NCL Nanotechnology Characterization Laboratory

NCI Alliance for Nanotechnology in Cancer

Preclinical Evaluation Strategies for Nanomedicines and Other Non-Biological Complex Drugs

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SAPhS
Swiss Academy
of Pharmaceutical
Sciences

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Overview

- **Definitions & Challenges**
  - Non-Biological Complex Drugs (NBCDs)
  - Critical Quality Attributes (CQAs)

- **Addressing Characterization Challenges**
  - Approaches to help identify CQAs early in the process
  - NCL characterization aims to identify CQAs

- **Therapeutic Equivalence**
  - Follow-on nanomedicines are emerging
  - Need methods to properly evaluate bioequivalence
  - NCL develops new method to test follow-on nanomedicine

Non-Biological Complex Drugs (NBCDs)

- Fully synthetic materials that are medicinal products but not biological medicines
- Active substance is not homomolecular but contains different closely related structures
- Cannot be fully characterized by physicochemical analytical means
- **Nanomedicines** are a type of NBCDs

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Issue: Annals *Reports*
CONCISE ORIGINAL REPORT

Equivalence of complex drug products: advances in and challenges for current regulatory frameworks

Leonie Hussaarts,[1] Stefan Mühlebach,[2] Vinod P. Shah,[3] Scott McNeil,[4] Gerrit Borchard,[5] Beat Flühmann,[2] Vera Weinstein,[6] Sesha Neervannan,[7] Elwyn Griffiths,[8] Wenlei Jiang,[9] Elena Wolff-Holz,[10] Daan J.A. Crommelin,[11] and Jon S.B. de Vlieger[1]

For more information:

Non Biological Complex Drugs working group

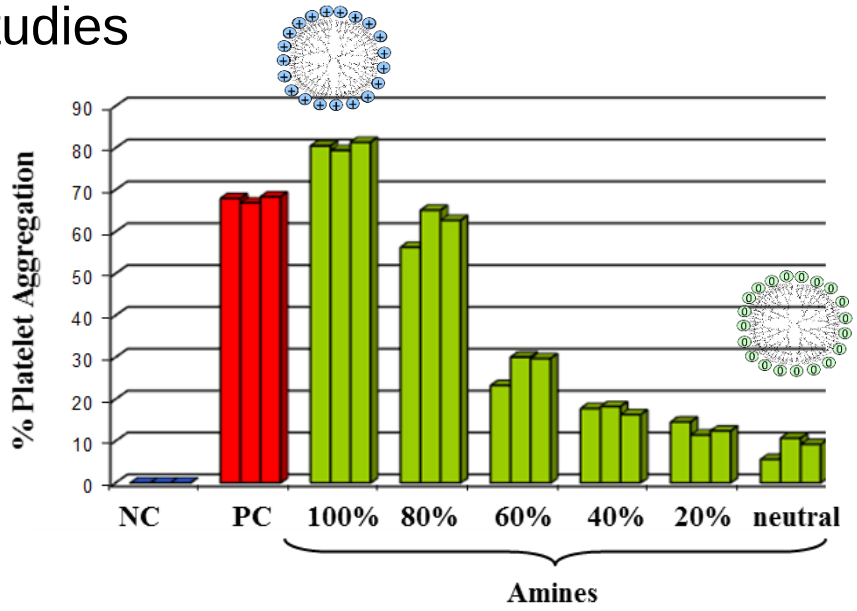
http://lygature.org/non-biological-complex-drugs-nbcd-working-group

Cannot be fully defined by physicochemical characterization

Critical Quality Attributes (CQAs)

A critical quality attribute is a chemical, physical, biological or microbiological property that should be within an appropriate limit, range, or distribution to ensure product quality.*

- Unique to each formulation
- Identify by evaluating physicochemical properties, sample heterogeneity, and batch-to-batch consistency against efficacy/other biological studies



Surface charge caused platelet aggregation.

Identifying critical attributes early in the process will speed development.

*** FDA Guidance for Industry: Q8(R2) Pharmaceutical Development, November 2009**

Dobrovolskaia, M.A., et al. Mol Pharm. 2012, 9(3), 382-393.

Challenges of Nano-based NBCDs

New Nano-based NBCDs

What are the critical quality attributes?

- How are critical attributes defined in a complex drug?
- What methods can inform critical attributes for a complex drug?

Follow-on Nano-based NBCDs

How is equivalence assessed for follow-on NBCDs?

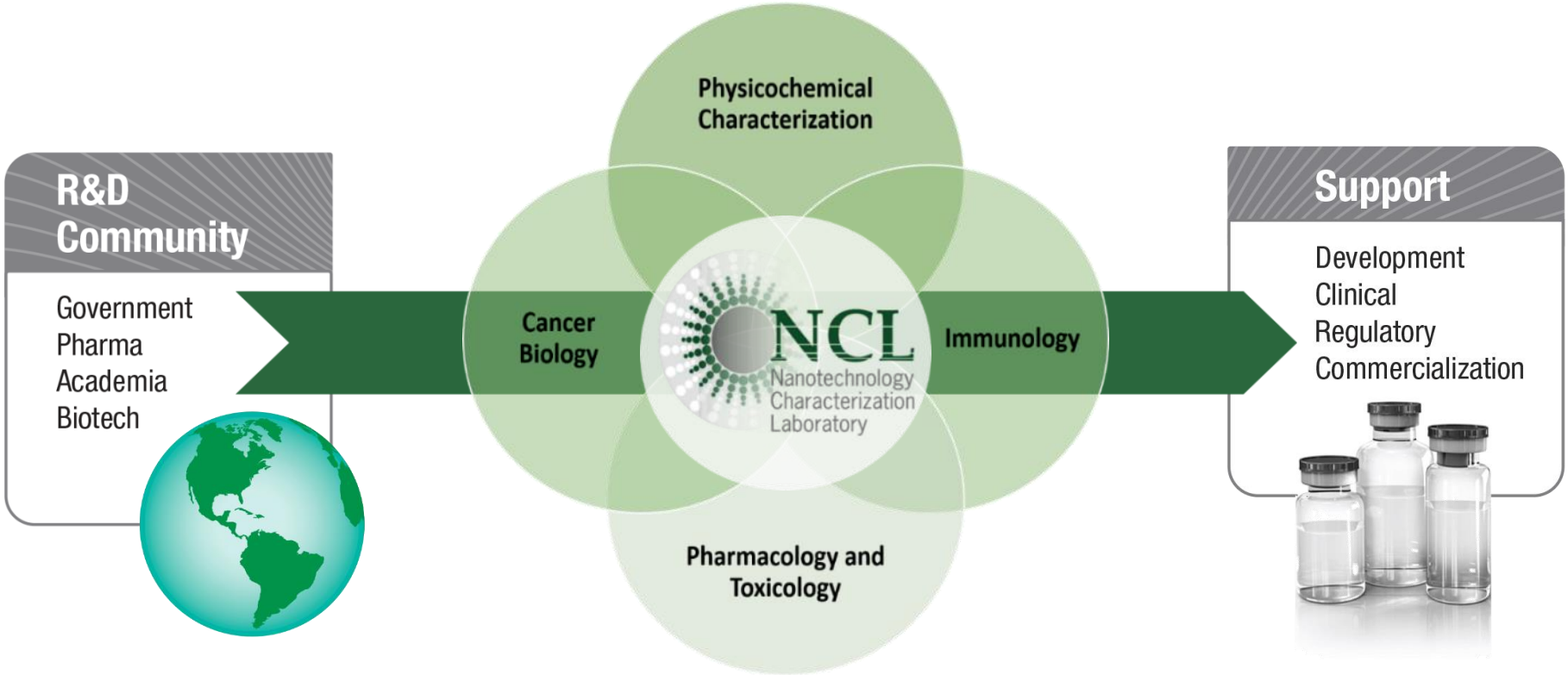
- How is drug release evaluated in a drug product that can have multiple drug fractions in vivo?
- How are multiple drug fractions quantitated?

NCL characterization aims to address these questions

Nanotechnology Characterization Lab

NCL Nanotechnology Characterization Laboratory | NCI Alliance for Nanotechnology in Cancer

Nanotech expertise & resources in multiple disciplines, brought together in one location.



NCL has 14 years of knowledge and expertise in nanoparticle characterization, and utilizes this to help accelerate the translation of promising nanotech drugs and diagnostics.

