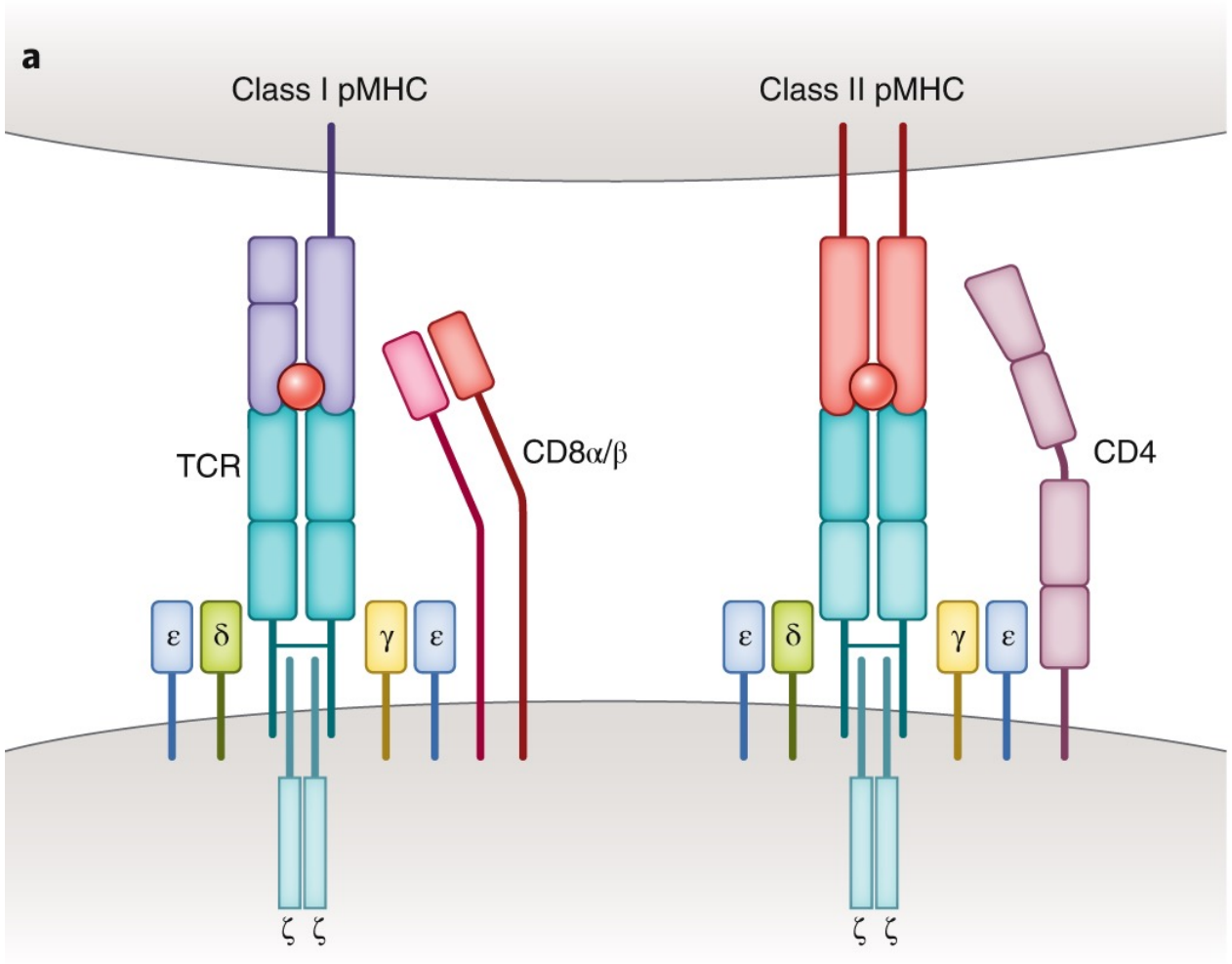
The immunology and science of CAR T cells

Leonardo M.R. Ferreira

Department of Microbiology and Immunology and Hollings Cancer Center

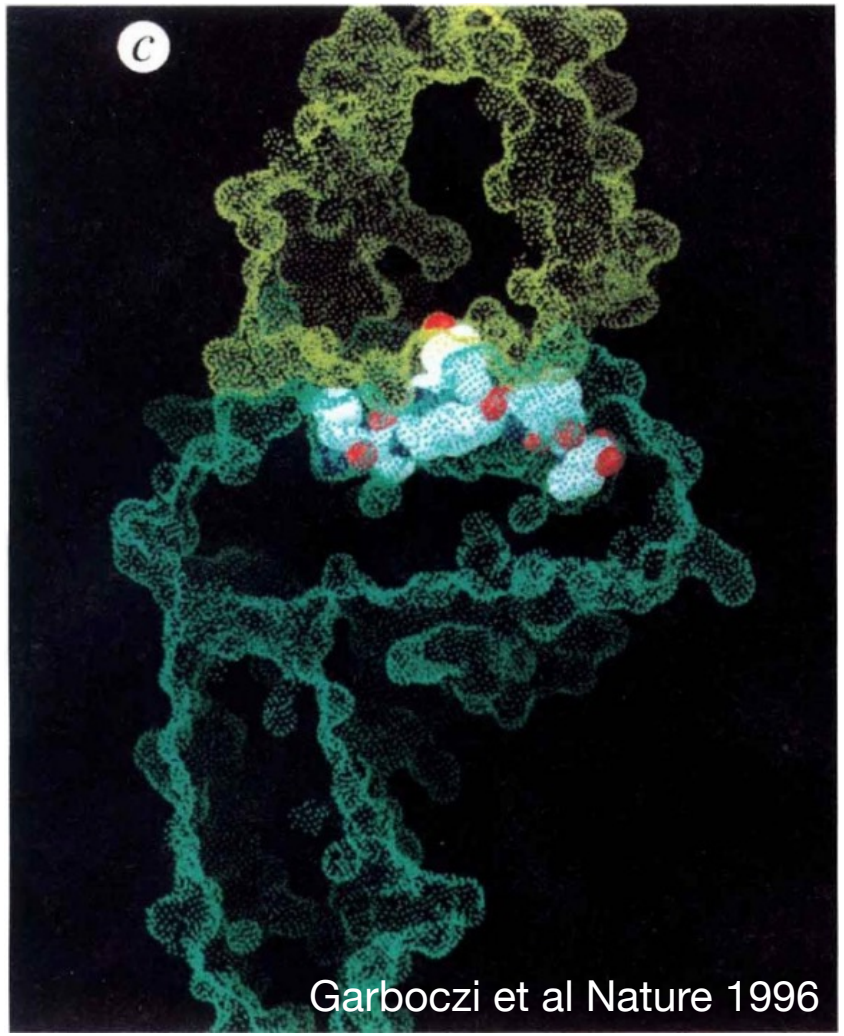
MUSC Rheumatology Grand Rounds 6/16/2023

How do T cells see?

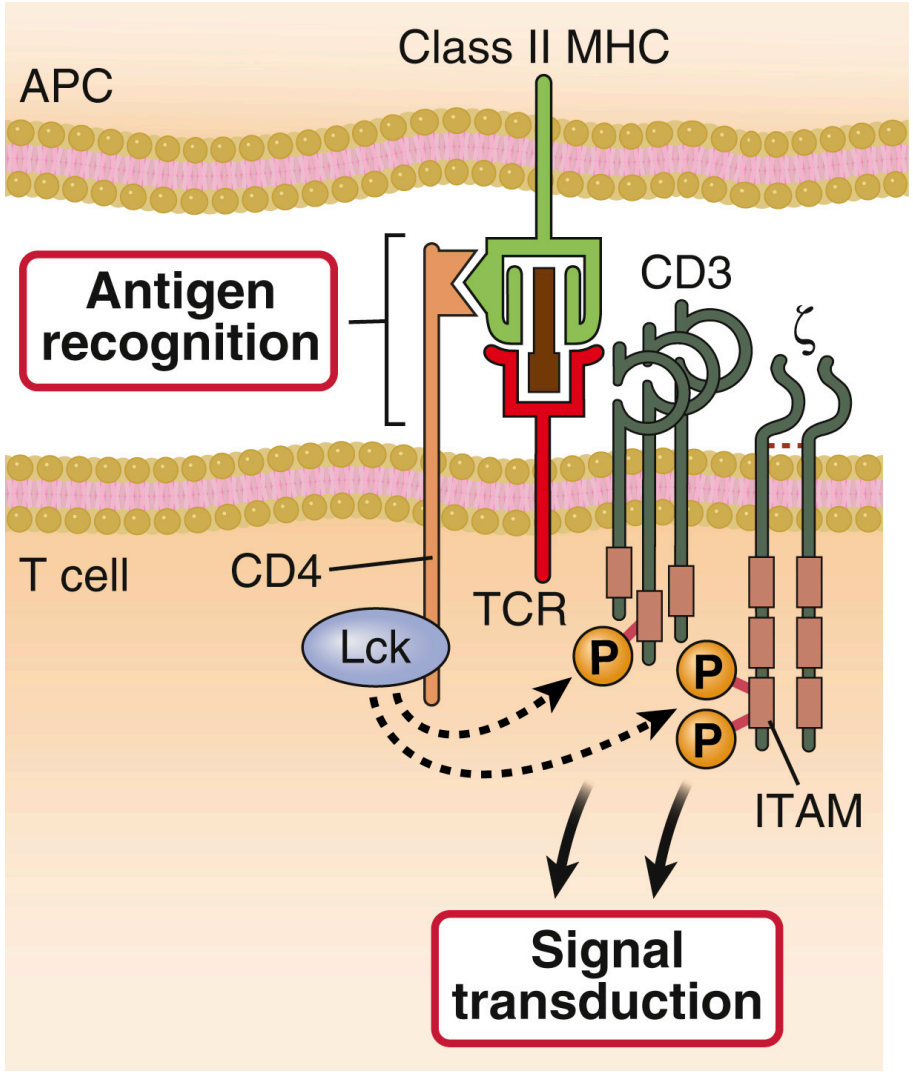


Joglekar & Li, Nature Methods 2020

TCR-peptide-HLA-A2 complex



T cell signal 1: TCR activation



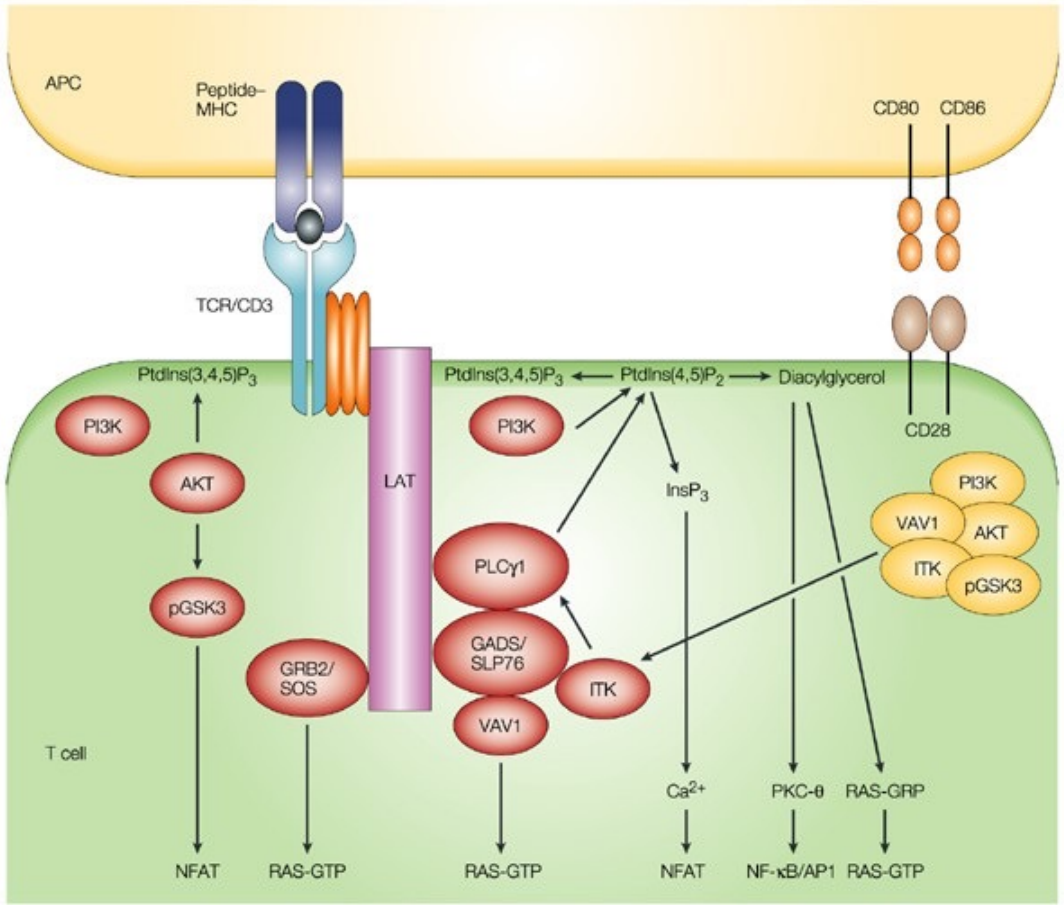
APC
antigen-presenting cell

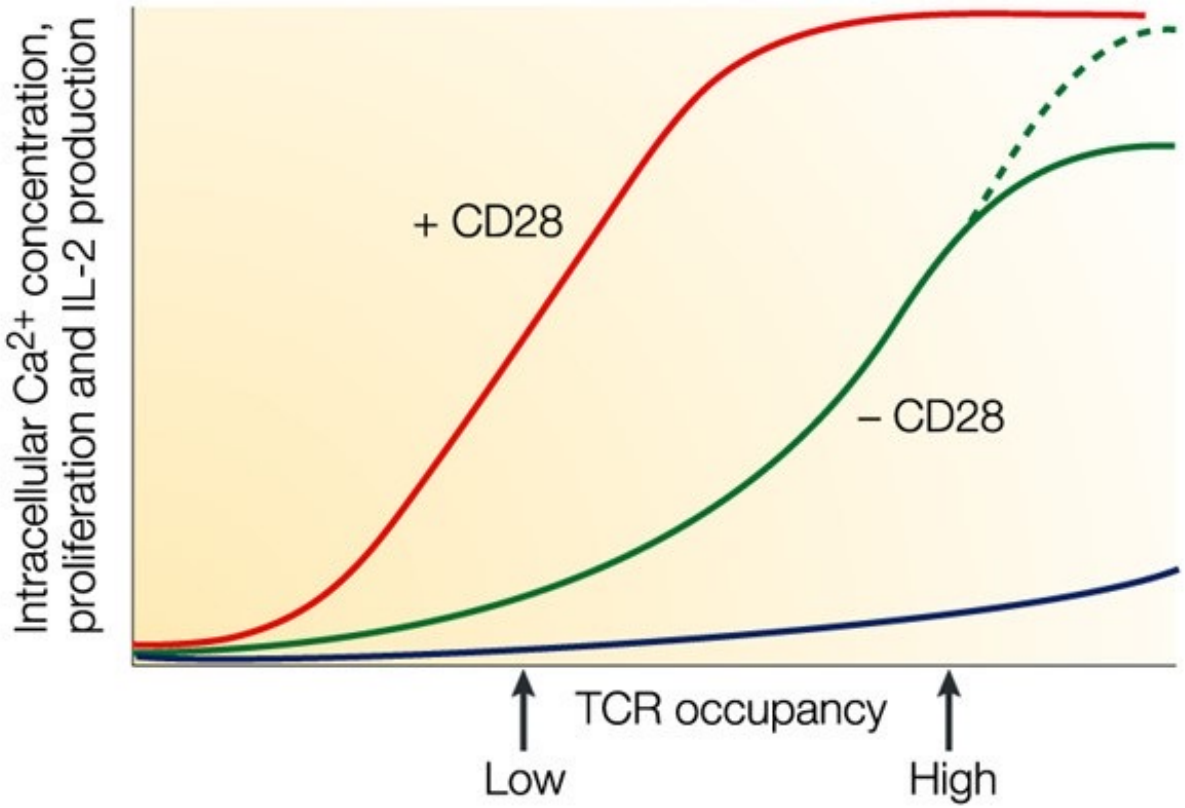
Lck
lymphocyte-specific protein tyrosine kinase

ITAM
immunoreceptor tyrosine-based activation motif

Abbas et al, Cellular and Molecular Immunology

T cell signal 2: CD28 co-stimulation





Acuto & Michel 2003

1987

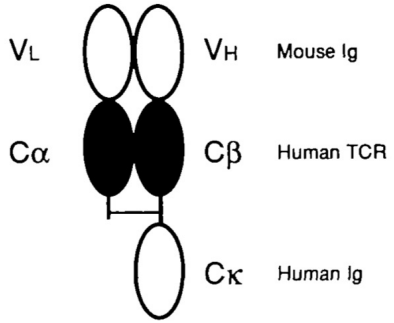
First chimeric receptor: V portion from antibody and C portion from TCR- “IgTCR”

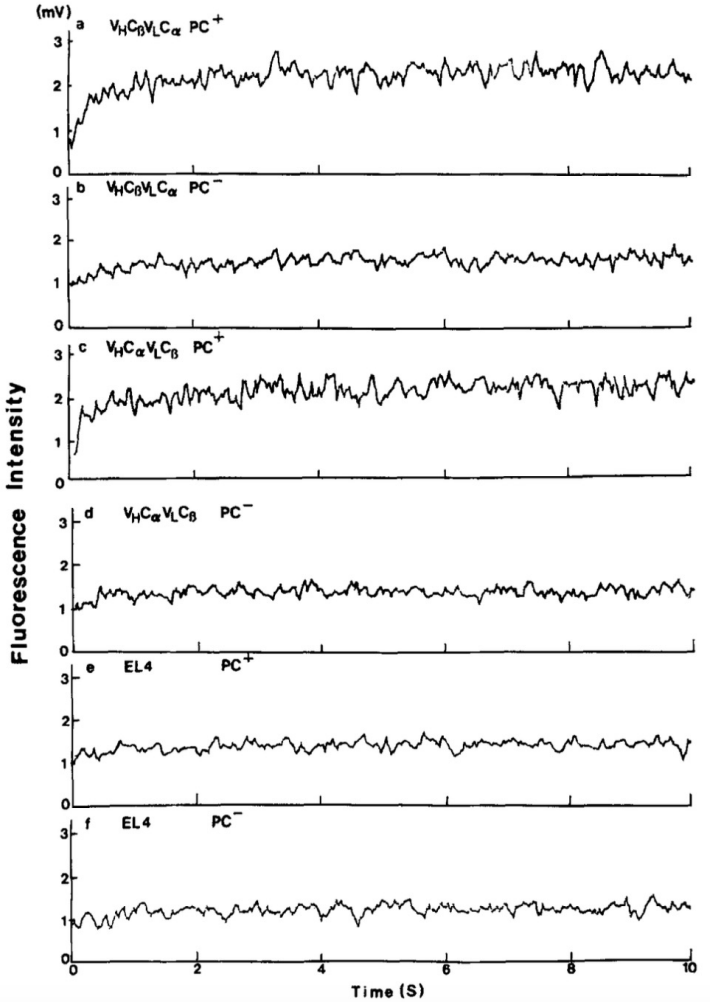
Kwana et al, BBRC **1987**

Yoshikazu Kurozawa

α and β chains of TCR are organized into immunoglobulin-like domains consisting of multistranded antiparallel β-sheet bilayers. Our present experiments indicate that the chimeric receptor composed of Ig-derived V regions and TCR-derived C regions has the capability to trigger T cell

activation with the antigen alone, without MHC molecules. Since calcium influx is an early transmembrane event (8), we are now examining whether helper and cytolytic functions occur with antigens. If this is the case, the ability of Ig to bind to free antigens could be transferred into T cells. In future, it might become possible for T cells recognizing any antigens without MHC restriction to be produced by the technique described in this paper.



