Best Practices in Cancer Nanotechnology: Perspective from NCI Nanotechnology Alliance

William C. Zamboni1, Vladimir Torchilin2, Anil K. Patri4, Jeff Hrkach3, Stephen Stern4, Robert Lee6, Andre Nel7, Nicholas J. Panaro4, and Piotr Grodzinski5

Abstract

Historically, treatment of patients with cancer using chemotherapeutic agents has been associated with debilitating and systemic toxicities, poor bioavailability, and unfavorable pharmacokinetics. Nanotechnology-based drug delivery systems, on the other hand, can specifically target cancer cells while avoiding their healthy neighbors, avoid rapid clearance from the body, and be administered without toxic solvents. They hold immense potential in addressing all of these issues, which has hampered further development of chemotherapeutics. Furthermore, such drug delivery systems will lead to cancer therapeutic modalities that are not only less toxic to the patient but also significantly more efficacious. In addition to established therapeutic modes of action, nanomaterials are opening up entirely new modalities of cancer therapy, such as photodynamic and hyperthermia treatments. Furthermore, nanoparticle carriers are also capable of addressing several drug delivery problems that could not be effectively solved in the past and include overcoming formulation issues, multidrug-resistance phenomenon, and penetrating cellular barriers that may limit device accessibility to intended targets, such as the blood–brain barrier. The challenges in optimizing design of nanoparticles tailored to specific tumor indications still remain; however, it is clear that nanoscale devices carry a significant promise toward new ways of diagnosing and treating cancer. This review focuses on future prospects of using nanotechnology in cancer applications and discusses practices and methodologies used in the development and translation of nanotechnology-based therapeutics. Clin Cancer Res; 18(12); 3229–41. ©2012 AACR.

Defining Oncology Applications for Nanotechnology Constructs

Moving a prospective new drug to the clinic is an arduous task. Despite decades of experience and well-defined best practices for evaluating small molecules as drug candidates, only 1 of every 5,000 to 10,000 prospective formulations reaches U.S. Food and Drug Administration (FDA) approval, and only 5% of oncology drugs entering phase I clinical trials are approved (1).

Recently, chemists, pharmaceutical scientists, biologists, biomedical engineers, and oncologists have turned to nanotechnology in their quest for innovation and improvement of the success rate in drug development. Nanotechnology is defined by the National Nanotechnology Initiative (http://www.nano.gov) as research and technology development at the atomic, molecular, or macromolecular scale leading to the controlled creation and use of structures, devices, and systems with a length scale of approximately 100 nm (Fig. 1). Examples of nanoparticle platforms are included in Figs. 1 and 2. The multifunctional constructs based on novel nanomaterials can be delivered directly to the tumor site and eradicate cancer cells selectively. An appropriate nanoconstruct design allows for improved drug efficacy at lower doses as compared with the small-molecule drug treatment, as well as a wider therapeutic window and lower side effects. In addition to established therapeutic modes of action, nanomaterials are opening up entirely new modalities of cancer therapy, such as photodynamic and hyperthermia treatments. Furthermore, nanoparticle carriers are also capable of addressing several drug delivery problems, which could not be effectively solved in the past and include overcoming multidrug-resistance phenomenon and penetrating cellular barriers that may limit device accessibility to intended targets, such as the blood–brain barrier, among others. Polyethylene glycol (PEG)ylated-
liposomal doxorubicin (Doxil; Janssen and Caelyx; Schering-Plough), liposomal daunorubicin (DaunoXome; Galen Ltd.), liposomal cytarabine (DepoCyt; Celgene), and paclitaxel albumin-bound particles (Abraxane; Abraxis Biosciences) are the only members of this relatively new class of agents that are approved in the United States (2–7).

The challenges in optimizing design of nanoparticles tailored to specific tumor indications still remain; however, it is clear that nanoscale devices carry significant promise toward new ways of diagnosing and treating cancer. This review focuses on future prospects of using nanotechnology in cancer applications and discusses practices and methodologies used in the development and translation of nanotechnology-based therapeutics.