Visit https://ncl.cancer.gov/

NCL Characterization – 50+ Standardized Protocols for Nanotechnology

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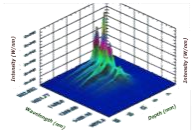


Physicochemical Characterization

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

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

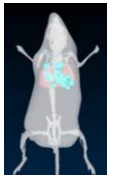
Sterility
- Bacterial/Viral/Mycoplasma
- Endotoxin

Cell Uptake/Distribution
- Cell Binding/Internalization
- Targeting

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

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

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

Single and Repeat Dose Toxicity
- Blood Chemistry
- Hematology
- Histopathology (42 tissues)
- Gross Pathology
- Immunogenicity

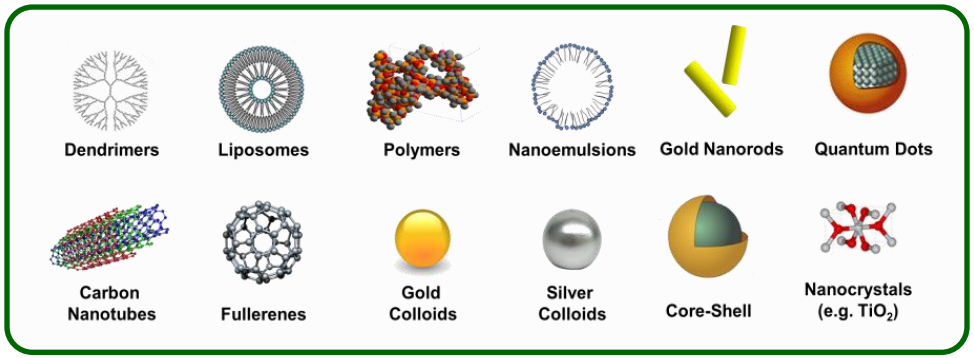
Efficacy
- Therapeutic
- Imaging

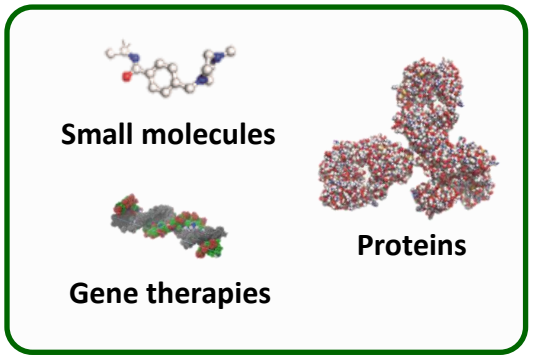
NCL testing links physicochemical properties to biological outcomes.

Protocols Available Online: https://ncl.cancer.gov/resources/assay-cascade-protocols

NCL by the Numbers

400 Different nanomaterials characterized with a wide range of nanotechnologies and APIs





14 NCL collaborators in clinical trials

>150 Extensive pharmacokinetic and toxicological preclinical studies

~200 Peer-reviewed publications

NCL testing is tailored to the platform properties, API, route of administration, and desired outcome of the individual nanomedicine.

NCL has in-depth experience at testing the wide variety of nanomedicines.

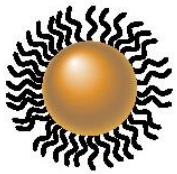
Importance of Defining Critical Attributes

Nanomaterial physicochemical properties greatly influence biocompatibility, but are often challenging to measure, identify.

- Size
- Composition
- Surface coating attachment/density/orientation
- Surface charge
- Shape/architecture
- Stability
- Purity

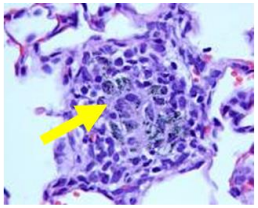
→

- Pharmacokinetics
- Biodistribution
- Clearance
- Toxicity
- Efficacy
- Bioaccumulation



Batch 1

In tox studies, 1st batch caused extensive lung lesions.



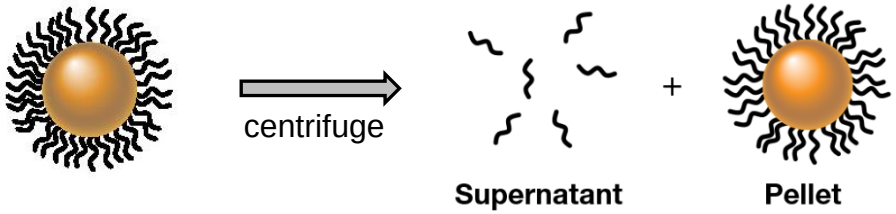
Pyogranulomatous Inflammation-Lung- H&E-40x

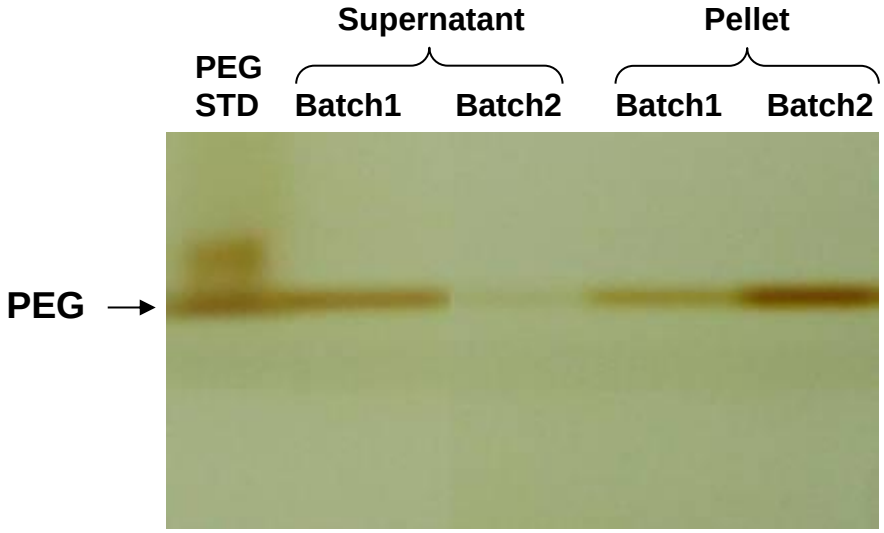
Batch 2

In repeat tox study, 2nd batch was largely benign. No apparent difference between batch 1 and batch 2 in terms of size, charge, or polydispersity.

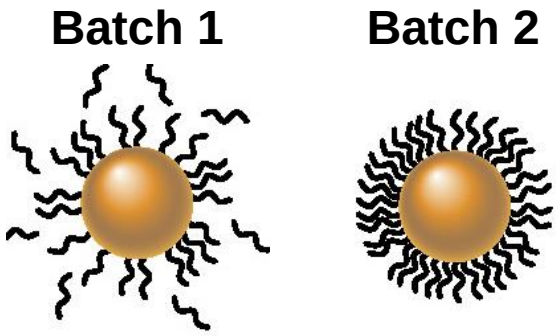
What are the material's critical attributes?
What is causing the dramatically different safety profiles of seemingly identical batches?

Critical Attributes Are Formulation Specific





Barium Iodine Gel Staining



Small differences in surface coating caused dramatically different in vivo outcomes.

PEG was dissociating from the particles over time, ending up in solution.

This difference in coatings was subtle enough not to be detected by routine PCC...but resulted in aggregation *in vivo.*

Critical attribute <u>for this formulation</u> = Surface ligand density

Critical attributes are unique to each formulation.

