NCL Nanotechnology Characterization Laboratory

NCI Alliance for Nanotechnology in Cancer

Achievements, Disappointments and Lessons Learned from Characterization of Nanotechnology-Formulated Complex Drugs

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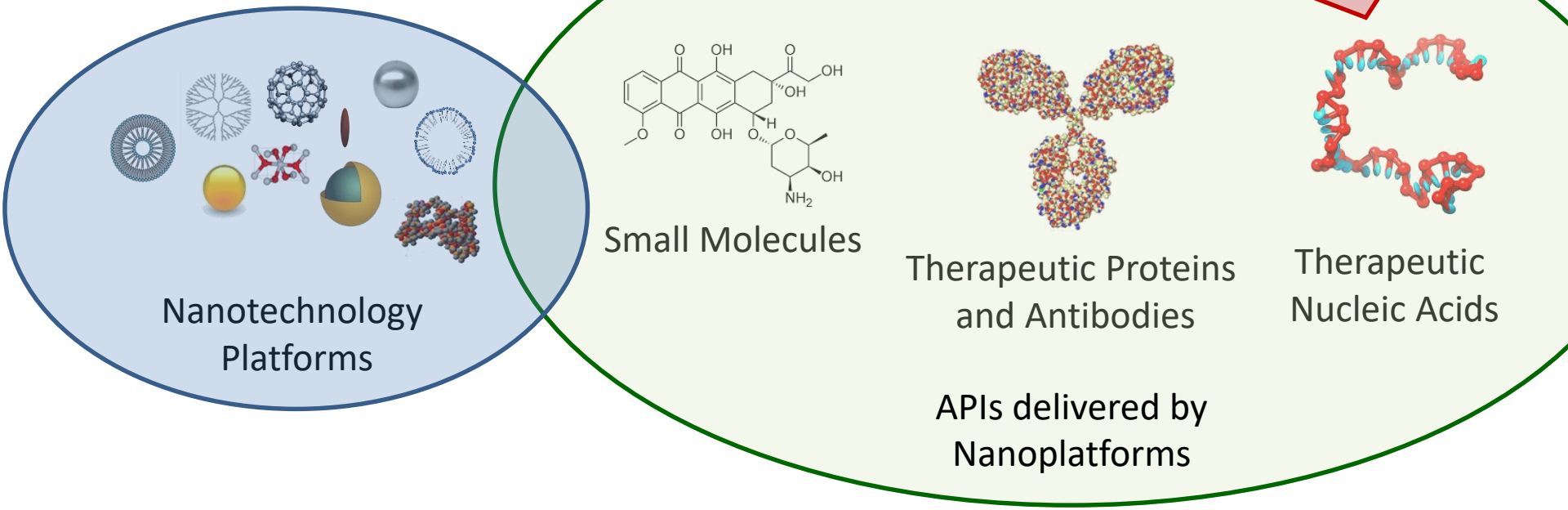
NATIONAL CANCER INSTITUTE

Frederick National Laboratory for Cancer Research

Presentation Outline

- Global Landscape of Nanotechnology Drug Products
- Achievements, Disappointments and Lessons Learned over past decade
- Challenges in Preclinical Characterization
  - Considerations for Platforms
  - Considerations for APIs
- Conclusion and THM
- Gaps and future directions

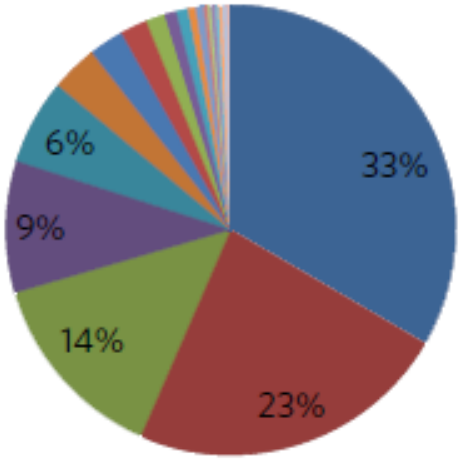
Case Studies
Lessons Learned



Evolving Landscape of Nanotechnology Products

- Liposome
- Nanocrystal
- Emulsion
- Iron-polymer complex
- Micelle
- Drug-protein complex
- Drug-polymer complex
- Dendrimer
- Polymeric NP
- Nanobubble
- Silica NP
- Drug-lipid complex
- Drug-metal complex
- Protein NP
- Drug NP
- Solid lipid NP
- Nanotube
- Metal-protein complex
- Metal-nonmetal complex
- Metal-polymer complex

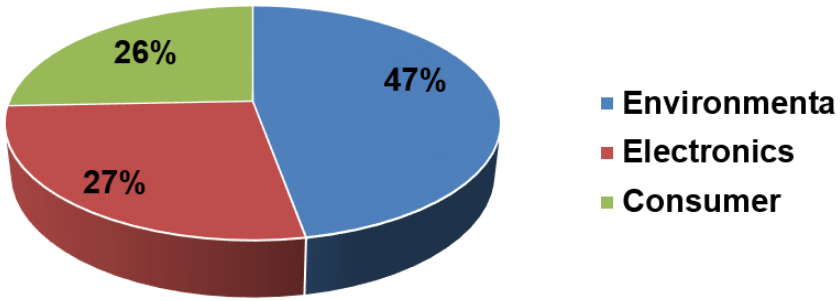
(1973-2015)



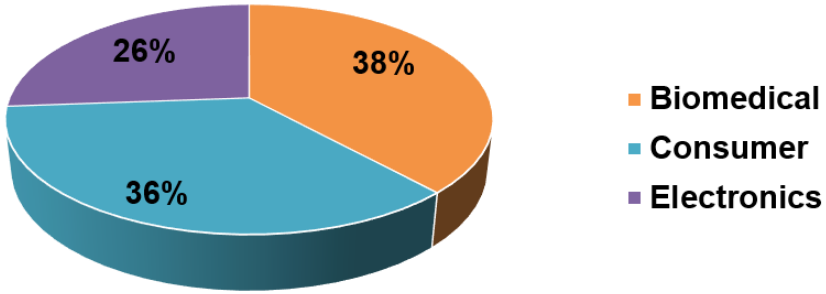
D'Mello S.R. et al., Nature Nanotechnology, June 2017

Liposomes, Nanocrystals and Emulsions dominate current nanomedicine landscape

Global Nanotechnology Market (2015)



CAGR rates (2016-2021)

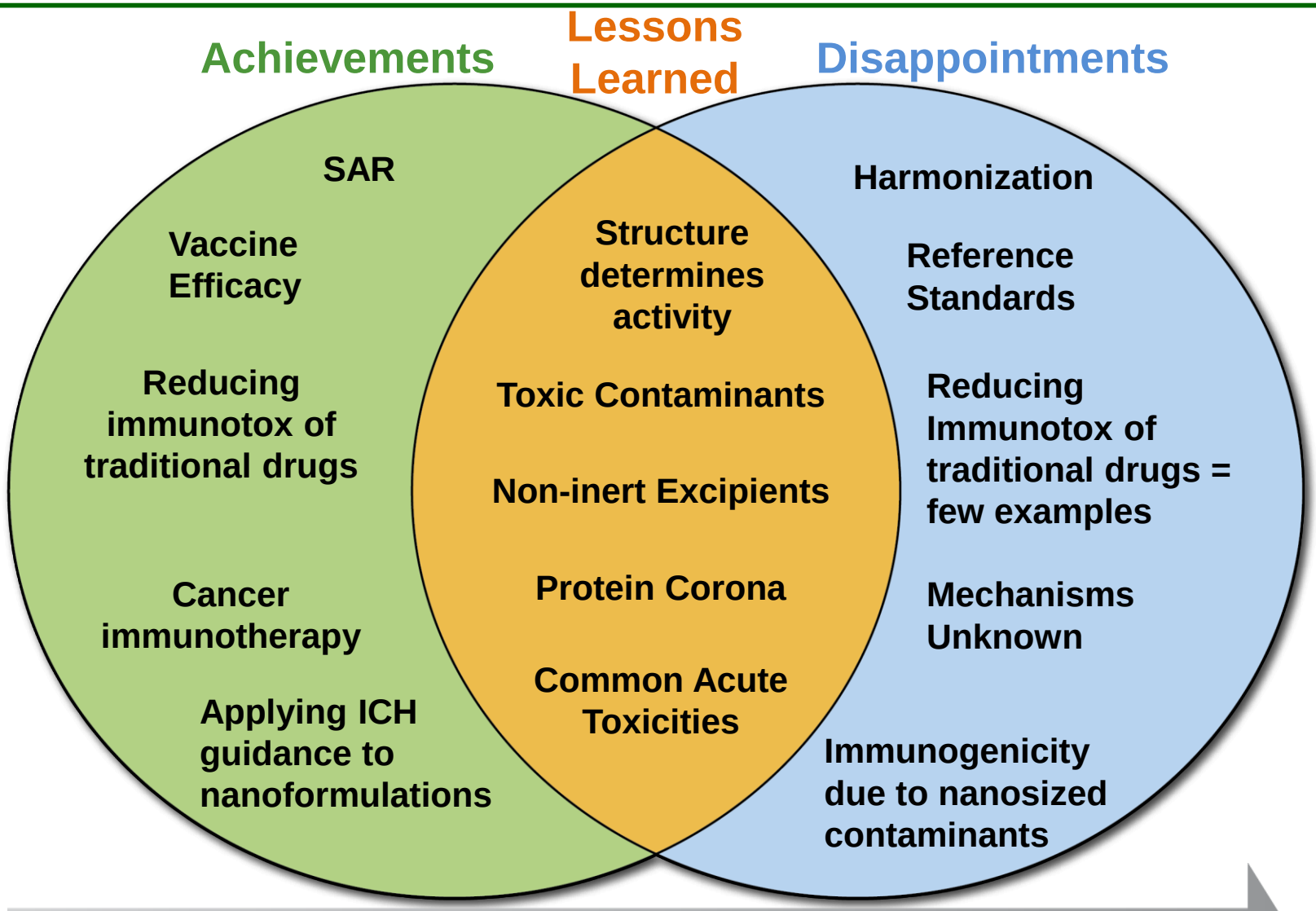


These graphs are prepared based on the business analytical report by Cumming S., BCC Research (201

Global Nanotechnology Market in 2015 was dominated by environmental, electronic and consumer products

Biomedical Applications of Nanotechnology are predicted to have the highest 5-year compound annual growth rate by 2021

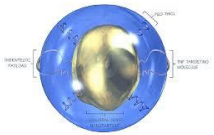
Achievements, Disappointments and Lessons Learned



Achievements

Data from A.M.M. Eggermont, MD

Female, 80 years old, large melanoma: Amputation?
Three months after TNF + melphalan ILP: > 98% tumor shrinkage, resection of residual tumor; no local recurrence



CytImmune

Disappointments

Contaminating particles induce protein aggregation

Biologicals 38 (2010) 602–611

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Meeting report

Meeting report on protein particles and immunogenicity of therapeutic proteins: Filling in the gaps in risk evaluation and mitigation

John Carpenter [a], Barry Cherney [b], Anthony Lubinecki [c,*], Stacey Ma [d], Ewa Marszal [e], Anthony Mire-Sluis [f], Thomas Nikolai [g], Jeanne Novak [h], Jack Ragheb [b], Jan Simak [e]

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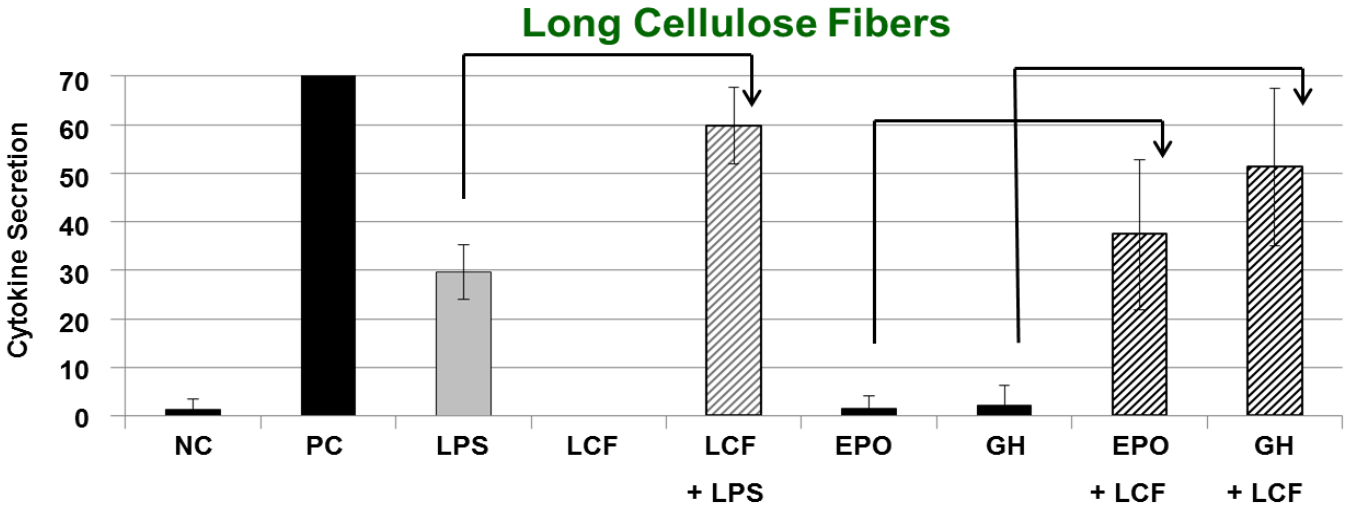
[b] Division of Therapeutic Proteins, Center for Drugs Evaluation and Research, Food and Drug Administration, Rockville, MD, USA
[c] Pharmaceutical Development & Manufacturing Sciences, Johnson & Johnson Pharmaceutical R&D, Radnor, PA, USA
[d] Genentech, Inc, South San Francisco, CA, USA
[e] Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville, MD, USA
[f] Amgen Inc, Thousand Oaks, CA, USA
[g] Abbott Laboratories, North Chicago, IL, USA
[h] CBR International Corp, Denver, CO, USA

