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Conscripts of the infinite armada: systemic cancer therapy using nanomaterials

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Abstract

The field of clinical nanomaterials is enlarging steadily, with more than a billion US dollars of funding allocated to research by US government agencies in the past decade. The first generation of anti-cancer agents using novel nanomaterials has successfully entered widespread use. Newer nanomaterials are garnering increasing interest as potential multifunctional therapeutic agents; these drugs are conferred novel properties, by virtue of their size and shape. The new features of these agents could potentially allow increased cancer selectivity, changes in pharmacokinetics, amplification of cytotoxic effects, and simultaneous imaging capabilities. After attachment to cancer target reactive-ligands, which interact with cell-surface antigens or receptors, these new constructs can deliver cytolytic and imaging payloads. The molecules also introduce new challenges for drug development. While nanoscale molecules are of a similar size to proteins, the paradigms for how cells, tissues and organs of the body react to the non-biological materials are not well understood, because most cellular and metabolic processes have evolved to deal with globular, enzyme degradable molecules. We discuss examples of different materials to illustrate interesting principles for development and future applications of these nanomaterial medicines with emphasis on the possible pharmacologic and safety hurdles for accomplishing therapeutic goals.

Introduction

In his 1959 lecture, “There's plenty of room at the bottom,” which is credited with initiating interest in nano technology, Richard Feynman discussed “the problem of manipulating and controlling things on a small scale,” including placing “the mechanical surgeon inside the blood vessel” to observe, report, and perform the surgery.¹ In the field of nanomedicine we are now successfully approaching solutions to this challenge (Figure 1). Many current systemic therapeutic approaches to cancer lack specificity and most of the actions of these agents cannot be controlled following injection. Some solutions may be found in the ability to create truly intelligent drugs.^{2,3}

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Anti-cancer agents that utilize novel materials to alter pharmacokinetics (PK), such as emulsified drugs and liposomal constructs, are already in development or approved by the FDA (Supplementary Tables 1 and 2) and many of these agents have been extensively reviewed. The first of a generation of complex, nanoscale, multifunctional medicines have already been designed or are in human use. These include the engineered targetable toxins that bind to cancer cells, enter their cytoplasm and deliver an enzyme capable of shutting down ribosomal synthesis,⁴ multistep targeting strategies to enhance and control the rate of drug delivery to a tumor;⁵ enzymes targeted by ligands to cells, which then act on pro-drug substrates to convert them on-site to the active agent,⁶ a nano-cell that sequentially delivers different drugs to the tumor⁷ or targetable atomic nanogenerators.⁸ Other newer multifunctional nanomaterial-based agents in development have reached human testing (Supplementary Table 1).

Here, we restrict the scope of our discussion to a relatively small segment of the novel, systemically administered nanomaterial-based cancer drugs, including polymers, dendrimers, carbon nanotubes, and various metallic and non-metallic nano-particles, in order to illustrate certain interesting features that make nanomaterial-based agents both appealing and problematic (Boxes 1 and 2). These properties, include size, shape, charge, surface patterning, polydispersity, multivalency and multicomponent structures, biocompatibility and biochemical stability.^{9–13} Due to space considerations, we will not discuss various nanoparticle or liposomal drug formulations or locally implantable devices or depots or detection systems^{14–18} nor engineered cells, viruses, aptamers or nucleotide agents,^{19–21} fusion proteins, or antibody–drug conjugates,⁴ which do display many of the above properties and have been extensively reviewed elsewhere.

The appeal of nanomaterial-based drugs in cancer is based on two factors. Firstly, the ability to control the synthesis of the agents in a manufacturing process to make multifunctional and multivalent molecules, or to alter PK, allowing changes in potency and safety, with structures arranged in distinct surface patterns exposed to the environment. In cancer cells, the diverse signaling and receptor changes that occur provide targets that might be templates for multivalent and multifunctional targeting ligands and cargo. Secondly, the sizes (up to several hundred nanometers in one of the dimensions) and shapes (hollow spheres, long rods, stars, etc.) of the molecules, which are quite distinct from traditional small molecule or protein-based drugs, yield very large surface to volume ratios or the possibility of containment for various cargo (Figure 2). For example, a single wall carbon nanotube (in which every atom is a surface carbon atom) with a volume similar to a typical large protein (for example, 100–150 kDa), will have more than 15 times the surface area available for ligand attachment or cell interactions than a large protein. Because of the non-biological sources of some materials, and the distinct sizes, valencies and shapes, the pharmacology, such as clearance and biodegradation, toxicology and possible safety of these agents in patients might be very different from current drugs; in order for some of the agents to be useful, it is likely that biocompatible coatings and ligands will be a part of their structure. Unfortunately, gaps in current knowledge about nanomaterial biology, toxicology and pharmacology, which have led to fears of possible toxic effects and environmental damage, have already put societal, ethical and political constraints on their successful application.^{22–24} In this regard, we will attempt to address the current benefits and hurdles

of these new agents and future directions for these key issues, especially those relevant to cancer therapy.

Multiple examples of nanoparticles have emerged as candidates for drugs or have reached FDA approval (Supplementary Tables 1 and 2). These include those with intrinsic medically useful properties to allow robust external imaging^{25–27} or promote tumor thermolysis^{28–31} (for example, quantum dots, silica nanoparticles, gold nanoparticles, carbon nanotubes), or which have noncovalent and covalent modifications with encapsulation of therapeutic agents (for example, liposomes, dendrimers, [co]polymers). More details can be found in recent articles.^{11,12,14,15,17,32–36}

Interaction with cells and tissues

The size, shape, and patterns of proteins presented to cells are important factors in their receptor binding and the signaling processes they control. As the physical scale of nanomaterials is of the same order as viruses, proteins, oligonucleic acids and macromolecular cellular machinery (Figure 2), one might expect they also will have distinctive and highly variable interactions with cells and tissues dependent on their size, shape, surface patterning, and charge.³⁷ These interactions will have a role in both the beneficial and potentially toxic effects of these drugs. The great appeal of nanomaterials as cancer drugs is the ability to control these properties, thus improving specificity towards malignant cells and PK properties.

Size

Early studies and modeling involving microscale and nanoscale materials focused on the effect of molecular weight and size on the biologic behaviors of micelles, liposomes, and polymers.^{17,32} Most organic nanoparticles (such as polymeric particles) have traditionally been synthesized using emulsion-based synthetic techniques that allow for encapsulation of various drugs, while inorganic nanoparticles (such as metallic particles) are often produced through controlled nucleation.³⁸ While these techniques allow particle sizes to be controlled, newer synthetic techniques and improved characterization through dynamic light scattering, electron microscopy, and atomic force microscopy, have attempted to provide more precise control over nanoscale sizes, and have allowed delineation of the biological effects of nanometer scale changes. For the same material, size changes as small as a few nanometers can lead to significant differences in cellular uptake, toxic effects, and fate within the cell.^{39–43} For example, engineered particles of sizes 40–60 nm were able to maximally bind and induce receptor-mediated endocytic processes, while smaller particles had an impaired ability to induce membrane receptor crosslinking.⁴⁰ Size also plays an important role in directing the fate of particles in the antigen presenting cells of the immune system.^{44,45} Overall, initial studies suggest that adjustable sizes could offer a means to direct different nanomaterials into different cellular pathways, maximize affinity of appended ligands, modulate uptake into malignant cells, and minimize adverse effects towards normal cells.^{40,46,47} Size also affects PK and renal clearance.^{48,49} Consistent with this, renal filtration and nonspecific uptake by the reticuloendothelial system (RES) has been shown to be dependent on particle size.^{50–55}

