Building the Interface of Nanotechnology and Cancer Imaging Research

National Cancer Institute Symposium

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Executive Summary

On January 28, 2004, the NCI convened a symposium "*Building the Interface of Nanotechnology and Cancer Imaging Research"* to explore how nanotechnology can foster strategies to target cancer. Nanotechnology refers to the interactions of cellular and molecular components and engineered materials – typically clusters of atoms, molecules and molecular fragments – with dimensions that are typically, though not exclusively, smaller than 100 nanometers. The NCI is currently developing a Cancer Nanotechnology Plan that will focus on developing platforms and standards for this growing field in addition to supporting innovative research of the type highlighted in this symposium. NCI-supported investigators have created nanotechnology platforms that hold promise for simultaneously diagnosing and treating cancer. Some of the fruits of these projects are in preclinical studies designed to lead to human clinical trials. The NCI is also developing programs that apply nanotechnologies as tools for systematic interrogation of cellular and molecular processes of basic cancer biology.

Although several specific technologies were discussed, the primary goals of the meeting were to identify state-of-the-art approaches to advance cancer imaging through nanotechnology, as well as pinpointing barriers and opportunities to accelerate progress of this promising field. The symposia was led by Mauro Ferrari, Ohio State University, and Lee Hartwell, Fred Hutchison Cancer Research Center, with the NCI leadership of Ann Barker, Daniel Sullivan, and Peter Choyke. Andrew von Eschenbach, Director, NCI addressed the experts representing 35 cancer cancers, engineering programs, and companies and provided an overview of NCI's vision for technology development programs to fight cancer.

Featured at the meeting were methods to develop nanoscale contrast agents for multi-modal imaging techniques including optical, radionuclide, ultrasonographic, and magnetic resonance techniques. Experts emphasized a need to establish standardized testing methods to enable comparisons of the applicability of various nanoparticles to a given imaging task – such as molecular tumor cell recognition. Meeting participants also noted that another important barrier to overcome is the lack of identified targeting molecules that would provide better specificity for targeted imaging agents. Attendees also noted that the most successful research groups are interdisciplinary teams (e.g., cancer biologists, engineers, physics, and chemists) and emphasized that progress will

depend on developing new models to reward collaboration in the academic, government, and private sectors.

Presenters and participants alike also identified the lack of an established commercialization pathway for clinically-applied cancer imaging agents as a major obstacle. The potential for nanoscale contrast agents to help elucidate basic mechanisms of cancer biology and improve clinical diagnostic and therapeutic management strategies represent new opportunities for multi-sector research and development investment and partnerships. As an example of the potential improvements in clinical care offered by nanotechnology applications in cancer imaging, researchers noted the recent success of superparamagnetic nanoparticles coupled with magnetic resonance imaging (MRI) to improve the detection of clinically occult lymph-node metastases in patients with advanced prostate cancer.[1]

Introduction

If the Nation is to meet the 2015 goal of eliminating death and suffering from cancer, clinicians will need new ways to detect cancer and metastases as early as possible, since early detection will continue to be the single best way to greatly improve therapeutic outcomes. One expectation of the cancer field is that non-invasive imaging technologies, such as MRI, optical imaging, PET and others, will play a critical role in early detection, but only with the development of new imaging and contrast agents that will improve the sensitivity of these technologies. At the same time, nanotechnology is rapidly making a mark among a small but growing group of NCI-funded cancer researchers as a potential tool for creating such imaging and contrast agents. Indeed, at least one nanotechnology based imaging product has demonstrated success in detecting micrometastases in humans and is awaiting FDA approval, and several other nanoparticulate imaging agents are either in or are close to human clinical trials.

As key component of its Cancer Nanotechnology Initiative, the NCI desires to boost the number of cancer researchers who are interested in applying nanotechnology to the problem of diagnosing – and treating – cancer. A goal is to increase the number of investigators who are interested in applying their skills with nanotechnologies to the problem of developing new diagnostics and therapeutics for cancer. To help accomplish this, the NCI is holding a series of symposia aimed at introducing nanotechnology and its potential uses to as broad an audience as possible within the cancer and nanotechnology communities. An ancillary goal of these symposia is to solicit broad scientific input to provide direction to research and engineering applications and to identify barriers that are or may impede progress.

