Drug Delivery in Oncology – Challenges and Perspectives

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Summary

To date, none of the approximately 60 anticancer drugs used in conventional chemotherapy exhibits a selective uptake in tumor tissue and generally only a very small fraction of the administered dose reaches the tumor site. If surgery or radiotherapy is not effective, cure rates are in the range of 10% and, as a consequence, 90% of chemotherapeutic agents are administered in the palliative setting to stabilize the disease or to improve the quality of life. With such a low rate of drug accumulation in the tumor it is in fact surprising that tumor remissions can be attained; admittedly, these are achieved in the fast-growing tumors where cytostatic agents alone or in combination therapy are most effective in killing the rapidly dividing tumor cells by inhibiting different specific targets of the tumor cell that are responsible for tumor proliferation. Generally, however, tumor doubling times are slow, the tumor cells are in different stages of their cell cycles, and vascularization in the tumors is heterogeneous with necrotic and hypoxic areas being present that respond poorly to anticancer agents. Last, but not least, late-stage tumors have mostly formed micro- and macrometastases that are characterized by the multidrug resistance phenotype that includes changes in the cellular target of the respective drug, alterations in enzymatic activation and detoxification mechanisms, defective apoptotic pathways, membrane changes as well as elimination of the drug from the tumor cell through the action of drug efflux pumps. For treating metastatic cancer, chemotherapy regimens applied alone or in combination with hormones or novel agents such as monoclonal antibodies and signal transduction inhibitors are to date the best option of inhibiting or reducing the size of the primary tumor and/or metastases. However, treatment is basically palliative and improvement in overall survival through the introduction of novel drugs has generally not been more than a few months. Anticancer agents have steep dose–response curves, which has the consequence that a critical toxic concentration of the drug must be exposed to the tumor cell for a sufficient time to induce cell killing. The dilemma of conventional chemotherapy as well as with low-molecular-weight targeted therapeutics is that due to an unfavorable biodistribution and a lack of accumulation in tumor tissue,
they exhibit poor therapeutic indices and tumor remissions are often not achieved. It is here where the potential of drug delivery in oncology resides. Any means of transporting and delivering anticancer drugs in higher concentrations to the tumor over a long period of time whilst sparing healthy tissue is a step to a more effective cancer chemotherapy. This goal has been pursued for approximately 60 years, and has encompassed encapsulating or conjugating drugs with vitamins, lipids, peptides, oligonucleotides, antibodies, serum proteins, synthetic or natural polymers, liposomes, or protein- or polymer-based nano- or microparticles. Aided by the advent of sophisticated diagnostic tumor imaging and analytical tools that have enabled a far more precise understanding of the biochemical and physiological characteristics of tumor cells and tissue, as well as the expression of tumor-associated receptors and antigens, scientists have more opportunities than ever for designing and validating new drug delivery systems. During this process, we are also learning that similar to the translation of targeted therapies into the clinic, drug delivery systems are probably most effective in the form of a personalized medicine and in combination with established chemotherapeutic regimens. This three-volume state-of-the art book gives an account of the different anticancer drug delivery systems realized to date, the products that have reached the clinical setting or have obtained market approval, and the challenges that lie ahead in translational research in the area of cancer drug delivery.

Introduction

Alle Ding’ sind Gift, und nichts ohn’ Gift; allein die Dosis macht, daß ein Ding kein Gift ist [All things are poison and nothing is without poison, only the dose permits something not to be poisonous]

When Paracelsus, a pioneer in the application of chemicals and minerals in medicine, wrote this theorem in the sixteenth century, he addressed a fundamental principle that the practicing oncologist faces every day: “How can I treat and hopefully cure a cancer patient with a drug at a nontoxic or acceptable dose without the risk of conversely overdosing and risking severe side-effects or even the death of my patient?”

Paracelsus (1493–1541; true name was Phillippus Aureolus Theophrastus Bombastus von Hohenheim) (Figure 1) went on to summarize his own views on drug development: “Many have said of Alchemy, that it is for the making of gold and silver. For me such is not the aim, but to consider only what virtue and power may lie in medicines” [1].

Scientists over the past 60 years have developed around 60 clinically established cytostatic agents, which are classified into alkylating agents, antimetabolites, anthracyclines, plant alkaloids, microtubule inhibitors or modulators, topoisomerase inhibitors, and other antitumor agents. Their modes of action are diverse and
Figure 1  Paracelsus (Philippus Aureolus Theophrastus Bombastus von Hohenheim, 1493–1541). “Paracelsus,” meaning “equal to or greater than Celsus,” refers to the Roman encyclopedist Aulus Cornelius Celsus from the first century, known for his tract on medicine. Paracelsus is also credited with giving zinc its name, calling it zincum, and is regarded as the first systematic botanist.

Figure 2  All available anticancer drugs, 1940s to June 2006, by source (N = 175). The major categories: B = biological, usually a large (greater than 45 residues) peptide or protein either isolated from an organism/cell line or produced by biotechnological means; N = natural product; ND = derived from a natural product and is usually a semisynthetic modification; NM = natural product mimic; S = totally synthetic drug, often found by random screening/modification of an existing agent; S* = made by total synthesis, but the pharmacophore is/was from a natural product; V = vaccine. (Adapted from [2].)

manifold, and sometimes overlap, resulting in cytotoxic and/or cytostatic effects by affecting cell division, DNA synthesis, or apoptosis.

