

# Exploring the potential of **single domain antibodies** for cancer immunotherapies and imaging

Patrick Chames



**CRCM**

Antibody Therapeutics and Immunotargeting

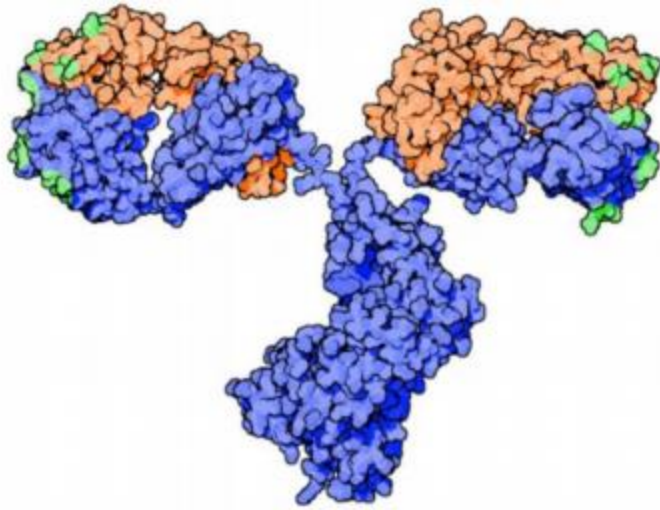
[Patrick.chames@inserm.fr](mailto:Patrick.chames@inserm.fr)

# Made in Marseille

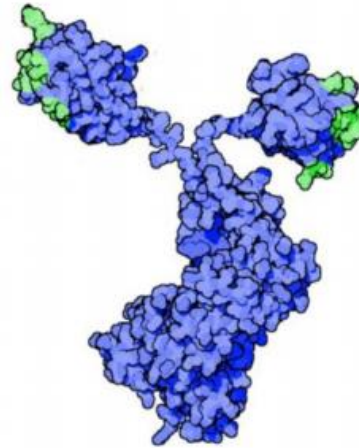




# Llama antibodies and Nanobodies



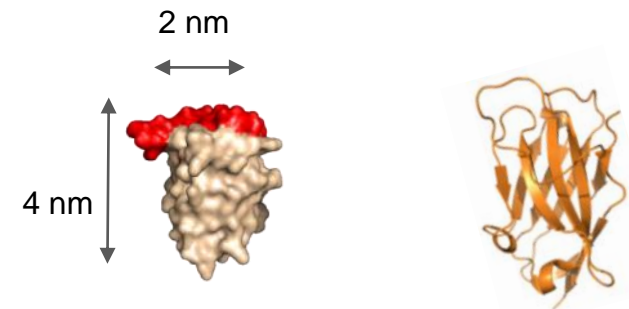
Conventional IgG



Heavy chain only IgG

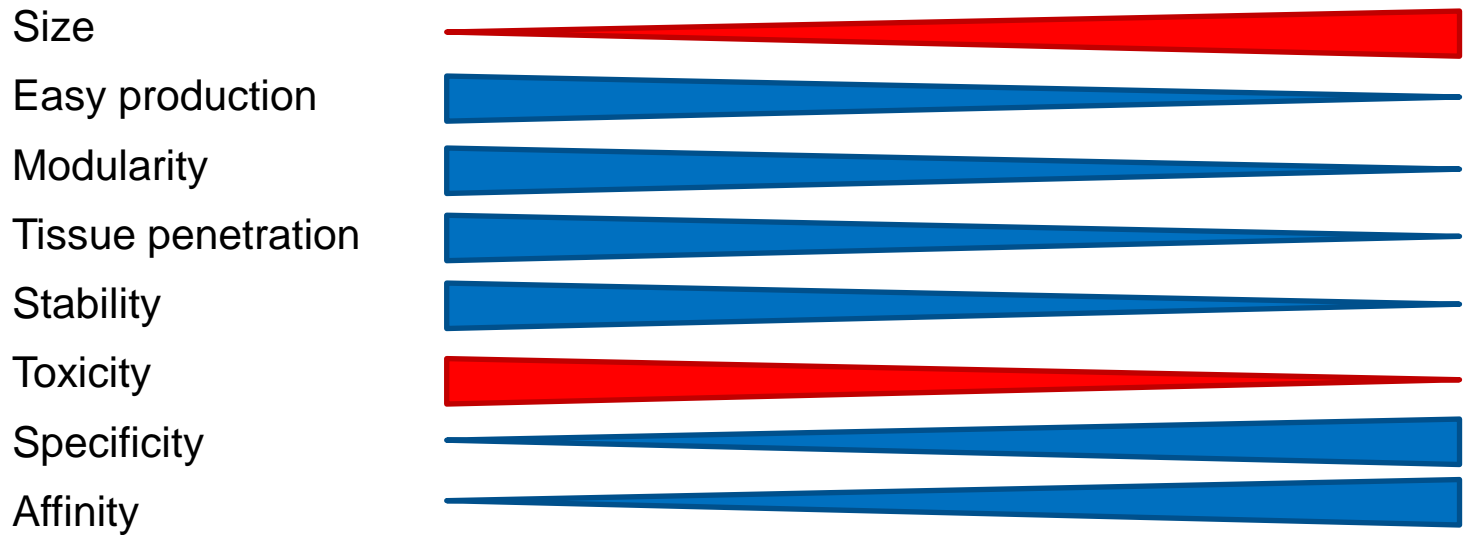
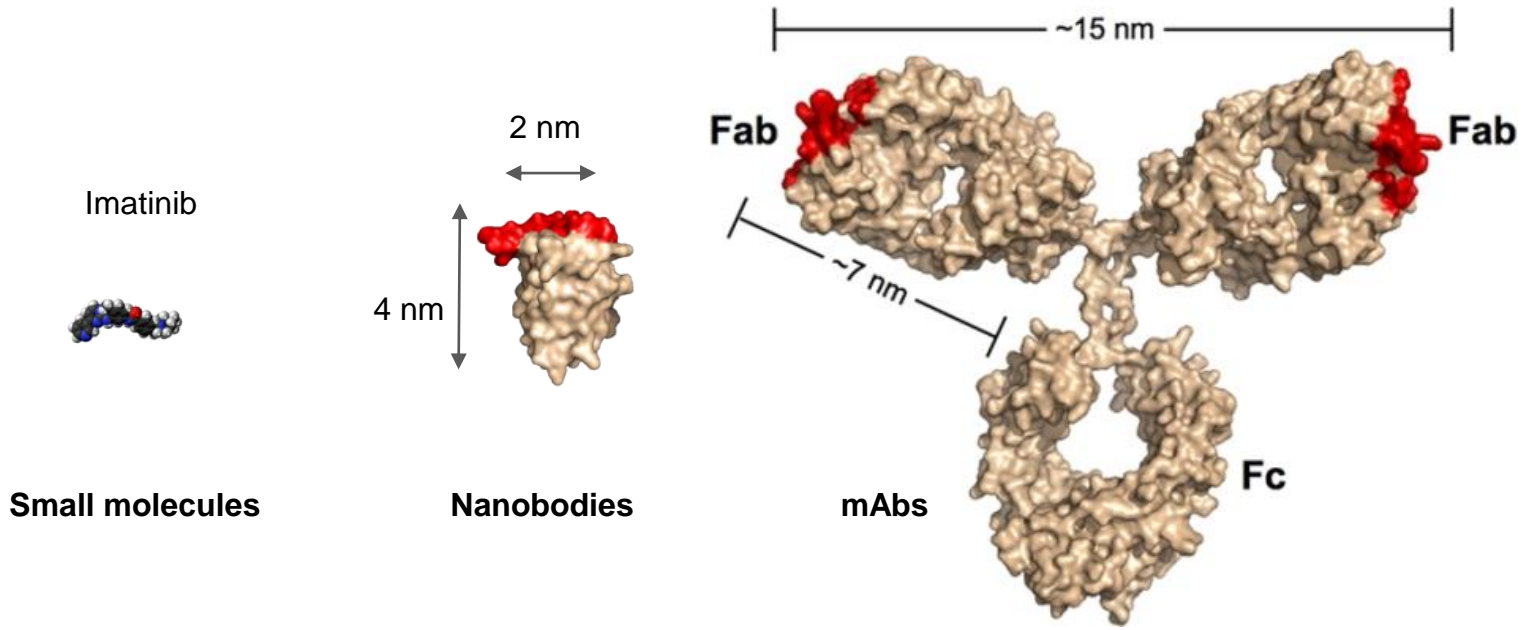


- **Small** (13 kDa), easy to clone and very efficiently produced (E. coli)
- Very **stable**
- Bind to **different epitopes** (cavities)
- **High** sequence **similarity** with **human** VHIII gene family



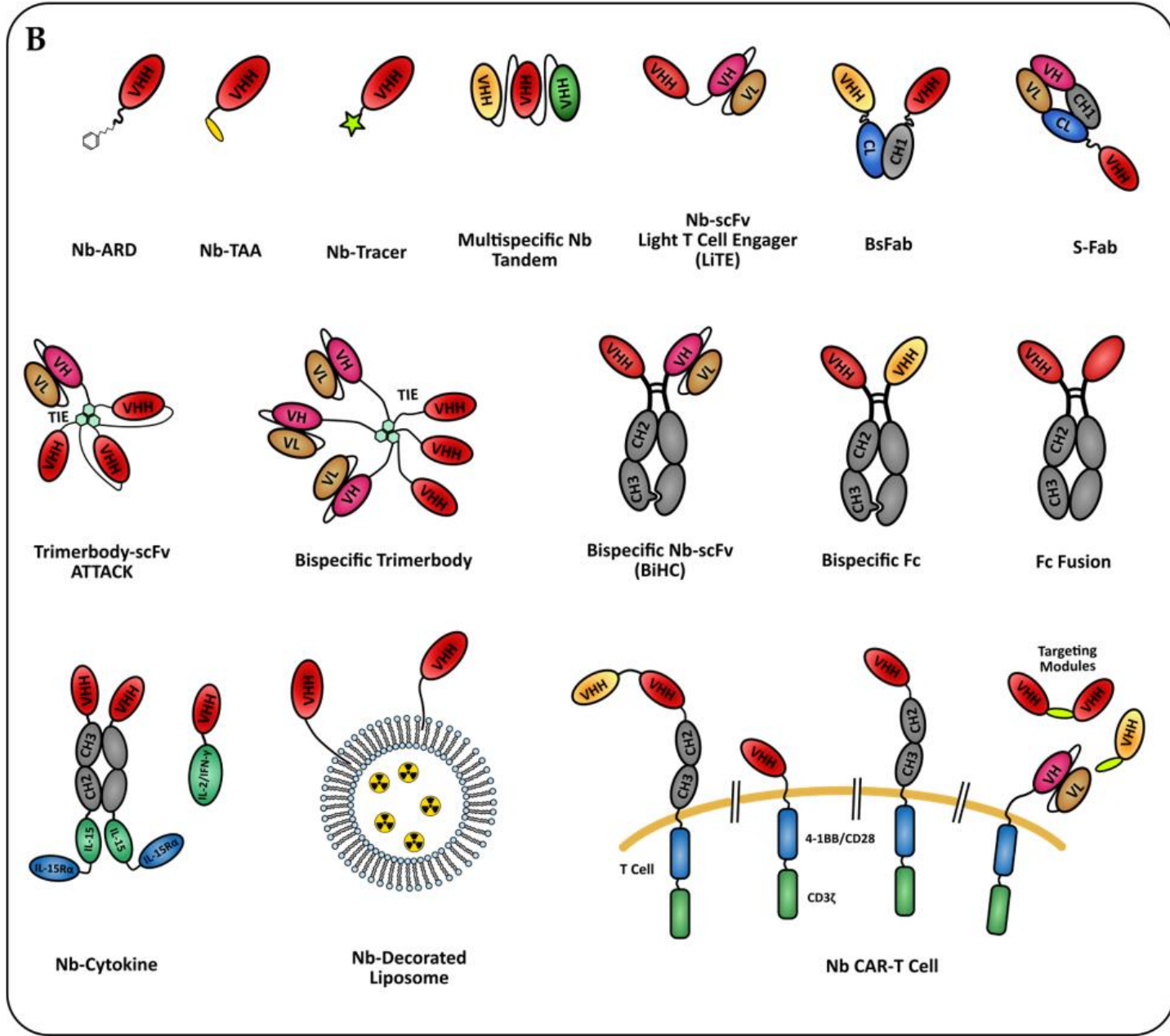
**Single domain antibodies (sdAb) or Nanobodies (Nb)**

# Nanobodies : best of both worlds ?

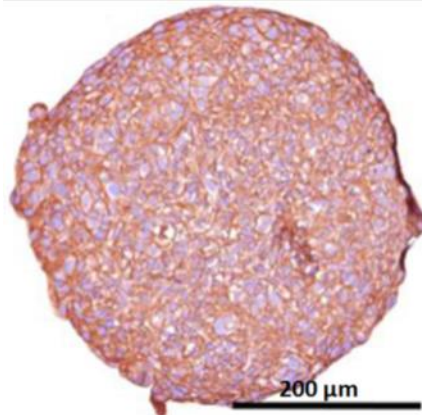


# Nanobody modularity

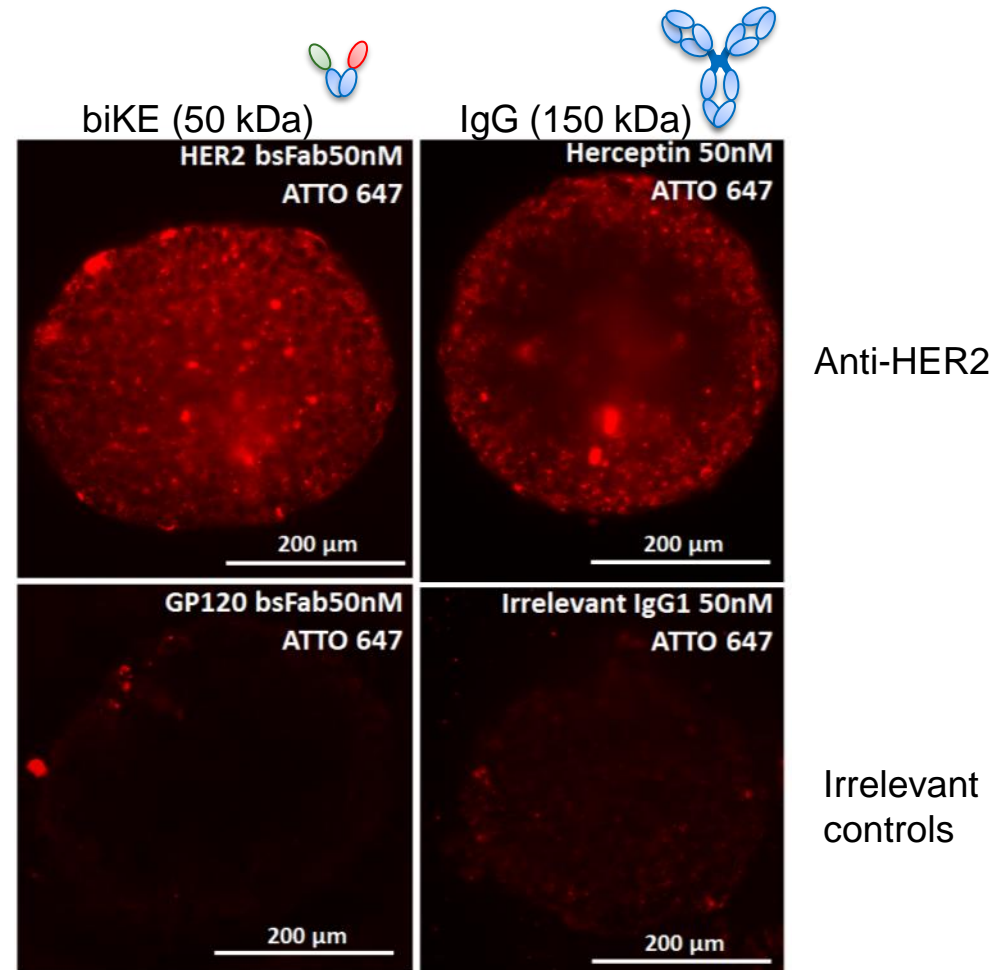
Chanier, T.; Chames, P.  
*Nanobody Antibodies (Basel)*  
2019, 8 (1).



