NCL
Nanotechnology
Characterization
Laboratory

Annual Report 2023

Nanotechnology Characterization Laboratory
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CONTENTS

NCL'S ASSAY CASCADE— APPLICATION TRENDS

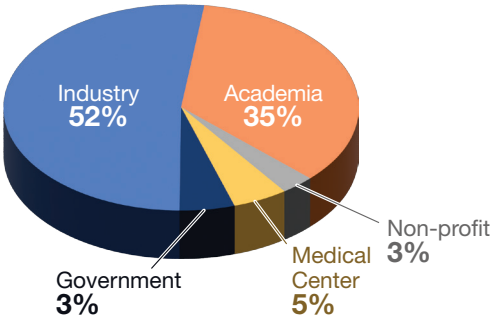
The NCL's Assay Cascade is a free characterization program providing physicochemical characterization and in vitro and in vivo immunology, pharmacology, and toxicology analyses to developers of novel cancer-based nanotechnology formulations. Funded through the NCI's Nanodelivery Systems and Devices Branch, the NCL's characterization service is available through a competitive application process.

The program accepts applications for a variety of applications, including chemotherapy, immunotherapy, vaccines, imaging agents, radiation enhancers, combination therapies, and more. In addition, the program also accepts concepts that are aimed at reducing any of the multitude of side-effects cancer patients experience as a result of their radiation, chemotherapy, or immunotherapy treatments, e.g., palmar plantar erythrodysesthesia (PPE), thrombocytopenia, neutropenia, etc.

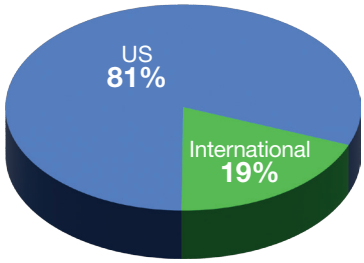
The program is open to all researchers, including academicians, government researchers, and pharma and biotech organizations. There is also no requirement for pre-existing NCI funding for your concept or US residency.

Though quite competitive, the program has a relatively high acceptance rate at nearly 50%. For those applicants not initially accepted, the review committee provides a concise summary of data they recommend to improve the application's chance of success. In fact, many applicants have successfully re-applied to the program after addressing the committee's questions and concerns. Regardless of application status, all applicants are offered, at a minimum, free consultation from the multidisciplinary experts of the NCL team on appropriate characterization strategies, in vivo study design, and more.

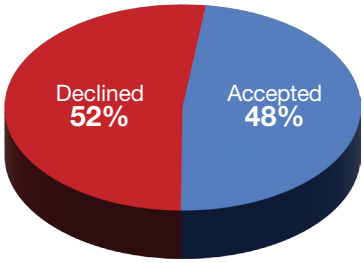
Applicants' Organizations



Global Interest



Overall Acceptance



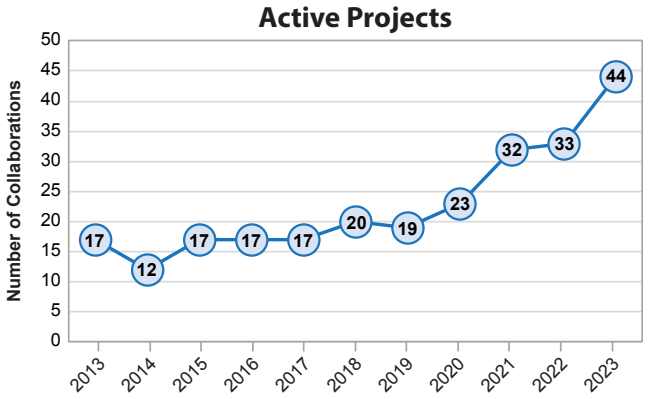
NCL is seeking applications for several leading technologies:

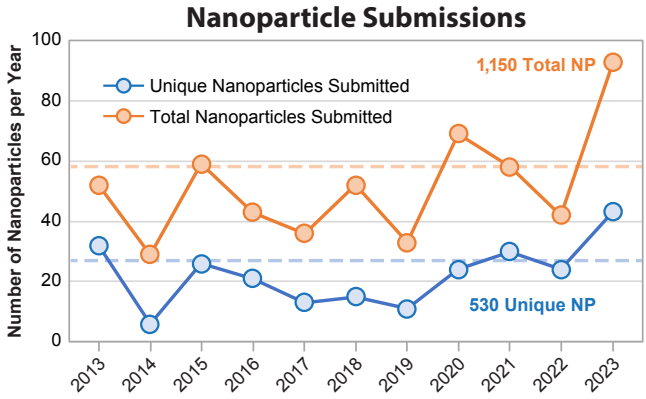
- Lipid nanoparticles
- Therapeutic nucleic acid nanotechnologies
- Imaging agents
- Immunotherapies

