Preclinical immunological characterization of nanoparticles

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NCL Immunology Team

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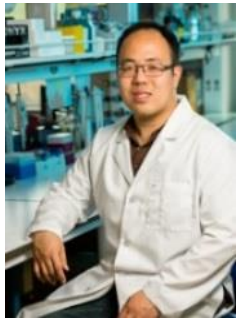
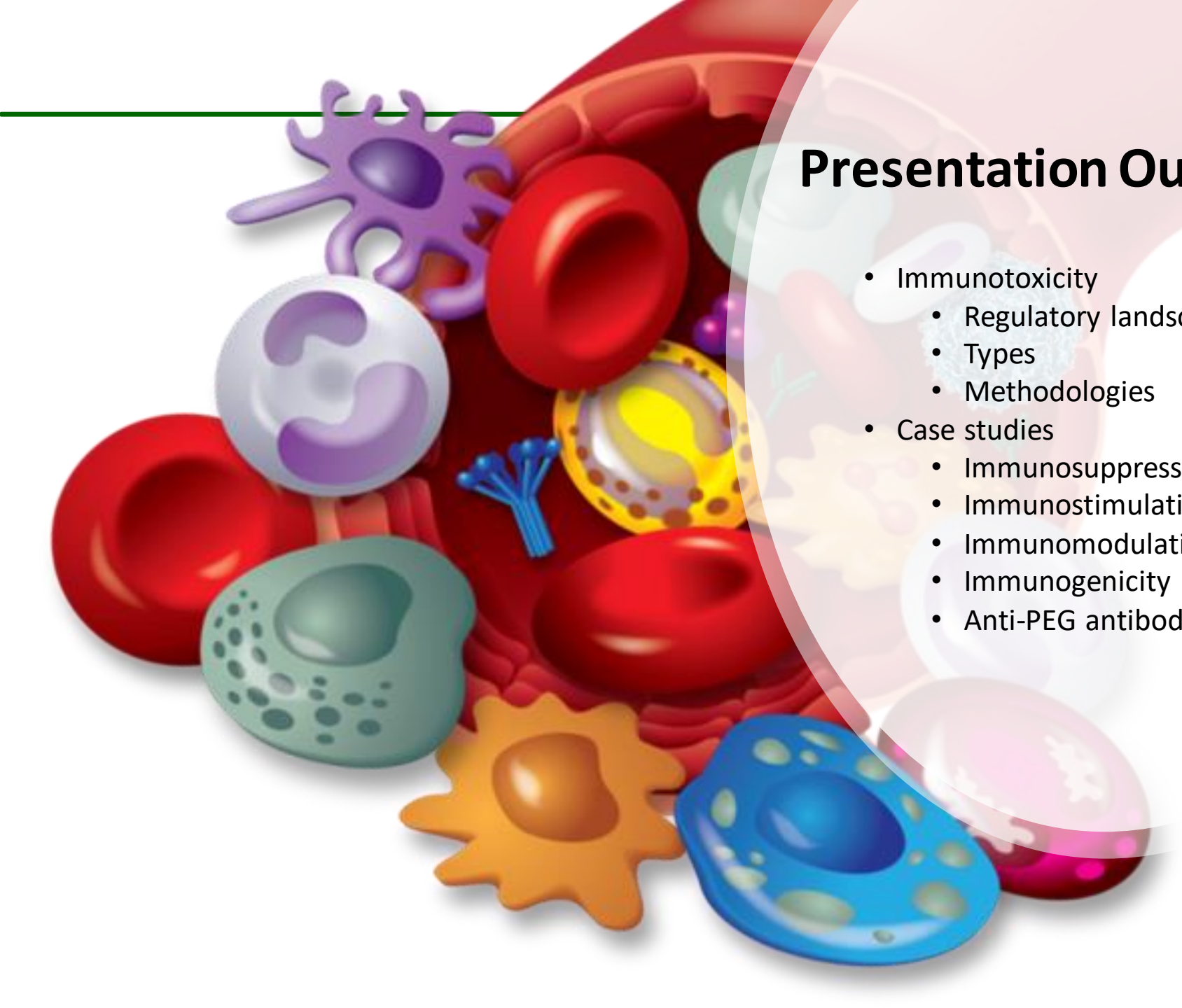


Enping Hong

Ankit Shah

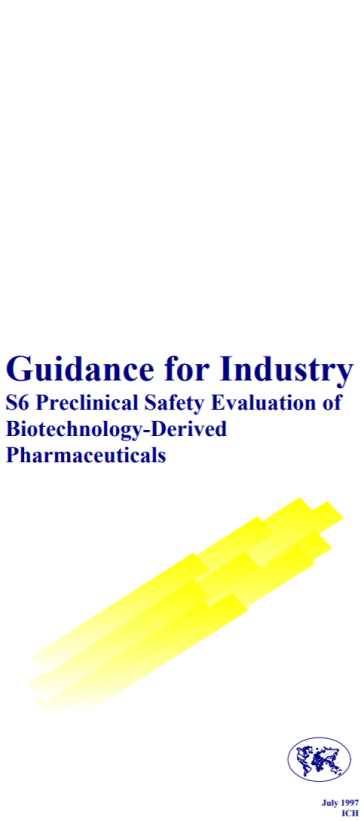
Developed Protocols for the NCL Assay Cascade and Improved Understanding of Nanoparticle Interactions with the Immune System

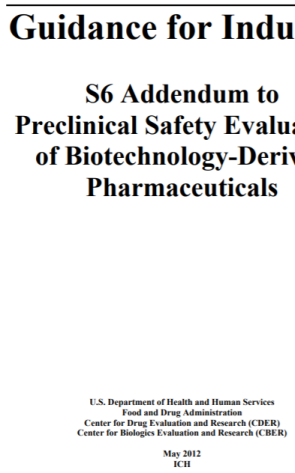


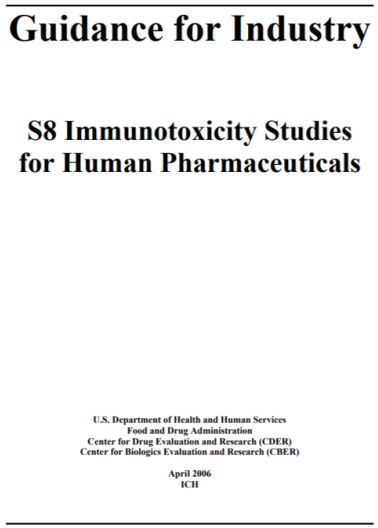
Presentation Outline

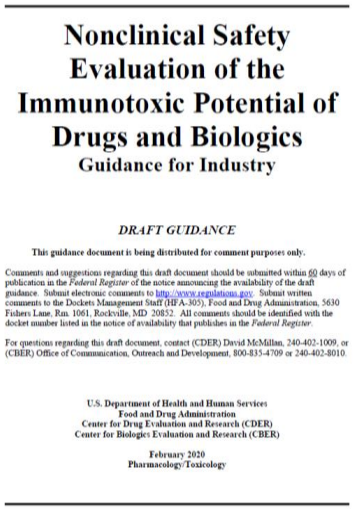
- Immunotoxicity
  - Regulatory landscape
  - Types
  - Methodologies
- Case studies
  - Immunosuppression
  - Immunostimulation
  - Immunomodulation
  - Immunogenicity
  - Anti-PEG antibodies


• Clinical studies can be halted due to immunotoxicity
• Drugs can be withdrawn from clinical use due to immunotoxicity
Immunotoxicity









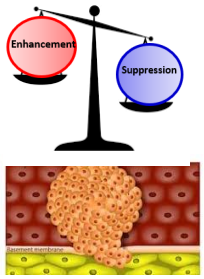
ICH harmonisation for better health

Regulatory landscape of immunotoxicity

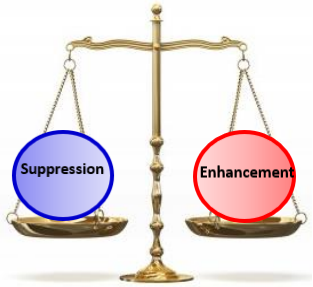
ICH = International Conference on Harmonization; International Council for Harmonization since 2015

General principles

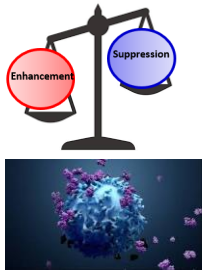
- Toxicity to the immune system encompasses a variety of adverse effects.
- These include **suppression** or **enhancement** of the immune response:



