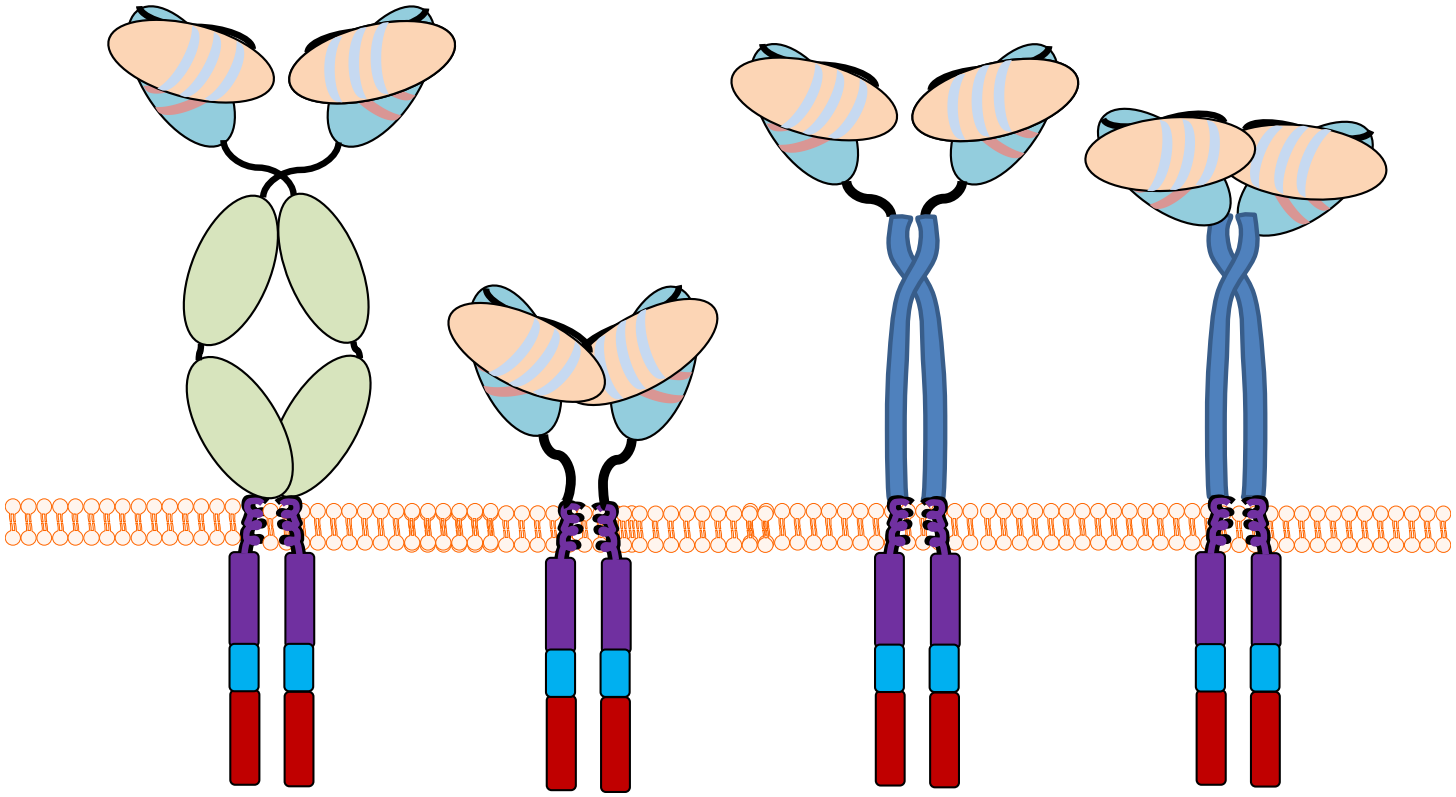
CAR T cell therapy – Modular approach to solid tumours



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Disclosure & Conflict of Interest

Dr Pule is the founder and Chief Scientific Officer of Autolus Therapeutics plc and a clinical senior lecturer in the Dept. of Hematology at UCL Cancer Institute. The views and opinions expressed in this presentation are of Dr Pule in his personal capacity. The views expressed are his own and do not necessarily represent the views of Autolus Therapeutics.

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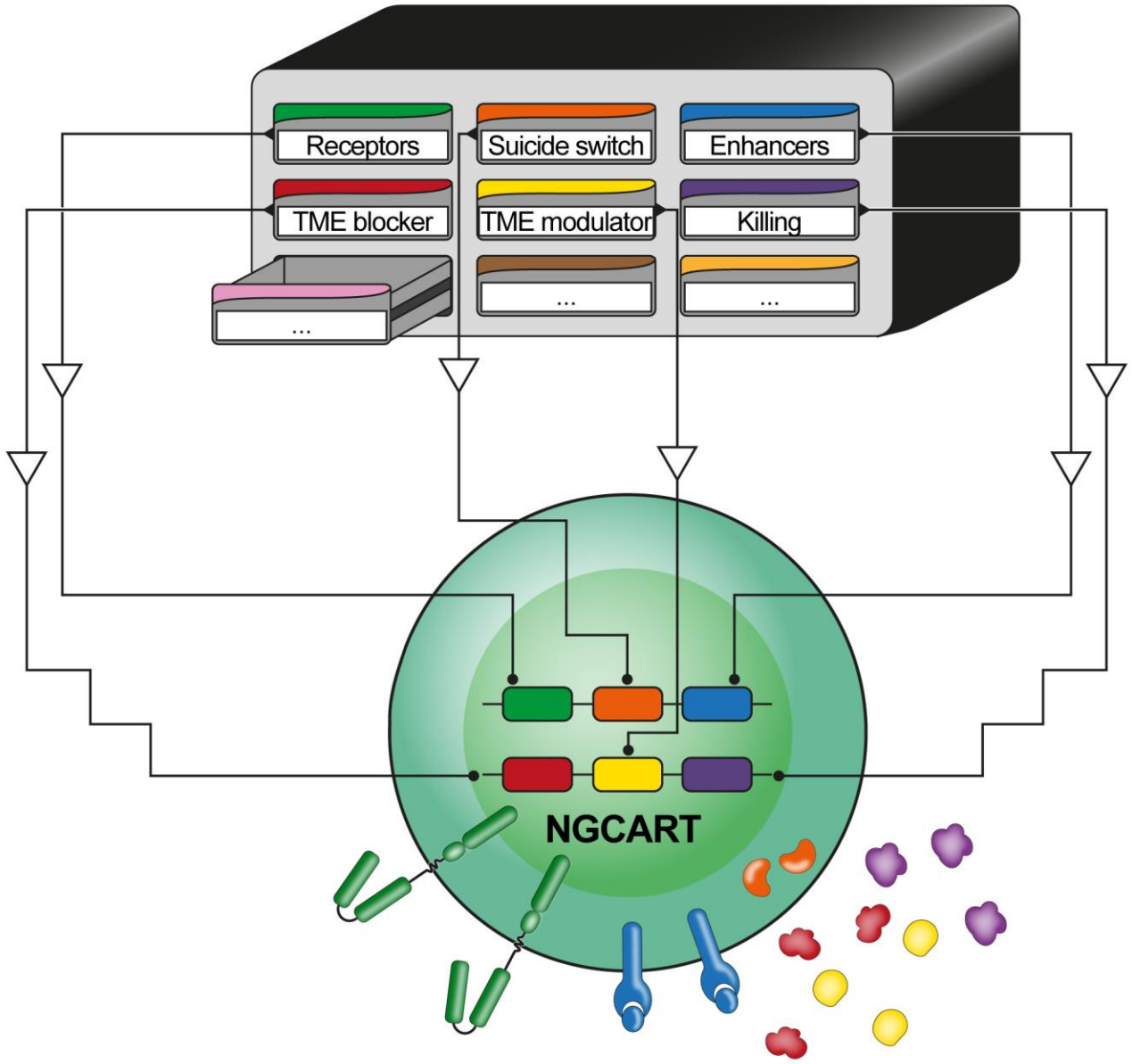
Modular engineering approach to CAR T cell therapy in solid cancer



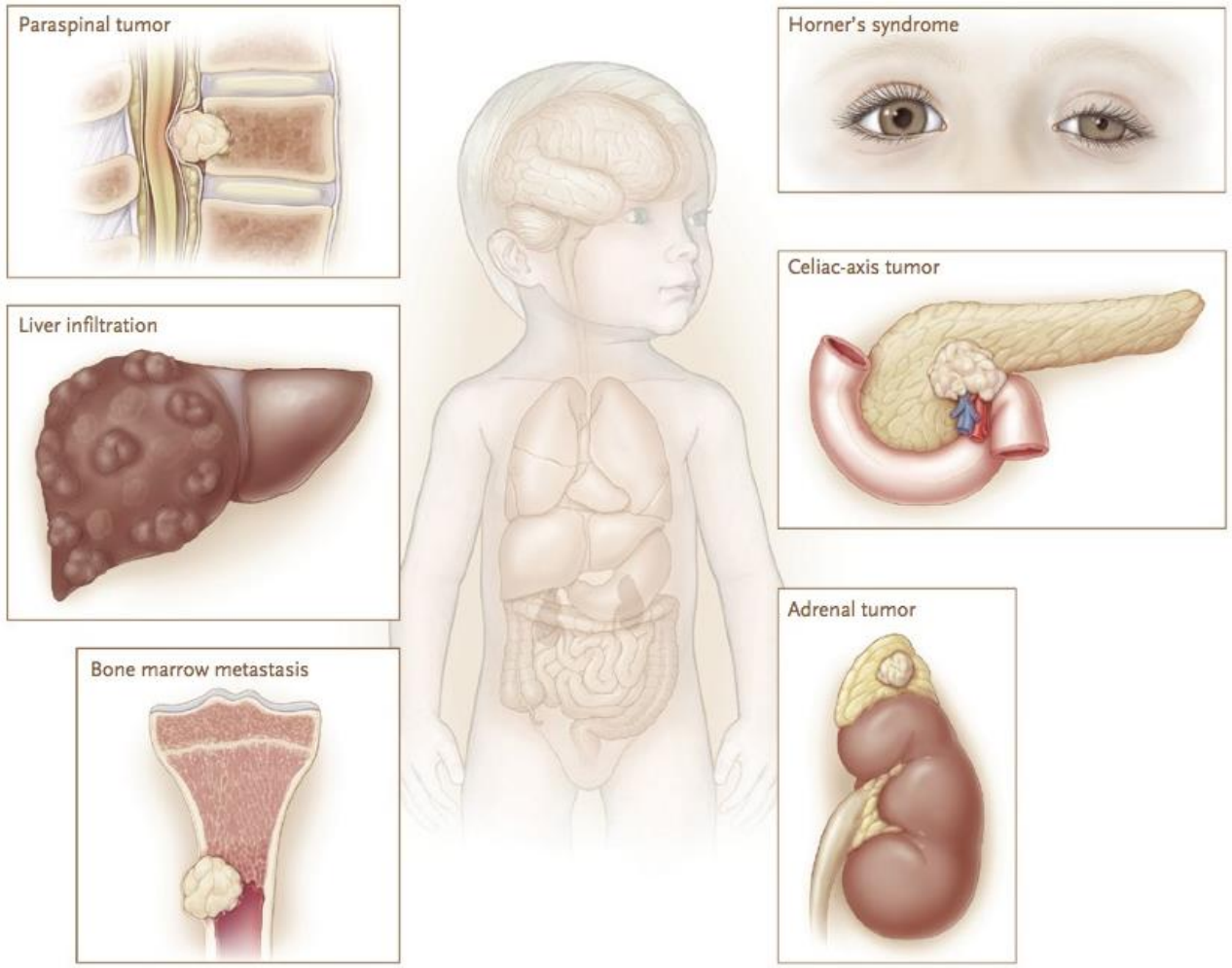
A Cancer Research UK Phase I trial of anti-GD2 CAR T-cells in patients with relapsed or refractory neuroblastoma (1RG-CART)

Karin Straathof, Barry Flutter, Rebecca Wallace, Simon Thomas, Gordon Cheung, Angela Collura, Talia Gileadi, Jack Barton, Gary Wright, Sarah Inglott, Lorenzo Biassoni, Kieran McHugh, David Edwards, Claire Barton, Karen Dyer, Nigel Westwood, Thalia Loka, Sarita Depani, Karen Howe, Giuseppe Barone, Martin Pule and John Anderson

CANCER RESEARCH UK

Straathof et al, Sci Trans Med, 2020

Childhood cancer neuroblastoma



From: Maris JM, N Engl J Med 2010;362:2202–2211

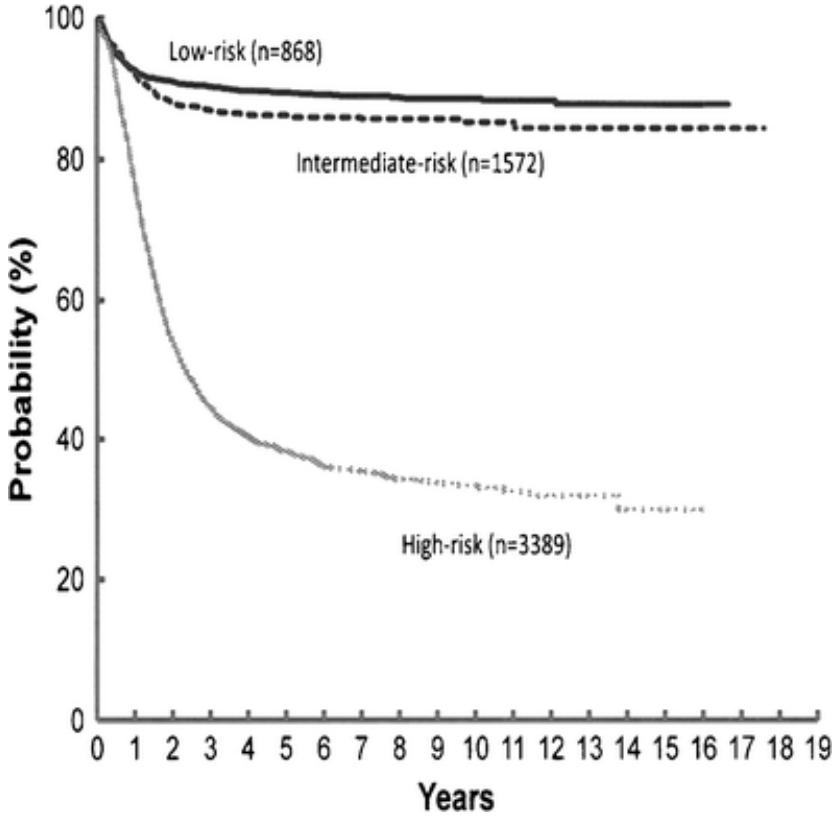
Most common solid tumour after brain tumours

