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CREATE CHANGE

Pre-targeting approaches for polymeric nanomedicine alpha therapeutics

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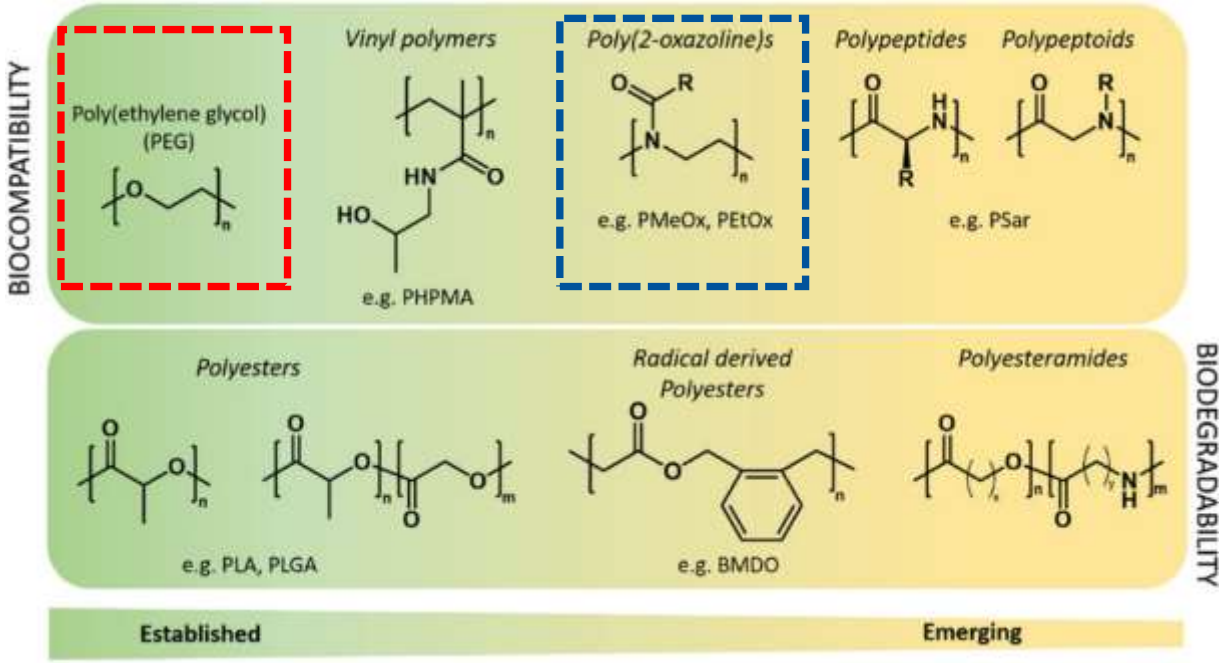
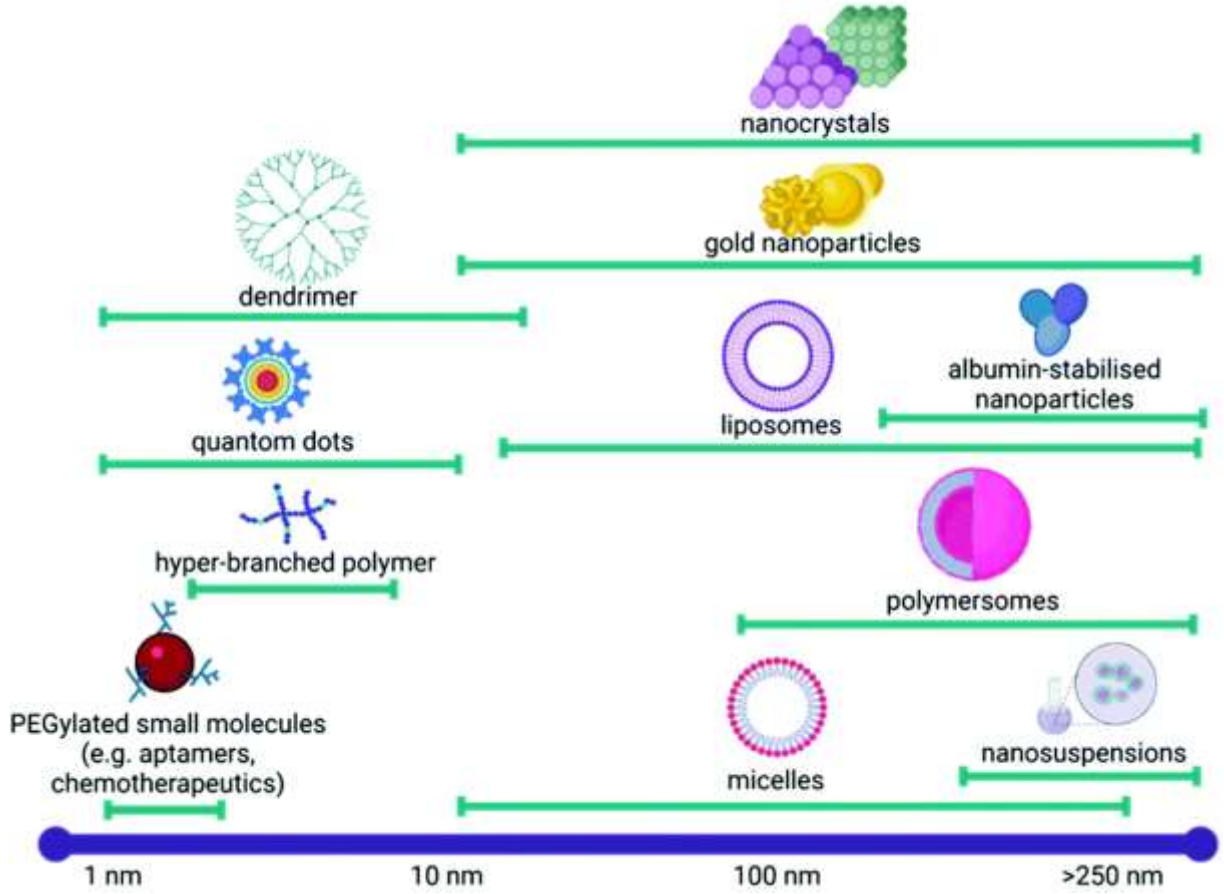
Nanomaterials for medicine

- Enormous efforts to material engineering
- Nearly ubiquitous approach is to produce from or coat with intelligent/“stealthy” polymers
- Reduce fouling and biological interactions

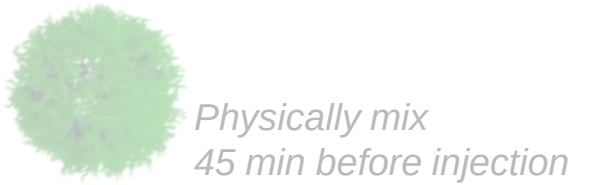




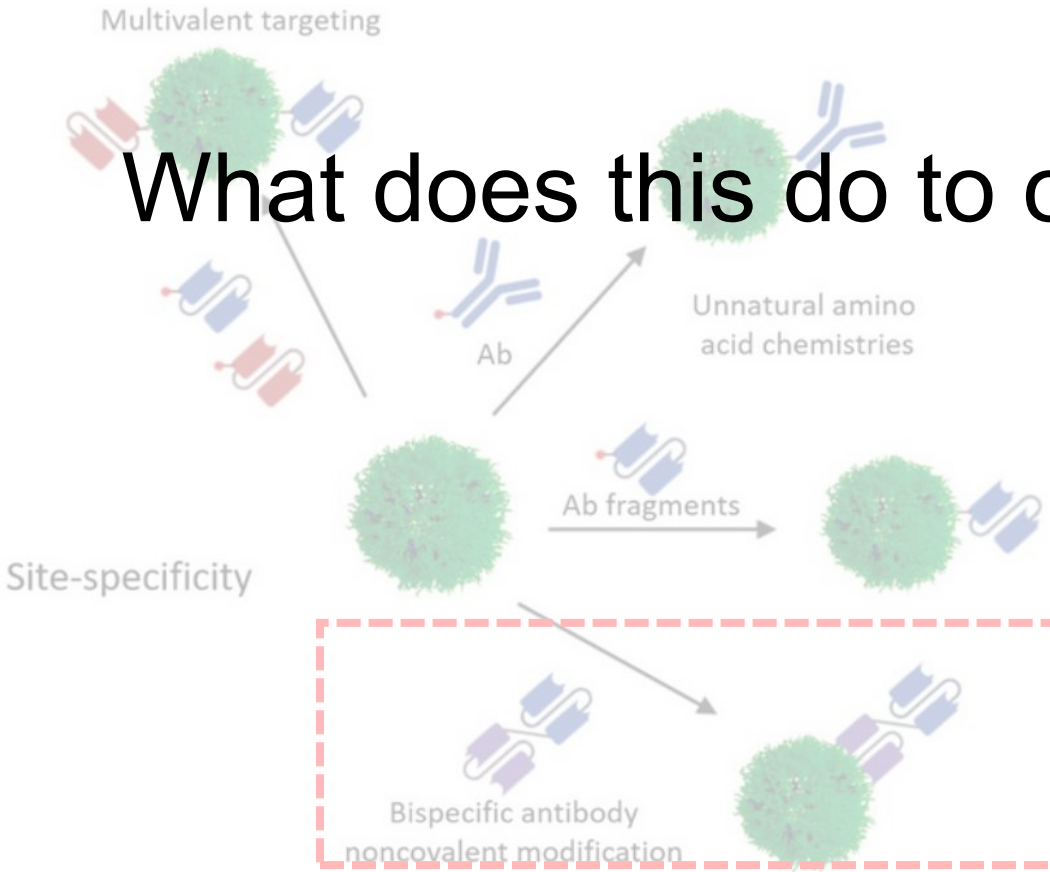
Mills, J., Liu, F., Jarrett, T., Fletcher, N., Thurecht, K., *Biomater. Sci.*, 2022
Fletcher, N. L., Kempe, K., Thurecht, K. J., *Macromol. Rapid Commun.* 2020

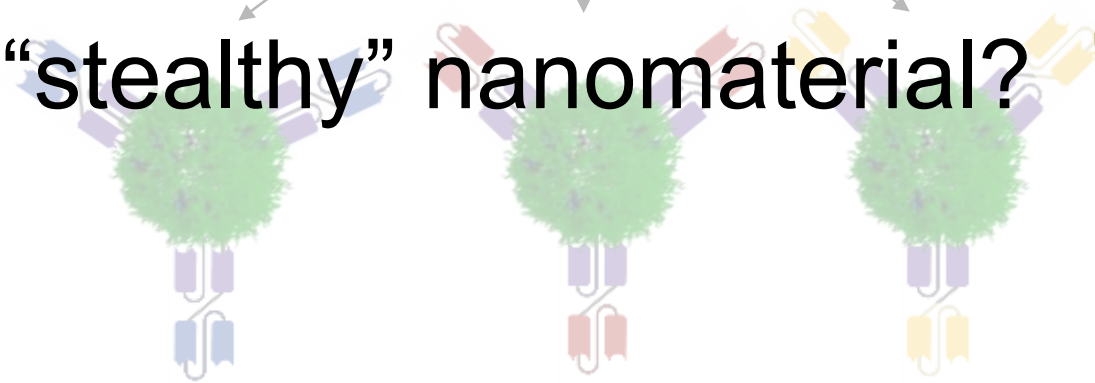
Nanomaterial targeting

- Desire nanomaterial selectivity (therapy, diagnostic etc)
- Attachment of targeting ligands – most commonly antibody fragments



Physically mix
45 min before injection





What does this do to our nice “stealthy” nanomaterial?

Well studied (imaging and therapy)
Ease of conjugation
Simple to vary targets
Minimal nanomaterial engineering

What about our “stealthy” nanomaterial?

PEG-based NP

We do a lot of imaging – picture worth 1000 words

[^{89}Zr]HBP

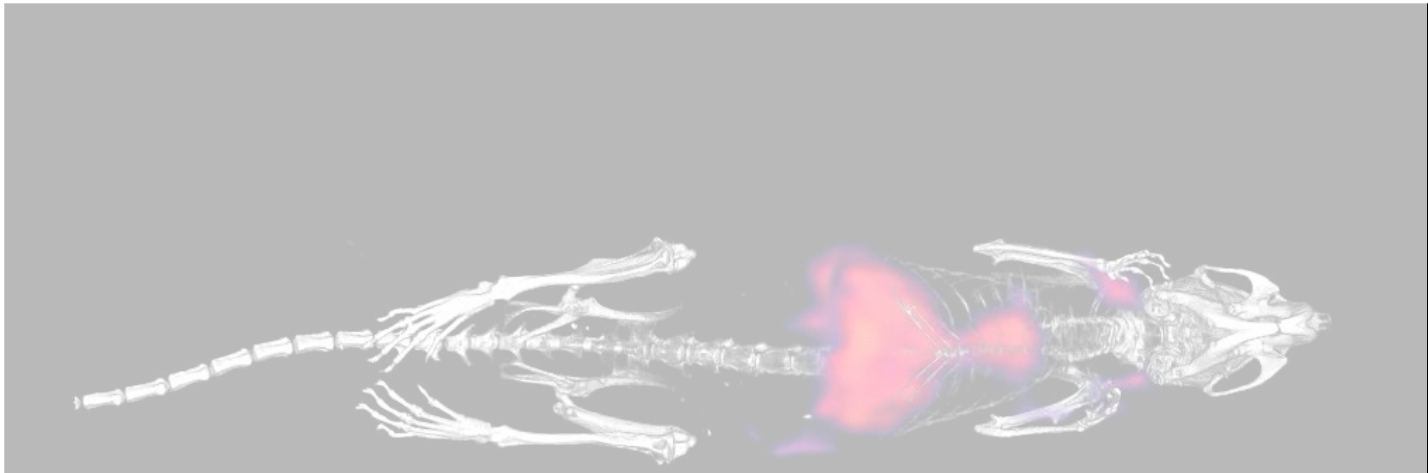
3 days

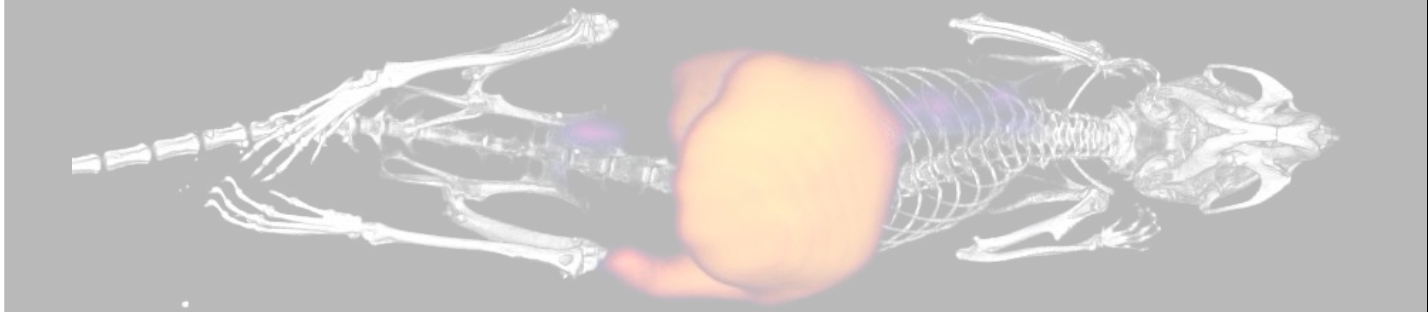
^{89}Zr PET imaging isotope

Is there another way?

