Introduction to CRISPR: Its application and potential for treating human disease

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NCCN National Comprehensive Cancer Network®
NCI CCC
A Comprehensive Cancer Center Designated by the National Cancer Institute

Disclosures

- **Consulting/Advisory Committees:** Rivervest, Bioline, CellIncyte, NeoImuneTech, Macrogenics
- **Employment/Salary:** Washington University
- **Ownership Investment** : Magenta, WUGEN

Endogenous DNA repair mechanisms

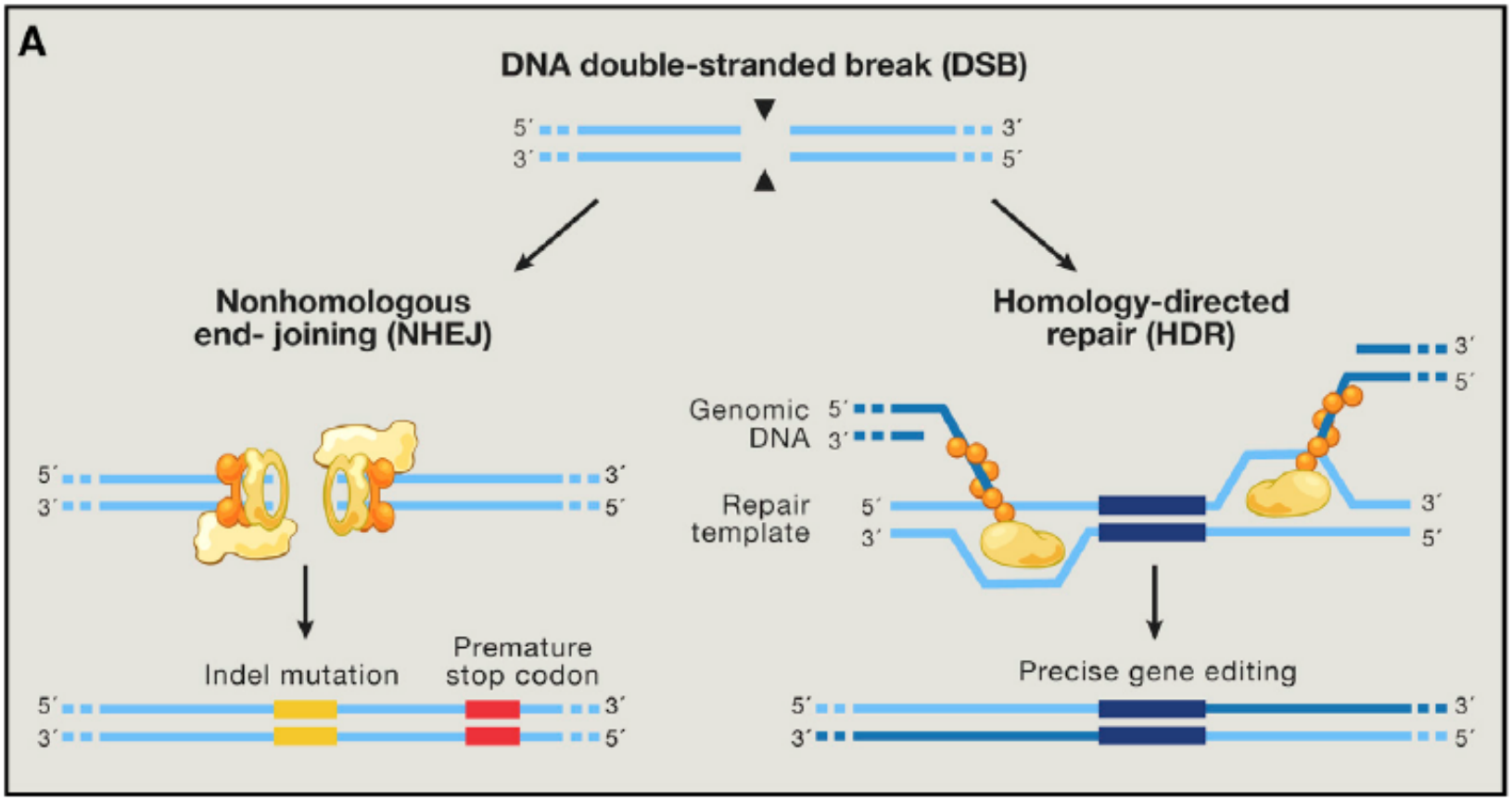
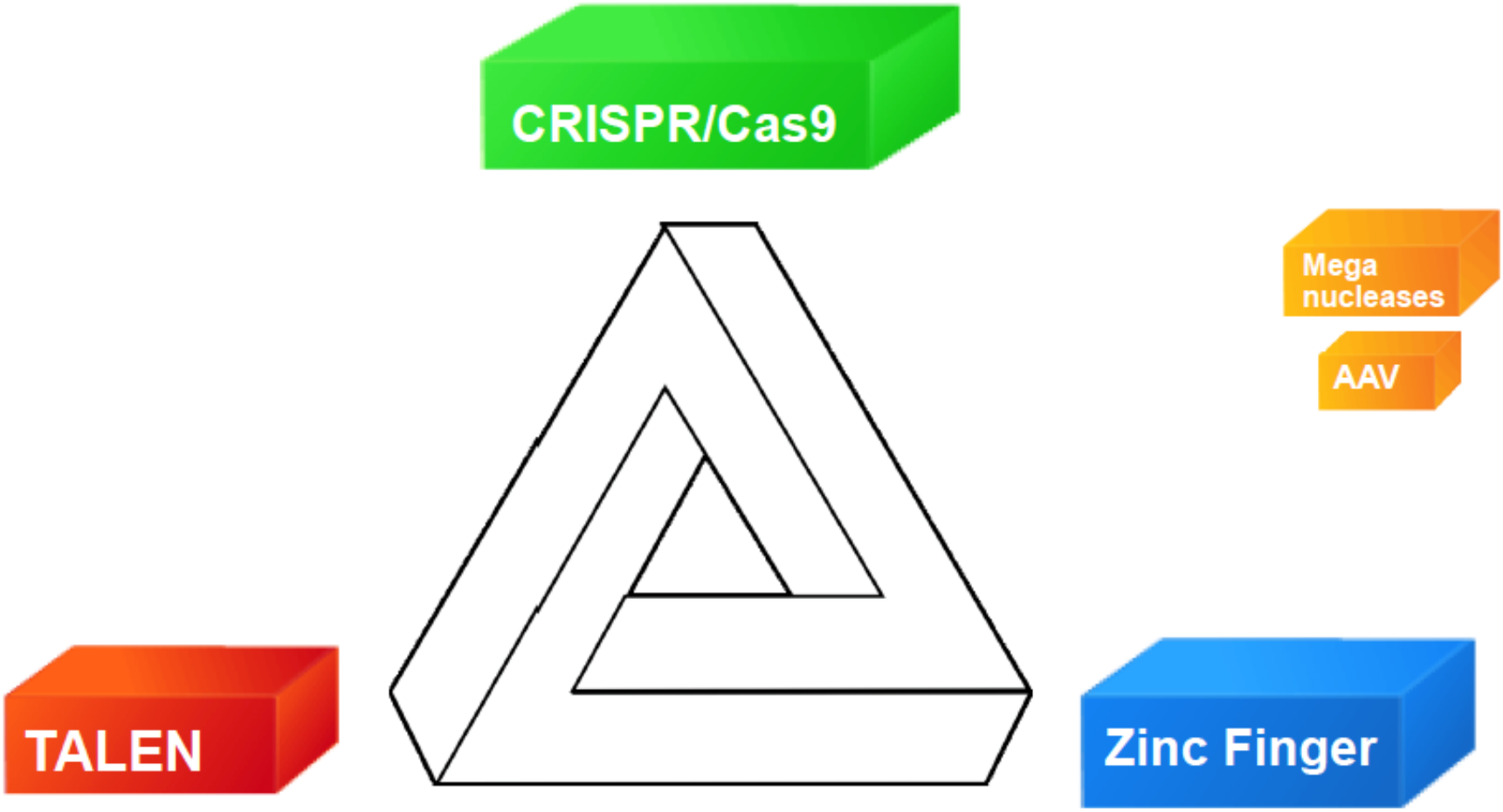


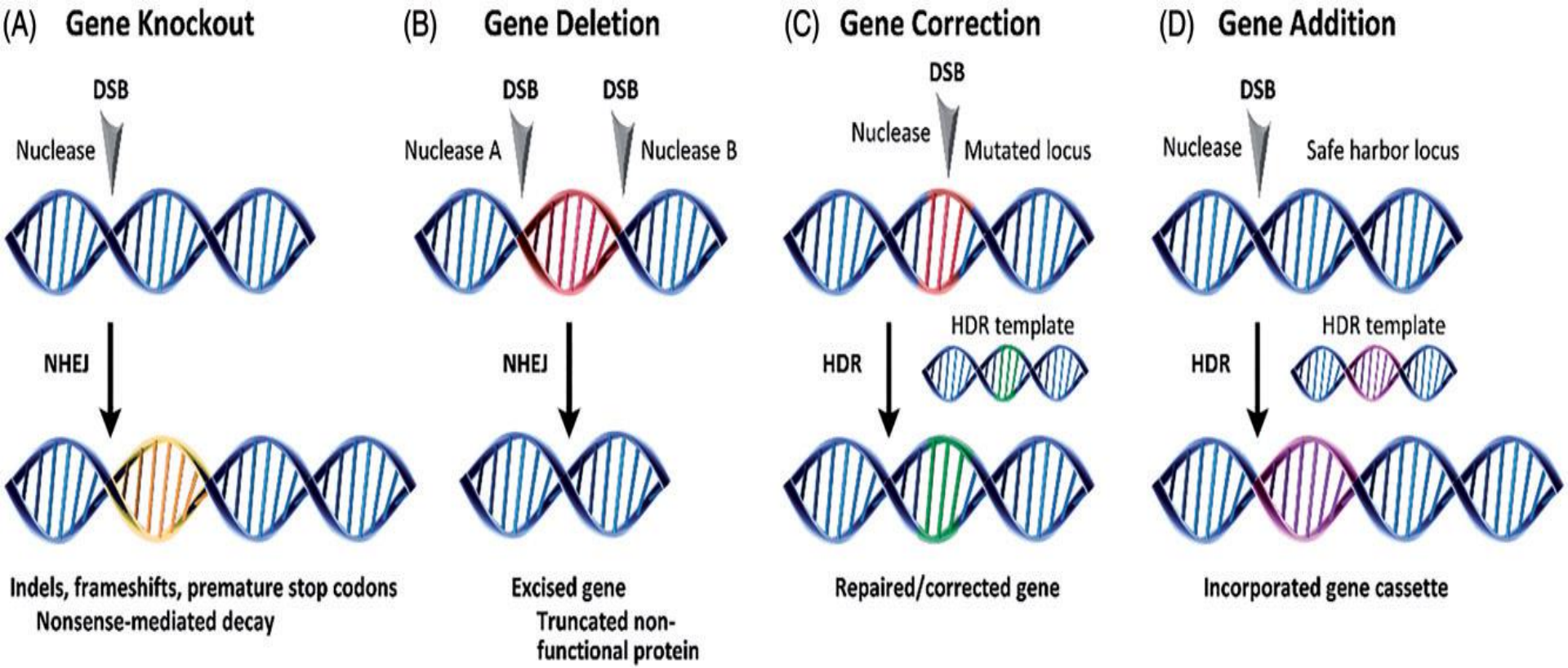
Figure: Hsu, Lander, Zhang: Development and Applications of CRISPR-Cas9 for Genome Engineering; Cell 157, June 5, 2014

The triumvirate of genome editing



Read: Gaj, Gersbach, Barbas III: Trends in Biotechnology, July 2013, Vol. 31, No. 7

Products of site-specific nuclease-based gene editing



Lino et al. Drug Delivery, 2018

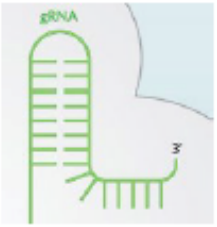
CRISPR loci and Cas nuclease nomenclature

CRISPR: Clustered Regularly Interspaced Palindromic Repeats
Loci in 40% of bacteria and 90% of archaea

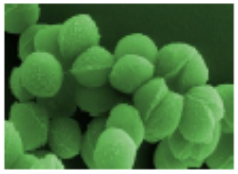
Cas9: CRISPR associated protein 9
a nuclease, an enzyme specialized for cutting DNA
Cas1..Cas10 exist

CRISPR/CAS: type I, type II and type III

gRNA: guide RNA – a construct/chimera of CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA)