On January 28, 2004, NCI convened the first of these symposia in suburban Washington, D.C., titled "*Building the Interface of Nanotechnology and Cancer Imaging Research.*" Over 30 investigators from academia, industry and government participated in this symposium. To provide the symposium participants with context for this meeting and to

[1] Harisinghani et al. (2003) "Noninvasive Detection of Clinically Occult Lymph-Node Metastases in Prostate Cancer." *NEJM*. 348(25) 2491-2499.

set its tone, Anna Barker, of the NCI, opened the proceedings with a summary of NCI's strategic initiatives, all of which are aimed to enable the cancer field to reach the NCI's goal of eliminating death and suffering from cancer by 2015. She then defined for the participants what NCI wanted to participants to accomplish in this meeting:

- Define the state of the art in the application and development of nanoparticles as contrast agents.
- Identify the bottlenecks in the use of nanotechnology in advanced biomedical imaging.
- Identify the most promising combinations of nanoparticle technology and targeting agents in the context of the various imaging modalities, e.g., optical imaging, MRI, PET, and others.
- Identify emerging imaging technologies that could become clinically significant with the help of nanotechnology

Session 1: Improving imaging performance for early detection of neoplastic disease

In this session, chaired by Lee Hartwell, of the Fred Hutchison Cancer Research Center, three speakers discussed how imaging agents made of nanoscale particles could be used to improve the diagnostic performance of magnetic resonance and optical technology. Ralph Weissleder, of the Massachusetts General Hospital and Harvard Medical School, began the symposium by presenting the results of ongoing clinical experience using dextran-coated, superparamagnetic iron oxide nanoparticles (Combidex™) to detect micro-metastases in lymph nodes using MRI. The presence of such micro-metastatic lesions blocks the particles from entering parts of the lymph nodes, allowing them to be easily distinguished in the MRI image from normal lymph node tissue, which fill with the particles. After compiling a data set comparing normal to abnormal scans, Weissleder and his team were as good at finding metastatic nodes as were surgeons who inspected lymph nodes manually.

Given that the cost of such exploratory surgery tops $40,000 per procedure, this technique, should it prove itself in further clinical trials, has the potential to significantly reduce medical costs, not to mention patient risk and discomfort. But Weissleder cautioned that despite this early promise, there are significant technical improvements that can be made and roadblocks in the path to developing these particles for a wide variety of cancer imaging applications. Safety concerns are paramount, though the latest particles being developed are stable, using synthetic coatings; nevertheless, taking Combidex from mouse to humans took 10 years of development to ensure that this product was nontoxic. Detection sensitivity, which would decrease the number of particles injected, can be improved by an order of magnitude by improving the magnetic properties of the particles. More selective tumor-targeting strategies are needed, but such targets are limited in number and the chemistry to attach targeting molecules to the nanoparticles is still poorly defined and will require attracting more chemists to the field. Along the same lines, he said combinatorial approaches using libraries of possible ligands and nanoparticles are needed to speed the development of particles for targeted delivery.

There is also a need for display and "fusion" software that would merge MRI data onto CT images, which would provide surgeons with an accurate 3-dimensional map of micro-metastases. Studies with higher-field magnets are needed as well to determine if future high-field MRI instruments will provide better spatial resolution. Weissleder also highlighted the need for the field, perhaps under NCI's leadership, to develop standards and reference materials that would help face the regulatory challenges that nanoparticles face, and to develop standardized acquisition models for analyzing normal and abnormal data sets.

Should these technical challenges be met, Weissleder predicted that nanoparticles will have uses that go beyond tumor and metastasis imaging. Among the potential future applications he listed were cell labeling and tracking, apoptosis detection, and angiogenesis targeting. As an example of this type application, he cited work that his group published early this year detailing the development of a novel magnetic nanoparticle capable of detecting telomerase activity in biological samples (elevated telomerase levels are characteristic of many types of cancer). He predicted that a year of work would be necessary to translate this initial work into a superparamagnetic nanoparticle suited for use in humans.

Rebecca Richards-Kortum, of the University of Texas-Austin, then talked about her laboratory's work with targeted gold nanoparticles as contrast agents for *in vivo* endoscopic optical imaging of three different cancer markers: over-expression of the epidermal growth factor receptor (EGFR), matrix metallo-proteases (MMPs), and oncoproteins associated with human papillomavirus (HPV) infection. Optical imaging, she said, is ideally suited to detecting epithelial cancer-related alterations because it can detect biochemical and morphologic alterations with sub-cellular resolution throughout the entire epithelial thickness. Optical techniques can be implemented non-invasively, in real time, and at low cost to survey the tissue surface at risk. She also mentioned briefly ongoing efforts to create inexpensive, fiber optic confocal imaging systems that would be small enough to travel through ducts and capillaries to reach, and therefore image, virtually any place in the body.

