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# Development of an Off-The-Shelf CAR-T Cell Therapy for T-ALL Without Gene Editing

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# Allogeneic anti-CD7 CAR-T Therapy for T-ALL

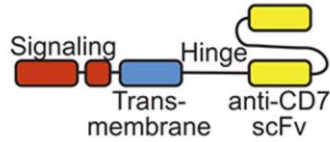
- Encouraging results are being obtained with autologous anti-CD7 CAR-T cells in T-ALL
- We generated an effective autologous anti-CD7 CAR T cell product<sup>1</sup>
  - To eliminate fratricide, CD7 expression on CAR-T cells was downregulated using PEBL
- In some patients it is difficult to obtain good quality lymphocytes for manufacturing autologous product
- Allogeneic CAR-T cells would be useful as a bridge to transplant or to autologous CAR-T cells

<sup>1</sup>Png, Y. T. et. al. *Blood Adv.* 2017

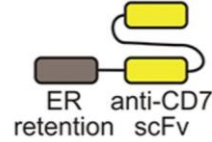


# Anti-CD7 PEBL to prevent fratricide

anti-CD7-41BB-CD3 $\zeta$  CAR

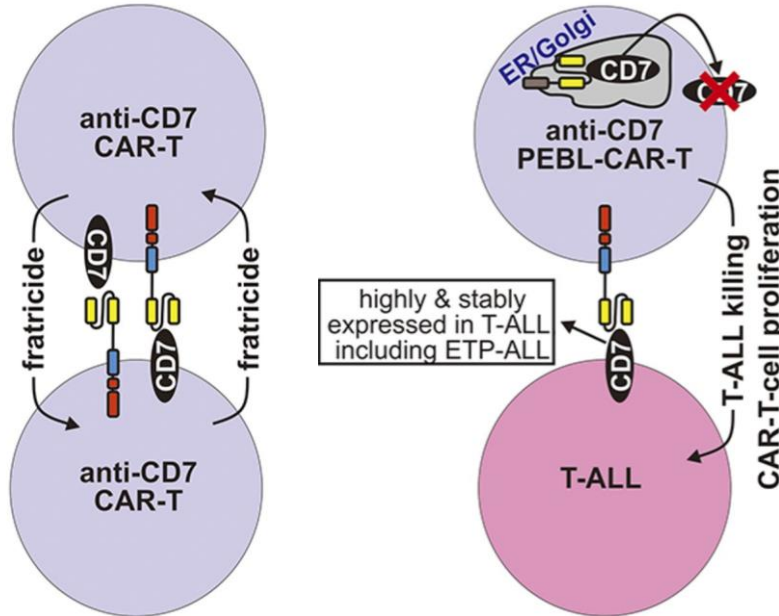


anti-CD7 PEBL



+

= PCART7

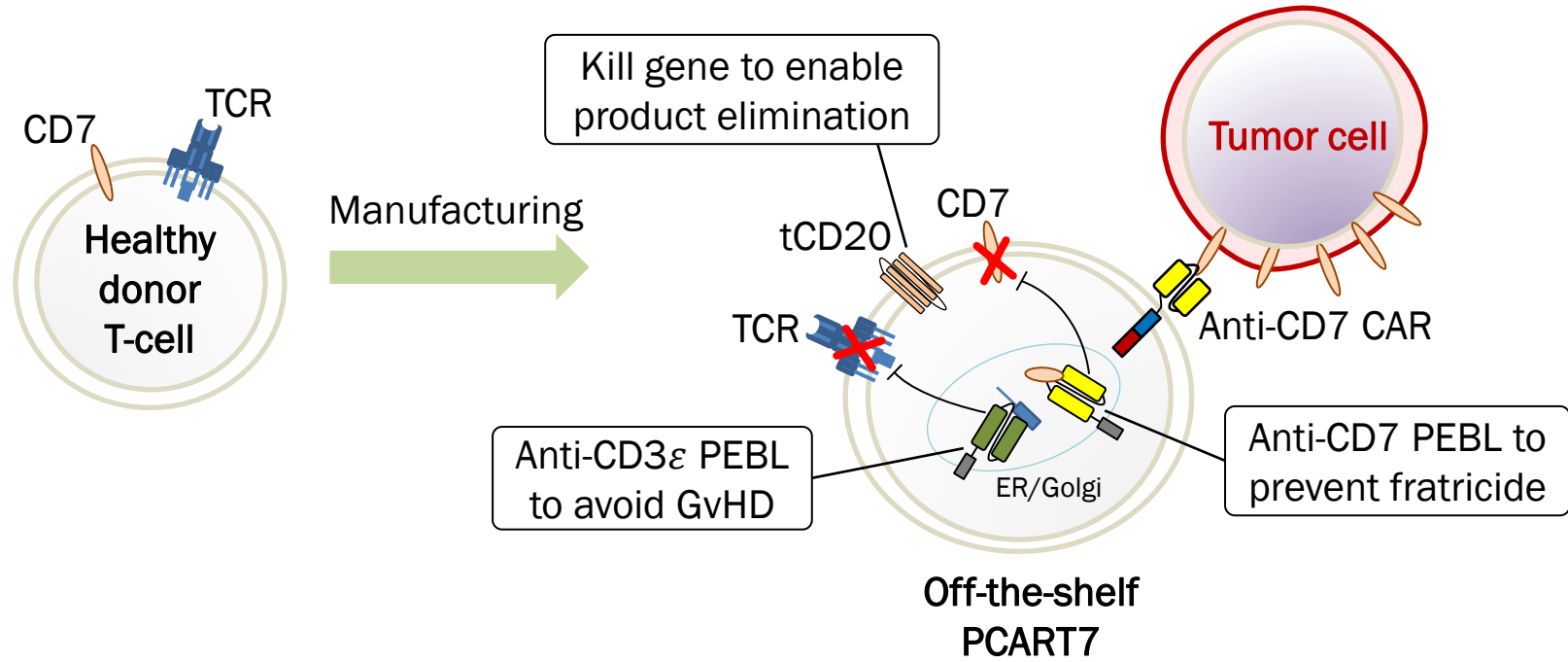


Advantages using PEBL-CAR:

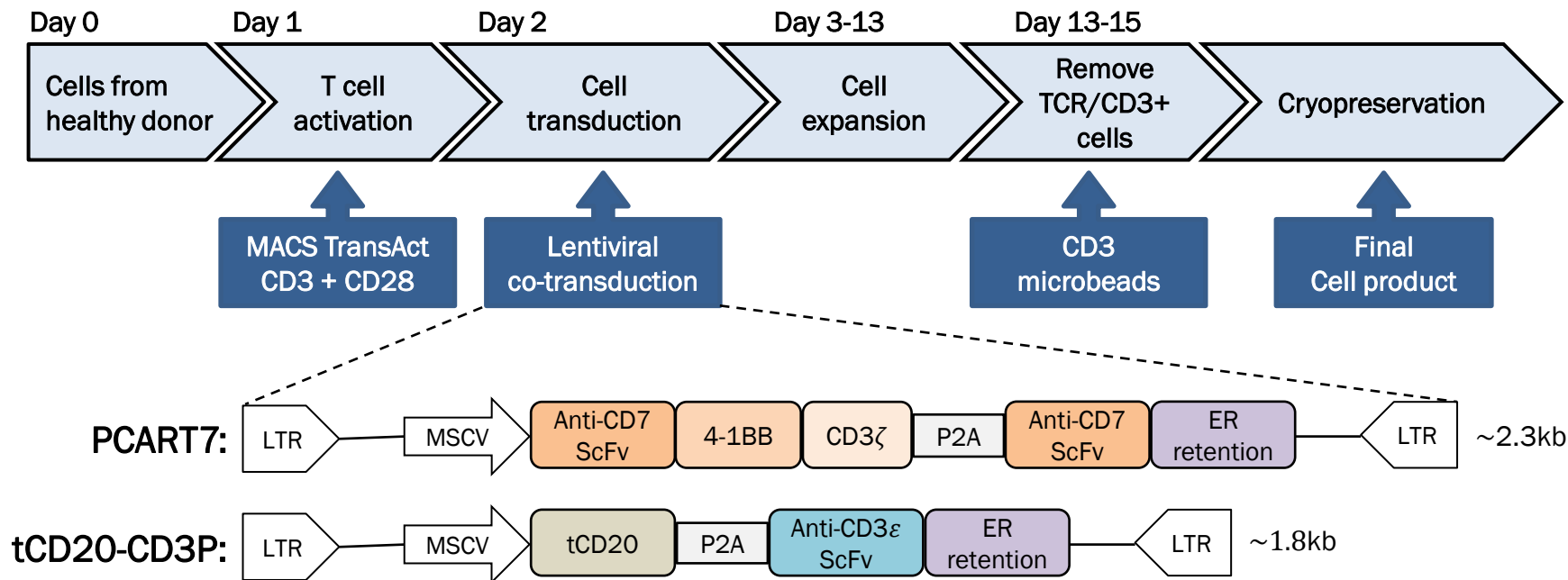
1. Highly efficient in downregulating surface protein
2. Fits seamlessly in current GMP cell manufacturing protocol
3. No risk of non-specific gene editing