[^{89}Zr]HBP-BsAb

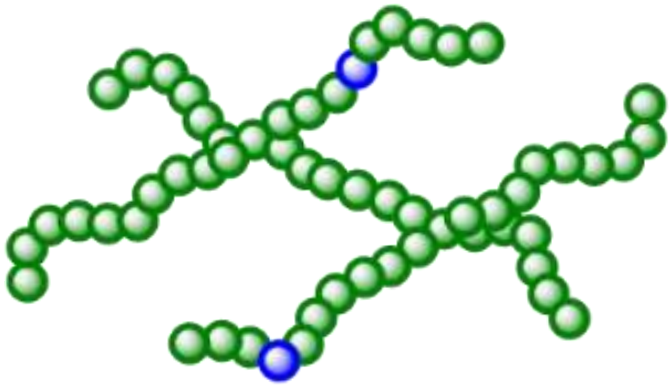
3 days

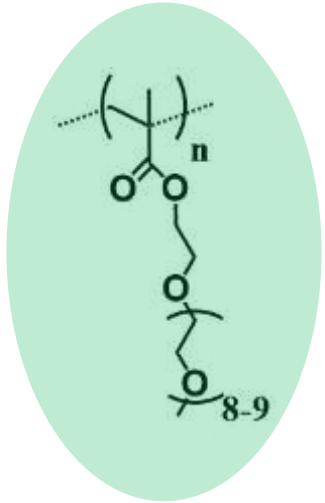




Hyperbranched Polymers (HBP) and targeting

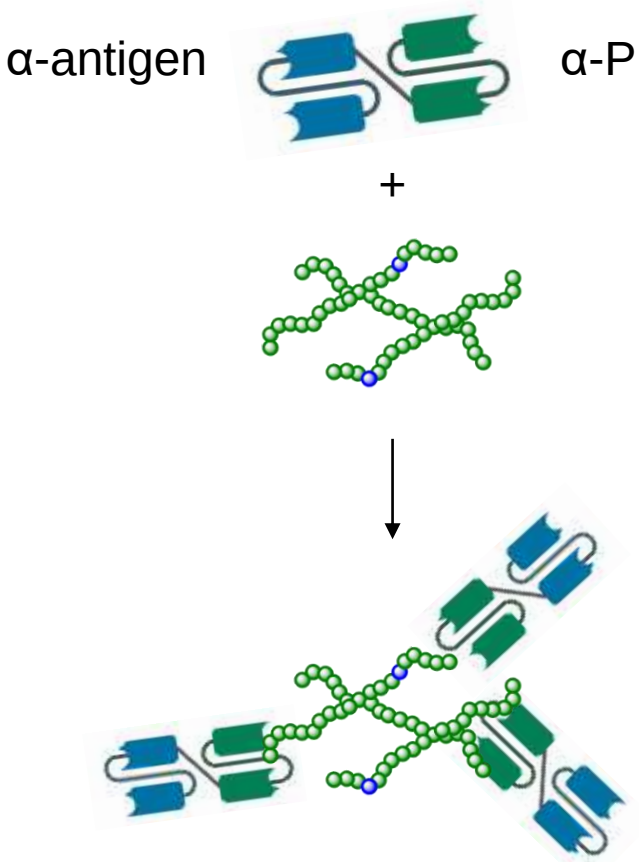
- PEG based Hyperbranched Polymers
- Biocompatible and engineerable
- RAFT polymerization of range of monomers
- Multifunctional
  - Imaging (Fluorescence, PET, MRI)
  - Therapy (Chemotherapy, biologics)





5-10 nm

Bispecific Antibody (BsAb) Targeting



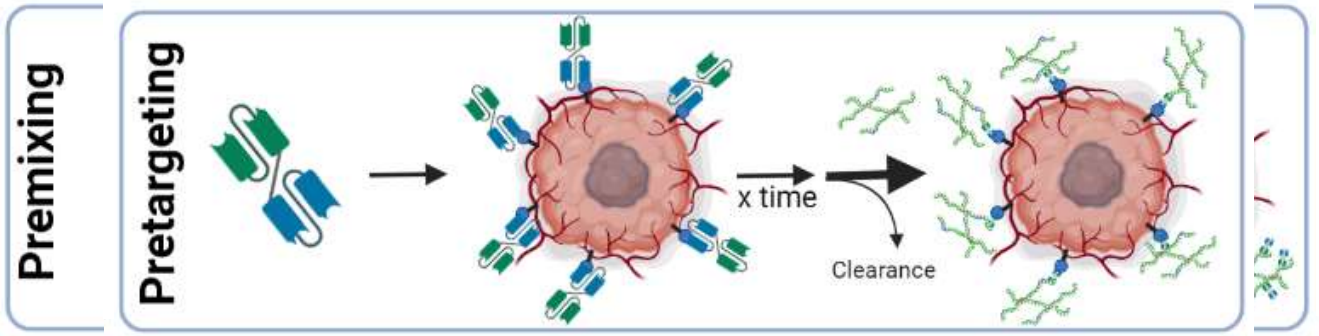
Targeted nanomedicine

Alternate strategy to control nanomaterial clearance

Protein coated
"stealthy" low-fouling material

Enhanced clearance
(largely Mononuclear Phagocytic System (MPS))

What if we can temporally separate these components?

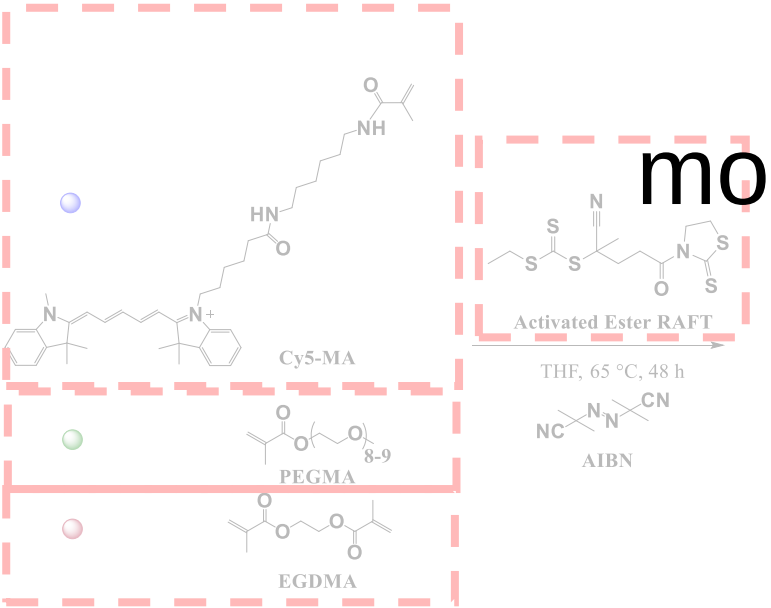


Does it work?

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

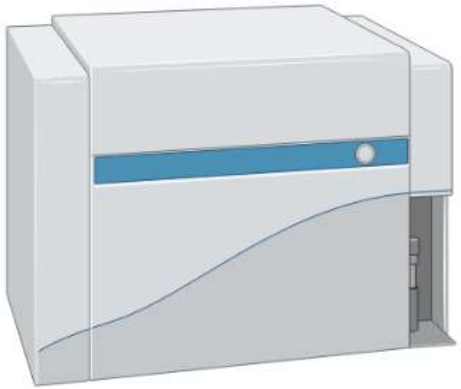
$M_{n(SEC\text{-}MALLS)}$ [a]	$M_{n(NMR)}$ [b]	Chain ends/particle [c]	Cy5/HBP (UV-Vis)
46 kDa	11.4 kDa	4.0	0.01

[a] Molar mass of HBP. [b] Estimated chain molecular weight based on NMR integration of PEGMA relative to RAFT end groups. [c] Number of arms/HBP.

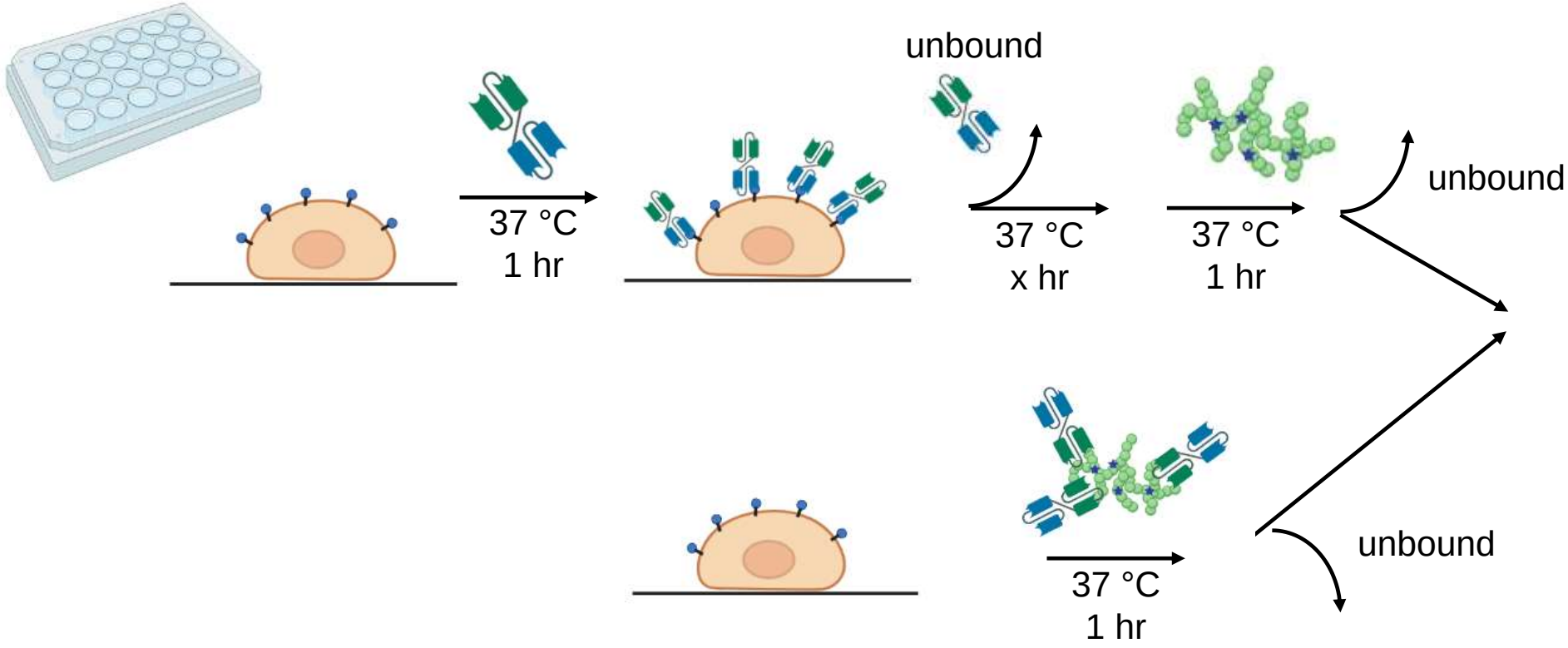


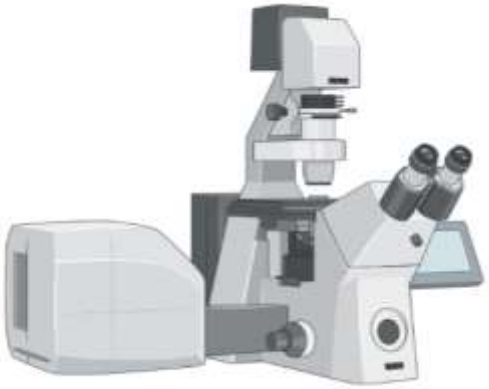
Well defined imageable model PEG-based nanomaterial

Initial *in vitro testing*



Flow Cytometry





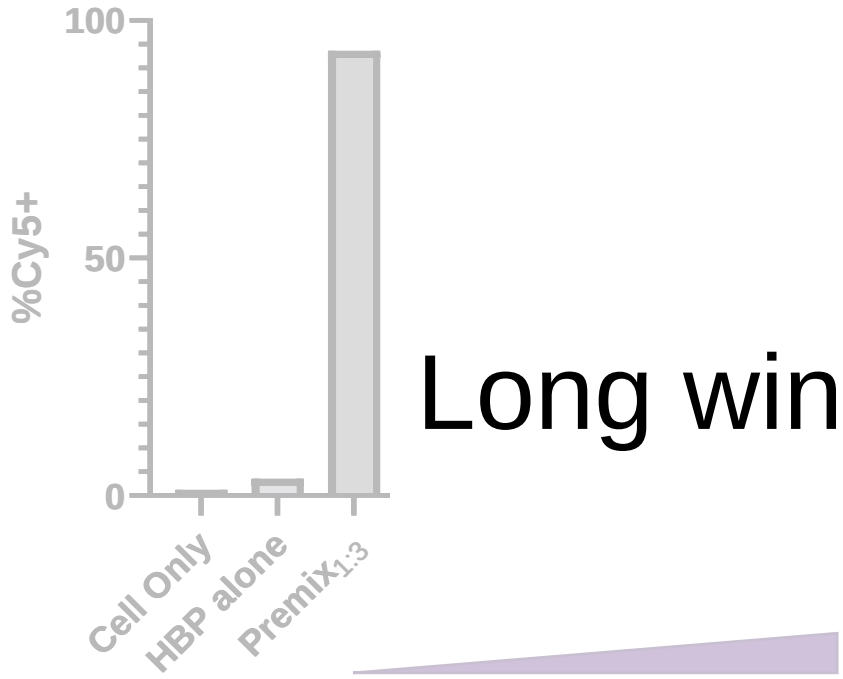
Confocal

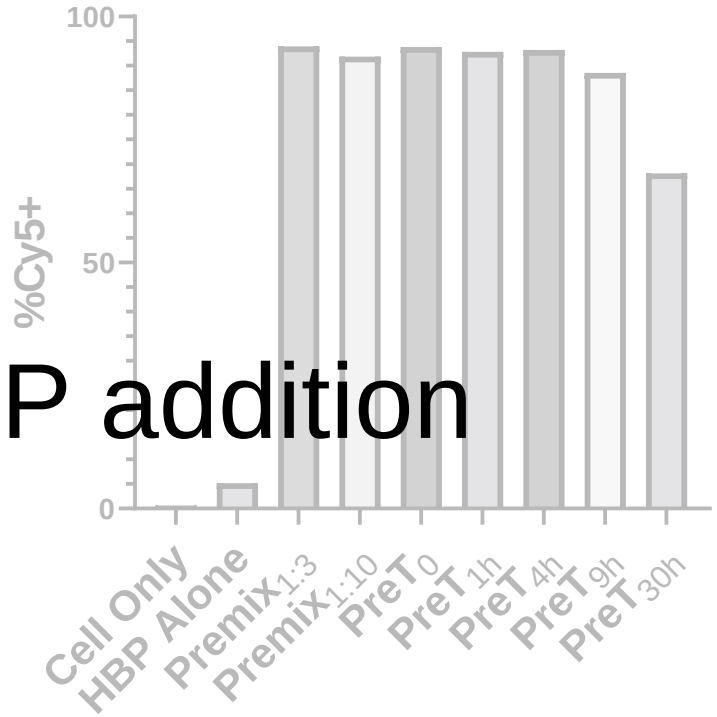
α-EGFR BsAb Cy5-HBP MDA-MB-468 (EGFR+) Breast Cancer Cells

Cy5-HBP

Does it work on cells?