Design Trends for a Successful In Vivo Carrier

General concepts

The therapeutic effects of many anticancer drugs and the outcome of anticancer therapies could be significantly improved if (i) delivery of the drug occurs specifically to tumors (cancer cells) or preferably inside specific organelles in cells and (ii) reduction of drug toxic side effects is achieved. In the case of poorly soluble drug candidates, the solubility and/or bioavailability problem could also be overcome. Various pharmaceutical nanocarriers (e.g., liposomes, polymeric micelles, and polymeric nanoparticles) have been used in preparing novel dosage formulations with good bioavailability and specific drug delivery to tumors. Zeta potential, size, cationic surface charge, and solubility are factors that affect the biocompatibility of these nanocarriers (Fig. 3). These factors influence the cytotoxicity
Multifunctional nanomedicines that are being developed can combine not only different biologic properties (e.g., increased circulation time in blood, ability to accumulate in tumors via the EPR effect, and stimulus sensitivity), but also carry a combination of several drugs and diagnostic labels and/or markers (8). Targeted nanosystems that have a capability of specifically targeting cell surfaces or intracellular components and, as such, contribute to enhanced accumulation of the drug in tumor are also emerging (9).

Currently, lipid-based nanomedicines (such as liposomes, lipid-core polymeric micelles, solid lipid nanoparticles) have gained increased interest owing to their good biologic compatibility, easy control over their composition and properties, and suitability for scale-up to large-scale production at reasonable costs (10).

### Active targeting with ligands and antibodies

Nearly all cancer nanomedicines use some aspect of targeting. Most rely solely upon “passive” targeting, also known as the EPR effect, which allows for the extravasation of nanoparticles from the circulation via abnormal fenestrations in tumor vasculature.

"Active" targeting of nanomedicines provides the additional targeting mechanism of receptor-mediated binding of nanoparticles to surface receptors expressed on tumor cells or blood vessels such as αvβ3-integrins (11), folic acid (12), and prostate-specific membrane antigen, also known as PSMA (13). Research has been conducted on several targeting ligands, including antibodies, aptamers, peptides, and small molecules. Studying transferrin-targeted nanoparticles, Choi and colleagues at the California Institute for Technology showed that actively targeted nanoparticles deliver a higher payload to cancer cells than their passively targeted counterparts (14).

A successful, actively targeted nanomedicine requires a delicate balance of ligand content and surface exposure that minimizes immunologic recognition and clearance to provide sufficient nanoparticle circulation time to reach the target cells, while achieving appropriate binding affinity to the surface receptors expressed on tumor cells or blood vessels. The presence of multiple targeting ligands per nanoparticle yields a binding affinity stronger than for the ligand alone, thus enhancing the ligand-receptor-binding interaction for the nanoparticles.

Gu and colleagues, at MIT and Harvard University, showed the importance of this balance with their aptamer-functionalized, actively targeted poly(lactic-co-glycolic acid)-PEG (PLGA-PEG) nanoparticles in which 5%...
aptamer surface coverage was ideal for optimal tumor accumulation (13). These fundamental findings have been advanced by BIND Biosciences, replacing the aptamer with a small-molecule–targeting ligand, better suited for pharmaceutical development, with their PSMA-targeted docetaxel (BIND-014) in a phase I clinical study for a range of solid tumor cancers (ClinicalTrials.gov identifier NCT01300533). The efficacy of BIND-014 PSMA-targeted docetaxel nanoparticles in a PSMA-expressing human LNCaP prostate cancer xenograft mouse model is presented in Fig. 4.

Design trends for nanoparticles intended for delivery of special agents or distinct indications: highly toxic agents

Because many anticancer drugs are highly toxic, it is very desirable to develop nanomedicines that limit their toxicity at tumor sites with a minimal toxicity toward normal tissues. Ideally, drugs should be developed that possess toxicity only against diseased or mutated cells. For example, some inhibitors of poly (ADP-ribose) are highly toxic only against cell lines exhibiting certain cancer-associated mutations and do not affect cells without such mutations (15). However, the development of such drugs with mechanism-based selectivity is in its infancy. Approaches that are more developed are associated with the specific targeting of drug-loaded nanoparticles to tumor cells or providing controlled or on-demand drug delivery. The paradox of targeted delivery is that even with effective targeting, the majority of the administered dose ends up in normal tissues throughout the body, resulting in pronounced nonspecific off-target toxicity. If targeting efficiency could be increased such that as little as 3% of the injected dose reaches the tumor site, this would allow for the dramatic decrease in the total administered dose, thus sharply diminishing the toxic effect to normal tissues and still providing more drug in the tumor than after “traditional” administration. Thus, the high cytotoxicity of many anticancer drugs can be predominantly localized only in the tumor. On-demand drug delivery by mesoporous silica nanoparticles functionalized with a pH-sensitive nanovalue that only opens in acidifying intracellular endosomal compartments is an example of a novel type of nanocarrier capable of controlled drug release (16).

