Memorial Sloan Kettering Cancer Center

Gene engineered cell therapies for cancer

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1. Basic design of CARs
   - How CARs target tumor antigens
   - Role of costimulation
   - Viral vectors

2. **Module #1: Explore CAR constructs**

3. Problems with current generation CAR T cells
   - Toxicity
   - Disease relapse

4. Next generations T cell therapies #1: Solid tumors

5. **Module #2: SynNotch CAR circuits manuscript**

6. Next generations T cell therapies #2: Off the shelf

Notes on these slides:

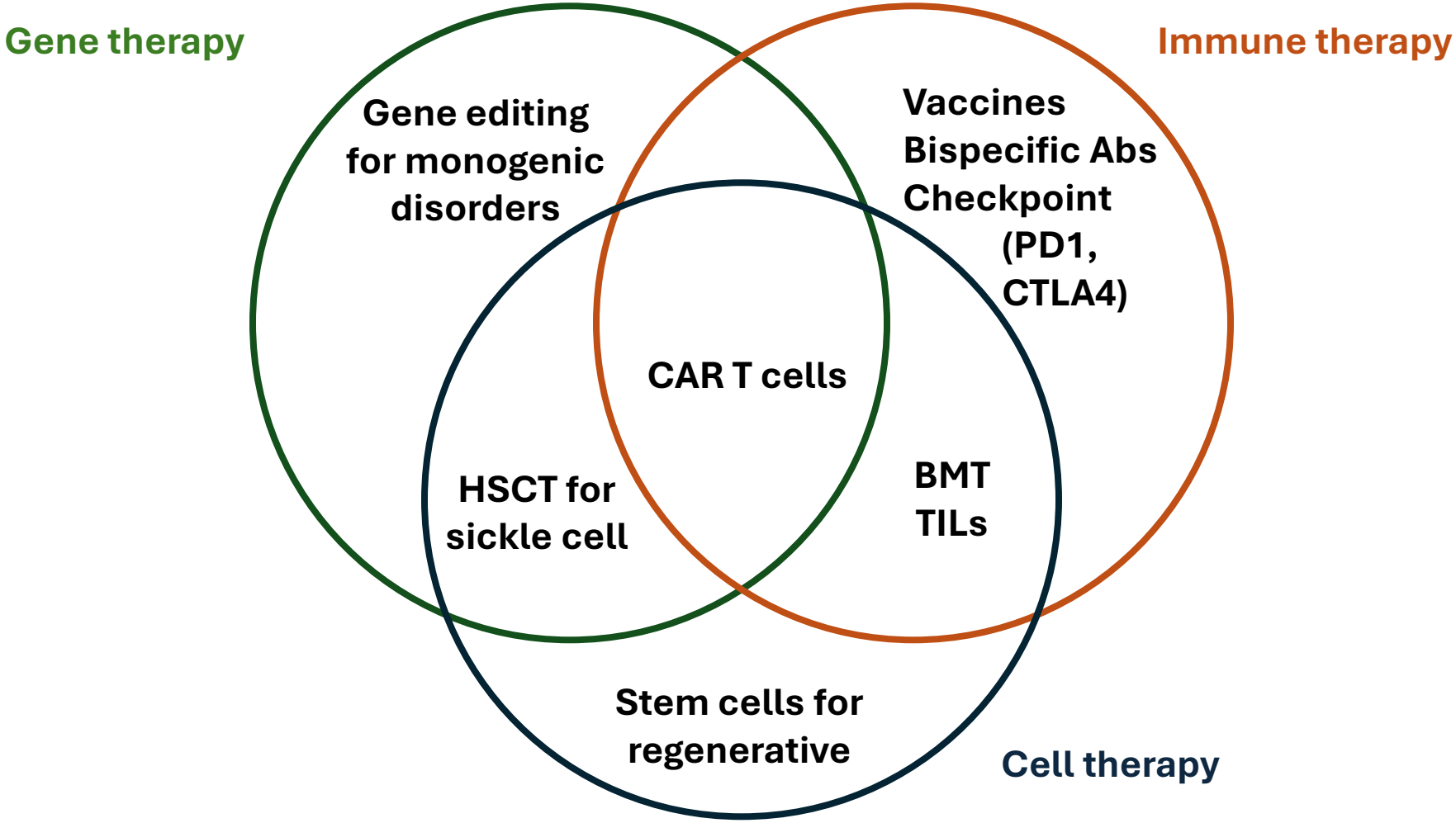
This is an applied and interdisciplinary topic that covers cellular immunology, cell signaling, gene engineering. Concepts will be more or less challenging based on your background.

There is a lot of dense information. Key slides are highlighted, and review slides emphasize the key concepts. You are not expected to know everything.

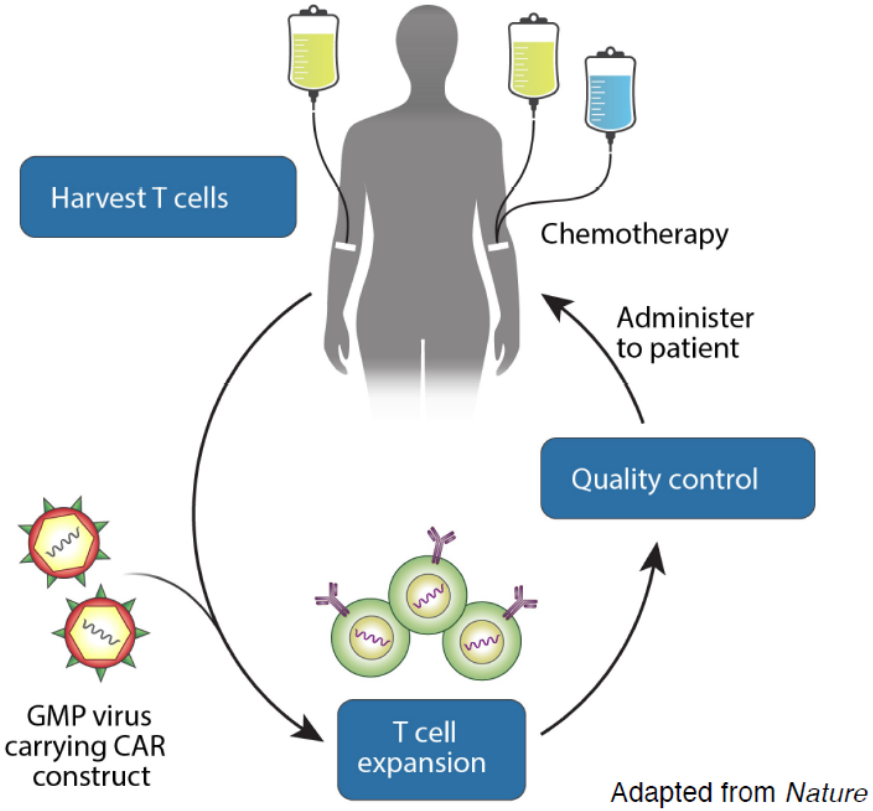
Many slides will contain copious text. That is intentional, for review later.

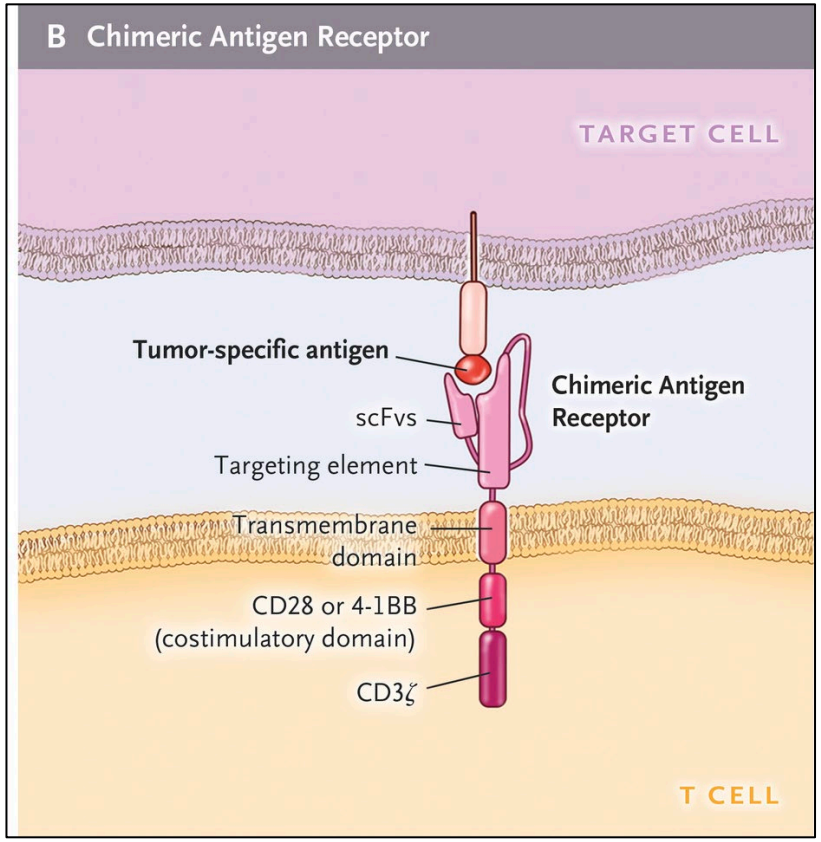
Terminology

Cell therapy is a treatment modality where the drug product is a cell (as opposed to a small molecule, protein, etc.)
Gene therapy is any treatment modality where the nucleic acids (typically DNA, but also RNA) are delivered and/or directly manipulated
Immune therapy is any treatment that acts directly on and primarily by using the immune system



CAR T cells are a gene engineered cell therapy in which a patients T cells are reprogrammed to recognize and kill cancer

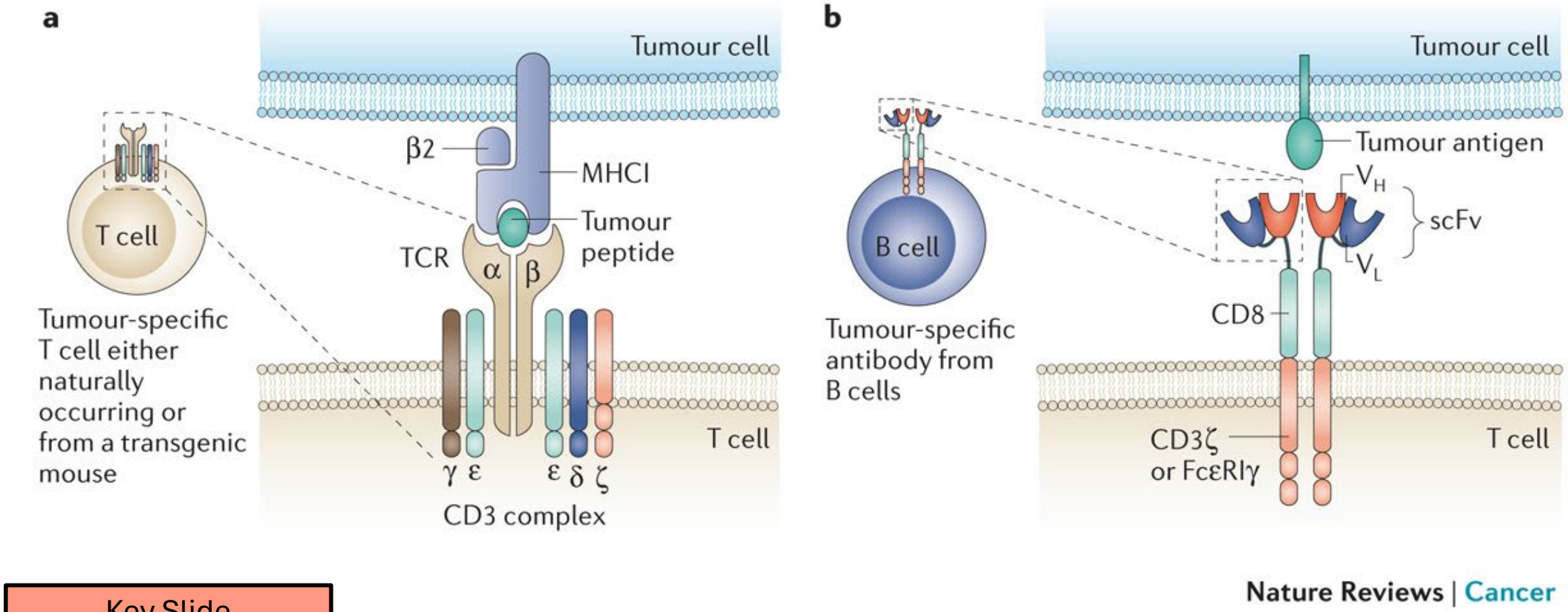




June and Sadelain, *NEJM*, 2018

T cell Receptor vs Chimeric Antigen Receptor

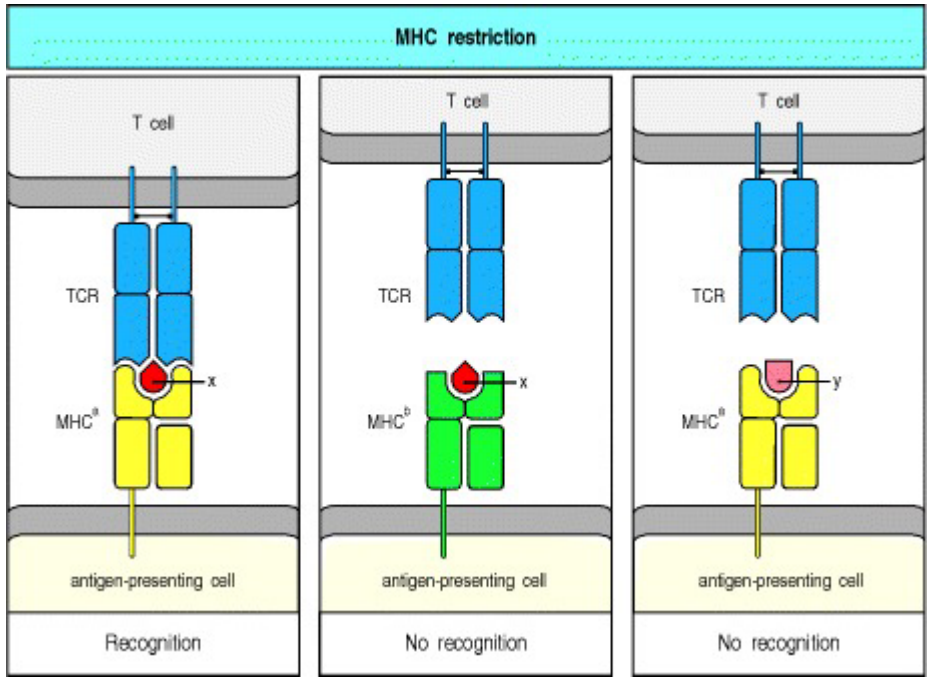
Kershaw et al., 2013, Nat Rev Cancer 13:525



Key Slide

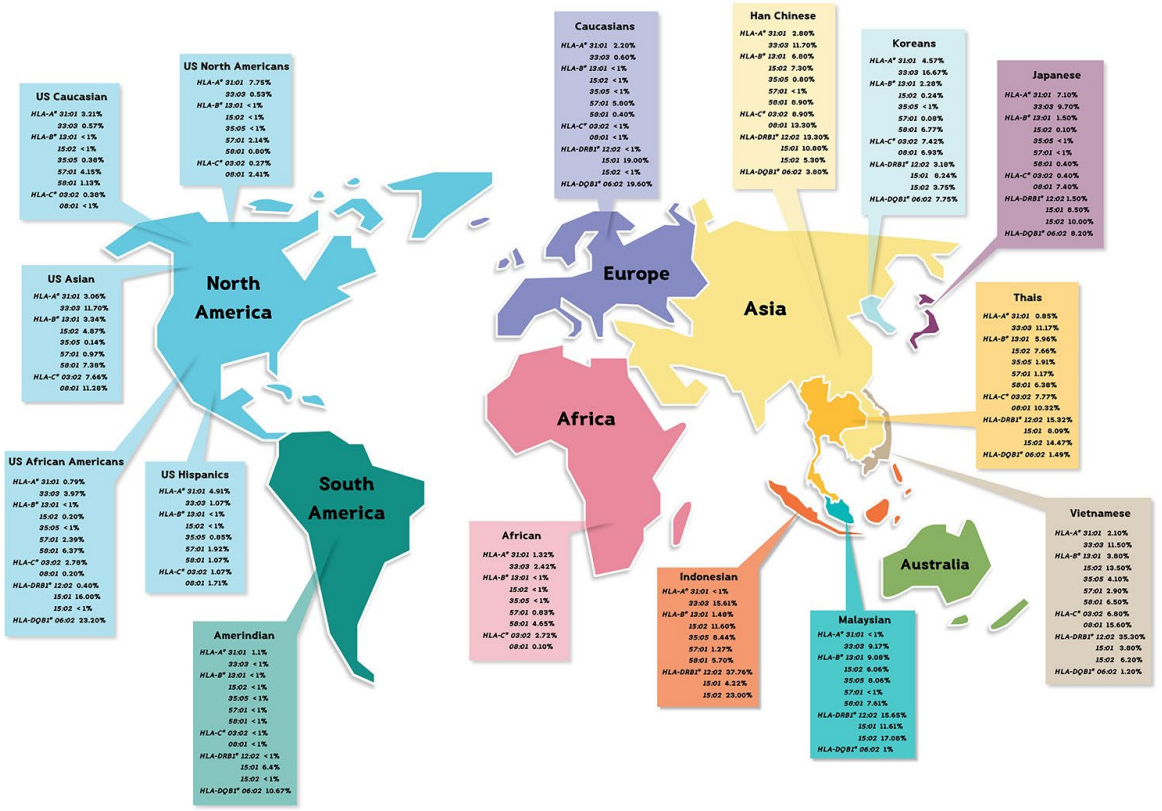
A **<u>Chimeric Antigen Receptor</u>** binds surface proteins using an antibody-based or other binding domain, **<u>not MHC-peptide.</u>** Because it is not MHC-restricted, it is universal.
It then delivers signals necessary for T cell activation: **Signal 1 (CD3ζ)** and **Signal 2 (Costimulation, ex CD28)**
It is a chimera of B cell-like antibody/antigen recognition and T cell signaling

MHC restriction makes it challenging to design universal T cell receptor therapies



TCR recognizes a specific peptide in the context of a specific MHC allele

Janeway 9th edition

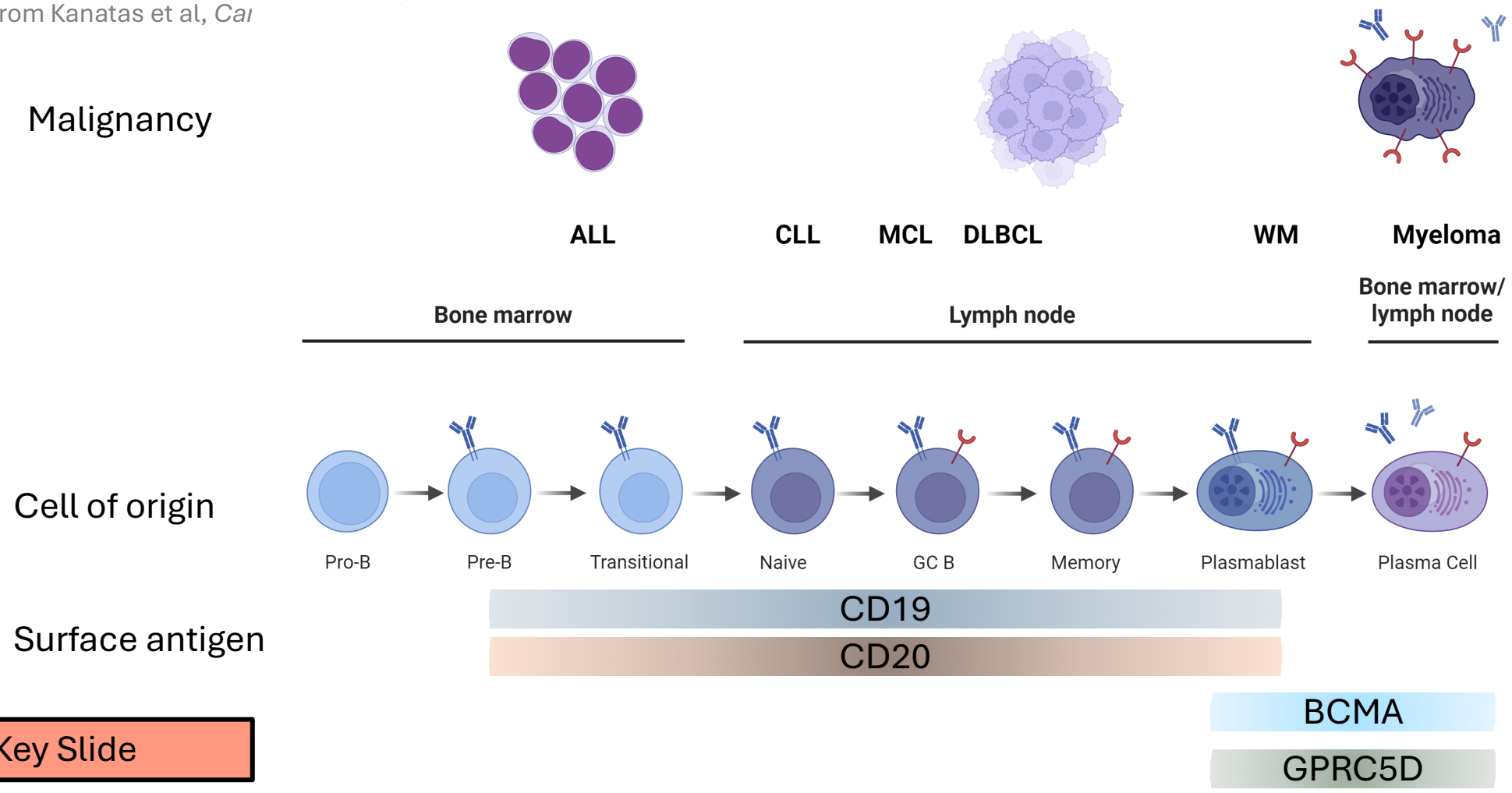


HLA is the most diverse gene locus in human populations (Sataporn pong Frontiers 2020)

Creating a universal TCR based therapy is therefore challenging...
But not impossible (more on this later)

Instead of targeting unique peptide-MHC, use a CAR target lineage markers

Adapted from Kanatas et al, *Cai*

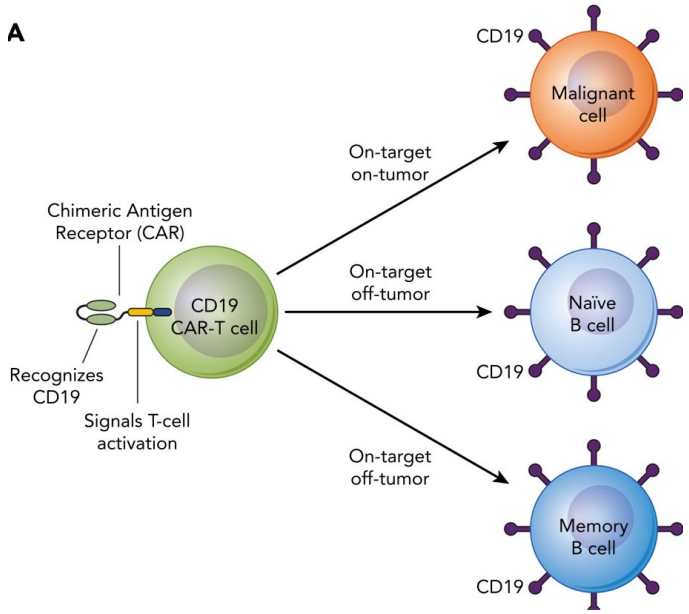


Key Slide

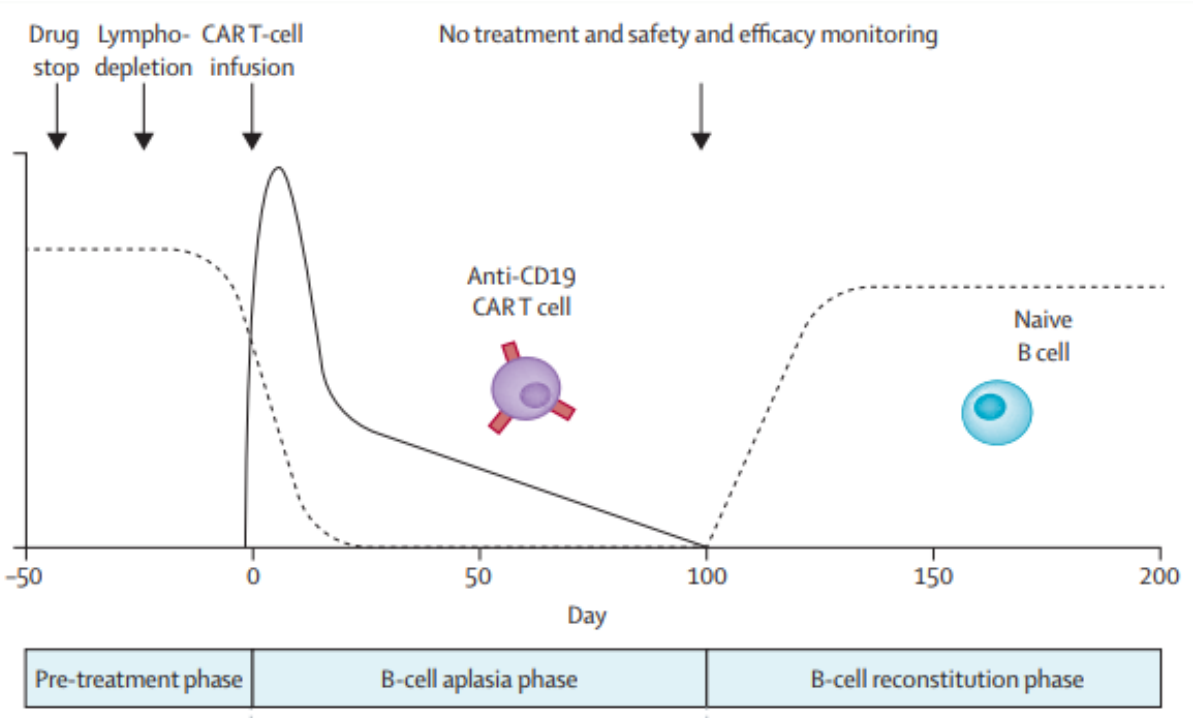
A lineage marker tumor antigen is expressed or overexpressed on a tumor cell **surface** and is **not found on any critical normal tissues**. B cell and plasma cell markers such as CD19 and BCMA are the best example as you can live without B cells.

CAR T cells target normal B cells and plasma cells in addition to cancer

Hill Blood 2020



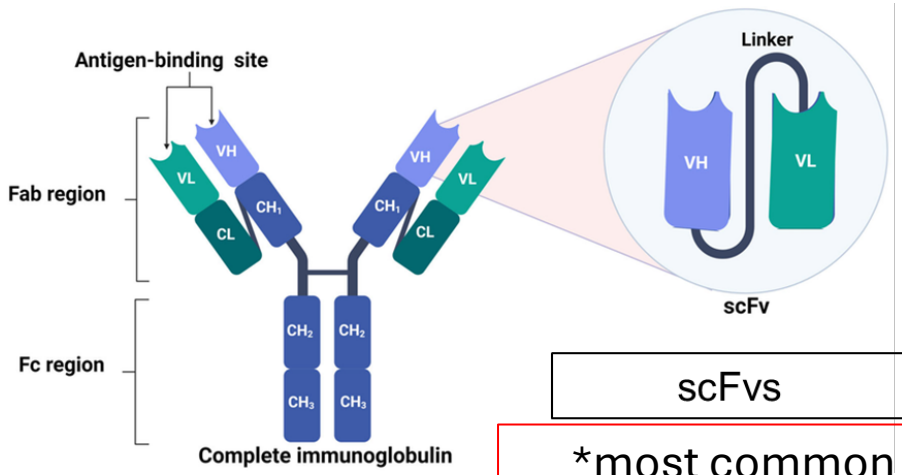
Schett et al. Lancet 2023



CD19 CAR T cells kill normal B cells, and BCMA CAR T cells kill normal plasma cells. Patients experience months of B cell aplasia and hypogammaglobulinemia after therapy. Infectious risk is present but managed with prophylactic antibiotics and, if indeed, intravenous immunoglobulin supplementation. Ability to kill B cells also motivates use of CD19 CAR T cells in autoimmunity. Targets like CD19 and BCMA are feasible because antigen expression is confined to a normal cell whose temporary loss can be tolerated.

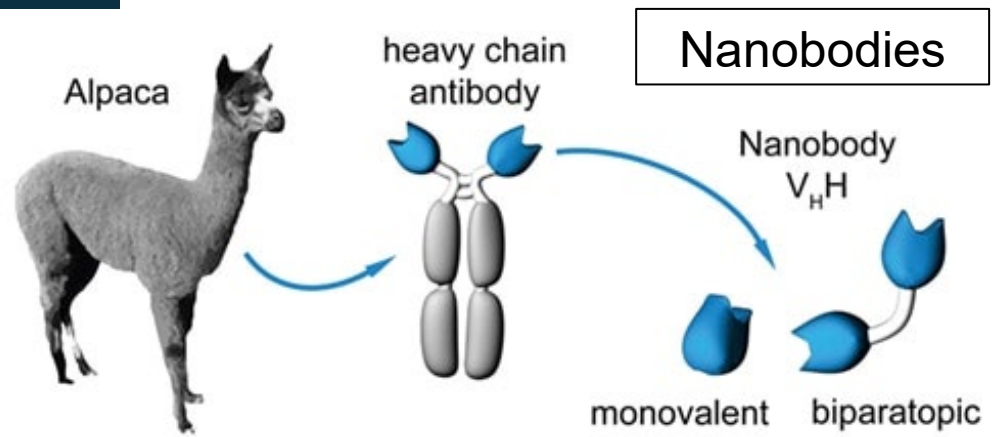
Sources of Binder Sequences

(adapted from Yvonne Chen)

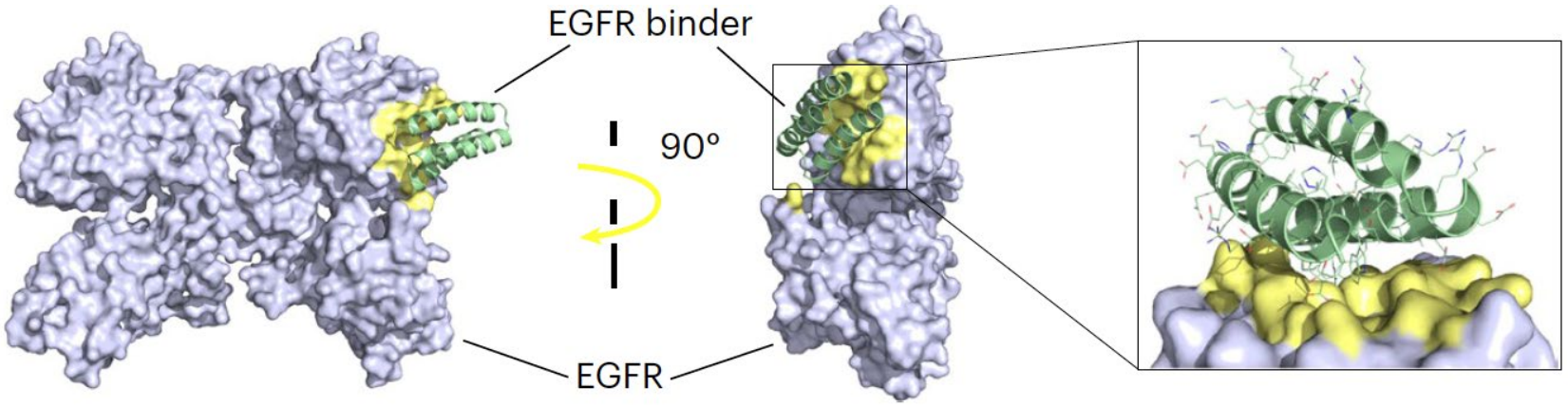


scFvs

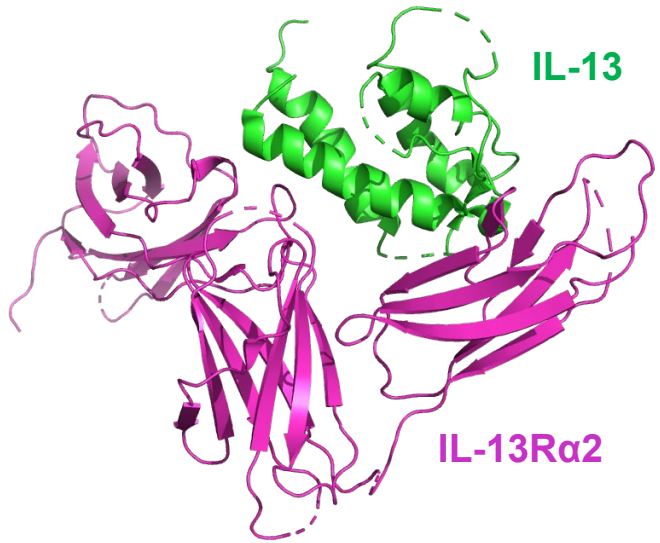
*most common



Nanobodies



Computationally designed binders



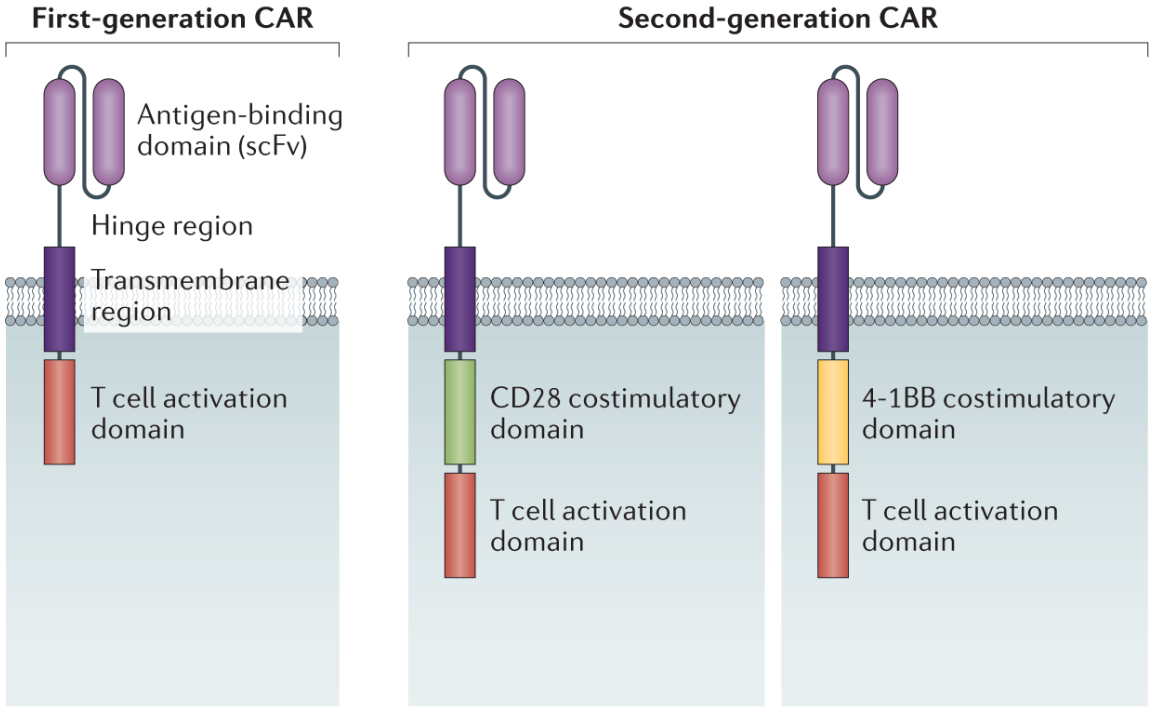
Natural ligands

Rodriguez-Nava et al., Biomedicines. 2023, 11(6):1610.
Xie et al., Nat. Biomed. Engr. 2024, https://doi.org/10.1038/s41551-024-01258-8
https://www.ptglab.com; https://www.rcsb.org/structure/3BPO

‘Generations’ of CAR T cells and the role of costimulation

Key Slide

First generation CAR has CD3ζ domain. This CAR can kill *in vitro* but does not expand or persist well, and does not work in animal models.



Cappell and Kochenderfer, *Nat Rev Clin Oncol*, 2021

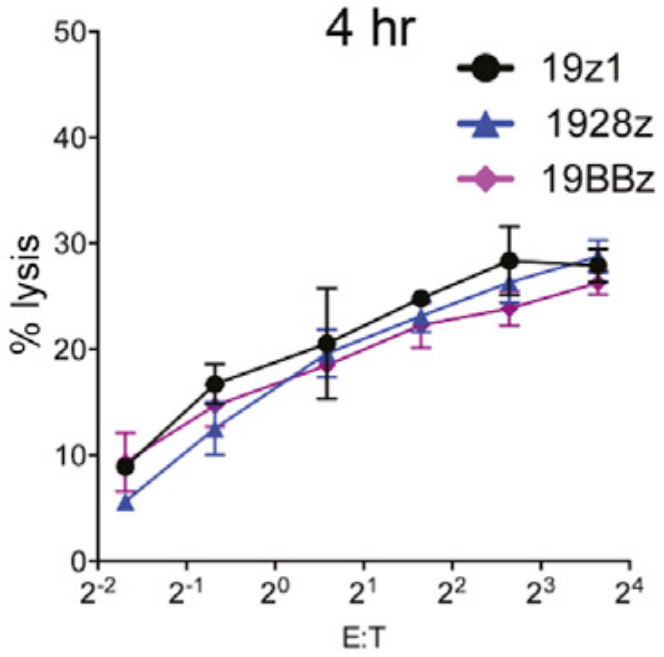
Second generation CAR adds a costimulatory domain like CD28 or 41BB. This CAR works *in vivo*. The CAR T cells expand, proliferate and proliferate.

Some people talk about third and fourth generation CAR T cells. I hate it. While there have been many interesting innovations in CAR design, nothing beyond second generation has proven superiority in patients, and we can’t add a generation every time someone publishes a paper.