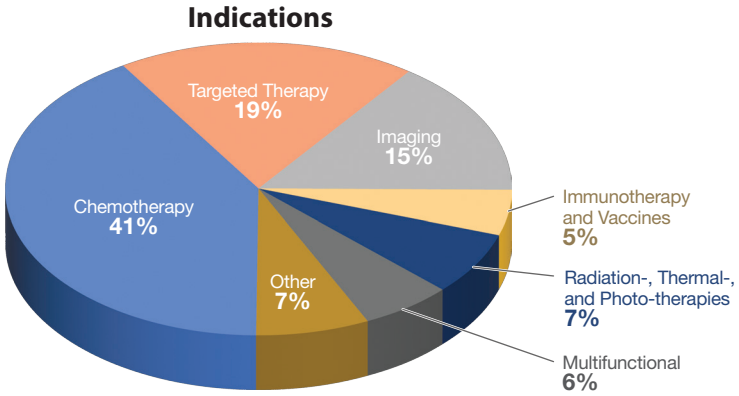
If you are developing one of these technologies, the NCL would be interested in receiving an application from you.

NCL'S ASSAY CASCADE—SUBMISSION TRENDS

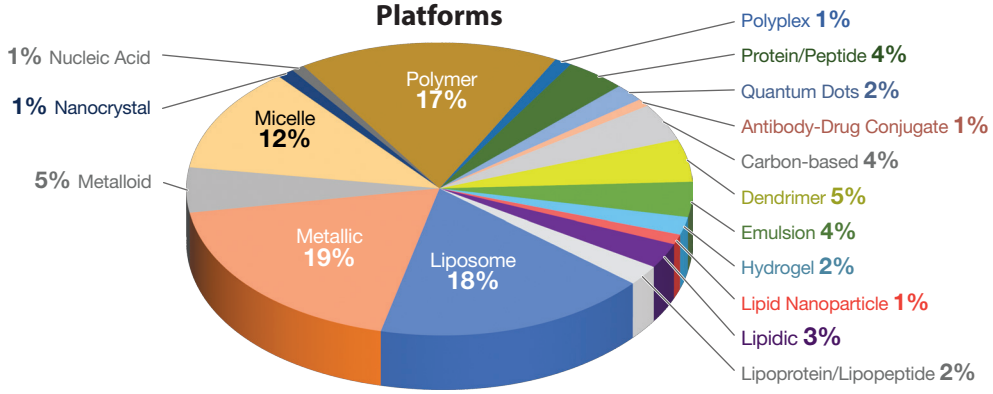
This year proved to be the busiest year for NCL in over a decade. The program had an unprecedented 44 active projects in 2023—more than double the annual average of 20 active projects. Additionally, the program received the highest number of both unique and total nanoparticles submissions since 2010 and 2011, respectively. The NCL received 42 unique nanoparticle submissions and 95 total nanoparticle submissions (accounting for multiple batches). As the graph below depicts, both metrics are well above average, with the average unique submissions being 28 nanoparticles and the average total submissions being 60 nanoparticle batches.



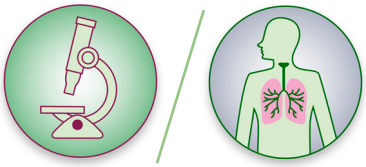




Traditional chemotherapies (e.g., formulations of doxorubicin, paclitaxel, docetaxel, etc.) have dominated the therapeutic submissions to the program for many years. However, in recent years, there has been a shift towards applications covering vaccines, immunotherapies, and cellular and gene therapies. Imaging agents, radiation-, thermal-, and photo-based therapies, as well as multifunctional formulations (e.g., cytotoxic plus imaging), also remain a consistent element of the yearly submissions. Among these, lipid-based, polymer-based, and metallic nanoparticles remain the most common formulation-types submitted and characterized in the NCL's Assay Cascade program.



