Chapter

The Principles behind Targeted Therapy for Cancer Treatment

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Abstract

The advent of molecular and genetic advancement in the field of oncology research has led to a shift in the treatment of various forms of cancer from traditional chemotherapeutics to targeted therapy. The principle behind targeted therapy is utilizing therapeutics designed to interfere with specific molecules that have a relatively specific or higher expression profile in cancer cells and are critical for cancer growth and progression. These were designed as mechanistic therapeutics in the form of small molecules and monoclonal antibodies. Currently, they have been modified to incorporate passive or active targeting delivery systems to improve their specific distribution and enhance cytotoxicity towards cancer cells while simultaneously reducing their systemic toxicity profile. Passive targeting employs encapsulated delivery systems to take advantage of the enhanced permeation and retention effect of the tumor microenvironment, while active targeting relies on receptor mediated interactions, such as cell surface ligands conjugated to the therapeutic moiety. A synergistic strategy for cancer therapy is evolving, where precision medicine acts as a diagnostic prerequisite for targeted therapy via prognostic biomarkers and tumor genotyping. Despite the plethora of research undertaken in targeted therapy, limited numbers were approved for clinical use, and significant challenges remain to be addressed.

Keywords: targeted therapy, cancer, chemotherapy, resistance, toxicity

1. Introduction

Various forms of cancers remain to be the leading cause of mortality worldwide. A recent article estimated the incidence and mortality of cancer in 20 world regions (using the GLOBOCAN 2018 estimates), and suggested approximately 18 million new cases and 9.6 million deaths in the year of 2018. Lung cancer was most commonly diagnosed, and the leading cause of cancer-related death followed by breast, prostate, colorectal, stomach, and liver cancer [1]. Although surgery and radiation therapy are considered the primary treatment for localized forms of cancers, chemotherapeutic agents must be used when cancer cells metastasize to the regional lymphatic vessels and bloodstream. This placed more emphasis on the development of drugs and biological molecules as chemotherapeutic agents to minimize the risk of cancer metastasis to other organs, which will lead to organ failure and death [2, 3].

2. Traditional chemotherapeutic agents

The era of cancer drug development was pioneered in the 1940s after using nitrogen mustard as a toxic treatment for cancer [2]. Traditional chemotherapeutic agents

mediate cytotoxicity by interrupting processes or inhibiting molecules required for rapid cellular division and DNA synthesis at the cell cycle level. They are categorized as either cell cycle specific (they target a specific phase in the cell cycle) or cell cycle non-specific (they target all cell cycle phases) agents [4]. Their main disadvantage relies on their relative non-selectivity in targeting rapidly dividing non-cancerous cells such as hair follicles, bone marrow and gastrointestinal epithelial cells. This commonly manifests as serious adverse effects on patients such as hair loss, anemia, infections (due to low white blood cell count), infertility, nausea and vomiting. As a result, the effective therapeutic dose is unattained and the efficacy of conventional chemotherapeutic agents is compromised. This is commonly experienced in the clinic, when a chemotherapy regimen is administered for a delimited period, but the dose has to be reduced or treatment is postponed as a safety precaution despite tumor responsiveness [5, 6]. Furthermore, many conventional chemotherapeutics do not accumulate in the tumor mass at effective therapeutic concentrations, thereby cannot effectively inhibit their proliferation and metastasis. This is particularly true at the core micro-regions of tumors; due to the disorganized intratumoral vasculature and high interstitial fluid pressure as a consequence of aberrant angiogenesis and poor lymphatic drainage. Some types of cancers such as brain gliomas, are also difficult to reach with traditional chemotherapeutics, as they are unable to penetrate the blood brain barrier. These factors play a paramount role in drug resistance [7]. Chemotherapeutic agents with inadequate bioavailability and pharmacokinetic profiles are more inclined to metabolism and excretion before reaching cancerous cells [8]. Cancerous cells not killed during treatment are likely to acquire resistance and eventually lead to a more aggressive form of tumors with high probability of organ damage and death [4]. In addition, various oncogenes and oncoproteins in a variety of cancers are able to inactivate chemotherapeutic agents and/or eliminate them from tumor cells (e.g., through the activity of multi-drug resistance; MDR) [7, 8].

Mechanisms that mediate resistance have been studied abundantly, and many are attributed to mutations of various oncogenes. They include altered transport of chemotherapeutics across the plasma membrane by ATP-dependent multi-drug transporters or upregulation of multidrug resistant gene, which encodes P-glycoprotein responsible for xenobiotic efflux out of cells [7]. Another example are defects in the apoptotic pathways leading to a loss of function in the tumor suppressor gene p53, allowing cells with damaged DNA to continue replicating and hence be unresponsive to DNA damaging effects of chemotherapeutics such as pyrimidine antagonist 5-fluorouracil and the mitotic spindle inhibitor paclitaxel [7, 8]. Enhanced action of DNA repair proteins in cancer cells also contributes to acquired forms of chemoresistance. This phenomenon has been observed with chemotherapeutics causing direct damage to the structural integrity of DNA such as intercalating agents like cisplatin and alkylating agents [7, 8]. While chemotherapeutics that mediate their action by binding to the topoisomerase-DNA complex to prevent DNA synthesis, such as doxorubicin, etoposide and camptothecin are rendered inactive by the altered activity of topoisomerase I and II, hence resistance facilitates the repair of topoisomerase-DNA complex [7, 8].

The design and development of conventional chemotherapeutics is prehistoric relative to recent findings in the complex cancer pathophysiology and tumor microenvironment. Recently, the heterogeneity of tumors is widely established as a challenge for traditional forms of cancer therapy. It is recognized to result from the higher genetic instability of uncontrolled cell division; increasing the likelihood of multiple mutations and replications errors. Ultimately it manifests as phenotypic and functional tumor heterogeneity that can occur within and between tumors [9]. Dynamic and regional variations in the tumor microenvironment in blood, lymphatic vasculature, extracellular matrix metalloproteases and cellular secretions

in the tumor stroma may greatly influence the diverse development of cancer cells. Furthermore, a distinct population of cells within tumors termed cancer stem cells with a capacity for self-renewal and differentiation are recognized to be responsible for cancer relapse, also contributing to this factor [9]. The heterogeneity of tumors has proved to be a limitation to treatment with traditional chemotherapeutics and restricts their use for a variety of cancer types, it also supports intrinsic resistance to cancer therapy. In order to combat these limitations which ultimately lead to cancer progression and reduces the survival rates of patients, more selective/targeted/efficient therapies are required. This lead to the discovery of new agents which are based on the investigation of molecular behavior, biomarkers, oncogenes and biological pathways used by cancer cells to determine specific key distinctions between them and normal cells, that are responsible for tumor cell proliferation, survival and progression. Once identified these distinctions are targeted "with precision" while sparing normal cells. This concept has been renowned the "magic bullet" of cancer therapy and is the fundamental principle behind targeted cancer therapy [10].

The advent of targeted cancer therapy was established by the development of molecular targeted therapeutics, also recognized as mechanistic or direct-targeted cancer therapy [11]. This strategy utilizes small molecules or monoclonal antibodies (MAb) designed to interfere with specific molecular targets that have a relatively specific or higher expression profile in cancer cells and are critical for their growth and metastasis [11, 12]. Small molecule targeted therapies are usually low molecular weight organic compounds (<800 Daltons), that have a higher rate of cell entry relative to MAb, and so are designed to interfere with the expression/activity of intracellular signaling molecules [4]. On the other hand, MAbs generally have high binding affinities to extracellular domains expressed on cancer cells and are commonly designed to target the extracellular surface of cancerous cells; they mediate their mode of action through receptor ligand interactions [4].

