Antibody-based PET tracers for Glioblastoma

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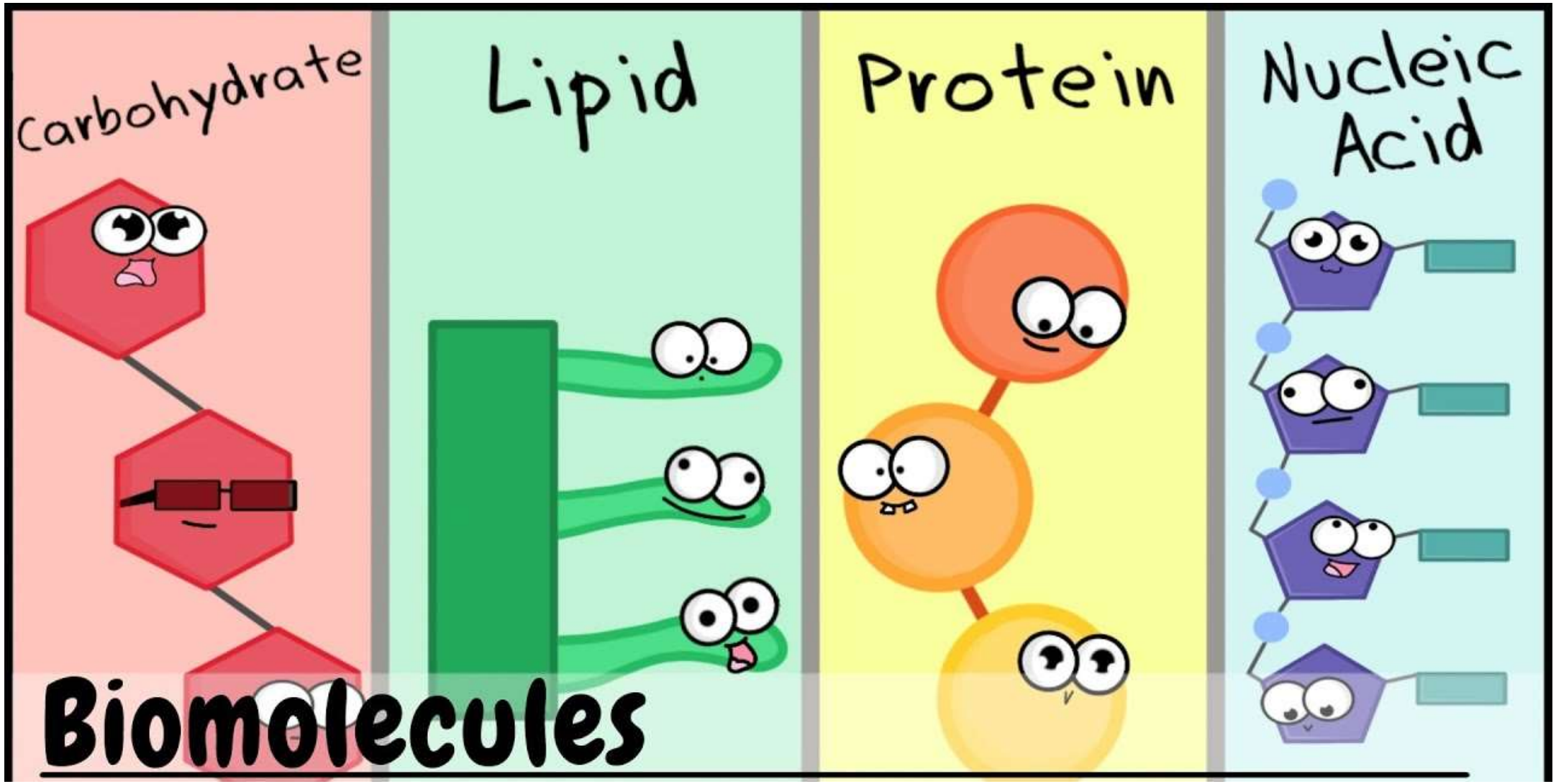
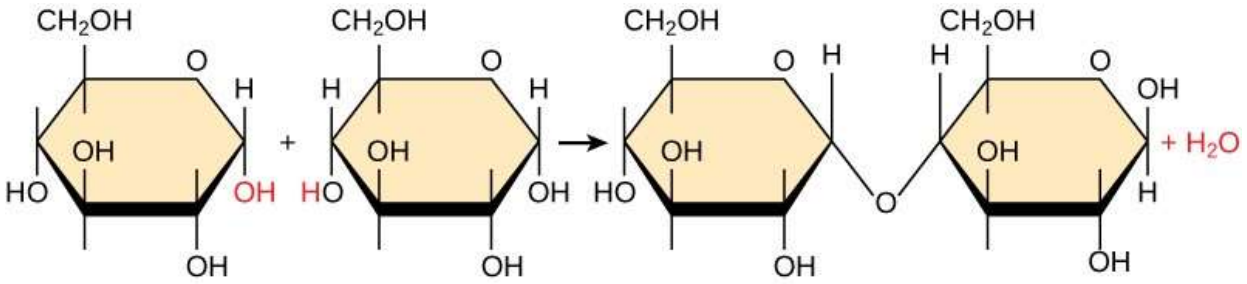
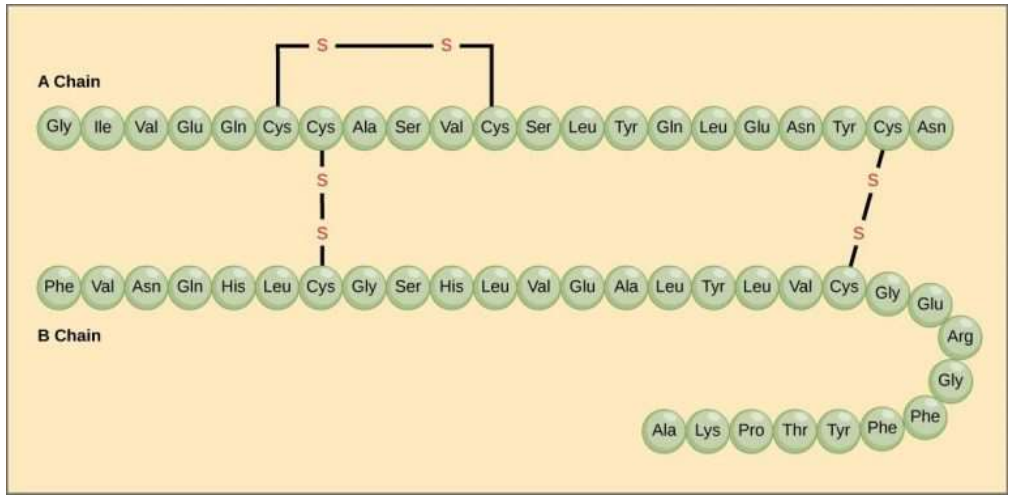
Polymers

