An “Off-the-Shelf” CD2 Universal CAR-T Therapy Combined with a Long-Acting IL-7 for T-Cell Malignancies

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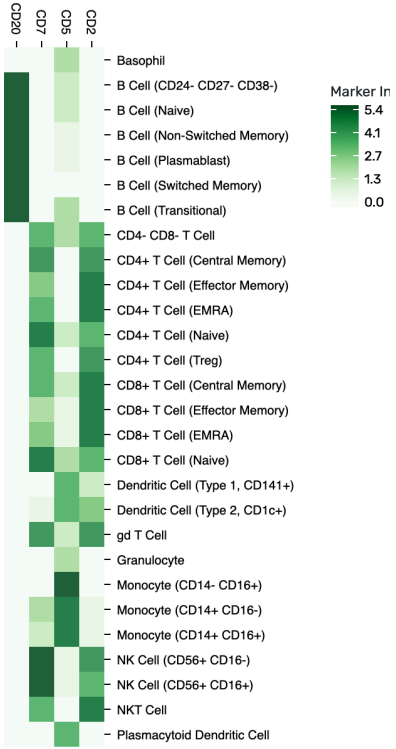
ASH2023 | 12/11/2023

Targeting CD2 in T-cell malignancies

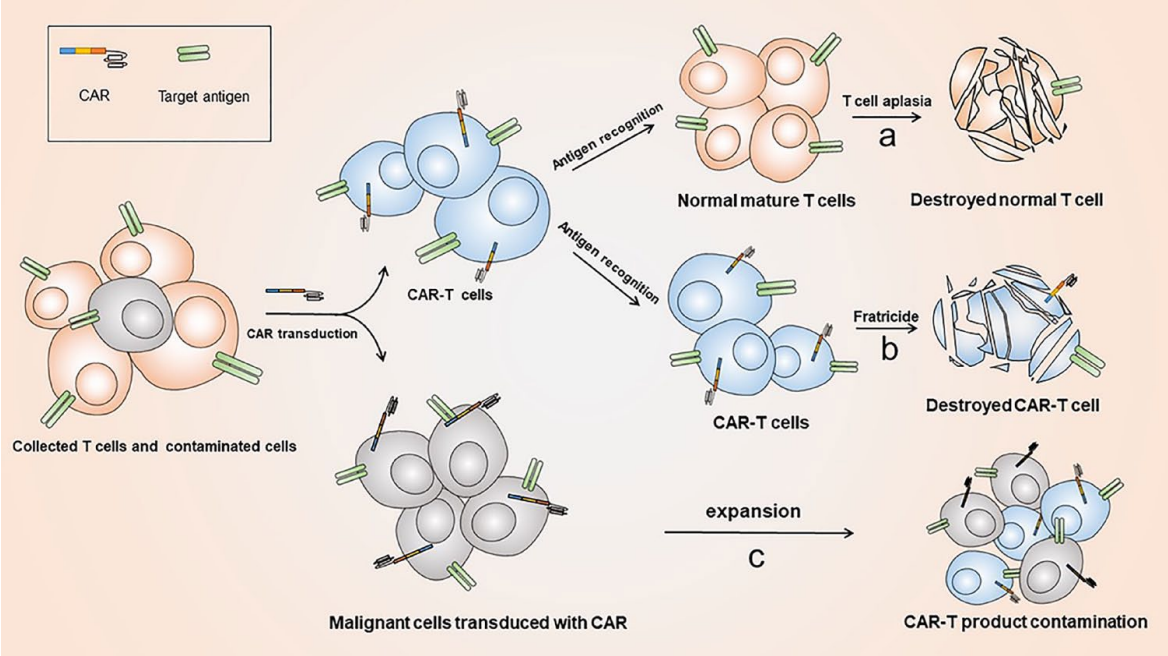
- CD2 is a surface glycoprotein restricted to hematopoietic cells with high expression in T cells and NK cells.
- In conjunction with its binding partner CD58, CD2 co-stimulation plays an important role in T cell activation and TCR signaling.
- CD2 is broadly expressed in T cell malignancies including T-cell acute lymphoblastic leukemia (T-ALL), Sezary Syndrome (SS), and adult T cell leukemia/lymphoma (ATL).
- Additionally, CD2 has been found to be downregulated at a lower frequency than other pan-T cell markers, providing an attractive therapeutic target.



