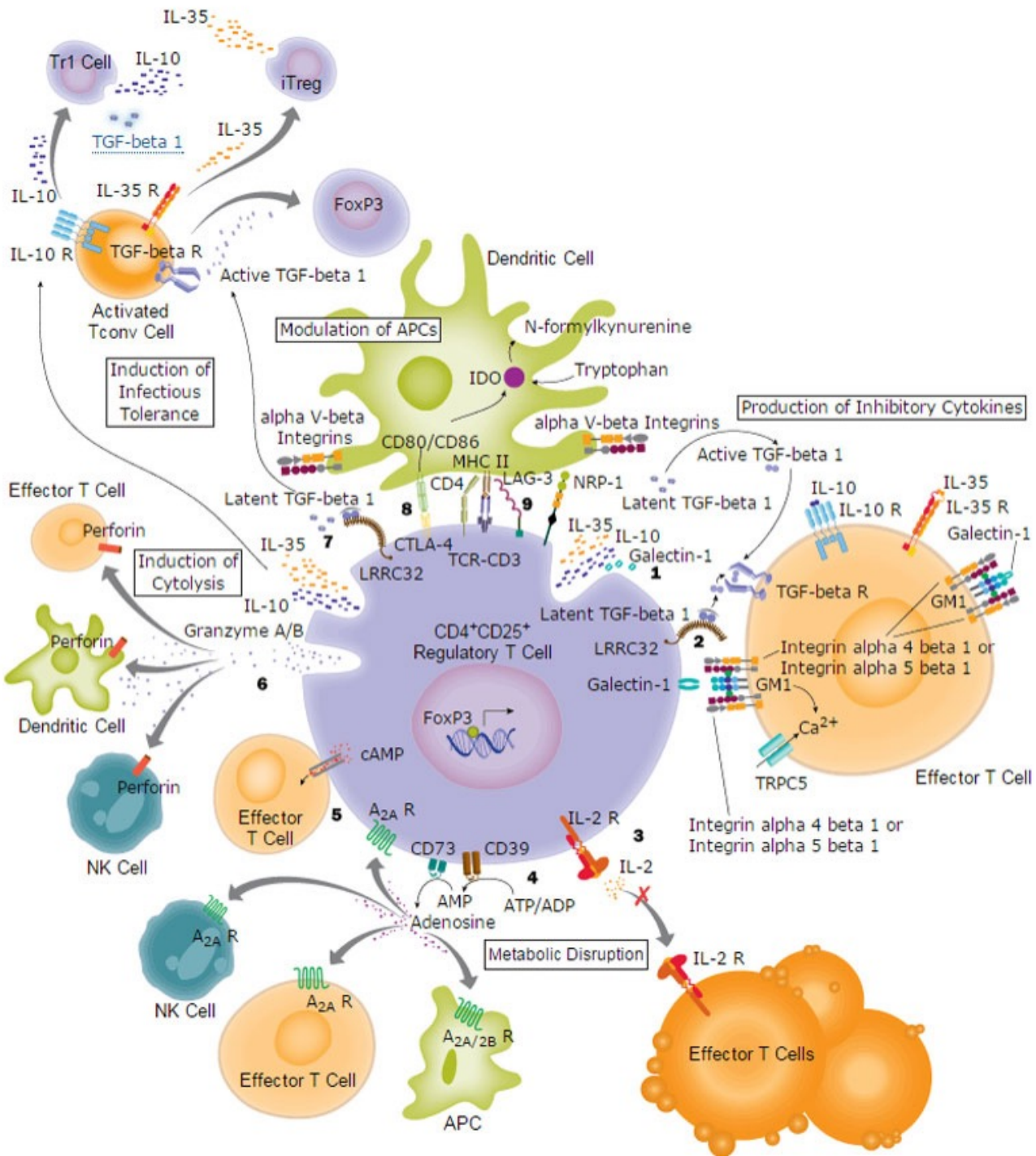


Treg therapy

MBIM735: Inflammation and Immunity

Leonardo M.R. Ferreira

Microbiology and Immunology

Regenerative Medicine and Cell Biology

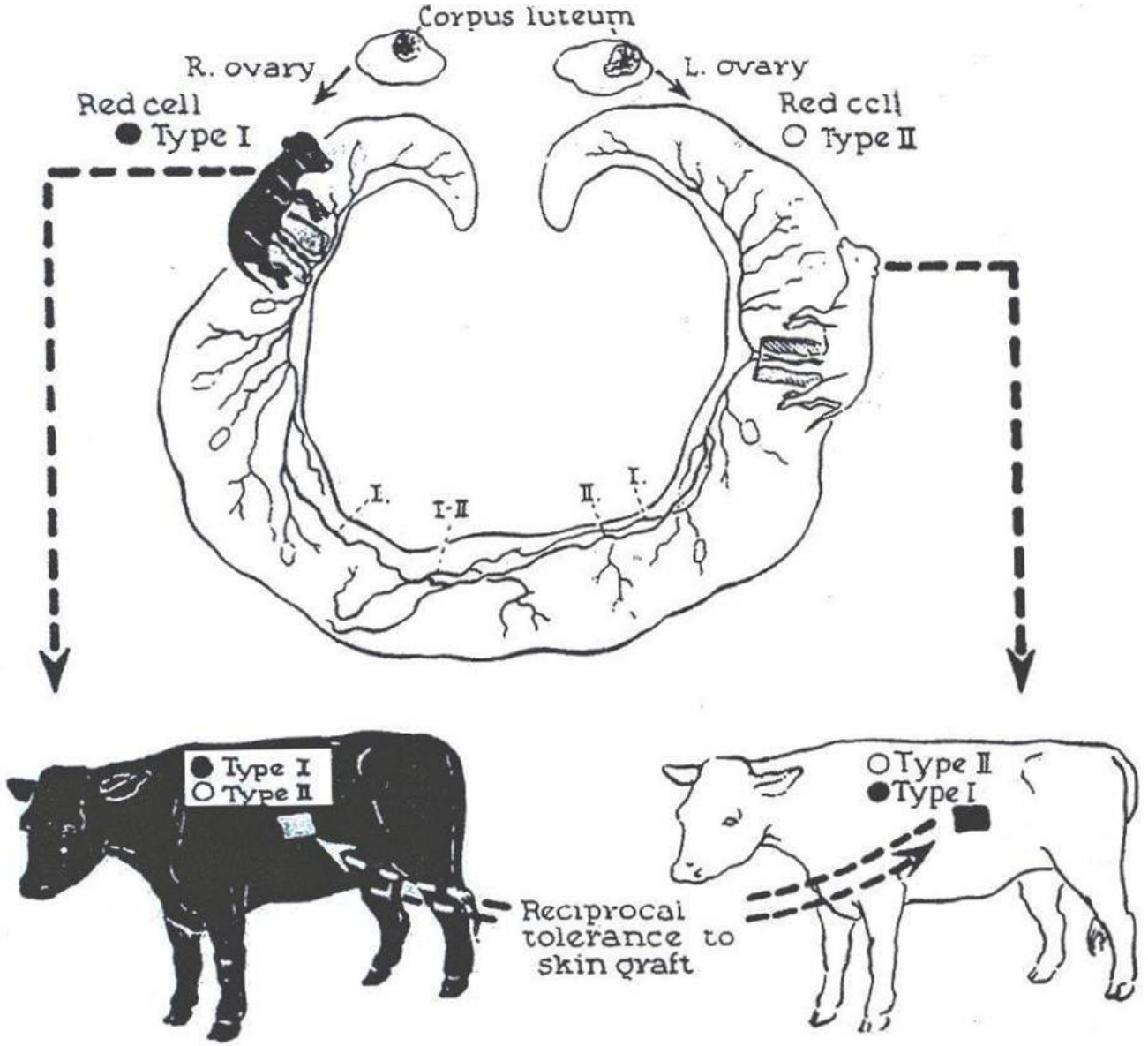
Hollings Cancer Center

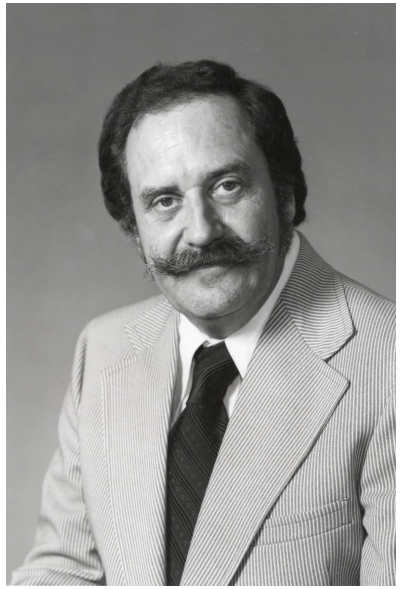
Medical University of South Carolina

Questions

- What is a regulatory T cell (Treg)?
- How do Tregs work?
- What diseases are possible indications for Tregs?
- Can Tregs be made into living drugs?
- How can one assess Tregs' therapeutic efficacy?
- What is the cutting edge of Treg biology and engineering?
- Will Tregs become a proven treatment?

