



Antibody-based PET tracers for Glioblastoma

SAIKAT GHOSH, PhD

The University of Queensland



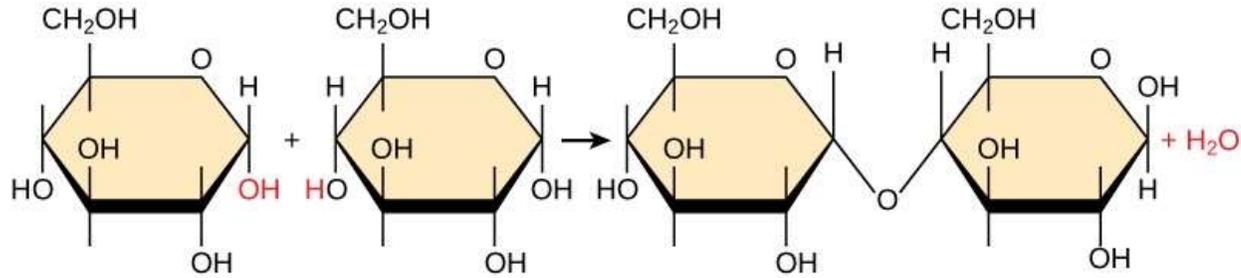
38APS

Polymers

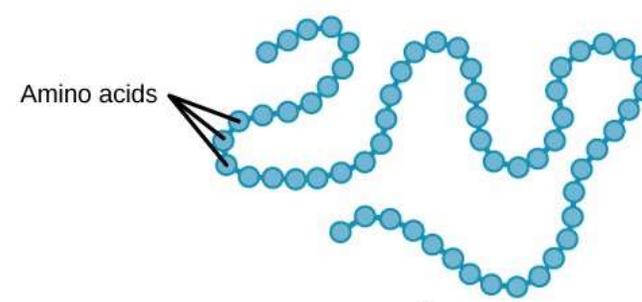
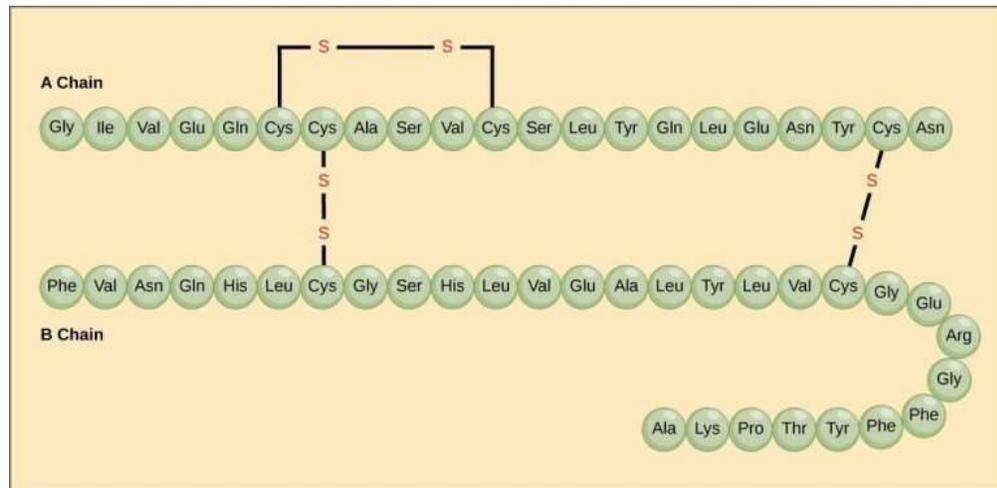
The diagram is divided into four vertical panels, each representing a different type of biomolecule. At the bottom of the entire diagram, the word "Biomolecules" is written in large, bold, black letters.

- Carbohydrate:** The top panel is pink. It is labeled "Carbohydrate" in the top left. It shows three red hexagons connected by a grey line. Each hexagon has a face with eyes and a mouth. The top hexagon has its tongue sticking out, the middle one wears glasses, and the bottom one has a neutral expression.
- Lipid:** The second panel is light green. It is labeled "Lipid" in the top center. It shows a thick green vertical bar on the left. Three green horizontal bars extend from the right side of the vertical bar, each with a face with eyes and a mouth.
- Protein:** The third panel is yellow. It is labeled "Protein" in the top center. It shows three orange circles of varying sizes connected by a red line. Each circle has a face with eyes and a mouth.
- Nucleic Acid:** The bottom panel is light blue. It is labeled "Nucleic Acid" in the top right. It shows a vertical chain of four purple pentagons connected by blue circles. Each pentagon has a face with eyes and a mouth. To the right of each pentagon is a small teal rectangular block.

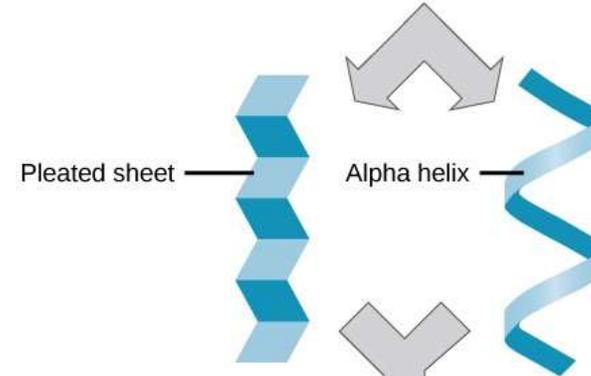
Proteins: Nature's polymers



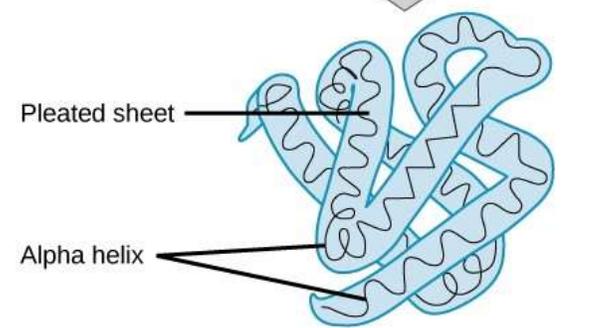
Dehydration synthesis reaction



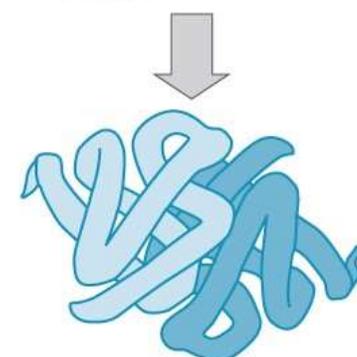
Primary Protein structure
sequence of a chain of amino acids