Immunogenicity of Recombinant Human Interferon Beta Interacting with Particles of Glass, Metal, and Polystyrene

MIRANDA M.C. VAN BEERS,[1,2] FRANCESCA GILLI,[3] HUUB SCHELLEKENS,[2] THEODORE W. RANDOLPH,[4] WIM JISKOOT[1]

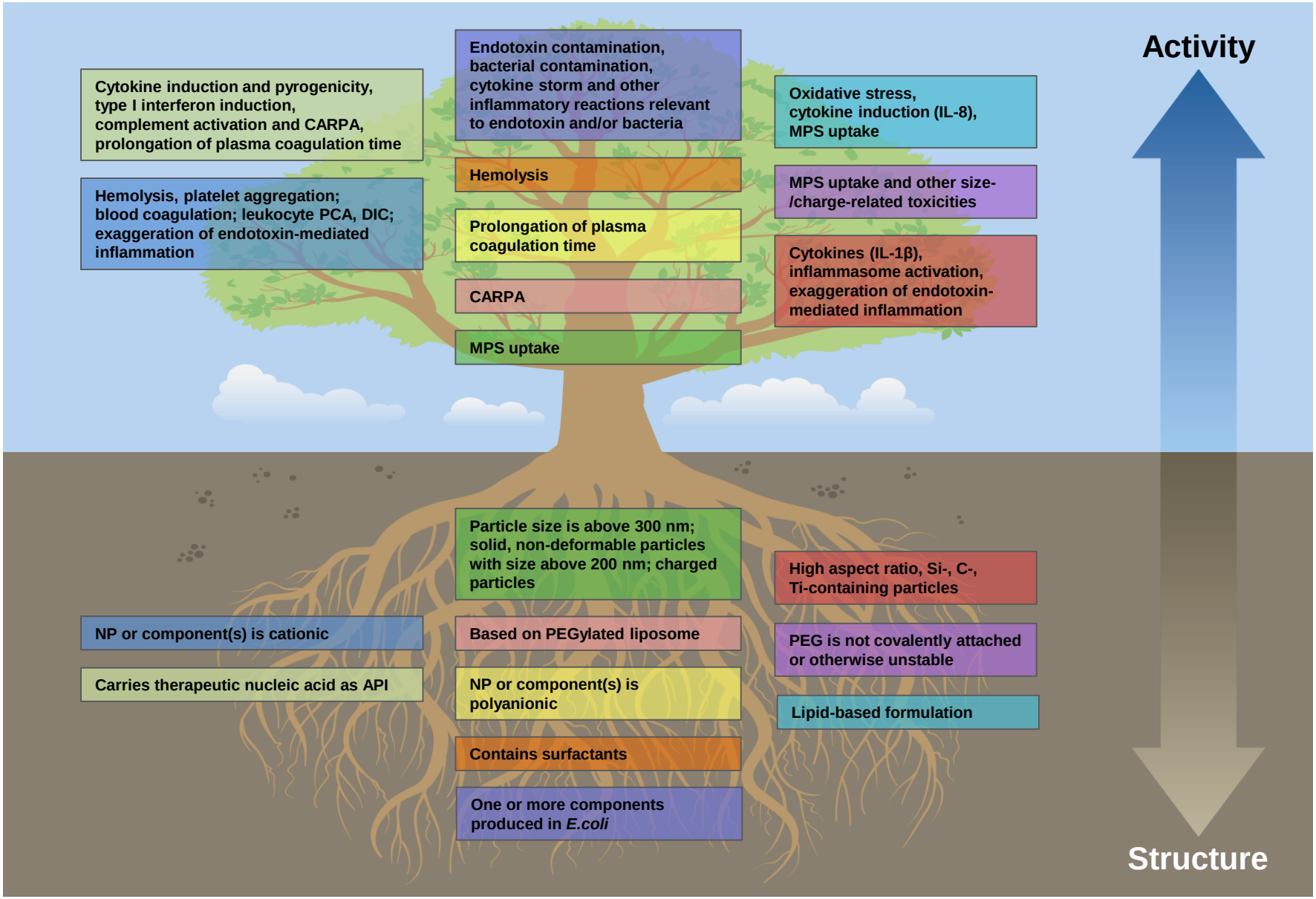
[1]Division of Drug Delivery Technology, Leiden/Amsterdam Center for Drug Research, Leiden University, Leiden, The Netherlands
[2]Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands
[3]Clinical Neurobiology Unit, Neuroscience Institute Cavalieri Ottolenghi, University Hospital San Luigi Gonzaga, Orbassano, Italy
[4]Department of Chemical and Biological Engineering, University of Colorado, Boulder, Colorado

Contaminating particles may exaggerate inflammation by traces of endotoxin in proteins and/or particles.



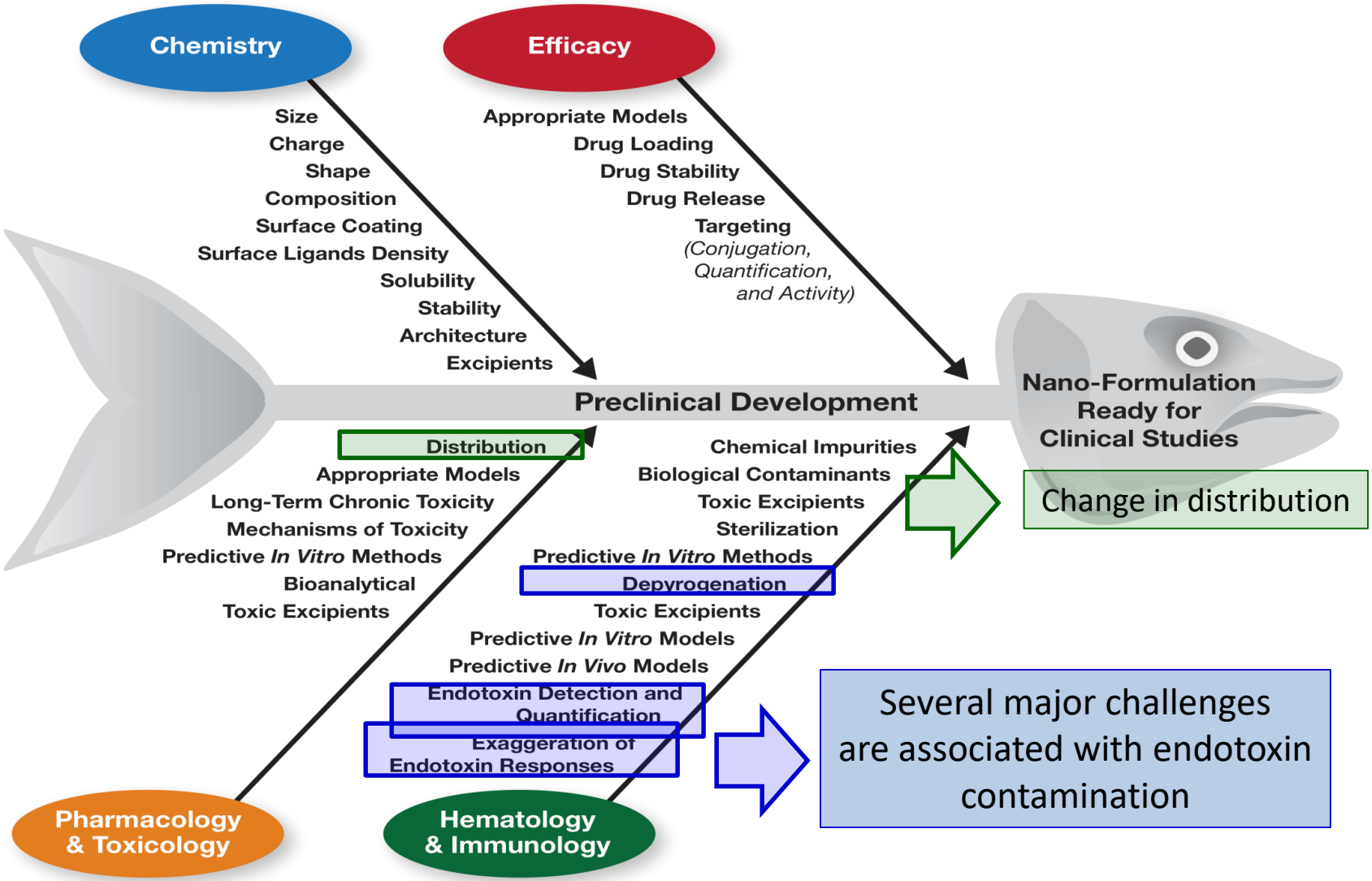
While monitoring protein aggregation due to contaminating particulate materials is important, traces of endotoxin contamination should not be ignored

Lessons Learned: Structure Activity Relationships



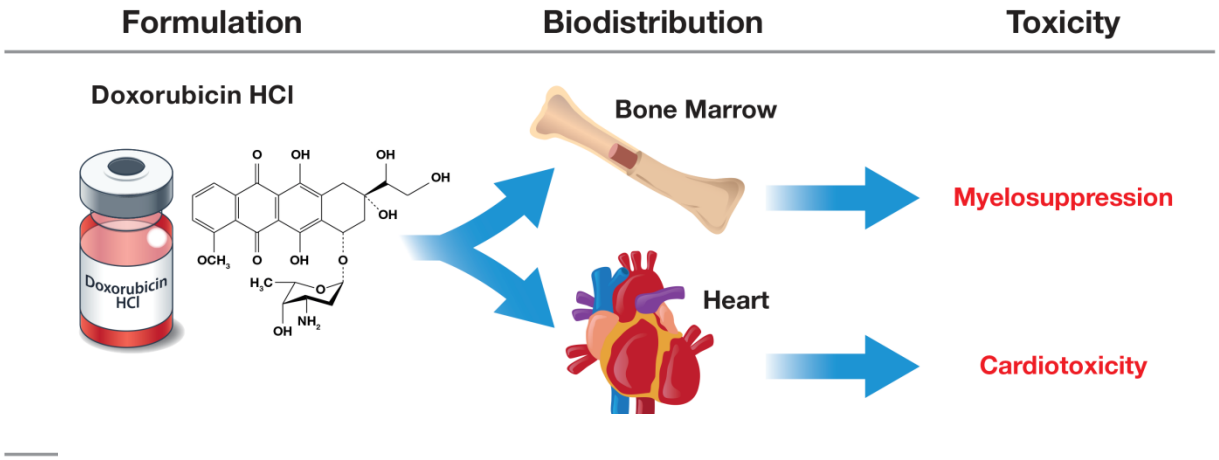
Dobrovolskaia MA., et al., Toxicol. Applied Pharmacol, 2016

Challenges in Preclinical Characterization



Preclinical characterization of nanomaterials has many assorted challenges in several areas: chemistry, toxicology, pharmacology, immunology, hematology and efficacy