Flow cytometry





Long window for HBP addition

How long does it work?

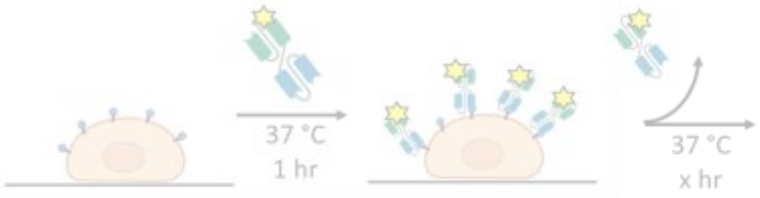
Nomenclature;

HBP pre-functionalized with BsAb ($Premix_{1:1}$)

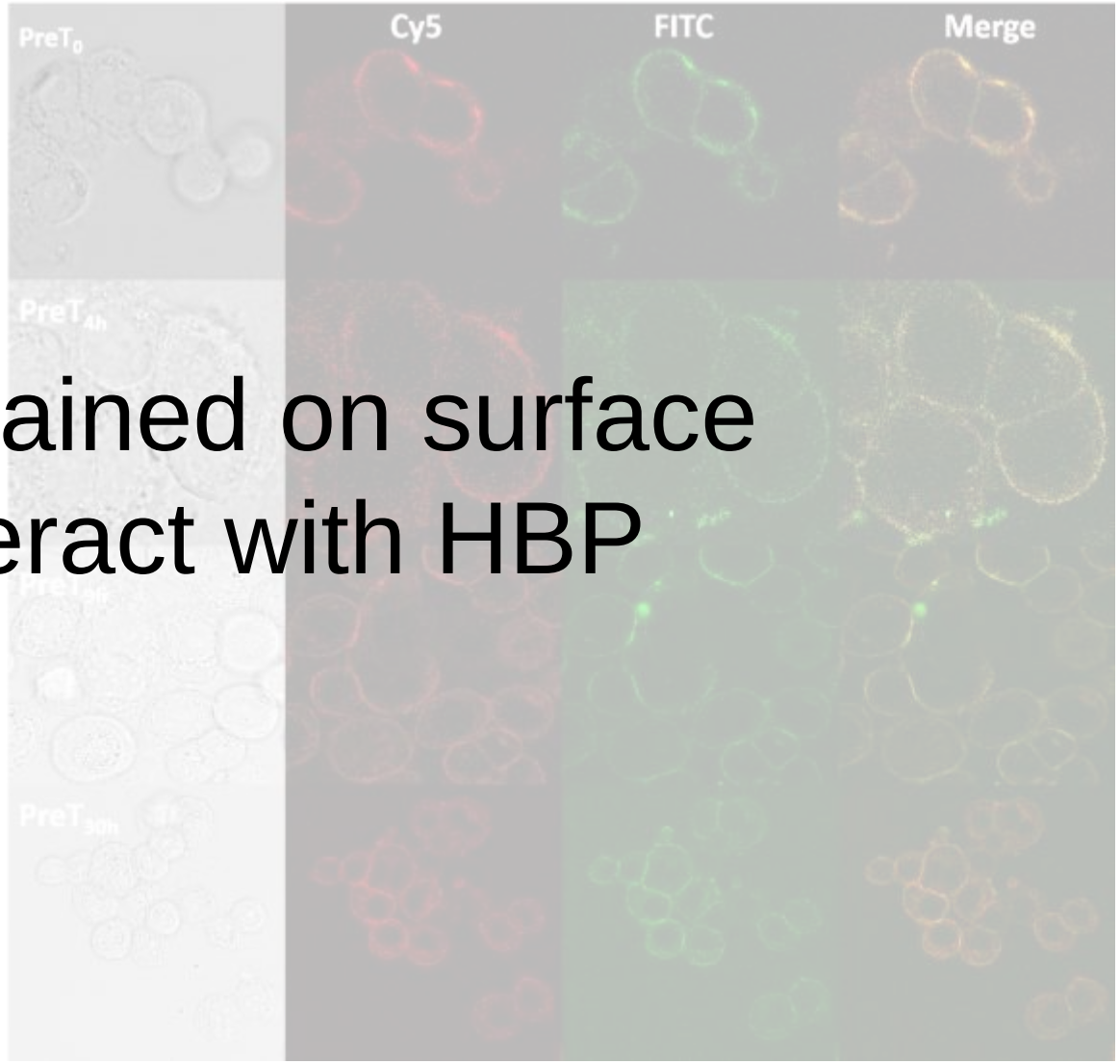
HBP pre-targeted by BsAb with excess BsAb washed off x hours before ($PreT_{xh}$)

What are the BsAb and HBP doing?

- FITC label BsAb directly

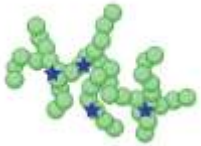


1 hr post-HBP addition



BsAb primarily retained on surface
Available to interact with HBP

Do they internalize?



Cy5 labelled

- Binding is great – but what do they do after?
- 4 hours post HBP addition

HBP alone

Premix$_{1:3}$

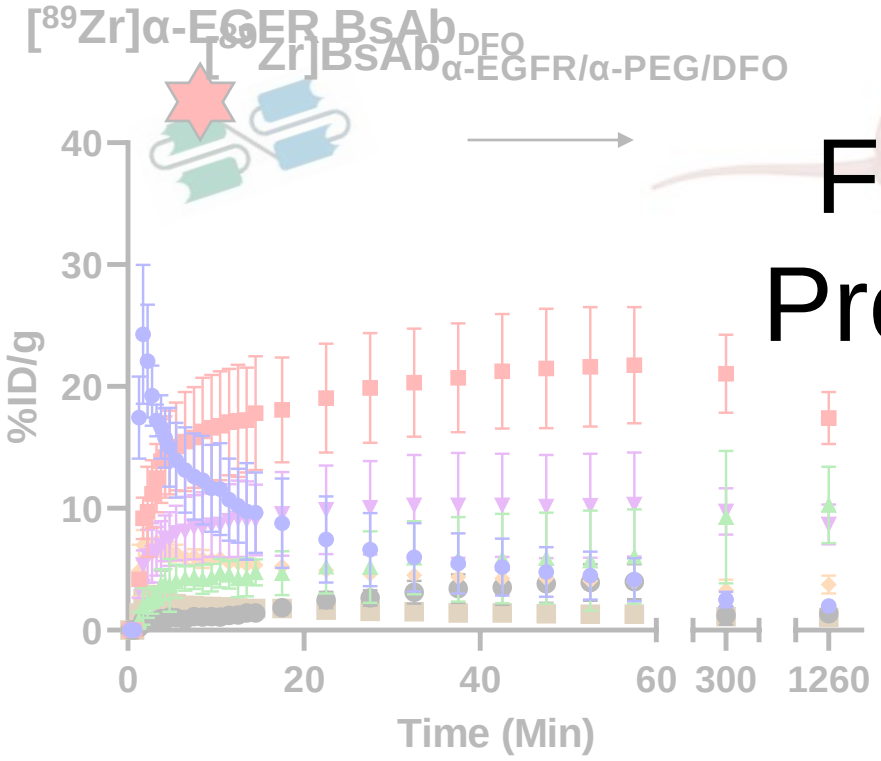
PreT$_{1h}$

Key for many potential therapeutic payloads

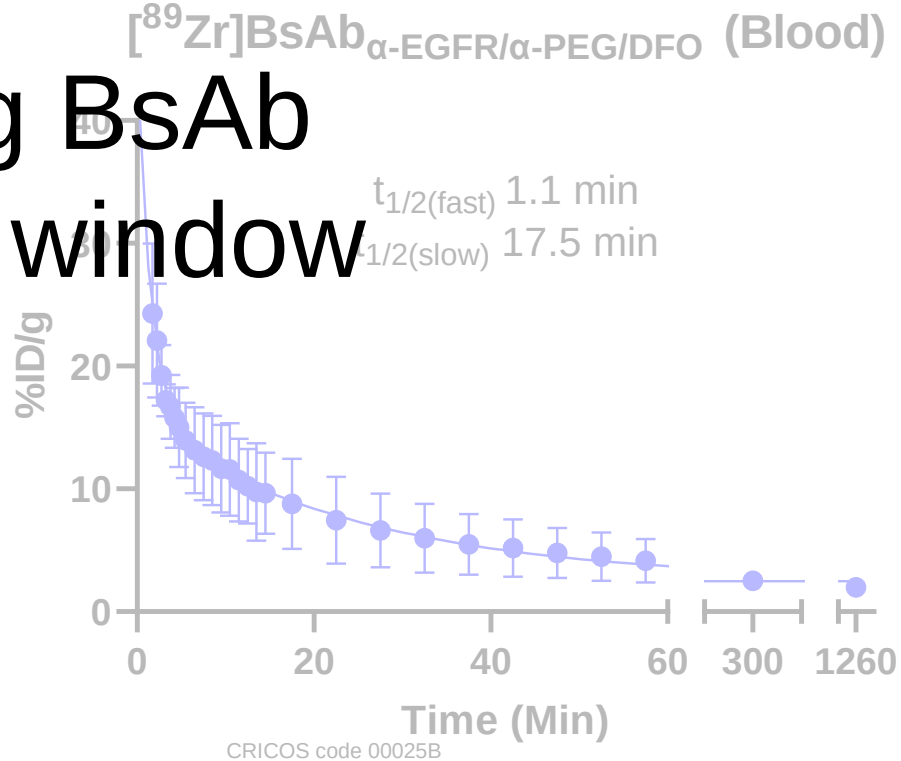
DFO label α-EGFR BsAb ($BsAb_{DFO}$)

What about in vivo?

- First – BsAb alone pharmacokinetics
- ^{89}Zr label $BsAb_{DFO}$ and inject naïve mice



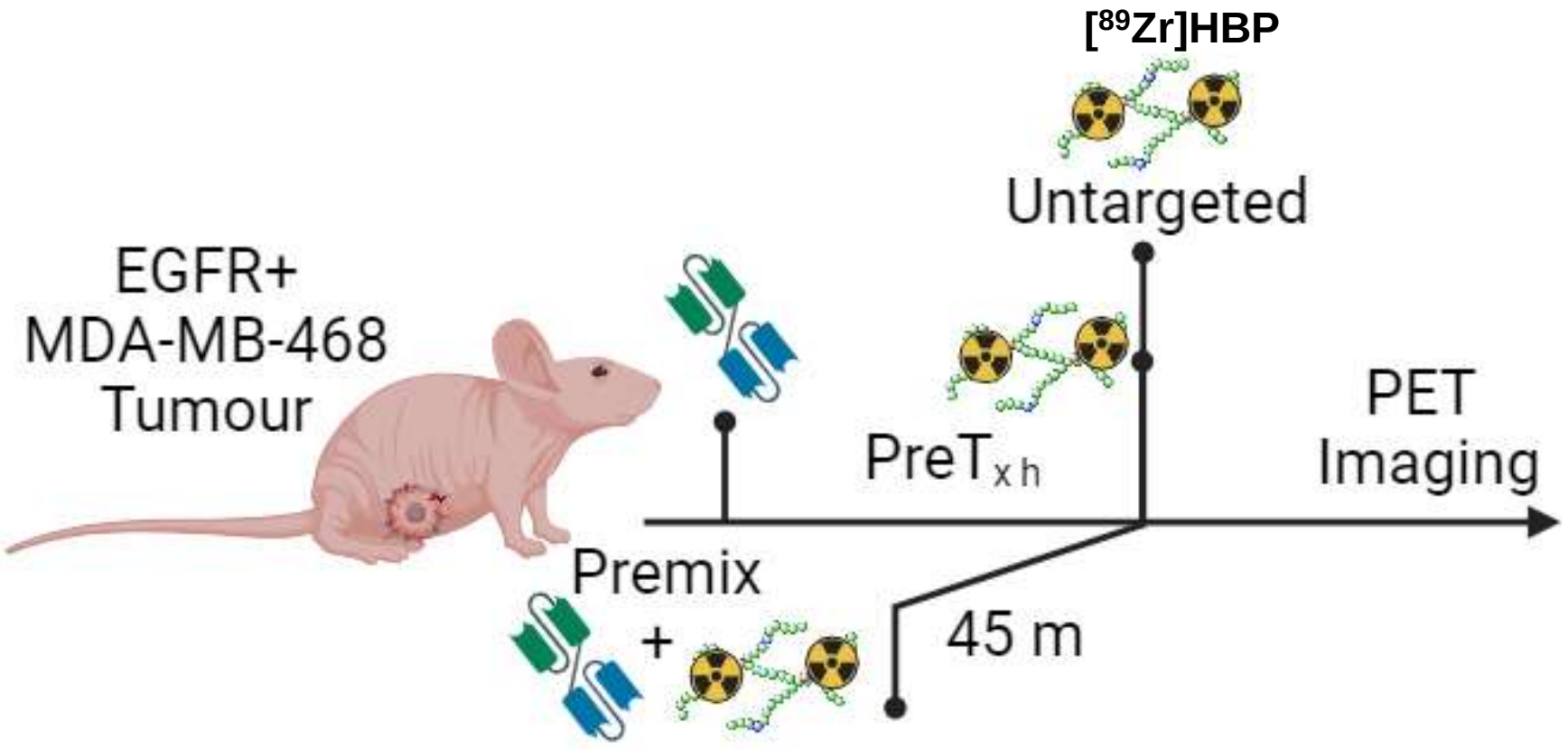
Fast clearing BsAb
Pre-targeting window



What about in vivo?