3. Types of targeted therapies

Various types of molecular target mechanisms have been identified for cancer therapy; these include signal transduction inhibitors, hormone therapies, gene expression modulators, apoptosis inducers, angiogenesis inhibitors, immunotherapies and toxin delivery molecules. Many of these mechanisms overlap and with the advancement in cancer research a single anticancer therapeutic agent can encompass multiple molecular mechanisms. This is further elaborated in the subsequent paragraphs of this chapter.

3.1 Signal transduction inhibitors

Signal transduction is considered the link between a ligand mediated activation of a receptor to the resultant cellular responses such as metabolism, gene expression, cell division and apoptosis, and motility/migration, etc. [13]. Hence it is a vital route to explore for molecular-targeted therapies. Many remarkably successful agents have been developed under this category, owning to the understanding of signal transduction pathways in cancer cells. Genetic and epigenetic changes that occur in cancer cells lead to uncontrolled cellular functions (such as proliferation) in part through aberrant/modulated activity of various signaling and metabolic networks [13]. While normal cells have redundant overlapping pathways that allow for alternative signal transduction when one is inhibited, cancer cells have placed more emphasis on distinct signaling pathways owning to various mutations [13]. As a result, they have a diminished signaling network with "hyper-active" pathways

to sustain cell proliferation and survival [13]. These "hyper-active" pathways are reflected in the overexpression of certain proteins in cancer cells. Together, these characteristics make cancerous cells more sensitive to stress and mutagens [13]. This forms the basis of signal transduction targeted molecular therapy. Therefore, theoretically depriving cancer cells from essential signaling elements needed for survival will lead to apoptosis and growth arrest [13]. Furthermore, some cancer cells are "addicted" to specific signaling proteins, as they are dependent on their activity for survival; a prime example is in chronic myeloid leukemia (CML) cancer. CML cells particularly in the early stage of cancer have absolute dependency on the kinase activity of Bcr-Abl fusion protein [13]. Bcr-Abl fusion protein results from an abnormal translocation in the Philadelphia chromosome (Ph) in 95% of CML patients. Fusion of Bcr and Abl genes leads to constitutive activation of Bcr-Abl tyrosine kinase, causing CML cells to grow and divide excessively [13]. Protein kinases are enzymes that regulate the biological activity of proteins by phosphorylation of specific amino acids with adenosine triphosphate (ATP) to induce conformational changes from the inactive to the active form [14]. Protein Kinases have been implicated in cell proliferation and many have transforming capacity making them oncoproteins [14]. Consequently, protein kinases have been extensively studied as a signal transduction inhibitor pathway in targeted drug therapy [13–15].

One of the most successful molecular targets of anticancer agents is the protein tyrosine kinase inhibitor imatinib (Gleevec®), for the treatment of Ph positive CML [4, 9, 10, 12–15]. Its success is based on the extreme "addiction" of CML cells to Bcr-Abl fusion pathway. Imatinib is designed as an ATP mimic which acts by blocking tyrosine phosphorylation, it competitively binds to the ATP binding site on Bcr-Abl fusion protein to disrupt tyrosine kinase activity [14]. It is reported to have up to 80% response rate in Ph positive CML patients [14]. This protein kinase inhibitor is also indicated for a subset of patients with gastrointestinal stromal (GIST) tumors, which have activated point mutations in the c-Kit proto-oncogene or platelet derived growth factor receptor (PDGFR)-α kinase [4, 12, 13]. Constitutive activation of kinase, as in the Bcr-Abl fusion protein, inhibits apoptosis and stimulates cell proliferation [3, 12, 13]. Although imatinib is a highly selective agent, it has been responsive for the inhibition of Bcr-Abl, PDGFR, cKit and FIt3 protein kinases [13]. Unfortunately, long term use of imatinib in some late stage GIST or CML patients may cause drug resistance leading to therapeutic failure. The dynamically complex oncogenic signaling network of cancer cells is able to "escape the addiction" of the Bcr-Abl oncogene as it becomes mutated. Furthermore, tyrosine kinase inhibitors cannot completely eradicate leukemic stem cells; both of these factors facilitate the progression of tumors [13]. Luckily, next generation tyrosine kinase inhibitors are available for imatinib resistant patients [13]. The broad-spectrum protein tyrosine kinase inhibitor Dasatinib (Sprycel®) not only binds to the mutated Brc-Abl kinase but also to Src tyrosine kinase. Despite the reduced selectivity of this inhibitor, the Src kinase family is also responsible for tumor progression, and their overexpression has been linked to several malignancies [13]. Nilotinib (Tasigna®) is another tyrosine kinase inhibitor, which shares imatinib's narrow tyrosine kinase selectivity profile, it inhibits the mutated Brc-Abl kinase activity, and is more efficient when compared to imatinib [13, 16]. Although lifelong therapy is expected with these next generation tyrosine kinase inhibitors, they are reported to achieve longer response rates [13, 16]. The FDA has approved both agents for first line use in patients with Ph positive CML in the chronic phase and for patients with resistance to imatinib [17]. Another dual Src and Bcr-Abl tyrosine kinase inhibitor called bosutinib (Bosulif®) has also been approved by the FDA for patients with Ph positive CML that have been treated with both imatinib and next generation tyrosine kinase inhibitors. It has been reported to have a superior

molecular response profile relative to imatinib in terms of inhibition potency to Bcr-Abl mutated tyrosine kinase and more selectivity, thereby decreasing its toxicity profile [18]. Imatinib indicated for GIST patients has partial response rates and differs between subset populations depending on the cKit point mutation. Imatinib is able to extend patient life but resistance usually emerges due to modified point mutations in the cKit gene on GIST stem cells, or mutations in other oncogenes and enhanced drug efflux transporters. A second line broad-spectrum tyrosine kinase inhibitor Sunitinib (Sutent®) is indicated for imatinib resistant GIST patients. This is a multi-target inhibitor that affects vascular endothelial growth factor receptor (VEGFR) and PDGFR protein tyrosine kinases; it has also been approved for the treatment of advanced renal cell carcinoma [13].