Pharmacologic Characterization and Properties

Pharmacologic nomenclature

The terms used to describe the pharmacokinetic disposition of carrier-mediated drugs are "encapsulated" or "conjugated" (drug within or covalently bound to the carrier), "released" (the active drug released from the carrier), and "sum total" (encapsulated or conjugated drug plus released drug; refs. 17, 18). The released drug has also been called the legacy drug, regular drug, or warhead (17–19). The released drug consists of a protein-bound or free drug. The pharmacokinetic disposition of these nanoparticle agents is dependent upon the carrier and not the parent drug until the drug is released from the carrier (20). The drug that remains encapsulated in nanoparticles or linked to a conjugate or polymer is an inactive prodrug and, thus, the drug must be released from the carrier to be active (17, 21). Whether the drug needs to be released outside of the cell in the tumor extracellular fluid (TCEF) or within the cell...
depends on the formulation of the carrier and the mechanism of release (17, 19, 22). After the drug is released from the carrier, the pharmacokinetic disposition of the drug will be the same as after administration of the noncarrier form of the drug (17, 18). In certain formulations, there may be unencapsulated drug along with the encapsulated drug; separation and quantitation of each of these species would help explain complex pharmacokinetics. Many formulations use a combination of drugs for cancer. In such cases, the encapsulation and/or conjugation efficiencies and release profiles of individual drugs may be different depending on the hydrophobicity and/or hydrophilicity of the drug and the formulation platform. For synergy, the appropriate concentration of drugs reaching the desired site of action, such as the tumor, is critical for efficacy. As such, carrying out appropriate pharmacokinetic studies to understand the individual release profiles and fine tuning the nanomaterial platform for enhanced efficacy are critically important. Thus, the pharmacology and pharmacokinetics of these agents are complex, and detailed studies must be done to evaluate the disposition of the encapsulated or conjugated form of the drug and the released active drug and metabolites in plasma, tumors, and tissues (21).

**Systemic, tissue, and tumor disposition of nanoparticles**

Nanoparticles can alter both the tissue distribution and the rate of clearance of the drug by making the drug take on the pharmacokinetic characteristics of the carrier (23–25). Pharmacokinetic parameters of the nanoparticles depend on the physiochemical characteristics of the nanoparticle, such as size, surface charge, shape, nature and density of coating, composition, stability, membrane lipid packing (in case of liposomal particles), steric stabilization, deformability, dose, and route of administration (23). The primary sites of accumulation of nanoparticles are the tumor, liver, and spleen compared with nonnanoparticle formulations (23, 24, 26–30). The development of PEGylated nanoparticles was based on the discovery that incorporation of PEG onto the surface of nanoparticles yields preparations with superior prolonged plasma exposures and tumor delivery compared with non-PEGylated nanoparticles (23, 26, 27).

The clearance of nanoparticles has been proposed to occur by uptake of the carrier by the RES, which is also called the MPS (Fig. 5; refs. 23, 27, 31). The MPS uptake of cationic or hydrophobic nanoparticles results in their rapid removal from the blood and accumulation in tissues involved in the MPS, such as the liver and spleen. Uptake by the MPS may result in irreversible sequestration of the encapsulated drug in the MPS, in which it can be degraded. In addition, the uptake of the nanoparticles by the MPS may result in acute impairment of the MPS and toxicity. The presence of the negatively charged coating on the outside of the nanoparticles does not prevent uptake by the MPS, but simply reduces the rate of uptake (Fig. 5; refs. 25–27, 32). The exact mechanism by which steric stabilization of nanoparticles decreases the rate of uptake by the MPS is unclear (23, 30, 32).

The development of effective chemotherapeutic agents for the treatment of solid tumors depends, in part, on the ability of those agents to achieve cytotoxic drug exposure within the tumor via the EPR effect (33, 34). In addition, studies suggest that the cells of the MPS may also play a role in the tumor disposition of liposomal agents and in the sensitivity of the tumors to liposomal agents (35–37). Once in the tumor, the nonligand-targeted PEGylated nanoparticles are localized in the ECF surrounding the tumor cell, but do not enter the cell (38, 39). Thus, for the nanoparticles to deliver the active form of the anticancer agent, the drug must be released from the nanoparticle into the ECF and then diffuse into the cell, or be taken up into the cell directly and then released (21). As a result, the ability of the nanoparticle to carry the anticancer agent to the tumor and release it into the ECF are equally important factors in determining the antitumor effect of nanoparticle anticancer agents. In general, the kinetics of this local release are unknown, as it is difficult to differentiate between the nanoparticle-encapsulated and released forms of the drug in solid tissue, although with the development of microdialysis, this is becoming easier (21).