<sup>1</sup>Png, Y. T. et. al. *Blood Adv.* 2017

# Generation of anti-CD7 CAR-T cells devoid of fratricide and graft-versus-host disease (GvHD) potential



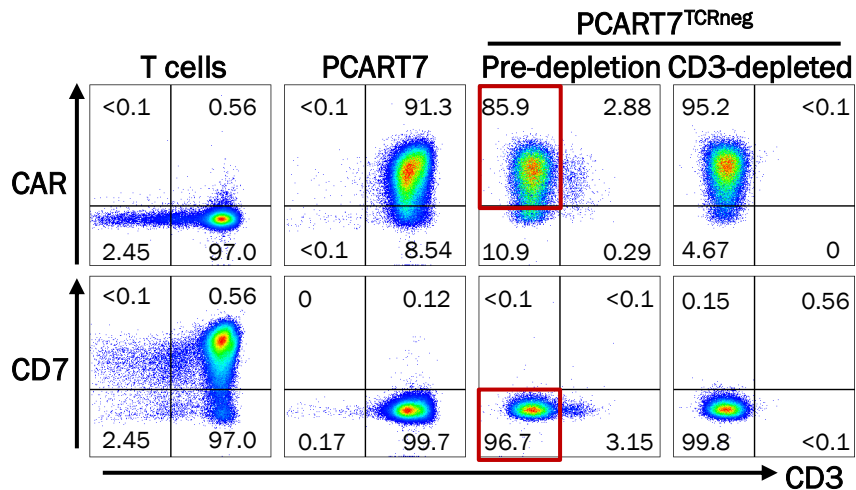
# Off-the-shelf PCART7 manufacturing process



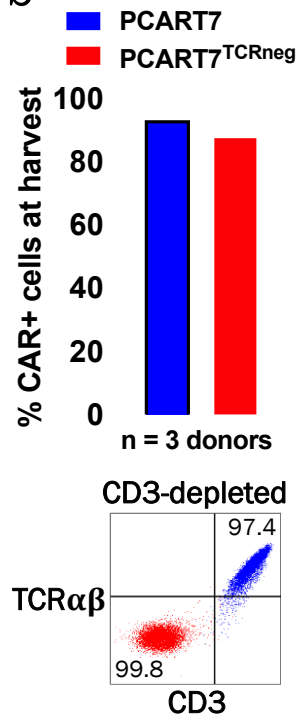
# Phenotype and expansion of PCART7<sup>TCRneg</sup>