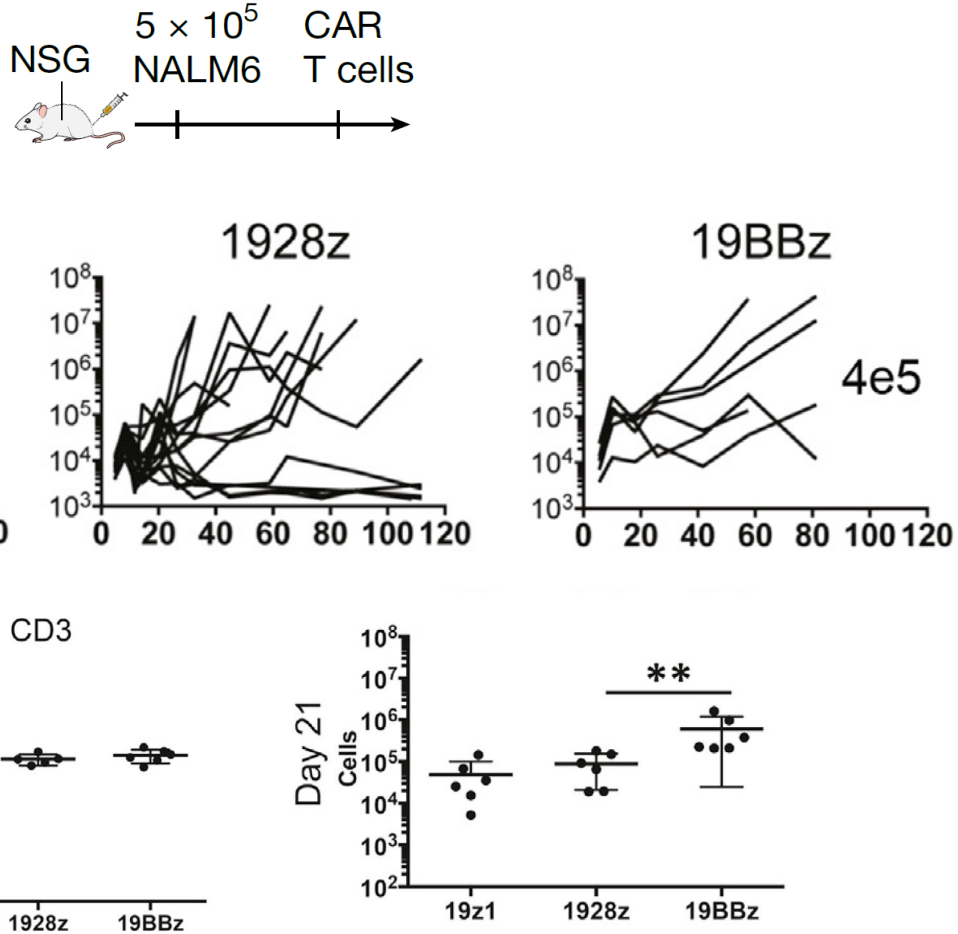
Costimulation is required foroptimal *in vivo* CAR function and proliferation

19z1 – no costimulation
1928z - CD28 costimulation
19BBz – 41BB costimulation

All 3 CARs kill tumor *in vitro*
1st Gen CAR T cells work *in vitro*



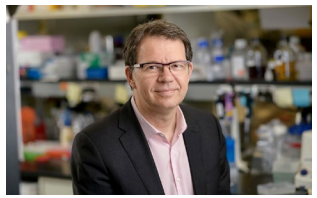
2nd Generation CAR T cells with costimulation control tumor in mouse models where you need CAR T cells to expand and persist

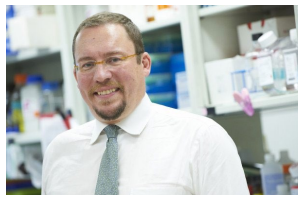


Zhao Cancer Cell 2015

History of CAR T cells at MSKCC


Isabelle Rivière


Michel Sadelain


Renier Brentjens

Chimeric antigen receptor (CAR) T cells developed

Phase I Trial of CD19 CAR for Acute Lymphoblastic Leukemia (ALL) opened (IRB#09-114)

First to report CD19 CAR T cells can achieve complete remissions in Acute Lymphoblastic Leukemia

First to develop academic BCMA & GPRC5D CAR T cells for Multiple Myeloma

Several myeloma CAR T products were developed at MSKCC

Cellular Therapy Service Created

2003 2006 2010 2013 2017 2020 2021

First to show CD19 CAR can eradicate human cancer in mice

First patient ever to receive CD19 CAR (1928z) T cells in the world happened at MSK (IRB#06-138)

Scientific Breakthrough of the Year:

Cancer Immunotherapy

Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15

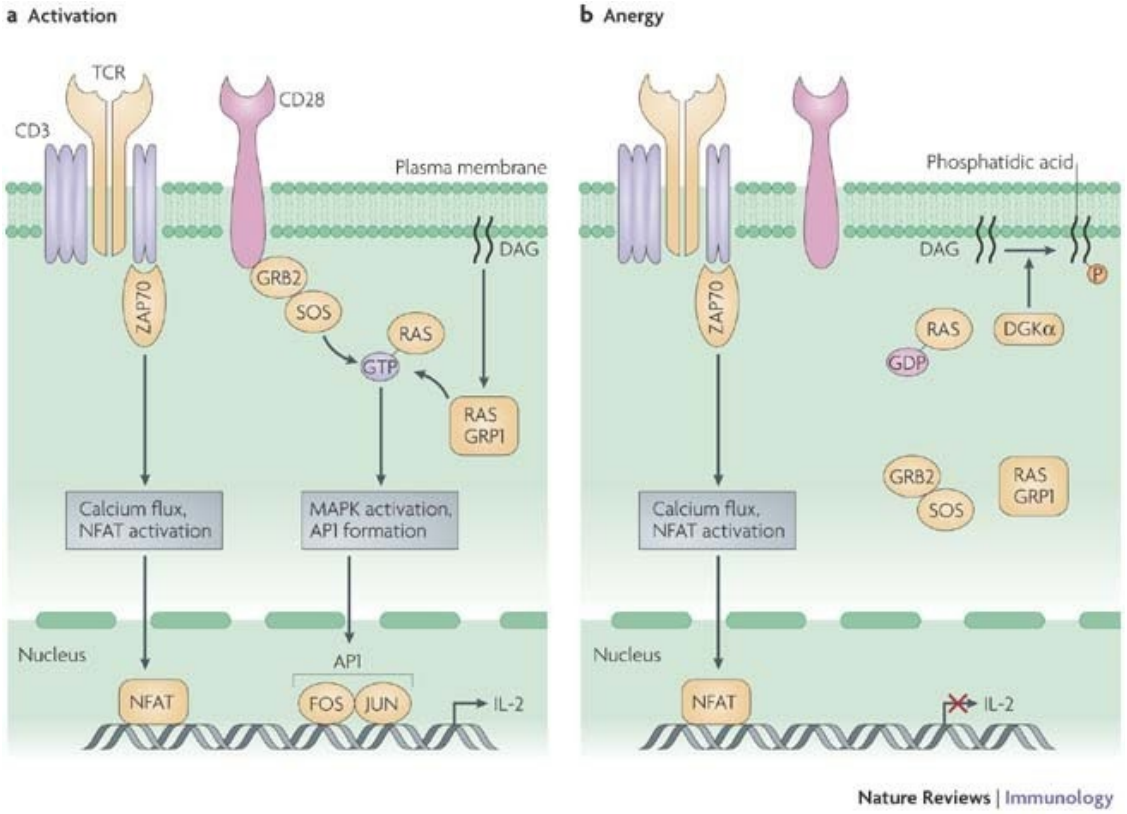
Renier J. Brentjens[1], Jean-Baptiste Latouche[1], Elmer Santos[1,2], Francesc Marti[5], Michael C. Gong[1], Clay Lyddane[1,3], Philip D. King[5], Steven Larson[2], Mark Weiss[1], Isabelle Rivière[1,3,4] & Michel Sadelain[1,3,4]

nature medicine

2nd Gen CAR T cells by addition of CD28 was one of major discoveries by Sadelain group at MSKCC.

What is the role of costimulation: Costimulation in normal T cells

Fatham Nature Reviews Immunology 2007



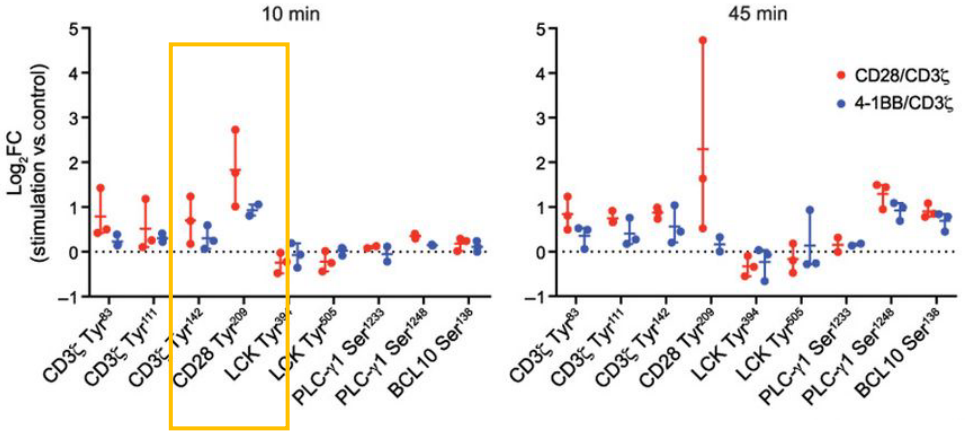
In normal T cells, CD28 and 41BB are required for optimal T cell proliferation, expansion and persistence. In the absence of costimulation, CD4 T cells T cells are ‘anergized,’ and become non-responsive. CD28 and 41BB costimulation can amplify Signal 1 from the T cell receptor, and also provide unique proliferative and anti-apoptotic signals. CD28 acts more directly to amplify TCR signals such as LCK and ZAP70, whereas 41BB additionally acts *via* TRAF family receptors and promotes anti-apoptotic pathways *via* ATK and mTOR.

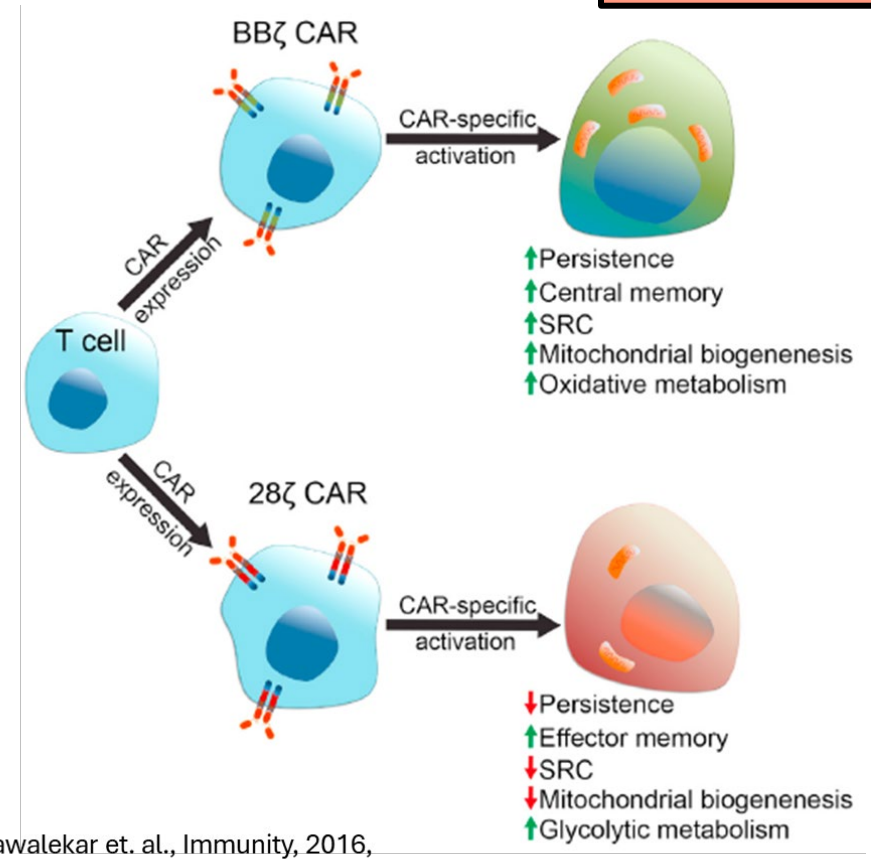
CD28 compared to 41BB induces stronger signaling, more T cell differentiation, and less persistence

Key Slide

Salter Science Signaling 2018
Salter Science Signaling 2021
Ritmesser-Loy Science Signaling 2024

Phosphoproteomics reveals that CD28 costim induces stronger CD3ζ phosphorylation and generally stronger downstream TCR signaling



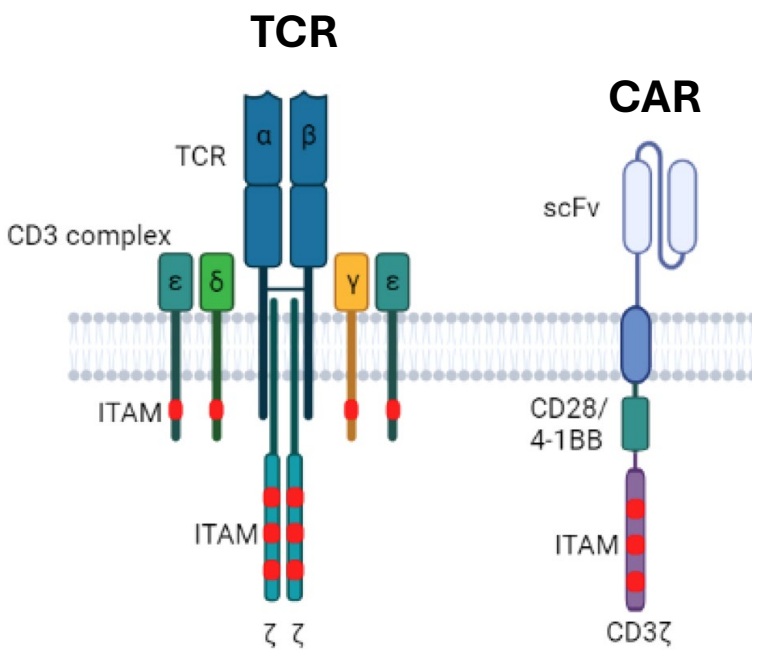


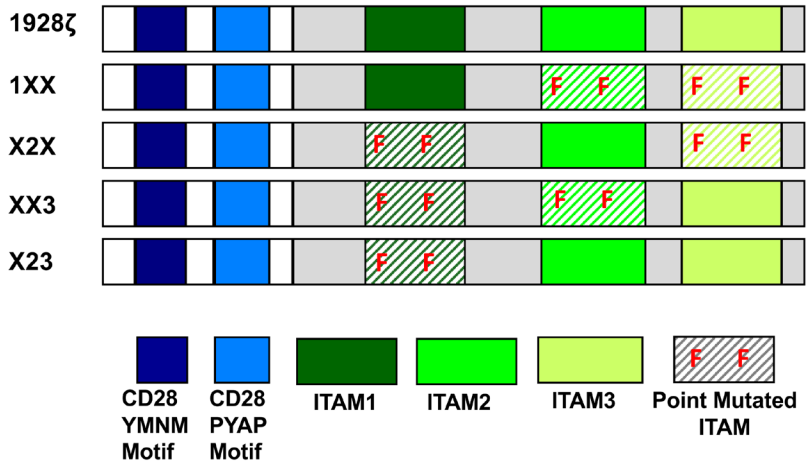
Kawalekar et. al., Immunity, 2016, 44:380–390.

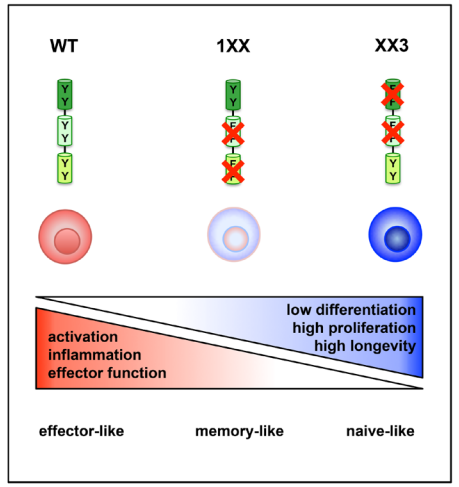
Stronger signaling from CD28 translates into faster expansion, more effector differentiation, and less persistence.

CAR signaling *via* ITAMS can be engineered to modulate behavior

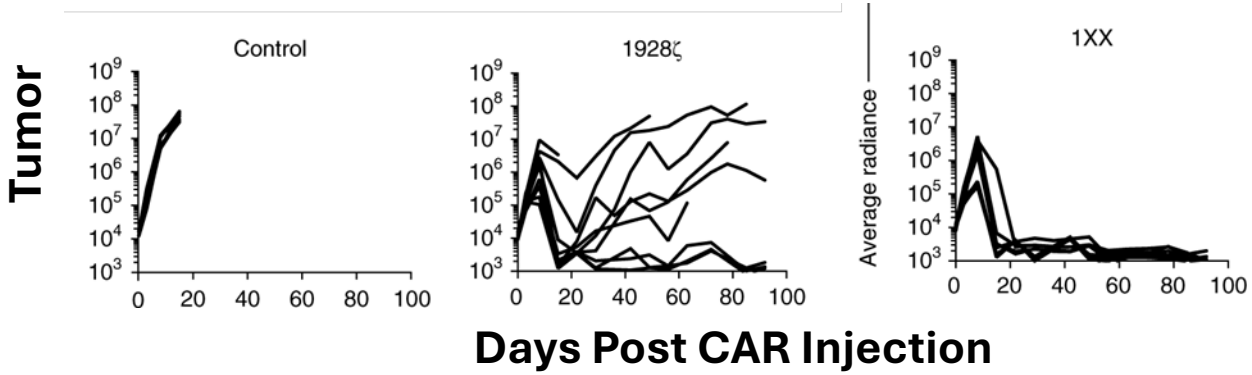
Yun Leukemia 2023







Feucht, Sun et al. *Nat. Med.* (2019)

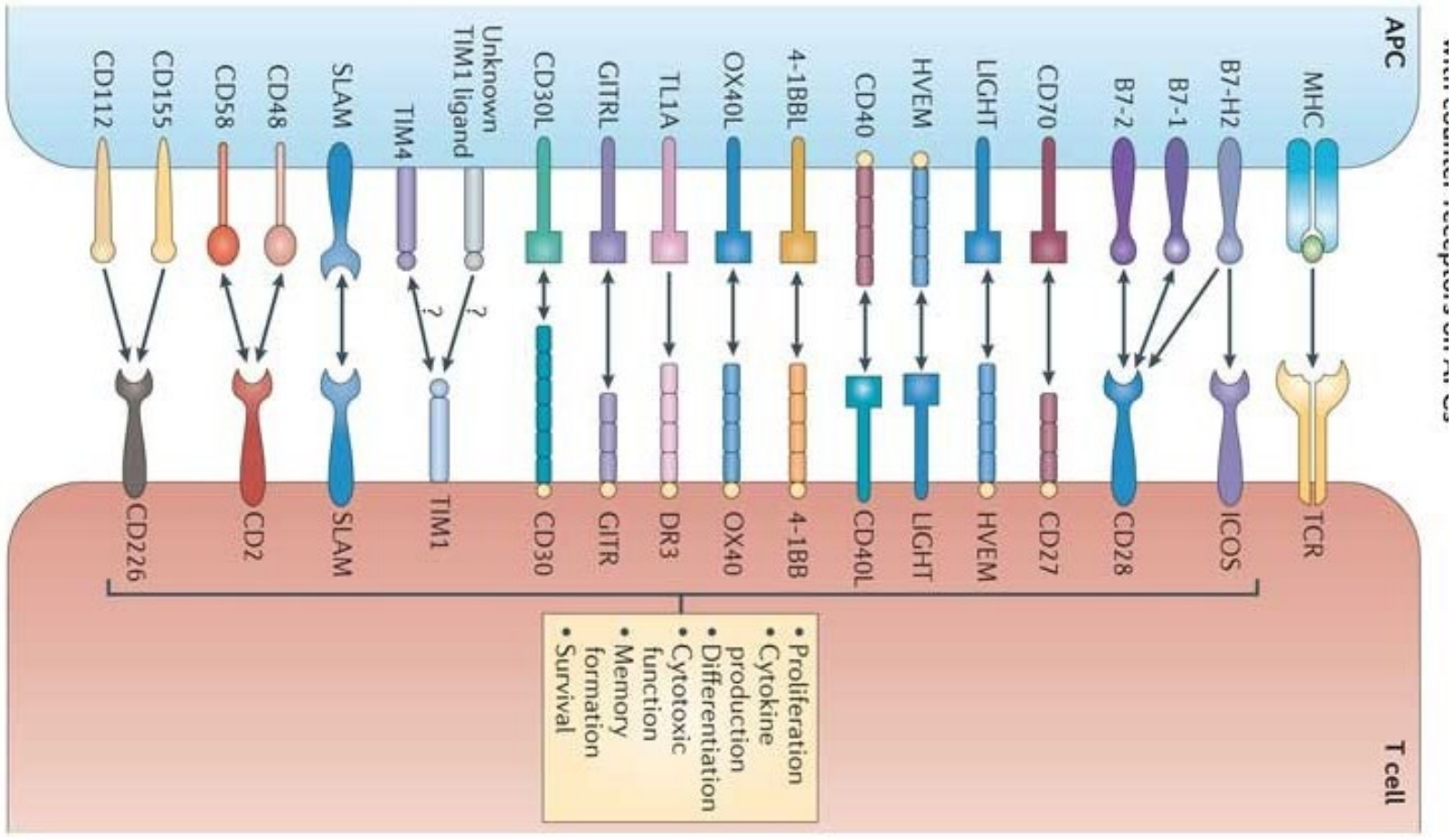


A TCR complex is composed of TCR and CD3 complex containing CD3γε, CD3δε, and CD3ζζ. A TCR complex has 10 ITAMs in total, with one in CD3γ, CD3δ, and CD3ε, and three in each CD3ζ chain. A single monomeric CAR molecule has three ITAMs in its CD3ζ domain.

Since 28z CARs have strong signaling, the Sadelain group hypothesized that attenuating the signaling would modulate CAR function. Eliminating the 2nd and 3rd ITAM of CD3ζ promotes 28 CAR survival and enhances function.

There are many potential T cell costimulation domains

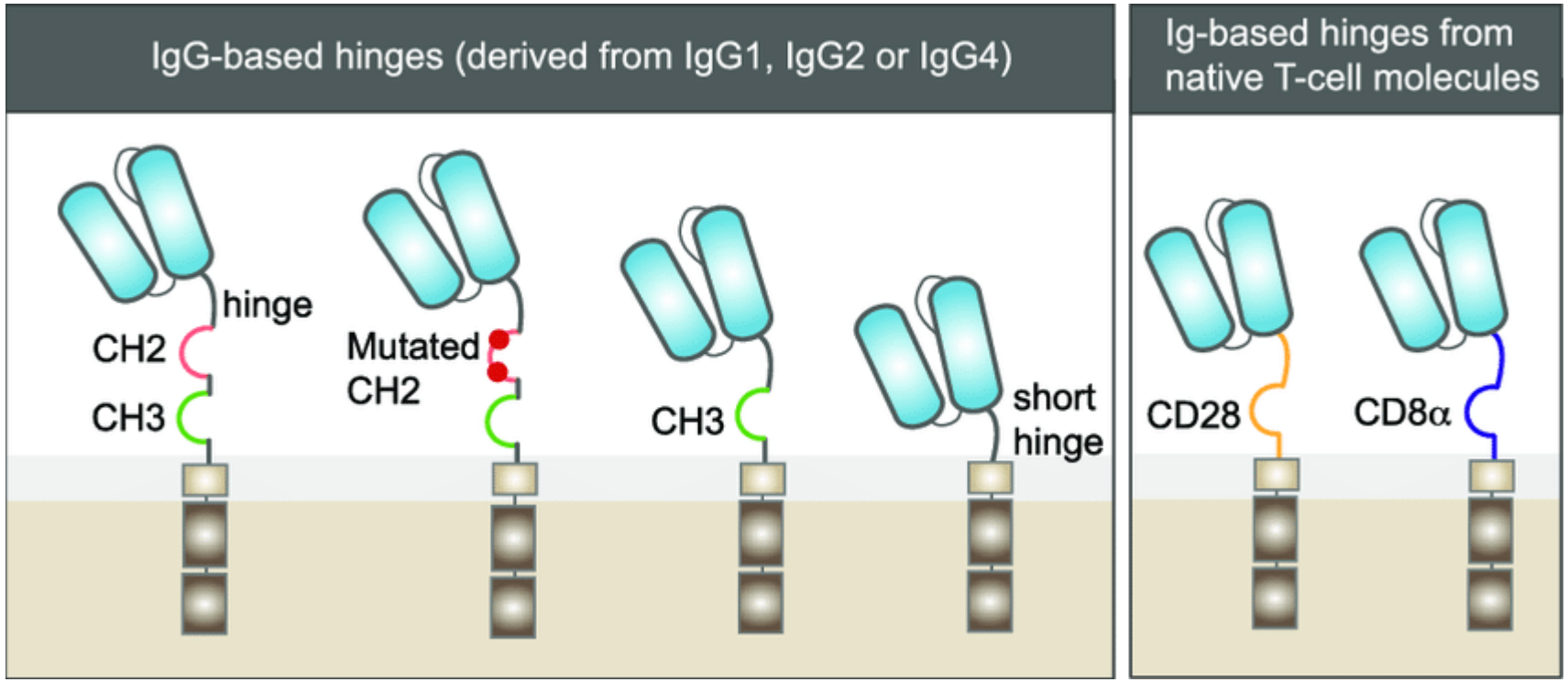
Chen Nature Reviews Immunology 2013



There are many surface proteins that can be said to ‘costimulate’ T cells. 41BB and CD28 are the main two used in CAR T cells, but all of the above have been/are being explored with different mechanisms and strengths/weaknesses.

The hinge and transmembrane portion of a CAR also affect CAR T cell function

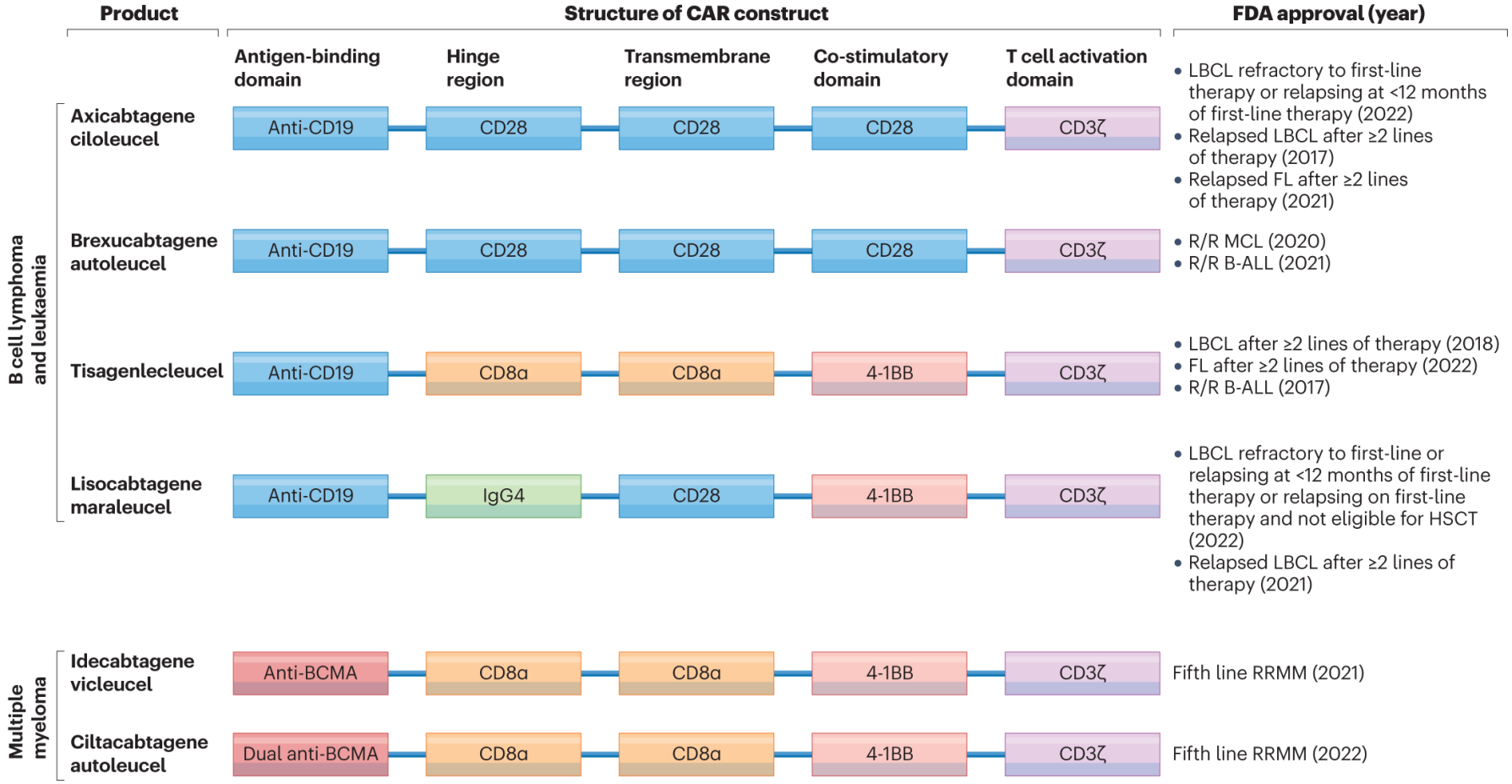
Guedan Molecular Therapy 2018



Initially, the transmembrane and hinge regions were felt to be a spacer without much activity.
We now know that the length, flexibility of the hinge can impact CAR sensing and detection.
There are no great first principles, you just have to empirically try different designs for your CAR and target antigen.

Putting it all together: FDA approved CAR T cells

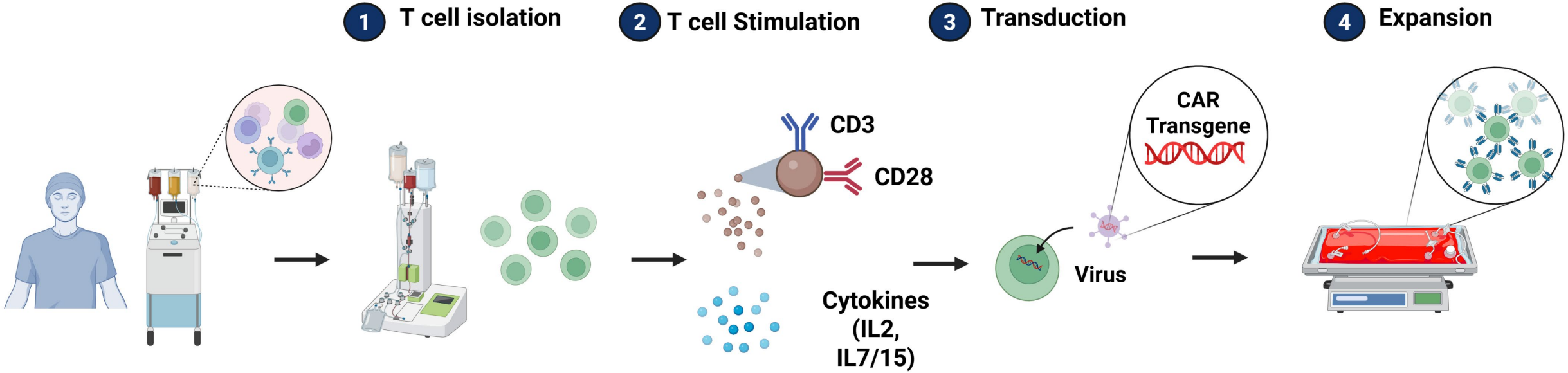
Cappel *NRCO* 2023



All target B cell (CD19) or plasma cell (BCMA) lineage markers
41BB is more popular than CD28 due to lower toxicity (more on this later)
Most use scFV designs, one (ciltacabtagene) uses a dual nanobody binder

CAR T cell production process

Key Slide



Peripheral blood mononuclear cells are collected by large volume apheresis. 1) Typically, but not mandatory, T cells are isolated for example by bead based magnetic purification, as monocytes can interfere with production. 2) Before transduction, T cells must be stimulated to induce cell cycling and proliferation. This is typically accomplished with anti-CD3 and anti-CD28, which deliver Signal 1 and Signal 2 (just like CARs deliver these signals to activate T cells). T cells also require cytokine support, such as IL2. 3) The CAR itself is delivered by viral vectors, typically a lentiviral or sometimes gamma retroviral system. 4) Cells continue expansion, now transduced with CAR, in large scale bioreactor systems.

Cells are typically shipped to centralized facilities for manufacturing, equipped with GMP grade clean process for cell processing

The end result is an infusion bag such as the ones below that are delivered intravenously to the patient

Tisa-cel

Brex-cel

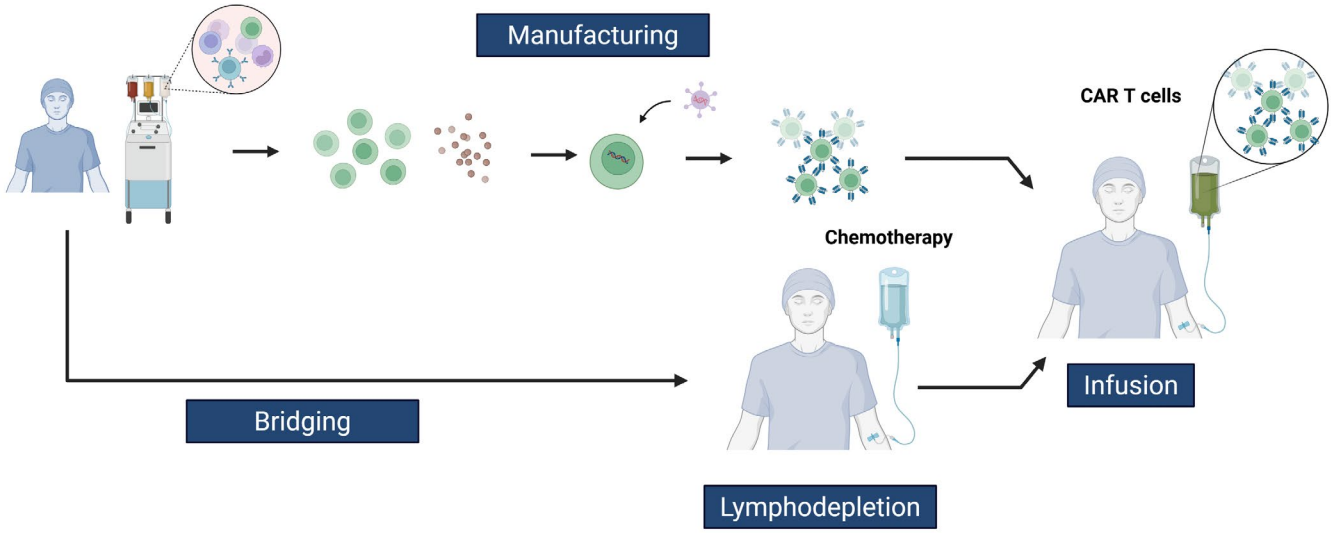
Ide-cel

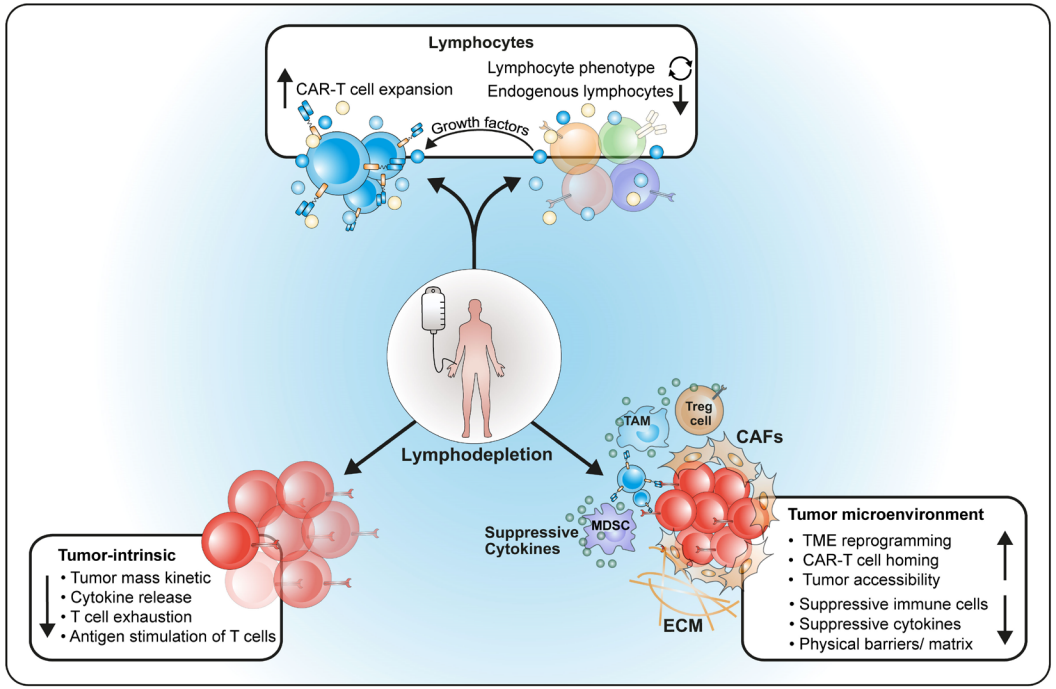
c/o lovance (facilities)

c/o Marco Ruella (infusion bags)

Before CAR T cell infusion: Lymphodepletion

Cappel *NRCO* 2023





Lickefett Front Imm 2023

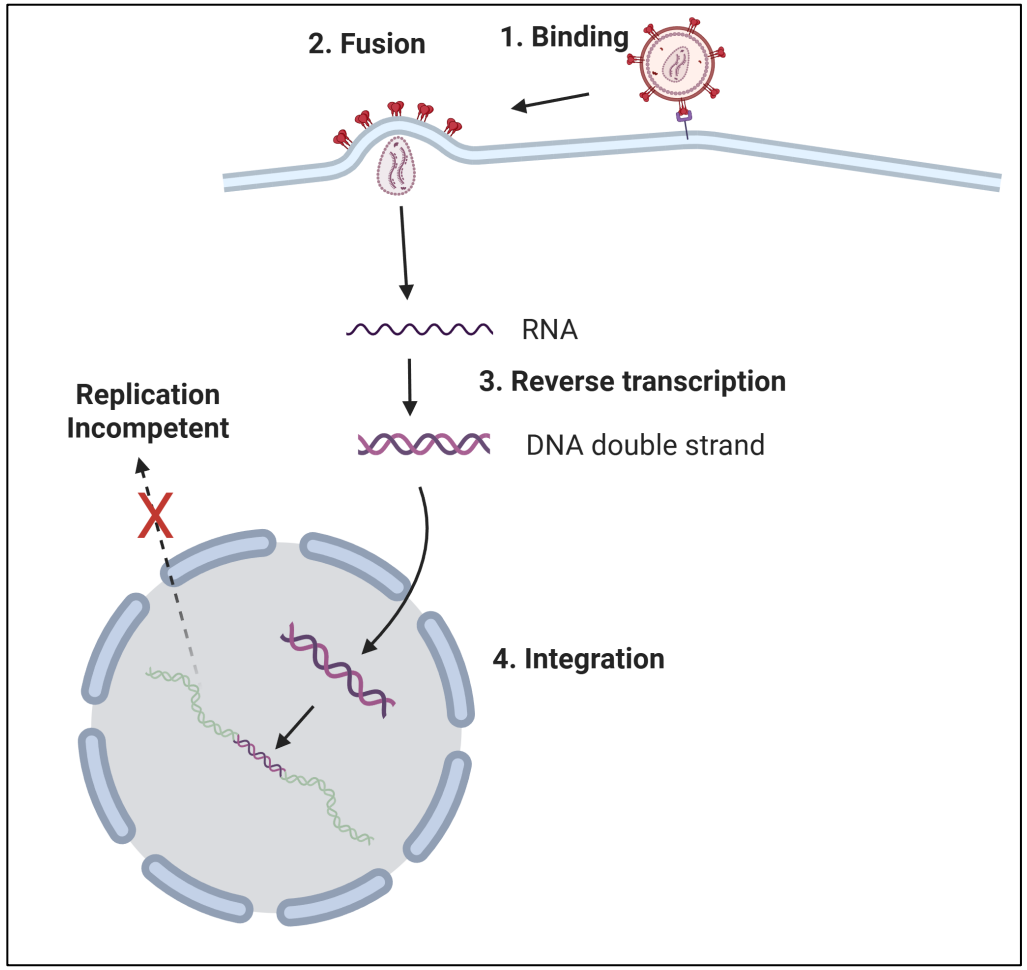
First, patient T cells are harvested by leukapheresis. These cells are sent to a manufacturing facility to be transduced with a viral vector (more on this later). Once the cells are ready (can take 1-2 months), the patient must be prepared for CAR T cell infusion with lymphodepleting chemotherapy (usually a combination of fludarabine and cyclophosphamide).