The era of cancer chemotherapy started with a great deal of optimism in the 1950s after alkylating agents, antimetabolites, and platinum complexes proved to be highly effective in the treatment of hematological malignancies and certain solid tumors. Often the word chemotherapy conjures fears in cancer patients due to the notion that they are being treated with toxic and synthetically designed chemicals. Without intending to play down the side-effects of anticancer therapy in any way, 60–70% of anticancer chemotherapeutic agents are in fact natural products or derived from them (Figure 2, as analyzed in depth by Newman and Cragg [2]).
Tumors that respond best to cytostatic or cytotoxic agents are those with fast doubling times of the order of a few days. These include chorioncarcinomas, lymphomas, leukemias, rhabdosarcomas, and testicular cancers. The response rates of the most common solid tumors (i.e., breast, lung, prostate, ovarian, liver, colorectal, gastric, and colorectal cancer) were far less encouraging. From around 1965 onwards, the mostly empirical approach of combining cytostatic agents improved the response rate, the overall survival, and the quality of life for many solid tumors. However, cure rates for the most common metastatic cancers (i.e., lung cancer, colon cancer, breast cancer, and prostate cancer) remained low, and the response rate for many cancers such as renal cell carcinoma, pancreatic carcinoma, gastric carcinoma, hepatocellular carcinoma, glioblastoma, and sarcoma was disappointingly poor.

Although oncologists were well aware of the narrow therapeutic windows of cytostatic agents, there was a continuing hope for many years that by using screening programs, improved preclinical animal models, and optimized combination therapies, new cytotoxic or cytostatic agents would emerge for treating solid tumors more effectively and eventually result in higher cure rates with side-effects being the price that had to be paid.

Despite several novel and effective cytostatic agents being discovered or developed in the past two decades, the advances in molecular and tumor biology from the 1980s onwards, which allowed a progressive elucidation of the genetic, cellular, subcellular as well as physiological mechanisms underlying cancer, continually made scientists working in drug development realize that cancer posed challenges that were not comparable to other fields of chemotherapy where major breakthroughs had been achieved, such as in the treatment of antibacterial, antifungal, or antiprotozoal diseases. Basically, two insights into the characteristics of malignant cells and tissue accounted for this recognition (see Chapter 1):

(i) Cancer cells essentially do not express any molecular targets, neither intra- nor extracellularly, that are unique and not present in healthy tissue.

(ii) The biochemical, cellular, and physiological nature of angiogenesis, proliferation, and invasion of cancer cells as well as the intricate steps involved in the formation of metastases results in tumors that hinder the penetration of therapeutic agents, and in addition a hostile microenvironment develops within the tumor characterized by necrotic, hypoxic, and acidic areas promoting chemoresistance.

In the following section the complexity and heterogeneity of malignant diseases is addressed, as well as the mechanisms of how tumor cells evade the cell-killing effect of drugs on a cellular, subcellular as well as physiological level and the challenges that lie ahead for improving the therapy of this worldwide disease, which according to the World Health Organization accounted for approximately 7.9 million deaths in 2010.
Dilemma and Challenge of Treating Malignant Diseases

A tumor is a neoplasm characterized by a failure in the regulation of tissue growth. The term “tumor” is not synonymous with cancer. A tumor can be benign, premalignant, or malignant, whereas cancer is by definition malignant. The abnormal proliferation of tissues is caused by mutations of genes that fall into two categories: oncogenes that promote cell growth and reproduction, and tumor suppressor genes that inhibit cell division and survival. Cancer develops through the formation of novel oncogenes, the overexpression of normal oncogenes, or the malfunction of tumor suppressor genes. Typically, changes in many genes are required to transform a normal cell into a cancer cell.

The transformation of normal cells into cancer has often been compared to a slow-starting chain reaction caused by initial genetic errors that progressively allow the cells to escape the controls that limit normal tissue growth until the formed cell cluster drives progression toward more invasive stages (Figure 3a). In order to do so, the cancer cell population must form new blood vessels – a process called angiogenesis – to satisfy their growing need for oxygen and nutrients. This is induced when proangiogenic molecules outweigh the effects of molecules with antiangiogenic activities. A so-called angiogenic switch takes place that can already occur when the malignant cell cluster reaches a size of merely 100–200 µm, and cancer cells, endothelial cells, stromal cells, and inflammatory cells secrete growth factors, permeability regulating factors, migration stimulators, proteolytic enzymes, extracellular matrix molecules, and adhesion molecules. The growth factors can be vascular-specific, such as the vascular endothelial growth factors (VEGFs) and their receptors, the angiopoietin family (Ang), Tie receptors, and the ephrins. Nonspecific molecules include platelet-derived growth factor (PDGF), basic fibroblast growth factors (bFGFs), transforming growth factor (TGF)-β, tumor necrosis factor-α (TNF)-α, and epidermal growth factor (EGF).

The process of angiogenesis is extremely complex and requires a series of steps in the “angiogenic cascade,” including (Figure 3b):

- Dilation of existing vessels.
- Activation, migration, and proliferation of endothelial cells.
- Hyperpermeability of postcapillary venules and vessel destabilization.
- Localized degradation of basement membrane by proteases such as matrix metalloproteases, cathepsins, urokinase, and plasmin.
- Extracellular matrix remodeling.
- Tube and sprout formation of vessels, and recruitment of pericytes and smooth muscle cells and vessel maturation.

Angiogenesis is not only a prerequisite for the transformation from a small, often dormant cluster of cancer cells to a solid tumor, but is also required for the spread of a tumor – the formation of metastases (the word originating from the Greek “angeion”, which means vessel, and “genesis”, which means birth). For metastases to form, a complex series of steps in which cancer cells leave the original tumor site and migrate to other parts of the body via the bloodstream or the lymphatic
Mutation inactivates tumor suppressor gene

Cells proliferate

Mutation inactivates DNA repair gene

Mutation of proto-oncogene creates an oncogene

Mutation inactivates several more tumor suppressor genes

Cancer

(a) (b)

**Figure 3** Development of cancer cell clusters due to a series of mutations in oncogenes or tumor suppressor genes ([http://en.wikipedia.org/wiki/Cancer](http://en.wikipedia.org/wiki/Cancer)), and (b) growth of the solid tumor due to tumor angiogenesis – the formation of blood vessels that supply the cancer cells with oxygen and essential nutrients (modified from [3], with permission).

