service center for cancer chemotherapy was established in 1955 for testing cancer drugs [42]. At that time monotherapy with corticosteroids was the choice to treat cancers, and the first cancer cured was choriocarcinoma in 1958 [34]. By 1960s, new trends of chemotherapeutic agents with alkaloids from vinca and ibenzmethyzin (procarbazine) were applied to leukemia and Hodgkin's disease [30].

The principal of chemotherapy is controlling tumor progression by halting the cell division and enforcing apoptosis majorly by genotoxic effect that is by production of reactive oxygen species [5]. Tumor cells continuously grow without apoptosis and with very high ratio of cell proliferation and cell death, however, it also effects the normal cells and has some side effects on the body. Out of total chemotherapeutics currently use, only 132 are approved by FDA. These cells also damage the normal body cells [47]. Chemotherapy is mainly systemic and can be used separately and in combination therapies [5, 16]. Mode of their action, the chemical structure, homology, and composition are the main factors while choosing and following up of chemotherapeutic agents. While starting the therapy order of drug given, time for which it is given and dosage that is administered are very crucial steps [47].

1.8 Chemotherapeutic Agent

Alkylating agents cause direct DNA damage by halting cell division. The major treating cancers using alkylating agents are leukemia, myeloma, lymphoma, sarcomas, and Hodgkin's disease along with ovary, breast, and lung cancers [2, 3], The side effects include damage to bone marrow and rarely causing acute leukemia after 5–10 years of treatment. Based on similarities in mode of action platinum drugs are also included in alkylating agent's family with reduced chances of causing leukemia after treatment. Some examples are

- Platinum drugs (cisplatin, carboplatin, and oxaliplatin)
- Alkylating agents are nitrogen mustard (mechlorethamine, chlorambucil, cyclophosphamide, ifosfamide, and melphalan)
- Alkyl Sulfonate (busulfan)
- Trizines (dacarbazine, temozolomide)
- Ethylenimines: thiotepa and altretamine
- Nitrosoureas (streptozocin, carmustine, lomustine).

Another class is **antimetabolites** which are analogs for the unit of DNA and RNA and halt their growth specifically effecting the S phase of cell cycle [28]. They are used for treating leukemia, ovary, breast, and intestinal cancers. Few examples of this class includes

- 5-Fluorouracil (5-FU)
- 6-Mercaptopurine (6-MP)
- Capecitabine
- Cladribine
- Clofarabine
- Cytarabine
- Floxuridine
- Fludarabine
- Gemcitabine
- Hydroxyurea
- Methotrexate
- Pemetrexed
- Pentostatin
- Thioguanine.

Anthracyclines are another important class of chemotherapeutic agents that target DNA replication enzymes, effecting all phases of cell life cycle. Different tumors are treatable with this class but with a limitation of damaging the heart permanently, by inhibiting the topoisomerase II, leading to post treatment acute myelogenous leukemia, after 2–3 years in most cases if dosage limit reaches the upper level [46]. These are

- Daunorubicin
- Doxorubicin
- Epirubicin
- Idarubicin.

Other than anthracyclines, mitoxantrone is an anticancerous antibiotics which is comparable to doxorubicin due to their similar mode of action and effect on heart damage at high dosages [28].

Topoisomerase inhibitors, another class of chemotherapeutic agents that unwind the DNA and stop its replication. Both topoisomerase I and II have their inhibitors separately like topotecan and irinotecan for topoisomerase I and etoposide, mitoxantrone and teniposide for topoisomerase II [46].

Other naturally derived inhibitors such as plant alkaloids act as **Mitotic inhibitors** because they halt cell division at mitotic phase of cell cycle by inhibiting the protein synthesis. Lungs, breast, ovaries, lymphomas and leukemias can be treated with them [46]. With the high dosages, this class of chemotherapeutic agents can damage peripheral nervous system. Some examples are

- Taxanes: paclitaxel and docetaxel
- Epothilones: ixabepilone
- Vinca alkaloids: vinblastine, vincristine, and vinorelbine
- Estramustine.

There are some chemotherapeutic agents like L-asparaginase, bortezomib (proteasome inhibitors) which have uncommon mode of actions and are included in uncategorized chemotherapeutic agents [28, 43].

1.9 Angiogenesis Inhibitors

These inhibitors works differently by blocking the development of blood vessels inside the tumor tissue. Tumor cells require separate blood supply to fulfill their nutrition requirement and growth. Angiogenesis inhibitors do not kill the tumors rather starve them and block their growth resulting shrinkage of tumor mass by preventing new blood vessels to form [48]. Chemotherapeutic agents kill normal cells as well but angiogenesis inhibitor do not kill normal cells but they need to be administered for the longer time periods. Usually, they inactivate VEGF receptors by binding to them and ultimately the receptors to stimulate growth of new vessels around tumor mass. Some examples are thalidomide, interferon, bevacizumab (Avastin), cilengitide (EMD 121,974), and cediranib (Recentin VB-111) [11, 46].

1.10 Radiotherapy

In late nineteenth century, Becquerel and Rontgen had discovered X-rays which were beneficial for radiation treatment. The first cancer case was cured using radiation in 1898. Later, the progress was made in the procedure gradually enabling scientists to use rotational linac radiotherapy known as "Clinac6" in which these charged physical particles are propelled to move through a vacuum tunnel called linear accelerator or linac [4]. The development of modern computers enabled three-dimensional X-ray therapy like intensity-modulated radiation therapy (IMRT) using mapping information from Computed Tomography (CT) scans. Marie Curie research in radium introduces radiotherapy in medicine. Now it is a separate specialized field in cancer treatments [19].

Radiotherapy is linked to the use of physical particles like electrons, protons, and different ions following the mechanism that high energy radiations alter and stop the process of cell division and proliferation by damaging the genetic material. It can also shrink the tumor growth if given prior the surgery [8]. Also, it can shrink the left over tumor cells after surgery, thus reducing the relapse of cancer.

As mentioned, the physical particles are charged electrically in biological bodies upon incidence, and energy is transferred from the rays to the body cells through which it passes [4]. These radiations either can directly kill the cancer cells

or genetically alter them to accede to apoptosis and cell death. The mechanism involved in genetic alteration with radiation lies in the fact that damaged DNA do not replicate, halting cell division and proliferation, resulting in cell death [19].

During treatment the major adverse effect of radiation therapy is that it also effect the normal cell growth in the body, especially those lying close to the main tumor mass [49]. However, cancer cell lacks effective repair system as compared to normal cells that minimizes the net damage done as a result of radiation therapy.

1.11 Radiation-Based Surgeries

Gamma Knife Systems are not actual surgeries yet they use gamma beam light emission for treating tumors and sores. The radiation beam combines to concentrate on tumor cellular mass giving high radiation doses on the affected area without any incision [31]. **Stereotactic Surgery** ionizing radiations at high doses are focused and concentrated on the damaged area in the body non-invasively [31]. The focusing of radiations is very important and crucial otherwise the adjacent normal tissues to the tumor area also gets effected [25]. This technique is advisable for brain tumors especially when the surgeries are considered unsafe for patients. **Linear Accelerator Systems (LINAC)** are the one which utilize high energy X-rays for cancer treatment like Cyber Knife®, X-Knife®, Novalis®, and Peacock® [25]. Another method is **Proton Beam Therapy** that utilizes radiation beams and is considered as molecular radiation treatment. It includes X beams/gamma beams and particles like proton and neutrons [21].

1.12 Techniques Used in Radiation Therapy

Fractionation is based on the radiobiological difference between normal and cancer cells, as normal cells have better tendency to regrow and repair the damage by only sub-lethal dosage of radiation. **3D Conformal Radiotherapy** includes CT scan-based 3D radiation therapy, which is now one of the primary detection method for cancer masses in the body [22]. Another one is **Intensity Modulated Radiation Therapy** that utilizes inverse planning software for modulating radiation intensity during therapy. The irregular intensity dosage targets the tumor mass differentially and shrink their size gradually [38]. **Image Guided Radiotherapy** is a technique that helps in positioning the radiation correctly away from the critical body organs and toward the tumor mass avoiding incorrect aiming and thus damaging the nearby cells [39].

1.13 Hormonal Therapy

Recent advances have revealed that hormones play crucial role in growth and proliferation of cells. Hormonal disturbances cause malignancy in nearly 25% males and 40% females. Unlike chemotherapy, hormonal therapy causes no cytotoxicity; hence, very less side effects are associated with it (EBCTCG) [17]. This therapy can be a better choice while treating lymphoma, multiple myeloma, and leukemia. If given before the chemotherapy treatment, it can mitigate the hypersensitivity caused by chemotherapy, and if given after chemotherapy, it can relief the nausea and vomiting in the patients [46].

1.14 Immunotherapy

It includes the treatment with antibodies, dendritic cells, vaccines, and cytokines. It has added new dimensions to clinical practices. This treatment method is more specific, efficient, less toxic, and with minimal side effects. Immunotherapy kills tumor cells either directly or indirectly by activating human immune system against the tumor cells [32]. Other treatment methods like surgery, chemotherapy, and radiotherapy also affect the healthy cells of the body. However, antibodies are specific and show a very less toxic effect. This property has also encouraged the production of therapeutic antibodies [4]. The very first evidence was dated back to 1982 with a lymphoma patient while treating with mouse mAb directed against B lymphocytes. However, it is also revealed that some have developed anaphylactic reactions when administered repeatedly [4]. The larger size of antibodies causes immunogenicity along with differences in glycosylation pattern between murine and human Abs, leading to cessation of antibody use in therapy. Development of complete human Abs is harder to develop yet required to avoid immune rejection [26].

1.15 Nanostructure-Based Therapeutics

From past two decades, the nanostructure-based therapeutics and diagnostic agents have been introduced in cancer treatment [33].

The main purpose for this therapy is to deliver a therapeutic moiety to treatment site that is tumor cells, depending upon the required pharmacokinetics, in a controlled manner with reduced side effects and drug resistance [51]. Nanostructured materials may also be used to detect cancer cells based on their associated biomarkers.

The main advantages of these structures are ability for specific size synthesis and penetration to tumor cell surface. They can overcome physiological barriers, target tumor specific cell markers, increase plasma half-life of chemotherapeutic drug, drug protection from biological degradation and synthesis of multifunctional platforms for combined therapeutic applications (theranostic nanoparticles) [4].

The nanoparticles can be coupled with biological agents like folic acid. The major concerns while designing nanostructures are heterogeneous distribution of reactants, insufficient mixing, variations in their physicochemical characteristics, and various post-synthesis purification steps. Adaptive immune response is another major concern with the repeated application of nanoparticles [12, 29].

1.16 Miscellaneous Treatment Modalities

Hyperthermia or thermotherapy is one of the form of cancer treatments in which body tissues are heated at high temperature almost equal to 113 °F to damage and kill cancer cells with very little or no damage to the normal body cells [27].

Another form is photodynamic treatment for cancer in which a drug called a photosensitizing agent is activated by a laser light or light emitting from LEDs to kill cancer cells. The treatable cancers are pancreatic, esophageal, lungs, and non-melanoma skin cancers [14].

In gene therapy, new genes are introduced into cancer cells or their surrounding tissues to cause cell death or slow down the cancer cell growth. It is a flexible technique in which wide range of genes can be introduced in cells using vectors to respond to various chemical or physical stimulants either internally or eternally to deliver therapeutic gene to the cell nuclei [45].

Various gene therapies, hyperthermia, and photodynamic treatments use engineered nanomaterials. They can be used in isolation or in combination to treat cancers.

Cancer development is also associated with viruses, and almost 15% of malignancies are associated with oncogenic viruses like human papillomaviruses, Epstein–Barr viruses, herpes virus, and hepatitis B & C virus [36]. Recent studies have revealed that related studies of these causative agents will help in understanding initiation and spread of cancer in the body [37].

Stem cell therapy is one of the options for cancer treatment as these are the undifferentiated cells in bone marrow and has the ability to differentiate into any type of cells in the body [40]. This therapeutic method is still under clinical trials. Mesenchymal stem cells are currently under use in these trials [46].

1.17 Conclusion

Cancer is still a threat to human lives and still it has high prevalence in human population. It involves complex alterations in morphological and physiological condition of the body. It is still a challenge to find a complete remedy, yet there are different treatment modalities used for curing cancer. Some are effective in certain type and stage of cancer yet some still have few side effects.

Throughout this book different treatment modalities are discussed with their effectiveness and side effects. Radiotherapy, immunotherapy, hormonal therapy, surgeries, chemotherapy, personalized medicine approach, gene therapy, viral

oncotherapy, nutritional assessments, psychological care for cancer patients, nanostructure practices, and some others.

However, it is much clear that fighting cancer will not be very easy task due to complex nature of cancer spread.

Although these new treatment modalities may open new era as a hope for so many cancer patients. As we proceed in this book, different modalities are discussed.

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2 Transforming Diagnosis and Therapeutics Using Cancer Genomics

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2.1 Background

Greek physician Hippocrates first categorized the cancer in two forms: ulcer-forming and non-ulcer-forming carcinomas. In the late nineteenth century, with the advent of microscope physicians reported numerous cellular forms of cancer. Advancement in the technology is transforming our understanding of cancer's origin and complexity suggesting it a complex disorder of abnormal cell growth and its progression is controlled by multifaceted interaction of multiple biological/signaling pathways and genetic events [1]. The over-expression of signaling/biological pathways is under control of various genes that leads to a unique type of cancer.

Genomics provides significant insight to understand that how cancer progresses in each individual and respond to the particular type of treatment [2]. Knowledge of cancer driver genetic components provides all the necessary treatment information that can save people's life. Such information cannot increase survival duration/life span but can open most promising treatment options as cancer-related genetic or epigenetic changes can be a potential target for drug development, even for those cancer types which have limited treatment choices available currently.

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2.1.1 Classical Genomics

There are more than three billion base pairs found in human genome. This information comes up after the human genome was mapped through an extensive collaboration of 13 years comprising human genome project [3]. Advances in the genome sequence technologies enabled to map genome within hours, which enhances the widespread applications of genome in early diagnosis through research [4]. Initially, each gene was sequenced exon by exon using classical Sanger sequencing method (capillary electrophoresis), that need considerable input for data interpretation and read despite of automation and speed. Such experiments typically provide data after 2–12 weeks subjected to clinical urgency [5]. With the advent of most recent sequencing era using WGS and targeted sequencing through static panels has revolutionized the diagnostic market with more accurate results.

Genomics have been utilized in different facet of research and clinical applications which range from diagnostics, pharmaceuticals, pharmacogenomics and disease prevention, gene therapy, developmental biology, comparative and evolutionary genomics. Whole Genomics Sequencing (WGS) data generation was initiated with human genome project (HGP), which was started in 1990 and completed in 2003. Over the time, the genome sequencing technology has evolved which changed the DNA sequencing industry [5, 6]. In the start of the twenty-first century, completion of WGS of a single haploid human genome took many years with spending of more than three billion dollars [7]. The advancement in the sequencing technology over the period of time has improved. Next-generation sequencing (NGS) techniques like nanopore (e.g., MinION), SMRT (single molecule real-time; e.g., sequel system) and semiconductor (e.g., Ion S5 sequencer) are the keys for reducing the cost and time [8, 9].

2.1.2 Rise in Genomics and Cancer

During the last decade, quest for the genetic predisposition carrying high risk for development of cancer in life has clarified several bases of cancer syndromes. Such genetic markers are key target for genetic diagnostic and screening projects. Similar genetic mutation screening strategies has great impact on chances of survival, genetic counseling, explaining genetic carriers and to adopt most appropriate personalized treatment options.

The more advanced approaches leading toward new genes and biomarkers discovery include third-generation sequencing provide further insight into disease mechanisms. The identification of new mutation by sequencing individual's entire genome [10], helps scientists in developing precision medicine and is transforming traditional cancer therapeutics to the advance level. The huge cancer-centric datasets are key for the development of bioinformatics tools for designing gene-based treatments and drugs against cancer [11].

The categorization based on the tumor's genomics, location and histomorphology significantly helps to classify and elucidate the pathogenesis of different

cancers. Nevertheless, complications about morphological ambiguities and poor knowledge of underlying causes necessitate the development of additional testing such as immunohistochemistry, flow cytometry and molecular testing/profiling. Different molecular approaches which provide detailed characterizations of genetic mutations that trigger or aid cancer development include genetic profiling through sequencing, molecular testing based on genetic mutations panels, antigen and DNA methylation groups [12]. The genetic alterations and mutations in tumor suppressor and/or DNA damage repair genes amongst other genes are linked to higher risk of familial cancers.

2.2 Genome-Based Cancer Diagnostic

The improvements in genome datasets led to more accurate and earlier diagnosis of cancer. Cancers are the expansion of abnormal clones of cells arises due to changes in hereditary material. Several studies have proven that specific genetic variations can lead to specific cancer type. For instance, genetic translocation between chromosomes: 9 and 22 (also known as Philadelphia translocation) is associated with chronic myeloid leukemia [12, 13]. Similarly, a study presents that transfer of total genomic DNA of cancer cell in normal NIH3T3 cell lines could convert cancer cells into normal cells. Identification of particular DNA sequence involved in transforming activity (single base pair change G > T in HRAS gene causes Glycine to Valine substitution in codon 12) lead to the marking of first cancer-causing sequence in the genome [14–17].

Some of the cancers causing genomic alterations interrupt the cell growth and death regulating genes also known as tumor suppressor genes that usually defend against the cancer. For example, mutations in BRCA1 and BRCA2 have been associated with high risk of breast cancer, prostate and ovarian cancer development [18]. Likewise, genetic alterations in the genes accountable for repairing and maintaining the DNA damages can also lead to cancer formation, as these genomic alterations in oncogenes actively convert normal cells into carcinoma cells. Similarly, HER2 is identified as another oncogene; mutations in this gene can enhance the production of protein that ultimately ends up in cancer development. BRCA1 and BRCA2 mutations can be inherited, but mostly other reasons may be involved. Identification of genomic alterations in oncogenes is necessary for cancer diagnosis, especially in case of hematological cancers. These mutations are not playing central role in cancer diagnosis as distinct cancer subtype is highly variable in its progression, clinical presentation and treatment options [1, 19, 20]. Overall, better classification of cancers on the bases of their origin and cell type somehow relies on genetic factors which might be its initial cause.

2.3 Limitations of Cancer Diagnosis and Treatment

Despite the sufficient cancer genomics data has been accumulated, actual benefits are still not clear. The oncogenes mutations overweigh the treatment options available for cancer patients. Though at small scale these genetic mutations facilitate cancer categorization but large scale impact is still not clear. There are certain limitations of applying genetic information for cancer treatment; some of them are summarized in Table 2.1.

2.4 Transforming Cancer Diagnosis from Classical to Advance

The conventional method for cancer detection includes examining the histological features of tumor biopsies with the help of tumor specific biomarkers/antibodies. Advances in biosciences have resulted in modernized and sophisticated techniques for cancer detection. These techniques possess critical part in the diagnosis of cancer as well as help in early disease detection, risk of recurrence, predict the future cancer risk.

2.4.1 Tumor's Location

The inspection and palpation can detect reformatted body parts with slight cutaneous or subcutaneous prominences, identify tumor formations and possible tumor-induced peculiarities in natural cavities and abdominal organs. The clinical examination also reveals the presence of penetrated plaques, identify any nodules present cutaneous or subcutaneously, erosions or ulcerations, masses made up of compact tissues, hemorrhages; lymph node hypertrophies which can be generalized, single or multiple; and deformations of bone lesions which may provide a clue for initiation of tumor growths. The symptoms and injuries with persistent or intermittent development are conceivably in neoplastic state and considered as doubts of cancer disease if coupled with weight loss, anorexia and constant or recurrent fever. The conventional cancer diagnosis is broadly categorized into three stages including identification of tumor location, evaluation of tumor extension and histological nature [35].

2.4.2 Histomorphology

An old approach that has long been used to identify and diagnose all forms of tumors including both malignant and benign is histomorphology. A range of cancer can be classified based on histology examine of characteristics such as cellular density, tissue architecture, mitotic activity, nuclear atypia, in combination with higher-order configurations and cytological details. It also improves patient care

Table 2.1 Cancer treatment strategies and their limitations

S. no	Cancer treatment method	Limitation/challenge	References
1	Genomics-based assays NGS	Intra-tumoral heterogeneity Intrinsic genetic complexity of cancer Multiple NGS-based assays data algorithmic analysis Widespread copy number alterations	[12, 21, 22]
2	Samples specificity	Quality and quantity of tumor cells can create technical challenge for NGS data analysis Low levels of tumor cells can create infiltration of non-malignant cells	[23–26]
3	Nonmalignant DNA analysis	For large sequence panel and comprehensive assays, availability of patients matched nonmalignant DNA sequence analysis is very important. It facilitates distinction between germ line variants from somatic mutations. False positive and/or false negative mutation calls in platforms may create limitation in this analysis	[27–29]
4	Algorithms/time requirement in digital data analysis	The harmony in NGS assay and computational data analysis pipeline is crucial for accurate results assessment as routinely generated large NGS data where creates scope also increase complexity equally. Examination of number of different algorithms and sufficient time is needed to deeply evaluate such big data, which is one of the limitations	[12, 30, 31]
5	Data sharing/variants interpretations	All the variants filtered by computational system as promising disease-causing variants may not be actually pathogenic in nature. For that comparative analysis of the variants filtered from big data for specific cancer type with the previous studies is necessary. There is lack of such repositories where all the clinical data can be shared	[32–34]

by detecting prognostic signs including necrosis, lymphovascular invasion, mitotic rate and infiltration of the neighboring tissues [36–39], and it's still a useful tool for predicting how a patient's illness will progress in the future [40]. The phenotypic data represents the cumulative influence of changes in the molecular structure on the behavior of cancer cell and serves as a useful visual indicator of disease aggressiveness. However, due to common subjective nature of human histological assessments, use of computational analysis for identification of histological

images has gotten a lot of interest. With the inclusion of computational methods to histomorphology, number of algorithms for grading of image analysis have been created [41, 42] based on classification [43–46] and identification of lymph node metastases in multiple cancer types [47].

2.4.3 Immunohistochemistry

Immunohistochemistry (IHC) uses monoclonal or polyclonal antibodies for detecting antigens of interest in the tissue samples. It is used as an influential tool in the diagnostic surgical pathology. The antigen of interest is analyzed for its distribution on the tissue in both health and disease. It can be used to differentiate cells, discover metastases, characterize a tumor's preliminary site, identify prognostic markers, predict targeted therapeutic response, and even identify organisms, structures and materials released by cells of interest [48, 49]. Certain tumor antigens are expressed de novo and/or up-regulated in certain tumors, and they are commonly employed for cancer diagnosis. In pathology, IHC is particularly essential in subspecialties of hematopathology, neuropathology, oncologic pathology and surgical pathology [50, 51]. In examination of the basic histological alterations, tissue sample is considered a helpful and important component of autopsy pathology and IHC may provide more information [52, 53]. To diagnose the benign or malignant nature of cancers, grading or staging of a tumor and identification of cell type and metastatic origin to discover the primary tumor location, physicians use prognostic tumor markers along with IHC. IHC is also utilized in the field of drug development and to assess efficacy of any drug by detecting the activity of disease targets or their down- or up-regulation [54].

2.4.4 Flow Cytometry

Flow cytometry (FCM) was first created in 1960s as an analytical tool for measuring various features of single cells in suspension after they were excited with a light source [55, 56]. It was first utilized in clinical oncology to investigate DNA content in order to determine cell ploidy and proliferative activity [57]. development of novel fluorescent dyes whose emission and excitation spectra is within narrow range, as well as the discovery of monoclonal antibodies (moAbs), allows for a broad application of this technology, particularly in haematological cancer [58]. FCM is also utilized for the analysis of rare events, like detection of the residual leukemic blasts existence in the bone marrow after therapy, dendritic cells of blood, or the cell types that are correlated with any metastatic incident, such as cells of circulating tumors and endothelial progenitor cells, has grown in popularity in recent years [59, 60].

2.4.5 Cancer Biomarkers Discovery

Biomarkers are advantageous for the diagnosis, monitoring, progression detection, prediction of chances of recurrence and therapeutic efficacy of various types of cancer disease. More importantly, the cancer biomarkers can be utilized in the widespread screening of asymptomatic individuals to detect the disease at a very early stage [40]. With the coming of better than ever genomic and proteomic advances combined with cutting-edge bioinformatics devices, it is feasible to foster biomarkers that can dependably and precisely foresee results during management of cancer and its treatment. Clinical utility of disease explicit biomarkers depends on its noninvasive and prompt accessibility in the samples of biological nature. Because of the great cell turnover, disease patients convey raised degrees of free DNA (∼200 ng/ml) in their blood [61], urine [62], bronchoalveolar lavage (BAL) and sputum [63], mammary aspiration fluids [64], saliva [65] and stools [66]. Proteins, high mutation burden, metabolites, epigenetic changes, cancer-specific mutations, chromosomal translocations and microRNAs are among the many molecular biomarkers now available for host tissues/cells. Tumor biomarkers aren't usually derived from the host. Essentially all cervical malignancies, and a subset of anal, genital and oropharyngeal cancers, are linked to high-risk HPV [67].

Imaging biomarkers cover a wide range of anatomic (location, size and calcification) and functional (tumor growth, phenotypes and rates of metabolism) characteristics [68, 69]. Molecular imaging and analysis tools like Totalys (Becton Dickinson) and CellCT (VisionGate) allow for the hundreds of captures and the morphological measurements in different cells in clinical samples, resulting in three-dimensional images. Mayer et al. (2015) used a Papanicolaou (Pap) test or human papillomavirus (HPV) genotyping for creating high-resolution biosignatures from sputum samples collected from intact cells and liquid-based preparations for lung cancer screening using low-dose CT and screening of cervical cancer using Pap test or HPV genotyping [70].

2.4.6 DNA Methylation Group Based Cancer Diagnosis

DNA methylation occurring at CpG residues is a potent epigenetic process and that adversely controls the gene expression. Every type of cancer has a specific DNA methylation markers and can easily be identified, and they are used in accurately diagnosing and monitoring the effect of therapies during or after treatment in different types of cancers [71]. The circulating DNAs from blood and/or other fluids in the body is used to determine the DNA methylation position at various gene promoters in cancer patients. The DNA hypermethylation of GSTP1 in urine samples for prostate [72]; p16 in the sputum of lung [63, 73]; promoters for p16, DAPK, RARβ and MGMT genes in bronchoalveolaer fluid and serum of lung [74]; RARβ, DAPK, E-cadherin and p16 in urine for bladder is detected for diagnosis of cancer patients.

2.4.7 Sequencing

The biological mechanisms and genetic events that affect the mechanism involved in tumor pathogenesis at several levels, encompassing initiation of carcinogenesis, persistent proliferation, suppression of apoptosis, metastasis and invasion, are numerous and exhibit intricate relationships. Specific genetic constituents or biologic pathways are expected to be overexpressed in each tumor which can be detected through molecular profile. The introduction of next-generation sequencing (NGS) technologies has broadened the genomic landscape of cancer. Some putative cancer driver-gene changes have been discovered using NGS [75–78]. The curation and exploration of huge data sets, the patient identification at higher risk for susceptibility of cancer based on inheritance, NGS-based panels for hereditary cancer, and the identification of patients at risk for inherited cancer susceptibility with clinically relevant cancer predisposition genes.

The NGC technology is being used to reinterpret the phenotypic spectra that have been well-studied for hereditary cancer susceptibility disorders [28, 79–81], pedigree analysis-based approaches [82], outlining moderate and high-risk cancer-causing genes through case–control [83–85] exome or genome sequencing-based studies [78, 86, 87] for the discovery of novel gene(s).

2.4.8 Panel Testing

The limited numbers of cancer-associated genes are tested using NGS for clinical use through gene panel testing and is considered as the most practical genome profiling method worldwide [88–90]. Gene panels are designed with comprehensive-targeted sequencing, validated and implemented that cover single nucleotide variants (SNV), copy number alterations (CNV), small insertions/deletions (indel) and fusion genes for cancers [91]. Over the last few years, genetic testing recommendations have evolved to include genes found on multigene panel testing (MGPT) for inherited cancer in clinical practice. Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High-Risk Assessment, for example, are published by the National Comprehensive Cancer Network (NCCN). NCCN Guidelines® for Genetic/Familial High-Risk Assessment: Breast and Ovarian Cancer: For a variety of genes included on multigene panel tests, colorectal provides information regarding risk of cancer and recommendations for its management. While great progress has been made in understanding the clinical significance and implications of these genes, testing criteria are still confined to genes linked to well-known cancer syndromes like TP53, BRCA1/2 and mismatch repair genes [92].

2.4.9 Microarray

One of the most powerful methods for studying global gene expression in all areas of cancer in human is DNA microarrays and is a widely accepted diagnostic and classification tool. It provides major understandings into advancement and prognosis of cancer and response to the therapy based on expression profiles of genes. Improvement in microarray methods along with providing differential gene expression also identifies small insertions or deletions in tumor-suppressor genes [93] GeneChip® (Affymetrix, USA) is one of the microarray technology whose use is widespread.

In situ synthesis of several thousand short oligonucleotides on glass wafers using a combination of photolithography and light-directed solid-phase DNA synthesis produces high-density oligonucleotide GeneChips [94, 95]. Capacity to monitor the levels of gene expression in cells and tissues is a benefit of the GeneChip technology. Its sensitivity allows it to detect mRNA with extremely low abundance [94]. Microarray technology necessitates prior knowledge of the gene sequences to be studied, which restricts its utility and makes gene prediction difficult.

SAGE technology is a comparatively more comprehensive and sensitive and can be helpful in gene expression analysis of uncharacterized genomes [96]. SAGE and microarray technologies are combined to investigate thousands of genes at once, revealing information on prognosis, diagnosis, treatment targets and clinical outcome [97]. Estrogen/progesterone receptor protein expression, 17q23 genomic amplifications, HER2 gene/protein alterations and cyclooxygenase-2 protein expression, vimentin protein expression, insulin-like growth factor (IGF) binding protein 2 protein expression and Myc and A1B1 protein expression are among the most important biomarkers discovered through microarray analysis [98].

2.4.10 Third Generation Sequencing

The analysis of natural RNA and DNA fragments without manipulating them through a real-time and single-molecule sequencing technology, the third generation sequencing (TGS), in parallel fashion using the single-molecule DNA sequencing (SMDS) and Direct RNA Sequencing (DRS) technologies. Epigenetic modifications play a crucial role in tumorigenesis and certain other diseases. TGS detects the epigenetic modifications in the real time. The TGS in combination with the single-molecule real-time bisulfite sequencing is used to target the long reads of CpG [99].

TGS in combination with other technologies, such as single-cell sequencing, NGS, or target genome editing, provides the genomic insight and allows for the development of new therapeutics. After large genes were inserted at the endogenous HBB, CCR5 and IL2RG loci by zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), or clustered regularly interspaced short

palindromic repeats CRISPR/Cas9 or RNA-guided endonucleases (RGENs) for SMRT sequencing assisted quantification of the genome editing outcomes [100].

2.5 Genomics Based Characterization of Cancer

Cancer is a wildly heterogeneous disease that usually originates from different molecular irregularities which contains mutational events in somatic cells such as changes in copy number, DNA methylations and single nucleotide mutations [24, 101, 102]. Characterization at genomic level, transcriptomic level and at epigenomic level, cancer is considered as a disease with diverse molecular oncogenic processes and its therapeutic responses.

2.5.1 Evaluating Genome for Mutations: Genetic Cancer Culprits

Through research studies, it is well known that genes and genetic alterations are the basis of cancer. Some of these mutations and genetic alterations disturb the normal functions of tumor suppressor genes- *BRCA1* and *BRCA2* which are associated with a higher risk of breast, ovarian and prostate cancer [1]. Mutations that interrupt the normal functioning of genes involved in repairing damaged DNA have also been involved in cancer, as have mutations that produce oncogenes. *HER2*-positive breast cancers are one of the examples involving a mutated *HER2* oncogene, thus produces a protein that increases the cancer cells growth [1].

2.5.2 Interruption of the Normal Functioning of Tumor Suppressor Genes

Two genes generally related to cancer are the proto-oncogenes and the tumor suppressor genes. Proto-oncogenes are linked with pathways that help in cellular growth; these genes, when activated either by mutations or by alterations transform normal cells into cancerous one [103]. A crucial gene, TSGs plays an important part in DNA damage repair, interference in cell division, metastasis suppression and initiation of apoptosis. For this reason, functional loss of genes which are involved tumor suppression would lead to the commencement of cancer and its development [104].

2.5.3 Gene Mutations and DNA Repair Systems

An efficient DNA repair system is important that leads to a survival without having cancer. Inherited cancerous syndromes are due to mutations in genes such as mutations in the DNA repair genes of the NER group, i.e., nucleotide excision repair (e.g., XP genes in patients with xeroderma pigmentosum), mutations

in DNA crosslink repair Fanconi anemia genes is such example, mutations in the MMR genes. People having inherited colorectal cancer (CRC) is because of mutations in mismatch repair genes. Other than these predispositions and many such mutations lead to inherited cancer syndromes [105].

2.5.4 Cancer Genome Analysis

Genetic susceptibility may favor the disease as cancer is developed in the presence of mutated genes. Cancer occurs due to mutations in specific tissues whose cells would divide and accumulate over time. Progress of the tumor depends on the evolution of the diverse cell types (clonal versus parallel evolution) in the tumor [106]. Based on the type of data and analysis purpose, characterization and genome analysis of cancer may concenter on type of cancer, its subtype or patient.

First step in genomic analysis approach is examination. A group of patients that have a specific type of cancer are examined. Next thing is to identify biomarkers. With the clinical or therapeutic knowledge, characterization of cancer subtype is done. These steps help in our better understanding of the progressing process of tumor. The other approach is about inspecting a patient's genome having a specific kind of cancer. This examination is held for finding the specific alterations for a directed therapy. Common experimental and bioinformatics techniques are applied in both these approaches, but they have different objectives and examine different types of information; thus, they present the results in distinct ways [107].

2.5.5 Primary Analysis of Data

Sequencing, alignment and variant calling are all included in data primary analysis. First, the samples are sequenced and then aligned to a genome which is used as a reference sequence to identify all differences through the variant calling process. The information that is obtained through variant calling gives a full record of genomic differences that are organized in accordance to their genomic location such as chromosome position and variant allele [107].

2.5.6 Secondary Analysis of Data

In the secondary analysis of data, identification of genetic alterations that may affect or alter the functionality of products of protein is done. For this purpose, the data of genomic or somatic variations that is obtained from primary analysis is examined. For this, mutated DNA is transcribed to RNA which is the translated into proteins that have altered amino acid sequences. These alterations affect the function of protein in different manner, as they could or could not affect any area of protein that is participate in binding or catalysis, or they might or might not change the protein structure and its solidity [96, 108]. Certain specialized tools

known as protein mutation pathogenicity predictors are used to check the severity of these alterations.

2.5.7 Squamous Cell Lung Cancers and Its Genomic Characterization

Lung squamous cell carcinoma (SCC) is a general kind of non-small cell lung cancer (NSCLC). About 85% of all lung cancer are of NSCLC type. Squamous cell carcinoma and the adenocarcinoma are the record famous subtypes of NSCLC, accounting for 30% and for 50% NSCLC cases [109]. About 178 lung SqCCs were profiled to provide a comprehensive outlook of epigenomic and genomic variations. Due to complex genomic variations, lung SqCC contains a mean of 165 genomic rearrangements with 360 exonic mutations and has 323 segments of copy number alteration per tumor. Hence, a mutation rate of 8.1 mutations/Mb is noticeable with increased genomic complexity. Almost all lung SqCCs possess similarity to high-grade serous ovarian carcinoma17 in exhibiting somatic mutation of TP53 [110].

2.5.8 Genomic Analyses of Ovarian Carcinoma

One of the main reasons of cancer death in women is because of ovarian cancer. For this analysis of mRNA expression, miRNA expression, DNA copy number, methylation of promotor in 489 high-grade serous ovarian adenocarcinomas (HGS-OvCa) and analysis of DNA sequences of exons from coding genes in 316 of these tumors is done by The Cancer Genome Atlas (TCGA) project. Analysis result shows that HGS-OvCa is marked by TP53 mutations in around 96% of tumors. The microarray analysis of 489 HGS-OvCa produces a high-resolution measurement of mRNA expression, the microRNA expression, methylation of the DNA promotor regions and DNA copy number while massively parallel sequencing combined with hybrid affinity capture [111, 112]. Information of the whole-exome DNA sequence of 316 samples shows that atleast 96% of HGS-OvCa has mutations in TP53, while 22% of tumors have mutated BRCA1/2 due to germline and somatic mutations [24].

2.6 Transforming Cancer Therapeutics from Classical to Advance

2.6.1 Evolution of Cancer Therapeutics: Traditional to Advance Level

Ancient documents of the times of early Egyptian and Greek civilizations provide shreds of evidence that show that oncology has its roots in much older times

[113]. Chemotherapeutic antitumor drugs usage was a first and a revolutionary approach toward cancer treatment. Though these used drugs are cytotoxic against different tumors but still they show lethal effects on normal tissues and one of the major barriers to overcome is that when these drugs are used against tumor cells, they develop a drug resistance mechanism. In the last 20 years, a new anti-tumor therapeutic strategy which includes monoclonal antibodies and new immunotherapeutic drugs has been developed. Due to the effectiveness of these treatments, cancer patients' survival rate has increased. New strategy of personalized medicine following proper therapeutic protocols has been really effective and also has a low toxicity for the cancer patients [114, 115]. For these reasons, oncological research consistently focuses at the development of novel and operative therapeutic approaches, that includes gene therapy and CAR-T cell therapy [116–120]. Combined therapeutic protocols that use different types of antitumor drugs to find the possible therapeutic strategy for reducing the drug resistance and increasing the effectiveness of the drug undergoes clinical trials [121, 122]. Till now, chemotherapy, radiotherapy and surgery are the methods used in the whole world against treating cancer.

2.6.2 Chemotherapy and Its Limitations

Out of all methods, the main method used against treating malignant tumors is chemotherapy. In recent years, clinical practices have improvised the cancer chemotherapy method [123], but still there are many hurdles that must be overcome:

(1) There is a shortage of effective treatments against metastatic tumors.
(2) The treatments available cannot still kill the tumor cells that have developed drug resistance.
(3) There is a deficiency of finding new aims that are based on the characteristics of neoplasm, much like tumor plasticity.

2.6.3 Alkylating Agents

In clinical practice, mechlorethamine the first- nitrogen mustard was used as an alkylating agent. Mechlorethamine shows its alkylating activity as the aziridinium group of the nitrogen mustard binds to nitrogen N7 of guanine, forming cross-links (ICLs) after the displacement of chlorine which is the basis of cytotoxic activity of nitrogen mustard and thus inhibits DNA replication which leads to cell death [124].

2.6.4 Antimetabolites

Some molecules that copy the structure of physiological metabolites have been developed for the treatment of tumors, acting by blocking the enzymatic chains that are essential for the formation of purines which leads to cell proliferation inhibition. These molecules or antimetabolites are purine analogs (mercaptopurine), pyrimidine analogs (fluorouracil, gemcitabine, capecitabine), and folate analogs (aminopterin and methotrexate) [125, 126].

2.6.5 Cytotoxic Antibiotics

There are some antibiotics and their derivatives among the standard chemotherapeutic drugs, with clear cytotoxic activity, that are more effective anticancer drugs used in diverse therapeutic strategies. A widespread variety of natural antibiotics show cytotoxic effects as they form covalent bonds with nucleic acids and thus interfere with DNA synthesis [127].

2.6.6 Revolution of Targeted Therapy

Monoclonal antibodies and cancer therapy
The idea of monoclonal antibodies arose with the discoveries in different biological fields like cell biology, molecular biology and immunology. Advance research gave researchers an insight into the molecular mechanism for the neoplastic transformation of cells by finding new molecular targets that can be blocked by small and selective inhibitory molecules or monoclonal antibodies. These inhibitors would only act against the cancerous cell with no or very few disruptions to the normal cell. This method is completely opposite to the conventional chemotherapy approach through which not only cancer cell, but normal cells are also affected [128]. Due to variations in the percentage of murine protein portion presence in immunoglobulin, 4 types of monoclonal antibodies are available which are murine, humanized, chimeric and human monoclonal antibodies.

2.6.7 A New Strategy of Immune Checkpoint Inhibitors for Cancer Treatment

Advancements in recent years have fully accomplished the strategy of cancer immunotherapy. After the year 2010, new monoclonal antibodies have been developed against tumor antigens or against T-cell protein receptors that suppresses the immune response [129]. The new drugs act as the inhibitors to the immune checkpoints and located on the membrane surface of cancer cells, and T-cells are the anti-programmed cell death protein 1 antibody (anti-PD1) and also monoclonal antibodies, anti-cytotoxic T-lymphocyte associated antigen 4 (anti-CTLA4) [130].

A human IgG1 antibody, Ipilimumab (Yervoy®) was the first authorized immune checkpoint inhibitor in 2011. It binds the membrane protein CTLA-4 expressed in regulatory T-cells. The overexpression of CTLA-4 is induced by the tumor microenvironment, which binds the stimulating proteins CD86 and CD80 present in the antigen-presenting cells to prevent their interplay with the T cell surface receptor, accountable for immune system activation against cancerous cells [131].

2.7 Genomic Study Transforms Cancer Therapeutics to an Advanced Level

With the passage of time, latest advancements in technologies are helping our understanding towards origin of cancer and the complexity linked to it. Now the disease can be categorized on the basis of the location of tumor cell to genome sequencing giving more details of the mutations in genes that trigger or aids cancer onset in an individual [1].

Detail mapping of the human genome can improve the future of cancer therapeutics by the fusion of genomics with pharmacology with the identification of patient who has been benefited from these specific therapeutic agents. In the pharmacogenomics research field, single-nucleotide polymorphisms (SNPs) are a beneficial tool used as a disease marker in cancer therapy. Furthermore, DNA and tissue microarray analyses also play a potent role in cancer therapy. For example, tumor classification systems can be improved through DNA microarrays, and it can also help in the examination of gene expression changes at the molecular level that takes place in cancer progression. Tissue microarrays help in the verification of the candidate genes, which are identified from the DNA microarrays, against the actual tumor patterns with known clinical outcomes. Furthermore, microarray may be combined to certify gene targets rapidly [132].

2.7.1 Multi-omics and Cancer

In the age of omics, high-throughput NGS, genomics, transcriptomics, proteomics and metabolomics have become the powerful tools in cancer research. Genomics is the study of the DNA infrastructure and function through sequencing and polymorphism analysis. Transcriptomics deals with study of total RNA at given conditions for the prediction of biomarkers. Proteomics is the study of total protein in the biological processes, their variations in expression, and PTMs which are thoroughly characterized in cancer patients. Metabolomics deals with the study of small molecules and metabolites responsible for prediction of cancer biomarkers [133]. In cancer multi-omics, multiple factors like malignant state, epigenetic modifications, genetic aberrations, changes in metabolism, and signaling pathways are involved [134]. So, for comprehensive understanding of multi-omics, we need to look into each factor individually.

To enhance the understanding of diagnostic and prognostic and stratification of patients, cancer biomarkers has been investigated, e.g., RAS mutations can be used for the risk prediction of cutaneous carcinoma for the patients treated with BRAF inhibitor [135]. Likewise, prostate cancer antigen (PSA) is being used for the screening of prostate cancer. Scientists have developed OPKO 4K score, which is a prostate health index using PCA3 biomarker for the prediction of prostate cancer [136]. For lung cancer several biomarkers like actinin-4 protein have been reported [137] in adjuvant chemotherapy of lung carcinoma [138]. Several biomarkers for cancer prediction have been reported from blood, urine and tissue. Blood is the main source of biomarker discovery including metabolites, proteins, DNA, circulating tumor DNA, platelet RNA, RNA and circulating tumor cells. Additionally, from serum endometrial and breast biomarkers have been identified. In most of the cancers types tissue biopsy is considered as the gold standard due to its direct analysis. Other bodily fluids including urine, sweat, sputum, feces, semen, tears, saliva and cerebrospinal fluid are the source of non-invasive discovery of cancer biomarkers [133].

2.7.2 Genomics

Generally, cancer is responsible for the cellular disruption which results in mutation or deletion of vital genes. In cancer regulation, three types of genes (oncogenes, gatekeeper and carekeeper genes) are involved. Oncogenes including HER2 and RAS mutation or deletion promote active cell proliferation. Gatekeeper genes are tumor suppressors including TP53, p53, BRCA1 and BRCA2. Carekeeper genes include all DNA repair genes along with p53, BRCA1 and BRCA2 [139]. With NGS, bulk data of cancer-related genome became freely available and provided opportunities for clinical discovery and practice of cancer through whole-exome and whole-genome sequences [140]. Initial cancer research investigated the inherited mutations and these reposts identified several mutations associated with high risk of cancer. For example, colorectal cancer is generally caused by mutations of PMS1, PMS2, MLH1, MSH2 and MSH6 genes [141]. Likewise, mutation of TP53 tumor suppressor gene is responsible for the development of sarcomas, leukemia and brain cancers [142]. Hereditary ovarian and breast cancers are associated with BRCA1 and BRCA2 genes mutations [143]. Genome sequencing enables discrimination of genetic modifications on the basis of TP53, PIK3CA and GATA3 genes, and results suggested that these genes are modified in more than 10% of breast cancer patients. BRCA1 and BRCA2 identification opened the paths for screening tests to identify different mutation points for hereditary BC. For early age diagnosis, BC screening is now recommended for females with family history of cancer [144]. Currently, BRACAnalysis® is the sole sequencing provider for the detection of mutations in BRCA1 and BRCA2 [145].

In 2005, NIH founder The Cancer Genome Atlas (TCGA) by characterizing 10 cancers and 33 tumors [146]. TCGA sequenced 7500 genomes of tissue samples and predicted DNA modifications, sequence variants, copy number and structural

variations [143]. The NGS impact on cancer treatment was reported in multiple studies including for biliary cancer, colorectal cancer and advanced melanoma [147]. Refractory and relapsed cancer patients under fluoropyrimidines, irinotecan, oxaliplatin, cetuximab, bevacizumab or panitumumab treatment has been reported for the presence of targeted mutations in multiple genes including TP53, KRAS, APC, PIK3CA, SMAD4, BRAF, SPTA1, PDGFRA, FAT1, ATM, ALK, ROS1, CDKN2A, TGFBR2, FBXW7, HER3 and NOTCH1 respectively [148].

Melanoma developments are also associated with multiple somatic mutations in AKT and MAPK pathways. The MAPK conforms 50–60% of all cancers via somatic mutation of threonine/serine kinase BRAF. MAPK pathways is also triggered by missense mutation NRAS which oncogene homolog of neuroblastoma RAS conforming 15–20% of all melanomas [149]. Stark et al. reported MAP3K somatic mutations leading to decrease in kinase activity associated with chemoresistance [150]. Likewise, MAP3K5 somatic mutations are also responsible for lesser pro-apoptotic activity. On the other hand, AKT pathway is associated with uncontrolled activity in cancers [151]. PTEN is function as tumor suppressor gene and in melanoma, PTEN mutation results in loss of function associated with BRAF mutations reported in about 44% cases [152]. In different cancer types, mutation in AKT isoforms (AKT1, AKT2 and AKT3) are reported which act as downstream regulator in signaling along with phosphorylation properties. Similarly, KIT gene somatic mutations conform 2–8% of melanomas [153]. NF1 is responsible for hydrolysis of GTP which inhibits the RAS which is a tumor suppressor protein and counts for 15% of melanomas [154]. Recently, a mutation model consisting immunotherapy score (ITS) has been developed to predict the immunotherapeutic responses. Scientists developed ITS through whole exomes sequencing. This ITS model has proven to benefit for the patients in terms of treatment efficiency and survival rate [155]. Carol Amato et al. reported the activation of NF-kB pathway due to function loss of NFKBIE gene therefore, can be included in predictive biomarker list against treatment response [156]. In breast cancer, key reported signatures are PR (progesterone receptor), ER (estrogen receptor) and HER2 (human epidermal growth factor receptor 2) [157]. For management, if a patient is PR+ or ER+ will probably receive endocrine treatment, while HER2 patients will likely receive trastuzumab. Triple-negative breast cancer (TNBC) covers all tumors which are PR, ER and HER2 negative. TNBC are more aggressive tumors and are associated with a poorer outcome to chemotherapy [8]. However, there is still no targeted therapy for TNBC [158]. Ki67 is another proliferative biomarker is currently being used to predict growth rate of tumor [159]. Combination of these four signatures (ER, Ki67, PR and HER2) are referred as protein-based biosignature. Even so, the data is still insufficient to predict risk for cancer development and their treatment response probability.

2.7.3 Transcriptomics

Transcriptomics deals with the study of the complete RNAs present under given conditions. Transcriptomics includes the discovery of noncoding RNAs, novel genes and splicing variants. NGS has enable scientists to study RNA sequence of single cell to analyze different transcripts in cancer patients [160]. It revealed the importance of intratumoral heterogeneity in cancer and its microenvironment [161]. Transcriptomics study has also led the discovery of miRNAs and lncRNAs melanomas patients representing integrated genome associated phenotype-based diagnosis of cancer [162].

Currently, microarrays and RNA sequencing are two hi-tech method being utilized for the transcriptomics molecular approach [149]. Through this approach, immuno-predictive score model has been developed to predict the ICIs response in patients. ICIs include pairwise 15 pairs of transcriptomics in association with 28 checkpoint genes which discriminate respondent and non-respondent cancer patients against immunotherapy [163]. However, due to complex heterogeneity of melanomas prediction ability of biomarkers are generally compromised. Hence, recent research is focusing more on microenvironment of the melanomas. In this context, a study has been conducted to analyze progression of melanoma in 94 samples collected from cancer patients at baseline followed by anti-PD1 treatment. Sequence results of RNA demonstrated downregulation of MHC-I in association with PD1 inhibitors resistance. This condition can be controlled by transforming TGF-β. Hence, combinations of PD-1 inhibitors and anti-TGF-β could provide potential therapeutic benefits [149, 164]. Alternatively, in a study, transcriptome samples from 23 females with cervical cancer were analyzed using whole-exome sequencing, and genetic mutations in neuroblastomas were identified [165]. Transcriptome data has procreated an innovative approach known as expression-quantitative-trait-loci (eQTL) analysis. eQTL is used to investigate functional mechanisms of genetic variation in sequences leading to variation in disease DNA sequence responsible for changes in gene expression [166]. TCGA consortium has also analyzed transcriptomics data to identify gene expression of individual samples from over 11,000 patients [146] in terms of tumor growth and formation.

Transcriptome-wide association studies (TWAS) combine the data from whole genome sequencing and microarray or RNA sequencing to get insights into the cancer management. Mancuso et al. [167] identified 1196 genes that were associated with 30 complex biological pathways in cancer using TWAS approach. At present, three TWAS studies have been reported by different groups. Gao et al., reported TP*53INP2* (tumor protein p53-inducible nuclear protein 2) to be efficiently linked with ER-negative breast cancer in African, European and Asian populations [168]. Similarly, Hoffmann et al. [169] identified significant links between cancer risk and the expression of *RCCD1* and *DHODH* in cancer tissue, along with *ANKLE1* association in trans-ethnic meta-analyses of U4C and UK Biobank data. Wu et al. [170] identified 48 genes from which 14 were novel using the data acquired for Genotype-Tissue Expression Project. Another group

identified 26 new target genes for breast cancer including 17 genes for estrogen receptor (ER)-negative breast cancer using eQTL [171]. These studies have reported 59 genes whose predicted expression levels are associated with high risk of cancer [171]. Mosig et al. reported transcriptome analysis of 22 ovarian cancer which showed overexpression of IGFBP-4 gene [172]. Xing et al. evaluated three serum biomarkers consisting CHI3L1, MMP13 and SPP1 as a diagnostic panel. This panel was identified in 90% of esophageal squamous cell carcinoma cases, while in non-cancerous samples the panel's detectability was 10–15% [173]. Additionally, several RNA molecules like snoRNAs and miRNA can also serve as cancer biomarkers. miR-221 miR-20a and miR-106b are also reported for early detection of gastric cancer [174]. Likewise, piRNAs, together with transcriptional and posttranscriptional silencing effects, can also be used as biomarkers for diagnosis and prognosis of renal, hepatocellular, glioblastoma and gastric cancer [174]. XIST (lncRNAs) is being used as active biomarker for early detection of gastric cancer [175].

Currently, six tests including Breast Cancer Index, EndoPredict, MammaPrint, OncotypeDX, Prosigna and Genomic Grade Index have been designed on the basis of the transcriptomic signatures for early diagnosis of cancer. The breast cancer index is designed on 60 ER+ tumor samples from patients previously treated with tamoxifen. It measures the ration of HOXB13 and IL17BR genes together with expression of the genomic grade index genes including BUB1B, NEK2, CENPA, RRM2 and RACGAP1. This test is used to determine the prognosis of the women with estrogen receptor positive and lymph node negative disease [176]. The EndoPredict is designed on 964 ER+ tumor samples from patients with LN± disease treated with tamoxifen. This test includes the expression of eight tumor-associated genes BIRC5, UBE2C, RBBP8, AZGP1, IL6ST, MGP, DHCR7, STC2 and three control genes OAZ1, CALM2 and RPL37A. This test is used to determining the prognosis of women with estrogen receptor positive and Lymph node ± disease [177]. MammaPrint is a 70-gene test which uses microarray technology for quantitative expression of the genes belonging to following processes cell-cycle dysregulation (15 genes), angiogenesis (12 genes), proliferation and oncogenic transformation (11 genes), invasion and metastasis (8 genes), growth factor signal transduction (6 genes), resistance to apoptosis (2 genes) and miscellaneous/unknown function (16 genes). This test determines the prognosis of women with ER+/− and LN− disease of stages 1 or 2 [178]. Oncotype DX has been evaluated on 447 ER+/− tumour samples from patients with LN ± disease registered in three distinct clinical trials, including from the tamoxifen only arm of NSABP B-20. This test measures genes for the proliferation (5), invasion (2), estrogen (4), HER2 (2), GSTM1, BAG1, CD68 and also five genes for reference. It is used to predict 10-year recurrence risk in patients with ER+ and LN− disease [179]. Prosigna test is designed on 189 ER± tumor samples from patients with LN± disease and 29 nonmalignant breast tissue biopsy samples. This test measures the expression level of 50-genes along with 5 reference genes to classify breast cancer into one of four intrinsic subtypes. Clinically, it has been utilized to Prosigna also determine the prognosis of postmenopausal women with ER+ and

LN± disease of stages 1 or 2 [180]. Although ample work has been done on the discovery of the biomarkers for cancer diagnosis, progression and treatment end point; further investigations are required to identify the biomarkers for divers forms of the other cancer types. Afirma® gene classifier is a microarray-based panel for diagnosis of thyroid cancer. Aziz et al. reported 19-gene biosignature as classifier to predictive colorectal cancer using microarray analysis [185]. Other diagnostic classifiers include ThyroidPrint® (10-gene) [188], ThyroSeq v3 (112-genes) [189], RosettaGX Reveal and ThyraMIR/ThyGenX™, expression tests [190, 191].

2.7.3.1 Proteomics

Proteomics deals with study of expressed proteins. Being functional representative molecules proteins provide detailed insight of the biological mechanism for cancer growth and expansion including genetic interplay and environmental factors. Proteomic analysis also includes the characterization and quantification expressed protein, their localization, translational modifications and interactions with other proteins. Study of all these parameters leads to the discovery of therapeutic targets and innovative biomarkers [133]. The 1st comprehensive profile of proteomics was presented by TCGA in the field of cancer proteomics. TCGA used reverse-phase protein arrays for the analysis which is target limited technique of a few hundred proteins. TCPA employed antibodies for the identification and quantification of approximately 200 proteins along with phosphoproteins from bulk of TCGA tumor samples [146]. Though, several scientists reported hi-tech MS analysis for the identification of biomarkers for several types of cancers [181]. Other studies used this proteomics data to identify resistance and sensitivity of the drugs [182]. These proteomics studies counterpart immunohistochemical classification of cancers, e.g., characterization of estrogen receptor expression in tumors [183]. Recently, with the start of high throughput "Orbitrap" MS instruments coupled MaxQuant with has simplified the identification and quantification of genome-wide expression of all proteins (18,000 approximately) collected from human tissues have surfaced the foundation for the development of first human proteome draft [184]. MS-based analysis of proteomes has also been extended to identify the proteins modification in some cancer types [185]. However, the potential of MS analysis is concluded gigantic for the identification of new diagnostic cancer biomarkers [186].

MS analysis also identified different peptide biomarkers including fragments of C3, C3adesArg, factor XIIIa, ITIH4, FPA, apoA-IV, fibrinogen, bradykinin and transthyretin [187]. Palacios et al. [188] reported 37 protein biomarkers using proteomics classification. Among these, BRCA2 mediated cancers are found to be associated with the D1 and D3 cyclins along with CDK4. Collectively, 97 breast cancer biosignature have been reported so far from pathological and proteomics studies including ER, p53, CK8/18, Ki-67, PR, cyclin D1, HER-2, CK5/6, cyclin E, BCL2, cyclin E and E-cadherin [187, 189, 190]. In another proteomic study, scientists reported the role of retinoic acid receptor alpha as potential biosignature in ER-positive patients. Brozkova et al. identified proteomic role of HSP27 and ANXV as biomarkers in cancer. He et al. [191] by using MS and ELISA reported

that serum CD14 could be an active biomarker for the prediction of cancer. Kabbage et al. reported the overexpression of the Hsp27 and Hsp5 in BC tissues which are known as α-B-crystallin. Moyano et al. [192] reported that α-B-crystallin can solely responsible for cancer transformation because it can induce the expression of EGF and anchorage-independent growth. α-B-crystallin can enhance cell invasion and migration along with activation of MAPK/ERK pathway. These reports suggest the oncoprotein nature of α-B-crystallin.

Hudelist et al. [193] performed MALDI-TOF and 2-DE comparative analysis of LCM from normal and tumor tissues of five cancer patients. Collectively, 32 proteins were expressed differentially and identified as tumor-suppressor genes, cytokines, signal-transducers structural proteins and cell-cycle regulators. Some proteins suggest their active role in tumor suppression as they are sub-regulated during cancer invasion including Maspin, DCC and DSG3.On the other hand, CATH, HER-3 and HSP-27 are overexpressed during cancer invasion. Some overexpressed proteins like CGG3 have significant role in in malignant transformation in cancer also termed as ALADIN [194]. A group of scientists compared the level of ubiquitin and calgranulin-A in 167 normal tissues with 122 tumor tissue and it was found that ubiquitin expression was decreased while the expression of calgranulin-A enhanced in tumor tissues. Schulz et al. [195] reported proteomic expression of TNBC compared with Her-2 positive tumors using MALDI-TOF/MS and 2D-DIGE. Through this technique, vimetin, L-plastin, glycolytic enzymes, fironectin, cytokeratins, annexin-1, annexin-2 and peroxiredoxin proteins were identified and validated by IHC and western blotting.

The study of cancer gene expression is not a straight forward process due to multiple protein modifications resulting in variations of immunotherapy [141]. Recently, a studied of four groups reported the survival patterns of breast cancer functional proteins [196] which revealed about 10 different protein biomarkers that might differentiate cancer subgroups biologically and clinically more accurately as compared to prognostic markers. Umar et al. [108] identified 09 tryptic peptides being differentially expressed by stromal and tumor analysis using laser capture microdissection. Afterward, Sanders et al. [197] reported the reduced expression level of S100-A8 and ubiquitin in cancer tissue as compared to normal tissue. In a study, 116 stage-IV cancer individuals having anti-PD or TILs therapies were investigated for proteome analyses using MS analysis. The results represented substantial changes in oxidative and lipid metabolism among respondent and non-respondent patients proteomes [198]. In another study, authors involved 46 cancer stage-IV patients undergoing targeted immunotherapy. He analyzed their plasma with ICIs using LC-MS/MS and antibody-targeted proteome analyses with PEAs to identify biomarkers. The authors found enhanced PD1 levels of only against anti-PD1 treatment. Conclusively, presence of plasma PD1 enhance the inhibition of endogenous PDL1 along with inhibition therapy [199]. The treatment adverse effects and drugs toxicity on normal cells in cancer patients can also be studied through proteome analysis via identification of representative biomarkers. For example, cytokines including IL1a, IL2 and IFNα2 might help in identification and management of immune-associated toxicity in metastatic cancers. A group of

authors reported upregulation of 11 different cytokines associated with immune toxicities in cancer patients treated with ICIs compared to the control group [200].

2.7.4 Metabolomics

Metabolomics is the study of cellular end products including low-molecular weight molecules and metabolites. These metabolites have key role in the cancer microenvironment and overall health condition of the cells. Metabolomics study can be targeted or untargeted. Targeted approach is a quantitative one along with the identification and localization of particular metabolites in a single analysis to predict role of metabolites in particular pathway or in a disease. Untargeted approach deals with the identification of metabolites only in a particular sample [133]. Bother approaches has its own merits and demerits and being used for the discovery of novel biomarkers like targeted approach was used for biomolecules identification of colorectal cancer while untargeted approach was employed in prostate and hepatocellular carcinoma [201, 202]. The blend of both approaches can be used to evaluate the high metabolite/biomolecules profile of tumors.

Serum and plasma analysis of cancer patients have been the main source of metabolomics studies so far for the identification and quantification of metabolite be used as key biomarkers for the diagnosis and treatment of invasive cancers [146]. These biomarkers are indicators of tumor progression, metastatic activity and drugs response. Among different serum identified biomarkers, LDH is considered as highly specific biomarker for cancer along with other prognostic and predictive biomarkers as it is being used for the prediction of drugs responses [203]. Studies showed reduced survival of patients with advanced melanoma and reduced ICIs response when their LDH baseline levels were high. It was later investigated that during hypoxia of the tumor glycolytic activity increases resulting in higher level of LDH in patients. Thus, in this category of patients VEGF growth factors in combination with glycolysis inhibitors and ICIs could be a new potential therapeutic approach [204]. Recently, clinical trials of KEYNOTE-001 were re-evaluated [205], and the result proposed that estimation of serum level LDH after the treatment of targeted therapy could be an efficient and valid biomarker for the determination of immunotherapy response. In pathology, another protein biomarker S100 is also reported whose elevated level is considered as indicator of metastasis, relapse of disease, treatment response and survival rate [206]. Additionally, basal level of protein subtype S100B is considered as trusted predictor for therapeutic choices in cancer patients undergoing immunotherapy against metastatic melanoma. In some cases, it has been reported that cancer patients receiving pembrolizumab alone or combination of pembrolizumab + ipilimumab treatments showed higher LDH and S100B baseline levels along with reduced survival rate compared with cancer patients having normal S100B level [207].

Cancer biology studies have identified few potential biomarkers, like modified carbohydrates moieties in leukemia and free unsaturated fatty acids in colorectal cancer [208]. In case of prostate cancer, citrate level and amino acids changes have

been reported [209, 210]. However, such changes are also reported of other diseases as well [211, 212]. So, the reports suggests that these biomarkers may not likely be specific to cancer due to shared disease pathological responses. Besides, it is still needed to investigate whether changes identified in serum or plasma metabolites are characteristic features in tumor cells or not [146]. For high throughput results metabolome profiling is being carried out by LC followed by MS analysis to provide identification and quantification data of metabolites changes in normal and disease state [213]. However, identification of unknown metabolite, diversity of putative metabolites and efficient reproducibility of target metabolites are key challenges which must be overcome for clinical level profiling. Still, metabolomics approach grips the high potential to be effectively used in the discovery of novel biomarkers for diagnosis and progression of cancer [184].

