In vivo CRISPR engineering to generate *TRAC*-targeted CAR-T cells

William A. Nyberg, Ph.D.

Assistant Professor

SciLifeLab Fellow

Center for Hematology and Regenerative Medicine

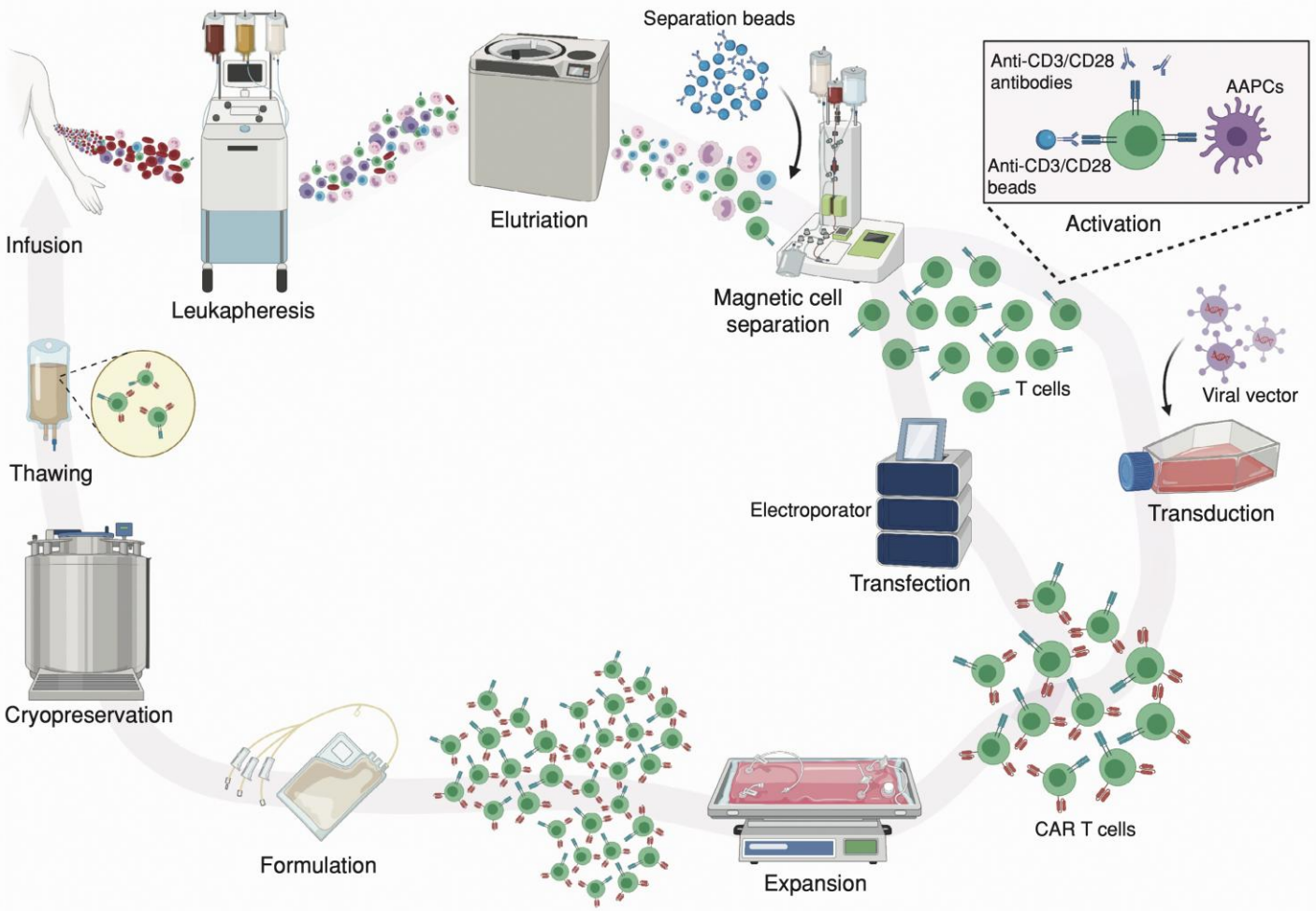
Department of Medicine Huddinge, Karolinska Institutet

Disclosures

- Three patent applications on work in presentation

- Two patents licensed by Azalea Therapeutics in 2025

CAR-T cell manufacturing is expensive and complex



<u>Limitations:</u>

- High cost (3-4M SEK)
- Donor-to-donor variation
- Logistics and cost reduce availability
- Time consuming (2 weeks – 2 months)
- Low throughput leads to limited access

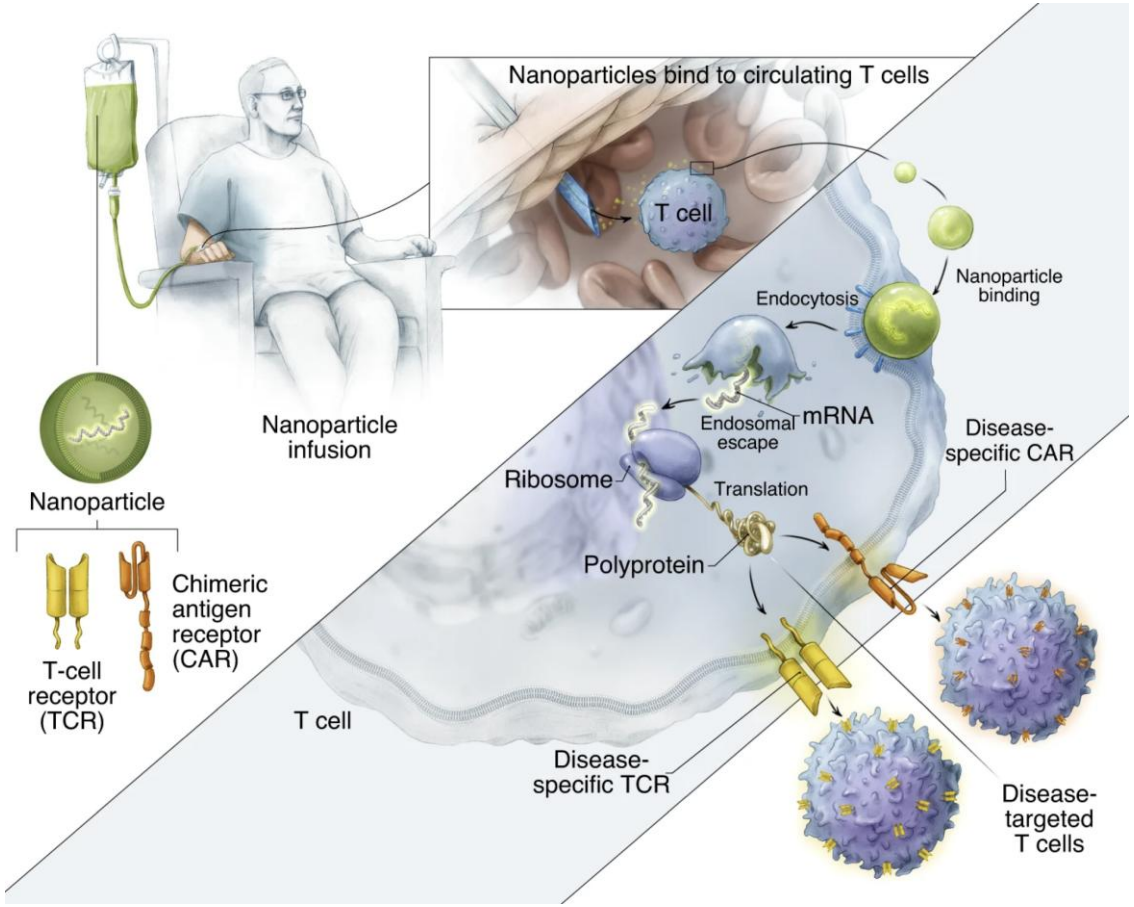
<u>Solutions:</u>

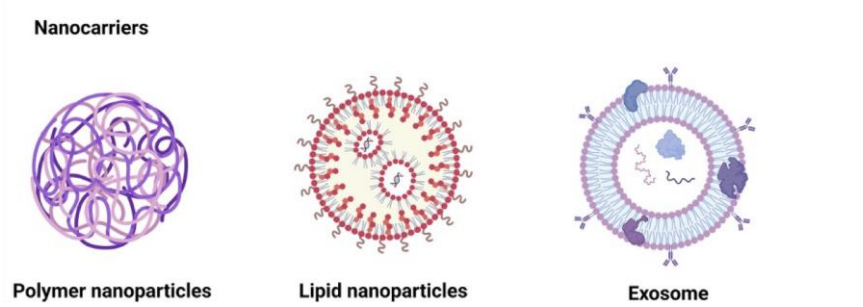
- Reduce manufacturing time
- More efficient CAR-T cells

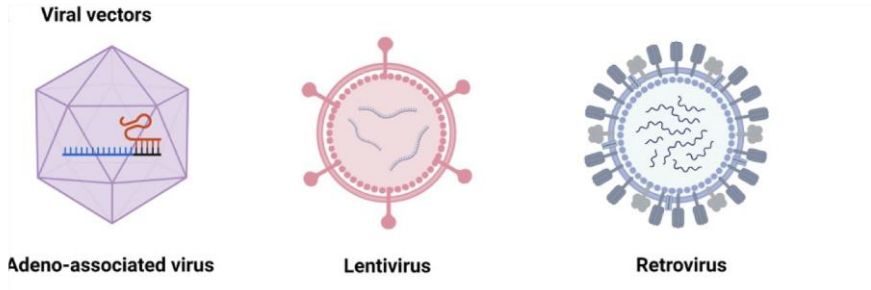
  -> Less cells needed
- Allogeneic solutions
- Gene therapy?

Blood Cancer Discov. (2021)

Generating cell therapies *in vivo* - Gene Therapy







First reported by Dr. Stephan's group in 2017 using nanocarriers.

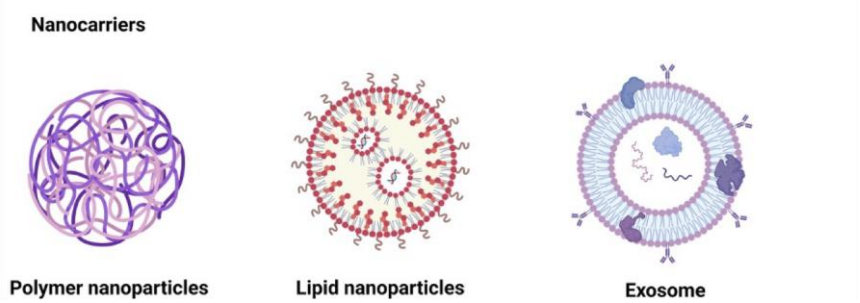
Generating cell therapies *in vivo* - Gene Therapy

LNP mRNA

- Transient delivery

⇒ Need to re-dose

⇒ Poor nuclear delivery

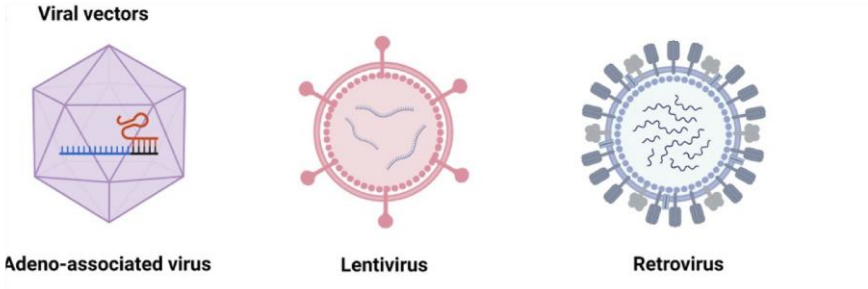


Engineered LVV

- Semi-random integration
- Constitutive promoter

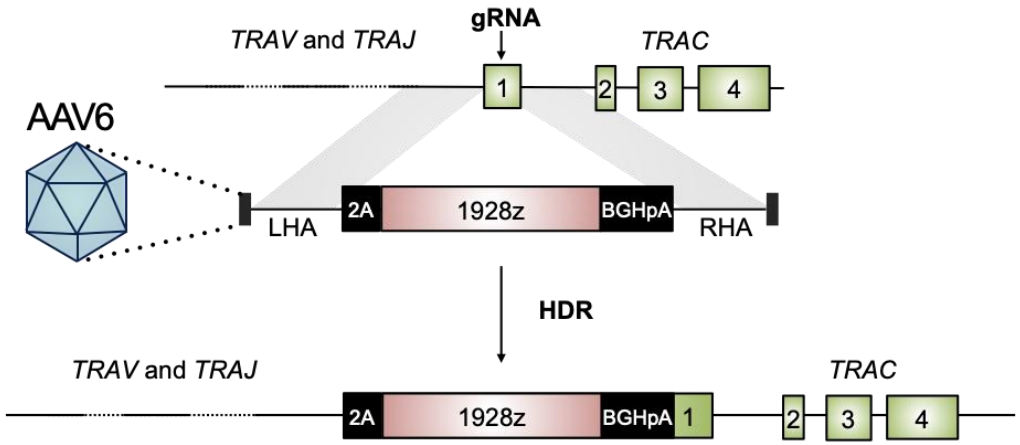
⇒ Every infected cell express CARs

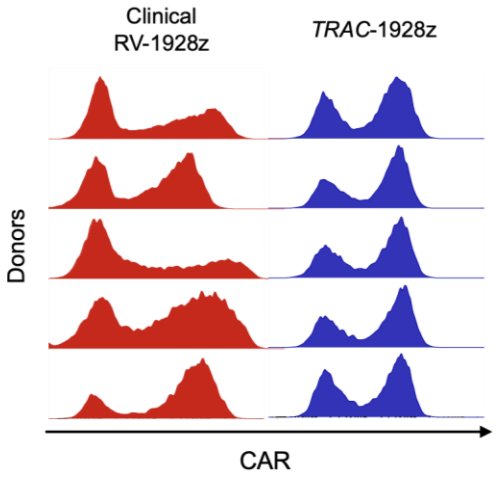
⇒ Expression not T cell restricted

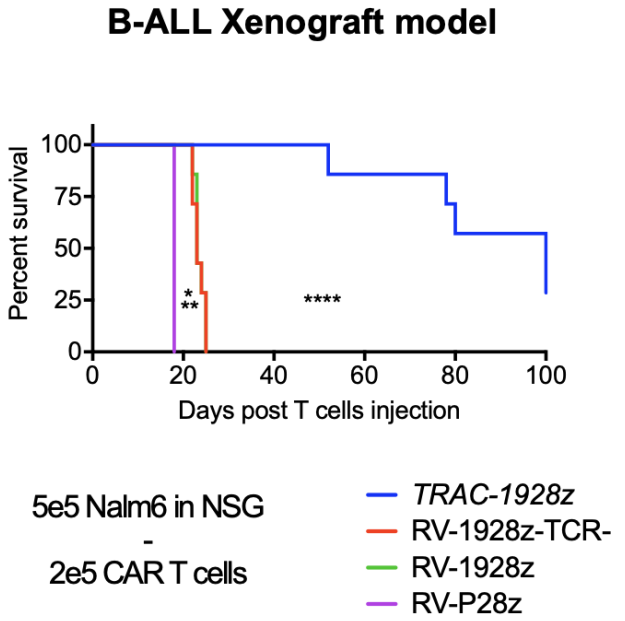


First reported by Dr. Stephan's group in 2017 using nanocarriers.