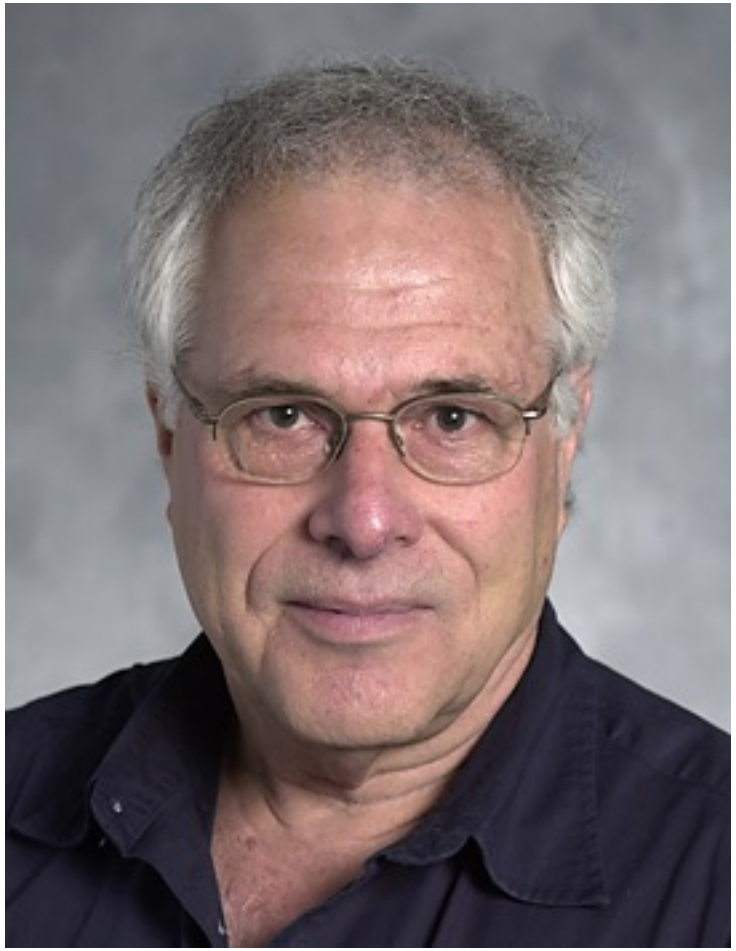


1989

"First" to add a single-chain variable fragment (scFv) to T cell-activating receptors – "T-bodies"

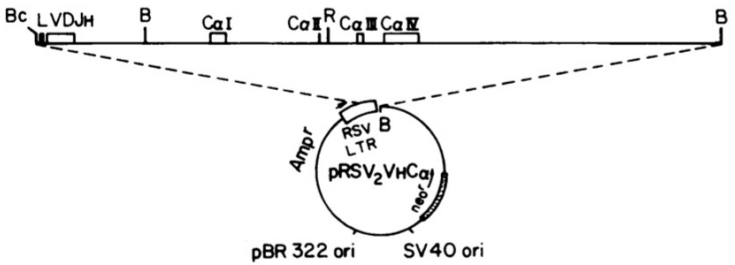
Zelig Eshhar

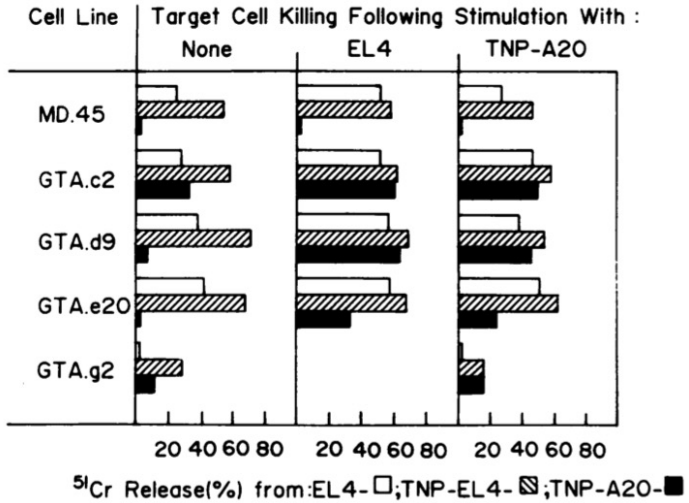
Gross et al, PNAS **1989**



Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity

(chimeric genes/antibody variable region)





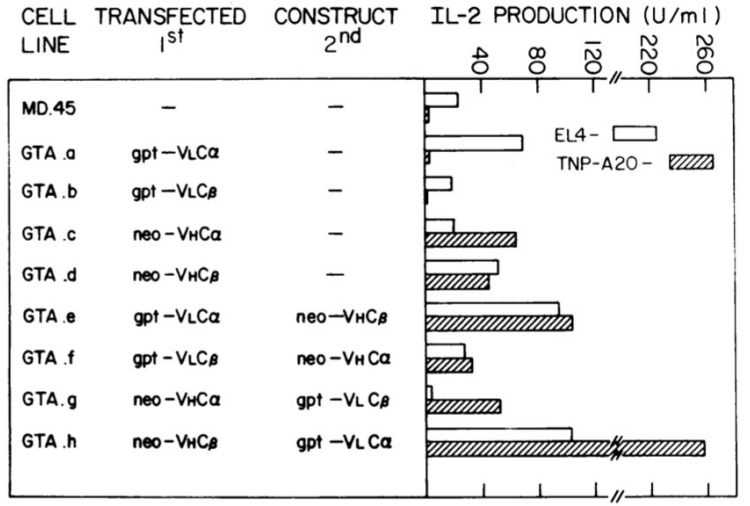
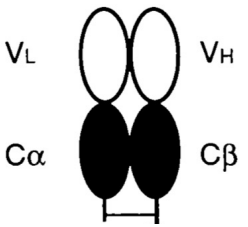


FIG. 3. Production of IL-2 by transfectants following stimulation with EL4 and TNP-modified A.20 cells.



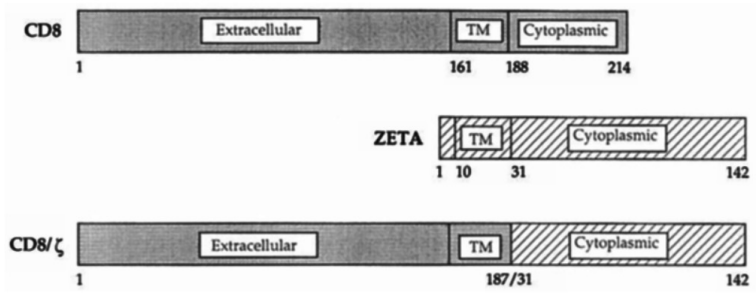
All mouse

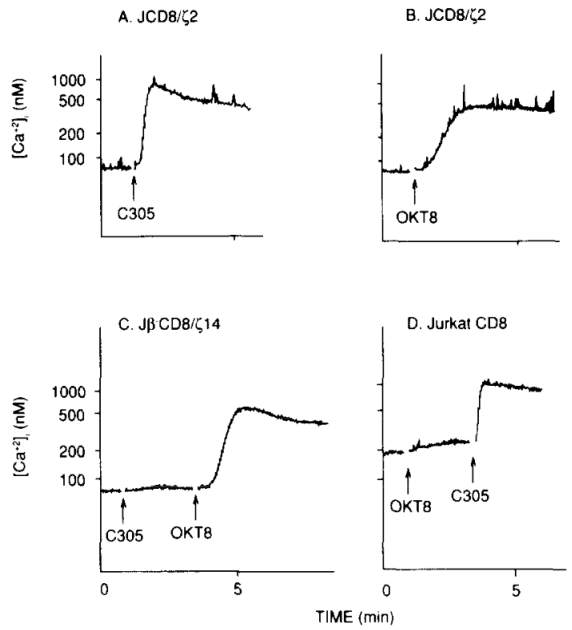
1991

First chimeric T cell-activating receptor

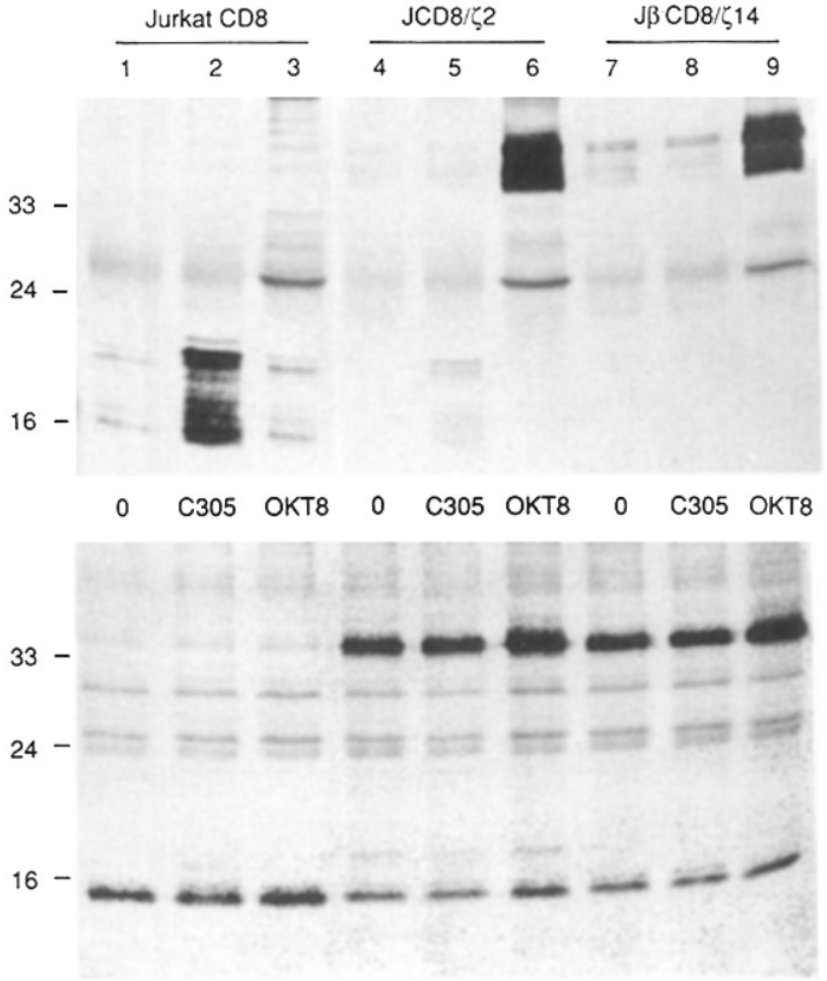
Art Weiss

The Cytoplasmic Domain of the T Cell Receptor ζ Chain Is Sufficient to Couple to Receptor-Associated Signal Transduction Pathways



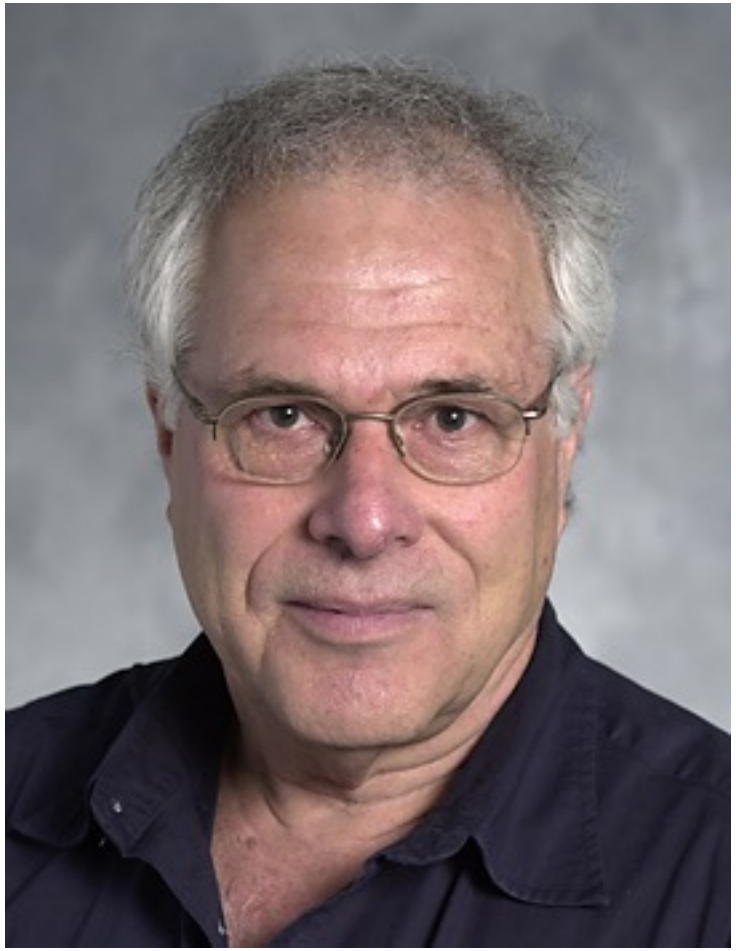


Irving & Weiss, Cell 1991



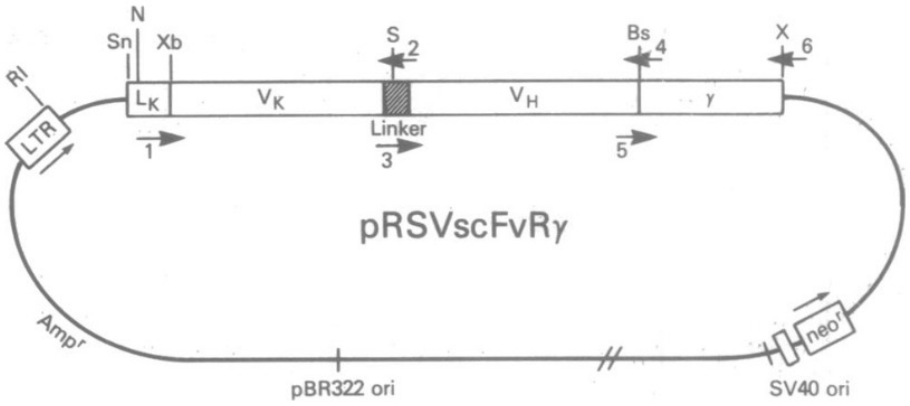
1993 First to add a single-chain variable fragment (scFv) to B cell-activating receptors

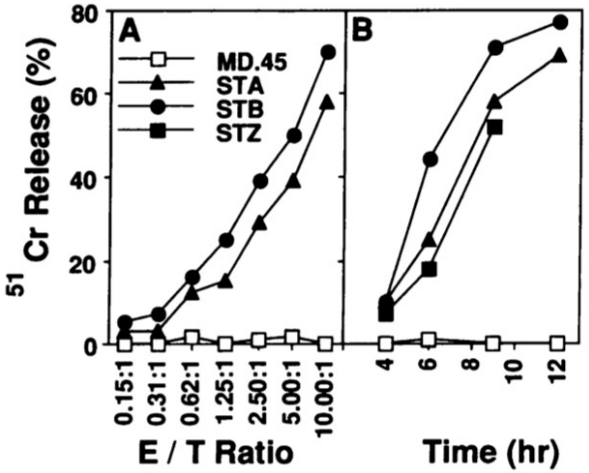
Zelig Eshhar

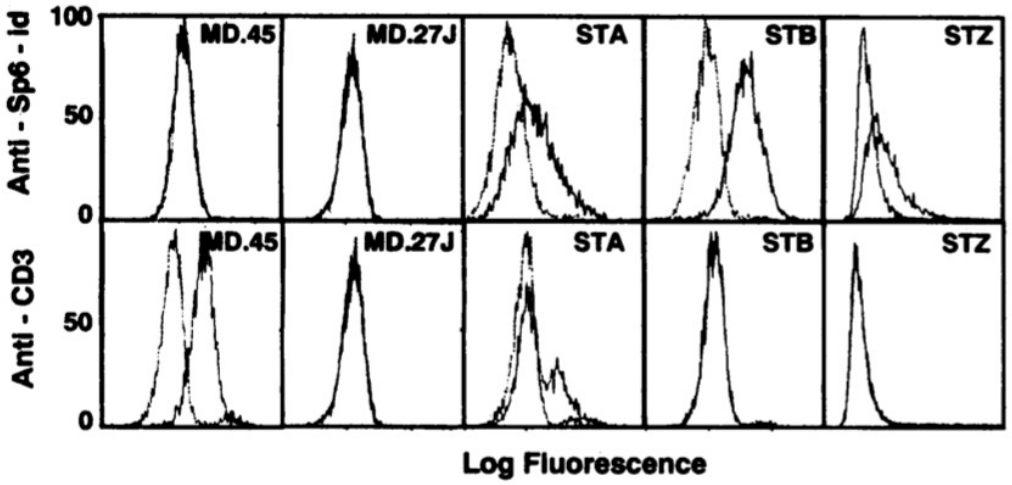


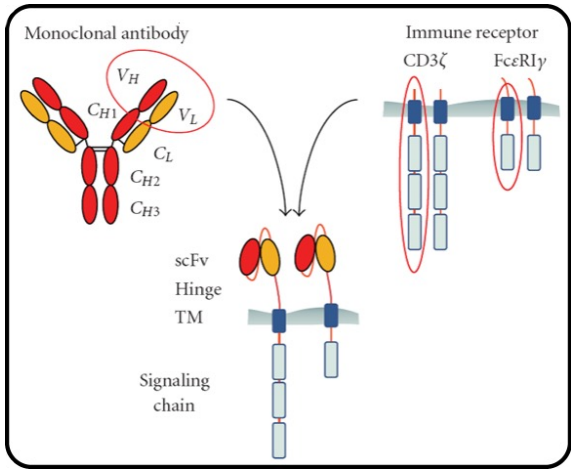
Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the γ or ζ subunits of the immunoglobulin and T-cell receptors