Chimerism and immune tolerance

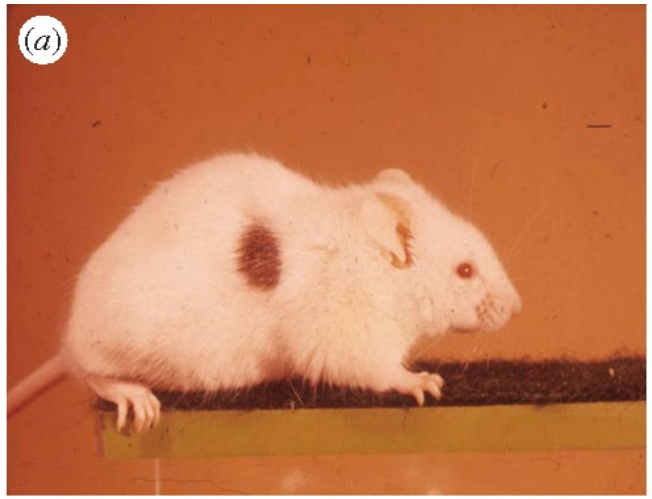


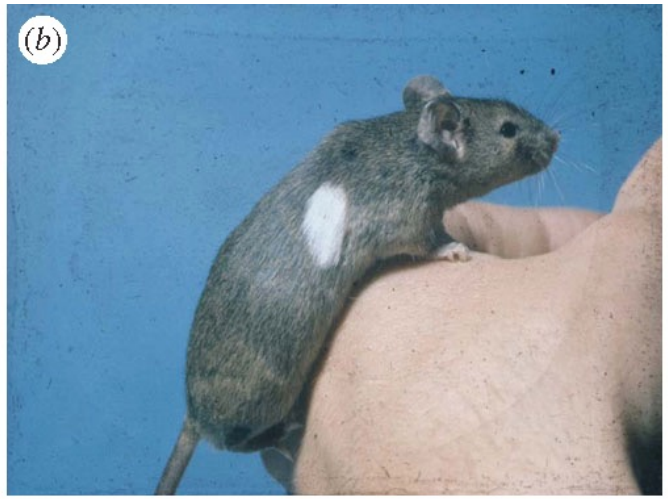


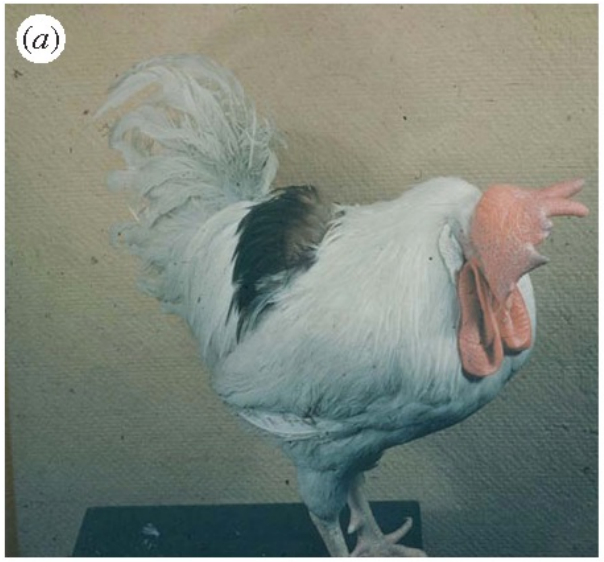
Ray Owen

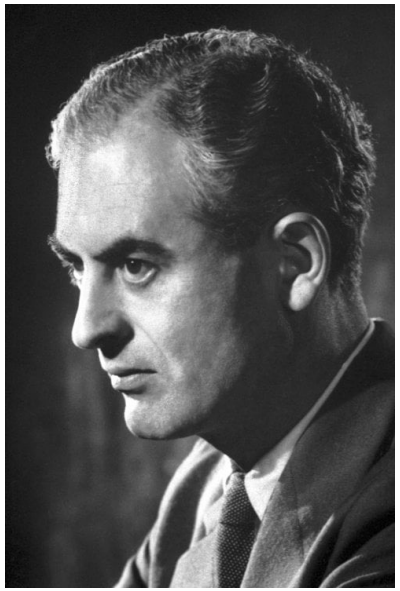
Owen 1945
(Figure from Starzl & Butz Surg Clin North Amer 1962)