2.8 Case Studies of Multi-omics

Multiple transcriptome and proteome analysis have been reported to interpret the abnormal molecular mechanisms of cancer in different tissues including ovarian, colorectal squamous, breast and lung carcinoma. Through proteomic profiling, TCGA established linkage of genomics with expression profiling like transcriptomics and proteomics in multiple cancers including breast, colorectal and ovarian cancers. In a proteome study, 19 protein biomarkers have been identified in Her2+, estrogen-receptor + and triple-negative + breast cancers [214]. Out of them nine genes together with ENO1, MAPK3, STMN1 and MCM5 showed consistent changes in mRNA and protein expression levels, suggestion their potential as therapeutic targets in breast cancer treatment [214]. In another study, HNF4A, TOMM34 and SRC protein coding gene in the 20q chromosomal region, were extremely affected by amplification of 20q hence suggesting their vital role diagnosis and identification of cancer hallmark of proliferation [215]. Proteogenomic analysis of cancer tissues led the identification PAK1, RIPK2, TLK2 and CDK12, protein candidates showing gene-amplification-driven proteogenomic patterns [216]. Likewise, in ovarian melanoma profiling, CNAs trans-effect on protein expressional changes were found independent of mRNA expression levels. Meanwhile, CNA was reported to have vigorous trans-effect on Chromosome-2 effecting >200 proteins, while this effect was very negligible in case of mRNA. These highly effected proteins were mainly involved in cell migration and invasion representing their expected role of CNA-induced proteo-genomic events in conquering these cancer hallmarks [217].

In RNA-based cancer therapy miRNA has been of prime interest due to its oncogenes and tumor suppression properties. OncomiRs (miR155/miR21) block the expression tumor suppressor genes by endorsing carcinogenesis, cell transformation and metastasis following the suppression of mRNAs translation involved in oncogenic pathways [218]. Several studies have reported that tumor suppressors miRNAs are downregulated in different types of cancers [218]. Another strategy is an application of locked nucleic acid (LNA) anti-miRNA oligonucleotides with

complementary sequences to target miRNAs [174, 219]. Currently, SOLAR and PRISM, two clinical trials are being carried out to evaluate the safety and efficiency of Cobomarsen (MRG-106) which is locked nucleic acid antimiR® inhibitor for miR-155 for the treatment of cutaneous T-cell lymphoma, chronic lymphocytic leukemia, adult T-cell lymphoma and diffuse large B-cell lymphoma [220]. Second approach known as miRNA replacement therapy is being focused for the restoration of down-regulated tumor suppressor miRNAs using viral or non-viral vectors [221]. Nevertheless, targeting OMICs in cancer therapeutics hold strong potential for future clinical practice. Therefore, a growth in OMICs therapeutics in the coming several years is highly expected.

2.9 Conclusion

The understanding of cancer origins has been changed in recent years. Now the researchers transform their approaches in cancer examination and treatment from broad characterization towards genomic-based characterization of cancer. Although universal cancer treatments like chemotherapy played a very important role in the cancer therapy field, there are still many obstacles in these drug treatments such as drug shortage, drug side effects to normal cells and drug resistance. The new designs and implementation of methodologies that integrate several multiomic approaches to produce robust models can lead to significant improvements in cancer biology. Despite the fact that this discipline is still in its early stages, many breakthroughs are being made with the invention of new updated algorithmic approaches.

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Chemotherapy

3

Mahabuba Binta Hossain and Aahil Hossain Haldar Neer

Abbreviations

5-FU	5-Fluorouracil
G-CSF	Granulocyte colony-stimulating factor
GIT	Gastrointestinal tract
IV	Intravenous

3.1 Chapter 1

3.1.1 Goals and Principles of Chemotherapy

One of the major pillars of cancer treatment is the systemic anti-cancer drugs in the form of chemotherapy. Chemotherapeutics are cytotoxic drugs that are used in a majority of cancers with an aim to kill the cancer cells to prevent further growth and improve the survival rate or quality of life [1].

As chemotherapy agents are cytotoxic, their use is highly depending on the potential intent on the patient's benefit, where the benefit outweighs the toxic effects. There is a wide variety of chemotherapy agents and the treatment is decided after careful consideration of the need of individual patients [1]. Chemotherapeutics are commonly used for three purposes as below:

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1. Curative: The primary goal of curative chemotherapy is to eradicate the cancer cells for improving the life-expectancy and achieving complete remission. They are generally used in the early stage of cancer without any distant metastasis in two ways.
   (i) Neo-adjuvant: Chemotherapy given prior to the definitive treatment such as surgery to reduce the size and extent of malignant cells to achieve better effectiveness.
   (ii) Adjuvant: Chemotherapy given following the primary treatment such as surgery to destroy any remaining microscopic cancer cells to maximize the disease-free survival.
2. Palliative: The intent of palliative chemotherapy is to reduce distressing cancer-related symptoms. They are commonly used in systemic metastatic cancer patients where no other curative treatment options are available.
3. Combination treatment: Chemotherapy is often used together with radiotherapy to increase the treatment efficacy and to have an improved outcome. Concurrent chemotherapy along with radiotherapy is now considered a standard of care for some cancers.

3.1.2 Chemotherapy Administration

Chemotherapeutics are mostly delivered via the intravenous route through a central or peripheral vein or in an oral form. Sometimes chemotherapy can be administrated via subcutaneous, intramuscular, intrahepatic, intraperitoneal, intrathecal routes depending on the disease status and treatment goals [2].

The dose of chemotherapeutic agents is generally calculated according to the individual patient's body surface area but is based on weight for infants.

3.2 Chapter 2: Chemotherapeutic Drugs

3.2.1 Mode of Action

Chemotherapy agents work on different phases of a cell cycle. In a nutshell, the cell cycle is the process through which both normal and cancerous cells proceed to replicate [3]. This cycle constitutes a continuous sequel of the following stages:

G0: Resting phase
G1:Post-mitotic gap
S: DNA synthesis phase
G2:Pre-mitotic gap
M: Mitosis phase

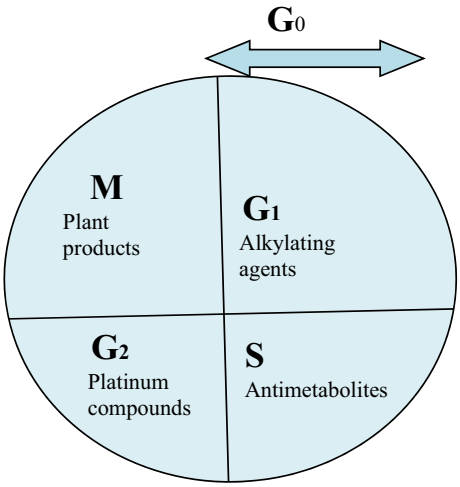

Fig. 3.1 Chemotherapeutic agents action on various phases of the cell cycle, alkylating agents on G1, antimetabolites on S, platinum compounds on G2, and plant products on M phase. (Picture adapted from Brown et al. [4])

Some chemotherapy drugs have activity on the resting phase, but the majority of chemotherapy drugs act on the other phases of the cell cycle [4]. These drugs disrupt normal cellular activity in one or more stages of the cell cycle and target the diving cells to inhibit further replication (Fig. 3.1).

3.2.2 Classification of Common Chemotherapeutics

There are varieties of chemotherapy drugs and most commonly they are divided into the following categories as described in Table 3.1.

3.2.3 Common Uses of Chemotherapeutics

Chemotherapy agents can be used as a single agent or as a combination of multiple drugs for improved outcome. For example, in breast cancer, a combination of epirubicin and cyclophosphamide (EC) or 5-fluorouracil, epirubicin, cyclophosphamide (FEC) followed by docetaxel or a combination of paclitaxel with carboplatin (PC) is generally used in adjuvant settings [6]. The common uses of chemotherapy according to the specific cancer type are described below in Table 3.2.

Table 3.1 Common chemotherapeutics and their mechanism of action [1, 3–5]

Types of chemotherapeutic agents	Mechanism of action	Common drugs
Anthracycline antibiotics	Natural compounds, derived from microorganisms, act by inhibiting DNA and RNA synthesis	Adriamycin Actinomycin-D Bleomycin Daunorubicin Doxorubicin Epirubicin Mitomycin Mitoxantrone Plicamycin
Antimetabolites (i) Folic acid antagonists (ii) Purine antagonists (iii) Pyrimidine antagonists (iv) Ribonucleotide reductive inhibitors	Structural analogs of natural metabolites, which either substitute or compete with key metabolites responsible for cellular function and thereby prevent DNA or RNA synthesis	(i) Methotrexate Trimetrexate (ii) 6-mercaptopurine Fludarabine (iii) 5-fluorouracil 6-thioguanine Cytarabine Capecitabine (iv) Hydroxyurea Gemcitabine

(continued)

Table 3.1 (continued)

Types of chemotherapeutic agents	Mechanism of action	Common drugs
Alkylating agents (i) Nitrogen Mustards (ii) Nitrosoureas (iii) Tetrazines (iv) Aziridines	Interacts with intra-cellular DNA, causing stand breaks and subsequently cross-linking of DNA to prevent replication	(i) Busulfan Chlorambucil Cyclophosphamide Ifosfamide Mechlorethamine Melphalan (ii) Lomustine Carmustine (iii) Dacarbazine Procarbazine Temozolomide (iv) Mitomycin C
Plant products (i) Taxanes (ii) Vinca Alkaloids	Derived from plants or plant extracts, bind with microtubules and prevent spindle formation to inhibit mitosis	(i) Docetaxel Paclitaxel (ii) Etoposide Irinotecan Vincristine Vinblastine
Platinum compounds	Produce antitumor activity through coordinating with DNA and forming DNA adducts to inhibit replication and transcription	Cisplatin Carboplatin Oxaliplatin
Miscellaneous (i) Topoisomerase 2 inhibitor (ii) Camptothecin analogs		(i) Etoposide (ii) Irinotecan Topotecan

Table 3.2 Common uses of chemotherapeutics in specific cancer type [7–13]

Cancer type	Cytotoxic drugs
Head and neck cancer	5-Fluorouracil (5-FU) Bleomycin Cisplatin Carboplatin Capecitabine Docetaxel Gemcitabine
Breast cancer	Doxorubicin Epirubicin Cyclophosphamide Docetaxel Paclitaxel Carboplatin
Lung cancer	Non-small cell lung cancer Paclitaxel Docetaxel Vinorelbine Pemetrexed Gemcitabine Etoposide Small cell lung cancer Cisplatin Carboplatin Gemcitabine Etoposide
Oesophageal cancer	Cisplatin 5-FU
Hepatobiliary and pancreatic cancer	Gemcitabine Capecitabine Irinotecan 5-FU with Folinic acid Nab-Paclitaxel
Colorectal cancer	Oxaliplatin Carboplatin Capecitabine 5-FU and Folinic acid Paclitaxel Nab-Paclitaxel
Gynecological cancer	Cisplatin Carboplatin Cyclophosphamide Gemcitabine Paclitaxel Pegylated Liposomal Doxorubicin

(continued)

Table 3.2 (continued)

Cancer type	Cytotoxic drugs
Neuroendocrine cancer	Carboplatin Etoposide Irinotecan
Sarcoma	Vincristine Vinorelbine Doxorubicin Cyclophosphamide Ifosfamide Etoposide Irinotecan Temozolomide Topotecan

3.3 Chapter 3

3.3.1 Side-Effects of Chemotherapy

3.3.1.1 Acute and Common Toxicity [3, 4, 6, 14]

1. **Myelosuppression**

(i) Anemia: Low hemoglobin count is generally observed with some anti-cancer drugs, and patients need to be monitored for signs and symptoms. Treatment depends on the severity of anemia and often needs red blood cells transfusion.

(ii) Leucopenia: Reduction in the number of white blood cells including neutropenia is common following chemotherapy which potentially increases the risk of developing infection which sometimes can be life-threatening. Depending on the severity, fever management requires protective isolation, immediate IV antibiotics, and G-CSF support.

(iii) Thrombocytopenia: Chemotherapies can lower the number of platelets and increase the risk of bruising and bleeding. Symptoms may characterize as petechiae or purpuric skin rash and bleeding from the nose, gums, urinary system, or GI tract, such as melena.

2. **GI Effects**

(i) Anorexia
(ii) Altered taste sensation
(iii) Nausea and vomiting
(iii) Mucositis: sensitive tongue, sore mouth, tender gum, oral candidiasis
(iv) Bowel alteration: Diarrhea, Constipation.

3. **Hair and Nail Changes**
(i) Hair thinning and hair loss
(ii) Brittle nail and color changes.

4. **Skin Reaction**
(i) Dry skin
(ii) Skin rashes
(iii) Palmar-plantar syndrome.

5. **Neurological Dysfunction and CNS Toxicity**
(i) Fatigue, tiredness
(ii) Headache
(iii) Peripheral neuropathy–generally observed as tingling sensation, numb feeling in hands and feet.

6. **Hypersensitivity and Allergic Reaction**

Common hypersensitivity reaction symptoms are sickness, flushing, chest tightness, back pain, and tachycardia. Those are generally managed with antihistamine and corticosteroids.

7. **Hepatic and Renal Toxicity**

Generally monitored with routine blood tests prior each cycle of chemotherapy.

8. **Blood Clotting Abnormalities**

Disturbance in the coagulation parameters commonly leads to deep vein thrombosis and pulmonary embolism.

3.3.1.2 Long Term Side-Effects [3, 14–16]

1. **Effects on the Reproductive System**

Sexual dysfunction and effects on fertility in both men and women may develop following the chemotherapy.

(i) In female:
Amenorrhea
Ovarian failure
Early menopause
Loss of libido, infertility.

(ii) In males:
Azoospermia
Impotence
Gynecomastia.

2. **Effects on Cognitive Function**

Some people may experience impaired cognitive function along with mental fogginess, poor concentration, and trouble with memory and intellectual impairment particularly in children.

3. **Effects on the Heart**

Anthracyclines drugs lead to cardiomyopathy with poor cardiac function, coronary artery disease, congestive cardiac failure, and arrhythmias that may be observed later on.

4. **Effects on Lung**

Late effects on the lung might be seen, such as reduced lung capacity, breathing difficulties, and pulmonary fibrosis.

5. **Effects on the Nervous System**

Peripheral neuropathy often persist for a long time following the chemotherapy.

6. **Eye Problems**

Some chemotherapy and its associated supportive medications including steroids can lead to dry eye and increase the chances of developing cataract.

7. **Hearing Problems**

Hearing difficulty or hearing loss can be developed following drugs like Cisplatin.

8. **Risk of Second Malignancy**

Certain chemotherapeutics such as alkylators and topoisomerase II inhibitors increase the risk of developing a second cancer.

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Radiation Therapies in Cancer

4

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4.1 Introduction

Radiation oncology is a multidisciplinary approach to treat cancer by prevention, medicine, and application of ionization radiation by understanding the knowledge of its cause at molecular level [1]. Biomedical sciences are devising ways through biotechnology to understand the cancer proteome and genome, its causative agents, gene expression, and biosynthesis. Today cancer cells are studied at molecular level by using different techniques such as modern radiobiology, molecular pathology, molecular pathophysiology, molecular imaging, and molecular targeting. Radiotherapy is practically more promising than any other techniques as it only acts on cancer stem cells (CSCs) [2].

Neoplastic cells are abnormal cells which stimulate tumor growth. There are ten "*Hallmarks of cancer*" which consists of proliferative signaling, evading of

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growth suppressors, activating Invasion and metastasis, enabling of replicative immortality, inducing angiogenesis, resisting cell death, deregulation of cellular energetics and metabolism, evading immune destruction, tumor-promoting inflammation and genome instability and mutation. These markers occur when there is genetic alterations or modification on oncogenes or tumor suppressor genes [3, 4].

The cancer cells are produced due to changes in cells at genetic or epigenetics levels. Some chemical agents and exposure to certain radiations also lead to development of tumor cells like papillomaviruses [HPVs] in humans. At genetic level, cancer genes are divided in two classes namely as *Oncogenes* which cause gain of function when dominant mutation occurs. Its function is stimulated by cell proliferation by activation of *ras* signals. Second class is *tumor suppressor genes* which cause loss of function when recessive mutation occurs. Cancer transmits to next generations called inherited cancer predisposition, having rare groups; cancer syndromes and familial cancers, other is frequent group named as weak predisposition cancers. Studies showed that about four to seven somatic gene alterations cause initiation of carcinogenesis [5]. Molecular Radiation Biology studies showed that disability of many functional and structural consequences which may lead DNA repair, cell cycle delay, cancer stem cells development, triggered antitumor immunity, instability of genome, mutations, and altered gene expression. In terms of functional disability, cell death is the most common and important in radiation oncology. These effects are today treated with radiotherapy and chemotherapy. Biological markers are also being under used to cure cancer. Brachytherapy is the other important technique of radiation therapy too.

4.2 Basis of Radiation Therapy

In treatment of cancer, the Ionizing Radiation (IR) is used to break targeted cells at molecular level. Radiotherapy (RT) damage depends upon "*Linear Energy Transfer (LET)*" that can be high or low influenced by many reactive species and free radicals like oxygen, superoxide, and hydrogen peroxide. The mechanism is initiated by oxidative stress such as ROS/RNS which is major cause of inflammation leading to physiological dysfunction and cell death in target site. The RT targets all cellular organelles, but main target is DNA recognized by sensors like ATM/ATR. In response to cancer RT, DNA has two major pathways to repair itself that are nonhomologous end joining (NHEJ) and homologous recombination (HR). The former is more efficient as it is error prone and not cell cycle specific. Further, it is difficult to repair due to diverse lesions, and complex DNA damage is slow and difficult. Other than DNA, other cells activate signals "Immediate Early Response" leading to redox activity. After irradiation, the radio sensors in cell determine its fate. It can be cell death which can occur in several ways like *Mitotic death* in M phase, *Apoptosis* in Interphase, *Necrosis* in pathogenic tissues caused by IR, *Autophagy and Senescence* leading to removal of damaged cell from reproducing pool and lowering the risk of radio carcinogenesis. Tissue damage responses are several but before that we should know that tissues are classified as rate of cell

loss and regeneration called "*Turnover*." Tissue damage responses are of three main types "Acute, Sub-acute, and Late effect" with changes leading to stenosis, fibrosis or necrosis, acute ulceration, etc. Acute response last about 6–8 weeks during RT with rapid turnover. Sub-acute responses are displayed about several months after RT causing cell loss and inflammation. Lastly, late responses are severe and with limited recovery as its effect can be both acute and chronic. It is also studied that late responses are due to dysregulated homeostasis.

In RT, the tolerance doses vary tissue to tissue determined by intrinsic radiosensitivity, repopulation of cells, and other factors. Even the dose volume called "Volume effects" is also important when applying dosage as larger tissues require low amount of it. Today oncologists use dose–volume histograms (DVHs) to develop treating plan. Regeneration or repopulation effect can be measured by split dose with two doses given in separate time, and the second dose is important for isoeffect. Growth factors also increase the regeneration like G-CSF of IL-11 in hematopoietic cells [6]. Tumor radiobiology shows us that cell death is important in radiocurability. And molecular markers of apoptosis and human curable tumor yield results showing that CSCs do not express proapoptotic phenotype, but cell undergo senesce or autophagy have high chance of surviving and its reprogramming. Tumors have high turnover rate with acute response in normal tissues while have slow turnover rate with late response in prostrate or breast cancer. Rate of cell loss is called *Tumor regression* symbolized by cell loss factor (ϕ), <1 required for tumor growth. The repopulation process will also be slow with decrease in ϕ in all tissues. Scientists are developing the standard regimens of dose fraction patterns to cure clinical and subclinical diseases. The patterns involve accelerated treatment, hyperfractionation, and spatial/low dose rate of continuous irradiation. Quality of radiation is measured by linear energy transfer (LET) and relative biological efficiency (RBE) [7].

4.3 Pathophysiology of Cancer

Tumors are produced by neoplastic cells which are like normal cells. In 2000s, the path to prevent cancer was suggested to be anti-angiogenesis strategies in renal cells, gastric cancer, etc. [8]. The vascularization in tumors has pathways such as co-option, intussusception, sprouting, vasculogenesis, vascular mimicry, and transdifferentiation [9]. The molecular mechanism of vascularization is dependent upon growth factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), etc. They help in blood vessel maturation and repopulation. This all can be stopped by various inhibitors and proangiogenic receptors. The product of pro- and anti-angiogenic molecules is triggered by several response which can be mechanical, metabolic, inflammatory, or genetic mutations. The tumor vessels have specific interconnection patterns that are dilated, saccular, tortuous, and chaotic. The branches of vessels are uneven in diameter and many trifurcations [10]. The blood flow in tumors is lower due to abnormalities in blood viscosity and vasculature increase flow resistance [11]. The understanding

of microenvironment and vascular permeability helps us to improve the delivery of molecular medicine to tumor site [12].

When the tumor cells are in extravascular region, there are both neoplastic cell and host cells resides in interstitial fluid. They can move across the wall of vessels like immune cells. In tumors, the basement membrane is defective [13], and transport is likely to be done by diffusion and convection [14]. Lymphatic vessels are also present in the marginal membranes of tumors. The metabolic environment of tumor cells is hypoxia (low oxygen level in cells), extracellular low pH (depend upon H^+ ions), and therapeutic consequences. There are two major clinical implications which we face physiologic barriers and inherent or acquired resistance of cancer cells [15, 16]. We have developed predictive biomarker implications and therapeutic implications to predict and destroy the cancer forming sites by targeting ECs. Further studies should show more complement strategies leading to better understanding of pathophysiology of tumors.

4.4 Radiation Therapy and the Immune System

A crucial element of cancer treatment is radiation therapy that is used to destroy tumors and cancer cells through radiation. Another essential component is immunotherapy that helps immune system to combat cancer. The combination of both radiation therapy and immunotherapy is being focused recently for the treatment of many tumors. Both collectively can enhance immune responses and change phenotype of tumor cells [17]. They also activate sustained antitumor immune responses. The motive for combining radiotherapy and immunotherapy can straightly cause cell death and start inflammatory response in tumor cells [18]. Radiation therapy is of two types, i.e., photon radiation therapy and particle radiation therapy. Photon radiation therapy is given using highly penetrating radiations like photons or X-rays which are quite effective in destroying local tumors [19]. Other type is particle radiation therapy which deposits lower energy when it enters tissues and when it stops at the tumor it releases a peak dose of energy called Bragg peak [20]. Both these types of radiation therapy can be used in various immunotherapies for the generation of antitumor immunologic memory and enhancement of effector immune responses. Methods of radiation treatment must be designed in a way that enhance anticancer immune response. Nowadays, this concept is common among radiation oncologists that the higher the radiation dose, the higher is the cell destruction based on Puck's seductive in vitro clonogenic cell survival curves [21]. Increasing radiation field size is another concept in this regard to eliminate macroscopic tumor and subclinical disease. Recent clinical trials differ from these concepts. Although it is necessary to apply a small dose of radiation, attempts to further increase the doses have failed to help in many types of cancer care or treatments [22]. Moreover, large radiation fields have less tolerance especially in concomitant chemotherapy. Cell survival curves created by Theodore Puck show that there is an exponential relation between radiation dose and cells killed by negating systemic effects and the role of the microenvironment.

After many years of fundamental research, the importance of the immune response in cancer treatment is becoming clearer and more obvious.

4.5 Chemotherapy and Irradiation Interaction

Chemotherapy includes the use of some chemical agent to control the growth of cancer, whereas irradiation involves the use of radiations of high energy to kill cancer cells. The union of both became the strongest practice in cancer treatment techniques. The chemoradiation therapy not only have been a clinical success using traditional drugs but also lead to new discovery of chemotherapeutic drugs for their interaction with radiations. To develop new and better treatment strategies and research the knowledge of basic principles for using chemotherapy, molecular targeted agents, and immunotherapy in combination with radiation treatments and mechanistic interactions between drugs and radiation is necessary [1–11].

Radiation and chemotherapeutic drugs both have a limitation that they are unable to prevent normal tissue cell damage. When their dose is increased, the damage of normal cells become more severe. This relationship between dose and impact helps to find therapeutic index. It is a quantitative measure of desiribility of a drug. It is defined as the ratio between the dose that produces desired effect and the dose that produces damage. This ratio should be more than 1 or positive to get therapeutic benefit. To get positive therapeutic results of treatment, there are many ways that can be adopted. Steel and Peckham grouped them into four groups: spatial cooperation, independent toxicity, enhancement of tumor response, and protection of normal tissues [12]. Spatial cooperation is the basis for combining chemotherapy and radiation therapy in which radiation is first used to control the primary tumor and then chemotherapy is used to confront micrometastases. Independent toxicity is another strategy in which chemotherapeutic drugs-induced toxicities are made not to overlap radiation-induced toxicities to increase therapeutic ratio of chemoradiation therapy. Enhancement of tumor response means to use the ability of chemotherapeutic agents to increase tumor radioresponse through interaction between drugs and radiation at the molecular, cellular, or pathophysiologic level, resulting in an antitumor effect. Protection of normal tissues can be achieved either by delivering radiation in an improved method or by selecting such chemical or biological agents that protect normal tissue cells.

Drug–radiation interaction is assessed by clinical trials prior to their usage in a combination for cancer treatment. This assessment can be done both in in vitro and in vivo to observe antitumor activity and normal tissue toxicity. Clinical chemoradiation therapy is being considered in devising the best timing of drug application regarding radiation therapy. Keeping the purpose of therapy in mind, drugs are applied before, during, or after the series of radiation therapy. There are different strategies used for chemoradiation sequencing: induction or neoadjuvant chemotherapy (before), concurrent or concomitant chemotherapy (during), and adjuvant chemotherapy (after). In induction or neoadjuvant chemotherapy, chemotherapeutic drugs are given before a course of radiation therapy. It makes

tumors more controllable by radiation with the objective to cope with primary tumors. It also uses smaller radiation field which leads to less exposure and damage to normal tissue cells. The other strategy is concurrent or concomitant chemotherapy in which chemotherapeutic drugs are given during a course of radiation therapy. It is used against dispersed lesions and the primary tumor. It also provides better results in controlling local tumors as compared to other forms of chemoradiation therapy combinations [13, 14]. In adjuvant chemotherapy, chemotherapeutic drugs are given after a course of radiation therapy. Its main target is disseminated disease.

Specific chemotherapies are combined with radiation in the treatment of cancer after proper preclinical assessment of their effectiveness. Some classes of compounds include platinum-based drugs, antimicrotubules (Taxanes), antimetabolites (5-Fluorouracil, Capecitabine, Gemcitabine, Pemetrexed), topoisomerase I inhibitors, alkylating agents (Temozolomide), and other agents (Mitomycin-C, Hypoxic Sensitizers, Nimorazole) [15].

More particular and effective chemotherapeutic agents are being produced to improve chemoradiation therapy. Also, the treatment of normal tissues is being minimized and application to tumor is being maximized by improving radiation therapy delivery techniques [16]. Protection of normal tissues from drug damage is another effort in the similar regard. Moreover, clinical experience of the combination of chemoradiation and radiation therapy has also highly increased in recent years [17]. Many molecular pathways have also been discovered that serve as targets for enhancing responses of radiation and chemotherapy. As there exist some important radiation issues pertaining to combination of immune agents with radiotherapy, there is need of a careful and organized approach to clinical trials. New techniques and trials in coming years must be potential enough for improvement in therapeutic effectiveness of combination of chemotherapy and radiation in the battle against cancer [18].

4.6 Clinical Radiation Oncology

Each kidney is encased by a fibrous capsule and encircled by perinephric fat. At the renal hilus are the pelvis, ureter, renal artery, and vein. The lymphatics of the kidney and renal pelvis drain along the renal vessels. The abrasions debated in this section are restricted to mature renal cell carcinoma and urothelial carcinoma of the renal pelvis and ureter. Acquired cystic kidney disease (ACKD) upturns 50-fold the risk of developing renal cell carcinoma (RCC). VHL disease causes hypoxia, with eminent heights of HIF-1-α, despite the existence of normal oxygen levels. Around 88% of solid renal multitudes are malicious, and the possibility of malevolence is comparative to the extent of the wound [19]. Cigarette smoking is the most chief element putting up to the universal prevalence of urothelial cancer in Western countries. Sufferer with Lynch's syndrome has an enlarged threat of evolving urinary tract cancer. Subsequently 2002, kidney cancer disease rates have decreased approximately 1% per year [20]. Arsenic-contaminated water has been

correlated with an inflated appearance of upper urinary tract carcinoma. About 45% of tolerant with RCC have restrained disease, 25% have regional disease, and about 30% have corroboration of remote metastases at the time of verdict.

Lymph node metastases occur with a prevalence of 9–27%, and utmost frequently involve the lymph nodes in the renal vein. Corpulence, diabetes, hepatitis C, and hypertension are likewise concomitant with a sophisticated comparative menace for enlargement of these tumors [21, 22]. Renal cell carcinoma (RCC) presents as an incidental mass on an indicative imaging study methodical for additional determinations. Patients with RCC may be contemporary with an occult prime tumor or through emblems and indications determinable to a local mass or inherent paraneoplastic syndromes. Constitutional chromosome 3 relocation is accompanying with numerous, bilateral vibrant cell RCCs [23]. The analysis of renal cell carcinoma (RCC) is reputable clinically and radiographically in furthermost circumstances. If a central renal mass suggests the presence of urothelial cancer, careful consideration should go into urine cytology, ureteroscopy, and biopsy. Patients with symptoms evocative of bone metastases should undergo a bone scan. Cystoscopy is very imperative because of the extraordinary incidence of manifold tumors. CT or MRI of the abdomen and pelvis previously and subsequently divergence supervision gives valuable gen. renal cell tumors (RCC) which comprise 90% of all malignancies in the kidney. About 40–50% of suffering people through better urinary region tumors will have a synchronous or metachronous bladder cancer [24, 25].

Vibrant cell RCC is the utmost corporate, monitored by papillary RCC and chromophobe RCC. Renal medullary carcinoma is a violent malignancy customarily associated with sickle cell trait. The 5-year survival rate of patients with kidney cancer has folded over the last 50 years. Extrapolative structures for RCC are tumor, sufferer, and test site allied. Tumor interrelated features embrace stage, tumor size, tumor grade, histologic type, necrosis, and sarcomatoid transformation. The intermediate endurance period of patients with sarcomatoid renal cell cancer (RCC) is merely 6.6 months, related to 19 months for histologic categories. In order to soothe the tenacity of cancer-free persistence in patients with RCC, nomograms and algorithms must be pronounced. A small percentage of RCC patients eventually develop metastatic infection [26]. There is no worth mentioning modification in extrapolation between urothelial carcinomas prompting in the ureter and those ascending in the renal pelvis. Finest tumors are escorting with a sophisticated frequency of metastases and endurance proportions. Grown-up patients and those cured with parenchymal-sparing measures had complex rates of relapse. Tumors in the ureters were further probably to continue than those in the renal pelvis. A radical nephrectomy comprises perifascial resection of the kidney, peri-renal, regional lymph nodes, and ipsilateral adrenal gland. An experienced squad would be intricate in the outlook of thrombus, as treatment-related transience can influence 10%. The furthermost numerous indicators concomitant with RCC is hematuria, either gross or microscopic, when there is assault of the assembling structure [26].

In patients undertaking nephron-sparing surgery, disease-free survival is restored than in patients undergoing radical nephrectomy. There is specific peril

that thrifty of the renal parenchyma may dispensation microscopic lingering tumor or defectively indulgence multifocal cancers. Until 2005, wide-ranging supervision for RCC was customarily inadequate to cytokine therapy. Cryoablation and radiofrequency ablation have arisen as potential treatment options. NCCN strategies list the following first-line systemic therapies for clear cell histology: pazopanib, bevacizumab + interferon, temsirolimus, sorafenib, high-dose IL-2 for selected patients. Chemotherapy has inadequate use in RCC because it is one of the supreme chemotherapy-resistant solid tumors. The resection of one or a restricted quantity of metastases in amalgamation with nephrectomy or at relapse has been associated with a 13–50% 5-year survival. Neoadjuvant radiotherapy did appear to be escalating the rate of complete respectability in patients with locally advanced tumors. An algorithm for the checkup of renal commonalities has remained anticipated [27]. No advantage was confirmed in patients receiving 30 Gy RT in 2 Gy fractions with respect to overall endurance or survival free of metastasis. Adjuvant radiation therapy (RT) is given after the primary treatment to lower the risk of tumor relapse or metastatic ailment. Several minor phase I and II trials are discovering the role of SBRT in the administration of renal tumors.

Some homework has exposed that intensifying the WBRT prescription elsewhere 3 Gy could repair the consequences in RCC patients with brain metastases. Overall actuality at 6 months was 29% after 3 Gy × 10 and 52% subsequently sophisticated doses (P = \r.003). The inclusive threat of lymph node metastases is 20% [28]. The authors settled an algorithm for the supervision of RCC intellect metastases. Good-response patients endured profoundly extended than the poor-response assembly after SRS, a study set up. The median subsistence for patients unloading radiotherapy for cranial cancer (RCC) is 16.6 months, associated with 7.2 months for those not reception conduct. A biologically effective dose (BED) > 50Gy10 was associated with an increased rate of response: 59% versus 39%. Patients with RCC are treated with single-dose, intensity-modulated radiotherapy (IMRT) to a prescription dose of 18–24 Gy. An erstwhile time gone by of bladder cancer has been conveyed to get worse the diagnosis of patients with subsequent urothelial cancers concerning the upper tracts [29, 30]. Toxicity rates were low and correspondent in both groups, with no grade 4 or 5 toxicity reported. Radical nephroureterectomy is the simply theoretically therapeutic management for supreme convalescent with urothelial carcinoma of the renal pelvis or ureter. Neoadjuvant chemotherapy may be considered in selected patients. Patients who have the uppermost risk of lymph node metastases have a high hazard of systemic disease. Adjuvant radiotherapy (RT) may weaken the probability of local tumor recurrence, but it does not appear to have an influence on survival or reducing future distant metastases. There are no randomized trials on the role of postoperative RT in convalescent with greater urinary region cancer. Radical nephrectomy-encouraged persistent renal inefficiency is additionally accompanied by an increased risk of cardiovascular death and death for any reason [31].

The pathologic similarity of urothelial carcinoma of the renal pelvis and ureter to bladder cancer partakes invigorated medical oncologists to use similar treatment regimens. Standard MVAC is no longer used because it is less efficacious and more

toxic. The dose to the stomach and small bowel should be kept below 45 Gy and the small bowel V45 < 195 cc when it is contoured as a bowel bag. Sparing at least 700 cc of the liver from radiation is another potential strategy to avoid complications [32].

Skin cancer is the most communal of all malignancies. Most skin cancers are accomplished surgically. Because of the functional and/or cosmetic deficits that may occur after surgery for skin cancers of the head and neck, this chapter will focus on lesions in this area. The most common carcinomas of the skin are basal cell carcinoma (BCC) (65%), SCC (30%), a variant of BCC or SCC, and the adnexal carcinomas. Merkel cell carcinoma (MCC) is an infrequent neuroendocrine malignancy arising in the skin that was first described in 1972 [33]. No reliable histologic criteria exist to discriminate the source of carcinomas of the sweat gland. MCC is a small cell neuroendocrine carcinoma ascending in the skin. Certain lacerations keep on circumscribed to the epidermis and could encompass an enormous expanse of skin [34]. Classifications of spindle cell tumors are documented. SCC occurs added repeatedly on the ears, preauricular and temporal area, scalp, and skin of the neck. Adenocarcinoma is a sporadic tumor. The eyelid is the primary site in about one-half of cases. Local re-emergence is common after excision. MCC was misjudged as BCC, lymphoma, adnexal carcinoma, or carcinoma metastatic to the former skin as of additional chief place [35].

Keratoacanthoma benign lesions start as a firm, round skin nodule and grow to 1–2 cm. Merkel Cell Carcinoma occurs primarily in white men between 60 and 80 years of age. Melanoma is more aggressive, and more than 1,665 suffering people were reported [36]. This is prone to metastasize to regional lymph nodes. Human polyomavirus DNA in the MCC cells may be accompanying with an upgraded projection. The menace of lymphatic metastases is predictable to be 10–15% for cutaneous SCC of the skin. The jeopardy upturns with the size of the graze, profundity of dissemination, histologic ranking, and reverberation. The prospect of remedy is analogous subsequently surgery or RT for early-step BCCs and SCCs [37]. Lymph node metastases, however, are seen on sporadic occasions. Patients with lymphocytic leukemia and skin cancer often have enlarged lymph nodes from both processes. Large lesions occurring on the free skin areas (i.e., not involving the eyelid, or periorbital areas) habitually can undergo biopsy and be treated with surgical excision. The possibility of cure is similar after surgery or RT for early-stage BCCs and SCCs. The menace of impediment is not as much of after RT of skin cancer linked fragment [38, 39]. Postoperative RT is added after surgery if pathologic examination reveals findings indicative of a high risk for local recurrence. 167 long-suffering persons cured at Westmead Hospital (Sydney, Australia) amid 1980 and 2000 for cutaneous SCCs metastatic to the parotid and/or cervical nodes [40].

Close or positive margins and/or invasion of nerve, cartilage, or bone may be considered. Postoperative RT may be withheld if the primary site is located on the free skin. Most early skin cancers are managed with orthovoltage RT with beam energies of 100–250 kVp. The supreme dosage is at the membrane exterior; bolus

is not obligatory, and there is fewer shaft of light tapering mutually at the superficial and at deepness so that reduced fields can be used. Suffering persons cured with orthovoltage RT for 1,267 skin cancers and monitored for an intermediate of 82 months [41]. Intensity-modulated radiotherapy (IMRT) may be used in selected patients to create a supplementary conformal prescription dissemination. Proton beam radiotherapy may also be used. The parotid gland and upper neck are cured with face varied beam of 6-MV X-rays and high-energy electrons. The probability of local control after irradiation for skin carcinomas of various shapes and sizes is mentioned. Treatment with orthovoltage irradiation yields indigenous control rates that are as upright as, or improved than, other conduct modalities. Adjuvant radiotherapy (RT) is an operative mode for the primary cure of skin cancer as well as in the adjuvant setting. It is used to treat the primary skin lesion when resection would result in an unacceptable functional and/or cosmetic outcome [42].

4.7 Radiation Oncology Education

Radiation oncology refers to a unique specialization in which one must have vast knowledge because it deals with patients of almost every age group and every gender and interferes with a wide range of medical specializations. A radiation oncologist must have the vital clinical experience and competence that is required to examine a new patient and effectively detect a tumor [43], analyze, and provide treatment plans to deal with the target volumes of the tumor in the specific organs that are at risk [44] and can assess the patients during their radiation treatments and after it to check for toxicities and chances for the occurrence of tumor [45]. The main objective of this chapter is to provide education to people learning to become radiation oncologist, and who want to provide their service to patients. Details for training programs on radiation oncology can easily be searched in the 1950s literature [46], and nowadays, such programs for radiation oncology commonly exist on global level [47]. To develop a better understanding for medical related education and its curriculum creation, it is must to search beyond the literature of just oncology and sometimes even further from medical literature. Many of the learning and teaching theories have their concepts established experiences related to transformation and behavior. The objective of any intervention related to education is to create an effect on the learner's change in behavior using a certain type of experience. A successful creation of a curriculum needs a method that is based on some social science as well as some business relating theories. Radiation oncology is a unique field that is initiated in the undergraduate time of the medical education. The students starting medical school all have different capabilities and varied experiences, and they have a lot of presumptions about which kind of specialty should they choose for their future. There are several practices reported for the development of radiation oncology experiences related to education in context of the basic UGME [48]. It is vital for a radiation oncologist who is in practice, to make sure that he is up-to-date with the recent innovations. Probably, it can be

said that radiation oncology is progressing even more than other fields of medical sciences and is also continuously evolving which we can say might be due to constant development of new innovative technologies and novel treatments for patients of cancer. The continuous maintenance of the new developed skills in the field of radiation oncology is a complicated procedure, and the practical application of those new and novel techniques while maintaining the level of competency can be a quite challenging job [49].

The evidence that supports the effectiveness of CME for how it can maintain and manage the competency level is mixed. Previous studies suggested that CME has a very strong optimistic effect on the performance of the practitioners and outputs of the patients [50]. The current results of many studies have failed to describe an important improvement in the outputs of patients for practitioners engaging in the current management of activities relating competency [51, 52].

The educators of radiation oncology must assess carefully all the trainings on national and international level and evaluate innovations to make sure on-going active revisions of the curriculum. Disruptive innovation is a technique used to ensure that variations. In the context of business, we can say that disruptive innovation is something that refers to "Prominent players focus on sustaining innovations in many markets. Which makes a market ready for new companies going to introduce disruptive innovations more convenient products or services." This concept that narrates what is disruptive innovation can be easily applied in the field of radiation oncology for the betterment of education. The recent oncology curriculum currently taught around the world that fulfill the requirements set by all the accredited bodies still cannot be considered as the greatest or efficient methods of for delivering a lecture on a selected topic or branch of radiation oncology. Education-related new innovations that are considered as efficient throughout the difficult curriculum inquiry, evaluation of a program, and assessment of learning have a great capability of making a revolution in the curriculum of radiation oncology having the ultimate objective improving the quality of service for patients on a great scale [53].

4.8 Legal Considerations in Radiation Therapy

Many medical schools now require students to take an introductory course in ethics, and most curricula now include ethics-related coursework. The term "bioethics" refers to the academic study and public policy movement concerned with the humanistic application of science and medicine [54]. Among the more influential theoretical approaches are utilitarianism, deontology theory, casuistry, theory of virtue, and feminism. Utilitarianism is defined as theory of action that can be applied to policy of health to ensure that decisions are made in the best interests of the greatest number of public. Deontology is a moral theory that focuses on the morality of activities rather than their outcome [55]. Casuistry is an ethical approach that stresses inductive analysis build on precedents, and it is widely used in law regulations and medicine. Virtue theory captures the way in which correct

ethical actions occur and explains why it is necessary to inform patients the reality about their diagnoses yet do so in a compassionate manner. Feminism does not focus solely on women's issues, but on those who are traditionally subject to discrimination.

A prima facie obligation is one that is enforceable unless it is superseded or outweighed by other moral responsibilities. Respect for autonomy, beneficence, nonmaleficence, and justice are the four basic concepts of bioethics. Most patient–physician relationships begin with respect for a patient's autonomy, which is predicated on a patient's decision to get medical care or agree with a referral to a specialist [56]. The nonmaleficence principle states that physicians should behave in the best interests of their patients and not hurt them. According to the distributive justice calculus, everyone should be able to get medical treatment based on their medical requirements and the medical system's ability to offer it. Many physician-authored declarations of medical ethics have been published. Each piece's voice and language reflects the social standards and historical incidents of the time in which it was written. The Hippocratic Oath is factor of the Corpus Hippocraticum, an ancient Greek collection of medical treatises [57]. Medical Ethics was written by Thomas Percival at a period when there was a lot of friction among professionals in his community [58]. Medical Ethics served as a model for the American Medical Association and other institutions' codes of ethics in the nineteenth century. According to the American Medical Association, a physician must respect professional norms, be truthful in all professional dealings, and seek to report a physician who takes part in fraud or dishonesty [59].

Conflicts of interest can arise from financial connections with hospitals and consulting physicians. The physician's desire for personal benefit can inadvertently influence treatment choices. Radiation therapy can be associated with a number of slighter difficult therapies that a physician could provide in his or her office, such as simple tests of blood or injections of antibiotic. Non-radiation oncologists may own linear accelerators and derive profit from the utilization of this technology on patients referred to them. Innovative treatment delivery methods can be intuitively appealing but cannot be assumed to provide meaningful clinical benefit unless tested against existing standard treatment [60]. Flagrantly, improper clinical research taints the annals of medical history. Exactly how much information should be conveyed to a potential clinical trial participant is debatable. The National Society of Genetic Counselors have published a guideline concerning genetic cancer risk assessment, counseling, and testing [61]. Physicians should be aware of any real or perceived influence on the patient–physician relationships resulting from their tacit compliance with such marketing. In-kind advertising items such as patient education pamphlets and anatomic models bearing a sponsor's name are commonly found in radiation oncology clinics.

Palliative medicine places emphasis on symptom management and on the goals of care. Palliative care can also be beneficial for patients with high symptom burden who are still receiving anticancer therapy. The first cases of malpractice involving the clinical use of radiation therapy were first tried in the US court system [62]. According to a recent study, medical errors may be the third largest

cause of death in the USA. Physicians are often reluctant to discuss medical errors because of fear of litigation and uncertainty about their accuracy.

The cost figure of cancer control in the USA is escalating precipitously from $24.7 billion in 1987 to $157.77 billion in 2010 dollars (in 2010 US dollars) assuming constant incidence, survival, and cost [63]. The US national health spending is projected to rise 1.2% points faster than GDP per year over the next five years [64]. Greater than 70% of cancer diagnoses will be made in Medicare-eligible people by 2030. With mounting care costs, concern in health policy and economics of health has flourished. Health policy as a discipline encompasses questions of access, cost, and quality of health care. Competition in health care should focus on the art of care's quality, such as how patient focused it is and how staff and clinicians connect with patients. Radiation oncology has been labeled as an outlier in its contribution to healthcare spending. Total Medicare payments for external beam radiation therapy grew from $256 million to $1.08 billion between 2000 and 2009 [65]. Radiation oncology plays fundamental role in the curative and palliative treatment of cancer. Its continued value in cancer care will depend on the advancement of evidence and research. Rising healthcare spending has led to calls for an increased focus on "high-value care" by policy makers, providers, payers, and patients in the context of healthcare reform. A "good value" product or service is one that is desirable and may be obtained at a reasonable cost. A working precision of value in health care has been developed by Michael Porter and Elizabeth Olmsted Teisberg. Value-based care shifts focus away from volume of services as is typical in traditional fee for service systems. Costs are defined to include the total costs of the complete cycle of care as opposed to individual services. The Accreditation Program for Excellence, developed by the American Society for Radiation Oncology (ASTRO), evaluates a practice of personnel, equipment, therapy planning, medical documentation, patient safety plans, and quality assessment activities against a set of performance standards [66]. The way care is delivered, with a focus on patient focus, accessible, and harmonized care, is referred to as the process. Review of medical data, interviews with patients, and direct inspection of healthcare visits can all be used to assess the process. The impacts of health treatment on patients or populations are referred to as outcomes. The medical community excels at evaluating objective results.

Advancement of accurate methods for risk assessment and risk stratification is an important step forward in the field of oncology, as well as in many other areas of medicine. In recent decades, patient-centered cancer care has gotten a lot of attention. Through a shared focus on high-quality treatment, patient-focused care and value-based look after are organically aligned [67]. However, it has distinct attributes not typically included in discussions of value such as patient experience and preference measures. Patients are left to make important healthcare decisions with a relative deficiency of information, unable to assess the original costs or quality of care afforded by different providers. Cost-effectiveness analyses assess the costs relative to health outcomes for a particular medical intervention. Decision analysis can help outline the trade-offs for different treatments in clinical situations when no good evidence is available. When there isn't enough evidence to decide,

decision analysis can help define the trade-offs between competing therapies. A set of mathematical methods is used in decision analysis to determine the most beneficial outcome for a given endpoint. Sensitivity analyses vary a parameter (for example, the chance of prostate cancer recurrence) over the whole range of possible values [68].

4.9 Smart Radiotherapy

The main objective of this study is to view a broader perspective of the new emerging concepts, which are being used in the field of oncology that are related to the practitioners and educators of radiation oncology. The main point probably can be considered as that radiation oncology is the base or center of all the cancer care science fields. In the previous edition of this chapter, there was a concept introduced in which radiation was referred to as a drug and a modifier for immune system. Luo changed the "cancer hallmarks" that was a model presented by Weinberg and Hanahan by making the non-cancer genes their specific target and the genes related to oncogene addiction. The metabolic and the biochemical reactions happening in the cell performs the functions of almost all the genes which as a result the cancer therapy does the main function of turning the genes off or on and responsible for disrupting the pathways of metabolism and biochemical reactions that are activated by it [69, 70].

Tumor microenvironmental variables, including as the amount of tumor oxygenation, tumor pH, and the interaction of tumor cells with host stroma and inflammatory cells, all influence the efficacy of radiation. Tumors have inadequate vascular perfusion, low pH, and low oxygen levels. This may create areas where cytotoxic drug delivery and activity are both possible in which cancer stem-like cells (CSCs) reside, therapies are reduced [71, 72]. Furthermore, specific tumor settings may favor more aggressive or resistant tumors oncogenes for tumor [73]. One aspect that has gotten little clinical attention but may be important is the impact of mechanical force on the environment provides an avenue for future advancements. Cancer cell change, progression, and therapy response [74]. Similarly, the interaction of stromal components with integrins causes signaling on cancer cells has the potential to alter tumor survival and give targets for treatment.

Vasculogenesis, unlike angiogenesis, involves the recruitment of cells from distant places, such as the bone marrow, to help build new blood vessels [75]. Although the precise contribution of the different recruited cell types is still a matter of debate, the process is thought to be an important contributor to the tumor vasculature [76]. Myeloid cells that express CD11b and matrix metalloproteinase-9 (MMP-9) appear to be at the center of this process [77]. A CD11b neutralizing antibody decreased myeloid cell migration to malignancies and delayed radiation-induced regrowth delay. Furthermore, mice with lower CD11b expression had better xenograft radiosensitivity [78]. The extracellular matrix, host fibroblasts, and immune cells in the tumor stromal compartment are known to play a role in tumor development and survival [79, 80]. The activation of quiescent stromal

cells, known as stellate cells, to myofibroblast-like cells engaged in extracellular matrix synthesis, contributes considerably to tumor growth in some tumors, such as pancreatic cancers [81]. Collagens are deposited by activated fibroblasts, causing stromal stiffness to alter. Both transformation and development can be hampered by stromal stiffness [82] Intercellular communication via integrins to prosurvival pathways, including PI3-kinase, is one method of stromal-induced tumor promotion.

Multifunctional nanoparticles enable simultaneous targeted delivery of diagnostic and therapeutic agents to tumor tissue, spawning a new area of theragnostic that blends diagnostic and therapeutic modalities [83]. Although such smRDEs can improve radiotherapeutic efficacy while reducing adverse effects, one of the primary obstacles to clinical applications is the safe and effective delivery of smRDE to target tissue [84]. Because of the quick dehalogenation mechanism in DNA, as well as the heterogeneity and limited expression of target receptors on cancer cells, iodine-attached tumor-targeting antibodies were utilized to increase their biodistribution, but their targeting and therapeutic efficacies were not met. Furthermore, due of the harmful side effects on the host organs, extended therapy with large doses of iodine compounds should be avoided. Using physics and engineering technologies to increase the radiation dose to cancers to prolong survival has not been particularly successful so far (GBM, lung, prostate), and in some cases has even been detrimental (GBM, lung, prostate). Tumor-treating fields are an exception, where the administration of low-energy electrical fields to the brain in conjunction with radio chemotherapy improved the survival of GBM patients. However, the mechanism of action is still unknown. Radium 223 was another positive spot, as it helped patients with metastatic CRPC live longer [85]. To be a SMART radiation oncology healthcare practitioner, one must have the desire, aptitude, and commitment to keep current not only in one's own area, but also in the rapidly changing field of cancer biology. Imaging, machine learning, and computer-assisted therapy planning, and delivery may allow us to spend more time with patients and on creative endeavors. Knowing one's toolkit's limit motivates one to explore other topics and take on new challenges. Radiation oncology works at the intersection of cancer biology, cutting-edge technology, imaging, economics, long-term outcomes from many cured patients, social anxieties of radiation, and the opportunity to provide care to millions of people who are underserved.

4.10 Conclusion

Cancer cells are studied at molecular level by using different techniques such as modern radiobiology, molecular pathology, molecular pathophysiology, molecular imaging, and molecular targeting. Radiotherapy is practically more promising than any other techniques as it only acts on cancer stem cells. Molecular Radiation Biology studies showed that disability of many functional and structural consequences which may lead DNA repair, cell cycle delay, cancer stem cells

development, triggered antitumor immunity, instability of genome, mutations, and altered gene expression.

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5 Traditional Treatment Approaches and Role of Immunotherapy in Lung Malignancy and Mesothelioma

Mirza Tasnia Tamanna and Christopher Egbune

5.1 Types of Lung Cancer and Where Mesothelioma Fits In

According to the histopathological changes of the tumour cell, lung cancers can be classified, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). According to American Cancer Society report reviewed by Maurie Markman on 25 August 2021, NSCLC covers around 80–85% of all lung cancer incidences. Lung carcinomas are then sub-classified further as bellow [4] (Flow Chart 5.1).

Other thoracic malignancies can include, mesotheliomas and metastatic lung cancer (MLC). MLCs are very frequently seen in the clinical setting. Although mesotheliomas are rare but, significantly growing concern as the incidences are sharply increasing. Mesothelioma is one of the causes of unnatural death across the world and often missed diagnosed in initial phase. Pleural mesothelioma develops in thin lining tissue of the lung called mesothelium, which separates the lungs in from chest wall. Mesotheliomas can occur in the abdominal mesothelium as well. However, that is even more rare and not as potentially life threatening as lung mesothelioma.

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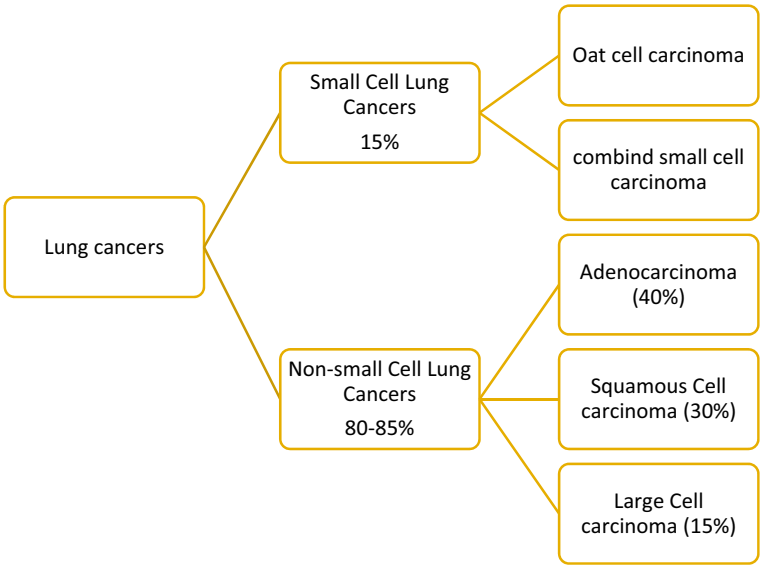

Flow Chart 5.1 Types of lung cancers with incidence rate

5.2 Diagnosis and Staging

Lung cancer and mesothelioma are diagnosed through series of tests and scans, confirmed by histopathology reports, and TNM staging is done before commencing treatment. However, diagnosis of malignant pleural mesothelioma (MPM) is greatly challenged even to this date and it almost always fatal when diagnosed [15]. To understand the staging of thoracic malignancies, it is essential to grab a clear knowledge about thoracic lymph drainage. Treatment and management plans vary upon the staging and histopathological types of the cancer cell.

Small cell lung cancers are commonly seen in people who have a long history of smoking cigarette. The reason it is called small cell cancer because during the histopathology of lung biopsy, the diagnostic feature of the tumour cell looks smaller than normal cells due to less cytoplasm inside. It also looks darker for the same reason. In small cell lung cancers, chromogranin, synaptophysin, CD56 and TTF are usually positive with high index of Ki67 which indicates high cell division rate. According to the American Cancer Society, SCLCs are staged broadly into two categories, limited stage and extensive stage and then further TNM staging is usually done. Limited stage indicates the cancer has involved in only one side of the lung with or without involving lymph nodes of the same side. In this stage, aggressive treatments are more beneficial and effective if tolerated by patients. Once cancer has spread to the other side which means when both the lungs have been affected by the SCLC, then it is considered as extensive stage and treatment are planned more corresponding with palliative management. It is worth to keep

in mind that, SCLCs have more potentiality to develop brain metastasis comparing other types of thoracic malignancies.

Non-small cell lung cancers: **Adenocarcinomas** has higher incident rate than others. They are also known as cancers of non-smokers and young people. It accounts almost 40–50% of all NSCLCs. Common mutation occurs with adenocarcinomas are EGFR, ROS1, ALK, BRAF, MET, RET and HER2. According to the mutation type, treatment can vary in different cases. Adenocarcinomas are usually detected before spreading due to its slow growth rate. According to the statistic report done by Dr. Ananya Mandal in May 2019, lung adenocarcinoma predominantly occurs is Asian population. Under the microscope, the adenocarcinoma looks like a glandular structure, which is usually mucinous stain prone to bleed easily. Low grade tumour indicates the carcinogenesis tissue looks almost similar to the healthy surrounding. The more it becomes undifferentiated with irregular margins, the more advanced the tumour is and the poorer the prognosis is and graded higher in histopathology.

Among all other types of lung cancers, **squamous cell carcinomas (SCCs)** are predominant. SCCs typically occur in the central part of the lung likely involving main airway branches. Sometimes this subtype of NSCLC is referred as bronchogenic carcinoma of the lung. Aetiology of tobacco smoking is strongly associated with SCC of lung. To confirm the diagnosis of SCC, the samples need to demonstrate transformation features like keratinization or intracellular bridges. If the differentiated squamous element of the tumour is less than 10%, a diagnosis of poorly differentiated SCC is made which is usually related to the poor prognosis as it means a large number of tumour cells are identical with the normal cells. In an immunohistochemistry (IHC) panel of SCC biomarkers, p63 and p40 proteins are often strongly expressed. SCCs are further subdivided into keratinizing, non-keratinizing and basaloid types according to the revised classification of WHO in 2015.

5.2.1 TNM Staging

To understand the TNM staging, knowing the thoracic lymph drainage is unavoidable, it describes the extent of the primary tumour in terms of the size, location and involvement of the lymph nodes (LNs). A brief summary of SCLC and NSCLC cancer staging has been drawn below in line with International Association for the Study of Lung Cancer (IASLC) which is adopted by the 7th edition of American joint committee on cancer staging system for thoracic malignancies [16] (Table 5.1 and Fig. 5.1).

Since the last decade radiological imaging took a big role in cancer staging, computed tomography (CT) with contrast, magnetic resonance imaging (MRI), whole body 18-fluorodeoxyglucose (FDG) positron-emission tomography (PET) and integrated PET-CT have added impulsive values in overall cancer staging. Although PET-CT scan is the readily available in the premium healthcare settings,

Table 5.1 TNM staging (Re-created with permission from MypathologyReport.ca)

Summary of TNM staging in thoracic malignancies	
T1-4	Locally invasive primary tumour
N1	Hilar LNs involvement of the same side of the disease
N2	Mediastinal lymph nodes involvement
N3	Hilar LNs of the opposite side or mediastinal nodes or supraclavicular nodes involvement
M1a	Intrathoracic metastasis
M1b	Distant metastases

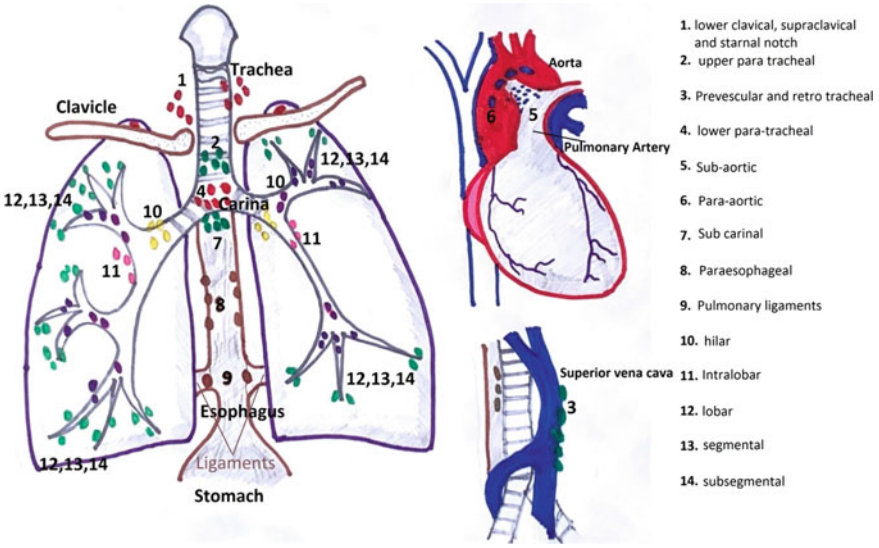

Fig. 5.1 Distribution of thoracic lymph nodes (Re-created with permission from MypathologyReport.ca) [4]

hence in limited use among the low-economic community due to high cost and remote availability.

Some invasive procedures like endobronchial ultrasound (EBUS), transbronchial needle aspiration (TBNA) are also occasionally used when the diagnosis and staging remain unclear even after detail investigations. EBUS-TBNA has outstanding specification in mediastinal LNs staging as well as in diagnostic staging of mesotheliomas [2].

5.2.2 Staging of Pleural Mesothelioma

Staging of pleural mesothelioma basically depends on the size of the tumour and its involvements. Mesothelioma takes decades to evolve since the initial exposure but once the tissue mutation take place they are mostly aggressive in nature with little clinical symptoms that till the advanced stage. Usually, size and site of the tumour have direct influence on disease symptom. Repeated pleural effusion is one of the clinical symptoms that increase suspicion towards mesothelioma. Thus, screening with pleural biopsy could escalate timely diagnosis. Early changes in mesothelium and expression of calretinin and WT-1 can indicate pre-disposer and worth regular follow up. Nowak et al. literature about staging and prognosis of mesothelioma has been summarised below (Table 5.2).

5.3 Traditional Treatment Approaches

Chemotherapy

The treatment of lung cancer hugely varies upon the staging of the disease. Traditionally surgical resection followed by systemic anti-cancer therapy is the primary and most ideal method of treatment where the tumour is fully resectable. However, in cases where surgery is not possible, chemotherapy and/or radiotherapy plus or minus immunotherapy can be used both for curative and/or palliative purposes depending upon risk-benefit ratios. Immunotherapy has shown promising results in thoracic malignancies by dramatically improving the overall survival rate (OS) and disease-free interval (DFI).

Table 5.2 Pathological staging of pleural mesothelioma [19]

Stages	Features		Prognosis/median life expectancy
Stage 1	1A	Unilateral tumour within the pleural layers	22.2 months after successful surgical removal
	1B	Stage 1A extends further into lungs or other tissue in thoracic rib cage	
Stage 2	Tumour spreads unilaterally beyond the mesothelial lining and nearby LNs		20 months after surgery
Stage 3	3A	Tumour has spread deeper into nearby organs and tissues with LNS	17.9 months, if surgical removal is possible
	3B	Stage 3A with more extensive LN involvement	
Stage 4	Tumour extended beyond its primary site and distant metastasis took place		14.6 months, however, it varies according to the rage of metastasis in this stage

Limited-stage lung cancers are typically treated with systemic chemoradiotherapy or chemo-immunotherapy or only chemotherapy depending on the histopathological sensitivity. In cases where certain biomarkers like EGFR, ROS, TTF1 are positive specific targeted therapy is used to slow down the spread of advanced NSCLCs [24]. Only if the tumour is resected fully with margins mediastinal nodes negative histopathology, mono therapies can be equally beneficial. Typical chemo protocol for adenocarcinoma of lung includes one of the platinum group chemo (Cisplatin or Carboplatin) with one of the following; Taxens, Gemcitabine, Etoposides or Pemetrexed combined with immunotherapy depending on PD1/PD-L1 expression status as first line treatment followed by maintenance treatment till further progression/up to 2 years according to Macmillan cancer research suggests [11]. Chemotherapy protocol for small cell lung cancer is boardly different, as radiotherapy plays vital role in other subtypes of lung cancers.