Shape

Similar to the study of nanomaterial size effects, the study of nanomaterial shapes began with polymers that demonstrated the significant impact of the molecular architecture of these materials on pharmacology and function.⁵⁶ As with improvement in particle size control, technology for control of shape has rapidly progressed.^{13,57–59} The advancement in synthetic techniques for non-spherical particles has shown that particle shape significantly impacts the cellular and tissue interactions of nanomaterials. While a variety of shapes (elliptical, spherical, and discoid) of model polystyrene particles can induce endocytosis, endocytosis of shapes that are non-spherical are highly dependent on the local shape at the interface of the cell with the nanomaterial or the tangential angle that the nanomaterial shape makes with the cell.^{39,60,61} These studies have predicted that when spherical particles bind or rod-like particles align perpendicular to the cellular membrane as apposed to aligning parallel to the surface, there is greater propensity for cellular uptake.^{62,63} More recently, ‘worm-like’ particles of high aspect ratio (that is, high length to width ratio) were shown to inhibit phagocytosis, and could only be engulfed when cells interacted specifically at particle ends.⁶⁴ By contrast, studies of particles with diameters >100 nm with varying aspect ratios (up to three) found that higher aspect ratios resulted in increased uptake by phagocytic cells.⁴² Interestingly, carbon nanotubes, which have unusually large aspect ratios (50–1,000:1) but are of very small diameters (1–2 nm) have highly rapid and efficient cellular uptake. Several mechanisms have been described for these observations.^{30,63,65,66} Investigators have found that filamentous micelles had circulation times about 10-fold longer than their spherical counterparts.⁶⁷ By contrast, circulation times of carbon nanotubes of a similar filamentous shape but smaller diameter (<2 nm) were demonstrated to be very short (1–3 h) with rapid renal clearance.^{68–70} In one example where novel shapes were used to mimic biological structures, discoid nanosheets were able to enhance spreading of platelet thrombi twofold as compared with their spherical counterparts.⁷¹

Surface patterning

Another component of nanoparticle structure is the geometric arrangement, or patterning, of surface groups. Varying patterning provides a valuable tool to investigate the importance of multivalency and ligand geometry in the targeting and avidity of cancer antigens.^{72,73} Thus, if targeting ligands were rationally patterned onto nanomaterials of particular shapes, there is potential for designing nanomedicines with ‘intelligent’ interactions with cancerous and normal tissues and enhancing specificity.

Charge

Biomacromolecules have different cellular and tissue interactions depending on the net charge of the chemical substituents. For example, cell-penetrating-peptides often contain a high density of cationic residues and are known to readily cross cellular membranes.^{74,75} On the tissue level, engineering the charge on ligands targeted by drugs, such as antibody fragments, results in dramatically altered biodistribution profiles.⁷⁶ Indeed, a common strategy to avoid RES uptake of nanomaterials is to introduce neutral, hydrophilic polyethylene glycol chains (pegylation) to reduce opsonization.⁷⁷ Unsurprisingly, surface

charge or zeta potential (the electrical potential at the hydro dynamic slipping plane of a particle) is also an important factor influencing the biologic behavior of nanomaterials.⁷⁸ It seems that negative or neutral surfaces could be preferable to avoid nonspecific cellular uptake.^{79,80} Furthermore, for nanomaterials that are filtered by the kidney, charge plays a major role in tubular reabsorption, with positive charge leading to increased retention in the renal cortex.⁸¹

Cancer targeting

An important goal in nanomedicine is to combine several of the special features of the materials to improve the agent's therapeutic index. The simplest way to achieve this is by combining the functions of targeting, often with specific ligands, and killing with a cytotoxic warhead, with both functions associated with the nanomaterial vehicle (Table 1 and Box 3). This might be achieved via active targeting with specific ligands (antibodies, peptides, etc.), passive targeting that takes advantage of physical interactions between the agents and the tumor microenvironment (blood flow, lymphatic drainage, etc), or more complex interactions with molecules at the tumor site that might serve to activate or release the therapeutic moiety (peptidases, pH, etc). Active (or ligand directed) targeting improves relative tumor localization; if the target is internalized it will also allow efficient cellular accumulation of the agent at specific sites, which can further increase efficacy.⁸² Effective cancer therapies that are selective for the expressed target are now routinely achieved with monoclonal antibodies specific to antigens overexpressed in cancer cells.⁸³ A large range of other targeting moieties has now become available ranging from macromolecular biologics to synthetic small molecules (Table 1).^{84–87} Most active targeting approaches in nanomedicine directly append one of these targeting ligands to a nanoparticle scaffold.

Types of tumor-associated targets

To date, hematopoietic cancer targets (Table 1) have yielded the most clinically successful targeting approaches,⁸³ in part because these malignancies are readily accessible due to their intravascular dispersion and are often more sensitive to chemotherapy and radiotherapy. By contrast, the delivery of nanoparticles to solid tumors is more challenging due to the poor penetration of the relatively large nanoscale particles through the interstitial tumor microenvironment, despite the fact many solid tumor antigens demonstrate great selectivity for cancer cells. One solution to this problem is the use of targets within the tumor vasculature including nascent vessel-associated markers or a variety of integrins.⁸⁸ Targeting the vessel has the pharmacologic advantage of immediate delivery for hematopoietic cancers, and also the benefit that the tumor vessels are derived from the host and are thus genetically stable (and thus unlikely to become drug resistant). Moreover, tumor vessels display a more uniform antigen profile thus allowing a single agent to target a large variety of tumor types. In some cases, targeting of the tumor vasculature has resulted in remodeling of the vessels to make them more-effective transporters of the therapeutic agent and of oxygen, which might in turn make the tumors more radiosensitive.^{88,89}

The accumulation of nanoparticles in tumors, termed the enhanced permeability and retention effect (EPR)^{90,91} was initially described over two decades ago, and has been

successfully applied to nanoparticles. Approved agents include re-engineered conventional therapeutics that take advantage of EPR to improve the therapeutic index⁷⁸ (Supplementary Tables 1 and 2). Some of the earliest ‘nano-medicines’, such as Abraxane[®] or Doxil[®], are novel macromolecular reformulations of paclitaxel and doxorubicin, respectively. EPR depends upon the leaky, disorganized, and tortuous nature of tumor neo-vasculature, which allows for selective retention of particles in the range of 60 to 500 nm due to their propensity to leak out of the vascular space more readily than they can permeate back into the circulation. These effects occur as a result of the deficient lymphatic drainage of the tumor environment, but the effect may not be fully reproduced in physiologic models such as in transgenic mouse tumors, orthotopic models, or ultimately in humans, especially in non-vascularized metastases.^{92–94}

Novel multifunctional agents are being developed such that passive targeting delivers the active agent near the surface of the cancer cell and a second event, such as the release of cargo for diffusion and entry into the cell, follows. While the EPR effect could improve the therapeutic index of nanoscale particles by improving relative accumulation in solid tumors, the most successful nanomedicines for cancer will likely still need to use an active targeting approach in conjunction with this approach to improve tumor-to-normal tissue ratios. Further improvements in therapeutic index can then be achieved by combining the platform with a cytotoxic agent appropriate for the particular cancer being targeted, or an oligonucleotide selective for a particular activated gene product within the cell. While each of the forms of targeting are not truly cancer specific, one could propose agents that provide sequential increases in cancer selectivity, first by EPR or vascular targeting, second by ligand directed delivery, and finally by cell pathway-selective cargo.

PK, manufacturing and regulatory issues

The unusual properties of the proposed nanomaterials, as well as their multivalency and multifunctionality pose challenges to understanding their PK because different components will have different features that affect the distribution, clearance and catabolism of nanomaterials. Typical PK studies examine the absorption, distribution, metabolism, and excretion (ADME) of a drug. These four factors and the administered dose determine the concentration of a drug at its sites of action, and thus, the intensity of its effects as a function of time. After prototype nanomaterials are modified with different cargoes, their ADME profile might change, requiring iterative testing to determine the new profiles. Multiple steps of synthesis and purification and a thorough physico-chemical characterization followed by the determination of the PK and the evaluation of tolerability (toxicity and immunogenicity) by the host will require developmental steps. For many of the new materials, both a detailed physicochemical description and PK data in animals or humans are limited and will be expected to vary significantly among different materials and the composition of the structures.