## HER2 expression by IHC



BT474 spheroid



mAb and biKEs labeled with **ATTO 647**

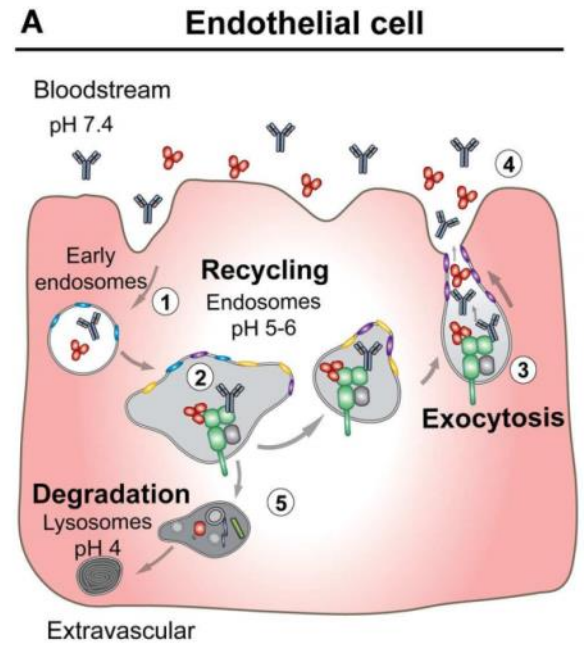
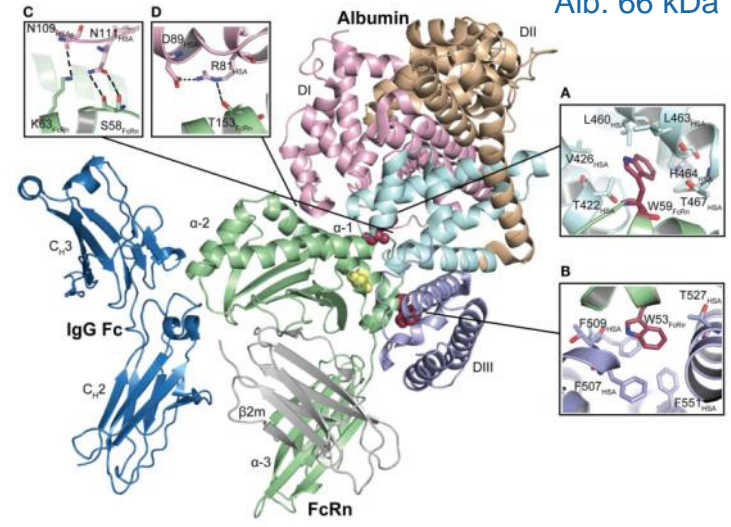
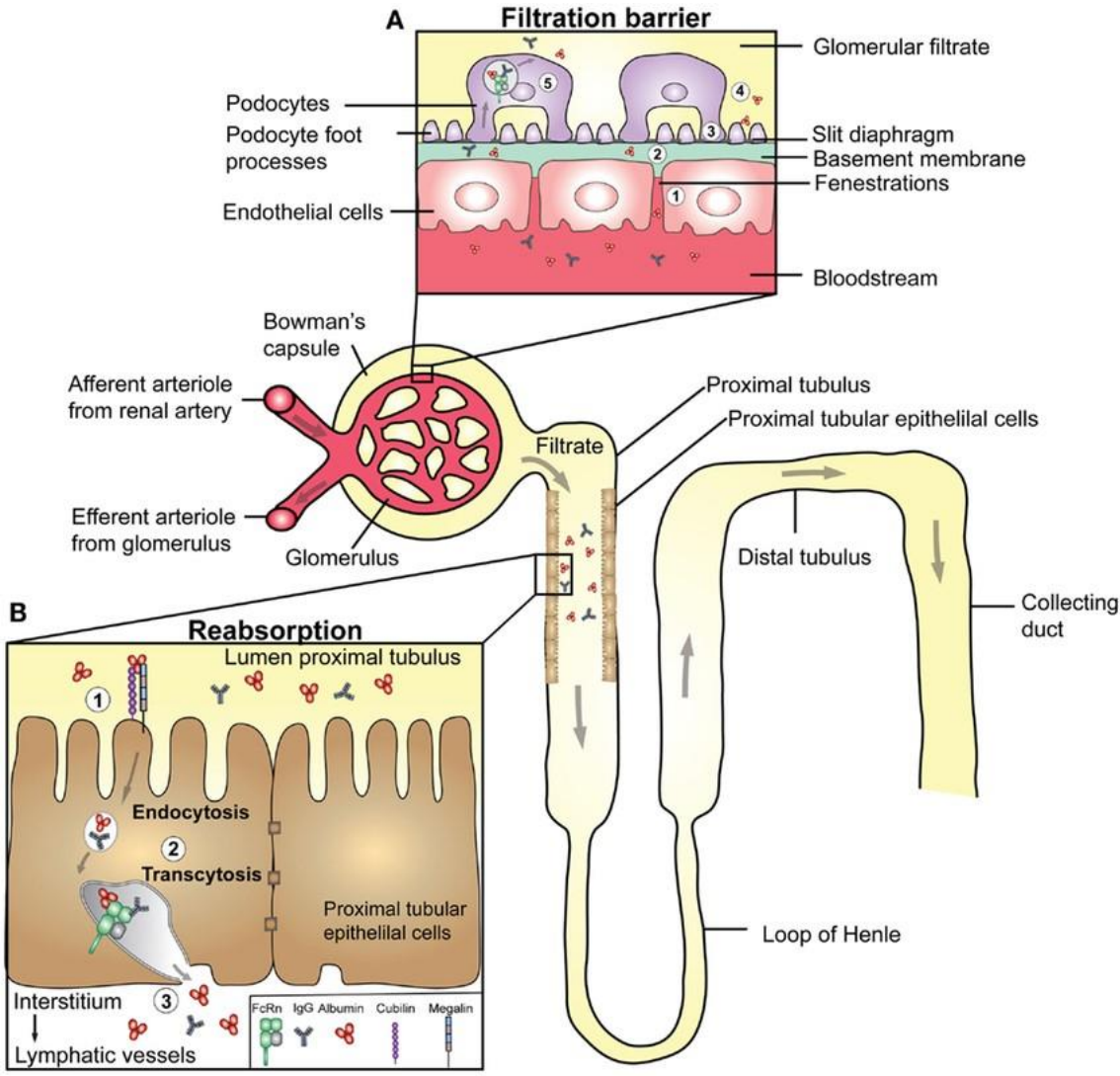
- ✓ **Specific retention** of **HER2 bsFab** and **Herceptin** into spheroid
  - ✓ BiKE accumulation is **more homogenous**



# FcRn, albumin, size and Serum Half Life

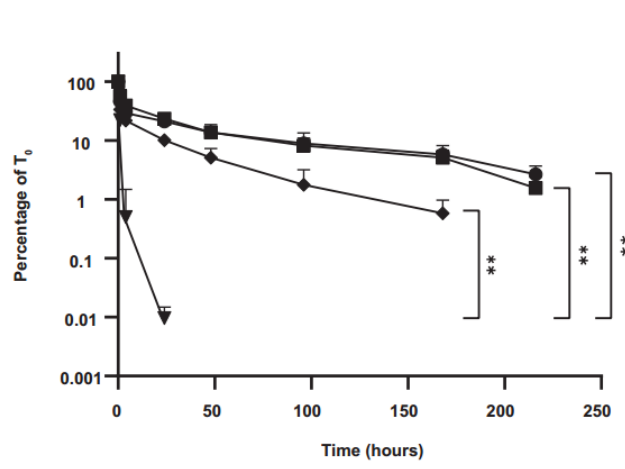
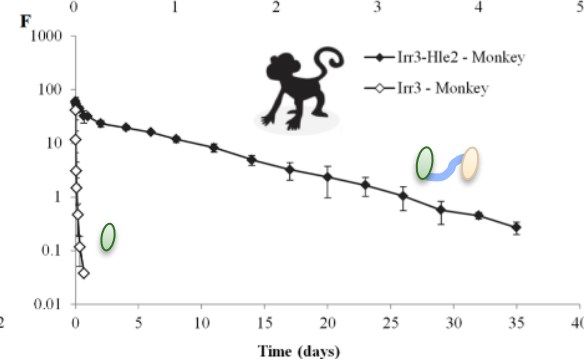
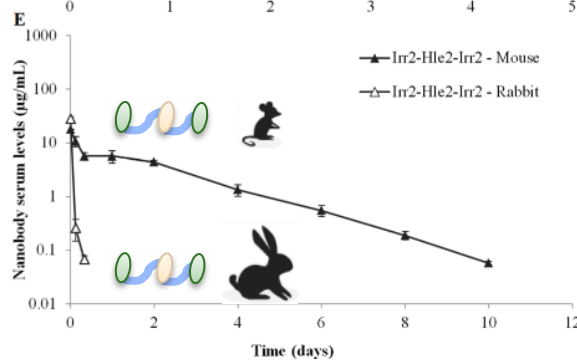
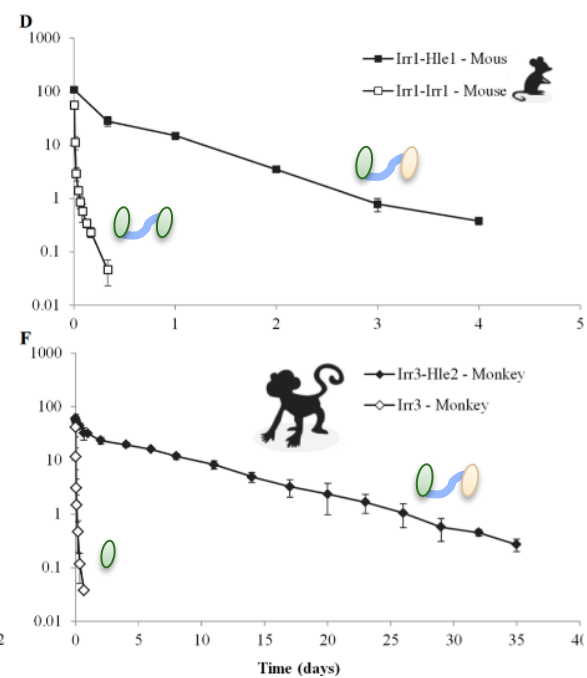
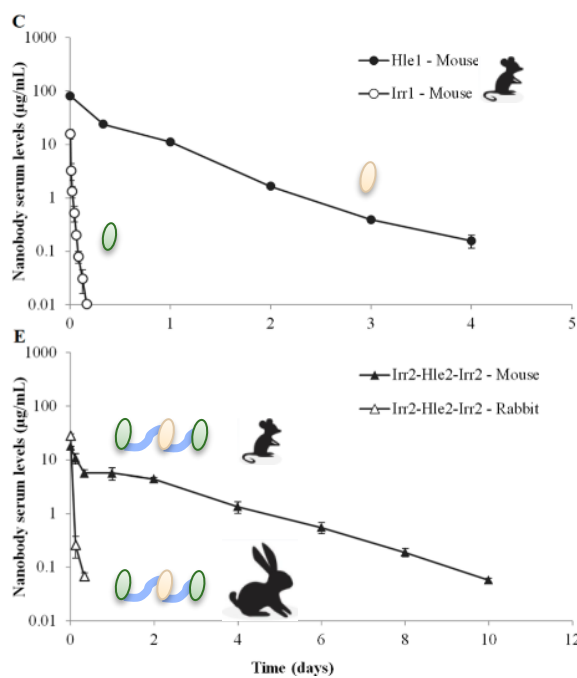
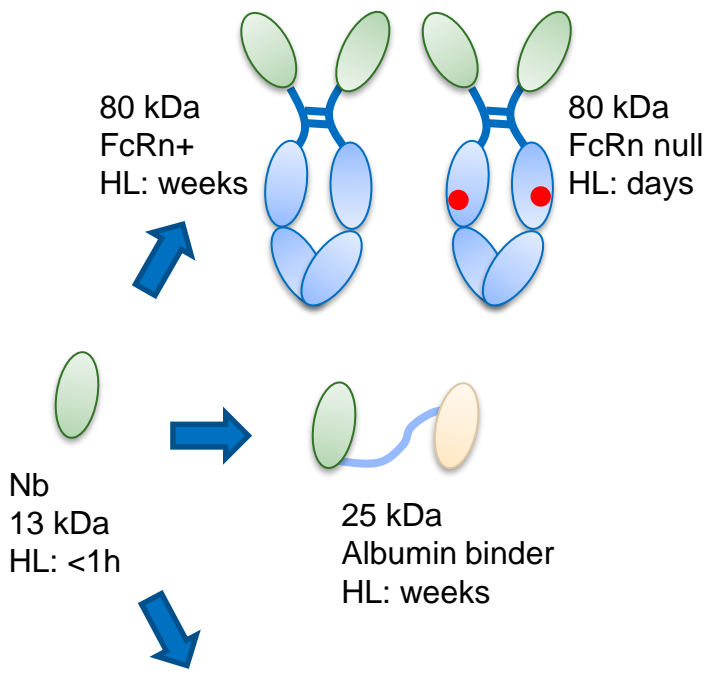
Renal clearance threshold : 60-70 kDa

Alb: 66 kDa



# Half life extension strategies

Hoefman et al., 2015



Construct	$T_{1/2}$ hours	$R^2$
LITE	~0.59	0.99
Albu-LITE-NB	18.68	0.99
Albu-LITE-WT	25.90	0.98
Albu-LITE-HB	37.20	0.98

human FcRn and human albumin tg mouse model (AlbuMus)

Construct	Anti-EGFR	Anti-CD3	Albumin	FcRn affinity	MW kDa
LITE bispecific	Yes	Yes	No	-	49
Albu-LITE-NB	Yes	Yes	Yes	Null	108
Albu-LITE-WT	Yes	Yes	Yes	Wild Type	108
Albu-LITE-HB	Yes	Yes	Yes	High	108





Can we manipulate the **cancer cell / NK** immune synapse ?

Can we manipulate the **cancer cell / T** immune synapse ?

Can we enhance cancer cell **detection ex vivo** ?

Can we enhance cancer cell **detection in vivo** ?



Can we manipulate the **cancer cell / NK** immune synapse ?

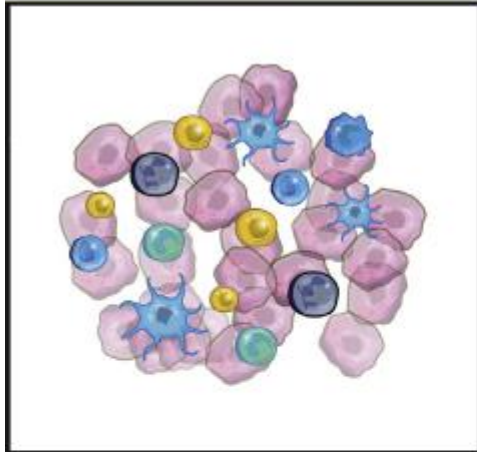
Can we manipulate the cancer cell / T immune synapse ?

Can we enhance cancer cell detection ex vivo ?

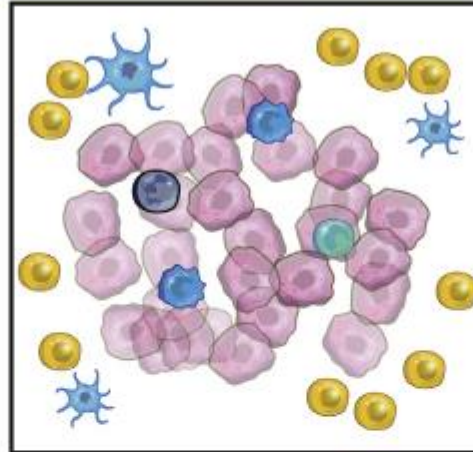
Can we enhance cancer cell detection in vivo ?

