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EXPERT REVIEW

Clinical Developments in Nanotechnology for Cancer Therapy

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ABSTRACT Nanoparticle approaches to drug delivery for cancer offer exciting and potentially "game-changing" ways to improve patient care and quality of life in numerous ways, such as reducing off-target toxicities by more selectively directing drug molecules to intracellular targets of cancer cells. Here, we focus on technologies being investigated clinically and discuss numerous types of therapeutic molecules that have been incorporated within nanostructured entities such as nanoparticles. The impacts of nanostructured therapeutics on efficacy and safety, including parameters like pharmacokinetics and biodistribution, are described for several drug molecules. Additionally, we discuss recent advances in the understanding of ligand-based targeting of nanoparticles, such as on receptor avidity and selectivity.

KEY WORDS clinical · nanoparticle · oncology · pharmacokinetics · targeting

INTRODUCTION

Despite continuing advances in cancer diagnosis and treatment in recent years, it is projected that there will be 562,340 cancer-related deaths in the United States in 2009 (1). Although there have been important steps forward in our understanding of cancer and its treatment (2), these statistics and projections are a clear indication that further advances are needed. Such advances include improved early screening and diagnosis, as well as treatment regimens that are more selectively taken up by tumor cells and have reduced off-target toxicity, two areas where nanoparticle approaches are likely to have significant future impact.

In this review, we discuss some of the unique and critical properties of nanoparticles that differentiate them from other types of cancer therapeutics and make them well suited for application to various types of cancer. We summarize nanoparticle-based approaches that are currently under clinical oncological investigation, highlighting the key findings and comparing them to each other and, when possible, to what has been observed with their precursor drugs alone.

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NANOPARTICLES FOR CANCER: CRITICAL PROPERTIES

A number of key properties of nanoparticles render them well suited for application to cancer and distinguish them from small molecule or nucleic acid therapeutics and/or their molecular conjugates. These important parameters include size, payload density, duration of effect, and surface properties/targeting.

Size

While the term *nanoparticle* generally refers to entities having diameters in the range of 1–100 nm, current understanding is that nanoparticle therapeutics for cancer ought to be within the 10–100 nm range (3). Indeed, a recent review of all nanoparticles being evaluated clinically found their published sizes to be between 20 to 120 nm (2). The 10-nm lower size limit is based upon experimental determina-

tion, using a variety of materials, of the removal (filtration) of material from plasma through pores within the glomerular capillary wall of the kidney. Materials with hydrodynamic diameters below ~5 nm are subject to rapid kidney clearance, whereas molecules or particles ~10 nm or larger are negligibly, if at all, removed from circulation via this mechanism (4,5). The 100-nm upper size limit is less well-defined and is the result of the leaky nascent vasculature known to exist within tumors. Owing to the poor lymphatic system in tumors, there is accumulation of macromolecules that leak out of the fenestrated vasculature; collectively, this mechanism has been termed the EPR (enhanced permeability and retention) effect (6,7). There is uncertainty around the upper size limit of nanoparticles that can effectively utilize the EPR effect because the sizes of these fenestrations are not constant; tumor vascular permeability is known to vary with tumor type and microenvironment and may even vary temporally for an individual tumor (8). Once EPR-mediated extravasation of nanoparticles to the tumor occurs, there is the additional issue of their restricted mobility within the extracellular milieu (9). An investigation of liposomes varying in size and surface charge revealed that vesicles ~120 nm, but not ~250 nm, in size with minimally negative surface charge (zeta potential −2 to −5 mV), but not strongly positive surface charge (+48 mV), were able to move through tumor tissue (10). Perrault *et al.* convincingly illustrated that gold nanoparticles surface-modified with poly(ethylene glycol) (PEG) must be less than 100 nm in diameter to move away from the vasculature and throughout the tumor (11). Additionally, recent investigations of systemically administered nanoparticles ~30–40 nm (12) and ~70 nm (13) in size, each also having a slightly negative surface charge, revealed tumor extravasation, movement away from the blood vessels, and internalization by tumor cells in mice. Taken together, our current understanding is that nanoparticles with minimal surface charge in the ~10 nm to sub-100 nm size range should generally be able to reach tumor tissue and disseminate within it upon systemic administration.

Payload Density

Nanoparticles have the ability to carry a large number of therapeutic molecules—including small molecules, peptides, nucleic acids, and proteins—and protect them from degradation. Loading levels of 10^4 drug molecules per liposomal nanoparticle have been reported for small molecules (14), while a 70-nm polymeric nanoparticle has been shown to contain 10^3 siRNA molecules (15). Thus, cellular uptake of a single such nanoparticle can achieve orders-of-magnitude higher intracellular drug concentrations than that of an individual drug molecule or its molecular conjugate. The therapeutic entities within these nanoparticles need not impact the nanoparticles' properties—doxorubicin-loaded liposomes and their drug-free analogue liposomes, for example, may be expected to possess the same particle size, surface charge, pharmacokinetic profile, biodistribution, etc. This stands in contrast to molecular conjugates, for which the properties of the individual drug molecule are often strongly altered by the presence of a covalently attached modifier, such as PEG and/or an antibody (16).

