Cancer Nanotechnology Plan

Office of Cancer Nanotechnology Research
Center for Strategic Scientific Initiatives (CSSI)

National Cancer Institute/ NIH

November 2010

Table of Content

Foreword

The NCI Alliance for Nanotechnology in Cancer (ANC) was launched on the premise that nanotechnology based materials and devices can strongly benefit cancer research and clinical oncology. They can also contribute to new solutions in molecular imaging and early detection, *in vivo* imaging, and multi-functional therapeutics for effective cancer treatment. The direction and strategy behind Phase I (funding period of 2005 to 2010) of the Alliance were derived from the Cancer Nanotechnology Plan (CaNanoPlan) published in 2004.

The new CaNanoPlan 2010 summarizes the present state of significant areas in the field and builds upon recent discoveries. We asked several investigators participating in Phase I of the program to contribute a chapter; we also drew on the opinions voiced at the series of Strategic meetings held at NCI. Each chapter presents the current status of development and also highlights avenues for growth and opportunity, elucidates clinical applications for the technologies, and forecasts what goals might be achieved in the next 3-10 years.

We, the NCI Office of Cancer Nanotechnology Research, would like to thank all who contributed to CaNanoPlan 2010. Establishing forward strategy is important – there are always multiple paths to take and optimizing the ones we do take will bring us all closer to the goal of achieving new and more effective ways of diagnosing, treating, and preventing cancer. These efforts will ultimately change the lives of cancer patients.

.

Office of Cancer Nanotechnology Research/ Center for Strategic Scientific Initiatives
National Cancer Institute/ NIH

Piotr Grodzinski

Dorothy Farrell, George Hinkal, Sara S. Hook, Nicholas Panaro, Krzysztof Ptak

Introduction

Sara S. Hook, Krzysztof Ptak, Dorothy Farrell, George Hinkal, Nicholas Panaro, and Piotr Grodzinski

Office of Cancer Nanotechnology Research, CSSI, National Cancer Institute, NIH, Bethesda, MD

The complexity of cancer as a disease

Cancer remains one of the most complex diseases affecting humans and, despite the impressive advances that have been made in molecular and cell biology, how cancer cells progress through carcinogenesis and acquire their metastatic ability is still widely debated. The idea that cancer might be attributed to inherent changes within the organism's own genome did not arise until after the discovery that retroviruses could transform host cells and often they contain variants of cellular genes which are necessary for oncogenic transformation. Consequently, for perhaps nearly twenty years, the field of oncology was synonymous with virology and a major focus was on identifying these proto-oncogenes or genes that could be turned into cancer-causing genes. Today, cancer is recognized as a highly heterogeneous disease and over 100 distinct types have been described with various tumor subtypes found within specific organs. It is now also recognized that genetic and phenotypical variability primarily determines the self-progressive growth, invasiveness, and metastatic potential of neoplastic disease and its response or resistance to therapy. It seems that this multi-level complexity of cancer explains the clinical diversity of histologically similar neoplasias.

Recent advances in other disciplines have uncovered that in addition to virus infection, disregulation of many normal cellular processes such as gene regulation, cell cycle control, DNA repair and replication, checkpoint signaling, differentiation, and apoptosis, etc. can lead to cancer. The mechanisms of transformation can be complex with multiple pathways affected. For example, genetic changes in the p53 gene resulting in loss of heterozygosity are known to affect the pattern of gene activation and repression, dampen cell cycle checkpoints, and incapacitate the induction of apoptosis (Farnebo *et al.*, 2010). In addition to multiple pathways being compromised in tumor cells, tumors can arise in a cell- or tissue-specific manner. For instance, mutations in the breast cancer susceptibility gene, *BRCA1*, are associated with approximately half of the inherited forms of breast and ovarian cancer, but they do not predispose carriers to most other forms of cancer even though the gene is ubiquitously expressed and is involved in the fundamental processes of transcriptional regulation and DNA repair (Linger and Kruk, 2010). While some times there are common mutations frequently associated with many cancers, the majority of cancers arise from a diverse array of malfunctions that result in a tumor that is unique to that patient. The complexity of cancer combined with an avalanche of basic science research uncovering the plethora of pathways that feed into cellular growth control reveals many potential therapeutic targets. As such, there is a critical need for cancer biologists with a broad knowledge of the mechanisms of tumorigenesis to team up with clinical oncologists to address just how this information can be utilized to advance clinical therapies.

The need to advance cancer clinical therapies

To this day, the mainstay of cancer treatment has been the same for nearly 40 years and consists of surgical resection, radiation, and/or chemotherapy. This approach involves physically removing as much of the tumor bulk as possible then subjecting the entire body to agents that kill cells by non-selectively damaging the DNA of both cycling tumor and healthy cells. These therapies have limited effectiveness, high cytotoxicity, and untoward side effects. Additionally, the nature of the disease is such that unless all tumor cells are destroyed the cancer will eventually return, often in a form more aggressive and more refractory to treatment. There is a distinct paucity of effective therapies for cancers such as pancreatic and ovarian, which have relatively lower survival rates compared with other types of cancers and where most patients present with advanced stages of the disease at the time of diagnosis. Thus, there is a critical need for not only specific, effective therapies without side effects, but also mechanisms for early detection to ensure that therapies have the best opportunity to be timely and effective.

Nanotechnology approaches for cancer

The National Cancer Institute (NCI) has recognized these critical clinical deficiencies and has been on the forefront of identifying and developing new and innovative ways to approach cancer diagnosis, treatment, and management. Having witnessed substantial technological advances in the field of nanotechnology in various disciplines including physical sciences, engineering, physics, and chemistry in developing new materials and devices to be used in electronics and energy conservation, the NCI recognizes nanotechnology as an exciting and promising approach to address cancer applications as well.

Nanotechnology involves research and technology development at the atomic, molecular, or macromolecular levels and allows the creation and use of functionalized structures, devices, and systems that take advantage of specific properties of matter that exist at the nanoscale. Nanoscale structures can be manipulated on the atomic scale and integrated into larger material components, systems, and architectures. The potential for using nanotechnology in medicine and especially in the area of cancer is vast. For example, nanoparticles targeting tumor cells, using the knowledge we have about cellular biology, will enable clinicians to deliver therapy specifically to the tumor while reducing unwanted side effects. In addition, increased capacity to image tumor cells will enable earlier diagnosis, confer increased accuracy for surgical resection, offer real-time assessment of treatment effectiveness, and enhance monitoring for metastasis or primary tumor re-growth. Furthermore, powerful chemotherapeutic agents that were abandoned due to toxic side effects can be resurrected using nanotechnology enabled delivery systems thus enabling them to become viable treatment options.

Establishment of the Alliance for Nanotechnology in Cancer (Phase I)

In the late 1990s, the NCI established the Unconventional Innovations Program (UIP) to work with university research groups and small companies to evaluate potential nanotechnology applications in cancer. Building upon the productive experience of the UIP program, NCI established the Alliance for Nanotechnology in Cancer (ANC) program in September 2004. The overarching goal of this program has been to discover and develop nanotechnologies for applications ranging from discovery through translation and delivery of innovative, clinically relevant technologies for cancer prevention, diagnosis, and treatment. The Alliance's development model calls for the most promising strategies discovered and developed by Alliance grantees to be handed off to private sector partners for clinical translation and commercial development. In its first five years, the program focused on basic research and developmental efforts in six major challenge areas: molecular imaging and early detection, *in vivo* nanotechnology imaging systems, reporters of efficacy, multi-functional therapeutics, prevention and control, and research enablers.

The Phase I funding period (2005-2010) involved funding a constellation of eight Centers for Cancer Nanotechnology Excellence (CCNEs) and twelve Cancer Nanotechnology Platform Partnerships (CNPPs), together with eleven Multi-disciplinary Research Training and Team Development awards. CCNE teams were focused on developing integrated nanotechnology solutions with future potential for clinical applications. The CCNEs evolved into research organisms having distinct area(s) of technical excellence and core resources (e.g. fabrication and materials development, diagnostic assays, toxicology, drug delivery, *in vivo* technology validation, informatics). The CNPPs were individual research projects. The CCNEs provided infrastructure and translational support to the CNPPs where appropriate. The Multi-disciplinary Research Training and Team Development program was dedicated to training graduate students and post-doctoral fellows. The NCI also formed an intramural laboratory, the Nanotechnology Characterization Laboratory (NCL), to serve as a centralized facility to characterize nanomaterials. The NCL is a formal collaboration with the National Institute of Standards and Technology (NIST) and U.S. Food and Drug Administration (FDA). The NCL's role in the Alliance was to perform standardized characterizations and safety evaluation of nanoscale materials developed by researchers from academia, government, and industry. The NCL will have a more integral role in the next funding phase (Phase II) of the program as more technologies advance towards clinical development. In addition, there are some slight shifts in the programmatic focus as well as additional funding mechanisms that will strengthen training and collaborative efforts.

Challenges to Developing New Nanomaterials

*Joseph M. DeSimone and Robert Petros**

Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill and North Carolina State University, Raleigh, NC; *Currently at University of Texas, Denton, TX

Engineered nanoparticles have the potential to revolutionize the diagnosis and treatment of many diseases; for example, by allowing the targeted delivery of a drug to particular subsets of cells. However, so far, such nanoparticles have not proven capable of surmounting all of the biological barriers required to achieve this goal. Nevertheless, advances in nanoparticle engineering, as well as the understanding of the importance of nanoparticle characteristics such as size, shape and surface properties for biological interactions, have created new opportunities for the development of nanoparticles for therapeutic applications. In the past two decades, several therapeutics-based on nanoparticles have been successfully introduced for the treatment of cancer, pain, and infectious diseases (Davis *et al*., 2008; Petros and DeSimone, 2010; Zhang *et al*., 2008). These therapeutics harness the opportunities provided by nanomaterials to target the delivery of drugs more specifically, improve solubility, extend half-life, improve therapeutic index, and reduce immunogenicity.

General nanoparticle characteristics

The size, surface characteristics and shape of a nanoparticle play a key role in its biodistribution *in vivo*. Spherically shaped, passively targeted, nanoparticles less than 5 nm in diameter are rapidly cleared from circulation via extravasation or renal clearance, and as particle size increases from the nanometer range to ~15 micrometers, accumulation occurs primarily in the liver, spleen and bone marrow. Nanoparticle behavior in the size range ~10 nm to ~15 micrometers varies widely in terms of biodistribution and cellular uptake of nanoparticles in this range is heavily dependent on cell type. Under normal circumstances, nanoparticles are mechanically filtered by sinusoids in the spleen and removed from circulation via cells of the reticuloendothelial system (RES). In addition, Kuppfer cells in the liver, also part of the RES, play a key role in particle removal (Petros and DeSimone, 2010).

The propensity for accumulation of nanoparticles in cells of the RES is dictated by specific proteins adsorbed *in vivo* to the particle surface, which can be influenced through modifications of surface characteristics. This process of protein adsorption, known as opsonization, begins immediately after particles come in contact with plasma. The exact nature of the types and quantities of proteins and their conformations dictate the body's reaction. The mechanisms involved in this process are not well understood; however, the major opsonins are known. Immunoglobulin (Ig) and complement proteins are the predominant contributors to the recognition of foreign particles by the cells of the RES (that is, macrophages). Complement activation can further complicate targeted drug delivery by inducing hypersensitivity reactions. Finally, particulate matter larger than ~15 micrometers is removed from circulation via mechanical filtration in capillaries and can be lethal depending on dose.

Current methods for addressing the negative attributes associated with opsonization have focused almost exclusively on slowing the process by rendering the particle surface more hydrophilic or by neutralizing surface charge. The predominant strategy has been to adsorb or graft a hydrophilic polymeric coating, such as polyethylene glycol (PEG) to the surface of the particle. These polymer chains, depending on density, act as a steric brush that imparts resistance to protein adsorption. However, the PEG effect is transient, so eventual opsonization and macrophage clearance still occur (Howard *et al*., 2008).

Although studies have demonstrated the positive effects that can be achieved by dictating which proteins adsorb to the surface of nanoparticles, methods that have been employed in the design of potential nanoparticle therapeutics to date are limited in scope (Petros and DeSimone, 2010). Particle size is also known to influence the mechanism of cellular internalization — that is, macropinocytosis, clathrin-mediated endocytosis, or caveolin-mediated endocytosis — which in turn dictates the microenvironments an engineered nanoparticle experiences upon internalization (Figure 1). Detailed knowledge of the mode of entry into the cell is invaluable because it could be used to design an engineered nanoparticle targeted to specific intracellular microenvironments, as discussed in more depth later. As noted above, so far, the impact of size on biodistribution

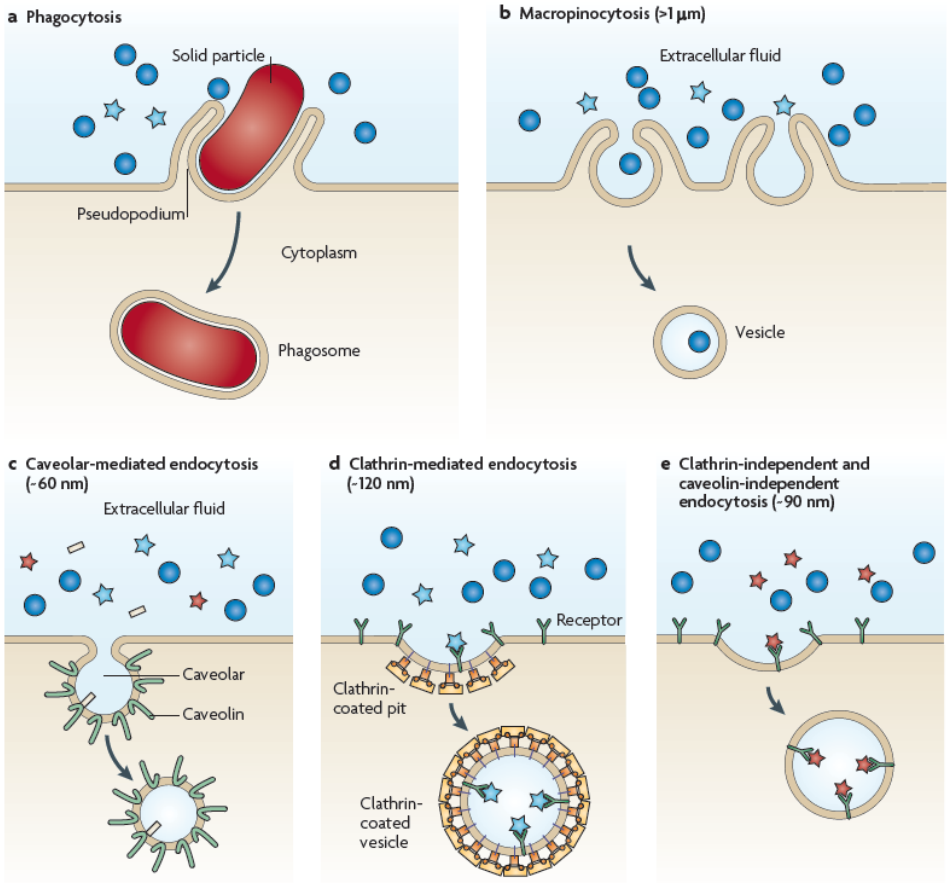

Figure 1 **Modes of cellular internalization of nanoparticles and respective size limitations. (a) Internalization of large particles is facilitated by phagocytosis. (b) Nonspecific internalization of smaller particles (>1 µm) can occur through macropinocytosis. (c) Smaller nanoparticles can be internalized through several pathways, including caveolar-mediated endocytosis, (d) clathrin-mediated endocytosis and (e) clathrin-independent and caveolin-independent endocytosis , with each being subject to slightly different size constraints. Nanoparticles are represented by blue circles (> 1 µm), blue stars (about 120 nm), red stars (about 90 nm) and yellow rods (about 60 nm) (reprinted with permission from Petros and DeSimone, 2010, Copyright, Nature Publishing Group).**

and cellular internalization has largely been elucidated using spherically-shaped particles. However, recent findings (Champion and Mitragotri, 2006; Decuzzi *et al*., 2010; Geng *et al*., 2007; Gratton *et al*., 2008) indicate that particle shape is as important, if not more so, than size in controlling key aspects of both these phenomena. For example, in HeLa cells there is a clear correlation between the rate of internalization and the shape and size of the particles (Gratton *et al*., 2008). Interestingly, they also showed that particles with similar volumes but different shapes were internalized at drastically different rates. In addition, the geometry of interaction between a cell and particle can induce or inhibit internalization (Champion and Mitragotri, 2006) and the shape has a significant impact on biodistribution (Geng *et al*., 2007) with filamentous engineered nanoparticles having single dimensions as long as 18 µm exhibiting circulation half-lives of ~5 days, which was much longer than even "stealth" liposomes.

Methods for incorporating cargo into engineered nanoparticles can be classified into two broad categories. In one category, the cargo is physically entrapped in or absorbed onto the nanoparticle through non-covalent interactions. The second category includes examples where the cargo has been directly attached to the nanoparticle matrix via degradable or non-degradable covalent bonds. The use of stimuli-responsive materials allows for release of cargo once the engineered nanoparticle reaches its intended location *in vivo*. The bulk composition of the engineered nanoparticle must be carefully chosen based on its biocompatibility, immunotoxicity (Dobrovolskaia and McNeil, 2007), and its ability to solubilize or sequester the cargo of interest. Beyond these basic features of nanoparticle design, a multitude of approaches for targeting specific cellular populations or altering the biodistribution of engineered nanoparticles *in vivo* are being developed. Targeting has been achieved using three predominant strategies that rely on either active or passive modes of

action, which can be further characterized as selective or non-selective.

General biological barriers

To achieve intracellular drug delivery, strategies for overcoming a variety of biological barriers — from the system level, to the organ level, to the cellular level — are needed. The initial barriers encountered depend on the mode of administration (that is, inhalation, oral, intravenous, or intraperitoneal injection). The degree of success in utilizing each of these modes of entry can be strongly influenced by attributes of the nanoparticles themselves. For example, size can be a major determinant for effective pulmonary delivery, whereas successful strategies for oral administration must address carrier stability during the harsh conditions in the gastrointestinal tract, while simultaneously targeting a specific site for entry. Intravenous injections must overcome the RES if prolonged circulation is to be attained and a method for escaping the endothelium is required in order to exit circulation into the desired tissue. Intraperitoneal injection allows tissue-specific delivery; however, nanoparticles can be rapidly cleared via the lymphatic system unless special steps are taken to avoid this.

Organ level: For intravenously injected engineered nanoparticles, avoidance of multiple organ-level clearance mechanisms, such as those operating in the spleen and liver, must be compensated for if the carrier is to reach its intended destination (Petros and DeSimone, 2010). Fenestrations in the spleen typically do not exceed 200-500 nm in width so particles larger than ~200 nm must be engineered to have some degree of deformability in order to remain in circulation. A method for attenuating the activity of cells of the RES is also usually necessary to prolong circulation times.

Several strategies can be employed to circumvent carrier removal by macrophages. First, decoy carriers can be pre-injected to saturate the phagocytic capacity of the RES, followed by injection of carriers containing the active ingredient. Second, altering the hydrophilicity of the carrier surface has been shown to reduce the rate of protein opsonization, which ultimately marks carriers for sequestration and removal. Third, specific proteins can be adsorbed or covalently linked onto the surface of the carrier that help minimize or avoid complement activation. Finally, markers-of-self can be attached to the surface of the carrier.

In view of these desired characteristics of engineered nanoparticles, red blood cells (RBCs) could be considered as a prototypical model (Petros and DeSimone, 2010). First, they are capable of traversing biological barriers that are impenetrable to objects less than one tenth their size and manage to avoid clearance by macrophages for up to three months. A number of factors are believed to contribute to their extended circulation, including their shape, deformability (which allows them to navigate through much smaller sinusoids in the spleen), and the presence of ligands, such as CD47 and CD200 that bind to inhibitory receptors expressed by macrophages (absence of these markers leads to immediate removal of RBCs by macrophages).

Cellular level: There are several biological barriers at the cellular level that an engineered nanoparticle must overcome. The cell membrane blocks diffusion of complexes larger than ~1 kDa. Several endocytic mechanisms can be engaged to facilitate internalization of a carrier. The details of the exact mode of endocytosis are important because they dictate the path of trafficking through various possible subcellular compartments. For example, engineered nanoparticles internalized via clathrin-mediated endocytosis are destined for lysosomal compartments, whereas those internalized via a caveolin-mediated process are not. In the former, endosomal escape must occur prior to fusion with a lysosome to prevent degradation of the cargo under harsh lysosomal conditions. In either case, endosomal escape is usually necessary to allow access of the carrier to the desired subcellular compartment whether it is the cytosol, mitochondria, or nucleus.

Ligands conjugated to the surface of engineered nanoparticles can influence the mode of cellular internalization. Ligands such as folic acid, albumin, and cholesterol have been shown to facilitate uptake via caveolin-mediated endocytosis whereas ligands for glycoreceptors promote clathrin-mediated endocytosis (Figure 1). Alternatively, macropinocytosis, a non-caveolin, non-clathrin-mediated process, can be engaged by incorporating cell-penetrating peptides, such as a TaT peptide (*trans*-activating transcriptional activator) into the design of engineered nanoparticles. What is not well understood is the interdependent role(s) of particle size, shape and flexibility with ligand type, density, multiplexing, and regio-specific labeling on the particles. The nuclear membrane is the final barrier for many engineered nanoparticles although recent advances have been made in the ability to target specific organelles (Petros and DeSimone, 2010).

Conclusions

Several particle characteristics have emerged as central to the function of engineered nanoparticles and should therefore be used to guide future design efforts.

Particle size: For rigid, spherical particles, the 100-200 nm size range has the highest potential for prolonged circulation because they are large enough to avoid uptake in the liver, but small enough to avoid filtration in the spleen. The design of non-spherical and/or flexible particles can, however, dramatically extend the particle's circulation time *in vivo*. The same general principles govern the biodistribution profile of these particles: for long-circulating particles, uptake by the liver and spleen must be avoided. This can be accomplished practically by engineering deformability into particles >300 nm or by keeping at least one dimension of the particle on a length scale >100 nm to prevent accumulation in the liver while maintaining at least two dimensions at <200 nm, thereby allowing the particle to navigate the sinusoids of the spleen.

Particle shape: In some instances, the effects of particle shape can be intimately coupled to particle size, as described for long-circulating non-spherical particles. Particle geometry also plays a key role in particle internalization. Although preliminary data exist demonstrating the marked effects of particle shape, the optimum parameters for engineered nanoparticles have yet to be determined.

Surface characteristics: This particle attribute has three vital roles in the function of engineered nanoparticles. First, surface chemistry is known to heavily influence the process of opsonization, which ultimately dictates RES response. Several methods designed to circumvent the activation of the immune system are described above. Second, to achieve cellular targeting, ligands known to bind cell-surface receptors of selected cells should be included in the design of engineered nanoparticles. Third, if organelle targeting is also required, those ligands must also be incorporated into surface design.

Release of therapeutics: Achieving tailored, activated release still represents a major barrier in the field of engineered nanoparticles. The predominant strategies to date incorporate materials that are enzymatically degradable, pH-sensitive, or reductively labile. The latter category facilitates either bond-breaking between drug and carrier or destabilization of the carrier upon reaching the intended site of action.

In summary, great strides have been made in the design and application of engineered nanoparticles over the last 50 years. However, significant challenges remain. Our ability to shepherd cargo to sites in the body to achieve precisely defined therapeutic effects is still in its infancy. Development of the requisite tools to dictate events occurring at the biotic/abiotic interface requires a highly interdisciplinary approach, which is benefiting tremendously from the increasing collaborations amongst scientists from the physical and life sciences. As this trend continues, the potential of appropriately engineered nanoparticles of increasing complexity and efficacy will be realized.

Milestones

3-year:

- Adopt standardized techniques for the characterization of nanoparticles both *in vitro* and *in vivo*.
- Design nanoparticle compositions with reproducible, activated, release properties *in vivo*.
- Conduct clinical trials of a variety of nanoparticles.

5-year:

- Determine the effects of surface regiochemistry on nanoparticle internalization and biodistribution.
- Expect the first polymer-based, nanoparticle therapeutic to be approved by the FDA.

10-year:

- Complete a map of nanoparticle biodistribution as a function of size, shape, deformability, zeta potential, and surface chemistry.
- Develop several cancer vaccines.
- Create long-circulating nanostructures via active strategies. Next generation methods should focus on engineering particle shape and modulus and the tailoring of particle surface chemistry to actively interact with the immune system.

In Vitro Multiplex Protein Assays and Sensors for Cancer Research and Clinical Applications

James R. Heath

Nanosystems Biology Cancer Center and Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA

Traditional *in vitro* measurements for cancer diagnostics have been single-parameter based. Examples include the measurement of prostate specific antigen (PSA) for prostate cancer, or measurement of Cancer Antigen 125 (CA125) for detecting the recurrence of ovarian cancer. However, a recent and growing trend has been to assess the levels of increasingly large panels of molecular biomarkers from ever smaller blood samples or tissue specimens. In this context, genome (DNA) and transcriptome (mRNA) measurements are playing important roles. However, for monitoring evolving health conditions, such as the response of a patient to a drug, assessing immune system status, or for monitoring evolving disease within a patient, measurements of protein biomarkers are the most informative.

In contrast with genome sequencing or mRNA profiling, the cost of protein biomarker measurements has remained relatively stagnant over time. This is for multiple reasons. First, the only reliable and broadly translatable assays for sensitively quantifying protein levels are based upon the use of affinity agents (antibodies). In fact, the gold standard, which is the Enzyme Linked Immunosorbent Assay (ELISA), requires two antibodies per detected protein. Antibodies are expensive, unstable, and often unavailable against their target proteins. The instability of antibodies, and the cross-reactivity of antibodies for non-cognate proteins can, in turn, make it difficult to reliably assess a large panel of proteins. In addition, the cost and time gains that are often achieved via miniaturization are non-trivial to realize for protein assays. For example, the use of microfluidics platforms within modern sequencing machines permits more sequencing more quickly and with less sample. However, antibody arrays are difficult to construct and maintain within microfluidics environments, since the fabrication of such platforms usually requires elevated thermal processing. As a result, even as sequencing technologies march towards (and beyond) sequencing a genome for under $1000, the cost of a single protein assay has remained around $50 per protein. However, there are a number of technology advances, many of them supported within the existing NCI-funded nanotechnology programs that have the potential to increase the flexibility of multiplex protein diagnostic measurements and dramatically decrease cost and performance time. These include (1) approaches that integrate blood and/or tissue handling onto the assay platform; (2) surface chemistries that permit antibody integration into microfluidics chips and that reduce non-selective protein adsorption; (3) miniaturized, multiplex and quantitative measurement platforms; and, perhaps most critical, (4) chemical technologies for the production of physically and chemically robust protein capture agents.

There are many benefits of multiplexed, integrated (blood/tissue handling are integrated onto the assay platform), and miniaturized diagnostic assays. An appropriately designed platform for clinical use can potentially serve as a point-of-care (POC) diagnostic tool, implying that the assay results are available to the patient during the same office visit. Most existing POC devices (pregnancy tests, developing world HIV and Hepatitis tests, etc.) are neither quantitative nor multiplex but they do yield a rapid and often reliable answer to a clinically relevant question.

Integrated assay devices

An integrated, multiplex diagnostic platform can minimize two of the key variables that most detrimentally impact biospecimen quality – handling by laboratory and clinical personnel, and the time between specimen collection and assay completion. Multiplex assays on small volume blood (*e.g.* pinprick) or tissue (*e.g.* skinny needle biopsy) samples can enable higher throughput of patient samples. When coupled with the right biomarkers, such approaches have the potential to accelerate clinical decision making regarding continuation of a therapy, adjusting dosing levels, etc. In addition, such assays can enable more information to be extracted from precious samples, such as circulating tumor cells, tumor infiltrating lymphocytes, cancer stem cells, small biopsy samples from tumor margins, etc. (Figure 2). Finally, highly multiplex assays can assist with the biomarker discovery process,

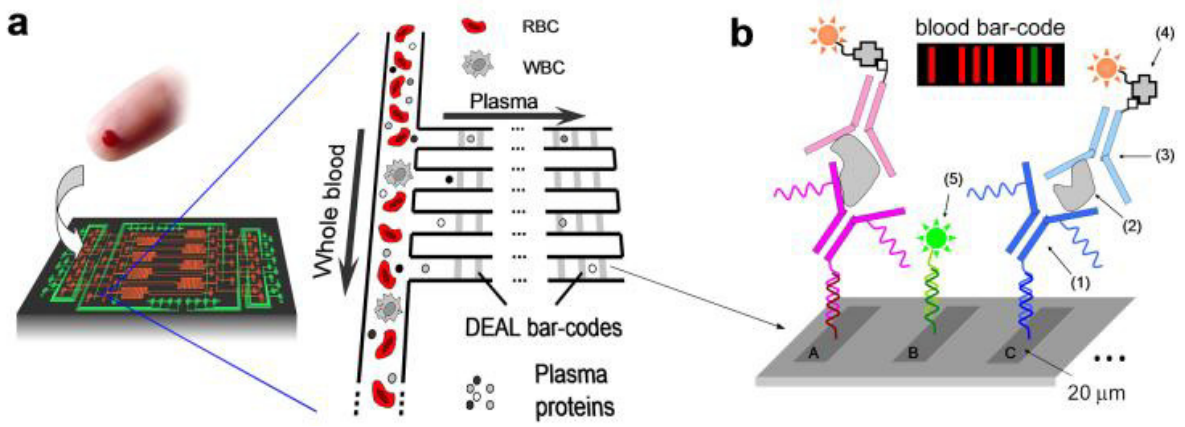

Figure 2 **Design of an integrated blood barcode chip (IBBC). (a) Scheme depicting plasma separation from a fingerprick of blood by harnessing the Zweifach-Fung effect. Multiple DNA-encoded antibody barcode arrays are patterned within the plasma skimming channels for *in situ* protein measurements. (b) Illustration of DEAL barcode arrays patterned in plasma channels for *in situ* protein measurement. A, B, C indicate different DNA codes. (1)-(5) denote DNA-antibody conjugate, plasma protein, biotin-labeled detection antibody, streptavidin-Cy5 fluorescence probe, and complementary DNA-Cy3 reference probe, respectively. The inset represents a barcode of protein biomarkers, which is read out using fluorescence detection. The green bar represents an alignment marker (reprinted with permission from Fan *et al*., 2008, Copyright, Nature Publishing Group).**

since they can permit many potential biomarkers to be assayed at a cost that is only incrementally greater than measuring a single assay. A number of relevant technology advances for multiplex protein cancer diagnostics have occurred over the past 5-10 years and, equally important, the goals of the technology developers have become increasingly aligned with the needs of the cancer biologists and clinical oncologists. Over this same period, certain technologies, such as nanotube (Chen *et al*., 2001; Besteman *et al*., 2003), nanowire or nanocantilever sensors, that were initially viewed as promising have failed to deliver for reasons of robustness, cost, or other practical considerations, although those technologies may still find non-clinical applications (Heath and Davis, 2008; Giljohann and Mirkin, 2009). By contrast, blood and tissue handling on chip (Heath and Davis, 2008) is becoming increasingly sophisticated and effective, even as the platforms have decreased in complexity (Qin *et al*., 2009; Nie *et al*., 2010) and likewise increased in robustness. Multiplexing via spatial (Fan *et al*., 2008) or colorimetric (Giljohann and Mirkin, 2009) encoding has been enabled by various nano- and micro- technologies. Quantitative protein assays with sensitivities far exceeding what was possible a decade ago have been developed (Armani *et al*., 2007; Heath and Davis, 2008), with some already in the clinic. Platforms that can execute multiplex protein assays from a variety of body fluids (Osterfeld *et al*., 2008; Gaster *et al*., 2009) and chip-based rare cell capture and analysis have been reported (Nagrath *et al*., 2007; Kwong *et al*., 2009). Microfluidics strategies that integrate highly multiplex protein assays (Bailey *et al*., 2007) and plasma separation from whole blood have also made it into human trials. In fact, it is likely that platforms that combine microfluidics, surface chemistry, and nanotechnology will dominate multiplex clinical protein biomarker measurements by the end of this decade.