TRAC-CAR-T cells; physiological expression and superior efficacy



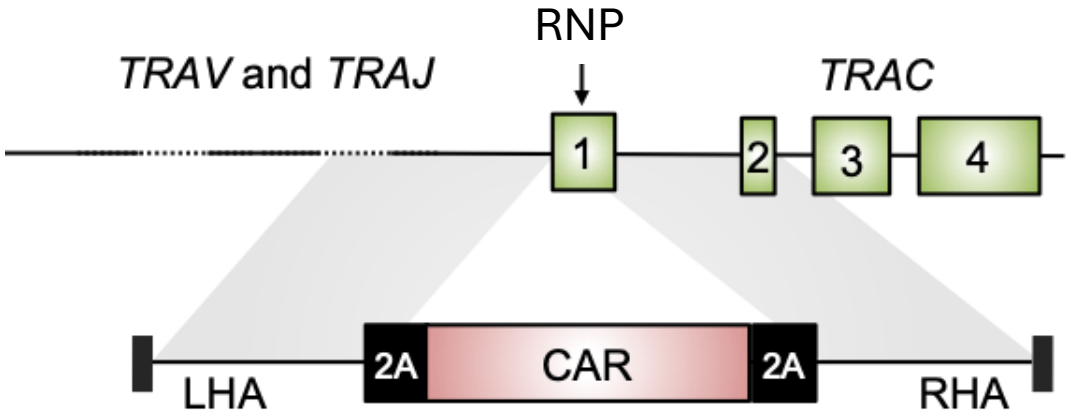




Eyquem et al., *Nature* (2017)

Is it possible to generate *TRAC*-CAR-T cells *in vivo*?

Challenges for generating *TRAC*-CAR-T cells *in vivo*



Cas9: specific and short expression (minimal immunogenicity)

HDRt: potent nuclear delivery

Advantages of TRAC targeting:

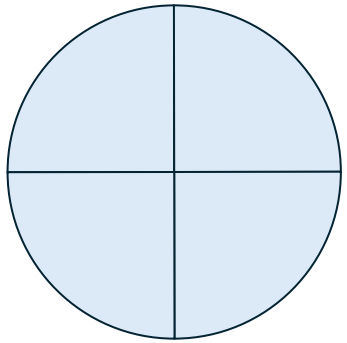
- ✓ *Precise*
- ✓ *Promoterless cassette*
- ✓ *CAR expression restricted to T cells*
- ✓ *Improved CAR-T cell function*

Problem:

- ✓ *In vivo HDR in primary immune cells?*
- ✓ *Potent (and non-toxic) In vivo DNA nuclear delivery*

Challenges for generating *TRAC*-CAR-T cells *in vivo*

1. DNA template delivery for homology directed repair



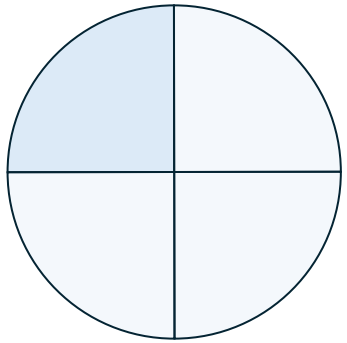
2. Gene editing of T cells *in vivo*

4. Sufficient CAR-T cell expansion for cancer therapies

3. CRISPR/Cas9 delivery to human T cells *in vivo*

Challenges for generating *TRAC*-CAR-T cells *in vivo*

1. DNA template delivery for homology directed repair



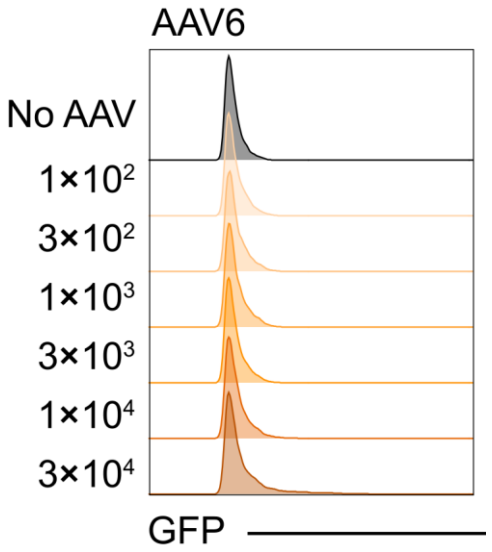
2. Gene editing of T cells *in vivo*

3. CRISPR/Cas9 delivery to human T cells *in vivo*

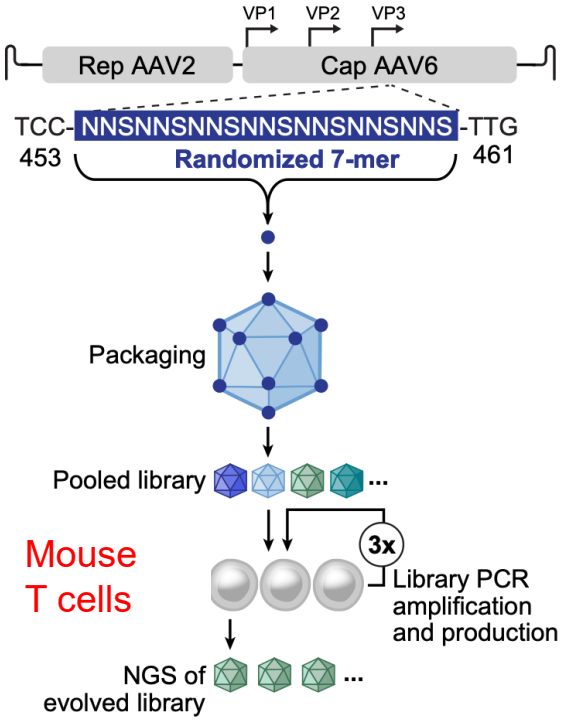
4. Sufficient CAR-T cell expansion for cancer therapies

Generating T cell evolved AAV seroptypes; mouse T cells

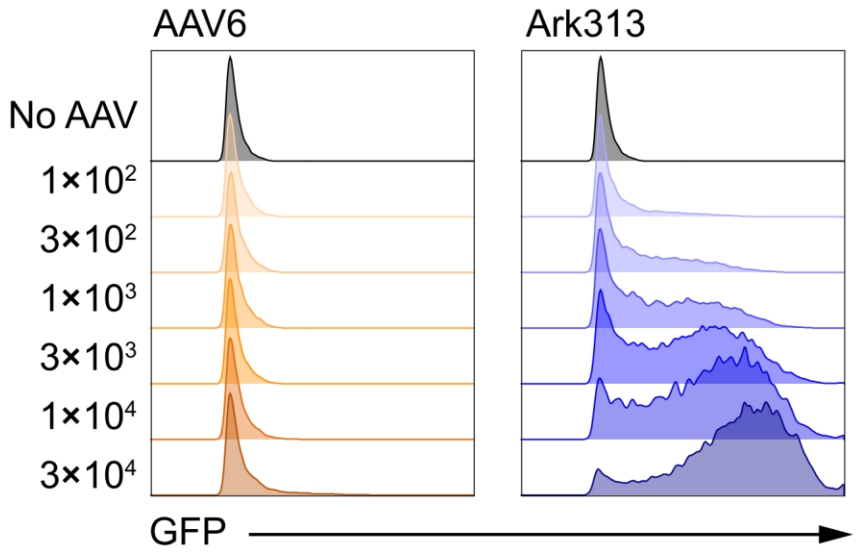
Limited transduction by AAV6



AAV6 library evolution

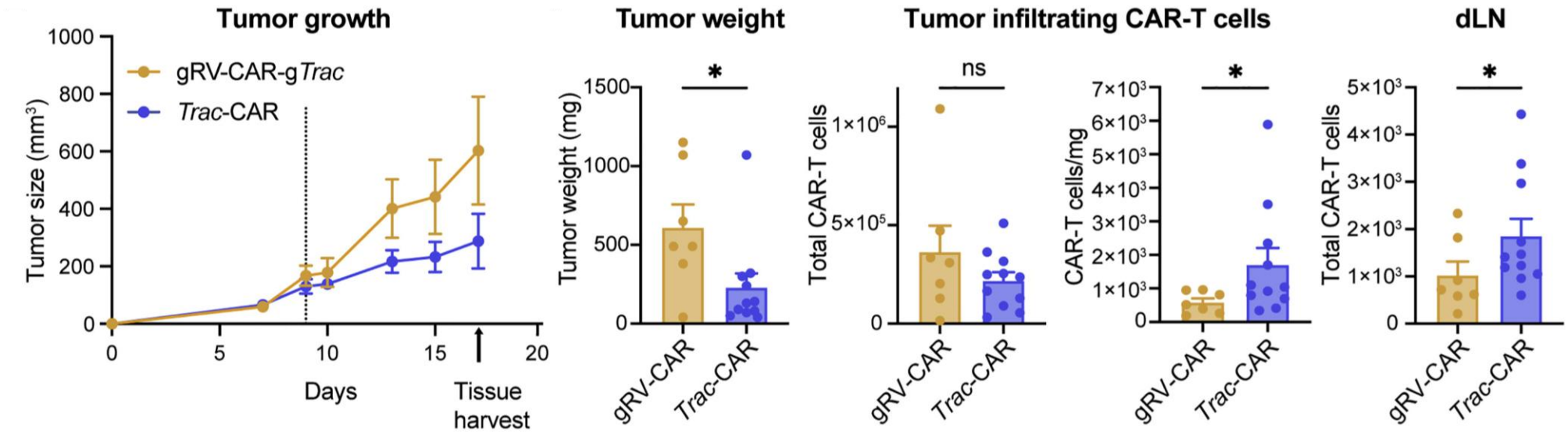


Improved T cell transduction



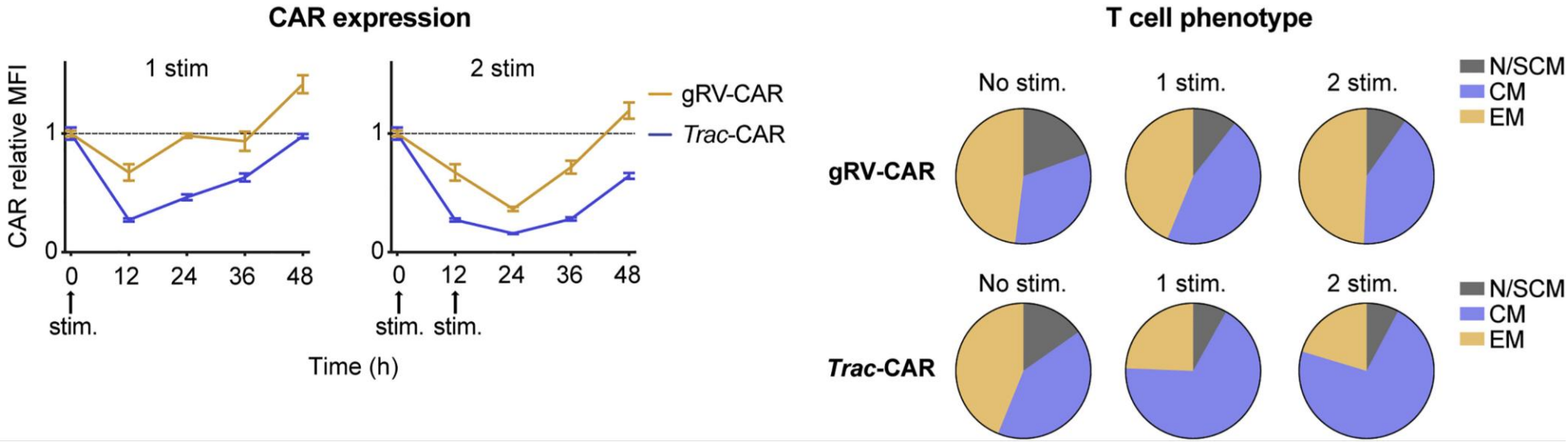
Nyberg et al., *Cell* (2023)

