NCL News

Nanotechnology Characterization Laboratory

Accelerating the transition of concepts to clinical applications

Volume 1, Issue 1

About the NCL

Working in concert with the National Institute of Standards and Technology (NIST) and the U.S. Food and Drug Administration (FDA), the National Cancer Institute (NCI) established the Nanotechnology Characterization Laboratory (NCL) to perform and standardize preclinical efficacy and toxicity testing of nanoparticles intended for cancer therapeutics and diagnostics. The NCL is a national resource and knowledge base for cancer researchers from academia, government and industry, facilitating the development and translation of nanoscale particles and devices for clinical applications.

The NCL's activities are expected to markedly speed the development of nanotechnology-based products for cancer patients, reduce the risk of doing so, and encourage private-sector investment in this promising area of technology development. The NCL will provide a comprehensive set of characterization parameters for nanomaterials and lay a scientific foundation enabling the FDA to make sound decisions concerning the testing and approval of nanoscale cancer diagnostics, imaging agents, and therapeutics. ■

Moving Nanotechnology Concepts to the Clinic

The mission of the National Cancer Institute's Nanotechnology Characterization Laboratory (NCL) is simple: to accelerate the transition of nanotechnology-based research into clinical applications for cancer. For many nanotechnology developers, the prospect of preparing, characterizing and submitting these products for regulatory approval can be daunting. Unlike the development of small molecule drugs or protein-based

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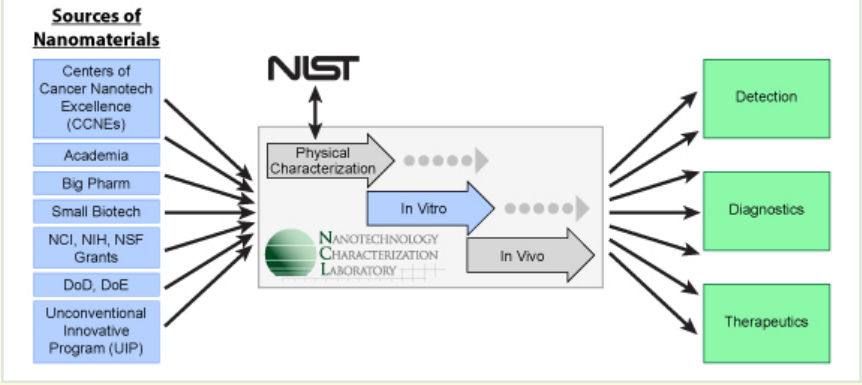

NCL conducts pre-clinical characterization in support of an IND submission to the FDA

Welcome to the inaugural issue of *NCL News*, the newsletter of the Nanotechnology Characterization Laboratory.

In each issue, you will find information on:

- Services provided by the NCL
- Processes for submitting your nanomaterials for evaluation by the NCL
- Assays developed by the NCL for evaluation of nanomaterials
- Upcoming nano-related conferences and publications

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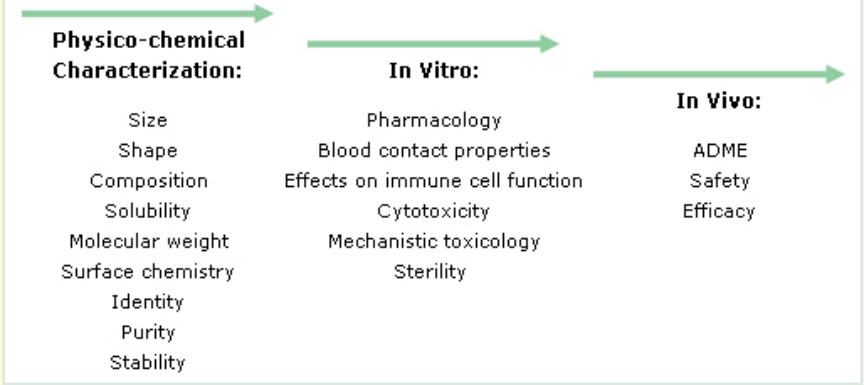
drugs, the protocols for characterizing new nanotechnology-based products for safety and efficacy are not well established. The NCL is a national resource that provides a smoother path to clinical trials by offering characterization of cancer diagnostics, imaging agents and therapeutics based on standard protocols of preclinical toxicology, pharmacology, and efficacy developed especially for nanoscale concepts and strategies.

be addressed in IND submissions of nanomaterials. Submission of a nanotechnology-based product to the FDA that has not been fully characterized increases the risk that the IND will be rejected.