Future developments

The biology of cancer, as well as the demands of clinical oncology, will likely serve as drivers for the further development of micro/nano technologies. As representative examples, drivers include protein biomarker development, understanding the tumor microenvironment, interrogating the functional status of the immune system of cancer patients, interrogating the interrelationship between the immune system and cancer, and stratifying patients and patient responses for molecularly targeted therapies. The best technology solutions will be cost effective, rapid, highly multiplex, and, of course, robust. It is likely that many of those technology solutions are at least already partially in hand. Some associated technology challenges have, as yet, no clear solution.

Practically all of the new nano/micro technologies that have emerged for quantitative, multiplex protein assays for clinical applications rely upon antibodies as the basic protein detection approach. This is a major limitation. The replacement of antibodies with alternative protein capture agents that exhibit the selectivity and affinity of good monoclonal antibodies, and yet are chemically and physically robust, is probably the toughest technology challenge today for multiplex protein diagnostics. Several approaches have emerged, ranging from nucleic acid aptamers (Proske *et al*., 2005) to peptides (Lam *et al*., 1993) to peptide multi-ligands (Agnew *et al*., 2009) assembled via *in situ* click chemistry. None of the approaches, however, has yet been demonstrated to compete effectively with monoclonal antibodies in terms of the combination of cost, ease of production, and selectivity/affinity for the cognate protein. If a solution to this problem does emerge, it will accelerate the development and deployment of many of the micro/nano technologies alluded to above.

Milestones

3-year:

- Develop and refine non-antibody-based methods to detect protein biomarkers.
- Devise mechanisms to incorporate antibodies into microfluidics chips.
- Increase the focus on developing and refining methods for blood and tissue processing within the assay platform.

5-year:

- Incorporate the methodologies developed above into multiplexed, integrated, miniaturized diagnostic assays. Hopefully these will be point-of-care tests.
- Conduct clinical trials on emerging diagnostic tests.
- Gain FDA approval for the first cancer nanotechnology-based diagnostic test.

10-year:

- Increase the use of multiplexed assays applicable to biomarker discovery research.
- FDA approval of various next generation diagnostic tests.

Nanotechnology in Tumor MicroRNA Profiling and Validation

Shanthi Ganesh and Mansoor Amiji

Northeastern University, Boston, MA

Tumor microRNA

MicroRNAs (miRNA) are a class of endogenous small, single stranded non-coding RNA molecules (about 22 nucleotides long) that play key roles in a variety of biological processes such as development, differentiation, proliferation, and cellular apoptosis. They generally function by blocking messenger RNA translation and/or affecting endogenous mRNA degradation (Figure 3). Accumulating evidence indicates that miRNAs are mechanistically involved in the development of various human malignancies, an observation which suggests these molecules represent a promising new class of cancer biomarkers and a significant target for cancer prevention and therapy (Paranjape *et al*., 2009). Many miRNAs function as oncogenes or tumor suppressors, hence they are often dysregulated in a variety of cancers (Ventura and Jacks, 2009). Although major advances have been achieved over the last several years in cancer biology and new targeted therapeutics, the development of early diagnostic methods are still inadequate leading to late diagnoses. The evidence that indicates alterations in miRNA expression levels in various tumor cells as compared to normal cells is considered indicative of the correlation with disease initiation and progression (Visone and Croce, 2009).

Current microRNA profiling technologies

Tumor miRNA profiling is one possible application towards establishing a cancer diagnosis. Two of the widely used high throughput techniques used for miRNA profiling are the solid-phase oligo microarray platform (Liu *et al*., 2004) and the bead-based flow cytometric method (Lu *et al*., 2005). The oligo microarray gene expression profiling technique is based on the development of a microchip containing gene specific oligonucleotide probes generated from hundreds of miRNAs. After immobilizing the microchip to the solid support, the sample containing RNA is hybridized to this chip to get the signal (Liu *et al*., 2004). In addition to using large quantities of material, this semi-quantitative method also carries another limitation of cross hybridization among miRNAs of a similar family. The bead-based profiling method involves both amplification and hybridization and requires flow cytometry for analysis (Lu *et al*., 2005). Capture probes for a specific miRNA are synthesized and attached to a bead that is coded by a mixture of two fluorescent dyes for identification. A cDNA library made from the RNA sample is amplified by a PCR reaction using biotinylated primers, which are then enzymatically reacted with streptavidin-phycoerythrin to emit light of a wavelength that can be registered by a flow cytometer. Although this method is technically demanding as it requires both amplification and hybridization steps during sample analysis which introduce sample variability, it has the advantage of increased specificity in differentiating the expression of closely related miRNAs as well as higher sensitivity. Data obtained from both methods need to be validated by a second method such as northern blot or quantitative real-time PCR to confirm the miRNA expression levels. Profiling hundreds of samples using both of these techniques clearly demonstrated aberrant miRNA expression in numerous tumors compared to their normal counterparts suggesting that a link does exist between miRNA and cancer (Iorio *et al*., 2005; Murakami *et al*., 2006; Leidinger *et al*., 2010).

Nanotechnology in microRNA profiling

Nanotechnology is slowly finding its way into the miRNA profiling world in a variety of highly sensitive novel methods. One system involves a combination of surface polyadenylation (polyA) enzyme chemistry and nanoparticle-amplified surface plasmon resonance imaging (SPRI). Briefly, the RNA sample is first hybridized to a complementary, single-stranded locked nucleic acid (LNA) array or capture probes followed by the addition of poly(A) tails to the surface-bound miRNA. Poly(T) coated gold

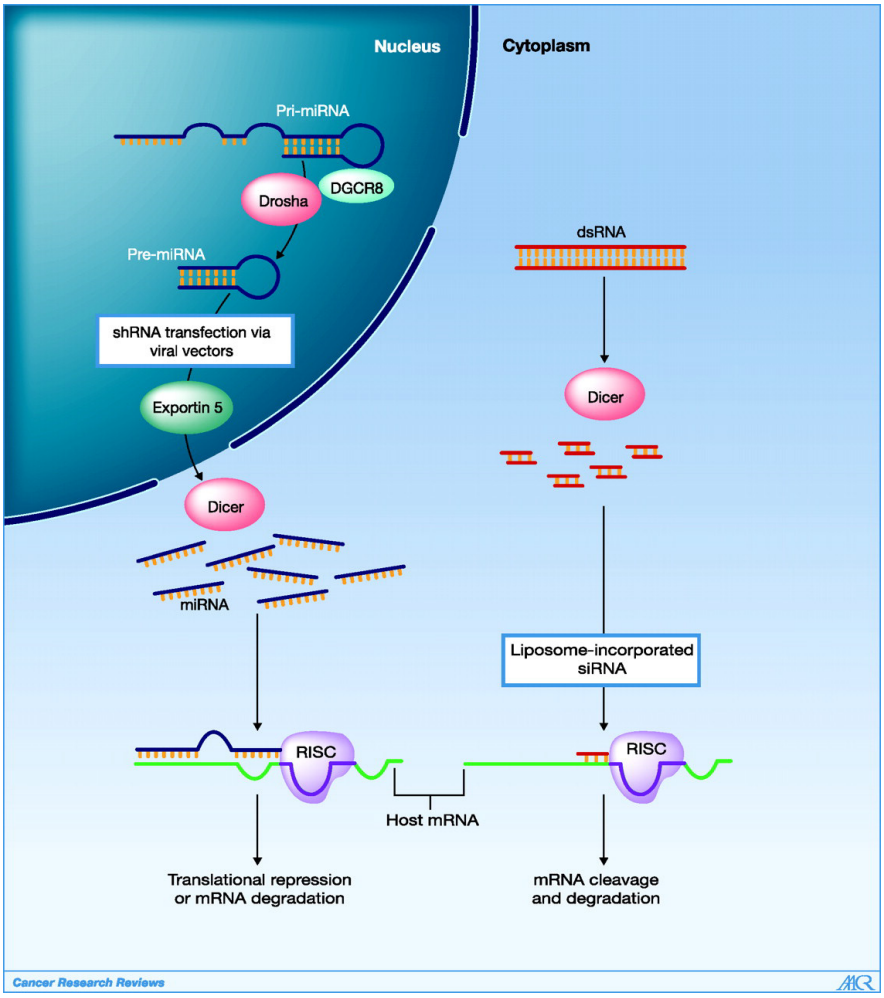

Figure 3 **Multiple components of the RNAi cascade are critical toward maturation of miRNA and siRNA complexes in humans. Altered expression of these entities is associated with poor outcomes and may limit RNAi function in cells. Introduction of exogenous RNAi sequences, such as siRNA, that bypass this machinery, may provide a novel pathway toward drug development in cancer therapeutics (reprinted from Merritt *et al*., 2010, Copyright, American Association for Cancer Research).**

nanoparticles are then hybridized with the poly(A)s present on the surface of bound miRNA for signal amplification and SPRI. A microarray image is obtained from a scanner that detects gold nanoparticles. This novel method is described to be very sensitive and reported to detect miRNAs down to a concentration of 10 fM, detecting a mere 5 attomoles of the miRNA (Fang *et al*., 2006).

Another reported nanotechnology-based method uses a biosensor that has the capacity to detect and quantitate miRNA in the fM range. It uses a microscopic platform made with gold and titanium microelectrodes interspaced with wells containing miRNA capture probes. The miRNA phosphate backbone uses its anionic nature to catalyze the reaction of polyaniline nanowire formation from a solution of cationic aniline particles. This closes an electrical circuit between gapped electrodes and results in an immediate digital readout. The recorded conductance correlates directly to the amount of hybridized miRNA (Fan *et al*., 2007). A method utilizing electrocatalytic nanoparticle tags for microprofiling has also been reported (Gao and Yang, 2006). This involves the generation of isoniazid (an antibiotic) capped OsO_2 nanoparticles and immobilization of oligonucleotide capture probes to an In_2O_3-SnO_2 electrode. After hybridizing the periodate-treated miRNA to the oligonucleotide capture probes, the nanoparticle tags (isoniazid-capped OsO_2 nanoparticles) are brought to the electrode to chemically amplify the signal. The addition of these nanoparticles to the hybridized miRNA molecules leads to the formation of electrocatalytic system generating a measurable current. Although the idea of amplified chemical ligation has been shown with only three miRNAs so far, it could be easily extended to wide range of miRNAs. As reported previously, the methods utilizing nanotechnology also need to be validated by a second method such as northern blot or quantitative PCR to confirm the miRNA expression levels.

These methods have been developed to address the sensitivity and specificity of existing profiling methods. They were also developed to reduce the total RNA required for the assay. Although they are very time consuming,

methods that require hybridization and polymerization steps are reported to be more specific and accurate. These nanotechnology-based procedures have been described to be sensitive to the fM range where previous technologies worked in the picomolar range. In summary, all of the methods used address a variety of specific needs, ranging from cost, sample size, sample quantity, speed, and ability to identify new miRNAs.

miRNA gene profiling, while providing important insights into plant and animal biology, have technical pitfalls associated with the current methodologies that need attention (Nelson *et al*., 2008). For example, various aspects of cellular processing, differential stability of specific miRNAs, and global miRNA expression regulation need special consideration when performing profiling experiments. Additional issues affecting profiling include the impact of pre-clinical variables, the substrate specificity of nucleic acid processing enzymes used in labeling and amplification, and the tissues used in new miRNA discovery and annotation. Another consideration is the cross-comparison between the results of different gene profile platforms. It has been shown previously that different cDNA-based miRNA profiling microarray techniques provide results with lack of reproducible comparability and low accuracy as there is presently no standardized methodology for hybridization-based profiling of miRNA (Yin *et al*., 2008). It is important, therefore, to focus more on technical parameters to increase the validity, reliability, and credibility of the assays.

In summary, a number of key issues need to be addressed to achieve meaningful and reproducible results in miRNA gene expression array studies. These include a well-defined clinical question, a statistically valid experimental design, consideration of tumor heterogeneity, identification of normal controls, and a robust platform using statistical and computational analysis of diagnostic predictors followed by independent validation (Tricoli and Jacobson, 2007). It was also suggested by the experts that accurate miRNA measurements are challenging due to dynamic miRNA expression, high miRNA sequence homology, and the lack of consensus on normalization methods (Tricoli and Jacobson, 2007). One recommendation would be to have probes with control probes with matching melting temperatures. Thus, the usefulness of using miRNA profiles for cancer detection and diagnosis depends on carefully designed translational studies taking into consideration the best methods for sample collection, miRNA isolation, miRNA quantitation, and data analysis.

Milestones

3 year:

- Develop a robust, clinically-relevant multiplexed assay system that can rapidly profile the tumor miRNA in patient samples and aid in early diagnosis of disease.

5 year:

- Complete characterization of tumor miRNA profiles in different types of human solid and hematological cancers as a function of disease progression, aggressiveness, and refractivity.
- Validate and correlate miRNA profiles with other methods of genetic and phenotypic tumor profiling (e.g., histology, western blot, etc.).

10 year:

- Develop a nanotechnology-based platform for rapid characterization of tumor miRNA profiles to allow for patient-specific clinical decision making. Ideally, this device or devices should be multiplexed and allow for small sample analysis such as tumor micro-biopsies.

Targeted Drug Delivery

Dong Moon Shin

Emory University School of Medicine, Atlanta, GA

Targeting tumor cells

The addition of targeting ligands mediates specific interactions between therapeutic nanoparticles (TNPs) and the tumor cell surface. Ligand-targeted therapeutic nanoparticles (TNP) are expected to selectively deliver drugs and especially cytotoxic agents specifically to tumor cells and enhance intracellular drug accumulation. Mechanisms of TNP internalization into target cells via receptor-mediated endocytosis have been well characterized.

Ligands targeting cell surface receptors can be natural molecules like folate or growth factors such as epidermal growth factor (EGF), which have the advantages of lower molecular weights and perhaps lower immunogenicities than antibodies (Figure 4). Modified antibodies can also be used as targeting moieties in an active targeting approach. Monoclonal antibodies (mAb) or antibody fragments, such as antigen binding fragments (Fab') or single chain variable fragments (scFv), are the most frequently used ligands for targeted therapies. Compared with mAbs, antibody fragments can reduce immunogenicity and improve the pharmacokinetic profiles of nanoparticles. In recent years, engineered antibody mimetics called affibodies, such as that against HER2, have been used to conjugate to thermosensitive liposomes (Affisomes) and to poly-(D, L-lactic acid) (PLA)-PEG-maleimide copolymer for delivery of paclitaxel (Alexis *et al*., 2008; Puri *et al*., 2008).

Once active targeting is achieved, the next important question is whether the targeted TNPs can be internalized in the target cells. Drugs released outside the cells can disperse or redistribute to the surrounding normal tissues rather than be delivered exclusively to the cancer

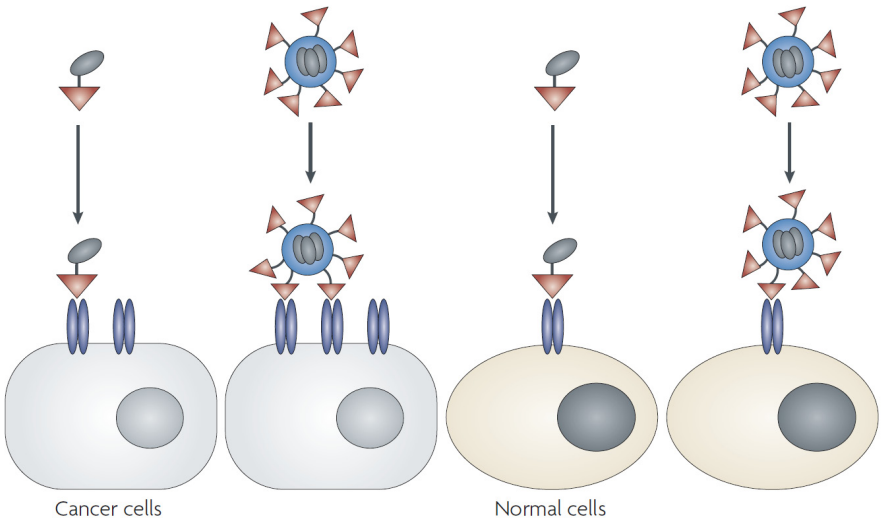

Figure 4 **Nanoparticles with numerous targeting ligands can provide multi-valent binding to the surface of cells with high receptor density. When the surface density of the receptor is low on normal cells, then a molecular conjugate with a single targeting agent and a targeted nanoparticle can compete equally for the receptor as only one ligand–receptor interaction may occur. However, when there is a high surface density of the receptor on cancer cells (for example, the transferrin receptor), then the targeted nanoparticle can engage numerous receptors simultaneously (multi-valency) to provide enhanced interactions over the one ligand–one receptor interaction that would occur with a molecular conjugate (reprinted with permission from Davis *et al*., 2008, Copyright, Nature Publishing Group).**

cells. *In vitro* and *in vivo* comparisons using internalizing or non-internalizing ligands have shown that the intracellular concentration of drug is much higher when the drug is released from TNPs in the cytoplasm after internalization. Several recent studies have demonstrated binding and internalization of targeted TNPs. Transmission electron micrographs have shown a polymer-based TNP containing human transferrin protein targeting agent bound to the cell surface, internalized into the cytoplasm and localized in the endosome. Using N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-doxorubicin-galactosamine (PK1, FCE28068), which has progressed to a phase II clinical trial, galactosamine moieties bind to the asialoglycoprotein receptor on hepatocytes (Duncan *et al*., 2005). These promising early clinical results suggest the potential of targeted TNPs as effective anti-cancer drug delivery systems. In an *in vivo* animal study, targeted TNP-delivered paclitaxel was mainly located in tumor cells, while non-targeted TNP-delivered paclitaxel was detected intercellularly (Wang *et al*., 2009).

Targeting the tumor microenvironment

There is an ongoing debate as to whether attaching a targeting ligand to a TNP is necessary, because the enhanced permeability and retention (EPR) effect is believed to play a major role in directing TNP accumulation into a cancer tissue area (Figure 5). When tumor vasculature is at a well developed stage, this might be true; however, for small tumors that lack a well-developed vasculature, targeting tumor cells or even the tumor microenvironment could be more effective. For example, the accumulation of Abraxane is in part due to endothelium transcytosis initiated by the binding of albumin to a cell surface glycoprotein gp60 receptor which induces formation of transcytotic vesicles *(*caveolae) (Petrelli *et al*., 2010). These data support the idea that targeting caveolae might provide a universal portal to pump drugs out of the blood and into nearby tissue. The addition of two tumor-homing peptides, LyP-1 and CREKA, selected from phage-display to Abraxane enhances accumulation of this TNP in tumor tissue (Karmali *et al*., 2009). LyP-1-Abraxane inhibits tumor growth in a breast cancer xenograft model significantly better than the nontargeted Abraxane. CREKA can bind to clotted plasma proteins present in tumor vessels and interstitium. As expected, in a xenograft model, the CREKA-conjugated TNPs can block tumor vasculature, reduce blood flow, induce necrosis, and therefore significantly inhibit tumor growth. Other ligands targeting endothelial cells include RGD and urokinase plasminogen activator (uPA). The RGD motif in many proteins has a strong affinity and selectivity for cell surface $\alpha_v\beta_3$ integrins, which are overexpressed on the surface of endothelial cells of neocapillaries and also in some types of tumor cells. Therefore, RGD has been used as a ligand for tumor tissue targeting of TNPs. A tumor-homing iRGD (CRGDK/RGPD/EC) on TNPs achieved binding to tumor vessels and spread into the extravascular tumor parenchyma, while the conventional RGD ligand only delivered nanoparticle to the blood vessels (Sugahara *et al*., 2010).

Targeting metastatic, recurrent, and drug resistant cancers

Cancer metastasis and recurrence are major prognostic factors. Advances in our understanding of the molecular mechanisms by which these aggressive tumor phenotypes develop have provided a solid basis for targeting metastatic cancer using TNPs, which is a new research emphasis in this field. Targeting a specific microenvironment, such as the tumor vasculature to inhibit the colonization of metastatic cancer cells in a new organ site is one application of TNPs in the treatment of metastatic disease. Targeting the extracellular signature of metastatic cancer cells is another task in the field. For example, a PEGylated liposome modified with a fibronectin-mimetic peptide has been developed to target metastatic colon cancer cells which overexpress integrins $\alpha_5\beta_1$, since fibronectin is one of the specific ligands binding to this integrin pair (Garg *et al*., 2009). In addition, as one of the factors contributing to bone metastasis of breast cancer, osteopontin is overexpressed in both osteoclast and breast cancer cells and may be responsible for the interaction between the bone and cancer cells that drives osteolysis. Osteopontin, therefore, serves as a target to prevent bone metastasis. A sustained delivery of polymeric nanoparticles carrying antisense DNA against osteopontin and bone sialoprotein in rats with breast cancer metastasis has shown significant reduction of bone metastasis, establishing this nanoparticle formulation as a promising therapeutic agent (Elazar *et al*., 2010). Currently there are no reports of the specific killing of recurrent cancer cells using targeted TNPs, due to the lack of ligands specific for this population. Similarly, though many studies have illustrated the potential of utilizing TNPs to minimize drug resistance, the lack of specific ligands for drug-resistant cancer cells limits the application of targeted TNPs to these aggressive populations.

Future challenges

These include: (1) Identify appropriate ligands specific to cancer cells from different tissue types and to metastatic, recurrent, and drug-resistant cancer populations. Of particular interest would be to identify ligands that can target both tumor cells and the tumor microenvironment simultaneously; (2) Develop organ-specific orthotopic animal models including those of metastasis and drug resistance, which are essential to evaluate TNPs in the treatment of specific phenotypes; (3) Conduct pre-clinical PD/PK and toxicology studies for Investigational New Drug (IND) filing; and (4) Collaborate with FDA to conduct the relevant clinical trials.

As mentioned, the debate is still ongoing as to the necessity of attaching a targeting ligand to a TNP, since the EPR effect is believed to play a major role in directing TNP accumulation in cancer tissues. To obtain a clear

answer, quantification methods should be developed to address tissue and intracellular drug accumulation when using TNPs for drug delivery. Tumor models representing different types and stages of cancer should then be used to evaluate targeted TNPs as compared with the non-targeted TNPs. Furthermore, catching and killing circulating metastatic cells or cancer stem cells which metastasize or are resistant to conventional cancer treatment by targeted TNPs is another attractive application for the treatment of aggressive cancer types. These studies will also require appropriate animal models.

Clinical potential

Accumulating evidence supports that TNPs, particularly targeted TNPs, have great potential in reducing toxicity and enhancing efficacy of currently used chemotherapeutic agents. In the next few years, more and more clinical trials using targeted TNPs are expected. Furthermore, theranostic nanoparticles will be used in the clinic for early detection and treatment of cancer, particularly metastatic cancers.

Milestones

3 year:

- Develop new targeted TNPs focusing on the tumor, microenvironment as well as metastatic disease.
- Conduct release and biodistribution animal studies for targeted TNPs to provide better insight into how targeted TNPs work *in vivo*.

5 year:

- Conduct phase 0/I/II clinical trials of some new TNPs therapies.

10 year:

- Evaluate the clinical application of TNPs *in vivo* to facilitate better understanding of TNPs in terms of their PK characteristics, tissue distribution, and long-term toxicity assessment.
- Carry out phase III clinical trials and gain FDA approval for TNPs therapies.

Nanotherapeutic Delivery Systems

Dong Moon Shin

Emory University School of Medicine, Atlanta, GA

Current status

Nanotherapeutic delivery systems can be used to deliver therapeutic entities such as small molecule drugs, peptides, proteins and nucleic acids either as single agents or as multiplexed combinations (Gindy and Prud'homme, 2009; Alexis *et al*., 2010; Ruoslahti *et al*., 2010). Increasing evidence indicates that the selective delivery of nanoparticle therapeutic agents into a tumor mass could minimize toxicity to normal tissues and maximize bioavailability and cell killing. These advantages are mainly attributed to changes in drug tissue distribution and pharmacokinetics. Furthermore, it has been demonstrated that nanoparticles can escape from the vasculature through the leaky endothelial tissue that surrounds the tumor and can accumulate in certain solid tumors via the EPR effect. After escaping from the vessel, non-targeted nanoparticles will typically be cleared from the tumor sites due to their lack of cellular uptake. In contrast, tumor-targeted nanoparticles can enter tumor cells from the extracellular space via receptor-mediated internalization (Figure 5). A variety of tumor targeting ligands, such as antibodies, growth factors, and cytokines have been used to facilitate the uptake of carriers into target cells (Dong and Mumper, 2010). Tremendous progress has been made and some tumor-targeted nanotherapeutics are already in clinical trials or have been approved by the FDA.

Diversity of delivery platforms

Many different types of nanoparticles have been widely studied for therapeutic delivery (Portney and Ozkan, 2006). These include polymers (polymeric nanoparticles, micelles, dendrimers), lipids, viruses and nanotubes. These therapeutic delivery carriers have many advantages, such as: 1) water solubility; 2) low or no toxicity; 3) biocompatibility or biodegradability; and 4) amenability of their surface to further modification for related applications (Table I) (Cho *et al*., 2008).

Polymers such as albumin, chitosan, and heparin are ideal carriers for the delivery of nucleic acids, protein and drugs, as demonstrated by nanometer-sized albumin-bound paclitaxel (Abraxane) which is already in clinical use (Fu *et al*., 2009; Kratz, 2008; Petrelli *et al*., 2010). The amphiphilic block copolymers of micelles can form a nano-sized core/shell structure in aqueous media (Venkatraman *et al*., 2010). Hydrophobic drugs can be loaded into the hydrophobic core region, whereas the hydrophilic shell region stabilizes the hydrophobic core and makes the polymers water-soluble. These nanoparticles are appropriate for intravenous administration. Genexol-PM is a cremophor-free polymeric micelle-formulated paclitaxel, which has been studied in a clinical trial in patients with advanced refractory malignancies. In addition, multi-functional polymeric micelles containing targeting ligands with imaging and therapeutic agents are being developed and have the potential to be used in the near future. A dendrimer is a synthetic polymeric macromolecule of nanometer dimensions, composed of multiple highly branched monomers that emerge radially from the central core; their monodisperse size and available hydrophobic internal cavity make them attractive for drug delivery, and the polyamidoamine dendrimer has been used as a cisplatin carrier for tumor therapy. Dendrimer-based multi-functional drug delivery systems consisting of imaging contrast agents, targeting ligands and therapeutic drugs can be engineered due to the modifiable surface characteristics of dendrimers. Liposomes are self-assembling closed colloidal structures composed of lipid bilayers and have a spherical shape in which an outer lipid bilayer surrounds a central aqueous space (Estella-Hermoso de Mendoza *et al*., 2009). Many cancer drugs have been loaded onto such lipid-based systems, including the anthracyclines doxorubicin (Doxil, Myocet) and daunorubicin (DaunoXome), which have been approved for the treatment of metastatic breast cancer and AIDS-related Kaposi's sarcoma. Several types of viruses including cowpea mosaic virus, cowpea chlorotic mottle virus, canine parvovirus, adenovirus, and bacteriophages have been developed for biomedical and nanotechnology applications that include tissue targeting and drug delivery (Farokhzad and Langer, 2009; Singh and Kostarelos, 2009). Additionally, a variety of ligands and antibodies have been conjugated to viruses for specific tumor targeting *in vivo*. Some viruses, such as canine parvovirus, have a natural

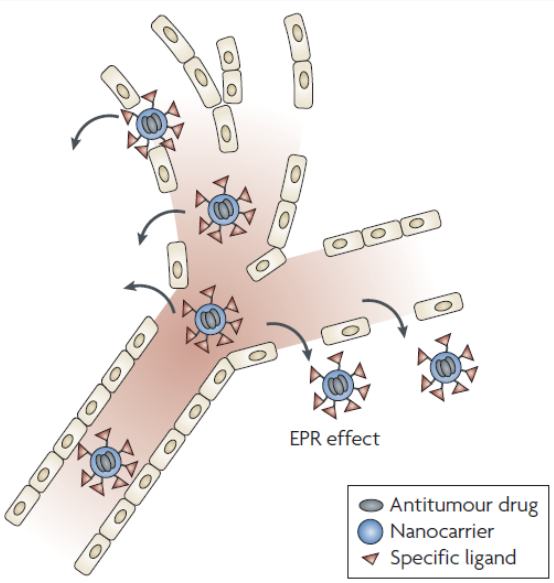

Figure 5 The enhanced permeability and retention (EPR) effect. Nanoparticle agents are designed to utilize the EPR effect to exit blood vessels in the tumour, to target surface receptors on tumour cells, and to enter tumour cells by endocytosis before releasing their drug payloads (reprinted with permission from Davis *et al.*, 2008, Copyright, Nature Publishing Group).

affinity for receptors that are upregulated on a certain tumor type, and thus can be used for targeted drug delivery. Carbon nanotubes are carbon cylinders composed of benzene rings which can be used as carriers to deliver conjugate peptides, proteins, nucleic acids, and therapeutic agents.