Infections & Tumors



Healthy Immune Function



Autoimmunity & Hypersensitivity

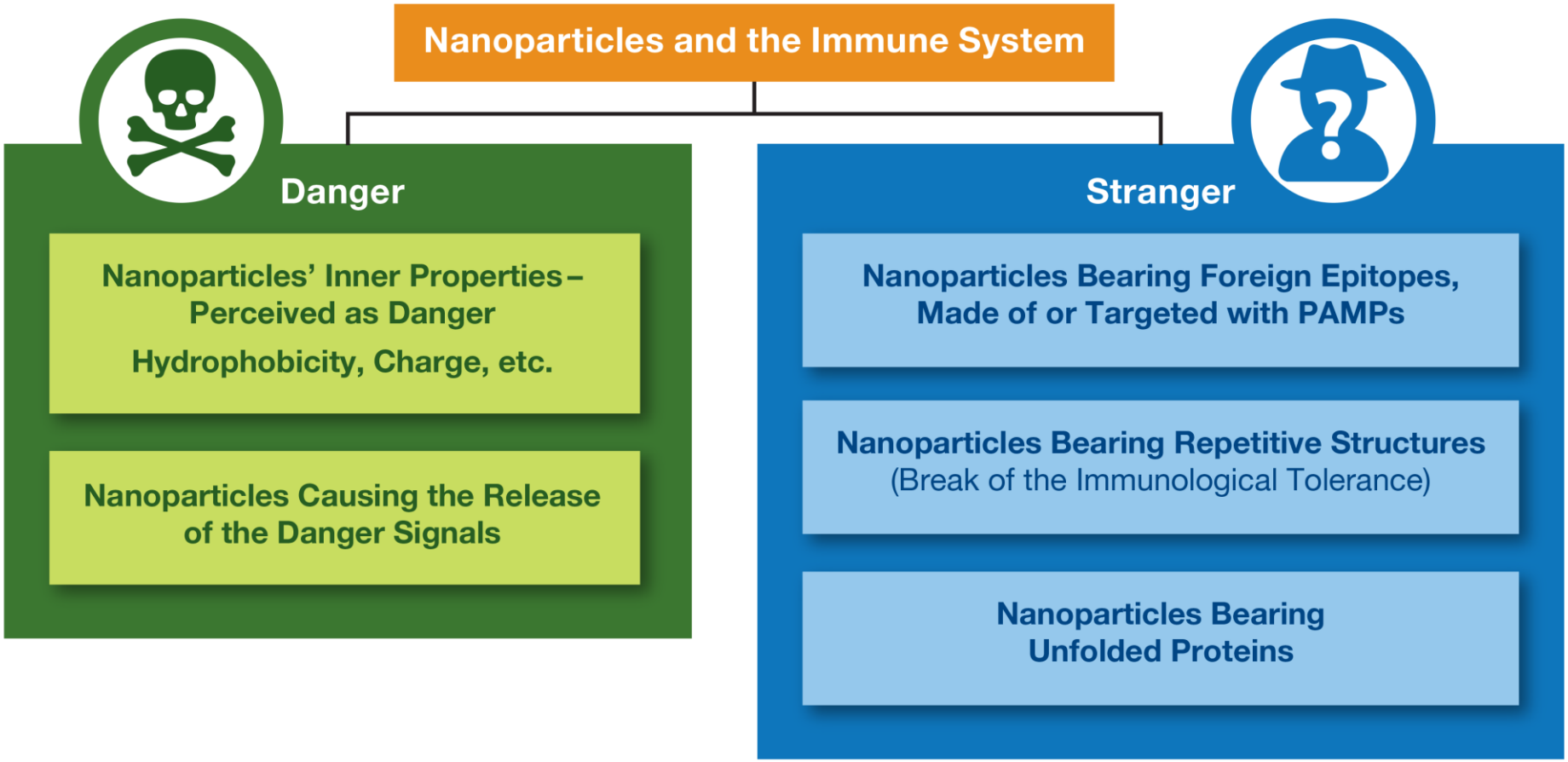
Immunosuppression or enhancement can be associated with two distinct groups of drug products:

(1) Intended to affect immune function for therapeutic purposes (e.g., to prevent organ transplant rejection); exaggerated pharmacodynamics

(2) Not intended to affect immune function but cause immunotoxicity (e.g., by causing apoptosis of immune cells)

Immunomodulation modifies the immune response; not overtly immunosuppressive or immunostimulatory; may have subtle or even mixed effects.

Nanoparticles and the immune system



Dobrovolskaia MA. Molecules. 2019 Dec 17;24(24):4620. doi: 10.3390/molecules24244620

- Nanoparticles can be immunosuppressive, immunostimulatory, and immunomodulatory
- These effects are due to either APIs or carrier

NCL Immunology Assay cascade

NCL Nanotechnology Characterization Laboratory | NCI Alliance for Nanotechnology in Cancer

In Vitro

Nanotechnology-Formulated Drug
(Precursors and Components of Formulation When Needed to Identify Source of Toxicity)

Tier I
To Verify Lack of Contamination

- **Endotoxin**
- **Sterility**

Tier II
To Assess Common Acute Toxicities

- **Hemolysis**
- **Complement Activation**
- **Thrombogenicity (Platelets, Plasma Coagulation, Leukocyte PCA)**
- **Cytokines**
- **Leukocyte Proliferation**
- **Total Protein Binding (If Feasible)**
- **Uptake by Macrophages (If Feasible)**

Tier III
To Assess Immune Cells and Their Function

- **WB Immunophenotyping**
- **CFU-GM**
- **Effects on Antigen-Induced Leukocyte Proliferation**
- **Effects on Mitogen-Induced Leukocyte Proliferation**
- **Effects on Macrophage Phagocytic Function**
- **Effects on NK Cytotoxicity**
- **Effects on DC Maturation**
- **Effects on CTL Activity**

Tier IV
Mechanistic Studies

- **Relevant Assays from Tiers II & III**
- **Additional Assays as Needed**

In Vivo

Inbred strains and transgenic models

Immunotoxicity

- Local lymph node proliferation assay
- T-cell dependent antibody response
- CFU-GM
- Rabbit Pyrogen Test
- Immunogenicity
- Psoriasis
- Lupus

Immunotherapy

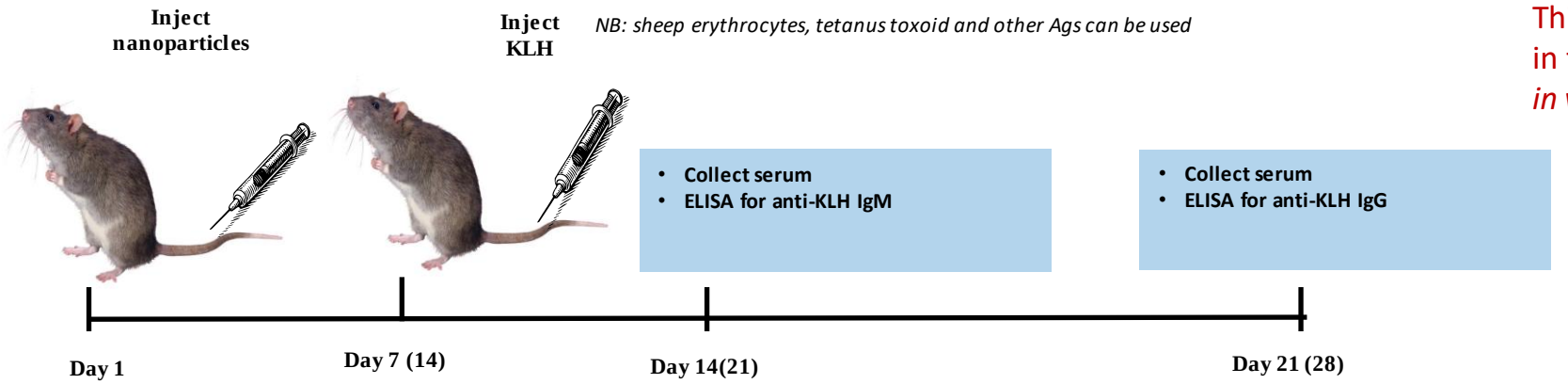
- Adjuvanticity
- Immunological milieu of the tumor

Relevant Guidance and Standards:

ICH S8
ICH S6
USP BET 85
USP 151
ISO 10993-4

Methods for the assessment of immunosuppression

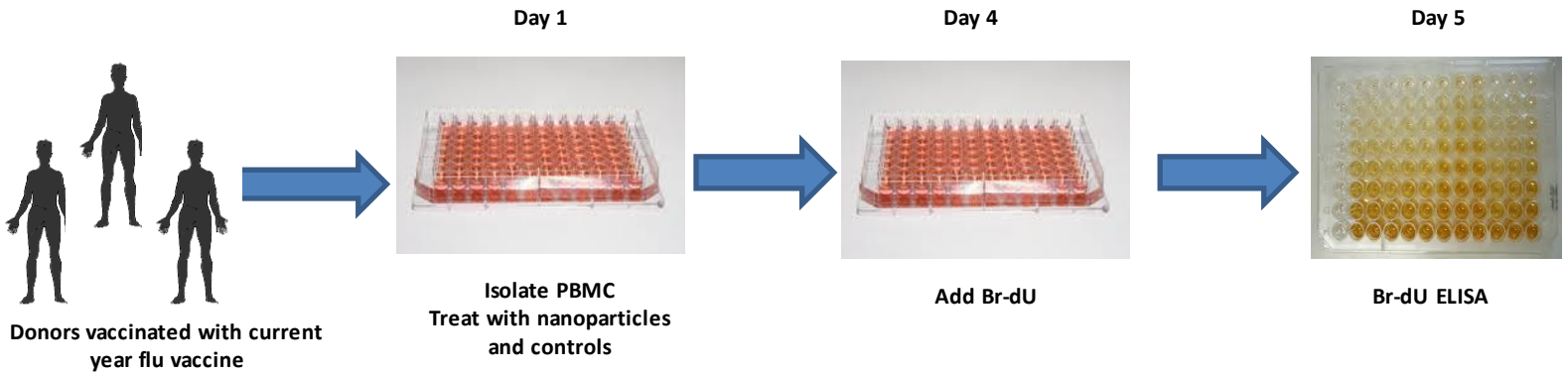
TDAR



This test is in the NCL *in vivo* AC

- **T cell dependent antibody response (TDAR)** is the traditional immune function test used to estimate materials' immunosuppression
- **Advantages** - effective, predictive, recommended by the FDA; **Disadvantages** - time- and material-consuming, expensive

