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T-cell engineering for CAR-T Therapy

Carl June

May 20, 2025

Consultant for: AC Immune, BluesphereBio, Cabaletta, Cartography, Cellcarta, Cellares, Celldex, Danaher, Decheng, Dispatch Bio, Genscript, Replay Bio, Verismo, ViTToria Bio, WIRB

Scientific co-founder and Stockholder in: Tmunity Therapeutics/Kite Gilead, Dispatch Bio, Capstan Therapeutics, Bluewhale Bio

First Idea of Gene Therapy

Gene Therapy for Human Genetic Disease?

Proposals for genetic manipulation in humans raise difficult scientific and ethical problems.

Theodore Friedmann and Richard Roblin

3 March 1972, Volume 175, Number 4025

SCIENCE

Chimeric

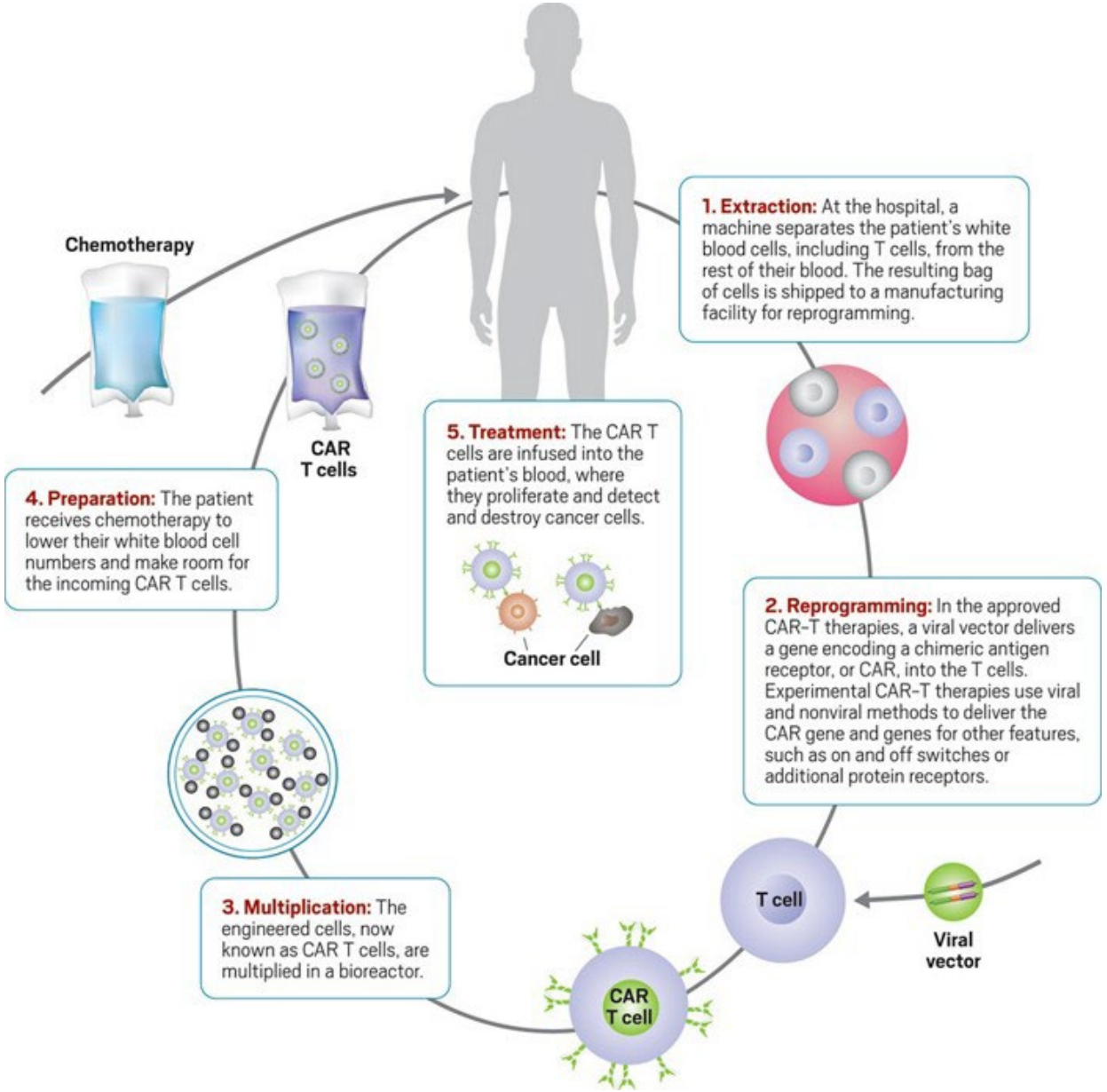
Antigen

Receptor

T Cells

CAR T Cells

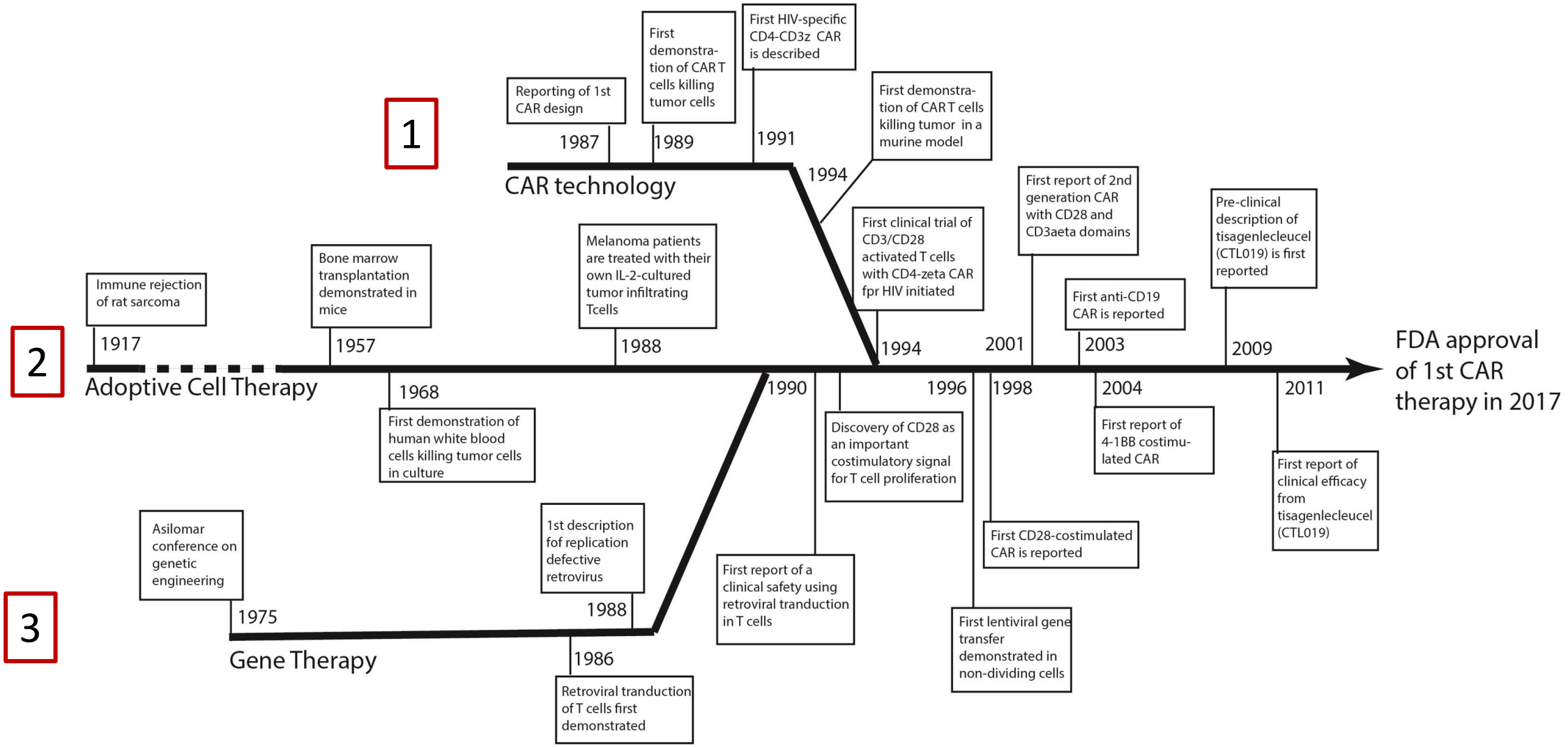
CAR T Cell Therapy: a process not a drug