Hydrodynamic diameter and positive charge in general are inversely related to glomerular filtration rate.⁵⁴ For example, Kobayashi and Brechbiel⁵⁰ used MRI to demonstrate that varying the size or hydrophobicity of non-targeting Gd-labeled dendrimer constructs altered the route of renal and liver excretion, respectively. Similarly, non-targeted, radiolabeled

quantum dots that were modified with metal-ion chelates and a 600 Dalton polyethylene glycol (PEG) moiety were rapidly cleared from the blood and accumulated in the liver within a few minutes.^{95,96} Similarly, the particle sizes of nanoparticle “C-dots” from Cornell University have been tuned for changes in filtration, or carbon nanotube charge properties were altered to make dramatic changes in their PK.^{68,69,97–99}

Typically a size cut off of about 6 nm is seen for filtration of globular materials. Interestingly, however, much larger carbon nanotubes with high aspect ratios are still filtered.^{68,69} Particle charge also affects total body clearance, mediated often by interactions with serum proteins and cells, as has been observed with Q-dots.⁵³ Knowledge of the physicochemical parameters and their interactions can allow design of particles, for example Q-dots, with ligand numbers and sizes that permit renal clearance.¹⁰⁰

The nature of the ligands and their attachments, and types of cargo will allow interesting approaches to drug design. For example, the intact agent may accumulate at the tumor site by the EPR effect, while its antibody ligand may selectively bind the tumor cell. A large particulate platform may be trapped in the liver and excreted while the released cargo might be cleared via the kidney; each of these processes may proceed at different rates. As a consequence of this complexity, studies of the PK of the intact particle as well as the components, cargo and metabolites may be required. An advantage to these agents might be that cargoes can be designed for triggered release under various conditions, such as those that exist inside the target cell. For example, pH, redox, protease-sensitive or esterase-sensitive linkers or crosslinkers that degrade over time or that loosen under cellular conditions can trigger release of the cargo. Cytotoxicity, therefore, could be focused directly on the cancer cell. One approach that might overcome the slow diffusion and clearance of certain nanomaterial platforms is to use multi-step pre-targeting approaches in which the slower material is administered first, coupled with a unique ligand (for example, avidin) followed later by a rapidly diffusing and clearing, small-molecule cytotoxic agent coupled with a second ligand specifically able to bind to the first ligand with high affinity (for example, biotin).

Developing a nanomaterial-based drug will be a complex process (Figure 3). It is anticipated that many of the nanotechnology products that will be regulated by the FDA will span the regulatory boundaries between pharmaceuticals, medical devices, and biologics. As a consequence, these products may be regulated as ‘combination products.’ In part, the FDA attempts to ensure that drugs, drug delivery systems, medical devices, and vaccines that reach the market are safe and effective. The FDA regulates products, not technology.¹⁰¹ Therefore, this distinction will determine the stage at which the FDA regulates a process to manufacture and market novel nanomaterial-based drugs. Because many of the new agents may behave similar to ‘devices’ and have aggregate large surface areas and multivalent moieties that are exposed to the blood stream, they may be expected to have similar effects as other full-scale medically implanted devices (for example grafts, catheters or valves) such as in the promotion of hemolysis, thrombosis, and platelet aggregation, which will need to be studied.¹⁰² The multi-component features of these agents might make the approval process complicated and possibly longer than for traditional drugs, for which more is known.

Key questions for nanomaterial development will be addressed by the use of animal models, the outcomes of which may not translate well for human use, and ultimately in human subjects.^{93,103} If possible, these PK experiments can be performed in both animals and humans using classic tracer methodology¹⁰⁴ in which a pure and well-characterized product, trace-labeled with a stable moiety should not alter the overall PK profile. Imaging of the agents will most likely be employed increasingly to provide real-time, whole-body bio distributions of the tracers, thus allowing far greater detail as to the possible clearance and toxic effects to be expected, than can be achieved with simple blood measurements.

As new toxicological risks arise from the uses of novel nanomaterials and their varied compositions, new characterization tests will be necessary. In principle, characterization of the ADME of the agent will be required as usual. The specific features of nanomaterial-based agents (large size, relative to small-molecule drugs, multiple component nature, polydispersity) will, however, require novel and additional methods of study and new standards.¹⁰⁵ The large size of nanomaterials can be seen directly with techniques such as transmission electron microscopy (TEM) and atomic force microscopy (AFM), which often brings insight into their shape, polydispersity and aggregation. Intrinsic features of the materials permit direct measurement *in vitro* and *in vivo* such as by fluorescence in the Q-dots or raman spectroscopy with carbon nanotubes, or by energy dispersive X-ray. Radio-tracing (see imaging modalities section) is also helpful for assessing the properties of these agents *in vivo*. Large, multivalent particles or their appended ligands are possibly immunogenic, in part, due to their poor solubility and because they are often opsonized with antigen-presenting cells and trapped in organs such as the liver, spleen and bone marrow. On the other hand, the immune system is designed to recognize biological peptides and carbohydrates with specific receptors. Thus, nanomaterials may not be recognized by these processes because of their chemical make-up. In addition to improved analytical methodologies, there is a clear need to devise a rational paradigm to characterize nanomaterials *in vitro* and *in vivo*.¹⁰⁵ Multiple components will need to have each component analyzed separately and also assessed as a final product. One solution to the complexity of evaluating the pharmacology of the materials for regulatory review may be to use components that by themselves are already well-characterized in humans with known toxic effect profiles. Imaging the agents *in vivo*, in particular by PET, which can provide real-time concentrations of the drugs in tissues, will also aid in describing the PK and potential for toxic effects in greater detail. Early trials that employ biopsies of target tissues can also provide invaluable information on local PK, especially if used in conjunction with full body imaging.

Toxic effects of nanomaterial drugs

Understanding and predicting the toxicities of new nanomaterial agents in humans is complicated by their multicomponent nature, novel structures and polydispersity. Some of the toxic effects of these multicomponent drugs will be attributable to the individual components. However, additional toxic effects may arise because the new PK properties of the materials described above may result in unexpected interactions with target and non-target tissues as well as with the organs involved in clearance. Predictions based on micron-scale particles (such as inhaled particles, asbestos, cells, bacteria or viruses) or from sub-

nanoscale molecules (such as typical pharmaceutical drugs) or from nanoscale agents (such as proteins) may not be generalizable to the nanomaterial-based agents discussed here because of large differences in the way cells respond to these novel agents. As a consequence, there may be changes in the magnitude, quality and tissue location of toxic effects compared with the original drugs. For example, the lack of biodegradation of some components could lead to long-term inflammatory problems. Indeed, considerable discussion has ensued about the possible environmental and ecological consequences of widespread nanomaterial uses.^{106,107}

While there has been considerable debate about the possible toxicities of nanomaterial-based drugs, it should be noted that drugs used in cancer patients have typically had low therapeutic indices. Patients have been willing to tolerate significant risks of morbidity and mortality during both the clinical development of the agents and after marketing approval. Data do not suggest that nanomaterial drugs are more toxic than their components (some of which are well-known cytotoxic agents) and in many cases the agents are designed to render the cytotoxic agent less toxic by altering its delivery and clearance. Therefore, in the development of these new agents, it seems reasonable to set the threshold for tolerability and therapeutic index to no higher than that used for anti-cancer agents in current use. In addition, for the cancer applications addressed here, quantities of injected materials are likely to be quite small (mg) so the large-scale manufacturing issues that may be seen with uses of these materials in the electronics or fabrication industries are less troublesome. Furthermore, some of the components of these agents (liposomes, antibodies, chemotherapy drugs, particulate albumin, PEG, super paramagnetic iron oxide, polylactic-co-glutamic acid) have been widely studied in humans for many years and have been approved by the FDA as safe and effective.

The possible toxic effects of carbon nanotubes have been the subject of much speculation, due to their profound chemical stability, which might confer a long life *in vivo* if these agents are not cleared. Moreover the high aspect ratio (length:width) of carbon nanotubes has prompted some researchers to compare them to asbestos.⁴⁶ As expected from inhalation studies, insoluble raw carbon nanotubes can cause variable amounts of inflammation (as measured by cytokine release, reactive oxygen species elevations, complement activation, cellular morphology changes) when inhaled or added to cell cultures.^{108–115} The relevance of these findings to cancer applications is not clear; cancer drugs would be formulated as soluble, intravenous forms, which do not seem to show systemic toxicity.^{68,69,98,116–118} Importantly, while insoluble micron-scale multi-wall tubes (with sizes larger than could be ingested by macro phages and thus mechanistically similar to asbestos) caused inflammation, nanoscale material caused no inflammation, exudate or granulomas, even in insoluble form.⁴⁶ Hence, at the nanoscale, these molecules seem to behave in a similar manner to proteins, which are of a similar size. In addition, a new study has shown carbon nanotubes to be biodegradable.¹¹⁹

Other particles under investigation for therapy and imaging such as quantum dots (Q-dots), gold-, iron-, or silica-based nanoparticles and nanoshells have associated toxicities *in vitro*^{120,121} including reactive oxygen species activation, inflammation and cytotoxicity.¹²² Iron oxide particles are safe and are widely used as imaging agents and sources of iron for

anemia.^{25,26} Q-dots are notable for their metallic cores and as a consequence, exposure of the core or its dissolution could result in toxicity, especially from heavy metals such as Cd, Pb, or Se.^{123–125}

