Presentation to

ASTM E56 Workshop

May 19, 2005

Scott McNeil, Ph.D.
Director, Nanotech Characterization Lab
SAIC-Frederick

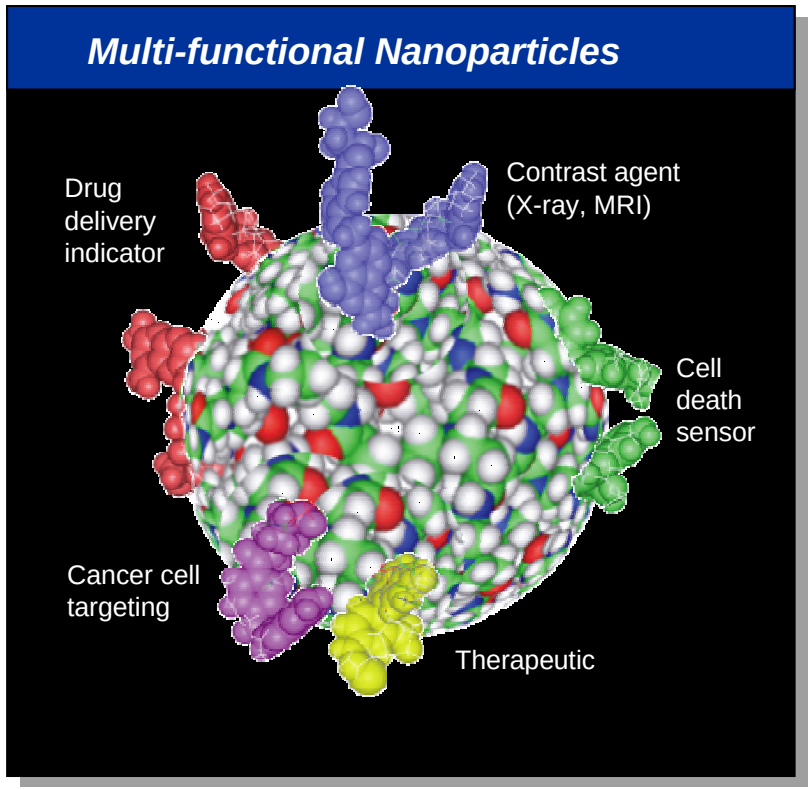
Outline

- Background
- NCL
- Assay Cascade
  - Physical Characterization (Dr. Patri)
  - In vitro and in vivo Pharm/Tox (Dr. Stern)

Why Nano?

Therapeutic Benefits

- Solubility
  - Carrier for hydrophobic entities
- Multifunctional capability
- Active and passive targeting
  - Ligands; size exclusion
- Reduced toxicity



Jim Baker, University of Michigan

Nanotech at NCI

Background

- NCI has funded exploratory work over the past 6 years on integrating nanotechnology into biomedical research
- Unconventional Innovations Program (UIP)
  - Diagnostics (Imaging)
  - Therapeutics
- Priority is to now transition that research into the clinical realm.

- Run by Office of Technology and Industrial Relations (OTIR)
  - Director: Dr. Greg Downing
  - Extramural Budget: $144M over 5 years
  - Launched on Sept 13th, 2004
  - Website: http://nano.cancer.gov/

- Consensus among cancer researchers that significant obstacles must be overcome in order to transition 'nano' to clinical realm
  - Critical lack of available standards
  - 1st principles characterization
  - Regulatory uncertainty

The Nanotechnology Characterization Laboratory

Mission Statement

- The Nanotechnology Characterization Laboratory (NCL) will perform and standardize the pre-clinical characterization of nanomaterials developed by researchers from academia, government, and industry.
- The NCL will serve as a national resource and knowledge base for cancer researchers, and facilitate regulatory review and translation of nanomaterials and devices into the clinical realm.

NCL Objectives

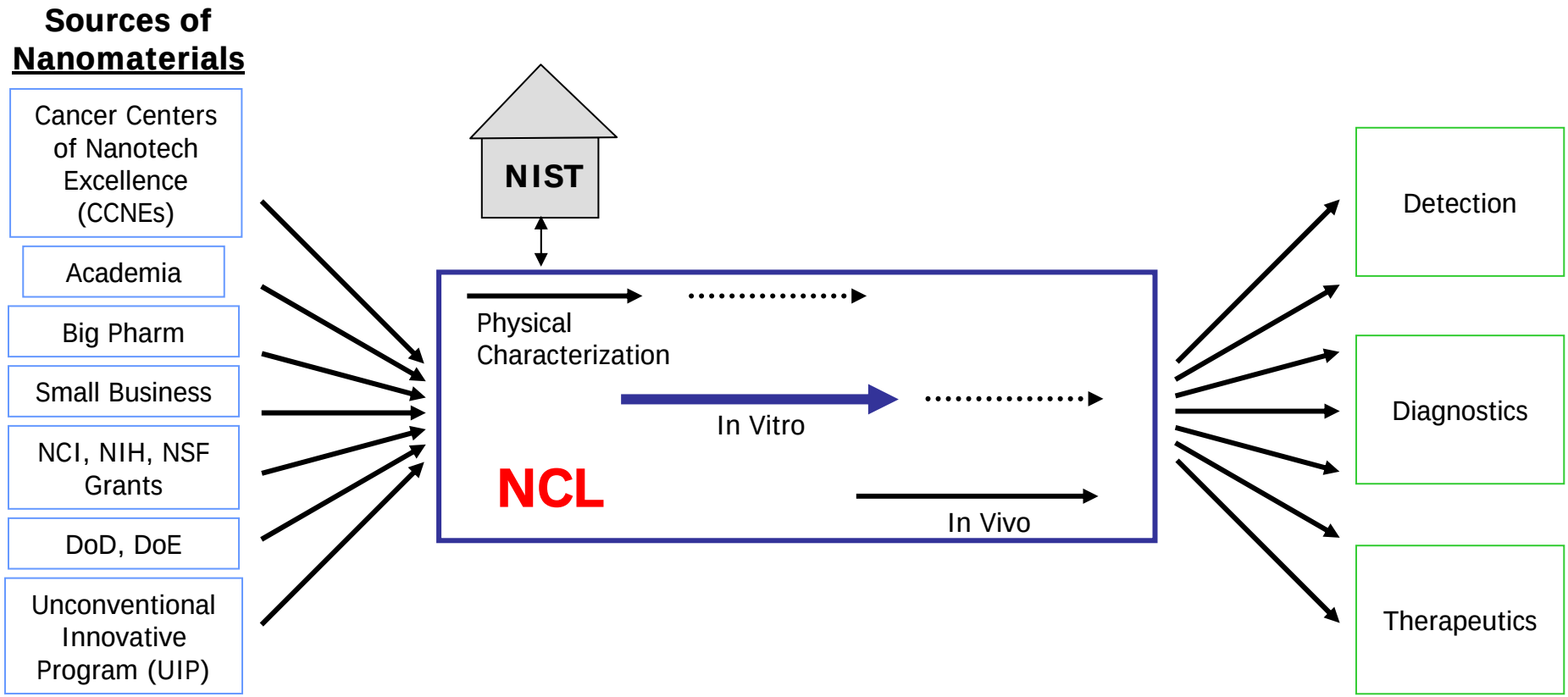
- Identify and characterize critical parameters related to nanomaterials' biocompatibility; structure-activity relationships.
- Establish and standardize an assay cascade for nanomaterial characterization.
- Examine the biological characteristics of multi-component/combinatorial platforms.
- Engage and facilitate academic and industrial-based education and knowledge sharing.