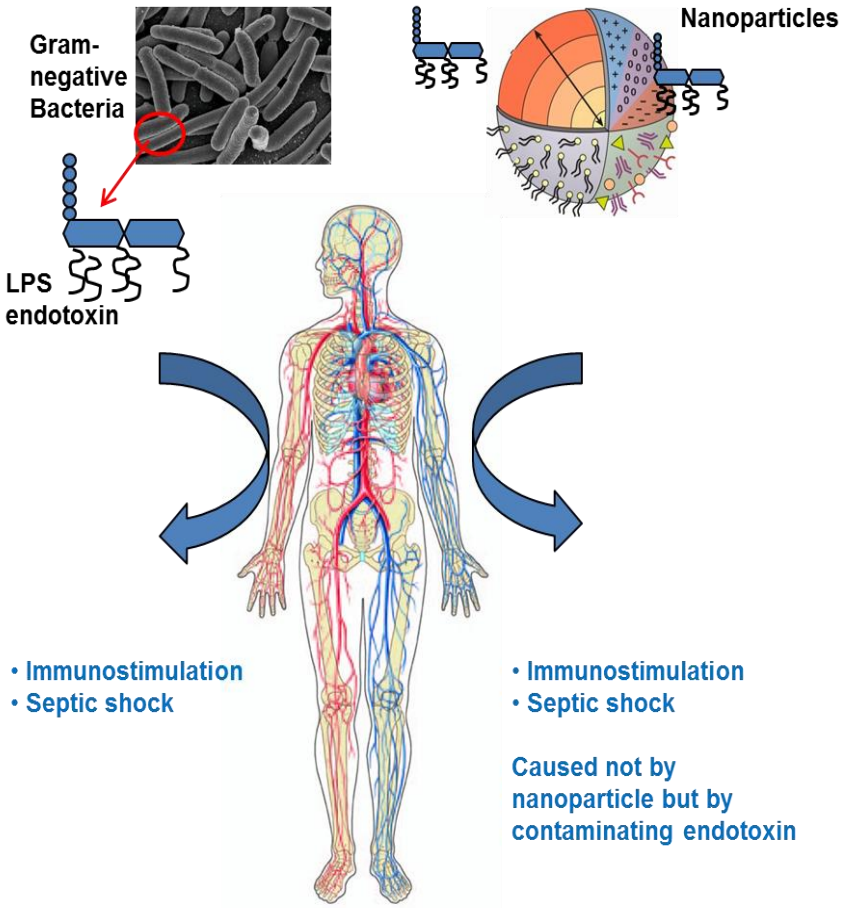
Change in biodistribution



Dobrovolskaia MA, 2017 Book Chapter in Pharmaceutical Nanotechnology: Innovation and Production, 2 Volumes

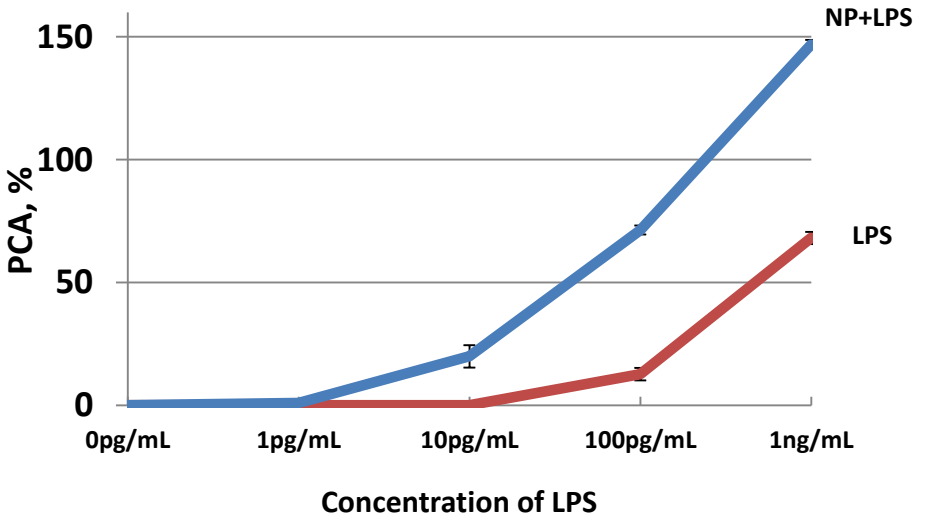
Altered distribution → altered toxicity profile

Importance of endotoxin screening



Endotoxin in Nanoparticles is bad because:

1. Erroneous data => wrong conclusions
2. Confound efficacy
3. Undesirable Toxicity
4. Exaggeration of endotoxin-mediated inflammation
5. Potential problems with immunogenicity of protein based APIs or targeting ligands

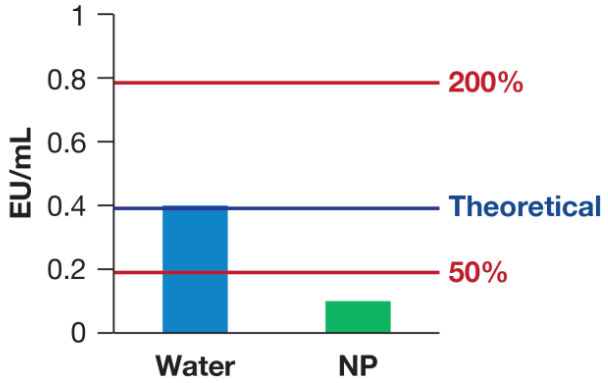
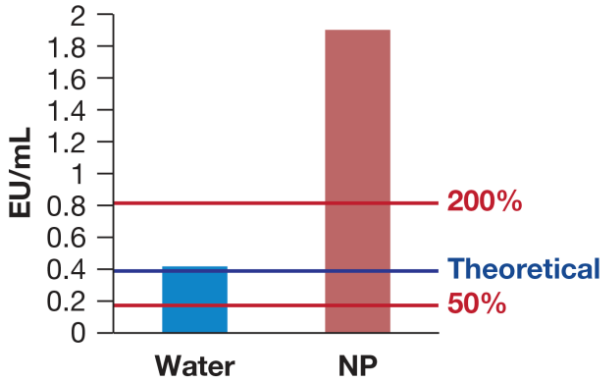
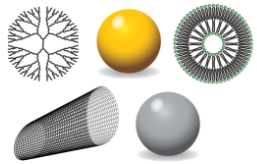
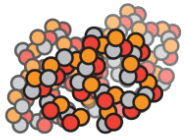


Dobrovolskaia MA et al, Nanomedicine 2012

- Endotoxin contamination is a common issue for engineered nanomaterials
- >30% preclinical nanoformulations fail due to endotoxin

Example: Cationic PAMAM dendrimers exaggerate endotoxin-mediated leukocyte procoagulant activity

Challenges in Endotoxin Detection

Type of Interference:	Inhibition	Enhancement
Evidenced by:	Spike Recovery <50%	Spike Recovery >200%
Typical IEC Result:		
Commonly Observed in the Following Nanoparticles:	 Cationic Nanoparticles, Unfunctionalized CNTs, Protein Rich Formulations,¶ Anionic Metal Colloids, Surfactant Containing Formulations¶	 Polymeric Nanoparticles, Protein Rich Formulations¶ Surfactant Containing Formulations¶

Dobrovolskaia MA, Journal of Controlled Release 220, 571-583

Nanomedicine Grand Challenge:
Nanoparticles that interfere with one or more LAL formats.

Immune response related challenges with clinical translation of therapeutic nucleic acids

This section will focus on one class of drug products, i.e. TNA, to highlight the existing challenges with their translation from bench to bedside

Immunological Barriers Halting Translation of Traditional TNAs

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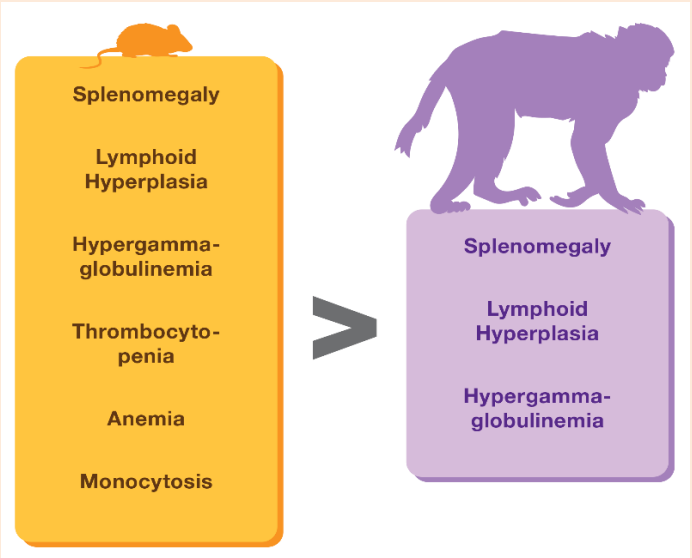
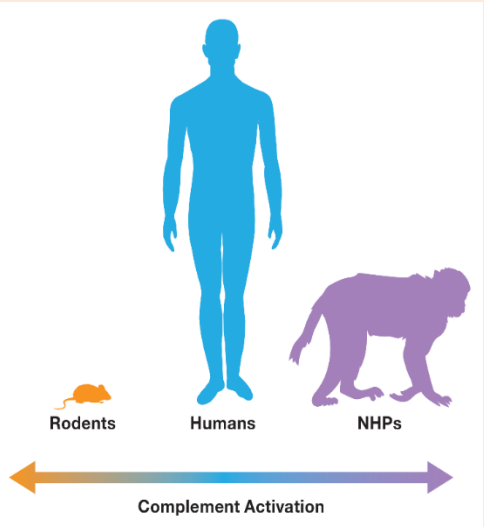
Three Top Immune-Mediated Toxicities

- Cytokine Storm
- CARPA
- Prolongation of Blood Coagulation Time

Common Preclinical Challenges

- Different sensitivity between human and preclinical animal species
- Delivery





Zamboni W., et al., ADDR, 2018

"Toll" of platform Selection

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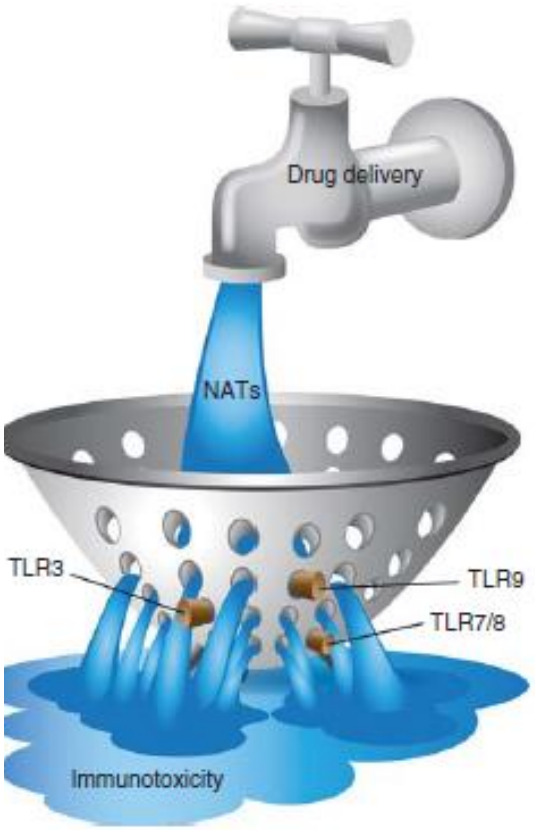
Receptor/protein	Species	Intracellular localization	Nucleic acid	Induces:
TLR3	Human, Mouse	Endosome Endolysosome	dsRNA	Cytokines Type I interferons
TLR7	Human	Endosome Endolysosome	ssRNA	Cytokines Type I interferons
TLR8	Mouse	Endosome Endolysosome	ssRNA	Cytokines Type I interferons
TLR9	Human, Mouse	Endosome Endolysosome	ssDNA Plasmid DNA Bacterial DNA dsDNA/RNA hybrids	Cytokines Type I interferons Type II interferons
TLR13	Mouse	Endosome Endolysosome	Bacterial RNA Ribozyme	Cytokines Type I interferons
RIG-1	Human, Mouse	Cytosol	Short dsRNA	Type I interferons
LGP-2	Human, Mouse	Cytosol	Long dsRNA ssRNA ssDNA	Regulatory role in MDA-5-initiated signaling; Type I interferons
MDA-5	Human, Mouse	Cytosol	Long dsRNA ssRNA ssDNA	Type I interferons
MAVS (IPS-1, CARDIF, VISA)	Human, Mouse	Mitochondria	ssRNA ssDNA	Cytokines Type I interferons
PKR	Human, Mouse	Cytosol	dsRNA	Type I interferons
OAS	Human, Mouse	Cytosol	dsRNA	Degradation of RNA and type I interferon induction through RIG-1
DNA-dependent RNA polymerase III	Human, Mouse	Cytosol	dsDNA	Conversion of DNA into 5'-triphosphate short dsRNA to initiate type I interferon response through RIG-1
STING	Human, Mouse	Endoplasmic reticulum Golgi	dsDNA	Type I interferons
cGAS	Human, Mouse	Cytosol	dsDNA	Type I interferons
DAI	Human, Mouse	Cytosol	dsDNA	Type I interferons
IFI16/p204	Human, Mouse	Cytosol	dsDNA	Type I interferons
DDX41	Human, Mouse	Cytosol	dsDNA	Type I interferons
DNA-PK	Human, Mouse	Cytosol	dsDNA	Cytokines Type I interferons
MRE11	Human, Mouse	Cytosol	dsDNA	Type I interferons
AIM-2	Human, Mouse	Cytosol	dsDNA	IL-1, IL-18, IL-33, IL-36, IL-37, IL-38



Blocking/Avoiding TLRs is insufficient

Dobrovolskaia MA & McNeil SE, EOBT, 2016

SAEs and Deaths in Clinical Trials

Battered and bruised, ProNAi Therapeutics drops PNT2258 completely

…no further investment in PNT2258 or the underlying DNAi platform by ProNAi is contemplated…

Dicerna Prioritizes Resources to Advance GalXC™ Product Candidates

…Clinical Development Discontinued for DCR-MYC in Oncology Indications…

Mirna Therapeutics Halts Phase 1 Clinical Study of MRX34

… following multiple immune-related serious adverse events (SAEs) observed in patients….. Three of these immune-related events resulted in the patient's death…

Alnylam shares crater after trial deaths force investigators to scrap PhIII RNAi drug

Alnylam shocked its investors with news that it has decided to scrap revusiran, its second most advanced RNAi therapy in the pipeline, due to a spike in the number of deaths among patients taking the drug in a late-stage trial. All dosing has been stopped and won't be resumed.