a

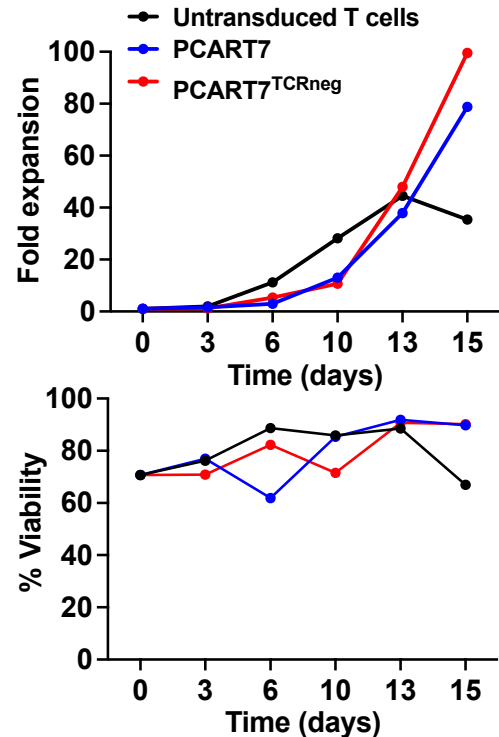
Day 15 post-manufacturing



b

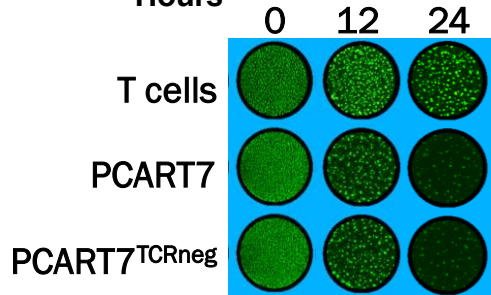
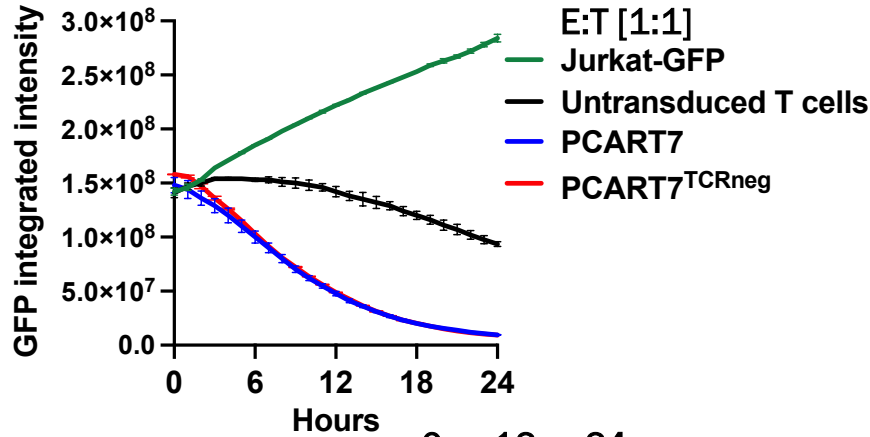


c

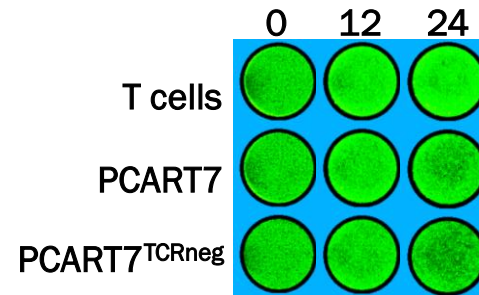
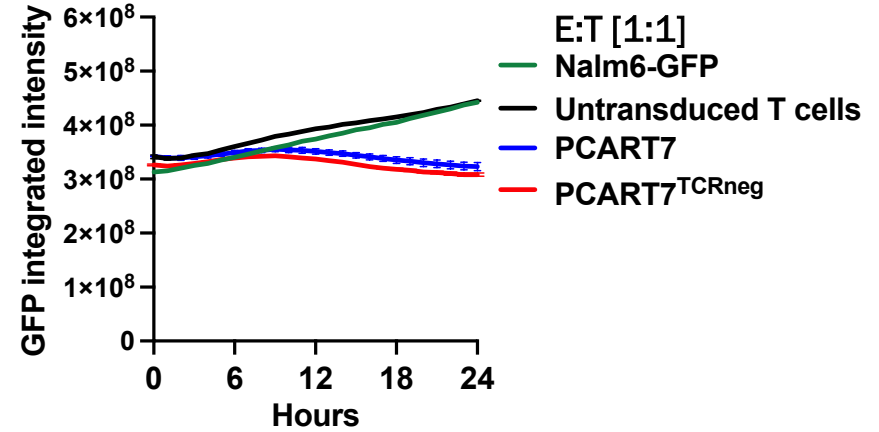