The ability to control the generation number of dendrimeric compounds (that is, the number of shells of chemical arms, each shell typically doubling the number of arms and consequently the molecular weight and sizes of the dendrimers from 1 to 20+ nm diameters) allows substantial alterations in charge and valency that can result in changes in cell surface crosslinking, aggregation or activation and thus possible increased toxic effects.^{47,79,126–129} Pegylation of the dendrimers reduces toxicity and improves the half-life of the agent in the plasma.^{79,130} These materials are also biodegradable. Although the toxicity potential for these agents is not well understood, there are already extensive efficacy and safety data with nano-formulations such as liposomes, albumin particles, and pegylated molecules, each of which has been used safely in thousands of patients after their FDA approvals. Moreover, recent data with novel nanomaterials are becoming increasingly available; this combination of information should allow design of safe drugs for the drug classes under development.^{17,32,33}

Imaging modalities

Imaging of nanomaterials can have a diagnostic role, dating back 40 years,¹³¹ or can be a method to characterize the material *in vivo* and a means to predict possible toxicity and therapeutic action.¹³² Nanomaterials are ideally suited as scaffolds to incorporate features (inherent or appended moieties) to report information and these features can result in considerable increases in signal-to-noise ratio relative to conventional imaging molecules. One advantage is that these materials could be designed to possess multimodal means to report information by having combinations of tracer features (for example, fluorescence, magnetic, and radionuclidic). Individually, these conventional imaging techniques have advantages and disadvantages: radionuclides yield high sensitivity, but suffer low spatial resolution; fluorescent labels can provide good spatial resolution *in vitro* but are difficult to quantify *in vivo* because of signal attenuation in tissue and high background noise; magnetic particles offer high resolution and good contrast but are not very sensitive. However, used in combination, these different modalities can be built into a single cancer targeting nanomaterial to simultaneously report PK, targeting, and clearance data *in vivo* and could also be used to report cellular and perhaps subcellular location of a biopsied sample *ex vivo*.

Another potential advantage of nanomaterials, based upon their size and surface area relative to smaller targeting scaffolds (such as peptides and proteins) is the potential to incorporate multiple copies of fluorophores, chromophores, Gd(III), FeO, and/or radionuclides, thus leading to amplification of signal-to-noise relative to conventional imaging agents.^{95,98,133} In one interesting example, the nanoscale dimensions and chemical environment of a carbon nanotube encapsulated imaging agent (in this case gadolinium for MRI) resulted in a dramatic improvement in sensitivity of the tracer, in principle allowing smaller tumors to be found.¹³⁴

Conclusions and future directions

A number of approaches for successful applications of nanomaterial-based cancer drugs will be possible because of their unusual characteristics. The unique pharmacological, biochemical and physicochemical properties will also require novel strategies to overcome the potential hurdles outlined. Control of size, shape, charge, and patterning of nanoscale therapeutic molecules offers potential for optimization of cellular and tissue interactions. Combining different modes of selective delivery offers the possibility of large increases in therapeutic index and the multivalent capability of the materials will provide higher potency and the possibility of simultaneous imaging and therapy, known as ‘theranostics’. There are already agents capable of multiple functions such as targeting and warhead delivery, dual warhead delivery, or imaging and therapy in trials. Some of the hurdles for future agents and ways to solve potential problems are shown in Table 2. Ultimately, we envision naturally available biomaterials combined with synthetic structures to create multifunctional agents, which are engineered to have defined diffusion, biodegradation and clearance rates, immunologic invisibility, and controlled actions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Feynman, RP.; Robbins, J.; Dyson, FJ. *The Pleasure of Finding Things Out: The Best Short Works of Richard P Feynman*. Perseus Books; Cambridge, MA: 1999.
2. Haberkant CA. Nanomedicine: destination or journey? *Nanotechnology*. 2002; 13:R9.
3. Whitesides GM. The once and future nanomachine. *Sci Am*. 2001; 285:78–83. [PubMed: 11524974]
4. Pastan I, Hassan R, Fitzgerald DJ, Kreitman RJ. Immunotoxin therapy of cancer. *Nat Rev Cancer*. 2006; 6:559–565. [PubMed: 16794638]
5. Zhang M, et al. Pretarget radiotherapy with an anti-CD25 antibody-streptavidin fusion protein was effective in therapy of leukemia/lymphoma xenografts. *Proc Natl Acad Sci USA*. 2003; 100:1891–1895. [PubMed: 12569172]
6. Xu G, McLeod HL. Strategies for enzyme/prodrug cancer therapy. *Clin Cancer Res*. 2001; 7:3314–3324. [PubMed: 11705842]
7. Sengupta S, et al. Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system. *Nature*. 2005; 436:568–572. [PubMed: 16049491]
8. McDevitt MR, et al. Tumor therapy with targeted atomic nanogenerators. *Science*. 2001; 294:1537–1540. [PubMed: 11711678]
9. National Research Council of the National Academies. *A Matter of Size: Triennial Review of the National Nanotechnology Initiative*. The National Academies Press; 2006.
10. Hartman KB, Wilson LJ, Rosenblum MG. Detecting and treating cancer with nanotechnology. *Mol Diagn Ther*. 2008; 12:1–14. [PubMed: 18288878]
11. Davis ME, Chen ZG, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat Rev Drug Discov*. 2008; 7:771–782. [PubMed: 18758474]
12. Ferrari M. Cancer nanotechnology: opportunities and challenges. *Nat Rev Cancer*. 2005; 5:161–171. [PubMed: 15738981]
13. Ferrari M. Nanogeometry: beyond drug delivery. *Nat Nanotechnol*. 2008; 3:131–132. [PubMed: 18654480]
14. Peer D, et al. Nanocarriers as an emerging platform for cancer therapy. *Nat Nanotechnol*. 2007; 2:751–760. [PubMed: 18654426]

15. Allen TM. Ligand-targeted therapeutics in anticancer therapy. *Nat Rev Cancer*. 2002; 2:750–763. [PubMed: 12360278]
16. Minko T, Pakunlu RI, Wang Y, Khandare JJ, Saad M. New generation of liposomal drugs for cancer. *Anticancer Agents Med Chem*. 2006; 6:537–552. [PubMed: 17100558]
17. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov*. 2005; 4:145–160. [PubMed: 15688077]
18. Heath JR, Phelps ME, Hood L. NanoSystems biology. *Mol Imaging Biol*. 2003; 5:312–325. [PubMed: 14630511]
19. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science*. 1990; 249:505–510. [PubMed: 2200121]
20. Simmel FC. Towards biomedical applications for nucleic acid nanodevices. *Nanomedicine*. 2007; 2:817–830. [PubMed: 18095848]
21. Cattaneo R, Miest T, Shashkova EV, Barry MA. Reprogrammed viruses as cancer therapeutics: targeted, armed and shielded. *Nat Rev Microbiol*. 2008; 6:529–540. [PubMed: 18552863]
22. Resnik DB, Tinkle SS. Ethical issues in clinical trials involving nanomedicine. *Contemp Clin Trials*. 2007; 28:433–441. [PubMed: 17166777]
23. Drexler, KE. *Engines of Creation*. Anchor Press/Doubleday; Garden City, N Y: 1986.
24. Crichton, M. *Prey*. HarperCollins; New York: 2002.
25. Stark DD, et al. Superparamagnetic iron oxide: clinical application as a contrast agent for MR imaging of the liver. *Radiology*. 1988; 168:297–301. [PubMed: 3393649]
26. Weissleder R, et al. Ultrasmall superparamagnetic iron oxide: an intravenous contrast agent for assessing lymph nodes with MR imaging. *Radiology*. 1990; 175:494–498. [PubMed: 2326475]
27. Eghtedari M, Liopo AV, Copland JA, Oraevsky AA, Motamedi M. Engineering of hetero-functional gold nanorods for the *in vivo* molecular targeting of breast cancer cells. *Nano Lett*. 2008; 9:287–291. [PubMed: 19072129]
28. Gannon CJ, et al. Carbon nanotube-enhanced thermal destruction of cancer cells in a noninvasive radiofrequency field. *Cancer*. 2007; 110:2654–2665. [PubMed: 17960610]
29. Burke A, et al. Long-term survival following a single treatment of kidney tumors with multiwalled carbon nanotubes and near-infrared radiation. *Proc Natl Acad Sci USA*. 2009; 106:12897–12902. [PubMed: 19620717]
30. Kam NW, Dai H. Carbon nanotubes as intracellular protein transporters: generality and biological functionality. *J Am Chem Soc*. 2005; 127:6021–6026. [PubMed: 15839702]
31. Georgakilas V, et al. Organic functionalization of carbon nanotubes. *J Am Chem Soc*. 2002; 124:760–761. [PubMed: 11817945]
32. Duncan R. Polymer conjugates as anticancer nanomedicines. *Nat Rev Cancer*. 2006; 6:688–701. [PubMed: 16900224]
33. Li C, Wallace S. Polymer–drug conjugates: recent development in clinical oncology. *Adv Drug Deliv Rev*. 2008; 60:886–898. [PubMed: 18374448]
34. Schluep T, et al. Preclinical efficacy of the camptothecin–polymer conjugate IT-101 in multiple cancer models. *Clin Cancer Res*. 2006; 12:1606–1614. [PubMed: 16533788]
35. Scheinberg DA, Strand M, Gansow OA. Tumor imaging with radioactive metal chelates conjugated to monoclonal antibodies. *Science*. 1982; 215:1511–1513. [PubMed: 7199757]
36. Langer R, Folkman J. Polymers for the sustained release of proteins and other macromolecules. *Nature*. 1976; 263:797–800. [PubMed: 995197]
37. Whitesides GM. The ‘right’ size in nanobiotechnology. *Nat Biotechnol*. 2003; 21:1161–1165. [PubMed: 14520400]
38. Euliss LE, DuPont JA, Gratton S, DeSimone J. Imparting size, shape, and composition control of materials for nanomedicine. *Chem Soc Rev*. 2006; 35:1095–1104. [PubMed: 17057838]
39. Chithrani BD, Ghazani AA, Chan WC. Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Lett*. 2006; 6:662–668. [PubMed: 16608261]
40. Jiang W, Kim BY, Rutka J, Chan WC. Nanoparticle-mediated cellular response is size-dependent. *Nat Nanotechnol*. 2008; 3:145–150. [PubMed: 18654486]