Eshhar et al, PNAS 1993









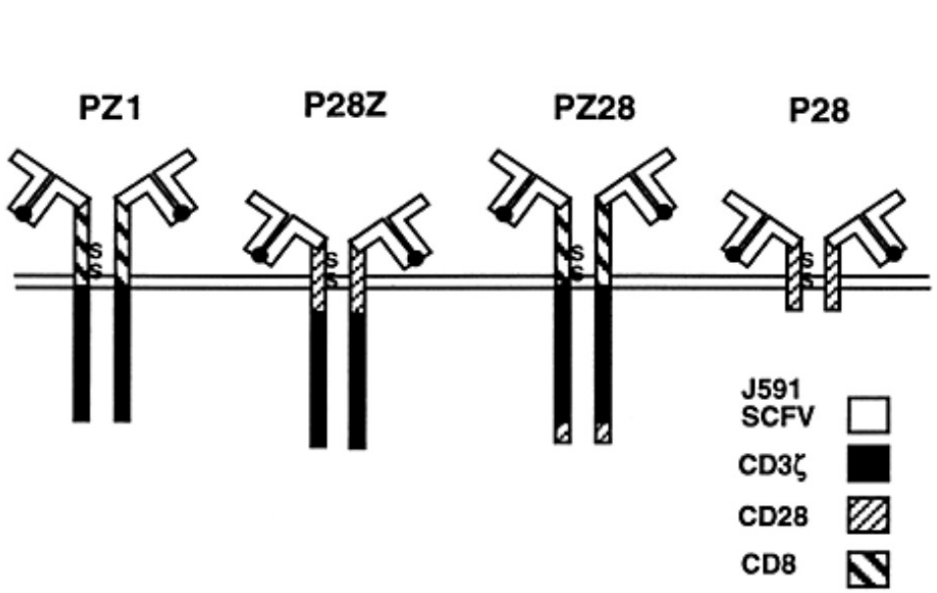
1997-2002

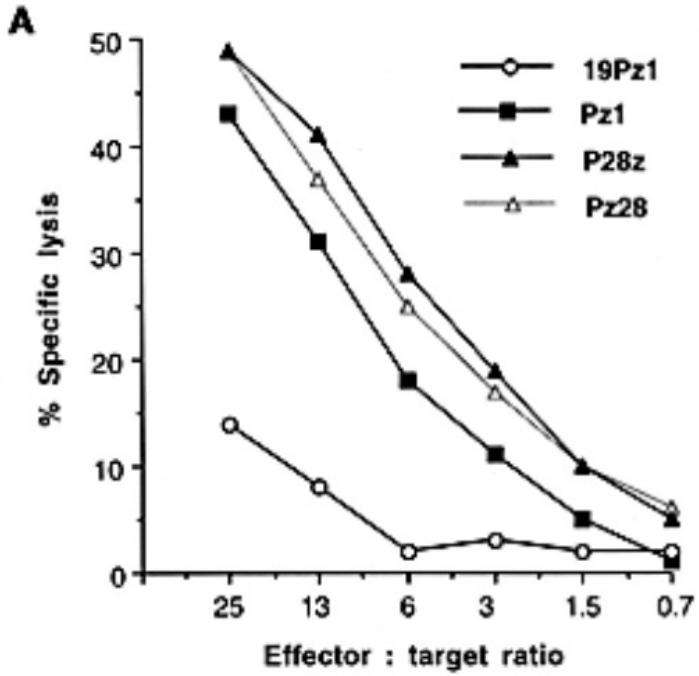
Michel Sadelain

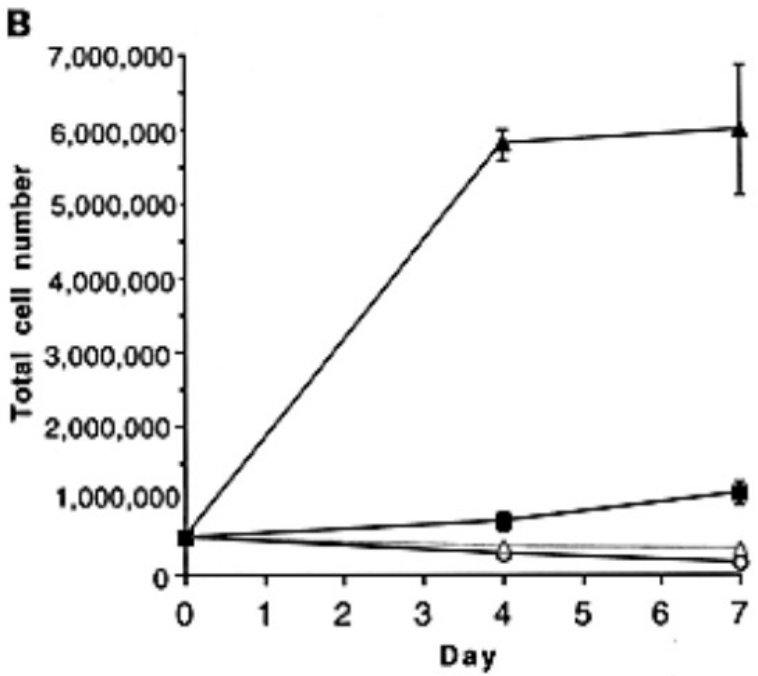
First to test chimeric antigen receptors (CARs) in primary human T cells (Gallardo et al, Blood 1997)

First to achieve sustained T cell expansion and function by adding the costimulatory molecule CD28 (second generation CAR – 28ζ) (Maher et al, Nat Biotech 2002)

Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRζ /CD28 receptor

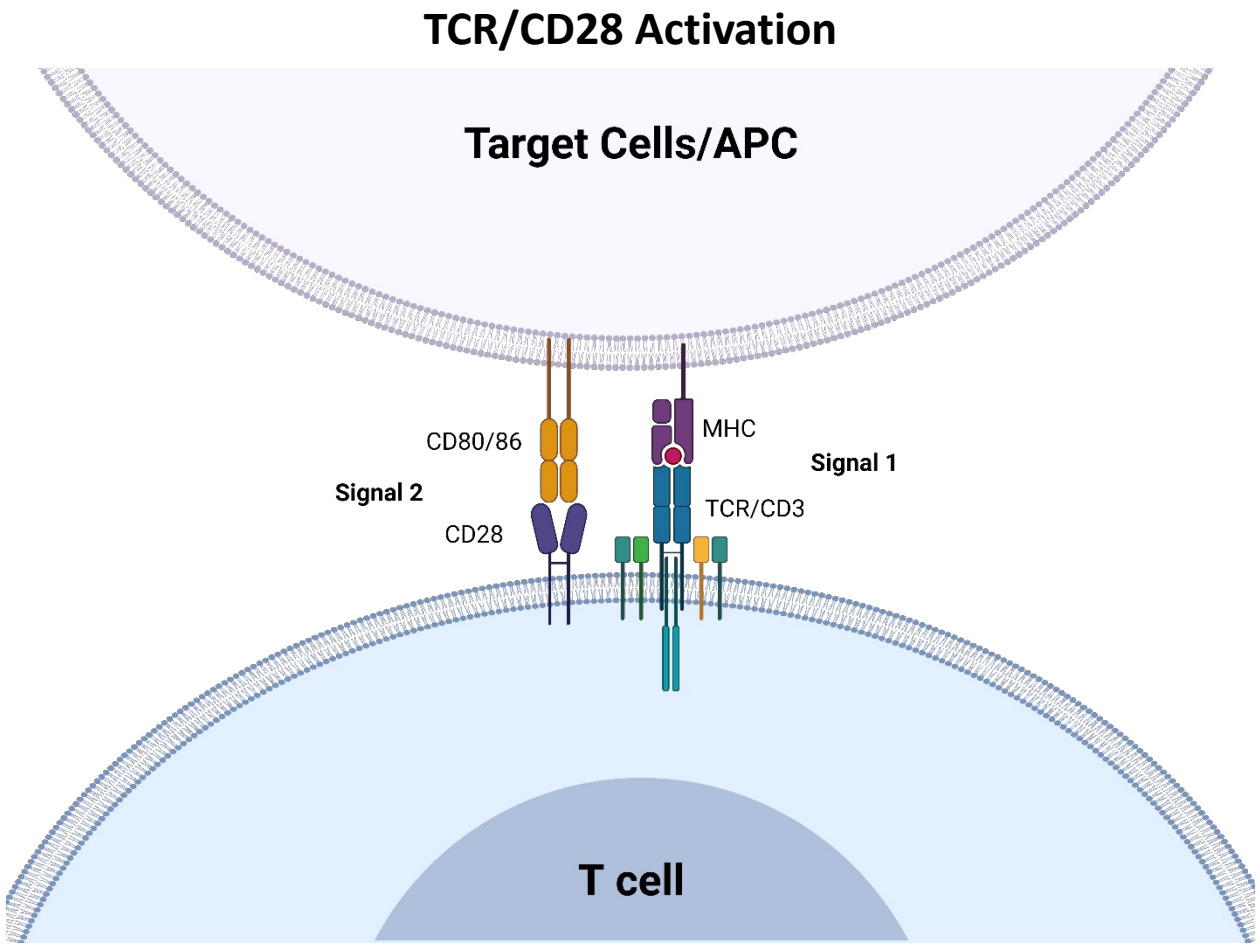


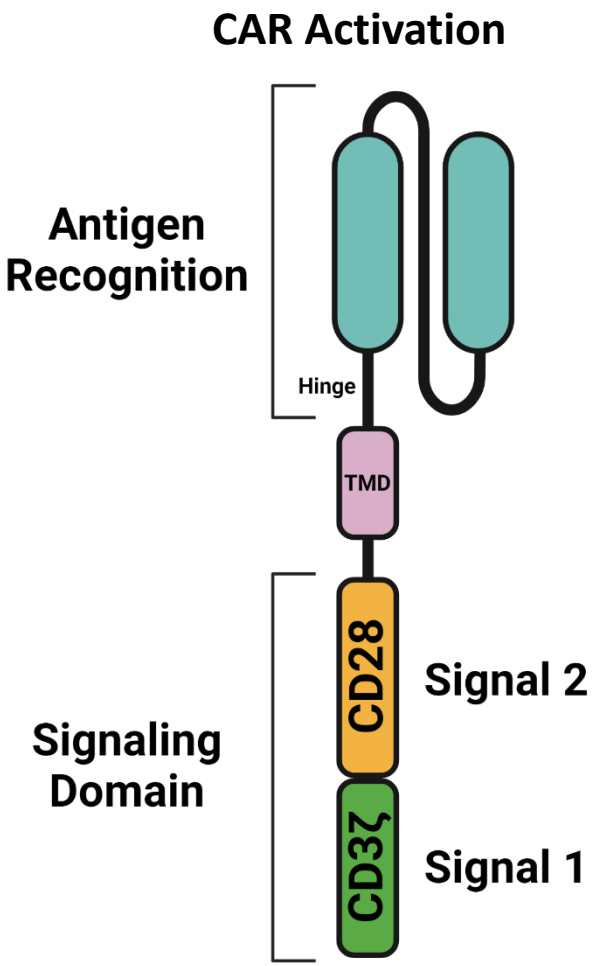




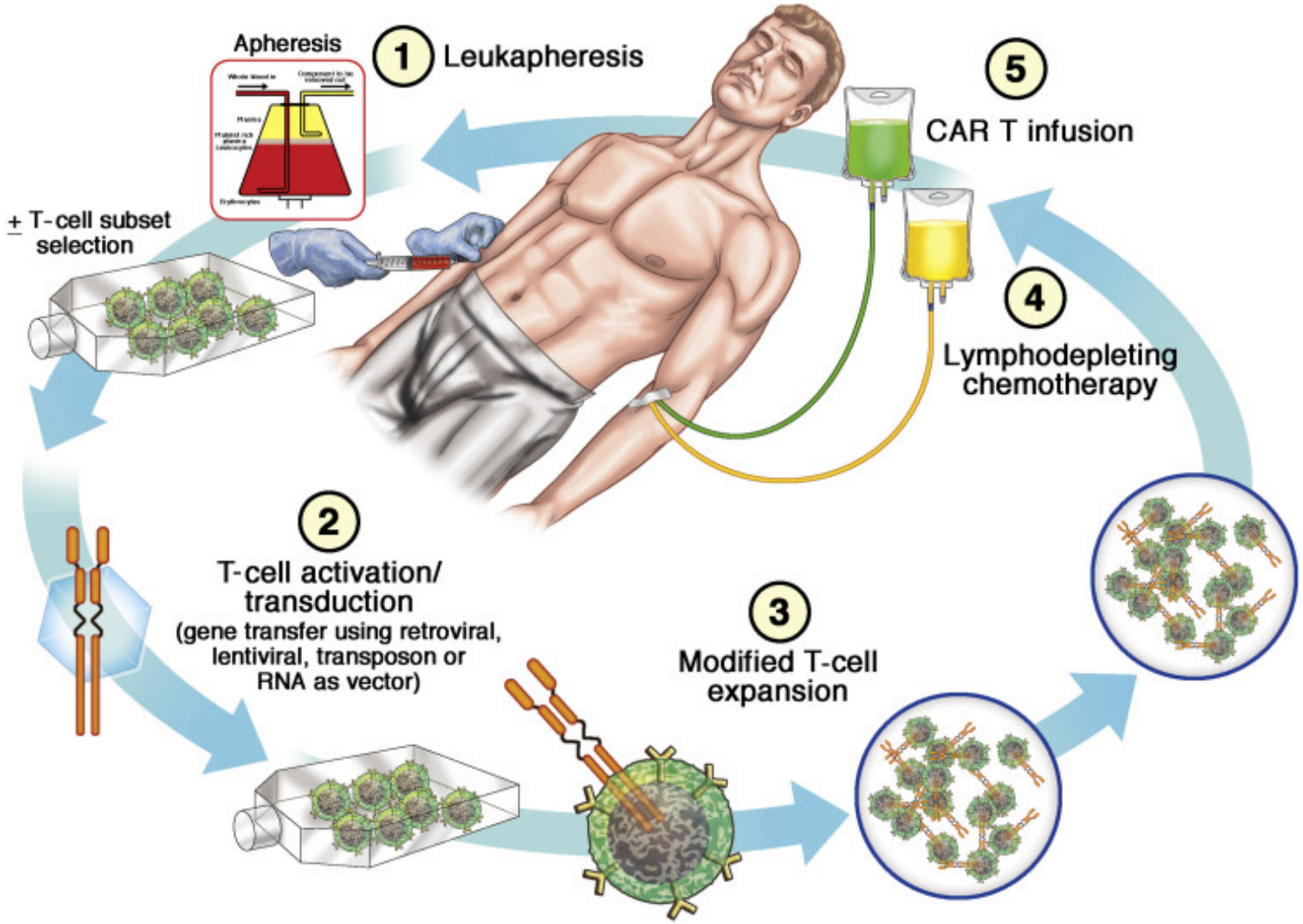
Maher et al, Nat Biotech 2002

Chimeric antigen receptor (CAR)

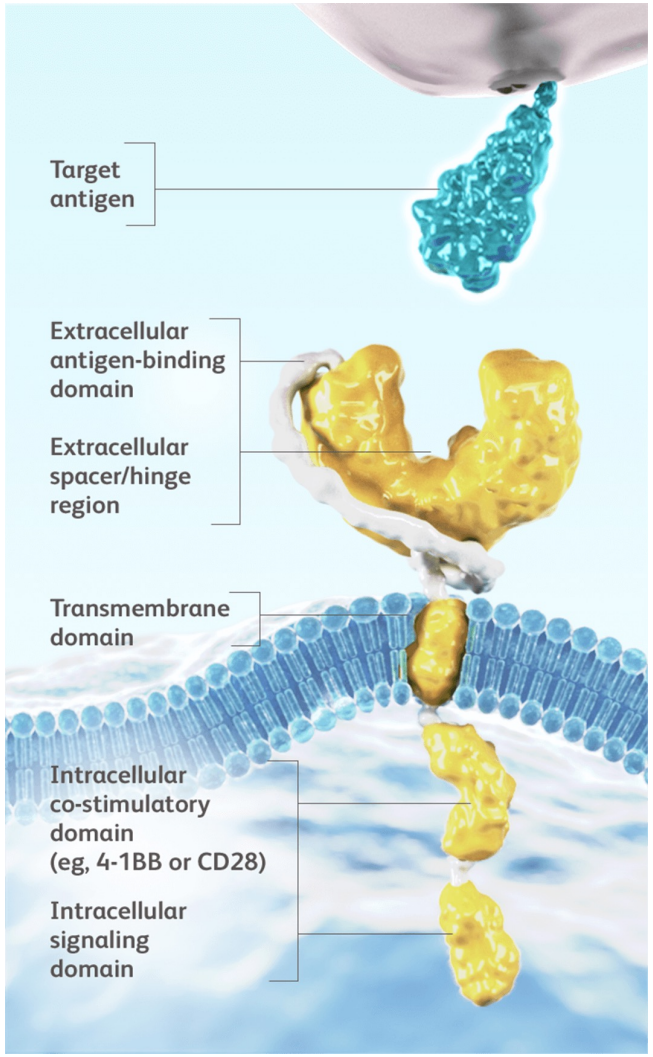




The CAR T cell therapy paradigm

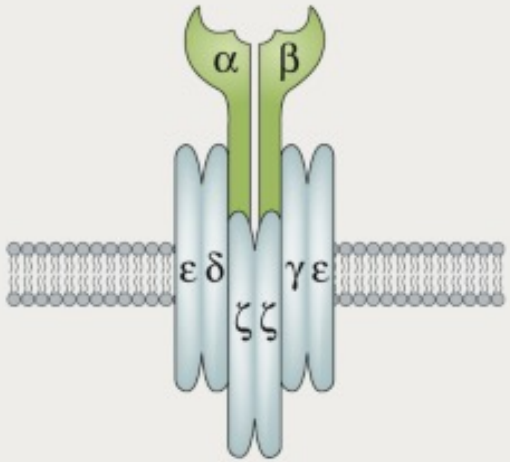
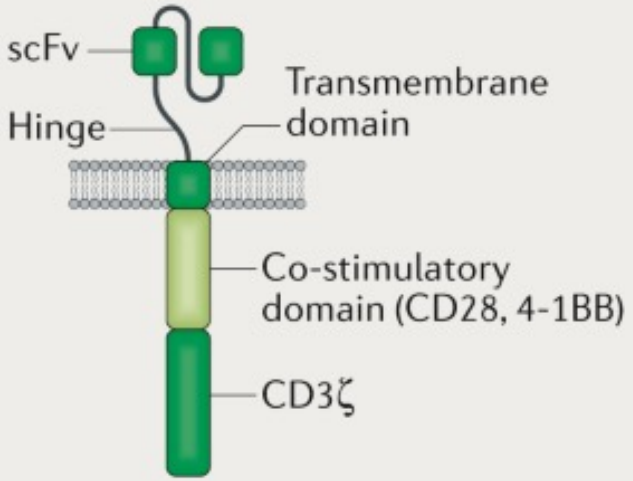