The NCL has developed and continues to evolve a set of characterization protocols (see figure below), from physicochemical studies to animal studies of efficacy and safety, which can satisfy most of FDA's



NCL assay cascade

Benefits to nanotechnology developers

Before a company can advance a biomedical nanotechnology product into clinical trials, it must submit either an investigational new drug (IND) application or investigational device exemption (IDE) to FDA. There is a standard package of data required for IND or IDE submission that includes various physicochemical characterizations as well as *in vitro* and animal studies that provide strong evidence for efficacy and safety. Without a methodical approach to collection, however, characterization data submitted to the FDA for a 'multifunctional' nanoparticle could be "exceptionally ambiguous…" in the words of Scott McNeil, Ph.D., Director of the NCL. "It would mean nothing."

Product sponsors today may not be aware of the important issues that should

current requirements. "At the data level, we have a very rigorous assay cascade that generates characterization data in support of the IND. We go above and beyond what is required" as outlined in FDA guidance documents, explains McNeil. Such rigor in assay development is critical, considering that nanomaterials can often interfere with conventional assays.

McNeil reminds us, however, "…there is no requirement to go through the NCL. We are a free resource available to developers of nanotechnology, specifically for cancer applications." The NCL does not see itself as the only resource, however, when it comes to conducting thorough characterization studies of nanomaterials. In fact, the laboratory would be elated to see other groups make use of the protocols it develops to conduct their own characterizations, from academic labs to contract research organizations to the internal R&D operations of a pharmaceutical company.

Submitting material for characterization

Once a cancer therapeutic, diagnostic or image contrast agent using nanotechnology is formulated and initial biological *in vitro* or *in vivo* studies indicate efficacy, the product sponsor should contact the NCL. The conversation with NCL should begin early in development in order to clarify whether the project can be submitted to the NCL for characterization. The first formal step in an NCL application is to submit a 3-4 page "white paper" describing the product concept and accompanied by initial characterization and efficacy data. Within 45 days after submitting the white paper, the NCL will inform the sponsor as to whether it will advance the application to the next stage – a phase II proposal.

The phase II proposal is an ~10 page document that expands upon the concepts presented in the white paper and includes additional criteria for evaluation, such as assessment of the inherent toxicity of the nanomaterial, and its amenability to scale-up. For the NCL to characterize material, particularly in animal studies, at least a half a gram of material is needed. Gram amounts of nanomaterial are not always easy to obtain, but the NCL can offer assistance, depending on resources available. Once the phase II proposal is submitted the NCL will review the new information within approximately 45 days. Once a proposal is formally accepted by the NCL, timelines, milestones, delivery dates, and intellectual property issues are discussed in close collaboration with the submitter.

A second way to submit materials to the NCL is through a structure activity relationship (SAR) study. This type of application option enables submission of material for collaborative research to examine how the

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particles' physical and chemical attributes influence their biological behavior.

The current acceptance rate for white papers is over 50%. Applications are accepted by the NCL every quarter. The next application deadline is December 1, 2006. For more information on the application process, please visit http://ncl.cancer.gov/working_application-process.asp

Keeping the goal in sight

The NCL not only provides characterization service to bio-nanotechnology researchers. Part of the NCL's mission is to use submitted material as an opportunity to develop and refine protocols for nanotechnology characterization and then disseminate those protocols to the entire research community. NCL's data and protocols will also help inform the FDA on parameters that may influence regulatory review.

With broad use of standards and characterization protocols, the goal of speeding the development of new cancer diagnostics and treatments will be that much closer. "The NCI saw that there were 'leap-ahead capabilities' from nanotechnology that were not just incremental improvements," said McNeil. Investment in helping nanotechnology "mature" as a biomedical technology will bring to light many more applications that will help make cancer a more easily detectable and manageable disease. ■

FAQs

Q. What does it cost to have nanomaterial characterized by the NCL?

A. If a nanotechnology strategy/material is selected for characterization, NCL's services are provided at no cost to the submitting investigator. As part of its assay cascade, the NCL will characterize the nanomaterial's physical attributes, its *in vitro* biological properties, and its *in vivo* compatibility using animal models. The time required to characterize nanomaterial from receipt through the *in vivo* phase is expected to be one year.