41. Osaki F, Kanamori T, Sando S, Sera T, Aoyama Y. A quantum dot conjugated sugar ball and its cellular uptake. On the size effects of endocytosis in the subviral region. *J Am Chem Soc.* 2004; 126:6520–6521. [PubMed: 15161257]
42. Gratton SE, et al. The effect of particle design on cellular internalization pathways. *Proc Natl Acad Sci USA.* 2008; 105:11613–11618. [PubMed: 18697944]
43. Rejman J, Oberle V, Zuhorn IS, Hoekstra D. Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. *Biochem J.* 2004; 377:159–169. [PubMed: 14505488]
44. Fifis T, et al. Size-dependent immunogenicity: therapeutic and protective properties of nano-vaccines against tumors. *J Immunol.* 2004; 173:3148–3154. [PubMed: 15322175]
45. Tran KK, Shen H. The role of phagosomal pH on the size-dependent efficiency of cross-presentation by dendritic cells. *Biomaterials.* 2009; 30:1356–1362. [PubMed: 19091401]
46. Poland CA, et al. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nat Nanotechnol.* 2008; 3:423–428. [PubMed: 18654567]
47. Malik N, et al. Dendrimers: relationship between structure and biocompatibility *in vitro*, and preliminary studies on the biodistribution of 125I-labelled polyamidoamine dendrimers *in vivo*. *J Control Release.* 2000; 65:133–148. [PubMed: 10699277]
48. Deen WM. What determines glomerular capillary permeability? *J Clin Invest.* 2004; 114:1412–1414. [PubMed: 15545991]
49. Deen WM, Lazzara MJ, Myers BD. Structural determinants of glomerular permeability. *Am J Physiol Renal Physiol.* 2001; 281:F579–F596. [PubMed: 11553505]
50. Kobayashi H, Brechbiel MW. Dendrimer-based nanosized MRI contrast agents. *Curr Pharm Biotechnol.* 2004; 5:539–549. [PubMed: 15579043]
51. Semmler-Behnke M, et al. Biodistribution of 1.4- and 18-nm gold particles in rats. *Small.* 2008; 4:2108–2111. [PubMed: 19031432]
52. De Jong WH, et al. Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. *Biomaterials.* 2008; 29:1912–1919. [PubMed: 18242692]
53. Choi HS, et al. Renal clearance of quantum dots. *Nat Biotechnol.* 2007; 25:1165–1170. [PubMed: 17891134]
54. Burns AA, et al. Fluorescent silica nanoparticles with efficient urinary excretion for nanomedicine. *Nano Lett.* 2009; 9:442–448. [PubMed: 19099455]
55. Joshi A, Vance D, Rai P, Thiyagarajan A, Kane RS. The design of polyvalent therapeutics. *Chemistry.* 2008; 14:7738–7747. [PubMed: 18553325]
56. Fox ME, Szoka FC, Fréchet JM. Soluble polymer carriers for the treatment of cancer: the importance of molecular architecture. *Acc Chem Res.* 2009; 42:1141–1151. [PubMed: 19555070]
57. Champion JA, Katare YK, Mitragotri S. Making polymeric micro- and nanoparticles of complex shapes. *Proc Natl Acad Sci USA.* 2007; 104:11901–11904. [PubMed: 17620615]
58. Gratton SE, Napier ME, Ropp PA, Tian S, DeSimone JM. Microfabricated particles for engineered drug therapies: elucidation into the mechanisms of cellular internalization of PRINT particles. *Pharm Res.* 2008; 25:2845–2852. [PubMed: 18592353]
59. Jana NR, Gearheart L, Murphy J. Seed-mediated growth approach for shape-controlled synthesis of spheroidal and rod-like gold nanoparticles using a surfactant template. *Adv Mater.* 2001; 13:1389–1393.
60. Champion JA, Mitragotri S. Role of target geometry in phagocytosis. *Proc Natl Acad Sci USA.* 2006; 103:4930–4934. [PubMed: 16549762]
61. Zhang K, Fang H, Chen Z, Taylor JS, Wooley KL. Shape effects of nanoparticles conjugated with cell-penetrating peptides (HIV Tat PTD) on CHO cell uptake. *Bioconjug Chem.* 2008; 19:1880–1887. [PubMed: 18690739]
62. Decuzzi P, Ferrari M. The receptor-mediated endocytosis of nonspherical particles. *Biophys J.* 2008; 94:3790–3797. [PubMed: 18234813]
63. Jin H, Heller DA, Sharma R, Strano MS. Size-dependent cellular uptake and expulsion of single-walled carbon nanotubes: single particle tracking and a generic uptake model for nanoparticles. *ACS Nano.* 2009; 3:149–158. [PubMed: 19206261]