Actively acquired immune tolerance





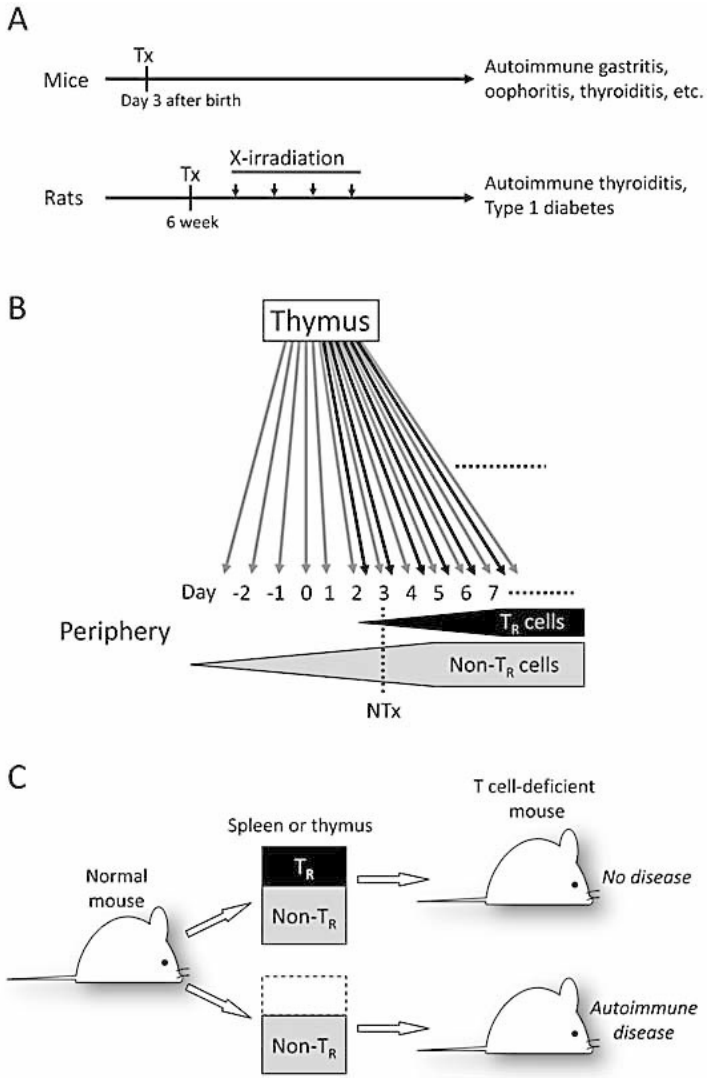


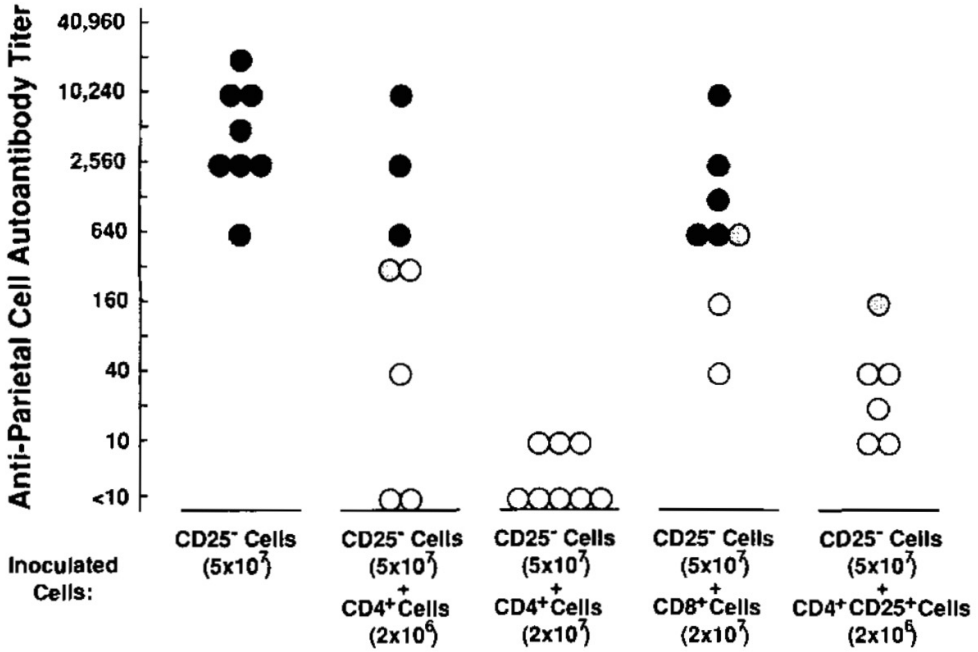


Peter Medawar

(Billingham, Brent & Medawar, Phil Trans Royal Soc B: Biol Sci 1956)

T cell-mediated immune tolerance





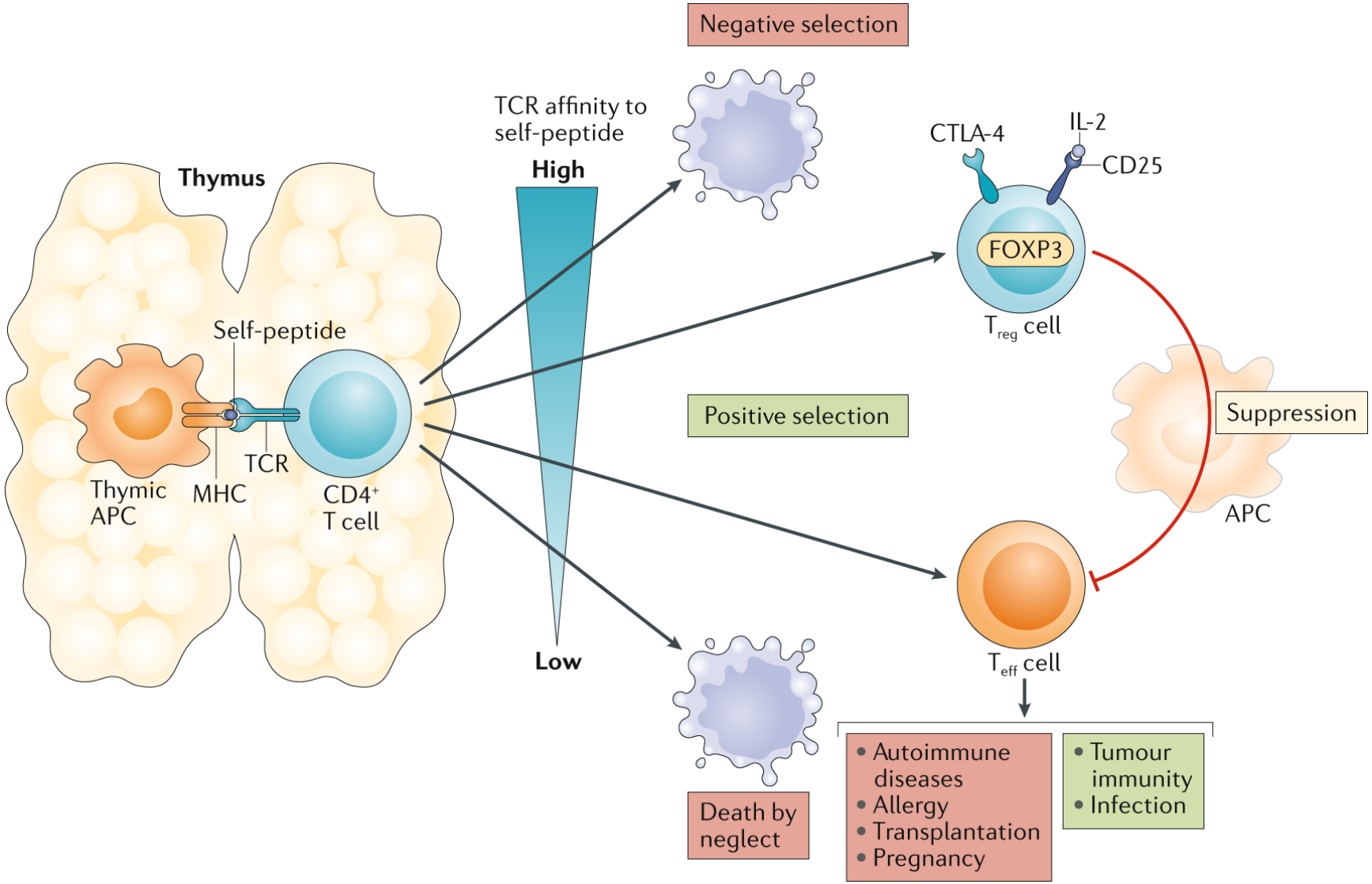
● Grade 2 gastritis
◉ Grade 1 gastritis
○ Intact mucosa
Tx – thymectomy
NTx – neonatal thymectomy
T_R – T regulatory cells

