Workshop on Characterization of Nanomaterials for Medical and Health Applications

National Cancer Institute Symposium
sponsored jointly by:
ASTM International
National Institute of Standards and Technology
U.S. Food and Drug Administration

Hilton Hotel
Reno, Nevada
May 19, 2005

INTRODUCTION

In September 2004, the National Cancer Institute (NCI) launched a new $144.3 million, five-year initiative – the NCI Alliance for Nanotechnology in Cancer – to develop and apply nanotechnology to cancer. In response to a need voiced by many in the scientific and clinical communities, the NCI also funded the creation of the Nanotechnology Characterization Laboratory (NCL), whose mission is to perform and standardize the preclinical characterization of nanomaterials developed by researchers from academia, government, and industry in support of the Alliance's goals. Already up and running, the NCL is serving as a national resource and knowledge base for cancer researchers, and facilitating the accelerated regulatory review and translation of nanomaterials and devices into the clinical realm.

To further NCL's mission, the NCI, together with ASTM International, the National Institute of Standards and Technology (NIST), and the U.S. Food and Drug Administration (FDA) co-sponsored a workshop to facilitate the development and use of nanomaterials for medical and health applications. This workshop, held in conjunction with the ASTM's semi-annual meeting of its standards setting committees, was organized to provide a forum for the development of standards for the characterization of nanomaterials and their behavior in biological systems. This standards development effort may also lay the groundwork for ascertaining and monitoring the environmental, health, and safety ramifications of nanotechnology for other applications.

The workshop provided a structured venue to address critical issues relevant to advancing nanotechnology into clinical applications. The primary focus of the workshop was on government programs currently in place to characterize nanomaterial for medical and health applications – especially the NCI's Nanotechnology Characterization Laboratory (NCL) and its collaboration with the U.S. FDA and NIST. The workshop addressed the need for consensus standards for physical, *in vitro* and *in vivo* assays to evaluate the efficacy, safety and toxicity of nanomaterial intended for medical applications, thereby enhancing the use and commercialization of nanotechnology in this field.

Workshop presentations and discussions focused on characterization of nanomaterials intended for use in the detection, diagnosis and treatment of cancer. A specific goal of the workshop was to elicit direct, detailed input from industry, academia and government agencies to identify areas in the nano-bio realm that would benefit from further standards development and to discuss potential solutions.

MORNING SESSION 1

The Workshop began with a brief welcoming address by **N. David Smith**, the 2005 ASTM International board chairman. He noted that ASTM is a standards-setting organization, not a professional society or an industry trade organization. He added that ASTM sees that cooperation among all stakeholders in efforts to develop standards is critical to the future of this developing field. ASTM's goal in this effort is to produce a global terminology standard to be used by all stakeholders worldwide. He closed by thanking the field for choosing ASTM as its venue of choice for these standards setting efforts.

Scott McNeil, Ph.D., director of the NCI's Nanotechnology Characterization Laboratory (NCL), then reviewed the four questions that Workshop speakers were asked to consider in developing their presentations. These questions, listed below, were developed by Dr. McNeil, Dr. Don Marlowe of the FDA, and ASTM staff.

1. Based on the history of the *in vitro* characterization of materials for drug applications, what characteristics are important for consideration of nanomaterials in this application? Are there any characteristics not previously important that are new for nanomaterials?

2. Given the literature available on characterization of nanomaterials for use as pharmaceuticals, what are the characteristics for which we should try to develop standard test methods for the *in vitro* characterization of nanomaterials for drug applications?

3. What infrastructure support do nanotech researchers need to better characterize their nanomaterial against these standard methods? (e.g., access to instrumentation, batch-to-batch analysis, experimental design, assay development/validation).

4. With respect to the above questions, how do we design the validation experiment for the methods we determine are important? What 3rd party entity will be acceptable/"recognized" to evaluate the validation experiment? Can we do the validation as an "ASTM E56 standards community"?

Pat Picariello, who oversees standards setting activities at ASTM as the organization's director of developmental operations, then outlined ASTM's activities in the nanotechnology field. He started his presentation with a brief overview of ASTM, whose primary objective, since its founding in 1898, has been "to be the foremost developer and

provider of consensus standards, related technical information, and services having globally recognized quality and market relevance." ASTM, in short, creates standards and does so by enabling industry and other interested parties to come together to work on a problem and create the relevant documents. These activities are carried out by 138 committees, staffed by volunteers, and have resulted in the development of over 12,000 standards. He added that the nanotechnology community has a rare opportunity to get involved in standards development as the technology is developing.

ASTM's nanotechnology efforts are carried out by ASTM committee E56, which was organized by industry in January 2005 after a wide range of companies involved in nanotechnology research and development made the appropriate request. Currently, this committee counts approximately 130 individuals and organizations as members. Mr. Picariello predicted that the membership of this committee will probably double by the end of the year.

ASTM committee E56 has six technical subcommittees:

1. Technology and Nomenclature
2. Characterization
3. Environmental and Occupational Health and Safety
4. International Law and Intellectual Property
5. Liaison and International Cooperation
6. Standards of Care/Product Stewardship

Mr. Picariello noted that more subcommittees may be created, while some may merge into others as time goes on. A hallmark of ASTM committees is a structure that is flexible and intended to evolve.

