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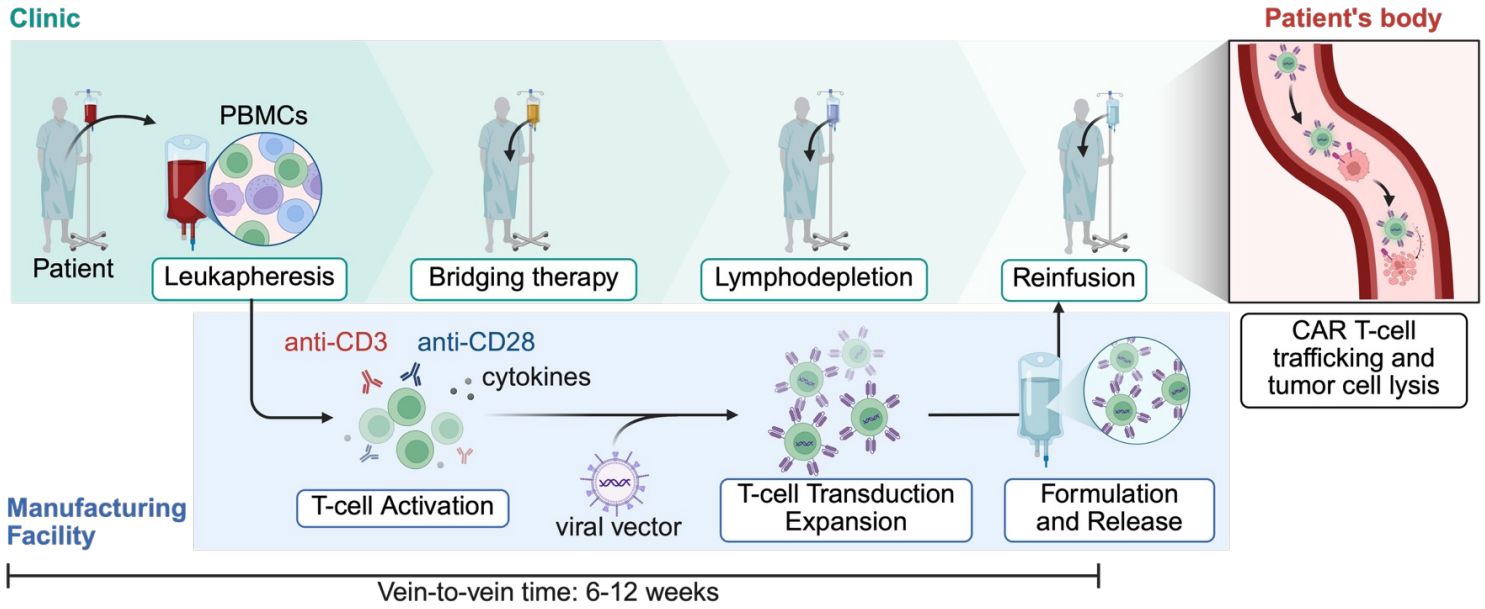
In Vivo CAR T-Cells

Laura Volta, PhD
Lab of Prof. Saar Gill, MD, PhD

Ex vivo generation of CAR T-cells

Traditional manufacturing



- **Autologous**
- **High manufacturing cost** → limitation to scalability, accessibility, and broader clinical implementation
- **Increased *ex vivo* culture** with supraphysiological levels of cytokines has been positively correlated with **decreased anti-tumor potency** (Ghassemi, S. et al. 2018. *Cancer Immunol. Res.*)

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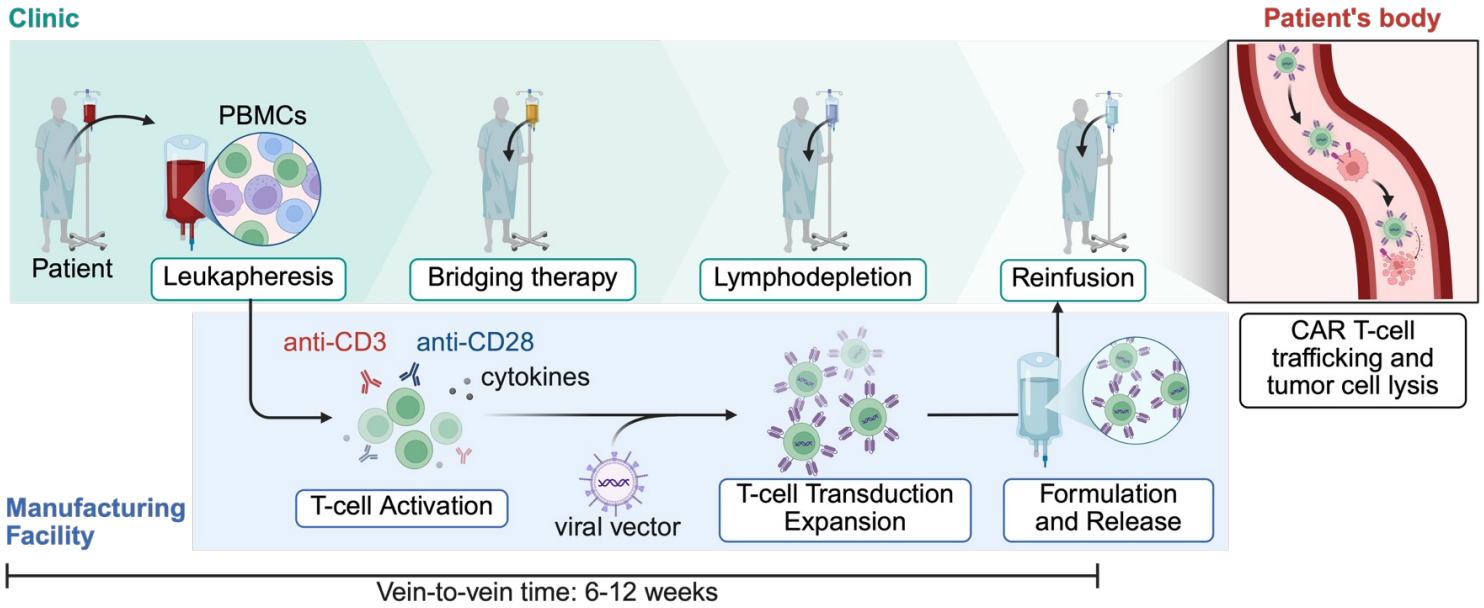
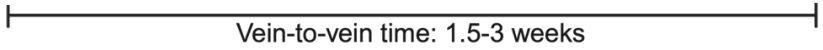


