Preclinical Characterization Resources for Cancer Nanomedicines

Anil K. Patri, Ph.D.

patria@mail.nih.gov
http://ncl.cancer.gov
Human Burden of Cancer

- 1,444,920 Americans were diagnosed with cancer in 2007
- 559,650 Americans died of cancer in 2007
- $206.3 billion was spent on healthcare cost for cancer in 2006

No data for 2007 was available. The data for 2007 is from previous years and does not reflect the current situation.

Unlike Other Major Disease Killers, Cancer Continues to Take the Nearly Same Toll As In 1950


Need Better Therapies!
Options for Cancer Treatment

- Surgery
- Radiation Therapy
- Chemotherapy
- Prevention
- Screening
- Early Detection
- Diagnosis

Advantages
Limitations

Key for Better Prognosis
Going Small for Big Advances
Cancer Nanotechnology

• Screening
  • Increased sensitivity
• Early Detection of Cancer
• Solubility
  • Carrier for therapeutics
• Improved PK and PD of Drug
• Multifunctional capability
  • Imaging and targeted drug delivery
• Active and passive targeting
  • Ligands, EPR
• Reduced systemic toxicity

↑Solubility ↑Stability ↑Specificity = ↓Toxicity ↑Efficacy
• NCI has funded exploratory work since 1999 on integrating nanotechnology into biomedical research
• Unconventional Innovations Program (UIP)
  – Diagnostics (Imaging)
  – Therapeutics
• Priority is to now transition that research into the clinical realm.
NCI Alliance Program Awards

Centers of Cancer Nanotechnology Excellence (8)

- University of Washington, Seattle, Wash.
- University of California, San Francisco, Calif.
- California Institute of Technology, Pasadena, Calif.
- The Sidney Kimmel Cancer Center, San Diego, Calif.
- University of North Carolina, Chapel Hill, N.C.
- University of Michigan, Ann Arbor, Mich.
- Northwestern University, Evanston, Ill.
- Emory University and Georgia Institute of Technology, Atlanta, Ga.

Cancer Nanotechnology Platform Partnerships (12)

- Massachusetts Institute of Technology, Cambridge, Mass.
- The University of Texas M. D. Anderson Cancer Center, Houston, Texas
- University of Missouir, Columbia, Mo.
- Washington University, St. Louis, Mo.
- Virginia Commonwealth University, Richmond, Va.
- State University of New York, Buffalo, N.Y.
- California Institute of Technology, Pasadena, Calif.
- University of California, San Diego, Calif.
- University of Missouri, Columbia, Mo.
- University of Washington, Seattle, Wash.
- University of California, San Francisco, Calif.
- The Sidney Kimmel Cancer Center, San Diego, Calif.
- University of North Carolina, Chapel Hill, N.C.
- University of Michigan, Ann Arbor, Mich.
- Northwestern University, Evanston, Ill.
- Emory University and Georgia Institute of Technology, Atlanta, Ga.
NCL provides infrastructure support to the Alliance and to nanotech researchers - to overcome obstacles and translate ‘nano’ into the clinical realm.

**NCL Objectives**

- Characterize nanoparticles using standardized methods
- Conduct structure activity relationships studies
- Facilitate regulatory review of nanotech constructs
- Engage in educational and knowledge sharing efforts

The NCL is a national resource available to investigators from academia, industry and government.

nci@ncifcrf.gov
http://ncl.cancer.gov
NCL Concept of Operations

NCL is a formal collaboration between NCI, FDA and NIST
NCL Assay Cascade

Physicochemical:
- Size
- Shape
- Composition
- Molecular weight
- Surface chemistry
- Identity
- Purity
- Stability
- Solubility

In Vitro:
- Pharmacology
- Blood contact properties
- Immune cell function
- Cytotoxicity
- Mechanistic toxicology
- Sterility

In Vivo:
- ADME
- Safety
- Efficacy

http://ncl.cancer.gov/assay_cascade.asp
Physicochemical Characterization

Small molecules
- Elemental analysis
- Mass Spec
- NMR
- UV-Vis
- IR
- HPLC
- GC
- Polarimetry

Physicochemical Parameters
- Composition
- Physical properties
- Chemical properties
- Identification
- Quality
- Purity
- Stability

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

Same parameters – different/additional characterization methods

http://ncl.cancer.gov/working_assay-cascade.asp
In Vitro Cascade

- **Sterility**
  - Bacterial/Viral/Mycoplasma
  - Endotoxin
- **Cell Uptake/Distribution**
  - Cell Binding/Internalization
  - Targeting
- **Blood Contact Properties**
  - Plasma Protein Binding
  - Hemolysis
  - Platelet Aggregation
  - Coagulation
  - Complement Activation
  - CFU-GM
  - Leukocyte Proliferation
  - Macrophage/Neutrophil Function
  - Cytotoxic Activity of NK Cells
- **Toxicity**
  - Phase I/II Enzyme Induction/Suppression
  - Oxidative Stress
  - Cytotoxicity (necrosis)
  - Cytotoxicity (apoptosis)

**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

http://ncl.cancer.gov/working_assay-cascade.asp
**In Vivo Cascade**

- **Initial Disposition Study**
  - Tissue Distribution
  - Clearance
  - Half-life

- **Immunotoxicity**
  - 28-day screen
  - Immunogenicity (repeat dose tox study)

- **Dose-Range Finding Toxicity**
  - Blood Chemistry
  - Hematology
  - Histopathology
  - Gross Pathology

- **Efficacy**
  - Therapeutic
  - Imaging

http://ncl.cancer.gov/working_assay-cascade.asp
Portfolio of Nanoparticles

- M. Kester (Penn State)
- E. Chang (Georgetown)
- R. Blumenthal (NCI)
- J. Nagy (Nanomed)
- J. Connor (Penn State)
- W. Zamboni (U. Pittsburgh)
- A. Miller (Imperial College London)