Critical Attributes Are Formulation Specific

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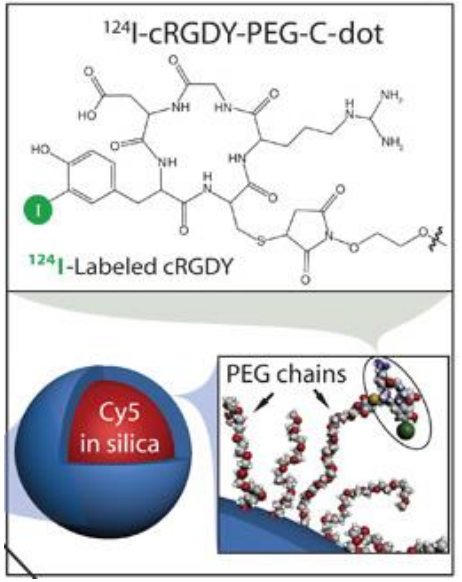
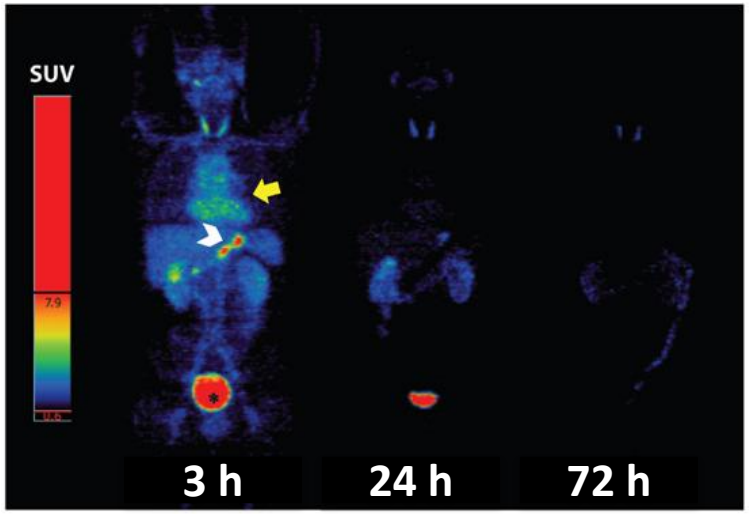
Cornell Dots (C dots)

- **Targeted core-shell hybrid silica nanoparticles as imaging probes**
- **Optical imaging NP and PET-optical NP currently in clinical trials**
- **Size <10 nm**

Size is tuned below effective renal glomerular filtration size cutoff

- **Enables bulk renal clearance**
- **Reduces nonspecific uptake in RES**
- **Enables high target-to-background ratios**





PET images of patient after intravenous injection of 124I-cRGDY-PEG-C dots

Critical attribute <u>for this formulation</u> = Ultrasmall size

RES: Reticuloendothelial system; SUV: Standard uptake value = [activity per gram of tissue]/[administered active per gram of body mass]

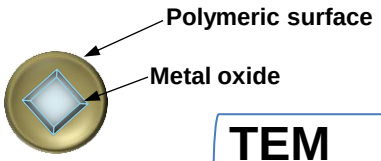
Phillips, E. et al. Clinical translation of an ultrasmall inorganic optical-PET imaging nanoparticle probe. *Science Translational Medicine* **6**, 260ra149-260ra149 (2014).

Identifying critical quality attributes during preclinical characterization

Test Multiple Batch Testing

Monitor batch-to-batch consistency for factors that influence biocompatibility, safety and efficacy.

Size differences can affect pharmacological and toxicological profiles.



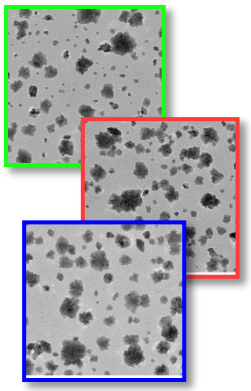
Size Characterization

TEM **Batch-mode DLS** **AF4-MALLS**

Lot 1

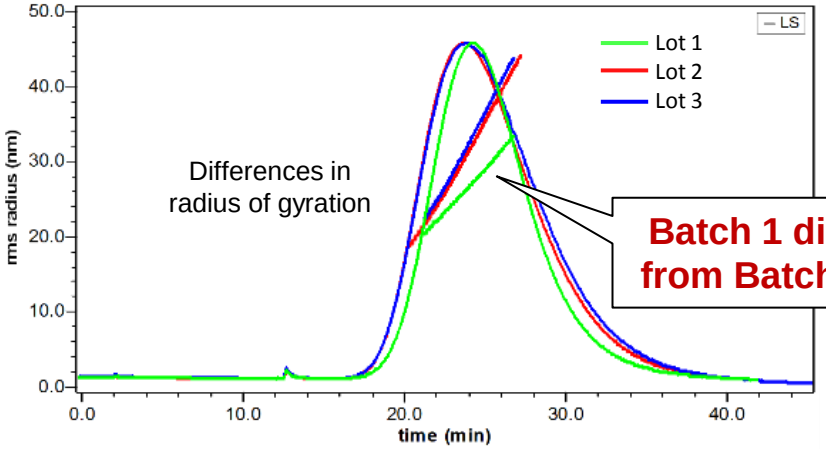
Lot 2

Lot 3



Avg. Diameter (nm)
20 ± 17
22 ± 16
23 ± 17

No difference in size by TEM, DLS.



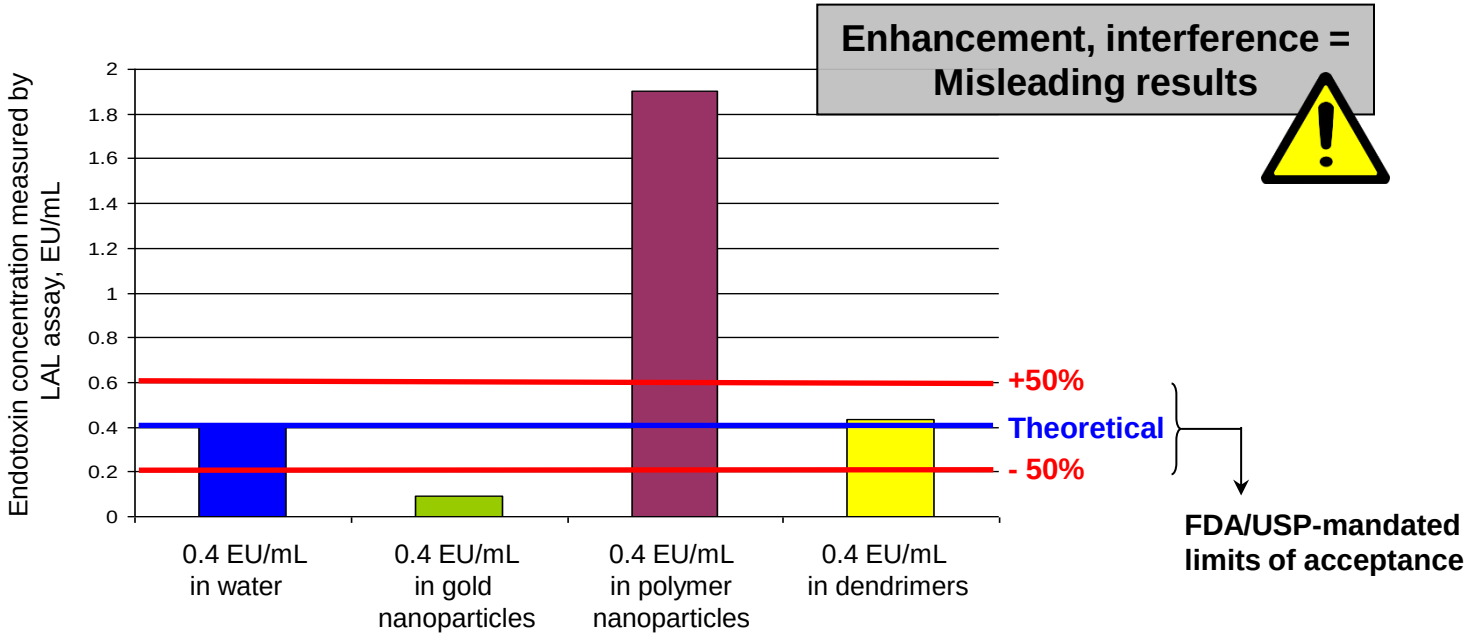
Flow-mode detects differences batch-mode cannot.

Drawing conclusions from only one batch can lead to false claims and irreproducible results.

Use Informative Controls

Proper controls will help identify when a formulation interferes with an assay.

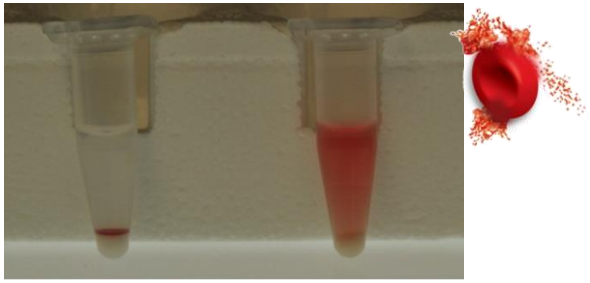
- Nanoparticles may interfere, inhibit, or enhance in vitro assays (e.g. hemolysis, complement activation, LAL assays).
- Assays need to be verified with proper inhibition/enhancement controls (IEC).



