The Role of Nanomaterials in Translational Medicine

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ABSTRACT There are a range of definitions for nanomaterials and a range of length scales that are considered nano, but one thing is consistent among fields: nanomaterials are small and special. Nanomaterials have the potential to have tremendous impact on medical treatments. In one example, nanomaterials are permitting the tracking of cells *via* magnetic resonance imaging (MRI) in clinical trials to assess the efficacy and safety of cellular therapies. In a second example, nanomaterials are acting as drug delivery vehicles for the targeted delivery of therapies to increase efficacy and to reduce side effects. However, there are distinct challenges that must be considered in the development and application of these materials, including careful analysis of the distribution and clearance of nanomaterials and their potential off-target effects. By carefully assessing materials early in their development at the bench, one may be able to move successful approaches through to the clinic more rapidly, which is indeed the goal of the field. For far too many conditions and diseases, the tools we have are less than adequate, and nanomaterials have the potential to fill that void. To realize this potential, investigators must be willing to invest time and resources to develop and to translate these technologies to the point where the risk is low enough that they have real commercial possibilities. Working collaboratively and leveraging resources and experience play important roles in moving technologies through preclinical and clinical testing. It requires incredible dedication of teams of researchers, but the result is new treatments and therapies.

For a technology to be described as nanoscale varies according to discipline from a few nanometers to hundreds of nanometers, but across a broad range of fields from battery research to catalysis and medicine, nanoscale materials exhibit special or unique behavior. One colleague, Prof. Ainissa Ramirez, describes nanomaterials as "small but special".[1] It is the latter quality that has the potential to impact medicine through improved translation and the development of clinically relevant therapies.

This Perspective is far from comprehensive but focuses on the potential impact and challenges of translating some nanomaterials. Two of the areas in which nanomaterials can have and are having therapeutic impact are the tracking and delivery of treatments. While nanomaterials open new doors and have the potential to improve patient care, there are fundamental challenges that we have to address to ensure that these therapies are as safe and effective as possible. These challenges include dealing with concerns about toxicity during the processing and delivery of materials, assessments of biodistribution over time as a function of route of administration, and the reality that the role these materials play in different tissues may lead to unanticipated findings because of their "special" nature. Looking at the biodistribution and clearance of nanomaterials at an early stage of research can help to provide insights that will, ultimately, lead to better technologies that are more likely to be successful therapies.

It is tremendously easy as scientists to spend our days talking about the potential of our new therapeutic paradigms. We often say there are 5 to 10 years between a breakthrough and a treatment. When one is suddenly moved to the patient side of the equation, the world often looks strangely medieval. For example, cisplatin, first described in 1845 and shown to reduce tumors in 1968, is still the first line of defense in a number of cancers.[2] As a patient, one expects to hear about a treatment that is more recent and less toxic than a systemically administered agent from 1968. There are treatments being developed, but if they are to achieve therapeutic impact, we have to be able to move them from discovery through to the clinic. More and more, investigators are finding that they need to take responsibility for this translation. The model of investors swooping in, developing the technology, and taking it all the way from preclinical testing through to approval and commercialization is becoming more rare. The further one can move a technology toward (or into) the clinic, the greater the reduction of risk and the potential for return on investment.

Can nanomaterials be translated, and can they help drive the development of therapies? The answer is an all too frustrating "perhaps", but there are some basic concepts that, when considered at the bench, help to move therapies to the clinic more efficiently.

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Using Nanomaterials To Track Treatments. Nanomaterials as contrast agents in imaging applications have a long and impressive history ranging from gadolinium-based systems for magnetic resonance imaging (MRI) to quantum dots for optical microscopy.[3] One of the areas in which nanomaterials can have and are having an impact in moving treatments to the clinic is in tracking treatments (Figure 1A). For example, cellular therapies including the administration of mesenchymal stem cells or mesenchymal stromal cells (MSCs) benefit greatly from the inclusion of nanomaterials to permit high-resolution tracking of the cells in clinical trials.