Other nanoparticles exploit their own inherent nature for their therapeutic effects. Plasmonic gold nanoparticles are very promising for photothermal cancer therapy because of their strongly enhanced radiative and nonradiative photothermal properties due to surface plasmon resonance; these nanoparticles absorb light $10^{5\text{-}6}$ times more strongly than the most strong light-absorbing dye molecules (Arvizo *et al.*, 2010; Cobley *et al.*, 2010). Thus, when gold nanoparticles are targeted to cancer cells, electromagnetic irradiation with an optical laser will induce heat capable of destroying the surrounding cells. However, most of these diverse nanoparticle carriers do not have inherent imaging properties to enable monitoring of their distribution *in vivo*. Magnetic iron oxide nanoparticles have emerged as a new generation of MRI contrast agents for imaging/guided drug delivery due to their long blood retention time, low toxicity, and biodegradability (Lin *et al.*, 2010; Sokolov *et al.*, 2009). Changes in MRI signals produced by drug-loaded iron oxide nanoparticles may be used to estimate tissue drug levels and facilitate real-time monitoring of the tumor's response to therapy.

There are several strategies to incorporate drugs into nanoparticles - drugs can be linked to the carrier coating, deposited on the surface layer, or trapped within the nanoparticles themselves. After a drug is loaded into the nanoparticle, it can usually be released by (1) diffusion out of the particles; (2) vehicle rupture or dissolution; (3) the process of endocytosis of the formulation; or (4) pH-sensitive or enzyme-sensitive dissociation. Anti-cancer agents such as paclitaxel, doxorubicin, and cisplatin are suitable for nanoparticle delivery, and tumor-targeted nanoparticles are also ideal carriers for systemic delivery of siRNA *in vivo*.

Recently, increasing concerns have focused on the safety of nanotherapeutic delivery systems. Although few studies have shown visible toxicities in animal studies, sub-chronic and chronic toxicity studies have yet to be conducted for most nanoparticles. Little is known about the long term fate of nanoparticles *in vivo*. Most nanotherapeutic delivery systems are non-targeted, thus more intensive studies using tumor-targeted nanoparticles as drug delivery carriers are needed. The precise mechanism by which nanoparticle-loaded drugs are released *in vivo* remains unclear. It will be helpful to label both the nanoparticles and the loaded drugs using special fluorescein dyes to perform real-time monitoring of their biodistribution and intracellular localization *in vivo*. In addition, quantification of nanoparticle and drug levels in different organs must be addressed.

Future challenges

There are still many challenges to overcome when constructing nanoparticles for drug delivery. These include: (1) evaluation and minimization of related toxicities induced by nanoparticles; (2) enhancement of drug loading efficiencies; (3) modification of the surface and control of the size and charge of nanoparticles for adequate delivery; (4) regulation of circulation duration; (5) controlled drug release; (6) nanotherapeutic stability; (7) specific accumulation in the tumor and minimal uptake in normal tissues and organs by selecting ideal tumor-targeted ligands; (8) selection of appropriate nanoparticles for particular drug delivery targets; (9) construction of smart tumor-targeted nanoparticles in which the loaded drug is released only within tumor cells; (10) pre-clinical pharmacokinetic/pharmacodynamic (PK/PD) and toxicity evaluation of nanotherapeutics; and (11) regulatory and approval issues related to nanoparticles.

Clinical potential

A selective increase in tumor tissue uptake of current anti-cancer agents would be of great interest for cancer chemotherapy given the lack of specificity of anti-cancer drugs for cancer cells. Nanotherapeutic delivery systems can be used to carry established drugs that have been widely used in the clinic, and can optimize their therapeutic index by increasing the drug concentration ratio in diseased tissue to normal tissue and by enhancing the anti-tumor effect while reducing side effects. In addition, new anti-tumor macromolecules such as peptides, siRNA, proteins, and small molecule inhibitors can potentially be systemically delivered using these targeted nanoparticle pharmaceuticals, an approach which may be explored in future clinical studies.

Table 1. Types of nanocarriers for drug delivery

System	Structure	Characteristics	Examples of compounds	Ref.
Polymeric nanoparticles (polymer-drug conjugates)	Drugs are conjugated to the side chain of a linear polymer with a linker (cleavable bond)	(*a*) Water-soluble, nontoxic, biodegradable (*b*) Surface modification (pegylation) (*c*) Selective accumulation and retention in tumor tissue (EPR effect) (*d*) Specific targeting of cancer cells while sparing normal cells—receptor-mediated targeting with a ligand	Albumin-Taxol (Abraxane) PGA-Taxol (Xyotax) PGA-Camptothecin (CT-2106) HPMA-DOX (PK1) HPMA-DOX-galactosamine (PK2)	(7) (11) (12) (14) (58)
Polymeric micelles	Amphiphilic block copolymers assemble and form a micelle with a hydrophobic core and hydrophilic shell	(*a*) Suitable carrier for water-insoluble drug (*b*) Biocompatible, self-assembling, biodegradable (*c*) Ease of functional modification (*d*) Targeting potential	PEG-pluronic-DOX PEG-PAA-DOX (NK911) PEG-PLA-Taxol (Genexol-PM)	(16) (17) (18)
Dendrimers	Radially emerging hyperbranched synthetic polymer with regular pattern and repeated units	(*a*) Biodistribution and PK can be tuned (*b*) High structural and chemical homogeneity (*c*) Ease of functionalization, high ligand density (*d*) Controlled degradation (e) Multifunctionality	PAMAM-MTX PAMAM-platinate	(64) (21)
Liposomes	Self-assembling closed colloidal structures composed of lipid bilayers	(*a*) Amphiphilic, biocompatible (*b*) Ease of modification (*c*) Targeting potential	Pegylated liposomal DOX (Doxil) Non-pegylated liposomal DOX (Myocet) Liposomal daunorubicin (DaunoXome)	(22) (23) (24)
Viral nanoparticles	Protein cages, which are multivalent, self-assembled structures	(*a*) Surface modification by mutagenesis or bioconjugation—multivalency (*b*) Specific tumor targeting, multifunctionality (*c*) Defined geometry and remarkable uniformity (*d*) Biological compatibility and inert nature	HSP-DOX CPMV-DOX	(29, 30) (27)
Carbon nanotubes	Carbon cylinders composed of benzene ring	(*a*) Water-soluble and biocompatible through chemical modification (organic functionalization) (*b*) Multifunctionality	CNT-MTX CNT-amphotericin B	(34) (33)

Abbreviations: PGA, poly-(L-glutamate); HPMA, *N*-(2-hydroxypropyl)-methacrylamide copolymer; PEG, polyethylene glycol; PAA, poly-(L-aspartate); PLA, poly-(L-lactide); PAMAM, poly(amidoamine); DOX, doxorubicin; MTX, methotrexate; PK, pharmacokinetics; EPR, enhanced permeability and retention; CNT, carbon nanotube; HSP, heat shock protein; CPMV, cowpea mosaic virus.

(reprinted from Cho *et al.*, 2008, Copyright, American Association for Cancer Research).

Milestones

3 year:

- Synthesize 20-30 tumor-targeted nanotherapeutic delivery systems with high quality and yield for cytotoxic agents such as doxorubicin, paclitaxel, cisplatin, and siRNA as well other small molecules.
- Demonstrate successful delivery of highly potent, toxic therapeutics using nanoparticle platforms. Enable widening of therapeutic window for these compounds through the nanoparticle delivery.

5 year:

- Perform PK/PD studies of the best nanotherapeutic systems in mice and rats (including human tumor xenografts) and in large animals.
- Determine the lowest non-toxic dose using the best nanotherapeutic system in humans. Study nanoparticle biodistribution and toxicity to identify those that are most efficacious and least toxic.
- Extend preclinical toxicology studies of the best nanotherapeutic systems from mice to rats and dogs. Conduct phase O, I, and II clinical trials.
- Gain FDA approval of at least one nanoparticle-based targeted therapeutic.

10 year:

- Gain FDA approval and commercialize several targeted nanotherapeutic delivery systems for cancer applications.

Nanotechnology Theranostics

Demir Akin and Sanjiv Sam Gambhir

Stanford University, Stanford, CA

Theranostic nanoparticles

Theranostics can be classified into two main subgroups based on historical origins: a) Classical theranostics and b) Nanotheranostics. Classical theranostics refers to a treatment platform wherein the therapy is guided by a specific diagnostic test, which stratifies the patients for treatment eligibility. For purposes of this review the focus is on nanotheranostics which will herein be referred to as "theranostics." These are multi-functional nanodevices with capabilities for simultaneous detection and drug delivery in a single device. Theranostics can further be subgrouped into two categories: a) Imaging Theranostics, (ITNs): nanodevices and nanomaterials with diagnostic imaging and therapy functionalities (e.g. optical or electromagnetic nanoparticles, such as drug functionalized Quantum Dots and magnetic nanoparticles) and b) Detection Theranostics (DTNs): theranostics with biodetection and biosensing capabilities and a therapy modality (e.g. polymeric nanomaterials/nanoparticles that sense and respond to their environment and modulate the release of a cargo drug or therapy modality). There are overlapping hybrid, multi-functional theranostics as well, such as the fluorophore-labeled imaging nanoparticles with environment responsive polymeric shells and a therapeutic magnetic core (Figure 6) (Vo-Dinh, 2007).

Theranostic nanoparticles are constructed using a variety of chemistries and come in an array of physical forms. These particles can be composed of metals, non-metals, synthetic polymers, dendrimers, lipids, nucleic acids, biologics (e.g. viral vectors), synthetic peptides, and combinations therein. Their shapes can take the form of solid spheres (e.g. quantum dots, iron oxide nanoparticles, etc.) or non-spherical geometries (e.g. nanorods, nanodiamonds, nanotriangles, nanocages, and hybrids of these forms). Each of these types of nanoparticles has shown to have unique advantages and disadvantages in diagnostic and therapeutic management of various cancers.

There are a number of ITN agents in use today. Encapsulated iron oxide core and polymeric nanoparticles are used for cancer detection via magnetic resonance imaging (MRI) or optical detection (fluorescence, Raman, near-infrared, luminescence) and to directly ablate tumors

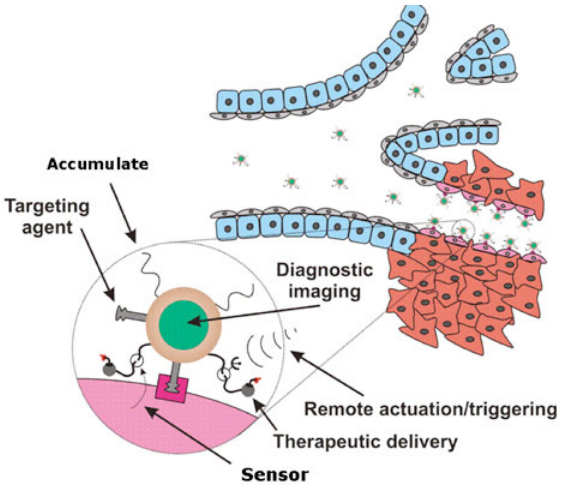

Figure 6 Schematic depicting multi-functional theranostic agents having properties of both the ITN and DTN classes. The nanoparticles interact with tumor cells via a targeting moiety and are capable of imaging, therapy, and sensing the microenvironment (Figure courtesy of Dr. Sangeeta Bhatia, MIT).

via either thermal or non-thermal means. Another available theranostic agent is cancer targeting aptamer-modified Quantum Dots conjugated with Doxorubicin (Ho and Leong, 2010). These agents are typically bio-passivated by incorporating them into liposomes or other polymer-based biocompatible matrices. A different class of theranostic device, such as plasmonic nanobubbles (Lukianova-Hleb *et al*., 2010), uses gold nanoparticles and transient photothermal excitation to create vapor-based nanobubbles for selective non-thermal, mechanical destruction of targeted cancer cells. Due to the photonic nature of the energy source, this theranostic modality is equipped with optical guidance to the desired anatomic location in addition to diagnostics via optical scattering and mechanical therapy.

One example of an up and coming class of DTNs is combining conventional PET imaging with the biomarker F-18 fluorodeoxyglucose (^{18}F-FDG) to monitor

the increased glucose metabolism common to many tumors. Response to imatinib treatment as well as recurrence can be assessed in patients with gastrointestinal stromal tumors using the high sensitivity and resolution capability of a PET camera (Goldstein *et al*., 2005).

Monoclonal antibodies (mAb) as well as engineered antibodies are being used to provide specific diagnostic information in conjunction with PET and other clinical imaging modalities with targeted-therapy for cancers (Wei *et al*., 2008). In a recent study, tumor targeting of radiolabeled-anti-CD20 diabodies, engineered antibody analogs of Rituximab, could detect low-grade B-cell lymphomas (Olafsen *et al*., 2010). The availability of good positron emitters, improvements in radiochemical labeling, and the development of scanners for advanced PET-computed tomography (PET-CT) are the crucial drivers of this theranostic imaging development. It is highly anticipated that immuno-PET will be playing an important role in the future improvements and tailoring of therapy and also in the expansion of the number of this class of theranostics.

Future challenges and clinical aspects

Despite the fact that many nanomedical tools have found great utility and application in *in vitro* studies, pre-clinical cancer models, and/or intra-operative investigational use, to date very few of these technologies have reached the clinical trial stage. Only a few of these platforms, such as the gold or iron oxide-based theranostics and the multi-functional-dendrimeric nanoparticles, are amenable for rapid translation into the clinical development cycle for in-patient use. Some of the issues impeding the progression of the theranostics into the clinic are centered on the lack of acceptable specificity of these theranostic modalities for the cancer target sites and the toxicity associated with these technologies. Our lack of adequate *in vivo* predictive capabilities for the ADME-Tox of these nanomedical tools are the major source of failure in the progression from the research and development phase to clinical use.

Currently, the efficacy of an anticancer treatment is evaluated by gross physical endpoint changes that occur in tumors following the therapy such as tumor volume changes, density/opacity changes, differential distribution pattern of a contrast reagent, and vascularization. Other indicators, such as cell death and apoptosis, occur on a cellular level and can instead provide a faster means of assessment of response to therapy via theranostic imaging using multi-modal nanoparticles equipped with treatment capabilities. This would change the timeframe of verifying the efficacy of a treatment from months to days. Nanotechnology offers the potential to develop highly sensitive imaging agents and *ex vivo* diagnostics that can determine whether a therapeutic agent is reaching its intended target and whether that agent is killing malignant or support cells, such as growing blood vessels. Such systems could be constructed using nanoparticles containing an imaging contrast agent and a targeting molecule that recognizes a biochemical signal only seen when cells undergo apoptosis. Further improvements of this type of system could provide clinicians with a way of determining therapeutic efficacy in a matter of days after treatment, rather than months. Targeted nanoscale devices may also enable surgeons to more readily detect the margins of a tumor prior to resection or to detect micrometastases in lymph nodes or tissues distant from the primary tumor. This information would inform therapeutic decisions and have a positive impact on patient quality-of-life issues.

Tumor and cancer cell phenotype heterogeneity and adaptive anti-cancer drug resistance are complex challenges in cancer necessitating our diagnostic and therapeutic response to be diverse and comprehensive. Future nanomedical interventions have to be safe, specific, affordable, and rapidly adaptive from the perspectives of both targeting as well as choice of therapy in order to tackle the formidable challenge presented by the fast developing drug resistance during the course of an anti-cancer treatment regime. These needs necessitate continued improvements in understanding cancer biology, clinical oncology, drug targeting and delivery, nanotechnology, biologically relevant engineering, and materials science.

Milestones

3 year:

- Accelerate the development of theranostics with improved targeting and biocompatibility, imaging contrast capability, controlled drug release, biobarrier breaching ability, ease of preparation, favorable cancer cell uptake, tumor distribution, reduced toxicity, and controllable clearance from body.
- Demonstrate several examples of preclinical to clinical stage nanoscale devices capable of reliable and validated earlier cancer signature and/or metastasis detection and simultaneous therapy by appropriate multi-faceted approaches. These theranostic devices will be able to interrogate and therapeutically target multiple (≥four) signaling pathways concurrently.

5 year:

- Work closely with the FDA and pertinent entities to facilitate the establishment of scientific framework and guidelines for a timely but properly regulated approval of nanoscale diagnostics, therapeutics, theranostics, and preventive agents.
- Submit at least three to five INDs in the area of multi-functional (≥four functions) nanotheranostics.

10 year:

- Demonstrate proof of concept intelligent nanomedical devices or integrated nanoscale comprehensive device systems that can simultaneously assess different types of genomic, transcriptomic, and proteomic level events involved in cancer predisposition, initiation, progression and metastasis in order to offer multi-faceted targeted therapy for these detected events. Ideally, these active nanomedical devices will be administered for a predetermined duration and operate

in vivo or embedded within the vicinity of target tissues and organs.

- Develop high impact molecular imaging approaches capable of detecting and imaging specific molecular activities that have the potential for clinical applications *in vivo*. These novel molecular imaging developments will focus on both of the following long-term translational goals: (1) imaging the characteristic markers and functions of normal cells in control human subjects and patients and (2) imaging the characteristic markers and biochemical or physiological abnormalities of cancer cells in patients.

siRNA Therapeutics

Sara S. Hook

Office of Cancer Nanotechnology Research, CSSI, National Cancer Institute, Bethesda, MD

Introduction

Often cancers arise due to overexpression of oncogenes or expression of inappropriate protein products produced by gene translocations, insertions, or rearrangements. For example, some types or chronic myelogenous leukemia, acute myelogenous leukemia, or acute lymphoblastic leukemia are caused by chromosomal translocations that fuse together portions of the BCR serine/threonine kinase and the ABL tyrosine kinase (Perrotti *et al*., 2010). The phenotypic effect is that the ABL kinase activity is uncontrolled due to the loss of regulatory protein sequences and addition of non-catalytic sequences from BCR. One approach to treating cancers that arise by these types of mechanisms would be to silence the incorrect gene and/or replace it with a normal copy. The later strategy would only be needed in cases of haplo-insufficiency, where one copy of the normal gene would not suffice and an additional copy is needed. A critical barrier, however, for gene silencing or gene replacement is efficient delivery mechanisms. The promise of nanoparticle-mediated delivery is well recognized and early clinical trials have already shown that double-stranded silencing RNAs or "siRNAs" are a feasible strategy for use in humans in the clinic (Davis *et al*., 2010).

The mechanisms for cellular siRNA processing (as well as for short-hairpin (sh) RNA) have been reviewed elsewhere and will only be briefly addressed here. These RNAs can be taken up by cells "as is" but most efficiently when packaged in either liposomes (siRNAs) or viral vectors (shRNAs). They are processed by the dicer family of enzymes to remove the hairpin sequences (if needed) and then both categories of RNAs are incorporated into the RISC complex which serves to further process them into single-stranded RNAs (Figure 3). According to their sequence homology they bind to endogenous RNAs and either facilitate their degradation or inhibit translation of the RNA into protein, thus effectively silencing gene expression (Morris, 2008). A major advantage to this approach is that once a gene is implicated in cancer initiation, progression, or metastasis, it can be targeted without an intrinsic knowledge of its function, regulation, pathway involvement, etc. In addition, with careful sequence design and validation, the approach can be very specific with little cross reactivity.

Aside from siRNA efficacy and specificity, two physiological factors loom large, those being stability/pharmacokinetics and cell and tissue targeting. There are a number of ongoing clinical trials addressing various diseases that utilize siRNAs and most of these are simple saline-based formulations for local or topical delivery for the eye, respiratory tract, and skin. Systemically, however, siRNAs injected intravenously are subject to rather rapid degradation and clearance via renal excretion. Despite this, some of these "naked" siRNAs have been shown useful in decreasing tumor growth and metastasis in a number of animal xenograft models (Vaishnaw *et al*., 2010). Modifications of the phosphodiester backbone, bases, or ribose ring have been reported to increase half lives in addition to chemical conjugation to cholesterol and protein moieties and undoubtedly research in this area will continue (Singh *et al*., 2010). In the area of targeting "naked" siRNAs, researchers have conjugated them to antibodies through a biotin-strepavidin linkage and successfully directed them to glial cells demonstrating the potential to penetrate the blood/brain barrier (Xia *et al*., 2007).

Delivery strategies for siRNA

In order to increase therapeutic benefit, it would be advantageous to protect the siRNA in "packaging" while specifically delivering the cargo to the intended target cell or tissue (Oh and Park, 2009). This goal in particular is where nanotechnology will shine (Figure 7). Due to the anionic, hydrophilic nature of RNAs, they are especially amenable to packaging within the cationic environment of lipid carriers such as liposomes, micelles, lipid-based nanoparticles, and emulsion formulations. Several examples of siRNA delivery via liposomes are entering phase I trials, including ALN-VSP, which simultaneously targets multiple transcripts of each VEGR and KSP (kinesin spindle protein) for liver tumors (Alnylam Pharmaceuticals website), and ATU027, which targets

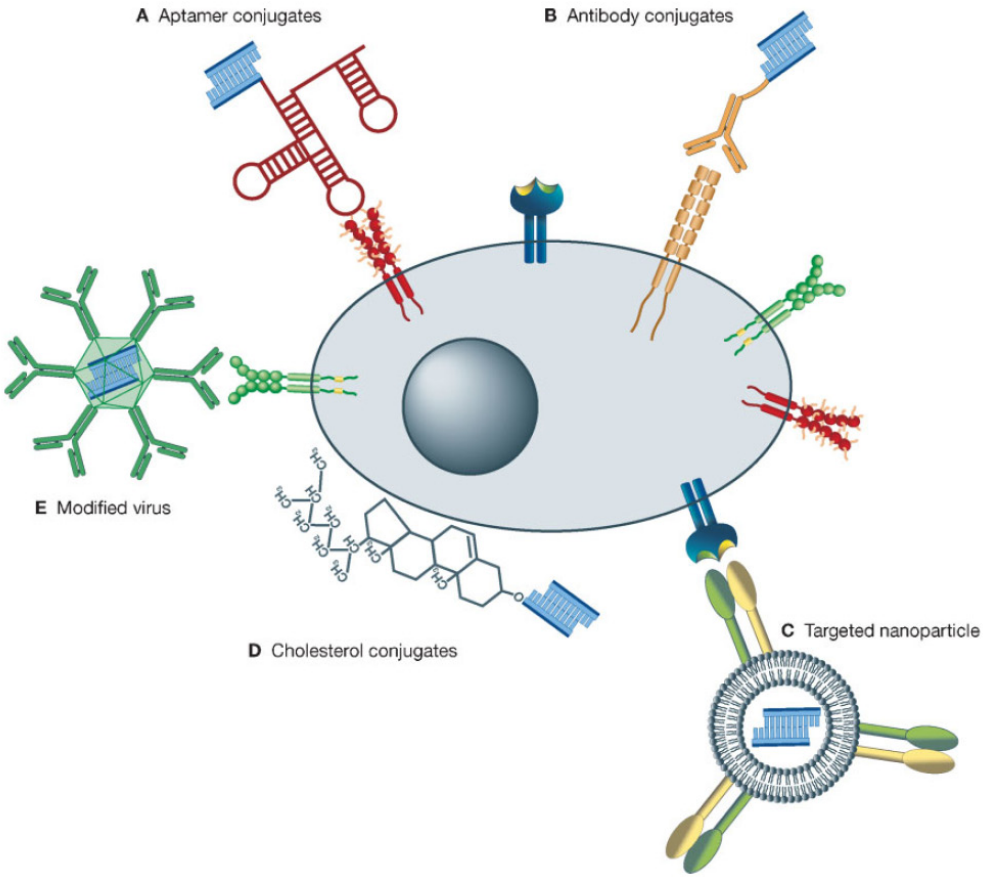

Figure 7 Delivery strategies for RNAi. The cell (grey ellipse) contains a nucleus (dark circle) and a cell membrane (dark ellipse). Cell surface molecules such as receptors are present on the cell surface (shown in color). RNAi therapeutics (mainly siRNA (blue)) can be targeted to the cell surface molecules via different delivery vehicles. They can be conjugated to aptamers (A), which can bind specifically to cell surface molecules and be internalized. siRNAs can also be conjugated to cell specific antibodies (B) and be delivered to the target cells via recognition of cell surface molecules by the specific antibody followed by internalization through endocytosis. Targeted nanoparticles (C) transport RNAi therapeutics to specific cells. The modifications of the nanoparticles (targeting ligand) can interact with receptors on the cell surface and the nanoparticle with its load can be internalized. Cholesterol conjugated siRNAs (D) can be delivered to cells and be internalized by the interaction of the cholesterol with the membrane through hydrophobic interactions, triggering clathrin-dependent endocytosis. Modified viruses (E) can also be used for cell specific delivery of RNAi therapeutics by cell specific cell surface interactions triggering endocytosis (Reprinted from Tiemann and Rossi, 2009, Copyright, Wiley and Sons).

protein kinase N3 (PKN3) and has shown promise in human xenograft tumors of pancreas and prostate in mouse models (Aleku *et al*., 2008). One study from Germany using one patient with CML resistant to both chemotherapy and the abl tyrosine kinase inhibitor imatinib found that siRNA to *BCR-ABL* packaged within liposomes decreased the fusion transcript and resulted in cellular death without adverse side effects (Koldehoff *et al*., 2007). All of these siRNA liposomal formulations, however, while showing promise do not appear to be equipped with a cell specific targeting mechanism. Calando Pharmaceuticals, however, is in the process of phase I trials using the first targeted siRNA for human cancers, CALAA-01 (Davis *et al*., 2010). They silenced the M2 subunit of ribonucleotide reductase (RRM2) by using nanoparticles directed to melanoma cells through a peptide targeting the transferritin receptor. Several lines of evidence indicate that RRM2 mRNA and protein levels are decreased following nanoparticle therapy and that the mechanism is through cellular action of the siRNA. Given the rapid pace in which the signaling pathways of various tumor types are being dissected as well as biomarkers being identified, we can expect to see an increase in this type of targeted, systemic nanoparticle therapy.

Clinical impact

Currently, 14 siRNA-based clinical trials have been initiated (Vaishnaw *et al*., 2010), four of which are for cancer and three of these are in liposomal formulations. Some remarkable features of nanoparticle delivery are the relatively low amount of immune system response (as discussed in a previous section) and decreased drug induced toxicity. Several clinical trials directed at other

diseases utilizing siRNA therapy that are not nanoparticle based have been terminated due to either no overall improvement of the condition (such as visual acuity), or due to non-specific effects of the treatment such as activation of innate immunity (Kleinman *et al*., 2008; Vaishnaw *et al*., 2010). The latter clinical outcome might be circumvented by nanoparticle formulations. Since cancer can arise by a vast array of mechanisms, some of which are more specific to tissue type and others that are integral pathways important for the life of all cells, the therapeutic strategy to combat it would be most advantageous if it were targeted to tumor cells and spared normal cells. This approach can be achieved using nanoparticle formulations.

As the research continues to develop siRNA-based nanotherapeutics, we expect an increasing number of diverse packaging systems for siRNAs (Gao *et al*., 2010). For example, siRNA has recently been incorporated into stimuli-responsive PEGylated nanogels which when subjected to the lower pH of the tumor intracellular environment enhances lysosomal and endosomal release (Oishi and Nagasaki, 2010). In addition, reports have described such concepts as delivering siRNAs via magnetic nanoworms (Agrawal *et al*., 2009), dendrimers (Ravina *et al*., 2010), nanocrystals (Namiki *et al*., 2009), and carbon nanotubes (Menard-Moyon *et al*., 2010). An alternative approach to siRNA but still targeting RNA degradation to decrease gene expression would be to employ DNAzymes. These are short synthetic DNAs with inherent enzymatic activity capable of cleaving target RNAs (Ravina *et al*., 2010). Nanoparticles containing DNAzymes could prove to be a valuable therapeutic approach in the future.

Beyond the potential value of siRNAs in therapy they can also be used for *in vitro* and *in vivo* diagnostics. They have already been used to screen for biological regulators as therapeutic targets and validate them for potential clinical applications. In addition, siRNAs can be useful for assay development and can serve as positive and negative controls to establish the relevant signaling pathways involved in cancer progression, angiogenesis, metastasis, etc. Recently, siRNAs have been tagged with fluorescent markers which can, in theory, be used to track which cells have received the siRNA in a living organism (Oishi and Nagasaki, 2010). In the future, we expect that more and more multi-functional nanoparticles will not only deliver siRNAs to the target tumor types but will also enable real-time imaging, thermal ablation, and/or small molecule drug delivery.

Milestones

3 year:

- Expand the repertoire of chemical modifications to the siRNAs themselves as well conjugation to other carbohydrates, lipids, proteins, etc. to increase stability, bioavailability, and intracellular processing.
- Increase research on catalytic oligonucleotides capable of cleaving the target RNAs.

5 year:

- Test new nanotechnology-based delivery vehicles for siRNA.
- Devlop formulations containing multiple siRNAs to target multiple signal transduction pathways.
- Conduct late stage clinical trials for siRNA delivery.

10 year:

- Increase focus on personalized therapies using tumor sequencing data to direct decisions on nanoformulations using multiple siRNAs specific to the patient's tumor genetic or proteomic profile.
- Gain FDA approval for nanoparticle-based therapies using siRNA delivery.