Lymphodepletion serves several purposes. Most importantly, it 'makes space' for incoming CAR T cells by killing normal lymphocytes and freeing up pro-survival cytokines such as IL-2, IL-7, IL-15. Secondly, it kills immune suppressive cell subsets like Tregs and MDSCs. Finally, it can have a small amount of direct anti-tumor cytotoxicity.

Retroviral vectors are the mainstay of CAR therapy

Vector Type	Expression	Genome	Packaging Capacity	Virus Size	Cells Infected	Genome Integration
Lentiviral	Stable	RNA	< 8 kb	80–130 nm	Dividing/Non-dividing	**Yes**
Gamma-retroviral	Stable	RNA	< 8 kb	80–130 nm	Dividing	**Yes**
AAV	Transient or Stable*	Single-stranded linear DNA	~4.5 kb	18–26 nm	Dividing/Non-dividing	No*
Adenoviral	Transient	Double-stranded linear DNA	5–36 kb	105 nm	Dividing/Non-dividing	No

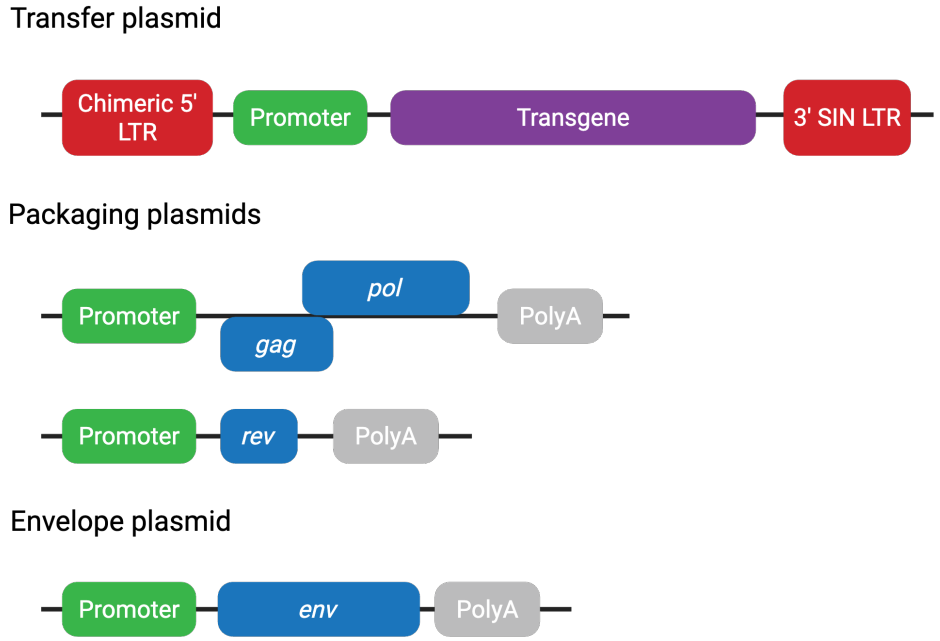
Adapted from Addgene

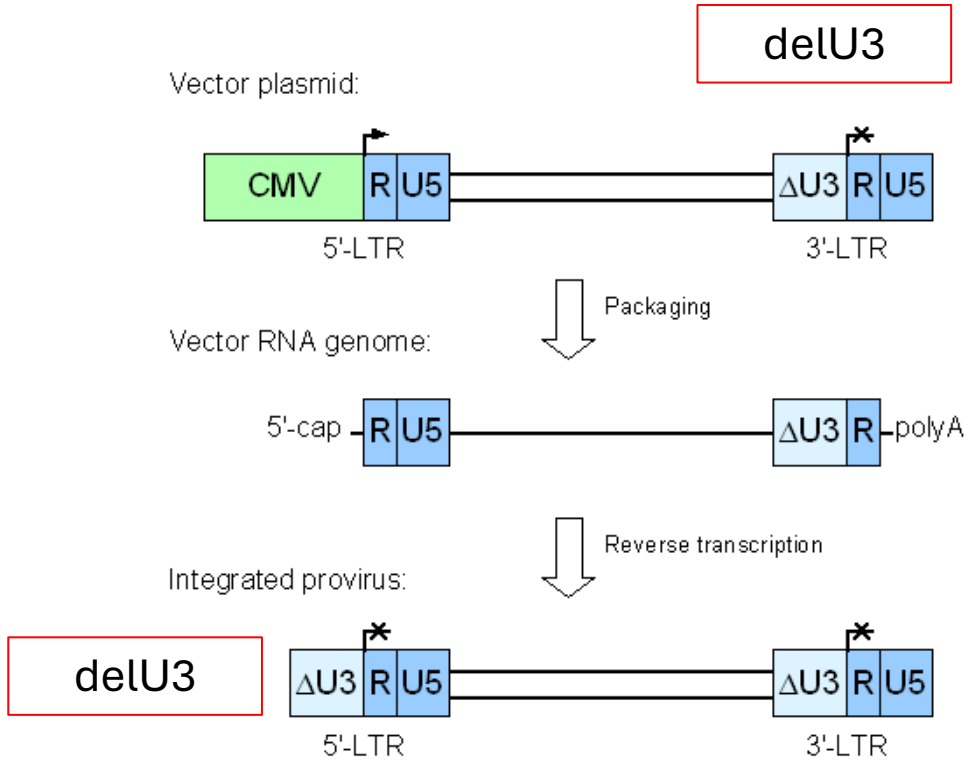


Retrovirus can mediate DNA integration. The two most commonly used for CARs are γ-retrovirus and lentivirus. Lentiviruses are the most common; these are enveloped retroviruses of the *Retroviridae* with an RNA genome that is reverse transcribed to DNA, which is subsequently integrated. Wild type viruses replicate to promote infection. Laboratory viruses (so-called 2nd or 3rd generation lentivirus) are rendered replication incompetent.

Retroviral vectors are the mainstay of CAR therapy

Third-generation lentiviral plasmids *via* Addgene
See also Milone Leukemia 2018





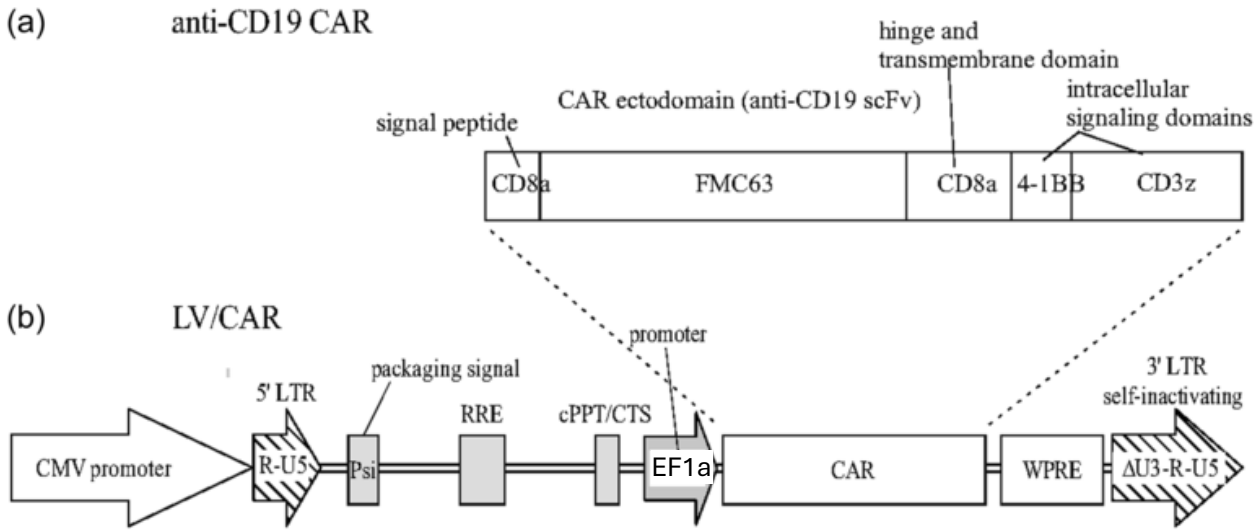
In 3rd generation lentiviral systems, the packaging system is split into two plasmids. The transfer plasmid contains a chimeric 5' LTR fused to a heterologous promoter (often CMV or RSV), eliminating the need for transactivation by Tat.
To render the virus replication incompetent, a self-inactiving (SIN) vector is uses. On the vector plasmid, enhancer/promoter sequences of the U3 region of the 3'-LTR have been deleted (ΔU3). During the reverse transcription, this "defective" U3-region will be copied to the 5'-end, resulting in a provirus with this enhancer/promoter-deletion in both LTRs. See also Miyoshi et al. (1998).

Key Slide

Because viral promoter is impaired, you choose add an additional promoter

The heterologous CMV promoter initial expression of the virus, and once the gene is integrated, the defective del3 promoter can no longer drive expression. You therefore need to insert one more promoter that will drive the transgene itself.
Strong constitutive promoters such as the human EEF1A1 gene that expresses the alpha subunit of eukaryotic elongation factor, are often chosen. These promoters induce high gene expression in a variety of cell types.

Key Slide



This promoter controls virus production and will be removed upon integration

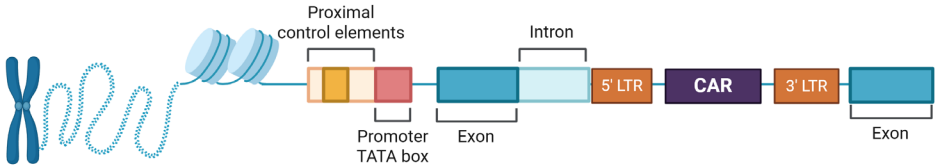
This promoter controls CAR expression

Keyer Biotech and Bioengineering 2024

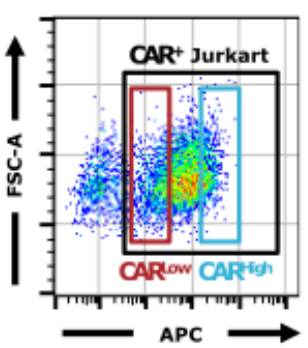
Lentiviral Problem #1: Variable expression of CAR

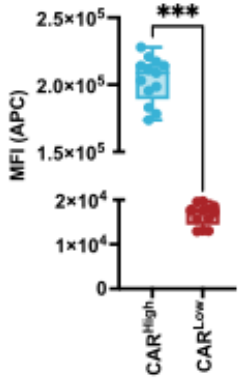
Rodriguez-Marquez Science Advances 2022

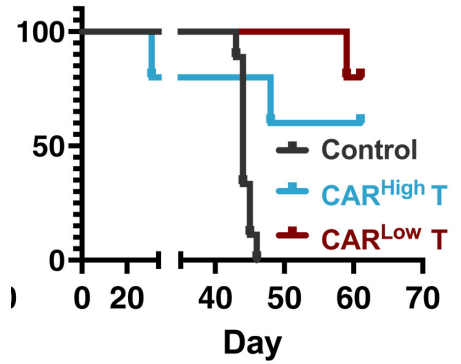
Semi-Random Insertion (retrovirus)



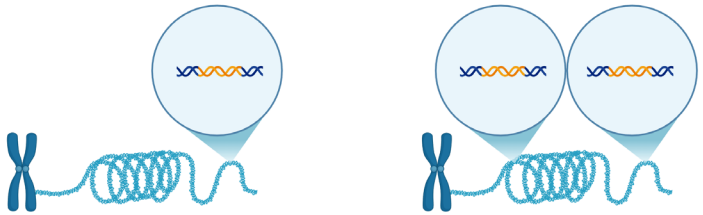
Unspecified Gene



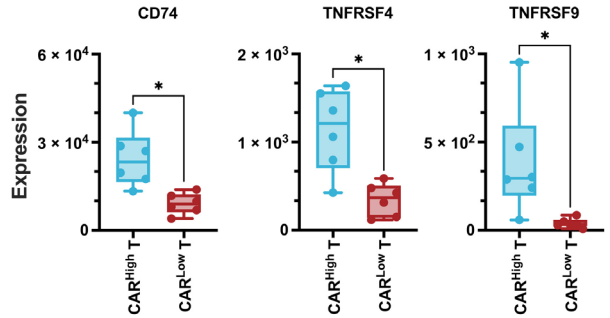




Multiplicity of Infection



Key Slide



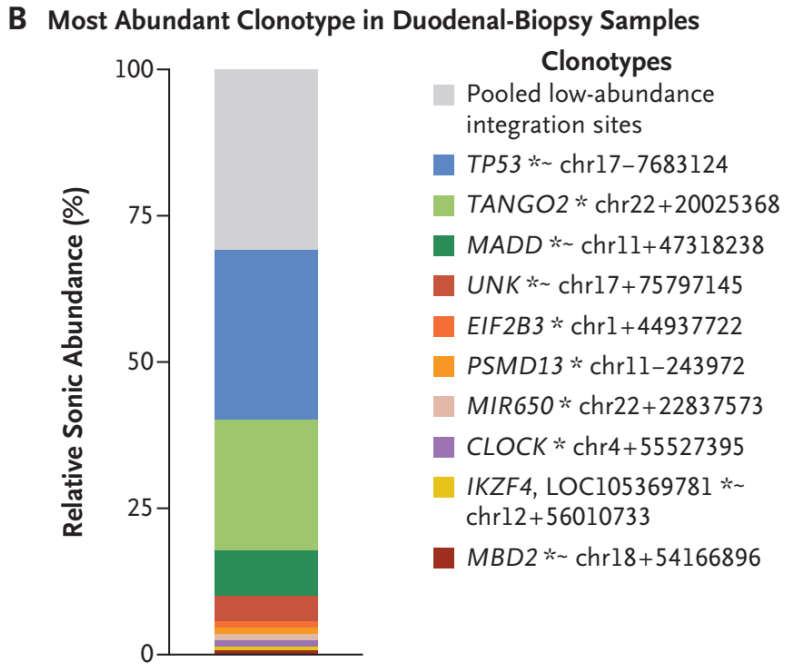
Even within a single product from a single patient using a single promoter, CAR expression from lentivirus is 'variegated.' Lentiviruses insert genes 'semi-randomly' across the genome, with a preference for open chromatin and intronic intragenic regions (so within highly transcribed gene bodies). Furthermore, the same cell can be infected with lentivirus multiple times, yielding high multiplicity of infection (MOI). The FDA requires low MOI, but even so, the combination of semi-random insertion and MOI leads to variable expression.

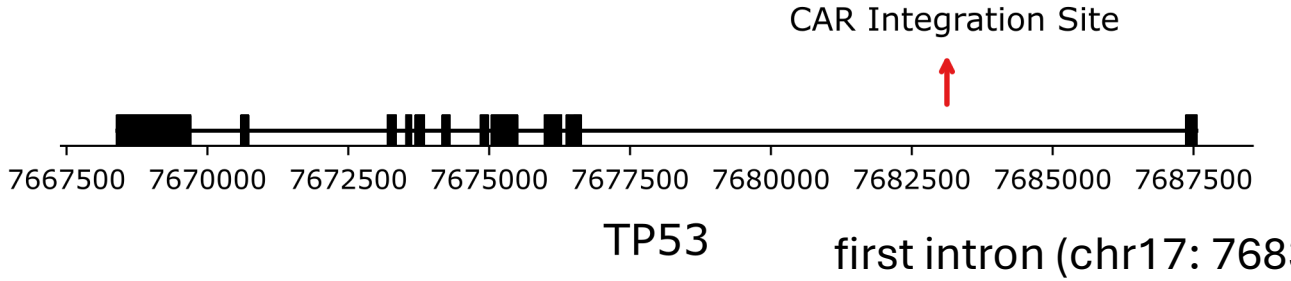
This variability has functional consequences. In one study, CAR T cell products enriched in CAR^{High} T cells show a significantly worse clinical response in several hematological malignancies (Rodriguez-Marquez et al).

Lentiviral Problem #2: Integration can disrupt normal gene function

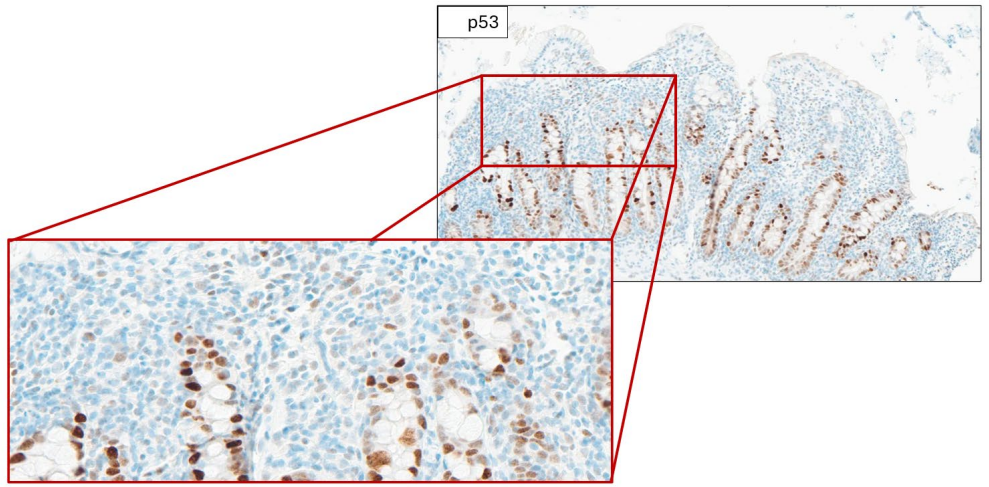
CAR integration into wrong gene can be deadly. A patient presented with copious diarrhea and was found to have a robust CAR infiltrate in the duodenum. The infiltrate was clonal; the CAR itself had transformed into a malignancy.

CAR integration site analysis revealed integration in first intron of *TP53*



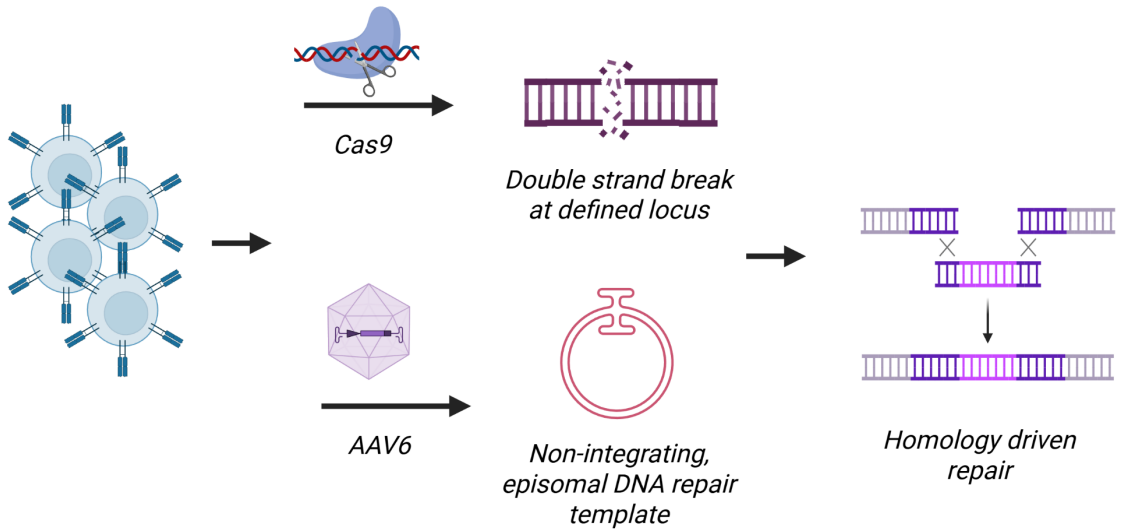


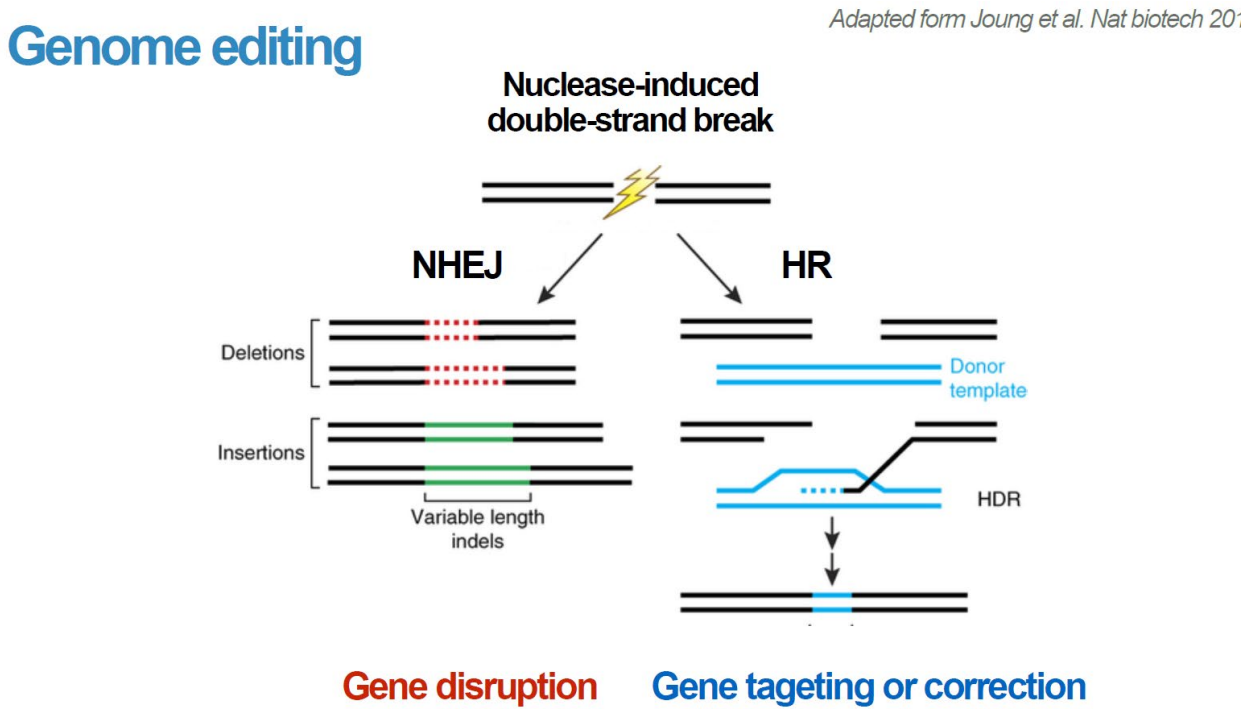
first intron (chr17: 7683124)



Perica et al., *NEJM* 2025

New Alternative: precisely deliver CAR to a specific gene locus





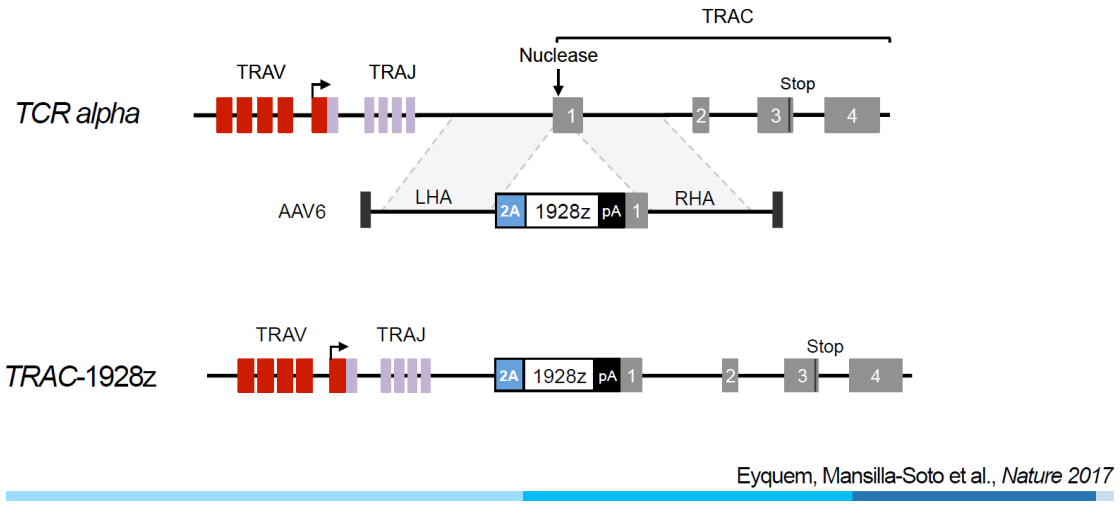
Key Slide

As an alternative to semi-random integration, site-specific insertion can be achieved using gene editing and homology driven repair (HDR).
CRISPR-Cas9 is used to specifically cut (induce a double strand break) at the location where we want the gene to be insert.
This break can be repaired in one of two ways: non-homologous end joining (the typical process, which often generates disrupted genes when you want a KO) or homology driven repair (HDR). HDR can occur when the cell has the right proteins expressed (usually during DNA replication, aka a proliferating cell) and a donor template with homology to the surrounding area. This donor template can be delivered for example by AAV, which does not frequently integrate and instead delivers an episomal donor template to the nucleus. This episome is used to smuggle CAR DNA in nucleus.

New Alternative: CAR targeting to *TRAC* locus

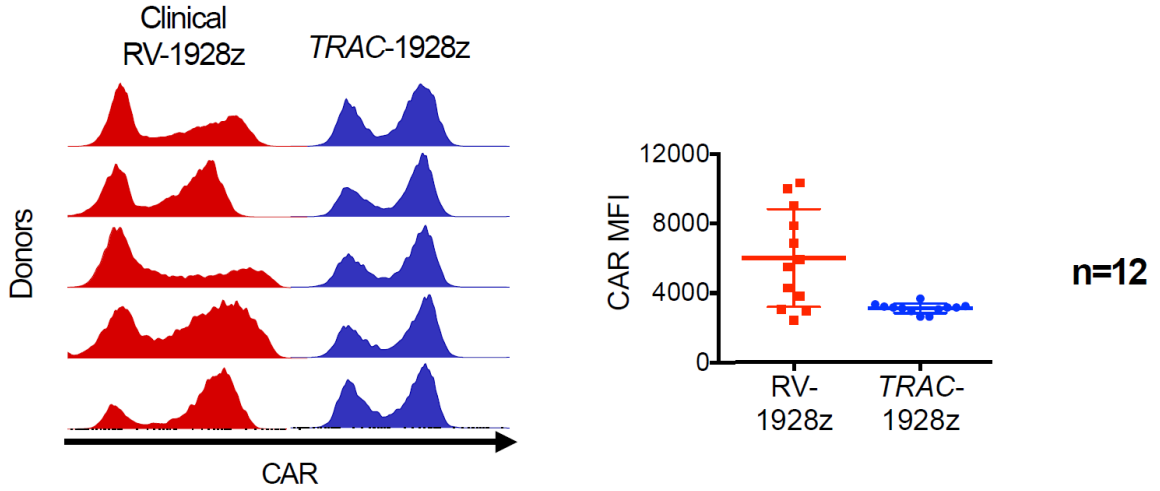
Which gene do we target for CAR integration? The T cell receptor alpha (TRAC) locus is a good place to start. CARs don't really need their TCR, so we knock it out with CRISPR. The donor AAV contains left and right homology arms (LHA, RHA) that trick the cell into repairing the genome with the CAR sequence in the middle. A single copy of the CAR gene is expressed under the control of the TRAC promoter*. This site specific insertion leads to lower, homogenous and predictable CAR expression compared to variegated retroviral (RV) expression.

Targeting the CAR transgene



Eyquem, Mansilla-Soto et al., *Nature 2017*

Homogeneous and Predictable CAR expression

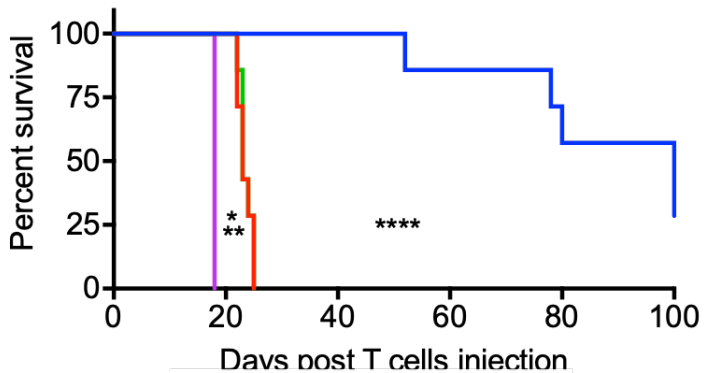


*this is a bit advanced, but it is usually a single copy because only one copy of the T cell receptor is typically recombined during T cell formation, so this is another reason to use TRAC locus as opposed to another gene.

Slide borrowed from Justin Eyquem

TRAC-CAR T cells display superior *in vivo* activity

B-ALL Xenograft model

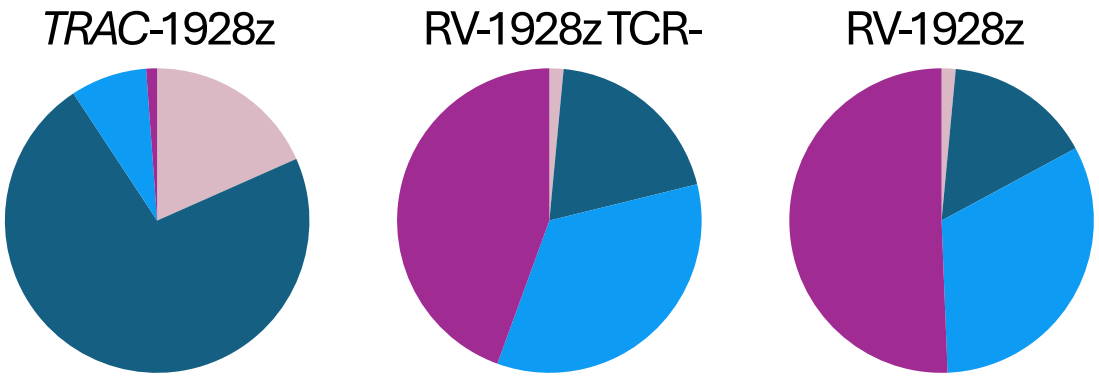


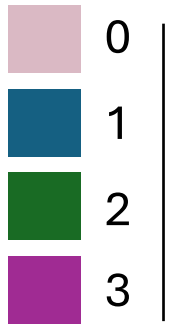
5e5 Nalm6 in NSG

-

2e5 CAR T cells

Bone Marrow T cell phenotyping





Exhaustion Markers

n=7 mice

Eyquem et al., *Nature* 2017

Slide borrowed from Justin Eyquem

Summary Part 1: AKA what you need to know

- A CAR consists of a binder recognizing a tumor antigen, a hinge/transmembrane region, a costimulatory domain and a CD3ζ T cell signaling domain
- CARs typically target lineage markers that ablate a tumor and its normal cell of origin (CD19 on B cells/B cell leukemias, BCMA on multiple myeloma/plasma cells)
- Second generation CARs include costimulation such as CD28 or 41BB which is required for *in vivo* CAR T cell persistence and function.
- CD28 compared to 41BB induces stronger signaling, which leads to greater sensitivity to antigen, more rapid expansion, and more effector function, but shorter persistence.

Summary Part 1: AKA what you need to know

- Retroviral vectors deliver CAR transgenes semi-randomly with variable MOI, leading to variable CAR expression

- Retroviral vectors are self-inactivating; they can produce virus once but should not replicate further due to a modification in their 3' LTR.

- In lentiviral systems, CAR is expressed off a promoter that you insert specifically for the purpose.

- Site-specific gene insertion can be achieved using gene editing following homology driven integration; you should know the general process, using a double strand break to target the integration and providing a homology template to promote repair.

Module 1: Exploring some CAR vectors

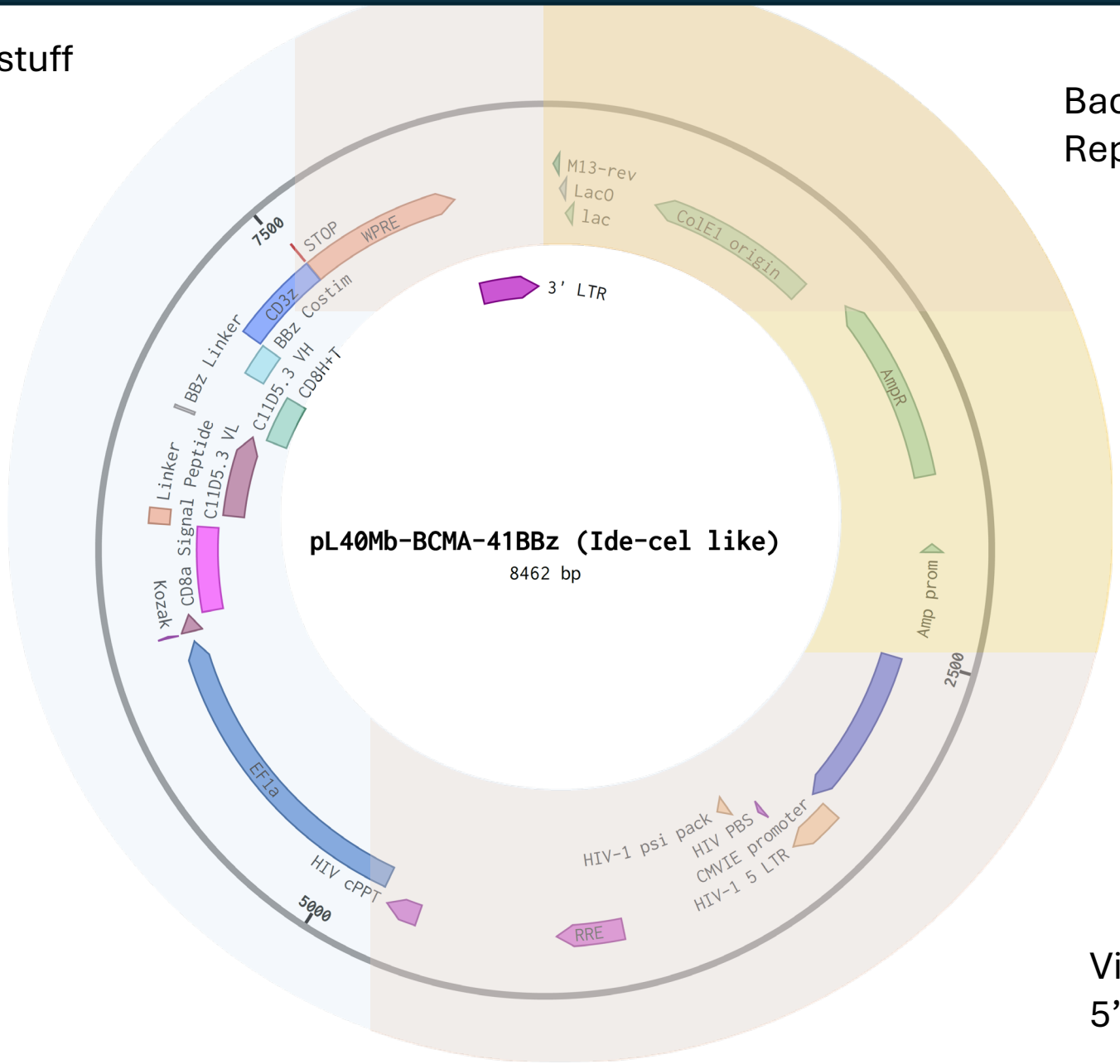
https://benchling.com/pericak/f_/HUqfe7QYGj-car-class/

Module 1: Exploring some CAR vectors

3’ Viral stuff

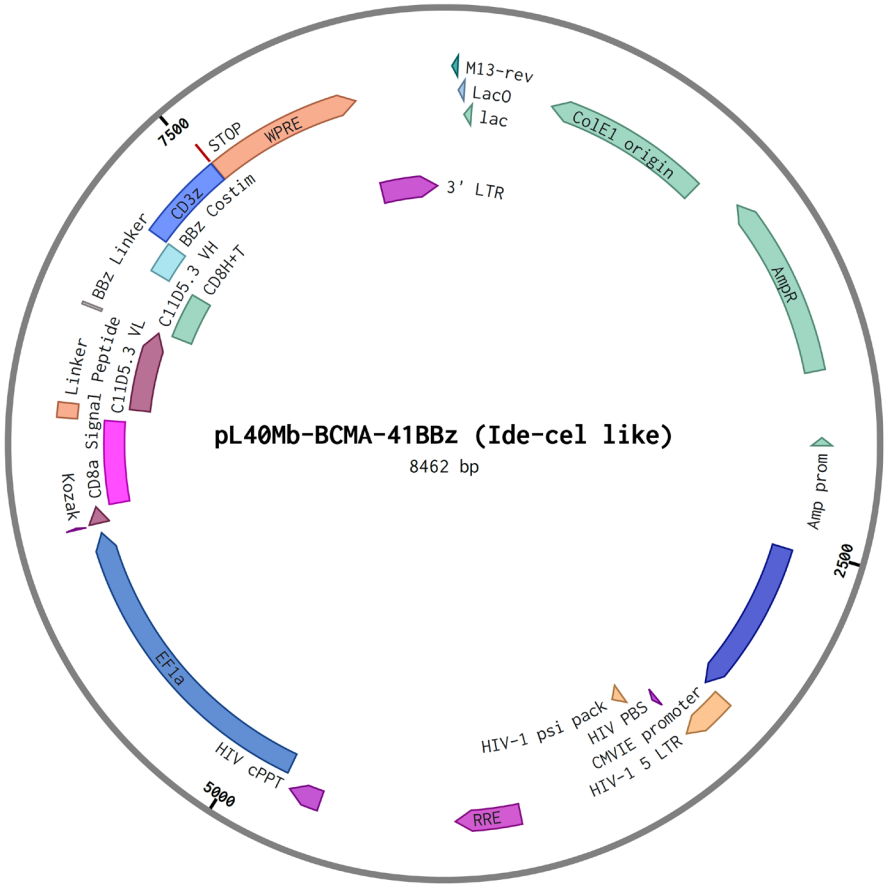
Bacterial stuff
Replication, Amp resistance

CAR promoter
CAR transgene



Viral stuff
5’ LTR

Some Definitions



Viral stuff

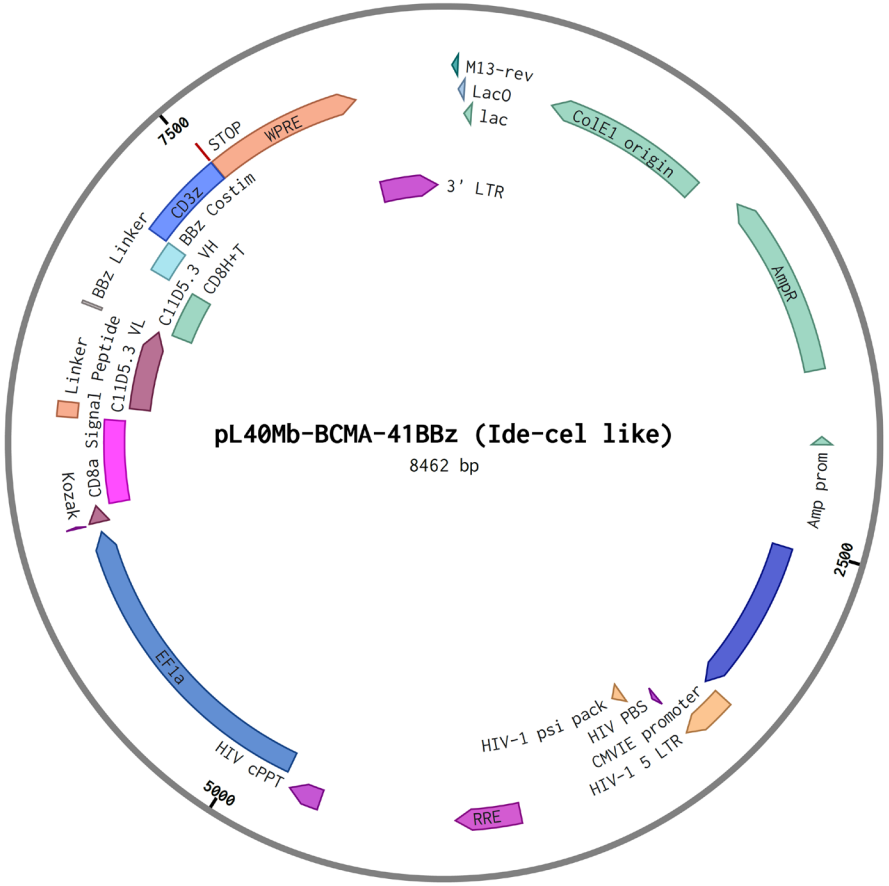
The retroviral psi (Ψ) packaging element is a cis-acting RNA element around 80–150 nucleotides in length that regulates viral capsid packaging.

