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From Composition to Cure: A Systems Engineering Approach to Anticancer Drug Carriers

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Abstract

The molecular complexity and heterogeneity of cancer has led to a persistent, and as yet unsolved, challenge to develop cures for this disease. The pharmaceutical industry focuses the bulk of its efforts on the development of new drugs, but an alternative approach is to improve the delivery of existing drugs with drug carriers that can manipulate when, where, and how a drug exerts its therapeutic effect. For the treatment of solid tumors, systemically delivered drug carriers face significant challenges that are imposed by the pathophysiological barriers that lie between their site of administration and their site of therapeutic action in the tumor. Furthermore, drug carriers face additional challenges in their translation from preclinical validation to clinical approval and adoption. Addressing this diverse network of challenges requires a systems engineering approach for the rational design of optimized carriers that have a realistic prospect for translation from the laboratory to the patient.

Graphical Abstract

Keywords

antitumor drugs; cancer; drug delivery; nanoparticles

1. Introduction

The global impact of cancer is staggering, with an estimated 14.1 million new cancer cases and 8.2 million cancer deaths worldwide in 2012.^[1] These numbers highlight the incredible effect that improved anticancer treatment could have. Although the death rate from cancer in the United States decreased by 26% from 1990 to 2015, most of this progress is attributed to preventive measures, including decreased tobacco use and increased screening by colonoscopy and mammography.^[2] Although major advances have been made in the successful prevention, diagnosis, and treatment of a variety of cancers, the prognosis for many types of cancer remains dismal, with pancreatic cancer being an example for which no improvement in survival has occurred over this 25-year period. Research is, therefore, still urgently needed to develop improved cancer therapies.

Administration of free drug, such as small-molecule chemotherapeutics, remains the standard of care for many cancers. However, there are inherent challenges presented by the administration of free drugs, primarily caused by their nonspecific action and nonspecific biodistribution. Many anticancer drugs exert some preferential cytotoxicity on cancer cells as a result of their rapid proliferation compared to most normal cells. However, this cytotoxicity is not completely restricted to cancer cells and is often apparent in healthy tissues, especially the heart,^[3] bone marrow,^[4] gastrointestinal tract,^[5] and nervous system.^[6] Even novel “targeted” chemotherapeutics and biologic drugs that act on dysregulated cancer-specific pathways exhibit off-target effects in healthy tissues.^[7] This nonspecific action, in combination with nonspecific accumulation of the drug in both the tumor and

healthy tissues, results in side effects that limit the maximum tolerated drug dose and, thus, ultimately hinder efficacy.

Improved means of drug delivery can enhance the safety and efficacy of existing anticancer therapeutics. The use of drug carriers—defined here as any integrated materials system that chaperones a drug cargo—can improve the safety and efficacy of the treatment by manipulating the drug's pharmacokinetics and biodistribution. Drug carriers serve to increase the amount of drug that reaches the tumor, while limiting the exposure of healthy tissues to the drug, thereby improving the therapeutic outcomes and minimizing undesirable side effects compared to the free drug. Decades of effort have produced diverse drug carrier designs ranging from protein or polymer conjugates, to self-assembled systems such as micelles and liposomes, to covalently stabilized nanostructures such as dendrimers and inorganic particles, and to macroscopic systems such as capsules, gels, and films. The appropriate choice of drug carrier from this growing inventory is largely dictated by the physicochemical properties of the anticancer drug that can be improved by a drug delivery system. These drug characteristics, such as insolubility and susceptibility to degradation, as well as the site and mode of therapeutic action, can be matched to a carrier whose design provides specific advantages to improve the drug's safety and efficacy.

To achieve antitumor efficacy, drug molecules must overcome numerous transport barriers that lie between their site of administration and their site of therapeutic action in the tumor. From the point of entry, for example, intravenous injection, a drug must traverse a pathway whose length scale is one billion times greater than its own dimensions before reaching its target. Along this arduous journey the drug encounters a series of barriers at the systemic, tissue, cellular, and subcellular level as it travels through the human body to its therapeutic site of action within the cancer cell (Figure 1).

Besides these pathophysiological barriers, there are additional challenges that must be overcome for the translation of drug carriers from their preclinical validation to their clinical testing and their ultimate adoption in clinical practice. These challenges are often insurmountable, as evidenced by the stark contrast in the abundance of drug carriers that achieve incredible preclinical success in the laboratory and the small handful of drug carriers that have been clinically approved as anticancer therapies. Translational challenges are less recognized as parameters in the design and validation of anticancer drug carriers, but are no doubt factors in their success or—more likely—their failure.

We thus suggest a systems engineering approach to drug carrier design that considers the challenges of both biological function and clinical translation (Figure 2). On a descending length scale, we first examine the pathophysiological challenges to drug delivery at the systemic, tissue, cell, and subcellular levels, while presenting approaches in which engineered drug carriers have been adopted to potentially overcome these obstacles. Then, in ascending levels of development, we consider the challenges in the translation of novel drug carriers for cancer therapy. We review this complex network of barriers with the goal of providing insight for the improvement of the next generation of drug carriers for clinical cancer treatments.

2. Pathophysiological Challenges

2.1. Systemic Circulation

Upon administration, anticancer drugs are immediately faced with challenges inherent to their in vivo environment. For systemically administered drugs that are delivered intravenously, the circulating blood presents barriers to drug delivery because it is an aqueous medium that is rich in cells and proteins and actively filtered by the spleen, liver, and kidneys. To overcome even the initial challenges of introducing a drug into the systemic circulation, there are distinct advantages to associating a drug with a delivery vehicle. Appropriately designed carriers can increase drug solubility, stability, and apparent molecular weight to safely prolong the presence of a drug in the circulation.

2.1.1. Solubility—For intravenous delivery, an anticancer drug must be soluble in the aqueous conditions of the blood to permit safe administration into the circulation. This is a major challenge for the many hydrophobic anticancer drugs that exhibit limited aqueous solubility (Figure 3; $\log D$). To permit their systemic administration, drugs with poor solubility must be administered in combination with potent surfactants. Paclitaxel, for example, is a small-molecule mitosis inhibitor that has an extremely low aqueous solubility of less than 0.03 mg mL^{-1} .^[8] Taxol, the solubilized pharmaceutical formulation of paclitaxel, utilizes a polyethoxylated castor oil surfactant (Cremophor/Kolliphor EL) to permit systemic administration by intravenous injection. This surfactant poses significant health risks, including anaphylactic hypersensitivity.^[9] This drug formulation, thus, requires prophylactic corticosteroids and antihistamines to mitigate serious side effects.

The sequestration of poorly soluble drugs in surfactant-free delivery systems can greatly improve drug safety and efficacy. The surfactant-free delivery of paclitaxel was first achieved in the clinic with Abraxane, a nanoparticle formulation of paclitaxel bound to the long-circulating blood protein albumin. Abraxane enhances the maximum tolerated drug dose and improves the therapeutic response in breast cancer compared to free paclitaxel delivered with Cremophor EL,^[10] while eliminating the dangerous hypersensitivity risks associated with the surfactant-based formulation. The surfactant-free delivery of paclitaxel has also been achieved in the clinic with micelle^[11] and liposome^[12] drug carriers.

2.1.2. Degradation—The complex environment of the blood presents another challenge to systemically administered drugs, in that the drug must resist degradation in an environment rich in cells and proteins. In particular, abundant enzymes in the blood can prematurely degrade or deactivate a drug. For example, gemcitabine, a nucleoside analogue, is rapidly converted into its inactive metabolite by deoxycytidine deaminase. This degradation leads to an extremely short half-life of only 8 min in humans after intravenous infusion over 30 min.^[13]

Drug carriers can provide protection to labile drugs by shielding them from interactions with environmental factors that lead to degradation. The conjugation of gemcitabine to a squalene carrier through its amine group—the site of enzymatic degradation—improves its stability, as shown by an 80% reduction in metabolized gemcitabine compared to free drug when exposed to blood plasma.^[14] The self-assembly of squalene–gemcitabine conjugates into

nanoparticles increases the terminal half-life of gemcitabine nearly fourfold over that of the free drug when delivered intravenously in mice.^[15] Protection can also be conferred by steric barriers to interaction with the local environment, for example, by the use of “stealth” polymers such as poly(ethylene glycol) (PEG). Modification with PEG has been used to successfully protect experimental anticancer protein therapeutics in mice, including tumor necrosis factor alpha (TNF- α)^[16] and asparaginase,^[17] from enzymatic proteolysis.

2.1.3. Clearance—The mechanisms of clearance from the systemic circulation present challenges in terms of the means by which, and the rate at which, a drug can be cleared from the blood. The kidney and the liver are primarily responsible for removing a drug from the circulation. Physicochemical drug properties including size, charge, and hydrophobicity dictate the speed of their clearance. Small-molecule drugs (Figure 3; molecular weight), for example, are often readily cleared from the systemic circulation by filtration in the kidney if their molecular weight is well below the glomerular filtration cutoff of approximately 60 kDa and/or their size is below approximately 6 nm.^[18] Renal filtration is, thus, a major contributor to the clearance of some small-molecule drugs, such as the alkylating agent cisplatin.^[19]

Drug carriers have been extensively explored as a means to increase the half-life of small-molecule drugs in the plasma by increasing the relative size of the drug to above the renal filtration cutoff. This has been achieved with macromolecular drug conjugates as well as drug-loaded nanoparticles. Cisplatin encapsulated in PEGylated liposomes, for example, exhibits greatly enhanced drug exposure, with an approximately 100 times greater area under the curve compared to the free drug.^[20]

Other physicochemical properties of the drug, including its charge, hydrophobicity, and immunogenicity also influence its clearance from the circulation. For small molecules, below the renal filtration cutoff, positively charged drugs may be preferentially cleared from the circulation through electrostatic interaction with the negatively charged capillary walls in the kidney glomerulus.^[21] Charge as well as hydrophobicity also influence clearance initiated by opsonization, in which adsorbed blood proteins tag drugs for degradation by the mononuclear phagocytic system (MPS).^[22] Shielding the solvent-accessible interface of the drug by a “stealth” polymer, such as PEG, can prevent protein adsorption and minimize opsonin-mediated uptake by macrophages.^[22]

2.2. Accumulation in the Tumor

The accumulation of drug in the tumor tissue remains the major challenge for achieving anticancer efficacy. Pathologic features of the tumor vasculature and extravascular space present both advantages and challenges for achieving accumulation in and distribution throughout the tumor following intravenous administration. Drug carriers have been extensively explored to improve accumulation and penetration in the tumor.