- **Several companies discontinued product development**
- **Immune-Mediated Serious Adverse Events are among the reasons**

http://www.fiercebiotech.com/biotech/battered-and-bruised-pronai-therapeutics-drops-pnt2258-completely http://pronai.investorroom.com/

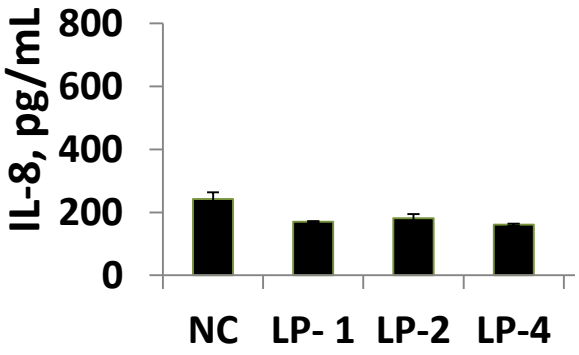
http://investors.dicerna.com/releasedetail.cfm?ReleaseID=990825

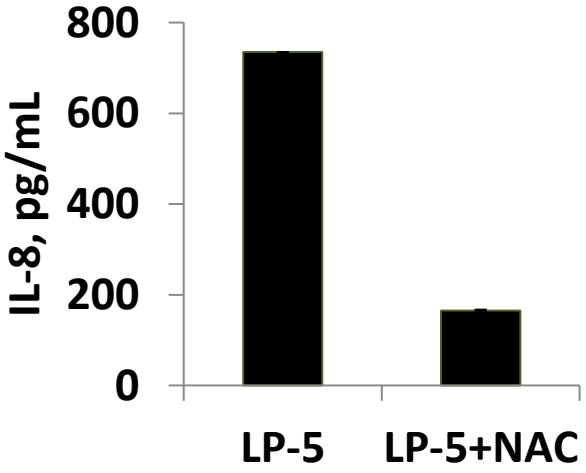
https://endpts.com/deaths-force-alnylam-to-scrap-its-no-2-drug-in-phiii-development/

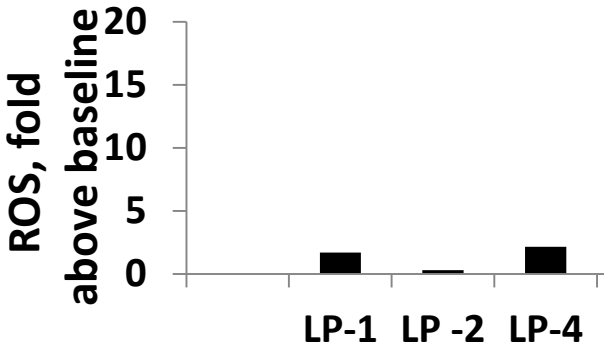
Immunotoxicity of Nanocarriers

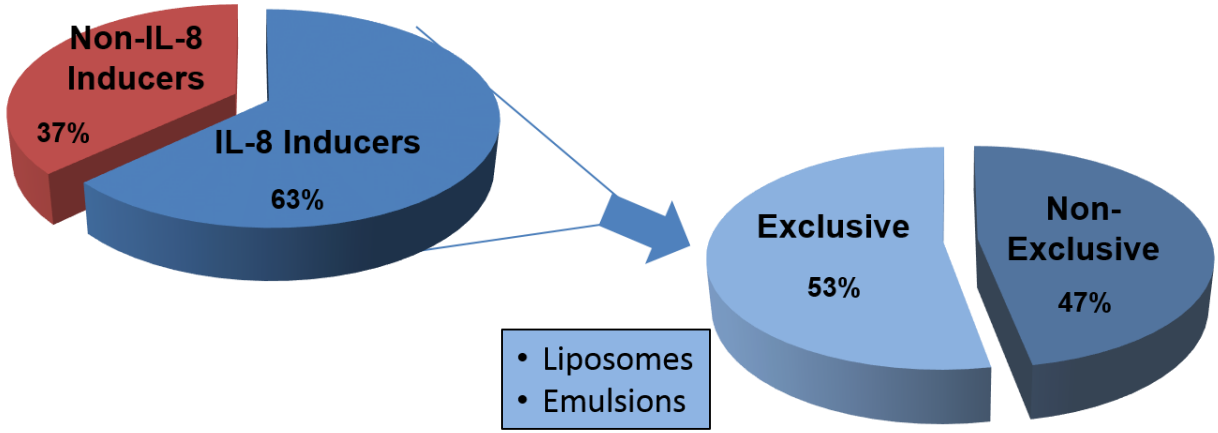
This section will discuss undesirable properties of nanocarriers and ways to overcome them

Case Study 1: Anionic and Neutral Liposomes





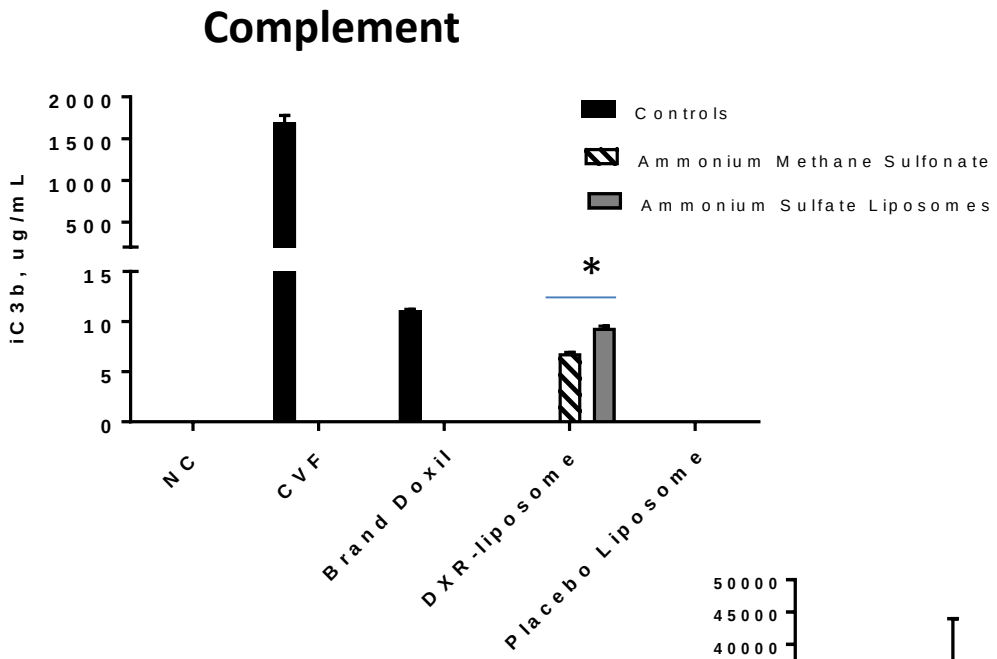


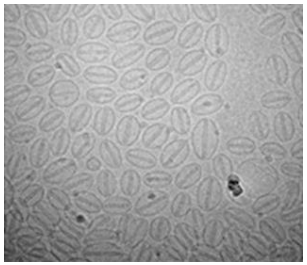


Exclusive means no concurrent induction other common cytokines (e.g., TNFα, IL-1β, IL-6, IL-10)

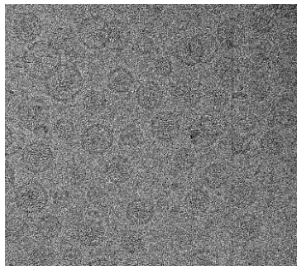
Chemokine induction by anionic and neutral liposomes is due to the oxidative stress and can be effectively managed by antioxidants

Case study 2: role of shape and interior cavity





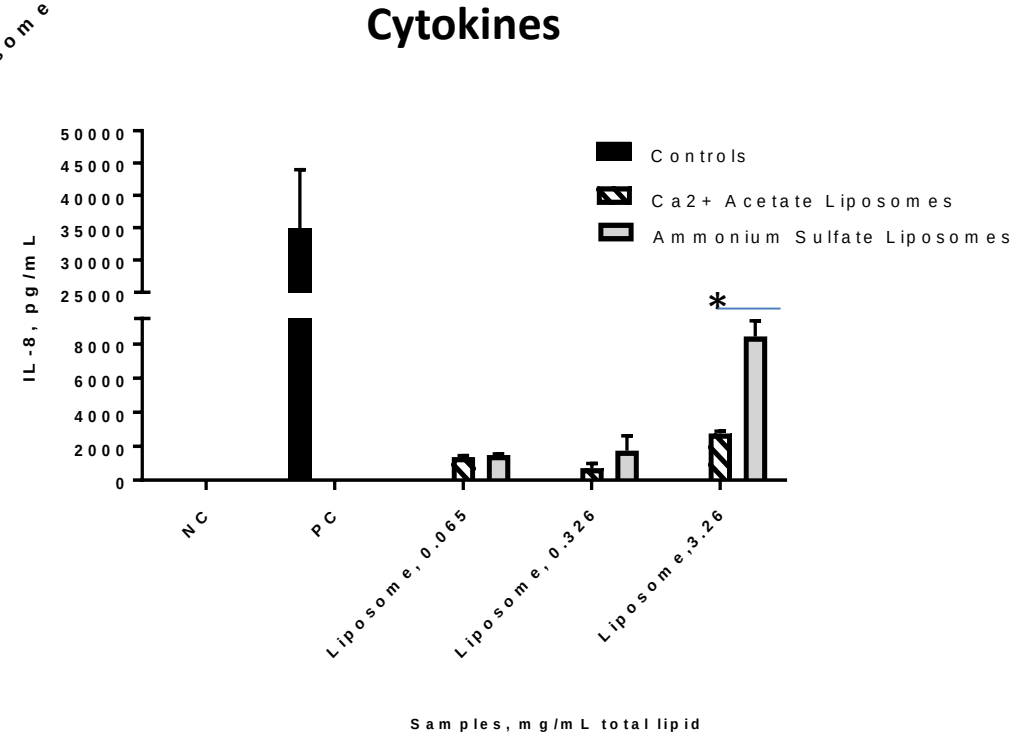
DXR-loaded "coffee-bean" shape liposomes



DXR-loaded Spherical shape liposomes

Shape of the liposome is important contributor

Ion content in the liposome **interior cavity** is important contributor



This study is conducted in collaboration with Chezy Barenholz, Hebrew University

Case Study 3: Cationic Liposomal Carriers

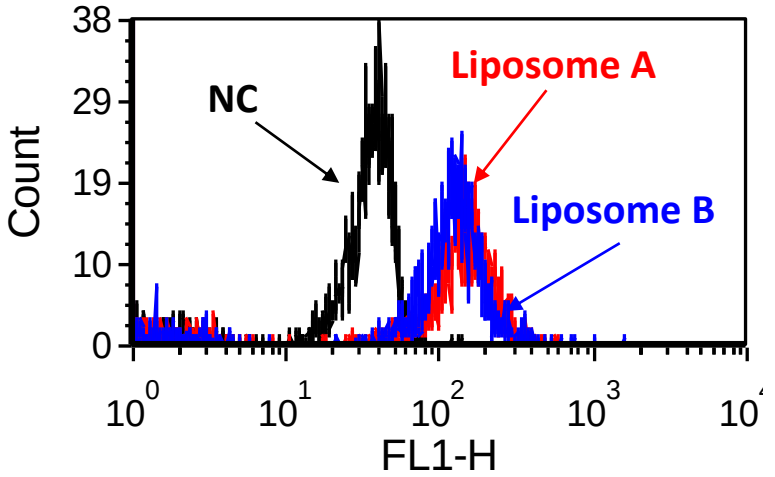
Cationic Liposomes

	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, preventing their systemic administration to avoid Cytokine Storm and other tox
- While cytokines are needed for adjuvanticity, excessive secretion of some of them (e.g. TNFα) often leads to side effects (necrosis at the injection site)



