Nanotechnology for cancer therapy: benefits, concerns and effects on the immune system

Marina A. Dobrovolskaia
Senior Principal Scientist, Immunology Section Head
Nanotechnology Characterization Lab (NCL)

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marina@mail.nih.gov
Presentation outline

- Nanotechnology Definitions
- Nanoparticles in Daily Life
- Nanoparticles in Medical Applications
- Nanoparticles for Cancer Diagnosis and Therapy
  - Benefits of nanotechnology
  - Toxicity concerns
- Nanomaterials and the Immune System
What is Nano?

Nanotechnology:
“Research and technology development at the atomic, molecular or macromolecular scale leading to the controlled creation and use of structures, devices and systems with a length scale of approximately 1 – 100 nanometers (nm).” (Source: National Nanotech Initiative)

“Whether a material or end product is engineered to exhibit properties or phenomena, including physical or chemical properties or biological effects, that are attributable to its dimension(s), even if these dimensions fall outside the nanoscale range, up to one micrometer (1,000 nm)” (US FDA)
Evolving landscapes

Evolving Landscape of Nanotechnology Products

Global Nanotechnology Market (2015)

- Environmenta: 26%
- Electronics: 47%
- Consumer: 27%

CAGR rates (2016-2021)

- Biomedical: 26%
- Consumer: 36%
- Electronics: 38%

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.

Liposomes, Nanocrystals and Emulsions dominate current nanomedicine landscape.

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

Examples of Clinical Grade NanoProducts
Cancer Nanotechnology

- Improve solubility; act as a carrier for hydrophobic drugs.
- Multifunctional capability
- Tumor targeting (reduced toxicity)
- Robotic tasks such as sensing, computation, and actuation; triggered responses.


Benefits

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
Immunotherapy Toxicity

Benefits: Immunotherapy

A

Athymic Nude

<table>
<thead>
<tr>
<th>Days after Cell Implantation</th>
<th>Tumor Volume (mm$^3$)</th>
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<tbody>
<tr>
<td>5</td>
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B

Balb/C

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Doxil Synergizes with Cancer Immunotherapies to Enhance Antitumor Responses in Syngeneic Mouse Models

Doxil improves efficacy of cancer immunotherapeutics in CT26 mouse model of colorectal cancer

The Immunotherapy Opdivo & Abraxane for Recurrent HER2-Negative Metastatic Breast Cancer

A Phase 1, Open-Label, Multicenter, Safety Study of Nivolumab (BMS-936558) in Combination With Nab-Paclitaxel Plus or Minus Gemcitabine in Pancreatic Cancer, Nab-Paclitaxel / Carboplatin in Stage IIIB/IV Non-Small Cell Lung Cancer or Nab-Paclitaxel in Recurrent Metastatic Breast Cancer (NCT02309177)

Abraxane is investigated in combination with a-PD-1 in clinical trials for metastatic breast cancer
Vaccines

Benefits: Vaccines

- Activates the Type I Interferon (IFN) pathway
- Recruits monocytes to injection site
- The cancer antigen is picked up by dendritic cells (DCs)
- Activated DCs produce key chemokines & cytokines
- DCs and Versamune® transport antigen to draining lymph nodes
- Matures and activates DCs
- Stimulates Type I IFN genes e.g., IFNa1, IFNb1
- Chemokine induced recruitment of T cells into lymph nodes
- Enhanced presentation of the antigen to CD8+ killer T-cells
- Proven activation and proliferation of potent multi-cytokine inducing/polyfunctional CD4 & CD8 T-cells
- Enhanced CD8+ killer T-cell migration to tumor sites

Versamune®: (R-enantiomer of a positively charged lipid)

- Killer-T cells enter tumor microenvironment.
- Decreased Treg and MDSC populations & activity
- Significantly increased ratio of Killer-T cells to Tregs induces effective killing of the tumor cells leading to tumor regression

- Nanoparticles (lipoplexes, polyplexes, liposomes) were shown to improve vaccine efficacy
  - One example of such platforms is shown on this slide
Toxicity

Concerns: Toxicity

- Both nanocarrier and API can be toxic
- API toxicity can “relocate” depending on the particle biodistribution
Immune system

Nanoparticles and the immune system

- Plasma Proteins
  - Biodistribution and MPS uptake
- Effects on erythrocytes
- Blood coagulation system
  - Platelets
  - Leukocytes
  - Endothelial cells
- Allergy
  - Complement activation
  - DTH
- Cytokines
- Immunogenicity
Bidirectional communication

Bidirectional Communication between Nanoparticles and Proteins

Opsonization and Uptake by MPS
Influence on Particle Distribution
Change in Physicochemical Characteristics
Dissolution
Interference with Targeting

Nanoparticle → Proteins

Interaction with Coagulation Factors
Activation of Complement
Formation of Amyloid Structures
Activity Gain or Loss
Changes to Protein Stability
Exposure to New Epitopes

Effect on Protein Conformation

Binding of proteins to nanoparticle surface result in changes in particle properties. Properties and function of some proteins may also change after binding to the nanoparticle.
Particle size

**Particle size influences protein binding**

Figure 2. Size of proteins in the corona compared to nanoparticles of varying diameter. Nanoparticles are represented in blue and the diameter is given by the number under each particle in nm. Serum albumin is shown in red and scaled relative to the nanoparticles. High-density lipoprotein is represented by orange spheres at a size of 12.5 nm diameter.