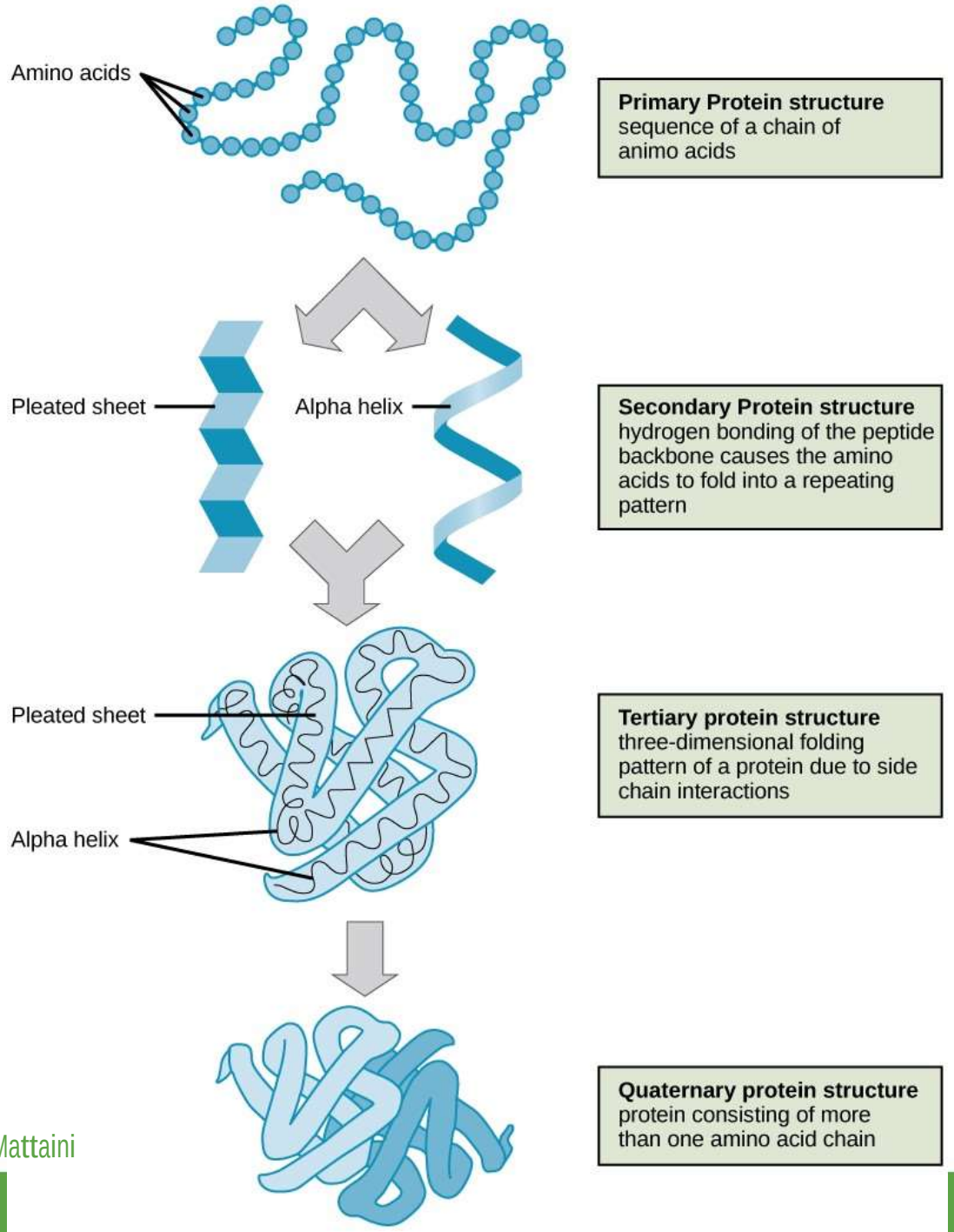


Proteins: Nature's polymers



Dehydration synthesis reaction





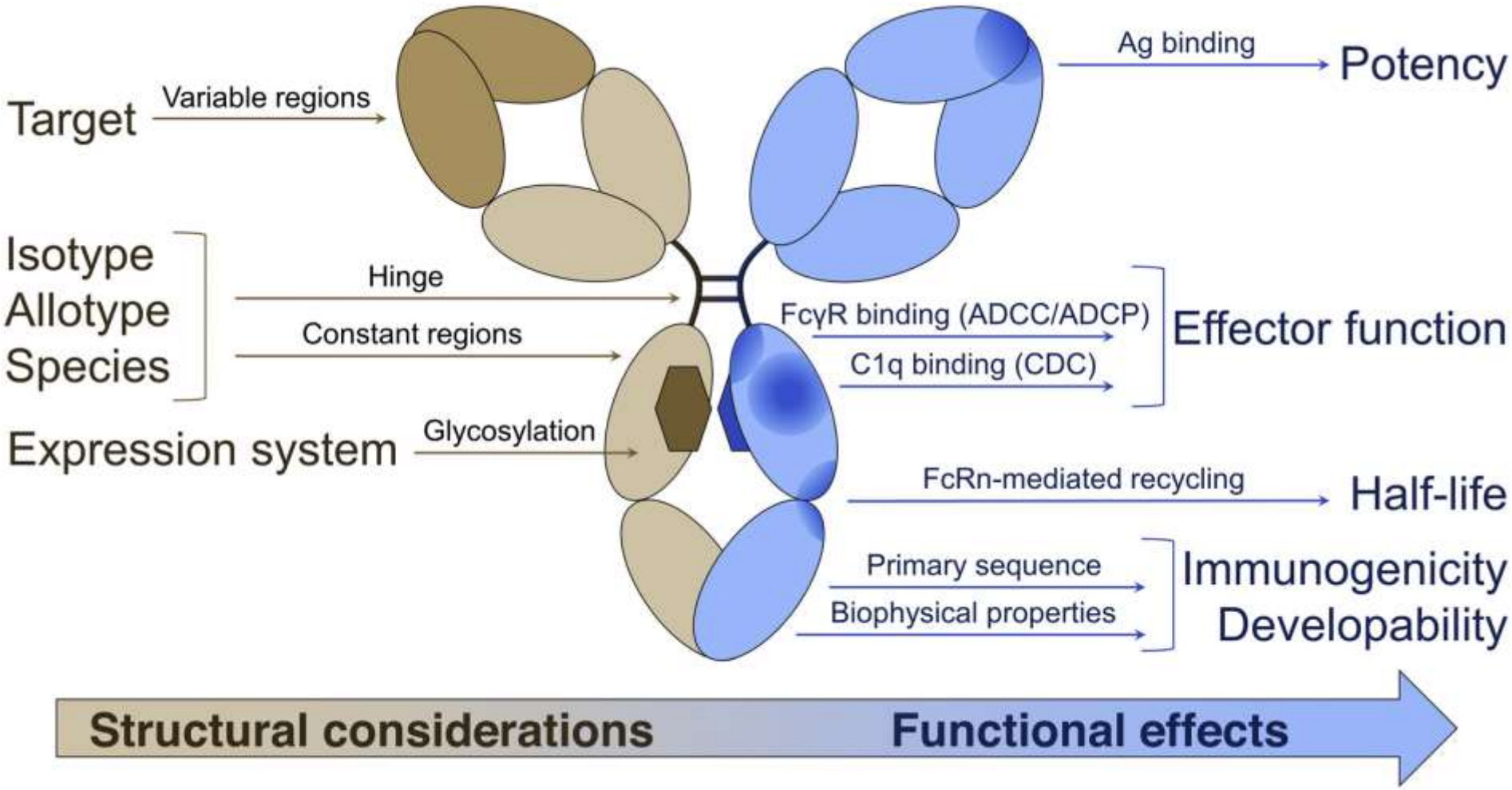
Primary Protein structure
sequence of a chain of animo acids