Trac-CAR-T cells superior in immunocompetent tumors



Nyberg et al., *Cell* (2023)

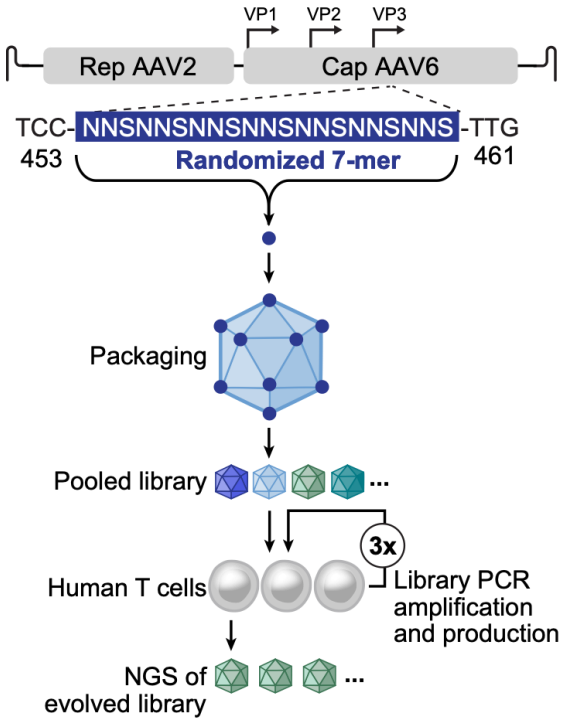
Increase in CM compartment of *Trac*-CAR-T cells



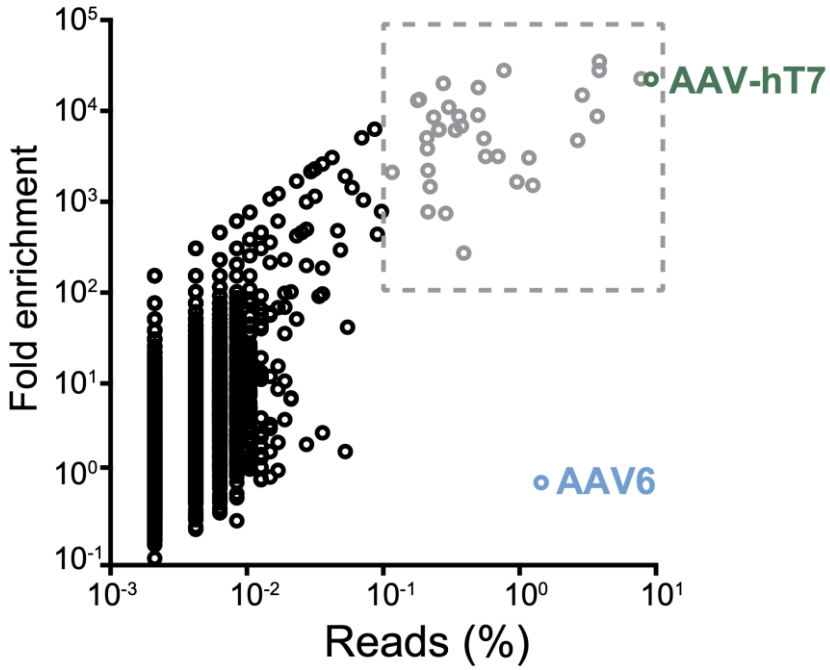
Nyberg et al., *Cell* (2023)

Evolving AAVs on human T cells

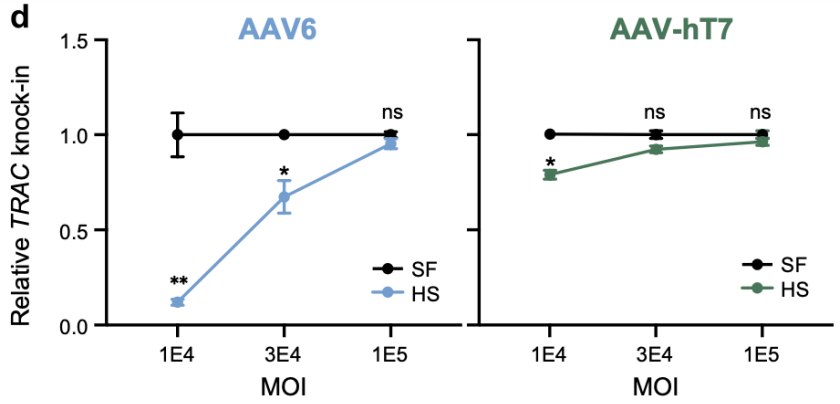
AAV evolution



Human T cell AAV



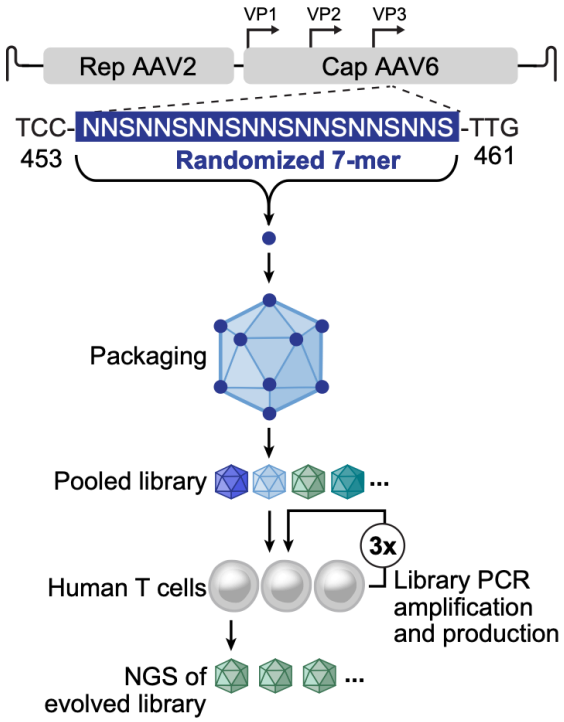
Serum resistance



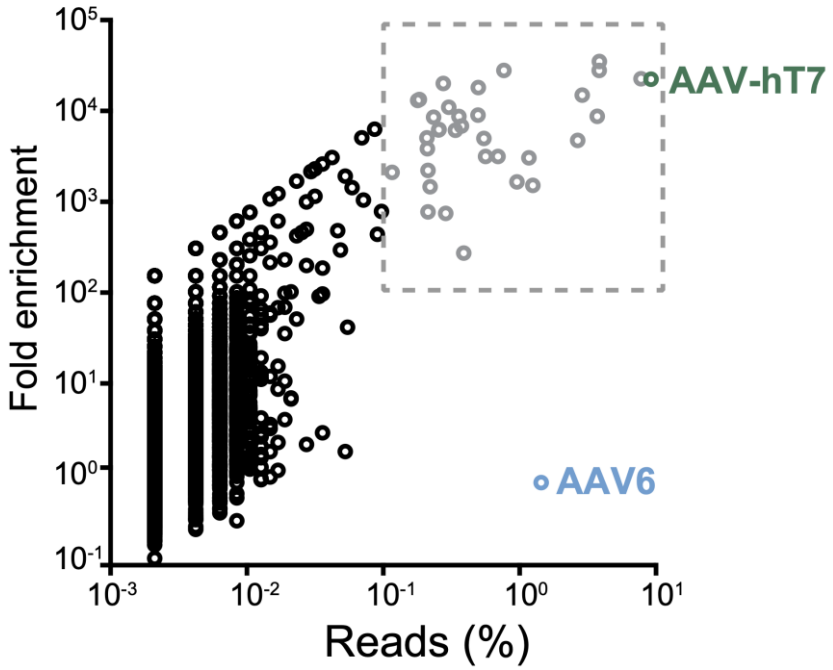
Nyberg et al., *Nature* (2026)

Evolving AAVs on human T cells

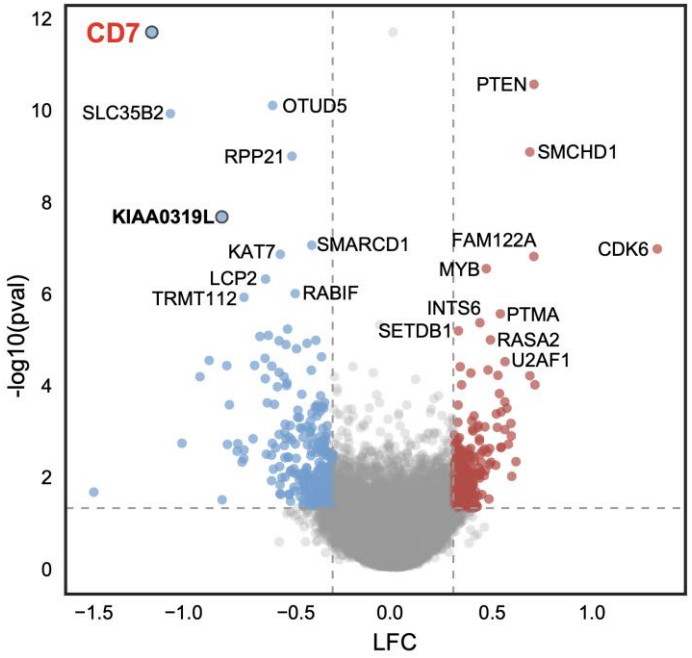
AAV evolution



Human T cell AAV



Receptor identification

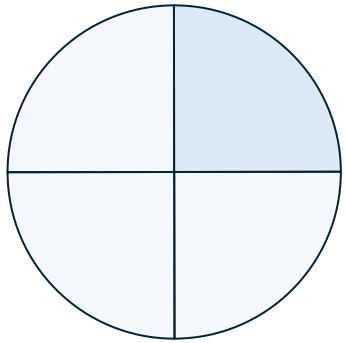


Nyberg et al., *Nature* (2026)