The scope of the E56 committee is twofold:

1. The development of standards and guidance for nanotechnology and nanomaterials, and
2. The coordination of existing STM standardization related to nanotechnology needs. This coordination shall include the apportioning of specific requests for nanotechnology standards through ASTM's existing committee base, as well as the maintenance of appropriate global liaison relationships with activities, both internal and external, related to this subject area.

The E56 committee, he noted, is charged with participating in the development of symposia, workshops, and other related activities to enhance the development of standards. He also listed the work items currently assigned to each of the subcommittees. For example, the characterization subcommittee (chaired by NCL staff member Martin Fritts) is currently in the process of identifying measurement techniques, developing standards for measurement methodology and metrology, and identifying reference and calibration standards, among its other work items. The nanotechnology nomenclature subcommittee has already developed a first draft of a nanotechnology terminology standard.

Going forward, Mr. Picariello expects the ASTM's activity in nanotechnology to evolve, particularly in terms of coordination with international efforts in the field, outreach, and

communications efforts. He closed by issuing an open invitation to attend the next E56 committee meeting, which will be held in Dallas, Texas, November 7-9, 2005.

In response to a question from the audience, Mr. Picariello also provided a brief explanation of ASTM's relationship with the American National Standards Institute (ANSI). ANSI is not a standards developer, as is ASTM. Rather, ANSI is an accreditor of standards organizations. In late 2004, ANSI created the nanotechnology steering panel (NSP), of which ASTM is a member. The NSP will function as a coordinating body to ensure there is no duplication of efforts or gaps in standards setting activities. ANSI also helps coordinate activities between ASTM and the International Organization for Standards (ISO), another standards developer based in Geneva, Switzerland.

The keynote speaker, **Gregory Downing**, **D.O., Ph.D.,** director of NCI's Office of Technology and Industrial Relations, presented the NCI's vision of nanotechnology as a disruptive technology with major potential to drive a new generation of cancer diagnostics and therapeutic products that address major market needs. He noted that the NCI also understands that realizing this potential requires systems-level changes and new product development approaches that will galvanize efforts to develop a new generation of nano-based products for cancer. He also stated that the NCI's efforts are actually being carried out in partnership with the FDA and NIST.

Aside from this innovative partnership, the NCI is realigning the way it does technology development in order to stress multidisciplinary team building, to build collaborations with the private sector into development projects, and to increase the transparency of opportunities available to the research and development communities. NCI is also establishing the infrastructure needed to catalyze activity in this field. Along those lines, the NCI has established caBIG for bioinformatics, the NCL for standards and characterization, and the Centers of Cancer Nanotechnology Excellence to integrate the physical and biological sciences and to speed the process to commercialization. In short, NCI wants input to bridge the gap from research to venture funding to product commercialization.

Why is this effort so important? Dr. Downing explained that cancer is now recognized as the major killer among people younger than 80 years old. With an aging population, cancer will trigger a major health care crisis unless significant progress is made soon in efforts to eliminate the suffering and death from cancer. Already, the health care costs attributable to cancer total some $189 billion annually.

Why is this the right time to look at nanotechnology as a disruptive technology? Dr. Downing cited five reasons.

1. The science of cancer is exploding, with major advances in genomics, proteomics, and computation and materials sciences generating a tidal wave of data on the molecular underpinnings of disease and an increased understanding of the mechanisms that trigger cancer.

2. The number of nanotechnology-based product candidates is expanding rapidly. Today, there are 61 nanotech-based drug and delivery systems and 91 devices or diagnostic tests in development.
3. The private sector is getting into the game, investing $1.7 billion in nanotechnology in 2004, which is about equivalent to Federal funding for nanotechnology. Over 88,000 patents were issued between 1976 and 2002, and 109 nanotech startups have secured venture funding since 1998.
4. Government investment has soared. Funding for the National Nanotechnology Initiative, for example, has grown from $46 million in 2001 to $1.08 billion in 2005.
5. State and regional commitment is high, with state and local governments investing over $864 million in nanotechnology research and development activity.

Dr. Downing then discussed the current view of cancer as a disease process. This process includes proliferation of cells, micro-invasion, immune invasion, cellular recruitment, dissemination, targeting, penetration, colonization and de-differentiation. He said that studying cancer in terms of the many processes that result in malignant disease requires a new set of tools that nanotechnology is uniquely capable of providing.

Seeing cancer as a disease process also affects the manner in which cancer detection and therapy are approached. Again, nanotechnology can enable the fundamental biomedical breakthroughs that are required to treat cancer as a disease. For example, novel multifunctional nanostructures are needed to detect and treat the very processes that have gone awry in malignancy. Nanotechnology-enabled devices may also yield the sensitivity needed to detect low-level cancer markers that have thus evaded discovery.

He then gave examples of the nanotech “toolbox” now available to researchers studying cancer and developing products to detect and treat cancer. Among these are cantilevers, carbon nanotubes, dendrimers, nanocrystals, nanoparticles, nanoshells, nanowires, and quantum dots. In the preclinical arena, nanotechnology can aid efforts to identify and validate new targets, as well as shorten the time needed to assess safety and efficacy in animal models and in human clinical trials. Products made from each of these “tools,” he said, are moving toward the clinic, but all could benefit from the development of standards and measurement technologies.