Most cell surface growth factor receptors have tyrosine kinase activity, and play an important role in cancer pathogenesis. One of the most notable for molecular targeted signal transduction cancer therapy is epidermal growth factor receptor (EGFR/ ErbB1/ HER1). It is a member of the epidermal growth factor receptors family (ErbB) of protein tyrosine kinases, which also include ErbB2/HER2, ErbB3/ Her3, and ErbB4/ Her4. Binding of a complementary ligand to these receptors induces receptor homo/hetero dimerization and subsequent tyrosine auto-phosphorylation, leading to activation of various downstream signaling molecules. In various types of cancers, the expression/activity profile of these receptors is increased leading to enhanced cell proliferation. Oncogenic mutations within the receptor kinases of this family have also been found in epithelial tumors, breast carcinomas, gliomas (glioblastoma multiforme) and in the case of EGFR in 10% of patients with non-small cell lung carcinoma (NSCLC) [13]. Currently, there are two FDA approved selective EGFR tyrosine kinase inhibitors gefitinib (Iressa®) and erlotinib (Tarceva®) and are indicated as first line therapy for NSCLC patients [13]. Initially, the response rates in patients after administration were not as efficient as expected, and it was later determined that the presence of EGFR overexpression alone does not predict effective therapy [13]. Instead, patients with particular types of EGFR substitution mutation, such as L858R mutation in the kinase domain are more likely to benefit from anti-EGFR therapy [13]. This was concluded from several clinical trials carried out on NSCLC patients comparing the administration of gefitinib to docetaxel in patients pretreated with platinum-based chemotherapy [19, 20]. Results of the trial concluded that gefitinib was either equally effective [19] or more effective [20] than docetaxel. A latter trial was conducted on patients of non-smoker Asian origin, and it was determined that they had a higher incidence of harboring the specific activating mutations in the EGFR kinase domain [13, 20]. Docetaxel is a well-established conventional chemotherapeutic agent which reversibly binds to microtubules with high affinity leading to inhibition of mitotic cell division. It is administered intravenously, and has been reported to cause dose-limiting toxicity and adverse effects of grade 3 and 4 neutropenia in 30% of patients, as well as edema and other common side effects shared by conventional chemotherapies [21]. Gefitinib, on the other hand, is administered orally, with reported adverse effects of grade 1 and 2 diarrhea and skin rash [22]. Therefore, in terms of patient compliance, therapy with gefitinib has greater efficiency than chemotherapy with docetaxel even if they provide the same therapeutic efficacy, since there is a lower incidence of severe adverse effects and oral administration is preferred over intravenous administration. Another important aspect is that not all EGFR mutations in the kinase domain are sufficient to determine patient response and instead only patients with specific EGFR mutations can benefit from certain molecular targeted treatments. In the case of gefitinib, the use of genetic screening is required prior to treatment. This prerequisite highlights the application of precision medicine in targeted cancer therapy and the need for diagnostic strategies involving prognostic biomarkers and tumor genotyping to

determine the choice of targeted therapeutic. Manufacturer AstraZeneca, of gefitinib as Iressa® had partnered with Qiagen to provide FDA-approved Therascreen® EGFR companion diagnostic test to identify eligible patients for this treatment, once they test positive for the specific EGFR substitution mutations [22].

Another member of the ErbB receptor family, HER2, has received wide recognition as a target for breast carcinoma, since it has been found to be upregulated in 20–30% of breast cancers [23]. HER2 tyrosine kinase activation is initiated by homo or hetero dimerization with other ErbB receptors, in turn phosphorylation leads to signaling through two main downstream cascades, phosphatidylinositol 3 kinase (PI3K)/Akt and mitogen- activated protein kinase (MAPK); both predominantly involved in promoting cell growth and survival [13, 23]. The first FDA approved targeted monoclonal antibody (Mab) for cancer was transtuzumab (Herceptin®), which interacts with the extracellular domain of HER2 (with high affinity) and is known to be one of the most successful recombinant humanized anti-ErbB- receptor family antibodies [13]. The advantage of transtuzumab as an antibody, over previously mentioned small molecular targeted therapies, is that it exerts its cytotoxicity by several mechanisms as well as binding to the extracellular domain of HER2 on cancer cells with high affinity. Antibodies generally bind to their complementary receptor with higher selectivity or even specificity compared to small molecule therapeutics. Transtuzumab binds HER2 on the surface of cancer cells with high affinity to hinder HER2 dimerization. This also leads to degradation of the receptor and prevents HER2 recycling. As a result, downstream signaling cascades of PI3K and MAPK are diminished, promoting cell-cycle arrest and apoptosis. Furthermore, transtuzumab is able to modulate the immune system by inducing antibody-dependent cellular cytotoxicity (ADCC) through its Fc domain [13]. It can also bind to Fc receptors on various immune cells, markedly natural killer cells, but also neutrophils, mononuclear phagocytes, macrophages and dendritic cells leading to immune cell recruitment to the tumor tissue. Cytotoxicity is mediated in various ways, natural killer cells inhibit cell proliferation and intratumoral angiogenesis by the secretion of cytokines and chemokines, and they also promote tumor immunogenicity by inducing the expression of major histocompatibility antigen on cancer cells [13]. Macrophages and phagocytes carry out phagocytosis by engulfing and eliminating tumor cells [13]. Complement dependent cytotoxicity is mediated after an antibody bound to the surface of a tumor cell also binds to a nearby complement protein C1q. This activates a series of enzymatic cascades in the classical complement pathway, ultimately facilitating the formation of a cytolytic membrane attack complex on the surface of tumor cells in the form of pores that generate an influx of ions and water molecules leading to tumor cell lysis [13]. Transtuzumab has also been identified to play a role in the inhibition of angiogenesis by interfering with pro-angiogenic and anti-angiogenic factors, and reverting tumor vasculature back into normal vasculature [13]. This is supported by the enhanced localization of paclitaxel in tumors during combination therapy with transtuzumab [24]. Unfortunately, one of the limitations of transtuzumab is that it is only able to block the dimerization of HER2 with unbound HER3. Consequently, dimerization of HER2 with EGFR and ligand-bound HER3 proceeds despite transtuzumab therapy, serving as one of the reasons of acquired resistance to transtuzumab. Concurrent therapy with another Mab pertuzumab (Perjeta®) is sometimes administered as it targets dimerization of HER2 with neuregulin ligand bound HER3 [13]. Resistance may also develop by mutations that prevent the complementary binding of transtuzumab to the HER2 extracellular domain. For instance, proteolysis leading to mutated isoforms of HER2 kinases, or elevation in the expression of Mucin-4 an O-glycosylated membrane protein that dimerizes with HER2 [23]. Overexpression of other ErbB members such as EGFR that are able to dimerize with HER2 is also common, in this case a small

molecule tyrosine kinase inhibitor lapatinib (Tykerb®), with dual action against HER2 and EGFR has been used [13, 23]. This further reinforces the challenges to treat patients with different molecular subtypes of breast carcinoma. As a result, transtuzumab is indicated for women that have been diagnostically found to strongly overexpress HER2 on their tumor cells and successful treatment response is more likely with early therapy. Transtuzumab has also been indicated as an adjuvant therapeutic particularly after surgery in order to eliminate micrometastases. It is also used in combination with various anticancer agents [13, 23, 24].

3.2 Hormone-based therapies

Various hormones are implicated in the pathogenesis of many forms of tumors such as breast, ovarian, and prostate. Hyperplasia and neoplasia can develop from excessive hormonal stimulation or loss of tumor suppressor genes that dictate hormonal secretions as they have a proliferating effect on target cells. Steroid hormone such as estrogen binds to estrogen receptors (ER) that belong to a superfamily of nuclear receptors. Upon binding, the receptor complex homo-dimerizes and interacts with sequence specific estrogen response elements in corresponding genes, leading to the activation of nuclear transcription factors that produce complementary mRNA. Elevated mRNA levels increase protein production in the endoplasmic reticulum, which ultimately result in various effector responses such as enhanced cell proliferation [25, 26]. While estrogen activity mainly regulates growth, development and physiology of reproductive systems in both sexes, ERs are also found in neuroendocrine, skeletal, adipose and cardiovascular systems [25]. Estrogen signaling pathways are complex due to its nuclear and non-genomic influences, and downstream transcriptional activities affect the biological function of different tissues expressing ERs [25]. Two types of ER have been identified ERα and ERβ, where ERα expression is considered the hallmark of hormone dependent tumor growth [25–27]. About 70% of breast cancer patients express the hormone receptors ER and/ or progesterone, making them susceptible to endocrine therapy [25–27]. These receptors have categorized breast cancer as intrinsic and molecular subtypes based on the genes the cancer cell expresses, and act as a blueprint for targeted breast cancer therapy [27]. The aim of hormone-receptor positive breast cancer therapy is to reduce the growth stimulatory effects of estrogen on breast cancer cells. A primary way to do this is by interfering with the ability of estrogen to bind to its receptor, via targeting estrogen receptors on breast cancer tumors that overexpress ER and competitively binding ERs to reduce the capacity for estradiol to bind. This concept actually paved the way for targeted cancer therapy, and was first demonstrated by the renowned non-steroidal derivative tamoxifen (Nolvadex®) [25–28]. After FDA approval in the 1970s, tamoxifen became one of the world's best-selling hormonal cancer drugs largely relating to its efficacy and short-term safety profile relative to traditional chemotherapeutics at the time [28].