Shimon Sakaguchi

Sakaguchi et al, Eur J Immunol 2007

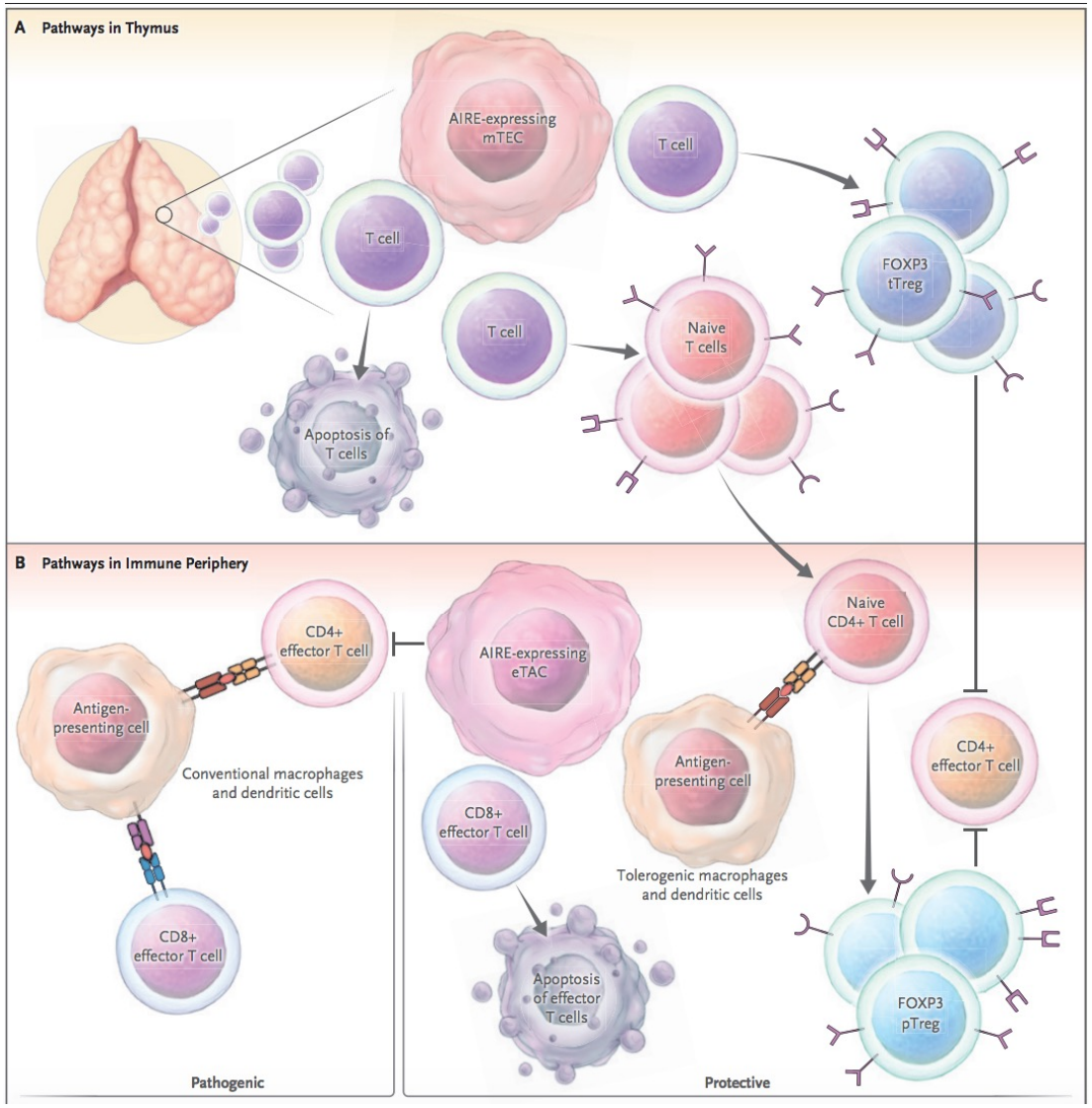
Sakaguchi et al, J Immunol 1995

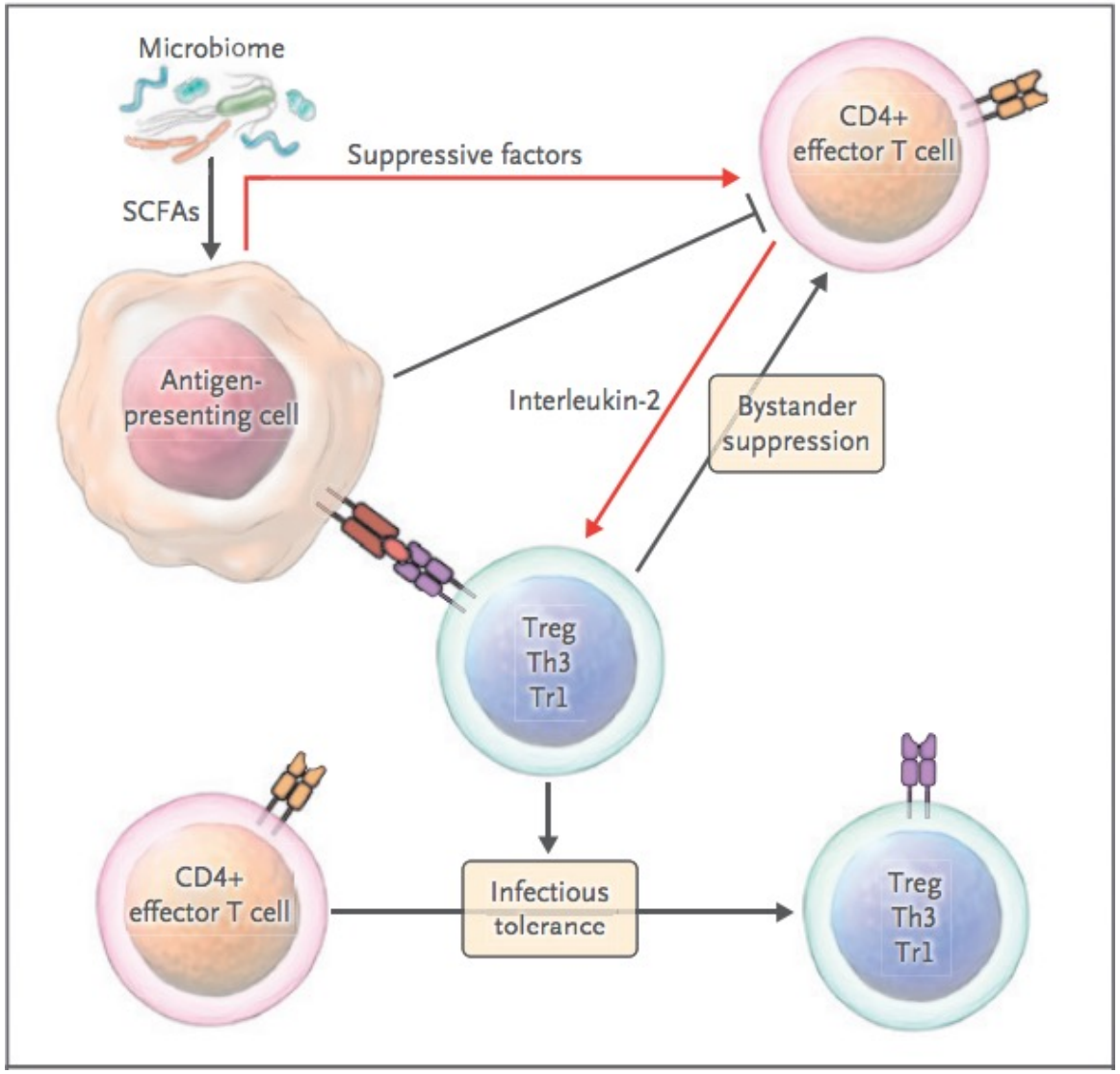
Regulatory T cell (Treg) development



Togashi et al, Nat Rev Clin Oncol 2019

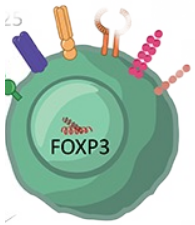
Central and peripheral immune tolerance





Bluestone & Anderson NEJM 2020

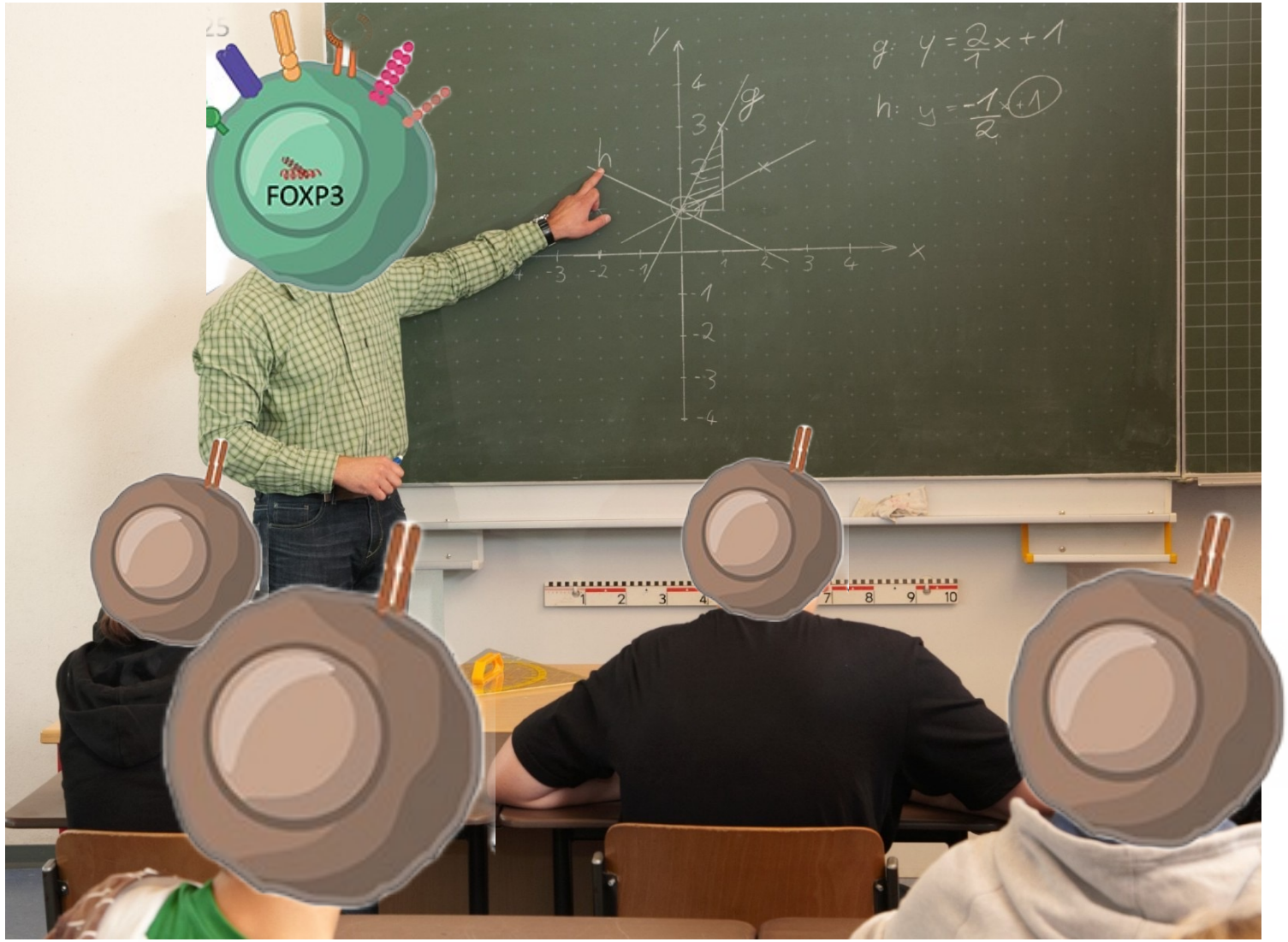
Tregs' first tenet: Bystander suppression

