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SPECIAL FOCUS: GOVERNANCE OF NANOBIOTECHNOLOGY

The bench scientist's perspective on the unique considerations in nanoparticle regulation

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Abstract The emergence and use of nanotechnologies in commercially available products, including nanotherapeutics, have necessitated the response of regulatory agencies to ensure that these products are safely employed. While bench scientists are at the forefront of nanoparticle development and design, many are unaware of the regulatory requirements necessary to transform their laboratory discoveries into marketable products. As bench scientists, we performed a "thought experiment" using multifunctional mesoporous silica nanoparticles synthesized in our lab, which we considered as a combination product, to try to understand the steps necessary for pre-clinical approval from the Food and Drug Administration. This thought experiment illuminated challenges associated with nanoparticle risk assessment and regulation.

Keywords Regulation · Silica · Nanotherapeutic · Nanotoxicology · Immunogenicity · Nanotechnology · Governance

Introduction

In a 2006 Senate Commerce Committee meeting, David Rejeski, the then-director of the Woodrow Wilson Center's Project on Emerging Nanotechnologies, testified that "the ability to reap the long-term benefits of nanotechnology—in areas from medicine to energy and food production—will depend heavily on how we manage the introduction of the first generation of nanotechnology products" (U.S. Congress 2006). This statement was made nearly 5 years ago, and the nanoscience community is still struggling with how to manage nanotechnology. As bench scientists, we find ourselves on the forefront of nanotechnology research, where we create new nanoscale materials and imagine their potential uses. Yet we, like most bench scientists, remain relatively naive and conflicted about oversight, as it can both advance and encumber our work. On the one hand, oversight helps identify critical areas in need of study and advancement and focuses scientific attention on areas of regulatory concern. There is also evidence that oversight encourages technology transfer by protecting intellectual property rights and creating stable economies, which, in turn, improve consumer confidence and increase technology investments. Oversight can also provide ethical boundaries for research and development. On the other hand, regulations may slow down or otherwise impede scientific and technologic advancement, especially exploratory scientific investigations that demonstrate a high degree of uncertainty. Many bench scientists see uncertainty about the regulatory process as an impediment to technology transfer. Many academic scientists would rather abandon a good idea with commercial potential than go through the hurdles necessary to get it

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past regulatory approval. Creating regulations that are specific enough to be effective, yet flexible enough to accommodate exploratory scientific research, has been a challenge for many areas of emerging technologies, such as gene therapy (Seymour 2006), leading many bench scientists to conclude that the same will be true for nanotechnology.

There are some aspects of nanotechnology research that could benefit from regulatory guidance. For example, oversight could enable the field of nanotoxicology, which focuses on the toxicological implications of nanomaterials and nanotechnology use, to achieve one of its major goals: creating nanoparticle design rules that allow for prediction and control of nanoparticle risks. This goal is currently hampered by a range of methodological challenges to nanoparticle toxicity assessment (Marquis et al. 2009a; Maurer-Jones et al. 2009; Teeguarden et al. 2007; Jones and Grainger 2009). Reaching this goal is further complicated by the ongoing debate over an appropriate definition of "nanoparticle," which impedes the ability to delineate what should and should not be included within the regulatory process. This issue of definition seems deceptively straightforward. For many years, common practice has been simply to use the definition originally put forward by the National Nanotechnology Initiative (NNI) of nanoparticles as those particles with "dimensions between approximately 1 and 100 nm, where unique phenomena enable novel applications." Many scientists now consider this 100 nm cutoff too limiting and arbitrary (Maynard 2009). There are emergent nanoscale phenomena that occur when individual features are larger than 100 nm or the particles are in assemblies. There are also many materials and many properties that are no different below the 100 nm threshold than above it. The absence of a concrete definition for nanomaterials is implicitly linked with the basic question of whether nanoparticles should be regulated differently than their bulk counterparts.

Another challenge of meeting the "design rule" goal lies in the fact that the benchmarks for nanoparticle safety are not well-defined. Most of the in vitro nanoparticle toxicity studies presented in the literature are live/dead assays that do not address the physiological impairments not predicted by cell death alone. Recently, more work has focused on assays that examine non-lethal changes in cell behavior (Marquis et al. 2008, 2009b; Maurer-Jones et al. 2010). While acknowledging a fuller array of physiological impairments is an important step forward, determining critical nanoparticle design criteria in the absence of nanoparticle-specific safety benchmarks remains problematic. Likewise, there are limitations with in vivo measurements where safety benchmarks would be beneficial. Figure 1 shows a flow diagram representing one way to design experiments to assess in vitro nanotoxicity. After choosing the relevant nanoparticle and a relevant model cell, the first step examines cell viability following some relevant exposure period. If the cells do not survive, the nanoparticles are suspected to be toxic to the chosen cell model at the exposure levels tested, and the assessment cycle need not continue without further investigation into the cause of decreased viability. If the cells are still viable following exposure, cell function should be considered to assess whether the cells are able to perform critical physiological tasks under the exposure range. This is a point where regulatory guidance can direct researchers toward the cell functions deemed critical for determining preclinical safety. If cell function is intact following nanoparticle exposure, then the nanoparticles can be considered non-toxic toward the model cell at the given exposure. If the in vitro tests show signs of toxicity, then the nanoparticles should be redesigned to combat the identified toxicity mechanism, including the often enlightening considerations of nanoparticle uptake by, association with, or proximity to cells. Then, the assessment cycle begins again. After a nanoparticle is designed to be non-toxic toward a model cell, different model systems can be used to explore the toxicity of the nanoparticles to cells from a wide variety of tissue types in order to reach generalizable conclusions that illuminate "design rules" for nanoparticle safety. While this approach will likely yield useful insight about nanoparticle/cell interactions, we recognize the limitations of examining a single cell function in a particular cell type with a narrow range of nanoparticle exposure conditions. From this cyclical approach to nanotoxicology, we see how regulatory oversight could help nanotoxicological scientists at the pre-clinical stage by creating common definitions and experimental protocols that allow for scientists to communicate and compare results from different labs. This standardization may take the form of a broader understanding of "Good Practice" quality guidelines (GxPs) and guidelines for their implementation.

