

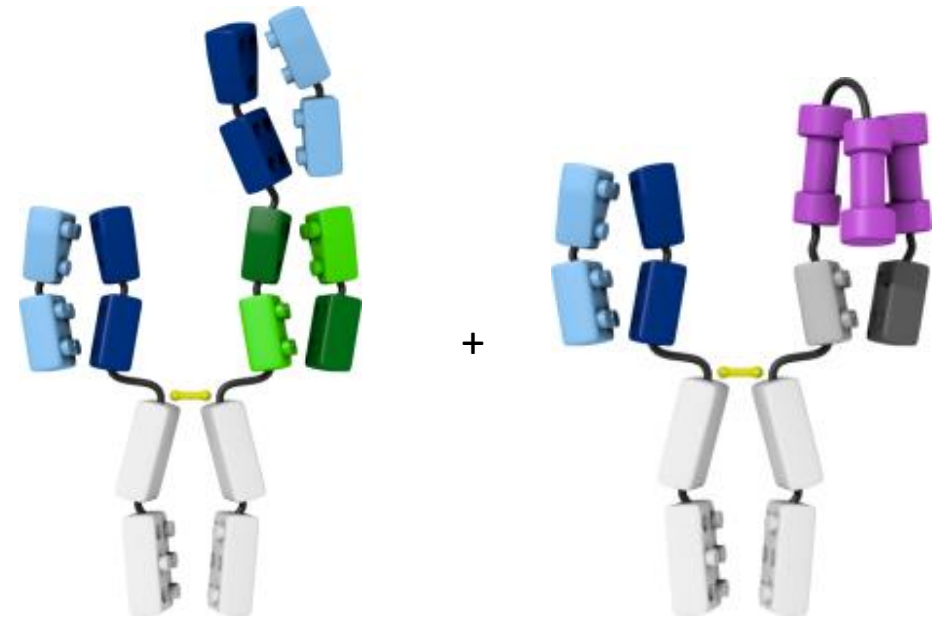
Webinar outline

- Discovery of 4-1BB and 4-1BB immuno-biology
 - **Byoung S. Kwon, PhD** - *Eutilex*
- 4-1BB as an important prosurvival signal for CAR T cells
 - **Michael Milone, MD, PhD** - *University of Pennsylvania*
- Novel antibody-based approaches targeting the 4-1BB pathway
 - **Christian Klein, PhD** - *Roche Innovation Center Zurich*
- Translational and reverse translational research in 4-1BB co-stimulation
 - **Ignacio Melero, MD, PhD** - *CIMA, Clinica Universidad de Navarra*

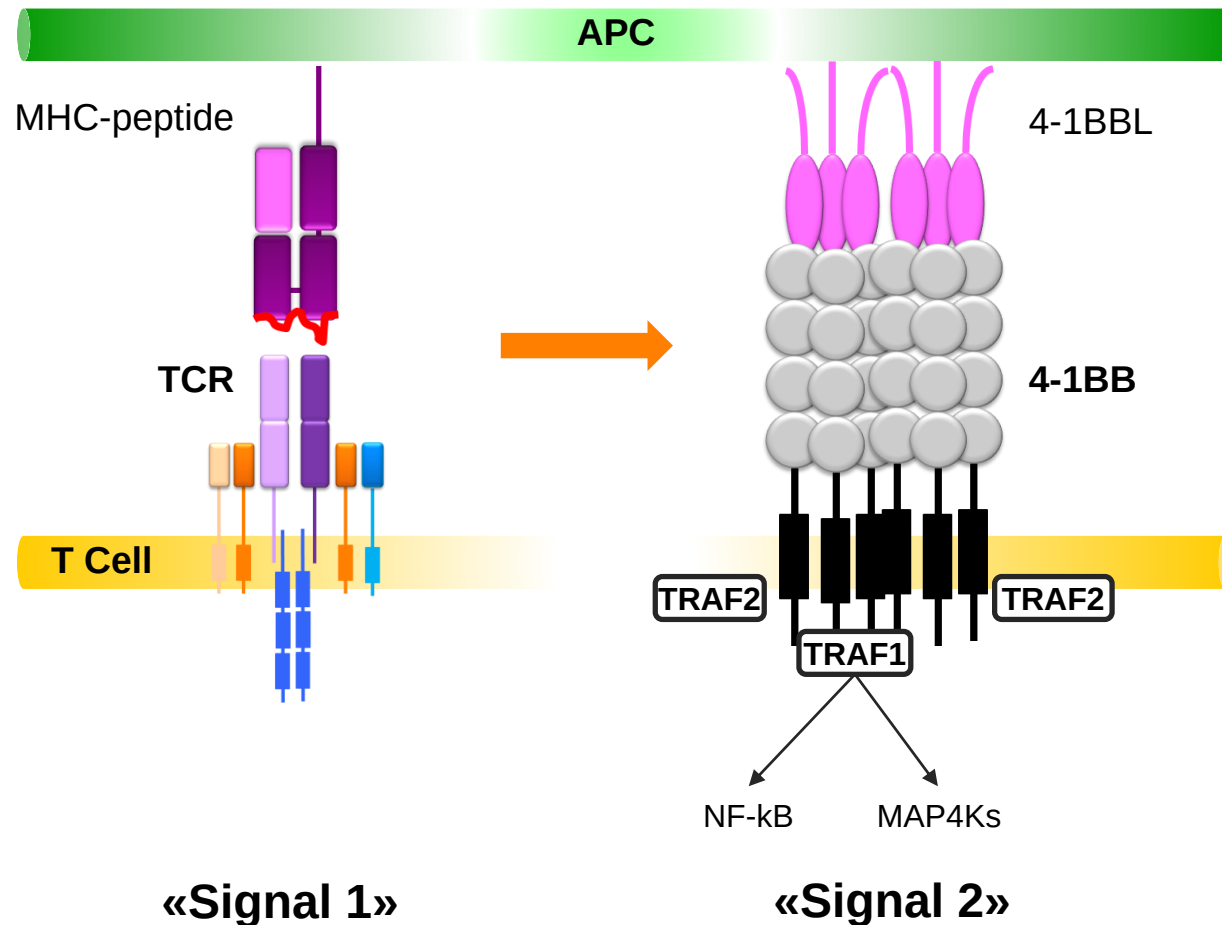
Novel antibody-based approaches targeting the 4-1BB pathway

Christian Klein

Roche Innovation Center Zurich



4-1BB (CD137) agonism enhances the strength and durability of T cell responses



4-1BB agonism enhances T cell

Proliferation

Cytotoxicity

Cytokine secretion
(e.g IFN γ , IL-2, TNF α , GM-CSF)

Th1 polarization

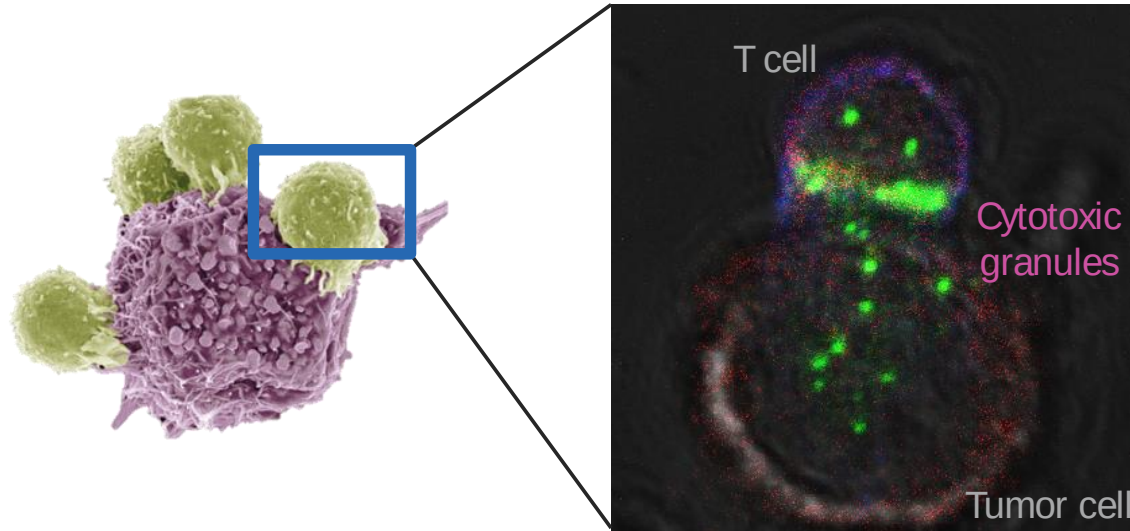
Memory formation

T cell survival (anti-apoptosis)

Resistance to exhaustion

Metabolic fitness

Mode of action of T cell bispecific antibodies (TCBs)



Simultaneous binding to tumor cell surface antigen and to common component of TCR leads to:

- TCR engagement (signal 1) and up-regulation of activation markers including 4-1BB/CD137
- T cell engagement, activation and killing of tumor cells
- T cell proliferation (expansion)
- Cytokine & chemokine secretion leading to recruitment of additional T-cells

Issues of first generation 4-1BB agonistic antibodies

- Hypercrosslinking of 4-1BB (> 3 receptor molecules) required for effective agonism
- 1st generation 4-1BB mAbs cannot achieve this unless they are themselves hypercrosslinked by Fc γ Rs on adjacent cells
- ✓ First generation 4-1BB agonistic antibodies (e.g., urelumab) are hampered by strong hepatotoxicity and subsequent requirement for dose reduction (0.1 mg/kg)
- ✓ Dose limiting toxicity restricts efficacy as single agent and in combination

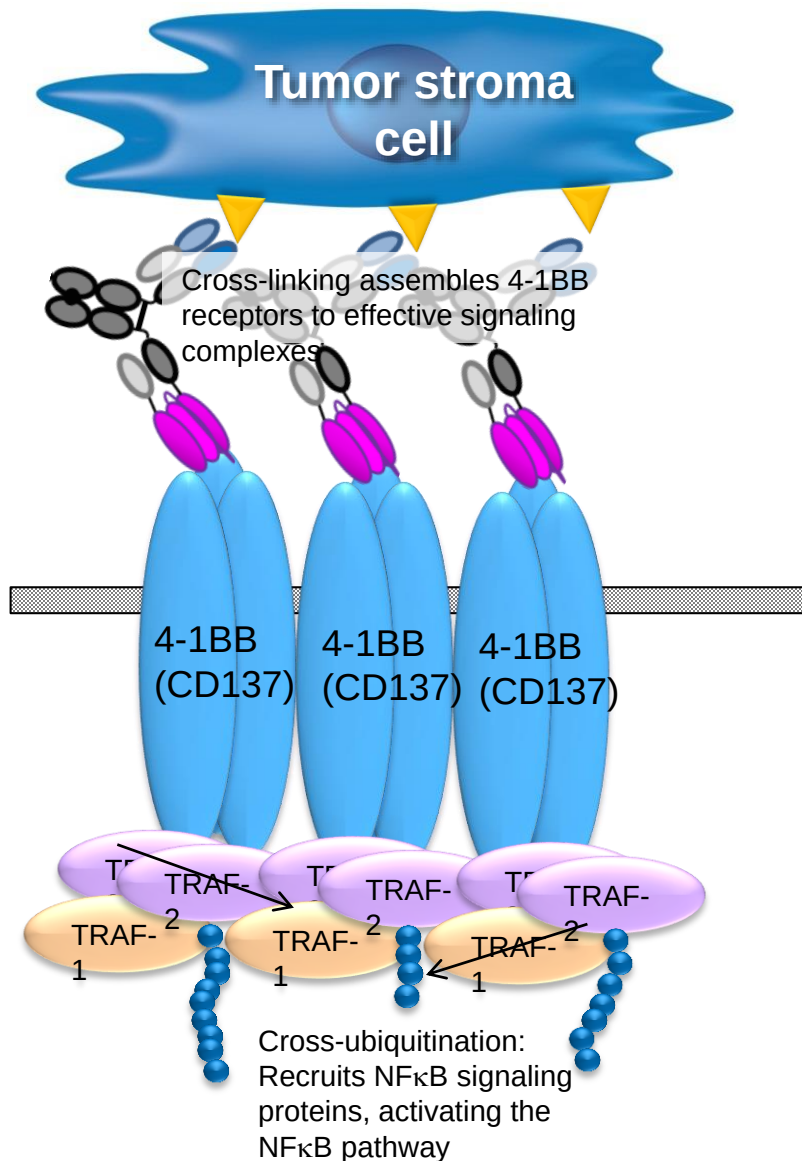
Cancer Therapy: Clinical

Clinical
Cancer
Research

Results from an Integrated Safety Analysis of Urelumab, an Agonist Anti-CD137 Monoclonal Antibody

Neil H. Segal¹, Theodore F. Logan², F. Stephen Hodi³, David McDermott⁴, Ignacio Melero⁵, Omid Hamid⁶, Henrik Schmidt⁷, Caroline Robert⁸, Vanna Chiarion-Sileni⁹, Paolo A. Ascierto¹⁰, Michele Maio¹¹, Walter J. Urba¹², Tara C. Gangadhar¹³, Satyendra Suryawanshi¹⁴, Jaclyn Neely¹⁴, Maria Jure-Kunkel¹⁴, Suba Krishnan¹⁴, Holbrook Kohrt^{15,†}, Mario Sznoi¹⁶, and Ronald Levy¹⁵