Freyer JACI 2020



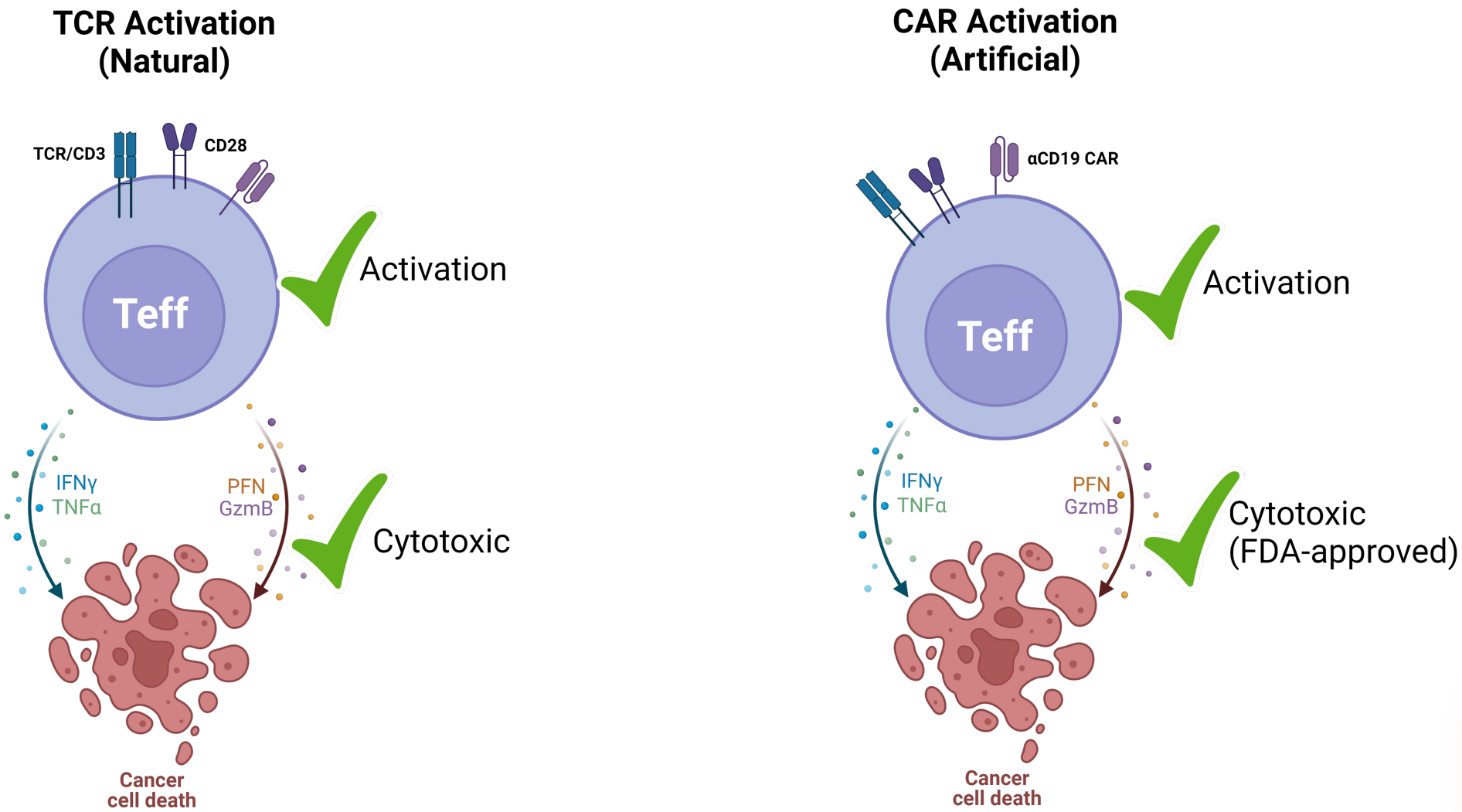
cartcellscience.com

TCR vs. CAR

Engineered TCRs	CARs
	
Multi-protein complex	Single protein
Low affinity	High and adjustable affinity
Recognition is MHC-restricted	Recognition is MHC-independent (broader applicability)
Recognize processed extracellular and intracellular antigens bound to MHC molecules	Recognize whole proteins, including MHC–peptide complexes and extracellular antigens present in the ECM
Risk of mispairing with endogenous TCR	CRS reported with CAR T cell ACT but unlikely with CAR T_{reg} cells

Raffin Nat Rev Immunol 2019

CARs redirect T cells to new targets



FDA approved CAR T cell therapies

FDA-Approved CAR T-Cell Therapies

Generic Name	Brand Name	Target Antigen	Targeted Disease	Patient Population
Tisagenlecleucel	Kymriah	CD19	B-cell acute lymphoblastic leukemia (ALL)	Children and young adults with refractory or relapsed B-cell ALL
			B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
Axicabtagene ciloleucel	Yescarta	CD19	B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
			Follicular lymphoma	Adults with relapsed or refractory follicular lymphoma
Brexucabtagene autoleucel	Tecartus	CD19	Mantle cell lymphoma (MCL)	Adults with relapsed or refractory MCL
			B-cell acute lymphoblastic leukemia (ALL)	Adults with refractory or relapsed B-cell ALL
Lisocabtagene maraleucel	Breyanzi	CD19	B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
Idecabtagene vicleucel	Abecma	BCMA	Multiple myeloma	Adults with relapsed or refractory multiple myeloma
Ciltacabtagene autoleucel	Carvykti	BCMA	Multiple myeloma	Adults with relapsed or refractory multiple myeloma

NCI

CAR T cell therapy regimens

Study Name	1st Author	Year	Clinical Phase	Generic Name	CAR Design	Indication	Number of patients infused	Age	Dose
Tisagenlecleucel in Adult Relapsedor Refractory Diffuse Large B-Cell Lymphoma	Schuster	2019	2	Tisagenlecleucel (CTL-019)	FMC63-8-8-41BBz	NHL (DLBC)	111	56 (22–76)	5.8 x 10^6/kg (3.7-8.9)
Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia	Maude	2018	2	Tisagenlecleucel (CTL-019)	FMC63-8-8-41BBz	Pediatric ALL	75	11 (3-23)	3.1 x 10^6/kg (0.2-5.4)
Chimeric Antigen Receptor T Cells in Refractory B-Cell Lymphomas	Schuster	2017	1	Tisagenlecleucel (CTL-019)	FMC63-8-8-41BBz	NHL	28	59 (25-77)	1.79-5 × 10^6/kg
Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia	Porter	2015	1	Tisagenlecleucel (CTL-019)	FMC63-8-8-41BBz	CLL	14	66 (51-78)	1.6 x 10^8 (0.14-11)
Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1–2 trial	Locke	2019	1/2	Axicabtagene ciloleucel (KTE-C19)	FMC63-28-28-28z	NHL	108	58 (34-69)	2 x 10^6/kg
End of phase I results of ZUMA-3, a phase 1/2 study of KTE-X19, anti-CD19 chimeric antigen receptor (CAR) T cell therapy, in adult patients (pts) with relapsed/refractory (R/R) acute lymphoblastic leukemia (ALL)	Shah	2019	1/2	Axicabtagene ciloleucel (KTE-C19)	FMC63-28-28-28z	ALL	45	46 (18 – 77)	0-5-2 x 10^6/kg
T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial	Lee	2017	1	Axicabtagene ciloleucel (KTE-C19)	FMC63-28-28-28z	Pediatric ALL	21	13 (5-27)	0.03-3 x 10^6/kg
Lymphoma Remissions Caused by Anti-CD19 Chimeric Antigen Receptor T Cells Are Associated With High Serum Interleukin-15 Levels	Kochenderfer	2017	1	Axicabtagene ciloleucel (KTE-C19)	FMC63-28-28-28z	NHL	22	53 (26-67)	2 x 10^6/kg (1-6)
Pivotal Safety and Efficacy Results from Transcend NHL 001, a Multicenter Phase 1 Study of Lisocabtagene Maraleucel (liso-cel) in Relapsed/Refractory (R/R) Large B Cell	Abramson	2019	1	Lisocabtagene maraleucel (JCAR17)	FMC63-I_4-28-41BBz-EGFRt	NHL	268	63 (18-86)	5 x $10^{7 - 8}$
Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults	Gardner	2017	1	Lisocabtagene maraleucel (JCAR17)	FMC63-I_4-28-41BBz-EGFRt	Pediatric ALL	43	12.2 (1-25)	10^6/kg (0.05-10)
Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of CD8+ and CD4+ CD19-specific chimeric antigen receptor–modified T cells	Turtle	2016	1	Fred Hutchinson Cancer Center (JCAR14)	FMC63-I_4-28-41BBz-EGFRt	NHL	32	58 (36-70)	2 x 10^6 /kg(0.2-20)
CD19 CAR–T cells of defined CD4+:CD8+ composition in adult B cell ALL patients	Turtle	2016	1	Fred Hutchinson Cancer Center (JCAR14)	FMC63-I_4-28-41BBz-EGFRt	ALL	30	40 (20-73)	2 x 10^6 /kg(0.2-20)
Durable molecular remissions in chronic lymphocytic leukemia treated with CD19-specific chimeric antigen receptor-modified T cells after failure of ibrutinib	Turtle	2017	1	Fred Hutchinson Cancer Center (JCAR14)	FMC63-I_4-28-41BBz-EGFRt	CLL	24	61 (40-73)	2 x 10^6 /kg(0.2-20)

Ferreira & Muller Front Immunol 2021

CAR T cell targets being pursued

Table 1. CAR-T-cell targets for the treatment of hematological tumors

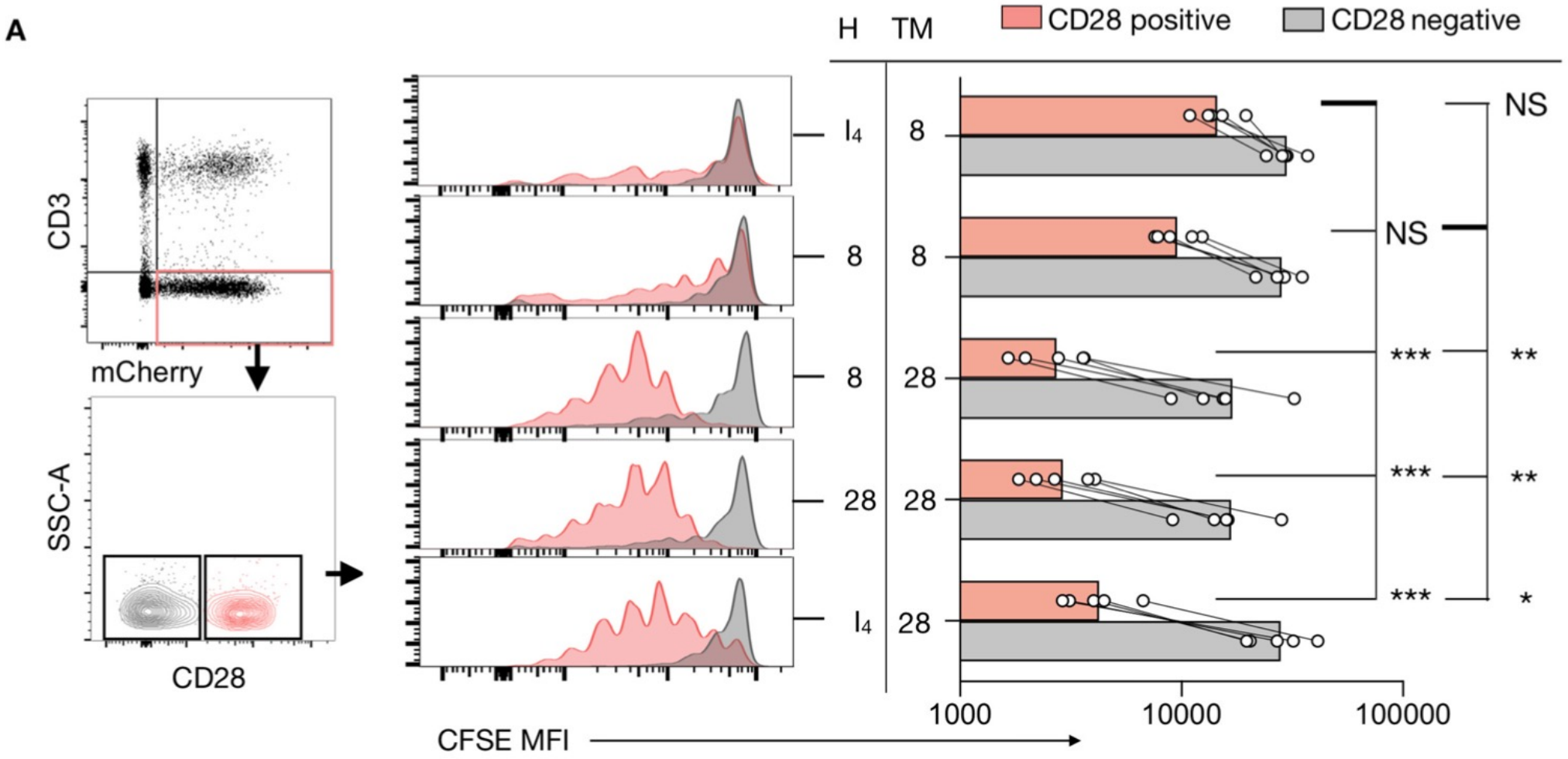
Target	CAR structure	Malignancy
BCMA	CD3ζ and 41BB	MM
CD19	CD3ζ and CD28; CD3ζ and 41BB KIR2DS2 and DAP12-	Lymphoma; Leukemia
CD22	CD3ζ and CD28	FL; NHL; DLBCL; ALL
CD20	CD3ζ; CD3ζ and 41BB-	CD20positive malignancies
CD138	CD3ζ and 41BB	MM
CD33	CD3ζ and 41BB	AML
CD123	CD3ζ and CD28	AML
CD19 CD20	CD3ζ and 41BB	Leukemia; Lymphoma
CD19 PSMA	CD3ζ and CD28 PD-1 or CTLA4	Leukemias
FITC-CD19 Ab	CD3ζ and CD28	CD19 positive cancers
Igκ	CD3ζ and CD28	CLL
LeY	CD3ζ and CD28	AML
ROR1	CD3ζ and 41BB	CLL; SLL

Table 2. CAR-T-cell targets for the treatment of solid tumors

Target	CAR structure	Malignancy
Biotin	CD3ζ, CD28 and 41BB	EGFRvIII positive cancer
CD171	CD3ζ and 4-1BB; CD3ζ, CD28 and 4-1BB	Neuroblastoma
EGFRvIII	CD3ζ and 41BB CD3ζ and ICOS-	Glioma
FAP	CD3ζ and CD28 KIR2DS2 and DAP12-	Mesothelioma; Lung cancer
FR	CD3ζ and CD27	Ovarian cancer; Breast cancer
Glypican-3	CD3ζ, CD28 and 41BB	Hepatocellular carcinoma
HER2	CD3ζ and CD28	HER2 positive cancer; Sarcoma
HER2 MUC1	CD3ζ and CD28	Breast cancer
HER2 IL13Rα2	CD3ζ and CD28	Glioblastoma
IL13Rα2	CD3ζ; CD3ζ and 41BB CD3ζ and CD28 CD3ζ, CD28 and 41BB CD3ζ, CD28 and OX40-	Glioma
Mesothelin	CD3ζ; CD3ζ and CD28 CD3ζand 41BB CD3ζ and ICOS KIR2DS2 and DAP12-	Mesothelioma; Pancreatic cancer; Non-small cell lung cancer
Mesothelin CD19	CD3ζand 41BB	Pancreatic cancer
MUC1	CD3ζ and 41BB	MUC1 positive solid tumor
NKG2D	CD3ζ; CD3ζ and DAP10 CD3ζ and 41BB CD3ζ and CD28	Ovarian cancer Ewing sarcoma
PSMA	CD3ζ and CD28	Prostate cancer
PD1 and CD19; PD1 and Mesothelin;	CD3ζ and CD28 CD3ζ, CD28 and 41BB	PD-L1 positive cells