Test in Biological Matrix

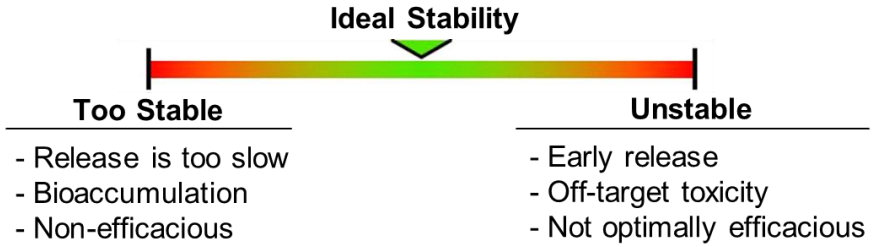
Testing only in buffer can be misleading.

- Some nanoformulations may be unstable in biological matrix, precipitating immediately after addition to plasma, serum or blood.
- Nanomedicines held together by non-covalent interactions (e.g. charge-charge, hydrophobic interactions) may not stay together in vivo as these interactions are environment dependent.
- Unstable nano platforms may not adequately shield the toxic payloads they carry, causing toxicities similar to that of free drug.



Blank Emulsion | Peptide Emulsion

Particles precipitated immediately after addition to whole blood, causing hemolysis.



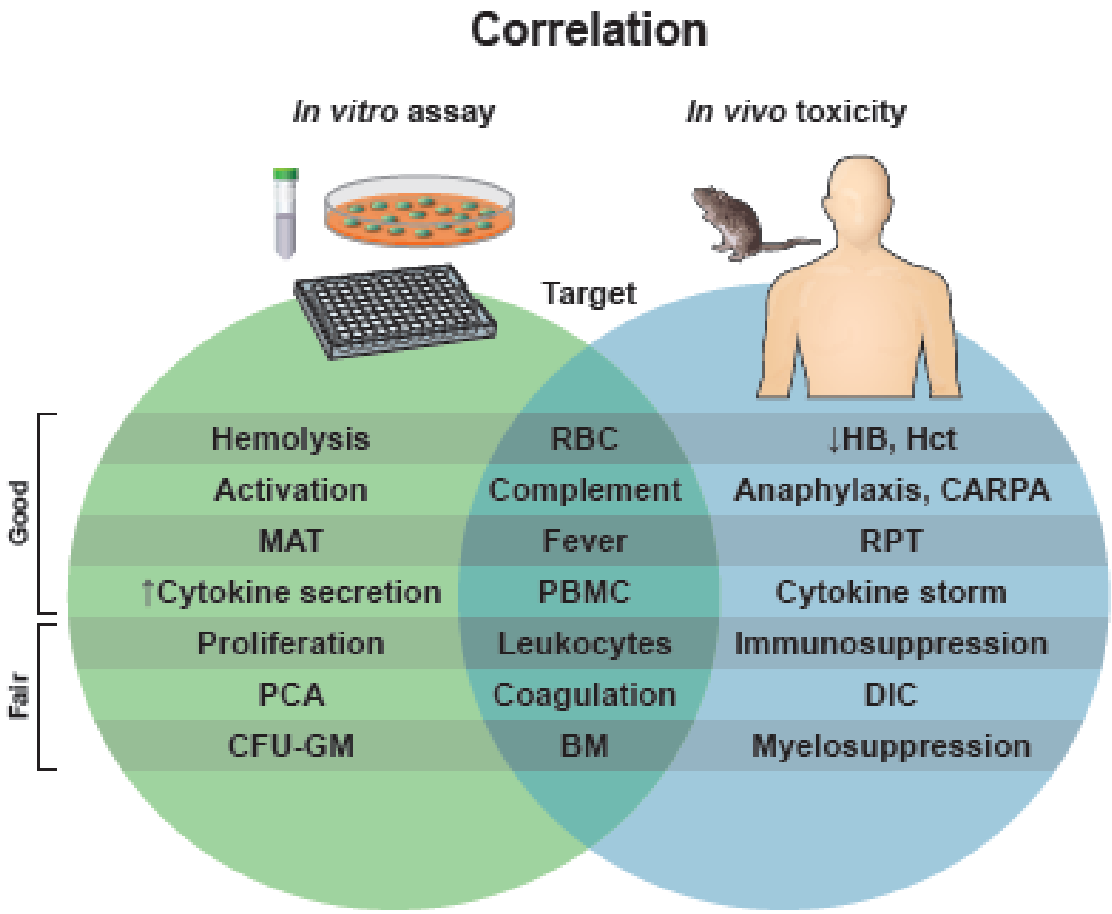
In vitro assays measuring drug release in plasma can be predictive of PK parameters in vivo.

Utilize Predictive In Vitro and In Vivo Models

- Animal models should be chosen based on intended clinical indication (e.g. orthotopic implant vs. xenograft).
- Studies should be performed using intended clinical route of administration (e.g. don't use i.p. for animal studies if planning to pursue i.v. clinically).
- Biology of the tumor can affect cancer nanomedicine distribution (e.g. tumor size, anatomical location, proliferation rate, vascular density, stromal composition, etc.).
- Toxicity and efficacy claims must match biological model (e.g. rodents are not sensitive to irinotecan delayed GI toxicity).
- Use sufficient animal numbers for toxicity and efficacy studies.

Careful design of animal models is critical to understand CQAs.

Immunoassays With Good IVIVC



NCL has protocols available for these in vitro assays:
https://ncl.cancer.gov/resources/assay-cascade-protocols

Dobrovolskaia MA, McNeil SE. J Control Release. 2013;172(2):456-66.

Surface Coating Quantitation

Nanomedicine surface properties are critical to biodistribution and toxicity, but challenging to measure.

Polyethylene glycol (PEG) is the most common type of coating polymer on nanoparticles for biomedical applications

- Chemically inert backbone
- Provides 'stealth property' to evade immune recognition
- End groups for covalent linkage (-SH, -NH_2, -COOH, etc.)
- Does not have chromophore
- Insensitive for UV-Vis or fluorescence detection


EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

22 May 2013
EMA/325027/2013
Committee for Medicinal Products for Human Use (CHMP)

Reflection paper on surface coatings: general issues for consideration regarding parenteral administration of coated nanomedicine products

Most coating polymers/lipids do not have chromophores/fluorophores — UV-Vis & fluorescence detection is not applicable.

Limited methods are available for accurate quantification of surface coatings.

Techniques for Coating Quantification

- **Charged Aerosol Detector (CAD)**
  - Good sensitivity for most lipids and polymers
  - More sensitive than UV-Vis

- **Thermogravimetric Analysis (TGA)**
  - Monitor weight loss as a function of increasing temperature
  - Dehydration, decomposition pattern, organic content analysis

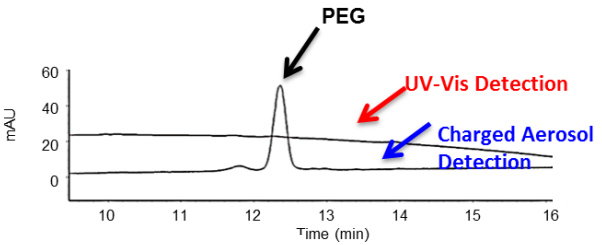
- **Chemical Methods**
  - Example: Thiol containing species - Ellman's Reagent

Multiple orthogonal techniques are best for analyzing physicochemical properties.

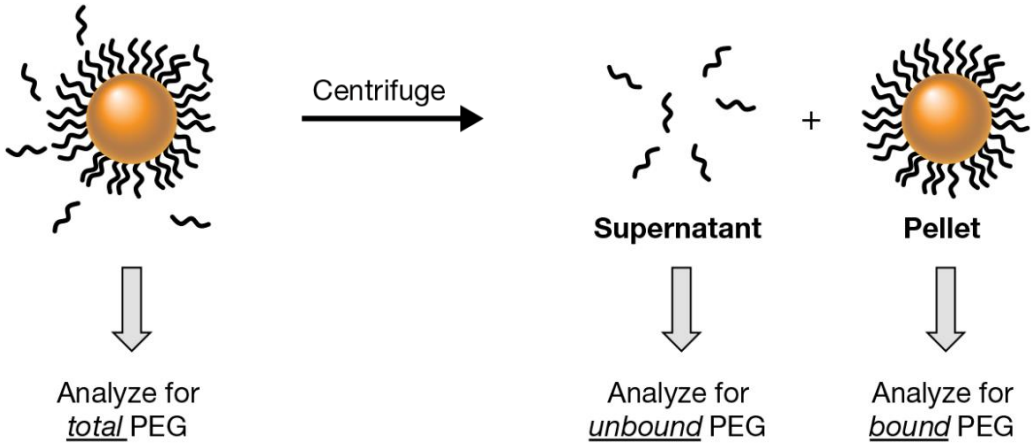
Smith, M.C., Crist, R.M., Clogston, J.D. & McNeil, S.E. Anal Bioanal Chem **407**, 3705-16 (2015).