Healthy PBMC
(Antibody Staining Data Set)

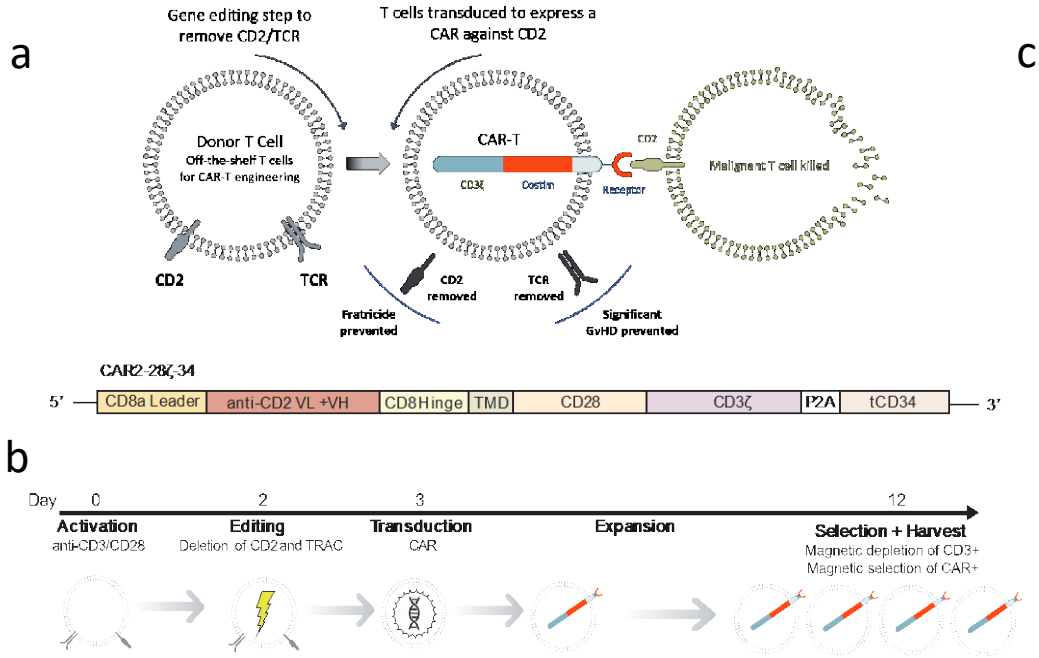
Current challenges of CAR-T therapy in T-cell malignancies

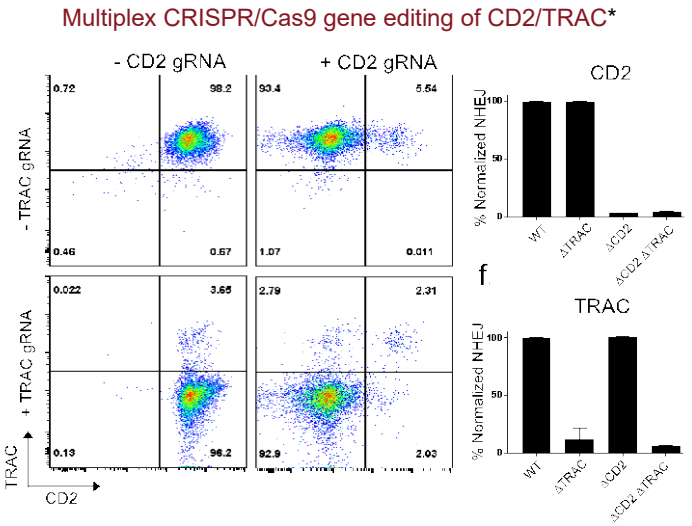
- CAR-T cell therapy is remarkable in treating CD19+ B-cell malignancies; however, its use in T-cell malignancies is restricted.



Luo *et al. Ther Adv Hematol, 2022*

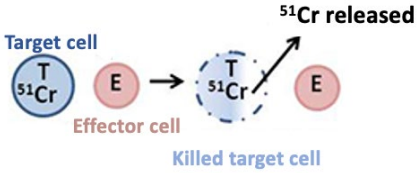
Production of allogeneic “universal” CD2 targeting CAR-T cells (UCART2)

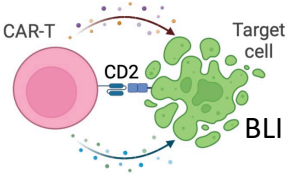




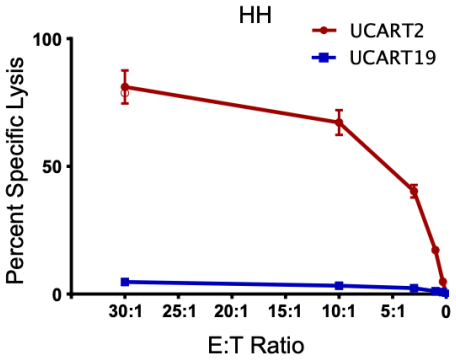
* Off-target sites of CRISPR/Cas9 gene editing was assessed by Guide-Seq

