CHAPTER SIXTEEN

Nanotechnology Therapeutics in Oncology—Recent Developments and Future Outlook

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Contents

1. INTRODUCTION

There has been intense interest in nanotechnology for the treatment of cancer, and numerous recent reviews have been published covering work in the field.[1,2] Understanding the underlying causes of cancer has lead to a new generation of chemotherapeutics.[3] However, these new "molecularly targeted" therapeutics suffer the drawback that only a very small fraction

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of the compound reaches the tumor site. Often following administration, the compounds are rapidly cleared and in a number of cases, even if the drug reaches the tumor, its residence time at the site of action is not sufficient to exercise a strong therapeutic effect. This drug delivery issue brings us to the key driver for using nanoparticulate formulations: improving small molecule drug levels at the tumor site (site-specific delivery), while keeping the drug away from healthy tissue (site-avoidance delivery).[4] Furthermore, the development of this technology offers the opportunity to reevaluate and expand the clinical use of classical chemotherapeutic agents. These potentially very useful cytotoxic drugs act by targeting fast growing cells, though differentiation between diseased and healthy cells is a critical issue, which contributes to the severe side effects observed, and leads to a narrow therapeutic index.

Nanoparticles for medical applications are typically defined as microscopic particles with a size between 1 and 1000 nm. They can be formulated from numerous materials and are optimally formulated to carry a variety of substances in a controlled and targeted manner. Nanoparticle distribution within the body is based on various parameters such as size, and surface coating, which can, in turn, be carefully controlled. In addition to this, nanoparticles offer considerable scope for engineering, thus enabling optimization of physicochemical characteristics.

2. PASSIVE TARGETING—THE EPR EFFECT

The most general method to target tumors relies on the enhanced permeability and retention (EPR) effect discovered by Maeda.[5] Most solid tumors have architecturally defective blood vessels and produce vascular permeability factors.[6] Therefore, they exhibit enhanced vascular permeability, which will ensure a sufficient supply of nutrients and oxygen to tumor tissues for rapid growth. In tumors, this defective architecture allows macromolecules (>40 kDa) to selectively leak out from tumor vessels and accumulate in the tumor tissue. In contrast, this does not occur in normal tissues. As such, this unique phenomenon is considered one of the cornerstones in tumor-targeting chemotherapy. All the currently approved nanomedicines, and the majority in clinical trials, rely on this passive accumulation within tumors.[7] Compared with conventional anticancer drugs (MW *ca.* 500), these macromolecular drugs have far superior pharmacokinetics (prolonged plasma half-life) and greater tumor selectivity. However, despite a focus on utilizing the EPR effect as a targeting mechanism,

there are drawbacks with this approach.[8] In particular, the random nature of the EPR effect makes it difficult to control the process and can induce multiple-drug resistance leading to the drug being expelled from the tumor tissue. Furthermore, many large tumors display a high degree of pathophysiological heterogeneity, which limits the penetration of the nanoparticulate formulation, and finally although often the formulation is effectively delivered to the tumor, internalization of the drug substance into the cell is an issue.

3. IDEAL CHARACTERISTICS OF NANOPARTICLES

Size and surface characteristics have long been recognized as key characteristics in the design and development of nanoparticles.[9] Effective drug delivery to tumor tissue requires that nanoparticles remain in the bloodstream for a considerable time. General trends on spherical nanoparticles have emerged. Particles less than 5 nm are rapidly cleared from the circulation through extravasation or renal clearance. The behavior of nanoparticles between 10 nm and 15 μm varies widely in terms of both biodistribution and cell uptake with the RES (reticuloendothelial system) being the primary mechanism for removal from the circulation. Particles of ~15 μm accumulate in the liver, spleen, and bone marrow. The ideal size of nanoparticles was found to be between 70 and 200 nm to reach tumor tissues through the EPR effect while avoiding first pass elimination by cells of the RES.[10]

However, size alone is not sufficient to ensure long circulation times in the bloodstream. The major issue observed is uptake by macrophages in the RES. The chief strategy to avoiding macrophage capture and increasing circulation time is incorporating a hydrophilic surface on the particle. This can be achieved either by coating the surface with a hydrophilic polymer (PEG) in order to protect it from opsonization or by formulating nanoparticles from block polymers with both hydrophilic and hydrophobic domains.