Accelerated manufacturing



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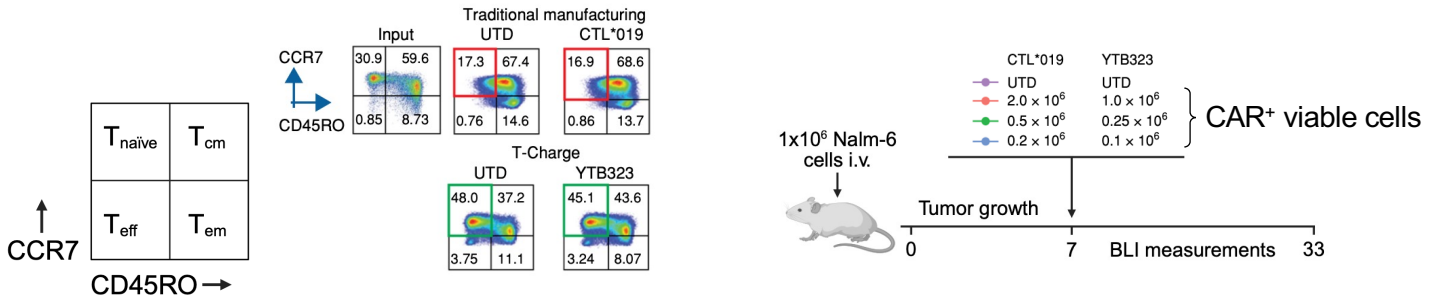
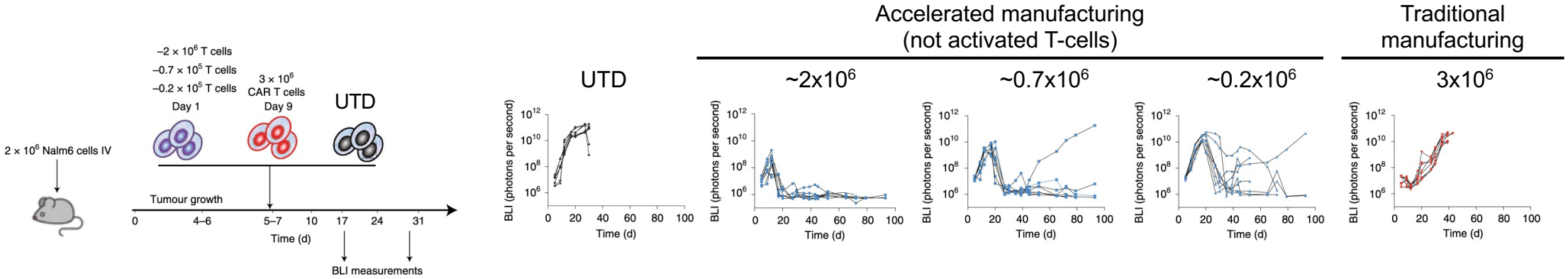
Accelerated CAR T-cell manufacturing

- **Novartis' T-Charge platform (<2d *ex vivo* culture, activation)**



Dickinson, M.J. et al. 2023. *Cancer Discov.*

- **UPenn platform (<1d *ex vivo* culture, no activation)**



Ghassemi, S. et al. 2022. *Nat. Biomed. Eng.*

→ **Reduced ex vivo expansion resulted in a superior product**

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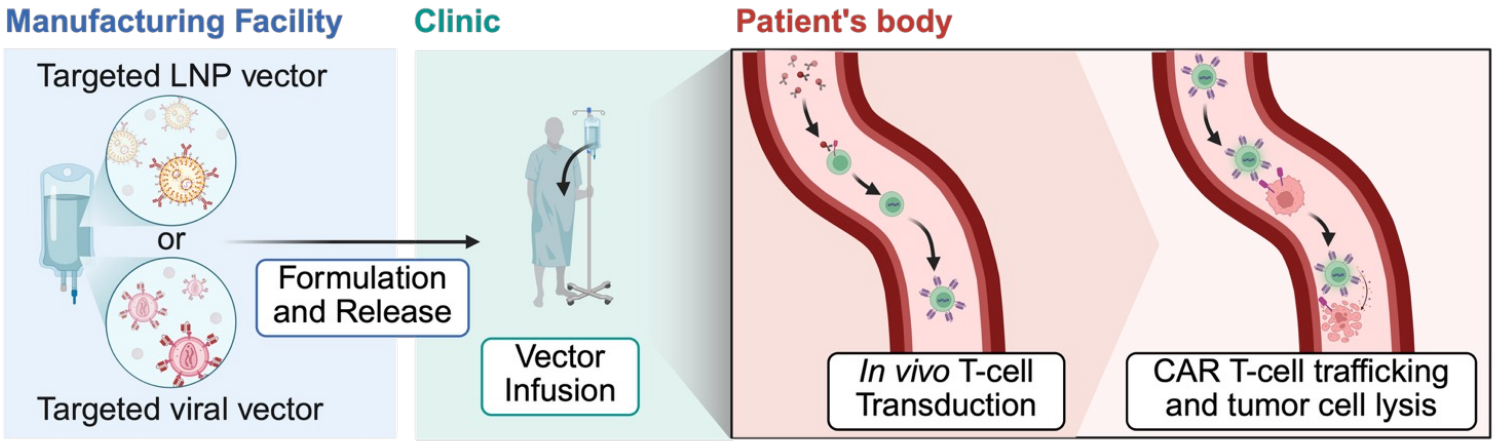
In vivo generation of CAR T-cells



Aim: Generate CAR T-cells by reprogramming T-cells directly within the patient, potentially enabling a more accessible and cost-effective therapeutic platform.

- **Specific delivery to a sufficient number of quality T-cells**

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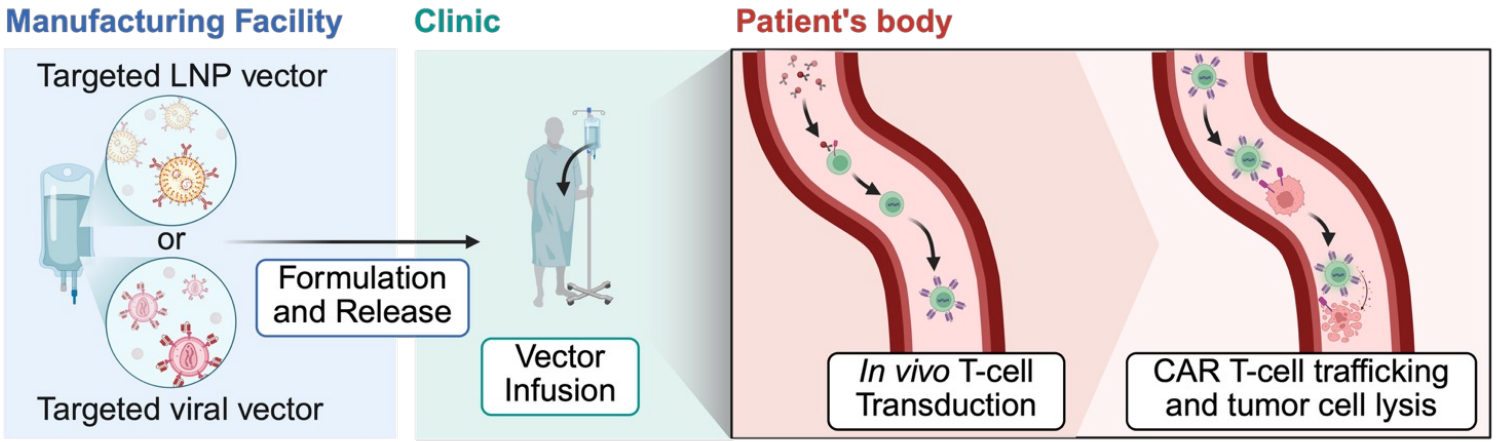
In vivo generation of CAR T-cells



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- **<u>Specific delivery</u> to a sufficient number of quality T-cells**

  → Safety considerations: avoid off-target transduction to germ, somatic, and cancer cells

  → Minimization of the competing mechanisms that reduce the effective on-target vector dose

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In vivo generation of CAR T-cells