MSCs have shown promise for the treatment of a number of diseases in clinical trials ranging from myocardial infarction[4] to the modulation of graft *versus* host disease[5] and multiple sclerosis.[6] In preclinical work, the hypothesis was that intravenous administration of MSCs would lead to homing of the cells to the sites of injury or disease.[7] While MSCs are often seen in higher numbers in compromised tissues, they have also been found to reside in a number of uninjured tissues, including the lungs, liver, and spleen.[8] By tracking the location of the cells over time, not only have investigators been able to determine the best routes of administration, but they have seen that the mechanism of the cells may have been at least in part one of immunomodulation related to paracrine (local) signaling.[9] Being able to track the cells is critical to understanding their safety and efficacy and, thus, is also critical for translation.

In preclinical models, it is typical to label the cells with green fluorescent protein or a similar molecule and to characterize their distribution histologically. This is not an option clinically, but just as we need to know in preclinical work where the cells go and what they do, we also need to be able to assess this movement in clinical trials. In phase I trials, it is critical to demonstrate safety, and it helps tremendously to be able to determine where the cells go and for how long.[10] Being able to track the cells greatly aids the demonstration of safety and provides insights into the mechanism of action that facilitates moving to phase II trials.

MRI is a particularly attractive imaging modality for clinical trials. It provides high-resolution, detailed structural images, three-dimensional spatial reconstruction, and no ionizing radiation. However, cells need to be modified to be visualized. Iron oxide nanoparticles have been used to track donor cells in preclinical trials,[11−13] and several clinical trials are underway. One clinical trial, based in Israel, focused on MSCs for the treatment of multiple sclerosis as well as amyotrophic lateral sclerosis.[14] The MSCs were labeled with Feridex, iron oxide nanoparticles approved by the FDA for use in humans. Feridex nanoparticles are nonfunctionalized and rely on either passive endocytosis or the use of cell permeation techniques to promote the internalization of the particles in the cells of interest.[15] The labeling has enabled tracking of the MSCs delivered either intravenously or intrathecally over 6 months.

A search through the NIH's clinicaltrials.gov Web site shows that several groups are running trials investigating relatives of Feridex, which is no longer being manufactured, for tracking cells for cell therapies. The ability to track cells longitudinally in clinical trials is tremendously exciting, but there are potential scientific issues as well as safety issues that must be considered. The early expectations of these particles were that, if the cells containing them died, the particles would be cleared readily by the immune system. This is likely in a number of tissues, but the particles may not always be cleared quickly, especially in the central nervous system with its microglial cells or under immune-compromised conditions.[16] This work raises concern over both the safety of the particles as well as whether, in some cases, there may be a positive MR signal that does not correlate well with the presence of the cells of interest. For nanoparticles to facilitate the development of cell therapies, the particles may have to be engineered to facilitate clearance when released from dying cells.

Using Nanomaterials To Deliver Treatments. Nanomaterials may accelerate the translation of therapies by improving their tracking, as in the case of cellular therapies. Nanomaterials may also deliver the therapy itself, improving the efficacy and localization of treatment to reduce side effects (Figure 1B−D). For many of the agents used to treat cancers, the side effects are often dose limiting. Systemic therapies are attractive for many cancers because they have the potential to reach tumors throughout the body in areas that are not amenable to other approaches. One of the tremendous areas of growth on the research side involves using nanomaterials both as imaging agents to identify tumor cells and as drug delivery vehicles. These techniques are tremendously exciting and build on the drug delivery approaches described here, often in conjunction with the some of the imaging approaches noted above.

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Delivering drugs with nanomaterials is far from new. For millions of years, viral nanoparticles have successfully delivered their payloads. In recent years, we have begun to catch up, albeit very slowly. One of the best-known nanoparticle delivery systems is Doxil, a PEGylated