Radiotherapy

Radiotherapy has seen much advances and technological luxury than any other field of cancer treatment. Stereotactic body radiation therapy (SBRT) can be delivered in highly specified manage both in early and advanced stage of lung cancer. Metastatic lung cancer including brain metastatic condition can be well manage with radiation therapy. In case of SCLC node positive resected/un-resected limited-stage disease, mediastinal radiotherapy with concurrent chemotherapy is common in practice [14].

Updated studies indicate inoperable Stage I-II NSCLCs patients, are the target population of SBRT with a total dose of 60 Gy in 3 fractions shows DFI of 26% and OS 40% in 4 years follow up [1]. The role of adjuvant radiotherapy is often significant to reduce the size of a solid cancer before surgery to minimize the volume of lung tissue to be resected. SBRT also plays a vital role in palliative management in metastatic bone disease for pain control and fragility facture prevention. Prophylactic cranial irradiation (PCI) radiotherapy offered in small cell lung cancer in prevention of brain metastasis has shown positive outcome [14].

Palliative Management

During the last decade, palliative care gained popularity in serious illness and life limiting conditions. In multidisciplinary cancer treatment, palliative care has occupied a fundamental place from the very beginning. It is a wide field for vast patient population in different medical aspects. However, in oncology and haematology, the palliative care is focused on comforting the patient by ensuring quality of life. Active main management including Palliative radiotherapy to the localised metastatic bone pain, bleeding control, reducing/preventing growth of solid tumour lesion/meatstatic lesion compressing nerve/spinal cord.

Integrated palliative manage can be affected by many factors and needs comprehensive assessment customized for individual patient. Each of these factors are interconnected with each other thus, successfully supporting every aspect of integrated care leads to interdisciplinary management plan [8].

Maintaining individual's dignity and prioritizing their will towards the end of life can be challenging where palliative team takes over with soft skills of management.

In advance stage of lung cancer, patient suffers immensely with breathing problems, infections as well as pain from the cancer itself and metastatic lesions. In cases where brain and bone are involved managing confusion and controlling pain becomes ultimatum for the clinical teams. Thus, a well backed up palliative care team can be very handy for cancer patient management in a multidisciplinary setting and it lighten the loads on medical team.

Extensive stage disease treatment initiates with chemotherapy followed by palliative radiotherapy. In the later-stage of cancer treatment, symptomatic management plays more important role than active cancer treatment [16]. Unlike adjuvant chemotherapy, there are very few data about neo-adjuvant chemotherapy in lung cancers. They are occasionally used to shrink a large tumour in order to surgical benefits, avoid early micro metastasis, possible down-staging and develop better tolerance. Few studies from 1990s showed neo-adjuvant chemotherapy improves OS rate. Notably, a number of meta-analyses of neo-adjuvant chemotherapy trials showed improved survival rate which were substantially similar to those seen with adjuvant chemotherapy. Thus, chemotherapy before the surgical removal of tumour is not encouraged unless there is a robust reason behind it [18].

5.4 Role of Immunotherapy

Most immune checkpoint inhibitors and monoclonal anti-body have shown significant effect on lung cancers and have approved over time [17]. Below is a list of Food and Drug Administration (FDA) approved monoclonal antibodies functioning as negative checkpoints for lung malignancies (Table 5.3).

Notably, SCLC cases are only about 10–15% of the total lung cancer pedigree; however, it is being reported as most fatal subtype. The traditional treatment of SCLC has remained the same for decades. But chemotherapy alone has failed to improve long term OS. Fortunately, development of immunotherapy has brought the initial change in both OS and progression free survival (PFS). The use of immune checkpoint inhibitors (ICIs); monoclonal antibodies targeting cytotoxic T-lymphocyte protein-4 (CTLA-4), PD-1 and PD-L1 triggered a hug modification in

Table 5.3 FDA approved monoclonal antibodies acting as negative checkpoints inhibitors in lung cancers [17]

Checkpoint inhibitors	Targeted monoclonal antibodies	Types of lung cancer	Approval year
Pembrolizumab	Humanized anti-PD-1 lgG4	NSCLC	2014
Nivolumab	Humanized anti-PD-1 lgG4	NSCLC, SCLC	2014
Atezolizumab	Humanized anti-PD-L1 lgG1	NSCLC, SCLC	2016
Durvalumab	Humanized anti-PD-L1 lgG1	NSCLC	2017

lung cancer treatment [17]. The PD-1 inhibitors Nivolumab and Pembrolizumab and the PD-L1 inhibitor Atezolizumab demonstrated high efficacy as combination therapy in multiple clinical trials and being approved the third-line treatment for SCLC as mono therapy [7]. Additionally, Zhang et al. delved into extensive stage of SCLC and demonstrated that chemotherapy with the combination of immunotherapy can significantly improve PFS and OS where used as first-line treatment [24].

In context of NSCLC, the treatment line varies upon types and stages of the disease. Commonly Stage I and II are treated with surgical removal of the tumour and LNs dissections, whereas platinum-based adjuvant chemotherapy is typically considered for stage II and III NSCLCs with 5% survival rate of 5 years [12]. Apparently, researchers have suggested combination therapy for these categories of patients, with significant positive effect. Arguably, these combination therapies have not been devoid of adverse side effects. Some documented data suggest that use of EGFR-TKIs targeted therapy after adjuvant chemotherapy could improve PFS without noticeable improvement in OS. Adjuvant immunotherapy; Pembrolizumab, Nivolumab and Atezolizumab demonstrated great significance in the treatment of metastatic NSCLC.

One of the significant on-going adjuvant clinical trials is the Adjuvant Lung Cancer Enrichment Marker Identification and Sequencing Trial (ALCHEMIST), aimed to compare the outcome of adjuvant targeted therapy and adjuvant chemotherapy verses adjuvant single agent immunotherapy in context of intermediate stage (IB-IIIA) NSCLC tumour. Patients in the ALCHEMIST trial will receive adjuvant Nivolumab followed by observation for up to one year and Erlotinib/Crizotinib followed by observation for up to two years. Another phase III, randomized, open-label trial to compare the efficacy and safety of Atezolizumab versus optimal supportive treatment in post-OP stage IB-IIIA NSCLC patients who have had adjuvant platinum-based chemotherapy [18].

5.4.1 Mesothelioma and Anti-cancer Therapies

Malignant pleural mesothelioma (MPM) is a rare but very wild type of malignant condition where surgical approaches fail in most cases. Currently, the only combination therapy using Cisplatin-pemetrexed plus or minus Bevacizumab is approved and practised clinically. According to recent PROMISE-Meso trial, single arm immunotherapy versus chemotherapy has shown almost show no difference on patient outcome [20]. Similarly, NSCLC combination therapy with CTLA-4 and PD-L1 demonstrated promising results in phase II-III studies. However, either combination with chemotherapy followed by maintenance immunotherapy has taken over previous combination treatments [6]. According to NCC guidelines 2018, Nivolumab with or without Ipilimumab or Pembrolizumab has been recommended as emerging systemic therapy for MPM. Consequently, expert opinion from arrays of trials suggests that local or national guidance is important to update the use of Atezolizumab combination standard first-line treatment, which may open

opportunity towards the assessment of the length of survival period for eligible patients [13]. More recently, the introduction of immunotherapy in MPM phasic treatment has paved way for the discovery of few anti-cancer agents that work in unique fashion. The study of Anagnostou et al. [3] pointed out the benefits of immune checkpoint blockade (ICD) which targets the cytotoxic T-lymphocyte-linked antigen 4 pathways and the programmed death 1 in a special fashion during the chemo-immunotherapy treatment of mesothelioma and lung cancer. Their work has not devalued the multifaceted studies highlighting the production of antitumor agents that blocks the attack of repopulation of normal cells in the body. Furthermore, the earlier chimeric antigen receptor T cell (CART-4) therapy was seen to add longer survival period for patients. The treatment approach is termed to block tumour growth between cycles of the therapy. An experimental study shows the tumour cell repopulation was measured by Ki67 by immunohistochemistry and including bromodeoxyuridine by flow cytometric estimation [22]. Molecular variations in determining gene expression of cytokine-linked reverse transcription, which has been reported within phase treatment. Most recently, PD-L1 has been identified as less toxic biomarker of interest in NSCLC and yet to be reveal for MPM [11]. The PD-L1 turns off the T cells and reduces the downstream effects of immune response activation, hence paving way for the tumour to escape immune system [23]. Notably, blocking the PD-L1 pathway provides opportunity for the immune effect to be restored. Atezolizumab can be a second line (2L), Nivolumab plus Ipilimumab in 2L show 2 years survival for un-resectable MPM. Although, immunotherapy has been claimed to be less toxic but it is a lengthy and expensive process. Many other studies suggest high dose of thoracic intensity-modulated radio therapy (IMRT) which is also beneficial for adjuvant use in MPM.

5.5 Challenges and Future Prospective

Many sequential clinical trials have confirmed combination chemotherapy regimens which have better effect than single agent chemotherapy. On the other hand, maintenance chemotherapy showed no significant effects on PFS and OS. Immunotherapies are potential durable, meaningful responses with very favourable toxicity profiles. Some other types of treatment are drawing attention of many scientist which includes nutrition-based/MLT in cancer therapy, cancer and nano therapeutics, targeted therapy and personalized medicine. Hopefully, in near future, these researches can bring out ideal treatment for cancer patients with very minimal side effect. Among the new anti-cancer therapies, dendritic cell (DC) immunotherapy has already gone through some trails in different cancer types. In lung cancers and mesothelioma, clinical studies showed remarkable anti-tumour activity with DC immunotherapy [6]. Establishing screening for mesothelioma in high risk population groups by monitoring calretinin expression in pleural tissue can lead to punctual diagnosis of mesothelioma. Similarly, detection of WT-1 and prompt treatment with immunotherapy can have potential benefit in

overall survival rate. More studies and clinical trials need to take place to establish immunotherapy and other new anti-cancer treatment options to take place in routine use.

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Hormonal Therapies in Cancers

6

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6.1 Introduction

The use of hormones for the treatment of cancers rather than opting for surgical and clinical practices has gained the popularity in the recent times. The comparison of virtues of one product to other is often seen in the newspapers, journals, and articles. The hormonal therapy for cancer has become a household name and the series of experiments performed leading to the discovery of hormones use in the treatments of breast cancer. How some of them led to toxic effects in the human bodies and other complications and how some proved to be beneficial therapeutic agents for cancer therapy. In the start of the chapter, the use of extremely powerful luteinizing hormone-releasing hormone (LH-RH) agnostic, antiandrogens, antiestrogens, and aromatase inhibitors in the treatment of cancers because of their proven ability to treat cancers will be discussed. It followed reviews of clinical trials done for the execution of tamoxifen in the advanced stage of breast cancer in association with the use of estrogens for the treatment of toxicity and emphasized the kind of toxicity and complication caused by the overuse of the recommended

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dose. The biological fundamental principle in the evasion of cancer with help of antiestrogens, including the risk factors in the breast cancer and their interactions, the use of estrogen replacement therapy (ERT) to keep a check on menopausal indications in cancer of breast is also discussed under this chapter as the ERT is progressively more requested by the survivors of breast cancer. The systematic therapies renown in prevention of breast cancer rather than the control with hormonal strategies and development models of breast cancer will be discussed.

The chapter also includes the clinical mode of action of luteinizing hormone-releasing hormone agonists for the cure of the cancer in breasts involving the consequences of Zoladex on histology of cervix in relation to hyposecretion of luteinizing hormone and chronic injection based on 3.6 mg Zoladex and hypersecretion leading to critical distribution of 3.6 mg Zoladex. The risk assessment of contemporary menopausal hormone therapy (MHT) in cancer patients is also an important part of the study. The increased risk in the patients subjected to postmenopausal hormonal therapy of the ovarian cancer in women, and the results of the assessment of the women having different hormonal therapies and postmenopausal treatment are also discussed in the study.

6.2 Sex Steroids for Cancer Treatment

The current prostate and breast cancer treatments act as a barrier against steroid action and minimize its circulation in the cancer tissue. The hormones like antiestrogen, aromatase restrictors, antiandrogens, and use of extremely strong luteinizing hormone-releasing hormone agonists to perform a "medical hypophysectomy" because of their ability of causing desensitization in the pituitary gland have proven their value in the treatment of cancers over the last two decades. However, the significant connection joining the prostate growth and tumors in breasts and the sex steroids took a hundred years to put the knowledge in one place. George Beatson (Fisher et al. [1]) in 1896 described the concept of removing the ovaries of some premenopausal women can prove beneficial for them from the inoperable advanced breast cancer, and his study proven the effect on the histology of mammary gland of the rabbits or other farm animals. The discovery that proved to be a major gain access for the future research was in ovaries of pig by Allen and Doisy (Costantino et al. [2]) in 1923 and the presence of estrogenic in the fluid of ovaries of pig. The crystallization of the first steroid hormone estrone was done by Doisy in 1929 [3] and the identification of estrogenic compounds in ovariectomized mice leading to establishment of vaginal cornification.

The discovery of the regulation of the follicle vitalizing hormone and secretion in relation to luteinizing by the luteinizing hormone-releasing hormone that is the release of a small peptide in a pulsatile manner was initially viewed as a way of therapeutically introducing androgen and stimulating spermatogenesis in infertile men and inducing infertile women with ovulation [4]. However, the prolonged stimulation of LH-RH resulting into rapid desensitization of the pituitary gland led to the possible development of contraceptive agents. The production of

medical oophorectomy and obstruction of synthesis of testicular androgen in men and ovarian-estrogen in women led to preparation of highly potent LH-RH in a sustained manner [5]. Various surgical operations and endocrine ablation are now avoided because of the therapy of prostate cancer and premenopausal cancer in breasts by using the applications of sustainable release devising [6, 7]. The blocking of hormonal action using antagonist drugs when the causing agent of growth of prostate and breast cancer is the androgen and estrogen hormone, and the use of the antagonist drugs can prove to be valuable therapeutic agents. The rational identification of non-steroidal estrogens was the basis of antiestrogen's discovery. MER25 was reported to be the first non-steroidal antiestrogen by Lerner and coworkers (Bergman et al. [8]). However, analogous to estrogen triphenylethylene were investigated later because of MER25 was too toxic for the clinical use. A potent antiestrogen was then pursued for the therapy of breast cancer in the advanced stage which was ICI 46,474 (tamoxifen) found in rats by Harper and Walpole. EGF is seemed to introduce cellular proliferation, and EGF receptors are found in prostate cancer. Therefore, the control of prostate cancer by potent inhibitors of EGF receptors tyrosine kinase is being done in early clinical trials. We can expect significant number of new research in the coming year because of the accelerated pace of research in prostate cancer, both in effective therapies in premalignant conditions and in early diseases condition.

6.3 Tamoxifen for Treatment of Breast Cancer

The association of structure with activity of estrogens was studied by many pharmaceutical companies during the 1960s. Laboratory rat used as an efficient antifertility vehicle but with the clinical perspective, those properties were not translated the way they were being observed in the animals. The relation between the growth of some breast cancer and estrogens led to the conduction of trials of breast cancer at the preliminary level with several new antiestrogens [1, 2]. In 1970s, the rational for clinical studies was strong because of the discovery that the radioactive estradiol could be found antiestrogens in the target tissues. In 1970s, extensive tests on new antiestrogens, nafoxidine, were conducted by MI, The UpJohn Company in Kalamazoo, the resultant toxic effects that patients had to encounter led to the abandonment of further development [3]. The patients who show positive ER show significant recurring reductions in response to tamoxifen that are ($2p < 0.00001$) and exhibits a significant deviant trend as ($\times 2 = 45.5$, $2p < 0.00001$). However, tamoxifen shows very minute effects in the patients that are ER negative. Also, the question to the relation to slight better response of the tamoxifen because of ER could be asked. The improved results of tamoxifen in response to progesterone receptor (PgR) could be questioned. Tamoxifen showed proportional reductions in a recurring manner in the last 5 years of trials. It can be concluded that tamoxifen response could be potentially predicted by ER stating tamoxifen as a potential antagonist in the treatment of breast cancer [4]. The credit of regarding tamoxifen as a strong palliative treatment in postmenopausal females

with breast cancer at advanced stage goes to late Dr. Arthur Walpole (Powles et al. [5]). Tamoxifen has been designated as the endocrine cure of option for the cancer of breasts at all stages over the last 20 years. However, the conclusions on the actions of tamoxifen are yet to be drawn because the evaluation of the results of adjuvant clinical trials was done vigorously.

New endocrine treatments like fulvestrant increase the choice of therapies for postmenopausal women with breast cancer and offer new options for combine therapies. In clinical trials, it is confirmed that tamoxifen-resistant tumors are inhibited by the fulvestrant, and this approach provides an efficient treatment and improves patients' survival.

6.4 Hormonal Scheme for the Therapy of Breast Cancer

About a century ago, Beatson described the first systematic therapy for cancer that was proven to be successful [1]. He stated the functioning of oophorectomy keeping the growth of cancer in breasts under check by translating the observation of relation of epithelial proliferation seen in humans with the ovarian check of proliferation of mammary epithelial in the sheep that is lactating. On June 15, 1895, the attempt to suppress breast cancer with oophorectomy in the patient showed excellent response. Biologic mammary carcinogenesis replicas are very crucial, and the strategy for the evasion of cancer in breast at the early stage is based on hormonal strategies. Carcinogenesis is a process with multiple number of steps. The very first step is an irreversible step that involves the damaging of DNA and is termed as the initiation step. The second step involves the mitogenic or hormonal stimulation of cell division, termed as the promotion stage. Finally, a frank neoplastic lesion is formed by the progression of a tiny duplicate of modified cells. The breast cancer initiation in humans due to the tumor viruses has not been seen. However, the exposure to radiation is known to become a major causal agent of initiation of cancer in humans because of one radiation induced mutations leading to tumors in humans [2–4].

The carcinogenic effects of DMBA have seen to be intermediate in the rats that were pregnant or lactating [5, 6]. It suggested that the development of mammary glands during the pregnancy is being affected due to the placental hormones. The administration of placental hormones in an exogenous manner has affected the development of mammary epithelium. The incidence of mammary carcinomas has seen the reduction in a dose dependent fashion in the human chorionic gonadotropin as compared to the reaction shown in the animals on administration prior to DMBA treatment. Thus, the number of carcinomas is seen to be increased significantly due to the administration of placental lactogen after the DMBA treatment [7]. The age occurrence of cancer of breasts as shown within Fig. 6.1.

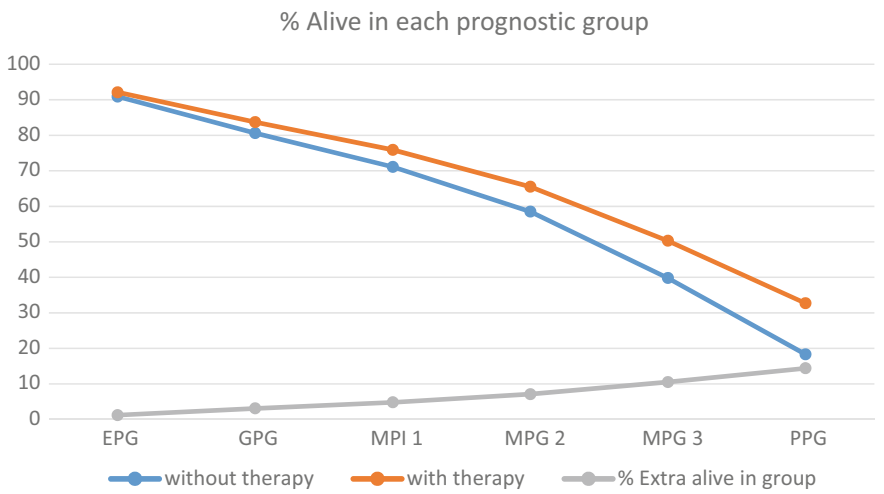

Fig. 6.1 Age occurrence of cancer of breasts in women

6.5 LH-RH Agonists Breast Cancer Therapy

In case study started by Paterson in 1948 [8] and Nissen-Meyer in 1957 [9], the use of ovarian ablation as adjuvant comprehensive remedy was examined which defines that collateral treatment with ovarian resection, ablation, or suppression improves long-term survival in women under the age of 50 [10]. Neither the surgical nor the radiation therapy procedures are considered a large intrusive procedure, yet both carry risks. Furthermore, ovarian radiation can take up to 6 weeks to decrease estrogen production and the suppression can be uneven. Whether the patient responds to treatment or not, both oophorectomy and ovarian transposition are irreversible and cause persistent menopausal situation [11]. ICI began searching for a way to suppress ovarian function with pharmaceutical methods and generated several luteinizing stimulating and releasing hormone (LH-RH) substitutes. ICI (118,630) called afterward as "Zoladex" which was the most promising and based on animal experiments (goserelin acetate) [12]. Zoladex was discovered to induce rises in plasma fluid mass of both gonadotrophic hormone (LH and FSH), but continuous therapy resulted in quick decreases in LH, estradiol, FSH, and progesterone. The levels of estradiol in blood were lowered to those seen in oophorectomized or menopausal women [13]. The action of Zoladex on the adenohypophysis is known as "receptor down-regulation" [14]. Pulsatile ejection of GnRH under the thalamus is the natural signal for the secretion of gonadotrophins (LH and FSH) from the pituitary gland. In menopausal women, the most important estrogen secreted by gonadotrophins is estradiol. Goserelin binds to all the LH-RH receptors on the pituitary cell's surface (Fig. 6.1a) and the working of LH-RH receptors form mass and progressively internalize into the cell and resulting in temporary rise of serum LH. Because of the goserelin, which is constantly released from Zoladex 3.6 mg depot, new receptors are

generated, but they are quickly occupied and internalized. As a result, persistent injection of the LH-RH match inhibits enough LH-RH sensors found on the cell surface, preventing LH production and excretion [15]. Resulting estrogen production and secretion reduced leading to menopause. In premenopausal women, the suppression of ovarian action prefers extensive treatment, while in postmenopausal women, an estrogen antagonist and tamoxifen (Nolvadex) prove the standard treatment. Because Zoladex therapy effectively makes women menopausal, it seemed logical to test the effectiveness of the combination of both medicines with respect to Zoladex alone.

Endocrinologically, the combination was found to be safe. The combined medication lowered serum gonadotropin, estradiol, and progesterone concentrations as effectively as Zoladex therapy alone. For advanced breast cancer treatment, premenopausal women were randomized either with Zoladex solely or tamoxifen with Zoladex as a primary treatment. Menopausal condition [14], weight, life period, energy-giving interval, hormone-receptor position, cytology, disease lot were all equivalent at the onset of the survey. No notable distinction in response rates (thirty-one percent for Zoladexvs thirty-five percent for the blend), however, the co-treatment had a considerable advantage in time to progression. Both trial groups had a very comparable side effect profile and there were no further immunity concerns related with the compound. The EBCTCG overview meta-analysis strongly supported the efficacy of oophorectomy, which is the oldest assisting extensive remedy studied in multiple experiment trials [3]. The extent of the impact appears to be comparable to that of tamoxifen [15] in climacteric women or cytotoxic drug treatment in parous women [16]. In women, who were additionally given cytotoxic therapy with cyclophosphamide, Adriamycin, and 5-fluorouracil (CAF), Zoladex plus tamoxifen significantly increased recurrence-free survival, whereas Zoladex alone didn't. This was another example of the efficacy of the two hormonal drugs in combinations. The trials with Zoladex-containing hormonal regimens as adjuvant therapy against CMF show significant uniformity of response in ER-positive tumors with considerably less aide effects. CMF's ovarian suppressive side effect accounts for a significant portion of its action in ER-positive cancers, and this hormonal effect is much stronger than the tumors pure cytotoxic effect. For women with ER-positive malignancy, hormone treatment possessing Zoladex protocols should now take the place of cytotoxic therapy as the optional adjuvant remedy.

Abarelix is a modified gonadotropin releasing hormone antagonists. The percentage decrease in PSA is greater in abarelix group after 15 days of treatment.

6.6 Breast Cancer Defense with Antiestrogens

In 1936, Lacassagne proposed that if breast cancer was a reason of a unique hereditary vulnerability to estrogen in the breast, then a therapeutic antagonist might be discovered to prevent the disease. During the last decade, research on the toxicity and pharmacology of tamoxifen has moved the notion of antiestrogens to treat breast cancer into drug trials. All women with breast cancer does not always have

known risk factors for the disease. Statistical analysis shows roughly 1/2 of the women who are diagnosed with breast cancer are not pre-selected for surveillance [17]. Based on this finding, new antiestrogen preventative tactics have been developed to widen the antiestrogen's applicability. Even though the etiology of breast cancer is unknown, certain factors have been linked to an increased chance of developing the disease. Hereditary and ancestral, hormonal variables, benign breast illness, and environmental factors can all be categorized as such. Furthermore, a significant risk factor of breast cancer is age, with 1/2 of a women's lifespan risk of development breast cancer happening after the period of 65 age [18].

The renowned probability factors for a breast tumor are likely to be a family history, two of the types of which are recognized. Since several women with an ancestral history of breast tumor do not have the hereditarily transmitted type of disease, their risk is substantially less than those of women who inherit a predisposition gene. A woman aged 30 with a mother or sister who has had breast tumor is susceptible to a 7–18% risk of having breast cancer by the age of 70. Endogenous hormones are clearly linked to breast tumor imperil, and several research have associated breast cancer risk to the menarche age, climacteric, and 1st gestation. Some authors claimed that abortion, whether accidental or planned have higher risks [19, 20], whether other studies have suggested no link between abortion and possibility of breast cancer.

Breast cancer women appear to be great candidates for chemoprevention programs, while the impact of culturalism on risk is mysterious, and the linkages among possibility factors and their fluctuation are rarely discussed. Finally, the number of women with high-risk factors will not suffer breast carcinoma except for those who have mutation in breast cancer predisposition genes. Just 21% of breast cancer cases in females aged 30–54 and 29% of cases in women aged 55–84 appeared with one of ten percent probability of breast tumor, according to a study by Seidman et al. (Daling et al. [21]). The study of tamoxifen and raloxifene (STAR) trial randomly assigned postmenopausal women to regular tamoxifen or five years raloxifene chemotherapy. The major goal of the STAR study is whether long-term raloxifene medication helps prevent incursive cancer of breasts in postmenopausal women being diagnosed having a great risk of illness. The known medication tamoxifen is used to compare cardiac data, orthopedic data, and general toxicities with concentrate to determine the net value of raloxifene therapy. Although it is obvious that tamoxifen and raloxifene activate or repress the same target areas around a woman's body, a comparison of drugs offers an essential raloxifene medical record. Raloxifene is an antiestrogen that has fewer estrogen like effects than tamoxifen [22–24]. The National Cancer Institute conducted random trials of raloxifene in high-risk premenopausal women because it had been shown to produce a small loss in bone density in premenopausal women [25]. Momentary raloxifene medication (5 or 28 days) generates systemic estradiol concentrations but doesn't inhibit ovulation hundred percent, which is similar with the documented rise of steroid hormone released by tamoxifen in dupe premenopausal breast cancer [26].

6.7 Hormonal Replacement Therapy of Menopausal Symptoms

Breast cancer survivors including all postmenopausal women suffering from infuriating and occasionally devastating menopausal symptoms like sudden sweating, dyspareunia, vaginal atrophy with urinary tract symptoms, sleep disruption, and mood swings. Fighters of breast cancer are frequently probing about or demanding estrogen replacement therapy (ERT) that help to resolve these issues [27, 28]. Tamoxifen has the potential to exacerbate or induce vasomotor and vaginal symptoms because these effects are severe, and several women stop taking tamoxifen adjuvant therapy. Coronary artery disease is a less apparent, but possibly lethal and side effect of menopausal estrogen loss. The reduction in coronary heart disease documented in ERT normally ranges from 30 to 70% [29, 30]. Another cause of weakness and temporality in parous women is osteoporosis. The decrease in the rate of future fracture in ERT users has been estimated to be between 30 and 60% [31].

Non-oncologic health issues are becoming more of a worry. Between 1982 and 1987, the incidence rate of breast cancer in the United States grew dramatically, owing to greater screening [32]. Premature menopause treated for breast tumor is a major subject of concern in women. Adjuvant chemotherapy is increasingly more common due to the positive outcomes for women with minor protruding but node negative cancer. Chemotherapy was one of the intermediation findings of large prospective and randomized clinical trials was reported in 1989 and recommended for the favorable variant more often breast cancer patient. Most of the studies aimed to the impact that women who reach menopause early have a higher risk of cardiovascular disease, and the age link is a comparative risk of severe heart disease that was 0.40 [33]. The leading death cause of node negative breast cancer survivors is non-neoplastic diseased and cardiac disease [34]. These figures are based on patients who didn't take drug medication and so did not reach early menopause. According to a recent global review, many patients with invasive cancers may recovered from tamoxifen, and long-term treatment may be better to shorter treatment durations [35]. Tamoxifen may be broadly applied in the future for women who are susceptible to breast cancer and its survivors. Three breast cancer prevention trials are presenting each testing tamoxifen prophylaxis prevention in healthy women at risk for breast cancer. Tamoxifen may be suggested as anticancer drug for a substantial number of pre- and postmenopausal women if the outcomes of these trials are favorable as stated in Fig. 6.2.

The use of ERT/HRT and tamoxifen in the combined form raises the possibility of tamoxifen effects on breast or tumor cells and will be counteracted by estrogen. On the other hand, premenopausal women have significant levels of estradiol flowing on sex hormone binding globulin, but they counter to tamoxifen. The elevated serum level of estradiol in postmenopausal women has been observed throughout menstrual cycle or follicular phase and throughout the rest of their cycle, premenopausal patients have substantially greater amounts [33–37].

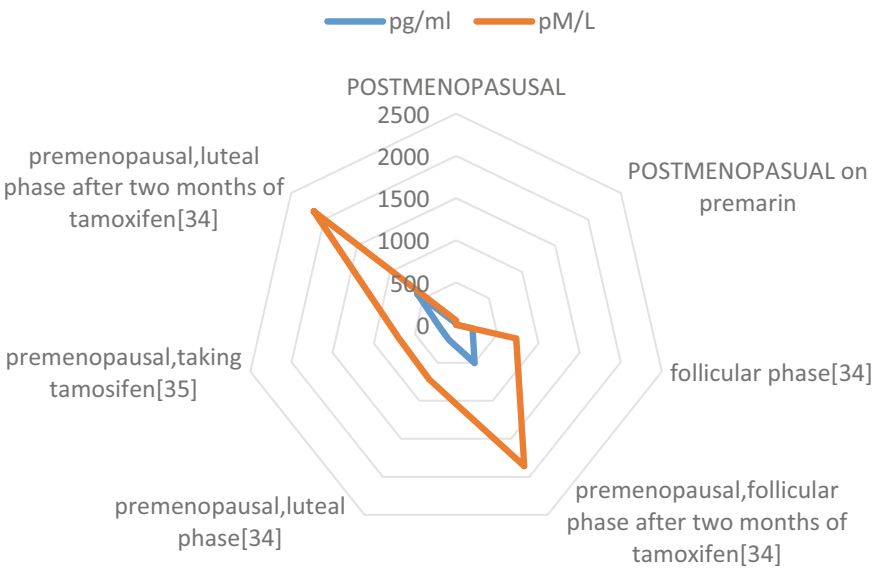

Fig. 6.2 Postmenopausal women tamoxifen enhances estrogen secretion

6.8 Hormonal Therapy in Post Menopause and Risk of Colorectal Cancer

The concept of endo and exogenous sex hormones may influence risk of colorectal cancer was first proposed in the early 1980s [38, 39] and most epidemiological data on HRT3's effects has just recently collected [40]. There is no significant link between colon cancer and HRT according to various research. Five investigations revealed no link between rectal cancer and obesity. Only Furner et al. (Miller et al. [35]) found that there was a significant reduction in risk and Calle et al. (Stampfer et al.[36]) discovered an unfavorable relationship with statistical trend and length of their use. This study, which is based on the biggest series of female colorectal cancer cases ever published on the subject, adds to the quantitative evidence that HRT consistently lowers the risks of colon and rectal cancer. Even after accounting for other known or suspected risk variables, a substantial inverse connection with the duration of usage was found. In studies, women who had ever used HRT are at minor chances to have colon cancer, with recent users having a higher level of protection [36].

When analyzing these observational studies, it is critical to keep in mind that women who use hormone replacement therapy may differ from women who don't in ways that lower their risk of colon cancer. Unregulated confounding is always a possibility in observational studies and it is more critical in this case because of the potential for differences in access to healthcare and colorectal cancer screenings. Colorectal cancer affected 470 women, and distal colorectal adenomas

struck 838 and use of postmenopausal hormones has been linked to a lower incidence of colorectal cancer. This link was weaker in previous users (RR; 0.84) and eliminated five years after hormone use was stopped (RR; 0.92). The chances of colorectal tumor were reduced in women who were on hormone therapy of post menopause, but the effect soon changed after the treatment was stopped. Hormone use was found to be inversely related to major colorectal adenomas, but not to small ones [37].

6.9 Menopause Hormone Therapy (MHT)

Millions of women still use hormonal therapy for menopause symptoms. Estrogen plus progestin (EPT) or estrogen separately utilized as a menopause hormonal therapy throughout the world. Since pill-controlled hormone therapy trials show that estrogen and progestin increase the probability of death from breast tumor and lungs cancer and decrease endometrial cancer but no clinical impact on colorectal cancer. On the other hand, estrogen alone has less risk of breast tumor, lungs carcinoma, and colorectal tumor [41].

A Nationwide Swedish population-based cohort study used controlled MHT during study period to observe 290,186 women aged ≥ 40 years. The dosage of ET and EPT is prescribed by Nationwide Prescribed Drug Registry. 16 different anatomical locations were grouped for cancer diagnoses, so that standardized incidence ratios (SIRs) and 95% confidence intervals (CIs) were deliberated: the results showed that the SIR of any cancer was 1.09 (95% Cl: 1.07–1.11) following ever MHT, 1.04 (95% Cl: 1.01–1.06) for E-MHT and 1.14 (95% Cl: 1.12–1.17) for EP-MHT. EP-MHT users of ≥ 70 years old had the highest SIR. The susceptibility of protruding breast, endometrial, and ovarian tumor is high for any MHT, however, invasive breast cancer (for EP-MRT users) risk increases with age. Therefore, EP-MRT was associated with increasing the risks of cancer [42]. Small amount of progestogen in estrogen therapy (ET) plays a role in endometrial protection. Uncontrolled estrogen therapy increases the risk of endometrial hyperplasia and glandular malignancy. A correct dose and duration of progestogen to ET lower this risk. US Food and Drug Administration (USFDA) approved; appropriate dose and duration of progestogen will give endometrial protection. The beneficial effect of ET on cardiovascular risk factors may be decreased by progestogen addition. Although, progestogen has increased mammographic density, but it decreased the effect of bone-enhancing action of ET and for the prevention of estrogen-induced endometrial hyperplasia, progestogen hormone should be added. However, the side effects are mild but may be sever in some women. Side effects can be minimizing by changing the type, regime, and route of progestogen [43].

6.10 Ovarian Cancer and Hormonal Therapy

The studies have shown that women receiving different premenopausal and postmenopausal hormonal therapies are on higher risk of having ovarian cancer. History of 8 women with ovarian cancer was not available, so these women were not included in association between hormonal therapy and epithelial cancer analysis, but their overall ovarian cancer analysis was done [44]. The tumors were categorized as epithelial and non-epithelial tumors. Two types of analysis were performed: one with women that carried out first hormonal therapy to remaining expose time and second for those women who had changed to another hormonal therapy during follow-up. The results had shown that out of 909,946 women 3068 had malignant ovarian cancer during the study period. Out of which, 2681 were epithelial tumors and 115 were unspecified epithelial tumors and 401 adenocarcinoma, 55 non-epithelial and 324 were unspecified tumors. The current users of hormonal therapy had higher risks of having ovarian cancer than the previous users and had never used. The risk of ovarian cancer did not increase with the increase of duration of hormonal therapy. In previous users, there were increasing risks of having epithelial ovarian cancer up to 2 years of seizing the hormonal therapy.

As the current users were on higher risks of cancer then never users similarly, the current users of EPT were on higher risks of ovarian cancer than never users of EPT. However, the duration of EPT users was highly association with increasing risks of ovarian cancer. Women who were taking cyclic EPT or long cycles of EPT were on higher risk of having epithelial ovarian cancer than whose who had never taken hormonal therapy or those who had taken continuous EPT. Epithelial ovarian cancer risks had also increased by combined therapy with norethisterone. The women treated with transdermal administration of ET were on higher risk of ovarian cancer as compared to those who had taken oral ET, but the difference was not statistically notable. Like oral estrogen, vaginal administration of ET was somewhat on higher risk of ovarian cancer. However, the oral users of EPT were on higher risk of epithelial cancer than the never users of hormonal cancer. The risks of ovarian cancer increased for short durational use of hormone (0–4 years) but no increased in risks for less than 5 years use of HT [45]. Nurses' Health Study had shown that in case of ET increasing the length of use the risk of cancer also increased [46]. One Danish Study had found that increasing dosage is more important than duration of ET [47] but in recent studies, it is shown that increasing dose had no impacts on risks of ovarian cancer. The recent studies had shown that the use of combined therapies for 5 or more years increased the risks of ovarian cancer [48]. The study shows that both combine HT and estrogen therapy increase the risk of ovarian cancer with small effect of type of progestin, length of procedure, routes of administration, and dosage. The risks of ovarian cancer must be considered while using hormones.

6.11 Conclusion

There are many choices for hormonal therapies, and patients could consider therapies at different points. Patients need to choose the appropriate therapy within the context of existing data. LH-RH agonists are still a standard of care. Some may use opt such as nutritional therapies or herbal supplements for alternative strategies. There is a dire need of new research in EGF to avoid prostate cancer, both in effective therapies in premalignant conditions, therapies in early diseases and in the new treatment options. Tamoxifen has been designated as the endocrine treatment for the breast cancer. However, the conclusions on the actions of tamoxifen are yet to be drawn because the evaluation of the results of adjuvant clinical trials was done vigorously. Raloxifene is an antiestrogen that has fewer estrogen like effects than tamoxifen. The use of ERT/HRT and tamoxifen combined raises the possibility that estrogen will counteract the effects of tamoxifen on breast or tumor cells. The risk of colorectal cancer was reduced in women who were on postmenopausal hormone therapy. Postmenopausal hormone use was found to be inversely related to major colorectal adenomas. The study shows that both combine HT and estrogen therapy increase the risk of ovarian cancer with small effect of type of progestin, usage length, routes of administration, and dosage. The risks of ovarian cancer must be considered while using hormones.

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Oncolytic Virotherapy

7

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List of Abbreviations

CAFs	Cancer-associated fibroblasts
cGMP-AMP	Cyclic guanosine monophosphate–adenosine monophosphate
CTLs	Cytotoxic T lymphocytes
GM-CSF	Granulocyte-monocyte colony-stimulating factor
HSV	Herpes simplex virus
IRF	Interferon regulatory factor
MDA-5	Melanoma differentiation-associated gene 5
MDSCs	Myeloid-derived suppressor cells
MHC	Major histocompatibility complex
NDV	Newcastle disease virus
OVs	Oncolytic viruses
PAMP	Pathogen-associated molecular pattern
Pexa-Vec	Pexastimogene devacirepvec
pRB	Retinoblastoma-associated protein
Reovirus	Respiratory enteric orphan virus
STING	Stimulator of interferon genes
T-VEC	Talimogene laherparepvec

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TAMs	Tumor-associated macrophages
TANs	Tumor-associated neutrophils
TK	Thymidine kinase
Tregs	Regulatory T cells
VPF	Vascular permeability factor
VSV	Vesicular stomatitis virus

7.1 Introduction

Oncolytic virotherapy has revolutionized the standard cancer treatments such as surgical treatment, chemotherapy, radiotherapy, and immunotherapy in the modern era, and it has the potential to become a primary treatment method for various cancers. Oncolytic virotherapy employs naturally existing or genetically modified viruses with the potential to replicate in and kill the cancerous cells without harming the healthy cells [68]. Oncolytic viruses (OVs) kill the cells in the tumor microenvironment either by direct lysis or by modulation of the antitumor immunity. The varying outcomes of these mechanisms are dependent on the type of cancerous cells, characteristics of the oncolytic virus, how the virus interacts with the tumor microenvironment, and also the immune response of the host [34]. The emergence and better understanding of virus molecular biology, genetic engineering, and tumor immunology has sparked a surge in interest in the area of oncolytic virotherapy. Several OVs including adenovirus, vaccinia virus, reovirus, herpes simplex virus 1 (HSV-1), Newcastle disease virus (NDV), poliovirus, etc., are being evaluated clinically for cancer treatments [62]. The clinical applications of OVs in cancer therapies began in the 1950s, after the rodent models and virus propagation methods were well-established. Hundreds of cancer patients were treated with multiple wild-type viruses, and tumor regression was observed in some of the cases over different time scales; however, the results were variable with overall limited success [35]. Later in the 1990s, oncolytic virotherapy evolved and began to exploit genetically modified viruses to enhance their antitumor specificity and efficacy in cancer therapeutics. In 1991, preclinical experiments with mice glioblastoma model, a thymidine kinase knocked out human HSV-1 was shown to be active in mice and inhibited the growth of glioma with excellent safety [52]. Since then, many different OVs are being tested in clinical trials for cancer therapies and thus far four OVs have been approved for clinical use globally (Table 7.1). In this chapter, we highlight the therapeutic abilities of OVs for the treatment of cancers. The characteristics of key oncolytic viruses, their mechanism of actions, and their clinical applications have also been discussed here.

Table 7.1 List of approved oncolytic viruses for cancer treatment

Oncolytic virus	Virus type	Genetic modification	Disease condition	Year/country
RIGVIR®	Picornavirus	Unmodified	Melanoma	2004/Latvia
Oncocrine (H101)	Adenovirus	E1B-55 K/E3-deletion	Nasopharyngeal carcinoma	2005/China
T-VEC (Imlygic™)	Herpes simplex virus type 1	ICP34.5 and ICP47 gene deletion, GM-CSF Insertion	Advanced melanoma	2015/United States & Europe
DELYTACT (G47Δ; teserpaturev)	Herpes simplex virus type 1	Deletion of ICP34.5, ICP6 and α47 genes)	Malignant glioma	2021/Japan

7.2 Mechanisms of Oncolytic Viruses

The use of oncolytic viruses is an emerging approach for antitumor therapeutics. These oncolytic viruses exist naturally or engineered in the laboratories with appropriate ability to use tumor cell's environment for their replication and production. The oncolytic viruses are generally induced immunological responses against tumor cells and increased frequency of expression of antitumor genes. The application of these oncolytic viruses in antitumor therapeutics is a fine addition along with existing therapies (radiotherapy and chemotherapy). Hence, it is very much important to understand the internal mechanisms of oncolytic viruses to be used for minimizing various types of tumors [82].

7.2.1 Tumor Lysis

Oncolytic viruses are the diversified biological agents having the potential to be used against various types of cancers. Generally, the tumor lysis has been divided into two categories based on the effectively—one is direct effect on cells while the other is its effect on vascular networking. The cellular machinery of cancerous cells is widely used by oncolytic viruses for the production of their new progeny. The cellular proliferation is disturbed by blocking of synthesis of protein and nucleic acid of the susceptible cell. This mechanism disrupts all major functions that are necessary for viability of tumor cell. The nucleus, mitochondria, endoplasmic reticulum, and other vital components of tumor cell are damaged due to increase concentration of viral progeny (Fig. 7.1).

Neovascularization is a natural process of new blood vessel formation. This neovascularization helps tumor growth and development as well due to which tumor maintains its integrity and stability [48]. An ample quantity of oxygen and other vital nutrients is provided to tumor cells through this neovascularization system. Hence, the inhibition of tumor neovascularization plays an important role

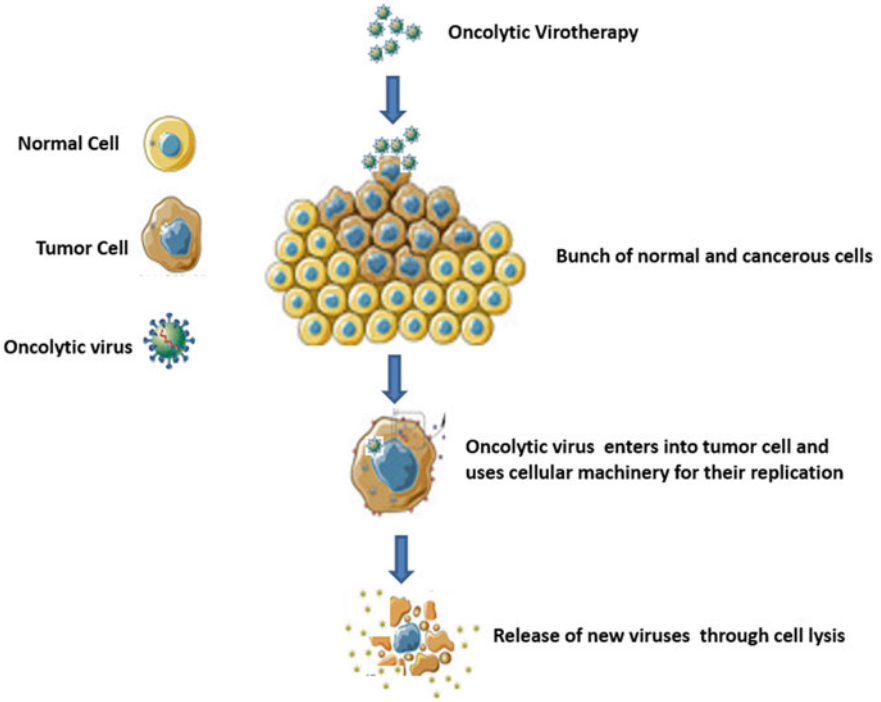

Fig. 7.1 Oncolytic viruses kill the cancerous cells through direct tumor lysis

for stopping metastasis and tumor growth [61]. Angiogenesis refers to the normal process of the development of new blood vessels as a result of any injury to support healing and often helps in oncogenesis as well. The antiangiogenesis is one of the most important abilities of OVs. These viruses can induce tumor cells to die through falling down cell's blood supply and vascular collapse [25]. For example, herpes simplex virus (HSV) has ability to develop local microthrombi by initiating inflammatory responses in tumor vasculature [8] and this process has been seen in ovarian cancer, sarcoma, and glioma. The studies of Breitbach et al. in [7] have reported that vaccinia virus has successfully replicated in vasculature of endothelial cells that were linked with tumor cells. Upon entrance of an oncolytic virus, the inflammatory neutrophils come to accumulate which results in the gradual decrease of blood supply toward tumor cells. This process declines tumor cell perfusion and induces ischemic death. The vascular permeability factor (VPF) is primarily a signal protein that participates to initiate blood vessel formation and it is specifically belongs to family of growth factors (cystine-knot growth factors). These growth factors are important in angiogenesis, tumor cell proliferation, and vascularization which ultimately add in tumor progression. The adenoviruses exhibit their oncolytic ability by expressing E1A protein which down

regulate VPF. This strategy affects angiogenesis which results in decrease blood supply for tumor cells and hence induces tumor cell death [83].

7.2.2 Antitumor Immunity

Tumor cells are recognized as aberrant by immune system while the tumor microenvironment performs a directive role in tumor development and immune evasion. The tumor microenvironment consists of tumor cell stroma, fibroblast, and inflammatory cells like microphages, lymphocytes and microglia, and endothelial cells and inflammatory molecules like cytokines. The extracellular material consists of fibronectin, laminin, hyaluronan, and collagen [31]. The mechanism of intercellular connection takes place through network of signaling. A number of signaling molecules like cytokines, inflammatory mediators, growth factors, and various enzymes are important in this process. The recent advancement has explored various new routes of interaction which include exosomes, circulating tumor cells, apoptotic bodies, and circulating tumor cells. These new horizons are also acting as information sources for tumor-associated and normal cells [3].

7.2.2.1 Innate Immunity

Cytokines are inflammatory molecules which are present in microenvironment, express immunosuppression, and reduced efficiency of effector immune cells. This process activates recruitment of immunosuppressive cells (TAMs, TANs, CAFs, MDSCs, Tregs, etc.) [6]. The "cold" microenvironment contains only a few immune cells. The oncolytic viruses are very capable in the immune conversion of such immunosuppressive microenvironment [50]. Through this process, the cold tumor is converted into hot tumor by altering cytokines milieu and maturing immune cells [20]. Upon interaction with oncolytic viruses, the tumor cells faced hard time to survive because these viruses induced death in tumor cells by facilitating immunogenic cells. The viral pathogen-associated molecular pattern (PAMP and tumor-associated antigen (TAAs) and tissue damaged-associated molecular patterns (DAMP) are released which are recognized by toll-like receptors (TLRs) and elicit MYD88-dependent and TRIF-dependent signaling [11, 84]. The other sensors for this activity are cytosolic pattern recognition receptors that are very important in recognizing viral genome and hence activating innate immune process. These receptors generally include retinoic acid-inducible gene-I (RIG-I) like receptors (RLRs, e.g., RIG-I & Melanoma differentiation-associated gene 5 (MDA5)) along with PKR and cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING). Among these receptors, RIG-I and MDA5 recognize viral RNA, single-stranded, and double-stranded, respectively. This recognition stimulates release of interferon regulatory factors (IRF3 & IRF7) and nuclear factor kappa light chain enhancer of activated B-cells (NF-κB). These both elements are very much important to act as key transcriptional regulator of interferon (IFN) in modulating innate immune responses [43]. In an infection induced by virus, cytosolic DNA sensor, the cGAS plays vital role in recognizing cytoplasmic DNA

while STING act as a connector to identify cGAMP. These recognitions, however, activate in-line signals to uplift interferon's production and corresponding cytokines [33]. In response to these cytokines, the innate immune system responds to active cells like dendritic cells (DCs) and nature killer (NK) cells. This recruitment reverts the microenvironment in tumors. For example, reovirus has the ability to reverse poor function of dendritic cells involved in melanoma-type tumor by inducing of higher concentrations of cytokines and co-stimulatory factors. The reoviruses are also induced release of pro-inflammatory factors (e.g., MIP-1α/β) while they minimized the release of IL-10 from immunosuppressive cells. This process initiates a diversity of immune responses [60].

7.2.2.2 Adaptive Immunity

The host immune system is responsive to detect and cast-off nascent tumor. The tumor-associated small peptides are recognized by receptors present on cytotoxic T lymphocytes (CTLs) [22]. Tumor cells can sometimes evade immune responses due to minimal expression of major histocompatibility complex (MHC) molecules [14]. The MHC molecules have a crucial value in escaping tumor from antitumor mechanism and presentation to T lymphocytes. These tumors do not persuade CTLs activation and antitumor mechanism [2]. The potential of oncolytic viruses to increase DCs induction, DCs maturation, and antigen presentation is worth interesting [63]. After maturation, the dendritic cells primarily functions in immune stimulation and show these peptide antigens present on MHC-I to cytotoxic (CD8+) and MHC-II to helper (CD4+) cells that ultimately co-stimulate signals to activate T cells [57]. Murphy and colleagues [55] have noticed a higher expression of tumor-associated MHC-I ligand by treating it with oncolytic reovirus while they were working on a model for ovarian cancer and a similar increased expression was observed in spleen tissue as well. The treating strategy in CMT64 lung adenocarcinoma cells through oncolytic adenoviruses has also exhibited similar results. In a different experimental model, the DCs and tumor cells were incubated without treating them with oncolytic reoviruses. These cells could not induce cytotoxic effects and cellular deaths while the same treatment with oncolytic reoviruses has resulted in vice versa. These results highlighted potential oncolytic ability of different viruses. Hence, the oncolytic viruses can be used for various tumors and/or at different stages of tumor development. The oncolytic virotherapy is also capable to reduce the chances of tumor reoccurrence through maintaining immunological memory. The Sindbis virus is used along with α4- 1BB monoclonal antibodies to treat in-vivo induced lymphoma that resulted in regression of lymphoma and long-lasting antitumor immunological memory [86]. Similarly, a recombinant oncolytic adenovirus containing two genes of human origin (IL-2 gene and hTNF-α gene) along with tumor-infiltrating lymphocytes to formulate a strategy to recover from pancreatic carcinoma in Syrian hamsters [28]. Through this strategy, higher frequency of helper T cells and cytotoxic T cells was detected along with their enhanced persistence. Moreover, this treatment with recombinant adenovirus vector has reduced the chances of recurrence of pancreatic cancer in Syrian hamsters [88].

7.3 Oncolytic Viruses

The advances that have recently been made in viral cellular and genomic structure and also the immune responses associated with cancer development have unrevealed the potential of oncolytic viruses as cancer therapy. Various virus families (DNA and/or RNA) effectively advanced from preclinical phase into clinical trials in the last two decades (Table 7.2). OVs may possess anti-neoplastic properties naturally or may be tailored for tumor-selective replication, and some of them are also armed with therapeutic transgenes to activate the immune system.

7.3.1 Herpes Simplex Virus (HSV)

HSV-1 is an enveloped, icosahedral virus from the alphaherpesvirus family, consisting of a large double-stranded DNA genome (150 kb). Around 30 kb of the genome is known to encode genes that may not be vital for viral infection [34, 49]. The large genome size significantly contributes to the improved oncolytic potential of the virus as it allows the transfer of large and/or multiple foreign genes. In addition, HSV-1 can infect a variety of tumor cells while not allowing the viral DNA to integrate into the host genome. Antiviral drugs against HSV are also available. These properties make HSV-1 an appealing target for the development of oncolytic virotherapy [72]. HSV has been considered promising therapeutic agent different tumor types since its development as oncolytic virus in 1991. HSV-1 was the first virus whose backbone was genetically altered for cancer treatment. A study in 1991 demonstrated that treatment with HSV-1 containing a deletion of thymidine kinase (HSV-dlspTK) led to the tumor growth inhibition as well as prolonged overall survival in a glioblastoma murine model [52]. Further advancements led to the development of many other oncolytic HSVs, and some are being clinically evaluated currently. The most effective among them was Talimogene Laherparepvec (T-VEC), the first OV approved by the US Food and Drug Administration (FDA) in 2015 for treatment of melanoma lesions in the skin and lymph nodes [59]. T-VEC contains mutations in genes resulting in the inactivation of neurovirulence factors as well as enhancement of virus replication and immunogenicity in the tumor microenvironment. These mutations include insertion of GM-CSF insertion and γ34.5 and ICP47 deletion, respectively [44, 75]. Another oncolytic HSV-1, named DELYTACT (G47Δ; teserpaturev) developed by Todo et al. in [73], was conditionally approved in Japan recently for the treatment of glioblastoma [70].

7.3.2 Adenovirus

Adenovirus, a non-enveloped virus, contains an icosahedral capsid encapsulating a 35 kb long, double-stranded linear DNA genome. The capsid comprises of multiple copies of three essential proteins, hexon, penton base, and fiber that are responsible for the antiviral response after recognition by the host immune

Table 7.2 Characteristics of oncolytic viruses

Oncolytic virus	Family	Genome	Size (kb)	Virion size (nm)	Cell receptor	Engineering for specificity
Adenovirus	Adenoviridae	dsDNA	35	70–90	CAR, CD46, VCAM-1	Deletion of E1A; E1B; E3; RGD; TJK, insertion of E2F1; GM-CSF; DM1; Kozak, replacement of KKTK with RGDK
Herpes virus	Herpesviridae	dsDNA	154	200	HVEM, Nectin 1 & 2	ICP34.5; ICP47 deletion; US11, human GM-CSF insertion, disruption of UL39, attenuation of HSV-1 mutant
Vaccinia virus	Poxviridae	dsDNA	190	70–100	Unknown	Deletion of TK;VGF, insertion of human GM-CSF, mutation in B18R
Parvovirus H-1	Parvoviridae	ssDNA	5	18–28	Sialic acid residues	None
Reovirus	Reoviridae	dsRNA	23	75	JAM-A	Wild type
Polio virus	Picornaviridae	ss(+)RNA	5	30	CD155	Substitutions of 11 amino acids located in L1 segment
Coxsackie virus	Picornaviridae	ss(+)RNA	7.5	28	CAR/ICAM-1/DAF	None
Measles virus	Paramyxoviridae	ss(−)RNA	16	100–200	SLAM, CD46	MV-CEA, MV-NIS

(continued)

Table 7.2 (continued)

Oncolytic virus	Family	Genome	Size (kb)	Virion size (nm)	Cell receptor	Engineering for specificity
Newcastle disease virus	Paramyxoviridae	ss(−)RNA	15	100–500	Unknown	None
Vesicular Stomatitis virus	Rhabdoviridae	ss(−)RNA	11	80	LDLR	Recombinant VSV, mutation in M; G; insertion of miRNA target sequences for safety and transgene for immunomodulation

CAR, coxsackie-adenovirus receptor; CD 46, cluster of differentiation 46; VCAM-1, Vascular cell adhesion molecule 1; HVEM, herpes virus entry mediator; JAM-A, junction adhesion molecule; ICAM-1, intracellular adhesion molecule 1; DAF, decay accelerating factor; LDLR, low-density lipoprotein receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICP, infected cell protein; TK, thymidine kinase gene; VGF, Vaccinia virus growth factor, MV-CEA, carcinoembryonic antigen-expressing measles virus, MV-NIS, measles virus engineered to express the sodium iodide symporter gene; M, matrix; G, glycoprotein

system [40]. The large genome of adenovirus allows incorporation of long DNA sequences and multiple gene modifications, thus making it an excellent viral vector for clinical development. Moreover, the ability of the virus to exploit multiple receptors, including the human coxsackie-adenovirus receptor, CD46, CD80, and CD86 for entry into host cells, the thermal and chemical stability outside the cell, and understanding of the biology of adenoviruses, they have been considered an efficient target for the development of novel immunotherapies for different cancers [76]. Adenoviruses can infect both humans and animals with over 50 serotypes which are known to cause infections in humans and some of them are associated with a range of pathological conditions ranging from minor respiratory infections in young children to life-threatening illnesses in immunocompromised patients. Among all the serotypes, serotype 5 backbone has been most commonly employed for oncolytic virus therapies [4]. Adenoviruses use the early genes that encode E1A and E1B for proliferation within the host cells which are crucial for viral replication in normal cells, however, redundant in cancer cells. E1A and E1B genes promote the cell cycle entry through suppression of the tumor suppressors P53 and retinoblastoma-associated protein (pRB). In healthy cells, pRB binds to another early protein E2F and inhibits cell cycle progression. E2F function as a transcription activator of critical genes that facilitate the entry of normal cells to S phase of the cell cycle. To support virus replication in tumor cells selectively without damaging the normal cells, a strategy consisting of a mutated E1A gene is adapted. Expression of a defective E1A harboring a deletion of 24 bps (E1A-d24) in cells lacking pRB thus blocks normal cells from entering S phase resulting in the suppression of virus replication [5, 74]. This strategy has been adopted in the development of an oncolytic adenovirus called H101 (Oncocrine) in which E1A/E1B genes are deleted. Oncocrine was the first recombinant oncolytic adenovirus approved in China after successful clinical trials against nasopharyngeal carcinomas in 2005 [42, 81]. Similarly, another oncolytic adenovirus Onyx-015 having complete deletion of E1B was developed by Onyx Pharmaceutical, South San Francisco, USA, and its safety was proven in phase 1 clinical trials for treating patients with head and neck cancer, premalignant oral dysplasia pancreatic and ovarian cancer [38]. Recently, safety and efficacy of CRAd-S-pk7, a conditionally replicative oncolytic adenovirus containing tumor-specific survivin promoter (S) and a fiber protein polylysine modification (pk7), has been established for treatment of glioma (high-grade) patients in phase I clinical trials [17].

7.3.3 Reovirus

Respiratory enteric orphan virus (reovirus) from the Reoviridae family is a non-enveloped virus having a double-stranded segmented RNA genome, an outer icosahedral capsid and an inner protein core [58]. Reoviruses have been known to cause respiratory and gastrointestinal infections in humans but unable to cause any serious illness, therefore widely exploited as oncolytic viruses in the unmodified form. The natural tropism of reoviruses for tumor cells and their replication

ability in the transformed cells with altered signaling pathways (Ras mutation) makes them an invaluable target for oncolytic virotherapy [27]. The mechanism of reovirus induced oncolysis include apoptosis along with autophagy and immunomodulation effects of the virus [80]. Treatment of mouse models having subcutaneous and intracerebral gliomas with oncolytic reovirus led to an intense reduction and in some cases total regression of the tumor through direct tumor lysis along with a substantial upsurge in T cell infiltration and Type 1 interferon secretion in the tumor microenvironment [69]. Among the reovirus strains, reovirus dearing strain (type 3) also known as Pelareorep or Reolysin, has been the most studied OV clinically for therapies in a number of carcinomas such as melanoma, gliomas, colorectal and ovarian cancer, particularly in breast cancers. Phase 1 clinical studies of advanced cancer patients using reovirus alone (Reolysin) for therapy showed promising antitumor activities and were proven safe and well-tolerated [26, 78]. The combinations of reovirus with chemotherapies, radiotherapies, and immunotherapies have shown promising results in various clinical trials including breast cancer patients [39].

7.3.4 Vaccinia Virus

Vaccinia virus, belonging to poxvirus family, is a large, enveloped virus with dsDNA genome of around 190 kb in length. The classic role of attenuated vaccinia virus in the small pox vaccination has been well-known particularly due to its safety profile and the generation of a potent cell-mediated and humoral immunity [77]. Therefore, it has been considered a promising oncolytic immunotherapeutic agent for cancer treatments as well. The potential of vaccinia to allow large transgene insertion, the capacity to infect a variety of cells, and a natural tropism for cancer cells makes it a highly suitable vector for oncolytic virotherapy [56]. Many vaccinia virus strains have been attenuated to improve their tumor selectivity and oncolytic potential and are currently being evaluated for clinical safety and efficacy. Pexastimogene devacirepvec (Pexa-Vec) also known as JX-594 is an attenuated oncolytic vaccinia virus which has been designed to contain granulocyte-macrophage colony-stimulating factor (GM-CSF) and a deleted thymidine kinase (TK) gene to allow selective replication in the tumor cells [37]. Pexa-Vec has recently been assessed alongside immune checkpoint inhibitors in phase 1/II trials in advanced colorectal cancer patients. The treatment was proven to be safe with possible antitumor activity and was well-tolerated in patients [54]. Other oncolytic vaccinia viruses developed for cancer therapies include TG6002, which contains a double deletion of thymidine kinase gene and viral ribonucleotide reductase to improve tumor specificity [18] and GL-ONC1, a modified vaccinia virus strain, which contains three insertional mutations (Ruc-GFP, β-glucuronidase, and β-galactosidase) [41].

7.3.5 Other Viruses

Numerous other OVs are under investigation for cancer therapies which are now in different phases of clinical development. These include measles virus [15], poliovirus [9], coxsackievirus [46], NDV [36], vesicular stomatitis virus (VSV) [29], Retrovirus [47], and parvovirus [23].

Clinical trials of different OVs against glioma, melanoma, pancreatic, and breast cancers have been summarized in Table 7.3. Clinical findings provided evidences that oncolytic viruses selectively infected tumor cells and have undergone viral replication and lysed tumor cells. Published data confirmed that the OVs are safe and well-tolerated in cancer patients.

7.4 Oncolytic Viruses and the Blood–Brain Barrier

The most common route for administering OVs to the tumor site has been the intratumoral delivery in preclinical and clinical trials. However, this has been the limiting factor in cases of inaccessible tumors such as that of brain tumors as the blood-brain barrier (BBB) blocks the efficient delivery of viruses or any other therapeutic agents to the target site [12]. Some OVs are known to have natural tropism for the central nervous system and are able to cross the BBB thus it is possible to deliver them to the target site in the brain intravenously. These viruses include Seneca Valley virus (SVV), chimeric VSV, vaccinia virus, Semliki Forest virus, and parvovirus H-1 [71]. Systemic administration of SVV-001 in preclinical studies demonstrated efficient delivery of the virus across the BBB in medulloblastoma [85] and pediatric glioma mouse models [45] and showed potent anticancer activity. Intravenous administration of REOLYSIN in phase 1 trials of glioma patients showed that REOLYSIN was able to cross the BBB and efficiently replicate in tumor [32]. For optimal efficacy of the OVs in CNS tumors, carrier cells have been utilized for loading the virus before intravenous administration. Neural and mesenchymal stem cells have been exploited as carriers for oncolytic virotherapy and have shown promising outcomes in terms of efficient delivery of OV in preclinical trials of malignant gliomas [87].