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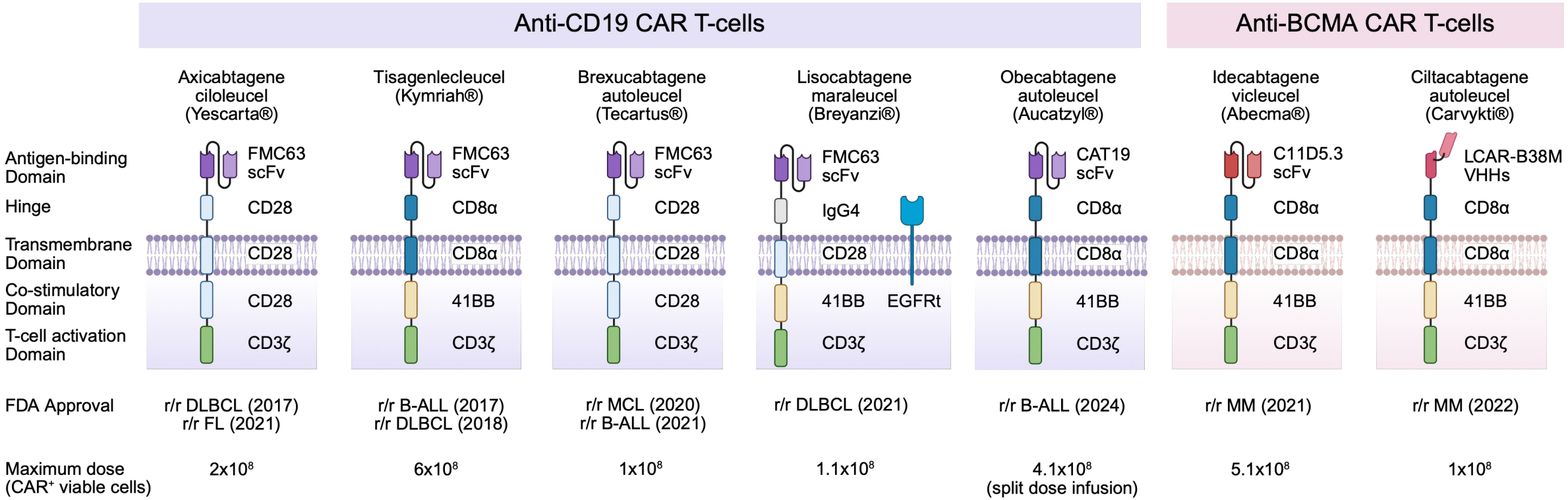

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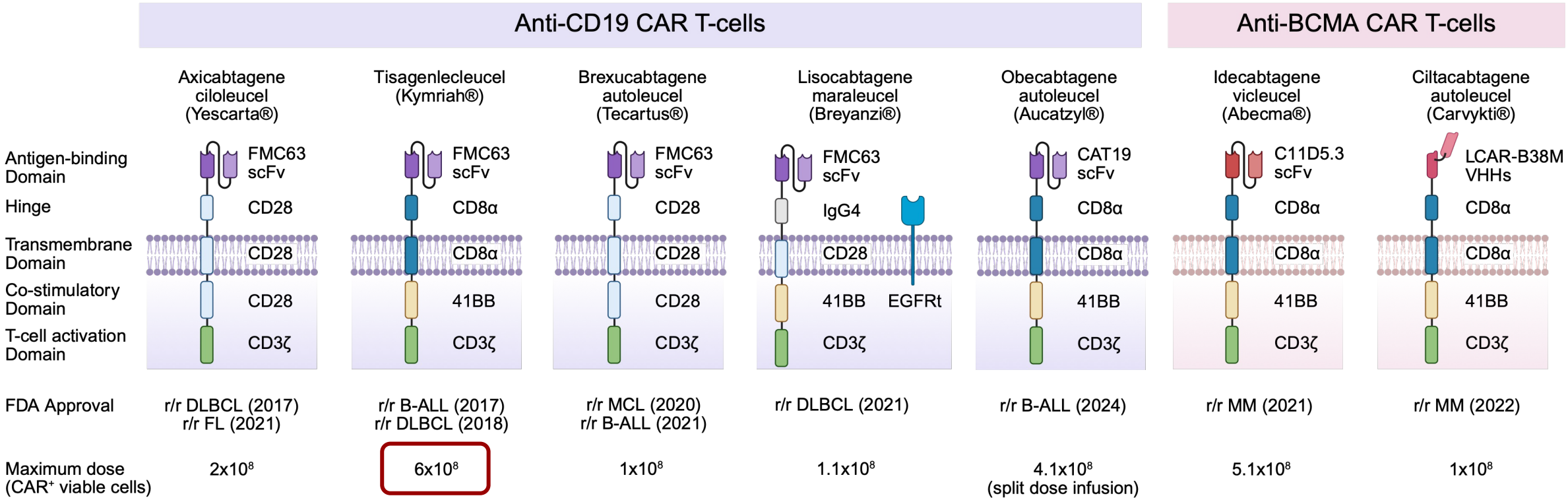

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In vivo generation of CAR T-cells

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Assuming that:

Max CAR T-cell administered dose = $6x10^8$

Total T-cells in 5L **blood** = ~$10x10^9$

} **~2%**

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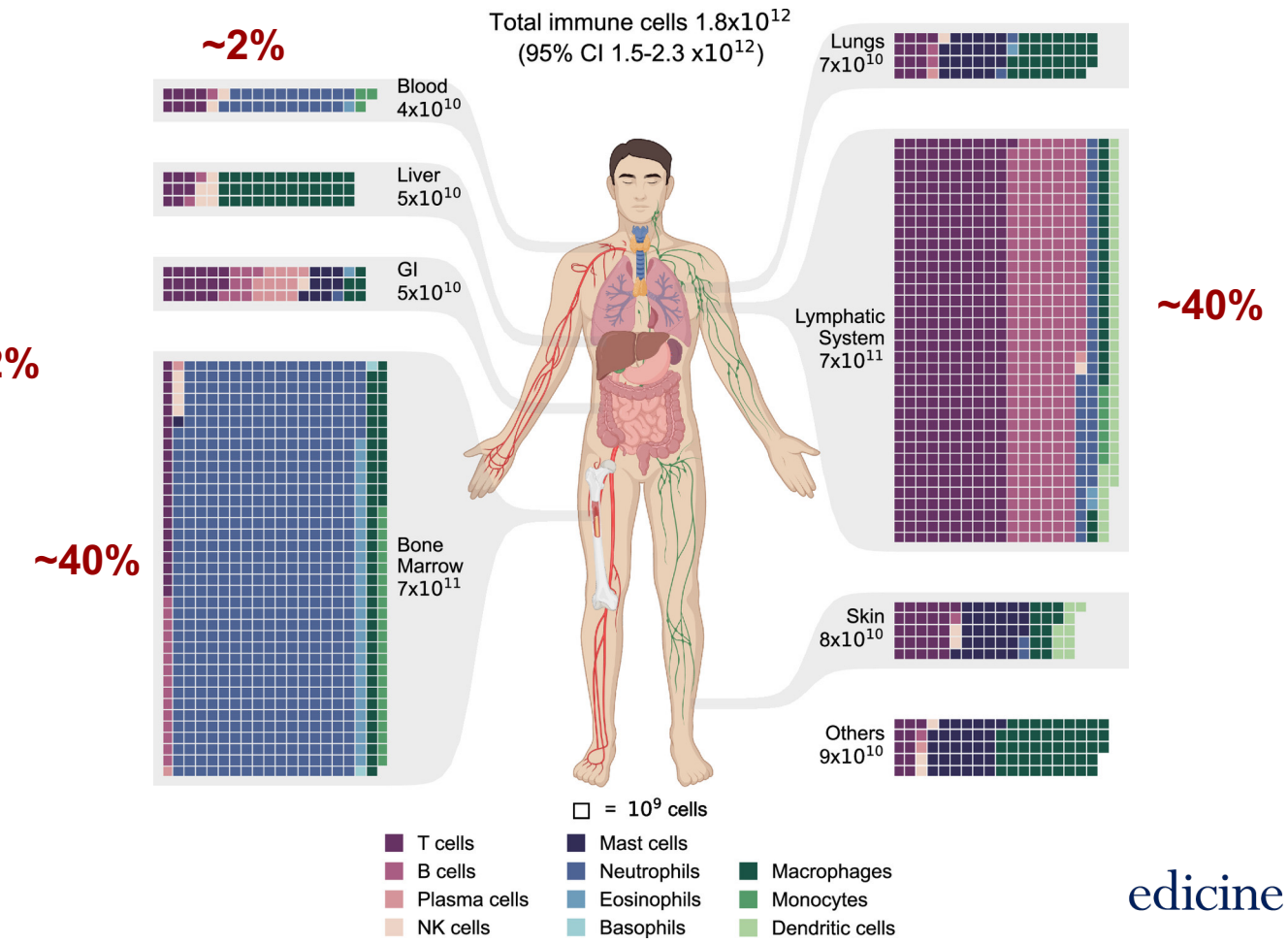
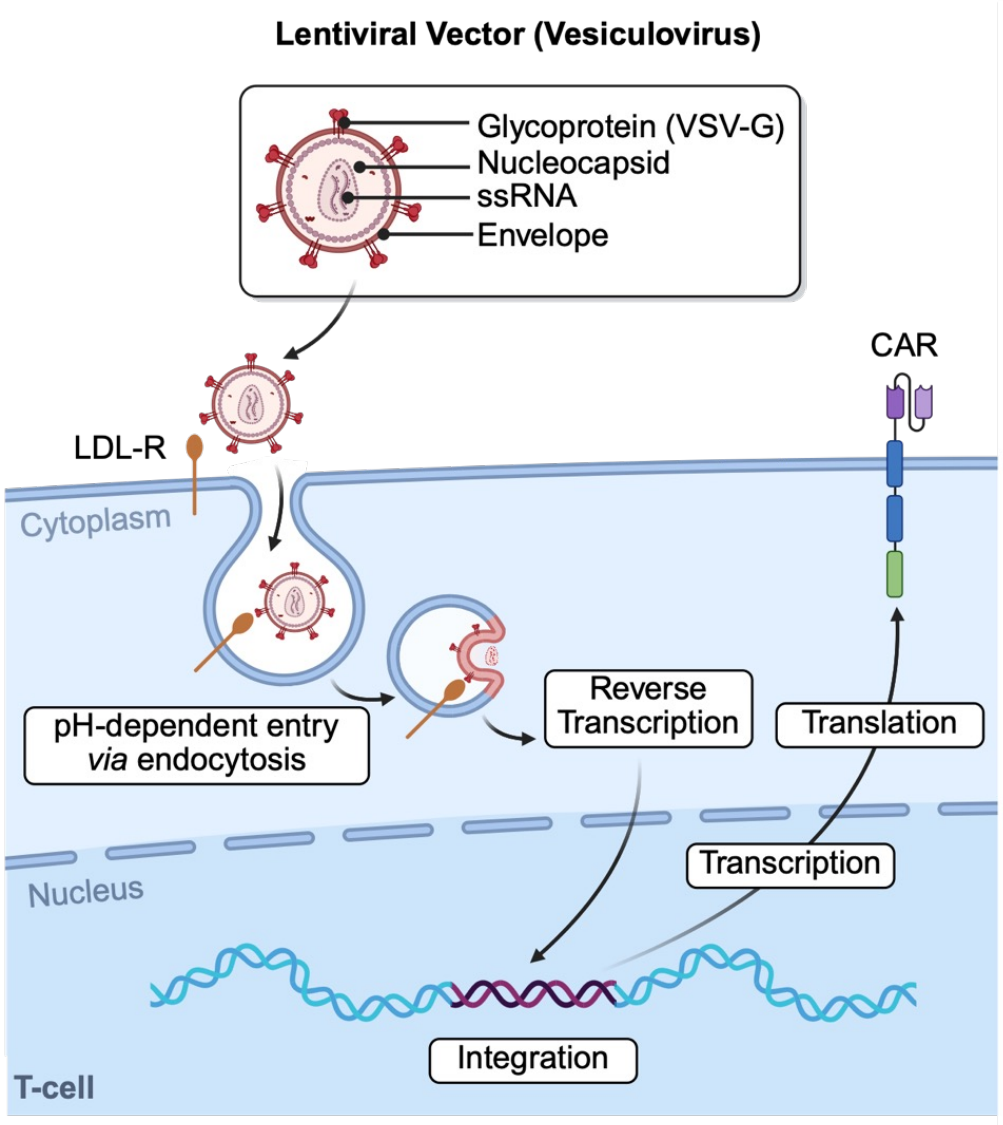
Max CAR T-cell administered dose = $6x10^8$

Total T-cells in the **human body** = ~$5x10^{11}$ } **~0.12%**

Sender, R. et al. 2023. *Proc. Natl. Acad. Sci.*



Viral surface engineering for cellular targeting



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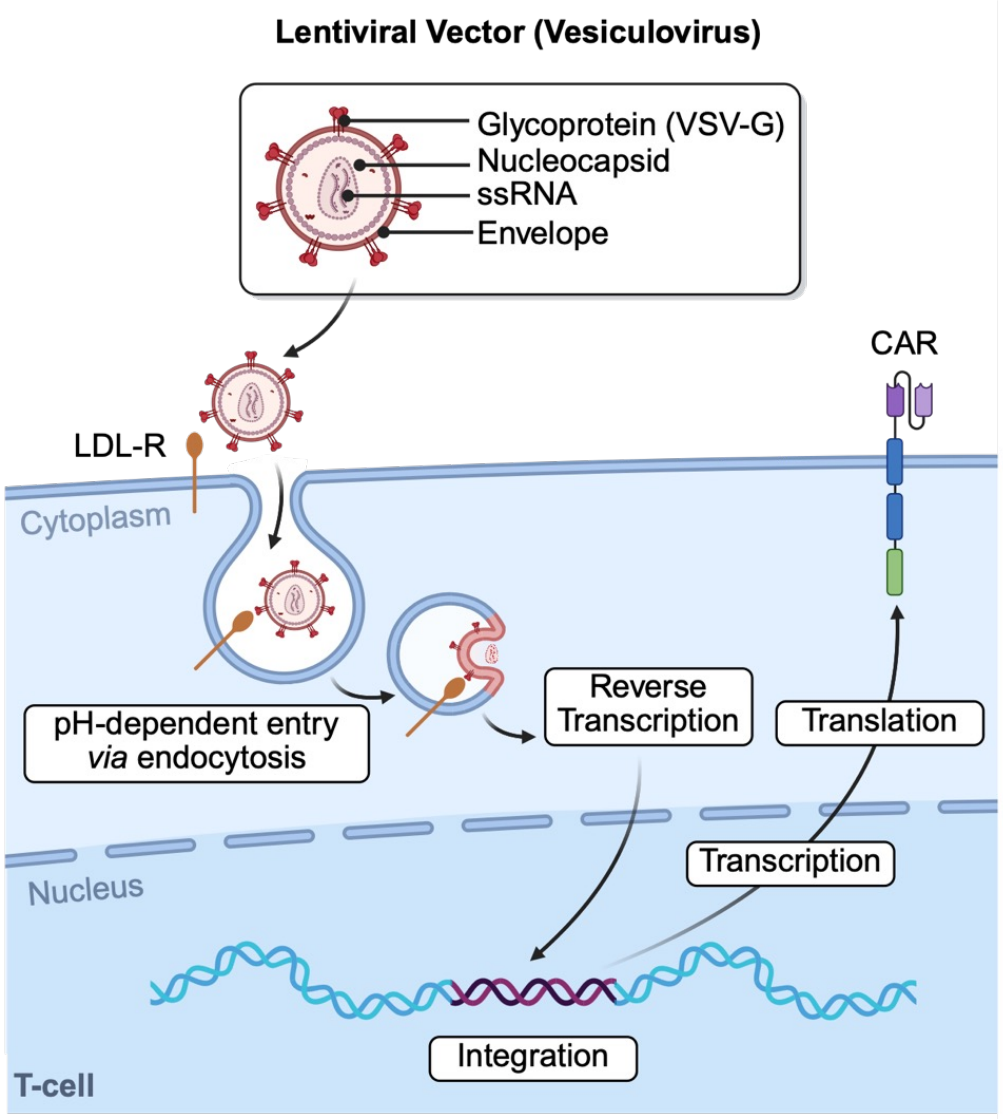