The clinical benefits of such an approach, she said, include earlier detection of cancer; the ability to use biological markers to predict the risk of progression for precancerous lesions; detection and characterization of tumor margins; the ability to select molecular therapies rationally; and the ability to monitor response to molecular therapy in real time. The chief challenges to realizing these benefits include issues of delivery, particularly for intracellular targets, and in putting together the cross-disciplinary teams and collaborations that are needed to move this field ahead. She also predicted that intellectual property issues will soon begin causing problems for the field, and she called for the field to begin thinking of ways to mitigate intellectual property fights that could stop the field in its tracks.

The session ended with a presentation by Shuming Nie, of Emory University, on multi-colored semiconducting quantum dots and their use in imaging multiple cellular processes simultaneously. The quantum dots he uses, which luminesce in the near-

infrared, are made of cadmium selenide coated with zinc sulfide. While he conceded that cadmium, because of its toxicity, is not an ideal substance for use in the human body, he noted that such particles could nonetheless prove useful for research studies or in topical applications. He then discussed how his laboratory has devoted a significant effort to developing a variety of chemistries for modifying the surfaces of these quantum dots to allow derivatizing the particles with targeting molecules and even MRI contrast agents.

Following these presentations, symposium participants joined in a vigorous discussion of the various barriers that are or may impede progress in using nanoparticles as imaging agents. The issues raised fell into four categories, and can be summarized as follows:

Science/Technology issues
- Single vs. multiplex approaches
- Better/more targeting strategies
- Delivery issues – inability of nanoparticles to enter tumors
- Standardized toxicology testing
- Versatile, reproducible surface chemistries for nanoparticles
- Combinatorial/high-throughput screening for nanoparticles/targeting agents
- Miniaturization of detection systems (e.g., optical)
- Targeting cancer processes – relationship to other processes (angiogenesis, apoptosis)

Regulatory issues
- Safety of the particles – toxicology, pharmacokinetics, metabolism, clearance
- Need to retest particle with each imaging application
- Difficulty combining diagnostic and therapeutic modalities in the same particle

Data issues
- Lack of clinical and data format standardization
- Need "learning" data sets for researchers and clinicians
- Need internal imaging reference, at least for research and animal studies

"Social/cultural" issues
- Collaboration among researchers, contrast agent developers and equipment manufacturers (is there a marketplace for contrast agents?)
- Lack of financial models that make diagnostic indications economically viable yet approvable

Session 2: Imaging the Tumor Microenvironment for Diagnostic Applications: Roadblocks and Promising Directions

This session, chaired by Daniel Sullivan of the NCI, began with Samuel Wickline, of Washington University in St. Louis, asking an important question: If you want to image the microenvironment of the tumor, what exactly do you want to image in that microenvironment: angiogenesis, lymphatic flow, matrix/stroma, necrosis, inflammatory cells, genes or RNA, cytokines or other messengers, tumor cells markers, or all of the

above? Certainly, he concluded, it would be best to image several of these features simultaneously, which means that the best imaging agents would be polyvalent for more than one of these markers. He then noted that the cancer environment is reminiscent of normal physiological processes with, in most cases, only subtle changes. Nonetheless, recent advances in "gene fishing" have identified important differences. He pointed out that the differences between malignant cells and normal cells occur in highly regulated physiological systems that have numerous checks and balances. Thus, any technology developed for imaging the tumor microenvironment must be able to answer questions about when, where, and in what order events unfold and with what time constants. As an example, he pointed out that it does not do much good to characterize levels of matrix metalloproteins (MMPs) without simultaneously delineating levels of TIMPS, the inhibitors of MMPs, that are active in cells. As a result, there is a critical need to characterize processes in time and space (fingerprinting) in order to differentiate normal process dynamics – the body's molecular pothole filling – from abnormal processes.

He continued by discussing issues relating to the design criteria for a multivalent or pluripotent targeting agent in order to perform *in vivo* image-based molecular fingerprinting. How many targets are enough, for example, to differentiate the different signals? Fluorine NMR spectroscopy might be a suitable technology since each fluorocarbon has a differentiable and quantifiable signal. There is a need for any imaging technique to be quantitative because the differences between a malignant and normal cell often comes down to one of quantitative differences in pathway activity rather than an all-or-nothing change. Targeting agents will have to be safe when administered over multiple doses, and they will have to be of a size sufficient to carrying the suitable imaging payload, yet be able to be taken up by cells. Finally, he asked whether such imaging agents should also be capable of delivering a therapeutic agent once it has reached and identified malignancy.