2.2.1. Passive Accumulation—Disorganized tumor growth leads to abnormalities in angiogenesis that result in aberrant vasculature that often exhibits increased permeability because of endothelial porosity. A range of vascular pore sizes from 200 nm to 1.2 μ m have been measured in murine and human tumors implanted subcutaneously in mice, with most

tumor vasculature exhibiting a pore size of 380–780 nm.^[23] Healthy vessels, in contrast, have tight endothelial junctions that prevent vascular permeability. Tumors also generally lack an organized lymphatic system, which inhibits the clearance of extravascular fluid from the tumor. The leaky tumor vasculature and lack of lymphatic vessels, thus, facilitates the passive extravasation and accumulation of a drug in the extravascular tumor tissue through the enhanced permeability and retention (EPR) effect.^[24]

Passive accumulation in the tumor by means of the EPR effect is influenced by the size and systemic circulation of the drug. The drug must be smaller than the size of the vascular pores and it must be maintained at a high concentration in the vasculature to enhance the passive accumulation that can be achieved over time. The prolonged circulation afforded by macromolecule and nanoparticle drug carriers that prevent rapid clearance can increase tumor accumulation by the EPR effect, provided the drug carrier is smaller than the vascular pores of the tumor (Figure 4A). Although drug carriers approximately 100 nm in diameter are commonly delivered to their target site by the EPR effect, nanoparticles smaller than 50 nm in diameter may be necessary to target poorly vascularized tumors with limited permeability,^[25] as is typical in tumor types with a dense fibrous stroma, such as pancreatic cancer.^[26]

An incredible variety of macromolecule and nanoparticle drug carriers have been designed to exploit the EPR effect as the primary mechanism of drug accumulation in the tumor after systemic injection. However, only a median of 0.6% of the administered dose of nanoparticle drug carriers has been reported to typically reach the tumor by passive accumulation.^[27] To improve the extravasation of these carriers, mechanisms have been exploited to temporarily increase the vascular permeability in the tumor. Increasing the vascular permeability by administration of vascular endothelial growth factor (VEGF), for example, significantly increases the extravasation of 158 kDa dextran from the tumor vasculature,^[28] but to a lesser extent from healthy vasculature. Similarly, the administration of transforming growth factor (TGF)- β type I receptor inhibitor (T β R-I) destabilizes the tumor vasculature by reducing the pericyte coverage, thereby enhancing the accumulation of doxorubicin-loaded liposomes (Doxil) and adriamycin-loaded polymeric micelles, and improving tumor regression of subcutaneous pancreatic adenocarcinoma xenografts in mice.^[29] Mild hyperthermia, in which the tumor is locally heated to 42°C for about 1 h, can also increase the vascular permeability by inducing endothelial gaps.^[30] Local heating can significantly increase the extravasation of liposomes as large as 400 nm in diameter in tumors that completely prohibit extravasation of liposomes greater than 100 nm in diameter at normal body temperature.^[31] This effect persists for up to 6–8 h^[30,32] and is limited to the tumor vasculature.

Despite its advantageous contribution to the EPR effect, the abnormal tumor vasculature also presents a significant barrier to accumulation, in that it contributes to the high interstitial fluid pressure exhibited in many tumors. The permeable tumor vasculature allows fluid to leak into the interstitial tumor tissue,^[33] which is not well-regulated because of the lack of organized tumor lymphatics. If elevated pressure in the interstitial space approaches the intravascular pressure, the transvascular pressure gradient decreases so that there is little convective driving force for drugs to leave the circulation and penetrate the tumor tissue.

Consequently, interstitial fluid pressure in the tumor has been negatively correlated with accumulation of drug carriers in tumors.^[34]

A number of methods have been explored to increase passive accumulation in tumors by combating the high interstitial fluid pressure in the tumor that arises from a leaky tumor vasculature. The systemic blood pressure or blood flow, for example, can be increased with drugs such as angiotensin or nitroglycerin^[35] to overcome the interstitial fluid pressure of the tumor. Alternatively, the interstitial fluid pressure can be reduced by normalization of the tumor vasculature^[36] with inhibitors of angiogenesis, such as anti-VEGF-receptor-2 antibody. A single intraperitoneal injection of anti-VEGF-receptor-2 antibody can reduce the interstitial fluid pressure and increase penetration of intravenously delivered bovine serum albumin into the tumor.^[37] Normalization of tumor blood vessels has been shown to enhance the efficacy of Abraxane (ca. 10 nm), but not Doxil (ca. 100 nm), thus demonstrating that the decreased pore size that accompanies normalization of the tumor vasculature limits application of this method to nanoscale drug carriers that are smaller than 100 nm.

Although the EPR effect is assumed by many to provide a nearly universal mechanism of passive accumulation in tumors for drug carriers, there is significant evidence of variability in the EPR effect in preclinical animal models. Vascular permeability varies with tumor type, location, and size.^[23,38] Furthermore, the vascular permeability can vary within a single tumor depending on factors such as the rate of blood flow.^[39] The EPR effect is, thereby, a heterogeneous characteristic of tumors that should not be taken for granted in every tumor model.

2.2.2. Active Accumulation—Beyond abnormal vasculature and lymphatics that promote passive drug accumulation by the EPR effect, distinctive features of tumor cells and their extracellular environment provide opportunities to direct drug accumulation to tumors by active tumor targeting. Distinctive characteristics of tumors may include upregulated cell-surface receptors on cancer cells, overexpressed extracellular enzymes, or a depressed pH value in the tissue. Active targeting relies on the functionalization of the drug or drug vehicle with a moiety that specifically recognizes or responds to a unique feature of the tumor environment (Figure 4B). The true value of active targeting may not be accumulation, but prolonged residence in the tumor or intracellular delivery that increases drug efficacy.^[40] Active targeting may, however, contribute significantly to accumulation in metastatic disease, where passive mechanisms of accumulation by the EPR effect may be limited by lack of angiogenesis. Active targeting may permit the identification of and homing to sites of systemic disease, provided the metastatic niche has a targetable feature.^[41]

In its simplest form, an actively targeted drug carrier is composed of a targeting moiety and a therapeutic cargo. Antibodies, for example, have been used extensively to confer highly specific binding to cancer-associated targets when conjugated to drug cargo. The anti-HER-2 antibody, which targets the HER-2 receptor on a subset of breast cancers and inhibits HER-2 signaling, has been covalently linked to DM1 (emtansine), a small-molecule microtubule inhibitor, to improve tumor-targeted drug delivery while preventing drug exposure in healthy tissues.^[42]

Other upregulated cell-surface receptors can also serve as targets for active drug delivery. The transferrin receptor, for example, is upregulated in a variety of cancers.^[43] Functionalization of liposomes with a single-chain anti-transferrin-receptor antibody permits receptor-mediated intracellular delivery in cancer cells that upregulate the transferrin receptor. The delivery of a p53-encoding plasmid, to restore the function of a tumor suppressor gene, with this targeted liposome can achieve exogenous p53 gene expression in metastatic tumors.^[44] The transferrin receptor can also be targeted by transferrin, its natural ligand.^[45] Transferrin-functionalized cyclodextrin nanoparticles are internalized by receptor-mediated uptake in cancer cells that upregulate the transferrin receptor. The cellular uptake of transferrin-functionalized nanoparticles is believed to be the key advantage of this active targeting mechanism, as the overall accumulation of the targeted nanoparticles in the tumor is not greater than unfunctionalized control nanoparticles.^[46] These nanoparticles can deliver antiproliferative small interfering RNA (siRNA) against ribonucleotide reductase subunit 2 (RRM2) and achieve intracellular accumulation in tumors where RRM2 is then reduced,^[47] thereby leading to slowed tumor growth in mice.^[48]

The cell-surface prostate-specific membrane antigen (PSMA) is a receptor overexpressed in prostate cancer which has also been targeted for drug delivery.^[49] Docetaxel-loaded micelles of poly(lactic acid) (PLA) and PEG functionalized with a small-molecule substrate analogue of PSMA achieve greater tumor regression in mice with PSMA-expressing tumors compared to nontargeted nanoparticle controls.^[50] PSMA has also been targeted with aptamer-functionalized self-assembled poly(lactic-co-glycolic acid)-PEG ((PLGA)-PEG) nanoparticles, where increasing the PSMA-targeting aptamer density on the PLGA-PEG nanoparticles increases accumulation in tumors of human prostate cancer xenografts in mice.^[51] However, increasing the aptamer density also increases accumulation in the liver, thus demonstrating the trade-off that often occurs with active targeting, where functionalization of drug carriers can expedite their clearance.