- Mutants K47Q and R354A render VSV-G unable to bind LDL-R, but are **not** required for fusion (Nikolic, J. et al. 2018. *Nat. Commun.*)

  → **It is possible to uncouple G fusion activity and receptor recognition**

- It is thus crucial to functionalize the virus with a ligand that triggers internalization upon binding to target cell receptors

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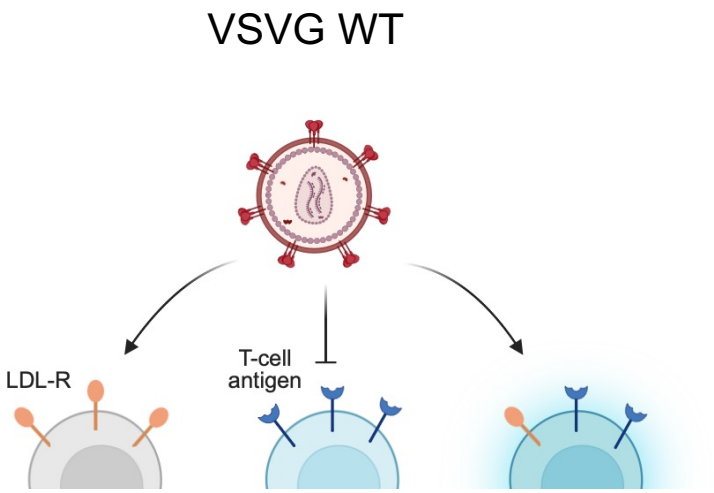
Viral surface engineering for cellular targeting



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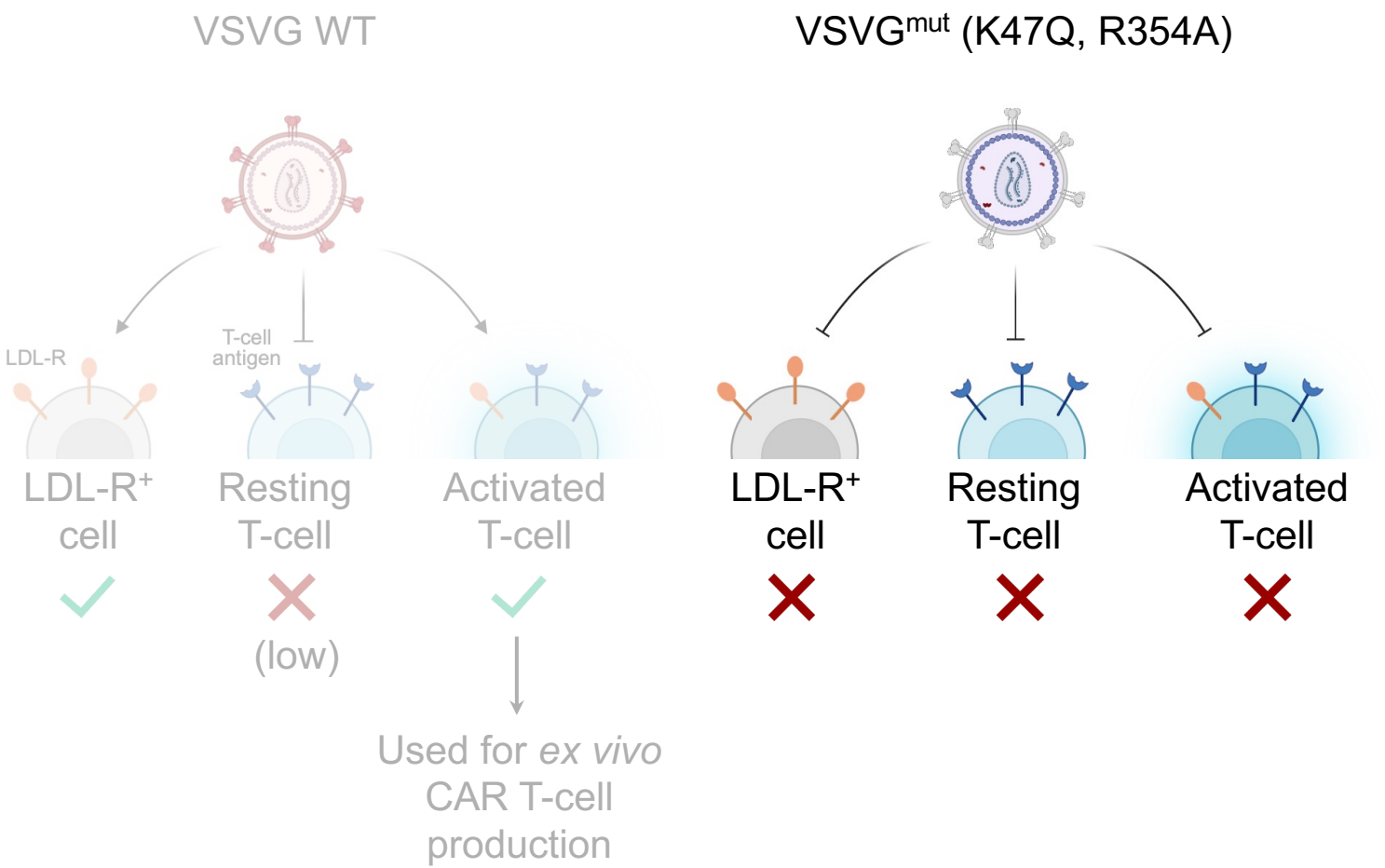
Viral surface engineering for cellular targeting



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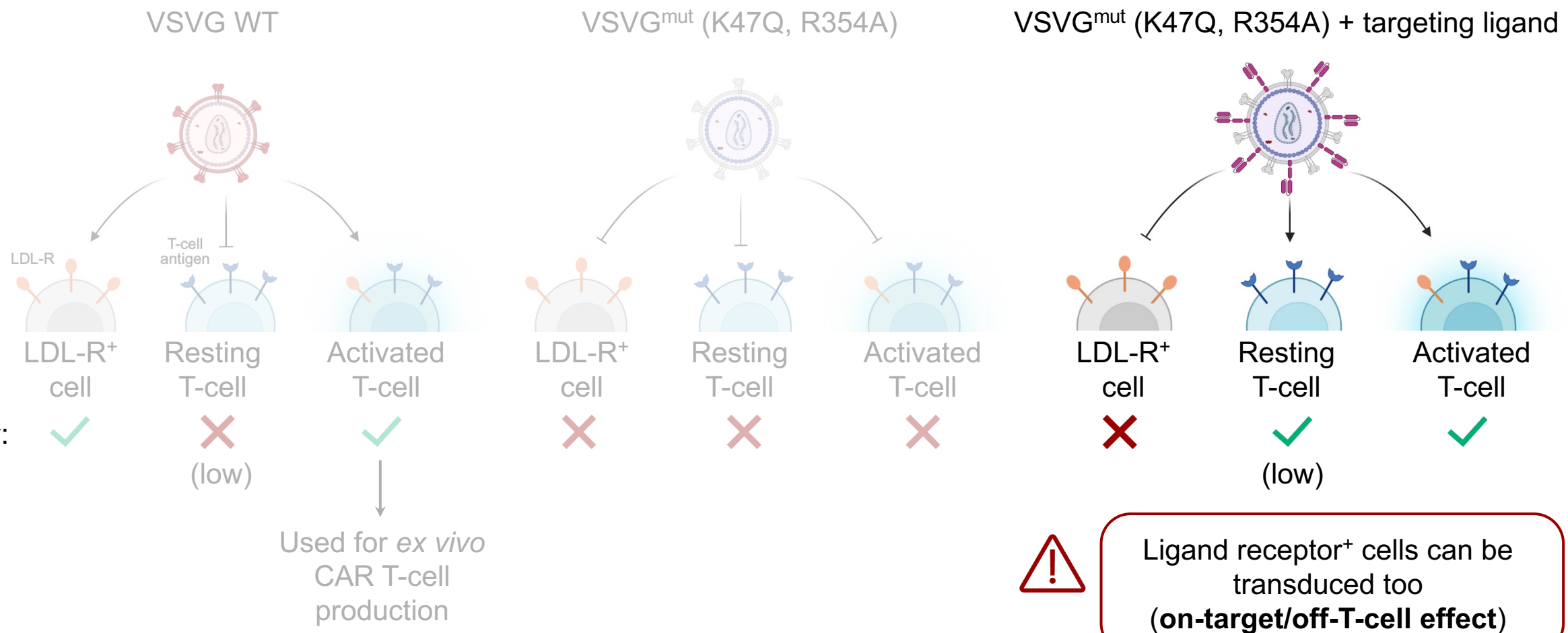
Viral surface engineering for cellular targeting