# Cold tumors vs hot tumors

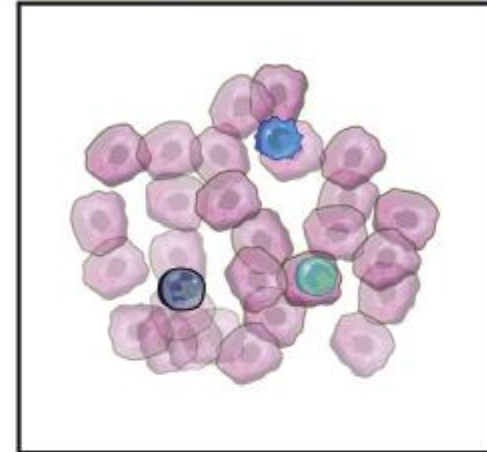
Hot (inflamed) tumor



Cold (excluded) tumor

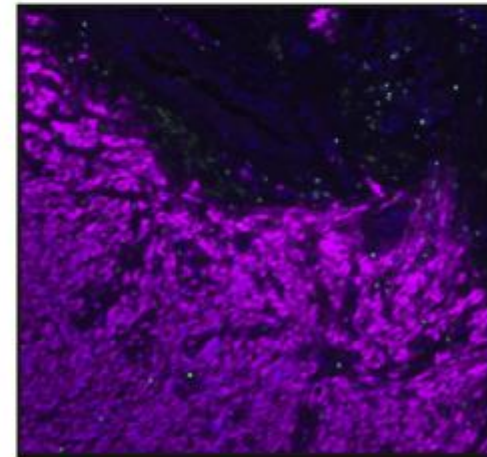
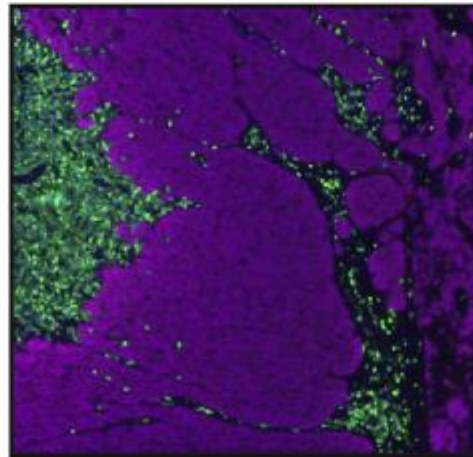
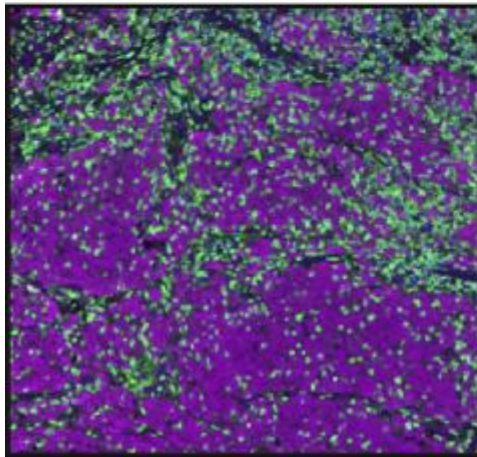


Cold (ignored) tumor



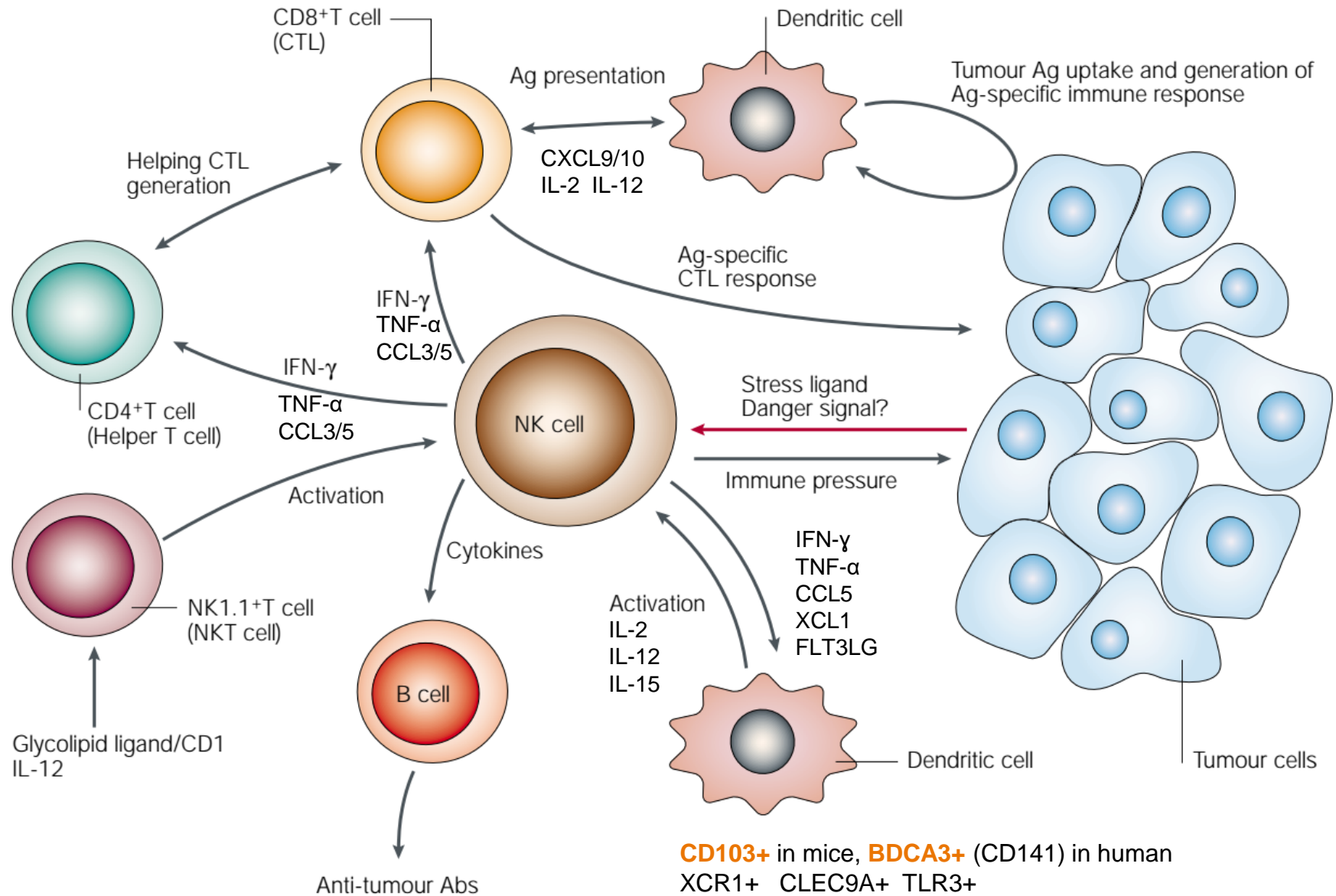
T cells

Tumor

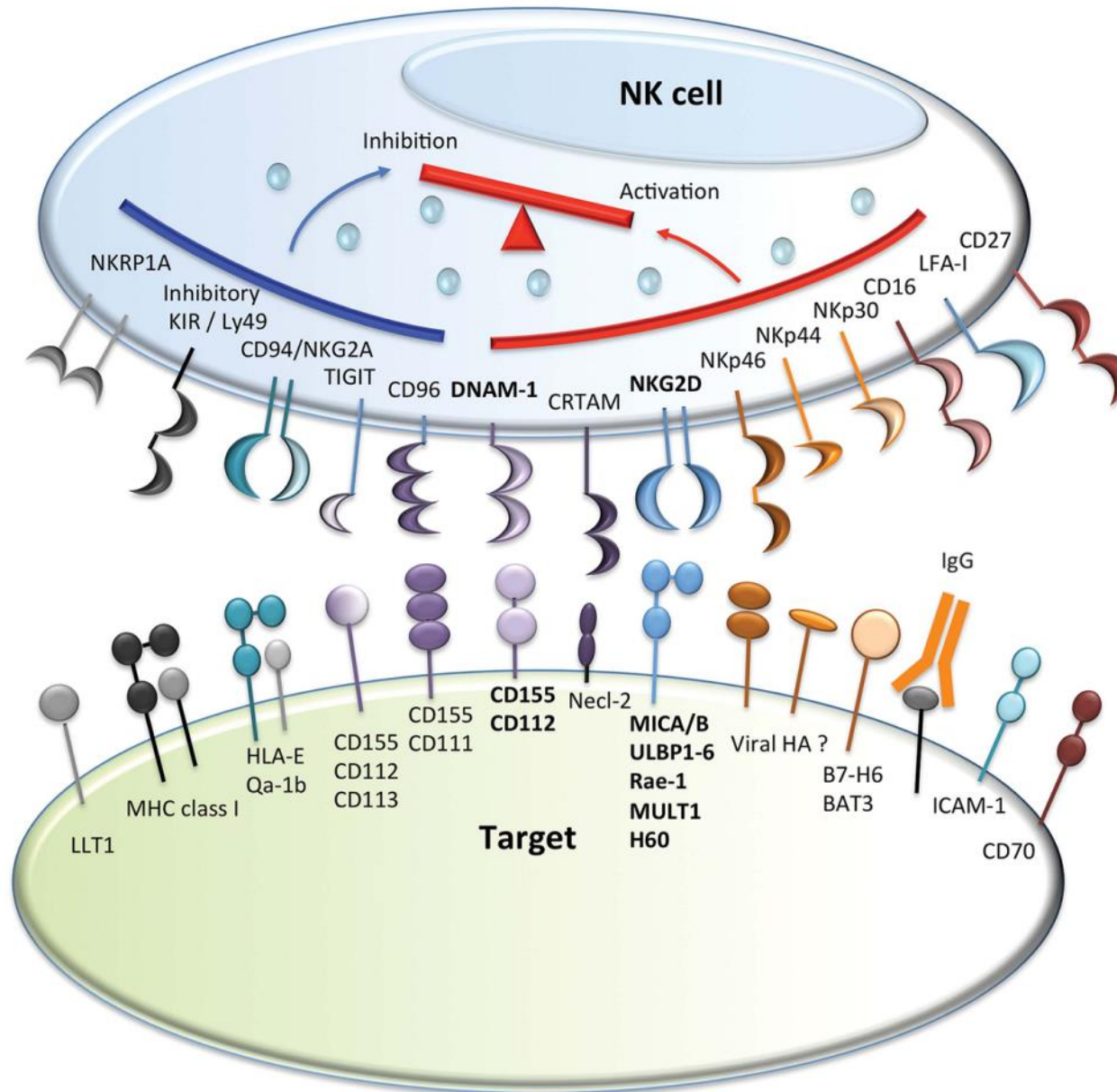




# NK cells and immune response to cancer



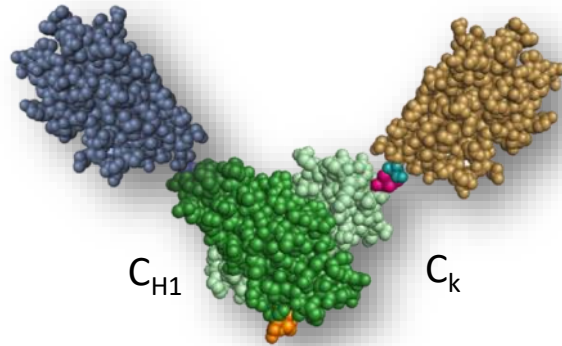
# Recruiting NK cells



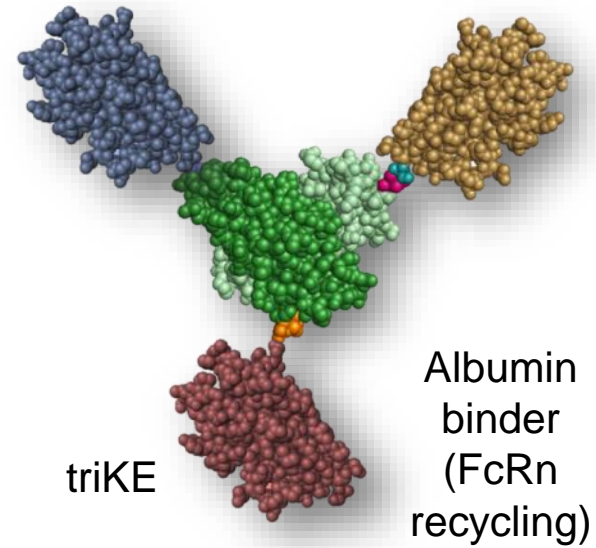
# Small NK Engagers

- Small format with long half life for efficient tumor penetration

Nb 1 Anti-tumor marker      Nb 2 Anti-activating NK receptor



$C_{H1}$        $C_k$   
biKE

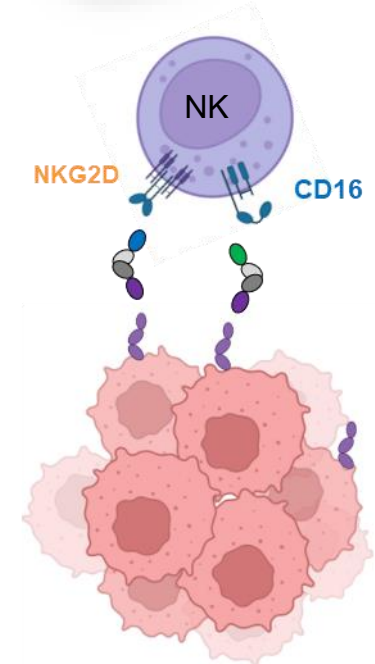
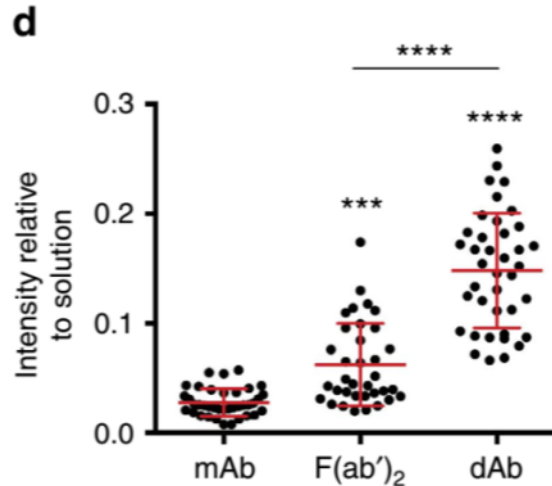
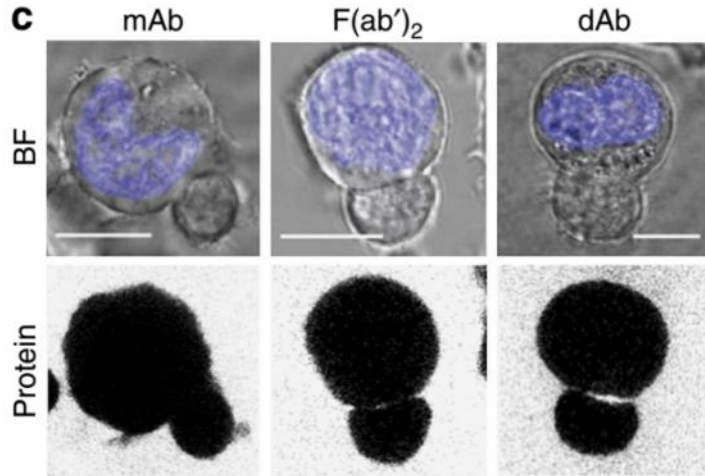


Albumin binder (FcRn recycling)  
triKE

WO/2006/064136.  
EP10306541.3  
US 61/428,294

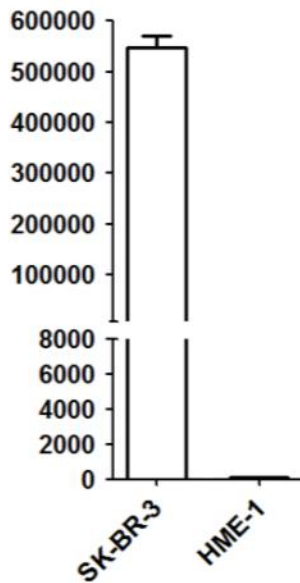
Rozan et al., Mol. Cancer Ther (2013)