- **Autologous T cells**
- **Allogeneic "3rd party" T cells**
  - **Cord blood**
  - **Healthy donor**
  - **iPSC**

https://cen.acs.org/pharmaceuticals/oncology/Controlling-CAR-T-scientists-plan/96/i19

The Genealogy of CAR T Cells



Ben Philipson, MD PhD

CT019 (tisagenlecleucel)

FDA approval August 30,2017

The NEW ENGLAND JOURNAL of MEDICINE

Porter, 2011
Grupp, 2013
Maude, 2014
Garfall, 2015
Schuster, 2017
Maude, 2018

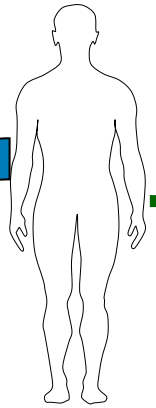
July 31, 2010
1st CART19 Infusion

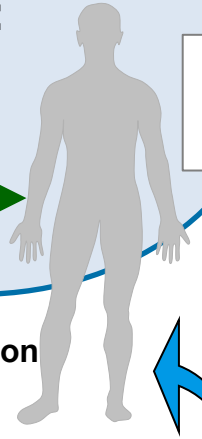
The New York Times

Patient Donates Cells

Pediatric Oncology: ALL

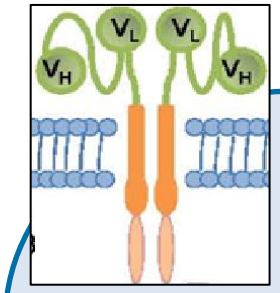
Cytokine Release Syndrome





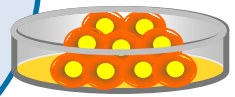
T cell transfusion

Adult Oncology: ALL, CLL, DLBCL, MCL, Myeloma

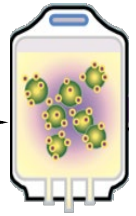


Genetic engineering

Synthetic Biology

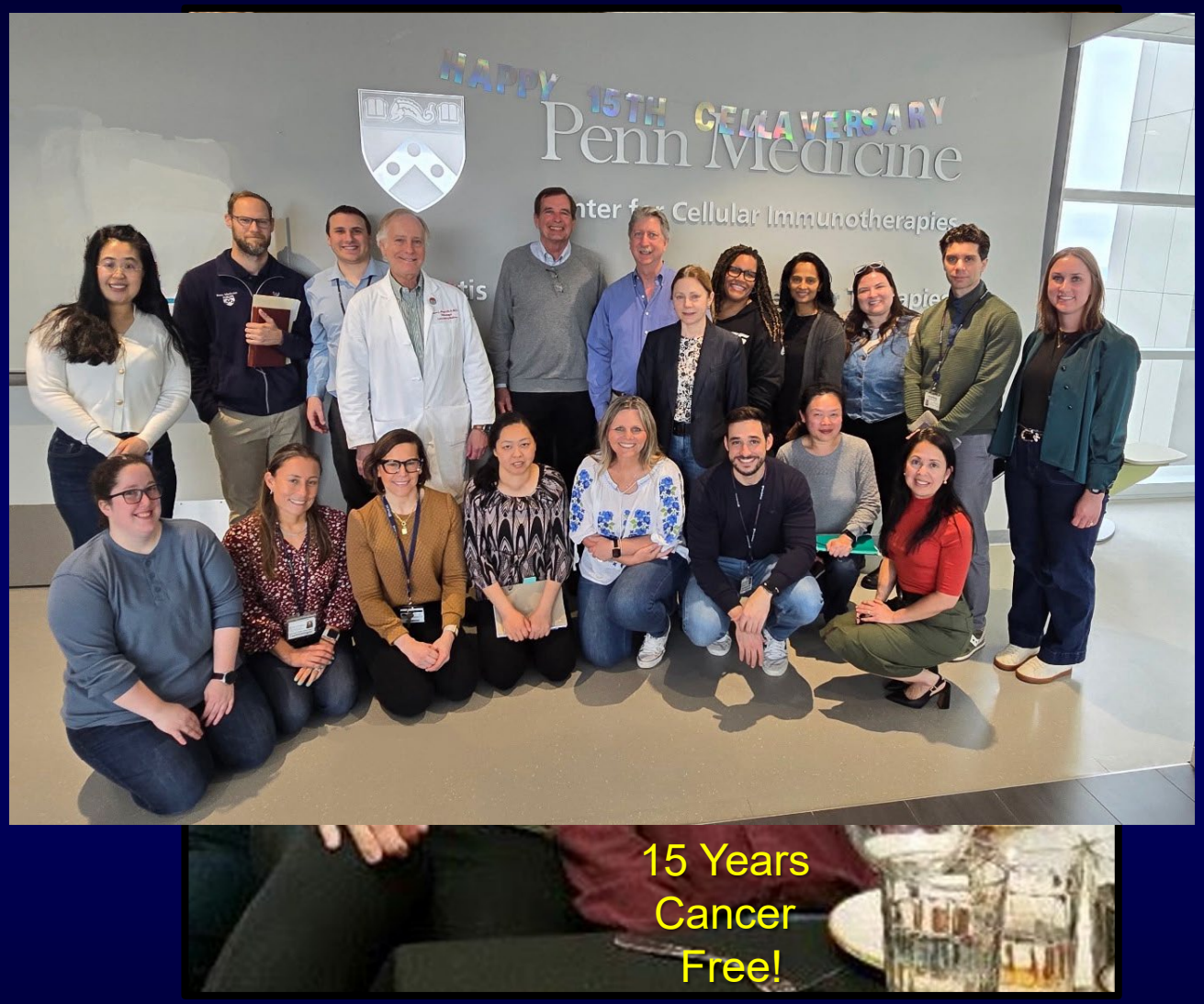


Expand T cells



CVPF

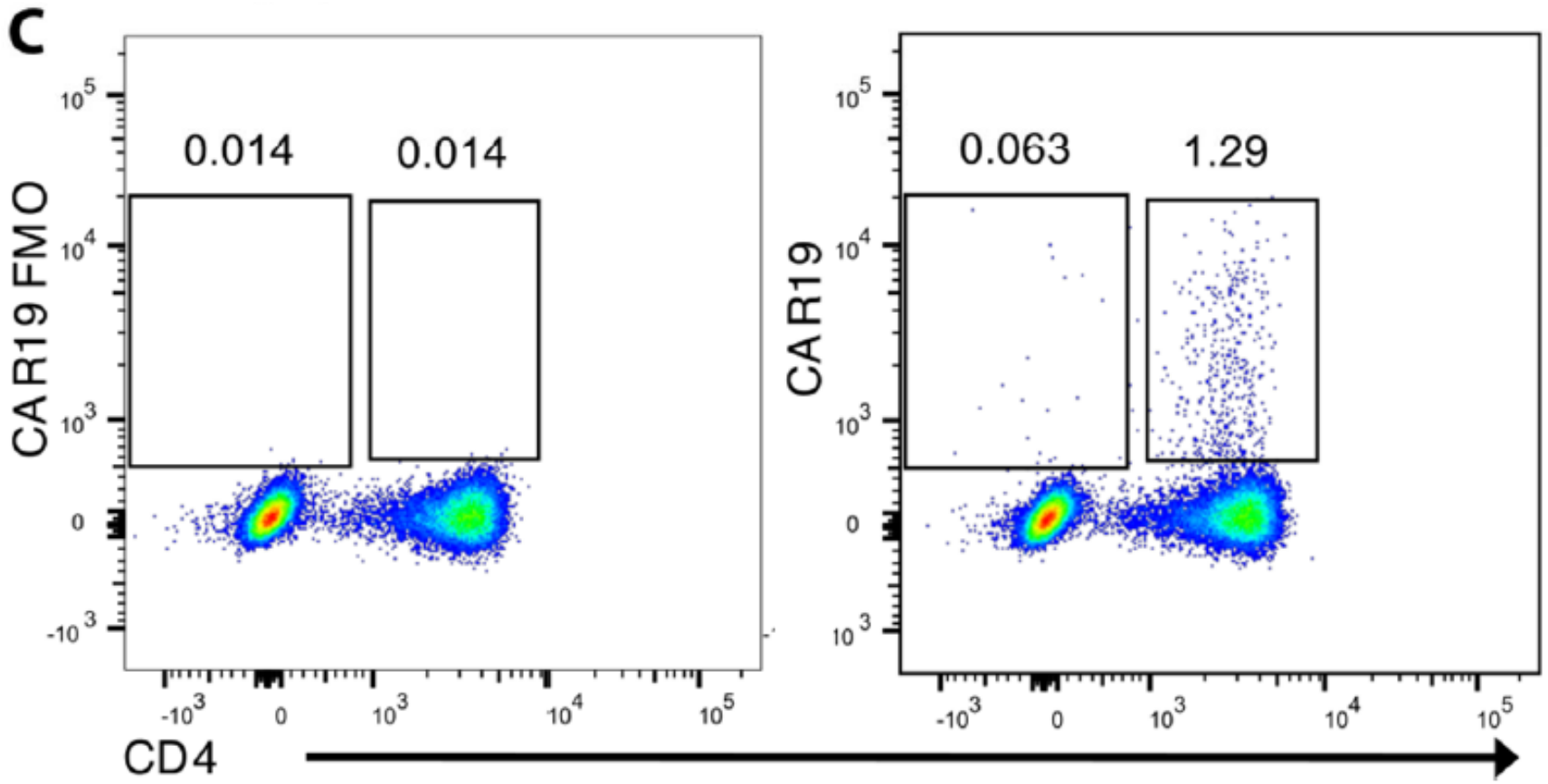
r/r CLL (2010)



r/r ALL (2012)

FDA approval 2017

CTL019 Is Expressed on the Cell Surface in CLL patient #1 10 years after infusion

