# Nanotechnology Characterization Laboratory

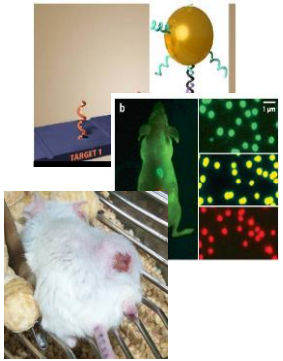
*Supporting Translation of Cancer Nanomedicines*

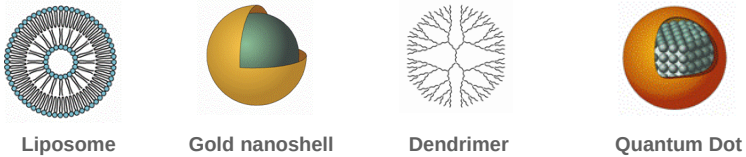
NIH NATIONAL CANCER INSTITUTE

Piotr Grodzinski, PhD
DCTD/CIP

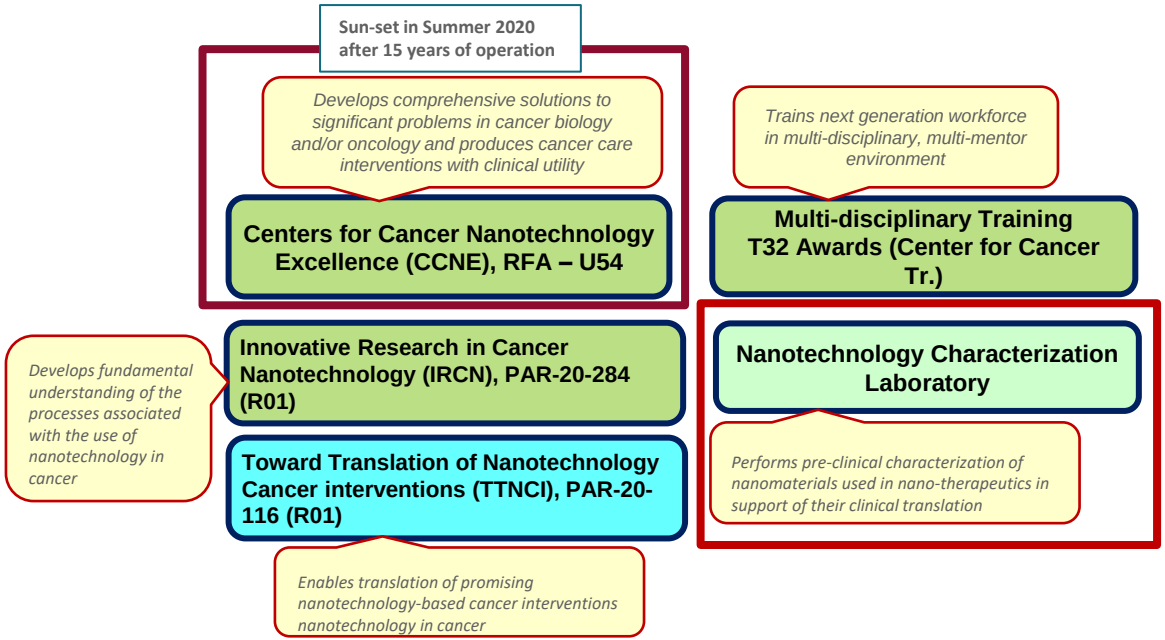
# Cancer Nanotechnology: The Opportunity

- Combine power of innovation in nano-materials and cancer biology to develop new solutions in cancer
- Detect Disease *Before* Health Has Deteriorated
  - Sensors
  - Imaging
- Deliver Therapeutics
  - Local delivery
  - Improved efficacy
  - Post-therapy monitoring
- Develop Research Tools to Enhance Understanding of the Disease



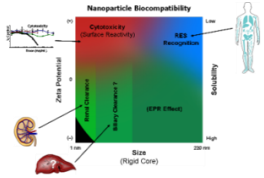



# NCI Alliance for Nanotechnology in Cancer (since 2005)




- Very strong scientific output demonstrated by large number of high-profile publications;
- Translating technologies through formation of start-up companies (over 130 to-date);
- Over 20 clinical trials have been pursued by start-ups.