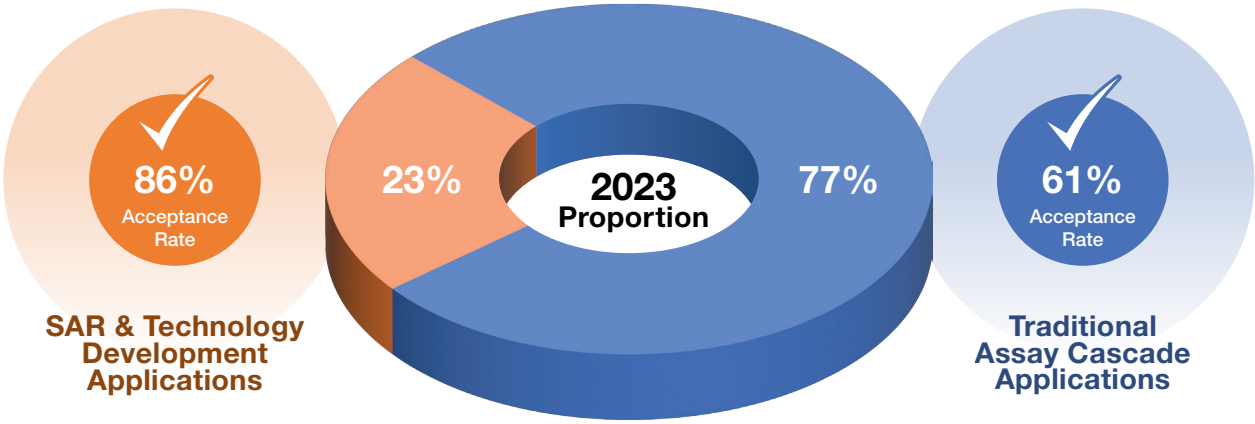
NEW NCL APPLICATION TO SUPPORT SAR STUDIES, TECHNOLOGY ADVANCEMENT & METHOD DEVELOPMENT



Beginning in June 2023, the NCL began accepting abbreviated applications intended to support limited-scope projects in several areas:

- Small scale SAR-type studies intended to help an investigator decide between two or more formulations for lead development or to address a question of broad interest to the nanotech community, for example in vitro cytokine studies on two or three related formulations or compositional characterization of several formulations to assess changes in manufacturing processes.
- A small set of assays critical to further development or understanding of the technology that may be beyond your in-house capabilities, for example asymmetric-flow field-flow fractionation studies or histology assistance.
- Assistance in developing an analytical or bioanalytical method critical for evaluation of the formulation, for example an LC-MS method to quantitate formulation components in biological matrices.

This new application route was met with great enthusiasm in its first year. Nearly one quarter of all applications received were for this new, abbreviated characterization support. The acceptance rate for this application was also significantly higher than the full Assay Cascade characterization application—86% of the SAR/technology advancement applications were accepted in the inaugural year. The abbreviated characterization support allows the NCL to stretch resources to help even more extramural researchers each year. The ultimate goal of these studies is to help investigators mature their concepts for a quicker path to application for full characterization via the traditional NCL Assay Cascade application.



To apply for SAR/Technology Advancement/Method Development

- Download the application here: https://www.cancer.gov/nano/research/ncl/assay-cascade/application-process#characteristics-of-proposed-sar-studies-technology-advancements-and-method-development-efforts-to-be-addressed-in-white-paper-submission (Note, this is a different application than for the full Assay Cascade characterization.)
- Application deadlines are the same as the full proposals—the first business days of March and September.
- If you have questions regarding the application, eligibility, etc., please reach out to us at ncl@mail.nih.gov, and a member of our team will be happy to provide consultation.

TTNCI AND IRCN AWARDEES RECEIVE NCL SUPPORT

Reminder for Toward Translation of Nanotechnology Cancer Interventions (TTNCI; issued as PAR 20-116 and 22-071) and Innovative Research in Cancer Nanotechnology (IRCN; issued as PAR 14-285, 17-240, 20-284, and 23-246) awardees, you are eligible to receive free NCL characterization to support your funded projects—without going through a separate application for NCL's services. There is a **100% acceptance** of these NCI-funded projects. Simply reach out to the NCL (ncl@mail.nih.gov) as a first step in initiating the collaboration; it is not necessary to wait for one of NCL's quarterly application cycles.

The NCL has worked with a dozen TTNCI and IRCN awardees—some have applied to the NCL prior to their NCI award, using NCL data to help secure funding, and some have approached the NCL following their funding award for NCL characterization of the awarded nanotechnologies.