The NCL

The Nanotechnology Characterization Laboratory

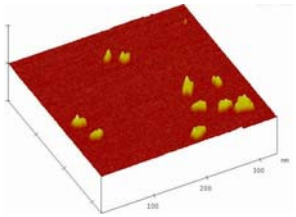
- Provides critical infrastructure support for Alliance
- Performs pre-clinical characterization of nanomaterials intended for cancer therapeutics and diagnostics
- Collaboration between NCI, NIST, US FDA

NCL Concept of Operations



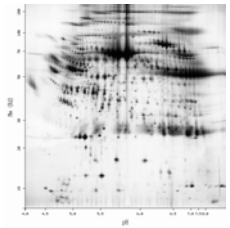
NCL conducts pre-clinical characterization in support of an Investigative New Drug (IND) submission to the FDA

NCL Assay Cascade



Physical Characterization:

- Size
- Size distribution
- Molecular weight
- Density
- Surface area
- Porosity
- Hydrophilicity
- Surface charge density
- Purity
- Sterility
- Surface chemistry
- Stability



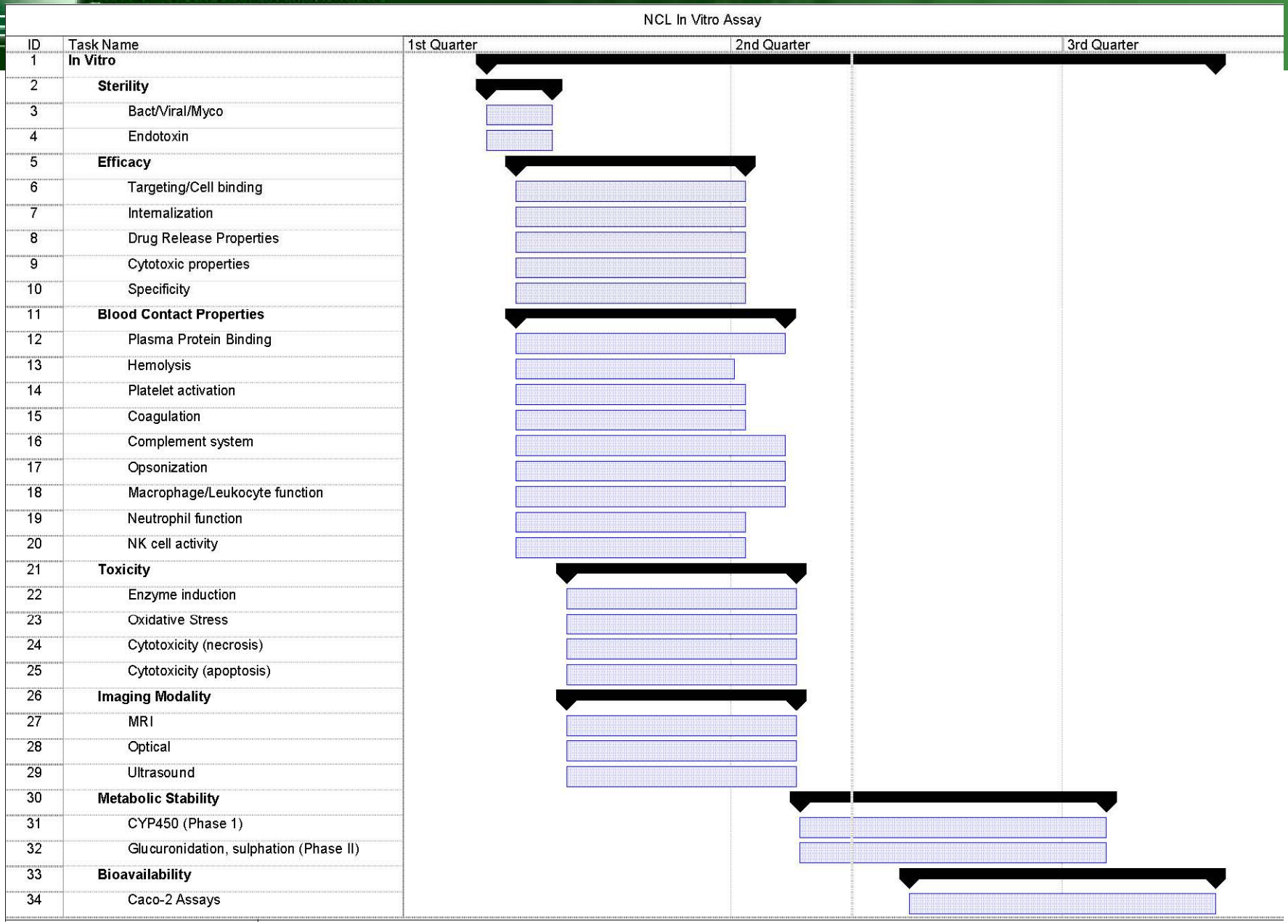
In Vitro:

- Binding
- Pharmacology
- Blood contact properties
- Cellular uptake
- In vitro absorption, distribution, metabolism, excretion

In Vivo:

- Absorption
- Pharmacokinetics
- Serum half-life
- Protein binding
- Tissue distribution
- Metabolism
- Excretion

In Vitro Cascade

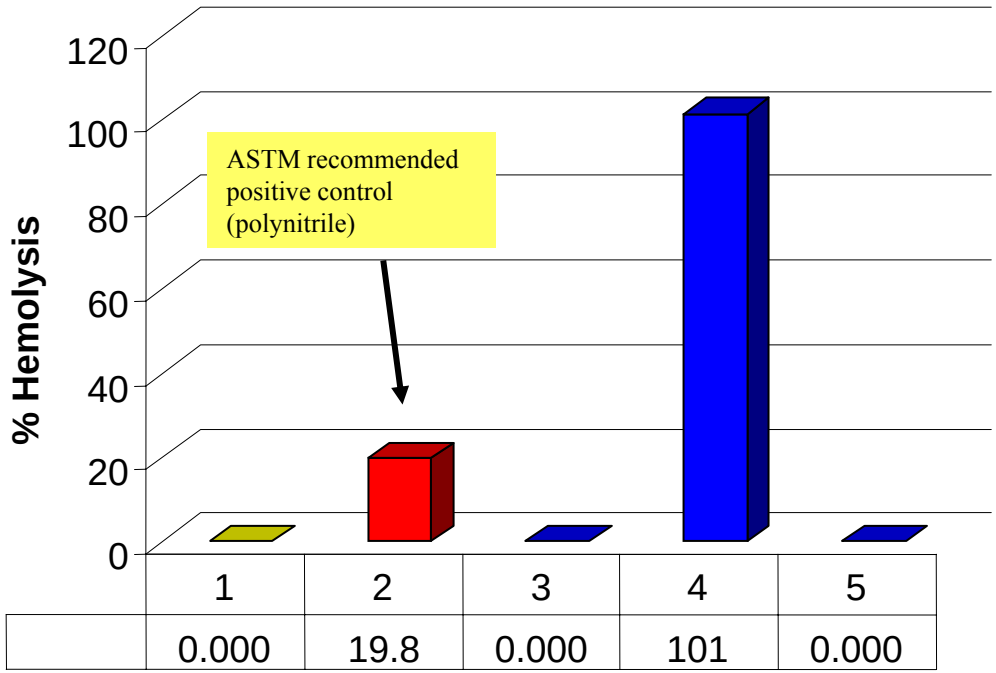


In Vitro
Example: Hemolysis Protocol

NCL Method ITA-1

Analysis of Hemolytic Properties of Nanoparticles

Nanotechnology Characterization laboratory
National Cancer Institute at Frederick
SAIC-Frederick
Frederick, MD 21702
(301)-846-6939



1-NC (polyethylene); 2- PC (nitrile); 3- dH2O; 4 - 50nm polystyrene NP; 5- silica coated glass beads

Physicochemical Characterization and Standardization of Nanoparticles

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Anil K. Patri, Ph.D.
Senior Scientist, Nanotech Characterization Lab
SAIC-Frederick

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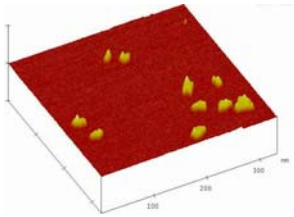
NCL Assay Cascade



Physical Characterization:

- Size
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- Surface charge density
- Purity
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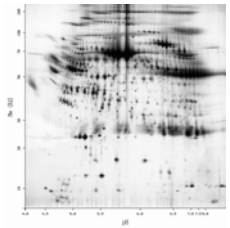
- Surface chemistry
- Stability



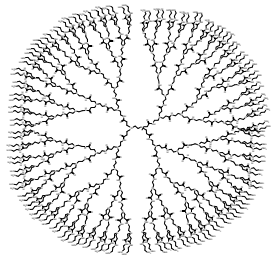
In vitro:

- Binding
- Pharmacology
- Blood contact properties
- Cellular uptake
- Toxicity

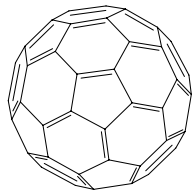
In vivo:

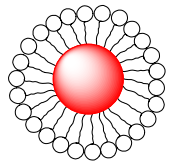
- Absorption
- Pharmacokinetics
- Serum half-life
- Protein binding
- Tissue distribution
- Metabolism
- Excretion

Nanoparticles by type for medical applications

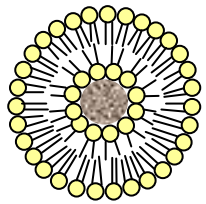


- **Organic Nanoparticles (e.g.: Polymers, Dendrimers)**
- **Inorganic Nanoparticles (e.g.: Iron oxide, gold nanoparticles)**
- **Organic/Inorganic hybrids (e.g.: Nanocomposites, core-shell type, Gd-chelates)**





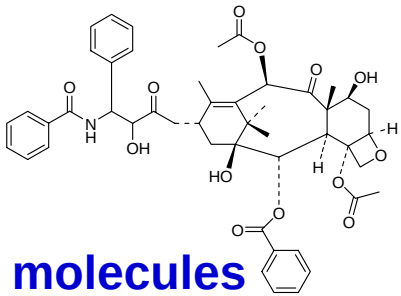
- **Carbon based (e.g.: Functionalized fullerenes)**
- **Liposomes (e.g.: Functionalized, inclusion complexes)**



- **Nanoemulsions (e.g.: Oil-water-surfactant mixtures)**
- **Biological nanoparticles (e.g.: Protein and peptide based nanoparticles with other biological components)**

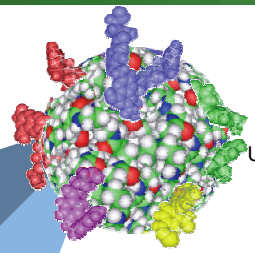
Terminology and Nomenclature will be addressed by E56 sub-committee

Core parameters to define physicochemical property of material



Small molecules

- Elemental analysis
- Mass
- NMR
- UV-Vis
- IR
- HPLC
- GC
- Polarimetry



James Baker
University of Michigan

Nanomaterial

- Microscopy (AFM, TEM, SEM)
- Light scattering (Static, Dynamic)
- SEC, FFF
- Electrophoresis (CE, PAGE)
- Zeta sizer
- Fluorimetry