Although most nanoscience researchers would like their discoveries to translate into viable products, many remain unaware of the regulatory requirements for this translation to occur. Since basic research often involves collaboration across disciplines and even across national borders, these collaborations are increasingly taxed by a lack of uniform regulatory guidelines and requirements that apply regardless of one's academic discipline or the location of one's research laboratory. The multiplicity of regulatory standards tends to hinder rather than encourage such collaborative efforts (see, for instance, an FDA document comparing GxP standards within the FDA, EPA, and OECD http://www.fda.gov/ICECI/EnforcementActions/BioresearchMonitoring/ucm135197.htm). The lack of awareness of oversight within the nanoscience community is slowly changing; there are now special issues of journals and discussion sections at conferences dedicated to the question of regulation and its implications for bench science. The field of nanotoxicology is beyond its infancy, with new conferences and funding opportunities on the topic regularly appearing. Yet, it is still the minority of scientists who think about regulation and its implications for science. As an example, for pre-clinical studies to have relevance during the regulatory approval process, the experiments need to have been conducted under GxP conditions (Hill 2002). The number of bench scientists who understand GxP conditions is rather small. As an unfortunate consequence, most of the work done by bench scientists to elucidate the safety and efficacy of these nanomaterials is inadmissible as pre-clinical evidence. This results in wasted resources and lost opportunities to translate laboratory discoveries to new products.

As practicing bench scientists in the field of nanoscience, the realization of the requirements necessary to move a product through the approval process, particularly in the pre-clinical phase, has been eye-opening. Accordingly, we began a thought experiment to navigate pre-clinical stages of the regulatory process for a promising nanoparticle made in our laboratory. During the process, we found the guidelines to be nebulous for this nanotechnology product, with clear holes where nanoparticle-specific behavior will likely deviate from traditional products. Herein, we detail this thought experiment as an intellectual foray into the hypothetical (Brendel 2004). From Einstein following a beam of light to Galileo's falling bodies to Schrödinger's cat, thought experiments have played an important role in the history of science and technology (Brendel 2004). These exercises do not require any new experimental data; they only frame what we already know in a new way to illuminate areas for further scientific work (Norton 1996). In this case, we recognize our bias in coming from an analytical and materials chemistry background, one that errs on the side of complete nanoparticle characterization. We also recognize our limitations in having no experience with the FDA regulatory process—a fact that should mimic well the perspective of the general bench scientist—and keep our focus on pre-clinical in vitro considerations for simplicity, knowing that in vivo work will certainly present further challenges.

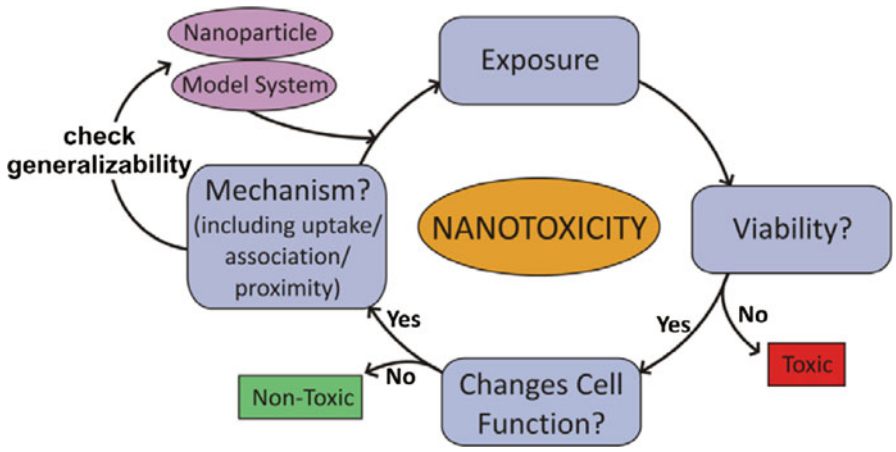

Fig. 1 An example of a bench scientist's approach to studying nanoparticle toxicity

Thought experiment nanoparticle

For this thought experiment, we focused on multifunctional mesoporous silica nanoparticles (MMSNs), a nanoparticle with great potential for translational use as a theranostic device. We considered the process to perform pre-clinical screening as an FDA combination product. For this thought experiment, the FDA classification of combination product was chosen based on the fact that the MMSNs consist of an imaging, a drug, and a delivery agent.

Multifunctional mesoporous silica nanoparticles are known as multifunctional because they are capable of delivering drugs to a targeted site and can provide magnetic and fluorescent contrast for imaging during treatment. The synthetic procedure to produce the MMSNs (Fig. 2) begins with fabrication of 11-nm-diameter superparamagnetic Fe_3O_4 particles capped with oleic acid. These Fe_3O_4 nanoparticles are then transferred from organic to aqueous solution using a cetyl trimethylammonium bromide (CTAB)/poly(vinylpyrrolidone) (PVP) co-surfactant system. Under appropriate conditions, the CTAB forms a micelle structure (around the Fe_3O_4 nanoparticles) and acts as a structure-directing agent during the silica condensation reaction, creating a mesoporous silica nanoparticle where the pore size is determined by the CTAB chain length. The silica condensation is accomplished from a mixture of tetraethylorthosilicate and a silanated fluorophore to achieve a highly fluorescent mesoporous structure with embedded Fe_3O_4 centers. It is straightforward to vary the overall diameter of the MMSNs by changing precursor ratios, as well as the surface chemistry using silane modification. Size control is critical to ensuring particle stability and long circulation times following intravenous injection.

For this thought experiment, we focus specifically on MMSNs with 42-nm-diameter that we have manufactured in our lab. The highly porous nanoparticles have very large total surface areas, about 20 times that of their solid counterpart and, thus, have the capacity to carry a significant therapeutic load within each nanoparticle. One potential translational use of the MMSNs is to load the nanoparticles with chemotherapeutic agents and target specific tumors. Magnetic resonance imaging (MRI) can be used diagnostically, based on the embedded superparamagnetic centers, to follow the progress of nanoparticle-targeted tumor treatment. As MMSNs are largely silica, they are generally considered to be biocompatible. Toward the goal of designing nanoparticles that are therapeutically relevant, we have performed full nanoparticle characterization, as well as some initial measures of in vitro cell toxicity with promising results in terms of providing strong magnetic contrast and the ability to load, and later release, doxorubicin (an FDA approved antitumor drug) (Lin and Haynes 2009, 2010).