Oxidative stress is underlying mechanism

Mechanism of IL-1 induction by cationic nanocarriers

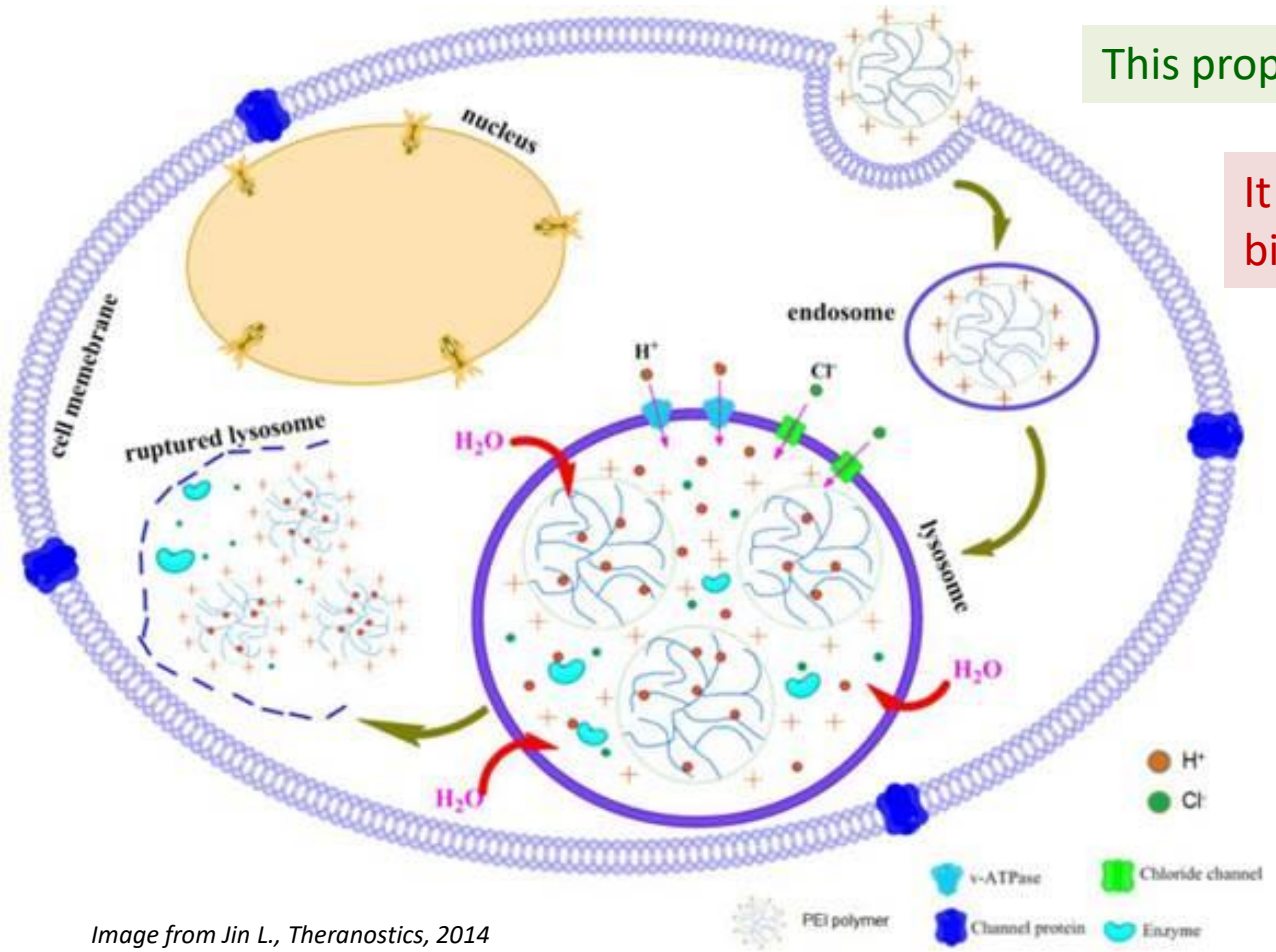


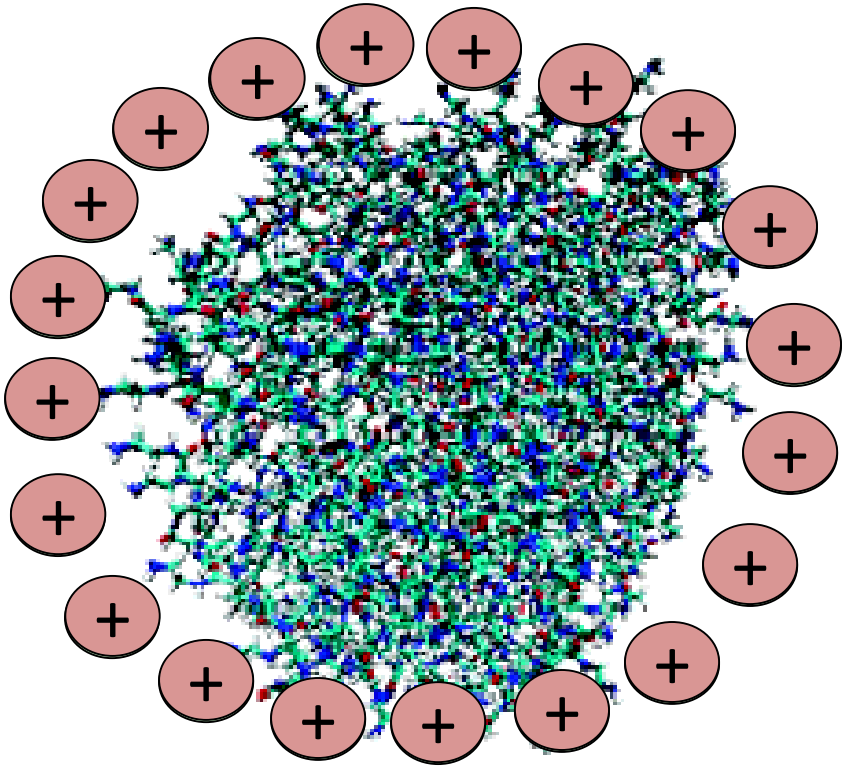
Image from Jin L., Theranostics, 2014

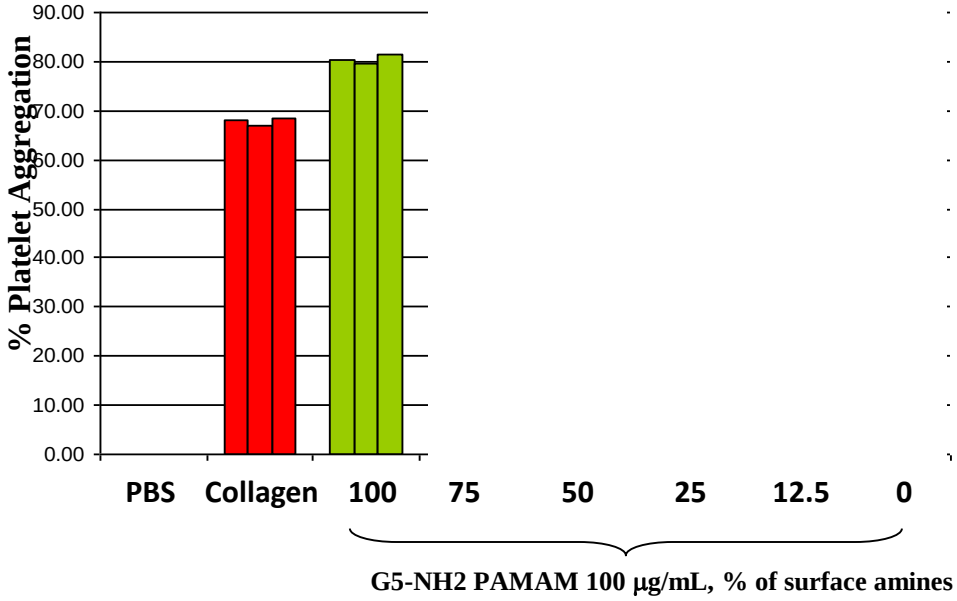
This property is beneficial for vaccines

It crates safety concerns for biotechnology therapeutics

Cationic particles induce IL-1β through activation of NLRP3 inflammasome triggered by a proton-sponge effect

Case Study 4: cationic polymeric platforms





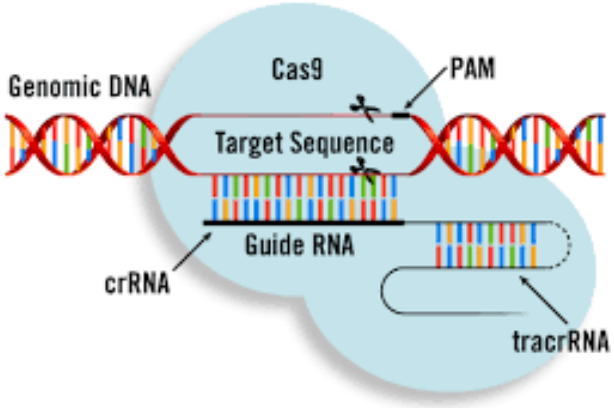
Cationic nanocarriers often are pro-thrombogenic
The toxicity can be reduced by masking cationic groups of the particle surface

Immunological recognition of new TNAs

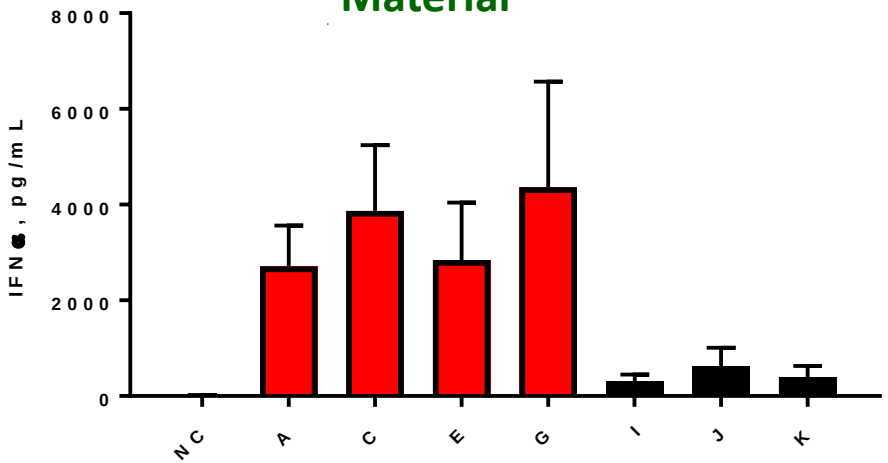
- *CRISPR gRNA*
- *DNA/RNA nanoparticles*

This section will discuss immunostimulatory properties of novel TNAs and ways for reducing undesirable immunostimulation

Case Study 5: sgRNA

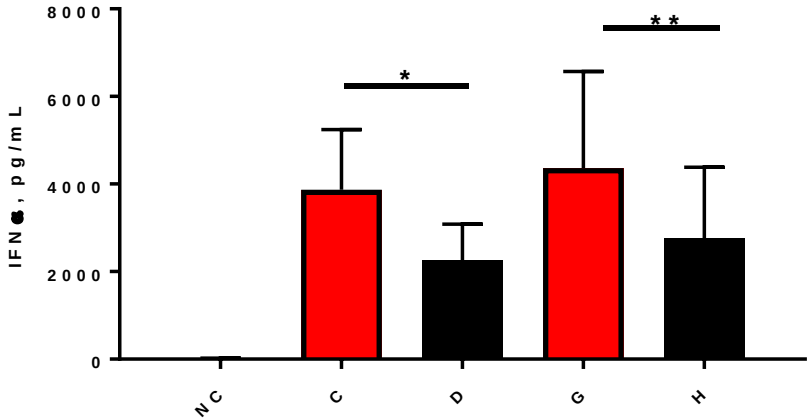


Material



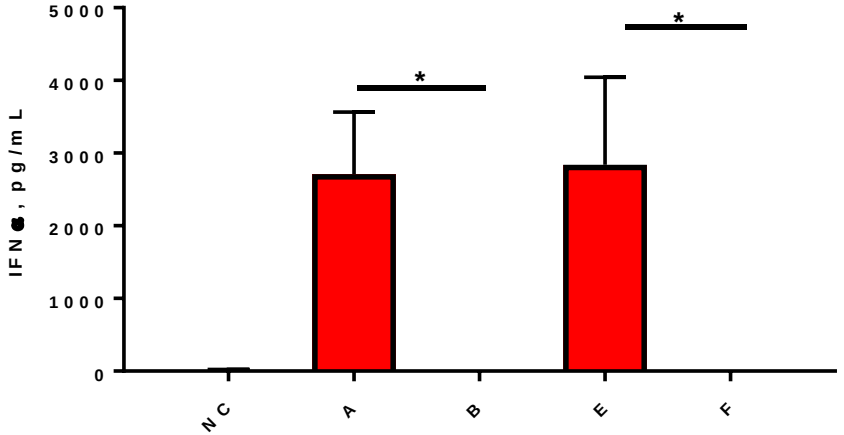
RNA > DNA

Presence of 5`-triphosphate



With 5'-PPP > without 5'-PPP

Chemical Modifications



Unmodified > 2'OMe+PS

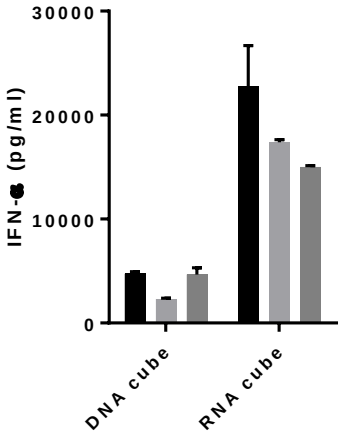
Schubert M., et a., J Cytokine Biol, Vol 3(1): 121