- **Composition**
- **Physical properties**
- **Chemical properties**
- **Identification**
- **Quality**
- **Purity**
- **Stability**

Same parameters – different characterization methods

NANOTECHNOLOGY CHARACTERIZATION LABORATORY

Standardization of Characterization methods

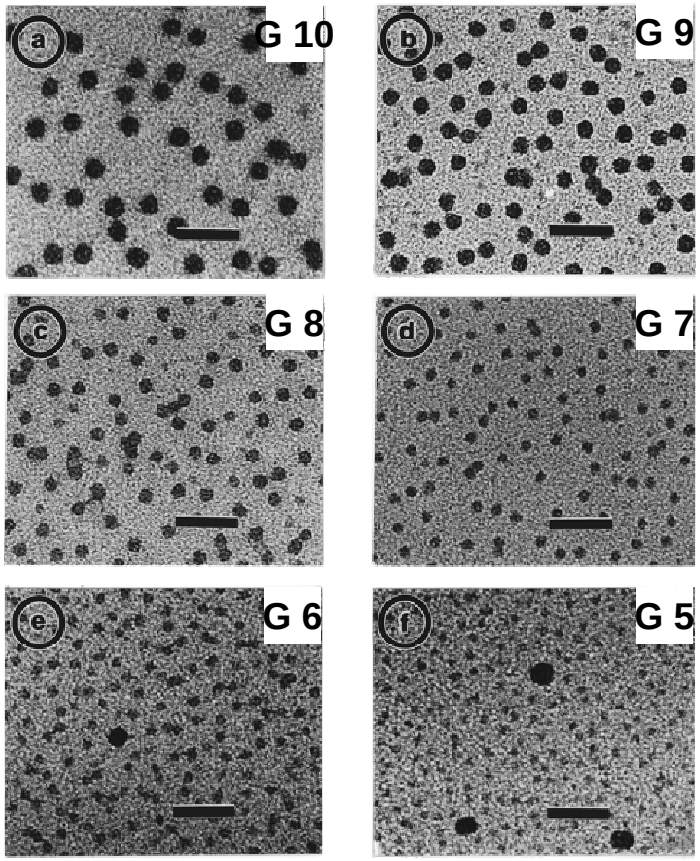
Property		Instrumentation
Size, Size Distribution, Topology	→	Light Scattering, Microscopy (SEM, TEM, AFM), SANS, SAXS
Purity, Composition, MW	→	HPLC, SEC, NMR, Mass Spectrometry, Light scattering, Atomic Absorption and Emission, PAGE, CE
Functionality, Charge Zeta potential	→	NMR, FTIR, UV-Vis, Fluorimetry, PAGE, Zeta sizer, CE, pH titrations
Stability, Reproducibility	→	Chemical analysis, above methods

Physical characterization in collaboration with NIST with input from academia, industry and government agencies

All properties of the nanomaterial should be defined for standardization

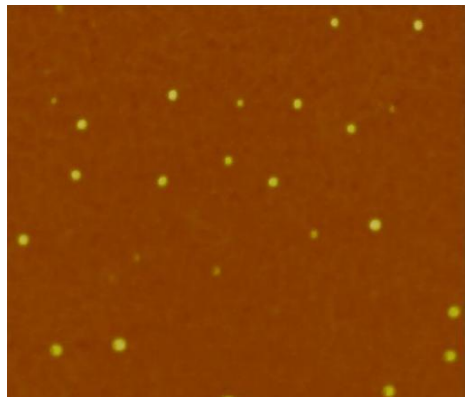
Size, size distribution with microscopy

Visualization of Poly(amidoamine)(PAMAM) Dendrimers by TEM

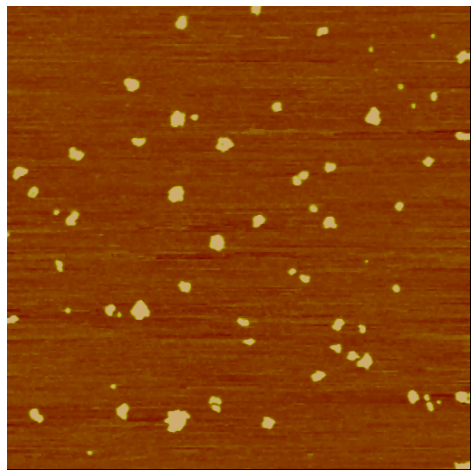


C.L. Jackson, H.D. Chanzy, F.P. Booy, B.J. Drake, D.A. Tomalia, B.J. Bauer, E.J. Amis, *Macromolecules*, 31(18), 6259, 1998