Tamoxifen is non-steroidal triphenylethylene derivative; it is classified as a prodrug, since its metabolites have a more pronounced effect on ERs [25, 28]. Tamoxifen and its metabolites act as selective estrogen receptor modulators (SERM) since they have both anti-estrogenic and pro-estrogenic activity contingents on the target tissue [25, 28]. On mammary epithelia, tamoxifen is able to bind competitively to ERα thereby disrupting the binding of estrogen and inhibiting the transcription of estrogen responsive genes that ultimately antagonize hormone dependent breast cancer cell proliferation and tumor development [25, 28]. Tamoxifen is metabolized hepatically by cytochrome P450 (CYP450) isoforms into pharmacologically active metabolites 4-hydroxytamoxifen (4-OHT), N-desmethyl tamoxifen and endoxifen. These metabolites have a higher affinity for ERs. 4-OHT

binds to ERs in breast tissue with an affinity similar to that of estradiol and inhibits ER-mediated gene transcription by recruiting co-repressors that modulate gene expression [29]. Their anti-tumorigenic activity is attributed to induction of apoptosis by downstream ER signaling pathways, inhibition of mitogenic growth factors activity and reduction of angiogenesis [30, 31]. Literature has demonstrated the complex mechanisms of action for tamoxifen and its metabolites and the difficulty in determining the molecule responsible for each mechanism, it is also hypothesized that the response to tamoxifen therapy is an aggregate of the parent and metabolites actions [29]. Pro-estrogenic activities of tamoxifen and its metabolites are demonstrated on bone density, as it decreases bone loss and inhibits osteoclasts in post-menopausal women but causes deleterious effects on bone density in healthy pre-menopausal women [32]. Another pro-estrogenic activity of tamoxifen and its metabolites is found in uterine epithelia, and it has been linked to endometrial cancer in some women restricting its use to 5 years and labeling tamoxifen as a carcinogen [25, 28]. Tamoxifen has been indicated for pre- and post-menopausal women and men diagnosed with hormone-responsive ER positive early stage breast cancer after surgery and as a chemopreventative for high-risk women [25, 27, 28].

Alternatives to SERMs exist, particularly for patients with advanced breast cancer or as a second line therapy to tamoxifen resistant tumors. These include selective estrogen receptor down regulators such as Fluvestrant (Faslodex®) [33]. Their molecular activity is also mediated by binding to ER, however, they function entirely as ER antagonists, causing downregulation and degradation of ER and ultimately inhibiting proliferation of estrogen dependent breast cancer cells. An advantage to the use of fluvestrant over tamoxifen as it is devoid of ER endometrial adverse effects [33]. It has been used in combination with docetaxel as it enhances the sensitization of breast cancer cells to chemotherapy [33].

Another group of endocrine therapy that has been indicated for postmenopausal women with hormone responsive ER breast cancer is aromatase inhibitor (AI) [26]. Their principle mode of action is to decrease circulating levels of estrogen and function by targeting and interfering with the enzyme responsible for the conversion of androgens to estradiol. CYP450 enzyme complex aromatase is responsible for catalyzing the final step in the biosynthesis of estradiol in both pre and post-menopausal women. In premenopausal women, the primary source of estrogen is from the ovaries, while in post-menopausal women adrenal and ovarian androgens are converted to estrogen by enzyme aromatase in peripheral tissues [26, 33, 34]. AIs have been categorized into two main groups, Type I and Type II inhibitors. Type I AIs are irreversible inhibitors of aromatase, they are also known as mechanism based inactivators [26, 34]. These AIs are designed to mimic the substrate of aromatase androstenedione and are recognized by the enzyme as alternate substrates. Hence they undergo irreversible chemical reactions and are converted into intermediates during catalysis; the intermediate however, is reactive and causes inactivation of the enzyme [26, 33, 34]. A successful example of a steroidal AI inhibitor is exemestane (Aromasin) [26, 33, 34]. It is an inhibitor of human placental aromatase and has shown relatively prolonged reduction of estrogen levels (4-5 days) in postmenopausal women with breast cancer due to irreversible binding to aromatase [34]. Exemestane has been found to decrease hormone dependent mammary tumors in hormone receptor positive metastatic breast cancer [34]. Type II inhibitors act by non-covalent competitive binding to the active site of aromatase to decrease the amount of estrogen formed. These inhibitors are designed to target aromatase selectively in order to avoid binding to other CYP450 enzymes [26, 34]. They contain a triazole ring, which aids in their selective binding to the haeme iron of aromatase [26]. As the binding is non-covalent it is reversible, hence therapy

with these inhibitors must be continued [26, 34]. Non-steroidal examples of these AI include anastrazole (Arimidex®) and letrozole (Femara®), they are found to inhibit more than 95% of estrogen biosynthesis in post-menopausal women with advanced breast cancer [26, 34]. The FDA has approved the use of AI for postmenopausal women diagnosed with hormone receptor positive breast cancer in the early stage as adjuvant therapy and for advanced and metastatic stages after tamoxifen treatment [26, 34].

3.3 Anti angiogenic therapies

Angiogenesis is a physiological process where new blood vessels are formed from preexisting mature vasculature [35]. This process allows the surrounding tissues to be supplied with nutrients and oxygen and simultaneously gets rid of metabolic waste products and carbon dioxide. In healthy tissue, angiogenesis is a temporary process and occurs during mensuration and embryogenesis, it is also an attribute of wound healing [35]. Prolonged angiogenesis however, is usually an indication of a pathogenic state such as cancer [35, 36]. The aberrant proliferation of cells during tumor formation in many types of cancers requires an extensive capacity of vasculature to manage the high demand in oxygen and nutrients and eliminate accumulated metabolic waste for tumor cells to grow and survive. Hypoxia in the tumor microenvironment leads to the production of hypoxia-inducible factor 1α; a stimulus for angiogenic switch, inducing the overexpression of pro-angiogenic factors mainly vascular endothelial growth factor (VEGF)- A, as well as fibroblast growth factor (FGF), placental growth factor (PlGF) and platelet derived growth factor (PDGF) by tumor and host cells [13, 35, 36]. Subsequently, proliferation of endothelial cells is stimulated and chemotaxis to tumor tissue facilitates the formation of the vascular lumen architecture and simultaneous disruption of surrounding vascular membrane structure [13, 36]. These factors contribute to the defective heterogeneous vasculature surrounding and within the tumor microenvironment, differentiating it from normal vasculature [35, 36]. Tumor vasculature has fenestrated blood vessels, with diminished pericyte coverage, and intratumoral blood vessels resemble immature perforated capillaries. As a result, vasculature is highly permeable, leaky, has poor blood perfusion and interrupted blood flow [35, 36]. Furthermore, the enhanced microvascular permeability leads to the aggregation of fibrin and other plasma proteins in the stroma of tumors, increasing the interstitial fluid pressure within tumors particularly in the absence of adequate lymphatic drainage [35]. This dynamically chaotic tumor microenvironment favors tumor progression in multiple ways. The high interstitial fluid pressure prompts the dissemination of tumor cells into intratumoral capillaries and neo-vessels providing a route for metastasis [36]. Elevated pressure in the tumor core resists the delivery of chemotherapeutics into these micro-regions of the tumor [35, 36]. The heterogeneous vascularization of the tumor microenvironment is dependent on the degree of angiogenesis inflected by tumor cells, so it can be different within and between tumor tissues, and obviously for different types of cancers [36]. These variations are amplified because intratumoral neo-vessel formation is mediated when pro-angiogenic factors outweigh anti-angiogenic factors within the tumor microenvironment. Luckily, the differential activation of angiogenesis in normal tissue compared to tumor tissue provides a means of targeting this pathophysiology selectively, based on the phenotypic and functional differences between intratumoral vasculature and normal vasculature. Therefore, anti-angiogenic strategies serve as attractive cancer therapy; with the aim of terminating the blood supply to tumor tissues and micro-regions in order to impose widespread hypoxia and necrosis selectively within solid tumors while sparing normal cells.