Designing nanoparticles that have either slightly negative or positive surface charges is also advantageous.[11] Charges serve to minimize self–self and self–non-self interactions. However, as the surface charge becomes larger, macrophage scavenging is increased and can lead to greater clearance by the RES. Thus, minimizing nonspecific interactions *via* steric stabilization and control of surface charge helps to prevent nanoparticle loss.

One additional characteristic that impacts nanoparticle delivery is the particle's shape.[12] The effect of shape and geometry has been investigated using both spherical and nonspherical polystyrene microparticles during

phagocytosis.[13] With elliptical disk-shaped microparticles, if the macrophage first contacted particles along the major axis, the particles were rapidly internalized (< 6 min). However, when first contact was along the minor axis, even after 12 h, the particles were not internalized. Spherical particles were rapidly and uniformly internalized because of their symmetry.[14]

4. LOADING OF NANOPARTICLES

Most oncology nanoparticles are highly engineered drug delivery devices. The formulation is utilized to improve the biodistribution and pharmacokinetic profile of the chemotherapeutic agent.[15] Given this, strategies can be divided into two broad categories. The first involves physically entrapping or absorbing the active substance onto the nanoparticle through noncovalent interactions. The second method involves directly attaching the drug substance to the nanoparticulate matrix by degradable or nondegradable covalent bonds. For both methods, it is key that the chemotherapeutic agent remains retained with the matrix until it reaches its ultimate site of action *in vivo*. Current research focuses on elucidating methods for stimulating release of the active drug at the tumor site. The bulk composition of the engineered nanoparticle must be judiciously chosen to take into account biocompatibility, immunotoxicity, and ability to solubilize or sequester effectively the drug substance.[16]

5. TYPES OF NANOPARTICLES

The most common types of nanocarriers are shown in Figure 16.1.

5.1. Liposomes

Liposomes are the most studied colloidal particles applied in antitumor therapy,[17] and their potential as drug carriers was rapidly recognized for anticancer treatment.[18] Two major issues emerged preventing the development of liposomes: (1) problems with robust loading and (2) preventing rapid clearance.[19] However, the development of technology to efficiently encapsulate ionizable products within liposomes (conventional liposomes) enabled the clinical application of liposomal doxorubicin (i.e., Myocet).[20] Myocet was approved in Europe and Canada in 2000 and is currently undergoing a phase 3 global trial. Myocet provides a limited degree of prolonged circulation compared to free drug (2–3 vs. 0.2 h), but this innovative encapsulation technology did not afford long-term stability in the bloodstream.