# NCL Timeline


NCL Lessons Learned Workshop

MOU & MDTA with NIST & FDA

NIH IDP Support

ASTM&ISO

PAR20-116
PAR20-284
Support

Formulation Capability Established

Community Outreach & Education

SAR Studies; Early NM Trends

Assay Cascade Development

MOU & MDTA with NIST & FDA

cCRADA

TSA

SITUA

FDA IAA

Organizational Changes

FDA IAA

NIEHS IAA

2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021

NCL Established

First Submission

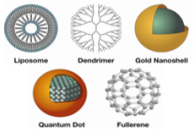
More Mature Concepts

NCL 2.0 EUNCL

Generic Nanomedicines

Assay cascade opened to COVID-19 applications

Nanotechnology Characterization Laboratory




NCL Nanotechnology Characterization Laboratory

Unique, Non-Traditional Concepts

EUNCL Nanomedicine Characterisation Laboratory

Planning & Gaps Analysis

100th Nanoparticle

200th Nanoparticle

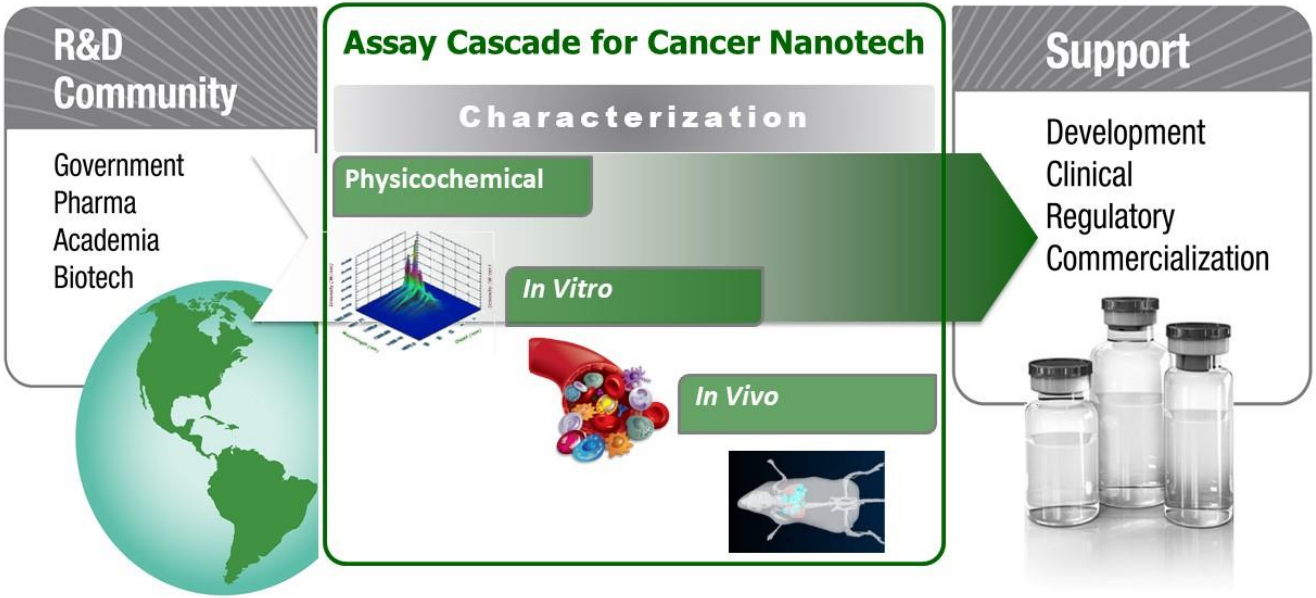
300th Nanoparticle

400th Nanoparticle

450th Nanoparticle

- **Over 15 years of experience in nanoparticle characterization**
- **Standardized assay cascade, method development, education & knowledge sharing**
- **Beyond assay cascade – cCRADA, TSA, IAA agreements**

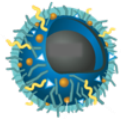
# Nanotechnology Characterization Lab (NCL)




- NCL was established in 2004 as an interagency collaboration among NCI, FDA, and NIST;
- Lab's primary mission is to advance the science and enable translation of promising nanomedicines with a support of a standardized "Assay Cascade";
- 'Assay Cascade' characterization is **FREE** of charge to submitting investigator.

# NCL's Assay Cascade

## FREE Service for cancer nanotechnology concepts, by application



### *Physicochemical Characterization*

Applicable to CMC section of IND

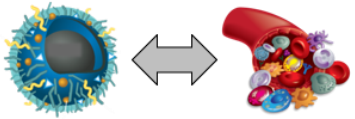
**Size/Size Distribution**
- Dynamic Light Scattering (DLS)
- Electron Microscopy (TEM, SEM, cryo)
- Atomic Force Microscopy (AFM)
- Field Flow Fractionation (FFF), SEC-MALS

**Composition**
- TEM with EDS
- Inductively coupled plasma-mass spec. (ICP-MS)
- Spectroscopy (NMR, CD, Fluorescence, IR, UV-vis)

**Purity**
- Chromatography
- Capillary Electrophoresis

**Surface Chemistry**
- Biacore
- Zeta Potential

**Stability**
- Stability can be measured with any number of instruments with respect to time, temperature, pH, etc.



### *In Vitro Characterization*

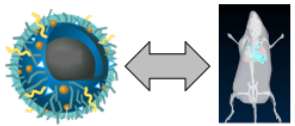
Applicable to vaccines, immunotherapies

**Sterility**
- Bacterial/Viral/Mycoplasma
- Endotoxin

**Hematology**
- Hemolysis
- Platelet Aggregation
- Coagulation
- Complement Activation
- Plasma Protein Binding

**Immune Cell Function**
- Cytokine Induction
- Chemotaxis
- Phagocytosis
- Leukocyte Proliferation
- Leukocyte Procoagulant Activity

**Toxicity**
- Cytotoxicity
- Autophagy



### *In Vivo Characterization*

Comprehensive animal studies

**Pharmacology**
- Clinical Tx cycle
- NP Quantitation methods
- PK Parameters

**Immunotoxicity**
- Local lymph node proliferation assay
- T-cell dependent antibody response
- Adjuvanticity
- Rabbit pyrogen test
- Immunogenicity
- Inflammatory response
- Autoimmunity

**Single and Repeated Dose Toxicity**
- Blood Chemistry
- Hematology
- Histopathology (42 tissues)
- Gross Pathology

Not GLP

**Inter-disciplinary approach to link physicochemical attributes to biological outcomes**

**72 protocols available online: https://ncl.cancer.gov/resources/assay-cascade-protocols**

# NCL's Assay Cascade Application Process

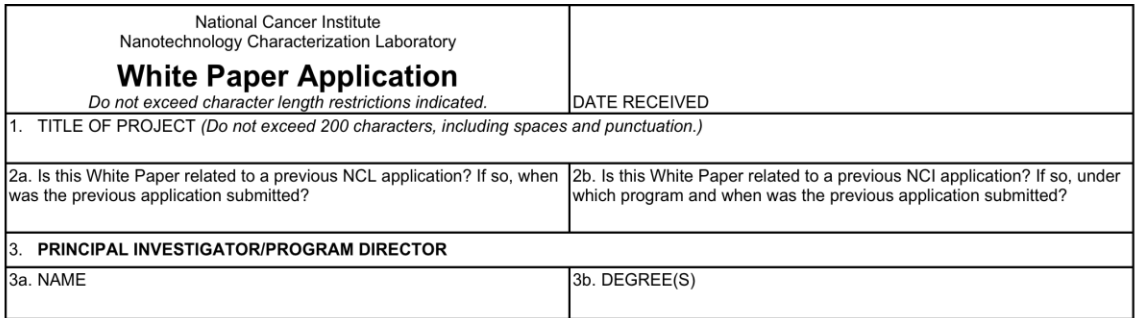
## Two-phase application process

**Phase 1: Brief (3-4 page) White Paper**

- Abstract, Background, Strategy/Concept, Data on Synthesis, Characterization, In Vitro & In Vivo Testing, Novelty, Clinical Impact & Scale Up.
- Applications are accepted and reviewed quarterly.
- Decisions are remitted within 45 days of the application deadline.

**Phase 2: Oral or Written Proposal**

- Expansion of data presented in White Paper; addressing reviewer questions.
- Inputs due within 3 months of receiving invitation letter.
- Decisions are remitted within 2 weeks.



National Cancer Institute
Nanotechnology Characterization Laboratory

**White Paper Application**

*Do not exceed character length restrictions indicated.*

DATE RECEIVED

1. TITLE OF PROJECT *(Do not exceed 200 characters, including spaces and punctuation.)*

2a. Is this White Paper related to a previous NCL application? If so, when was the previous application submitted?

2b. Is this White Paper related to a previous NCI application? If so, under which program and when was the previous application submitted?

