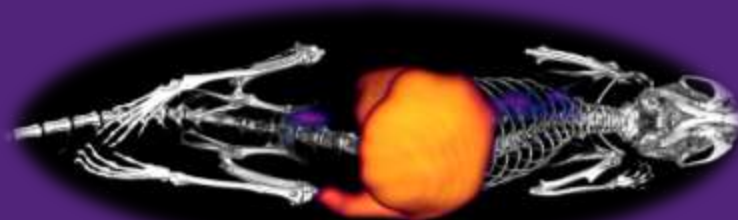


Responsive Polymers and Bioorthogonal Chemistry to Probe Biological Processes

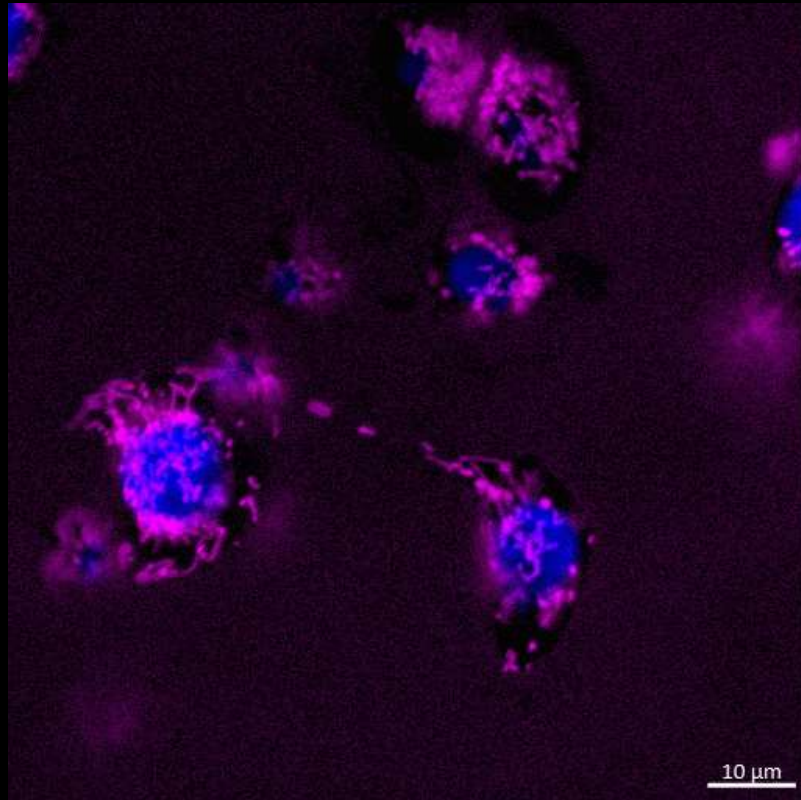
Kristofer J Thurecht

Australian Institute for Bioengineering and Nanotechnology and Centre for Advanced Imaging
The University of Queensland

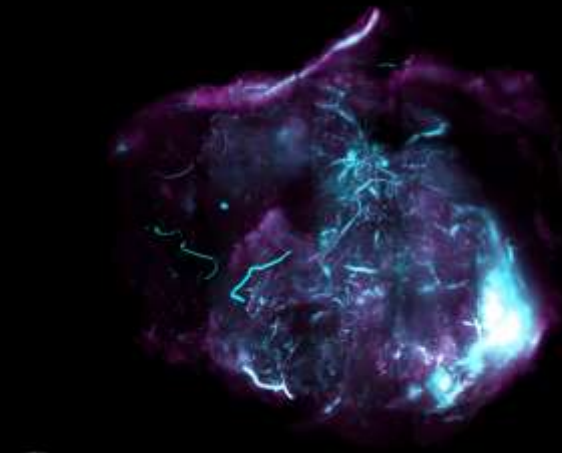
ARC Training Centre for Innovation in Biomedical Imaging Technology
ARC Research Hub for the Advanced Manufacture of Targeted Radiopharmaceuticals



Developing polymeric materials to probe biological systems



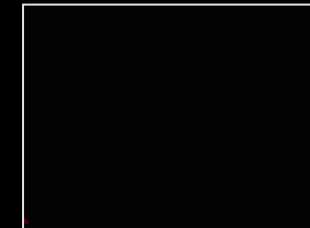
Material-driven cellular trafficking



Material-driven tissue interactions



liver



heart

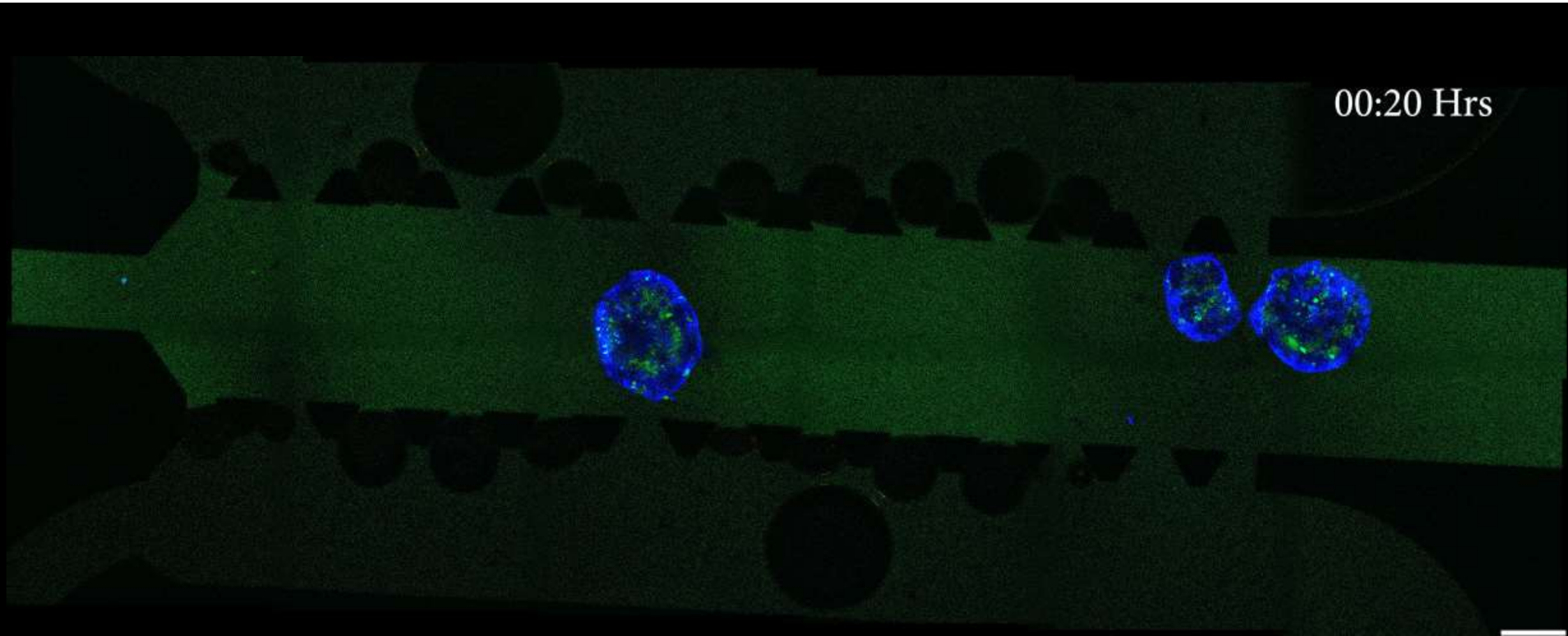


kidney

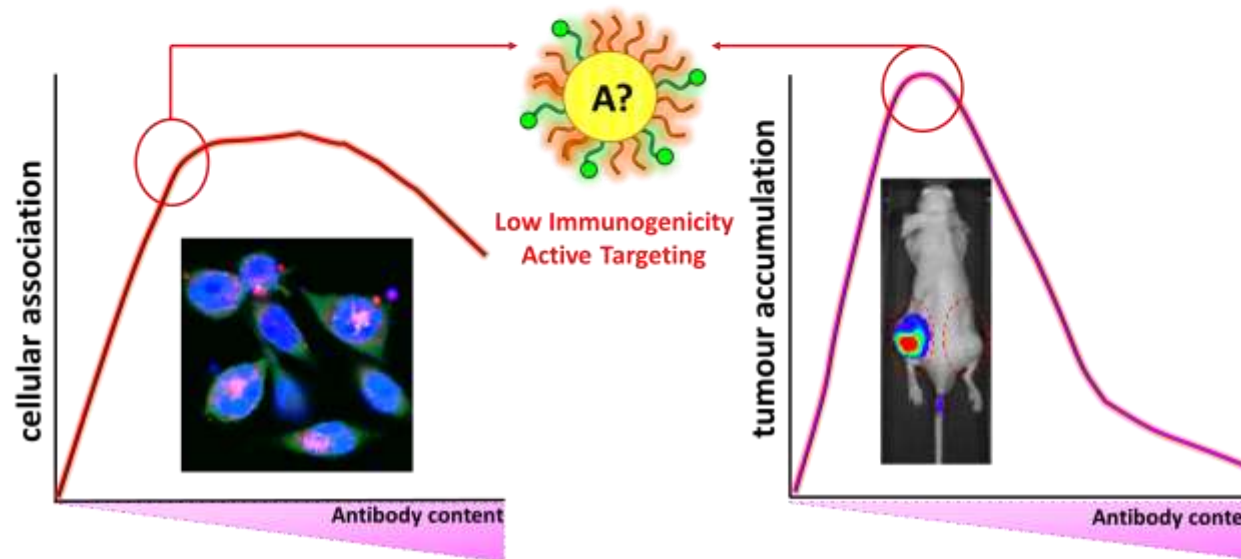
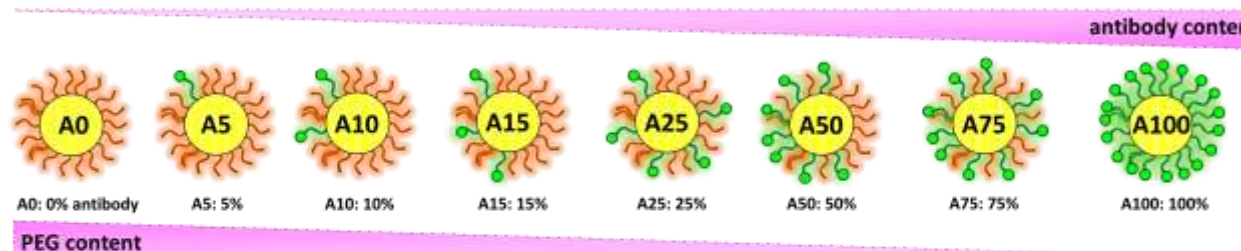
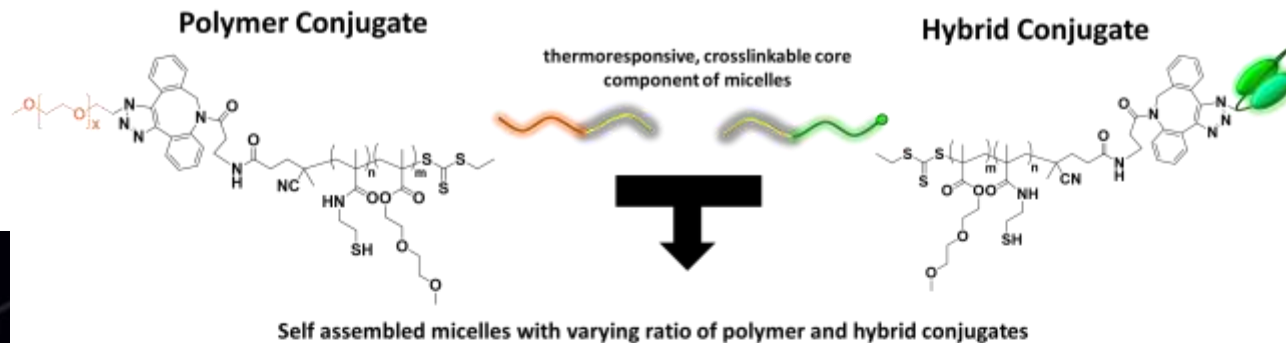
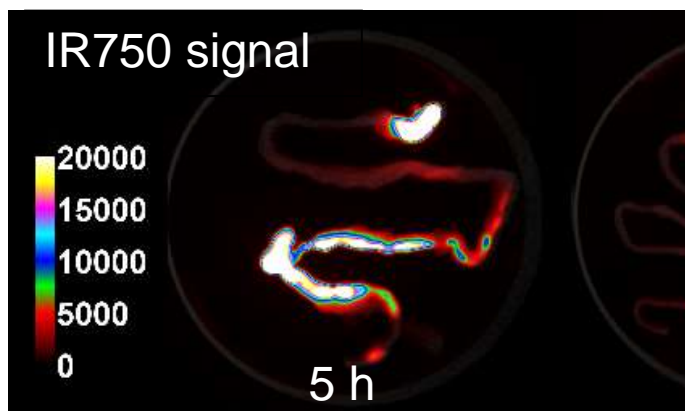
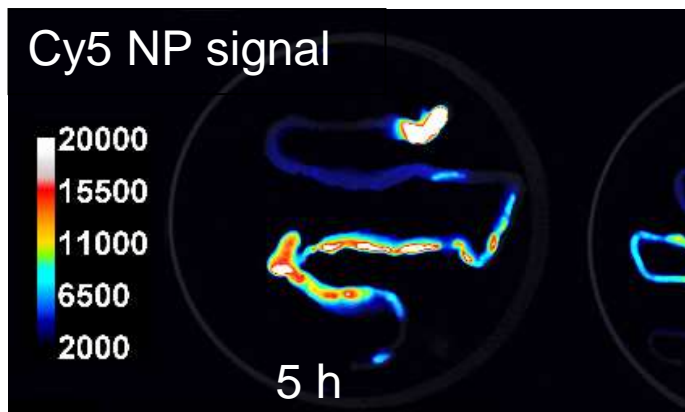


Material driven whole animal interactions

Developing polymeric biomolecules to probe biological systems



Polymeric nanomedicines to address biological questions



[S]: W 1215 L 798.6
1]: W 1482.4 L 1914.7
Scale: 4.15

1406.07

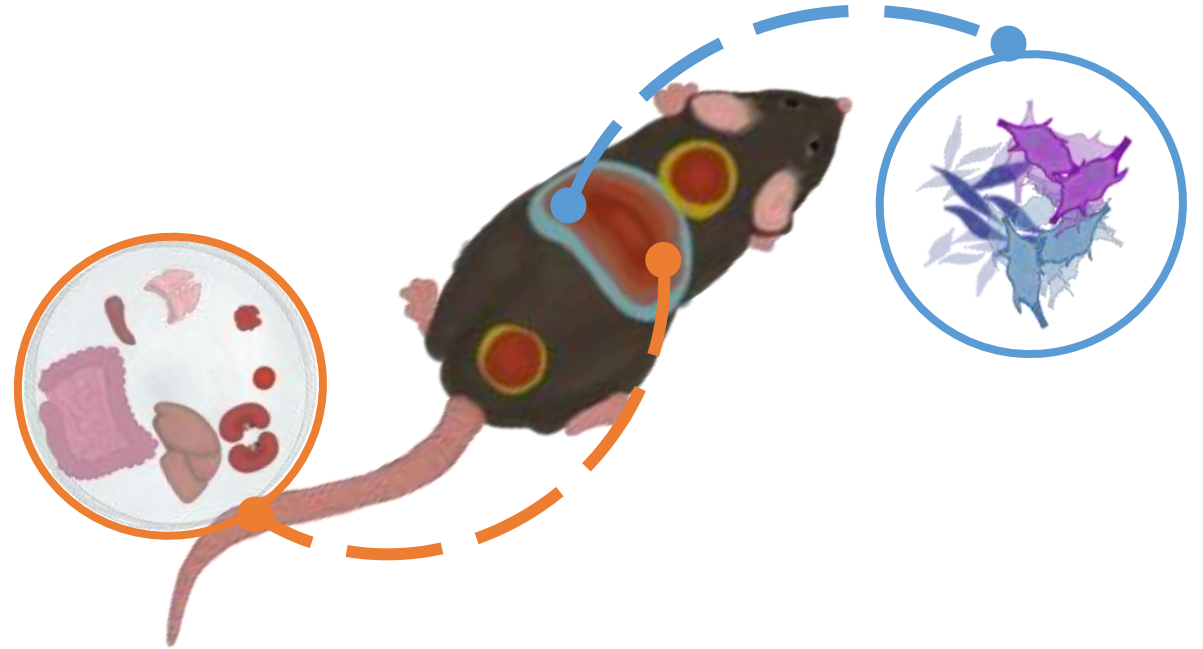
191.07

MIP
Source
Target 1
Angle: 231°

Current research focus – our challenges in nanomedicine

Biodistribution, retention, clearance...