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

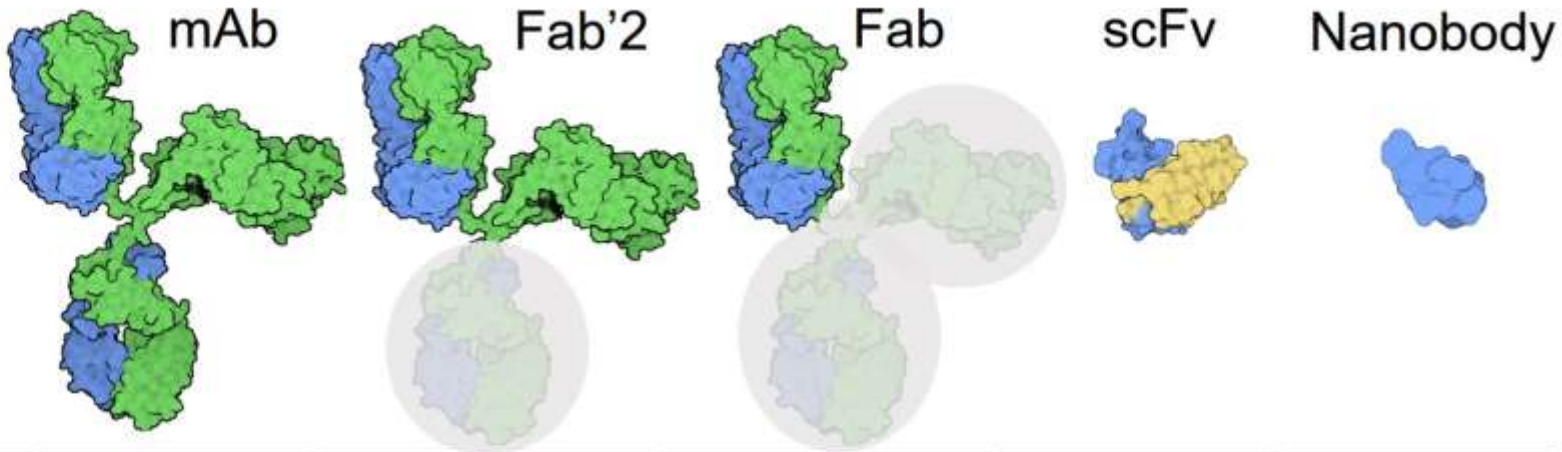


Goulet et al, 2019. https://doi.org/10.1016/j.xphs.2019.05.031

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)

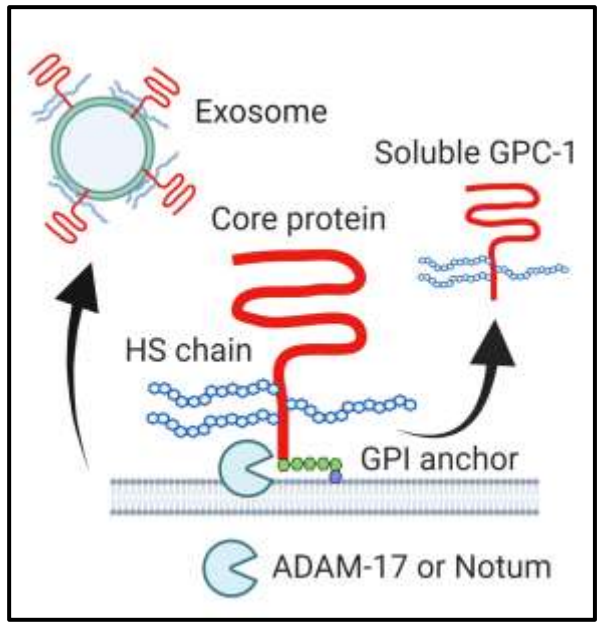
Antibody-based formats & properties



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	+++	–	–	–	–

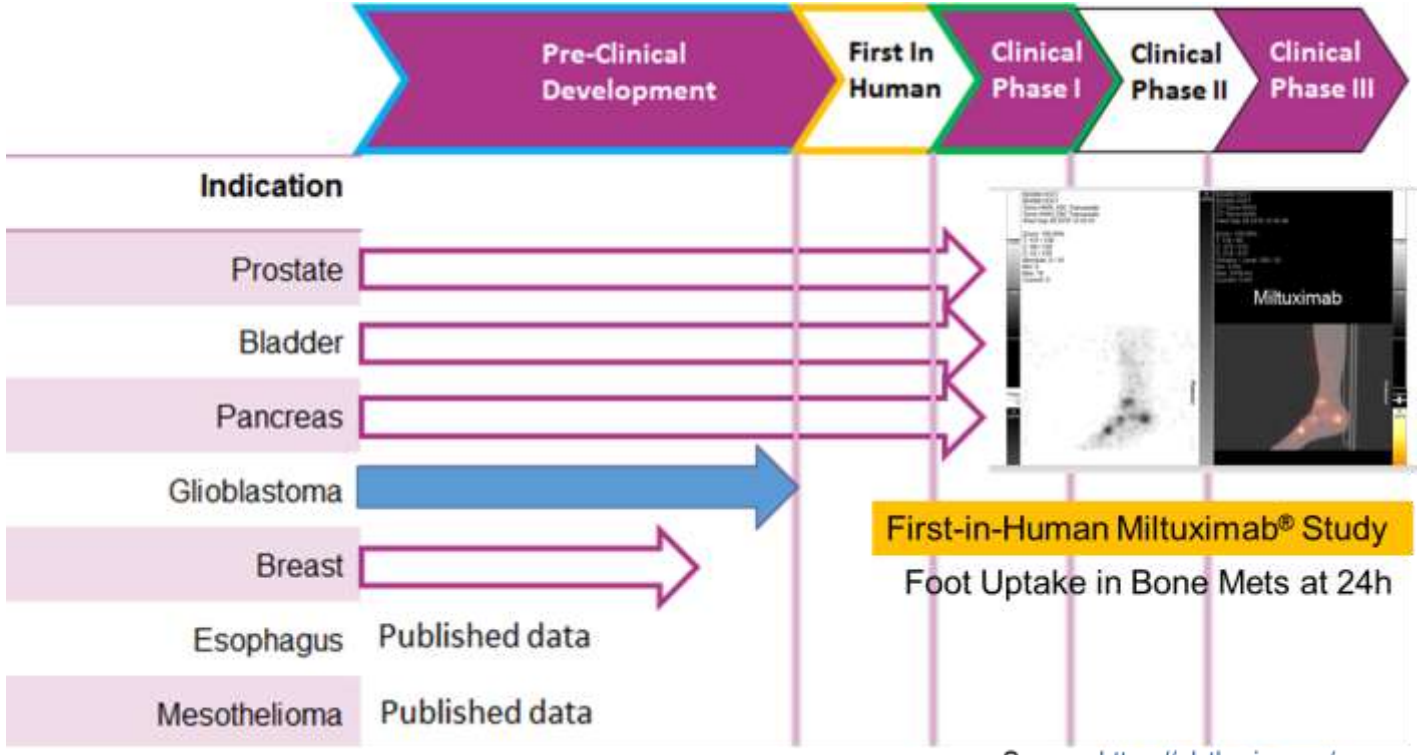
Ghosh et al., Mol Pharm, 2022, 19, 5, 1233–1247

Glypican-1 targeting antibody: Miltuximab®



Glypican-1: A cell-surface Heparan Sulphated Proteoglycan

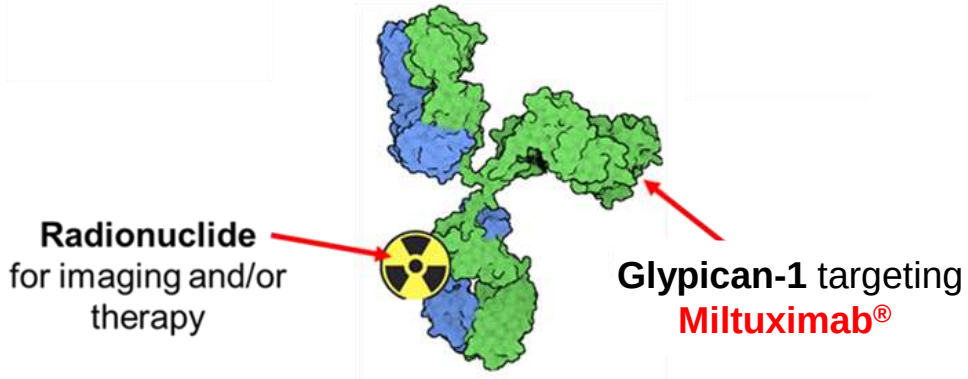
Ghosh et al., EOBT (2022); doi.org/10.1080/14712598.2022.2033204