Secondary Protein structure
hydrogen bonding of the peptide backbone causes the amino acids to fold into a repeating pattern



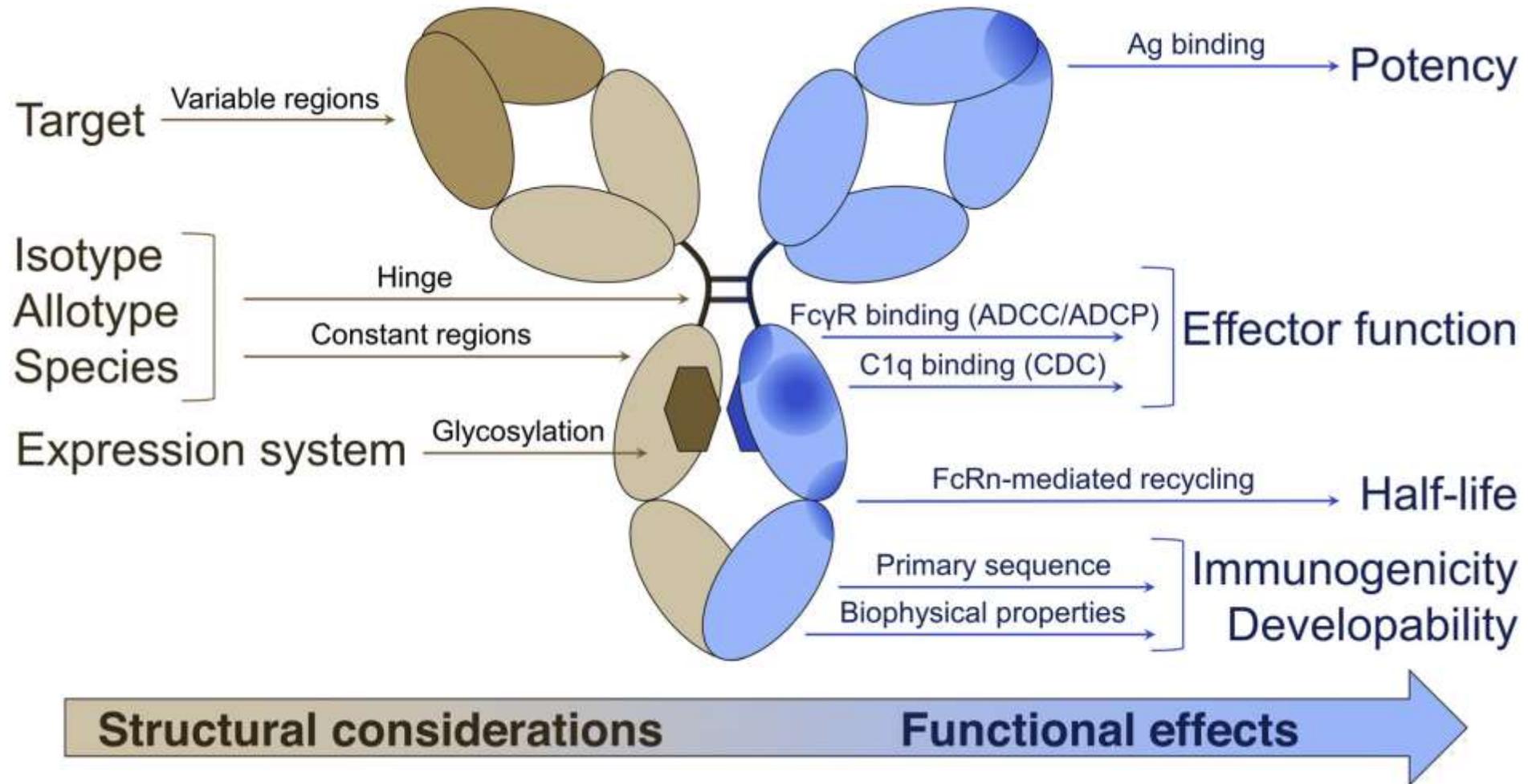
Tertiary protein structure
three-dimensional folding pattern of a protein due to side chain interactions



Quaternary protein structure
protein consisting of more than one amino acid chain