Tumor targeted 4-1BB agonism: Mechanism of Action



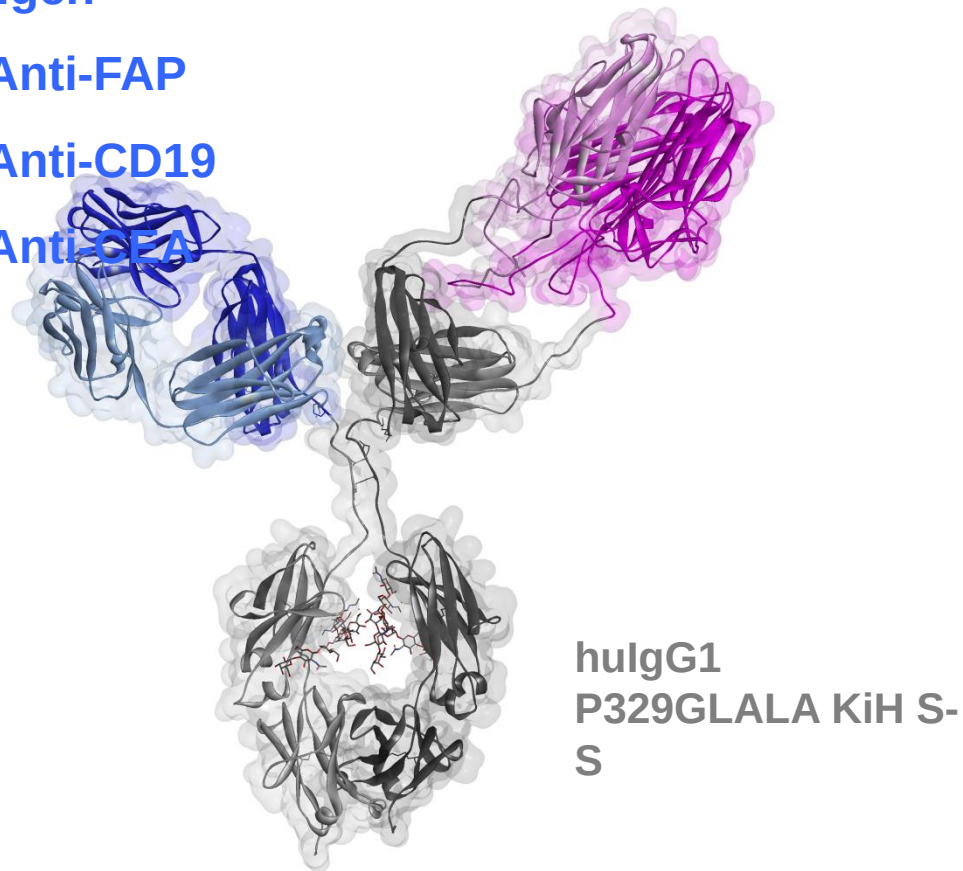
- 4-1BB is inducible and is expressed on activated T and NK cells
- Cross-linking is required for effective signaling (enables cross-ubiquitination)
- Co-stimulatory signal 2: Effects on T-Cells:
 - Enhanced effector functions and cytokine release
 - Increased proliferation
 - Anti-apoptosis

Agonistic Tumor-targeted 4-1BBL: Developed to specifically activate T cells at the tumor site but not in periphery

Tumor (stroma) antigen

- Anti-FAP
- Anti-CD19
- Anti-CEA

Trimerized split 4-1BBL

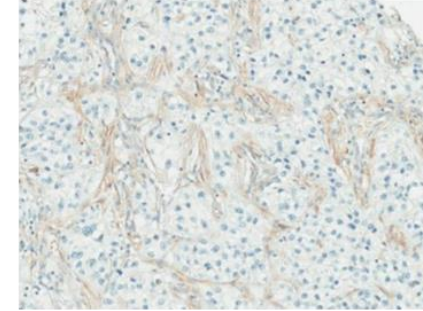


- Reduce toxicity by eliminating FcγR-binding
- Deliver 4-1BB agonist to the tumor stroma (FAP) or tumor (CD19)
- Re-introduce hypercrosslinking via bispecific binding to FAP in tumor stroma
- Strictly targeting dependent via tumor antigen binding

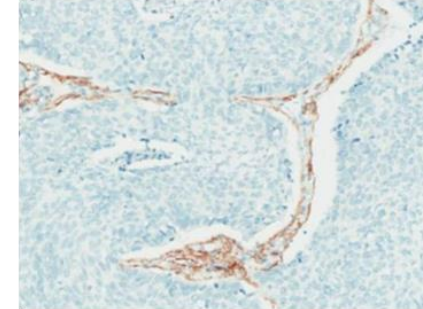
Broad FAP prevalence in major solid tumor indications by IHC

Tumor Type	IHC (n)
	H > 15
BC HER2+	91% (46)
BC TNBC	81% (48)
CRC	44% (45)
DLBCL	38% (39)
FOLLICULAR LYMPH	0% (16)
GASTRIC	62% (47)
HEAD AND NECK	66% (47)
LUNG NSCLC	83% (47)
PANCREATIC	82% (45)
RENAL CELL CARCINOMA	8% (40)
SARCOMA*	45% (40)

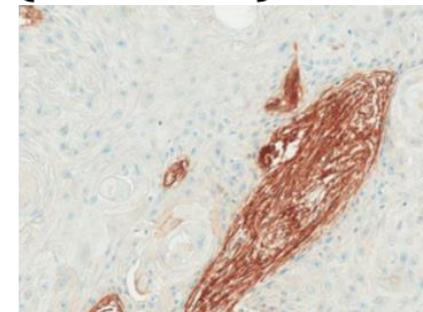
(H-score 15)



(H-score 25)

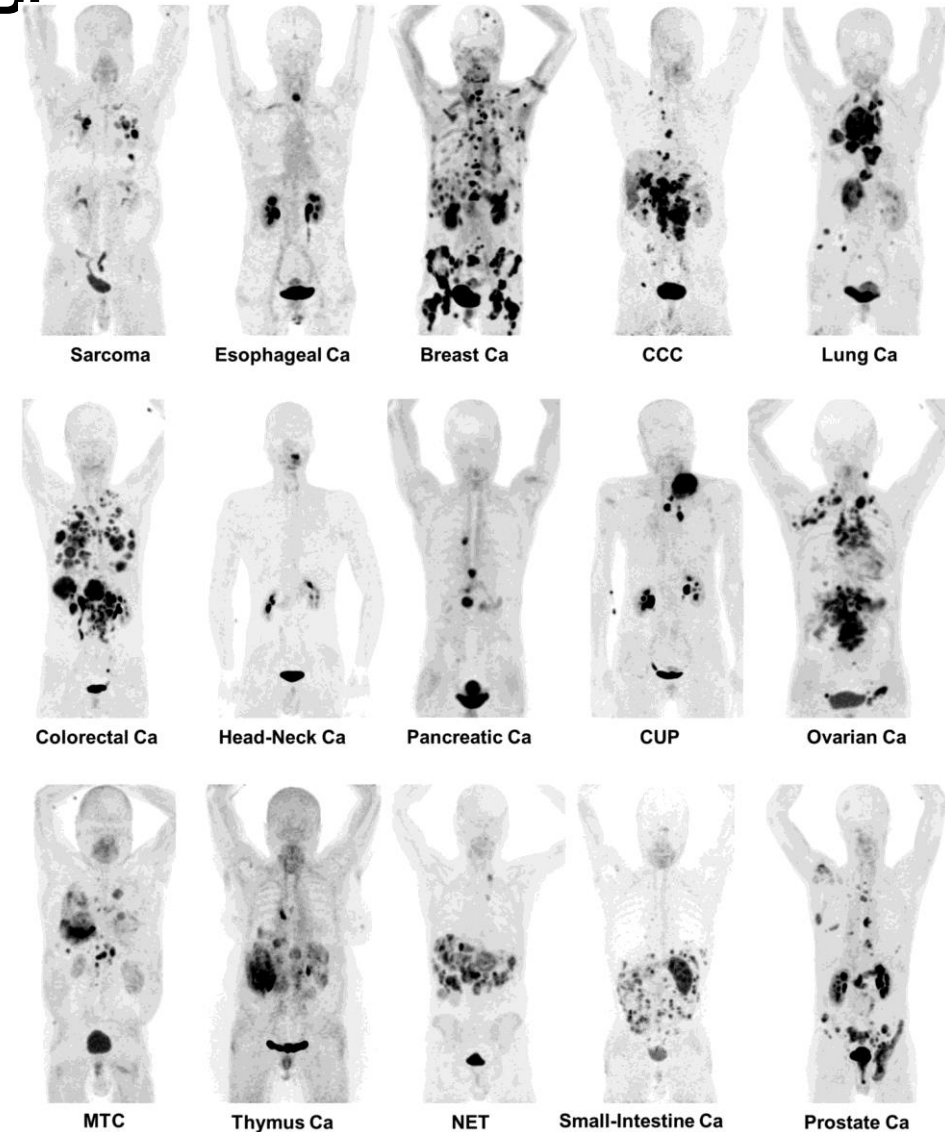
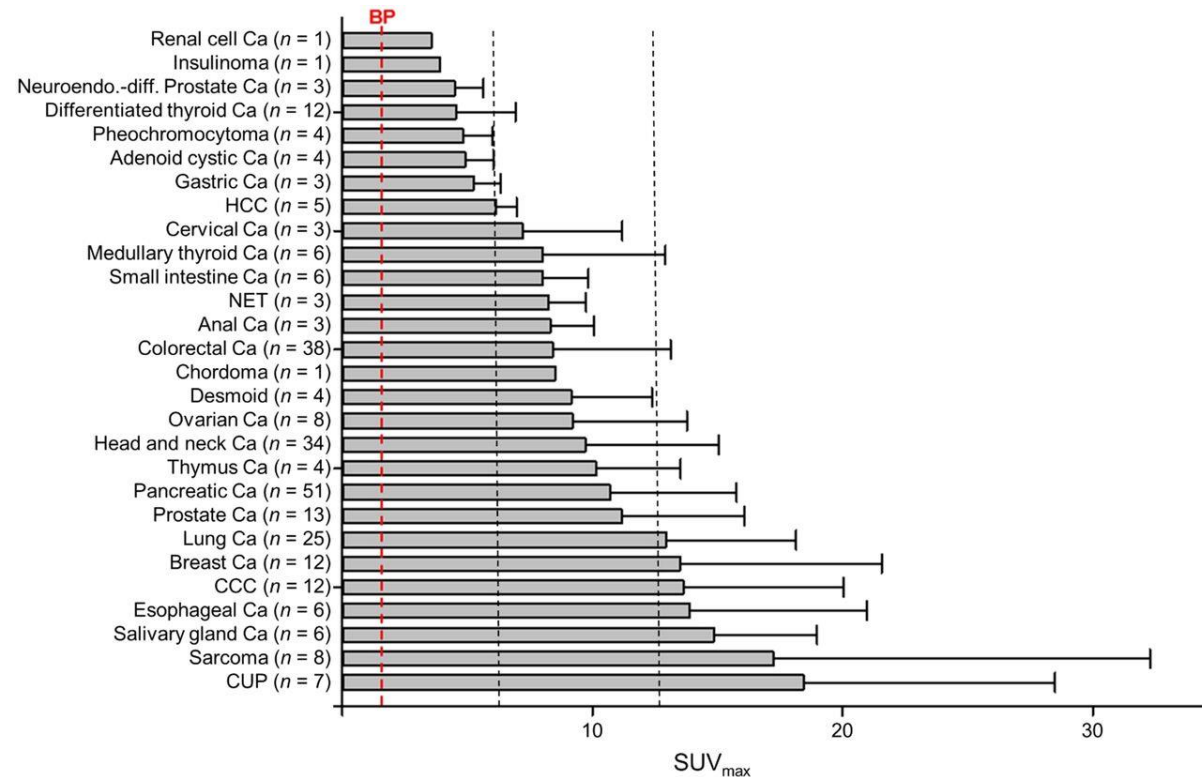


(H-score 60)



H-score” combines the staining intensity with the percentage of positive cells

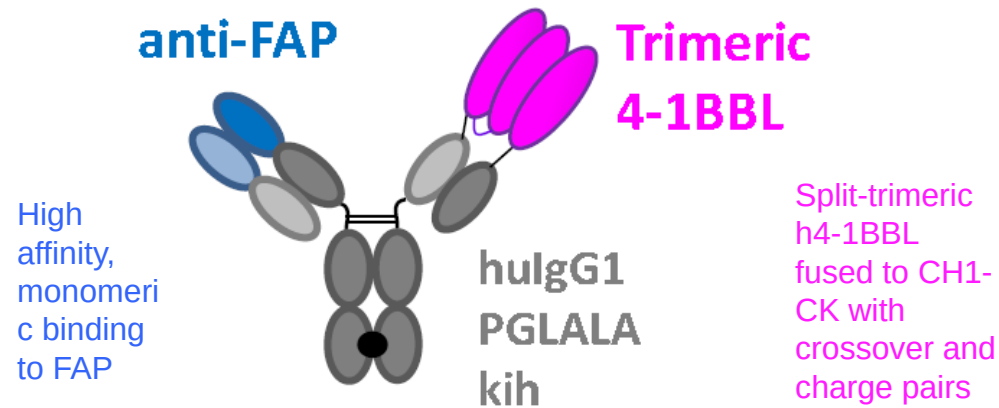
Imaging FAP in cancer patients using FAPi



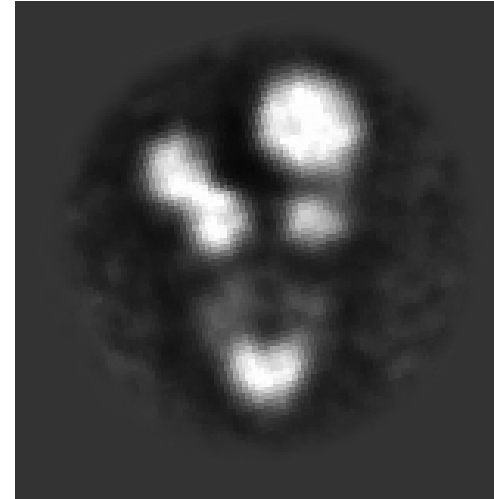
Average SUVmax of 68Ga-FAPI PET/CT in various tumor entities

Clemens Kratochwil et al. J Nucl Med 2019;60:801-805

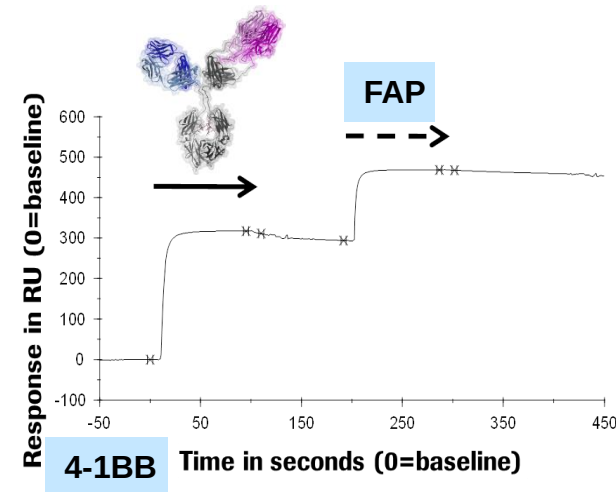
Tumor-stroma FAP-targeted split-4-1BBL: Developed to specifically activate T cells at the tumor site