**Factors affecting pharmacokinetic and pharmacodynamic variability in patients**

There is significant interpatient variability in the pharmacokinetic disposition of nanoparticle and liposomal encapsulated agents in patients (17, 37, 40–42). It seems that the pharmacokinetic variability of the carrier formulation of a drug is several-fold higher compared with the nonnanoparticle formulation of the drug (17, 41, 42). Thus, there is a need to identify factors associated with the significant pharmacokinetic variability. Most of the studies evaluating the factors that affect the pharmacokinetic variability of nanoparticle agents in patients have involved liposomal agents. The factors associated with altered pharmacokinetics of PEGylated liposomal agents are age, body composition, gender, presence of tumors in the liver, and changes in and the function of monocytes in blood (Fig. 5). There is a 2- to 3-fold lower clearance of PEGylated liposomal doxorubicin (Doxil) and PEGylated liposomal CKD-602 (S-CKD602) in patients ≥60 years of age compared with patients <60 years of age (43). Results also suggest that monocytes in blood engulf S-CKD602, which causes the release of CKD-602 from the liposome and toxicity to the monocytes, and that the effects are more prominent in patients <60 years old (44). Patients with a lean body composition have an increased plasma exposure of encapsulated drug after administration of S-CKD602 (P = 0.02; Fig. 4). It has also been reported that women have a lower clearance of encapsulated drug after administration of PEGylated liposomal agents compared with men. Population pharmacokinetic studies have reported that patients with refractory solid tumors who have primary or metastatic tumors in the liver have a higher clearance of S-CKD602 compared with patients who do not have tumors in their liver (45). In theory, the presence of tumors in the liver may induce the MPS cells in the liver and, thus, increase the clearance of nanoparticles.
The sequestering of the liposome in the liver, which would lead to an increase in systemic clearance of encapsulated drug (45).

Gabizon and colleagues reported that the clearance of Doxil decreased by approximately 25% to 50% from cycle 1 to 3 (Fig. 5; ref. 46). In addition, La-Beck and colleagues reported that this reduction in clearance from cycle 1 to cycle 3 was associated with a reduction in pre-cycle monocyte count (47). These studies suggest that there is a reduction in the clearance of liposomes over time, and, thus, dose reductions may be needed in subsequent cycles to minimize the risk of toxicity (46). Interestingly, repeat dose studies of PEGylated liposomal doxorubicin in mice and rats did not report accumulation of drug in plasma, suggesting that these preclinical models may not accurately reflect the disposition of PEGylated liposomal agents after repeated dosing (48, 49). Thus, there is also a need to develop better preclinical animal models for pharmacology and toxicology studies of liposomal and nanoparticle agents.

Translation of nanotherapeutics: perception and reality

The successful translation of anticancer nanomedicines from bench to bedside requires significant efforts that
include the development of reasonably simple, inexpensive, and scalable protocols to prepare such medicines and control over the biologic behavior and pharmacologic properties of these preparations. In general, the real use of nanomedicine as an essential part of personalized medicine will also require the development of multiple regulatory guidelines and appropriate training and education (54). The creation of an “industrial culture” of making nanomedicines, including their standardized testing and characterization, is another challenge. It seems that nanomedicines currently under development will target the most common cancers, such as breast and prostate cancer (55). The first generation of anticancer nanomedicines is based on their “passive” (EPR-mediated) delivery into tumors, and drugs (such as Doxil) and diagnostic agents (such as superparamagnetic iron oxide nanoparticle-based nuclear magnetic resonance contrast agents) are now approved and in active clinical trials, respectively (56).

Still, despite a clear understanding that effective anticancer nanomedicines should specifically recognize the disease (diseased cells), provide an imaging signal from the affected zone, and effectively deliver drugs in this zone, a clear set of uniform requirements to such preparation is still absent (57). Despite receiving significant attention (58), rational design of such systems has yet to suggest clear guidelines for clinical translation. Another important, but unresolved issue for the translation of nanoparticle-based medicines is the role of nanoparticle shape and architecture in their biologic behavior and therapeutic properties (59).