Characterization will be individually tailored to each project based on the specific aims and objectives. A minimum set of characterization is provided for all interested awardees, including sterility, endotoxin and beta-glucan testing, as well as limited physicochemical characterization of the concept. Other, e.g., a more expanded physicochemical characterization, in vitro, and/or in vivo biological studies, are decided in collaboration with the submitting investigator and NCI personnel.

Those interested in applying to the TTNCI and IRCN programs can find additional information at the following websites:

PAR 22-071, Toward Translation of Nanotechnology Cancer Interventions:
https://grants.nih.gov/grants/guide/pa-files/PAR-22-071.html

PAR 23-246, Innovative Research in Cancer Nanotechnology:
https://grants.nih.gov/grants/guide/pa-files/PAR-23-246.html

Applications are accepted twice per year, with due dates in May and November.

TRAINING SUPPORT FOR STUDENTS & POST-DOCS

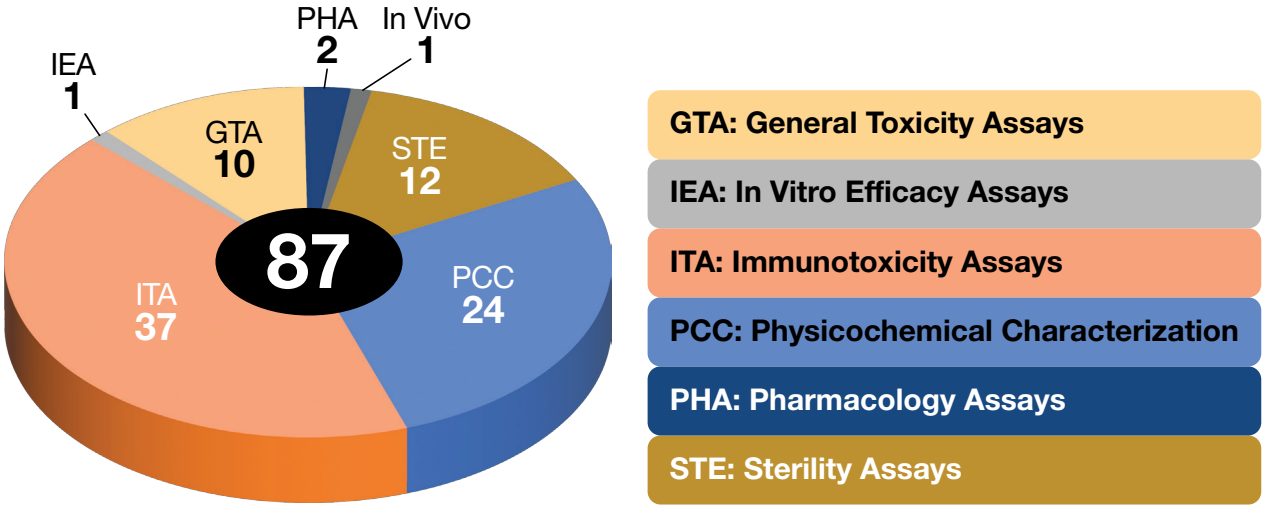
In addition to characterization support, as part of NCL's education and knowledge-sharing mission, the NCL also offers training for your undergraduate and graduate students and post-doctoral fellows. There are a limited number of in-person training opportunities each year, but virtual training opportunities are also available. Trainings are offered in any of the scientific disciplines within the NCL, e.g., endotoxin, physicochemical characterization, immunology, pharmacology, and toxicology. Trainees can gain experience in one or more assays by characterizing their own nanomaterials under the guidance and supervision of NCL staff. In-person training varies from as little as 1–2 weeks up to 1 year depending on the scope of activities.

If you are interested in discussing this opportunity, please let us know what your interests are. Reagents and access to instrumentation are covered by the NCL; however, you are responsible for covering the costs associated with housing, transportation, food and other daily expenses, and health insurance.

PROTOCOLS & NEW TECHNOLOGIES

The NCL now has an Assay Cascade portfolio of protocols, techniques, and guides covering a plethora of topics, with 87 protocols and guides now available for download from the NCL website (https://www.cancer.gov/nano/research/ncl/protocols-capabilities). Many additional capabilities are also available, but as product-specific procedures, developed for each unique concept that is accepted into the characterization program. Once optimized, the NCL provides collaborators the detailed procedure and will work with the CRO of your choice to help transfer the capabilities needed in your regulatory pursuit.

Highlighted below are a few of the most recent additions to NCL's Assay Cascade.