...system have to take place. New evidence suggests that is not only the properties of the metastatic cancer cells, but also of the endothelial progenitor cells that allow single cancer cells to break away from a primary tumor and enter the blood vessels. This mosaicism of endothelial cells and tumor cells together with the secretion of proteases that degrade proteins of the extracellular matrix of the primary tumor allows for substantial shedding of tumor cells into the vasculature. Although the numbers of cells that leave a primary tumor can be of the order of many millions per day, the process of metastasis formation is in fact a very inefficient process because only a small fraction of the cells that leave a tumor are able to survive in the blood or lymphatic vessels, and only a few will have the intrinsic property to find a suitable location to settle and re-enter the tissues and form new tumors. Nevertheless, the formation of metastatic tumors is very common in the late stages of cancer due to the increasing number of tumor cells that are shed from the growing primary tumor. The most common places for the metastases to occur are the lungs, liver, brain, bones, and peritoneal or pleural cavities (see Figure 4 as an example of liver metastases originating from a pancreatic cancer).
The successful treatment of metastases represents such a vital challenge because they are responsible for approximately 90% of cancer-related deaths as well as for the many devastating symptoms that emerge and progress rapidly. In contrast, a primary tumor, such as a prostate cancer, can grow extremely slowly for many years without causing any symptoms at all.

**Narrow Therapeutic Window of Cytostatic Agents**

One of the main dilemmas of treating solid tumors is that they are not detected early enough and once diagnosed have often formed metastases. If they cannot be treated by surgery in combination with radiotherapy or neoadjuvant chemotherapy, the prognosis for curing the patient, mostly expressed in the literature as at least a 5-year tumor-free interval, remains highly unsatisfactory. Current chemotherapy regimens applied alone or in combination with hormones or novel agents such as monoclonal antibodies and signal transduction inhibitors are to date the best option for inhibiting or reducing the size of the primary tumor and/or metastases. Chemotherapy regimens are generally applied intravenously in cycles (ranging from a 1- to 4-week interval), with the frequency and duration of treatments limited by the toxicity to the patient. Most commonly, chemotherapy acts by killing cells that divide actively – one of the main properties of most cancer cells. As a consequence, cytostatic agents also harm cells that divide rapidly under normal circumstances, such as cells in the bone marrow, digestive tract, and hair follicles, producing side-effects in these organs. In most cases the cytostatic agents have distinct toxicity profiles, such as neurotoxicity, nephrotoxicity, dermatotoxicity, ototoxicity, and cardiotoxicity. These can be difficult to treat, are often dose-limiting, and in some cases are irreversible.

Despite these drawbacks, it is obligatory that repeated and optimized chemotherapy cycles be administered in order to obtain the best therapeutic outcome and to continuously reduce the size of the tumors or metastases. Only a fraction of the cells in a tumor die with each treatment cycle. This principle is known as the *log-cell-kill hypothesis*, which is a generally accepted hypothesis for hematological
cancers that states that during every cycle of chemotherapy the same fraction of tumor cells is killed, but not the same number. When mice with leukemia are treated with constant doses of anticancer agents, the number of leukemia cells diminishes logarithmically; if, for example, 99% of leukemia cells are killed after the first administration, this is equivalent to a decrease of $10^9 - 10^7$ cells, which corresponds to 2 orders of magnitude (two log steps). A second administration will also result in a 99% cell kill, but the number of tumor cells is only reduced from $10^7$ to $10^5$, which is only 10 million cells compared to the billion cells in the first cycle. In other words, in this idealized model, the fraction of cells that are killed remains constant, but the number of cells killed over time constantly decreases.

Transferring the log-cell-kill hypothesis to solid tumors is not as straightforward as it appears at first glance (Figure 5). With modern diagnostics, a tumor is detectable when it reaches a size of 1 cm$^3$ after 30 doubling cycles, which

![Figure 5](image)

**Figure 5** Tumor growth curve of a solid tumor. Once the tumor comprises approximately 1 billion tumor cells, its size is around 1 cm$^3$ (1 g) and it becomes detectable. The initial tumor cell has to perform 30 doubling steps to reach this size (which can take months to years considering that the tumor doubling times for human tumors lies in the range of 5–200 days), and merely further 10 doublings are needed to reach a mass of 1 kg assuming tumor growth occurs exponentially. This generally does take place because of an insufficient growing vasculature in large tumors leading to a lack of supply of nutrients and tumor necrosis. Of note is that according to the log-cell-kill hypothesis many cycles of chemotherapy are necessary to eliminate all of the tumor cells and only in 10–20% of cases are cures achieved. Palliative treatment is particularly disappointing with large tumors where only a relatively small fraction of tumor cells respond to anticancer agents.
corresponds to 1 g (i.e., $10^9$ cancer cells). Only 10 further doubling steps are necessary for the tumor to reach a size of 1 kg (i.e., $10^{12}$ cancer cells). In this time interval, tumor symptoms start emerging.

These insights are the reason why during curative, adjuvant, or palliative chemotherapy the doses and cycles of anticancer agents should not be reduced or discontinued even if the tumor or tumor lesions are no longer detectable, assuming that the treatment is tolerated by the patient. The log-cell-kill hypothesis can additionally be viewed as a theoretical basis for further treating patients for longer periods even though a complete remission has apparently been achieved.