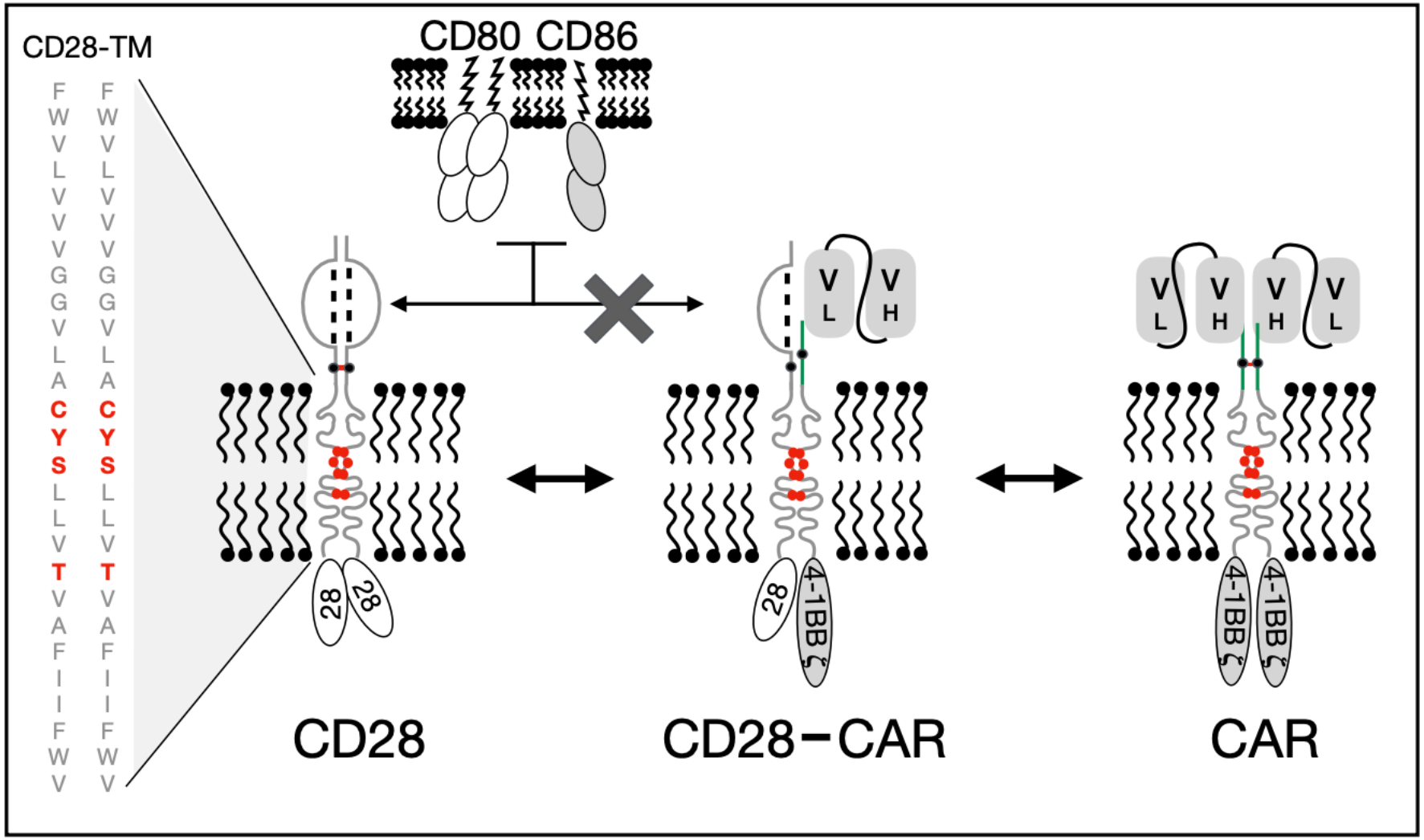
CD28 TM domain CARs interact with CD28

Anti-CD28 activation



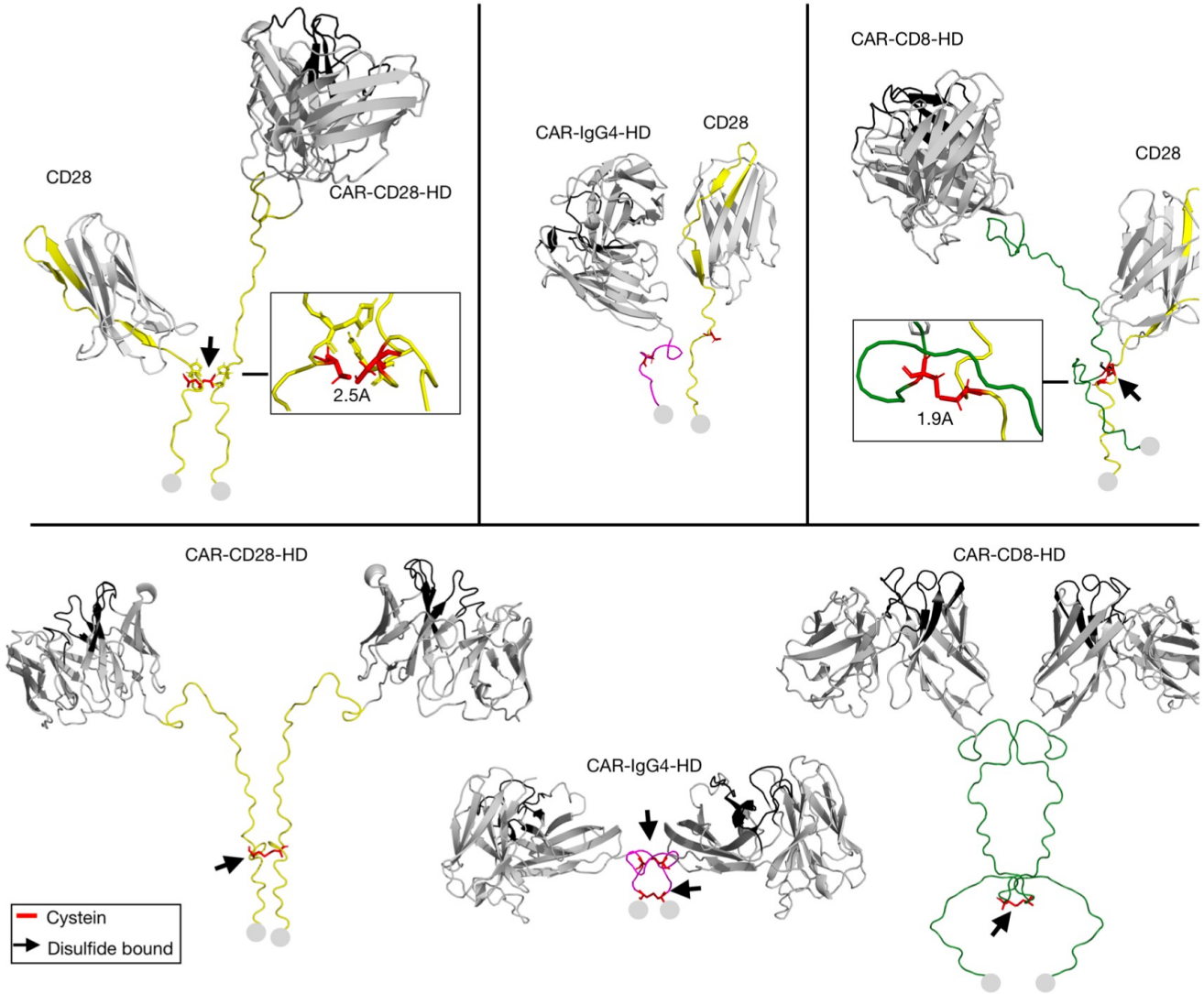
Muller et al Front Immunol 2021

CAR transmembrane domains matter



Muller et al bioRxiv 2020

CAR hinge domains matter



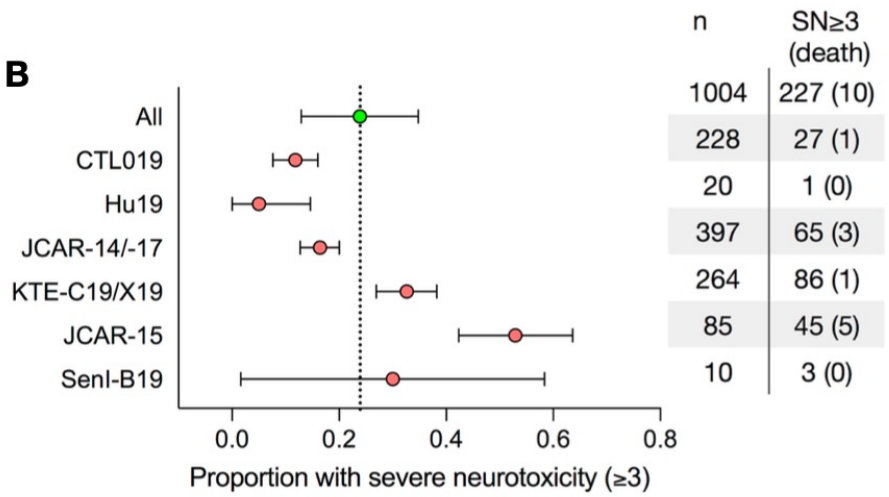
Muller et al Front Immunol 2021

CAR transmembrane and hinge domains are associated with CAR T cell toxicity

A

	scFV	HD	TMD	ICD	Reporter	CD28-CAR H_eD_i
CTL019	FMC63	8	8	41bbζ		NO
Hu19	Hu19	8	8	28ζ		NO
JCAR-14/-17	FMC63	I_4	28	41bbζ	EGFRt	YES inefficient
KTE-C19/X19	FMC63	28	28	28ζ		YES
JCAR-15	SJ25C1	28	28	28ζ	EGFRt	YES
SenlB19	SJ25C1	28	28	41bbζ	EGFRt	YES

B



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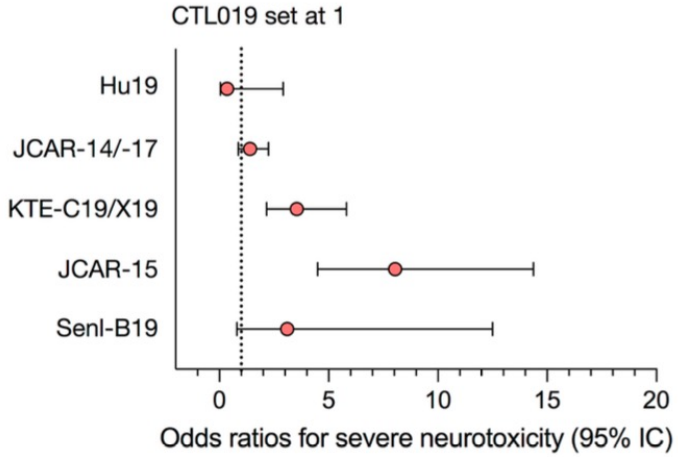

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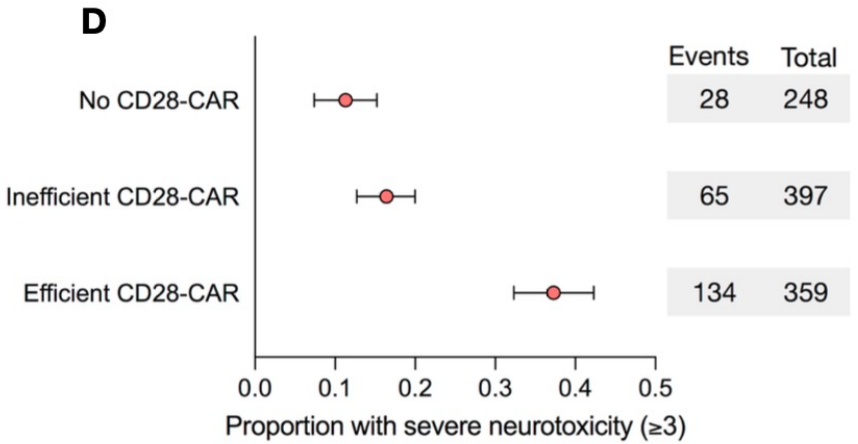
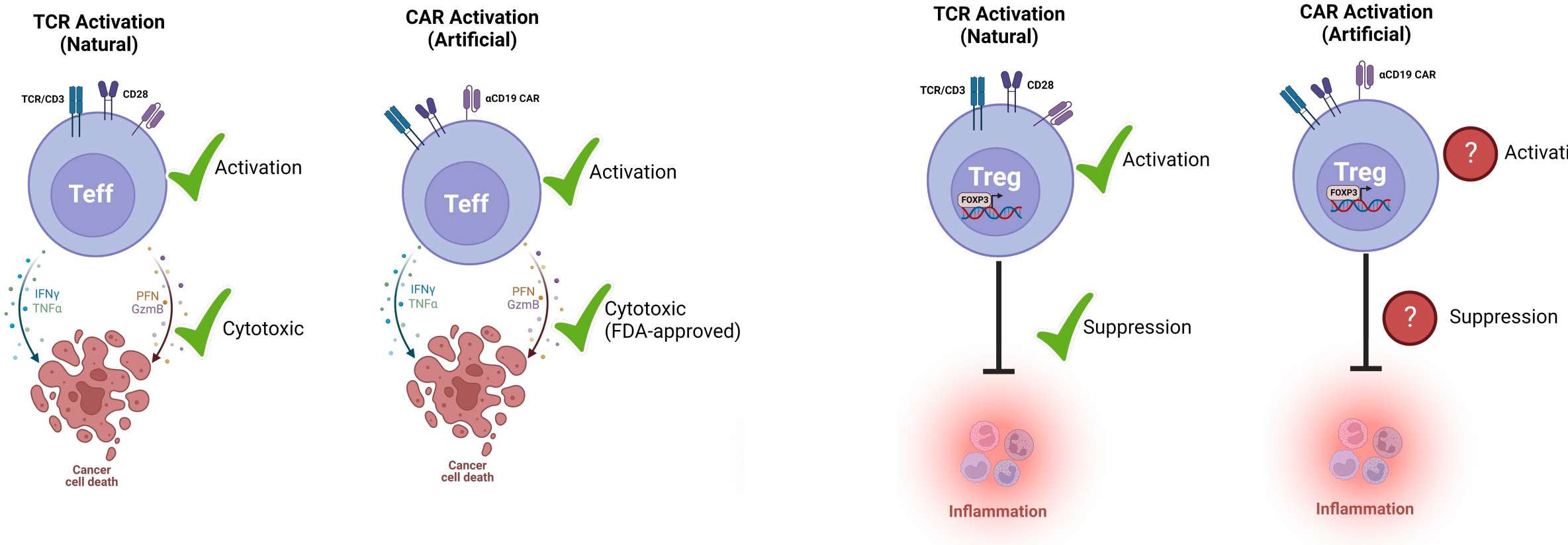
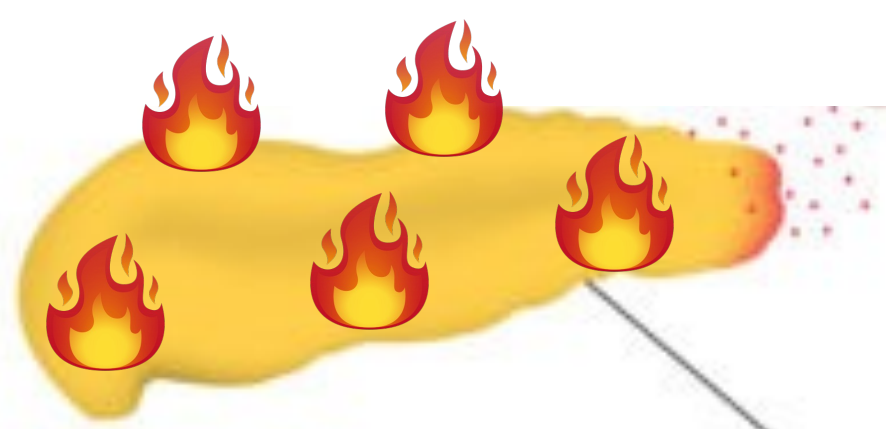


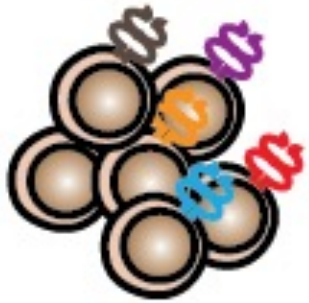
Ferreira & Muller Front Immunol 2021

How well do CARs work in Tregs?