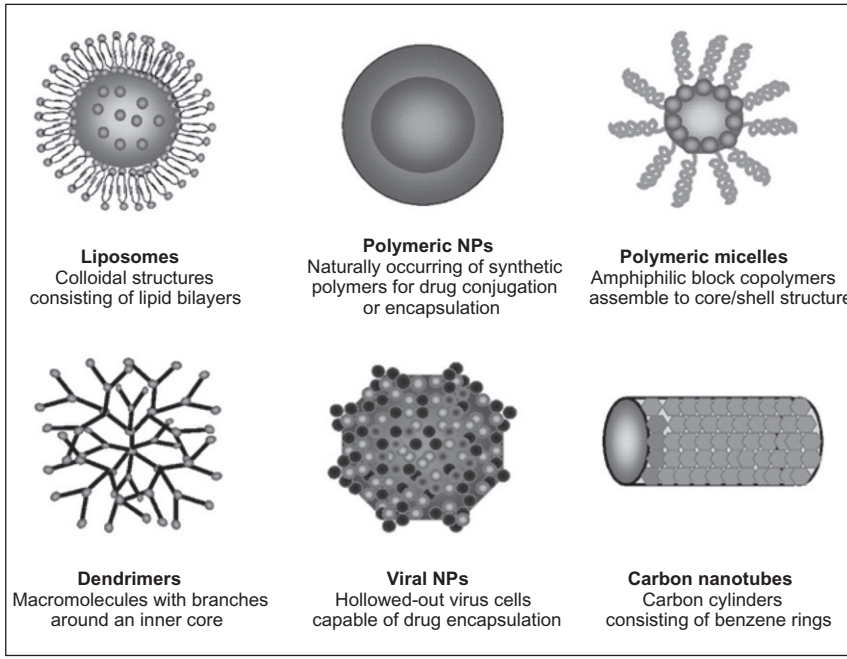

Figure 16.1 Common nanocarriers for drug delivery (reprinted by permission from the American Association for Cancer Research: Cho, K.; Wang, X.; Nie, S.; Chen, Z.; Shin, D.M. Therapeutic Nanoparticles for Drug Delivery in Cancer, *Clin. Cancer Res*. **2008**, *14* (5), 1310–1316. http://dx.doi.org/10.1158/1078-0432.CCR-07-1441).

Second-generation liposomes are referred to as "long circulating liposomes," and achieve this property through two key modifications to the liposome structure. First, their size is tightly controlled (< 100 nm), and second, saturated lipids are more stable than unsaturated lipids in plasma. DaunoXome (a liposomal formulation of daunorubicin) was approved in 1996 for the treatment of Kaposi's sarcoma.[21] DaunoXome provides extended circulation (half-life of 5 h against 45 min for the free drug) due to its small size (~45 nm) and is efficacious against numerous tumors while limiting cardiotoxicity observed with free drug.

Another branch of liposome research emerged during this time which investigated the effects of coating liposome surfaces with inert materials. A major advance in the surface-modified liposomes was the development of the coated liposomes using polymers like polyethylene glycol (PEG) which has many attractive properties. Pegylated-liposomes circulated for remarkably long times after intravenous administration and evaded

interception by the immune system, thus terming them "stealth" liposomes.[22] Currently, the only approved stealth liposome is pegylated liposomal doxorubicin (PLD, Doxil), which treats Kaposi's sarcoma, ovarian, and breast cancer.[23] The PEG surface coating substantially extends the half-life of doxorubicin *in vivo* with a half-life of approximately 55 h in humans versus 0.2 h for the free drug. Intravenous injection of doxorubicin allows less than 1% of the free drug to reach the tumor cells, whereas Doxil shows 10-fold higher levels in cells. Total plasma levels of doxorubicin are relatively high for several days after PLD administration; however, the majority of the dose is not available to healthy tissues thus protecting these from any toxic effects. Presumably, this is because the majority of doxorubicin in plasma (95–99%) remains encapsulated within the liposomes. Patients on PLD exhibit minimal cardiotoxicity even after receiving high cumulative doses. The major disadvantage of PLD is that acute infusion reaction, mucositis, and skin toxicities are often observed as dose-related toxicities.[24] The most notable of these is palmar-plantar erythrodyesesthesia ("hand-foot" syndrome), which is the usual dose-limiting toxicity of PLD, and causes issues with any substantial dose escalation that the liposomal formulation might provide. However, PLD still displays a higher therapeutic index than the free drug. Interestingly, these skin toxicities are not observed with Myocet, and as such are associated with the PEG surface coating.

5.2. Polymeric nanoparticles (polymer–drug conjugates)

The development of polymeric nanoparticles from concept to a commercial product for oncology has mirrored that of liposomes in that, despite initial rapid progress, the first protein-based nanoparticle did not receive commercial approval until 2005.[25] Numerous naturally occurring and synthetic polymers have been evaluated as materials for the delivery of drugs. Poly-L-glutamic acid, which was the first biodegradable polymer to be used for conjugate synthesis, is commonly used in drug delivery because the free γ-carboxylic acid enhances water solubility and can be utilized as a chemical handle for derivatization.[26] To be successfully employed in controlled drug delivery formulations, a material must be (1) chemically inert, (2) nontoxic, (3) free of leachable impurities, (4) biodegradable, and (5) degraded into molecules that can be metabolized and eliminated *via* normal metabolic pathways. During drug delivery, the hydrophobic core of the polymeric nanoparticle encapsulates a wide range of drug molecules with high loading

efficiency, while the hydrophilic shell provides steric protection. Numerous techniques are available to formulate polymeric nanoparticles, with PRINT (particle replication in nonwetting templates) being of interest as it controls the precise size, composition, and shape of particles.[27]