Efficient target-specific killing of CD2+ tumor cells by UCART2 *in vitro*

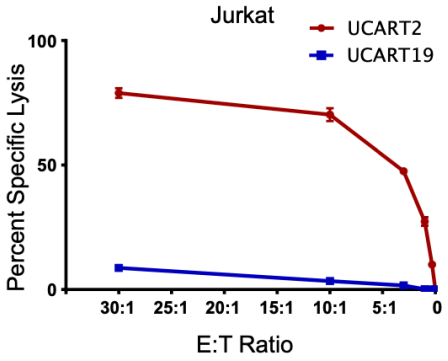




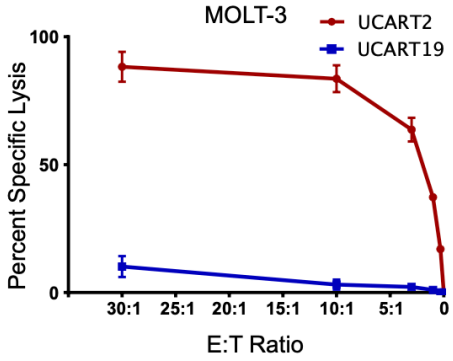
^{51}Cr release cytotoxicity assay
(4 hr post co-culture)



CTCL

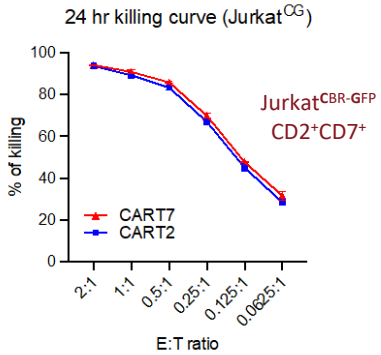


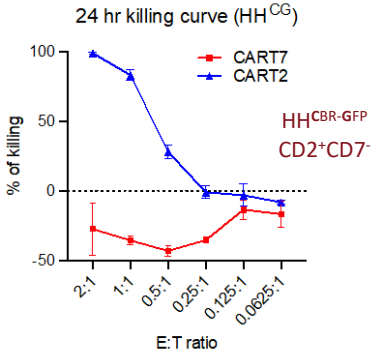
T-ALL



T-ALL

BLI killing assay (24 hr)

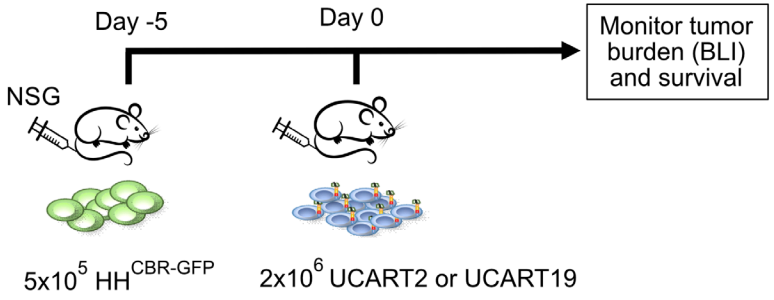




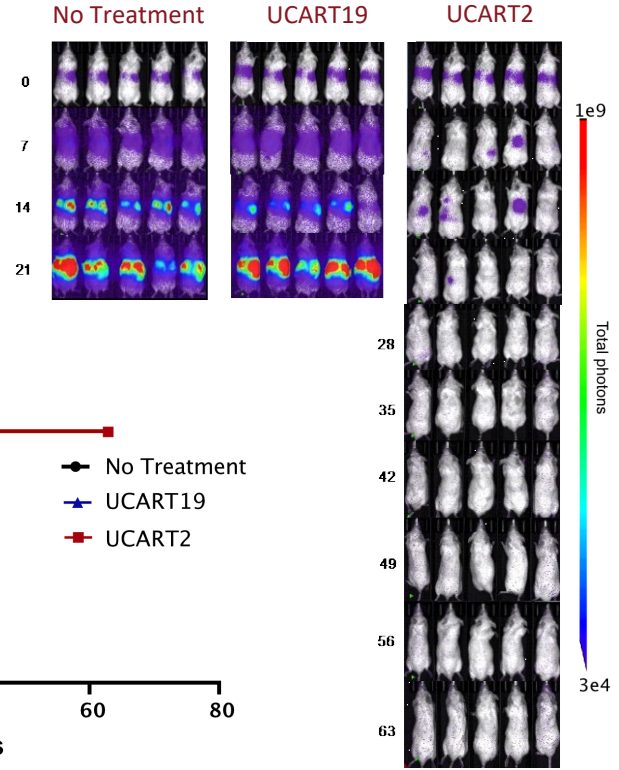
UCART2: CD2 targeting CAR-T cells (TRAC/CD2 KO)
UCART19: CD19 targeting CAR-T cells (TRAC KO)

In vivo efficacy of UCART2 against HH$^{CBR-GFP}$ CTCL tumor (CD2+CD7-)

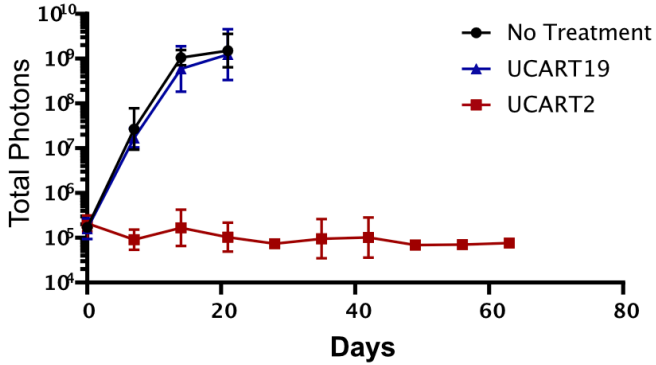
a.