Nanotechnology to Overcome Tumor Drug Resistance

Lara Milane and Mansoor Amiji

Northeastern University, Boston, MA

Tumor microenvironment, hypoxia, and cancer stem cells

The tumor microenvironment contributes to the development of **m**ulti-**d**rug **r**esistant (MDR) cancer and affects a patient's response to treatment. The microenvironmental selection pressures that contribute to the development of MDR include abnormal tumor vasculature, hypoxia, decreased pH, increased interstitial fluid pressure, and alterations in the expression of tumor suppressors and oncogenes. MDR cells often have increased DNA repair mechanisms, up-regulation of ABC transporters, and a decreased apoptotic response (Figure 8) (Dong and Mumper, 2010; Gottesman *et al*., 2002). Abnormal tumor vasculature is the most defining characteristic of the tumor microenvironment; the vasculature of a tumor is highly disorganized and inefficient relative to normal vasculature. These fluctuating states of vascularization lead to regions of acute and chronic hypoxia. Cancer cells undergo a complex phenotypic transformation under hypoxic conditions. This survival cascade is initiated when the alpha subunit of Hypoxia Inducible Factor (HIF) translocates from the cytoplasm to the nucleus where it complexes with the beta subunit of HIF, forming an active transcription factor. The HIF complex binds to hypoxia responsive elements (HRE's) on target genes, inducing transcription (Harris, 2002; Semenza, 2003; Depping *et al*., 2008). The vast array of HIF targets include genes involved in invasion, proliferation, metabolism, drug resistance, and glycolytic pathways. (Denko, 2008; Semenza, 2010a; Semenza, 2010b). In fact, with less oxygen available for energy acquisition through oxidative phosphorylation, these hypoxic cancer cells revert to aerobic glycolysis for the production of ATP (the Warburg effect) (Guppy, 2002).

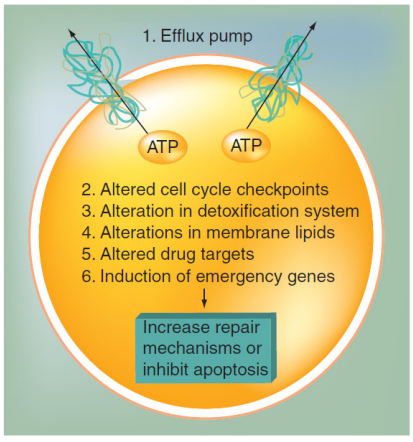

Figure 8 Summary of the mechanisms in which cultured cancer cells have been shown to become resistant to cytotoxic anticancer drugs. The efflux pumps at the plasma membrane include P-glycoprotein, multi-drug resistance protein family members and breast cancer resistance protein (reprinted with permission from Dong and Mumper, 2010, Copyright, Future Medicine Ltd.)

The relationship between MDR, cancer stem cells, and hypoxia is only beginning to be understood (Barnhart and Simon, 2007). There are two primary cancer stem cell theories: (1) cancer stem cells are regular stem cells that have gone awry and cause cancer and (2) cancer stem cells arise from a subpopulation of cancer cells. Probably both of these concepts are correct and vary on the particular tumor. Recently it has been shown that a subpopulation of precancerous cells can acquire stem-like properties, becoming cancer *derived* stem cells (Mani *et al*., 2008; Morel *et al*., 2008). Importantly, many of the mutations that can cause this phenotypic change also facilitate MDR. Different studies have shown that cell stressors such as hypoxia and activation of an epithelial to mesenchymal transition (EMT) are efficient inducers of cancer aggression and MDR phenotypes and induce stem-like properties in cancer cells such as the expression of stem cell factor (SCF) (Jewell *et al*., 2001; Harris, 2002; Kizaka-Kondoh *et al*., 2003; Semenza, 2003; Shannon *et al*., 2003; Brahimi-Horn *et al*., 2007; Cosse and Michiels, 2008; Han *et al*., 2008; Nanduri *et al*., 2008; Semenza, 2008; Ansieau *et al*., 2010). Inhibiting SCF or EMT in MDR cells may increase the effectiveness of treatment by reducing the apoptotic threshold of these putative cancer stem cells, thereby removing the repopulating source of a tumor.

Multi-pronged strategy to overcome MDR – enhancing delivery efficiency and altering cellular phenotype

As our understanding of cancer deepens, one concept that becomes increasingly evident is that cancer is a heterogeneous disease on both the intra- and inter-patient levels. As such, a therapy that treats only one phenotype is not slated for success. For a cancer therapy to be effective the therapy must be multi-faceted, simultaneously treating multiple aspects of the disease.

Nanocarriers serve as ideal delivery solutions for combination therapy which is required for effectively treating MDR cancer. The benefits of nanocarriers include, (1) they can be engineered to achieve multiple effects using one system; (2) nanocarriers improve the therapeutic index of drugs and can alter the pharmacokinetic profile of agents; (3) they preferentially accumulate in the tumor environment thanks to the EPR effect and their capacity to be conjugated to targeting moieties; and (4) nanocarriers avoid drug efflux by preferentially localizing agents in the peri-nuclear region of a cell, away from membrane localized efflux pumps.

The most effective treatment for MDR should address multiple MDR phenotypes which can be facilitated using the multi-functional platforms available through nanotechnolgy. As such, combining a traditional cytotoxic chemotherapeutic agent with one or more of the following strategies could prove effective:

1. inhibiting ABC-transporter mediated drug efflux
   a. small molecule inhibitors such as verapamil
   b. siRNA/shRNA silencing
2. lowering the apoptotic threshold
   a. inhibiting the Warburg effect (aerobic glycolysis)
   b. increasing intracellular ceramide
      i. exogenous delivery
      ii. siRNA silencing of glucosylceramide synthase
   c. stimulating cytochrome c release (mitochondrial permeability transition pore complex)
   d. increasing pro-apoptotic Bcl2 family members
   e. decreasing anti-apoptotic Bcl2 family members
3. increasing tumor suppressor activity (such as p53 gene therapy)
4. decreasing oncogene activity
5. decreasing the stem-like properties of MDR cells (*exploratory, e.g.* silencing stem cell factor)

Tumor-targeted multi-functional nano-delivery systems

Although nanocarriers passively target cancer through the EPR effect, using active targeting can increase the specificity of nanocarriers for MDR cells. It is relatively simple to modify the surface of nanocarriers with targeting residues. Common targeting residues include antibodies for cancer antigens, ligands for over-expressed cell-surface proteins, and lectins for carbohydrate targeting. Active targeting can further improve the therapeutic index of an agent by decreasing off-target accumulation. Common targets include EGFR receptors, transferrin receptors, and folate receptors.

Active targeting also alters the mechanism of uptake of nanocarriers. Non-targeted nanocarriers are taken up by non-specific endocytsosis whereas targeted nanocarriers are internalized via their target-specific mechanism. For example, nanocarriers that target the EGFR receptor are internalized via a flip-flop mechanism, a rapid process compared to endocytosis. Active targeting, therefore, not only decreases the residual toxicity of a system, it can further alter the pharmacokinetic profile of a system. Some nanocarrier systems are designed to target more than one MDR phenotype, further increasing their specificity to MDR cells. However, the *in vivo* effects of active targeting are inconclusive and need to be validated and explored.

Milestones

3 year:

- Develop animal models of refractory disease that recapitulate the human disease in terms of location, genotypic and phenotypic heterogeneity, etc.
- Characterize tumor microenvironmental factors (i.e., soluble and insoluble) on the development of clinically-relevant refractory disease.
- Identify and validate drug targets and strategies to overcome resistance through a multi-factorial approach that utilizes efficiency in drug delivery, residence, and intracellular penetration as well approaches to overcome cellular resistance.

5 year:

- Establish robust pre-clinical programs to develop and test multi-functional nanoparticulate drug delivery systems in appropriate models of refractory diseases.
- Evaluate the toxicological properties of nanoparticulate formulations under GLP conditions.

10 year:

- Establish collaborations with pharmaceutical industries and clinical centers to rapidly facilitate the transfer of technologies from academia to cancer patients.
- Establish a clinical development program for multi-functional nanoparticulate systems using the appropriate guidance from regulatory agencies.

New Contrast Agents with Improved Spatial and Temporal Resolution

Gregory Lanza

Siteman Cancer Center, Washington University, St. Louis, MO

Current status

Molecular imaging agents promise new unprecedented opportunities to assess changes in tumor microanatomical and physiological character with greater spatial and temporal resolution in cancer patients. Until recently, the majority of advancements in cancer imaging have favored improved detectability of minute masses. Today, we can detect minute lesions with high resolution CT and MRI, and the challenge has become deciding whether a lesion is a benign fascinoma or an early malignancy. Early categorization of a pathology as benign stable disease, an inflammatory lesion, or a malignancy has dramatic implications in medical management, but commonly minute tissue anomalies cannot be characterized, necessitating a conservative "wait and see" management approach. Re-evaluation in three to six months is common to assess gross morphological changes that would point to cancer but an aggressive tumor may have already disseminated beyond the original primary site.

Delineation of an unknown pathology suspected of cancer requires biopsy for microscopic and biochemical characterization. Although such procedures are routinely performed, the acquisition of tissue specimens by surgical resection or fine-needle aspiration still presents challenges due to lesion accessibility, tissue sample quality and artifacts, and a patient's willingness to undergo the procedure. Biopsy procedures become particularly troublesome when the lesion is small (< 1 cm) and centrally located. Molecular imaging offers a noninvasive mechanism to assess microanatomical changes, for example the development of a neovasculature, or the expression of important biochemical markers, such as HER-2/neu. These pathological signatures serve not only as an aide in tumor diagnosis and grading, but also as responsive biomarkers to treatment efficacy. Improved noninvasive characterization will lead to definitive diagnoses sooner, and because the lesion is "visualized" *in vivo*, key anatomical and metabolic information destroyed or nonassayable by excising the tissue is retained.

Microanatomical and biochemical measurements of tumors require robust, quantitative techniques with high spatial and temporal resolution, but what constitutes high resolution is often a matter of perspective and dependent on the medical question posed. For example, nuclear "hot spot" imaging with PET or SPECT tracers are detected with very high sensitivity per tracer concentration but low spatial resolution (millimeters) when compared with MRI. PET has high temporal resolution for kinetic studies given adequate nuclear tracer counts, which allows convenient and rapid assessments of probe "wash-in" or "wash out" of a target tissue. Moreover, in some situations, low spatial resolution may be adequate for noninvasive tissue characterization when a boolean answer based on the presence or lack of radioactivity for a pathognomonic receptor or biochemical pathway is sought. Unfortunately, ^{18}FGD is completely nonspecific except for a prevalent accumulation in cells with high metabolic rate and receptor specific ligands are foiled by nature's utilization of the same receptors and pathways for many cell types. For example, radiolabeled RGD peptides (arginine, glycine, aspartate) and antibodies, particularly directed to the $\alpha_v\beta_3$-integrin, have been used to target and characterize tumor angiogenesis by PET (Haubner *et al*., 1999; Beer *et al*., 2007) and SPECT (Liu *et al*., 2007). However, these small molecules, despite exquisite chemistry, readily permeate beyond the tumor and bind many cell types, including macrophages and tumor cells, which diminishes the signal specificity for angiogenesis per se (Zitzmann *et al*., 2002; Liu *et al*., 2007).

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) can detect changes in tumor microvasculature permeability to MR blood pool contrast agents and some studies have correlated these kinetic estimates with traditional measures like MVD, but initial clinical trials have yielded inconsistent results either due to insufficient standardization of the endpoints or technique issues (Jayson *et al*., 2002; Liu *et al*., 2005; Schmieder *et al*., 2008). However, MR molecular imaging with paramagnetic nanoparticles facilitates high-resolution 3D mapping of angiogenesis (Schmieder *et al*., 2008; Winter *et al*., 2008). Such *in vivo* studies clearly indicate that angiogenesis is peripherally distributed nonuniformly around a tumor in a heterogeneous pattern associated with

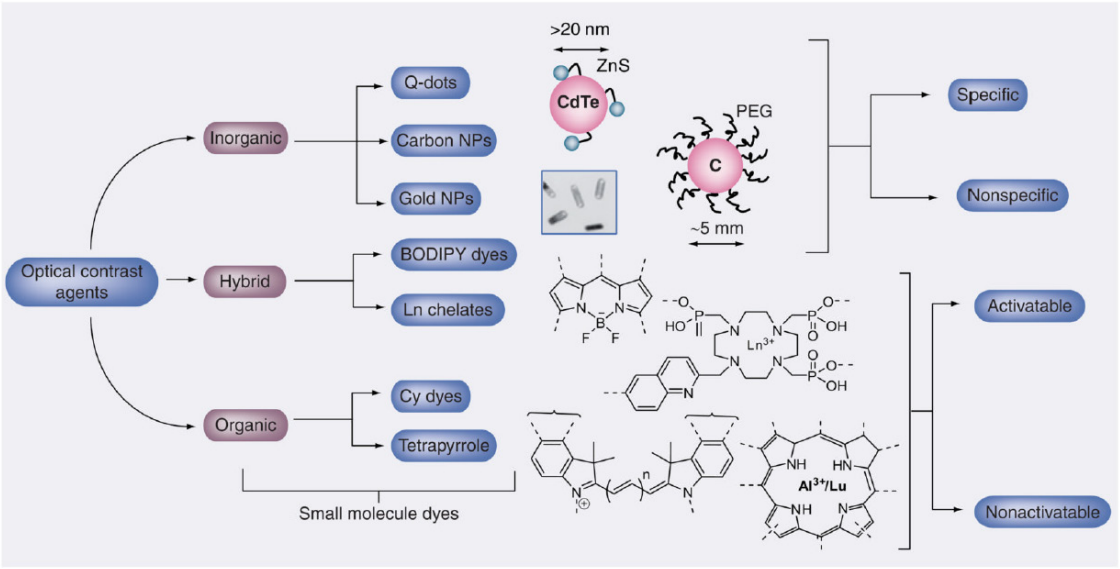

Figure 9 **General classification of optical contrast agents (reprinted from Pan *et al.*, 2010, Copyright, Future Science).**

rapidly proliferating cancer growth fronts. Clearly, neither fine needle aspiration into a tumor core nor routine histology sections randomly oriented on resected tumors are severely prone to sampling error and cannot provide reasonable quantitative estimates of neovascularity, that could be used to risk-stratify patients for anti-angiogenesis treatment.

Like MRI, CT offers tomographic imaging with very high spatial and temporal resolution, which overcomes the issues of motion in many tissues including pulmonary and gut. However, the inherent tissue x-ray contrast is low, necessitating the use of iodinated low molecular weight contrast agents. Although like gadolinium-based DCE, CT can be used for kinetic modeling, the data provide no biochemical and limited pathological prognostic information. New nanoparticle based homing agents have been reported that overcome the marked insensitivity of CT to contrast, but the majority of pre-clinical applications studied to date have been directed toward targets with high epitope density or to passive accumulation in macrophages, liver, or spleen.

Ultrasound is another important clinical imaging modality with moderately high spatial (mm to micron, dependent on frequency) and very high temporal resolution (real-time). Once a planar technique, the advent of 3D ultrasound provides improved spatial registration. Ultrasound is the clear favorite with regard to cost, portability, and ease of use, but it has significant limitations. The most common problems are derived from the limited “acoustic windows” available where bone, gas (bowel or lung), or depth of tissue do not preclude or compromise imaging results. Moreover, achieving high imaging resolution is dependent upon increasing the transducer insonification frequency. While high frequency transducers, 25 MHz and up, offer the best temporal – spatial resolution, but sound penetration decreases with increasing frequency, requiring these targets to be near the skin or accessible with intravascular ultrasound catheters. Ultrasound molecular imaging with microbubbles (Klibanov *et al.*, 1999), echogenic liposomes (Alkan-Onyuksel *et al.*, 1996), and PFC nanoparticles (Lanza *et al.*, 1996) have been demonstrated *in vivo*, but the microbubbles due to their highly amplified ultrasound reflectance, offer the greatest contrast and the greatest noise, even a single bubble, targeted or random, is detectable.

Temporal resolution is becoming an important factor in the clinical use of ligand-targeted molecular imaging agents, particularly with respect to drug delivery with theranostic agents. Initially, molecular imaging will play a role in stratifying patients into optimal treatment plans, but soon thereafter, the effectiveness of treatment, particularly for small tumors in asymptomatic patients, will utilize molecular imaging follow response and manage the pharmacologic strategy. With the advent of theranostic agents (as discussed previously), now demonstrated repeatedly in pre-clinical models, imaging will be used not only to stratify patients to best treatment regimen, but also to confirm dosing of targeted therapy using the coupled imaging feature. While repeat imaging with ultrasound and MRI will pose no known health threats, recurrent use of ionizing radiation (PET and CT) may predispose to unwarranted health side effects. This issue gains significance as younger patients with cancer identified and treated earlier.

A concern of temporal resolution will be inherent in the contrast agent used. For instance, with MRI paramagnetic nanoparticles (and the like), imaging occurs with one to three hours after injection and there is no residual contrast signal at the target site 24 hours after treatment. Repeat imaging can easily occur within two days. In contradistinction, most targeted iron oxide contrast

agents cannot be imaged until 24 or 48 hours after treatment due to blood pool induced magnetic artifacts and the persistence of the iron oxide nanoparticles at the target site can last variably from weeks to months, limiting timely reinterrogation. Ultrasound microbubbles have very short blood half-life and tissue persistence, making them an excellent choice for serial imaging, but the acoustic rupture of microbubbles for perfusion-reperfusion targeting techniques or for acoustically enhanced drug delivery, may alter the presentation of bioepitopes for homing and confound serial imaging results. Both CT and MRI agents dependent upon heavy elements and repeat dosing must address the possibility of toxic accumulation. Metal administered for contrast must be chemically stable *in vivo* and predominantly eliminated from the body in a few days with virtually all of the remaining metal excreted in a few weeks.

Future challenges

The clinical utility of molecular imaging with high spatial and temporal resolution depends on the quantitative reproducibility of signal estimates derived within an individual patient. Contrast imaging must be quantitatively correlated with target expression and be repeatable. To date, the depiction of a tumor hot-spot PET or angiogenic map with MRI are dependent on thresholding techniques, which must be optimized for pathologic correlation and normalized for serial within patient comparison over time. Today's clinical imaging techniques present have 20 to 30% variability related to performance technical issues (e.g., MR coil or nuclear detector placement) and manufacturer provided internal hardware recalibration routines. The current drive to quantitative, reproducible imaging must continue with the institution of more stringent operational standards, development of National Institute of Standards and Technology (NIST) calibration phantoms, and rigorously validated imaging software and hardware capable of absolute measurements. Without meeting this essential challenge, molecular imaging with or without drug delivery will not achieve its potential and could fail to become a proven, clinically relevant and reimbursable procedure to improve cancer management. Fortunately, these goals are more or less engineering accomplishments that can be achieved with determined effort.

Milestones

3 year:

- As nanotechnologies reach the clinic, the potential for early application for molecular imaging will become known as will the challenges of signal detection, reconstruction, and calibration within the human body. This information will be critical as clinical trials proceed. We expect each new agent reaching the clinic will elucidate new problems and uncover unexpected opportunities which will enhance formulation of the global and specific issues governing efficacy, safety, and clinical use compatibility.

5 year:

- The information achieved in clinical trials must drive hardware-software vendors to implement improved validated software to optimize image acquisition and presentation to physicians for clinical interpretation. Molecular imaging literally means detecting, presenting and characterizing nascent cancers, which is akin to finding the proverbial "needle in a haystack" with robust quantitative rigor.
- Concurrently, basic and clinical scientists must work together to devise guidelines for utilizing the imaging information alone and with drug delivery in an effective, cost-responsible manner leading to the improved health care management of cancer patients.
- Because the information developed over the first five years of the clinical molecular imaging revolution will be "first of its kind data in man", dogmatic views and perceptions of the past will need to be revisited, revised and often discarded. Willingness to accept new molecular imaging data and to discard our preconceived notions will be the greatest achievement of this period.

10 year:

- Expanded use of first generation molecular imaging technologies combined with new generation systems, which must robustly overcome the transendothelial barrier to nanoparticle delivery and expand opportunities for direct to cancer cell theranostic medicine. Insight into these pathways and mechanisms to utilize nature's machinery has already been achieved and our understanding is rapidly increasing.
- Next generation product candidates created over the next five years will reach the IND stage for clinical testing in five to eight years with the homing and size specificities needed overcome this targeting obstacle.
- During the last two years of this decade, these new generation nanomedicines should clear phase I safety and proof of concept hurdles and begin focused clinical study toward efficacious cancer applications considered intractable today.

Multi-modal Imaging

Paras N. Prasad

Department of Chemistry, SUNY Buffalo

Introduction

Currently a number of advanced imaging modalities are available in the pre-clinical and clinical setting, including magnetic resonance imaging (MRI), positron emission tomography (PET), computed tomography (CT), and optical imaging (OI) (Willmann *et al*., 2008). However, all these modalities vary in their limits of sensitivity, resolution, and depth profiling. Therefore, it is unlikely that a single imaging modality will provide conclusive evidence of a biological process or therapeutic response. In this regard, a synergistic combination of multiple non-invasive imaging technologies will play a critical role in the early detection of cancer and other diseases. The choice of these imaging techniques is driven by their ability to provide complimentary (structural and functional) information and enable cross-validation of imaging signals along with differences in resolution, sensitivity and clinical application. For example, OI approaches are valuable for *in vitro* and *in vivo* evaluation in pre-clinical model systems but are not ready for 'prime-time' in the surgical setting. In contrast, advanced imaging techniques such as MRI are widely used in clinical diagnosis and monitoring the response of patients to therapy. Therefore, the combined OI-MRI approach is likely to provide valuable information on the diagnostic/staging potential of our nanoplatform. In addition, combined with a therapeutic modality, such a system will facilitate the monitoring of therapy in real time. Such real-time monitoring would allow patients with 'non-responsive' tumors to avoid the side effects of ineffective treatment by enabling them to be switched in a timely manner to more appropriate therapies that are likely to offer better survival benefit (Prasad, 2004). However, successful realization of these objectives will require the development of novel multi-modal and biocompatible agents, along with multi-imaging instrumentation and software capable of co-registering the signals obtained from the various imaging modalities.

Nanoparticle-based probes have several advantages over traditional molecular agents because: (1) they provide a tunable, optically traceable (fluorescence, NIR and/or bioluminescence) chassis upon which targeting agents (antibodies, peptides, small molecules, etc.) can be added or changed to suit a specific need; (2) they enable multi-modality (e.g., optical, MR and radionuclide) imaging thus permitting concurrent evaluation for the same nanoparticle across different imaging platforms; (3) they enable targeted and sustained delivery of potent chemotherapeutic agents specifically to diseased sites, avoiding normal organs; (4) they can be functionalized with both imaging and therapeutic abilities (i.e., "theranostic" nanoparticles); (5) they are of sufficient size to permit multi-valency and therefore the potential for higher affinity binding than standard molecular agents; and (6) they enable imaging from the molecular level, to single cells, and to the entire, intact organism. This attribute further enables validation of the imaging marker by correlating results obtained *in vitro*, e.g., relying on the optical (fluorescence/near-infrared [NIR]) aspects of the probe, with those obtained *in vivo*, which may also rely on optical, radionuclide or MR imaging. Therefore, targeted multi-modal nanoparticles are expected to play a pivotal role in the development of the "next generation" of clinical agents for cancer diagnosis and treatment, as they will facilitate detection of both structural and functional anomalies which are characteristic of the early stages of cancer. Furthermore, the ability to simultaneously deliver chemotherapeutic agents specifically to tumor sites would greatly improve patient survival and post-treatment quality of life.

Current status

The rapid growth of *in vivo* multi-modal imaging arises from the convergence of established fields of *in vivo* imaging technologies, along with nanotechnology, as well as molecular and cell biology (Caruthers *et al*., 2007). The major hallmark of nanomedicine is the fabrication of multi-modal nanoprobes, which would not only incorporate multiple image-contrast agents, but also therapeutic probes and targeting molecules for site-specific delivery. Multi-modal nanoprobes can provide both structural and metabolic information specifically from diseased sites, thus leading to significantly improved imaging techniques for the detection of a variety of human cancers (e.g. breast,

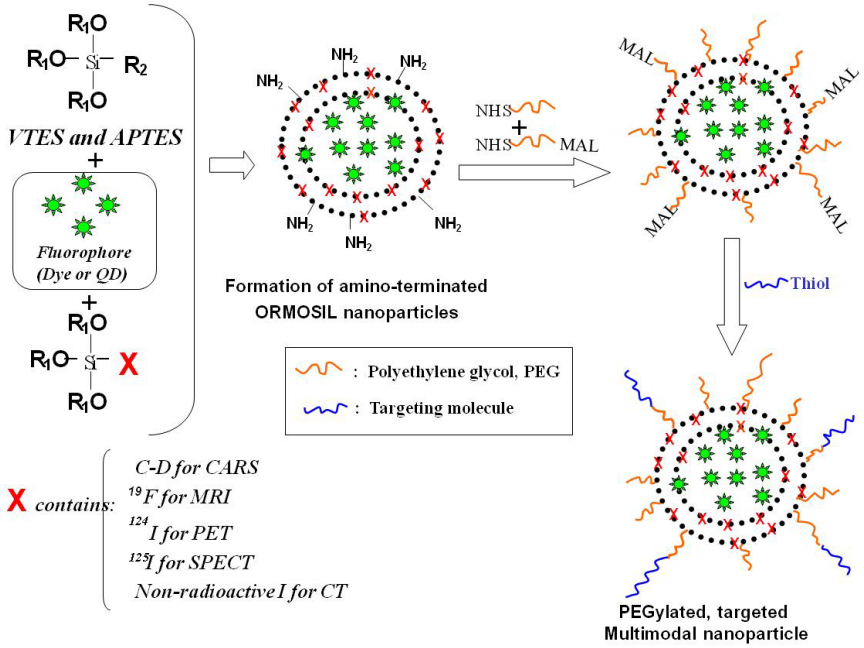

Figure 10 **Synthetic strategies for a ORMOSIL nanoparticle incorporating probes for different imaging modalities like optical, MR, SPEC, CT, and PET (Figure courtesy of P.N. Prasad).**

pancreas, lung, and prostate) including improved staging for occult metastases. In this regard, the combination of optical and MR contrast agents within a nanoparticle have gained much popularity owing to the feasibility of both *in vitro* (mainly using OI) and *in vivo* (mainly using MRI) imaging, without the involvement of any radioisotopes.

Optical imaging further facilitates image-guided surgery, which is an active area of current pre-clinical research. A number of such nanoformulations are currently in active developmental stage in several laboratories. NIR fluorophores have been combined with ultrasmall iron oxide nanoparticles and their effectiveness in imaging of cancer and other diseases, such as atherosclerosis has been shown (McCarthy and Weissleder, 2008). The feasibility of co-encapsulation of iron-oxide nanoparticles and optical probes within a silica shell (multi-functional 'nanoclinics'), which can be targeted specifically to cancer cells has also been demonstrated (Prasad, 2003). In addition, combined optical and MR imaging capability using upconverting nanophosphors with co-incorporated gadolinium has also been developed. Recently, polymeric nanomicelles incorporating optimal amounts of NIR phosphorescent optical probes and gadolinium have demonstrated specific target delivery and combined optical and MR imaging, both *in vitro* and *in vivo* (Kumar *et al*., 2009). All these multi-modal nanoformulations are currently undergoing advanced pre-clinical trials in orthotopic and transgenic cancer models.

In addition to OI and MRI, the rapid evolution of PET-SPECT and PET-CT scanner hybrids in the clinic encourages the fabrication of multi-modal nanoparticles co-incorporating OI, PET and SPECT probes (Nunez *et al*., 2010). In the pre-clinical set-up, there is also a growing interest in building and designing dedicated devices for specific applications, such as high-resolution scanners for imaging small animals in various molecular imaging centers worldwide. Fluorescence and bioluminescence optical imaging will provide a cheaper alternative to the more expensive and specialized microPET, microSPECT, and microMRI scanners. Along with volumetric tomographic imaging technologies such as SPECT and CT, which offer deep tissue penetration and high spatial resolution, noninvasive small animal optical imaging facilities will meet the growing needs of comprehensively imaging specialized animal models. These might include highly metastatic 'transgenic' tumor-model animals as well as larger non-human primates where the pathological anomalies are akin to that observed in humans. In this perspective the versatility of ORMOSIL nanoparticle platform for multi-modal imaging, incorporating a NIR fluorophore and ^{124}I PET imaging probes has been established. In addition, the ease of surface modification of the ORMOSIL based nanoparticles bolstered the conjugation of several imaging probes on the surface of the nanoparticles which includes ^{19}F for MR imaging as well as ^{124}I for SPECT/CT imaging. Figure 10 shows the application of multi-modal ORMOSIL nanoparticles developed for different imaging techniques.

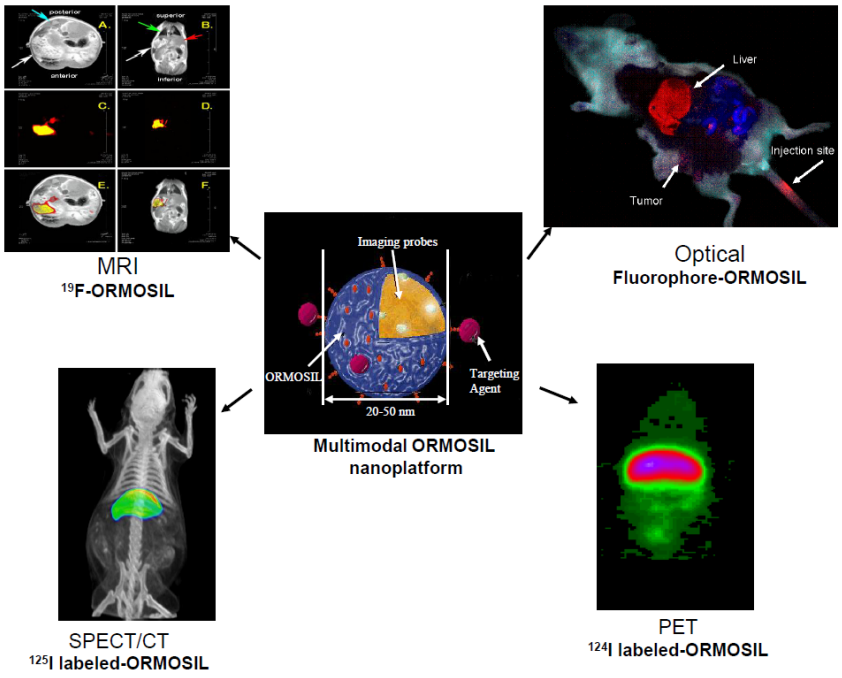

Figure 11 Multi-modal *in vivo* imaging using the ORMOSIL nanoplatform (Figure courtesy of P.N. Prasad).