Source: https://glytherix.com/

GlyTherix

Miltuximab®
Clinical stage
anti-GPC-1 antibody



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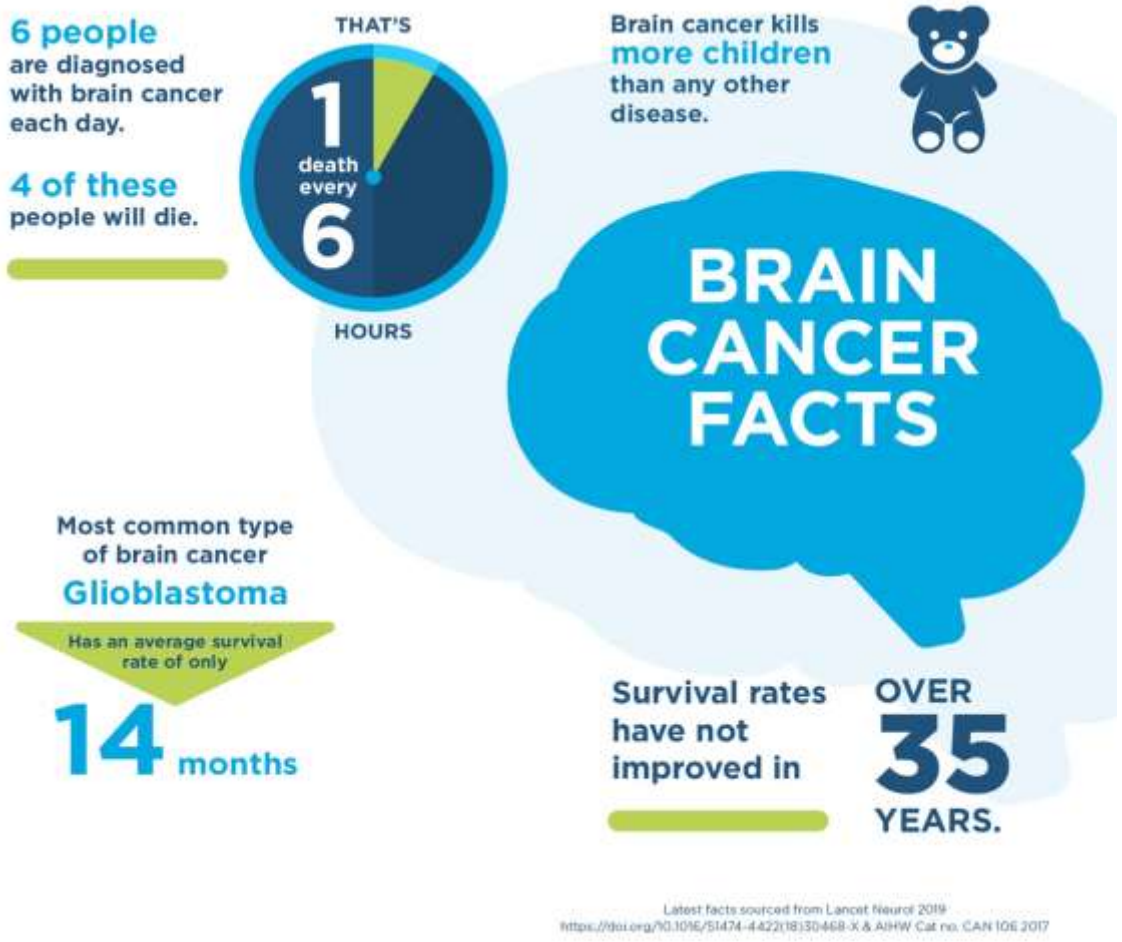
Glioblastoma: Challenges to treatment



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

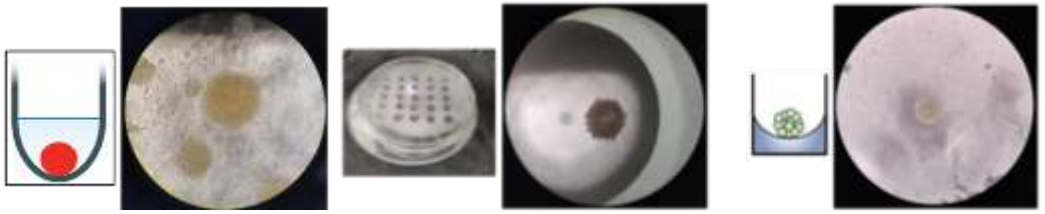
3D GBM Tumour Spheroid Model

Tumour Spheroids: Advantages

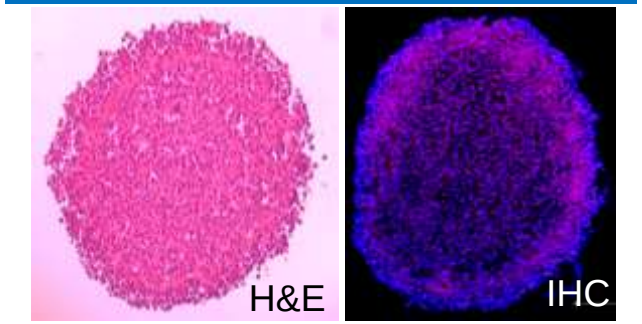
- Resembles *in vivo* tumours
- Mimics drug interactions
- Allows HT drug screening
- Reduces animal testing