Active targeting can also be used to direct drug delivery to cancer stem cells. These cells, which represent a subpopulation of the tumor, are believed to be responsible for tumor initiation and act as mediators of drug resistance, metastasis, and cancer recurrence.^[52] CD133, a transmembrane glycoprotein, is overexpressed on cancer stem cells from several tumor types.^[53] Targeting these cancer stem cells by functionalization of paclitaxel-loaded PLGA nanoparticles with anti-CD133 antibody can inhibit tumor-initiating cells in vitro and suppress the recurrence of breast cancer tumors in mice better than nontargeted nanoparticles.^[54]

Finally, active targeting of tumor lymphatics can be used to deliver drug to sites of metastasis in lymph nodes. PEGylated liposomes functionalized with LyP-1, a cyclic peptide that binds to tumor lymphatics as well as tumor cells in some cancers,^[55] increase accumulation in tumor lymph nodes when administered subcutaneously. When loaded with doxorubicin, these functionalized liposomes inhibit the growth of lymphatic metastases of lung adenocarcinoma in mice.^[56]

Other unique features of the extracellular tumor environment, such as overexpression of extracellular enzymes or the mildly acidic extracellular pH value of tumors, have also been

exploited for active tumor targeting. These intrinsic features of the tumor tissue are often used to actuate stimulus-responsive drug carriers to localize drug delivery specifically in the tumor. In an example of this approach, cationic cell-penetrating peptides (CPPs), which exhibit nonspecific, receptor-independent cell uptake, are coupled with anionic inhibitors through a matrix metalloproteinase (MMP) cleavable linker that prevents cellular uptake when the linker remains intact. Cleavage of the linker only in tumors that overexpress MMP frees the CPP and leads to enhanced accumulation in the tumor compared with an uncleavable control.^[57] This mechanism has been explored extensively for tumor imaging^[58] as well as for drug delivery.^[59] Alternatively, the depressed pH value of tumors (pH $\approx$ 6.8) can also serve as an intrinsic trigger for the targeted activation of CPP-functionalized drug carriers.^[60]

These approaches to active targeting, however, are limited by the heterogeneity of the intrinsic tumor targets, whose expression varies between cancer types, individual patients, and within single tumors.^[61] To circumvent these limitations of intrinsic targets, active targeting can alternatively exploit extrinsic triggers (namely, applied externally to the body) to control the accumulation of drug in the tumor. Mild hyperthermia, the heating of tumors to 40–42°C,^[62] has a long history of clinical use, whereby localized heating is achieved by focused ultrasound, microwaves, or radiowaves.^[63] Mild hyperthermia can be used to influence accumulation in tumors by controlling the in situ behavior of temperature-responsive drug carriers as they circulate in the tumor vasculature. The lower critical solution temperature (LCST) phase-transition behavior of elastin-like polypeptides (ELPs), within a precisely tuned thermal window of 39–42°C, can be exploited in vivo, as local heating triggers the transition of ELPs from a soluble state into insoluble micrometer-scale aggregates within the tumor vasculature. When the thermal stimulus is removed, the resolubilization of the aggregates creates a large vascular–extravascular ELP concentration gradient, which drives the soluble ELP into the tumor tissue by diffusion through the leaky tumor vasculature.^[64] Thermally cycling the tumor between physiological temperature and 42°C leads to repeated cycles of ELP aggregation and dissolution which increase accumulation in the tumor compared to unheated tumors or temperature-insensitive controls.^[64,65]

Despite the passive and active targeting of drug carriers, only a small fraction of drug is likely to accumulate in the tumor. A median of less than 1% of the administered dose has been reported to typically reach the tumor by either passive or active targeting, while 99% of the dose accumulates in off-target organs or is cleared by the liver, kidney, and spleen.^[27] Thus, while passive and active targeting of drug carriers can improve the efficacy and safety of their drug cargo, it is important to recognize that off-target organs are exposed to the majority of the drug dose.

2.2.3. Penetration—Once a drug extravasates into the tumor tissue, it faces an environment rich in cancer cells, stromal cells, immune cells, and extracellular matrix (ECM),^[66] which together prohibit the movement of drug throughout the tumor. Dense cell packing, of which tightly packed epithelioid-derived cancer cells are an example, decrease the penetration of small-molecule chemotherapeutics compared to loosely packed cell variants.^[67] This decreased drug transport can lead to pathological symptoms such as drug

resistance, as demonstrated by the decreased cell death of densely packed cells in multilayered culture treated by chemotherapy compared to loosely packed cells.^[67] The organization of cells within the tumor thus presents the first extravascular barrier in the local transport of anticancer drugs.

The ECM surrounding cancer cells presents the second transport barrier within the tumor tissue and is often heavily remodeled in tumors, as cancer cells modify their local environment.^[68] Collagen is a component of normal ECM, whose high concentration in some cancers may magnify the barrier to drug transport, as increased collagen content is correlated with decreased macromolecular mobility.^[69] Ultimately, the decreased drug mobility in tumors with excessive ECM may limit drug accumulation, as collagen content is negatively correlated with accumulation of systemically administered 40 nm diameter nanoparticles in tumors.^[34]

These barriers often result in limited drug penetration throughout the tumor. Intravenously injected doxorubicin has a penetration length of 40–50 μm (defined as the distance at which the fluorescence intensity of doxorubicin decreases to half its perivascular value) and is dependent on the tumor type.^[70] As the average distance from the tumor vasculature to hypoxic regions is approximately 90–140 μm in these tumors, it is clear that a significant volume of the tumor is not exposed to the drug. This challenge of limited penetration extends to drug carriers, where 90 nm diameter liposomes, for example, extravasate and accumulate primarily in the perivascular space of tumors and largely remain within 30 μm of the tumor vessel wall.^[71]

The size of the drug carrier is a critical factor that influences penetration throughout the tumor tissue. A decrease in the molecular weight of dextran correlates with enhanced penetration throughout the tumor. Large dextrans (2 MDa) cannot extravasate significantly from the vasculature, while 70 kDa dextran remains localized within 15 mm of the blood vessel wall, and 3.3 kDa dextran is observed homogeneously in the extravascular space of the tumor within 30 min of systemic administration.^[72] Similar trends have been observed for nanoparticle drug carriers, where polymeric micelles with diameters of 60 nm remain proximal to the tumor vasculature, while micelles with a diameter of 25 nm achieve more homogeneous distribution throughout the tumor by penetrating further from the tumor vessels.^[73]

To enhance tumor penetration and distribution, stimulus-responsive drug carriers have been designed to disassemble and thereby reduce their dimensions upon reaching the tumor. These stimulus-responsive drug carrier systems can thus reap the benefits of larger carriers, including long circulation and accumulation by the EPR effect, but then reduce their size by programmable disassembly to enable penetration of the drug throughout the tumor (Figure 5).

Intrinsic tumor-specific cues can be exploited to trigger changes in a drug carrier to enhance local tissue penetration. The extracellular enzyme MMP-2 that is overexpressed in tumors has been exploited to trigger a change in the size of gelatin nanoparticles, as gelatin is a natural substrate for MMP-2.^[74] The degradation of 100 nm gelatin nanoparticles in the

tumor can release 10 nm quantum dots that serve as model nanoparticles and can in principle be replaced by drug vehicles of similar dimensions. After intratumoral injection, the MMP-2-cleavable quantum dot gelatin nanoparticles exhibit penetration of up to 300 μm from the injection site, whereas a nondegradable control exhibits little distribution away from the injection site.

Alternatively, the depressed pH value of tumors provides an intrinsic cue that can trigger changes in the size of the drug carrier by dismantling self-assembled nanoparticles in the acidic tumor. Micelles composed of a diblock that includes histidine residues in the hydrophobic domain have demonstrated pH-triggered disassembly. At pH 7.4 the neutral histidine residues pack into the stabilized micelle core, but at pH 6.4, below the $\text{p}K_{\text{a}}$ value of histidine, protonation of the histidine residues leads to disassembly of the micelles by increasing the hydrophilicity of the core component.^[75] This stimulus response allows a transition from a micelle of 70 nm in diameter at a physiologic pH value to 14 nm unimers in the acidic conditions of the tumor. After systemic injection, these pH-responsive micelles accumulate in the tumor at levels equivalent to nonresponsive controls without histidine residues. However, the pH-responsive nanoparticles exhibit a more homogeneous distribution throughout the tumor tissue, whereas nonresponsive controls exhibit localized accumulation primarily at the tumor periphery.

Tumor penetration may become increasingly difficult when the drug is actively targeted to the tumor. Functionalization of the drug or drug carrier with targeting moieties can increase the interaction with the tumor tissue, but at the cost of compromising penetration throughout the tumor. A classic example of the effects of targeting on tumor penetration is the binding-site barrier originally described for antibody-targeted delivery.^[76] Once the antibody extravasates and interacts with its tumor target, it is immobilized and prevented from moving deeper into the tumor tissue, thereby leading to limited penetration away from the vasculature. This phenomenon has also been observed with actively targeted polymeric micelles, where micelles targeting the epidermal growth factor receptor have decreased penetration distance from tumor blood vessels compared to nontargeted micelles.^[73] The penetration of actively targeted drug is, thus, dependent on both diffusion and affinity for the tumor target.

Analogous to the challenge posed by interstitial fluid pressure, the challenge posed by the interstitial ECM can be overcome by modulating the tumor environment. Controlled degradation of the tumor ECM is one approach that could improve drug penetration by breaking down physical barriers within the tumor. Recombinant relaxin, a peptide hormone responsible for upregulating ECM-degrading MMPs, increases the diffusion of antibodies and dextran within dorsal fold sarcomas in mice.^[77] Furthermore, in situ expression of relaxin from virally transduced hematopoietic stem cells successfully reduces the collagen content of tumors and improves breast tumor regression with anti-HER-2 antibody therapy.^[78]

2.3. Cellular Uptake

After a drug has successfully reached the tumor, it can safely exert its cytotoxic effects on the cancer cells. For most therapeutics, this requires cellular uptake of the drug such that it

can reach an intracellular therapeutic target. Many drugs, therefore, must face the challenge of traversing the cell membrane. The cell membrane is readily permeable to small hydrophobic drugs, thereby allowing these drugs to easily move from the extracellular to the intracellular space. For other drugs, the cell membrane is an impermeable barrier because of their size, charge, or hydrophilicity.