Size/geometry

The FDA guidance document (von Escherbach 2007) suggests that the only unique information required for nanoparticle-based product approvals pertains to their size. There are two issues with this guideline. First, as it has been pointed out in the literature, the definition of nanoparticle remains outstanding (see, for instance, Lindquist et al. 2010 for a review of the historical evolution of the term). Second, the concept of "size" as applied to nanoparticles is riddled with ambiguities. The size of a spherical nanoparticle can be measured as the size of an individual particle or of an ensemble. In

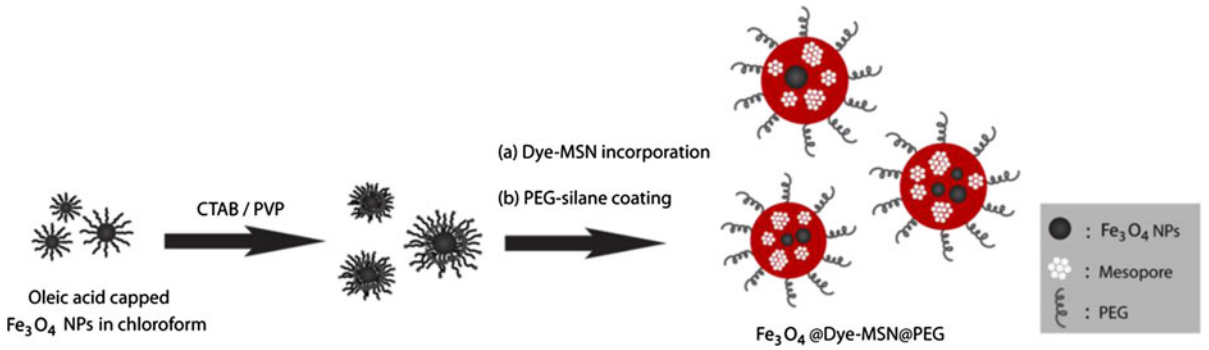

Fig. 2 Synthetic procedure to produce MMSNs. Adapted and reprinted from Lin and Haynes 2009

addition, the size can be measured from dried material or from material in solution as the hydrodynamic radius. The hydrodynamic radius may be important because it includes the proteins and solvent sphere at the surface, both of which are relevant in physiological conditions. For example, the dry diameter of our MMSNs is 42 nm but the hydrodynamic diameter is 57 nm. Since nanoparticles may form agglomerates and aggregates in solution, spontaneous grouping of nanoparticles that are reversible and irreversible through suspension, the choice of media in which measurements are made is important. Finally, the stability of these particles may change over time in media, so the time at which measurements are made may be important. Particles with complex geometry may require more in-depth descriptions of size characteristics. In addition, the engineered nanomaterial may have inherent geometric properties such as crystallinity, which has been shown to affect toxicity profiles (Warheit et al. 2007). Some nanoparticles, such as nanosilver, are commonly produced in a mixture of sizes and geometries. A standard description for such mixtures will be required to adequately describe these materials. An added consideration is that any one of these geometric nanomaterial properties may change based on the environment in which the nanoparticle resides. The body contains a complex variety of environments, begging the question of whether these geometric properties should be assessed and described in the environment in which the nanoparticles would be found in the body. The FDA guidance document also neglects to offer statistical guidelines on the number of particles/agglomerates/aggregates to measure. Such ambiguity is not only an impediment to expedient and repeatable measures, but also has the potential to create loopholes in the regulatory process. For instance, in the approval of pharmaceuticals, this ambiguity would allow those with vested interests to pick the version of their nanomaterial that helps it get through the process, rather than the one that is most relevant.

Even with the seemingly simple criteria of specifying nanoparticle size, there are significant complications when considering nanoparticle-enabled technology as compared to molecular technology. This led our thought experiment to the question: how might nano-enabled drugs be different from traditional drugs? In Table 1, we list the drug-like characteristics and device-like characteristics where nanoparticle drug delivery combination devices may differ significantly from those traditionally screened by the FDA. This table is not meant to be exhaustive, but a starting point for the unique considerations warranted by nanomaterials.

Table 1 Drug and device-like characteristics that may warrant additional consideration during nanoparticle therapeutic preclinical approval

Drug-like considerations	Device-like considerations
Dose/biodistribution	Surface chemistry
Assay interference	ζ-potential
Immunogenicity	Catalytic properties
Liberation/absorption	Size
Metabolism	Geometry
Excretion	Degradation

Surface chemistry

The high surface area to volume ratio of nanomaterials contributes to many of the unique and advantageous properties of engineered nanomaterials. Multifunctional mesoporous silica nanoparticles have extremely high total surface areas (885 m^2/g, approximately 20 times their solid counterpart with the same diameter) and, thus, the potential to transport a large payload of drugs, contrast agents, and other cargo. However, the large surface area that is advantageous for drug delivery applications may be a disadvantage if the nanomaterial surface induces toxicity. For this reason, characterization of the nanotherapeutic surface chemistry is critically important to describing both the nanoparticle's function and risk. Recently, we have demonstrated that it is important to distinguish total surface area from the "cell-contactable" surface area (i.e., the area that is accessible to the cell surface) in the context of nanotoxicity, as the latter provides a more accurate prediction of surface area-dependent effects on cellular function (Maurer-Jones et al. 2010; Lin and Haynes 2010). While the total surface area of nanomaterials may be difficult to model/measure for rough, porous, aggregated, or agglomerated nanomaterials, this challenge can often be overcome by employing N_2 adsorption/desorption measurements with Brunauer–Emmett–Teller (BET) modeling.