Median age at diagnosis:
17 months

New diagnoses/year:
100 in UK, 800 in USA

Need for new treatment modality

low risk | intermediate risk | high risk



Extra chemotherapy if needed

Induction chemotherapy

~ 3-4 months

Surgery

Myeloablative chemotherapy with stem cell rescue

~1 month

Radiotherapy

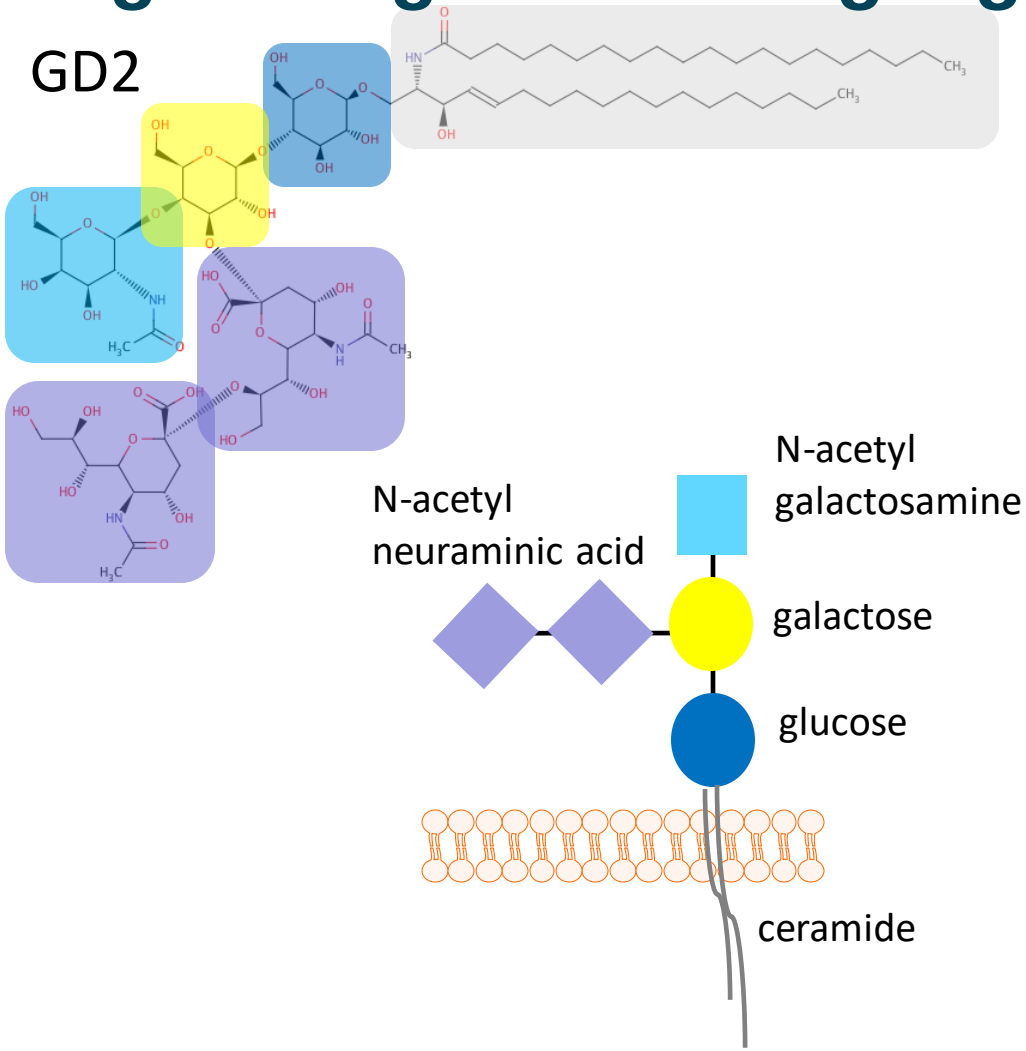
Immunotherapy (anti-GD2)

Differentiation therapy (cisRA)

~4-5 months

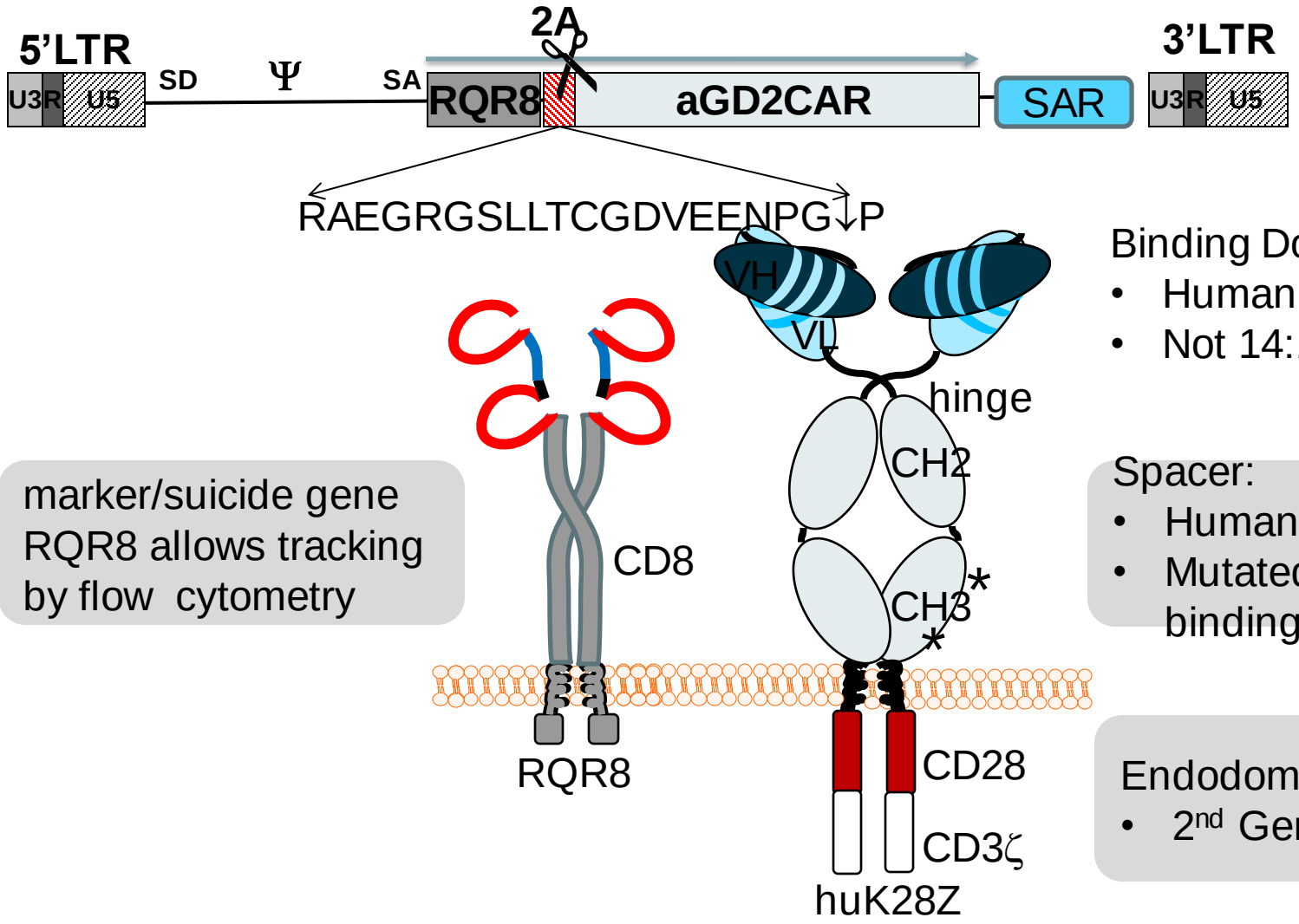
Straathof et al, Sci Trans Med, 2020

Target antigen: disialoganglioside GD2



- Abundantly expressed on neuroblastomas
- Dim expression on normal tissue restricted to neurons
- Experience targeting GD2 with therapeutic monoclonal antibodies
- CART approach to induce durable anti-GD2 immunity

GD2-CAR with humanized novel binder



Transfer Vector
- SAR enhanced MoMLV
- Bi-cistronic with 2A peptide

Binding Domain:
- Humanized scFv
- Not 14:18 GD2 mAb derived

marker/suicide gene RQR8 allows tracking by flow cytometry

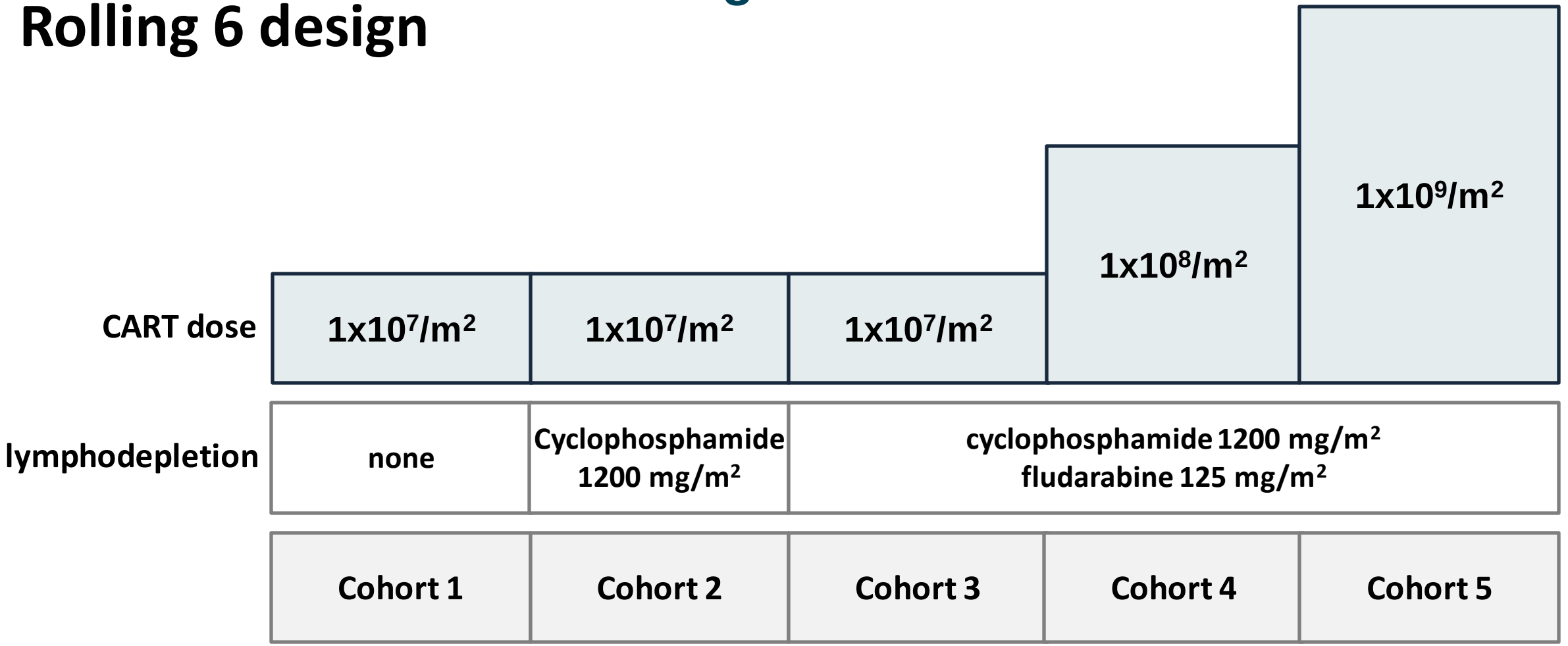
Spacer:
- Human IgG1 Fc
- Mutated to avoid FcR binding

Endodomain
- 2nd Generation CD28-ζ

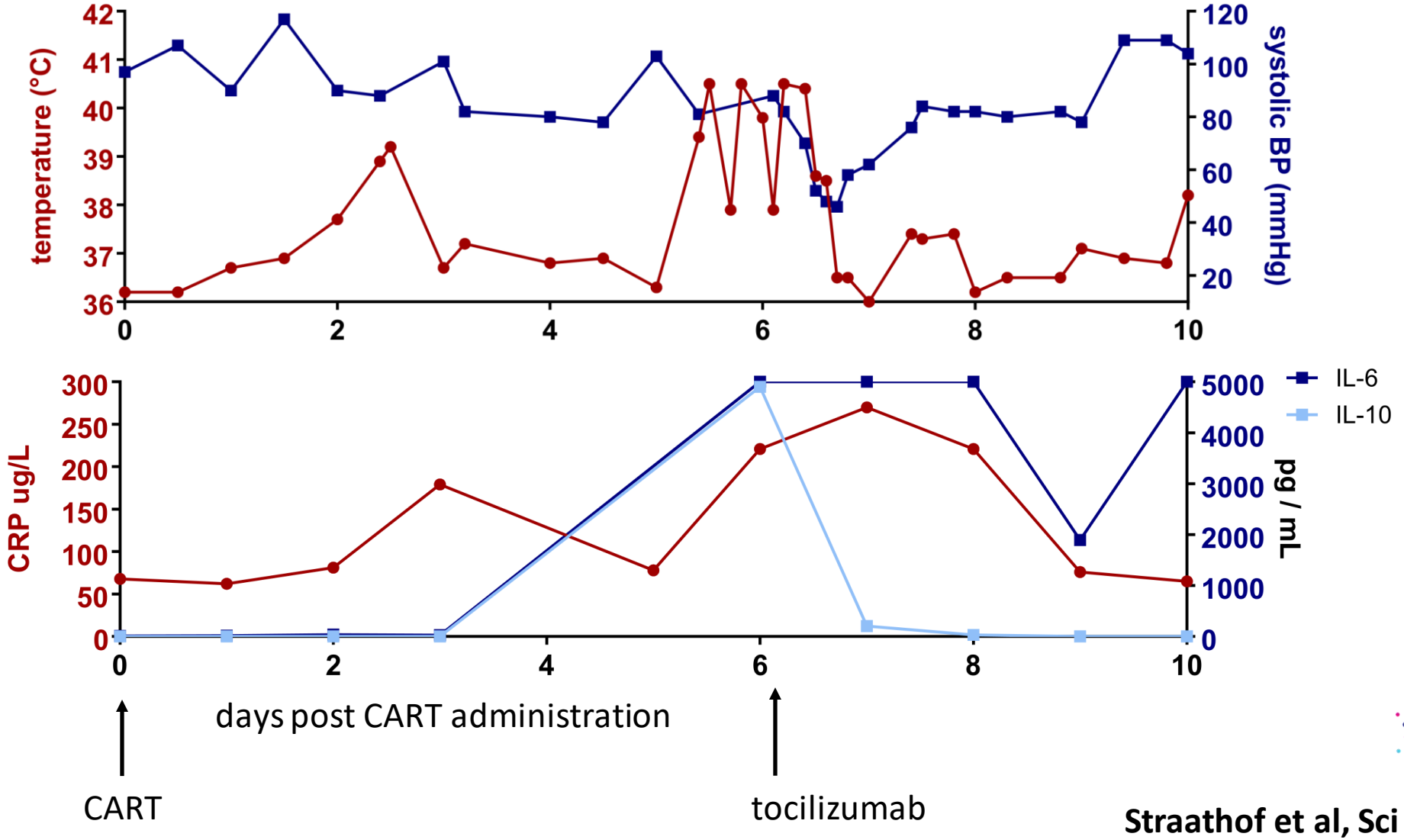
Thomas S et al, PLoS-One 2016;11;e0152196
Philip B et al, Blood 2014;124:1277–1287

Phase I dose escalation design

Rolling 6 design



Week 1: cytokine release syndrome



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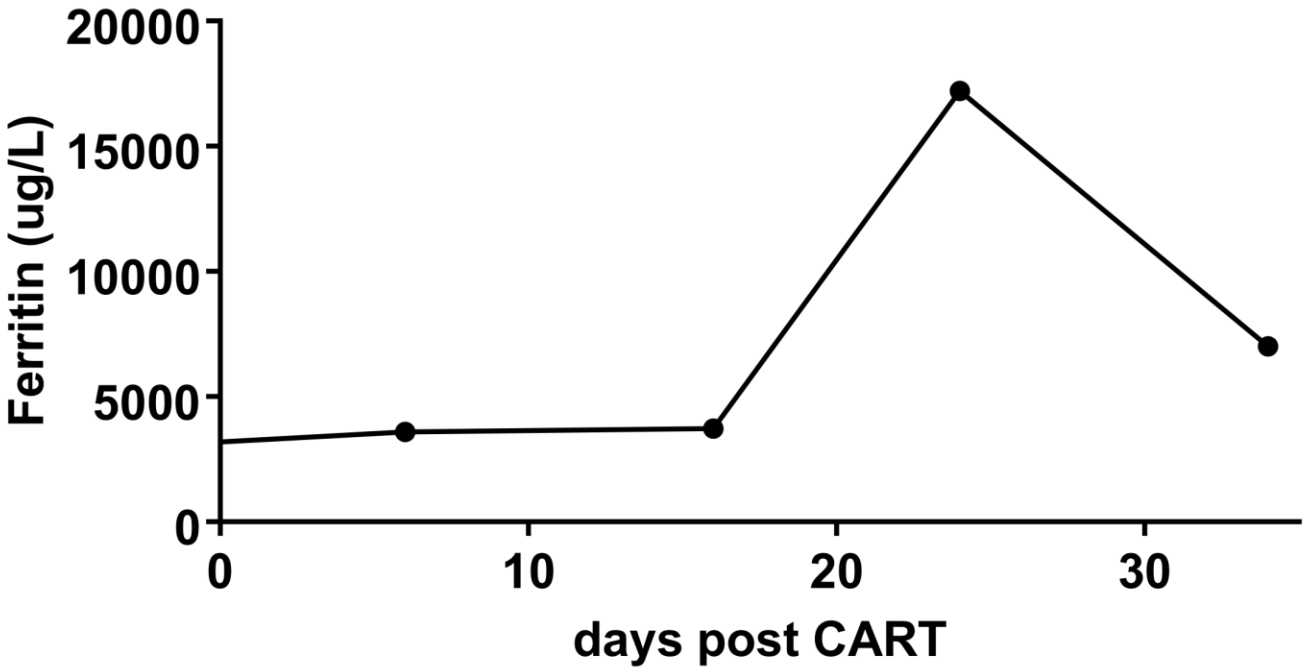
Straathof et al, Sci Trans Med, 2020h