3. **PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR**

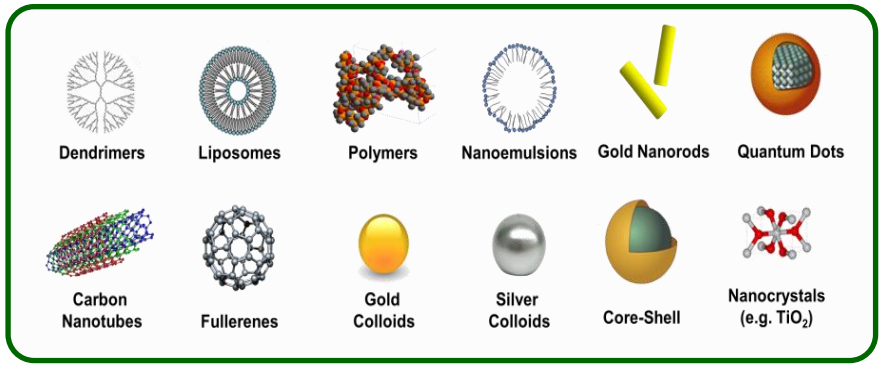
3a. NAME

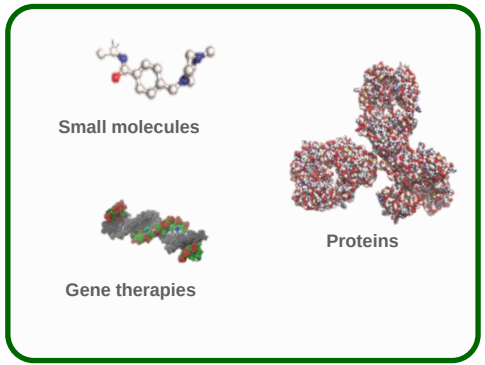
3b. DEGREE(S)

***https://ncl.cancer.gov/working-ncl/ncl-assay-cascade-application-process***

# NCL by the Numbers

**>450** Different nanomaterials characterized with a wide range of nanotechnologies and APIs



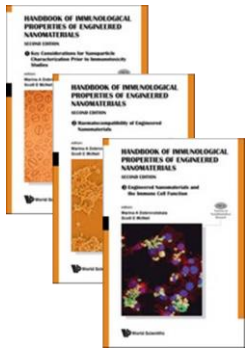




**>200** Peer-reviewed publications covering nanoparticle characterization, immunotoxicity and safety

**>250** Collaborations with academia, industry, and government labs

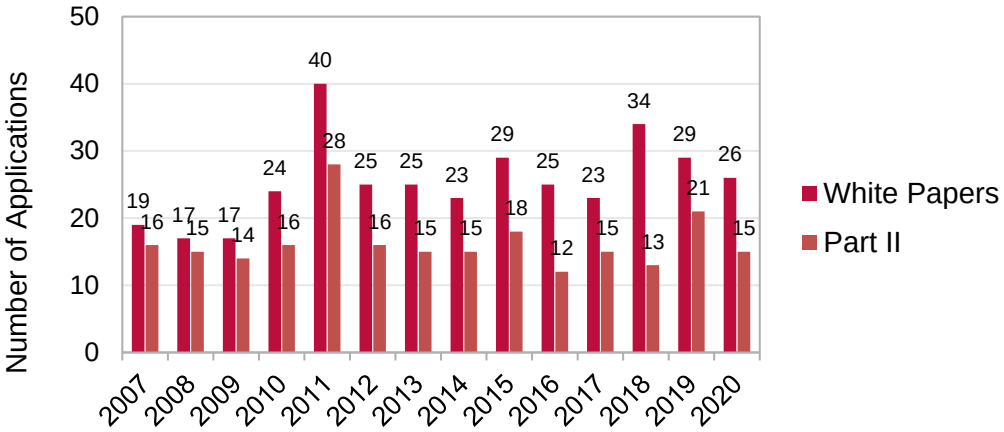
**>70** Protocols standardized for various nanoparticles

**17** NCL collaborators reached clinical trials



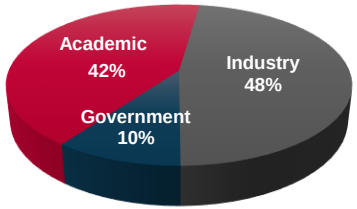

**Cumulative experience of providing NCL Assay Cascade for 16 years has made NCL a unique resource**

# Assay Cascade: Applications and Acceptance Rates

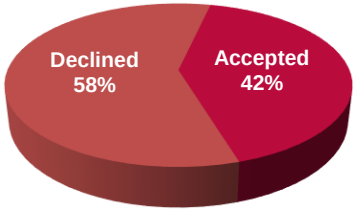



- **On average ~20 applications/cycle;**
- **Majority of white paper applications come from industrial and academic researchers;**
- **Overall acceptance rate ~40%.**