A. Glioblastoma Spheroid Formation

1. U-bottom wells
2. Hanging drop
3. Agar bed

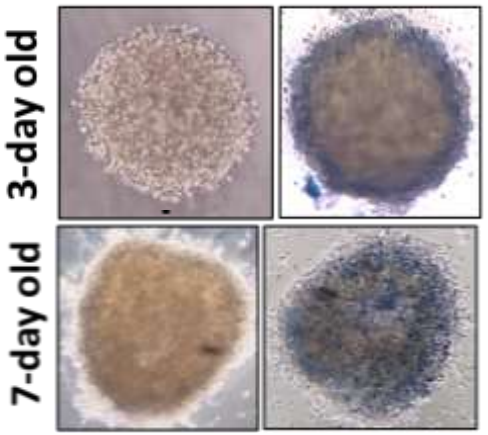


D. GBM Spheroid Histology

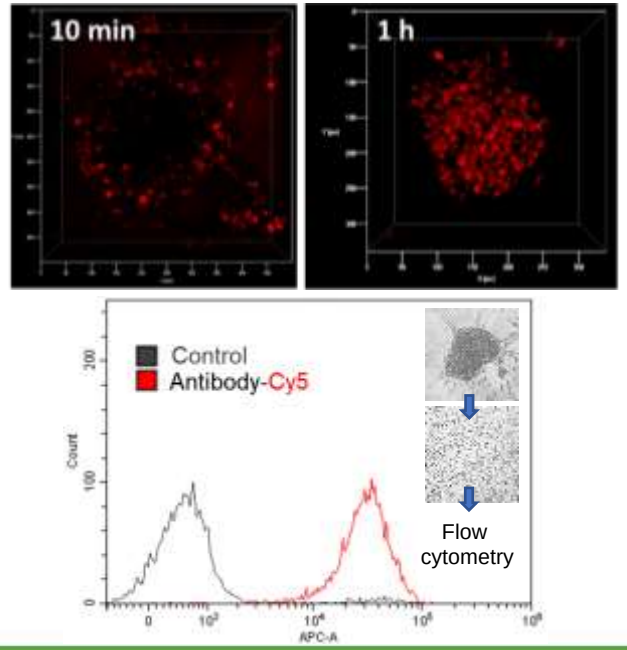


Antibody-Cy5

B. Spheroid Viability

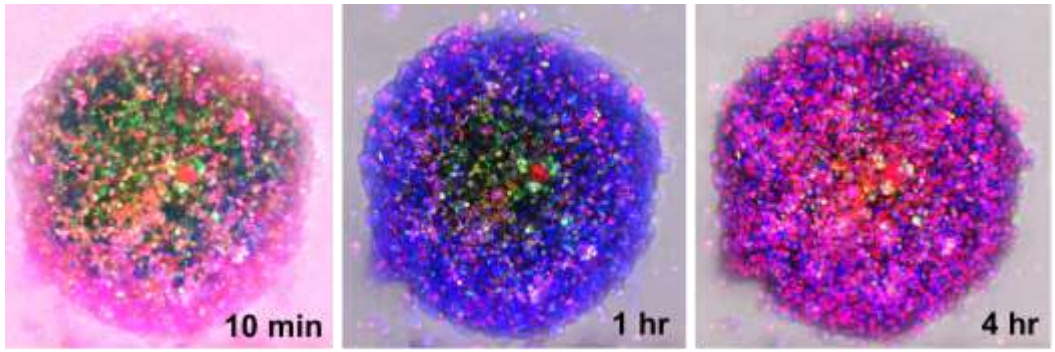


C. Antibody-Cy5 uptake



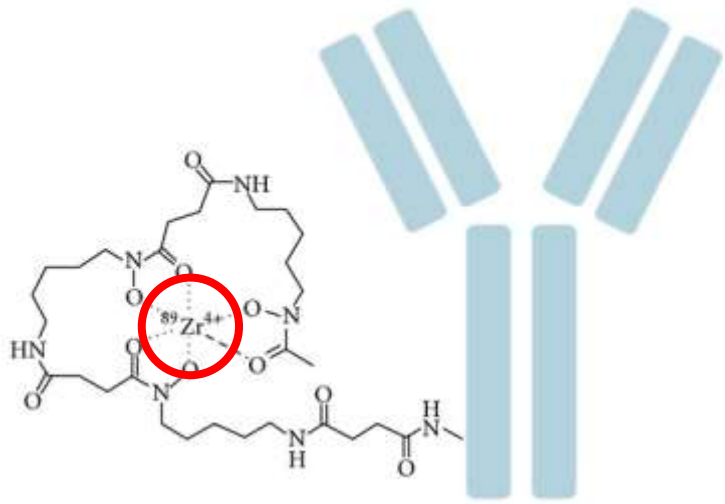
E. GBM-Microglia Multicellular Spheroids in a dynamic microfluidics model

Thanks: Amber Prior



GBM cells– Antibody-Cy5
Cell nucleus – Hoechst
Microglia – CellTrace CFSE
Dead cells – Propidium Iodide

Chelation of Miltuximab® with DFO

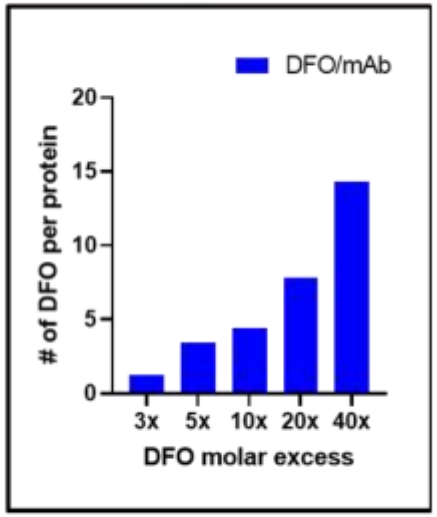


Antibody-Desferoxamine (DFO) Complex with [^{89}Zr]