This study was done in Collaboration with Dr. Mark Behlke, IDT

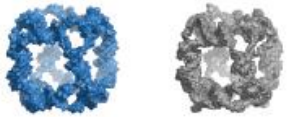
Case Study 6: DNA/RNA Nanoparticles

UNC CHARLOTTE | NCL Nanotechnology Characterization Laboratory | NCI Alliance for Nanotechnology in Cancer

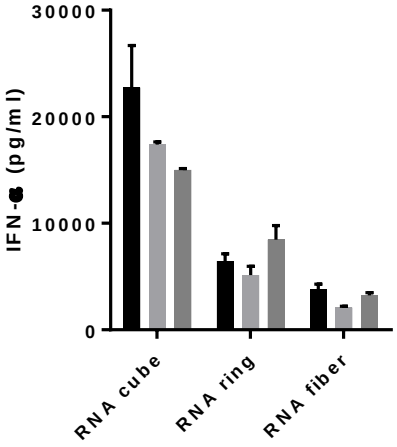
Material

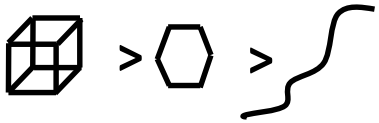


DNA < RNA

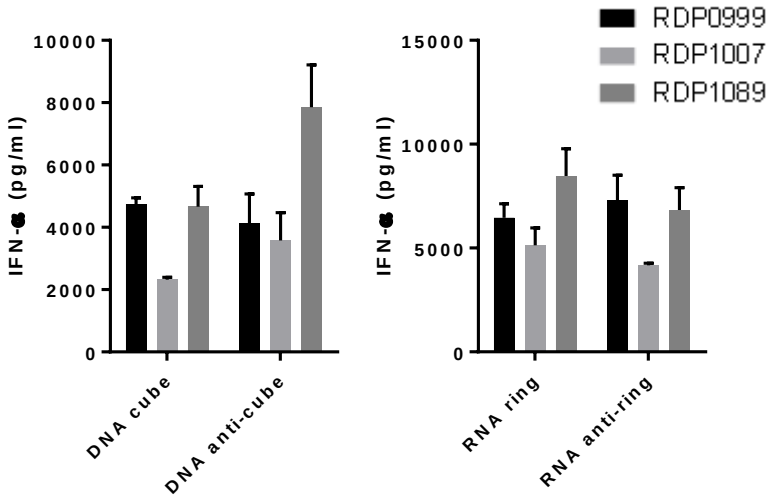


Structure





Sequence complementarity



RNA nanoparticles are more potent than DNA counterparts

Globular particles are more potent than planar than fibrous stuctures

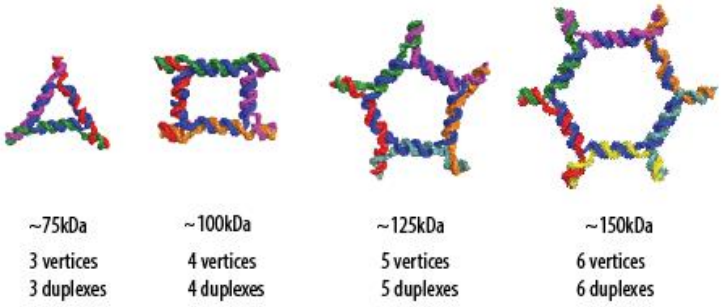
No significant difference between sense and anti-sense

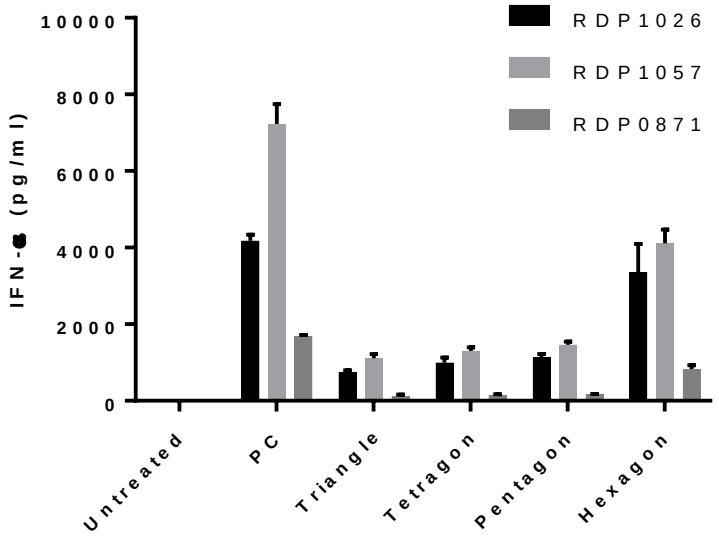
Hong E., et al., NanoLetters, 2018

This study was done in collaboration with Dr. Kirill Afonin, UNCC

Case Study 6: DNA/RNA Nanoparticles (Continued)

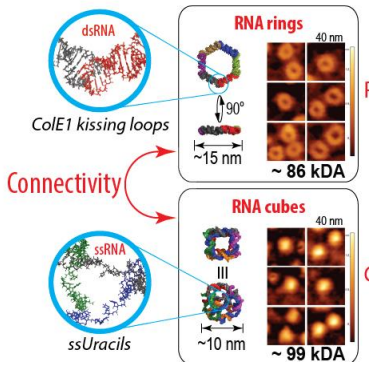
Size

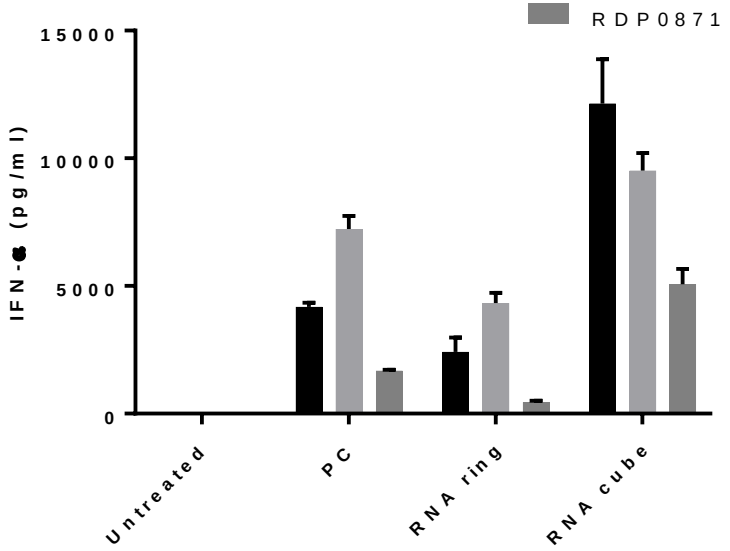




Larger particles are more potent their smaller counterparts

Connectivity

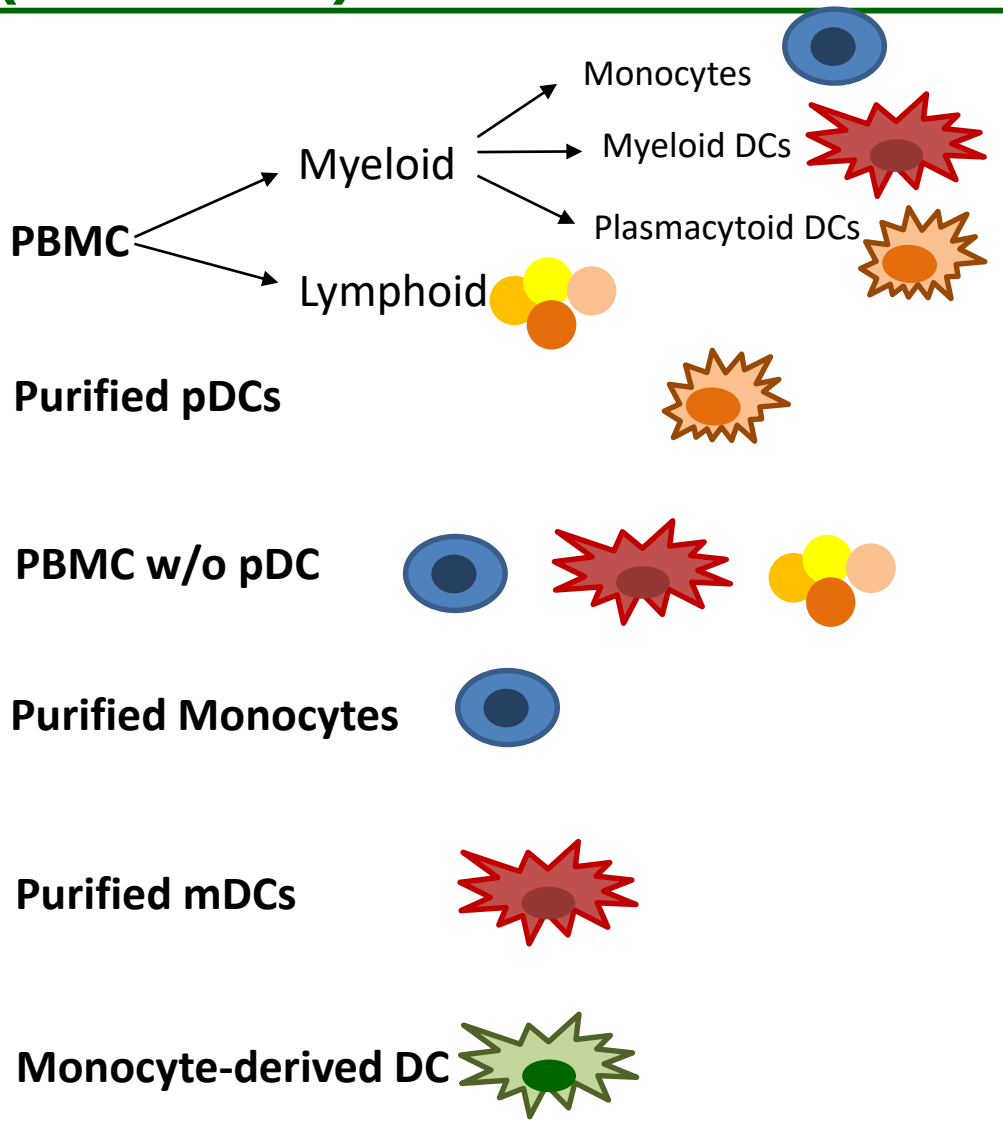




Connectivity contributes (more studies are in progress)

Hong E., et al., NanoLetters, 2018

Case Study 6: DNA/RNA Nanoparticles (Continued)

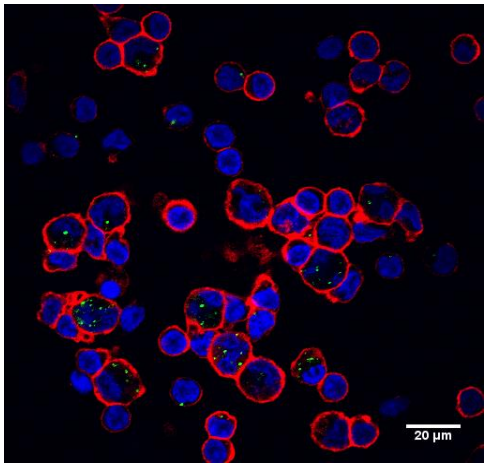


Plasmacytoid DCs are the primary cell type responsible for interferon induction by DNA/RNA nanoparticles

Hong E., et al., NanoLetters, 2018

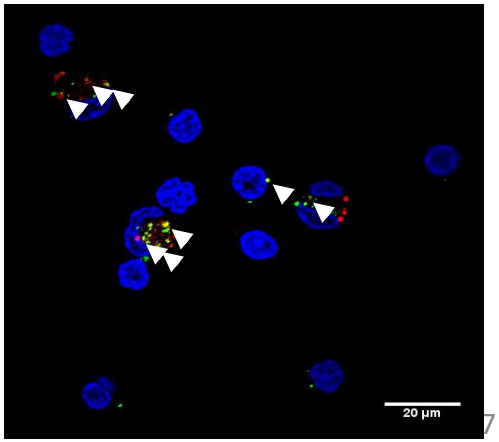
Case Study 6: DNA/RNA Nanoparticles (Continued)