Heterodimeric hlgG1 Fc with PGLALA & «knob-into-holes» + engineered disulfide bridge mutations

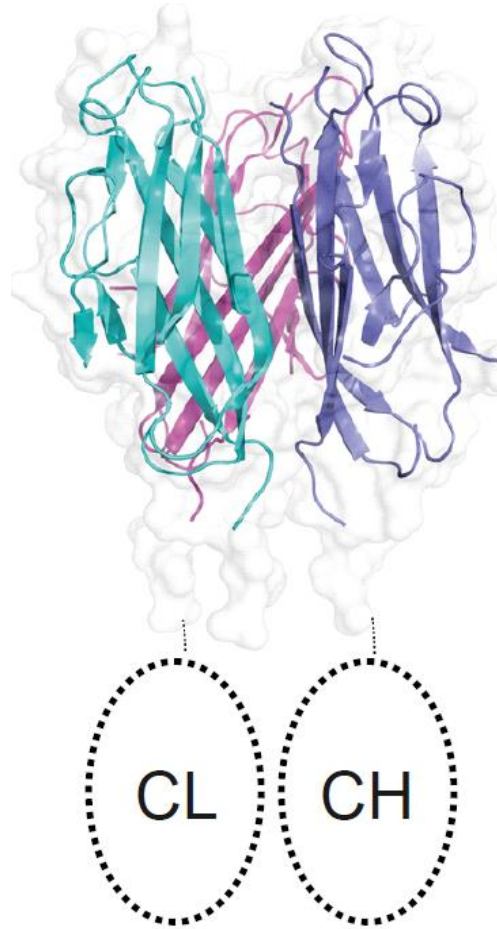


FAP-4-1BBL is a **highly flexible** molecule, able to assume **different conformations**

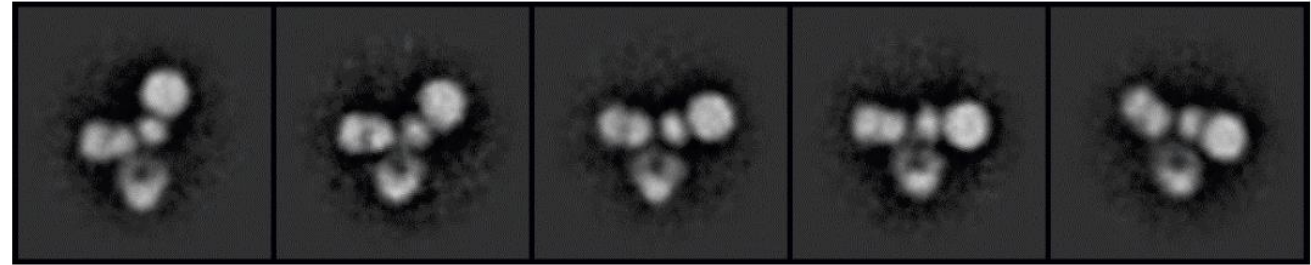


FAP-4-1BBL **binds simultaneously** to 4-1BB and FAP

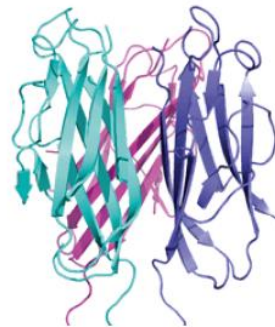
Structural analysis of 4-1BBL shows identity to published structures and TNFR superfamily



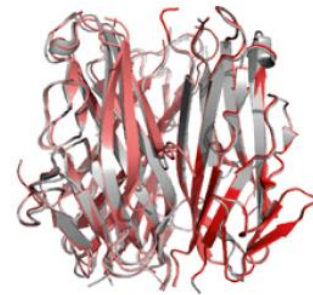
A



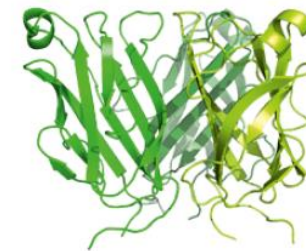
B



4-1BBL trimer
in this study



4-1BBL trimer as
published previously
(6CPR red, 6BWV grey)

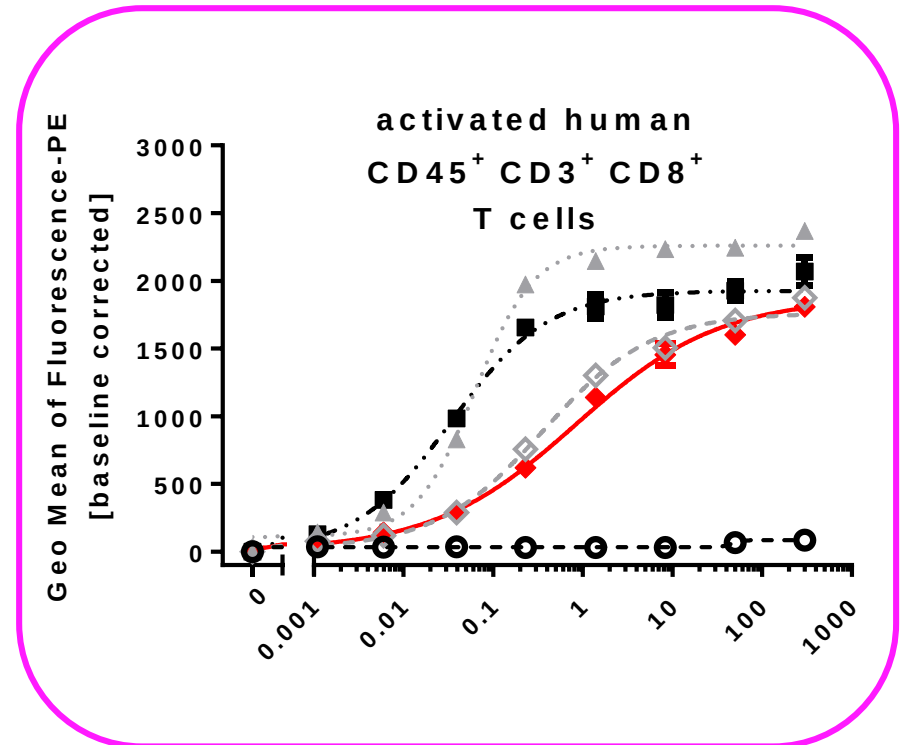
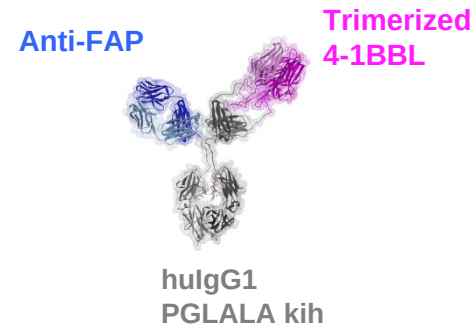
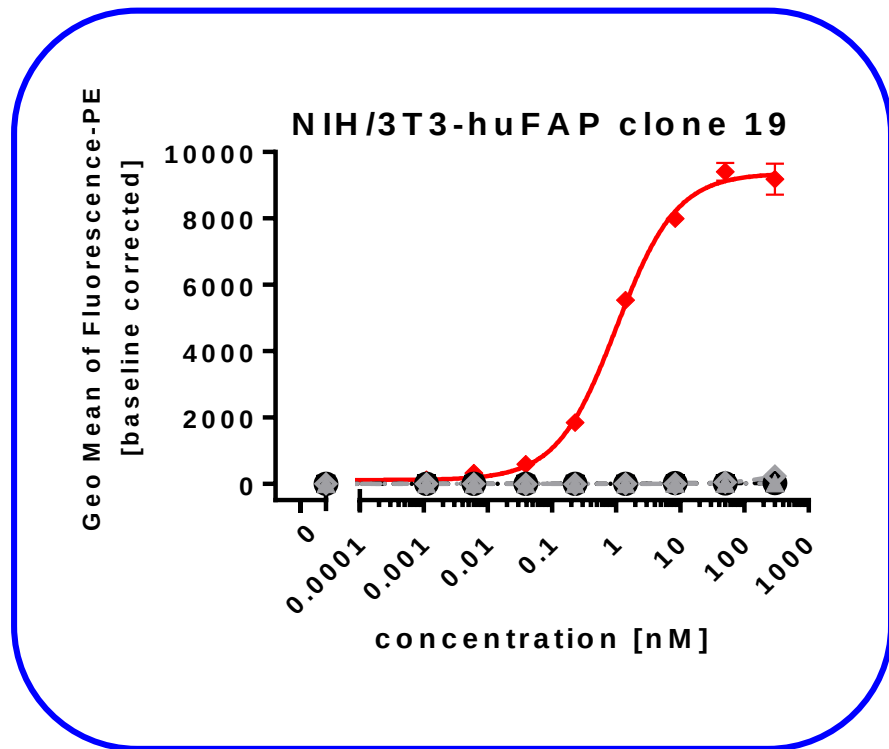


OX40L trimer (2HEW)



TNF trimer (1TNF)

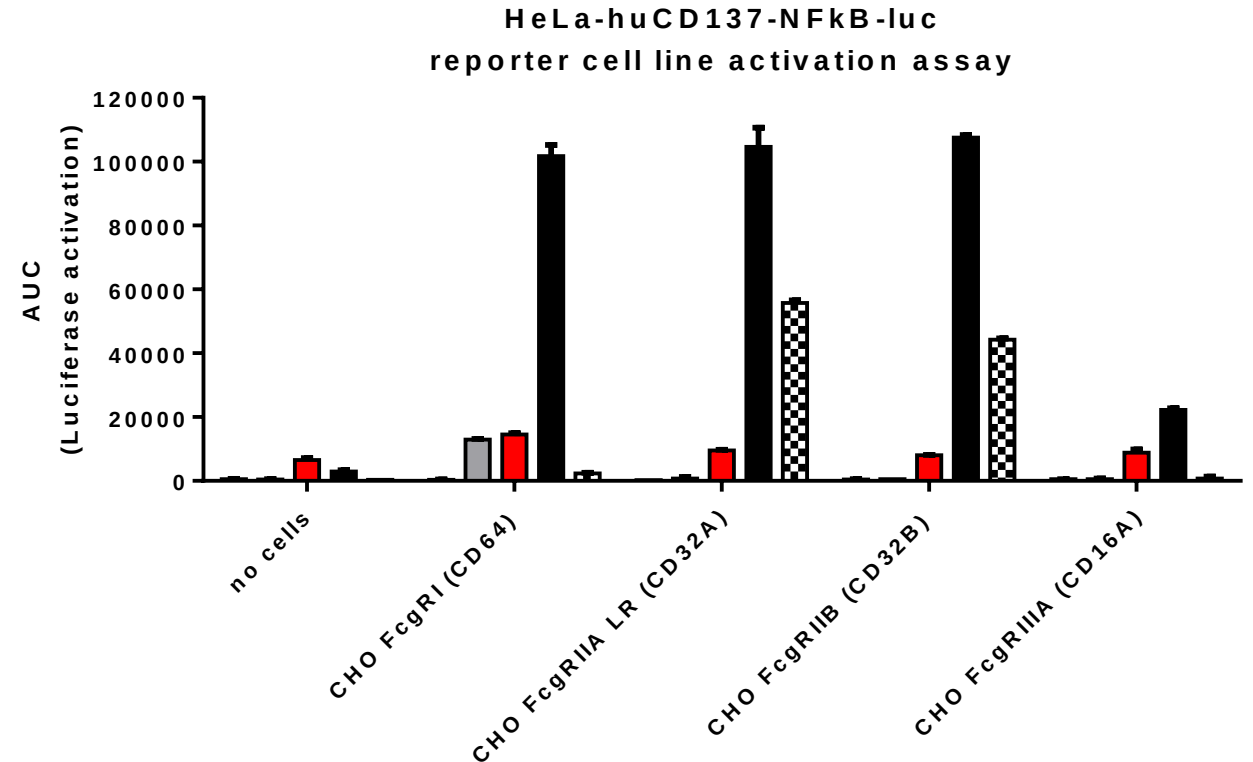
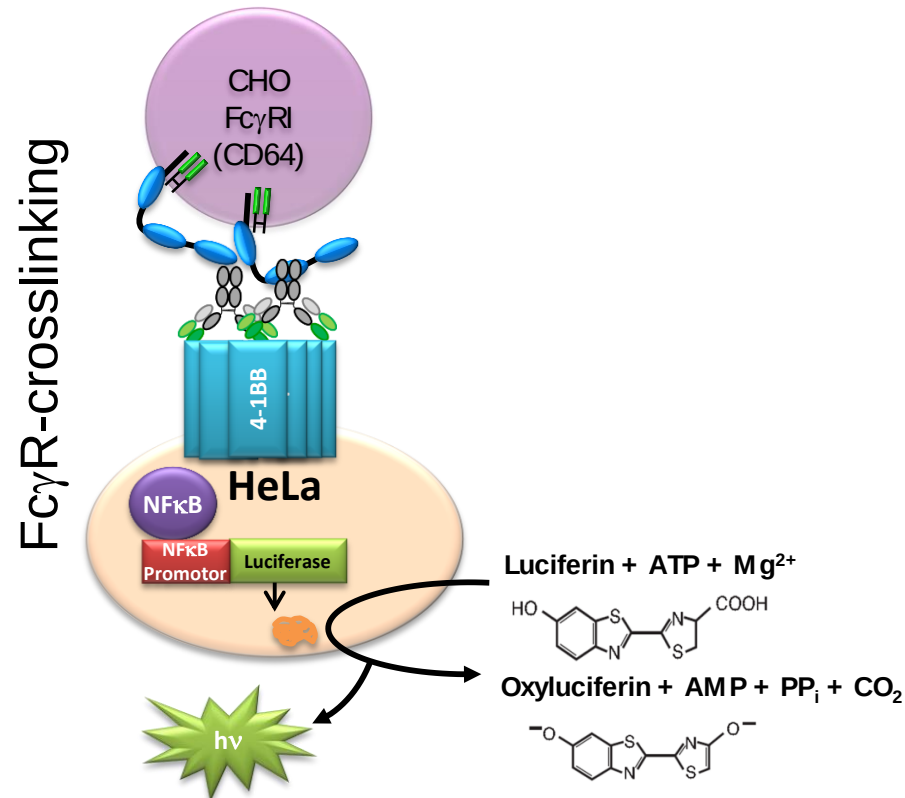
FAP-4-1BBL binds FAP expressed on fibroblasts and 4-1BB expressed on activated human CD8 T cells