HuLa

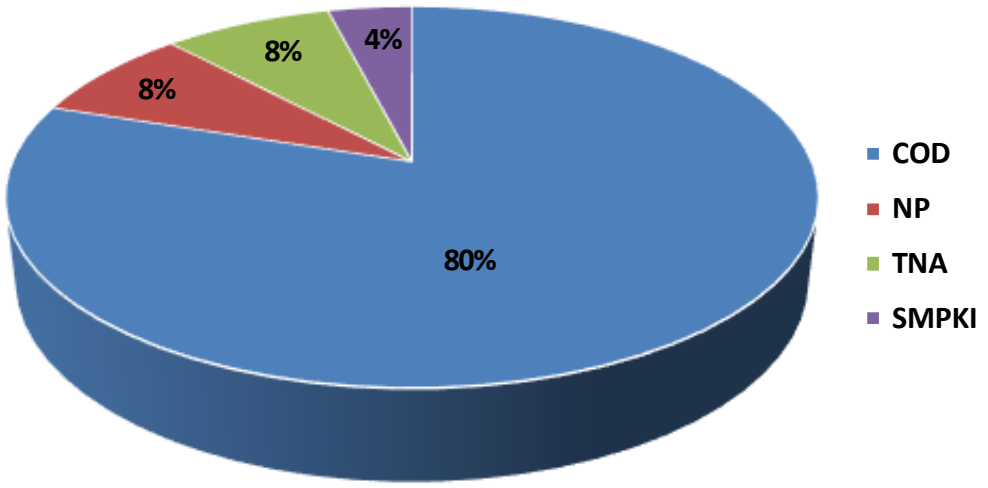


This test is in the NCL *in vitro* AC

- **Human Leukocyte Activation (HuLA),** originally developed by Mark Collinge at Pfizer and adapted by us to test nanomaterials, is an in vitro surrogate of TDAR
- **Advantages** – proven IVIV correlation with TDAR for immunosuppressants with various MOA; **Disadvantages** – requires donor prescreening and current year flu vaccine which is not always available for laboratory usage

NCL experience with assessing immunosuppression

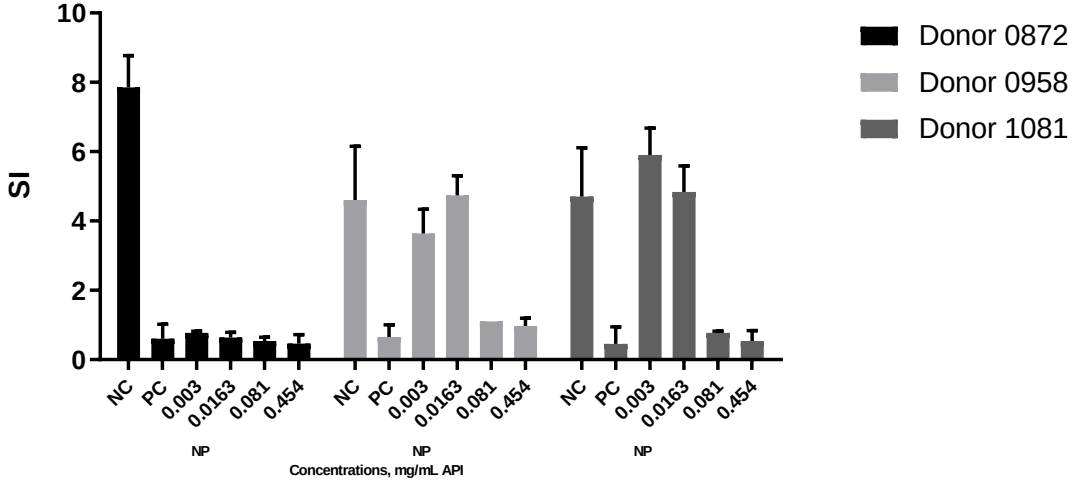
- The majority (> 90%) of formulations were immunosuppressive due to the API (small molecules cytotoxic oncology drugs (COD), therapeutic nucleic acids (TNA), small molecule protein kinase inhibitor (SMPKI))
- Small proportion (8%) were immunosuppressive due to nanocarrier (NP).



NCL in vitro assays to screen for Immunosuppression:

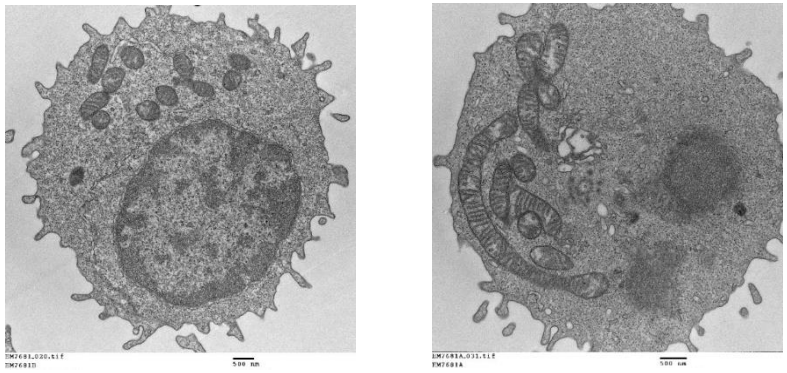
- ITA-6
- ITA-18
- ITA-3

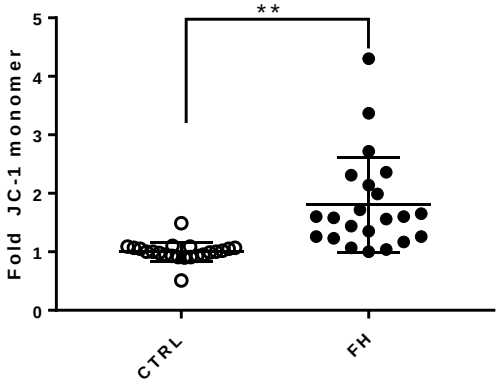
https://ncl.cancer.gov/resources/assay-cascade-protocols



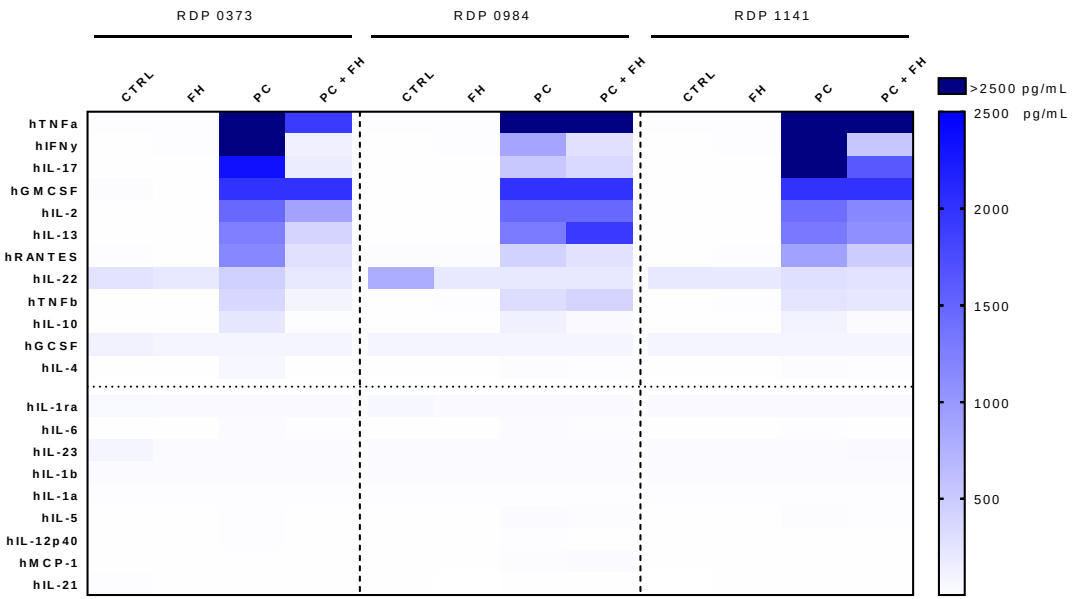
Example of in vitro analysis of nanoparticle immunosuppressive properties using HuLa assay

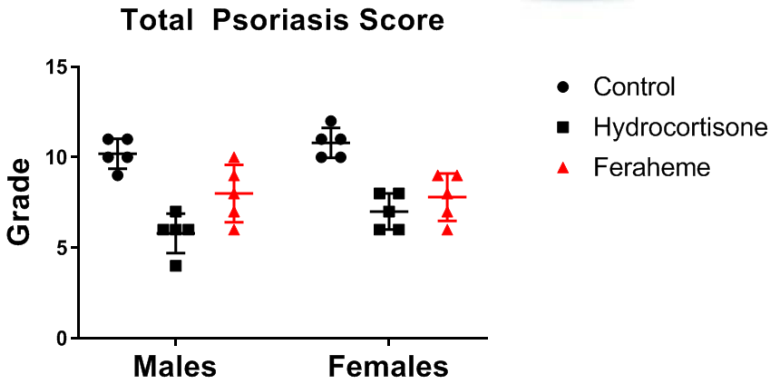
Immunosuppression: case study





Ankit Shah





Iron oxide nanoparticles (Feraheme) suppresses activation of T-cells via a mechanism involving mitochondrial ROS in vitro

Topical application of Feraheme inhibits development of skin lesions in a mouse model of psoriasis

Shah et al., Toxicology and Applied Pharmacology, 2018

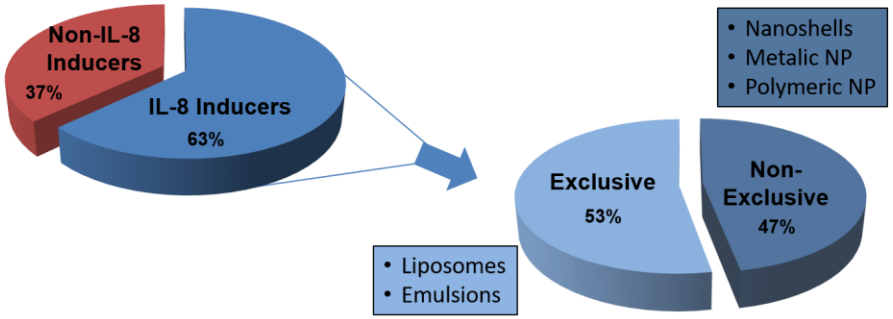
Shah et al., Precision Nanomedicine, 2019

NCL experience with assessing immunostimulation

Many markers exit; cytokines are reliable and have good in vitro-in vivo correlation