Future challenges

Multi-modality imaging not only will facilitate the early diagnosis of diseases, but will also have the potential to monitor in real time the progress of a therapeutic intervention. In addition, it also enhances the precision of surgical intervention techniques. For instance, comprehensive surgical removal of cancer necessitates the removal of all cancerous cells surrounding the tumor site. Bioconjugated nanoparticles can be used as sensitive biomarkers to label only the cancerous cells and aid the surgeons in visualizing and safely resecting the tumors while reducing damage to adjacent healthy tissues. The future of multi-modality and nanomedicine would extensively involve efficient packaging of both diagnosis and therapy components within one biocompatible nanoprobe, leading to the fabrication of an ideal 'theranostic' agent.

The biggest challenge that nanotechnology faces at present is meeting all the safety guidelines required for gaining clinical acceptance, particularly those required by the FDA. Over the past decade, several nanoparticles, including polymeric, inorganic, and hybrids have been modified in terms of their size, shape and surface properties in order to meet these guidelines. Remarkable among them are the development of (1) 'stealth' nanoparticles, which can evade capture by the RES, (2) 'target-specific' nanoparticles, which accumulate only in the diseased organs/sites, bypassing normal ones, (3) ultrasmall iron-oxide nanoparticles, as well as cadmium-based quantum dots, which can eliminate themselves from the body through the renal filtration system, and (4) biocompatible nanoparticles, made up of natural polymers/biomolecules, such as chitosan, albumin, and calcium phosphate which are unlikely to evoke an immune response and will be well tolerated by the body. The introduction of Abraxane, the first nanoparticle-based clinical drug delivery system for the treatment of certain human cancers, has strongly mobilized nanotechnology researchers in the pursuit for other, more improved nano-based drug delivery systems (Miele *et al*., 2009). However, despite of all these developments, the non-specific accumulation and long-term persistence of nanoparticles *in vivo* continues to pose serious roadblocks toward their clinical acceptance. This challenge is particularly daunting in regards to multi-modal nanoparticles, where a number of components need to be assembled within a single nanosystem, potentially making the overall nanocomposite cumbersome and large in size. Therefore, the issues that need to be immediately addressed are properly balancing the necessary payloads during the fabrication of a multi-modal nanoparticle.

The significance of nanotechnology in multi-modal imaging relies on the efficient packaging of the different imaging probes and targeting molecules on a single nanoparticle system. There have been several reports mentioned earlier which combine OI as well as MRI efficiently but the foremost challenge still remained unanswered when combining OI and MRI with clinically relevant PET, SPECT/CT imaging. In this context there is speculation that the ORMOSIL nanoparticle which has shown a promise in combining the different modalities together may open a pathway into multi-modal imaging, combining all aspects of the clinically accepted imaging techniques (Figure 11). A systematic titration and assessment of the surface functionalities of the OMROSIL nanoparticles and their conjugation with different imaging

probes will result in a multi-modal nanoparticle platform for efficient *in vivo* imaging.

Clinical potential

There is a dire clinical need for agents that can provide comprehensive diagnostic information, initiate targeted and preferentially externally activated therapy, and assess the progression of therapy in real time. In this regard, multi-modal nanoparticles are ideal candidates that can address all the above challenges comprehensively. However, as stated earlier, meeting the safety requirements for clinical acceptance continues to be a huge challenge. Encouragingly, the incorporation of NIR fluorophores within clinically used iron oxide nanoparticles can potentially pave the way for faster clinical translation of such multi-modal agents. In addition, incorporation of NIR optical imaging probes with radioisotopic imaging probes such as SPECT and PET, within targeted, biocompatible nanoparticles is another attractive approach. Combining multi-modal imaging probes with a clinically acceptable nano-drug delivery system, such as Abraxane, will lead to the development of 'theranostic' agents, where the tumor response to the administered drug can be monitored in real time via non-invasive imaging in the clinic.

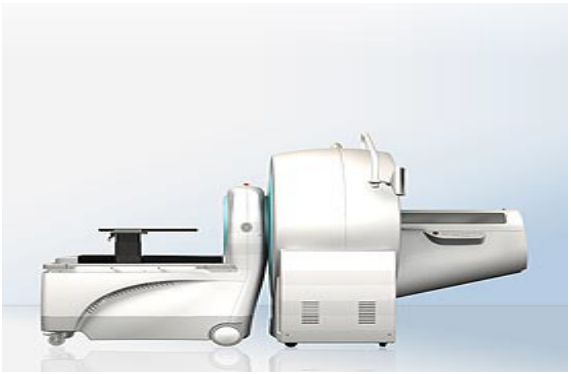

Figure 12 A clinically accepted multi-modal imaging system. The Siemens Inveon Docked PET•SPECT•CT system combines a Docked PET scanner with a SPECT•CT system (Figure courtesy of P.N. Prasad).

The other immediate clinical application of fluorescent nanoparticles is in the intra-operative delineation of the tumor boundary during surgery. Such optical guidance will enable surgeons to accurately resect the tumor mass and any metastatic spread while sparing normal cells/tissues and avoiding the risk of recurrence due to leftover neoplastic cells. The availability of clinically accepted multi-modal imaging systems (Figure 12) has bolstered the need for multi-modal nanoparticle imaging agents. Further development in instrumentation technology combining other modalities like optical and MR in the same instrument will pave the way for additional opportunities in imaging.

Milestones

3 Year:

- Develop multi-imaging scanners for multi-modal imaging of small animals.

5 Year:

- Translate these scanners into human applications in the clinic.
- Complete successful large animal studies such as dogs and non-human primates of at least five formulations of multi-modal nanoparticles.

10 Year:

- Complete successful clinical trials involving at least three formulations of multi-modal nanoparticles. The essential parameters to evaluate will include: (1) low or absent acute and chronic toxicity; (2) early diagnosis of cancer and other diseases, including non-invasive visualization of occult metastases; and (3) non-invasive, real-time monitoring of therapy.

Nanotechnology for Image-Guided Interventions

Shuming Nie

Emory University and Georgia Tech, Atlanta, GA

Overview

There are major opportunities and challenges in developing nanotechnology and advanced instrumentation for image-guided cancer surgery and biopsies (Singhal *et al*., 2010). The ability to visualize tumors in real-time will help the surgeon to delineate tumor margins, to identify residual tumor cells and micrometastases, and to determine if the tumor has been completely removed. This would apply to tumors of many organ sites, especially aggressive lung, pancreatic, ovarian, and metastatic breast cancers. Nanometer-sized particles such as quantum dots, colloidal gold, and biodegradable nanoparticles have functional and structural properties that make them appealing for tumor imaging. When conjugated with targeting ligands such as monoclonal antibodies, peptides or small molecules, these nanoparticles can be used to target malignant tumor cells and the tumor microenvironments (such as tumor stroma and tumor vasculature) with high specificity and affinity. In the "mesoscopic" size range of 10-100 nm diameter, nanoparticles also have large surface areas available for conjugating multiple diagnostic and therapeutic agents, opening up new possibilities for integrated cancer imaging and therapy (Nie *et al*., 2007). Similarly, advanced optical instrumentation provides unique advantages for intraoperative cancer detection that are not available from other imaging modalities. In the visible spectrum, optically labeled tumors are visible to the human eye, and can be seen and resected by the surgeon without any visual aid. In the near-infrared spectrum, standard fiber optics and silicon-based CCD cameras can be used for tumor visualization at high sensitivity and low costs (De Grand and Frangioni, 2003).

Nanotechnology is well suited for image-guided interventions because several problems that are often associated with nanoparticles and optical instrumentation are circumvented under surgical or biopsy conditions. For example, optical methods have relatively limited penetration depths due to tissue scattering and blood absorption, but this is no longer a major limitation during intraoperative cancer detection because the tumors are surgically exposed and are accessible to optical illumination and detection. Another common problem in using nanoparticles and macromolecules for cancer therapy is that they are unable to deeply penetrate solid tumors. This is not an issue as defining the tumor's external margin is the actual goal for surgical resection and internal staining is inconsequential. For intraoperative detection of small and residual tumors, deep penetration is also not required because the small tumors do not have high intra-tumoral pressures or hypoxic/necrotic cores, two main factors in limiting tumor penetration of nanoparticle imaging and therapeutic agents (Lunt *et al*., 2009). Thus, the combined use of nanoparticle contrast agents and imaging technologies is expected to improve the sensitivity and specificity of detecting microscopic tumors and residual tumor cells after resection, with important applications in both image-guided surgery and image-guided biopsy.

Clinical significance

Most human cancers are treated by surgical resection, chemotherapy and/or radiation. Surgery cures approximately 45% of all patients with cancer, and provides a dramatic survival advantage (http://seer.cancer.gov/). To cure a patient with surgery, the surgeon must remove the entire tumor at the time of surgery. A complete resection is the single most important predictor of patient survival for almost all solid tumors. This includes removing the primary tumor and draining lymph nodes that may contain tumor cells and small adjacent satellite nodules. In lung, breast, prostate, colon, and pancreatic cancers, a complete resection has a three to five fold improvement in survival compared to a partial or incomplete resection. Clearly, it is important to maximize the efficacy of surgical procedures because it is the most important method that exists to cure people of their cancer.

Minimally invasive cancer surgery

One of the most important changes in surgical oncology has been the development of minimally invasive surgery, which promises to alter the delivery of cancer care in the U.S. and in the world. Historically, one challenge of cancer surgery has been the loss of six to eight weeks that

occurs following an open procedure. After surgery, there can be a lengthy recovery time during which no adjuvant therapies can be given. Many common set backs including a urinary tract infection, pneumonia or arrhythmia, can delay the start of chemotherapy or radiation an additional month, during which the disease can still progress. Another problem is that many patients do not qualify for open procedures due to frail health and advanced age. The development of minimally invasive surgery has solved these challenges. Lung cancers are now removed by thoracoscopic lobectomy, colon cancers by a laparoscopic colectomy, and prostate cancers by robotic surgical instruments. Consequently, recovery time has dramatically decreased. These surgical techniques have translated well into other realms making rapid diagnoses and specimen retrieval possible with minimal patient duress.

Furthermore, minimally invasive surgery has largely replaced open surgery as an important tool to obtain rapid diagnostic information and specimens. For example, laparoscopic examination of the abdomen is used to evaluate and obtain diagnostic material for ovarian cancer, gastric cancer, and pancreatic cancer. Similarly, thoracoscopic (chest) surgery is used to obtain pleural biopsies in metastatic breast cancer, lymphomas, and mesothelioma. These procedures require only three to four small ports on a patient's chest, and can take place as an outpatient with costs under $5000 (vs. $30,000 for open surgery).

Nanoparticle contrast agents

As advancements in the field of nanoparticle imaging science are made, one of the first theatres for their use will be open and endoscopic conditions. There is considerable evidence indicating that the use of injected contrast agents can improve the detection of tumor margins and small metastases (Sajja *et al*., 2009). New and innovative targeting and contrast agents including small molecules, antibodies, and nanoparticles should be developed for a broad range of tumor types such as breast, brain, pancreatic, and ovarian cancers. At present, a number of organic dye molecules have been approved for human use including (1) indocyanine green (ICG), a near-infrared fluorescent dye; (2) fluorescein, a green fluorescent dye; (3) photofrin, a mixture of fluorescent protoporphyrin oligomers approved for photodynamic therapy, and (4) 5-aminolevulinic acid (ALA), a small molecule that is preferentially taken up by tumor cells leading to biosynthesis and accumulation of protoporphyrin IX, a natural fluorophore with red fluorescence emission. On the other hand, nanoparticles have not received FDA approval for clinical tumor imaging.

A major task is, therefore, to develop biocompatible and nontoxic nanoparticle contrast agents with the potential for FDA approval and human use. Such agents need to show improved sensitivity and specificity for tumor imaging in comparison with small-molecule dyes. In this regard, it is highly promising to develop "smart" or activatable nanoparticles with improved pharmacokinetic, tumor-targeting, and organ clearance properties, based on the use of natural, biodegradable polymers (dextran and heparin). Dextran-based particles are sensitive to pH, and can be rapidly broken down under acidic conditions. Under neutral or slightly basic conditions, on the other hand, the dextran nanoparticles are stable and are able to circulate systemically in blood for 14-15 hours (Gaur *et al*., 2000). In contrast, self-assembled heparin nanoparticles have much shorter blood circulation half lives (about 60-80 min) (Chen *et al*., 2009). For intraoperative use, this short circulation time could be beneficial because the probes will be cleared from the blood quickly, so that surgical operations can start without much delay or waiting. For near-term clinical applications, it is important that both the dextran and heparin particles are able to trap an FDA-approved dye (such as indocyanine green), leading to a new class of imaging contrast agents with improved biodistribution and photophysical properties. Figure 13 shows a class of "nano-ICG" contrast agents that are quenched in their initial state but are activated under *in vivo* conditions (Mohs *et al*., 2010). This class of nanoparticle contrast agents could also be conjugated with tumor targeting ligands such as folate, EGF, or RGD for improved sensitivity and specificity.

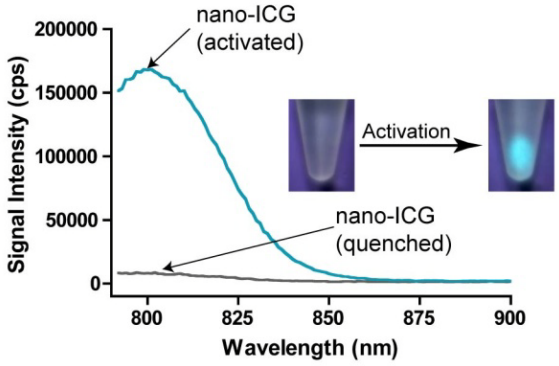

Figure 13 Optical properties of nano-ICG, a new class of biodegradable and self-assembled particles with physically trapped indocyanine green (ICG) molecules. In this type of "nano-ICG" imaging agent, the ICG fluorescence is quenched in the trapped state, and is activated when the dye is released under *in vivo* conditions (reprinted from Mohs *et al*., 2010, Copyright, American Chemical Society).

Milestones

3 year:

- Generate polymer-coated nanoparticles using current FDA-approved fluorescent dyes for residual tumor and metastases labeling. Incorporate tumor-targeting ligands for increased sensitivity and specificity.
- Develop novel nanoparticle imaging dyes that are not subject to photobleaching with targeting moieties to differentiate tumor from normal tissues and precisely delineate tumor margins.

5 year:

- Study *in vivo* toxicity in model organisms.

- Begin clinical trial evaluation of the most successful nanoparticles, coupled with minimally invasive delivery procedures.

10 year:

- Commercialize several targeted nano-imaging particles.

Development of Imaging Hardware Based on Nanotechnology

Otto Zhou
University of North Carolina, Chapel Hill, NC

Introduction

X-ray radiation is widely used today for *in vivo* cancer detection and for radiotherapy. For example, mammography is the most common modality for breast cancer screening and over 50% of the cancer patients in the U.S. undergo radiation therapy. For x-ray based imaging and radiotherapy techniques there is a constant demand to increase resolution to detect tumors at an early stage, minimize the imaging dose to reduce side effects, improve the accuracy of dose delivery during treatment, and minimize normal tissue damage. The new carbon nanotube based x-ray source technology enables the design of new imaging and radiotherapy devices with improved performances in these areas.

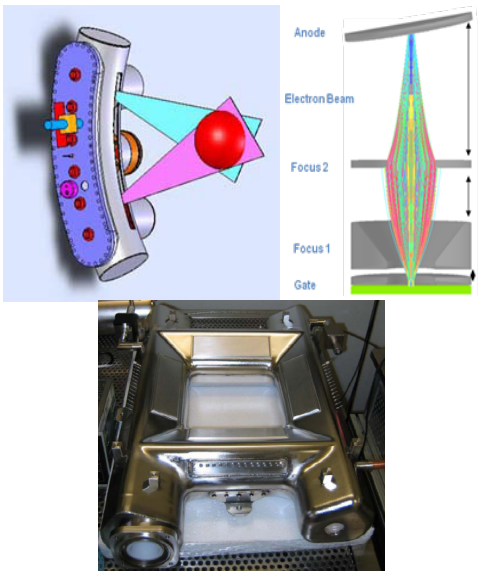

Figure 14 **Schematics showing a nanotube x-ray source array (top) and a square-geometry nanotube x-ray source array with 52 individually controllable x-ray beams (bottom; XinRay Systems).**

Utilizing the recent advances in nanomaterials a new x-ray source technology has been developed. Carbon nanotubes instead of the conventional thermionic filaments are used as the "cold" electron sources for x-ray generation. The technology is capable of generating *temporally* and *spatially* modulated x-ray radiation that can be readily gated and synchronized with physiological signals. The spatially distributed x-ray source array technology opens up new possibilities for designing *in vivo* imaging systems with increased resolution and imaging speed and expanded functionalities. By distributing the x-ray power over a large area, the technology can generate a significantly higher dose rate for certain radiotherapy applications. Since its invention this nanotechnology enabled x-ray source technology has moved from a simple academic curiosity to commercial production (Figure 14). The applications of this new technology for cancer detection and treatment are being actively investigated in academic institutions and in industry. Below are some examples of the *in vivo* imaging systems currently under development with the support of the NCI Alliance for Nanotechnology in Cancer program.

High-resolution micro-CT for *in vivo* imaging of small animal cancer models

Utilizing the electronic programmable capability of the nanotube x-ray source a physiologically gated micro-computed tomography (CT) scanner has been developed for *in vivo* imaging of small animal cancer models (Figure 15) (Cao *et al*., 2009; Cao *et al*., 2010). By synchronizing x-ray exposure and data collection with the non-periodic respiratory and cardiac motions high resolution CT images with minimum motion blurs can be obtained from free-breathing mice. The scanner is used routinely by a large number of cancer researchers at the University of North Carolina-Chapel Hill (UNC) for *in vivo* imaging of their small animals. Additional systems are being constructed and will be installed at UNC and the University of Iowa for cancer research.

"Real-time" tomosynthesis image guidance for radiation therapy

Utilizing the distributed x-ray source array technology, Siemens and XinRay Systems developed a high-speed tomosynthesis scanner to provide real-time image guidance for radiation therapy (Maltz *et al*., 2009). The development won the team the 2010 Sorkin Award from the American Association of Physicists in Medicine. The technology will enable the oncologists to "see" tumors in real time during treatment and will allow more accurate radiation delivery. The scanner has been integrated with the Siemens Artiste treatment system. It is currently under testing at the UNC Cancer Hospital. Clinical tests are scheduled for this year and Institutional Review Board approval has already been obtained.

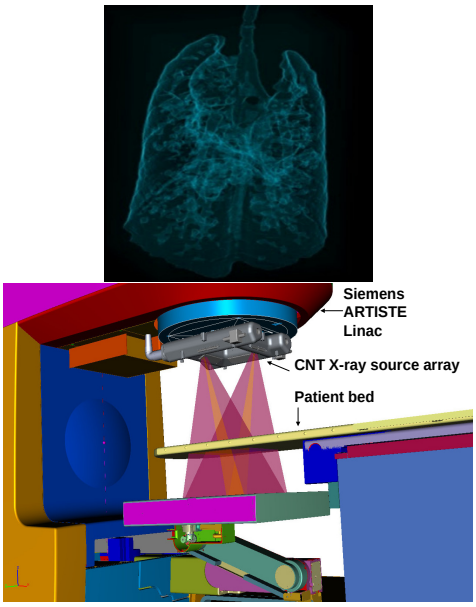

Figure 15 Prospective-gated micro-CT image of a mouse lung tumor model (top; UNC data. Mouse model from Dr. B. Kim). An illustration of CNT x-ray source array mounted on a radiotherapy machine (bottom; image courtesy of J. Maltz of Siemens and P. Lagani of XinRay).

Digital tomosynthesis for early stage detection of human breast tumors

Digital breast tomosynthesis (DBT) has the potential to become the next generation screening tool for breast cancer, replacing the current two-view mammography scanners. This limited-angle tomography technique provides quasi 3D views of the breasts which help radiologists differentiate breast tumor from the surrounding tissues. Utilizing the spatially distributed nanotube x-ray source array technology, a proof-of-concept stationary DBT scanner increases the imaging resolution, improves the detectability of micro-calcification, and reduces the imaging time which reduces the patient discomfort from breast compression, compared to the rotating DBT scanners from commercial vendors that are currently under clinical trials for FDA approval (Qian *et al*., 2009). Encouraged by the initial results a second generation, clinical test ready, scanner is currently under development which will integrate the nanotube x-ray source with a commercial mammography scanner.

Future challenges

From the engineering perspective, the reliability, consistency, and lifetime durability of the devices need to be demonstrated to be comparable or even better than the existing systems before they can be adapted in the clinics. Since imaging and radiotherapy devices are complicated, new device development requires a large multi-disciplinary team with complementary expertise in a wide range of fields as well as close collaborations with industry. The question as to how to organize and finance the research and development effort is always a challenging one.

Clinical potential

Recent research has clearly demonstrated the potentials of the nanotube x-ray based systems for clinical *in vivo* cancer imaging and radiation therapy applications. Some examples include early detection of breast cancer, image guidance for radiation therapy, and novel radiotherapy techniques.

Milestones

3 year:

- Develop stationary tomosynthesis scanners for applications such as breast imaging and image-guided radiation therapy and conduct clinical tests.
- Commercialize imaging systems for small animal models.

5 year:

- Develop microbeam radiation therapy using the nanotube x-ray source array technology for small animal models.
- Conduct studies of their therapeutic effects on small animal brain tumor models.
- Commercialize tomosynthesis imaging systems.

10 year:

- Develop a new generation of CT scanners based on this technology and utilize it in radiotherapy for human patients (for example, microbeam radiation therapy).

Nanotechnology and Cancer Prevention

Sara S. Hook

Office of Cancer Nanotechnology Research, CSSI, National Cancer Institute, NIH, Bethesda, MD

Patient prevention strategies

There are several possible approaches to cancer prevention. Patients can decrease behaviors that put them at risk, be more vigilant in screening and surveillance, opt for surgical pre-intervention, and/or utilize "medicinal" approaches. The latter three areas in particular can benefit from the advances that nanotechnology can offer.

It is well recognized that several factors contribute to and enhance cancer prevention including dietary and lifestyle changes. The field of epidemiology has long been examining what types of risk factors are correlated with certain types of cancers. For instance, probably one of the best documented and most studied behavioral risk factors is that smoking increases the incidence of lung cancer. In fact, smoking also greatly increases the risk of many types of cancers as well as heart attacks (Khan *et al*., 2010). A second well documented example is increased exposure to UVB rays from sunlight clearly damages DNA and can result in an increased risk of various types of skin cancer including the most deadly, melanoma (Cooper and Bowden, 2007).

Patients themselves can also implement mechanisms of surveillance. This would include performing breast self-exams to detect lumps and nodules, monitoring the skin for changes in moles, and seeing a doctor for routine physical exams. For those with a family pre-disposition to cancer, additional monitoring may be in order. For instance, patients who have a primary relative such as a mother or sister with breast cancer might want to undergo genetic testing to determine whether they are carriers of the familial breast cancer susceptibility genes, *BRCA1* and *BRCA2*. Additionally, imaging such as mammography has played an important role in screening at risk women and those over 40 for breast cancer. The areas of diagnostic imaging and molecular *in vitro* screening are areas in which nanotechnology can play a significant role. For example, Dr. Otto Zhou's group at UNC-Chapel Hill is developing a stationery digital breast tomosynthesis scanner using carbon nanotube (CNT) multi-pixel field emission x-ray (MBFEX) technology. This approach will increase image resolution and decrease both patient discomfort and radiation exposure times (Qian *et al*., 2009). The advances and future challenges in cancer imaging have been outlined in several previous sections. Likewise, *in vitro* genomic and proteomic testing strategies based on nanotechnology, such as those outlined earlier in this document, can be more sensitive, more cost effective, more rapid, and possibly more accurate than technologies currently in clinical use. Surgical intervention for "pre-cancerous" lesions detected during routine colonoscopies, or prophylactic breast, ovary or complete hysterectomies for patients at high risk for reproductive cancers likely also play a role in primary and secondary cancer prevention. As previously discussed in other sections, nanotechnology offers the physician increasing ability for image-guided surgical resection of tumors and possibly also pre-cancerous lesions. In fact, one example of this is from Dr. Sanjiv Sam Gambhir's research group where they have used single-walled carbon nanotubes (SWCNTs) combined with Raman imaging to visualize tumors in live small animal models (Keren *et al*., 2008). They are pursuing applications for this technology such as clinical colonoscopy and have already built a flexible endoscope capable of Raman imaging.

"Medicinal" prevention strategies

Many might hope that one day cancer could be prevented using some type of vaccine or pill to ward off the disease. The etiology, however, makes this a huge task due to the myriad of mechanisms by which the disease arises, the ability of cancer cells to escape immune system detection (due to recognition as "self"), the tissue specificity of some tumor types, the altered cellular growth and metabolism pathways, etc. Thus the concept of medical prevention in terms of vaccines and drugs is extremely challenging.

There are strong indications that avenues of medical prevention of cancers may be successful. One approach that is very promising is in the area of human papillomavirus (HPV) vaccines to prevent genital warts and hopefully also cervical, vulvar, and vaginal cancers. Two FDA approved vaccines, Cervarix (GlaxoSmithKline) and Gardasil (Merck), are recombinant versions of virus

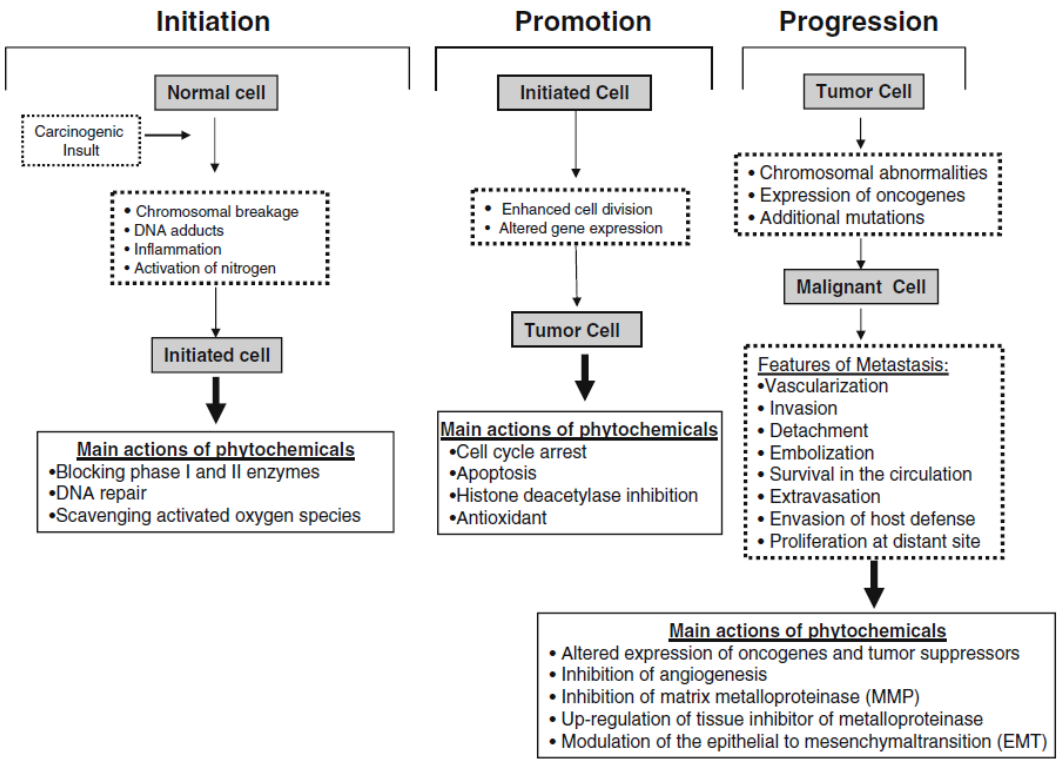

Figure 16 Areas in which neutraceuticals or phytochemicals can intervene in the process of carcinogenesis (reprinted from Mehta *et al*., 2010, Copyright, Springer Science).

like particles of the most common types of HPV, strains 16 and 18. Since up to 75% of cervical cancer cases are caused by HPV-16 and HPV-18 these vaccines might eliminate most of these cases in the future. Additionally, Cervarix has also been shown to decrease infection rates of other cancer causing HPV strains including 31, -33, -45, and -52 (D'Andrilli *et al*., 2010). Clearly these vaccines have shown effective in dramatically decreasing genital HPV infection, but a long term follow-up is needed to establish the efficacy of decreasing cancer incidence and mortality. A randomized, double blind, Phase III trial did show Cervarix decreases the risk of developing CIN2+ pre-cancerous lesions (Paavonen *et al*., 2009). Vaccines, however, for the prevention of cancer might be the exception and not the rule. An alternative approach being pursued by PDS Biotechnology is developing nanoparticles that contain an antigenic peptide to an essential protein component of HPV, E7. These particles target dendritic cells to produce antigens to E7 and promote a cytotoxic response from killer T cells. This approach is unique in that current vaccines only work if the patient is not already infected with strains of HPV, whereas this approach can target patients who are already infected (Chen *et al*., 2008).