7.5 Conclusion

Globally, cancer is the major cause of mortality, and currently employing radiotherapy and chemotherapy have many side effects as these therapies are not specifically targeting cancer cells. Oncolytic virotherapy has become a promising immunotherapy against cancers. Oncolytic viruses are capable of multiplying in tumor cells selectively allowing them to target and destroy cancerous cells without harming normal cells. Oncolytic viruses that belong to diverse families of viruses can be naturally occurring or modified through genetic manipulations. Engineered oncolytic viruses have enhanced tumor-targeting ability and oncolytic

Table 7.3 Clinical trials of various oncolytic viruses

NCT number	Intervention	Title	Conditions	Phase	Study results
NCT01844661	Biological: **CELYVIR**, bone marrow-derived autologous mesenchymal stem cells (MSCs) infected with an oncolytic Adenovirus, ICOVIR5	Safety and efficacy of repeated infusion of CELYVIR in children and adults with metastatic and refractory tumors	Solid tumors in Children, Metastases	1, 2	Celyvir has been confirmed to be safe for use in oncolytic virotherapy and warrant further study in the phase 2 setting. It enhances anticancer effects while avoiding unacceptable toxicities [53]
NCT01598129	Genetic: **ONCOS-102** (CGTG-102), Adenovirus armed with a potent stimulator of immunological cells, granulocyte-macrophage colony-stimulating factor (GM-CSF)	ONCOS-102 (previously CGTG-102) for therapy of advanced cancers	Malignant solid tumor	1	ONCOS-102 is well-tolerated and induces local and systemic CD8 + T cell immune response with immune cell-poor and treatment refractory tumor patients [64]
NCT02365818	Biological: **CG0070**, an oncolytic Adenovirus having an E2F promotor and GM-CSF	Safety and efficacy of CG0070 oncolytic virus regimen for high-grade non-muscle invasive bladder cancer (NMIBC) after BCG failure (BOND2)	Bladder cancer	2	CG0070 produced an overall 47% complete response in all patients and 50% for carcinoma-in-situ patients, with an acceptable level of toxic effects for patients with high-risk BCG-unresponsive NMIBC [79]

(continued)

Table 7.3 (continued)

NCT number	Intervention	Title	Conditions	Phase	Study results
NCT02053220	Biological: ColoAd1 Oncolytic virus	Mechanism of action trial of ColoAd1 (MOA)	Resectable colon cancer, resectable renal cell carcinoma resectable bladder cancer resectable non-small cell lung cancer	1	The enadenotucirev (ColoAd1) delivery by both IV and IT methods was found well-tolerated and produced high local CD8 + cell infiltration in 80% of samples, resulting in a potential enadenotucirev-mediated immunity [21]
NCT03072134	Biological: Neural stem cells loaded with an oncolytic Adenovirus	Neural stem cell-based virotherapy of newly diagnosed malignant glioma	Glioma, anaplastic astrocytoma, anaplastic oligodendroglioma, anaplastic oligoastrocytoma, glioblastoma multiforme, astrocytoma grade III & IV, Brain cancer	1	Administration of engineered oncolytic virus (NSC-CRAd-S-pk7) in malignant glioma patients showed promising results in terms of survival. This trial provides basis for further studies in phase 2/3 settings [16]
NCT00769704	Biological: Talimogene laherparepvec (**OncoVEX^{GM-CSF}**), genetically engineered oncolytic herpes simplex virus type 1 (HSV-1) Biological: GM-CSF	Efficacy and safety study of Talimogene laherparepvec compared to granulocyte–macrophage colony-stimulating factor (GM-CSF) in melanoma	Melanoma	3	Talimogene laherparepvec resulted in an enhanced longer-term efficacy as compared to GM-CSF with good tolerability. Further investigation revealed that Talimogene laherparepvec resulted in complete response, and prolonged survival [1]

(continued)

Table 7.3 (continued)

NCT number	Intervention	Title	Conditions	Phase	Study results
NCT02263508	Biological: Talimogene laherparepvec Drug: Anti-programmed death protein 1 PD-1 (pembrolizuma; MK-3475)	Pembrolizumab with or without Talimogene laherparepvec or Talimogene laherparepvec placebo in unresected melanoma (KEYNOTE-034)	Melanoma	3	Treatment of Talimogene laherparepvec in combination with pembrolizumab was found well-tolerated without dose-limiting toxicities. Response was not associated with baseline CD8 + T cell infiltration or IFN- γ signature suggesting an improvement of the efficacy of anti-PD-1 therapy with the help of oncolytic virotherapy by altering the tumor microenvironment [65]
NCT00402025	Biological: Talimogene laherparepvec	Talimogene laherparepvec in patients with unresectable pancreatic cancer	Pancreatic cancer	1	T-VEC was found feasible and tolerable up to doses of 10^7 PFU/mL and showed biologic activity [10]
NCT01017185	Biological: **HF10**, a replication competent Herpes Simplex virus type 1	Study of HF10 in patients with refractory head and neck cancer or solid tumors with cutaneous and/or superficial lesions	Refractory head and neck cancer, malignant melanoma squamous cell carcinoma, skin, carcinoma of the breast	1	In HSV + and HSV- patients with refractory/superficial cancers, HF10 was found to be safe and well-tolerated. The viral antitumor activity of HF10 suggests that it could be a promising oncolytic virus against cancer [19]

(continued)

Table 7.3 (continued)

NCT number	Intervention	Title	Conditions	Phase	Study results
NCT02272855	Biological: **HF10** plus Ipilimumab	A study of combination treatment with HF10 and Ipilimumab in patients with unresectable or metastatic melanoma	Malignant melanoma	2	HF10 + ipi has shown favorable benefit/risk profile, and the combination has substantially improved the antitumor activity; thus, it could be a novel therapeutic option for metastatic melanoma [67]
NCT00429312	Biological: **JX-594**, Thymidine kinase-deleted Vaccinia virus plus GM-CSF	A Study of recombinant Vaccinia virus to treat malignant melanoma	Melanoma	1, 2	JX-594 (Pexa-Vec) was found safe and effective. JX-594 has replicated, expressed transgenes, and induced tumor regression. These findings have implications for future clinical products from this new therapeutic class [30]
NCT01227551	Biological: Coxsackievirus A21 (CVA21)	A study of intratumoral CAVATAK™ in patients with stage IIIc and stage IV malignant melanoma (VLA-007 CALM) (CALM)	Malignant melanoma	2	Oncolytic Coxsackievirus A21 (V937) was well-tolerated and suggests further studies for the treatment of unrespectable melanoma patients. Furthermore, investigations are underway in combination with V937 and immune check inhibitors [66]

(continued)

Table 7.3 (continued)

NCT number	Intervention	Title	Conditions	Phase	Study results
NCT00984464	Biological: **REOLYSIN**, Reovirus serotype 3 Dearing strain Drug: Carboplatin Drug: Paclitaxel	Study of REOLYSIN® in combination with Paclitaxel and Carboplatin in patients with metastatic melanoma	Metastatic melanoma	2	REOLYSIN in combination with carboplatin and paclitaxel was found safe and effective for the treatment of malignant melanoma patients [51]
NCT01301430	Drug: Parvovirus H-1 (ParvOryx)	Parvovirus H-1 (ParvOryx) in patients with progressive primary or recurrent glioblastoma multiforme. (ParvOryx01)	Glioblastoma multiforme	1, 2	Trial showed an evidence of H-1PV safety and tolerability. H-1PV has been able to breach the blood–brain/tumor barrier and establish an immunogenic tumor microenvironment, suggesting H-1PV as a clinically potential therapy for future development [24]
NCT01491893	Biological: Recombinant nonpathogenic polio-rhinovirus chimera (PVSRIPO)	PVSRIPO for Recurrent Glioblastoma (GBM) (PVSRIPO)	GBM, glioblastoma, glioma, malignant glioma	1	Intratumoral infusion of PVSRIPO in recurrent malignant glioma patients indicated no evidence of neurovirulence and also showed an increased survival of patients who have received PVSRIPO immunotherapy than that of historical controls [13]

activity. The mode of action of oncolytic viruses has been discovered after years of research, and it was found that in addition to killing tumor cells directly through virus replication, oncolytic viruses also enhance the antitumor immune response through modulation of the immune-related molecules and promote the maturation, migration, and infiltration of immune cells. Oncolytic viruses have been shown to generate potent anticancer immune responses in animal models and clinical trials in cancer patients after decades of extensive research findings. Hopefully, the comprehensive knowledge regarding oncolytic virotherapy and the cancer biology will lead to promising treatment modality in the near future.

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Osteosarcoma and Its Advancement

8

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Cancer is a leading cause or mortality which is just second to mortality caused by cardiac conditions. New advance techniques are developed in treatment of cancer over the past time; it still continues to be a major health threat, and extensive work trial is being done to develop new therapeutic lines.

The past trial methods of the treatment have proven that cancer can be effectively treated with surgery, hormonal, chemotherapy, immunotherapy, and radiotherapy. These treatment methods have been used either alone or combined to bring the maximum benefit of the treatment, which have significantly benefited to control tumor growth and even to produce cures for the cancers which were considered incurable in the past. The metastasized cancer still have treatment challenges as currently developed treatment methods are not strong enough alone or in combination to treat complicated metastasized cancer. Still, treatment development challenges remain for certain types of cancer.

Diagnosis and staging of cancers is important to choose from the possible treatment options developed at present for which biopsy is of most importance for identifying cancer stage, histology, and the presence of metastases for staging. Cancer characteristics differ among different patients with same class of cancer. Recent advancement in genetic analysis has shown that each patient expresses different subtype of cancer cells because of new genetic mutations causing irreversible changes in the cell structure [1].

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Advancement in new image guidance biopsy has improved with the safety, for example, true-cut biopsy in breast cancer does not require patient to undergo surgical excision biopsy in many cases [2].

Lab-based analysis of cancer cells is necessary to understand it to decide therapeutic approach for each patient, which helps in drug development with precision. Research work on biopsies serves as a basic foundation stone for development and improvement in precision medicine. Due to its importance in cancer treatment approach, it have become a mandatory component in research clinical trials which are aimed at studying drug effects on specific cancer cell type and identifying relevant biomarkers. Hence, in this chapter, the focus will be on recent advances and the current challenges in osteosarcoma.

To bring a successful treatment approach of patients with osteosarcoma, it requires multidisciplinary team approach including pediatric, medical and surgical oncologist, surgeons, pathologists, pain management, orthopedic oncologist, endocrinologist, and radiologists. Therefore, cancer treatment is to be performed in specialized hospitals able to provide access to all approaches with multidisciplinary team care. Therapy aims to include surgical resection of all detectable tumor sites where excision is possible as well as combination of chemo, radio, and hormonal therapies. A genetic screening is advisable where possible to understand possible mutations. The basic chemotherapy is to include several or all of the following drugs: doxorubicin, high-dose methotrexate with Leu-k-ovorin-rescue, cisplatin, and ifosfamide. Preoperative and postoperative polychemotherapy should be considered because it gives safe surgery and preparation of the suitable prosthesis for the individual need. The choice to postpone definitive surgical procedure should be influenced by the anatomical site of the primary tumor, its relationship to surrounding structures like blood vessels and nerves, the age and growth potential of the patient. A major challenge remains facing problem in prognosis for patients with unresectable, advances, and relapsed osteosarcomas. New therapeutic approaches are needed to improve their prognosis outcome. Before the development of polychemotherapy, >90% of patients with osteosarcoma died from pulmonary metastases [3].

8.1 Epidemiology

Osteosarcoma is a mesenchymal origin, advance bone sarcoma. It comes as in malignant form, cartilage producing bone cancer. It is less common, with an estimated occurrence of one in 200,000 persons [4]. Still osteosarcoma occurs in the general population with average of two to three million each year, but is higher in adolescence, in which the annual incidence peaks at 8–11/million/year at 15–19 years of age. Osteosarcomas account for 15% of all solid extracranial cancers in this age group. Males are affected 1.4 times more frequently than females [5, 6]. The most frequent sites of origin are the distal femur, proximal tibia, and proximal humerus. Patients typically present with pain, swelling, localized enlargement

of the extremity and occasionally, pathologic fracture. Most patients present with localized disease.

8.2 Etiology and Pathogenesis

In most patients, the etiology of osteosarcoma remains obscure. The predilection of osteosarcoma for the age of the pubertal growth spurt and the sites of maximum growth suggest a correlation with rapid bone proliferation. A minority of osteosarcomas is caused by radiation exposure. The incidence of osteosarcoma is increased in several well-defined hereditary disorders associated with germline alterations of tumor suppressor genes such as hereditary retinoblastoma [1] and the Li–Fraumeni cancer family syndrome [7].

Before 1970, osteosarcomas were treated with amputation. Survival was poor: 80% of patients died from metastatic disease. With the development of induction and adjuvant chemotherapy protocols, advances in surgical techniques and improvements in radiologic staging studies, 90–95% of patients with osteosarcoma can now be treated with limb-sparing resection and reconstruction. Long-term survival and cure rates have increased to between 60 and 80% in patients with localized disease [8].

8.3 Biologic and Genetic Behavior of Osteosarcoma

An osteosarcoma grows in a radial manner, forming a ball-like mass. When it penetrates the bony cortex, it compresses the surrounding muscles into a pseudocapsular layer referred to as the "reactive zone." Tumor nodules representing microextensions of the primary mass invade the reactive zone (satellites)." The entire tumor mass, including the satellites, must be resected to ensure removal of all gross tumor. Thus, the surgical margin must be wide.

Genetic aberrations or changes in gene expression in osteosarcoma tissues have not identified common or recurrent genetic lesions or pathway alterations that explain the development of this tumor type [9, 10]. Rather, osteosarcoma is best characterized by its disorganized genome. Indeed, the most consistent genetic finding in osteosarcoma, beyond dysregulation of p53 and Rb (retinoblastoma), is significant aneuploidy and some evidence of massive disruption in the control of chromosomal structure (i.e., chromothripsis) [10, 11]. This has suggested the possibility of an early defect in DNA repair/surveillance as a mechanism for osteosarcomagenesis and the resultant bizarre aneuploidy. Irrespective of the actual cell of origin, it is commonly agreed that the gene expression and the cellular phenotype of osteosarcoma are related to bone [12].

8.4 Diagnosis and Staging

Patients typically present with dull, aching pain of several months' duration that may suddenly become more severe, followed by localized swelling and limitation of joint movement, are the typical signs and symptoms of osteosarcoma. In rare cases, particularly in patients with osteolytic tumors, a pathological fracture can be the first sign of disease. The tumor may metastasize regionally or systemically. With metastasis, the prognosis worsens dramatically [13]. Systemic metastases have a predilection for the lungs. The bones are the second most common site of metastasis and usually become involved only after pulmonary metastases have occurred. Distant bone metastases represent the latest stage of disease and are associated with the poorest prognosis [14–16].

8.5 Recommended Diagnostic Work-Up for Osteosarcoma Patients

See (Table 8.1).

Table 8.1 Shows the routine steps taken for the diagnosis of osteosarcoma patient

Primary tumor	
X-ray	Tumor localization in two planes Whole extremity p/a
Magnetic resonance imaging	Whole extremity/tumor region
Metastases	
^{99m}Tc bone scan	Whole skeleton
Computed tomography	Chest
Organ function	
Heart	Echocardiogram, electrocardiogram
Hearing	Audiogram
Kidney	Creatinine (including estimated clearance) Tubular function tests
Liver	Liver function tests
Other laboratory	
Alkaline phosphatase i.S.	
Lactate dehydrogenase i.S.	

8.6 Plain Radiography

The basic radiology is helpful in primary detection of osseous appearances like osteoblastic, osteolytic, or mixed appearance. They can identify calcifications patches resulting from new bone formation or spiculae. Codman triangle is a radiologic sign seen most commonly on musculoskeletal plain films, a periosteal reaction that occurs when bone lesions grow so aggressively they lift the periosteum off the bone and do not allow the periosteum to lay down new bone. The sunburst appearance occurs when the lesion grows too fast and the periosteum does not have enough time to lay down a new layer and instead the Sharpey's fibers stretch out perpendicular to the bone. It is frequently associated with osteosarcoma but can also occur with other aggressive bony lesions [17].

8.7 Magnetic Resonance Imaging

MRI is superior to the other imaging modalities in detecting bone marrow lesions and tumoral tissue which are unclear on plain X-rays. MRIs can help determine the exact extent of a tumor, as they can show the marrow inside bones and the soft tissues around the tumor, including nearby blood vessels and nerves. MRIs can also show any small bone tumors and the skip metastases. In MRI, T1- and T2-weighted and fat-suppressed images are obtained to demonstrate the affected region [18–20]. An MRI scan taken at the time of surgical resection allows for precise planning of the osteotomy site, which is 2–3 cm away from the tumor [21].

Once cancer has been diagnosed, a metastatic work-up is required, which includes a CT scan and a bone scan. Body bone scintigraphy in three phases can help establish metastatic disease sites, polyostotic involvement, and the tumor's intraosseous extension. CT scanning of the affected limb is helpful in determining the tumor's extraosseous and intraosseous extent. Although osteosarcomas lack particular tumor markers, some patients have elevated lactate dehydrogenase or, more commonly, alkaline phosphatase levels in their blood. Both have been linked to negative results. Patients should have baseline examinations such as echocardiography, an audiogram, and liver and kidney function tests since polychemotherapy for osteosarcoma can cause cardiac and auditory impairment as well as substantial renal and liver toxicity.

8.8 Histopathology

The most important step in diagnosing an osteosarcoma is a biopsy. Biopsies conducted incorrectly are a common cause of misdiagnosis, amputation, and local recurrence, and they can reduce survival. Osteosarcoma diagnosis must always be confirmed histologically. Because of the wide range of histological appearances

and the tumor's rarity, it is strongly suggested that biopsies be performed in specialized centers, where suitable biopsy techniques and histological examination of the acquired material, including genetic evaluation, are ensured.

The most suitable procedure for obtaining sufficient material for histological assessment and subsequent studies is open biopsy. True-cut needle biopsy can also be utilized if enough material can be recovered; however, fine-needle biopsies are not recommended.

The biopsy specimen should not be fixed before being sent to a qualified pathologist. The proliferation of malignant mesenchymal tumor cells, as well as their formation of osteoid and/or bone, is diagnostic features. The degree of osteoid and/or bone formation varies a lot between tumors and even within one.

8.9 Pathology and Staging

Depending on cellularity, pleomorphism, anaplasia, and the number of mitoses, osteosarcomas can be classified as high grade or low grade. Each kind of tumor has its own osteoid production pattern. Conventional osteosarcomas are made up of malignant-looking spindle cells that create osteoid under the microscope. The Enneking system is the most widely used orthopedic staging method [22].

Enneking System for Staging Osteosarcomas

Stage Grade and anatomic extent
I Low-grade tumor
IA Intracompartmental
IB Extracompartmental
II High-grade tumor
IIA Intracompartmental
IIB Extracompartmental
III Either grade with metastases

8.10 WHO Classification

The current World Health Organization (WHO) classification [23] divides conventional osteosarcoma into three major subtypes: osteoblastic, chondroblastic, and fibroblastic, based on the predominant matrix type within the tumor. Other histological types of osteosarcoma recognized by the WHO classification include telangiectatic osteosarcoma, small cell osteosarcoma, parosteal and periosteal osteosarcomas, as well as low-grade central and high-grade surface osteosarcomas [23]. Surface osteosarcomas are generally low-grade I or intermediate-grade II tumors, whereas central subtypes are almost always WHO grade III high malignant tumors.

8.11 Multidisciplinary Treatment Approach

If chemotherapy is not included as part of the multidisciplinary treatment, 80–90% of all patients with seemingly limited disease will develop metastases, primarily in the lungs, and will die [24]. Chemotherapy's efficacy has been proven in two randomized trials in North America [25]. Currently available treatment regimens that include primary (preoperative; neoadjuvant) induction chemotherapy, definitive surgery, and then postoperative (adjuvant) chemotherapy cure about two-thirds of patients with ostensibly confined disease [16, 24]. Chemotherapy normally takes at least 6–8 months to complete.

8.12 Treatment

8.12.1 Chemotherapy

In the treatment of osteosarcoma, chemotherapy is essential. Over the last 30 years, advances in chemotherapy have resulted in improved limb salvage and increased survival rates [26]. Chemotherapy has also been proven to minimize the amount of lung metastases or postpone their emergence, thereby making surgical removal easier.

Induction and adjuvant chemotherapy are now standard treatments. Induction chemotherapy causes tumor necrosis in the primary tumor, allowing micrometastatic disease to be treated early. It aids in wide-margin surgical resection and has thus been one of the key factors contributing to increased limb salvage rates.

Doxorubicin (Adriamycin), cisplatin (Platinol), ifosfamide (Ifex) with mesna (Mesnex), and high-dose methotrexate (Rheumatrex) with leucovorin calcium rescue have all been demonstrated to be successful against osteosarcoma. Table 8.2 summarizes the mechanisms of action and side effects of different chemotherapeutic drugs.

In patients with localized (nonmetastatic) disease, modern multiagent, dose-intensive chemotherapy regimens have resulted in long-term disease-free survival rates of 60–80%.

8.13 Surgery

A successful removal of a tumor and reconstruction of a viable, functional extremity is referred to as "limb salvage." In 90–95% of patients, limb-sparing resection and repair, rather than amputation, can be safely performed during induction chemotherapy.

Complete tumor excision must always be the goal of osteosarcoma surgery. According to Enneking's definition [2], margins should be at least broad, implying that the tumor, including the biopsy scar, must be removed surrounded by an

Table 8.2 Chemotherapeutic agents used in the treatment of osteosarcoma

Agent	*Mechanism of action*	*Side effects*
Doxorubicin (Adriamycin)	Doxorubicin intercalates at points of local uncoiling of the DNA double helix; it also inhibits the synthesis of DNA and RNA.	Cardiomyopathy, transient electrocardiographic abnormalities, emesis, alopecia, mucositis, myelosuppression
Cisplatin (Platinol)	Cisplatin inhibits the synthesis of DNA through the formation of DNA cross-links; it binds directly to tumor DNA and denatures the DNA double helix.	Acute renal failure, chronic renal failure, peripheral neuropathy, ototoxicity, emesis, myelosuppression, alopecia, hypomagnesemia
Ifosfamide (Ifex), with mesna (Mesnex)*	Ifosfamide causes cross-linking of DNA strands, inhibiting the synthesis of DNA and protein.	Hemorrhagic cystitis, renal failure, myelosuppression, alopecia, emesis, encephalopathy
High-dose methotrexate (Rheumatrex), with leucovorin calcium rescue†	Methotrexate is a folate antimetabolite; it inhibits the synthesis of purine and thymidylic acid by binding dihydrofolate reductase.	Renal failure, mucositis, mild myelosuppression; rarely, central nervous system effects

inviolate cuff of healthy tissue. Advances in imaging and biomedical engineering, as well as the beneficial effects of preoperative chemotherapy, have resulted in a significant movement away from amputation and toward limb-salvage surgery [7, 24, 27, 28]. Endoprosthetic devices [27–29], biological reconstruction, or a mix of both are all options for reconstruction after limb-sparing tumor resections.

Another well-established biological reconstruction procedure for tumors around the knee, rotation-plasty, can produce functional and psychological outcomes that are comparable to or even better than endoprosthetic reconstruction [21], but it is visually difficult.

Surgery for axial skeleton sarcomas is very difficult, both because local recurrence is a significant risk and because problems following reconstruction are common [19, 22, 23]. Surgeons must be familiar with all surgical approaches and use the one that is most appropriate for each patient after consulting with the multidisciplinary osteosarcoma team. Total en bloc spondylectomy for spinal tumors [23] and hip transposition for pelvic sarcomas are two recent developments [24].

8.14 Radiotherapy

Because osteosarcoma has long been thought to be a radioresistant tumor, local treatment with radiotherapy for osteosarcomas has been limited [30]. Patients treated with multiagent chemotherapy who are unable to perform complete resection or who have microscopic remaining tumor foci following attempted

resection may benefit from radiation [31]. In some cases, targeted irradiation with Samarium-153-ethylendiame tetramethylene phosphonate may be considered, while the role of this therapeutic method is not well characterized and would require further research in controlled clinical studies [32].

8.15 Polychemotherapy

The most active drugs against osteosarcoma are now doxorubicin [33], cisplatin [34], high-dose methotrexate with leucovorin-rescue [35] and ifosfamide [36]; however, the best combination has yet to be determined. A period of neoadjuvant chemotherapy is included in most current programs [37]. The amount of preoperative chemotherapy's histological response [38], on the other hand, provides crucial prognostic information. Current prospective trials are examining if changing postoperative chemotherapy improves outcomes in poor responders. The use of high-dose chemotherapy followed by autologous hematopoietic stem cell retransfusion has not improved results [39, 40].

8.16 Immunomodulation

A preliminary uncontrolled Swedish series of single-agent a-interferon treatment showed promising results [41]. In the present EURAMOS 1 Intergroup Study, a-interferon maintenance after chemotherapy is being evaluated in a randomized trial [42]. A follow-up report of a recent randomized trial found that adding the immunomodulator liposomal muramyl tripeptide phosphatidyl ethanolamine (MTP) to postoperative chemotherapy was associated with a statistically significant advantage in overall survival and a non-significant trend in event-free survival [43].

A variety of medicines have been developed to help minimize chemotherapy-related toxicity, in conjunction with therapeutic advancements in osteosarcoma management using polychemotherapy. Chemotherapy-induced emesis has been considerably reduced with the introduction of serotonin antagonists [44].

8.17 Treatment of Metastatic Disease and Relapse

Primary metastatic osteosarcoma is treated similarly to localized osteosarcoma, with the exception that all known metastatic foci must be surgically removed, usually via exploratory thoracotomy with full lung palpation [45]. Long-term survival is possible for about 30% of individuals with primary metastatic osteosarcoma and >40% of those who achieve complete surgical remission [45].

Therapeutic treatment relapse is treated mostly surgically, either locally or within the lungs. The prognosis is bleak, with only 20% of patients surviving long after a relapse. Because the condition is almost always deadly, complete excision

of all metastases must be undertaken. CT scans, on average, undercount the number of lung metastases and may miss contralateral involvement in patients with seemingly unilateral pulmonary metastasis [46]. As a result, open thoracotomy with bilateral examination and palpation of both lungs is indicated.

8.18 Follow-Up and Effects of Treatment

The orthopedic oncologist and medical oncologist should regularly monitor the patient after chemotherapy is finished.

Most trials advocate follow-up every 6 weeks to 3 months in the first two years following diagnosis, every 2–4 months in the third and fourth years, every 6 months in years 5–10, and every 6–12 months thereafter. A history and physical examination, as well as a chest X-ray, should be performed at each appointment. There is no commonly agreed cutoff point for tumor surveillance because late metastases might arise up to ten years after diagnosis. Cure patients, like those with other childhood malignancies, require lifelong monitoring [47].

Polychemotherapy for osteosarcoma has been linked to irreversible changes in cardiac [48], renal [49], auditory, and reproductive function, as well as orthopedic issues and other side effects such as secondary cancers [50]. As a result, suitable studies should be included in regular follow-up to discover these late effects as early as possible.

8.19 Conclusion

Therapeutic targets that may significantly improve patient outcomes in osteosarcoma, and other solid tumors with high rates of metastases have been identified as those that target metastatic progression and, as a result, may not have significant activity on measurable main tumors.

Finally, the therapy of osteosarcoma has advanced significantly during the previous 40 years. Initially, the treatment consisted of ablative surgery, which was linked with a high rate of morbidity and a low chance of long-term survival.

With the diagnosis and knowledge of the prevalence of micrometastatic disease, as well as the introduction of chemotherapy and complicated surgical treatments, enormous progress has been made in curing a larger percentage of patients while improving their quality of life. The rapid growth observed in the 1970s and early 1980s, however, has since stopped. Understanding the intricate biology of osteosarcoma and selecting innovative medicines with the best possibility of success when efficacy is assessed are currently top priorities. Collaboration programs like the PPTP, TARGET, and EURAMOS have aided these efforts.

Using a novel prioritization schema [51] to identify compounds with the strongest rationale for efficacy testing should help to simplify resources and allow for faster progress in therapy development. Furthermore, sustained international collaboration through the EURAMOS group will be important to the future design

of studies in order to achieve speedy and efficient accrual while minimizing redundancy and combining efforts to better the prognosis for this rare disease. These new medicines will be evaluated in ongoing and future clinical studies, raising hopes that we may be able to effectively navigate the maze and witness the increase in survival that has been so elusive in the past.

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9 Pharmacogenetics of Anticancer Drugs: Clinical Response and Toxicity

Ammara Siddique, Samra Bashir, and Mateen Abbas

9.1 Introduction

Pharmacogenetics is the study of heritable diversified traits on subject's genome that explicate their pharmacological variability in drug response and toxicity. Pharmacogenetics became major bioscience of concern in the 1950s when clinical observations of tangled drug response in individuals were found in the relevance of inheritance and were postulated on simple Mendelian inheritance patterns [76]. Friedrich Vogel in 1959 was the first who coined the term pharmacogenetics [107]. Genetic polymorphism is the basic unit of pharmacogenetics. Over the past two decades, plenty of research and discoveries have been made since DNA sequencing has gotten persuasive and generally accessible. Around 30–90% of DNA in the human genome discovered has regions of repetitive DNA that are highly polymorphic in nature [116]. It is estimated that there are 3 billion nucleotides pairs on DNA in the double helix of 23 pairs of chromosomes, the likelihood of polymorphic DNA and diversity in people is so obvious.

Although the distinct drug responses among individuals are multifactorial and include both genetic and environmental factors which were remarkably found to be linked with genetic polymorphisms in drug metabolizing enzymes, drug-molecule transporters, drug receptor as well as target sites [26]. Since the launch of the human genome project, a massive amount of data on the occurrence of various polymorphisms on the human genome has been obtained [142].

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9.2 Genetic Polymorphism

Polymorphism is a Greek word comprised of "poly" means many/multiple and "morph" means forms/variety. The term "polymorphism" was used during the early stage of clinical observations as altered behavior of drug and was explained as subgroups of the population which are displaying a distinct drug metabolic behavior from the majority and encompassing at least 1% or more in the given population [62, 100]. Phenotypes are observable differences in organisms controlled by DNA. These variations in DNA sequence or polymorphism (genotypes) make the individuals unique in appearance, risk of development of various diseases, response to drugs (xenobiotics), and the pathogens, etc. [135]. The altered gene expression may produce proteins that may correlate to individual's phenotypic status, disease idiopathy and a different response to drugs.

The human genome is 99.9% identical with minute difference of 0.1%. This difference is accountable for the adapted phenotypic diversity within the geo-environmental range. Single nucleotide polymorphisms (SNPs) are most frequent type of polymorphism, involving variations in a single base pair. Other polymorphic variants can be substantially bigger, involving extensive regions of DNA [1].

9.2.1 SNPs and Polymorphism

SNPs are the simplest form of genetic difference among individuals with an alteration in a DNA structure building block unit, the nucleotide; i.e., A, T, C, or G (adenine, thymine, cytosine, and guanine, respectively). More than 325 million SNPs are known on the human DNA strands out of which 15 million are present at frequencies of 1% or higher across the populace of the world [135]. SNPs are used for gene discovery and mapping, evolutionary biology studies, prediction of drug activity, diagnostic tests, and pharmacogenomic studies [57, 121]. Example of SNP-induced diseases is sickle cell anemia and cystic fibrosis [72]. Due to complex inheritance patterns of many genetic diseases like diabetes, cardiovascular and many cancers, many geneticists like to focus on SNPs in order to understand the inheritance pattern and pathogenic pathway of the disease as well as involvement of genetics in drugs responses which allows liberty to design therapeutic strategies with respect to genetic differences at individual level. SNPs in the regulatory and coding regions of a gene shown functional consequences. Linkage between a phenotype and a functional variant in individuals can be identified directly through SNP markers. This characteristic makes SNPs ideal for high-density genetic-markers maps essentially to separate complex genetic traits. SNPs occur in the human genome at the frequency of every 300–2000 base pairs where every individual carries two copies of each gene [90]. Most of the SNPs in non-coding regions of the genome are functionally silent. However, few SNPs are biologically functional which can alter protein structure or expression during sequencing. Such SNPs are the soul of human diversity in both health and disease.

The identification procedure of these SNPs is an ongoing process that particularly are relevant to any disease. Genetic profiling based on such SNPs may act as a fingerprint that gives complete information about a subject's susceptibility to different diseases and response to different drugs [7].

9.2.1.1 SNPs in Pharmacogenetics

SNPs are key factors in determining an individual's vulnerability to a variety of diseases like high blood pressure, diabetes, cardiovascular diseases, neurological disorders, and especially cancers, despite the fact that these disorders are multifactorial and include genetic and nongenetic factors [78]. Individual responses to drugs, including efficacy and adverse drug reactions (ADRs), are also highly variable, however, these variations may be influenced by the age, nutritional state, pathophysiology and severity of the disease, renal/hepatic function, medication interactions, and coexisting disorders [6]. Since the first documented occurrence in the 1950s, it has been well established that changes in drug metabolism and disposition at drug target receptors are caused by genetic polymorphism and are evidently inherited [48]. SNPs are abundant on the human genome and have become significant biological markers for mapping human maladies, heritable traits of population, diseases, and drug disposition as well as in developmental investigations.

9.2.1.2 Importance of SNPs in Cancer Pharmacogenetics

Cancer is a disease notorious for abnormal cell growth. The cancer patients show a heterogeneous pattern of response to many of the chemotherapeutic agents [47]. Scrutinized personalized anticancer therapy paired with biomarker-based regimen has promising potential for optimal cure from chemotherapy with reduced chemotherapy-related fatality [119]. Clinically, prognostic and predictive biomarkers are used simultaneously for the facilitation of cancer diagnosis, cancer treatment, and determination of possible toxicity [8]. For example, pharmacogenetic markers are extensively used for predicting therapeutic responses to methotrexate and 5-fluorouracil, the drugs that target folate metabolism [146]. Many cancer biomarkers depicted predictive and prognostic characteristics but with the variety of prognostic, predictive, toxicity, or pharmacological biomarkers, there are fewer clinically useful predictive biomarkers for solid tumors [25]. Different researches found a correlation between SNPs and cancer. *Gemignani* et al. studies show that polymorphism in the dopamine receptor gene, i.e., DRD2 is linked with an increased risk of colorectal carcinoma [52]. Some other examples include response to gefitinib treatment in non-small cell lung cancer patients with an EGFR mutation, ERCC1 polymorphisms and cisplatin activity, UGT1A1 gene polymorphic forms and irinotecan neutropenia, thymidylate synthase (TS) gene polymorphisms and 5-FU sensitivity, and cytidine deaminase (CDA) genotype and response to gemcitabine [32].

9.2.2 Somatic Versus Germline Mutations in Oncology

Cancer is a diversified by-product of mutations occurring in the germline and somatic (tumor) genome. The germline and somatic cells' genetic variations are the main factors for the prognosis of cancers [2]. Genetic factors influencing the response to a drug include both pharmacokinetic and pharmacodynamics factors (Fig. 9.1). Variations in tumor DNA influence the drug choice for chemotherapy and germline mutations influence the efficacy of a drug chiefly through alteration of pharmacokinetic parameters [112, 153].

Any cell in the body other than the germ cell (i.e., sperm and egg) is a somatic cell. Mutations in the somatic cells are acquired mutations that can be caused by intrinsic factors such as DNA damage, oxidative stress, replication errors, or mutations in proto-oncogenes or oncogenes, or extrinsic factors such as direct DNA gene damage by ultraviolet rays, X-rays, radiation exposure, toxic chemicals, heat variability, and/or further environmental variables. These changes are non-inheritable and are useful in research studies of different disease conditions, e.g., cancer or neurological disorder. Some somatic mutations damage the DNA and accumulate in the cancerous cells which serve as drug targets or prognostic markers [2, 113]. Somatic cell mutations are extremely rare as they do not provide atmosphere or enhance cell division or expansion to new genotype [28].

Somatic mutations affect the response of drugs as they keep changing due to pressure from cytotoxic therapy and genetic instability. For the assessment of drug efficacy, genetic sequencing for somatic mutations from tumor cells is regularly done throughout the treatment span as a distinct mechanism of resistance emerges from these mutations that may require the recommendation of different therapy.

Germline variations are constitutional mutations. These mutations, particularly SNPs, are valuable biomarkers for predicting drug-induced toxicity and response to medication. Tumor cell markers may identify possible germline mutations for inheritable cancer, however, confirmatory germline testing is indicated for genetic cancer prediction [21].

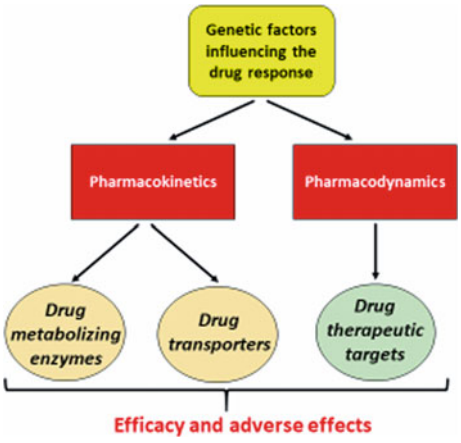

Fig. 9.1 Genetic factors influencing the efficacy and toxicity of the drugs

Cancer genetics hold a risk of inheritance and is also an important preventive factor. The oncologists and cancer geneticists utilize the information generated from genomic testing for risk reduction and prevention strategies. Currently, parallel sequencing of tumor and germline DNA is performed, which can provide information about therapeutic options and cancer predisposition [149].

9.2.3 Pharmacogenetic Biomarkers and Their Significance

Biomarkers are check-points or indicators in the biological system that are powerful tools to portray the entire picture of disease from the earliest manifestation to the terminal stages [98]. They are either predictive, prognostic, or surrogate biomarkers. Predictive and surrogate biomarkers are important in decision-making strategies of treatment [9]. Predictive and prognostic biomarkers are proteins or bio-molecules, that are measured in body fluids, used as indicators that help in the diagnosis, progression, or reappearance of cancer, and are important for designing personalized chemotherapy regimen by identifying response to therapy and progression of the disease [94]. Biomarkers are usually well categorized according to the demand. Biomarkers used during the drug discovery stages for the assessment of drug effects in preclinical and/or early clinical studies are known as pharmacological biomarkers [8]. SNPs act as genetic biomarkers because they are polymorphic alleles on DNA sequencing which are useful for pinpointing a progression of disease instead the cause of disease [135].

The main purpose of PGx knowledge is to optimize drug therapies. For this purpose, many DNA-based pharmacogenetic tests are performed for the detection of genetic variations associated with the risk of drug disposition. Biomarkers counterparts for therapy selection in targeted chemotherapeutics which are intended to attack tumor cells with specific identifiable protein structure considerably different from the normal cells. As a result, individual-specified chemotherapy which is carefully guided by PGx biomarkers is more selective for cancer cells than normal cells which results an improvement in prognosis of disease condition in cancer patients' and reduce toxic effect events from chemotherapy in normal cells. Epidermal growth factor receptor EGFR, KRAS, HER2, and c-Kit are some of the examples [10]. The FDA labeled alleles that influence drug efficacy and toxicity as pharmacogenetics biomarkers [66]. The FDA has incorporated pharmacogenetics information into drug labels and provided a detailed list of drugs entitled with genetic biomarkers. The FDA has also emphasized the importance of pharmacogenetics for personalized medicine. For example, dosing recommendations based on *CYP2D6* and TPMT polymorphism have been given for mercaptopurine, as these enzymes affect the drug metabolism [110].

PGx (pharmacogenomic and pharmacogenetic) labeling in FDA-approved drugs includes biomarkers as biomarker-drug pairs as either predictive or prognostic markers. These PGx biomarkers can be labeled according to their nomenclature systems, for example, cytochrome P450 (CYP) may be labeled main family of

enzymes, subfamily, gene levels, and alleles variants, etc. including wild-type and mutant one with the allele also labeled for functional status (i.e., decreased, increased, normal or nonfunctional) [99].

9.2.4 Anticancer Drugs and Pharmacogenetics of Enzymes

Alteration in proteins or enzymes involved in drug targets (i.e., pharmacodynamics–PD) and drug metabolism and transport (i.e., pharmacokinetics–PK) are key factors for drug response variation [33, 45].

All drugs undergo pharmacokinetic changes before imparting pharmacodynamic effects. The drug pharmacokinetics primarily involves phase I modification oxidation, reduction, and hydrolysis reactions primarily by CYP-450 family of enzymes, conjugation in phase II modification occurs through enzymes like glutathione-S-transferases (GSTs) and uridine diphosphate glucuronosyltransferases (UGTs), and elimination usually through bile or urine [74]. SNPs in drug-metabolizing enzymes can be classified as the phenotype of the extensive-metabolizer to the poor-metabolizer which either intensifies the toxicity or nullifies the drug response [5].

Enzymes of anabolic and catabolic pathways for purine and pyrimidine analogues, such as TPMT and DPD, and drug transporters are important targets in cancer therapy. Polymorphism in these targets greatly influences the pharmacokinetics of drugs by affecting the enzyme activity and may alter the drug response and extent of toxicity [15].

Genotyping an individual allows selecting the effective drug and dose for prophylactic or therapeutic use to optimize and enhance the effectiveness of therapy. Pharmacogenetics by identifying the hereditary variabilities that influence the drug responses helps to create proper phenotyping and genotyping tests. Utilization of this information is helpful to anticipate the effective and positive clinical results from dose-related toxicity [110, 136].

Adverse drug reactions are therapy-limiting factors that are due to interference with the target protein or due to activation or inhibition of therapeutic drug targets. A proper screening method and diagnostic biomarker can work wonders to control individual variability of drug toxicity and drug hypersensitivity [92].

9.3 Applying Pharmacogenetic Knowledge in Clinical Oncology

PGx research mainly emphasizes the genetic variations found in germline genome as well as in tumor genome. Germline variations help to forecast drug efficacy and toxic effects while somatic mutations help in selection of a better chemotherapeutic drug with improved therapeutic efficacy. Mainly these genetic variations are studied relative to gene expression for chemotherapy response and tumorigenesis [153]. Contemplative genetic and pharmacological knowledge has given

profound benefits in cancer therapy by increasing therapeutic index and reducing toxicity and tumor response [23]. It is evidently well-recognized that DNA damage is a vital factor for cancer prognosis. Damaged DNA during repairing procedure leads to mutations that affect oncogenes and tumor suppressor genes. These genetic defects predispose various cancer types. However, this damaged DNA also provides an important avenue for chemotherapy [144]. Patient-genome and tumor-genome polymorphic variations not only affects the regulation of transport, retention, efflux of drugs, also determine the penetration into tumor tissue. Hence, drug-related toxicities depend on the germline mutations whereas tumor mutations play key role in dose-limiting factors in cancer management [79].

In clinical oncology, PGx information about genetic variations and polymorphism that affects the drug related toxicity, treatment response and survival from chemotherapy is utilized to develop safer and effective patient-tailored or targeted therapy. Various of the targeted patient-tailored approaches include hormone treatments, inhibitors of signal transduction, gene expression modulators, apoptosis enhancers, angiogenesis inhibitors, immune-therapies, and toxin delivery agents [46]. However, drug response alteration is not monogenic polymorphisms that encode for PK- or PD-related proteins, it is in fact a complex multifactorial and multigenetic process. For example, patients with wild-type KRAS tumors show response to EGFR-targeted therapies while mutated KRAS tumors and non-responsive to monoclonal antibodies targeting EGFR receptors [37]. FDA has included gene expression studies in PGx studies in relevancy of tumorigenesis and chemotherapy response [153].

Pharmacogenetics is gene-drug interaction and pharmacogenomic is all-genes in genome-to-drug response which imparts great significance in oncology. Chemotherapeutic agents have a narrow therapeutic index and a high risk of the development of drug toxicity. Both somatic and germline genetic variations have role in cancer treatment however germline variations impart a key role in cancer risk and treatment outcome [102].

Genetic biomarkers (SNPs from somatic or germline mutations) are the alterations in the nucleotide sequences of DNA. These genetic traits are readily identifiable and are helpful to detect predisposed risk of heritable diseases in individuals of populace, ethnically different populations, and/or different species [135]. Some examples from somatic and germline cells are available in Table 9.1 with their respective pharmacogenetic biomarkers.

9.4 Tools and Techniques for Pharmacogenetics Studies

To identify the genetic variations and understand their significance in tumorigenesis and treatment response several techniques and tools are available. Genome-wide association studies (GWAS) of cancer have databased huge number of variants associated with cancer susceptibility and/or genetics of cancer risk [133]. Similarly, advancement in genetic and molecular technologies helped to understand the molecular basis of genetic alteration and tumorigenesis. The Cancer Genome

Table 9.1 Cancer pharmacogenetic biomarkers in FDA drug labeling

Somatic variant		Germline variant	
PGx biomarker	Drug	PGx biomarker	Drug
PML-RAR alpha	Arsenic trioxide	DPYD	5-Fluorouracil/ Capecitabine
EGFR	Cetuximab Panitumumab Erlotinib Afatinib	TPMT	6-Mercaptopurine Thioguanine Cisplatin
		UGT1A1	Irinotecan Indacaterol Nilotinib
		Estrogen receptor, *CYP2D6*	Tamoxifen Gefitinib Rucaparib
KRAS	Cetuximab Panitumumab Sotorasib	G6PD	Rasburicase
HER2/neu	Pertuzumab Lapatinib Nivolumab Neratinib Trastuzumab Ado-trastuzumab emtansine		
Philadelphia chromosome, C-kit, and PDGFRA	Imatinib Bosutinib		
BRAF V600E	Trametinib Dabrafenib Vemurafenib		
ALK	Crizotinib Ceritinib		

Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) are serving well by providing comprehensive data on the nature of tumors from different cancers [152]. Dynamic and explorative efforts are being made from the data of both types of mutagenic risk-alleles to identify the associative link in carcinogenetic risk and/or may influence the development of any cancerous tumor or cancer type. Preliminarily, the identification, association, and influential relation of cancer and tumor are done on basic principles that analyze tumor data with germline cancer risk variant, the relationship of the patient genome to particular tumor type, and finally relationship between the tumor molecular data for germline association studies [50].

The Next Generation Sequencing (NGS) has performed sequencing of millions of DNA fragments and identified novel and rare mutants of cancer, also detects the carrier of cancer mutation in family, and provides the molecular rationale of precisely targeted therapy [58]. NGS technologies provide economical testing for

genetic sequencing and are now widely applied in the diagnosis of genetic disorders, especially in cancer [95]. Germline variants are mostly either identified through candidate-gene approaches or genome-wide association studies (GWAS). The Cancer Genome Atlas (TCGA) and International Cancer Genome Consortium (ICGC) are vigilant in the identification of somatic mutations from genetic materials of cancer cells. NGS has enabled the incorporation of genomic data into the clinical utility [24]. For example, high-throughput genotypic technology can identify genes with germline mutations associated with susceptibility of cancer, e.g., there is elevated risk of breast and ovarian cancers with BRCA1 and BRCA2 genes inheritance. Genetic testing for mutations in these genes is done for the identification of the individuals susceptible to heritable cancers.

Cancer is also known as a disease of altered pathways. Mutations show inconsistent behavior, as sometimes high-impact coding site mutations show no functional significance while some somatic mutations from high-ratio of rare variants to common ones show clinical functional impact. Differential mutation analysis, another method to identify the significance of mutant genes in cancer, analyzes the significant driver mutations along with pathway analysis which potentially can uncover novel cancer pathways instead of addressing known cancer pathways [114].

Catalogue of Somatic Mutations in Cancer (COSMIC) provides a comprehensive database for studying the consequences of somatic cell mutations in human malignancies (https://cancer.sanger.ac.uk). It encompasses all genetic pathways by which somatic mutations cause cancer, including non-coding mutations, gene fusions, copy-number variants, and drug-resistant mutations, in addition to coding mutations [140].

9.5 Pharmacogenetics and Drug Development

The annotation of the human genome sequence with concomitant advancement in genomic technologies has facilitated the drug discovery and development process along with precise genetic data availability which can outline individual risks and benefits for specific therapeutic strategies. The clinical observational data, molecular information of targets and drugs, and understanding of biological pathways of disease are driven together to design and develop a better therapeutic regimen [118]. This knowledge is applied within the pharmaceutical industry along with research engines, in the relevance of genetic tools for the drug discovery process. The goal of developing a beneficial drug in the pharmaceutical industry is a well-outlined process and is termed the pharmaceutical pipeline [70]. SNPs are favorite molecular-markers in pharmacogenomics investigations because they affect drug-metabolizing pathways and/or ADRs, as well as, are linked to a variety of disorders, including cancer, autoimmune diseases, neuropsychiatry, and infectious diseases [7].

Pharmacological understanding of the drug disposition, i.e., pharmacokinetic parameters (PK) and pharmacodynamic parameters (PD) along with SNPs (pharmacogenetics; drug transporters and metabolizers) and advancement in gene expression technologies has opened new doors for drug development that can have high efficacy and selectivity to target and low toxicity. This is especially significant in cancers where both the germline and somatic genotypic variations that correlate with adverse events and drug efficacy profiles have been identified [49]. The new drug discovery process has evolved drastically over the past half-century from pharmacological effects process to chemically-modulated drugs to more precisely biologically-driven process, i.e., identification of biological pathway of disease as well as drug responses toward the development of the new drug as a forward drug discovery process [44].

9.5.1 Pharmacogenetics in Anticancer Drug Development

Cancer drugs development also follows the same traditional pathway which approximately takes 15 years although considerable efforts have been made by the research industry and FDA to reduce the timeline and cost of this procedure. Almost 90% of the potential new drugs that have passed through discovery and preclinical phase fail in clinical trials due to efficacy, safety, and toxicity issues. Implementation of PGx (pharmacogenomics) biomarkers in drug development have progressed the oncology therapeutics to more tailored treatment from the paradigm of fit-for-all to a more targeted and histology-oriented approach toward, namely Precision Medicine (PM) [139].

The application of PGx principles in treating certain cancers has beneficial outcomes and has a crucial role in cancer drug development [41, 154]. An integration of PGx principles with various phases of drug development is shown in Fig. 9.2.

There is a major discordance in phase II and phase III results in terms of efficacy, safety, and toxicity which imparts huge time and money losses. In 2014, AstraZeneca's scientists proposed five "R's", as shown in Fig. 9.3, being the most important technical determinants for drug discovery and development success and pipeline quality which recently has shown considerably improved success rates in phase III trials [104].

Imatinib, a tyrosine kinase inhibitor used to treat chronic myelogenous leukemia, is a classic example of anticancer drug development utilizing PGx knowledge [153]. Similarly, crizotinib which is a tyrosine kinase inhibitor targeting ALK, demonstrated substantial results in phase I trials. This granted its accelerated approval to phase II trials by the FDA and is recently approved for ALK-positive relapsed or refractory, systemic anaplastic large cell lymphoma (ALCL) in pediatric patients of 1-year and older (www.fda.gov).

The advancement and development in strategic drug discovery technologies including high-throughput screening and structure-based design strategies has made it possible to design small-molecule targets that are target-based therapeutic

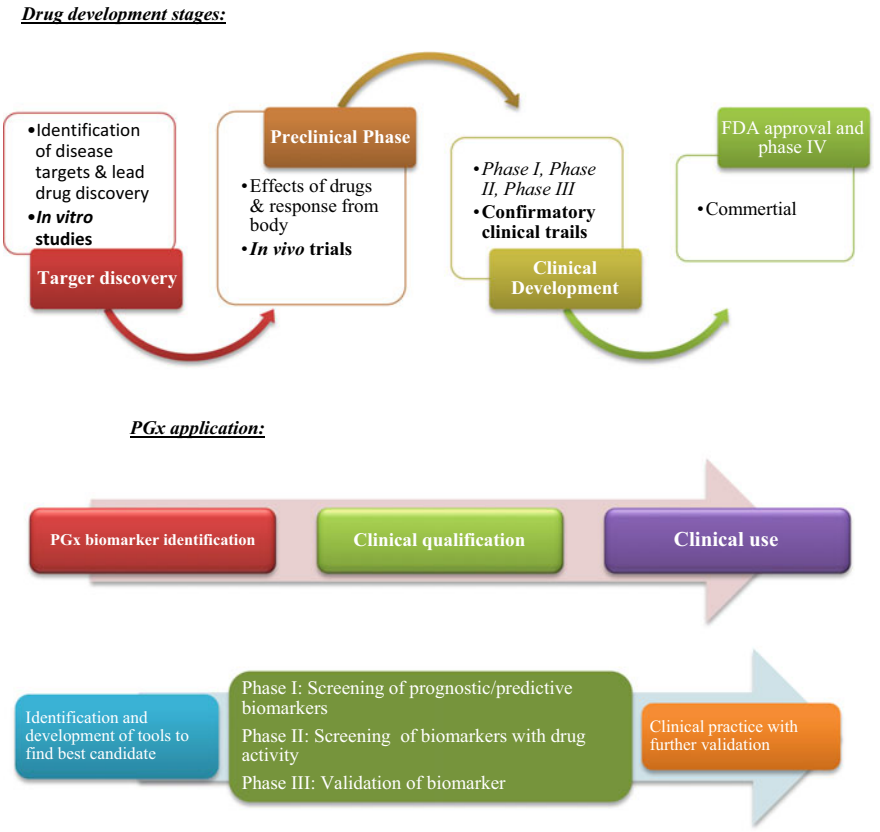

Fig. 9.2 PGx application in each R&D phase of drug development

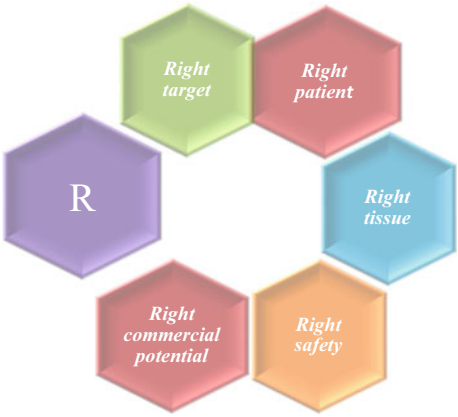

Fig. 9.3 Proposal of a "5-Rs" framework applied to cancer drug development

agents. Such agents are expected to have high efficacy, sensitive to target selectivity, and exhibits less toxicity than the previous versions of anticancer agents [138].

Identification of prognostic, diagnostic, and predictive biomarkers will accelerate the process of drug development and approval with the possibility of post-approval risks management [86]. However, PGx incorporation along with drug development has added costs burdens to the already high-budget drug development process [40]. However, drug regularity authorities realized the potential benefits of PGx technologies, encourage the pharmaceutical industry to integrate PGx knowledge into drug development [87].

9.6 Genetic Factors Influencing Efficacy and Toxicity of Individual Chemotherapeutic Agents

The concept of "Chemical individuality of Man" was given by Sir Archibald Garrod that's based on clinical observation and the concept of erring in the drug-metabolizing pathways that alters pharmacological effects of the drugs are inherited [101]. The potential overlapped linkage of metabolic pathways of disease (receptors), genetics (SNPs), and pharmacological effects (i.e., pharmacokinetic and pharmacodynamic effects) can be utilized to determine the peculiarities of a disease based on the differential response of drugs,as the drug targets the disease-causing gene [27].

In cancer, the remarkable inter-individual unpredictability in drug response was found to be linked with polymorphisms in drug-metabolizing enzymes, drug-molecule transporters, drug receptors sites, and drug target sites [26, 118]. Although inter-individual drug responses are influenced by numerous factors including genetic and environmental aspects. With the advancement of the Human Genome Project, substantial information, and data has been compiled for multiple polymorphic alleles and heritable variables on the human genomic DNA [142].

In the GWAS catalogue, about 93% of registered SNPs are in the non-coding regions and are known as regulatory SNPs that can affect gene splicing and transcription factor binding. Approximately 10 M SNPs are in coding or non-coding regions. For example, elevated levels of HDL are associated with a defect in cholesteryl ester transfer protein (CEPT), deactivating mutations in the Janus kinase 3 (JAK 3) gene results in the severe combination of the immune-deficient syndrome [106, 126].

Pharmacogenetic factors influencing pharmacokinetic and pharmacodynamics properties of anticancer drugs are summarized in Table 9.2.

9.6.1 Tamoxifen and Polymorphic CYP2D6

Tamoxifen, a selective estrogen-receptor modulator, has successfully been used for treatment and prevention of breast cancer. Oral tamoxifen citrate has been approved as endocrine therapy of breast cancer and has sustained since for over

Table 9.2 Genetic polymorphisms altering PK and PD of anticancer drugs

Drug	Genetic enzymes	Gene mutation	Target	Clinical PK Altered Parameter	Phenotypic actions
Tamoxifen (Chemotherapeutic)	*CYP2D6*	CYP2D6, CYP3A5, CYP3A4, CYP2C9, CYP2C19, CYP1B1, UGTs	Estrogen receptor	PM with reduced plasma concentrations of active metabolite (endoxifen)	Dose adjustment is needed in poor metabolizers and/or alternative therapy is recommended
Irinotecan (Chemotherapeutic)	UGT1A1 ABCC1	UGT1A1*6 (c.211G > A) UGT1A1*28a	topoisomerase I inhibitor	Reduced clearance of SN38; increased levels of SN-38	Severe diarrhea, Neutropenia PK parameters
6-Merceptopurine (Chemotherapeutic)	TPMT	TPMT*2, *3A, *3B, *3C	DNA/RNA	TPMT deficiency results increased levels of 6-MP	Altered plasma concentration of active drug, GI toxicity & myelotoxicity
5-Florouracil (Chemotherapeutic)	DPD *TYMS* MTHFR	Splice site mutation at IV14 + 1G > A	DNA/RNA	Increased levels of 5-FU due to decreased activity of DPD	Reduced clearance, increased neurologic, hematological toxicities
		TSER*2/*2 TSER*3/*3	DNA/RNA	low TYMS-mRNA expression	Cytotoxicity
		677C > T, 1298A > C variant	DNA/RNA	Genotype associated decreased drug response and increased toxicity	Cytotoxicity
Methotrexate (Chemotherapeutic)	SLCO1B1 MTHFR	rs1051266 rs2306283 A > G	DNA/RNA	Transporter (SLC, MDR1)	Altered clearance
		677C > T, 1298A > C variant	DNA/RNA	Low expression of TYMS gene	Cytotoxicity

(continued)

Table 9.2 (continued)

Drug	Genetic enzymes	Gene mutation	Target	Clinical PK Altered Parameter	Phenotypic actions
Platinum compounds (cisplatin, carboplatin & oxaliplatin) (Chemotherapeutic)	GST, XRCC1, ERCC1 *ERCC2/XPD*	Deletion, Ile105Val, Arg194Trp, Arg280His, Arg399Gln, K751Q	DNA/RNA	Increased DNA damage Endonuclease Non-Catalytic Subunit ATP-dependent helicase	Drug toxicity Increased, Thrombocytopenia
Vemurafenib (Chemotherapeutic)	BRAF V600E	BRAF V600E mutation	BRAF	Somatic mutation in BRAF	BRAF V600E wild-type show toxicity
Crizotinib (Tyrosine kinase inhibitor)	CYP3A4, CYP3A5	EML4-ALK	ALK	EML4-ALK mutation	Reduced CNS penetration
Erlotinib (Tyrosine kinase inhibitor)	CYP3A4 ABCB1		EGFR	EGFR mutation	Rare rhabdomyolysis
Imatinib Nilotinib (Tyrosine kinase inhibitor	PDGFR BCR-ABL C-kit	Ph + ALL c-KIT mutation FIP1L1-PDGFRα	EGFR	Ponatinib for BCR-ABL mutation	Edema, musculoskeletal pain, diarrhea
Cetuximab Panitumumab (Biologics)	KRAS, BRAF, PIK3CA, or AKT over expression	KRAS mutation-negative (wild-type)	EGFR	Poor treatment prognosis with mutant genes	Rash, sensory neuropathy, neutropenia Poor objective response rate, lower OS and PFS
Trastuzumab (Biologics)	ErbB2 *FcgR3A*		Her2/neu	Trastuzumab with anthracycline interaction	Cardiac toxicity Higher response rate

25 years in estrogen-receptor (ER) positive breast cancer treatment and related conditions. Tamoxifen binds and thus block the ligand-binding domain of an ER from interaction with estrogen. This inhibits the ER associated transcriptional activation and subsequent tumor growth [96].

Hepatic phase I and II enzymes are responsible for the complex metabolism of tamoxifen. Tamoxifen is metabolized to its major primary metabolites: N-desmethyltamoxifen and 4-hydroxytamoxifen by hepatic cytochrome P450 enzymes, (CYPs) *CYP3A4* (and *CYP3A5*), and highly polymorphic *CYP2D6*, respectively [39]. When the primary metabolites are oxidized, a pharmacologically active metabolite called 4-hydroxy-N-desmethyltamoxifen (endoxifen) is formed in large amounts. The binding affinities of endoxifen and 4-hydroxytamoxifen to ER and ER, as well as the reduction of ER-dependent gene expression and breast cancer cell proliferation, are similar. Tamoxifen and 4-hydroxytamoxifen stabilize ERα, and endoxifen reduces ERα protein levels in breast cancer cells by tagging it for degradation by proteasomes. These data, together with the fact that endoxifen plasma concentrations are 5–10 times higher than those of 4-hydroxytamoxifen, indicate that endoxifen is an important metabolite having approximately 50% high affinity for the estrogen receptors of tamoxifen [17, 89]. The efficacy of tamoxifen can be affected by the genetic polymorphism in CYP2D6 exhibiting two major alleles for poor metabolizers (PM of drug and extensive metabolizers (EM, where PM's have low levels of endoxifen and need dose adjustments [71]. Hence, pharmacogenetics testing with pharmacokinetic consideration is required for achieving tamoxifen efficacy in breast cancer, however, there is a controversy for pre-emptive CYP2D6 genotyping due to conflicting results of the studies concerning the association between *CYP2D6* genotype and tamoxifen-related clinical outcome [20].

Alternative anti-estrogen medication, such as letrozole, anastrozole, or exemestane, could be administered to postmenopausal women with early stage ER positive breast cancer who are anticipated to not react to tamoxifen based on their cytochrome P450 2D6 (CYP2D6) genotype. By blocking the aromatase enzyme CYP19A1, these drugs restrict the production of estrogenin extragonadal tissues [67].

9.6.2 Irinotecan and Polymorphic UDT1A1

Irinotecan is a topoisomerase inhibitor that has displayed anticancer effect in a range of solid tumors. It is a first-hand choice to treat colorectal, pancreatic, and small cell lung cancer [68]. Carboxylesterase metabolize irinotecan to its active form of 7-ethyl-10-hydroxy-camptothecin (SN-38). Elevated levels of SN-38 cause the side effects of irinotecan, such as diarrhea and leucopenia. Allelic variations on the genomic structures cause divergent expression of enzymatic functional capacity as metabolizers and transporter proteins which may interfere with the pharmacokinetics and pharmacodynamics of irinotecan [35]. SN-38 glucuronide

is a polar product of SN-38 which is excreted in urine and bile after being inactivated by UDP-glucuronosyltransferase 1A1 (UGT1A1) in the liver. Thus, the rate of glucuronidation is a vital predictive factor in the development of toxicity with irinotecan. The enzyme UGT1A1 is highly polymorphic. UGT1A1*6 and UGT1A1*28 are the most commonly investigated polymorphisms in relation to irinotecan metabolism. In variant allele UGT1A1*28, an extra TA repeat sequence is present within the T-A-T-A box of the CGT1A1 gene promotor. Allelic form UGT1A1*28 is linked with reduced transcription of UGT1A1 protein with lower titer of SN-38 glucuronidation. The patients homozygous for UgT1A1*28 variant have significant high levels of the active metabolite SA-38, and consequently are at elevated risk to present with adverse events like diarrhea and leucopenia with irinotecan therapy. Similarly, homozygous patients with UGT1A1*6 mutant allele are on the higher risk zone for adverse events with an increased systemic exposure to irinotecan and SN-38 [73, 151].