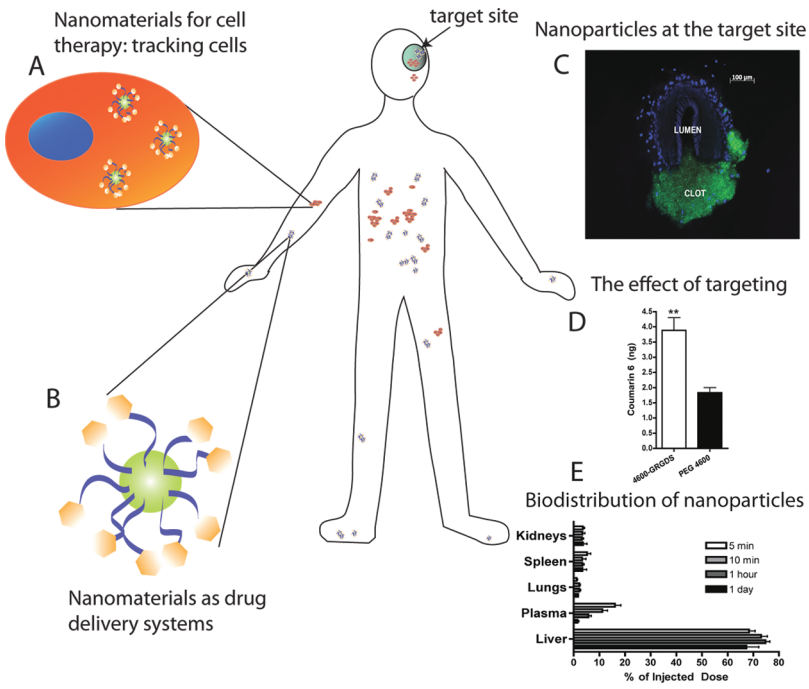

Figure 1. (A) Nanomaterials for therapy: tracking cells. Nanomaterials can be designed to be endocytosed by cells. The most common ones for MRI cell tracking are iron oxide based. The particles generate a signal on MRI that correlates with the cell location and permits non-invasive longitudinal tracking of cell therapies. (B) Nanomaterials as drug delivery systems. Nanoparticles can be administered systemically to deliver a drug to a target site such as a tumor through either passive or active targeting. (C) Nanomaterials at a target site. PEGylated PLGA nanoparticles labeled with Coumarin 6 (green) are seen participating in a clot following intravenous administration.[32] (D) Nanoparticles that were designed to bind to the cells involved in the clot show greater accumulation at the target (injury) site than untargeted nanoparticles.[32] (E) Biodistribution of the nanoparticles over time. No nanoparticles were detected in any organs or plasma at 3 days post-administration.[32] Images C, D, and E are reprinted with permission from ref 32. Copyright 2009 American Association for the Advancement of Science.

liposomal formulation of doxorubicin. The encapsulation of the drug in the PEGylated nanoparticles leads to longer circulation time than the free drug and greater efficacy than the free drug.[17] The longer circulation time, size of the liposomes, and PEGylation are designed to facilitate passive diffusion through the enhanced permeation and retention (EPR) effect associated with the leaky vessels in tumors. Doxil is approved for the treatment of ovarian and breast cancers as well as Kaposi's sarcoma. While in many ways it is an excellent example of the benefits of nanomaterials for therapy (longer delivery, greater efficacy, and simple intravenous administration), it is not without its challenges. The longer circulation time and ability to circulate through the capillary beds of the hands and feet can lead to hand-foot syndrome, in which the skin breaks down on the hands and feet.[17,18] The condition is found with the nanoparticle formulation and is not seen with the free drug. The condition is painful and can be dose limiting. Doxil represents both the promise and challenges of nanomaterials for delivery.

Nanomaterial delivery systems that have moved into clinical trials more recently have focused on targeting the particles to increase the concentration of the drug at the site of interest while reducing the systemic side effects. BIND Biosciences is using aptamer targeting prostate cancer cells in their nanoparticle delivery system for docetaxel (clinical trial identifier NCT01300533). In animal models, these particles were shown to reduce tumor size and to increase survival compared to either untargeted nanoparticles or the free drug.[19] In subsequent work, they showed that the majority of the particles went to the liver and spleen as has been seen previously with nanoparticles administered systemically, but with the targeting moiety, a greater number of particles were seen in the tumor.[20]

Aptamers are attractive targeting molecules because they exhibit reasonably high affinity and specificity while being more stable than antibodies under a number of conditions.[21] Their development has played an important role in developing targeted nanoparticles for therapeutic interventions, particularly for conditions like cancer, as seen in the BIND Biosciences clinical trial. The scientific challenge lies in finding a specific surface ligand on the cancer cells for targeting. In the case of prostate cancer, there are well-known surface ligands on the cancer cells. The same is true for breast cancers. However, for many cancers, we do not have good, specific markers. It is critical from a basic research perspective that we investigate targeting molecules for other cancers and apply these targeting moieties to therapies.