Challenges for generating *TRAC*-CAR-T cells *in vivo*

1. DNA template delivery for homology directed repair

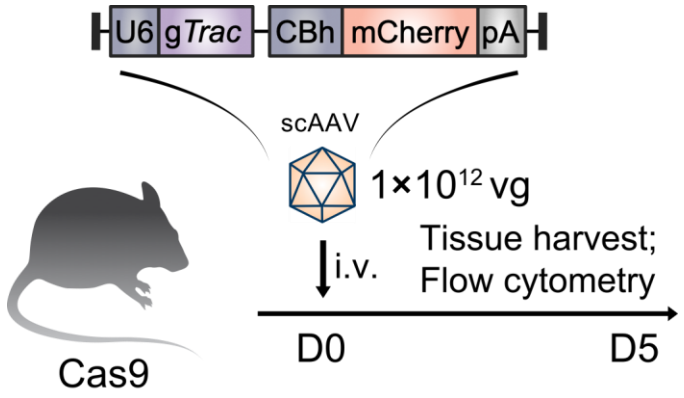
2. Gene editing of T cells *in vivo*

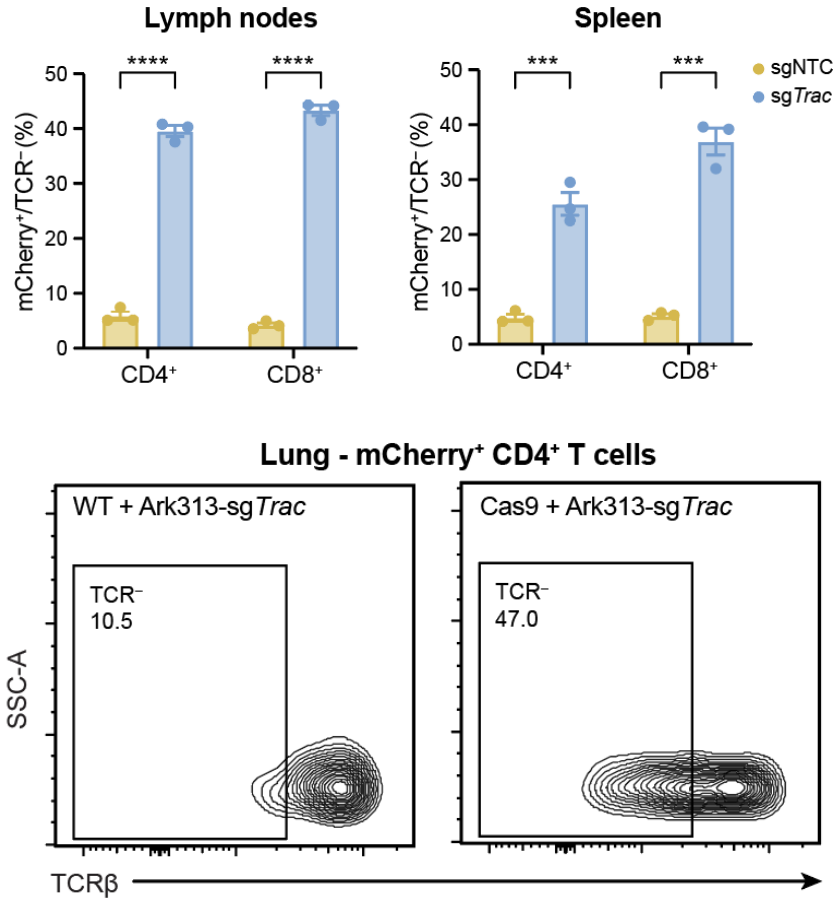


4. Sufficient CAR-T cell expansion for cancer therapies

3. CRISPR/Cas9 delivery to human T cells *in vivo*

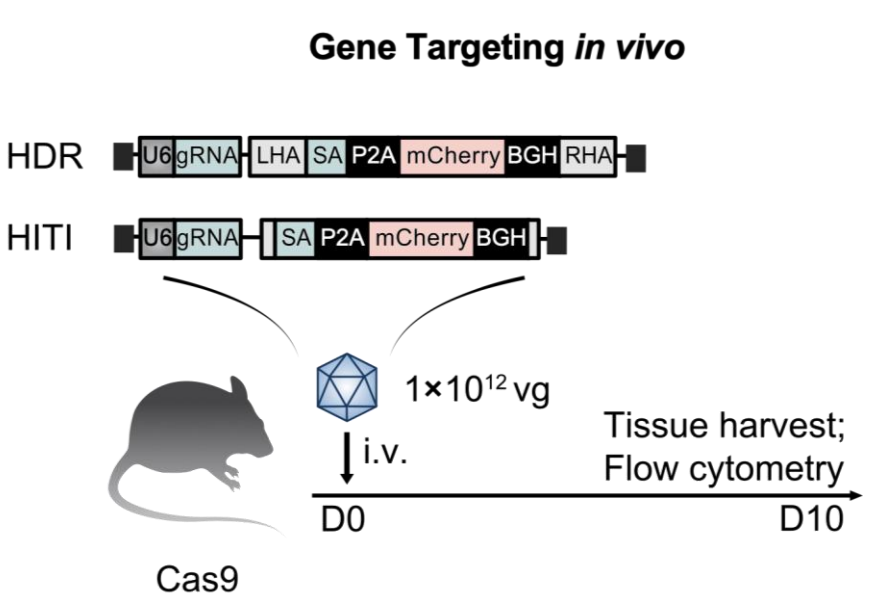
Gene editing of T cells *in vivo*

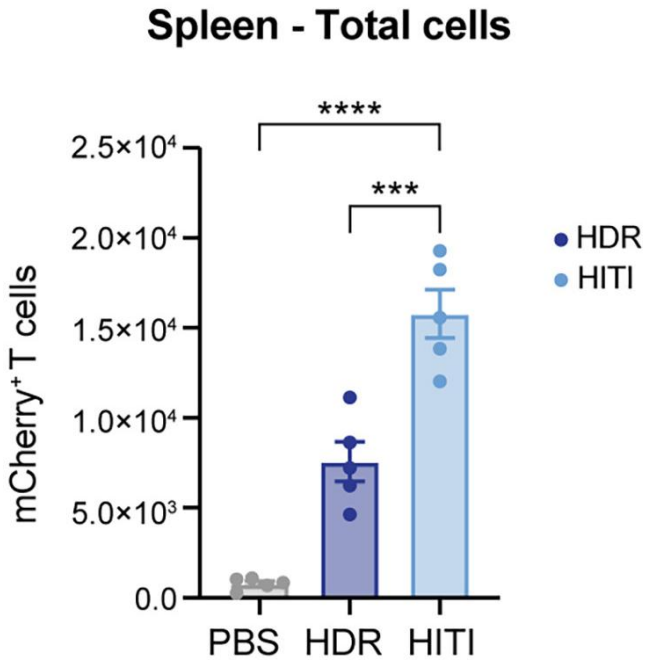




Nyberg et al., *Immunity* (2025)

Targeting *Trac* for site-specific integrations *in vivo*





1 x 10^4 cells in 25 g mouse -> 3 x 10^7 cells in a 80 kg human

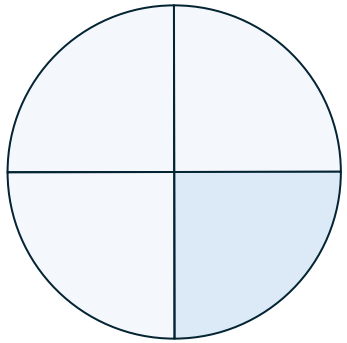
Enough for cell therapy?

Nyberg et al., *Immunity* (2025)

Challenges for generating *TRAC*-CAR-T cells *in vivo*

1. DNA template delivery for homology directed repair

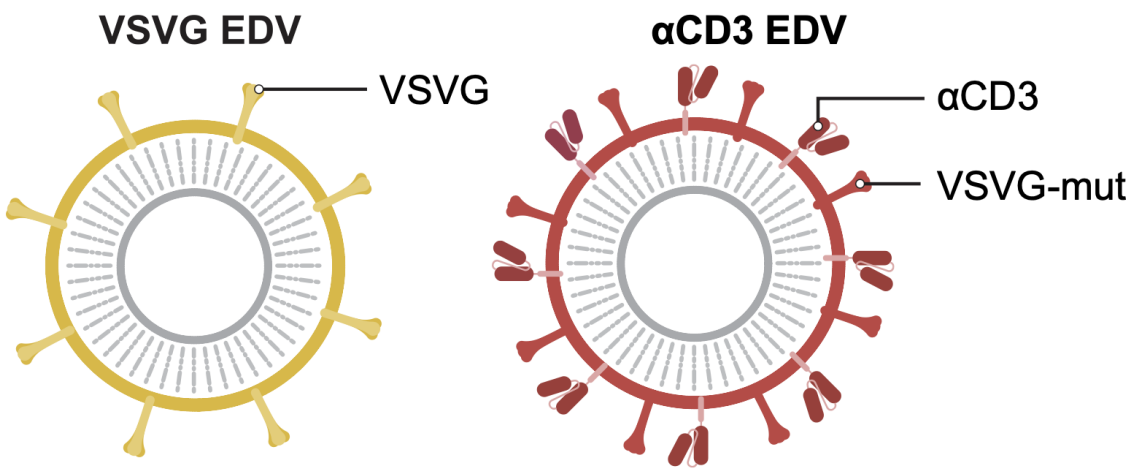
2. Gene editing of T cells *in vivo*

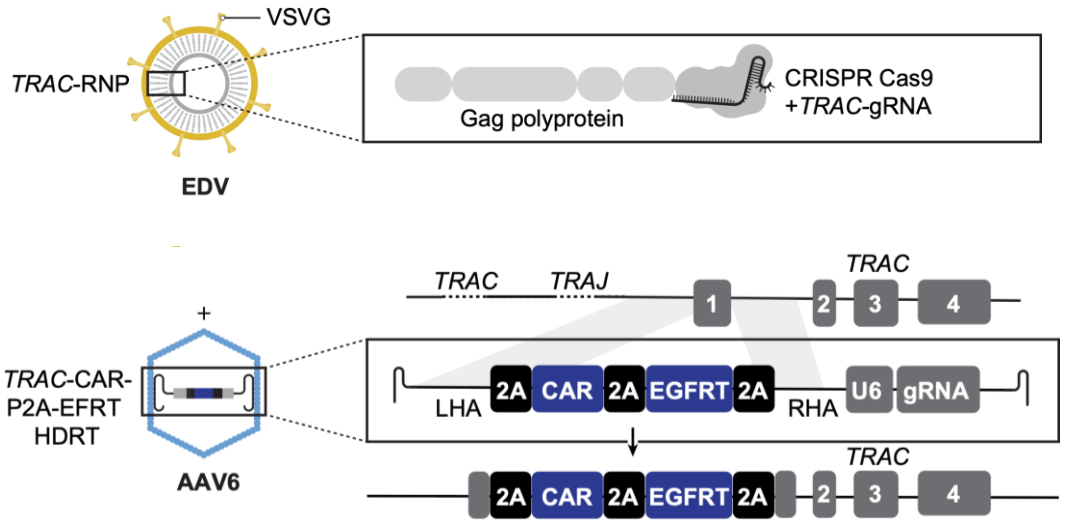


4. Sufficient CAR-T cell expansion for cancer therapies

3. CRISPR/Cas9 delivery to human T cells *in vivo*

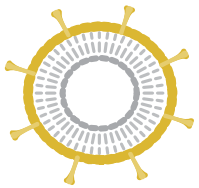
Enveloped **D**elivery **V**ehicles for Cas9 delivery *in vivo*

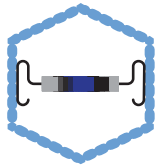


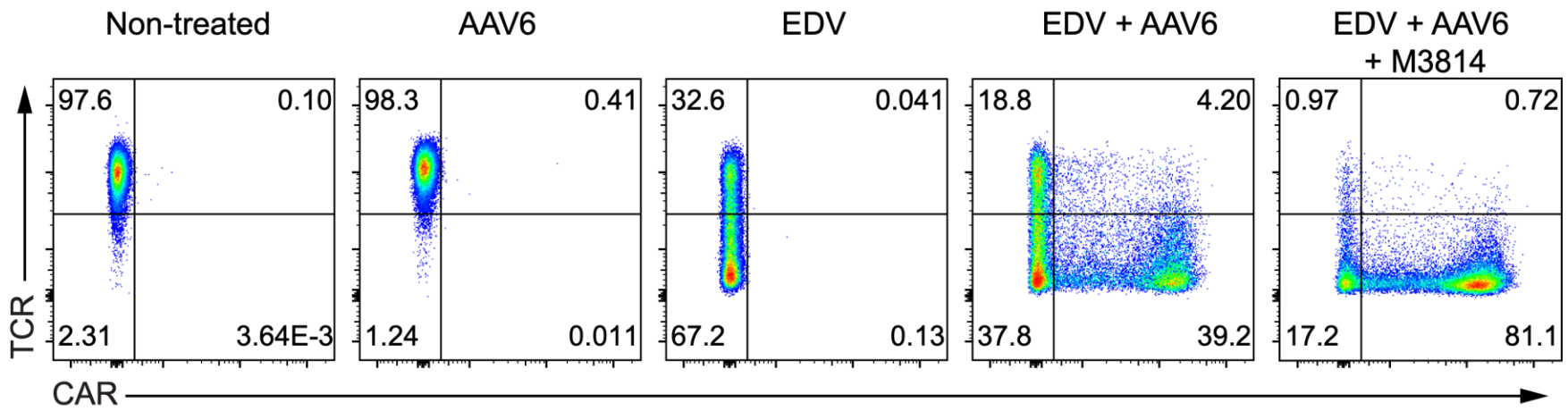


In collaboration with the Doudna Lab (UC Berkeley):
Doudna, Hamilton and Ngo

EDV and AAV gene editing efficiency across cell types

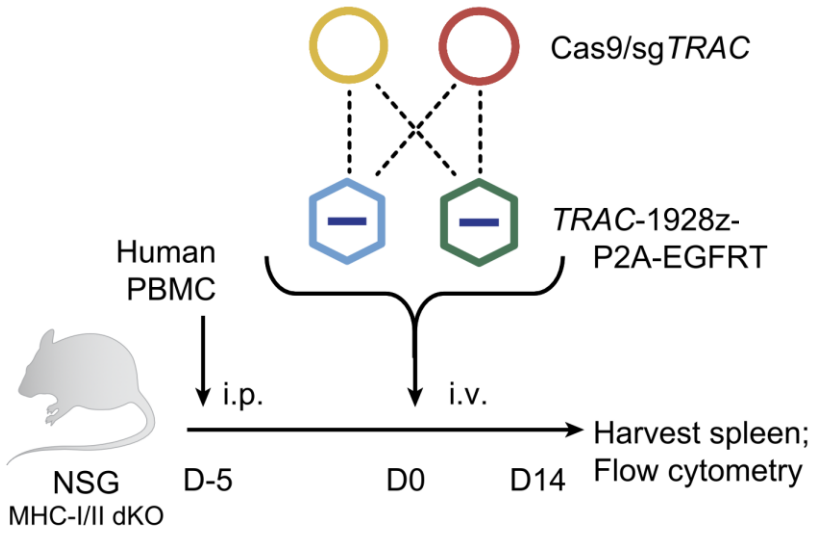


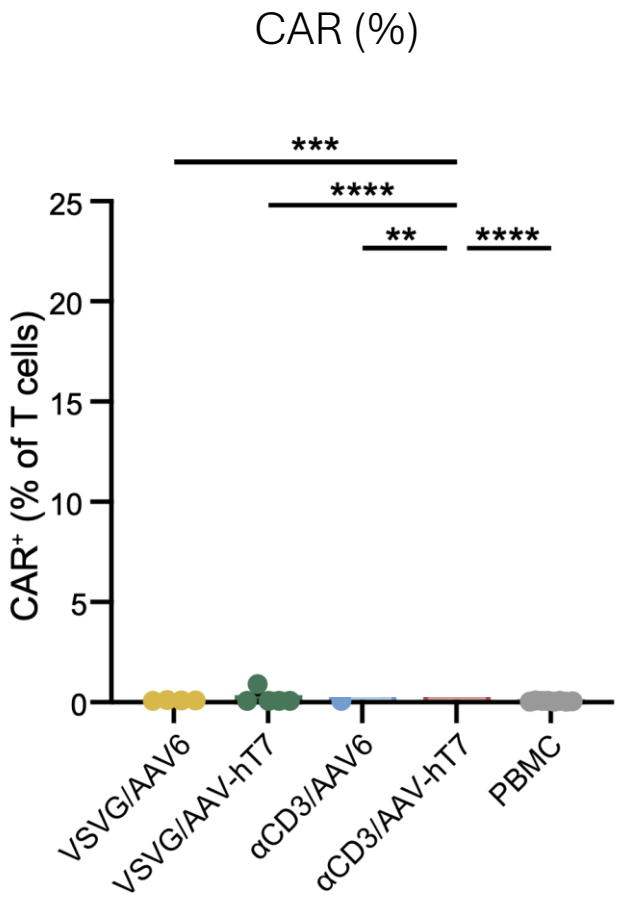


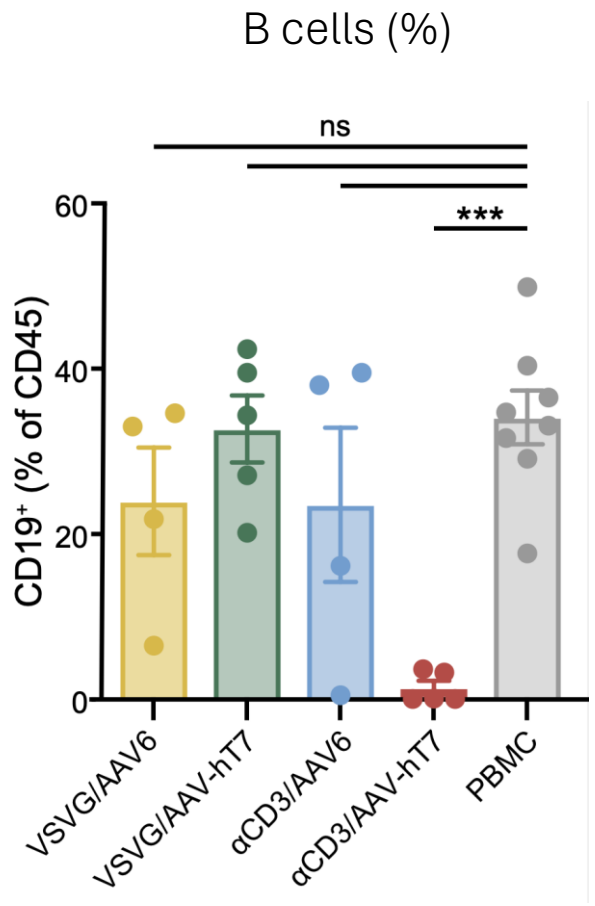


Nyberg et al., *Nature* (2026)