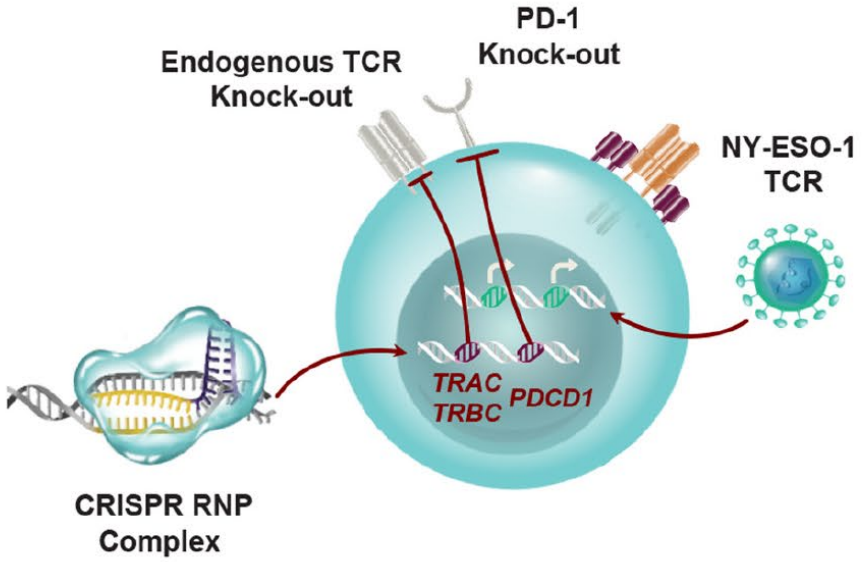


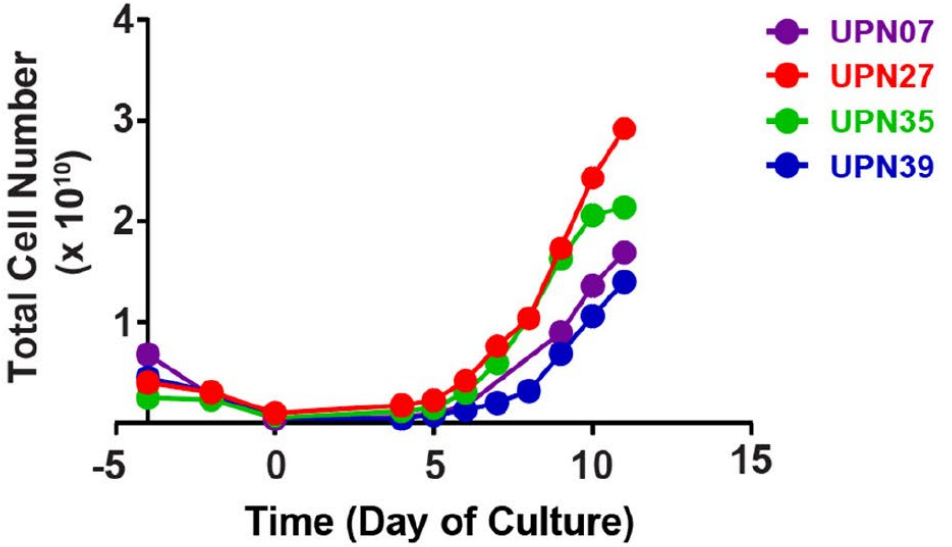
Melenhorst, Nature 2022

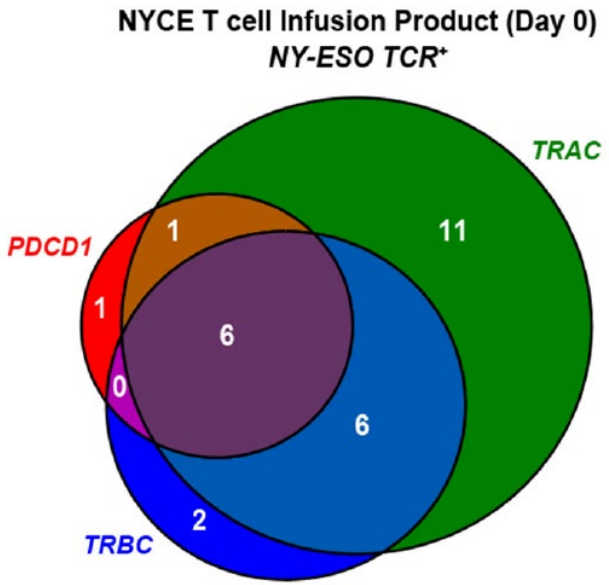
CAR T in Hematologic Malignancies: summary

1. Many FDA approvals for many blood cancers using CD19 and BCMA specific CAR T cells
2. More than 50,000 patients have been treated world-wide with commercial and academic products. Rare cases of T cell transformation with autologous CAR T cells
3. Allogeneic CAR T cells and NK CAR T are in early stage trials. Safety profile of allogeneic cells remains to be established
4. Combinations of CAR T w targeted agents: CD19 CAR T + ibrutinib example
5. Progress in commercial scale out of cell manufacturing
    1. Automation
    2. Vein to vein time
    3. Multiplex human genome editing safe and feasible

Defining the Role of Multiplex Genome Editing in CAR T and TCR T





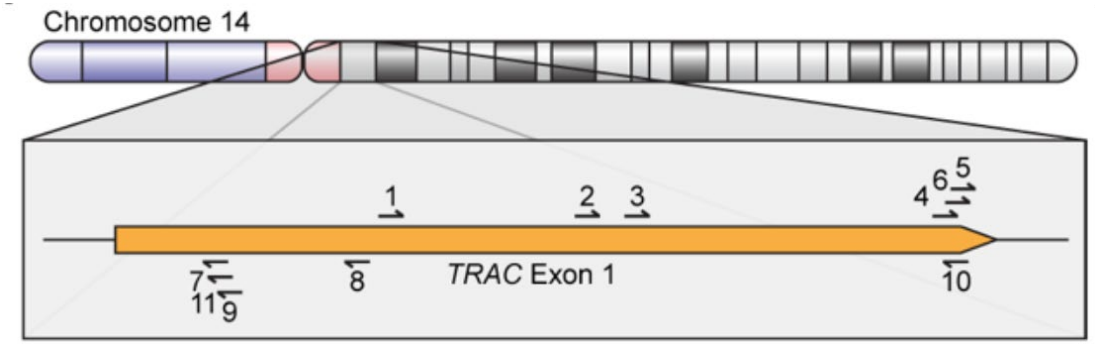


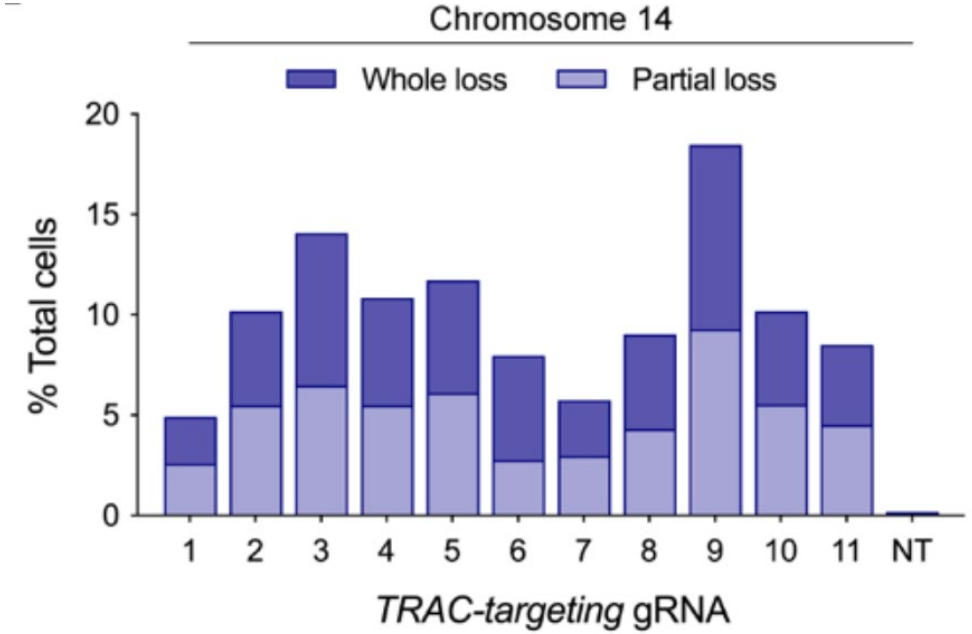
- Feasibility of large-scale manufacture with lentiviral modification and CRISPR/Cas9 RNP electroporation was demonstrated
- Multiplex editing at the level of a single human genome is safe and feasible in T cells
- Off-target edits were rare, however chromosomal translocations were detected.

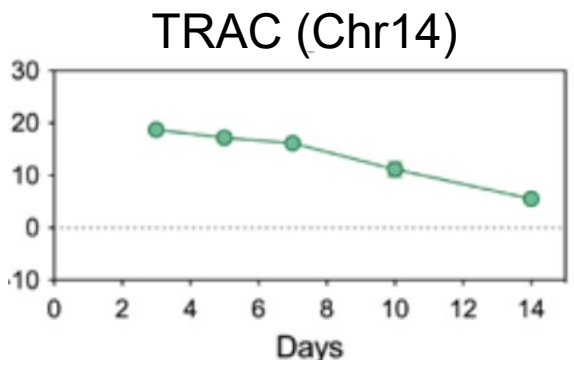
  => Cells with translocations had decreased fitness