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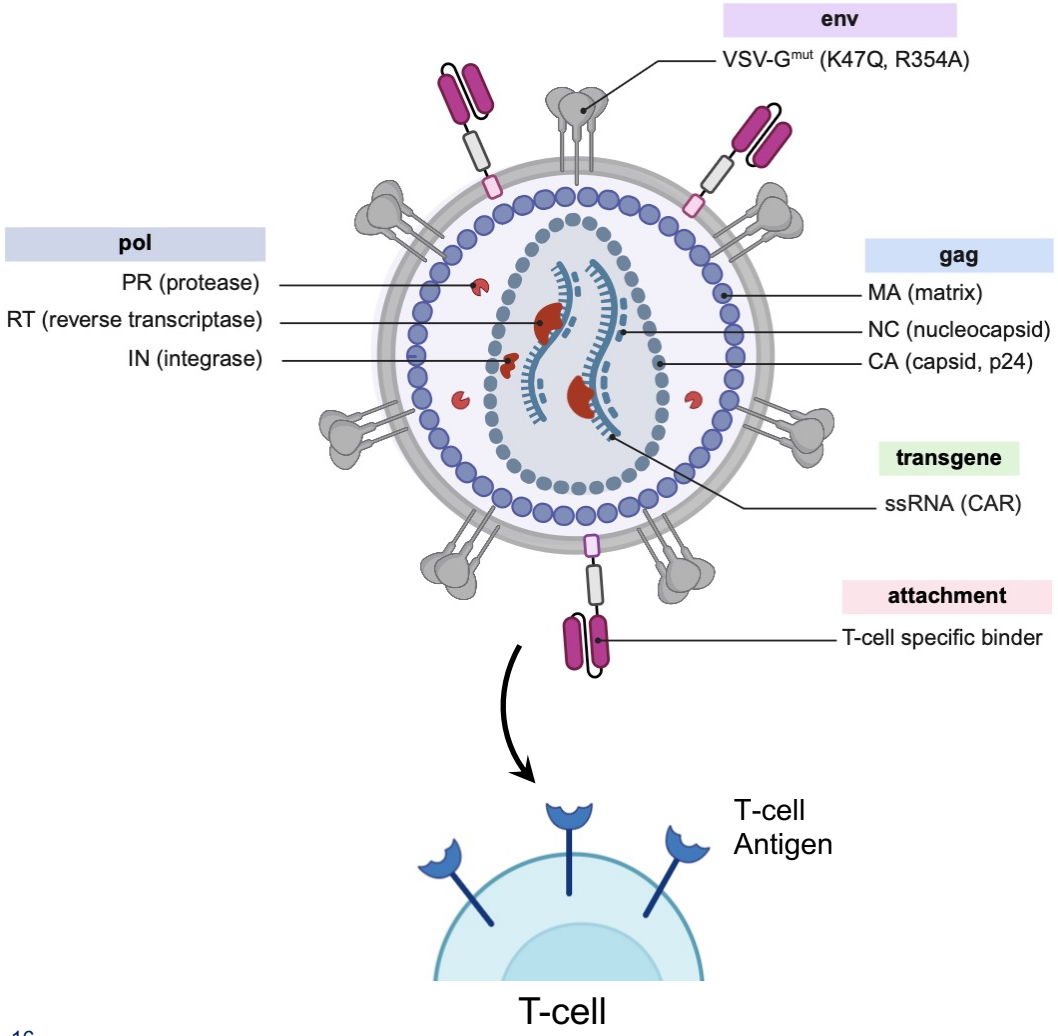
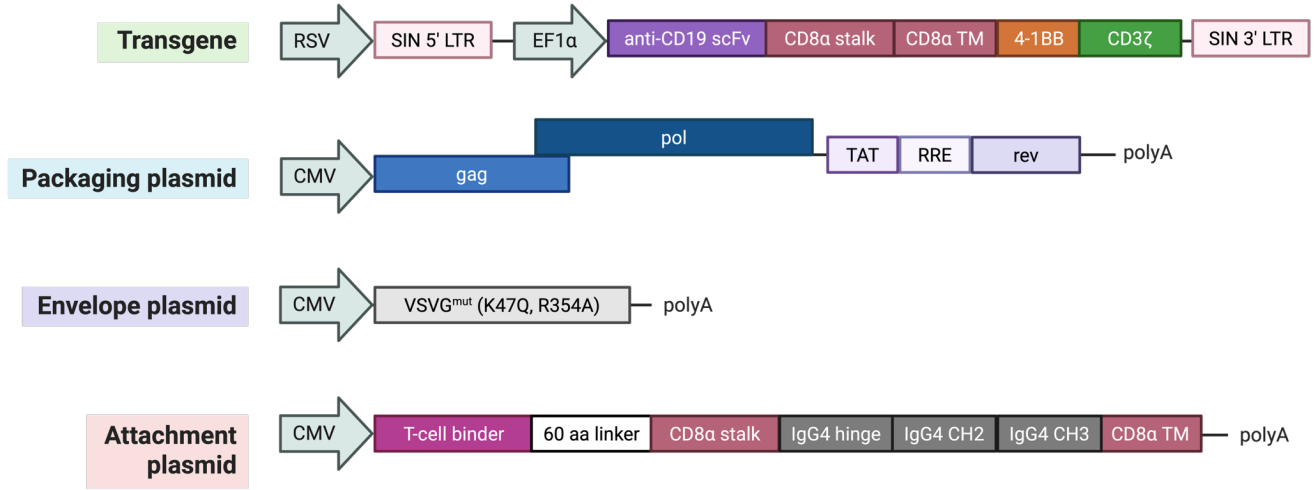
In vivo editing of T-cells: our system

Miroslaw Kozlowski

2nd generation SIN LV vectors (*in vivo* CAR T-cells generation)





Preclinical efficacy data for a similar platform have already been reported in

- Dobson, C.S. et al. 2022. *Nat. Methods.*
- Hamilton, J.R. et al. 2024. *Nat. Biotechnol.*