Dr. Downing then discussed how nanotechnology can help improve drug discovery efforts, which struggle to cope with the complexities of cancer. Nanoparticles, for example, can have multiple functionalities that can provide detailed information on numerous processes simultaneously. Similarly, nanoscale devices can deliver multiple therapeutic agents to a tumor in order to simultaneously attack multiple points in the pathways involved in cancer. Nanotechnology is also generating *in vivo* biosensors that have the capability of detecting and pinpointing the location of tumors and metastatic lesions that are far smaller than those detectable using conventional technologies. Detecting cancer before it spreads completely changes the game when it comes to treating cancer, since non-metastatic cancer is rarely fatal. Nano-enabled *in vivo* sensors may also provide rapid information on whether a given therapy is actually working as

expected, and targeted nano-based therapeutics that hone in on tumors stand to increase the efficacy of drugs while dramatically reducing potential side effects.

The NCI is trying to serve as a catalyst for turning the promise of nanotechnology into clinically useful products. Thanks to six years of funding nanotechnology applications in cancer, there are now at least three nanotechnology-based based products being readied for human clinical trials, with more in the wings. Moving forward, the NCI Alliance for Nanotechnology in Cancer will spend $144.3 million to ignite nanotechnology-enabled product development. The Alliance places heavy emphasis on team-building and on leveraging both private investment and NCI funding for its comprehensive cancer centers and Specialized Programs of Research Excellence (SPOREs). The Alliance also includes support for the NCL, which will pre-qualify new materials and provide standards for this developing field.

In the final talk of the first session, **James Baker Jr.**, **M.D.,** director of the newly endowed Michigan Nanotechnology Institute for Medicine and Biological Sciences at the University of Michigan, spoke about his group's work developing dendrimeric nanodevices. He began his presentation by addressing the question, "Why is nanotechnology so important?" The answer, he said, is that the scale of nanotechnology is the same as the scale at which biology works, and it is a scale that has been difficult to probe in molecular detail until recently. Science, he said, has done well developing tools that probe the micro world, down to the 100 nanometer range, and at the Angstrom level, so nanotechnology provides a powerful bridge for that 1 to 100 nanometer scale, which covers the realm of biology. The lipid membrane, for example, is about 5.5 nanometers across and the DNA-histone complex is 6.7 nanometers by 8.5 nanometers.

From a practical perspective, explained Dr. Baker, working at the nanoscale has the potential to allow therapeutic devices to surmount two important biological barriers: particles larger than 20 nanometers will not be able to escape the bloodstream, and particles larger than 150 nanometers will not be able to enter cells. A variety of nanoparticles, including fullerenes, gold nanoshells, iron oxide nanocrystals, starch nanoparticles, quantum dots, and dendrimers all fall into the sub-20 nanometer size range.

Since 1999, he then noted, the NCI has been funding efforts to develop nanoparticles that can target tumors and enter malignant cells, image a tumor, sense pathological changes in cells, select therapeutic agents based on those changes and deliver those agents into tumor cells, and then report on the efficacy of the chosen therapy. Though a huge challenge, Dr. Baker says that the promise of nanotechnology is that you can actually envision creating such a multifunctional nanoscale device. His team's approach to achieving this goal has been to use dendrimers, spherical, uniform polymers of size and solubility similar to that of proteins. Dendrimers are made in a layer-by-layer process that produces nanoparticles of defined size that can be readily seen under the microscope. The uniform nature of dendrimers is an advantage because uniformity leads to consistency and reproducibility when it comes to developing a desired nanoparticulate formulation, a key consideration for the FDA. Dr. Baker's group uses what is known as a G5 dendrimer,

which is about the same size as hemoglobin and which is just small enough to avoid being filtered by the kidney yet large enough to carry targeting agents, therapeutic drugs, imaging agents, and other molecules.

Dr. Baker then described the work his group has done using folate as a tumor targeting agent – a wide variety of tumor types over-express a high-affinity folate receptor on their cell surfaces. He recounted how the initial attempts at targeting tumors with folate were a failure because the dendrimers they were using folded in such as way that all the folate was buried internally and not accessible for receptor binding. Computer modeling studies identified this problem and provided a solution, which entailed modifying the dendrimer with acetamide groups. Subsequent experiments showed that folate targeting using this second generation dendrimer was successful and that folate-targeted dendrimers could saturate tumor cells within three minutes. More importantly, when the cytotoxic drug methotrexate was also attached to this dendrimer and the resulting multifunctional nanoparticle was administered to tumor-bearing mice, the effect was dramatic – one third of the animals survived to old age. In comparison, no mice treated with methotrexate alone survived.

Dr. Baker said that his group has now prepared 300 grams of GMP material and is hoping to file an IND for this dendrimeric formulation in early 2006. He also pointed out a lesson learned in making the GMP material – one batch, prepared by an inexperienced chemist, did not work in animal tests. This result, he said, points to the importance of having standards.

Additional work has added imaging capability to this dendrimer. The first step was to add fluorescein to the dendrimer, which enabled his group to image how much folate-labeled dendrimer was taken up by tumor cells. Next, his group added gadolinium chelates to the dendrimer surface, which allows tumors to be imaged using MRI. Such a formulation could enable clinicians to determine if a given patient's tumor was one that over-expressed folate, and thus, would be expected to respond to the dendrimeric therapeutic. Using DNA as the linker, Dr. Baker's group has also created a linking mechanism that can join monofunctional dendrimers to create a mix-and-match multifunctional device. This approach works, he said, but he worries that the regulatory issues associated with such an approach might be difficult to surmount. He then closed his talk by noting that he has licensed this research to a newly funded company, Avidian Therapeutics, which will handle the clinical development of folate-targeted dendrimers.