AFM



Gold colloids



Functionalized gold particles

Molecular weight determination

Mass spectrometry

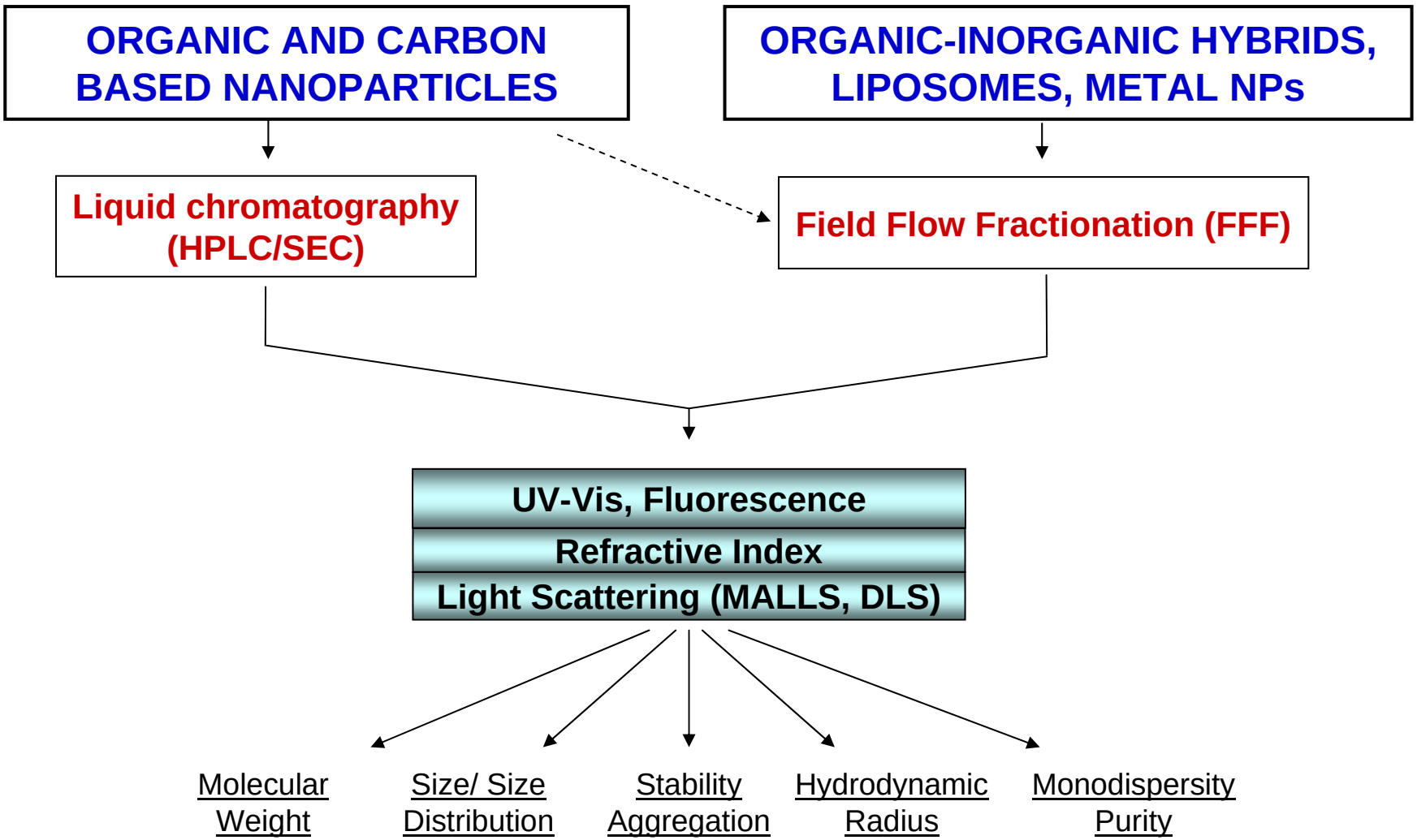
- Small organic molecules
- Peptides and proteins
- Low M.Wt. polymers
- Lower generation dendrimers

❖ Not suitable for very high molecular weight material

Light scattering

- Ideal for nanomaterial
- Gentle method
- Size, size distribution
- Hydrodynamic size
- Batch mode
- Flow mode

Flow mode analysis of Nanoparticles



Quality, Purity, Functionality

UV-Vis Spectroscopy

- Qualitative and quantitative analysis
- Kinetic studies
- Stability studies

Infrared spectroscopy

- Functional group analysis
- Surface characteristics

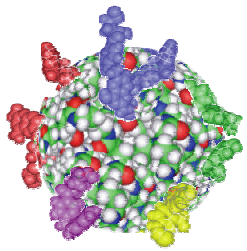
NMR spectroscopy

- ^{1}H and ^{13}C NMR
- Identity
- Purity
- Functionality
- Quantitation from integration

Electrophoresis CE and PAGE

- Separation based on charge/mass
- Purity
- Component distribution

Multifunctional Nanomaterial



James Baker, University of Michigan

- Targeting, Imaging, therapeutic, reporter functions
- Complex nanomaterial for analysis
  - Control particles with each component or control components shall be established and characterized
  - Reproducibility
    - authenticated by physical, in vitro and in vivo test results
- Analysis to confirm the homogeneity in ligand distribution (e.g.: CE)
- Limitations in physical and chemical characterizations will be defined
- Detection limit of impurities or free individual components in the preparation

NCL Pharmacology and Toxicology Preclinical Protocols

Presentation to
ASTM E56 Workshop
May 19, 2005

- Stephan Stern, Ph.D.
- Scientist, Nanotech Characterization Lab
- SAIC-Frederick

NCL Pharmacology and Toxicology Preclinical Protocols

Outline

I. In Vitro Pharmacology and Toxicology

II. In Vivo Pharmacokinetics

III. In Vivo Toxicology

NCL Pharmacology and Toxicology Preclinical Protocols

Objectives of NCL Preclinical Testing Program

- **Develop Standardized Assay Cascade for Nanoparticle Characterization**

ASTM Guidance

ASTM F 1904-98: Standard practice for testing the Biological Responses to Particles *in vivo*

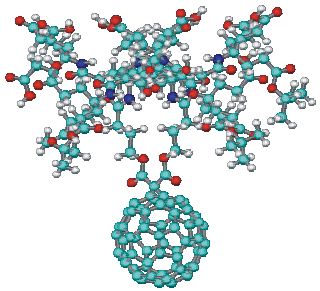
ASTM F 1903 – 98: Standard Practice for Biological Responses to Particles *in vitro*

NCL Nanotherapeutics and Diagnostics

Nanoparticle Applications

- **Cytotoxic oncology drugs**
  - **ex. dendrimer/cytotoxic drug formulation, liposome/cytotoxic drug formulation**
- **Imaging/Diagnostic Agents**
  - **ex. Denrimer/gadolinium MRI agent, Quantum dot diagnostic agent**

NP Structure-Activity Relationship



I. Surface hydrophobicity

- Hydrophobic surface- taken up by RES system

- Hydrophilic surface- Increased systemic half-life, enhanced permeability and retention in tumors (EPR)

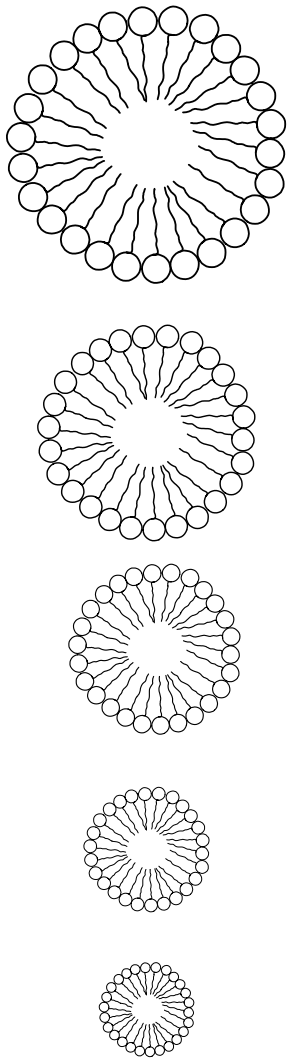
II. Surface Charge

- Toxicity: cationic>anionic>neutral

III. Surface reactivity

- More reactive (↑ROS)= ↑Toxicity

NP Structure-Activity Relationship



IV. Size (aggregation/disaggregation tendency)

- Lung deposition

  (titanium dioxide, Oberdorster *et al.*, 1994)