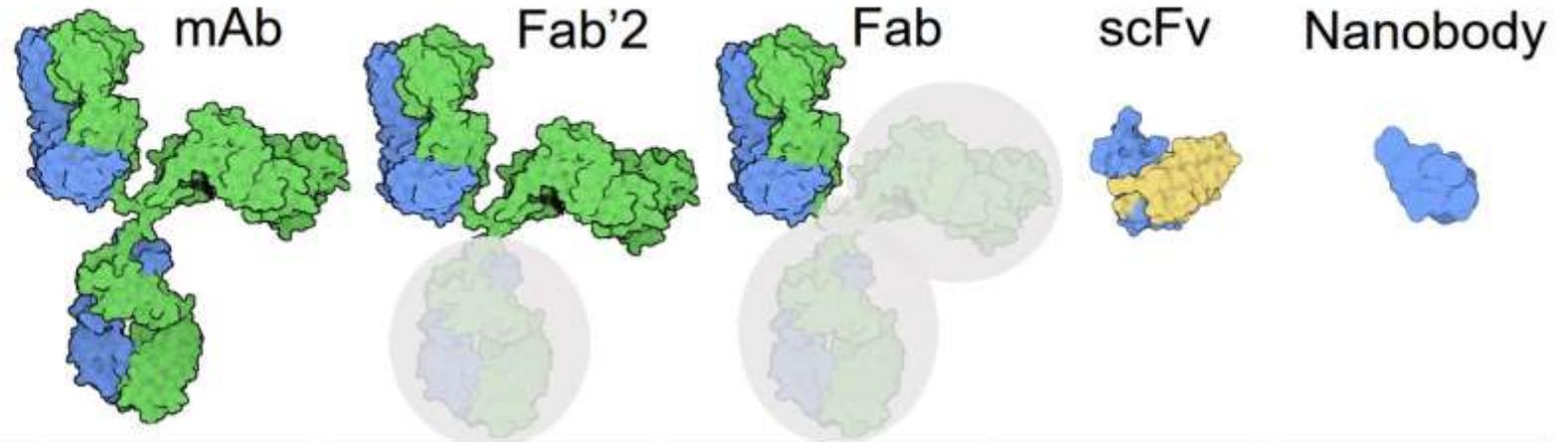
Antibodies: The Precision Polymers



Antibodies: Highly tuneable biopolymers

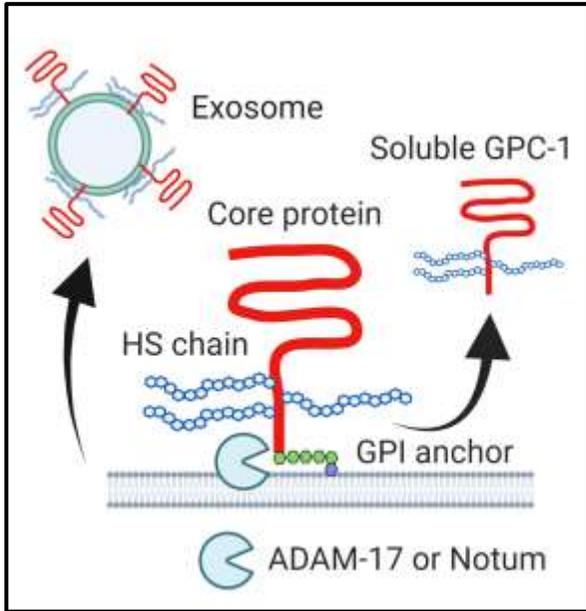
Modifications	Biological effects/ Outcomes
CDR (Fab) polymorphisms	Binding affinity
Fab & Fc-domain grafting	Reduced immunogenicity
Fc-domain polymorphisms & glycosylation	Clearance & PK (FcRn) Immunomodulatory (Fc-receptors)
Reformatting	Size, avidity & PK (Fab, Fab'2, scFv, nanobody, Bispecifics)
Hydrophilicity	Stability
Conjugation	Fluorophores, IR dyes etc.
	Antibody Drug Conjugates (ADC)
	Antibody Radionuclide Conjugates (ARC)

Antibody-based formats & properties



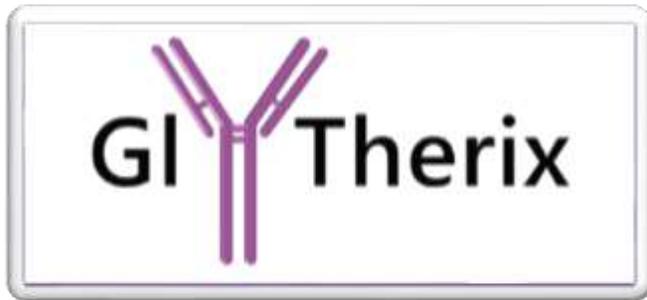
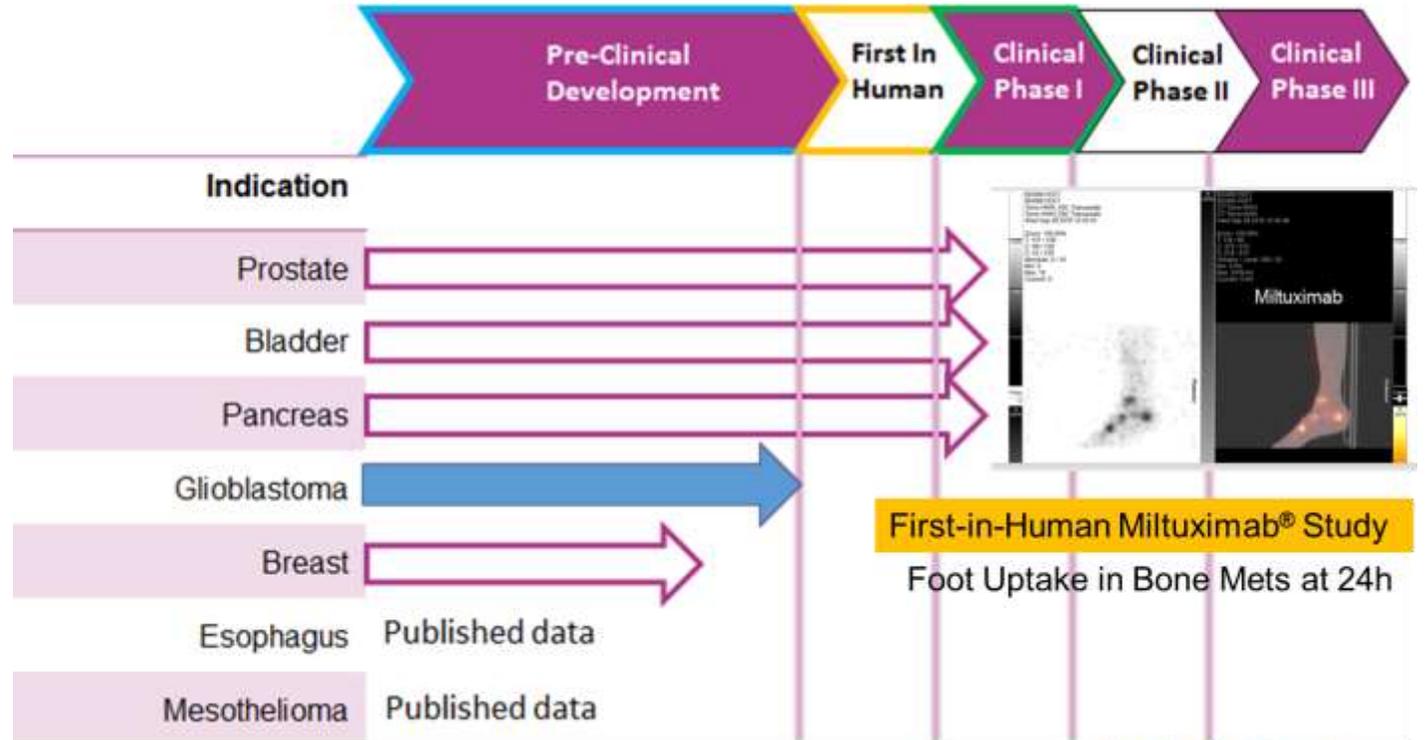
Production	Recombinant	Pepsin digestion of mAb	Papain digestion of mAb	Recombinant	Recombinant
Size (kD)	~150	~100	~50	~25	12-15
Valency	Bi-	Bi-	Mono-	Mono-	Mono-
Major clearance organ	Liver	Liver	Kidneys	Kidneys	Kidneys
Serum half-life	12-20 days	2-4 days	0.5 – 4 hours	< 1 hour	< 1 hour
Tissue penetration	+	++	++	+++	+++
Tumour uptake & retention	+++	++	+	+	+
Immune engagement	+++	-	-	-	-