Internalization

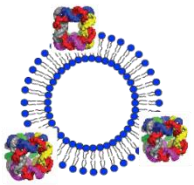


Red: Cell membrane (wheat germ agglutinin AF594)
Green: Nanoparticles (RNA cube, AF488)
Blue: Nucleus (DAPI)

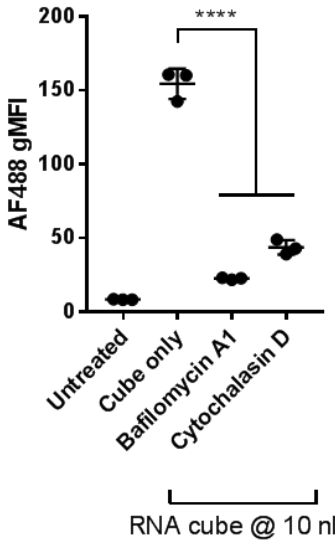
Co-localization with endolysosomal pathway

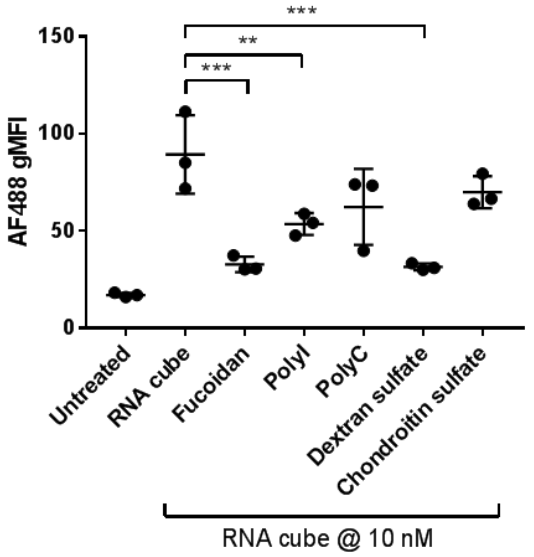


Red: Endolysosomes (Lyso-ID Red)
Green: Nanoparticles (RNA cube, AF488)
Blue: Nucleus (DAPI)

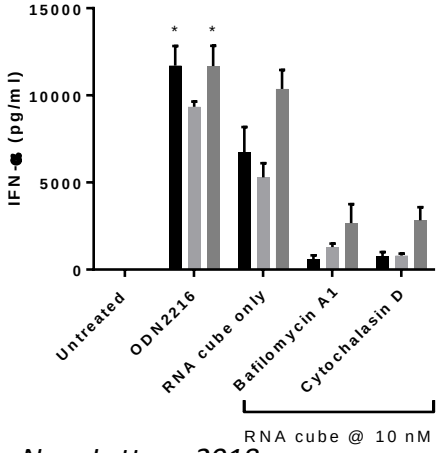


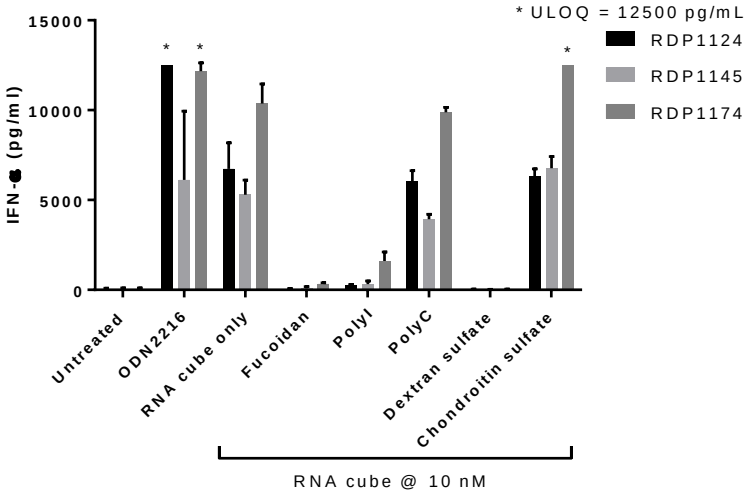
Inhibition of particles uptake by SR-mediated endocytosis….





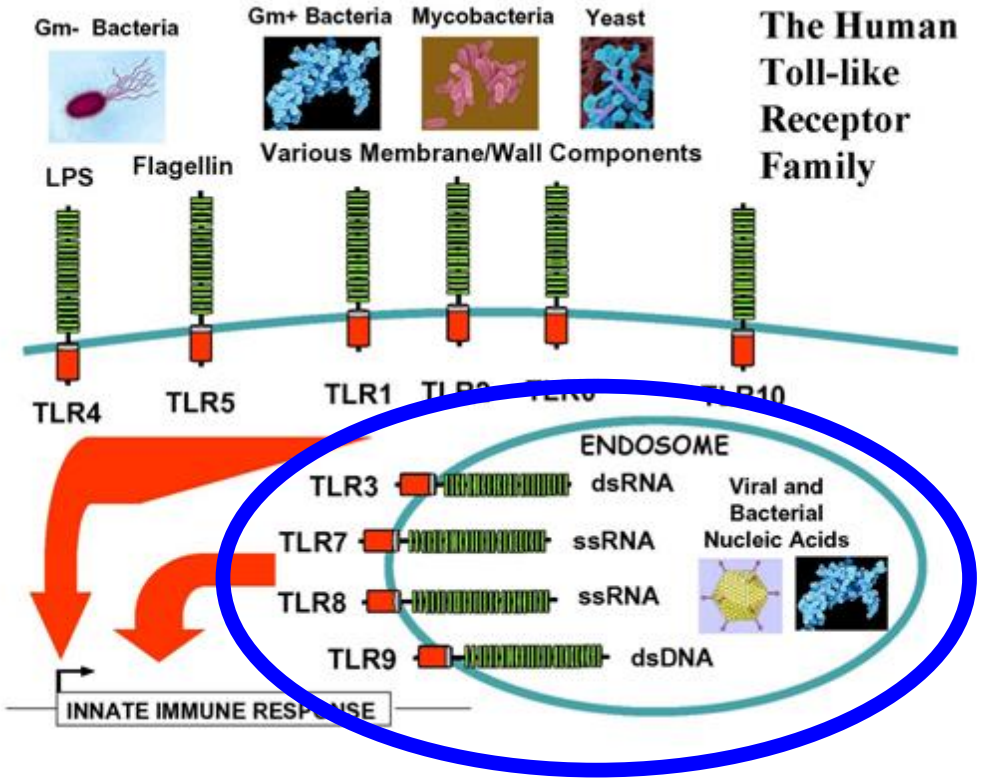
…. correlates with inhibition of IFN production



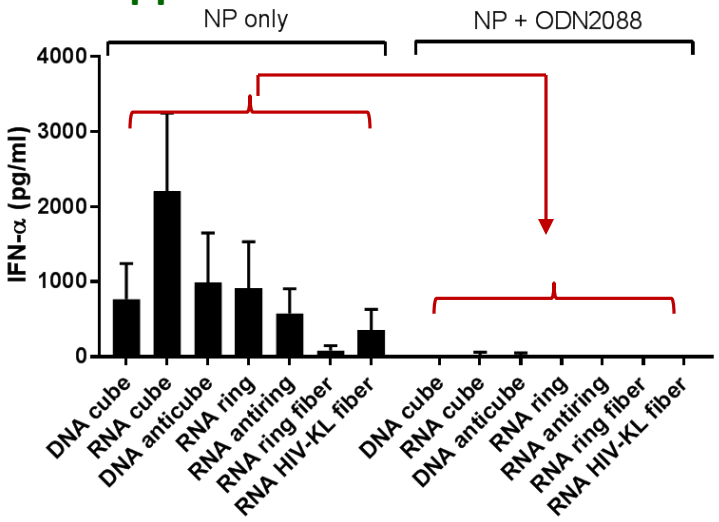


Hong E., et al., NanoLetters, 2018

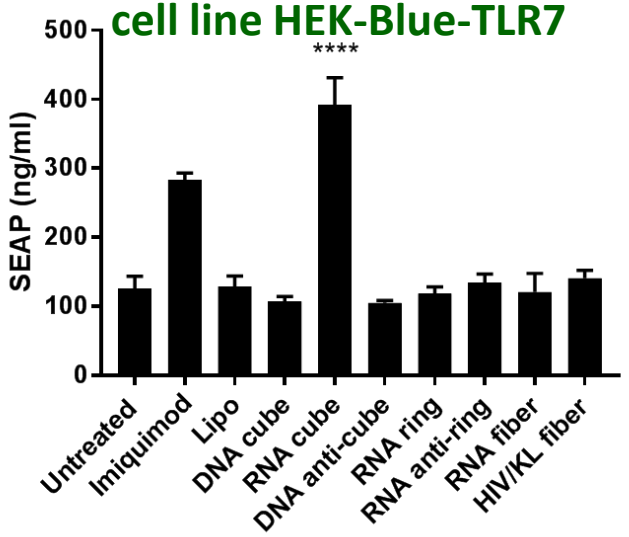
Case Study 6: DNA/RNA Nanoparticles (Continued)



Pan endosomal TLR inhibitor suppresses IFN induction



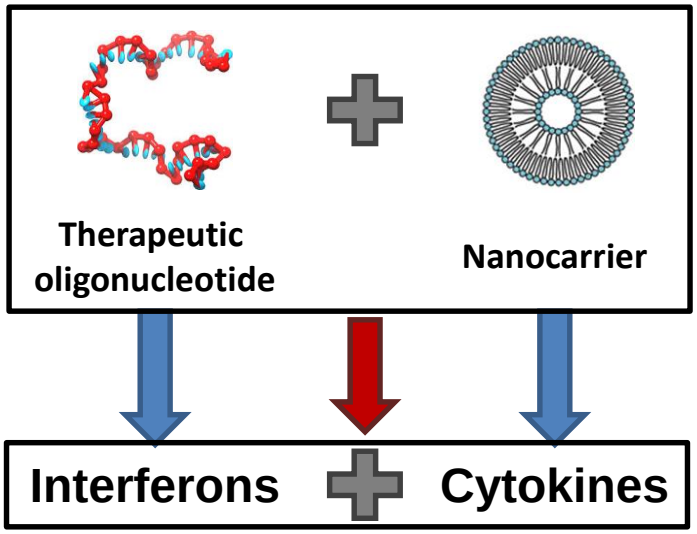
RNA cube activates TLR7 in reporter cell line HEK-Blue-TLR7

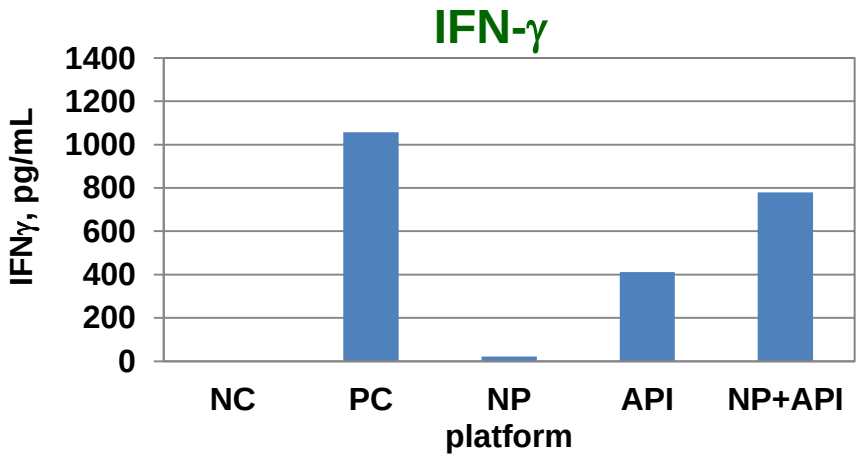


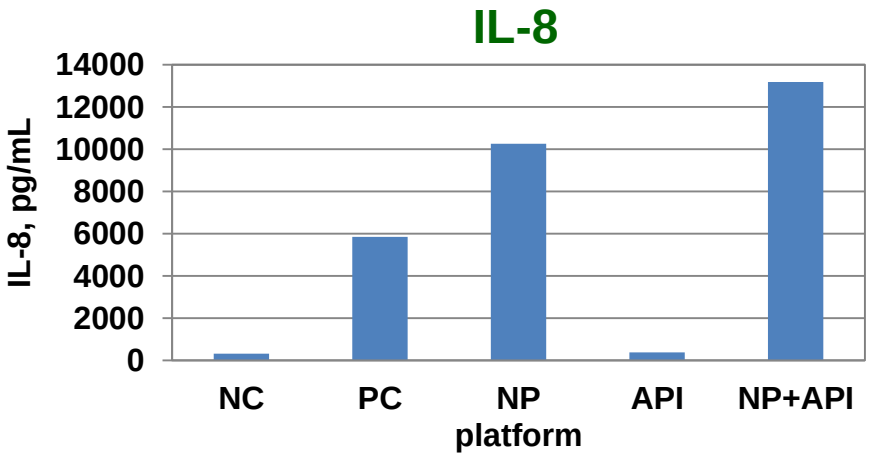
- Intracellular TLRs (3,7,8 and 9) are involved in recognition of DNA/RNA nanoparticles
- TLR7 appears as sensor for RNA cubes