Animal models for preclinical evaluations of nanomedicines

As the translation of nanomedicines into clinical practice is still in the early stages, the concordance of preclinical and clinical data with regard to pharmacokinetics, toxicology, and efficacy are still unknown. However, the developing paradigms underlying nanoparticle distribution and biocompatibility offer perspective about what preclinical models are likely to have the greatest translational utility. One important determinant of nanoparticle distribution is the MPS, previously referred to as RES, which is responsible for particle sequestration (60). It seems that the primary MPS organ(s) of particle sequestration is species dependent, with more common laboratory animals (e.g., dog, rat, and rabbit) having particle distribution primarily to Kupffer cells of the liver and splenic macrophages, similar to man (61). In less commonly used preclinical animals (e.g., goat and pig), distribution of particles to pulmonary intravascular macrophage is primarily observed (61). For example, i.v. injection of nanoparticulate iron oxide resulted in high pulmonary intravascular uptake (>85% of dose) in sheep, calf, pig, goat, and cat, whereas uptake was primarily observed in hepatic Kupffer cells (>65% of dose) in monkey, hyrax, hamster, rabbit, guinea pig, rat, mouse, and chicken (62). This finding would support the use of the more traditional species for toxicologic and pharmacokinetic evaluation of nanomedicines owing to the similar MPS distribution profile. This correspondence in nanoparticle distribution for common laboratory species and humans is also supported by a recent allometric analysis of clearance of a PEGylated TNF-bound gold nanoparticle in rats, rabbits, and humans (63). Across these species, the TNF clearance–brain-weight product was found to scale proportional to body weight, as has been found for many macromolecular therapeutics, and would suggest common mechanisms of nanomedicine disposition in these species. However, in a recent study Caron and colleagues found that the clearance of a series of PEGylated liposomal anticancer agents did not allometrically scale from mice, rats, and dogs to patients (64). Thus, the ability to scale the pharmacokinetic disposition of nanoparticle agents across species may be nanoparticle and model specific. Caron and colleagues found that the physiologic cofactors that produced the best scaling of clearance across animal models and patients were factors associated with the MPS, such as monocyte count in blood. Thus, new methods of allometric scaling of nanoparticle agents and the use of MPS characteristics and function need to be evaluated and developed.

The issue of selective tissue distribution and accumulation of nanoparticles is a concern (65). Accumulation of nanoparticles in organs of the MPS, or selectively targeted tissues, is a common occurrence for nanomedicines. For this reason, repeat-dose tissue distribution studies may be required to identify such tissues, which may then be subjected to greater scrutiny in subsequent toxicity studies (65). Similarly, nanoparticle biopersistence is also a concern, especially for metals and nonbiodegradable polymers, and may necessitate lengthy toxicology studies to identify potential chronic toxicities.

In addition to general pharmacokinetic evaluation, assessing tumor distribution and efficacy of oncology nanomedicines in relevant preclinical cancer models is also crucial. One issue unique to nanomedicine tumor distribution in comparison with small molecules is the dependency upon long systemic circulation and vascular permeability for uptake into the interstitial space. Studies have identified nanoparticle properties associated with long circulation, such as PEGylation and small size, which correlate with increased tumor concentration maximum and total exposure (area-under-the-time-concentration curve; ref. 66). However, there have been no systematic studies evaluating the clinical relevance of vascular permeabilities found in these animal models. Studies have shown that tumor vascular pore size can be highly variable in animal xenografts, ranging from hundreds of nanometers to microns (67). As nanomedicine tumor permeability is at least partially dependent upon vascular pore size, it is important that the tumor model vasculature resemble the clinical case (68). In addition to histologic type, tumor vascular permeability has also been shown to vary depending on site of tumor implantation, with orthotopic brain tumors, for example, having lower permeability than peripherally implanted tumors (69). This finding would suggest a role for the tumor microenvironment in vascular permeability.
Preliminary studies suggest that some clinical tumors contain ultrastructural features, such as fenestrations, similar to those found in animal models (70). However, other human cancers, such as certain brain tumors, seem to be devoid of these pores (71). Because the vascular permeability of human cancers in comparison with animal models have not been thoroughly evaluated, the selection of animal models most appropriate for specific cancer types with regard to nanoparticle permeability is difficult. At this time, the same recommendations for small-molecule oncology animal model selection would apply to nanomedicines. Researchers in the National Cancer Institute (NCI) Developmental Therapeutics Program, after analyzing the clinical and preclinical data sets of several small-molecule chemotherapeutics, found that medicines that were successful in multiple xenograft models were more likely to succeed in the clinic (72). Because histologic correlations of treatment success between clinical and preclinical cancers were not observed in most cases, this would suggest that xenograft cancer models are not predictive of specific cancer activity, but rather activity in general. Together with the variability in tumor vascular architecture and nanomedicine permeability discussed above, this would suggest that evaluation of nanomedicines in multiple models is prudent. Although veterinary cancers and transgenic models may be more physiologically relevant than xenograft and/or syngeneic models, they often suffer from low availability or prolonged time in generating tumors. For this reason, xenograft and/or syngeneic models are the most commonly used models. Syngeneic models, with intact immune systems, would conceivably be an improvement over xenograft models in athymic nude or severe combined immunodeficient mice, especially for evaluation of nanomedicines, which are prone to immunologic interactions (see below). Likewise, orthotopically implanted tumors, with relevant microenvironments, may also have advantages.