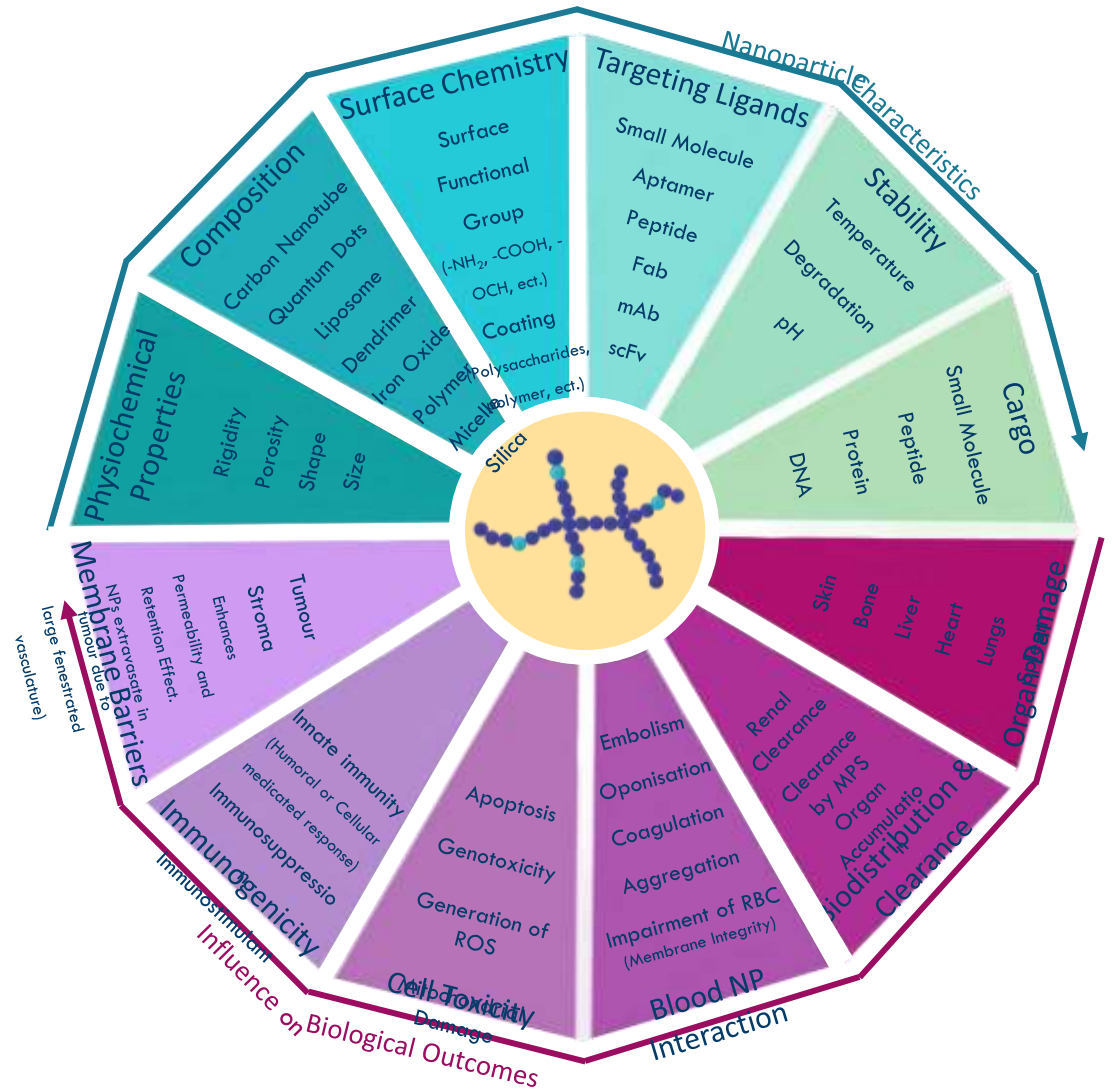
1. The immune system; how it affects our development of nanomedicines and how it can be exploited



Current research focus – challenges in nanomedicine

Biodistribution, retention, clearance...

1. The immune system; how it affects our development of nanomedicines and how it can be exploited
2. What does “stealthy” mean - decoration of polymers with biological molecules intrinsically modulates “stealthiness” of the particle.

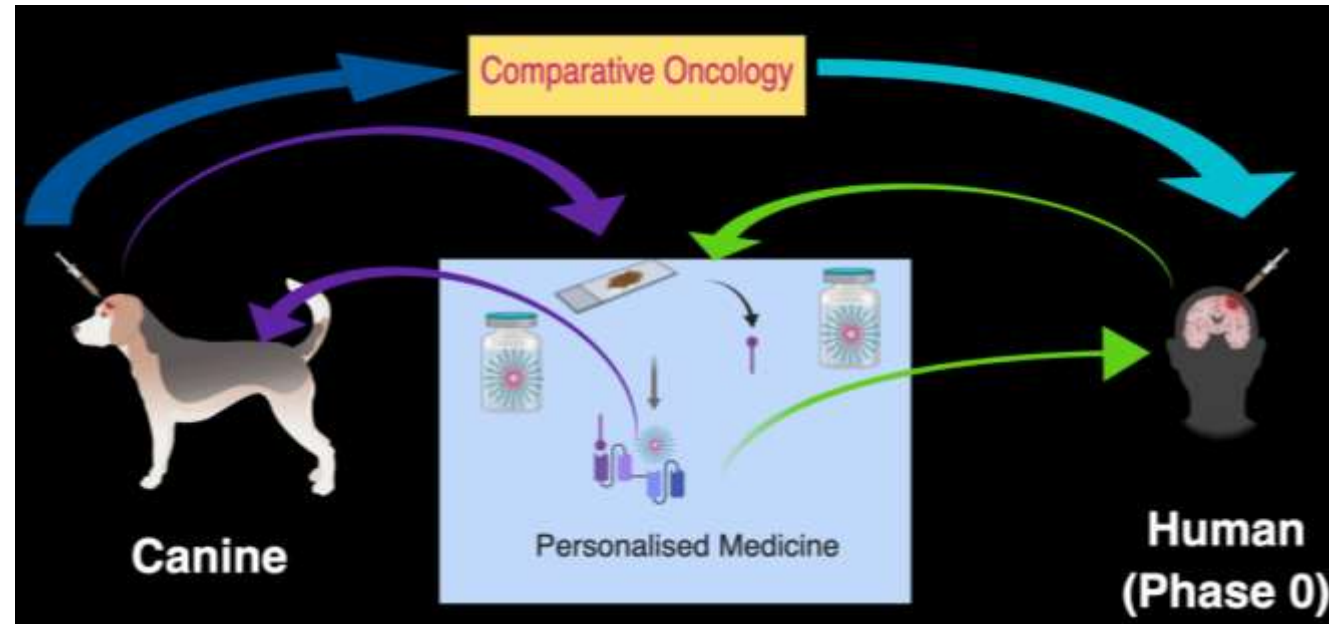


Sivaram et al. ACS Nano, 2021
Balaji et al. Biomaterials, 2023
Moles et al. Sci Trans. Med. 2023

Current research focus – our challenges in nanomedicine

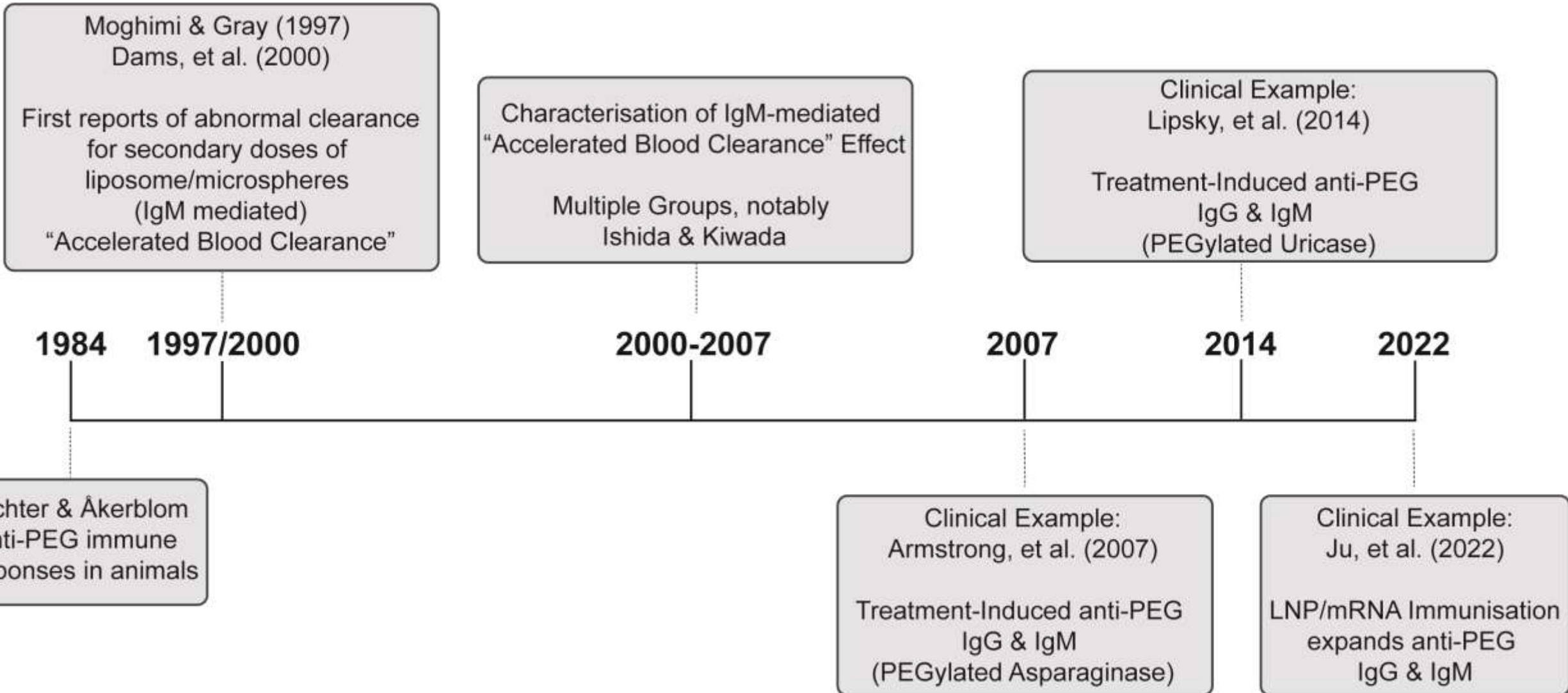
Biodistribution, retention, clearance...

1. The immune system; how it affects our development of nanomedicines and how it can be exploited
2. What does “stealthy” mean - decoration of polymers with biological molecules intrinsically modulates “stealthiness” of the particle.
3. Our translational pathway → clinical personalised nanomedicine.



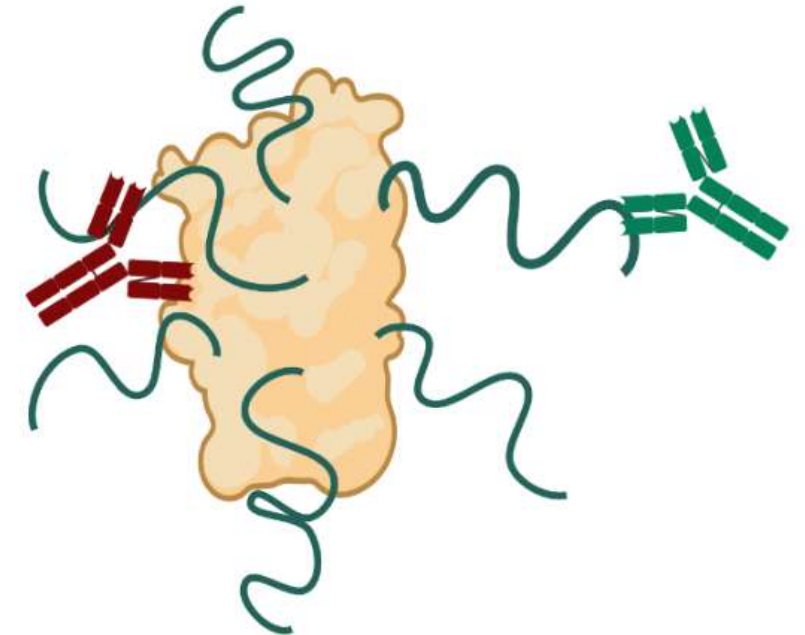
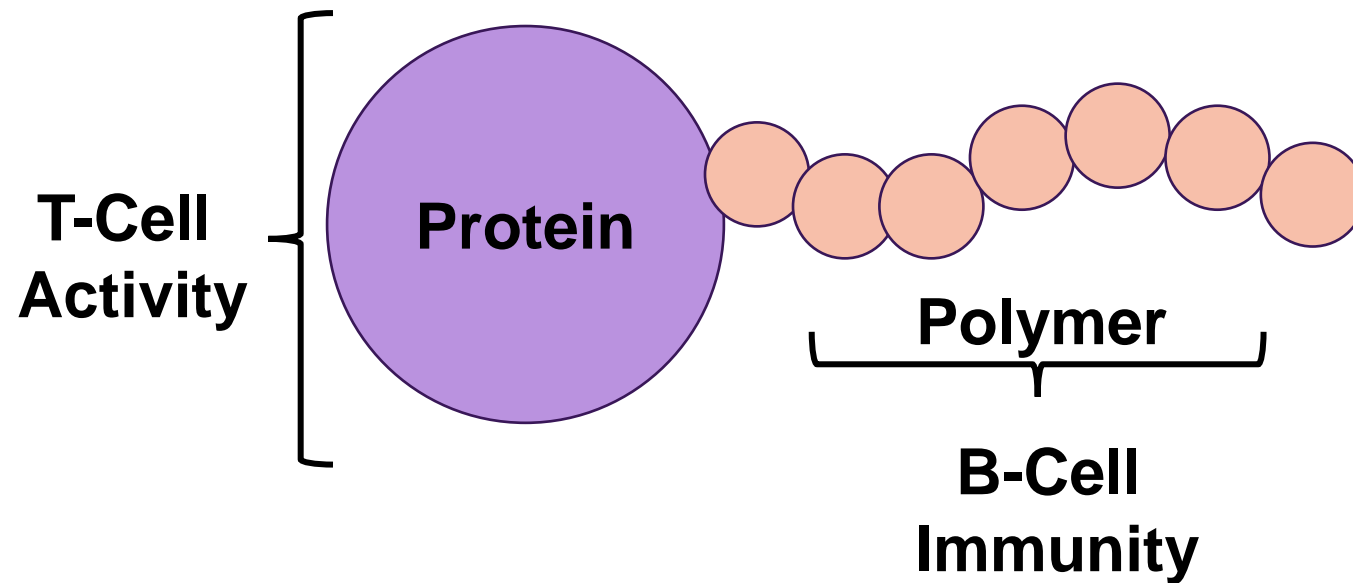
Houston et al. ACS Central Science, 2020
Janowicz et al. Biomaterials, 2022
Daniel et al. J Nuc Med, 2020
CRICOS code 00025B

PEG – Clinical reality of an all-purpose polymer



Understanding Anti-PEG responses in the clinic

- Seroconversion to IgG is significantly more problematic than IgM (Fc recycling)
- eg. Oncaspar^{®1}, Krystexxa^{®2,3}, Palynziq^{®4}
 - Non-host, evolutionarily divergent (xenogeneic) proteins conjugated with PEG.