MORNING SESSION 2

Following a short break, **Eric Steel**, acting director of the program office at NIST, opened the workshop's second session with a review of the role that measurements and standards play in the development of bionanotechnology. He began by noting that NIST has a long history of looking at innovative technology areas and developing enabling measurement technologies to further their commercial development. In the area of nanotechnology, he said, the goal is to develop the science of measurement in the sub-100 nanometer region to better understand the three-dimensional elemental, chemical, structural, and physical properties of nanoscale materials intended for use in biological

systems. Developing these measurement technologies will allow the field to better understand and predict unforeseen consequences. Standards and measurement can also help alleviate fear and uncertainty among the public.

There are many challenges in developing new measurement technologies. There is the issue of determining if a given measurement is real, if the measurement can be distinguished from artifacts that depend on the instrument used to make the measurement, for example. The precision, accuracy and variability of a measurement are important considerations, because they determine uncertainty. There is also the question of what to measure – particle numbers versus mass measurements, for example. For polydisperse, multimodal specimens, a small fraction of particles with large mass may control the bulk characterization results, while in nanoscale analysis the results will tend to be weighted by particle number. Once characterization data is collected, there is the matter of how to portray what is measured. In the nano realm, depictions are more likely to be in the form of schematics rather than actual images.

Making measurements at the nanoscale, said Mr. Steel, raises the issue of what size actually means – is it the average diameter of all particles or the actual size of an individual particle. What does size mean? The size distribution, shape, surfaces, crystalline form and chirality of nanoscale materials comes into play, too, given that such materials are rarely perfectly defined and of symmetrical structure. Characterizing each of these facets of a nanomaterial is therefore a pressing challenge, as is the need to understand how the interactions among nanostructures can affect measurement and characterization across wide range of size scales.

Then, he said, there is biology, which means that there are an incredible number of interrelated variables to measure with hard to control parameters. Biological systems also are hard to model, and when measuring biological systems it is hard to run experiments to verify measurement techniques and it is difficult to gather sufficient population data. Biological systems are also easy to perturb from equilibrium.

Mr. Steel then reviewed the different types of standards that need to be developed, each of which must be measurement-based. Documentary standards include guides, methods, glossaries and specifications. Reference materials can include in-house standards, consensus standards and absolute standards. Calibration standards are needed to ensure that different instruments or methods yield the same answer. Quality assurance standards are also important to develop for they help us know if a given analysis is generating the correct answer. Standards also include reliable reference data that can be used to test new theories and models. Algorithms and models themselves can serve as standards.

The timeline for developing standards is well established, explained Mr. Steel. The process starts with research. This research leads to a draft proposal using in-house materials, inter-laboratory comparisons using consensus materials, guidelines development using reference materials, and finally creation of a standard method, which requires a certified reference material for calibration. Once a standard is applied to a

given material or field, there is a need for quality assurance materials that allow for accreditation and proficiency testing, which requires its own set of materials.

In closing, Mr. Steel discussed the relationship of measurements and standards to new technology. Measurements, he said, play a role in the growth and development of a technology spanning the period from scientific discovery to maturation of a technology. The nanotechnology field is still in the scientific discovery/new invention period, which affords the uncommon opportunity to engage in standards development in close coordination with technology development.

Don Marlowe, who oversees standards development at the FDA, spoke about the use of standards to enable product approval, an important consideration given that the goal of biomedical nanotechnology research is to develop products that help patients. He noted that the FDA has been using voluntary consensus standards to help guide the agency's regulatory decisions for over 30 years. In fact, various Federal laws and regulations have been developed to enable FDA to use voluntary consensus standards as often as possible in fulfilling the agency's mission. Today, over 160 standards developing organizations (such as ASTM) work with FDA to develop such standards, and FDA is one of the few Federal agencies to be a member of ANSI.

Two FDA offices play major roles in standards management at the agency. The Office of Science and Health Coordination, which is Mr. Marlowe's office, is responsible for coordinating consensus standards development and developing internal FDA procedures. The Office of International Programs is responsible for coordinating international activities, including standards participation, as they relate to the agency's international policy issues.

The FDA, explained Mr. Marlowe, even has a strategic standards vision that calls for the agency to develop and operate a unified technical standards system and that embodies a strategic standards manager to ensure greater internal coordination and communication. He then explained the rationale behind this vision:

1. It addresses elements of the FDA's Performance Plan because standards enable the FDA Critical Path Initiative by supporting the provision of clear standards, guidance, and predictive analytic tools.
2. It optimizes FDA's and a manufacturer's resources by reducing the overlap between standards and FDA regulatory development and by encouraging standards development organizations to avoid overlapping activities. This optimization, in turn, translates into a company and the FDA devoting fewer resources to participate in multiple standards setting venues.
3. It accomplishes the agency's international trade commitment by increasing FDA's recognition of globally recognized standards, the backbone of international trade agreements.
4. It strengthens cooperation among governments through FDA's participation in multinational regulatory harmonization forums. Standards also provide a common starting point on regulatory cooperation between governments.