Drug carriers act as a double-edged sword with respect to intracellular drug delivery, in that they can either inhibit or promote cellular uptake of a drug depending on the physicochemical properties of the drug cargo. Small hydrophobic drugs must often be delivered by hydrophilic drug carriers to achieve sufficient solubility for systemic administration, and although these small hydrophobic drugs can easily cross the cell membrane, this sequestration in hydrophilic drug carriers prevents the easy penetration of hydrophobic drugs across the cell membrane. For this class of drug, a carrier can enable intracellular drug delivery by releasing its drug cargo into the extracellular space of the tumor to allow its diffusion across the cell membrane. Alternatively, drug carriers can significantly increase the intracellular delivery of drugs that do not easily penetrate the cell membrane if they are functionalized with receptor ligands or cell-penetrating peptides that promote cellular uptake by a variety of endocytic pathways.

2.3.1. Extracellular Drug Release—For drugs that can easily cross the cell membrane to reach their intracellular therapeutic target, all that is needed is for the drug carrier to release the drug in the tumor tissue by passive or active mechanisms. If the drug is physically sequestered by a carrier, the drug can be passively released from the carrier by diffusion, which can be further facilitated by the inherent degradation of the carrier through processes such as hydrolysis. Alternatively, if the drug is loaded into a stimulus-responsive drug carrier, it can be actively released as the carrier responds to the local tumor environment (Figure 6a–c).

Drug release from stimulus-responsive carriers can occur in response to intrinsic properties of the tumor tissue, such as tissue acidity and extracellular enzyme activity. Self-assembled micelles composed of PEG–poly(β -amino ester) diblocks have been designed to release their drug cargo in acidic tumors. Ionization of the amine groups in the core-forming poly(β -amino ester) block of the micelle under mildly acidic conditions causes disassembly of the micelle, which in turn releases the encapsulated drug.^[79] The release of doxorubicin in the acidic tumor environment by this mechanism improves regression of subcutaneous tumors in mice compared to free drug, although comparison with pH-insensitive micelles is not reported.

Tumor acidity can alternatively be exploited to change the morphology of self-assembled carriers to trigger local drug release within a tumor. This mechanism of extracellular drug release has been achieved with micelles containing a thermally responsive polymer segment in the corona that is functionalized with 6-aminocaproic acid.^[80] Protonation of 6-aminocaproic acid in an acidic environment increases the hydrophobicity of the thermally responsive polymer and induces a change in the conformation of the micelle, presumably because of the collapse of the micelle corona. This pH-induced effect decreases the size of the carrier and expels drug cargo that is encapsulated in the core of the micelle. The delivery

of doxorubicin by this pH-sensitive micelle enhances regression of subcutaneous melanoma tumors, compared to free doxorubicin or pH-insensitive doxorubicin-loaded micelle controls, when administered intravenously in mice.^[80]

Overexpressed tumor enzymes provide an alternative intrinsic trigger for drug release. For example, liposomes have been fabricated with a lipopeptide component wherein the peptide is a substrate for MMP-9, which is overexpressed in many tumors.^[81] Cleavage of this peptide induces defects in the lipid bilayer that then permit release of encapsulated drug. Incubation of these liposomes in conditioned media from MMP-9-overexpressing cancer cells enhances the release of the model cargo carboxyfluorescein compared to liposomes incubated with conditioned media from cells with a low level of MMP-9 production.^[81]

Extrinsic triggers have also been exploited to enable drug release from carriers within tumors and are an attractive alternative to intrinsic triggers because they provide spatial as well as temporal control of drug release. Focused mild hyperthermia of tumors has been extensively investigated to release drugs from temperature-sensitive liposomes. Temperature-sensitive doxorubicin-loaded liposomes (ThermoDox) release encapsulated doxorubicin in tumors heated to 42°C, a temperature that is greater than the melting temperature of grain boundaries in the gel phase of the liposome.^[82] This temperature-triggered drug release greatly enhances the extracellular accumulation of drug compared to liposome delivery without tumor hyperthermia.^[83] Optimal drug accumulation by this approach is achieved when the tumor is preheated prior to liposome delivery, such that drug release in the tumor vasculature creates a concentration gradient that drives the drug into the extravascular space.^[84]

2.3.2. Internalization—For drugs that cannot penetrate the cell membrane sufficiently on their own, a drug carrier can provide a mechanism by which the drug can be internalized into the cancer cell. Drug carriers facilitate internalization by various endocytic pathways, which often depend on the functionalization of the drug carrier. For example, functionalization of drug carriers with transferrin, folate, or CPPs has been shown to induce uptake by clathrin-mediated endocytosis, caveolae-mediated endocytosis,^[85] and macropinocytosis,^[86] respectively (Figure 6d). Cellular uptake by endocytic mechanisms can also serve to evade mechanisms of drug resistance, whereby drug that accumulates in endosomes and lysosomes is retained in the cell rather than being expelled from the cell by efflux transporters, such as P-glycoprotein (Pgp).^[87] Furthermore, a nanoparticle drug carrier that co-delivers a chemotherapeutic and siRNA-targeting Pgp can overcome multidrug resistance of breast cancer tumors in mice.^[88]

Drug carriers can, hence, increase cellular uptake of their drug cargo when functionalized with moieties that facilitate their endocytosis, but exposure of these ligands in systemic circulation may cause toxicity if they induce cellular uptake in off-target tissues. It is thus desirable to mask these ligands for cellular uptake until the carrier has reached the tumor. This has been accomplished with stimulus-responsive drug carriers decorated with “stealth” polymers such as PEG, which effectively prevent premature cellular uptake, but are selectively removed in the tumor to expose the underlying functionality that induces cellular uptake (Figure 6e,f).

To achieve selective cellular uptake in the tumor, functionalized drug carriers can be masked by “stealth” polymers attached to the carrier through a cysteine-cleavable linker. Folate-functionalized liposomes have been modified with PEG through a disulfide bond that is cleaved by exogenous administration of cysteine.^[89] Cysteine-cleaved unmasked-doxorubicin-loaded folate liposomes induce significant cell death, while cells exposed to masked doxorubicin-loaded folate liposomes are spared. Furthermore, masking of the folate ligand leads to longer circulation in vivo compared to folate-functionalized liposomes without PEG, which allows the PEGylated folate-functionalized liposomes to passively accumulate in the tumor prior to the administration of cysteine, thereby leading to significant specific internalization in cancer cells.

A similar approach has been employed to take advantage of CPPs as a means to induce cellular uptake by receptor-independent mechanisms. The unmasking of CPPs has been achieved with “stealth” polymers that are released by linkers that are cleaved in response to tumor acidity^[90] or upregulated tumor enzymes.^[91] In a complementary approach, “stealth” polymers can mask cationic CPPs when they are attached to anionic inhibitors that electrostatically bind to CPPs. The CPP is unmasked in the acidic environment in the tumor, where the depressed pH value can protonate the anionic inhibitor and disrupt its binding to the CPP.^[60a] Furthermore, mechanisms of extrinsic activation have also been developed for the controlled cellular uptake of CPPs, such as the modulation of CPP density by thermally triggered nanoparticle self-assembly.^[92] In this approach, CPP-functionalized stimulus-responsive diblock copolymers achieve enhanced cellular uptake only when a high density of CPPs is presented on the micelle corona after self-assembly is triggered by mild hyperthermia.

Some carriers exhibit efficient cellular uptake as a result of their inherent material properties, but must be functionalized with “stealth” polymers to ensure that uptake does not occur at off-target locations prior to reaching the tumor. Cationic drug carriers, for example, typically demonstrate excellent cellular uptake as a consequence of their strong interaction with negatively charged cell surfaces. PEGylation of cationic carriers greatly increases their stability and circulation time, but often at the cost of eliminating their cellular uptake at the tumor site. PEGylation through stimulus-responsive functionalization provides a means to selectively remove the PEG shield in the tumor. This has been demonstrated by the pH-responsive removal of PEG from cationic carriers such as siRNA polyplexes that are electrostatically functionalized with diblocks of PEG and anionic polymers whose pendant groups are negatively charged. Amide bonds within these pendant groups are degraded at depressed pH values, thus conferring a positive charge that causes removal of the PEG from the polyplex surface as a result of electrostatic repulsion.^[93] Such an approach provides pH-triggered PEG removal, which increases uptake in the acidic conditions of the tumor compared to normal tissues at a physiologic pH value.

2.4. Subcellular Targeting

Once internalized into the cell, there are additional barriers that separate the drug cargo from its therapeutic target. Directing a drug to its site of molecular action at the subcellular level is the final physiological challenge in anticancer drug delivery. Drug carriers can help direct

the drug in the intracellular space in several ways, such as releasing drug cargo within the cell, promoting escape from endolysosomal compartments, and promoting the interaction of the drug with its intracellular therapeutic target.

2.4.1. Intracellular Drug Release—For those drugs that enter the cell still associated with their drug carrier, the release of the drug must occur in the intracellular space. The intrinsic triggers for intracellular drug release include the acidic and enzyme-rich environment of the endosome and lysosome as well as the reducing environment of the cytosol. In the simplest approach, drug release can be triggered by the selective cleavage of a drug linker inside the cell (Figure 7d). For example, short (3 or 4 amino acid) peptide linkers have been investigated for release of doxorubicin from PEG conjugates in response to concentrated proteases in the endosome and lysosome.^[94] The antitumor activity of cleavable carriers in subcutaneous melanoma tumors in mice appears to correlate with the rate of doxorubicin release measured in vitro, thus suggesting that local release of drug in the tumor cell can improve therapeutic outcomes.