In vivo generated TRAC-CAR-T cells produce B cell aplasia





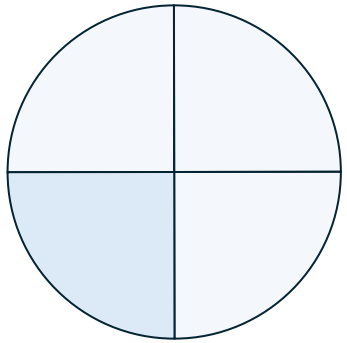


Nyberg et al., *Nature* (2026)

Challenges for generating *TRAC*-CAR-T cells *in vivo*

1. DNA template delivery for homology directed repair

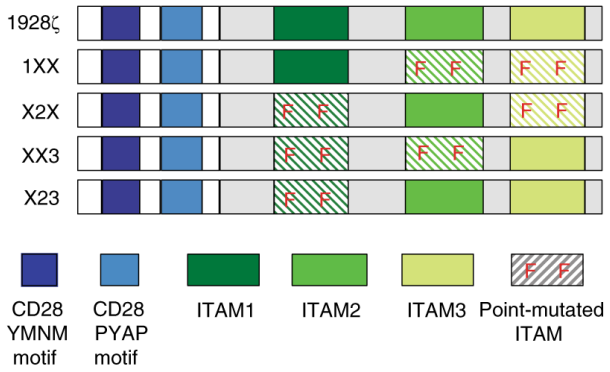
2. Gene editing of T cells *in vivo*

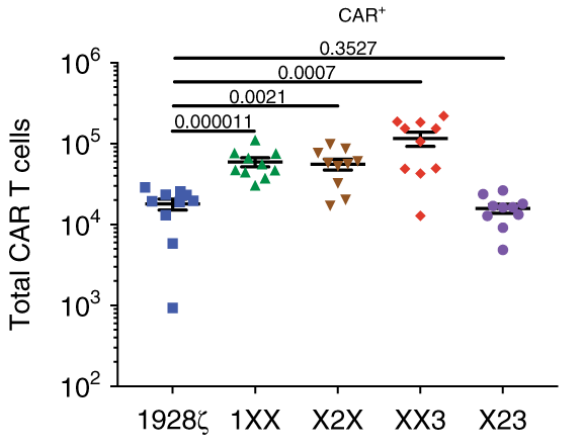


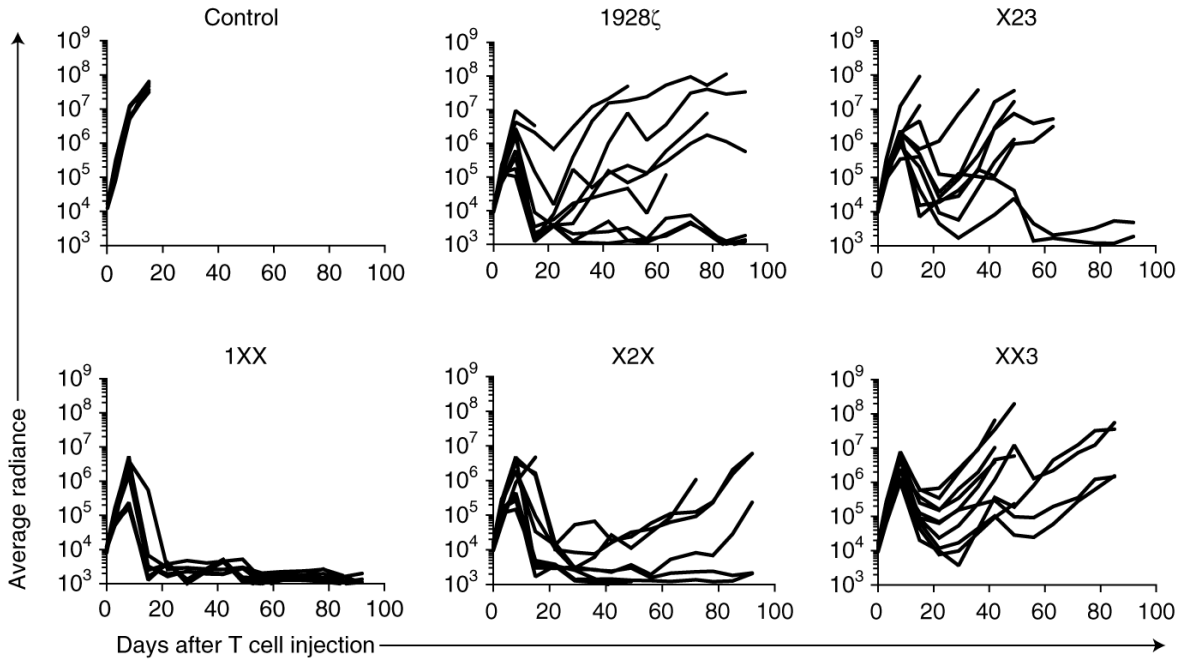
4. Sufficient CAR-T cell expansion for cancer therapies

3. CRISPR/Cas9 delivery to human T cells *in vivo*

Calibrated signaling domains improve function and expansion



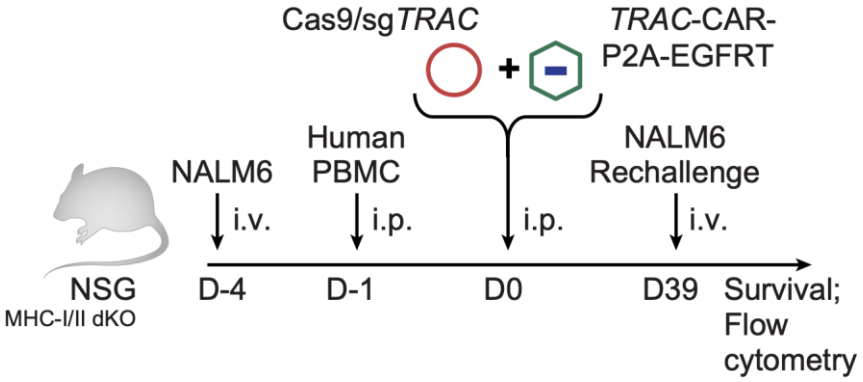


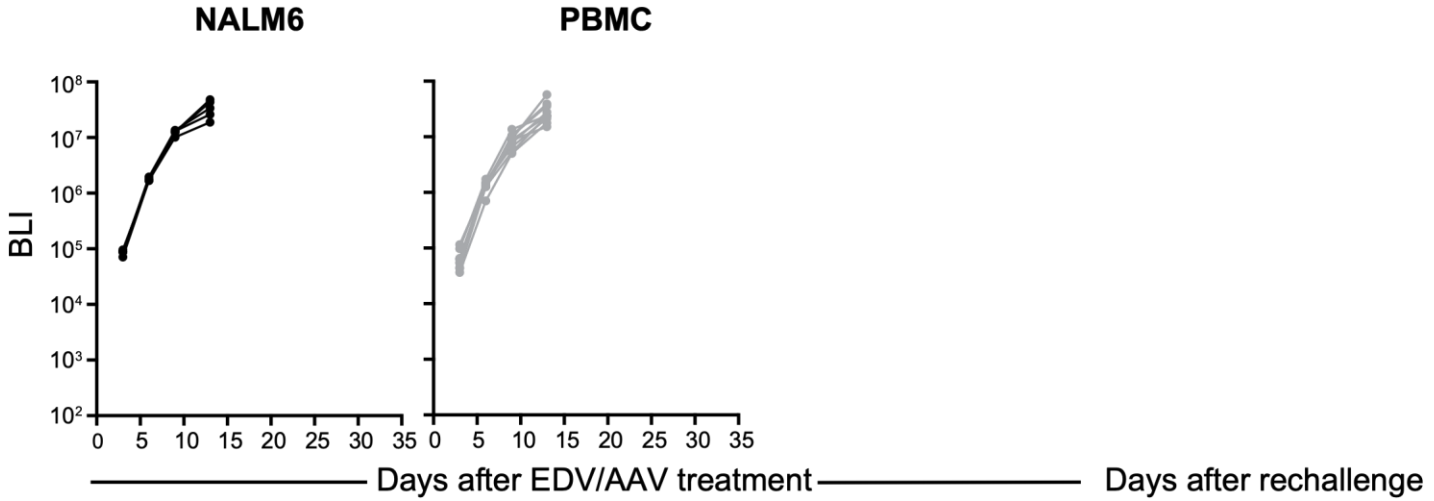


TRAC-1928z-1XX have cured leukemia patients with 1 x 10^7 CAR-T cells

Feucht et al., *Nature Medicine* (2019)

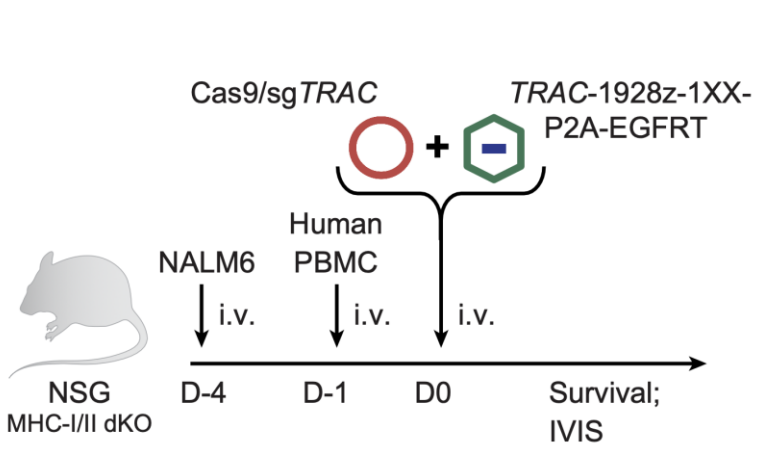
In vivo generated *TRAC*-CAR-T cells in treatment of B-ALL

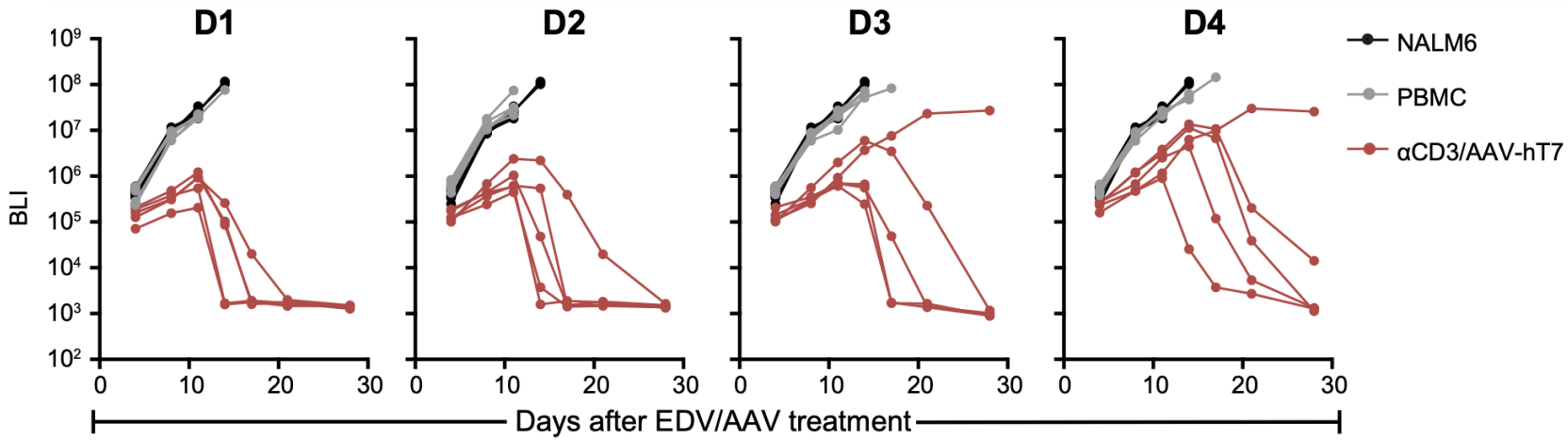




Nyberg et al., *Nature* (2026)