The HIV-1 Rev response element (RRE) is a highly structured, ~350 nucleotide RNA segment that regulates nuclear export.

The polypurine tract (PPT) is a structure in HIV that is involved in viral replication, specifically the initiation of plus-strand DNA synthesis during reverse transcription.

WPRE is the Woodchuck Hepatitis Virus Post-Transcriptional Regulatory Element ,a DNA sequence that, when inserted into the 3' untranslated region (UTR) of a gene, enhances the expression of that gene. It does this by promoting mRNA export from the nucleus and increasing the stability of the resulting mRNA.

Some Definitions



CAR stuff

The **Kozak sequence** is a specific short DNA sequence in eukaryotic messenger RNA (mRNA) that enhances the initiation of protein translation at the start codon, AUG.

A signal peptide is a short sequence of amino acids at the N-terminus of a protein that acts as a "homing device" to direct the protein to its correct destination. CARs can ‘borrow’ the signal sequence from CD8α, but other sequences can increase expression.

A ‘linker’ joins the VH and VL segments of an antibody. Frequently, the G4S flexible linker is used.

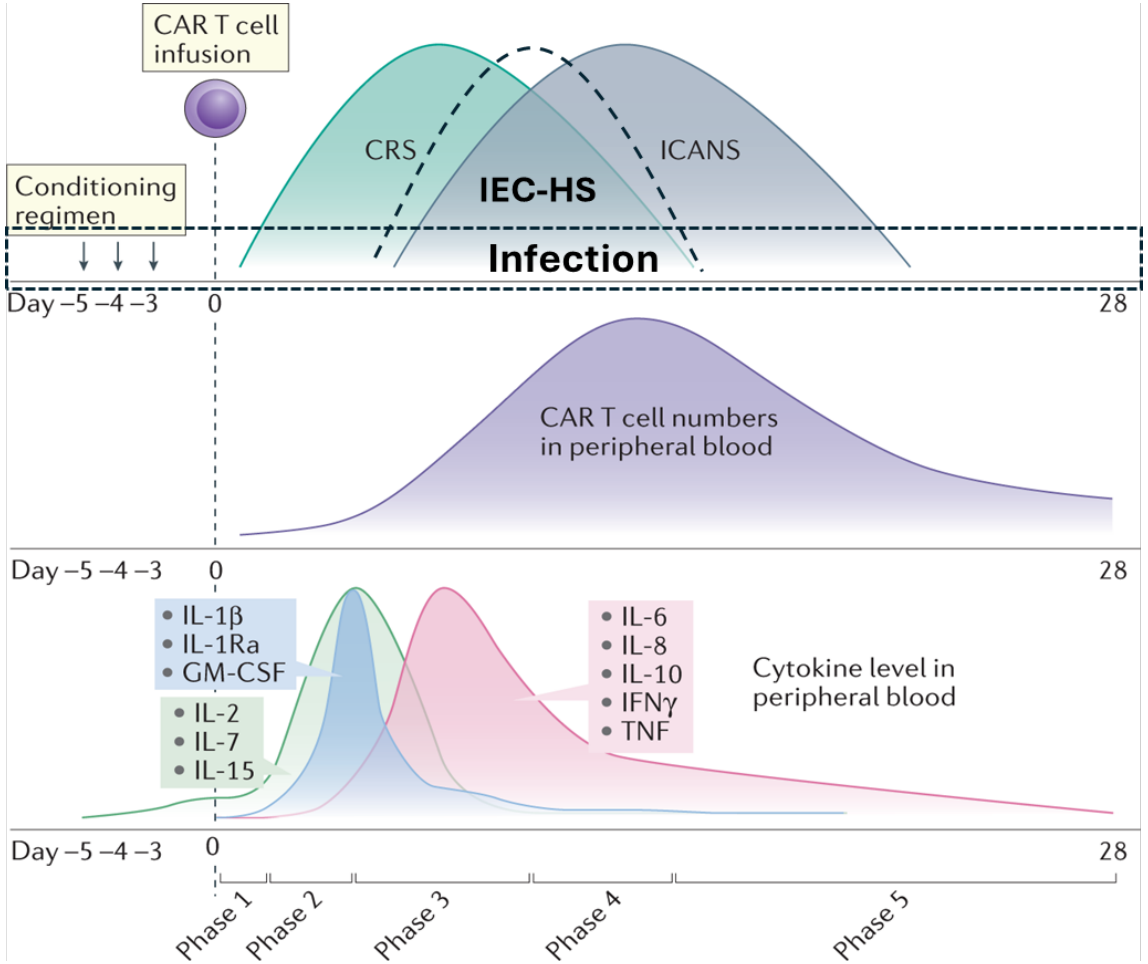
P2A sequences generate 2A peptides that are a class of 18–22 aa-long peptides which can induce ribosomal skipping during translation of a protein. They are useful for generating bicistronic vectors that express two genes off a single promoter.

LNGFR is a transmembrane protein frequently used as a neutral ‘marker’ in bicistronic vectors.

Problems with current CAR T cells #1: Toxicities

Acute Complications of CAR T cell Therapy

Adapted from Morris Nat Reviews Immuno 2021



CRS = cytokine release syndrome

ICANS = IEC-associated neurotoxicity syndrome

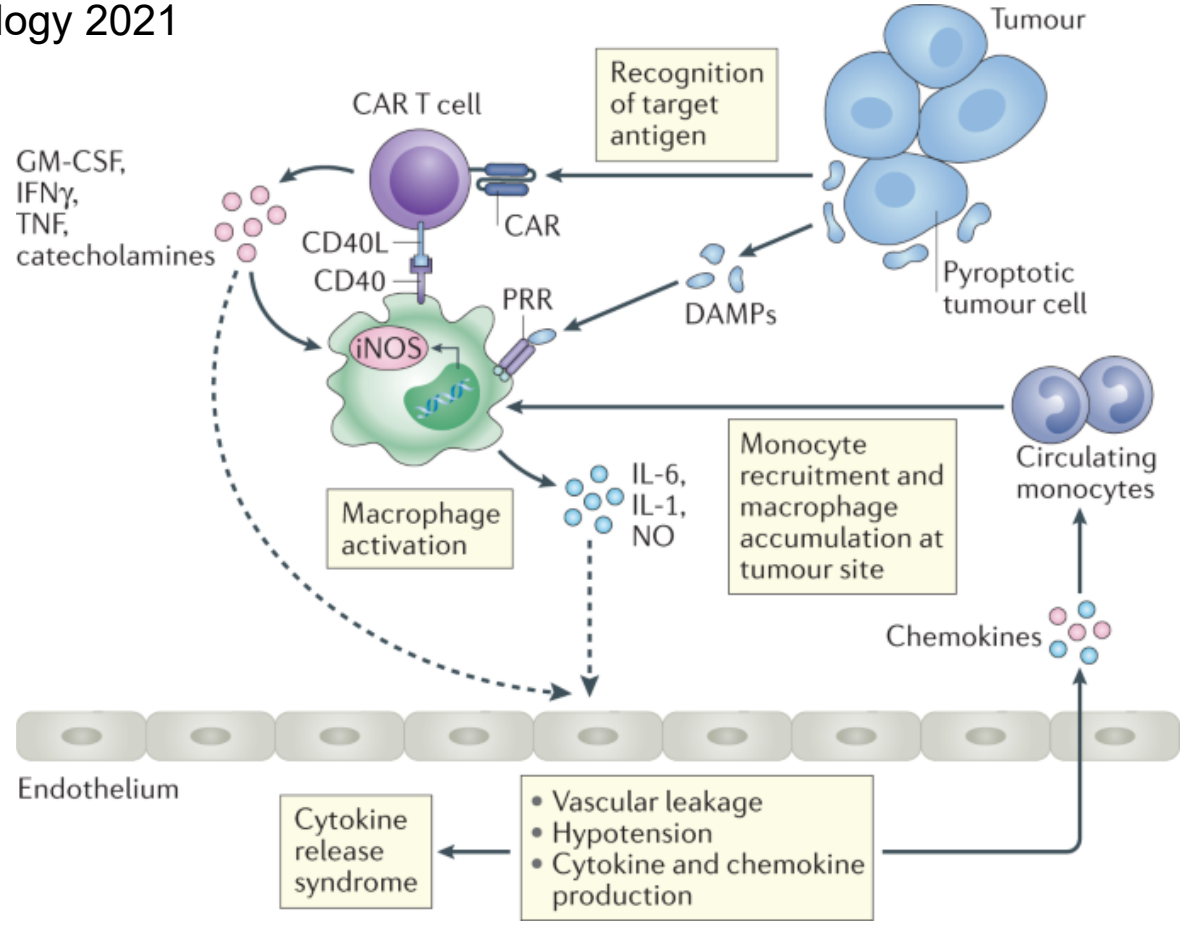
IEC-HS = IEC-associated HLH-like syndrome

CRS is systemic inflammation associated with release of cytokines such as IL6 leading to fever, hypotension, and hypoxia.
ICANs is a neurologic syndrome associated with blood brain barrier breakdown leading to loss of speech and consciousness.
They occur at specific times and with specific products, and they are related but distinct syndromes.

CRS pathophysiology

Morris Nature Reviews Immunology 2021

Key Slide



CRS depends on cross-talk between tumor, T cell, and macrophage/monocytes
Monocytes are major source of IL-1 and IL-6 (Norelli Nat Med 2018, Giavridis Nat Med 2018)
IL-6 is a systemic inflammatory cytokine that regulates acute phase response
IL-6 blocking drugs like Tocilizumab are used in combination with immune suppressing steroids like dexamethasone to treat CRS

Grading and Treatment CRS: ASTCT Concensus

CRS Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever	Temperature ≥38◦C	Temperature ≥38◦C	Temperature ≥38◦C	Temperature ≥38◦C
Hypotension	None	Not requiring vasopressors	Requiring 1 vasopressor	Requiring multiple vasopressors
Hypoxia	None	Low-flow nasal cannula	High-flow nasal cannula or equivalent	Intubation & mechanical ventilation

h/t Natalie Brunwell

Lee DW, et al. *Biol Blood Marrow Transplant*. 2019;25:625-38.

Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS)

Santomasso JCO 2019
Neelapu NRCO 2017

Day 4, MMSE 29/30

I love Shawnee, KS.

Day 5, MMSE 27/30

Day 6, MMSE 29/30

I miss my kids.

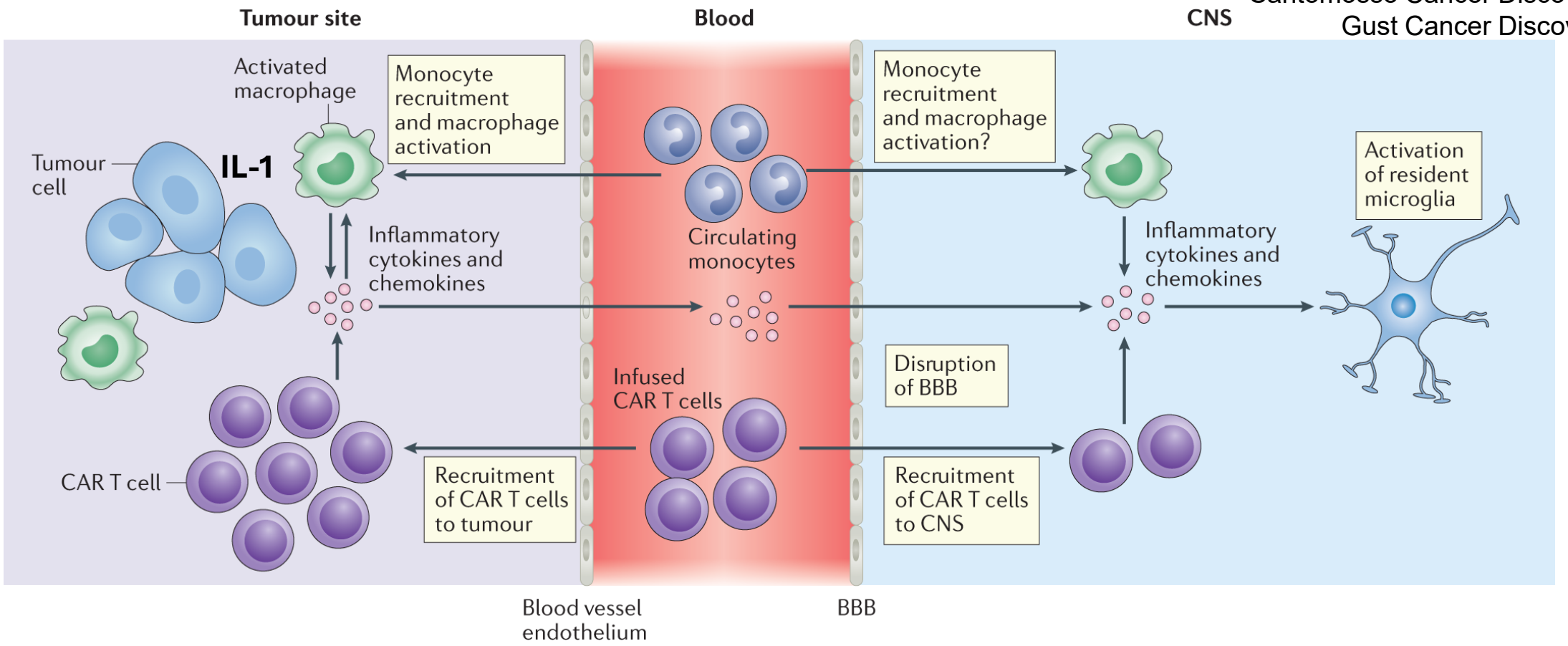
Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE score	7–9	3–6	0–2	0 (patient is unarousable and unable to perform ICE)
Depressed level of consciousness	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse; stupor or coma
Seizure	NA	NA	Any clinical seizure focal or generalized that resolves rapidly; or nonconvulsive seizures on EEG that resolve with intervention	Life-threatening prolonged seizure (> 5 min); or repetitive clinical or electrical seizures without return to baseline in between
Motor findings	NA	NA	NA	Deep focal motor weakness such as hemiparesis or paraparesis
Raised ICP/cerebral edema	NA	NA	Focal/local edema with or without hemorrhage on neuroimaging	Diffuse cerebral edema on neuroimaging; decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing triad

Abbreviations: ASBMT, American Society of Blood and Marrow Transplantation; EEG, electroencephalogram; ICANS, immune effector cell–associated neurologic syndrome; ICE, Immune Effector Cell Encephalopathy screening tool; ICP, intracranial pressure; NA, not applicable.

ICANS is a neurologic syndrome occurring after CAR T cell infusion which typically manifests with speech disturbances such as aphasia, and can progress to in rare cases to obtundation, coma, cerebral hernation and death.

Pathobiology of ICANS involves inflammatory cytokines and endothelial injury

Morris Nature Reviews Immunology 2022
Santomosso Cancer Discovery 2018
Gust Cancer Discovery 2017



- Pathophysiology of ICANS starts with the production of pro-inflammatory cytokines by CAR T cells and the activation of bystander immune cells such as macrophages in the tumor microenvironment.
- Inflammatory cytokines and chemokines such as IL-1β, IL-6, IL-10, the chemokines CXCL8 and CCL2, interferon-γ, granulocyte–macrophage colony-stimulating factor and tumor necrosis factor — diffuse into the bloodstream and, eventually, result in disruption of the blood–brain barrier (BBB), with accumulation of cytokines and CAR T cells in the central nervous system (CNS) together with activation of resident microglial cells.

Class Effects of the Cell-Mediated Immune Response: CRS and Neurotoxicity

Disease	ALL	DLBCL		MCL	MM	
Trial	ZUMA-3[2]	ZUMA-1[4]	TRANSCEND[5]	ZUMA-2[6]	KarMMa[7]	CARTITUDE-1
CAR T-cell agent	Brex. autoleucel	Axicabtagene ciloleucel	Lisocabtagene maraleucel	Brex. autoleucel	Idecabtagene Vicleucel	Ciltacabtagene
Construct	Anti–CD19-**CD28**-CD3z	Anti–CD19-**CD28**-CD3z	Anti–CD19-**41BB**-CD3z	Anti–CD19-**CD28**-CD3z	Anti-BCMA	Anti-BCMA
N treated	55	101	269	68	128	97
CRS, %	89†	93†	42†	91†	84†	95
Grade ≥3 CRS, %	24†	13†	2†	15†	5†	5
NT, %	60	64	30	63	18	17
Grade ≥3 NT, %	25	28	10	31	3	2

CD28 costimulated CAR T cells have much higher rates of serious Grade 3 or higher Cytokine Release Syndrome and ICANS than 41BB costimulated CAR T cells

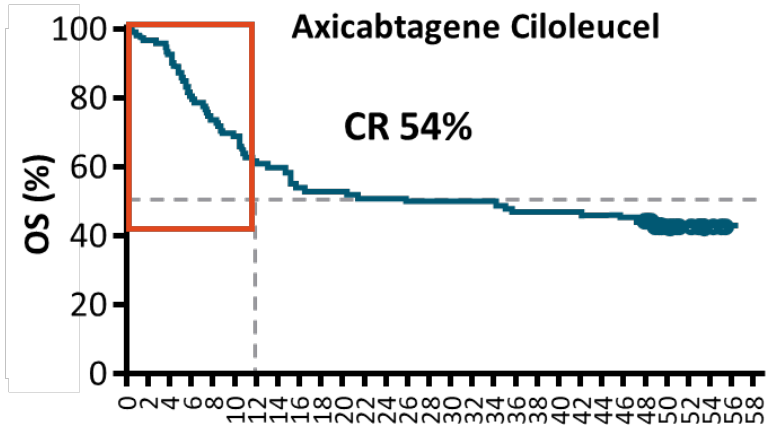
Key Slide

1. Maude. NEJM. 2018;378:439. 2. Shah. Lancet. 2021;[Epub]. 3. Schuster. NEJM. 2019;380:45. 4. Neelapu. NEJM. 2017;377:2531.
5. Abramson. Lancet. 2020;396:839. 6. Wang. NEJM. 2020;382:1331. 7. Munshi. NEJM. 2021;384:705.

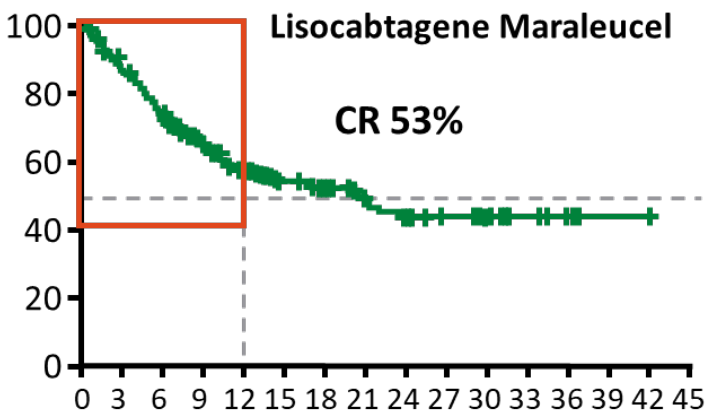
Problems with current CAR T cells #2: Relapse

Response and Survival in CAR T cell therapy

Lymphoma

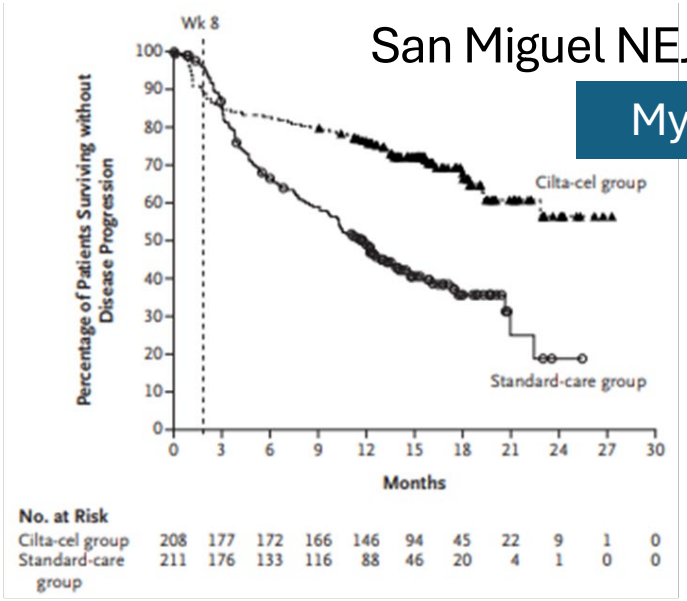


Lymphoma



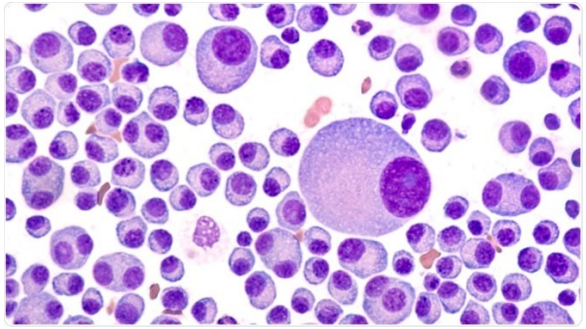
San Miguel NEJM 2023

Myeloma



For both lymphoma and multiple myeloma, most patients (50-80%) will have a response, that is, tumor initially shrinks or even completely disappears (complete response – CR). However, most patients will also relapse. We think the best CARs cure about 40% of patients with lymphoma, and maybe 25% of patients with myeloma (Jagganath JCO 2025). This is impressive, as these patients failed all other treatments, and myeloma was thought to be incurable. But the fact remains that the majority relapse.

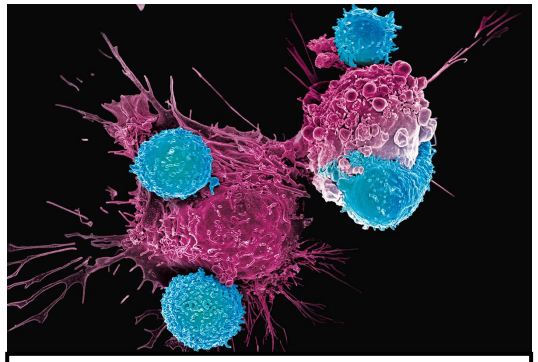
Mechanisms of Resistance to CAR T cell therapies



Cancer cell related

Loss or decrease in antigen expression

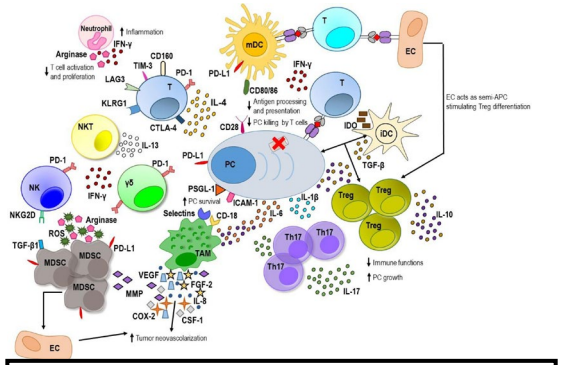
- CAR T constructs targeting low antigen density
- **Dual targeting**



T cell related

Lack of persistence or exhaustion of T cells

- T cell therapies at earlier stages of disease
- Allogeneic cell therapies
- CAR design
- **T cell gene engineering**



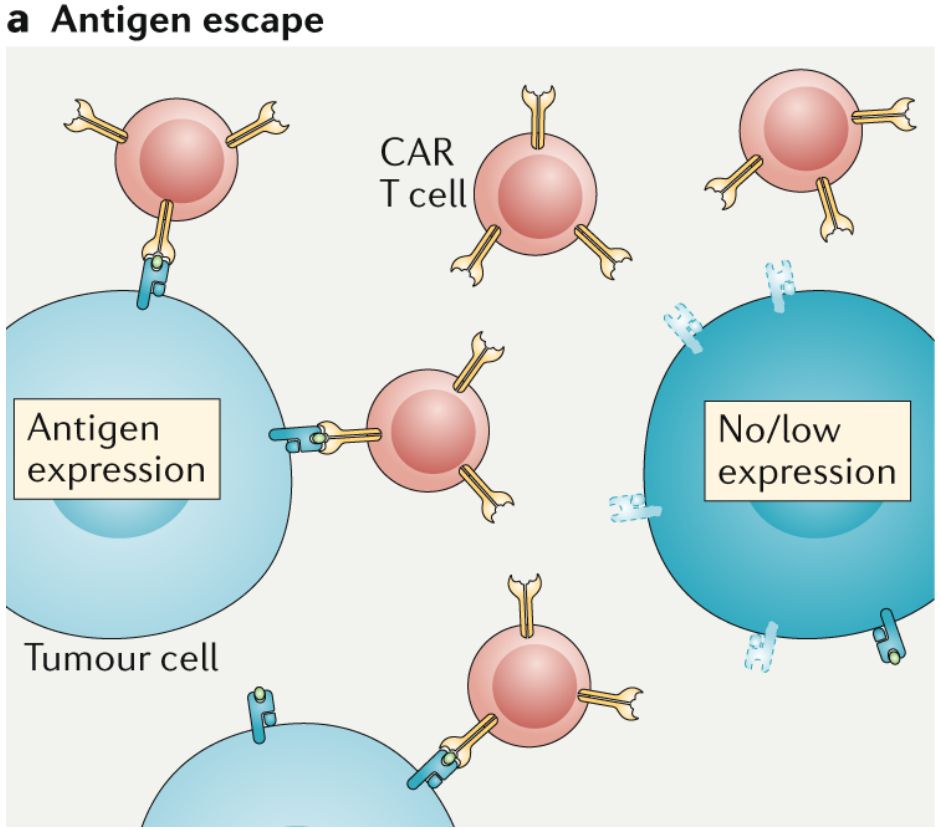
Microenvironment related

Extramedullary disease

- CAR armoring
- Radiation therapy

Rate of antigen loss varies by disease and construct

Brown Nature Reviews Immunology 2019



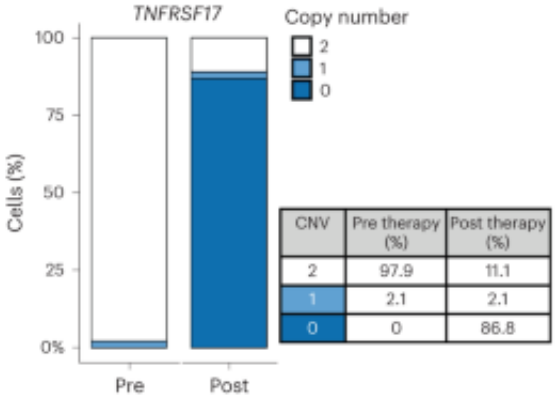
Adapted from Majzner Cancer Discovery 2018

Disease	CAR construct	Antigen Low/Negative relapse	References
Pediatric ALL	CD19-BBζ (Tisa-cel)	25% (15/61)	Maude NEJM 2018 ELIANA
Pediatric ALL	CD19-BBζ (Seattle)	18% (7/40)	Gardner Blood 2016
Adult ALL	CD19-28ζ (MSKCC)	9% (4/44)	Park NEJM 2018
Adult ALL	CD19 (FCCRC)	7% (2/29)	Turtle JCI 2016
Adult LCL	CD19 (Axi-cel)	5% (5/100)	Plaks Blood 2021 ZUMA-1
Myeloma	BCMA-BBζ (Ide-Cel)	**4% (3/71)**	Munshi NEJM
Myeloma	GPRC5D-BBζ (MSKCC)	**50% (6/12)**	Mailankody NEJM

There are a number of ways that tumor can resist CAR T cells, including expression of anti-apoptotic proteins. However, the most straightforward way is to lose expression of the antigen that CAR targets, such as CD19 or BCMA. Antigen loss varies by disease and CAR construct. It is not the majority of relapses but is a sizeable fraction.

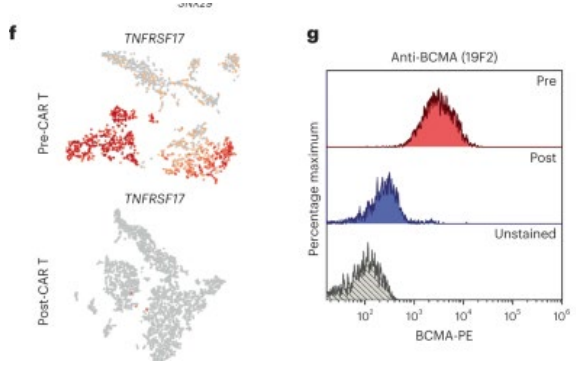
Mechanisms of antigen loss in multiple myeloma

BCMA: 3/71 patients



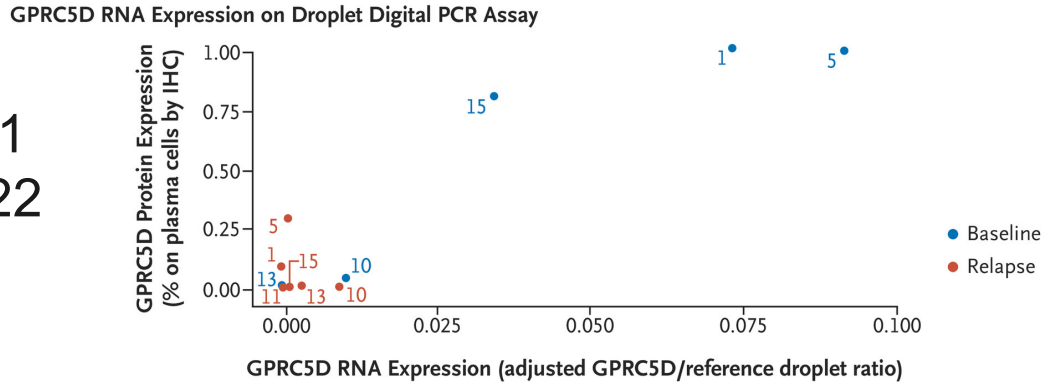
CNV	Pre therapy (%)	Post therapy (%)
2	97.9	11.1
1	2.1	2.1
0	0	86.8

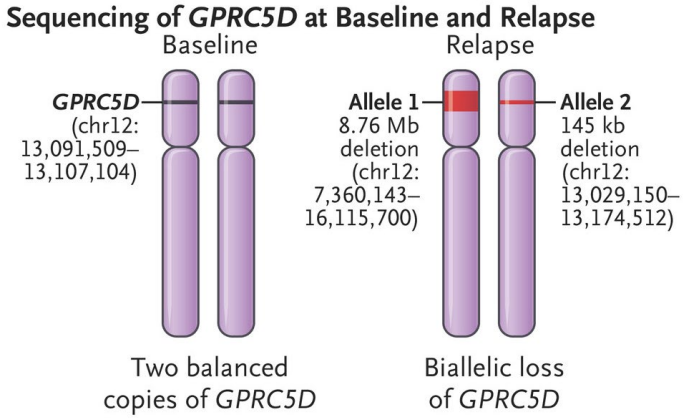
Lee Nat Med 2023
Munshi NEJM
Truger Blood Adv 2021
Samur Nat Comm 2022



GPRC5D: 6/12 patients

Mi NEJM 2023

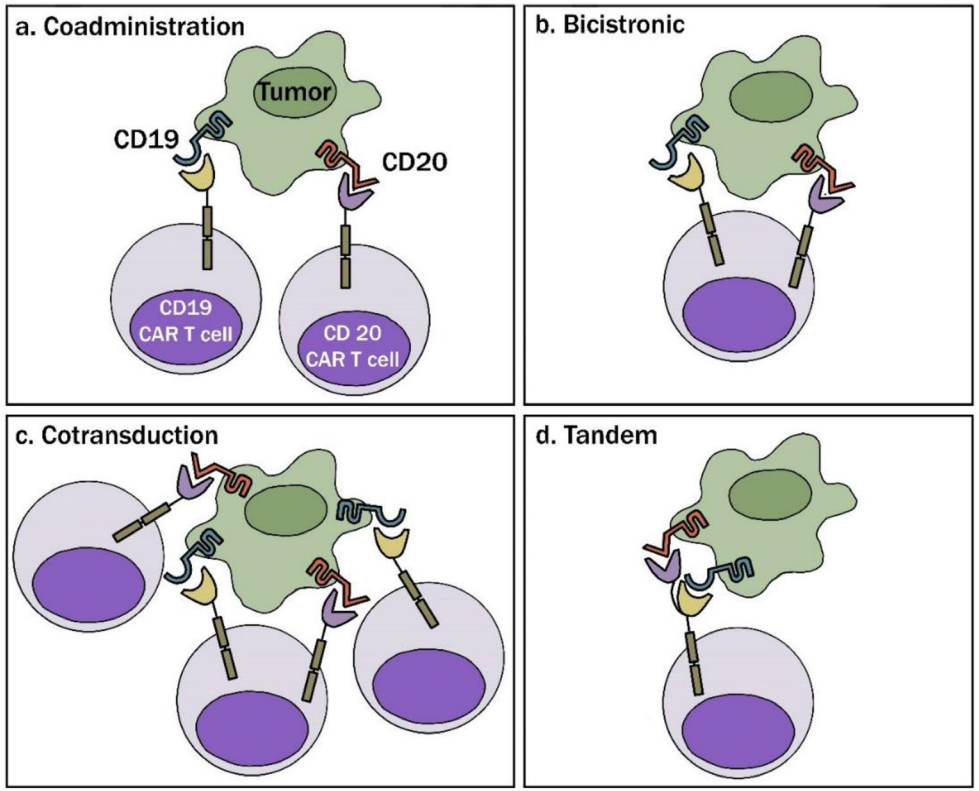




The myeloma antigen BCMA is rarely lost, whereas loss of GPRC5D appears more common. The current thinking is that BCMA is more central to myeloma biology and progression than GPRC5D. Thus there is a greater cost to BCMA loss. BCMA is downregulated and adjusted in some patients, but GPRC5D frequently shows biallelic deletions at relapse.

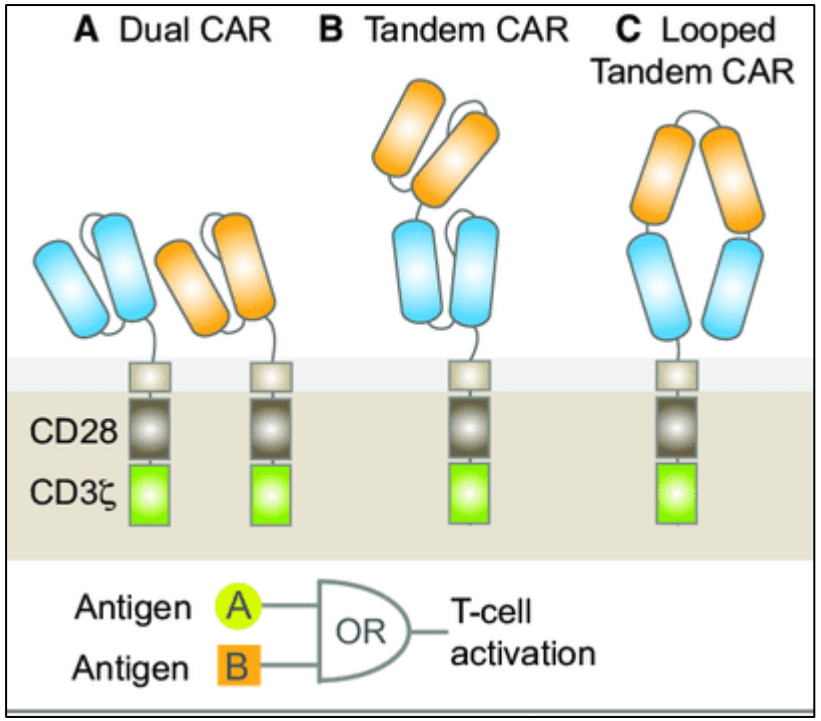
Potential Solution: Target two antigens at same time.

Cronk Cancers 2020



Guedan Mol Therapy 2018

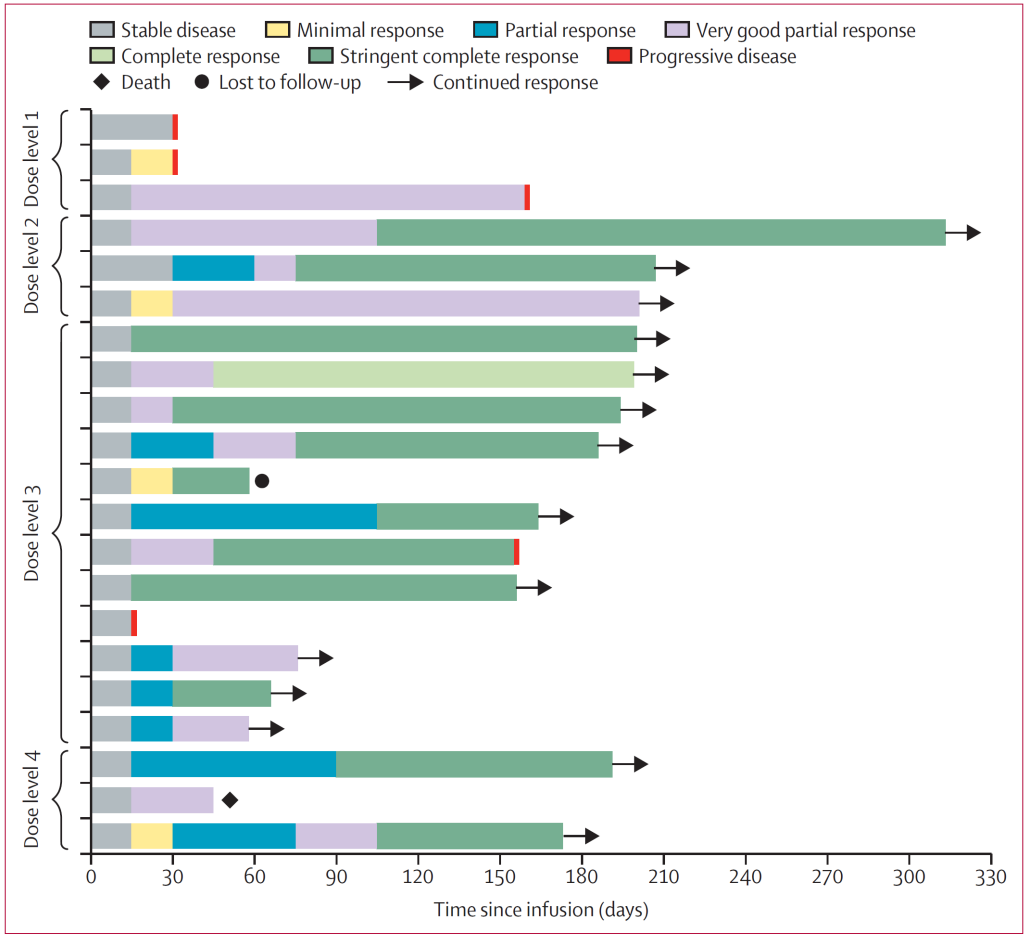
Key Slide



If loss of a single antigen drives relapse, can you target two antigens simultaneously?
Dual antigen chimeric antigen receptor CAR T approaches. (**a**) Coadministration: involves production of two separate CAR T cell products infused together or sequentially. (**b**) Bicistronic: allows expression of two different CARs on the same cell. (**c**) Cotransduction: encodes two CAR constructs with multiple vectors. With this process, one will also obtain cells that express each CAR alone. (**d**) Tandem: encodes two CARs on same chimeric protein using a single vector.
These designs generate an OR gate logic; expression of either antigen activates CAR T cell to kill.