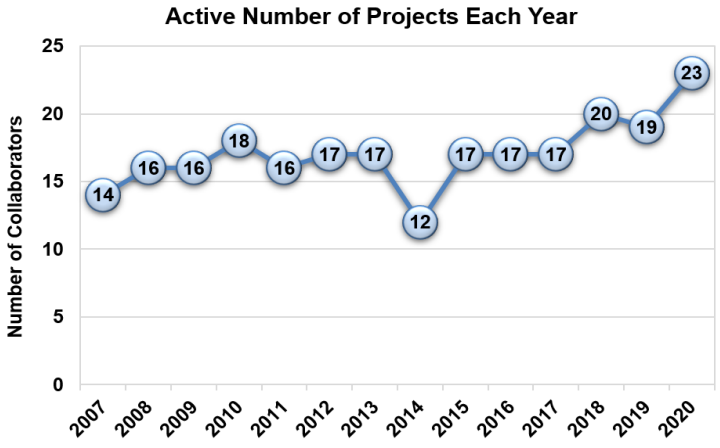
## Applicants

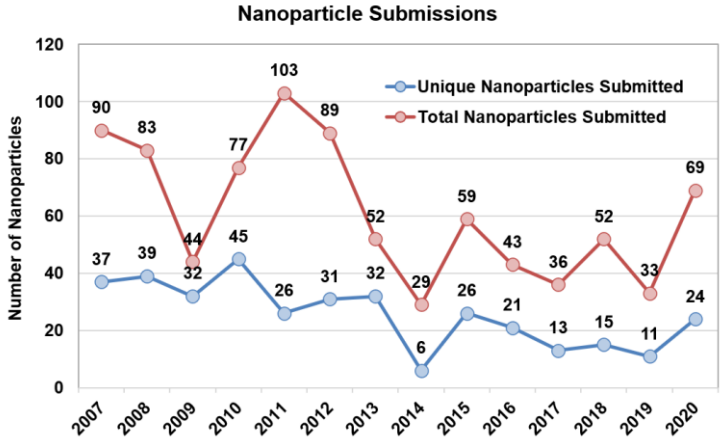


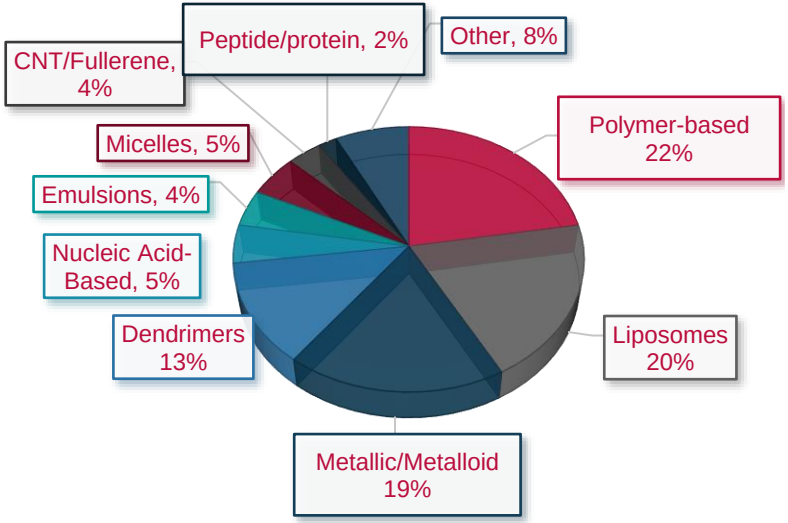

## Overall Acceptance Rate



# Assay Cascade: Nanoparticles Metrics



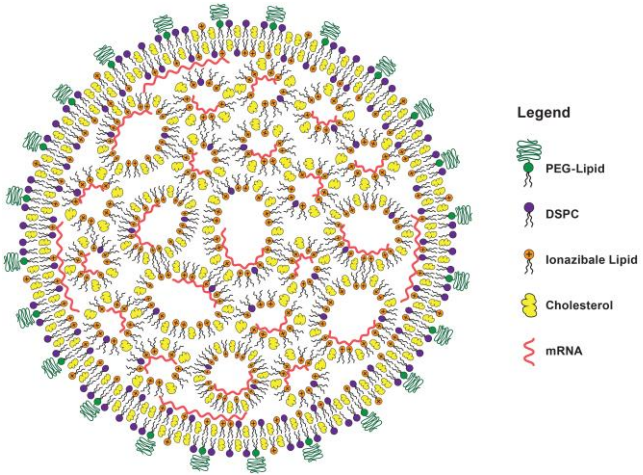



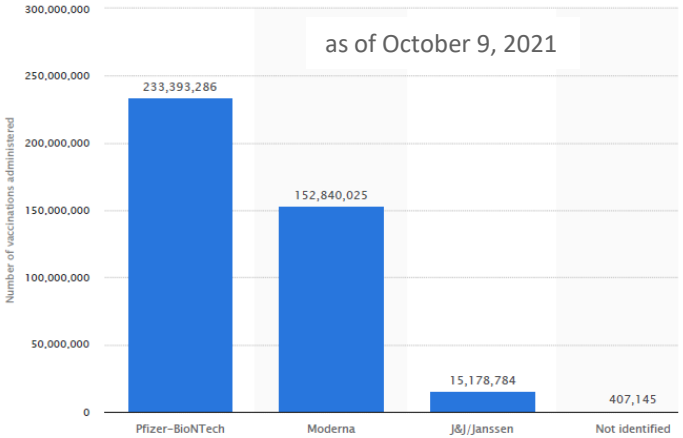

**Polymer-based nanomaterials, liposomes and metal-based nanoparticles dominate the NCL nanoparticle portfolio.**

- **Average number of projects per year - 15-16**
- **Average number of total nanoparticles per year - 61**
- **Average number of unique nanoparticles per year – 26**

# Lipid Nanoparticles for mRNA-based COVID-19 Vaccines

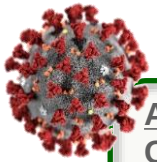



Structure of Pfizer/BionTech nanoparticle;
drawing by NCL



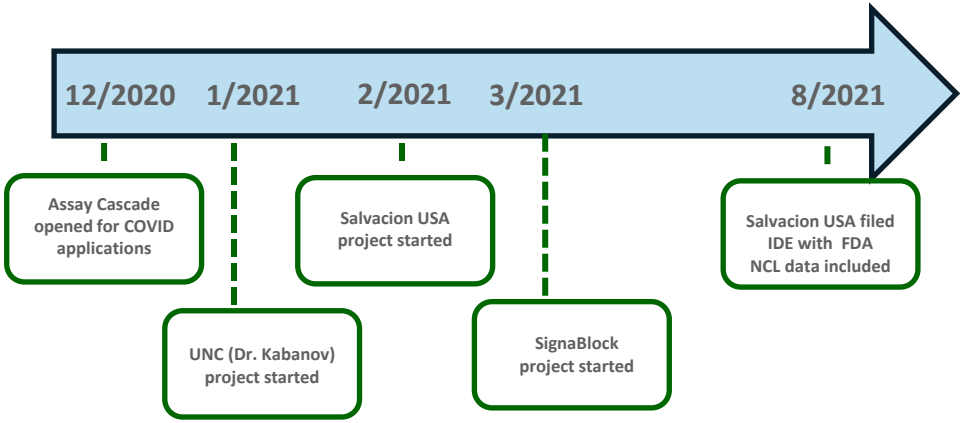

https://www.statista.com/statistics/1198516/covid-19-vaccinations-administered-us-by-company

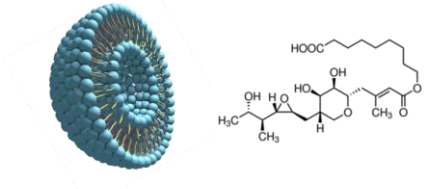
# Assay Cascade: Response to COVID-19



**Assay Cascade opened to COVID-19 Concepts**

- December 2020, first application cycle
  - **SignaBlok**
  - **Salvacion USA, LLC –** *IDE filed*
  - Gerrit Borchard, University of Geneva
  - **Alexander Kabanov, University of Norther Carolina-Chapel Hill**




- Repurposing cancer formulations

NCL321 (Dr. Barenholz)