In 2005, the FDA approved Abraxane (ABI-007) for the treatment of metastatic breast cancer.[28] Abraxane is a cremophor-free protein-stabilized nanoparticle formulation of paclitaxel. Paclitaxel is traditionally formulated with the micelle-forming vehicle cremophor EL (polyoxyethylated castor oil) and ethanol. The nanoparticle albumin-bound platform (nab) uses albumin as a therapeutic carrier for the delivery of hydrophobic chemotherapeutics.[29] Abraxane is a colloidal suspension of albumin-bound paclitaxel in which the particles are approximately 130 nm. Direct comparison of efficacy demonstrated that Abraxane had significantly higher response rates compared with conventional paclitaxel (33% vs. 19%), and longer time to disease progression (23 vs. 16.9 weeks). Overall, side effects were lower and no premedications were required using Abraxane. This is the first demonstration of a higher therapeutic efficacy for a nanoparticle therapeutic over the normal formulation.[30]

5.3. Polymeric micelles

The core/shell segments of polymeric micelles (PM) control the functionalities of the nanoparticle structure.[31] The hydrophilic outer shell region stabilizes the hydrophobic core region and controls the *in vivo* pharmacokinetic behavior by rendering the polymers water soluble to enable facile intravenous administration. The hydrophobic inner core is responsible for drug loading capacity, stability, and drug release behavior. Size control of the PM is simple through control of the block copolymer chain lengths. PM can also alter the drug internalization route through interaction and binding of the block copolymer to the cell membrane to facilitate endocytosis. The polymers also have been shown to avoid P-glycoprotein (P-gp) expressed in membranes of multidrug resistant cells, thus increasing drug absorption. In addition, they possess a higher drug loading capacity as well as improved stability compared to other nanoparticle systems.[32] Due to these and other favorable characteristics, interest in PM has been rapidly increasing and several formulations are currently undergoing clinical evaluation (NK105, a PM formulation of Paclitaxel is currently in phase III). Previously, NK105 has demonstrated complete tumor effacement after a single dose in HT-29 colon cancer cells in female nude mice.[33]

5.4. Dendrimers

Dendrimers are macromolecular compounds that comprise branches around an inner core. Their key advantage is that they can be tailored for specific applications and are ideally suited as drug delivery systems as it is easy to tailor their topology, functionality, and size.[34] The terminal groups can be utilized for bioconjugation, as targeting moieties, for signaling purposes, or simply to modify the dendrimer surface. Through judicious selection of the surface functional groups, biodistribution, permeability, and other pharmacokinetic properties can be modulated. No dendrimer-based products are currently in clinical development for oncology, but several promising studies have been carried out both *in vitro* and *in vivo*.

5.5. Viral nanoparticles

Viral nanoparticles are hollowed out virus cells, which can be utilized to target drugs directly to cancer cells.[35] Numerous viruses have been developed for nanotechnology applications, and in general, plant viruses are utilized as they are the easiest to produce in large quantities. These virus cells readily self-assemble into a nanoparticle with approximately a 10-nm core capable of encapsulating a high load of drug substance.[36] Researchers have demonstrated *in vivo* the enhanced recognition of prostate tumors by a PEG-coated cowpea mosaic virus particle modified with bombesin.[37] The major challenges for the development of viral nanoparticle-based systems are enhancement of physicochemical properties to prevent rapid clearance and limiting toxicity.

5.6. Carbon nanotubes

Carbon nanotubes are tubular materials consisting of benzene rings with nanometer-sized diameters, and axial symmetries, which leads to unique properties in the treatment of cancer. The high surface area of carbon nanotubes allows multiple functionalization sites to attach therapeutic agents and targeting ligands, or to aid solubility.[38] However, toxicity problems related to chronic insolubility have inhibited development, though functionalization can enhance solubility and alleviate these effects.[39] Interest in this drug delivery modality is focused on their ability to cross biological barriers.[40] However, despite the fact that modified carbon nanotubes have been evaluated with numerous therapeutic agents, the issue of biodegradability remains unresolved.