Surface charge (ζ-potential) also has a strong influence on both the stability of a solution-phase

nanoparticle suspension and the uptake of nanoparticles by cells and tissues (Verma and Stellacci 2010). This critical surface chemical characteristic can be measured via phase-analysis light scattering. In this case, these MMSNs are known to have a ζ-potential of -24 mV in deionized water, indicating a stable colloidal suspension (to promote long circulation times) and a charge that promotes interaction with naturally charged cell membranes. In addition to ζ-potential influencing nanoparticle behavior and interactions, the surface-bound chemical species can play a large role in toxicity; MMSNs have silanol groups on the surface, and the concentration of silanol groups on the surface correlates with induced hemolysis (Lin and Haynes 2010). This fact is the basis for the mesoporous silica nanoparticles being less toxic than their solid silica counterparts—the mesoporous voids mean there are fewer cell-contactable silanol groups (Fig. 3). Surface chemistry can be purposefully controlled for therapeutic applications. For example, if the MMSN is covalently modified with polyethylene glycol (PEG), they appear compatible with red blood cells (e.g., no induced hemolysis) even at very high exposure concentrations (up to 1.6 mg/mL). The surface-bound chemical species can have a large influence on the function, influencing circulation and localization, as well as reactivity. Unfortunately, there are limited techniques for characterizing this surface speciation, such as surface-enhanced Raman spectroscopy or advanced mass spectrometry techniques, and these limited techniques are not compatible with many types of nanomaterials. Similar to nanomaterial size/geometry, the local chemical environment may have a profound effect on the surface chemistry. In biological samples, unmodified nanomaterials are often found coated in a layer of adsorbed protein dubbed the "protein corona." It is currently an open question whether this corona should be characterized in order to predict the biological function of nanotherapeutics in vivo.

Dose/biodistribution

Since it is likely that nanoparticle therapeutics behave differently than molecular therapeutics, new challenges arise for determining the proper dose that effectively treats a disease without inducing unintentional toxicity. That is, when describing dose, it is necessary to consider not only the exposure to the molecular pharmaceutical loaded within the multifunctional nanoparticle (which may only partially release from the drug delivery nanoparticle), but also the exposure to the nanoparticle carrier itself, and this dual consideration makes it difficult to define dose. Dose is related to biodistribution, as it is necessary to know where a nanotherapeutic localizes to accurately determine the effectiveness of the nanotherapeutic to treat a specific disease and to account for unintentional treatment and potential toxicity to other areas.

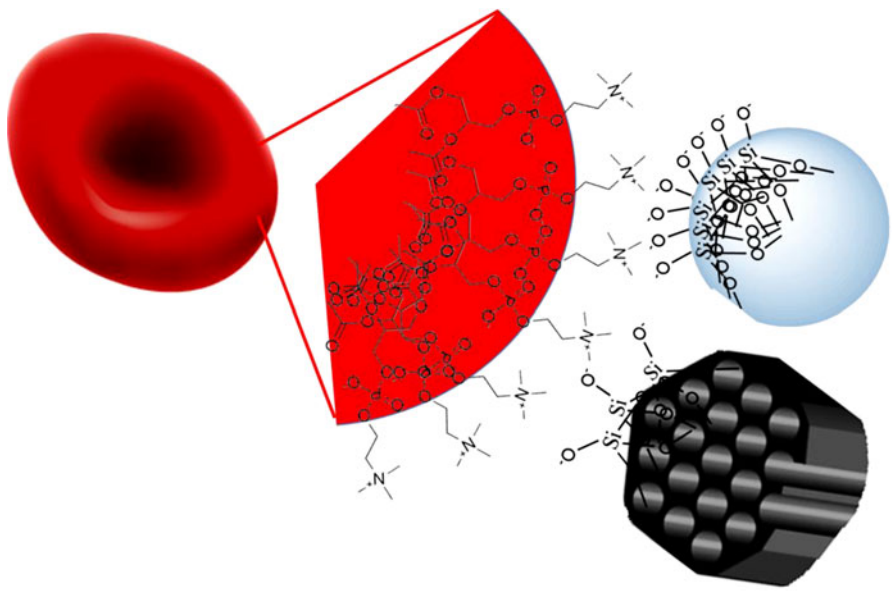

Fig. 3 Proposed interaction of silica nanoparticles' surface with human red blood cells. The silanol groups of the silica surface interact with the cell membrane, causing the cell to rupture. The more silanol groups available for cell contact induce greater hemolysis. Reprinted with permission from Lin and Haynes 2010. Copyright 2010 American Chemical Society

There are multiple literature examples demonstrating various implications of in vitro toxicity as nanoparticles are localized in (Marquis et al. 2009b; Maurer-Jones et al. 2010; Love and Haynes 2010), adherent to (Lin and Haynes 2010), or simply near (George et al. 2009) cells of interest.

Within our thought experiment, we consider an intravenous injection of the MMSNs where we initially know the dose of the drug and the dose of the nanoparticle, and we can assume that the drug is localized within the pores of the silica nanoparticle upon injection. Early experimental work where doxorubicin is loaded into the MMSNs shows that we can load up to 3 wt%. However, if we were to introduce these nanoparticles into the blood stream, effective dose would quickly become unclear. First, the nanoparticles begin circulating, and while we may be able to control circulation time to some extent with size and surface coatings (He et al. 2008; Kreyling et al. 2009), it is not always clear how long they will persist within the blood stream. During circulation, some of the loaded drug may be liberated from the nanoparticle (Fig. 4a). Already, ambiguity arises when describing dose, because it may be challenging to know how much drug stays within the nanoparticle and/or the rate at which it is being released as the nanoparticle is in a dynamic environment where its properties, particularly surface characteristics, are likely to be changing (Oberdorster 2010; Lynch et al. 2007). Tracking the biodistribution of the silica nanoparticle and the molecular therapeutic independently using imaging probes may enable us to determine this information, but there are few studies to date that have successfully done this and we are, therefore, limited in our understanding of the nanotherapeutic behavior within the blood stream. An additional complication is that each different molecular therapeutic loaded into the MMSNs will have different interactions with the nanoparticles based on hydrophobicity and molecular interactions. Different