- ^{89}Zr label $HBP_{Cy5/DFO}$ for PET imaging
- Probe the effect of x hours

Tested x;
1 h (early, BsAb in circulation)
4 h (mid, BsAb clearing)
9 h (late, BsAb largely cleared)

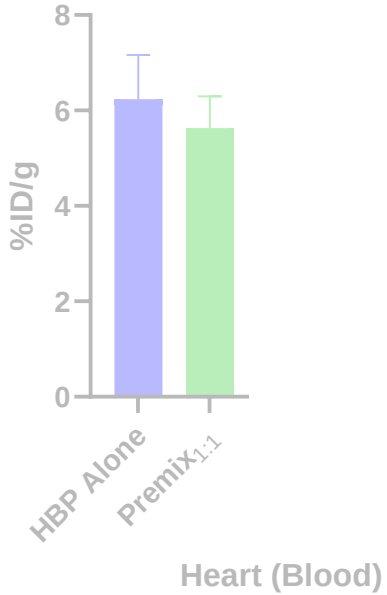


In vivo pre-targeting

- Image mice from 8 hour to 6 days post injection
- Compare HBP alone, Premixed with BsAb or pre-targeted at 1, 4 or 9 hours
- A lot of data – some interesting trends

Clearly a balancing act

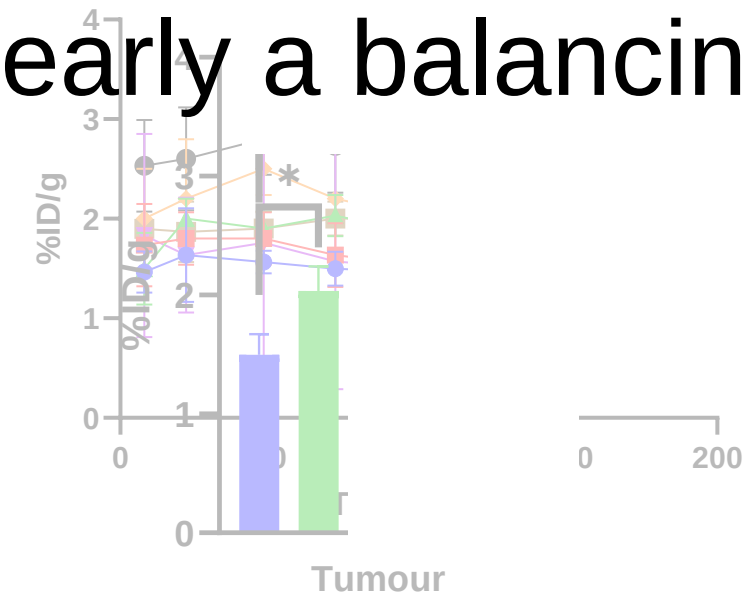
8 hours post HBP injection



Circulating BsAb enhanced clearance

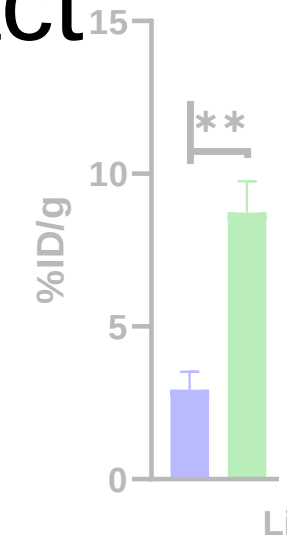
3 days post HBP injection

Tumours over time (all)



Premix + PreT >1 hr enhance tumour accumulation

3 days post HBP injection

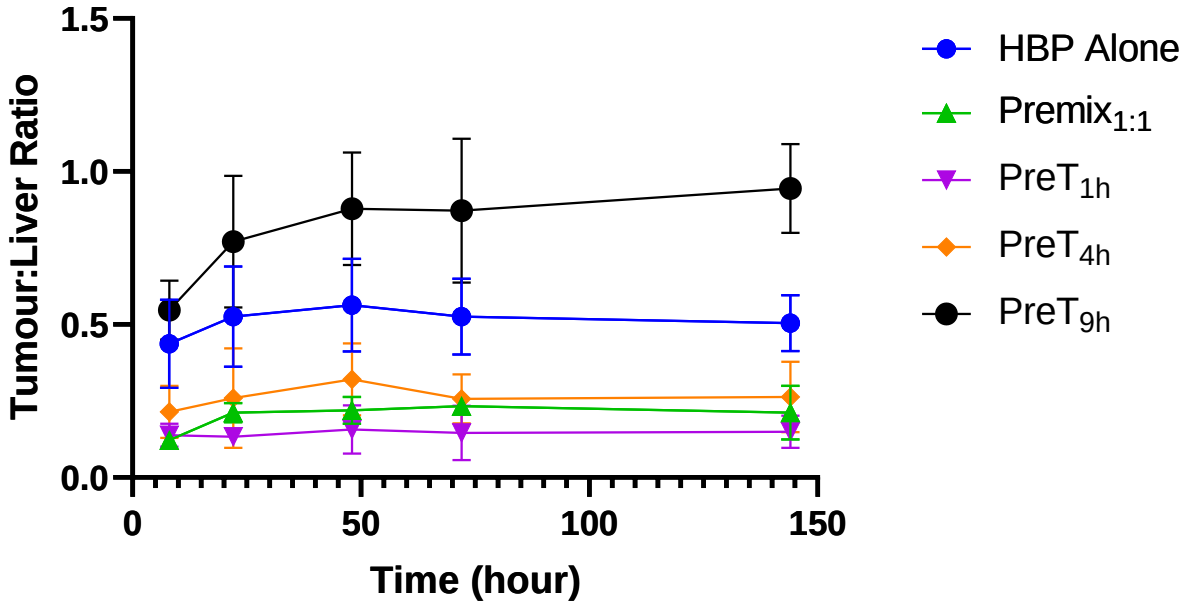


PreT >4 hr reduce liver accumulation

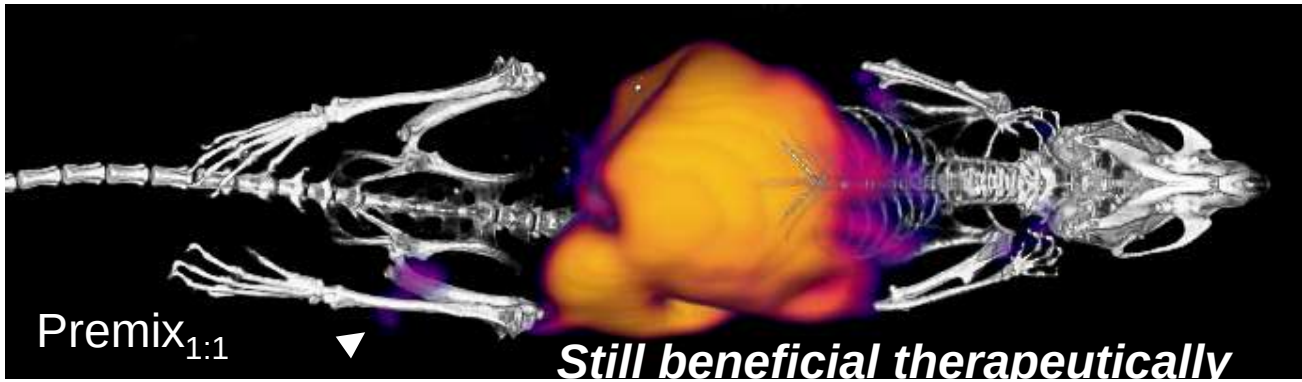
Fletcher, N. L., et al. *Chem Commun* 2022 doi: 10.1039/d2cc02443h

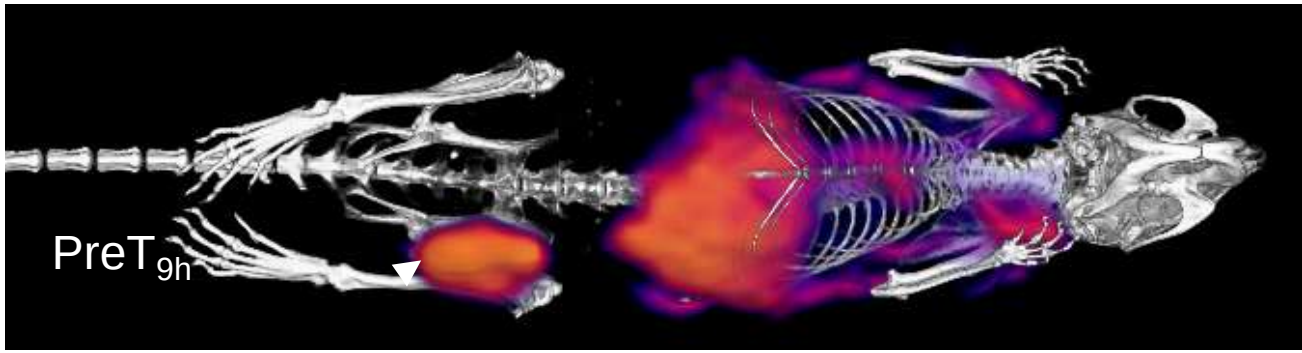
A balancing act

- Tumour:Liver ratio as a measure of target:clearance balance



What does that look like?



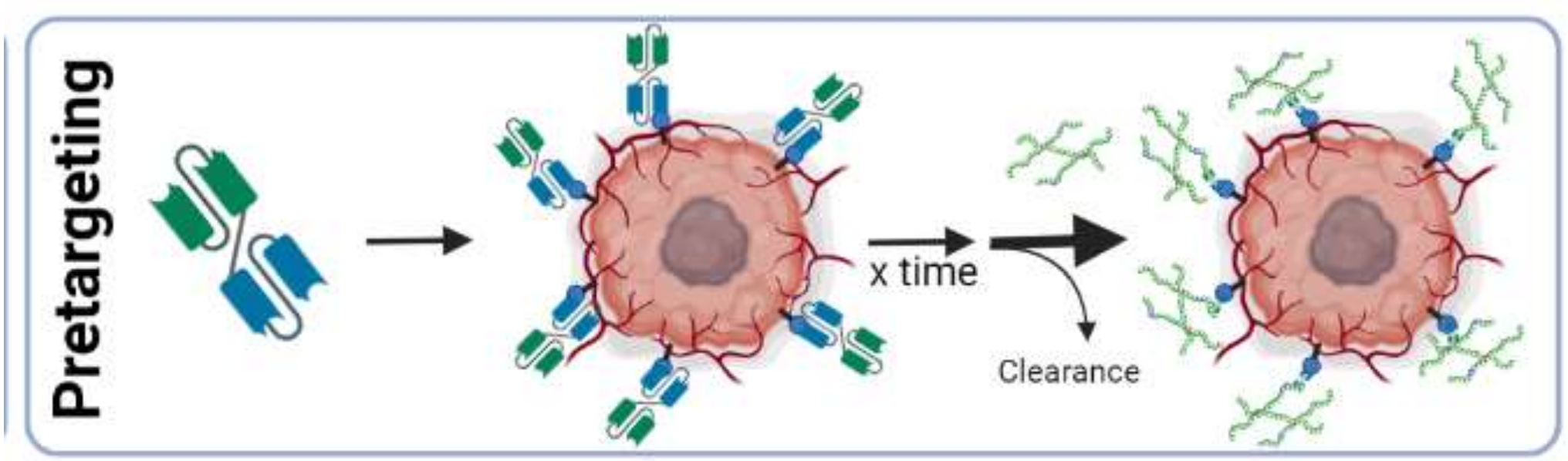


So what?

BsAb pre-targeting of PEG-based materials works

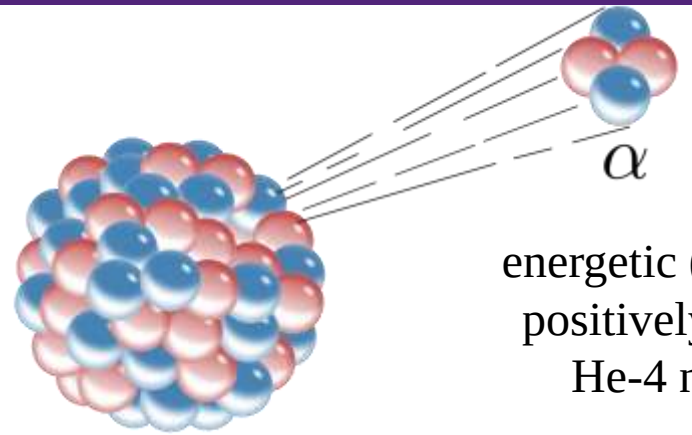
What can we do with it?

Therapy

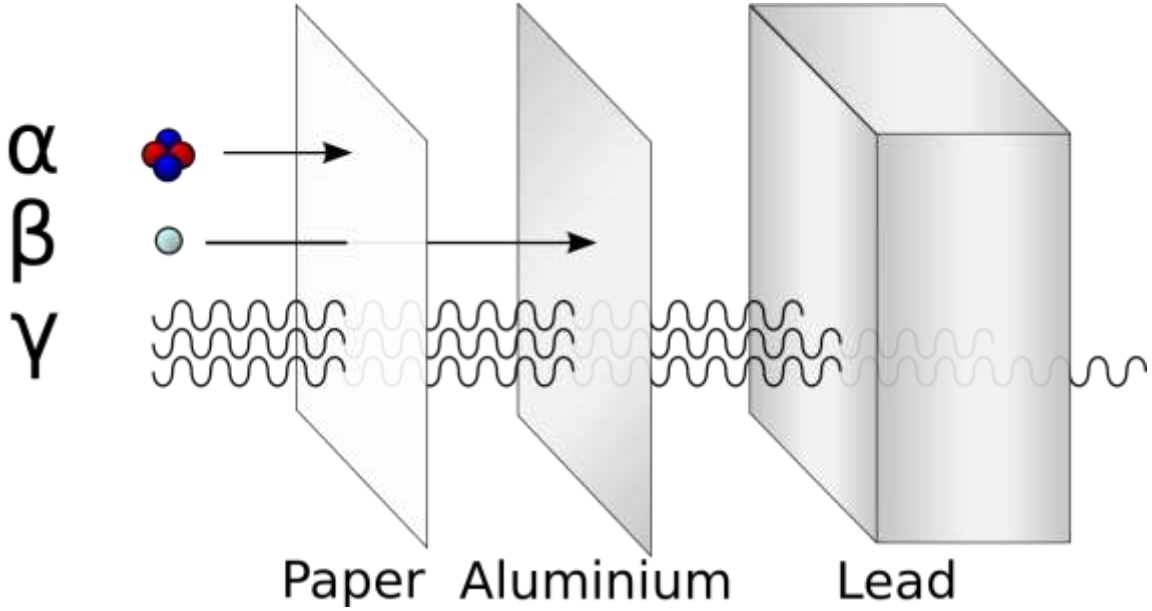


Payload Choice - Alpha therapy

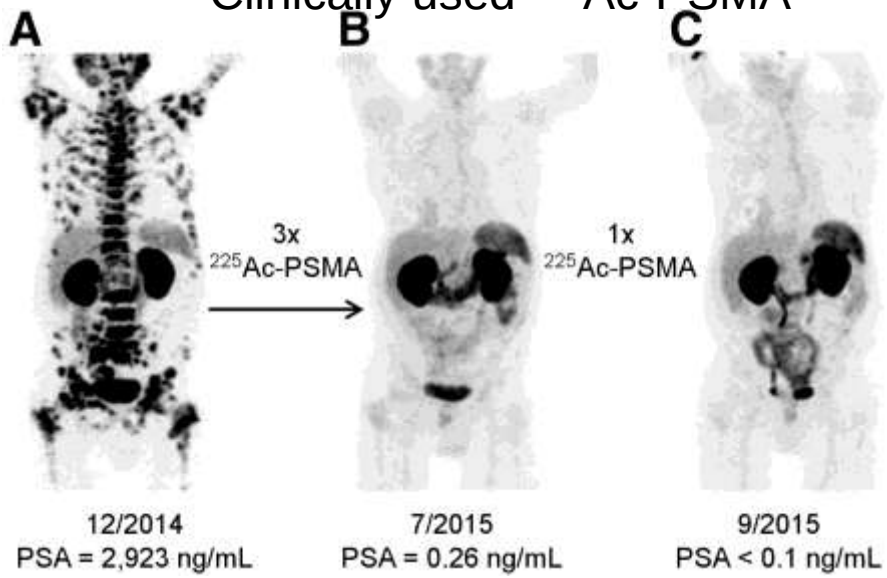
- Emerging class of molecular radiotherapy
- 100-1000 times more potent (β^- particles, e.g. ^{177}Lu)
- Limited penetration depth (<100 µm) – precision therapy
- E.g. ^{225}Ac, ^{223}Ra, ^{211}At, ^{212}Bi, ^{212}Pb