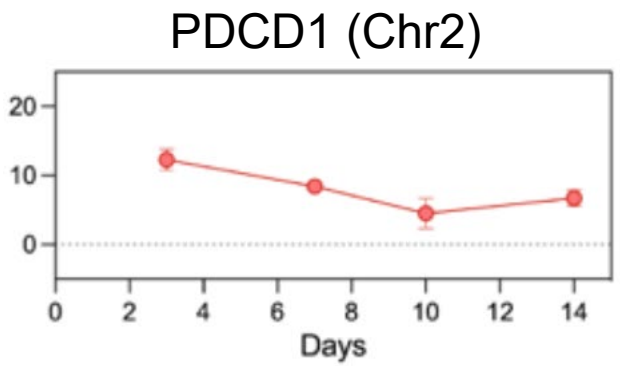
Stadtmauer et al, Science 2020

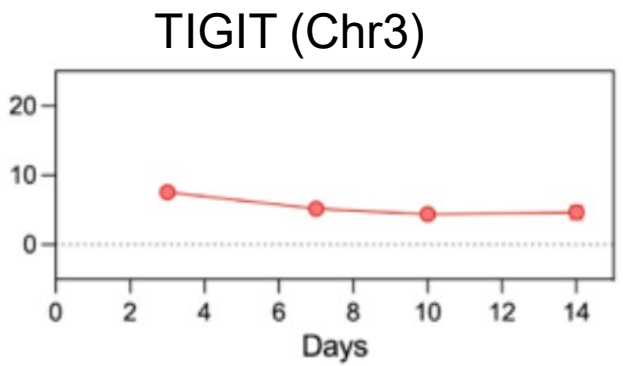
CRISPR-Cas9 genome editing of TRAC results in chromosome loss





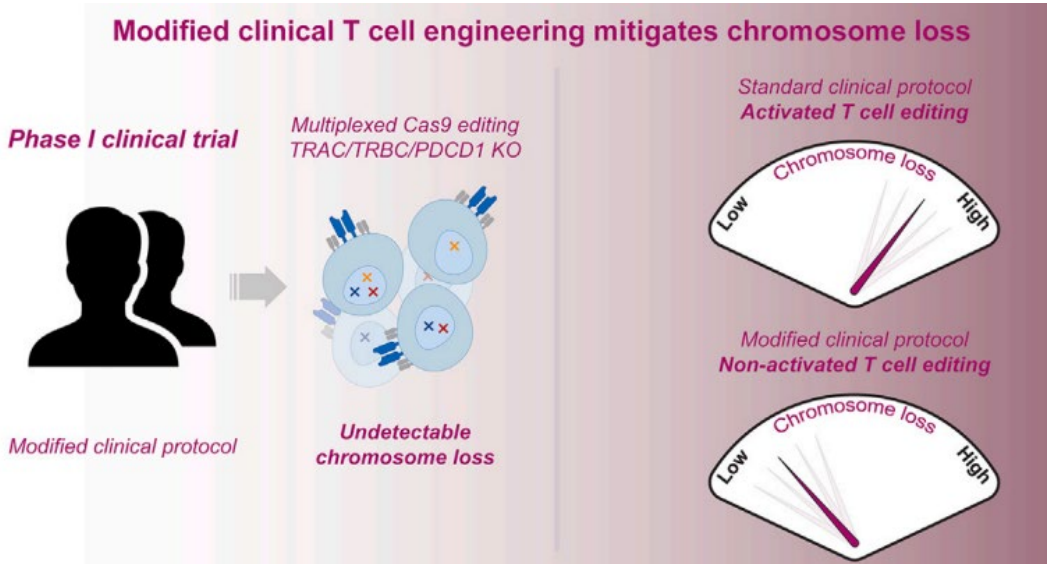






Tsuchida et al, Cell, 2023

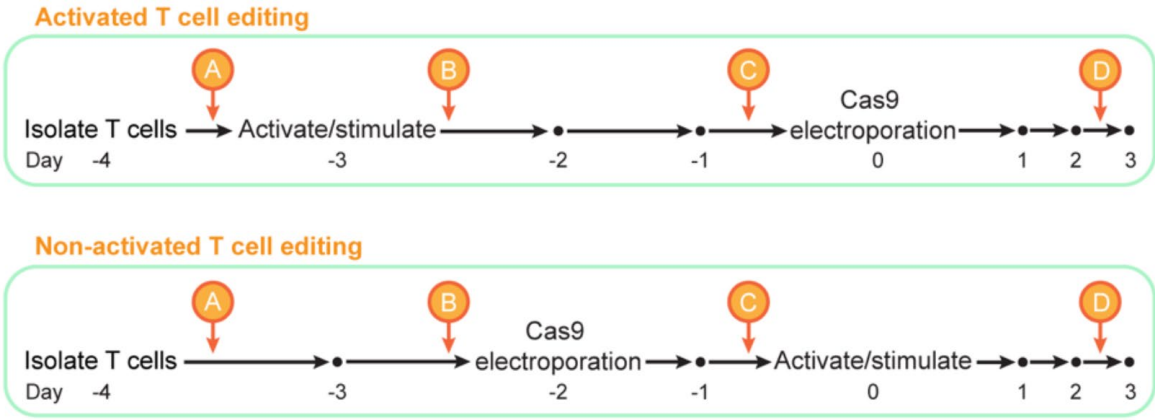
Engineering T Cells: Details Matter

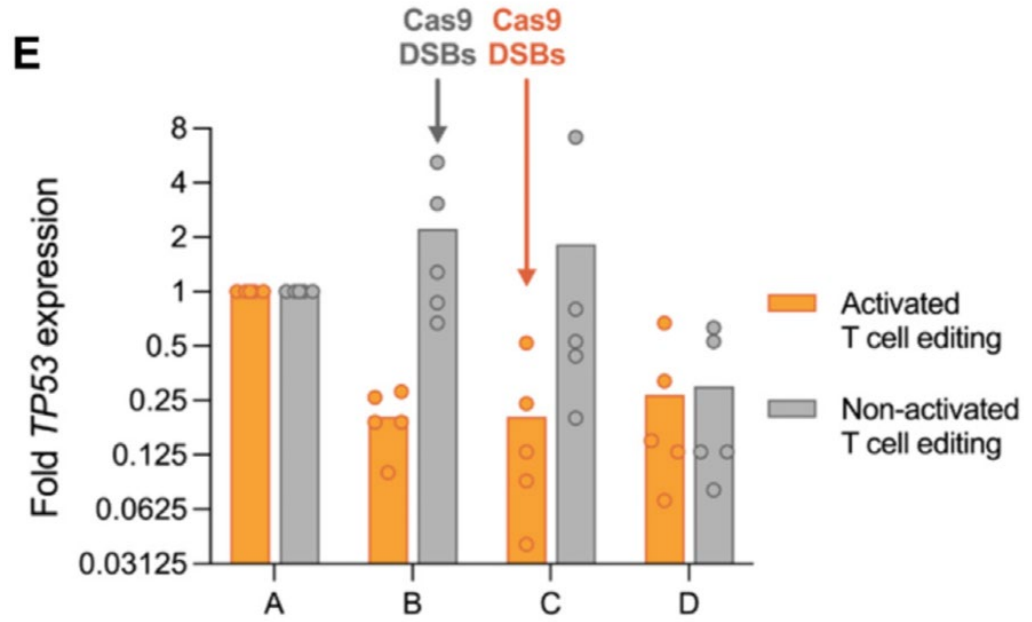


Highlights

- Cas9 genome editing in T cells results in unintended but targeted chromosome loss
- Chromosome loss from T cell genome editing is generalizable across target sites
- Cas9-induced chromosome loss persists for weeks in cultured T cells
- A modified protocol mitigates chromosome loss in T cells used for a clinical trial