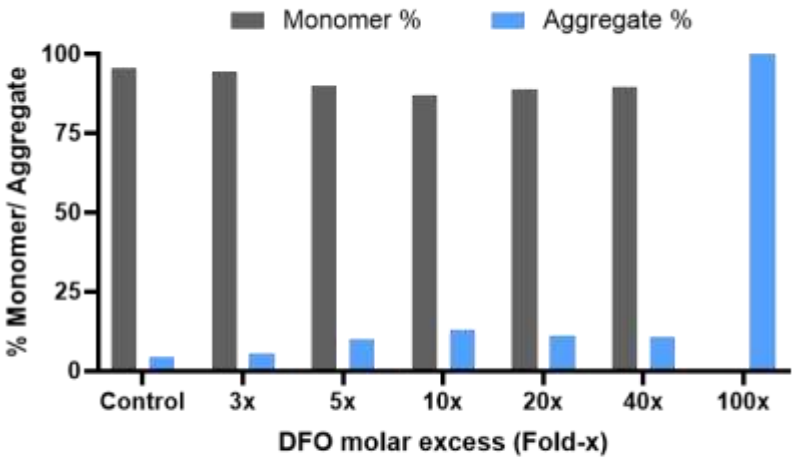
^{89}Zr - Characteristics & Advantages

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

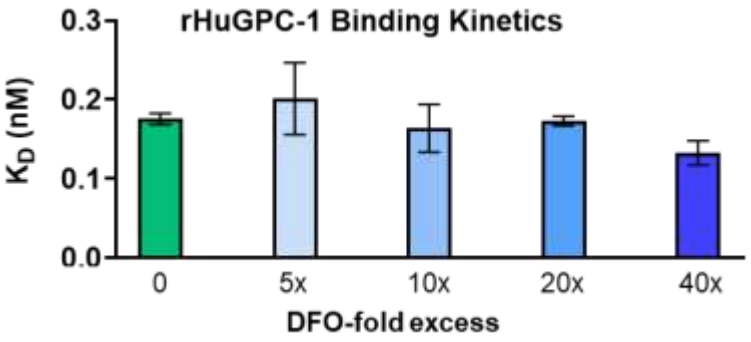
1. Antibody-DFO chelation



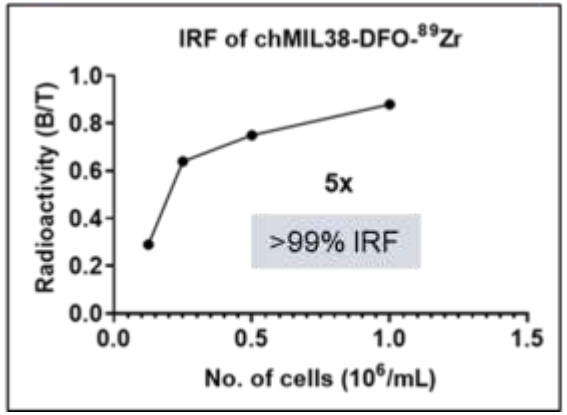
2. Stability – SEC-HPLC



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

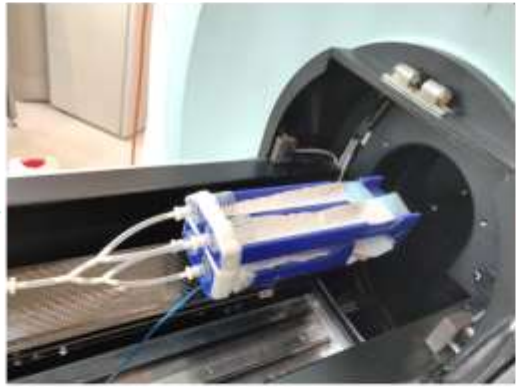
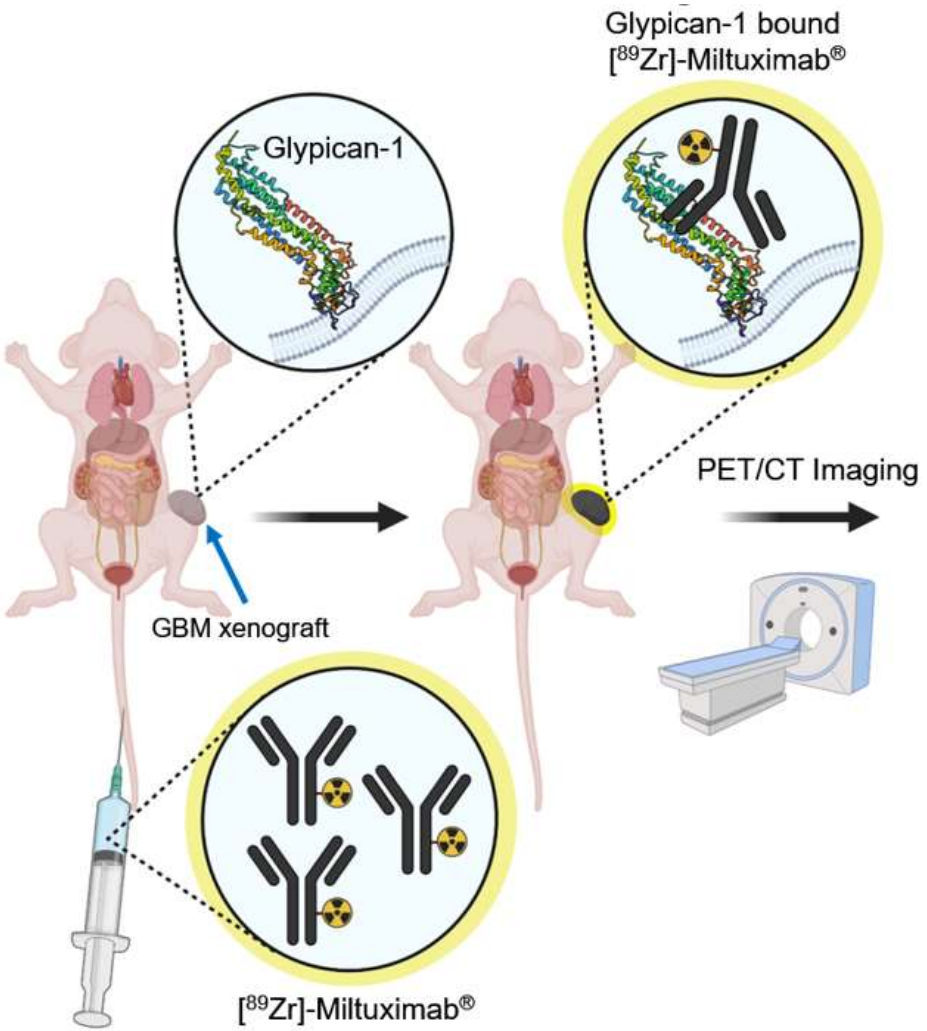