Quantitate Polymer Coatings: CAD

- Charged Aerosol Detector (CAD)
  - Polyethylene Glycol (PEG), Lipids
  - More sensitive than UV-Vis for most polymers



Applicable to a variety of platforms and coatings



Gold Nanoparticle Sample	Measured Total PEG µg/mL
2 kDa PEGylated	3.4 ± 0.2
5 kDa PEGylated	3.2 ± 0.1
10 kDa PEGylated	7.9 ± 0.3
20 kDa PEGylated	31.4 ± 1.1

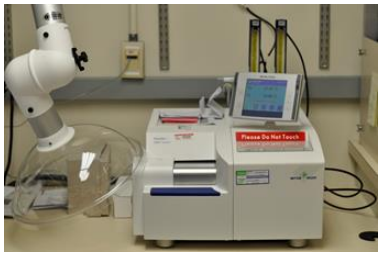
BLOD = below limit of detection; BLOQ = below lower limit of quantitation

Good agreement between measured total PEG and summation of bound and unbound PEG.

Smith, M.C., Crist, R.M., Clogston, J.D. & McNeil, S.E. Anal Bioanal Chem **407**, 3705-16 (2015).

Quantitate Polymer Coatings: TGA

- Thermogravimetric Analysis (TGA)
  - Measure total protein on metal oxide
  - Monitor weight loss as a function of increasing temperature
  - Dehydration, decomposition pattern, organic content analysis

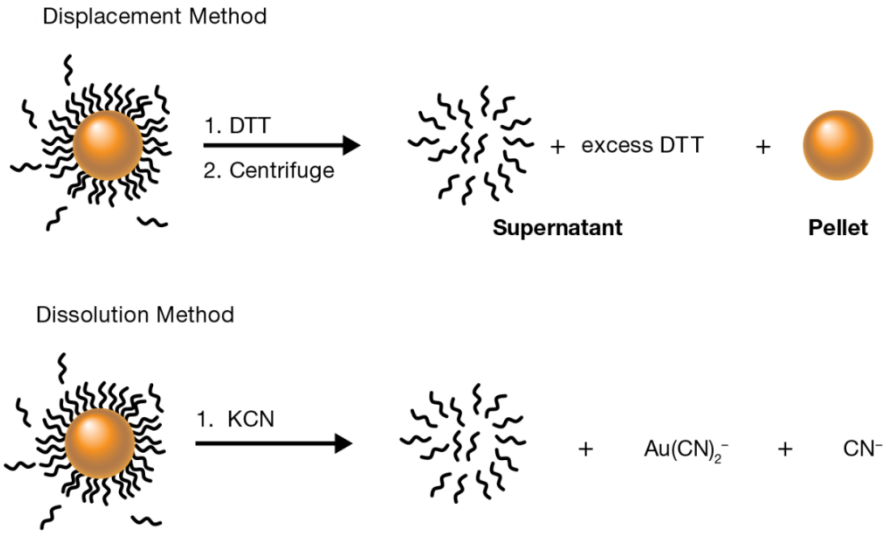


Water loss

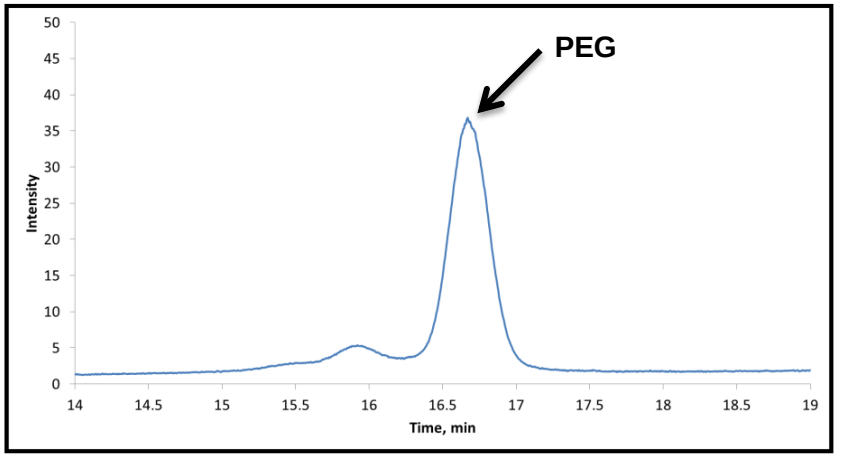
Single TGA run gives both protein and metal oxide concentrations.

Quantitate Polymer Coatings: Chemical Methods

- Chemical Methods
  - Thiol containing species - Ellman's Reagent
  - Dissolution of gold nanoparticle



HPLC
CAD Detection



Gold Nanoparticle Sample	Total PEG Concentration µg/mL	
	Displacement Method	Dissolution Method
2 kDa PEGylated	3.3 ± 0.1	3.4 ± 0.2
5 kDa PEGylated	3.3 ± 0.1	3.2 ± 0.1
10 kDa PEGylated	7.9 ± 0.3	8.4 ± 0.4
20 kDa PEGylated	31.4 ± 1.1	34.5 ± 1.1

Good agreement between displacement and dissolution methods.

Robust, reproducible methods for quantifying total PEG.

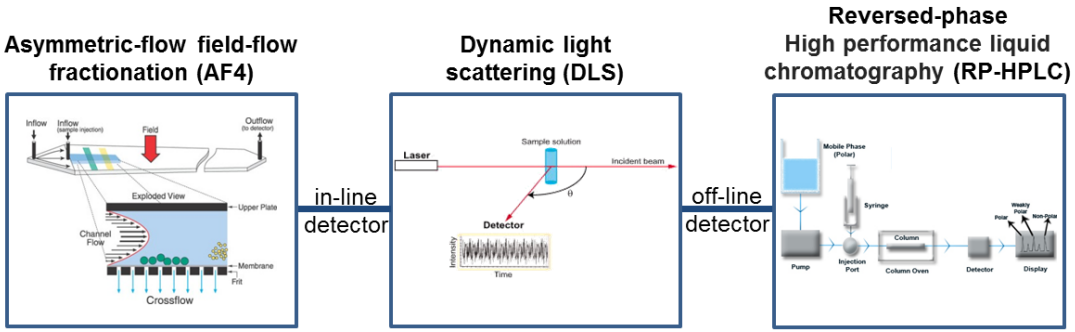
Smith, M.C., Crist, R.M., Clogston, J.D. & McNeil, S.E. Anal Bioanal Chem **407**, 3705-16 (2015).

Nanoparticle Heterogeneity

Nanomaterials are inherently heterogenous; what are acceptable limitations in heterogeneity?

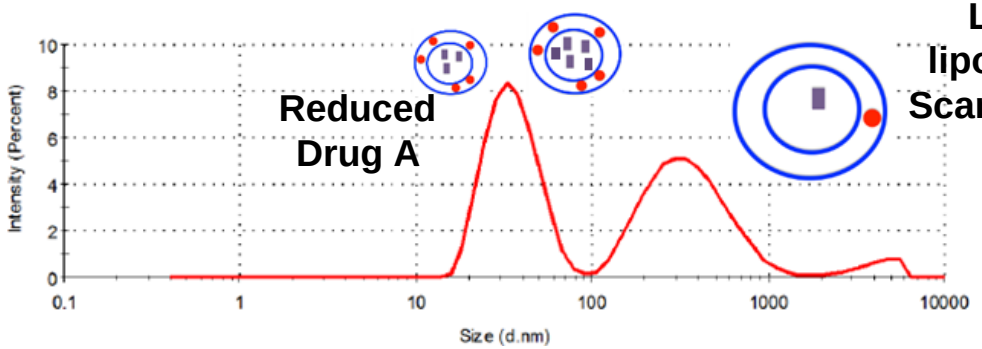
Asymmetric Flow Field Flow Fractionation (AF4)

- AF4 separates size populations, defines polydispersity
- Various in-line/off-line detectors can define drug/ligand distribution



Dual drug loaded liposomes

Where are the drugs?