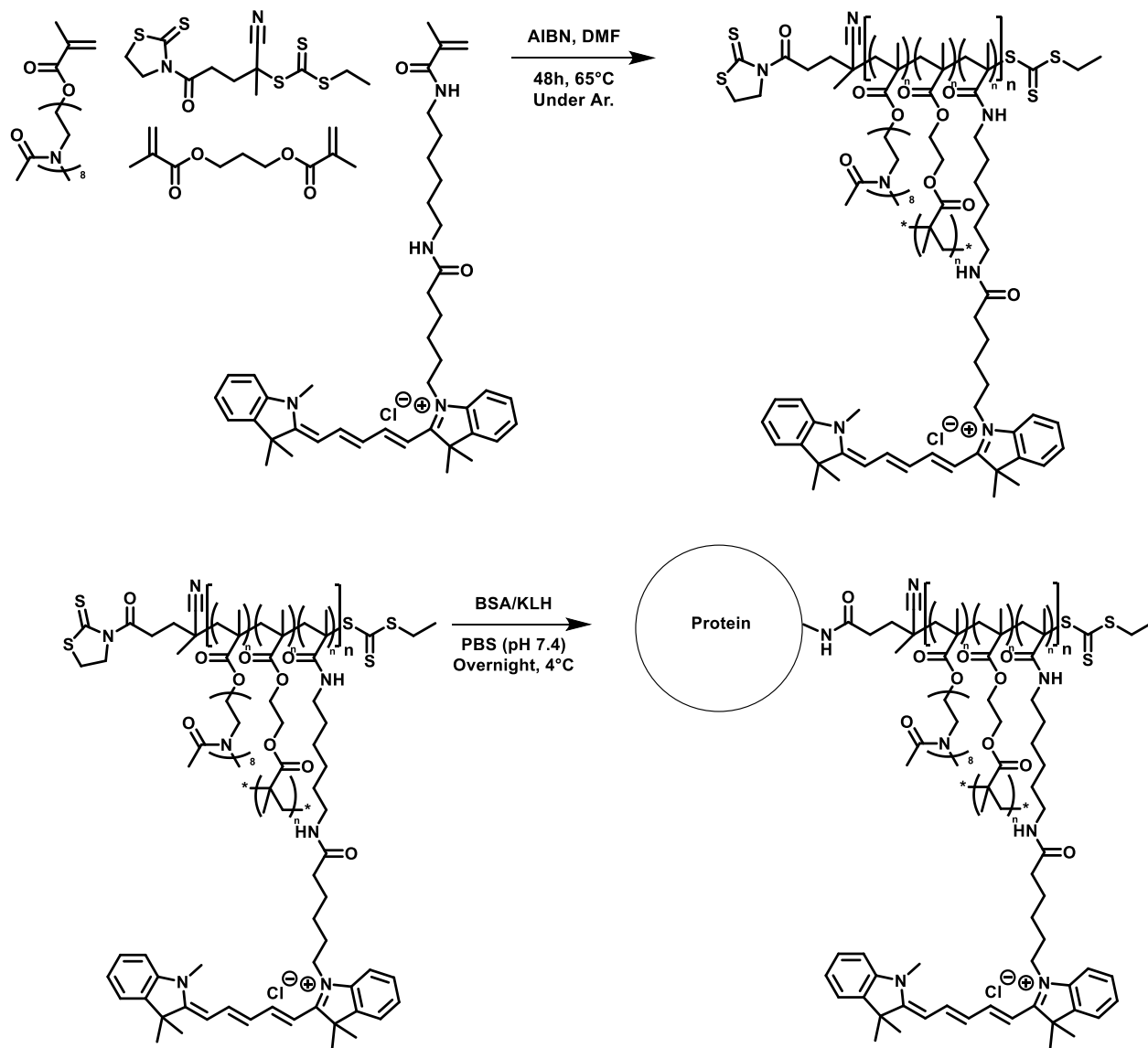


1. Armstrong, J. K., et al., *Cancer* **2007**
2. Hershfield, M. S., et al., *Arthritis Res. Ther.* **2014**
3. Lipsky, P. E., et al., *Arthritis Res. Ther.* **2014**
4. Gupta, S., et al., *EBioMedicine* **2018**

F

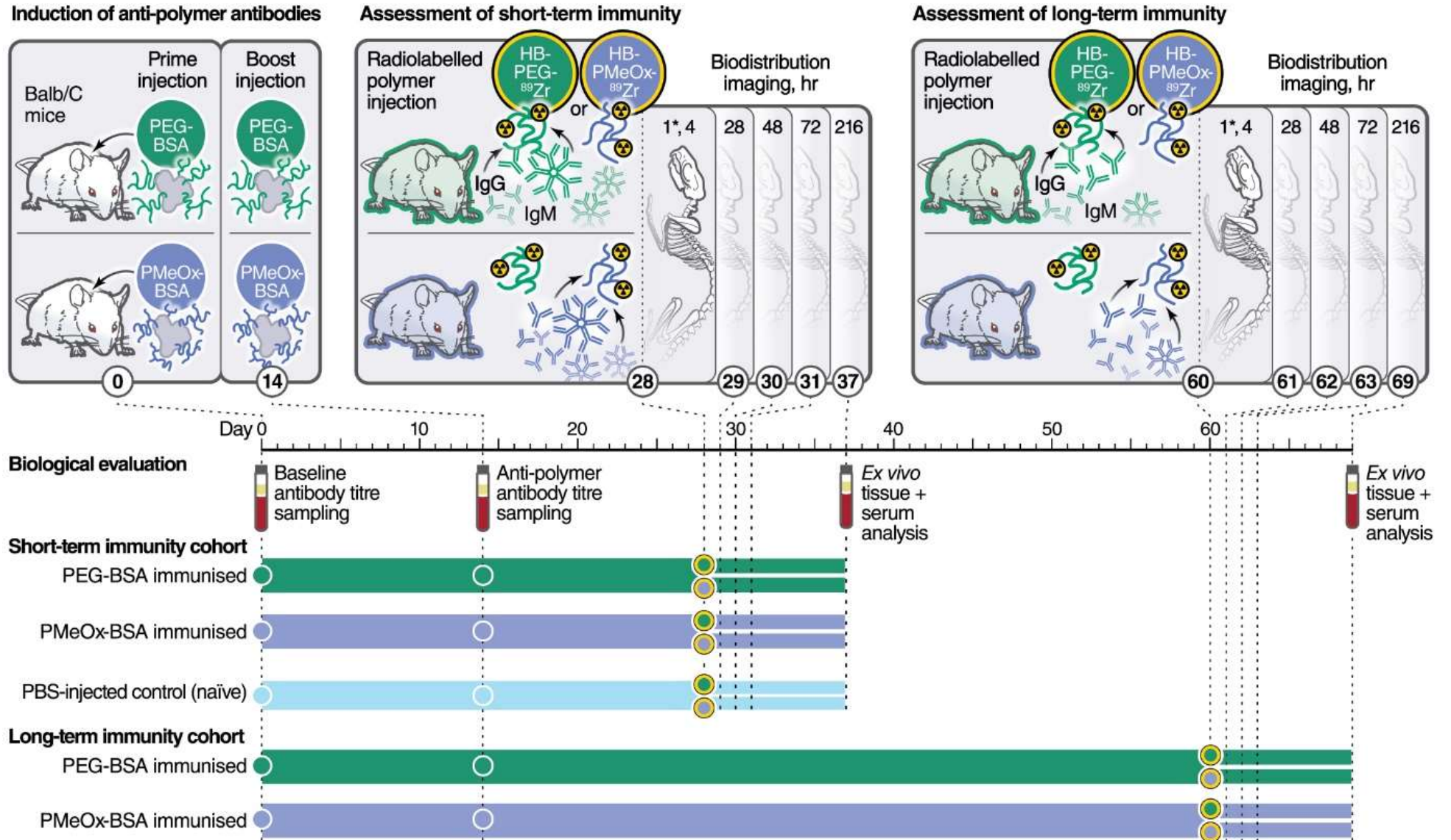
+tbody-

2



Anti-Polymer Immunity Model

A. Induction of an anti-polymer model for pharmacokinetic assessment



*Imaging performed at multiple intervals within first 60 minutes for time-activity analysis (see Figure 4A).



Magnitude of this effect depends on many factors:

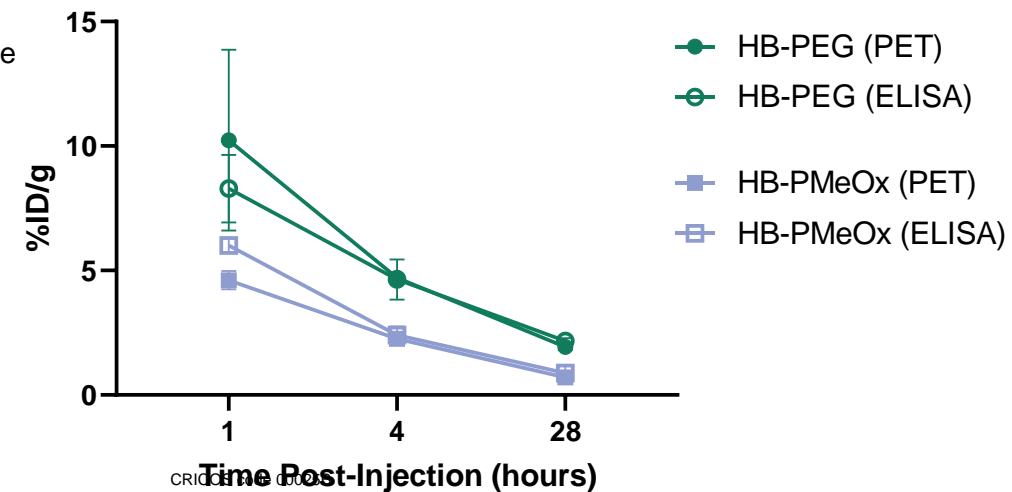
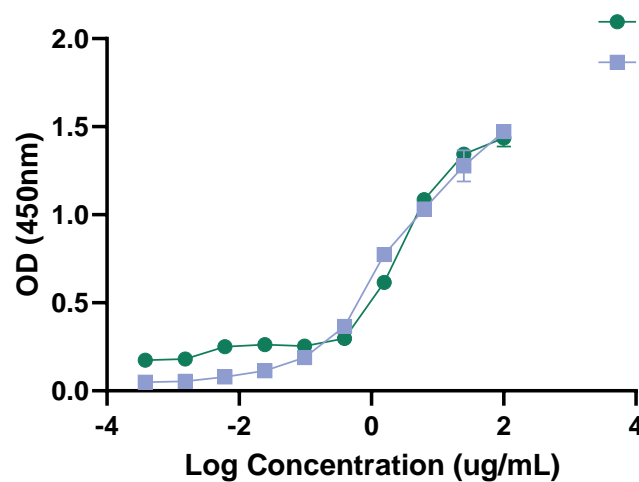
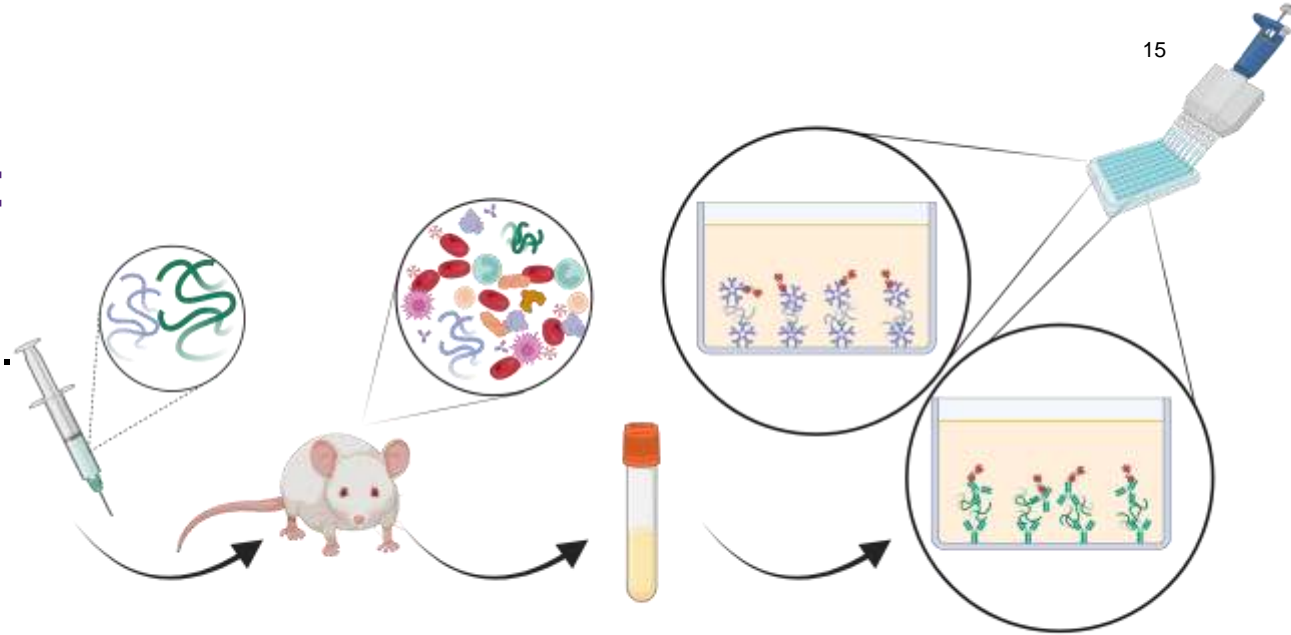
- Size of polymer (D_h) ie. faster naïve $t_{1/2}$ leads to less time for opsonisation/deposition into liver/spleen
 - Model is currently applicable to therapeutics with a narrow therapeutic index (**lower doses**)
 - Future work: Investigate this in the context of **large doses** to visualise Fc Recycling

Alternating between antigenically distinct polymers is a viable strategy to evade detection by the adaptive immune system.

Can we use this knowledge to establish anti-Polymer mAbs in scalable therapeutic drug monitoring assays?