# NCL Advances Science and Promotes Clinical Translation

CytImmune Sciences Inc — NCL74

Bexion Pharmaceuticals — NCL229

E-Gen — NCL254

BIND Therapeutics — NCL142

Azaya Therapeutics — NCL151

AADI bioscience — NCL399

NCL182 Celator Pharmaceuticals

US authorization - 2017
EU authorization - 2018

PDS Biotechnology — NCL140

Intezyne Technologies — NCL359

ProNAi — NCL246

Keystone Nano — NCL170

MonTa Biosciences — NCL423

NanoBiotix — NCL311

EU authorization - 2019

Nanospectra — NCL61

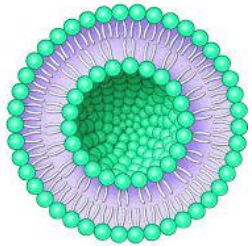
Merrimack Pharmaceuticals — NCL328

האוניברסיטה העברית בירושלים
The Hebrew University of Jerusalem — NCL321

We Make Lives Better
UT Health Science Center San Antonio — NCL303

- **17 Applicants in clinical trials with novel nanomedicine therapies**

PDS Biotechnology
Protein Delivery Sciences



**PDS0101/Versamune®**

| PRODUCT | INDICATION | COMBINATION | PC | P1 | P2 | P3 | R | PARTNER(S) |
|---|---|---|---|---|---|---|---|---|
| **Oncology** | | | | | | | | |
| PDS0101 (HPV16) | First line treatment of recurrent / metastatic head and neck cancer | KEYTRUDA® | | | | | | MERCK |
| PDS0101 (HPV16) | Advanced HPV-associated malignancies | M7824<br>NHS-IL12 | | | | | | NIH National Cancer Institute |
| PDS0101 (HPV16) | Stage IIb-IVa cervical cancer | Chemo-radiation | | | | | | MD Anderson Cancer Center |
| PDS0102 (TARP) | Prostate and breast cancer | Immunotherapy | | | | | | NIH National Cancer Institute |
| PDS0103 (MUC-1) | Breast, colorectal, ovarian and NSCLC cancer | Immunotherapy | | | | | | NIH National Cancer Institute |
| PDS0104 (TRP2) | Melanoma | Immunotherapy | | | | | | |

PDS Biotech Funded — Partner Co-Funded

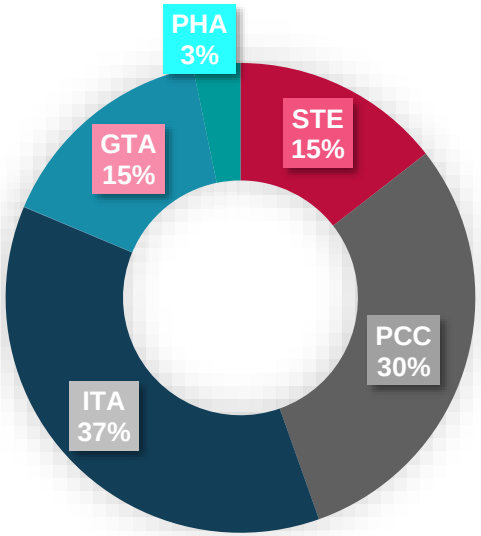
# Education and Knowledge Sharing: Method Dissemination

**72 total protocols on the NCL website**
https://ncl.cancer.gov/resources/assay-cascade-protocols

**2243 protocols downloaded last year**

**All protocols have unique DOI**

## Protocol Downloads by Category




- STE = sterility;
- PCC = physicochemical characterization;
- ITA = immunotoxicity assay;
- GTA = general toxicity assay;
- PHA = drug release assay

# Education and Knowledge Sharing: NCL/NCI-Sponsored Workshops & Training


WORKSHOP

"MOLECULAR AND CELLULAR IMMUNOLOGY: FROM BASICS TO APPLICATIONS IN PRECLINICAL DEVELOPMENT OF NANOTECHNOLOGY-FORMULATED DRUGS"

## Immunology Workshop Series

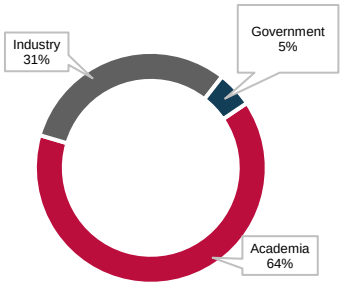
- December 2019 & May 2020
- ~175 participants
- ~20% from outside the US
- ~60% were PI
- *Stimulated T32 and RO1 grant submission*

## NCL Bootcamp

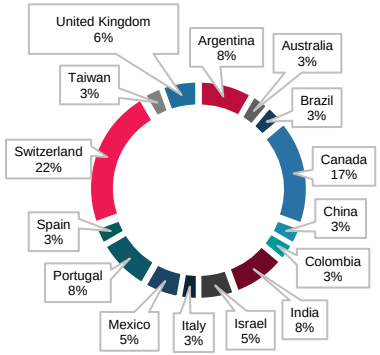
NCI had approved in-person training opportunities for graduate students and post-doctoral fellows to gain hands-on experience with NCL assays;

Originally planned for Spring 2021; delayed due to pandemic.

**Breakdown of Institution Types**




**Non-US Countries Participating**




NIH NATIONAL CANCER INSTITUTE

## Technology-Development Seminars

- NCI & FNL Technology Showcase
- Drug delivery seminar on lymphatic targeting
- Platform technology for monitoring progression and treatment of cancer

*Drew interest from many participants.*

**This is in addition to attending and presenting at National and International research conferences**

# Education and Knowledge Sharing: Client Reports and Publications

## Client Reports

**121 Assay Cascade reports written to date**

**Avg. 7-8/year**


**Report details:**

- **Every experiment** conducted on **every batch** of material submitted
- Methods, results, analysis & discussion
- **Connecting** physicochemical and biological data

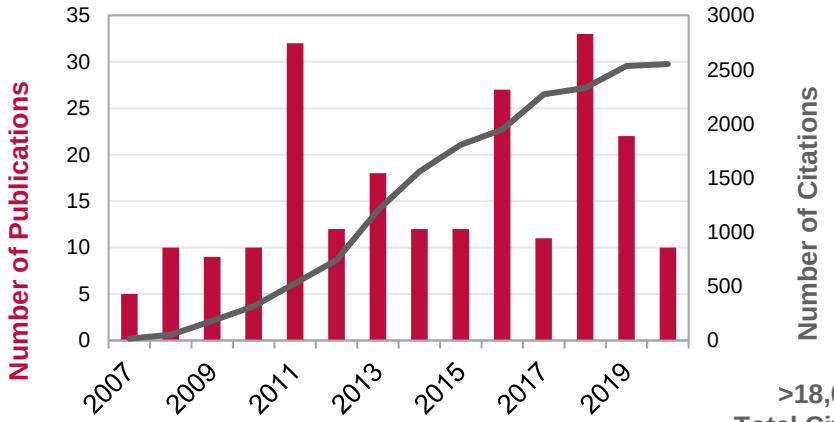
**Data is used for:**

- IND filings, other regulatory questions
- Publications, presentation
- Venture capital
- Verification of internal results
- Transfer to CRO

## Publications

Though not a priority for NCL's mission, NCL publishes several manuscripts focused on:

- Method development efforts;
- Areas of focus per SOC recommendation, e.g., nucleic acid nanoparticles
- Review articles to attract new clientele, e.g., imaging constructs, pharmacokinetic interpretation.