Duration of Effect

One of the principal benefits of incorporation of a therapeutic molecule within a nanoparticle is to extend the duration of effect. A large number of factors—including composition, size, core properties, surface modifications and targeting ligand functionalization (discussed separately below)—have been shown to significantly impact the clearance and biodistribution of nanoparticles (17,18). Indeed, the pharmacokinetic profile of nanoparticle-incorporated drugs often includes a dramatic increase in circulation half-life ($t_{1/2}$) compared to the drug alone (see comparisons of drug *vs.* nanoparticle formulations of the drug below). For example, IT-101, a nanoparticle containing a polymer-bound conjugate of camptothecin (CPT), increased circulating plasma concentrations and area-under-the-curve by ~100-fold compared to CPT alone (19). This is particularly important for therapeutic molecules with very poor stability in circulation, such as unmodified nucleic acids (20) and highly cytotoxic small molecules and peptides (21,22) for which nanoparticle formulation permits the use of lower doses to achieve similar, if not enhanced, efficacy with sharply reduced side effects. In addition to extended circulation half-life, nanoparticles can be prepared to have extended release of the drug payloads. That is, the nanoparticles can release their therapeutic payloads in designed fashions. For example, the mechanism of action of CPT would suggest that a slow, continuous release from the nanoparticle in the tumor would optimize its effectiveness while minimizing toxicity. IT-101 was designed to slowly release CPT when it is located within the acidic compartments of tumor cells. In mouse models of lymphoma, the tumor concentrations of IT-101 and its released CPT were constant over several days, while the concentrations of CPT-11 and its therapeutic product, SN-38, declined several orders of magnitude in the 24 h after systemic injection (23). Thus, nanoparticles can extend the duration of therapeutic effects in several ways.

Surface Properties/Targeting

As mentioned above, the surface charge (zeta potential) of nanoparticles has been shown to influence the particles' disposition within tumor. Indeed, control of the surface

properties of nanoparticles is critical, particularly given the large surface:volume ratios that these particles possess (24). Minimization of nanoparticle surface charge is often achieved by incorporation of a neutral polymer, such as PEG, that reduces aggregation caused by particle-particle interactions as well as limits potential electrostatically induced interactions with other components within the circulation (many of which are negatively charged, including the plasma membranes of cells). As the nanoparticle surface charge is increased, whether it is positive or negative, the probability that the particle will be removed from circulation by macrophage scavenging grows concomitantly (25).

In addition to zeta potential, the nanoparticle surface is also the site at which many nanoparticles are modified to include targeting ligands. The rationale behind the inclusion and selection of a targeting ligand is that the cell surface density of the cognate receptor is elevated on target cancer cells relative to other cell types. Thus, ligand incorporation may provide a measure of cell type selectivity and employment of receptor-mediated endocytosis as a means of cell entry and avoidance of multi-drug resistance. Ligands can be any of a variety of molecular types, including small molecules (26), aptamers (27), peptides (28), proteins (29), or antibodies (30). Nanoparticles can benefit from the avidity achieved by multiple ligands on a particle surface engaging multiple cell surface receptors, but currently the optimal ligand density for a given nanoparticle:ligand:receptor combination cannot be predicted and must be determined empirically. The affinity of the ligand for its receptor can strongly influence the impact of multivalency, with evidence indicating that relatively low-affinity ligands have the potential to create strong effective affinities within the context of a multivalent nanoparticle (31). For example, increasing the number of transferrin molecules on a 70-nm PEGylated gold nanoparticle up to 144 gave a K_d of the nanoparticle to the surface of Neuro2A cells (which have upregulated transferrin receptors) of 0.13 nM, compared to 64 nM for transferrin alone (13). Thus, one of the useful features of nanoparticles is their ability to significantly increase avidity via multivalency. Molecules that do not have sufficient binding affinity for use as a drug or individual targeting ligand (as with drug conjugates) can be employed with nanoparticles. Thus, many drug candidates that failed because of low binding to the target can be used on the surface of nanoparticles as targeting agents, and the avidity of the nanoparticle is significantly enhanced by multivalency.

Interestingly, it has been shown on numerous occasions, in the context of polymer-based (32), lipid-based (30,33,34), and gold nanoparticle (13) systems, that the presence of a targeting ligand does not alter the overall biodistribution of particles to the tumor but, rather, increases the extent of cellular internalization by particles that reach tumor tissue—and does so in a ligand density-dependent fashion (13). The inability of a targeting ligand to significantly increase tumor deposition (relative to untargeted nanoparticles) is consistent with a recent modeling analysis of the roles of molecular size and affinity on tumor uptake as well (35). Consequently, the term *targeting ligand* might well be replaced by *internalization ligand* to better reflect its actual role based upon a growing body of literature. In addition, modeling analysis revealed that intermediate-sized ligands (MW ~25 kD) achieve the lowest tumor uptake levels, while both smaller ligands (that require high receptor affinity to be retained) and larger ligands (that can achieve similar retention as smaller ligands with > 100-fold weaker binding) can achieve enhanced tumor uptake (35). This information is consistent with several published examples of ligand-containing nanoparticle systems and will likely prove valuable to drug developers moving forward with respect to the role and selection of targeting ligands.