Cedervall Tet al, 2016. Handbook of Immunological properties of engineered nanomaterials
Protein binding

Protein binding affects particle size

BEFORE

30 nm colloidal gold nanoparticle

AFTER

30 nm colloidal gold nanoparticle

“Protein Corona”

DLS

Size Distribution by Volume

33 nm

DLS

Size Distribution by Volume

76 nm

Incubation with human plasma increases hydrodynamic size of nanoparticles

Biodistribution

Protein Binding and biodistribution

- Particles which bind proteins are eliminated by MPS
- Particle surface protection (e.g. with PEG) reduces protein binding and MPS
- Good correlation between in vitro and in vivo
MPS uptake

- Two theories about nanoparticle distribution to the MPS
  - Capture – uptake by phagocytic cells in the tissue
  - Hijacking – uptake by circulating phagocytic cells which then take the particle to tissue

Hemolysis

Main symptoms of Acute hemolytic reaction

- Systemic
  - Chills
  - Fever
- Vascular
  - Hypotension
  - Uncontrollable bleeding
- Transfused vein
  - Heat sensation
- Lumbar region
  - Pain
- Heart
  - Increased heart rate
- Chest
  - Constricting pain
- Urinary
  - Hemoglobinuria
  - Hyperbilirubinemia

Role of Nanoparticle Surface Charge

- Anionic
- Neutral
- Cationic

Role of Nanoparticle Size
Hemolysis

- Cationic dendrimers are more hemolytic than their anionic and neutral counterparts of the same size.
- Larger dendrimers are more hemolytic than smaller.

NC = negative control; PC = positive control; EDA = ethylenediamine; PAMAM = poly(amideamine)

Effects of Size

Effects of Surface Charge

EDA core
PAMAM
Dendrimers
G3-G6

Terminal groups

Succinamic Acid

Amine

Ethanolamine

% Hemolysis

% Hemolysis

NCL data
Coagulation system

Nanoparticles can be engineered to avoid or specifically interact with coagulation system.
Undesirable effects on coagulation

- Coagulation factors:
  - Contact activation
  - Binding and depletion of coagulation factors

- Endothelial cells:
  - Cytotoxicity
  - Induction of inflammation and oxidative stress

- Platelets:
  - Direct activation
  - Influence on agonist induced activation
    - Exaggeration
    - Inhibition

Ilinskaya A & Dobrovolskaia MA. Handbook of Immunological properties of Engineered Nanomaterials (2016), Vol 2
Zeta potential

Platelets: role of zeta potential

Zeta Potential is important
Less surface amines = less platelet aggregation
Platelets

Platelets: effect of composition

Triazine dendrimers are less potent in inducing platelet aggregation than their PAMAM counterparts.

Triazine dendrimers were kindly provided by Dr. Eric Simanek, Texas Christian University.
## Allergenicity

**Table 2. Main characteristics of the different types of hypersensitivity reactions.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Type I (anaphylactic or immediate hypersensitivity)</th>
<th>Type II (cytotoxic hypersensitivity)</th>
<th>Type III (immune complex mediated hypersensitivity)</th>
<th>Type IV (delayed hypersensitivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main mediators</td>
<td>IgE, mast cells</td>
<td>Complement</td>
<td>IgG, IgM, complement</td>
<td>T helper cells and macrophages</td>
</tr>
<tr>
<td>Antigen</td>
<td>Exogenous</td>
<td>Exogenous</td>
<td>Soluble antigens</td>
<td>Bacteria, tissues</td>
</tr>
<tr>
<td>Time</td>
<td>15–30 min</td>
<td>15–30 min</td>
<td>3–8 h</td>
<td>48–72 h</td>
</tr>
<tr>
<td>Skin reaction</td>
<td>Skin prick positive wheal and flare</td>
<td>Skin prick-negative</td>
<td>Intradermal injection (swelling and redness)</td>
<td>Mantoux-positive (erythema and induration)</td>
</tr>
<tr>
<td>Clinical manifestations</td>
<td>Allergic asthma, hay fever, anaphylactic shock</td>
<td>Urticaria, angioedema</td>
<td>Serum sickness, fever, glomerulitis, vasculitis</td>
<td>Tuberculin test, poison ivy, contact dermatitis, maculopapular rashes, granuloma</td>
</tr>
</tbody>
</table>