In the meantime, other groups and companies are looking to provide localized therapy by combining nanomaterials with other modalities. Nanospectra Biosciences, Inc. has a phase 1 clinical trial underway for patients with head and neck cancers based on gold nanoshell technology (NCT00848042). The particles (~120 nm in diameter) consist of a dielectric material, silica in this case, coated with a thin gold shell that is then PEGylated to increase circulation time like the liposomes in Doxil. They accumulate in the tumor through the EPR effect associated with leaky vessels. A near-infrared laser is used to heat up the particles and to kill the tumor. The specificity is driven by the need to combine the particles with the laser to achieve toxicity.[22,23] It is a different approach to killing cells that has the potential to work in tumors where targeting moieties are not available.

Much of the work on nanomaterials for drug delivery is extremely promising, but not every targeted formulation has performed well in clinical trials. Cisplatin, the long-used chemotherapy agent, is associated with a host of dose-limiting side effects including nephrotoxicity. Providing a targeted, localized delivery system could make this drug more effective and safer. A liposomal formulation of cisplatin, lipoplatin, has been through phase 3 trials for non-small-cell lung cancer. Lipoplatin is a relative of Doxil in that it is a PEGylated liposomal system with no specific targeting components. The liposomal formulation permitted higher dosing (200 mg/m^2 for the lipoplatin *versus* 75 mg/m^2 for cisplatin) and showed significantly less nephrotoxicity (6% *versus* 40%), but the median survival time was not affected.[24] Nonetheless, this is an encouraging finding. The nephrotoxicity of cisplatin is devastating, and being able to administer it with the same efficacy without the side effects is a step forward, but one would still like something that is more effective. One of the arguments for the limited efficacy has been that the liposomes do not effectively release the drug at the tumor sites. To address this issue, liposomes were fabricated with an enzymatically degradable unit sensitive to phospholipase A_2, which exists in high concentrations around tumors.[25] The concept behind delivery of an enzymatically targeted system is intriguing, and preclinical work showed reduction in tumors with minimal side effects noted beyond weight loss (5% in the liposomal group *versus* 3% in the control on day 1).[26] *In vitro* toxicity studies looked promising.[26] The preclinical work motivated a phase 1 study. The study was halted when patients exhibited acute infusion reactions and renal toxicity (33% of patients).[27] Just as nanomaterials can augment therapy, they can lead to increased side effects. It is not easy to find articles on failed trials, and it is incredibly helpful when investigators share what does *not* work. We all need the opportunity to learn from failures.

> Looking for the biodistribution as well as off-target effects early in the bench studies helps to shorten the time to translate the technology.

In all of these systems, even with excellent targeting moieties or combination technologies to localize the treatment, the majority of the carriers do not get to the tumor. Demonstrating efficient clearance of the unbound particles becomes critical for safety and for the translation of the therapy to patients. Early bench studies need to study the biodistribution of the nanomaterial carefully and not just focus on what is happening at the tissue of interest. Doing so helps to identify the most promising candidates that can make it through preclinical testing and into clinical trials. A therapy may be tremendously effective in the tissue of interest, but if there is a build up in another part of the body, the efficacy may be overshadowed by safety concerns or complications. Looking for the biodistribution as well as off-target effects early in the bench studies helps to shorten the time to translate the technology (Figure 1E).

THE CHALLENGES FACED IN TRANSLATING NANOMATERIALS

It is tremendously exciting to see more nanomaterials moving into clinical trials. Nonetheless, for many patients looking for treatments, nanomaterial therapies are a lifetime away. The question we all face is: What do we need to do to develop approaches that can get to the clinic faster?

For most of us at the bench, proof-of-principle is an exciting goal on which we spend most of our time. Focusing on how a material will be scaled up, or what else a material may do, or where it may go seems unimportant without signs that the technology has promise. However, if we look at these issues from day 1 at the bench, then successful technologies will be more likely to move forward quickly. This is true with any technology, but the challenges become greater as the materials become smaller and more carefully engineered. If one designs a material that is 10 nm with 10 targeting moieties that is successful but the 20 nm version is not, one needs to be able to scale up the relatively precise 10 nm system. This is easier said than done. The techniques most of us use at the bench are not typically industrially friendly. A low yield of a system for a proof-of-principle study is fine, but if it takes another 10 years to get the system to volumes needed for large animals much less patients, the technology can lose a great deal of its initial potential and value.