Week 2: macrophage activation syndrome



- From day +7 hepatomegaly, ascites, weight gain
- Raised ferritin, and sIL-2R triglycerides
- Supportive management
- Clinical symptoms resolved by day +22

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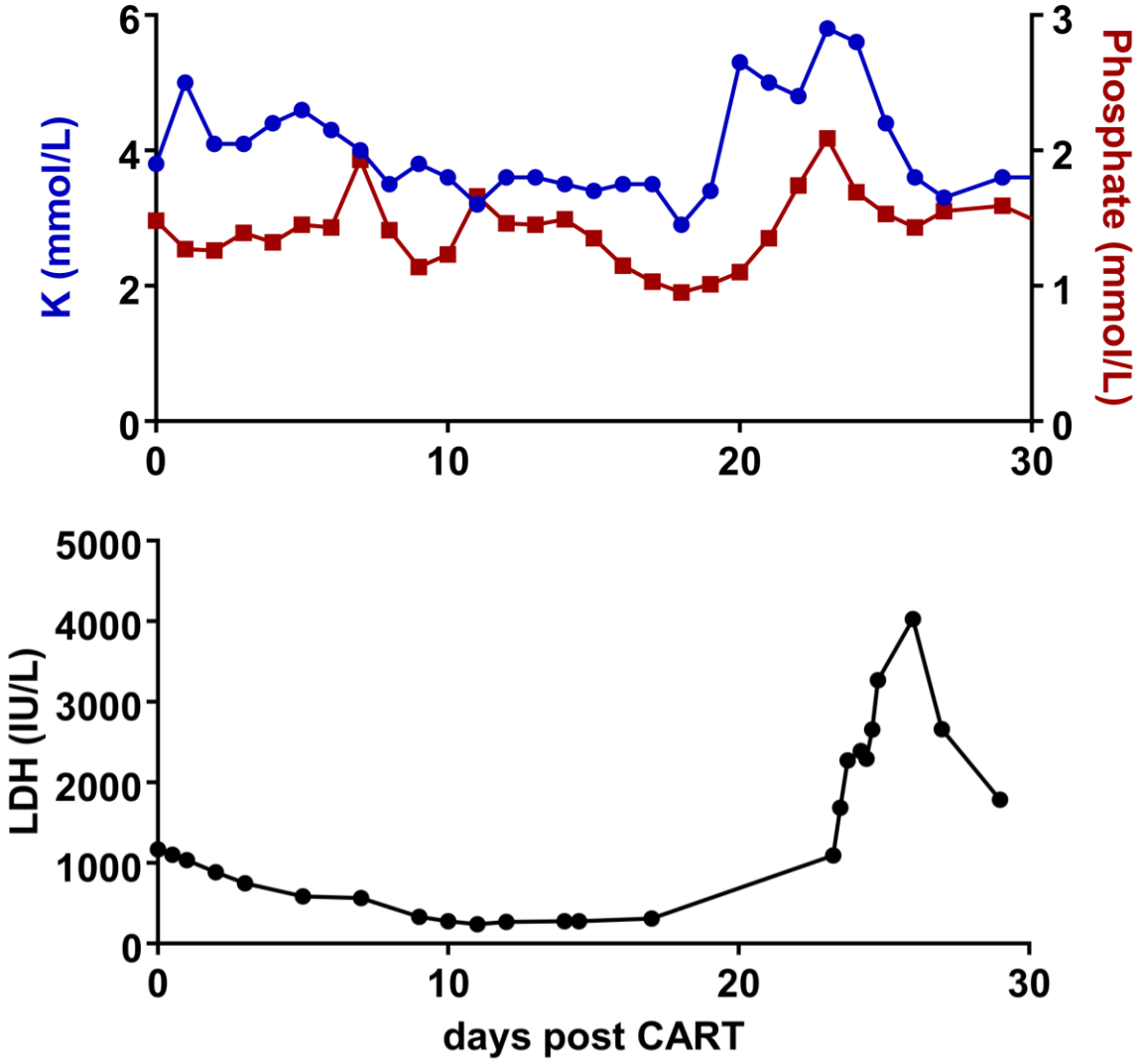
Straathof et al, Sci Trans Med, 2020

Week 3: secondary tumour lysis



- Back pain and recurrence fever from day +21
- Rise in LDH, K and PO_4
- Supportive management
- Clinical symptoms resolved by day +24

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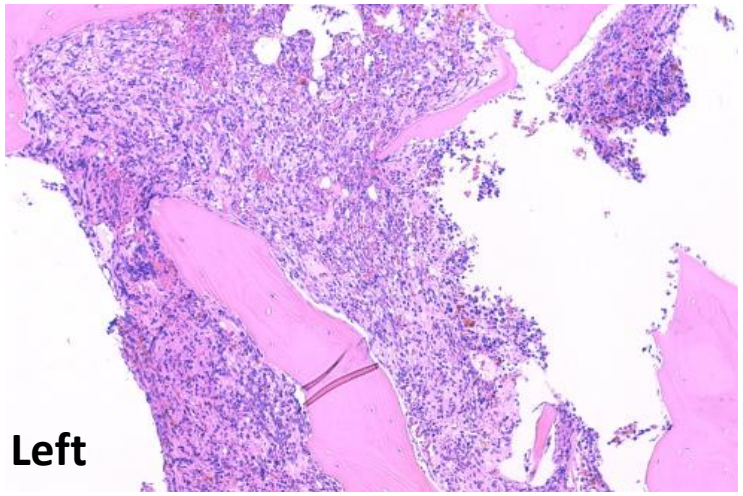
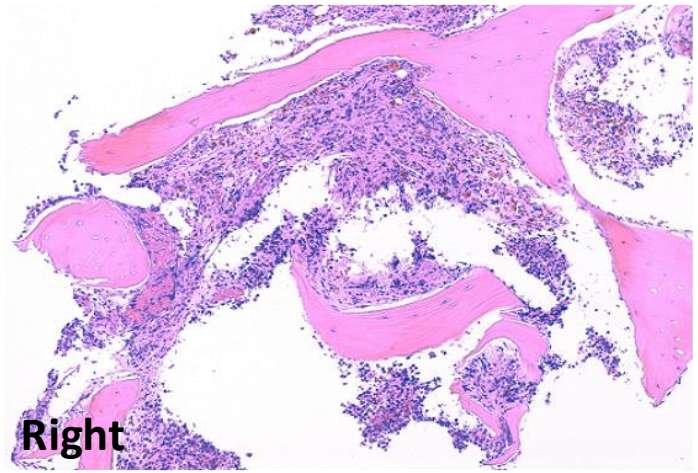
Straathof et al, Sci Trans Med, 2020