Quantifying label-free POx and PEG:

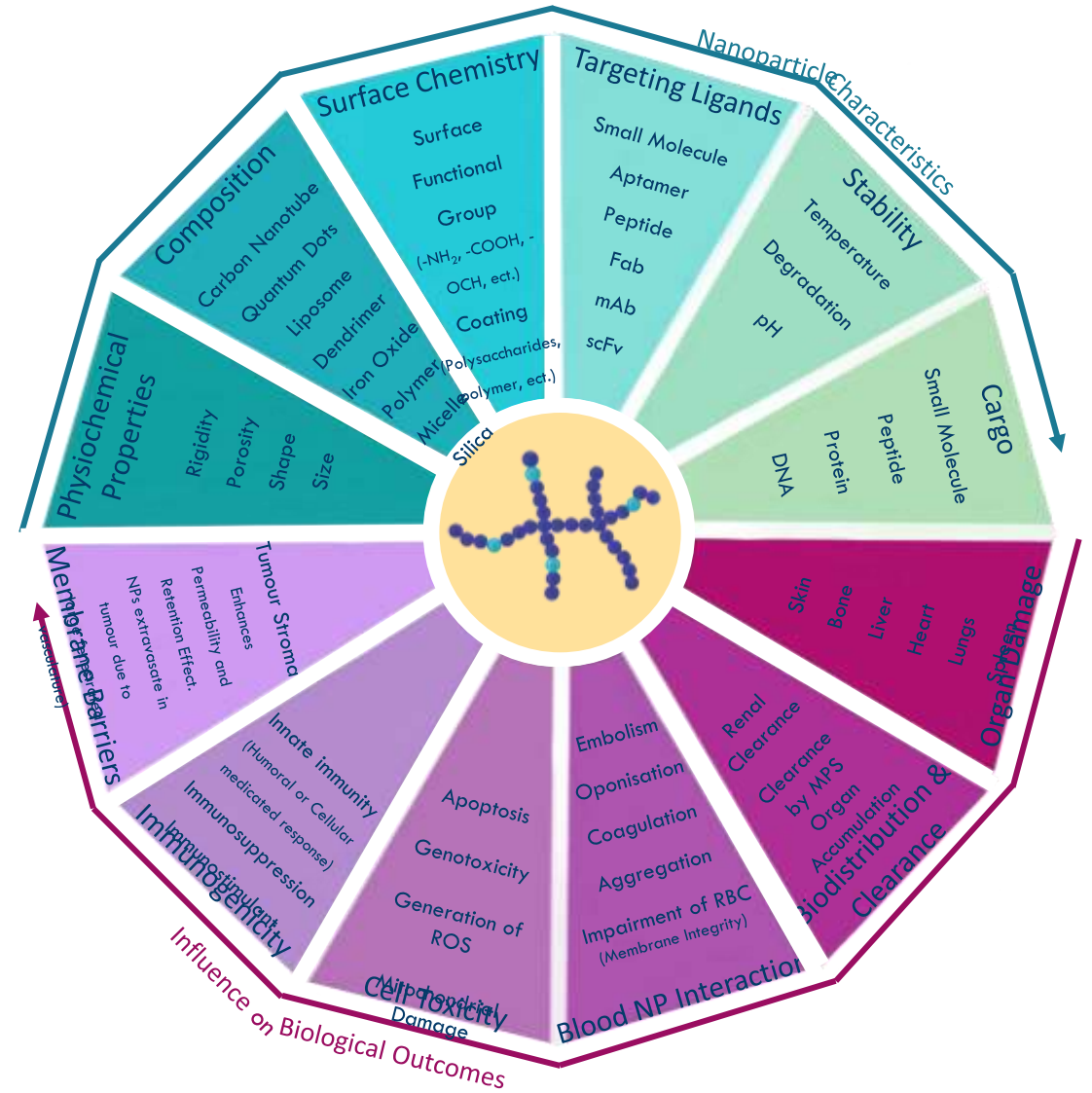
- Sandwich ELISA based format for multiplexing label-free polymer therapeutics.
- What this is useful for:
 - Applications where multiple polymer therapeutics are employed to deliver synergism (e.g. metronomic multi-drug chemotherapy)



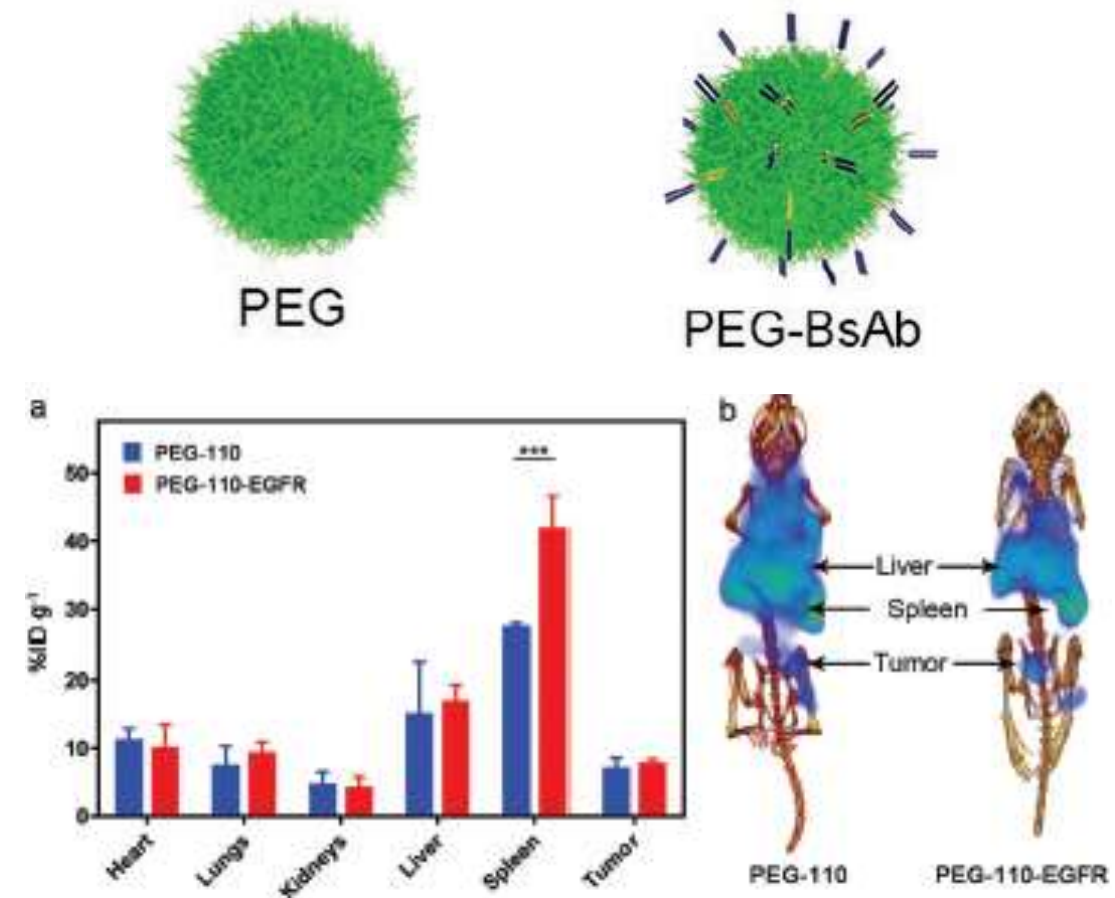
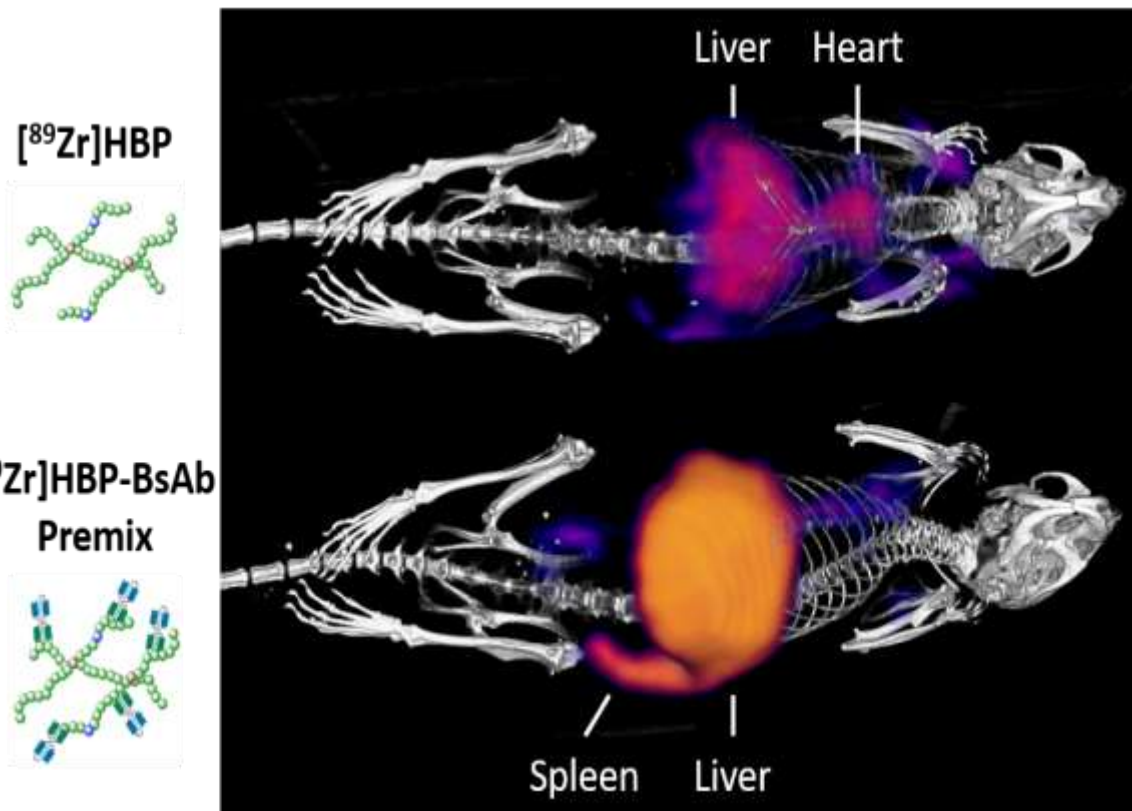
Current research focus – our challenges in nanomedicine

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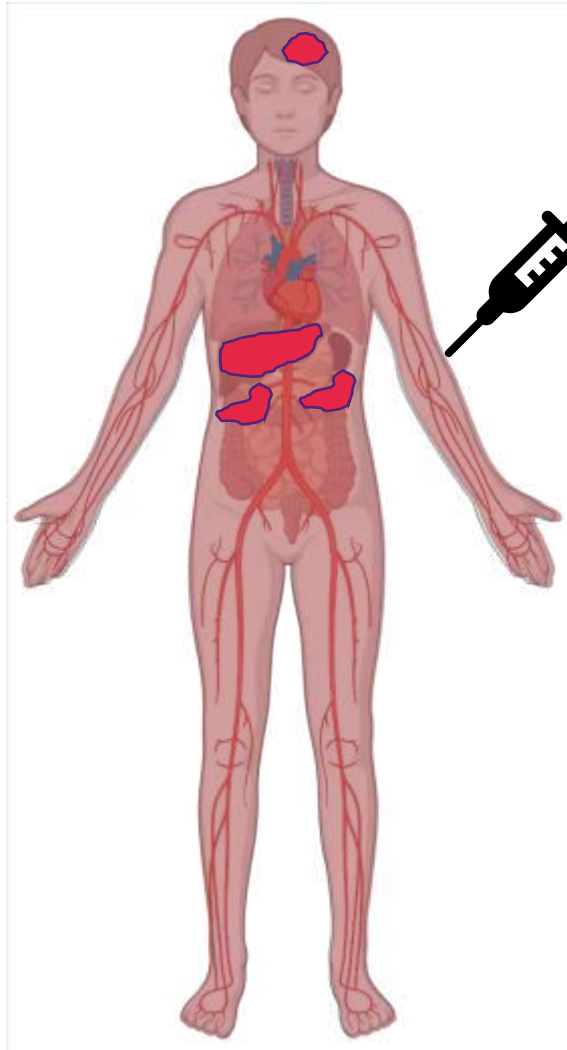
The effect of targeting ligand on biodistribution



Fletcher et al., Chem Commun. 2022

Cui, Thurecht, Caruso et al., ACS Nano 2019

Pretargeting: Strategies to overcome clearance of nanoparticles

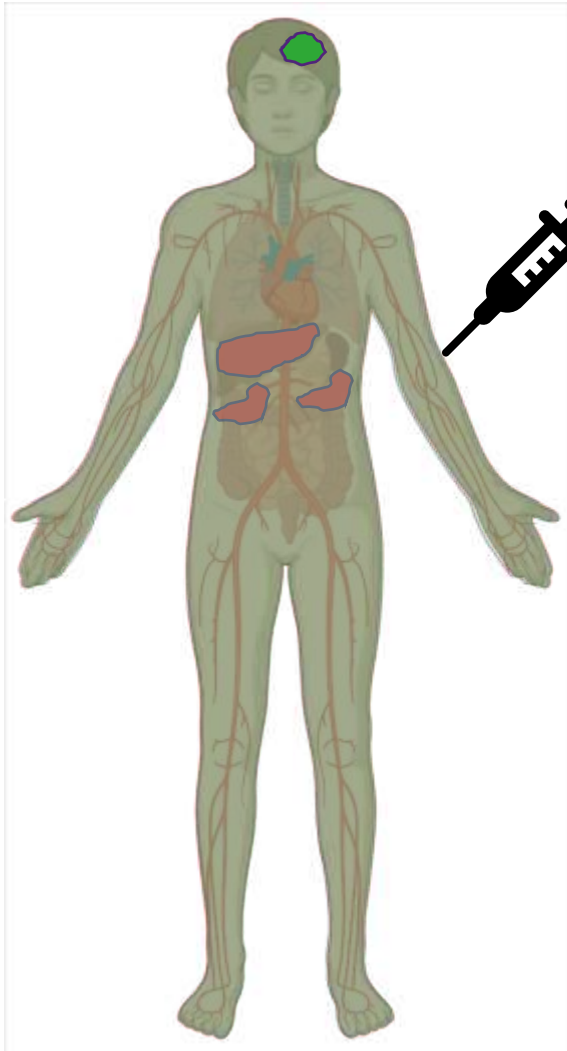


mAb imaging

- Usually good target accumulation
- Long circulation
- Long retention in clearance organs