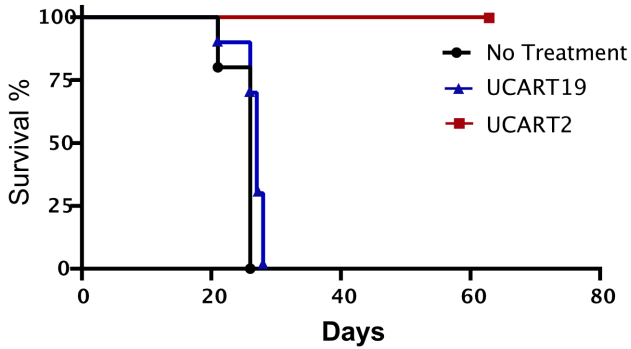
b.



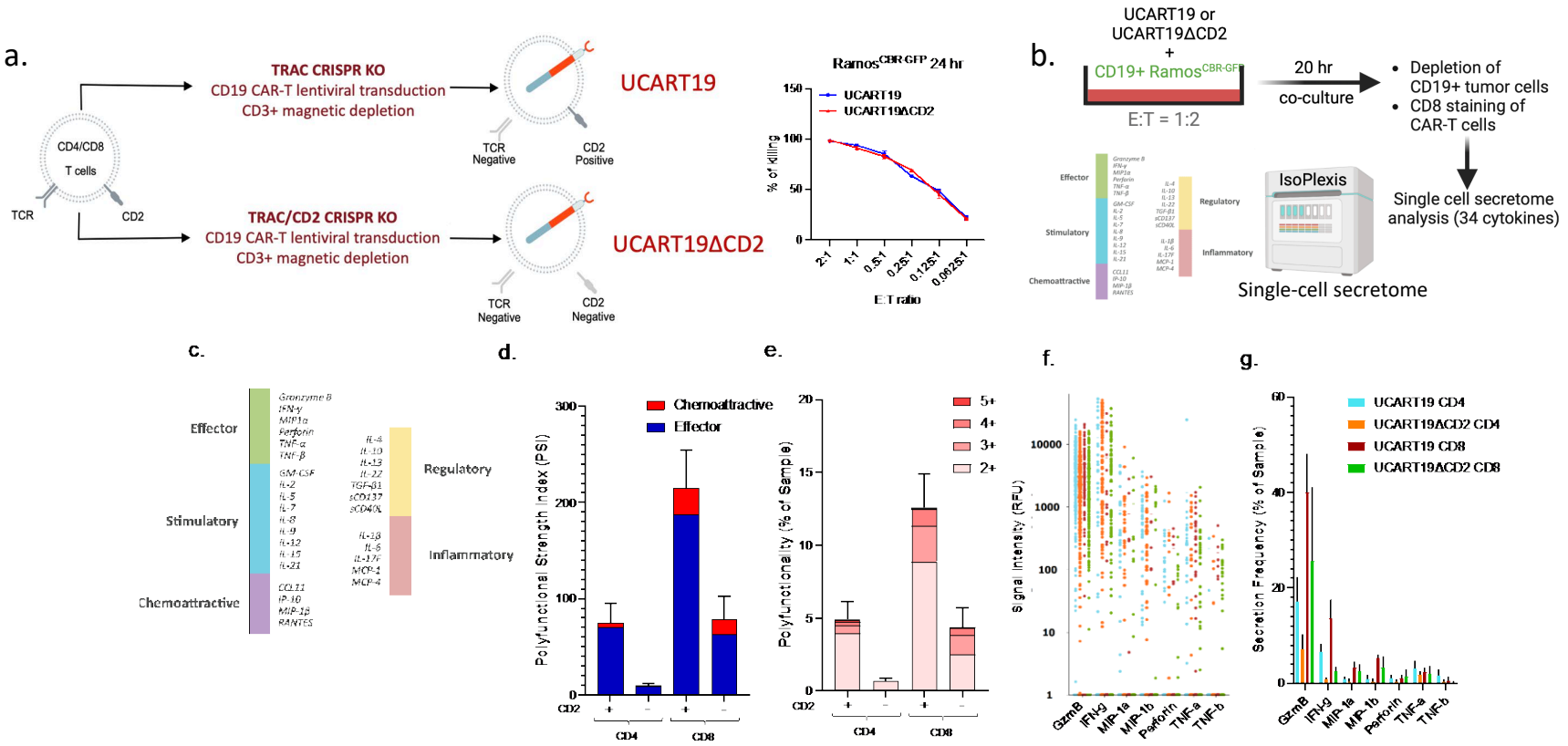
c.