- Biodistribution (dendrimers, Malik *et al.*, 2000)

- Clearance mechanisms (i.e. renal, biliary)

- Subcellular distribution

  e.g. < 9 nm access nucleus via nuclear pores

  (QD, Lovri *et* al., 2005)

- Particle surface area/cellular interaction

Mechanisms of Nanoparticle Toxicity

Toxic mechanisms attributed to NP's

- Oxidative stress

  (fullerenes, Oberdorster 2004; polystyrene, Fernandez-Urrusuno *et al.*, 1997)

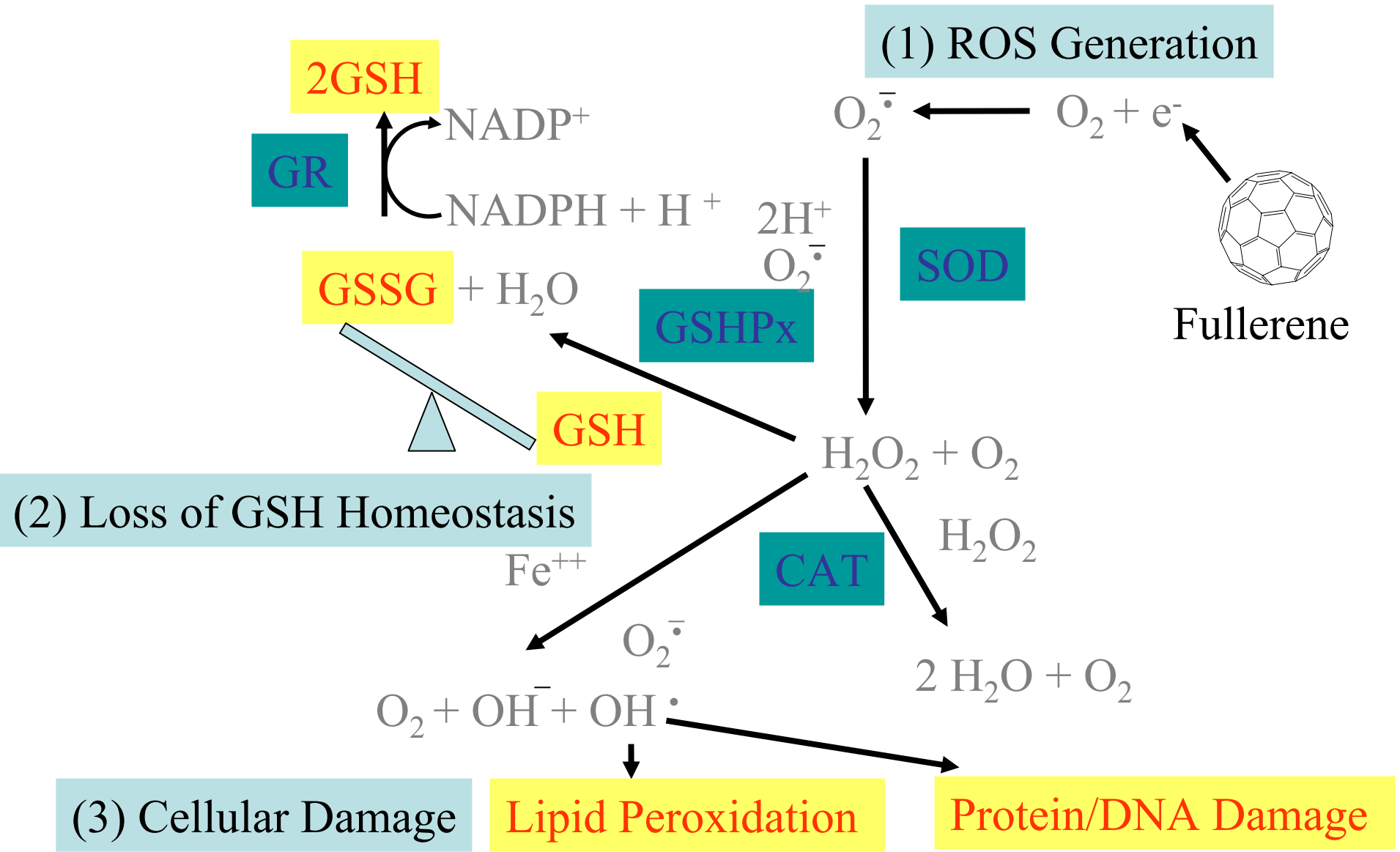
- Induction of apoptosis

  (cationic dendrimers, Kuo *et al.*, 2005)

- Protein binding (quantum dots, Lovri *et al.*, 2004)

- Macrophage dysfunction (liposomal doxorubicin, Daemen *et* al., 1995; titanium dioxide, Renwick *et al.*, 2001)