BCMA/GPRC5D tandem CAR for multiple myeloma

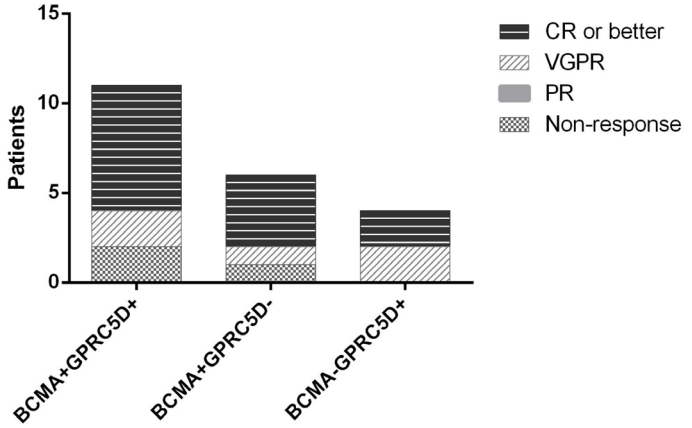
Zhou Lancet Hematology 2024



	All dose levels (n=21)	Dose level 1 (n=3)	Dose level 2 (n=3)	Dose level 3 (n=12)	Dose level 4 (n=3)
Overall response	18 (86%)	1 (33%)	3 (100%)	11 (92%)	3 (100%)
Very good partial response or better	18 (86%)	1 (33%)	3 (100%)	11 (92%)	3 (100%)
Complete response or better	13 (62%)	0	2 (67%)	9 (75%)	2 (67%)
Measurable residual disease negativity in bone marrow	17 (81%)	1 (33%)	3 (100%)	10 (83%)	3 (100%)

Data are n (%). Dose level 1: $0{\cdot}5 \times 10^6$ CAR T cells per kg. Dose level 2: $1{\cdot}0 \times 10^6$ CAR T cells per kg. Dose level 3: $2{\cdot}0 \times 10^6$ CAR T cells per kg. Dose level 4: $4{\cdot}0 \times 10^6$ CAR T cells per kg. CAR=chimeric antigen receptor.

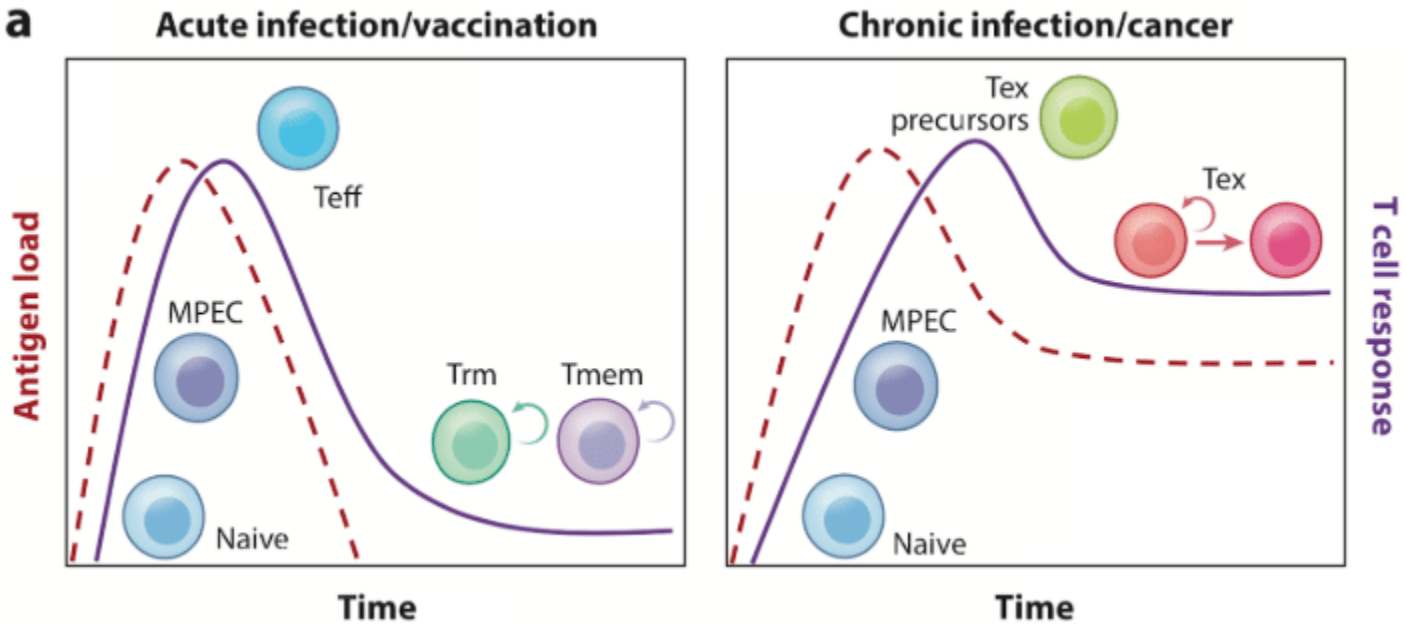
Table 3: **Clinical responses at different doses**

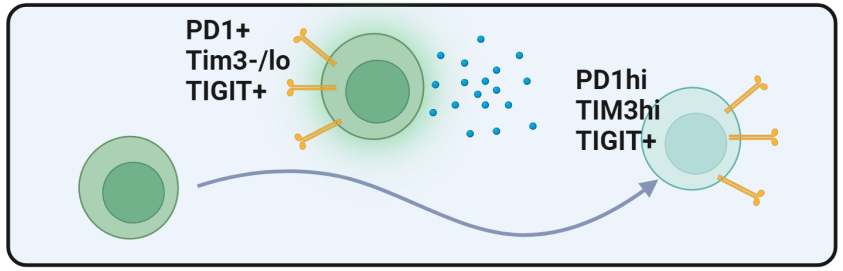


Encouraging activity including against single antigen expressing disease
Short follow up, and not yet clear if/what will drive relapse

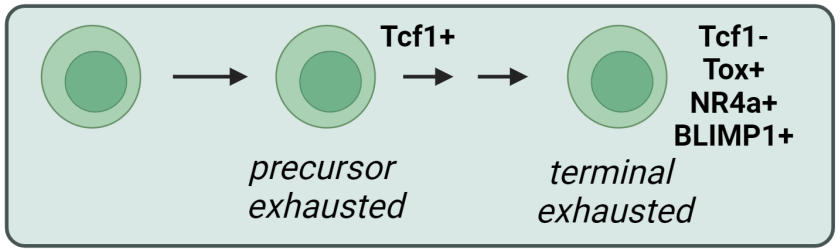
T cell factors

McLane Annual Reviews 2019
Giles *Immunity* 2023

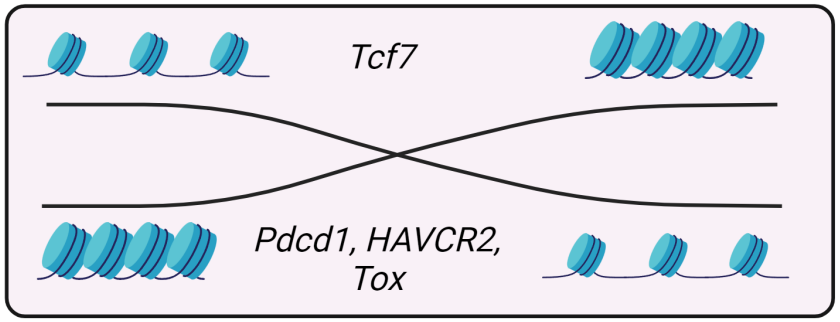




Transcriptional program



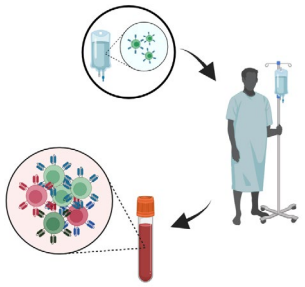
Epigenetic program

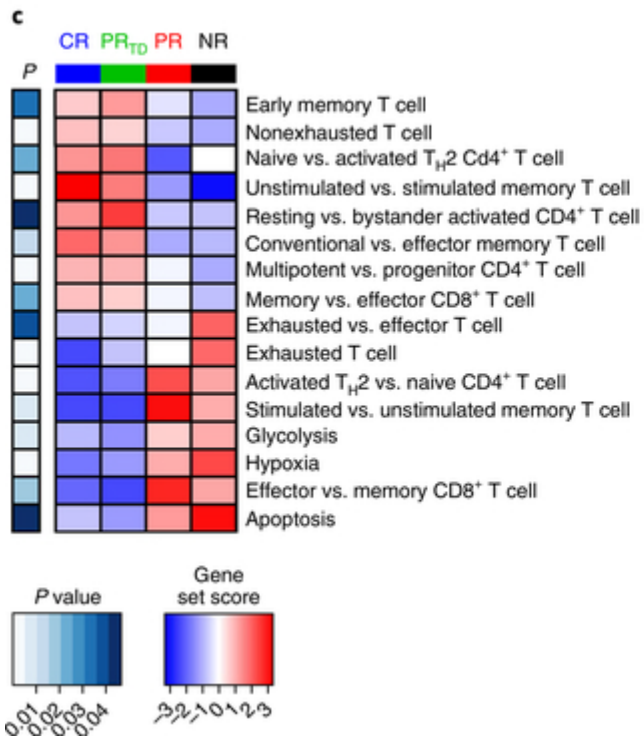


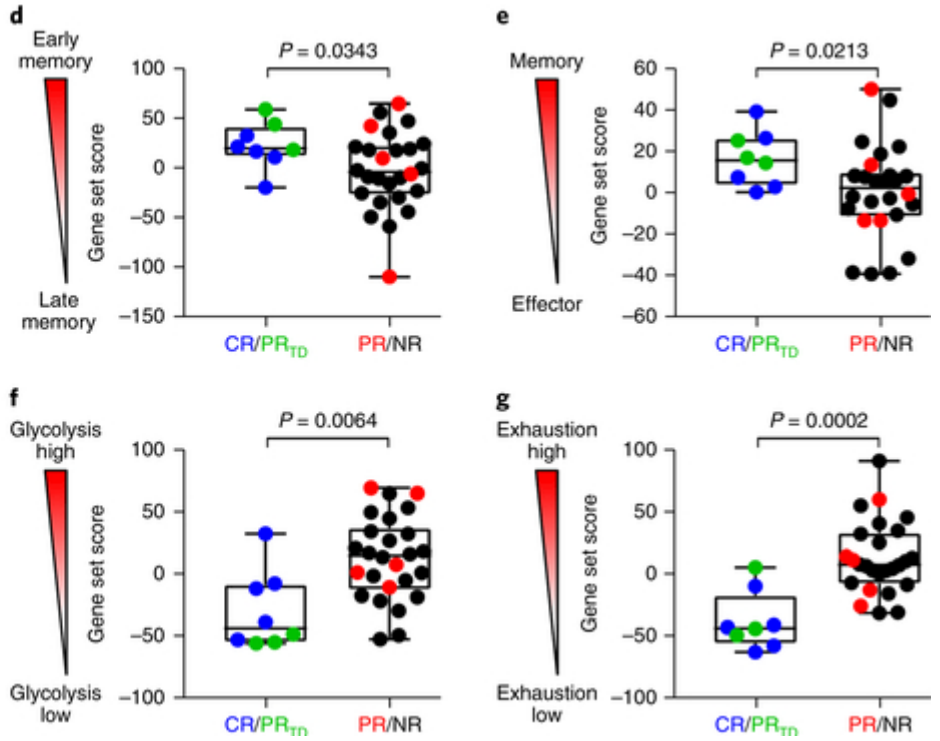
T cell exhaustion is a state of persistent dysfunction under chronic antigen stimulation. Exhaustion is associated with characteristic epigenetic, transcriptional, and functional changes. The term is probably overused, and inhibitory receptor expression (PD-1, TIM3 etc) cannot readily distinguish active and exhausted cells. See Masopust et al. Nature Reviews Immunology 2025 for current best practice on T cell states and naming.

Preinfusion T cell quality

Fraietta JA et al. Nat. Med. 2018.







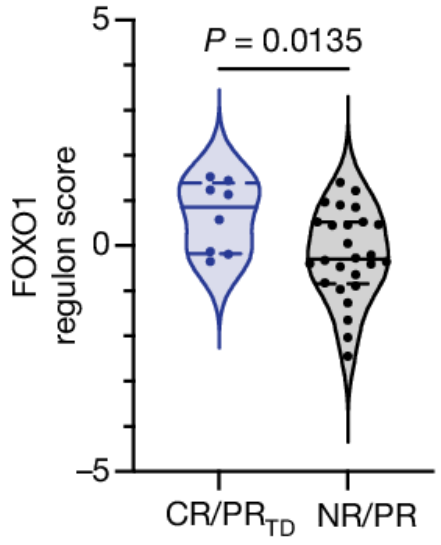
- **Fraietta Nat Med 2018**
  CD27+ PD1- CD8+, IL6/Stat3 signatures
- **Locke Blood Advs 2020**
  CCR7+CD45RA+ cells
- **Rossi Blood 2018**
  Polyfunctionality index
- **Deng Nat Med 2020**
  scRNA: exhaustion
- **Monfrini Clin Canc Res 2022**
  CD8+ central memory
- **Singh Sci Trans 2016**
  CCR7+ CD45RO-
- **Bai Sci Adv 2022**
  CCR7+ TCM

The patients T cell 'quality' can massively influence CAR T cell efficacy. **Even before cells are infused**, there is variability in T cell phenotypes.
Quality can be defined in a number of ways, although memory-like markers seem to predominate
Older, heavily pre-treated patients may be at risk of poor cell quality

Can we engineer T cell quality in patients that lack it?

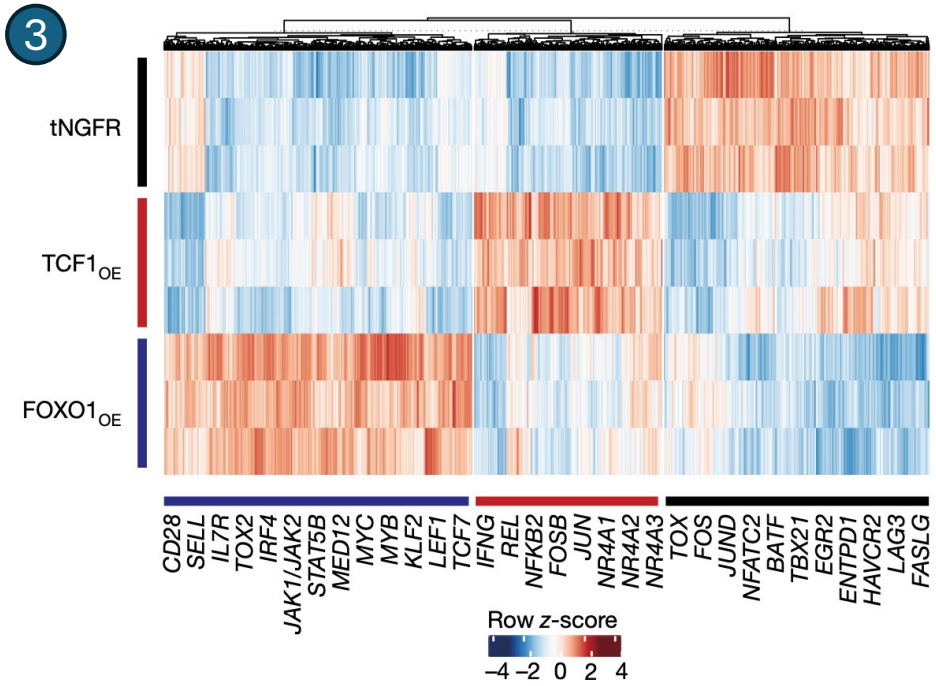
Doan Nature 2024
Chan Nature 2024

1



2

3



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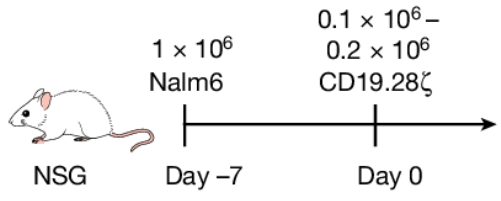
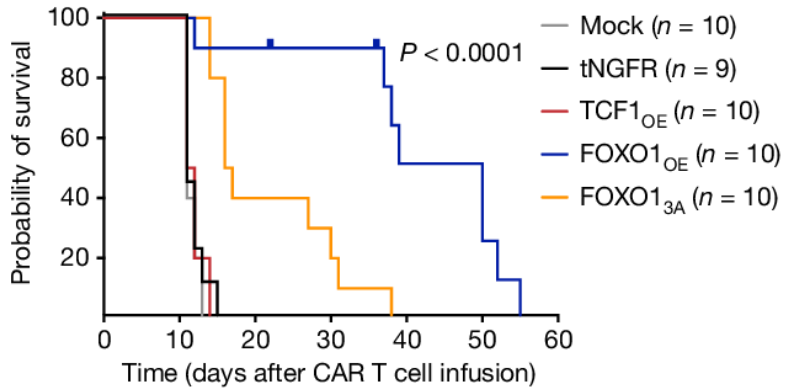


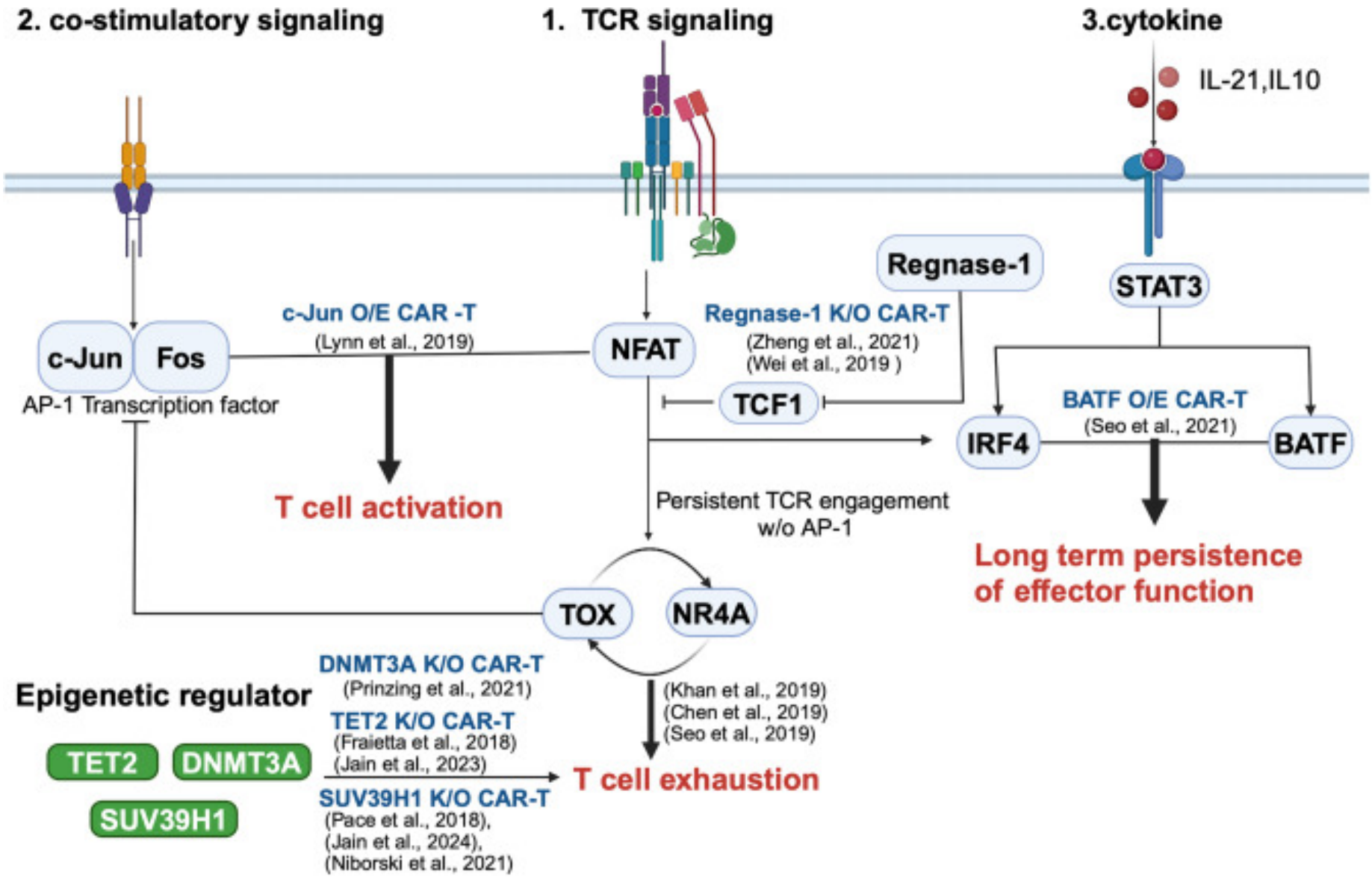


Key Slide

1) The authors notice that FOXO1 regulon is upregulated in patients responding to CAR T cells. FOXO1 is known to be involved in T cell memory and persistence. So they hypothesize overexpression will enhance CAR function. 2) They engineer a bicistronic vector that expresses a CAR and FOXO1 (linked with p2A). 3) It remodels the T cell to enhance quality and 4) improves CAR function *in vivo*.

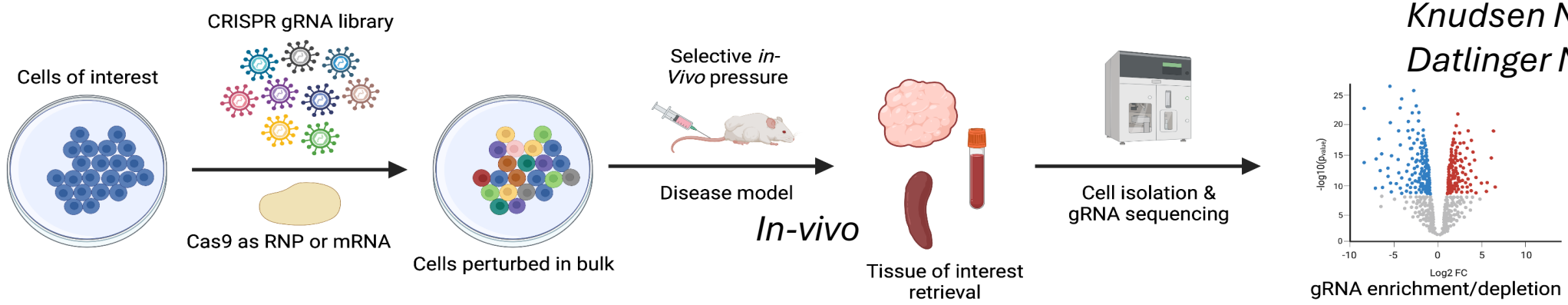
There are many proposed targets for gene editing to enhance CAR T cell function

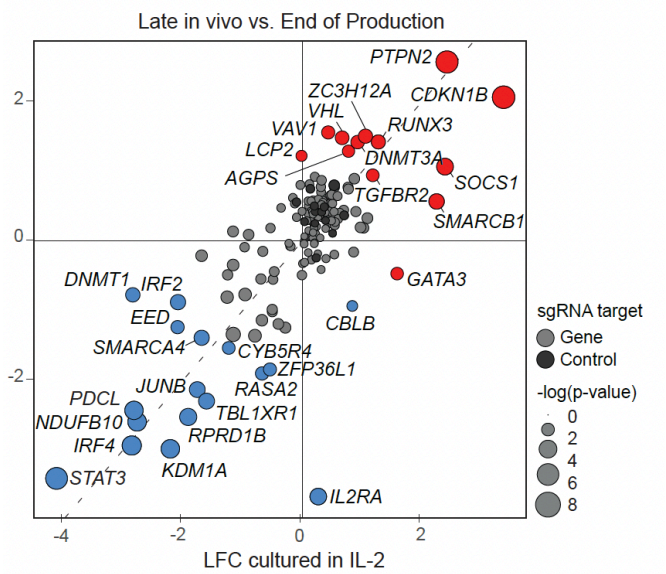
Ahn Molecular Therapy 2024

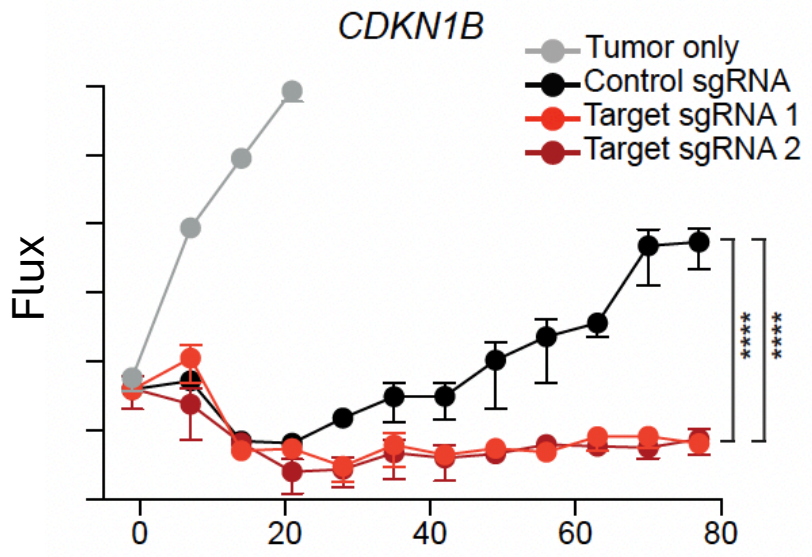


Genetic screens are an alternative strategy to define potential edits to enhance CAR therapy

Knudsen Nature 2025
Datlinger Nature 2025

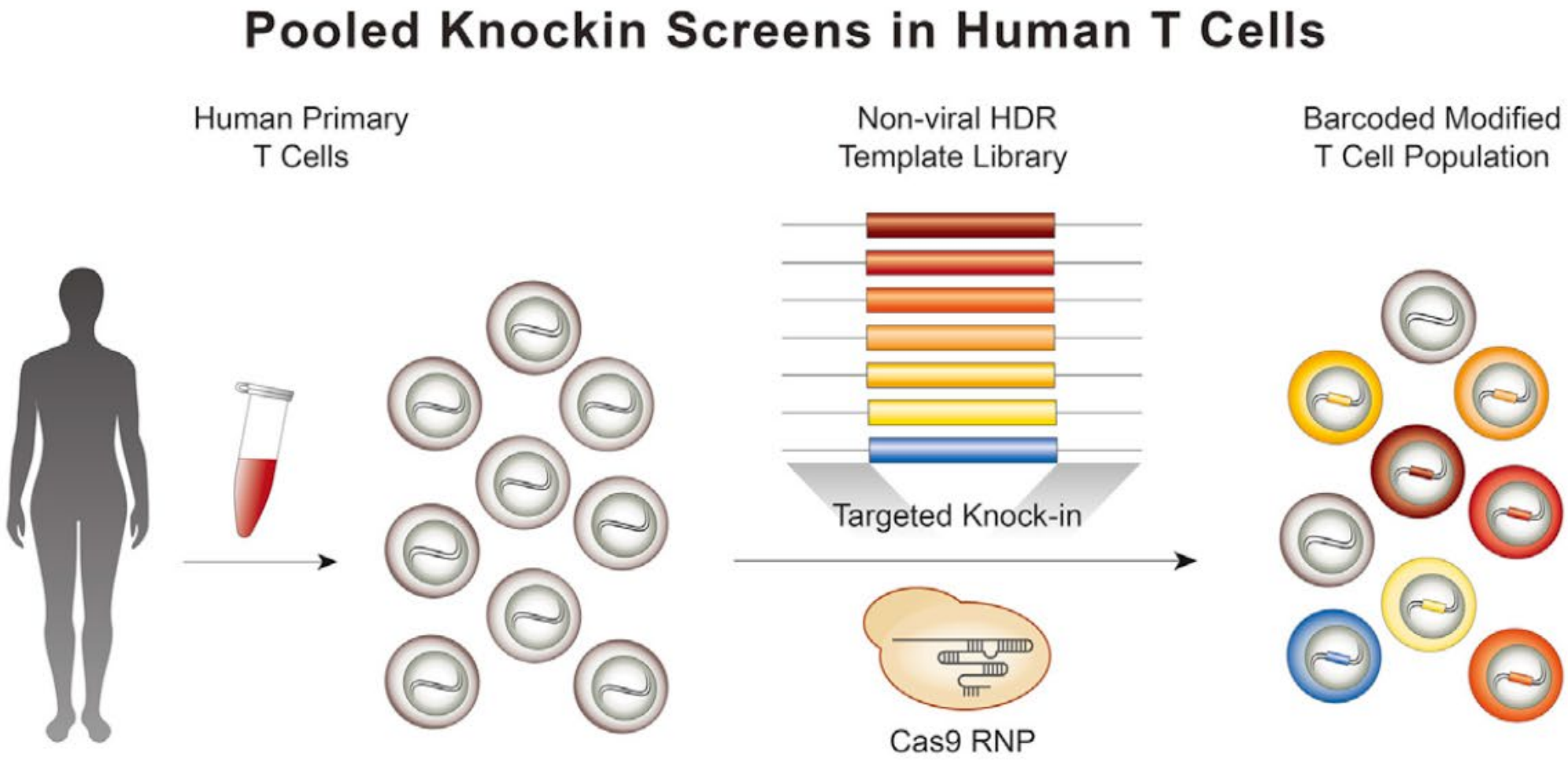






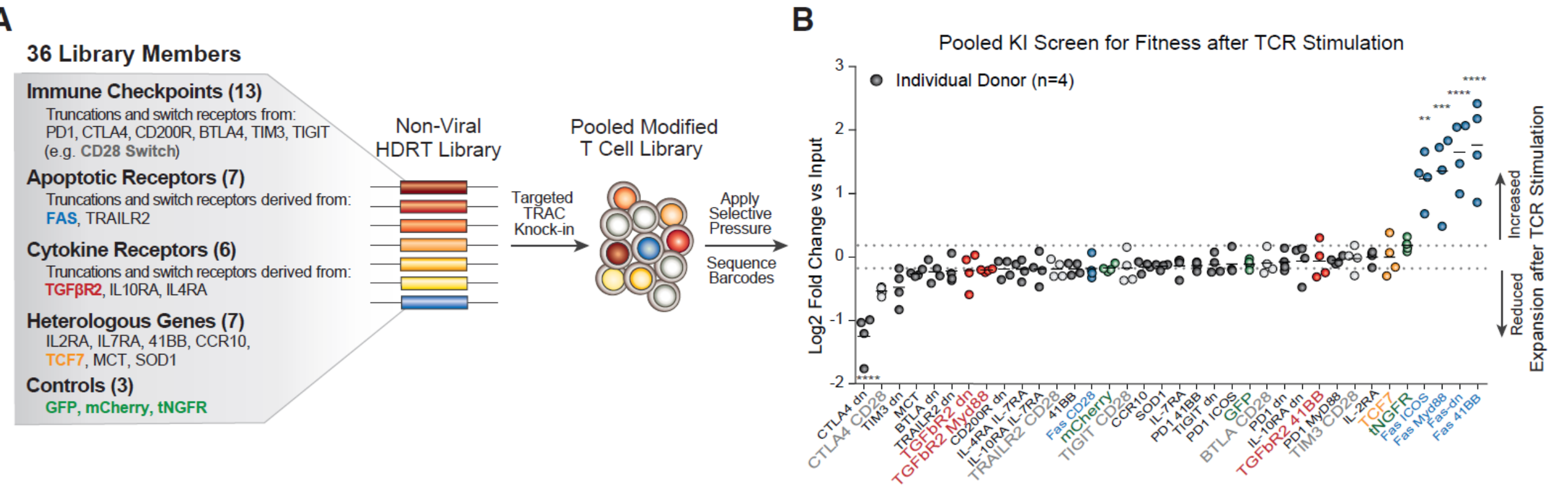
Candidate genes for KO can come from analysis of patient samples, or knowledge of T cell immunology. Alternatively, new candidates can be nominated using screening methods. Screening, particularly *in vivo* in mouse models, is a technical challenge but is increasingly being done. Screens can be done with Cas9 or alternatives such as base editors. The assumptions underlying screens must be carefully examined; is the most abundant cell always the best?

‘CARpool’ screening strategies can also be used to identify optimal CAR designs



Roth et al., *Cell* 202

Pooled Knockin Screen to Enhance T Cell Function



Summary: Relapse from CAR

Antigen loss

- Rate varies by tumor and T cell product
- Some antigens (GPRC5D) more likely to be lost than others
- Can potentially be overcome by combinatorial CARs targeting multiple antigens
- Tandems CARs are most common combinatorial strategy

T cell exhaustion and lack of memory

- T cell responses can be limited or low quality in some patients
- Engineering strategies such as FOXO1 overexpression can be used to overcome
- Screening methods can be used to nominate pathways for editing

Beyond B cell and plasma cell malignancies: How to target solid tumors

The ideal CAR target

Expressed on tumor surface at high levels (1000s molecules/cell)

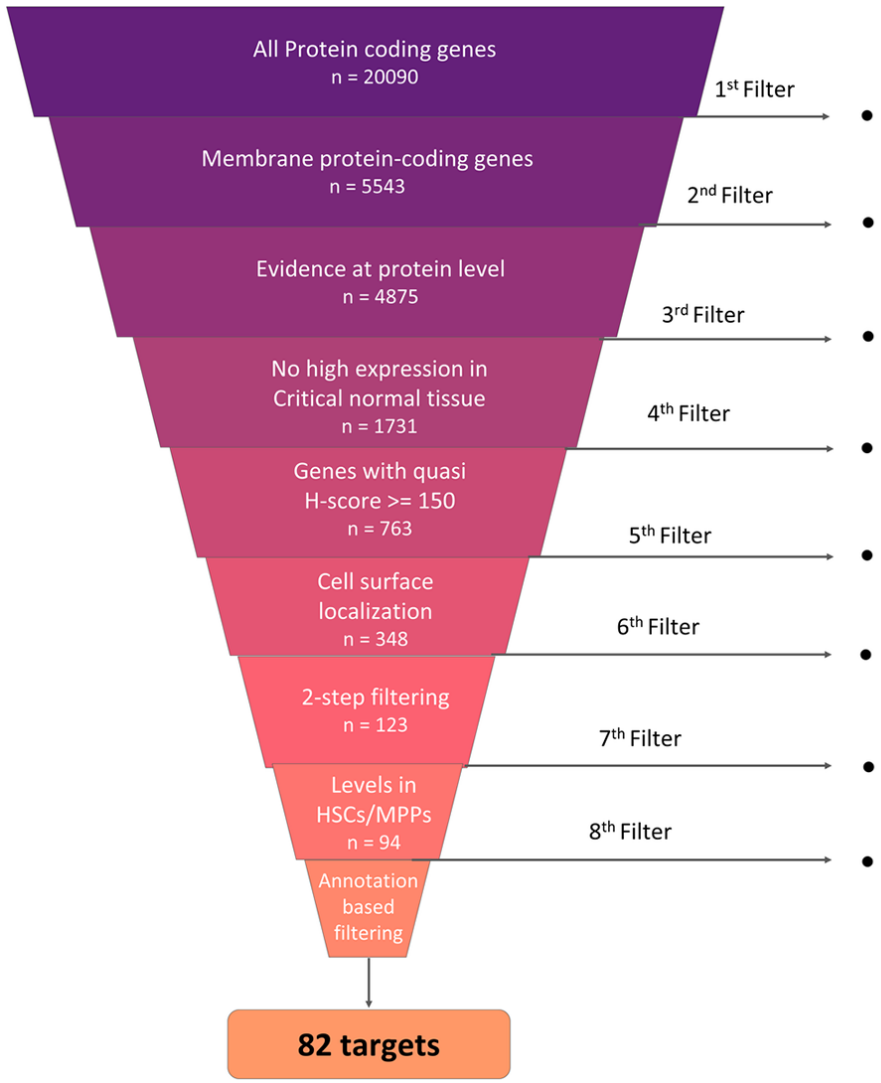
Expressed on tumors from all patients (inter-patient heterogeneity)

Expressed on all tumor cells within the same patient (intra-patient heterogeneity)

Not expressed on normal tissue, or at least not expressed on tissues critical to life

The surface proteome is quite limited

Key Slide



- Non membrane genes (removed 14547 genes)
- No evidence at protein level (removed 668 genes) - Evidence of protein existence for all protein-coding genes
- Removed genes having high expression in one / more critical normal tissue (removed 3144 genes)
- Removed genes having quasi H-score (proxy of the tumor overexpression) of < 150 (removed 968 genes)
- Removed genes with non-surface localization (removed 415 genes) encoding surface protein
- Consistency in the data (removed 225 genes)
  - Consistency between mRNA and IHC data
  - quasi.H score and TCGA FPKM correlation
- Remove the genes with high expression in Hematopoietic Stem Cells (HSCs) and Multipotent Progenitor cells (removed 29 genes)
- Annotation of Literature, Antibody, Protein family, Clinical and Preclinical level

The universe of membrane proteins is actually quite small. Once proteins with normal tissue expression are excluded, you are left with very few potential targets. This is particularly true for common epithelial cancers where the normal tissue is critical for survival.