Irinotecan is a hydrophilic molecule with a large volume of distribution (Vd) in steady state, estimated over 400 L/m^2 [157]. The lactone ring of 7-ethyl-10-hydroxy-camptothecin (SN-38) can be hydrolyzed to a carboxylate isoform at physiological pH. As a result, there is a pH-dependent equilibrium between these types [35]. Because only the lactone form of irinotecan has anticancer activity, a modest alteration in physiological pH could affect the drug's pharmacokinetics and effectiveness [111].

9.6.3 6-Mercaptopurine and TPMT

6-Mercaptopurine (6-MP) is a very well-known cytotoxic, purine analogue agent from thiopurine family, frequently used in conditions like autoimmune diseases, inflammatory bowel disease as well as certain cancers like acute lymphoblastic leukemia (ALL) especially in children and chronic myeloid leukemia (CML). 6-MP is an inactive-prodrugs that requires activation to thioguanine nucleotides (TGN) that incorporate into DNA to cause cytotoxicity. 6-MP undergo conversion to 6-thioguanine nucleotides (6-TGN) by hypoxanthine guanine phosphoribosyl transferase enzyme (HGPRT) once it enters leukemic cells. The integration of 6-TGN into DNA or RNA causes the cytotoxic and immunosuppressive effects of 6-MP. The other two pathways involved in the inactivation of 6-MP are oxidation to thiouric acid catalyzed by xanthine oxidase enzyme and S-methylation of thiol moiety of 6-MP by thiopurine methyltransferase (TPMT), thus opposing the action of HGPRT.

Intracellular metabolism is key factor to keep a balance between efficacy and toxicity. Inter individual variation in clinical response and toxicity of 6-MP may result from variability in intracellular 6-MP disposition due to DNA point mutations affecting gene expression and/or protein functions of the genes involved in 6-MP metabolism.

The TPMT polymorphism is important in inter-individual variability in 6-MP response, with over 90% of population exhibiting high activity with only about

10% having intermediate, and 0.3% having low-to-undetectable enzyme activity. The important genetic variants accounted for the majority of the intermediate and low enzyme activity cases include TPMT*2 (239G > C),*3A (460G > A and 719A > G,*3B (460G > A). Traditional thiopurine doses have been linked to a high risk of developing hematological toxicity in TPMT-deficient patients, according to studies. Heterozygous patients for TPMT mutant variant require 30–50% lower thiopurine dosages than standard doses, and homozygous patients for TPMT deficient mutation require up to tenfold lower doses or the use of alternate medicines [134]. TMPT-deficient patients are at elevated risk of suffering from hematological toxicity if given frequent thiopurine dosages, according to several studies. The efflux transporters of the ATP-binding cassette superfamily, particularly the multidrug resistance associated protein 4 and 5, are another genetic component responsible for inter-individual variability in response to thiopurines treatment (MRP4 and MRP5). These two proteins regulate the intracellular quantities of cyclic nucleotides at the physiological level [3]. Overexpression of these transporters has been linked to thiopurine treatment resistance [155].

9.6.4 5-Flurouracil and TYMS, MTHFR, DPD

5-fluorouracil (5-FU), a fluorinated analog of uracil, is a key component of chemotherapeutic medicines used to treat solid tumors, such as colorectal (CRC), gastric cancer, and breast cancer, for palliative and adjuvant purposes [19, 56]. 5-FU, as well as its oral prodrugs capecitabine and tegafur have narrow therapeutic index, although tolerable develop severe toxicities like neutropenia, stomatitis, diarrhea, and hand-foot syndrome which may result in prolonged hospital stay and death may result in 0.5–1% of patients. The cytotoxic action of 5-FU requires its activation to 5-fluoro-2-deoxyuridine monophosphate (5-FdUMP). By inhibiting thymidylate synthase (TS), an enzyme essential for de novo pyrimidine production, 5-FdUMP reduces tumor cell proliferation. Many patients experience poor outcome from 5-FU treatment as a result of tumor recurrence after 5-FU therapy [38]. 5-FU is concomitantly used as 5-FU-based therapy like in FOLFOX therapy which includes 5-FU, leucovorin, and oxaliplatin or FOLFIRI consisting of 5-FU, leucovorin, and irinotecan, although empirically has provided boosted response rates to therapy to about 40–50% in CRC patients but unfortunately clinical studies showed this has not effectively prolonged disease-free survival in such patients [42, 55].

9.6.4.1 5-Fluorouracil and DPD

In the liver, the enzyme dihydropyrimidine dehydrogenase (DPD) converts at least 85 percent of 5-FU to dihydrofluorouracil. Several allelic variants in the DPD gene have been associated with reduced DPD enzymatic activity. Generally, 3–5% of carriers are heterozygous individuals and 0.1% are carriers are homozygous individuals of allelic variants of DPD gene that are probably associated with inactivation of enzyme function. A deficiency of DPD causes increased levels of 5-FdUMP leading to hematopoietic, gastrointestinal (GI) and neurological toxic

events with standard doses of 5-FU. Previous investigations have shown significant results in determining the relationship of DPD enzyme deficiency and 5-FU toxicity; where 55% of patients with DPD-deficient enzyme suffered from grade-4 neutropenia compared to 13% of patients with a normal DPD activity ($P = 0.01$). Reduced DPD activity was also identified in peripheral blood mononuclear (PBM) cells in 39–59% of these patients. Furthermore, individuals with low DPD activity developed toxicity symptoms 10-days sooner than patients with normal DPD activity [22]. Studies on *DPYD*, the gene that encodes DPD, from the patients who developed severe 5FU-associated toxicity have identified 11 mutations including a splice-site mutation, a nonsense mutation, i.e., E386X, four missense mutations and five polymorphic alleles. The most common mutation altering DPD activity is the splice-site mutation which is IVS14 + 1G → A. As 5-FU is a widely used chemotherapeutic agent in cancer patients and the associated toxicity is severe; analysis of the DPD activity in PBM cells or screening for the IVS14 + 1G → A mutation has been routinely suggested as pharmacogenetic protocol prior to the start of 5-FU treatment [147].

9.6.4.2 5-Fluorouracil and TYMS

The cytotoxic effect of 5-FU is due to an impairment of de novo nucleotide synthesis. In the presence of methylene tetrahydrofolate reductase, 5-FdUMP inhibits the activity of thymidylate synthase (TS) enzyme by inhibiting the methylation of deoxyuridylic acid (dUMP) to deoxythymidylic acid (dTMP) by creating a stable covalent complex. 5-FU limits purine synthesis via decreasing TS activity, resulting in decreased DNA replication and repair and tumor cell growth suppression. Nucleotides can be synthesized by cells in two ways: de novo synthesis and salvage synthesis. Nucleosides and nucleobases must first be transferred across the cell membrane by nucleoside transporter proteins before they can be salvaged. Human equilibrative nucleoside transporter 1 (hENT1) is the most common nucleoside transporter which is also closely linked to transport and pharmacological activity of numerous other drugs [123]. The in vitro cell viability studies on colorectal tissues have exhibited high expression of hENT1 demonstrated and resistance to 5-FU treatment [132].

9.6.4.3 5-Fluorouracil and MTHFR

Methylenetetrahydrofolate (i.e., MTHFR) is an enzyme pivotal for the regulation of intracellular pool of folate for nuclei acid and protein synthesis. It irreversibly catalyzes the conversion of 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate, which assists in homocysteine re-methylation to methionine. The pyrimidine and DNA synthesis are halted by the complex produced by FdUMP which is an active metabolite of fluorouracil, thymidylate synthetase, and 5,10-MTHF. It is hypothesized that MTHFR enzyme activity is indirect proportional to 5,10-MTHF and fluorouracil cytotoxicity. 677C > T variant of MTHFR polymorphic alleles is predominantly linked to clinical outcome where 677TT genotype showed more toxicity to 5-FU in advanced colorectal cancer

[36]. According to studies, advanced gastric cancer patients receiving fluorouracil-based therapy should have their TS and MTHFR polymorphisms genotyped as pharmacogenetic protocols. Individuals with MTHFR 677TT had a much greater survival rate than patients with CT or CC, according to the findings [125]. Another study demonstrated that MTHFR polymorphisms are not clinically relevant for cancer treatment as only homozygous carriers of MTHFR polymorphisms probably exhibit functional variations in folate metabolism if folate levels are low in vivo [4].

9.6.5 Methotrexate and SLCO1B1, TYMS

For the treatment of rheumatoid arthritis (RA) and acute lymphoblastic leukemia (ALL), methotrexate (MTX) is the most commonly utilized medication. Nonetheless, MTX can cause serious dose-limiting side effects and organ toxicity. The reduced folate carrier (SLC19A1) or the solute carrier organic anion transporter B1 (SLCO1B1) transports MTX, a structural analogue of folic acid, into the cell [53]. A polymorphism (rs1051266) at codon 27 of the gene coding for SLC19A1 protein with resultant substitution of arginine for histidine has been identified, although the functional significance of this SNP is not yet established.

Homozygous patients for the rs1051266 variation exhibited greater plasma MTX levels compared to patients of other genotypes in one trial of 204 children with leukemia receiving MTX [85]. Despite this, genome-wide association studies have found no evidence of a link between the SLC19A1 polymorphism and MTX pharmacokinetics.

The SLCO1B1 gene is almost entirely expressed in the liver. SLCO1B1 has been identified as the only gene linked with MTX clearance in genome-wide association studies. Endogenous chemicals like bilirubin and estrogens, as well as medicines like statins and MTX, are substrates of the SLCO1B1 transporter, which is situated at the basolateral membrane of the hepatocyte. The SLCO1B1 SNP rs4149056, which encodes a T521C change, has been linked to lower MTX clearance across the board. The rs4149056 common variant is found in two ∗ alleles: *5 (no additional coding variants) and *15 (with rs2306283 A > G). *23 and *31 are two further reduced function alleles linked to poor MTX clearance and in vitro transport. *14 and *35, respectively, were alleles linked to enhanced MTX clearance and transporter expression. Many ∗ alleles carry the rs2306283 A > G mutation, which is linked to enhanced transporter expression [141]. Thymidylate synthase (TYMS) is a crucial enzyme in DNA synthesis that produces purines de novo. The enzyme is inhibited by MTX, which hinders the synthesis of deoxythymidine monophosphate. Low TYMS expression in lymphoblastic leukemia cells is linked to lessened MTX's antileukemic effect and an increased risk of relapse. Within the TYMS gene's 5′-untranslated region,a tandem repeat sequence with a variable number of 28 base pair repeats has been discovered [95]. These repeats appear to function as enhancers, as more repeat sequences boost both mRNA expression and enzyme activity [115]. Individuals homozygous for three

copies of the repeat required larger doses of MTX (>6 mg/week) than patients homozygous for two copies, according to a Japanese study [93].

9.6.6 Platinum Compounds and ERCC1,2, ATP7B

Platinum compounds, i.e., cisplatin, carboplatin, and oxaliplatin are popular antitumor drugs. They are standard first-line choice-of-chemotherapy agents for advanced lung cancer, breast cancer, and colorectal cancer, etc. They cause cell apoptosis through formation of DNA adducts by crosslinking DNA strand and abates cell growth in active tumors. The cell replication system has an active DNA repair system. The genes responsible for DNA repair show genetic variations. Due to presence of polymorphic genes, this DNA repair pathway is important and promising predictor to determine the effective treatment outcomes from these agents as well as the various pathways responsible in drug resistance development. As for instance, excision repair cross-complementing enzyme group 1, i.e., ERCC1, compared with other nucleotide excision repair, i.e., NER genes, is prominently involved in developing cisplatin resistance. As ERCC1 is responsible in NER pathway for DNA repair through single-stranded DNA breakage and forms a complex which is structure-specific endonuclease with xeroderma pigmentosum complementation group-F (XPF, also known as ERCC4) [158]. ERCC1 has been identified with several polymorphic forms with varied DNA repair capacities.

Platinum compounds are standard chemotherapy regimen for advanced non-small cell lung cancer (i.e., NSCLC). The patients having K751Q-ERCC2 genotype showed slower disease progression with cisplatin-treated NSCLC than those harbored the K751Q-ERCC2 genotype. The KK-type homozygous patients showed significant chemotherapy benefits and significant longer survival than those patients with heterozygous KK-type or QQ-homozygous genotype. However, the reported data shows confliction of ERCC2/XPD variant alleles responsible for decreased overall survival in NSCLC patients treated with cisplatin. Similarly, a silent C118T SNP is reportedly associated with lowered mRNA production in ovarian carcinoma cell lines. Studies have reported that homozygous carrier patients of ERCC1-118C allele have shown suggestively better survival rate. Also in colorectal carcinoma, SNP-K751Q of the ERCC2-XPD (xeroderma pigmentosum group D gene) in peripheral blood lymphocytes was of prognostic significance in patients treated with combination of 5-FU and oxaliplatin. Also the presence of a nonsynonymous SNP, replacing a lysine with glutamine at codon 751 of the XPD protein, was reportedly associated with treatment outcome in patients with metastatic colorectal cancer [120].

ATP7A and ATP7B are important copper-transporting ATPases that contribute to platinum compounds resistance by regulating drug efflux. ATP7B overexpression show poor prognosis of platinum-agent treated patients in different cancerous conditions including epidermoid carcinoma cells due to increased rate of cisplatin efflux and cisplatin resistance [122].

Mutations in the genes of DNA repair system leads to defective mismatch repair (i.e., MMR). This also hinders with cisplatin binding to DNA. Also, this MMR defectiveness causes microsatellite instability (i.e., MSI) which is useful to demonstrate genome instability. The MSI + positive shows significant resistance to chemotherapy and poor prognosis of disease in metastatic germ cell tumors [65].

Tumor protein 53 (TP53) gene makes a tumor suppressor protein. Better expression of p53 protein (wild-type) is directly related to the level of chemotherapeutic response from DNA damaging agents. Mutations in TP53 weaken the of apoptotic pathways and significantly decrease the levels of the BAX gene (Bcl-2 associated X-protein, the apoptosis agonist), with the resultant inability to commence programmed cell death by damaging cell DNA and develops chemo-resistant phenotype. Whereas TP53/MDM2 alterations reportedly coexisted frequently in patients with adverse clinical outcomes from GCT tumor tissue [97].

9.6.7 Vemurafenib and BRAF V600E

Vemurafenib is a BRAF inhibitor which was approved in 2011 by FDA in United States for the treatment of unresectable or metastatic melanoma. A combination of vemurafenib and MEK inhibitor cobimetinib is given to the patients with BRAF mutant V600E. Compared with vemurafenib monotherapy, its combination therapy with cobimetinib showed significant promising progression-free survival chances in patients along with decreased incidence of secondary cutaneous cancers [84]. The coefficients of variation of systemic exposure of vemurafenib-treated patient is reported from 32 to 55% which is significant for determining the exposure-response relationships with regards to toxicity and response [80].

Interpatient pharmacokinetic variations of vemurafenib are considerably due to polymorphic drug-metabolizing enzymes and drug transporters hence provides sound grounds to consider pharmacogenetic protocols by genotyping the patients and dose adjustments as an effective approach to optimize the interpatient variability and drug response and combat drug toxicity. CYP3A4, enzyme of cytochrome family readily metabolizes vemurafenib. The drug efflux transporters system of ATP-binding cassette subfamily B member 1 (ABCB1), P-glycoprotein and ATP-binding cassette subfamily G member 2 (ABCG2) have reportedly polymorphic forms that are potentially responsible for acquired resistance against vemurafenib in breast cancer and BRAF (v600E) mutant cancers [69, 156]. Polymorphic forms of CYP3A4, ABCB1, and ABCG2 hinders the protein expression and clarify the significant interpatient differences of vemurafenib bioavailability and response to toxicity relationship. As CYP3A4*22 variant (rs35599367, 15389C > T) reportedly reduced the CYP3A4 mRNA expression and functional enzyme activity [150]. This was postulated due t observable reduced pazopanib clearance in heterozygous patients for CYP3A4*22 allele [16] as well as also in patients treated for breast cancer with drug docetaxel, a CYP3A4*22 positive allelic status was showing increased events of grade 3/4 toxic effects [128]. Increased exemestane plasma concentrations are reported for patients with positive CYP3A4*22 allele status with

early stage breast cancer [63]. Furthermore, peripheral neurotoxicity either acute or chronic is more often common in patients carrying the CYP3A4* 22 allele on paclitaxel exposure [34].

9.6.8 Crizotinib and ALK

Crizotinib was approved in 2011 by FDA and is a first clinically designed and synthesized as an antitumor agent with multiple targets that inhibits of receptor tyrosine kinase including hepatocyte growth factor receptor (HGFR, MET) and Recepteur d'Origine Nantais (RON) [159]. Mutations in the ALK gene can result in altered expression of oncogene fusion proteins. This agent showed marked antitumor activity in patients with advanced, ALK-positive NSCLC [18, 124].

Oral dose of crizotinib is 250 mg given twice daily. The maximum tolerated dose with a steady-state concentration reached in 15 days when given to 167 patients with cancer [60]. Average bioavailability is 43% (range: 32–66%) with minimal influence of other factors like ethnicity, food, age, sex or body weight on the single-dose crizotinib [88]. PK parameters for crizotinib treated ALK-positive NSCLC patients including anaplastic large cell lymphoma, neuroblastoma, and inflammatory myofibroblastic tumor ware similar. In Asian ethnic patients, peak plasma concentrations (C-max) and area under the plasma concentration-time curve of crizotinib was greater than patients with non-Asian ethnicity [109].

Mutant ALK gene fusion plays an active role for underlying development of NSCLC. ALK gene fusion defines a subgroup of tumors that are susceptible to targeted molecular therapy. It is extremely essential to precisely identify ALK gene fusion for the use of ALK inhibitors for NSCLC treatment. Crizotinib has shown significant reduction in the proliferation of cells carrying genetic alterations in ALK in phase I and II clinical trials [82].

Crizotinib is primary metabolized by highly polymorphic CYP3A4/5 enzymes. Drug interactions and resistance is most likely. Crizotinib like small-molecule tyrosine kinase inhibitors (TKIs) including imatinib, erlotinib, and gefitinib have low penetration of the cerebrospinal fluid and requires alternative treatment regimen. Studies on cell lines overexpressing showed that a novel agent PF-06463922 which is a multitargeted ALK and ROS1-TKI, a low-efflux substrate potentially had better CNS penetration in advanced ALK-positive NSCLC [11]. PF-06463922 also exhibited better efficacy and potency against brain metastases compared with crizotinib and alectinib in Phase I and II clinical trials. This agent can have promising results in terms of treatment effectiveness in ALK-rearranged NSCLC patients with CNS disease in the crizotinib resistance [30].

9.6.9 Cetuximab, Panitumumab and KRAS, BRAF

Cetuximab and panitumumab are commonly used monoclonal antibodies for the treatment of metastatic colorectal cancer as well as head and neck cancers. These

agents bind to extracellular EGFR. However, cancer patients with mutation of KRAS oncogene do not benefit from this treatment which also act as powerful negative predictive biomarker for resistance to EGFR-inhibitory therapy [30]. KRAS gene is a member of RAS family of oncogenes that activate proteins for intracellular EGFR signaling pathways which plays an important role in cell proliferation, differentiation, and apoptosis. KRAS mutation downregulates the signaling pathways. Hence, treatment regimen with cetuximab or panitumumab will have no combined benefit in curing KRAS-mutated tumors [77].

Many retrospective researches and assessments have shown that clinical benefits from EGFR-inhibitor treatment benefits the cancer patients with unmutated KRAS oncogene. The 60–65% of the population with wild-type or neutral KRAS oncogene respond well with cetuximab or panitumumab while rest of 35–40% of the population with cancers are poor responders to this regimen [127]. Therefore, FDA has recommended a pharmacogenetic testing on the KRAS gene before advising cetuximab and panitumumab in the treatment of colon, lung as well as head and neck cancers and is indicated only for patients with KRAS mutant negative, i.e., the wild-type KRAS [81].

In addition to KRAF-mutation, BRAF mutations also show predictive biomarker characteristics for EGFR-inhibitors as serine-threonine-kinase BRAF is major effector of KRAS. KRAS mutation resistant patient were also non-responders to treatment with BRAF mutation. However, patients of colorectal cancer cells with mutant V600E allele showed response to BRAF inhibitor sorafenib [108].

9.6.10 Trastuzumab and HER2/neu

Trastuzumab humanized monoclonal antibody which are highly effective in breast cancers. These agents target the human epidermal growth factor receptor type-2, i.e., HER2. It is registered U.S. and European Union (EU) for the curing of human epidermal growth factor receptor HER2 positive metastatic breast cancer, for adjuvant treatment of localized HER2 breast cancer as well as for rare type of gastroesophageal junction adenocarcinoma. In patients with breast cancers, HER-2 gene amplification is significantly related to poor disease prognosis, overall cancer-free periods, and survival of patients [129, 130]. However, it is noted that only 25–30% of HER-2/neu + breast cancer patients are responsive to this agent [131]. In addition to its direct anti-proliferative and pro-apoptotic effects, various immune mechanisms including antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-mediated cytotoxicity (CMC) along with antiangiogenic activity are contributor mechanisms [131]. There is a considerable improvement in prognosis of HER2 + breast cancers since the development of targeted therapies such as trastuzumab. Lapatinib is the other approved regimen other than trastuzumab for HER2 + breast cancer. It works intracellularly against trastuzumab-resistant patients [75].

The trastuzumab showed induced cardiotoxicity in clinical trials. The combination therapy trastuzumab with anthracycline have showed increased incidences of cardiac toxicities [130]. Several explanations are postulated to determine the trastuzumab-induced cardiac toxicity. Trastuzumab is a monoclonal antibody initiates a mechanism of antibody-dependent cell cytotoxicity and complement-dependent cytotoxicity which may result in induced cardiac cell toxicity [54]. Other debatable explanations is evidence-based experimental data which supports a major role for HER2 signaling in cardiac myopathies [64]. Various HER2 gene polymorphic variants have been reported [51]. At clinical levels, the extensively investigated germline polymorphism is codon 655A > G (rs1136201, Ile/Val) of transmembrane domain of the HER2 protein associated with a high risk for breast cancer. The presence of polymorphic Val allele may act as predictive biomarker to determine trastuzumab-induced cardiac toxicity that possibly render cardiomyocytes dependent upon HER2 signaling pathway [13, 14, 61].

9.6.11 Imatinib and BCR-ABL, C-Kit

The discovery of molecular mechanisms and chromosomal anomaly responsible for chronic myeloid leukemia was a hallmark achievement. This also promised advancement and development of more targeted therapies. The Philadelphia chromosome abnormality was due to translocation at chromosome 22 and 9. This lead to juxtaposition of protooncogene between the breakpoint cluster region (BCR) and the c-ABL oncogene [31]. This additionally triggered numerous other signal transduction pathways associated with cell proliferation, differentiation, survival and resistance to programed cell death or apoptosis [145].

Imatinib is an original tyrosine kinase inhibitor (TKI) widely used for the targeted treatment of chronic myeloid leukemia (CML) as well as a first-line treatment for multiple other BCR-ABL-, c-KIT-, and PDGFR-driven cancers.

There is interpatient variability in plasma concentrations after oral administration of imatinib is associated with drug transporters. These drug transporters may act as liable predictive biomarkers for imatinib distribution into CML cells. Therefore, PGx parameters expected to play potentially important role in personalized imatinib dosing to improve treatment outcomes. Imatinib is metabolized by CYP2C8 and CYP3A4 in vitro studies, however, genotype CYP2C8 showed significant effect on imatinib metabolism in CML patients when exposed systemically. There is no considerable clinical evidence for CYP3A4 or CYP3A5 genotypes to modify imatinib metabolism and pharmacokinetics [12]. Imatinib has showed a promising efficacy in CML patients with failed interferon-alfa therapy in phase I-II trials [43].

Imatinib is a substrate for several drug influx and efflux transporters in liver and/or CML cells. Polymorphic forms in genes of drug transporters expressed in CML cells may influence intracellular distribution of drug as well as plasma concentration to drug response relationship [12]. Imatinib being a potent selective inhibitor of BCR-ABL, also targets c-kit and the platelet-derived growth factor

receptor. Mutations in c-kit are found to be quite common in Asian ethnics therefore c-kit inhibitors are priority-based investigational material in this population. The phase II, open-label, single-arm trial studies for disease progression free survival, overall response to drug rate, and survival of the patient were measured. These patients with metastatic melanoma were screened for c-kit mutation. The study advocated treatment of metastatic melanoma with imatinib was especially favorable in patients with genetic variances in c-kit gene [59].

Imatinib is a first-line of choice tyrosine kinase inhibitor (TKI) for the treating of CML, which has improved overall survival (OS) in these patients. The discovery of newer TKIs for Ph + chronic-phase (CP) CML prognosis was compared for imatinib versus second-generation dasatinib, nilotinib, bosutinib, and third-generation TKIs ponatinib for efficacy and safety in terms of overall survival period, progression-free survival period of patient, hematological and nonhematological toxicities [148]. Among all of these agents, imatinib showed advantage for patients with comorbidities. However, ponatinib is an oral TKI showed potent in vitro activity against natural BCR-ABL as well as mutant BCR-ABL, including T315I in phase II trials. This can potentially be a drug-of-choice for patients who experience failure to TKI therapy and with T315I BCR-ABL mutation [29].

9.6.12 Erlotinib and EGFR

Erlotinib is a small-molecule tyrosine kinase inhibitor (TKI) that specifically binds to the epidermal growth factor receptor (EGFR) approved as a drug-of-choice in the second-line treatment setting. However, phase III trials made it the backbone for the treatment of mutated-EGFR NSCLC patients. Exon-19 deletion and at exon-21 L858R are most common EGFR mutations that show higher response to EGFR-TKIs [103]. Erlotinib at standard daily oral dose is well tolerated in NSCLC patients. The reported adverse effects are skin rash and diarrhea generally. Others are mild, reversible, and manageable [117, 143]. Erlotinib has showed clear evidence to benefit the younger Asian nonsmoker patients with adenocarcinoma of the lung especially patients carrying mutated EGFR [137].

Rhabdomyolysis syndrome is muscle injury due to myoglobinuria, electrolyte abnormalities, and acute nephrotoxicity. This can be associated with the use of lipid-lowering agents and alcohol consumption but still it is an uncommon complication in chemotherapeutic treatment [91]. Only one case is reported till now with erlotinib treatment as single-agent for occurrence of rhabdomyolysis as an adverse event [105]. Erlotinib is mainly metabolized by CYP3A4 isoenzyme and follows P-glycoprotein (MDR1/ABCB1) and ABCG2 drug transporter system. This system potentially interacts transporter-mediated drug–drug interactions especially with CYP3A4, P-glycoprotein, and ABCG2 inhibitors. Therefore, inter-individual pharmacokinetic variability due to genetic variance at drug targets affects erlotinib's disposition [83].

9.7 Conclusion

Treatment of cancer is challenged by drug resistance, moderate tumor selectivity, narrow therapeutic index, severe side effects, significant inter-individual variability to drug response, and frequent relapse. Genetic polymorphism predominately involving SNPs has largely explained the inter-individual differences in drug response. Therefore, the knowledge of pharmacogenetics has the potential to address the issues of variable drug efficacy and toxicity by individualizing cancer therapy based on a patient's genetic profile to improve treatment outcomes and reduce adverse events. Genotyping for critical SNPs should be an integral part of clinical workup to identify the patients who could benefit from a specific regimen without developing extensive toxicities. Controlling the genetic factors responsible for treatment failure and side effects has also been one of the main goals in the search for new anticancer drugs tailored to individual genetic profiles for personalized medicine.

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Targeted Therapy and Personalized Medicine

10

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Abbreviations

Acute lymphocytic leukaemia	ALL
Acute myeloid leukaemia	AML
Adenosine triphosphate	ATP
Anaplastic lymphoma kinase	ALK
Chronic lymphocytic leukaemia	CLL
Chronic myeloid (or myelogenous) leukaemia	CML
Colorectal cancer	CRC
Computed tomography	CT
Cyclin dependent kinase 4 and 6	CDK 4/6
Cytotoxic T-lymphocyte antigen 4	CTLA-4

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Epidermal growth factor receptor	EGFR
Fms-related receptor tyrosine kinase 3	FLT3
Folate-receptor	FR
Hepatocellular carcinoma	HCC
Human epidermal growth factor receptor-2	HER
Internal tandem duplications	FLT3-ITD
Magnetic resonance imaging	MRI
Metastatic CRC	mCRC
Multidrug resistance	MDR
National cancer institute	NCI
Non-small cell lung cancer	NSCLC
Oestrogen receptor	ER
Overall response rate	ORR
Platelet derived growth factor receptor	PDGFR
Poly-ADP ribose polymerase	PARP
Positron emission tomography	PET
Precision and personalized medicine	PPM
Progesterone receptor	PR
Programmed death-ligand 1	PD-L1
Progression-free survival	PFS
Prostate-specific antigen	PSA
Receptor tyrosine kinase	RTK
Relapsed/refractory	R/R
RNA interference	RNAi
Small interfering RNA	SiRNA
Tyrosine kinase domain	FLT3-TKD
Tyrosine kinase inhibitors	TKIs
Vascular epidermal growth factor receptor	VEGFR

10.1 Introduction to Cancer Therapy

10.1.1 Overview

Cancer is a foremost public health-issue that affects individuals all over the world. Cancer caused 19.3 million new diagnoses in addition to 10.0 million deaths in 2020, according to GLOBOCAN [1]. Lung cancer continued to be the leading cause of cancer death, with a predictable 1.8 million deaths/year (18%) [1]. Global cancer prevalence is forecast to climb to 28.4 million cases by 2040, with a substantial increase rise in transitioning (64%), than in transitioned (32%) countries, albeit this could be worsened further by increased risk factors linked with globalization and flourishing economies [1]. For global cancer control, developing a sustainable infrastructure for the distribution of cancer prevention measures and the provision of cancer care in transitional countries is crucial. Many research

investigations over the last decade have concentrated on identifying novel therapies to lessen the negative effects of traditional therapies. The most significant impediment to targeted cancer therapy is the emergence of drug resistance [2]. As a result, a thorough understanding of the complicated events is essential for developing accurate and effective treatments. The major goal of "translational research" is to carry investigation from "bench to bedside" by combining advancements in molecular biology with clinical studies [3]. This continuous feedback encourages the detection of disease biomarkers and pharmacological targets, leading to more rational drug design, improved treatment efficacy, and quicker clinical modification of chemical entities [4]. Personalized research may aid in the development of future medicines by decreasing the period between drug target identification and clinically useful therapy options.

10.1.2 Chemotherapy

Despite the fact that cancer treatment's ultimate goal is to eradicate or destroy all cancer cells from the body, virtually abnormal cells can often persist after treatment. Chemotherapy is a typical treatment approach that focuses on many aspects of cell proliferation (Fig. 10.1). Over 100 cancer drugs are currently accessible, and many of them are regularly used in conjunction with other therapies or techniques. Traditional treatments have proven to be beneficial in the short term, but many patients relapse over time [5]. The probability of cancer recurrence, on the other hand, is dependent on initial therapy, cancer type, and time when preliminary treatment is given, among other things. Furthermore, studying the mechanisms driving therapeutic response is critical for developing treatments that are both efficacious and personalized.

10.1.3 Proteomics and Genomics

Advancements in biotechnology have opened up a few new possibilities for cancer prevention, diagnosis, and treatment. The way we think about cancer prevention, diagnosis, and therapy has evolved significantly throughout the time [3, 6, 7]. This comprises "omic" investigations, including proteomic and genomic studies, which are valuable in finding genes in addition to proteins that might have a part in cancer biology by defining considerable heterogeneity in gene expression and activation in healthy and disease situations [8]. Prominently, the reaction to targeted therapy might be tracked using proteomic approaches to assess the targeted therapy's success as well as the efficacy of prospective future therapeutics targeting the same protein pathway [9].

Fig. 10.1 Illustration of the main types of cancer treatment

10.1.4 Plant-Based Therapy

Plant-based therapy offers a viable alternative to conventional cancer treatment. This refers to the anti-proliferative and pro-apoptotic effects of active phytochemicals in battling diverse cancers cell lines for instance, breast, oral, lung, stomach, colon, hepatic, and cervical cancers [10]. Polyphenols, flavonoids, alkaloids, quercetin, brassinosteroids, and a variety of other plant-derived bioactive chemicals have demonstrated significant anti-cancer activity and have been used as complementary medicines [11–13]. Regardless of the benefits of employing natural medications, their clinical use is limited due to their low bioavailability and/or toxicity.

10.1.5 Nanotechnology

Because nanoparticles have shown a momentous role as a drug delivery mechanism, nanotechnology has been widely used in cancer treatment. It has a number of advantages over traditional drug delivery, including better biocompatibility and stability, increased permeability, and precise targeting [14]. Nanoparticle drug delivery systems have also been found to aid in the treatment of cancer-related drug resistance. Furthermore, scientists have lately begun to look into the role of

nanoparticles in immunotherapy, which is becoming increasingly crucial in cancer treatment [15].

10.1.6 Gene Therapy

The discovery of the cellular apoptosis mechanism has opened up new treatment options for cancer, and gene therapy exploiting that suicidal system was proven to allow cancer cells to self-destruct without triggering inflammation. Despite its modest clinical success, gene therapy aiming the apoptotic machinery brings a lot of promise for patients with life-threatening cancers, assuming that effective and targeted gene delivery is available [16]. The discovery that small interfering RNA (siRNA) can influence gene expression over a mechanism known as RNA interference (RNAi) is one of the most noteworthy advances in biology [17]. Because of its capacity to suppress explicit genes in a selection of hereditary illnesses, simultaneous RNAs (siRNAs) has attracted thoughtfulness as a potential therapeutic reagent. siRNAs are appealing in the treatment of cancer and other disorders, because they can be used to investigate the activity of a single gene [18].

10.1.7 Radiomics

Radiomics is a new field that combines image data from tests like computed tomography (CT), positron emission tomography (PET), and magnetic resonance imaging (MRI) to make medical decisions. Clinicians can, for example, confirm therapy efficacy, anticipate the location of tumour metastases, relate results with a histological investigation, or further precisely describe the cancer type. When radiomics is combined with other testing procedures, each patient receives a unique treatment plan, which is critical for enhanced assessment and therapy [19]. As a result, when various approaches are integrated, they can deliver the greatest individualized cancer therapy, emphasizing the importance of merging several disciplines to achieve the best results. The main focus is on improving and lowering the treatment cost from bench to bedside. Clearly, the drug development process is in a state of flux and some of the most major obstacles in drug development are certain requirements that must be met, such as safety, efficacy, and quality. Science is pushing for the creation of targeted therapies, so you can have the proper treatment for the right population or person. The pharmaceutical industry is transitioning to developing these types of drugs, but understanding the underlying causes of disease remains major obstacles. Bringing an anti-cancer drug to market still costs a lot of money, and firms need to return their investment in a niche market. Hence, we may need to construct smarter, leaner, and less expensive clinical trials employing patient classification and enrichment procedures that allow us to get just as much information about a therapy from a much smaller study.

10.2 Strategies of Targeted Therapies

10.2.1 Traditional Treatment

At the cell cycle level, traditional chemotherapeutic drugs cause cytotoxicity by disrupting or suppressing processes that are essential for the fast cellular division and DNA synthesis [20]. Their biggest drawback is their non-selectivity when it comes to targeting quickly dividing non-cancerous cells including bone marrow and epithelial cells. Patients typically experience anaemia, hair loss, infertility, and other major side effects in response to cancer therapy. As a result, the effective therapeutic dose is not reached, reducing the efficiency of standard chemotherapy medications.

10.2.2 Targeted Therapies

Despite advances in cancer biology, many drugs are still in preclinical research. Many specific chemicals are being investigated in both preclinical and clinical settings, making targeted treatment one of the utmost favourable areas of cancer therapy. Targeted therapy is characterized by the National Cancer Institute (NCI) as "***A type of treatment that uses drugs or other substances to identify and attack specific types of cancer cells with less harm to normal cells***". This definition explains that some drugs inhibit particular enzymes or other substances associated with the growth and spread of cancer cells. Other varieties boost the immune system involved in the destruction of cancer cells or send toxic compounds directly to cancer cells, killing them. It might have less side effects compared to conventional cancer treatments. The advance targeted therapy is to inhibit cancer cell proliferation via interfering definite molecules essential for tumour growth, is an excellent example of translating basic research into practical application (Fig. 10.2).

Targeted cancer therapies can be broadly categorized as either small molecules or monoclonal antibodies [21]. Monoclonal antibodies, which bind to a large number of targeted antigens with excellent specificity, are the subject of significant research in the area of cancer therapies. For example, for acute myeloid leukaemia treatment, gemtuzumab (Mylotarg®) is an explicit monoclonal antibody of CD-33 conjugated with calicheamicin [22]. They are used for the delivery of active medicines in targeted therapy [23] or chemotherapy toxins [24]. Small drugs are usually directed at specific molecular targets involved in cancer cell proliferation, metastasis, and angiogenesis. Many cancer patients' lives have been improved as a result of molecularly targeted cancer therapy. For example, erlotinib, which inhibits EGFR in patients with non-small cell lung cancer (NSCLC) or sorafenib, which inhibits growth factor receptor, are among such inhibitors [25].

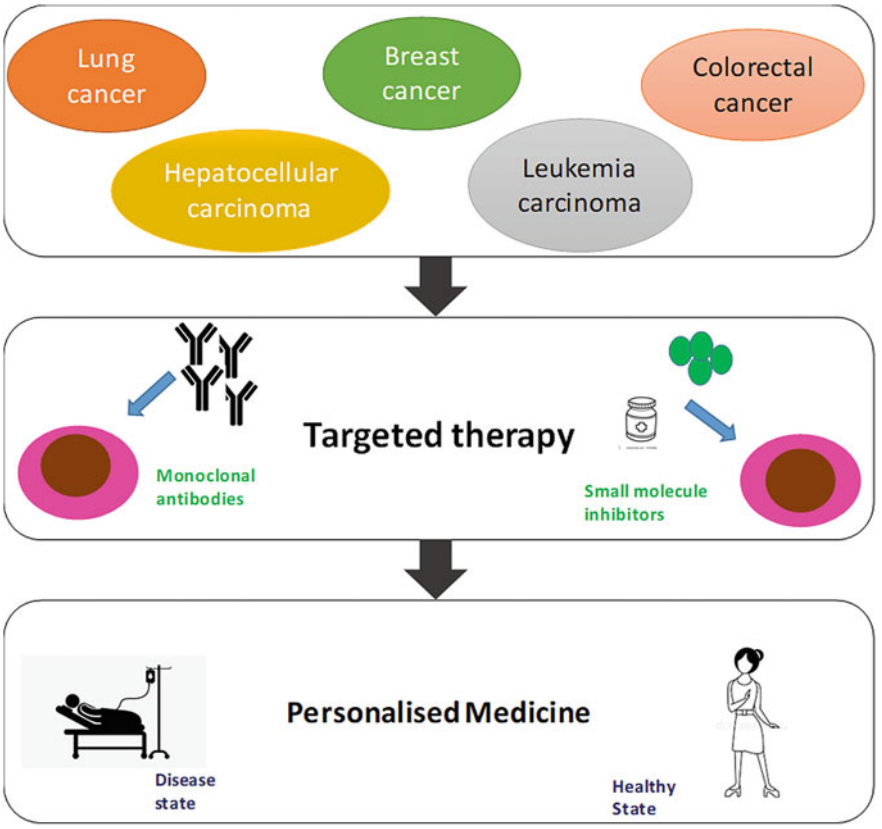

Fig. 10.2 Schematic presentation of various cancers with targeted therapy applications implemented in clinical practice to achieve a personalized medicine

10.2.3 Targeted Therapy Versus Traditional Therapy

The following is a summary of the distinctions between targeted therapies and conventional chemotherapy as defined by the NCI: standard conventional chemotherapy designed to kill rapidly dividing tumour cells, may also affect dividing normal cells, and is cytotoxic, whereas targeted therapies act on specific molecular targets and are often cytostatic. In actuality, these distinctions are not black and white, as it has been evident that most targeted medicines can cause toxicity in normal cells, which can be fatal. The traditional chemotherapies are often designed to inhibit a single target, for example methotrexate, only inhibits dihydrofolate reductase; however, many new antifolates, for example folate-receptor (FR)-targeted drugs that deliver their cytotoxic load specifically to tumour cells with high FR expression, can be considered targeted cytotoxic therapy [26]. Tumour heterogeneity has been shown to represent a barrier to traditional chemotherapeutic treatment, restricting its use in a range of cancer forms while

also promoting intrinsic resistance to cancer therapy. More targeted and efficient medicines are needed to fight these constraints, which eventually lead to cancer growth and lower patient survival rates. Multidrug resistance (MDR) includes increased xenobiotic metabolism, growth factors, higher drug efflux, greater DNA repair capacity, and genetic variables (mutations). A growing number of biomedical research investigations are focusing on developing chemotherapeutic agents that can avoid or reverse (MDR). Furthermore, numerous oncogenes and oncoproteins in a range of malignancies have the ability to inactivate and/or remove chemotherapy drugs from tumour cells [27]. Unlike chemotherapy, targeted therapy targets specific molecules within cells, subsequently fewer and milder side effects and does not work for all cancer patients. Certain medications may be helpful during the first year of treatment, but if cancer cells become resistant to targeted therapy drugs, a biopsy must be performed again to find a new targeted therapy drug.

10.2.4 Personalized Therapy for Cancer

There have been substantial breakthroughs in identifying and treating cancer over the last twenty to thirty years, with early detection being one of the most crucial elements of successful therapy. With new approaches being created all the time, the promise of newly customized therapies is born of a greater understanding of cancer biology. Moreover, with the advent of high throughput sequencing, this field has been revolutionized and now it is relatively easy to sequence genetic information. Genetics information obtained through genomics is now being integrated with other omics technologies such as genomic, transcriptomic, proteomic, and metabolomic to get relevant clinical information [28, 29]. These advancements in the field of personalized medicine are also influencing cancer treatment. Drug discovery in different types of cancer is influenced by omics technologies [30]. New personalized therapies are now being developed that are specific for each subtype of cancer patients based on their DNA, RNA, protein, and/or metabolic profiles [29]. Detection methods for circulating DNA and tumour cells are not only used for the diagnosis purposes but have a potential to be used for identifying personalized therapies. The patients selected through biomarkers-based approach showed better outcome with targeted therapies compared to those that were selected via this approach. Hence, phase I clinical trials can be enriched with biomarkers-based approach for targeted therapies [31]. Another useful approach is the growth of individual tumour in vitro. This method is being used to define safest and greatest effective therapy before it is administrated to patients. However, this is just the beginning of the field, and there is still much to do for the improvement of personalized therapies in cancer management. In addition, this field must be developed in accordance with a country's legislation.

10.2.5 Current Targeted Therapies Approved by the FDA

As mentioned above that targeted therapies are aimed for cancer prevention. Much effort is being put into finding treatments that target cancer-specific cells, while causing the least amount of damage to healthy tissues. This strategy also tries to develop therapies that can be used to treat an extensive range of tumours instead of just those that affect a specific tissue. A number of targeted pharmaceutical cancer therapies have been approved by the FDA, and many more are being tested in clinical studies, either alone or in combination with other treatments as shown in Table 10.1. The EGFR gene mutation is one of the most notable examples [32, 33]. The following is a list of currently authorized targeted therapy for solid tumours, along with their molecular targets.

Table 10.1 List of currently approved some cancer targeted medicines and their molecular targets. Modified from [34]

Targeted therapies	Target (s)	FDA-approved suggestion(s)
Afatinib	EGFR	NSCLC (EGFR with exon 19 deletions or exon 21 substitution (L858R) mutations)
Alectinib	ALK	NSCLC (with ALK fusion)
Bevacizumab	VEGF ligand	Colorectal cancer NSCLC
Blinatumomab	CD3/CD19	Acute lymphoblastic leukaemia (precursor B-cell)
Brigatinib	ALK	NSCLC (ALK+)
Ceritinib	ALK	NSCLC
Cetuximab	EGFR	Colorectal cancer
Crizotinib	ALK, MET, ROS1	NSCLC
Durvalumab	PD-L1	NSCLC
Enasidenib	IDH2	Acute myeloid leukaemia
Gefitinib	EGFR	NSCLC (exon 21 substitution (L858R) mutation or with EGFR exon 19 deletions)
Lapatinib	HER2, EGFR	Breast cancer (HER2+)
Midostaurin	FLT3	Acute myeloid leukaemia (FLT3+)
Nivolumab	PD-1	Colorectal cancer Hepatocellular carcinoma NSCLC
Panitumumab	EGFR	Colorectal cancer (KRAS wild type)
Pertuzumab	HER2	Breast cancer
Ribociclib	CDK4, CDK6	Breast cancer
Ziv-aflibercept	PIGF, VEGFA/B	Colorectal cancer

Abbreviations EGFR—epidermal growth factor receptor; ALK—anaplastic lymphoma kinase; NSCLC—non-small cell lung cancer; VEGFR—vascular epidermal growth factor receptor; PD-L1—programmed death-ligand 1; and FLT3—Fms-related receptor tyrosine kinase 3

10.3 Targeted Therapies and Personalized Medicine in Cancer

Advances in cancer biology have given us a better knowledge of the complexity and variety of a tumour's genotypic and phenotypic expression, as well as the complicated cancer transformation. To better understand and treat this category of illnesses, there is still much study to be done. Due to the intricacy of the tumour's and patient's genetic profiles, cancer treatment has initiated to move towards a more individualized, precise, and focused strategy for the patient's malignancy. Surgery, radiation therapy, chemotherapy, and immunotherapy are the four basic types of traditional cancer treatments. To battle cancer's resistance, most people will require a combination of medications. This has signalled the start of a new era of targeted therapeutics and precision medicine, and the beginning of the journey is exemplified by the evolution of various cancer therapies. This section provides an overview of the history of targeted therapy as well as the importance of personalized medicine in cancers such as lung, breast, colorectal, hepatocellular carcinoma, and leukaemia.

10.3.1 Lung Cancer

With 1.8 million deaths/year, lung cancer (i.e. small and non-small cells) is the most common malignant neoplasm and the leading cause of cancer death in both men and women globally [1]. Some of the risk factors include patient age and history, poor diet, air pollution, smoking intensity/duration, and age of smoking initiation/cessation [35–37]. Despite the fact that targeted therapy has substantially improved prognosis of advanced lung cancer, the advanced-stage patients continue to have a terrible prognosis, with just 4% of those with stage IV disease surviving [38]. As a result, early detection is critical for undergoing aggressive resection before it spreads.

10.3.1.1 Targeted Therapies in Lung Cancer

Until customized therapy was discovered, chemotherapy was the most essential aspect of treatment for advanced-stage patients. Patients with advanced stage were only able to live for several years after the introduction of individualized therapy based on molecular type. This includes tyrosine kinase inhibitors (TKIs) (afatinib) for active EGFR mutations, ALK rearrangement (crizotinib), or monoclonal antibodies against VEGF (bevacizumab).

10.3.1.1.1 EGFR inhibitors

Overexpression of EGFR has been seen in 50–80% of patients with NSCLC, making it a great candidate for targeted therapy [39]. The loss of amino acids at locations 747–750 in exon 19 and the L858R mutation in exon 21 are the most common sensitive alterations [40]. Gefitinib meaningfully prolongs progression-free survival (PFS) in patients with EGFR mutations, according to the first big randomized controlled trial, published in 2009 [41]. Afatinib (an EGFR-TKI of the

second generation) is effective, and FDA has approved it for use with rare EGFR mutations, such as G719X, S768I, and L861Q [42]. In patients with NSCLC who have a T790M mutation, osimertinib (third-generation EGFR-TKI), was shown to be significantly superior to chemotherapy, by means of an overall response rate (ORR) of 77% and a PFS of 10.1 months [43].

10.3.1.1.2 ALK inhibitors

In 3–7% of NSCLC patients, modifications in ALK gene are discovered [44]. Crizotinib was the first ALK inhibitor to be approved by receive FDA approval and is among the most important clinical and beneficial clinical drugs. Crizotinib was the first ALK inhibitor to be approved by the FDA, and it is one of the most essential and helpful therapeutic medications available. Choi et al. [45] discovered secondary mutations in the EML4-ALK kinase domain in patients with acquired resistance. Alectinib, ceritinib, and other second-generation ALK-TKIs were designed to overcome crizotinib resistance as they are more potent than crizotinib [46]. Remarkably, altogether three second-generation ALK medicines improved survival in first-line NSCLC patients who tested positive for ALK. However, second-generation ALK-TKIs, like EGFR-TKIs, encountered resistance issues.

10.3.1.1.3 Emerging molecular targets

ROS1 is an insulin receptor that is occasionally seen in NSCLC (1–2%) [47]. Because the tyrosine kinase domains of ROS1 and ALK are so similar, crizotinib was tested on NSCLC with a ROS1 rearrangement and showed ORRs of 72 and 80%, respectively, in a US cohort [44]. MET amplification, RET fusions, BRAF/HER2 mutations, all have been identified as targetable oncogenic drivers.

10.3.1.2 Drug Resistance

The causes of drug resistance are numerous. In cancer patients, drug resistance is most frequently caused by the T790M mutation of the EGFR, while MET or HER2 amplification and small cell histologic transformation are less prevalent [48]. As a result, some researchers feel that combining a c-MET inhibitor with an EGFR will be a novel strategy for combating drug resistance. Even though lung cancer has been controlled with target therapy, the tumours will eventually acquire drug resistance. For better treatment outcomes, a greater knowledge of resistance mechanisms and the development of combination medications are required.

10.3.1.3 Personalized Medicine in Lung Cancer

All patients with advanced stage with varied histologies should currently be screened for mutations of EGFR, ALK rearrangements, and ROS1 fusion. Personalized medicine confronts challenges and restrictions, despite its successes and promises. The establishment of specific therapeutic resistance mechanisms is another constraint of personalized medicine. The sequential use of drugs and/or their combinations will have to be carefully regulated to keep the tumour receptive to treatment for long periods of time. In future, a cancer treatment that targets

tumour cell genes and proteins may aid in early identification and assist clinicians in designing the most effective treatment package for each individual patient.

10.3.2 Breast Cancer

According to the GLOBOCAN 2020 report, breast cancer is the most frequently diagnosed cancer, with an estimated 2.3 million new cases identified each year (11.7%). Breast cancer is a biologically and clinically highly heterogeneous disease, involving many genetic and environmental factors which make the treatment highly complex. Unfortunately, the many of the breast cancer patients are diagnosed at an advanced stage when curative treatment becomes difficult. Furthermore, nearly 30% of women diagnosed with early stage cancer progress to metastatic cancer, for which treatment choices are minimal. As a result, breast cancer continues to be one of the top causes of illness and mortality around the world [49]. Genes linked to breast cancer BRCA1 and BRCA2 are tumour suppressor genes whose mutations increase the risk of developing breast cancer. Many features such as tumour size, its type, histological score, metastasis, and receptor status influence the prognosis of breast cancer and treatment response. Thus, each patient would be different from the other in respect of type and drug targets.

10.3.2.1 Targeted Therapies in Breast Cancer

10.3.2.1.1 Estrogen Receptor (ER) positive

Nearly 75% of breast cancer are HR+and HER2− , making endocrine therapy the backbone of treatment for these patients [50]. Tamoxifen is the good example of targeted endocrine therapy and is approved by FDA [51]. Fulvestrant is an antagonist of ER, competitively binds to oestrogen receptor (ER) and down regulate its expression. Multicenter and randomized Phase 2 trials (Trial 0020 and 0021) compared the intravascular injection of fulvestrant (250 mg; once monthly) and an oral dose of anastrozole (1 mg; once daily) in postmenopausal women suffering from advanced breast cancer. The majority of patients progressed on fulvestrant. The ORR for fulvestrant was 19.2% and for anastrozole 16.5%. Median overall survival was comparable among both groups at an extended follow-up. Trials documented the non-inferiority of fulvestrant to anastrozile, and fulvestrant was approved as a treatment option [52–54].

10.3.2.1.2 Human epidermal growth factor receptor 2

Trastuzumab is a monoclonal antibody, inhibits the extracellular domain of HER2. For early patients, Yin et al. conducted a meta-analysis to examine the advantages of concurrent or sequential trastuzumab with adjuvant chemotherapy (HER2-positive). The efficacy of trastuzumab in the adjuvant context to improve disease-free survival and overall survival was confirmed in this trial. Inhibition of HER2 via trastuzumab results in inhibition of HER2 signalling and thus inhibits tumour growth and vice versa [55]. Phase III clinical trial of TANDEM reported

that combined therapy using trastuzumab and anastrozole improved the progress free survival but with increased adverse effects [56].

Targeted inhibition of HER2: HER3 dimerization using pertuzumab has dramatically affected the clinical outcomes in HER2-positive metastatic breast cancer patients. The HER2: HER3 complex increases growth and survival of cancer cells via modulation of protein kinase C, MAPK, and Akt signalling [57]. This antibody inhibits tumour growth as significantly as trastuzumab. The combination of trastuzumab and pertuzumab along with chemotherapy resulted disease-free progression rate of 94.1% compared to 93.2% in the placebo group. Three-years rate of invasive disease-free progression in node-positive breast cancer patients was 92.0% in pertuzumab group in comparison with 90.2% [58].

Lapatinib is an inhibitor of tyrosine kinase and targets the adenosine triphosphate (ATP) binding site positioned on the intracellular kinase domains in both HER2 and EGFR [59]. In the clinical setting, targeting HER2+ with lapatinib plus trastuzumab gives better results than trastuzumab or lapatinib only in treating HER2+ breast cancer [60]; because lapatinib exhibits distinctly different mechanisms of action than trastuzumab [61]. Lapatinib and trastuzumab combination in phase II trial improved the median overall survival to 14 months vs. 9.5 months in patients on trastuzumab only [61, 62].

10.3.2.1.3 Triple negative breast cancer

Preclinical studies show that in cells with BRCA1/2 dysfunction targeted inhibition of poly-ADP ribose polymerase (PARP) lead to profound cytotoxic results [63]. PARP inhibitors provide the genotype-specific treatment with BRCA mutations, including those with metastatic TNBC patients [64]. Olaparib and talazoparib are PARP inhibitors, and a concept trial has shown the overall response rate of 41% and 50%, respectively, in metastatic breast cancer [65]. Targeting DNA repair system is one of the promising targeted approaches for basal like TNBC with BRCA mutations. Platinum is among DNA cross-linking agents and therapeutic option for sporadic or germline DNA-repair-deficient breast cancers. Preclinical studies show that BRCA-mutant breast cancer cells respond to carboplatin in a dramatic way than BRCA intact ones [66]. In a phase III trial, paclitaxel + carboplatin was utilized as an adjuvant therapy in women with operable TNBC, against a standard-dose CEF-T (cyclophosphamide, fluorouracil, and epirubicin, followed by docetaxel). After a median of 62 months of follow-up, those randomized to PCb had a longer disease-free survival time than those assigned to CEF-T. The data suggested that paclitaxel-plus-carboplatin in combination is a beneficial treatment option for breast cancer patients [67].

10.3.2.1.4 Emerging targeted therapies

Targeting intracellular molecular pathways is one of the promising strategies in personalized patient care. Mammalian target of rapamycin (mTOR) pathway induces resistant endocrine breast cancer [68], and combined targeting of mTOR and ER had led to improved survival. Baselga et al. documented the synergistic effects of mTOR and aromatase inhibitors with hormone receptor positive

patients and verified the practice of mTOR inhibitors [69]. Ribociclib, abemaciclib, and palbociclib are known for targeted inhibition of cyclin dependent kinase 4 and 6 (CDK 4/6). Combined use of CDK 4/6 with aromatase inhibitors is shown synergy in hormone receptor–positive breast cancer. Finn et al. showed in PALOMA-1/TRIO-18 that the use of palbociclib and letrozole improved the disease-free survival of 20.2 months as compared to 10.2 months in letrozole alone [70]. Remarkably, three new CDK 4/6 inhibitors, comprising palbociclib (Ibrance, PD0332991), abemaciclib (Verzenio, LY2834219), and ribociclib (Kisqali, LEE011), over a three-year period (2015–2018) obtained FDA clearance.

10.3.2.2 Drug Resistance

In the beginning, systematic therapy remains active in 90% of primary and 50% of metastatic breast cancer. To overcome resistance to single agents, the strategy of combinational theory has been used [71, 72]. Research revealed the involvement of several mechanisms that might underlie resistance. These include drug efflux due to increased ATP-binding cassette expression including breast cancer resistance proteins, enhanced metabolic enzymes production, deactivation of cytotoxic drugs by CYP2C9∗2, and targets for cytotoxic drugs [73–76]. Similarly, doxorubicin, the most effective chemotherapeutic treatment for treating breast cancer, has been shown to cause drug resistance, resulting in a poor prognosis and survival rate for patients [77].

10.3.2.3 Personalized Medicine in Breast Cancer

Personalized classification is required due to heterogeneity of breast cancer and induction of different mechanisms of resistance, that can be characterized by distinct molecular aberrations [78]. The molecular stratification of breast cancer aids in the decision to personalized treatment and selection of specific targets. Different molecular assays and multigene microarrays are in clinical use for stratification and applied routinely for targeted therapy. Multigene arrays including Oncotype DX, MammaPrint, and PAM50 are individualizing the choice for chemotherapy among patients. Further developments in targeted therapy may become feasible using analysis of patient outcomes through array of gene expression and high-throughput analysis. Overall, personalized treatments for breast cancer are evolving rapidly and these advanced achievements in the field of drug discovery are changing the standard of care and have reduced the breast cancer-related mortality.

10.3.3 Colorectal Cancer

Colorectal cancer (CRC) is the world's third most frequent cancer and the second major cause of cancer-related deaths [79]. Lifestyle, body fatness, and food patterns all have a role in the rise in morbidity [79]. It is assessed that 2–8% of CRC cases arise as a result of inherited syndromes. Patients with CRC have been treated the same way for decades and have received the same "standard care". However, surgery is no longer beneficial for late-stage CRC patients, who make

about 25% of all CRC patients [80]. As a result, novel CRC therapy alternatives are desperately needed to enhance overall survival and reduce the severity of the disease.

10.3.3.1 Targeted Therapies in CRC

Chemotherapy and targeted therapeutic advancements have resulted in a dramatic surge for metastatic CRC (mCRC) patients, who now have an average survival of nearly 40 months [81]. As per BOND trial, which helped the FDA approve cetuximab for mCRC in 2004, the first monoclonal antibody targeting EGFR (cetuximab) showed significant promise in improving PFS in patients who had a poor response to single-agent treatment [82]. In the PRIME study, FOLFOX with panitumumab was compared in patients, and the combination regimen achieved a superior PFS and OS than FOLFOX alone, with additional proven significance in the survival analysis in mCRC [83]. Cetuximab and panitumumab are FDA-approved first-line treatments for CRC. Anti-EGFR medicines may be a low priority for second-line or beyond CRC treatment because cetuximab and panitumumab have been revealed in multiple studies and fail to achieve a reasonable PFS or OS for CRC patients [84]. Aflibercept, Regorafenib (multikinase inhibitor), and Ramucirumab (mab) are three more antiangiogenic agents that have been approved for mCRC after bevacizumab based on the findings of a clinical trial [85, 86]. In 2012, Zaltrap regimen along with FOLFIRI was approved by the US for resistant patients [85].

10.3.3.2 Drug Resistance

Advances in molecular technology have led to a greater understanding of how to create targeted medicines, which has helped people with mCRC live longer. Resistance to contemporary targeted therapy, on the other hand, is still a substantial issue in clinical practice. BRAF, RAS, and PIK3CA mutations, among others, have been linked to anti-EGFR therapy resistance, and it has been shown that these drugs will benefit 40% of all mCRC patients [87]. Understanding resistance mechanisms, as well as developing new biomarkers and other targetable pathways, is critical for optimizing therapy options and improving patient survival in resistant patients.

10.3.3.3 Personalized Medicine in CRC

Treatments for CRC have improved dramatically, due to the use of monoclonal antibody therapies (bevacizumab). On a molecular level, however, tumours differ greatly, which could affect prognosis and treatment response. KRAS mutation testing is now widely used to guide anti-EGFR monoclonal antibodies, cetuximab, and panitumumab, as personalized medicine to customize the treatment based on patient's specific characteristics [88]. Anti-EGFR treatment, on the other hand, does not function for all KRAS wild type individuals. Hence, new indicators are needed to predict response to existing and experimental treatments.

10.3.4 Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC), the sixth most prevalent tumour, is the fourth foremost reason of cancer death globally, with an increasing frequency [89]. In most of the cases, HCC is linked to a chronic liver illness. Its prevalence is rising as hepatitis C virus infection, chronic hepatitis B virus infection, obesity, non-alcoholic fatty liver disease, and metabolic syndrome are becoming more common [90]. Due to the lack of obvious symptoms of early HCC, a large percentage of cases are detected at an advanced phase, removing the prospect of curative treatment approaches. As a result, the treatment of advanced HCC via systematic route is a huge concern, and its critical role and potential have sparked a lot of studies in the last ten years [4]. Correct treatment choice and specific treatment modalities directed by personalized medicine provides the best possible clinical outcomes for every HCC patient [91]. Various conventional chemotherapy regimens have been attempted in multiple trials in the past, with disappointing results in the treatment of HCC due to drug resistance and toxicity [92].

10.3.4.1 Targeted Therapy in HCC

Sorafenib is an oral small molecule that inhibits VEGFR and platelet derived growth factor receptor (PDGFR), as well as tumour cell development, via blocking signalling pathways [93, 94]. This is the first molecular targeted drug to show a benefit in nonresectable HCC patients in terms of survival. Both the trials, SHARP [95] and the ORIENTAL [96] found that sorafenib has improved HCC patients survival. In 2010, the FDA authorized sorafenib for advanced HCC based on the findings of these trials [97]. Despite the fact that numerous medicines were produced during 2007 and 2016, the majority of which were flopped in the clinical trials, and in clinical practice, exceptional molecular medications have turned out to be the first and second-line systemic therapy for advanced HCC [4].

Lenvatinib is an oral small molecule multikinase inhibitor that suppresses tumour angiogenesis and growth by inhibiting tyrosine [98]. The REFLECT trial assessed the effectiveness of lenvatinib plus sorafenib for first-line treatment showed that lenvatinib provided a greater overall survival benefit than sorafenib [99]. Lenvatinib was licenced by the FDA for the advanced HCC in 2018 and 2019, the NCCN guidelines proposed lenvatinib for advanced HCC management as the second-line therapy [100]. The safety profile of regorafenib (a small molecule multitarget inhibitor) was evaluated by Bruix et al. as a second-line systematic therapy for HCC. Interestingly, the FDA has approved ramucirumab, a recombinant IgG1 monoclonal antibody and VEGF receptor-2 antagonist, for the management of NSCLC, gastric and colorectal cancer [101, 102].