# PCART7<sup>TCRneg</sup> effectively and specifically kill T-ALL cells

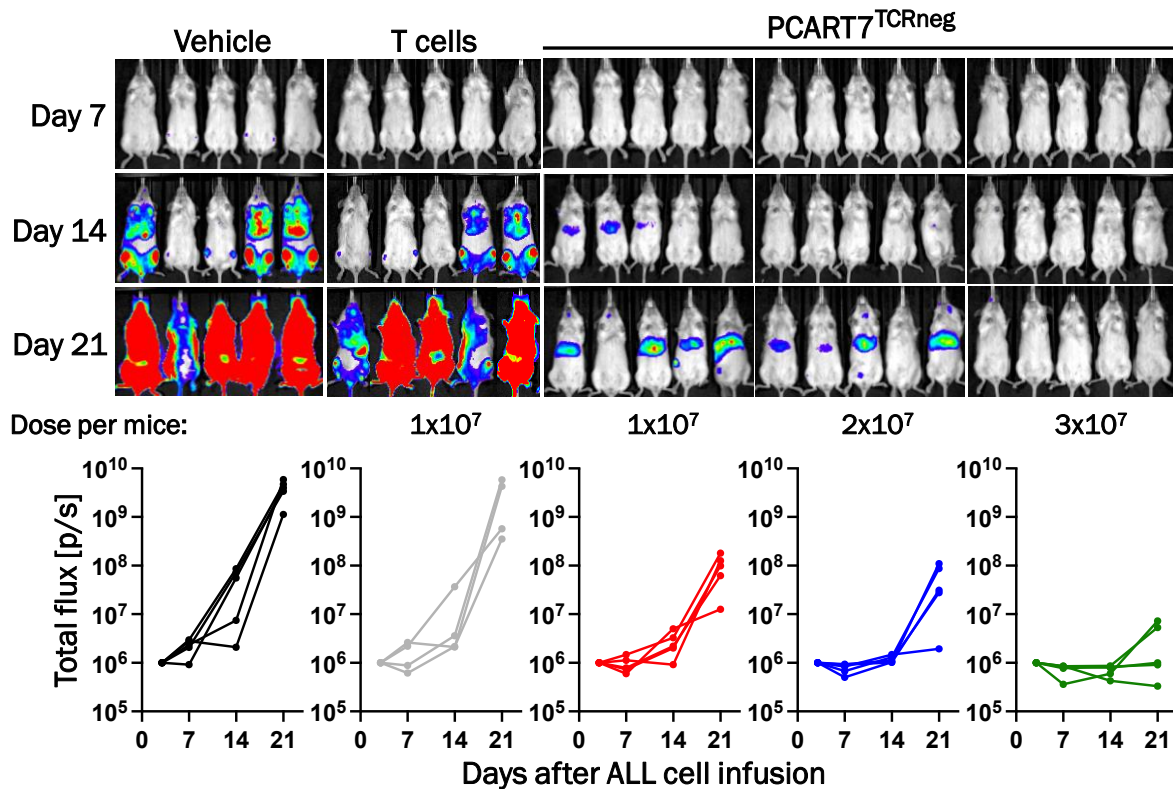
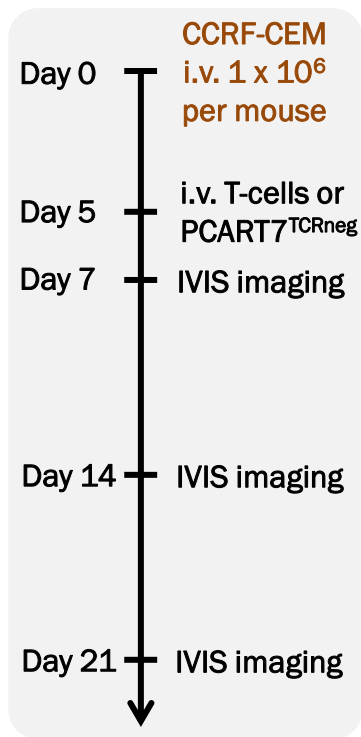
## CD7+ Jurkat cells