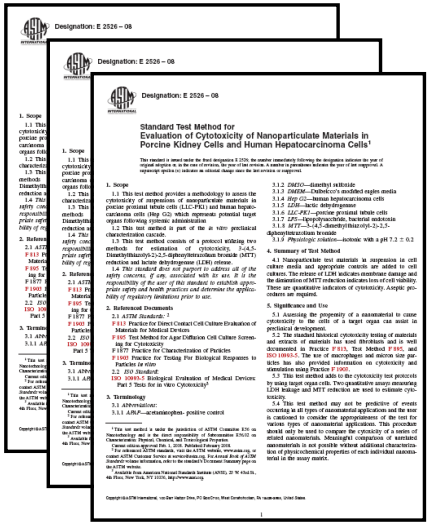



**224 Total Publications**

**>18,000 Total Citations (h-index=52)**

# Standards Development at NCL

- Standards development with ASTM and ISO
  - Three NCL protocols are ASTM standards
  - Multiple NCL protocols are ISO PASs
- NCL supported the production of NIST's colloidal gold RM
  - Gold selected for calibration and biocompatibility
  - 10 nm, 30 nm, and 60 nm diameters
- NCL participates in inter-laboratory studies (ILS)


Designation: E 2526 – 08

Standard Test Method for
Evaluation of Cytotoxicity of Nanoparticulate Materials in
Porcine Kidney Cells and Human Hepatocarcinoma Cells[1]


# Standards Development

ASTM INTERNATIONAL

1. **WK76862** Guide for the Identification of Nanoparticles Ability to Induce Infusion Reactions

1. **WK76861** Method for the In vivo analysis of nanoparticle-mediated physiological changes accompanying hypersensitivity reactions
2. **WK76860** Method for the Preparation and Analysis of Culture Supernatants for the Presence of Cytokine Biomarkers by Nanoparticles in Human Whole Blood Cultures
3. **WK76878** Method for the analysis of nanoparticle effects on human platelets in vitro
4. **WK76821** Practice for the Synthesis and Assembly of Nucleic Acid Nanoparticles
5. **WK76822** Method for the Preparation and Analysis of Culture Supernatants for the Presence of Cytokine Biomarkers by Nucleic Acid Nanoparticles in Human Peripheral Blood Mononuclear Cells
6. **WK76823** Guide for the Evaluation of Immunostimulatory Properties of Nucleic Acid Nanoparticles (NANPs)
7. Method “In vitro Analysis of Nanoparticle Hemolytic Properties” (a revision of **ASTM E2524-08** (2013) previously developed by the NCL)
8. Method “In vitro Analysis of Nanoparticle Effects on CFU-GM” (a revision of **ASTM E2525-08** (2013) previously developed by the NCL)
9. Method “Evaluation of Cytotoxicity of Nanoparticulate Materials in Porcine Kidney Cells and Human Hepatocarcinoma Cells” (a revision of ASTM **E2526-08** (2013) previously developed by the NCL)

ISO

- Member of ISO’s Technical Committee 229, Nanotechnologies
- Liposomes Terminology working group (JWG 1)


Support FNL STAR TREC initiative

## Nanomaterials and Nanotechnology IQ Working Group

Part of the International Consortium for Innovation and Quality (IQ) in Pharmaceutical Development.

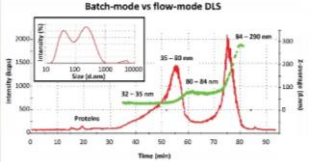
This working group consists of industry members from:

- Sunovion Pharmaceuticals
- Boehringer Ingelheim
- AstraZeneca
- GlaxoSmithKline
- Celgene/Bristol Myers Squibb
- Biogen
- Pfizer
- Abbvie
- Merck
- Eli Lilly

# Parameters, Methods & Considerations for the Physicochemical Characterization of Liposomal Nanoparticles

## Size/Size Distribution

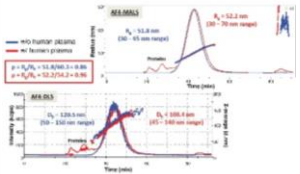
- Dynamic light scattering (DLS)
- Multi-angle light scattering (MALS)
- Laser diffraction
- Cryogenic-transmission electron microscopy (Cryo-TEM)
- Resistive pulse sensing
- Asymmetric-flow field-flow fractionation (AF4) – MALS/DLS




Batch-mode (inset) versus flow-mode DLS measurements of dual-drug loaded liposomes. Multiple size populations are observed by both techniques and indicate a polydispersed sample. However, flow-mode DLS can better resolve the size distribution of each population. Adapted from Anal Bioanal Chem, 2020, 412(2), 425-428.

## Surface Characteristics

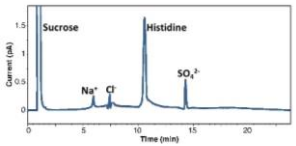
- Zeta potential
- Protein binding assessment by AF4-MALS/DLS
- Quartz crystal microbalance with dissipation monitoring (QCM-D)




Flow-mode AF4-MALS (top) and AF4-DLS (bottom) of PEGylated irinotecan liposomes before and after incubation in human plasma. The increase in the ratio (ρ) of the measured MALS ($R_g$) and DLS ($R_h$) sizes after human plasma incubation suggests protein binding to the surface of the liposomes. Adapted from Anal Bioanal Chem, 2020, 412(2), 425-428.

## Composition

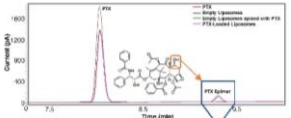
- Drug concentration: total, free & encapsulated
- Drug distribution as a function of size
- Targeting ligand concentration: total, bound & unbound
- Individual lipid concentrations
- Counterion concentrations: interior & exterior
- Excipient concentrations
- Particles per mL concentration
- Osmolality, viscosity measurements




Counterion and excipient concentrations for PEGylated liposomal doxorubicin measured by RP-HPLC with charged aerosol detection (CAD). Adapted from J Pharm Biomed Anal, 2019, 165, 41-46.