Previous reviews have suggested that the current regulatory framework for assessing the safety of small molecules, biologics, and devices is considered sufficient for nanomedicines (65). Although that suggestion seems to be true at this time, the choice of the most relevant preclinical models for toxicologic evaluation has yet to be identified. Major toxicologic issues commonly observed with cancer nanomedicines are development of immunologic and hematologic complications (73). As an example, cationic dendrimers have recently been reported to induce disseminated intravascular coagulation in mice (74). Anaphylactoid reactions are a primary concern for translational development of iron oxide nanoparticle magnetic resonance contrast agents, which may be related to the polymer coatings used and has resulted in removal of many from the market (75, 76). Additionally, endotoxin contamination and associated immunologic complications, such as complement activation and pyrexia, are a common issue for nanomedicines (77). For this reason, the use of immunologically sensitive species, such as rabbits, in addition to historic models (e.g., rodents and dogs), which tend to be less sensitive, is warranted early in preclinical development to identify these possible concerns. A meta-analysis comparing study design issues of nanoparticle and small-molecule anticancer agents in preclinical models and in phase I clinical trials was done by Morgan and colleagues (78). In this study, the degree of dose escalation from starting dose to maximum tolerated dose, number of dose levels, and time to complete the phase I clinical studies were significantly greater for nanoparticle agents compared with small-molecule drugs. These data suggest that the standard animal models and cross-species–scaling paradigms employed to define the starting dose in the phase I clinical study for small-molecule agents may not be optimal for nanoparticle agents.

In addition to the suggestions about animal model selection made above, proper study design is very important for evaluation of nanomedicines. Whereas the use of intraperitoneal (i.p.) administration in place of i.v. administration may have little consequence when evaluating preclinical efficacy or toxicity of small molecules in rodents, owing to the high tissue permeability of these agents, this is not likely the case for many nanomedicines. Because of their size, nanomedicines do not freely diffuse across tissue barriers, and for this reason, i.p. administration cannot be thought of as a parenteral route equivalent to intravenous administration. Indeed, studies have shown substantial differences in distribution when comparing i.p. and i.v. routes of administration (79). Thus, it is important to use the intended clinical dosing route when evaluating nanomedicines. Another issue is the use of proper controls. There have been many examples of both nanoparticle-dependent toxicities and distribution-related shifts in drug toxicities (80, 81). Consequently, it is important to include both empty (drug-free) nanoparticles and a nonnanoparticle (small molecule) formulation of the drug as controls to identify toxicities related to the nanoparticle platform and shifts in drug-related toxicity.

**Role of Nanotech Characterization Laboratory**

The Nanotechnology Characterization Laboratory (NCL) is part of the Alliance for Nanotechnology in Cancer at NCI’s federally funded research and development center (managed by SAIC Frederick, Inc.) and is a resource for preclinical development of nanomaterial-based drug delivery and imaging agents that are beyond a proof-of-principle stage of development, with proven biologic efficacy. The facility was established to accelerate clinical translation of promising nanotechnology-derived formulations. This NCI-funded resource, available to academic investigators, industry collaborators, and government laboratories, was established by NCI in collaboration with the FDA and the National Institute of Standards and Technology. Once a project is approved through a simple submission and material transfer agreement process, a large-scale batch is obtained from the collaborator for testing at NCL facilities. The laboratory conducts preclinical assessment through an established assay cascade that includes thorough physicochemical assessment, relevant in vitro studies to investigate biocompatibility, and in vivo absorption, distribution, metabolism, excretion, toxicity, efficacy, and imaging.
studies in rodent models as appropriate. The outcome of the NCL studies is a client report that is provided to the collaborators to further their concept toward an investigational new drug (IND) submission or an investigational device exemption (82) with the FDA.