Glypican-1 targeting antibody: Miltuximab®

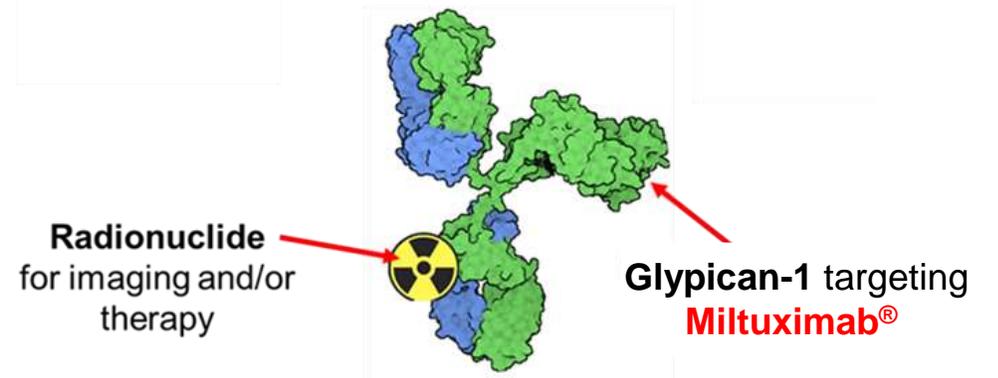


Glypican-1: A cell-surface Heparan Sulphated Proteoglycan

Ghosh et al., EGBT (2022); doi.org/10.1080/14712598.2022.2033204



Miltuximab®
Clinical stage
anti-GPC-1 antibody



Glioblastoma: Challenges to treatment

6 people are diagnosed with brain cancer each day.

4 of these people will die.



Brain cancer kills more children than any other disease.



BRAIN CANCER FACTS

Most common type of brain cancer
Glioblastoma

Has an average survival rate of only

14 months

Survival rates have not improved in **OVER 35 YEARS.**

Latest facts sourced from Lancet Neurol 2019
[https://doi.org/10.1016/S1473-4422\(18\)30468-X](https://doi.org/10.1016/S1473-4422(18)30468-X) & AIHW Cat no. CAN 106 2017



Drug	Median Survival Improvement
Temozolomide (Temodar)	2.5 months
Lomustine wafers (Ceenu); Carmustine wafers (Gliadel)	2.3 months
Bevacizumab (Avastin)	No definitive data yet

3D GBM Tumour Spheroid Model

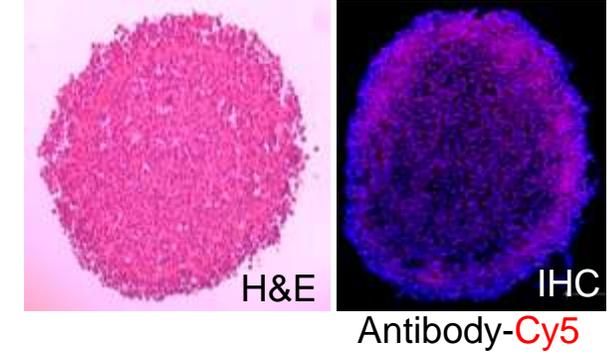
Tumour Spheroids: Advantages

- Resembles *in vivo* tumours
- Mimics drug interactions
- Allows HT drug screening
- Reduces animal testing