Oxidative Stress



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In Vitro Protocols

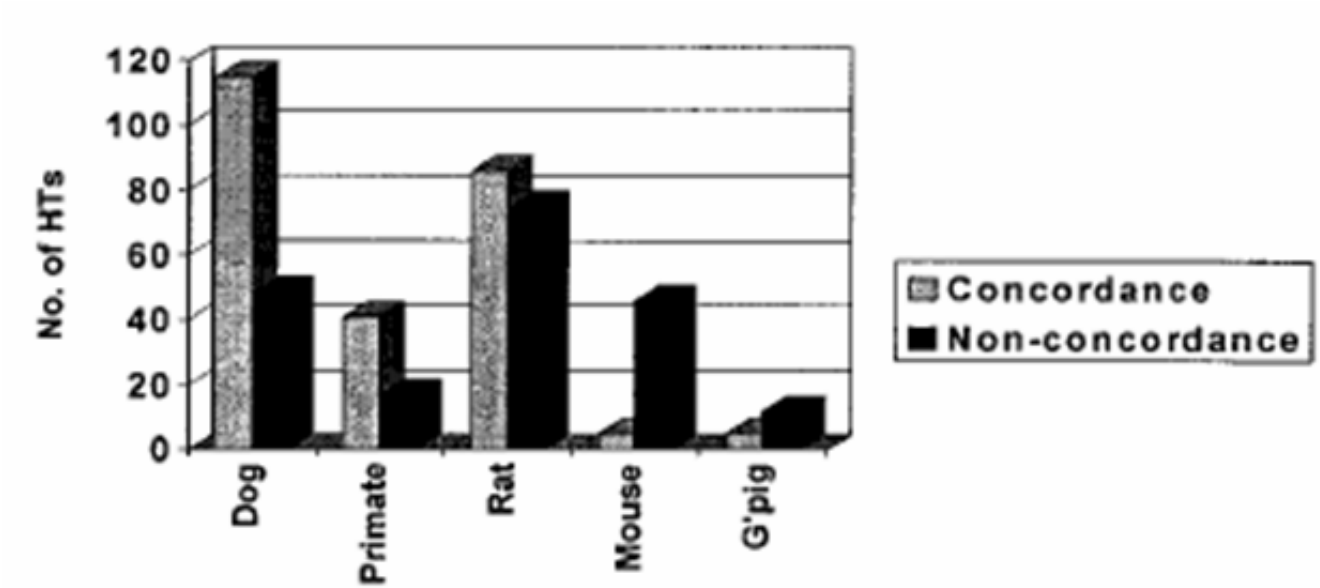
Study	Cell line	Parameter	Specific Assay
Pharmacology	Rat/human hepatic microsomes	Metabolic stability	Oxidative/Conjugative *in vitro* metabolism
	Leukemia-(HL60) Non Small Cell Lung (NCI-H460) Breast-MCF7 CNS-SF268	Efficacy-Necrosis (Cytotoxicity)	MTT Assay
		Efficacy-Apoptosis (Cytotoxicity)	Caspase-3 activation
Toxicology	Rat hepatic primaries (Sprague Dawley)	Necrosis	MTT Assay (viability), LDH (membrane integrity)
		Apoptosis	Caspase-3 activation
		Oxidative Stress	GSH homeostasis (DTNB assay), lipid peroxidation (TBAR assay)
		Cytochrome P450 induction	CYP 1A1 (EROD), CYP 1A2 (MROD), CYP 2B1 (PROD)
	Pig renal proximal tubular cells (LLC-PK1)	Necrosis	MTT Assay (viability), LDH Assay (membrane integrity), γ-GT assay (membrane integrity)
		Apoptosis	Caspase-3 activation

In Vivo PK and Toxicity Studies

Rats are the preferred rodent species :

-Large blood volume for serial blood sampling

-Predictive of human toxicities (HTs)



(Olson *et al.*, 2000)

In Vivo Pharmacokinetics

- **Single/repeat-dose PK/TK/tissue distribution**

- **Clinical Tx cycle**
  -Schedule
  -Duration
  -Route
  -Formulation

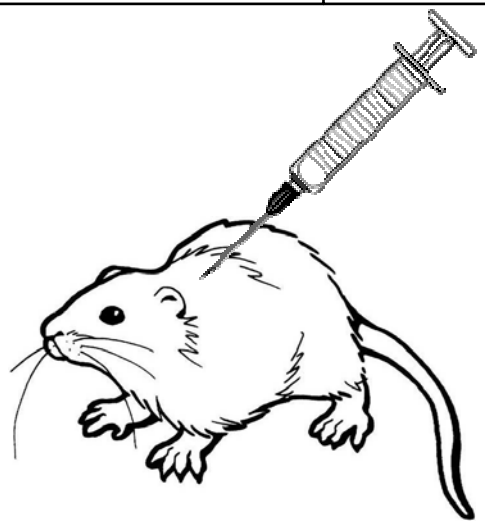
- **Quantitation method**
  -radiolabeled nanoparticle (Scintillation)
  -Imaging
  -ELISA

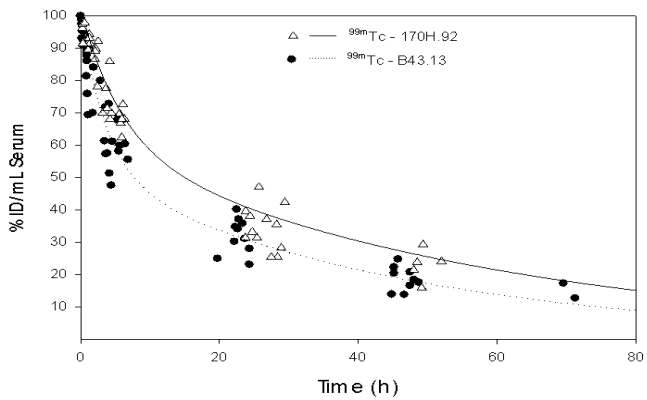
- **PK Parameters**
  -AUC, Cmax, CL, t ½, tmax

Based on FDA Pre-clinical Guidance

In Vivo Pharmacokinetics

Purpose	Duration	Time Point's	Groups	Tests	Comments
Plasma PK profile/ Tissue distribution (Liver, lungs, kidney, heart, spleen brain)	24 hrs	8	1X, 10X (5 F SD Rats/Tx)	scintillation counting of plasma and tissue samples (NCL)	Dosing, blood draws by Jugular catheter, cardiac puncture (final tp)





In Vivo Toxicology Studies

- **Single/Repeat-Dose Acute/Subacute Toxicity**
  -Rats (determine STD10/NOAEL/Lethal dose)