However, the log-cell-kill hypothesis is strictly valid only for solid tumors, if at all, and only for those that are fast growing; however, in most cases the effect of cytostatic or cytotoxic agents on tumor growth can be described by the so-called Gompertz growth curve. This implies that tumor growth diminishes with increasing size of the tumor, which is noted in the semilogarithmic plot by a decreasing slope of the tumor growth curve as depicted in Figure 5. With increasing tumor size, many tumor cells remain in the G phase (quiescent phase) of the cell cycle because of an insufficient growing vasculature leading to a lack of supply of nutrients and tumor necrosis. In this phase, the response to treatment with anticancer agents is significantly reduced and the initial cycle of chemotherapy only manages to kill a fraction of the tumor cells, mostly those proliferating in the periphery of the tumor. As a consequence, the tumor mass is reduced, and quiescent cells are reactivated to enter the cell cycle and multiply. This is the reason why the response in the second or third cycle of palliative treatment is often better than in the first cycle because a higher percentage of tumor cells are killed.

Unfortunately, in this advanced stage of the disease further reduction of tumor size is seldom achieved because a population of tumor cells that has developed chemoresistance and/or micrometastases has already formed. Intrinsic or acquired chemoresistance is a major problem in cancer therapy. In the majority of cases the cancer cells develop resistance against a spectrum of anticancer agents – a phenomenon called multidrug resistance (MDR). A number of biochemical mechanisms have been described that are responsible for the MDR phenotype, which include changes in the cellular target of the respective drug, alterations in enzymatic activation and detoxification mechanisms, defective apoptotic pathways, membrane changes as well as elimination of the drug from the tumor cell through the action of drug efflux pumps such as P-glycoprotein, multiple resistance protein (MRP), and breast cancer resistance protein (BCRP), which belong to the ATP-binding cassette (ABC) transporter family. Hence, the concentration of the anticancer agent in tumor cells remains too low and cannot counterbalance the diverse mechanisms of chemoresistance. In addition, there are a number of physiological mechanisms that are responsible for resistance to chemotherapy as well as an impaired accessibility of anticancer drugs and drug delivery systems to all parts of the malignant tissue due to the heterogeneity of the tumor mass, as will be described below (for details, see Chapter 2).
Heterogeneity of Solid Tumors: Abnormal Blood Vessel Networks, Tumor Physiology, and Tumor Environment

Once a tumor cell cluster, whether in its initial stage as a primary tumor or in later stages when forming metastases, induces an angiogenic switch, its vasculature and microenvironment change dramatically, and an abnormal cellular organization, vessel structure, and physiological function develops. As an example, in contrast to the unbranched, nearly parallel vessels of healthy tissue (Figure 6A, right) of a murine brain, the vasculature of a brain tumor is dense, chaotic, and highly branched (Figure 6A, left).

The new tumor vessels formed during angiogenesis differ markedly from those of normal tissue and the neovasculature is characterized by an irregular shape, high density, and heterogeneity (Figure 6B). In addition, the endothelial cells are poorly aligned or disorganized with large fenestrations (Figure 6C, b–c). Other differences affect the perivascular cells, the basement membrane, and the smooth muscle layer that are frequently absent or abnormal. As a consequence, solid tumors are heterogeneous and form a complex society of cells in different microenvironments that can hinder the penetration not only of low-molecular-weight anticancer compounds, but also of macromolecular drug delivery systems through the same or different mechanisms.

These pathophysiological properties of tumors that influence the delivery of drugs to tumor tissue include (for details, see Chapter 2):

(i) Abnormal structure of tumor vasculature. The variable vascular density restricts the anticancer drug from reaching all parts within the tumor. This is due to the abnormal branching patterns and intercapillary distances in growing tumors. Tumor vessels are dilated, tortuous, and heterogeneous in their

Figure 6  (A–C) Differences in the architecture of microvessels and endothelial cells between healthy and tumor tissue. (A) Normal vasculature in the brain of a mouse (right) is very orderly, compared with the extremely branched vasculature of a mammary brain tumor (left). (B) Scanning electron microscopy (SEM) imaging of a polymer cast of normal microvasculature (vasa vasorum of rat carotid sinus, left) and tumor microvasculature (xenograft of a human head and neck cancer of a nude mouse, right). Marked differences are found in the degree of organization and an apparent lack of conventional hierarchy of blood vessels in the tumor sample. (C) SEM images of the luminal surface of healthy blood vessels (mammary gland, left) and tumor (MCa-IV mouse mammary carcinoma, right) blood vessels. While the healthy vessels are smooth and have tight endothelial junctions, the tumor vessels show widened intercellular spaces, overlapping endothelial cells, and other abnormalities. SEM images: (a) luminal surface of normal blood vessel, which is smooth and has tight endothelial junctions (arrowheads, mouse mammary gland); (b) tumor blood vessel, which has widened intercellular spaces, overlapping endothelial cells, multiple cellular processes, and other abnormalities (arrowheads, MCa-IV mouse mammary carcinoma); and (c) high magnification of a hole in the endothelium (arrows) showing the underlying basement membrane filaments (arrowheads). Scale bar: 5 µm in (a); 2 µm in (b); 0.5 µm in (c). (Reproduced kind permission of M. Konerding, University of Mainz, modified from [4].)
healthy vasculature
Tumor vasculature

Healthy
Tumor

(A) (B) (C)
spatial distribution. Intervessel distances in solid tumors can vary between 10 and 1000 µm, and thus many viable tumor cells are not exposed to detectable concentrations of low-molecular-weight drugs following a single injection. In these tumor regions the anticancer drugs do not achieve sufficient concentrations to kill all of the cancer cells. In addition, the concentrations of essential nutrients in these tumor regions are also low, leading to hypoxic, acidic, and necrotic areas that can partially or completely reduce the cytotoxicity of the anticancer agent.