- Better access to immunological synapses

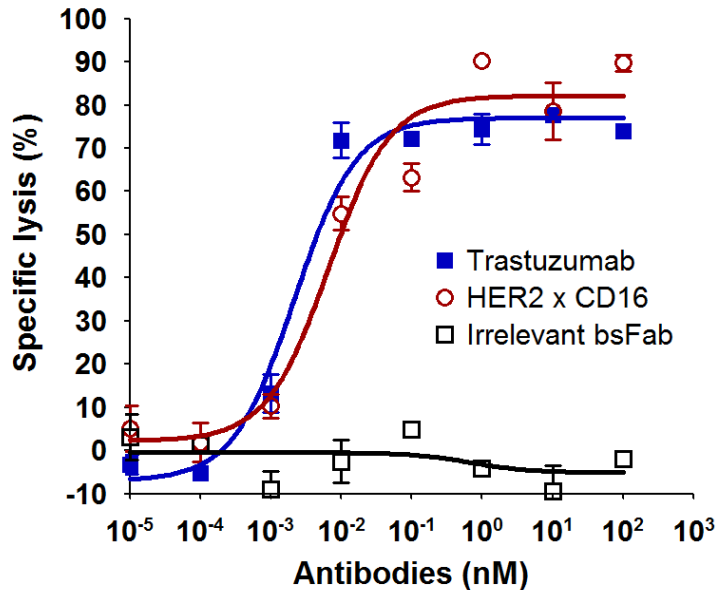




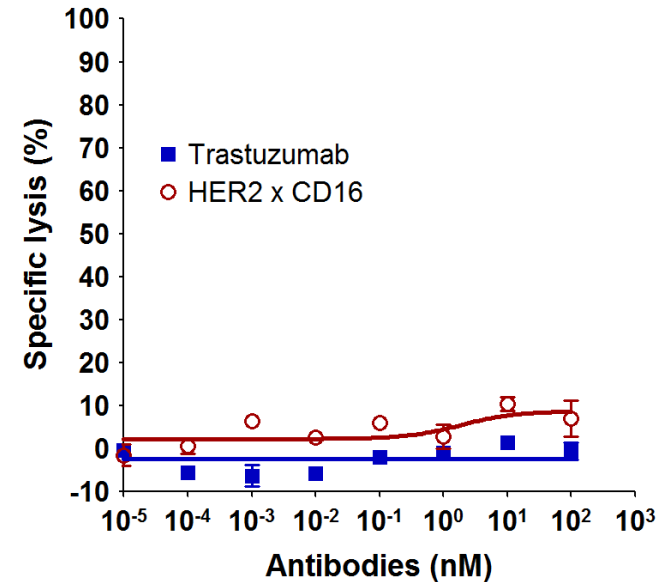
## HER2 binding capacity



## SK-BR-3 (HER2<sup>High</sup>)



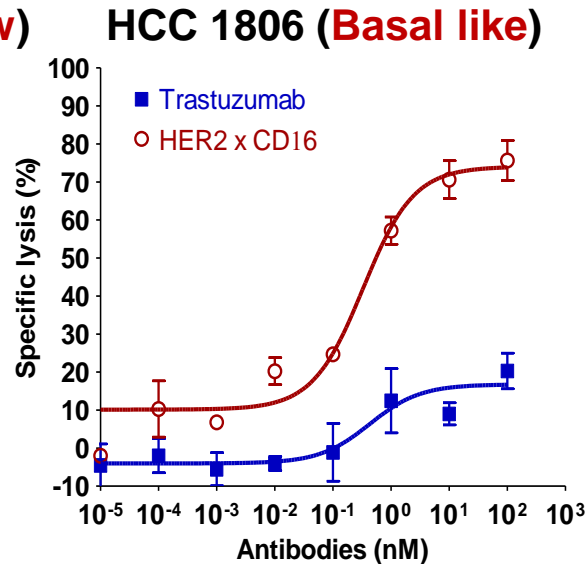
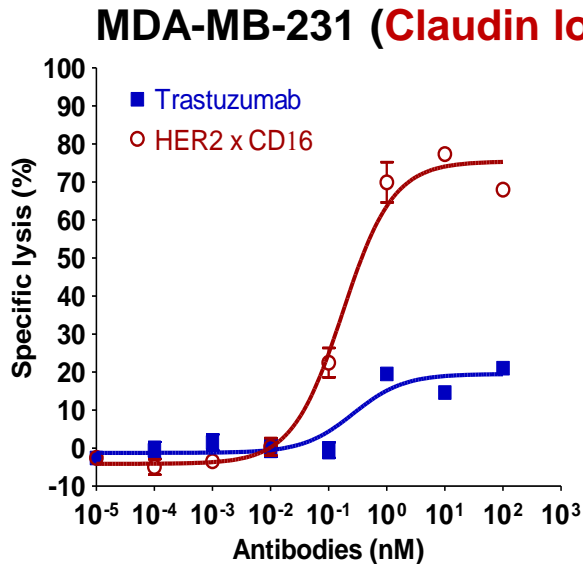
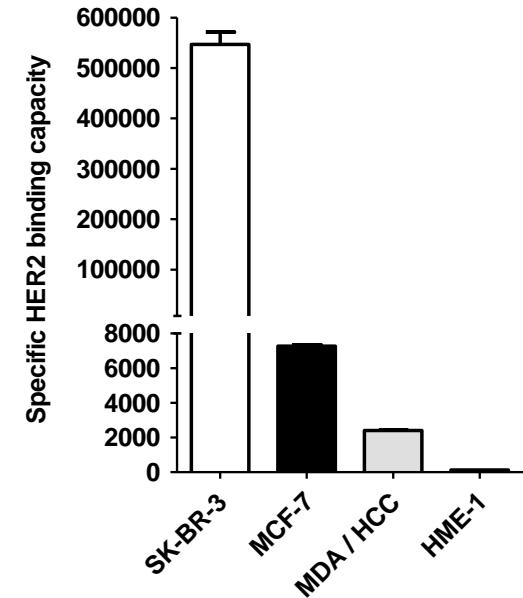
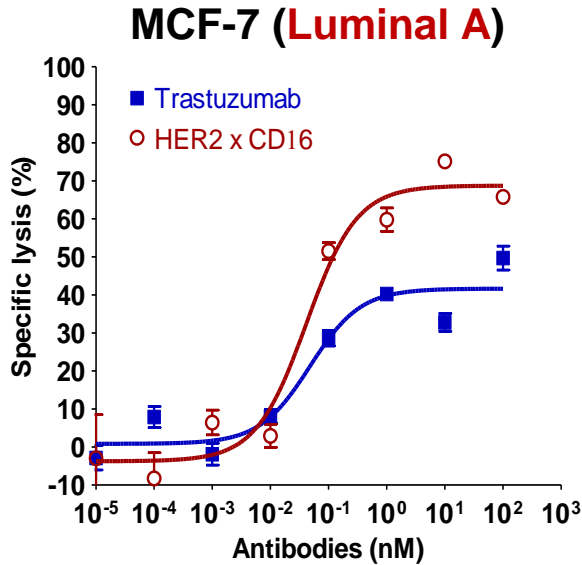
## HME-1 (normal breast epithelial)



HER2 x CD16 mediates similar ADCC than Trastuzumab on HER2<sup>high</sup> breast cancer cells in vitro

# In vitro cytotoxicity assay on $HER2^{low}$ breast cancer cells

ATP release assay

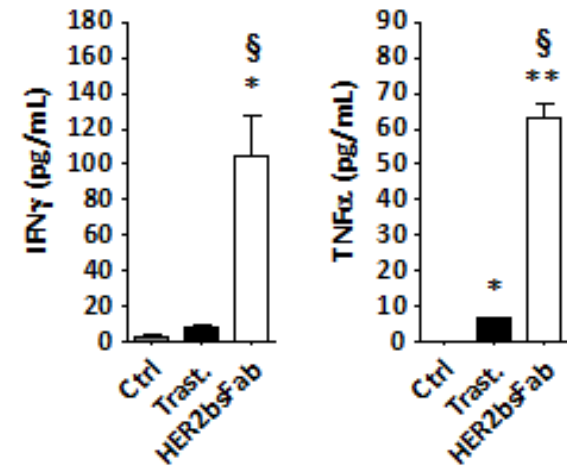
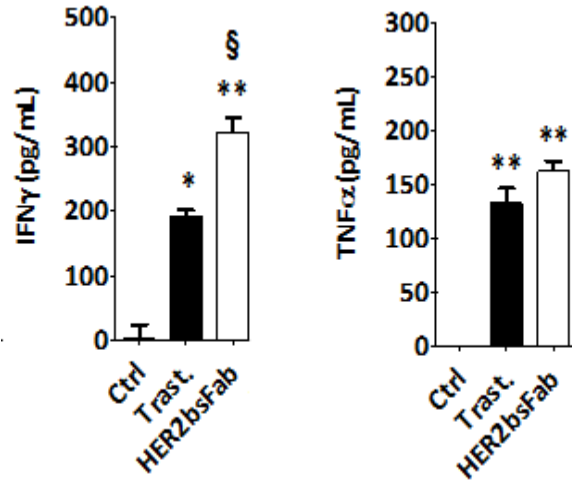


HER2 x CD16 elicits a stronger ADCC on  $HER2^{low}$  cell lines compared with Trastuzumab

# BiKEs lead to stronger cytokine release

## SK-BR-3 (HER2<sup>High</sup>)

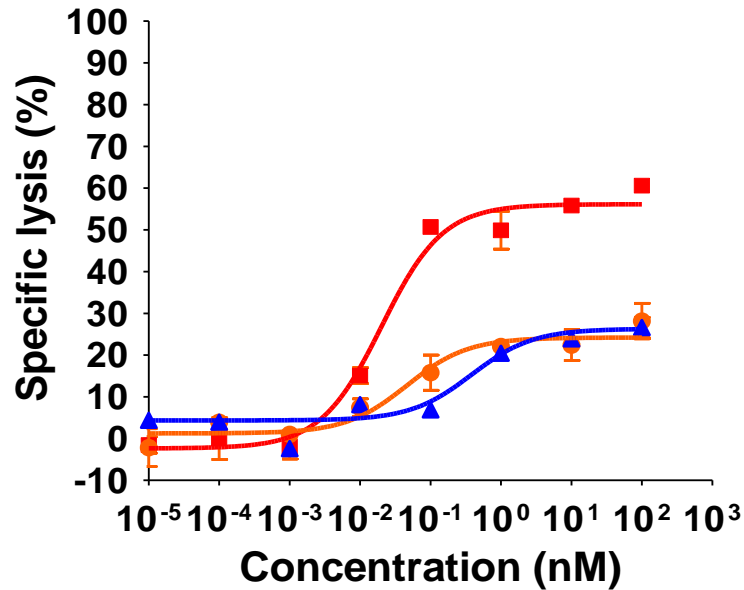
## MCF-7 (HER2<sup>Low</sup>)



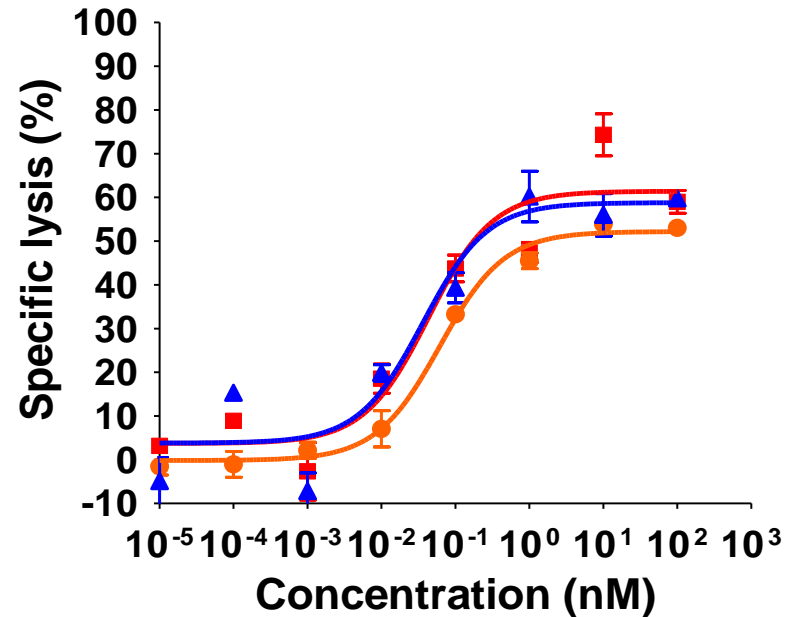


# BiKE Insensitivity to FcγRIIIa polymorphism

Trastuzumab



HER2 bsFab



Donor phenotype

■ FcγRIIIA-158 V/V

● FcγRIIIA-158 V/F

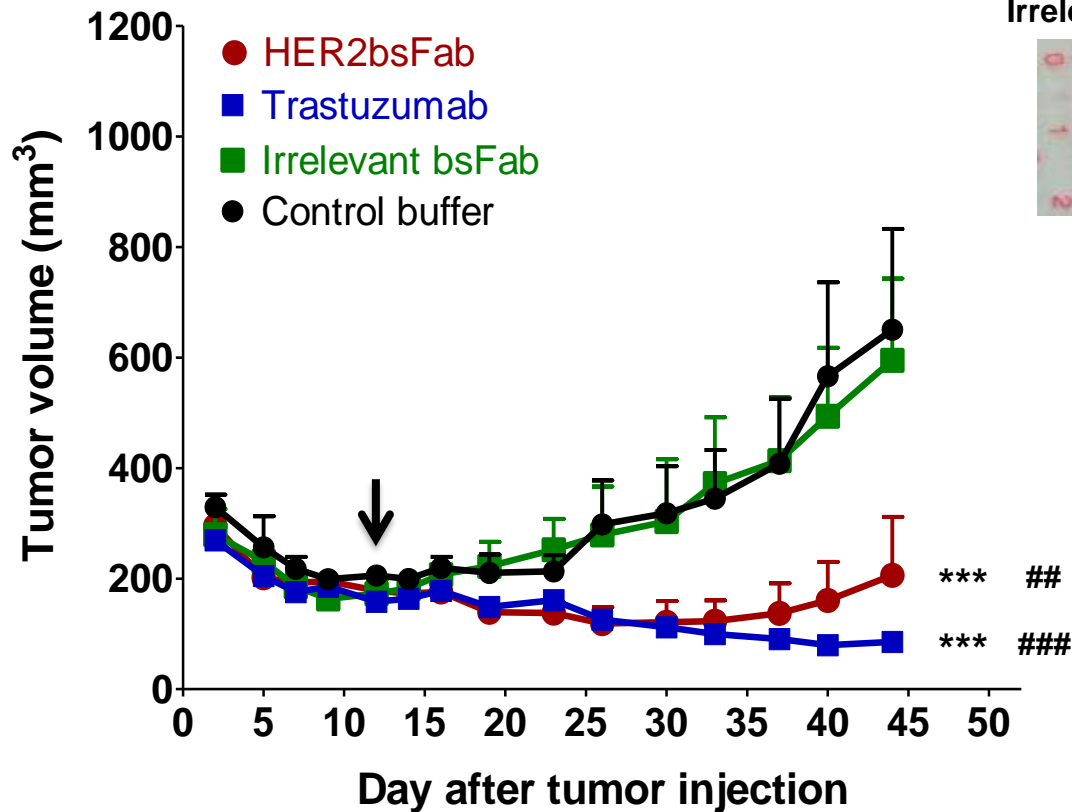
▲ FcγRIIIA-158 F/F



ADCC mediated by biKEs is independent of FcγRIIIa polymorphism

# Tumor growth inhibition assays (HER2<sup>high</sup>)

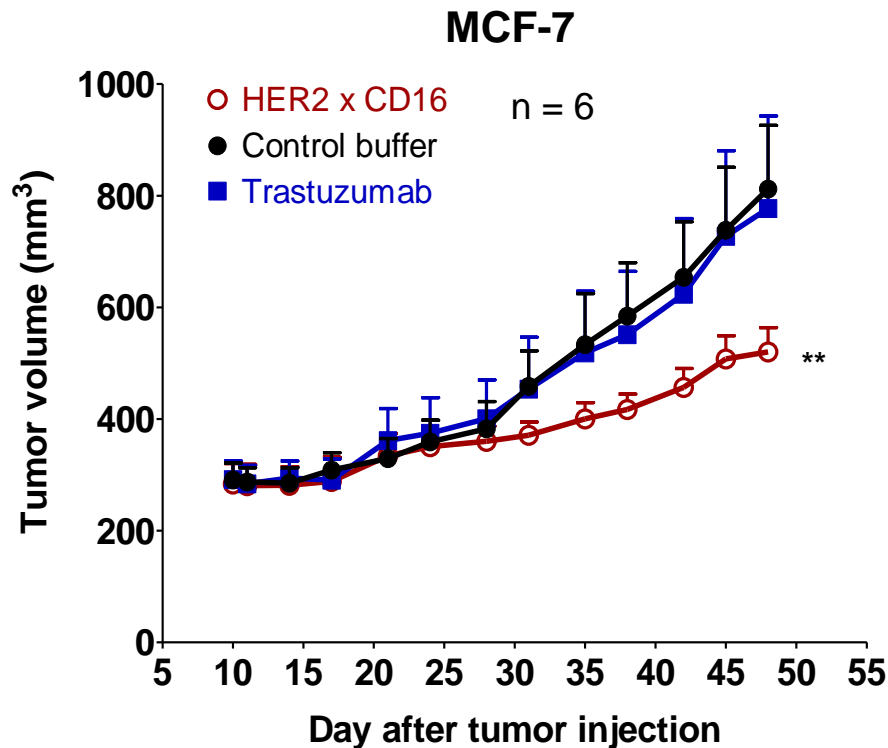
- Weak binding of BiKE to murine NK and MΦ (nude mice)
- 10<sup>7</sup> BT474 (sc) until tumors reach 250 mm<sup>3</sup>
- Trastuzumab (ip, 5mg/kg, 2/week), or bsFab (ip, 5mg/kg, 3/week) from day 12



Representative examples

# Tumor growth inhibition assays (HER2<sup>Low</sup>)

- 10<sup>7</sup> MCF-7 cells (sc) → Trastuzumab (ip, 5mg/kg, 2/week)  
→ HER2 x CD16 (ip, 5mg/kg, 3/week)



