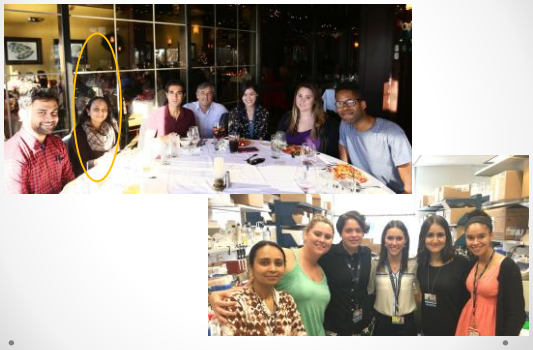
Systemic exposure to nanoparticles effects anti-cancer immune stimulation for metastatic breast cancer: A study in mouse models

Robert Ivkov, Ph.D.



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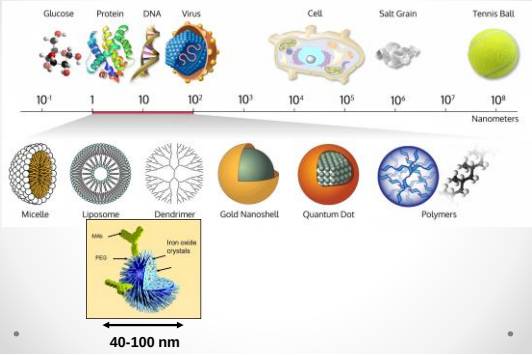
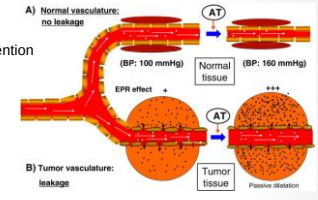
- Robert Ivkov, Ph.D.

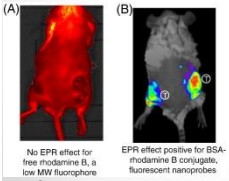
Nanoparticles are colloids that can resemble biological agents



Nanometer-scale anti-cancer agents can exploit aberrant properties of cancer tumors

Enhanced Permeability and Retention (EPR)



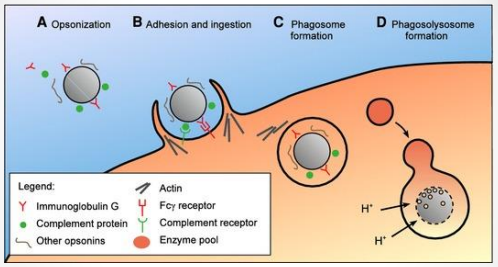


No EPR effect for free rhodamine B, a low MW fluorophore

EPR effect positive for BSA-rhodamine B conjugate, fluorescent nanoprobes

Maeda, et al. Adv. Drug Del. Rev. 2013

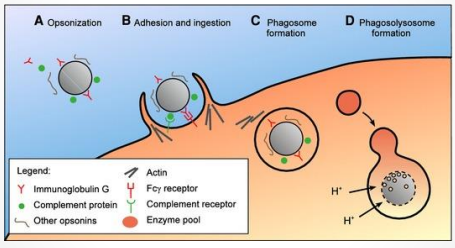
Innate and adaptive immune systems intercept nano-scale invaders



Nanoparticle blood circulation kinetics (PK) is decided by interactions with blood proteins, with rapid clearance reducing access to tumor extravascular spaces.

The 'Stealth' nanoparticle paradigm

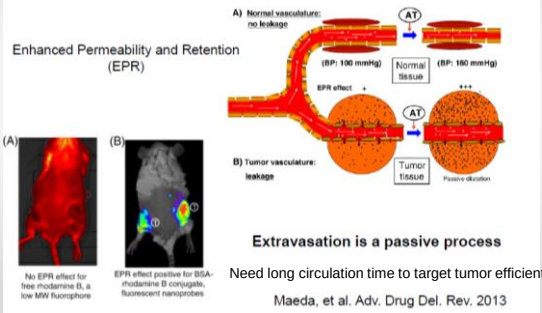
Immune cells survey blood and tissues to internalize or destroy opsonized materials