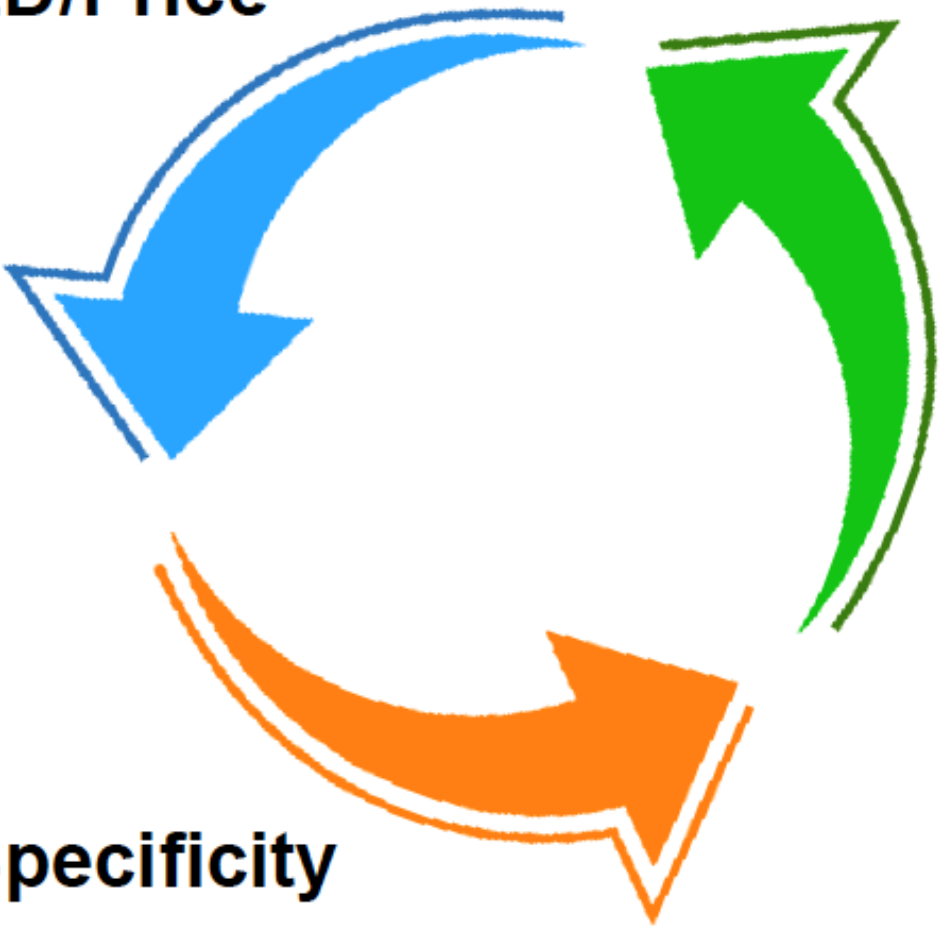


PAM: protospacer adjacent motif with sequence
NGG (any, guanine, guanine) specific to *Streptococcus pyogenes*
and 5′-NAG (any, adenine, guanine) PAM tolerated in human cells

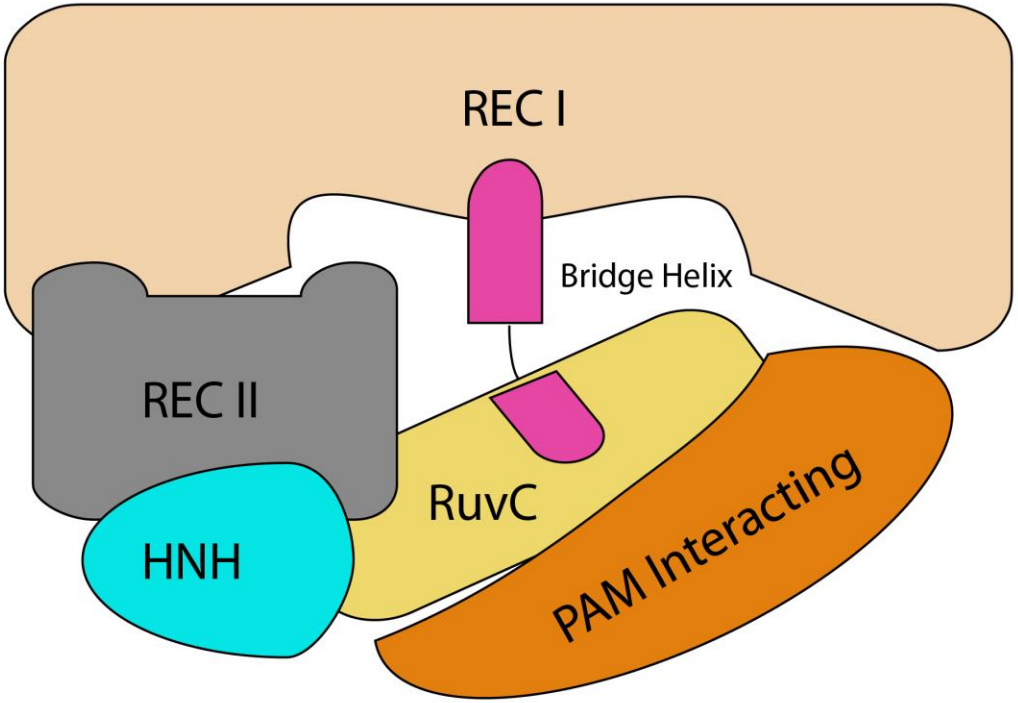


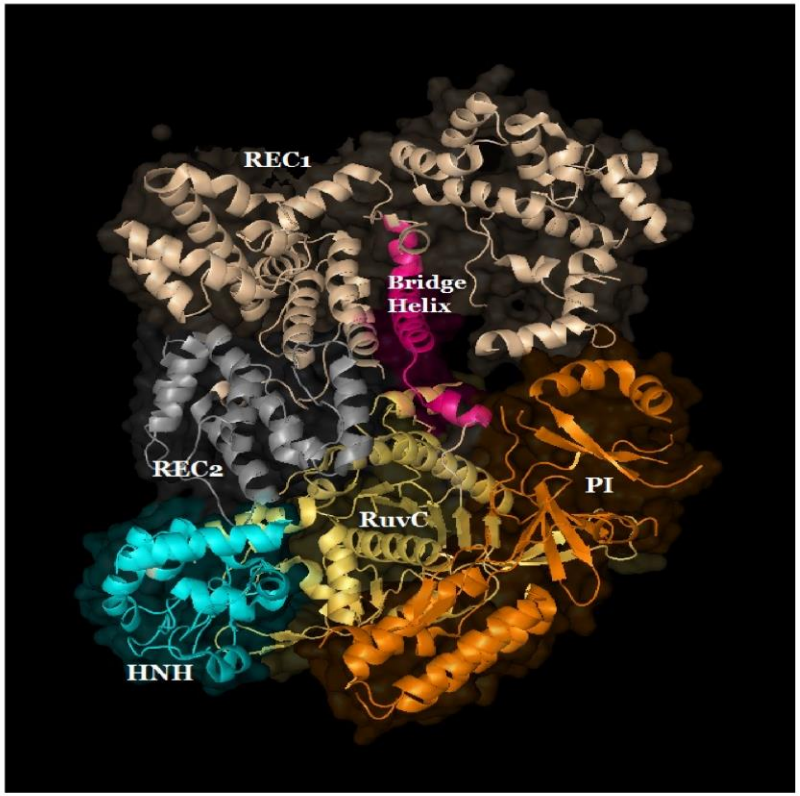
Figures: WIKI and HZI/Rohde

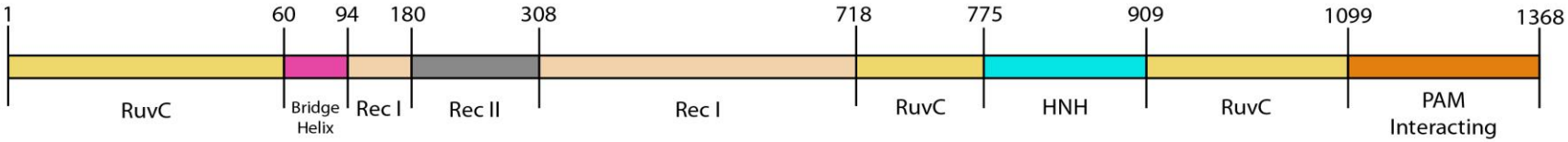
Why is CRISPR/Cas9 better?



Cas9 Complex unbound

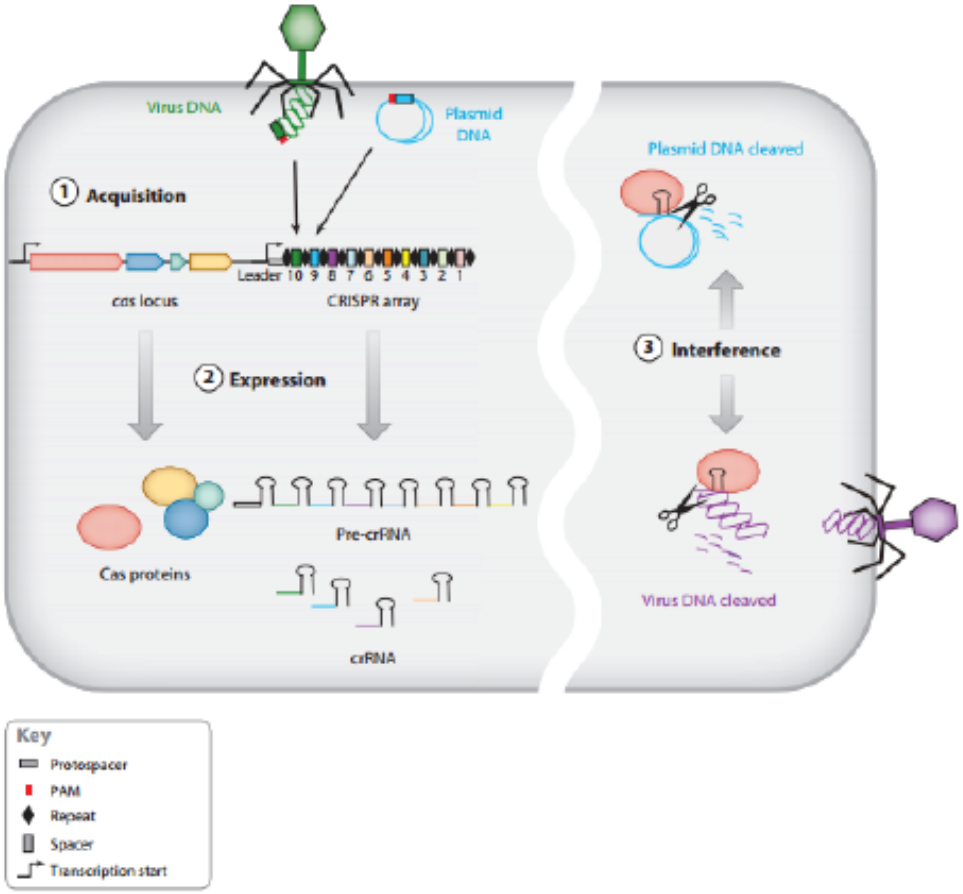






Cas9 Protein. The Cas9 protein is comprised of six domains: Rec I, Rec II, Bridge Helix, RuvC, HNH, and PAM Interacting. Domains

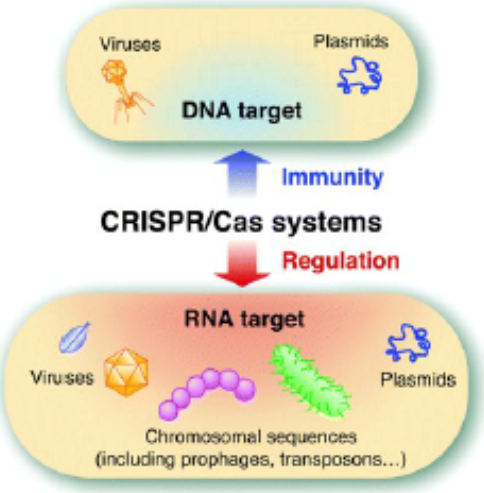
CRISPR/Cas the immune system of bacteria