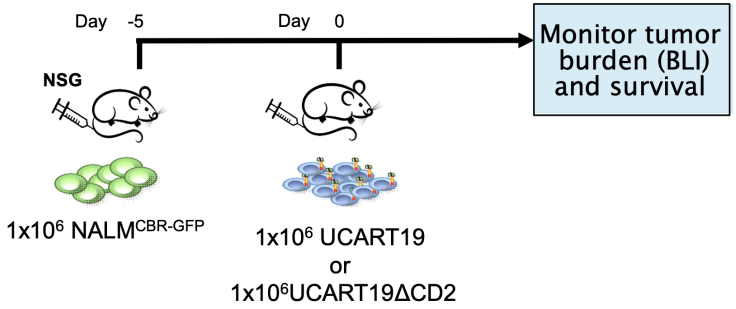
d.

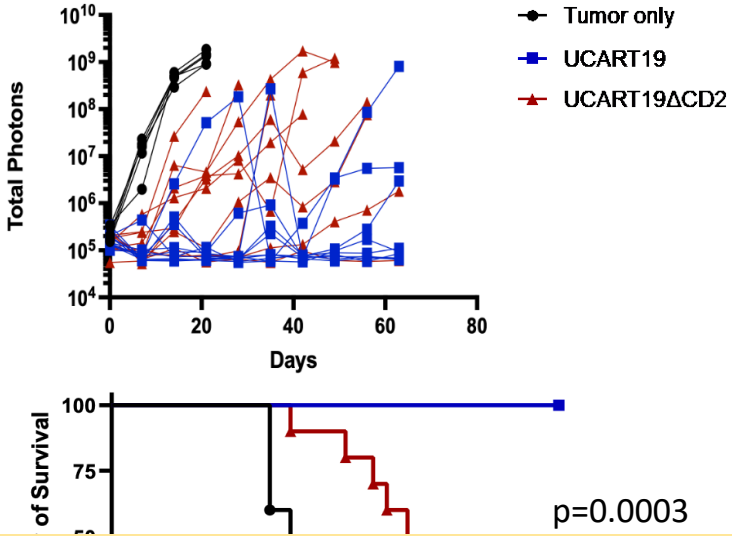


What's the impact of CD2 deletion on CAR-T cell function?
Deletion of *CD2* decreased the effector cytokine production in UCART19 cells



Deletion of *CD2* in UCART19 leads to reduced *in vivo* CAR-T function

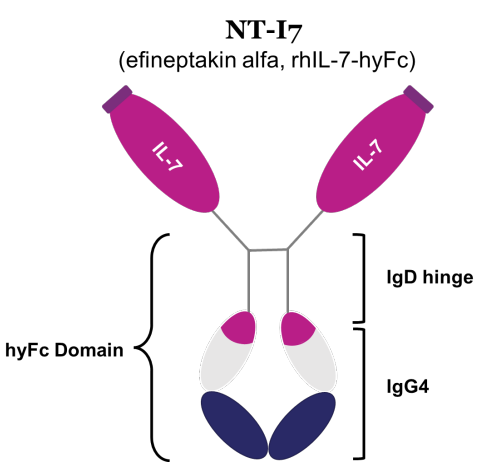




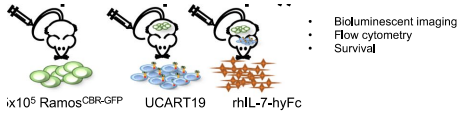
- **CD58 Aberrations Limit Durable Responses to CD19 CAR in Large B Cell Lymphoma Patients Treated with Axicabtagene Ciloleucel but Can be Overcome through Novel CAR Engineering.** (Majzner *et al. Blood* 2020)
- **CD58 loss in tumor cells confers functional impairment of CAR T cells.** (Yan *et al. Blood Adv* 2022)
- **The CD58-CD2 axis is co-regulated with PD-L1 via CMTM6 and shapes anti-tumor immunity.** (Ho *et al. Cancer Cell* 2023)

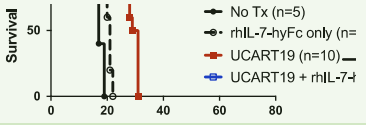
Can we overcome the reduced CAR-T function due to CD2 loss?