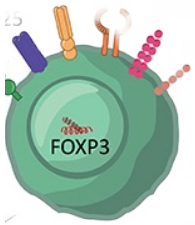


Treg

Teff

Tregs' second tenet: Infectious tolerance

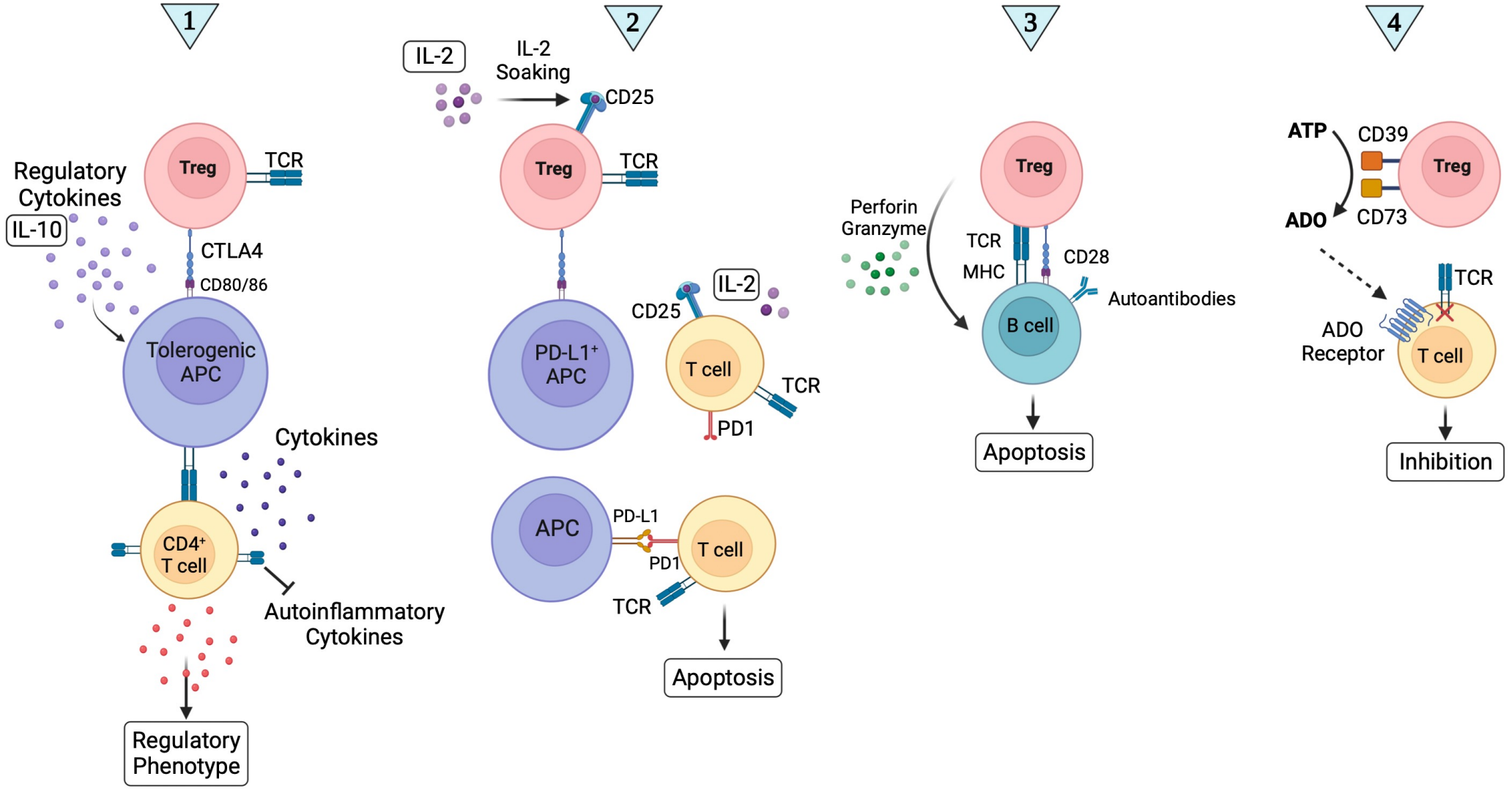




Treg

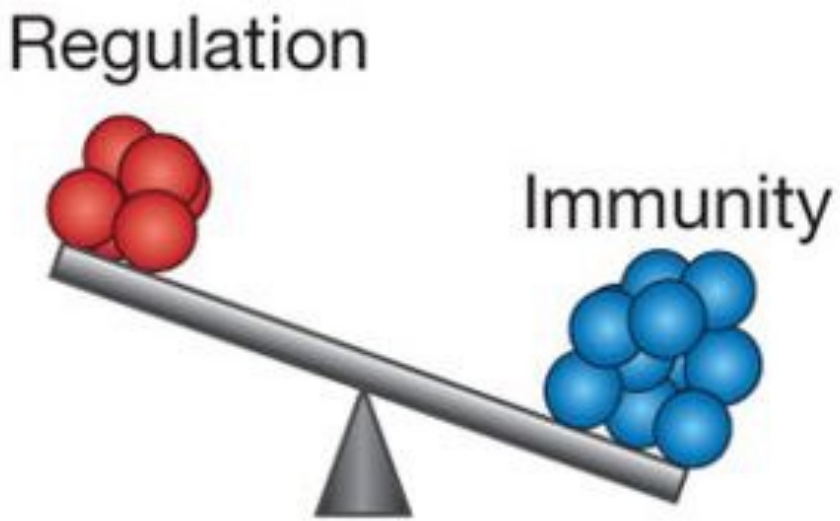
Naïve T cell

Treg-induced tolerance mechanisms

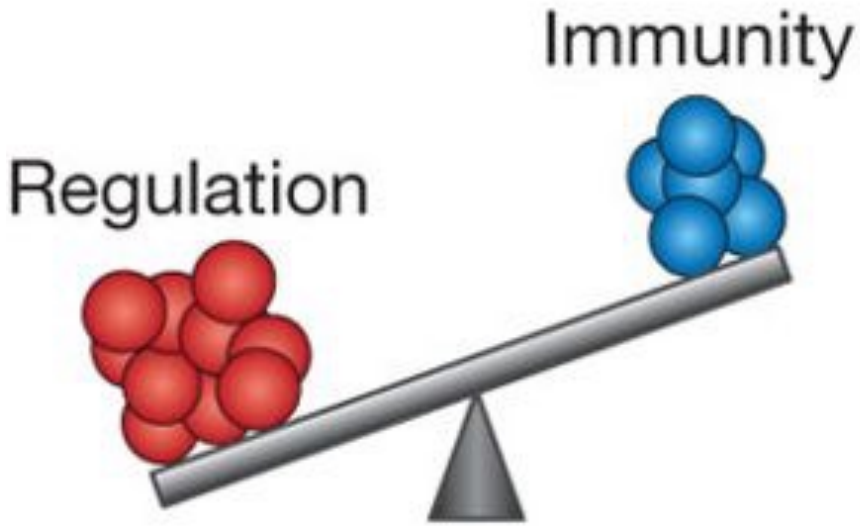


Ghobadinezhad et al Front Immunol 2022

Can Tregs be used as living drugs?