(1) acquisition of foreign DNA

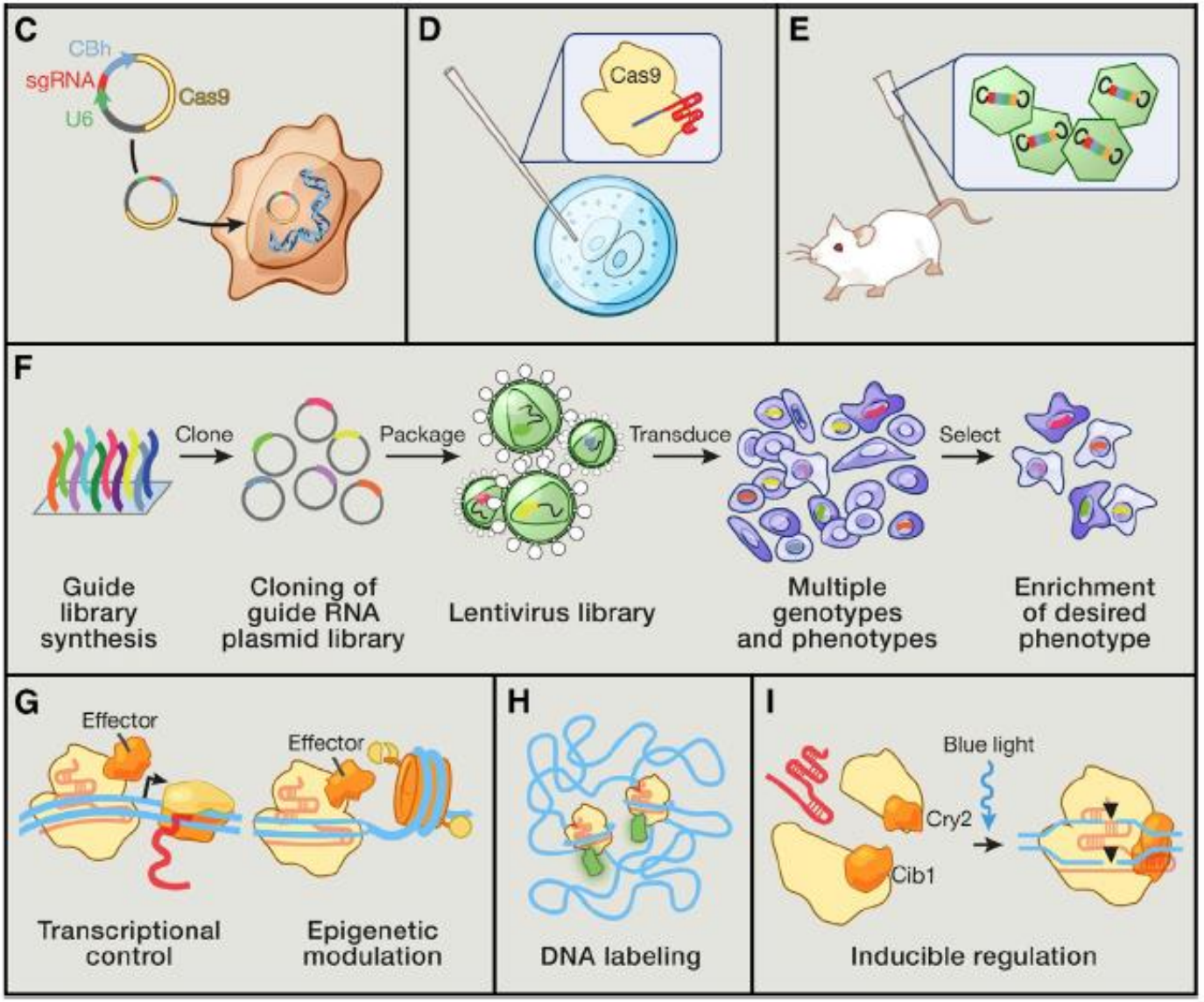
(2) synthesis and maturation of CRISPR RNA (crRNA) followed by formation of RNA-Cas nuclease protein complexes

(3) target recognition by crRNA and destruction of foreign DNA by Cas nuclease cleavage



Figures: Bhaya et al., Annu. Rev. Genet. 2011. 45:273-97 **and** Horvath: Science (2010) Vol. 327; 167-170: CRISPR/Cas, the Immune System of Bacteria and Archaea
Text: Singh et al: A Mouse Geneticist's Practical Guide to CRISPR Applications; Genetics. 2015 Jan; 199(1): 1–15.

Application examples



Source: Hsu, Lander, Zhang: Development and Applications of CRISPR-Cas9 for Genome Engineering; Cell 157, June 5, 2014

Problems, problems, problems

Delivery to cells in humans

lentivirus vectors

Via stem cells

Via liposomes

Off-target effects and unintended DNA cleavage

paired nickases
(single-strand DNA break)
double-nicking

Check with whole genome sequencing

Variable DNA cleavage efficiencies

modified Cas9 variants
paired nicking
modified sgRNAs

Use DBS and Cas9 to correct

inter- and intrachromosomal rearrangement

Chen et al. Dual sgRNA-directed gene knockout using CRISPR/Cas9 technology in Caenorhabditis elegans;
Smith et al: Whole-Genome Sequencing Analysis Reveals High Specificity of CRISPR/Cas9 and TALEN-Based Genome Editing in Human IPSCs
Ranganathan: Expansion of the CRISPR-Cas9 genome targeting space through the use of H1 promoter-expressed guide RNAs
Mali et al: CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering.

Examples of current clinical and translational efforts to modify human disease with CRISPR

1. Remove Malaria from Mosquitos (genetic modification to prevent carrier state)
2. Eliminate a patient's cancer (Cellectis/ALL)
3. Treat Muscular Dystrophy (in vivo correction of dystrophin gene defect)
4. Altering size and milk production of cows (China)
5. Giving pig organs to humans (G Church et al deleted 62 genes in pigs at once)
6. Treat HIV (delete CCR5 in T cells and in stem cells)
7. Modify the immune response (C June/U Penn and PD-1 in CART19)
8. In vivo delivery of CRISPR-like drugs (Bayer)
9. Make super plants (grapes resistant to mildew- Rutgers)
10. Make "mini-pigs for pets (China)
11. Cure retinitis pigmentosa (Editas Medicine Biotechnology IND)
12. Cure Sickle Cell Disease (Bluebird Bio, BCL11A edit (Vertex and CRISPR) and HR-HBSS correction Graphite Bio HbSS)
1. Edit Humans.

VIDEO: Genome Editing with CRISPR-Cas9

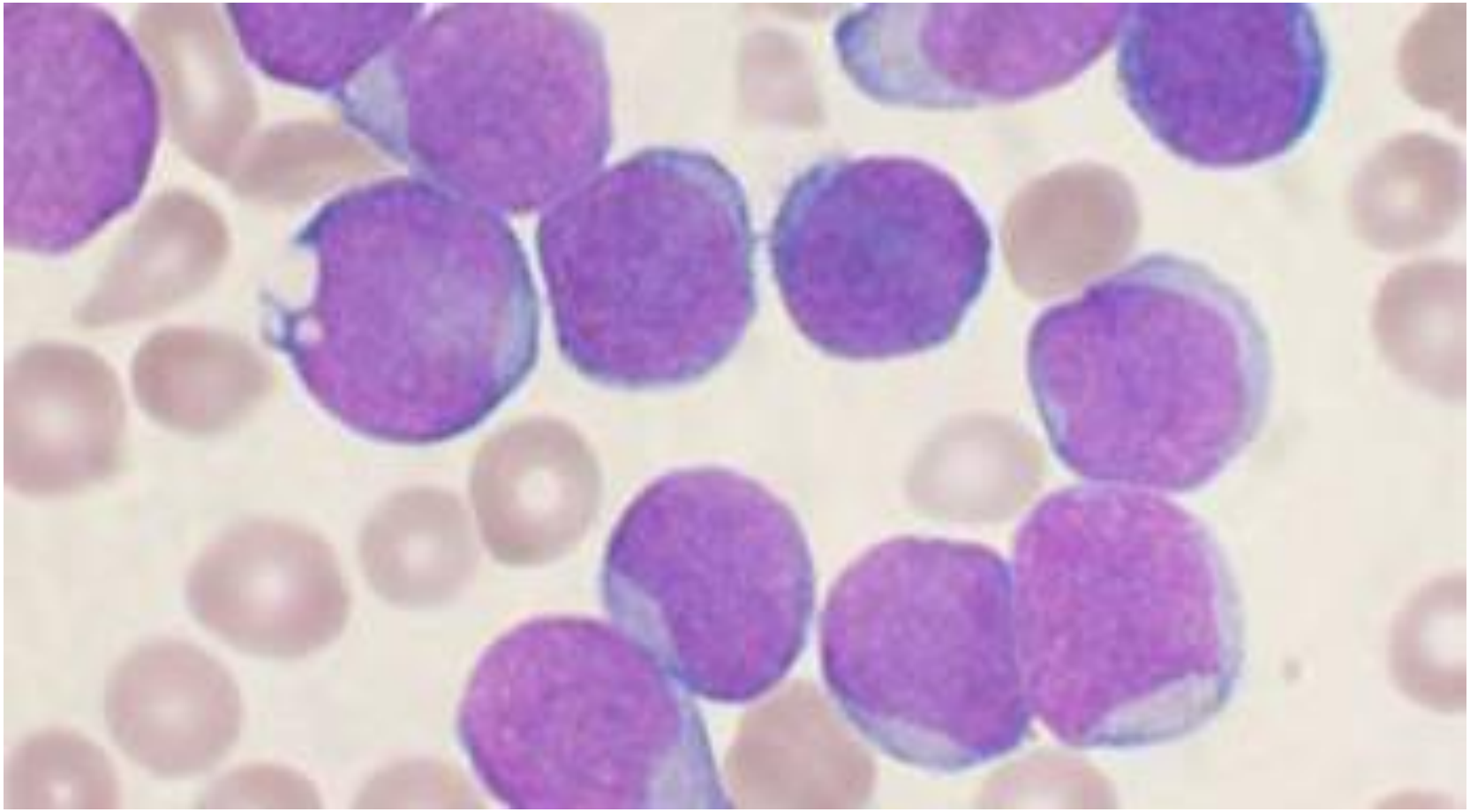
Click to watch

Source: McGovern Institute for Brain Research at MIT; https://www.youtube.com/watch?v=2pp17E4E-O8

Example of CRISPR/Cas9 Gene Editing for Treatment of Acute Leukemia

CD19 CARs for B-ALL

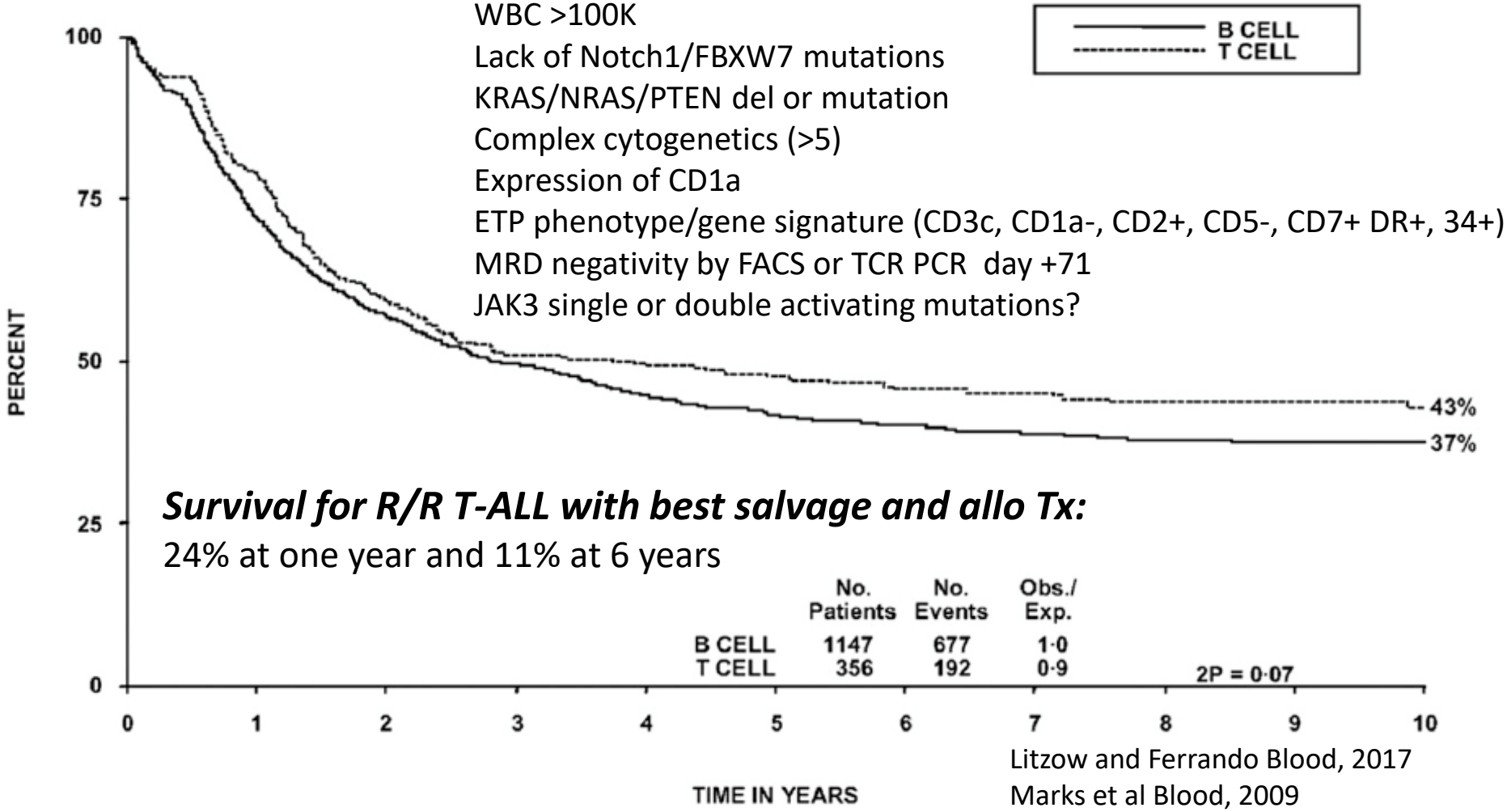
What about T-ALL



Overall survival from diagnosis for patients with B-ALL and T-ALL treated on UKALLXIIE2993 trial

Poor Prognostic markers for T-ALL:

WBC >100K
Lack of Notch1/FBXW7 mutations
KRAS/NRAS/PTEN del or mutation
Complex cytogenetics (>5)
Expression of CD1a
ETP phenotype/gene signature (CD3c, CD1a-, CD2+, CD5-, CD7+ DR+, 34+)
MRD negativity by FACS or TCR PCR day +71
JAK3 single or double activating mutations?