Think about scale-up early in the process. The value is many-fold; not

only does it mean that one may have more material as one moves through preclinical testing and one may have a process more amenable to good manufacturing practice (GMP) conditions, a prerequisite for clinical testing, but making a material in a variety of ways allows one to ask some important basic questions about what factors or features of the material are critical for its function. Translating technology does not mean abandoning basic research and the fundamental questions posed. Basic and translational research can move in parallel and thrive on each other. Looking at the results from preclinical or early clinical testing in conjunction with basic work can give new insights into the materials and mechanisms of the technology.

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The elephant in the room with nanomaterials are the concerns regarding safety. In 2007, the FDA released a report of the nanotechnology task force that sought to address how nanomaterials should be treated in the regulatory process.[28] There have been a number of papers looking at the toxicity of a host of nanomaterials from gold nanoparticles[29] to carbon nanotubes[30] and PLGA nanoparticles.[31] The very things that make nanomaterials so exciting, from their ability to be delivered systemically with preference for specific tissues to their ability to provide a label for cells through being endocytosed, can also be risks that must be assessed. The FDA task force recommendations are broad but include awareness of the potential changes in pharmacokinetics and pharmacodynamics as the length scales of materials change as well as the potential for novel biological interactions. They recommended careful characterization of nanomaterials with respect to surface chemistry, crystallinity, and aggregation with a particular note that the last could fundamentally change the behavior of nanomaterials *in vivo*. As noted above, looking carefully at biodistribution and clearance is an important first step toward determining the potential biological interactions nanomaterials may have *in vivo*. Just as nanomaterials can augment imaging modalities, new imaging approaches may allow more efficient longitudinal analyses of nanomaterials, which could lead to faster and greater insight into the potential of these materials and their risk assessment.

Ultimately, one of the biggest issues with translation is the need for financial resources. Investors are generally more conservative than a decade ago, and if one is seeking to move technology to the clinic, one is likely to have to do more of the preclinical and, potentially, clinical work before being able to license technologies successfully. It is a tremendously intimidating and time-consuming process that often has far less appeal than the initial proof-of-principle work. It would be wonderful to develop a new technology, then to hand it off and to move to the next exciting idea, but if we want to see new therapies in the clinic, as a community, we need to build the infrastructure to support translation and we need to shepherd technologies further along the pipeline and to reduce the risk. It requires the commitment of the investigators and the support of their institutions, but the payoff is being able to offer new and better therapies to patients.

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REFERENCES AND NOTES

1. Ramirez, A. Personal Communication, New Haven, 2008.
2. Cavaletti, G. Peripheral Neurotoxicity of Platinum-Based Chemotherapy. *Nat. Rev. Cancer* 2008, *8*, 1 page following 71; author reply 1p following 71.
3. Hahn, M. A.; Singh, A. K.; Sharma, P.; Brown, S. C.; Moudgil, B. M. Nanoparticles as Contrast Agents for *In-Vivo* Bioimaging: Current Status and Future Perspectives. *Anal. Bioanal. Chem.* **2011**, *399*, 3–27.
4. Hare, J. M.; Traverse, J. H.; Henry, T. D.; Dib, N.; Strumpf, R. K.; Schulman, S. P.; Gerstenblith, G.; DeMaria, A. N.; Denktas, A. E.; Gammon, R. S.; *et al.* A Randomized, Double-Blind, Placebo-Controlled, Dose-Escalation Study of Intravenous Adult Human Mesenchymal Stem Cells (Prochymal) after Acute Myocardial Infarction. *J. Am. Coll. Cardiol.* **2009**, *54*, 2277–2286.
5. Auletta, J. J.; Cooke, K. R. Bone Marrow Transplantation: New Approaches to Immunosuppression and Management of Acute Graft-*versus*-Host Disease. *Curr. Opin. Pediatr.* **2009**, *21*, 30–38.
6. Miller, R. H.; Bai, L.; Lennon, D. P.; Caplan, A. I. The Potential of Mesenchymal Stem Cells for Neural Repair. *Discov. Med.* **2010**, *9*, 236–242.
7. Chamberlain, G.; Fox, J.; Ashton, B.; Middleton, J. Concise Review: Mesenchymal Stem Cells: Their Phenotype, Differentiation Capacity, Immunological Features, and Potential for Homing. *Stem Cells* **2007**, *25*, 2739–2749.
8. Barbash, I. M.; Chouraqui, P.; Baron, J.; Feinberg, M. S.; Etzion, S.; Tessone, A.; Miller, L.; Guetta, E.; Zipori, D.; Kedes, L. H.; *et al.* Systemic Delivery of Bone Marrow-Derived Mesenchymal Stem Cells to the Infarcted Myocardium: Feasibility, Cell Migration, and Body Distribution. *Circulation* **2003**, *108*, 863–868.
9. Meirelles Lda, S.; Fontes, A. M.; Covas, D. T.; Caplan, A. I. Mechanisms Involved in the Therapeutic Properties of Mesenchymal Stem Cells. *Cytokine Growth Factor Rev.* **2009**, *20*, 419–27.
10. FDA Investigational New Drug (IND) or Device Exemption (IDE) Process (CBER); http://www.fda.gov/BiologicsBloodVaccines/DevelopmentApprovalProcess/InvestigationalNewDrugINDorDeviceExemptionIDEProcess/default.htm (accessed April 7, 2011).
11. Shapiro, E. M.; Sharer, K.; Skrtic, S.; Koretsky, A. P. *In Vivo* Detection of Single Cells by MRI. *Magn. Reson. Med.* **2006**, *55*, 242–249.
12. Chen, C. L.; Zhang, H.; Ye, Q.; Hsieh, W. Y.; Hitchens, T. K.; Shen, H. H.; Liu, L.; Wu, Y. J.; Foley, L. M.; Wang, S. J.; *et al.* New Nano-Sized Iron Oxide Particle with High Sensitivity for