In addition to the preclinical assessment, NCL is actively engaged in standard protocol development and reference material standards development through collaborations. The NCL also plays an active role in educational and knowledge sharing efforts to advance the nanomedicine field. For further information, visit http://ncl.cancer.gov.

**Scale-up and manufacturing issues**

A critical element for successful development and commercialization of any pharmaceutical product is a scalable, reproducible manufacturing process. Besides typical considerations and challenges with scale-up and commercial manufacture, there are additional challenges for nanotechnology-based products for treating cancer. Some of the most critical aspects of the manufacture of cancer nanomedicines include sterility (most will be administered intravenously), nanoparticle size and polydispersity, encapsulation efficiency, removal of free drug, and drug-release rate. In addition, for actively targeted cancer nanomedicines that employ receptor-mediated binding of nanoparticles to tumors, the amount and appropriate surface exposure of targeting ligand must be addressed.

The most important quality parameter for an injectable product is sterility. Achieving sterility may be quite difficult because terminal heat sterilization could disrupt nanoparticle size and polydispersity, negatively affecting trafficking and biodistribution. Also, if nanoparticle size is much greater than 100 nm and polydispersity is broad, sterile filtration may not work. This leaves few options, including use of sterile raw materials and aseptic processing, which is very costly, or gamma irradiation, which many nanoparticle products also may not withstand.

The removal of unencapsulated drug from nanoparticle drug products is often difficult, yet free or nonencapsulated drug contamination will compromise both efficacy and safety. Likewise, if the manufacturing process cannot control the drug-release rate from nanoparticles, performance will be unreliable and potentially unsafe if there is a rapid or high burst of drug released from the carrier. Finally, actively targeted nanomedicines require a well-controlled process providing consistent ligand exposure on nanoparticle surfaces to fully benefit from effective nanoparticle binding to yield higher tumor-drug concentrations and/or cellular trafficking.

**Interaction with Regulatory Agencies**

**Pathway to the clinic: preinvestigational new drug-- and investigational new drug--related studies**

Although the FDA and the pharmaceutical industry have developed standards to assess drug and material biocompatibility, immune reactivity, purity, and sterility, the unique properties of nanomaterials often hamper the execution of these standardized protocols and require special consideration. Thus, although the FDA has criteria for the preclinical data that should be presented in an IND for small-molecule drugs, there is no standardized set of characterization methods for engineered nanomaterials. In consideration of the novel properties and often multicomponent nature of nanoparticle-based therapeutics, a rational characterization strategy comprises 3 elements, namely physicochemical characterization, in vitro assays, and in vivo studies. For the physicochemical characterization, reproducible synthesis and characterization assays are needed for batch-to-batch consistency, which are predictive of in vivo fate. Although the basic criteria for the chemistry, manufacture, and control section of an IND filing are the same, the methodologies and instrumentation should be appropriate to the type of nanomaterial being assessed. Additional physicochemical properties that need to be considered include particle size, size distribution, polydispersity, surface ligand density, surface area, surface charge, surface functionality, shape and confirmation, composition, purity, and stability.

Although the number of properties to assess seems substantial, once a set of characterization assays and tools have been identified that are predictive of components that cause variation in safety, efficacy, and potency profiles, the methodologies can be standardized to qualify lots for batch-to-batch consistency. Nanomaterial size and surface characteristics are critical for predictive biodistribution and toxicity. For example, most nanomaterials have PEG coating. Differences in polydispersity of the PEG used and the density of the ligands on the surface of the nanoparticles will result in significantly different toxicity profiles. For core-shell nanoparticles, impurities can come from the core and shell reagents that are used. They need to be appropriately assessed to see if residual free components are present in the drug product. If so, they need to be quantified using appropriate methods. Purity in the nanomedicine sense can be affected by the presence of residual solvents, bound and free components (such as unchelated gadolinium or free drug), and finally, homogeneity, inhomogeneity, and heterogeneity in the ligand distribution that will have significant biologic impact.