Current cancer drug paradigm motivates development of stealth nanoparticles that evade detection by the immune system

By relying on a generalized biologic concept, attention is focused on materials engineering

'Stealthy' particles exploit aberrant vascular properties of tumors



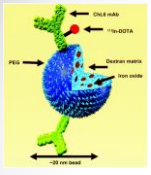
Need long circulation time to target tumor efficiently

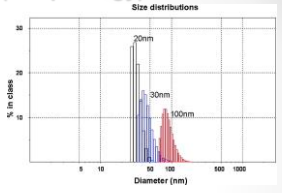
Maeda, et al. Adv. Drug Del. Rev. 2013

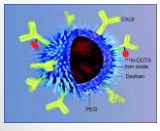
Targeting nanoparticles to tumors?

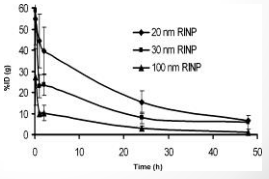
- Without adequate targeting – nanoparticles are unlikely to be beneficial and toxicity profile may dominate.
- Mode of 'targeting' is determined by indication
  - Local primary tumor or palliation – percutaneous or local intravascular
  - Metastatic disease (direct) – systemic treatment, e.g. intravenous
  - Metastatic disease (combination indirect) – local/systemic nanoparticle treatment combined with other local/systemic therapies.

Effect of size (morphology) on PK



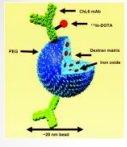


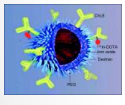


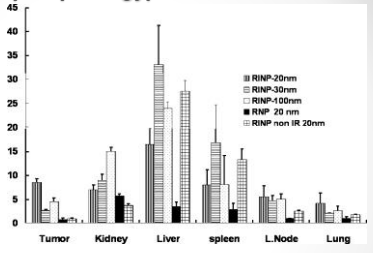


Natarajan, et al. *Bioconjugate Chem.* 2008, vol 19, pp 1211-1218

Effect of size (morphology) on biodistribution





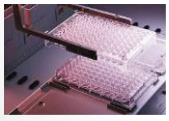


Pharmacokinetic biodistribution of the RINPs (20, 30, and 100 nm size), negative controls of RNP (20 nm) NP without targeting molecules, and RINP (20 nm) non-immunoreactive were measured as %ID/g in various organs after 48 h injection. The difference between targeted (RINP) and nontargeted particles (RNP) and non-immunoreactive RINP (5) should be noted. The mean ± SD (error bars) were calculated based on three animals (n = 3) in each study group. IR = immunoreactive.

Natarajan, et al. *Bioconjugate Chem.* 2008, vol 19, pp 1211-1218

Nanoparticles interact with the tumor immune microenvironment

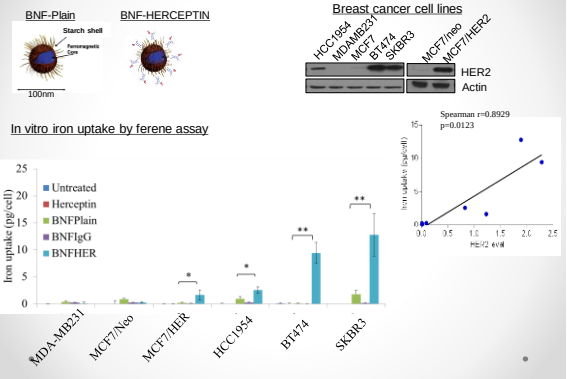
- Is there a fundamental flaw in our approach to develop nanoparticle therapies for cancer?
- Unlike small molecules, nanoparticles are engineered foreign particles that can actively interact with the immune system



In vitro drug screening

Xenografts in immunodeficient model

Antibody-labeled nanoparticles demonstrate activity with a positive correlation between iron and HER2 protein expression