Reardon et al. 1985; Goodwin et al. 1986; Hnatowich et al. 1987

Pretargeting: Strategies to overcome clearance of nanoparticles

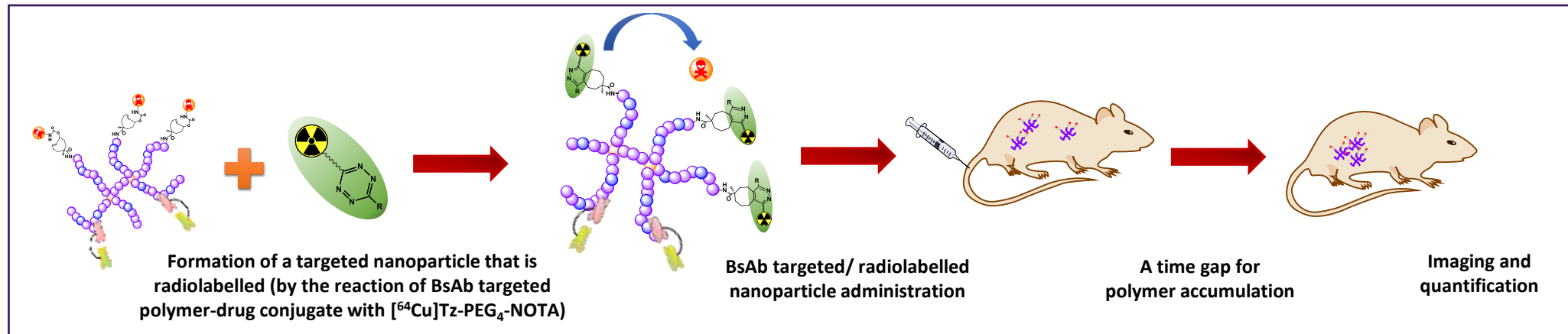


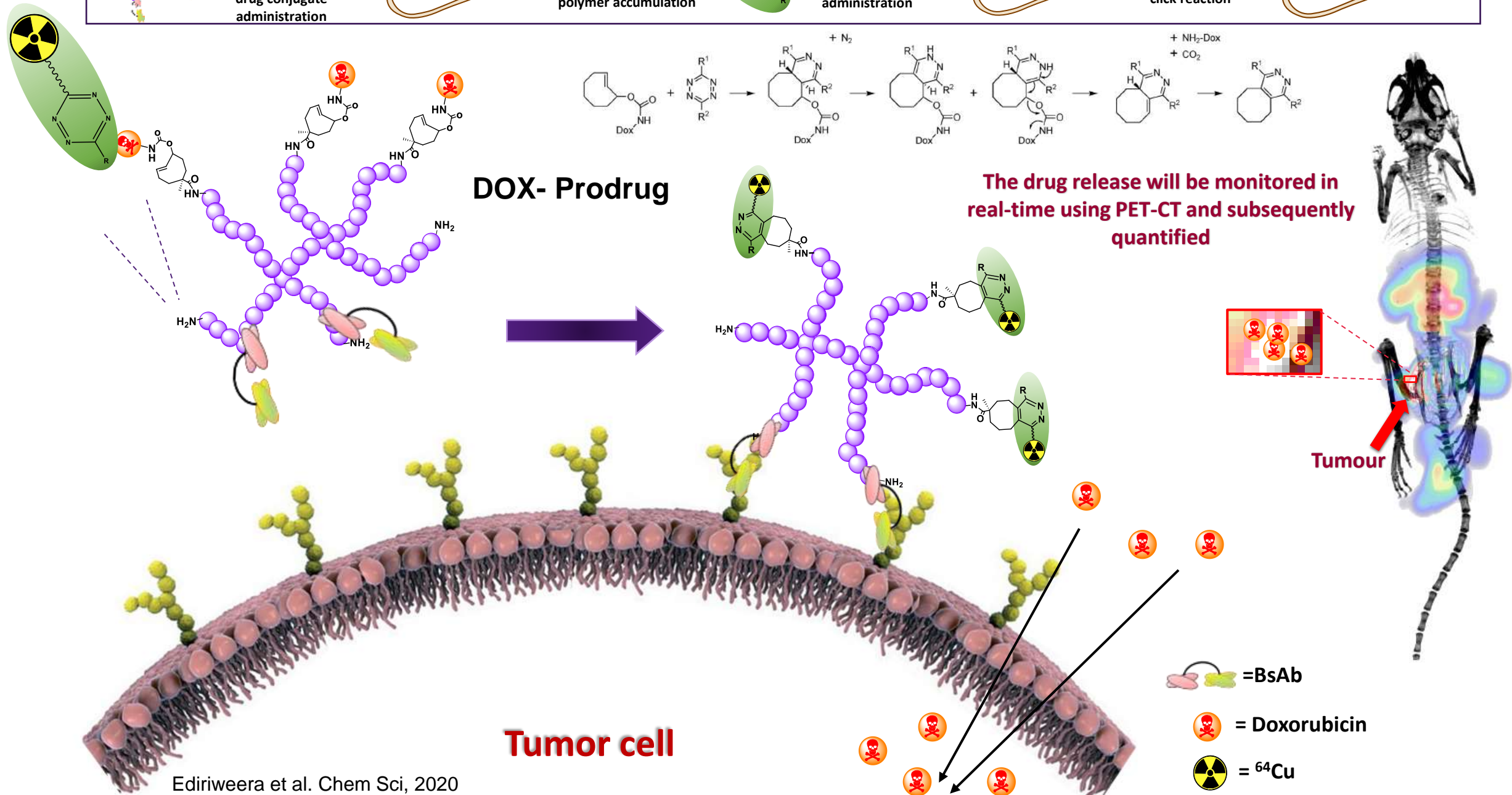
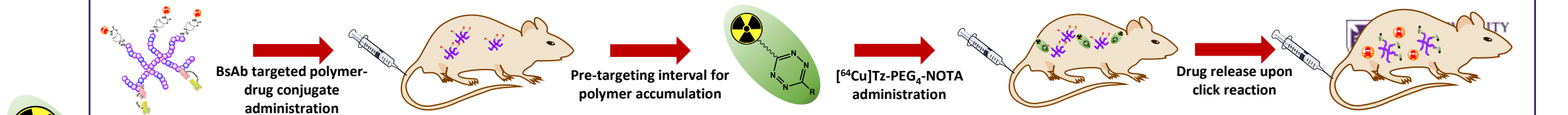
mAb pre-targeting approach

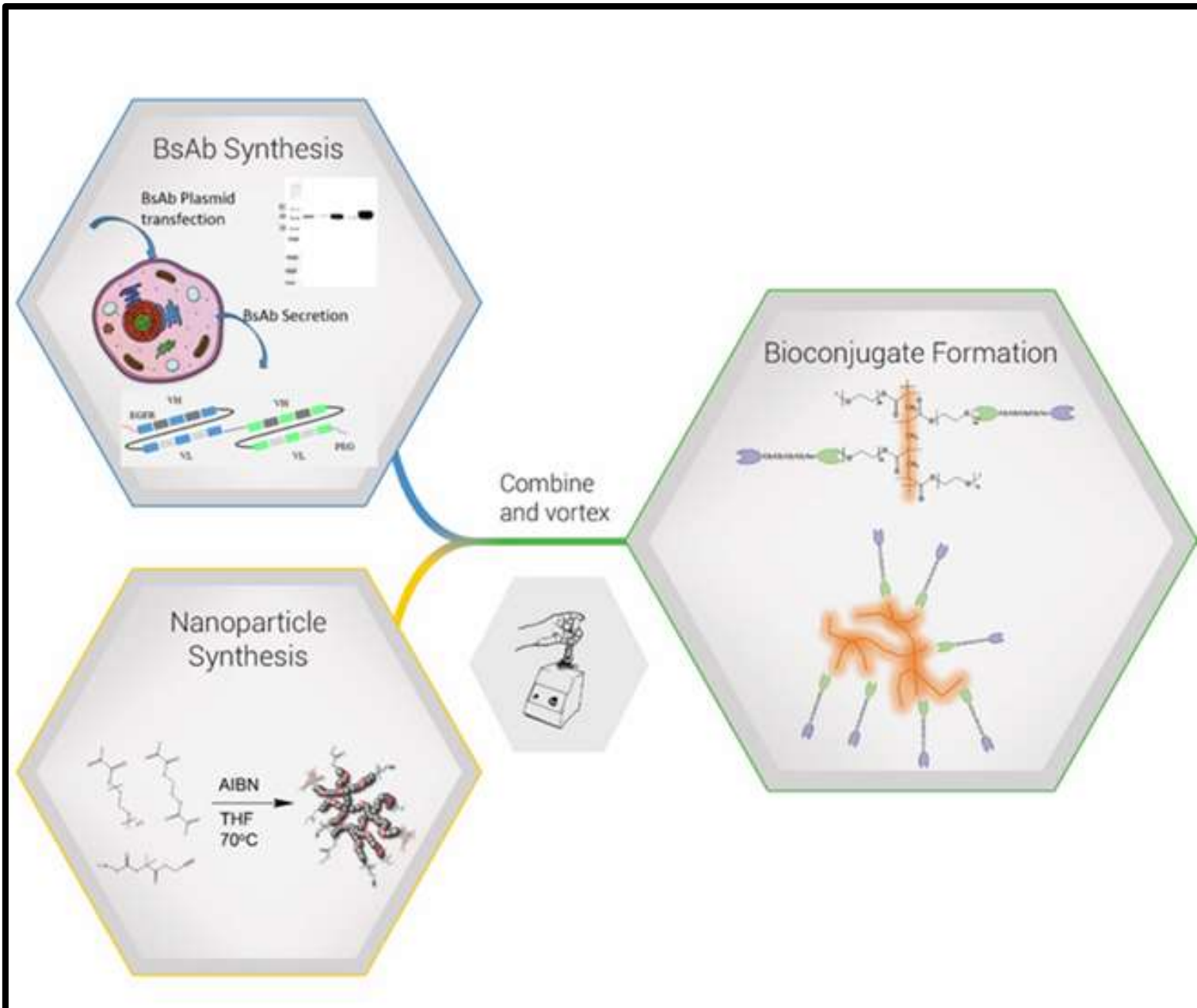
- 2-step process
- Bio-orthogonal click reaction occurs between pre-injected mAb and chase molecule
- PK of the chase molecule dictates S/N and



Conventional approach







Bispecific Antibody – BsAb

- Dual targeting
- Rapid conjugation to synthetic polymers
- Targeting antibody conjugation through affinity ($K_d = 10 \text{ nM}$)



Howard et al. Adv. Healthcare Mater. 2016

Pretarging using direct antigenic interactions (bispecific antibodies)

- First need model system
- PEG-based hyperbranched polymer
- Incorporate Cy5 fluorophore (*in vitro*) and Deferoxamine chelator (^{89}Zr PET *in vivo*)



The power of alpha therapeutics

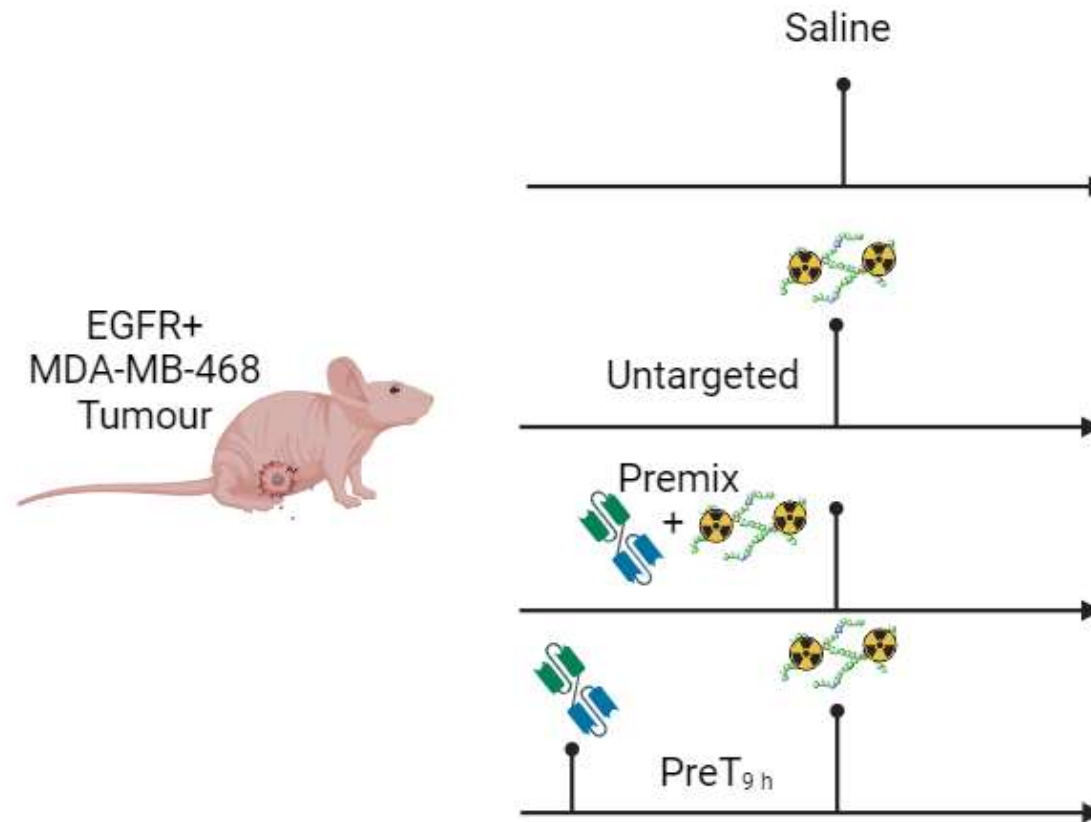


12/2014

PSA = 2,923 ng/mL

MeV)
arged
us

Pre-targeting [^{212}Pb]HBP Therapy

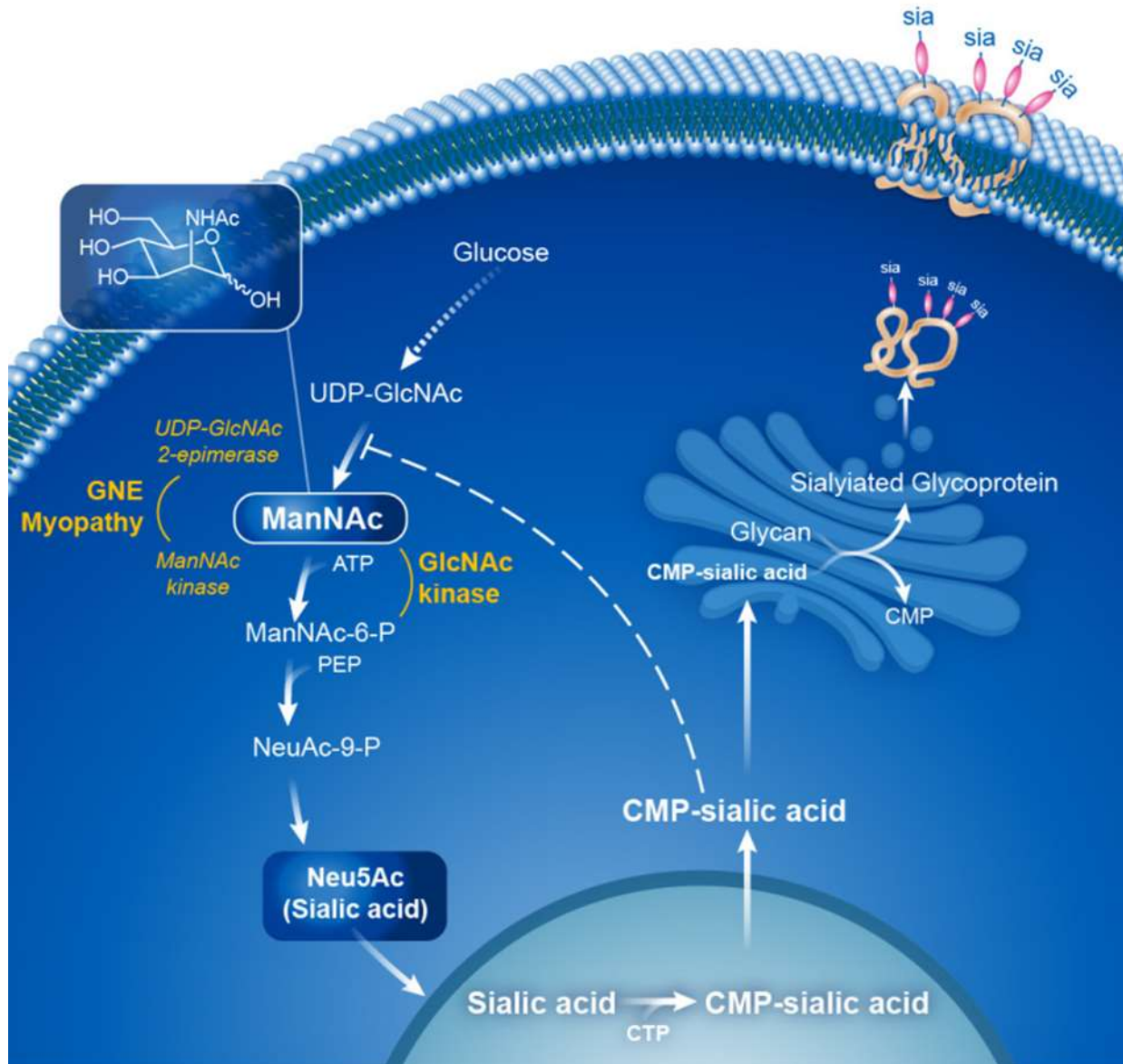


MDA-MB-468 (EGFR+) breast cancer tumours in Balb/c nude mice (n=5 per group)
1 MBq [^{212}Pb]HBP dose (Untargeted, Premixed with BsAB or Pretargeted)

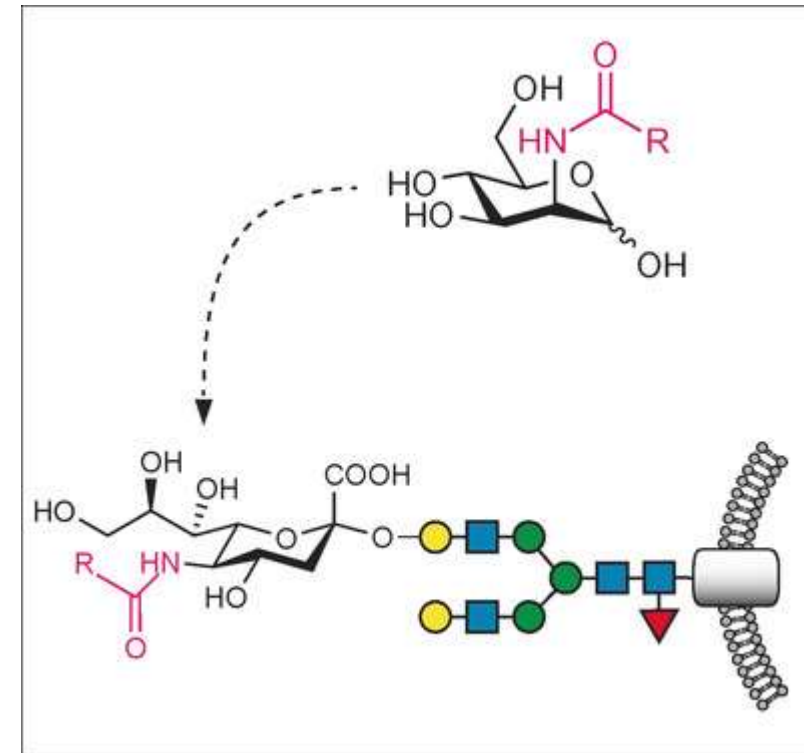
Pre-targeting [^{212}Pb]HBP Therapy (this is the only efficacy slide in my talk!)