He then enumerated a number of roadblocks to nanotechnology initiatives in the imaging field. There are few examples of ground-breaking applications of nanotechnology in the imaging area, though his work using superparamagnetic iron oxide nanoparticles to image metastases is close. There are still concerns and open questions about biocompatibility, pharmacokinetics, degradation, elimination, and overall safety issues that must be answered before we know if nanoparticles will work in humans. He predicted that intellectual property issues are going to become a problem that the field will have to address sooner rather than later. Also, there is the issue of the meager profit margins associated with diagnostic as opposed to therapeutic products, especially targeted diagnostic agents. Scale-up and manufacturing issues are still unresolved, particularly concerning the eventual cost of nanoparticulate imaging agents, and what the regulatory and reimbursement policies will be for any diagnostic agent that also delivers a therapeutic load. There is also a need for new approaches to funding research and training in this cross-disciplinary field, though he cited the NCI's Unconventional Innovations Program as an effective model for this particular field. Finally, he noted the academic mindset that discourages forming the large cross-disciplinary teams needed to develop nanoparticles for biomedical imaging is a major impediment to the field. So, too,

is the dichotomy between translational research and basic science in terms of the bias that the academic reward system at most institutions shows to the latter over the former.

In closing, Wickline called on the FDA to allow investigators to prove the safety of a given nanoparticulate platform once rather than with each new ligand or payload. In other words, he said, if you show that a given nanoparticle is safe, there would be a fast-track process to test the effectiveness of the new construct. Such an attitude on the part of regulators would have a great positive, exciting impact on the field. The contrary attitude currently in place is a major impediment to commercial development of nanoparticulate imaging systems.

King Li, of the NCI, noted in his talk that the biggest obstacles to imaging the tumor microenvironment are delivery and uptake, since the human body seems designed to isolate and eliminate particulate matter. The tumor environment itself is also hostile to the uptake of particles, which he predicted would dominate any favorable kinetics derived from targeting a particle to a tumor with a tumor-specific ligand. As a result, it may be more effective to target the host response to a tumor, such as angiogenesis. It should also be easier to target endothelial cells, something that Li's lab is pursuing by targeting integrins on the surface of angiogenic cells.

He then discussed various multiplexing strategies that would capitalize on the fact that once a nanoparticle has imaged a tumor, it should be possible to modulate physiological properties to enhance tumor uptake of the particle and then dose the tumor with a therapeutic agent carried by the particle. Li reported on the use of high intensity focused ultrasound (HIFU) on a tumor to increase tumor leakage thereby doubling nanoparticle uptake without any histological changes to individual cells. Subsequent studies identified a number of genes that are consistently upregulated by HIFU, including genes for a heat shock protein. It may be possible to use this protein to modulate targeted expression of a therapeutic gene and have it expressed only in the tumor, which would be advantageous given that it is likely that organs such as spleen and liver will accumulate particles nonspecifically.

Kathy Ferrara, of the University of California at Davis, also discussed the use of nanoparticles as contrast agents for ultrasound imaging. Her laboratory has developed thick-shelled nanoparticles with a gas core and a PEG-stabilized lipid shell that she calls acoustically activated lipospheres. Using very low energy ultrasonic pulses forces these particles are pressed up against the vascular cell wall, where they then burst. Depending on the contents of the liposphere, this can release an imaging agent, an inflammatory mediator, or even a therapeutic agent at the site of compression. Using a high-resolution ultrasound imager, it is possible to detect very small tumors with spatial resolution of 0.5-1.0 millimeters, and a temporal timeframe of several images per second, allowing for an exam on a person to be completed in three minutes. The procedure can be repeated often and quickly to image the temporal response to therapy. Though the normal tissue movement that occurs in the body degrades performance, Ferrara said that new wide-bandwidth ultrasound devices should improve performance enough to compensate for tissue movement factors.

She then listed several possible applications for this technology, including imaging molecular targets within the vasculature, imaging sentinel nodes, measuring tissue permeability, quantifying vascular density and viable tumor density, measuring flow and flow rate, and as dual imaging and therapeutic systems. She added that it is possible to correlate vascular density measurements between ultrasound and CT images, providing picomolar sensitivity in very small vessels. It is possible, too, to characterize vessel blood flow by destroying the particles and measuring reperfusion of the agent. Experiments have shown that this technique can detect irregular flow as well as flow volume through tumors using area under the curve measurements. For drug delivery, her group has shown that they can achieve a 10-fold increase in local concentrations of an anti-tumor drug.