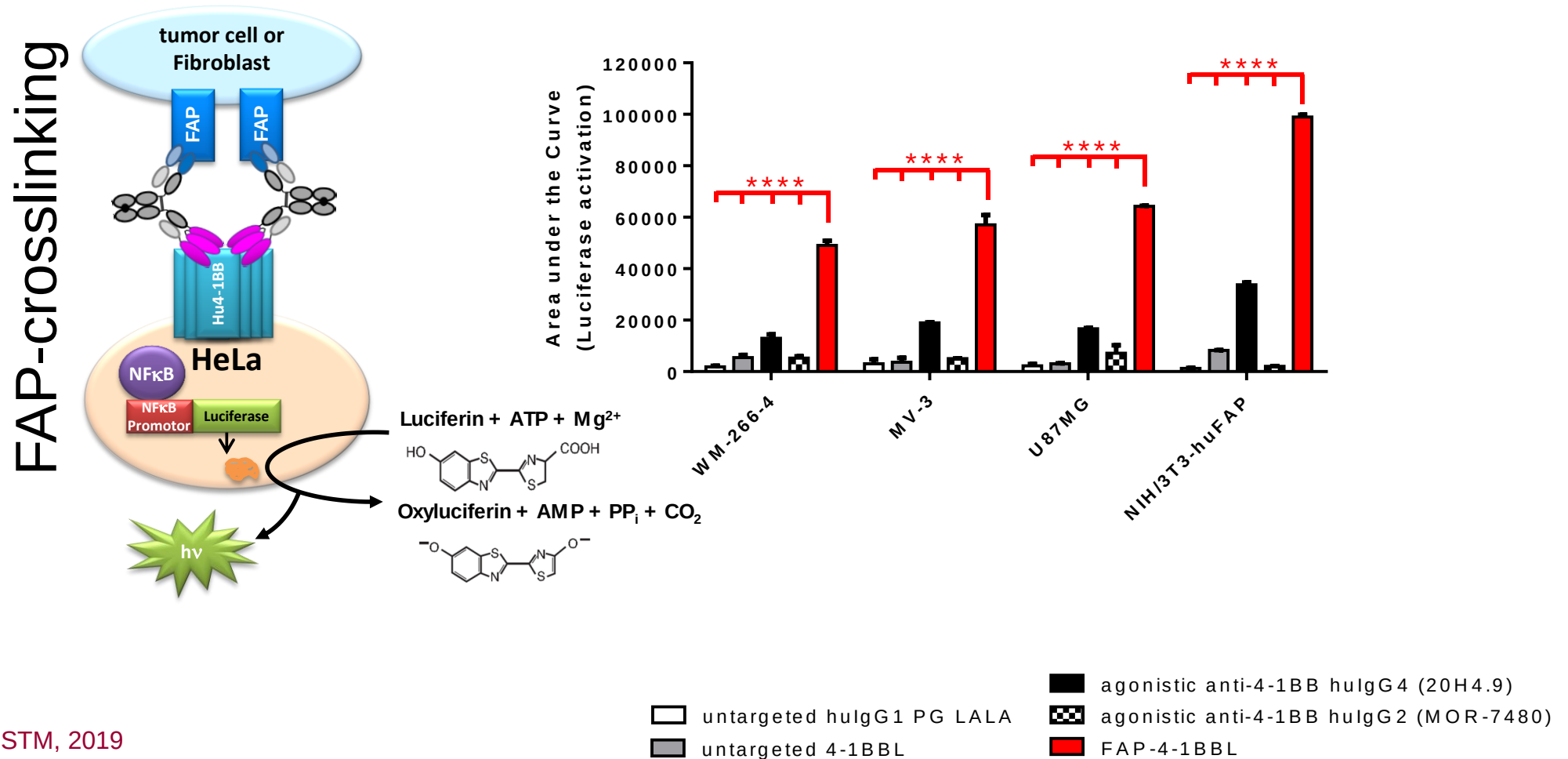
● untargeted hulgG1 PG LALA
 ◆ untargeted 4-1BBL

▲ agonistic anti-4-1BB hulgG4 (20H4.9)
 ■ agonistic anti-4-1BB hulgG2 (MOR-7480)
 ◆ FAP-4-1BBL

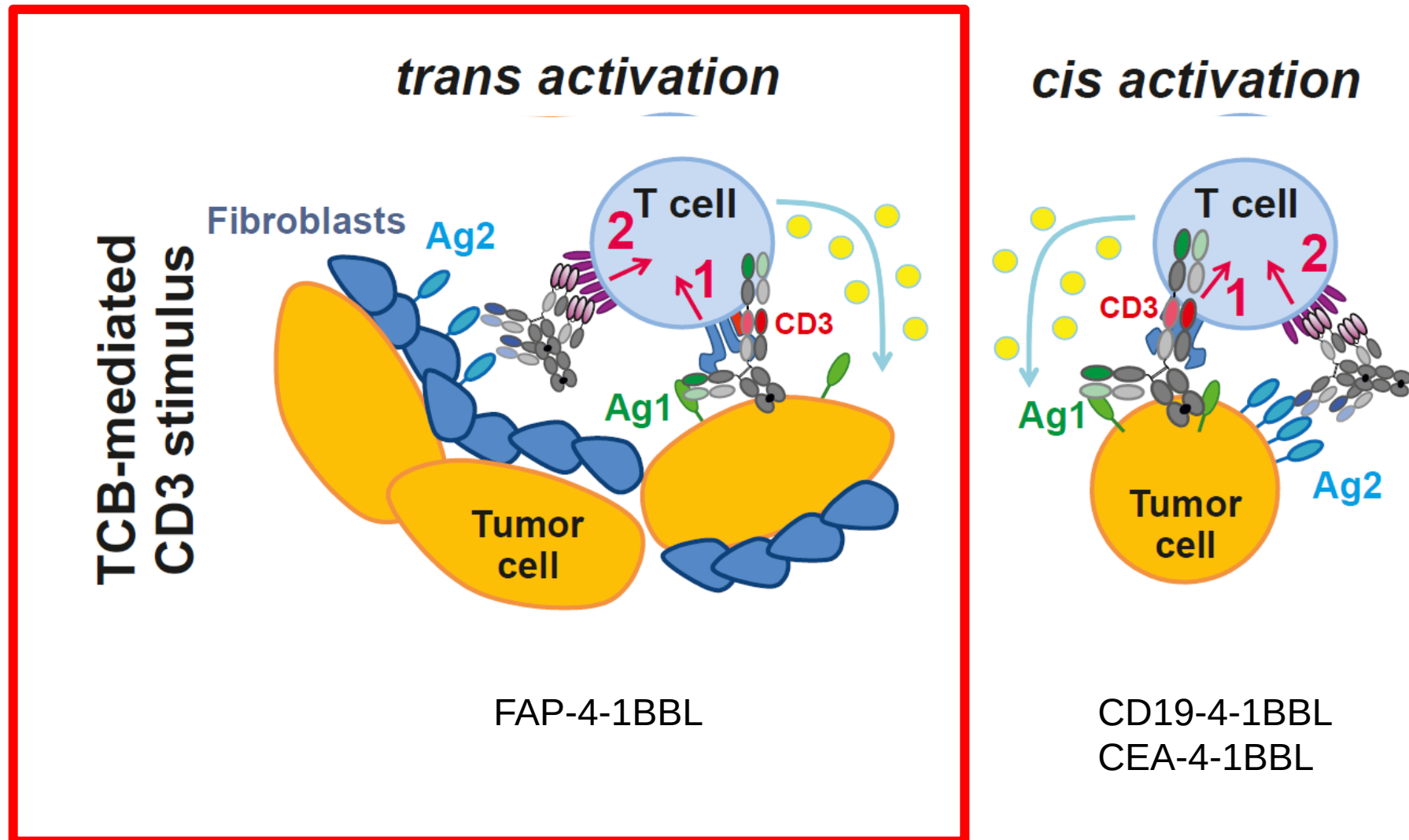
Classical 4-1BB antibodies require Fc γ R crosslinking for their activity



FAP-4-1BBL is only active upon FAP crosslinking

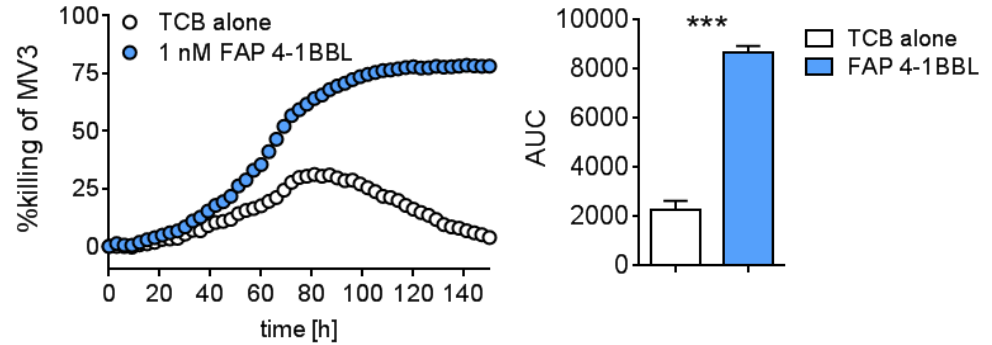


TA-4-1BBL provides signal 2 in presence of T cell bispecific antibodies

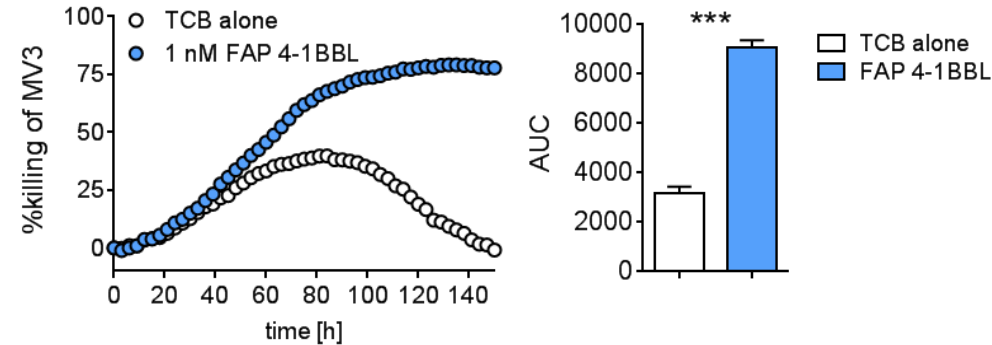


FAP 4-1BBL significantly increases TCB mediated target cell killing by pan-PBMC T cells

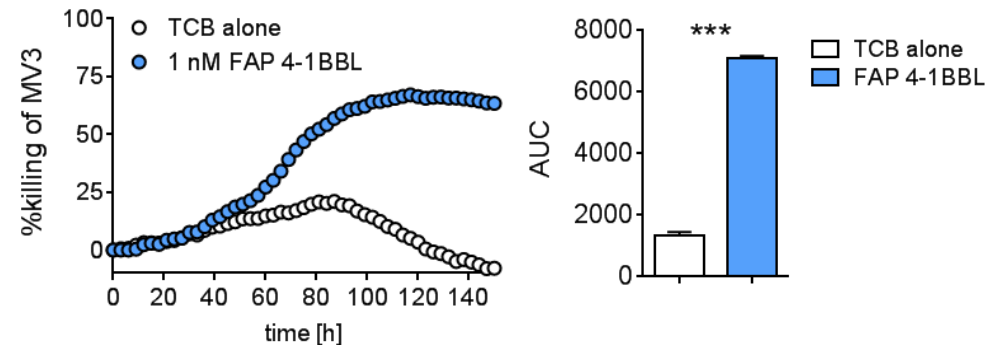
Donor 1



Donor 2



Donor 3

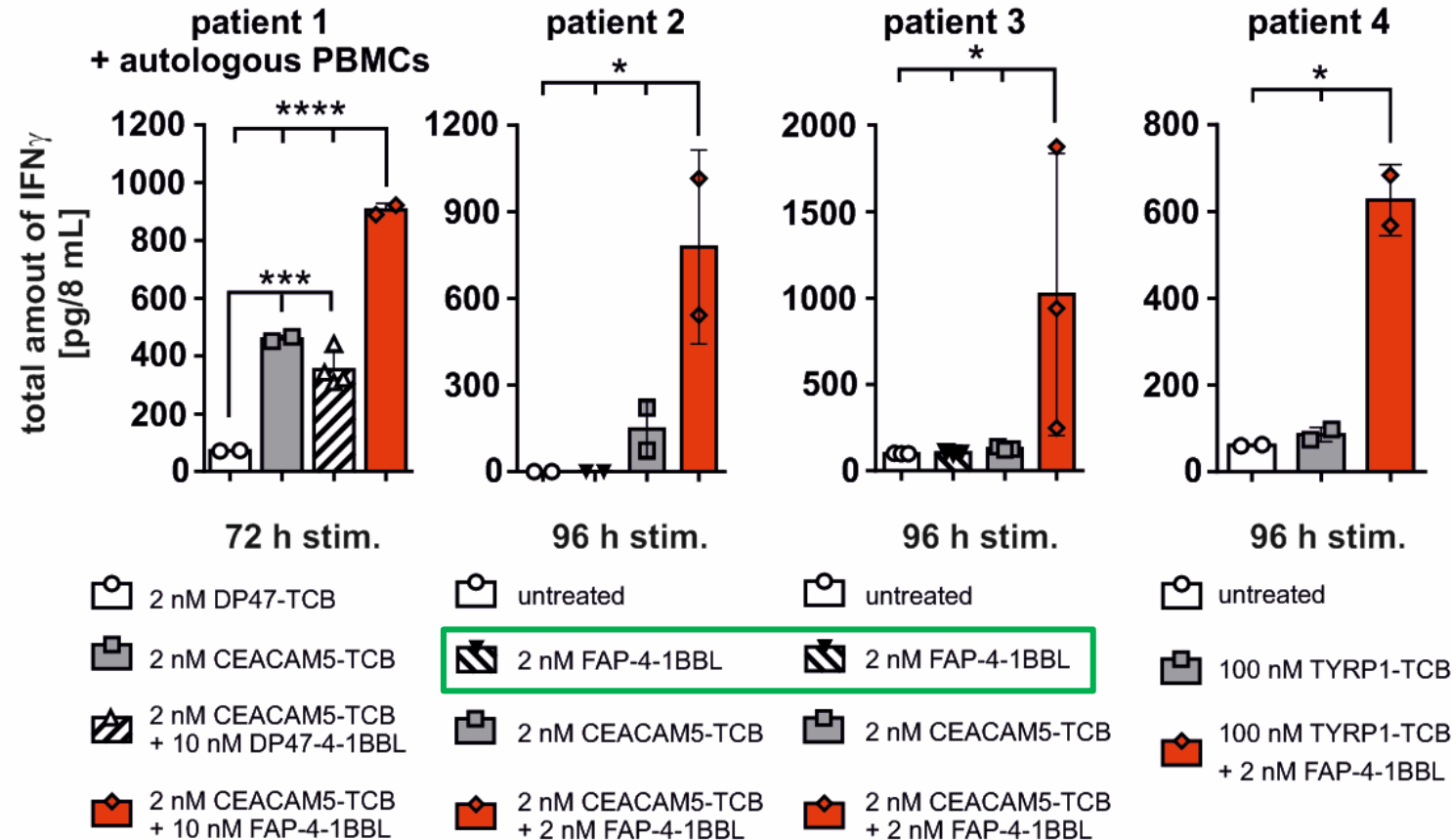


FAP-4-1BBL enhances TCB-mediated T cell activation in primary human tumor explants in 3D bioreactor