Kathad PLOS ONE 2024

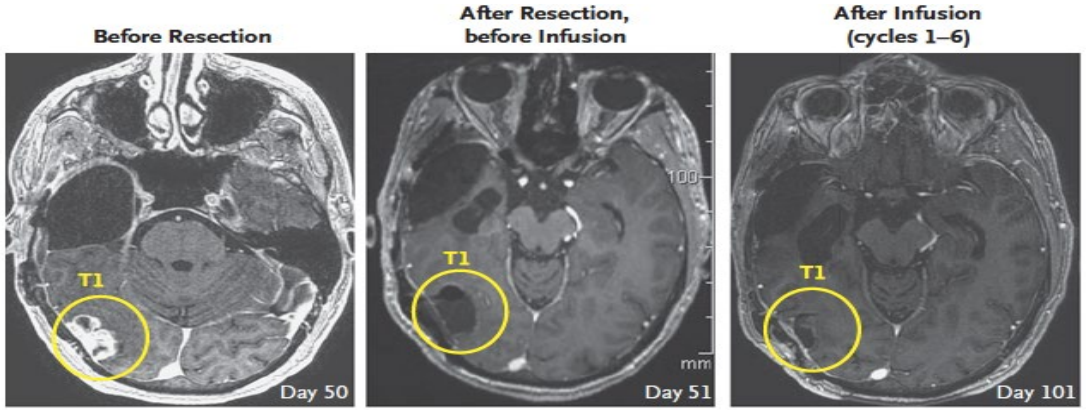
Antigen Loss / Heterogeneity

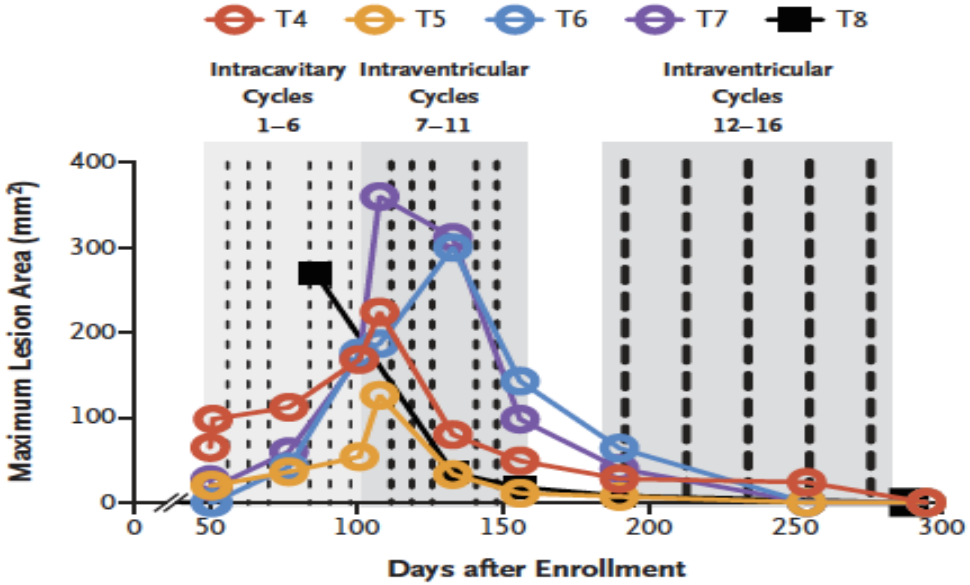
Brown, CE et al., *NEJM.* 375;26 (2016)

BRIEF REPORT

Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy

- **IL13Rα2 CAR with 4-1BB costimulation**
- **Intracavitary + Intraventricular CAR T administration (16 doses)**
- **Regression of intracranial and intraspinal tumors x 7.5 months**
- **Decreased expression of IL13Rα2 at recurrence**

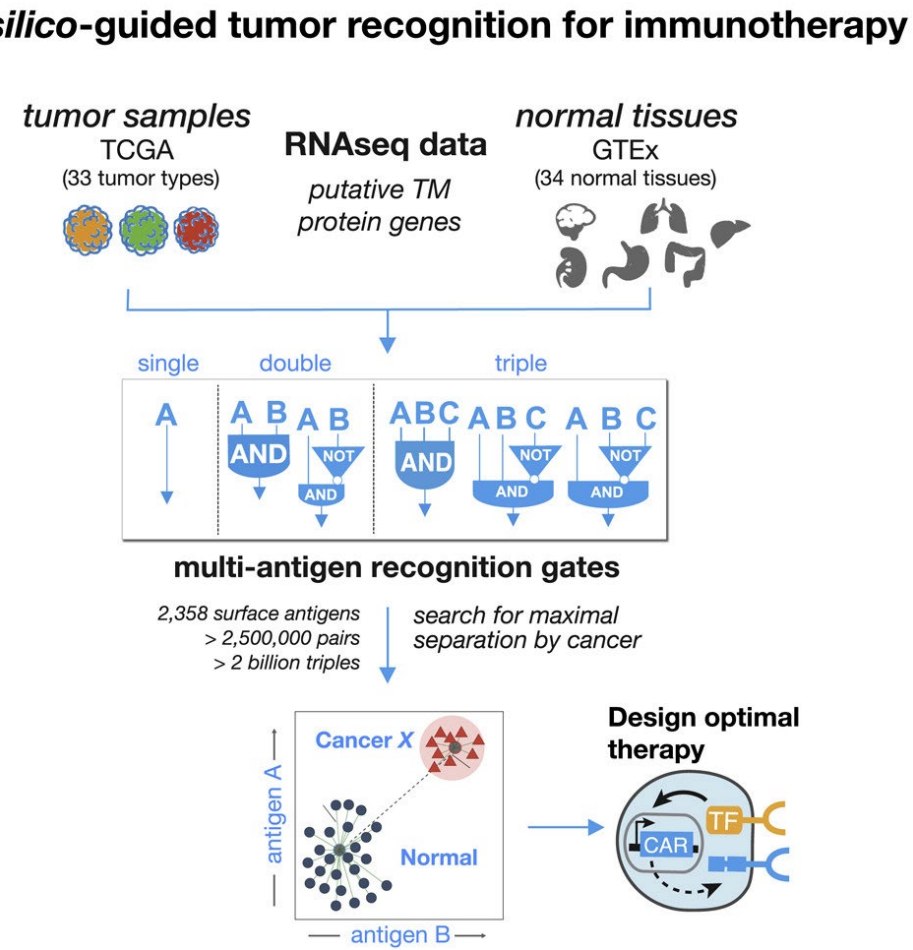




Surface antigens for solid tumors are not only limited in number, but have poor expression characteristics. For example, they have high intrapatient heterogeneity and low surface expression, such that tumor escape is possible.

If a single target can't work, can we create logic gates to target multiple

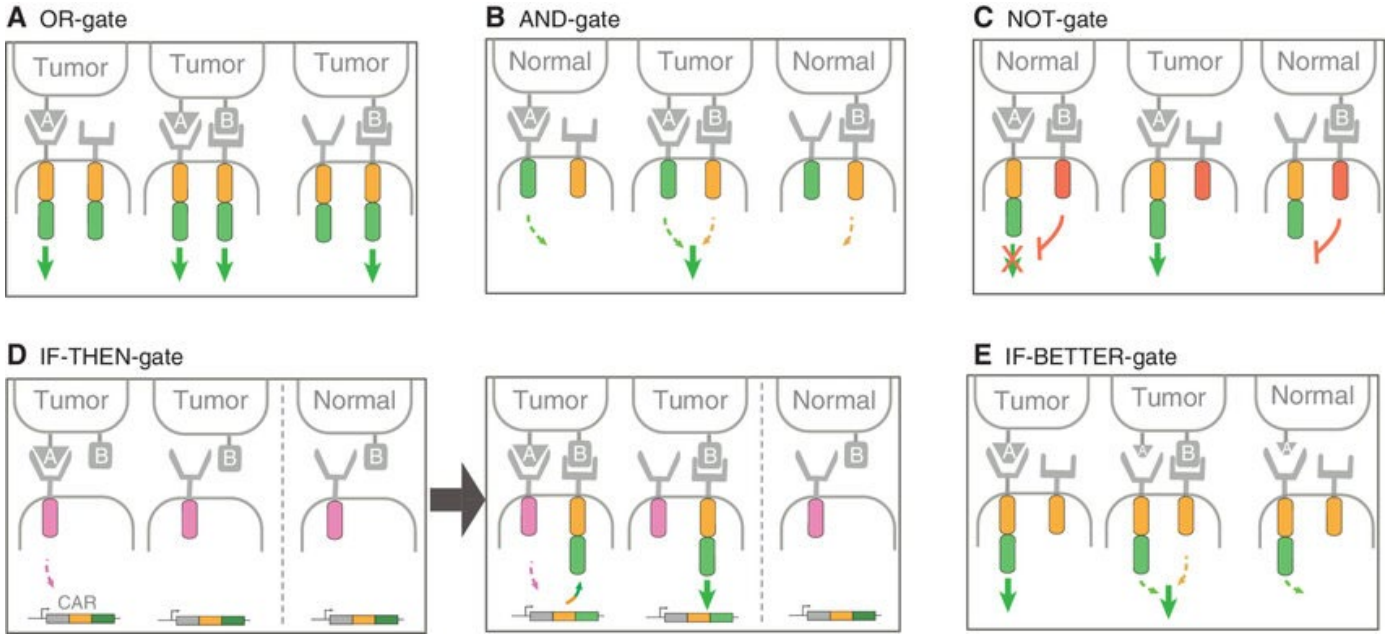
Dannenfelser Cell 2020



2- and 3-antigen AND or NOT logic gates improve tumor discrimination of CAR T cells. Adding antigens improves precision at the cost of recall; 2–3 is optimal.

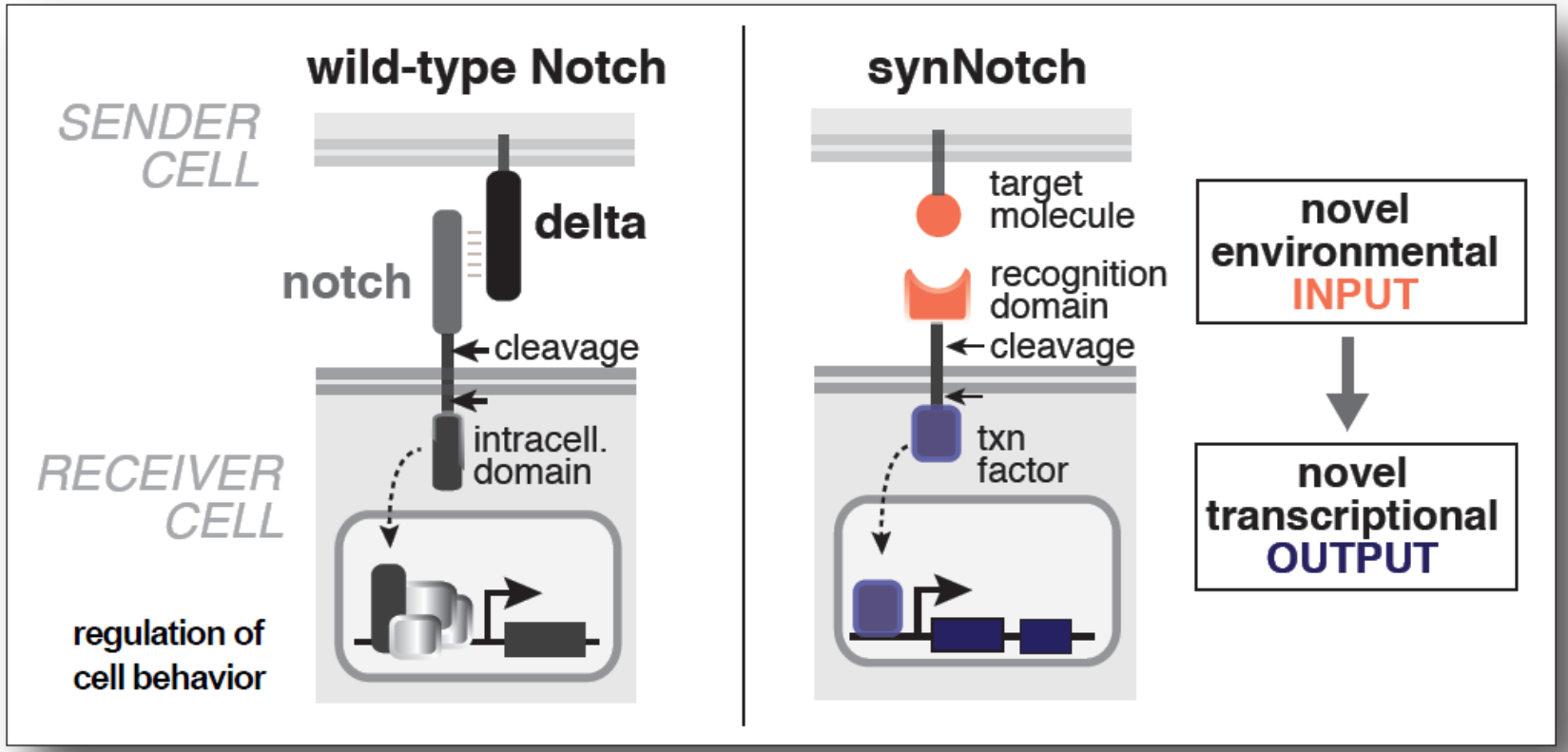
Potential CAR design architectures

Hamieh Cancer Discovery 2023



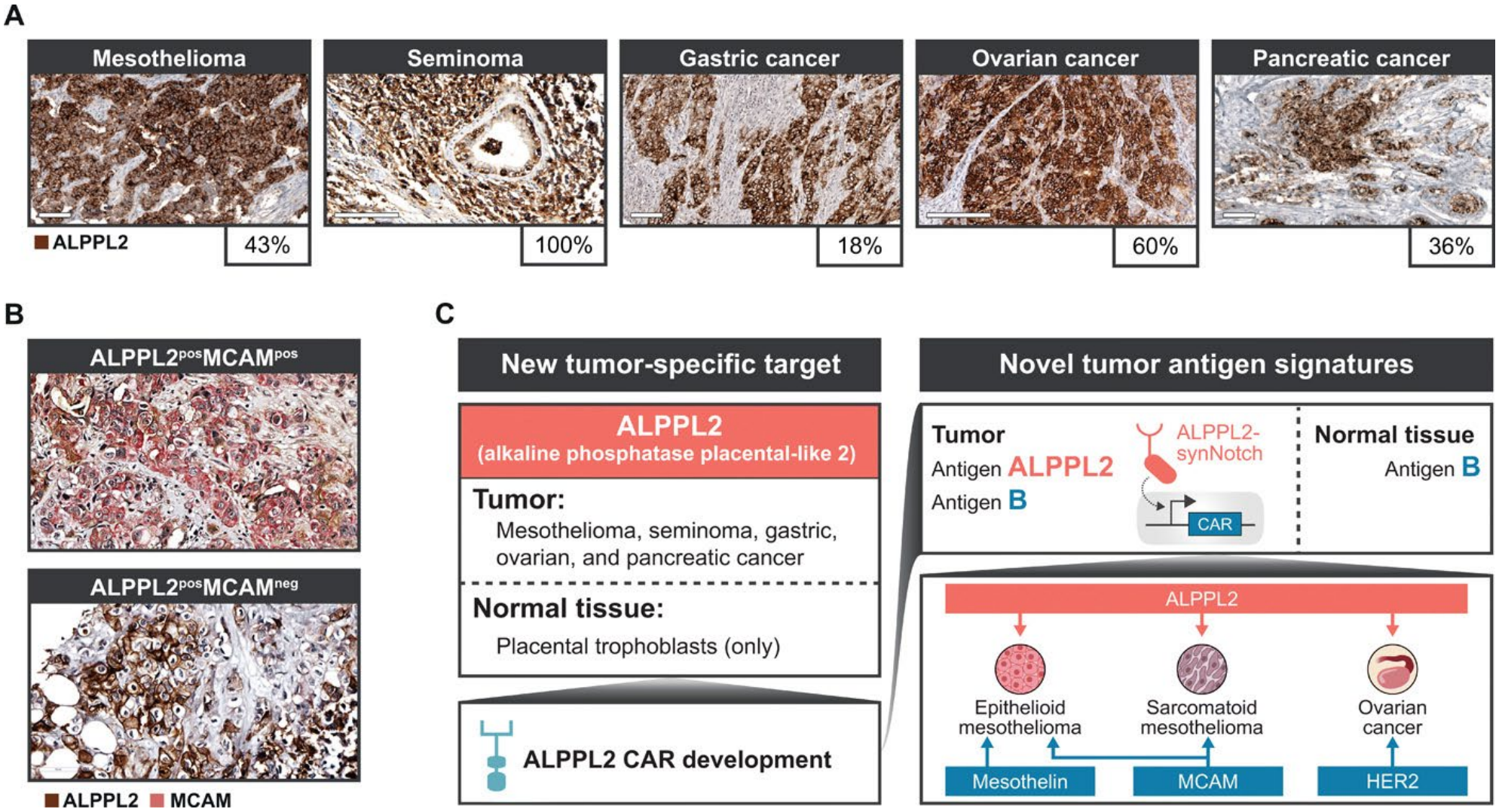
A variety of combinatorial CAR designs are theoretically possible to design using various principles of synthetic biology. Designing with good performance characteristics is challenging. The syn-notch system is perhaps the most developed method of engineering combinatorial CAR behavior.

The synNotch system can be used to generate response circuits



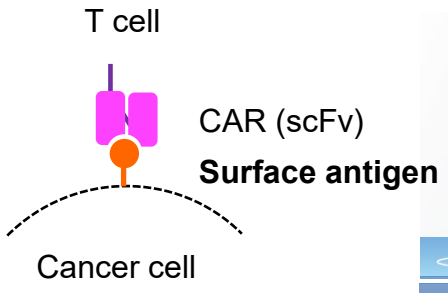
Roybal and Morsut et al. Cell. 2016

Module 2: SynNotch CAR circuits enhance solid tumor recognition and promote persistent antitumor activity in mouse models

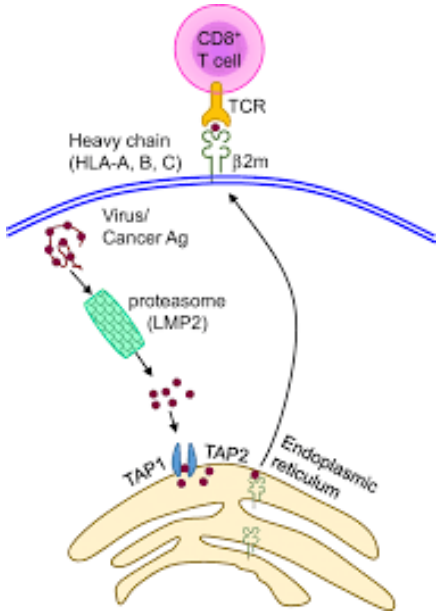


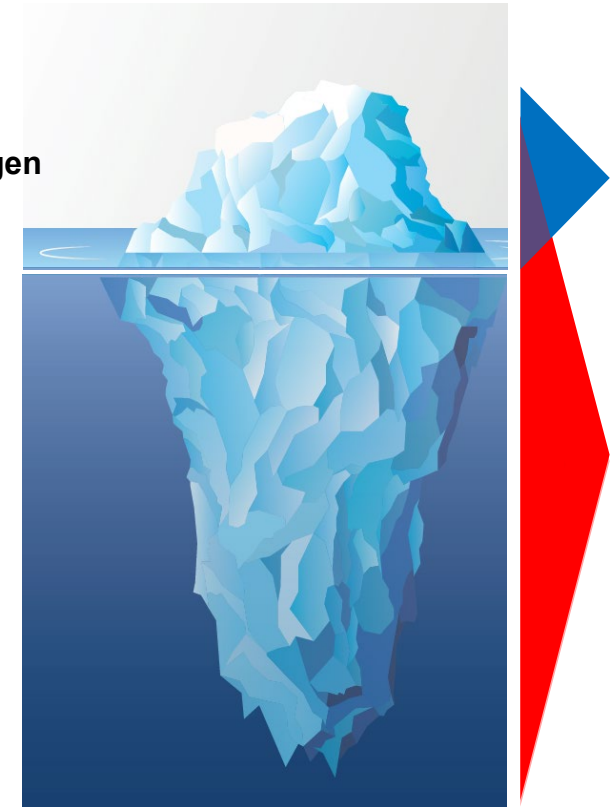
Let's return to using the T cell receptor

CAR can only recognize surface antigen



TCR can target intracellular antigen → expand applicability & disease subtypes





Membrane-associated proteins

~11% of the proteome

- Potential **antibody** and **CAR** targets
- Examples:

  CD19, **BCMA**, mesothelin, HER2, etc

Intracellular proteins

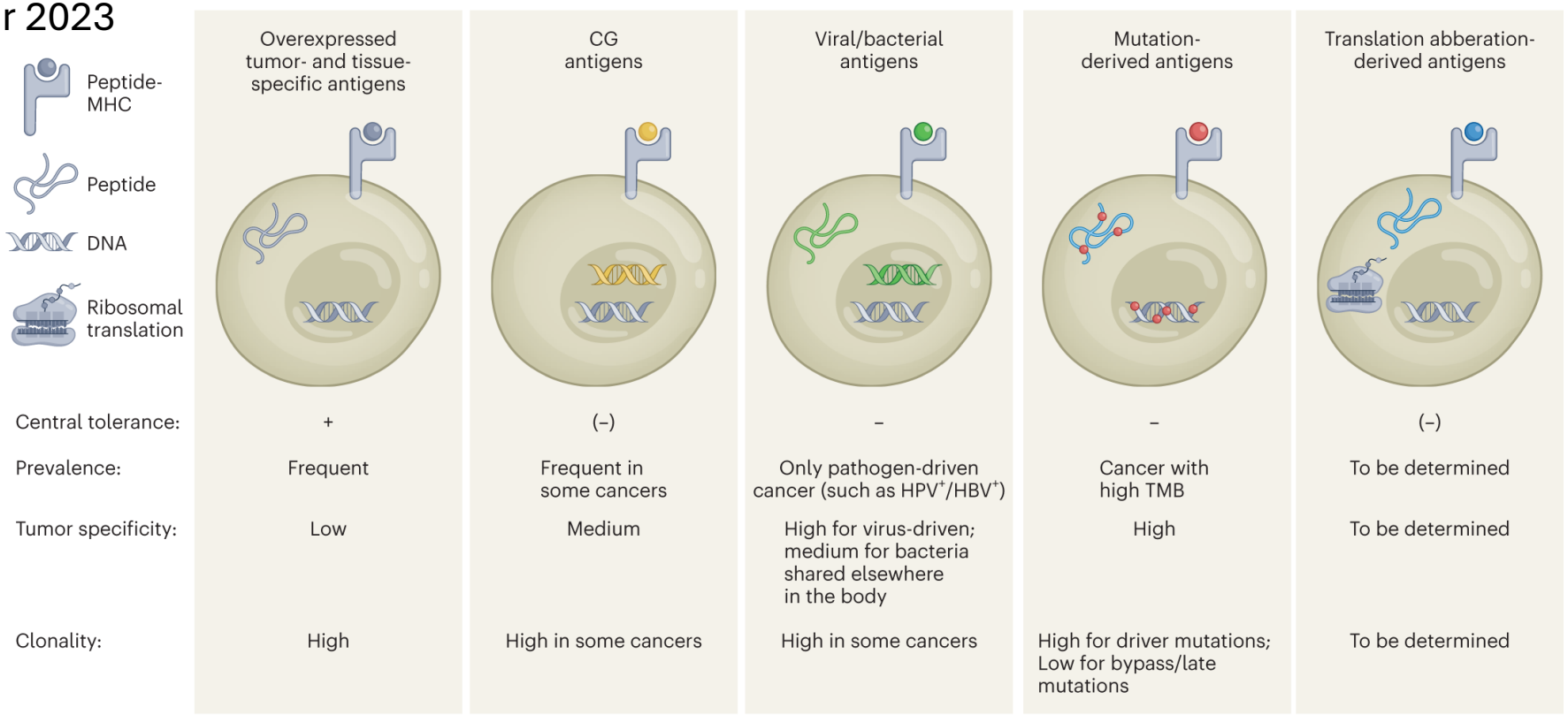
~89% of the proteome

- Exclusively **TCR** targets because intracellular proteins must be presented by MHC for T cells to recognize

Adalted from Chris Klebanoff

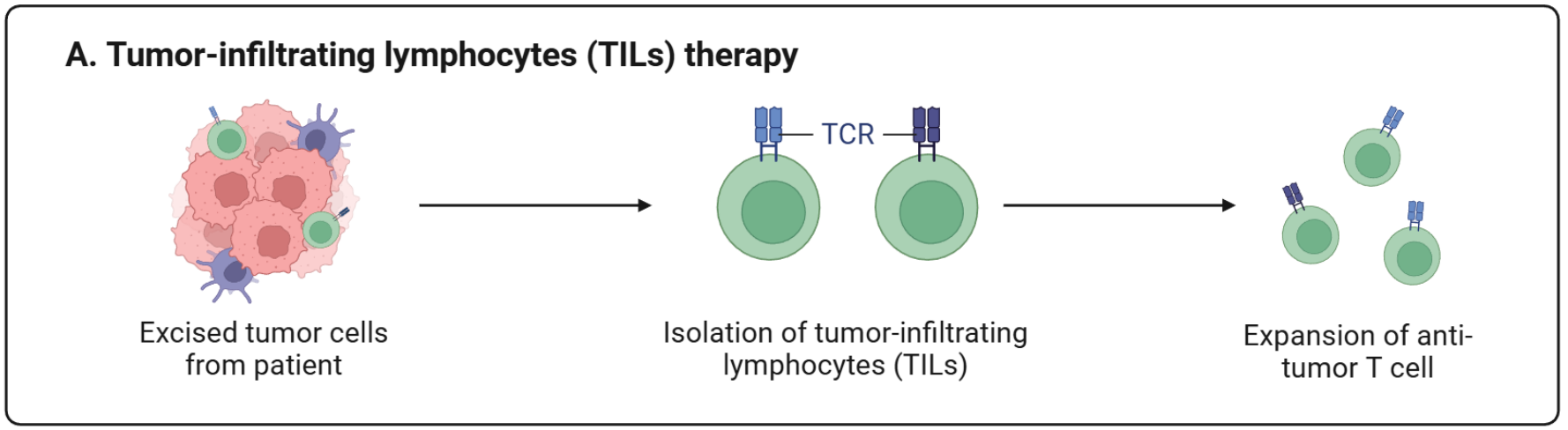
The landscape of T cell antigens for cancer immunotherapy

Peri Nature Cancer 2023



Overexpressed tumor- and tissue-specific antigens are ubiquitously present in tumor cells; however, they are shared with healthy tissues and thus have low tumor specificity and are hampered by central tolerance. Cancer Germline (or tesists) antigens are solely expressed in the germline and become re-expressed in tumor cells, providing them with a medium tumor specificity and subjecting them to central tolerance. Viral and bacterial antigens stem from former oncogenic pathogen infection, which renders them highly tumor-specific with no expression in healthy tissue and a lack of central tolerance. Neoantigens arising from mutation, for example single-nucleotide variations, indels or fusion genes arise from oncogenesis and are exclusively present in cancer cells, harbor a high tumor specificity and no central tolerance. Aberrant translation-or transcription-derived neoantigens are a result of malfunctional cellular transcription and translation machinery in cancer and are not encoded by the genome. As a rather new class of TSAs, their prevalence and tumor specificity largely remain to be explored.

Two strategies for exploiting TCR to target tumor

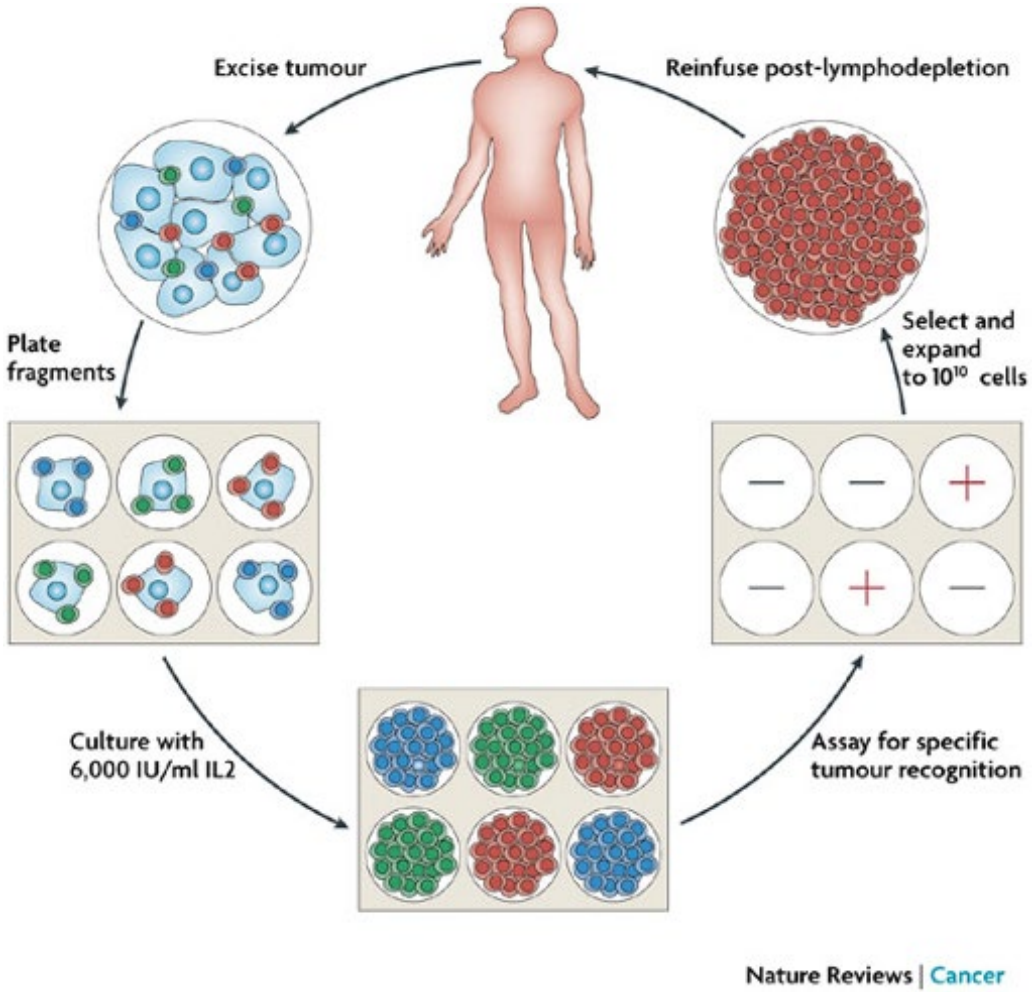


Not gene edited
Polyclonal
Private
Custom

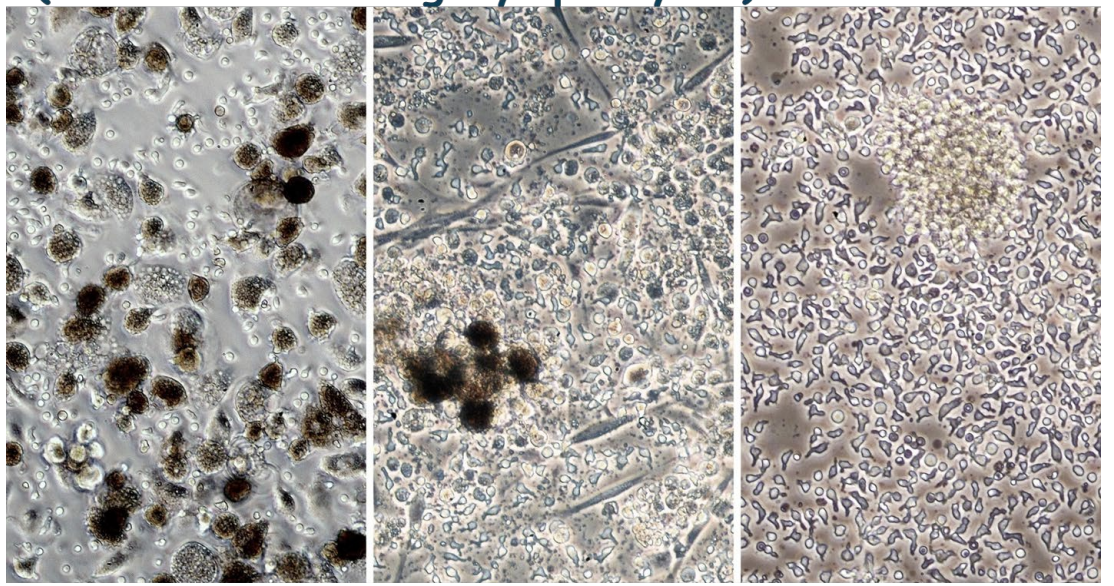
Gene edited
Monoclonal
Public
HLA restricted

Adapted from BioRender Want, et al (2023) Vaccines

Tumor Infiltrating Lymphocyte as treatment for cancer



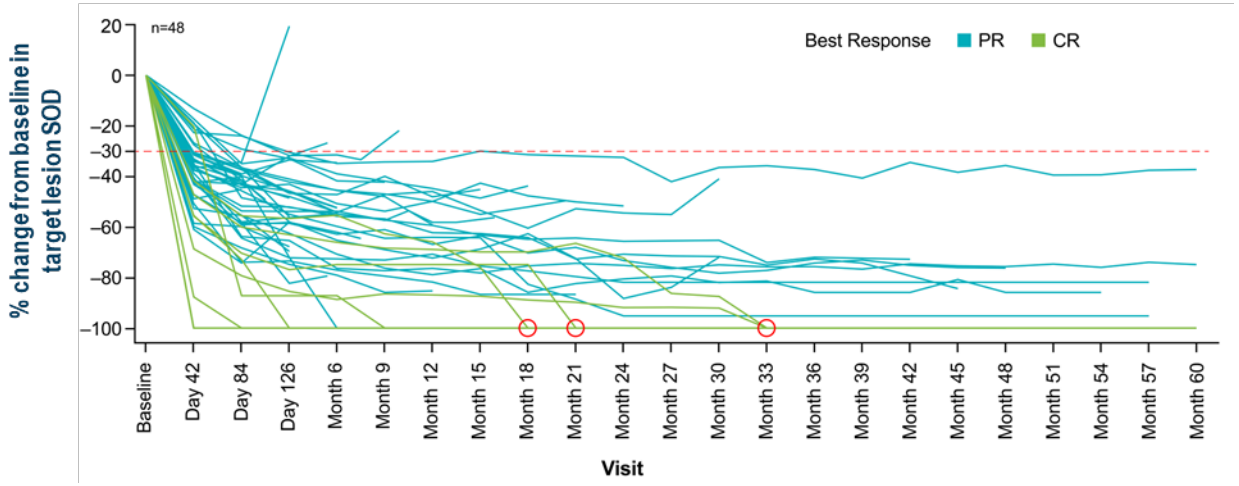
Melanoma TIL
(Tumor Infiltrating Lymphocytes)



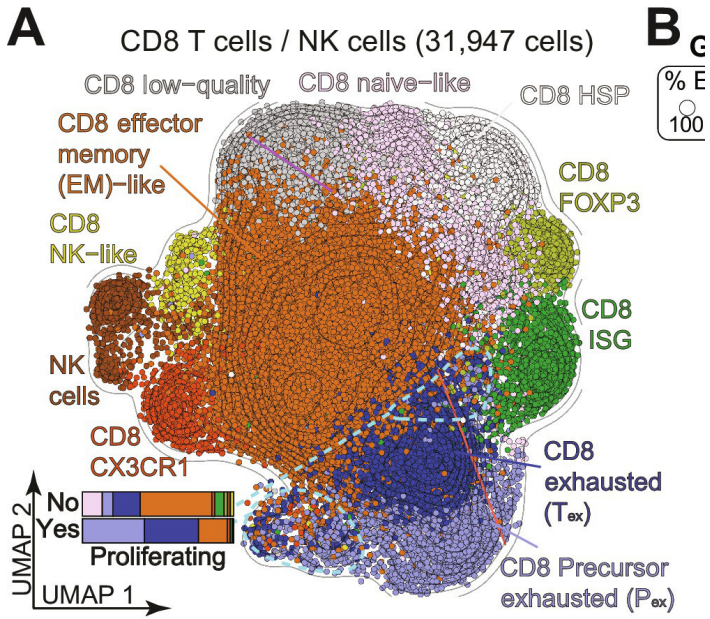
Fresh digest **One week** **Two weeks**

Some tumors are full of T cells with variable specificity, a fraction of which are tumor specific. This is most common in tumors with high mutation burden, such as NSCLC and melanoma. These cells can be excised, re-expanded *ex vivo*, and reinfused, with some efficacy. Notice that TILs are **NOT** gene edited. The therapy relies on intrinsic polyclonal T cell responses present in tumor.

Lifileucel is an FDA approved TIL product for metastatic melanoma

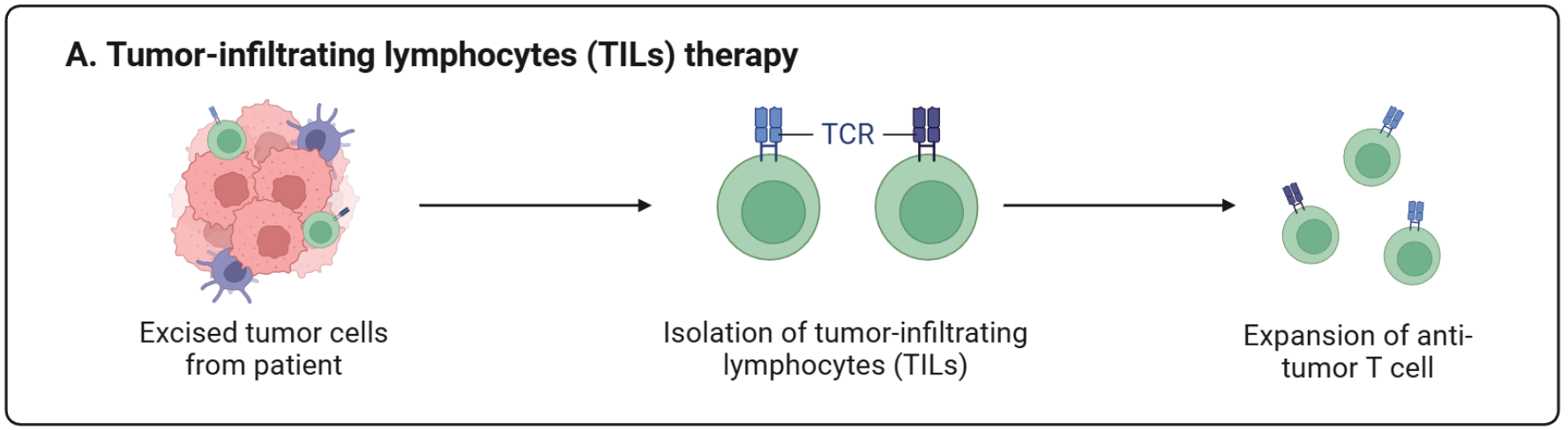


Barras Science Immunology 2024



Non gene modified TILs can induce responses in patients with melanoma, and potentially non-small cell lung cancer (NSCLC). However, these cells are fundamentally dysfunctional and exhausted, limiting their long term persistence an ability to truly clear tumor.

Two strategies for exploiting TCR to target tumor



Not gene edited
Polyclonal
Private
Custom

Gene edited
Monoclonal
Public
HLA restricted

Adapted from BioRender Want, et al (2023) Vaccines

Target antigens for T-cell cancer immunotherapy

1. **Tissue differentiation antigens (analogous to CD19)**
   Eg. CEA, MART-1, gp100, HER-2, thyroglobulin, etc.