CLINICAL APPLICATION OF NANOTECHNOLOGY FOR CANCER

Clinical trials of nanoscaled entities for cancer therapy have been conducted for decades; e.g., Doxil®, a PEGylated liposomal formulation of doxorubicin, was approved in 1995. While the term *nanoparticle* was not used during these early days of lipid-based formulations, those formulations which form ~100-nm liposomes are the first examples of nanoparticles being used in humans for cancer treatments. We will present information on nanoparticle formulations of small-molecule therapeutics first, discussing both ligand-containing and non-ligand-containing approaches, followed by an overview of nanoparticles incorporating other types of therapeutics, including proteins and nucleic acids.

Nanoparticle/Nanoscaled Formulations of Small Molecules

Small-molecule drugs often are extremely effective at killing cancer cells they reach, but their small size leads to rapid clearance from circulation and, consequently, significant uptake by non-cancer cells with concomitant side effects that are, at best, undesirable and, at worst, prohibitive of use. For this reason, current and potential small-molecule therapeutics are prime candidates for exploration within nanoparticle formulations.

Doxil® is one of several nanoparticle formulations of doxorubicin that have been investigated clinically. A product of Centocor Ortho Biotech, Doxil® (marketed as Caelyx® outside of the United States) is a PEGylated liposomal formulation of doxorubicin. Initially approved for chemotherapy-refractive AIDS-related Kaposi's sarcoma, Doxil® has since been approved for other indications,

including ovarian cancer and multiple myeloma. Myocet® (a product of Sopherion Therapeutics in the U.S. and Canada) is an alternative, non-PEGylated liposomal formulation of doxorubicin—it is approved in Europe and Canada but not yet in the U.S. Still other nanoparticle formulations of doxorubin have been developed, including SP1049C, a micelle formulation of doxorubicin with pluronic (also known as poloxamer, a triblock copolymer consisting of two hydrophilic poly(ethylene oxide) chains flanking a central hydrophobic poly(propylene oxide) chain), and NK911, a micelle containing PEG and poly(aspartic acid).

As expected, incorporation of doxorubicin within these liposomal or micellar formulations significantly alters the drug pharmacokinetics (PK). With respect to circulation half-life ($t_{1/2}$), while free doxorubicin alone has a $t_{1/2}$ of less than an hour (36), the micellar formulations extended the plasma half-life by approximately three-fold (37,38), while the liposomal formulations extended it further by more than an additional ten-fold (36,39,40) (see Table I). It is difficult to quantitatively compare these half-life numbers given the differing models use to generate them, however, so evaluation of additional PK parameters, such as clearance rate, is instructive (see Table II). These results further illustrate the strong impact all of these formulations have on doxorubicin PK and also indicate differences between the micellar and liposomal approaches. For the two micelle-containing formulations, SP1049C (12.6 ml/(min•kg)) has a similar clearance rate to free doxorubicin (14.4 ± 5.6 ml/(min•kg)), and NK911 (6.7 ± 1.1 ml/(min•kg)) reduces the clearance rate only minimally (approximately two-fold). These results are consistent with the hypothesis that these micelles may disassemble shortly after administration. By contrast, Myocet® (2.57 ml/(min•kg)) reduces the clearance rate by nearly six-fold, and Doxil® (0.02 ml/(min•kg)) has a nearly one-thousand-fold reduced clearance rate. These liposomal results suggest that PEGylation plays a key role in reduced clearance of these nanoparticles.

Nanoparticle formulation of doxorubicin can significantly alter the PK properties of the drug as well as its biodistribution, safety, and efficacy. Significantly higher drug levels in tumor tissue have been observed with Doxil® than free doxorubicin in multiple cancer models (42,43). Just as importantly, Doxil® has shown the ability to clinically reduce cardiotoxicity, a hallmark of free doxorubicin treatment (41,44). For non-PEGylated liposomes (such as Myocet®), the cardiac-sparing effect is believed to occur because these liposomes generally extravasate in areas that lack tight junctions, found in the vessels that supply the myocardium (44). For PEGylated liposomes, the blunted peak plasma levels of free drug combined with the presumed biounavailability of liposome-entrapped drug circulating through the myocardium are hypothesized for the reduced cardiotoxicity (44). Because of their reduced cardiotoxicity, liposomal formulations of doxorubicin (unlike free doxorubicin) can also be used in combination with other cardiotoxic drugs, such as docetaxel and trastuzumab (45), allowing for clinical exploration of additional potential therapeutic options.

Doxorubicin has been clinically evaluated as nanoscaled polymer conjugates using an *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer, although it is unknown whether these conjugates form nanoparticles in the circulation. These can be untargeted (FCE28068, also known as PK1) or contain a galactosamine ligand (FCE 28069, also known as PK2) for targeting of asialoglycoprotein receptor (ASGPr) on the surface of hepatocellular carcinoma cells. In a Phase I evaluation, FCE28068 had a maximum tolerated dose of 320 mg/m^2, and pharmacokinetic evaluation revealed a much-extended plasma half-life and three orders-of-magnitude decrease in clearance compared to free doxorubicin (46). FCE28068 demonstrated antitumor activity in refractory cancers, showed no polymer-related toxicity, and provided proof of principle that polymer-drug conjugation decreases doxorubicin dose-limiting toxicities (46). Phase II investigations of FCE28068 were performed in patients with breast, non-small-cell lung, or colorectal cancers (47); overall, a few (6 of 62 total) partial responses were seen along with evidence of tumor accumulation in two subjects with metastatic breast cancers. FCE28069, the galactose-targeted variant nanoparticle, showed some antitumor activity in patients with hepatocellular carcinoma (48) but required a reduction in infusion rate in response to pain, perhaps due to the increased drug concentration and concomitant lower solubility than FCE28068 (49).