Survival for R/R T-ALL with best salvage and allo Tx:

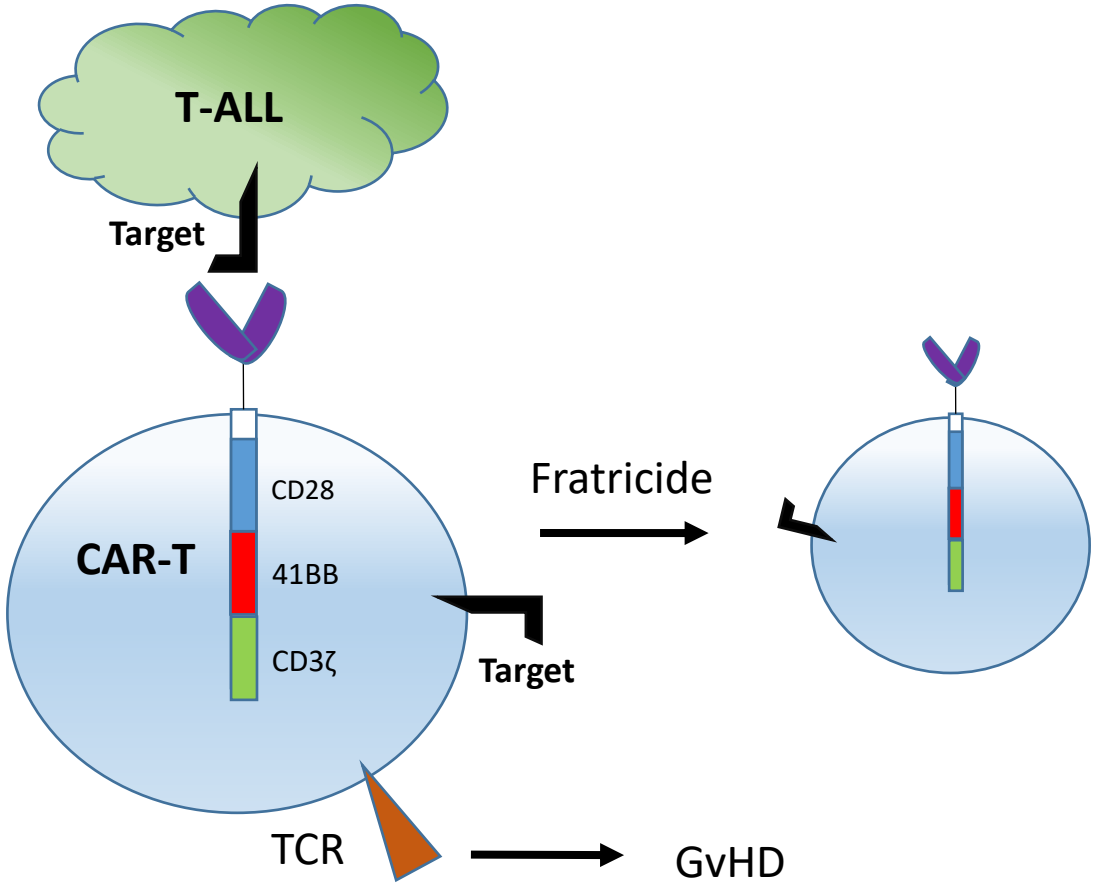
24% at one year and 11% at 6 years

Litzow and Ferrando Blood, 2017
Marks et al Blood, 2009
Asnafi et al Blood, 2009
Trinquand et al JCO. 2013

Complications of using CAR-T against T cell malignancies

- Target antigen shared between T cells and target resulting in fratricide
- Harvesting adequate numbers of autologous T cells, without contamination by malignant cells is challenging and expensive.
- T cells from allogeneic donor's risks causing significant GvHD

Matt Cooper



Complications of using T cell therapies against T cell malignancies

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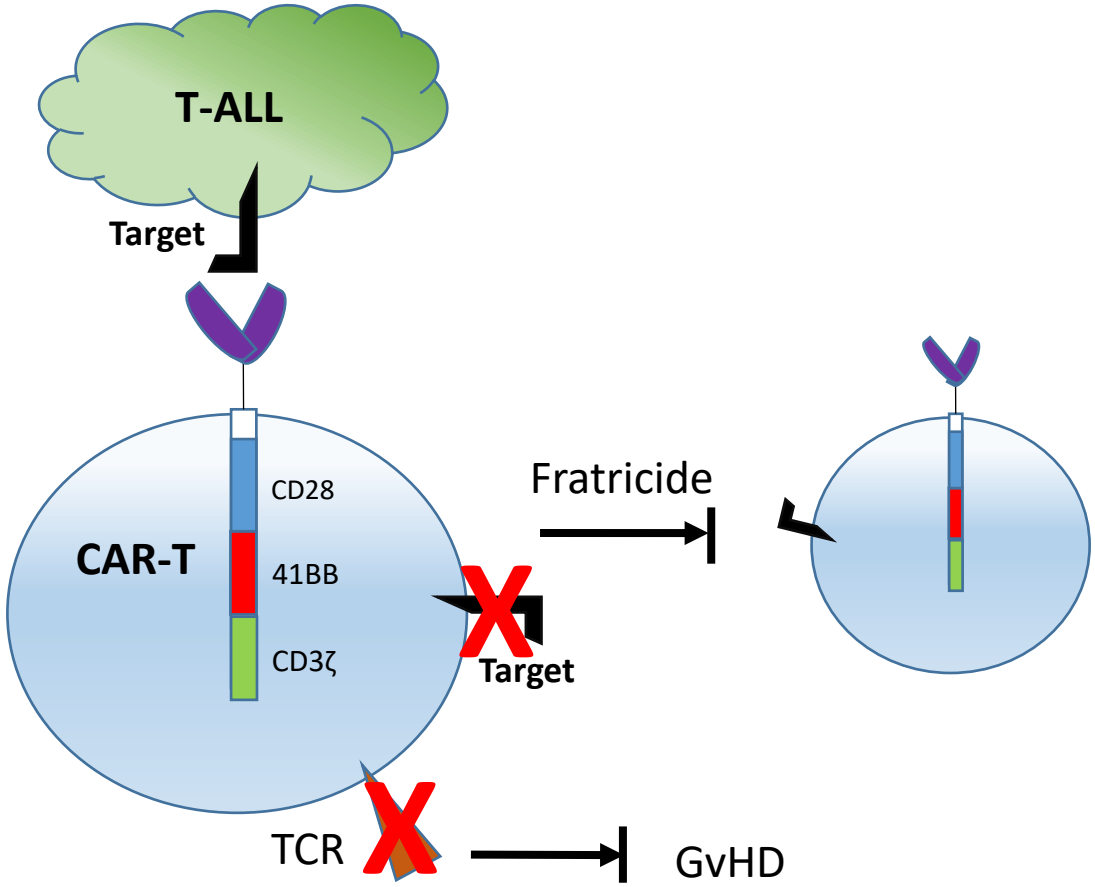


Hypothesis

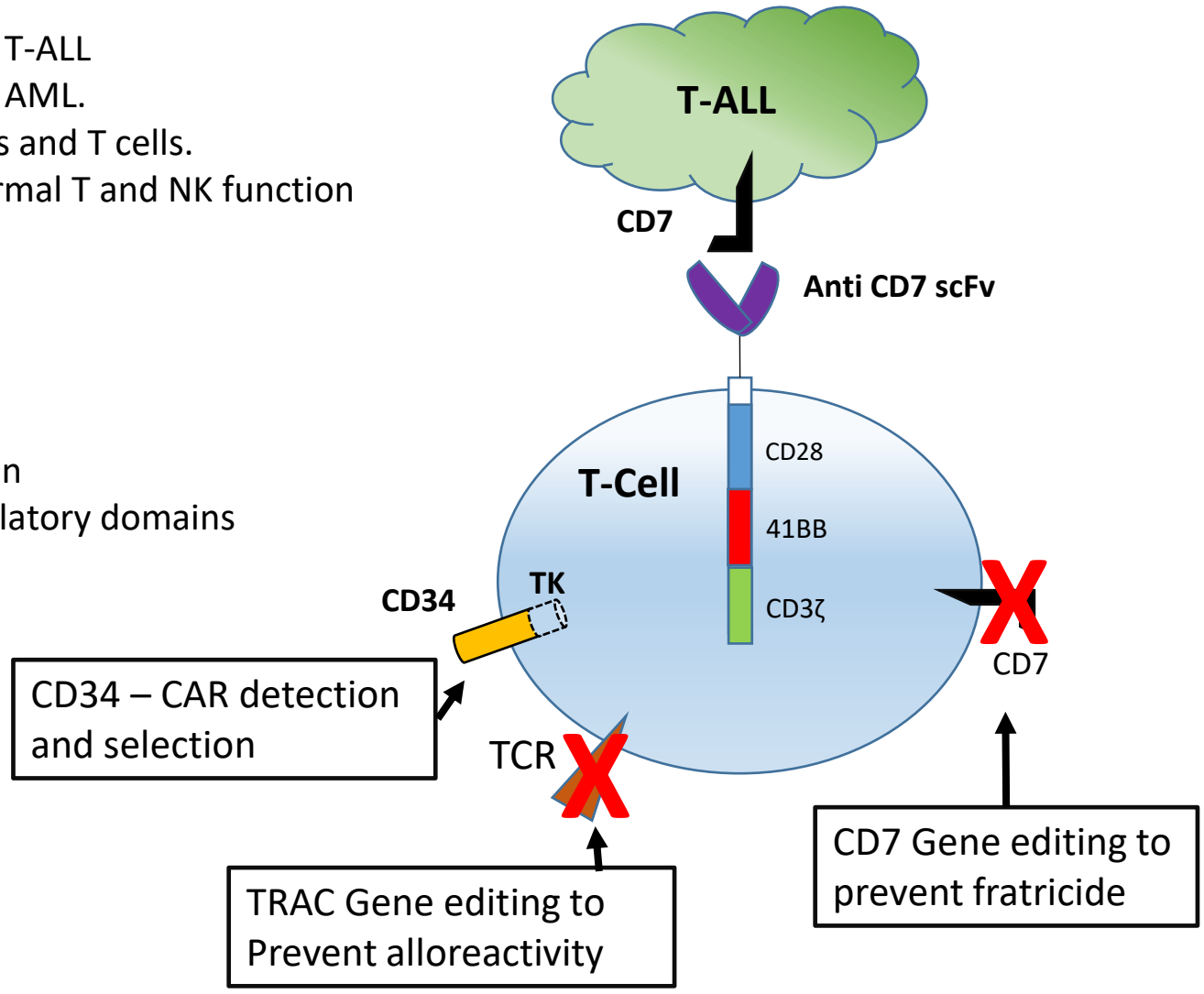
Gene editing of CAR-T to delete target antigen and T cell receptor alpha chain will mitigate fratricide and prevent GvHD