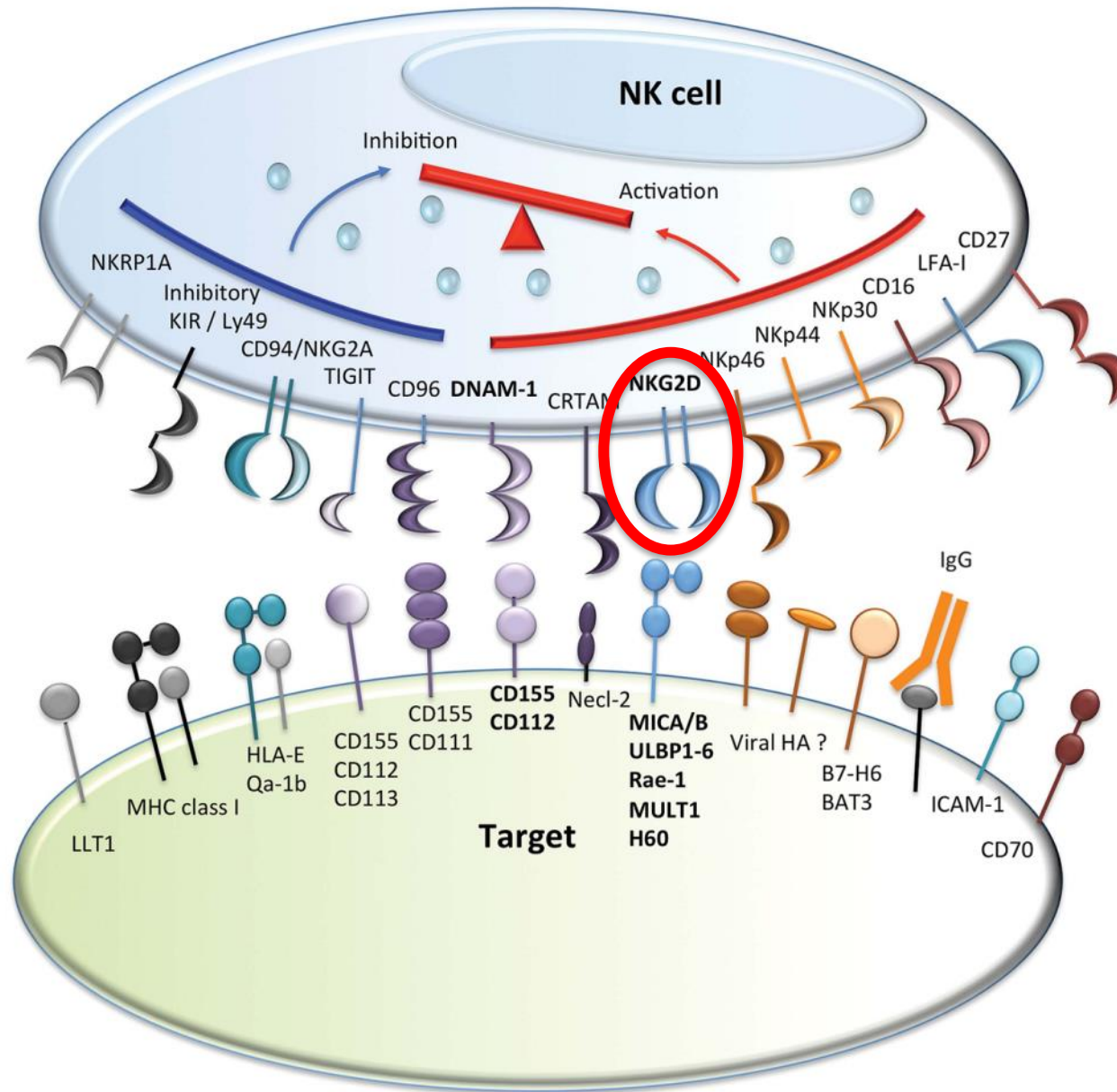
□ Despite moderate binding to murine NK and macrophage effector cells



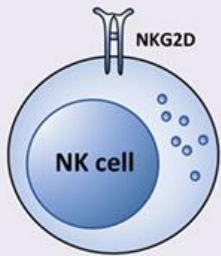
BsFab inhibits moderate-HER2-expressing tumor growth in Nude mice



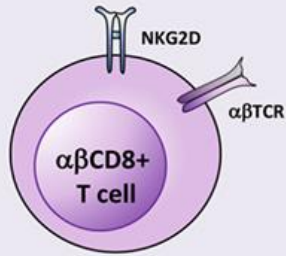
# Beyond CD16 : NKG2D based cell engagers ?



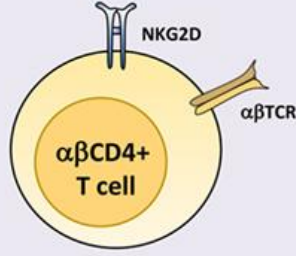
# NKG2D : toward T and NK engagers



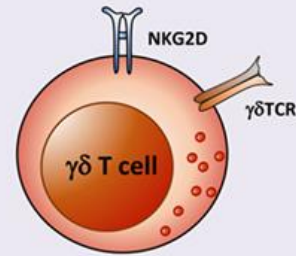
- All NK cells
- DAP10 and DAP12 adaptors
- Mouse: NKG2D-short isoform (DAP10 and DAP12) and NKG2D-long isoform (only DAP10)
- Human: NKG2D-long isoform (DAP10 adaptor)



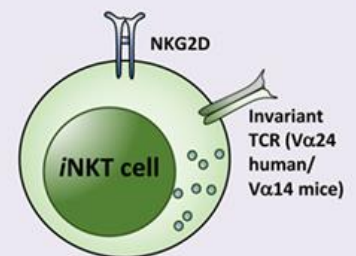
- Human: all CD8+ T cells
- Mouse: activated CD8+ T cells
- DAP10 adaptor



- Normal conditions: **not** expressed
- Induced in some pathological conditions:
  - Rheumatoid arthritis
  - Crohn's disease
  - Wegener granulomatosis
  - Multiple sclerosis
  - MIC+ tumors, invasive cervical carcinoma
  - CMV infection

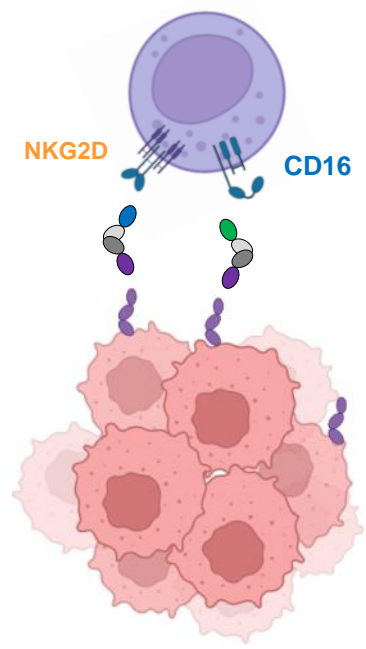
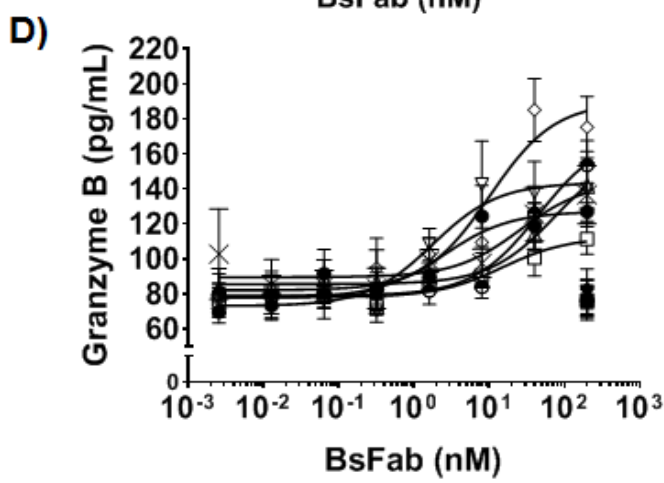
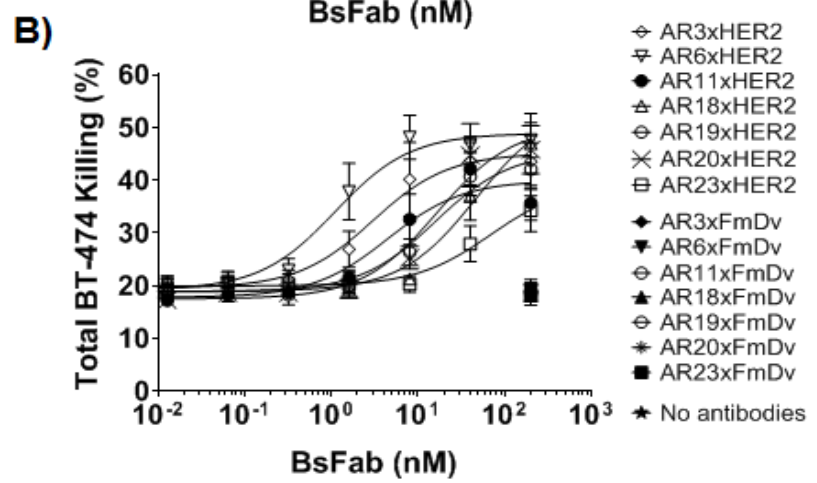
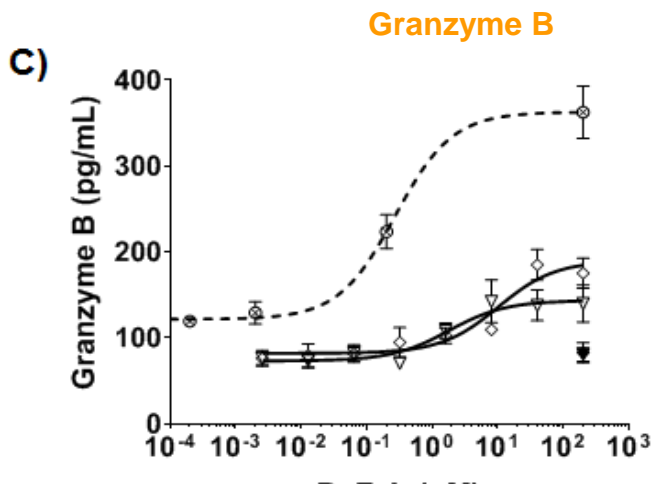
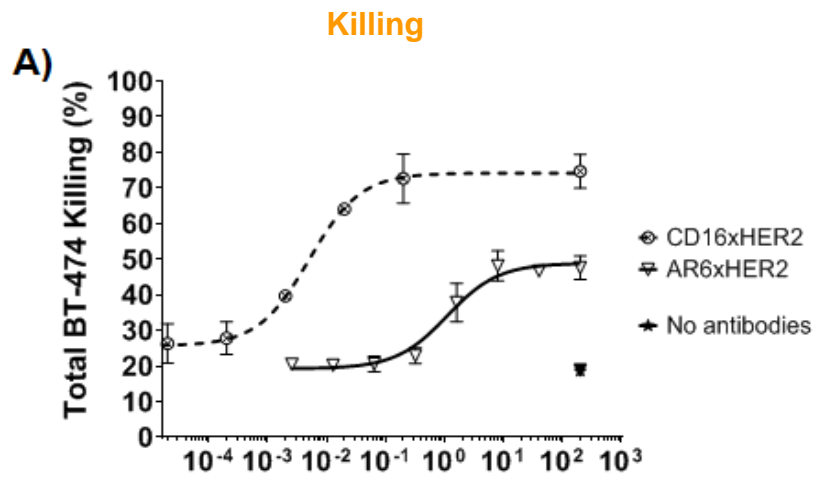


- Vγ9Vδ2T (peripheral blood)
- Vδ1 (intestine, skin)
- DETC (mice, skin)



- CD4- iNKT cells

# Anti-NKG2D cell engagers trigger cytotoxicity



BsFab	EC50 (nM)	max BT-474 killing (%)
CD16xHER2	0,005	74 +/- 2,2
AR3xHER2	3	45 +/- 1,9
AR6xHER2	1	49 +/- 1,7
AR11xHER2	4	40 +/- 1,5
AR19xHER2	16	45 +/- 1,3

Non-stimulated human NK cells (E:T 6:1) vs BT474 breast cancer cells



Can we manipulate the cancer cell / NK immune synapse ?

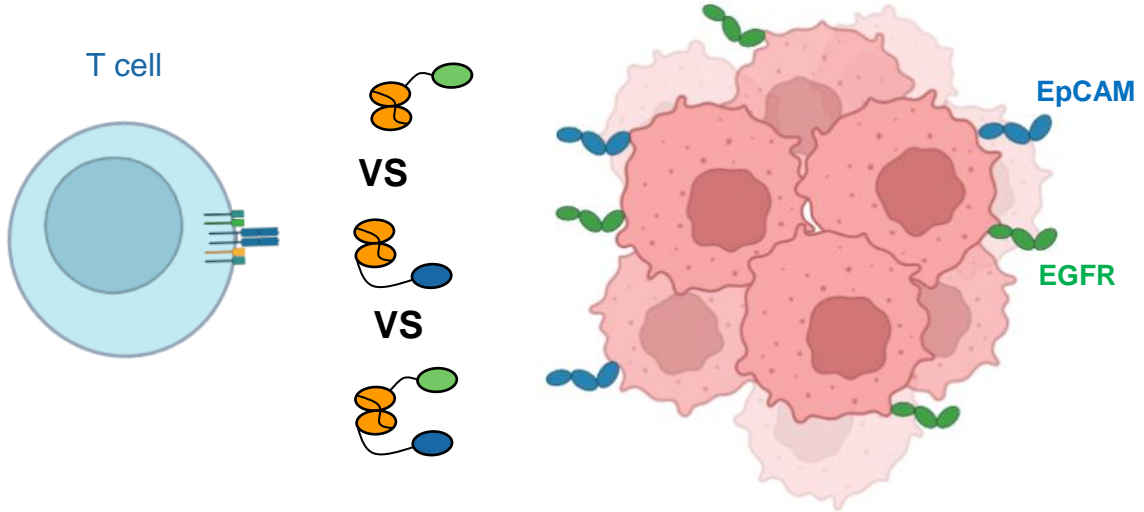
Can we manipulate the **cancer cell / T** immune synapse ?

Can we enhance cancer cell detection ex vivo ?

Can we enhance cancer cell detection in vivo ?



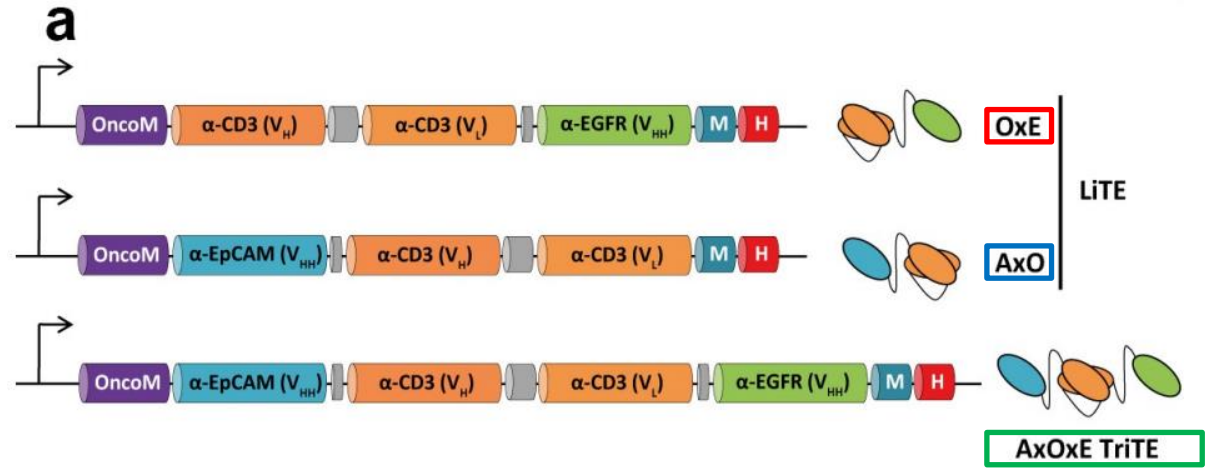
# Multispecificity : Toward better T Cell Engagers ?