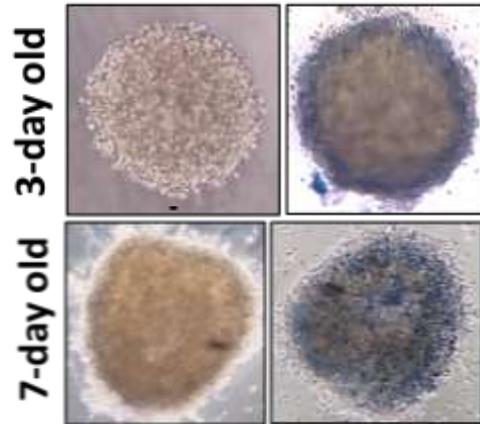
A. Glioblastoma Spheroid Formation



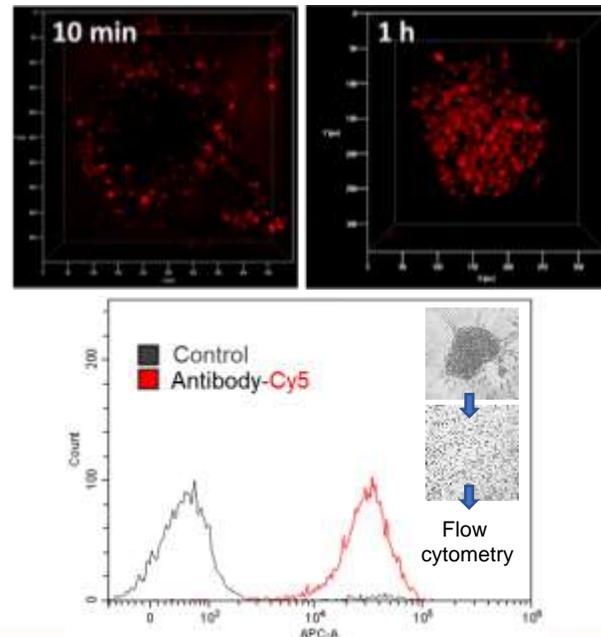
D. GBM Spheroid Histology



B. Spheroid Viability

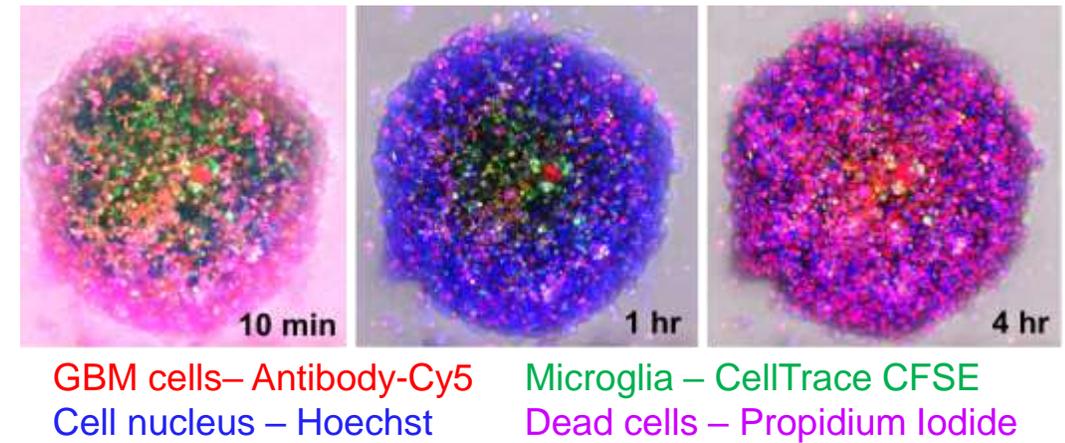


C. Antibody-Cy5 uptake

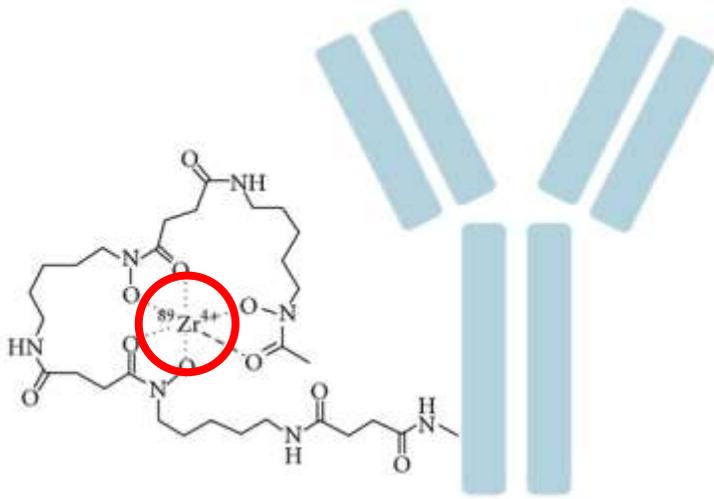


E. GBM-Microglia Multicellular Spheroids in a dynamic microfluidics model

Thanks: Amber Prior



Chelation of Miltuximab[®] with DFO

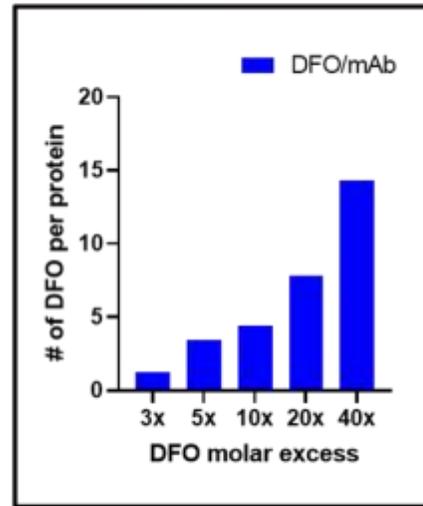


Antibody-Desferoxamine (DFO)
Complex with [⁸⁹Zr]

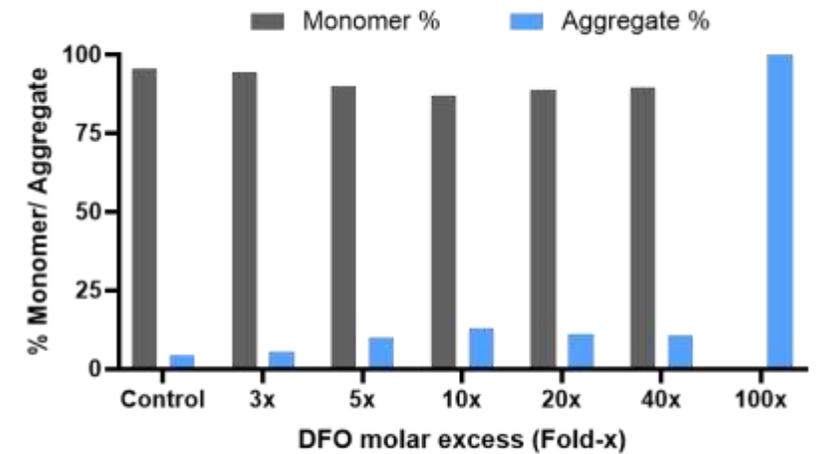
⁸⁹Zr - Characteristics & Advantages

- Long half-life of ~78.4h
- Compatible with $t_{1/2}$ of antibodies
- Low positron energy of 395.5 keV
- High image resolution

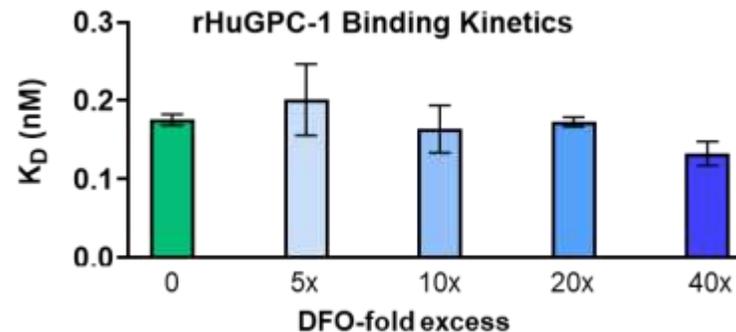
1. Antibody-DFO chelation



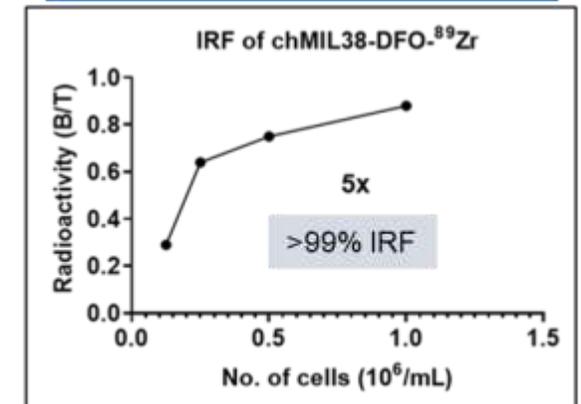
2. Stability – SEC-HPLC



3. Ligand Binding Kinetics



4. Cell Binding Assay



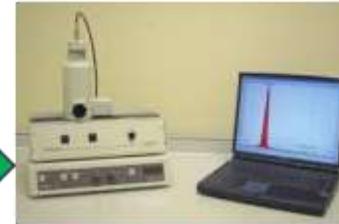
Workflow: Radiochemistry, QC and in-vivo Imaging