5. It helps manufacturers of regulated products to reduce risk. Identifying and reducing risks to acceptable levels is the principle underlying good standards development. Standards development also strengthens FDA's partnership with industry and other stakeholders in efforts to identify risks and address their management. If necessary, FDA can adopt and use standards but still develop additional requirements through guidance to meet the agency's specific needs.
6. It enables productivity improvements by a regulated industry by basing FDA requirements on accepted global standards that promote health and facilitates commerce.
7. It improves the quality of FDA's operations.

The importance of the FDA's involvement in setting standards is that FDA regulates products that account for one dollar of every four generated by the U.S. economy. FDA bases its regulation on a product-by-product basis and does not regulate technology *per se*. Mr. Marlowe closed his talk by noting that the FDA anticipates that many nanoproducts will be "combination products," in which case the FDA will consider the primary intended mode of action. He added that the agency believes that nanoproducts will be important in every product area that the agency regulates, and that the existing battery of pharmacotoxicity tests is probably adequate for most nanotechnology products that the agency regulates.

The last speaker of the morning session was **Kenneth Watkin**, **Ph.D.,** founder and chief executive officer of Ultra-Imaging LLC, who spoke about nanoparticles and targeted delivery using ultrasound. His company has developed a small device, about the size of a human hand that can deliver ultrasonic pulses. This device may be useful for interacting with nanoparticles and getting them to open once they have reached their target.

Dr. Watkin's research on controlled-release nanoparticles began with an effort to identify lipids that could be used to make liposomes that were susceptible to rupture when subjected to ultrasound. The resulting liposomes can entrap drug molecules. Working with several corporate partners, Dr. Watkin and his colleagues assessed whether loaded liposomes, targeted to tumor cells, would adhere to tumor cells and then release their contents when irradiated with ultrasound. The results to date, he said, have been promising. This research has now moved to more advanced *in vitro* testing using a variety of tumor cells.

He ended his presentation by briefly discussing work using silica nanoparticles containing gadolinium. These particles could be useful in small animal imaging studies, he said, but how they would be used in humans is unclear.

In the following panel discussion, an audience member asked the morning presenters how to best interface with Federal agencies. Dr. Downing responded by acknowledging that this can be challenging when it comes to technology development. NCI, he said, is reaching out to the engineering communities using different funding mechanisms. Among these are contracts with deliverables, milestones and timelines. The NCI's

contract-based projects concentrate on building teams, and the Institute is working diligently to link technologists with clinicians and biologists.

Dr. Downing also explained that the NCI uses a two-step system in its technology development portfolio in which the first phase does not require substantial data, but is an experimental, proof-of-concept stage that can evolve into larger funding mechanisms that are also tied to SBIR grants. The NCI has a requirement, he told the audience, that a certain percentage of its funding must go to SBIR awards. The NCI even has special review panels for these types of proposals, as differentiated from applications for RO1 grants. The NCI also takes potential projects to many of the groups, institutions and cancer centers that the Institute believes might participate in these projects.

Mr. Steel then commented that NIST's research labs are open to guest researchers who can come in for days to weeks, or even years, to work on specific projects. All that is needed is sponsorship from a NIST researcher. He offered his help and his email address (eric.stell@nist.gov) to anyone who needs to find a NIST sponsoring researcher. He added that NIST also has a special grant program for developing innovative technologies, though there is no money left in that program for the rest of this fiscal year. NIST also has instrumentation and measurement groups, as well as consulting staff, to work with small businesses on technology-related projects.

Speaking for the FDA, Mr. Marlowe said that the agency wants researchers to come in for a talk as early as the concept development stage. Such a discussion can raise issues that the FDA is concerned about, which can lead to new research or clarification. There are designated contact people in each of the agency's Centers. He closed by stating firmly that anyone who is developing a product that will eventually need FDA approval should approach the agency long before submitting an IND application.

AFTERNOON SESSION 1

After lunch, three speakers discussed their nanotechnology-based efforts that are driving products toward human clinical trials. The first speaker was **Barrett Rabinow, Ph.D.,** senior director of BioPharma Solutions at Baxter Healthcare, who discussed his company's NANOEDGE technology for solubilizing water-insoluble drugs. This technology, he said, is not something that a drug developer would use unless absolutely needed. He presented a decision tree showing how to make this choice.

The NANOEDGE platform, broadly applicable as long as a drug is insoluble, achieves a high level of loading, between 10 and 20 percent. The platform is scalable, stable for at least two years, and has a tunable pharmacokinetic profile. To formulate a drug using NANOEDGE, an insoluble drug is first reconstituted with a solvent in the presence of a surfactant, yielding a precipitate that by itself is unusable. This initial suspension is passed through a homogenizer, producing a stable crystalline material. When the solvent is removed, the surfactant coats the surface of the freshly prepared crystals and prevents aggregation.

The resulting water-soluble formulation is then characterized using over two dozen tests. Particle coating, for example, is analyzed using transmission electron microscopy. Of particular interest, said Dr. Rabinow, is the distribution of particles at the high end of the particle size range, which is measured using laser diffraction. This method is fast, but not that accurate and not useful for measuring particle size accurately above 1 micron. However, this technique is useful for determining particle size distribution stability over time. Dr. Rabinow added that before undertaking a formulation study, it is useful to investigate what the pharmacokinetic profile would be. This estimate is done using *in silico* dissolution analysis, which provides good agreement between the *ab initio* results and measured results.