CD7 CAR Design

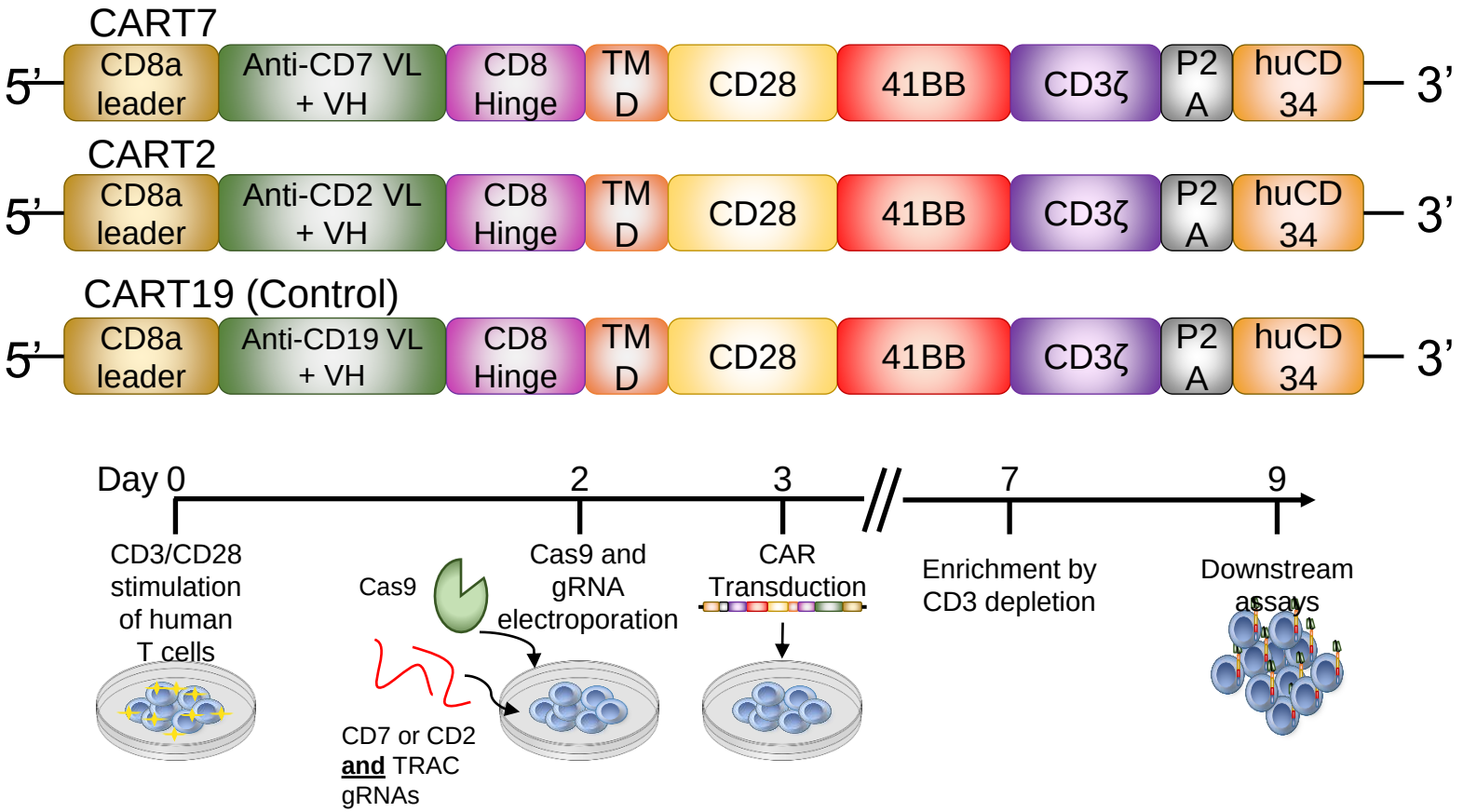
- **CD7 as a target.**
  - Expressed on 98% of T-ALL
  - Expressed on 24% of AML.
  - Expressed on NK cells and T cells.
  - CD7-/- mice have normal T and NK function

- **CAR Design**
  - 3rd generation CAR
  - Anti CD7 scFv
  - CD3ζ signaling domain
  - 4-1BB, CD28 costimulatory domains
  - CD34

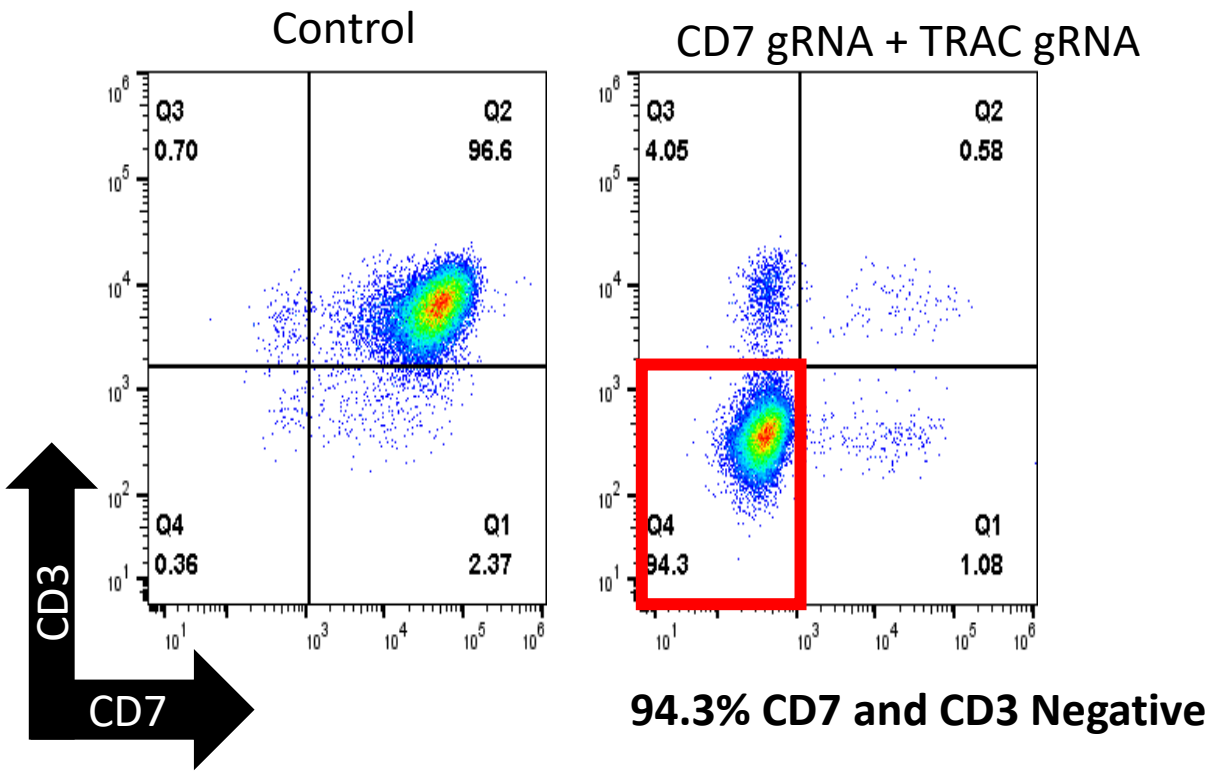
- **Gene editing**
  - CRISPR/Cas9



CAR construct and multiplexed CRISPR/Cas9 transfection strategy



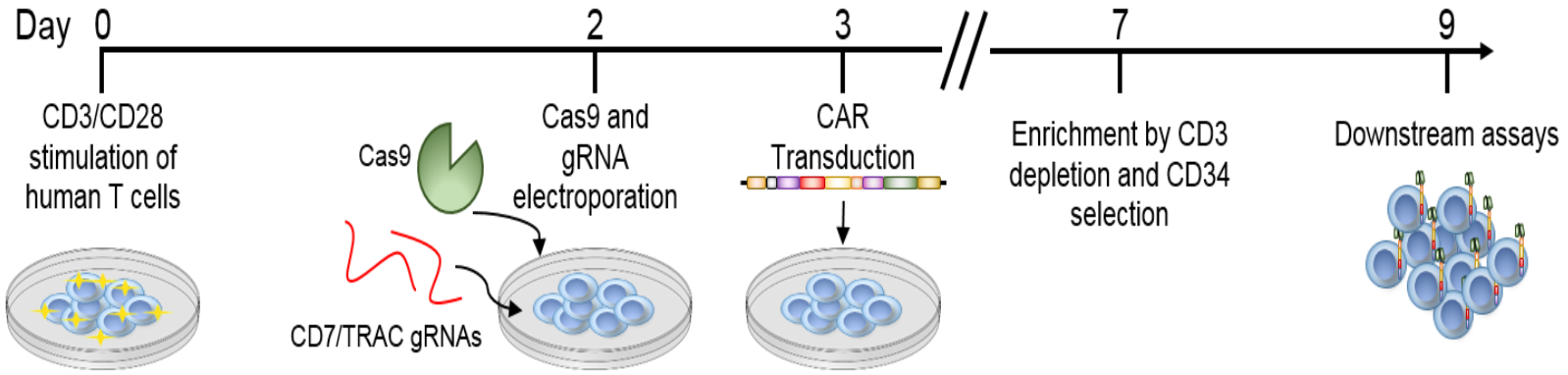
UCART7: Simultaneous deletion of CD7 and TRAC

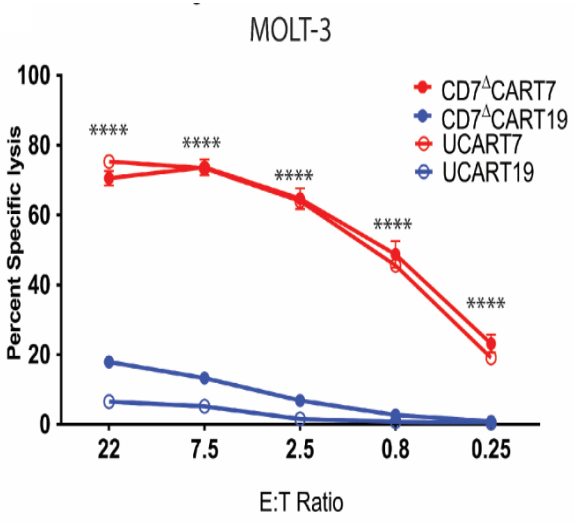


94.3% CD7 and CD3 Negative

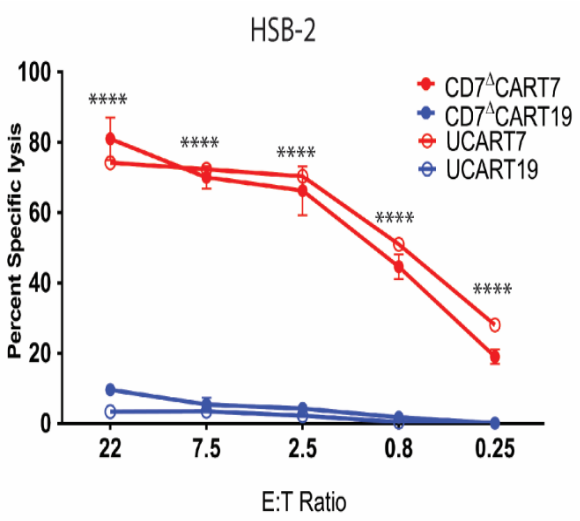
MaxCyte GT clinical grade electroporation