In addition to the aforementioned approaches, a nanoparticle for delivery of doxorubicin, doxorubicin Transdrug® (BioAlliance Pharma SA), is under clinical development. Doxorubicin-containing nanoparticles are formed with PIHCA (polyisohexylcyanoacrylate), a biodegradable polymer; these particles have been shown to avoid the efflux pump (multidrug resistance) mechanism (MDR, discussed further below) (50), presumably due to ion-pair formation between doxorubicin and soluble polymer hydrolysis products (51). In December 2009, BioAlliance Pharma SA announced results from a Phase II clinical trial in patients with advanced hepatocellular carcinoma that included a significantly increased survival rate (88.9% after 18 months of treatment *vs.* 54.5% survival for standard-of-care treatment). Despite this positive outcome, this trial was suspended due to pulmonary adverse events in July 2008.

Daunorubicin—a member of the anthracycline class of small molecules, like doxorubicin—has also been investigated within nanoparticle formulations. In particular,

Table I Pharmacokinetic (Plasma Half-Life) Data for Doxorubicin and Its Nanoparticle Formulations

Formulation	Description	Plasma half-life	Reference
Free doxorubicin		$t_{1/2,\alpha} = 0.07$ h, $t_{1/2,\beta} = 9.6$ h[a]	(34)
SP1049C	Micelle, Pluronic	$t_{1/2,\alpha} = 0.11$ h, $t_{1/2,\beta} = 2.83$ h, $t_{1/2,\gamma} = 48.8$ h	(35)
NK911	Micelle, PEG and poly(aspartic acid)	$t_{1/2,\alpha} = 0.08 - 0.13$ h, $t_{1/2,\beta} = 1.6 - 4.7$ h, $t_{1/2,\gamma} = 29.4 - 241.4$ h	(36)
Doxil®	PEGylated liposome	$t_{1/2,\alpha} = 2.3$ h, $t_{1/2,\beta} = 45.6$ h[a]	(34)
Myocet®	nonPEGylated liposome	$t_{1/2} = 50.95$ h[b]	(37)

[a] Data is average of that presented for 25 and 50 mg/m^2 dose levels in ref. (36)

[b] Data is median presented in Table 2 of ref. (39)

the DaunoXome® formulation, a nonPEGylated, 35–65-nm liposome containing DSPC (disteroylphosphatidylcholine) and cholesterol that has been approved for treatment of AIDS-related Kaposi sarcoma, has been widely studied in both pediatric and adult cancer patient populations. While early clinical results in both populations indicated reduction of daunorubicin-mediated cardiotoxicity in both population types (52), a more recent study in pediatric patients which contained a longer follow-up time period revealed similar cardiotoxicity to conventional (i.e., not formulated within nanoparticles) anthracyclines (53). Ongoing and future studies will continue to shed light on the safety and efficacy profiles of DaunoXome® in various cancer patient populations, both alone and in combination with other drugs.

In addition to anthracyclines, taxanes, such as docetaxel and paclitaxel, are some of the most investigated small-molecule drugs for incorporation into nanoparticles. Sparingly water soluble (0.7 μg/ml for paclitaxel, 6–7 μg/ml for docetaxel) (54), these molecules kill cells primarily through microtubule stabilization, including impedance of microtubule depolymerization during mitosis. Both of these taxanes are approved for treatment of various types of cancer. Paclitaxel is commercialized by Bristol Myers Squibb as Taxol®, a formulation containing Cremophor® EL, a low-molecular-weight surfactant that forms micelles in aqueous media. Docetaxel, commercialized as Taxotere® by Sanofi Aventis, employs a Tween® 80 (polysorbate 80)-based surfactant formulation in a similar fashion. These formulations have minimal stability in circulation and cause significant side effects, such as neurotoxicity and nephrotoxicity (Cremophor® EL), peripheral edema (Tween® 80), and acute hypersensitivy reactions (both) (54). Consequently, alternative nanoparticle formulations for taxanes have been developed and studied clinically—these include Abraxane®, OPAXIO™, and Genexol®-PM.

Abraxane® is a ~130-nm, albumin-based nanoparticle formulation of paclitaxel; it was developed to eliminate the toxicities caused by Cremophor® EL (within Taxol® and its generic equivalents) but retain the potency of paclitaxel (55). The success of Abraxane® in achieving the goal of enhanced tolerability was realized in nearly doubling the maximum tolerated dose (MTD) seen with Taxol® when administered once every 3 weeks (300 mg/m^2 for Abraxane® (56) *vs.* 175 mg/m^2 for Taxol® (57)). In a subsequent Phase III study, in which Abraxane® (260 mg/m^2) and Taxol® (175 mg/m^2) were each dosed in the same fashion (once every 3 weeks) in metastatic breast cancer patients, a significantly higher response rate was seen for Abraxane® (33% *vs.* 19%) along with significantly longer time to tumor progression and significantly fewer incidences of grade 4 neutropenia (58). As expected, this enhanced efficacy corresponds to an altered pharmacokinetic profile—Abraxane® exhibits greater clearance (21.13 *vs.* 14.76 l/h/m^2) and volume of distribution at steady state (663.8 *vs.* 433.4 l/m^2) than Taxol® (59). Despite all of these findings, a recent crossover study involving Abraxane® and Taxol® indicated that Abraxane® simply allows for higher circulating concentrations of paclitaxel (60). Since Abraxane is a physical mixture of the drug and albumin