He noted that there are several different ways to characterize injectable nanosuspensions:

1. Rapid delivery: particles dissolve rapidly in the bloodstream, which is useful for moderately insoluble drugs and provides tissue distribution equivalent to that for solution formulations.
2. Sustained release: sustained delivery by depot mechanism, allowing for high dose administration in a low volume of injected material.
3. Extended Release: particles are captured by macrophages over 20 minutes and released from the macrophages before the drug can dissolve completely in the bloodstream, which is useful for highly insoluble drugs.

He then detailed how one particular formulation of the antifungal drug itraconazole was developed to maximize antifungal activity while minimizing toxicity by taking advantage of macrophage uptake and release. *In vitro* testing showed that macrophages did sequester the nanosuspension in a dose-dependent manner and that the macrophages remain capable of taking up bacteria even when heavily loaded with drug. Nanosuspension formulation also enabled dogs to safely receive 100 times more drug, which when administered produced steady release that lasts for days. Tests in immune compromised rats achieved total elimination of fungal cells, while treatment with itraconazole alone did not eliminate fungal cells completely. This formulation also successfully saved half of the animals infected with an itraconazole-resistant fungal strain while untreated animals died within three days and animals treated with itraconazole alone all died within 10 days.

Dr. Rabinow concluded his talk by stating that nanosuspensions provide a generally applicable approach to the preclinical testing of water-insoluble drugs. They enable high loading levels without interfering excipients and can modulate disposition of drug in the body and potentially improve the safety and efficacy profile of a drug. He noted, too, that current characterization methodology is applicable to nanosuspensions with appropriate modifications.

The next speaker was **Gregory Lanza, M.D., Ph.D.,** professor of medicine at Washington University School of Medicine and cofounder of Kereos, which is advancing much of the technology invented by Dr. Lanza and his colleague at Washington University, **Sam Wickline, M.D.** Dr. Lanza's presentation highlighted the potential of using ligand-targeted perfluorocarbon nanoparticles to enable personalized medicine.

This work, he said, was supported initially by the NCI's Unconventional Innovations Program, and its goal is to create nanoparticles capable of imaging and delivering drug to tumors.

Perfluorocarbon emulsion nanoparticles, he told the audience, can be decorated with a variety of agents. One such agent is the integrin $\alpha_v\beta_3$, an important biomarker for the newly developing vasculature that sprouts around tumors. The particles that Dr. Lanza and his colleagues use are relatively large, at 200 nanometers in diameter, and are thus constrained to the vasculature by design. The particles are not taken up by macrophages, either. Loading these particles with various imaging contrast agents allows for *in vivo* imaging of new vasculature using modalities such as ultrasound, SPECT, and MRI. In addition, the perfluorocarbon core can be imaged using ^{19}F NMR.

It is also possible to load these nanoparticles with drug, though unlike the case with many nanoparticles, drug is loaded on the surface of the perfluorocarbon emulsion nanoparticles. This allows for contact-facilitated drug delivery, in which the lipids and drugs in the particle's outer layer are passed over the contacted cell, delivering the drug in the process.

Preparation of the nanoemulsions particles looks simple, Dr. Lanza said, but it is actually complicated, involving some 32 steps to create a particle with a homing ligand and payload. Characterization assays are extremely important for determining the three-dimensional dispersal of ligands and payload over the surface of the nanoparticle. If the targeting ligands and payload are not dispersed properly, the nanoparticles are not functional. Assays are also critical for determining the final polydispersity of the particles as well as monitoring the presence of lipid degradation products, which can render a nanoparticle non-functional, too. In short, he said, the development of analytical methods was critical to the success of this project.

The final speaker of the session was **Mauro Ferrari, Ph.D.,** professor of internal medicine at the Ohio State University, special advisor on nanotechnology to the NCI, and cofounder of iMEDD, a company commercializing some of his work. Dr. Ferrari and his colleagues have been developing silicon-based nanotechnologies for cancer detection and therapy. In the diagnostics area, silicon-based nanotechnologies may find use in micro- and nanoarrays, micro- and nanofluidic devices, nanowire sensors, nanocantilever sensors, and in selective molecular enrichment substrates that may be able to collect and enrich rare biomarkers from blood. Silicon-based nanotechnologies may find use as therapeutics as part of biocompatible implants, transdermal delivery devices, microneedle arrays for injection, inhalation delivery devices, and in nanoparticulates delivery vehicles for both oral and intravascular administration. In general, said Dr. Ferrari, what is micro today in the silicon world will be nano tomorrow. He added that one of the advantages of silicon-based technologies, which originated in the semiconductor industry, is that mass production is rarely an issue.

As silicon-based nanotechnologies continue to develop, Dr. Ferrari foresees a day when a microfluidic device, comprising multiple nanoscale features, can store multiple drugs,

and based on input from on-chip sensors, deliver finely controlled doses of therapeutic agents in response to certain biochemical and genetic signals. Such a personal reconstitution and delivery system, he said, would be one of the technologies that will enable the era of personalized medicine. Already, truly implantable silicon nanosystems that Dr. Ferrari and his coworkers have developed can produce time-controlled, sustained release of drugs with zero-order kinetics, that is, drug release quickly reaches a steady state and remains constant over time. This device uses a nanopore membrane structure etched onto the surface of a silicon wafer to achieve zero-order kinetics. Other investigators, he explained, are developing nanoporous wafer implants for both drug delivery and brachytherapy (the use of radioactive pellets to treat solid tumors). Nanoporosity renders materials biodegradable with release kinetics that can be tailored to timescales of minutes to months.