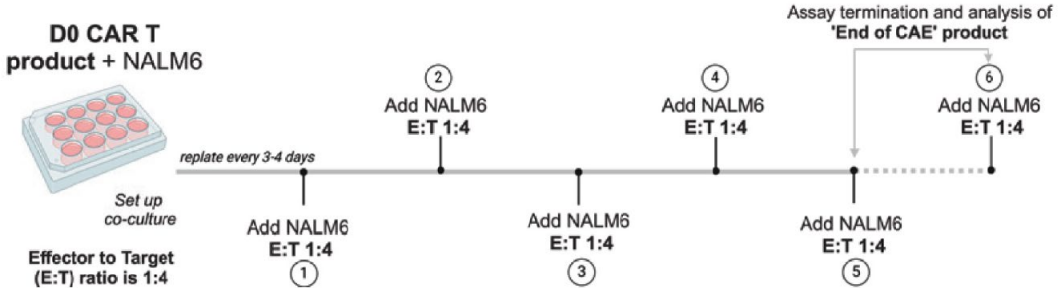
Expression of p53 correlated with protection from chromosome loss

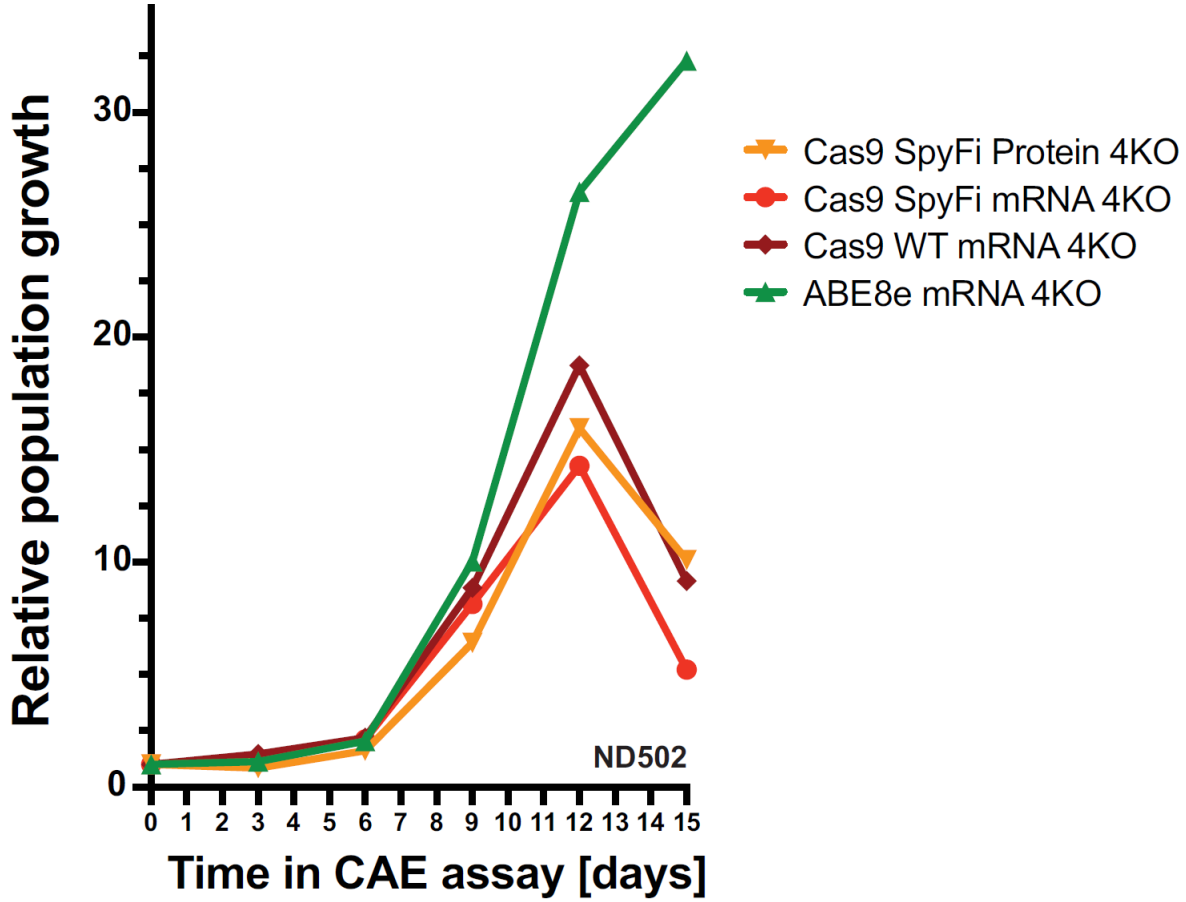




Tsuchida et al, Cell (2023)

Quadruple Adenine-Base Edited CAR T Superior to CRISPR/Cas9





PNAS RESEARCH ARTICLE | IMMUNOLOGY AND INFLAMMATION

Quadruple adenine base–edited allogeneic CAR T cells outperform CRISPR/Cas9 nuclease–engineered T cells

Nils W. Engel[a], Israel Steinfeld[b], Daniel Ryan[b], Kusala Anupindi[a], Samuel Kim[a], Nils Wellhausen[a,c,d], Linhui Chen[e], Katherine Wilkins[b], Daniel J. Baker[a,f,g], Philipp C. Rommel[a,f], Danuta Jarocha[a], Mercy Gohil[a], Qian Zhang[a,f], Michael C. Milone[a,f], Joseph A. Fraietta[a,f,h,i], Megan Davis[a], Regina M. Young[a,f], and Carl H. June[a,f,1]

CAR Cells Move Beyond Oncology

- **CAR T Cells for HIV/AIDS**

JCI The Journal of Clinical Investigation

CCR5-edited CD4⁺ T cells augment HIV-specific immunity to enable post-rebound control of HIV replication

Pablo Tebas, … , Carl H. June, James L. Riley

J Clin Invest. 2021;131(7):e144486. https://doi.org/10.1172/JCI144486.