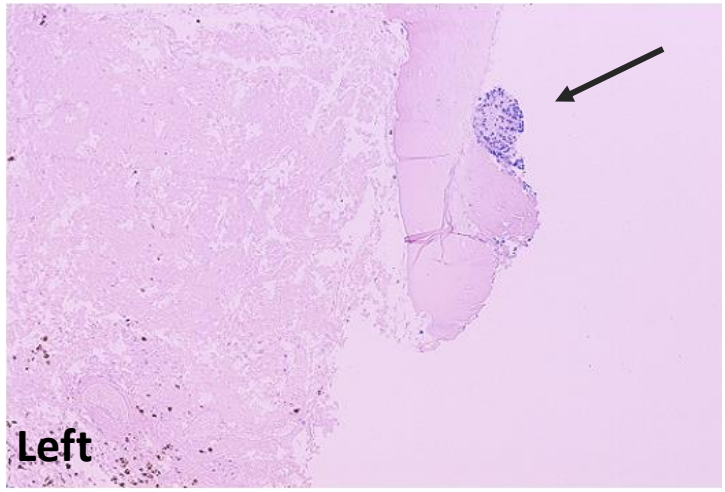
Patient 10: decrease BM tumour burden

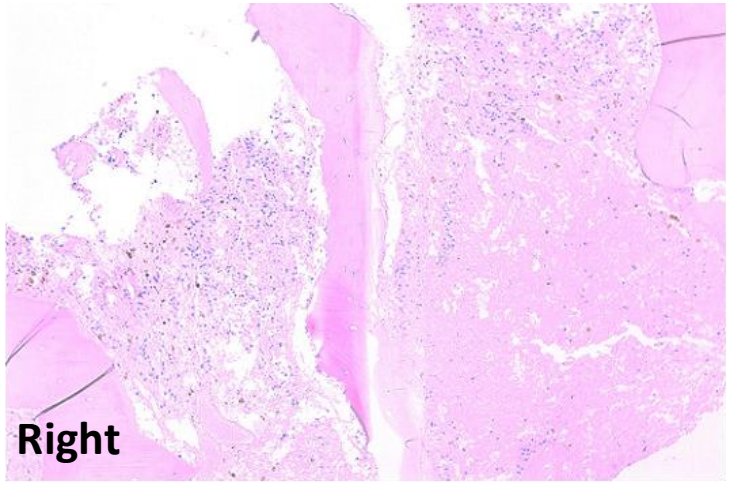
Day 0





Day +28

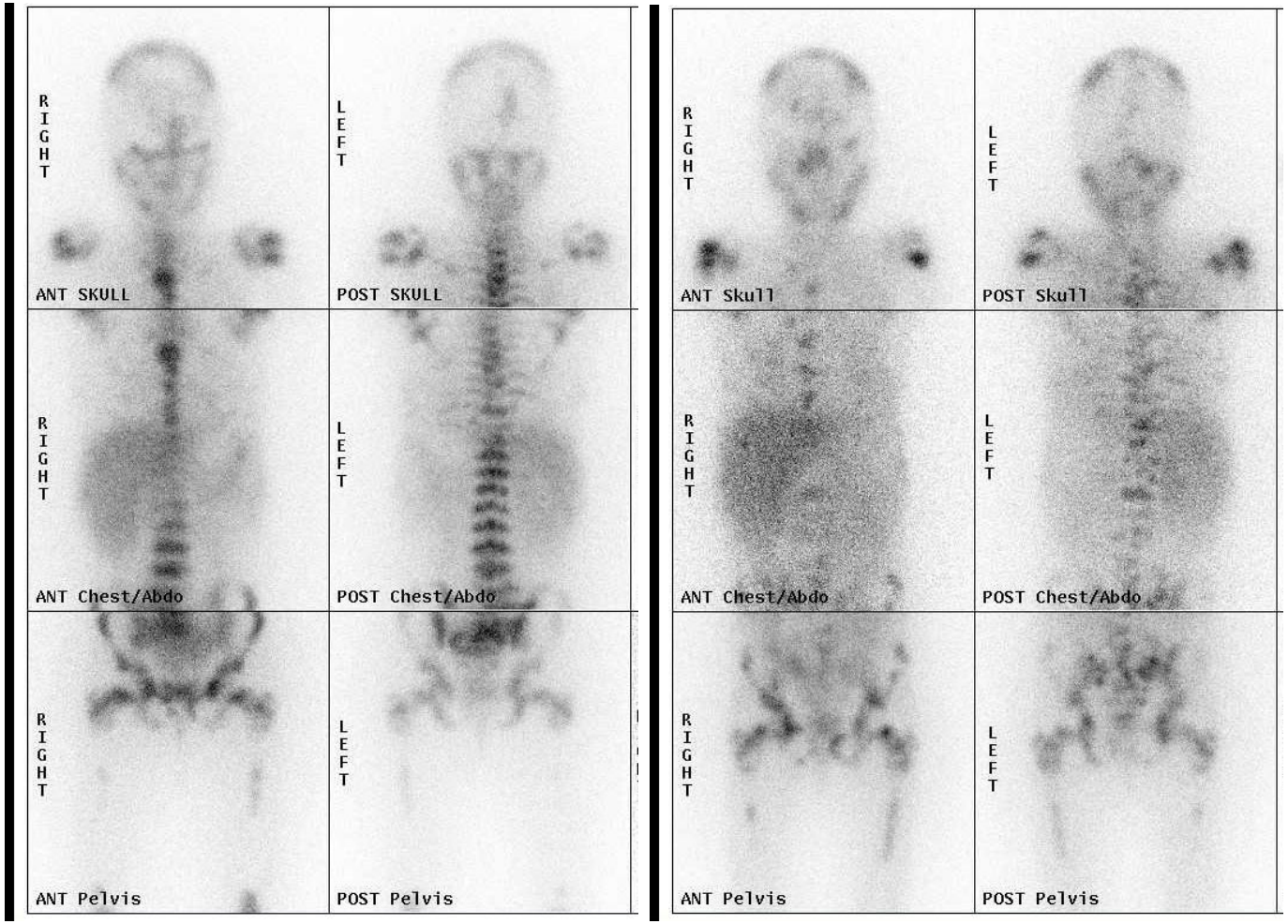




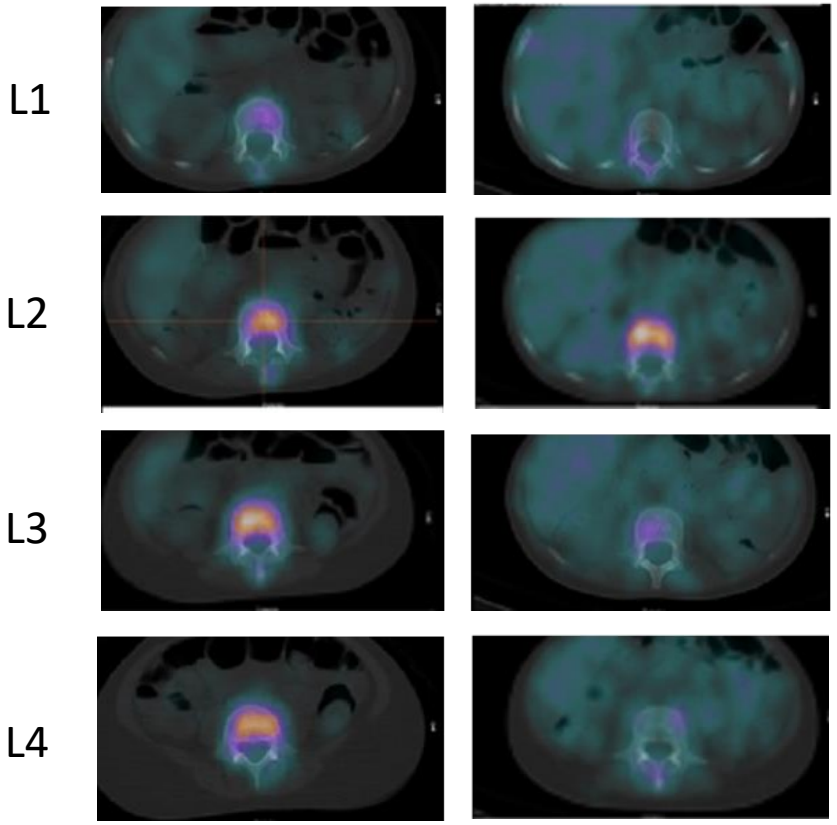
Straathof et al, Sci Trans Med, 2020

Pt 10: reduced MIBG uptake/SPECT activity

Day 0 Day +28



Day 0 Day +28

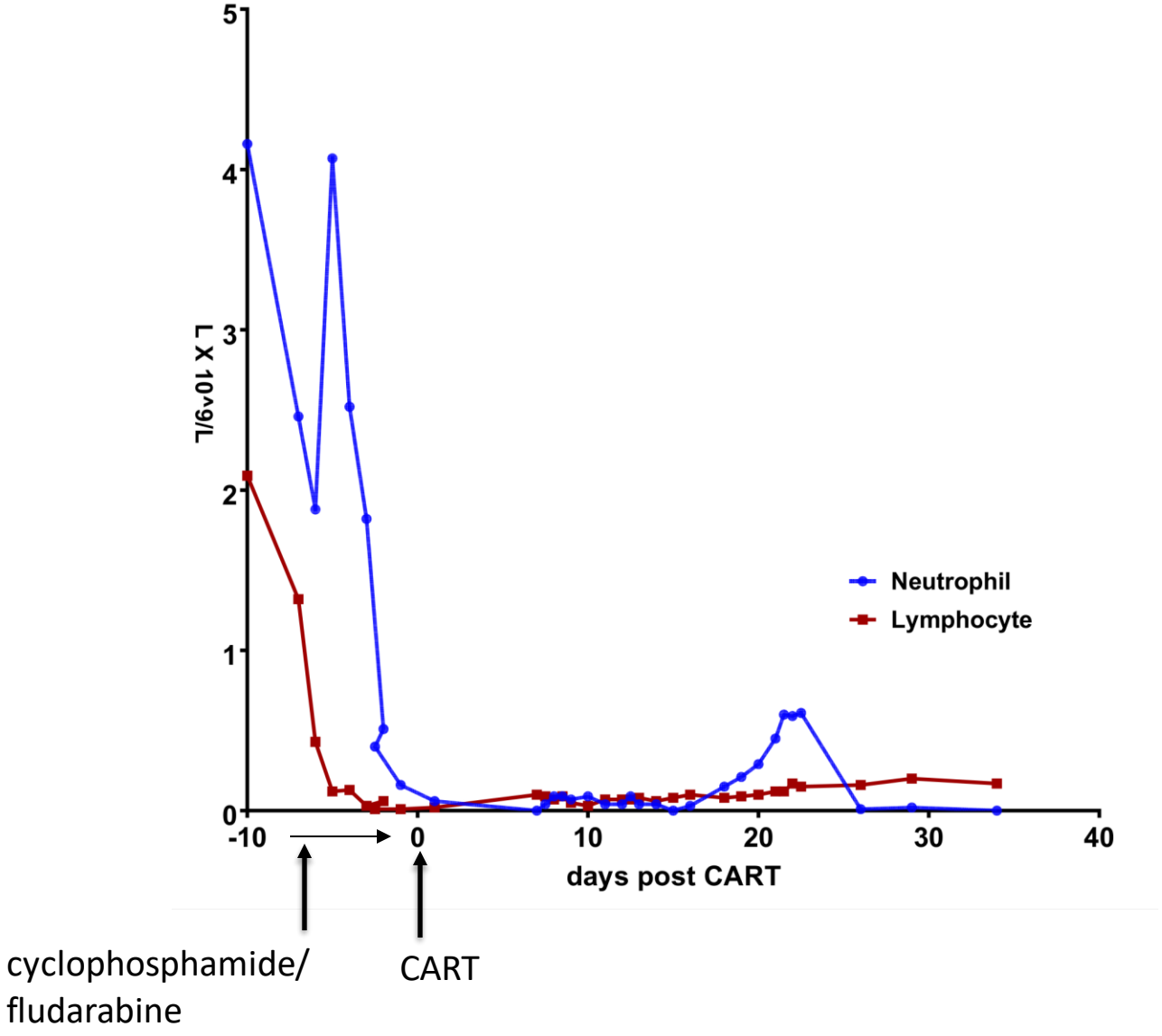


MIBG: iodine-123-**m**eta-**i**odo**b**enzyl**g**uanidine

SPECT:
Single **p**hoton emission **c**omputed **t**omography

Straathof et al, Sci Trans Med, 2020

Patient 10: prolonged pancytopenia



- Patient with pre-existing limited bone marrow reserve and bone marrow involvement neuroblastoma
- Profound lymphopenia following conditioning
- Apparent neutrophil recovery at day +20
- Recurrence of pancytopenia
- Highly blood product dependent

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Straathof et al, Sci Trans Med, 2020

- Repeat bone marrow aspirate at day +45 showed hypoplasia and early disease regrowth
  - 1RG-CART detected in blood on day +42 by PCR, undetectable by flow
- Patient died of pseudomonas septicaemia on day +50

- Two further patients had signs of CAR T cell activity

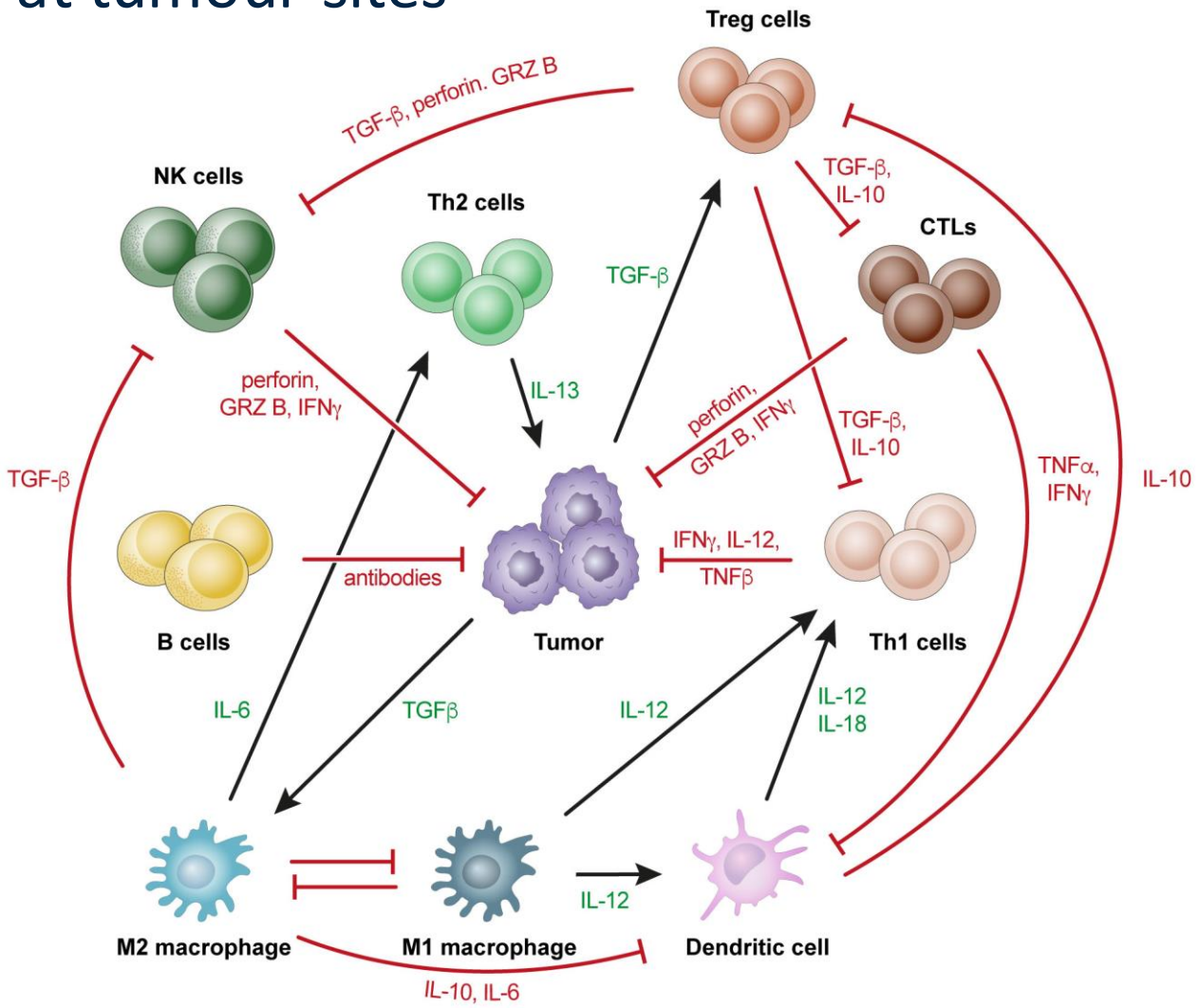
CONCLUSION: GD2 CAR can work without on-target toxicity but effect is transient.

- Further clinical data from Stanford and Rome support use of D2 as a solid tumour bCAR T target

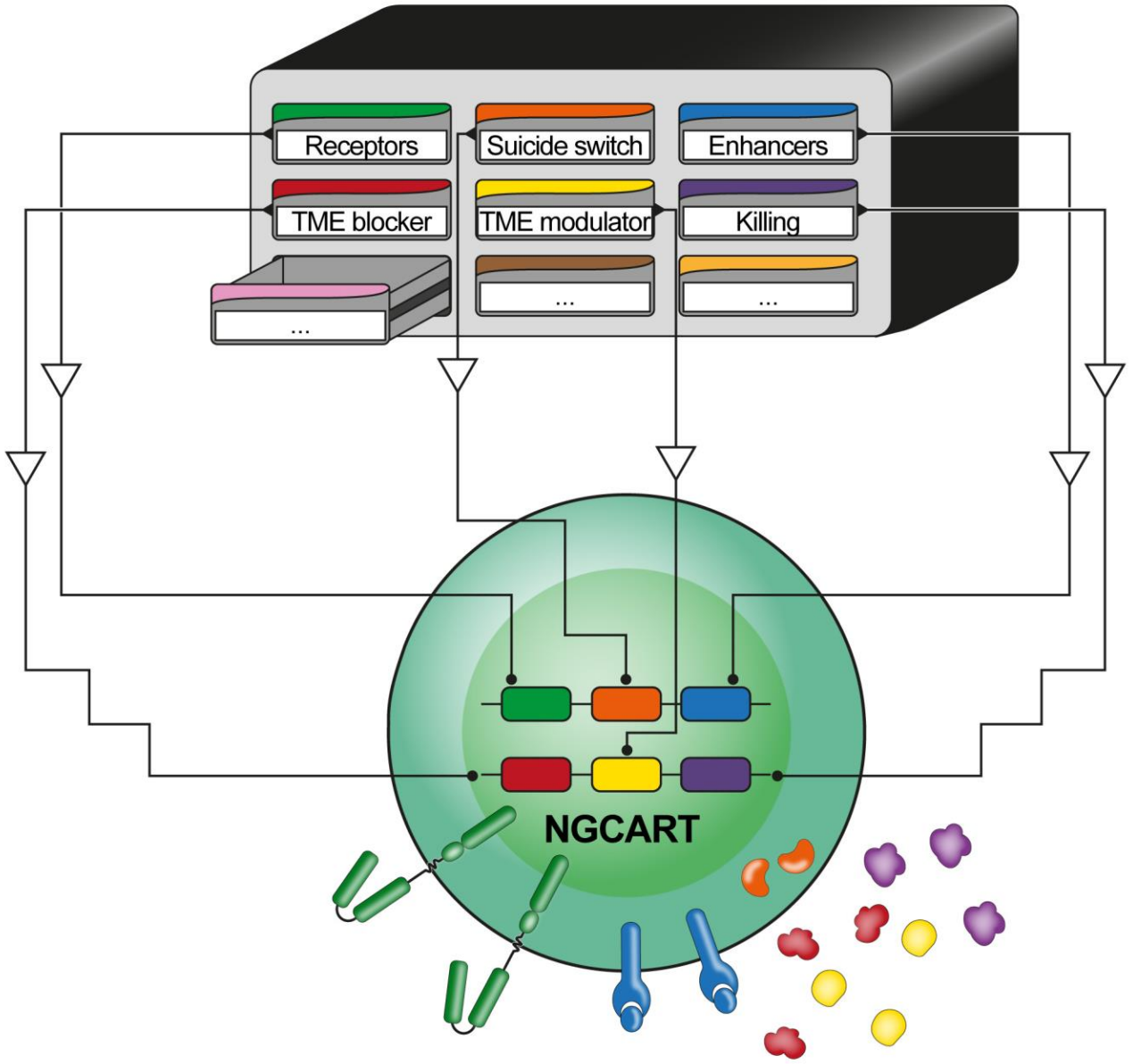
The problems (compared with lymphoid malignancies)…

1. Antigen sequestered at tumour sites

2. Microenvironment



The solution…



Modular GD2 CAR-T design (MCARGD2): to achieve durable activity

Improve persistence and function in the hostile tumour microenvironment (TME)

GD2 CAR

Shows anti-tumor activity in patients in the absence of neurotoxicity

dSHP2

Truncated SHP2 to block multiple checkpoint signals

dTGFβ Receptor

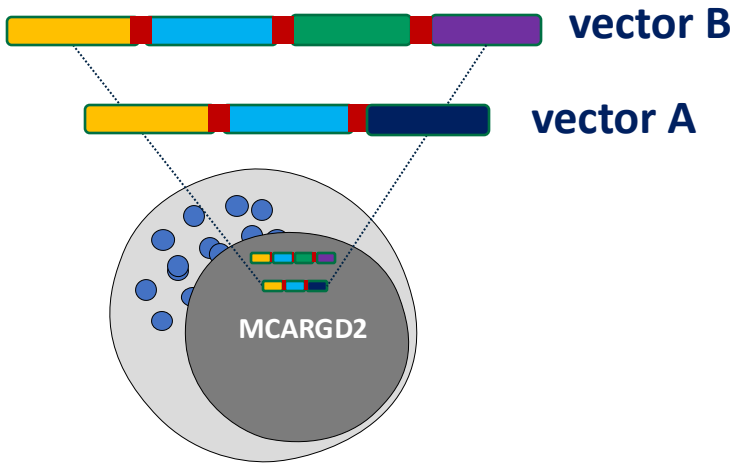
Truncated TGFβ receptor to render CAR-T cells resistant to TGFβ inhibition

Safety Switch

To deplete CAR-T cells in the event of unacceptable toxicities

Cytokine signal

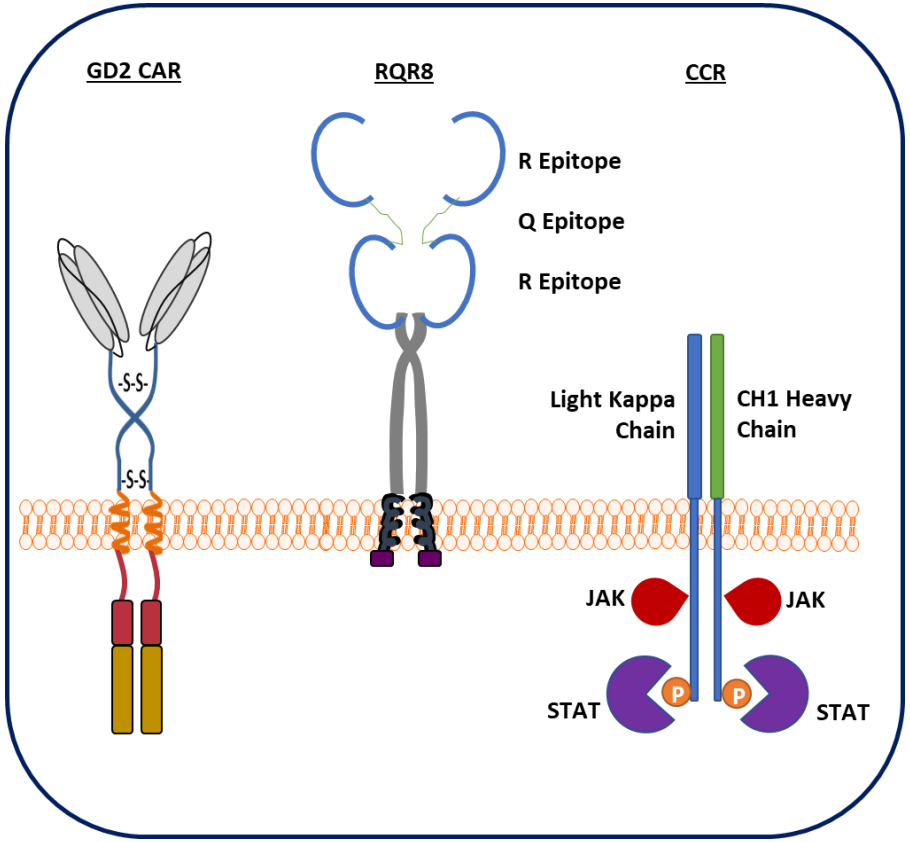
Constitutively active cytokine signal to improve persistence