For more information on the NCL assay cascade, visit http://ncl.cancer.gov/working_assay-cascade.asp

Q. I'm studying nanomaterials but my application is not in the area of cancer. Can I still submit a proposal for characterization by the NCL?

A. The NCL serves as a national resource and knowledge base for researchers to facilitate the regulatory review of nanotechnologies intended for cancer therapies and diagnostics. The NCL generally does not accept proposals for characterization of nanomaterials intended for application in areas other than cancer. Cancer-related nanostrategies proposed to the NCL for characterization are ranked according to their projected impact on clinical cancer applications and/or furthering nanotechnology's compatibility with biological systems. Specific evaluation criteria include, but are not limited to:

- Previously demonstrated efficacy *in vitro* and/or in animal models
- Advantages offered by the strategy over existing cancer therapies or diagnostics
- Previous physical characterization of the nanomaterial such as purity and stability
- The nanostrategy's manufacturing process and compatibility with scale-up
- The material's inherent toxicity and/or environmental concerns
- Plans or approach to transition the strategy to clinical trials such as filing the follow-on IND, IDE or pre-IDE

For detailed information on submitting a proposal for a cancer-related project, please visit http://ncl.cancer.gov/working_application-process.asp

Q. Does the NCL provide funding?

A. No. The NCL is a resource enabling researchers in academia, industry, and government to transition their nanotechnology strategies to clinical applications. The NCL provides critical infrastructure and characterization services but does not fund research grants.

Q. If my proposal is accepted, how much material will I be required to submit?

A. The NCL requires ½ to 1 gram of your nanomaterial in order to complete all assays. Depending on the availability of resources during the application process, the NCL may be able to assist researchers with scale-up in order to have enough nanomaterial to enter the assay cascade.

To share and safeguard research material and proprietary information, the NCL's interaction with researchers is normally conducted under a Material Transfer Agreement (MTA), permitting the collaborative exchange of materials and associated information.

For more information on Material Transfer Agreements and intellectual property protection, please visit http://ncl.cancer.gov/working_intellectual-property.asp. ■

NCL Protocols

This regular feature will describe the process of protocol development at the NCL and highlight specific protocols and how they have been adapted for use in the presence of nanomaterials.

Nanomaterials characterized by the NCL are intended for in vivo diagnostic and therapeutic use. Before nanoparticles can be used in patients, however, they must first be evaluated for safety and efficacy by the FDA. To this end, the NCL has developed and performs a standardized analytical cascade that tests the preclinical toxicology, pharmacology, and efficacy of nanoparticles and devices. In order to foster the study and evaluation of nanomaterials for clinical applications throughout the research community, assay protocols developed and validated by the NCL are freely available via the NCL website (http://ncl.cancer.gov).

Establishment of Protocols

An extensive set of protocols for the characterization of nanoparticles is being developed and validated by the NCL. It is expected that these protocols will become "best practices" by which nanomaterials are evaluated for clinical applications. The figure below shows the process by which these protocols are established and made available to the research community through collaboration with a number of agencies.