Dr. Ferrari then reviewed the growing use of silicon-based nanoparticles as targeted drug delivery vehicles. For example, nanoshells, comprising a silica core within a gold shell, injected into the general circulation accumulate in tumors. When irradiated with near-infrared light, the nanoshells become hot enough to kill tumors in laboratory animals. Dr. Ferrari's group has developed flan-shaped particles that are decorated with a ligand that recognizes the endothelium of angiogenic blood vessels and that can deliver anti-angiogenic compounds directly to the targeted blood vessels.

Silicon-based nanodevices may also enable targeted oral delivery of drugs. Silicon-based particles coated with lectin will bind to the inner lining of the lower intestines. As the particle hydrates, it releases a permeation enhancer that disrupts the tight junctions that exist between cells of the intestinal lining. At the same time, drug is released, and because the tight junctions are disrupted, drug can cross the intestinal lining and enter the bloodstream. Because this enhanced permeation is extremely localized, this method of intestinal drug delivery should not produce systemic disruption to the intestinal mucosa and, thus, should not interfere with its protective role. Such a delivery route could serve as a model for crossing other biological barriers.

Multifunctionality, said Dr. Ferrari, is the defining advantage of nanovector delivery. Silicon-based nanotechnologies, as well as the other materials discussed by other speakers, can provide preferential, effective concentrations of therapeutic agents and imaging enhancers at lesion sites by combining multimodal targeting, an ability to surmount biological barriers, and the ability to provide co-localized combination therapy. However, there are numerous issues that remain to be addressed before such multifunctional nanoscale devices impact clinical medicine.

The central issue, he said, is that it is still unclear what the regulatory pathway will be for successfully developing multifunctional nanoscale delivery vehicles. To help address the uncertain regulatory pathway, Dr. Ferrari said that there is an immediate need for a master file approach to nanovectored therapeutics and for clarity on whether such vectors will be treated as devices, drugs or biological agents. The FDA, he suggested, should develop a guidance document and mechanisms for communications between the agency and potential applicants. He also noted that the lack of clear guidance impedes the field

by inducing skepticism within the private sector that anyone will be able to successfully market a multifunctional nanoscale device. In addition, the lack of guidance encourages a "low-hanging fruit" approach that is not in the public's best interest and it diminishes enthusiasm for nanotechnology-based projects among funding agencies.

Other important issues facing the field include standardization – how do we compare one type of particle to another? The NCL's primary mission, he added, is to focus squarely on this issue. Another concern rests on the issue of combinatorial approaches to drug development along the lines that Dr. Baker raised in his talk. Preparing various subunits for targeting, drug delivery, imaging, and efficacy reporting would be an efficient path to personalized medicine, but again, the regulatory methods for approving such constructs may be too difficult to overcome. Dr. Ferrari also expressed concern that there is not enough work being done to integrate mathematical modeling into the field. Such models could serve to guide nanoparticle design and selection for a given task and help with manufacturing decisions. Finally, there remains a great deal of work to be done characterizing the toxicology of nanoscale materials. Again, this is an issue that the NCL is addressing.

AFTERNOON SESSION 2

The final workshop session was devoted to describing the NCL's efforts in standardization and characterization, featuring three presentations by NCL staff scientists. Dr. McNeil led off the talks by quickly addressing the question, "Why nano?" As Dr. Rabinow showed, the ability to modify the surface chemistries of nanoscale materials offers tremendous opportunities to address solubility issues, a key consideration in drug formulation. The multifunctional capabilities of nanomaterials and their ability to target tumors and other tissues, either actively or passively, and deliver drugs with reduced toxicity are also promising attributes.

Dr. McNeil noted that the NCI is not a newcomer to the nanotechnology field, having funded exploratory work over the past six years on integrating nanotechnology into biomedical research, largely through the Unconventional Innovations Program. The NCI's priority now is to move those technologies into the clinical realm and to foster adoption of nanotechnologies throughout the cancer research enterprise where appropriate. The primary vehicle for catalyzing this transition from basic research to widespread use is the Alliance for Nanotechnology in Cancer, which is run by the NCI's Office of Technology and Industrial Relations, directed by Dr. Downing. The extramural budget for this initiative is $144 million over five years.

The NCL is intended to have a core mission in this initiative through its efforts to address the consensus opinion among cancer research that there are significant obstacles that must be overcome in order for nanotechnology to successfully advance to the clinical realm. The three primary obstacles that the field has identified are:

1. A lack of available standard for comparison among nanoscale materials;
2. A need for first principles based on physical characteristics; and
3. Regulatory uncertainty.