Type 1 diabetes



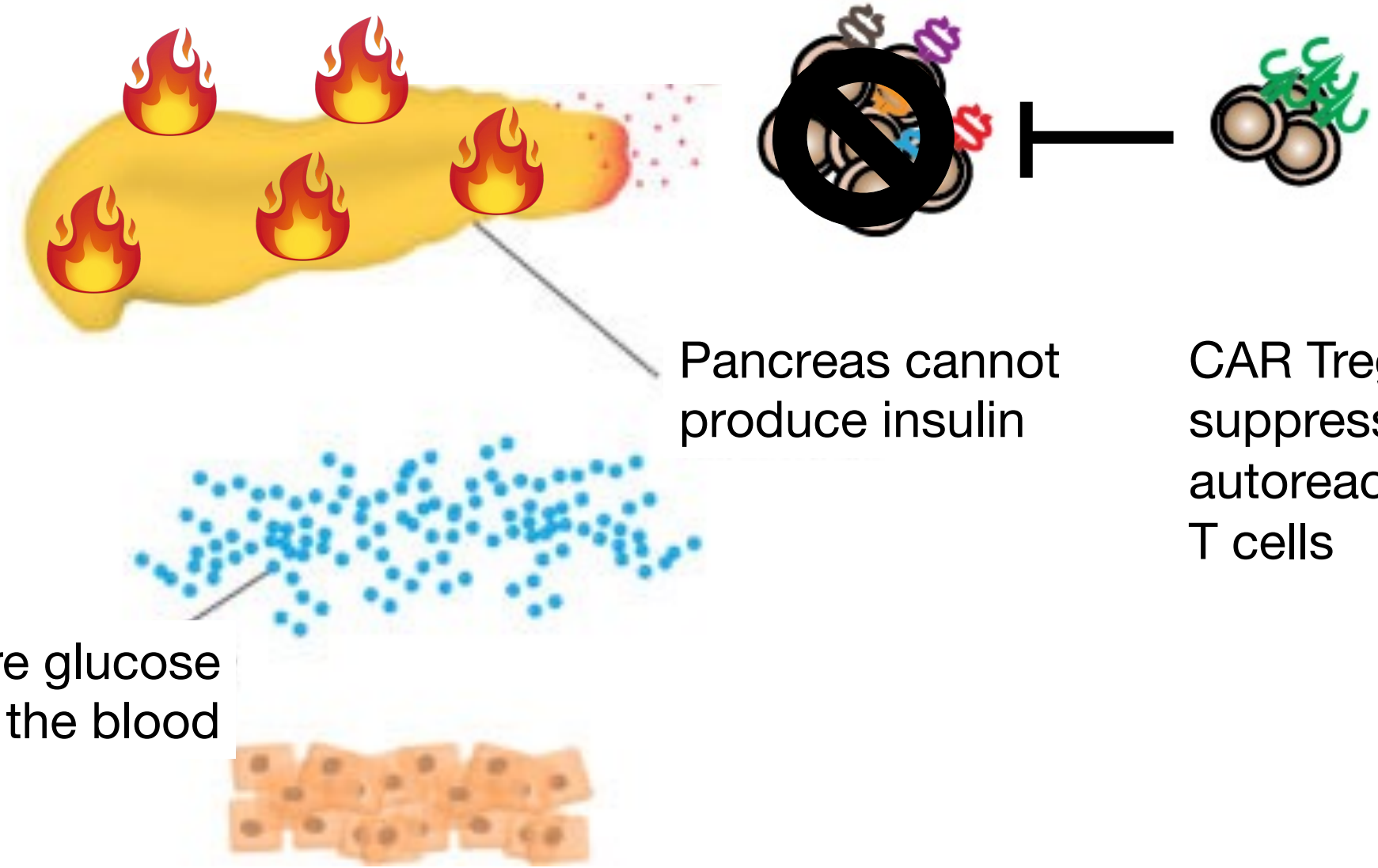


Autoreactive T cells destroy the pancreas

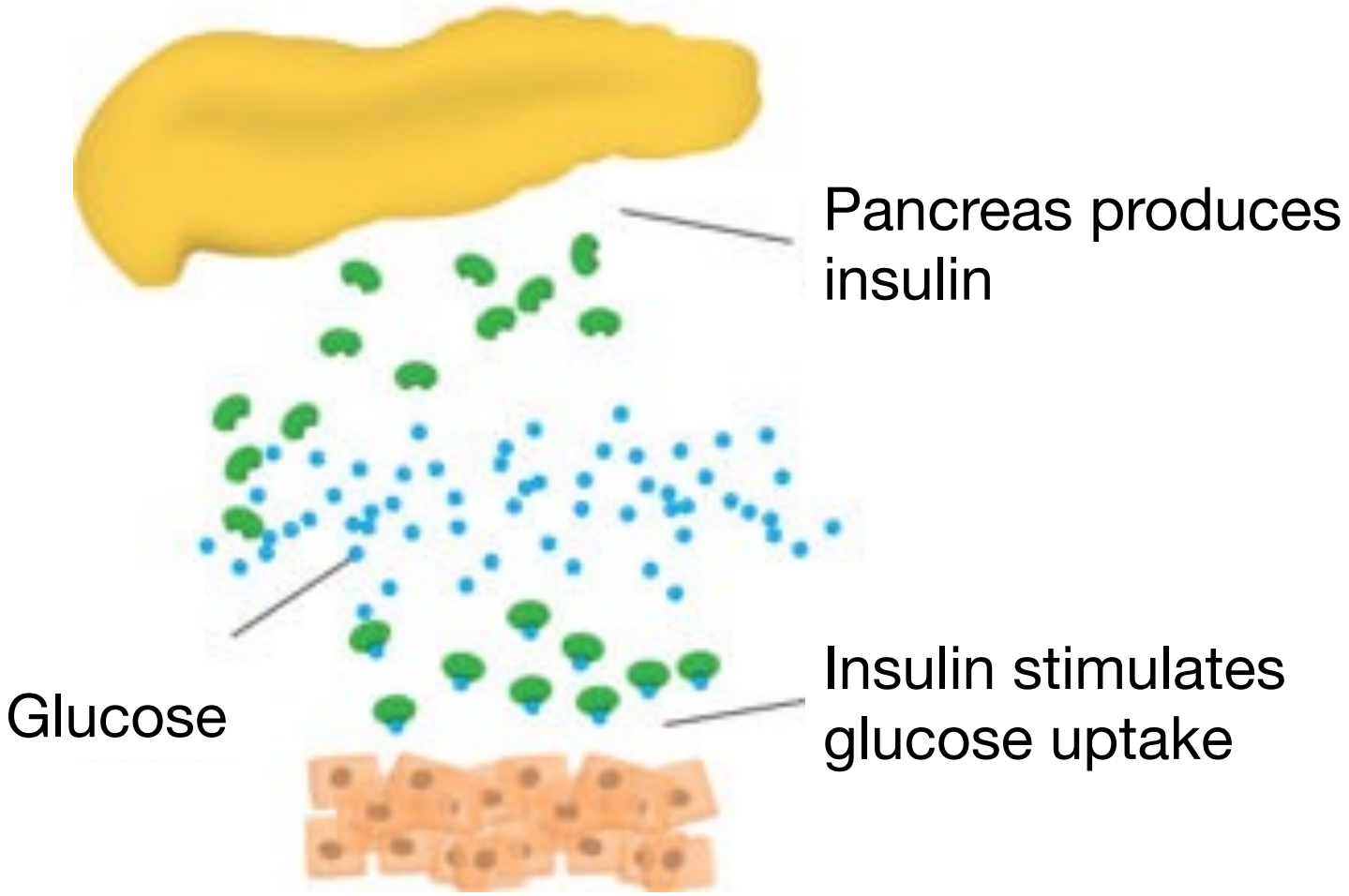
Pancreas cannot produce insulin

More glucose in the blood

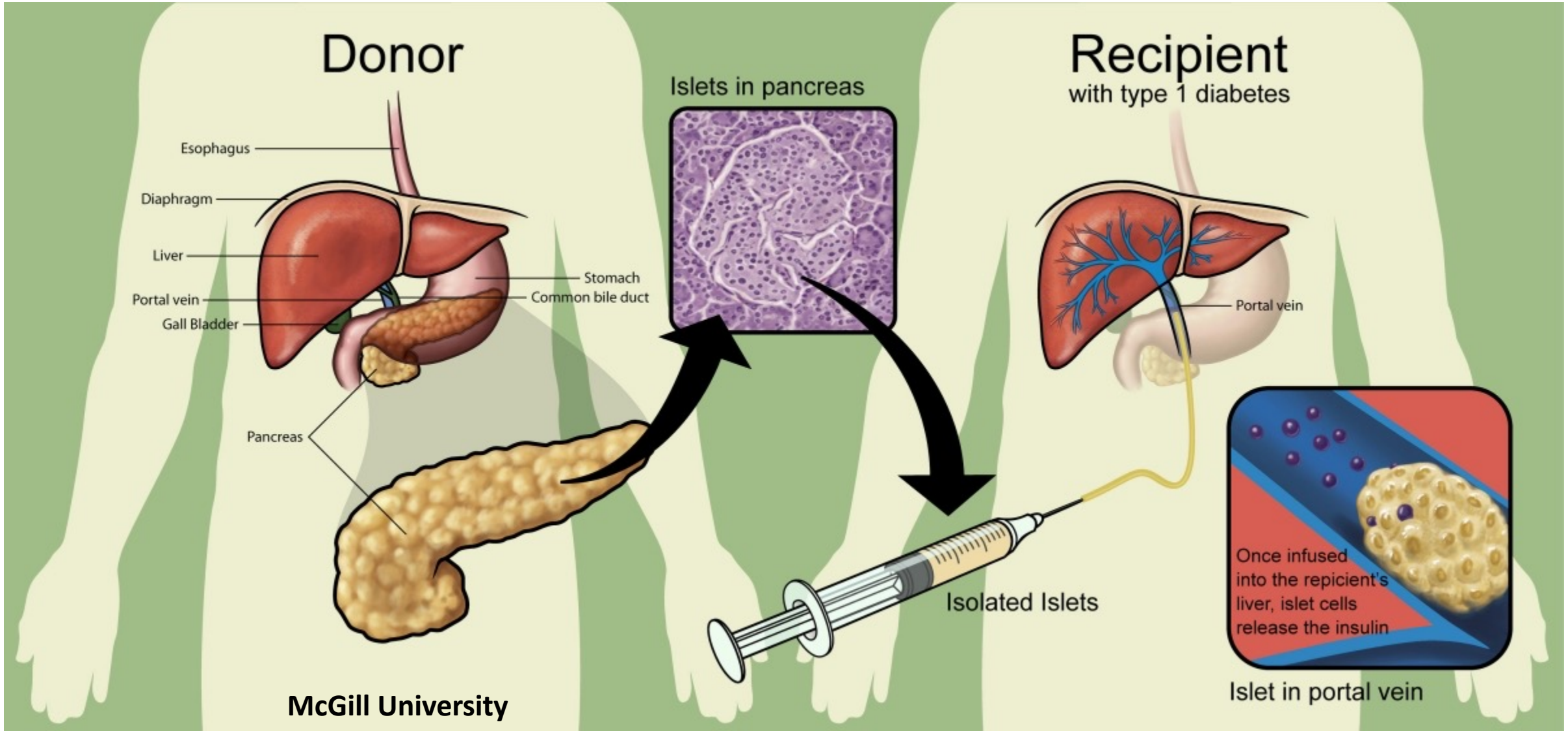
Treating type 1 diabetes using CAR Tregs



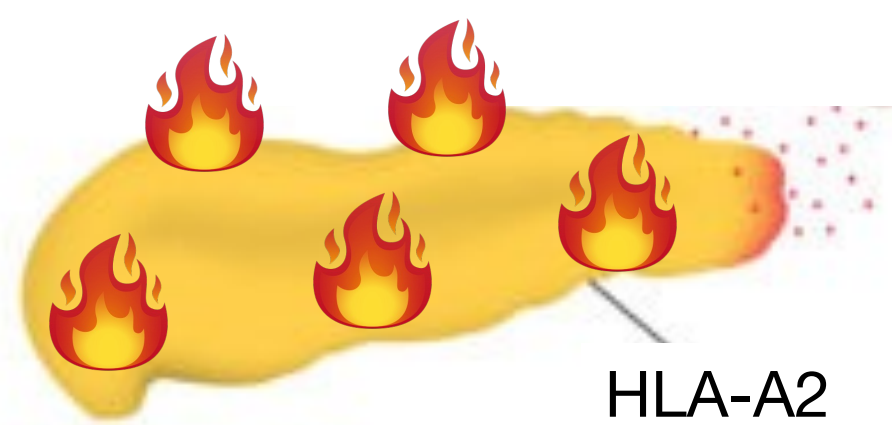
Treating type 1 diabetes using CAR Tregs



Islet transplant as a treatment for T1D



Anti-HLA-A2 CAR Tregs for HLA-A2^{+} islets transplants to treat T1D



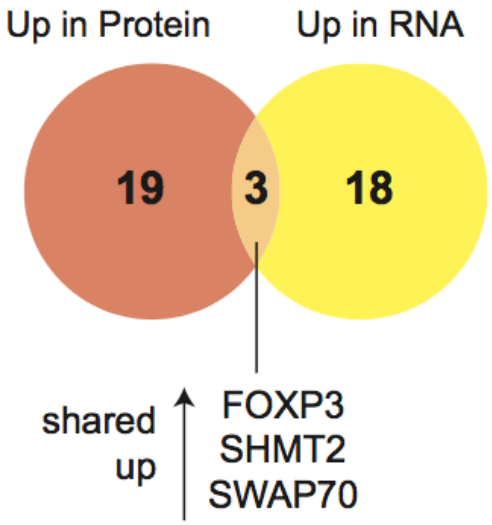
HLA-A2

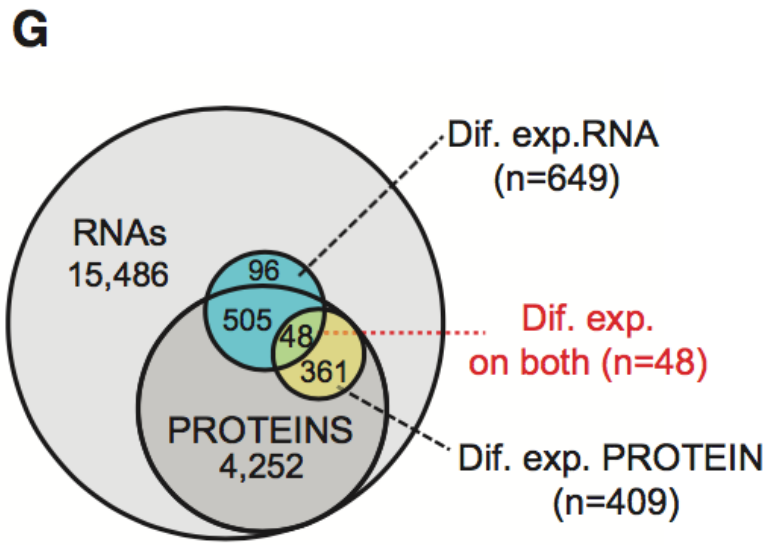
Alloreactive & autoreactive T cells destroy the islets

A2-CAR Tregs suppress aggressive T cells

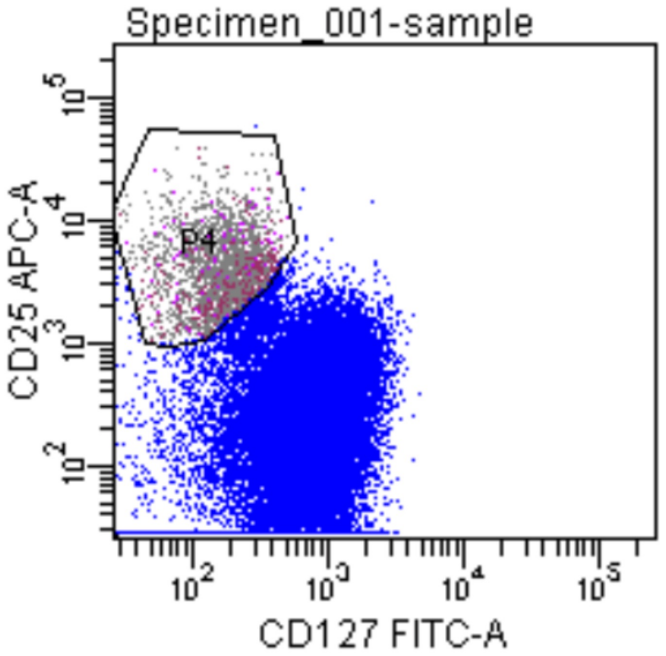
Human Treg purification from peripheral blood

Treg signature





Treg sorting

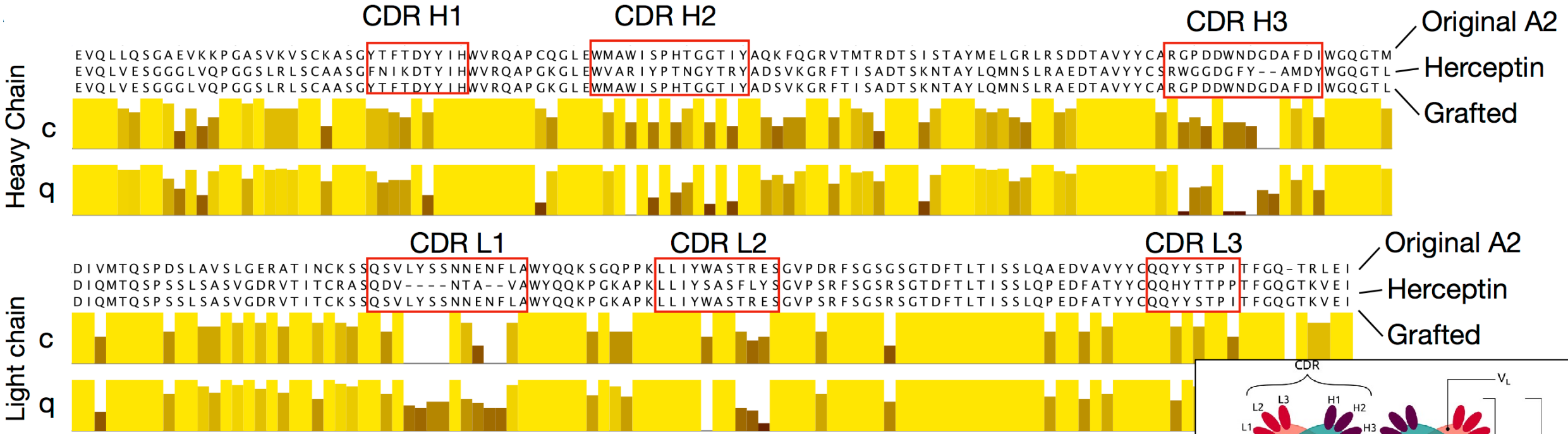


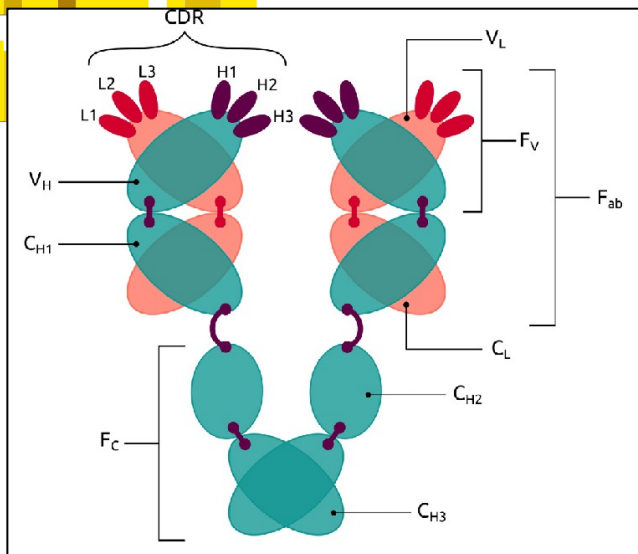
FOXP3 – forkhead box protein 3 (Treg development, suppresses IL-2 expression)
SHMT2 – serine hydroxymethylase 2 (respiration, controls formylmethyonine tRNA production, 1C metabolism in T cells)
SWAP70 – switch associated protein 70 (binds PIP3, mediates membrane ruffling signaling, associates with cytoskeleton)

Cuadrado et al Immunity 2018

Sorting based on Liu et al J Exp Med 2006

Grafting A2-CAR scFv specificity





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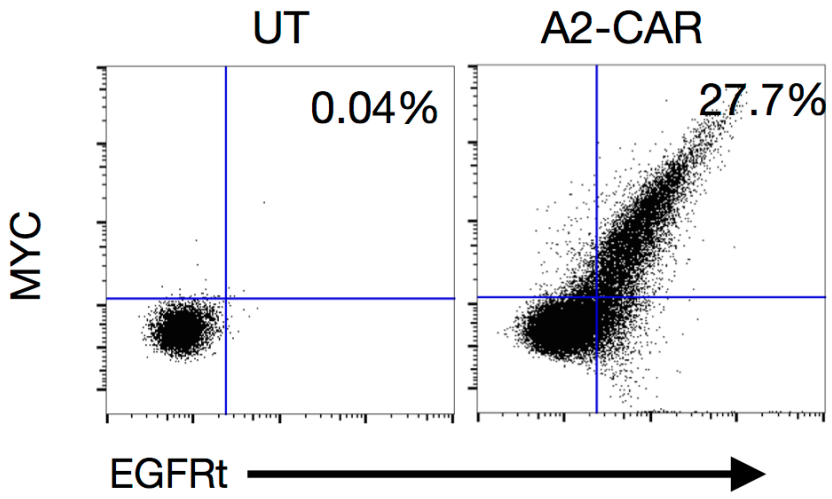
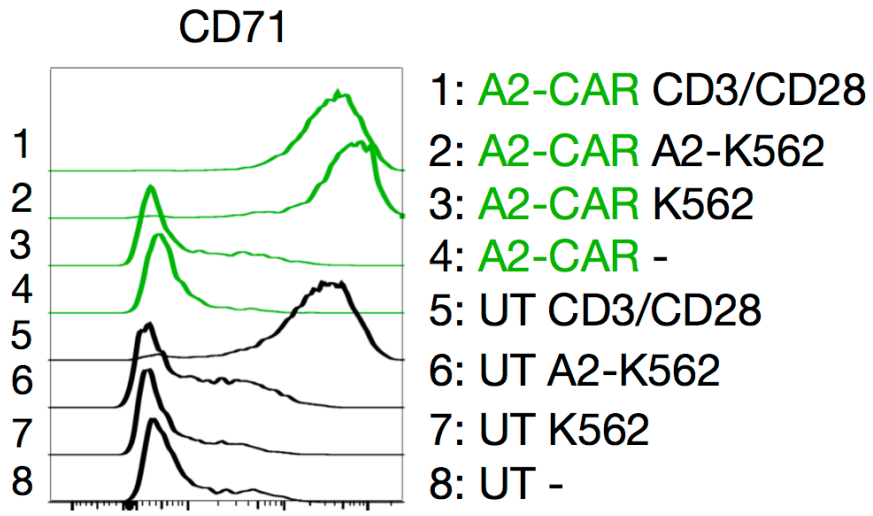
A2-CAR Tregs recognize HLA-A2