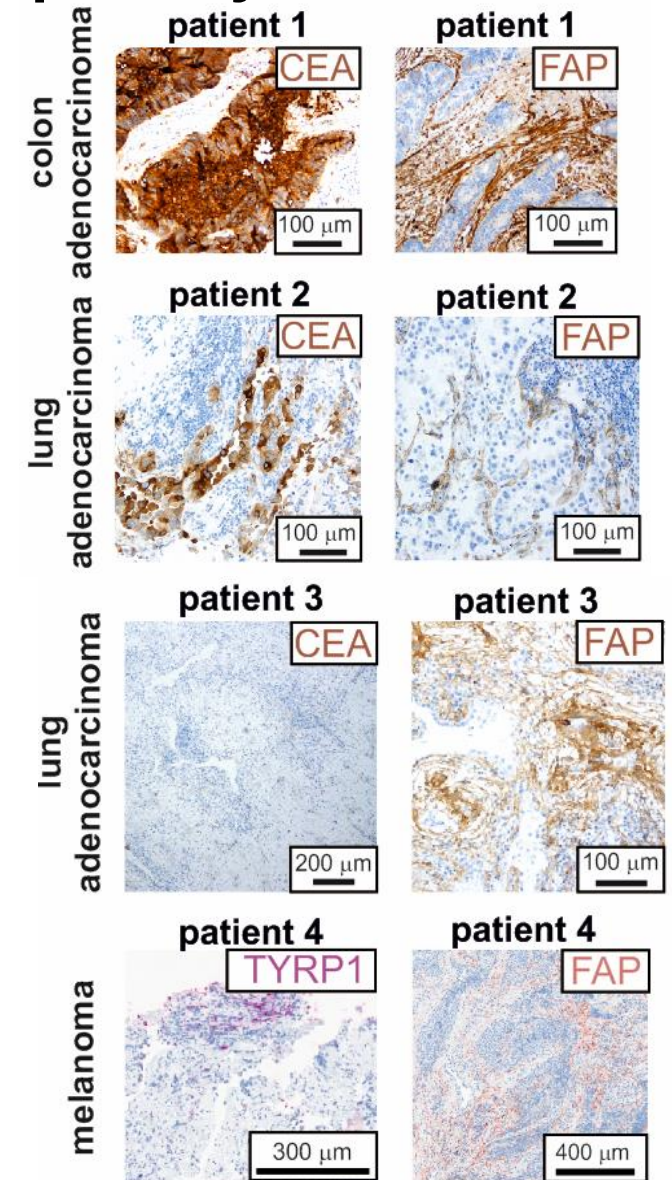


bioreactor for on condition containing 4-6 tumor slices

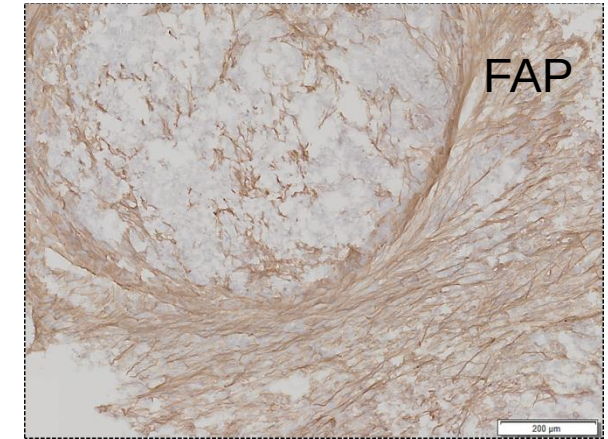
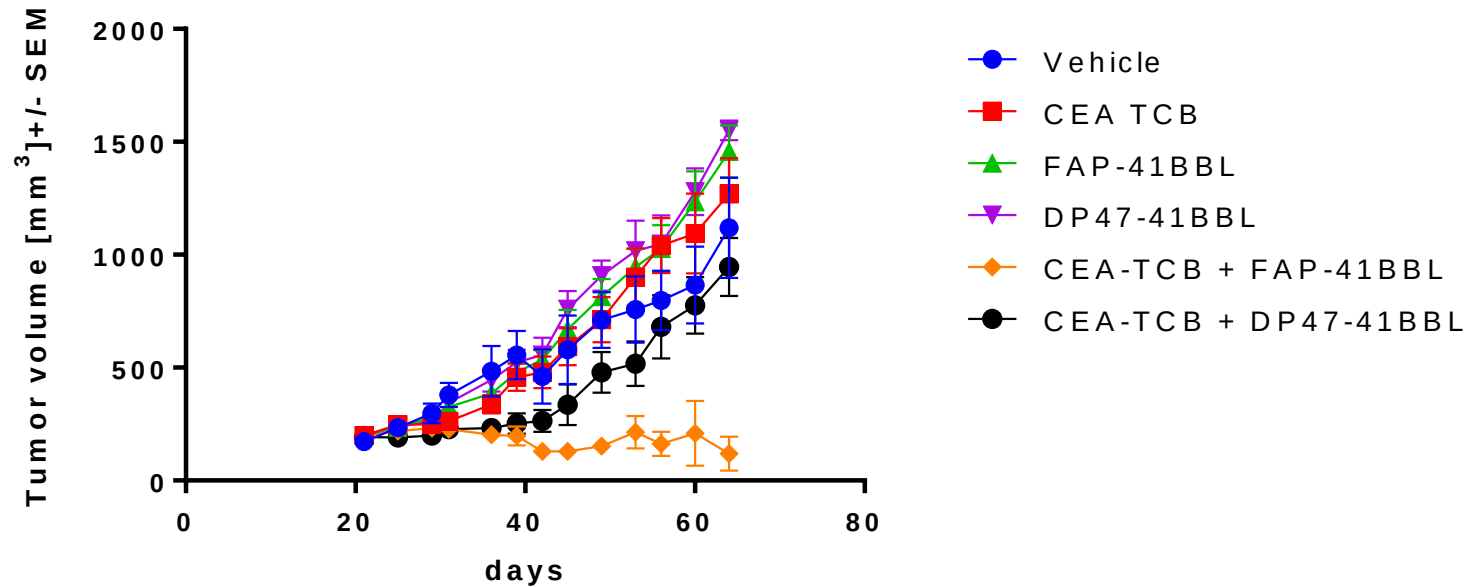
each symbol in the graphs indicates a different bioreactor with 4-6 tumor slices, number of bioreactors depends on tumor size (available tumor slices)



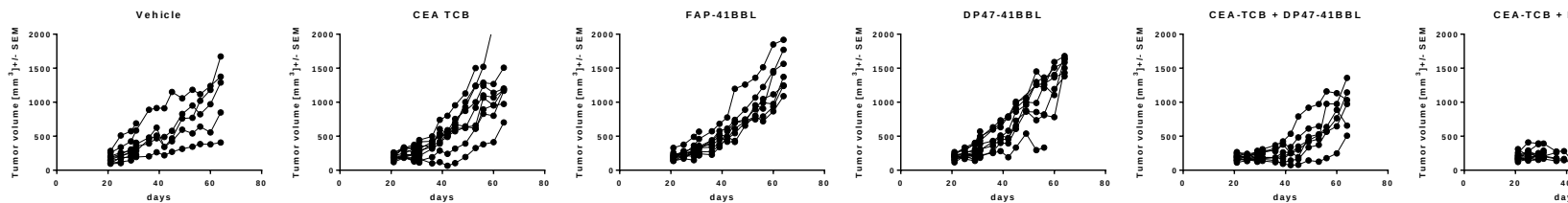
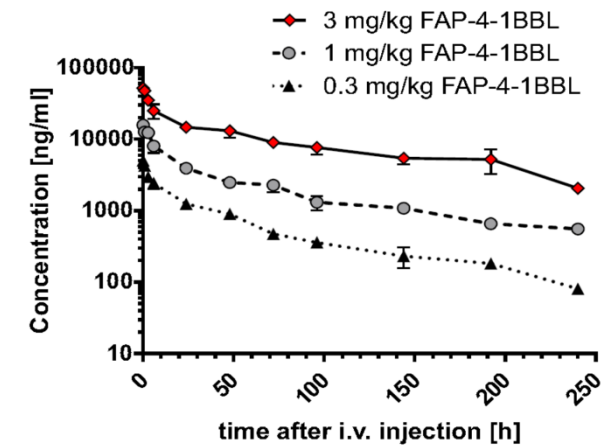
in two experiments a "FAP-4-1BBL only" condition was included, no immune activation above untreated control was seen.



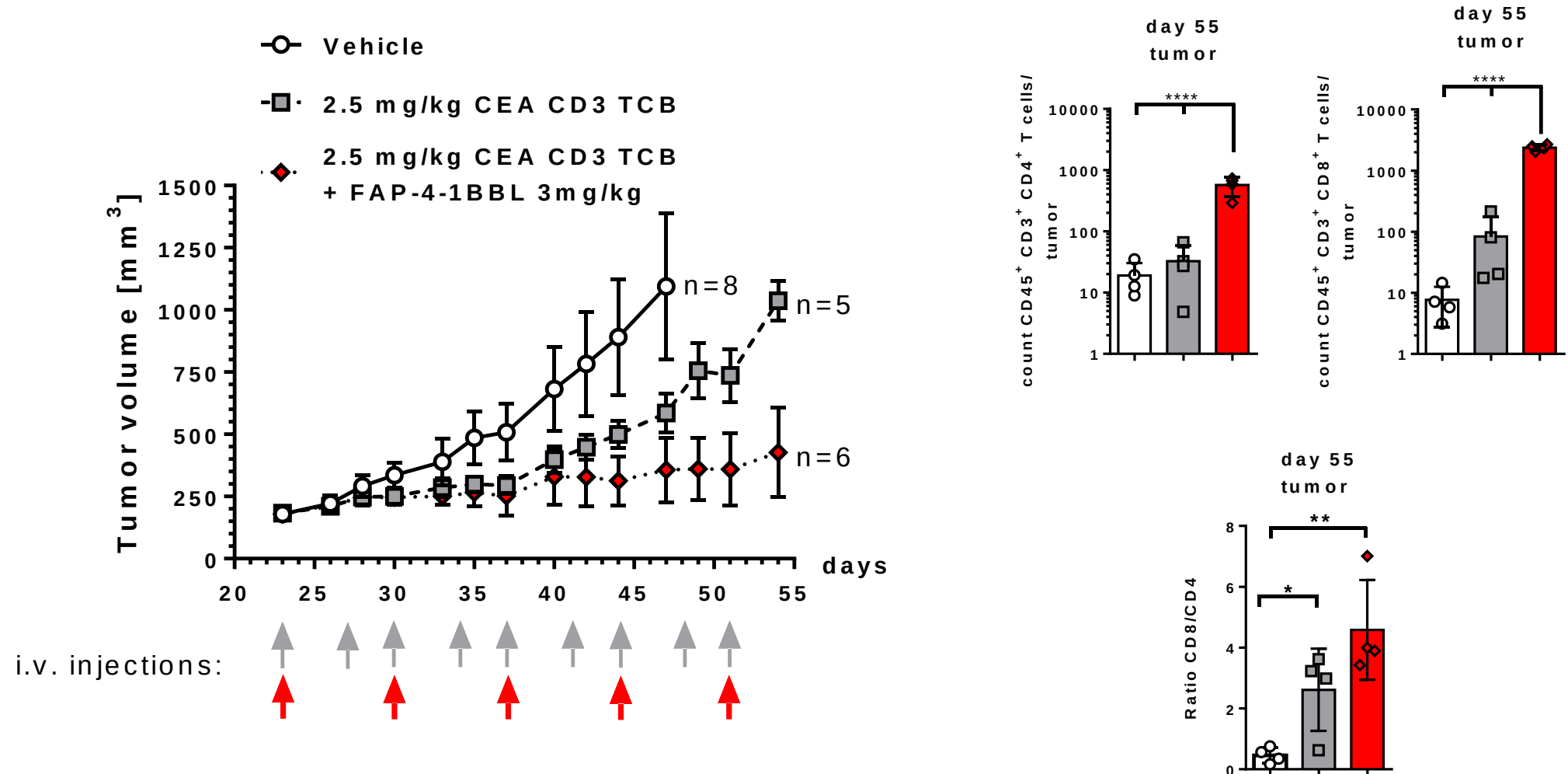
FAP-4-1BBL enhances efficacy of CEA-TCB in MKN45-3T3 fibroblast model in HSC-NSG mice