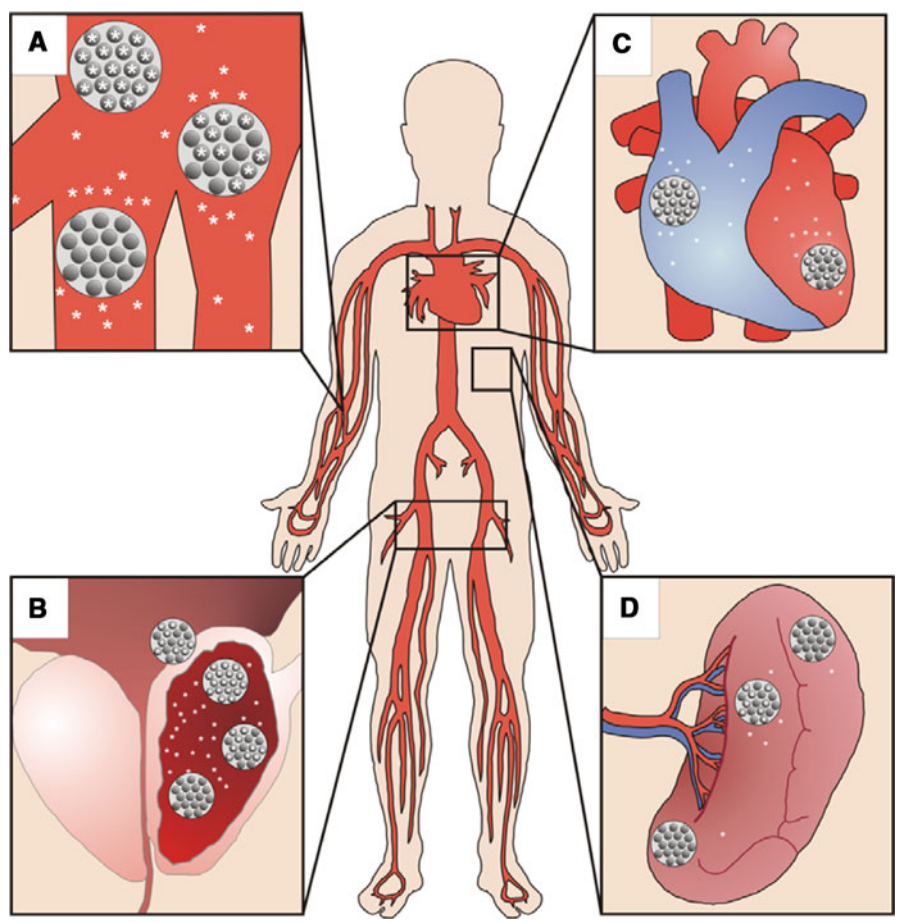

Fig. 4 Dose determination has many challenges **a** after initial injection, the MMSNs begin circulating and some nanoparticles may begin release stored drug (*asterisk*) into the blood stream. **b** Nanoparticles may localize within the targeted prostate tumor and release their drug, though some nanoparticles may have already liberated some drug during circulation. **c** Either the nanoparticle or drug may have non-specific interactions with other tissues like the heart, where it would be important to discern the dose to understand the nanoparticle and/or drug toxicity. **d** The excretion or clearance of the nanoparticle and drug should also be considered in determining dose because areas such as the spleen may be dosed unintentionally by the nanoparticle carrier, remaining drug within the nanoparticle, or both

drugs will be liberated with different dynamics, making generalization difficult. In addition, surface modification of the internal surface of MMSNs will also influence the drug loading and release profiles.

From the blood stream, the nanoparticles can access a large portion of the body (Oberdorster et al. 2005), and it is common in nanotherapeutic design to target them to a specific area (Pangburn et al. 2009), a prostate tumor for this example (Fig. 4b). The effective dose in this targeted area is dependent on the efficiency of the nanoparticle in reaching the tumor, the rate of drug release, and nanoparticle clearance rate (Zamboni 2008; Hagens et al. 2007). The amount of nanomaterial carrier reaching the prostate tumor or targeted tissue, however, may not indicate the amount of drug that reaches the same tissue because some of the drug may have already been liberated from the nanoparticle prior to localizing in the tumor. This must be considered when calculating the effective dose. Studies have determined the dose of a molecular therapeutic within a targeted tissue by tracking the drug itself (van Vlerken et al. 2008; Dong et al. 2010), but it is also necessary to consider the amount of nanoparticle colocalized in that tissue as the carrier may contribute to cellular toxicity or immunogenicity (see below). As with all drugs, there may also be some non-specific association of the nanoparticle and/or drug in non-targeted tissue (e.g., the heart) (Fig. 4c), which again is difficult to quantify based on aforementioned variables.

Finally, we must consider the rate and mechanism of clearance for the nanotherapeutic to accurately define dose. If a particle accumulates within a tissue faster than it is excreted, this greatly affects the exposure concentration within that particular tissue which, in turn, would alter the administered dose of nanotherapeutic throughout the disease treatment. In addition, there may be drug still confined within the pores of the silica nanoparticle as the carrier is cleared by the body. As many nanoparticles have been shown to be taken up by the reticuloendothelial system (RES), including the liver and the spleen, these organs may unintentionally receive a dose of drug and the MMSN carrier (Fig. 4d). If, again, the nanoparticle localizes in the RES organs faster than they are cleared, these areas may have significant bioaccumulation of the material.

Immunogenicity

In developing novel nanotherapeutics, it is critical to consider their immunogenicity, which is the ability of the material to elicit an immune response. The scope of immune system response ranges from phagocytosis of nanoparticles within the innate arm of the immune system to antibody-mediated pathogen destruction within the acquired immune system. While the sheer multiplicity of response mechanisms and their timing (i.e., acute vs. chronic; primary vs. secondary) confer survival advantage to the host organism, they represent monumental challenges from an experimental design perspective in terms of what, when, and where to observe immune responses in vivo. There are many ways in which nanoparticles may act as immunogens, where the nanoparticle invokes a direct immune response on its own accord. As a matter of fact, deliberate immunogenicity is the principle behind certain cancer therapies and vaccinations (O'Hagan et al. 2001). Nanoparticles may also induce responses indirectly by behaving as haptens, species that are normally non-immunogenic and that become immunogenic when attached to a larger protein (Larsen et al. 2010). In light of experimental evidence that nanoparticles acquire a protein corona upon in vivo exposure (Cedervall et al. 2007; Lundqvist et al. 2008), it is especially important to study the nanoparticle–macromolecule combination vis-à-vis the immune system. Whereas freshly manufactured and administered small nanoparticles may very well fit the hapten classification ex vivo, aggregation and agglomeration subsequent to administration are likely to change the immunogenic profile of these manufactured particles in vivo. In this regard, observing immunogenicity along the exposure timeline may yield results that map to the aggregation status of nanoparticles once in the host system. Finally, nanoparticles may act as an adjuvant, or carriers for adsorbed antigens, causing an antigenic response where, after repeated exposure to the nanoparticle, the body becomes sensitized to the material (Staroverov et al. 2009; Inoue and Takano 2010).