6. THE NEXT GENERATION OF NANOMEDICINES FOR ONCOLOGY

The major downside to current nanotechnology delivery systems is that the efficacy of cancer treatment is reduced because nanoparticles release their payload *via* a slow and passive mechanism. Although passive targeting has succeeded in reducing off-target toxicity effects (and as a result the formulations often show increased therapeutic index), polymer–drug carriers have shown limited increases in therapeutic efficacy. Large tumors are not homogeneous and nanoparticles cannot disperse uniformly through the tumor. In addition, the negative pressure gradient within the tumor limits the nanoparticle's effective range and can force them out of the tumor. Finally, although the EPR effect localizes the nanoparticles to the tumor, release of the drug substance and its subsequent internalization into the cells is still an issue. Thus, the next generation of nanoparticles has focused on incorporation of "smart" technologies that are responsive to environmental stimuli. Two major areas have emerged. The first involves active-targeting, whereby particles search for and subsequently attach themselves to diseased cells.[41] The second involves active drug payload that is triggered to release at the desired site of action owing to chemical or physical changes in the environment.[42,43] The ultimate goal in nanooncology would involve combining site-targeting and site-specific release into the same nanoparticle.[44]

6.1. Active targeting of nanoparticles

The most common approach to achieve this goal is to attach targeting ligands to the surface of the particle, taking advantage of receptor overexpression on specific tumor cell surfaces compared to healthy cells. Coupled with the EPR effect, targeted particles should increase the interaction time between the particles and the tumor and increase the likelihood of the particles being taken up by the tumor cells *via* endocytosis. Numerous ligands have been utilized to take advantage of targeting mechanisms,[45] and a tabulated summary of the more common approaches has been compiled.[46]

The folate receptor, which is overexpressed in many tumors, has been widely exploited for this purpose.[47] Attaching folic acid and related derivatives to the particle surface creates a targeted drug delivery vehicle.[48] Folic acid-functionalized dendrimers conjugated with methotrexate show a 10-fold increased efficacy and significantly lower toxicity when compared to the free drug at an equivalent cumulative dose in a mouse model of human

epithelial cancer. This approach shows great promise as the folic acid particles can be produced at low cost.

Monoclonal antibodies (mAbs) have also been aggressively pursued as targeting moieties owing to their exquisite ability to target specific disease processes.[49] One key problem with mAbs is that they are targeted by the immune system. In addition, mAbs are large, complex molecules, expensive to manufacture, and require significant engineering at the molecular level to be effective.[50] One potential solution involves using antibody fragments as targeting molecules. Antibody fragments retain high affinity for the target antigens but have less immunogenicity (due to the lack of an Fc region, and relatively short circulation times); being smaller in size, they are better suited for molecular targeting.[51]

Aptamers are small nucleic acid ligands that bind to targets with both high sensitivity and specificity. One major advantage of aptamers is that they are identified using an *in vitro* evolutionary process called systemic evolution of ligands by exponential enrichment (SELEX).[52] This process uses a library of 10^{15} random oligonucleotides, and enrichment identifies aptamers that bind with high affinity and specificity to the target.[53] This approach allows scale-up without batch to batch variation. Further advantages are their small size ($\sim$15 kDa) and low immunogenicity which leads to controlled biodistribution. Several aptamer-targeted nanoparticles are in preclinical development. Unfortunately, aptamers are unstable in serum and have relatively high production costs.

Peptides represent an extremely attractive targeting moiety because of their small size, low immunogenicity, high stability, and relative ease of manufacture. Development of peptide phage libraries ($\sim 10^{11}$ different peptide sequences) and efficient screening technologies has made selection of potential targeting ligands extremely facile.[54] They also have the ability to bind to their targets with high affinity and specificity.[55]

6.2. Target-controlled release

Efficient release of drugs from nanoparticles is critical to bioavailability. Controlling the timing and degree of release are the two major challenges. First, stability is key to avoiding discharge of the cargo into healthy tissues. Second, specificity and efficiency of the trigger are essential so that nanocarrier instability and release of cargo are done in the desired tumor environment. Triggers can be divided into two categories based on where the stimuli originates. Internal triggers rely on exploiting subtle differences within the tumor environment such as pH or specific enzymes, while

external triggers release the drug cargo prompted by stimuli from outside the body such as temperature, ultrasound, or radiation.