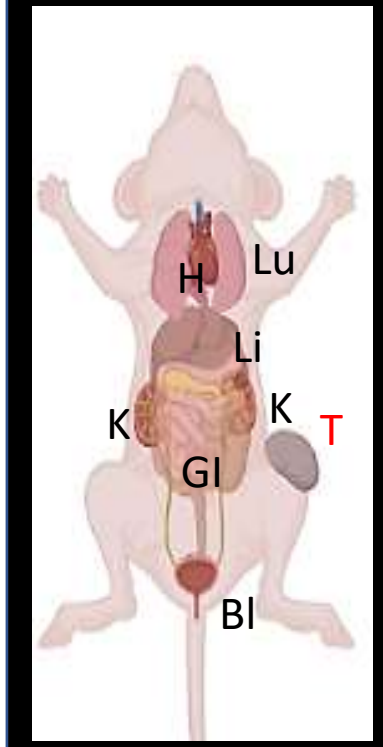
Image acquisition & processing

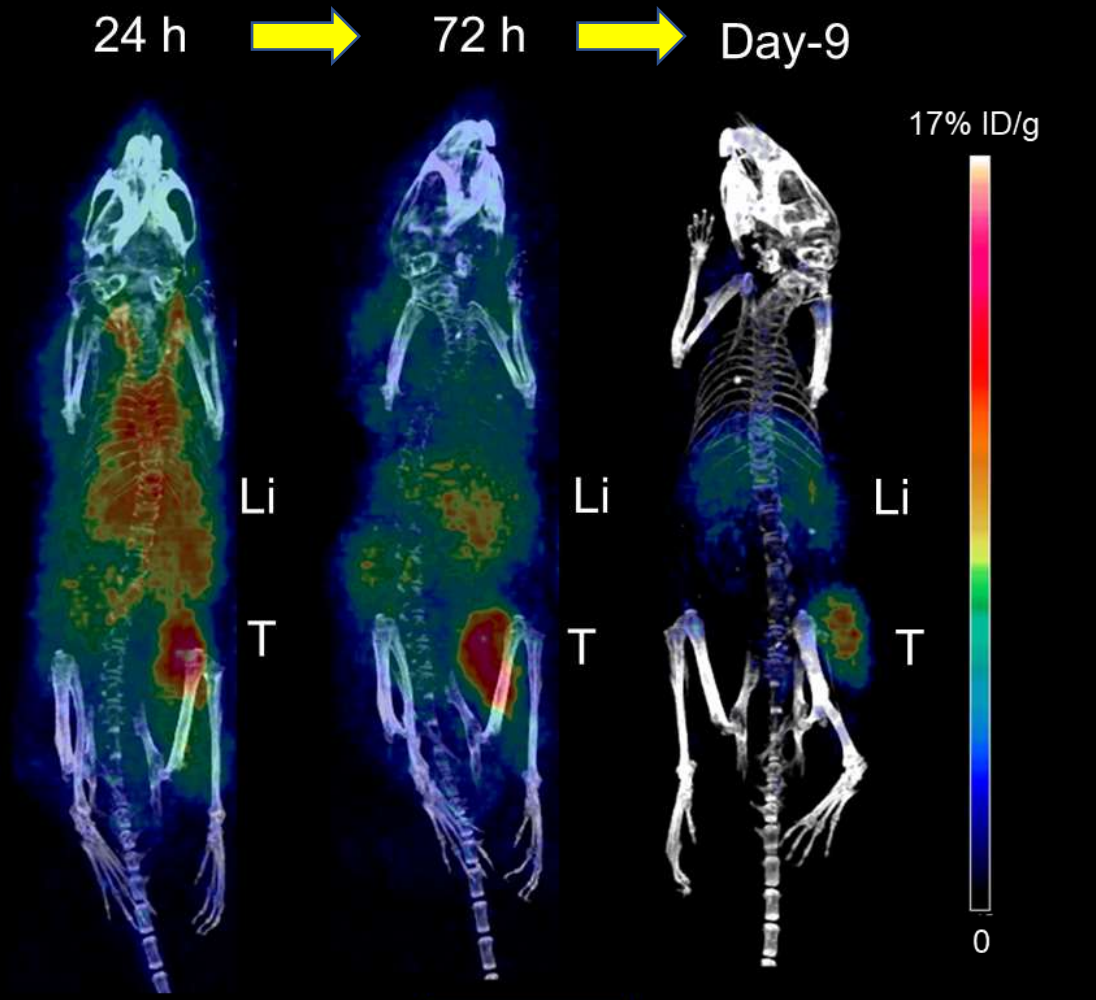
Ex-vivo organ harvest

Gamma counter for BioD

In vivo biodistribution of [^{89}Zr]-Miltuximab®

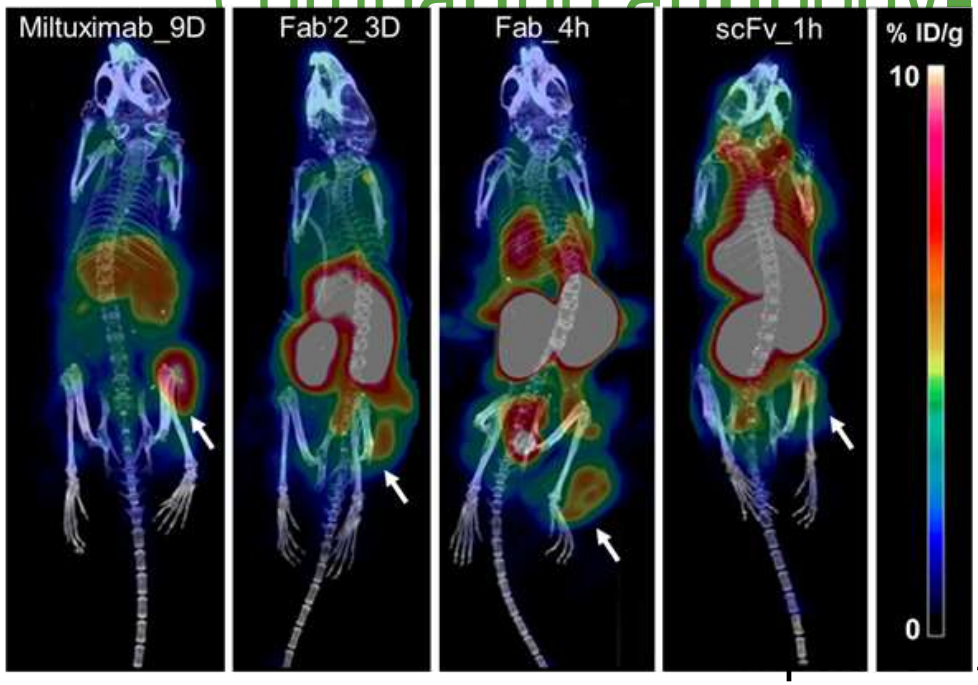


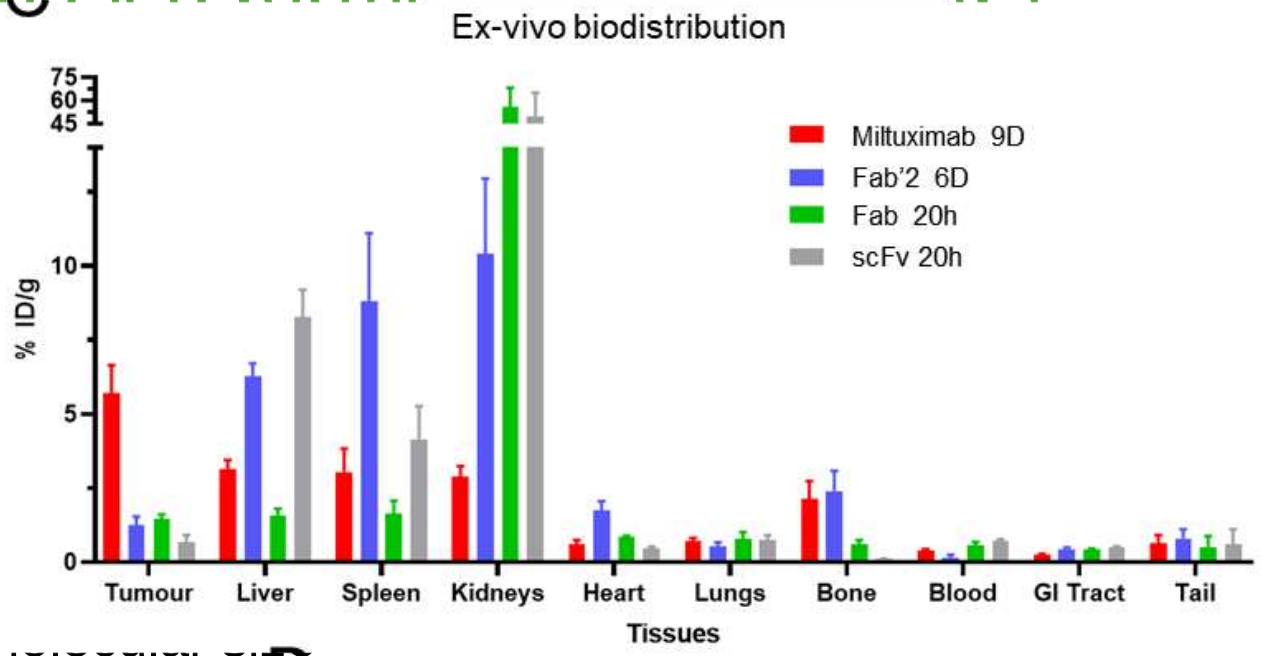


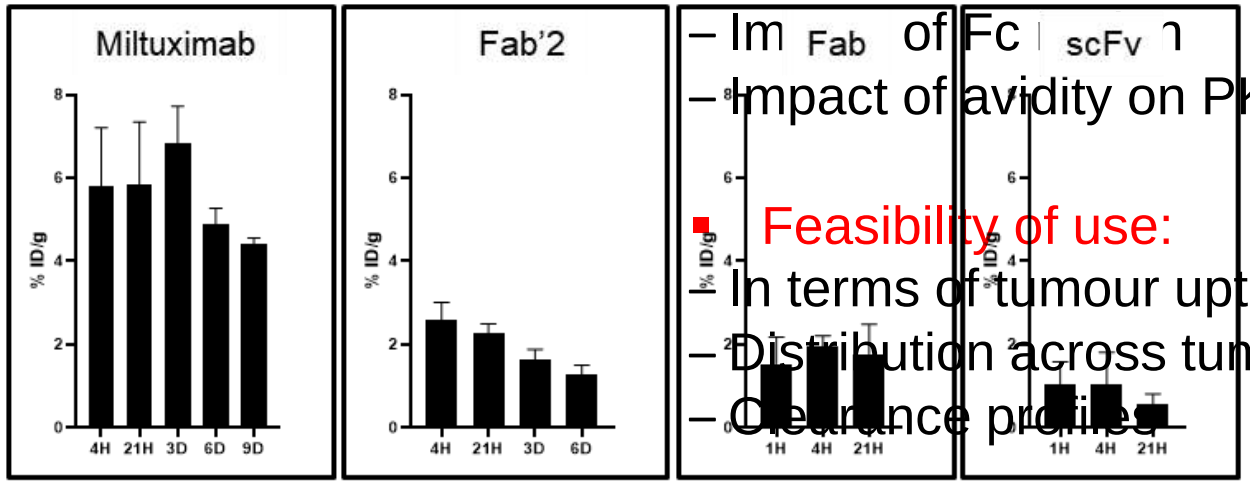


Ghosh et al., Mol Pharm 2023

Comparing antibody-based formats as PET agents

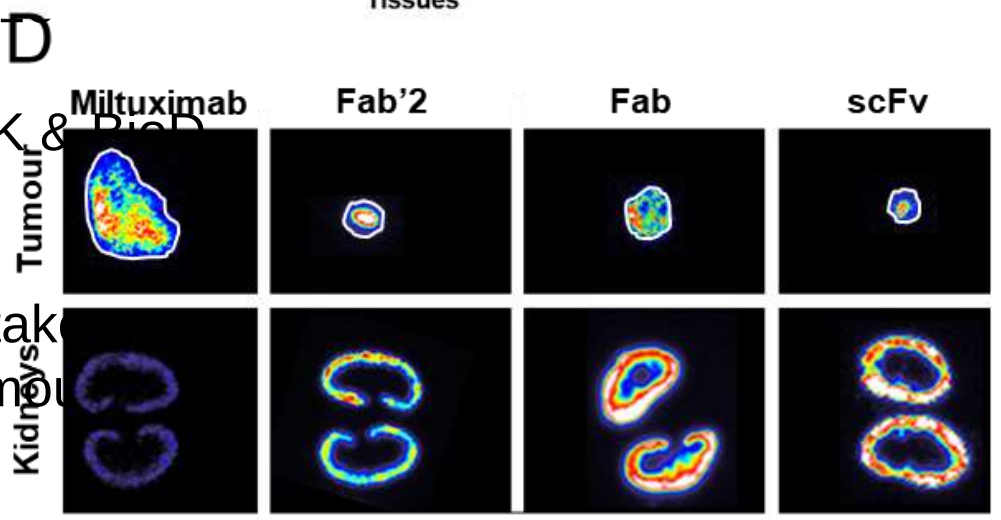






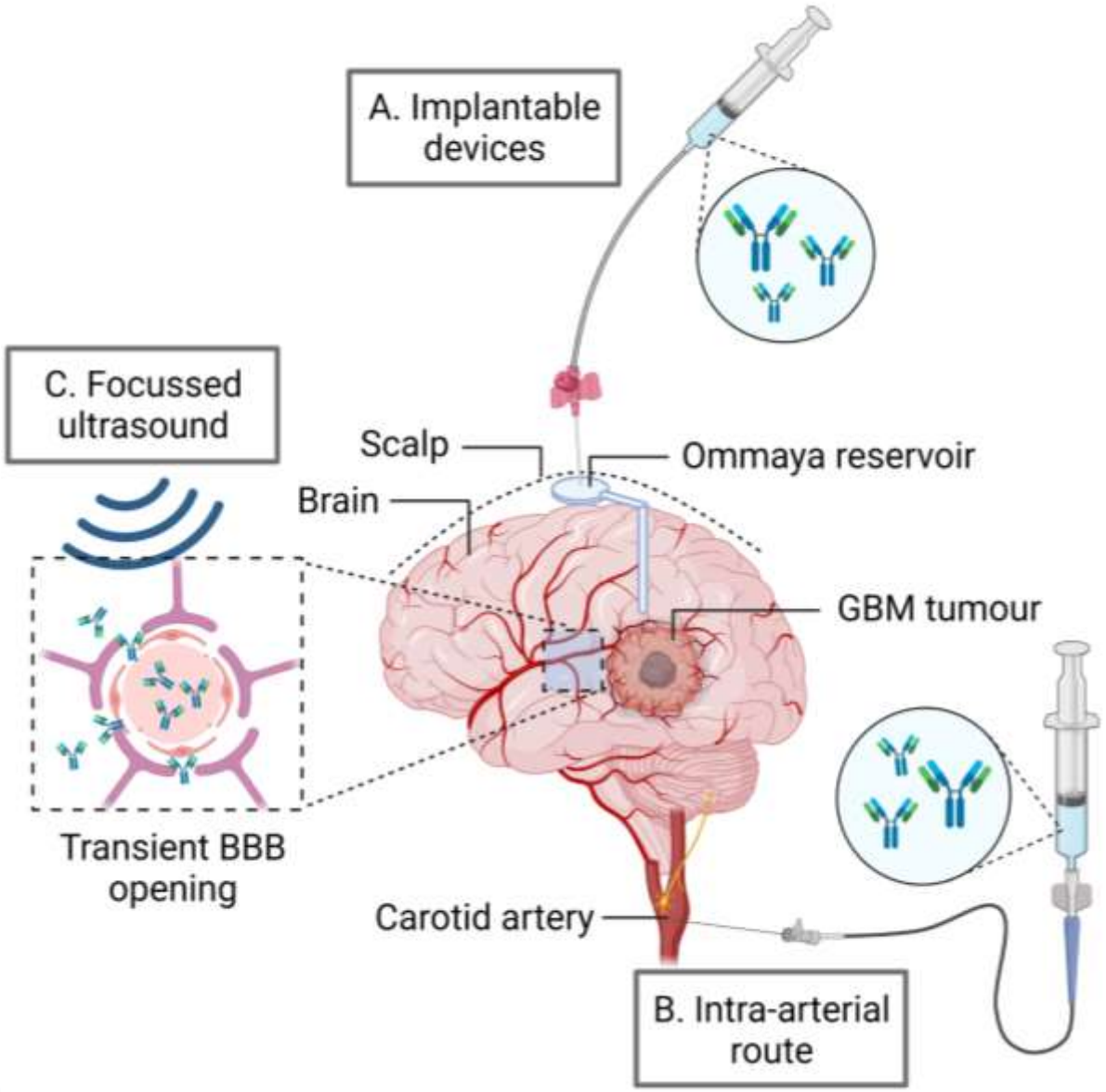
- Im Fab of Fc scFv n