UCART7 effectively kills T-ALL cell lines in vitro

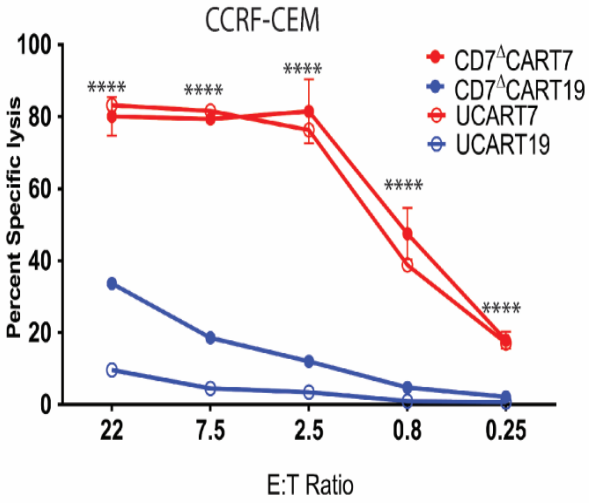




96%
CD7+

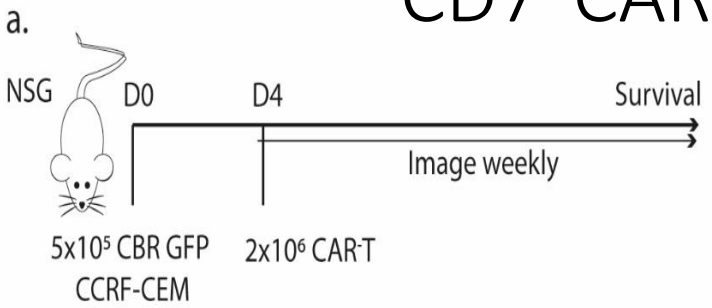


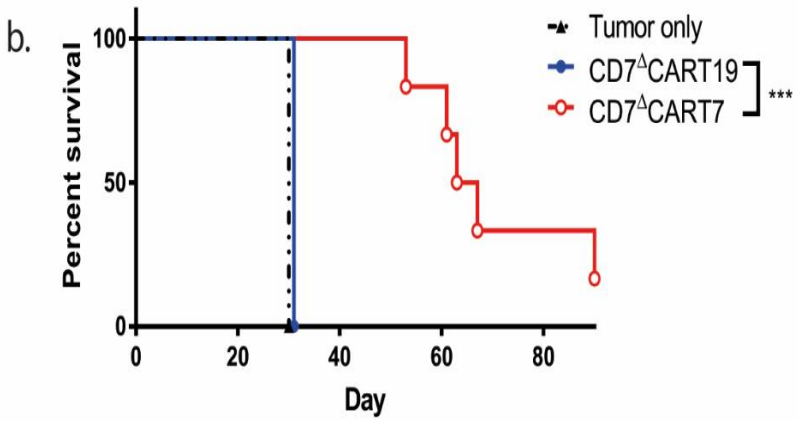
99%
CD7+

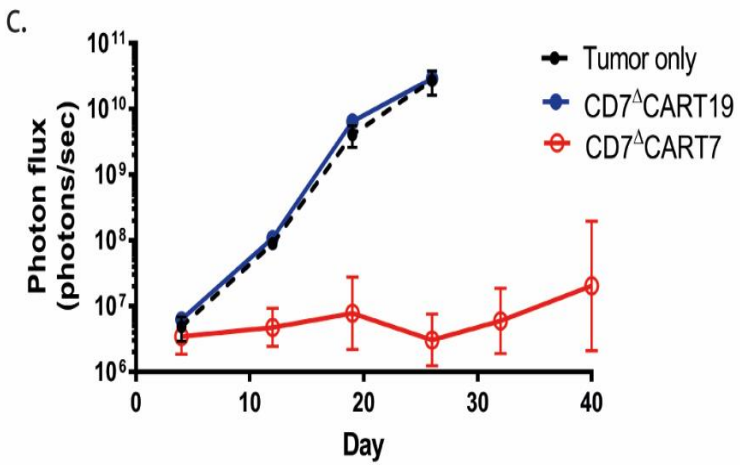


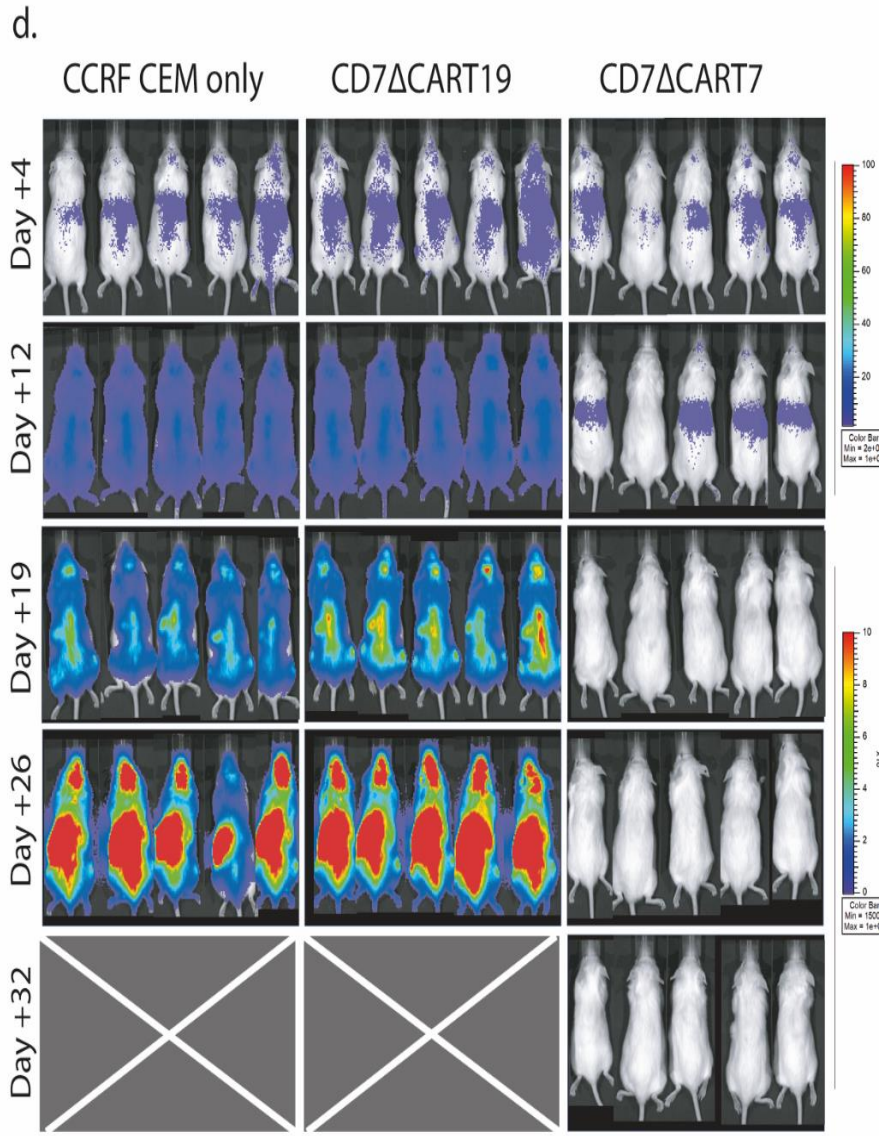
99%
CD7+

CD7ΔCART7 kills CCRF-CEM in vivo

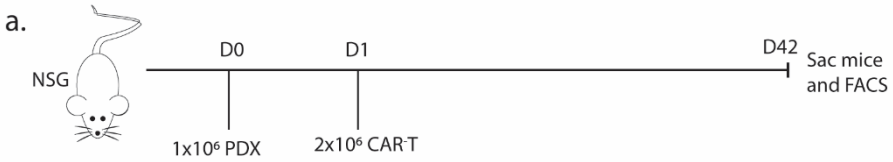


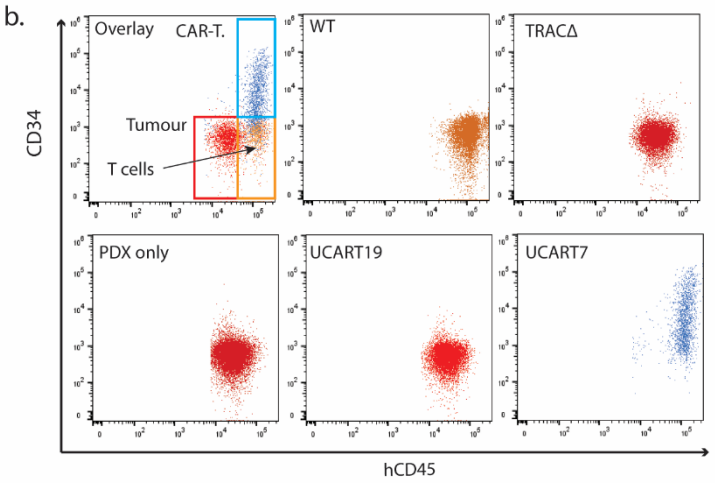


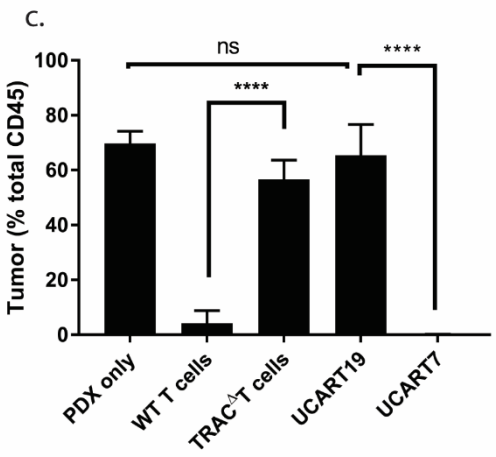


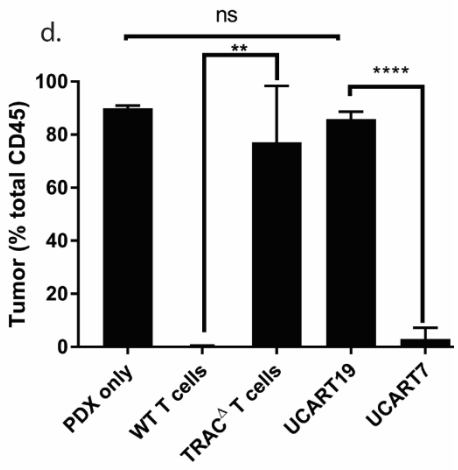


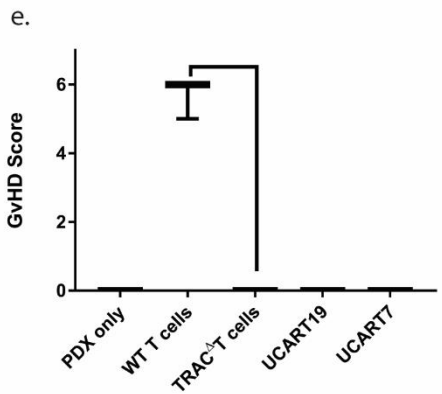
UCART7 effectively kills primary T-ALL in vivo

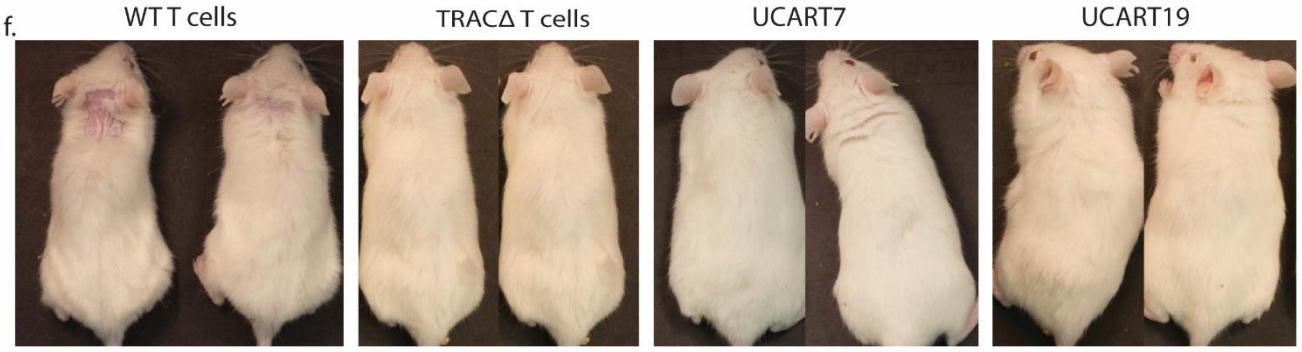












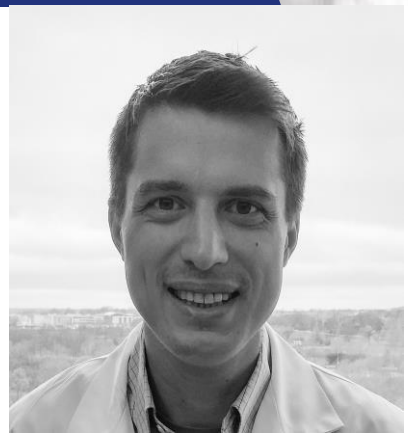
Unbiased genome wide off-target nuclease activity

GUIDE-seq

		CD7g4		TRACg		CD7g+TRACg	
position	Gene	total reads (mean)	# of replicates	total reads (mean)	# of replicates	total reads (mean)	# of replicates
Chr17:80274575	CD7	501	3	-	-	326	3
Chr14:23016519	TRAC	-	-	2843	3	1171	3
Chr7:155492215	RBM33 (Intron)	-	-	-	-	739	3