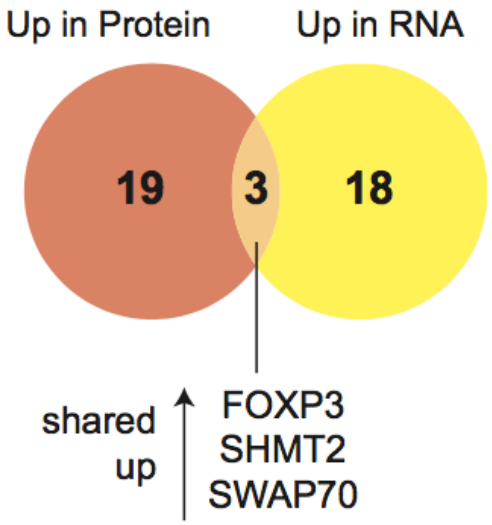


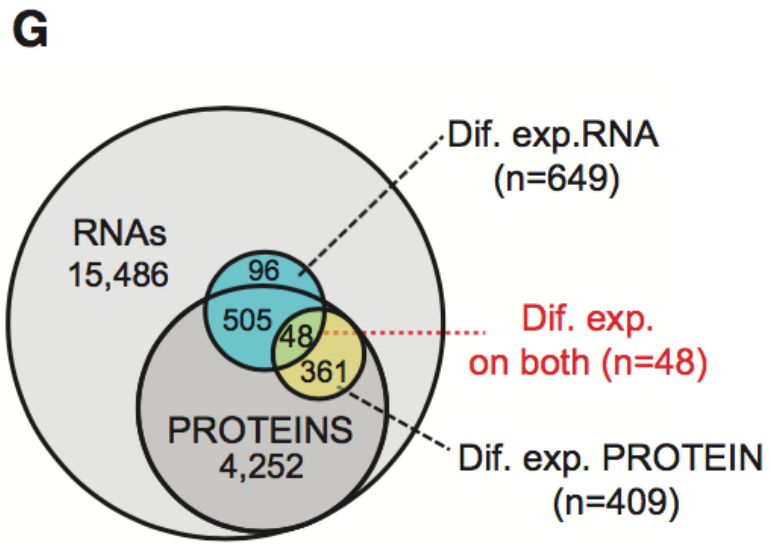
Can Tregs be used as living drugs?



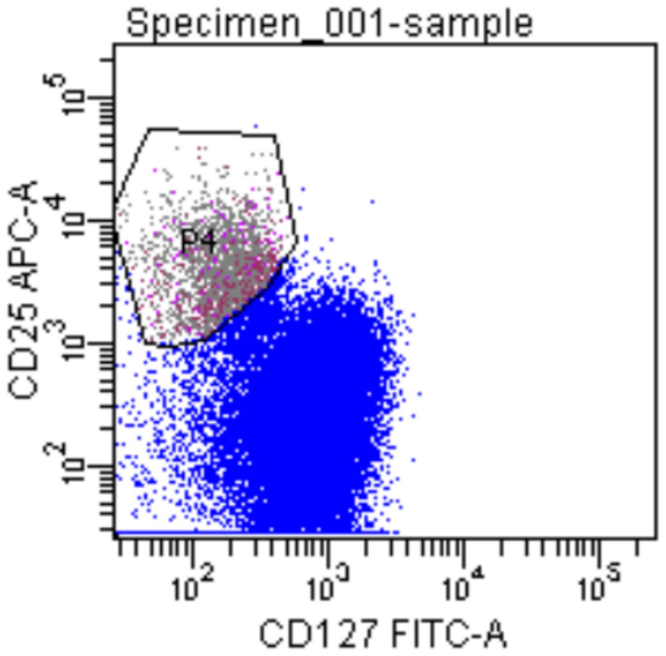
Human Treg identification remains an active field

Treg signature





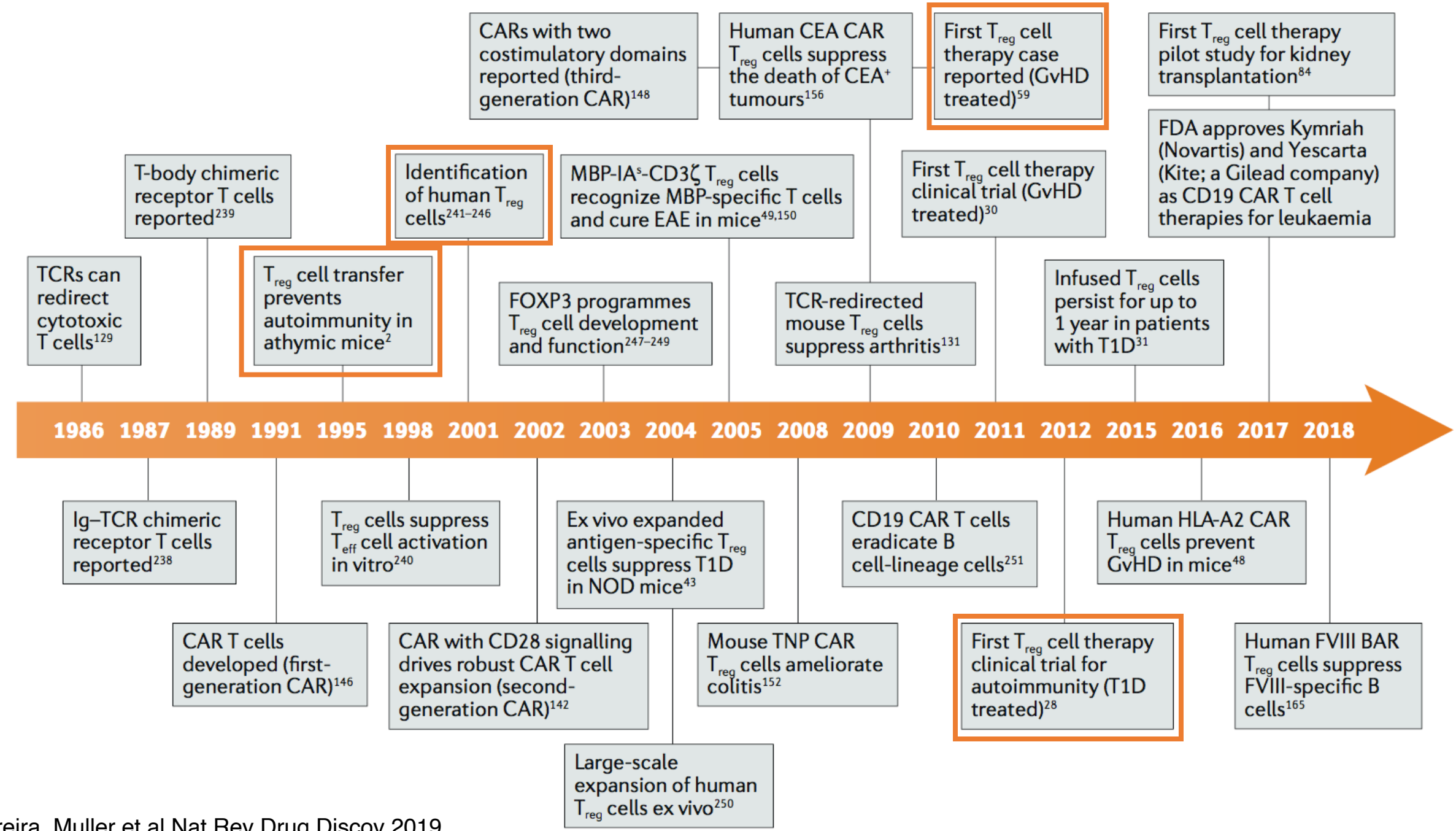
Treg sorting



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)