Larger liposomes; Scarce drugs

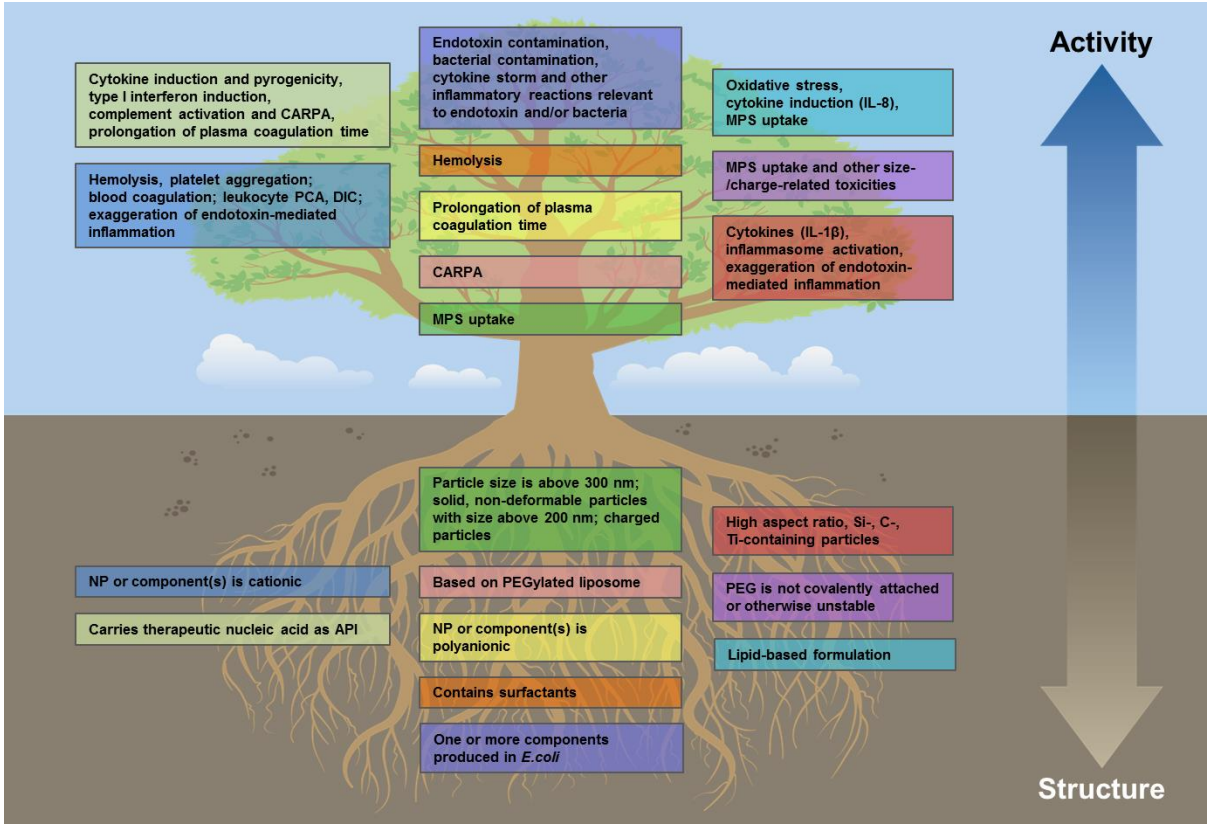
Defining sample heterogeneity with respect to efficacy can help identify limits.

Immunosafety

Common reasons for preclinical and early clinical failure of nanoformulated drugs:

- Endotoxin contamination
- Cytokine storm
- Hypersensitivity Reactions
- Complement activation
- Thrombogenicity (DIC)
- Exaggeration of API immunotoxicity by nanoparticle carrier

Lessons Learned: Structure Activity Relationships

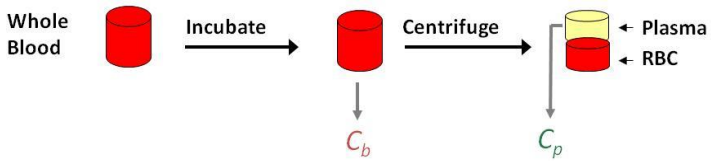


Most toxicities can be assessed rapidly using in vitro models, many with good in vitro-in vivo correlation.

Dobrovolskaia MA & McNeil SE, J Control Release. 2013 172(2), 456-466.
Dobrovolskaia, M.A., et al. Toxicol Appl Pharmacol. **2016**, 299, 78-89.

Methods for Estimating Drug Release

- Blood partitioning assay



$$F_p\% = (C_p / C_b) \times (1 - H_c) \times 100$$

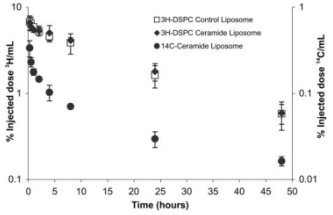
Plasma Fraction

Hematocrit

Concentrations in plasma (C_p) and blood (C_b)

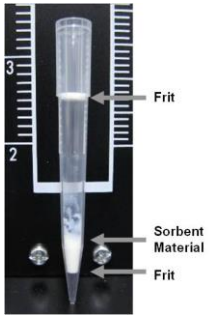
Zolnik et al., *Drug Metab Dispos.* 2008, 36(8):1709-15.

- Dual labeling/complementary analysis in vivo

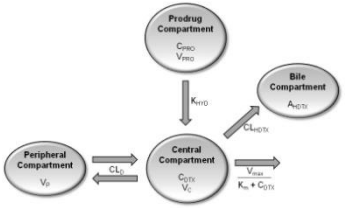


Zolnik et al., *Drug Metab Dispos.* 2008, 36(8):1709-15.

- New extraction methods to separate free and encapsulated

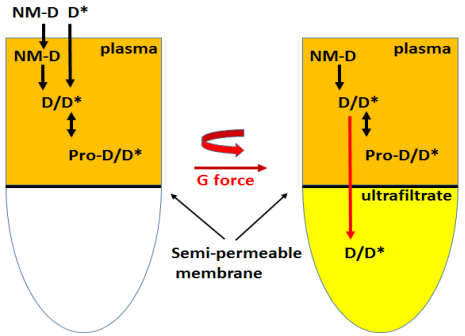


- Metabolite modeling to predict free drug



Stern ST et al., *J Control Release,* 2013, 172(2), 558-567.

- Measurement of fraction unbound to quantify drug encapsulated/released



Stern et al. J Control Release. 2013 Dec 10;172(2):558-67.

NCL has several tools to evaluate drug release in vitro before spending resources on costly animal studies.

Analytical Toolbox to Identify Critical Attributes

Quantitation of Nanoparticle Coating

- Measuring total, bound and unbound PEG, lipids and proteins on nanoparticles

Probing Nanoparticle Heterogeneity

- Resolving nanoparticle size populations
- Measuring drug/ligand loading in different fractions

Immunosafety Assessment

- Identifying physicochemical parameters responsible for immunotoxicities

Nanomedicine Fractionation

- Quantitating free/unbound drug, protein-bound, and formulation-bound drug fractions

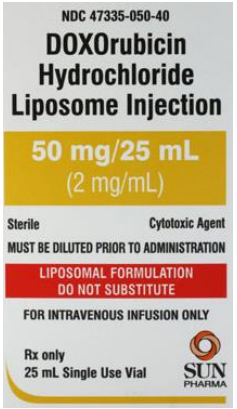
How is equivalence assessed for follow-on NBCDs?

Follow-on Nanomedicines

NCL Nanotechnology Characterization Laboratory | NCI Alliance for Nanotechnology in Cancer

The First Follow-on Nanomedicine

- Doxorubicin HCl Liposome Injection, a generic version of Doxil, was the first generic nanomedicine approved by the FDA (2013).



Nanomedicines are complex formulations, and there will always be some degree of polydispersity and batch-to-batch variation. The challenge is to identify meaningful differences between the follow-on and the reference/innovator product.

More Follow-on's are Coming

IPSEN Innovation for patient care

- Merrimack/Actavis(Teva subsidiary)'s generic doxorubicin hydrochloride liposome injection is currently under FDA review.
  - Rights to generic liposomal doxorubicin sold to Ipsen in 2017.

MERRIMACK PHARMACEUTICALS

- Sorrento Therapeutics completed a bioequivalence study of Cynviloq against nab-paclitaxel.
  - NantWorks acquired rights to Cynviloq in 2015.