A long-acting interleukin-7, NT-I7, enhances CAR T cell expansion, persistence, and anti-tumor activity

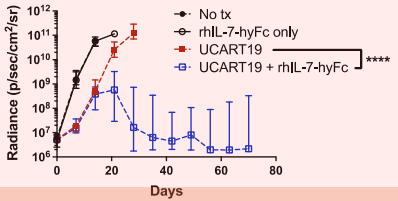


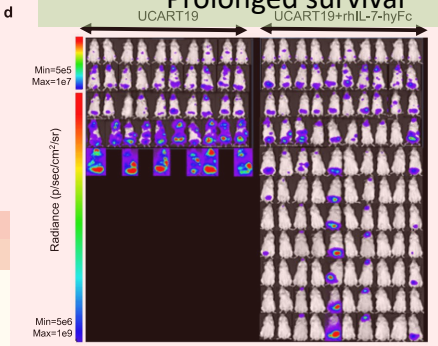
hyFc domain significantly extends serum half-life of NT-I7 ~ 10-20 fold



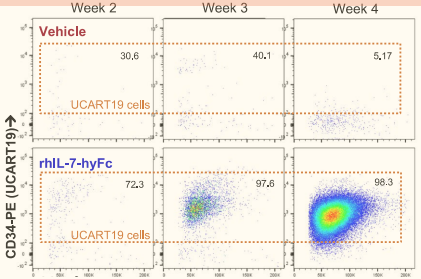


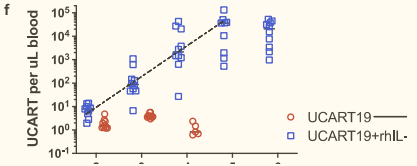
Prolonged survival





Enhanced anti-tumor activity