Co-transduction results in high percentage of double positive CAR-T

Vector 1

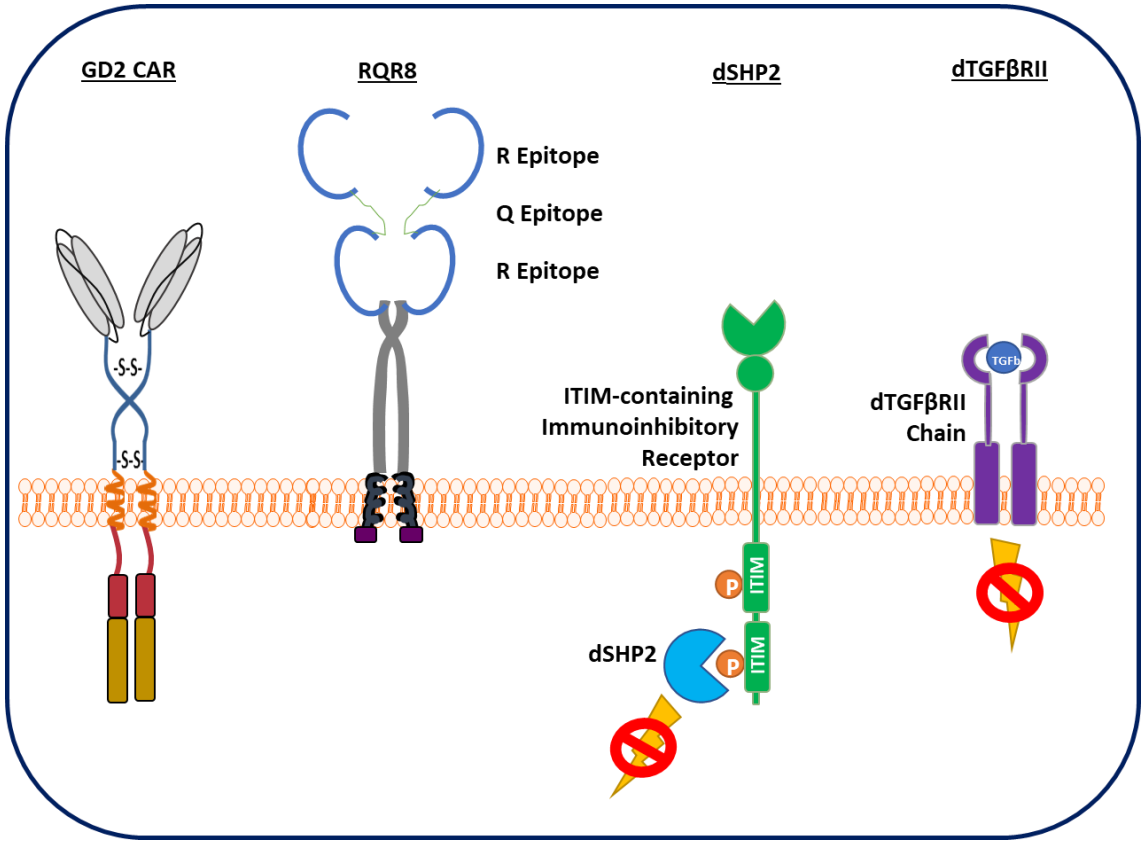
Designed to improve CAR T cell persistence



GD2 CAR ◆ RQR8 ◆ CCR

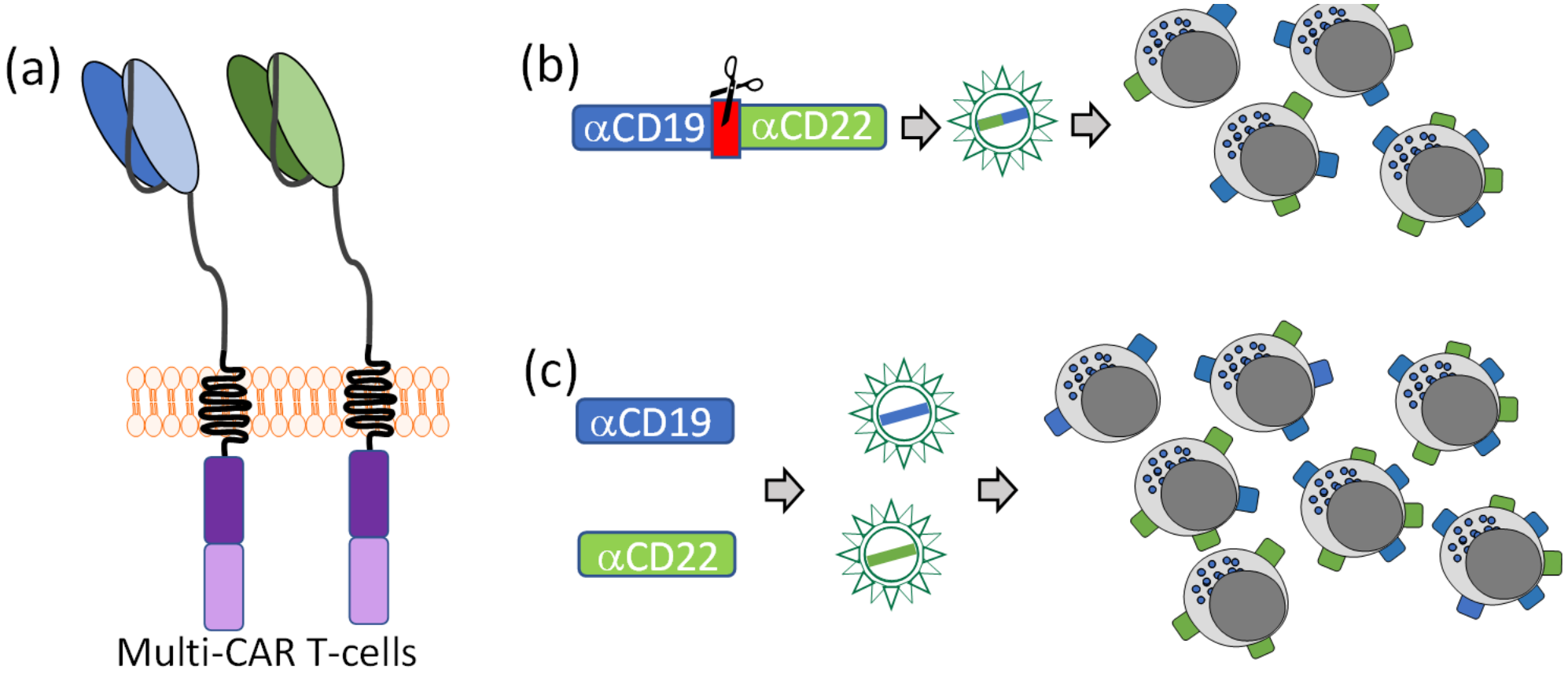
Vector 2

Designed to support CAR T cells function in TME



GD2 CAR ◆ RQR8 ◆ dSHP2 ◆ dTGFβRII

UCL current CD19/CD22 strategy: co-transduction



- Co-expression of transgenes can be achieved using FMD 2A like peptides
- Alternatively, T cells can be co-transduced with multiple vectors
- Co-transduction lacks a fixed stoichiometry which may allow extra insight into in vivo behavior

Co-transduced CAR T products are in the clinic

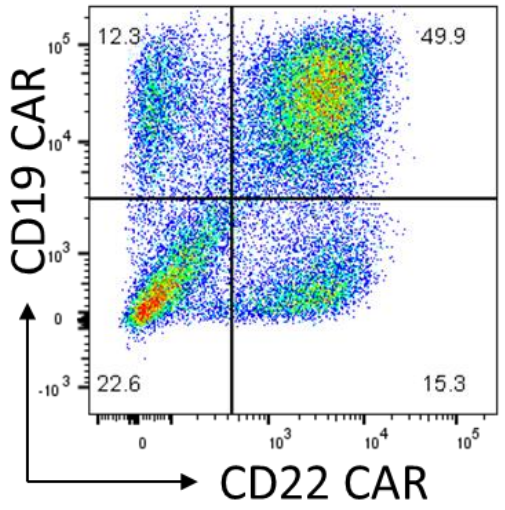
CARPALL V3
NCT02443831
(Ghorashian et al, ASH, 2021)

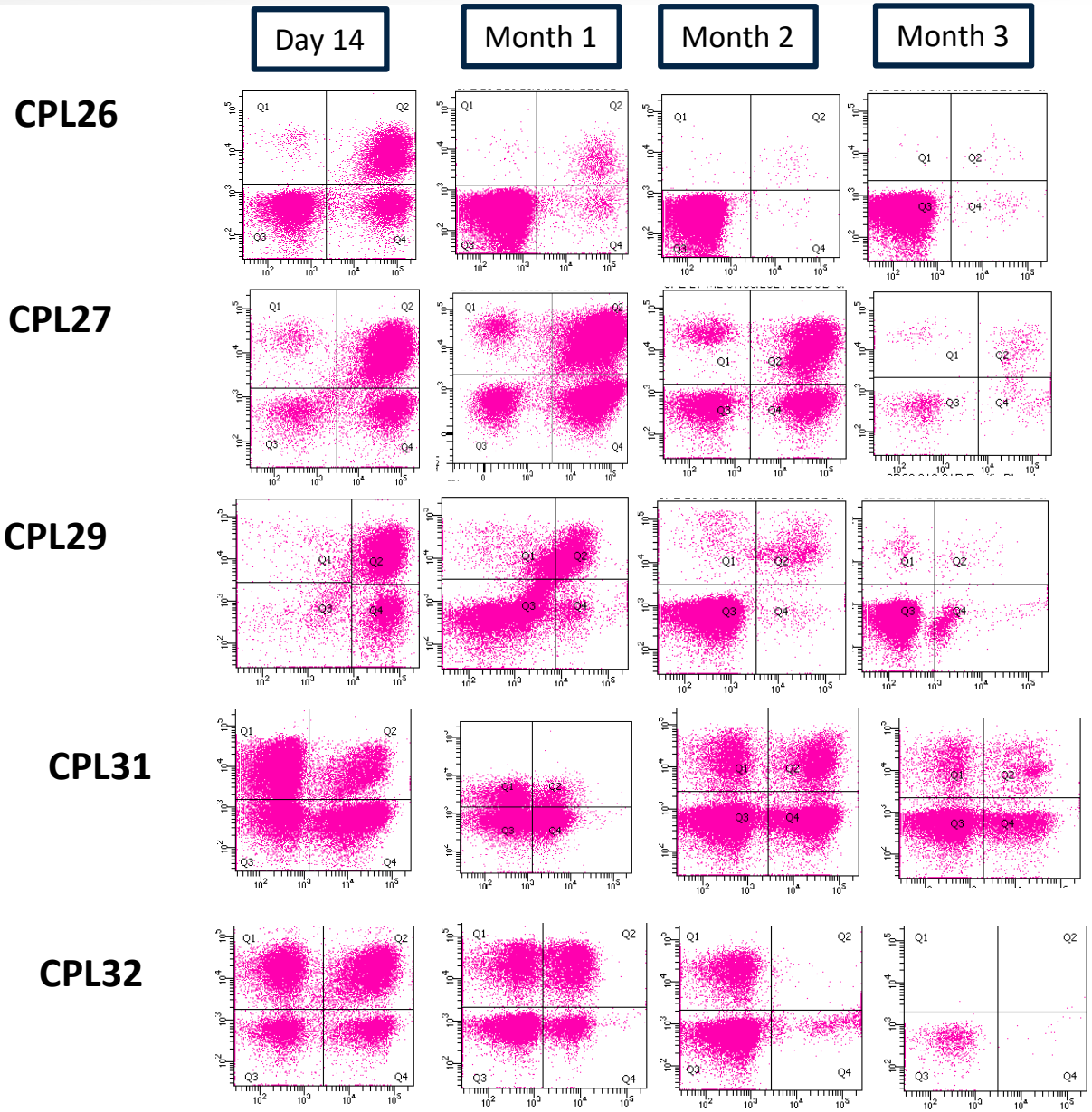
CAR T product

CPL-26

20-CARPALL-02

Transduction efficiency





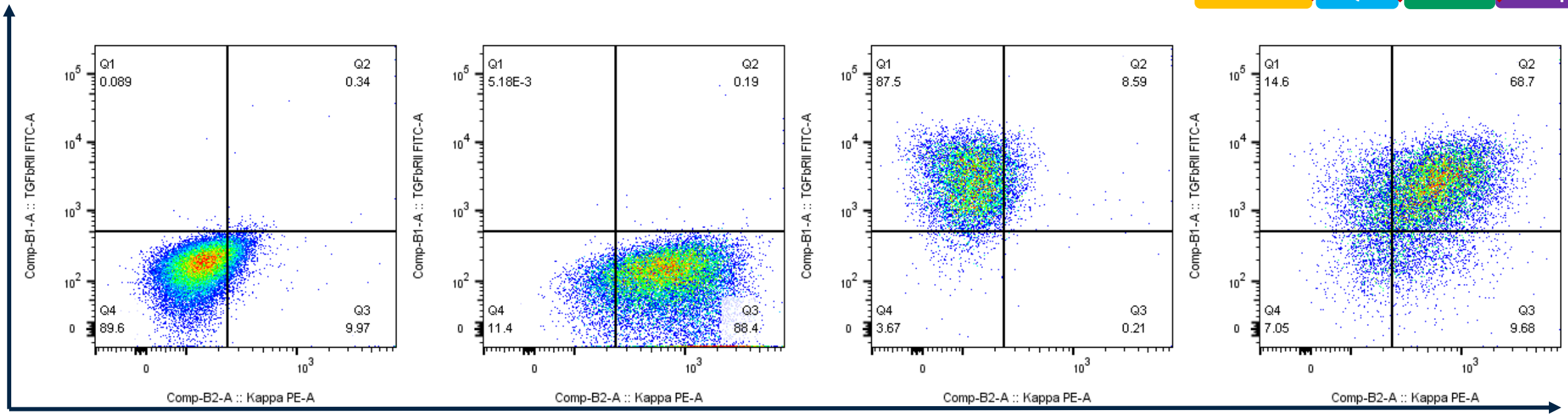
Modular GD2 CAR-T design (MCARGD2): to achieve durable activity

GD2-CAR

Product A

Product B

MCARGD2 Product A/B/AB



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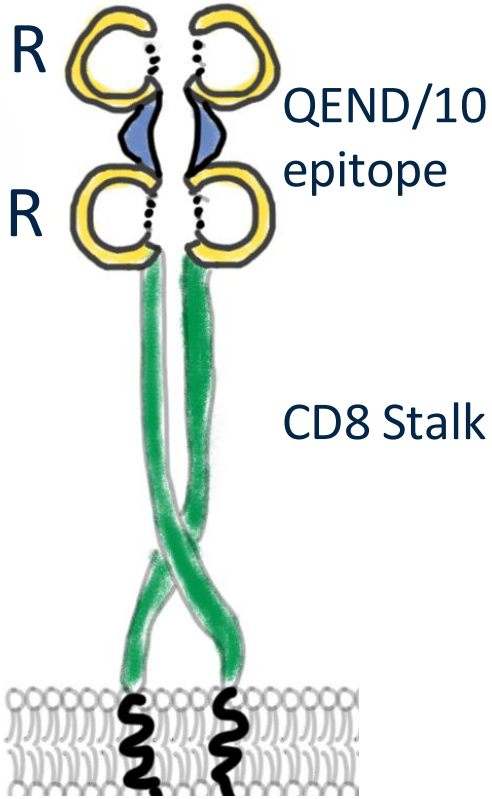
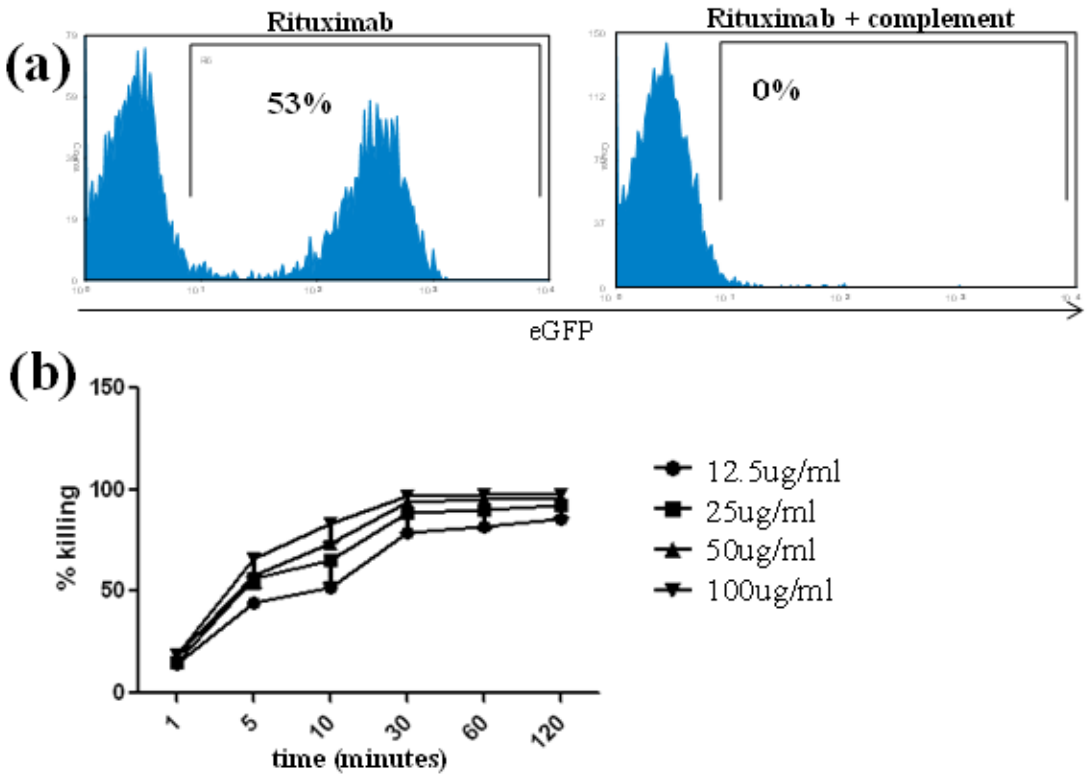
Remote control of engineered T cells



In vitro Rituximab mediate lysis



RQR8 is a sort suicide gene which renders T cells susceptible to Rituximab lysis

Philip et al, Blood 2014 Aug 21;124(8):1277-87.