Radioisotope production



Antibody-Radio-Conjugate generation



Quality Control testing



Pre-clinical Molecular imaging

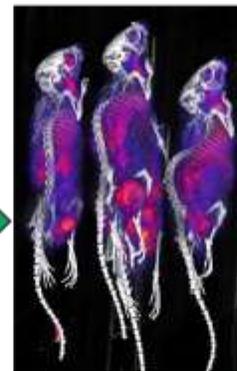


Image acquisition & processing

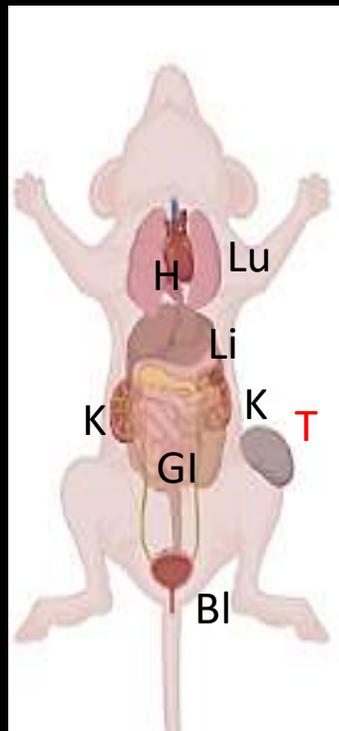
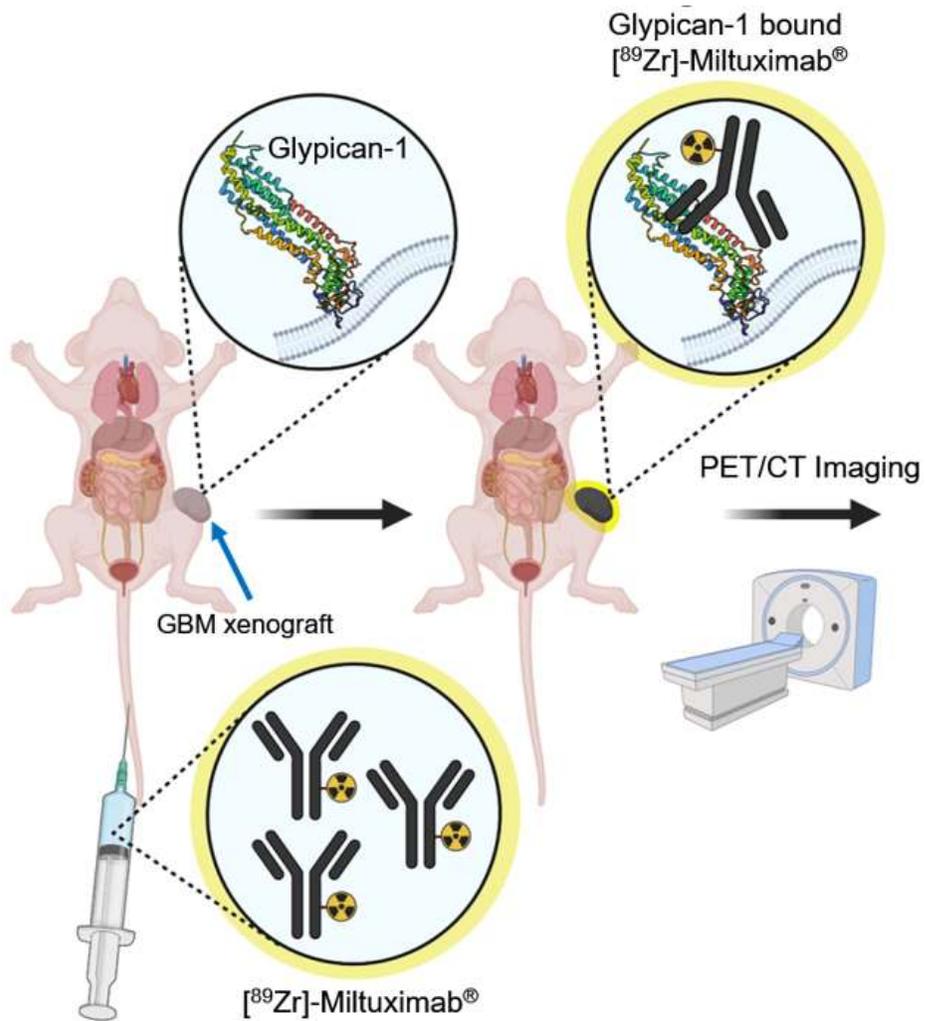


Ex-vivo organ harvest



Gamma counter for BioD

In vivo biodistribution of [⁸⁹Zr]-Miltuximab[®]