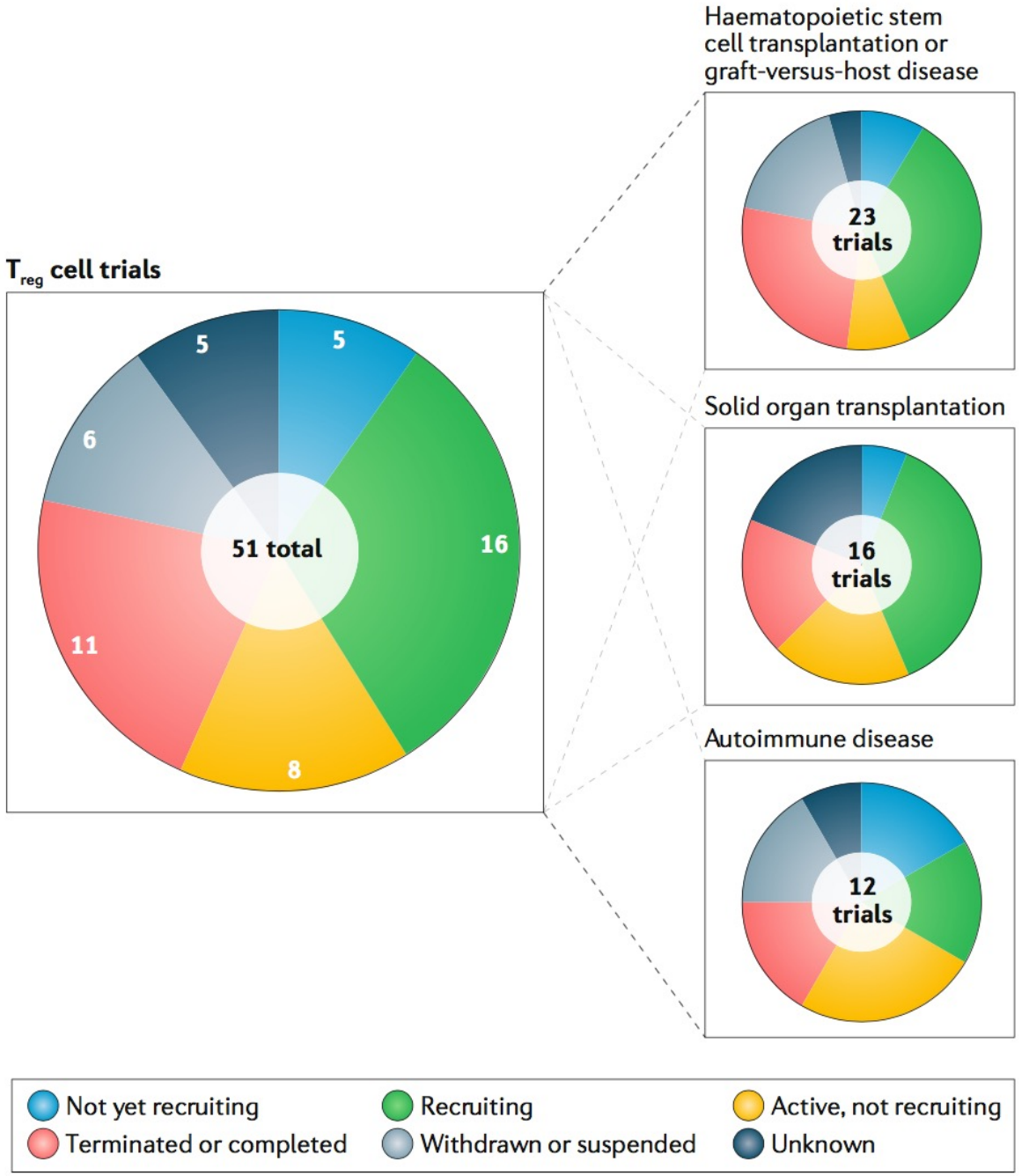
Cuadrado et al Immunity 2018

Sorting based on Liu et al J Exp Med 2006



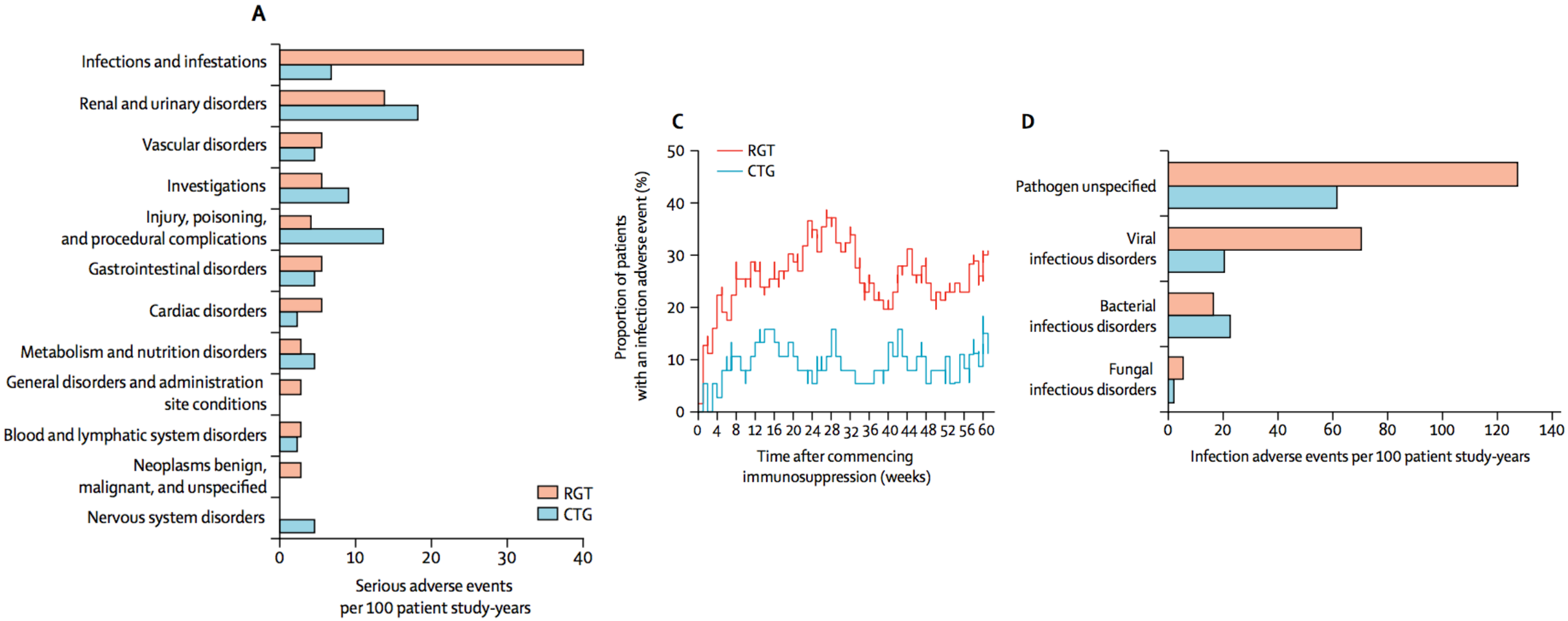
Ferreira, Muller et al Nat Rev Drug Discov 2019