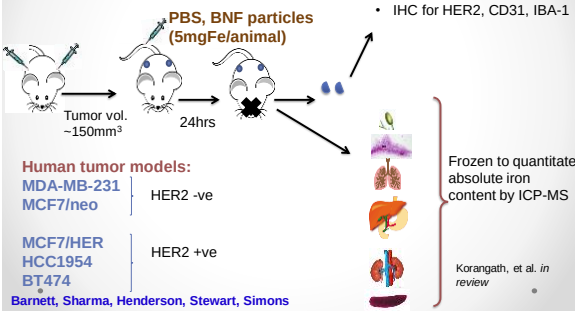
Mouse models chosen to present varied host immune status

Immune system components	Syngeneic FVB/N (Intact immune system)	Athymic nude (Least Immunodeficient)	NOD SCID Gamma (NSG) (Highly Immunodeficient)
Mature B cells	Present	Present	Absent
Mature T cells	Present	Absent	Absent
Dendritic cells	Present	Present	Defective
Macrophages	Present	Present	Defective
Natural killer cells	Present	Present	Absent
Complement	Present	Present	Absent

Overall study design (xenografts)

Mouse strains:
NSG (Highly immunodeficient)
NUDE (Least immunodeficient)

End points:
- Prussian blue (iron distribution)
- ICP-MS
- Quantitative MRI
- IHC for HER2, CD31, IBA-1



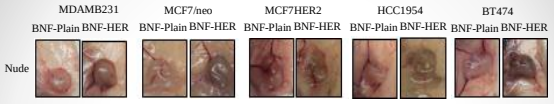
Human tumor models:
MDA-MB-231
MCF7/neo
HER2 -ve

MCF7/HER
HCC1954
BT474
HER2 +ve

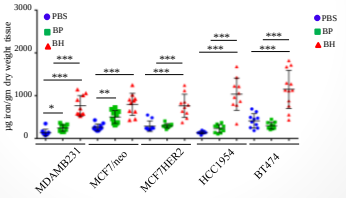
Korangath, et al. *in review*

Barnett, Sharma, Henderson, Stewart, Simons

Herceptin-labeled nanoparticles are retained by tumors irrespective of HER2 status; whereas much less of plain nanoparticles remain in tumors



Tumour ICP-MS from athymic nude mice



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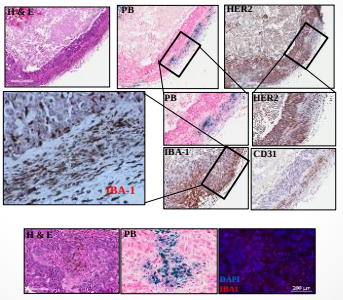
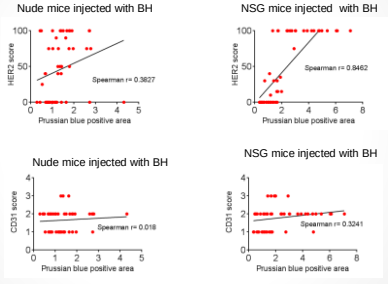
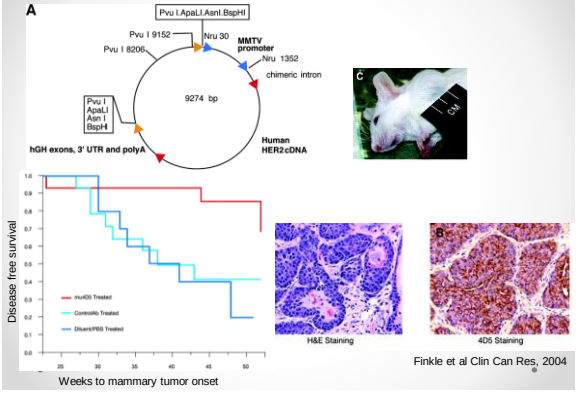
Histopathology analysis of tumors reveals antibody-labeled nanoparticles localize to regions rich with immune cells.



Histopathology analysis of tumors reveals little or no correlation of antibody-labeled nanoparticle localization to HER2 positive or CD31 positive regions.