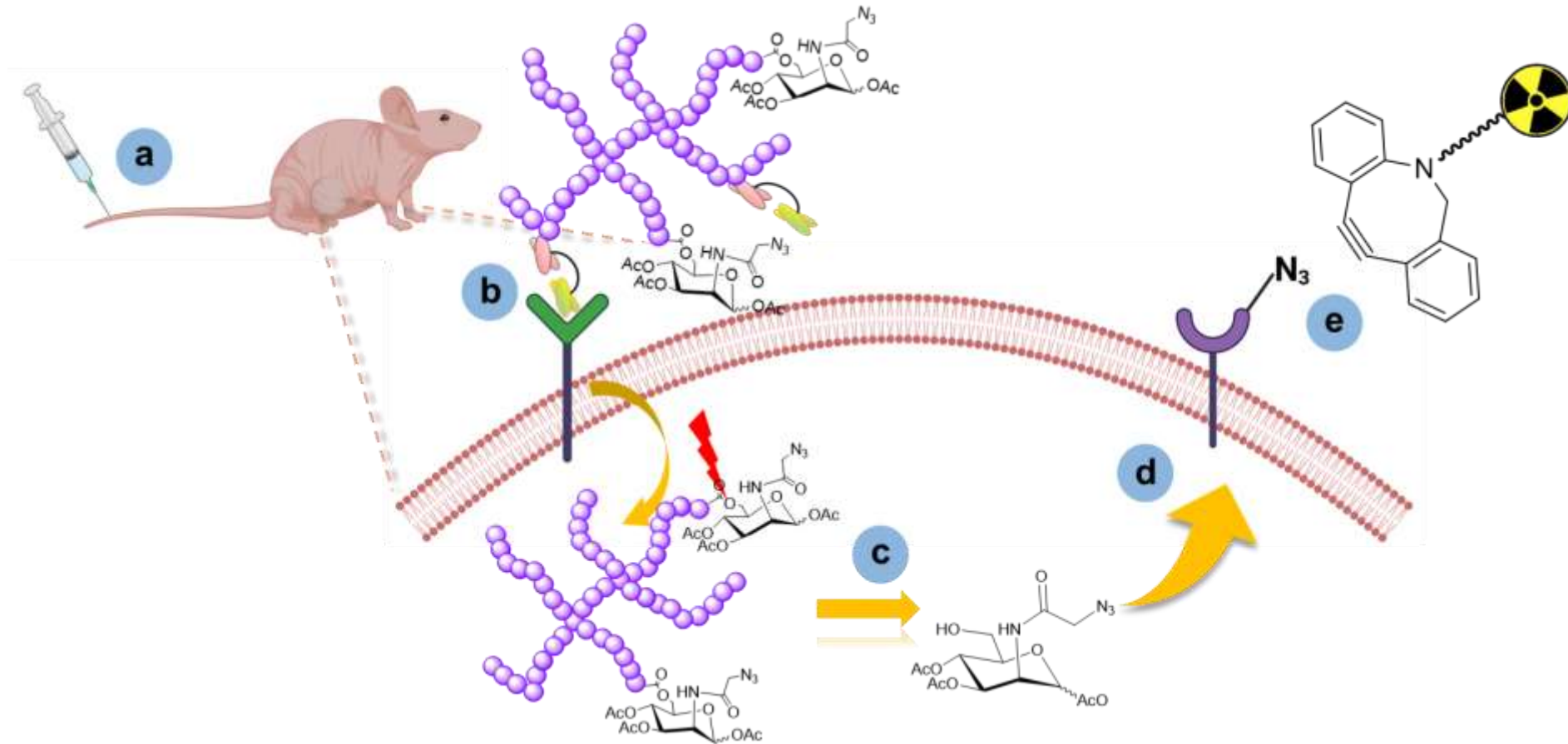
Monitor tumour volume as efficacy measure

Pretargeting using bispecific antibodies improves polymer radiotherapeutics through enhanced efficacy and decreased off-target toxicity



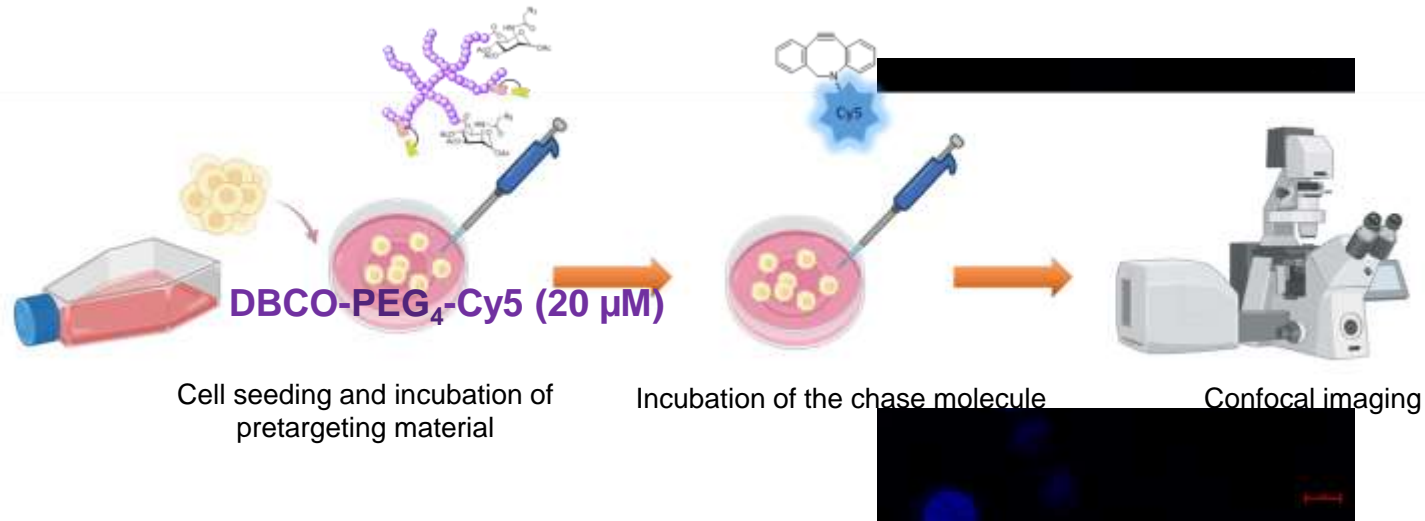
Metabolic glycoengineering (MGE) is a method used to modify glycan structures by treating cells with unnatural derivatives of monosaccharides



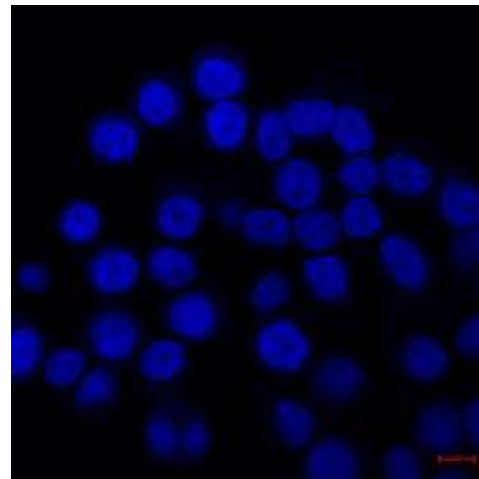


- a** Intravenous administration of targeted HBP- Ac₃ManNAz
- b** Receptor targeting and internalization (tumour cells)
- c** Release of metabolic precursors containing bioorthogonal groups from nanoparticles

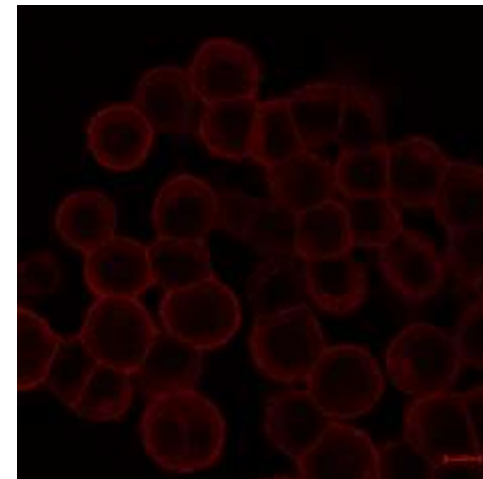
- d** Metabolic glycoengineering to produce artificial receptors containing bioorthogonal groups on the cell surface
- e** *In vivo* bioorthogonal click reaction with radiolabelled chase molecule and PET/CT



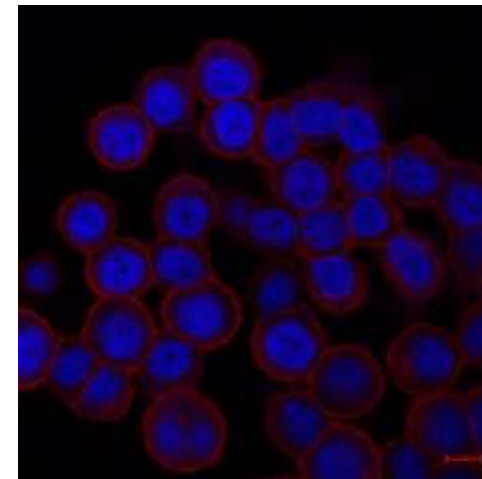
Ac₃ManNAz (10 μM) + DBCO-PEG₄-Cy5 (20 μM)



Hoechst (Nuclei)

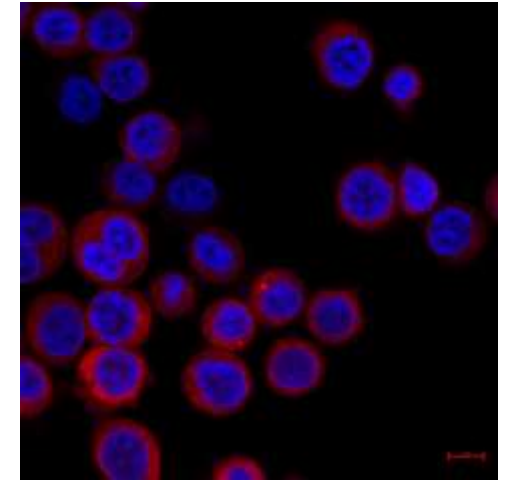
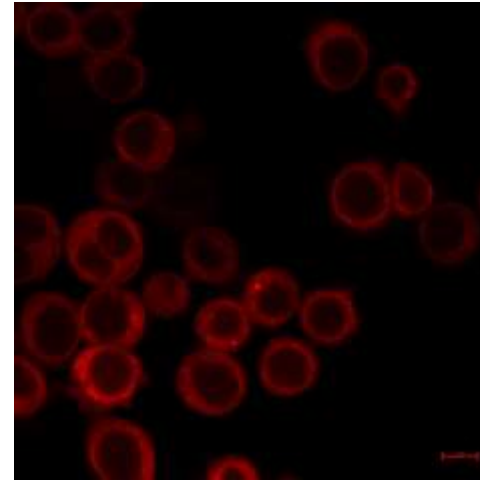
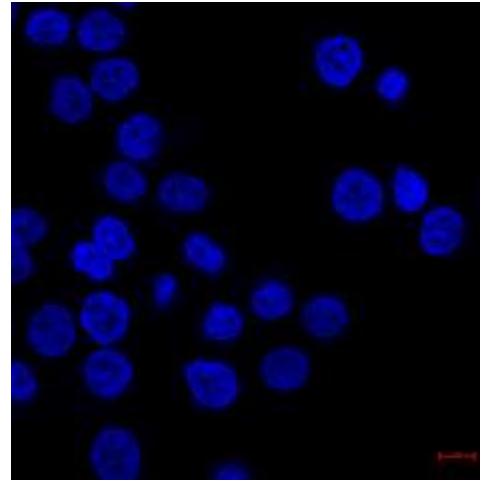


Cy5 (Chase molecule)



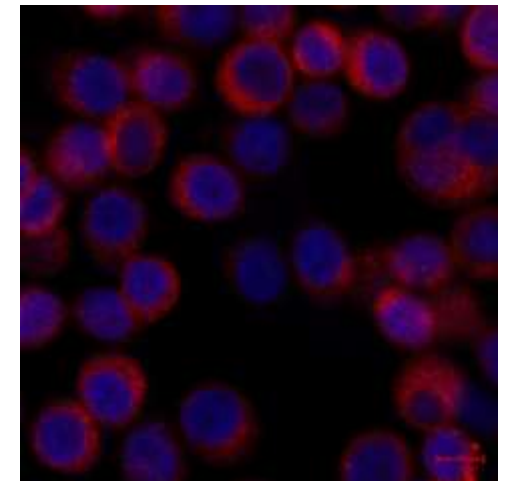
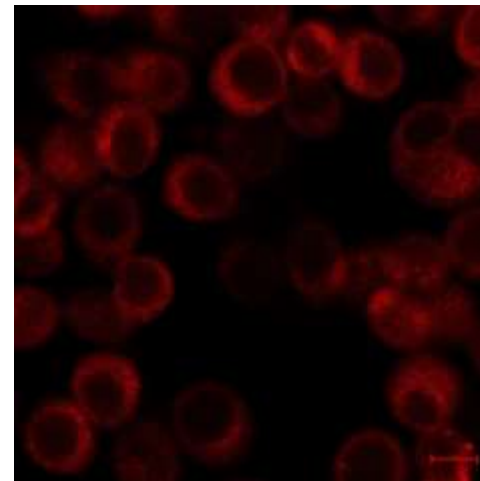
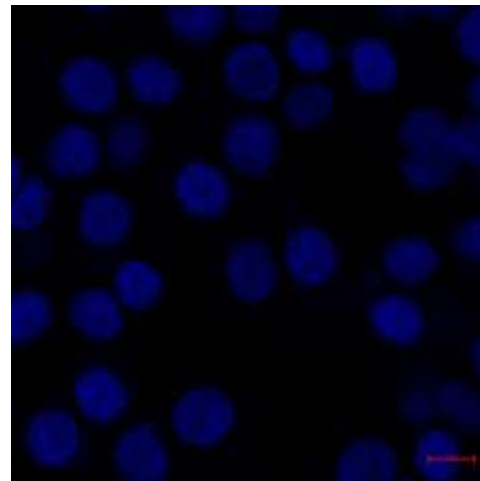
Merge

48 h incubation



EGFR/HBP-Ac₃ManNAz (10 μM) +
DBCO-PEG₄-Cy5 (20 μM)

72 h incubation



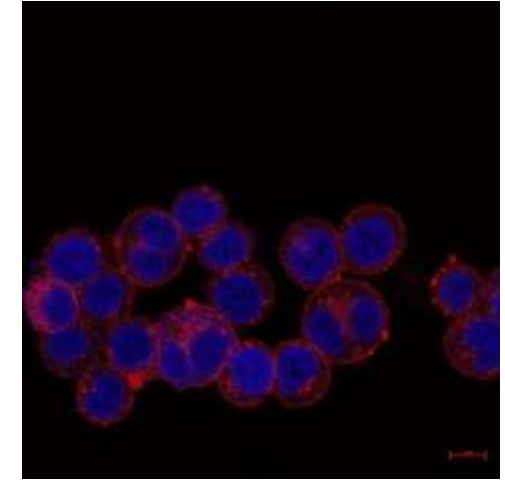
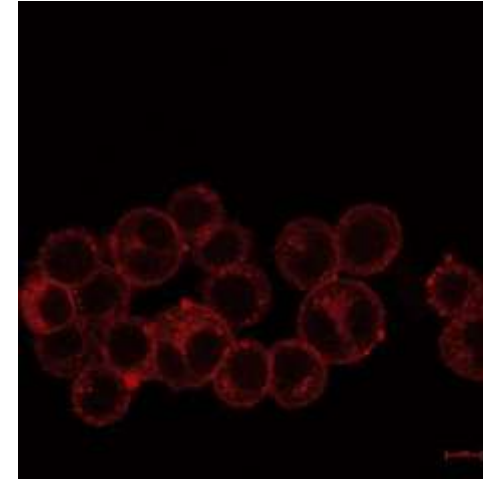
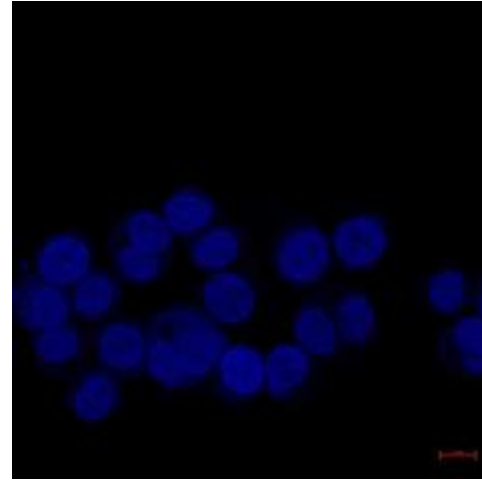
Hoechst (Nuclei)