The low pH value within endosomes and lysosomes can also be exploited for intracellular drug release. Acid-labile linkers, such as hydrazone bonds, can be used to selectively release drug from a carrier in these acidic intracellular compartments.^[95] Alternatively, pH-sensitivity can be imparted to the drug carrier itself, such that the local acidic environment triggers drug release by means of a material response. “Expansile” nanoparticles, for example, exploit a pH-dependent nanoscale modulation in the volume of the drug carrier to selectively release drug in the lysosome. In one example of “expansile” nanoparticles, cross-linked nanoparticles formulated with 2,4,6-trimethoxybenzaldehyde groups undergo hydrolysis at a low pH value, which triggers a hydrophobic-to-hydrophilic transition that causes the nanoparticles to swell and thereby release over 80% of their physically loaded paclitaxel cargo over 24 h.^[96]

If the drug carrier can bypass or escape endosomes, then drug release can also be triggered by the reducing environment of the cytosol, because of its high glutathione levels, by using reducible linkers such as disulfide bonds.^[97] Alternatively, the reducing environment of the cytosol can also trigger drug release by disassembly of the drug carrier. This has been demonstrated with self-assembling diblock copolymers whose hydrophilic and hydrophobic segments are connected through a reducible disulfide linker.^[98] Although these cleavable linkers provide a valuable means to release free drug by disassembly of the carrier, they can also decrease the stability of the drug carrier as it travels in systemic circulation because of premature breakdown of the carrier.^[99] The choice of linker can thus have an impact on the pharmacokinetics and biodistribution of drug cargo.

2.4.2. Endosomal Escape—Most drug carriers are internalized into cells by endocytosis, which sequesters the carrier and its drug cargo in endosomes and lysosomes. Entrapment in these intracellular compartments physically separates the drug carrier from its therapeutic target in a degradative environment that is rich in enzymes and has a low pH value. Drug carriers have been designed to achieve endosomal escape to prevent degradation of drug cargo and promote drug access to its intracellular therapeutic target (Figure 7a–c). Endosomal escape is of particular importance in anticancer gene delivery, as it serves to

protect RNA and DNA drug cargo from degradation and promotes access to RNA and DNA targets in the cytosol and nucleus.

Endosomal escape can be achieved by the proton sponge effect, which is the most common mechanism exploited for the release of drug and gene cargo from endolysosomes. Materials with a high buffering capacity at low endosomal pH values can increase the osmotic pressure within endosomes, which leads to their rupture and the escape of cargo into the cytosol. Materials that are weak bases and susceptible to protonation can escape the endosome by this mechanism, including polyethyleneimine (PEI), histidine-functionalized polymers, and imidazole-conjugated cyclodextrins.^[100] Cyclodextrins formulated for gene delivery have demonstrated enhanced transfection efficiency after the incorporation of imidazole groups, in part as a result of their endosomolytic nature.^[100b]

Endosomolytic peptides have also been incorporated into drug carriers to induce lysis of the endosome membrane through the formation of pores. This approach has been demonstrated with pore-forming peptides such as melittin. Covalent modification of a gene delivery vehicle based on *N*-(2-hydroxypropyl)methacrylamide (HPMA) and poly(lysine) with melittin achieves a 35-fold increase in transgene luciferase expression compared to the gene carrier without melittin.^[101]

Controlled endosomal escape can alternatively be achieved with peptides that induce membrane fusion. For example, the GALA peptide (WEAALAEALAEALAEHLAEALAEALAA) induces pH-dependent membrane fusion under acidic conditions. GALA-functionalization of a gene carrier composed of a cationic core enveloped in a lipid coating induces pH-triggered fusion with the endosomal membrane, thereby releasing gene cargo into the cytosol and enhancing the transfection of a model luciferase gene.^[102] Furthermore, GALA-mediated endosomal release of siRNA that targets the model luciferase gene improves gene silencing in vivo after intratumoral administration compared to a carrier without GALA functionalization.^[103]

2.4.3. Intracellular Targeting—If the drug exerts its therapeutic action intracellularly, its final challenge is to localize at its therapeutic target within the cell. Drug carriers have been developed to direct drug cargo intracellularly, with a focus on two important organelles: the nucleus and the mitochondria (Figure 7e). The nucleus is an important target for many anticancer therapies due to its role in gene regulation and proliferation, while mitochondria are an important target due to their role in metabolism and cell survival.

Targeting the nucleus has been achieved with drug carriers functionalized with ligands that target nuclear components. Silica nanoparticles, for example, have been targeted to the nucleus of cancer cells by functionalization with dexamethasone, a ligand for the nuclear glucocorticoid receptor.^[104] Alternatively, gold nanostars have been trafficked to the nucleus in cancer cells by functionalization with an aptamer that binds with high affinity to nucleolin, a nuclear protein that in cancer cells is proposed to recycle from the cell surface to the nucleus.^[105]

The nucleus can alternatively be targeted by electrostatic interactions by using a positively charged drug carrier. Charge-reversal materials have been formulated from poly-(lysine) and polyamidoamine (PAMAM) dendrimers whose positively charged primary amine groups are modified to pH-sensitive amides. This material is negatively charged at a physiological pH value, but reverts to its positively charged state when its amide groups are hydrolyzed into positively charged amines in the acidic conditions of the lysosome.^[106] The negatively charged amides exhibit minimal nonspecific interaction outside the cell, while conversion into positively charged amines within the acidic environment of lysosomes provides electrostatic targeting to the negatively charged nucleus after escape into the cytosol. The delivery of camptothecin conjugated to a charge-reversal polymer increases cytotoxicity in cancer cells in vitro compared to free drug.^[106]

Mitochondrial targeting has been achieved with peptides that preferentially accumulate intracellularly at this target. The tumor-homing peptide CGKRRK, for example, is internalized in cancer cells and localizes at the mitochondria.^[107] CGKRRK-functionalized iron oxide nanoparticles localize at the mitochondria in vitro and, compared to nontargeted drug, drastically enhance the cytotoxicity of a conjugated proapoptotic peptide drug that acts on the mitochondria. This mitochondrial targeting furthermore improves regression of orthotopic glioblastoma in mice after intravenous injection compared to nontargeted carriers,^[107] thus demonstrating that intracellular targeting may have important implications for therapeutic outcomes in vivo.

Materials that are both cationic and lipophilic, such as those functionalized with triphenylphosphonium (TPP) cations, also localize at the mitochondria and can cross the mitochondrial membrane to reach the negatively charged mitochondrial matrix.^[108] The incorporation of TPP into liposomal formulations leads to the accumulation of drug at the mitochondria and increases the toxicity of ceramide drug cargo compared to delivery with nontargeted liposomes.^[109] Similarly, TPP-modified polymer nanoparticles increase the in vitro cytotoxicity of lonidamine and α -tocopheryl succinate drug cargo compared to nontargeted controls.^[110]

3. Translational Challenges

Pathophysiological challenges present significant barriers to selectively deliver therapeutics to the tumor and minimize off-target side effects. Drug carriers provide significant improvements to the pharmacokinetics and biodistribution of their drug cargo, which often leads to improved therapeutic performance and safety compared to the free drug. Perusing the scientific literature, one could easily conclude that drug carriers have overcome the physiological challenges of anticancer drug delivery, with a plethora of reports of tumors in mice being cured with merely a single dose of drug. However, drug carriers that have long been expected to revolutionize cancer therapy have yet to significantly improve clinical efficacy. A large part of this gap between preclinical success and lack of clinical adoption are the often insurmountable challenges that face anticancer drug carriers in their translation from the laboratory to the clinic. These challenges are discussed in the following sections.

3.1. Formulation of the Drug Carrier

The physicochemical properties of free drugs, such as their size and solubility, have clearly been identified as barriers in the successful administration of anticancer therapeutics. Drug carriers provide means to address these challenges. The formulation of drug-loaded carriers is, however, not without its own challenges to incorporate the therapeutic cargo in a way that provides stability as well as spatiotemporal release of the functional drug. Both physical and covalent mechanisms have been used to address these challenges in loading drugs into carriers (Figure 8).

3.1.1. Physical Entrapment—The simplest method to load a drug into a carrier is by physical entrapment, whereby the intrinsic physicochemical properties of the drug cause it to inherently sequester within the structure of the drug carrier. This approach is most commonly used with drug carriers that are self-assembled from amphiphilic components, such as micelles and bilayer vesicles. Micelles can entrap drugs in their hydrophobic core, whereas vesicles can entrap drug in either their aqueous core or hydrophobic bilayer.

Despite the simplicity of physical entrapment, it has its limitations. First, it is dependent on the physicochemical properties of the drug, so that not all drugs are amenable to physical encapsulation. Second, it requires that the physicochemical properties of the drug are suitably matched to properties of the carrier to provide a driving force for physical loading. Third, the driving forces that induce drug loading by physical entrapment may impede the release of drug from the carrier, such that carriers that readily load their drug cargo may be reluctant to eventually release it.

Self-assembled micelles with sufficiently hydrophobic cores can physically entrap hydrophobic drugs. For example, the hydrophobic chemotherapeutic paclitaxel can be encapsulated into the core of polymeric micelles that are self-assembled from amphiphilic PEG–polyaspartate diblock copolymers. To make the core hydrophobic enough to permit drug encapsulation, a fraction of the carboxy groups in the polyaspartate block are modified with 4-phenyl-1-butanol.^[11b] This micellar formulation of paclitaxel (NK105) achieves 23 wt% drug loading in the core of micelles with a diameter of approximately 85 nm.