Cancer immunotherapy aims to increase immune cell activity in order to attack cancer cells. In HCC, several inhibitory checkpoints including PD-L1 and human cytotoxic T-lymphocyte antigen 4 (CTLA-4) have been intensively examined and linked to a faulty immunological mechanism. CTLA-4, expressed on regulatory T cells, inhibits the activation and multiplication of T lymphocytes that identify tumour antigens in anti-cancer immunity [103]. The FDA-approved nivolumab,

a PD-1 inhibitor, for advanced HCC with sorafenib resistance in 2017. Peptide-based vaccination is another type of immunotherapy. GPC3 belongs to the glypican family of heparin sulphate proteoglycans, which are glycosylphosphatidylinositol-anchored to the cell membrane's outer surface [104]. Because GPC3 is overexpressed in 72–81% of HCC patients, it is a prospective target for antigen-specific immunotherapy. Sorafenib is the first-line therapy for HCC patients, and regorafenib is the second-line therapy; nevertheless, HCC is commonly resistant to chemotherapy due to genetic instability, necessitating the adoption of additional management strategies.

10.3.4.2 Drug Resistance

Several trials examining the efficacy of various medications have yielded disappointing results, indicating that HCC is resistant to chemotherapy and is mostly caused by MDR. In HCC cells, several scientists discovered that the respective MDR pathways such as EMT, Hif1 signalling, and many more. Other researchers investigated the mechanisms of sorafenib resistance that are tightly connected to intracellular calcium [105] and fibroblast growth factor 19 (FGF19) [106]. In the treatment of HCC, FGF19 is required for sorafenib efficacy and resistance [107].

10.3.4.3 Personalized Medicine in HCC

Most HCC cases are discovered when the disease has progressed beyond the point where curative treatment is available. Suitable patient selection and specialized treatment strategies directed by personalized medicine should be used to provide the best potential treatment outcome for each HCC patient [108]. Various conventional chemotherapy regimens have been tried in the past with varied results in the treatment of HCC in multiple trials [109].

10.3.5 Leukaemia

With an estimated 309,006 deaths in 2018, leukaemia is the ninth furthermost cause of cancer death worldwide [110]. Leukaemia is a heterogeneous disease with several distinct subgroups. Arrays of factors lead to somatic mutations in hematopoietic pluripotent stem cells and progenitor cells resulting in development of leukaemia. Leukaemia comprises of different types, but the four major kinds include acute myeloid leukaemia (AML), chronic lymphocytic leukaemia (CLL), chronic myeloid leukaemia (CML), and acute lymphocytic leukaemia (ALL). The prognosis of disease and treatment outcomes in the majority of leukaemia patients remains poor.

10.3.5.1 Targeted Therapies in Leukaemia

Fms-like tyrosine kinase 3 (FLT3) is a receptor tyrosine kinase and plays a role in the development of both myeloid and lymphoid lineage, at an early stage. Internal tandem duplications (FLT3-ITD) (25%) and or missense mutations in the tyrosine kinase domain (FLT3-TKD) (7–10%) are two common mutations found in

nearly 30–35% of newly diagnosed AML. Some of the targeted agents are pan inhibitors of FLT3, while others are multi-targeted tyrosine kinase inhibitors. A multicentre clinical trial RATIFY evaluated midostaurin induction and consolidation in patients not having allogeneic transplant. After maintenance therapy of one year, in midostaurin group survival (4 years) was 51.4% versus placebo 44.2% in patients with either ITD or TKD FLT3 mutations [111–113]. After the completion of RATIFY trial, which took 13 years, midostaurin was approved by US FDA.

In a phase III trial, a single-agent gilteritinib (tyrosine kinase inhibitor) treated 34 patients. In relapsed/refractory (R/R) FLT3-mutated patients, the gilteritinib also improved the survival and enhances percentage of complete remission compared to conventional therapy. To estimate the efficacy of gilteritinib in combination with chemotherapy, many trials are in progress [113, 114]. Gilteritinib was approved by the FDA in 2018, for AML patients suffering from R/R FLT3 mutations, as the new standard therapy [115].

Crenolanib is a potent pan-FLT3 inhibitor (Type I) and is used as targeted therapy against both ITD and TKD mutations. Several studies report the transient responses and relapse using crenolanib monotherapy in greatly pre-treated R/R AML patients [116]. Quizartinib is a potent inhibitor (Type II) and was used as an effective therapy for patients with FLT3-ITD AML through inhibition of FLT3. Quizartinib reduces the oncogenic drive and thus inducing apoptosis. Efficacy is documented when used as monotherapy for maintenance and combination with induction chemotherapy [117, 118]. Sorafenib is an inhibitor of multiple kinases and studies demonstrate its efficacy as monotherapy in $FLT3^+$ AML. Sorafenib and sunitinib have similar therapeutic effects in AML [119]. However, resistance is reported if used as single agent [120].

Imatinib was the first tyrosine kinase inhibitor to receive FDA approval as a first-line treatment for CML. In IRIS clinical trial, which included almost 1106 CML patients they reported overall survival was 86.4%, and progression-free survival was 81.2% with imatinib [121]. Ruxolitinib regulates gene transcription and inhibits JAK-STAT cell signalling cascade. Ruxolitinib with nilotinib combination typically inhibits the proliferation of leukaemia cells, particularly in Ph + ALL [122]. Pacritinib, on the other hand, inhibits JAK2/FLT3, and phase I and II studies show its efficacy in advanced myeloid malignancies [123].

Multiple monoclonal antibodies that have been developed for the treatment of various leukaemia include alemtuzumab, blinatumomab, gemtuzumab ozogamicin, inotuzumab ozogamicin, obinutuzumab, ofatumumab, and tisagenlecleuce, which target specific antigens. Overall, the personalized targeted therapies are replacing toxic chemotherapy in leukaemia. The precise targeted therapy has the potential to increase not just the disease's cure rate but also the patients' quality of life.

10.3.5.2 Drug Resistance

Conventional chemotherapy in most of the leukaemia cases leads to resistance that hinders the conventional therapy and leads to relapse of the disease and ultimately patient death [124]. Several factors and mechanisms may account for resistance

to leukaemia therapy. The resistance results from failure of the cells to undergo apoptosis and drug failure to reach the target.

10.3.5.3 Personalized Medicine in Leukaemia

Molecular characterization has provided deep understanding and information for diagnosis, disease types, and therapeutic strategies for leukaemia. In recent years, targeted drugs and targeted immunotherapies have improved the outcomes of the patients. In-depth identification and characterization of different types of leukaemia, precise clinical diagnosis, gene mutation-targeted new drug therapies, and stratification of risk factors have made the breakthrough and progress in treatment of different types of leukaemia. The personalized targeted therapies used for treatment of leukaemia include inhibition of tyrosine kinase, inhibition of histone deacetylase, inhibition of hypermethylation, and inhibition of proteasome.

10.4 Advancements in Targeted Therapies and Personalized Medicine

Personalized medicine has changed the way cancer is treated, replacing the "one size fits all" approach with the development of individualized medicines for each subtype of cancer, established on the measurement and modification of critical patient genetic and omics data (transcriptomic, metabolomics, and proteomics) [125], identifications of key post-translational modifications, and analysis of temporal expression of a precise protein [126]. Personalized medicine improved cancer diagnostics by using molecular assays to analyse quantities of proteins, genes, or specific mutations to reveal a specific, effective prescription for an individual's disease [127]. These CDx enable everyone to receive a more effective treatment depending on the distinct characteristics of their tumours. The FDA has backed the personalized medicine strategy by authorizing this and other technologies since 1998, when trastuzumab was licenced for the treatment of breast cancer (HER2). Furthermore, the Precision Medicine Initiative, which was established in 2015 and mandates the FDA to create new platforms to examine personalized medicine diagnostics and medicines, has propelled this industry forward [128]. Immunotherapy is among the advanced remedies that hitches a patient immune system and piloted the concept of personalized medicine to combat cancer. The treatments include checkpoint inhibitors, monoclonal antibodies (mAbs) and vaccines, and hematopoietic stem cell transplants (HSCTs), cytokines, and chimeric antigen receptor (CAR) T-cell usages [129]. The genetic mutations in ALK and BRCA led to the development of FDA-approved drugs. For instance, crizotinib and ceritinib [130] for patients who test positive of genetic mutations. Sorafenib is another PPM drug used for HCC patients. The development of checkpoint regulators has helped in a new age of cancer immunotherapy.

10.5 Challenges for Oncology Targeted Approaches and Personalized Therapy

Personalized cancer medicines are becoming more widely available, yet they still face substantial obstacles in medical oncology. These challenges include lacking evidence to guide practical biomarker implementation, knowledge of the functional implications of the variant alleles, and consensus on the level of evidence required to choose patients for customized medicines [131]. The number of treatment methods for which good predictive biomarkers exist is limited and selected genetic alterations can only be seen in a small number of cancer types, restraining the use of biomarker-targeted medicines in ordinary clinical practice. There is a paucity of robust evidence that genetic variations within a disease have an effect on tumour behaviour [132, 133] and a lack of agreement on what constitutes the lowest amount of evidence required to qualify a variant allele as a viable biomarker, as well as insufficient data to determine the functional value of variant alleles, all of which obstruct the development of targeted medicines [133]. Another key difficulty in personalized medicine is establishing the relationship between biological data, disease, and clinical translation in order to make meaningful medical decisions. The use of a big data method to reconcile omics components allows for the creation of predictive models of human physiology that may be employed in experimental design and clinical trial development [134–136]. As a result of the growth of modern molecular biology and the deployment of several new technologies, small molecule targeted anti-cancer therapies have entered a rapid development stage. Despite tremendous advancements, anti-cancer medicines based on small molecules confront a number of difficulties. Drug resistance is the first major issue to be tackled. Almost all targeted anti-cancer medicines acquire resistance over time in clinic. Drug resistance has been linked to gene mutation, amplification, efflux transportation, apoptotic dysregulation, and autophagy, to name a few mechanisms [27, 137]. Gene mutation is the key cause of anti-cancer medication resistance in general. Tumour can be kept receptive to treatment for prolonged periods of time through the sequential use of medications and/or careful regulation of their combinations. The requirement to standardize tests, for laboratory techniques and their assessment for positive, is a major difficulty when doing personalized medicine. Similarly, stringent tests and thresholds for clinically significant positivity in other approaches, such as molecular biology, must be developed. It is important to perform numerous methodological quality assurance processes before implementing biomarkers in clinical settings. Another important issue with focused anti-cancer medications is their inefficiency. Targeted anti-cancer medications are only existing in a small proportion of individuals, e.g. EGFR inhibitors like gefitinib and erlotinib are only effective in about 20% of NSCLC patients [138]. Because of cancer's complexity and interpatient variability, it is evident that incorporating a PPM approach into cancer research and therapy might lead to significant gains. PPM research and products with FDA support have all begun to acknowledge this; nonetheless, there are several broader, social problems that must be

addressed and overcome before PPM can be fully integrated into standardized therapy [29].

10.6 Conclusion and Future Perceptive

Because personalized medicine focuses on three topics: detecting disease indices in people, selecting the optimal therapeutic strategy, and predicting disease relapse, it appears that additional study is needed in the area of cancer. Even though several mutations and signalling pathways linked to the disease have been discovered, the importance of customized medicine studies in this condition is undeniable. Because present classifications have various flaws, the overall findings of these studies could aid in the efficient classification of cancer patients. Considering the wide range of responses to cancer treatments, more research into patient classification is required. Drug resistance in these patients shows that the molecular information currently available about this disease process is insufficient to comprehend its intricacies. Extensive research on customized medicine in the treatment of lung and breast cancer has resulted in a particular classification of the disease, and treatments now are more targeted and efficient. As a result, the risk of death and drug resistance during treatment has been reduced to a bare minimum. Current molecularly stratified umbrella clinical trials allow panel for next-generation sequencing analysis and bio-guided treatment recommendations. The most important component in treating cancer patients with any chemotherapy or targeted medicine is to focus on early diagnosis and the development of research on early diagnosis biomarkers. The incorporation of multiple populations has lately become necessary as a result of pharmacogenomic and pharmacogenetic discoveries. All of this necessitates the development of molecular methods, as well as their accessibility and affordability. On the other hand, using only cellular approaches and focusing on heterozygous tumour cells in the presence of multiple therapy regimens based on sophisticated molecular technologies, it is feasible to locate the effective medicine and the suitable dosage for the patient's tumour. In a wide range of areas related to cancer detection, diagnosis, treatment, and survivorship; traditional endpoints, such as survival and toxicity outcomes, will continue to be gathered, while novel endpoints that allow quick assessment of these methodologies will be required for validation. This is one of the most exciting times in oncology's basic science and translational research, with the potential to speed up the final search for a cancer cure.

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Smart Nanocarrier-Based Cancer Therapeutics

11

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Abbreviations

ADME	Absorption, Distribution, Metabolism, and Excretion
Ag	Silver
Au	Gold
BBB	Blood–Brain Barrier
CDER	Center for Drug Evaluation and Research
CNTs	Carbon Nanotubes
CT	Computed Tomography
DOX	Doxorubicin
ECM	Extracellular Matrix
EGFR	Epidermal Growth Factor Receptor
EMEA	European Medicine Agency
EPR	Enhanced Permeability and Retention Effect
FDA	Food and Drug Administration
Fe	Iron
GLOBOCAN	Global Cancer Incidence, Mortality and Prevalence
GNPs	Gold Nanoparticles
HER-2	Human Epidermal Receptor 2
IP	Intellectual Property
MDR	Multidrug Resistance

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MMP	Matrix Metalloproteinase
MRI	Magnetic Resonance Imaging
MSNs	Mesoporous Silica Nanoparticles
NCE	New Chemical Entity
NDA	New Drug Application
nm	Nanometers
NPs	Nanoparticles
OX	Oxaliplatin
PAMAMs	Polyamidoamine Dendrimers
PEG	Poly (Ethylene Glycol)
PTX	Paclitaxel
RES'	Reticuloendothelial System
ROS	Reactive Oxygen Species
SAR	Structure–Activity Relationship
siRNA	Small-Interfering Ribonucleic Acid
TME	Tumor Microenvironment
VEGF	Vascular Endothelial Growth Factor
WOR	Wortmannin

11.1 Introduction

11.1.1 Cancer, Challenges in Diagnosis and Therapeutics

According to GLOBOCAN 2020, cancer mortality and incidence are rising worldwide, with 19.3 million new diagnoses and around 10.0 million cancer-related deaths [1]. By 2030, it is estimated that over 30 million people will die from cancer each year [2]. Cancer is defined by cellular transformation that spreads from a primary focal point to various parts of the body, eventually killing the patient. Early detection is critical for successful cancer therapy in the fight against cancer which dramatically reduces cancer-related mortality [3]. Morphological study of cells (cytology) or tissues (histopathology) and imaging techniques are currently used for early cancer detection. The most commonly used imaging procedures, such as X-ray, computed tomography (CT), magnetic resonance imaging (MRI), ultrasound, and endoscopy, are able to only identify cancer when there is a visible alteration in the tissue [4, 5]. Millions of cancerous cells might well have proliferated and possibly spread to other tissues by that time. Furthermore, existing imaging technologies are unable to discriminate between benign and malignant tumors [6]. Complex genetics and diversity in phenotypic levels are the major causes of clinical variation and drug resistance. For the treatment of cancer, a range of techniques are used, each with its own set of restrictions and adverse effects [7]. Surgical removal, chemotherapy, radiation, and hormone therapy are all

options for cancer treatment. Chemotherapy is a traditional and widely used treatment that involves administering anticancer medications to patients via a systemic route in order to stop malignant cells from proliferating uncontrollably [8].

Nevertheless, off-target effect of anticancer agents causes adverse effects including hair loss, bone marrow suppression, and gastrointestinal problems as well as inadequate delivery of drug cannot bring about the desired outcome in the majority of cases. As a result, a substantial percentage of cancer-related research in recent decades has focused on producing treatments which more precisely target cancerous cells rather than healthy cells. Despite the fact that the considerable progress has been reported by targeted therapy in the field of precision medicine, still there are numerous inevitable side effects, and drug resistance has been a major concern [9].

Separating malignant cells from healthy cells is the most difficult component of cancer treatment. As a result, the main goal is to create a drug that can recognize cancer cells and stop them from growing and multiplying. Chemotherapy is unable to target cancer cells selectively without causing harm to healthy cells in the body. As a result, they have serious side effects such as organ failure, which leads to poor treatment with lower doses and, ultimately, poor survival rates [10].

11.1.2 Rational for Nanotechnology in Cancer Therapeutics

Nanotechnology deals with particles ranging in size from a few nanometers (nm) to several hundred nm, depending on the application, which is one of the most extensively employed technologies in cancer research today [11]. It has gained interest in the last decade for its potential applications in cancer diagnosis and treatment, including detection/diagnosis, molecular imaging, drug/gene delivery [12], drug carriage, targeted therapy, and biomarker mapping, as it provides several advantages over traditional approaches [13, 14].

Nanotechnology has been utilized to generate nanomaterials [15], like gold-based nanoparticles (NPs), magnetic NPs, and quantum dots, employed for molecular cancer diagnostics. Cancers can be detected reliably and promptly using molecular diagnostics based on nanotechnology, such as the development of biomarkers [16]. Treatments based on nanotechnology, like the designing of nanoscale drug delivery system, can assure accurate malignant tissue targeting with minimum adverse effects [17, 18]. Nanomaterials can easily bypass cell barriers due to their biological nature and have been employed in the treatment of various tumors for many years due to their targeting capabilities [19].

In cancer treatment, NPs-based drug delivery systems achieved several advantages like precise targeting, better pharmacokinetics, decreased adverse effects, and drug resistance [20, 21]. The size and properties of NPs utilized in drug delivery systems are generally fabricated by considering the pathophysiology of tumor. After being absorbed, nanocarriers target cancerous cells mechanically via the positioning effect of the targeted chemical and the carrier action of NPs. Subsequently, these NPs discharge the drug to cancer cells so as to kill them.

Traditional chemotherapeutic agents, as well as some poorly soluble drugs, can be encapsulated into nanocarriers and successfully transported into the blood [22]. In the meantime, the targeting system shields healthy cells from the cytotoxic effect of drug, reducing the side effects of cancer treatment. In comparison to free doxorubicin, a study demonstrated that the doxorubicin-loaded PEGylated liposomes showed decreased cardiotoxicity [23]. Furthermore, when compared to solvent-based taxanes, NPs' albumin-bound paclitaxel had fewer adverse effects with greater tolerable doses [24]. Nanomaterials can extend drug half-life and increase drug accumulation in tumor tissues, due to their size and surface features, as well as their role of boosting permeability and retention [25]. According to several studies, NPs have been used in cancer immunotherapy, chemotherapy, ablation treatment, and gene therapy [26, 27]. The NPs-based delivery technology is thought to improve immunotherapy and counteract the immunosuppressive milieu found in tumors [28]. Drug delivery systems based on hybrid NPs have gained much attention as they integrate the features of many NPs to improve the stability and functionality of drug delivery system [29]. Furthermore, in anti-tumor multidrug resistance (MDR), NPs have demonstrated some advantages, since they give platforms for drug combination therapy and hinder the operation of specific drug resistance mechanisms [30].

11.2 Active and Passive Targetings of Solid Tumors With a Focus on Potential Nanotechnology Applications

Targeting cancer cells is an important aspect of nanocarriers for medication delivery since it boosts therapeutic potential while protecting normal cells. A variety of research has been conducted to investigate the targeted design of NPs-based medicines. To properly address the challenges of tumor targeting and nanocarrier system design, one must first understand tumor biology and the interactions between nanocarriers and tumor cells. Passive and active targetings are the two most frequent forms of targeting systems.

11.2.1 Passive Targeting

The drugs are efficiently transported to the desired region to perform a therapeutic effect, and passive targeting is based on the different features of tumor and normal tissue. High cancer cell propagation causes neovascularization which causes tumor vessels to have worse permselectivity than normal vessels [31]. Macromolecules, including NPs, enter into the blood stream through leaky vasculature and accumulate within tumor tissue due to fast and defective angiogenesis. In the meantime, cancer patients' poor lymphatic drainage causes NPs to be retained, enabling the nanocarriers to liberate payload to the target tumor tissue. Each of these processes improves the enhanced permeability and retention effect (EPR), which is one of the passive targeting's key driving forces [32].

The EPR effect is influenced by the size of the NPs, with studies showing that smaller NPs have superior penetrability but do not leak into normal capillaries [33]. Larger particles, in contrast, have a higher chance of being removed by the immune system [34]. The tumor microenvironment, besides EPR effect, is a crucial element in the passive distribution of nanomedicines. The main metabolic feature of cancerous cells such as glycolysis is the primary source of energy for their growth which lowers the pH of the tumor microenvironment by yielding acidic environment [35]. Larger particles, on the other hand, have a higher chance of being removed by the immune system [36]. However, passive targeting has a number of limitations, including non-specific drug distribution, the EPR effect's non-universal frequency, and varied blood vascular permeability across tumors [37].

11.2.2 Active Targeting

By interacting directly with ligands and receptors, active targeting specifically targets cancerous cells. The ligands on the surface of NPs are chosen to target molecules that are elevated on the surface of cancer cells, allowing them to differentiate between cancerous and healthy cells [38]. Receptor-mediated endocytosis occurs when the ligand on NPs interacts to receptors on the surface of cancer cells, allowing NPs to be internalized and release therapeutic payloads [39]. As a result, active targeting has been customized specifically for macromolecular medication delivery, such as siRNAs and proteins. Amino acids, peptides, antibodies, vitamins, and carbohydrates are examples of targeting moieties [40]. The transferrin receptor, folate receptor, glycoproteins, and the epidermal growth factor receptor (EGFR) are among the best researched of these ligands, which bind to receptors on targeted cells.

11.2.2.1 Targeting to Cancer Cells

Transferrin receptors, responsible for transport of iron into the cells, are highly expressed in most solid tumor cells compared to healthy cells. As a result, transferrin-conjugated NPs are being employed to deliver anticancer medicines as an active targeting approach [41, 42]. Transferrin-modified NPs exhibited higher cellular absorption efficiency and enhanced intracellular drug delivery compared to unmodified NPs [43]. Furthermore, research suggests that transferrin-conjugated polymeric NPs aid in the treatment of drug-resistant cancers [44].

Folic acid, which is necessary for nucleotide synthesis and absorbed by a folate receptor located on just a few types of normal cells, is essential for nucleotide synthesis. The alpha isoform of folate receptor (FR-a) is found on the surface of hematological cancers and is highly expressed in roughly 40% of human malignancies [45]. As a result, targeting folate receptors using folate-conjugated nanomaterials has become a popular cancer treatment option [46].

Additionally, cancer cells produce a variety of glycoproteins, especially lectins, which are non-immunological proteins that identify and adhere to specific carbohydrates. The direct lectin targeting approach involves utilizing lectins coupled to

NPs to target cancer cell surface carbohydrates, while the reverse lectin targeting pathway involves using carbohydrates moieties integrated into NPs to target lectins on cancer cells [47].

The ErbB family of tyrosine kinase receptors includes the EGFR, which is highly expressed in a variety of malignancies, is involved in a number of tumor growth and progression processes, and has previously been used as a cancer therapeutic target [48]. In the human epidermal receptor 2 (HER-2)-positive breast and stomach cancer, targeting the HER-2 is a frequent treatment. Hence, NPs that have been developed to integrate modified EGFR ligand to target EGFR-upregulated cancer cells are a viable delivery strategy. Another method of active targeting is to combine two cancer-specific ligands into a single NP, which can assist in improving target specificity [49].

11.2.2.2 Targeting to Endothelium

Rather than directly targeting cancer cells, certain NPs have an effect on angiogenesis, which is a different approach to cancer treatment. The association between vascular endothelial growth factor (VEGF) and VEGF receptors (VEGFRs) is crucial in vascularization [50]. Additionally, liposomes that target two important VEGF receptors, VEGFR-2 and VEGFR-3, have been shown to improve therapeutic efficacy [51]. Integrins are extracellular matrix protein receptors that play an important role in tumor cell motility and penetration. Overexpression of vascular cell adhesion molecule-1 (VCAM-1) has been seen in a variety of malignancies [52] indicating its potential role as a therapeutic target. In a breast cancer model, Pan et al. found that VCAM-1-targeted NPs were highly effective [53]. Furthermore, matrix metalloproteinase (MMP) is involved in extracellular matrix remodeling and tumor neovascularization. MMP-sensitive NPs have been linked to anticancer activity in a variety of malignancies, including breast cancer, pancreatic cancer, and melanoma [54].

11.3 Multifunctional Nanomaterials for Cancer Therapy—Novel Concepts

In cancer study and research, mostly colloidal NPs are used which usually possess three structural domains, a core, coating, and targeting moiety. Core consists of either organic such as polymeric or inorganic like metals surrounded by coating which provides protection against chemical or physical damage in the diverse biological environment like proteins that can interfere with its functions [55]. Moreover, the coating of NPs also minimizes toxic effects toward cells by providing an inert biophysical interface [56]. There are different surface functionalization agents, such as various polymers which are flexible and electrostatically neutral, which can easily bind to the surface of NPs to provide long-term chemical stability by hindering non-specific protein binding [57]. The targeting NPs can be designed by conjugating some targeting molecule which is usually an antibody or ligand for a cell surface receptor that can bind to a specific molecular or cellular target [58].

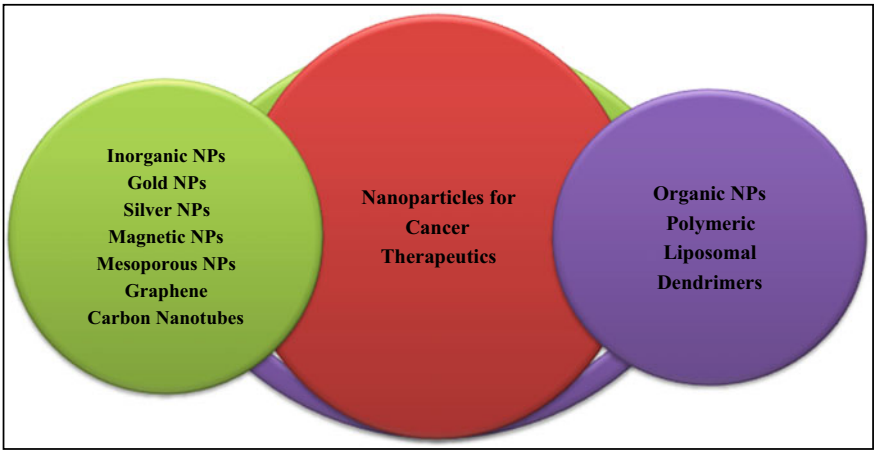

Fig. 11.1 Different types of nanoparticles to be used for cancer therapeutics

Below, we briefly describe the unique emergent properties and smartness of four key classes of core materials used in cancer applications as described in Fig. 11.1. We also discuss the clinical applications such as imaging, sensing, and therapeutics of each material as well as a very brief description of their sub-classes depending on size, morphology, and chemical properties [59]. The multiple components of NPs can be fused together to achieve multifunctionality [60].

11.3.1 Organic Nanoparticles

Preference for natural products in biomaterial research is increasing due to their increased biocompatibility. Organic NPs are fabricated with natural or synthetic organic molecules with limitation to control size below certain values [61]. Organic NPs such as micelles or vesicles have a dynamic character and can change size and shape with time through self-assembly or fusion among them [62]. The use of non-covalent (weak) interactions for molecular self-assembly and design aids in the transformation of organic NPs into desirable structures such dendrimers, micelles, liposomes, and polymers. Organic NPs with intriguing features can load molecules either by conjugation on the surface or in the core or by physical encapsulation, which makes them appealing systems for the delivery of molecules and more specifically for drug delivery and biomedical applications [61].

11.3.1.1 Polymer

In cancer therapeutics, most commonly used organic nanomaterials are composed of polymers or amphiphilic molecules because they can self-assemble as a larger and more stable particles in aqueous solution due to hydrophobic molecular domains [63]. The Food and Drug Administration (FDA) of the USA approved

synthetic and natural polymers have already reached the medical and pharmaceutical markets [64]. Few biocompatible polymers such as poly(lactic-co-glycolic acid) (PLGA), poly (ethylene glycol) (PEG), polycaprolactone (PCL), poly(lactic acid) (PLA), and chitosan deserve special note among the many biocompatible polymers utilized for cancer therapeutics. With the rise of polymer conjugate systems, the diversity of such polymer-based systems has reached unprecedented heights. The PEG, a hydrophilic polymer, is the most often utilized polymer in many therapeutic systems because it allows for longer circulation duration and prevents enzymatic destruction of encapsulated agents by the reticuloendothelial system (RES) in the biological system [65].

11.3.1.2 Liposomes

Liposomes are naturally occurring phospholipid-based amphipathic nanocarriers that have been most extensively used for delivering anticancer agents as they have clinically proven ability to enhance treatment efficacy by encapsulating lipophilic and hydrophilic drugs while reducing complications associated with off-target toxicity [66]. The major advantage of liposomes is that its composition depends on components of the human physiological membrane, which facilitated its approval by the FDA [67]. Liposomal drug nano-formulations such as liposome-encapsulated doxorubicin are clinically approved for cancer chemotherapy. Clinical trials on liposomes have shown that they are pharmacologically and pharmacokinetically more efficient than drug-alone formulations in treating various cancers [68]. Different moieties can be grafted on liposome such as peptides, proteins, carbohydrates, glycoproteins, vitamins, monoclonal antibodies, antibody fragments, and radioligand. Liposome can be used simultaneously as therapeutic and imaging agent for theranostics, for co-delivery of drugs or genes [67].

11.3.1.3 Dendrimers

Dendrimers are monodispersed, bifurcated three-dimensional nanocarriers comprising a branched network of chemical bonds around an inner core. Due to their globular shape, their surface can easily be functionalized with various moieties, and this characteristic feature makes them ideal candidate as therapeutic carrier [69]. Dendrimers functionalized with targeting agent, fluorophore, and anticancer drug have been fabricated for synchronized targeting, imaging, and drug delivery in cancer cells. Covalently linked imaging and therapeutic substances can be enclosed within the dendrimer's core or covalently connected with dendrimer's many surface locations and the functionally active groups that facilitate solubilization of hydrophobic anticancer drugs. Polyamidoamine dendrimers (PAMAMs) are the most popular dendrimers because it has terminal amine functional groups for conjugation of a divers surface moieties; moreover, it is non-immunogenic and water-soluble and, therefore, can been used to encapsulate drugs [70]. Rapid clearances and lower cellular uptake are the limitations associated with conventional dendrimers.

11.3.2 Inorganic Nanoparticles

Inorganic NPs including those derived from metals (silver, gold) or oxides (iron oxide) and carbon-based (graphene, nanotubes) have been deeply explored recently for diagnostic and therapeutic purposes in oncology [71]. Because of their structural features, inorganic NPs are an effective mechanism for targeting and penetrating cancer-related aberrant cell proliferation. They have the ability to infiltrate aberrant cells, cause DNA damage, and determine gene abnormalities. They help in drug distribution, imaging of aberrant cells, and the release and monitoring of cancer treatment agents 68, in addition to targeting cancer cells. Inorganic NPs have various advantages and unique qualities for improved imaging and drug delivery as compared to organic NPs [72].

11.3.2.1 Metallic NPs

Metals transmit electricity because of the free mobility of electrons, but their optical properties change when they shrink from macroscopic to nanoscale, which are lesser than the typical distance that an electron travels unobstructed [73]. In this lower size range, the electrons which absorb energy from the light would communally vibrate with frequency in the visible spectrum on the surface of NPs, known as surface plasmon. Depending on the shape, size, and composition of the NPs, this effect enhances the absorption and scattering intensity of light with frequency tunable over a large spectrum of colors. These unique optical properties enable a high sensitivity of sensing, changes in the local surroundings, and the capacity to produce hyperthermia because absorbed light is turned to heat [74]. Clinical trials have been conducted as a result of this hyperthermic action to use as photothermal ablation agents for solid tumors [75]. Gold (Au) and silver (Ag) are the most often utilized metal particles (Ag). Metallic NPs are being extensively utilized in the basic research for electronics and nanosensors, and they have also been studied as injectable medicines and imaging moieties, but they have not so far been licensed to be use for human [61].

11.3.2.2 Magnetic NPs

Many tiny domains make up magnetic materials, each with its own coherent magnetic moment. When magnetic particles like iron (Fe) and ferrite iron oxide (Fe_3O_4) are shrunk to a size of the single magnetic domain, they exhibit distinct magnetic properties [76]. Although magnetic NPs only contain one magnetic moment, it has a surprisingly vast magnetic field. Magnetic NPs have been widely used to generate a local magnetic field for imaging such as MRI, to heat tissue with an oscillating magnetic field because of this unique characteristic, and to detect the presence of molecules and cells [77]. Iron-based magnetic NPs had previously been certified for clinical purposes and were sold under brand names such as Lumirem and Feridex due to a good safety profile, but after additional assessment, due to safety concern, these were removed from the American market [61].

11.3.2.3 Carbon Nanotubes

Nanotubes (CNTs), a group of carbon allotropes that comes in a variety of shapes such as hollow spheres, ellipsoids, tubes, and other shapes. And is a graphene sheet that has been rolled up into a seamless cylindrical tube [78]. Due to its appealing qualities like as high surface area, high drug loading capacity, and ease of modification, carbon-based nanomaterials have been widely researched in cancer imaging, delivery, and diagnostics [79]. CNTs and graphene have been the most well-studied carbon nanomaterials in cancer therapy applications. CNTs can be used as a drug carrier systems to target cancerous cells more effectively. Functionalized carbon nanotubes have been shown in recent investigations to be able to cross the BBB. It can be used for both thermal ablation of a cancer site and gene transfer. CNTs have showed potential in transporting small-interfering ribonucleic acid (siRNA) and plasmid DNA, and they can be employed as cancer diagnostic tools [80].

Cellular imaging, drug delivery, bio-sensing, and photothermal therapy are all applications for graphene and its derivatives. Cellular imaging and drug delivery in cancer treatment have been developed using multifunctional graphene smart nanomaterials [81]. For instance, tumor imaging and photothermal therapy were performed using nano-graphene oxide complexed with upconverting nanoparticles, demonstrating the multifunctional graphene's potential for clinical anticancer treatments. PEGylation is an essential step in increasing solubility, and when CT-26 murine colorectal cells were treated with the combination of chemotherapy and photothermal therapy showed to be helpful using magnetic graphene oxide modified with PEG and cetuximab [82].

11.4 Smartness of NPs as Drug Delivery Carrier for Cancer Therapeutics

Chemotherapy is an important part of cancer treatment since it employs chemicals to kill or stop cancer cells from growing. Because of the limitations of traditional chemotherapy, smart drug delivery systems based on nanocarriers have previously been created and show potential to deliver therapeutic agents to precise and targeted areas [83]. Moreover, this smart drug delivery system based posses various properties like high selectivity, stability, enhanced biocompatibility, and increased drug release at the target site (Fig. 11.2). Combining nanomaterials and chemotherapeutic drugs was proven to improve tumor control and lessen unwanted side effects by optimizing pharmacokinetics and targeted distribution of drug payloads in nanomaterials-based cancer therapy (Fig. 11.2) [84]. Combination therapy appears to be a regular practice in typical chemotherapy to overawed cross-resistance and provide a synergistically increased therapeutic effect while reducing toxicity. Monotherapy, in general, is constructed on the use of a single medicine, which is insufficient to cause tumor regression. As a result, combination therapy is a viable and alternate modality of treatment, as well as a potential way to improving cancer treatment efficacy. Multiple therapeutic substances with distinct physiochemical and pharmacological properties would be delivered in single

Fig. 11.2 Designing of nanodrug delivery system

NPs via NP-mediated combination treatment. Nanotechnology has gotten a lot of attention in the previous two decades and has helped with clinical therapies. The ability of NPs to transport hydrophobic and/or hydrophilic drug molecules, small molecule medicines, or peptides, to the site of tumor with minimal harm to adjacent tissues makes them favorable drug carriers; this safe delivery is predicated on their penetrating capacity [85]. Polymeric NPs and liposomes have been employed as nanoscale drug carriers to deliver a variety of medications more efficiently and safely. The principal self-assembled materials used for medication administration were lipids (such as micelles and liposomes) [86]. Liposome-mediated drug delivery is a step forward because of its effectiveness, non-immunogenicity, biocompatibility, enhanced solubility of chemotherapeutic drugs, and the ability to encapsulate a wide range of pharmaceuticals [87]. Liposomes coated by polyethylene glycol (PEG) are also useful for certain drug delivery activities due to surface modification by means of functional ligands and changes in charge and size Furthermore, liposomes have demonstrated enormous therapeutic promise as payload carriers for delivery to specific areas [88]. Lammers et al. verified that N-(2-hydroxypropyl) methacrylamide (HPMA)-based polymer drug conjugates carrying gemcitabine, DOX, and tyrosinamide may transport several chemotherapeutic drugs to tumors in vivo [89].

To improve localization selectivity, formulations with many functions and components have been developed, enabling for specific targeting of various tissue. Chen and colleagues loaded oxaliplatin (OX) into a liposome which was modified with glycyrrhetinic acid (GA) for hepatic-targeted biodistribution experiments and found that liposomes are quite good at targeting specific tissues and organs [90]. Liposomes could be utilized to change antibiotics, anticancerous medicines, and DNA in addition to increasing medication intracellular uptake. Another interesting technique for increasing treatment efficacy is the use of RNA liposomes [91]. Liposomes that have been functionalized become smarter as traditional liposomes have a number of flaws, including deficient drug loading, instability, rapid drug release, in addition shorter blood circulation periods [92]. Some of these challenges have been overcame, including liposomes that have been PEGylated have a longer blood circulation period. More interestingly, various biological molecules (such as carbohydrates, lipids, peptides, antibody body fragments, and many more) grafted onto a liposome in order to actively target the cancerous location [93].

Wang et al. proliferated the chemotherapy therapeutic index using the combination of paclitaxel (PTX) and a self-assembled nano-preparation containing PEG-PE micelles which caused targeted toxicity in cancerous cells only. The micelles were taken up by cells successfully, and the newly created dual-drug co-delivery technique allowed them to release pharmaceuticals into the cells at the same time [94]. Bae et al. used a PEG-poly(aspartate hydrazide) conjugation with wortmannin (WOR) and DOX in a unimodal micelle structure. As a result, micellar-mediated combination therapy may be useful as a safe and effective chemotherapeutic modality for the management of breast cancer [95]. The cytotoxic medication doxorubicin (DOX) has been attached to PEGylated polylysine dendrimers to promote the regulated and prolonged exposure of lung-resident malignancy to the cytotoxic agent. The findings demonstrate that PEGylated polylysine dendrimers have a lot of potential as inhalable chemotherapeutic nano-formulations, increasing lung tumor exposure to lethal drugs [96].

Mesoporous silica nanoparticles (MSNs) are a material that has piqued the interest of many researchers due to their simplicity of large-scale production, variable homogeneous pore size, and enormous surface area and pore volume. Micelles were taken up by cells successfully, and the newly created dual-drug co-delivery technique allowed them to release pharmaceuticals into the cells at the same time. Thus, MSNs have good drug encapsulation and delivery efficiency because of these features. Since the FDA declared silica-based materials to be safe, the significant efforts have been made to use MSNs to build nano-platforms for chemotherapy and drug delivery [97]. Hsiao and colleagues developed and built an MSN-based theranostic drug delivery system that can be used for both cancer imaging and therapeutic delivery [98].

Brown et al. produced an anticancer medicine using platinum, which linked the active component of oxaliplatin to an GNPs using PEG to overcome the negative effects induced by anticancer treatments [99]. Interestingly, capecitabine, cisplatin, and DOX conjugated to L-aspartate-stabilized GNPs had stronger cytotoxicity against hepatocellular carcinoma cells compared to free capecitabine, cisplatin,

or doxorubicin [100]. Widder et al. published the initial study using magnetic NPs as drug carriers in 1979. DOX was encapsulated in albumin magnetic NPs in this work. Following this publication, numerous researchers concentrated on utilizing the magnetic characteristics of MNPs to develop several targeted medication deliveries in addition to imaging systems [101]. Liu et al. created graphene oxide that had been improved with chitosan and then conjugated with hyaluronic acid and the anticancer medication SNX-2112, which was effective in suppressing and killing A549 cells while causing less harm in normal human bronchial epithelial cells [102]. All of these findings are inspiring and have the potential to transform the way cancer is treated and managed.

11.5 Tumor Microenvironment-Targeted Nanotherapy

Evidences give insight that cancer growth is not solely dependent on tumor cells themselves, but tumor microenvironment (TME) plays a pivotal role in growth of tumor cells, tumor proliferation, resistance development to therapeutic agents, invasion, and metastasis [103]. Thus, proper understanding of TME is crucial for designing the therapeutic interventions with maximum efficiency. The TME involves different cell types, like myofibroblasts, immune cells, fibroblasts, adipocytes, extracellular matrix (ECM), and blood and lymphatic vasculature. The complex microenvironment contributes resistant to many conventional therapies and poor overall survival. The traditional medication delivery mechanism fails to deliver chemotherapeutics in effective concentrations and is linked to serious side effects. Nanomedicine offers a technique to get around or take use of the TME's features. Passive diffusion with improved EPR has been discovered to enable tumor localization of nano-chemotherapeutics. Nanotherapeutics that target TME has proven to be a potential strategy for combating medication resistance. Various endogenous factors are taken into consideration for delivery of nanodrug composites to the TME. These include high interstitial fluid pressure, enzyme activity, acidosis, hypoxia, hyperthermia, redox potential, oxidative stress, and ATP. Additionally, pathophysiological conditions in TME, such as functional proteins levels, amino acids, DNA fragmentations and inflammatory cells, like mast cells, neutrophils, macrophages or lymphocytes [104].

Lipid-, polymeric-, carbon-, metal-, surfactant-, silica-, or metal oxide-based TME-targeted nano-chemotherapeutics are also possible. In preclinical models, stimuli-responsive nanocarriers, PEGylated nanocarriers, and multifunctional nanocarriers have proved thriving outcomes in tumor growth inhibition by targeting TME. Many acid-sensitive polymeric NPs are explored to targeting the acidic TME [105–107]. Min et al. designed and studied the effects of pH-sensitive micelles comprised camptothecin and methyl ether PEG-ploy(β-amino ester). They proposed that in acidic TME of MDA-MB231, the micelle disintegrated to release the chemotherapeutic in mice [108]. They proposed that tumoral acidic pH-responsive polymeric micelles are highly useful for cancer targeting therapy. Amphiphilic

polymer-based nanocarrier provides another approach and in acidic TME undergoes protonation-induced hydrophobic–hydrophilic switch which leads to release of therapeutic agent. The EPR effect has been employed as the foundation for delivering nanomedicines to tumor areas for passive targeting, and it is dependent on the degree of tumor angiogenesis and vascularization. Pathophysiology of vasculature has unique features which play role in induction of EPR effect [109, 110]. Higher tumor interstitial fluid pressure due to leaky vasculature serves as a barrier for drug uptake [111]. NPs targeting the vasculature have emerged as promising approach. Sengupta et al. used PLGA nanocarriers and attached DOX to inner core and encapsulated combretastatin outside lipid envelope. The delivery system enabled the efficient release of the two drugs to the site of action. Mechanistically, the combrestatin release from the outer envelope triggered the vascular shutdown, through disruption of cytoskeletal structures. The NPs remained trapped in the TME due to EPR effect and were efficiently taken up the tumor cells. Afterward, the DOX was efficiently released within the cells. The delivery system improved the therapeutic index with reduced toxicity [112]. Lodamin, a polymeric micelles-based delivery system, loaded with TNP-470 (angiogenesis inhibitor), was developed by Benny et al. The intervention decreased the tumor growth more efficiently as compared to inhibitor alone [113]. Moreover, ECM serves as another obstacle for the efficient delivery of chemotherapeutic agents. Studies and clinical trials showed that collagenase and hyaluronidase, which are ECM-degrading enzymes, can improve NPs' penetration into solid tumors. The PEGylated recombinant human hyaluronidase (PEGPH20) shown therapeutic effects in metastatic pancreatic cancer patients [114]. Unfortunately, side effects led to withdrawal of the treatment, but the therapeutic effects warrant additional studies. Moreover, studies investigated the enzymes and proteases, involved in remodeling of ECM, like LOX and MMPs, respectively. Interestingly, LOX-coated polymeric nanoformulation inhibited the growth of mammary tumor cell after selective binding to ECM [115]. Furthermore, to target fibronectin, one of the important components of ECM, paclitaxel-loaded PLA nanocarriers were designed and therapeutic effects were studied in mice. Results showed that NPs conjugated with drugs exhibited almost 70% greater survival than drug alone [116]. NPs may also offer continuous, precise drug delivery of cytokines. The TNFα-encapsulated liposomes were recommended for cytokine anticancer therapy after evaluating their in vitro cytotoxicity [117]. Doxil and liposomes containing TNFα, cytokines exhibited the synergistic effect and resulted in significant inhibition of tumor growth in rat [118].

Thus, targeting TME using NPs serves as the promising strategy in which multi-functionalized nanocarriers are used which ensure the efficient delivery of chemotherapeutics to the target site. However, many challenges, especially stability and toxicology, are highly required to make this successful form bend to bedside. Moreover, it is still debatable if the EPR effect is adequate to dramatically increase the survival of cancer patients using nanomedicine.

11.6 Nanotoxicology and Possible Solutions

The NPs have found in wide biomedical applications due to their physicochemical and behavioral uniqueness. However, the toxicities do exist and serious health effects might occur when body is exposed to these NPs without caution. Several factors contribute to extent of toxicity including size and nature of NPs, shape, surface area and property, crystallinity, surface coating, dispersion, and dissolution. Size is an important factor and studies have shown that smaller nanomaterials have higher toxicity in comparison to the bigger particles with similar compositions. Size-depended toxicity of silver nanoparticles was investigated by Chao et al. The study proposed that the smaller NPs exhibit acute toxicity in mice. They found that 10-nm silver NPs led to histopathological changes than 60-nm and 100-nm silver NPs. Similarly, other group investigated the toxic effects and sub-cellular localization of Au NPs of different sizes. The particles of size 10, 30, and 60 nm were administrated into the rat. The results showed that 10- and 30-nm NPs crossed the nuclear membrane-induced breaks in DNA. The 10- and 30-nm Au NPs were seemingly accumulated in kidney, liver, and intestine as compared to 60-nm particles. The greater accumulation of 60-nm particle was found in the spleen [119, 120]. Like size, the shape of NPs plays pivotal role in toxicity, for example, silver nanospheres were reported to be safer than silver nanoplate [121]. Less accumulation of autophagosome by gold nanorods was observed than gold nanospheres [122]. Similarly, star-shaped gold NPs were more toxic than rods and spheres [123]. In RAW 264.7 cells, the Fe_2O_3 NPs having rod shape produced higher cytotoxicity than sphere shape and initialized the inflammatory response and leakage of lactate dehydrogenase along with induction of reactive oxygen species (ROS) [124]. Moreover, aspect ratio also contributes in toxicity of NPs such as higher the aspect ratio, the greater the toxicity [125]. Li et al. reported that the mesoporous silica NPs' toxicity depends on aspect ratio. Reduction of aspect ratio led to increased systematic absorption through organs and reduced excretion through urine [126]. The type of crystalline structure is also the contributing factor in toxicity of nanomaterials [127]. Xu et al. performed experiments to assess the effects of surface coatings, using HeLa and A549 cell lines. The study revealed that iron oxide NPs coated with silica resulted in decreased iron homeostasis and oxidative stress [128]. Thus, the overall toxicity can be reduced by surface coating of NPs. Furthermore, two identical NPs having same size and composition may have totally different behaviors in dissolution, depending on different surface modifications [129]. Agglomeration of NPs also contributes to toxicity. The well-dispersed carbon nanotubes have been reported as having less toxicity than the agglomerated carbon nanotubes [130].

Mechanistically, most of the NPs elicit toxicity via direct cell surface interaction. The formation of ROS and the oxidative stress that results has been intensively studied as a nanotoxicity mechanism [131, 132]. The NPs mostly generate imbalance between the oxidants and free radicals' intermediates which may lead to drastic effects in terms of cytotoxicity. NPs-induced ROS can cause damage to genetic materials, which includes either breakage and/or cross-linking of

DNA strand, and mutations [133]. Different NPs are reported to induce the side effects via different mechanisms such as zinc oxide NPs which are documented to induce oxidative stress which leads to damage on a cellular level. Hou et al. reported that zinc oxide NPs induce the failure of chromosomal maintenance and DNA replication disorder leading to cell cycle arrest at G1, M, and G2 phase [134]. Silver NPs mediated alterations and genetic material and DNA damage at several tissues [135]. Ahamed et al. presented that in MCF-7 cells, Bi_2O_3 NPs cause dose-dependent apoptosis and cytotoxicity. Interestingly, supplementation of external antioxidants reverted the cytotoxic effects, suggesting that redox homeostasis is responsible for Bi_2O_3 NPs medicated cytotoxicity [136]. In addition to physiochemical toxicity and toxicity induced by ROS, the toxicity persuaded by NPs can be caused by various biochemical and molecular mechanisms [132]. For safer implementation and designing of the nanocarriers, in-depth understanding of the interactions of NPs with biological system is highly required. Based on the mode of nanotoxicity, the redesign approaches need to be chosen, but also considering the change should not impact the ability to perform in its intended application. Many strategies could be used to limit the toxic effects of NPs. As mentioned above, the variations in size and shape contribute to possible toxic effects so by changing shape and size, and especially, surface modification can lead to desired nanocarriers with less toxicity [137]. Negative surface charge can limit the interaction of NPs with the cell surface [131]. Use of ligand such as PEG also led to reduced interaction of NPs with the cell surface [131]. Moreover, the use of biological methods like green synthesis of NPs is becoming more popular due to less toxic effects. The use of fungi, plants, algae, and fungi led to formation of biocompatible products [138, 139]. Bioactive compounds from natural sources such as hydrocolloids, saccharides, and polyphenols found in plant extracts can be for improving the biocompatibility of metal NPs and reduction of toxicity [140].

11.7 Bench to Bedside: Translational Prospective

Extensive research on nanocarrier has revealed their potential in translational biomedical applications. Many nanocarrier-based drugs are available in market and many more are in the clinical development. Usually, such medicines include an already approved drug with a nanocarrier-based drug delivery platform such as liposomes, dendrimers, polymeric micelles, and inorganic nanoparticles [67]. In fact, liposomes are indisputably dominating in nanomedicine market and it is because liposomes-based system allows formulation of poorly soluble or highly toxic drugs such as amphotericin and paclitaxel [141]. The first FDA-approved NPs-based drug Doxil®/Caelyx® also contain liposomes. It is a PEGylated liposomal formulation of DOX with a long intravascular circulating half-life, reduced volume of distribution, and slow plasma clearance compared with free DOX. It has been approved by the FDA and European Medicine Agency (EMEA) as a medication for the treatment of AIDS-related Kaposi's sarcoma [142]. More recently, it is also approved for recurrent epithelial ovarian cancer. It has been

used in numerous studies for the treatment of breast cancer, soft tissue sarcoma, malignant gliomas, squamous cell cervical carcinoma, squamous head and neck cancers, and prostate cancers [143]. Ongoing clinical trials will shed more light on its role in the treatment of advanced solid malignancies. Another FDA-approved formulation Abraxane® is formed using NPs' albumin-bound technology that complexes human albumin with PTX without forming covalent bond. In 2005, it was initially approved for the treatment of metastatic breast cancer; later, it was approved for the local treatment of advanced or metastatic non-small cell lung cancer. Additionally, it was added to gemcitabine for the treatment of patients with metastatic adenocarcinoma of the pancreas. In this way, Cremophor EL was eliminated from the formulation which has shown several advantages, for example, eliminating the need of pre-medication, reducing the infusion volume and administration time (30–40 min), and avoiding the use of special infusion sets. Other nanocarrier-based formulations in which paclitaxel is used include Lipusu® (liposomes), Genexol®, Nanoxel®, and Paclical® (polymeric micelles) [144]. Every year, many more nanocarrier-based medicines are expected to advance to clinical setting (Table 11.1).

However, the clinical translation of nanocarrier-based drugs is a time-consuming and expensive process. The development of such drugs is way more complex than the development of conventional drugs. Biological problems, biocompatibility and safety, large-scale manufacturing, intellectual property (IP), overall cost-effectiveness in contrast to present medicines, and government regulations are all major concerns in the development of NPs-based medications. These factors are major barrier to the introduction of NPs-based drugs to the market, regardless of their therapeutic efficacy.

11.8 Preclinical Characterization of Engineered Nanoparticles Proposed for Cancer Therapeutics

Nanomaterials with altered biological and physicochemical properties allow for more accurate pharmaceutical targeting and administration, resulting in increased efficacy and specificity with fewer side effects. Despite the fact that the combinatorial possibilities of these multifunctional platforms have attracted a lot of attention and funding, their use in clinical trials is heavily reliant on rigorous preclinical characterization to meet regulatory requirements and well-understood structure–activity relationship (SARs). The characterizations including physicochemical, in vitro, and in vivo are three critical components of a rational characterization strategy for biomedical NPs (Fig. 11.3) [159]. Physicochemical characterization of different properties of NPs, like size, surface area, charge, shape, surface chemistry, and aggregation state, can help us comprehend SARs better [160].

Under controlled settings, in vitro experiments allow the identification and investigation of various mechanistic and biological pathways, whereas for nanomaterials, other in vitro assays, such as oxidative stress, will be similar to those used for traditional drugs, which will focus on mechanisms unique to NPs. Non-cellular

Table 11.1 Nanoparticles' formulations with positive results in the recent investigations

Type of nanoparticle	Anticancer agent	Target tissue/outcomes	References
Polymeric	Paclitaxel	Selective targeting of cancer cells	[145]
	Paclitaxel	Improved drug accumulation in tumor cells	[146]
	Cystatin	Inhibit tumor metastasis	[147]
Polymer micelle	Genexol-PM plus carboplatin	Well-tolerated toxicity in ovarian cancer	[148]
	Doxorubicin	Enhanced uptake and cytotoxicity	[149]
	Doxorubicin	Increased endocytotic cellular uptake	[149]
Liposome	Doxorubicin	Favorable risk–benefit profile in ovarian cancer	[150]
	Paclitaxel	Considerable disease response and resection rate, with acceptable toxicity in non-small cell lung cancer	[151]
	Amphotericin	Effective in acute lymphoblastic leukemia	[152]
	Daunorubicin	Effective in myeloid leukemia	[153]
	Eribulin	Effective in solid tumors	[154]
	Lipovaxin-MM	Effective in malignant melanoma	[155]
Dendrimer	Small interfering RNA (siRNA)	High specificity for tumor cells	[156]
Gold nanoparticles	5-Fulorourcil	Enhance the curative effect for hepatocellular carcinoma cells	[157]
Magnetic mesoporous silica	Folic acid	Inhibiting proliferation of HeLa cell lines higher cytotoxicity effect	[158]

tests that measure processes like protein adsorption will be a valuable addition to in vitro cellular assays. For example, evaluating the blood protein profile that absorbs to NPs in an in vitro environment may help us better understand how NPs interact with RES components in vivo [161]. Proteomics and toxicogenomics can also be used to detect biomarkers of toxicity associated with nanomaterial exposure [162].

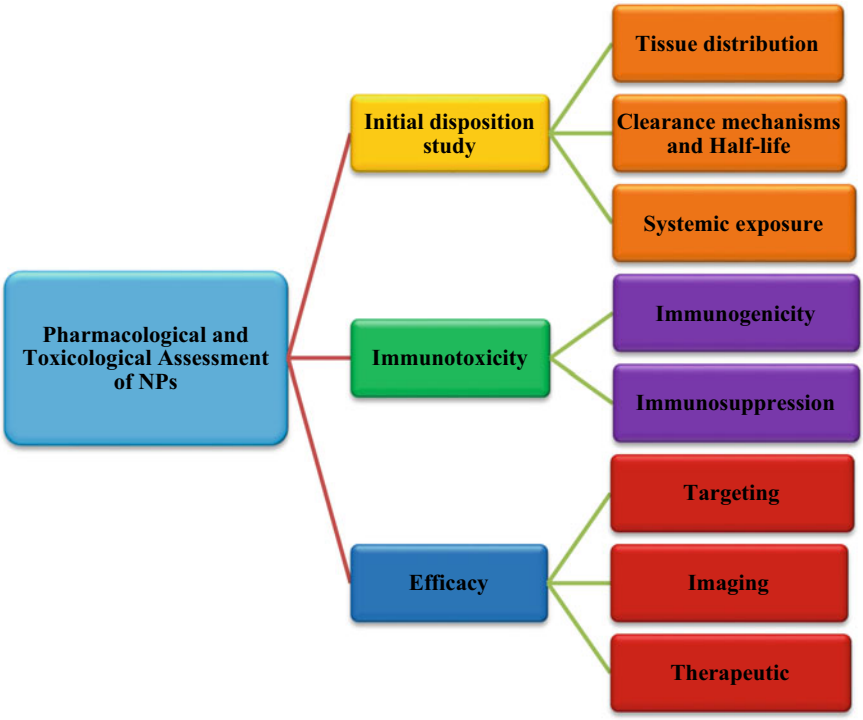

Fig. 11.3 In vivo pharmacological and toxicological assessment of NPs

In order to fully comprehend the safety and behavior of NPs in living organisms, in vivo studies are essential. The pharmacological and toxicological properties of NP formulations as shown in Fig. 11.3 must be thoroughly studied, much like any other new chemical entity (NCE). The impact of NPs on diverse organs and systems, like the heart, liver, kidneys, and immune system, should be investigated in vivo.

11.9 Nanotechnology: Regulatory Perspective for Drug Development in Cancer Therapeutics

The lack of existing safety information in biological systems for the vast majority of these novel nano-sized materials and nano-particlized pharmaceuticals further adds to the need for more investigation into any potential hazardous processes in humans [161]. The following are the most often asked questions when determining the safety of any NP: Are these NPs and associated technology new to the FDA? How does the FDA plan to deal with nanotechnology and how do existing rules enable the creation of safe and effective nanotechnology-based drugs? What are the scientific issues about nanotechnology-based treatments that are unique?

Many goods containing particle materials, such as nanomedicines or nano-products (e.g., small size, mechanism of delivery, increased surface area, specialized function related to size and increased surface area, etc.), have been encountered and approved by the FDA [162]. Even though the field of nanomedicine is relatively new, this agency is not unfamiliar with the applications submission for products comprising novel drug dosage forms or delivery methods, such as nanomaterials, NPs-based medicines, and medical devices. Because products are examined one at a time, numerous risk management principles (such as risk identification, risk analysis, and risk control) are frequently used to aid the drug review process. The question whether nanotechnology goods would be classified as pharmaceuticals, devices, biologics, or a combination of the three for regulatory process and assignment of work has piqued people's curiosity. Internally within the FDA, this has been extensively addressed, and the current assumption is that many of these medications will be deemed combination products. Combination products (drugs–devices, drugs–biologics, and devices–biologics) are progressively embracing cutting-edge, innovative technologies that have enormous potential for improving patient care. Innovative medication delivery technologies, for example, have the potential to make patient treatments safer, more effective, or more convenient.

For most products, the FDA has specific guidance/requirements in place that apply to all products, whether or not they contain nanomaterials. For the time being, existing criteria are thought to be sufficient for most nanotechnology medicinal products. The preclinical requirements for approval to commercialize pharmaceutical goods in the Center for Drug Evaluation and Research (CDER) include both short-term and long-term toxicity testings. Particularly, different types of studies like pharmacology (mechanism of action and safety profile), absorption, distribution, metabolism, and excretion (ADME), genotoxicity, immunotoxicology, carcinogenicity, and other possible studies are conducted by pharmaceutical campiness before the submission of New Drug Application (NDA). Because of the following factors, this set of tests is deemed adequate: the drug under the study is utilized in high-dose multiples (with low, medium, and high toxicity), as a minimum two different animal species are employed (rodent and non-rodent), many functional tests and thorough histopathological investigations are performed to see if there are any effects on specific organ systems, and to evaluate any carcinogenic effect, the drug treatments in animals are done for extended periods of time [162]. On a case-by-case basis, additional studies may be needed depending on drug-specific factors. Nanotechnology products, like other drugs, will be treated on a case-by-case basis, depending on the characteristics of the specific product being produced. As more information on the toxicity of nanomaterials becomes available, the FDA may demand further toxicological studies to assure the safety of the items it regulates [162]. All of these efforts have been aimed at gaining a better grasp of the science and determining whether existing laws are enough for the types of items that the FDA is expected to oversee.

11.10 Conclusion

Advances in nanomedicine have brought up new possibilities for upgrading the anticancer arsenal. Both targeted and non-targeted NPs are in the preclinical and clinical stages right now, highlighting the impact of delivery mechanisms on the domain. It has had a significant impact on selective cancer cell recognition, targeted drug administration, and overcoming the limits of traditional therapies. A few NPs-based formulations have hit the market already, while others are still in the research and trial stages. Traditional chemotherapies' adverse effects can be drastically reduced by these radical passive or active targeting strategies, which can significantly decrease death rates. Because cancer is one of the most fatal diseases, nanotechnology's contribution to precision therapy while avoiding life-threatening side effects has the potential to contribute to a favorable shift in clinical practice toward a life-saving strategy. Further nanomedicine research will increase the therapeutic window of drugs with drastically fewer side effects, resulting in better patient outcomes.

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Cancer Chemoresistance; Recent Challenges and Future Considerations

12

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12.1 Introduction

Cancer, the second major cause of global mortality, accounts for virtually 1 out of every 6 human fatalities across the world [13]. Moreover, the number of new cancer patients is expected to rise up to 23.6 million by 2030 [94]. The most frequent types of cancer in women include breast, cervical, pulmonary, thyroid and colorectal cancers, whereas, men are usually affected by prostate, pulmonary, colorectal, hepatic and gastric cancers. Despite the availability of diverse treatment methods, including surgery, radiotherapy, hormonal therapy, gene therapy and immunotherapy, chemotherapy still constitutes the mainstay of anticancer therapeutics. Nevertheless, the resistance of malignant cells rigorously obliterates the effectiveness of anticancer chemotherapy. Resistant neoplastic cells exhibit the capacity to persist and grow despite the repeated exposure to previously effective chemotherapeutic agents. Cancer chemoresistance and resultant therapeutic ineffectiveness have been implicated in causing almost 90% of the cancer-induced mortalities [1, 60, 63].

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12.2 Types of Chemoresistance

Chemoresistance is defined as reduction in the efficacy and potency of a chemotherapeutic drug to produce the potential outcome, thus representing a key barrier to cancer treatment and patient survival. Based upon the nature of occurrence, chemoresistance is classified into intrinsic and acquired forms (Fig. 12.1).

12.2.1 Intrinsic Chemoresistance

Intrinsic or innate resistance occurs prior to the use of chemotherapeutic agents, on account of mutation(s) in genes associated with tumor growth and/or apoptosis. The effectiveness of chemotherapeutic drugs can be decreased through the stimulation of intrinsic pathways that are primarily applied for defense against the environmental toxins, including antineoplastic agents. For instance, human epidermal growth factor receptor 2 (HER2) overexpression upregulates the transcription factor Snail and eventually impaired the clinical outcome of cisplatin therapy in gastric cancer patients [45].