64. Champion JA, Mitragotri S. Shape induced inhibition of phagocytosis of polymer particles. *Pharm Res.* 2009; 26:244–249. [PubMed: 18548338]
65. Wong Shi Kam N, Dai H. Single walled carbon nanotubes for transport and delivery of biological cargos. *Physica Status Solidi B.* 2006; 243:3561–3566.
66. Kostarelos K, et al. Cellular uptake of functionalized carbon nanotubes is independent of functional group and cell type. *Nat Nanotechnol.* 2007; 2:108–113. [PubMed: 18654229]
67. Geng Y, et al. Shape effects of filaments versus spherical particles in flow and drug delivery. *Nat Nanotechnol.* 2007; 2:249–255. [PubMed: 18654271]
68. McDevitt MR, et al. PET imaging of soluble yttrium-86-labeled carbon nanotubes in mice. *PLoS One.* 2007; 2:e907. [PubMed: 17878942]
69. Singh R, et al. Tissue biodistribution and blood clearance rates of intravenously administered carbon nanotube radiotracers. *Proc Natl Acad Sci USA.* 2006; 103:3357–3362. [PubMed: 16492781]
70. Villa C, et al. Synthesis and biodistribution of oligonucleotide-functionalized, tumor-targetable carbon nanotubes. *Nano Lett.* 2008; 8:4221–4228. [PubMed: 19367842]
71. Okamura Y, et al. Novel platelet substitutes: disk-shaped biodegradable nanosheets and their enhanced effects on platelet aggregation. *Bioconjug Chem.* 2009; 20:1958–1965. [PubMed: 19788180]
72. Joshi A, Vance D, Rai P, Thiyagarajan A, Kane RS. The design of polyvalent therapeutics. *Chemistry.* 2008; 14:7738–7747. [PubMed: 18553325]
73. Verma A, et al. Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles. *Nat Mater.* 2008; 7:588–595. [PubMed: 18500347]
74. Jones SW, et al. Characterisation of cell-penetrating peptide-mediated peptide delivery. *Br J Pharmacol.* 2005; 145:1093–1102. [PubMed: 15937518]
75. Costantini DL, Hu M, Reilly RM. Peptide motifs for insertion of radiolabeled biomolecules into cells and routing to the nucleus for cancer imaging or radiotherapeutic applications. *Cancer Biother Radiopharm.* 2008; 23:3–24. [PubMed: 18298325]
76. Kobayashi H, et al. The pharmacokinetic characteristics of glycolated humanized anti-Tac Fabs are determined by their isoelectric points. *Cancer Res.* 1999; 59:422–430. [PubMed: 9927057]
77. Faure AC, et al. Control of the *in vivo* biodistribution of hybrid nanoparticles with different poly(ethylene glycol) coatings. *Small.* 2009; 5:2565–2575. [PubMed: 19768700]
78. Alexis F, Prigden E, Molnar LK, Farokhzad OC. Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Mol Pharm.* 2008; 5:505–515. [PubMed: 18672949]
79. Duncan R, Izzo L. Dendrimer biocompatibility and toxicity. *Adv Drug Deliv Rev.* 2005; 57:2215–2237. [PubMed: 16297497]
80. Yamamoto Y, Nagasaki Y, Kato Y, Sugiyama Y, Kataoka K. Long-circulating poly(ethylene glycol)-poly(D, L-lactide) block copolymer micelles with modulated surface charge. *J Control Release.* 2001; 77:27–38. [PubMed: 11689257]
81. Gotthardt M, et al. Indication for different mechanisms of kidney uptake of radiolabeled peptides. *J Nucl Med.* 2007; 48:596–601. [PubMed: 17401097]
82. Bartlett DW, Su H, Hildebrandt IJ, Weber WA, Davis ME. Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles measured by multimodality *in vivo* imaging. *Proc Natl Acad Sci USA.* 2007; 104:15549–15554. [PubMed: 17875985]
83. Albrecht H, Denardo S. Recombinant antibodies: from the laboratory to the clinic. *Cancer Biother Radiopharm.* 2006; 21:285–304. [PubMed: 16999595]
84. Oldham RK, Dillman RO. Monoclonal antibodies in cancer therapy: 25 years of progress. *J Clin Oncol.* 2008; 26:1774–1777. [PubMed: 18398141]
85. Boyiadzis M, Foon KA. Approved monoclonal antibodies for cancer therapy. *Expert Opin Biol Ther.* 2008; 8:1151–1158. [PubMed: 18613766]
86. Castillo J, Winer E, Quesenberry P. Newer monoclonal antibodies for hematological malignancies. *Exp Hematol.* 2008; 36:755–768. [PubMed: 18565392]
87. Tassev DV, Cheung NK. Monoclonal antibody therapies for solid tumors. *Expert Opin Biol Ther.* 2009; 9:341–353. [PubMed: 19216623]

88. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science*. 2005; 307:58–62. [PubMed: 15637262]
89. Shaked Y, et al. Rapid chemotherapy-induced acute endothelial progenitor cell mobilization: implications for antiangiogenic drugs as chemosensitizing agents. *Cancer Cell*. 2008; 14:263–273. [PubMed: 18772115]
90. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumorotropic accumulation of proteins and the antitumor agent smancs. *Cancer Res*. 1986; 46:6387–6392. [PubMed: 2946403]
91. Yuan F, et al. vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. *Cancer Res*. 1995; 55:3752–3756. [PubMed: 7641188]
92. Boven E, et al. Phase II preclinical drug screening in human tumor xenografts: a first European multicenter collaborative study. *Cancer Res*. 1992; 52:5940–5947. [PubMed: 1394220]
93. Voskoglou-Nomikos T, Pater JL, Seymour L. Clinical predictive value of the *in vitro* cell line, human xenograft, and mouse allograft preclinical cancer models. *Clin Cancer Res*. 2003; 9:4227–4239. [PubMed: 14519650]
94. Minko T, Kopeckova P, Pozharov V, Jensen KD, Kopecek J. The influence of cytotoxicity of macromolecules and of VEGF gene modulated vascular permeability on the enhanced permeability and retention effect in resistant solid tumors. *Pharm Res*. 2000; 17:505–514. [PubMed: 10888300]
95. Michalet X, et al. Quantum dots for live cells, *in vivo* imaging, and diagnostics. *Science*. 2005; 307:538–544. [PubMed: 15681376]
96. Gao X, Cui Y, Levenson R, Chung L, Nie S. *In vivo* cancer targeting and imaging with semiconductor quantum dots. *Nat Biotechnol*. 2004; 22:969–976. [PubMed: 15258594]
97. Liu Z, et al. Drug delivery with carbon nanotubes for *in vivo* cancer treatment. *Cancer Res*. 2008; 68:6652–6660. [PubMed: 18701489]
98. McDevitt MR, et al. Tumor targeting with antibody-functionalized, radiolabeled carbon nanotubes. *J Nucl Med*. 2007; 48:1180–1189. [PubMed: 17607040]
99. Bhirde AA, et al. Targeted killing of cancer cells *in vivo* and *in vitro* with EGF-directed carbon nanotube-based drug delivery. *ACS Nano*. 2009; 3:307–316. [PubMed: 19236065]
100. Choi HS, et al. Design considerations for tumour-targeted nanoparticles. *Nat Nanotechnol*. 2009; 5:42–47. [PubMed: 19893516]
101. US Department of Health and Human Services. FDA Regulation of Nanotechnology Products. 2009. [online], <http://www.fda.gov/ScienceResearch/SpecialTopics/Nanotechnology/default.htm>
102. Dobrovolskaia MA, Aggarwal P, Hall JB, McNeil SE. Preclinical studies to understand nanoparticle interaction with the immune system and its potential effects on nanoparticle biodistribution. *Mol Pharm*. 2008; 5:487–495. [PubMed: 18510338]
103. Eckelman W, Kilbourn MR, Joyal JL, Labiris R, valliant JF. Justifying the number of animals for each experiment. *Nucl Med Biol*. 2007; 34:229–232. [PubMed: 17383571]
104. Wilson, BJ., editor. *The Radiochemical Manual*. 2nd. The Radiochemical Centre; Amersham: 1966.
105. Hall JB, Dobrovolskaia MA, Patri AK, McNeil SE. Characterization of nanoparticles for therapeutics. *Nanomedicine*. 2007; 2:789–803. [PubMed: 18095846]
106. Boxall AB, Tiede K, Chaudhry Q. Engineered nanomaterials in soils and water: how do they behave and could they pose a risk to human health? *Nanomedicine*. 2007; 2:919–927. [PubMed: 18095854]
107. Nel A, Xia T, Mädler L, Li N. Toxic potential of materials at the nanolevel. *Science*. 2006; 311:622–627. [PubMed: 16456071]
108. De Jong WH, Borm PJ. Drug delivery and nanoparticles: applications and hazards. *Int J Nanomedicine*. 2008; 3:133–149. [PubMed: 18686775]
109. Elgrabli D, et al. Induction of apoptosis and absence of inflammation in rat lung after intratracheal instillation of multiwalled carbon nanotubes. *Toxicology*. 2008; 253:131–136. [PubMed: 18834917]