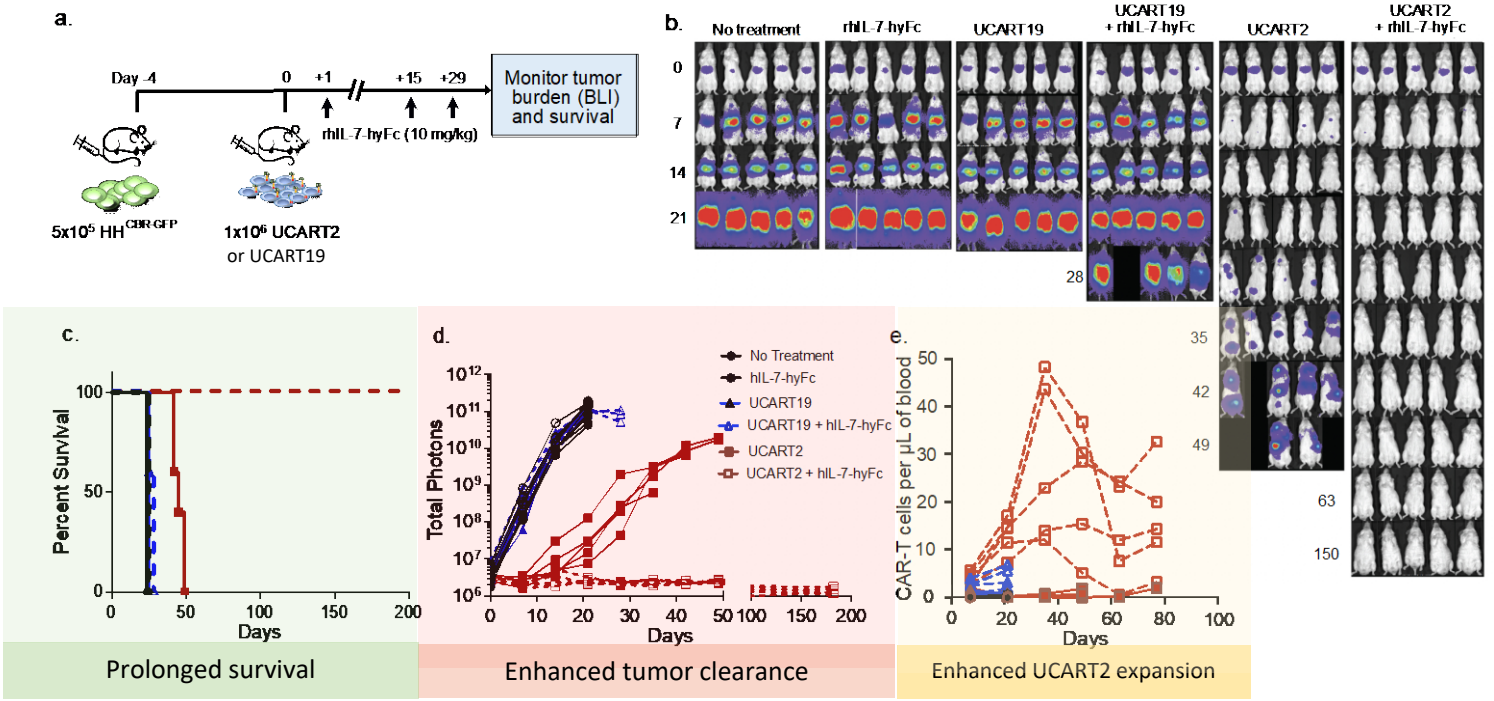




Enhanced CAR-T cell expansion and persistence

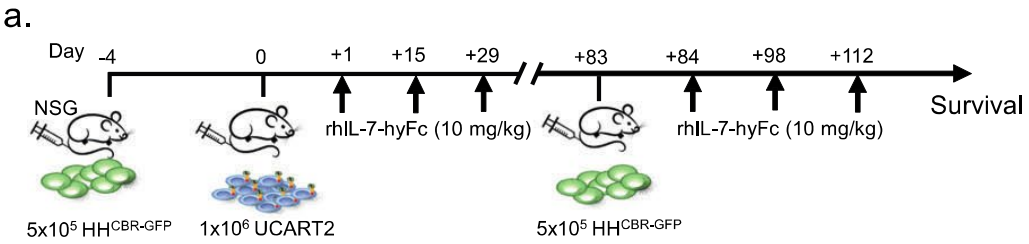
Kim *et al. Nature Communication*, 2022

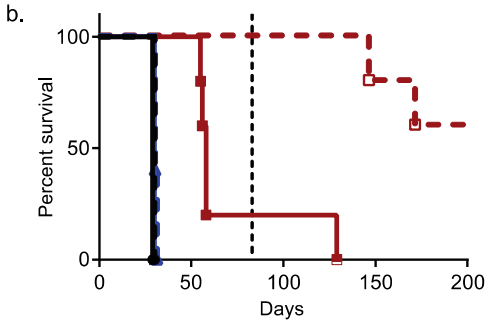
UCART2 and NT-I7 combination completely abolish CTCL tumor *in vivo*

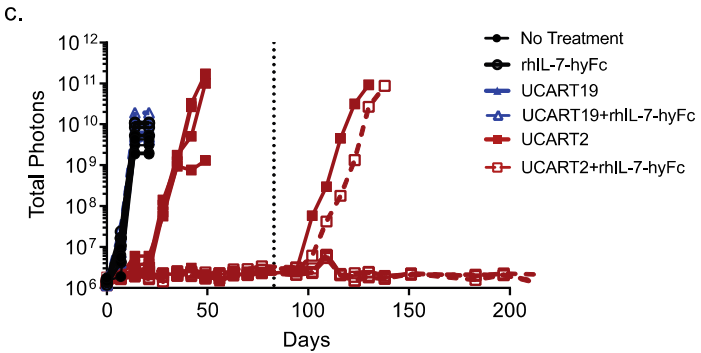


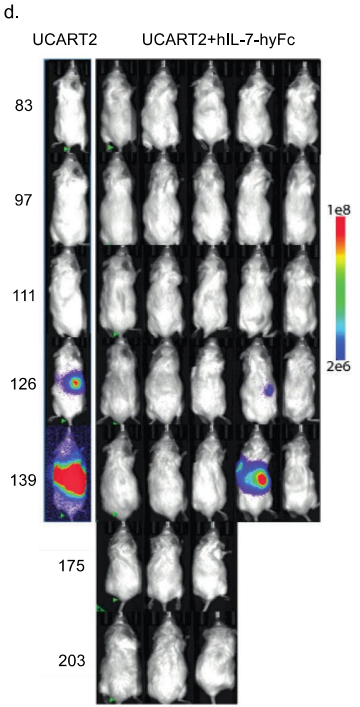
* Sub-optimal doses of CAR-T cells were used