## Dual TAA targeting

Avidity

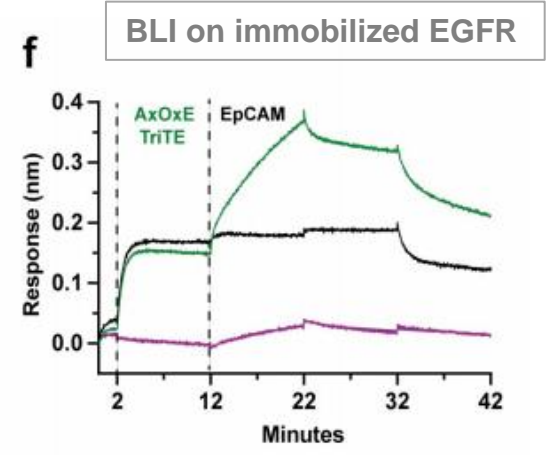
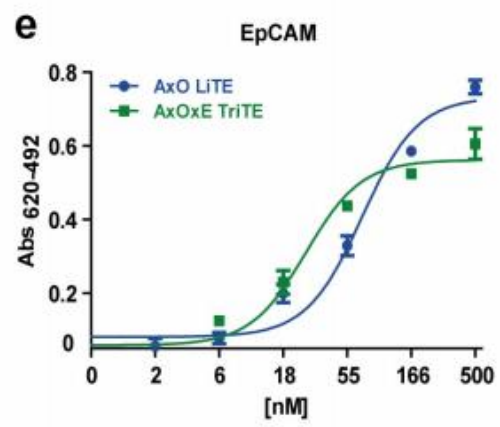
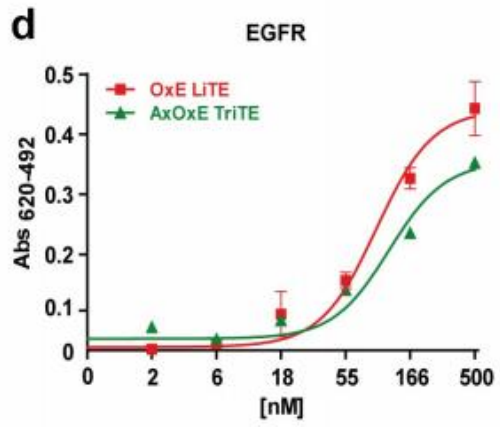
Antigen downregulation



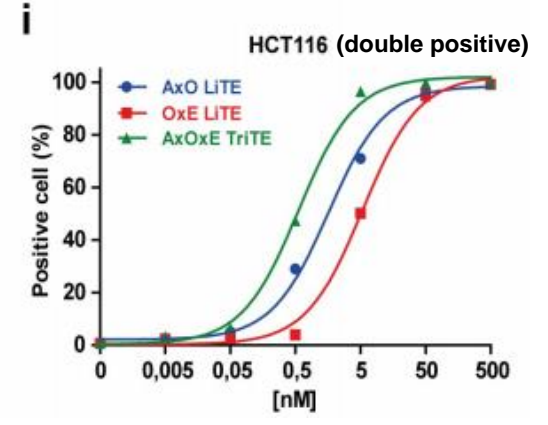
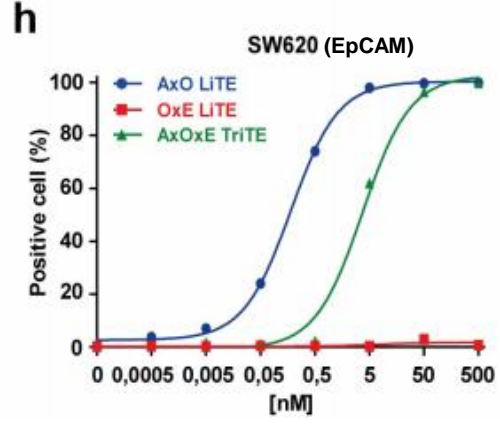
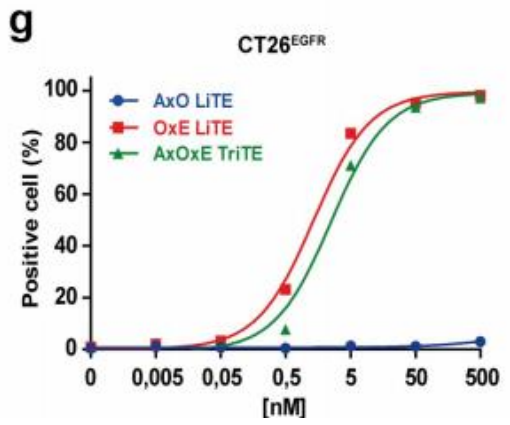
# A Trispecific T Cell Engager : binding



ELISA



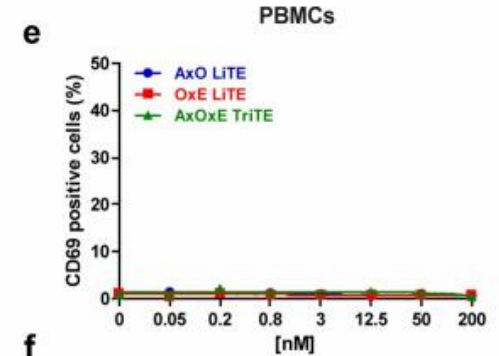
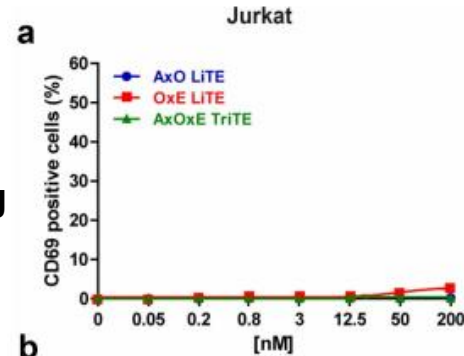
Flow



# Avidity effect on T cell activation (CD69) ?



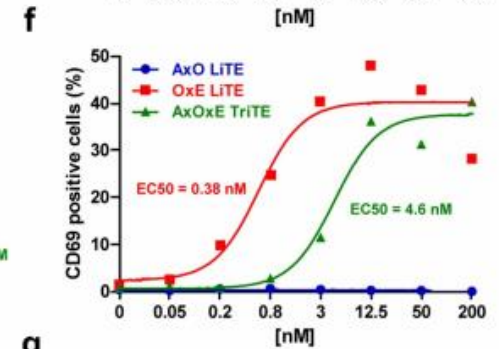
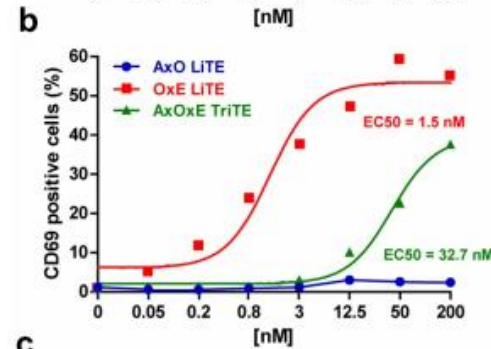
Double Neg



CT126



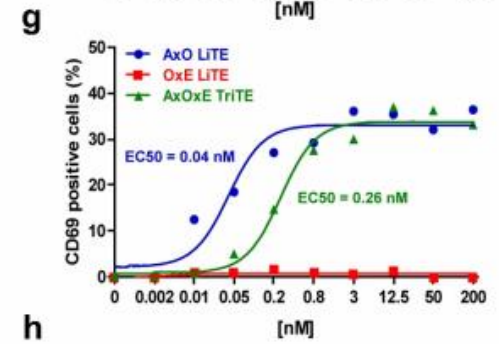
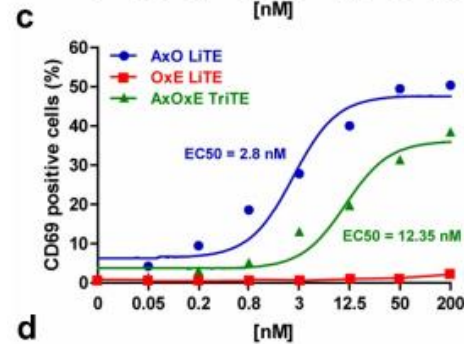
EGFR +



CT126<sup>EGFR</sup>

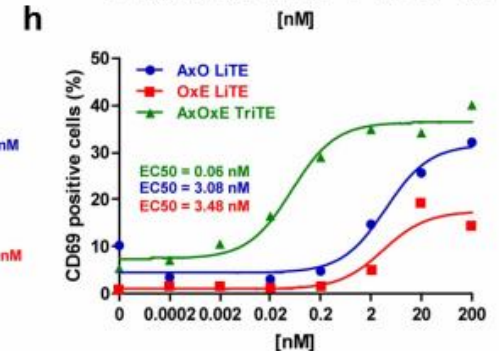
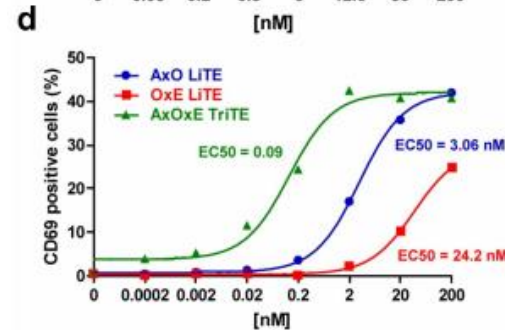


EpCAM +



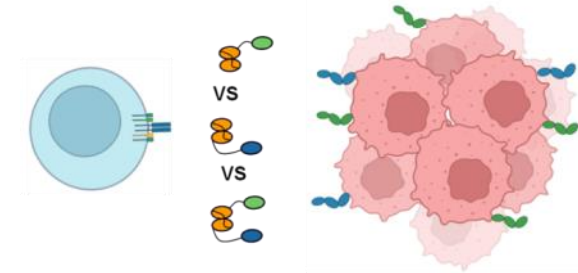
SW620

EGFR +  
EpCAM +



HCT116

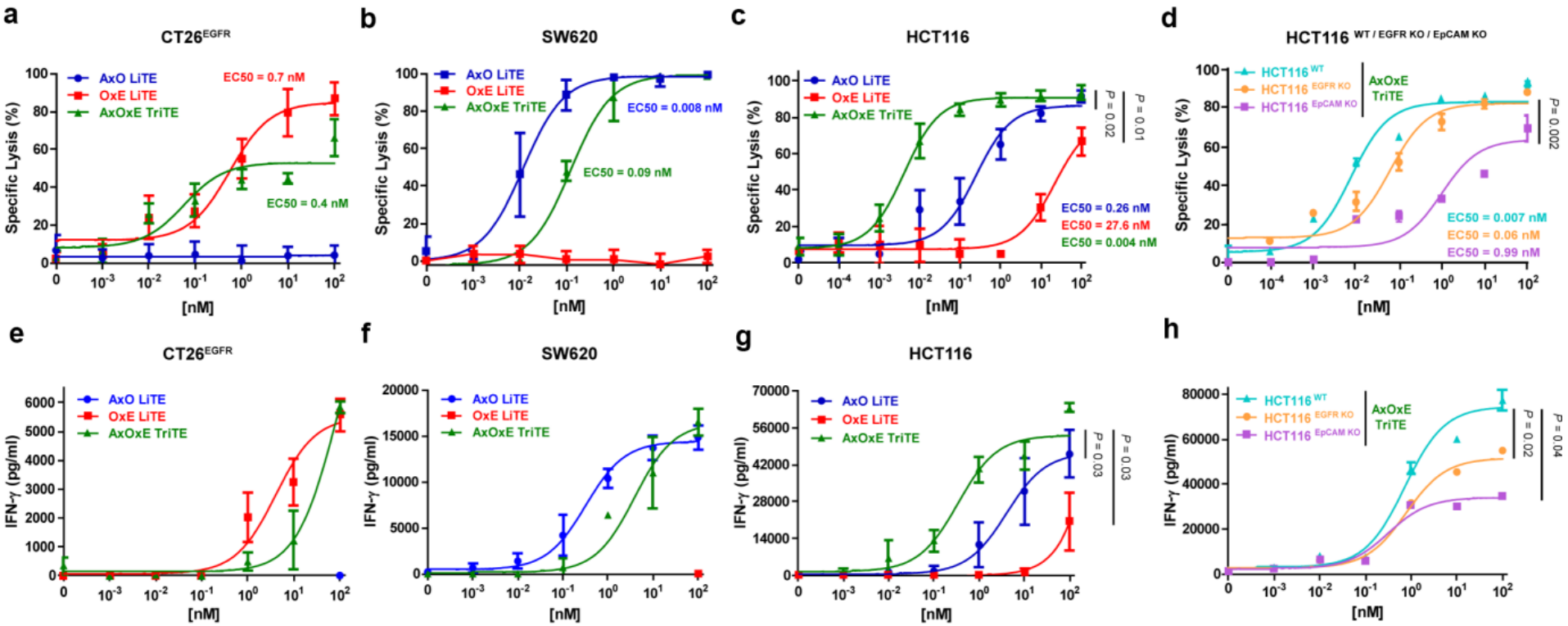
# Translation into a better cytotoxicity ?