While the general immunogenicity of the MMSNs has not been fully characterized, there is clear evidence that they impact mast cells, important immune system cells involved in inflammatory response. Exposure to MMSNs decreases cell viability and alters a cell's chemical messenger molecule

secretory process (Maurer-Jones et al. 2010). Given the wide range of possible immune responses, a claim of non-immunogenicity for these particles, or any others, is highly contextual and presents a significant challenge for pre-clinical assessment.

Metabolism/degradation

As the multifunctional nanoparticle fits the definition of a combination therapeutic, we must consider both the metabolism of the drug loaded within the nanoparticle and the degradation of the nanoparticle carrier itself. The liberated drug will be metabolized and will follow the same pharmacokinetic pathway that a non-nanoparticle therapeutic would experience (Zamboni 2005). The mechanism and rate of degradation of the nanoparticle carrier, however, are poorly understood. For some nanotherapeutics, such as liposomes, it is less important to understand the degradation as the components of the nanoparticle cause little toxicity. Metal-containing nanoparticles, such as semiconductor quantum dots, are of greater concern, because toxic concentrations of metal ions could result upon nanoparticle degradation in vivo. Herein, both the silica and the incorporated Fe_3O_4 magnetic centers represent possible source of metal ions.

It is evident from various studies that silica nanoparticles will degrade in in vitro physicological conditions (Lin and Haynes 2010; Liu et al. 2010), but the mechanism and the extent to which degradation occurs in vivo are yet to be determined for most nanotherapeutics. In this thought experiment, we know that the MMSNs undergo some degradation within 6 days in a buffer solution (Fig. 5) as the pore structure collapses and silicon ion is released (Lin and Haynes 2010). In vitro toxicity of these nanoparticles increases drastically as this degradation and liberation of new chemical species occurs, although experiments show that the increased toxicity cannot be attributed to the liberated content. Most nanotoxicology work characterizes nanoparticles in solution prior to either in vitro or in vivo exposure only; as the nanoparticle localizes within targeted or untargeted tissue (see dose section above) and the nanoparticle degrades, there could be inadvertent toxicity. While fluorescent or radio-labels could be used to track the nanoparticles' distribution throughout the body, technology is currently insufficient to follow the nanoparticle degradation and metal ion release in vivo.

Perspective

Depending on the physiological implications of the emergent properties, the possible deviations detailed above in both drug- and device-like characteristics of nanoscale therapeutics may require significant effort and cost for drug developers. However, much of this cost may be mitigated down the road if the FDA concept of "similarity" is applicable to nanotherapeutics as it is for molecular therapeutics. Could the concept of similarity be applied if only a single characteristic of the nanotherapeutic is changed? For example, if all other characteristics of an approved MMSN combination product remained the same except for the drug loaded, would the product be exempt from complete characterization? This is a simple example where one could easily imagine that the concept of similarity would apply and that only the drug-specific characteristics need be reported (i.e., drug dose and biodistribution). What if other and less straightforward aspects of the MMSN (e.g., size, surface chemistry, or composition material) were modified? For example, how much does size have to change before the nanoparticle is a "new" size: 10 nm, 1 nm, or even 0.1 nm? Is it possible for basic safety criteria established for particular nanoparticles to traverse a variety of nanotherapeutics? For example, could it be established that all nanomaterials with the same coating within a given size regime will biodistribute to the same locations? As bench scientists working in the area of nanotoxicology, we are driven by the goal of determining these "design rules" that govern the biological behavior of nanomaterials. It can be seen from this example that the goals of the bench scientist and FDA regulator have significant overlap.

In an ideal situation, the regulator and bench scientist have a symbiotic relationship where regulations influence and are influenced by basic science research. However, for the average bench scientist working in a nano-bio field, this relationship may often seem a one-way street. The FDA may invite scientists from across a spectrum of organizations to draw on their expertise in drafting documents regarding