Table II Pharmacokinetic (Clearance Rate) Data for Doxorubicin and Its Nanoparticle Formulations

Formulation	Description	Clearance Rate	Reference
Free doxorubicin		14.4 ± 5.6 ml/(min•kg)	(38)
SP1049C	Micelle, pluronic	12.6 ml/(min•kg)	(38)
NK911	Micelle, PEG and poly(aspartic acid)	6.7 ± 1.1 ml/(min•kg)	(38)
Doxil®	PEGylated liposome	0.02 ml/(min•kg)	(38)
Myocet®	nonPEGylated liposome	1.216 ml/(min•kg)[a]	(39)

[a] Data is median presented in Table 1 of ref. (41) (3.05 l/(h•m^2)), converted to units of ml/(min•kg) using values of 70 kg and 1.6 m^2

(that is, no covalent cross linking of the albumin to form the nanoparticle and no covalent linkage to the drug), a more proper way to describe this product is a nanoparticle formulation. It is extremely difficult to believe that this nanoparticle formulation remains a nanoparticle in circulation. It most certainly must dissolve and the drug partition onto innate albumin in circulation. Thus, it appears that the main advantage of this formulation is the elimination of Cremophor® EL.

OPAXIO™ (also known as PPX and CT-2103, formerly known as XYOTAX; Cell Therapeutics) is a nanoparticle formulation of paclitaxel; it is a macromolecular drug conjugate where paclitaxel is covalently linked to a biodegradable polymer, poly-L-glutamic acid. The conjugation site is through the 2′ hydroxyl of paclitaxel, a critical site for tubulin binding; consequently, the OPAXIO™ conjugate itself does not interact with β-tubulin and is biologically inactive (61,62). These conjugates, which most likely aggregate into some form of nanoparticles (although no size measurements are available in the open literature), have been shown to be resistant to hydrolysis (<14% hydrolysis upon 24 h incubation at 37°C in plasma) and are believed to be endocytosed intact and subject to enzymatic (cathepsin B-mediated) degradation of the polymeric backbone within lysosomes, releasing the active paclitaxel drug (63,64). Phase I evaluations of OPAXIO™ in patients with advanced solid malignancies have revealed MTDs of 233 mg/m^2 (dosed once every 3 weeks) (62), 177 mg/m^2 (dosed once every 2 weeks) (62), and 70 mg/m^2 (dose once weekly) (65), all of which are enhancements over observed MTDs for Taxol® given at the same schedule. While there are indications of antitumor activity in preclinical models and some early clinical studies, more recent single-agent Phase III studies have failed to show an OPAXIO™-induced significance enhancement in the duration of overall survival for patients with non-small-cell lung cancer (66–68).

Genexol®-PM is a biodegradable polymeric micellar system made with paclitaxel and a low-molecular-weight amphiphilic diblock copolymer, methoxy PEG-block-poly (D,L-lactic acid (mPEG-PLA)) (69). These micelles are 20–50 nm in size and, in Phase I studies in patients with advanced malignancies, yielded MTDs of 390 mg/m^2 (dosed once every 3 weeks) (70) and 180 mg/m^2 (dosed once weekly) (71). The most common toxicities seen were neuropathy and myalgia; acute hypersensitivity reactions (a hallmark of Taxol® and Taxotere® treatment) were not observed (72). Further, like Abraxane® and OPAXIO™, Genexol®-PM has the advantage that it does not require premedication as is given prior to administration of Taxol® and Taxotere® (such as dexamethasone, diphenhydramine, and cimetidin) (55). In Phase II studies, Genexol®-PM has demonstrated efficacy, both alone in patients with breast cancer (72) and in combination with cisplatin in patients with advanced non-small-cell lung cancer (73). Recently approved in South Korea, Genexol®-PM is the first nanoparticle of this type to receive approval anywhere for the treatment of cancer; it remains in clinical testing in the United States.

In addition to the aforementioned examples, numerous other nanoparticle delivery approaches for these and other small molecules have been developed. Camptothecin, along with its analogues and/or prodrugs, has been clinically investigated in the context of liposomes (LE-SN-38) (74) or polymer-based nanoparticles containing poly-L-glutamate (CT-2106) (75), poly(1-hydroxymethylethylene hydroxylmethyl formal) (PHF; MER-1001) (76), HPMA copolymer (MAG-CPT or PNU166148) (77,78), or a β-cyclodextrin-containing polymer (IT-101) (79). Of this group, IT-101 is unique in that it is a multifunctional nanoparticle that dramatically extends circulations times and, upon entering the target cells, facilitates a slow release of the drug (23) giving good antitumor results in many different cancer types (80). Platinum agents, such as oxaliplatin and cisplatin, have been investigated clinically within nanoparticle formulations as well, such as MBP-426 (a transferrin-targeted liposomal formulation of oxaliplatin) (81), Lipoplatin™ (a liposomal formulation of cisplatin) (82,83), ProLindac™ (AP5346; a covalent conjugate of HPMA to an oxaliplatin analogue) (84), and AP5280 (a covalent conjugate of HPMA to a cisplatin analogue) (85). A nanoparticle formulation of the small molecule mitoxantrone with polybutylcyanacrylate (PBCA), a biodegradable polymer, has completed Phase II investigation in patients with hepatocellular carcinoma and showed a statistically significant improvement in response (reduced progressive disease and increased stable disease) compared to treatment with the small molecule alone (86). Clinical development of such formulations and additional novel, small-molecule-containing nanoparticles that are currently being investigated preclinically will surely continue in the years ahead.