(ii) **Abnormal blood flow in tumors.** Blood flow rates in many tumors are generally lower than those in many normal tissues and can vary considerably, ranging from around 0.01 to around 3.0 ml/g/min. The heterogeneity of tumor blood flow directly hinders the delivery of therapeutic agents to tumors and additionally causes interstitial pressure that in turn compromises the effectiveness of various therapies, and selects for more aggressive and metastatic cancer cells.

(iii) **Interstitial fluid pressure in tumors.** The interstitial compartment of tumors is significantly different to that of normal tissues. Primarily, as a result of vessel leakiness and hyperpermeability with a concomitant bulk flow of free fluid into the interstitial space that cannot be removed effectively due to a lack of functional lymphatics, most solid tumors have an increased interstitial fluid pressure. Increased interstitial fluid pressure within solid tumors above all inhibits the extravascular transport of larger molecules and nanoparticles because they rely more heavily on convection as opposed to simple transport by diffusion of low-molecular-weight drugs; exceptions being the transport into the core of the tumor through receptors expressed on the tumor endothelium by transcytosis such as for albumin (see Chapters 4 and 35).

The interstitial fluid pressure can, however, also hamper the efficacy of low-molecular-weight anticancer drugs because, although it is fairly uniform within the center regions of the tumor, the interstitial fluid pressure is significantly reduced at the tumor periphery, and interstitial fluid oozes out of the tumor and subsequently removes anticancer agents from the tumor tissue.

(iv) **Pathophysiological tumor microenvironment as an obstacle in tumor therapy.** As mentioned above, abnormal blood vessels are formed during tumor growth and blood flow in these vessels is heterogeneous, thus the intermittent blood supply leaves portions of the tumor with regions where the oxygen concentration is significantly lower than in healthy tissues. As a consequence, the lack of oxygen promotes an anaerobic metabolism of tumor cells and an extracellular acidosis in tumor tissue in the range of pH 6.0–6.8 prevails, primarily due to excessive production of lactic acid and CO₂. The hypoxic tumor cells as well as acidosis present in many solid tumors manifest a pathophysiological microenvironment that is often resistant to radiotherapy and chemotherapy. On the one hand, the mode of action of several anticancer agents (e.g., cyclophosphamide, methotrexate, 5-fluorouracil (5-FU), etoposide, carboplatin, bleomycin, and anthracyclines) is oxygen-dependent and thus hypoxia protects
tumor cells from damage by chemotherapy. On the other hand, extracellular acidosis in tumors reduces the tissue and cellular uptake of weakly basic drugs such as anthracyclines, bleomycin, mitoxantrone, and vinca alkaloids because their cellular uptake by diffusion is primarily efficient only for the nonionized form of the molecule. In addition, various mechanisms may additionally be involved in the acidosis-induced resistance to anticancer drugs, including an increased efflux of drugs, resistance to apoptosis, and an increased activity of DNA repair enzymes. That regions of hypoxic, acidic, and necrotic influence tissue penetration of a drug such as doxorubicin is shown impressively for three different preclinical tumors in mice in Figure 7. The immunofluorescence images after administration show the blood vessels in red, hypoxic areas in green, and doxorubicin in blue. The penetration lengths for doxorubicin

Figure 7  Representative three-color composite images showing the perivascular distribution of doxorubicin (blue) in relation to blood vessels (red) and hypoxic regions (green) in three different tumors growing in the right flank of mice: (a) human prostate PC-3 carcinoma, (b) mouse mammary sarcoma EMT-6, and (c and d) 16/C mammary carcinoma. Bar: 100 µm. (From [5].)
from the nearest blood vessels vary considerably within a 100 µm range and doxorubicin is unable to accumulate in hypoxic areas.

**Drug Treatment for Cancer Diseases: State-of-the-Art**

Current drug treatment for cancer diseases is based on therapy with cytostatic agents, hormones, cytokines, targeted therapeutics (monoclonal antibodies, tyrosine kinase inhibitors, proteasome inhibitors, histone deacetylase (HDAC) inhibitors), drug delivery systems (liposomes, albumin nanoparticles), and supportive care (pain therapy, hematopoietic growth factors, alternative therapies). Figure 8 gives a historic overview of the major classes of drugs and representative examples of the global cancer market. Sales for cancer-treating drugs increased by 10–14% in the past 3 years and is predicted to expand to approximately US$100 billion by 2012.

It is apparent when interpreting Figure 8 that the largest number of new drugs belong to so-called targeted therapy, which is defined as a medication that blocks the growth of cancer cells by interfering with specific intra- or extracellular molecular targets needed for carcinogenesis and tumor growth rather than by simply interfering with rapidly dividing cells. This term is somewhat misleading because most cytostatic agents used in chemotherapy also act on one or several molecular targets. The major difference is that many of the drugs that act as cytostatic agents were discovered by serendipity or in screening programs of natural products against a panel of tumor cell lines and were at the time often developed without any notion of their mode of action or cellular targets. In contrast, a new generation of targeted therapeutics was designed with an isolated target in hand, allowing for the generation of rationally designed drugs that had predetermined modes of activity. These agents were often derived using such techniques as high-throughput screening, molecular modeling, and structure-based design.

From the 1980s onwards this was a logically consistent step to take. Molecular and genetic approaches uncovered entirely new signaling networks of intra- and extracellular kinases, growth factor receptors, and antigens that regulate activities of tumor cells and tumor tissue, such as their epigenetic nature, their proliferation and survival as well as angiogenesis. As a result, the pharmaceutical and biotech industry invested heavily into the generation of targeted therapeutics.