- **Clinical Tx Cycle**
  -Schedule
  -Duration
  -Route
  -Formulation

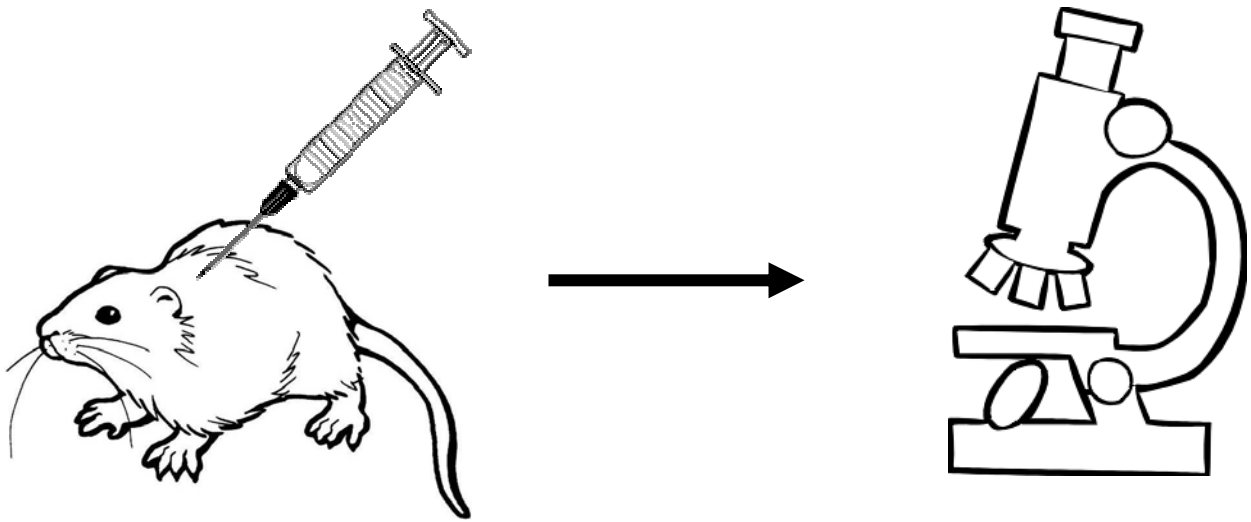
- **Endpoints monitored**
  -Hematology
  -Clinical chemistry
  -Gross pathology
  -Histopathology
  -Clinical signs

Based on NCI DTP toxicology protocols

In Vivo Toxicology Studies

Dose Range-Finding Toxicity Study

Purpose	Duration	Groups	Tests	Comments
determine dose at which toxicity is observed	14 days	ctrl, 10X, 50X, 100X (5 M+F SD Rats/Tx)	Clinical chemistries, histopathology, hematology, gross pathology, clinical observation (PHL)	BW measured daily, euthanasia criteria (decrease in body weight ≥ 20%)



Comprehensive Toxicology

Histopathology

Brain	Pancreas	Salivary gland
Lymph node	Esophagus	Parathyroid
Thyroid	Trachea	Adrenal
Pituitary	Heart	Kidney
Thymus	Gall Bladder	Liver
Spleen	Lung	Duodenum
Ileum	Rectum	Stomach
Cecum	Colon	Jejunum
Lymph node	Epididymis	Ovary
Prostate	Seminal vesicle	Testis
Urinary bladder	Uterus	Eye
Hardian gland	Nasal Sections	Femur
Femur	Vertebra	Spinal cord
Mammary gland	Skin/Subcutis	Tongue

Comprehensive Toxicology

Hematology

Erythrocyte count (RBC)
Hemoglobin (HGB)
Hematocrit (HCT)
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)
Platelet count (Plate)
Reticulocyte count (RETIC)
Total leukocyte count (WBC)
Differential leukocyte count
Nucleated red blood cell count

Comprehensive Toxicology

Clinical Chemistry

BUN	AST	ALT	GGT
GLUC	Creatinine	total protein	Albumin
Globulin	A/G	Sodium	Potassium
Chloride			

Environmental Aspects

Studies Applicable to Environmental Risk Assessment

- **General Cytotoxicity Assays-** determining concentration-response relationships.
- **Mechanistic Studies-** Identifying apoptosis, oxidative stress and cytochrome P450 induction/suppression as potential mechanisms
- ***In Vivo* Toxicology Studies-** Identification of target organs
- **General ADME-** define t1/2, clearance mechanisms (i.e. metabolism, biliary excretion, renal clearance, etc.)

NCL Pharmacology and Toxicology Preclinical Standards

References

1 DeGeorge *et al.* (1998) Regulatory considerations for preclinical development of anticancer drugs. *Cancer Chemother Pharmacol* 41: 173-185.

2 Guideline for Industry: Pharmacokinetics: Guidance for Repeated Dose Tissue Distribution Studies, FDA:CDER: March 1995

3 Guidline for Industry: Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies, ICH:S3A:March 1995

4 Guidance for Industry: S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals

5 Guidance for Industry: Single Dose Acute Toxicity Testing for Pharmaceuticals, FDA:CDER: August 1996

6 ASTM F 1904-98: Standard practice for testing the Biological Responses to Particles *in vivo*

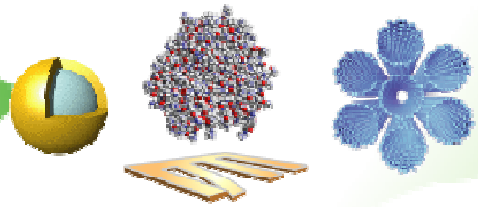
7 Guidance for Industry: Developing Medical Imaging Drug and Biological Products Part 1: Conducting Safety Assessments, FDA:CDER:June 2004

8 Guidance for Industry: S7A Safety Pharmacology Studies for Human Pharmaceuticals, FDA:CDER: July 20-01

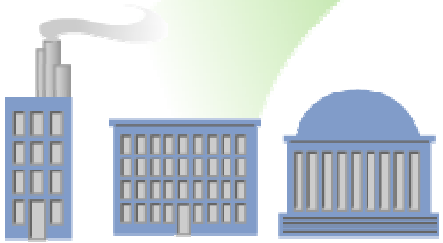
9 ISO 10993-5, Biological evaluation of medical deivices-part5: tests for *in vitro* cytotoxicity

10 ASTM F 1903 – 98: Standard Practice for Biological Responses to Particles *in vitro*

NCI Alliance for Nanotechnology



Nanotechnologies



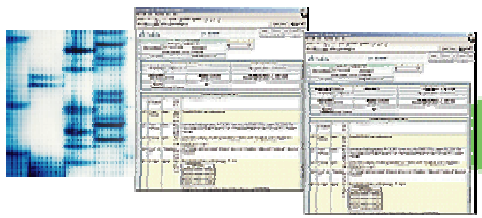
- University and Medical Centers
- Technology Centers of Excellence
- National Labs
- NCI Technology
- Development Programs

Clinical Applications

Nanotechnology Characterization Laboratory

NCI

NIST FDA



Protocols and Data

Questions/Comments

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