In vivo performance of RQR8 with muRitux

F1: C57BL/6 x Balb/c

Day 1 | Day 7 | Day 10 | Day 12 | Day 14

A. 5Gy irradiation

B. $1.5x10^6$ T-cells/mouse

A. Peripheral blood analysis

B. 150µg mRtx (IV)

150µg mRtx

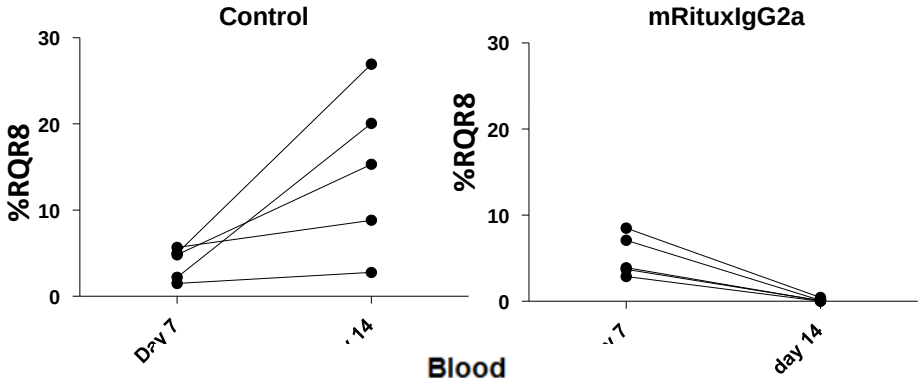
150µg mRtx

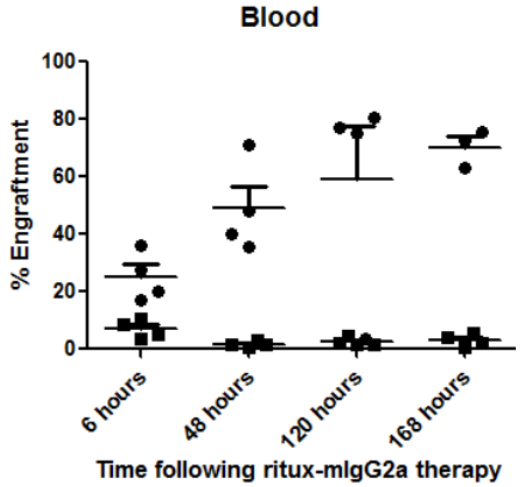
Culled

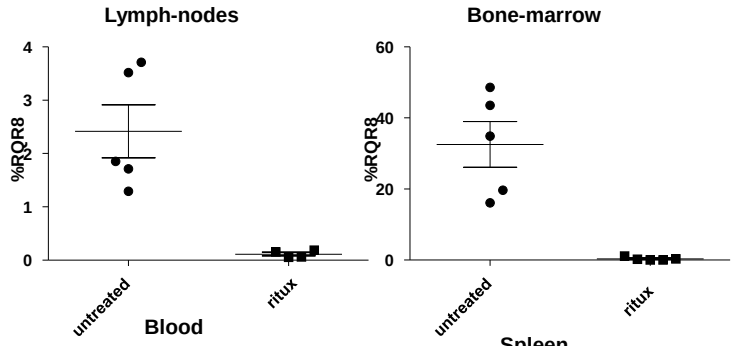
Transduction & MACs sort

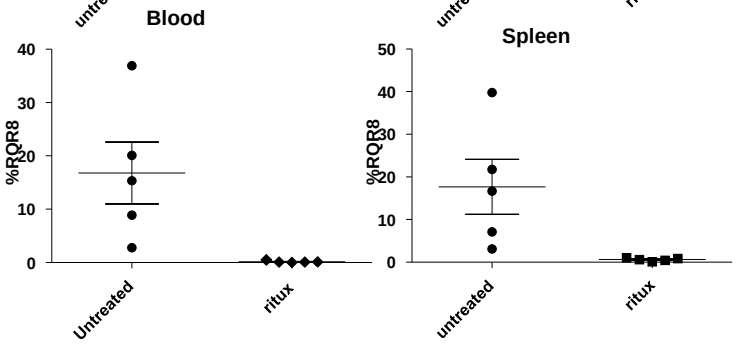
Isolation of spleenocytes

C57BL/6





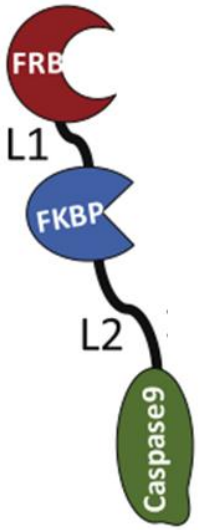


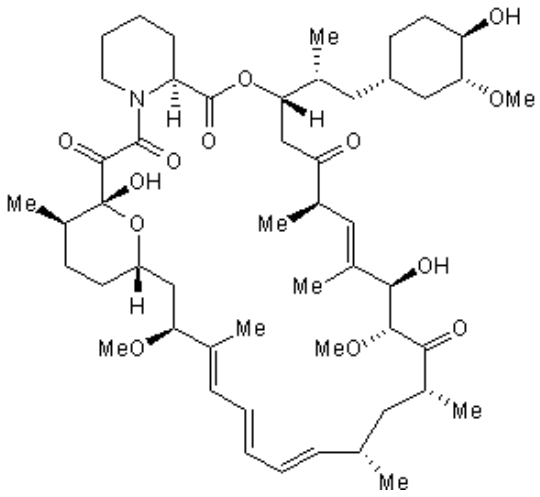


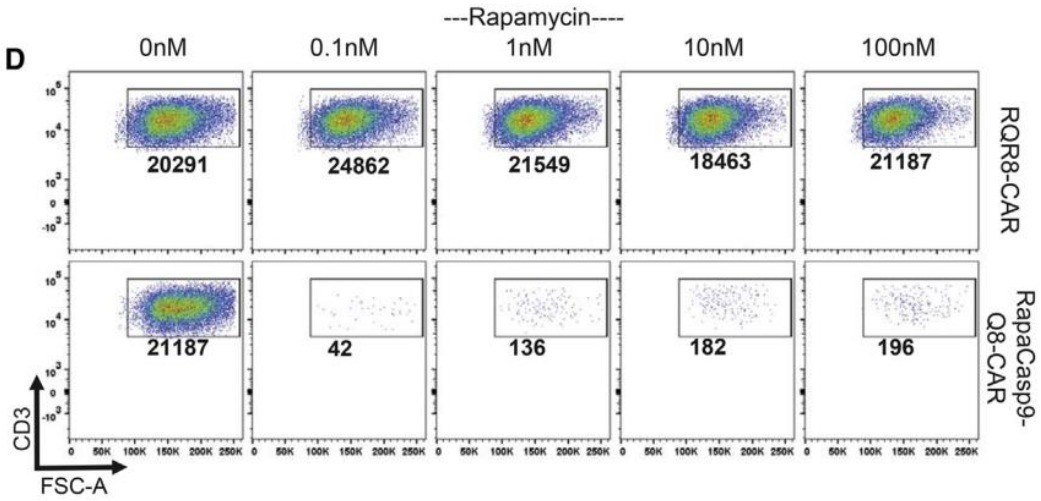
Philip et al, Blood 2014 Aug 21;124(8):1277-87.

Other control systems

RapaCasp9

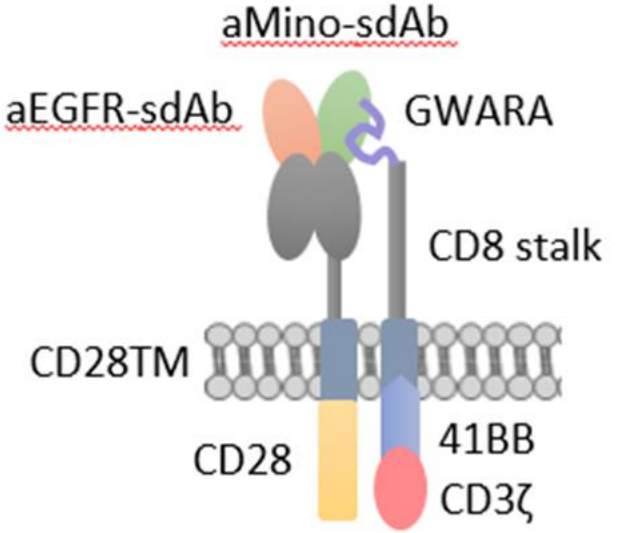


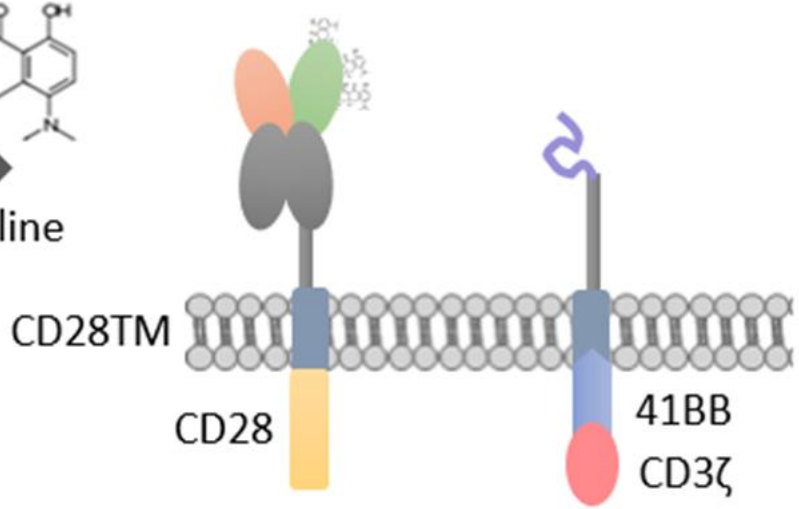


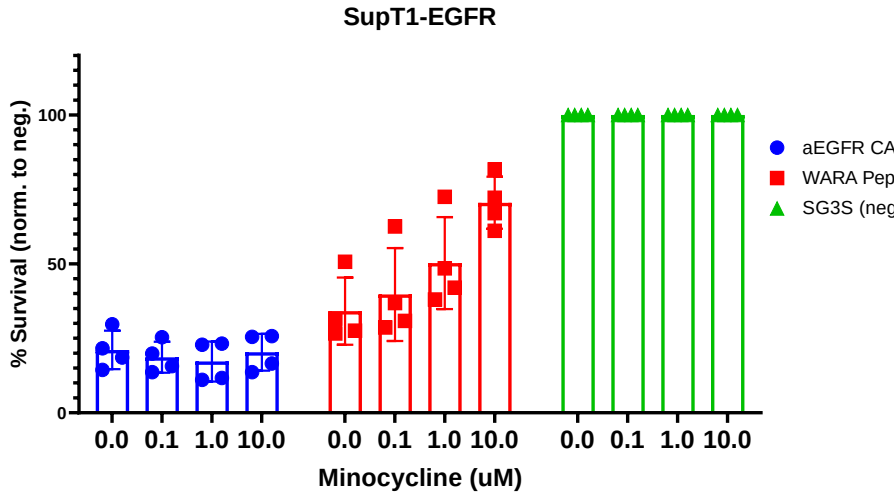


Stavrou et al, Mol Ther, 2018 May 2;26(5):1266-1276.