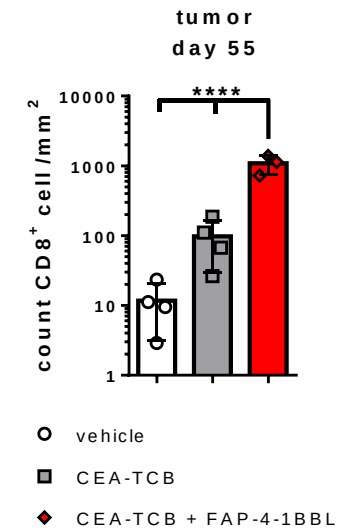
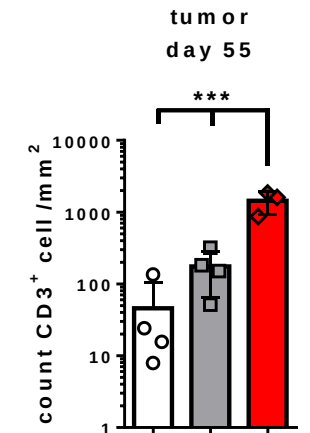
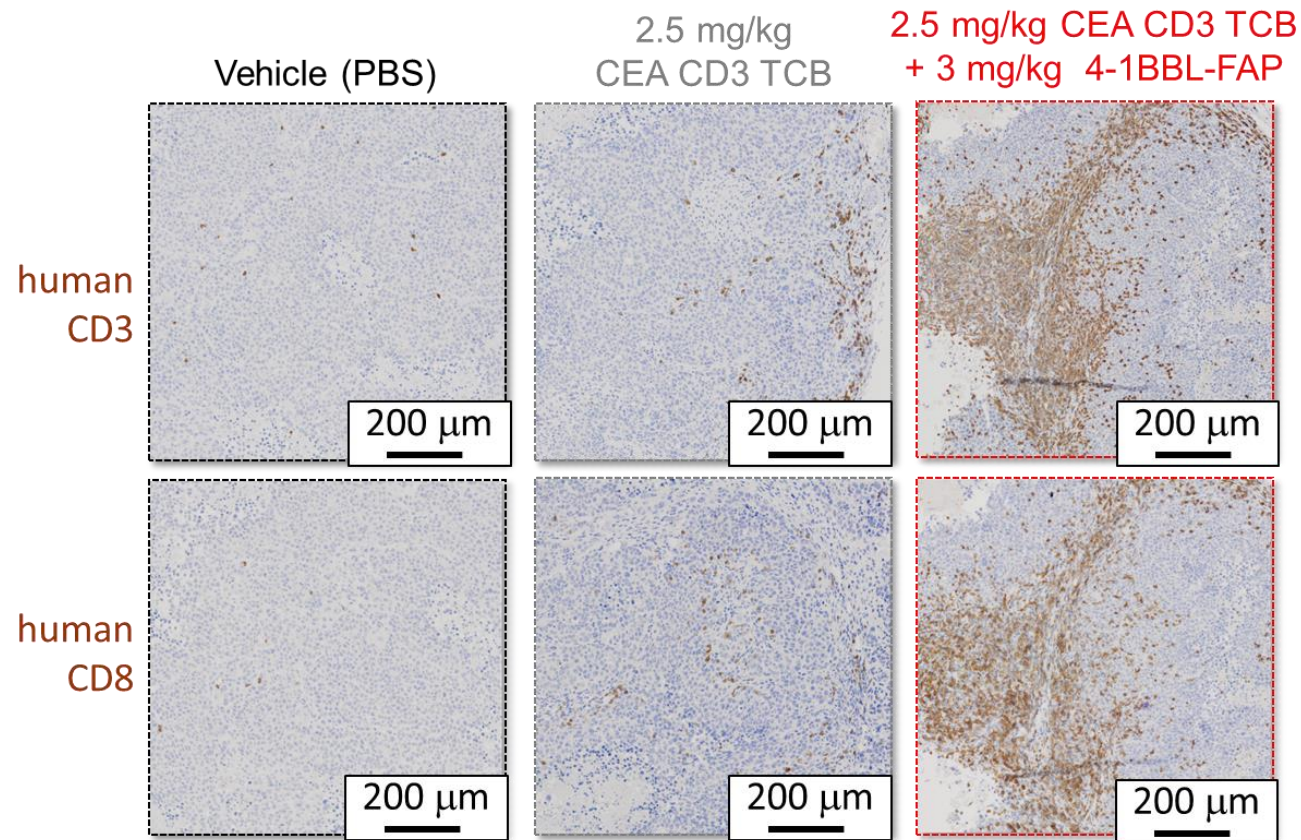
IgG-like pharmacokinetics



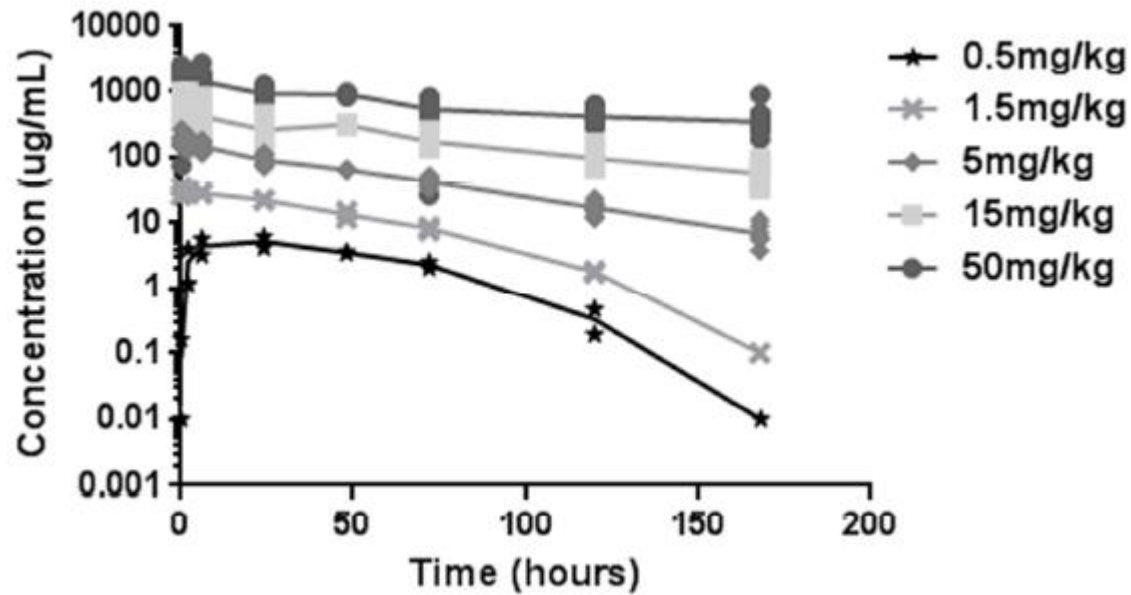
FAP-4-1BBL strongly enhances activity and T cell infiltration in combination with CEA-TCB in MKN45/3T3 HSC-NSG mice



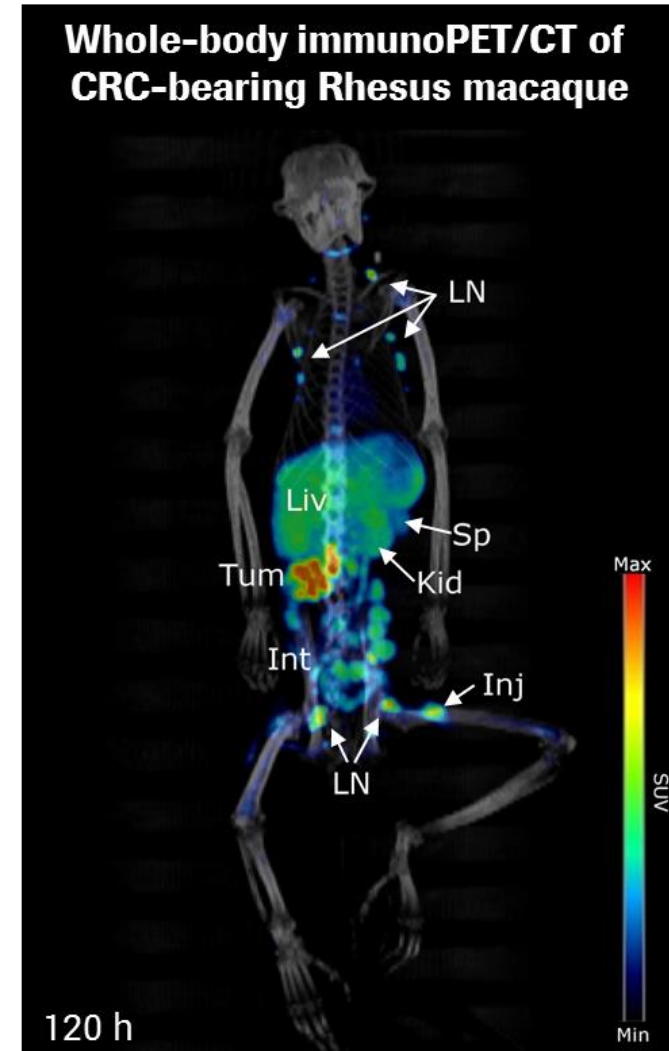
FAP-4-1BBL strongly enhances activity and T cell infiltration in combination with CEA-TCB in MKN45/3T3 NSG mice



FAP-4-1BBL has IgG1-like PK in cynomolgus monkeys & targets to tumor & lymph-nodes in macaque with CRC

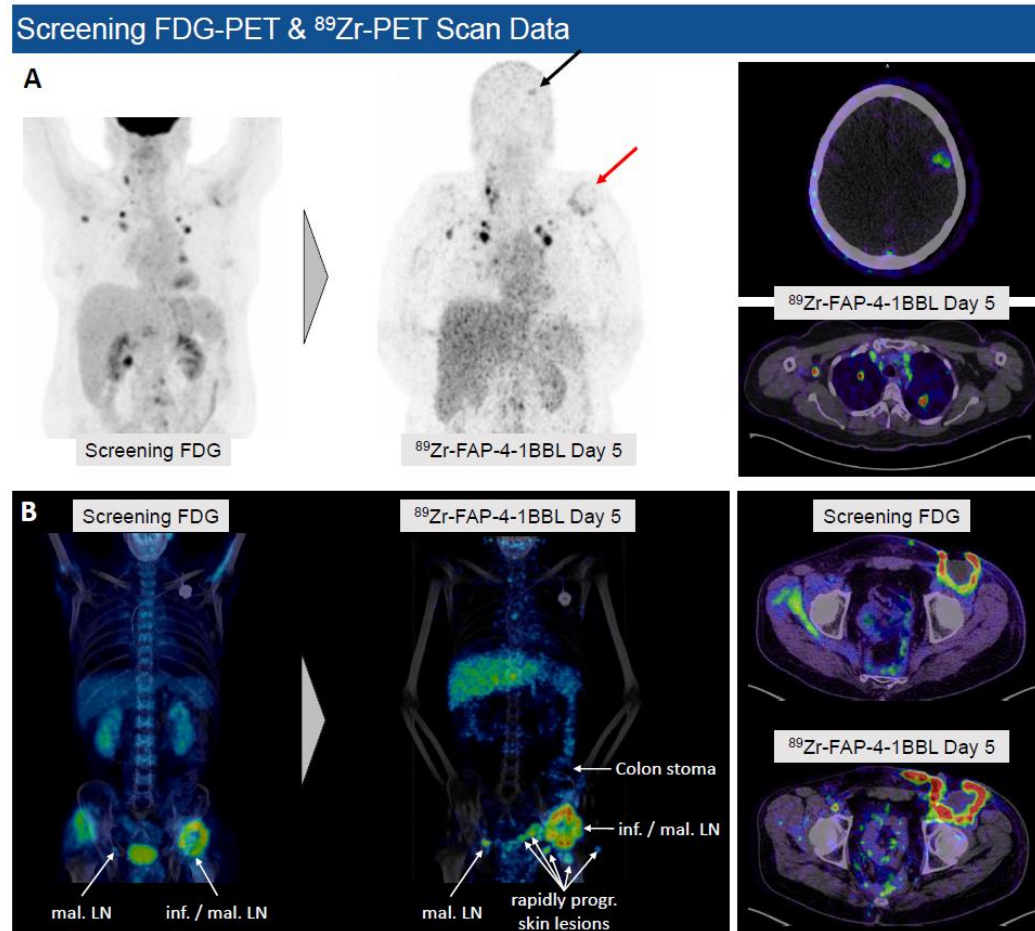


Accumulation in tumor, lymph nodes (FAP-mediated) and liver, kidney, and spleen (excretion and perfusion)



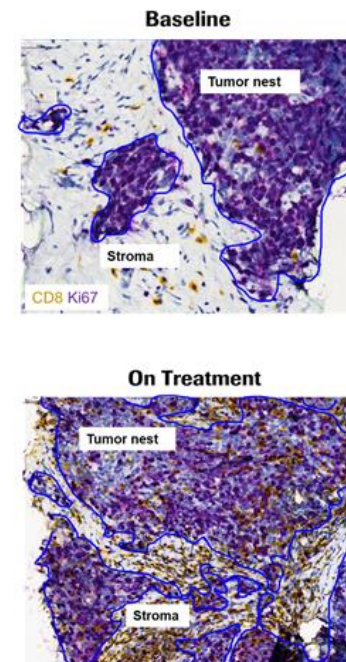
FAP-4-1BBL imaging and biomarker findings from Phase 1 study