The most extensively studied target to inhibit the angiogenesis process is the VEGF and its receptor (VEGFR). VEGF is a soluble glycoprotein with pro-angiogenic activity, which is overexpressed in tumor tissue, and also by host fibroblasts and inflammatory cells. It is a ligand for the soluble and membrane bound tyrosine kinase VEGFR expressed on endothelial cells. Upon binding, a signaling cascade is activated leading to endothelial cell proliferation, maturation and migration to tumor tissue and neo-vessel formation. Different isoforms of VEGFR and ligands of VEGF exist; the most influential interaction for intratumoral angiogenesis is VEGF-A/VEGFR-2. The first and currently most successful anti-angiogenic cancer therapeutic approved by the FDA is the humanized monoclonal anti-VEGF-A antibody bevacizumab (Avastin®). It contains complimentary-determining regions of a mice antibody that selectively binds to circulating VEGF-A to neutralize it and inhibit its interaction with VEGFR2 [13, 35, 36]. Hence a unique feature of bevacizumab is that unlike most antibodies that bind to receptors, it binds and traps the ligand VEGF-A, limiting its availability to bind to VEGFR2. This initially leads to vascular normalization, which involves reduction in the overall intratumoral vasculature and hence limits the blood supply to tumors. The interstitial fluid pressure is lowered, hypoxia decreases and intratumoral perfusion is enhanced in the core of tumor tissues. Combination therapy is usually administered with bevacizumab to take advantage of the localization of chemotherapeutics deep into the micro-regions of solid tumors [35, 36]. Bevacizumab has been approved for the treatment of renal cell carcinoma, metastatic colorectal cancer, advanced NSCLC and recurrent glioblastoma [35, 36].

Another anti-angiogenic agent which has a similar mode of action to bevacizumab is ziv-aflibercept (Zaltrap®), acts as a decoy receptor. It is a recombinant fusion protein designed by fusion of extracellular VEGF-A binding domain portions of two isoforms of VEGFR (VEGFR1 & VEGFR2) with Fc portion of human IgG1 immunoglobulin [37]. Incorporation of the two VEGFR isoform binding domains in aflibercept allows this angiogenesis inhibitor to trap VEGF-A, VEGF-B and PlGF [36]. Although VEGF-B is not implicated in the stimulation of angiogenesis, it is overexpressed in metastatic tumors and findings suggest it has a role tumor progression by maintaining existing vasculature, while PlGF is required for inflammation-associated angiogenesis in cancer progression. The FDA approves ziv-aflibercept for metastatic colorectal cancer in combination with chemotherapeutic agents 5-Fluorouracil, irinotecan and leucovorin.

Many receptors that mediate angiogenesis are activated by the tyrosine kinase motif attached to their intracellular domain, most prominently is VEGFR, but also FGF and PDGR. Therefore, small molecule receptor tyrosine kinase inhibitors have been utilized as angiogenesis inhibitors; these include sunitinib (mentioned previously) and sorafenib (Nexavar®). Like sunitinib, sorafenib has multiple receptor tyrosine kinase inhibition activity, so its mode of action is not limited to the inhibition of angiogenesis, which it does by binding to VEGFR-1, VEGFR-2 and PDGF-B receptors, leading to morphological vascular normalization of tumor tissue [35, 36]. It also inhibits activation of signaling pathway Raf kinase (Raf/MEK/ERK) which is found to be activated in renal cell carcinoma among other cancer types, and c-KIT and Flt-3 kinases; also implicated in different cancer types [35, 36]. Multiple receptor tyrosine kinase inhibitors can be prescribed as mono-therapy since they act on multiple targets in cancer cells. In fact, their co-administration with chemotherapeutics was not found to improve the drug accumulation in tumor tissues [35]. However, it was found less likely to develop resistance to multiple tyrosine kinase inhibitors compared to selective single targeted anti-angiogenic therapies [36]. The opposite is true for bevacizumab and is the reason why it is co-administered with chemotherapeutic agents [35, 36]. During the initial phases of anti-angiogenic

therapy, the intratumoral vasculature undergoes vascular normalization. However, continuous administration of bevacizumab and several other anti-angiogenic therapies causes vascular shutdown and regional tumor tissue necrosis, leaving tumor cells adjacent to normal vasculature viable and prone to resistance [35, 36]. The turning point between vascular normalization and vascular shutdown for anti-angiogenic therapies is called the normalization window and it is this duration that is found to provide optimal intratumoral drug accumulation of chemotherapeutics [35, 36]. Therefore, several factors are taken into account to maximize cancer therapy using angiogenic inhibitors. These include the type of cancer; whether it is dependent on angiogenesis, type of angiogenic inhibitor; hypertension is a common adverse effect for bevacizumab while multi-tyrosine kinase inhibitors can cause more adverse effects. The type of chemotherapeutic agent to insure it will be effective once accumulated in tumor tissues and will not counteract the angiogenic inhibitor. In addition to their temporal sequence and the time lapse between administrations that would define the normalization window; so that the chemotherapeutic agent will be able to localized and accumulate within the tumor core after vascular normalization is induced by the angiogenic inhibitor to increase the overall tumor cell exposure to cytotoxic drugs [35, 36].

In order to define the vascular normalization window and success of anti-angiogenic therapy, predictive detection of vascular parameters is required to enable precision and personal therapy for each patient. Microvascular density analysis is significant to determine vascular integrity during patient treatment and the sensitivity of the cancer to anti-angiogenic therapy; it can be monitored *in vivo* by magnetic resonance imaging and vessel architectural imaging. These techniques have led to the finding that HER2-negative (triple negative) breast cancer patients have a variable response to bevacizumab therapy and the FDA withdrew its approval for breast cancer [35, 36]. Hypoxia detection is also a significant parameter to consider not only for angiogenesis but also since hypoxia is an indication of cancer aggressiveness, its metastatic potential and it can also contribute to tumor resistance particularly during radiotherapy. This parameter can be evaluated by monitoring oxygenation status during therapy. The use of positron emission tomography for hypoxia imaging has been implemented to select patients that will benefit from specific therapies. Tracers, biomarkers and genetically encoded fluorescent sensors have also been utilized as predictors of vascular normalization. **Table 1** summarizes examples of molecular targeted therapeutic agents and the main target they affect.