EGFR +

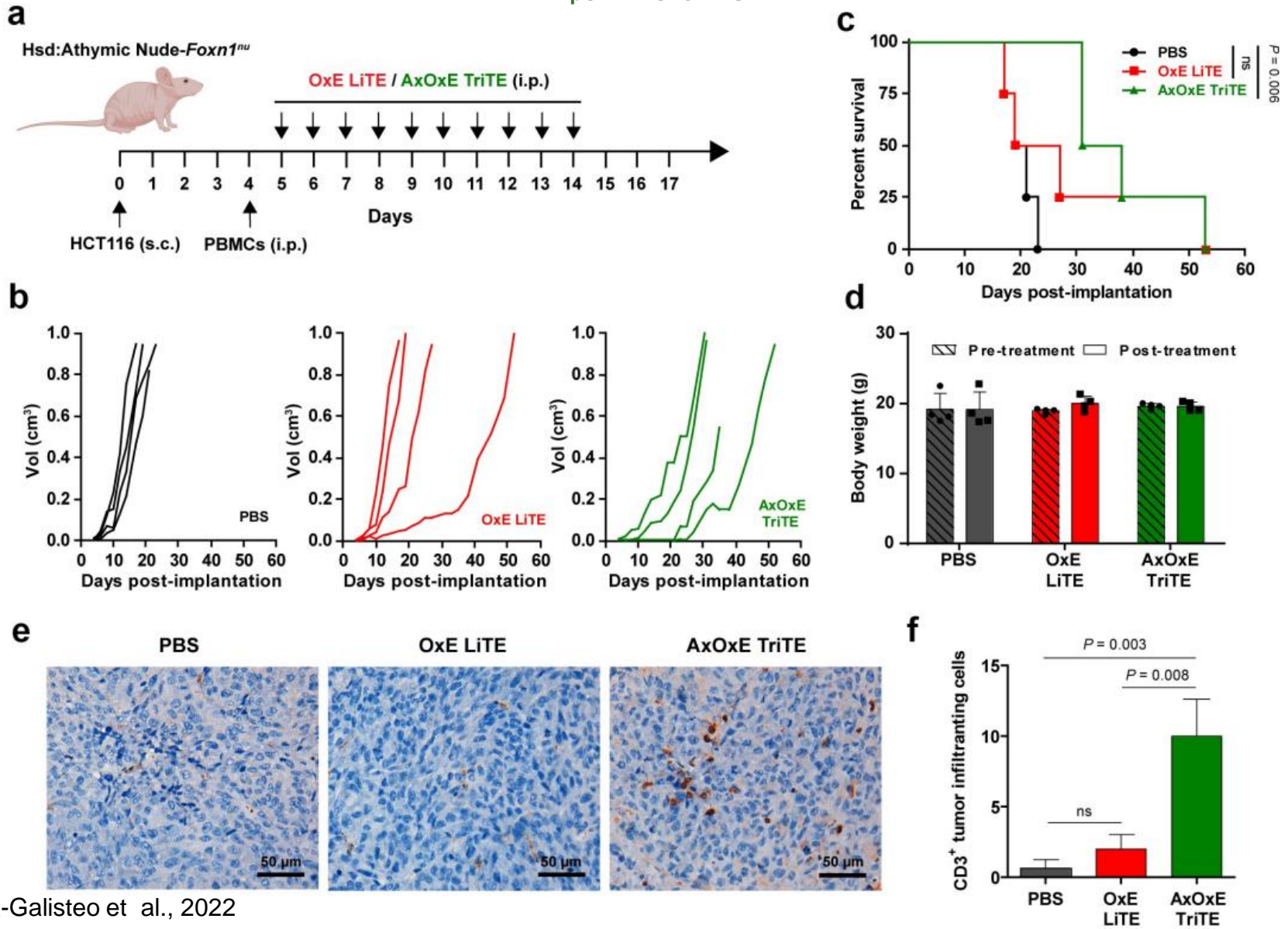
EpCAM +

EGFR +  
EpCAM +

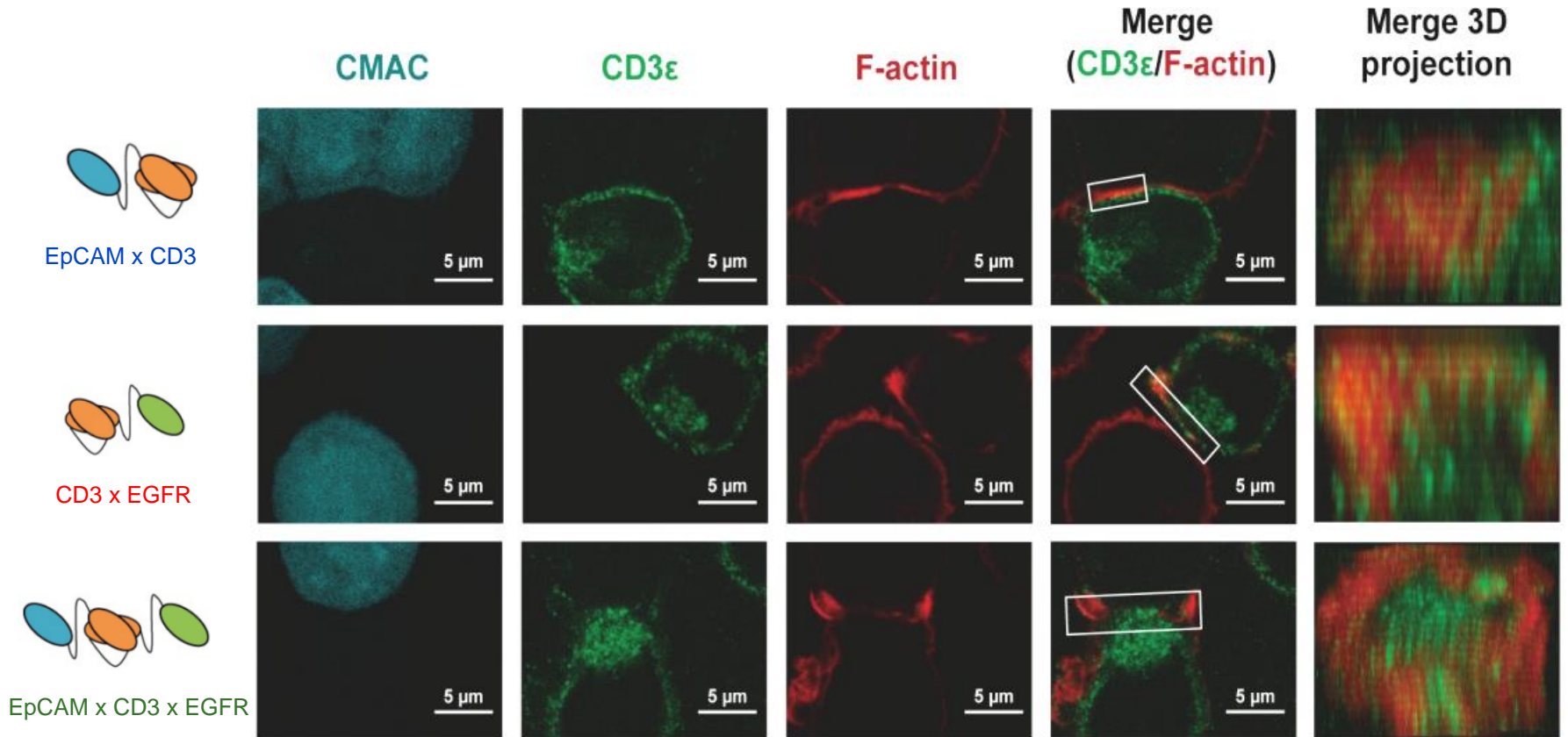




# A better efficacy of dual targeting in vivo ?



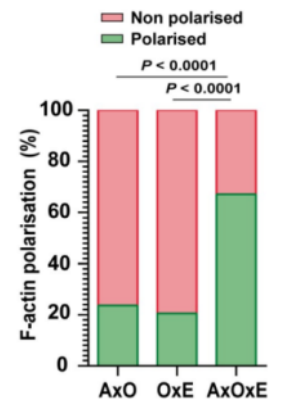
# Trispecific TCE: canonical synapse formation ?



HTC116 cells (CMAC : cyan dye tracker)

T cells (anti CD3ε A488)

F-actin (phalloidin A647)





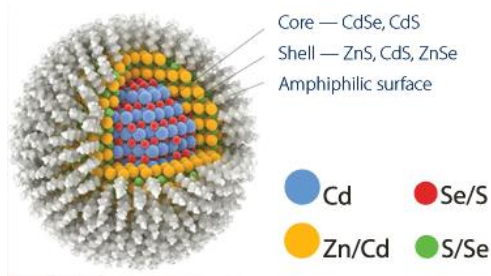
Can we manipulate the cancer cell / NK immune synapse ?

Can we manipulate the cancer cell / CTL immune synapse ?

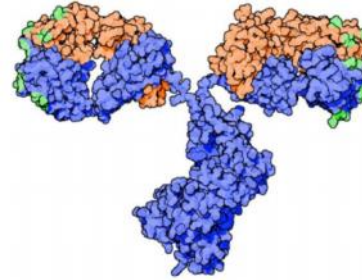
Can we enhance cancer cell **detection ex vivo** ?

Can we enhance cancer cell detection in vivo ?

# Deep tissue imaging by 2P-Laser Scanning Micro.

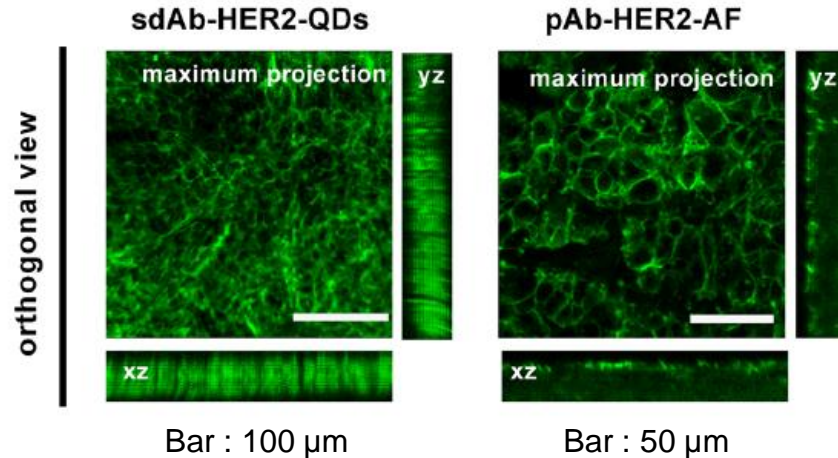
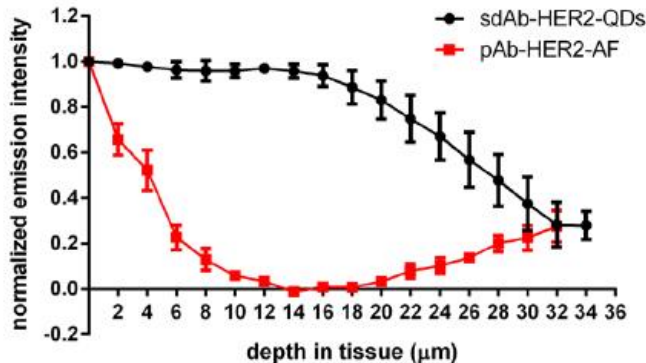


Quantum Dots



50  $\mu$ M thick breast tumor sections

Bars : 25  $\mu$ m

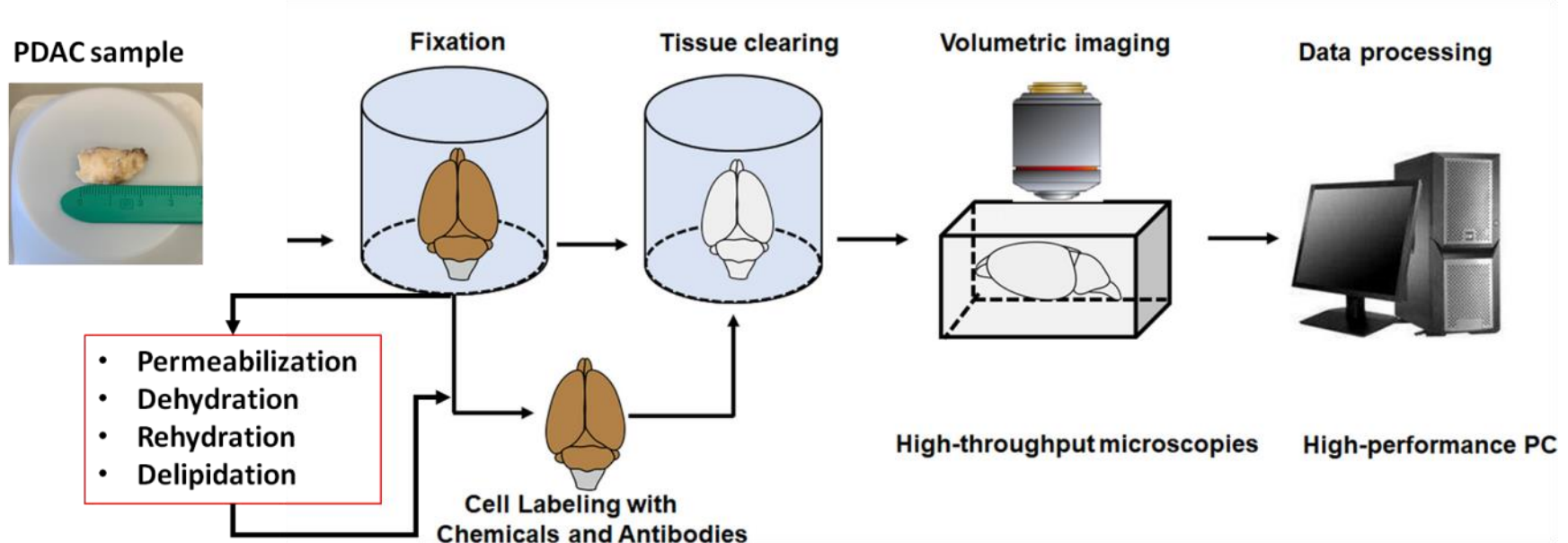


# Going deeper and deeper...

## Tissue clearing protocols

Matching of **refractive indices** of tissue components leading to **transparency**

Emitted light can travel through the tissue



3 – 50 days tissue processing depending on protocol and type of labelling

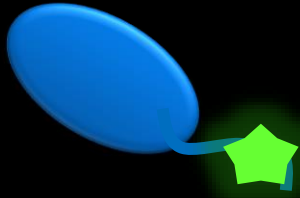
**Bottleneck** : Poor penetration of full length antibodies : the **smaller** the better



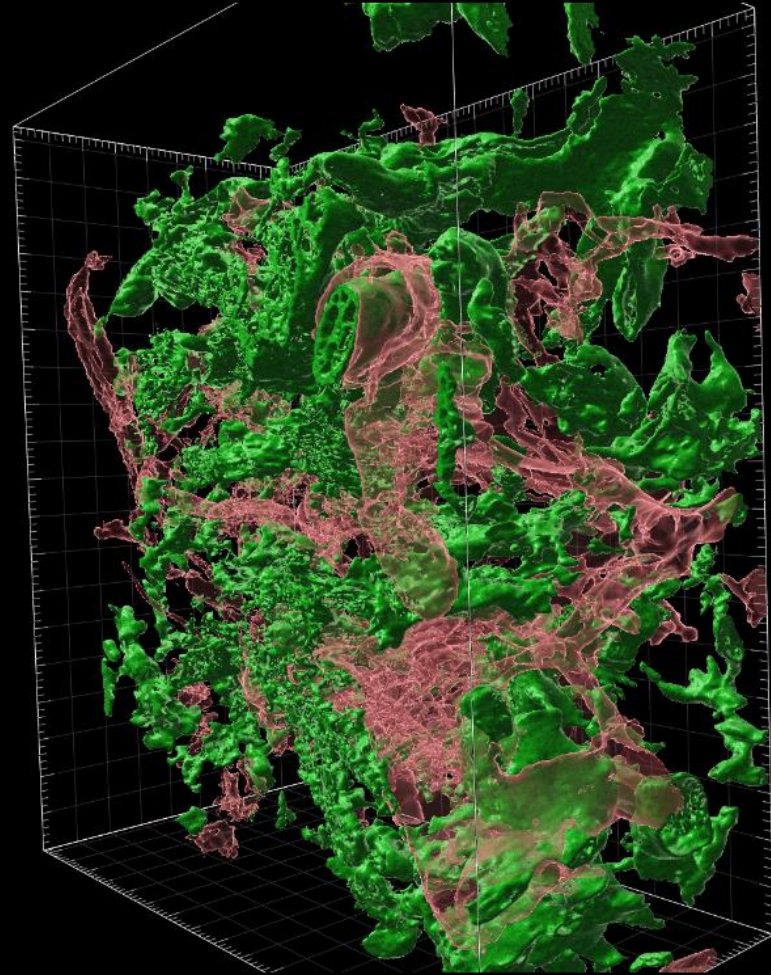
# Detection of CEA<sup>+</sup> cancer cell in PDAC

Detection of **C**arcino **E**mbrionic **A**ntigen (**CEA**)  
in Pancreatic Ductal Adenocarcinoma Cancer  
(**PDAC**) samples

**2500 μM thick** tumor sections



site specific enzymatic labeling  
(BTG) using ATTO 647 substrate  
on the C-myc tag



**Red** = autofluorescence (vessels, ECM sheets)  
**Green** = CEA



Can we manipulate the cancer cell / NK immune synapse ?

Can we manipulate the cancer cell / T immune synapse ?

Can we enhance cancer cell detection ex vivo ?

Can we enhance cancer cell **detection in vivo** ?



Small size  
High affinity



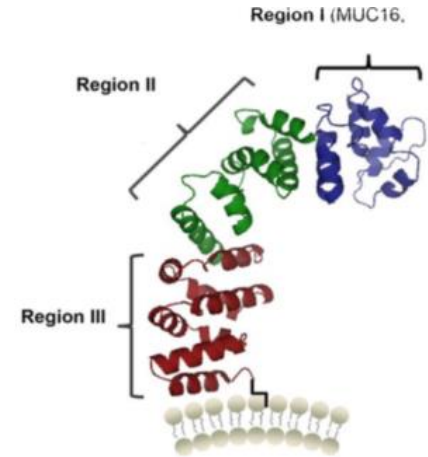
High tissue penetration  
Short serum half life



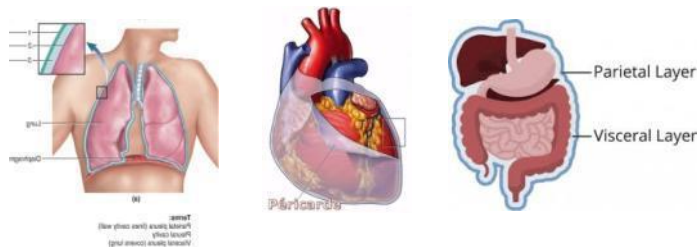
High contrast  
Early after injection

# Mesothelin, TAA with high potential

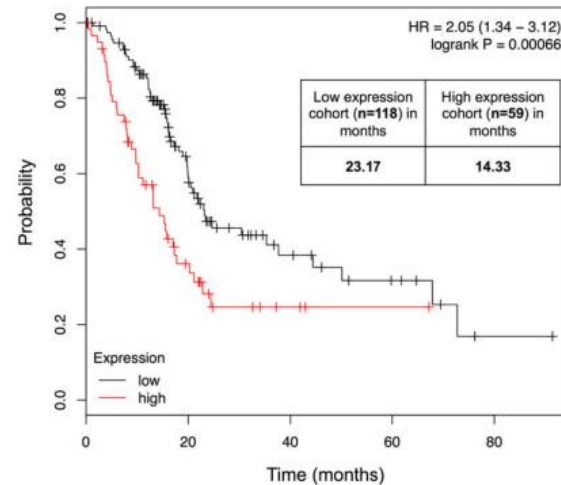
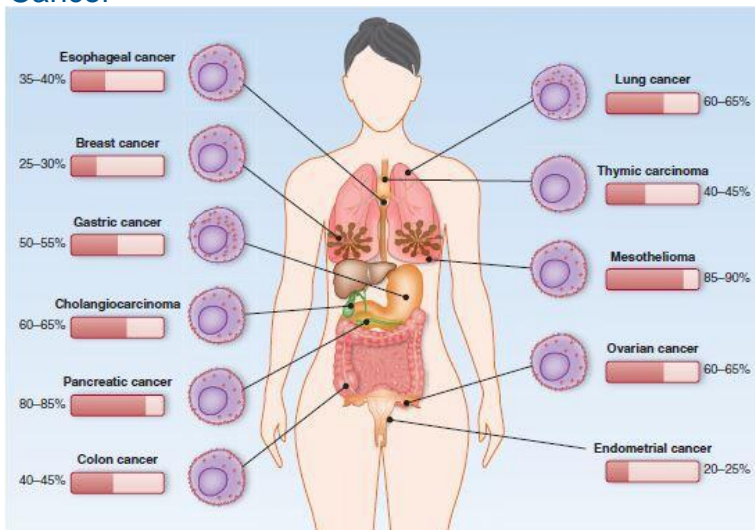
- 40 kDa GPI-anchored membrane glycoprotein
- Role in cell adhesion (known ligand MUC16)
- Normal expression: **weak** and **restricted** to mesothelial cell lining (pleura, pericardium and peritoneum = **dispensable tissues**)
- **Highly overexpressed** in several cancers (bad prognosis)