- **CAR T Cells for autoimmunity and organ transplantation**

nature biotechnology LETTERS
https://doi.org/10.1038/s41587-020-0462-y
Check for updates

- **CAR macrophages for cancer**

Human chimeric antigen receptor macrophages for cancer immunotherapy

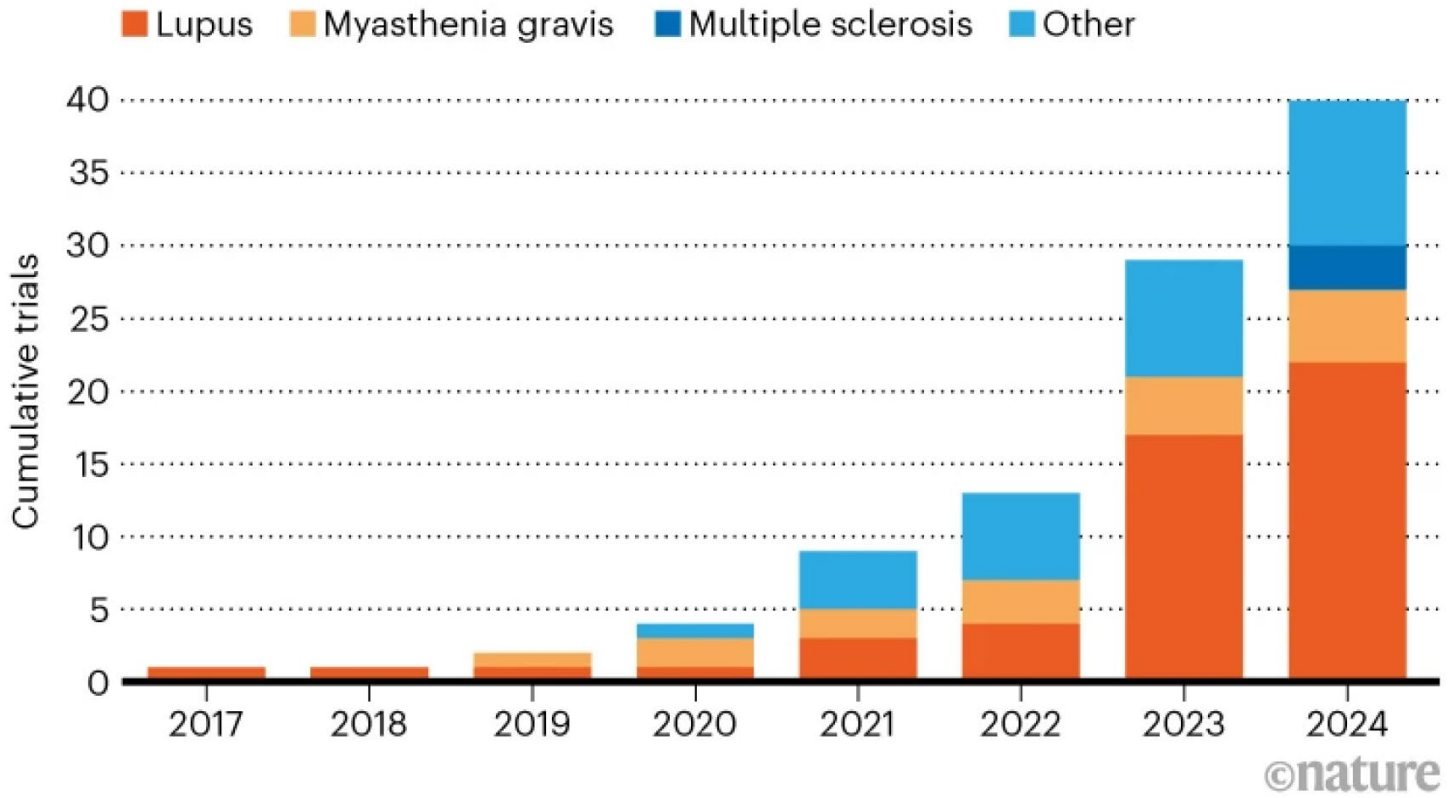
- **CAR T Cells for heart failure and fibrosis**

2024: Year of CAR T in Autoimmune Disease?

Daniel Baker

ENLISTING IMMUNE CELLS TO TREAT AUTOIMMUNE DISEASE

The number of clinical trials of CAR T cells — engineered immune cells — used to treat autoimmune disorders has grown rapidly over the past seven years. Testing of CAR-T therapy for the autoimmune disorder lupus accounts for the bulk of the trials.



Baker et al, Nature 2023

CAR T Lymphomas: FDA warning November 2023

U.S. FOOD & DRUG ADMINISTRATION

Home / Vaccines, Blood & Biologics / Safety & Availability (Biologics) / FDA Investigating Serious Risk of T-cell Malignancy Following BCMA-Directed or CD19-Directed Autologous Chimeric An

FDA Investigating Serious Risk of T-cell Malignancy Following BCMA-Directed or CD19-Directed Autologous Chimeric Antigen Receptor (CAR) T cell Immunotherapies

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Safety & Availability (Biologics)

Biologic Product Security

November 28, 2023

Summary of the Issue

The Food and Drug Administration (FDA) has received reports of T-cell malignancies, including chimeric antigen receptor CAR-positive lymphoma, in patients who received

The NEW ENGLAND JOURNAL of MEDICINE

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Secondary Cancers after Chimeric Antigen Receptor T-Cell Therapy

Nicole Verdun, M.D., and Peter Marks, M.D., Ph.D.

Article | Figures/Media | Metrics

January 24, 2024
DOI: 10.1056/NEJMp2400

5 References

SINCE THE FIRST SUCH PRODUCT WAS approved in 2017, chimeric antigen receptor (CAR) T-cell therapies have become important treatments for relapsed or refractory hematologic cancers, and the six products involving autologous CAR

NEJM CareerCente

PHYSICIAN JOBS

- 22 cases/8,000 in FAERS
  - Maybe >11,000 AER's
- 3 cases/11,345 in CIBMTR
- Cases in 5/6 approved CAR's
- 1 to 19 months post CAR
- FAERS and CIBMTR are voluntary
- >34,400 commercial CAR's
  - 27K in US
- 3 are CAR positive
- New "Black Box" warnings

Overall Low Risk of Secondary Malignancy Occurring from T-cell Engineering

Paper	Year	Number of Patients Studied	Number of Malignancies Linked with Probable Transgene Insertional Mutagenesis
Cordeiro et al. (PMID: 31419568)	2020	86	0
Ghilardi et al. (PMID: 38266761)	2024	449	0
Hamilton et al. (PMID: 38865660)	2024	724	0
Ozdemirli et al. (PMID: 38865661)	2024	1	1
Barone et al. (PMID: 38877876)	2024	651	0
Perica et al. (PMID: 39908432)	2025	1	1
Dulery et al. (PMID: 39779930)	2025	3066	1
Jadlowsky et al. (PMID: 39833408)	2025	783	0
Braun et al. (PMID: 39984633)	2025	1	1