Cy5 (Chase molecule)

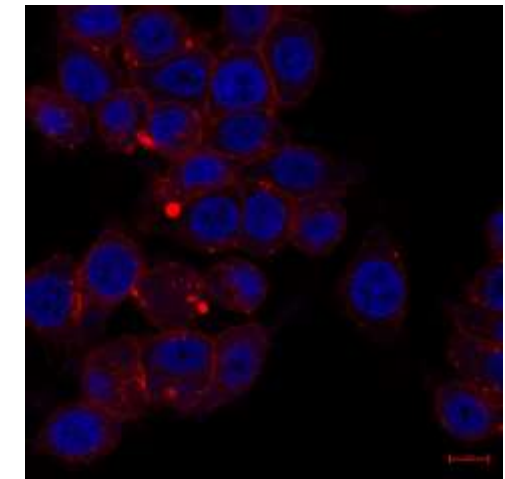
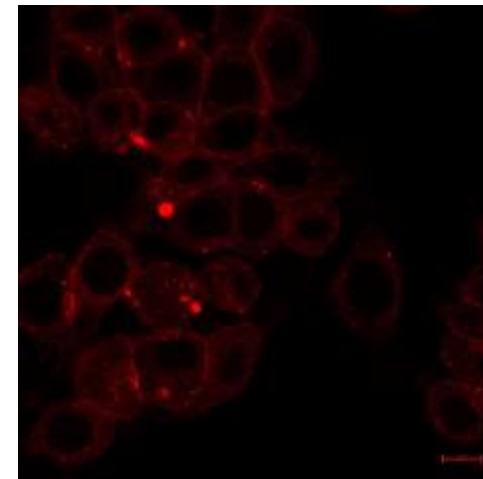
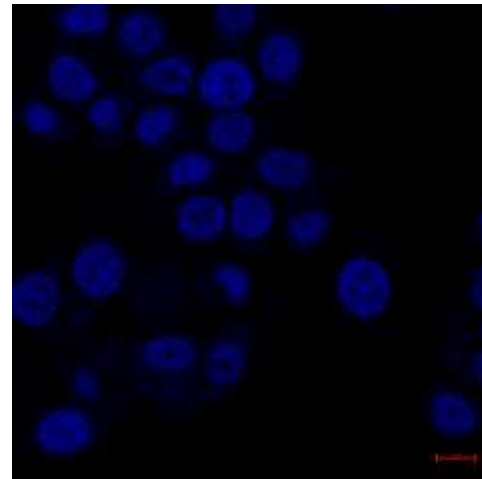
Merge

EGFR/HBP-Ac₃ManNAz (10 μM) +
DBCO-PEG₄-Cy5 (20 μM)

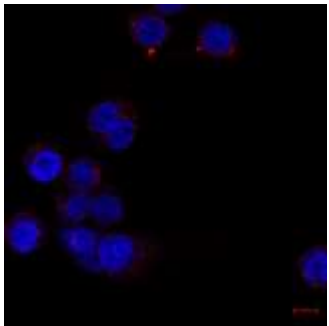
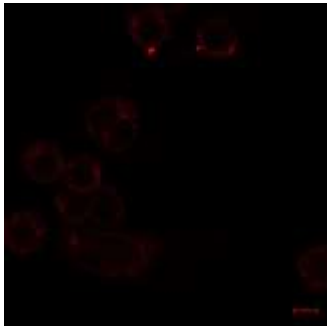
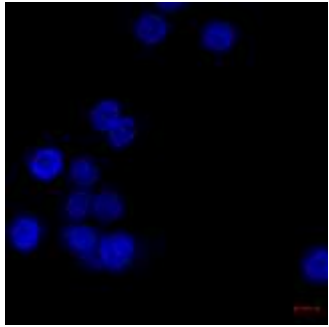
5 days incubation



6 days incubation



Control (EGFR/HBP-Az)
DBCO-PEG₄-Cy5



Hoechst (Nuclei)

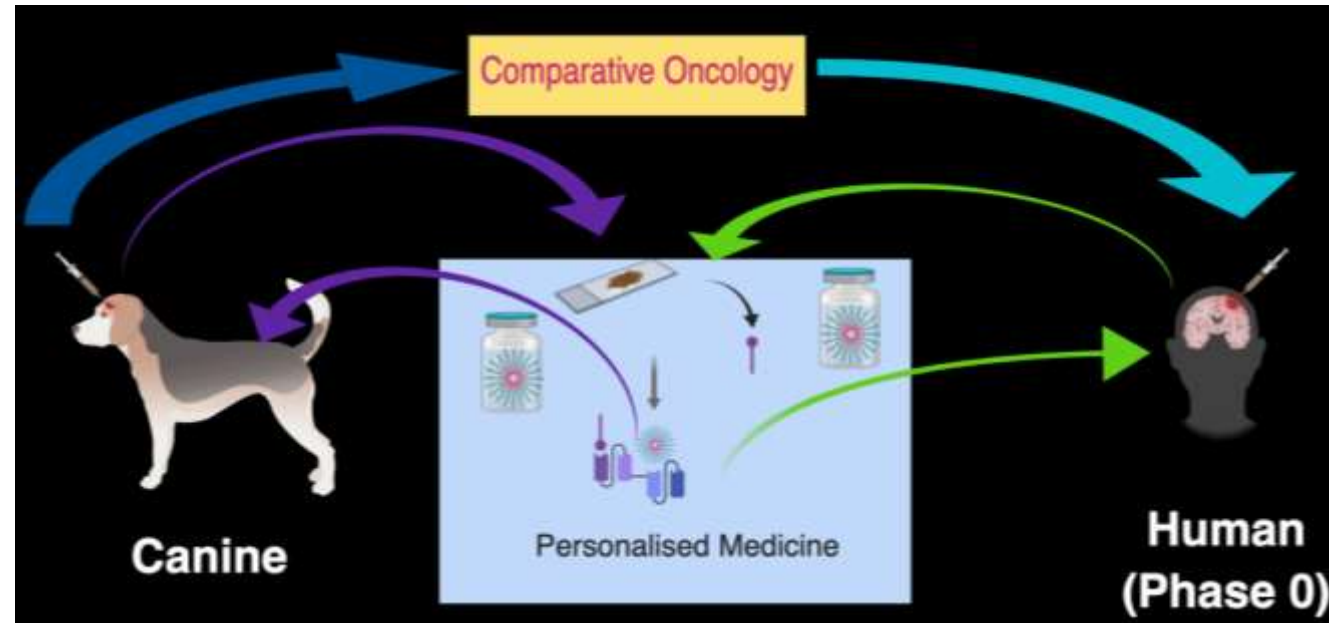
Cy5 (Chase molecule)

Merge

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2. The stealth conundrum; decoration of polymers with biological molecules intrinsically modulates “stealthiness” of the particle.
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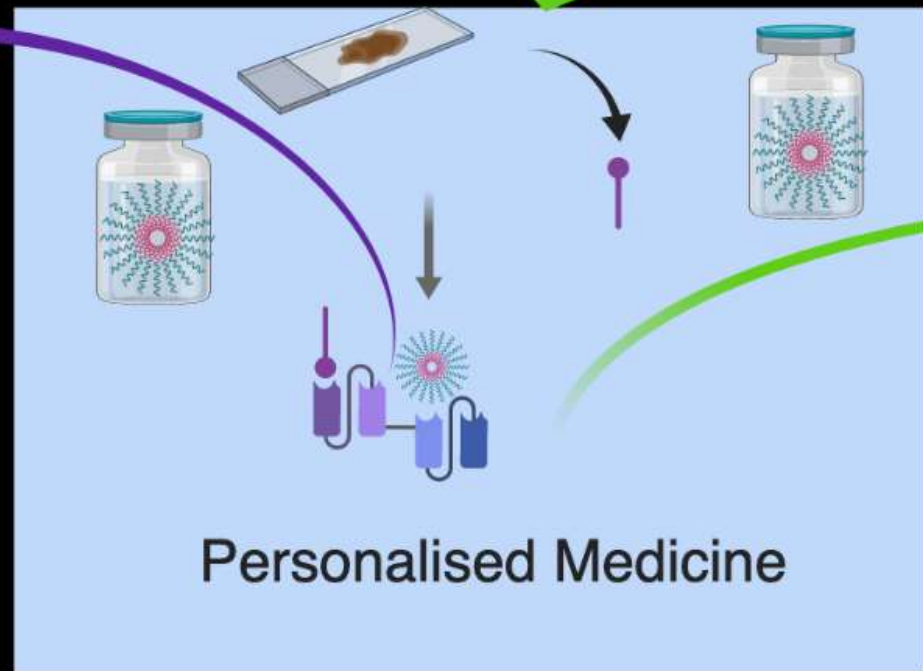
Canine trial starting in 2024 with for GBM

Comparative Oncology

Human study proposed in 2024 for GBM



Canine



**Human
(Phase 0)**

Milestones to Clinical Translation of Nanomaterials



1

Get a lead candidate

- Easy to synthesize
- High yield
- Tuneable



2

Therapeutic Profile

- Cytotoxicity
- *In Vivo* Toxicity
- Biodistribution
- Targeting



3

Safety & Tolerability

- Maximum Tolerated Dose
- Systemic Toxicity
- Safety for Medical Staff

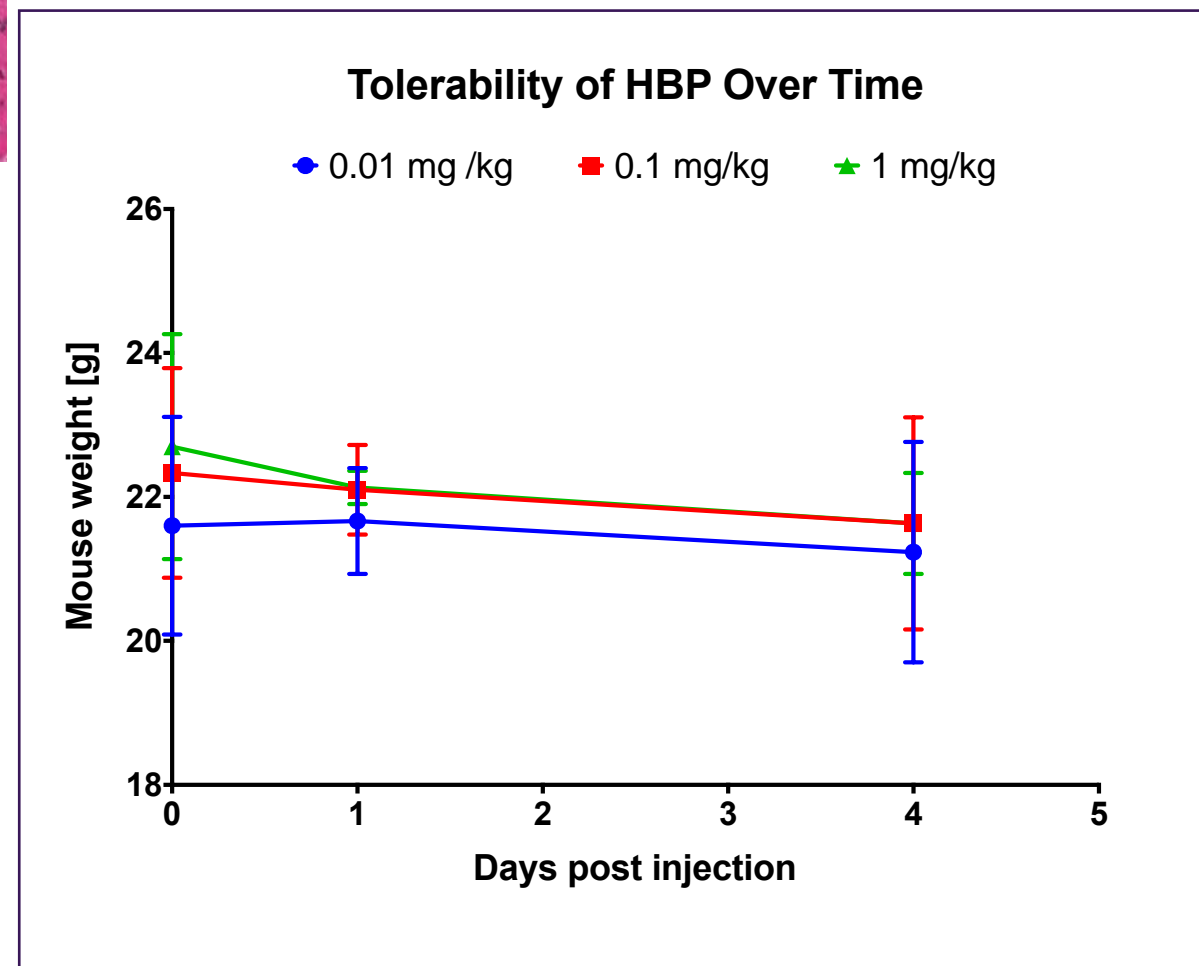
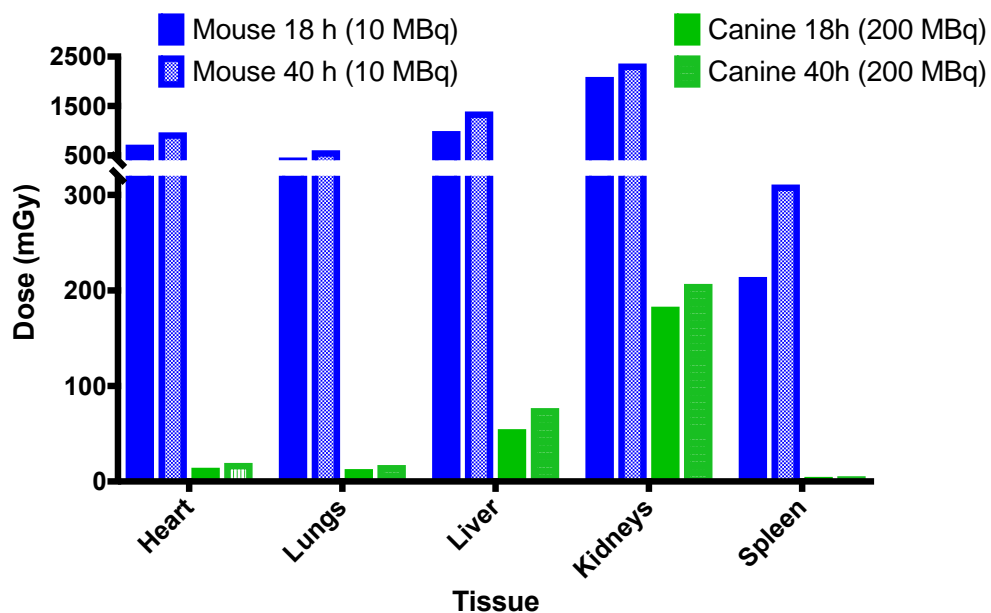
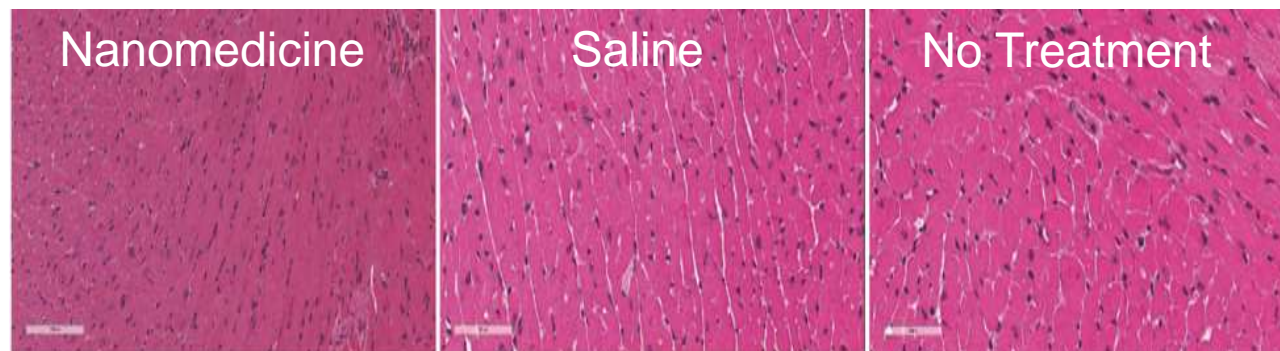