12.2.2 Acquired Chemoresistance

Acquired resistance can be determined by the perpetual diminution of cytotoxic efficacy of a chemotherapeutic drug following its administration. Altered expression levels or mutation in target-coding genes can also trigger the resistance of cancer cells to targeted drugs. Almost 20–30% of the chronic myelogenous

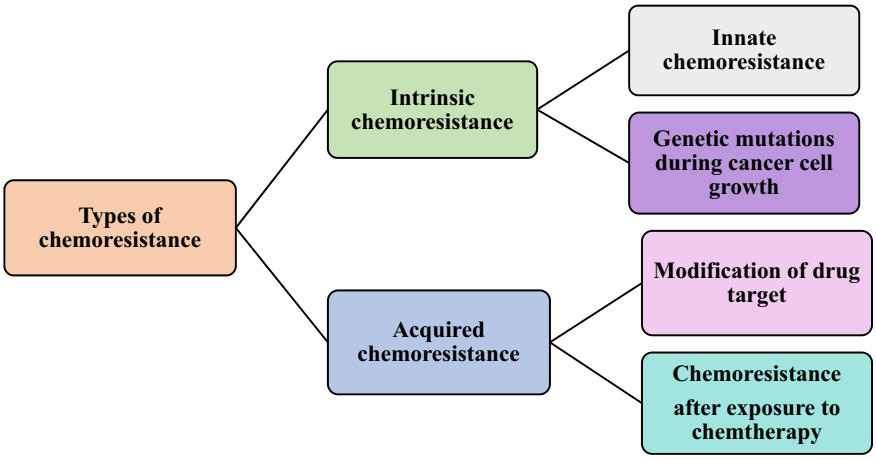

Fig. 12.1 Types of chemoresistance

leukemia patients, receiving tyrosine kinase inhibitor, imatinib, suffer from relapse or acquire resistance on account of point mutation in fusion tyrosine kinase protein [51, 77]. Vigorous changes of the tumor microenvironment arising during the treatment course can also result in drug resistance. Tumor microenvironment may contribute to chemoresistance through exosome-based communication between tumor-associated macrophages and tumor cells, as well as the exchange of exosomal microRNAs between stromal cells and cancer cells [16].

The afore-mentioned types of intrinsic and acquired chemoresistance can concurrently exist during cancer progression and therapy. Acquired drug resistance may arise through the mechanisms that are entirely different from those associated with the intrinsic form. Conversely, the selective extension of intrinsic drug resistance can lead to acquired mode. The sensitivity of a specific cancer to an antineoplastic drug can be predetermined by the extent of intrinsic drug resistance. Biochemical and genomic examinations are prerequisites for designing the therapeutic plan to circumvent the pre-existing chemoresistance [93]. Moreover, therapeutic adjustment is recommended following the detection of acquired drug resistance. A major aim of chemotherapy should be the efficient deceleration or inhibition of tumor growth without provoking the acquired, or at least the irrepressible form of acquired drug resistance [92].

12.3 Potential Mechanisms of Cancer Chemoresistance

Cancer chemoresistance can arise through several different mechanisms (Fig. 12.2), and the simultaneous involvement of multiple pathways further confounds the circumvention of this phenomenon.

12.3.1 Enhanced Drug Efflux

Enhanced efflux of antineoplastic drugs leads to intrinsic or acquired chemoresistance by significantly reducing the intratumor drug concentration [87]. The drug efflux has been primarily attributed to transmembrane transporter proteins representing the adenosine triphosphate binding cassettes (ABC) superfamily. Overall, 48 human ABC genes, categorized into seven subfamilies (ABCA to ABCG) have been recorded [95]. Out of these, ABCB1, ABCC1 and ABCG2 extensively contribute to the acquisition of multidrug resistance. By virtue of its several binding sites, the ABCB1 (P-glycoprotein) is capable of pumping a wide range of substrates including drugs like doxorubicin, vinblastine, etoposide and paclitaxel [88]. Likewise, the ABCC1 also referred to as multidrug resistance-associated protein 1 (MRP1) can also carry out the efflux of multiple drugs such as anthracyclines, methotrexate, vinca alkaloids, camptothecins and epipodophyllotoxins [20].

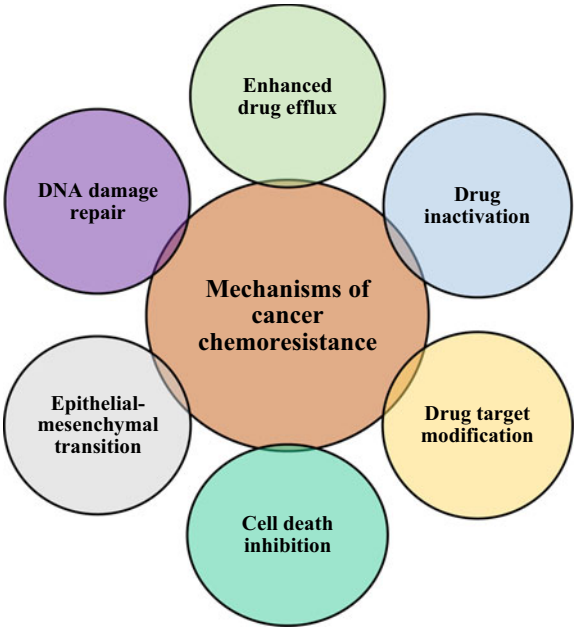

Fig. 12.2 Mechanisms of cancer chemoresistance

12.3.2 Drug Inactivation

Several antineoplastic drugs undergo metabolic activation, and tumor cells can become resistant to them through impaired drug activation. For instance, the nucleoside analogue, cytarabine that is used to treat the acute myelogenous leukemia, requires activation through several phosphorylation steps [100]. Defective activation caused by mutation or downregulation of the metabolic pathway may induce resistance to cytarabine. Platinum-based anticancer drugs are combined with taxanes for the post-operative treatment of advanced ovarian cancer. However, ovarian cancer cells may acquire resistance to platinum analogues through metabolic inactivation attributed to glutathione and metallothionein [68].

12.3.3 Modification in Drug Target

With reference to structure–activity relationship, altered drug targets lack the affinity for pharmacological ligands and consequently fail to support the elicitation of therapeutic response. Therefore, modified targets can be no longer pharmacologically exploited by means of previously effective drugs. For example, the genomic amplification of androgen receptors occurring in almost 30% of prostate cancers impairs the antagonistic action of drugs such as bicalutamide and leuprolide. Besides, the modifications in signaling transduction pathways associated with

drug activation can also induce drug resistance. Accordingly, the complicated interaction of estrogen receptor signaling with growth factor receptor pathway reverses the typical antagonistic and antineoplastic actions of tamoxifen in HER2 positive breast cancer cells [80].

12.3.4 Cell Death Inhibition

The destruction of tumor cells triggered by antineoplastic drugs occurs in different forms including apoptosis, necroptosis, autophagy and necrosis [55]. However, the several types of cell death profoundly differ in the degree of damage that is necessary for cell destruction. Autophagy is capable of promoting either the sensitivity or resistance of tumors to chemotherapeutic agents [72]. Although the involvement of necroptosis in chemoresistance is still paradoxical, its induction may lead to the blockade of anti-apoptotic pathways [83]. The evasion of apoptosis supports the development as well as the continued chemoresistance of cancer [63]. The chemoresistance associated with apoptosis inhibition, primarily results from the avoidance of cell death through survival signaling. Besides, the activation of prosurvival proteins, mutation or downregulation of proapoptotic proteins can also lead to chemoresistance through the circumvention of apoptosis [33]. Moreover, the cyclosporine-binding proteins, cyclophilins, have also been implicated in suppressing the apoptotic pathway in some types of neoplastic cells [85]. The failure of first-line therapy predominantly leads to the acquisition of chemoresistance, most probably caused by the preclusion of cell death in prostatic, ovarian and pulmonary cancer cells [43].

12.3.5 Epithelial-Mesenchymal Transition

In addition to its pivotal role in the metastasis of solid tumors, the epithelial to mesenchymal transition has also been linked with the development of chemoresistance [79]. While expediting the metastasis of cancer cells, the epithelial to mesenchymal transition may promote drug resistance in certain cells through the provision of signals for enhancing the cellular survival. However, the contribution of the epithelial to mesenchymal transition to drug resistance varies with the degree of tumor metastasis. This is exemplified by the relatively high degree of resistance to trastuzumab associated with the overexpression of β1 integrins in HER2 positive breast cancer [59]. Besides, the signaling pathways of differentiation that are critical for the epithelial to mesenchymal transition, may also facilitate the phenomenon of drug resistance. Thus, the upregulation of transforming growth factor β (TGFβ) during the epithelial to mesenchymal transition, that is triggered by the overexpression of integrin αvβ1, provides survival signals to colon cancer cells against chemotherapy-induced cytotoxicity [70].

12.3.6 DNA Damage Repair

DNA damage repair exerts a distinct role in antineoplastic drug resistance, and damage response mechanisms can defuse the drug-mediated impairment. For instance, platinum-based anticancer drugs like cisplatin result in detrimental DNA crosslinks that can give rise to apoptosis. Nevertheless, resistance to platinum analogues usually occurs as a result of homologous recombination and nucleotide excision repair, which are known for resolving the platinum-induced cell damage [12]. Therefore, the efficiency of DNA-damaging antineoplastic drugs depends upon the malfunction of DNA damage response pathways in tumor cells. The therapeutic efficiency of anticancer drugs can be improved through the concurrent blockade of DNA repair mechanisms for sensitizing the tumor cells [28].

12.4 Role of Biological Factors in Cancer Chemoresistance

12.4.1 Epigenetics, MicroRNAs and Stem Cells

Epigenetic mechanisms have been implicated in the initiation and development of multiple diseases including cancers. Owing to the significance of epigenetic mechanisms in the regulation of several genes and pathways, epigenetic changes exhibit a pivotal role in cancer chemoresistance. The two major forms of epigenetic alterations occurring in carcinogenesis, histone modification and DNA methylation, have been successfully targeted for the restoration of chemosensitivity in heterogeneous multiple myeloma [46], whereas, the demethylation of DNA promoter region induces chemoresistance through the overexpression of oncogene [91]. Therefore, several drugs that are capable of interaction with cancer progression like DNA methyltransferase blockers and histone deacetylase antagonists can help to circumvent the drug resistance.

MicroRNAs being too short are unable to carry out transcription, but exert a distinct role in the regulation of several genes particularly those that are linked with drug resistance. Hence, the aberrant expression of microRNAs triggers the acquisition of chemoresistance. Moreover, damage to candidate microRNAs also impairs the sensitivity of tumors to typical cytotoxic drugs as well as newly developed biological agents. However, some other microRNAs have been implicated in the avoidance of cancer chemoresistance [67].

Cancer stem cells are primarily found in certain solid tumors and leukemias. But latest research infers the co-occurrence of stem cells and proliferative cells in virtually all cancers. However, tumor stem cells constitute merely up to 1% of the entire cancer cells and are therefore hard to identify and subsequently examine. These cells form a reservoir for relapse and metastasis of cancer. Of particular significance is the capacity of tumor stem cells to preserve the constitutive feature of auto-protection by means of multidrug resistance transporters [30]. Similar to normal stem cells, many cancer stem cells are also equipped with the expression of ABCB1 and ABCG2 genes [64]. Hence, the dormant cancer stem cells

characterized by constitutive multidrug resistance present a major hurdle to the effectiveness of chemotherapy. Discriminating the normal and tumor stem cells based on immunological and biological attributes may help to devise eradication strategies for cancer stem cells with least or no impact on normal stem cells.

12.4.2 Cell Signaling Pathways, Endoplasmic Reticulum and Exosomes

Several signaling pathways with potential significance in cancer transformation, apoptotic inhibition, tumoral metastasis and chemoresistance have been explored inside the cell. Current studies have linked the drug resistance with aberrant stimulation of one or many of these signaling cascades. For example, the dysregulation of Wnt/B-catenin, Ras/Raf/MAPK, TGF-B, EGFR and Notch signaling pathways has been implicated in cancer development and conferring the resistance of tumors to cytotoxic agents [62, 73].

The pathway of endoplasmic reticulum stress response is known to facilitate the survival and drug resistance of cancer cells through an adaptive mechanism. Three distinct sensor proteins including ATF6, IRE1a and PERK regulate the endoplasmic reticulum stress response in normal cells [31], whereas the dysregulation of this pathway in cancer cells, due to oncogenic stimulation or tumor suppressor genes depletion, supports the cell survival during extreme metabolic stress and high rate of translation [90]. PERK signaling attributed to endoplasmic reticulum stress response also contributes to chemoresistance, but the underlying molecular mechanism of this association is still unclear [6].

Exosomes, the bilayered, smaller molecules are released from the luminal membrane of extracellular vesicles which act as carriers for the transport of genetic information [98]. Additionally, a key role in enhancing the post-chemotherapy survival of tumor cells has also been assigned to exosomes. Exosomes-induced chemoresistance occurs through direct or efflux-mediated drug transport, DNA repair as well as the delivery of anti-apoptotic signaling molecules and ABC transporters [8, 82]. Besides, drug resistance elements are also transferred by means of exosomal long coding RNAs [101]. Chemoresistance and therapeutic failure in myeloid leukemias have been attributed to stromal cell-derived exosomes [48, 97].

12.4.3 Tumor Heterogeneity and Microenvironment

Tumor heterogeneity is based on cellular type (such as immune cells, stromal cells), genetic, metabolic status and temporal progression [18]. The co-occurrence of heterogenic cancer cells has been established in several types of primary tumors including breast cancer [71], ovarian cancer [7], chronic lymphocytic leukemia [57] and renal cell carcinoma [36]. Tumor heterogeneity also contributes to chemoresistance and therapeutic failure. Since, the clonal cellular subgroups

with diverse genetic profiles exhibit significant variations in terms of chemosensitivity, only the susceptible fraction of cancer cells can be destroyed, while the resistant cellular clones survive, multiply and grow, thereby confounding the sensitivity of tumor to initial chemotherapy. Additionally, same treatment may lead to incongruent responses in genetically heterogenic patients.

As tumors usually comprise of heterogeneous cancer cells, thus the tumor environment is composed of extracellular matrix, normal stromal cells and soluble substances like growth factors and cytokines [67]. Current research has indicated an integral role of tumor microenvironment in chemoresistance. Several components of tumor microenvironment such as cell adhesion molecules, cytokines and extracellular adenosine triphosphate (ATP) contribute to the signal transduction mechanisms associated with the survival, growth and resistance of cancer [26, 58, 67]. Likewise, the typical acidic pH, oxidative stress and varying hypoxia of cancer microenvironment also support the development of chemoresistance [17, 84]. Besides, an extracellular matrix protein, integrin, has been demonstrated to facilitate antineoplastic drug resistance in prostatic, hematological, breast and small cell lung cancers [4].

12.5 Cancer Multidrug Resistance

Cancer multidrug resistance refers to cross-resistance or lack of sensitivity of cancer cells to the antineoplastic actions of unrelated chemotherapeutic drugs exhibiting different molecular structures or target sites [34, 39]. The underlying mechanisms of multidrug resistance encompass increased efflux of drugs, evasion of apoptosis, epigenetic alterations, gene mutations, enhanced DNA damage repair and modification of xenobiotic biotransformation [35, 63]. Multidrug resistance is considered as the primary cause of therapeutic failure and main barrier to the treatment of metastatic cancers [21, 37, 39, 54]. Besides, majority of the cancer-related deaths in patients using either typical chemotherapeutic agents or novel targeted drugs have been attributed to multidrug resistance.

12.6 Prognostic Markers and Diagnosis of Cancer Chemoresistance

Appropriate monitoring of drug resistance not only determines the effectiveness of chemotherapy but also helps to preclude the potential adverse effects [61]. Cancer chemoresistance is typically detected during an extended phase of chemotherapeutic drug administration. Currently, two laboratory techniques and one clinical technology including cancer biomarker test, fresh tumor cell culture and whole-body positron emission tomography scanning can be used for the rapid diagnosis of cancer chemoresistance. These tests are based upon the metabolic functions of tumor cells. Fresh tumor cell culture enables the evaluation of resistance during in vitro conditions, prior to drug administration. A positive uptake on positron

emission tomography scan, post-chemotherapy from residual or persistent disease can be employed in vivo for an indirect assessment of drug resistance, whereas, the emerging cancer biomarkers tests have been projected to yield maximum diagnostic potential.

12.6.1 Cancer Biomarkers

Cancer biomarkers also known as tumor markers gained earlier attention for the prediction of cancer [27]. Initially, alpha-fetoprotein and carcinoembryonic antigen were focused as the presumptive tumor markers. Serum markers are considered more attractive on account of their convenient, non-invasive and repeated sampling. However, clinical studies have determined the predictive role of only few biomarkers such as prostate-specific antigen, human chorionic gonadotropin, CA 125 and thyroglobulin in prostatic, chorionic, ovarian and thyroid cancers, respectively [47]. Moreover, successful treatment with antineoplastic drugs tremendously declines the levels and resultant diagnostic value of these tumor markers. Besides, the pathobiological aspects of cancer markers are still partially understood. More recently, overexpressed growth factor receptors are exploited for the development of targeted anticancer agents. Nevertheless, growth factor receptors can predict the therapeutic outcomes in only discrete cases. For instance, HER2 in breast cancer, C-kit in gastrointestinal stromal cell cancer and BCR-ABL fusion protein in chronic and myeloid leukemia exhibit overexpression and may thereby reflect the therapeutic efficacy of some antiangiogenic agents [56].

12.6.2 Fresh Tumor Culture Test

Fresh tumor cell culture technique is applicable to several types of cancer, as they exhibit the essential cell reaction. This assay involves the culturing of freshly isolated tumor cells followed by incubation with sequentially varying drug concentrations and the appraisal of cell survival. The range of chemotherapeutic doses usually corresponds to in vivo drug concentrations during treatment. Cellular growth or viability is analyzed following the incorporation of thymidine into cell DNA [11, 50]. Drug resistance is indicated by the lack of any reduction in either cellular ATP or thymidine uptake into cellular DNA [61].

12.6.3 Positron Emission Tomography

Although formerly meant for detecting the cancer localization, the application of this nuclear medicine technique has now been expanded to the metabolic activity assessment of tumors. Cancer cell glycolysis is determined by means of dynamic imaging following the administration of a radiopharmacon, 18-fluorodeoxyglucose [32]. The cellular uptake of radiopharmacon is correlated with the

degree of glycolysis and remains comparatively higher in neoplastic cells. Both pre- and post-chemotherapy scans are required for the assessment of therapeutic response. The growing development of novel radiotracers indicates the potential value of tomographic imaging in quantifying the levels of tumor hypoxia, cellular multiplication, apoptosis and specific growth factor receptors [66].

12.7 Strategies for Overcoming Cancer Chemoresistance

Despite the multifaceted nature of cancer chemoresistance, elucidation of the underlying mechanisms has gradually improved together with the development of experimental conditions for predicting the likelihood of drug resistance in clinical milieu. In vitro techniques such as target-directed mutagenesis, loss/gain of resistance screens, utilization of isogenic cancer cell lines and comprehensive analysis of chemoresistant tumors at cellular, molecular and genomic levels can be applied for this purpose. Besides, ex vivo cell culture methods, patient-derived xenografts and genetically-engineered, cancer animal models can considerably contribute to better understanding and subsequent control of chemoresistance [22]. Different strategies can be employed for overcoming the phenomenon of chemoresistance (Table 12.1).

The genetic heterogeneity and multiclonal nature of tumors provides a rationale for combination therapy to overcome cancer chemoresistance. Single agent-based chemotherapy most probably suffers from treatment failure owing to the selective survival and proliferation of resistant cancer cells. Accordingly, combination therapies using two or more anticancer drugs are suggested to simultaneously target

Table 12.1 Potential strategies for the circumvention of cancer chemoresistance

• Combination therapies using different anticancer drugs for simultaneously targeting the independent driver pathways
• Suppression of drug resistance genes through specific small interfering RNAs
• Inhibition of P-glycoprotein-mediated drug efflux
• Metronomic chemotherapy and treatment holidays
• Development of semi-synthetic analogues of classical chemotherapeutic drugs
• Designing nanoparticle-based formulations of anticancer drugs
• Blockade of vascular endothelial growth factor using antiangiogenic agents
• Concomitant use of proton pump inhibitors with anticancer drugs for inhibiting the acidification of tumor microenvironment
• Reduction of intracellular glutathione concentration
• Glycolysis suppression through glucose transport blockers or glycolysis inhibitors

the independent driver pathways and thereby restrict the development and dissemination of resistant cellular clones. Despite being developed on empirical grounds, such drug combinations have demonstrated clinical success in a wide range of cancers. However, further development and validation of combination therapies necessitate the conduction of expensive and time-consuming clinical trials [22]. Although cell lines have been successfully employed for designing the drug combinations, the altered cancer cell behavior of passaged cell lines is a major drawback [2, 24]. Moreover, the clinical outcomes of such chemotherapeutic cocktails primarily depend upon the distinct resistance pattern of respective cancer and toxicity tolerance of patients.

ABC transporters have gained substantial attention on account of their pivotal role in the phenomenon of drug resistance. Therefore, several P-glycoprotein inhibitors including verapamil are currently used as an adjunct to cytotoxic agents for combating efflux-mediated drug resistance. Nevertheless, the application of such P-glycoprotein antagonists has also resulted in certain undesirable pharmacokinetic consequences. For instance, the intracranial accumulation of P-glycoprotein substrate drugs has been associated with unpredicted toxicity in knockout mice characterized by a genetic MDR1a disruption [41]. Besides, in vitro studies have also demonstrated the blockage of P-glycoprotein-induced drug efflux and subsequent reversal of multidrug resistance by means of anti-MDR1 monoclonal antibodies [23, 29, 99]. The suppression of drug resistance genes via mRNA damage constitutes an innovative and promising molecular technique [29]. More recently, antisense oligonucleotides or ribozymes have been introduced for the inhibition of multidrug resistance at transcriptional or translational levels [38, 75]. The susceptibility of HL-60 cell line to etoposide was markedly improved following the suppression of MDR1 gene through specific small interfering RNAs [49]. Nonetheless, majority of the former investigations on microRNAS have used either cancer cell lines or animal models. Thus, translational studies and those focusing the circulating microRNA in human plasma are recommended [3].

Besides, the overexpressed growth factor receptors in breast and gastrointestinal cancers have been efficiently targeted by specific antibodies (such as trastuzumab and cetuximab) for either enhancing or restoring the susceptibility of previously refractory tumors to anticancer drugs [69, 76, 78, 81]. For example, co-treatment with cetuximab restored the effectiveness of irinotecan in patients bearing the irinotecan-resistant tumors and may help to circumvent the chemoresistance [25]. The blockade of vascular endothelial growth factor in tumor-bearing mice reestablished the efficacy of vinblastine even at a submaximal tolerated dose [52]. Likewise, antiangiogenic agent significantly improved the efficacy and diminished the adverse effects of concomitantly given cytotoxic drugs [40]. Furthermore, adjuvant therapy with suramin is also currently being analyzed for reversing the fibroblast growth factor-induced resistance in tumors [86].

The acidic microenvironment of solid tumors provides yet another target for combating chemoresistance. Therefore, acid secretion inhibiting drugs may potentially enhance the cytotoxic effect of concurrently administered antineoplastic agents. This approach has already been supported through in vitro as well as

in vivo evidence, wherein the co-administration of a proton pump inhibitor, lansoprazole led to synergistic effects with paclitaxel in melanoma cells [5].

Metronomic chemotherapy involving the repeated administration of cytotoxic agents at considerably low doses can be quite beneficial in avoiding the development of drug resistance but requires further confirmation through extended research [10, 74]. Treatment holiday offers yet another strategy to circumvent anticancer drug resistance, wherein the discontinuation of chemotherapy helps to preclude the selection resistant tumor cells [15]. Semi-synthetic analogues of certain typical chemotherapeutic agents such as vinca alkaloid-derived drugs have also shown comparatively high antineoplastic potency against the resistant tumors, but this technique enhanced the toxicity profile in some cases [9, 42]. The remarkable intratumor diffusion and accumulation tendency of nanoparticles may reduce the likelihood of chemoresistance by potentiating the cytotoxic effect of anticancer drugs [3].

Certain cancer cells preferably utilize glucose to fulfill their energy requirement, and glucose deprivation prompts the death of these so-called, glucose-addicted cells [14, 19, 53, 69, 89]. Therefore, the concurrent use of glucose transport blocker or glycolysis inhibitor with a typical anticancer drug may provoke the death of cancer cells. For instance, glycolysis suppression in hypoxic condition deteriorated the drug resistance by enhancing the cellular death in lymphoma and colon cancer [96]. Cancer progenitor cells often exhibit drug resistance and persist for prolonged periods thereby leading to cancer remission at the original or distant locations. Therefore, despite of its complexity, the elimination of this small proportion of cancer progenitor cells may likely help to diminish the occurrence of anticancer drug resistance [44]. The circumvention of drug resistance has also been attributed to reduced production and intracellular levels of glutathione. Both in vitro and in vivo studies have linked the reduction in intracellular glutathione levels with improved cytotoxic efficacy of several antineoplastic drugs [35]. Finally, the phenomenon of cancer chemoresistance can be employed for useful purposes like inducing chemoprotection and selecting the regenerating stem cells via transfer of MDR1 gene-expressing vectors into cancer patients [65].

12.8 Conclusion and Recommendations

In a nutshell, as cancer cells could always acquire alternative ways to withstand the current therapy, the evasion of chemoresistance seems like an endless battle. Nevertheless, the underlying mechanisms of cancer chemoresistance require comprehensive elucidation for determining novel therapies against cancers that are refractory to typical cytotoxic drugs. Furthermore, cancer progenitor cells should be properly eradicated for preventing their potential contribution to chemoresistance and cancer relapse. Besides, the beneficial aspect of cancer chemoresistance can be also exploited by clinically investigating the potential delivery of MDR1 gene-expressing vectors into cancer patients for selecting the regenerating stem cells or imparting chemoprotection.

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Psychological Support for Cancer Patients

13

Shazia Khalid, Imran Abbas, and Saira Javed

13.1 Overview

Cancer is a complex chronic disease that is accompanied by a multitude of intense medical and psychosocial challenges. From the time of screening, to diagnosis, treatment, and survivorship or palliative care, the journey is replete with distress and adjustment to changing circumstances that affect the quality of life of patient as well as caregivers and other family members. It is also noteworthy that the stress related with cancer is not limited to the patient and family but also has a trickle-down impact on the psychological and interpersonal well-being of health care practitioners. Thus, it is not only the cancer patient who need psychological support and care, though may need it at priority, the family members especially caregivers and oncology medical staff may also benefit from it.

The statistics related to incidence and prevalence of cancer are quite daunting. According to World Health Organization [137], in every single minute, six individuals are diagnosed with some type of cancer in the world. In year 2020 only, approximately 10 million people all over the world expired due to cancer, among which lung, breast, gastrointestinal, skin, and prostate cancer were the most common sites, while the incidence rate for cancer is projected to rise to 21 million by 2030 [36]. On the other hand, with incredible advancements in medical field related to oncology, early disease detection, better prognosis, and evidence-based treatment plans have emerged, which have generally resulted in longer survival

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period for the cancer patients. Consequently, there are around 300 million cancer survivors estimated in the world presently. These facts have led practitioners and patients to appreciate the significance of addressing psychological challenges that cancer patient, cancer survivor, and terminally ill patients and their caregivers face throughout the experience.

Psychological support and care in the domain of oncology is termed as psycho-oncology, which has been an expanding field since 1970s [62] bringing about a model that incorporates psychological domain into oncological field. The prime purpose of psycho-oncology is to identify psychological factors in multi-faceted notion of carcinoma disorders and provide evidence-based interventions and therapies to provide psychological support to the patient and family [39]. Two major concerns psycho-oncology deals with are (1) how patient, patient's family, and the caregivers respond psychologically to cancer and (2) how the survival, treatment, its detection, and risk occurrence are affected by psychological, behavioral, biological, and social factors [47]. Thus, each concern requires specialized set of psychological support, a service to be provided by psychologists. Table 13.1 presents target areas in oncology where the support of psychologists might be required to enhance the quality of life of patient, caregivers, and oncology medical staff.

Different studies have highlighted the psychosocial determinants and factors that can affect the whole process of psychological impact of cancer. For instance, factors that can have an effect on the occurrence of oncological ailments might include stressful life events, traumatic events, lack of support from others, certain personality characteristics, dysfunctional coping skills with disease, suppressing

Table 13.1 Potential target areas for psychological support and interventions in cancer

	Domain	Subcategories
Patient	Physical and medical	Pain, fatigue, treatment management such as treatment adherence, improvement in general health such as nutrition, exercise
	Psychological distress	Emotional problems, anxiety, depression, denial, anger, suicidality, shame, guilt, fear of death and recurrence, quality of life
	Personal and social adjustment	Adjustment to changes, meaning in life, religious and spiritual concerns, life style changes, relationship improvement, management of finances, quality of life
Caregivers/ family	Psychological and physical	Caregiver burden, stress, sleep disturbances, health issues, physical exertion
	Socio-economic	Reduced social interaction, economic burden, job stress
Oncology medical staff	Occupational	Job stress, job burnout, workload, lack of resources
	Family and marital relationships	Family-work balance, emotional disturbances, stressful relationships

feelings, negative emotional reactions, social relationships, and neurological disorders [39]. Psychological effects of being diagnosed with cancer and receiving treatment can be very grim. On psychological level, the patients might experience emotional challenges, changes in perception and expectations about future, feelings of being left out, stigma and marginalization, and interpersonal problems, whereas on physical level, it can lead to body changes, hair loss, and how patient start perceiving their body. Both problems can occur during any phase or stage of cancer diagnosis and treatment [9]. Other predisposing factors for mental health challenges among oncology patients are related to the nature of disease, fertility problems, pre-existing physical and psychiatric ailments, etc. [9].

13.2 Psychological Reactions and Mental Health: Interventions for Cancer Patients and Survivors

Psychological reactions are considered secondary disorders to carcinoma. A person diagnosed or suspecting oncological problems suddenly find themselves drowned in a plethora of emotional reactions; these reactions might range from denial, anger, fear, uncertainty to disabling psychological disorders such as anxiety, death anxiety, posttraumatic stress, and depression. Collectively, these responses are termed as psychological distress [92]. Estimates indicate that approximately 50% of cancer patients exhibit psychological distress at some point in the course of illness, which adversely affect their quality of life and functionality [55], leads to poor prognosis [33], low treatment adherence, and might intensify the experience of pain [56]. However, it is worthwhile to note that not all individuals become distressful to the point of developing serious mental health issues. Some individuals use the adversity to change their life style, find new meaning in their existence, and develop appreciation for life. In fact, research indicates that at least 60% cancer patients exhibit moderate to high positive changes [119, 122] following diagnosis and/or during treatment. Such changes are associated with better adjustment to new circumstances, increased hope and quality of life [22, 104], increased adherence to treatment regimens, and improved prognosis for cancer patients [28].

The question that needs to be addressed here is why for some individuals the experience leads toward negative consequences while for others it might result in positive changes? In the late 1980s, Lazarus and Folkman presented Transactional Model of Stress and Coping, which has proven its utility in understanding unique responses to the traumatic experience of oncology related problems. The model states that the cognitive appraisal of the stressor and coping resources, not the stressor itself, will determine whether the trauma will lead to psychological distress or positive changes. According to the model, the stressor can be perceived as harmful ('It is catastrophic'), threatening ('It will destroy my future and social relationships'), or challenging ('This means I have to change my health habits') (primary appraisal) depending upon personality traits (optimism, hopeful, neuroticism, etc.), and perception of social and financial resources and coping styles

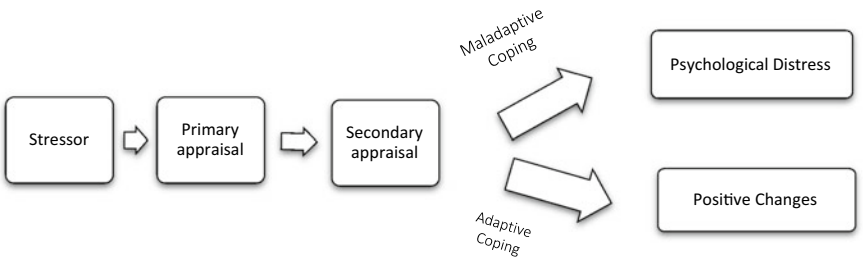

Fig. 13.1 Transactional model of stress and response

(secondary appraisal). For instance, the patient is more likely to respond maladaptively and in consequence develop psychological distress if the patient evaluates the cancer diagnosis as harmful or threatening and believes that he or she lacks required resources for coping, whereas positive changes will result if the patient interprets the stressor as challenging and possess adaptive coping skills (Fig. 13.1).

Psychological Distress and Disorders. The experience of cancer implicates psychological distress and extreme fear. In the field of oncology, National Comprehensive Cancer Network (NCCN) has defined psychological distress as 'a multidetermined unpleasant emotional experience of a psychological, social, and/or spiritual nature that may interfere with the ability to cope effectively with cancer, its physical symptoms, and its treatment' (p. 6; NCCN [92]). Studies have shown that women [84], single, less educated, unemployed [33], and younger [44] or older [84], cancer patients are more at a risk of high level of psychological distress, while individuals in last stage [56] and hospitalized patients [15] report higher distress compared to patients in early stages and outpatients. However, the relationship between type of cancer and psychological distress remains ambiguous [105].

In addition, recent researches and clinical practice suggest that psychological distress puts about 30–35% of oncology patients at a risk of psychiatric/psychological comorbidity disorders. Li et al. [68] have presented a pathway for the progression of psychological distress into psychiatric illnesses (Fig. 13.2).

Some psychiatric or psychological disorders that have been frequently observed among cancer patients are adjustment disorders, depressive disorders, anxiety disorders, and posttraumatic stress disorder [54].

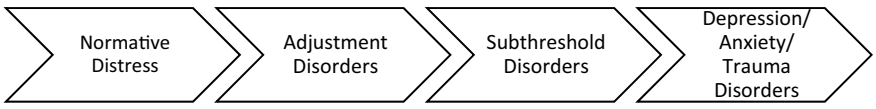

Fig. 13.2 Continuum of psychological distress

1. ***Adjustment Disorders***. Adjustment disorders refer to a 'transient and often self-limiting response to stress' [35] and have been reported in around 19.4% of oncology patients [88]. It is characterized by emotional and behavioral symptoms that lie in between normative psychological distress and pathological mental disorders such as anxiety and depression. According to DSM-V, the adjustment disorders are diagnosed when the emotional response to experience of trauma such as cancer does not correspond to normal distress symptoms, significantly affects social and occupational functioning, appear within three months of stressor, and does not meet the criteria of other mental disorders.
2. ***Depressive Disorders***. Depression is one of the common reactions associated with oncology experience. While some researches associate depression in 58% cases of cancer patients [141], others report 11% prevalence rate of depression in the group [83] depending upon type of cancer and prognosis. It worsens pain bearing ability, exacerbates fatigue, leads to low compliance, and low desire for long time therapy. Major depression in cancer patients is marked by social withdrawal, feelings of worthlessness or guilt, loneliness, anhedonia, dysphoric mood, low self-esteem, and suicidal thoughts and is diagnosed if the symptoms persist for at least two years. On the other hand, subthreshold depressive disorders have been reported in around 22% of cancer patients and are diagnosed if fewer symptoms of depression are observed for at least two weeks. Compared to major depression, subthreshold depressive disorders are more amenable to psychological support in cancer patients.
3. ***Anxiety Disorders***. The prevalence rate of anxiety varies from 10 to 34% in cancer patients [83, 141] and includes disorders such as generalized anxiety disorder, phobias, panic disorders, and social anxiety. Anxiety problems interlinked with oncology disorders can include excessive worrying, financial concerns, panic attacks, disturbed sleeping patterns, nausea, vomiting, which in all greatly affect the quality of life of the cancer patient [124]. According to DSM-V, the most common characteristic of different anxiety disorders is the 'heightened focus on perceived danger and efforts to avoid or escape from such threats.' Anxiety symptoms can arise on the genesis as well as during the treatment of the disease, be it any decision patient has to take. In some cases, if not properly handled, anxiety can lead to severe consequences which can take patient's life as well.
4. ***Fear of Cancer Recurrence (FCR) and Death Anxiety***. Fear of recurrence of cancer in the same or another part of the body is a common emotional experience of cancer patients and their caregivers. It has been estimated that 73% respondents typically report some level of FCR, while approximately 49% report moderate and high level of FCR. Compared to other groups, the fear of death has been observed higher in cancer patients, which might be accompanied by suicidal ideation.
5. ***Post-Traumatic Stress Disorder (PTSD)***. The prominent features of posttraumatic stress include intrusive and recurring thoughts and images, flashbacks, and frightening dreams, which persist for at least 30 days after the traumatic experience. The prevalence rate for the disorder has been reported to range from

10 to 20%. It appears to affect certain populations more such as minority. In addition, gender and education level are identified as risk factors for the onset of posttraumatic stress disorder and is frequently accompanied by or leads to depression, anxiety and substance use disorders. For instance, studies indicate that women are more likely to develop PTSD as compared to men [63].

Screening. Cancer distress is considered as the sixth sign in oncology. Thus, early detection and management plays a vital role in cancer care, which requires short and psychometrically reliable and valid measurement tools. The Distress Thermometer (DT) is a single item rating tool, anchored on a 10-point scale ranging from 'no distress at all' to 'extreme distress' (NCCN, 2019 as cited in Ownby [99]. The cutoffs vary depending upon gender, age, and time of administration of the scale. Another tool is the NCCN Problem Checklist (2019), which comprises 39 items used to identify the sources of cancer distress. It is important to highlight that during the assessment of the connection between cancer and distress symptoms, there is a risk for under diagnosis or over diagnosis, where in former, there is a non-recognition where depression is seen as normal symptom of cancer while later deals with the over-recognition where normal symptom is connected to cancer as a part of depression [5]. It is commonly recommended that the distress should be measured on receiving the diagnosis and in every subsequent visit to hospital.

In similar vein, instruments for the measurement of unmet needs of cancer patients and survivors have received much attention. Such instruments help psychologists and medical staff comprehend the physical, medical, and emotional needs of the patients and devise psychological support accordingly. On the basis of meta-analysis conducted by Wang et al. [133] on all articles appearing since inception of ten major global search engines till 2016, it was concluded that Supportive Care Needs Survey and Problems and Needs in Palliative Care Questionnaire were most commonly used to assess the needs in the advanced cancer care. On the other hand, there are numerous scales available for the measurement of depression, anxiety, PTSD, and FCR applicable for cancer patients. Some of the frequently used instruments are Beck Depression Inventory (BDI), Center for Epidemiologic Studies Depression Scale (CES-D), General Health Questionnaire-28 (GHQ-28), Psychosocial Screen for Cancer (PSSCAN), PTSD Checklist, Depression, Anxiety, and Stress Scale (DASS), etc.

Growth in Adversity. The positive changes that occur due to traumatic illnesses have been interchangeably referred to as 'adversarial growth,' posttraumatic growth,' or 'benefit finding.' According to Tedeschi and Calhoun [127], a life-threatening illness, like cancer, trigger ample stress so that the very core of personal beliefs and meaning of life are shaken. The theorists have argued that it is not the event itself but the struggle one has to go through, which instigates the patient to critically evaluate one's place in the world, general worldview, and what is more valuable to them. Within these deliberate reflections and ruminations, a revised set of beliefs in congruence with new reality and changed circumstances is generated, making

their lives enriched and aligned with their values [127]. Studies have shown that individuals who undergo such changes exhibit a renewed urge to find meaning and purpose in life, a marked tendency to prioritize relationships, high sense of coherence, personal strength, spiritual and existential development, and adoption of compatible health-related behaviors.

Menger et al. [85] conducted a comprehensive scoping review of 28 qualitative and mixed-method studies published 1996 onward. Most of these studies were carried out in US, UK, Australia, and Iran and included cancer patients and survivors of breast, head and neck, hematology, bones, mixed, etc. The participants reported that the traumatic experience prompted them to prioritize and build constructive relationships, review their health-related behaviors and lifestyle, made them feel more self-efficacious, and as a result experienced spiritual change. Earlier, Shand et al. [118] performed a systematic review and meta-analysis on 119 published articles in between 1990 and 2012. Their findings showed a significant association of posttraumatic growth with positive re-assessment of events, optimism, social support, and religious and spiritual coping. Several studies have attempted to investigate the determinants of positive changes in cancer patients and survivors. For instance, clinical determinants emphasize upon the trajectories of posttraumatic growth in cancer patients over different time span after diagnosis. Generally, an increasing trend in growth is observed in patients till 18 to 24 months after diagnosis, flattening off thereafter [23], while few studies yielded a curvilinear relationship in growth of oncology patients [65]. On the other hand, investigations focusing on demographic determinants found considerable positive changes in young, married, more educated cancer patients, whereas significant psychosocial predictors of positive changes included 'active-adaptive' coping, optimism, enhanced social support, and higher emotionally expressive [23, 77, 118].

Screening. Positive changes in oncology can be assessed through various measures that will help the psycho-oncologist determine the needs of the patients. Posttraumatic Growth Inventory (PTGI) is a reliable and valid tool to determine five dimensions of positive changes including: 'new life possibilities,' 'relating to others,' 'appreciation of life,' 'personal strength,' and 'spiritual change' [127]. It consists of 21-items with 5-point response options. Higher scores indicate higher positive changes and posttraumatic growth. Other scales that can be used are Miller Hope Scale (MHS; [87]), Optimism Scale [94], Meaning in Life Scale (ML; [125]) etc.

Interventions. Considering the complexity of the disease, several evidence-based interventions and psychotherapies are available to provide psychological support to the cancer patient. An overwhelming literature indicates the efficacy of specific psychological modules in decreasing cancer distress, depression, and anxiety [144], improving health-related behaviors, treatment compliance [28], quality of life and well-being, better pain management [53] and neuro-immunological response [3], and in some instances slowing progress of disease and reducing risk of death [2].

Research also suggests that the unmet psychological needs of the cancer patients can add to the burden of utilizing medical services and increase medical costs in the process. Thus, it becomes imperative that the interventions should address every facet of psychological impact of cancer on patient's life.

Accordingly, psychotherapists and psychiatrists should be properly and fully trained on how to provide therapeutic services. A holistic approach would include understanding the beliefs of the patients associated with treatment method and preferences, fears related with hospitalization, life circumstances and liabilities, etc. [40]. In some cases, being knowledgeable about psychoanalytic and psychosexual development phases help psycho-oncologists to teach sustainable coping skills to the cancer patients. Psycho-analytic knowledge allows the psychologists to appreciate how memories of experiences and desires and unique defense mechanisms affect distinctive responses of the patients in the face of stresses [87].

Hutchison et al. [50] have presented a tiered model of psychosocial intervention for cancer based on community approach (Fig. 13.3). Each tier represents guideline for screening and intervention opportunities for cancer patients, survivors, and individuals in last stages of cancer.

Broadly, the evidence-based interventions can be categorized into two groups: traditional approaches that emphasize cancer distress management such as Cognitive Behavioral Therapy (CBT), Supportive Expressive Therapies, Mindfulness-Based Stress Reduction (MBSR) Therapy, Progressive Relaxation Techniques, and Pharmacotherapy approaches and contemporary approaches that target not only coping of negative emotions but also assist the patient in adjusting to new circumstances and improving quality of life such as Self-Management Intervention, Meaning Making Therapies, and Acceptance and Commitment Therapy

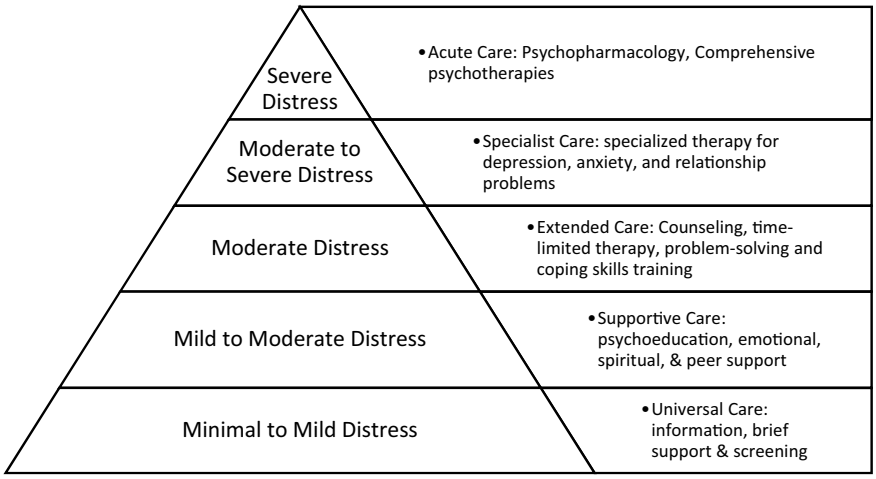

Fig. 13.3 Tiered model of psychosocial intervention in cancer: a community-based approach [50]

(ACT). Most of these interventions are provided in hospitals, clinics, or through E-services, in individual or group setting or family sessions by trained psychologists, psychiatrists, or medical staff. Description of the most frequently used interventions is presented below.

Cognitive Behavioral Therapy (CBT). Cognitive Behavioral Therapy (CBT) is considered as the most effective for the treatment of depressive and anxiety disorders [47] with a moderate effect size maintainable for 12–18 months and as efficacious as medication [36]. In addition, studies indicate that patients exposed to CBT have 50% less chances of relapse as compared to those receiving medication only. CBT has also shown efficacy for the management of fatigue, sleep disturbances, and physical symptoms in cancer patients.

CBT encompasses a variety of intervention methods including stress management, coping skills, problem-solving techniques, etc. The central focus of the psychotherapies is to confront the irrational thoughts and actions that underlie dysfunctional cancer depression and anxiety. For instance, a person who already holds rigid and dysfunctional beliefs such as 'If I can control my life, I will be safe' or 'The world is always predictable' is more likely to be shattered by the experience of cancer and may develop feelings of helplessness and hopelessness. Accordingly, CBT practitioners help the patient to recognize erroneous cognitions and learn constructive behavioral and cognitive strategies to deal with cancer distress. Though the steps involved in CBT are same for psychiatric anxiety and depression with and without cancer comorbidity, the CBT for cancer patients is flexible according to the type and stage of the disease, goals of the therapy are adjustable, and duration is trimmed as per physical requirements of the patient.

A typical CBT program for depression includes three step process: (a) *behavioral activation* (patients are trained to engage in pleasurable activities as depression restricts will to act), (b) *changing automatic negative thinking* (patients are taught to recognize depressive thinking co-occurring with negative moods and are trained in problem-solving or effective communication skills, etc.), and lastly (c) modify underlying irrational core beliefs. Brothers and colleagues (2011) incorporated elements of CBT with 'biobehavioral intervention (BBI)' to alleviate depressive symptoms in cancer patients, whereas BBI includes components of muscle relaxation, developing skills to communicate with oncological staff, psycho-education training, understanding barriers to changing, and maintaining healthy life style. The combined program is administered in 12–20 sessions and has been found to effectively reduce symptoms of major depression and improve quality of life in cancer patients.

Similarly, CBT programs for the management of anxiety are applied in successive steps but unlike CBT for depression, irrational beliefs are not addressed in this case. In the first step, the patients are trained to analyze the preceding activating events for excessive worrying, followed by the second step in which the patients are given training in muscle relaxation techniques to manage daily symptoms. In the third step, coping skills for identifying and dealing with worrying

behavior are taught, which the patient rehearses in the counseling setting through imagery and homework assignments. Greer et al. [38] developed a short CBT program comprising of six to seven sessions as an intervention strategy for anxiety in advanced cancer patients. The program includes four components: (a) the patient is provided information about the features of anxiety, CBT, and goals are set for therapy, (b) the patient is trained in relaxation therapy, (c) the patient receives training in coping with fears related with cancer through learning problem-solving or emotion-focused coping skills, and lastly (d) the patient learns to plan activities and tasks for functional independence. An RCT study indicated that relative to control group, the patients who received the short CBT program reported 35% less anxiety.

Recently research institutes are evaluating online cognitive behavioral therapy approaches, techniques that could both expand access and help cancer programs alleviate some of the stresses they face from an overwhelming number of patients needing emotional support. A stand-alone platform, i.e., beating the Blues, is a computerized cognitive behavioral therapy which incorporates eight online sessions that mirror what a patient review would be if he or she went to a psychologist [38].

Self-management Interventions. In the wake of cancer care paradigms, which have shifted the emphasis from didactic interventions to partnership between practitioners and patients, new models and strategies have been evolving to empower cancer patients and their families. Self-management (SM) programs, embedded in the cancer care continuum, have a history of nearly four decades of scientific investigation before their functional significance have been accepted [112]. These programs endorse active participation of patient and family for better physical and psychological health consequences. Accordingly, a substantial number of randomized control trial studies on oncology patients have established the effectiveness of SM in enhancement of quality of life, emotional regulation, psychological well-being, and commitment to treatment regimens [49, 120]. SM training has proven its utility for different age groups, types of cancer, stages of cancer, etc. (references need to be included). Another advantage of the programs is its potential as a preventative measure.

Also termed as ‘psychoeducation’ or ‘cognitive-behavior interventions,’ various definitions of SM are available, which are broader and inclusive in scope. For instance, for oncology patients, SM pertains to ‘awareness and active participation by the person in their recovery, recuperation, and rehabilitation, to minimize the consequences of treatment, promote survival, health, and well-being’ (cited by Brown et al. [13]).

Thus, the major philosophy behind SM is to change the perspective from ‘illness to wellness’ [72] through empowering the patient and family to become self-efficacious in the management of disease and changing life circumstances [114]. The model of SM was originally developed by Corbin and Strauss [18], which identifies three tasks relevant to cancer survivorship or terminal illness.

These include 'medical management,' 'daily life-role management,' and 'emotional management,' whereas each task is achieved through six skills: 'problem-solving, decision-making, resource utilization, forming partnerships with health care providers, and taking action' [72]. Because of its flexibility and diversity in goals, presently, there are various empirically valid SM programs available.

McCorkel et al. [81] reviewed the efficacy of different SM models for different phases of cancer treatment. For instance, they found that programs such as PRO-SELF Pain Control Program (PSCP; Miaskowski et al. [86]), Standard Nursing Intervention Protocol (SNIP, ref), Nurse Assisted Symptom Management (NASM; Giese-Davis et al. [34]) prompted patients to self-manage disabling symptoms experienced during chemo and radiotherapy. These programs were specifically designed to be administered by the nurses after appropriate training. Braden et al. [7] formulated an SM program to address emotional, psychological, and existential concerns originating from treatment experiences among women patients of breast cancer. The program titled as Self-Help Intervention Protocol (SHIP) has shown high effectiveness for increased knowledge about cancer, better adjustment, and self-care in patients. On the other hand, evidence indicates that the cancer survivors may also benefit from SM training after completing primary treatment. Psychoeducational programs have been found to effectively assist the patients to deal with fatigue, improve level of energy, and cancer-associated distress [123], habit of engaging in physical exercise and activity [21], and adjustment to new life demands [14] during post-treatment phase. Some studies also suggest including Internet-based SM programs in the management of psychological symptoms after surgery in oncology. For example, an Internet-based intervention, Project Onward, has also shown efficacy in reducing emotional dysregulation and depressive symptoms among cancer patients [26]. These interventions, though are limited to individuals who have internet facilities and are literate, provide an opportunity for individuals in working hours or who live in rural areas. Similarly, for advanced cancer care specific self-management modules provide means for coping with emotional dysfunctions, distress caused by severity of symptoms, negative thinking, and spiritual forebodings. One such program, labeled as ENABLE, has been formulated for advanced cancer patients living in rural areas. The program is targeted at helping the patients to improve their quality of life and relationships implemented through palliative care nurses. Recently, there is a general trend in SM to include cancer survivors as coaches and facilitators in the programs, which has found to be effective in the success of interventions.

Meaning Making Therapies. Among the most immediate emotional reactions, which emerge upon diagnosis of cancer and might prevail thereafter, include loss of hope and meaning in life and experience of debilitating fear of recurrence, death anxiety, etc. On one hand, hopelessness and meaninglessness are associated with loss of desire to live, suicidal ideation, and demand for euthanasia especially among terminally ill patients [12, 93], in contrast high hope and meaningfulness in adversity have been implicated in high tolerance for symptom severity [8] and improved quality of life, psychological well-being, and adjustment to disease and related challenges

[49, 102]. In a recent qualitative analysis, [90] explored the search for and process of meaning making in 119 oral-digestive cancer survivors whose ages ranged from 41 to 88. Their results indicated that around 43–73% of their participants had undergone the process of finding meaning in the adversity, with 53% accepting that cancer had changed their worldview while 43% reported the desire to understand the reasons for developing cancer.

The urge to find meaning and hope is recognized as a drive and mechanism to reduce the stress accompanying traumas such as cancer [18, 118]. Researches indicate that the probability for the experience of distress and growth may co-occur in cancer patients, if they find valuable meaning in their suffering, it will result in personal growth and well-being, however, if the patient engages in dysfunctional ruminations and meanings, the result may be distress and despair [118]. Other psychologists working in the domain of meaning making and trauma have also attempted to understand the processes involved in it. For instance, according to Frankl [30], the originator of Logotherapy or meaning making psychotherapy, suffering creates not only the need but also the means to search meaning in life so that the individual moves from a position of feeling worthless to worthwhile through appreciating the remaining life and valuing it. Thus, regarding meaningfulness as a state rather than a trait, Frankl's work suggested the significance of meaning making amendable through psychotherapy. In similar vein, Park and Folkman's [101] model enumerates sequential steps in the process of meaning making beginning with positive re-assessment of an event, to finding reasons for the occurrence of an event, to listing how the events have changed life, ending with discussing the meanings derived from the experience.

In the last couple of decades, several meaning making interventions for cancer patients, survivors, and terminally ill patients have been empirically evaluated and their effectiveness established [45]. For example, Meaning-Centered Psychotherapy (MCP), developed by Breitbart et al. [11], is a brief intervention applicable for cancer patients in curative as well as palliative care and can be administered in individual and group format by psychologists, psychiatrists, and even by Ph.D. Psychology scholars [128]. Ranging from 7 to 8 weekly sessions, MCP expands upon Viktor Frankl's theory of meaning searching to teach patients to find what is most valuable and meaningful to them and help connect with those sources of meaning. RCT studies have shown efficacy of MCP for increasing meaningfulness, feelings of hope, existential well-being, and quality of life and reducing depression and anxiety in advance cancer patients [10, 45, 111, 132] and stage III and IV solid tumor cancers patients [69]. Similarly, Managing Cancer and Living Meaningfully (CALM) therapy is also an evidence-based brief intervention especially effective for patients with metastatic cancer [40]. CALM is usually administered in six sessions, each comprising of 45–60 min, applied over 3–6 months. The intervention addresses four aspects including (1) 'symptom management and communication with healthcare providers,' (2) 'changes in self and relations with close others,' (3) 'sense of meaning and purpose,' and (4) 'the future and mortality.' Different trial studies have provided substantial empirical evidence for CALM in alleviating

death anxiety, depression, and improving existential and spiritual meaning in life [71, 109]. It is also delivered by trained professionals. Like MCP, CALM also address fears related to death.

Acceptance Commitment Therapy (ACT). Another intervention, which has swiftly made its place in the contemporary psychological approaches especially designed for oncology patients, is Acceptance Commitment Therapy (ACT), authored by Steve (1982). ACT provides a framework not merely for tackling distress but mechanisms for activating positive psychological health [43]. The intervention has received sufficient empirical support for its application in adversarial growth among cancer patients. ACT has been associated with increased quality of life, meaning making, living valued life, and relationship improvement. Some of the most recent researches, which provide evidence base for ACT in psycho-oncology are mentioned below.

Of particular interest is the study by Zhao et al. [145] who conducted a meta-analysis of 25 studies (eight non-randomized and 17 randomized control trial studies) to ascertain the impact of ACT on psychological and physical health in cancer patients. The findings indicated large effects for high psychological flexibility, sense of hope, quality of life, and low psychological distress. The analysis also suggested higher efficacy of ACT for younger people, countries belonging to eastern hemisphere, and longer duration sessions. Another psychological variable which appears to benefit from ACT is fear of cancer recurrence (FCR). For instance, [52] conducted randomized control trials on 91 female patients of breast cancer survivors falling in stages 1–3. Compared to usual care and survivorship programs, a weekly 2-h sessions for one and a half month of ACT yielded significant reductions in fear of recurrence. The results remained stable even after 6-month time period. In addition, ACT has also shown utility in emotional regulation of terminally ill cancer patients [116].

Grounded in relational frame theory (RFT; Hayes et al. [43]), which espouses language and development from behaviorists perspective, ACT consider suffering, grief, pain as inescapable experiences of being human and associated experiences of distress and emotional problems as healthy reactions to such experiences. The theory asserts that how much ever agonizing and tormenting the reality is, it cannot be changed. Therefore, the goal of ACT is not to avoid dysfunctional feelings and reality or change perception toward them but to achieve *psychological flexibility*. Psychological flexibility is described as the 'the ability to stay in contact with the present moment regardless of unpleasant thoughts, feelings, and bodily sensations, while choosing one's behaviors based on the situation and personal values' [47]. Putting in other words, ACT enables clients to become consciously aware and rooted in the present and to re-evaluate meaning in life developing a repertoire of responses which serves long term goals and not respond on the basis of their distressing emotions and thoughts. For example, the diagnosis of cancer may prompt a psychologically flexible person to embrace the feelings of anxiety

and stress, consciously re-evaluate what is valuable for him/her in life and respond accordingly.

The Acceptance and Commitment Therapy (ACT), also referred to as hexaflex model, comprises six components, which may be subsumed in three categories including (a) contact with the present moment and acceptance (client becomes attentive to present meaningful activities rather than ruminating about past or future fears of relapse; client acknowledges negative emotions such as anxiety, sadness, and becomes empowered), (b) cognitive defusion and self-as-context (client learns to differentiate between inner experiences and the truth, e.g., the thought that 'the doctor might have misdiagnosed' may be rephrased as 'I have the thought that doctor might have misdiagnosed', 'or 'I am devastated' may be substituted with 'I am having a thought that I am devastated'), and (c) values and committed action (clients reviews meaning in life and values and chooses actions, e.g., 'I am going to take care of myself in order to be a good parent').

One of the strengths of ACT lies in its transdiagnostic approach [57], that is, ACT has applicability for various types of cancers and in different time line of the disease, it does not need to be modified as per requirements of causes and/or diagnoses. Another strength of ACT is that it can be administered in psycho-oncological milieus by trained psychologists, mental health counselors, and social workers either in individual or group setting. However, certain limitations have also been reported for ACT for oncology patients. Most of the RCT studies lack heterogeneity in the characteristics of patients and generally include small sample size further partitioned in different groups. Thus, it is recommended that further studies including large and less homogenous samples will provide additional support for the applicability of ACT in this segment of population [67].

Mindfulness-Based Interventions (MBIs). Mindfulness-Based Interventions are increasingly being used in management of cancer distress, depression, anxiety, insomnia, fatigue, death anxiety, and pain associated with chemotherapy. Embedded in the Buddhism philosophy, the interventions are designed to equip the patients with a skill that helps them to live and be attentive in the present moment in an accepting and nonjudgmental way. The participants are trained to understand that their mental energy is being used in ruminating about the past or obsessing about the future, which is not letting one live in present and is causing depression or anxiety. Mindfulness-Based Stress Reduction (MBSR) Therapy and Mindfulness Based Cognitive Therapy (MBCT) are among some of the widely used interventions that have proved efficacy in psycho-oncology. Generally comprising of 8 weeks, the interventions include meditation, yoga, and exercises.

Different meta-analysis studies have attempted to establish the effectiveness of MBIs in emotional problems experienced by cancer patients through controlling inflammation and other biological processes. For instance, Haller et al. [42] in a meta-analysis of ten RCT studies consisting of 1709 breast cancer patients found significant impact of MBIs in reducing their depression, anxiety, fatigue,

and sleeping problems. However, the outcomes were not found to be effective after 6 months.

Pharmacotherapy. Pharmacotherapy has been traditionally employed to manage moderate to severe psychological distress. Clinical practice suggests the benefits and needs of antidepressants in oncology for treating anxiety disorders, depression, stress, adjustment disorders, or any other medications that can cause or imitate anxiety or depression. For depression treatment only few antidepressants (Mianserin and Fluoxetine) have been tested in oncology. Depression and anxiety can be linked to cytokine or immune system as evidences identifies how, for instance, antidepressants help prevent or reduce depressive symptoms after therapy interferon alpha.

The symptoms of chemotherapy, like insomnia, decreased appetite, are reduced by antidepressants, and it also acts as analgesics and treats depressive disorder. Also, it affects level of prostaglandin responsible for regulation of every component of cell microanatomy and physiology [70]. Recent researches have shown how antidepressants have the characteristics of an ideal anticancer medication which inhibits production of prostaglandin in a way to stop pathogenesis. Antidepressant improves sleeping pattern, appetite problems, and energy, and it has autonomous analgesic effect and enhances narcotic effect. Moreover, antidepressants aid in treating infections after radiation or chemotherapy by the help of its immune stimulative and antimicrobial effect [9].

13.3 Caregiver Burden and Fatigue: Interventions for Caregivers

Caregiving of cancer patients involve a variety of responsibilities in cancer care. The caregiver who might be a parent, spouse, sibling, child, or an extended family member not only has to look after the physical and emotional needs of the patient, accompany the patient for hospital visits, make sure that the treatment methods are properly followed but also has to take care of household responsibilities, and often have to go to work; the situation may be aggravated by the age, gender, economic resources, health issues, and severity of disease of caregiver and patient. All these activities for which the caregiver is usually untrained add to emotional baggage that the caregiver is already carrying because of cancer patient in family. Collectively, these experiences are termed as caregiver burden leading to fatigue and reduced quality of life.

Caregiver burden pertains to ‘distress that caregivers feel as a result of providing care’ [108]. According to Xia et al. [139], the psychological needs of the caregivers are often unattended, who themselves become ‘invisible patients.’ For instance, studies have yielded heightened levels of anxiety, depression, and sleep disturbances among caregivers (see, for instance, Jaarsma et al. [51] and Yang et al. [142]). Li et al. [67] has reported incidence rate of 12–59% of depression and 30–50% of anxiety in cancer caregivers. Moreover, these psychological reactions have

been observed more in caregivers as compared to the patient him/herself [37]. Other studies have found that caregiving also puts pressure on financial resources of the family and their physical health [75, 108] studied quality of life of caregivers of 212 cancer patients. They found that caregiving burden reduced almost 30% of their quality of life, especially caregivers of cancer patients whose mobility was perpetually declining while the education level of caregivers helped them to have a better quality of life. Yabroff and Kim [140] estimated the time spent by family members in caregiving activities of patients of different cancers. Based on a survey of more than 600 caregivers, the data indicated that on average they spent approximately 8 h daily, whereas 25% spent more than 16 h per day in cancer care. These studies recognize the need for psychological support for cancer caregivers.

Screening. Assessment of caregiver burden is steadily becoming the focus of psychological support in oncology as the significance of emotional distress of cancer caregivers is being recognized. One of the renowned scales used in this regard is Needs Assessment of Family Caregivers-Cancer (NAFC-C) Scale [57]. NAFC-C is a 27-item scale, with each item anchored on 5-point Likert scale. The scale assesses two dimensions, namely the 'importance of needs' and 'satisfaction of need fulfillment in the past 4 weeks.'

Interventions. Treanor [130] carried out a systematic review of 15 studies conducted from 2019 to 2020 on psychosocial interventions for cancer caregivers. His analysis suggested that psychoeducation intervention was mainly found to be useful in improving the quality of life and reducing distress among caregivers. Freudenberger [31] meta-analyzed 22 RCT studies, which had assessed the effectiveness of different interventions for cancer caregivers. Their findings indicated success rate for paired-intervention (including patient and caregiver), individual intervention, and group intervention. In addition, they found that for anxious caregivers, music therapy appeared to be quite effective in alleviating their symptoms while for depressed caregivers paired-intervention worked for improving their quality of life and reducing symptoms. Another meta-analysis of 29 RCT studies on family-oriented interventions for cancer caregivers obtained a small to medium beneficial effects. The results indicated small effects for reducing anxiety and caregiver burden, whereas for coping and family and marital relationships medium effects were observed [96]. However, another meta-analysis of 36 intervention studies using cognitive behavioral psychotherapies yielded non-significant effects for controlling the caregivers' mood disturbances [98]. Thus, it is being recognized that cancer caregiving is a complex experience, which needs a holistic model to understand the needs of caregivers of cancer patients. Another concern of such interventions is the duration till which it remains effective [6].