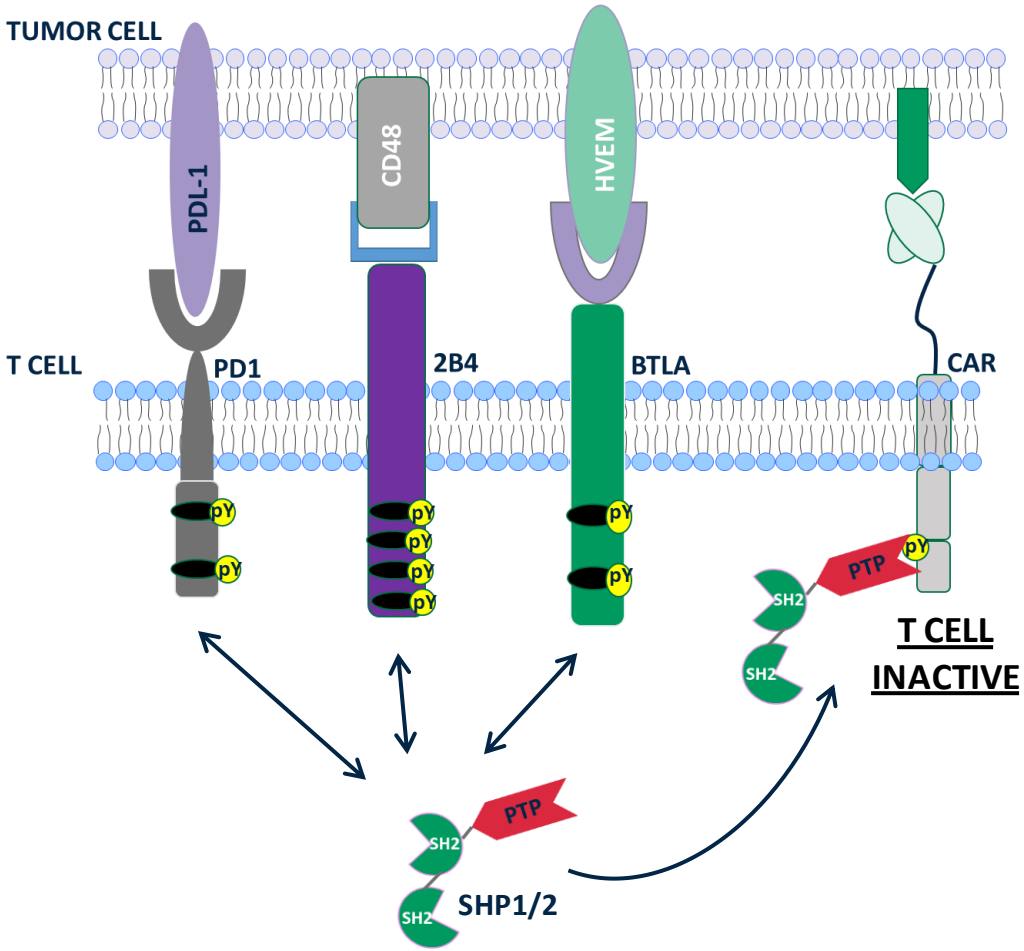
MinoDabCar





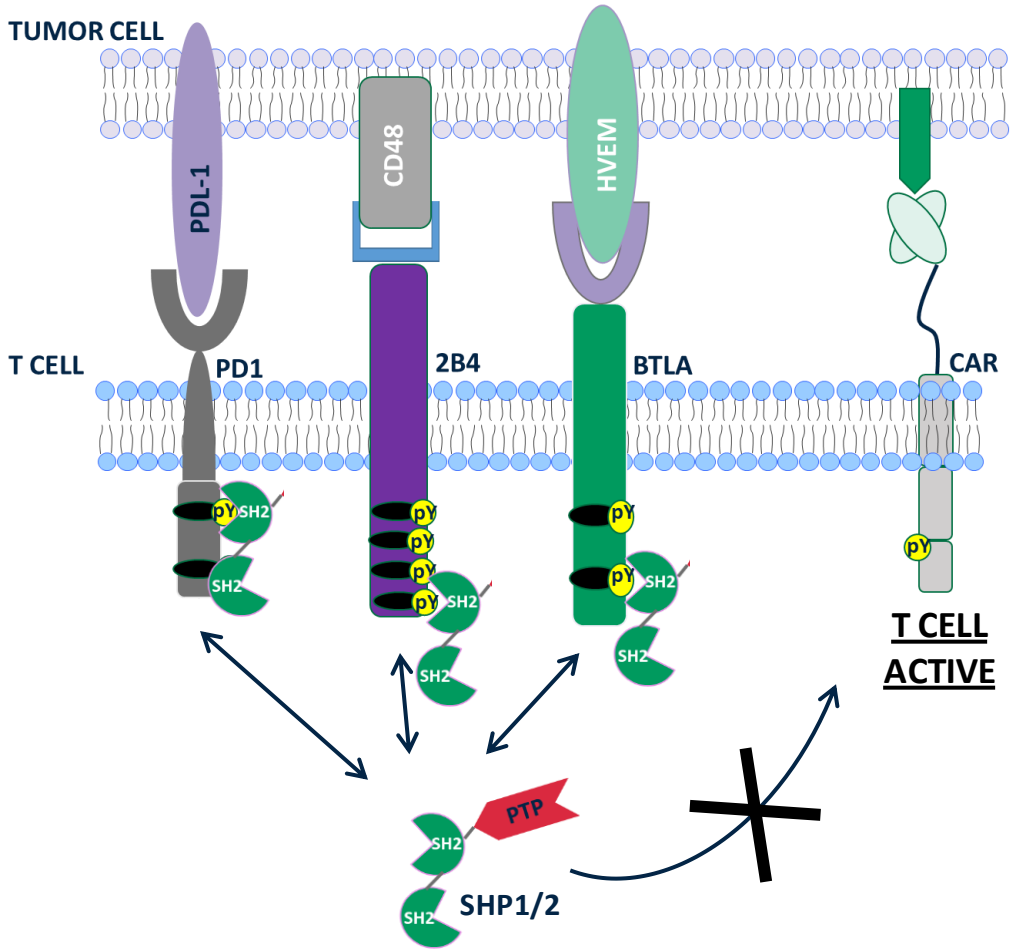


ITIM - SHP1/2 is an inhibitory signaling bottle-neck



- Ligation of inhibitory receptors on the surface of tumor cells impair a T cell's ability to kill the tumor
- Many immune checkpoints act through a common T cell signaling pathway:
  - PD1 (PDL1 ligand)
  - 2B4(CD48 ligand)
  - BTLA (HVEM ligand) etc
- Ligation of these inhibitory receptors leads to the recruitment and activation of SHP1/2 phosphatases
- Active SHP1/2 dephosphorylates the CD3z domain of the CAR or TCR and inactivates the T cell

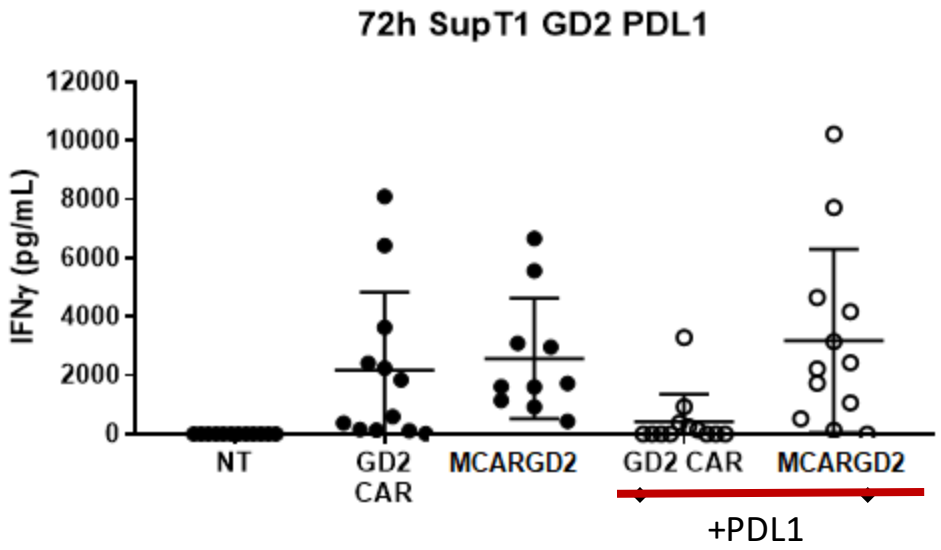
Truncated SHP2 can inhibit SHP1/2 signaling



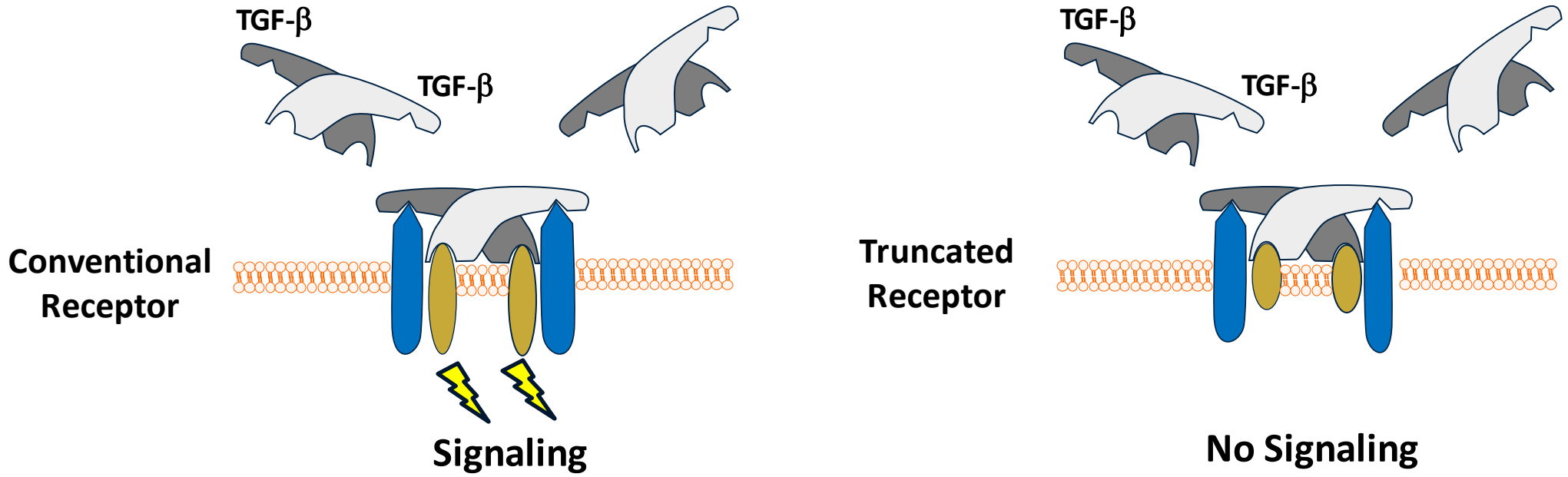
- Other approaches may only tackle one inhibitory receptor at a time using antibodies or gene editing
- We have designed a truncated SHP2 module that lacks the phosphatase domain and is unable to inactivate the T cell

Truncated SHP2 can inhibit SHP1/2 signaling

IFNγ production



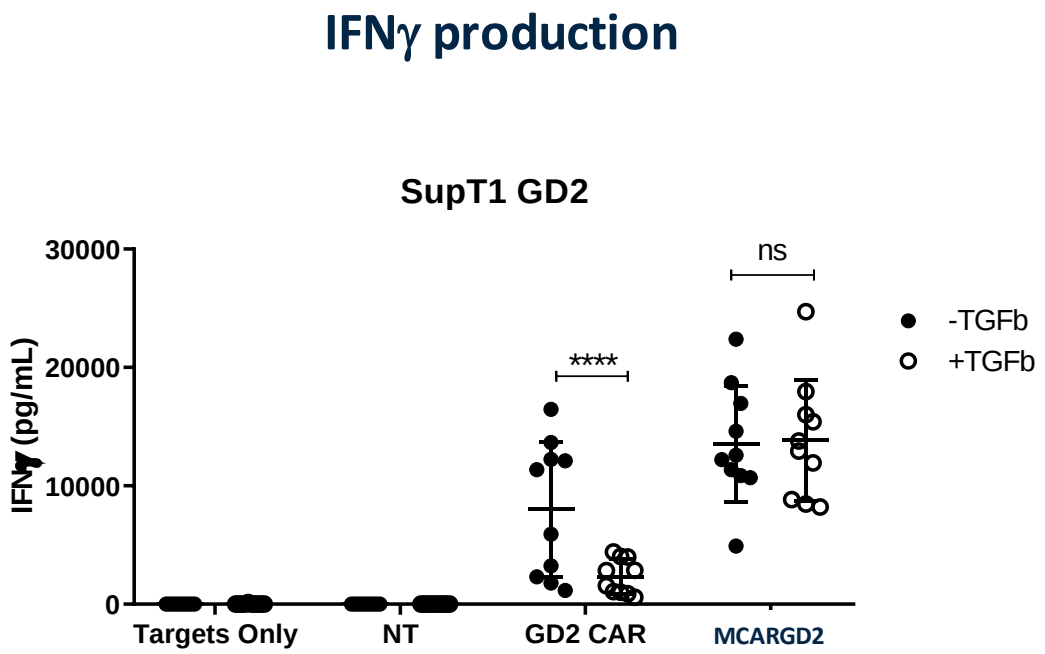
Blocking TGFβ Signaling Using a Truncated TGFβ Receptor (dnTBR2)



- TGF-β is a key negative regulator of the immune microenvironment
- dTBR2 lacks signaling endodomains and when over-expressed acts in a dominant negative manner

Bollard, C. M. *et al. J. Clin. Oncol.* **36**, 1128–1139 (2018).

Blocking TGFβ Signaling Using a Truncated TGFβ Receptor (dnTBR2)



Heterodimeric constitutive cytokine receptor

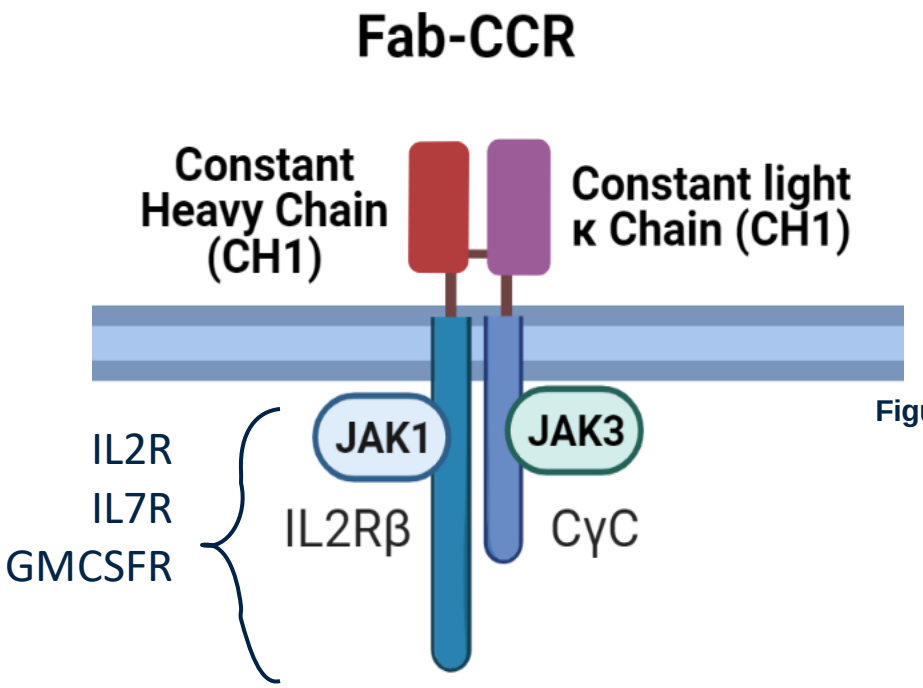
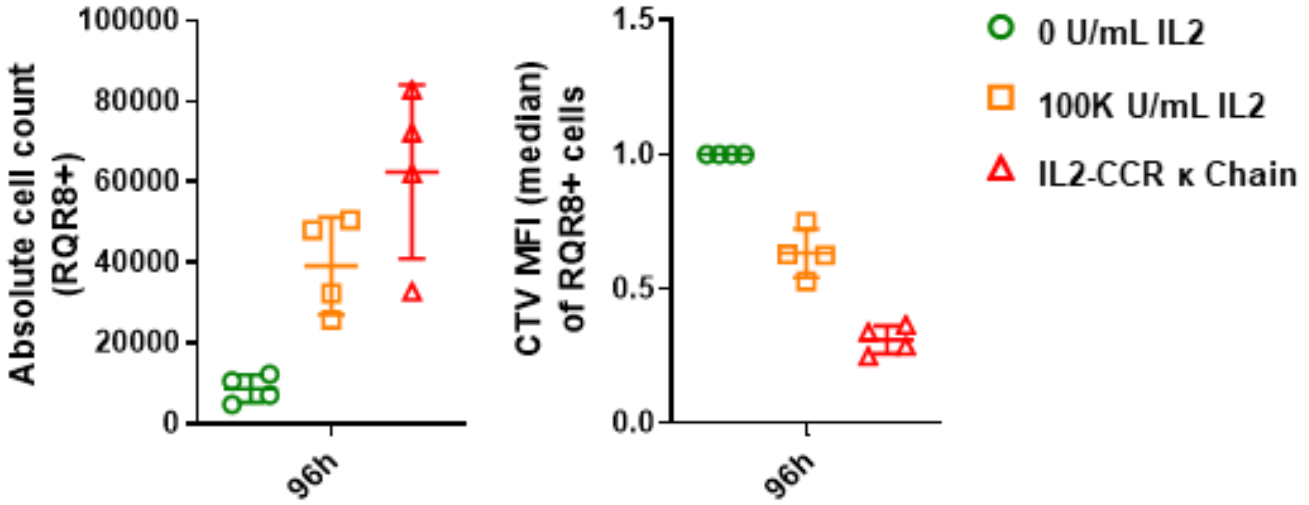
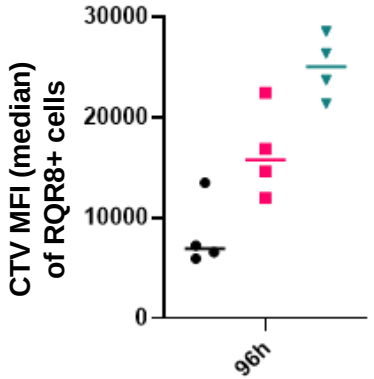
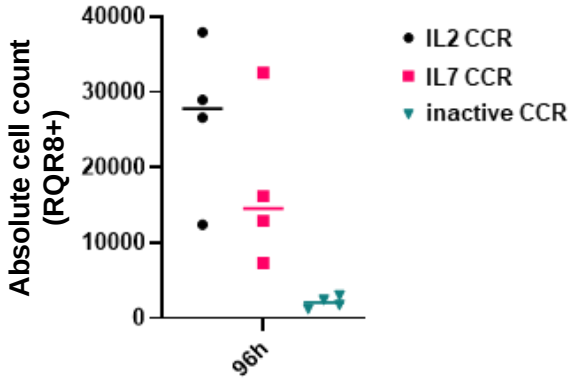
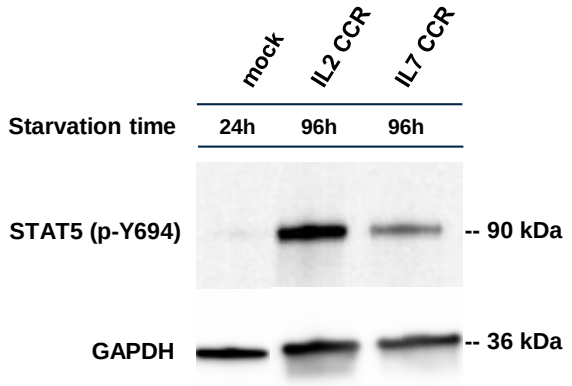