When examining Figure 8, it is logical to ask whether the development of these new drugs in the past decade has translated into a reduction in cancer mortality or 5-year relative survival. In developed countries, approximately one in four deaths are due to cancer. If we take the American Cancer Society’s Cancer Facts & Figures report of 2010 for the United States as a guideline, the 5-year survival rate for all cancers diagnosed from 1999 to 2005 in the United States was 68%, up from 50% in 1975–1977. This improvement is primarily due to earlier diagnosis and conventional chemotherapy, but above all due to the refinement in surgery and radiotherapy, which have for many indications reached an optimal technical endpoint.
<table>
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<tr>
<th>Year</th>
<th>Cytostatic agents</th>
<th>Hormones</th>
<th>Antimetabolites</th>
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<th>Vinca-alkaloids</th>
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Figure 8  Major classes of drugs for treating cancer since 1950 and representative examples.
Table 1  Cancer incidence and 5-year prevalence trend from 2005 to 2009 in the United States\textsuperscript{a}.

<table>
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\textsuperscript{a}http://www.cancer.org.com.

In contrast, as shown in Table 1, an analysis of the cancer incidence and 5-year prevalence trend from 2005 to 2009 reveals that the overall 5-year-survival rate increased by merely around 5.7%, with the number of new cases increasing from 1 372 910 in 2005 to 1 437 180 in 2009 owing basically to an aging population.

It should be emphasized that nearly all targeted therapeutics, whether antibodies or small molecules, are used in combination with conventional chemotherapy and it is largely these combination protocols that account for the increase in overall survival rates for cancer patients. This analysis is not in any way meant to discredit the efforts and successes that have been achieved with so-called targeted therapy, and R&D in this field should certainly be continued. Rather, the analysis shows how difficult it is to treat cancer even with rationally designed drugs with defined targets. In many cases the actual therapeutic advantages gained were suboptimal. This underscores how progress in cancer treatment is achieved in small steps.

If there is room for criticism, it is that the scientific community, the cancer funding organizations as well as the pharmaceutical industry are prone to follow new trends and easily forget that an empirical as opposed to a rational approach in drug design can be equally successful. For example, cisplatin has made testicular cancer in young men a curable cancer. The drug is a metal complex discovered fortuitously by Barnett Rosenberg when he noticed that during an electrolysis experiment with platinum electrodes the growth of the common bacteria \textit{Escherichia coli} was inhibited. In contrast, Gleevec\textsuperscript{\textregistered} is a selective inhibitor of an aberrant, constitutively active enzyme, the BCR–ABL tyrosine kinase, that was developed by rational drug design and screening chemical libraries with subsequent lead optimization. The drug is highly effective in treating chronic myelogenous leukemia and gastrointestinal stromal tumors, and is currently being investigated in other tumor indications. Rational design of targeted therapeutics can even take a paradox turn. Although sorafenib (marketed as Nexavar\textsuperscript{\textregistered} by Bayer) was developed as a specific kinase type II inhibitor against Raf kinase, it was subsequently found to inhibit a variety of kinase receptors, including VEGF, EGF, and PDGF receptors. As it turned out, sorafenib was not approved in tumors with high Raf kinase expression such as melanoma or colorectal cancer, but is now approved as a multikinase inhibitor for advanced renal cell carcinoma and advanced hepatocellular carcinoma, (i.e., tumor indications for which it was never originally intended). Conversely,
Drug Delivery in Oncology – Challenges and Perspectives

Micro- and macro-particulate drug delivery systems

- Liposomes
- Nanoparticles
- Hydrogels
- Micelles

Macromolecular drug conjugates

- Drug conjugates with:
  - Antibodies
  - Synthetic polymers
  - Natural polymers
  - Serum proteins

Low-molecular weight drug conjugates

- Drug conjugates with:
  - Vitamins
  - Targeting peptides
  - Cell-penetrating Peptides
  - Aptamers
  - Fatty acids
  - Prodrugs

Figure 9  Classification of drug delivery systems.

Even after intensive investigations and thousands of publications on this topic, we still do not know why cisplatin is so highly effective against testicular cancer and not against other solid tumors.

Principles of Tumor Targeting

Drug delivery systems can be classified as micro- and macroparticulate drug delivery systems, macromolecular drug conjugates, and low-molecular-weight drug conjugates (Figure 9).

Whereas the drug is physically encapsulated in liposomes, nanoparticles, hydrogels, or micelles, it is covalently bound to the diverse low- and high-molecular weight drug carriers when developing drug conjugates. Transporting the drug cargo to the tumor site relies on two principles – defined as active and passive targeting, which are described below.

Active Targeting: Receptors and Antigens on Tumor Cells

Active targeting is based on cellular differences between normal and cancer tissue. From 1975 onwards the field of drug targeting received an important impetus with the development of monoclonal antibodies by Köhler and Milstein. Using this technology, it was now possible to derive pure antibodies that bound specifically to targets that were overexpressed on tumor cells. Thus, it seemed that the realization
of Paul Ehrlich’s early twentieth century vision of “the magic bullet” was at hand (Figure 10).

Although Paul Ehrlich is often regarded as the father of chemotherapy and drug targeting that is based on the concept of affinity, he was not involved in the concepts of drug delivery as is often mistakenly cited in the literature [6]. The concept of cancer drug delivery implies transporting the anticancer drug to the tumor tissue and cells with subsequent release, either intra- or extracellularly. Drug conjugates developed for active targeting comprise mostly high-molecular weight carriers, but low-molecular weight compounds are also used. A suitable carrier combines optimal loading and release properties, long-term circulation, low toxicity, and high affinity for the receptor or antigen without increasing drug levels in healthy tissue.