Anti-inflammatory drugs directed to the cox-2 (cyclooxygenase-2) family of enzymes responsible for prostaglandin synthesis have shown promise in cancer prevention. Two of these, however, Vioxx (Merck) and Bextra (Pfizer), have been pulled from the market due to side effects related to heart attack, stroke, and gastrointestinal bleeding. Studies initiated well before these drugs were removed from the market have indicated that these drugs significantly reduce the risk of developing cancer of the colon, breast, lung, and prostate (Harris, 2009). The side effects, however, limit their therapeutic value. The remaining cox-2 inhibitor, celecoxib (Celebrex manufactured by Pfizer), has been approved by the FDA for use to prevent colon cancer but only in the extreme case of patients with Familial Adenomatous Polyposis (Half and Arber, 2009). Additionally, Celebrex was found to reduce the growth of basal cell carcinomas by 50% in some patients with a rare genetic condition, Gorlin syndrome, which makes them highly susceptible to tumorigenesis (Tang *et al*., 2010). An additional study suggests that patients who took Celebrex daily for nine months had 60% fewer non-melanoma skin cancers than people who did not take the drug (Elmets, 2009). Numerous studies also suggest that cox-2 inhibitors when given in combination with other therapies can potentiate cancer cell death. In terms of prevention, these types of drugs could be reformulated into targeted nanoparticles to make use of their protective effects in preventing the formation of colon and rectal polyps or skin carcinomas without the unwanted cardiovascular side effects. Although attempts at microemulsion formulations are underway (Margulis-Goshena *et al*., 2010), nanoparticle encapsulation would be most beneficial to circumvent the unwanted side effects of these drugs.

Another avenue that could potentially be exploited for prevention would be the area of anti-inflammatory nutraceuticals (Nair *et al*., 2010). Research

has shown that part of the cancer progression phenotype is chronic inflammation (Grivennikov and Karin, 2010). Quite a number of natural products have been shown to decrease inflammation but in almost all cases, the bioavailability of these compounds is limited. Thus, nanoparticle delivery of such agents as curcumin, green tea polyphenols, coenzyme Q, etc. could be very useful. For example, a catechin, epigallocatechin-3-gallate (EGCG) found in green tea, has chemopreventive potential for human breast, pancreatic, colon, esophageal, and lung cancers, but its oral absorption rate is only 1% (Nair *et al*., 2010). Consequently, more than 5 cups of green tea would need to be consumed for a health benefit (Johnson *et al*., 2010). Nanoparticle delivery of EGCR then would be beneficial. In fact, the formulation of EGCG into PLA-PEG nanoparticles offered a more than 10 fold decrease in the IC50 over free EGCG when monitoring tumor cell viability (Siddiqui *et al*., 2009). EGCG can inhibit tumor cell growth and decrease angiogenesis in mouse xenograft models (Siddiqui *et al*., 2009) on its own but it can also sensitize tumors to growth inhibition by other agents such as interferon-α2b (Nihal *et al*., 2009). In addition to this compound's anti-inflammatory properties, it also decreases signaling of several kinase pathways, insulin-like growth factor, and androgen receptor signaling. In fact, clinical studies in men with prostatic intraepithelial neoplasia (PIN), a pre-cancerous lesion of the prostate, revealed a 90% reduction in the progression to prostate cancer when taking ECGC containing supplements (Bettuzzi *et al*., 2006). Additional studies have, however, indicated that the controlled formulation of nutritional supplements is quite important for biologically efficacious effects (Johnson *et al*., 2010). Green tea catechins are just one example within many that are being evaluated for their chemopreventative potential in similar research studies (Nair *et al*., 2010). Although a great deal of discussion was devoted to natural products, researchers could also build upon these chemical structures using rational drug design approaches to improve upon what nature has given us.

The main focus here has been on prevention meaning before malignant growth has started. Confirmation of whether a compound has this potential is usually through prospective studies where patient cohorts are followed over a long period of time to correlate behavioral risks with cancer development. Neutraceuticals can, however, have an impact with other chemotherapeutic agents even after malignancy has been diagnosed to enhance the effectiveness of these treatment regimes (Mehta *et al*., 2010). As depicted in Figure 16, neutraceuticals can by a vast variety of mechanisms feed into the processes of apoptosis, cell cycle arrest, DNA repair, protection against free radicals, etc., all processed known to be important in preventing cancer formation. Thus, future research will undoubtedly include an increasing focus not only on neutraceutical effects by themselves but also in combination with other therapeutic strategies.

Milestones

3 year:

- Publish more studies on characterizing natural products and their chemopreventive potential.
- Develop nanotechnology delivery systems for neutraceuticals and other chemopreventive agents.
- Carry out more prospective studies to identify genetic, behavioral, and environmental risks for various types of cancers.

5 year:

- Incorporate natural products with more standard therapeutic approaches in an increasing number of clinical trials.
- Conduct rational design experiments to improve on the potential therapeutic effects of existing neutraceuticals.
- Identify other potential targets for cancer vaccine development.

10 year:

- Follow-up studies with patients vaccinated with HPV vaccines will reveal whether they actually decrease the development of cervical, vulvar, and vaginal cancers.
- Develop nanotechnology mechanisms to limit exposure to environmental toxins.

NCI's Nanotechnology Characterization Laboratory

Scott E. McNeil

Nanotechnology Characterization Laboratory, National Cancer Institute, Frederick, MD

Mission

The NCI's Nanotechnology Characterization Laboratory (NCL) provides infrastructure support to NCI's Alliance for Nanotechnology in Cancer. The lab's mission is to provide pre-clinical characterization to its sponsors, and to accelerate the translation of promising nanotechnology-derived cancer treatments into clinical applications. The NCL was founded in 2004 as a formal interagency collaboration among NCI, NIST, and the FDA and is operated through the NCI's Federally Funded Research and Development Center (FFRDC) at SAIC/NCI-Frederick.

NCL has a number of key objectives which include characterizing nanoparticles using standardized methods and conducting structure activity relationship (SAR) studies to identify and delineate critical parameters related to nanomaterial pharmacological properties and toxicology. Additionally they facilitate the regulatory review of nanotechnological constructs and engage in educational and knowledge sharing efforts.

The NCL's services are available for free to researchers developing a nanotechnology cancer therapy or diagnostic. Nanomaterials accepted by NCL are subjected to a three-tiered Assay Cascade of scientific tests, including physic-chemical characterization, *in vitro* assessment, and *in vivo* evaluation for safety and efficacy. The data generated from the NCL characterization are intended for use in support of IND or Investigational Device Exemption (IDE) applications to the FDA. As such, the NCL serves as a bridge to take promising cancer nanotechnology research to regulatory approval.

Achievements

In just six years of operation, the NCL has become a recognized authority in nanotechnology for biomedical applications. The Lab has over 50 collaborations with researchers from academia, industry, and government and has characterized almost 200 different nanomaterial samples – including liposomes, metal colloids, dendrimers, polymers, quantum dots, metal oxides, and fullerene derivatives. Multiple NCL collaborators have now submitted an IND or IDE application and one collaborator has begun Phase II clinical trials.

Lessons learned

One of the ways that the NCL contributes to the Alliance and to the nanotechnology research community in general is by sharing the observations made in its Assay Cascade. Investigators benefit from these "Lessons Learned" thus accelerating the progress of the entire community.

Stability and Scalability. The Lab now has several examples where stability issues negatively impact the rapid development of nanoparticle-based therapies. Particles that release their payload within seconds to minutes of administration offer minimal advantage over traditional small molecule drugs. On the other end of the stability spectrum are nanoparticle formulations that are *too* stable – that is, the drug is not released from the nanoplatform and is generally inefficacious. In the case where drugs are covalently linked to the carrier, it is essential that this linkage is cleavable or otherwise degradable by the intracellular environment. Scale-up is also a common hurdle in the development process. In the case of nanoparticle formulations, early-stage planning can easily circumvent obstacles in this path to commercialization. An obvious example of this pitfall is found in the misunderstanding that academic studies are simply smaller versions of large-scale production.

Sterility. Another problem common to small-scale synthesis is contamination. Academic labs often use glassware not dedicated to aseptic procedures, and generally do not utilize "best practices" to prevent endotoxin contamination. On numerous occasions, investigators have submitted material to the NCL that is rife with endotoxin or other microbial contamination. This type of contamination severely impedes *in vitro* and *in vivo* studies, as it perturbs cell signaling pathways and may induce an immune response.

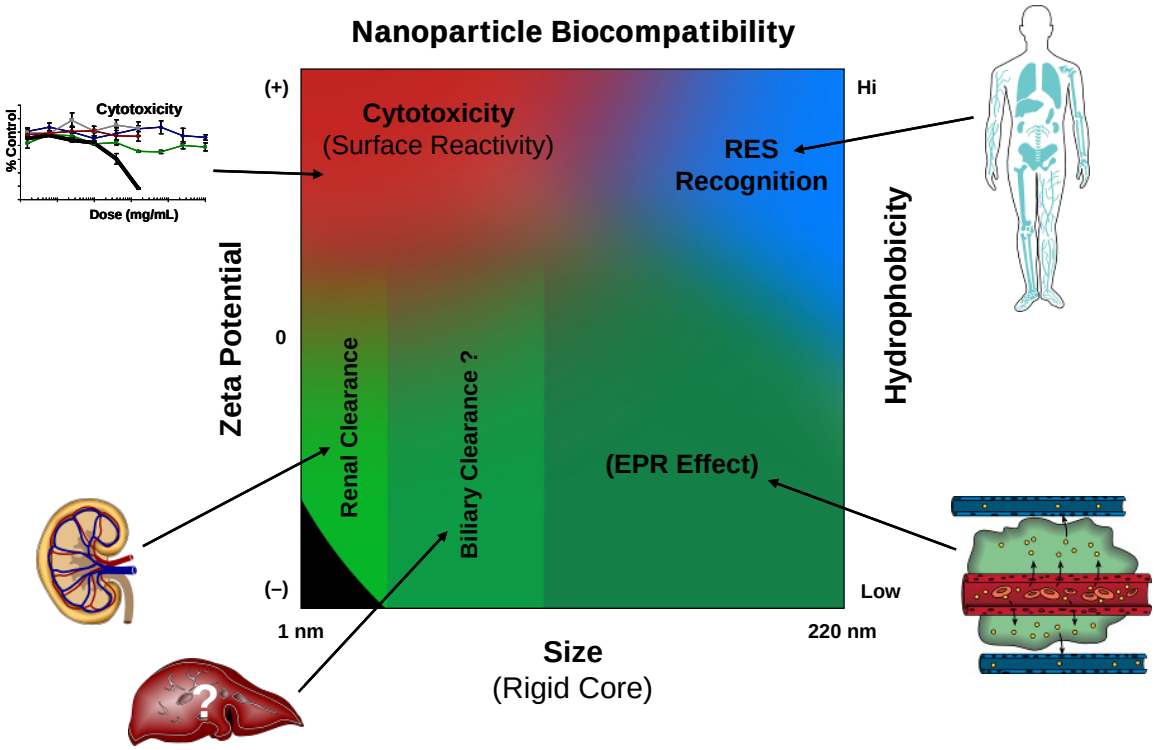

Figure 17 **Nanoparticle Biocompatibility. This plot shows trends the NCL has observed in the relationship between nanoparticle physico-chemical properties and biological responses. The independent variables in this plot are the zeta potential (related to surface charge), size, and hydrophobicity which are plotted versus the dependent variable of biocompatibility, manifested in such biological responses as cytotoxicity, clearance, the EPR effect, and RES recognition. (Adapted from McNeil, 2009).**

Predicting Toxicology Based on Nanoparticle Structure. NCL-generated data has elucidated trends in nanomaterial characteristics and identified critical parameters that influence nanomaterial biocompatibility (Figure 17). This data has contributed substantially to the current understanding of the "nano-bio interface." For example, particles must be smaller than approximately 200 nanometers to transverse the architecture of the liver and spleen. Particles that are hydrophobic (e.g. without a PEGylation layer) will quickly be removed from circulation by macrophages in the RES. With respect to elimination, particles and/or their breakdown products must be less than 10 nm to be excreted through the kidneys, otherwise they may reside in the RES organs for the lifetime of the animal. However, particles as large as 30 nm may be excreted in the bile. Finally, cationic (strongly positively charged) particles are cytotoxic, with or without the chemotherapeutic agent onboard. Investigators should engineer particles with these parameters in mind – exploiting the advantageous particle characteristics to hopefully avoid repeating trial and error studies of the past.

Milestones

3 year:

- Conduct more in-depth structure-activity relationship (SAR) studies. Coordinated efforts to address research gaps are critical to advancing our understanding of the nano-bio interface. In mathematical terms, it is imperative to obtain the *total derivative* (all the parameters that influence biocompatibility) by examining many *partial derivatives* (varying one parameter while holding the others constant). While a few academic labs are attempting this, e.g. varying PEGylation density and observing phagocytosis, a comprehensive study of multiple parameters is desperately needed. Direct resources towards the synthesis of these reagents and testing them in the NCL's assay cascade.
- As the nanotech concepts submitted to the NCL mature, more NCL collaborators are seeking access to IND-enabling pre-clinical characterization resources such as good laboratory practice (GLP)-certified toxicology studies, large animal studies, and good manufacturing process (GMP)-certified manufacturing/synthesis capabilities. Collaboration with other government laboratories (e.g. FDA's National Center for Toxicological Research) will

allow the NCL to leverage such resources without great expense.
- Establish collaborations with industry to reformulate discontinued cancer drugs using nanotechnology.

5 Year:

- Increased interaction with Contract Research Organizations (CROs) will facilitate the scale-up process and transition to GMP manufacturing. NCL will endeavor to make contacts at the CROs that have experience with nanoparticle formulations and to increase our visibility to these organizations.
- Devise analytical methods to differentiate nanoparticle-bound vs. free drug. To support regulatory review of nanoformulations, analytical methods that can determine the free, and therefore "active" drug component of a nanoparticle drug profile are needed.

10 year:

- As NCI's Alliance moves into its second iteration and the nanotech concepts submitted to the NCL continue to mature, the NCL's relationship with the FDA will necessarily evolve as more NCL-characterized concepts enter the regulatory process. Specifically, we expect increased interaction with FDA reviewers. NCL will continue to seek input from the FDA on its assays and to collaborate with the FDA on the regulatory aspects of nanotechnology and SAR studies. In 10 years, NCL aims to facilitate three to five IND filings.

Safety Issues in Pre-clinical and Clinical Evaluation of Nanotechnology-based Products

Subhas Malghan and Carlos Pena

U.S. Food and Drug Administration

Nanotechnology allows scientists to create, explore, and manipulate materials in the nanoscale range. Behavior of such materials in terms of chemical, physical, and biological properties may differ from those of their larger counterparts. A general finding of the "FDA Nanotechnology Task Force Report 2007" is that nanoscale materials present regulatory challenges similar to those posed by products using other emerging technologies. However, distinct challenges may also arise because at the nanoscale, properties of a material might change in ways that could affect the performance, quality, safety and/or effectiveness. While applications of nanoscale materials in cancer treatment are continuing to evolve, one needs to consider the potential unintended health impact of these materials. One reason for this potential is that some of these materials will eventually come into contact with biological structures and processes that frequently occur at the nanoscale.

Understanding interactions of nanoscale materials with biological systems

To assess the interaction of nanoscale material with biological surfaces, reliable and reproducible screening methods are needed. Achieving this goal has become a challenge because of the large variety of new nanoscale materials that are under development, their unique set of novel physico-chemical properties, and uncertainty of how those properties relate to biological outcomes. There is a possibility of a vast number of physico-chemical interactions with biological surfaces when nanoscale materials of different size, composition, shape, surface area, aggregation, crystallinity, surface coating and functionality, and hydrophilic/hydrophobic interactions come in contact with biological fluids, proteins, lipids, DNA, cell membranes, lysosomes, mitochondria, and biological processes (Nel *et al*., 2009). Therefore, a comprehensive physico-chemical characterization as well as pharmacokinetic and biodistribution studies are required to evaluate safety as well as efficacy. Currently, there is considerable discussion on nanomaterial toxicity testing, with the major discussion centering around which toxicological end points to screen for, the adequacy of the screening effort, and the correct balance of *in vitro* (cellular and molecular) versus *in vivo* (animal or whole organism) testing (Oberdorster *et al*., 2005; Borm and Berube, 2008; Nel *et al*., 2009). Attempts to use traditional toxicological assays and models have resulted in conflicting and sometimes irreproducible results.

Additional important questions exist concerning the transport of nanoscale particles in the human body and mechanisms of interaction at the sub-cellular and molecular levels. The unique and diverse physico-chemical properties of engineered nanoscale materials suggest that their toxicological properties may differ from materials of similar composition but larger size. Studies also suggest that particle size, surface area, and surface chemistry of engineered nanoscale materials can impact toxicity equally, if not more so, than chemical composition (Nel *et al*., 2009). Research is in progress to evaluate toxicity of nanoscale materials that represent a cross-section of composition, size, surface coatings, and physico-chemical properties. Many of these studies are designed to investigate fundamental questions concerning how nanoscale materials are absorbed and distributed *in vivo* and whether they can adversely impact biological systems. More studies are needed to detect and quantify nanoscale particles in tissues, mechanisms of nanoscale material absorption, distribution in the body, and subsequent up take by cells. These studies have the potential to develop a better understanding of biological and toxicological interactions.

Different uses may have different requirements with regard to nanoscale material

While biocompatibility and toxicity would be important for devices, absorption, distribution, metabolism, and excretion are relevant in the evaluation of safety of nanoscale materials contained in drugs. Concepts that have been applied in the micron size range may be usefully applied to the nanoscale range, but new challenges are presented based on the small size and possible change in the dissolution-translocation relationship (Nel *et al*., 2009). Solute concentration, surface area, surface morphology, surface energy, dissolution layer properties, adsorbing species, and aggregation are some relevant parameters when considering dissolution at the nanoscale. With regard to the etiopathology caused by nanoscale particles, the metrics of dose (particle number, surface area, mass or shape) is not yet well defined. Analytical procedures for assessing dissolution and translocation include chemical assay and particle characterization. Leaching of components from particle surfaces as well as compartmentalization within the respiratory tract may add another dimension of complexity. Dissolution may be a critical step for some nanoscale materials in determining their fate within the body. An integrated approach combining particle toxicology, material science, and analytical chemistry is required to provide a useful basis for developing relevant dissolution assay(s) for nanoscale particles.

Studies have indicated that various attributes of a particular nanoscale material, including increased specific surface area, morphology, surface features, and charge, can affect the distribution of that material in the body, that material's toxicity, and/or its biocompatibility. In addition, current testing approaches may need to be evaluated and new approaches developed to assess safety, effectiveness, and quality of a product that uses a nanoscale material.

A conclusion of some studies in this area is that current risk assessment methodologies require some modification to address hazards associated with nanoscale materials and in particular that existing toxicological and biocompatibility methods may not be sufficient to address all issues related to nanoscale particles. For exposure evaluation, dose determination requires information on the number of nanoscale particles and/or their surface area in addition to the traditional mass concentration characterization. Equipment for routine measurements in various media for representative exposure to free nanoscale particles is inadequate. In addition, existing assessment methods may not be appropriate to determine the fate of nanoscale particles. While an understanding of general risks of products using nanoscale materials is continuing to evolve, there is greater need for understanding the risks of free or "unconjugated" nanoscale materials because they are likely to behave differently from the same material/compound in a complex nanoparticle which may result in altered biological and toxicological behavior. Nanoscale materials may exhibit unique physico-chemical properties due to surface coatings or other nanotopographical features.

Summary

Inclusion of a nanoscale material in an FDA-regulated product or a change in the nanoscale material(s) used may affect the quality, safety, and effectiveness of that product and may raise questions regarding appropriate testing methods. Accordingly, additional data and testing methods may be needed for assessing the effects of a nanoscale material on a product, whether subject to premarket authorization or not (FDA Nanotechnology Task Force Report 2007). In some cases, the presence of a nanoscale material may also affect the regulatory requirements applicable to a product.

The FDA is available to assist manufacturers and sponsors in identifying and addressing regulatory issues raised by specific uses of particular nanoscale materials, including issues with regard to safety, effectiveness, good manufacturing practices, and possible changes in the regulatory classification or pathway for product approval. Both research and development groups are encouraged to contact the FDA to discuss the proposed use of specific nanoscale materials in an FDA-regulated product even if no legal requirement to notify the Agency applies.

Regulatory Aspects Related to Products Containing Nanoscale Materials

Subhas Malghan and Carlos Pena

U.S. Food and Drug Administration

The FDA regulates a broad range of products under the Federal Food, Drug, and Cosmetic Act (FFDCA) and the Public Health Service Act (PHS Act). The Agency's statutory authorities subject some types of products to premarket authorization requirements, either individually or by category, while permitting other products to be marketed without prior Agency authorization (FDA Nanotechnology Task Force Report 2007). The term "premarket authorization" refers to a number of regulatory actions that the FFDCA, the PHS Act, and agency regulations may refer to by other names, including "approval," "clearance," "licensing," and "listing." Most, if not all, laws and regulations under which the FDA operates are by design general in nature. Therefore, the agency's authorities usually are able to accommodate products made with the use of emerging science, new technologies, or containing new kinds of materials. The use of nanoscale materials in an FDA-regulated product may raise questions regarding which regulatory requirements apply and how they can be satisfied. Nanoscale materials are of particular interest to the FDA, since there is significant potential for their application to a large number of products regulated by the FDA. Nanoscale materials can have physical or biological properties that are different from those of their larger counterparts because of their small size and high specific surface area. Such differences may include altered magnetic properties, altered electrical or optical activity, increased structural integrity, or increased chemical or biological activity. Because of some of their special properties, these materials may present different safety and efficacy issues than their larger counterparts.

Medical products

Drug products (FDA Nanotechnology Task Force Report 2007): New drugs for humans, as well as new animal drugs, are subject to premarket authorization on a product-by-product basis. Information on the identity of products such as the type of product, the size of the components, and the manufacturing protocol is required as part of marketing applications if it is relevant to safety or effectiveness. In the case of replacing a current drug substance or excipient with a nanoscale version, the resulting product may be considered a new product for which a new approval would be needed.

Biological products (FDA Nanotechnology Task Force Report 2007): With regard to human cell and tissue products that might otherwise be subject to regulation only under section 361 of the PHS Act and, therefore, not subject to premarket authorization, we encourage manufacturers to contact the FDA before marketing any version that incorporates nanoscale materials or is otherwise modified at the nanoscale, to confirm whether these features trigger premarket authorization requirements.

Devices (FDA Nanotechnology Task Force Report 2007): Medical devices are regulated according to a tiered classification system that is largely based on the degree of risk posed by the product. Devices that are low risk, for which safety and effectiveness are generally well-established, are designated as Class I devices. These device types are subject to general controls, such as labeling, good manufacturing practices and adverse event reporting. Class II devices are more complex and carry a higher risk than Class I devices. For certain Class I devices and most Class II devices, manufacturers must submit to the FDA a premarket notification to demonstrate that their device is as safe and effective as another legally marketed device in order to obtain FDA clearance before marketing. Class III devices are the most complex, high risk devices and are reviewed under a premarket approval application (PMA). In a PMA, pre-clinical and clinical data, in addition to manufacturing information, are typically used to support the agency's determination that the device provides a reasonable assurance of safety and effectiveness.

Nanoscale material manufacturing issues

Products regulated under the FD&C and PHS Acts must be manufactured to conform with applicable requirements concerning, for example, safety, quality, and purity, and so as to avoid being adulterated. Some are additionally subject to current good manufacturing practice requirements (FDA Nanotechnology Task Force Report 2007). In some cases, the use of nanoscale materials in the development of an FDA regulated product may raise new safety issues that require new or different testing methods. Since there may be some uncertainty in the use of nanoscale materials and its impact upon such products, questions regarding safety may not be specifically addressed in existing guidance. Accordingly, manufacturers may have questions regarding how to ensure sound manufacturing practices for products that use nanoscale materials and they are encouraged to consult with the relevant FDA product center to ensure that new technologies do not present any new safety issues.

Contact FDA

There is a possibility that the presence of certain nanoscale materials used in the manufacture of medical products may affect the safety or effectiveness. Therefore, we encourage applicants to clearly indicate in regulatory submissions the presence of nanoscale materials.

If you are considering using a nanoscale material in your product, contact the FDA to confirm whether the product contains nanoscale material by FDA standards. In addition, the FDA should be contacted to discuss appropriate manufacturing practices and developing testing methods for assessment of product safety, effectiveness, and quality. Communications with the FDA regarding new nano-products will help ensure compliance with all legal obligations and will help the FDA to regulate products effectively and to address regulatory and patient safety issues proactively and efficiently. Following these recommendations will minimize delays to market entry and avoid evoking enforcement authorities to protect the public health.

Clinical Translation of Nanotechnologies: From Academic Laboratory to Start-up Company

Jeff Hrkach

Pharmaceutical Sciences, BIND Biosciences, Cambridge, MA

Developing a successful model of translation

At the highest level, the key elements to successfully translating a technology from academic research into clinical development are: technology, team, innovation and financing. These basic elements hold true for any start-up company, but even more so for the field of cancer nanomedicine given the challenges, complexities, and consequences of optimizing nano-scale technology for the treatment of people suffering from cancer.

When a start-up company is founded based upon academic research, the initial scientific efforts focus on the transfer of the technology from the academic labs into the hands of the company to develop an in-depth understanding of the technology's strengths, weaknesses, and potential when viewed from the very different lens of drug development. From the outset, the regulatory requirements dictated by the FDA for pharmaceutical development of the drug product candidate (as discussed previously) must be taken into consideration along with the pharmaceutical development considerations of product candidate optimization through rigorous pre-clinical evaluation, development of appropriate and robust analytical characterization methods, and of critical importance, manufacturing process development and scale up. The optimization approach requires evaluation of nanoparticle performance using *in vitro* cell-based assays (particle binding interactions, uptake and toxicity, drug activity), *in vivo* pre-clinical evaluation (PK, biodistribution, targeting, tolerability/toxicity, efficacy) as well as several CMC (Chemistry, Manufacturing, and Controls) requirements mandated by current good manufacturing practices (cGMP) and the FDA. These requirements assure among other things batch to batch reproducibility and shelf-life stability based on testing a variety of properties (particle size, drug content and purity, drug release rates, targeting ligand content and activity [if applicable], stability of nanoparticles and drug under storage and in-use conditions). Through the course of pharmaceutical development, the CMC requirements become more stringent; however, it is at this early stage where the company first begins testing these critical parameters.

Innovation and financing are the remaining key elements for successful clinical translation. Not all technologies are created equal, so matching your technology to the right drug and indication and the required technical and clinical innovation to make it happen are critical. A start-up company cannot afford to get it wrong with their first product candidate, as second chances are very difficult to come by. Financing is extremely challenging, with venture capital being the most common funding mechanism for start-up companies. Economic climate has strong impact and over the last few years venture funding has been extremely competitive and sparse making it very challenging to raise the capital required to fund the significant early development costs for pre-clinical testing, GLP pharm/tox studies, process scale-up and GMP clinical drug product manufacturing. Unfortunately, government funding of start-up companies is also quite limited and extremely competitive, often with grant opportunities pitting academic research and start-up early development as competitors in what can be difficult projects to fairly assess against one another given their potentially very different scope and goals. Ironically, venture and government funding are sometimes at odds with each other. If one assumes that venture firms will often fund the most promising companies, then these companies are typically ineligible for SBIR funding, which limits the government from providing additional key funding to reach the clinic.

The two most notable nanotechnology-based drugs are DOXIL® (PEGylated-liposomal doxorubicin, approved in 1995, developed by SEQUUS) for the treatment of ovarian cancer and ABRAXANE® (albumin-bound paclitaxel, approved in 2005) for the treatment of metastatic breast cancer. DOXIL is more potent than doxorubicin and decreases cardiac-related side effects whereas ABRAXANE eliminates the use of the toxic excipient cremophor, allowing a higher dose of paclitaxel. Despite these successes, several nanotechnology start-up

companies have struggled to navigate the clinical translation of their technologies with process scalability and lack of robust analytical characterization leading to some failures, while other companies have appeared to match either the wrong drug or cancer indication with their technology resulting in disappointing clinical outcomes.

Future steps

An exciting opportunity for the future of nanomedicine is the targeting of nanoparticle drugs to specific disease cells through specific binding interactions between ligands on the nanoparticle surface and cell surface receptors present only on or at highly upregulated levels on cancer cells or tumor neovasculature. As is the case with DOXIL, this approach will also require optimization of particle characteristics to take advantage of the enhanced permeability and retention effect to allow for particle circulation in the bloodstream and extravasation through the irregular tumor neovasculature. It is the added impact of the specific nanoparticle binding as well as potential nanoparticle and drug uptake to provide intracellular delivery that offers very exciting possibilities. Early leaders in this area are Calando, which has recently reported early clinical data for their transferrin-receptor targeted siRNA demonstrating dose-dependent accumulation of drug in the melanoma cancer target tissues as well as BIND Biosciences which intends to initiate clinical studies for their prostate specific antigen-targeted docetaxel in multiple solid tumor indications in 2010.

In order to drive these promising nanomedicine technologies and others into clinical development it is essential to build start-up teams that possess the right dynamics. Having the appropriate skills is an obvious requirement, so that the team of scientists, engineers, clinicians and management are equipped to do the job. Early stage drug development presents many obstacles, so recruiting people who have experienced the challenges, failures and successes puts the company in an excellent position. From a culture perspective, individually and collectively, there must be a tremendous work ethic and enthusiasm, a willingness to put the team goals as top priority knowing that if the team wins individuals will win. There also needs to be an understanding that they are facing a marathon and not a sprint with respect to the number of achievements and time required to accomplish the ultimate goal of treating patients with cancer.

Training Programs in Cancer Nanotechnology: Preparing the Next Generation of Researchers and Clinicians

Carolyn J. Anderson

Washington University, St. Louis, MO

Introduction

An important consideration when contemplating the potential that nanotechnology holds to treat cancer and other diseases is how we can best train and educate our young people to meet the challenges of doing research, establishing start-up companies, translating knowledge to the clinic and the like. Harnessing the power of nanomedicine will require scientists and clinicians with inter- and multi-disciplinary training in key aspects of chemistry, physics, biology, medicine, computer science, engineering, and clinical sciences. Interdisciplinary science requires a departure from a parallel-processing model in which individual investigators worked alone. The best scientists in nanomedicine will not be experts in all fields of research, but they will comprehend the role each discipline plays and will competently communicate across fields to achieve better solutions. As most scientists are not trained in an interdisciplinary fashion, it is imperative to develop training programs in nanoscience that fulfill the goals of offering interdisciplinary nanoscience courses and research experiences where trainees will learn many aspects of nanoscience, with a focus on one particular area in this discipline.