When the micelle core does not impart sufficient hydrophobicity, the covalent attachment of a few copies of hydrophobic drug to the hydrophobic block of a micelle-forming amphiphile can impart the necessary driving force for physical encapsulation of the drug. Micelles composed of PEG–polyaspartic acid diblock copolymers can encapsulate doxorubicin by this approach.^[111] The covalently conjugated doxorubicin stabilizes these 40 nm diameter micelles, but does not contribute to the therapeutic effect, as only the physically encapsulated doxorubicin is passively released from the micelle.

Liposomes are another nanoscale delivery system that enables physical encapsulation in their aqueous core or hydrophobic lipid bilayer. The large aqueous core is predominantly used for drug loading due to its much greater volume fraction of the liposome compared to the interior of the bilayer. Encapsulating a drug in the aqueous liposome core can be achieved by methods such as reverse-phase evaporation, in which the drug is dissolved in an aqueous buffer and then mixed with the lipid solubilized in an organic solvent. Evaporation

of the organic solvent drives the self-assembly of liposomes, which retain a portion of the aqueous phase in their core. This technique has been used to achieve a loading efficiency of up to 45% of [^3H]cytosine arabinoside in liposomes with diameters of 200–500 nm.^[112] Loading by solvent evaporation, however, can result in unstable liposomes that exhibit premature release of the loaded cargo, especially in the presence of serum.^[113]

An improved stability of liposome-encapsulated drug has been realized by drug loading that exploits a gradient between the external and internal environment of the liposome to drive accumulation of the drug in the aqueous core. A pH gradient can drive the encapsulation of lipophilic drugs into the liposome when the drug is uncharged and capable of traversing the lipid bilayer, but becomes trapped in the core where the altered pH value induces protonation of the drug, which then prevents its movement across the lipid bilayer. This approach is suitable for drugs whose lipophilicity can be controlled by the pH value. Doxorubicin, for example, can be loaded with high efficiency by pH gradients created by citrate^[114] or ammonium sulfate.^[115] Clinically approved Doxil, Myocet, and DaunoXome liposomes all encapsulate drug by this approach.^[116]

Polymersomes offer an alternative to liposomes, whereby polymer amphiphiles (e.g. PEG–PLA, PEG–PCL, or PEG–PBD) self-assemble into bilayer vesicles. The bilayer thickness of polymersomes is greater than that achieved with lipid-based vesicles,^[117] so that this hydrophobic environment of the bilayer can be used to sequester drugs with low aqueous solubility. This feature of polymersomes has been exploited to create dual-loaded polymersomes, wherein the hydrophobic drug paclitaxel is incorporated in the bilayer and the more hydrophilic drug doxorubicin is encapsulated in the aqueous core.^[117,118] The paclitaxel is loaded simply by partitioning the drug from solution into the bilayer of self-assembled polymersomes, whereas doxorubicin is loaded by the pH-gradient-driven method described for liposomes.

Dendrimers, although not self-assembled structures, also enable drug encapsulation because of their unique structure. The highly branched architecture of poly(glycerol-succinic acid) dendrimers creates hydrophobic pockets into which drugs with poor water solubility can be sequestered.^[119] The encapsulation of camptothecin in these dendrimers is achieved by solvent evaporation. In this case, the drug carrier and drug are solubilized in methanol, water is added, and the methanol is evaporated to drive association of the drug and dendrimer.

Alternative approaches also exist for the physical loading of drugs into carriers that are fabricated by top-down methods, whereby the drug is encapsulated by simple mixing with the carrier matrix. For example, PLGA nanoparticles can encapsulate high levels of anticancer drugs when synthesized by methods such as particle replication in non-wetting templates (PRINT). This technique exploits soft lithography to create nanoparticles by filling a mold of controlled shape and size with heated PLGA, which solidifies as it cools. There is no aqueous phase associated with the loading of PRINT nanoparticles, which leads to a maximum encapsulation efficiency of over 90%. Docetaxel can be encapsulated in 200 nm cylindrical PLGA particles with this approach, achieving 40 wt% drug loading.^[120] The PRINT process has also been optimized to physically encapsulate doxorubicin,^[121] as well as therapeutic proteins and oligonucleotides.^[97b,122]

3.1.2. Electrostatic Complexation—Electrostatic complexation is a variant of physical loading in which the ionic character of the drug and carrier are exploited for encapsulation in the delivery vehicle. This approach is particularly useful for loading negatively charged therapeutic DNA and RNA into carriers, as their negative charge allows them to be easily condensed with positively charged materials. Drug loading by electrostatic complexation is achieved in its simplest means by mixing negatively charged therapeutics with positively charged homopolymers. Poly(lysine) and PEI are classic examples of cationic polymers that can condense with negatively charged DNA through electrostatic interaction of their positively charged amine groups along the polymer backbone.^[123] This approach, however, leads to drug carriers whose size and surface properties can only be poorly controlled. Polyplexes condensed with excess PEI typically exhibit positive surface charge that can contribute to aggregation in blood, adsorption of plasma proteins, and nonspecific cellular interactions.^[124] To solve this problem, these electrostatically loaded assemblies are often sheathed with a protective “stealth” coating of PEG.^[125]

A better control of electrostatic drug loading can be achieved by using well-defined multifunctional polymer constructs. For example, stable DNA-loaded nanoparticles are assembled from a triblock copolymer composed of PEG, poly(aspartamide), and poly(lysine).^[126] The positively charged poly(lysine) terminus of the triblock copolymer is responsible for condensation of negatively charged plasmid DNA, while the PEG confers a long plasma circulation time upon systemic administration. Physical mixing of the triblock copolymer with DNA induces self-assembly of nanoparticles with a diameter of 80 nm. The middle poly(aspartamide) block is functionalized with 1,2-diaminoethane to provide pH-dependent protonation, which confers endosomal escape properties.

siRNA has also been loaded into drug carriers through electrostatic interactions. A mixture of anionic siRNA with cationic cyclodextrin polymers and adamantine-PEG results in spontaneous assembly of nanoparticles that are approximately 75 nm in diameter.^[127] Alternatively, siRNA can be loaded into liposomes through electrostatic interactions with ionizable lipids.^[128] Cationic lipids, below their pK_a values, can interact with siRNA during vesicle formation. Raising the pH value above the pK_a value of the cationic lipids results in neutralization of the lipid, thereby releasing siRNA from the liposome surface while siRNA remains trapped in the aqueous liposome core.

3.1.3. Covalent Conjugation—An orthogonal approach to physical drug loading is to covalently attach the drug to the carrier. This approach has the advantage of not being limited by the physicochemical properties of the drug or carrier. Covalent conjugation is, however, limited in several ways by the nature and number of conjugation sites on the drug and drug carrier. First, a reactive functional group on the drug molecule that is compatible with a corresponding reactive group on the drug carrier is required for covalent conjugation. Second, the conjugation site on the drug should not interfere with its therapeutic function, thus site-specific attachment of the drug to the carrier is desirable. Third, drug loading by covalent conjugation is limited in that the number of conjugated drug molecules cannot exceed the number of accessible reactive sites on the drug carrier. Finally, covalent conjugation can greatly diminish the therapeutic efficacy of the attached drug; therefore,

drug-release mechanisms must often be incorporated into the carrier design when exploiting this approach to drug loading.

Drug molecules do not always contain the suitable functionality to react with a drug carrier, but in some cases an appropriate reactive group can be incorporated by a derivatization reaction. Camptothecin, for example, can be appended with a glycine at a hydroxy group to enable an ester linkage to cyclodextrin-PEG copolymers.^[129] In most cases, however, the drug must be cleaved from the carrier within the tumor environment to ensure the efficacy of the drug. Cleavable linkers are very useful in this regard, as they simultaneously provide a means to covalently attach the drug and incorporate a mechanism for drug release. Paclitaxel, for example, has been covalently loaded on mesoporous silica nanoparticles by functionalizing the drug at its 2'-hydroxy group with a reducible disulfide linker whose carboxy component reacts with the amine-functionalized surface of the nanoparticle.^[130] Covalent attachment both on the surface and within the pores of the nanoparticle can result in paclitaxel loading of up to 13 wt% with this approach.

Cleavable linkers have also been used for covalent conjugation to dendrimeric drug carriers. Bow-tie dendrimers, with one half of their branches terminating in a reactive hydroxy group, have been conjugated to doxorubicin through an acid-cleavable hydrazone linker.^[131] The degree of drug loading can be controlled by the number of reactive sites on the dendrimer, which is dictated by the number of generations in its branched structure. Fourth generation (G4) hydroxy-terminated branches on these bow-tie dendrimers can achieve a drug loading of 8–10 wt%.

For biologic therapeutics, such as proteins, reactive lysine residues are most commonly used for covalent conjugation. These residues, however, are indiscriminately distributed on the surface of proteins, which leads to heterogeneous conjugation with drug carriers, such as PEG. PEGylation of the experimental therapeutic protein TNF- α , for example, has been achieved with the reactive amine groups present on 18 lysine residues distributed on its surface, thereby resulting in a heterogeneous product with varying degrees of polymer functionalization.^[16] An optimal degree of PEG functionalization prolongs the circulation time and decreases the enzymatic degradation of TNF- α . However, PEG functionalization in excess of this optimal degree of conjugation abrogates TNF- α activity. As this example demonstrates, one challenge of conjugation is to control the stoichiometry of the functionalization.