FDG-PET & ^{89}Zr -PET scan data



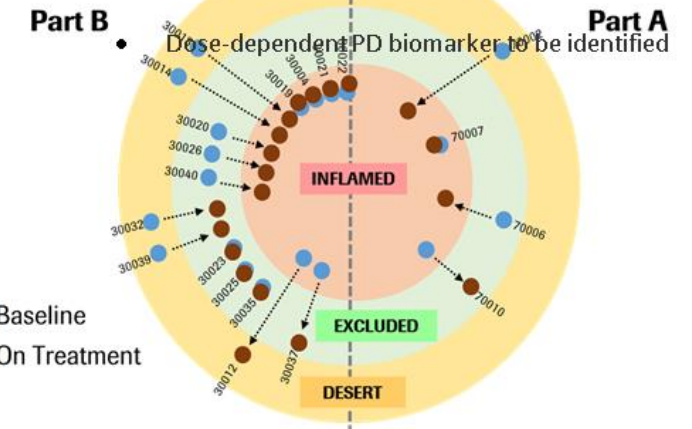
CD8 T cell infiltration and proliferation
in stroma and tumor nests

CD8 Increase in both stroma and tumor nests

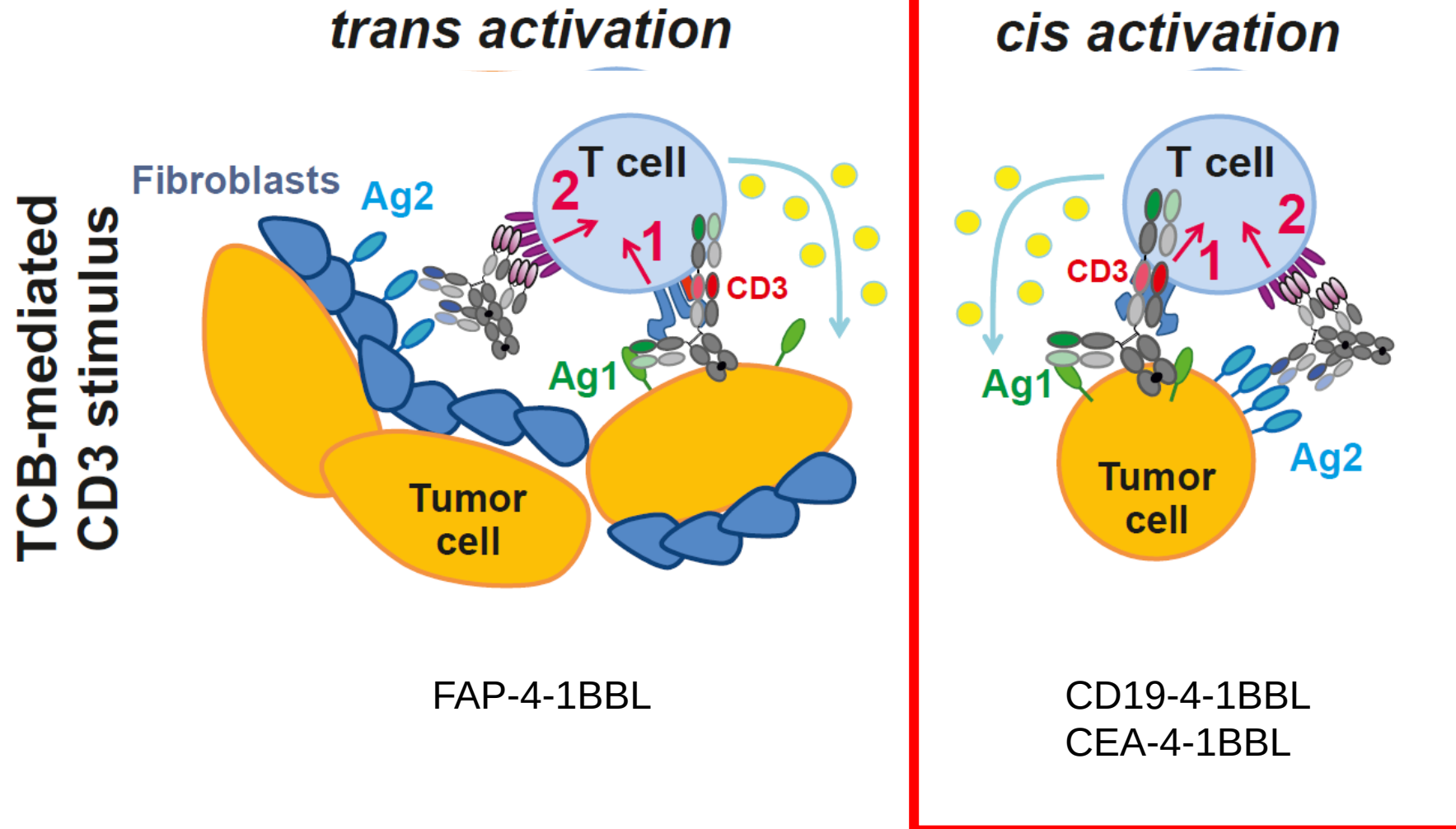


ATZ combo
n=16

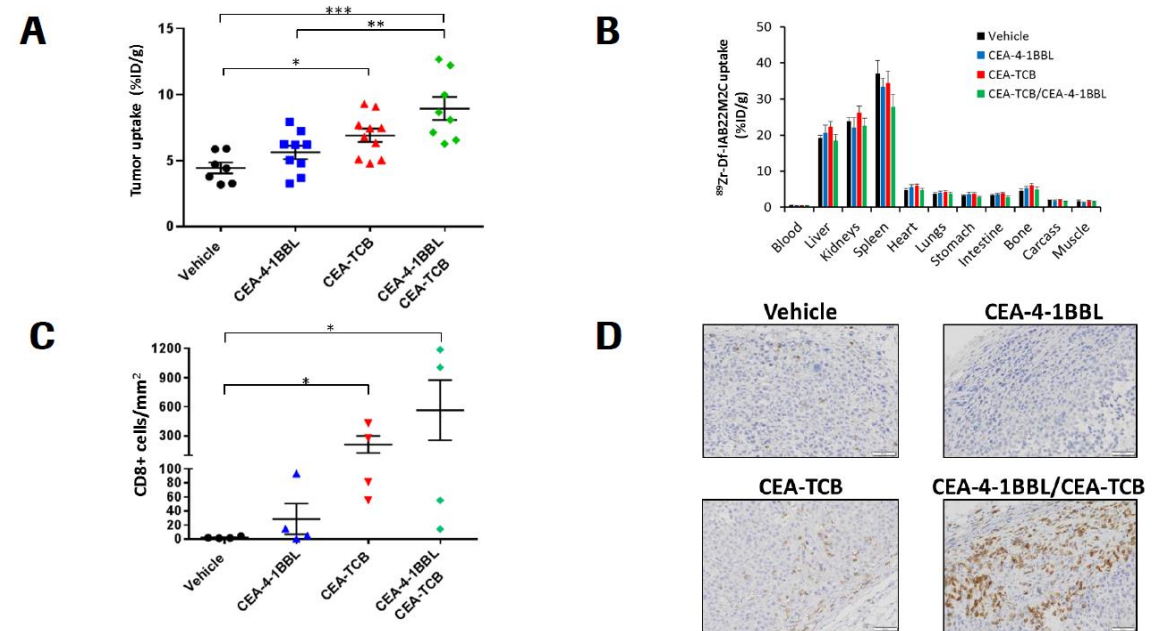
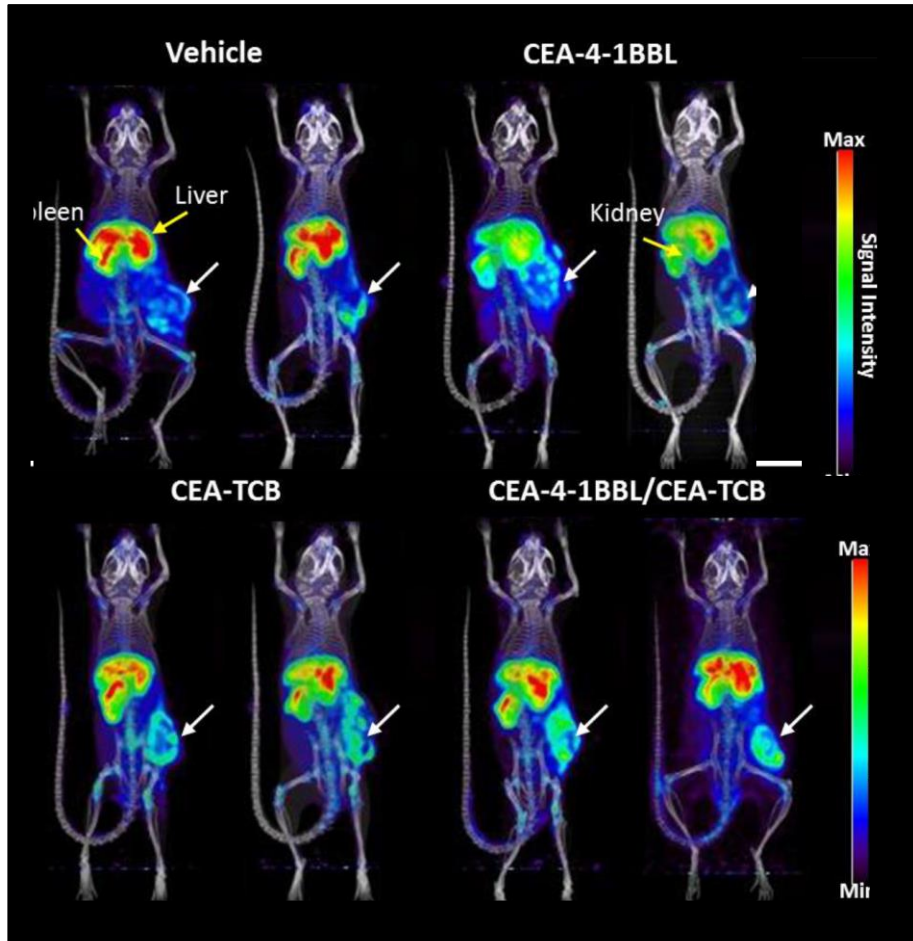
Mono
n=4



TA-4-1BBL provides signal 2 in presence of T cell bispecific antibodies

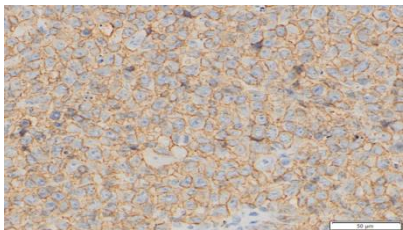


Imaging enhanced intratumoral CD8 infiltrates combining CEA-TCB with CEA-4-1BBL in HSC-NSG using the CD8 PET-tracer ^{89}Zr -Df-IAB22M2C

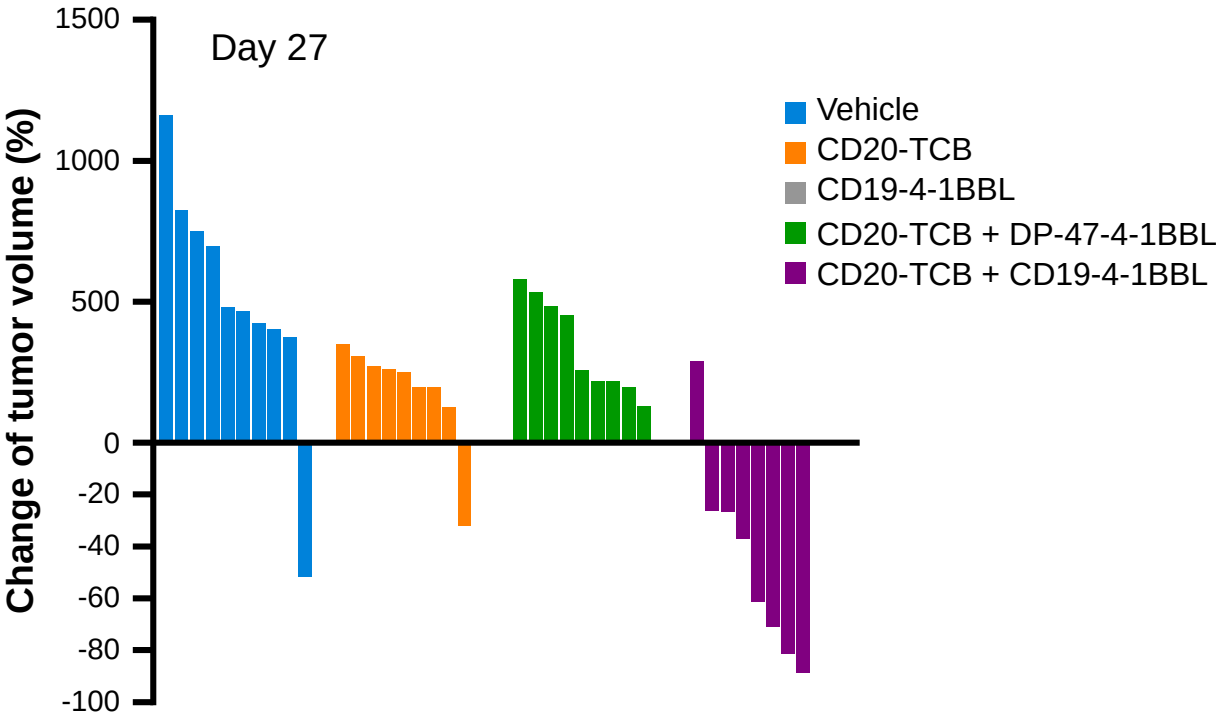
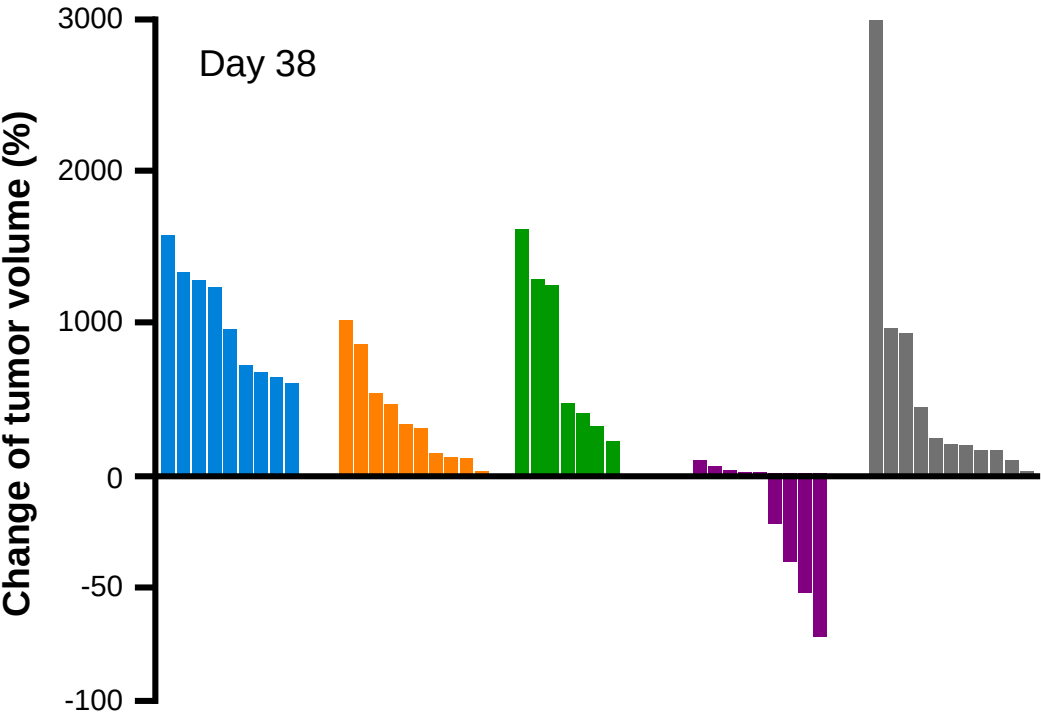
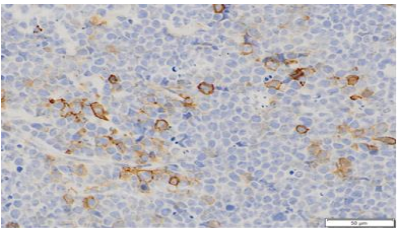