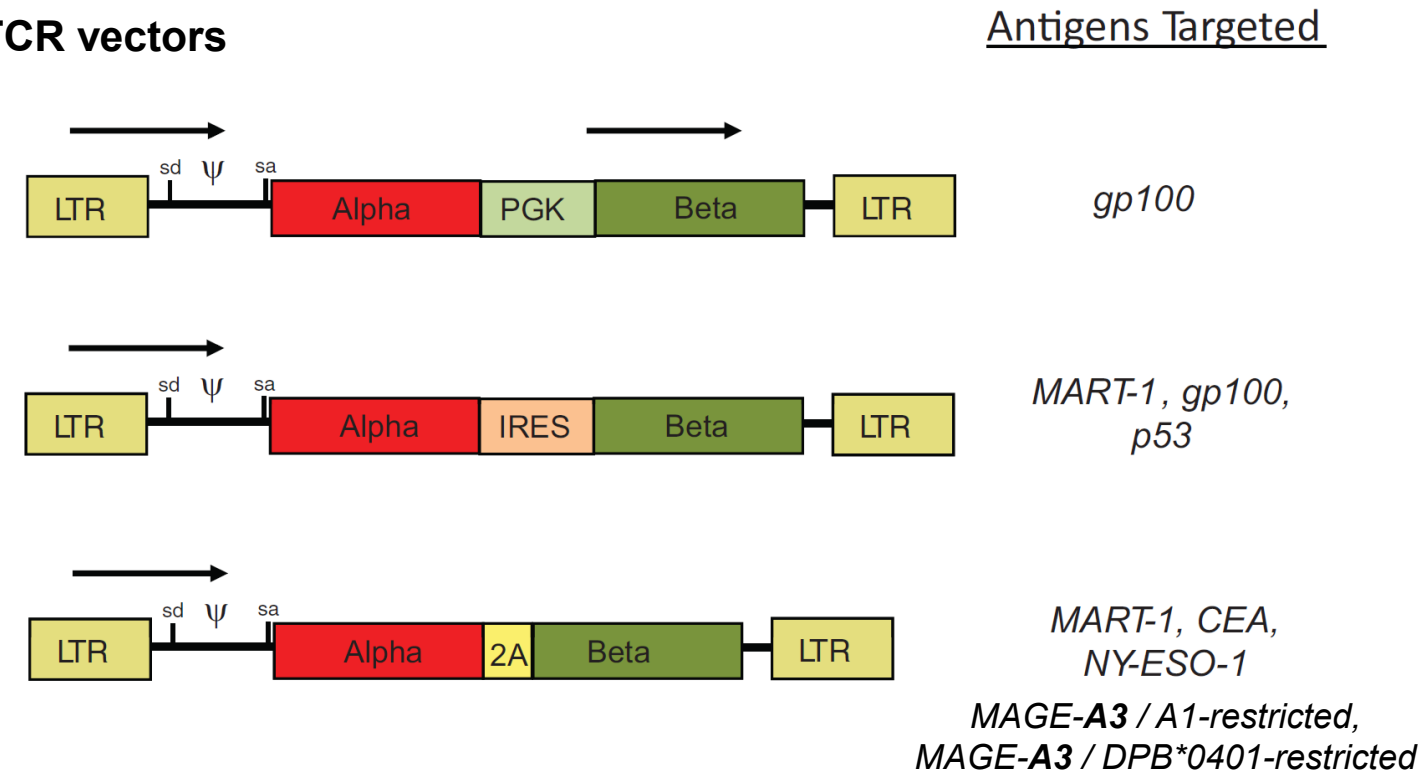
2. Shared non-mutated antigens unique to cancer (cancer-germline antigens)
   Eg. NY-ESO1, MAGE, SSX2, etc

3. Viral-associated antigens from oncogenic viruses
   Eg. HPV E6/E7, EBV LMP1/2

4. Random or driver mutations unique to each individual cancer

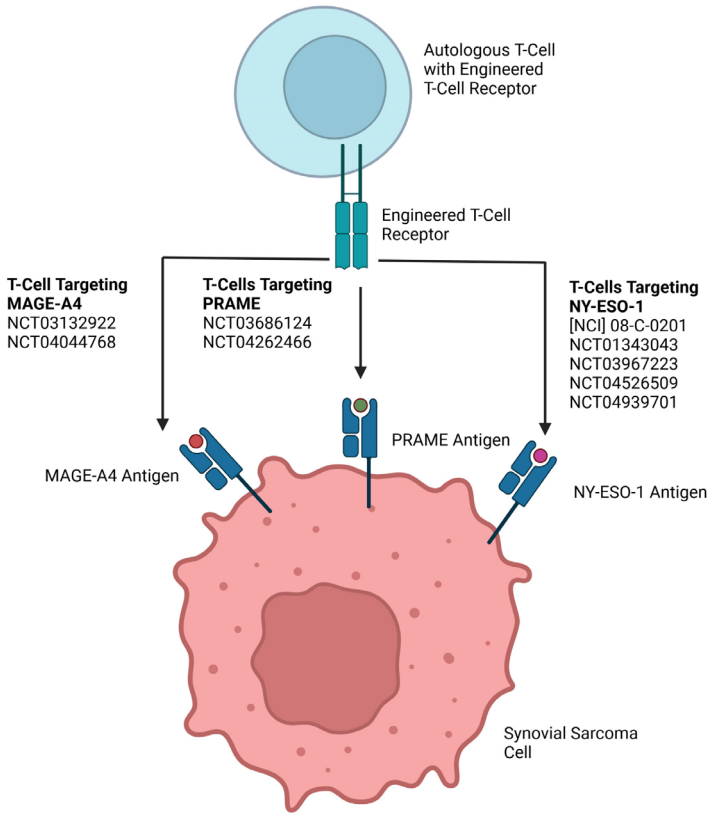
Genetic redirection of peripheral T cells using gamma retroviral vectors encoding exogenous TCRs

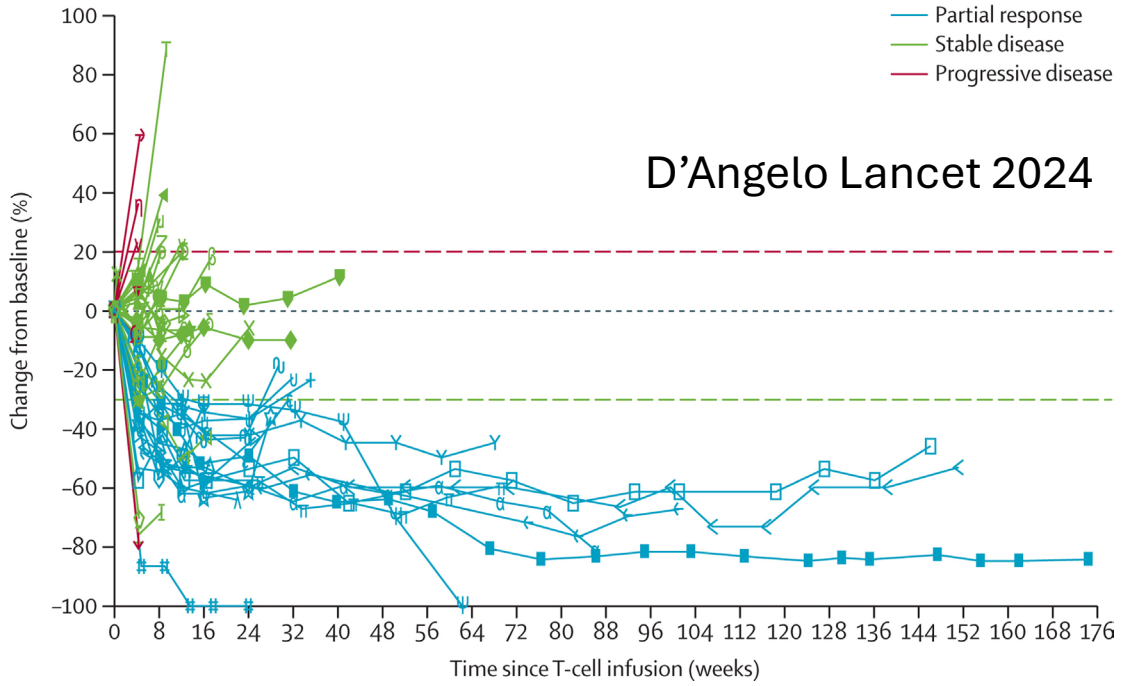
Modified from: Morgan RA *et al.*, *Cancer J.*, 2010.



Just as a CAR can be inserted into a T cell, a new T cell receptor can be genetically delivered with a lentiviral vector. TCR recognize peptide in the context of MHC, so this TCR is specific for the combination. The tumor must express the antigen or have the specific mutation, and the patient must express the given HLA.

Afamicel is an engineered TCR therapy for synovial sarcoma





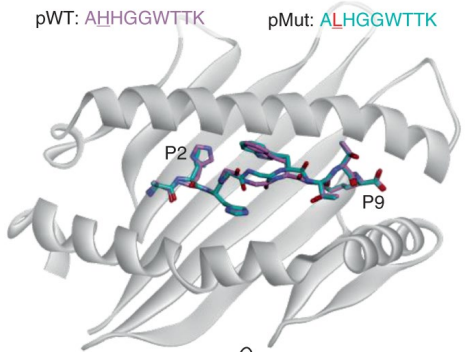
Cancer-testis antigens (CTAs) are a category of tumor-associated antigens. Normally silenced in adult tissues, except testis and sometimes placenta. They are aberrantly re-expressed in some cancers such as synovial sarcoma, a soft tissue tumor. They include MAGE-A4, PRAME, and NYESO-1.

Afamicel is an engineered TCR that recognizes an antigen from MAGE-A4 expressed in the context of HLA-A2 (the most common HLA allele in people of northern European heritage.

Target neoantigens for T-cell cancer immunotherapy

There are common mutations that generate neoantigens. However, not all antigens bind all HLA alleles, so again the patient must have both the HLA allele and the mutation to qualify for therapy.

Most common recurrent mutations X **Most common HLA alleles** = **Potential eTCR therapies**



A common (public) PI3K neoantigen is presented in HLA-A03:01
Chandran Nat Med 2022

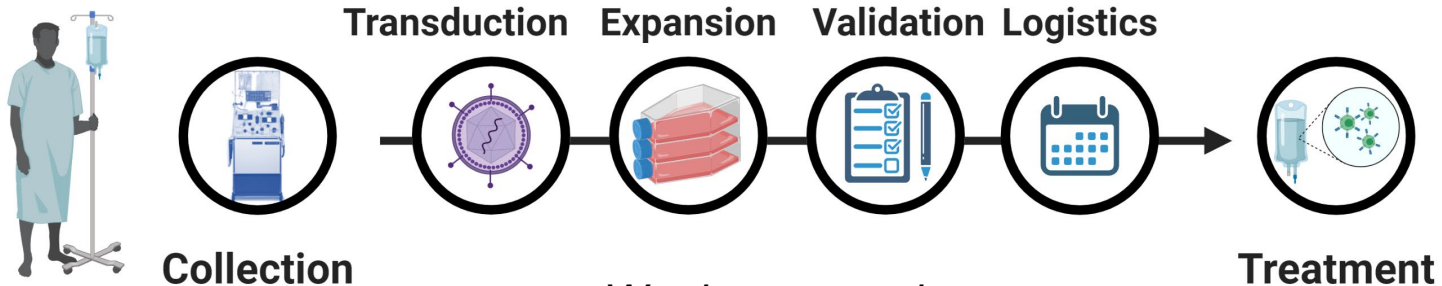
Gene	Mutation	HLA allele	Expected frequency	Observed frequency
BRAF	V600E	A03:01	1.55%	1.51%
KRAS	G12D	A02:01	1.01%	1.06%
PIK3CA	H1047R	C07:01	0.73%	0.80%
PIK3CA	E545K	A03:01	0.68%	0.70%
PIK3CA	E542K	A03:01	0.44%	0.44%
TP53	R248W	A02:01	0.33%	0.34%
TP53	R273C	A02:01	0.29%	0.31%
TP53	R248Q	C07:02	0.25%	0.23%
TP53	Y220C	A02:01	0.24%	0.19%
PIK3CA	R88Q	C07:02	0.16%	0.17%

Note: Expected frequency indicates the frequency of shared neoantigens predicted by recurrent mutations in combination with highly frequent HLA alleles. Observed frequency, the frequency of shared neoantigens in 7748 tumor samples.

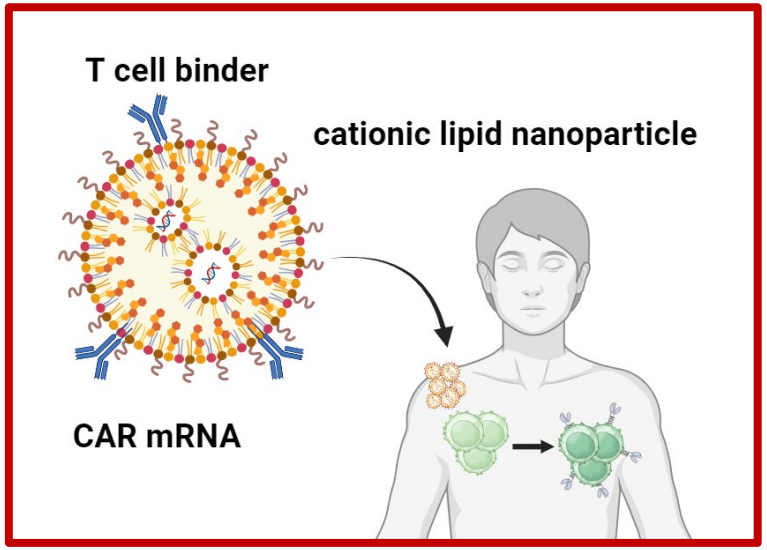
Off the Shelf cell therapies: *In Vivo* gene delivery

Autologous CAR T cells

Kourelis JCO 40, 2022
Mikhael JCO Practice 2022

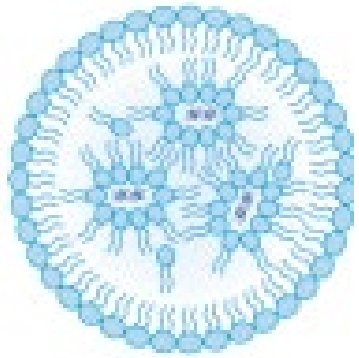


In vivo editing of CAR T cells



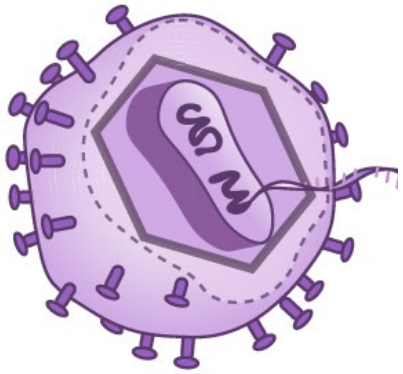
Gene Delivery Platforms for *in vivo* Editing

Non-viral



Transient mRNA delivery

Lentivirus



Stable DNA Integration

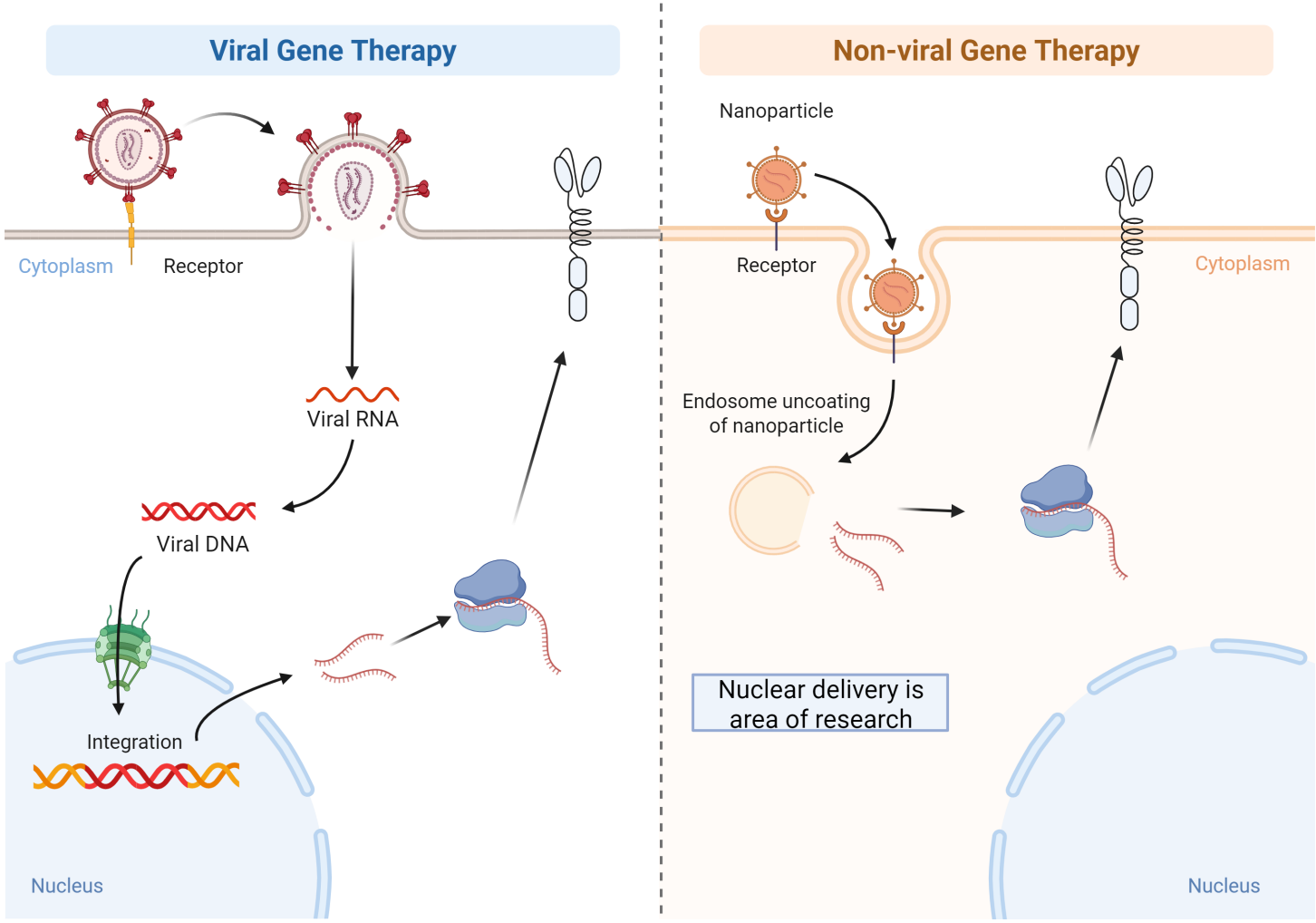
AAV

Episomal

Mitchell Nat Rev Drug Discovery 2020
Haasteren Nat Biotech 2020

Viral delivery allows for stable DNA integration

Key Slide



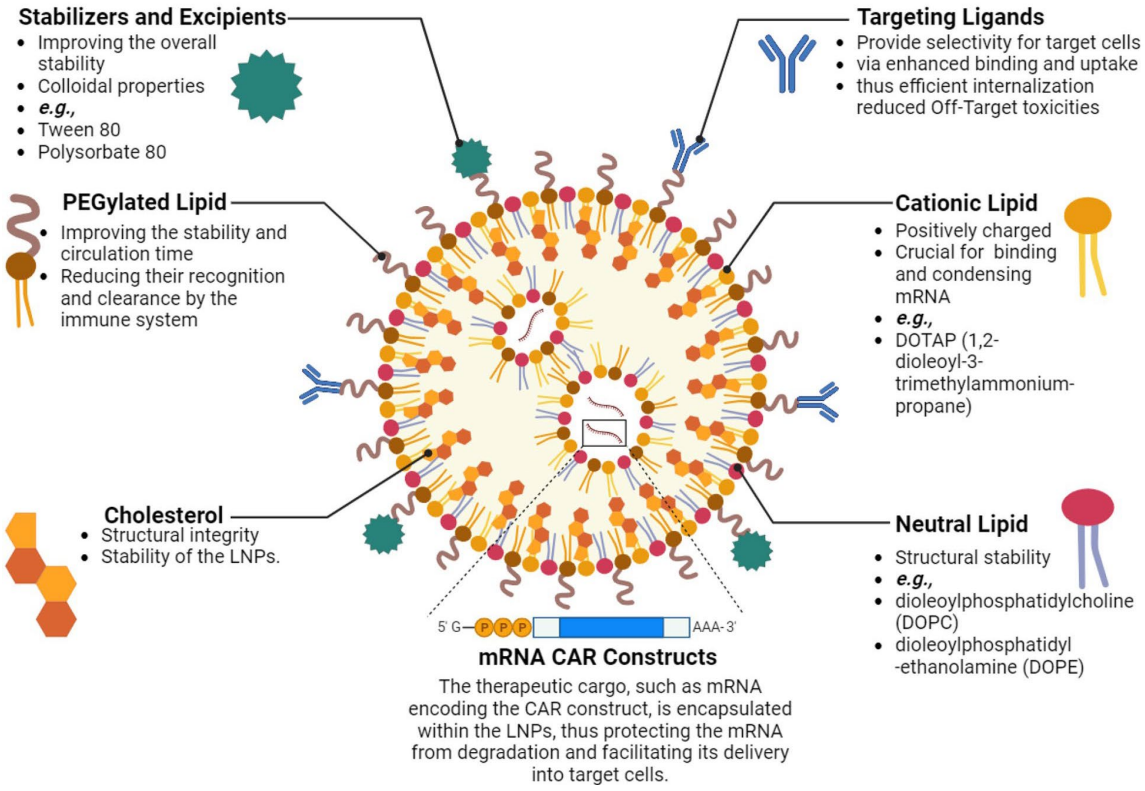
Viral platforms mediate nuclear delivery and DNA integration

Existing nanoparticle platforms lack nuclear delivery capacity

Biorender. Adapted from “Viral vs Non-viral delivery.”

Part 1: Cationic Lipid Nanoparticles for Delivery of mRNA CAR

Key Slide



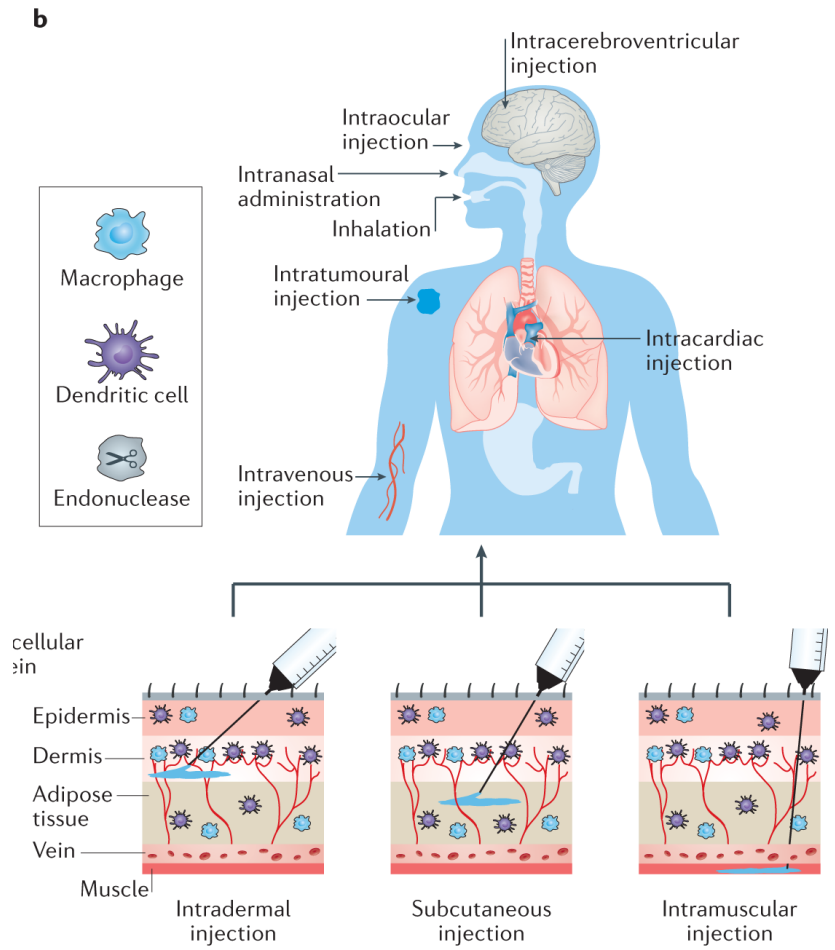
Khawar J Nanobiotech 2024

Non-viral, nanoparticle delivery systems include biodegradable polymers (PGA, PLGA), poly(ß-amino esters), inorganic platforms, and lipid-based platforms

Ionizable cationic lipid nanoparticles (LNPs) have numerous favorable properties including self-assembly, robust *in vivo* delivery, and existing manufacturing infrastructure

Intravenous delivery is most common route for T cell targeting

Hou Nature Rev Materials 2021

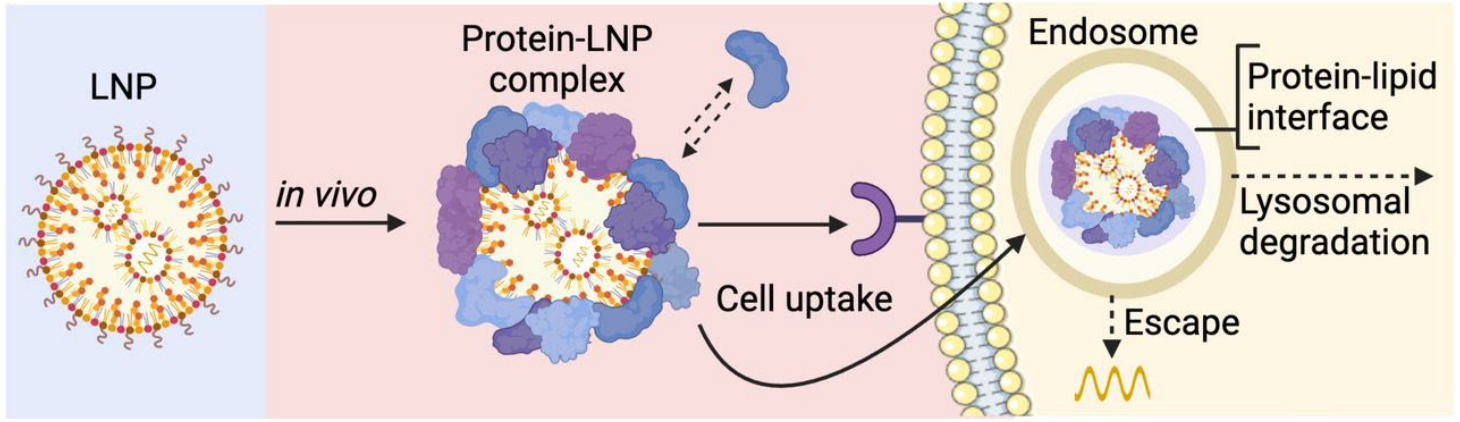


T cells are readily accessible in circulation, whereas vaccines target APC present in subcutaneous tissue.

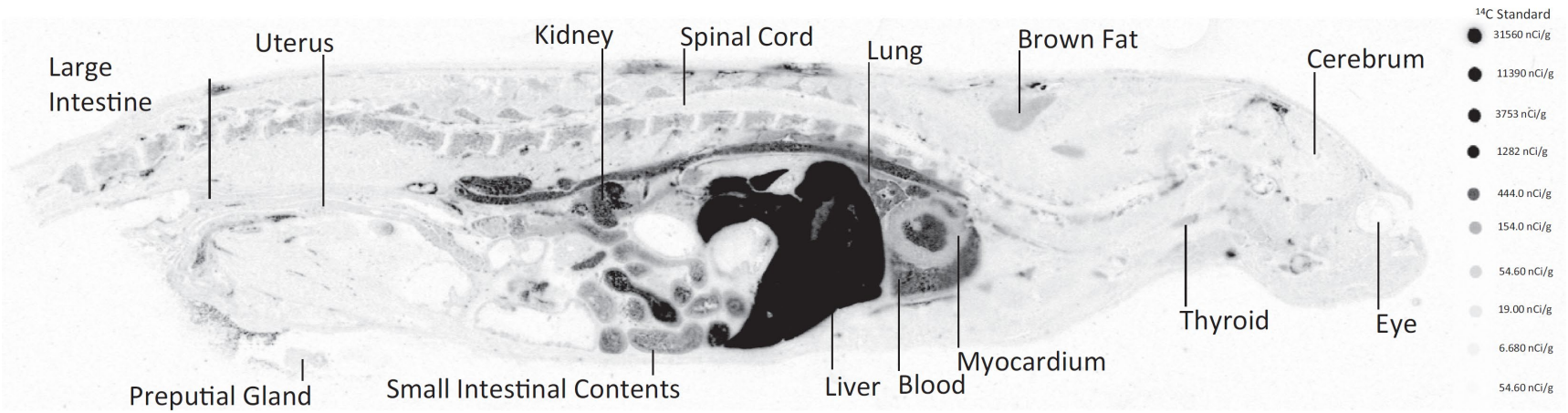
LN (Chen PNAS 2022) and intratumoral delivery have also been explored.

Intravenously injected LNPs have strong preference for liver

Adsorption of serum proteins ("protein corona") such as ApoE promotes liver uptake (Dilliard PNAS 2021, Hosseini-Kharat Mol Therapy 2019, **Voke Nat Comms 2025**)

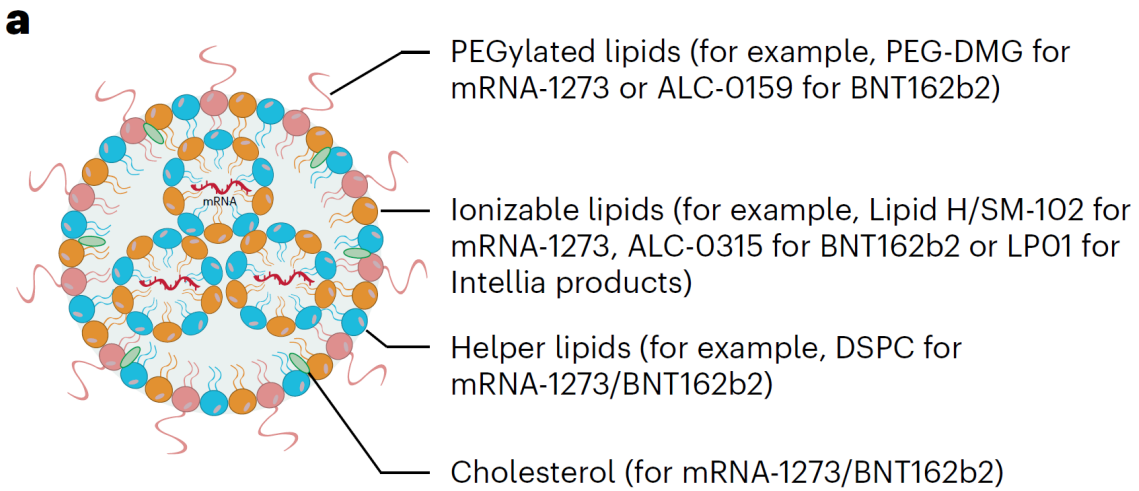


LNP radiography in a rat 1 hour after LNP administration IV (Ci Drug Metab Dispos 2023)



Two approaches to target LNPs to T cells

Lipid Formulation



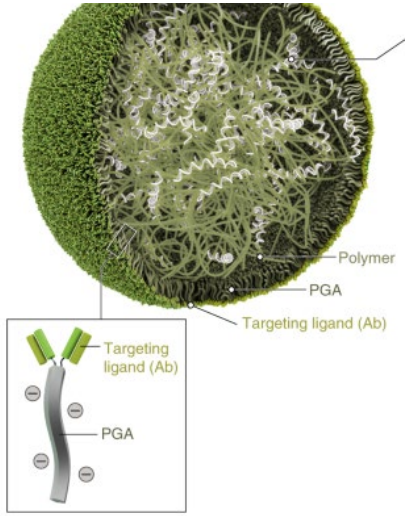
Huang *Nat Med* 2022

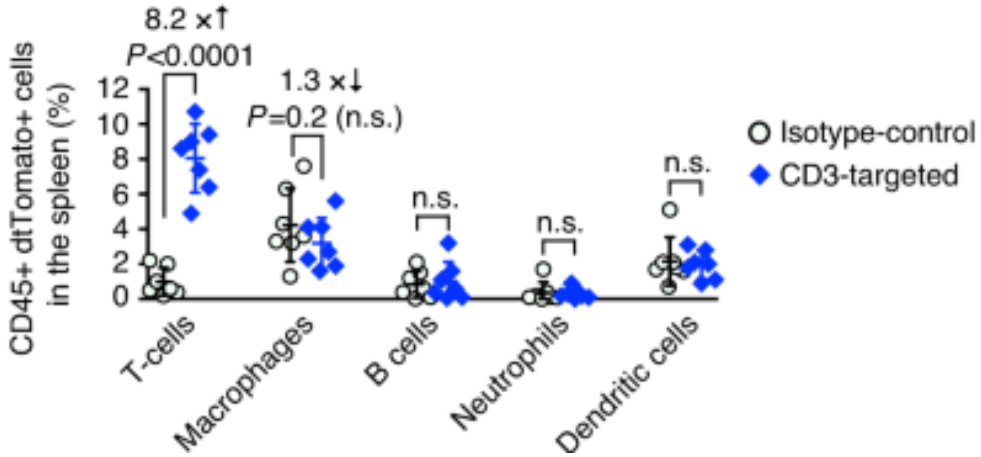
Lipid formulation affects charge, stability, and size indirectly impacts T cell vs. hepatic delivery

Key Slide

Surface Functionalization

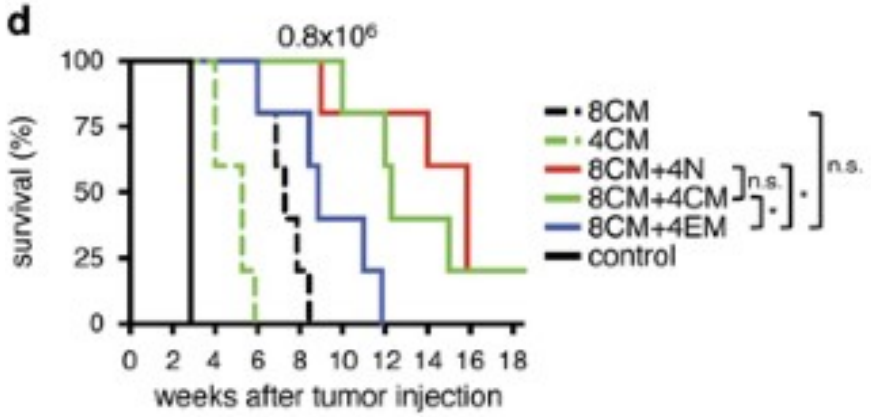
Parayath Nat Comms 2020





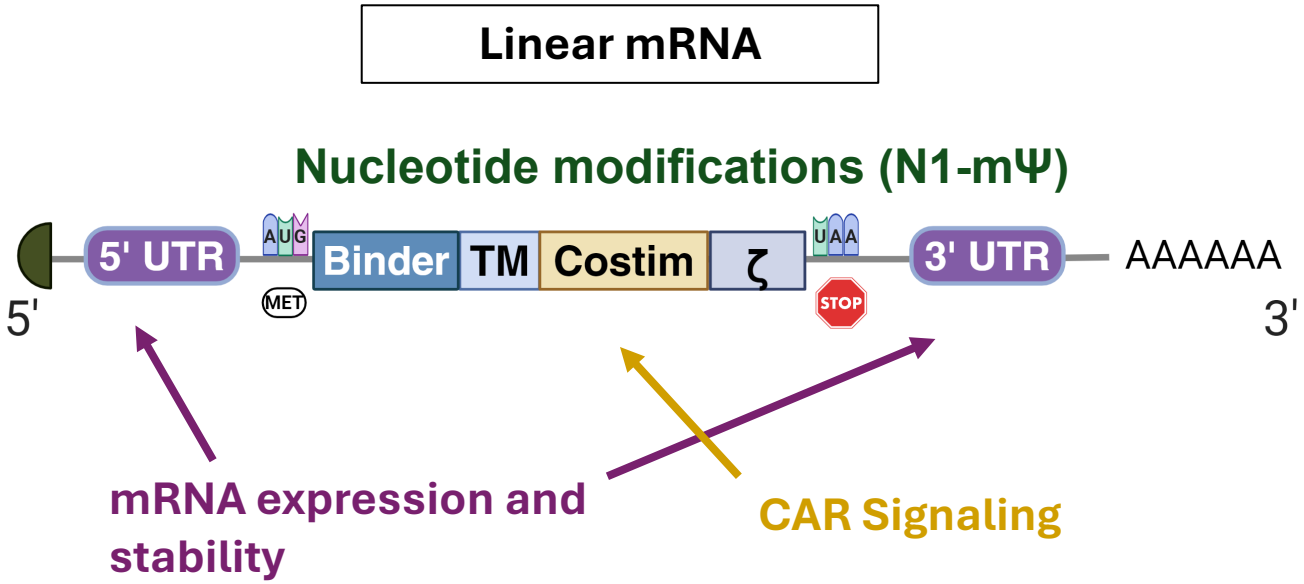
Potential T cell delivery targets

CD4 CD8	Both subsets required in literature**
CD3	T cell specific, triggers T cell activation Can promote transduction and internalization
CD5 CD7	Predominantly expressed on T cells, but some off-target hematopoietic expression
CD32a CD2 CD58	Fc and adhesion proteins with high expression but significant off-target expression



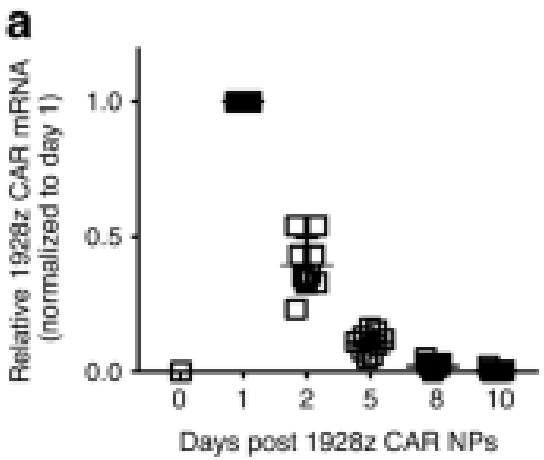
**Sommermeyer Leukemia 2016

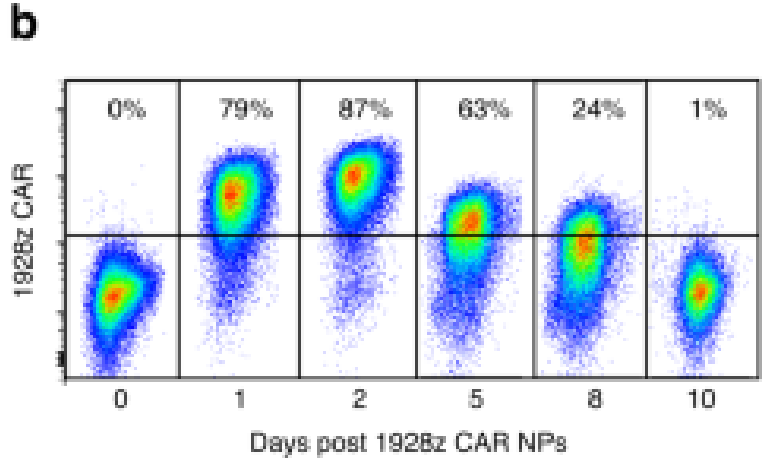
CAR mRNA design is a key area of study and innovation

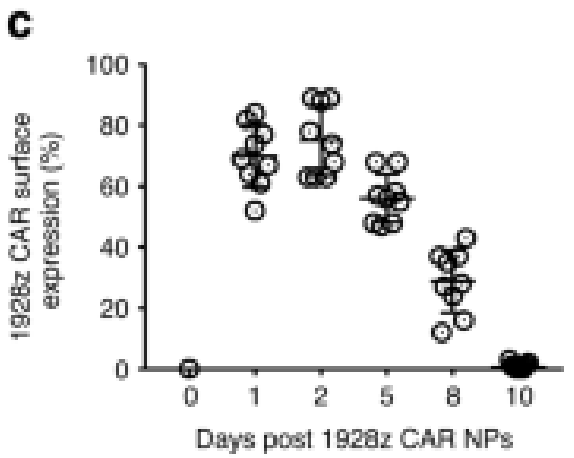


mRNA delivery results in transient CAR expression on cells

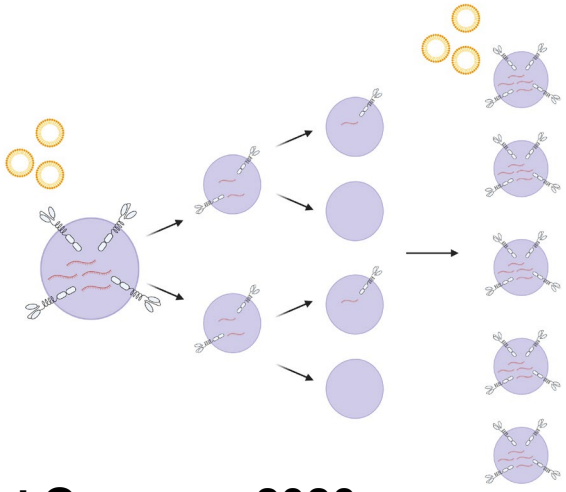
Robust and high but transient CAR expression can be achieved *in vivo*