- Impact of avidity on PK & BioD

- Feasibility of use:
- In terms of tumour uptak
- Distribution across tumou
- Clearance profiles



Ghosh et al., Mol Pharm 2023

Novel antibody delivery strategies to the brain



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

Thank you!!

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An ARC Industrial Transformation Training Centre

GlyTherix

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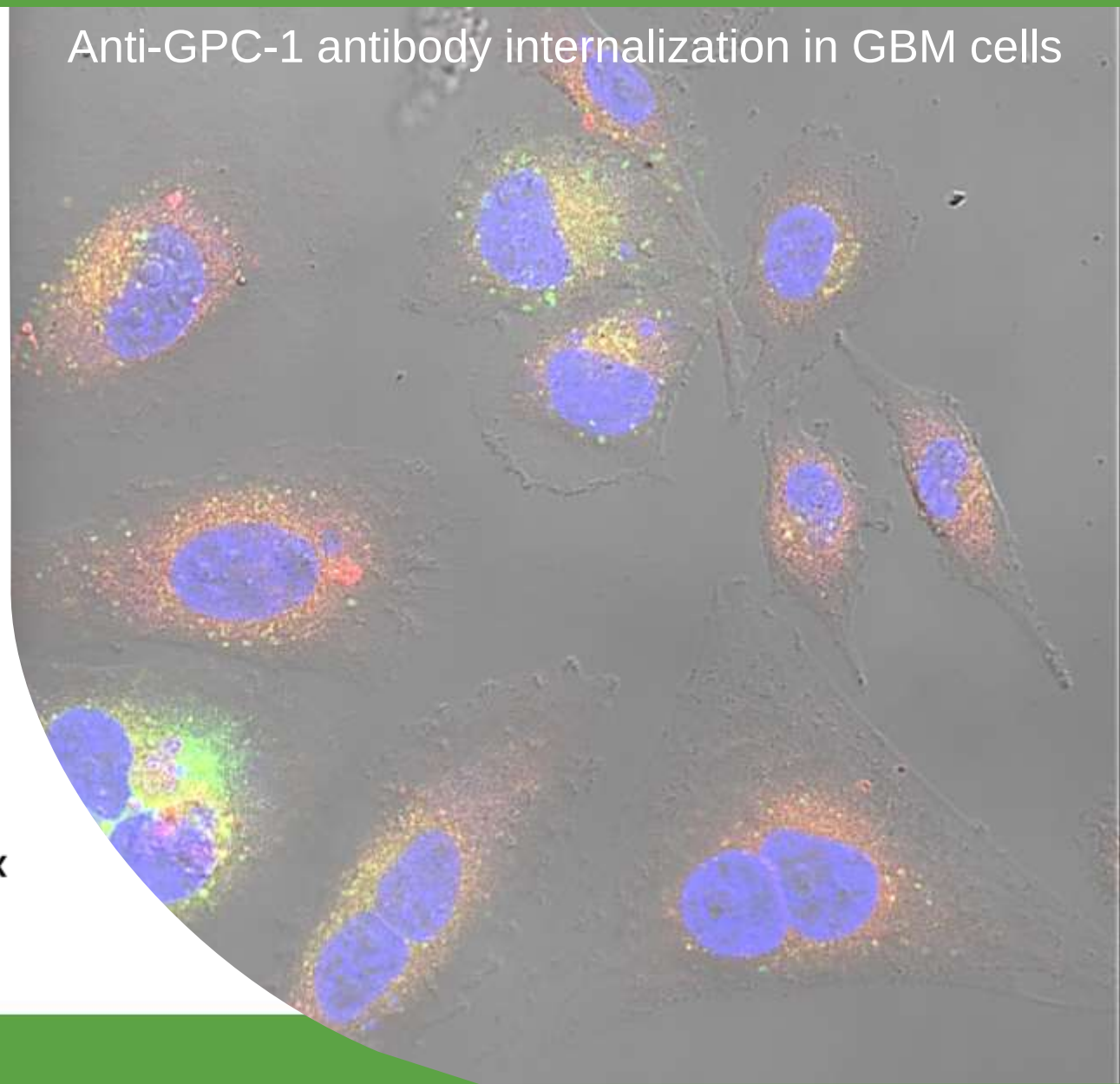
Anti-GPC-1 antibody internalization in GBM cells