Cellular Magnetic Resonance Imaging. *Mol. Imaging Biol.* 2010, DOI: 10.1007/s11307-010-0430-x.
13. Yang, Y.; Schumacher, A.; Yang, Y.; Liu, J.; Shi, X.; Hill, W. D.; Hu, T. C. Monitoring Bone Marrow-Originated Mesenchymal Stem Cell Traffic to Myocardial Infarction Sites Using Magnetic Resonance Imaging. *Magn. Reson. Med.* 2011, DOI: 10.1002/mrm.22735.
14. Karussis, D.; Karageorgiou, C.; Vaknin-Dembinsky, A.; Gowda-Kurkalli, B.; Gomori, J. M.; Kassis, I.; Bulte, J. W.; Petrou, P.; Ben-Hur, T.; Abramsky, O.; *et al.* Safety and Immunological Effects of Mesenchymal Stem Cell Transplantation in Patients with Multiple Sclerosis and Amyotrophic Lateral Sclerosis. *Arch. Neurol.* **2010**, *67*, 1187–1194.
15. Matuszewski, L.; Persigehl, T.; Wall, A.; Schwindt, W.; Tombach, B.; Fobker, M.; Poremba, C.; Ebert, W.; Heindel, W.; Bremer, C. Cell Tagging with Clinically Approved Iron Oxides: Feasibility and Effect of Lipofection, Particle Size, and Surface Coating on Labeling Efficiency. *Radiology* **2005**, *235*, 155–161.
16. Berman, S. C.; Galpoththawela, C.; Gilad, A. A.; Bulte, J. W.; Walczak, P. Long-Term MR Cell Tracking of Neural Stem Cells Grafted in Immunocompetent *versus* Immunodeficient Mice Reveals Distinct Differences in Contrast between Live and Dead Cells. *Magn. Reson. Med.* **2011**, *65*, 564–574.
17. Lorusso, D.; Di Stefano, A.; Carone, V.; Fagotti, A.; Pisconti, S.; Scambia, G. PEGylated Liposomal Doxorubicin-Related Palmar-Plantar Erythrodysesthesia (`Hand-Foot' Syndrome). *Ann. Oncol.* **2007**, *18*, 1159–1164.
18. von Moos, R.; Thuerlimann, B. J.; Aapro, M.; Rayson, D.; Harrold, K.; Sehouli, J.; Scotte, F.; Lorusso, D.; Dummer, R.; Lacouture, M. E.; *et al.* PEGylated Liposomal Doxorubicin-Associated Hand-Foot Syndrome: Recommendations of an International Panel of Experts. *Eur. J. Cancer* **2008**, *44*, 781–790.
19. Farokhzad, O. C.; Cheng, J.; Teply, B. A.; Sherifi, I.; Jon, S.; Kantoff, P. W.; Richie, J. P.; Langer, R. Targeted Nanoparticle-Aptamer Bioconjugates for Cancer Chemotherapy *In Vivo*. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 6315–6320.
20. Gu, F.; Zhang, L.; Teply, B. A.; Mann, N.; Wang, A.; Radovic-Moreno, A. F.; Langer, R.; Farokhzad, O. C. Precise Engineering of Targeted Nanoparticles by Using Self-Assembled Biointegrated Block Copolymers. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 2586–2591.
21. Cerchia, L.; de Franciscis, V. Targeting Cancer Cells with Nucleic Acid Aptamers. *Trends Biotechnol.* **2010**, *28*, 517–525.