10% of NCL tested nanomaterials induced cytokines

↳ > 60% of these particles induced IL-8



> 50% of IL-8 inducing nanocarriers did so exclusively
(i.e. w/o inducing other common pro-inflammatory cytokines)

↳ These were typically liposomes and nanoemulsions

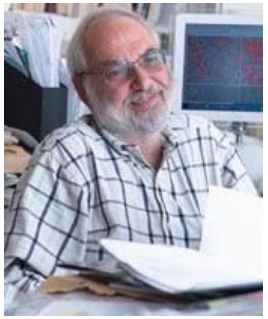
Induction of chemokines (IL-8, MIP-1a, MCP1/2) is commonly observed with lipid nanocarriers and liposomes (neutral and anionic)

NCL in vitro assays to screen for immunostimulation:

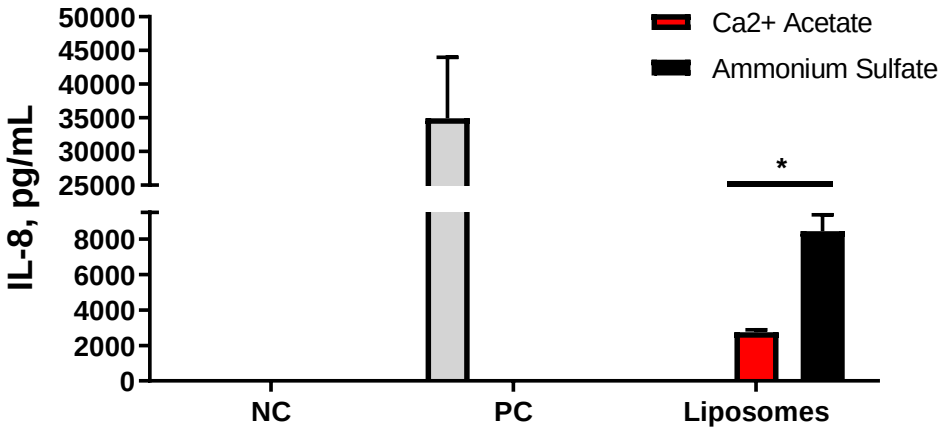
- ITA-10
- ITA-22
- ITA-23
- ITA-25
- ITA-27

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

האוניברסיטה העברית בירושלים
THE HEBREW UNIVERSITY OF JERUSALEM



Prof. Barenholz



Chemokine induction by liposomes can be controlled by optimizing ion content in the liposome cavity

Biomarkers of immunostimulation depend on the type of nanomaterial and API

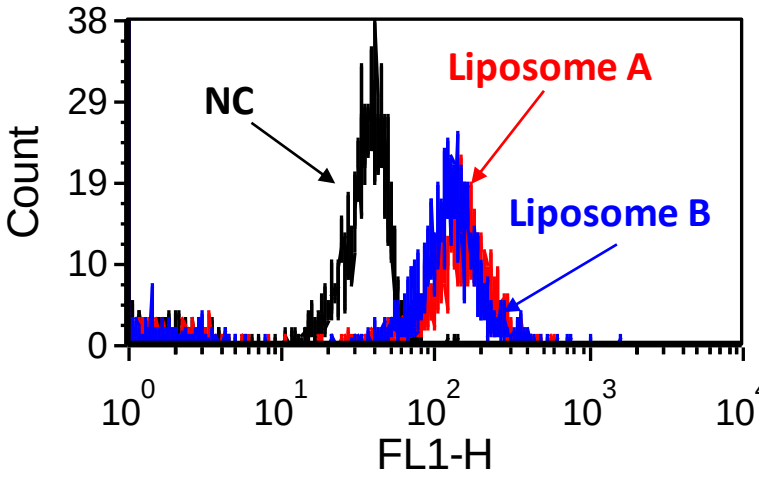
Cytokine induction by cationic liposomes

	IFN-γ	IL-1α	IL-1β	IL-6	IL-8	IL-10	MCP-1	MIP-1α	MIP-1β	RANTES	TNF-α
donor #1	-	++	++	+++	+++	+	+++	+++	++	++	++
donor #2	-	++	++	+++	+++	+	+++	+++	++	++	++
donor #3	-	++	++	+++	+++	+	+++	+++	++	+++	++
donor #4	-	++	++	+++	+++	+	+	+	+	++	++
donor #5	-	++	++	+++	+++	+	++	++	++	++	++
donor #6	-	++	++	+++	+++	+	++	+++	++	++	++
donor #7	-	+	+	++	+++	+	++	+++	+	++	++

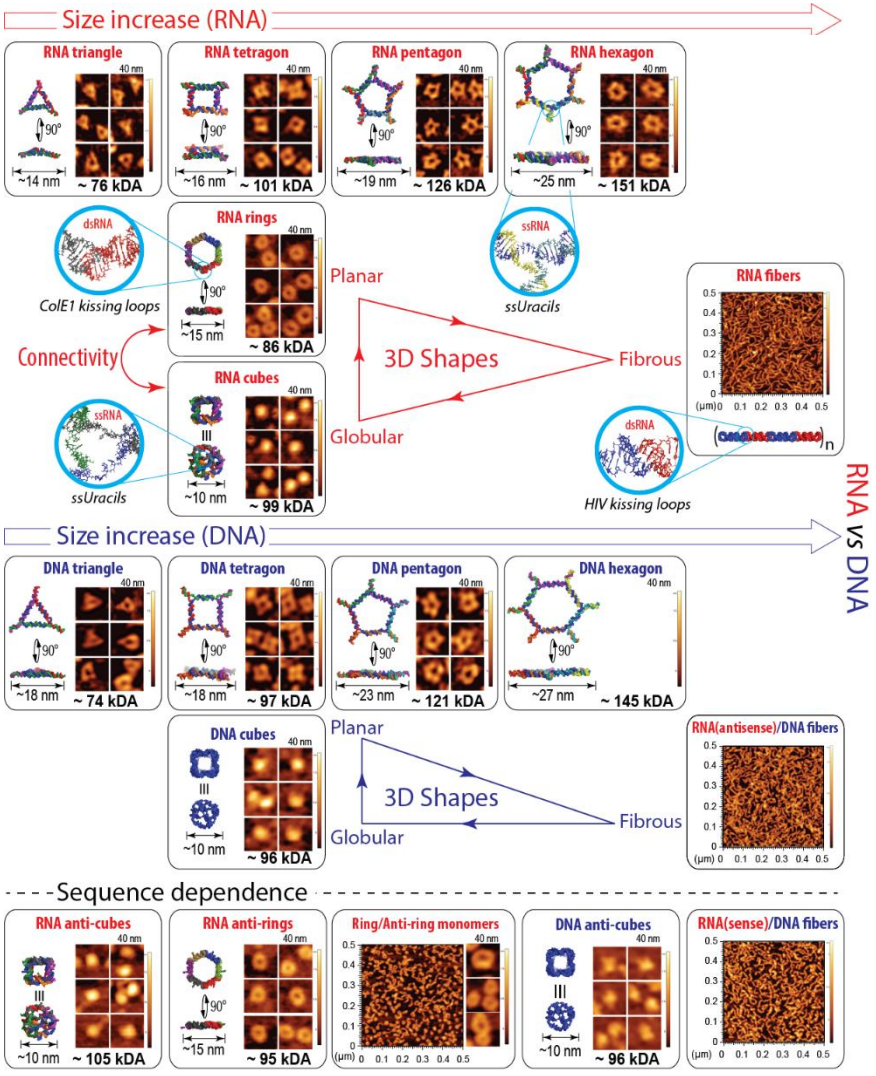
Detected cytokines	IL-1α	IL-1β	IL-6	TNF-α	IL-10	IL-8	MCP-1	MIP-1α	MIP-1β	RANTES
Group:	cytokines					chemokines				

Detected danger signals	MMP-1	MMP-7	MMP-9
Group:	metalloproteinases		

- Cationic liposomes induce wide range of pro-inflammatory responses
- Oxidative stress is the underlying mechanism

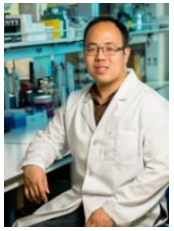


Cytokine induction by Nucleic Acid Nanoparticles



Kirill Afonin

Justin Halman



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- The study involved over a library of NANPs and PBMC from over 100 donors
- Mechanistic study involved siRNA/PBMC and HEK-TLR reporter cell lines

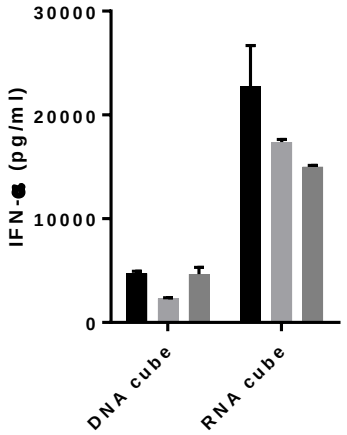
Biomarkers of immunostimulation by TNA are Interferons

Hong et al., Nano Lett. 2018 Jul 11;18(7):4309-4321
Hong et al., Molecules. 2019 Mar 20;24(6):1094

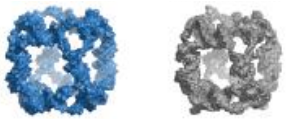
Cytokine induction by Nucleic Acid Nanoparticles

NCL Nanotechnology Characterization Laboratory | NCI Alliance for Nanotechnology in Cancer