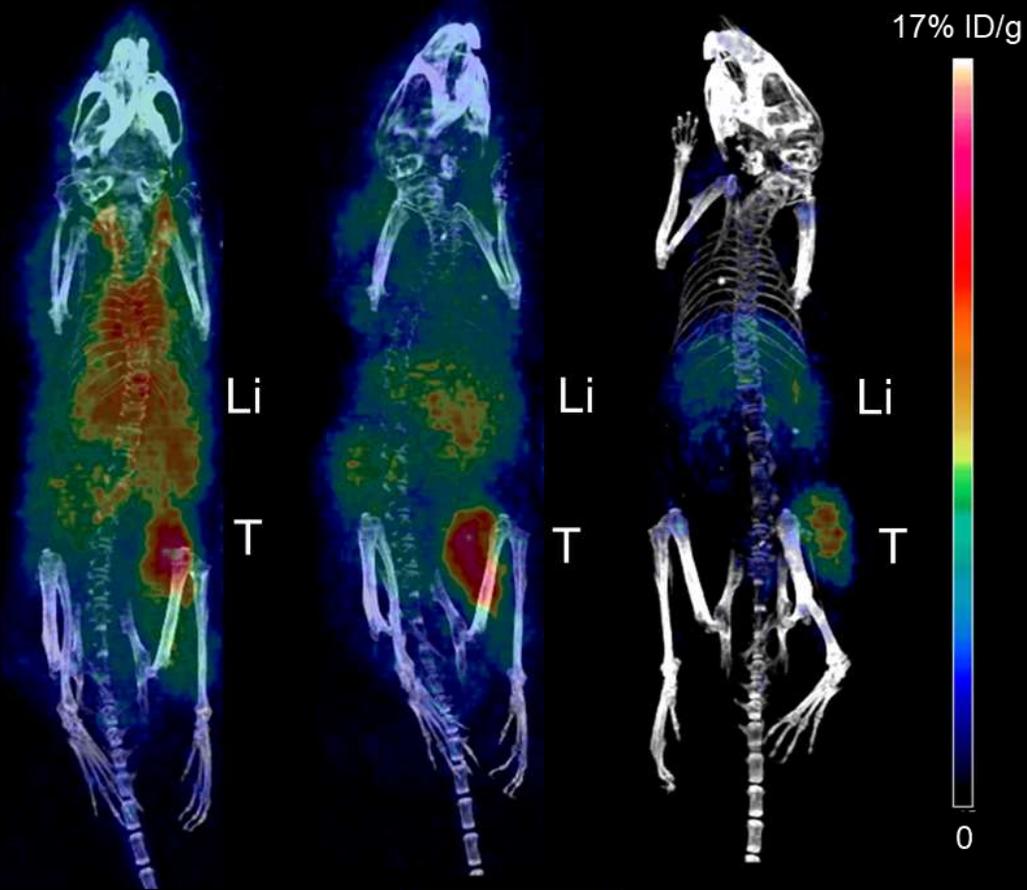
24 h



72 h



Day-9

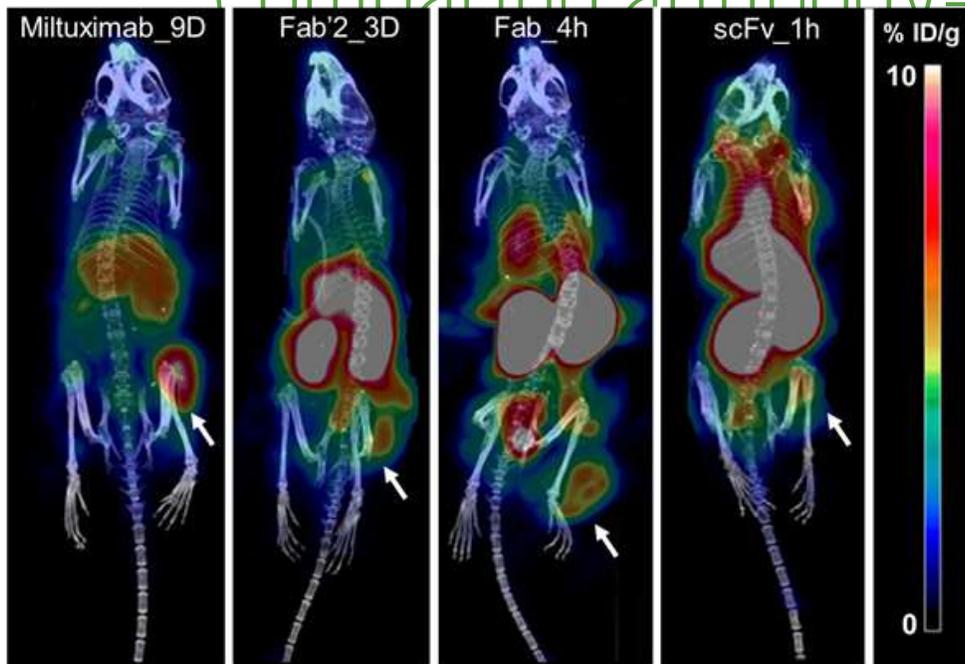


3D MIP

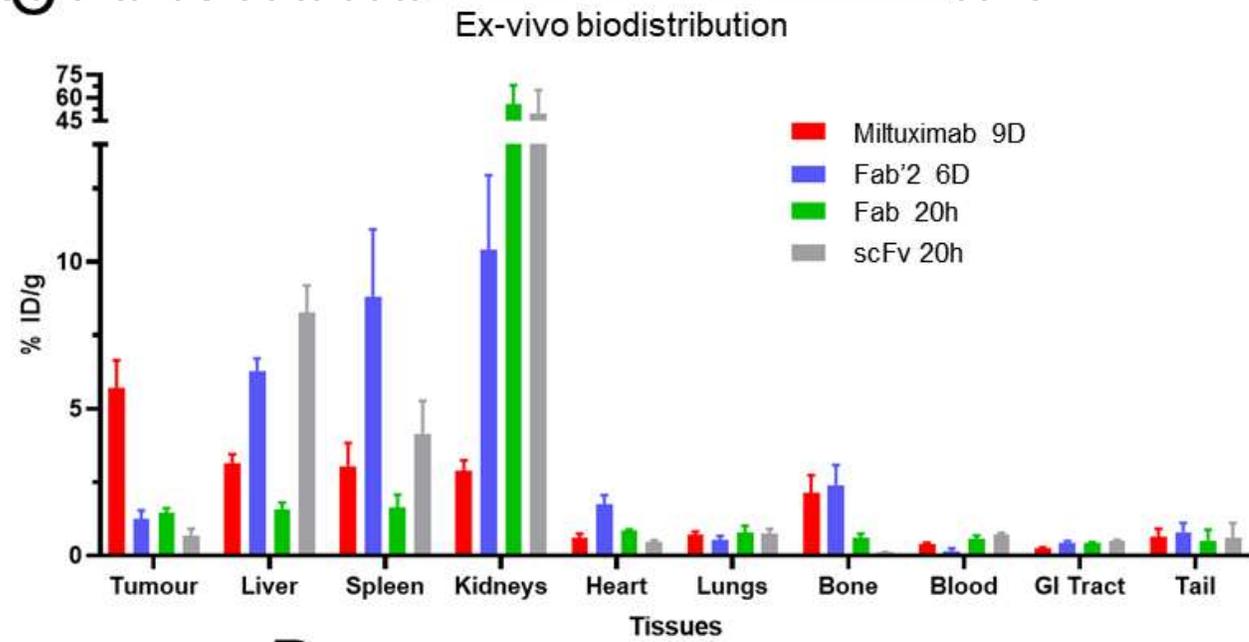
Ghosh et al., Mol Pharm 2023

Comparing antibody-based formats as PFT agents

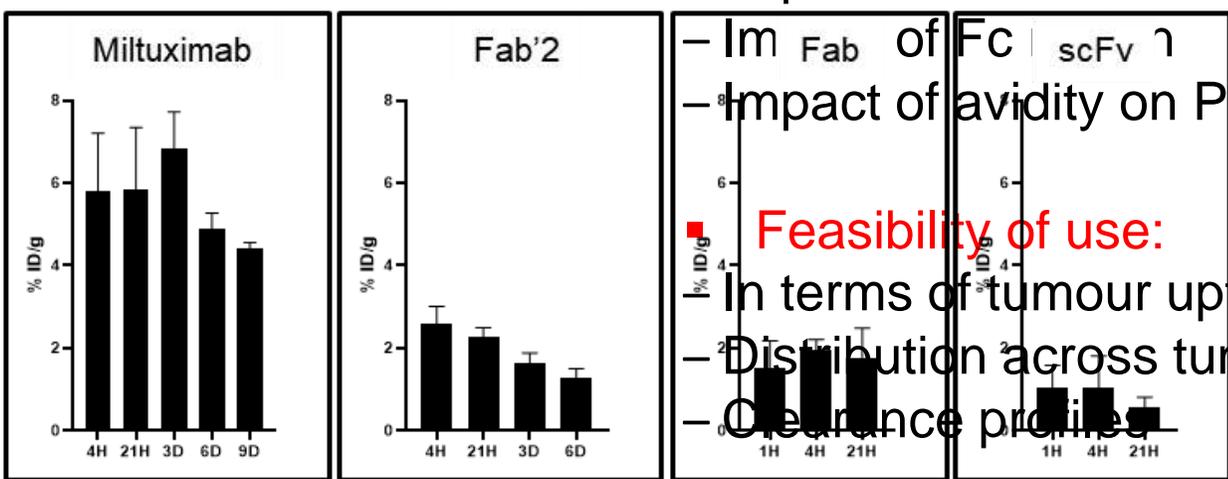
A



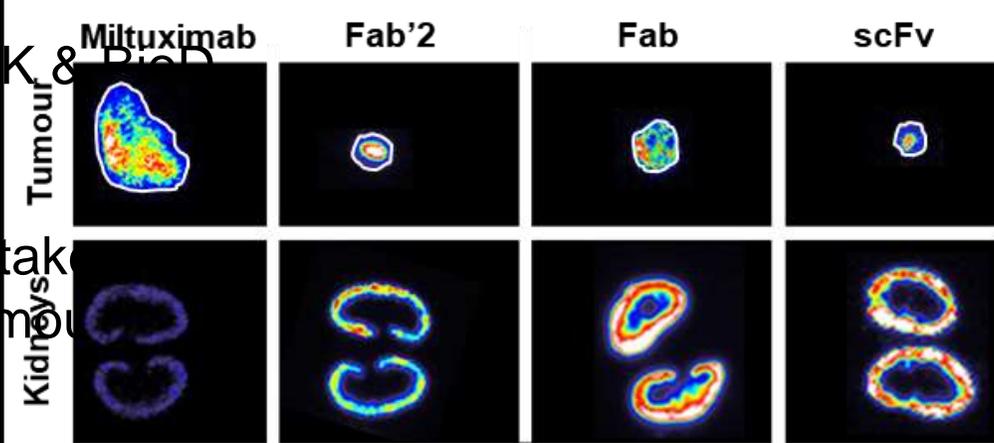
C



B



D

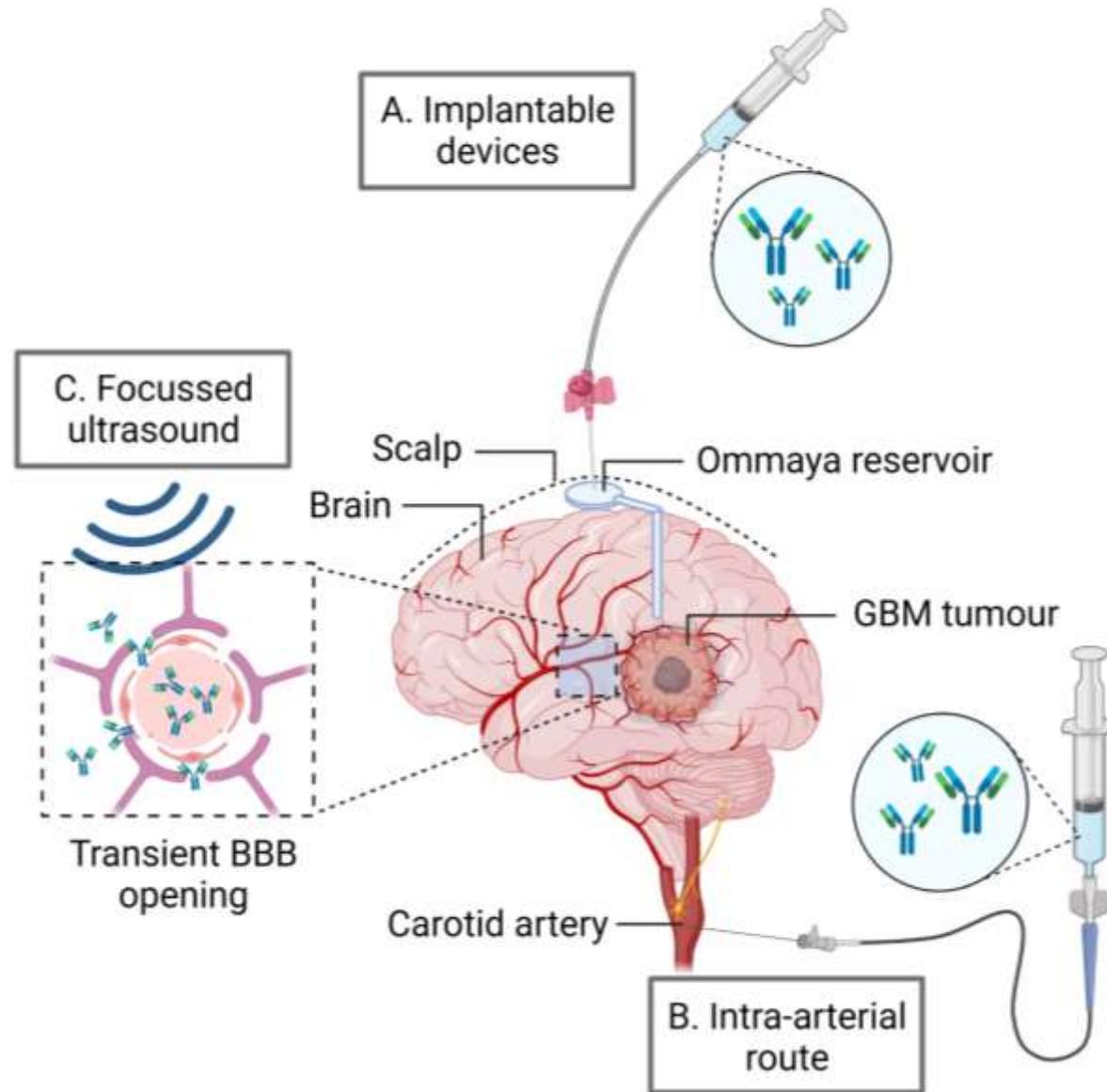


Impact of avidity on PK & PK/PD

Feasibility of use:

- In terms of tumour uptake
- Distribution across tumour
- Clearance profiles

Novel antibody delivery strategies to the brain



Ghosh et al., Mol Pharm, 2022, 19, 5, 1233-1247

Thank you!!

Saikat Ghosh, PhD

Industry Postdoc, AMTAR Hub

saikat.ghosh@uq.edu.au

<https://www.amtarhub.com.au>

Thurecht Group
members....



Kris Thurecht



Brad Walsh



Chris Howard



Doug Campbell



Nick Fletcher



Pie Huda



Yanling Lu



**Centre for Innovation in
Biomedical Imaging Technology**

An ARC Industrial Transformation Training Centre



Anti-GPC-1 antibody internalization in GBM cells