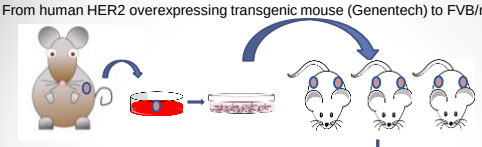
Human HER2 overexpressing transgenic mouse model



Finkle et al Clin Can Res, 2004

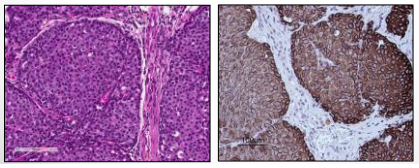
HuHER2 allograft model development

From human HER2 overexpressing transgenic mouse (Genentech) to FVB/n animals



Subsequent transplantation to FVB/N

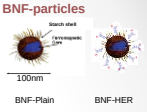
Confirmed tumor architecture by H&E and
HER2 protein by HER2 Immunohistochemistry in allograft tumors



Overall study design

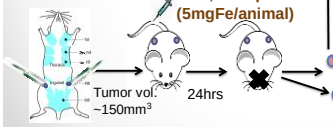
Transgenic allografts grown in mammary fat pad of FVB/N mice (Donor)

BNF-particles



Mouse strains:
NSG (Highly immunodeficient)
NUDE (Least immunodeficient)
FVB/N (Immune competent)

PBS, BNF particles (5mgFe/animal)

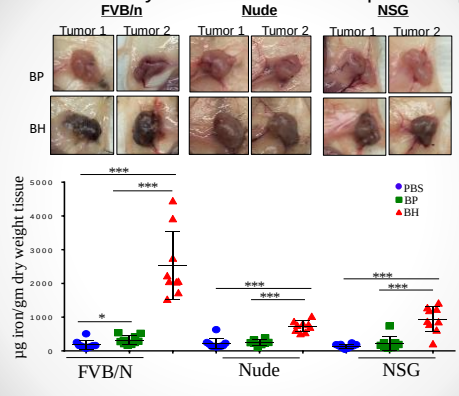


End points:

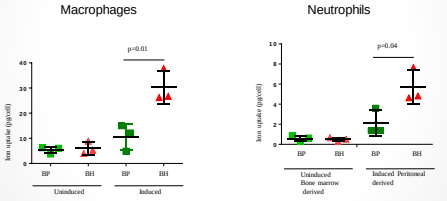
Histology
- Prussian blue
- IHC for Tumor marker (HER2)
  Vasculature (CD31)
  Immune cells
  - o CD45
  - o PAX5 (B cells)
  - o CD3 (T cells)
  - o IBA-1 (Mono/ macrophage)
- ICP-MS for iron content

James Barnett, Anirudh Sharma, Elizabeth Henderson, Jacqueline Stewart, Simons

Host immune system influences nanoparticle uptake



In vitro data demonstrate that innate immune cells displaying 'inflammatory' phenotype (T_H1-type induction) preferentially retained Herceptin (antibody) labeled nanoparticles.



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Korangath, Barnett, Henderson, Yang, Armstrong, Jaffee, Sukumar, Simons, et al.

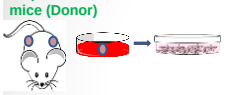
Summary

- The tumor immune microenvironment preferentially retains antibody-labeled nanoparticles over plain nanoparticles.
- EPR paradigm fails to account for observed 'targeting' of antibody-labeled nanoparticles.
- A pro-inflammatory phenotype of phagocytic immune cells in the tumor immune microenvironment preferentially take up antibody-labeled nanoparticles.
- Is there any potential effect on tumor growth upon exposure?

What is the immune effect of systemic exposure to nanoparticles, and how does this affect cancer tumors?

Flow cytometry of cells isolated from treated tumors

Transgenic allografts grown in mammary fat pad of FVB/N mice (Donor)



FVB/N (Immune competent n=8)

PBS, BNF particles (5mgFe/animal)

Tumor vol. ~150mm^3

24hrs

Dissociate in digestion media to get single cells for 1 hr

Put on magnet for 1hr

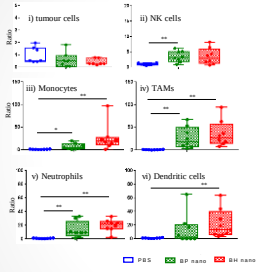
Nanoparticle associated cells

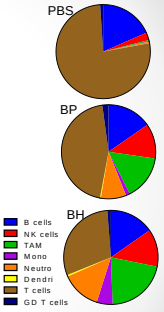
Separated supernatant

Flow cytometry

- T cells
- GD T cells
- B cells
- NK cells
- Macrophages
- Monocytes
- Neutrophils
- Dendritic cells
- Tumor cells