Recently, there is a trend in caregiver interventions to address the negative symptoms such as distress and anxiety while simultaneously focusing on growth-oriented variables such as self-efficacy and coping mechanisms [31]. In a study of 112 caregivers of gastrointestinal cancer patients, Nouzari and colleagues [97]

found significant positive changes in the caregivers associated with challenging circumstances. Accordingly, different intervention programs aim at providing psychological support to cancer caregivers, which can help them grow and cope effectively. Furthermore, these interventions vary across phases of disease and the relationship of the caregiver with the cancer patient or survivor.

Family members are definitely affected hugely by the patient as ailment of one family member affects every other family member and family as whole emotionally and mentally. Every family member reacts with fear and reinforces the interdependence of family in the situation of serious disease of one of the members. Families have difficulties coping to accept someone who is close to heart writhing in pain, so after the initial diagnosis, family is put under therapeutic interventions to develop cooperation between family members on different stances like dietary regime, medication, or possible disabilities associated with the ailment or death of patient. The patient is reconnected with the family, re-identifying their partner or parent's needs, developing a healthy relationship with children needs, overall learning along in the way how to live again and have a positive outlook on life.

13.4 Stress and Burnout: Interventions for Oncologists and Staff

According to Lazarus and Folkman [64], 'psychological stress is a particular relationship between the person and the environment that is appraised by the person as taxing or exceeding his or her resources and endangering his or her well-being.' Stress is described as 'the feeling of being overwhelmed or unable to cope with mental or emotional pressure' (mentalhealth.org.uk). Freudenberger in early 1970s introduced and described the term burnout as a work-related stress syndrome resulting from chronic exposure to job stress [31]. Stress and burnout are the most studied issues as compared to the compassion fatigue and moral distress in workplace [131].

Oncology hospital professionals (HPs) work under stressful conditions while recommending diagnosing and delivering complex treatments. Although oncological practice could be rewarding and deeply meaningful work, it is also a demanding and stressful area of medicine. Significance of burnout is indicated by its prevalence among oncology HPs as one of the most common manifestation of distress. For instance, it has been observed that 28–36% surgical oncologists, 35 and 38% medical and radiation oncologists, respectively, report job burnout [117]. In addition, research suggests that at least one in three physicians experience burnout at any given time which not only interfere with their own well-being but also may impact the quality of delivered care [24]. The correlation between job related stress and ill health of the HPs is frequently recorded, and poor health of HPs can lead to lack of focus and absenteeism leading to reduced efficiency. Existence of a correlation between work-related stress and poor health is indicative of the importance of providing effective stress-free environment for the HPs [59].

Christina [78] has presented a three-dimensional model of burnout comprising emotional exhaustion (EE), depersonalization (DP), and lack of personal accomplishment (PA), while emotional exhaustion refers to 'depleted emotional resources,' depersonalization is defined as 'the negative, cynical, or excessively detached response to other people,' and lack of personal accomplishment signifies 'decline in feelings of competence at work and productivity at work.' Maslach observed that these three types of responses reduce social and occupational functioning and impact psychological well-being of the employee.

Table 13.2 presents a summary of the symptoms and risk factors of job burnout.

Screening. Maslach Burnout Inventory (MBI) was based on Maslach's main three dimensions of burnout: EE, DA, and PA. It was developed as a research tool [79, 80] and was being used widely [113]. Not only researchers have been using MBI as a clinical tool, in Netherlands, MBI has been used as a diagnostic tool and is clinically validated [110]. Several new adapted versions of MBI as screening tools are now available to diagnose burnout in different groups and settings. For instance, Human Services Survey (MBI-HSS), General Survey (MBI-GS), Human Services Survey for Medical Personnel (MBI-HSS(MP), Educators Survey (MBI-ES), and General Survey for Students (MBI-GS(S) are some of the adapted versions. MBI-HSS(MP) and MBI-HSS use the nine-item EE scale to measure overextension and exhaustion, the 5-item depersonalization scale to assess impersonal response toward the care, treatment and service to the patient, and an eight-item PA scale to measure the feelings of competence. All items are scored on a frequency scale, from 0(never) to 6 (every day) [24]. MBI-HSS is the most commonly used and validated tool to diagnose burnout in clinicians and was found to be more accurate when compared with its abbreviated version of MBI, (Lim WY).

Table 13.2 Summary of symptoms and risk factors of burnout

Burnout symptoms*	**Physical**: Fatigue, physical and emotional exhaustion, headaches gastrointestinal ailments, sleeplessness, weight loss, hypertension, and myocardial infarction
	Psychosocial: Anxiety, depression, boredom frustration, low morale, irritability, and alcoholism and drug addiction could also be the manifestations of psychological burnout, marital difficulties, relationship problems
	Occupational: Emotional exhaustion, depersonalization, job turnover, impaired job performance, deterioration in the physician–patient relationship, and a decreased quantity and quality of care
Burnout risk factors**	**Individual**: Age (younger), gender (females), problematic relationships, personality factors (emotional dysregulation), spiritual concerns
	Organizational: Lack of control in decision-making, lack of support or conflicts with the colleagues, fairness, ethical issues, emotional distress because of work with sick and dying patients, workload exhaustion, lack of rewards (financial or appreciation), not enough personal time, conflicts with administration

Note *Vashon and Butow [131],** Yates and Samuel [143]

Interventions. Schaufeli and Enzmann [113] have classified the occupational stress management interventions in following categories:

1. Primary interventions aim to prevent burn out from developing
2. Secondary interventions aim to help staff members at high risk of burnout
3. Tertiary interventions aim to provide help to the staff already suffering from burnout and reduce the adverse effects.

To decrease the probability of burnout various individual strategies have been proposed. These may include developing an approach to dealing with end of the life care and death, creating balance between personal and professional life, taking time for recreation and hobbies, spiritual practices and personal reflection, keeping positive outlook, identifying professional goals and optimizing career fit, identifying and managing specific stressors to specific areas, identifying and reflecting on the personal priorities, and spending time on research [131]. Mackereth et al. [73] have proposed a 3C model for the HP working in the oncology and palliative care, where C refers to clinical supervision, counseling of the staff, and complimentary therapies.

1. Clinical supervision. Clinical supervision can offer time, space and supportive relationship to help the practitioners to reflect on their practice and professional development. It can be used as primary intervention in the organizations when it is considered mandatory for the staff and should not be offered as a tertiary intervention.
2. Counseling for the staff. It is suggested that in some cases staff is referred for counseling as a tertiary intervention, but gradual changes in attitudes have allowed accepting counseling as a helpful way of dealing with professional and personal stressors.
3. Complementary therapies like aromatherapy, acupuncture, massage, chair massage, and reflexology can provide a more acceptable physiological and psychological intervention for the staff [73].

By addressing burnout and fostering social support in the workplace, and programs like arranging staff retreats can help to improve coping skills, to reduce burnout and ensure the psychosocial wellness of the oncology nursing staff and can help in the retention of the staff [82]. Staff retreats has also been found to improve teamwork and leadership skills [60]. Structured journaling can be used as a psychosocial wellness tool. Adams and Putrino [1] conducted expressive writing workshops for patients with cancer and oncology health professionals and reported positive response from the participants. West et al. [134] analyzed 15 randomized trials, including 715 physicians and 37 cohort studies including 2914 physicians to analyze interventions to prevent and reduce physician burnout ascertained that both individual and organizational level strategies are needed for the prevention and reduction in burnout and for effective interventions for specific populations [134]. The individual-based strategies attempt to address the issues related

to person-job mismatch such as work load, financial benefits, discrimination followed by discussion with organization and colleagues, while in organization-based strategies, the management identifies the mismatches and involves the individuals to rectify the problems. Kleiner and Wallace have investigated time pressure as a predictor of oncologist burnout and its link to work-family conflict. It's suggested that to improve oncologist mental health, work-family conflict can be reduced by addressing time pressure in the work place. Interventions to improve mental health of oncologists, along with high workload, subjective time pressure should also be taken into account. Further investigation into greater flexibility to access personal time for oncologists is recommended.

Recently, mindfulness-based stress reduction programs have emerged as effective intervention to improve relaxation, self-care and work, and family relationships, although at times it generated feelings of restlessness, difficult emotions and pain [16]. Mindfulness-based stress reduction can have a positive effect on the work-related stresses suffered by the HPs [61]. This technique has been adapted from Buddhist tradition and is being used to alleviate a variety of mental and physical conditions [58] including burnout and improved mood. As defined by Epstein, a mindful clinician is the one who 'attends in a nonjudgmental way to his or her own physical and mental processes during ordinary everyday tasks to act with clarity and insight.'

To address the root cause of burnout, a change in the culture and stigma attached to it has to be brought about, which can be achieved by addressing burnout, depression and physician suicide, and it should start with the medical education [121]. The unique stressors associated with oncology clinicians are in addition to the stressors causing burnout and depression in medicine. Communication skills training (CST) can help the oncology professionals to enhance communications with their patients, which has a positive effect on their well-being of patients and health care professionals [89]. The review conducted to compare the CST programs and their effectiveness indicated that various CST courses are effective in improving HCP communication skills, but were unable to determine whether the results are sustained over time or whether consolidation sessions are necessary. Furthermore, no evidence to support the effects of CST programs on HPs burnout was found. Although CST courses have been found to significantly improve self-efficacy of HPs, who considered the acquired skills to be important [76].

Empathy is the backbone of patient-physician relationship and its importance in patient-physician communication is unquestionable. Enhanced empathy and mindfulness can increase physician's well-being which can lead to more effective patient care [25]. Empathy can be taught and nurtured within a system that is also empathic to the physician's needs [66]. Empathy is considered as the fundamental determinant of quality in medical care and empathic communication can improve the patient outcomes [95]. Research suggests that empathy protects against burnout and depression [106]. Burnout not only has a negative impact on the work quality, job commitment, and the health of the sufferer, but can also result in poorer outcomes. To protect against the deleterious effect of burnout, along with the causes,

the factors that can protect from it are also important. Positive emotions like empathy can be associated with decreased burnout and increased job satisfaction [4]. A study in oncology nursing staff in Iran has found that nurses with high work experience in the oncology ward had higher empathy score, official nurses had higher empathy score and lower burnout. The findings of the study revealed the importance of interventional programs to prevent burnout, and to increase empathy, which will improve patient-centered communication and outcome [126].

The increased awareness and acceptance of the underlying causes resulting in stress and eventually burnout has prompted researchers to find creative solutions and strategies. That, in turn, empowers the individuals and organizations to implement those strategies, to pursue well-being of its staff, which in turn is beneficial for the patients. It is recommended that interventions should be directed at both the individual and institutional levels, and in a balance between personal training and service responsibilities. The individual-prevention training should start early in the physician's careers to develop skill sets which make them less prone to burnout and perform better [135].

13.5 Conclusion

The impact of cancer on the individual, family, and practitioners is enormous. This chapter attempts to provide a precise understanding of cancer stress-related trajectories and evidence-based intervention strategies for the practitioners and mental health workers for the support of the cancer patients. However, it must be noted that the intervention plans need to take into account cultural requirements and barriers that might affect the effectiveness of psychological support.

Another area of upcoming concern is cancer care for children and adolescents. According to an estimate, 400,000 children and adolescents are diagnosed with cancer per annum in the world [138]. It has been further observed that while the survival rate for this age group is less than 30% in low to middle income countries, it is quite high in developed countries, which is more than 80%. This has resulted in a specialized field of 'Pediatric Psycho-oncology' that addresses not only the emotional needs of the child patients and their psychosocial care, but includes dedicated intervention programs for parents and related cancer caregivers.

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Nutritional Assessment in Cancer Patients

14

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14.1 Introduction

Cancer is a multifarious disease affecting the quality and expectancy of life through its deteriorating effects on physical and mental health. A cancer diagnosis significantly increases the malnutrition risk in patients. In a study, the incidence of malnutrition in cancer patients was reported as 51% of patients had nutritional impairment at the time of their initial medical oncology visit, and 9% were visibly malnourished [1]. Despite the abundance of research on the risk of malnutrition, focus on nutritional status assessment and nutritional intervention in cancer patients is an often overlooked part of medical care plans which leads to serious consequences and poor prognosis in patients.

The severity of malnutrition may vary dependent on the type, stage, and site of the cancer and the treatment regime type. Most commonly manifested forms

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of cancer-induced malnutrition are muscle wasting, weight loss, physical impairment, and sarcopenia. The etiology of malnutrition in cancer is complex. Primarily, it is due to reduced food intake, metabolic impairments, and increased energy and protein requirements because of cachexia [2]. These factors contribute to reduced therapeutic response and tolerance for the treatment (comprising of surgery, anticancer drugs, chemo- and radiotherapy), ultimately leading to negative clinical outcomes, longer hospital stays, side effects, adverse reactions, and toxicity to chemotherapy, post-operative complications, compromised immune system causing delayed healing of wounds, elevated risk of infections and increased mortality and morbidity [2].

Therefore, it is essential to recognize the changes that follow after cancer diagnosis to detect malnutrition early on in patients, and initiate nutrition therapy timely to ensure better treatment outcomes and survival rates [1]. Unfortunately, oncologists often neglect to screen cancer patients for risk of malnutrition. Currently, there is a pressing demand to integrate nutritional assessment as part of standard clinical care practices so oncologists can identify nutritional risks promptly and by referral to a dietitian, individualized intervention plan could be planned and implemented.

14.1.1 Nutritional Implications in Cancer

Cancer has severe implications in patients newly diagnosed or those undergoing treatment [3]. Reduced physiological and biological function, malnutrition, weight gain/loss, tiredness, and psychological disturbances are all common side effects of cancer treatment. The underlying explanation for high susceptibility toward malnourishment is the accelerated catabolism that is triggered by cancer and loss of appetite which leads to loss of muscle mass resulting in weight loss. On the surface, the explanation sounds simple; however, cancer malnutrition is a result of multiple and interrelated mechanisms and pathways. Additionally, there are more than hundred types of cancers, each of which impact the metabolic pathways and nutritional status differently. To gain a better understanding, we take a look into the effect of tumor and cancer treatments/therapies on body's metabolism.

14.1.1.1 Cancer-Induced Metabolic Impairments

The most prevalent indicators of malnutrition are weight loss and cachexia [2]. Reduced nutritional intake, metabolic inefficiency, and increased energy demand all contribute to unintended weight loss in cancer patients. Reduced food intake is the consequence of anorexia, which is induced by the tumor's altered appetite signals. The tumor disrupts hormone metabolism (ghrelin and leptin), which could lead to a decrease in appetite and dietary intake. It could also be linked to physical difficulties that limit intake in particular types of cancer. In addition, cancer causes metabolic changes such as elevated muscle proteolysis, altered carbohydrate, protein, and lipid metabolism, and a slowed inflammatory response.

Catabolism and Inflammatory cytokines
The tumor causes the body to be in a catabolic state, causing an imbalance in muscle protein production and degradation. Inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor (TNF) are elevated, have been found to possess a key function in the nutritional metabolism in cancer patients. IL-6 and TNF are inflammatory cytokines that are evidenced that they can have a direct catabolic effect on skeletal muscle and adipose tissue through the ubiquitin proteasome pathway [4]. Cancer is characterized by impairment of human inflammatory regulation process. Via the ubiquitin-proteasome pathway and autophagy, protein synthesis and degradation are regulated by hormones and cytokines [2]. The upregulation of the proteasome pathway by catabolic stress has been linked to muscle atrophy in various animal tumor model. Muscle protein's myofibrillar components are primarily broken down in the ubiquitin- Many hormones and cytokines influence production of proteins and their break down via ubiquitin-proteasome pathway and autophagy [2]. In many animals' tumor models, catabolic stress has been connected to the activation of the proteasome pathway, which has been associated to muscle atrophy. Muscular strength declines as a result of this pathway [5]. TNF-α is thought to activate nuclear factor (NF-kB) and induce protein degradation through a route independent from the ubiquitin-proteasome system. In cachexia, uncontrolled inflammation can result in a higher resting metabolic rate, which raises the demand for sources of protein and energy even more [6]. In this sense, IL-6 is also thought to have a significant function. According to one study, elevated IL-6 levels were linked to reduced skeletal muscle and fat mass, and also the elevated tumor burden [7]. IL-6 raises acute phase reactants like CRP in cancer patients and is linked to muscle atrophy [8].

Impaired Carbohydrate metabolism: Carbohydrate metabolism dysfunction commonly occur in cancer patients. Cancer cells require a constant supply of glucose to grow uncontrollably, which is provided through liver gluconeogenesis, which uses lactate formed by tumor cells. The cancer cells use the majority of this glucose. This explains why cancer patients require a high level of energy. Higher levels of insulin-like growth factor-1 are caused by cancer-induced muscle atrophy, which can lead to insulin resistance [9]**.**

Impaired fat metabolism: Cancer patients' lipid metabolism has been demonstrated to be affected in studies. Cancer-related cachexia is caused by the loss of adipose tissue as a result of metabolic changes [2]. Triglycerides accumulate in adipocytes' cytoplasm. Patients with cancer-related cachexia had higher levels of free fatty acid and glycerol from triglycerides [10]. A study found that cancer patients who were losing weight had higher levels of lipolysis (due to hormones, pro-inflammatory cytokines, and lipid mobilization factor) and fat oxidation [11].

Cachexia develops as a result of these conditions (anorexia and metabolic abnormalities). Cachexia is characterized by rapid weight loss, loss of fat and muscle, and an increase in catabolism. Even with increasing food quantities, weight

loss induced by cancer cachexia results in greater weight loss, muscular wasting, and disability. To put it in other words, cancer cachexia has always been irreversible. Lymphomas, leukemia, breast cancer, and soft tissue sarcoma have the least prevalence for weight loss, whereas pancreatic and stomach cancer have the highest occurrence, with roughly 85 percent of patients developing cachexia [12]. Sarcopenia, or the slow loss of muscle mass, is a closely linked condition. It's thought to be a crucial factor in cancer patients' prognosis. It's also linked to physical dysfunction, chemotherapeutic toxicity, and prolonged hospital stays after surgery [2].

Etiological factors of unintended weight loss	Side effects and implications
Tumor presence	• Anorexia • Abnormal hormonal (leptin and ghrelin) metabolism • Compromised GI tract integrity (perforation, obstruction etc.) • Disease induced anxiety and depression • Imbalances in fluid and electrolyte levels • Malabsorption • Maldigestion
Impaired metabolism	• Changed Resting Energy Expenditure (REE) • Increased Proteolysis • Increased Gluconeogenesis to meet high glucose demands • Impaired fat metabolism • Increased inflammatory cytokine
Treatment	Depending on treatment type (surgery, radiation, chemotherapy): • Dysphagia • Gastrointestinal complications like constipation, nausea, diarrhea etc. • Mucositis • Side effects caused by infections

14.1.1.2 Metabolic Changes Caused by Cancer Treatment

Therapy and treatment regimen of cancer have been seen to create a negative effect on patient's nutritional status. Surgery, radiation therapy, chemotherapy, and immunotherapy are the commonly used treatment methods. Cancer treatment plan is individualized for patients dependent on the type, site, and stage of the cancer. Most cancers, however, require surgery in the end [13].

Malnutrition prior to surgery has been linked to lower survival rates. It is therefore crucial to manage malnutrition before surgery in patients. Research has found patients of head, neck, stomach, and bowel cancer as being more likely to be malnourished [13]. Furthermore, surgery has nutritional implications and side effects that greatly affect gastrointestinal function [13]. Use of cytotoxic agents also has toxic side effects in patients, making them weak and reducing their appetite. The

table below provides an overview of the nutritional complications and side effects of different types of cancer treatments [13].

Treatment type	Location/site	Nutritional implications (side effects)
Surgery	Oropharyngeal resection Esophagectomy Gastrectomy Intestinal resection (Jejunum, Ileum)	Chewing and swallowing difficulties Early satiety, regurgitation Malabsorption, Vit. B12, Vit. D deficiency, early satiety, dumping syndrome, hypoglycemia Maldigestion, malabsorption
Radiation	Oropharyngeal area Lower neck Abdomen/pelvis region	Anorexia, fatigue, Xerostomia (dry mouth), dysphagia, Taste and smell alterations, mucositis, osteoradionecrosis, odynophagia, trismus Esophagitis, dysphagia, esophageal reflux, nausea, vomiting Acute/chronic bowel damage, diarrhea, maldigestion, malabsorption, bloating, abdominal cramps, gas, nausea, vomiting, lactose intolerance, fatigue, colitis, strictures, ulcerations, fistulization, hematuria
Chemotherapy	Targets rapidly dividing cells of cancerous organ Effects GI tract	Nausea, early satiety, Anorexia, altered smell, vomiting, constipation or diarrhea, Xerostomia Myelosuppression mucositis, esophagitis, stomatitis and infection
Immunotherapy	Immune cells	Fever, nausea, vomiting, anorexia, asthenia

14.2 The Need for Nutritional Assessment

Several studies reveal that most patients presenting with cancer to an oncologist are at malnutrition risk or are malnourished already [1]. Cancer related malnutrition can present serious consequences if left untreated. According to one research, Malnutrition causes around 20% of cancer patients to die rather than the disease itself [14]. A study conducted between 1972 and 2017 investigated 3,779 patients having colorectal cancer. Results showed that the underweight group had a substantially poor survival rate in general in comparison to the patient group with normal weight in advanced stage (III and IV) of cancer [15]. As discussed above, cancer-caused malnutrition is attributed to multiple factors, including the metabolic alterations caused by the tumor and the effects of the treatment methods. A 2019 report on cancer in Europe estimated that around 50% of patients with gastrointestinal cancers, over 45% with cancers of head and neck region, and over 40% of patients with lung cancer are malnourished [16].

Cancer treatments also have their side effects which inevitably places the patient at an increased risk for malnutrition and poor treatment outcomes. An Ethiopian study that assessed 281 patients receiving chemotherapy found that 58.2% had malnutrition [17]. Up to 80% of the patients undergoing radiotherapy treatment to the head, neck, or esophagus developed mucositis, and experienced reduced food intake and loss of weight. Likewise, pelvic region radiotherapy was accompanied by symptoms of GI in up to 80% of patients [18]. The importance of nutritional assessment and nutrition interventions in cancer is underestimated by patients and in clinical settings. Only one out of every three cancer patients received nutritional assistance in Europe, according to Hospital Research [1]. However, recent research has brought the risk and impact of cancer-induced malnutrition into spotlight. There have been calls for action all around the globe to pair nutritional therapy with oncologic treatments for better outcomes and survival rates.

The World Health Organization (WHO) report states that appropriate nutritional care helps cancer patients respond to treatment better and overcome their illness [19]. A study from the Netherlands revealed that cancer patients receiving nutritional interventions ended up surviving around 6 months longer than patients who did not [20]. Even today, the use of nutritional therapy in oncology is inconsistent in oncological practice around the world. One possible factor being the lack of uniform standards and guidelines for cancer nutrition. Recently, nutritional therapy application in Hungary has been legally regulated. Updated European guidelines on cancer patient nutrition published in 2017 are available for physicians' assistance [21]. Moreover, ESPEN-based nourishment sustenance rules for grown-up disease patients and rules gave by the Academy of Nutrition and Dietetics (AND) on healthful administration for malignant growth patients in escalated care and walking settings bring issues to light of expanded hunger and unhealthiness. consequences for treatment reaction, forecast, and endurance. Such information assists doctors with remaining educated regarding progressed disease care techniques.

Furthermore, malnutrition is associated with higher healthcare costs since malnourished patients usually require longer hospital stays. Multiple studies have shown nutritional therapy to be cost effective. Hence, nutritional support addressing individual needs of cancer patients is not only an effective strategy for improving prognosis but also for reducing the economic burden. Therefore, there is a dire need for better nutritional screening and assessment practices so that malnutrition can be prevented, detected, and managed at the earliest possible opportunity. A thorough patient's nutritional status assessment should be made an integral part of the treatment and care plan in cancer. The physician, or dietitian, then must critically interpret the information obtained from the assessment to determine nutritional risks and issues, and design individualized interventions to address those issues.

14.3 Nutritional Assessment

The combined processes used for collection of data related to nutrition are known as the nutritional assessment. Its primary goal is identification of the nutritional state, risks of nutrition-related complications, and the early detection of any changes in the dietary patterns and nutritional health. A through, in-depth evaluation strategy in cancer patients is required to give a complete picture of the nutritional state in individuals. Multiple components of nutritional assessment are anthropometric measurements, clinical evaluation, laboratory assessment, biochemical markers assessment, and dietary evaluation. The following sections discuss these in detail.

14.3.1 Anthropometric Measurements

Anthropometric measurements analyze normal growth and development of an individual in all age groups. It is a non-invasive technique for the nutritional status assessment of a person and is helpful in detecting both the nutritional inadequacies and excesses [22]. It is a quantitative measure of all the body composition, body size, as well as that of body proportion. Though its use in cancer patients is debatable as its results can be affected by multiple factors such as edema which may result in an increase in body weight, yet it is considered an indispensable part of the assessment of the nutritional state. Commonly applied and validated anthropometric measures for nutritional status evaluation in cancer include weight, height, BMI, skinfold thickness, Midarm muscle circumference, and calf circumference (for older population).

14.3.1.1 Body Mass Index (BMI) and Weight Loss %

The usual method for the weight assessment is BMI which is calculated through the formula

$$BMI = \frac{Weight\,(in Kilograms)}{Height(m)^2}$$

Classification of weight status	BMI values (Kg/m^2)
Underweight	<18.5
Normal	18.5–24.9
Overweight (pre-obese)	25–29.9
Obesity class I	30–34.9
Obesity class II	35–39.9
Obesity class III	>40

The final malnutrition score in BMI is <23 kg/m^2, a BMI score of <21 kg/m^2 represents a risk of possible malnutrition, whereas the BMI of <19 kg/m^2 shows an even greater risk of malnutrition. The cut-off point of malnutrition is <18.5 kg/m^2. Measurement of weight and height to find out BMI, combined with weight loss percentage is an effective way to discern the nutritional status and the severity of the metastases. A study was conducted by comparing the BMI and WL% of the cancer patients, and it was concluded that the chances of survival increased with the increase in BMI and decreased with increase in the weight loss percentage. This shows that a nutritional intervention plan to increase the weight of the person may help in increasing the chances of survival [23].

>10% of WL in three to six months is a critical indicator of malnutrition, whereas a WL% between 5 and 10% is also clinically significant and requires continuous observation, especially if it is accompanied by loss of function [24].

14.3.1.2 Skinfold Thickness

A non-invasive method for body fat estimation is the skin fold thickness (SFT); the skin consists of two layers of subcutaneous fat where almost 50% of the body fat is. The subcutaneous fat is measured on multiple sites including the bicep, tricep, subscapular, and suprailiac region, with the best site of assessment being the triceps region because of low risks of edema. The Lange skinfold caliper is used for the measurements [22]. National Health and Nutrition Examination Surveys I and II give the standard measurements of skinfold thickness; however, the measurements may vary depending on the body type and may increase with age.

The following table shows standard minimum values for SFT, MAC, and MAMC.

	Triceps skinfold thickness (mm)	Muscle arm circumference (mm)	Midarm muscle circumference (mm)
Men	12.5	29.3	25.3
Women	16.5	28.5	23.2

14.3.1.3 Mid-Arm Muscle Circumference/Calf Circumference

Lean body mass is calculated by mid-arm muscle circumference (MAMC) which suggests muscle mass and caloric adequacy. It is measured by a flexible tape of fiberglass at the midpoint marked on the left upper arm. SFT and MAC can be used for the calculation of the true muscle mass since the total circumference comprises of two layers of skinfold. The formula for the MAMC calculation is [22].

$$MAMC = MAC - (3.14 \times SFT)$$

Protein deficiency and a negative nitrogen balance can be indicated by a decrease in muscle mass due to muscle wasting. A reference graph is available for age and gender for comparison [22]. Sarcopenia can be detected by changes in the calf circumference. The standard for measuring the calf circumference by

the International Society for Advancement of Kinanthropometry is that the subject's knee should be flexed at 90°, the subject should either be seated or right foot could be placed at an elevated point to allow flexion, and the calf muscle should be relaxed when taking the measurement.

Optimal cut off values of calf circumference for predicting Sarcopenia [25].

	Men			Women		
	Class II	Class I	AWGS sarcopenia	Class II	Class I	AWGS sarcopenia
SMI (Kg/m^2) reference values for sarcopenia	6.87	7.77	7	5.46	6.12	5.4
Optimal cut-off values of calf circumference (cm)	34.1	36.8	34.3	32.8	33.6	32.8

14.3.2 Clinical Evaluation

Signs specific to malnutrition and overall nutritional health can be revealed via clinical examination. Individuals with apparent characteristics of malnutrition may be identified by clinical assessment; conversely, those with subclinical or borderline malnutrition may be disregarded. Medical history and physical examination are the two basic components of clinical evaluation [22].

14.3.2.1 Medical History

Medical history reveals issues that cause organ failure, chronic diseases, changed GI function and absorption capacity, increased metabolic demands, and level of physical activity [26]. Malnutrition implications may be comprehended when current drugs are evaluated. It also investigates the patient's complications as a possible consequence of chemotherapy or radiation, etc.

14.3.2.2 Physical Examination

This entails a thorough examination of the physical indications and symptoms of muscle wasting, low body fat, evidence of chronic liver illness, nutrient deficiency signals, and physical endurance.

Chronic Protein Energy Malnutrition (PEM) can be identified through physical examination: Elia and Stratton [24].

- **Muscle**: Muscle wasting is frequently seen in several regions of the body, including face (temporalis muscle), arms (deltoids), and the legs (quadriceps in the proximal leg).

- **Fat**: The presence of loose skinfolds and little or no clinically observable subcutaneous fat indicates fat loss.
- **Fat and muscle**: The presence of hollow buttocks and cheeks, as well as noticeable bony outlines and bulges, is due to fat and muscle loss.
- **Skin**: The skin is delicate, dry, and flaky, with indications of dehydration or edema.
- **Specific nutrients**: Clinically, specific nutrient, vitamin, and trace element deficiencies can also be observed.

The following table outlines some common nutrient deficiencies and their physical signs and symptoms. Table adopted from [24].

Physical signs	Nutrient deficiency	Condition
Frail hair, depigmentation of skin	Protein	Kwashiorkor
Dry scaly skin	Essential fatty acids	
Easily bruised skin	Vitamin C Vitamin K	
Blurry vision Corneal scars Bitot's spots/white spots on conjunctiva	Vitamin A	Keratomalacia, Xeropthalmia
Swollen wrists, knock knees, bone pain, Muscle weakness, muscle cramps, paresthesia	Vitamin D	Rickets in children, Tetany, Proximal myopathy, Hypocalcemia
Uncoordinated, staggered gait Blindness	Vitamin E	Ataxia Retinopathy
Numbness, tingling in feet, legs, hands, and arms	Vitamin B1	Peripheral neuropathy
Atrophic tongue, smooth, shiny, and dry appearance of tongue	B vitamins	Atrophic glossitis
Swelling/puffiness under skin in arms or legs	Vitamin B1/protein	Edema
Ulcers, blisters, red patches in mouth; burning sensation in mouth	B vitamins/iron	Atrophic stomatitis
Fatigue, Lethargy, Paresthesia, difficulty in producing coordinated body movements	Folic acid Vitamin B12	Anemia (megaloblastic and macrocytic) Peripheral neuropathy, degeneration of spinal cord
Poor stamina, lethargy, pale skin	Iron	Hypochromic anemia
Peristomal rashes, impaired taste, and smell, diarrhea	Zinc	
Swelling in neck, fatigue	Iodine	Goiter
Weakness, fatigue	Copper	Anemia with megaloblastic features

Additionally, there are multiple screening tools that have been developed and utilized for initial clinical evaluation. These tools provide an insight into the nutritional status of the patient and indicate if there's a need for a detailed nutritional evaluation.

14.3.2.3 Use of Screening Tools for Clinical Examination

There are several screening tools available, each with its own set of characteristics. ESPEN guidelines for patients with cancer recommends and validates these tools. Commonly used screening tools include MST, PG-SGA, NRS (2002), and the Mini- Nutritional Assessment Short form. Patients can be classified as well-nourished, moderately malnourished, or severely malnourished using a systematic bedside subjective global assessment, SGA, of nutritional status [26]. However, **MST and the PG-SGA** are the foremost validated screening tools for use in cancer patients. The PG-SGA is a tool for assessment which includes screening components; on the other hand, the MST is a screening tool which makes it fast and easy in usage.

14.3.2.3.1 *Malnutrition Screening Tool (MST)*

Questions about appetite, food intake, and recent weight loss are included in it. The sum of these categories yields a score ranging from 1 to 5, with a 2 indicating that action is required. The MST has been thoroughly verified in both inpatient and outpatient settings [27].

MALNUTRITION SCREENING TOOL

1. Have you lost weight without trying?	
No	0
Unsure	2
2. If yes, how much weight (in kilograms) have you lost?	
1-5	1
6-10	2
11-15	3
>15	4
Unsure	2
3. Have you been eating poorly because of a decreased appetite?	
No	0
Yes	1

Total MST Score:

14.3.2.3.2 *Nutrition Risk Screening (2002)*

It is a screening tool proposed by ESPEN. The objective behind its development was to determine the probability of malnutrition in hospitalized patients. This information helps identify patients who require nutritional support for better treatment outcomes. It is easy to use, which makes it practical in clinical settings.

It consists of an initial screening that includes BMI, weight loss history, nutritional intake, and disease stage. Final screening is done if any one of the components of initial screening is positive. It involves assigning scores (ranging from 0 to 3) based on severity of nutritional status impairment, disease severity, and age. Out of a total of 7 points, a score of ≥ 3 means the patient is at "nutritional risk" [27]. A study showed the NRS 2002 score to be sufficient for oncology patients in relation to the recognized prognostic factors (type of cancer, symptoms, and performance status) [28].

14.3.2.3.3 *Mini Nutritional Assessment Short (MNA) Form*

It is a smaller and quicker-to-use version of the MNA. It was originally developed to evaluate the nutritional condition and risks in the older aged population living in nursing homes or hospitals. The MNA short form has a total of six items that assess BMI, stress level, dietary intake, mobility, weight loss and anthropometric measurements. A score of ≤ 11 out of 0–14 indicates malnutrition risk. It has a "fair" validity in the oncologic population [27].

14.3.2.3.4 *Patient-Generated-Subjective Global Assessment (PG-SGA)*

It was created particularly for cancer patients and has been acknowledged by the American Dietetic Association's Oncology Nutrition Dietetic Practice Group as the gold standard for nutrition assessment of these patients. Stress, GI symptoms, functional capacity, physical symptoms, weight and diet history and so on are all factored [27]. Despite PG-SGA being more user-friendly and providing benefits such as identifying treatable nutrition-related symptoms via patient involvement, it might not be viable because the patient-generated section tends to rely on patient literacy who might be hesitant to fill out extra documentation. Furthermore, in order to avoid conducting the physical exam and adding to their workload clinical professionals usually avoid using these tools. The following images show the PG-SGA forms that are filled by collaborative efforts of patient and nurse/physician.

Scored Patient-Generated Subjective Global Assessment (PG-SGA)

Patient ID Information

History: boxes 1-4 are to be completed by the patient.

[1-4 are referred to as the PG-SGA short form]

1. Weight

In summary of my current and recent weight:

I currently weight about _____ pounds.

I am about_____ feet _____ tall.

One month ago I weighed about _____ pounds.

Six months ago I weighed about _____ pounds.

During the past two weeks my weight has:

- ☐ decreased (1)
- ☐ not changed (0)
- ☐ increased (0)

BOX 1 ☐

2. Food Intake: As compared to my normal intake, I would rate my food intake during the past month as:

- ☐ Unchanged (0)
- ☐ More than usual (0)
- ☐ Less than usual (1)

I am now taking:

- ☐ Normal food but less than normal amount (1)
- ☐ Little solid food (2)
- ☐ Only liquids (3)
- ☐ Only nutritional supplements (3)
- ☐ Very little of anything (4)
- ☐ Only tube feedings or only nutrition by vein (0)

BOX 2 ☐

3. Symptoms: I have had the following problems that have kept me from eating enough during the past two weeks (check all that apply):

- ☐ No problem eating (0)
- ☐ No appetite, did not feel like eating (3)
- ☐ Vomiting (3)
- ☐ Diarrhea (3)
- ☐ Nausea (1)
- ☐ Dry mouth (1)
- ☐ Constipation (1)
- ☐ Smells bother me (1)
- ☐ Mouth sores (2)
- ☐ Feel full quickly (1)
- ☐ Things taste funny or have no taste (1)
- ☐ Fatigue (1)
- ☐ Problems swallowing (2)
- ☐ Pain; where? (3) _____
- ☐ Other** (1) _____

(**Examples: depression, money, or dental problems)

BOX 3 ☐

4. Activities and Function:

Over the past month, I would generally rate my activity as:

- ☐ Normal with no limitations (0)
- ☐ Not my normal self but able to be up and about with fairly normal activities (1)
- ☐ Not feeling up to most things, but in bed or chair less than half the day (2)
- ☐ Able to be little activity and spend most of the day in bed or chair (3)
- ☐ Pretty much bed ridden, rarely out of bed (3)

BOX 4 ☐

Additive score of boxes 1-4 ☐ **A**

The remainder of this form is to be completed by your doctor, nurse, dietitian, or therapist.

Scored Patient-Generated Subjective Global Assessment (PG-SGA)

Worksheet 1-Scoring Weight (Wt.) Loss

To determine score, use 1-month weight data if available. Use 6-month data only if there is no 1-month weight data. Use points below to score weight change and add one extra point if patient has lost weight during the past 2 weeks. Enter total points:

Wt. loss in 1-month	*Points*	*Wt. loss in 6-month*
10% or greater	4	20% or greater
5-9.9%	3	10-19.9%
3-4.9%	2	6-9.9%
2-2.9%	1	2-5.9%
0-1.9%	0	0-1.9%

Numerical Score from Worksheet 1 ☐

Additive score of the Boxes 1-4 (See page 1) ☐ A

5. Worksheet 2- Disease and its relation to nutritional requirements

All relevant diagnoses (specify) _____

Primary disease stage (circle if known or appropriate) I II III IV Other_____

One point each:

☐Cancer ☐AIDS ☐Pulmonaryorcardiac cachexia ☐Presence of trauma

☐ Presence of decubitus, open wound or fistula ☐ Age greater than 65 years

☐ Chronic Renal Insufficiency **Numerical score from Worksheet 2** ☐ B

6. Worksheet 3- Metabolic Demand

Score for metabolic stress is determined by a number of variables known to increase protein & calorie needs. The score is additive so that a patient who has a fever of >102degrees (3 points) and is on 10mg prednisone chronically (2 points) would have an additive score for this section of 5 points.

Stress	**none (0)**	**low (1)**	**moderate (2)**	**high (3)**
Fever	no fever	>99 and <101	≥101 and <102	≥102 [**Numerical score** C
Fever duration	no fever	<72 hours	72 hours	>72 hours **from Worksheet 3**] ☐
Corticosteroids	no corticosteroids	low dose (<10 mg prednisone Equivalents/ day)	moderate dose (>10 and <30mg prednisone equivalents Per day)	high dose steroid (≥30mg prednisone equivalents/day)

7. Worksheet 4- Physical Exam

Physical exam includes a subjective evaluation of 3 aspects of body composition: fat, muscle, & fluid status. Since this is subjective, each aspect of the exam is noted for degree of deficit. Muscle deficit impacts point score more than fat deficit. Definition of categories: 0=no deficit, +1=mild deficit, +2=moderate, +3=severe.

Muscle Status:				
Clavicles (pectorais&deltoids)	0	1+	2+	3+
Interosseous muscles	0	1+	2+	3+
Thigh (quadriceps)	0	1+	2+	3+
Global muscle status rating	**0**	**1+**	**2+**	**3+**
Orbital fat pads	0	1+	2+	3+
Triceps skin fold	0	1+	2+	3+
Global fat deficit rating	**0**	**1+**	**2**	**3+**

Fluid Status:				
ankle edema	0	1+	2+	3+
sacral edema	0	1+	2+	3+
Global fluid status rating	0	1+	2+	3+

Numerical Score from Worksheet 4 ☐ D

Total PG-SGA Score

(Total numerical score of A+B+C+D above) ☐

(see triage recommendations)

Global PG-SGA Rating (A, B, or C) = ☐

Clinician Signature_____ **RD RN PA MD DO Other** ____ **Date**_____

Worksheet 5-PG-SGA Global Assessment Categories

	Stage A	**Stage B**	**Stage C**
Category weight	Well-nourished	Moderate/ suspected Malnutrition	Severely malnourished
	No weight loss OR recent non fluid wt. gain	≤5% loss in 1 month (≤10% in 6 months) OR progressive weight loss	>5% loss in 1 month (>10% in 6 months) OR progressive weight loss
Nutrient Intake	No deficit OR significant recent improvement	Definite decrease in intake	Severe deficit in intake
Nutrition Impact Symptoms (NIS)	None OR significant recent improvement allowing adequate intake	Presence of NIS (Box 3 of PG-SGA)	Presence of NIS (Box 3 of PG-SGA)
Functioning	No deficit OR significant recent improvement	Moderate functional deficit OR recent deterioration	Severe functional deficit OR recent significant deterioration
Physical Exam	No deficit OR chronic deficit but with recent clinical improvement.	Evidence of mild to moderate loss of muscle mass &/ or muscle tone on palpation &/or loss of SQ fat	Obvious signs of malnutrition (e.g.: severe loss of muscle, fat, possible edema).

Nutritional Triage Recommendations: Additive score is used to define specific nutritional interventions including patient and family education, symptom management including pharmacologic intervention, and appropriate nutrition intervention (food, nutritional supplements, enteral or parenteral triage).

First line nutrition intervention includes optimal symptom management.

Triage based on PG-SGA point score

0-1 No intervention required at this time. Re-assessment on routine and regular basis during treatment.

2-3 Patient & family education by dietitian, nurse, or other clinician with pharmacologic intervention as indicated by symptom survey (Box 3) and lab values as appropriate.

4-8 Requires intervention by dietitian, in conjunction with nurse or Physician as indicated by symptoms (Box 3).

≥9 Indicates a critical need for improved symptom management and nutrient intervention options.

Besides the screening and assessment tools discussed above, some other frequently used nutritional screening tools include, Nutrition Risk Index (NRI), Malnutrition Universal Screening Tool (MUST), and. The table below provides an overview of the factors evaluated by these tools and their validity for use in cancer patients [2].

Screening/assessment tool	Evaluvation items	Recommended for	Validity for use in oncology
Malnutrition Screening Tool (MST)	Appetite, weight loss, weight loss percentage	Adults	Good
Mini Nutritional Assessment (MNA) short form	BMI (Kg/m^2), calf circumference (cm), food intake, mobility, stress, neuropsychological problems	Elderly	Fair
Malnutrition Universal Screening Tool (MUST)	BMI (Kg/m^2), food intake, weight loss percentage	Adults	Good (for patients undergoing radiotheraphy) Poor (for inpatients)
Nutrition Risk Screening (2002)	Initial screening: BMI (Kg/m^2), nutritional intake, weight loss, disease severity	High risk hospitalized patients	Fair to good
Patient-generated-subjective-global-assessment (PG-SGA)	Patient: weight, food intake, symptoms, activities and function. Clinic staff: diagnosis, metabolic demand, physical exam	Oncology patients	Good
Nutrition Risk Index	Serum albumin levels, weight NRI = 1.519 (serum albumin g/dL) + 41.7 (current weight/usual weight)	Adults and elderly	–

The approach in screening cancer patients for nutritional risk should be adapted to each medical institution's specific needs, taking into account the applicability of each tool [2]. Despite the lack of strong data indicating the clinical benefits of screening methods for nutritional risks in cancer patients, no research has proven their ineffectiveness either. In reality, based on the cancer type and treatment, there are certain advantages to assessing for nutritional risk. Consequently, the ESPEN suggests that BMI, weight change and nutritional intake, be evaluated periodically beginning with the cancer diagnosis and continuing until the clinical situation is stable [2].

14.3.3 Laboratory Assessment

Laboratory tests may indicate certain nutrient deficiency conditions, such as anemia, iron insufficiency, or protein deficiency. Before clinical or anthropometric symptoms, biochemical examinations provide the earliest indication of dietary abnormalities [29]. Because these tests are unique to the nutrient under investigation, a clinical suspicion of a deficiency must be present in order to perform the proper evaluation. The most critical concern and predictor of the likelihood or severity of malnutrition for cancer patients is an assessment of protein and calorie status. As a result, a nutritional profile of cancer patients should include measurements of body protein status (visceral and somatic), body fat, immune-competence, and inflammatory markers [29].

14.3.3.1 Assessment of Body Protein Status

Body protein can be equally split between the substantial and non-instinctive pieces of a wholesome enhancement. It is useful to have a proportion of both physical and instinctive protein parts while evaluating the seriousness and sort of unhealthiness (e.g., marasmus or calorie-denied versus kwashiorkor or protein-depride) [22].

14.3.3.1.1 *Visceral (Non muscle) Protein Component*

Protein-acting proteins, covers, and immunologically dynamic proteins structure this part. Irritation can influence the biochemical markers that are usually used to survey dietary status. Notwithstanding, egg whites, prealbumin, and transferrin ought to be in every way appraised as death expectations [30].

Serum Albumin Levels

In human serum, egg whites are the most plentiful protein. Its capacity to tie to numerous synthetic compounds in the blood (bilirubin, unsaturated fats, iron, cortisol, and headache medicine) and its commitment to the osmotic concealment of intravascular liquid, which advances sufficient liquid dissemination in the tissues, the two jobs are known. Diminished serum egg whites might be because of an absence of fundamental amino acids because of hunger or malabsorption or strange liver creation [22]. Incendiary circumstances, especially elevated degrees of cytokines, IL-6 and TNF-alpha, have been recognized as two significant reasons for low serum egg whites levels. The arrangement of egg whites lessens foundational aggravation, which likewise increments egg whites corruption and further develops trans-narrow spillage [31].

Reference Ranges: Egg whites levels in serum ought to be somewhere in the range of 3.5 and 5.5 g/dL. The centralization of egg whites somewhere in the range of 2.8 and 3.5 g/dL is remembered to demonstrate a diminishing in instinctive protein, 2.1 and 2.7 g/dL shows a moderate lessening, and under 2.1 g/dL demonstrates a critical reduction [22]. Egg whites, then again, have been disposed of as a sign of sustenance because of its exceptional lack and long half-life (around 20 days). The

grouping of egg whites in serum diminishes not just because of diminished focus because of aggravation of cytokines (high during disease) or liver brokenness, yet in addition because of kidney misfortune and loss of GI lot [31].

Serum Pre-albumin Levels

Pre-albumin, or PA, is named from the fact that it migrates ahead of albumin in standard serum or plasma protein electrophoresis [22]. Transthyretin is another name for it. It is a thyroid hormone transport protein that is produced by the liver and partially degraded by the kidneys. Its half-life is roughly two days, which makes it more beneficial than serum albumin levels [31].

Reference Ranges: Dad levels in the blood range from 15.7 to 29.6 mg/dL; 10–15 mg/dL shows a slight diminishing, 5–10 mg/dL shows a moderate decline, and under 5 mg/dL demonstrates a critical abatement in protein [22]. Pre-albumin has been ensnared in foreseeing guess (particularly endurance) in an assortment of clinical illnesses, including stomach malignant growth [32].

Serum Transferrin Levels

Serum transferrin is a β-globulin that transports iron to plasma and has a half-existence of 8–10 days; hence, it better addresses basic changes in the condition of instinctive protein. The absolute iron-restricting limit (TIBC) estimation can be utilized to gauge it in standard lab research. The accompanying condition is utilized for estimation [22]:

$$Transferrin = 0.8TIBC - 43$$

Concentrates on involving serum transferrin and serum PA as markers in light of nourishing help in patients with esophageal disease [33]. Serum levels diminished in extreme lack of healthy sustenance, nonetheless, this imprint was viewed as temperamental in surveying low unhealthiness and sans fat load in more established investigations of Italian patients [34].

Reference Ranges: Ordinary transferrin levels are 250–300 mg/dL plasma. A portion of 150–250 mg/dL proposes a little decrease, 100–150 mg/dL advances moderate weight reduction, and under 100 mg/dL advances a huge lessening in instinctive protein. Transferrin levels are steady with nitrogen equilibrium and in this manner are not useful in observing patients getting dietary help [22].

Serum Retinol Binding Protein

The liver creates a protein that ties retinol, which conveys retinol from the liver to the objective organs. It is in the molar reach unaltered with PA [22]. Instinctive protein has an exceptionally short half-life (around 12 h), which is the reason it can address huge changes in lack of protein [31], in any case, it is extremely delicate and can change even with insignificant pressure. Low serum RBP levels can likewise be brought about by a lack of vitamin An and zinc. Therefore, RBP measures are not broadly utilized in clinical practice [31].

The table below shows a summary of serum protein markers

	Albumin	Prealbumin	Transferrin	Retional binding protein
Reference range	3.30–4.80 g/dL	16–35 mg/dL	0.16–0.36 g/dL	3–6 mg/dL
Molecular weight	65,000	54,980	76,000	21,000
Half life	20 days	2 days	10 days	1.5 days
Advantages	• Easy to measure & low cast • Reliable predictor of post-surgical outcomes	• Shorter half life • Easily available • Levels respond rapidly to dietary intake changes	• Short half-life (compared to albumin) • Rapidly affected by differences in protein status	• Effective to predict short term effect of nutritional interventions
Disadvantages	• Long half life • Levels may drop due to infection, hepatic failure, fluid overload or burns etc.	• Levels may increase in kidney dysfunction • Levels may drop due to infection, physiologic stress, or hepatic dysfunction	• Influenced by iron status • Levels may drop due to infection, stress, liver disease, or fluid status	• Influenced by vitamin A and zinc levels • Affected by renal insufficiency • Very sensitive, can drop by even minor stress

14.3.3.1.2 *Somatic (Muscle) Protein Component*

The identification of the loss of lean muscle mass is a significant and reliable predictor of cancer related muscle wasting leading to sarcopenia. For studies on ageing and chronic diseases, methods for measuring human body composition have been developed and validated. The gold standard procedures for determining body composition are dual energy X-ray absorptiometry (DXA), magnetic resonance imaging (MRI), and computed tomography (CT scan). Bioelectrical impedance analysis (BIA) is an accessible method used for body composition measurement [31]. Laboratory assessment of creatinine excretion in urine is another practiced approach.

24 h Urinary Creatinine Clearance

Customarily, the creatinine length file has been utilized as a feature of a proportion of muscle misfortune [26]. 24 h pee creatinine discharge is a biochemical measure that is broadly utilized in deciding an individual's body weight. Creatinine is accessible in a strong structure from the dynamic muscle with respect to how much muscle a patient has. Pee creatinine will diminish with extent to weight reduction in a malnourished individual [22]. Creatinine remove is communicated as far as the creatinine level record (CHI), which joins biochemical and anthropometric estimations and is utilized to survey persistent protein-calorie lack of healthy sustenance in grown-ups. As body weight diminishes, creatinine levels and level will diminish. The file is determined as the normal rate as follows:

$$CHI = \frac{mg\ of\ creatinine\ excreted\ by\ the\ subject\ in\ 24\ hours\ \times\ 100}{mg\ of\ creatinine\ excreted\ by\ a\ normal\ subject\ of\ the\ same\ height\ and\ sex}$$

A mild muscle mass deficiency is defined as 80–90% of normal, a moderate muscular mass deficit is defined as 60–80% of normal, and a severe muscle mass deficit is defined as less than 60 percent of normal [22]. The value of urine creatinine may be affected by dietary creatinine from meat consumption. In people with renal disease, CHI is ineffective.

24 h 3 MH Urine Excretion

How much 3-methyl histidine (3MH) delivered in the pee in 24 h is one more manner to decide the all-out body weight of protein. 3MH is a fundamental amino corrosive found exclusively in myofibrillar protein. The 3MH created during the debasement of this protein isn't recovered and totally discharged in the pee. Albeit the sum delivered is equivalent to the heaviness of the muscle, it doesn't show a total decay of muscle protein. Disintegration of sarcoplasmic proteins affects them. Its estimation requires the utilization of an amino corrosive impetus, which isn't accessible all the time.

Dual X-Ray Absorptiometry

DXA is currently being utilized in a number of clinical settings to diagnose osteoporosis, obesity, and sarcopenia [35]. DXA works by transmitting a low-energy X-ray beam through your body that accounts for two different compositions: soft tissue and bone. The DXA's main purpose is to offer an in-depth analysis of body's key components: fat, muscle, and bone.

DEXA-measured lean mass has an independent predictive value for mortality. Long-term mortality has been linked to muscular function impairment, disability, and comorbidity, as measured by subjective evaluation, mid-arm anthropometry, calf circumference, and DEXA (appendicular lean and fat mass and BMD) [36].

14.3.3.1.2.1 CT Scan

In the analysis of body composition and muscle loss detection, CT scan provides valuable insights.

According to a report, in which cachexia and sarcopenia was diagnosed using computed tomography scan in oncology patients; CT scans are a valuable method in the evaluation of the extent of muscle wasting because of availability. Despite this, in the clinical oncology settings, body composition analysis is still required to truly exploit the CTs use [37].

In research, CT scans are performed on the basis of diagnostics or physiological analyzes. With the help of advanced CT scans, the Cross-sectional muscle area was analyzed at the third level of the lumbar vertebra. In length, the area of the Muscle was multiplied and divided by the length of a square meter, which was then considered an indicator of the skeletal muscle (cm^2/m^2). To diagnose muscle weakness, this method was used [37].

14.3.3.1.2.1 Bio Impedance Analysis (BIA)

During the last 10 years, bioelectrical impedance analysis (BIA) has been extensively used for body composition analysis [26]. Kyle and his colleagues describe

the method to be based on the bioelectrical principle of impedance i.e., vector sum of resistance and reactance [38]. As a result, when a square of height is divided by the resistance that will be directly proportional to the hydrated body part (i.e., lean body mass plus total body water). The non-hydrated body portion (fat mass) can be obtained by subtracting the lean body mass from the total body weight. Hence, BIA gives us the clear estimation of body fat %age, lean body mass %age, as well as total body protein and water. For the prognostic evaluation in oncology patients, accuracy of BIA derived phase angle has been reported by several studies. In another study which was done on advanced colorectal cancer patients, study participants investigated and demonstrated that phase angle is a strong survival predictor [39].

14.3.3.2 Assessment of Body Fat

For the prognosis of morbidity and mortality, body-fat mass is an important biomarker. Furthermore, for evaluating the efficacy of therapeutic interventions, assessment of changes in body composition provides an objective understanding [26]. By measuring the impedance, body fat can be determined. The conductivity qualities of an electrolyte-based media are used to determine total body electrical conductivity. It is determined by the electrical characteristics of fat mass and fat-free mass. Lean body mass demonstrate higher electrical conductivity due to more water content and with electrolytes. Therefore, leaner implies lesser resistance on electrical current [22]. Body composition analyzer works by passing a moderate electric current through the body by connecting electrodes to the hands and feet. The body fat percentage is then calculated using the measured electrical resistance. This method is only valid for individuals who do not have significant changes in fluid and electrolyte distribution, i.e., edema and dehydration, as they change the resistance value [22].

14.3.3.3 Lipids

Total serum cholesterol is used as a malnutrition indicator in nutritional screening tools. A U-shaped relationship has been observed between serum cholesterol and mortality, showing increased risk of mortality with low levels [31]. For the indication of fat nutrition, the concentrations of cholesterol and triglycerides are obtained from blood samples after a 12-h fast. Lipid metabolism impairment is indicated by high cholesterol levels, i.e., above 220–240 mg/dL. Lipid handling abnormality occurs by the individual's sensitivity to dietary fat and/or carbohydrate. Triglycerides above 150 mg/Dl imply lipid metabolism insufficiency [22]. For cancer patients, this method is less frequently used in the nutritional assessment; however, it is a good indicator of nutritional risk because cancer impairs lipid metabolism, which could be revealed by biochemical assessment.

14.3.3.4 Evaluation of Immune Function/Immune Competence

Cancer treatments like radiation therapy and chemotherapy affect the immune system adversely. This effect is amplified by the patient's inability to consume

adequate nutrients, digest and absorb them, making malnutrition inevitable. Malnutrition, in turn, weakens the immune system further. This leads to serious consequences for cancer patients.

Subsequently, it is expected to test for resistant capacity. Lymphocyte counts and skin tests are frequently used to evaluate healthful status [22]. All out lymphocyte count is determined by all out blood count. Ordinary worth surpasses 1500/mm3. The absolute lymphocyte consider diminishes constantly PEM advances. The presence of an enormous ulcer or a reaction to significant pressure might add to it [22]. The patient's reaction to antigen review is utilized to analyze postponed cutaneous touchiness. Streptokinase/streptodornase (SK/SD), mumps, Candida, and filtered protein subsidiary (PPD) are broadly utilized antigens [16]. Direct skin testing is characterized as the presentation of 5 mm or more at the infusion site 24–48 h after the organization of any of these antigens. PEM is related with an absence of response or redness, as well as variances of under 5 mm, demonstrating resistance or energy (Protein Energy Malnutrition). A significant thought while utilizing these markers is that both of these side effects answer marginally to the advancement of a healthful status [31].

14.3.3.5 Measurement of Inflammation Markers

Cancer-related cachexia is affected by the interactions of multiple signaling substances rather than a single cytokine. TNF- α and interleukin-6 (IL-6) play crucial roles in the nutritional metabolism of cancer patients, as previously discussed [2].

14.3.3.5.1 *C-Reactive Protein*

A side effect of circuitous aggravation can build up to multiple times because of irritation [33]. Basic stage proteins, including CRP, as a rule ascend simultaneously as basic or ongoing ailments, while serum protein levels move, for example, prealbumin or egg whites falls. For dietary testing, ordinary utilization of CRP isn't suggested. This is because of the uncertainty of CRP and the capacity of clinical preliminaries to distinguish the presence of fiery circumstances [13]. IL-6 upregulates basic stage reactors like CRP in malignant growth patients with signal transducer and record activator 3 (STAT3), and is connected to muscle squandering [2].

14.3.3.5.2 *TNF-α*

TNF-α levels ascend because of disease [40]. Investigations have discovered that malnourished patients have expanded degrees of IL-6 and TNF-α (16.7 and 28.0 pg/ml, individually). As per the PG-SGA, a 8.72 pg/ml TNF-alpha slice off was viewed as related with an expanded gamble of ailing health. There was no relationship with perioperative entanglements [40]. Other safe go between, like interleukin-1 (IL-1), growth corruption factor (TNF), and interleukin-2 (IL-2) impact body craving and dietary changes, as well as immediate consequences for skeletal muscle [26].

14.3.3.5.2 *IGF-1*

It is an ordinary development factor while its flowing structure is emitted by the liver. It was previously known as Somatomedin C. Its delivery is animated by the pituitary development chemical. It has a short serum half-life (around 24 h) and is profoundly restricting to plasma proteins (particularly IGF BP 3). Fasting lessens plasma IGF-1 levels by multiple times, while supplementation with expanded nourishment builds IGF-1 focus [31]. There is a connection between energy admission (yet not protein) and the convergence of plasma IGF-1 [31]. IGF-1 levels were all around upheld by a sign of protein-energy unhealthiness in older patients recuperating from hip break a medical procedure; in any case, this marker was likewise impacted by aggravation. Conversely, irritation meaningfully affected IGF-1 levels in certain gatherings of careful patients. In liver infection, kidney disappointment, and extreme injury, for example, consumes, IGF-1 levels are impacted. IGF-1 is viable in egg whites and transferrin in checking protein and energy status during sustenance restoration [31]. The detriments of estimating IGF-1 serum focuses are that they are impacted by an assortment of different factors like a basic stage response. As of late, interest in free IGF-1 has developed, with the possibility to act as a quality food brand. IFG-1 evaluations are not suggested in that frame of mind, aside from great past reports.

14.3.3.6 Biochemical Evaluation of Nutrient Levels

Estimations of supplement levels in liquids or tissues, atomic containing particles (e.g., iron hemoglobin), or utilitarian markers are utilized in the lab to decide the situation with explicit supplements (e.g., thiamin-based compound, action of thiamin transketolase or the collection of metabolites because of glitch of different catalysts) [22].

At an advanced stage of the deficiency, signs of specific nutrient deficiencies usually appear. It is essential for the clinician to connect the dots and check if there are signs of nutrient deficiency correlating or hinting at the underlying disease and other clinical evaluation findings. During chemotherapy, deficiencies in vitamins K, B1, B2, niacin, folic acid, and thymine were found to be common.

14.3.4 Dietary Evaluation

It is impossible to underestimate the value of obtaining a nutrition history in assessment of nutritional status. The dietitians cannot accurately diagnose nutrition problems unless they are aware of the types and quantities of foods consumed by the patient. Prior to diagnosis, it is necessary to know not just food intake patterns, but also the appropriateness of intake and the changes in intake connected to the condition and its treatment [13]. Dietary evaluation typically focuses on assessment and interpretation of the patient's dietary intake, including foods types and quantities, dietary intake pattern changes, and food intolerances. Dietary evaluation methods include the following:

14.3.4.1 24 h Recall

Under the direction of a doctor, a patient ought to recall each of the 24 h of admission of food and drink. This technique enjoys its benefits, for example, minimal expense, short administration time, and okay to the patient. It is additionally more regrettable as patients might pass or report less of taking, and records might be incorrect on the grounds that they depend on the patient's memorable's capacity [13].

14.3.4.2 Food Diary

The patient records his eating regimen at the designated time. Records cover the two work days and ends of the week and ought to be saved for three or five days. Since information isn't completely subject to patient memory, it very well might be more exact and viable. Revealing and changing dietary patterns during recording are two significant obstructions to this methodology. For this procedure to work, the patient should commit oneself to finishing the eating regimen plan precisely [13].

Important factors to incorporate in dietary evaluation

After medical procedure or radiation therapy, malignant growth patients might encounter actual constraints and issues that diminish craving and retention of supplements like mouth injuries, the runs, regurgitating, torment, obstruction, or malabsorption. These effects can also be because of cancer-induced metabolic changes. This results in **food intolerances** as well as **impaired chewing** and **swallowing** abilities. This very important risk factor of poor nutrition status should be considered in dietary evaluation. Anorexia caused by treatment procedures is another significant element to address in nutritional screening and assessment because cancer is a chronic condition [30].

14.4 Conclusion

The nutritional status in cancer is a significant determinant of the patient's quality of life, response to treatment, and overall survival chances. There is plenty of evidence that confirms the positive effect of nutritional care on treatment outcomes; however, the importance of nutritional screening and assessment is still neglected in oncological practice. Knowledge of the nutritional condition of a patient through an in depth nutritional assessment is a crucial factor in providing effective care to the patients. It is essential to make nutritional screening practices a mandatory part of cancer management and treatment plan so that the patients' nutrition status could be monitored at every stage of the disease and nutritional risks could be detected and addressed timely. Currently, there are no particular protocols for improving the nutritional status in cancer patients, therefore, it is important that each patient is given customized treatment to improve the nutritional status. In conclusion; the only viable option to avert the risk of malnutrition in cancer patients in the dearth of a standardized and regulated nutritional assessment

and intervention strategy is to adopt a multi-step nutritional assessment that evaluates all aspects of nutritional implications including anthropometric measurements, clinical evaluation, laboratory assessments, and comprehensive dietary evaluation.

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