Direct PEGylation of small molecules to create nanoscaled therapeutics has been explored as well. While these are not strictly nanoparticles (do not contain multiple polymer strands), they do create nanoscaled therapeutics that can be larger that 10 nm. For example, camptothecin was conjugated to PEG (linear; molecular weight of 40 kD) to yield Pegamotecan, which was evaluated in Phase I (87) and Phase II (88) studies in cancer patients and retained the potency of the nonPEGylated drug. PEGylated SN-38 (EZN-2208) (89) has been studied in two Phase I trials and is currently in a Phase II investigation in patients with metastatic breast cancer (see www.clinicaltrials.gov). Nektar has advanced PEGylated camptothecin (NKTR-102) and PEGylated docetaxel (NKTR-105) into the

clinic for applications including ovarian cancer and hormone-refractory prostate cancer, respectively (see www.clinicaltrials.gov, www.nektar.com). These examples are a strong indicator that interest and development in the area of PEGylated small molecules are active and are likely to remain that way in the near future.

Finally, a class of nanoscaled molecular conjugates in the clinic is antibody-drug conjugates (ADC). These molecular conjugates use antibodies to target cell surface receptors while carrying a small number of drug molecules (~2–4 molecules is typical). Recent reviews on ADCs are available (90,91). For example, SGN-35 (Seattle Genetics) is being investigated in several Phase II clinical trials, and trastuzumab-DM1 (Genentech) is currently in a Phase III clinical trial (see www.clinicaltrials.gov).

Nanoparticle/Nanoscaled Formulations of Proteins

Proteins represent a second class of therapeutic molecules that has been widely investigated within the context of creating nanoscale properties and/or nanoparticle formulations. These molecules can be quite potent but suffer from three interrelated pharmaceutical issues: *in vitro* and *in vivo* instability, immunogenicity, and relatively short half-lives (92). Consequently, approaches that reduce or prevent these from occurring, including covalent attachment of a polymer—such as PEG—and nanoparticle systems (including polymers and/or lipids), are being explored.

Covalent attachment of PEG ("PEGylation") to proteins has been shown to prolong their circulation because of reduced kidney clearance (by increasing the size of the molecule) and/or decreased proteolysis and opsonization (a process which leads to uptake and clearance by the reticuloendothelial system (RES)) (93). Because of these properties and the fact that PEG is generally well-tolerated, PEGylation has been studied for decades. A number of PEGylated protein products have already been approved. Marketed PEGylated proteins are listed in Table III.

Oncospar® (Enzon), PEGylated L-asparaginase, became the first FDA-approved PEGylated protein for cancer in 1994 when it received approval for acute lymphoblatic leukemia (94). A Phase I study revealed that, as expected, PEGylation dramatically decreased the plasma disappearance of the protein (95). While active in children with acute lymphoblastic leukemia (96), PEGylated L-asparaginase does not eliminate the neutralizing antibody response (Oncospar® contains L-asparaginase derived from *E. coli*) (97) and partially, but not completely, reduces the occurrence of hypersensitivity reactions that accompany administration of this protein (96). PEGASYS® and PEG-INTRON®—PEGylated interferon-α2a and -α2b, respectively—have demonstrated antitumor activity in a variety of solid and hematologic malignancies, including chronic myelogenous leukemia and metastatic renal cell carcinoma (98). Because of the prolongation of plasma half-life afforded by PEGylation, these drugs can be administered much less frequently than their nonPEGylated analogues while maintaining similar safety and tolerability profiles. Similarly, Neulasta® (PEGylated granulocyte-colony stimulating factor (G-CSF)) allows a reduced schedule of administration (*vs.* non-PEGylated G-CSF) that facilitates greater treatment compliance and improved patient quality of life (99). Treatment with these PEGylated biological response modifiers (interferon-α and G-CSF) reduces some of the toxicities (e.g., neutropenia) associated with concomitant chemotherapy (100). In addition to PEGylation, others are investigating modification of proteins with polysialic acid (PSA) to achieve similar effects on pharmacokinetics and tolerability with the potential for reduced toxicity (101). For example, ErepoXen® (Lipoxen), a PSA-conjugated erythropoietin, is currently in clinical evaluation as a potential treatment of chemotherapy-induced anemia (see www.lipoxen.com).