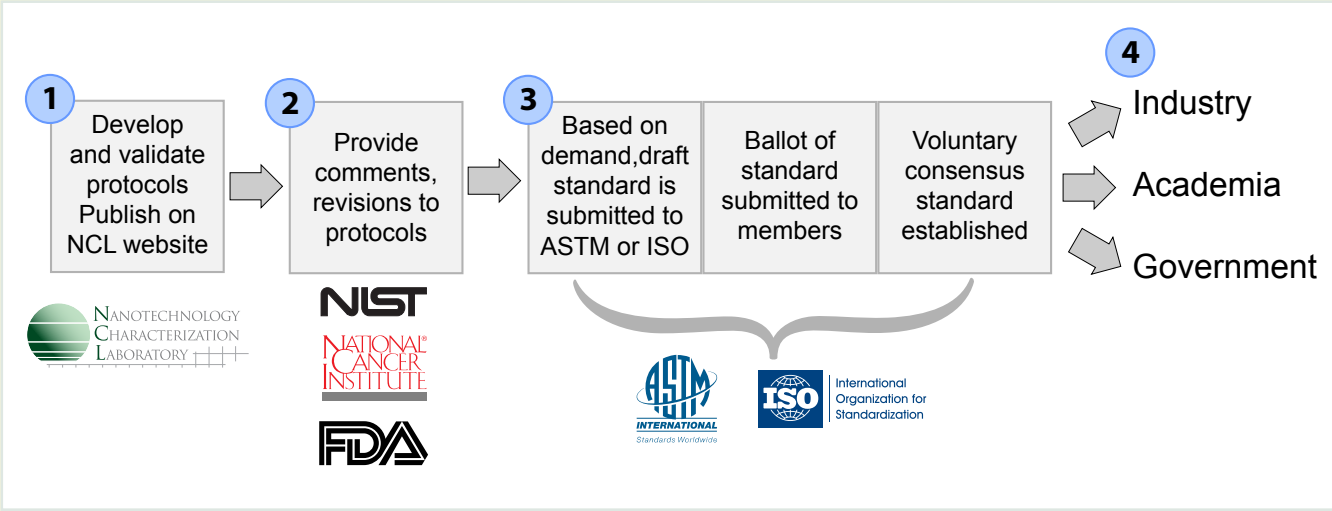
In step one, NCL develops and validates protocols with the intent to leverage existing methods and/or standards when possible. Method validation includes experiments aimed at evaluation of parameters critical for reproducible and reliable results, such as inter- and intra-assay variability, establishment of positive and negative controls, testing for potential nanoparticles' interference, number of allowable freeze/thaw cycles, and reagents' storage instability under given experimental conditions. Another aspect of the validation process is establishment of acceptance criteria; i.e. test results for a given nanomaterial are considered reliable only if certain criteria reflecting assay performance are met.

Once protocols have been validated by NCL scientists, they are made available to the public via the NCL website. Protocols validated by scientists at NCL are then submitted to NIST, NCI, FDA and other collaborators for comment and revision (step two).

Based on demand from the research community, the NCL will submit certain protocols to ASTM (originally known as the American Society for Testing and Materials) or the International Standards Organization (ISO; step three) for development of voluntary consensus standards. Founded in 1898 and 1919 respectively, ASTM International and ISO are not-for-profit organizations providing a global forum for the development and publication of voluntary consensus standards.

At the ASTM, a "draft of standard" is submitted to the committee on nanotechnology (E56) for review. The ASTM nanotechnology committee was formed in 2004 and addresses issues related to standards and guidance materials for nanotechnology and nanomaterials. Once comments and revisions on the draft of standard are received and addressed, a ballot of standard is submitted to ASTM members for consensus.

Once established, Voluntary Consensus Standards from ASTM and ISO are made available to industry, academia and government groups. Both ASTM and ISO standards can then be referred to by regulatory entities such as the FDA.



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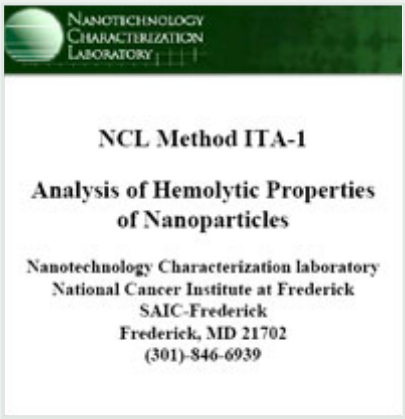
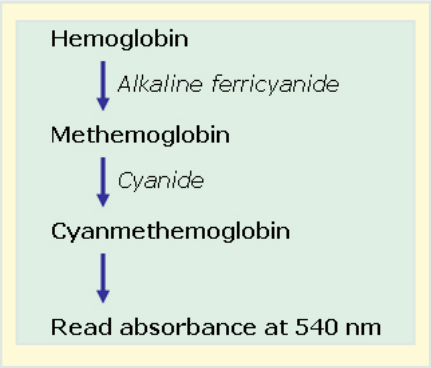


Analysis of Hemolytic Properties

Nanoparticles used for therapeutic or diagnostic purposes should not have toxic effects on blood elements when injected into the patient. Hemolysis (damage to red blood cells resulting in the release of hemoglobin) can lead to life-threatening anemia, hypertension, arrhythmia, and renal failure. To assess the effect of nanoparticles on blood elements, NCL has developed an *in vitro* protocol as a first order approximation of their hemolytic properties.