- NanoSpectra (Rice)

- Liposomes
- Gold nanoshells
- Colloidal gold
- Fullerenes
- Iron Oxide

- Dendritic Nanotechnologies (DNT)
- Avidimer Therapeutics
- M. Brechbiel (NCI)
- E. Simanek (Texas A&M)

- S. Weiss (UCLA)
- Evident Technologies
- J. Barchi (NCI)

- M. Amiji (Northeastern)
- Nanoscan

- US FDA

- J. Ljubimova (Mt. Sinai)
- M. Amiji (Northeastern)
- N. Tarasova (NCI)
- D. Ferguson (U. Wisc)
- V. Torchilin (Northeastern)
- Carigent Therapeutics
Trends from NCL Data

- Controlling the Chemistry
  - Reproducible synthesis
  - Stability
  - Surface Chemistry Characterization Challenges

- Parameters Influencing *In Vivo* Efficacy and ADME/Tox
  - Biodistribution
  - Biocompatibility
  - Biodegradability

- Critical Parameters in Early Phase *In Vitro* Immunological Characterization
  - Challenges related to Endotoxin/Sterility
  - Complement Activation
Size Matters

Dendrimer-Based MRI Contrast Agents


A difference in size as little as 2nm can influence route of clearance
Assessing Tumor Accumulation

LS-174T human colon cancer xenograft, treated with Fe$_2$O$_3$ NP-therapeutic

MRI imaging to evaluate tumor accumulation of Fe$_2$O$_3$ NP
Size in a Biological Context

30 nm Gold colloids

30 nm Gold colloids incubated in plasma

31 nm

69 nm
Surface Characteristics Matter

LDH Leakage Assay

% Total LDH Leakage
Mean ± SE

G4 PEG
G6 PEG
G6 OH
G6 NH2
G6-COOH

MTT Viability Assay

% Control
Mean ± SE

PEG
PL
G6-OH
G6-NH2

Hemolysis

Surface Characteristics are Important for Biocompatibility
Interaction with Immune System

- In general, cationic species are more toxic than anionic and neutral ones.
- Modification of particle surface decreases their toxicity to immune cells.
Trends: Surface characteristics

Phagocytosis

More than one method is often required to draw conclusions
Interference

False Negative Results

- Particles adsorb assay specific test-substance (e.g. PSN – hemolysis assay; cAu-NP – LAL test)
- Surfactants in NP formulation (e.g. Polystyrene NP – LAL test)
- Particles quench luminescence, fluorescence or interfere with absorbance (e.g. gold nanoshells, liposomes – multiple assays)
- Particles “block” protein interaction & binding (e.g. dendrimers – some ELISA-based assays)

False Positive Results

- Surfactants in NP formulation (e.g. Polystyrene NP – hemolysis assay)
- Particles’ absorbance at assay wave length (e.g. cAu-NP, some C60 derivatives, Dox-loaded liposomes; some NE – multiple assays)
- Oxidation of assay reagents (e.g. iron oxide- hemolysis assay)
- Particles have intrinsic fluorescence (e.g QD – multiple fluorescent assays)
- Particles catalize enzymatic reactions (e.g. dendrimers – LAL test)
Trends: Biocompatibility

Nanoparticle Biocompatibility

- **Cytotoxicity** (Surface Reactivity)
- **RES Recognition**
- **Renal Clearance**
- **Biliary Clearance?**
- **(EPR Effect)**

- **Zeta Potential**
  - (+)
  - (-)
  - 0
  - 1 nm
  - 220 nm

- **Size** (Rigid Core)
  - Low
  - High

- **Solubility**

- **Dose (mg/mL)**

- **Trends: Biocompatibility**

- **NCI Alliance for Nanotechnology in Cancer**

- **Renal Clearance**
- **Biliary Clearance?**

- **Cytotoxicity**
- **Zeta Potential**
- **Solubility**

- **Trends**

- **Graph**
  - Cytotoxicity
  - % Control
  - Dose (mg/mL)
Acknowledgments

NCL
Scott E. McNeil, Ph.D.
Nick Panaro, Ph.D.
Marina Dobrovolskaia, Ph.D.
Jeffrey D. Clogston, Ph.D.
Hari Devalapally, Ph.D.
Jennifer B. Hall, Ph.D.
Sarah Skoczen, M.S.
Chris B. McLeland, B.S.
Barry W. Neun, B.S.
David Parmiter, B.A.
Martin Fritts, Ph.D.
Stephan T. Stern, Ph.D.
Pavan Adiseshaiah, Ph.D.
Jiwen Zheng, Ph.D.
Denise Johnson, Ph.D.
Sonny Man, M.S.
Matthew Hansen, M.S.
Tim M. Potter, B.S.
Jamie Rodriguez, B.S.
Lisa Sheffield

NCI-Frederick
Joseph Kalen, Ph.D.
Raul Cachau, Ph.D.
Tim Veenstra, Ph.D.
Jack Simpson, Ph.D.
Haleem Isaaiq, Ph.D.
King Chan, Ph.D.
Kunio Nagashima, M.S.

Intramural Collaborators
Robert Blumenthal, Ph.D.
Anu Puri, Ph.D.
Joseph Barchi, Ph.D.
Larry Keefer, Ph.D.
Joseph Saavedra, Ph.D.
Martin Brechbiel, Ph.D.
Pete Choyke, M.D.

Contact Info:
Anil Patri
(301) 846-6939
patria@mail.nih.gov
http://ncl.cancer.gov

Funded by the National Cancer Institute Contract HHSN261200800001E