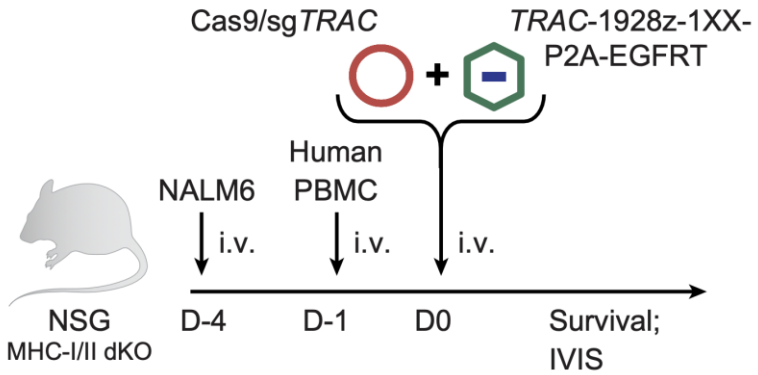
In vivo generated *TRAC*-CAR-T cells in treatment of B-ALL

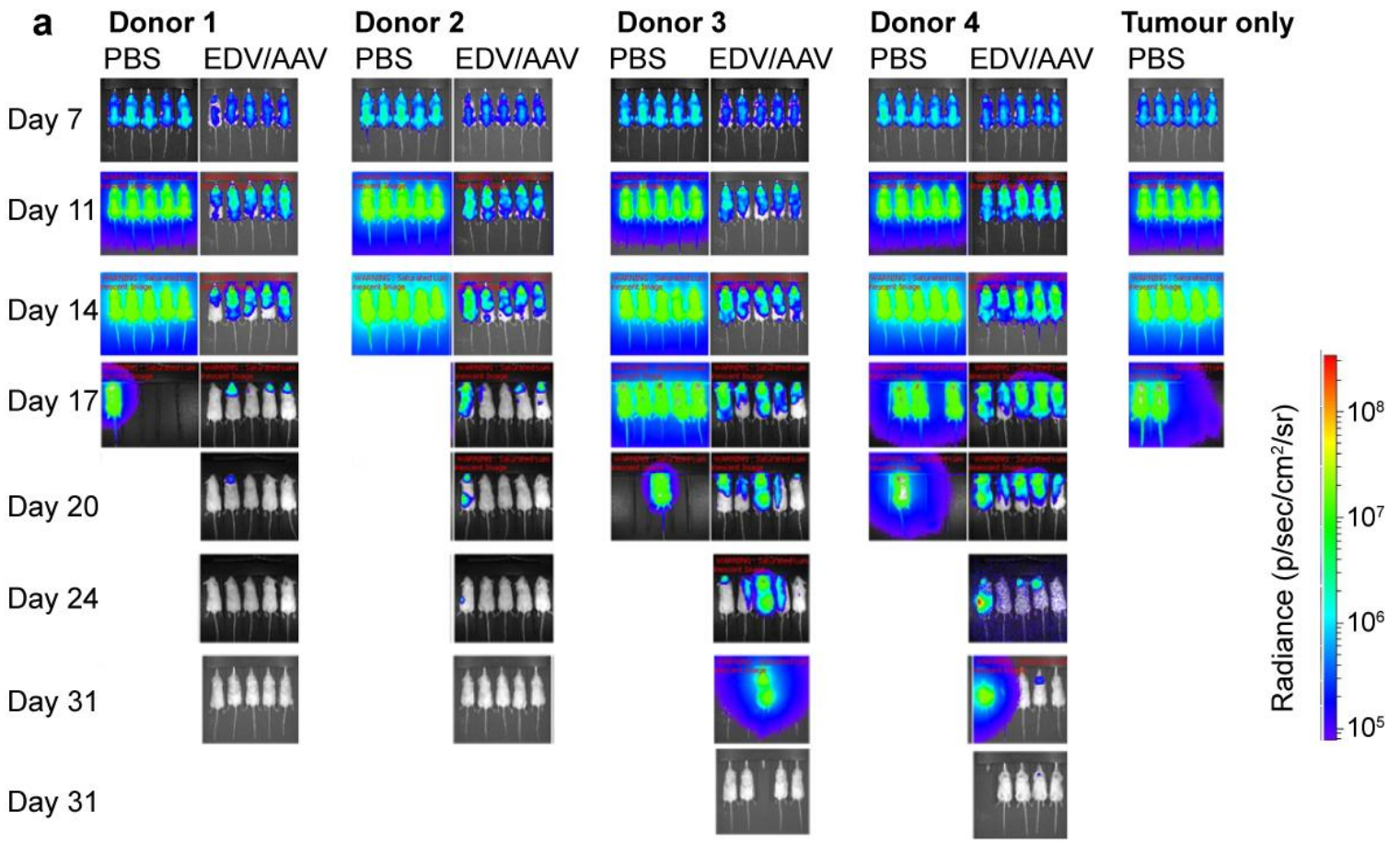




Nyberg et al., *Nature* (2026)

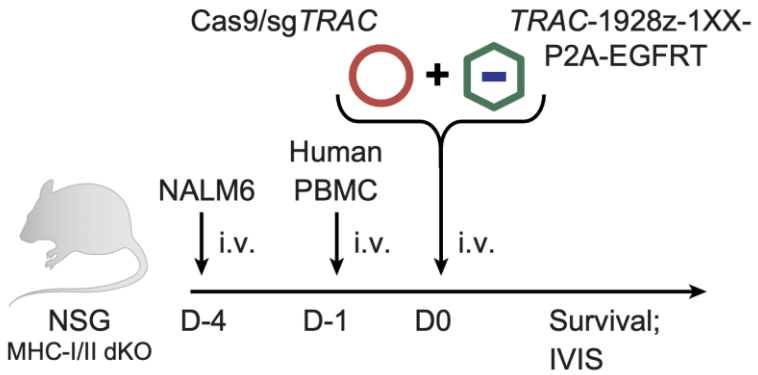
In vivo generated *TRAC*-CAR-T cells in treatment of B-ALL

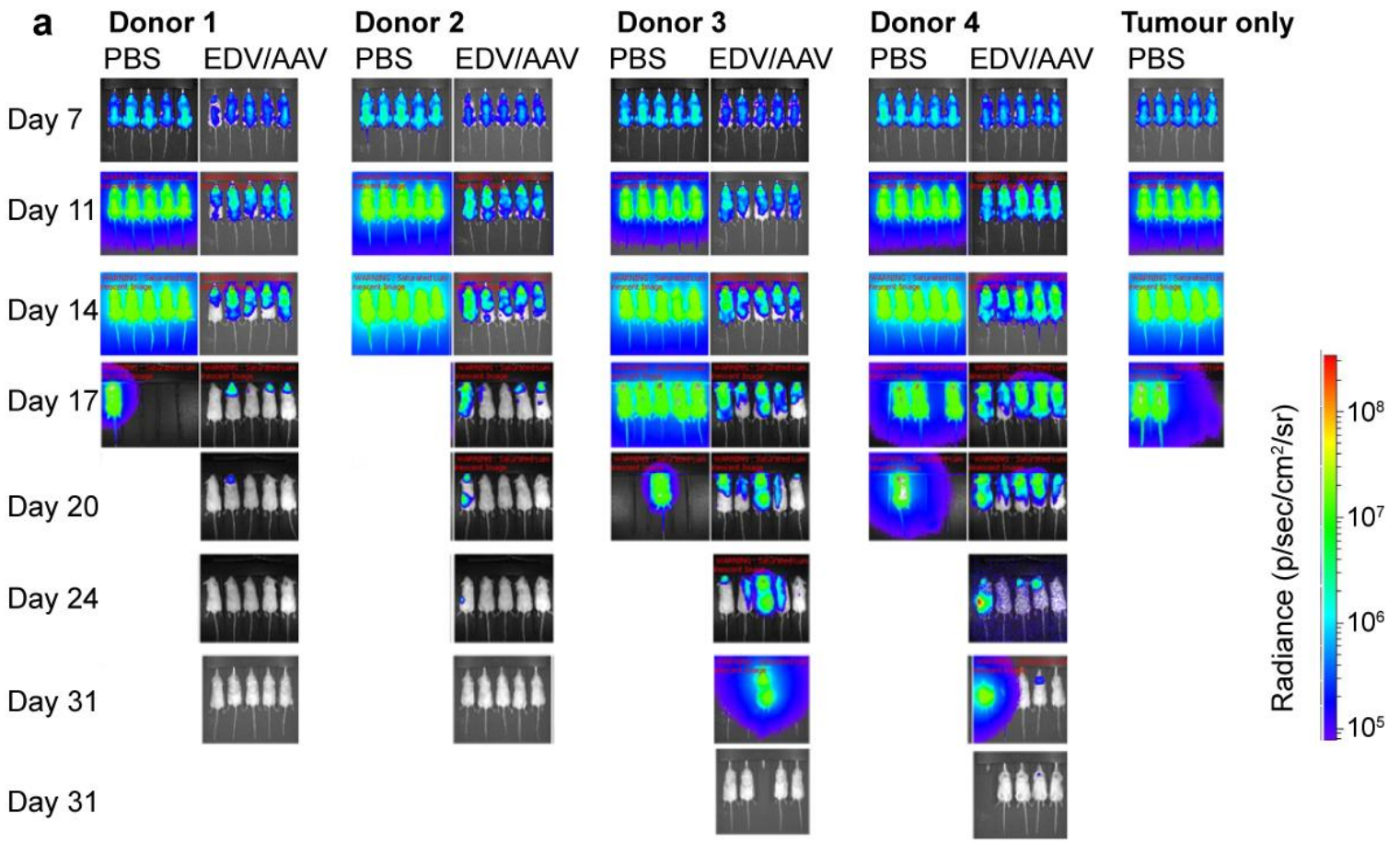




Nyberg et al., *Nature* (2026)

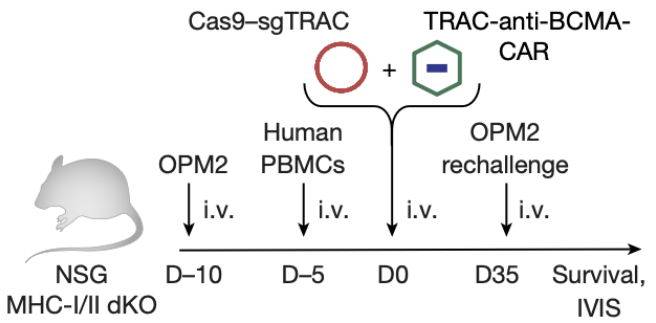
In vivo generated *TRAC*-CAR-T cells in treatment of B-ALL

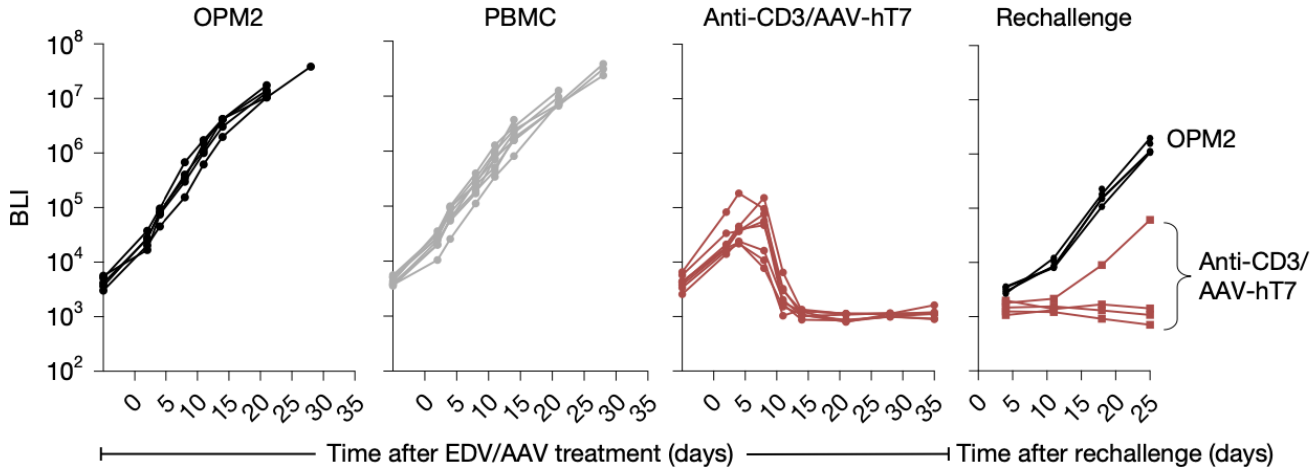


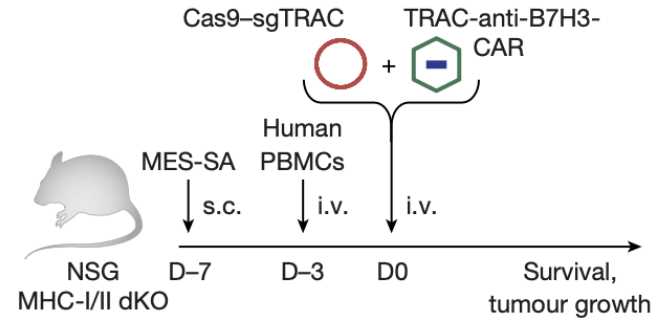


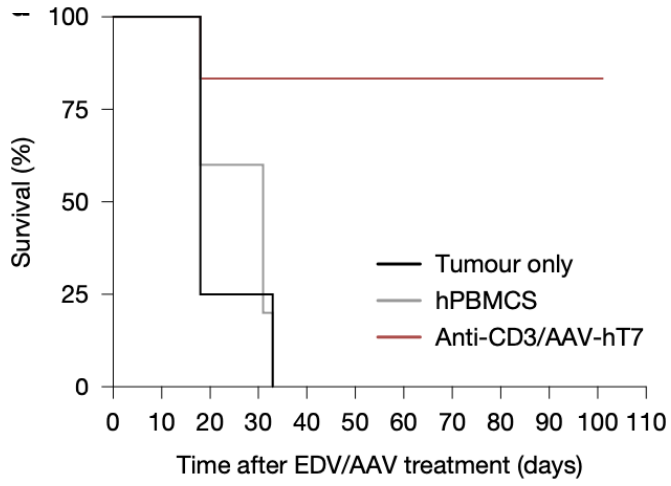
Nyberg et al., *Nature* (2026)