Physicochemical Characterization

Expanding on the analysis of **residual organic solvents**, NCL now has protocols to evaluate for the presence of 14 of the most commonly used solvents (PCC-22 and PCC-23). This information provides insight on the suitability of your nanomaterial purification strategy to remove/reduce levels of these impurities below the ICH-recommended limits.

Cholesterol is a one of the most widely used components in lipid-based formulations. Long-term storage of these formulations can result in the production of oxysterols, leading to the formation of more than 60 different cholesterol oxidation products. NCL now has UHPLC-UV-MS methods available to screen for common contaminants such as 7-ketocholesterol, one of **cholesterol's major oxidation products**.

In Vitro Immunotoxicity

Further expanding the NCL's immunology portfolio, a method for detection of **mast cell degranulation** was recently developed. Mast cells are immune cells that, upon activation, release pro-inflammatory markers—a process known as degranulation. As part of degranulation, mast cells release β-hexosaminidase. The technique provides quantitation of the released β-hexosaminidase using a simple UV-vis approach.

An **ELISpot assay** has also been developed for the detection of cytokines of a per-cell basis. Single- and multi-plex ELISAs offer affordable costs, high throughput, and a broad spectrum of analytes. However, limitations of these techniques can make them less-than-ideal for certain analyses. For example, detection of cytokines produced in low quantities are better detected using the ELISpot assay.

Through a partnership with the Sequencing Core Facility at the Frederick National Lab, the NCL has begun offering **single-cell sequencing** services to our Assay Cascade collaborators. The next-generation sequencing technology identifies the genomes of individual cells and provides a measure of the variability among the cells. Researchers can use the data to identify genes which are upregulated or downregulated.

PUBLICATION OF METHODS

The focus of NCL publications has centered around publication of trends and observations from the Assay Cascade submission and characterization process, collaborative manuscripts with applicants to the program, reviews of timely interest, and protocols and methodology of broad applicability to the nanomedicine community. Two recent manuscripts highlighting methodology available within the Assay Cascade's 80+ protocols are highlighted below.

Multicolor Flow Cytometry-Based Immunophenotyping for Preclinical Characterization of Nanotechnology-Based Formulations: An Insight into Structure Activity Relationship and Nanoparticle Biocompatibility Profiles.
Newton HS, Zhang J, Donohue D, Unnithan R, Cedrone E, Xu J, Vermilya A, Malys T, Clogston JD, and Dobrovolskaia MA.
Front Allergy. **2023**;4:1126012. PMID: 37470031.

Introduced into the immunology's Assay Cascade portfolio in 2022, immunophenotyping has become an important tool in the overall understanding of a nanomaterial's biological properties. Available as ITA-37 on the NCL website, immunophenotyping allows for identification of the immune cells that are activated as a result of exposure to a test-nanomaterial through use of multicolor flow cytometry. Two immunophenotyping panels are available, one for lymphocytes and one for monocytes.

The referenced publication highlights findings from assessment of the clinical formulations Doxil (PEGylated liposomal doxorubicin), AmBisome (PEGylated liposomal amphotericin B), and Feraheme (superparamagnetic iron oxide with a carbohydrate coating) and research-grade PAMAM dendrimer formulations of varied size (G3, G4, and G5) and surface chemistry (hydroxy-, carboxy-, and amine-terminated).

Detection of Nanoparticles' Ability to Stimulate Toll-Like Receptors Using HEK-Blue Reporter Cell Lines.
Cedrone E and Dobrovolskaia MA.
Methods Mol Biol. 2023;2709:241–251. PMID: 37572285

With the rise of nanomedicine development for immunotherapy and vaccine applications, use of toll-like receptor agonists like CpG DNA are likewise being increasingly utilized. The described protocol (also available as ITA-29 on the NCL website) describes the use of HEK-blue reporter cells lines to identify formulations with TLR activity as part of their immunostimulatory mechanism.

The NCL recently completed the third edition of their protocol book series. The third edition has 28 total chapters, describing topics such asymmetric-flow field-flow fractionation, single particle inductively coupled plasma mass spectrometry, PEG antibodies, immunosuppression, hypersensitivity, in vitro drug release, and more. Be on the lookout for the upcoming release in Q2 2024:

Characterization of Nanoparticles Intended for Drug Delivery, 3rd edition
Jeffrey D. Clogston, Rachael M. Crist, Marina A. Dobrovolskaia, Stephan T. Stern (Editors)
Methods in Molecular Biology Series, Springer Publishing Group

NON-CHARACTERIZATION SUPPORT

The NCL is most notably known for the interdisciplinary characterization that has become known as the Assay Cascade. However, the NCL provides a plethora of support opportunities beyond characterization of nanoformulations.