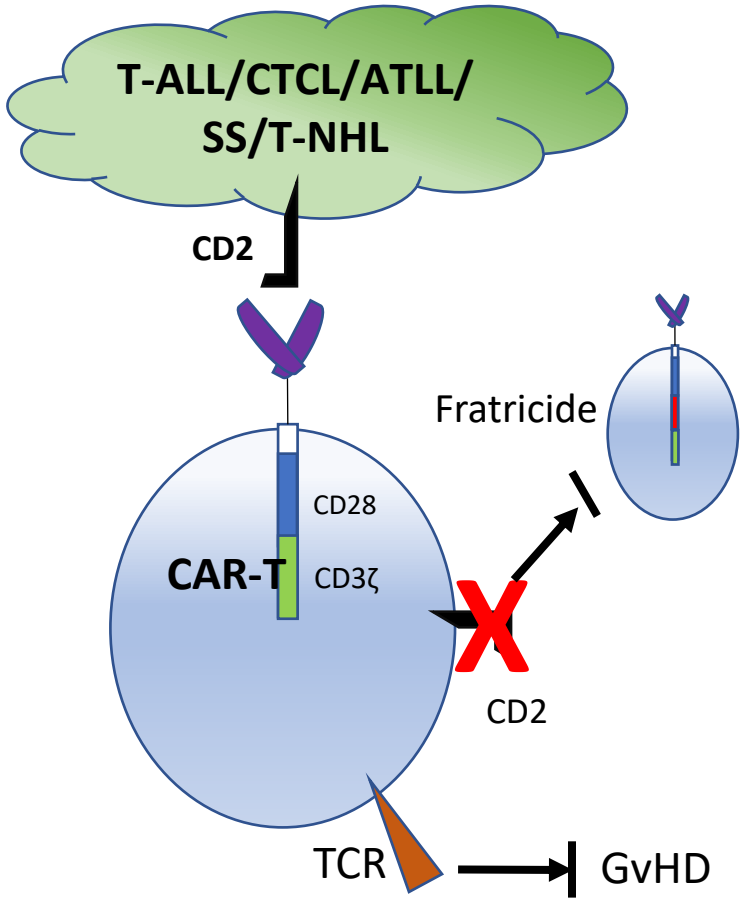
dsODN capture

		CD7g4		TRACg		CD7g+TRACg	
position	Gene	total reads (mean)	# of replicates	total reads (mean)	# of replicates	total reads (mean)	# of replicates
Chr17:80274575	CD7	2268	3	-	-	2163	3
Chr14:23016519	TRAC	-	-	22632	3	22810	3



Karl Staser

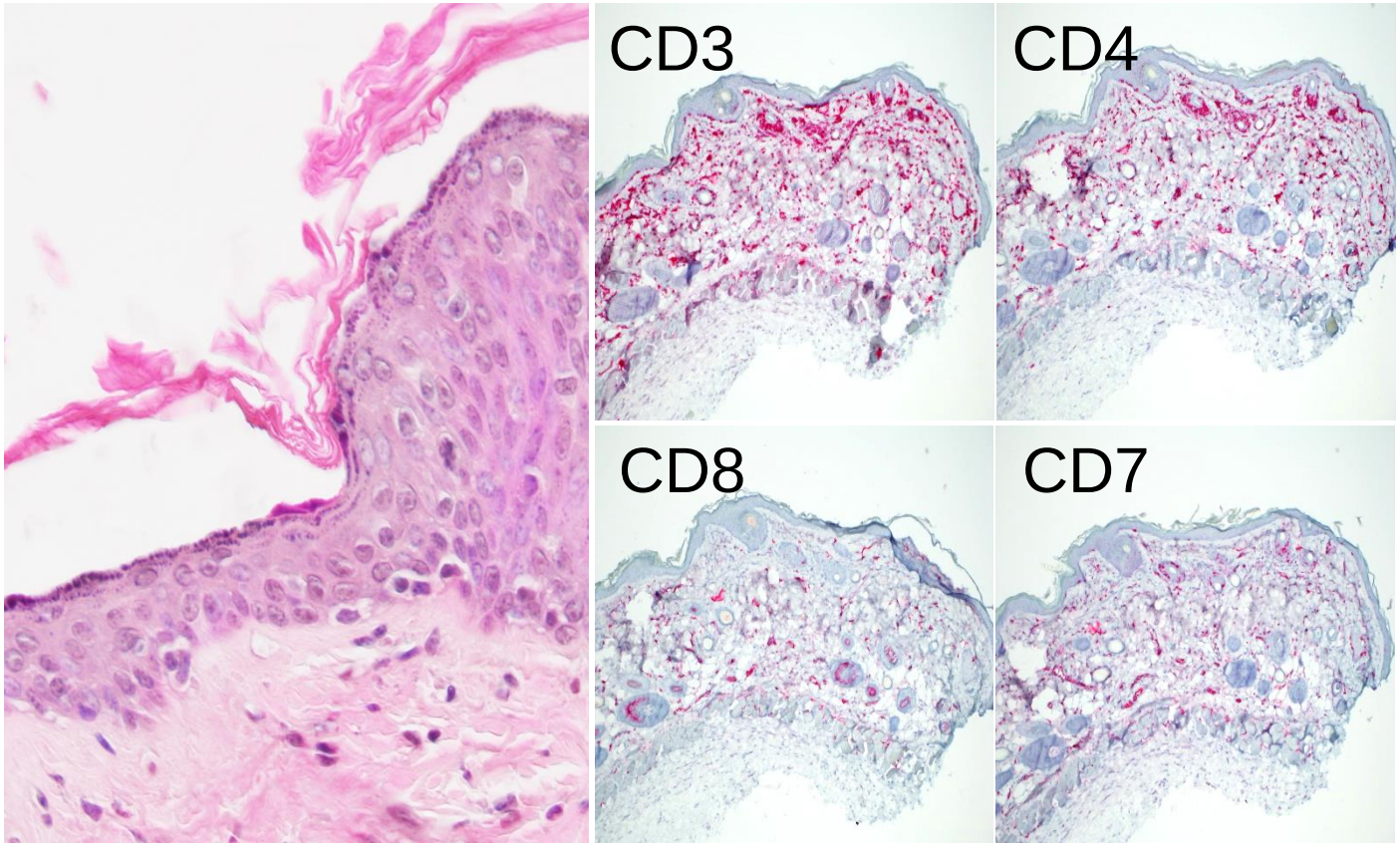
Phase 2: UCART2



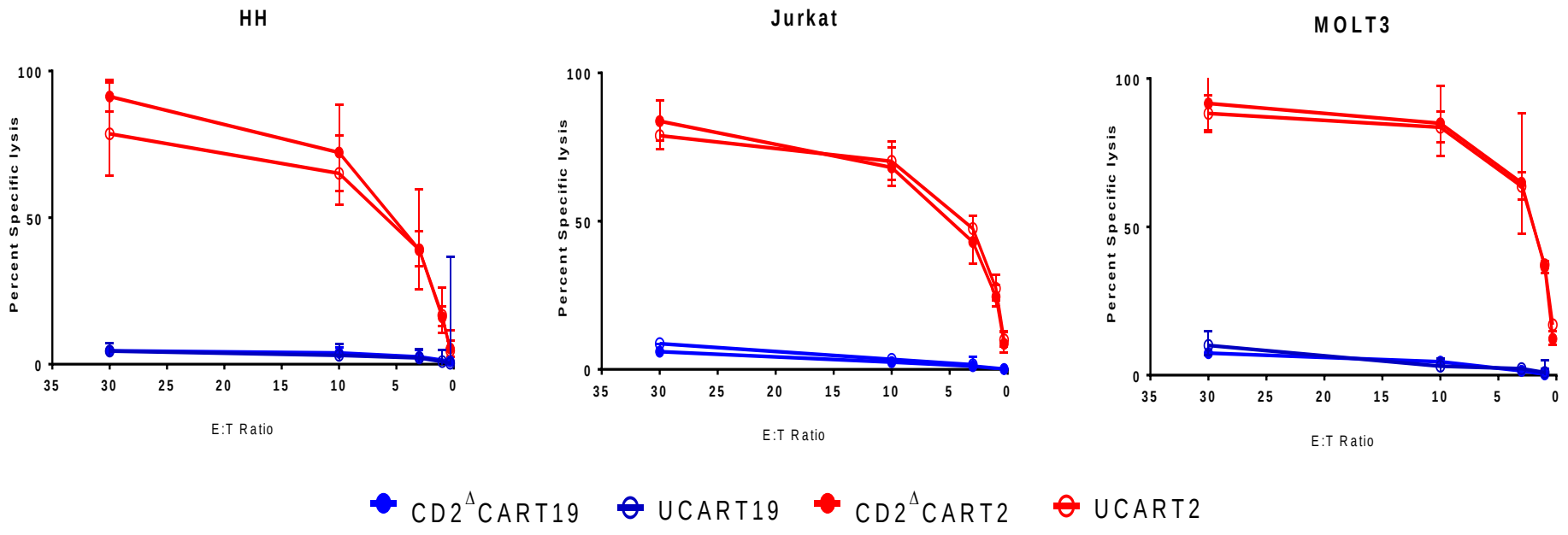
- Develop ‘off the shelf’ fratricide and GvHD resistant CAR-T targeting CD2 (UCART2).
- CD2 is highly expressed on T-ALL and unlike CD7, CD2 is highly upregulated on mature T cell and NK cell malignancies.
- Alternative therapy should UCART7 result in the selection of CD7 negative, UCART7 refractory, escape variants.

PDX phenotype recapitulates human SS

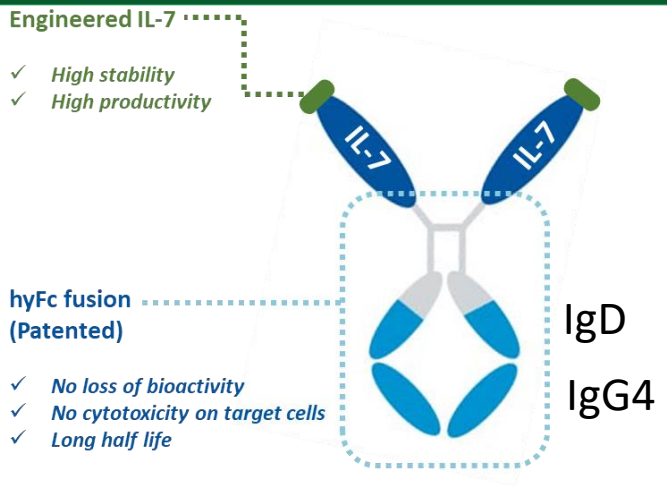
$CD3/4^{+}CD7/8^{lo/-}$ cells engraft PDX skin

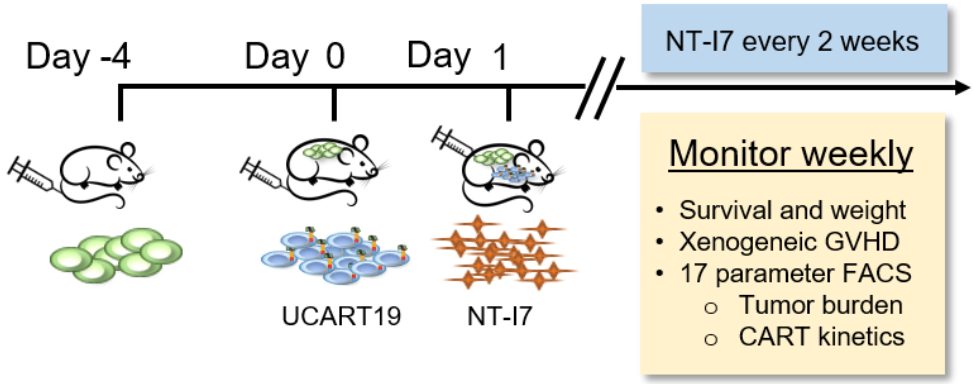


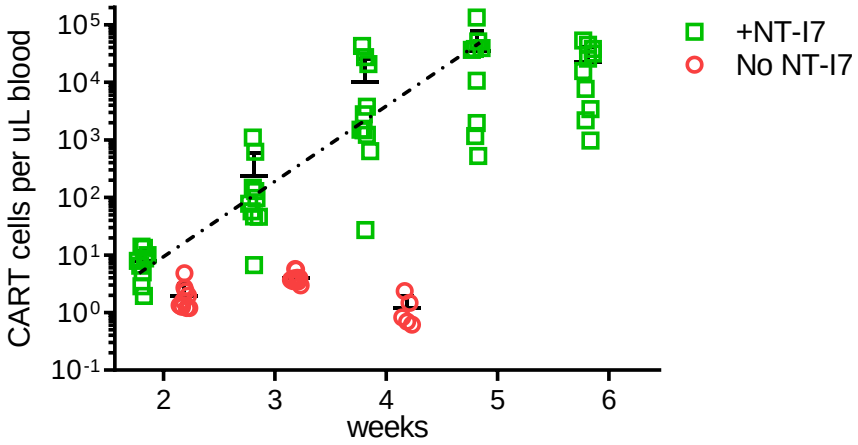
UCART2 effectively kills T cell malignancies in vitro.