NANTWORKS

SORRENTO THERAPEUTICS

As the number of FDA-approved nanomedicines continues to grow, developing a framework for evaluating follow-on products becomes increasingly critical.

Bioequivalence for Follow-On NBCDs

Generic drug products, **including non-biological complex drugs (NBCDs)**, are approved based on therapeutic equivalence to the reference/innovator product

Therapeutic Equivalence =

Pharmaceutical Equivalence + **Biological Equivalence**

Same dosage form and excipients

Equivalent clinical safety and efficacy

- Bioequivalence is surrogate marker for biological equivalence to study generic or follow-on products.
- Assumption is that equivalent clinical pharmacokinetics results in equivalent biological effects.
- Investigating bioequivalence of nanomedicines and other NBCDs is not an easy task.

Clinical Trials of Bioequivalence

Nab-Paclitaxel (Abraxane)

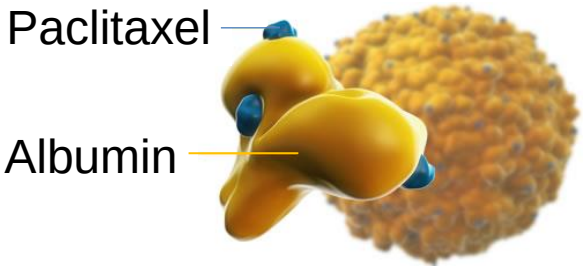


Image: http://www.abraxane.com/hcp/about/overview/

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via FDA 505(b)2

Cb-Paclitaxel (Cynviloq, Genexol-PM)

In co-development by Sorrento and NantWorks in US

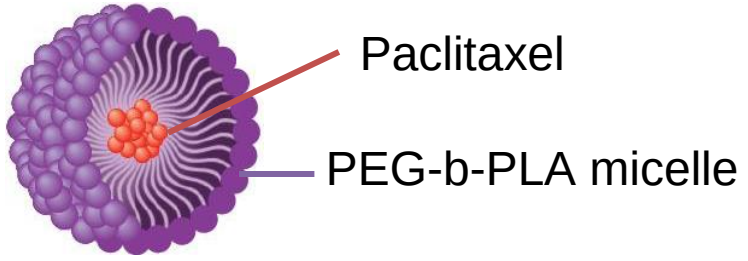
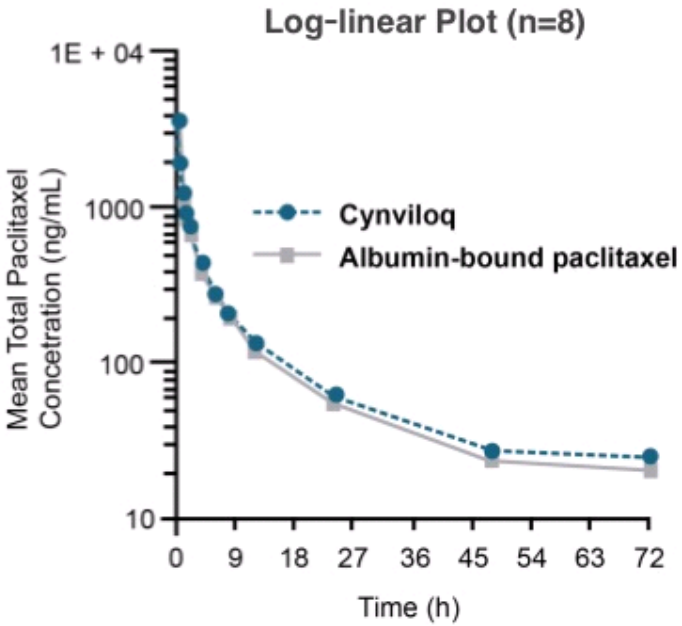


Image: Sorrento, Investor Presentation, June 2014

Pharmacokinetic Equivalence

First 8 patients enrolled in clinical trial demonstrated similar PK profile with albumin-bound paclitaxel

Ref: Sorrento, Investor Presentation, February 2015



- What is the [encapsulated drug] vs. [unencapsulated drug] fractions?
- How do those drug fractions compare to albumin-bound paclitaxel?

Bioequivalence Study of IG-001 Versus Nab-paclitaxel in Metastatic or Locally Recurrent Breast Cancer (TRIBECA)
ClinicalTrials.gov Identifier: NCT02064829

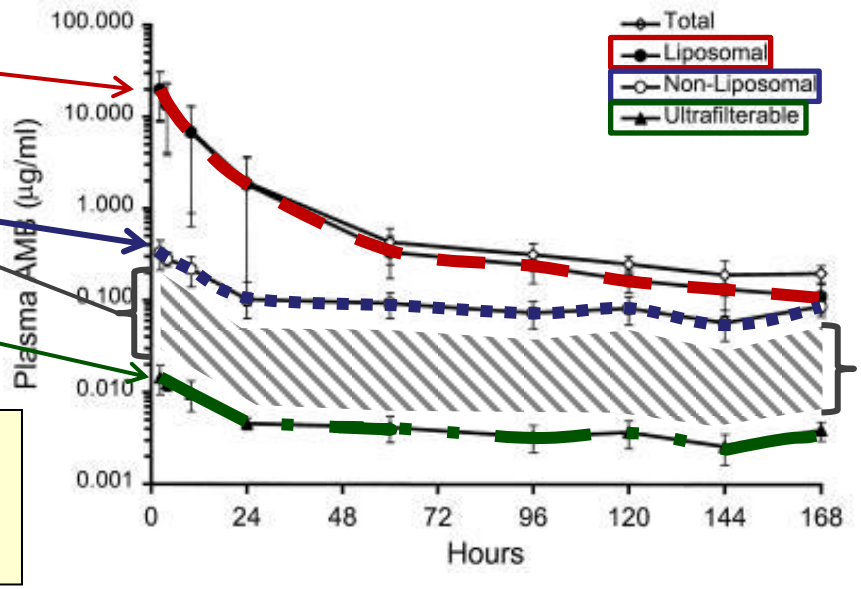
Nanomedicine Pharmacokinetics

Nanomedicine PK is more complex than small molecules; multiple nanomedicine drug fractions in circulation:

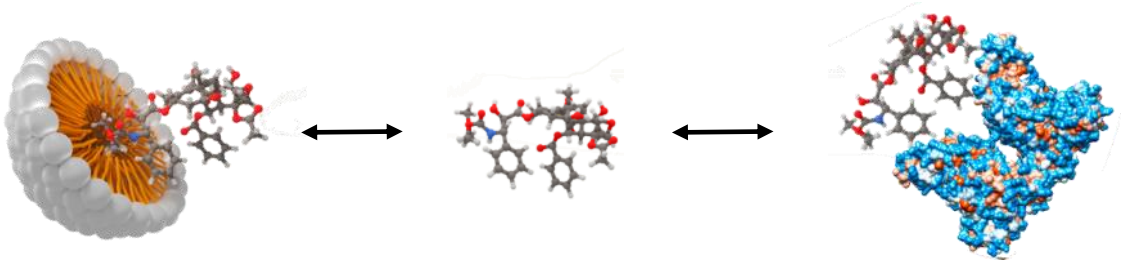
I. NM encapsulated fraction

II. Unencapsulated fraction

- 1-fu: protein bound fraction
- fu : unbound fraction



Evaluation of drug release and quantitation of unencapsulated drug fraction are critical for bioequivalence studies.