Material

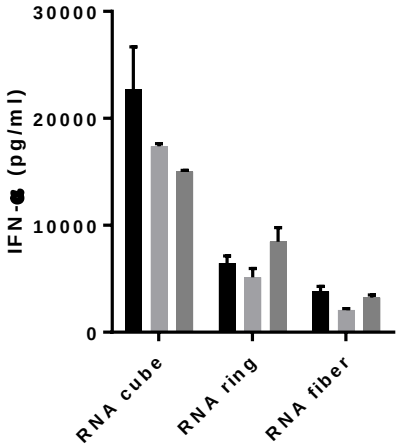


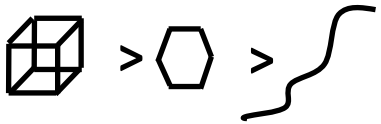
DNA < RNA



RNA nanoparticles are more potent than DNA counterparts

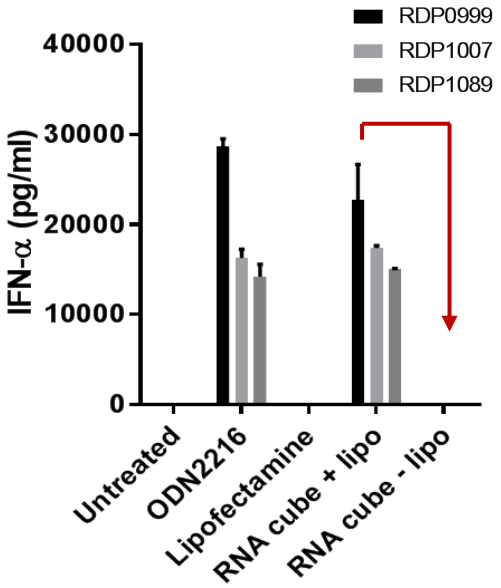
Structure



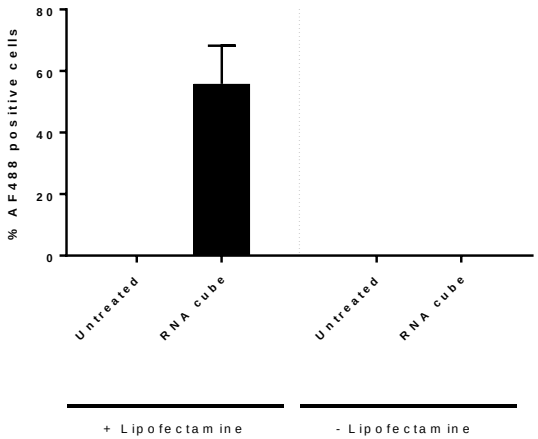


Globular particles are more potent than planar than fibrous structures

Interferon Response to NANPs…

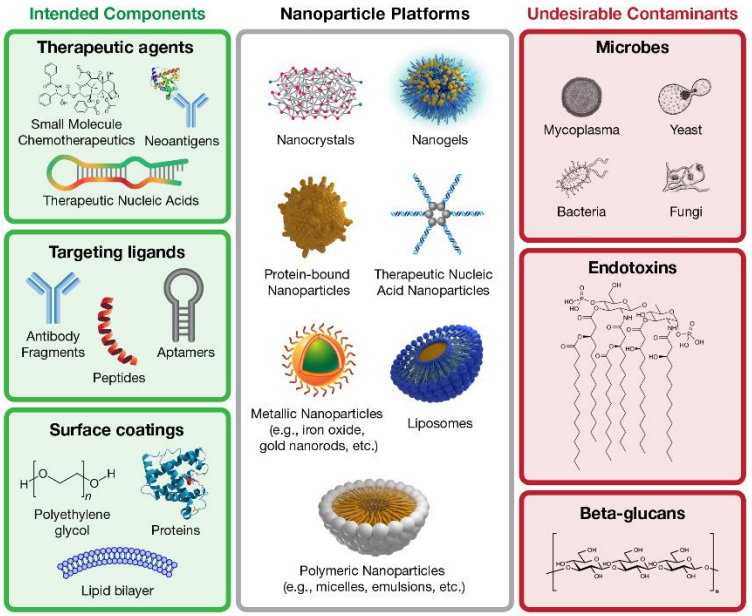


…Correlates with Uptake by Cells



Hong et al., Nano Lett. 2018 Jul 11;18(7):4309-4321

Biological impurities and immunostimulation



NCL in vitro assays to screen for sterility and IIMI contamination:

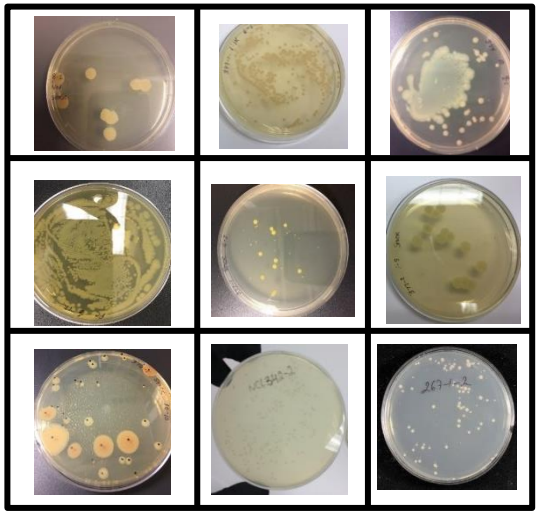
- STE-1 (.1, .2, .3 and .4)
- STE-2
- STE-3
- STE-4

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

- **~30 %** of preclinical formulations fail due to endotoxin contamination
- Common sources of endotoxin: **water and process**

- **< 5 %** fail due to bacterial contamination
- Common sources of bacterial contamination: **water, dust and handling**

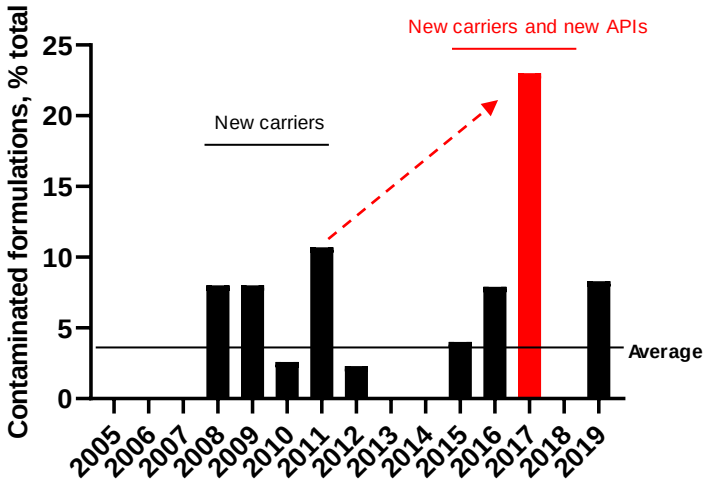
Examples of prescreen plates



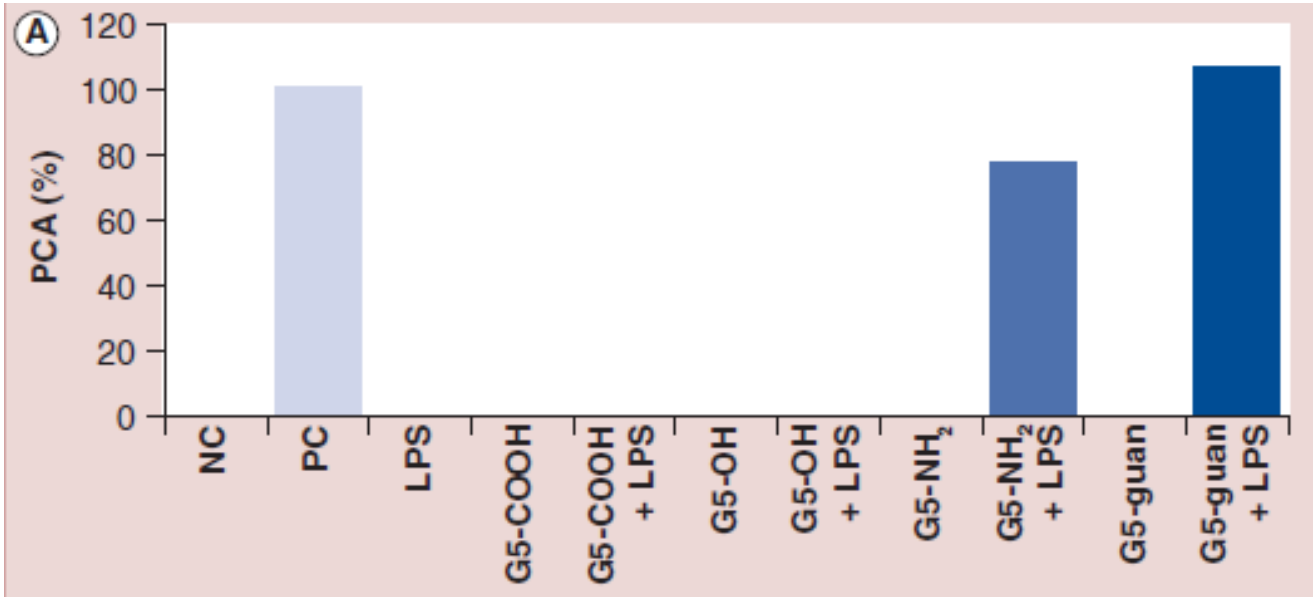
Environmental monitoring is important

- ***Phreatobacter oligotrophus***
- *Ralstonia pickettii*
- *Citrobacter freundii*
- *Ochrobactrum anthropi*
- *Achromobacter marplatensis*
- *Pseudomonas beteli*
- *Sphingomonas aeria*
- *Sphingomonas zeae*
- *Burkholderia contaminans*
- *Burkholderia cepacian*
- *Burkholderia cenocepacia*
- *Burkholderia metallica*
- *Staphylococcus haemolyticus*
- *Leifsonia lichenia*
- ***Rothia terrae***
- *Caulobacter segnis*
- *Rhizobium halotolerans*