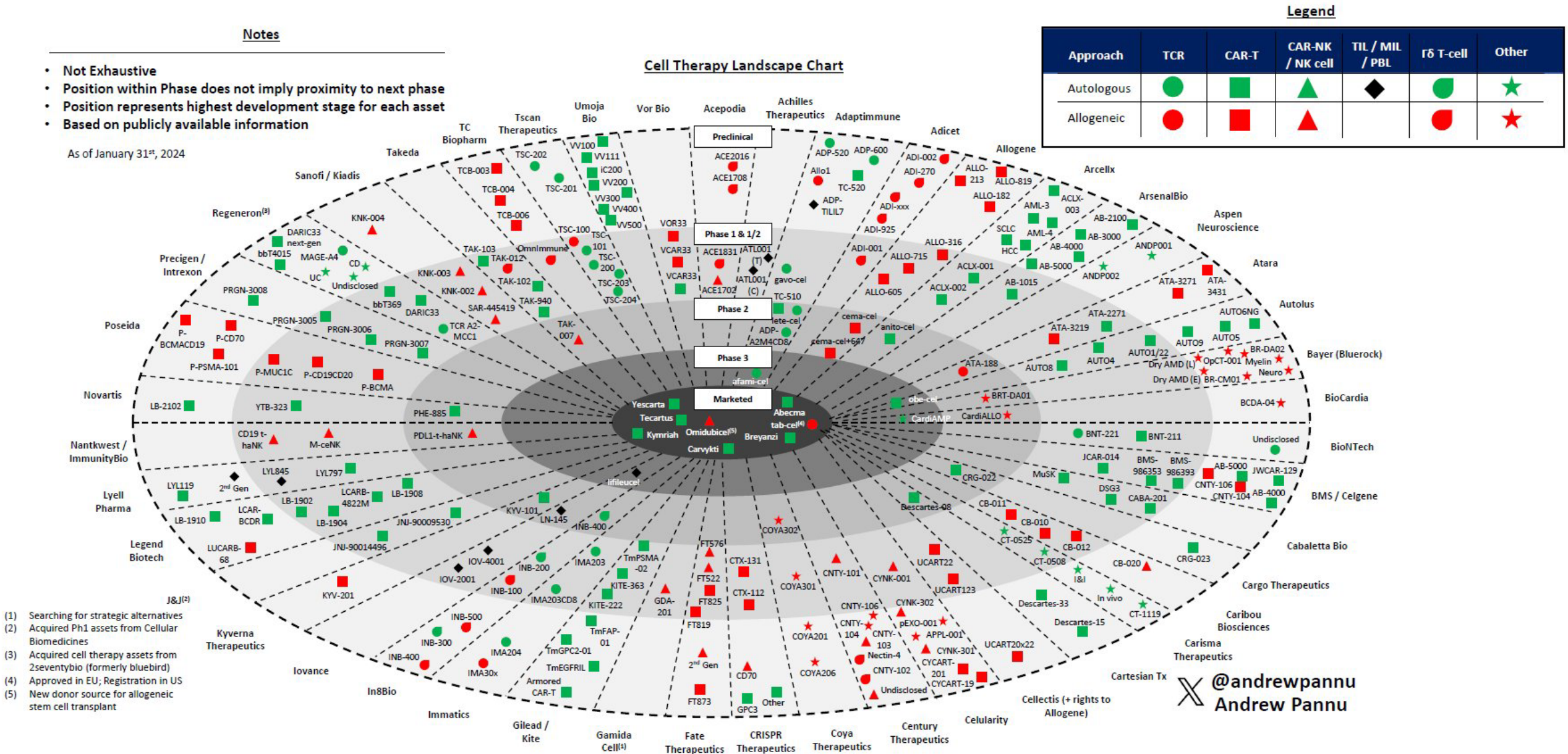
$$\text{Absolute Risk} = \frac{4 \text{ malignancies}}{5762 \text{ patients}} \approx 0.000694 \,(\text{about } 0.07\%)$$

***1** in **1440** patients treated with modified T cells may develop a secondary malignancy attributed to probable transgene insertional mutagenesis*
(true rate is almost certainly much lower)

- Now ~40-50 thousand patients treated with CAR T cell products and trillions of engineered cells infused
- Rare examples of clonal expansion associated with insertional mutagenesis
- 2025: two cases with vector integration in tumor suppressors and T cell malignancy
- T cells are relatively resistant to genotoxicity, but mature T cell transformation is possible under unusual settings

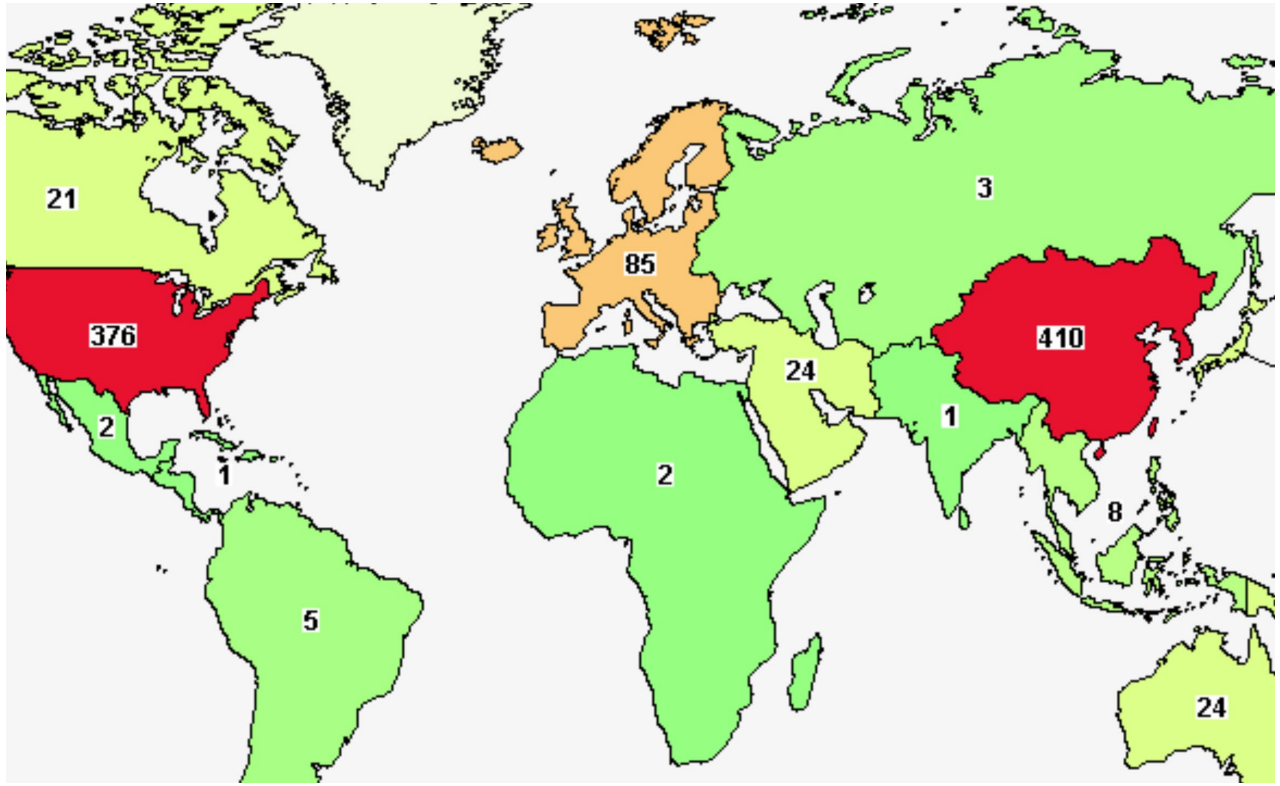
Fraietta, Levine, Ruella, Hexner, Jadlowski

Engineered Cell therapy: a diversity of CAR T, TCR T, CAR NK and TIL



Cell and Gene Therapy: A Global Disruptive Therapy

- The rate of FDA approvals is more rapid than the previous disruptive therapy
- There are 976 clinical trials testing CAR T cells therapy (clinicaltrials.gov): many more are on the way!



Thank you: Colleagues in Philadelphia

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