The previous sections discussed targeted therapeutics based on their molecular mechanisms of action. Although many have proved successful and more efficient than conventional chemotherapeutics, there still remained limitations in terms of toxicity and resistance. The next section addresses a new field in targeted cancer therapy that aims to improve on molecularly targeted therapies by embedding further selectivity into therapeutics. The general principle behind this new and growing field is utilizing the selective delivery of therapeutics to target tumor tissue as well as the selective molecular mechanisms observed in molecularly targeted therapeutics. Targeted delivery therapeutics can be classified into passive targeting which takes advantage of the enhanced permeation and retention effects of the tumor microenvironment or active targeting, which is based on incorporating targeting moieties that will guide the cancer agents to their targets. The following section discusses these two categories in more detail.

3.4 Passive targeting delivery therapeutics

Chemotherapeutic agents are often low molecular weight molecules, with characteristically unfavorable pharmacokinetic profiles usually having short half-lives,

• Tyrosine kinase inhibitors:
○ Imatinib (Bcr-Abl/c-kit/PDGFR)
○ Dasatinib (Bcr-Abl/Src)
○ Nilotinib (Bcr-Abl)
○ Bosutinib (Bcr-Abl)
○ Sunitinib (VEGFR/PDGFR)
○ Gefinitib (EGFR)
○ Erlotinib (EGFR)
○ Sorafenib (VEGFR-1/2.PDGF-B/Raf/MEK/ERK)
○ Lapatinib (HER2/EGFR)
• Monoclonal antibodies:
○ Transtuzumab (HER2)
○ Bevacizumab (VEGF-A)
• Hormonal therapies:
○ Tamoxifen (ER)
○ Fluvestrant (ER)
○ Exemestane (aromatase enzyme)
○ Anastrazole (aromatase enzyme)
○ Letrozole (aromatase enzyme)
• Anti-angiogenic therapies:
○ Ziv-aflibercept (VEGF-A)

Table 1.
Examples of molecular targeted therapeutic anticancer agents with their main target.

large volumes of distribution in healthy tissue but suboptimal biodistribution in tumors. The systemic circulation also influences their plasma protein binding strength limiting the free drug available for therapeutic action. Polar, and low molecular weight chemotherapeutic molecules (less than 30 kDa) generally have short systemic circulation exposure as they are cleared by glomerular filtration in the kidneys. On the contrary, macromolecules are commonly recognized by macrophages and cleared from the circulation by the reticuloendothelial (RES) system in the liver. Both of these mechanisms of clearance pose a threat to the drug concentration required to produce the desired therapeutic effect in tumor tissue. In turn, higher concentrations of these drugs that usually have a low therapeutic index, need to be administered making non-selective toxicity inevitable. Furthermore, the inability of conventional chemotherapeutics to sufficiently localize and accumulate in the core of tumors contributes to severe adverse effects and therapeutic resistance, rendering many incompetents for cancer therapy.

Passive targeting of cancer therapeutics aims to improve the pharmacokinetic properties of anticancer agents and tailor them to take advantage of the characteristics and architecture of the tumor microenvironment. The principle behind passively targeted chemotherapeutics is to design delivery systems with improved pharmacokinetic profiles complimentary to the tumor microenvironment. So that encapsulated chemotherapeutics can be transported in the circulation safely for longer durations with minimal toxicity to surrounding healthy tissue. Once they reach the tumor microenvironment they passively accumulate in tumor tissues and are released at therapeutic concentrations to exert their cytotoxicity against cancer cells. As mentioned previously, tumor vasculature is different from normal

vasculature. These variations have a unique impact on the behavior of substances in the vicinity of the tumor microenvironment; the phenomenon is known as the enhanced permeation and retention (EPR) effect of tumors. It is attributed to the abnormal vasculature and impaired lymphatic drainage in the tumor microenvironment [37]. The imbalance in angiogenic factors and matrix metalloproteinases leads to the formation of highly disorganized dilated vessels with fenestrations, due to pores and wide gap junctions between endothelial cells that lack sufficient pericytes and a basement membrane [37]. Intratumoral vessels usually lack the smooth muscle layer surrounding endothelial cells and hence remain dilated [37]. As a result, intratumoral vasculature is leaky enough to extravasate macromolecules >600 nM in diameter into tumor tissues as opposed to normal vasculature where tight junctions restrict the permeability of molecules sized >4 nM [37]. Furthermore, the impaired lymphatic system in the tumor microenvironment retains extravasated molecules allowing them to accumulate [37]. Therefore, the enhanced permeation of macromolecules in neoplastic vasculature and their retention and accumulation into tumor tissues lead to the application of nanotherapeutics as delivery systems for targeted cancer therapy.

The design of delivery systems with improved pharmacokinetic profiles and biodistribution that complement the pathophysiology of the tumor microenvironment is made possible by utilizing nanocarriers. The field of nanomedicine, derived from nanotechnology is extremely broad with a deluge of components synthesized and investigated for various diseased states. Nanocarriers are colloidal drug delivery systems with sizes in the nanometer range (generally <500 nM) imparting a high surface to volume ratio to them and their cargo. This is a significant feature for their characteristics. Variations in size, shape, and synthetic constituents have been investigated to determine the ideal nanocarriers for enhanced bioavailability and therapeutic efficiency of anticancer agents. The principle behind nanocarriers is to allow drugs to behave as though they have a larger molecular weight. Much research has gone into determining the ideal size of nanocarriers for cancer therapy and it was found to range from 10 to 100 nM [38]. Justified as nanocarriers >10 nM are more likely to escape rapid excretion by glomerular filtration in the kidneys. While nanocarrier >100 nM are more likely to be absorbed by proteins for opsonization prior to hepatic uptake and clearance by RES [38]. Surface charge of nanocarriers is another modifiable property to manipulate the pharmacokinetic profiles of therapeutics. It is attributable to the various types of nanomaterial formulations available to synthesis these carriers. Neutral and anionic nanocarriers are favorable in evading renal elimination whereas cationic nanocarriers form better interactions with the negatively charged cell membrane and enhances their cellular uptake [38]. Another way to modify the surface of nanoparticles is by a process called PEGylation. It involves coating the surface of nanoparticles with an inert polymer polyethylene glycol (PEG) so that they are shielded from interactions during systemic circulation, particularly from protein blood components and even aggregation with one another. This imparts stealth properties to nanoparticles and has been effective in increasing circulation times of nanoparticles as they avoid surface absorption and opsonization, reducing the frequency of clearance by phagocytosis and the RES system [39].

Nanocarriers can be categorized into 3 types based on their constituents, organic, inorganic, and hybrid [40]. Organic carriers include liposomes, solid lipid nanoparticles, dendrimers, polymer nanoparticles and polymeric micelles. Inorganic nanocarriers include carbon nanotubes and mesoporous silica nanoparticles, while hybrids are a combination of both. This part of the chapter discusses the most prevalent organic nanocarriers as only two have been approved by the FDA to date for anticancer use.