energetic (4-9 MeV) positively charged He-4 nucleus

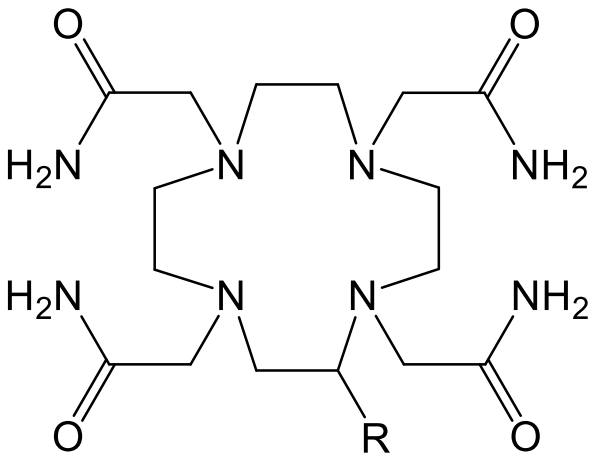


Clinically used ^{225}Ac-PSMA

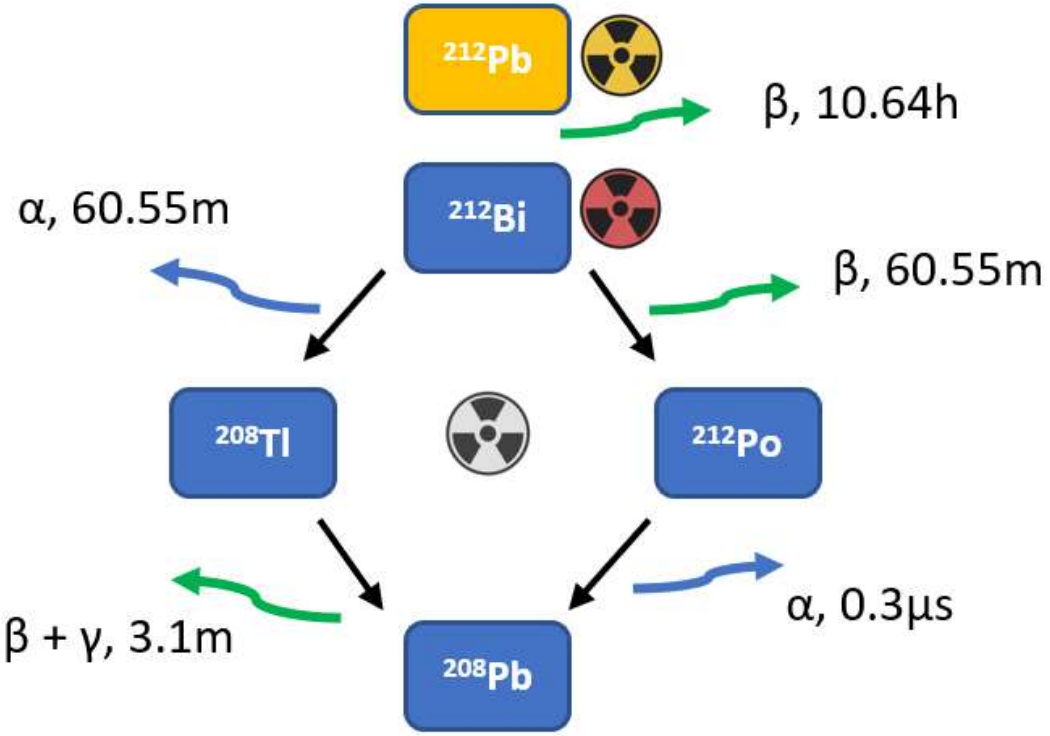


Alpha therapeutic nanomedicines and ^{212}Pb

- ^{225}Ac the most developed targeted therapy
- Long half-life, decay chain and supply make problematic
- ^{212}Pb one alternate alpha therapeutic isotope
- Established chelator chemistries

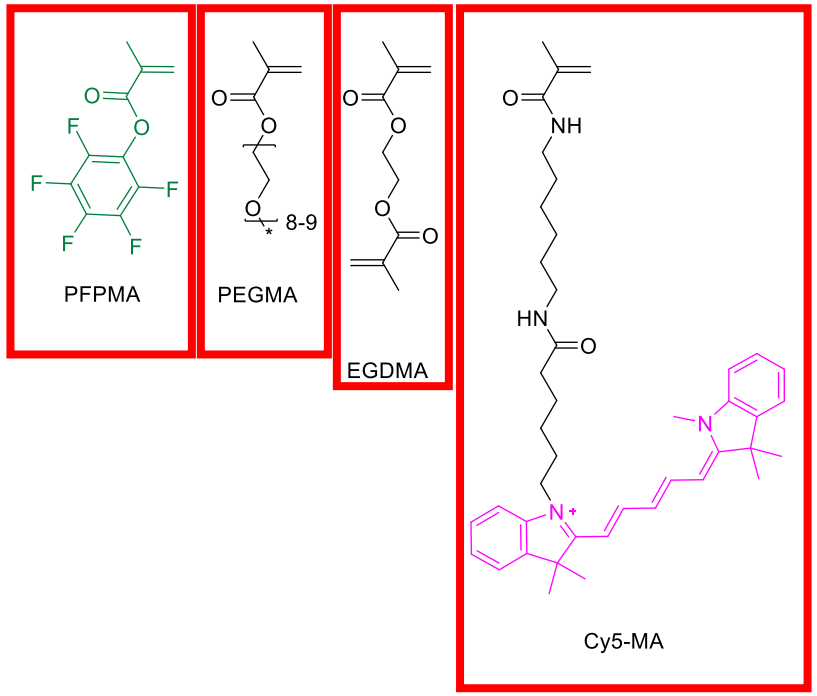


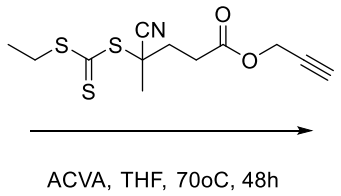
DOTAM/TCMC

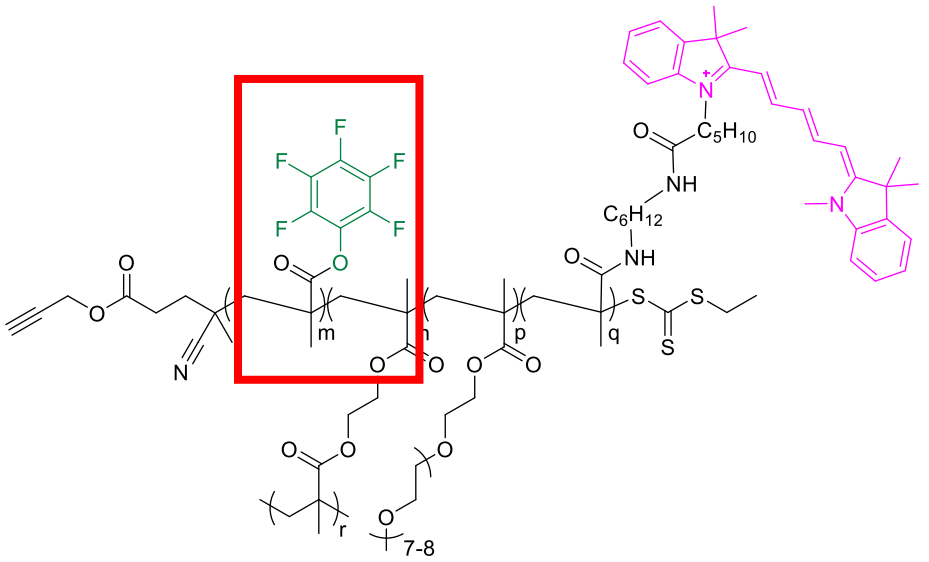


AdvanCell

Carrier synthesis





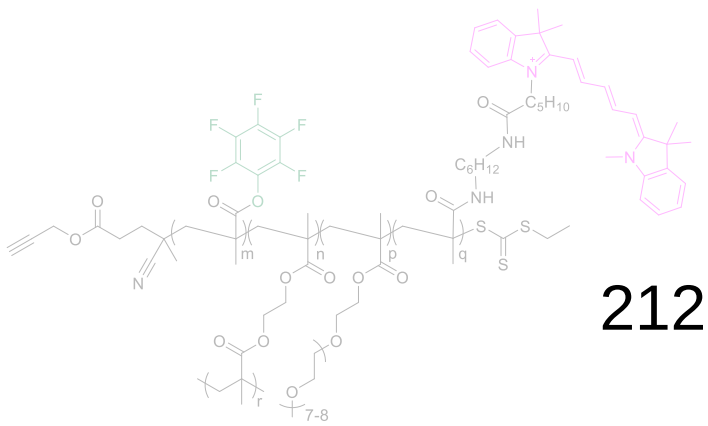


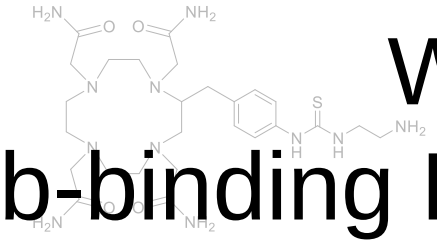
Monomer	RAFT	EGDMA (brancher)	ACVA
100	5	6.5	1

M_n ^{1}H NMR	M_n($Đ_M$) GPC-MALLS	Branches	Cy5/polymer
8.67 kDa	53.74 kDa (1.53)	6	1/20