Site-specific covalent conjugation is also needed for precise placement of drug attachment to sites that are known to not interfere with the function of the therapeutic cargo. Recent advances in site-specific functionalization have been made, particularly in the synthesis of protein conjugates to polymer carriers. Proteins can be functionalized solely at their N-terminus by means of the N-terminal amine, whose specific pK_a value differentiates it from the amine group of the lysine side chain.^[132] The specific reactivity of the N-terminus can be used to conjugate PEG to this terminus^[133] or to install a polymerization initiator, from which poly-(oligo(ethylene glycol) methyl ether methacrylate) (poly-(OEGMA))—a nonfouling alternative to PEG—has been polymerized in situ.^[134] Alternatively, the protein C-terminus can be used for site-specific conjugation when it is genetically appended with a

self-cleaving intein peptide, which upon cleavage leaves a residual thioester for initiator conjugation and in situ polymerization.^[135]

Recombinant strategies are also emerging for the site-specific covalent conjugation of small-molecule drugs to therapeutic or targeting proteins. One such approach exploits the natural capacity of the Sortase A transpeptidase from *Staphylococcus aureus* for native protein ligation, which provides site-specific cleavage of a peptide recognition sequence and subsequent attachment of moieties containing a triglycine donor by nucleophilic attack. In a demonstration of its utility, camptothecin has been specifically conjugated to the C-terminus of recombinant TNF-related apoptosis-inducing ligand (TRAIL) by this approach to create a drug conjugate with both a small-molecule and therapeutic protein component.^[136]

An additional advantage of site-specific covalent conjugation is that it can be exploited to drive the self-assembly of drug carriers. For example, covalent attachment of multiple copies of doxorubicin to reactive cysteine residues located at the C-terminus of a hydrophilic elastin-like polypeptide biopolymer triggers self-assembly into spherical micelles, where the drug is sequestered in the hydrophobic core of the micelle, while the hydrophilic biopolymer creates a solvated micelle corona.^[137] This approach is restricted to drugs above a threshold hydrophobicity defined by an octanol–water distribution coefficient ($\log D$) greater than 1.5.^[138]

Hydrophilic therapeutics can, however, also induce covalent conjugation-driven self-assembly of drug carriers when attached to a sufficiently hydrophobic segment. Site-specific conjugation of hydrophobic PLGA polymer to the 3'-end of a hydrophilic siRNA through a degradable disulfide linker can drive the self-assembly of micelles by the segregation of PLGA into the micelle core, with siRNA decorating the micelle corona.^[139] Such self-assembled carriers can be further stabilized by complexation with linear PEI on the particle surface.

3.2. Preclinical Testing

After its successful formulation, a drug carrier is next evaluated by in vitro testing in cancer cell lines and in vivo testing in animal models. Such testing is required by regulatory standards prior to entry into clinical studies, despite the low correlation of preclinical experiments with clinical performance. Shortcomings in the standard operating procedures for preclinical evaluation are, thus, a considerable challenge in the translation of novel drug carriers.

In vitro evaluation serves as a preliminary checkpoint of drug efficacy. The 60-cell line screen of the National Cancer Institute represents a gold standard for evaluating the in vitro cytotoxicity of new drug entities. Although this collection of cell lines represents major cancer types, it cannot encompass the genetic diversity of cancers seen in human patients. This limitation is becoming more apparent with the growth of targeted chemotherapeutics, whose molecular targets are present only in a small subset of cancers and are not represented in common cell lines.^[140] Furthermore, testing in cancer cell lines predominantly evaluates cytotoxicity, and current in vitro testing lacks the complexity to test new anticancer agents such as immunotherapies. Most importantly, in vitro testing provides no information about

the differential activity of drugs on cancer versus healthy tissues and, thus, provides little insight into the potential safety of new drugs when administered to patients.

Preclinical in vivo testing serves to understand the pharmacokinetics and biodistribution of new drugs and drug carriers to better predict safety and efficacy in human patients. The vast majority of these preclinical studies are performed in murine cancer models, which overwhelmingly fail to predict clinical outcomes. When comparing the performance of anticancer agents on xenografts in mice versus human tumors in phase II clinical trials, the response of only one tumor type—non-small cell lung cancer—out of ten investigated was predictive of activity in humans in an equivalent tumor type.^[141] It may, thus, be of no surprise that as few as 8% of the drugs evaluated in preclinical animal models successfully complete phase I clinical trials.^[142]

Preclinical mouse models largely fail to predict performance in human studies because of the many ways that these models cannot replicate human cancer. First, most preliminary drug testing is performed in immunocompromised mice implanted with human cancer cell lines. The lack of a fully functional immune system, to permit growth of a human tumor in the murine host, creates an artificial environment that eliminates a key factor in tumor development. With the rise of anticancer immunotherapy, it is clear that the immune system should be recognized as a significant player in cancer.

Second, many preclinical studies are performed with subcutaneous tumors, with cancer cell lines injected into a physiologic location that is vastly different from the organ in which these cancers originate or spread to in human patients. This drastically changes the local environment of the tumor, which can affect the accumulation and distribution of a drug. Alternatively, cancer cell lines can be introduced at their natural site of origin to create orthotopic models that better reflect their physiological environment in human patients. However, major differences between the orthotopic tumor models and human cancers still exist, as the composition and architecture of the local environment of orthotopic tumors is composed primarily from the murine host,^[143] rather than human stromal and immune cells. These differences in the local tumor environment may correlate with differences in mechanisms of tumor drug delivery in the mouse and humans.

Third, the fast growing tumors implemented for most subcutaneous and orthotopic models do not reflect the typically slow development of cancer, which in humans is predominantly a disease of older individuals. To better mimic the process of natural disease progression, genetically engineered mouse models have been employed whose mutations can drive cancer development. What they lack, however, is the slow accumulation of genetic mutations that contributes to the unique disease in each individual patient, thereby failing to replicate the heterogeneity of disease seen in humans.^[144]

These differences between murine cancer models and human disease culminate in distinct variability in the performance of drug carriers. Perhaps the most prominent example of this is the discrepancy of the EPR effect between mice and humans. In mice, the EPR effect has been used universally as a passive mechanism to target drug carriers to solid tumors. Understanding the role of the EPR effect when treating human tumors, however, has been

much more elusive.^[145] It may be that features of mouse tumor models, such as the type, size, and location of xenografts, facilitate an EPR effect that is not reflected in established human tumors. For example, subcutaneous implantation of xenografts may induce vasculature that is different from that in natural tumors, which may lead to an overestimation of the EPR effect.^[146] However, studies suggest that long-circulating drug carriers such as liposomes can accumulate in human tumors, although with heterogeneous results between tumor types.^[147]

Insight into the role of the EPR effect in spontaneously occurring tumors has been gained from large animal models. In dogs with naturally occurring solid tumors, the EPR effect is highly variable between tumor types, as liposomes appear to passively accumulate in carcinomas at much higher levels than in sarcomas.^[148] Furthermore, the uptake of liposomes in these tumors is variable across different areas of a single tumor. These studies of tumors more closely resembling human disease confirm the heterogeneity of the EPR effect between tumor types and within individual tumors, which suggests that the EPR effect should be carefully considered when relying on this phenomenon for the delivery of drug carriers to human tumors.

3.3. Clinical Testing

A financial barrier must be overcome for a novel drug carrier to transition from preclinical to clinical testing. The incredible cost of drug development, estimated at approximately \$1 billion per new approved drug,^[149] creates a prerequisite that sufficient intellectual property cover the new drug carrier. Intellectual property ensures the right to commercial exclusivity, such that profits from sales—should the novel drug succeed—can recoup the extraordinary costs of research and development, clinical trials, manufacturing, and marketing. Thus, to entice the backers that can supply the finances necessary for clinical testing, the novel drug carrier must be covered by strong intellectual property.

Compared to free drug, a drug carrier may require a family of many patents to cover the drug cargo, carrier composition, combined carrier and drug properties, and the therapeutic application of the drug-loaded carrier.^[150] This collection of intellectual property becomes difficult in a research area whose recent birth and rapid growth has flooded the field with broad patents that encompass large areas of technology relevant to drug carriers.^[151] Abraxane, for example, was found guilty of infringement of a broad patent regarding surface-modified nanoparticles that lists over 40 types of particles for drug modification by over 80 potential therapeutics.^[152] In the field of drug carriers it is likely that we will not fully appreciate the consequences of these broad and overlapping patents until the technology realizes more commercial success, thereby providing financial incentive for infringement law suits.^[153]

Despite the cost to take new drugs into the clinic, new drug carriers do provide a unique financial incentive for investment, in that they may face less generic competition, compared to free drug. Generic formulations may have difficulty in proving bio-equivalence to complex drug carrier designs, which is required for approval of generic alternatives.^[154] If generic competition is thereby reduced, then the developer of a new drug carrier may have prolonged commercial exclusivity beyond the expiration date of the original patents.^[155]

This is an advantage for drug manufacturers, but this could be a cause for concern for patients and healthcare systems. This lack of generic competition will likely prevent the cost-lowering effect created by patent expiration, and could, furthermore, limit the accessibility of therapeutics in the interest of global public health needs if costs cannot be driven down by compulsory licensing to generic manufacturers.^[149]

With patent protection and the promise of commercial exclusivity, a new drug carrier can in some cases entice the financial support necessary to undergo clinical trials. At this point, the drug carrier faces a critical challenge of translating its performance from animal models to humans. A major hurdle in this task is the difference between the physiology of mice and humans. Furthermore, differences in experimental parameters, such as dosing schedule and treatment controls, may in part be accountable for the stark contrast in performance that is often observed between animal models and human patients.