Beyond direct conjugation of PEG (or PSA) to proteins to aid in their delivery, incorporation of native proteins within polymer- or lipid-based nanoparticles has been widely investigated. Perhaps the most widely studied material for this purpose is the biodegradable polymer, PLGA (poly(D,L-lactic-co-glycolic acid)) (102). Adjustment of the ratio of the lactic acid and glycolic acid components of the copolymer yields a predictable alteration in degradation kinetics such that the release of internalized drugs can be "tuned" from hours to months. One PLGA-containing formulation of triptorelin pamoate, Trelstar™ Depot, was approved for the treatment of prostate cancer in 2008; these particles are in the micron size range and,

Table III Approved PEGylated Proteins for Cancer

Brand name	Drug name	Parent drug	Indication	Approval year
Oncospar®	Pegaspargase	Asparaginase	Leukemia	1994
PEG-INTRON®	Peginterferon-α2b	IFN-α2B	Hepatitis C	2000
PEGASYS®	Peginterferon-α2a	IFN-α2A	Hepatitis C	2001
Neulasta®	Pegfilgrastim	Granulocyte-colony stimulating factor (GCSF)	Neutropenia	2002

Table adapted from ref. (92)

therefore, are not nanoparticles. Numerous protein-containing, PLGA-based nanoparticles for cancer are currently in pre-clinical development, and it is reasonable to believe that some will progress to initial clinical investigations in the near future. Flamel Technologies has developed a platform for protein delivery based upon self-assembly of proteins with a polymer containing glutamic acid and vitamin E to yield 20–50 nm particles; therapeutic candidates using this approach in clinical development include those containing interferon-α2b and interleukin-2 for the treatment of hepatitis C infection and renal cell carcinoma, respectively (103).

Nanoparticle/Nanoscaled Formulations of Nucleic Acids

Nucleic acids—such as plasmid DNA (pDNA), antisense oligodeoxynucleotides, and small interfering RNA (siRNA)—are, like small molecules, good candidates for inclusion within nanoparticle formulations. The rationale is different, however; unlike small molecules that can diffuse throughout tissues and into cells, nucleic acids are macromolecules that are not prone to rapid cellular uptake. Rather, nucleic acids are highly susceptible to nuclease-induced degradation in circulation; without protection within nanoparticles (and/or chemical modification to reduce nuclease susceptibility), these therapeutics will be rendered inactive shortly after administration unless they are highly chemically modified for stability.

To date, Macugen® is the only approved nanoscaled formulation of a nucleic acid. A PEGylated aptamer targeting vascular endothelial growth factor (VEGF), Macugen® (Pegaptanib) was approved in 2004 for a non-oncological application: age-related macular degeneration (104). Multiple nanoparticle formulations of siRNA for oncology are currently under clinical development. CALAA-01, a PEGylated, transferrin-targeted nanoparticle comprised of a β-cyclodextrin-containing polycation and an siRNA targeting the M2 subunit of ribonucleotide reductase, is currently being investigated clinically in patients with solid cancers in the U.S. (105,106). Very recently, this system was shown to localize in tumor cells of melanoma patients in a dose-dependent manner from systemic administrations (107). Additionally, reductions in the target mRNA and protein were observed, and most importantly, the correct mRNA cleavage fragment was identified to prove that RNAi was occurring (107). This first proof of dose-dependent nanoparticle delivery and RNAi function will likely stimulate further work in this area. Atu027 is a liposomal formulation of siRNA against a kinase (PKN3) and is currently being investigated in a Phase I clinical trial in Germany (see www.silence-therapeutics.com). ALN-VSP, a non-targeted liposomal formulation of two siRNAs targeting kinesin spindle protein (KSP) and VEGF, is in clinical development in the U.S. for the treatment of liver cancers (see www.clinicaltrials.gov and www.alnylam.com). A plasmid encoding the p53 gene is contained within a transferrin-targeted liposome to give SGT-53, a nanoparticle formulation currently in a clinical trial for patients with advanced solid tumors (see www.clinicaltrials.gov) (108). Owing to the interest in new, potent oligonucleotides and other nucleic acid therapeutics, such as siRNA, and the need to protect these macromolecules from degradation within circulation and the tumor microenvironment, it is likely that interest and research into nanoparticle formulations of nucleic acids will continue to grow.

NANOPARTICLES FOR CANCER: KEY FEATURES, CONCERNS, AND BENEFITS

In reviewing the body of literature concerning clinical investigations of nanoparticle formulations, a number of trends emerge about the role that these nanoparticles, and particular features of them, play in their efficacy and safety.

Virtually all nanoparticle formulations investigated clinically—whether they are PEGylated drug conjugates or polymer- or lipid-based vesicles—dramatically extend the circulation times of the drugs they contain. This alone is sufficient to boost tumor uptake of the drug via the EPR effect discussed above. As many of the nanoparticle formulations discussed are PEGylated to extend circulation, it should be noted that there is evidence that these PEG moieties can also provide undesirable responses. An investigation of plasmid-containing, PEGylated liposomes in mice revealed hypersensitivity and loss of disease site targeting as a result of antibody responses to the PEG component of these nanoparticles (109); accelerated blood clearance of repeated injections of PEGylated liposomes has been reported by others as well (110,111). Subsequent studies with doxorubicin- (112), siRNA- (113), and plasmid-containing (114) PEGylated liposomes suggest that the encapsulated drug itself (which may act as an adjuvant) and the magnitude of the dose of the first administration to a given animal can play significant roles in modulating this effect. These effects are not limited to PEGylated liposomes, as PEGylated polymer nanoparticles have also elicited these responses in animal models (115). It seems possible that a methoxy PEG terminus may promote this antibody response, while a hydroxyl terminus may not, as protein-PEG conjugates with hydroxyl termini exhibit less antigenicity than those with methoxy termini (116,117). This phenomenon should continue to be monitored and reported as *in vivo* evaluations of PEGylated nanoparticles progress.