4. Organic nanocarriers

4.1 Liposomes

These microscopic lipid bilayers have become very popular after the success of the reformulated anticancer drug Doxil (Caelyx®) [41]. They are spherical vesicles with an aqueous core surrounded by single or multiple lipid bilayers, composed from natural or synthetic lipids such as phospholipids and cholesterol that can enter cells by endocytosis. The structure of liposomes allows them to encapsulate both water and lipid soluble drug payloads [41]. Schematic of liposome arrangement is shown in **Figure 1**. Hence liposomes can improve the pharmacokinetic properties of a range of drugs with different solubilities. Moreover, they can be coated with polymers or PEGylated to provide stealth properties. Doxil for instance is a polyethylene glycol coated liposome with the chemotherapeutic doxorubicin as the drug payload. Currently, it is the sole liposomal cytotoxic agent approved for solid tumors, and indicated by the FDA for ovarian cancer and multiple myeloma [41]. The chemotherapeutic doxorubicin is an anthracycline, which mediates its cytotoxicity by intercalating with DNA and inhibiting topoisomerase I and II activity, leading to apoptosis induction in cancer cells [42]. However, one of its main drawbacks is cardiotoxicity which can be fatal or lead to congestive heart failure, hence patients are only permitted a limited dose in their lifetime [41, 42]. The reformulation of doxorubicin as doxil has overcome this adverse effect; in fact, it has a very different toxicity profile from doxorubicin [41, 42]. While being dose limited by mucocutaneous toxicities, its adverse effects are much less severe. Furthermore, the bioavailability of doxil is preferential to doxorubicin as it is more stable in systemic circulation, has a longer half-life and slower clearance rate [42]. It is also able to extravasate into leaky intratumoral vasculature where its concentration is low compared to normal vasculature where its concentration is higher, by passive diffusion and accumulate substantially more in solid tumors [42]. As a result, it has a better therapeutic efficacy and index compared to doxorubicin [42].

4.2 Nanoparticles

Nanoparticles have been synthesized in a variety of forms from various constituents. Solid lipid nanoparticles constitute organic solid lipids such as mono- di- and

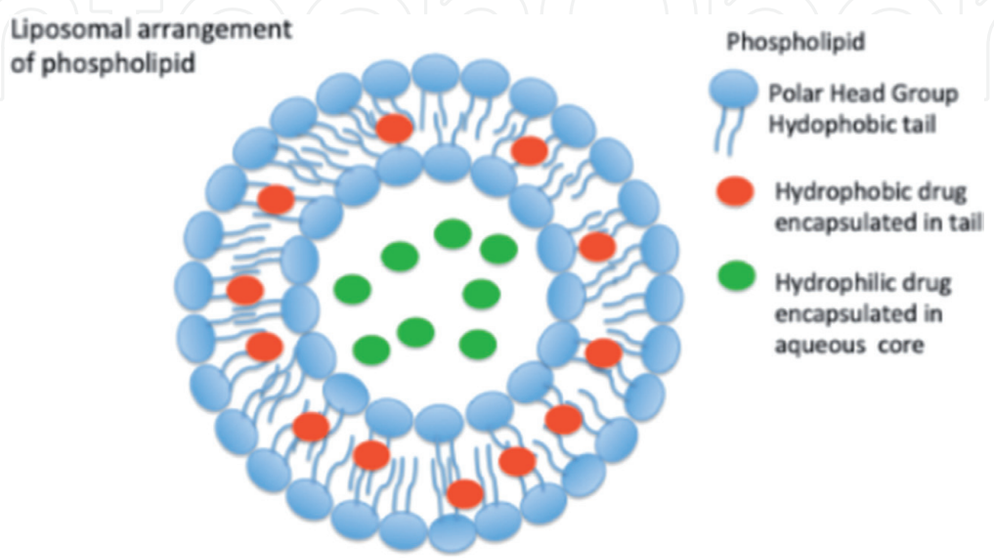

Figure 1.
Schematic diagram of liposomal arrangement of phospholipid (not to scale).

triglycerides, free fatty acids and alcohols or waxes and steroids [40]. These lipids are dispensed in water to form a nanosized colloidal dispersion with a large size range of 50–1000 nM, an emulsifier is added to stabilize the formulation [40]. Lipid constituents offer great advantages as nanocarriers for anticancer drugs classified as class II and class IV in the Biopharmaceutical classification system, where aqueous solubility is low [40]. This is because they increase the solubilization of lipophilic drugs and enhance their bioavailability. These delivery systems also provide controlled drug delivery, they are biocompatible and biodegradable and have greater drug payloads as well as improved stability and are feasible for large-scale manufacture [40]. Another type of nanoparticles is polymeric nanoparticles these are solid nanosized colloidal particles that are formulated as nanospheres or nanocapsules depending on the structural organization [40]. They are made from both synthetic biodegradable polymers such as polylactic acid, polyglycolic acid, PEG and N-(2-hydroxypropyl) methacrylamide, or natural polymers such as albumin, alginate, collagen, chitosan and heparin [40]. The advantage of using polymeric nanoparticles is that they are usually biocompatible and biodegradable, hence reduce toxicity and are degraded in the body by normal metabolic routes [40]. They also offer greater drug stability in the circulation and during storage, are formulated to be homogenous compared to liposomes and retain the enhanced bioavailability characteristic of nanocarriers [40].

A successful example of an anticancer polymeric nanoparticle that has been approved by the FDA is Abraxane®, for the treatment of metastatic breast cancer and NSCLC. It constitutes the chemotherapeutic taxane paclitaxel bound to the natural polymer albumin in a solvent free formulation, forming a colloidal suspension of 130 nM particles. This formulation has several advantages over the use of the traditional chemotherapeutic paclitaxel limited by its poor aqueous solubility. Previously, paclitaxel was administered in a formulation with Cremophor® a solubilizing agent to enhance its solubility in the systemic circulation, however, many patients suffered hypersensitivity reactions from Cremophor® and pretreatment with steroids or antihistamine is recommended for these cases [43]. Albumin acts as a carrier for hydrophobic paclitaxel without Cremophor®, and is found to accumulate in tumors along with its bound constituents [42]. It is also able to enhance the endothelial cellular uptake of paclitaxel by the formation of caveolaes during transcytosis, this is reflected in clinical trials where the permeation and antitumor activity of Abraxane® was higher compared to paclitaxel. It is also found to have a higher maximum tolerated dose and the adverse effects of Abraxane® were reported to be less severe and frequent than the general taxane associated adverse effects [41, 43].

4.3 Nanocarriers for gene therapy and smart delivery systems

The enhanced stability during systemic circulation offered by nanocarrier to their cargo has served as a key advantage to the application of targeted gene delivery for cancer therapy. Gene therapy has been implicated to replace or knock out mutations commonly detected in cancer or insert new genes into cancerous cells to induce apoptosis. Therefore, by targeting endogenous tumor gene expressions, a highly potent and specific therapeutic effect can be instigated with minimal off-target toxicity. This is widely applicable to cancer therapy due to its dependence on oncoproteins and oncogenes. Moreover, multiple genes can be targeted to inhibit tumorigenesis, recurrence and resistance [44]. However, challenges with regard to toxicity and instability have rendered gene therapeutics immature for cancer therapy. The introduction of naked genetic material such as DNA, plasmid DNA, small interfering RNA, nucleotides, and peptides in the systemic circulation is limited due to instability and degradation by serum nucleases in the plasma as well as rapid renal clearance and phagocytosis by immune cells [44].

In addition, not only is cellular uptake restricted but nuclear delivery of genes into non-dividing target cells is inefficient and discrepancies exist on long term incorporation of genetic DNA information into the host cell, as it is likely to have unforeseen implications on patient's safety and toxic immune responses have been reported [44].

Nanocarriers have the potential to be delivery vectors for genetic material as cargo to their target tissue. Their versatility allows them to be designed in a manner that allows high gene delivery efficiency and payload capacity [44]. Genetic material can be incorporated and attached into nanocarriers by electrostatic interactions or surface conjugation [44]. Cationic nanocarriers like liposomes synthesized from cationic and neutrally charged lipids are able to condense DNA, siRNA, nucleotides, peptides and proteins to form complexes of plasmid DNA lipids that self-assemble into lipoplexes. Similarly, polymer nanocarriers are able to form polyplexes with nucleic acids [44]. These complexes protect the genetic cargo from enzymatic degradation in the systemic circulation and aid in cellular interactions that facilitate endocytosis. Hence they are able to deliver their cargo intracellularly increasing the likelihood of efficient transfection.