NT-I7 prolongs UCART2 persistence *in vivo* and overcomes tumor re-challenge



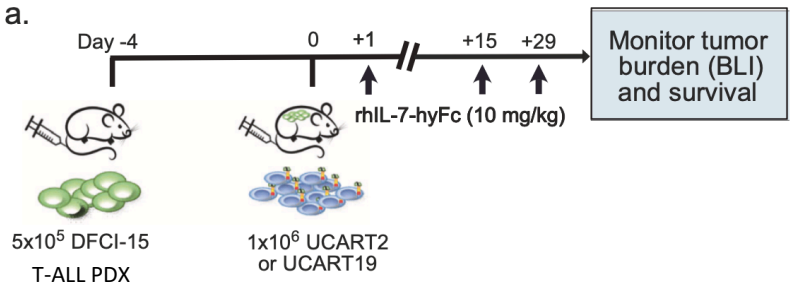


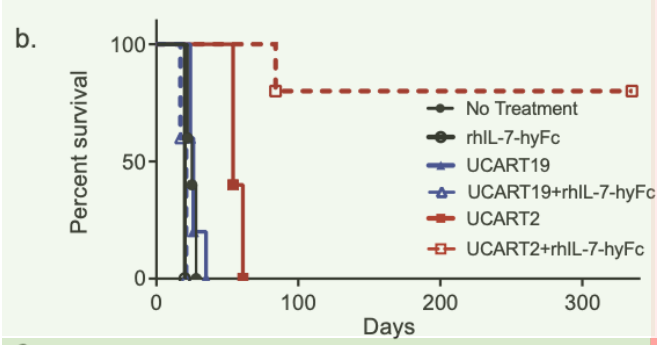




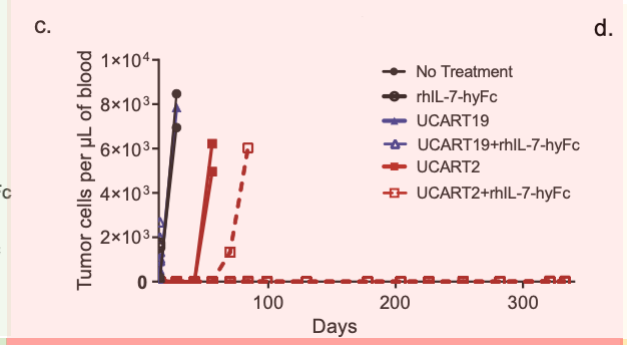
* Sub-optimal doses of CAR-T cells were used

UCART2, in combination with rhIL-7-hyFc (NT-I7), kills primary patient T-ALL *in vivo*

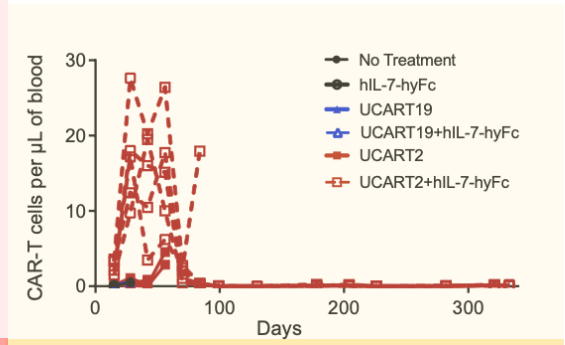




Prolonged survival



Enhanced tumor clearance

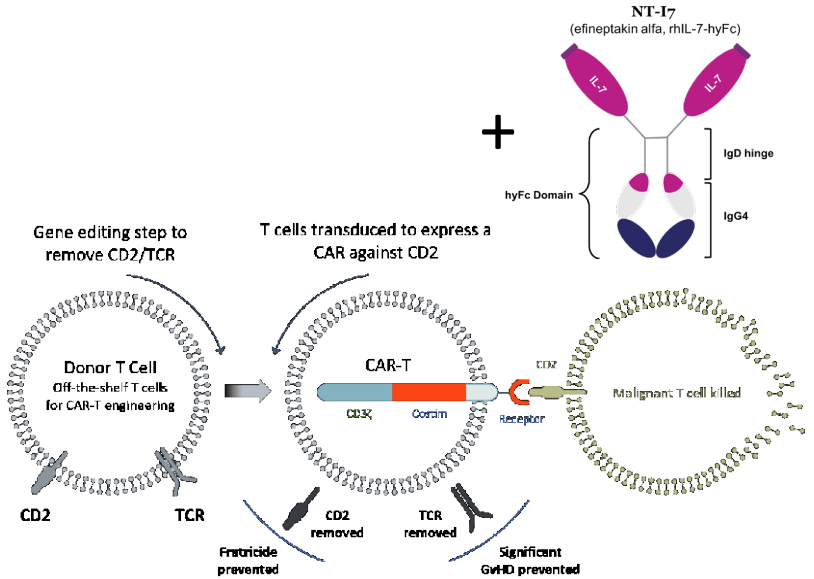


Enhanced UCART2 expansion and persistence

* Sub-optimal doses of CAR-T cells were used

Summary

- We have developed UCART2, a fratricide-resistant, allogenic “universal” CD2-targeting CAR-T cell, which is effective against T-ALL and CTCL.
- CD2 deletion in CAR-T cells resulted in reduced production of effector cytokines and reduced anti-tumor activity in CAR-T stress models *in vivo*, which was rescued by a long-acting recombinant IL-7, NT-I7.
- When combined with NT-I7, UCART2 induced durable complete responses in both primary and tumor rechallenge tumor models *in vivo*.



Acknowledgments

DiPersio Lab

- **John DiPersio**
- **Matthew Cooper**, **Jessica Devenport**
- Julie Ritchey, Dilan Patel, Parmeshwar Amatya, Steve Persaud, Peter Ruminski, Ramzi Abboud, Omar Ibrahim, Julia Wang
- Mike Rettig, Ezhil Chendamarai, Stephanie Christ
- Miriam Kim, Rubina Thomas, Jieun Kim, Nadim Abboud
- Darja Karpova, John Reed, Mya Cineus
- Julie O'Neal, Susan Gladney
- Gayla Hadwiger, Linda Eissenberg
- Alun Carter

Siteman Flow Core

- Bill Eades
- Dan Schweppe

Siteman BQSR

- Feng Gao

NeoImmuneTech

- Byung Ha Lee
- Alexandra Wolfarth
- Sara Ferrando-Martinez

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