The worldwide workforce necessary to support the field of nanotechnology is estimated at two million by 2015 (http://www.nano.gov/html/edu/home_edu.html). Questions arise as to how the U.S. educational system can train technicians, scientists, and clinicians, and how to assure that the students choose the appropriate educational path. Raising awareness and educating K-12 school children hopefully prompts students to study nanoscience at the undergraduate and graduate levels. Formal, didactic degree programs for undergraduate students, as well as strong graduate education and research in nanomedicine are also essential. There are currently many educational programs in nanotechnology at all levels of training, from K-12 to postgraduate experiences. However, the vast majority of the educational programs in place focus on the materials science and engineering aspects of the field. We should encourage programs that combine the physical sciences/engineering aspects with biology and/or medicine to foster the groundbreaking discoveries in the chemistry and materials fields that can be applied towards life-saving cancer treatments.

Current status

The field of nanotechnology has grown exponentially over the past 10 years, in part through government initiatives. The National Nanotechnology Initiative (NNI) was established in 2001 to coordinate Federal nanotechnology research and development. Today the NNI consists of the individual and cooperative nanotechnology-related activities of 25 Federal agencies with a range of research, regulatory roles, and responsibilities (http://www.nano.gov/html/about/home_about.html). The NNI does not fund research; however, it informs and influences the Federal budget and planning processes. One of the key goals of the NNI is to “Develop and sustain educational resources, a skilled workforce, and the supporting infrastructure and tools to advance nanotechnology.” The Education Center on the NNI website provides information on K-12 activities as well as listings of undergraduate and graduate programs in nanotechnology.

Resources for teaching nanotechnology to K-12 children

Several websites have nanoscience resources for classroom teachers and students’ families including the The

National Science Foundation (NSF) (http://www.nsf.gov/news/classroom/nano.jsp), The Nanobiotechnology Center (http://www.nbtc.cornell.edu/), Rice University (http://nanokids.rice.edu/), and the University of Albany (SUNY) College of Nanoscale Science and Engineering (http://cnse.albany.edu/Nano_for_Kids/K_12_links.html).

In addition to web-based resources several other resources for hands-on experience for youth are also available. The Nanobiotechnology Center sponsors such things as field trips for middle school children to learn about scanning electron microscopy and visits to the Strong Museum in Rochester, NY. Likewise the Nanoscale Informal Science Education (NISE) Network has sponsored NanoDays since 2008. NanoDays combine simple hands-on activities for young people with exploration of current research for adults at over 200 science museums, research centers and universities across the country. Through the Program of Excellence in Nanotechnology (PEN) and the Siteman Center of Cancer Nanotechnology Excellence (CCNE) at Washington University, researchers are participating at NanoDays at the St. Louis Science Center by hosting two booths with hands-on activities.

Undergraduate training

Currently, several community colleges working with larger universities offers Associate degrees in Nanotechnology. For instance, the University of Pennsylvania collaborates with Pennsylvania community colleges to offer an Associate degree in Nanobiotechnology. Dakota County Technical College (Rosemount, MN) in conjunction with the University of Minnesota offers an Associate degree in Applied Science in Nanoscience Technology. The North Seattle Community College offers an Associate of Applied Science-T degree in nanotechnology.

At this time, there are no advertised bachelor's degree programs in nanoscience. However, there are several institutions that offer either a minor or a concentration in nanoscience or related discipline. At the University of Texas at Dallas, undergraduates can minor in nanoscience by taking three core NANO-designated courses, the content of which is exclusively related to nanoscience and nanotechnology. Yale University has an undergraduate minor in nanotechnology, where students are required to take an Introduction to Nanotechnology course and five other courses from a selection of engineering and biotechnology electives. Neither of these undergraduate minors require courses related to biology or medicine.

At the University of Wisconsin-Stout, students can obtain a B.S. in Applied Science with a Nanoscience concentration, and a B.S. in Engineering Technology with a concentration in Nanotechnology. Michigan Technological University offers an interdisciplinary minor in Nanotechnology. Several institutions have courses on nanotechnology, targeted towards either undergraduates or graduate students, including Cornell, Florida Institute of Technology, George Mason University, Rice University, University of Central Florida, University of Maryland, University of Texas at Austin, University of Washington, Washington University, and University of Wisconsin.

The majority of these programs emphasize the area of the physical sciences and engineering. There is definitely a need to see more education in nanoscience that incorporates biology and medicine, which will provide a larger pool of trainees for graduate programs, as well as provide a background for students studying medicine to have knowledge of how nanotechnology can be used to treat diseases such as cancer.

Graduate training

There are numerous institutions in the U.S. that train graduate students to do research in the area of nanotechnology, nanoscience, or nanomedicine. There are fewer universities that have formal programs that offer coursework and either a degree, certificate, or specialization. The majority of these programs are focused in the physical sciences and engineering, and there are few that combine the physical sciences and engineering with biology and medicine. One of the more innovative and interdisciplinary programs is at Northeastern University, where they have a Nanomedicine program funded by the NSF IGERT (Integrative Graduate Education and Research Traineeship) initiative and the NCI. There are over 20 faculty involved from Northeastern University, with collaborations with other Boston-area researchers and scientists from neighboring hospitals and industries. Students are enrolled in a Ph.D. program in Biology, Chemistry, Physics, or one of their Engineering programs, and then graduate with a specialization in Nanomedicine Science and Technology. This is one of the best examples of a graduate program that allows students to obtain an interdisciplinary education, learning the science and/or engineering, as well as the biomedical applications.

The University of Michigan has the Michigan Nanotechnology Institute for Medicine and Biological Sciences (http://nano.med.umich.edu/). This program has several talented scientists with expertise in fields ranging from chemistry, biology, medicine, and engineering. Students can earn a Ph.D. in a typical field of study and obtain a certificate in NanoBiology. Coursework is selected from biology, physical sciences, and engineering. The nanoscience courses appear to be explicitly in the areas of the physical sciences and engineering rather than incorporating biology and/or medicine. The University of Texas Health Science Center at Houston opened a Department of NanoMedicine and Biomedical Engineering in 2009 whose mission is "to introduce students to the field of Nanomedicine and the vast opportunities it provides for enhanced therapeutics, personalized medicine, medical diagnostics, imaging, screening, prevention, and regenerative medicine." This program is unique in that it is probably the only one that educates and prepares *medical* students to learn emerging new technologies in biomedical nanotechnology and engineering. Students are required to complete a scholarly research project and present the data at a scientific meeting, as well as prepare a manuscript to

obtain the certificate of completion. There are also journal clubs and other meetings, but at the time of this writing, there were no formal courses described on the website.

Clinical potential

For nanomedicine to reach its full potential, there needs to be more training centers like the ones at Northeastern, University of Michigan, and University of Texas Health Science Center at Houston. Having top-notch researchers in nanomedicine at institutions is obviously important for training the future scientists in the field. However, combining the research with didactic training will provide another level of skill for these future scientists and clinicians. Incorporating nanomedicine into medical student training will also ensure that these students understand how nanomaterials and nanodevices can be applied in medicine, particularly cancer treatments and diagnosis. Additionally, post-graduate training of research residents would also fulfill this role.

Obtaining the support of the NCI cancer centers in promoting nanotechnology education will also be key for future success. Of the Centers for Cancer Nanotechnology Excellence (CCNE) that were funded in 2005, the Siteman at Washington University had outreach and education cores that promoted education to medical specialists, the general public, as well as students at the K-12 through graduate levels. A course in Nanomedicine was offered yearly to graduate and undergraduate students. Outreach events to promote nanomedicine to the public at the St. Louis Science Center were also sponsored by the CCNE.

Future challenges

Federal grants have provided resources for the infrastructure of several educational programs in nanotechnology and have sustained them for the past decade or more. One of the challenges will be to maintain these programs when the funding expires, in particular the K-12 outreach programs. Many of these initiatives are for a limited time, are not renewable, and it is apparent that many programs have ceased over the past few years. Novel ways to maintain K-12 education in nanoscience, possibly through school teachers themselves, as well as alternative funding sources, such as private donors or foundations should be investigated. Encouraging universities and institutions that have strong nanotechnology research and education programs to engage in outreach activities to K-12 school children and the general public would be an inexpensive way to expand the awareness of nanotechnology and nanomedicine and increase the pool of future trainees.

One of the major concerns in undergraduate and graduate education in nanomedicine is that aside from the few programs described above, the vast majority of existing programs offering minors, certificates and/or specializations in nanotechnology are highly focused in the areas of materials science and engineering, with little or no emphasis on combining this with biology and/or medicine. Some of the programs that are focused in the physical sciences and/or engineering are affiliated with strong medical schools and/or cancer centers, and these institutions should be encouraged to collaborate with the cancer biologists and oncologists in educating nano-scientists regarding these medical applications.

As the NNI funding initiatives phase out, funding of research in nanomedicine will likely continue and hopefully expand as the nano grants are submitted to NIH through the traditional mechanisms (e.g. R01, P01, etc.). Unfortunately, requesting funds for educational initiatives through these mechanisms is not allowed. Finding the resources to develop new educational programs in nanomedicine, or even maintenance of existing programs will be a significant challenge. For example, currently only the University of Texas Health Science Center at Houston has a program to train medical students in nanomedicine. Mechanisms for funding the development of similar programs at other institutions should be investigated.

Milestones

3-year:

- Encourage more universities with strong nanotechnology/nanomedicine programs to reach out to the general public and/or K-12 school children and/or their teachers.
- Sponsor a workshop on nanomedicine education, with sessions and panel discussions on education at all levels (general public, K-12 school children, school teachers, undergraduates, graduate students, medical students, and post-graduate education).

5-year:

- Three to five of the existing undergraduate minors/specialities in nanoscience will incorporate biology and medicine into their curriculum.
- An additional two to four graduate programs in nanoscience will add a focus on nanobiology and/or nanomedicine.
- Using the University of Texas Health Science Center at Houston's program for training medical students as a model, there will be one to two more of these programs offered at major universities.

10-year:

- There will be more medical students and graduate students graduating from existing and recently developed programs in nanomedicine, thus increasing the number of qualified scientists working in academia, industry and possibly even private medical practices.
- Due to advances in research and education in nanomedicine, there will be more nano-based agents approved for the diagnosis and/or treatment of cancer as well as other diseases.

Maximizing Research and Technology Development Effectiveness Through a Team Approach

Dorothy Farrell, George Hinkal, Sara S. Hook, Nicholas Panaro, and Krzysztof Ptak, and Piotr Grodzinski

Office of Cancer Nanotechnology Research, CSSI, National Cancer Institute, NIH, Bethesda, MD

In order to develop effective devices and treatments for cancer using nanotechnology, the NCI recognizes that it is imperative for diverse professionals to unite toward this common goal. “Team science is about developing new ideas, forging new partnerships, and collaboratively using new tools to understand cancer as a disease process and a highly complex system," explained Dr. Anna D. Barker, the former deputy director of NCI. "The model includes teams of experts who can not only view the many elements of the cancer process, but can integrate that knowledge and design an innovative and targeted strategy of drugs, biologics, and even devices that can be used at all phases of the cancer process in an integrated fashion. Although the individual investigator will continue to drive innovation, the old model of cancer research taking place in isolated silos is fading away." As an illustration, chemists and engineers have the expertise to design and synthesize the best types of nanoparticles with physical properties that will solubilize drugs, RNAs, and proteins and ensure transport across the blood/brain barrier if needed. Meanwhile, the expertise of biologists and clinicians is imperative to know what tumor type to target, through which molecular mechanism, and which biological read-out to use to monitor the effectiveness of treatment. In 2004 the NCI established the Alliance for Nanotechnology in Cancer to foster this type of interdisciplinary collaboration. One of the avenues they used was to establish CCNEs through an open competition. These centers were lead by multiple program directors (PD) and primary investigators (PI) coming from the areas of medicine, biology, chemistry, physics, and engineering. The power of team science can best be illustrated by stories of the program participants themselves.

Dr. Dennis Carson, the director of the University of California, San Diego’s (UCSD) Moores Cancer Center, had a vision for incorporating aspects of engineering into cancer research, and he knew there was significant talent and interested faculty at UCSD to carry out the large scale multi-disciplinary effort necessary to establish a CCNE. He needed to identify a director at UCSD, however, who could lead this diverse talent to success. In consultation with Dr. Roger Tsien, UCSD’s leading biochemist in the field of nanotechnology, and Dr. Andrew Kummel, a chemist and materials scientist very familiar with the engineering faculty, they quickly reached a bold and unusual decision. Their choice to lead the effort was Dr. Sadik Esener, a professor of Electrical and Computer Engineering and of Materials Sciences at the Jacobs School of Engineering with a strong expertise in electronics and photonics but surprisingly little involvement with cancer or nanoparticle research at UCSD. However, Dr. Esener had the key attributes required for successful leadership of the new center: (1) respect of his colleagues and proven success in running large scientific projects, (2) multiple successes in commercializing medically related chip-based technologies, (3) the ability to work with scientists of different backgrounds and personalities on their ideas, and (4) speed in learning new fields of science.

When Dr. Carson contacted Dr. Esener to ask him if he would agree to serve as the PI, Dr. Esener’s first reaction was there must be a mistake. Dr. Esener was eventually won over and concludes, “Nothing comes close to the fulfillment one feels as a researcher to know that you are wrestling with a problem that if resolved would eliminate so much pain and suffering in the world. Although, I had some doubts before I accepted this position that entails tremendous responsibility, I am now so grateful to have been given this remarkable opportunity to bring a new perspective to this disease as a result of NCI’s bold undertaking and Dennis’ courageous decision. I cannot imagine how I could have been involved with leading edge cancer research without this center and the team science approach.”

Since its inception, the Alliance program has demonstrated that multi-disciplinary teams can synergize to develop clinically translatable technologies and therapies for cancer. The research groups involved in the Alliance have published over 1000 research articles, generated 250

patent applications and disclosures, and started more than 30 companies by which the technologies will be developed and marketed. Currently, 10 clinical trials are ongoing using therapies that have been developed using funds from the program. These innovative technologies and therapies would not have been possible had it not been for the willingness of scientists from divergent fields coming together to lend their expertise, ideas, vision, and passion. With the recent renewal of the Alliance for Nanotechnology in Cancer program, there should be even more outstanding contributions to cancer diagnosis, imaging, treatment, and management in the years to come.

Interdisciplinary collaboration is critical to effectively train young scientists in the area of nanotechnology (as discussed in the previous section). Program efforts to foster a collaborative spirit in the first phase of the Alliance resulted not only in research projects and publications, but in exchanges of personnel and materials. This personnel exchange was particularly important for the program's training components, as numerous graduate students and postdoctoral researchers were able to use network connections formed at investigator meetings to establish their next positions. The next five years of the program, Phase II, has increased research training funding to include Cancer Nanotechnology Training Centers (CNTCs) and Pathway to Independence Awards in Cancer Nanotechnology Research (K99/R00). The funded CNTCs will target graduate student and post-doctoral researchers of broad background (in medicine, biology, and other health sciences as well as in the physical sciences, chemistry, and engineering). The program of multi-disciplinary research education in cancer nanotechnology will primarily focus on mentored training, usually from multiple investigators in different disciplines, through laboratory-based research projects. In addition, centers will offer both short courses and workshops as wells as outreach experiences. Given the challenges more senior post-doctoral fellows face in finishing projects and establishing themselves as independent investigators, the program has invested in funding several Pathway to Independence Awardees. These trainees will benefit not only from their direct mentors but from the more informal mentoring and interaction at PI meetings across the Alliance.

The bread and butter of the program remain the CCNEs and CNPPs. The CCNEs of this new program edition will have a greater focus on clinically-worthy technologies as compared to Phase I. The new program will emphasize more heavily cancers having particularly poor outcomes, including brain, lung, pancreatic, and ovarian cancers. The science will continue to pursue basic discovery and innovation, but will also explore the clinical utility and translation development of the technologies. The collaborative effort then between the physical and basic scientists will be driven by those pressing questions facing clinicians. Collaborations benefit from complimentary skills, experience, perspective, and the use of diverse methodologies, as such the right mix of expertise is crucial for a highly effective interdisciplinary research team. When basic and physical scientists realize, for instance, that one of the important aspects of pancreatic tumorigenesis is the microenvironment, they can begin to address how to develop interventions and therapies to intercede in relevant pathways. The "begin with the end in mind" approach can save valuable time and resources by honing in on the most profitable research direction, foreseeing possible roadblocks, and planning for alternate avenues. Likewise, it is important to consider what data is needed for pre-clinical testing and characterization of various nanoparticles and devices so that the proper experiments can be done early and the process of clearing institutional, legal, and regulatory hurdles may be initiated. It may be wise to seek the advice and guidance of institutional and federal regulatory bodies such as the FDA so that applications for INDs, IDEs, and patents will progress unhampered.

As part of NCI's commitment to clinical translation the NCL will continue to work with investigators as a hub for the pre-clinical characterization of nanomaterials and to assist in the process of bringing nanotechnologies to the stage of IND or IDE submission. The NCL has established protocols for bio-nanoparticle characterization and is currently expanding these protocols as well as working on others pertaining to GMPs such as scale-up process, purity, and batch-to-batch consistency. The lab will continue basic discovery and innovation, but it will also take great care in the evaluation of clinical utility of the technology and put strong emphasis on the translation.

The cross Alliance activity of the investigators can be enhanced by using the Alliance's Cancer Nanotechnology Laboratory (caNanoLab) where researchers and NCL are able to deposit, store, and retrieve nanoparticle characterization data. To date it has primarily been used to house *in vitro* data (physico-chemical properties and biological assays) and protocols but it is expanding to include *in vivo* characterizations of nanoparticles and their functional components. Data relating to the toxicity, pharmacokinetics, and ADME (absoption, distribution, metabolism, and excretion) in vertebrate animals will be collected. Another important aspect of caNanoLab is its contribution to nanotechnology ontology through standardizing vocabulary terms relating to the physical, chemical, and functional characteristics of nanotechnology.

The idea of data sharing usually makes scientific researchers uneasy. After all they have invested huge amounts of time and resources to generating this data. In addition, graduate students and post-doctoral fellows realize the importance to their graduate committees and careers of making an intellectual contribution to a project that results in several high quality, first author publications. However, it is important for trainees to recognize that they can obtain a significant benefit from working with a group of individuals to produce co-authored publications, promote idea exchange, and develop a network of colleagues within their field.

In order for effective data sharing to become a reality, there needs to be trust between all parties involved. First of all, there needs to be trust within each CCNE. Strong committed leadership breeds trust as well as motivation. "Within our own consortium, trusting relationships between people have already been established," noted Dr. Sanjiv Sam Gambhir of the Stanford CCNE. "Indeed, the whole process of building

and applying for the CCNE grant built a great deal of trust between members, and between the university and companies involved." As the leadership development website, http://www.thelearningcenter.net/, states "There are two parts to trust: a feeling part that indicates trust and a performance track record that confirms trust." Many of the investigators within established CCNEs have collaborated and published together thus "confirming" their trust with a previous track record. Trust within new CCNEs and across the Alliance program could be more difficult to establish. Through various programmatic mechanisms, not the least of which is the annual PI meeting, a large number of cross Alliance collaborations have been built. A key to building trust is effective communication. Physical scientists, for instance, know the language and acronyms of their field. Oncologists, however, do not know that specialized language. As Phase II of the Alliance takes shape, sensitivity to communication style, scientific "language," and effective listening strategies becomes crucial for building productive teams and collaborative efforts.

The Alliance has demonstrated that a multi-disciplinary approach to research can catalyze scientific developments and enable clinical translation. Alliance investigators have advanced diagnostic technology, using both *in vitro* assays and novel imaging methods, and offered improved therapies and therapeutic efficacy measures. Many of the technologies developed and clinically translated have applied novel engineering to existing cancer biology strategies. The next stage of cancer nanotechnology research should enable new avenues of cancer care through revolutionary diagnostic tools, imaging techniques, treatment options, and *in situ* tumor characterization.

The scientific strategy for the 2010-2015 segment of the program was formulated based on the lessons learned from Phase I, the evolving strategy of the NNI, and, most importantly, the input of the extramural community. Phase II of the program will promote early diagnosis and better monitoring of therapeutic efficacy using emerging *in vitro* diagnostic techniques and novel imaging technologies such as multiplexed, multi-modal molecular contrast agents. It will be important to correlate outcomes from both approaches. On the therapeutic front, an increasing number of treatments will exploit tumor targeting via cell surface ligands and enhanced formulations for chemotherapeutics that reduce systemic toxicity and improve therapeutic index. Cooperative treatment regimes in which drug delivery is combined with tumor microenviroment engineering to improve treatment response will emerge. In addition, despite early hopes that gene therapy approaches would change the face of medicine, virtually no success has been garnered to date. There are glimpses that silencing genes and hopefully also replacing mutated genes will become routine modalities of treatment due to nanoparticle delivery options. In conclusion, while we do not want to over speculate or promise what we cannot achieve, we feel confident that patients facing this disease will have many more options in their arsenal due to the concerted effort, commitment, dedication, and ingenuity of those in the cancer nanotechnology research field.

References

Agnew, H. D., Rohde, R. D., Millward, S. W., Nag, A., Yeo, W. S., Hein, J. E., Pitram, S. M., Tariq, A. A., Burns, V. M., Krom, R. J., *et al.* (2009). Iterative *in situ* click chemistry creates antibody-like protein-capture agents. Angew. Chem. Int. Ed. *48*, 4944-4948.

Agrawal, A., Min, D. H., Singh, N., Zhu, H., Birjiniuk, A., von Maltzahn, G., Harris, T. J., Xing, D., Woolfenden, S. D., Sharp, P. A., *et al.* (2009). Functional delivery of siRNA in mice using dendriworms. ACS Nano *3*, 2495-2504.

Aleku, M., Schulz, P., Keil, O., Santel, A., Schaeper, U., Dieckhoff, B., Janke, O., Endruschat, J., Durieux, B., Roder, N., *et al.* (2008). Atu027, a liposomal small interfering RNA formulation targeting protein kinase N3, inhibits cancer progression. Cancer Res. *68*, 9788-9798.

Alexis, F., Basto, P., Levy-Nissenbaum, E., Radovic-Moreno, A. F., Zhang, L., Pridgen, E., Wang, A. Z., Marein, S. L., Westerhof, K., Molnar, L. K., and Farokhzad, O. C. (2008). HER-2-targeted nanoparticle-affibody bioconjugates for cancer therapy. Chem. Med. Chem. *3*, 1839-1843.

Alexis, F., Pridgen, E. M., Langer, R., and Farokhzad, O. C. (2010). Nanoparticle technologies for cancer therapy. Handbook Exp. Pharmacol. *197*, 55-86.

Alkan-Onyuksel, H., Demos, S. M., Lanza, G. M., Vonesh, M. J., Klegerman, M. E., Kane, B. J., Kuszak, J., and McPherson, D. D. (1996). Development of inherently echogenic liposomes as an ultrasonic contrast agent. J. Pharm. Sci. *85*, 486-490.

Ansieau, S., Morel, A. P., Hinkal, G., Bastid, J., and Puisieux, A. (2010). TWISTing an embryonic transcription factor into an oncoprotein. Oncogene *29*, 3173-3184.

Armani, A. M., Kulkarni, R. P., Fraser, S. E., Flagan, R. C., and Vahala, K. J. (2007). Label-free, single-molecule detection with optical microcavities. Science *317*, 783-787.

Arvizo, R., Bhattacharya, R., and Mukherjee, P. (2010). Gold nanoparticles: opportunities and challenges in nanomedicine. Expert Opin. Drug Deliv. *7*, 753-763.

Bailey, R. C., Kwong, G. A., Radu, C. G., Witte, O. N., and Heath, J. R. (2007). DNA-encoded antibody libraries: a unified platform for multiplexed cell sorting and detection of genes and proteins. J. Am. Chem. Soc. *129*, 1959-1967.

Barnhart, B. C., and Simon, M. C. (2007). Metastasis and stem cell pathways. Cancer Metastasis Rev. *26*, 261-271.

Beer, A. J., Grosu, A. L., Carlsen, J., Kolk, A., Sarbia, M., Stangier, I., Watzlowik, P., Wester, H. J., Haubner, R., and Schwaiger, M. (2007). [18F]galacto-RGD positron emission tomography for imaging of α5β3 expression on the neovasculature in patients with squamous cell carcinoma of the head and neck. Clin. Cancer Res. *13*, 6610-6616.

Besteman, K., Lee, J. O., Wiertz, F. G. M., Heering, H. A., and Dekker, C. (2003). Enzyme-coated carbon nanotubes as single-molecule biosensors. Nano Letters *3*, 727-730.

Bettuzzi, S., Brausi, M., Rizzi, F., Castagnetti, G., Peracchia, G., and Corti, A. (2006). Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. Cancer Res. *66*, 1234-1240.

Borm, P. J. A., and Berube, D. A. (2008). Tale of Opportunities, Uncertainties, and Risks. Nano Today *3*, 56-59.

Brahimi-Horn, M. C., Chiche, J., and Pouyssegur, J. (2007). Hypoxia and cancer. J. Mol. Med. *85*, 1301-1307.

Cao, G., Calderon-Colon, X., Burk, L., Lee, Y. Z., Sultana, S., Lu, J., and Zhou, O. (2010). Desktop micro-CT with a nanotube field emission x-ray source for high-resolution cardiac imaging. Proc. of SPIE *7622*, 76222B.

Cao, G., Lee, Y. Z., Peng, R., Liu, Z., Rajaram, R., Calderon-Colon, X., An, L., Wang, P., Phan, T., Sultana, S., *et al.* (2009). A dynamic micro-CT scanner based on a carbon nanotube field emission x-ray source. Phys. Med. Biol. *54*, 2323-2340.

Caruthers, S. D., Wickline, S. A., and Lanza, G. M. (2007). Nanotechnological applications in medicine. Curr. Opin. Biotechnol. *18*, 26-30.

Champion, J. A., and Mitragotri, S. (2006). Role of target geometry in phagocytosis. Proc. Natl. Acad. Sci. USA. *103*, 4930-4934.

Chen, M. C., Wong, H. S., Lin, K. J., Chen, H. L., Wey, S. P., Sonaje, K., Lin, Y. H., Chu, C. Y., and Sung, H. W. (2009). The characteristics, biodistribution and bioavailability of a chitosan-based nanoparticulate system

for the oral delivery of heparin. Biomaterials *30*, 6629-6637.

Chen, R. J., Zhang, Y., Wang, D., and Dai, H. (2001). Noncovalent sidewall functionalization of single-walled carbon nanotubes for protein immobilization. J. Am. Chem. Soc. *123*, 3838-3839.

Chen, W., Yan, W., and Huang, L. (2008). A simple but effective cancer vaccine consisting of an antigen and a cationic lipid. Cancer Immunol. Immunother. *57*, 517-530.

Cho, K., Wang, X., Nie, S., Chen, Z. G., and Shin, D. M. (2008). Therapeutic nanoparticles for drug delivery in cancer. Clin. Cancer Res. *14*, 1310-1316.

Cobley, C. M., Au, L., Chen, J., and Xia, Y. (2010). Targeting gold nanocages to cancer cells for photothermal destruction and drug delivery. Expert Opin. Drug Deliv. *7*, 577-587.

Cooper, S. J., and Bowden, G. T. (2007). Ultraviolet B regulation of transcription factor families: Roles-of nuclear factor-kappa B (NF-kappa B) and activator protein-1 (AP-1) in UVB-induced skin carcinogenesis. Curr. Cancer Drug Tar. *7*, 325-334.

Cosse, J. P., and Michiels, C. (2008). Tumour hypoxia affects the responsiveness of cancer cells to chemotherapy and promotes cancer progression. Anticancer Agents Med. Chem. *8*, 790-797.

D'Andrilli, G., Bovicelli, A., and Giordano, A. (2010). HPV vaccines: state of the art. J. Cell Physiol. *224*, 601-604.

Davis, M. E., Chen, Z. G., and Shin, D. M. (2008). Nanoparticle therapeutics: an emerging treatment modality for cancer. Nat. Rev. Drug Discov. *7*, 771-782.

Davis, M. E., Zuckerman, J. E., Choi, C. H., Seligson, D., Tolcher, A., Alabi, C. A., Yen, Y., Heidel, J. D., and Ribas, A. (2010). Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nature *464*, 1067-1070.

De Grand, A. M., and Frangioni, J. V. (2003). An operational near-infrared fluorescence imaging system prototype for large animal surgery. Technol. Cancer Res. Treat *2*, 553-562.

Decuzzi, P., Godin, B., Tanaka, T., Lee, S. Y., Chiappini, C., Liu, X., and Ferrari, M. (2010). Size and shape effects in the biodistribution of intravascularly injected particles. J. Control. Release *141*, 320-327.

Denko, N. C. (2008). Hypoxia, HIF1 and glucose metabolism in the solid tumour. Nat. Rev. Cancer *8*, 705-713.

Depping, R., Steinhoff, A., Schindler, S. G., Friedrich, B., Fagerlund, R., Metzen, E., Hartmann, E., and Kohler, M. (2008). Nuclear translocation of hypoxia-inducible factors (HIFs): involvement of the classical importin alpha/beta pathway. Biochim. Biophys. Acta *1783*, 394-404.

Dobrovolskaia, M. A., and McNeil, S. E. (2007). Immunological properties of engineered nanomaterials. Nat. Nanotechnol. *2*, 469-478.

Dong, X., and Mumper, R. J. (2010). Nanomedicinal strategies to treat multidrug-resistant tumors: current progress. Nanomedicine (Lond.) *5*, 597-615.

Duncan, R., Vicent, M. J., Greco, F., and Nicholson, R. I. (2005). Polymer-drug conjugates: towards a novel approach for the treatment of endrocine-related cancer. Endocr. Relat. Cancer *12 Suppl 1*, S189-199.