Moreover, nanocarrier can be synthesized from polymers that are able to respond to specific stimuli based on physiochemical differences between cancer and non-cancerous cells, leading to the development of smart drug delivery systems [45]. Their aim is to reduce dosage frequency in a spatially controlled manner and facilitate the delivery and accumulation of the therapeutic system to the target tumor tissue. Followed by the release of anticancer agent at the specific location at a precise concentration based on internal or external stimuli [45]. Smart polymer nanocarriers can respond to stimuli such as changes in pH, enzyme configurations, redox reactions and light [45]. The application of passive targeted therapy in cancer has proved to dampen down adverse effects experienced with traditional chemotherapeutics. In addition, the improved pharmacokinetic profiles and enhanced efficiency of passive targeted cancer delivery systems has increased the response and survival rates of patients. However, this strategy of targeted cancer therapy is not devoid of challenges as non-selective toxicity and resistance remains to be an issue that impedes cancer therapy. Hence a further extension to passive targeted cancer therapy was explored that pioneered the birth of a different strategy for targeted delivery it is active targeted therapy and is discussed in the final section of this chapter.

4.4 Active targeting delivery therapeutics

Active targeting anticancer therapeutics aims to further increase the selectivity of chemotherapeutics to tumor tissue via delivery strategies through preferentially potentiating their affinity towards cancer cells and escalating endocytic internalization. The principle mechanism of active targeted cancer therapy is based on receptor-mediated interactions. Their framework is established through targeting moieties such as small molecule ligands or antibodies that bind to receptor of proteins, sugars or lipids on the surface of target cells. As a result, these ligands act as delivery agents for the anticancer therapeutic system, prompting its tumor accumulation and enhancing its residence time.

Many of the same receptors targeted in molecular targeted therapeutics have been utilized as active targeted delivery therapeutics, imparting a second functionality to active targeted delivery cancer therapy, whereby one part of the system acts by targeted delivery and the other part mediates molecular mechanisms of cytotoxicity. Commonly targeted cell surface receptors include EGFR and HER2. Widely explored cell surface receptors include folic acid receptor, transferrin, and prostate

specific membrane antigen [11]. The scope of this targeting strategy is endless and a heterogeneous array of targeting components have been utilized and reported as drug-targeting conjugates such as antibodies in antibody drug conjugates (ADC), proteins, peptiodomimetic and small molecules.

Much of the success of active targeted delivery therapeutics for cancer therapy has been achieved with ADC. They constitute extremely potent chemotherapeutic agents that are not administered alone, due to their narrow therapeutic window and extreme non-selective toxicity, examples include maytansinoids and auristatins. Maytansinoids are second-generation microtubulin polymerization inhibitors similar to vinca-alkaloid traditional chemotherapeutics, that have been modified to bind to tubulin with >100-fold higher affinity [46]. The ADC transtuzumab emtansine (Kadcyla®/T-DM1) has been synthesized with a derivative of maytansin (DM1) conjugated to transtuzumab, previously mentioned, a MAb for breast cancer patients that overexpress HER2. This design allows targeted delivery of a highly potent cytotoxic agent to tumor cells that express HER2 with a favorable therapeutic window that would otherwise not be achieved by the cytotoxic agent alone. Many factors need to be taken into account with the design of ADC, particularly the binding affinity of the targeting ligand to the receptor after conjugation with the therapeutic agent, in the case of T-DM1, it binds to HER2 with a similar binding affinity to free transtuzumab [46]. This is not always the case as conjugation and linkers connecting the targeting moiety and therapeutic agent can cause steric hindrance or altered structural configurations, which restricts or dissolves binding. Linkers are designed to stably carry the therapeutic system in systemic circulation and insure the therapeutic agent will not dissociate until it has reached the target site. This is achieved to a higher degree with non-cleavable linkers, but cleavable linkers are utilized more commonly as complete cleavage of the therapeutic agent from the system ensures efficient cytotoxic action. Binding of the targeting moiety to the receptor usually induces cellular internalization of the therapeutic system. Targeting moieties and linkers are also designed to facilitate intracellular release of the cytotoxic agent. Transtuzumab in T-DM1 aids in the internalization of DM1 into cancer cells, it contains a non-cleavable linker that keeps the system stable in circulation but may compromise the cytotoxic activity of DM1 if proteolytic lysosomal degradation of transtuzumab is inefficient [46]. In addition to the cytotoxic action of DM1 after intracellular release, T-DM1 is also able to inflect the antitumor action of transtuzumab regarding inhibition of HER2 signaling and marking HER2 overexpressing cells for ADCC [46].

Although no small molecule active targeting delivery therapeutics for cancer therapy have been approved by the FDA to date, research in this field is growing exponentially. Many of these potential therapeutics in clinical stages of development utilize nanoparticles that are decorated with targeting ligands on their surface and encapsulate anticancer drugs as payloads [11].

5. Conclusion

The origins of targeted therapy started by challenging cytotoxic chemotherapy with an alternative approach to treatment, achieved by adopting the "magic bullet" theory of selectivity between pharmacological principles of cancer and non-cancer cells. Targeted anticancer therapy is an exponentially growing class of chemotherapeutic agents with advantages over conventional anticancer drugs. The advantage is a result of selective targeting of cytotoxic agents towards cancer cells over normal cells. Selective targeting is based on variations in genes, proteins and

pathophysiology of cancer cells compared to non-cancerous cells. This has been explored and achieved in molecular mechanism therapeutics as well as passive and active targeting delivery strategies for cancer therapy. The progress in cancer therapy stems from the understanding cancer biology leading to detailed distinctions between the pathophysiology of tumors and the physiology of normal tissue.

Strategies of targeted anticancer therapy have advanced by applying meticulous selectivity to chemotherapeutic. This commenced with variations in molecular mechanisms of action in mechanistic therapeutics with much success in targeting signal transduction pathways specifically tyrosine kinase proteins. Subsequently, a greater degree of selectivity is investigated by passive targeting mechanisms, which utilizes nanocarriers to take advantage of the enhanced permeation and retention effects of the tumor microenvironment. This strategy has also shown that not only is the pharmacodynamic profile of the anticancer agents significant for the success of cancer therapy but so is their pharmacokinetic profile. Further selectivity has also been explored by active targeting delivery via receptor-mediated interactions with cancer cells. All these targeting strategies can be combined and tailored to achieve efficient response rates for patients.

The application of targeted therapeutics has shifted therapy protocols for cancer patients towards precision medicine; hence various aspects need to be considered with targeting cancer therapy. This approach involves determining diagnostic biomarkers and genotyping tumors to choose the relevant targeted therapeutic for the patient. Furthermore, treatment needs to be designed and tailored for patients in terms of duration, dose and monitoring of adverse effects. Careful selection of combination therapies and dosing regimens are also critical to the success of cancer therapy.

Targeted cancer therapy has proven more effective than conventional chemotherapeutics as the maximum tolerated dose is higher so patients are able to tolerate therapeutic doses with less severe adverse effects. This resulted in improved patient response rates and survival. Although resistance is still an issue with cancer therapy, the strategies employed by targeted therapy have widened the scope of therapeutics available if resistance occurs. Although much of this field is still under development, the progress made with targeted cancer therapy is changing the perspective of cancer from a fatal disease to a chronic one that can be managed throughout the patient's lifetime.

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