## Purity

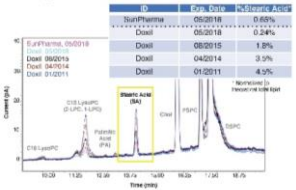
- Drug impurities
- Lipid impurities
- Free drug/lipid/targeting ligand concentrations
- Residual solvents and reagents




Purity assessment of PEGylated liposomal paclitaxel (PTX) as defined by the presence of drug impurities. The drug epimer concentration was measured by RP-HPLC and its identity confirmed by mass spectrometry.

## Stability

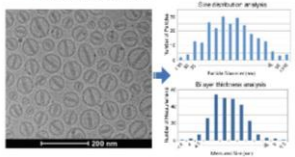
- Size/Size distribution; aggregation
- Drug leakage and degradation
- Hydrolysis of lipids
- Drug release in plasma
- Solvent, thermal, pH, photo, freeze-thaw, lyophilization, centrifugation, filtration
- Storage conditions/shelf-life




Stability assessment of PEGylated liposomal doxorubicin as defined by the hydrolysis of phospholipids. The formation of free fatty acids and lysophospholipids of several batches with varying expiration dates were measured by RP-HPLC with charged aerosol detection (CAD).

## Morphology

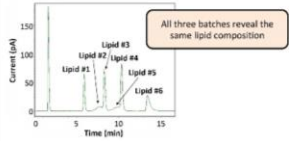
- Cryo-TEM can be used to evaluate:
  - Size distribution
  - Liposome morphology
  - Internal liposome volume
  - Bilayer thickness
  - Number of lamellae
  - Drug appearance/state




Representative cryo-TEM image of PEGylated liposomal doxorubicin. Cryo-TEM was used to determine size and morphology of the liposomes.

## Batch-to-Batch Consistency

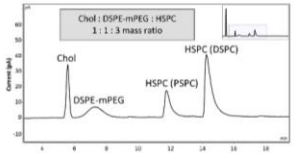
- Assessed by choosing relevant parameters (i.e., lot release criteria) that relate to a desired in vivo outcome



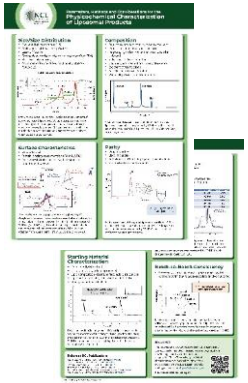

Batch-to-batch consistency for lipid nanoparticles with siRNA was assessed by quantitation of the lipid composition. Six individual lipid concentrations for three batches were determined by RP-HPLC with charged aerosol detection (CAD).

## Starting Material Characterization

- Drug identity (structure)
- Drug purity (degradation products)
- Lipid composition (structure, fatty acid distribution)
- Lipid purity (free fatty acid, lysophospholipids)
- Storage conditions/shelf life




Lipid composition (identity and individual lipid concentrations) and purity (presence of free fatty acid and lysophospholipids) were determined by RP-HPLC with charged aerosol detection (CAD) for a commercially available lipid mix. The theoretical mass ratio was confirmed.



**Handout available for download on NCL website**

https://ncl.cancer.gov/sites/default/files/PCC_Liposomes.pdf

# PCC Highlights:
# Nucleic Acid Quantitation and LNP Characterization

**Nucleic Acids as APIs and LNPs as carriers**

- Seeing increased application of nucleic acid  and LNP technologies
- NCL has seen concepts with mRNA, miRNA, siRNA, DNA, RNA
- Many collaborations with pharma, biotech, academia to develop characterization methods

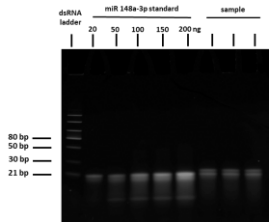
Lilly UREVAC the RNA people®

gsk

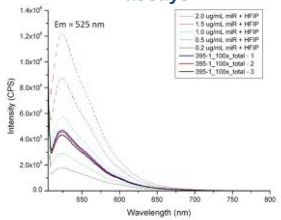
FRED HUTCH CURES START HERE®

JOHNS HOPKINS BIOMEDICAL ENGINEERING

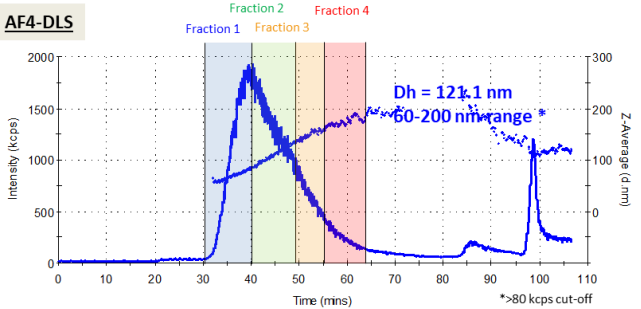
**Gel-based assays**




**Fluorescence-based assays**




**AF4-DLS**




**NCL is developing methods to accurately quantitate total, free and particle-bound nucleic acid components**

**NCL is developing AF4-based methods that are being used to improve the production process of LNPs**

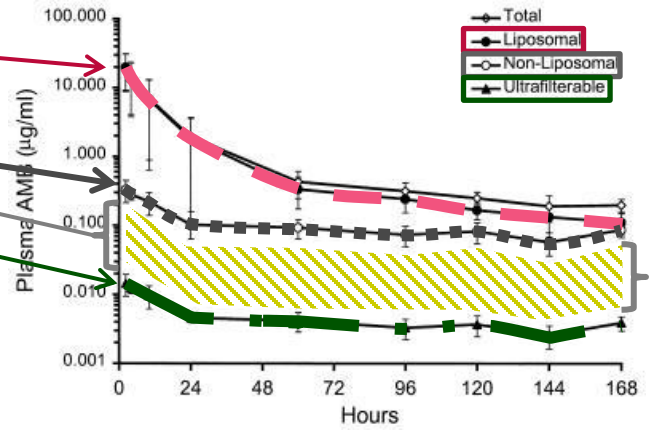
# Nanomedicine Pharmacokinetics

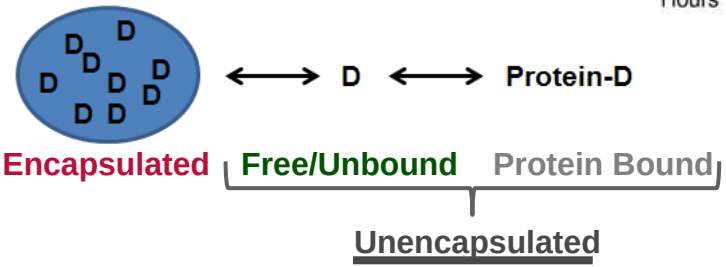
**Nanomedicine drug fractions in circulation:**

I. NM encapsulated fraction

**II. Unencapsulated fraction**

- protein bound fraction
- unbound fraction



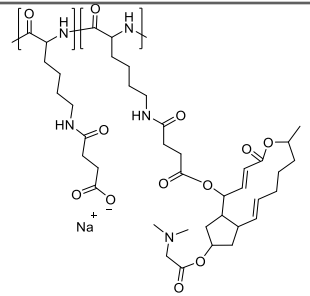




**All drug fractions can be important for characterizing nanomedicine PK.**

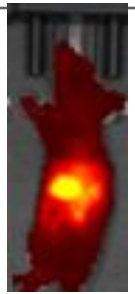
Bekersky et. al, *Antimicrob Agents Chemother* 2002, 46(3):834-40.