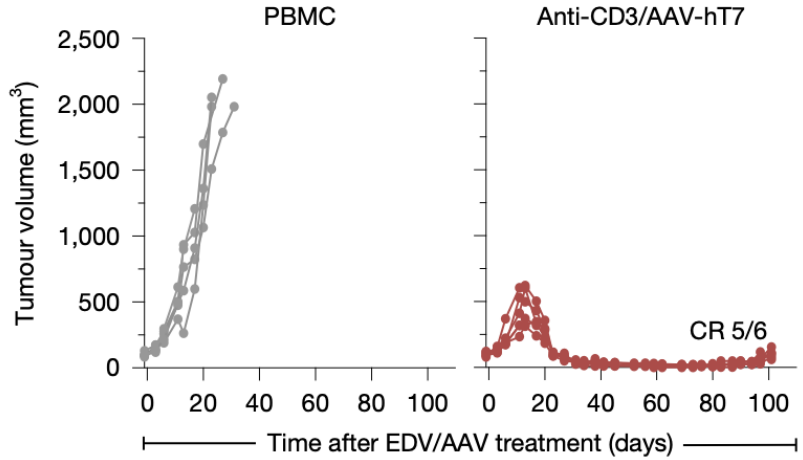
In vivo generated *TRAC*-CAR-T cells in treatment of multiple myeloma





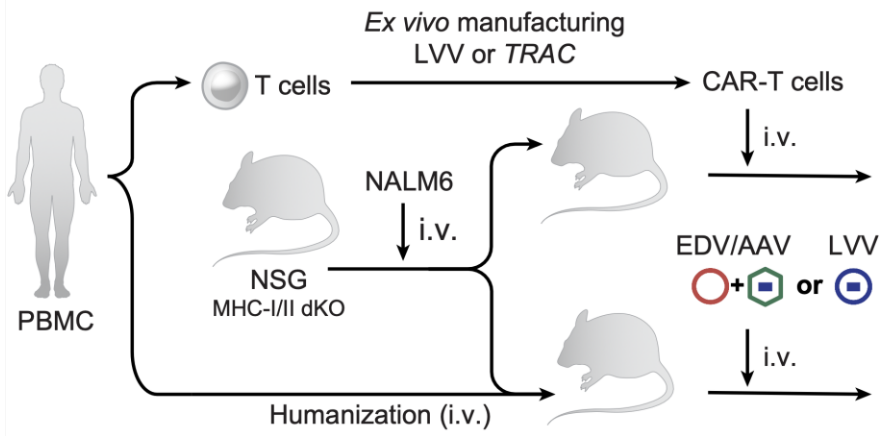


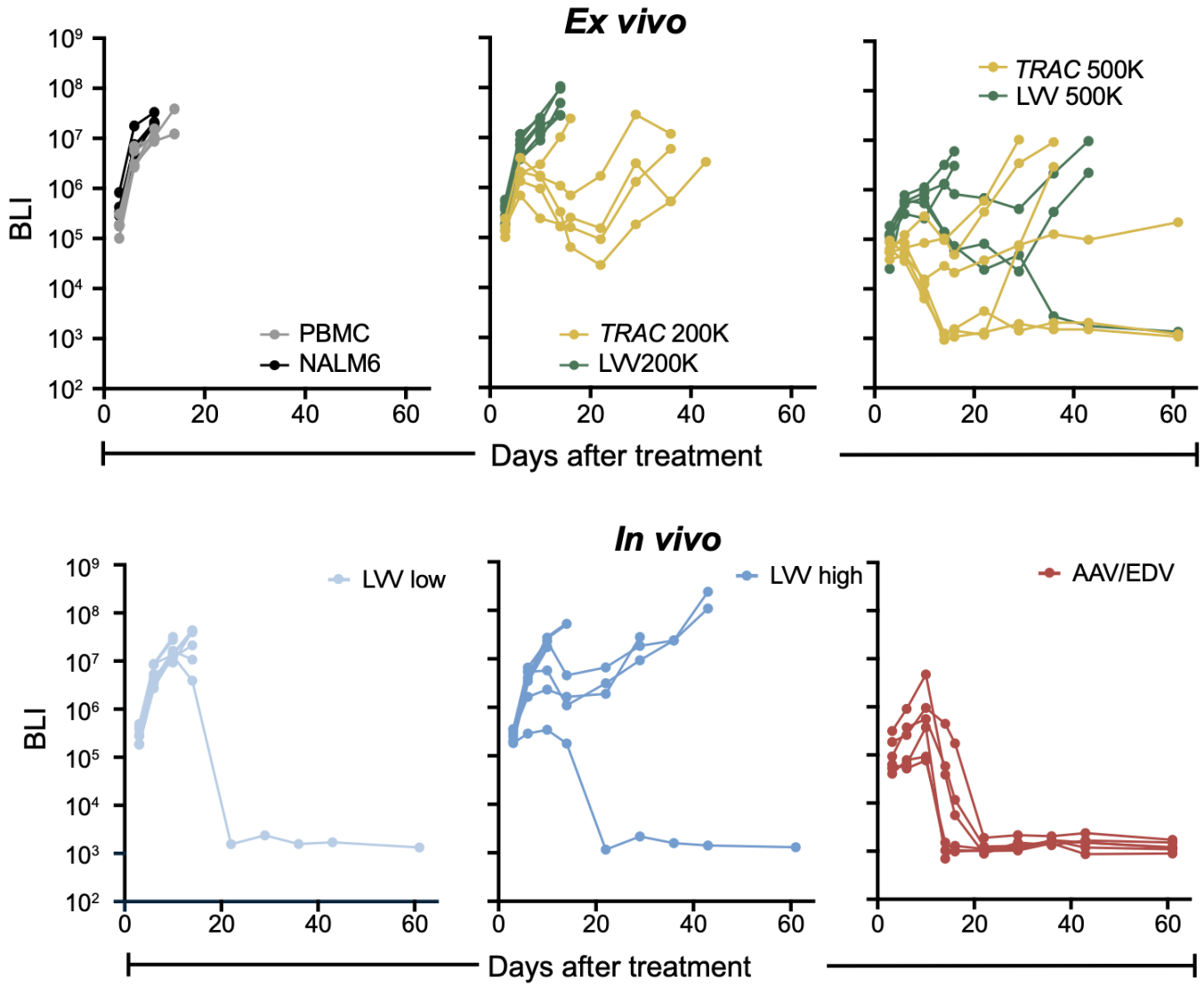




Nyberg et al., *Nature* (2026)

Comparing *ex vivo* manufactured cell products with *in vivo* generated CAR-T cell

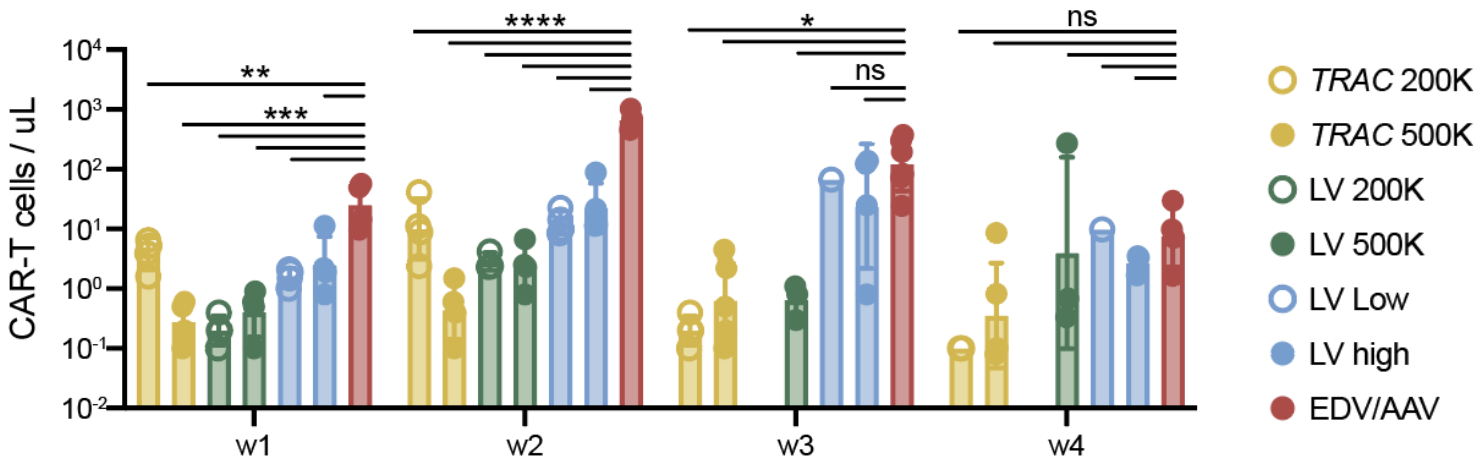




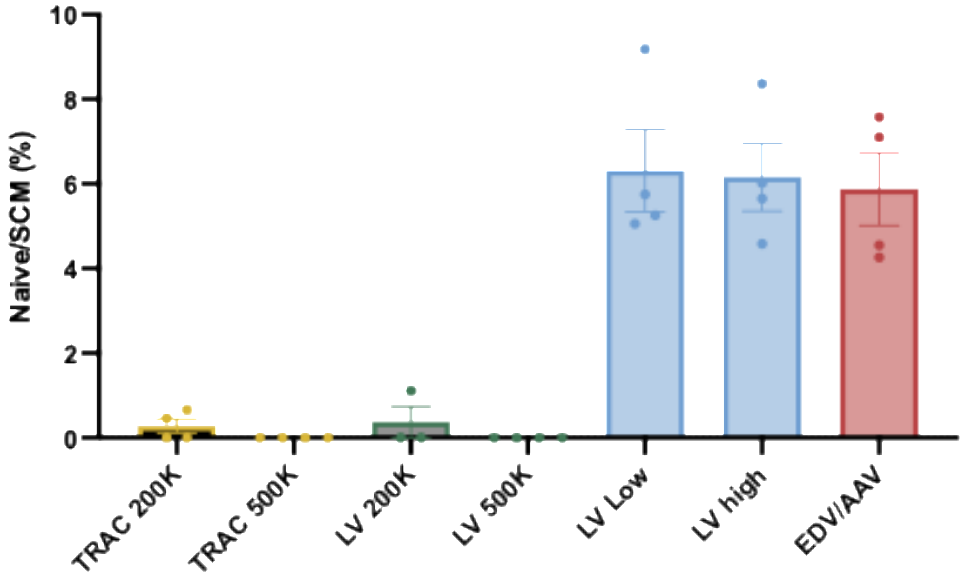
Nyberg et al., *Nature* (2026)

In vivo targeting produce more proliferative SCM CAR-T cells

CAR-T cells numbers



Naïve/SCM



Nyberg et al., *Nature* (2026)

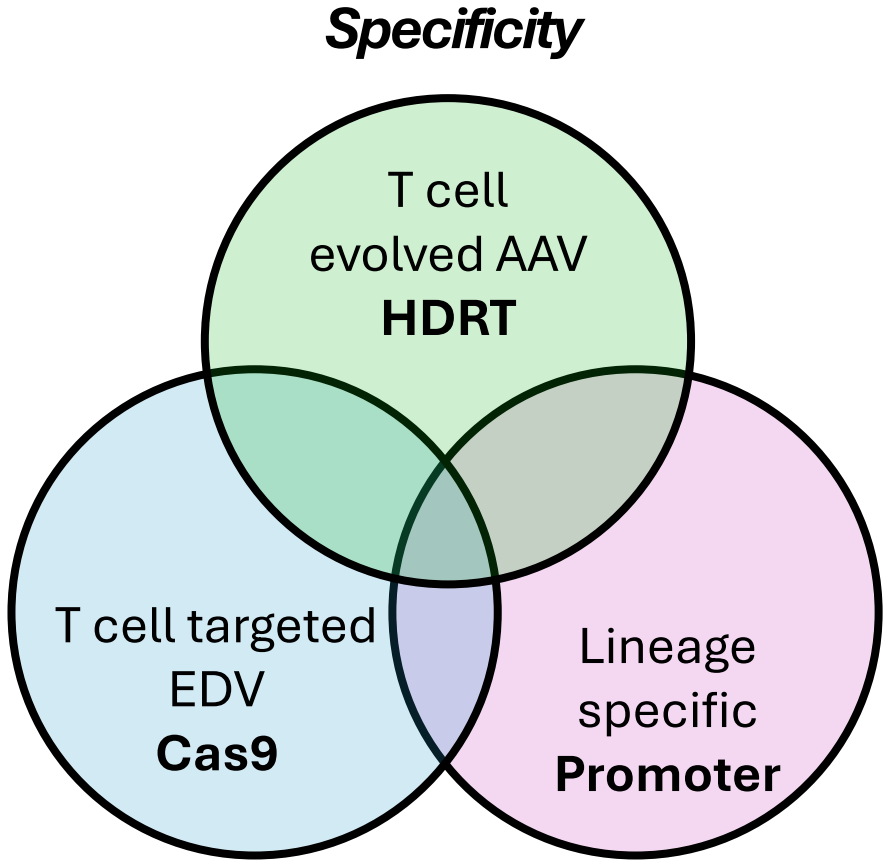
Conclusions

Targeting T cells *in vivo* with evolved AAVs

- Evolved AAVs offer T cell specific targeting of both mouse and human T cells
- AAVs allow for gene engineering of T cells in vivo

TRAC*-CAR-T cell generation *in vivo

- Combining AAV for HDRT delivery with Cas9-delivery allows for *in vivo* site-specific integrations
- *In vivo TRAC*-CAR-T cells are functional against hematological and solid tumor cancers
- *In vivo*-generated CAR-T cells produce more highly proliferative SCM T cells



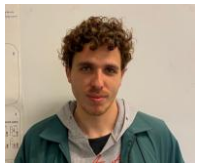
Acknowledgments

UCSF University of California San Francisco

GLADSTONE INSTITUTES

Justin Eyquem, Ph.D.