Bacterial Contamination Rates

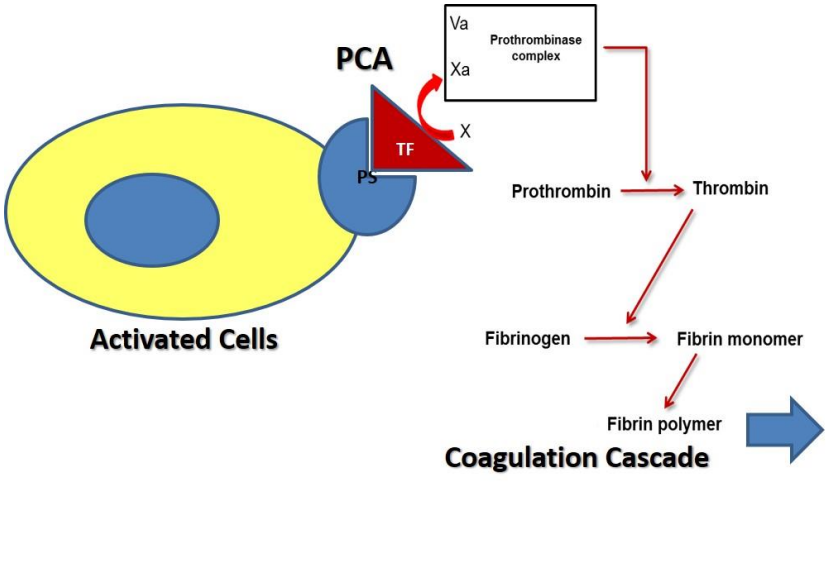


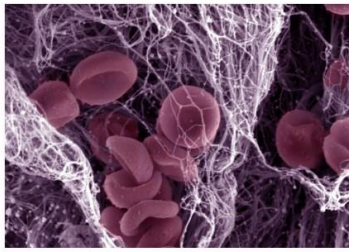
Immunomodulation



Anna Ilinskaya

Ilinskaya, A. N., et al., Nanomedicine (London, England), 2013, 9(9), 1311–1326.



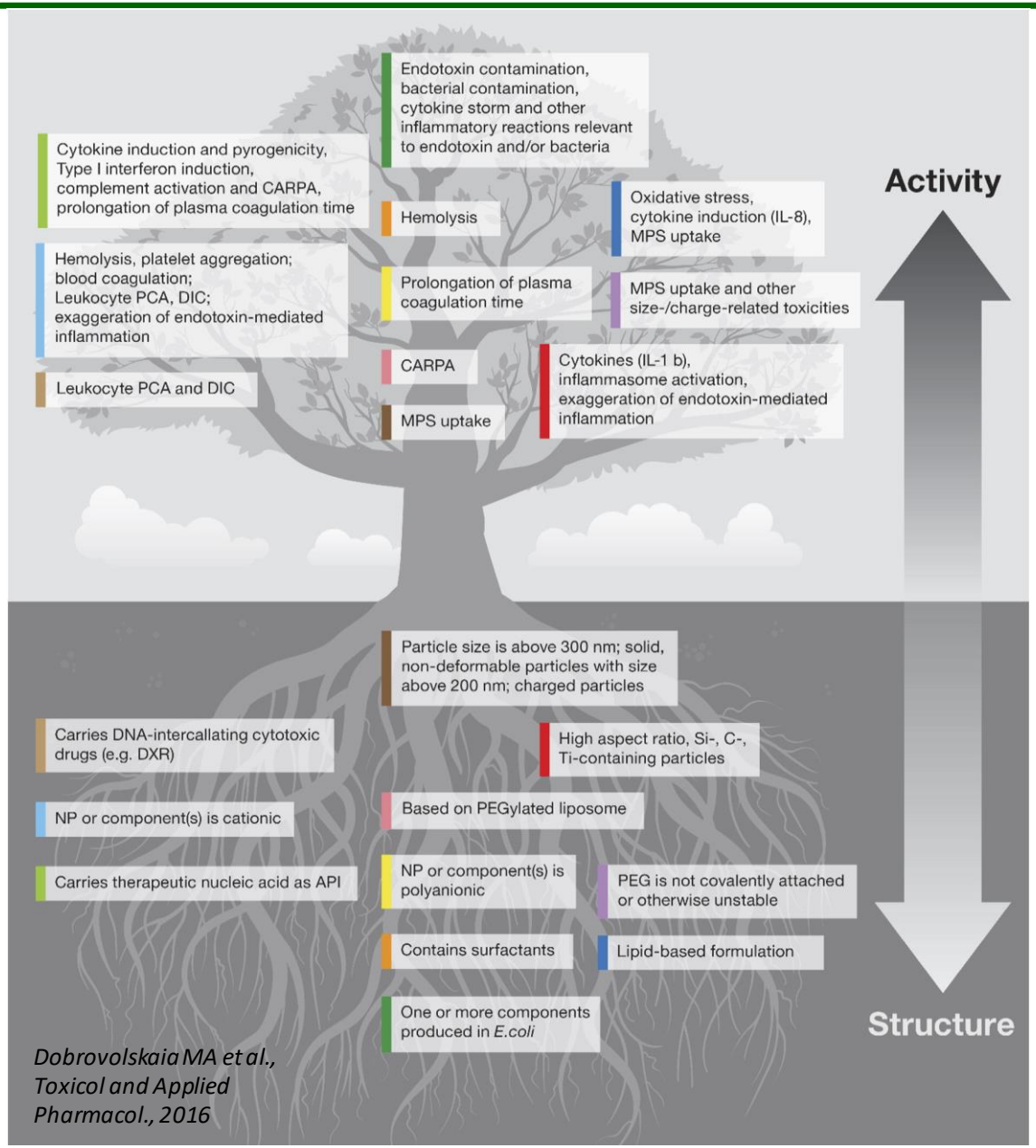


Blood Clot

Challenge is required to determine this type of toxicity

NCL in vitro assays to assess immunomodulation
https://ncl.cancer.gov/resources/assay-cascade-protocols

Other common immune reactions to nanomaterials



Dobrovolskaia MA et al., Toxicol and Applied Pharmacol., 2016

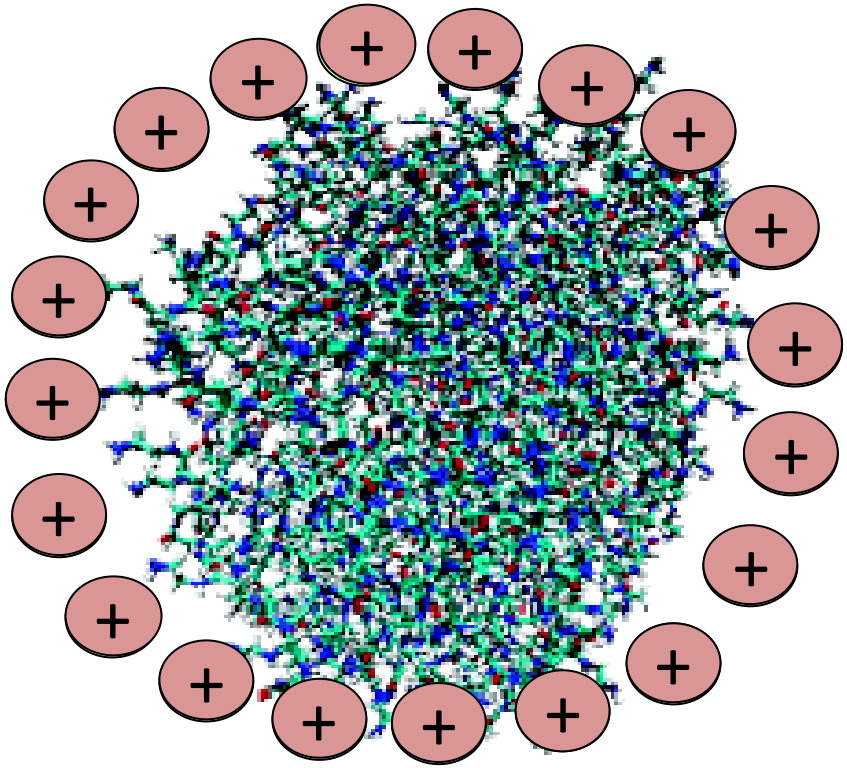
- Hemolysis
- Complement activation
- Change in plasma coagulation time
- Platelet aggregation
- Leukocyte Procoagulant Activity

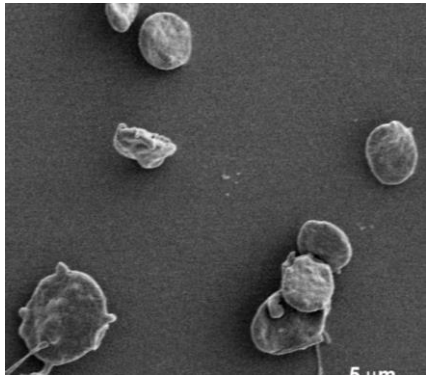
NCL in vitro assays to screen for these toxicities:

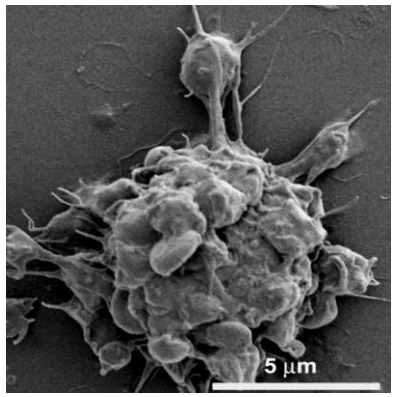
- ITA-1
- ITA- 2
- ITA-5
- ITA-12
- ITA-17