^{212}Pb carrying HBP

Amenable to functionalization with any NH_2-Chelator

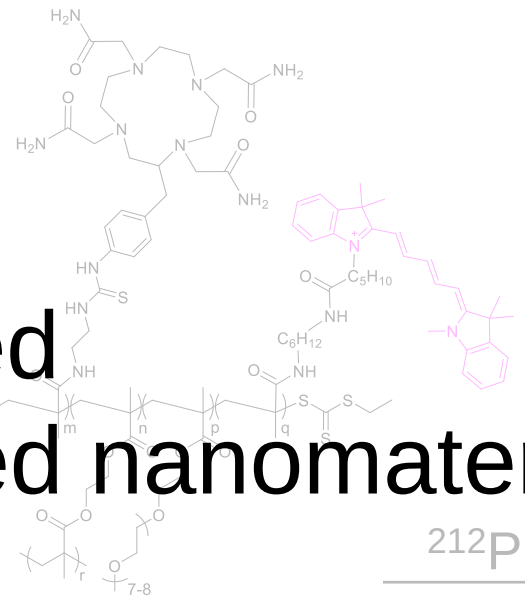




TCMC-NH_2

Well defined

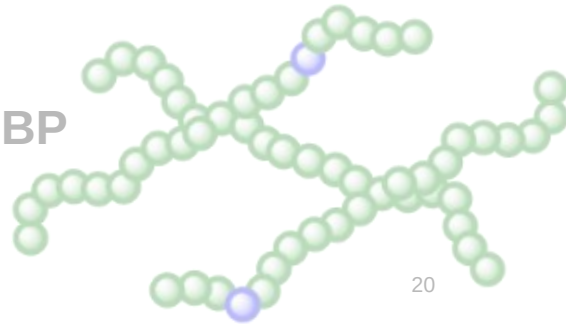
^{212}Pb-binding PEG-based nanomaterial



HBP-TCMC

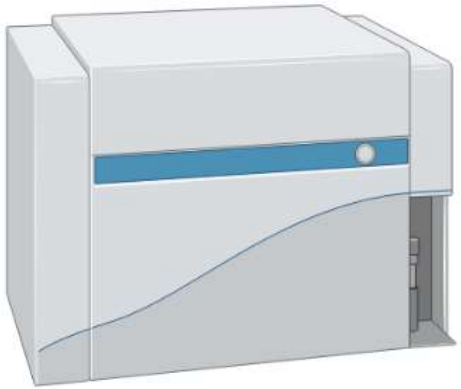
^{212}Pb

pH 5 NH_4OAc

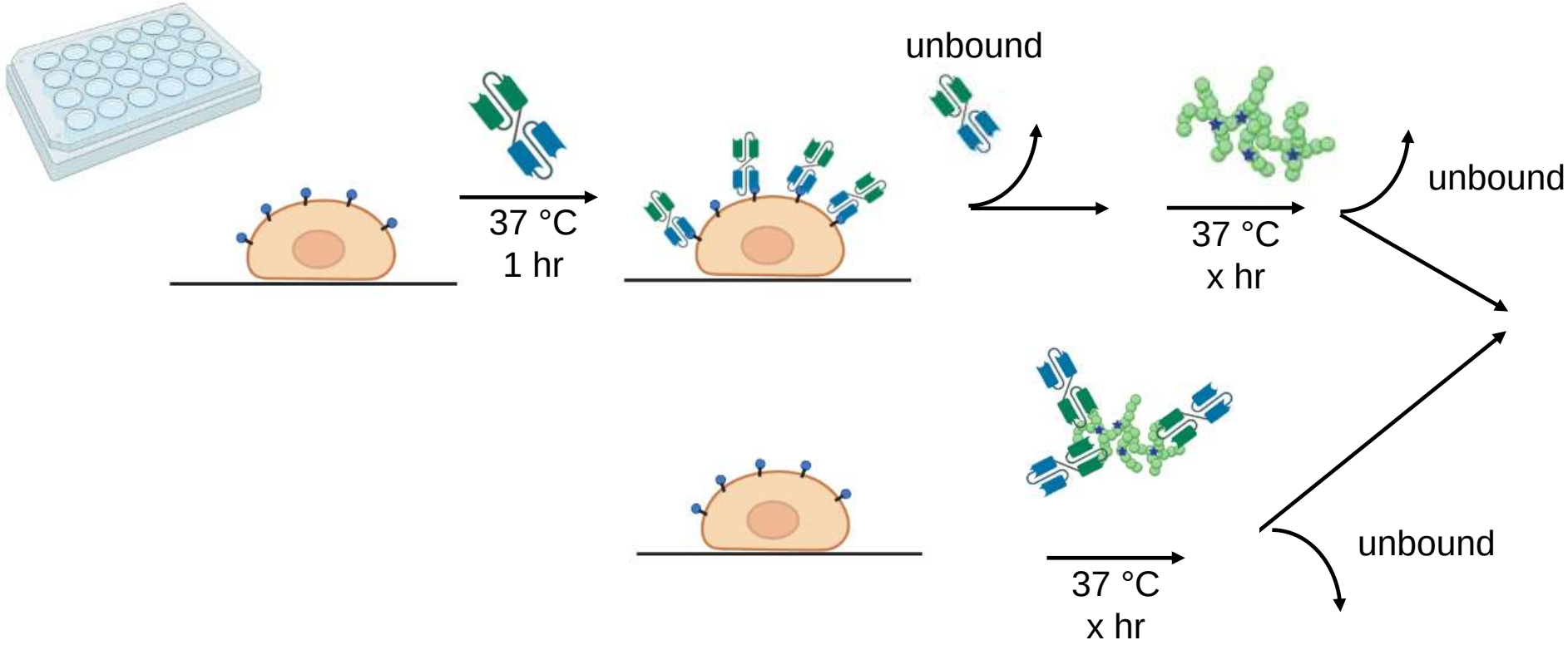


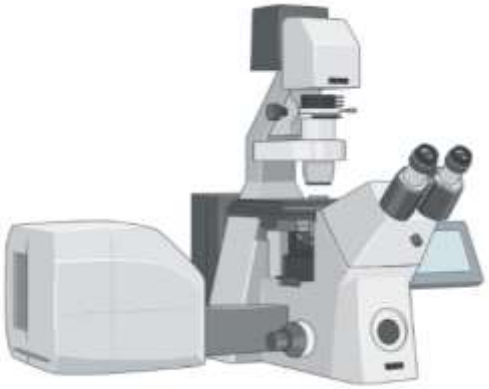
[^{212}Pb]HBP

Initial *in vitro testing*



Flow Cytometry





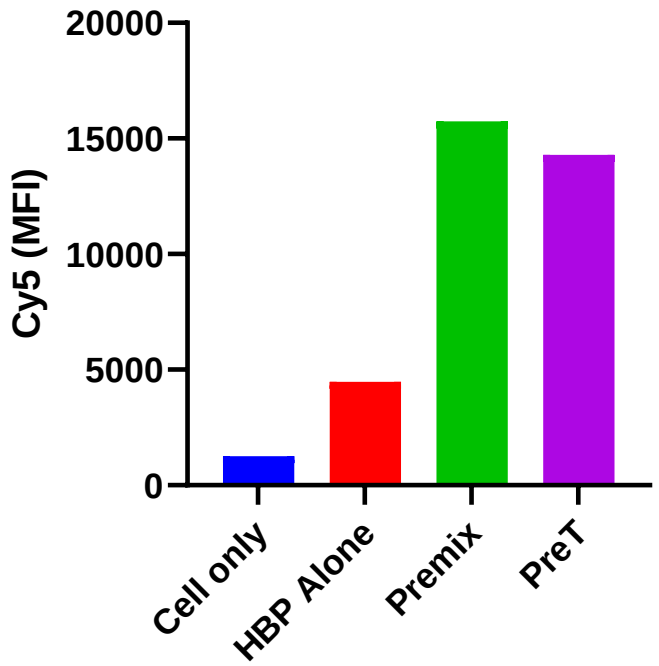
Confocal

α-EGFR BsAb Cy5-HBP MDA-MB-468 (EGFR+) Breast Cancer Cells

In vitro pre-targeting

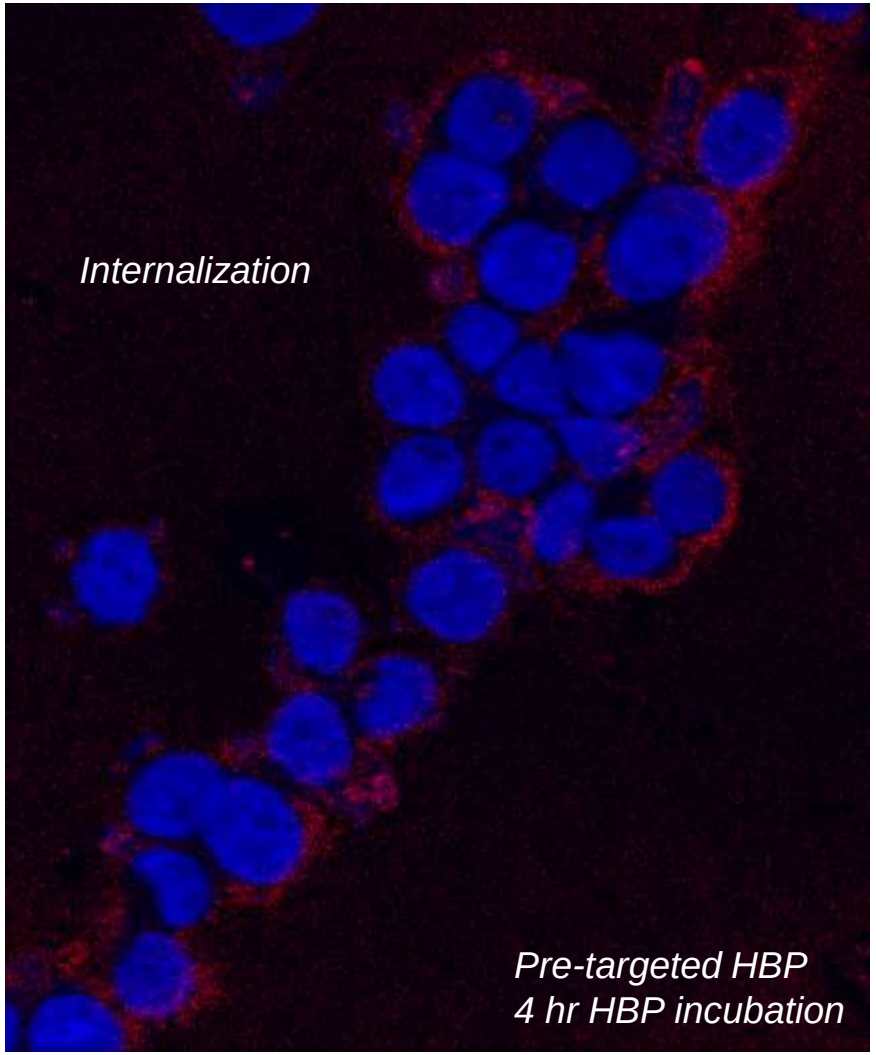
- Initial fluorescence assays

Flow Cytometry

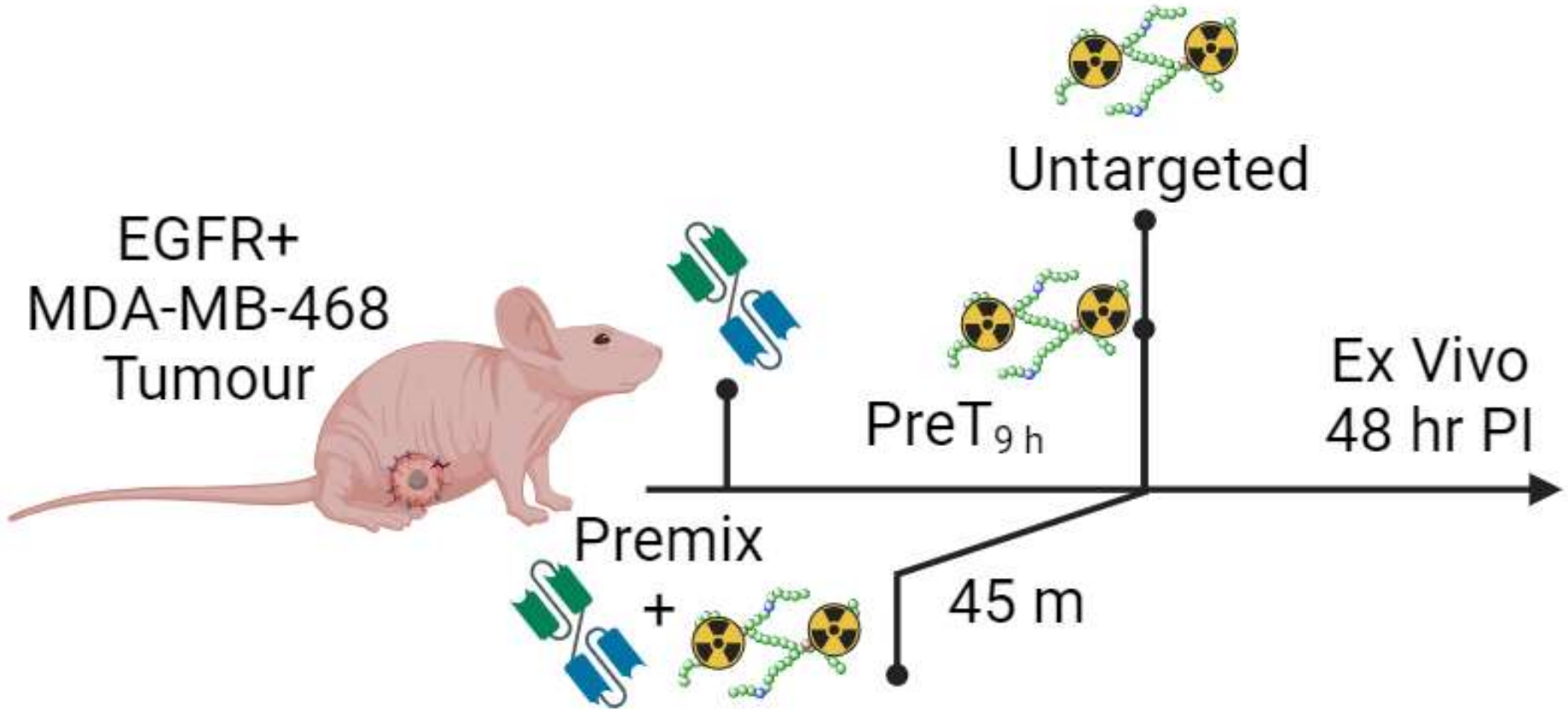


1 hr HBP incubation

Confocal Microscopy



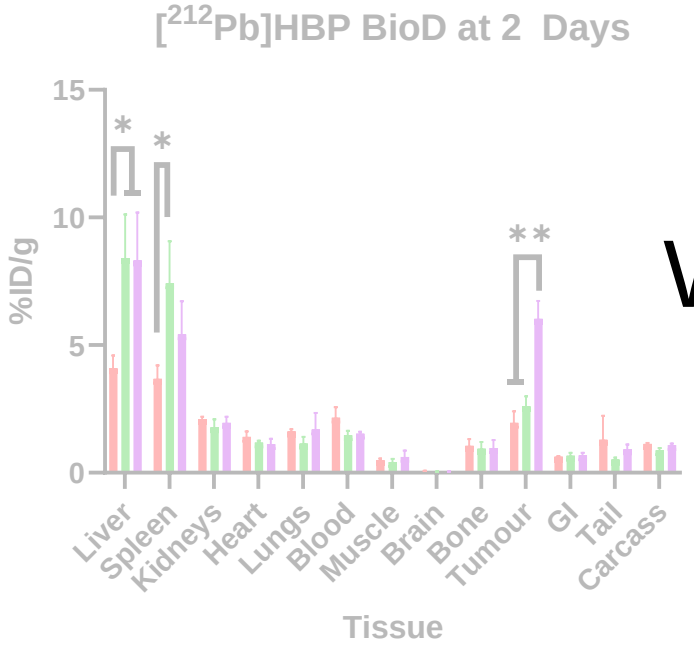
Pre-targeting [^{212}Pb]HBP



1 MBq ^{212}Pb/mouse – Therapeutic Dose

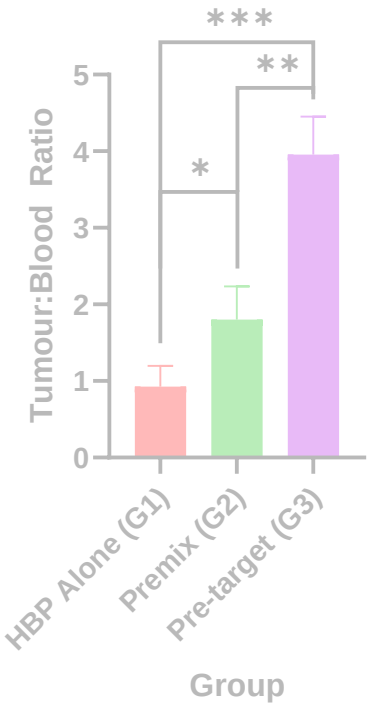
Pre-targeting [^{212}Pb]HBP

Hematological toxicity
Often dose limiting
In molecular radiotherapy



What does that look like?

>3-fold increase in tumour uptake



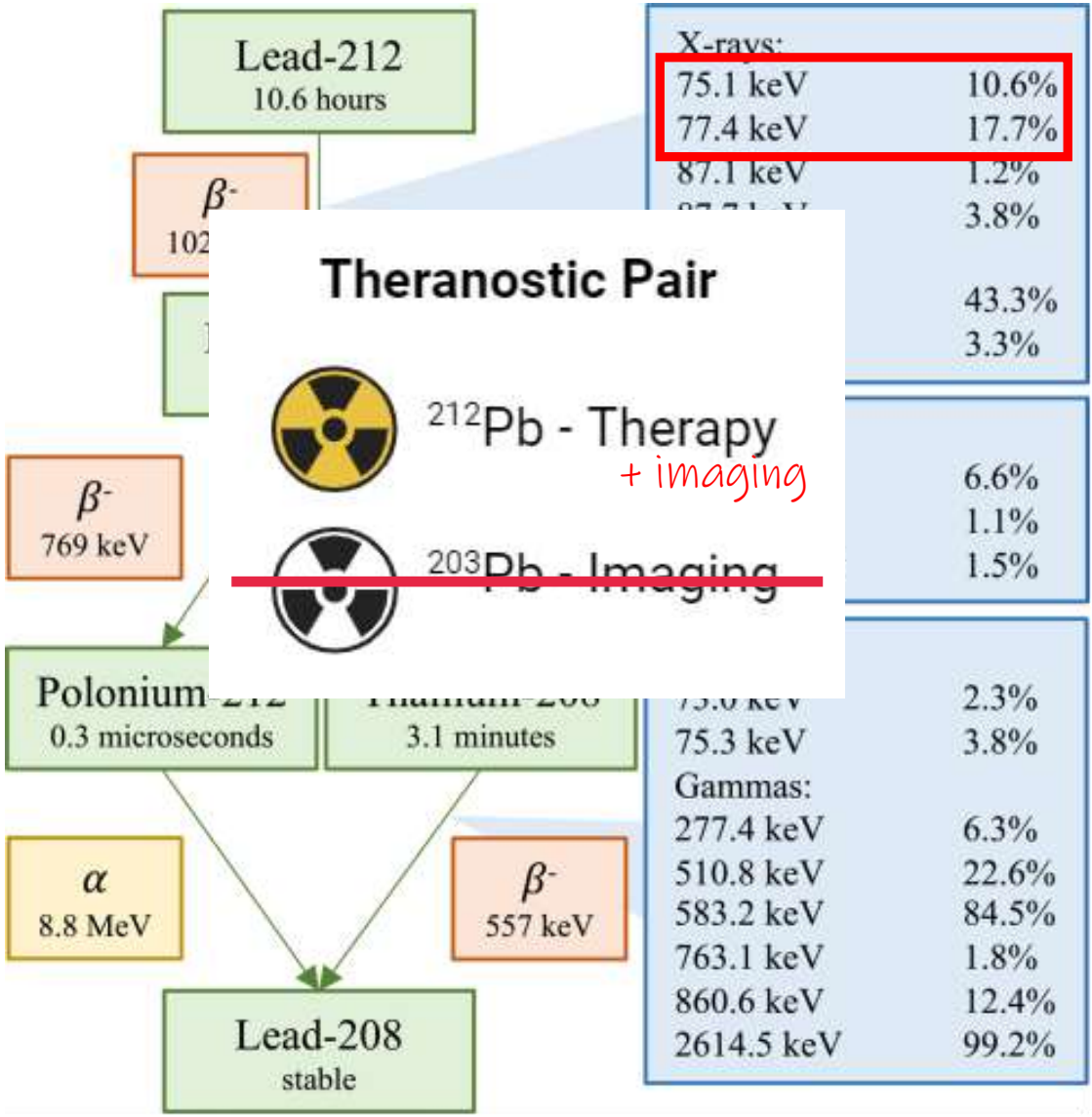
Also improved
Tumour:Liver
Tumour:Spleen