Nanoparticles are associated with Immune cells - not tumor cells





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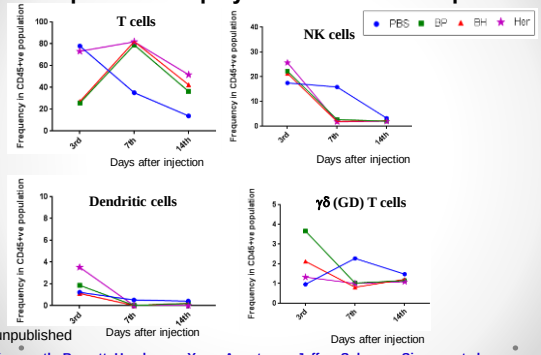
In vivo data demonstrate that immune reaction to nanoparticles displays 'infection-like' response.



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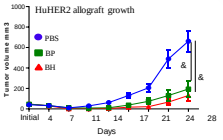
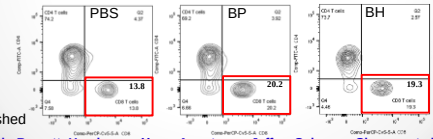
In vivo systemic exposure to NPs inhibits tumor growth and leads to CD8⁺ T cell infiltration



Exposure to plain NPs induces tumor growth suppression similar to Herceptin-labeled NPs



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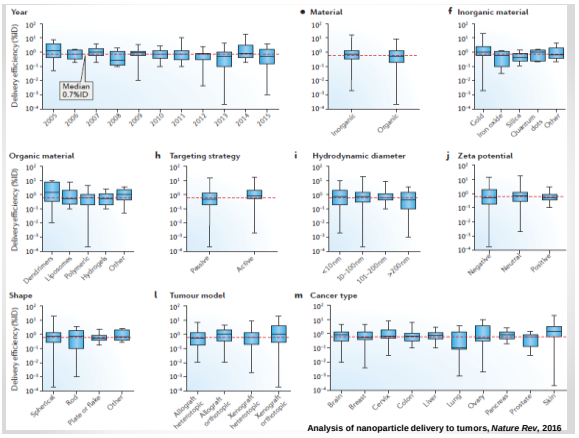
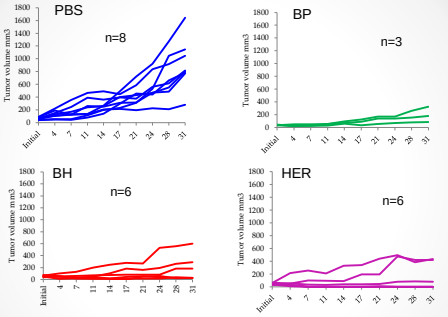
Summary and Conclusion

- Nanoparticles present an interesting and unique class of materials for biomedical research and clinical applications.
- Nanoparticle characterization requires multiple techniques to measure their properties.
- Their size may predispose them to interact with host immune systems, raising the question that small-molecule drug PK/BD models are valid and providing an opportunity to 'reprogram' the immune system to recognize malignant tumors as 'foreign'.
- Systemic exposure to nanoparticles induces a complex systemic host immune responses that can lead to tumor growth suppression.
- Conversely, the host immune system in *tumor microenvironment* influences retention of antibody conjugated nanoparticles, particularly when displaying an inflammatory phenotype - but this retention may not be associated with therapeutic activity.

Thank you for your attention



Autochthonous HER2⁺ tumors respond equally to BP, BH, or free Herceptin



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Korangath, Barnett, Henderson, Yang, Armstrong, Jaffee, Sukumar, Simons, et al.

Herceptin-labeled nanoparticles are retained by tumors irrespective of HER2 status; whereas much less of plain nanoparticles remain in tumors