Bringing these particles to the clinic will require a new focus on nanoparticle manufacturing techniques in order to achieve consistent particle size distribution, shell thickness, yield and purity. Further work is also needed to get reproducible surface and labeling chemistries, and to develop methodology to quantify the number of binding sites on a particle's surface and then reliably synthesize particles with a consistent number of binding sites. Another need is for high bandwidth ultrasound systems. Finally, she noted, because there is no FDA approved indication for the use of ultrasound diagnostic agents in the cancer area, pharmaceutical company interest in this technology is lagging. Indeed, it has been difficult to get some of these agents into human clinical trials. In contrast, she says, researchers working with nuclear imaging have done a good job of developing approved mechanisms for small, quick clinical trials. Nonetheless, she predicted that a system for testing in humans should be ready shortly, though the first clinical trials may be to detect angiogenesis in macular degeneration rather than in cancer.

Roger Tsien, of the Howard Hughes Medical Institute and the University of California at San Diego, finished the presentations in Session 2 by discussing the use of leashed cationic uptake sequences to create nanoparticles that selectively accumulate in disease tissue. Polycationic sequences, such as *tat* and poly-arginine, are known to drag payloads fused to that sequence onto and into cells. To create a delivery system, he fuses one end of the polycation to short sequence of amino acids that are cleaved by enzymes found on the surface of cancerous cells, such as matrix metalloproteins, urokinase plasminogen activator, and prostate membrane-specific antigen. Other potential cleavage mechanisms include taking advantage of acidic pH due to increased glycolysis in cancer and infarcts; free thiol reduction related to hypoxia in cancer and infarcts; beta-lactamase in antibiotic-resistant bacteria; photolysis using photodynamic therapy of skin of endoscopically accessible tissues, and by phosphatases in osteoporosis and bone malignancies. This cleavable sequence also contains a polyanion "vetoing" sequence that duplexes with the polycation, reducing uptake in cell culture by 86-99%. However, when the linker sequence is cleaved, the polycation and its payload are then taken up as usual, with high efficiency. He compared this construct to double-sided tape, with which removing the wax paper allows the tape to stick.

He concluded by listing some of the advantages of this approach, including its applicability to major disease targets such as cancer and thrombosis without gene

therapy. In that the active agents are relatively simple small molecules, reducing the chances of immunogenicity; and that the vetoing sequences might reduce stickiness of cationic sequences and improve pharmacokinetics. He noted that using these sequences as diagnostic agents may provide a simplified route to clinical utility and regulatory approval, particularly since only trace quantities are injected. He also predicted that the same mechanism of cleavage-activated uptake should deliver contrast agents for PET, SPECT, MRI, NIR imaging, as well as radiation sensitizers (^{10}B, ^{157}Gd) and chemotherapeutic agents for therapy. He added that for *in vivo* imaging, it shouldn't matter whether contrast agent enters the cytosol and nucleus as long as it sticks to target cells.

Following these presentations, symposium participants once again joined in a vigorous discussion of the various barriers that are or may impede progress in using nanoparticles as imaging agents. In this discussion, the issues raised fell into five categories, and can be summarized as follows:

Science/Technology issues

- What targets to image? Relevance to cancer? No systematic approach has evolved.
- What spatial and temporal resolution is needed to differentiate between normal and malignant state
- For multiplexing, how many targets are enough? How do we differentiate the signals?
- Particle delivery into tumor is problematic
- Is an agent give in a single dose or a multiple dose?
- What are the pharmacokinetics, half-life and clearance rates of different types of nanoparticles?
- Do nanoparticle-based agents accumulate over time and with repeated doses?
- Quantification is difficult; need to work with industry to obtain the ability to gain access to the internal workings of the sensors and processing equipment in various imaging devices in order to modify and upgrade both the hardware and software
- Need reference standards for probes – standardize measurement of biological processes
- New nano delivery approaches (e.g., leashed cationic uptake sequences)

Regulatory issues

- Approvability – what is needed?
- Demonstrated safety
- Multiple dose approaches add significant regulatory complexity over single dose
- Repeat use of the same nanotech platform with different ligands without repeating regulatory process for the platform/conjugate each time

Manufacturing issues

- Cost effectiveness
- Reproducibility
- Scale-up

Economics issues
- Financing
- Cost per dose
- Reimbursement for diagnostics in the absence of therapeutics
- IP

Collaboration issues
- The need for team building - difficult in academia
- Intellectual property sharing
- Need for specialized facilities for probe development, integrated platforms (gene expression arrays, etc.)