The hemolytic properties of nanoparticles are assessed using quantitative colorimetric determination of hemoglobin in whole blood and hemoglobin released into plasma when blood is exposed to nanoparticles. Hemoglobin and its derivatives (except sulfhemoglobin) are oxidized to methemoglobin by alkaline ferricyanide. Cyanmethemoglobin is then formed from the methemoglobin by its reaction with cyanide.

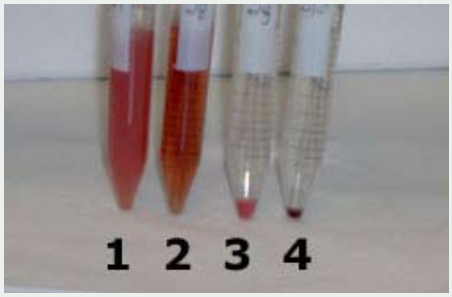


The cyanmethemoglobin can then be detected by a spectrophotometer set at 540 nm. Assay results are expressed as percent hemolysis and used to evaluate the acute, *in vitro* hemolytic properties of nanoparticles.

Special Adaptations for Nanoparticles

A number of adaptations have been incorporated into the hemolysis protocol to address and screen for possible interference resulting from the presence of nanoparticles.

- Specific controls are used to address interference. As a general precaution, a sample is run with all reagents and nanoparticles but without blood. These "minus blood" controls are used to evaluate potential interference of nanomaterial within the assay such as absorbance at or close to 540 nm or reactivity with reagents.
- If nanoparticles have absorbance at or close to 540 nm, removal of the particles from supernatant will be required during the assay. 10-50 nm colloidal gold nanoparticles, for example, have absorbance at 535 nm. The method used for nanoparticle removal from supernatant is nanoparticle-specific and when applied, appropriate validation experiments should be conducted to insure the removal procedure does not affect assay performance.
- In some cases, removal of nanoparticles by centrifugation is not feasible. In this situation, assay results obtained for particles incubated with blood are adjusted by subtracting the result obtained for the same particle in "minus blood" controls.
- When colored nanoparticles are part of the assay, precautions must be taken because they will absorb light. In addition, some nanoparticles may themselves oxidize hemoglobin thus interfering with the assay.
- The figure below demonstrates the importance of recording the appearance of a sample following centrifugation to avoid false negative results. Samples in tube 1 (20 nm polystyrene nanoparticles) and tube 2 (50 nm polystyrene nanoparticles) show hemolytic activity as demonstrated by the color of the supernatant. 80 nm polystyrene nanoparticles (tube 3) were also hemolytic; however, the 80 nm nanoparticles absorbed hemoglobin that can be confirmed by the pellet size and color. When read by the spectrophotometer at 540 nm, the supernatant will demonstrate negative results. Tube 4 is the negative control – no hemolytic activity was observed in the supernatant. Intact red blood cells formed a tight, dark red pellet.



Find the entire protocol for analysis of hemolytic properties of nanoparticles (NCL Method ITA-1) at http://ncl.cancer.gov/NCL_Method_ITA-1.pdf. ■

Important Dates

December 1, 2006 - Next due date for submission of proposals to the NCL.

NCL Connections

In order to develop and establish standards for characterization of nanomaterials, the NCL works closely with a number of agencies including the National Cancer Institute (NCI), the U.S. Food and Drug Administration (FDA) and the National Institute of Standards and Technology (NIST). In each issue of NCL News, the "Connections" feature will describe the interface of NCL with another agency and highlight how scientists are bringing together the critical knowledge, experience and skills necessary to facilitate the characterization and development of nanotechnology for clinical applications.