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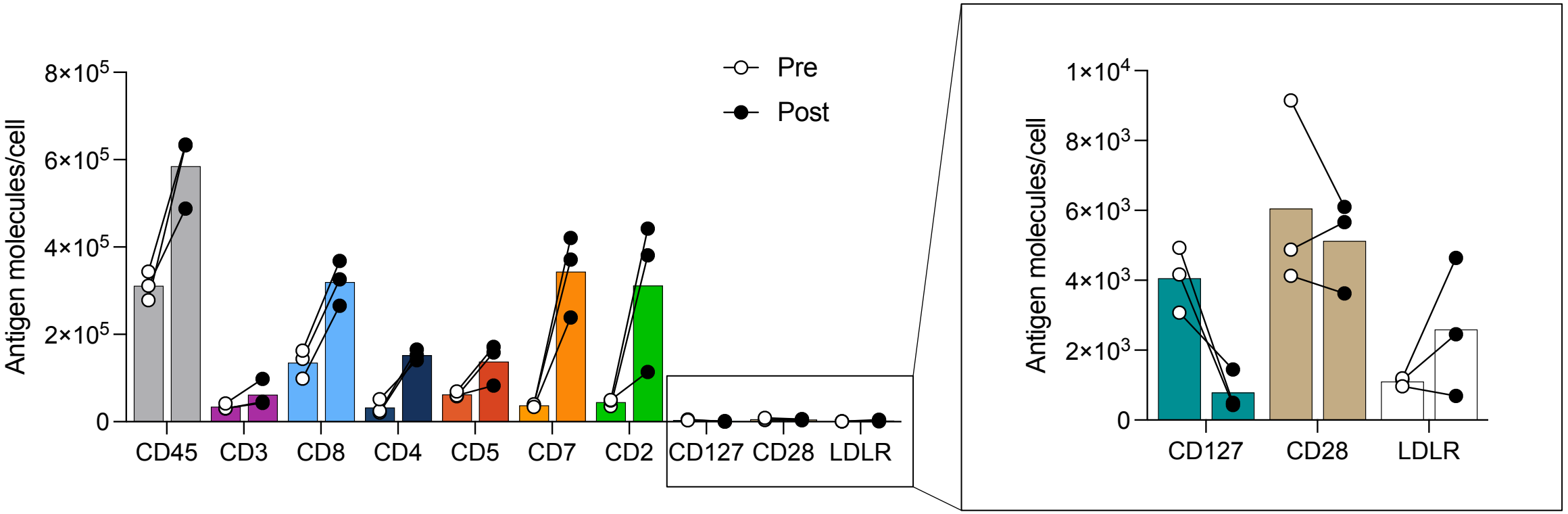
Antigen expression on T-cells pre and post activation

- Cells and Quantum™ Simply Cellular beads were processed as per manufacturer's instructions after collection and upon 3-day-long activation with CD3/CD28 beads (beads removed prior to staining)



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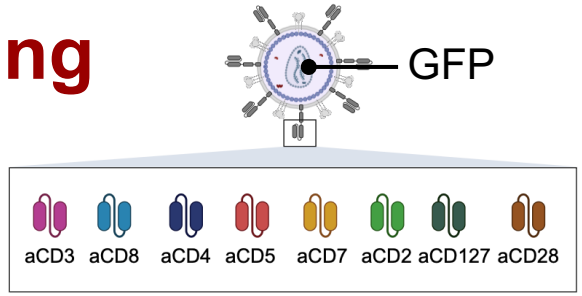
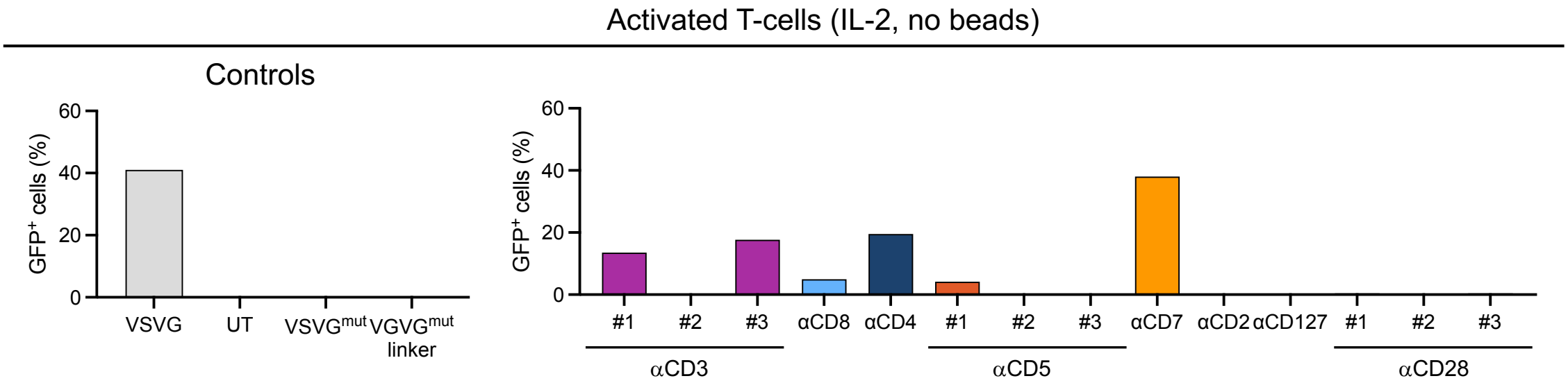
Screening of T-cell binders for VSVG^{mut} pseudotyping





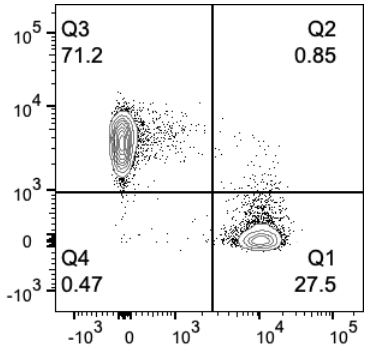
→ Lower transduction efficiency for VSVG^{mut} LVPs functionalized with scFvs compared to VSVG WT (100uL not upconcentrated virus suspension; LVPs batches produced in parallel)

Specificity of the platform

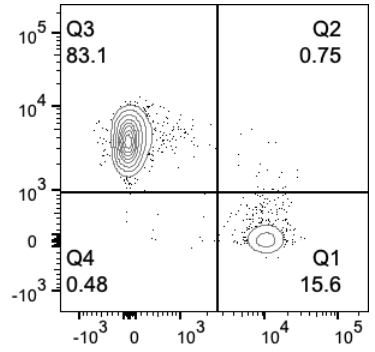
Pregating on live/GFP⁺ cells (+IL-2, no beads):

Pan T-cell Antigens

αCD7 scFv



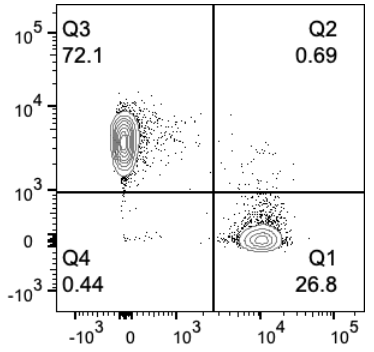
αCD5 scFv



Clone 1

Signal 1

αCD3 scFv



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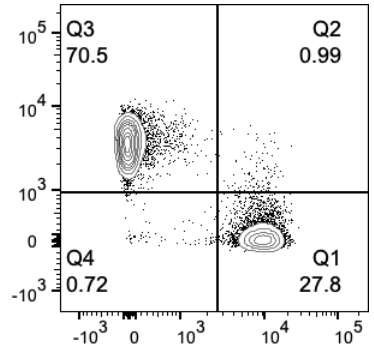
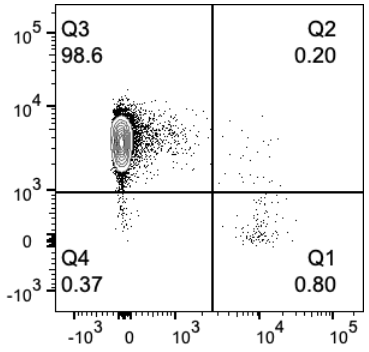