Indeed, the mission statement of the NCL calls for the lab to perform and standardize the preclinical characterization of nanomaterials developed by researchers from academia, government and industry, to serve as a national resource and knowledge base for cancer researchers, and to facilitate the regulatory review and translation of nanomaterials and devices into the clinical realm. To fulfill this mission statement, Dr. McNeil explained, the NCL's objectives include:

1. Identifying and characterizing critical parameters related to the biocompatibility and structure-activity relationships of biomaterials;
2. Establishing and standardizing an assay cascade for nanomaterial characterization that includes physical characterization as well as *in vitro* and *in vivo* characterization of toxicity, pharmacokinetics and pharmacodynamics;
3. Examining the biological characteristics of multi-component and combinatorial platforms;
4. Engaging and facilitating academic- and industrial-based education and knowledge-sharing;
5. Providing critical infrastructure support for the Alliance;
6. Performing preclinical characterization of nanomaterials intended for cancer therapeutics and diagnostics in support of IND submissions to the FDA; and
7. Serving as a nexus for collaboration among the NCI, NIST and FDA.

The NCL, noted Dr. McNeil, is a free national resource available to anyone producing nanomaterials with applications in clinical oncology.

Anil Patri, Ph.D., senior scientist at the NCL, then provided some details on the NCL's approach to developing a physiochemical characterization and standardization of nanoparticles. He noted that this effort is not designed to standardize nanoparticles, but the methods that are used to characterize nanoparticles and thus create standards against which the properties or behaviors of any nanoparticle can be compared.

The core properties that define the physiochemical properties of any material, whether it is a small molecule or a nanomaterial, are its chemical composition, physical properties, chemical properties, identification, quality, purity and stability. Numerous methods are available for determining these properties for molecules and particles up to about 5 nanometers in diameter. For particles larger than 5 nanometers, the corresponding analysis requires a new set of tools. Dr. Patri discussed some of the different types of instrumentation that will be used to characterize different properties of nanoparticles. For example, light scattering and various forms of electron microscopy will be used to characterize the size, size distribution and topology of nanoparticles. Much of the physical characterization of nanoparticles will be done in collaboration with NIST.

It will be important, said Dr. Patri, that the NCL will be developing multiple analytical techniques for assessing nanoparticle characteristics, because often there is more than one way to characterize a given property, each of which provides unique information. Gold nanoparticles, for example, are spherical, but not after they are functionalized. As a result, a measurement technique that assumes a particle is spherical would not be useful for studying functionalized gold nanoparticles. Similarly, under the microscope dendrimers can look very uniform in size, but mass measurements will reveal a broader

distribution of sizes. Thus, different characterization methods are needed to look at the same property in order to confirm results. He finished his presentation by noting that once the NCL has compiled a database of experimental results, he and his colleagues will attempt to define simple methods that are more widely affordable across the research community that nonetheless provide data that correlate well with the more detailed and expensive methodology that is required to develop standards.

In the final presentation of the day, **Stephan Stern, Ph.D.,** staff scientist at NCL, reviewed the NCL's preclinical pharmacology and toxicology program. The objective of this program is to develop a standardized assay cascade for nanoparticle characterization. This cascade will conform to available ASTM and ISO standards and FDA guidance.

While the immediate goal of this program is to characterize nanoparticles, a far-reaching goal is to use the data generated to start making predictions about structure-activity relationships that account for such properties as surface hydrophobicity, surface charge, surface reactivity, size, and the tendency to aggregate or disaggregate. Another goal is to identify mechanisms by which toxicity results and correlate various toxicities to nanoparticle characteristics. For example, certain properties of a particular type of nanoparticle may trigger oxidative stress or apoptosis, but this association may be more general so that all types of nanoparticles with a given physical property may trigger the same pathways that lead to toxicity.

Dr. Stern then went through many of the individual assays that will be part of the assay cascade. He noted that rats will be the preferred rodent species for study, as it is for most *in vivo* toxicology studies. *In vivo* pharmacokinetics panels in the assay cascade will include single-dose and repeat-dose pharmacokinetic and tissue distribution assays, clinical therapeutic cycle assays, and quantitation assays. Toxicity assessments, based on the NCI's developmental therapeutics program toxicology protocols, will involve determining single and repeat-dose assays of both acute and subacute toxicity, as well as a thorough examination of hematology, clinical chemistry, and histopathology parameters. Dr. Stern added that the NCL will also examine the metabolism of nanoparticles as part of its studies. Data applicable to environmental risk assessment will also be included as part of the panel of general cytotoxicity assays, for example. In closing, Dr. Stern noted that the data generated by the NCL's assay cascade will be usable in IND applications to the FDA.

In closing, Dr. McNeil reiterated that the NCL is open to receiving materials from any laboratory. He suggested that anyone interested in submitting materials should send in a 3-4 page white paper summarizing whatever is known about the material of interest, including any preliminary characterization data. If NCL staff believes the material is suitable for study, the NCL will request a more complete proposal. Applications are accepted every quarter – the next deadline is September 1, 2005. The NCL will protect intellectual property associated with all materials it receives, but data generated at NCL will eventually be added to a public database. The intent is to advance materials to clinical application and to aid the field at large in efforts to better understand the *in vivo*

activity of nanoparticles. Dr. McNeil added that he expects the entire assay cascade to take 18 months to complete.

Mr. Marlowe of the FDA added a final comment. He noted that one of the most useful things that the NCL will do is to create a knowledge base that can benefit the entire field of biomedical nanotechnology. He also commended the NCL for its commitment to the concept of standardizing the process of nanomaterial characterization.