## CD7- Nalm6 cells



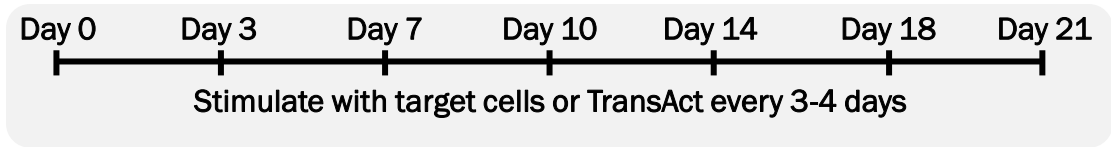
# PCART7<sup>TCRneg</sup> effectively kill T-ALL cells in mice



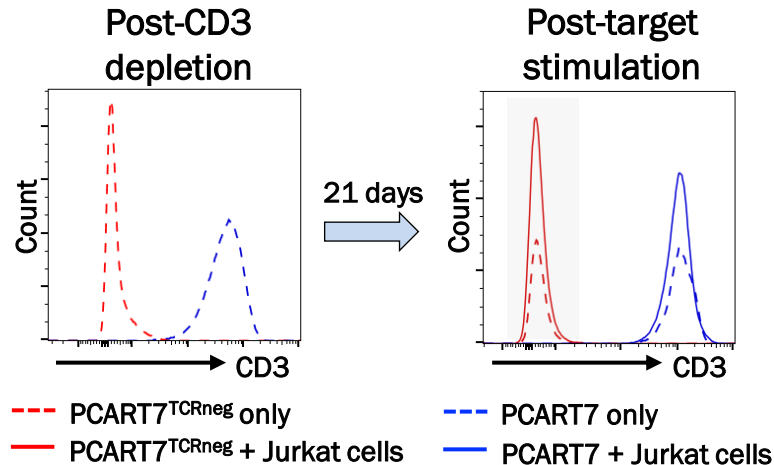


# CD3 can be downregulated in PCART7<sup>TCRneg</sup> cells

- CD3 remain suppressed in PCART7<sup>TCRneg</sup> upon repeated target stimulation
- PCART7<sup>TCRneg</sup> do not respond to CD3/CD28 stimulation