Consultations

Regardless of whether you are an active collaborator or even an applicant to the program, the NCL offers free consultation services to all researchers in the cancer nanomedicine field. Topics can range from nanoformulation advice to relevant characterization strategies and more.

Scientific Discussions

NCL's senior leadership team participates in more than 60 scientific discussions a year, providing data interpretation and discussion of biological significance.

Aid in Study Design

The NCL conducts dozens of in vivo studies every year, including pharmacokinetics, general toxicity, immunogenicity, adjuvanticity, and efficacy. The NCL supports study design by providing guidance on animal number, timepoints, suggested controls, appropriate statistical analyses, etc.

Relevant CRO/CDMO Expertise

Our program has a long list of CRO and CDMO with expertise in the various characterization required of nanomedicine formulations, as well as those with expertise in manufacturing nanomedicine formulations. If you are looking for specific capabilities, please reach out and we can provide a list of possible organizations who may be able to fill those needs.

Connections & Introductions

Having nearly 20 years of experience in the cancer nanomedicine field and working with hundreds of investigators around the world, the NCL may be able to make connections for researchers in need of specific expertise or collaboration opportunities.

Letters of Support

Interested in applying for funding? The NCL provides dozens of letters of support each year to researchers applying to programs such as NCI's TTNCI and IRCN grants, NCI's Experimental Therapeutics Program (NExT), the SBIR and STTR funding awards for startup companies, and more.

Lectures & Training

NCL staff provide seminars and training to dozens of institutions every year. Interested in having NCL present at your institution? Please reach out to us with your topic of interest.

SUCCESS OF NCL COLLABORATORS' REGULATORY PURSUIT

The preclinical characterization data generated by the NCL is often used in pursuit of regulatory approval. Many past NCL collaborators have used the data generated at NCL and knowledge gleaned from routine data discussions to enhance their IND and IDE applications. Comprehensive reports generated by the NCL can be attached—partially or completely—to regulatory filings in support of a product's translational efforts. Further, NCL staff can attend meetings with regulatory bodies in support of NCL data generated on any formulation that comes through the Assay Cascade characterization program.

Several collaborators have provided testimonials in support of NCL's efforts, including Dr. Chong-xian Pan, Brigham and Women's Hospital and VA Boston Healthcare System, who initiated a phase one clinical trial in 2023 with a concept that went through the NCL's Assay Cascade program in 2015; Dr. Len Pagliaro, Sona Nanotech, who recently began pre-IDE submission discussions with the FDA; and, Pauline Lau, Suntec Medical, who is in preparation for pre-IND discussions anticipated later this year.

3
US Market Authorizations

2
EMA Market Authorizations

1
US Over-the-Counter Market

4
Global Over-the-Counter Markets

> NCL was extremely helpful during the drug development of my bladder cancer-specific nanoparticles. They performed the in vitro characterization of my nanoparticles, and I used this data to support an IND application and currently have an ongoing Phase I trial (Clinicaltrials.gov identifier No: NCT05519241; PI: Pan). The NCL staff are very friendly, responsive and knowledgeable. I am highly satisfied with the service.
>
> **Chong-xian Pan, MD, PhD**
> Associate Professor, Brigham and Women's Hospital, Harvard Medical School
> Staff Physician, VA Boston Healthcare System

> Siva Therapeutics was first accepted into the NCL program several years ago. A few years later we completed the Assay Cascade program, and the data, learnings, contacts, and advice we received during this time were invaluable to us as an early-stage company in the cancer nano-device space. More recently, Siva was acquired by Sona Nanotech, which had become our nanomaterial manufacturing partner. After acquisition and further development of the manufacturing process for our nanomaterial, the NCL team continued to support our project with ongoing work to validate physical chemical properties, sterility, endotoxin, and other key parameters of the improved material. Recently, we were very pleased to have NCL team members participate in an FDA Pre-Submission meeting, and the NCL team provided valuable input as well as important clarification of next steps in our development process. The NCL has played a key role in our ability to move ahead in the cancer nano-therapy space.
>
> **Len Pagliaro, PhD**
> Chief Scientific Officer
> Sona Nanotech, Inc.