110. Hamad I, et al. Complement activation by pegylated single-walled carbon nanotubes is independent of C1q and alternative pathway turnover. *Mol Immunol*. 2008; 45:3797–3803. [PubMed: 18602161]
111. Kagan VE, Bayir H, Shvedova AA. Nanomedicine and nanotoxicology: two sides of the same coin. *Nanomedicine*. 2005; 1:313–316. [PubMed: 17292104]
112. Magrez A, et al. Cellular toxicity of carbon-based nanomaterials. *Nano Lett*. 2006; 6:1121–1125. [PubMed: 16771565]
113. Simon-Deckers A, et al. *In vitro* investigation of oxide nanoparticle and carbon nanotube toxicity and intracellular accumulation in A549 human pneumocytes. *Toxicology*. 2008; 253:137–146. [PubMed: 18835419]
114. Zhang LW, Zeng L, Barron AR, Monteiro-Riviere NA. Biological interactions of functionalized single-wall carbon nanotubes in human epidermal keratinocytes. *Int J Toxicol*. 2007; 26:103–113. [PubMed: 17454250]
115. Lam CW, James JT, McCluskey R, Arepalli S, Hunter RL. A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. *Crit Rev Toxicol*. 2006; 36:189–217. [PubMed: 16686422]
116. Dumortier H, et al. Functionalized carbon nanotubes are non-cytotoxic and preserve the functionality of primary immune cells. *Nano Lett*. 2006; 6:1522–1528. [PubMed: 16834443]
117. Lacerda L, et al. Tissue histology and physiology following intravenous administration of different types of functionalized multiwalled carbon nanotubes. *Nanomedicine*. 2008; 3:149–161. [PubMed: 18373422]
118. Schipper ML, et al. A pilot toxicology study of single-walled carbon nanotubes in a small sample of mice. *Nat Nanotechnol*. 2008; 3:216–221. [PubMed: 18654506]
119. Allen BL, et al. Biodegradation of single-walled carbon nanotubes through enzymatic catalysis. *Nano Lett*. 2008; 8:3899–3903. [PubMed: 18954125]
120. Costigan, S. The toxicology of nanoparticles used in healthcare products. 2006. http://www.mhra.gov.uk/home/idcplg?IdcService=SS_GET_PAGE&nodeId=996
121. De Jong WH, Borm PJ. Drug delivery and nanoparticles: applications and hazards. *Int J Nanomedicine*. 2008; 3:133–149. [PubMed: 18686775]
122. Chang JS, Chang KL, Hwang DF, Kong ZL. *In vitro* cytotoxicity of silica nanoparticles at high concentrations strongly depends on the metabolic activity type of the cell line. *Environ Sci Technol*. 2007; 41:2064–2068. [PubMed: 17410806]
123. Hardman R. A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. *Environ Health Perspect*. 2006; 114:165–172. [PubMed: 16451849]
124. Hoshino A, et al. Physicochemical properties and cellular toxicity of nanocrystal quantum dots depend on their surface modification. *Nano Lett*. 2004; 4:2163–2169.
125. Lovric J, et al. Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots. *J Mol Med*. 2005; 83:377–385. [PubMed: 15688234]
126. Chen HT, Neerman MF, Parrish AR, Simanek EE. Cytotoxicity, hemolysis, and acute *in vivo* toxicity of dendrimers based on melamine, candidate vehicles for drug delivery. *J Am Chem Soc*. 2004; 126:10044–10048. [PubMed: 15303879]
127. Heiden TC, Dengler E, Kao WJ, Heideman W, Peterson RE. Developmental toxicity of low generation PAMAM dendrimers in zebrafish. *Toxicol Appl Pharmacol*. 2007; 225:70–79. [PubMed: 17764713]
128. Roberts JC, Bhalgat MK, Zera RT. Preliminary biological evaluation of polyamidoamine (PAMAM) Starburst dendrimers. *J Biomed Mater Res*. 1996; 30:53–65. [PubMed: 8788106]
129. Plank C, Mechtler K, Szoka FC Jr, Wagner E. Activation of the complement system by synthetic DNA complexes: a potential barrier for intravenous gene delivery. *Hum Gene Ther*. 1996; 7:1437–1446. [PubMed: 8844203]
130. Kaminskas LM, et al. The impact of molecular weight and PEG chain length on the systemic pharmacokinetics of pegylated poly l-lysine dendrimers. *Mol Pharm*. 2008; 5:449–463. [PubMed: 18393438]
131. Larson SM, Nelp WB. Radiopharmacology of a simplified technetium-99m-colloid preparation for photoscanning. *J Nucl Med*. 1966; 7:817–826. [PubMed: 5955361]

132. Escorcia FE, McDevitt MR, Villa CH, Scheinberg DA. Targeted nanomaterials for radiotherapy. *Nanomedicine*. 2007; 2:805–815. [PubMed: 18095847]
133. Cai W, Chen X. Multimodality molecular imaging of tumor angiogenesis. *J Nucl Med*. 2008; 49(Suppl. 2):113S–128S. [PubMed: 18523069]
134. Sitharaman B, et al. Superparamagnetic gadonanotubes are high-performance MRI contrast agents. *Chem Commun*. 2005; 21:3915–3917.

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Box 1**Definition of nanomaterial cancer drugs**

- Size: 1–100 nm in one dimension
- Agents are composed of synthetic materials, at least in part
- The size and shape confer unique properties
- The agent is multifunctional
- The agent is multimeric

Box 2**Rationale for developing nanomaterial medicines**

- Multifunctionality
- Increased potency and multivalency
- Increased selectivity for targets
- ‘Theranostic’ potential (imaging and therapy together)
- Altered pharmacokinetics
- Controlled syntheses
- Controlled agent release and kinetics
- Novel properties and interactions
- Lack of immunogenicity
- Enhanced physical stability

Box 3**Examples of cancer-associated targets****Hematologic antigens**

- B cells: CD19, CD20, CD21, CD22, CD23
- T cells: CD4, CD25, CD30
- Myeloid precursors: CD33, CD66

Solid tumor antigens

- Colon: Integrins, A33 glycoprotein, Tag72, epithelial cell adhesion molecule (EpCAM)
- Breast: HER2/neu, Lewis-Y, carcinoembryonic antigen (CEA)
- Prostate: Prostate-specific membrane antigen (PSMA), prostate stem-cell antigen (PSCA)
- Other: GD2 ganglioside (glioblastoma), MUC1 mucin-like glycoprotein (pancreatic cancer), folate receptor (FR), epidermal growth factor receptor (EGFR), transferrin receptor (TFR)

Vascular antigens

- vascular endothelial cadherin (VE-cadherin), vascularendothelial growth factor receptor (VEGFR), integrins(such as alpha-V-beta-3)

Key points

- Therapeutic uses of novel materials have become widespread; many newer nanoparticles have emerged as candidates for drugs, each with distinctive chemical and biological compositions, and diverse *in vivo* behaviors
- Newer nanomaterials are garnering increasing interest as potential multifunctional therapeutic agents, which by virtue of their size, geometric patterning and shape are conferred novel properties
- The synthesis of nanomaterials allows multifunctional and multivalent molecules to be generated, which may enhance potency, therapeutic index or selectivity
- The various sizes and shapes of nanomaterials yield very large surface to volume ratios or the possibility of containment for various cargo
- The accumulation of nanoparticles in tumors, termed the enhanced permeability and retention effect was initially described over two decades ago, and has been successfully applied to nanoparticles
- The unusual properties of nanomaterials pose challenges to understanding their pharmacokinetics as different components will have different features that affect their distributions, clearance and catabolism

Review criteria

Articles included in the Review were chosen after an exhaustive search on the PubMed, Google Scholar and Science Direct databases for full-text papers published in English without a time restriction. The search terms included each of the relevant nanomaterials by name, cancer antigens and therapeutic drugs and modalities, and more general terms for the fields under study. Recent reviews and papers, many of which are cited in the text, were consulted for additional references.

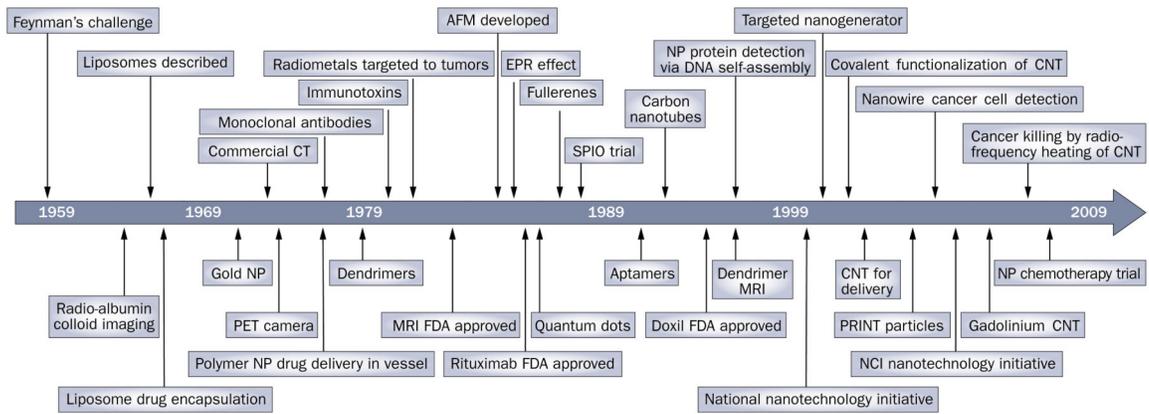


Figure 1.

50 years of nanomedicine development for cancer. The timeline is not to scale.

Abbreviations: AFM, atomic force microscope; CNT, carbon nanotubes; EPR, enhanced permeability and retention; NCI, National Cancer Institute; NP, nanoparticle; PRINT, particle replication in nonwetting templates; SPIO, superparamagnetic iron oxide.