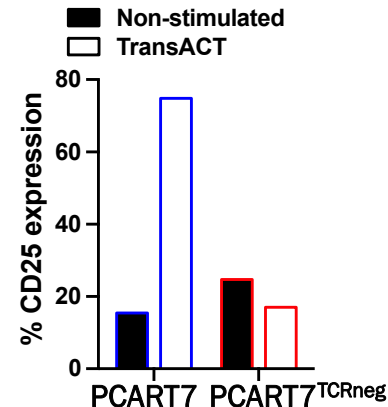


a



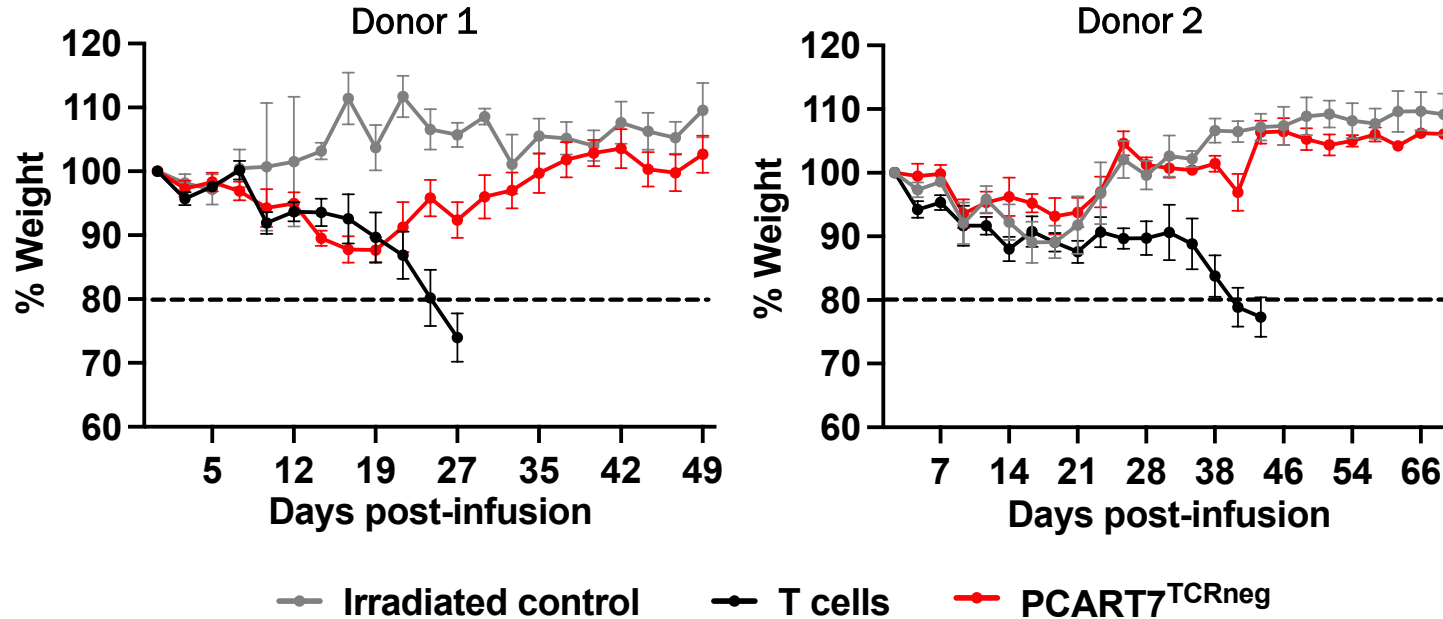
b

Day 21 post-stimulation



# PCART7<sup>TCRneg</sup> cells are not xenoreactive

- No weight loss observed in mice engrafted with PCART7<sup>TCRneg</sup> cells

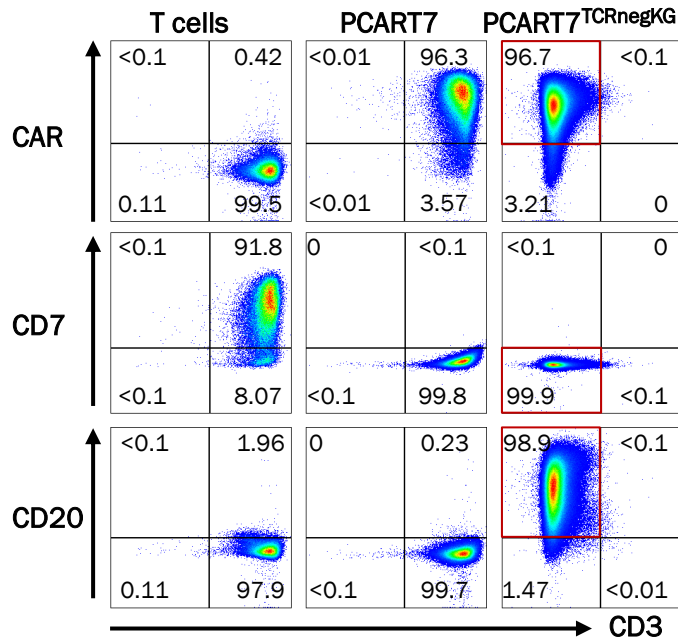


# Increasing the safety of PCART7<sup>TCRneg</sup> with a kill gene

- Expression of CD20t kill gene (KG) in PCART7<sup>TCRneg</sup> cells does not affect CAR T cell function