Combination of CD20-TCB and CD19-4-1BBL induces complete tumor regression in DLBCL models

WSU-DLCL2 (CD20 high)



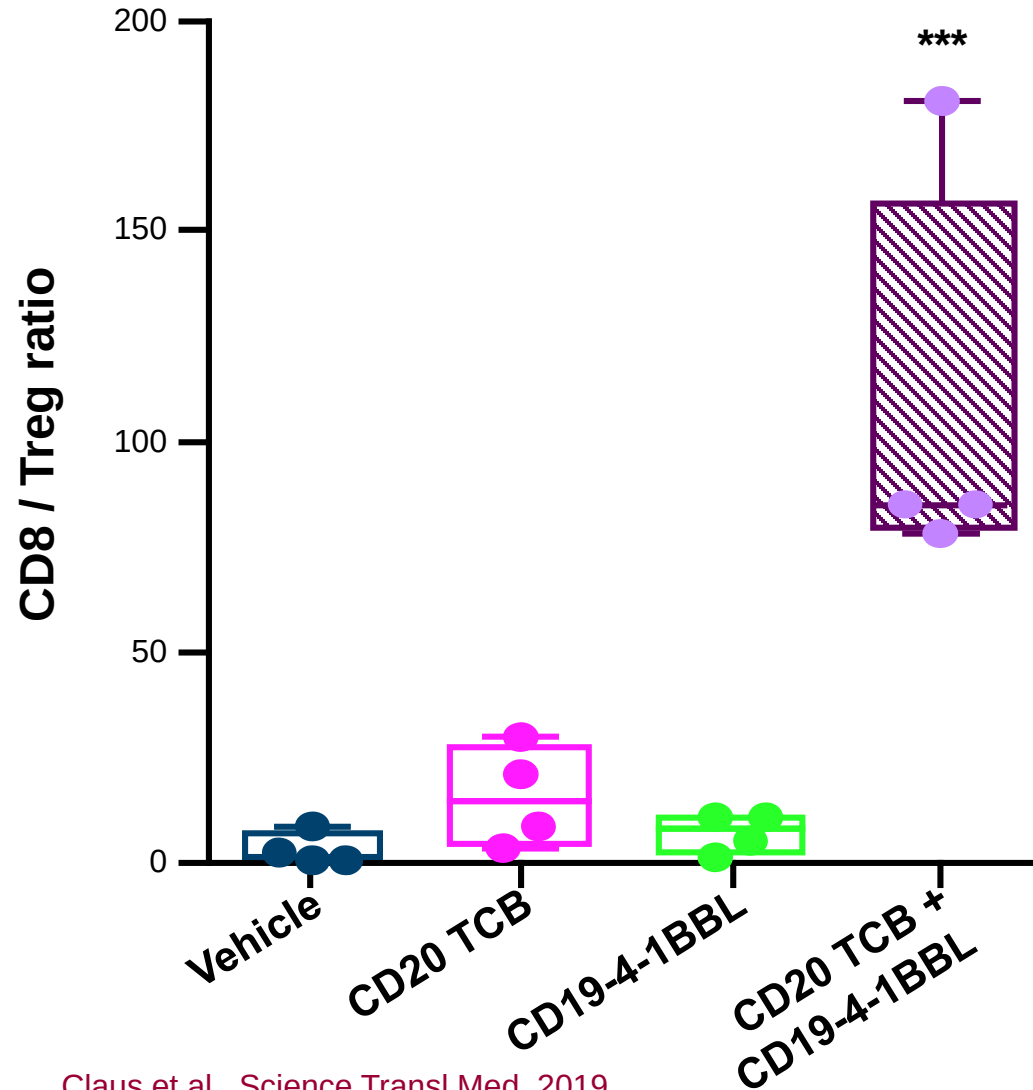
Nalm6 (CD20 low)



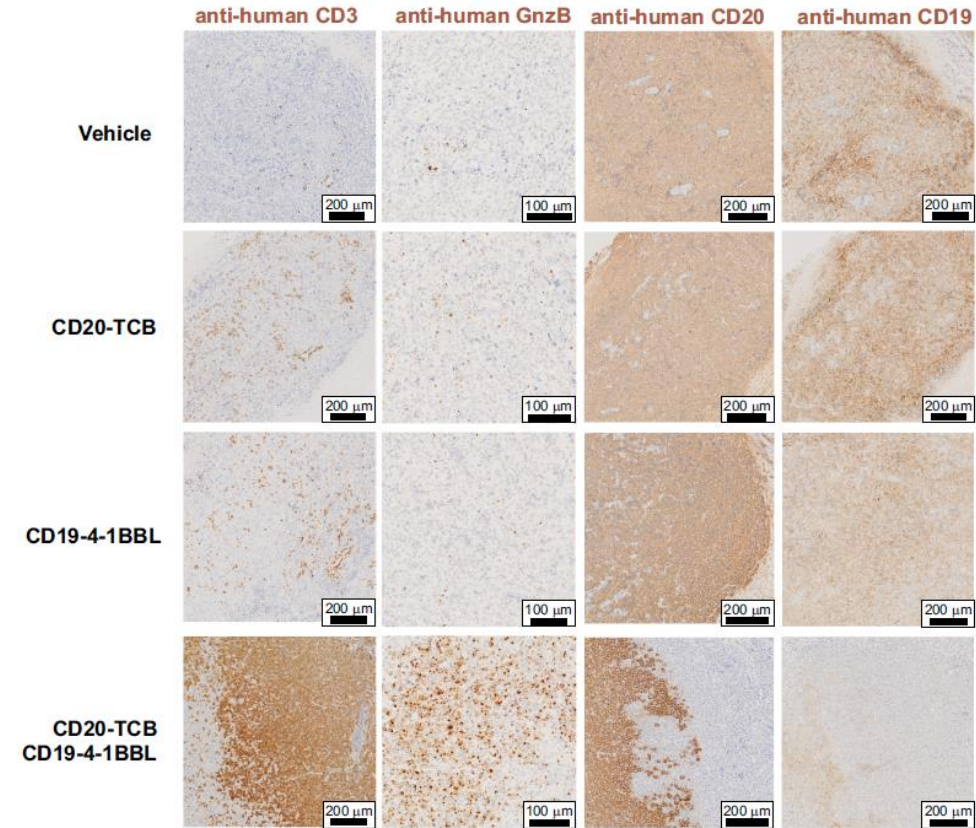
Claus et al., Science Transl Med, 2019

CD20-TCB used at suboptimal dose

Combination of CD20-TCB and CD19-4-1BBL induces massive intratumoral T cell infiltration (WSU-DLCL2)

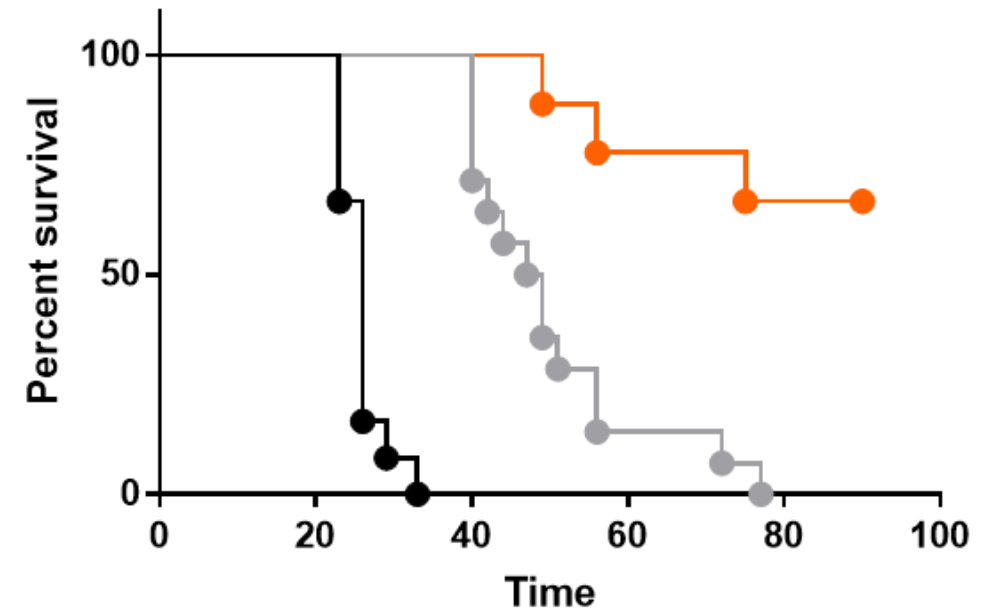
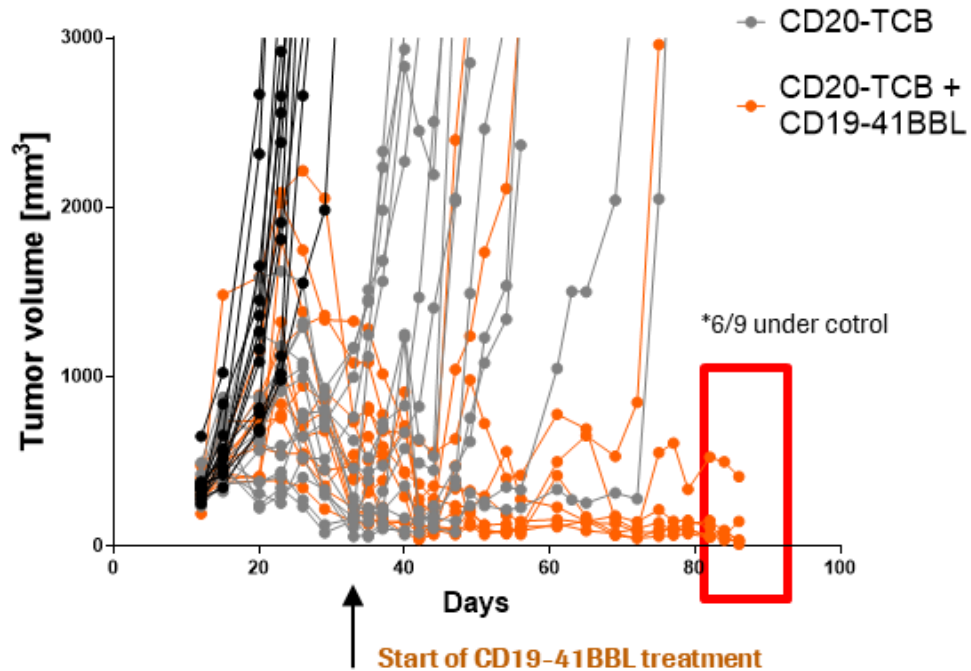


Claus et al., Science Transl Med, 2019



CD20-TCB used at suboptimal dose

Combination of CD20-TCB and CD19-4-1BBL prevents tumor escape in the aggressive OCI-LY18 DLBCL model



TA-4-1BBL: FAP-4-1BBL & CD19-4-1BBL

- TA-4-1BBL induces efficient 4-1BB hyper-crosslinking on T cells in a strictly target dependent fashion
- TA-4-1BBL combined with a TCR stimulus provides co-stimulation to T cells resulting in T cell proliferation, differentiation and activation, and cytokine secretion
- TA-4-1BBL are potent combination partners for TCBs and strongly enhances T cell infiltration and activity =>TA-4-1BBL combined with TCBs provide a safe and effective alternative off-the-shelf approach to CAR T-cells
- Based on these data clinical Phase 1b studies are ongoing testing FAP-4-1BBL in combination with cibisatamab (CEA-TCB) (NCT04826003) and CD19-4-1BBL in combination with glofitamab (CD20-TCB) (NCT04077723)



Acknowledgements

- TCB team: Christiane Neumann, Anne Freimoser, Tanja Fauti, Tina Weinzierl, Marina Bacac, Ekkehard Mössner, Peter Brünker
- CEA-TCB and CD20-TCB teams: Tanja Fauti, Sylvia Herter, Sara Colombetti, Johannes Sam
- FAP/CD19-4-1BBL teams: Claudia Ferrara, Christina Claus, Sabine Lang, Johannes Sam, Stella Tournaviti, Stephane Leclair, Wouter Driessen, Flavio Crameri, Anna Maria Giusti, Mauricio Ceppi, Volker Teichgräber, Stefan Evers, Wei Xu, Michael Hettich, Maurizio Ceppi
- All contributors from Roche pRED: Large Molecule Research, Pharmacology, Translational Medicine Oncology, Clinical Operations, Pharmaceutical Sciences, Clinical Safety & Regulatory
- Academic collaborators, clinical Investigators, patients & their families



Claudia Ferrara



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Johannes Sam



Sylvia Herter



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