Charlotte Wang



Pierre-Louis Bernard, Ph.D.

GLADSTONE INSTITUTES

UCB/Gladstone:
Jennifer Doudna
Wayne Ngo
Jennifer Hamilton, Azalea Tx

KAROLINSKA INSTITUTET ANNO 1810 Karolinska Institutet

Nyberg lab (KI)

Julian Fischbach, M.Sc.
Nikolaos Kyriakidis, Ph.D.
Alexandra Helleux, Ph.D.
Tom Pieper, Ph.D.
Matthias Huyghe, Ph.D.
Catarina Graca, Ph.D.

SciLifeLab

Funding:

Swedish Research Council

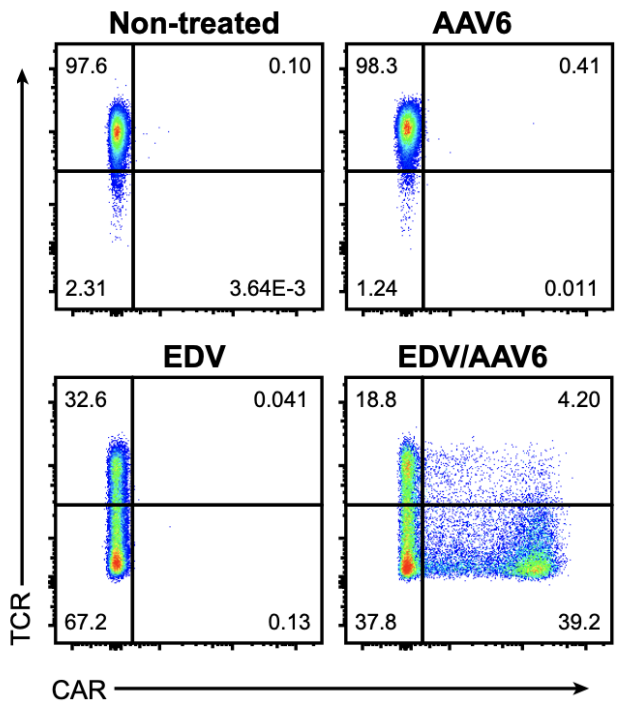
SVENSKA SÄLLSKAPET FÖR MEDICINSK FORSKNING
GRUNDAT 1919

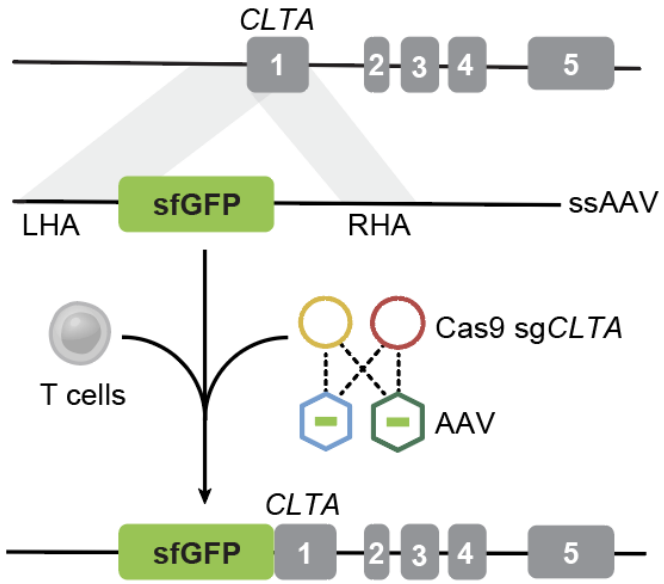
Funded by the European Union

erc European Research Council
Established by the European Commission

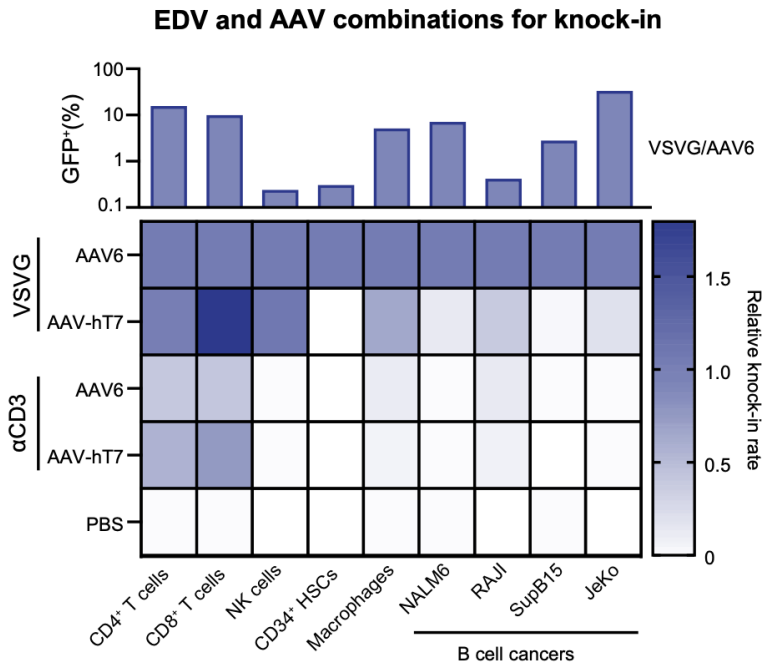
EDV and AAV gene editing efficiency across cell types

T cells in vitro



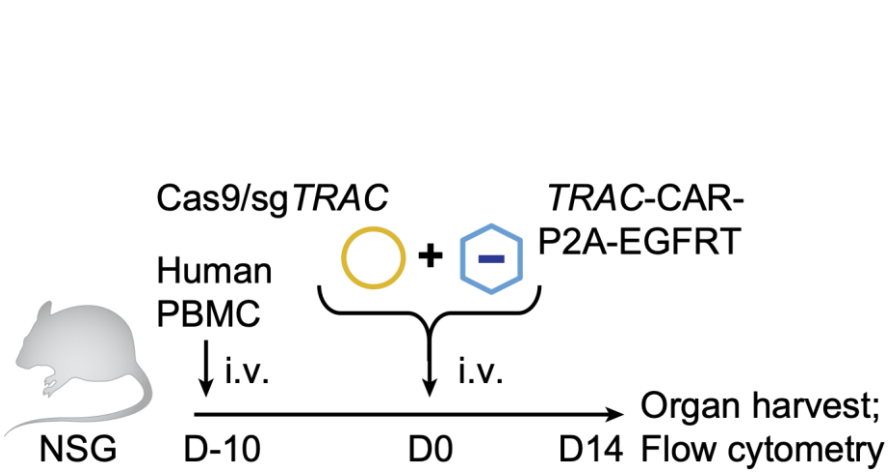


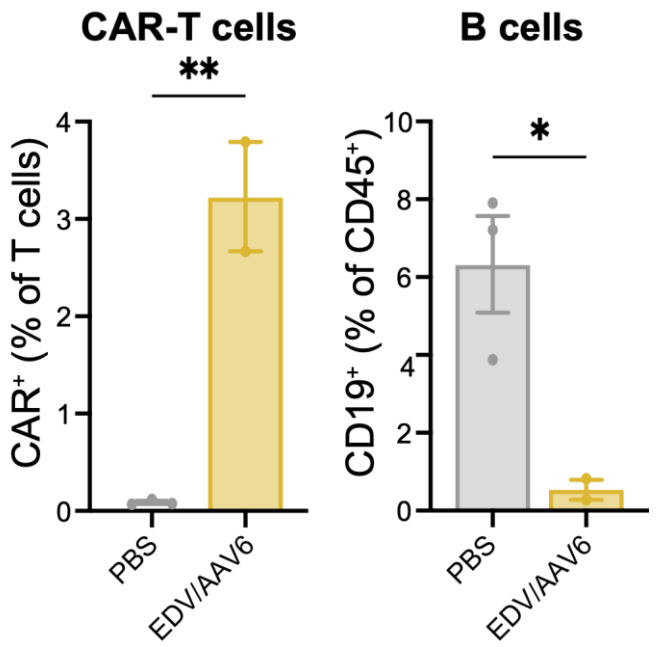
Immune cell panel in vitro

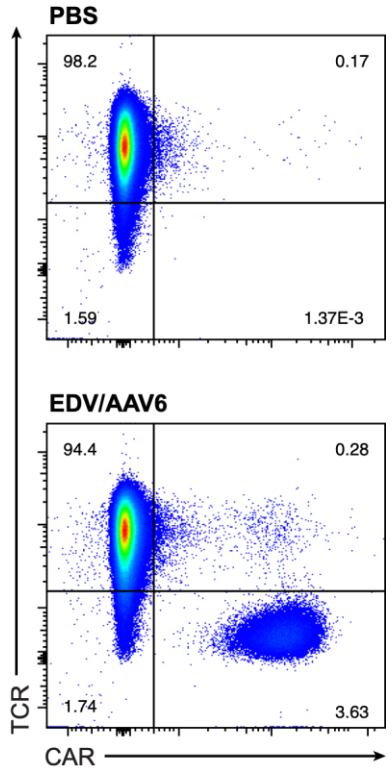


Nyberg et al., *manuscript in revision* (2025)

In vivo generated *TRAC*-CAR-T cells induce B cell aplasia

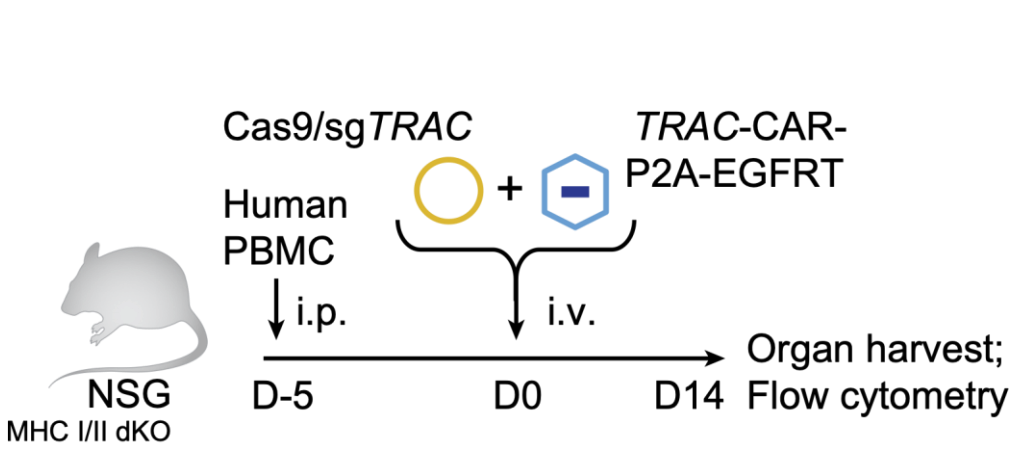


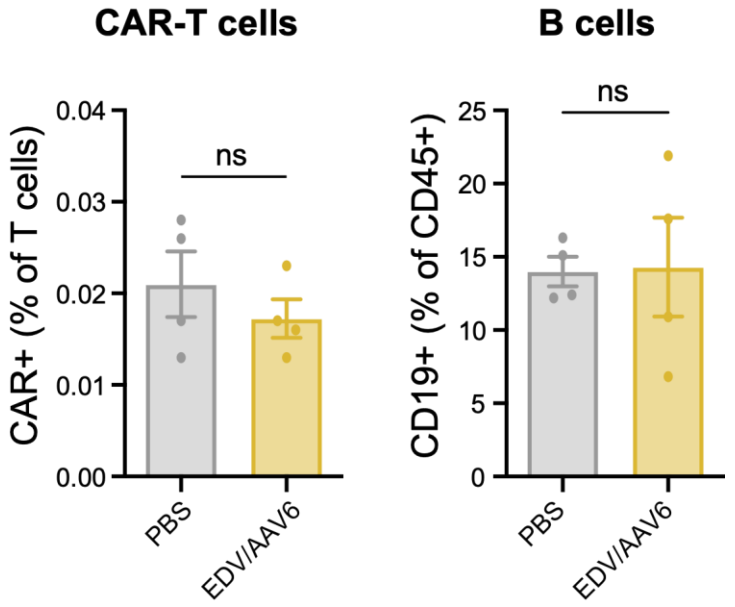




Nyberg et al., *manuscript in revision* (2025)

No TRAC-CAR-T cells without GvHD





Nyberg et al., *manuscript in revision* (2025)