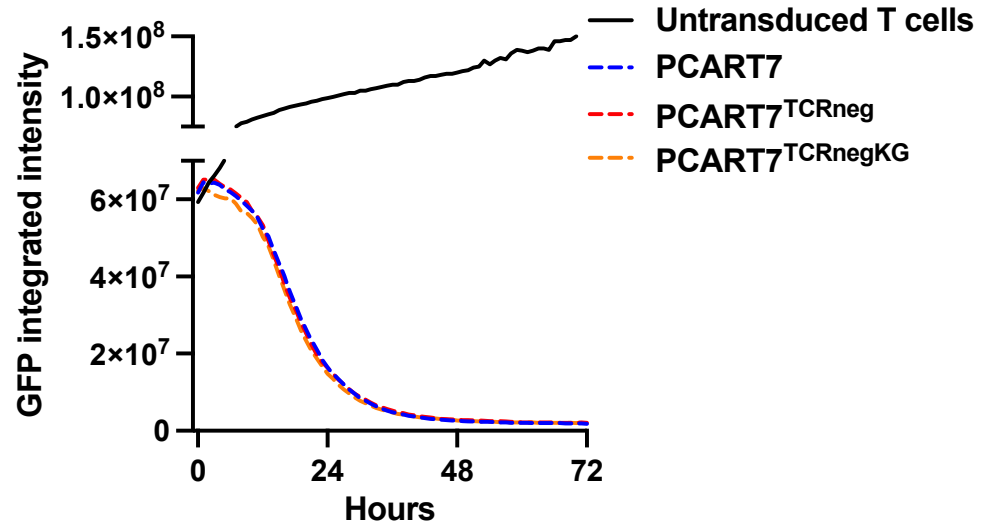
a

Day 15 post-manufacturing



b

CD7+ Jurkat cells

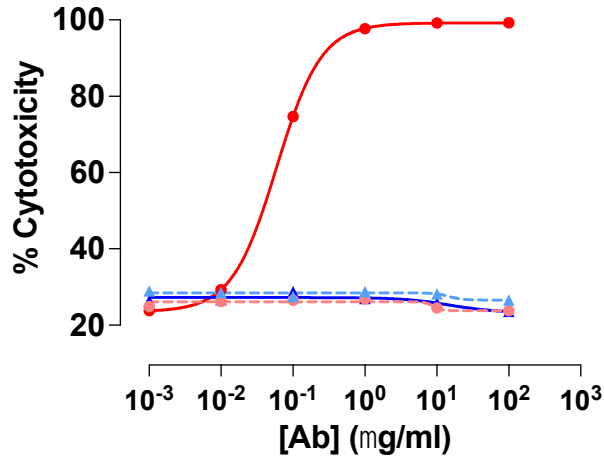


# Functional kill gene in PCART7<sup>TCRneg</sup>

- PCART7<sup>TCRneg</sup> cells with kill gene can be eliminated using Rituximab

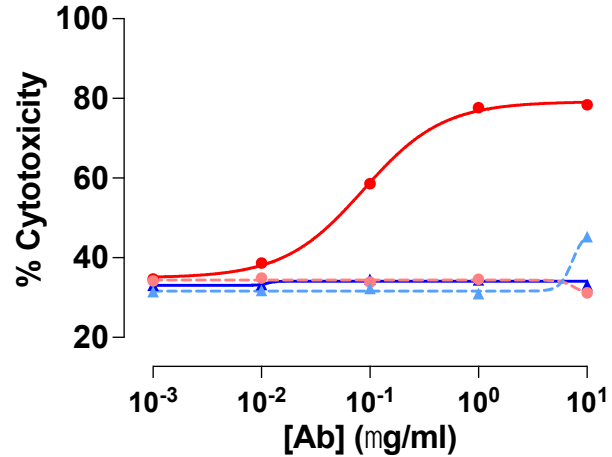
a

CDC assay



b

ADCC assay



---▲--- PCART7<sup>TCRneg</sup> [+Trastuzumab]

---●--- PCART7<sup>TCRneg</sup>KG [+Trastuzumab]

---▲--- PCART7<sup>TCRneg</sup> [+Rituximab]

---●--- PCART7<sup>TCRneg</sup>KG [+Rituximab]



# Summary

- TCR can be downregulated in cells expressing anti-CD7 CAR and PEBL
- Anti-CD3 $\epsilon$  PEBL and kill gene can be expressed without affecting anti-leukemic function
- PCART7<sup>TCR<sup>neg</sup></sup> demonstrate potent anti-leukemic efficacy without xenoreactivity
- Platform for development of allogeneic CAR-T therapy for T-ALL and other CD7-positive T cell malignancies

**Poster #1967:** Development of Anti-CD3 CAR-T Cells for Allogeneic Cell Therapy of Peripheral T-cell Lymphoma (PTCL)