NCL and NIST

The NCI's Nanotechnology Characterization Laboratory is drawing extensively from expertise at the National Institute of Standards and Technology (NIST) as it develops new assays and protocols for characterization of nanomaterials. NIST has over 100 years of history in setting national standards in physical and chemical measurement, but recently life sciences have become a very important focus for the agency. The ability to measure biological elements and response precisely is extremely important for clinical applications, where critical medical decisions rely on accurate, calibrated, reproducible measurements.

Such a mission has historical roots that go back many decades at NIST. In its earliest days, as the United States was just becoming an industrial world power, thousands of train derailments were caused by broken rails, wheels, flanges, and axles. Steel materials and components were inconsistent in quality and the industry had not established uniform practices in manufacturing. NIST (known at the time as the National Bureau of Standards) analyzed the failed parts using chemical, microscopic, and mechanical tests and investigated manufacturing processes. By 1930, with NIST's help in standardizing materials and processing, better steel went into rails and trains resulting in dramatic improvements in safety and reliability.

Today, a variety of manufacturing processes and protocols is creating similar challenges to ensuring the reliability and consistency of nanotechnology products. The partnership between NCL and NIST seeks to resolve many of those issues.

The interaction between NCL and NIST occurs at multiple levels. At the scientific level, NIST assists the NCL in conducting physicochemical characterization of nanomaterials. For example, NIST helps with x-ray diffraction studies, inductive coupled plasma optical emission spectroscopy studies or other types of measurement that might require specialized equipment or expertise. These capabilities complement those established in-house at the NCL. NIST scientists also work together with NCL and the FDA to determine the best measurement tools and protocols that may inform the regulatory process. At the programmatic level, NIST is actively engaged in program planning and execution for the NCL and associated workshops and meetings.

NIST has a seat, along with the FDA, on the NCL scientific oversight committee, which conducts technical reviews. Scott McNeil, Director of the NCL, describes these reviews as the time when "we roll up our sleeves and talk about scientific issues. We discuss data and what it means for the standards community, and how it may be important to the regulatory community." Interactions between the agencies also take place on a more informal level, with conversations frequently occurring directly between NCL, NIST and FDA scientists.

We have come a long way since the industrial age and the standardization of the rail industry, but the body of knowledge and expertise at NIST will help put today's revolutionary technology – nanotechnology – on a faster track to addressing the challenge of cancer. ■

Upcoming Conferences and Publications

Conferences in which NCL staff will participate:

Imaging as a Biomarker: Standards for Change Measurements in Therapy
Location: NIST, Gaithersburg, MD
Dates: September 14-15, 2006
Website: http://usms.nist.gov/workshops/bioimaging.htm

Samsung International Symposium of Molecular Medicine (SISMM)
Location: Seoul, South Korea
Dates: September 21-24, 2006
Website: http://sismm.sbri.or.kr/about_welcome.htm

Cambridge Healthtech's Targeted Nanodelivery
Location: Sheraton Inner Harbor, Baltimore, MD
Dates: October 12-13, 2006
Website: http://www.healthtech.com/2006/nno/index.asp

14th North American ISSX Meeting
Location: Westin Rio Mar, Puerto Rico
Dates: October 22-26, 2006
Website: http://www.issx.org/

Principal Investigators of NCI Nanotechnology Alliance
Location: San Diego, CA
Dates: October 25-26, 2006
Website: http://nano.cancer.gov

ASTM F04 and E56 Workshop Nanotechnology and Medical and Surgical Devices and Materials
Location: Hyatt Regency, Atlanta, GA
Date: November 14, 2006, 1:00 – 5:00 PM
Website: www.astm.org

Modern Drug Discovery and Development Summit
Location: Marriott Downtown, Philadelphia, PA
Dates: December 4-6, 2006
Website: http://www.gtcbio.com/confpage.asp?cid=8

International Conference on Nanotechnology Occupational and Environmental Health and Safety: Research to Practice
Location: Duke Energy Center, Cincinnati, OH
Dates: December 4-7, 2006
Website: https://www.uc.edu/noehs/index.html

Publications:

Preclinical characterization of engineered nanoparticles intended for cancer therapeutics. To be published in "Nanotechnology for Cancer Therapy", Taylor & Francis Group, LLC. December 2006