> We extend our sincere appreciation to the NCL for their pivotal role in advancing our STM-001 project at Suntec Medical. The NCL Assay Cascade provides crucial insights into the physical and chemical properties of STM-001 with studies of high scientific standards and great technical expertise. With NCL's support, STM-001 is ready to enter clinical developments for brain cancers sooner than we expected. We highly recommend NCL for organizations seeking top-tier nanotechnology characterization services.
>
> **Pauline Lau, PhD**
> Founder
> Suntec Medical

NANOTECH FORMULATION

The NCL has had a vast exposure to a unique array of nanomedicine formulations over the years—including almost every platform being explored for biomedical applications. This exposure has afforded valuable insight into the utility of each platform in combination with the various therapeutic agents being explored in oncology research, e.g., small molecule cytotoxic agents, therapeutic nucleic acids, neoantigens, etc. Using this unique insight, the NCL provides nanotechnology formulation services to support both intramural and extramural researchers reformulate their therapeutic moieties to overcome poor pharmacokinetic properties, dose-limiting or off-target toxicities, and improve therapeutic index. In addition to formulation, NCL can provide physicochemical, in vitro, and in vivo characterization to help test and validate the potential of developed formulations.

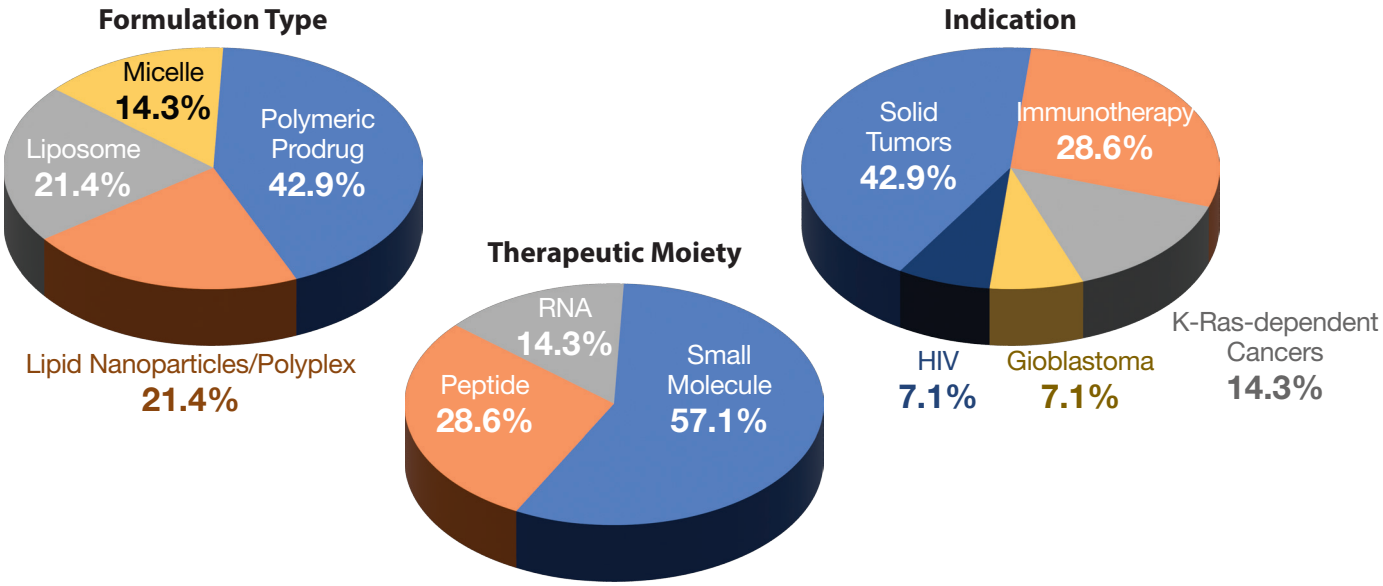
Intramural Collaborators

Intramural researchers interested in utilizing NCL's nanoformulation services can reach out to schedule an introductory call to assess formulation options for their therapeutic. Services are provided to you at cost, simply by providing a project ID for charge of materials, supplies, and labor.

Extramural Collaborators

Formulation work for extramural organizations is conducted under a Cooperative Research and Development Agreement with Leidos Biomedical Research, Inc. (cCRADA). After an initial assessment of feasibility, a statement of work is collaboratively developed which can include not only formulation, but also characterization of interest (e.g., pharmacokinetics). The NCL will put together a cost estimate based upon the statement of work, and funds are required before the initiation of any work. Notably, the NCL—as a contractor for the National Cancer Institute—cannot make a profit from these contracts. Therefore, a collaborator is charged solely for materials, supplies, and labor and associated overhead fees. Any funds remaining upon completion of the project are returned to the provider.