- Nanoparticles can be engineered to inhibit allergy (tolerogenic and drug-carrying nanoparticles)
- Some nanoparticle can exaggerate allergy to traditional allergens
- Pseudoallergy is the most common and best studied reaction to nanomaterials
- Rare example of cell-mediated allergy to dendrimers
Allergenicity: CARPA to PEG-Liposomes

Complement activation is dose limiting toxicity of PEGylated liposomes

Allergenicity

Allergenicity: DTH to dendrimers

A case of toxic epidermal necrolysis-like dermatitis evolving from contact dermatitis of the hands associated with exposure to dendrimers

Contact Dermatitis 2008: 59: 122–123
Department of Dermatology, Course of Integrated Medicine, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

- Only one case of necrotizing dermatitis (type IV reaction) in response to dendrimers is reported in the literature: fever, chills, exudative erythema and fused bullae (Nikolsky’s reaction)
- The mechanism is unknown
Cytokine storm

Cytokine storm: Lessons from biotechnology products:

Preclinical studies in NHP and rodents did not reveal cytokine storm

Phase I clinical trial: 6 of 6 volunteers experienced cytokine storm which lead to multiple organ failure

In vitro experiments using human PBMC showed high TNF levels in response to TGN1412

TGN1412 = CD28 Super-MAB
Understanding carrier properties

**Cationic Liposomes**

<table>
<thead>
<tr>
<th></th>
<th>IFN-γ</th>
<th>IL-1α</th>
<th>IL-1β</th>
<th>IL-6</th>
<th>IL-8</th>
<th>IL-10</th>
<th>MCP-1</th>
<th>MIP-1α</th>
<th>MIP-1β</th>
<th>RANTES</th>
<th>TNF-α</th>
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<tr>
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**Detected cytokines**

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<tr>
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<th>TNF-α</th>
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<th>MIP-1α</th>
<th>MIP-1β</th>
<th>RANTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group:</td>
<td>cytokines</td>
<td>chemokines</td>
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**Detected danger signals**

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<tr>
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<th>MMP-1</th>
<th>MMP-7</th>
<th>MMP-9</th>
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<tbody>
<tr>
<td>Group:</td>
<td>metalloproteinases</td>
<td></td>
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</table>

- Cationic liposomes induce wide range of pro-inflammatory responses
- 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)
IL-8 induction

Mechanism of IL-8 induction: Oxidative stress

- Induction of IL-8 by liposomes follows induction of oxidative stress and can be prevented by antioxidant N-acetyl cysteine
IL-1 Immunogenicity

**Mechanism of IL-1 induction:**
Proton sponge effect

Cationic particles induce IL-1β through activation of NLRP3 inflammasome triggered by a proton-sponge mechanism.
API properties

Understanding API properties

TNA-based APIs are known to induce IFN response

Presence of 5'-triphosphate

Material

RNA > DNA

Chemical Modifications
Immunogenicity

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

Accidental Nanoparticles ≠ Nanomedicines

ENM approved for clinical use which resulted in antigenic response
None

ENM carrying ThPr and resulting anti-ENP response
None

ENM carrying ThPr and resulting in anti-ThPr response
None

Accidental particles contributing to antigenicity of ThPr
Glass fibers
Cellulose fibers
Tungsten
Silicon Oil
Rubber
Stainless steel
Fluoropolymers

ANTM = engineered nanomaterials; ThPr = therapeutic protein; SWCNT = single wall carbon nanotubes; PAMAM = polyamidoamine; TNF = tumor necrosis factor

* - antibodies were generated ONLY after conjugation to protein carrier and injection in the presence of strong adjuvants

Anti-PEG antibody

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

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.
Future directions

- Identify reliable biomarkers and assess their relation to clinical outcome
- Establish in vitro and in vivo models to screen nanomaterials for IR potential
- Conduct mechanistic verification of selected biomarkers and model validation

**Combined Strategies**
- Current (addressing the symptoms)
- New (addressing the cause)

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

*Szaboni J., et al., Nature Nanotechnology, 2018*
Take home message

- Nanotechnology can benefit cancer therapy by improving formulation of traditional drugs (SM, biotechnology products and immunotherapeutics)
- Nanoparticles physicochemical properties determine particle toxicity
- Nanoparticles can be engineered to either specifically interact with or avoid the immune system
- Nanoparticle interaction with the components of the immune system can be desirable or undesirable
- Desirable interactions can benefit therapies of many disorders including cancer
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Gloryvee Rivera, B.S.
Wendi Custer, B.A.
Kelly Benauer

Contact Info about this presentation:
Marina Dobrovolskaia
(301) 228-4935
marina@mail.nih.gov
http://ncl.cancer.gov

Collaborators
Jan Simak, Ph.D.
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Kirill Afonin, Ph.D.
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