Treatment models used for human cancer patients vary significantly from those employed in animal models. The majority of studies in mice rely on the response of a primary tumor as the metric of successful treatment. In humans, however, the primary tumor is rarely the cause of mortality and is often removed or reduced by surgery or radiation therapy. Therefore in humans, treatment is most needed to address disseminated disease. Models of primary tumor removal, followed by treatment of established disseminated metastasis, should become more prevalent in small animal cancer models, as a surrogate for the actual treatment regiment in humans.^[156]

Furthermore, drug carriers are not tested on human patients with previously untreated cancers nor are they compared against their equivalent free drug, as they frequently are in preclinical animal models. The patients enrolled in clinical trials are most commonly suffering from advanced cancers with disseminated disease that has evaded extensive previous treatments and is now refractory to therapy.^[157] In clinical trials, novel drug carriers will thus face the most difficult cancers to treat. It is important we pursue preclinical models that can better mimic the acquired drug resistance faced in human patients, by employing methods such as metronomic treatment of tumors in mice,^[158] to induce drug resistance in vivo prior to the testing of drug carrier therapies. Furthermore, in clinical trials the performance of drug carriers will be compared against a standard of care, which may include combinations of multiple drugs or orthogonal methods of treatment.^[159] The clinical performance of many novel drug carriers will, therefore, be compared to treatments that they were never evaluated against in preclinical studies.

Finally, the dosing or duration of treatment in human clinical trials greatly extends beyond that investigated in preclinical studies. It is not uncommon that preclinical studies claim to cure solid tumors in a single dose, but without analysis of repeat dosing, a great deal of information regarding the efficacy of the drug carrier may be lost. An excellent example of the consequences of differences in dosing between human clinical trials and preclinical studies is the discovery of accelerated blood clearance of PEGylated drug carriers with repeat dosing. In human patients, the consequences of accelerated clearance have been seen with PEGylated asparaginase, used to treat acute lymphoblastic leukemia. Up to one third of these patients experience rapid clearance of PEG–asparaginase, which is correlated with the

presence of anti-PEG antibody levels and a lack of asparaginase activity.^[160] The particulate nature of many drug carriers may increase uptake in antigen-presenting cells in the liver and spleen, and the drug carrier or cargo may serve as an adjuvant to increase immune responses upon repeat dosing.^[161] This effect, so clearly seen in clinical trials, is easily missed in preclinical studies that do not replicate human dose schedules, do not characterize the pharmacokinetics and biodistribution of repeat doses, and may use immunocompromised mice in which immune responses will be dampened. Furthermore, in humans, pre-exposure to PEG as an additive in food and cosmetic products has been suggested to elicit anti-PEG immune responses, with up to 25% of healthy individuals having been found to possess anti-PEG antibodies.^[162] Thus, in humans, in contrast to mice, even an initial dose of PEGylated drug carriers may face accelerated clearance in the presence of pre-existing anti-PEG antibodies.

For those drug carriers that successfully navigate through clinical trials, regulatory approval represents an additional challenge. The complexity of drug carriers presents unique requirements for regulatory approval beyond those that are required for free drug. Each component of the drug carrier must be evaluated, as well as the function of the combined drug-loaded carrier. Drug carriers composed of clinically approved biomaterials and drug cargo may, thus, face the least resistance in regulatory approval.^[163] In contrast, novel stimulus-responsive drug carriers may face the greatest regulatory challenges, where their approval must include characterization of their changes in material properties as well as the resulting impact on safety, pharmacokinetics, and biodistribution. These regulatory challenges may in part restrict first generation clinical drug carriers to those that use pre-approved biomaterials and therapeutics.

Lastly, the lack of sufficient reporting to the public on the results of clinical trials is an enormous impediment to the field. Without these results being readily available soon after the conclusion of a clinical trial, the broader scientific community loses the opportunity to learn from the success or failure of novel drug carriers. The outcomes of only 20% of phase II to phase IV cancer drug clinical trials have been publically reported in journals or by posting the results on [ClinicalTrials.gov](https://clinicaltrials.gov) within a year of the completion of the clinical trial. This reporting improves to only 55% at 3 years after the clinical trial has ended.^[164] Increased transparency of clinical results is needed to educate the entire research community on the potential and shortcomings of the novel drug carriers that are making it to clinical trials.

3.4. Clinical Adoption

Despite successful clinical approval, novel therapies face additional challenges to achieve widespread use. For drug carriers, a significant challenge may be their price. The cost of complex drug carriers, such as Doxil and Abraxane, far exceed the cost of treatment with their free drug counterparts, doxorubicin and paclitaxel, respectively.^[154] As these drug carrier formulations decrease toxicity, but do not in many instances improve efficacy, one may question if increased safety alone is worth the high price tag. However, when decreased toxicity translates to a decreased need to treat side effects, drug carriers may reduce overall medical costs compared to their free drug alternatives.^[165]

Beyond cost, there are yet other factors that can cause clinically successful anticancer drugs to fail commercially. Bexxar, an antibody–radionuclide conjugate, was clinically successful in treating patients with non-Hodgkin’s lymphoma who are nonresponsive to the standard of care,^[166] but was discontinued due to declining sales.^[167] Several factors contributed to the lack of clinical adoption that led to the failure of this drug. Since it is a form of radiation therapy, administration required patient referral to a radiation oncologist. Prescribing the alternative traditional chemotherapy was, therefore, preferred for both its simplicity of administration in the oncologist’s clinic and continued revenue provided to the oncologist’s practice.^[168] Furthermore, reports of inadequate insurance reimbursement for this costly therapy further discouraged the use of this drug carrier.^[166] Even effective drugs must, therefore, overcome the inherent biases in referral and challenges in reimbursement to succeed in the clinic.

4. Summary and Outlook

Anticancer drugs face an incredible set of transport challenges from their site of administration to their eventual action at their therapeutic target. Drug carriers have provided improvements to nearly every challenge that has been identified to enhance the success of anticancer drug therapy. Despite the growing number of drug carriers that are demonstrating success in preclinical research, the promise of improved clinical treatment of cancer has not truly been realized thus far with carrier systems. This is likely due to our incomplete understanding of the complex interactions between physiological challenges and drug carrier design, as well as incomplete appreciation of the manufacturing, regulatory, marketing, and reimbursement challenges along the developmental path from preclinical to clinical use. Overcoming these barriers requires—in our view—a systems engineering approach to anticancer drug delivery. This perspective is needed to better enable the rational design of optimized vehicles that navigate the trade-offs of this complex system to create next generation anticancer drug carriers that have a realistic prospect for translation from the laboratory to the patient.

While it is important to consider the potential hazards at any point in the journey of anticancer drug delivery, we realize that it is unrealistic to address each and every barrier with the design of a single drug carrier. Is it feasible to formulate a drug carrier that provides long circulation, passive accumulation, active targeting, cellular uptake, endosomal release, and subcellular localization of a therapeutic? Probably not, as the surface area, volume, and chemical reactivity of a drug carrier is limited and excessive functionalization is likely to diminish the successful function of any one component of such a multicomponent system. Ultimately, design is a compromise of trade-offs, but too many competing trade-offs can reduce a design to irrelevance. It is thus advisable to look closely at the drug one wishes to deliver and determine the most critical challenges that a particular drug will face in achieving efficacy with an acceptable safety profile. Understanding the acceptable trade-offs will guide the design of a carrier that can best address critical challenges that are specific to that drug.

Academic research on the design of drug delivery vehicles for cancer is moving toward increasing complexity. This trend compels us to sound a final cautionary note: the design of

drug delivery systems is, at its core, an exercise within a systems engineering context. Much of the work in this field has ignored the precepts of minimal design—that parts should not be appended unless they bring additive value—and that the entire system must be manufacturable, capable of achieving regulatory approval, and likely to gain traction with prescribing clinicians. Building ever more sophisticated systems with multiple components runs the risk of failure at the manufacturing, regulatory, and clinical level. In our view, the field of anticancer drug carriers must focus on a minimalist design philosophy to translate academic research into viable products that save lives at reasonable cost. To end with an analogy: all travelers pack a suitcase before a long journey. It is the veteran traveler who will inevitably ask: What do I really need for this trip? The answer invariably is: much less than one thinks.

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Biographies

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Figure 1.

After systemic administration, successful delivery of anticancer therapeutics must overcome pathophysiological barriers.

Figure 2.

A systems engineering approach to the design of anticancer drug carriers recognizes both pathophysiological and translational challenges.

Figure 3.

The octanol–water distribution coefficient ($\log D$) and the size (molecular weight) of small-molecule anticancer therapeutics.

Figure 4.

Tumor accumulation following systemic administration of drug carriers is facilitated by passive (A) or active targeting (B). inc: increased, dec: decreased.

Figure 5.

- a) Decreasing the size of drug carriers increases their penetration into the interstitial space.
b,c) Tumor penetration of drug carriers can be enhanced when disassembly is triggered by overexpressed tumor enzymes (b) or a depressed tumor pH value (c).

Figure 6.

Membrane-permeable drugs may be released extracellularly in the tumor tissue by a) carrier disassembly, b) carrier collapse, or c) carrier permeabilization. d) Cellular internalization of drug carriers predominantly occurs by endocytic mechanisms, while controlled cellular uptake of drug carriers can be achieved by the selective removal of “stealth” polymers in response to e) the depressed pH value of the tumor or f) overexpressed tumor enzymes.

Figure 7.

Drug carriers internalized by endocytosis (a) are sequestered in endocytic vesicles (b) or released into the cytoplasm by endosomolytic drug carriers (c). Free drug may be released intracellularly by cleavage of labile drug linkers, disassembly of the carrier, or swelling of the carrier (d). Intracellular localization can be achieved with carriers that home to their therapeutic target (e).

Covalent conjugation

Physical loading

Electrostatic complexation

Figure 8.
Mechanisms of drug loading in delivery vehicles.