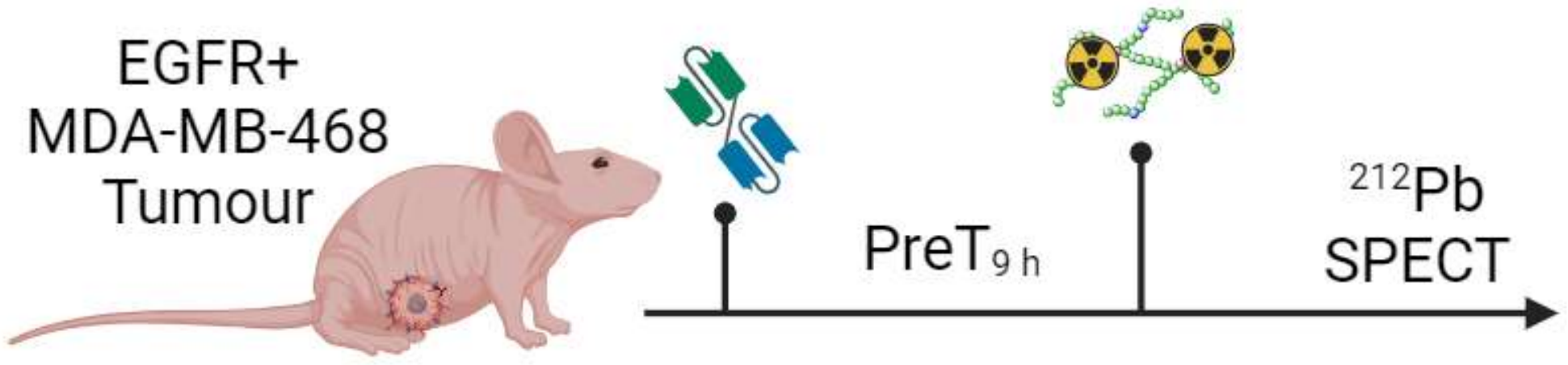
Preclinical Imaging

- Ex vivo biodistribution is great
- Longitudinal imaging improved understanding
- Single Photon Emission Computed Tomography (SPECT)

Energy window 10% 79 keV

Kvassheim, 2022; https://doi.org/10.1186/s40658-022-00481-z

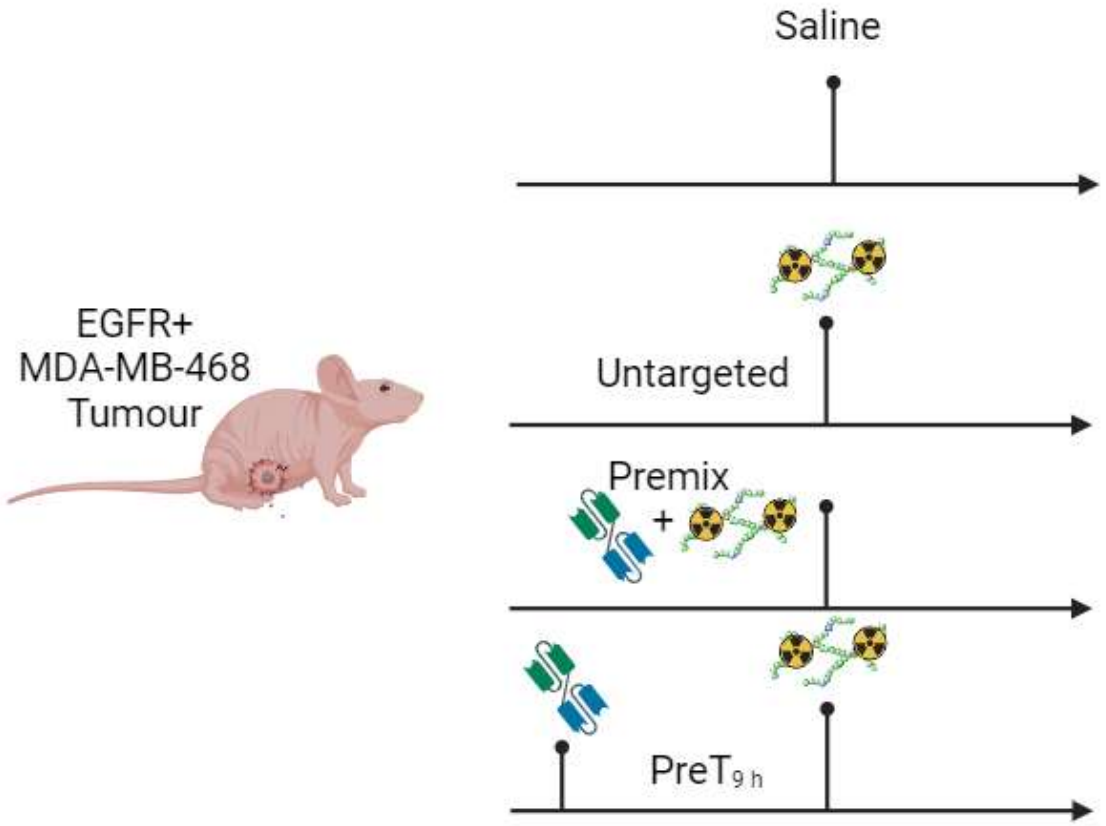



EGFR+
MDA-MB-468
Tumour
PreT9 h
212Pb
SPECT

48 H post injection

What about efficacy?

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

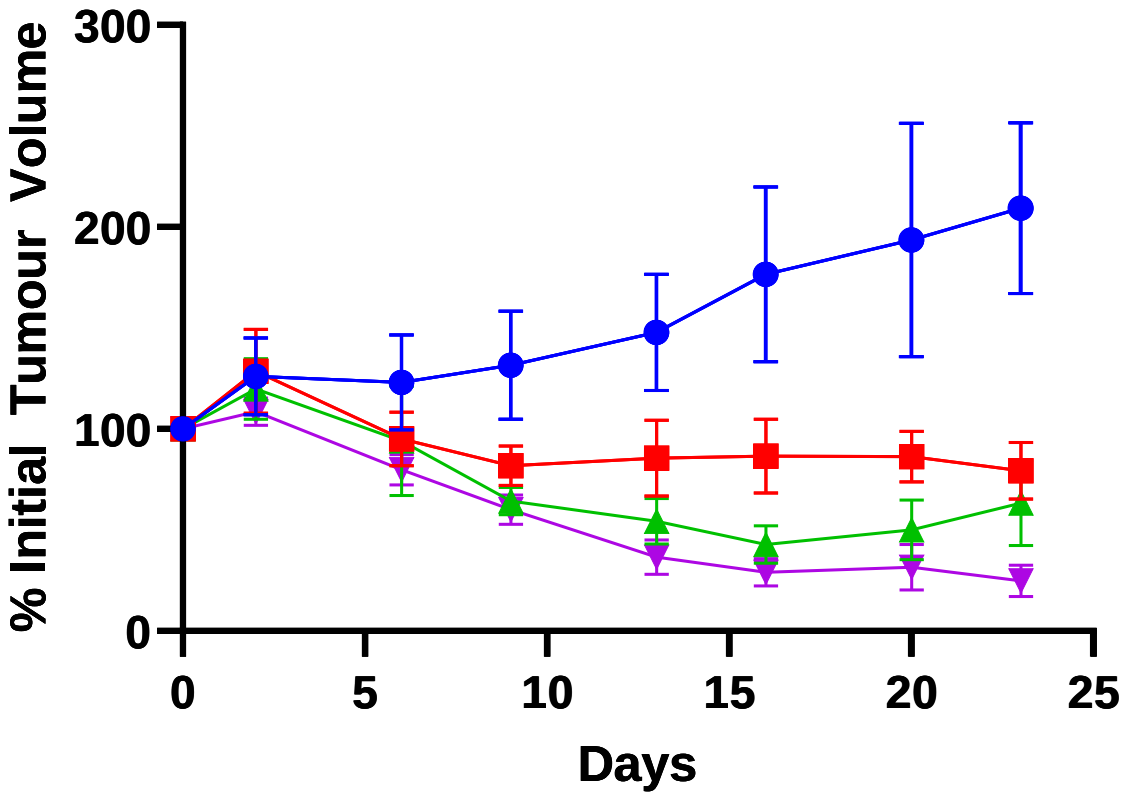


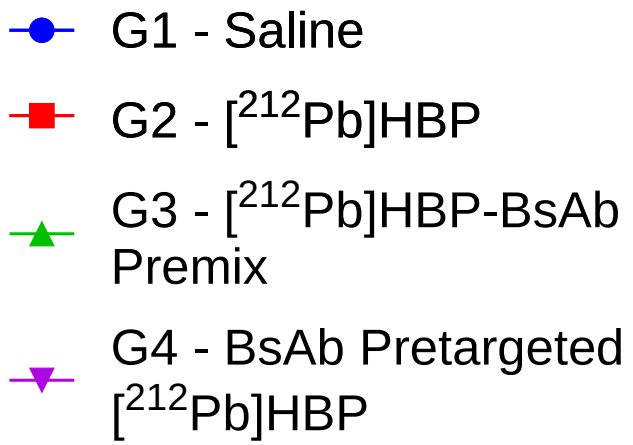
MDA-MB-468 (EGFR+) breast cancer tumours in Balb/c nude mice (n=5 per group)

1 MBq [^{212}Pb]HBP dose (Untargeted, Premixed with BsAB or Pretargeted)

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

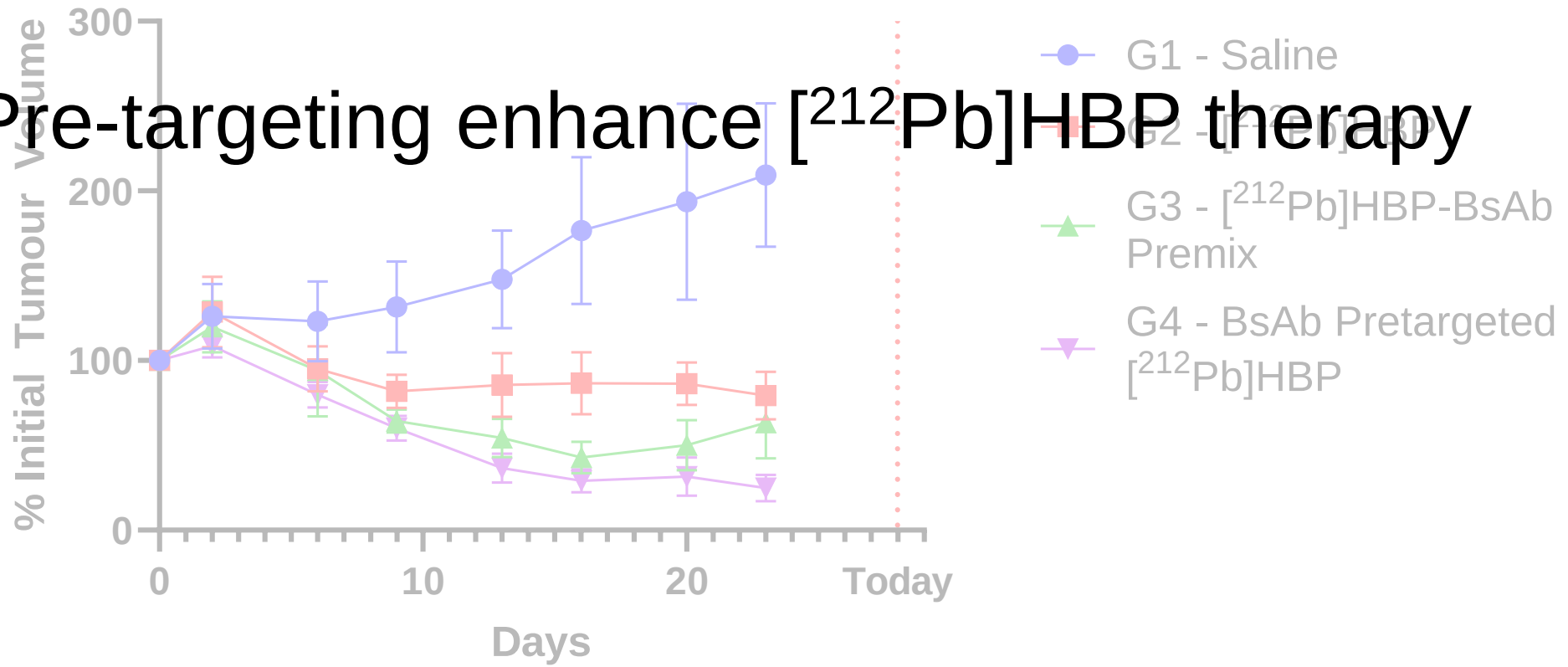
Monitor tumour volume as efficacy measure