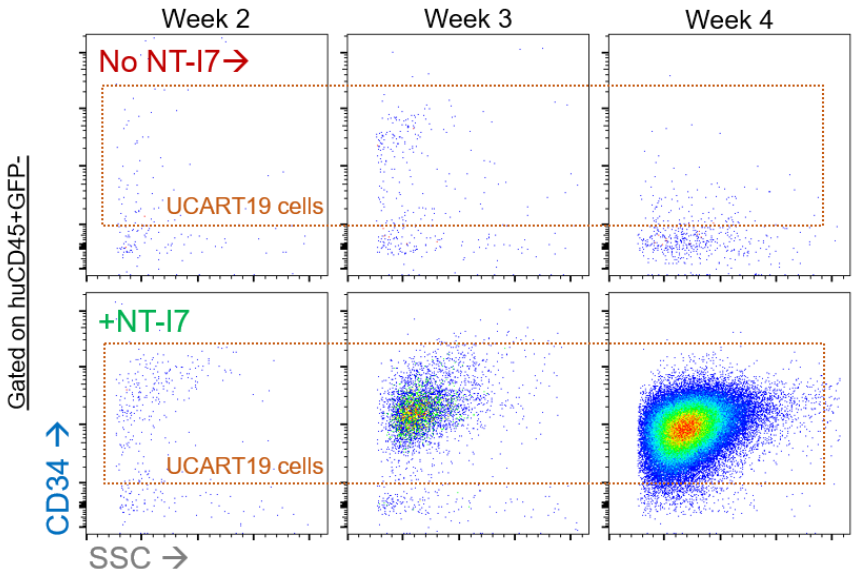


Enhancing persistence and efficacy of CART

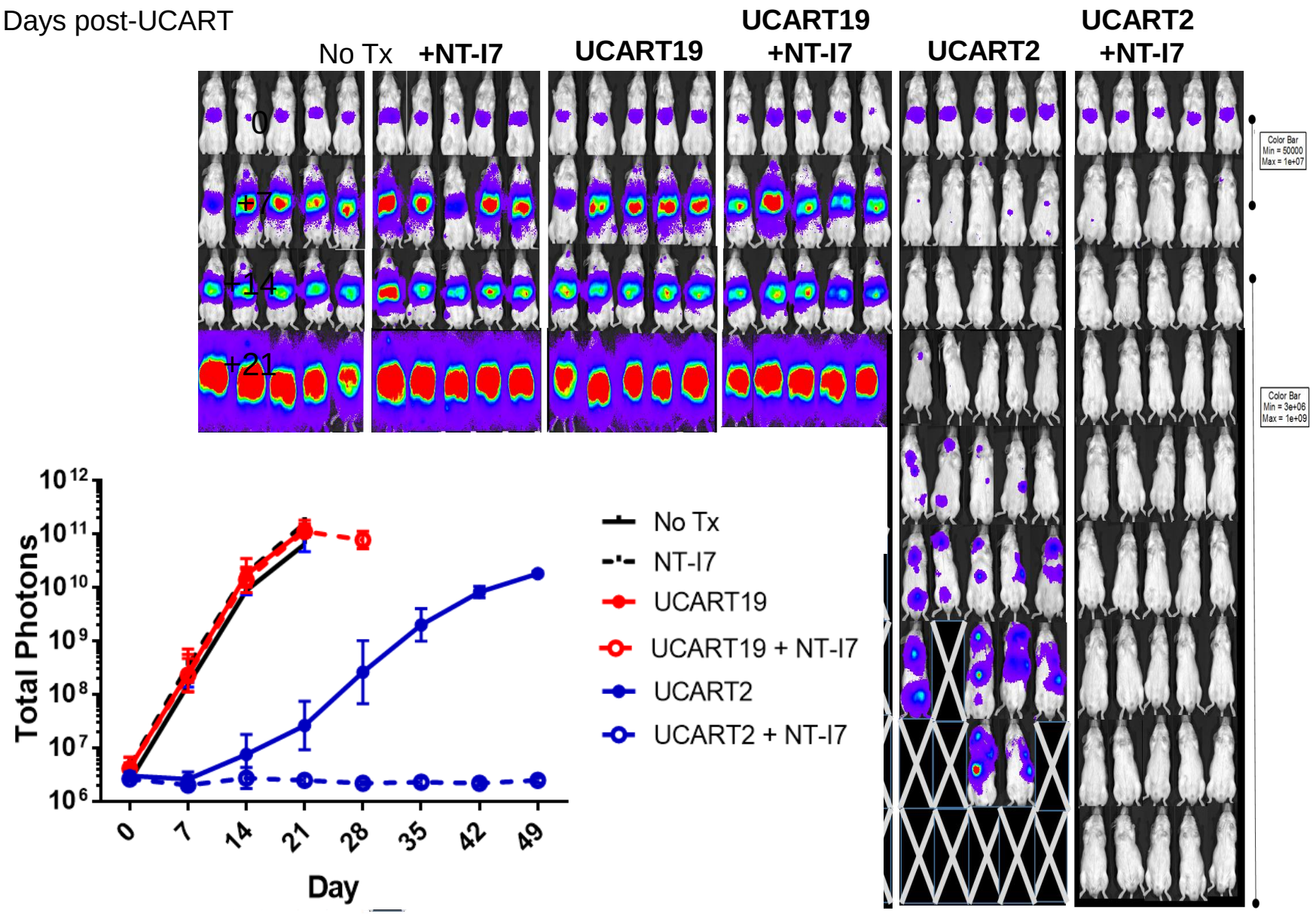






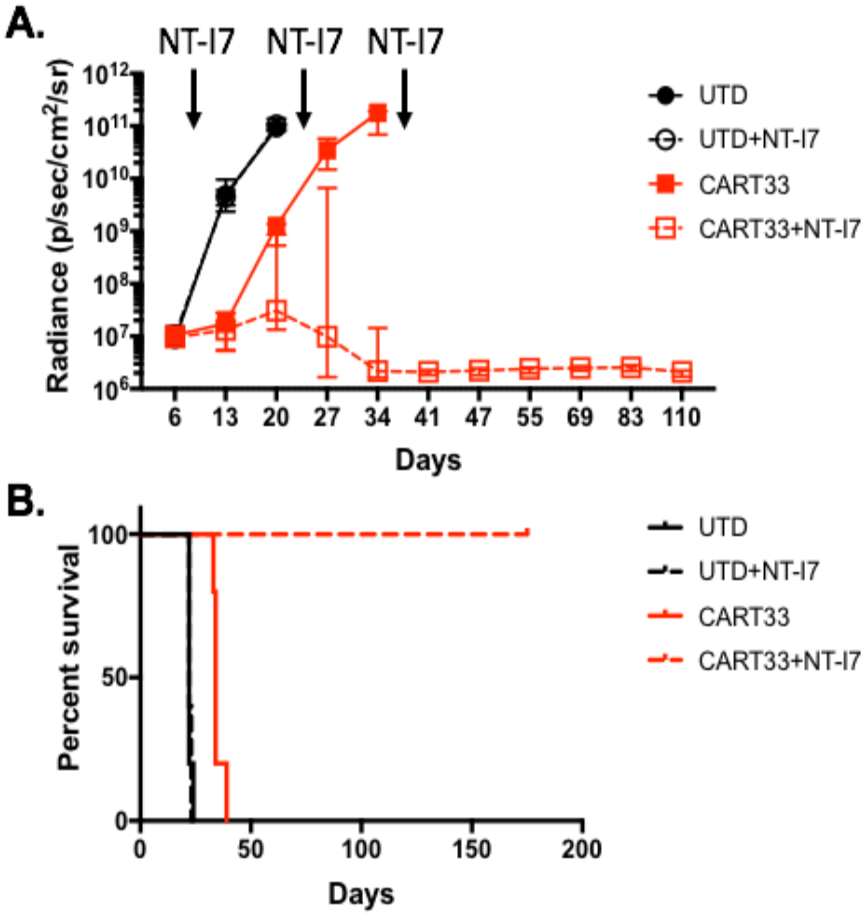


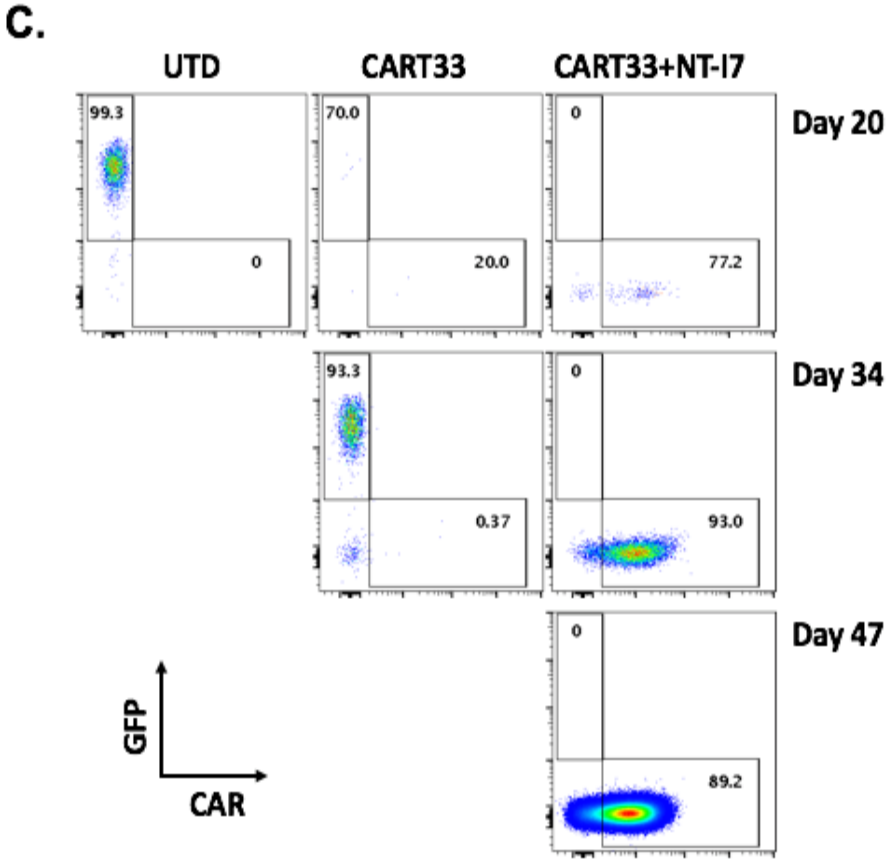
UCART2 and NT-I7 completely abolish CTCL tumor



In vivo effect of NT-I7 on UCART33

NSG mice were injected with 5x10^4 luciferase+ U937 AMLcells





Kim et al, unpublished

Phase 1b Trial of NT-I7 + Yescarta in Patients with r/r DLBCL

Screening & Enrollment → CAR-T Production → YESCARTA Infusion → On Treatment

SOC YESCARTA Management

Baseline | Wk 1 (D7) | Wk 2 (D9, D12, D14) | Wk 3 (D21) | Wk 4 (D28) | Wk 5 (D30, D35) | Wk 6 (D42) | Wk 7→ (D49, D100)

NT-I7 (rhIL-7-hyFc) IM injection

FDG PET CT and plasma Ig-HTS

Specimen Collection (FACS, qPCR, Cytokines)

Dose Level	NT-I7 Dose
-2	60 mcg/kg
-1	120 mcg/kg
1 (*start)	240 mcg/kg
2	360 mcg/kg
3	540 mcg/kg
4	720 mcg/kg

Standard 3+3 Design

*Dose Level 1

No DLTs → Dose Level 2

Excess DLTs → Dose Level -1

MTD or Dose Level 4

Conclusions

- High efficiency multiplex CRISPR/Cas9 gene editing of human primary T cells.
- UCART7 is effective at killing T-ALL cell lines, and primary T-ALL in vitro and in vivo.
- UCART7 does not cause xenogeneic GvHD.
- UCART7 overcomes barriers of autologous T cell collection, without risk of creating therapy resistant malignant clones.
- This platform can be adapted to target other T cell antigens
- Single traditional CART product is autologous and treats one patient from one apheresis. A single apheresis for preparation of universal UCART can treat 150-200 patients.

Acknowledgments

DiPersio Lab

- Mike Rettig
- Matt Cooper
- JaeBok Choi
- Karl Staser
- Matt Christopher
- Darja Karpova
- Linda Eissenberg
- Julie Ritchey

McDonnell Genome Institute

- Tim Ley
- Christopher A. Miller
- Allegra Petti

Dana Faber Cancer Institute

- David M. Weinstock
- Alejandro Gutierrez

NeoImmunetech, Macrogenics

- Jaehan Park, Ezio Bonvini

NIH/NCI P50 Molecular imaging grant CA094056-09
NIH/NCI Leukemia SPORE P50 CA171063-01
NIH/NCI R35 CA152329
NIH/NCI R01 CA 141523-01
NIH/NCI SCC CCSG P30 CA91842-01
NIH/NCIAML P01 CA101937
NIH/NCI U54 CA199092

Amy Strelzer Manasevit Research Program
St Baldrick's Foundation
Siteman CC Investment Program (SIP)
Gabrielle's Angel Foundation
Siteman Innovators Award
Children's Discovery Institute
Leukemia Lymphoma Society of America