CAR expression



Treg activation



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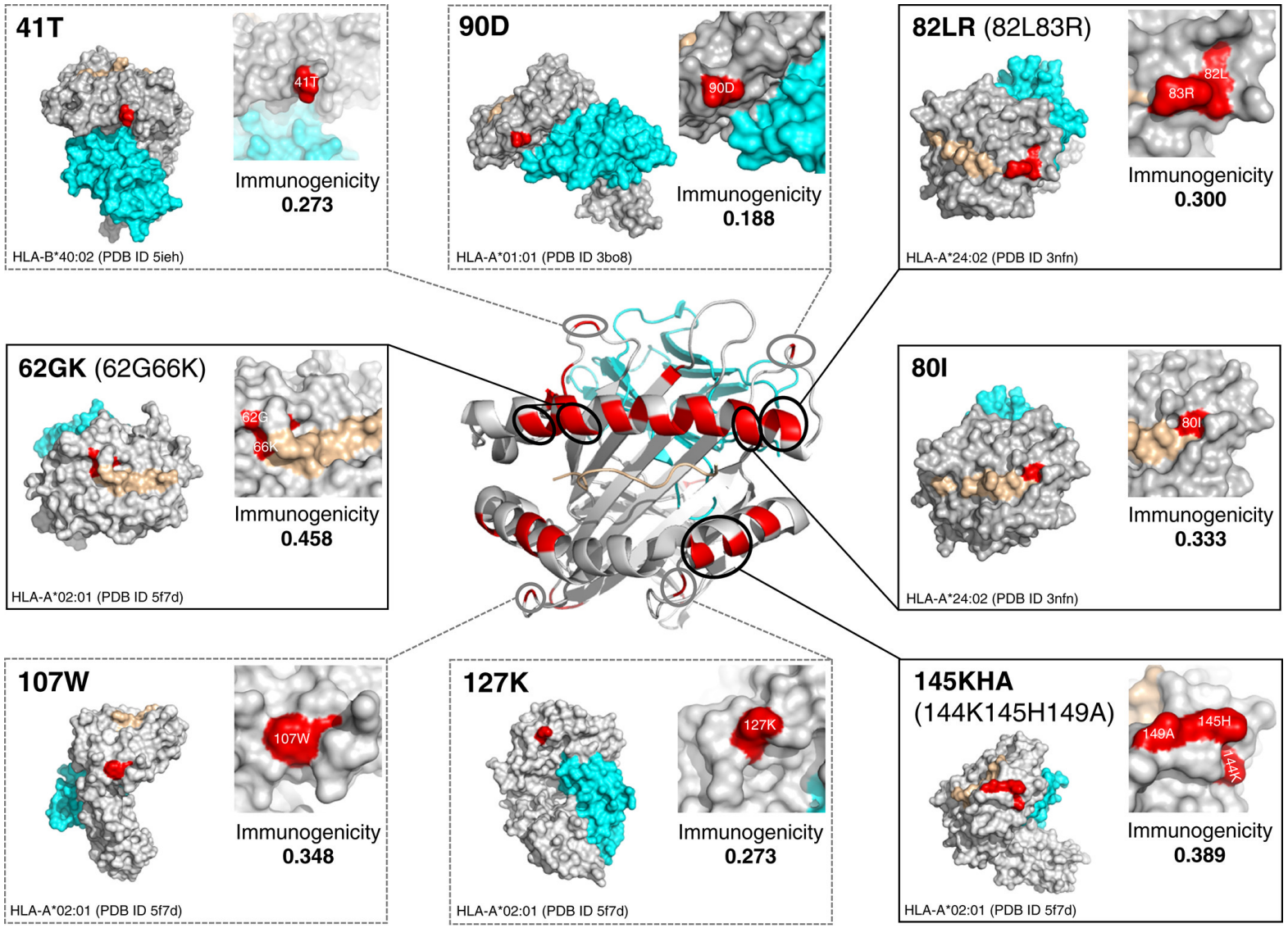
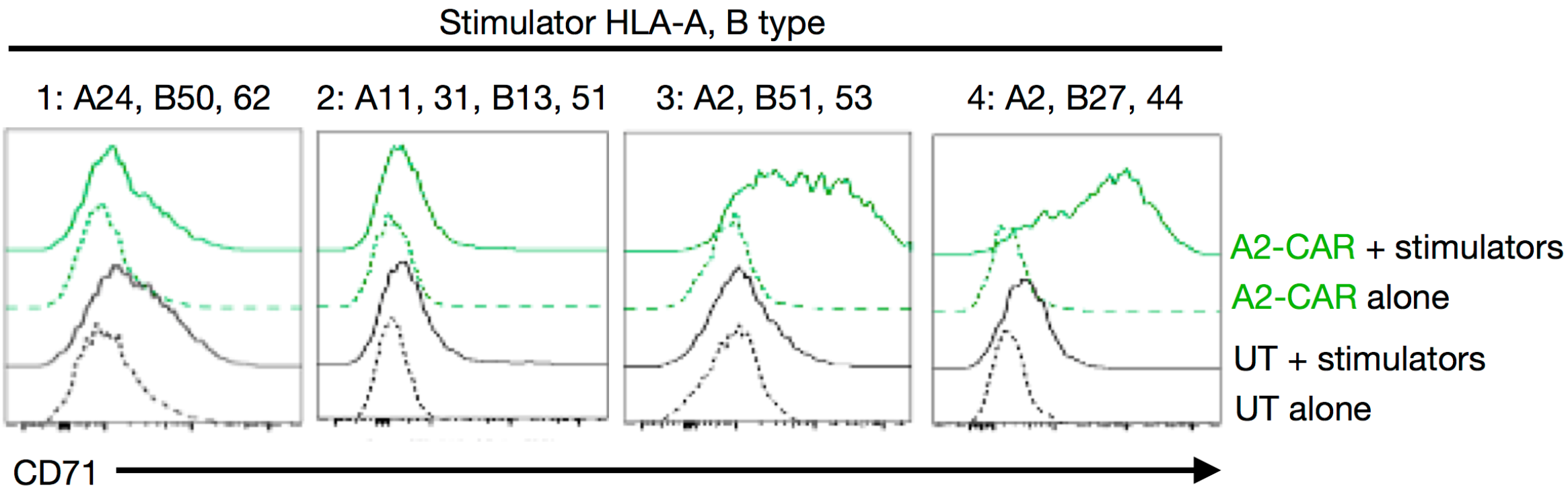
Immunogenic eplets in HLA-A2



Hönger et al HLA 2020

A2-CAR Tregs recognize the 144TKH eplet, not 44RME, 105S or 127K



44RME: A11, A24, A31
105S: A24, A31
127K: A24

A2-CAR Tregs co-incubated with allogeneic human islets

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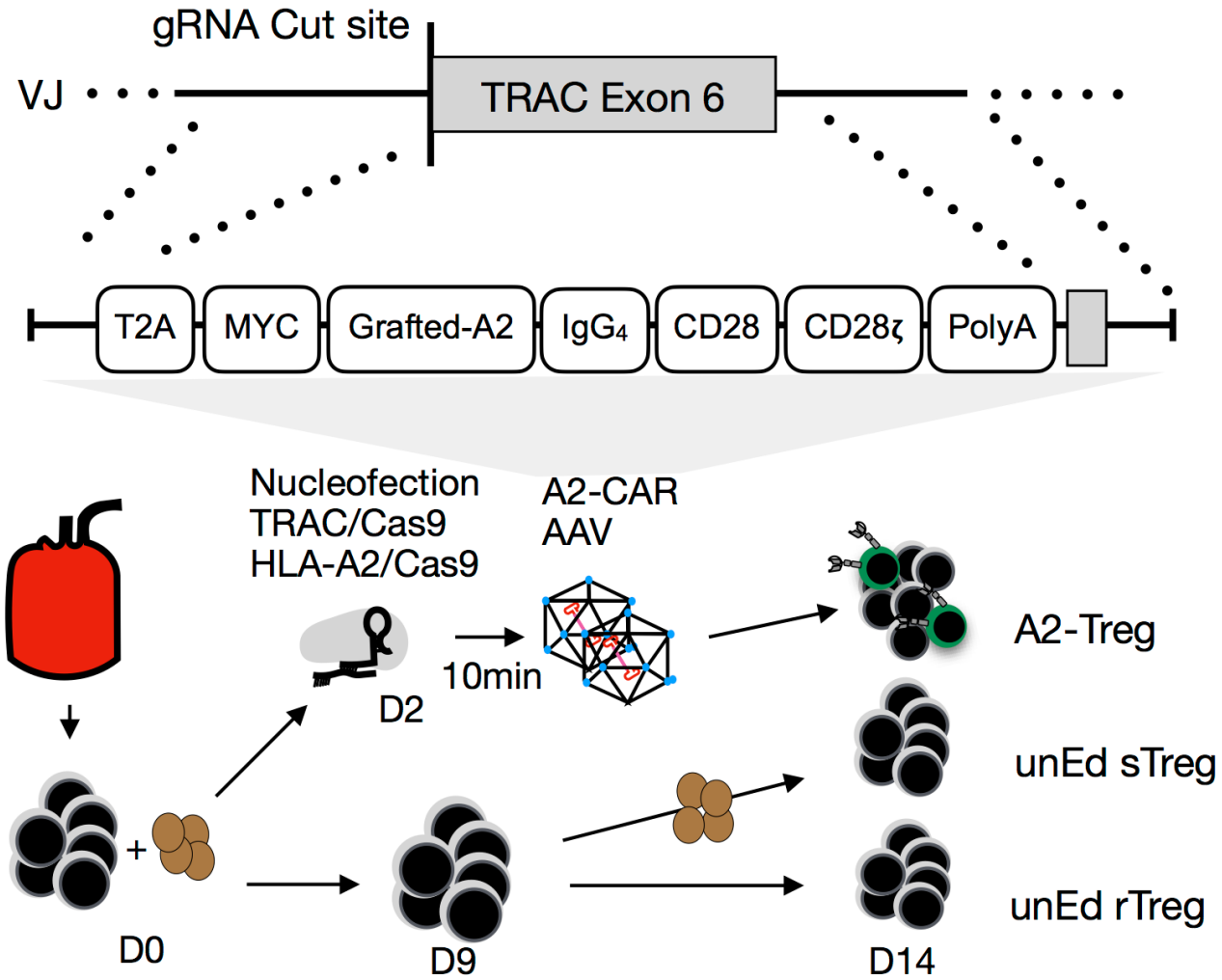
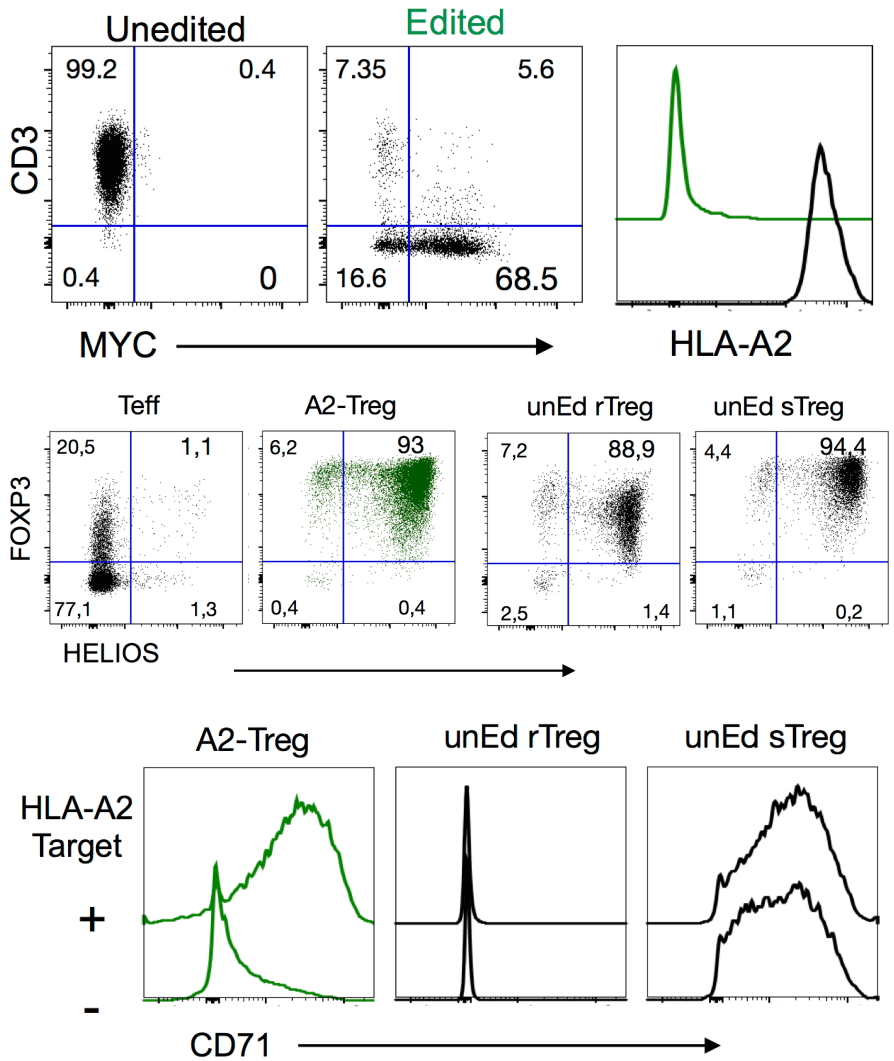
Reprogramming Treg specificity





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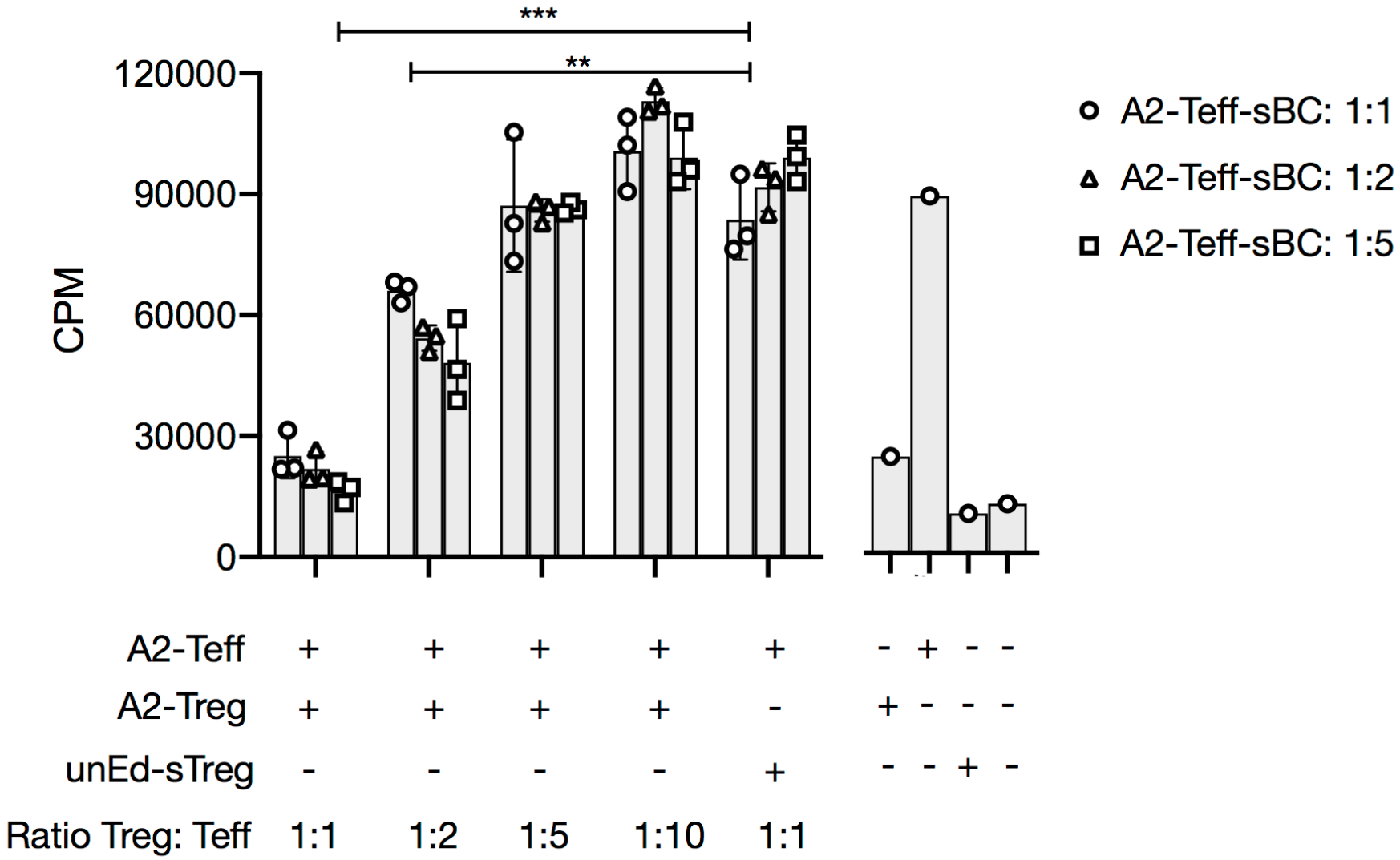
A2-CAR Tregs suppress Teff cell proliferation