Session 3: Imaging Diversity in Molecular Expressions and Their Time Evolution in Cancer Patients and the High-Risk Population

This session, chaired by Mauro Ferrari of the Ohio State University, began with a presentation by Lee Josephson, of the Harvard Medical School, who spoke about combinatorial approaches to synthesizing magnetic and optical nanoparticles based on cross-linked dextran-coated iron oxide amine particles known as amino-CLIO. These particles, about 20 nanometers in diameter, have amino and carboxy moieties on their surfaces, allowing them to be modified easily. The starting point is to create one bulk intermediate already derivatized with a fluorophore on the particle surface. Using thiol chemistry, the particles are then derivatized further in microtitre plates, which then allows the particles to be screened for cellular uptake using *in situ* cell-based fluorescence microscopy. Those particles that pass this first screen can then be screened rapidly *in vivo* using small animals and near-infrared fluorescence imaging. After this screen, the particles can be screened further using MRI, taking advantage of the magnetic core of the nanoparticle. The result would be particles that allow pre-operative MRI scanning to pinpoint a tumor's location along with real-time near-infrared imaging during surgery to allow the surgeon to ensure removing all of a tumor. Josephson concluded his talk by predicting that amino-CLIO nanoparticles may have a straightforward path to regulatory approval because of the already demonstrated safety of other superparamagnetic nanoparticles.

Jean Frechet, of the University of California at Berkeley, then discussed using dendrimer-based particles for targeted delivery of anticancer drugs, which in principle could be used to deliver contrast agents as well. Dendrimers, noted Frechet, target tumors based on particle size, with larger particles accumulating in tumor tissue via enhanced permeation and retention. Animal studies of doxorubicin accumulation have shown that dendritic carriers have a near ideal size. Working with libraries of dendritic carriers, which are polyvalent and easily modified to carry receptor-specific ligands, drugs and imaging agents, it is possible to create particles with a variety of molecular weights, architecture and functionalization that strongly affect pharmacokinetic and targeting properties. It also possible to build multimolecular assemblies with degradable crosslinking, creating nanoparticles that will fall apart under specified physiological conditions to release their

payloads. In summary, Frechet noted, it is possible to create a very wide variety of particles, each with tailorable properties. He added, though, that a lack of interest by the pharmaceutical industry is hampering the development of these nanoparticles for human clinical use.

In the ensuing discussion, symposia participants lauded the availability of many different types of nanoparticles and labeling schemes as a potential boon for studying complex cellular systems. For example, it may be possible to use different colored quantum dots, each labeled with a different ligand, to simultaneously measure the expression of multiple genes. Such nanoparticles could then be used to perform large-scale gene expression imaging in real-time. Participants also noted that industry is hesitant to invest in this area until it is clear that the intellectual property surrounding a particular nanotechnology is sound and well-developed. They also commented that it is important to remember that while the area of *in vitro* cancer diagnostics is expanding greatly, with good development efforts, the situation in *in vivo* applications is more difficult to justify given the long-term development issues and higher risk profile. Another important factor to consider is that the imaging agents used today are non-specific, meaning the market is relatively large. The more specific an imaging agent, the smaller the market, the smaller return, and the less likely it will be for industry to develop it.

Discussion then turned to ways of motivating development, noting that there are two ways to motivate industry: opportunity and necessity, i.e., keeping up with technology development. Today, we are not impacted by necessity, but if the approval process is expedited to speed up approval for new nanoparticles, then that would create a much better system where industry would be willing to work with others who develop targeting molecules. Another factor that would motivate development is the development of imaging agents that can identify those patients who can benefit from a particular therapy, i.e., that imaging can select patients for the most appropriate therapy. As an example, the NCI has helped with early stage clinical trials for Combidex because of its ability to do nodal staging, which the NCI concluded was critically important to therapy. One participant from industry noted that if an imaging agent can't make a difference in disease management, why bother developing it, for if it can, the product will not only have a big impact in the market, but an easier road of development and acceptance.

When the discussion turned to fundamental scientific roadblocks, one participant from academia said that most researchers in academia never worry about FDA, about business models and economic return. But having said that, to move this field forward NCI needs to encourage interdisciplinary teams that can work together to do great science. Another critical roadblock is that there isn't a good enough scientific understanding of the natural history of these diseases and of the biomarker history of these diseases. Participants also agreed that NCI should create a toxicity appraisal service similar to its drug screening program that is open to all. Could be a place for toxicity testing on diagnostic agents in order to get a quick vetting or "no" on an agent. Such a service would also create a publicly accessible database of what works and what doesn't in terms of toxicity.