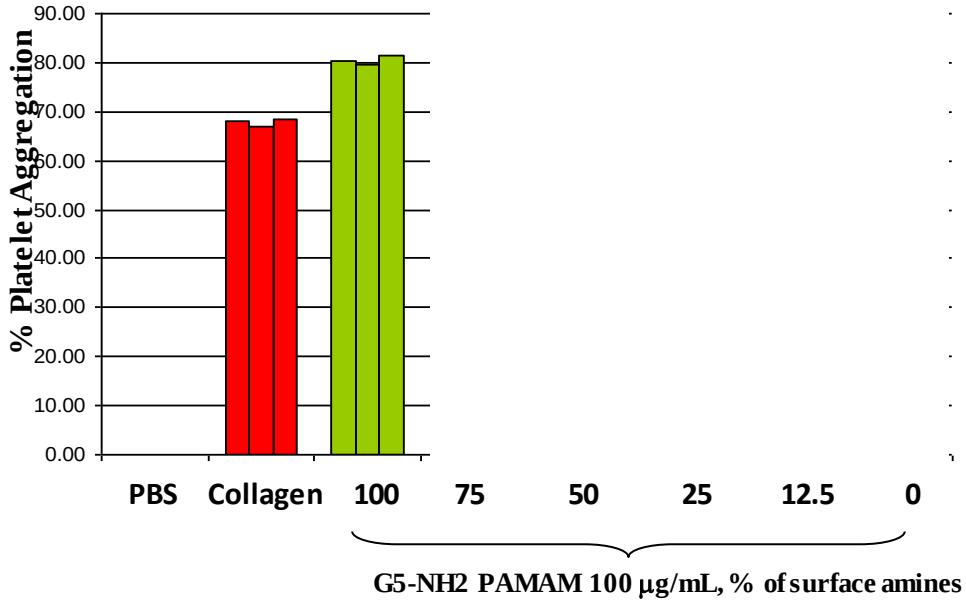
https://ncl.cancer.gov/resources/assay-cascade-protocols

Platelets: role of zeta potential









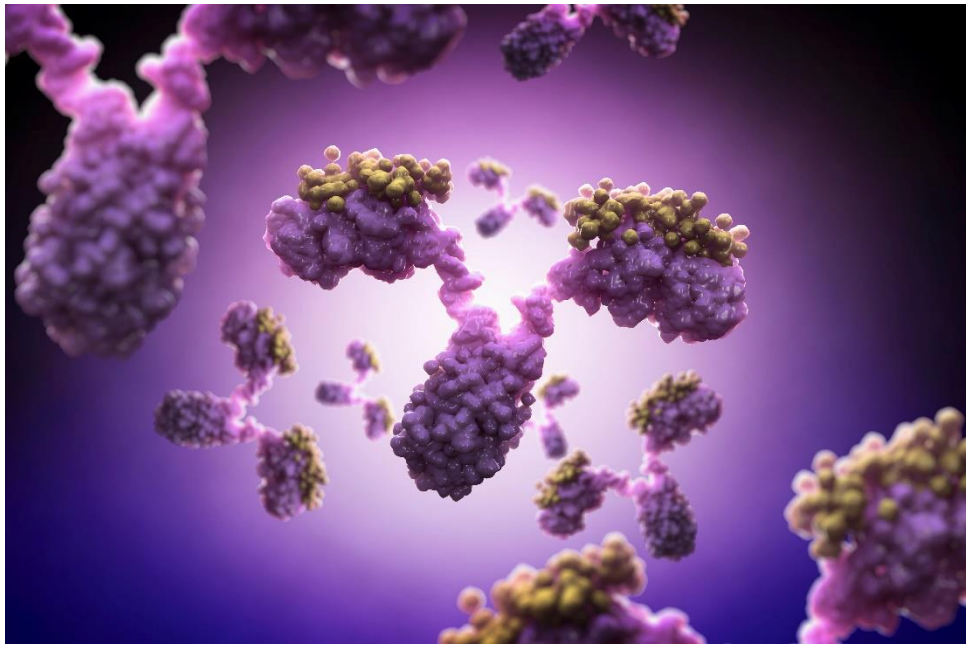
Surface charge and density of terminal groups affect platelet aggregation

Dobrovolskaia et al., Mol.Pharm, 2011

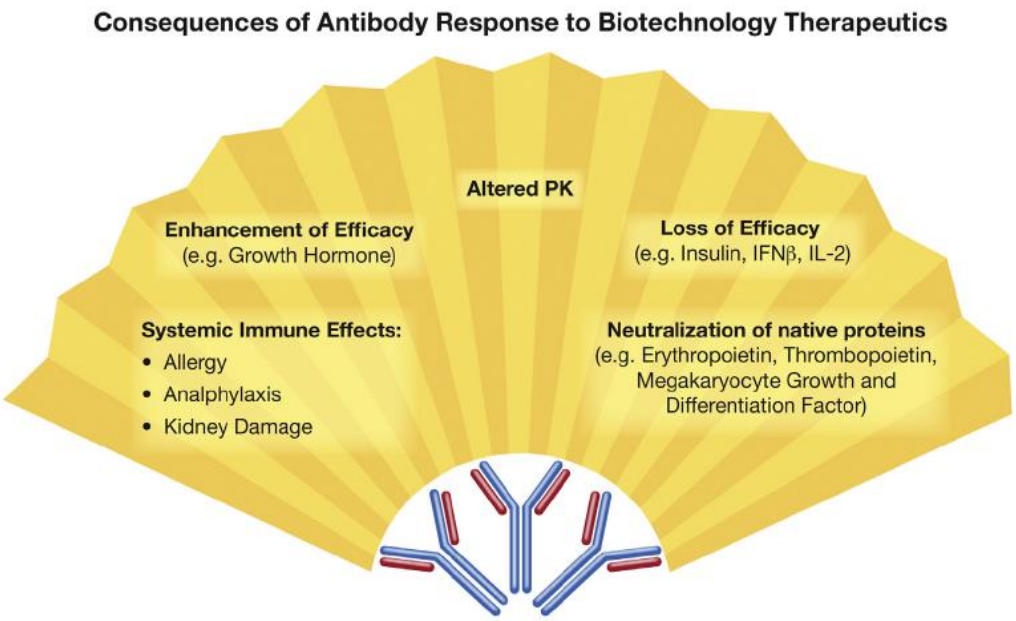
Immunogenicity

- Drug (common in biologics) or drug-protein adducts (common in small molecules) might also be recognized as foreign and stimulate an antidrug response (ADA)

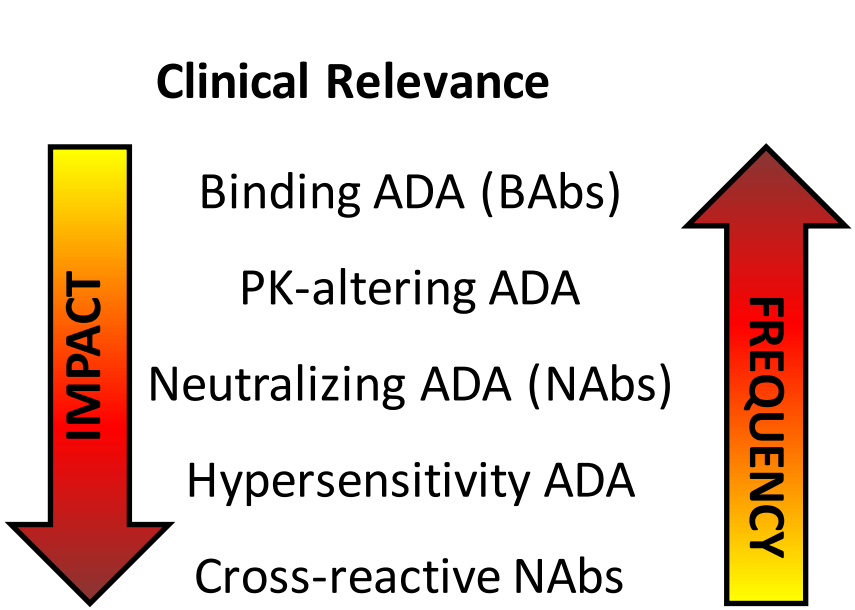
- **ADA = immunogenicity**



Immunogenicity

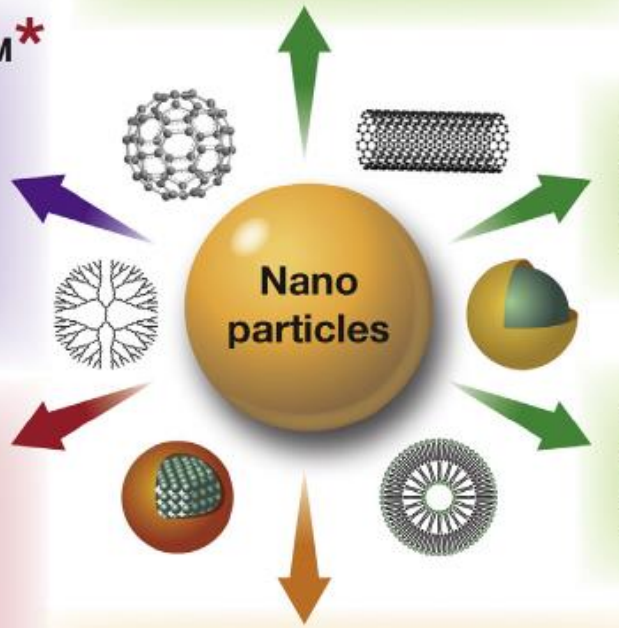


Ilinskaya AN & Dobrovolskaia MA, TAAP, 2015



- In vitro, in silico and in vivo methods, when used separately, can not accurately predict immunogenicity
- A combination of these methods help to get an insight and inform the design of clinical studies
- IG in animals may reveal IG difference between biosimilar and reference product; help to interpret the results of preclinical PK and Tox studies
- The information about a drug's immunogenicity is currently obtained from clinical studies

Nanoparticles' immunogenicity



ENM Approved for Clinical Use Which Resulted in Antigenic Response
- None

Research-Grade ENM* for Which Antibody Was Generated
- C60
- C70
- SWCNT
- PAMAM-Dendrimers
- Liposomes