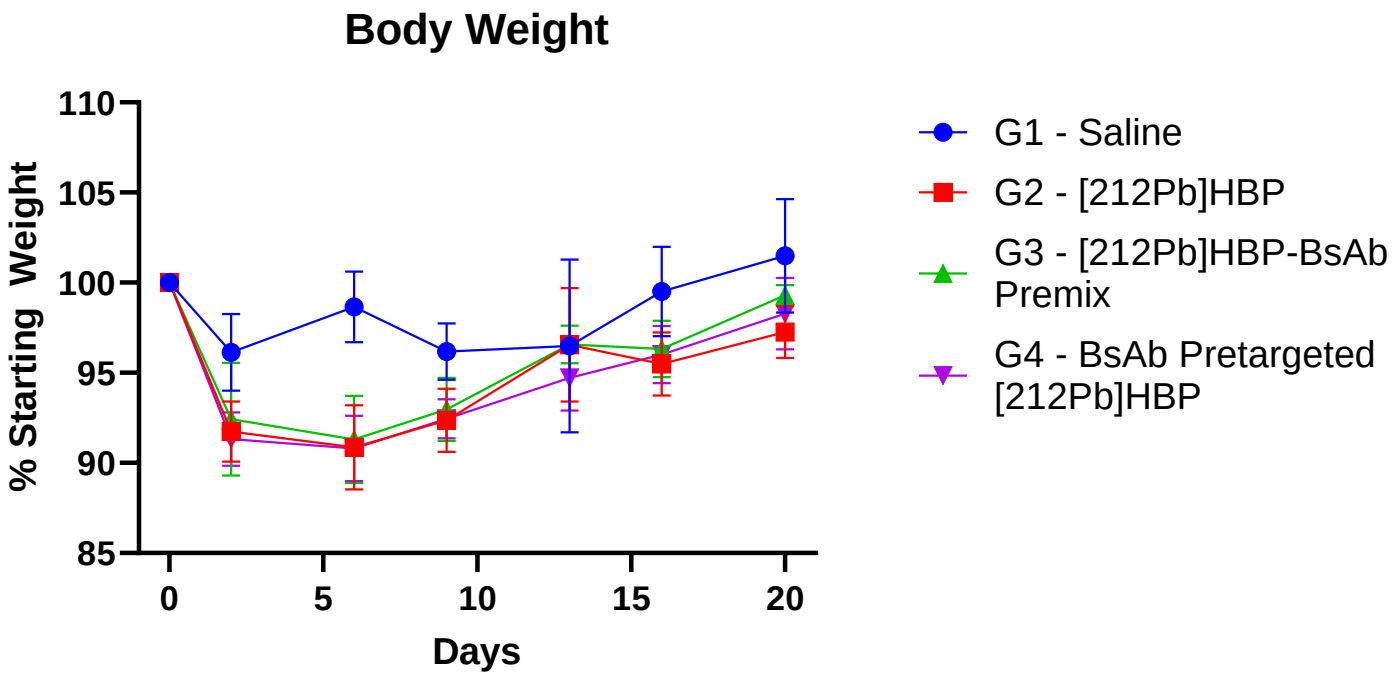
Pre-targeting [^{212}Pb]HBP Therapy

Monitor tumour volume as efficacy measure



Pre-targeting enhance [^{212}Pb]HBP therapy

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

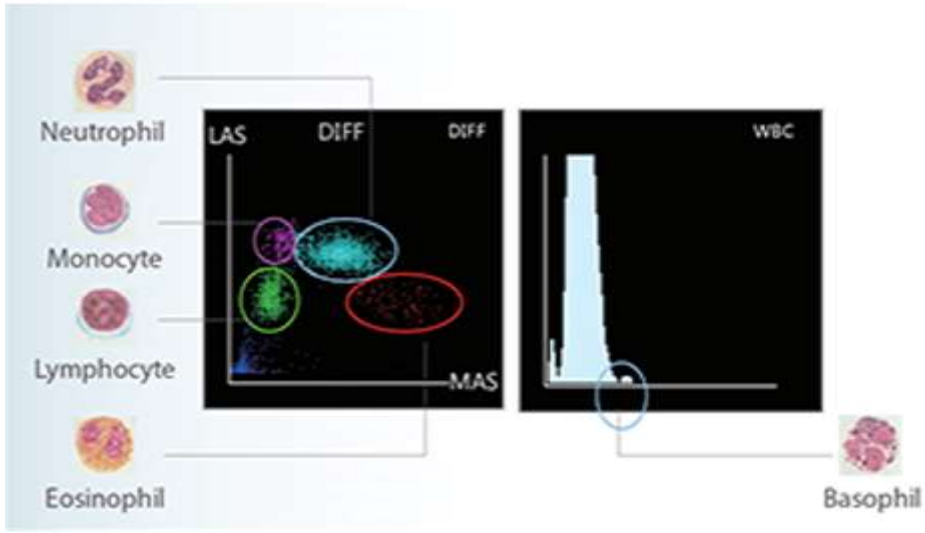


Hematological toxicity
Often dose limiting
In molecular radiotherapy

[^{212}Pb]HBP Hematology

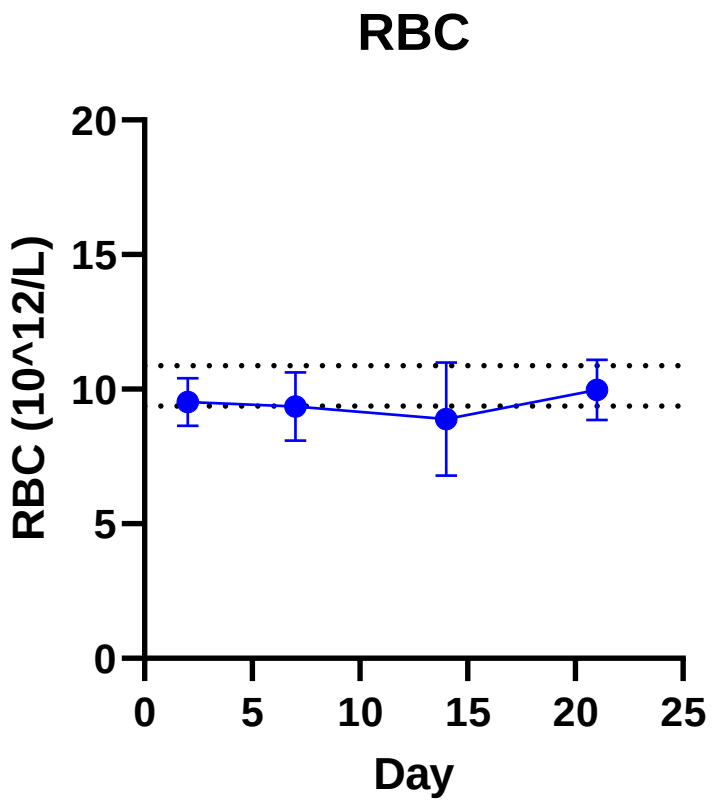
Key potential dose-limiting toxicities

Radiobiology Facility - Monitor bloods during therapy



23 parameters: WBC, Lym%, Mon%, Neu%, Bas%, Eos%, Lym#, Mon#, Neu#, Eos#, Bas#, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, MPV, PDW, PCT

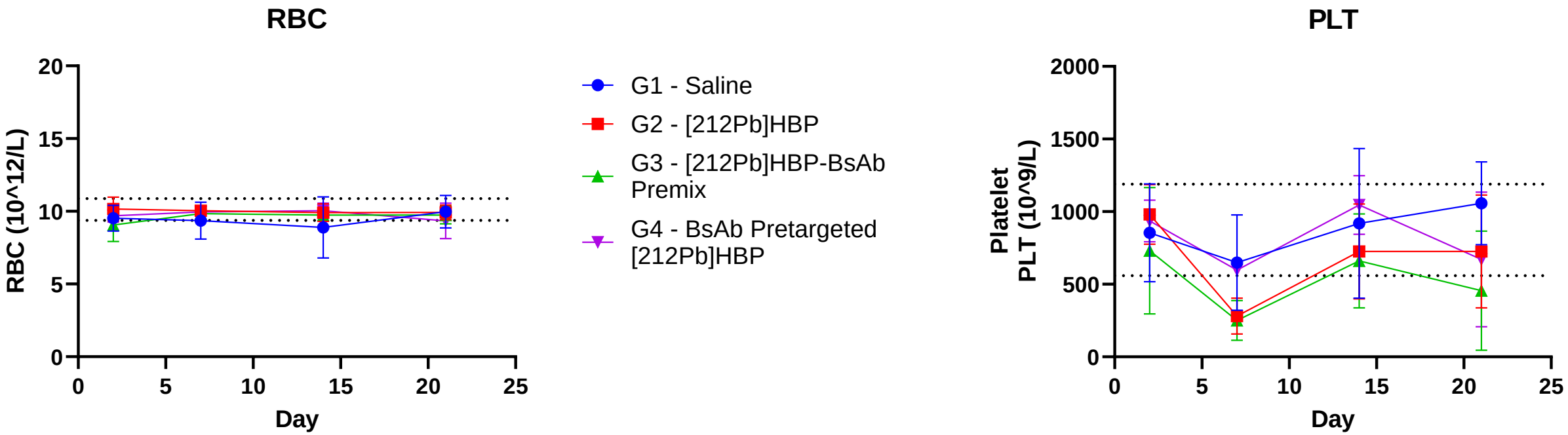
[^{212}Pb]HBP Hematology



Guidelines mean +/- SD of whole cohort pre-dose

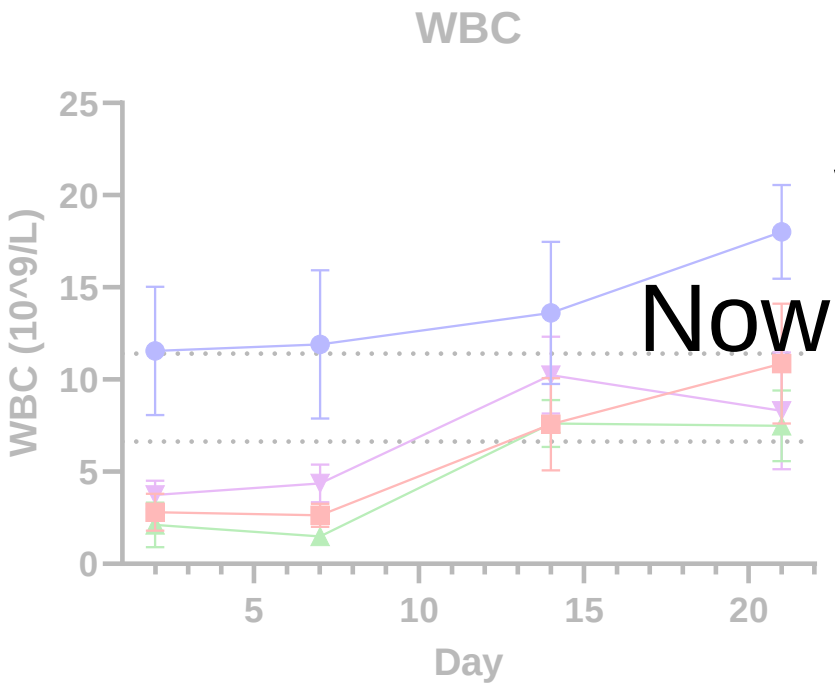
[^{212}Pb]HBP Hematology

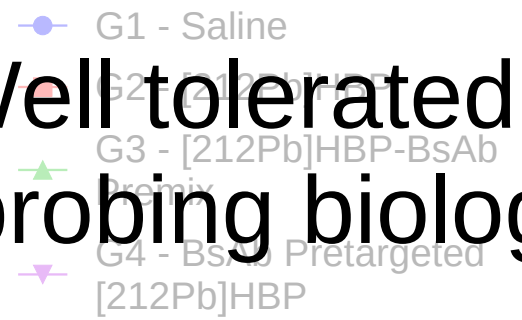
Broadly well tolerated



[^{212}Pb]HBP Hematology

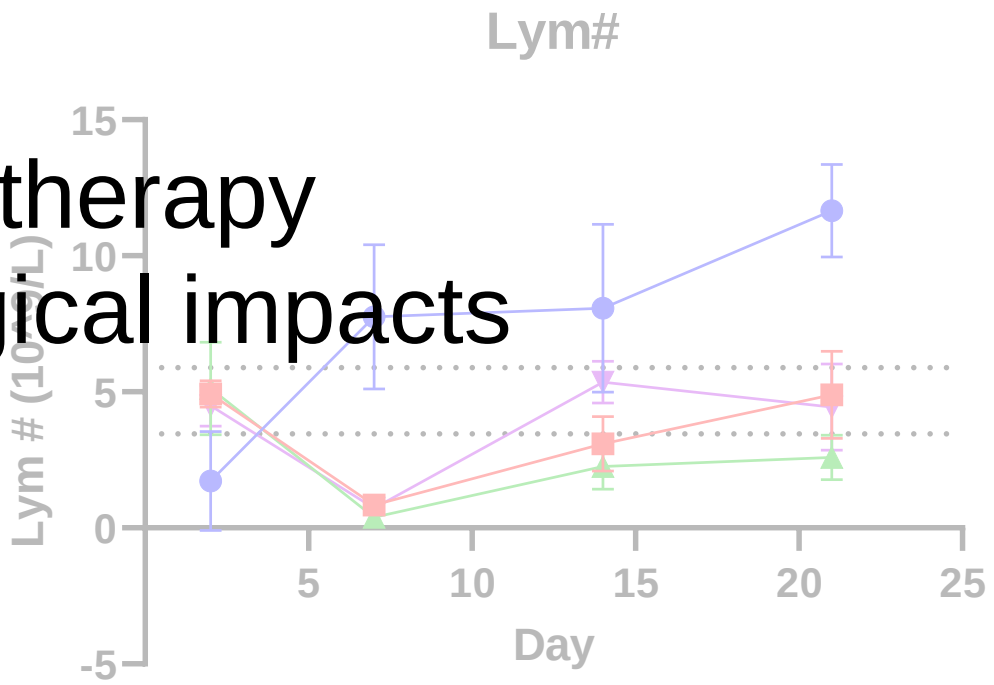
White Blood Cell counts





Well tolerated therapy

Now probing biological impacts

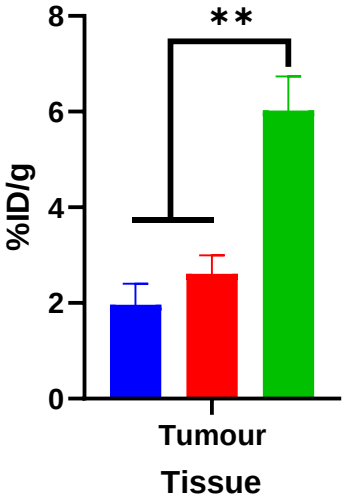


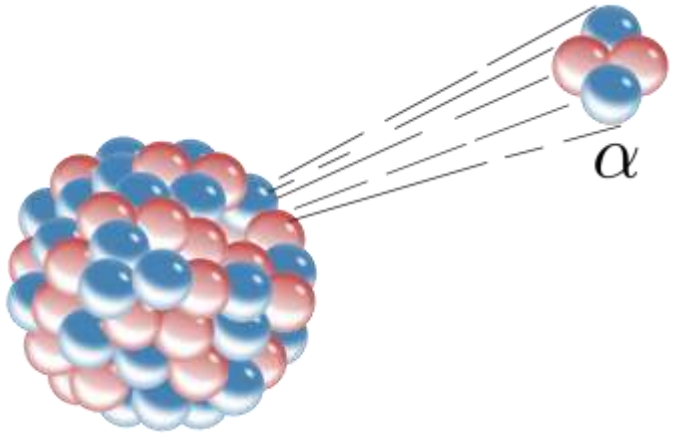
Trends in cell counts comparable to clinical observations

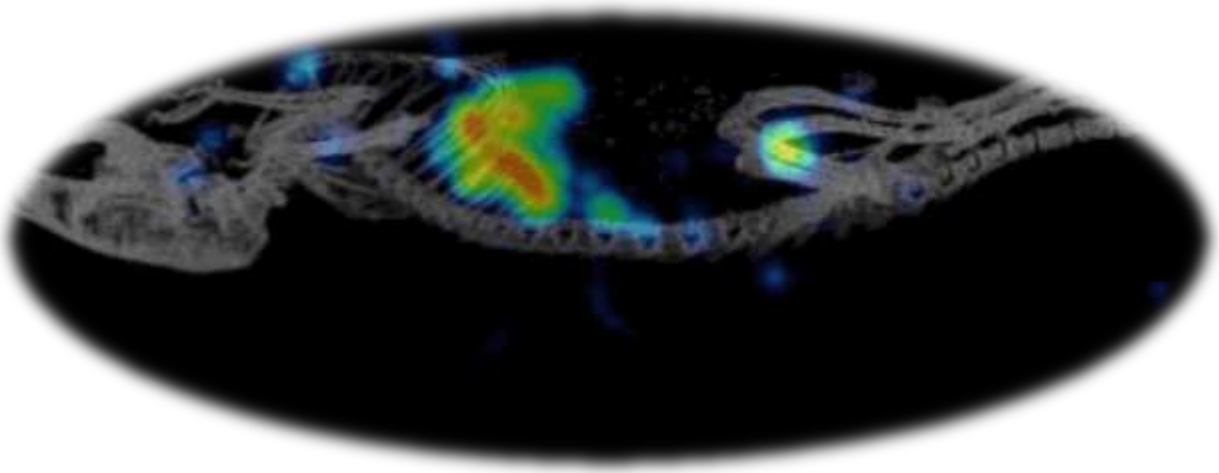
NK cells? Tumour Burden?

Conclusions

- [^{212}Pb] polymeric nanomedicines promising alpha therapeutic platform
- Pre-targeting potentially balances targeting and clearance
  - Broadly applicable to PEG-based materials
- Developing range of tools for monitoring behaviours and responses to [^{212}Pb]HBP in biological systems
- Promising therapeutic and tolerability results







Thank you

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