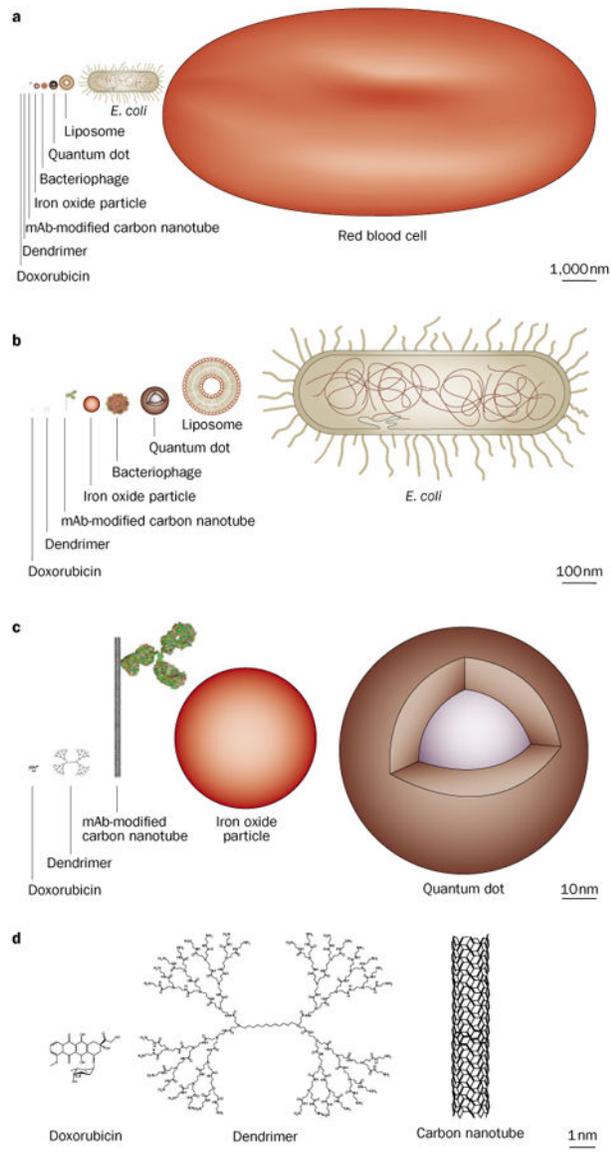


Figure 2. Representation of nanomaterials with comparison to biologics, drawn to scale.

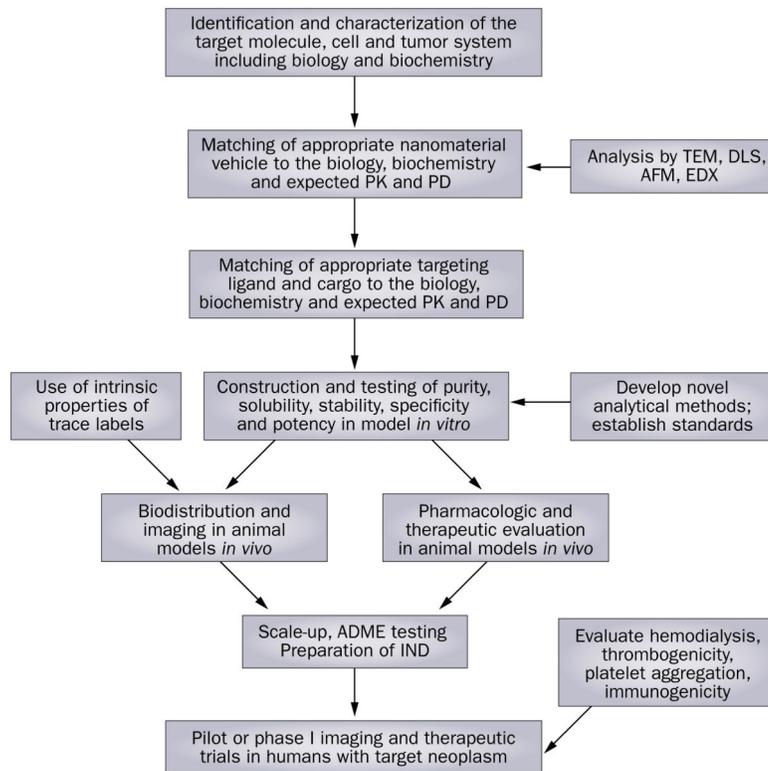


Figure 3.

Some proposed steps in the development of a nanomaterial anti-cancer agent. Abbreviations: ADME, absorption, distribution, metabolism and excretion; AFM, atomic force microscope; DLS, dynamic light scattering; EDX, energy dispersive X-ray; IND, investigational new drug application; TEM, transmission electron microscopy.

Table 1
Ligands for active cancer targeting

Type	Mw (kDa)	Diameter (nm)	Features
<i>Monoclonal antibodies</i>			
Whole antibodies	150	15–20	High affinity, divalent, many clinically approved examples, contains biologically active constant (Fc) region, long circulation
<i>Engineered fragments (monovalent)</i>			
ScFv	25	3–5	Lowered affinity, rapid clearance from circulation, renal retention, reduced stability, reduced immunogenicity
Fab'	50	5–10	Can be produced genetically or enzymatically by cleavage of monoclonal antibodies
Nanobody	15	2–3	Smallest antigen-binding fragment, single domain, can bind cryptic epitopes
<i>Engineered fragments (divalent)</i>			
F(ab') ₂	100	10–15	Improved affinity, can be engineered to a variety of sizes and arrangements of protein domains
Diabodies	50–80	5–10	Mono-specific or bi-specific dimer of ScFv
Minibodies	80	10	Can be produced genetically
<i>Aptamers</i>			
RNA	10–30	2–3	Rapid clearance, automated chemical synthesis, susceptible to nucleases without chemical modification
DNA	10–30	2–3	Rapid clearance, automated chemical synthesis, susceptible to nucleases without chemical modification
<i>Receptor ligands</i>			
Peptides	0.5–10	variable	Facile synthesis and modification, diverse libraries and screening technologies, susceptible to peptidases, renal retention
Whole proteins	30–150	variable	Produced using recombinant DNA technologies, can be biologically active, susceptible to proteases
Small molecules	0.1–1.0	0.5–2.0	Chemical synthesis, simple modification and coupling chemistries, can be biologically active, highly variable affinities

Table 2
Specifications and solutions for therapeutic nano-devices for cancer

Specification or hurdle	Possible solutions
Not detected by human immune system	Use of human-derived antibodies, proteins, receptors, ligands or enzymes for functionalization of platforms. Study of the antigen presentation characteristics of the new materials to understand how to reduce recognition of haptens.
Resistance to proteases, nucleases and esterases	Use of aptamers with altered DNA backbone chemistry, peptide ligands with unnatural amino acids, pegylation. Take advantage of the processes to engineer desired clearance and delivery of cargo.
Biocompatibility, catabolism and clearance issues	Engineered sizes, charge, and shape compatible with desired clearance; biofunctionalization to be soluble and biocompatible; use of structures that degrade or release cargo within cells. Preference for degradable components or linkers with small renally cleared agents; controlled degradation after use of agent by enzymatic cleavage.
Specificity for or retention near tumors and cancer cells	Platforms contain peptide or small molecule ligands, aptamers or antibodies/fragments; agent takes advantage of enhanced permeability and retention effect to increase selective delivery; multistep pre-targeting approaches are used to increase therapeutic index.
Potency	Surface multivalency to increase affinity and cargo delivery; contained spaces for multiple copies of cargo; use of enzymes to multiply effects, such as prodrug conversion at the tumor site.
Reporting and accountability	Use of theranostics: radiotraced; MRI active agents, optical or infra-red tracers; caged reporters that signal upon binding, activation or delivery to or into the cell; use of genetic reporters.
Controllability	Control by infused second ligands, by focused radio-frequency or magnetic fields; use of intrinsic sensors or tumor-activatable agents; use of artificial cells or viruses; suicide genes; controlled design of degradation pathways.
Intrinsic dynamics and information content	Use of addressable DNA sequences, proteins or sugar polymers; design multivalent ligands to encode avidity for the target. Use of built-in mechanical or enzymatic machinery or pirating such activities from the target cell.
Safety and environmental concerns	Science should be built on known agents and components already in use <i>in vivo</i> , then developed individually after pharmacologic study.
Political and social issues	Enhanced efforts to educate the public; careful toxicologic analyses. Avoid overstating the promise.