Elazar, V., Adwan, H., Bauerle, T., Rohekar, K., Golomb, G., and Berger, M. R. (2010). Sustained delivery and efficacy of polymeric nanoparticles containing osteopontin and bone sialoprotein antisenses in rats with breast cancer bone metastasis. Int. J. Cancer *126*, 1749-1760.

Elmets, C. A. (2009). The cyclooxygenase-2 inhibitor celecoxib effectively suppresses the development of nonmelanoma skin cancers: Results of a phase II/III clinical trial. SID *Abstract 325*.

Estella-Hermoso de Mendoza, A., Campanero, M. A., Mollinedo, F., and Blanco-Prieto, M. J. (2009). Lipid nanomedicines for anticancer drug therapy. J. Biomed. Nanotechnol. *5*, 323-343.

Fan, R., Vermesh, O., Srivastava, A., Yen, B. K. H., Qin, L. D., Ahmad, H., Kwong, G. A., Liu, C. C., Gould, J., Hood, L., and Heath, J. R. (2008). Integrated barcode chips for rapid, multiplexed analysis of proteins in microliter quantities of blood. Nat. Biotechnol. *26*, 1373-1378.

Fan, Y., Chen, X., Trigg, A. D., Tung, C. H., Kong, J., and Gao, Z. (2007). Detection of microRNAs using target-guided formation of conducting polymer nanowires in nanogaps. J. Am. Chem. Soc. *129*, 5437-5443.

Fang, S., Lee, H. J., Wark, A. W., and Corn, R. M. (2006). Attomole microarray detection of microRNAs by nanoparticle-amplified SPR imaging measurements of surface polyadenylation reactions. J. Am. Chem. Soc. *128*, 14044-14046.

Farnebo, M., Bykov, V. J., and Wiman, K. G. (2010). The p53 tumor suppressor: a master regulator of diverse cellular processes and therapeutic target in cancer. Biochem. Biophys. Res. Commun. *396*, 85-89.

Farokhzad, O. C., and Langer, R. (2009). Impact of nanotechnology on drug delivery. ACS Nano *3*, 16-20.

Fu, Q., Sun, J., Zhang, W., Sui, X., Yan, Z., and He, Z. (2009). Nanoparticle albumin-bound (NAB) technology is a promising method for anti-cancer drug delivery. Recent Pat. Anticancer Drug Discov. *4*, 262-272.

Gao, W., Xiao, Z., Radovic-Moreno, A., Shi, J., Langer, R., and Farokhzad, O. C. (2010). Progress in siRNA delivery using multifunctional nanoparticles. Methods Mol. Biol. *629*, 53-67.

Gao, Z., and Yang, Z. (2006). Detection of microRNAs using electrocatalytic nanoparticle tags. Anal. Chem. *78*, 1470-1477.

Garg, A., Tisdale, A. W., Haidari, E., and Kokkoli, E. (2009). Targeting colon cancer cells using PEGylated liposomes modified with a fibronectin-mimetic peptide. Int. J. Pharm. *366*, 201-210.

Gaster, R. S., Hall, D. A., Nielsen, C. H., Osterfeld, S. J., Yu, H., Mach, K. E., Wilson, R. J., Murmann, B., Liao, J. C., Gambhir, S. S., and Wang, S. X. (2009). Matrix-insensitive protein assays push the limits of biosensors in medicine. Nat. Med. *15*, 1327-1332.

Gaur, U., Sahoo, S. K., De, T. K., Ghosh, P. C., Maitra, A., and Ghosh, P. K. (2000). Biodistribution of fluoresceinated dextran using novel nanoparticles evading reticuloendothelial system. Int. J. Pharm. *202*, 1-10.

Geng, Y., Dalhaimer, P., Cai, S., Tsai, R., Tewari, M., Minko, T., and Discher, D. E. (2007). Shape effects of filaments versus spherical particles in flow and drug delivery. Nat. Nanotechnol. *2*, 249-255.

Giljohann, D. A., and Mirkin, C. A. (2009). Drivers of biodiagnostic development. Nature *462*, 461-464.

Gindy, M. E., and Prud'homme, R. K. (2009). Multifunctional nanoparticles for imaging, delivery and targeting in cancer therapy. Expert Opin. Drug Deliv. *6*, 865-878.

Goldstein, D., Tan, B. S., Rossleigh, M., Haindl, W., Walker, B., and Dixon, J. (2005). Gastrointestinal stromal tumours: correlation of F-FDG gamma camera-based coincidence positron emission tomography with CT for the assessment of treatment response--an AGITG study. Oncology *69*, 326-332.

Gottesman, M. M., Fojo, T., and Bates, S. E. (2002). Multidrug resistance in cancer: role of ATP-dependent transporters. Nat. Rev. Cancer *2*, 48-58.

Gratton, S. E. A., Ropp, P. A., Pohlhaus, P. D., Luft, J. C., Madden, V. J., Napier, M. E., and DeSimone, J. M. (2008). The effect of particle design on cellular internalization pathways. Proc. Natl. Acad. Sci. USA. *105*, 11613-11618.

Grivennikov, S. I., and Karin, M. (2010). Inflammation and oncogenesis: a vicious connection. Curr. Opin. Genet. Dev. *20*, 65-71.

Guppy, M. (2002). The hypoxic core: a possible answer to the cancer paradox. Biochem. Biophys. Res. Commun. *299*, 676-680.

Half, E., and Arber, N. (2009). Colon cancer: preventive agents and the present status of chemoprevention. Expert Opin. Pharmacother. *10*, 211-219.

Han, Z. B., Ren, H., Zhao, H., Chi, Y., Chen, K., Zhou, B., Liu, Y. J., Zhang, L., Xu, B., Liu, B., *et al.* (2008). Hypoxia-inducible factor (HIF)-1 alpha directly enhances the transcriptional activity of stem cell factor (SCF) in response to hypoxia and epidermal growth factor (EGF). Carcinogenesis *29*, 1853-1861.

Harris, A. L. (2002). Hypoxia--a key regulatory factor in tumour growth. Nat. Rev. Cancer *2*, 38-47.

Harris, R. E. (2009). Cyclooxygenase-2 (cox-2) blockade in the chemoprevention of cancers of the colon, breast, prostate, and lung. Inflammopharmacology *17*, 55-67.

Haubner, R., Wester, H. J., Reuning, U., Senekowitsch-Schmidtke, R., Diefenbach, B., Kessler, H., Stocklin, G., and Schwaiger, M. (1999). Radiolabeled alpha(v)beta3 integrin antagonists: a new class of tracers for tumor targeting. J. Nucl. Med. *40*, 1061-1071.

Heath, J. R., and Davis, M. E. (2008). Nanotechnology and Cancer. Annu. Rev. Med. *59*, 251-265.

Ho, Y. P., and Leong, K. W. (2010). Quantum dot-based theranostics. Nanoscale *2*, 60-68.

Howard, M. D., Jay, M., Dziublal, T. D., and Lu, X. L. (2008). PEGylation of nanocarrier drug delivery systems: State of the art. J. Biomed. Nanotechnol. *4*, 133-148.

Iorio, M. V., Ferracin, M., Liu, C. G., Veronese, A., Spizzo, R., Sabbioni, S., Magri, E., Pedriali, M., Fabbri, M., Campiglio, M., *et al.* (2005). MicroRNA gene expression deregulation in human breast cancer. Cancer Res. *65*, 7065-7070.

Jayson, G. C., Zweit, J., Jackson, A., Mulatero, C., Julyan, P., Ranson, M., Broughton, L., Wagstaff, J., Hakannson, L., Groenewegen, G., *et al.* (2002). Molecular imaging and biological evaluation of HuMV833 anti-VEGF antibody: implications for trial design of antiangiogenic antibodies. J. Natl. Cancer Inst. *94*, 1484-1493.

Jewell, U. R., Kvietikova, I., Scheid, A., Bauer, C., Wenger, R. H., and Gassmann, M. (2001). Induction of HIF-1alpha in response to hypoxia is instantaneous. FASEB J. *15*, 1312-1314.

Johnson, J. J., Bailey, H. H., and Mukhtar, H. (2010). Green tea polyphenols for prostate cancer chemoprevention: a translational perspective. Phytomedicine *17*, 3-13.

Karmali, P. P., Kotamraju, V. R., Kastantin, M., Black, M., Missirlis, D., Tirrell, M., and Ruoslahti, E. (2009). Targeting of albumin-embedded paclitaxel nanoparticles to tumors. Nanomedicine *5*, 73-82.

Keren, S., Zavaleta, C., Cheng, Z., de la Zerda, A., Gheysens, O., and Gambhir, S. S. (2008). Noninvasive molecular imaging of small living subjects using Raman spectroscopy. Proc. Natl. Acad. Sci. USA. *105*, 5844-5849.

Khan, N., Afaq, F., and Mukhtar, H. (2010). Lifestyle as risk factor for cancer: Evidence from human studies. Cancer Lett. *293*, 133-143.

Kizaka-Kondoh, S., Inoue, M., Harada, H., and Hiraoka, M. (2003). Tumor hypoxia: a target for selective cancer therapy. Cancer Sci. *94*, 1021-1028.

Kleinman, M. E., Yamada, K., Takeda, A., Chandrasekaran, V., Nozaki, M., Baffi, J. Z., Albuquerque, R. J., Yamasaki, S., Itaya, M., Pan, Y., *et al.* (2008). Sequence- and target-independent angiogenesis suppression by siRNA via TLR3. Nature *452*, 591-597.

Klibanov, A. L., Hughes, M. S., Villanueva, F. S., Jankowski, R. J., Wagner, W. R., Wojdyla, J. K., Wible, J. H., and Brandenburger, G. H. (1999). Targeting and ultrasound imaging of microbubble-based contrast agents. MAGMA *8*, 177-184.

Koldehoff, M., Steckel, N. K., Beelen, D. W., and Elmaagacli, A. H. (2007). Therapeutic application of small interfering RNA directed against *BCR-ABL* transcripts to a patient with imatinib-resistant chronic myeloid leukaemia. Clin. Exp. Med. *7*, 47-55.

Kratz, F. (2008). Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles. J. Control. Release *132*, 171-183.

Kumar, R., Ohulchanskyy, T. Y., Roy, I., Gupta, S. K., Borek, C., Thompson, M. E., and Prasad, P. N. (2009). Near-infrared phosphorescent polymeric nanomicelles: efficient optical probes for tumor imaging and detection. ACS Appl. Mat. Interfaces *1*, 1474-1481.

Kwong, G. A., Radu, C. G., Hwang, K., Shu, C. J., Ma, C., Koya, R. C., Comin-Anduix, B., Hadrup, S. R., Bailey, R. C., Witte, O. N., *et al.* (2009). Modular nucleic acid assembled p/MHC microarrays for multiplexed sorting of antigen-specific T cells. J. Am. Chem. Soc. *131*, 9695–9703.

Lam, K. S., Lebl, M., Krchnak, V., Wade, S., Abdul-Latif, F., Ferguson, R., Cuzzocrea, C., and Wertman, K. (1993). Discovery of D-amino-acid-containing ligands with selectide technology. Gene *137*, 13-16.

Lanza, G. M., Wallace, K. D., Scott, M. J., Cacheris, W. P., Abendschein, D. R., Christy, D. H., Sharkey, A. M., Miller, J. G., Gaffney, P. J., and Wickline, S. A. (1996). A novel site-targeted ultrasonic contrast agent with broad biomedical application. Circulation *94*, 3334-3340.

Leidinger, P., Keller, A., Borries, A., Reichrath, J., Rass, K., Jager, S. U., Lenhof, H. P., and Meese, E. (2010). High-throughput miRNA profiling of human melanoma blood samples. BMC Cancer *10*, 262.

Lin, M. M., Kim, H. H., Kim, H., Dobson, J., and Kim do, K. (2010). Surface activation and targeting strategies of superparamagnetic iron oxide nanoparticles in cancer-oriented diagnosis and therapy. Nanomedicine (Lond.) *5*, 109-133.

Linger, R. J., and Kruk, P. A. (2010). BRCA1 16 years later: risk-associated BRCA1 mutations and their functional implications. FEBS J. *277*, 3086-3096.

Liu, C. G., Calin, G. A., Meloon, B., Gamliel, N., Sevignani, C., Ferracin, M., Dumitru, C. D., Shimizu, M., Zupo, S., Dono, M., *et al.* (2004). An oligonucleotide microchip for genome-wide microRNA profiling in human and mouse tissues. Proc. Natl. Acad. Sci. USA. *101*, 9740-9744.

Liu, G., Rugo, H. S., Wilding, G., McShane, T. M., Evelhoch, J. L., Ng, C., Jackson, E., Kelcz, F., Yeh, B. M., Lee, F. T., Jr., *et al.* (2005). Dynamic contrast-enhanced magnetic resonance imaging as a pharmacodynamic measure of response after acute dosing of AG-013736, an oral angiogenesis inhibitor, in patients with advanced solid tumors: results from a phase I study. J. Clin. Oncol. *23*, 5464-5473.

Liu, S., Hsieh, W. Y., Jiang, Y., Kim, Y. S., Sreerama, S. G., Chen, X., Jia, B., and Wang, F. (2007). Evaluation of a (99m)Tc-labeled cyclic RGD tetramer for noninvasive imaging integrin α5β3-positive breast cancer. Bioconjug. Chem. *18*, 438-446.

Lu, J., Getz, G., Miska, E. A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebert, B. L., Mak, R. H., Ferrando, A. A., *et al.* (2005). MicroRNA expression profiles classify human cancers. Nature *435*, 834-838.

Lukianova-Hleb, E. Y., Hanna, E. Y., Hafner, J. H., and Lapotko, D. O. (2010). Tunable plasmonic nanobubbles for cell theranostics. Nanotechnology *21*, 85102.

Lunt, S. J., Chaudary, N., and Hill, R. P. (2009). The tumor microenvironment and metastatic disease. Clin. Exp. Metastasis *26*, 19-34.

Maltz, J. S., Sprenger, F., Fuerst, J., Paidi, A., Fadler, F., and Bani-Hashemi, A. R. (2009). Fixed gantry tomosynthesis system for radiation therapy image guidance based on a multiple source x-ray tube with carbon nanotube cathodes. Med. Phys. *36*, 1624-1636.

Mani, S. A., Guo, W., Liao, M. J., Eaton, E. N., Ayyanan, A., Zhou, A. Y., Brooks, M., Reinhard, F., Zhang, C. C., Shipitsin, M., *et al.* (2008). The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell *133*, 704-715.

Margulis-Goshena, K., Kesselmanb, E., Daninob, D., and Magdassia, S. (2010). Formation of celecoxib nanoparticles from volatile microemulsions. Int. J. Pharm. *393*, 231-238

McCarthy, J. R., and Weissleder, R. (2008). Multifunctional magnetic nanoparticles for targeted imaging and therapy. Adv. Drug Deliv. Rev. *60*, 1241-1251.

McNeil, S. E. (2009). Nanoparticle therapeutics: a personal perspective. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. *1*, 264-271.

Mehta, R. G., Murillo, G., Naithani, R., and Peng, X. (2010). Cancer chemoprevention by natural products: how far have we come? Pharm. Res. *27*, 950-961.

Menard-Moyon, C., Kostarelos, K., Prato, M., and Bianco, A. (2010). Functionalized carbon nanotubes for probing and modulating molecular functions. Chem. Biol. *17*, 107-115.

Merritt, W. M., Bar-Eli, M., and Sood, A. K. (2010). The dicey role of dicer: implications for RNAi therapy. Cancer Res. *70*, 2571-2574.

Miele, E., Spinelli, G. P., Tomao, F., and Tomao, S. (2009). Albumin-bound formulation of paclitaxel (Abraxane ABI-007) in the treatment of breast cancer. Int. J. Nanomedicine *4*, 99-105.

Mohs, A. M., Mancini, M. C., Singhal, S., Provenzale, J. M., Leyland-Jones, B., Wang, M. D., and Nie, S. (2010). Hand-held spectroscopic device for *in vivo* and intraoperative tumor detection: contrast enhancement, detection sensitivity, and tissue penetration. Anal. Chem. *82*, 9058–9065.

Morel, A. P., Lièvre, M., Thomas, C., Hinkal, G., Ansieau, S., and Puisieux, A. (2008). Generation of breast cancer stem cells through epithelial-mesenchymal transition. PLOS One *3*, e2888.

Morris, K. V. (2008). RNA-mediated transcriptional gene silencing in human cells. Curr. Top. Microbiol. Immunol. *320*, 211-224.

Murakami, Y., Yasuda, T., Saigo, K., Urashima, T., Toyoda, H., Okanoue, T., and Shimotohno, K. (2006). Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. Oncogene *25*, 2537-2545.

Nagrath, S., Sequist, L. V., Maheswaran, S., Bell, D. W., Irimia, D., Ulkus, L., Smith, M. R., Kwak, E. L., Digumarthy, S., Muzikansky, A., *et al.* (2007). Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature *450*, 1235-1239.

Nair, H. B., Sung, B., Yadav, V. R., Kannappan, R., Chaturvedi, M. M., and Aggarwal, B. B. (2010). Delivery of anti-inflammatory nutraceuticals by nanoparticles for the prevention and treatment of cancer. Biochem. Pharmacol. *80*, 1833-1843.

Namiki, Y., Namiki, T., Yoshida, H., Ishii, Y., Tsubota, A., Koido, S., Nariai, K., Mitsunaga, M., Yanagisawa, S., Kashiwagi, H., *et al.* (2009). A novel magnetic crystal-lipid nanostructure for magnetically guided *in vivo* gene delivery. Nat. Nanotechnol. *4*, 598-606.

Nanduri, J., Yuan, G., Kumar, G. K., Semenza, G. L., and Prabhakar, N. R. (2008). Transcriptional responses to intermittent hypoxia. Respir. Physiol. Neurobiol. *164*, 277-281.

Nel, A. E., Madler, L., Velegol, D., Xia, T., Hoek, E. M., Somasundaran, P., Klaessig, F., Castranova, V., and Thompson, M. (2009). Understanding biophysicochemical interactions at the nano-bio interface. Nat. Mater. *8*, 543-557.

Nelson, P. T., Wang, W. X., Wilfred, B. R., and Tang, G. (2008). Technical variables in high-throughput miRNA expression profiling: much work remains to be done. Biochim. Biophys. Acta *1779*, 758-765.

Nie, S., Xing, Y., Kim, G. J., and Simons, J. W. (2007). Nanotechnology applications in cancer. Annu. Rev. Biomed. Eng. *9*, 257-288.

Nie, Z., Nijhuis, C. A., Gong, J., Chen, X., Kumachev, A., Martinez, A. W., Narovlyansky, M., and Whitesides, G. M. (2010). Electrochemical sensing in paper-based microfluidic devices. Lab Chip *10*, 477-483.

Nihal, M., Ahsan, H., Siddiqui, I. A., Mukhtar, H., Ahmad, N., and Wood, G. S. (2009). Epigallocatechin-3-gallate (EGCG) sensitizes melanoma cells to interferon induced growth inhibition in a mouse model of human melanoma. Cell Cycle *8*, 2057-2063.

Nunez, R., Erwin, W. D., Wendt, R. E., 3rd, Stachowiak, A., Mar, M., Stevens, D., Madewell, J. E., Yeung, H. W., and Macapinlac, H. A. (2010). Acquisition parameters for oncologic imaging with a new SPECT/multislice CT scanner. Mol. Imaging Biol. *12*, 110-138.

Oberdorster, G., Oberdorster, E., and Oberdorster, J. (2005). Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ. Health Perspect. *113*, 823-839.

Oh, Y. K., and Park, T. G. (2009). siRNA delivery systems for cancer treatment. Adv. Drug Deliv. Rev. *61*, 850-862.

Oishi, M., and Nagasaki, Y. (2010). Stimuli-responsive smart nanogels for cancer diagnostics and therapy. Nanomedicine (Lond.) *5*, 451-468.

Olafsen, T., Sirk, S. J., Betting, D. J., Kenanova, V. E., Bauer, K. B., Ladno, W., Raubitschek, A. A., Timmerman, J. M., and Wu, A. M. (2010). ImmunoPET imaging of B-

cell lymphoma using ^{124}I-anti-CD20 scFv dimers (diabodies). Protein Eng. Des. Sel. *23*, 243-249.

Osterfeld, S. J., Yu, H., Gaster, R. S., Caramuta, S., Xu, L., Han, S. J., Hall, D. A., Wilson, R. J., Sun, S. H., White, R. L., *et al.* (2008). Multiplex protein assays based on real-time magnetic nanotag sensing. Proc. Natl. Acad. Sci. USA. *105*, 20637-20640.

Paavonen, J., Naud, P., Salmeron, J., Wheeler, C. M., Chow, S. N., Apter, D., Kitchener, H., Castellsague, X., Teixeira, J. C., Skinner, S. R., *et al.* (2009). Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet *374*, 301-314.

Pan, D., Caruthers, S. D., Chen, J., Winter, P. M., Senpan, A., Schmieder, A. H., Wickline, S. A., and Lanza, G. M. (2010). Nanomedicine strategies for molecular targets with MRI and optical imaging. Future Med. Chem. *2*, 471-490.

Paranjape, T., Slack, F. J., and Weidhaas, J. B. (2009). MicroRNAs: tools for cancer diagnostics. Gut *58*, 1546-1554.

Perrotti, D., Jamieson, C., Goldman, J., and Skorski, T. (2010). Chronic myeloid leukemia: mechanisms of blastic transformation. J. Clin. Invest. *120*, 2254-2264.

Petrelli, F., Borgonovo, K., and Barni, S. (2010). Targeted delivery for breast cancer therapy: the history of nanoparticle-albumin-bound paclitaxel. Expert Opin. Pharmacother. *11*, 1413-1432.

Petros, R. A., and DeSimone, J. M. (2010). Strategies in the design of nanoparticles for therapeutic applications. Nat. Rev. Drug Discov. *9*, 615-627.

Portney, N. G., and Ozkan, M. (2006). Nano-oncology: drug delivery, imaging, and sensing. Anal. Bioanal. Chem. *384*, 620–630.

Prasad, P. N. (2003). Introduction to Biophotonics, (New York: Wiley).

Prasad, P. N. (2004). Nanophotonics, (New York: Wiley).

Proske, D., Blank, M., Buhmann, R., and Resch, A. (2005). Aptamers--basic research, drug development, and clinical applications. Appl. Microbiol. Biotechnol. *69*, 367-374.

Puri, A., Kramer-Marek, G., Campbell-Massa, R., Yavlovich, A., Tele, S. C., Lee, S. B., Clogston, J. D., Patri, A. K., Blumenthal, R., and Capala, J. (2008). HER2-specific affibody-conjugated thermosensitive liposomes (Affisomes) for improved delivery of anticancer agents. J. Liposome Res. *18*, 293-307.

Qian, X., Rajaram, R., Calderon-Colon, X., Yang, G., Phan, T., Lalush, D. S., Lu, J., and Zhou, O. (2009). Design and characterization of a spatially distributed multibeam field emission x-ray source for stationary digital breast tomosynthesis. Med. Phys. *36*, 4389-4399.

Qin, L., Vermesh, O., Shi, Q., and Heath, J. R. (2009). Self-powered microfluidic chips for multiplexed protein assays from whole blood. Lab Chip *9*, 2016-2020.

Ravina, M., Paolicelli, P., Seijo, B., and Sanchez, A. (2010). Knocking down gene expression with dendritic vectors. Mini Rev. Med. Chem. *10*, 73-86.

Ruoslahti, E., Bhatia, S. N., and Sailor, M. J. (2010). Targeting of drugs and nanoparticles to tumors. J. Cell Biol. *188*, 759-768.

Sajja, H. K., East, M. P., Mao, H., Wang, Y. A., Nie, S., and Yang, L. (2009). Development of multifunctional nanoparticles for targeted drug delivery and noninvasive imaging of therapeutic effect. Curr. Drug Discov. Technol. *6*, 43-51.

Schmieder, A. H., Caruthers, S. D., Zhang, H., Williams, T. A., Robertson, J. D., Wickline, S. A., and Lanza, G. M. (2008). Three-dimensional MR mapping of angiogenesis with α5β1(αvβ3)-targeted theranostic nanoparticles in the MDA-MB-435 xenograft mouse model. FASEB J. *22*, 4179-4189.

Semenza, G. L. (2003). Targeting HIF-1 for cancer therapy. Nat. Rev. Cancer *3*, 721-732.

Semenza, G. L. (2008). Hypoxia-inducible factor 1 and cancer pathogenesis. IUBMB Life *60*, 591-597.

Semenza, G. L. (2010a). Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. Oncogene *29*, 625-634.

Semenza, G. L. (2010b). HIF-1: upstream and downstream of cancer metabolism. Curr. Opin. Genet. Dev. *20*, 51-56.

Shannon, A. M., Bouchier-Hayes, D. J., Condron, C. M., and Toomey, D. (2003). Tumour hypoxia, chemotherapeutic resistance and hypoxia-related therapies. Cancer Treat. Rev. *29*, 297-307.

Siddiqui, I. A., Adhami, V. M., Bharali, D. J., Hafeez, B. B., Asim, M., Khwaja, S. I., Ahmad, N., Cui, H., Mousa, S. A., and Mukhtar, H. (2009). Introducing nanochemoprevention as a novel approach for cancer control: proof of principle with green tea polyphenol epigallocatechin-3-gallate. Cancer Res. *69*, 1712-1716.

Singh, R., and Kostarelos, K. (2009). Designer adenoviruses for nanomedicine and nanodiagnostics. Trends Biotechnol. *27*, 220-229.

Singh, Y., Murat, P., and Defrancq, E. (2010). Recent developments in oligonucleotide conjugation. Chem. Soc. Rev. *39*, 2054-2070.

Singhal, S., Nie, S., and Wang, M. D. (2010). Nanotechnology applications in surgical oncology. Annu. Rev. Med. *61*, 359-373.

Schmieder T., Aaron, J., Harrison, N., Emelianov, S., and Johnston, K. (2009). Cancer imaging and therapy with metal nanoparticles. Conf. Proc. IEEE Eng. Med. Biol. Soc. *2009*, 2005-2007.

Sugahara, K. N., Teesalu, T., Karmali, P. P., Kotamraju, V. R., Agemy, L., Greenwald, D. R., and Ruoslahti, E. (2010). Coadministration of a tumor-penetrating peptide enhances the efficacy of cancer drugs. Science *328*, 1031-1035.

Tang, J. Y., Aszterbaum, M., Athar, M., Barsanti, F., Cappola, C., Estevez, N., Hebert, J., Hwang, J., Khaimskiy, Y., Kim, A., *et al.* (2010). Basal cell carcinoma chemoprevention with nonsteroidal anti-inflammatory drugs in genetically predisposed PTCH1(+/-) humans and mice. Cancer Prev. Res. *3*, 25-34.

Tiemann, K., and Rossi, J. J. (2009). RNAi-based therapeutics-current status, challenges and prospects. EMBO Mol. Med. *1*, 142-151.

Tricoli, J. V., and Jacobson, J. W. (2007). MicroRNA: Potential for Cancer Detection, Diagnosis, and Prognosis. Cancer Res. *67*, 4553-4555.

Vaishnaw, A. K., Gollob, J., Gamba-Vitalo, C., Hutabarat, R., Sah, D., Meyers, R., de Fougerolles, T., and Maraganore, J. (2010). A status report on RNAi therapeutics. Silence *1*, 14.

Venkatraman, S. S., Ma, L. L., Natarajan, J. V., and Chattopadhyay, S. (2010). Polymer- and liposome-based nanoparticles in targeted drug delivery. Front. Biosci. *S2*, 801-814.

Ventura, A., and Jacks, T. (2009). MicroRNAs and cancer: short RNAs go a long way. Cell *136*, 586-591.

Visone, R., and Croce, C. M. (2009). MiRNAs and cancer. Am. J. Pathol. *174*, 1131-1138.

Vo-Dinh, T. (2007). Nanotechnology in biology and medicine: methods, devices, and applications, 1st edition (Boca Raton: CRC Press).

Wang, X., Li, J., Wang, Y., Cho, K. J., Kim, G., Gjyrezi, A., Koenig, L., Giannakakou, P., Shin, H. J., Tighiouart, M., *et al.* (2009). HFT-T, a targeting nanoparticle, enhances specific delivery of paclitaxel to folate receptor-positive tumors. ACS Nano *3*, 3165-3174.

Wei, L. H., Olafsen, T., Radu, C., Hildebrandt, I. J., McCoy, M. R., Phelps, M. E., Meares, C., Wu, A. M., Czernin, J., and Weber, W. A. (2008). Engineered antibody fragments with infinite affinity as reporter genes for PET imaging. J. Nucl. Med. *49*, 1828-1835.

Willmann, J. K., van Bruggen, N., Dinkelborg, L. M., and Gambhir, S. S. (2008). Molecular imaging in drug development. Nat. Rev. Drug Discov. *7*, 591-607.

Winter, P. M., Schmieder, A. H., Caruthers, S. D., Keene, J. L., Zhang, H., Wickline, S. A., and Lanza, G. M. (2008). Minute dosages of αvβ3-targeted fumagillin nanoparticles impair Vx-2 tumor angiogenesis and development in rabbits. FASEB J. *22*, 2758-2767.

Xia, C. F., Zhang, Y., Boado, R. J., and Pardridge, W. M. (2007). Intravenous siRNA of brain cancer with receptor targeting and avidin-biotin technology. Pharm. Res. *24*, 2309-2316.

Yin, J. Q., Zhao, R. C., and Morris, K. V. (2008). Profiling microRNA expression with microarrays. Trends Biotechnol. *26*, 70-76.

Zhang, L., Gu, F. X., Chan, J. M., Wang, A. Z., Langer, R. S., and Farokhzad, O. C. (2008). Nanoparticles in medicine: therapeutic applications and developments. Clin. Pharmacol. Ther. *83*, 761-769.

Zitzmann, S., Ehemann, V., and Schwab, M. (2002). Arginine-glycine-aspartic acid (RGD)-peptide binds to both tumor and tumor-endothelial cells *in vivo*. Cancer Res. *62*, 5139-5143.