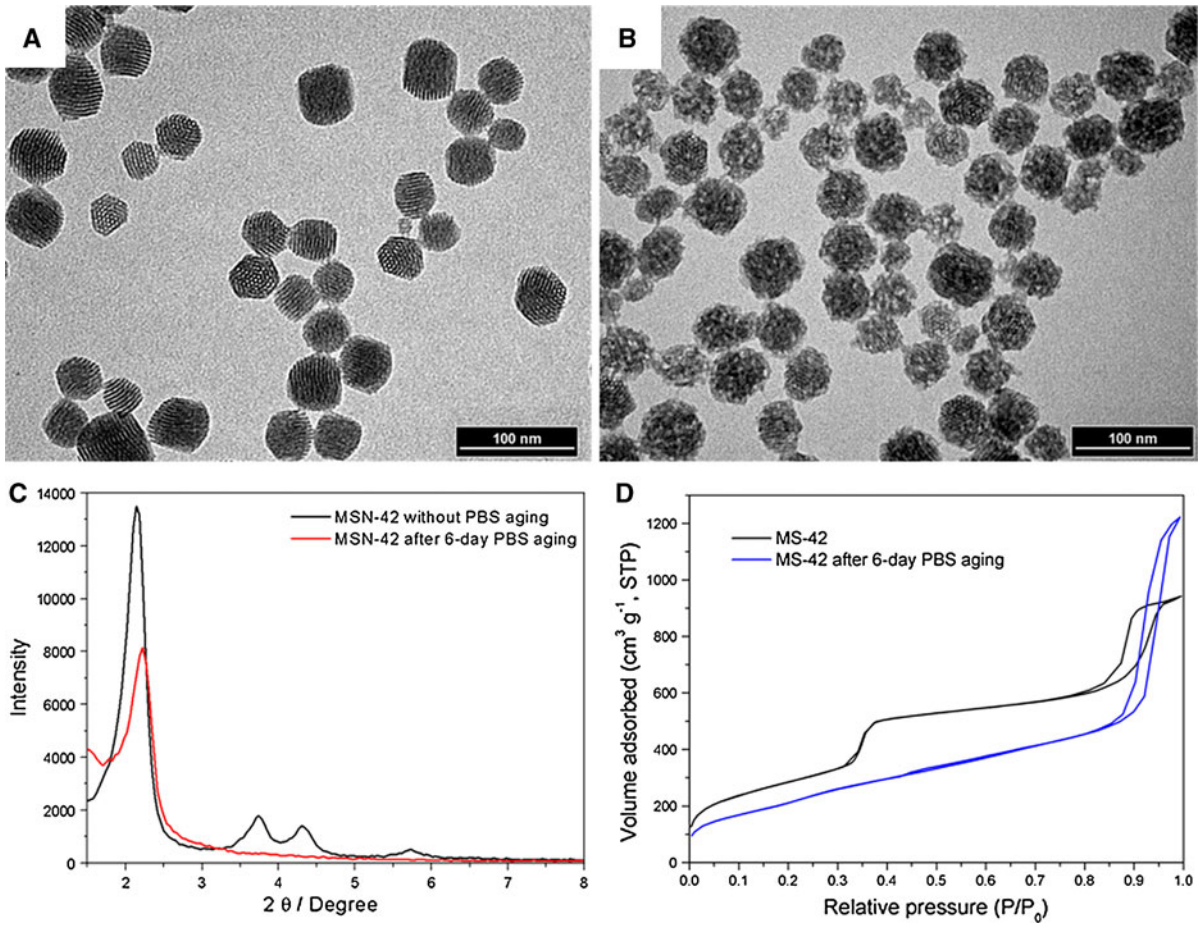

Fig. 5 Evidence of pore structure collapsing in MMSNs after 6-day aging in phosphate buffered saline (PBS). TEM images of nanoparticles before (**a**) and after (**b**) PBS aging show differences in nanoparticle appearance as it is harder to see well-defined mesopores after aging. **c** Powder x-ray diffraction patterns of nanoparticles before (*top line*) and after (*bottom line*) 6-day PBS aging indicate a collapse of the pore structure as the primary peaks caused by pores disappear after aging (*circled area*). **d** N_2 adsorption/desorption isotherms of nanoparticles before (*top line*) and after (*bottom line*) PBS aging also provide evidence for pore collapse as the inflection due to the pores is greatly diminished (*circled area*). Adapted and reprinted with permission from Lin and Haynes 2010. Copyright 2010 American Chemical Society

emerging nanotherapeutics, as was evident in 2007's report from the Nanotechnology Task Force (von Escherbach 2007). However, there appear to be few official and consistent venues for bench scientists to benefit from the accumulated expertise of the FDA regulators at early stages of nanotherapeutic development (i.e., pre-clinical or earlier), except for some sparsely prepared reports. Well-established therapeutics, such as molecular therapeutics, have a vast library of information for pre-clinical trials that can inform and direct future development at the basic science level. There is no such library of information for emerging technologies such as nanotherapeutics. While the need to protect intellectual property of nanotherapeutics is critically important, the process of nanotherapeutic pre-clinical evaluation could be made more transparent without divulging trade secrets. The same may be said for other regulatory bodies where the process of regulation of emerging technologies seems opaque to the bench scientists who are developing the technology. On the other hand, bench scientists need to seek out more involvement in the regulation process. We need to invite regulators to interact in nanotoxicity meetings more regularly to give them a conspicuous presence and larger influence in scientific discussion. The reaction panel featuring representatives of many of

the major U.S. oversight agencies at the recent "Governing Nanobiotechnology: Reinventing Oversight in the 21st Century" conference hosted at the University of Minnesota in April 2010 is a welcome step toward this type of interaction. The speed of technological development in this area requires more avenues for more frequent dialog to occur between scientists and regulators in order to establish this symbiotic relationship, to fully take advantage of the billions in investment already made by the government and private investors in nanotechnology development, and for us all to reap the long-term benefits of nanotechnology.

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References

Brendel E (2004) Intuition pumps and the proper use of thought experiments. Dialectica 58(1):89–108. doi:10.1111/j.1746-8361.2004.tb00293.x

Cedervall T, Lynch I, Lindman S, Berggard T, Thulin E, Nilsson H, Dawson KA, Linse S (2007) Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. Proc Natl Acad Sci USA: 2050–2055. doi:10.1073/pnas.0608582104

Dong L, Xia S, Wu K, Huang Z, Chen H, Chen J, Zhang J (2010) A ph/enzyme-responsive tumor-specific delivery system for doxorubicin. Biomaterials 31:6309–6316. doi:10.1016/j.biomaterials.2010.04.049

George S, Pokhrel S, Xia T, Gilbert B, Ji Z, Schowalter M, Rosenauer A, Damoiseaux R, Bradley KA, Maîdler L, Nel AE (2009) Use of a rapid cytotoxicity screening approach to engineer a safer zinc oxide nanoparticle through iron doping. ACS Nano 4:15–29

Hagens WI, Oomen AG, de Jong WH, Cassee FR, Sips A (2007) What do we (need to) know about the kinetic properties of nanoparticles in the body? Regul Toxicol Pharmacol 49:217–229. doi:10.1016/j.yrtph.2007.07.006

He XX, Nie HL, Wang KM, Tan WH, Wu X, Zhang PF (2008) In vivo study of biodistribution and urinary excretion of surface-modified silica nanoparticles. Anal Chem 80:9597–9603. doi:10.1021/ac801882g

Hill HM (2002) Regulatory aspects of bioanalysis in preclinical and clinical studies. Chromatographia 55:S83–S84. doi:Unsp0009-5893/00/02

Inoue K, Takano H (2010) Adjuvanticity of nanoparticles on Th immunity. Basic Clin Pharmacol Toxicol 106:445. doi:10.1111/j.1742-7843.2010.00584.x

Jones CF, Grainger DW (2009) In vitro assessments of nanomaterial toxicity. Adv Drug Deliv Rev 61:438–456

Kreyling WG, Semmler-Behnke M, Seitz J, Scymczak W, Wenk A, Mayer P, Takenaka S, Oberdorster G (2009) Size dependence of the translocation of inhaled iridium and carbon nanoparticle aggregates from the lung of rats to the blood and secondary target organs. Inhal Toxicol 21:55–60. doi:10.1080/08958370902942517