In summarizing this session, meeting participants noted the following:

- Parallel, combinatorial approaches are possible for creating nanoparticles
    - Screening for imaging properties, not activity
    - Looking for <u>any</u> difference between two parts of the body, not exactly what is different
- There is a lack of corporate interest in developing imaging agents, particularly with the move toward more specific applications
    - Need to reduce risk for industry
- "Killer app" in imaging can be imaging agents/combinations that improve therapeutic decisions and outcomes.
- There should be a focus on PET imaging
- Fundamental science is key
    - Interdisciplinary teams
    - Establish biomarker history of disease
- Toxicity appraisal/diagnostic screening service at NCI for imaging nanoparticles, which would reduce risk and increase standardization
- Need to determine the smallest surgical margin that a given imaging modality can detect and what impact imaging can have on detecting undiagnosed primary tumors.
- Possible applications that should be further studied:
    - Protein profiling using microarrays with imaging
    - High-throughput screening of targets, compounds, ligands and particles

Session 4: Engineering the Biodistribution of Imaging Nanoprobes *In Vivo*

The final session, chaired by Peter Choyke, of the National Institutes of Health, began with a discussion by Michael Knopp, of the Ohio State University, that highlighted some of the factors to consider when developing a nanoparticle-based diagnostic agent. At the top of the list is the need to remember that these are probes as well as devices, and that it is important to work out the details of how to use them, quantify them, visualize them and interpret data from them. Software is important, as are imaging database repositories, both of which will play a critical role not only in diagnostic but in monitoring response to therapeutic interventions. Important components that are often neglected in a development project include validation and standardization, regulatory processes, and intellectual property. Knopp proposed that the NCI might write protective patents to keep the field open for future development.

He then discussed the idea of using nanoprobe-based diagnostics to monitor therapy in breast cancer. The goal, he said, is to use such probes to understand not only what is happening in "the tumor" but also in the heterogeneity within the tumor. This would provide critical information on small areas of a tumor that are not responding to therapy, which would not only inform further therapeutic decisions but also provide a key opportunity to better understand the molecular and genetic heterogeneity within a tumor by applying proteomics and other analytical technologies. But as such technology is developed, it is important to consider the "real" world, which raises issues of tissue motion, reproducibility, and heterogeneity. He also promoted the approach of using multiple scales of imaging – first doing relatively low-resolution whole body imaging

followed by high-resolution imaging of small areas of the body singled out in the whole body scan.

In discussing the challenges facing the field, he noted that there are hurdles to rapid translation from basic proof of principles to clinical utilization. Sensitivity of detection in patients is a tremendous obstacle, with a need to understand the technical requirements for improving sensitivity. Interaction with non-targeted tissues is a concern, particularly early in development, as is potential toxicity of small particles. As he mentioned earlier, there are intellectual property issues, and he again called on Federal agencies to take a leadership position in preventing intellectual property issues from blocking interest and progress in the field. He noted that diagnostic agents have a lengthy approval process, and that while accelerated regulatory evaluations are well established for therapeutic cancer agents, there are no similar precedents for non-radioactive diagnostic agents in oncology. Also, current commercial clinical development for new probes and technology focuses more on cardiovascular than oncology applications. In large part, companies are shying away from oncology relative to cardiology, he stated, because of the higher risk profiles in treating cancer and higher likelihood of adverse events that would then be associated with the agent under development.

The penultimate presentation was given by Jennifer West of Rice University, who discussed potential diagnostic and therapeutic applications of gold-coated nanoparticles with a dielectric silicon core. She began by discussing how it is possible to tune the optical properties of these particles by varying the thickness of the gold coating on the amino silane derivatized silicon core. She also noted that these particles can then be modified further using PEG and a sulfide linker to add molecules such as antibodies or other targeting ligands. Using such particles it is possible to image whole breast and brain using near-infrared imaging, with the potential, too, for imaging at the molecular level. Mouse experiments have shown that Her-2 conjugated nanoshells can image breast carcinoma cells *in vivo* even with very small amounts of nanoshell. *In vitro* studies using cultured breast cells showed that once bound to the cells, the nanoshells could be used to kill the cells via photothermal ablation, since the nanoshells will heat up upon irradiation. Subsequent animal studies also demonstrated the same effect with no apparent toxicity. In this case, the nanoshells were administered and used therapeutically 10 days later, which allowed the particles to accumulate in breast tumors While no control animals survived, 100 percent of the test animals did, a promising result. Nanospectra Biosciences has licensed this technology and has constructed a GMP manufacturing facility to produce the nanoshells in quantity. Preliminary toxicity studies have shown that the particles are cleared intact over a 6-week period though the feces, not through the kidney.