22. Morton, J. G.; Day, E. S.; Halas, N. J.; West, J. L. Nanoshells for Photothermal Cancer Therapy. *Methods Mol. Biol.* **2010**, *624*, 101–117.
23. Gobin, A. M.; Lee, M. H.; Halas, N. J.; James, W. D.; Drezek, R. A.; West, J. L. Near-Infrared Resonant Nanoshells for Combined Optical Imaging and Photothermal Cancer Therapy. *Nano Lett.* **2007**, *7*, 1929–1934.
24. Stathopoulos, G. P.; Antoniou, D.; Dimitroulis, J.; Michalopoulou, P.; Bastas, A.; Marosis, K.; Stathopoulos, J.; Provata, A.; Yiamboudakis, P.; Veldekis, D.; *et al.* Liposomal Cisplatin Combined with Paclitaxel *versus* Cisplatin and Paclitaxel in Non-Small-Cell Lung Cancer: A Randomized Phase III Multicenter Trial. *Ann. Oncol.* **2010**, *21*, 2227–2232.
25. Scott, K. F.; Sajinovic, M.; Hein, J.; Nixdorf, S.; Galettis, P.; Liauw, W.; de Souza, P.; Dong, Q.; Graham, G. G.; Russell, P. J. Emerging Roles for Phospholipase A2 Enzymes in Cancer. *Biochimie* **2010**, *92*, 601–610.
26. Jensen, S. S.; Andresen, T. L.; Davidsen, J.; Hoyrup, P.; Shnyder, S. D.; Bibby, M. C.; Gill, J. H.; Jorgensen, K. Secretory Phospholipase A2 as a Tumor-Specific Trigger for Targeted Delivery of a Novel Class of Liposomal Prodrug Anticancer Etherlipids. *Mol. Cancer Ther.* **2004**, *3*, 1451–1458.
27. de Jonge, M. J.; Slingerland, M.; Loos, W. J.; Wiemer, E. A.; Burger, H.; Mathijssen, R. H.; Kroep, J. R.; den Hollander, M. A.; van der Biessen, D.; Lam, M. H.; *et al.* Early Cessation of the Clinical Development of LiPlaCis, a Liposomal Cisplatin Formulation. *Eur. J. Cancer* **2010**, *46*, 3016–3021.
28. *Nanotechnology: A Report of the U.S. Food and Drug Administration Nanotechnology Task Force*; FDA: Rockville, MD, 2007.
29. Zhang, X. D.; Wu, H. Y.; Wu, D.; Wang, Y. Y.; Chang, J. H.; Zhai, Z. B.; Meng, A. M.; Liu, P. X.; Zhang, L. A.; Fan, F. Y. Toxicologic Effects of Gold Nanoparticles *In Vivo* by Different Administration Routes. *Int. J. Nanomed.* **2010**, *5*, 771–781.
30. Shvedova, A. A.; Kagan, V. E. The Role of Nanotoxicology in Realizing the `Helping without Harm' Paradigm of Nanomedicine: Lessons from Studies of Pulmonary Effects of Single-Walled Carbon Nanotubes. *J. Intern. Med.* **2010**, *267*, 106–118.
31. Semete, B.; Booysen, L.; Lemmer, Y.; Kalombo, L.; Katata, L.; Verschoor, J.; Swai, H. S. *In Vivo* Evaluation of the Biodistribution and Safety of PLGA Nanoparticles as Drug Delivery Systems. *Nanomedicine* **2010**, *6*, 662–671.
32. Bertram, J. P.; Williams, C. A.; Robinson, R.; Segal, S. S.; Flynn, N. T.; Lavik, E. B. Intravenous Hemostat: Nanotechnology To Halt Bleeding. *Sci. Transl. Med.* 2009, *1*, 11ra22.