# DCTD Brefeldin Macromolecular Prodrug Formulation

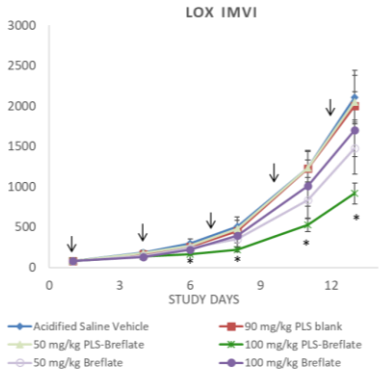
| Brefeldin Characteristics | | | |
|---|---|---|---|
| **Active Pharmaceutical Ingredient (DTP identifier)** | **Stage of Development** | **Basis for Halting Development** | **Project Goal for Nanotechnology** |
| (+) Brefeldin-A (NSC 089671) | Preclinical | DLT neurotoxicity | An IV dose of the API-nanoformulation achieves and maintains a plasma concentration ≥ 1 μM for 72 hours. |



**Brefeldin PLS prodrug**



Tumor accumulations of Cy7.5 labeled PLS

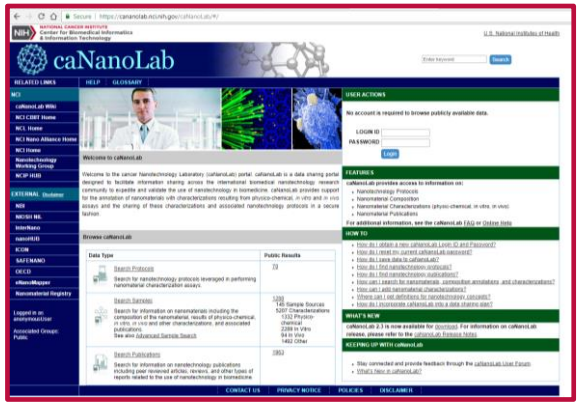



- **Tumor-targeted PLS polypeptide prodrug w/ 13% brefeldin w/w loading**
- **Improved anti-tumor activity in comparison to breflate (brefeldin small molecule prodrug)**

# caNanoLab Data Portal: A Resource for Data Sharing

## caNanoLab Goal

**To provide a nanotechnology resource that facilitates data sharing in the community to expedite and validate the use of nanomaterials in biomedicine**



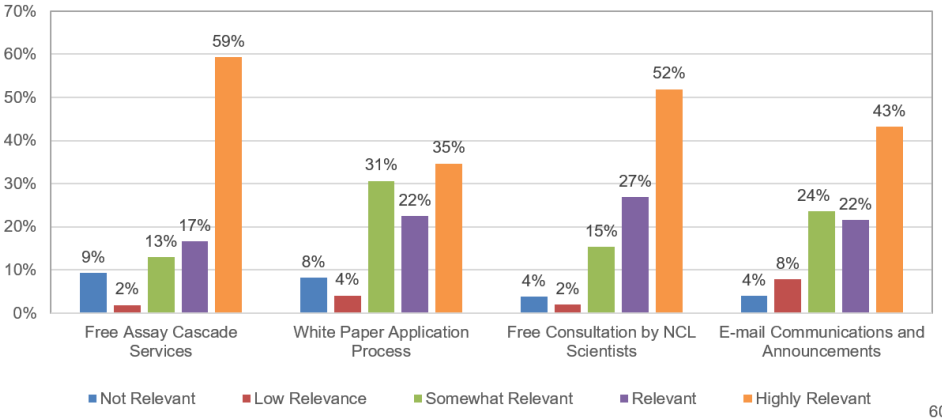

Home Page

- Provides support for the annotation of nanomaterials with composition information, and physico-chemical, *in vitro*, and *in vivo* characterizations
- Provides access to curated information from nanomaterial and nanomedicine samples, protocols, and publications

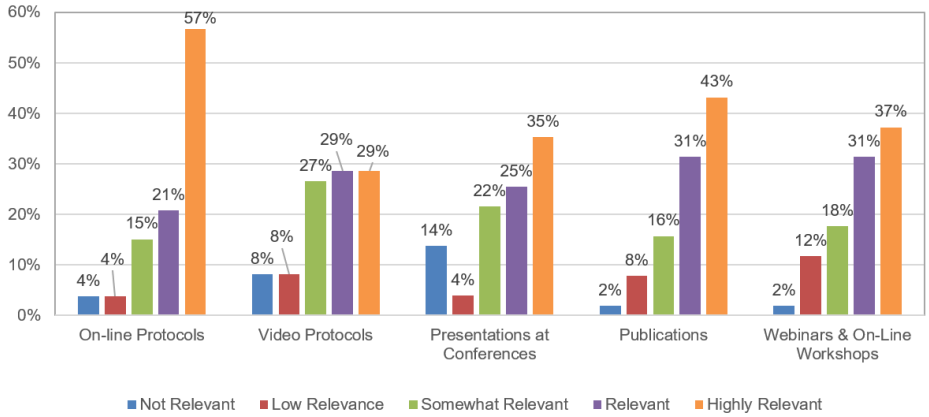
https://cananolab.nci.nih.gov/caNanoLab/

# NCL Operations and Provided Resources are Highly Relevant
# KPI Survey

**Please rank various aspects of NCL operations as they relate to your research needs**




**Please rank the importance of resources provided by the NCL to your research needs**



# Nanotechnology Characterization Lab (NCL)
# Summary

- NCL is a laboratory with unique set of capabilities supporting characterization of nanomaterials and nano-devices used in nanotechnology-based medical interventions
- Over last 16 years, NCL characterized over 450 different nanomaterials and established body of literature on nanoparticle designs and their design correlations with safety and toxicity
- NCL educates research community and disseminates knowledge on evaluation of nanomedicines
- NCL has worked with a diverse pool of researchers representing academia, industry, and government
- NCL is involved in standardization of nanomedicine characterization tools and nanomaterial standards

# Acknowledging the NCL Team



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