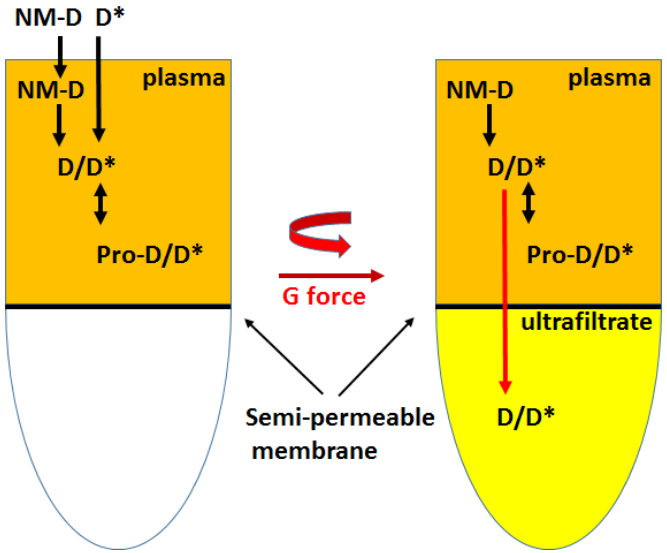


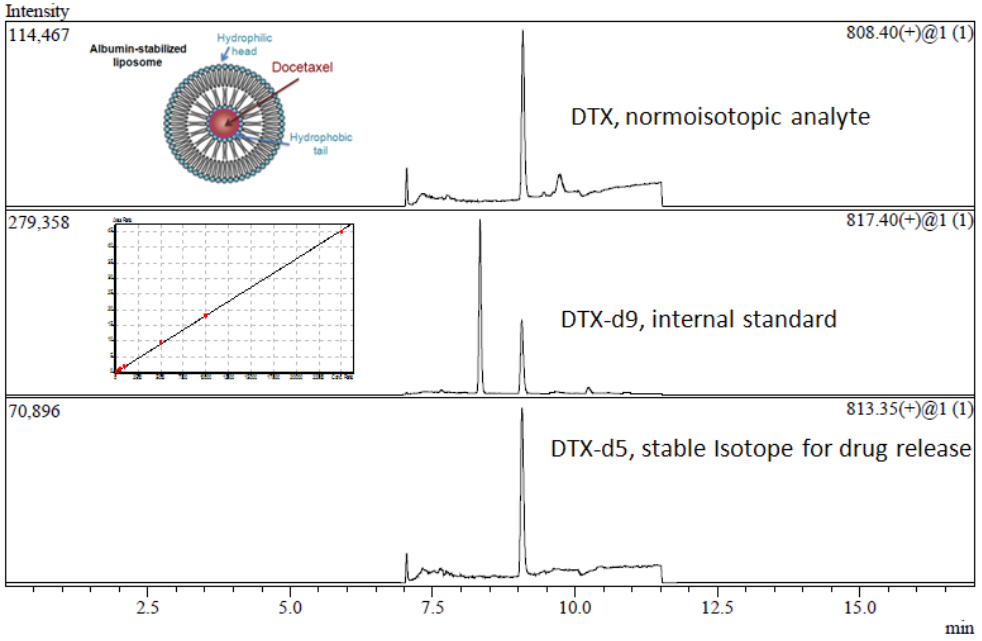
Nanomedicine Encapsulated | **Free/Unbound** | **Protein Bound**

Unencapsulated

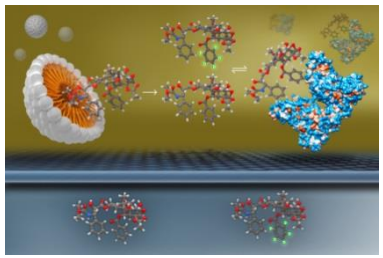
Bekersky et. al, Antimicrob Agents Chemother 2002, 46(3):834-40.
Skoczen et al. J Control Release. 2015, 220(Pt A):169-74.

Novel Stable Isotope Tracer Method to Measure Nanomedicine Drug Fractions





- Stable isotopically labeled drug (**D***) equilibrates with protein and unlabeled, normoisotopic drug (**D**) released from nanomedicine (NM) formulation.
- % **D*** bound estimation gives reliable prediction of %**D** bound.



$$\textbf{\%Bound} = \frac{([\text{Total D*}] - [\text{Ultrafilterable D*}]) * 100}{[\text{Total D*}]}$$

$$\textbf{[Unencapsulated D]} = \frac{[\text{Ultrafilterable D}]}{(1-(\%\text{Bound D*}/100))}$$

$$\textbf{[Encapsulated D]} = [\text{Total D}] - [\text{Released D}]$$

Cover Article: J Control Release, 2015, 220(Pt A):169-74.

Improved Ultrafiltration Method to Measure Drug Release from Nanomedicines Utilizing a Stable Isotope Tracer In S. McNeil (ed) *Characterization of Nanoparticles Intended for Drug Delivery*. Methods in Molecular Biology. Vol. 1628, 2018, Humana Press, New York, NY. p. 223-239.

Summary – NCL Findings

- NBCDs cannot always be fully defined by physicochemical characterization
- Bioequivalence studies may require evaluation of drug release
- New methods can improve evaluation of drug fractions in NBCDs for bioequivalence studies

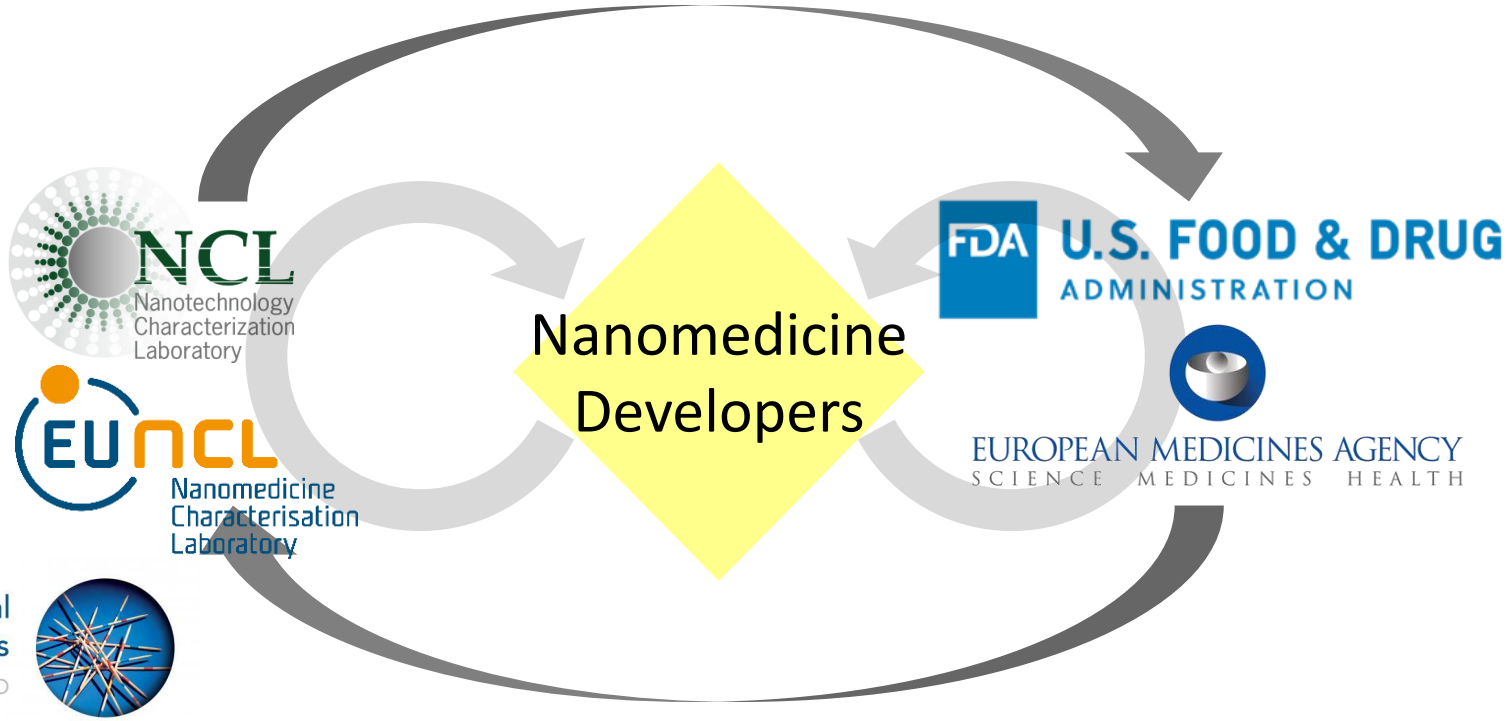
➢ NBCDs require new or improved analytical methods

➢ NCL is developing assays to evaluate bioequivalence among NBCDs

Informing the Regulatory Process

NCL/EUNCL develop new methods and bioanalytical tools that can inform regulatory decisions.

FDA/EMA engage nanomedicine community and NCL/EUNCL to identify best methods to evaluate nanoformulations and NBCDs.



NCL/EUNCL (and NBCD) WG identify developmental challenges from nanomedicine developers and produce IND-enabling data.

FDA/EMA are learning from the influx of nanomedicines under clinical review.

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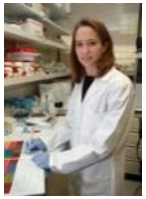

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