Apart from these parameters, the stability of the formulation has to be measured as a function of time, storage, temperature, pH, light (photo stability), diluent, vehicle, lyophilization, and centrifugation with appropriate methods that will be predictive of biologic effects. These characterization methodologies become much more challenging for multifunctional nanomaterials intended for drug delivery and imaging. Some of these challenges can be addressed during the synthesis, purification, and characterization steps in a multistep synthetic methodology, which would allow for purification of unreacted components and have controls in place to assure uniformity from multiple lots. For self-assembly methodologies, for example, in the case of liposomes or emulsions, the characterization for a multicomponent system is significantly more challenging. Thus, optimization of the methodology itself with appropriate controls is critical for its success in translation. In the case of targeted drug delivery systems, such as...
those with active targeting ligands that bind to overexpressed receptors on tumor, a bioassay predictive of in vivo behavior is critical to have as part of the analytical techniques to assure activity.

In vitro characterization is principally done to elucidate mechanisms of biologic interaction and toxicity and not strictly to screen for biocompatibility. Nonetheless, these in vitro studies could be very useful to identify areas requiring attention during execution of in vivo animal studies. Although a host of in vitro studies can be carried out with primary and transformed tissue culture cells to assess features such as nanoparticle uptake, subcellular localization, intracellular drug delivery, cytotoxic killing, reactive oxygen species generation, and proinflammatory effects, biocompatibility can also be evaluated ex vivo, using blood or blood cells to discern effects on coagulation, hemolysis, platelet aggregation, complement activation, and phagocytosis. Many in vitro assays can be used to elucidate potential problems that might be encountered during the in vivo assessment phase. Although all in vitro assays are not predictive of in vivo outcome, a set of relevant in vitro assays may be used to characterize potential issues with the nanoparticle formulation. Because most rodent animal models are not predictive of human immunotoxicity, the use of human blood, albeit in an in vitro setting, would point to potential issues during the clinical phase of development and can be used as a screening tool and to optimize the formulation. An important in vitro analysis is also checking sterility and endotoxin contamination. Preclinical pharmacology and toxicity studies in animals should be conducted in the most clinically relevant animal model, as discussed above.

Regulatory agencies are becoming increasingly stringent about characterization of the particle size distribution of nanotechnology-based products. Because the particle size distribution may have a significant influence on the biodistribution and biologic efficacy of the formulation, this parameter is critical to measure and track. This task is not trivial and needs to be well understood to generate meaningful and actionable data. A review of particle size analysis is also beyond the scope of this article, and the reader is referred to the literature (83).

Summary and future directions

Historically, treatment of patients with cancer using chemotherapeutic agents has been associated with debilitating and systemic toxicities, poor bioavailability, and unfavorable pharmacokinetics. Nanotechnology-based drug delivery systems that can specifically target cancer cells while avoiding their healthy neighbors, avoid rapid clearance from the body, and be administered without toxic solvents hold immense potential in addressing all of these issues. Such drug delivery systems will lead to cancer therapeutic agents that are not only less toxic to the patient, but also significantly more efficacious.

With Doxil and Abraxane (84, 85) approved by the FDA, and several new agents undergoing clinical trials, confidence is growing that nanotechnology-based therapeutics will become an important addition to currently available treatments. It is possible that, initially, these new formulations will have limited use because of their incremental improvement in performance and high cost. However, emerging research efforts indicate that nanotechnology can address uniquely serious cancer problems that do not have existing solutions. For example, effective systemic delivery of siRNA has been shown to date only using nanoparticle delivery vehicles (86). An additional host of examples includes reduction or elimination of multidrug resistance (16, 87, 88), broadening of the therapeutic index of existing drug formulations (89–91), and development of antimetastatic drugs (92–96). Thus, persistent further development of nanoparticle drug delivery technologies will continue, and, thus, these approaches will eventually become an important part of contemporary cancer care.

Disclosure of Potential Conflicts of Interest

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Authors' Contributions

Conception and design: W.C. Zamboni, P. Grodzinski, N.J. Panaro, A.K. Patri

Development of methodology: W.C. Zamboni, P. Grodzinski, N.J. Panaro

Acquisition of data: W.C. Zamboni, V. Torchlin, A.K. Patri, J. Hrkach, S. Stern, R. Lee, A. Nel, N.J. Panaro, P. Grodzinski


Writing, review, and/or revision of the manuscript: W.C. Zamboni, V. Torchlin, A.K. Patri, J. Hrkach, S. Stern, R. Lee, A. Nel, N.J. Panaro, P. Grodzinski

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