4

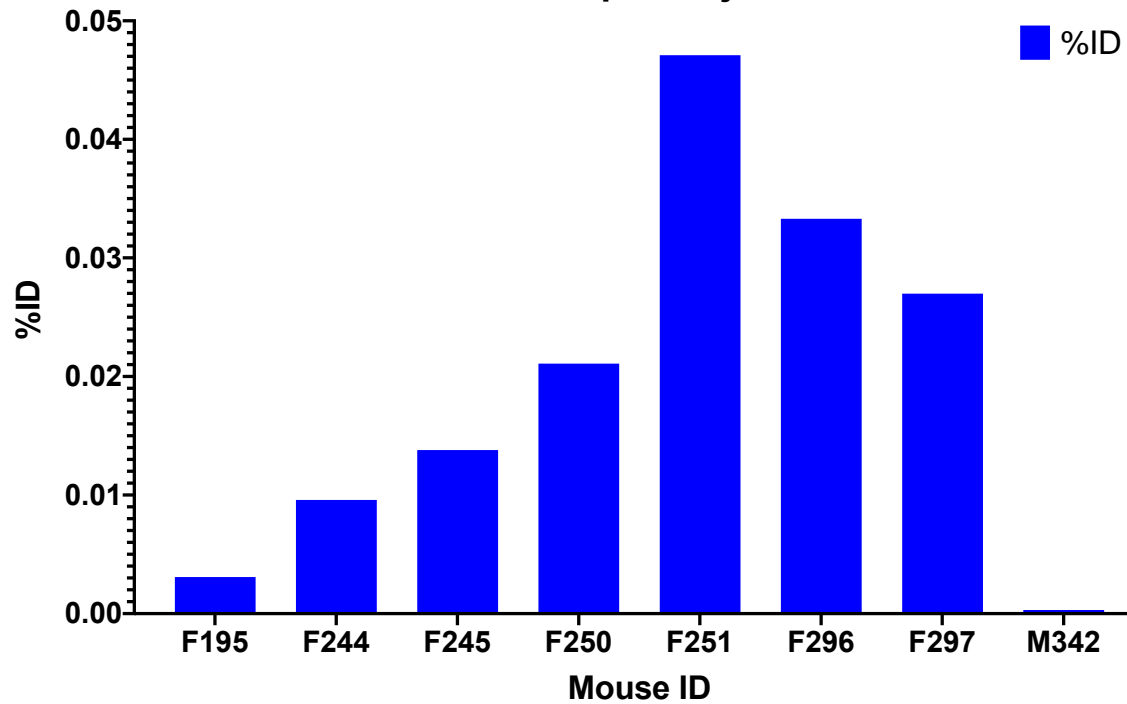
QC & Manufacturing

- Scale-up
- Purity
- Reproducibility
- Consistency
- GMP Process

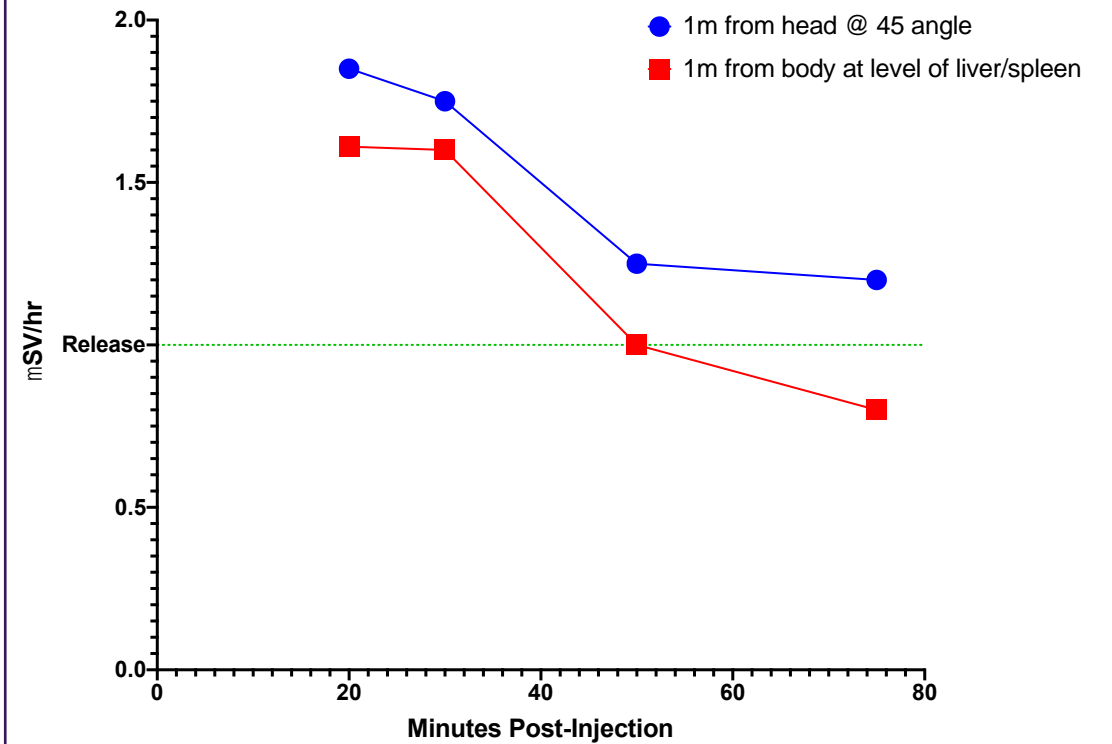
Talelli, M. *et al. Nano Today* **10**, 93-117, doi:10.1016/j.nantod.2015.01.005 (2015).



Radioactivity remaining in Brain Tumour in Mice at 36 hours post-injection



External Dose of Cu-64 nanomedicine after 132 MBq injected dose



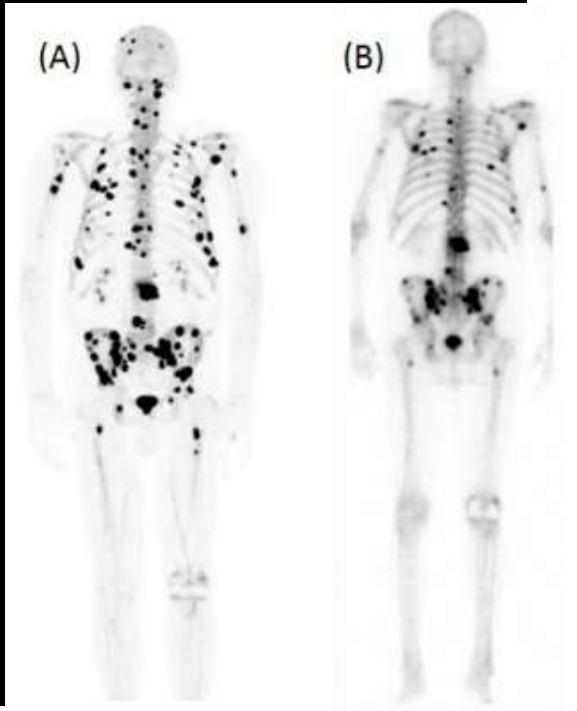
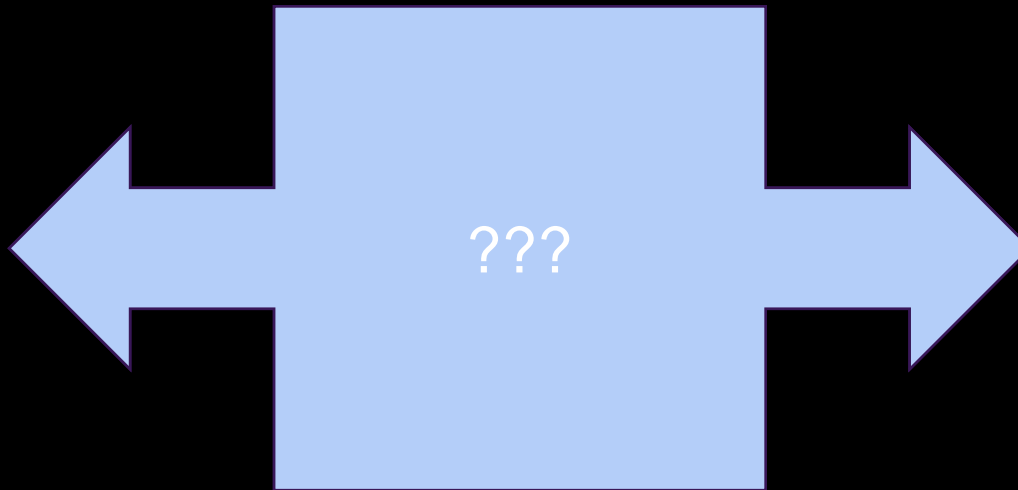
Comparative Oncology

2

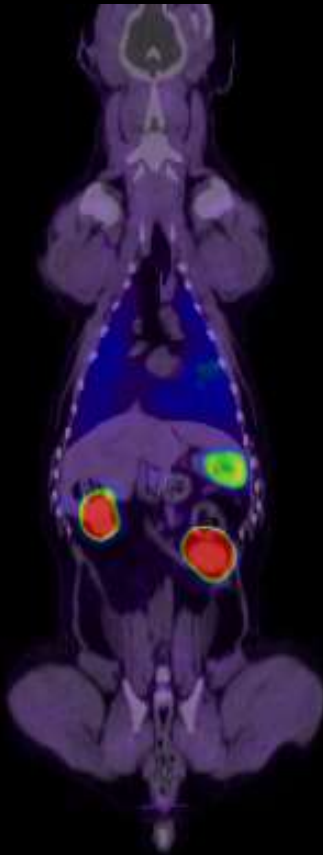
Therapeutic Profile

Canine

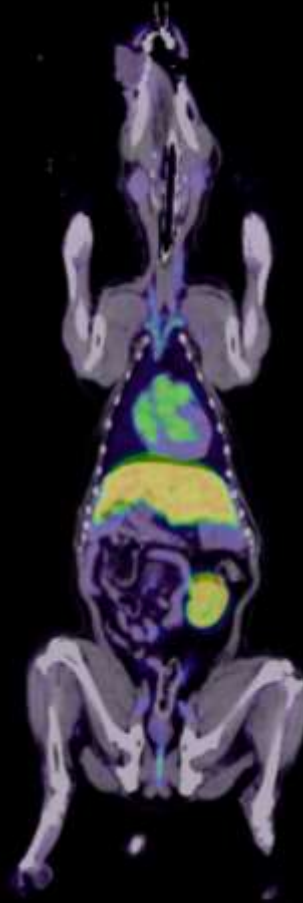
Human



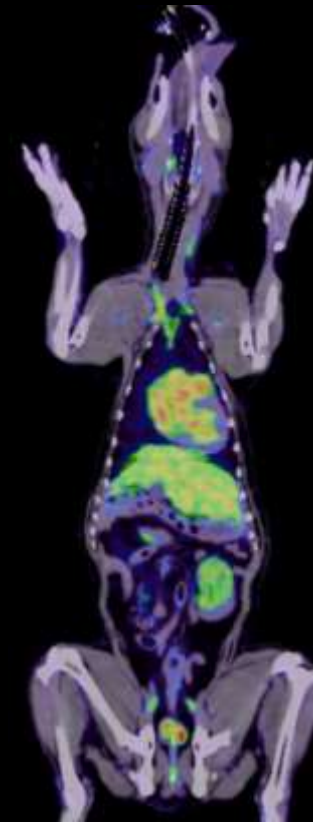
Clinical
Standard



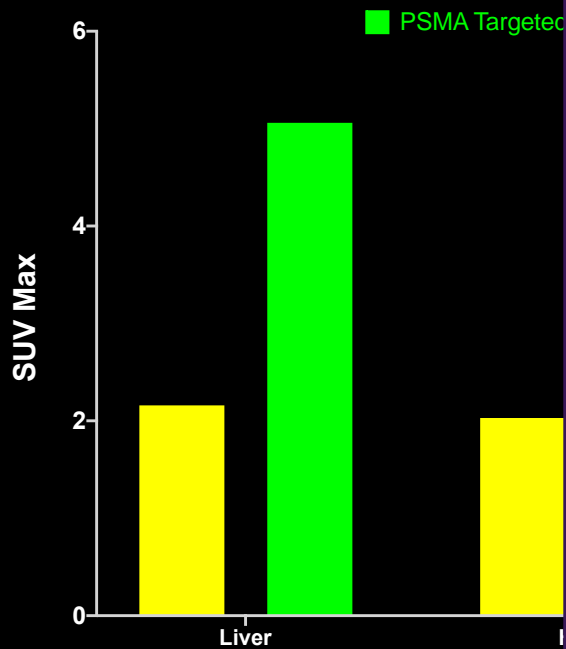
Nanomedicine with
Clinical Target



Personalised
Nanomedicine



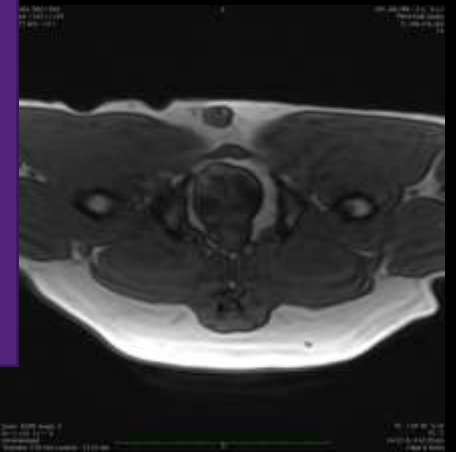
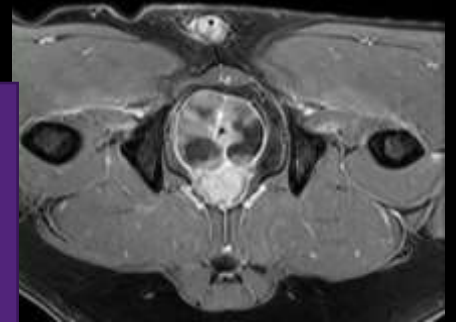
Biodistribution and Tumour Accumulation for Hoover
24h post injection



Prostate Cancer Tumour:Liver Ratio



Cross-species dosimetry provides a degree of prediction for human dose. Canine comparative oncology offers a unique system to validate and test safety and efficacy.



Acknowledgements

QLD Uni T
Prof

Uni of Queensland

Imaging

Assoc. Prof Idriss Blak

Dr Barbara Rolfe

Dr Zach Houston

Dr Nick Fletche

Dr Craig Bell

Dr James Hur

Dr Gayathri

Drug Deliver

Dr Chris Howa

Ms Amber Prio

Dr Amal Sivara

Dr Arun Balaji

Clinical

Mark Pinkham (PAH)

Dr Sunil Lakhani (RBW)

Rod Straw (BVSC)

Facilities

NIF, ANFF, NBF, TIA



in Australia
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