Fostering this kind of research, West noted, requires strong interdisciplinary teams. Various policies and reward systems at Rice University encourage that its faculty work in such teams, which enabled West to interest students and postdoctoral fellows from five different departments to work on this project.

In the last presentation of the day, Kam Leong of the Johns Hopkins School of Medicine discussed his work using DNA nanoparticles to achieve non-viral gene delivery and as

potential imaging probes. Under the right conditions, he said, polycationic lipids or other cationic polymers will condense with DNA to create nanoparticles. It is also possible to co-encapsulate bioactive compounds within the resulting nanoparticle. The same approach, he said, should work with an imaging contrast agent as long as it is a macromolecular charged compound, since the larger the compound the easier it is to encapsulate it. This work is still in its early stages, though the results so far show that it is possible to reproducibly produce nanoparticles of given size and properties.

The ensuing discussion identified a number of barriers to developing nanoparticles as imaging agents for cancer:

- Hurdles to rapid translation from basic proof of principles to clinical utilization
- Sensitivity of detection in patients – need to understand technical requirements
- Interaction with "non-targeted" tissues, particularly early in development
    - Tumor heterogeneity
- Toxicity of nanoparticles is still poorly defined.
- Develop a database for comparison and speeding up the safety/toxicity testing for compounds
- Intellectual property Issues – Federal agencies must take a leadership position in preventing intellectual property issues from blocking interest and progress
- Diagnostic agents have a lengthy approval process
- No precedents for rapid approval of non-radioactive diagnostic agents in oncology
- Current commercial clinical development for new probes and technology focuses more on cardiovascular than oncology applications
- There is a need for novel nanoparticle development
- Multidisciplinary teams are essential, and there is need for new academic models to incentivize team science
- Environmental issues – what becomes of these particles?

In wrapping up the meeting, the four session chairs made the following comments. Lee Harwell commented about how one systematically develops technology. The discovery phase ought to be one in which all flowers bloom, but the development and application stages in this field could benefit from identifying major opportunities for imaging to guide therapy and to focus on a few big opportunities, given the financial and regulatory constraints. Dan Sullivan noted that many of these issues are generic to imaging systems and agents. He also noted a concern specifically having to do with PET – GMP manufacturing has to occur on site, which must concern any diagnostic company.

Mauro Ferrari said that there is a tremendous amount of diversity coming from the innovation pathway that involves those who are developing nanosystems then working with investigators from other fields, including cancer. He added that fundamental science is key – interdisciplinary teams and need to establish biomarker history of disease. He also noted that risk-reduction is important, too, and that a toxicity appraisal and diagnostic screening service at NCI for imaging nanotechnologies could reduce risk and create standards for analysis that would benefit the field. Finally. Peter Choyke commented that intellectual property issues appear to be key. A tradeoff between highly targeted and non-targeted agents has to be considered, too. There is a great deal of

innovation in this field, taking experiences from other fields and bringing them into the imaging field. Multidisciplinary team building is key, and there are good existing models for accomplishing that kind of interdisciplinary effort. Finally, there are potential environmental issues associated with nanoparticles, and we need to look at this in advance, preemptively.

Anna Barker closed the meeting with her take-away impressions of what is important to the future development of this field. Her sense was that current business models for commercialization of imaging agents need improvement and wondered if the NCI might work with the FDA and Center for Medicare and Medicaid Services to develop such models. She also wondered if NCI's participation might reduce the risk of development, which would improve the business case for developing these agents. Along those lines, she suggested that NCI may be able to play a bigger role in studying the natural history of biomarkers and disease, in standardization, developing new chemistries, development of needed bioinformatics systems, and testing for toxicity, biodistribution and clearance of nanoparticles. NCI may also be able to better encourage interdisciplinary team building. She wasn't sure what role NCI could play in clarifying intellectual property issues and she noted concern that intellectual property could prove to be a decisive and divisive issue for this highly promising field. She closed the session by voicing her sense from this symposium that this field holds much promise and that NCI needs to consider how to best push this field along as quickly as possible.