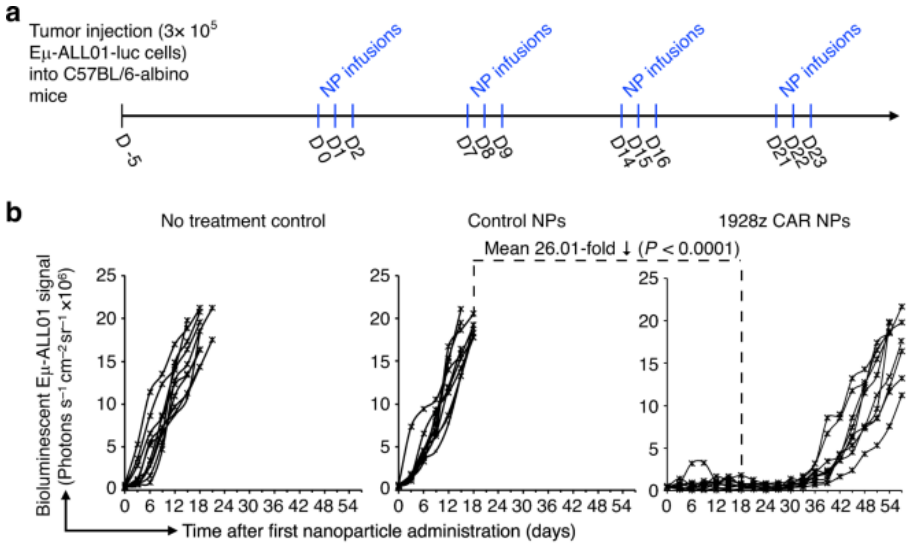




mRNA is both degraded and diluted

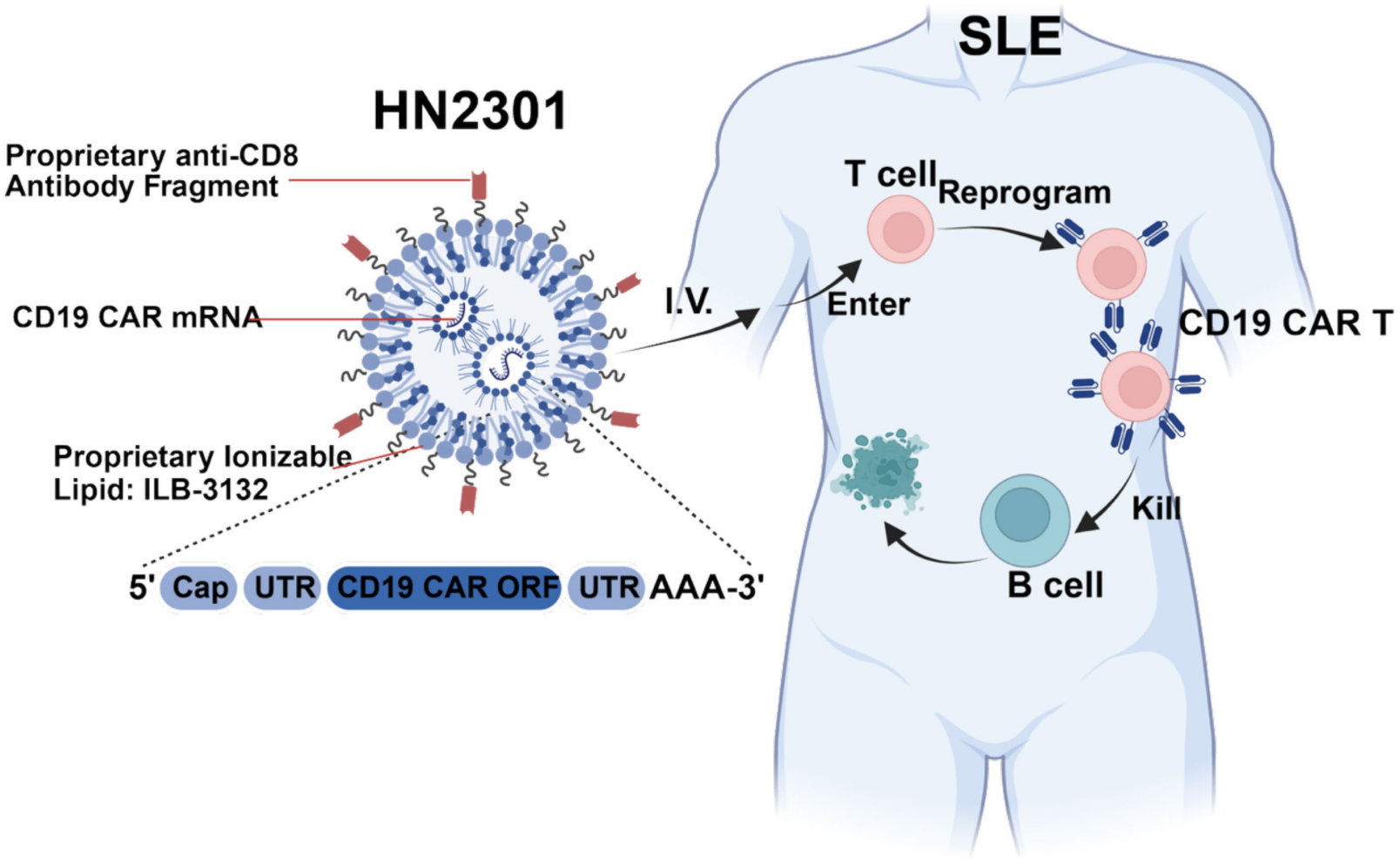


Repeat administration leads to potent effects



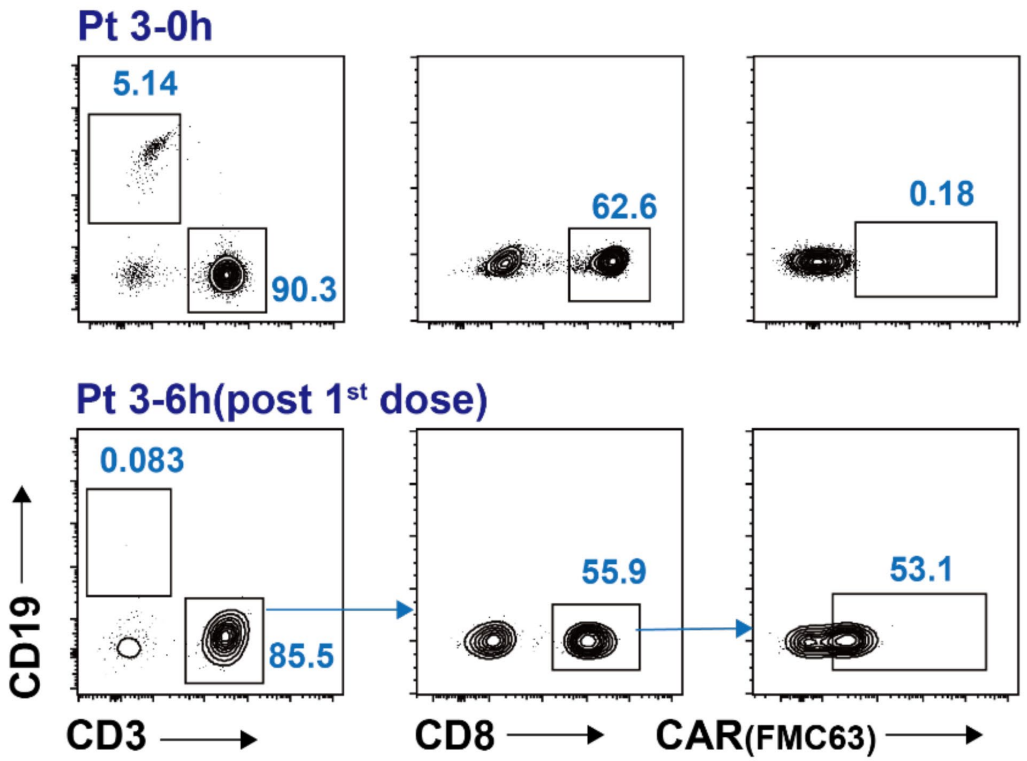
Parayath Nat Commun 2020

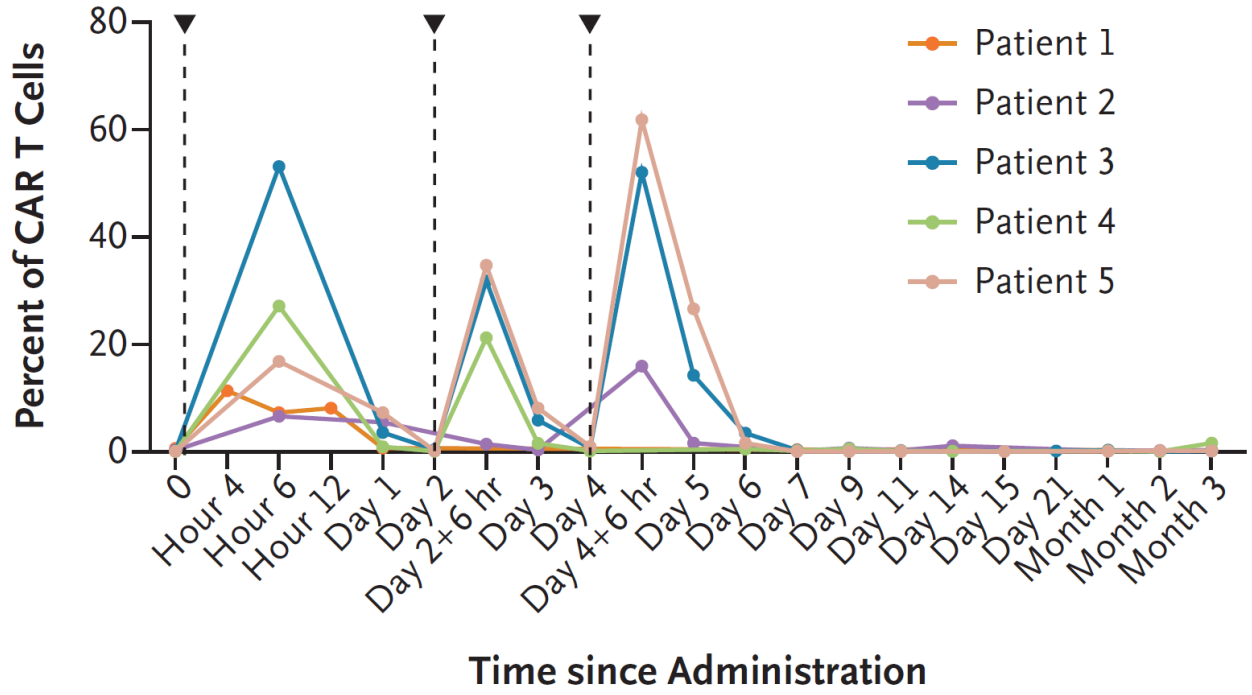
Case Study #1: HN201, a CD8-targeted CAR-LNP for SLE



Wang NEJM 2025

Rapid appearance and loss of CAR expression





Transient CAR expression is a different paradigm

Autologous CAR T cells

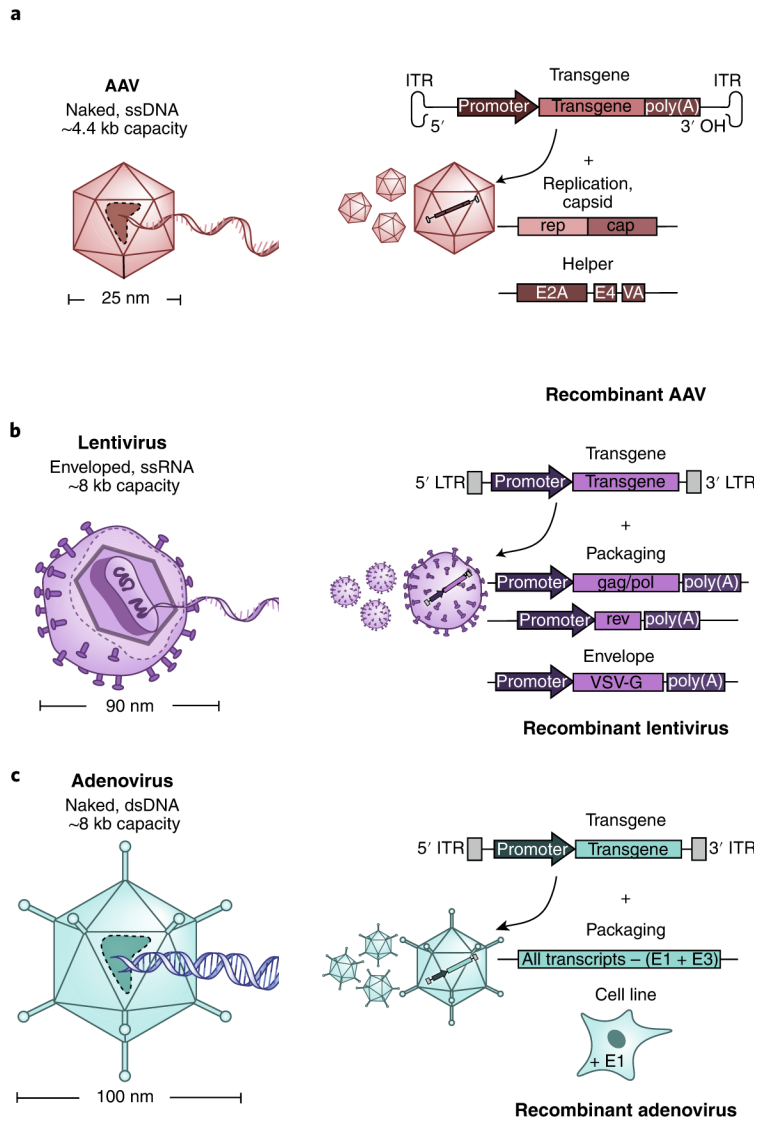
- Minimal/no off-target delivery
- Expansion and persistence drive response (Chow NRCO 2022)
- Dose thresholds
- Limited re-dosing

In Vivo CAR-LNP

- Transient delivery, both off-target and to T cells
- Dosing will be key
    - frequency
    - fixed duration
    - therapeutic index

Dosing, biological behavior, and trial design for CAR-LNP may resemble bispecifics/TCEs more than *ex vivo* autologous CAR T cells

Part 2: Viral platforms for DNA delivery



Episomal, non-integrating
Replicates with cell, durable expression
20-60% of patients have limiting ab titers
Immune reactions can occur

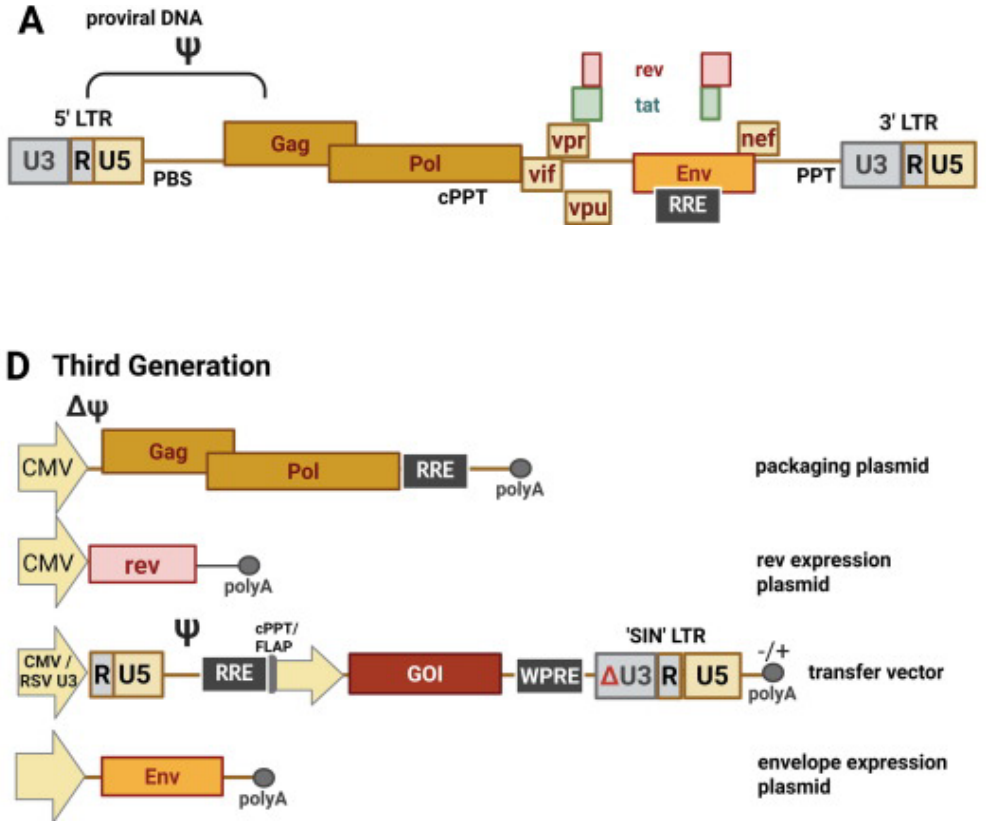
Current platform of choice for ex vivo and in vivo delivery
Integrates genome directly in host DNA

Immune reactions require suppression

Haasteren Nat Biotech 2020

Therapeutic SIN lentiviruses are replication-incompetent

Ali Jaballah JMB 2025

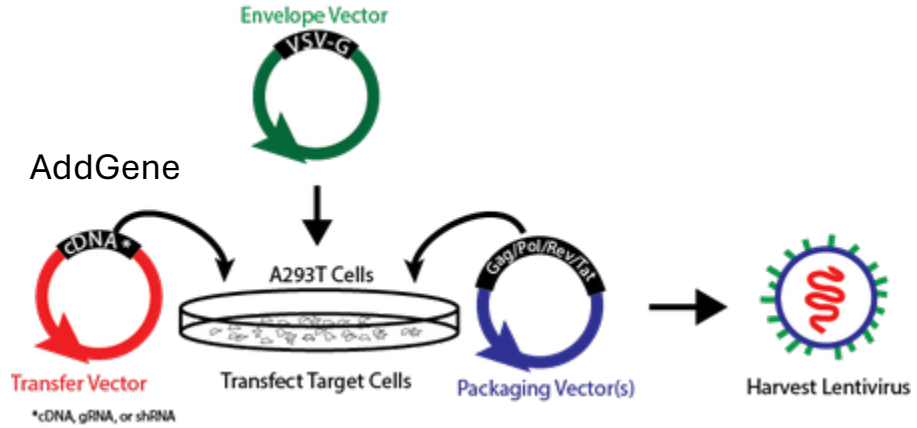


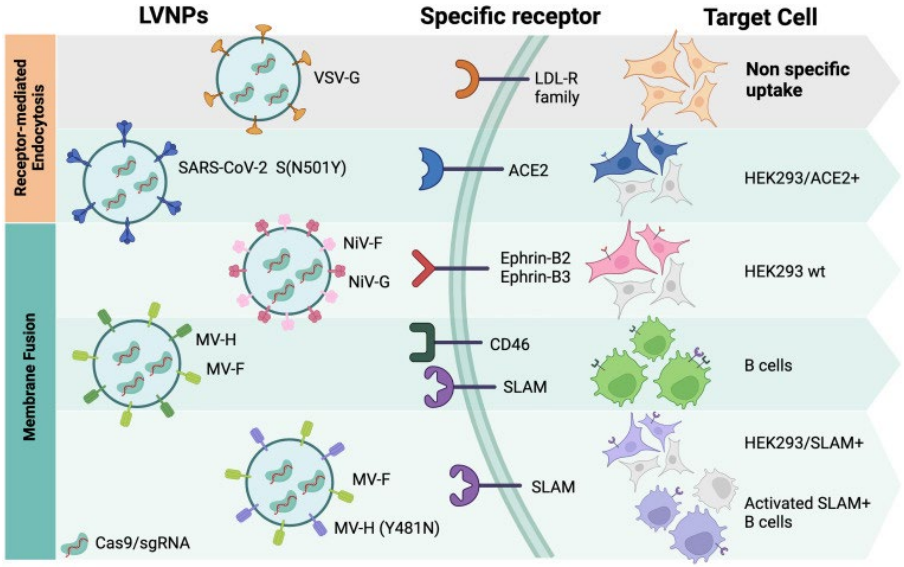
Deletion in 3'LTR loses ability to drive replication once integrated

Each virion can integrate once and (hopefully) should not drive replication of additional virus

Pseudotyped and functionalized lentivirus for targeted delivery

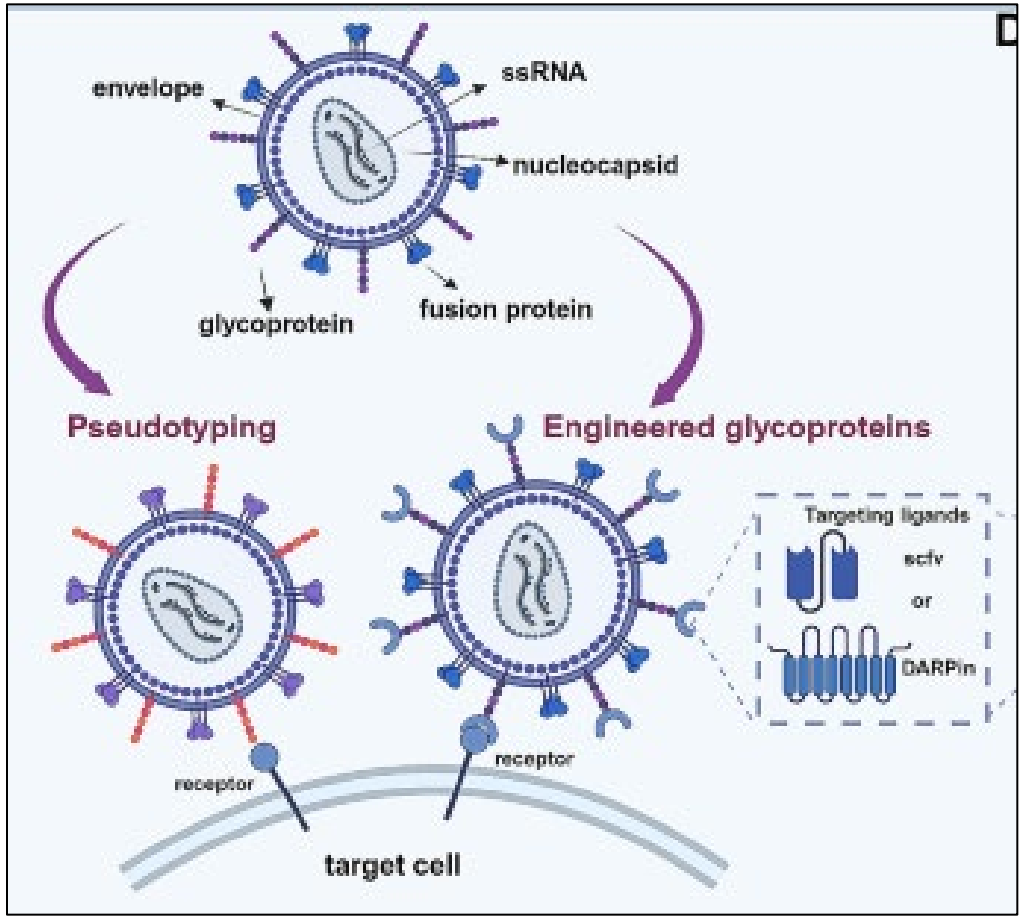
Viral envelope controlled during manufacture





Covo-Vergara Mol Therapy 2023

Pseudo-typing + functionalization control specificity



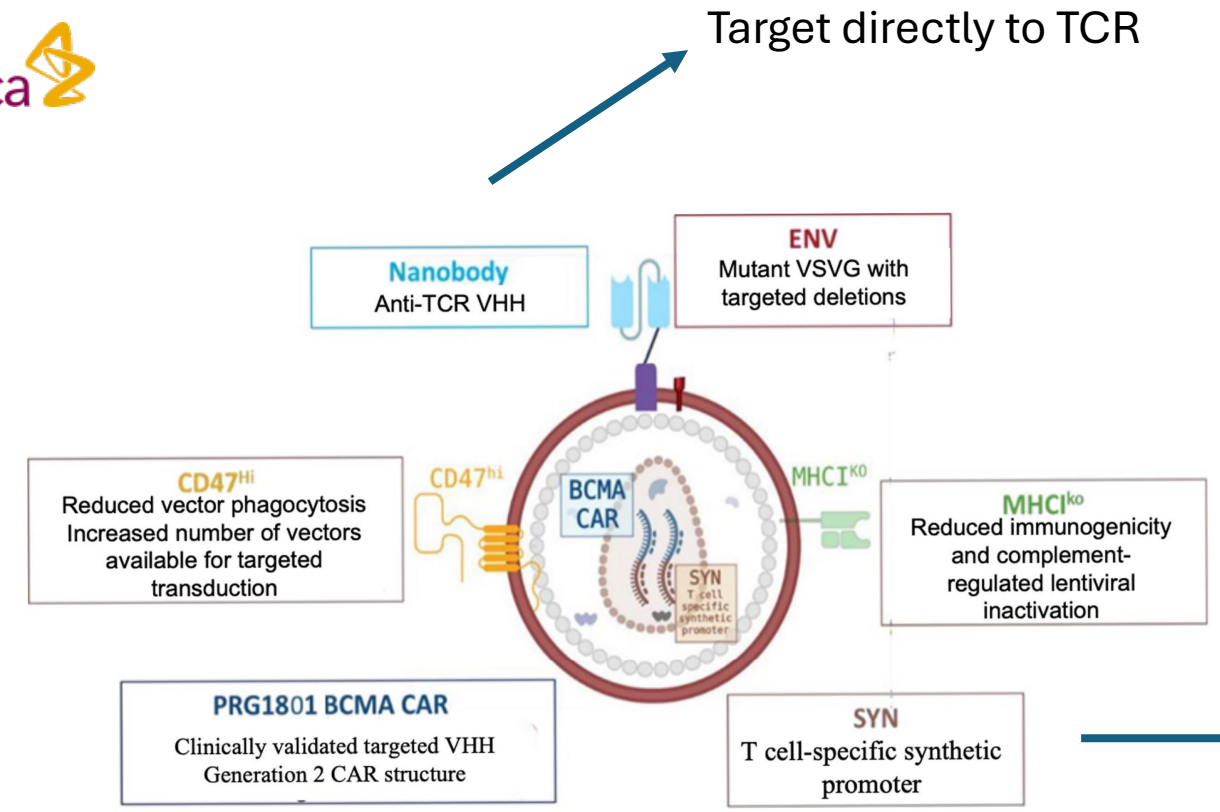
Wang Cellular Oncology 2024

Case Study 2: ESO-T01 lentiviral *in vivo* platform

EsoBiotec

AstraZeneca

Xiu et al. *Lancet* 2025



Licensed from Chromatin Bioscience
T cell specific synthetic promoter
Mitigate off-target CAR *expression*
Would not affect off-target *integration*

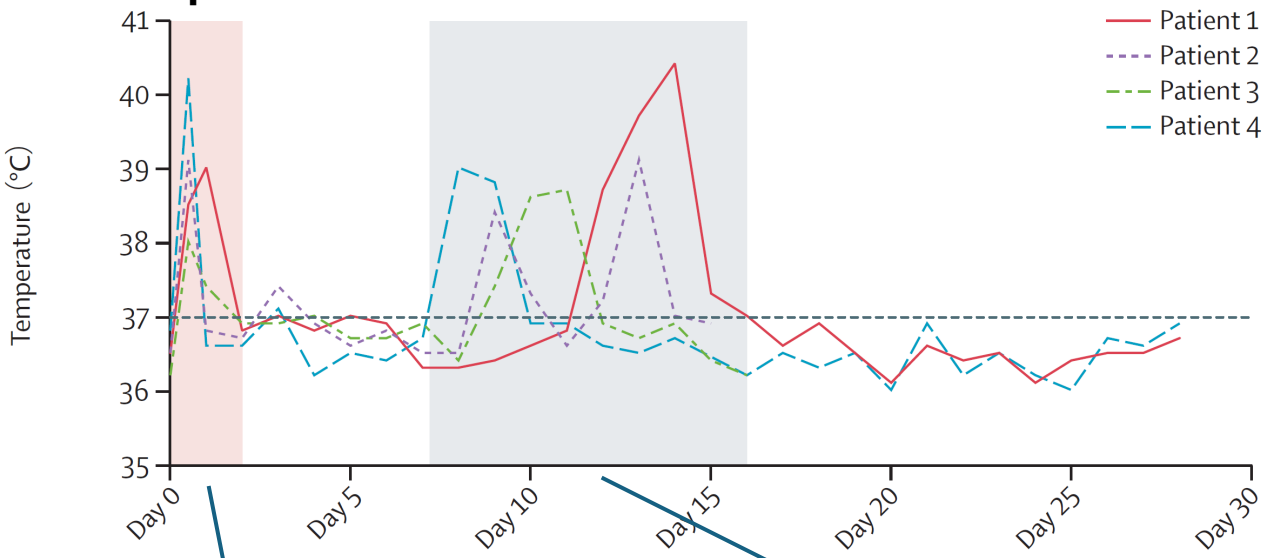
Figure S1: CAR construct and design mechanisms

(A) CAR construct of ESO-T01. (B) Design mechanisms of ESO-T01.

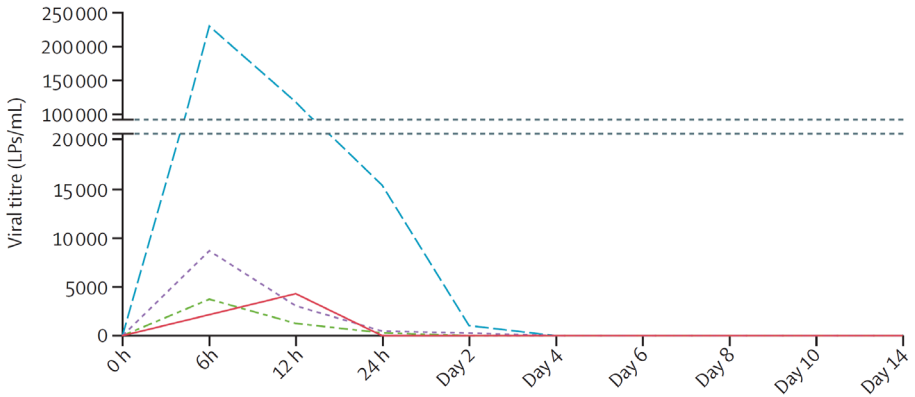
ESO-T01 lentiviral *in vivo* platform

Xiu et al. *Lancet* 2025

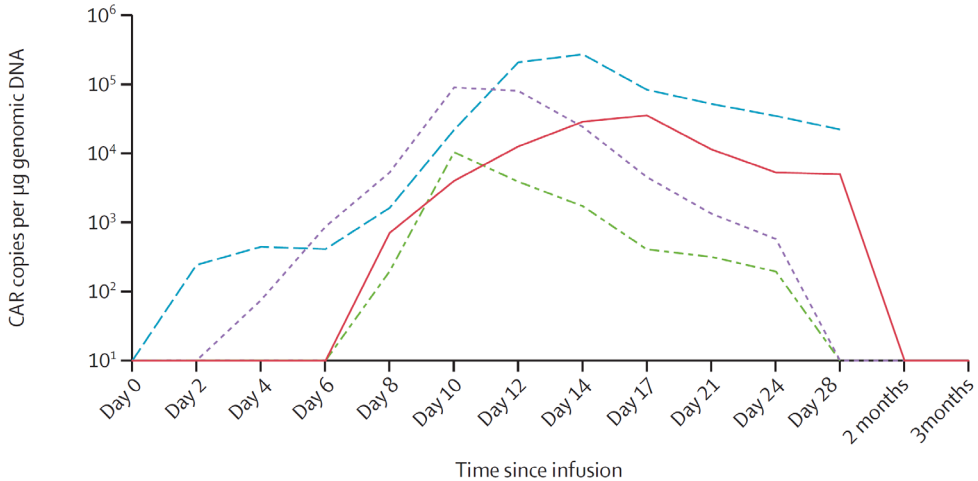
3/4 on pressors **Gr1 CRS**



High viral titer

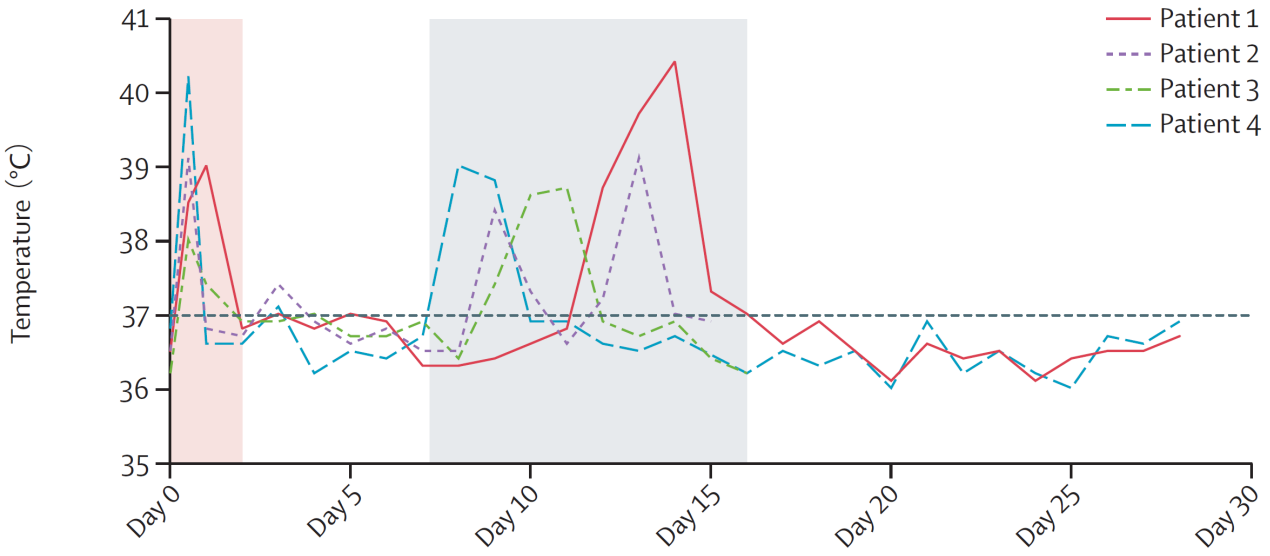


CAR integration, expression, and proliferation



ESO-T01 lentiviral *in vivo* platform

Xiu et al. *Lancet* 2025



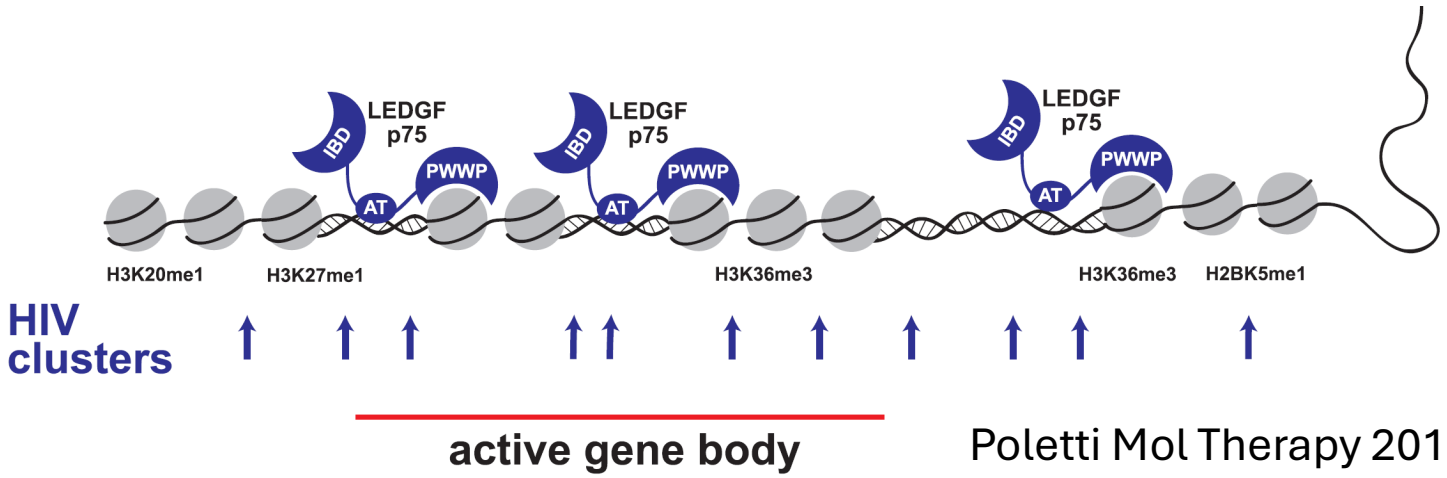
Pt 1: sCR, clearance of EMD
Pt 2: sCR
Pt 3: clear marrow, persistent EMD
Pt 4: Prior CAR, + CSF

Awaiting more patients, longer follow up, and s/sx of off-target integration

What is the risk of viral integration

We don't know. But…

1. Lessons from HIV (***in vivo* 'delivery', replicating**)
    1. HIV *can* integrate in non-immune cells
    2. HIV is rarely <u>directly</u> oncogenic but can drive clonal expansion in T cells (Maldarelli Science 2014)



Poletti Mol Therapy 2018

What is the risk of viral integration

We don't know. But…

1. Lessons from HIV (***in vivo* 'delivery', replicating)**
    1. HIV *can* integrate in non-immune cells
    2. HIV is rarely directly oncogenic

2. Lessons from gene therapy (***ex vivo* delivery, non-replicating**)
    1. Secondary malignancies in lentivirally transduced HSPC
    2. CAR integration in tumor can be an escape mechanism (Ruella Nat Med 2018)
    3. Secondary malignancies can occur in CAR+ cells

Current *in vivo* editing landscape

Mullard *Nat Rev Drug Disc* 2024

Table 1 | In vivo CAR immune cells in and approaching the clinic

Drug name	Company	Vector (cell-targeting mechanism)	Therapeutic payload	Lead indication	Planned phase I start
INT2104	Interius	Lentivirus (CD7 scFv)	CD20 CAR	B cell cancers	2024
INT2106	Interius	Lentivirus (CD7 scFv)	CD19 CAR	Autoimmune	2025
UB-VV111	Umoja/Abbvie	Lentivirus (CD3 scFv, CD80 and CD58)	CD19 CAR; RACR	B cell cancers	2024
UB-VV400/410	Umoja/IASO	Lentivirus (CD3 scFv, CD80 and CD58)	CD22 CAR; RACR	B cell cancers	2024
UB-VV300/310	Umoja	Lentivirus (CD3 scFv, CD80 and CD58)	CD20 CAR	NHL/Autoimmune	2026
KLN-1010	Kelonia	Lentivirus (CD3 antibody)	BCMA CAR	Multiple myeloma	2025
Discontinued	Sana	Lentivirus	Various CARs	Discontinued	Discontinued
CPTX2309	Capstan	LNP (CD8 antibody)	CD19 CAR (mRNA)	Autoimmune diseases	"Near future"
Undisclosed	Orbital	LNP (undisclosed cell-targeting moiety)	CD19 CAR	Autoimmune diseases	"Near future"
ORN-145	Orna	LNP (no cell-targeting moiety)	CD19 CAR (circular RNA)	B cell cancers	Undisclosed
ORN-252	Orna	LNP (no cell-targeting moiety)	CD19 CAR (circular RNA)	Autoimmune diseases	2026
ORN-328	Orna	LNP (no cell-targeting moiety)	BCMA CAR (circular RNA)	Multiple myeloma	2026
MT-302	Myeloid	LNP (no cell-targeting moiety)[a]	TROP2 CAR (mRNA)	Epithelial tumours	2023
MT-303	Myeloid	LNP (no cell-targeting moiety)[a]	GPC3 CAR (mRNA)	Liver cancer	2024
Undisclosed	Carisma/Moderna	LNP (no cell-targeting moiety)	GPC3 CAR (mRNA)	Liver cancer	Undisclosed

[a]To reduce off-tissue activity, Myeloid's uses a CAR-CD89 fusion payload, requiring FcRγ to be active. CAR, chimeric antigen receptor; NHL, non-Hodgkin lymphoma; RACR, rapamycin activated cytokine receptor; scFv, single-chain variable fragment.

Delivery platform	Non-viral	Viral
Most Common Platform	LNP	Lentivirus
T cell targeting	Lipid formulation Surface Functionalization	Pseudotyping
Gene delivery	mRNA, non-integrating	Integrating
Dosing	Repeat dosing	One-time infusion
Safety	Off-target uptake in liver	Viral infusion syndromes Risk of integration

Memorial Sloan Kettering Cancer Center

Questions?