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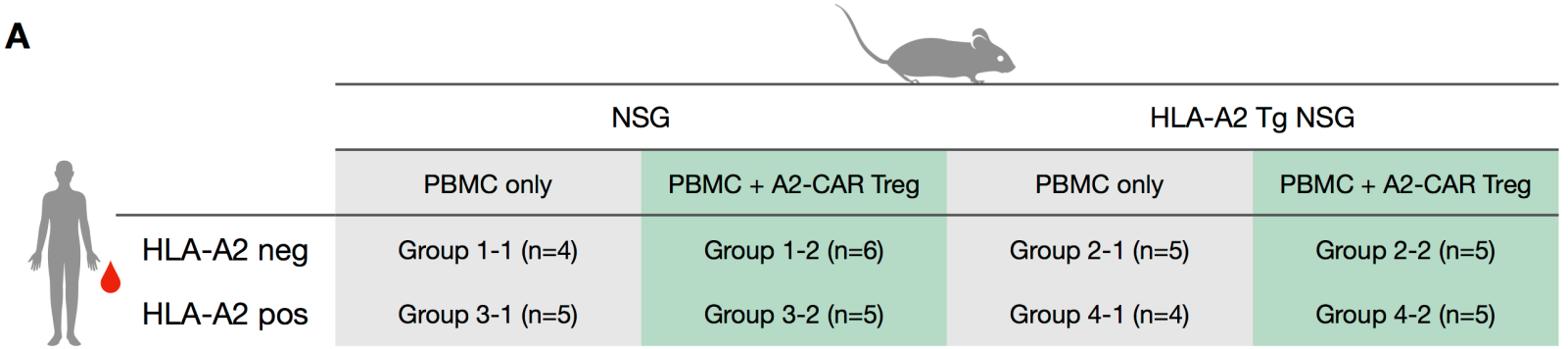
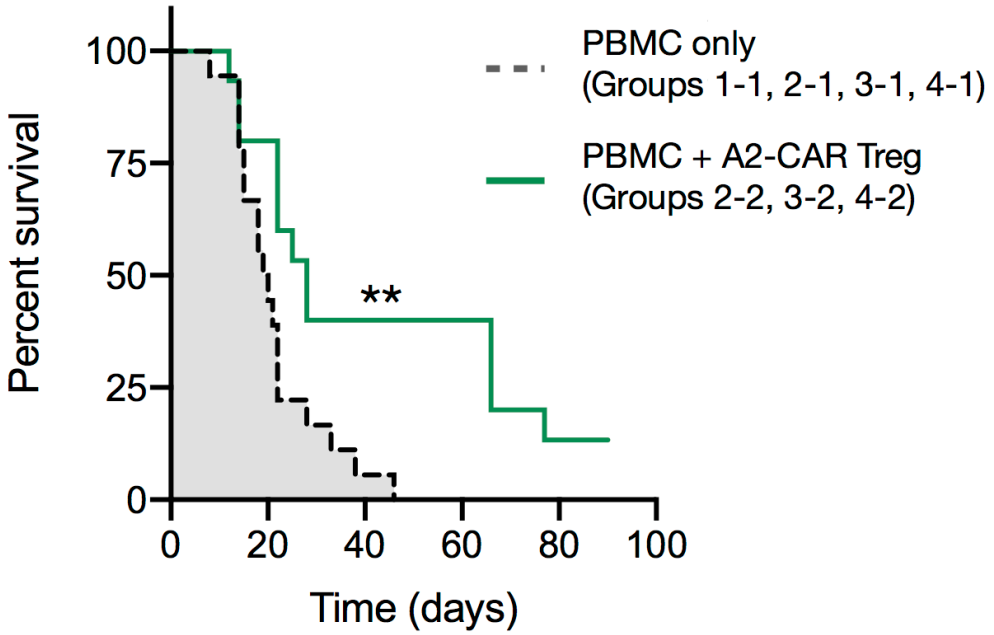
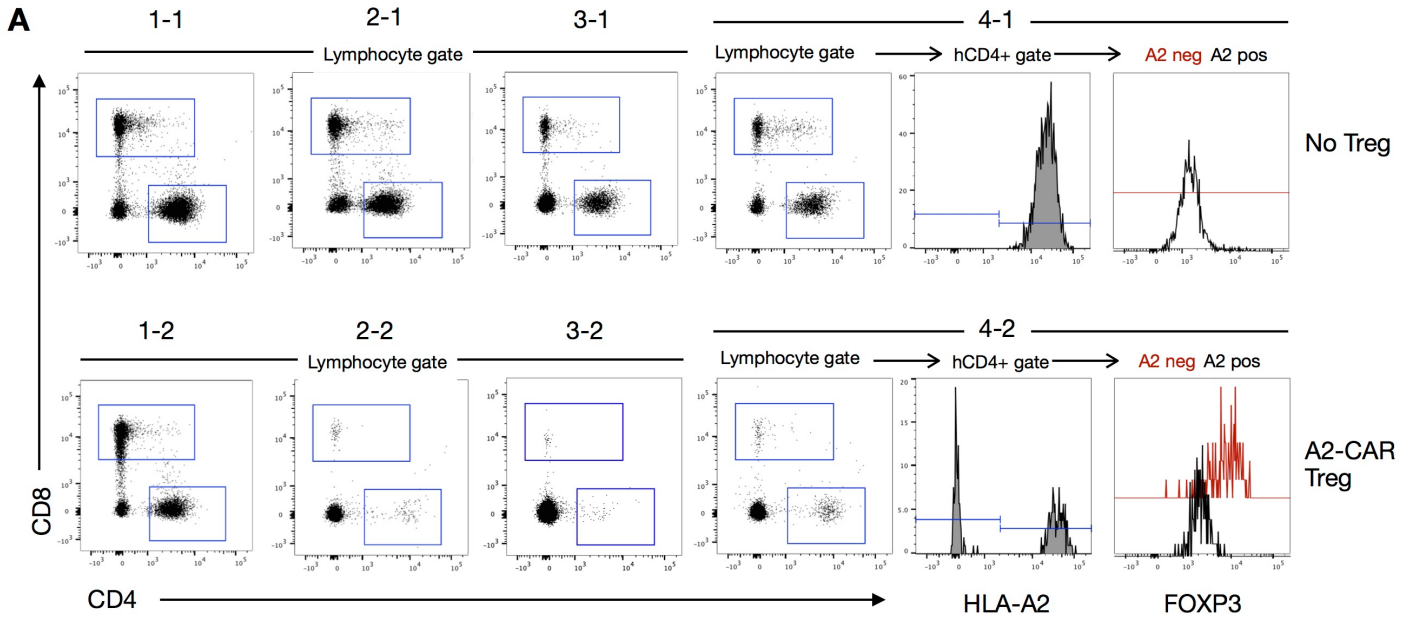
A2-CAR Tregs prevent graft-vs-host disease

A



	NSG		HLA-A2 Tg NSG	
	PBMC only	PBMC + A2-CAR Treg	PBMC only	PBMC + A2-CAR Treg
HLA-A2 neg	Group 1-1 (n=4)	Group 1-2 (n=6)	Group 2-1 (n=5)	Group 2-2 (n=5)
HLA-A2 pos	Group 3-1 (n=5)	Group 3-2 (n=5)	Group 4-1 (n=4)	Group 4-2 (n=5)





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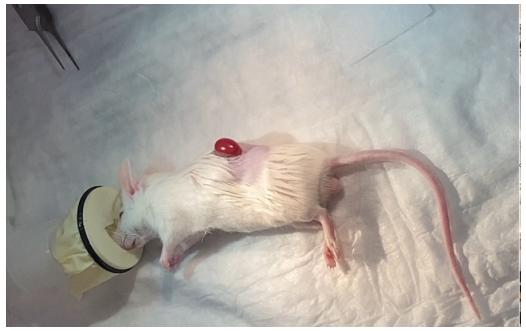
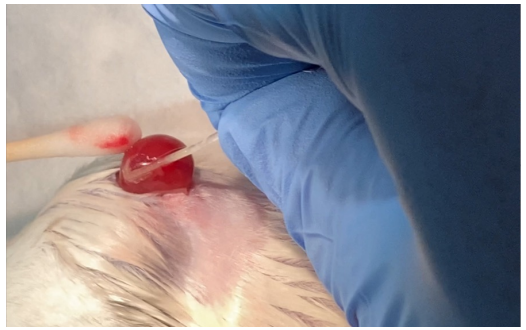
A2-CAR Tregs traffic to A2⁺ human islet grafts

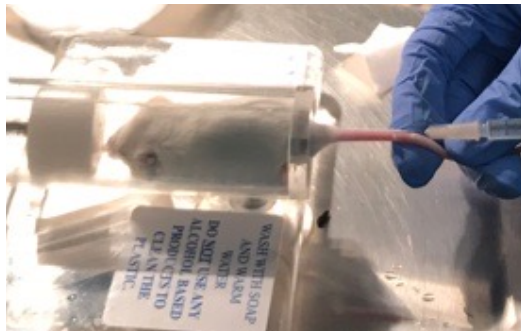
Kidney capsule exposure

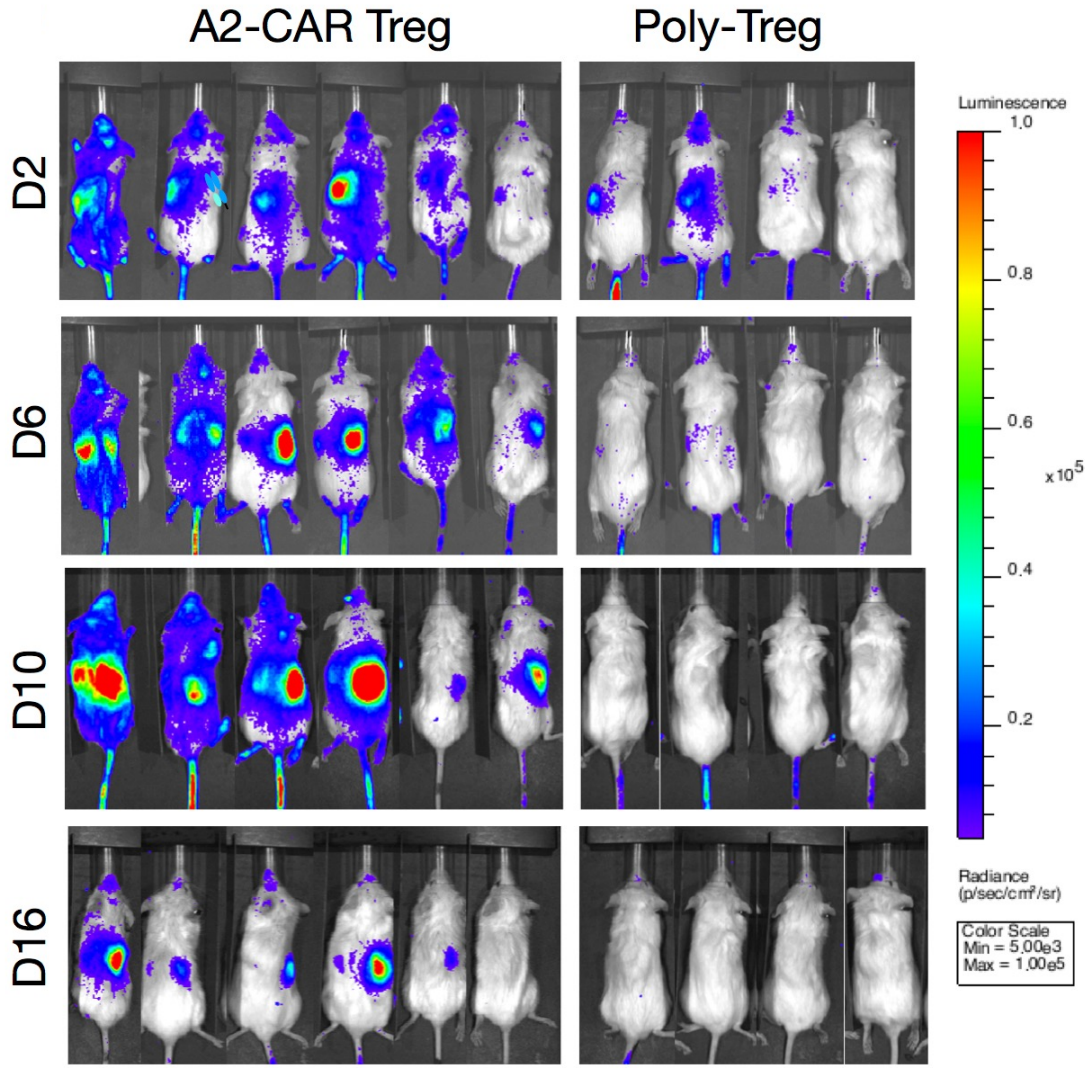


Human A2⁺ islet transplant



Luciferase⁺ A2-CAR Treg i.v. injection





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ARTICLES
https://doi.org/10.1038/s41591-022-02017-5
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Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus

Andreas Mackensen[1,2,8], Fabian Müller[1,2,8], Dimitrios Mougiakakos[1,2,3,8], Sebastian Böltz[2,4], Artur Wilhelm[2,4], Michael Aigner[1,2], Simon Völkl[1,2], David Simon[2,4], Arnd Kleyer[2,4], Luis Munoz[2,4], Sascha Kretschmann[1,2], Soraya Kharboutli[1,2], Regina Gary[1,2], Hannah Reimann[1,2], Wolf Rösler[1,2], Stefan Uderhardt[2,4], Holger Bang[5], Martin Herrmann[2,4], Arif Bülent Ekici[6], Christian Buettner[6], Katharina Marie Habenicht[7], Thomas H. Winkler[7], Gerhard Krönke[2,4,8] and Georg Schett[2,4,8] ✉

Systemic lupus erythematosus (SLE) is a life-threatening autoimmune disease characterized by ada activation, formation of double-stranded DNA autoantibodies and organ inflammation. Five patients v and one man) with a median (range) age of 22 (6) years, median (range) disease duration of 4 (8) yea (median (range) SLE disease activity index Systemic Lupus Erythematosus Disease Activity Index: 16 (8) immunosuppressive drug treatments were enrolled in a compassionate-use chimeric antigen receptor (Autologous T cells from patients with SLE were transduced with a lentiviral anti-CD19 CAR vector, expan dose of 1×10^6 CAR T cells per kg body weight into the patients after lymphodepletion with fludarabine a CAR T cells expanded in vivo, led to deep depletion of B cells, improvement of clinical symptoms and no tory parameters including seroconversion of anti-double-stranded DNA antibodies. Remission of SLE ac teria was achieved in all five patients after 3 months and the median (range) Systemic Lupus Erythemat Index score after 3 months was 0 (2). Drug-free remission was maintained during longer follow-up (me months after CAR T cell administration) and even after the reappearance of B cells, which was observed of 110 ± 32 d after CAR T cell treatment. Reappearing B cells were naïve and showed non-class-switched T cell treatment was well tolerated with only mild cytokine-release syndrome. These data suggest that CD is feasible, tolerable and highly effective in SLE.

"Cabaletta has established an exclusive translational research partnership with Dr. Georg Schett, a pioneer and global leader in the application of CD19-targeting cell therapies in autoimmunity."

Cabaletta Bio Announces CABA-201, a Newly Designed CD19-Targeting CAR T Cell Therapy Engineered to Address a Broad Range of Autoimmune Diseases

October 11, 2022 7:00am EDT

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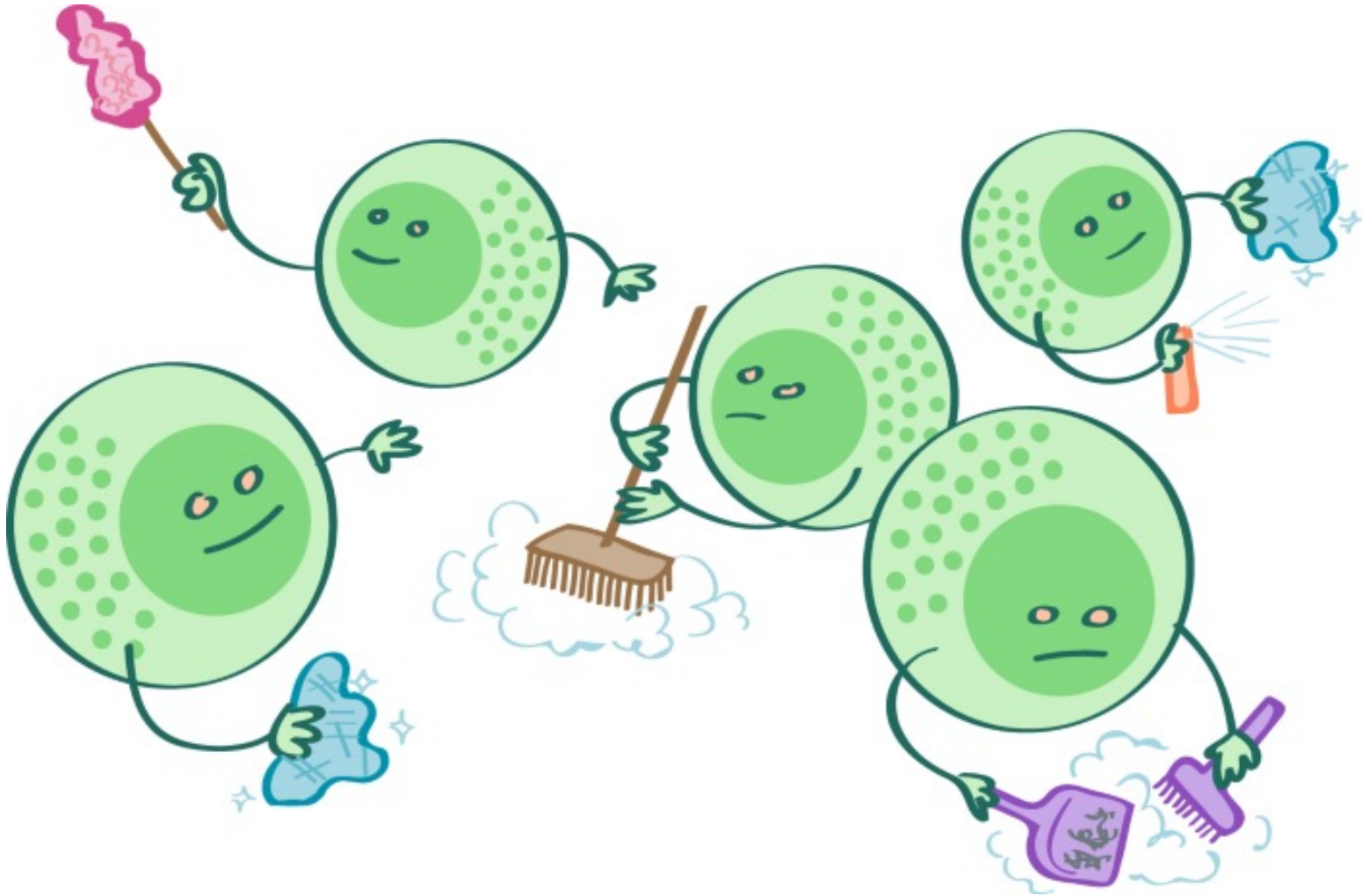
CAR T cells as programmable living nanobots to keep every disease at bay



Lara Crow/Springer Nature Limited