Hong E., et al., NanoLetters, 2018

Case Study 7:
Platform Contribution to API Immunotoxicity

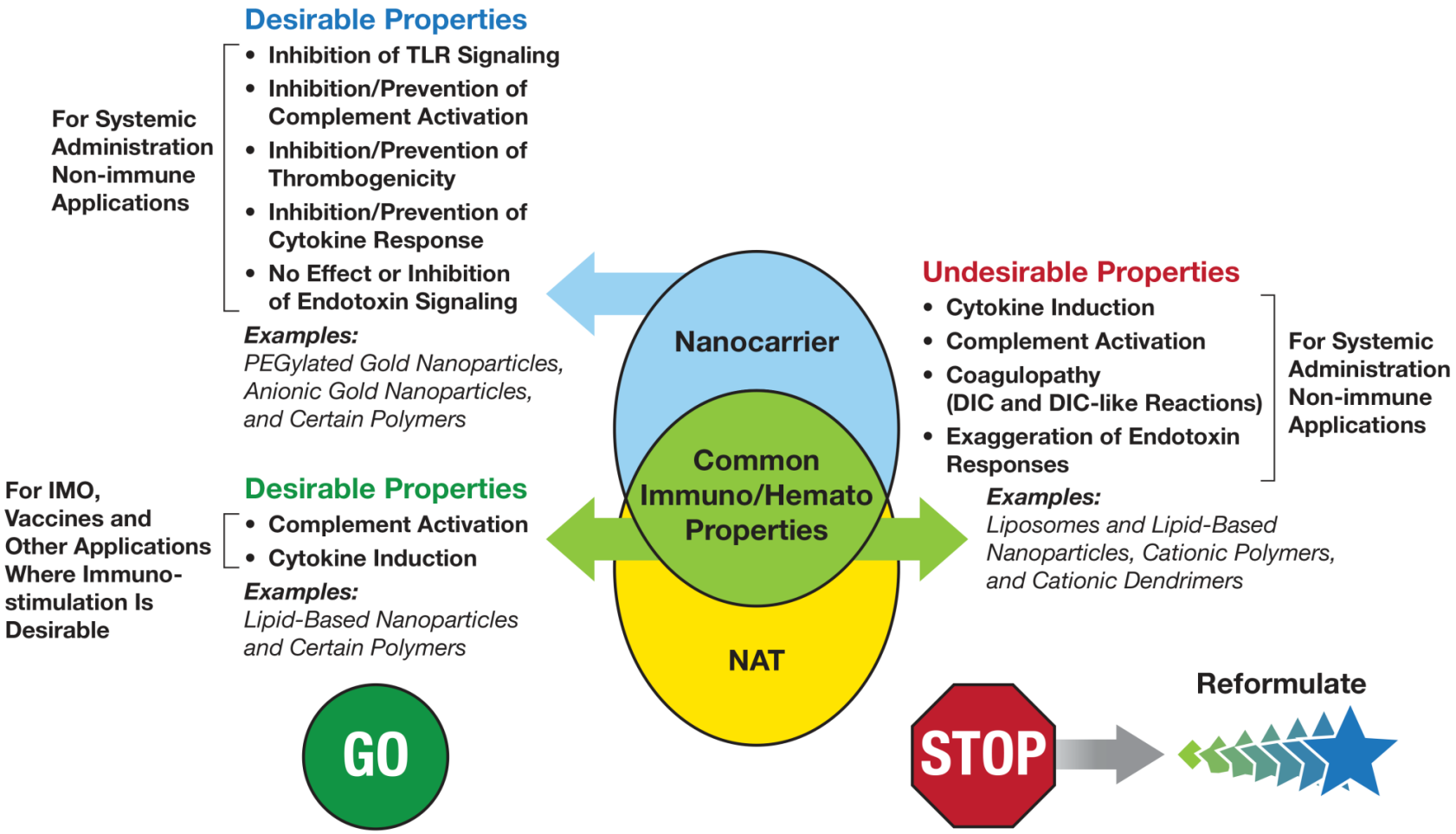






Some nanocarriers contribute to immunostimulation profile of nucleic acid API.

Conclusion



- **Immunotoxicity of both nucleic acid API and nanocarrier should be considered**
- **Use immunologically reactive carrier when immunomodulation is wanted**
- **Avoid such platforms when immunoreactivity is undesirable**

Dobrovolskaia MA& McNeil SE, EODD, 2015.

Take Home Message

- **Immunotoxicity can be GOOD or BAD**
- **Depends on whether it is desirable (intended) or undesirable (unintended)**

- Nanoparticles can be engineered to improve desirable properties or to reduce undesirable ones

- Understanding SAR and mechanisms of toxicity can inform creation of safe and efficient complex drug systems

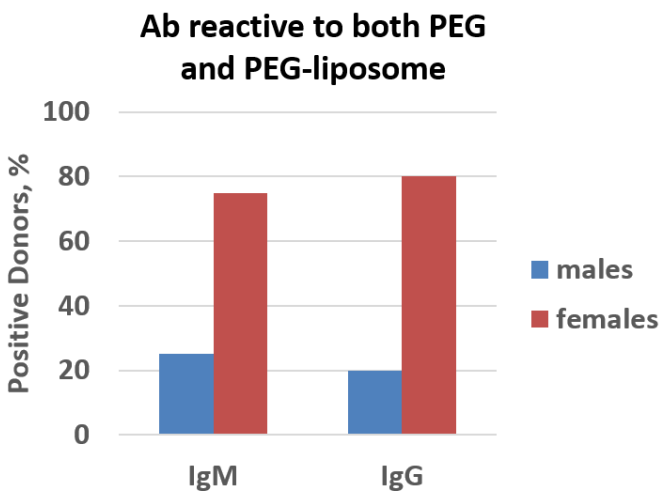
Gaps

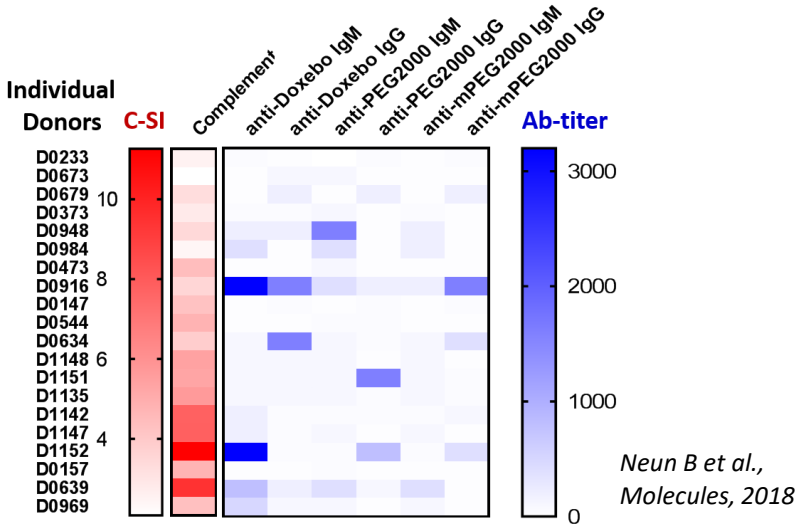
- **This section will discuss a case study with emphasis on the assay details**

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 incompletely understood

"a high level of pre-existing anti-PEG antibodies was a major, but not the sole, factor necessary for triggering first-exposure allergic reaction to pegnivacogin, a PEGylated RNA aptamer" Ganson et al., J ALLERGY CLIN IMMUNOL MAY 2016



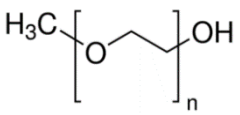


Neun B et al., Molecules, 2018

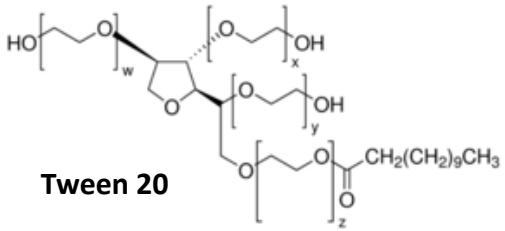
High (> 800) titer PEG-reactive antibodies are detected in both healthy males and females, but are more prevalent in females

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

Assay Note: Tween-20 commonly used in ELISA can't be used for the assay detecting anti-PEG Ab because it interferes with accurate Ab detection

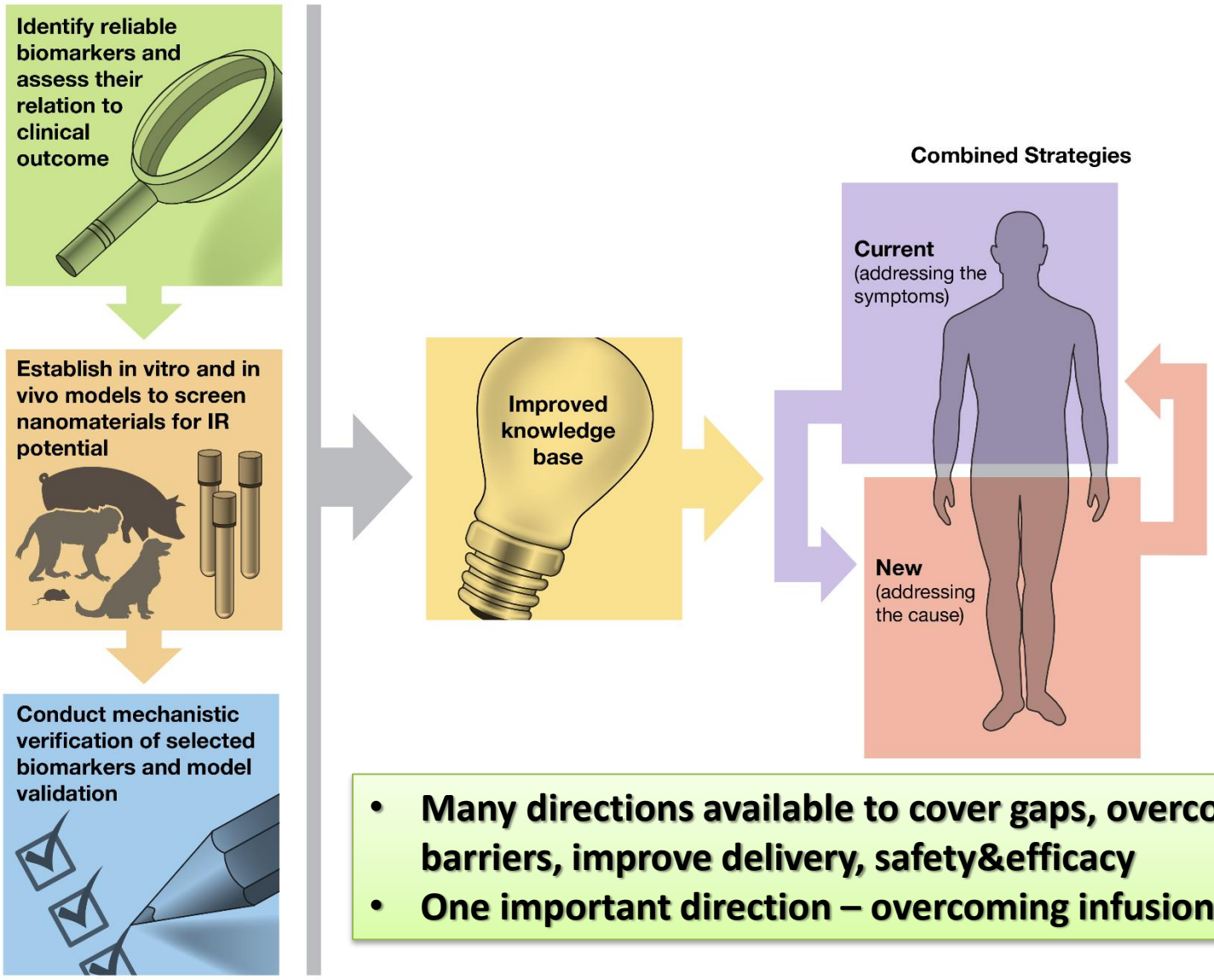


mPEG



Tween 20

Future Directions



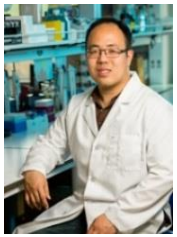
- **Many directions available to cover gaps, overcome biological barriers, improve delivery, safety&efficacy**
- **One important direction – overcoming infusion reactions**

Szebeni J., et al., Nature Nanotechnology, 2018

Acknowledgements

Funded by NCI Contracts N01-CO-12400 and HHSN261200800001E

NCL Immunology Team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Janos Szebeni

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