Of the potential triggers, pH is the most common.[56] Mildly acidic conditions exist in tumor and inflammatory tissues due to hypoxia and cell death, where the pH in tumor tissues is ~6.5 versus physiological pH of 7.4. A more pronounced pH change is encountered if the nanoparticles enter into cancer cells where the pH is ~5. Development of acid-labile linkers and acid-sensitive nanocarrier shells releases their cargo on a drop in pH. Numerous variations on these themes have also been investigated. Several *in vivo* studies have demonstrated the viability of this approach, but these have yet to be translated into the clinic.

Enzyme-triggered release uses localization of enzymes in specific areas. The most exploited enzymes are matrix metalloproteinases (MMPs) which are often overexpressed in tumor sites. Researchers have incorporated a MMP cleavage site within both liposomes and dendrimers to trigger release of the encapsulated cargo.[57] The main disadvantage of enzyme-triggered release is that no enzyme is solely expressed in the target region; as such nonspecific release occurs throughout the body. Work utilizing dendrimers bearing cell-penetrating peptides that were exposed upon cleavage to MMP indicated 4- to 15-fold higher uptake than the corresponding dendrimers without the peptides.

Two other approaches are being investigated utilizing heat[58] or ultrasound waves to trigger release. The heat-activated liposome formulation, Thermodox, is currently undergoing clinical evaluation. Use of ultrasound as a triggering mechanism is still at an early stage of development, though proof of concept has been achieved.[59]

6.3. In development

Numerous nanoparticle formulations are currently in development. A selection of these with comments regarding the key differentiating features is presented in Table 16.1[60,61]

7. CONCLUSIONS

There continues to be explosive growth in oncology research focused on utilizing nanoparticles as drug delivery vehicles. Although the development of both a commercial liposomal and a polymeric product within the field took place 30 years ago, there are many areas that still need

Table 16.1 Selection of nanoparticle formulations currently in clinical development

Name	Company	Indication	Status	Comments	Improvement
Genexol-PM	Samyang	Pancreatic cancer/ metastatic breast cancer/NSCLC	Phase 2/3 (US)	Approved in Korea for treatment of metastatic breast cancer. Polymeric micelle cremophor-free formulation of Paclitaxel	38% partial response versus 24% with Paclitaxel. Formulation enables higher MTD
Thermodox	Celsion	Hepatocellular carcinoma	Phase 3	Encapsulated doxorubicin in a heat-activated liposome. Activated by radio frequency ablation (RFA)	Significant tumor size decrease. 26.1% with RFA as opposed to 12.1% without
CDP 791	UCB Pharma	NSCLC	Phase 2	A PEGylated, humanized di-Fab fragment specifically inhibiting VEGFR-2 activation and blocks all signaling through this receptor	Six ascending doses once every 3 weeks. Well tolerated and met safety endpoint. No dose limiting toxicity or immunogenicity
CALAA-01[62]	Calando Pharm.	Solid tumors	Phase 1	Polymer formulation of siRNA using transferrin as a targeting ligand for binding to transferrin receptors	Active safety study trial
Lipoplatin	Regulon	NSCLC and pancreatic cancer	Phase 2/3	Liposomal formulation of cisplatin with significantly reduced toxicity	Half-life observed to be 60–117 h as opposed to 6 h for cisplatin. 11.1% partial response in patients, who had failed all previous chemotherapy
BIND-014[63]	BIND bioscience	Prostate cancer/ solid tumors	Phase 1	Prostate-specific membrane antigen-targeted docetaxel-encapsulated polymeric formulation. First targeted nanoparticle to enter clinical trials. Also displays controlled release of drug	Evidence for antitumor activity in cancers for which conventional docetaxel is ineffective. Well tolerated

improvement. The lessons learned from the first generation of products, investment in the field, and our increased understanding should lead to rapid progress.

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