Also of note, for work conducted under the cCRADA mechanism, the therapeutic is not required to be for an oncology application. If you are interested in discussing formulation potential for your therapeutic, please reach out to us to schedule a feasibility call, ncl@mail.nih.gov.



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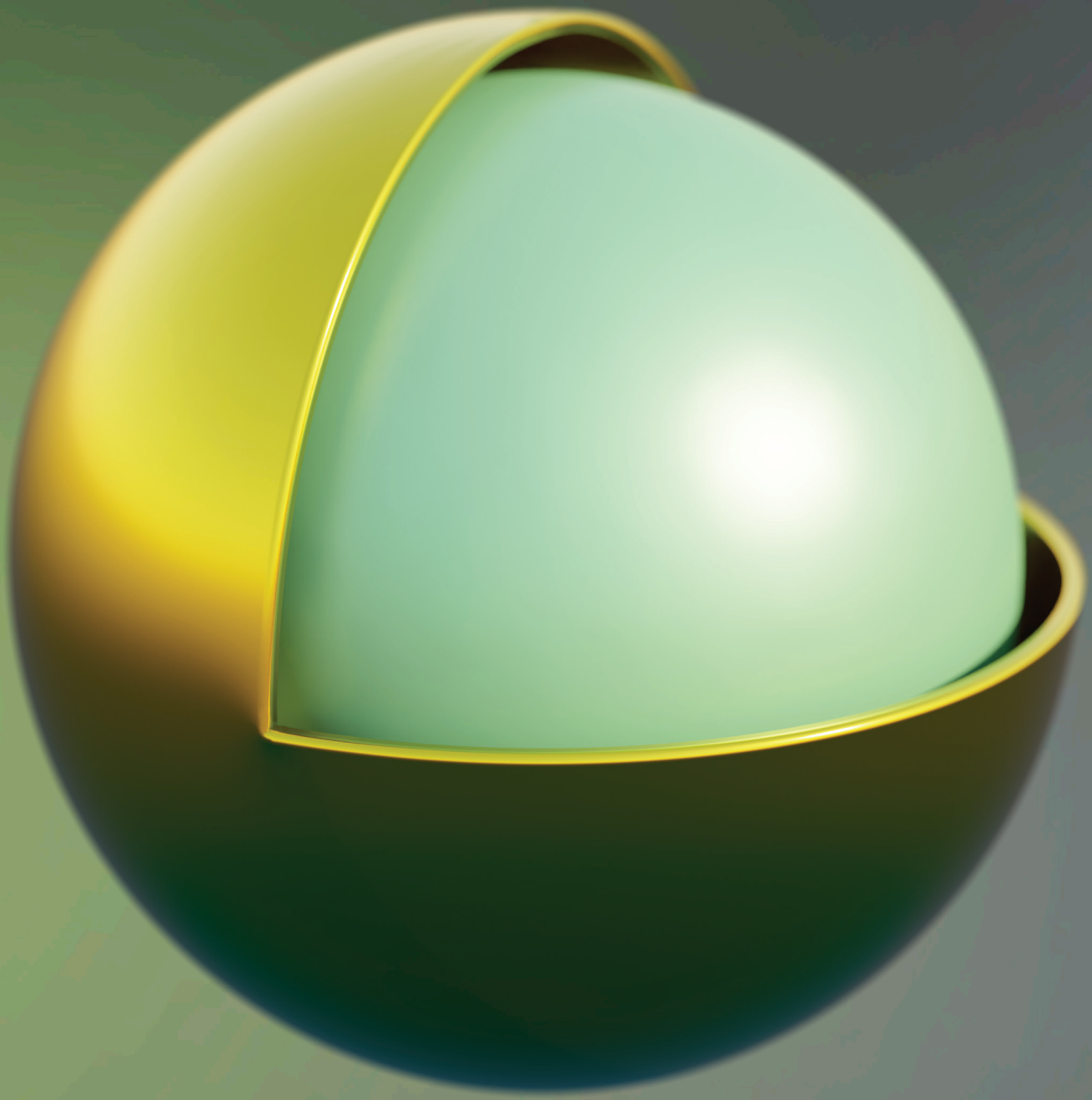

The NCL is part of the Frederick National Laboratory for Cancer Research (FNLCR). FNLCR is operated by Leidos Biomedical Research, Inc., for the National Cancer Institute, under contract 75N91019D00024.