The incorporation of targeting ligands has been shown to promote endocytosis of nanoparticles by tumor cells but

not to significantly alter the fraction of the administered dose that reaches tumor tissue. Of the nanoparticle formulations discussed above, only three contain a targeting ligand and are currently under clinical investigation: CALAA-01 (a siRNA-containing, polymer-based nanoparticle), MBP-426 (an oxaliplatin-containing liposome), and SGT-53 (a liposome containing a plasmid encoding the gene for p53, a tumor suppressor). All three of these target the transferrin receptor (TfR), which is known to be upregulated on many different cancer cell types, by incorporation of either the transferrin protein (CALAA-01 and MBP-426) or an anti-TfR single-chain antibody fragment (SGT-53) (118,119). Previously, FCE28069 (also called PK2)—a conjugate of HPMA copolymer, doxorubicin, and galactose as a targeting ligand for the asialoglycoprotein receptor (ASGPr)—was the first ligand-targeted nanoparticle to reach the clinic (48). ASGPr is a cell-surface receptor expressed specifically on hepatocytes in healthy subjects, and the rationale administration of this drug to patients with liver cancer is the belief that ASGPr levels remain high on cancer cells in these patients. Understandably, perhaps owing to the ASGPr expression on non-cancerous hepatocytes, biodistribution results indicated that, of the 16.9±3.9% of the total dose that accumulated in the liver region, only 3.3±5.6% was found to have localized in areas of hepatic tumor (48). Nonetheless, efficacy was observed in some of these patients with primary hepatocellular cancer, including two partial responses of 26+ and 47+ months, respectively (48). As our understanding of the roles of ligand density, linker chemistry, and affinity for the receptor in nanoparticle targeting/uptake continues to improve (31,35,91,120), we can expect more rationally designed, ligand-containing nanoparticle systems with improved potency to be developed in the years ahead. A major point of knowledge that is lacking at this point is whether or not targeting agents that, on their own, do not elicit an immune response will do so when multiple copies are displayed on the surface of nanoparticles. Clinical data will be necessary to address this issue.

Multidrug resistance (MDR) is a phenomenon which sharply limits the potency of numerous anti-cancer therapeutics (121). There are microenvironmental factors that contribute to inefficiencies of reaching cancer cells in tumors, such as high interstitial pressure, reduced microvascular pressure, and poorly vascularized regions of tumors, which act to limit drug extravasation and movement within the tumor. At the cellular level, MDR is mediated via a variety of pathways, including expression of transport proteins like P-glycoprotein which promote drug efflux out of tumor cells, protection from induced apoptosis or other forms of cell death (including development of resistance to host immune mechanisms), and genetic mutation of the drug target. By virtue of the fact that they enter cells by endocytosis, nanoparticle therapeutics have the potential to avoid MDR-mediated limitations in efficacy (in particular, by avoiding P-glycoprotein, a transmembrane drug efflux pump). Formulation within nanoparticles may also prevent drugs from being degraded within the tumor microenvironment, which is known to contain nucleases and be acidic. Indeed, in preclinical models, multiple nanoparticles have shown the ability to be effective in overcoming MDR. For example, a nanoparticle conjugate of a cyclodextrin-containing polymer and camptothecin, IT-101, demonstrated strong antitumor activity in mice in numerous tumors which have shown resistance to treatment with small molecule chemotherapeutics, such as irinotecan (80). Doxorubicin bound to HPMA copolymer yielded nanoparticles that were shown to overcome MDR in a doxorubicin-resistant human ovarian carcinoma model in mice, including higher antitumor activity and reduced toxicity compared to doxorubicin alone (122). Ligand-containing nanoparticles have also demonstrated the ability to overcome MDR by exhibiting enhanced antitumor activity compared to the free drugs they contain, including transferrin-targeted, oxaliplatin-containing liposomes (123) and folate-targeted, doxorubicin-containing polymeric micelles (124). Clinically, evidence of nanoparticle therapeutics overcoming MDR is manifested in patients who had previously failed traditional chemotherapeutic therapy but responded to treatment with a nanoparticle. For example, a Phase I study of Genexol®-PM yielded a partial response in a patient who had previously received paclitaxel and carboplatin and in another taxane-refractory patient who had received five prior chemotherapy regimens (70). While such results are not unequivocal evidence that nanoparticles overcome drug efflux pump-mediated MDR in humans, they offer indirect support that motivates continued development of nanoparticle therapeutics for cancer.

CONCLUDING REMARKS

With several nanoparticle formulations FDA-approved already, many more currently in clinical development, and even greater numbers being conceived and developed preclinically, the future of nanoparticle medicines for cancer therapy appears to be thriving. As the understanding of key nanoparticle features—such as size, surface properties, and targeting ligand function—continues to improve, this fundamental understanding will facilitate the improved rational design of nanoparticle approaches for specific applications. Additionally, as the fundamental knowledge of disease pathologies of various cancer types and subtypes

increases in parallel, it can be expected that expedited development of candidate nanoparticle therapeutics is likely to come in the years ahead.

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