Larsen ST, Roursgaard M, Jensen KA, Nielsen GD (2010) Nano titanium dioxide particles promote allergic sensitization and lung inflammation in mice. Basic Clin Pharmacol Toxicol 106:114–117. doi:10.1111/j.1742-7843.2009.00473.x

Lin YS, Haynes CL (2009) Synthesis and characterization of biocompatible and size-tunable multifunctional porous silica nanoparticles. Chem Mater 21:3979–3986. doi:10.1021/cm901259n

Lin YS, Haynes CL (2010) Impacts of mesoporous silica nanoparticle size, pore ordering, and pore integrity on hemolytic activity. J Am Chem Soc 132:4834–4842. doi:10.1021/ja910846q

Lindquist E, Mosher-Howe K, Liu X (2010) Nanotechnology. What is it good for? (absolutely everything): a problem definition approach. Rev Policy Res 27:255–271. doi:10.1111/j.1541-1338.2010.00441.x

Liu XY, Hurt RH, Kane AB (2010) Biodurability of single-walled carbon nanotubes depends on surface functionalization. Carbon 48:1961–1969. doi:10.1016/j.carbon.2010.02.002

Love SA, Haynes CL (2010) Assessment of functional changes in nanoparticle-exposed neuroendocrine cells with amperometry: exploring the generalizability of nanoparticle-vesicle matrix interactions. Anal Bioanal Chem 398:677–688

Lundqvist M, Stigler J, Elia G, Lynch I, Cedervall T, Dawson KA (2008) Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. Proc Natl Acad Sci USA: 14265–14270. doi:10.1073/pnas.0805135105

Lynch I, Cedervall T, Lundqvist M, Cabaleiro-Lago C, Linse S, Dawson KA (2007) The nanoparticle–protein complex as a biological entity; a complex fluids and surface science challenge for the 21st century. Adv Colloid Interface Sci 134–35:167–174. doi:10.1016/j.cis.2007.04.021

Marquis BJ, McFarland AD, Braun KL, Haynes CL (2008) Dynamic measurement of altered chemical messenger secretion after cellular uptake of nanoparticles using carbon-fiber microelectrode amperometry. Anal Chem 80:3431–3437. doi:10.1021/ac800006y

Marquis BJ, Love SA, Braun KL, Haynes CL (2009a) Analytical methods to assess nanoparticle toxicity. Analyst 134:425–439

Marquis BJ, Maurer-Jones MA, Braun KL, Haynes CL (2009b) Amperometric assessment of functional changes in nanoparticle-exposed immune cells: varying Au nanoparticle exposure time and concentration. Analyst 134:2293–2300. doi:10.1039/b913967b

Maurer-Jones MA, Bantz KC, Love SA, Marquis BJ, Haynes CL (2009) Toxicity of therapeutic nanoparticles. Nanomedicine 4:219–241

Maurer-Jones MA, Lin YS, Haynes CL (2010) Functional assessment of metal oxide nanoparticle toxicity in immune cells. ACS Nano 4:3363–3373. doi:10.1021/nn9018834

Maynard A (2009) Ten things everyone should know about nanoparticle safety. http://2020science.org/2009/08/29/10things/. Accessed July 2010

Norton JD (1996) Are thought experiments just what you thought? Can J Philos 26:333–366

O'Hagan DT, MacKichan ML, Singh M (2001) Recent developments in adjuvants for vaccines against infectious diseases. Biomol Eng 18:69–85. doi:10.1016/S1389-0344(01)00101-0

Oberdorster G (2010) Safety assessment for nanotechnology and nanomedicine: Concepts of nanotoxicology. J Intern Med 267:89–105. doi:10.1111/j.1365-2796.2009.02187.x

Oberdorster G, Oberdorster E, Oberdorster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113:823–839. doi:10.1289/ehp.7339

Pangburn TO, Petersen MA, Waybrant B, Adil MM, Kokkoli E (2009) Peptide- and aptamer-functionalized nanovectors for targeted delivery of therapeutics. J Biomech Eng 131:074005. doi:10.1115/1.3160763

Seymour LW (2006) The future of gene therapy in the UK. Trends Biotechnol 24:347–349. doi:10.1016/j.tibtech.2006.06.002

Staroverov SA, Aksinenko NM, Gabalov KP, Vasilenko OA, Vidyasheva IV, Shchyogolev SY, Dykman LA (2009) Effect of gold nanoparticles on the respiratory activity of peritoneal macrophages. Gold Bull 42:153–156

Teeguarden JG, Hinderliter PM, Orr G, Thrall BD, Pounds JG (2007) Particokinetics in vitro: dosimetry considerations for in vitro nanoparticle toxicity assessments. Toxicol Sci 95:300–312

U.S. Congress (2006) Hearing of the senate committee on commerce, science and transportation, "Promoting economic development through nano commercialization." Text from the Woodrow Wilson international center for scholar's project on emerging nanotechnologies. http://www.nanotechproject.org/publications/. Accessed July 2010

van Vlerken LE, Duan Z, Little SR, Seiden MV, Amiji MM (2008) Biodistribution and pharmacokinetic analysis of paclitaxel and ceramide administered in multifunctional polymer-blend nanoparticles in drug resistant breast cancer model. Mol Pharm 5:516–526. doi:10.1021/mp800030k

Verma A, Stellacci F (2010) Effect of surface properties on nanoparticle-cell interactions. Small 6:12–21. doi:10.1002/smll.200901158

von Escherbach AC (2007) Nanotechnology: a report of the U.S. Food and Drug Administration nanotechnology task force. U.S. Food and Drug Administration, Rockville

Warheit DB, Webb TR, Reed KL, Frerichs S, Sayes CM (2007) Pulmonary toxicity study in rats with three forms of ultrafine-T_iO_2 particles: differential responses related to surface properties. Toxicology 230:90–104. doi:10.1016/j.tox.2006.11.002

Zamboni WC (2005) Liposomal, nanoparticle, and conjugated formulations of anticancer agents. Clin Cancer Res 11:8230–8234. doi:10.1158/1078-0432.ccr-05-1895

Zamboni WC (2008) Concept and clinical evaluation of carrier-mediated anticancer agents. Oncologist 13:248–260. doi:10.1634/theoncologist.2007-0180