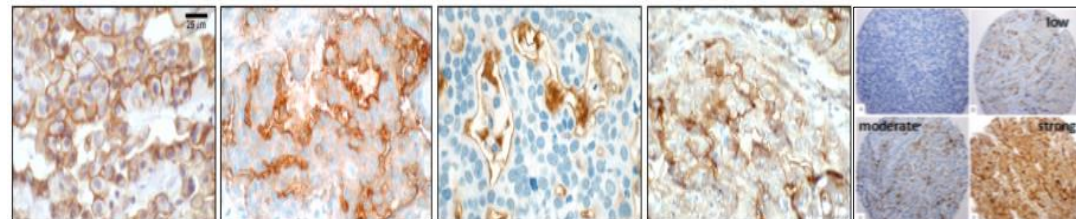
## Normal



## Cancer



Survival probability in PDAC



Mesothelioma  
>90 %

Ovarian  
70%

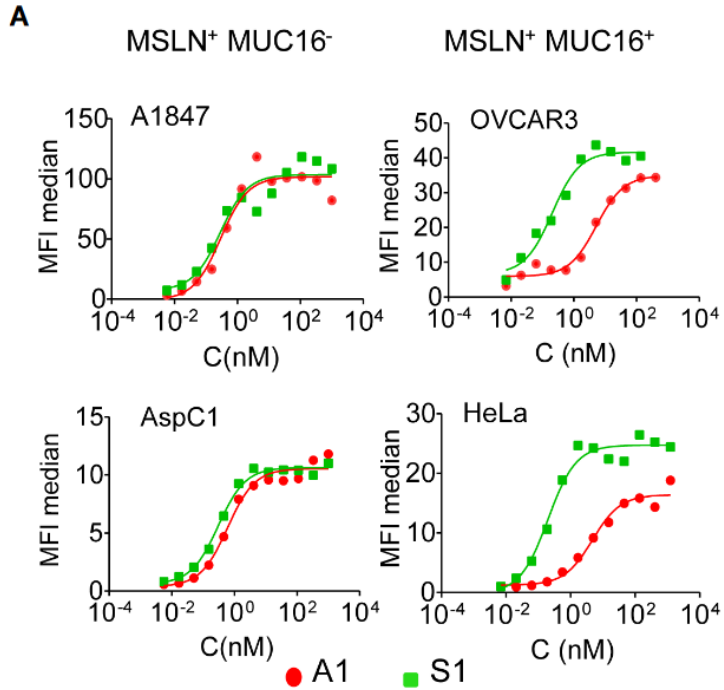
Pancreatic  
>85 %

Lung  
50%

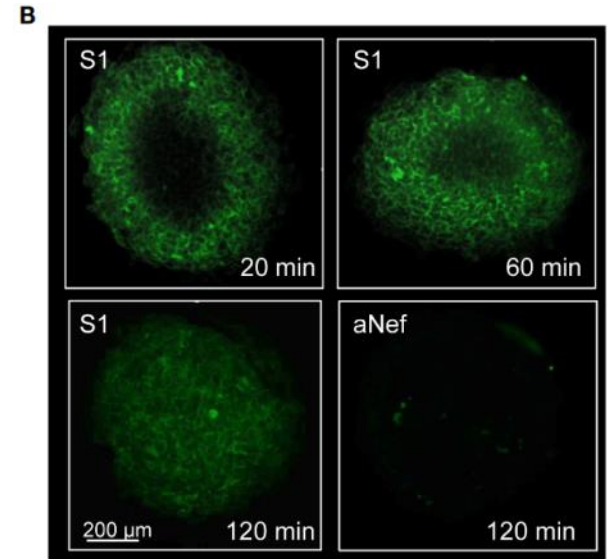
TNBC  
40-70%

# Anti Mesothelin binders

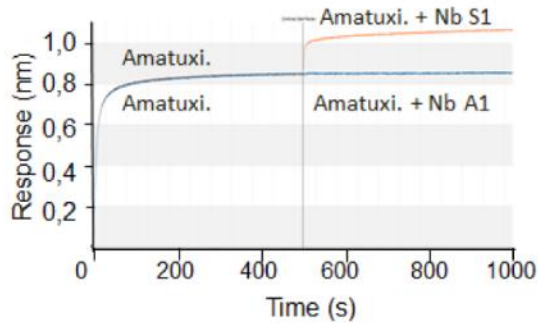
Sub nanomolar apparent affinity



Rapid spheroid penetration

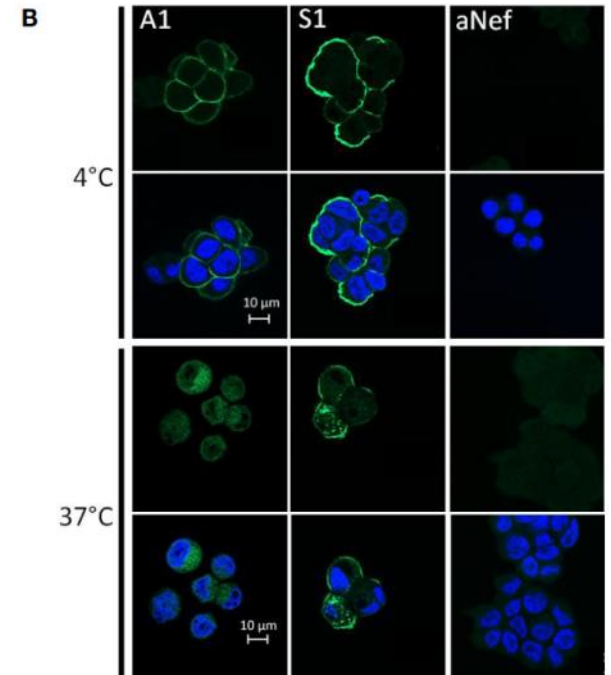


Nb S1 do not compete with blocking Amatuxilab

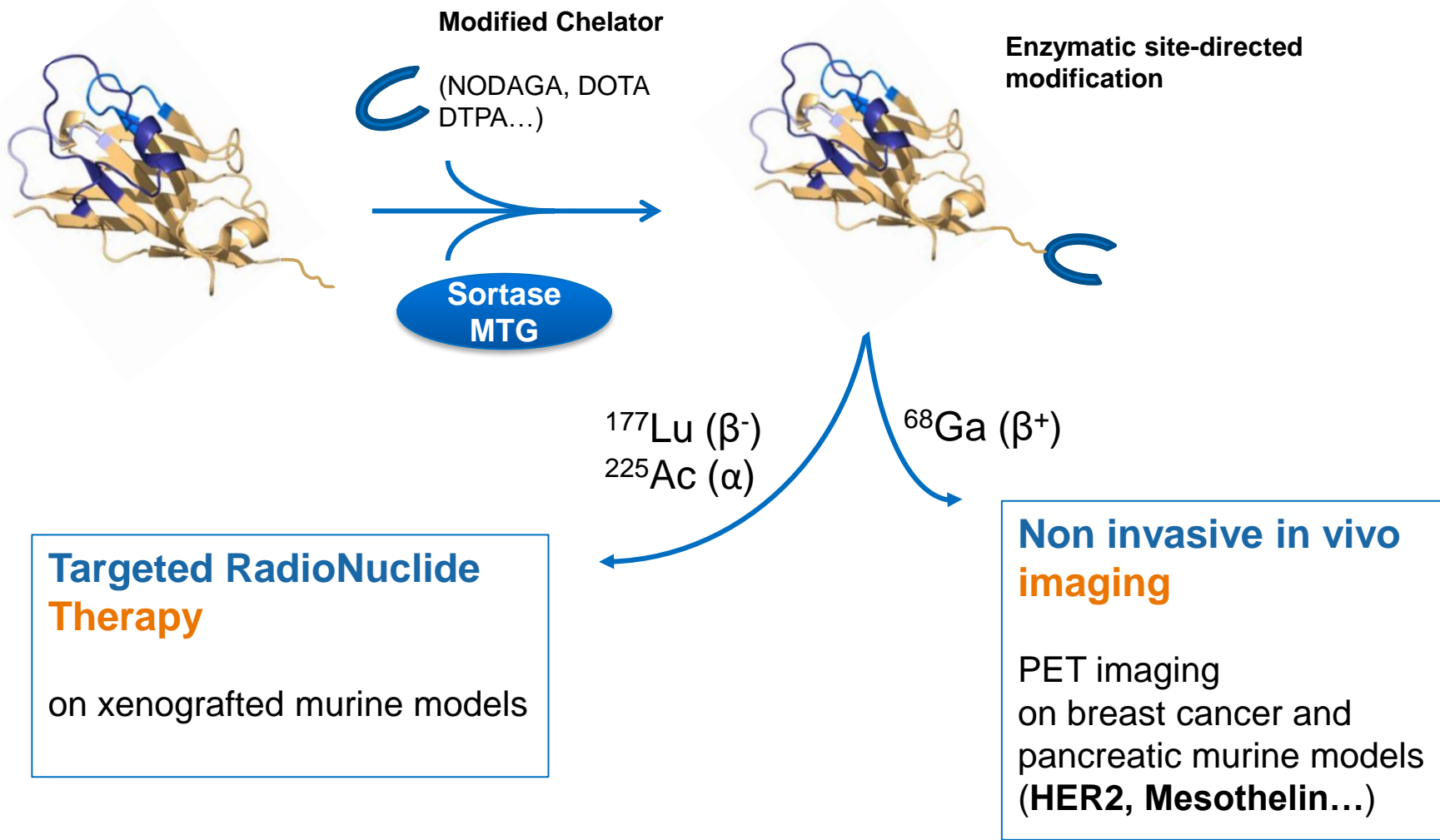


BLI competition assay

Efficient Internalisation (60 min)

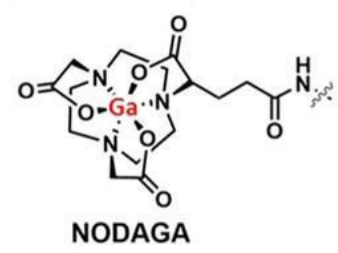
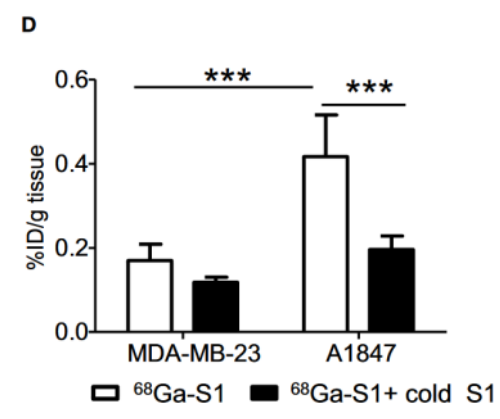
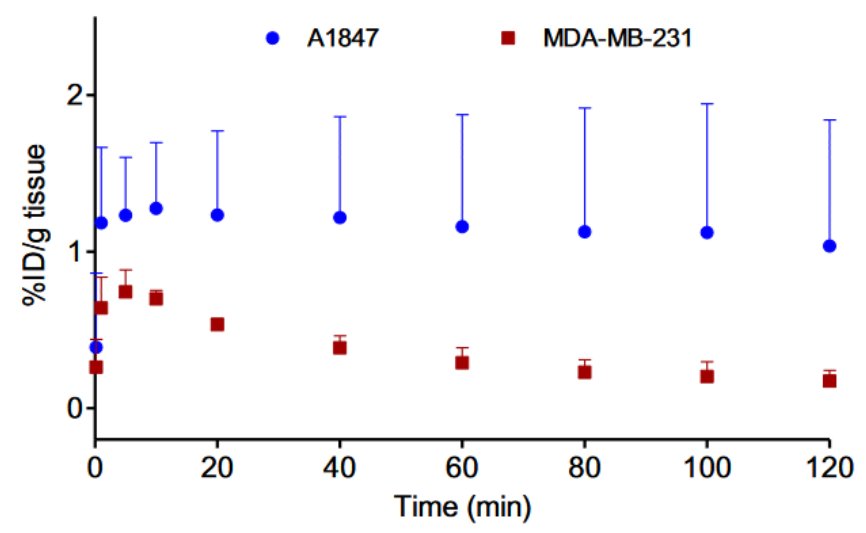
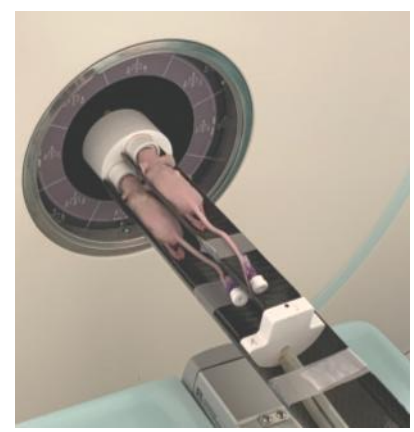
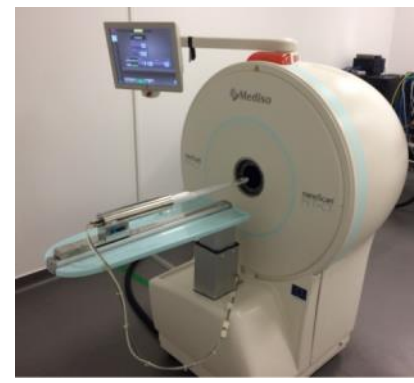
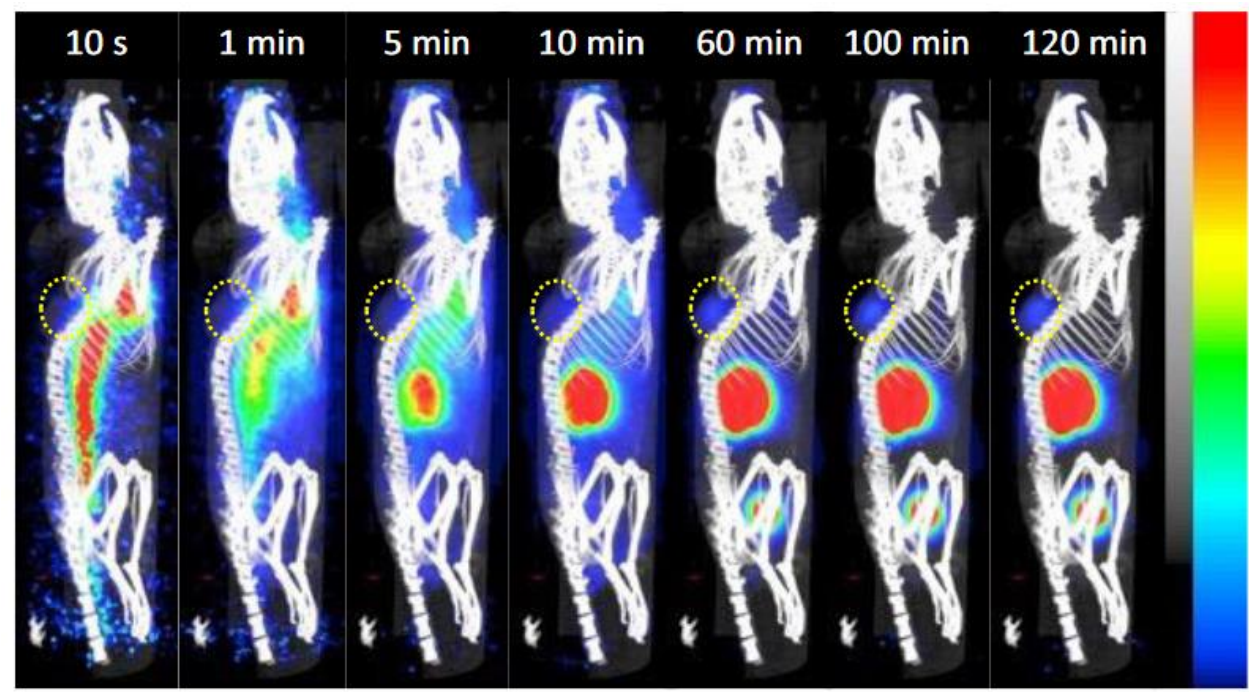


# Nanobodies as **theranostic** tools





# PET imaging (Ga<sup>68</sup>) of A1847 tumors



# Thanks for your attention !



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# Questions ?