The elucidation of suitable membrane-associated targets and the subsequent targeting properties of carriers and validation in preclinical models in the clinic have been expedited by the advances in immunohistochemistry, fluorescence-activated cell sorting analysis, and ultimately the refinement of tumor imaging techniques that can be routinely applied in the preclinical as well as clinical setting (see Chapters 7, 8, 9, and 15).

Selected cellular targets together with appropriate carriers that have been investigated for active targeting in cancer therapy are shown in Table 2.

### Passive Targeting and the Enhanced and Permeation Effect in Relation to Tumor Targeting

In same year as Köhler and Milstein reported on their pioneering work on the production of monoclonal antibodies, Helmut Ringsdorf proposed a general scheme of designing a drug delivery system using synthetic polymers for low-molecular
Table 2  Examples of membrane-associated targets and drug carriers for active targeting.

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Representative drug carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular receptors</td>
<td>linear peptides, cyclic peptides, antibodies, immunoliposomes</td>
</tr>
<tr>
<td>Integrins (αvβ3, αvβ5), Nucleolin, Aminopeptidase N, Endoglin, VEGF receptor (VEGF1–4)</td>
<td></td>
</tr>
<tr>
<td>Receptors of plasma proteins, Low-density lipoprotein receptor, Transferrin receptor, Albondin (gp60)</td>
<td>lipoproteins, transferrin, albumin antibodies</td>
</tr>
<tr>
<td>Peptide receptors, Somatostatin receptor, Bombesin receptor, Neuropeptide Y receptors, Luteinizing hormone receptor</td>
<td>linear peptides</td>
</tr>
<tr>
<td>Receptors for growth factors and vitamins, Folate receptors (e.g., EGF1, EGF2, HER2), TGF receptor, FGF receptors</td>
<td>folic acid, antibodies</td>
</tr>
<tr>
<td>Carbohydrate recognizing receptors, Asialoglycoprotein receptor, Galectins (e.g., galectin 1, galectin 3), Selectins (e.g., E-selectin, P-selectin), Hyaluronic acid receptors (CD44, glucose uptake transporters</td>
<td>lactosaminated albumin, glycoside clusters, natural polymers, sugars</td>
</tr>
<tr>
<td>Antigens</td>
<td>antibodies, immunoliposomes</td>
</tr>
<tr>
<td>Cluster of differentiation (e.g., CD20, CD33), Carcinoembryonic antigen, Blood group carbohydrates, Mucin-type glycoproteins (MUC1, CanAg), Lewis Y, Lewis X Cancer testis antigens (CT7, MAGE-A3), Prostate-specific membrane antigen</td>
<td></td>
</tr>
</tbody>
</table>

Ringsdorf’s model for a polymeric drug containing the drug, solubilizing groups, and targeting groups bound to a linear polymer backbone.

weight drugs (Figure 11). One to several drug molecules are bound to a polymeric backbone through a spacer that incorporates a predetermined breaking point to ensure release of the drug before or after cellular uptake of the conjugate. The system can also contain solubilizing groups or targeting moieties that render water solubility and targeting properties to the carrier.

Ringsdorf’s visionary model for developing drug delivery systems was basically ignored for many years, and it was not until Hiroshi Maeda laid the foundations for
passive targeting in 1986 that drug conjugates with synthetic polymers were intensively synthesized and evaluated for their antitumor efficacy. In 1986, he reported on a simple animal experiment. He intravenously injected the albumin-binding dye Evans blue into mice bearing subcutaneously growing tumors and to his surprise found that the Evans blue–albumin complex accumulated within tumors very efficiently (Figure 12). As an explanation for this phenomenon, Hiroshi Maeda coined the expression “enhanced permeability and retention” in relation to passive tumor targeting – the so-called EPR effect. In contrast to active targeting that proceeds on a cellular level focusing on the specific molecular interactions with tumor-associated cell receptors or antigens, passive targeting represents a more universal strategy of tumor targeting that exploits anomalies of malignant tissue resulting from the tumor’s pathophysiology. As described above, blood vessels differ markedly from those of normal tissue, characterized by an irregular shape and the endothelial cells are poorly aligned or disorganized with large fenestrations having diameters in the range of around 100–500 nm. These anatomical features make the vasculature
of tumor tissue permeable for macromolecules such as albumin or even larger nano-sized particles. Once the macromolecules have permeated into the tumor bed, a second effect is responsible for their tumor accumulation. Whereas smaller molecules are rapidly cleared from the tumor interstitium, large molecules are retained due to an impaired or absent lymphatic system (Figure 13).

A number of factors influence the EPR effect in preclinical animal models: the size and type of the tumor, and the tumor model (subcutaneously growing, intramuscular growing, spontaneously growing, orthotopically implanted, or chemically induced) all affect vascularization and the extent of hypoxic and necrotic areas. Indeed, techniques such as intravital imaging have provided a detailed insight into the tumor microcirculation and microenvironment confirming hyperpermeability, a heterogeneous and compromised blood flow, and an absence of functional lymphatic vessels resulting in elevated interstitial fluid pressure that hinder the delivery of therapeutic agents to tumors. It is therefore likely that although the EPR effect is universal to all tumors, the extent of the EPR effect can vary considerably within the tumor. Interestingly, there are a number of strategies emerging that enhance the EPR effect, including raising blood pressure or coadministering drugs that act as vascular mediators and release nitric oxide (see Chapter 3 for details).