Figure 2



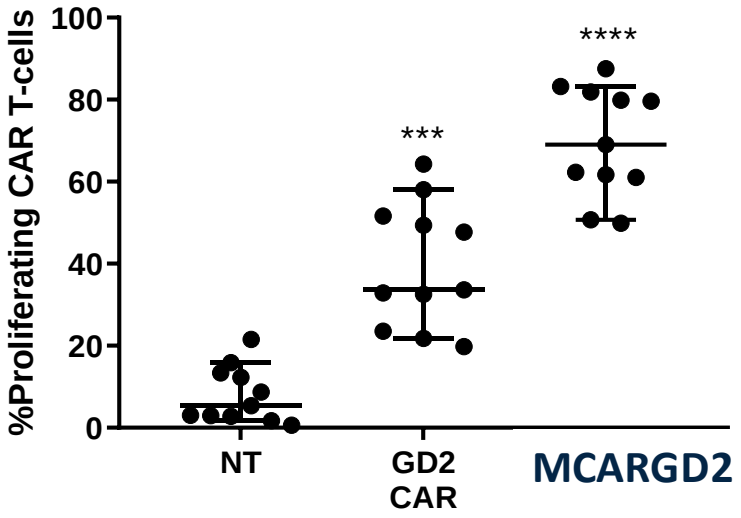




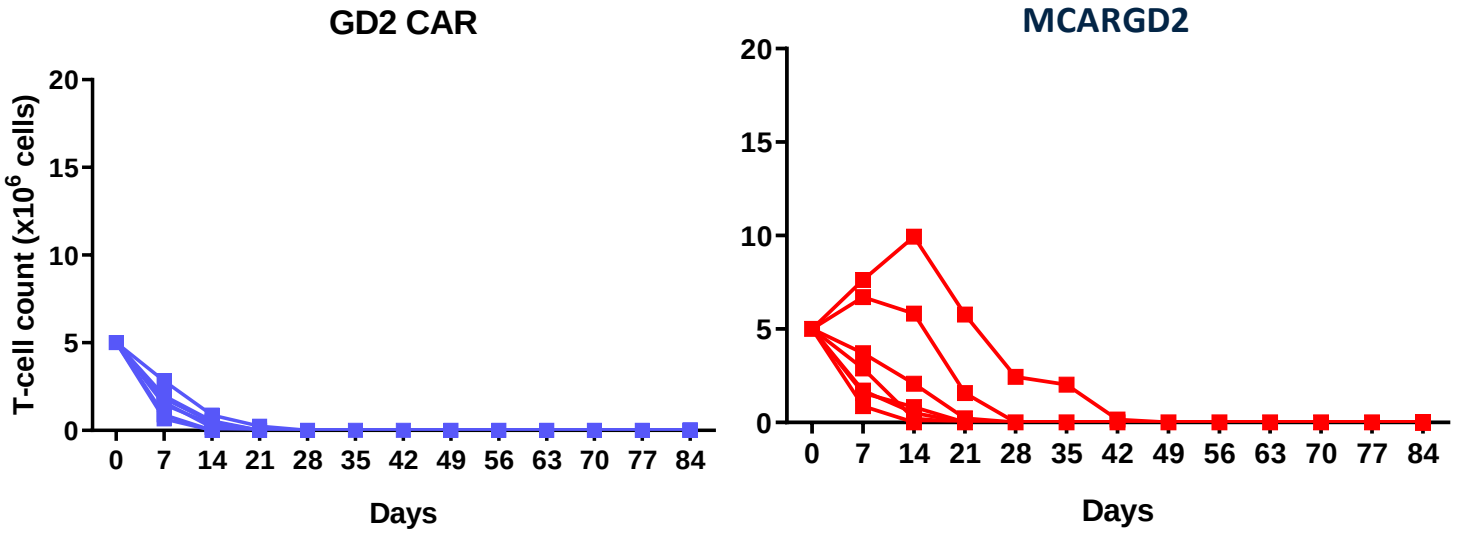


IL7 CCR promotes low-level cell turnover

CAR-T proliferation in absence of exogenous cytokine

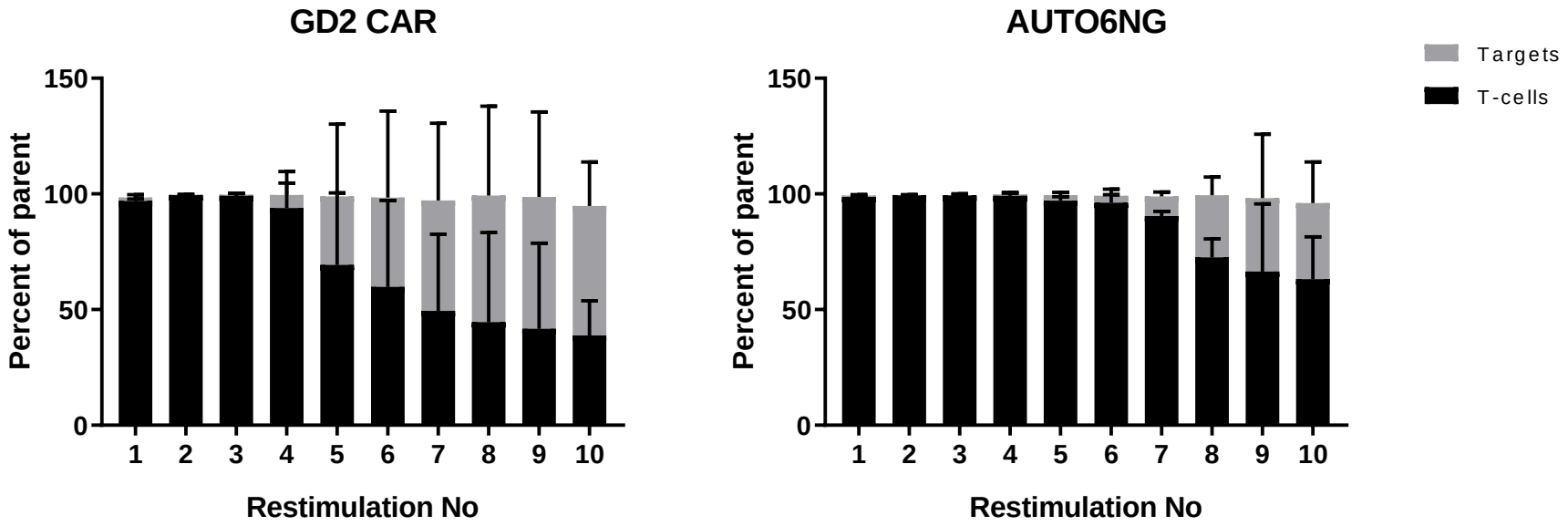


CAR-T proliferation remains depended on CAR ligand



IL7 CCR promotes low-level cell turnover

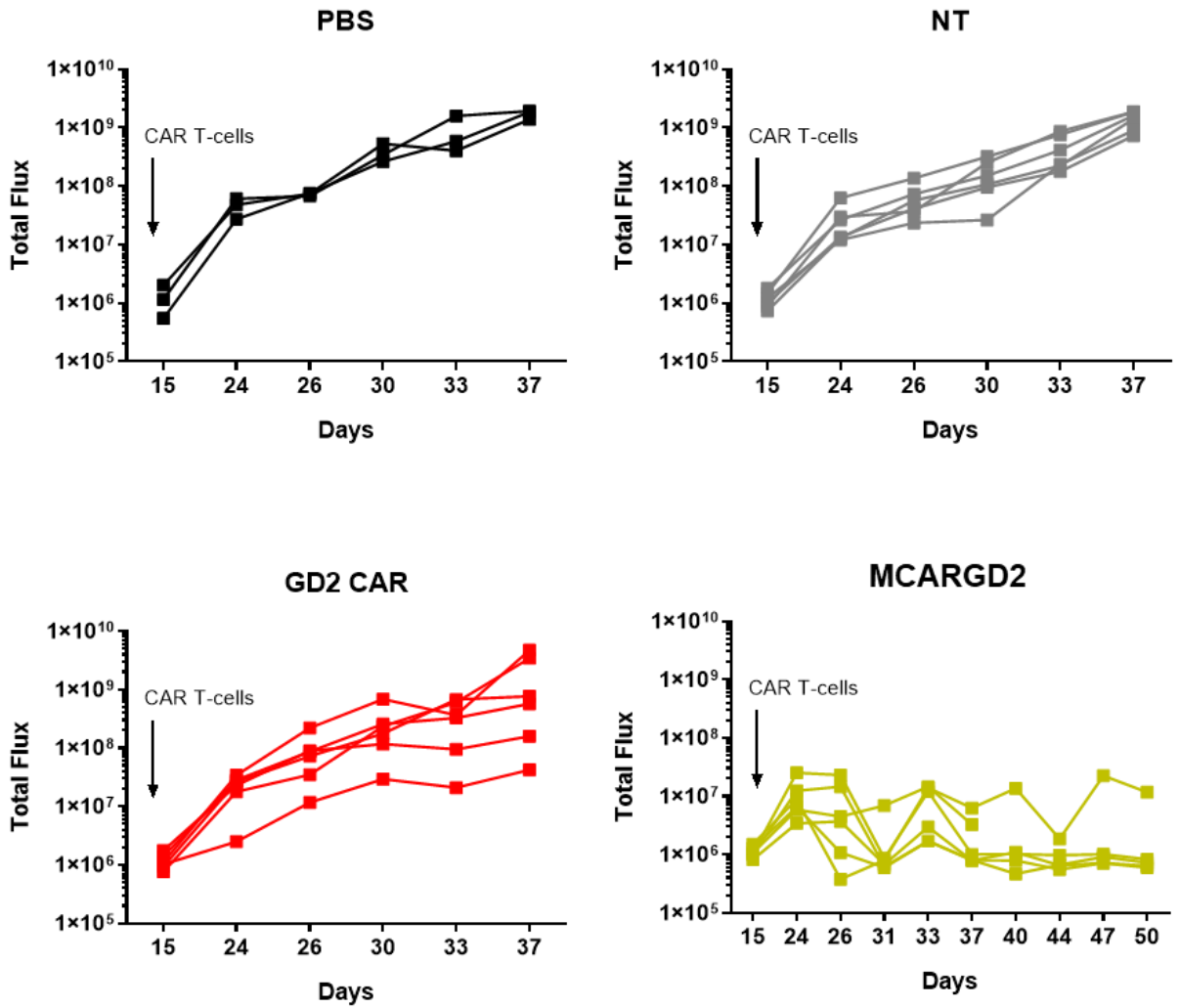
E:T =1:1
n = 4



Co-culture of CAR T cells + SupT1 GD2 re-challenged with 50,000 targets every 3 or 4 days

In vivo activity of MCARGD2 in pre-clinical neuroblastoma model

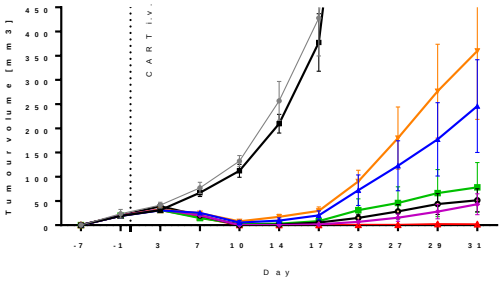
In vivo activity in established CHLA-255 xenografts in NSG mice

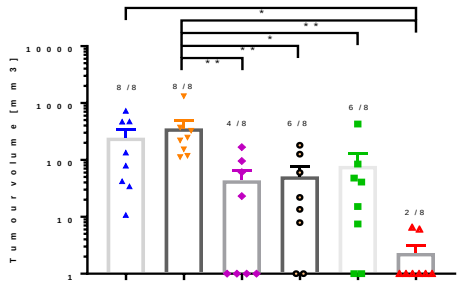


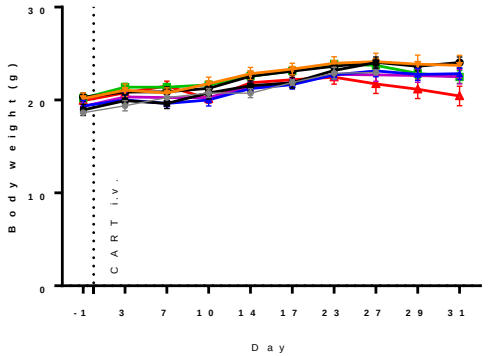
In vivo activity of MCARGD2 in pre-clinical SCLC model

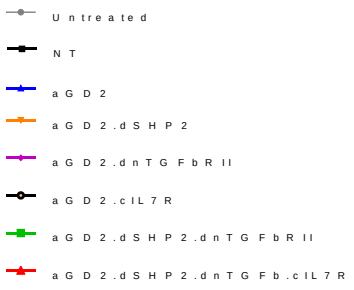
H446 s.c. model

Tumor growth

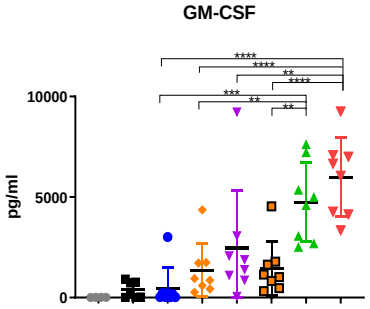


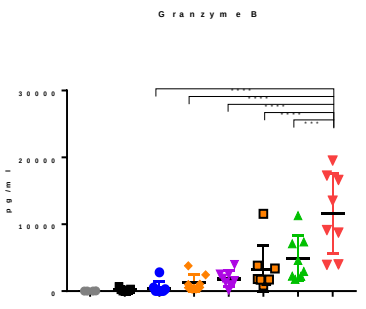


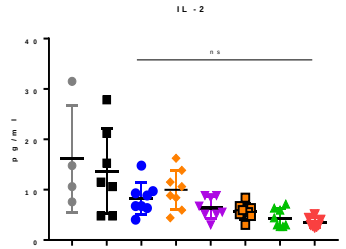


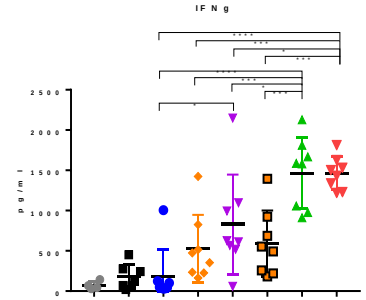


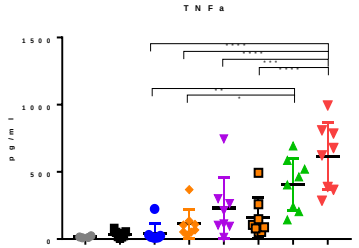
Serum cytokines











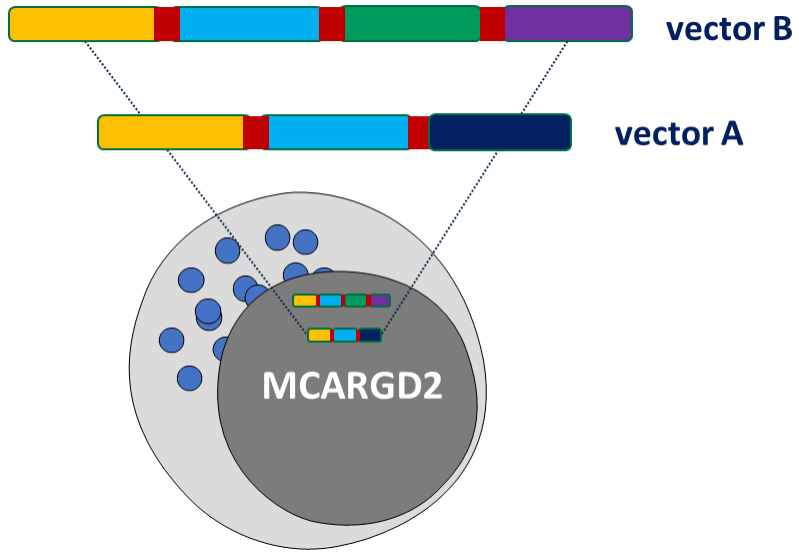
Multi modular GD2-CAR (MCARGD2) clinical trial design

Phase I:

- r/r neuroblastoma
- co-transduction autologous apheresate with vector A and B
- establish optimal CAR vector (combination) and cell dose

Possible Expansion cohorts:

- Include other GD2+ childhood solid tumours (DIPG and Osteosarcoma)



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AUTO1 study investigators

AUTO3 study investigators

AUTO4 study investigators

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