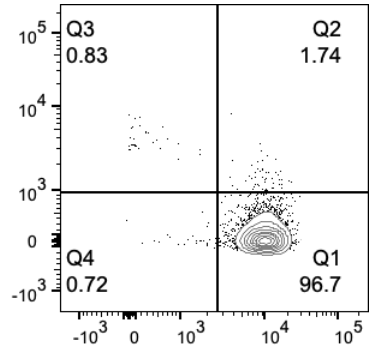
Clone 3

Co-Receptors

αCD4 Darpin

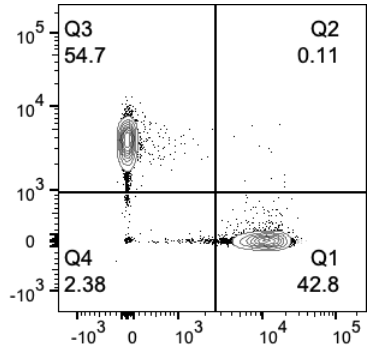


αCD8 scFv

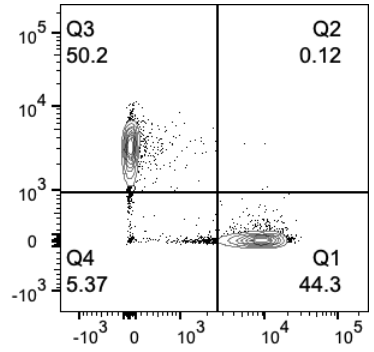


Controls

VSVG +ve ctrl



UT -ve ctrl



Pregated on live

CD4

CD8

Acknowledgments

Prof. Saar Gill

The *in vivo* team

Miroslaw Kozlowski
Federico Rossari
Bruno Casino Remondo
Shanay Desai
Conor Dickson

The whole lab!!

Orlando Arevalo
Joanne Baek
Anand Bhagwat
Astrid Beerlage
Asuncion Borrero
Dan Brown
Bryan Ciccarelli
Mara Davis
Elliot Goepfert-Waterman
Tim Grob
Neel Nabar
Winnie Nguy
Fiona O'Connell
Ryan O'Connell
Krystal Oon
Brandon Simone
Sara Sleiman
Max Shestov
Olga Shestova
Feng Shen
Taylor Tredinnick
Leo Torres
Nils Wellhausen

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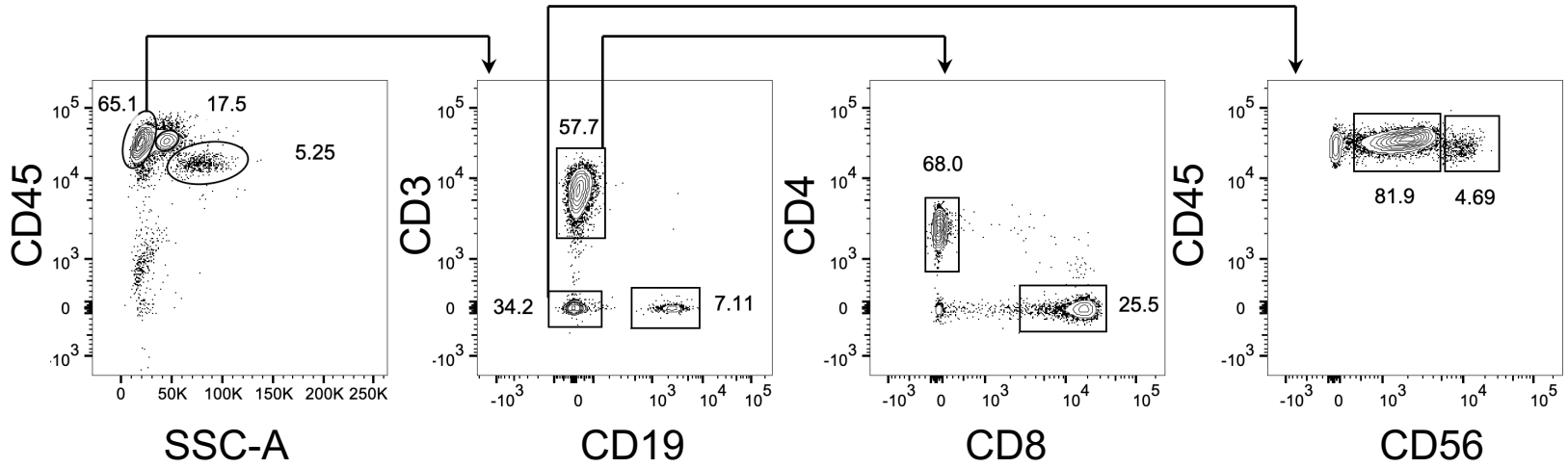
Supplementary Slides

Laura Volta, PhD
Lab of Prof. Saar Gill, MD, PhD

Antigen expression on T-cells

- PBMCs and ACK'd Ficoll pellet were mixed 1:1 ratio (n=3 donors)
- Cells were incubated with FcR blockers
- Cells and Quantum™ Simply Cellular beads were processed as per manufacturer's instructions after collection and upon 3-day-long activation with CD3/CD28 beads (beads removed prior to staining)

Gating strategy



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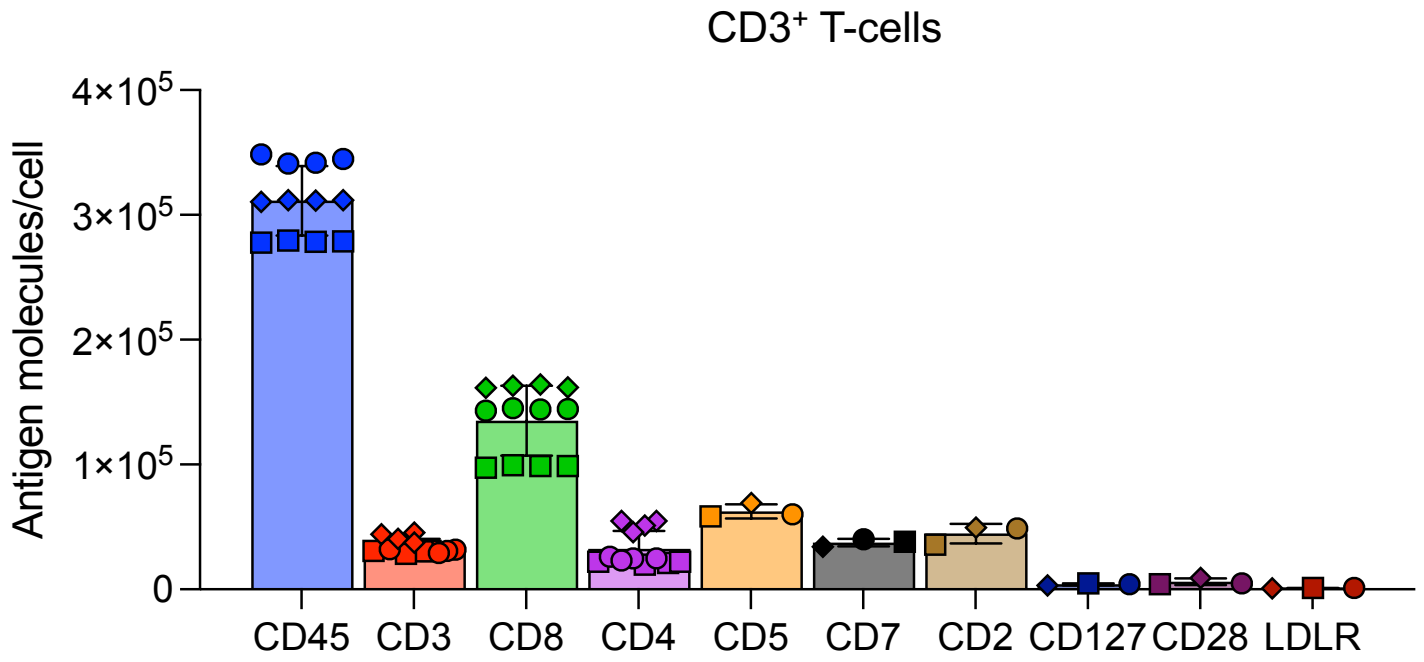
Additional Slides

- Data with technical replicates from ND587 (squares), ND615 (circles), and ND365 (diamonds).



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