In summary, the EPR effect has laid the foundation for developing a spectrum of drug delivery systems ranging from micro- and macroparticles, liposomes, drug conjugates with synthetic polymers to serum proteins.
Design and Development of Drug Delivery Systems

The principle structures of drug delivery systems that have been developed during the past six decades are depicted in Figures 14 and 15. While the drug conjugates with different carriers illustrated in Figure 14 can be small molecules as well as macromolecular drug delivery systems usually between 5 and 20 nm in size, the micro- and macroparticulate drug delivery systems are by nature all large particles with diameters exceeding 50 nm. These drug delivery systems encompass encapsulating or conjugating drugs with vitamins, lipids, peptides, aptamers, antibodies, synthetic or natural polymers, liposomes, or protein- or polymer-based nano- or microparticles. Related approaches have also been realized for the drug delivery of DNA and RNA as illustrated in Figure 16.

Both the covalent coupling of a drug or the physical encapsulation of a drug inside a carrier allow active or passive targeting drug delivery strategies to be realized. When designing drug delivery systems, the drug bound to the carrier should have sufficient stability in the bloodstream, but allow the drug to be released effectively at the tumor site by enzymatic cleavage, by reduction, or in a pH-dependent manner. Release of the free drug can occur extra- and/or intracellularly. Low- and high-molecular weight drug delivery systems that interact with a tumor-associated antigen or receptor are taken up by the tumor cell through antigen- or receptor-mediated endocytosis, drug delivery systems that follow a passive targeting approach by adsorptive or fluid-phase endocytosis. As depicted in
Figure 15 Examples of micro- and macroparticulate systems ranging from liposomes, hydrogels, micelles, aptamer nanoparticles, and albumin–drug nanoparticles.
Figure 16  Examples of drug delivery systems for gene delivery (see Chapters 42–44).

Figure 17, invaginations occur at the cell surface during endocytosis and endosomes are formed that migrate into the cytoplasm. Depending on the drug carrier and the kind of endocytosis process involved, a series of sorting steps take place in which the endosome is either transported to certain cell organelles (e.g., the Golgi apparatus), returns to the cell surface (recycling), or forms primary and secondary lysosomes, respectively. The pH drop during endocytosis is considerable – from 7.2 to 7.4 in the extracellular space to pH 6.5–5.0 in the endosomes and to around pH 4.5–4.0 in primary and secondary lysosomes. In the lysosomes a large number of enzymes such as esterases, proteases, or lipases become active.

There has been considerable research toward developing tailor-made cleavable linkers that exploit the endosomal/lysosomal pathways for prodrug activation. Additional efforts include extracellular cleavage of carrier-linked prodrugs that is mediated through the activity of proteases that are secreted by the tumor cells.

A further option for releasing the conjugated or encapsulated drug in the tumor tissue or tumor cells is by hydrolysis or diffusion.

Major challenges in the development of drug delivery systems include designing tailor-made cleavable linkers and defining the precise chemical modification of the drug, isolating and purifying macromolecular drug delivery systems from unbound
Figure 17 Cellular uptake of drug delivery systems as illustrated for carrier-linked prodrugs by either fluid-phase, adsorptive, or receptor-mediated endocytosis.

drug, achieving a stable and efficient encapsulation, and finally manufacturing the drug delivery systems and preparing sterile clinical trial samples. Also of critical importance is the precise characterization of the drug delivery system. Although this does not pose an obstacle for low-molecular-weight drug conjugates such as drug conjugates with vitamins, peptides, or fatty acids, the physicochemical characterization of macromolecular and nano-sized drug delivery systems can be cumbersome. In contrast to low-molecular-weight prodrugs, macromolecular drug delivery systems are not uniform, having molecular weight dispersities, charge distributions, and a range of drug loading ratios. While the heterogeneity of macromolecular drug delivery systems, which include drug conjugates with antibodies, synthetic polymers or liposomes, and nanoparticles and microparticles, creates additional complexities with respect to reproducibility and analytical characterization, the technology has been put into place to address these issues.

For the vast majority of drug delivery systems that are described by the authors in this book, technical and manufacturing issues have been solved and convincing in vivo proof of concepts have been obtained in tumor-bearing animal models. Although only a few drug delivery systems have reached market approval, such as liposomes (Doxil®, Daunosome®, Myocet®), the albumin taxol nanoparticle Abraxane®, a drug–polymer conjugate SMANCS (a conjugate of poly(styrene-co-maleic acid/anhydride) and the antitumor agent neocarzinostatin)
(Figure 8), phase I–III trials have been performed with the majority of drug delivery approaches. These translational efforts are vital steps in the development of drug delivery systems in oncology and provide important clinical information, including efficacy, toxicity issues, biodistribution, tumor targeting, and pharmacokinetics. These clinical data together with further preclinical R&D will guide us through the challenges that lie ahead of adding new drug delivery systems to the routine treatment of cancer diseases and will help to answer pivotal questions such as:

- Which drug delivery systems are suitable for which tumor indication?
- How can we avoid the uptake of macromolecular drug delivery systems in the reticuloendothelial system (macrophages, liver, and spleen)?
- What are the optimal dosing schedules for the individual drug delivery systems?
- How can potential cumulative toxicity be avoided?
- Which drug combinations with drug delivery systems are most effective?
- At what stage of cancer should we begin with administering drug delivery systems?

The development of drug delivery systems is a relatively new field of research compared to conventional chemotherapy. However, interest in this area is greatly expanding, as can be seen by the continual increase in publications on drug delivery concepts and cancer since 1945 (Figure 18).

With nearly 8000 publications appearing between 2005 and 2010, and the clinical experience achieved to date, there is reason for optimism that drug delivery systems will play a significant role in clinical cancer medicine. In addition, there is now considerable evidence that these systems can be combined with conventional therapies and add to the repertoire of agents used for cancer chemotherapy. It is highly likely that many of the new macromolecular-based approaches described in this book will eventually lead to approved drugs that will make differences in the lives of patients suffering from cancer.
References