ENM Carrying ThPr and Resulting in Anti-ENP Response
- None

Accidental Particles Contributing to Antigenicity of ThPr
- Glass Fibers
- Cellulose Fibers
- Tungsten
- Silicon Oil
- Rubber
- Stainless Steel
- Fluoropolymers

ENM Carrying ThPr and Resulting in Anti-ThPr Response
- None

ENM Carrying ThPr and Not Resulting in Anti-ThPr Response
- PEG-Gold-TNFα (Cyt6091)
- Liposome-Streptokinase
- PS-Liposome-Factor VIII

- **Nanoparticles Can Be Engineered To:**
  - Be (non)immunogenic
  - Reduce immunogenicity of therapeutic proteins
  - Enhance immunogenicity of proteins/peptides

Dobrovolskaia MA, Handbook of Immunological properties of engineered nanomaterials, 2016

Natural (pre)existing anti-PEG antibody

- PEGylation of nanoparticles is common to improve circulation time
- Several studies reported existence of naturally occurring antibody
- Functional significance of these antibodies is not well understood

analytical chemistry
Article
pubs.acs.org/ac

Measurement of Pre-Existing IgG and IgM Antibodies against Polyethylene Glycol in Healthy Individuals

Bing-Mae Chen,[†] Yu-Cheng Su,[†] Chia-Jung Chang,[†] Pierre-Alain Burnouf,[†] Kuo-Hsiang Chuang,[‡] Chien-Hsiun Chen,[†,§] Tian-Lu Cheng,[∥] Yuan-Tsong Chen,[†,⊥] Jer-Yuarn Wu,[*,†,§] and Steve R. Roffler[*,†,#]

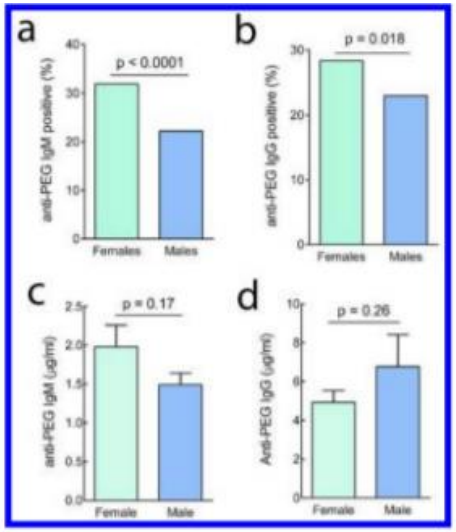
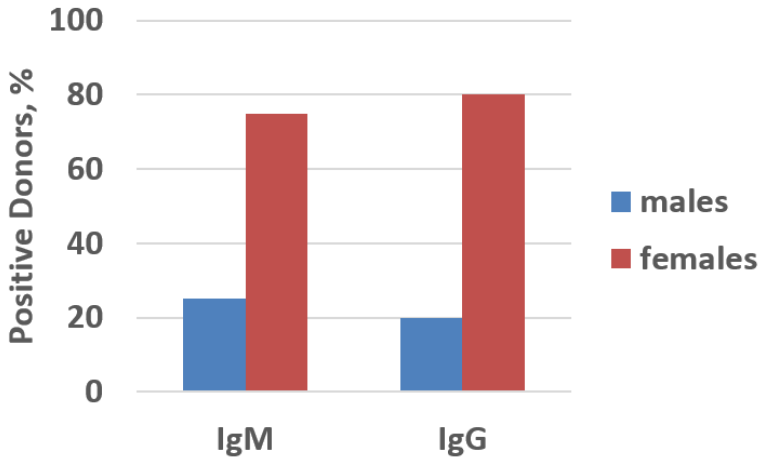


Figure 4. Anti-PEG antibodies are more prevalent in females than males. (a) The percentage of females (239 of 748) and males (168 of 756) with positive anti-PEG IgM. (b) The percentage of females (212 of 748) and males (174 of 756) with positive anti-PEG IgG. (c, d) The mean anti-PEG IgM (c) or anti-PEG IgG (d) concentrations in females and males among donors that were positive for anti-PEG IgM or anti-PEG IgG, respectively. Error bars, SEM.

molecules
MDPI
Article

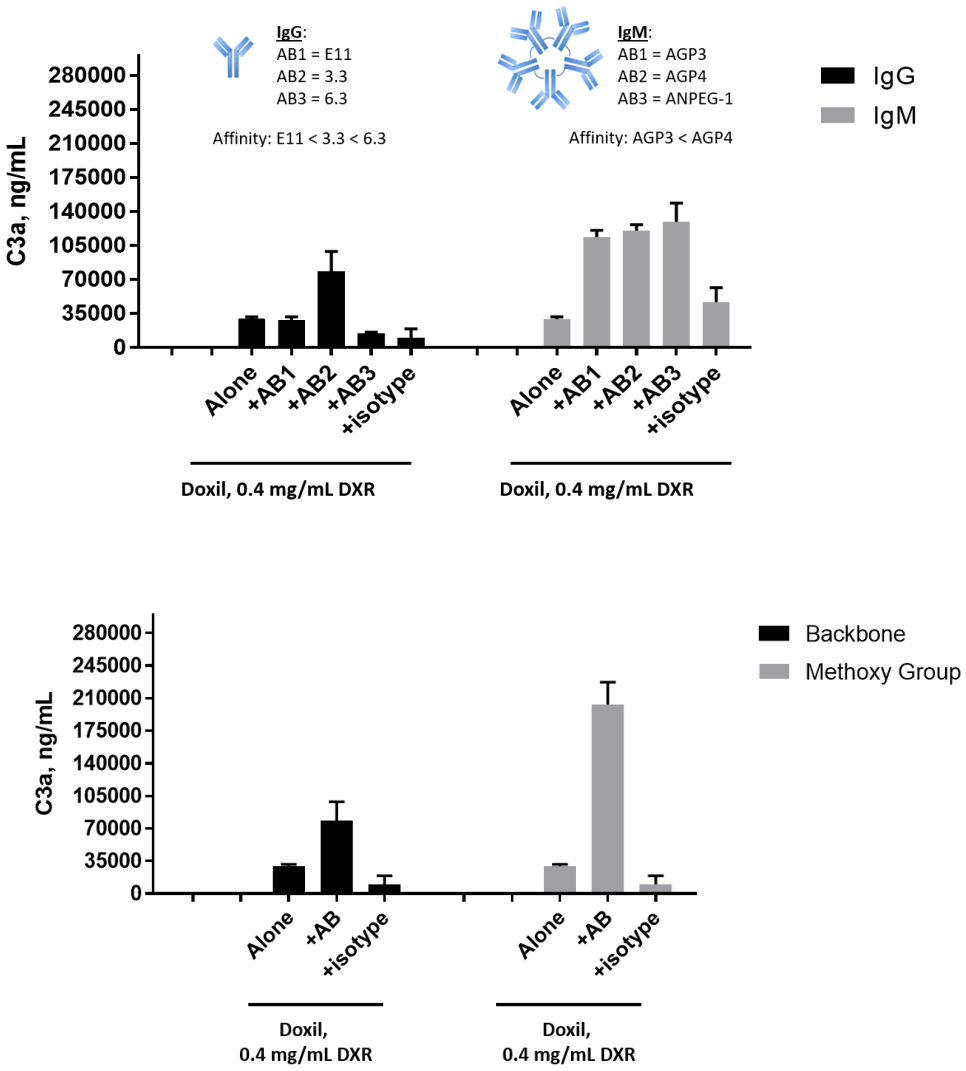
Understanding the Role of Anti-PEG Antibodies in the Complement Activation by Doxil in Vitro

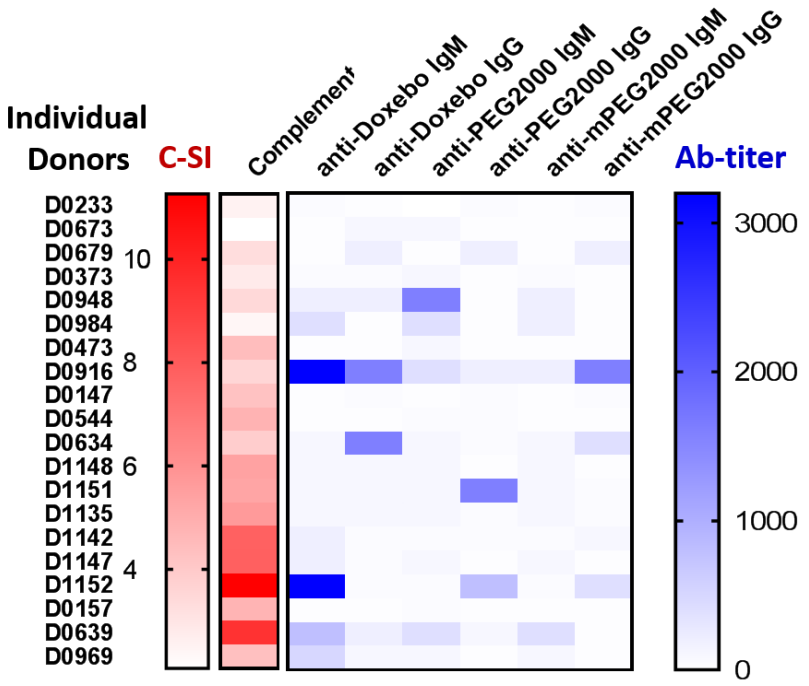
Barry W. Neun [1], Yechezkel Barenholz [2], Janos Szebeni [3,4,5] and Marina A. Dobrovolskaia [1,*]



High (> 800) titer PEG-reactive antibodies are detected in both healthy males and females, but are more prevalent in females

Anti-PEG antibody and complement activation





PEG Ab titer does not correlate with complement activation by PEGylated liposomes. The Ab suggest greater risk but can't predict the reaction and its magnitude. Functional assay, e.g. C3 ELISA, should be used instead

Purified anti-PEG antibodies contribute to the complement activation by Doxil

Thank you for your attention!