Clinical trials with Tregs



Ferreira et al, Nat Rev Drug Discov 2019

Treg therapy for organ transplant

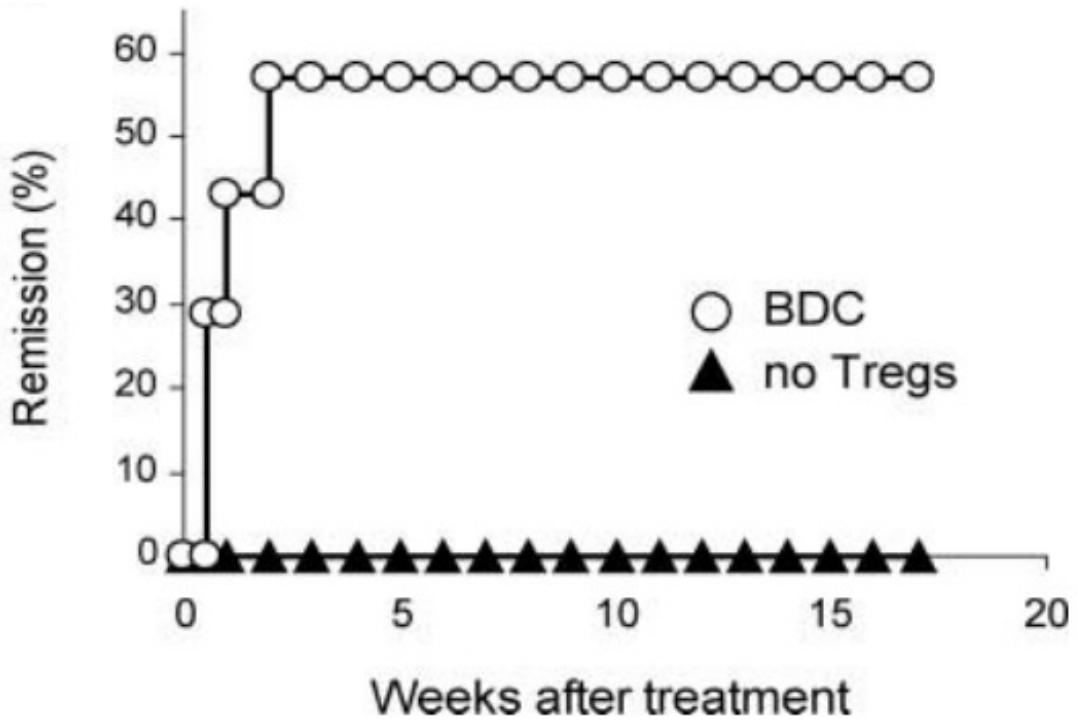


Regulatory cell therapy allows for lower doses of immunosuppressive drugs and leads to less infections

Sawitzki et al Lancet 2020

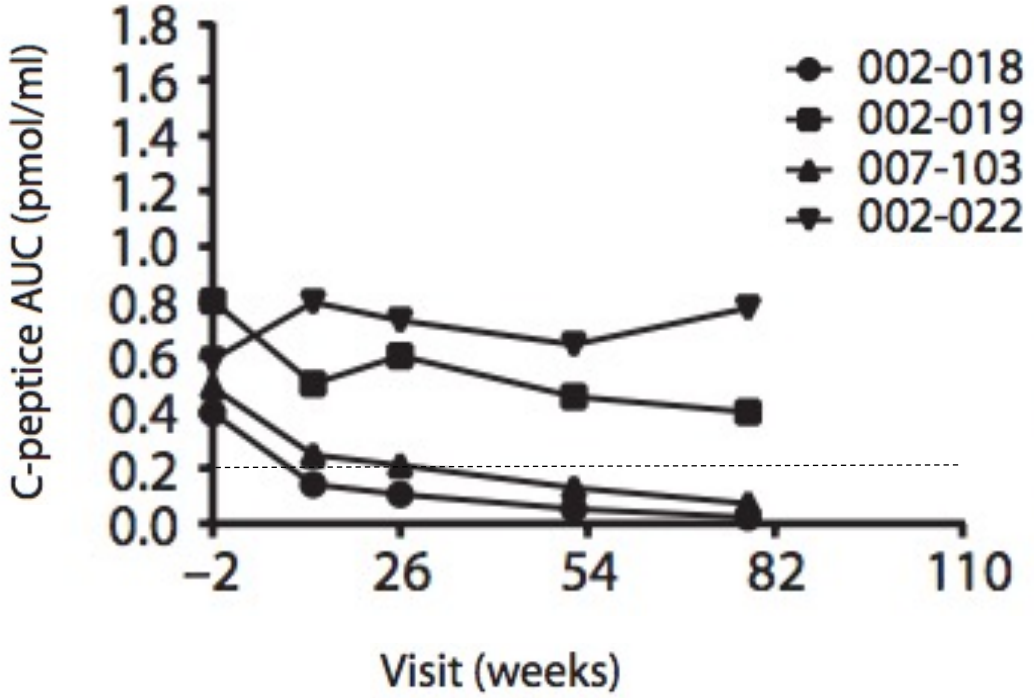
Treg therapy for type 1 diabetes (T1D)

In mice: 2 x 10^6 BDC2.5 Ag-specific Tregs together with syngeneic islet transplant revert autoimmune diabetes



Tang et al J Exp Med 2004

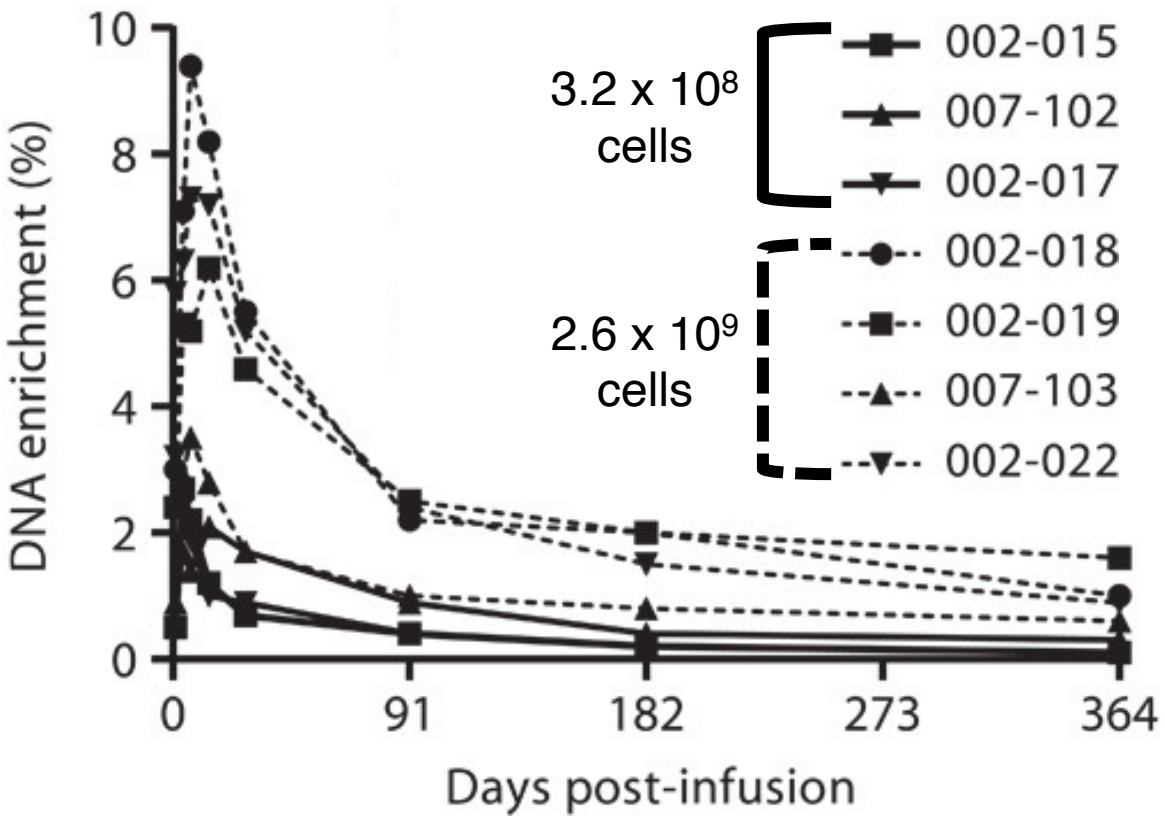
In humans: 2.6 x 10^9 polyclonal Tregs are safe for type 1 diabetes patients, but do not ameliorate disease



Bluestone et al Sci Trans Med 2015

Human Tregs infused in T1D patients can be detected 1 year later and are stable

Infused Treg cell detection



Bluestone et al Sci Trans Med 2015

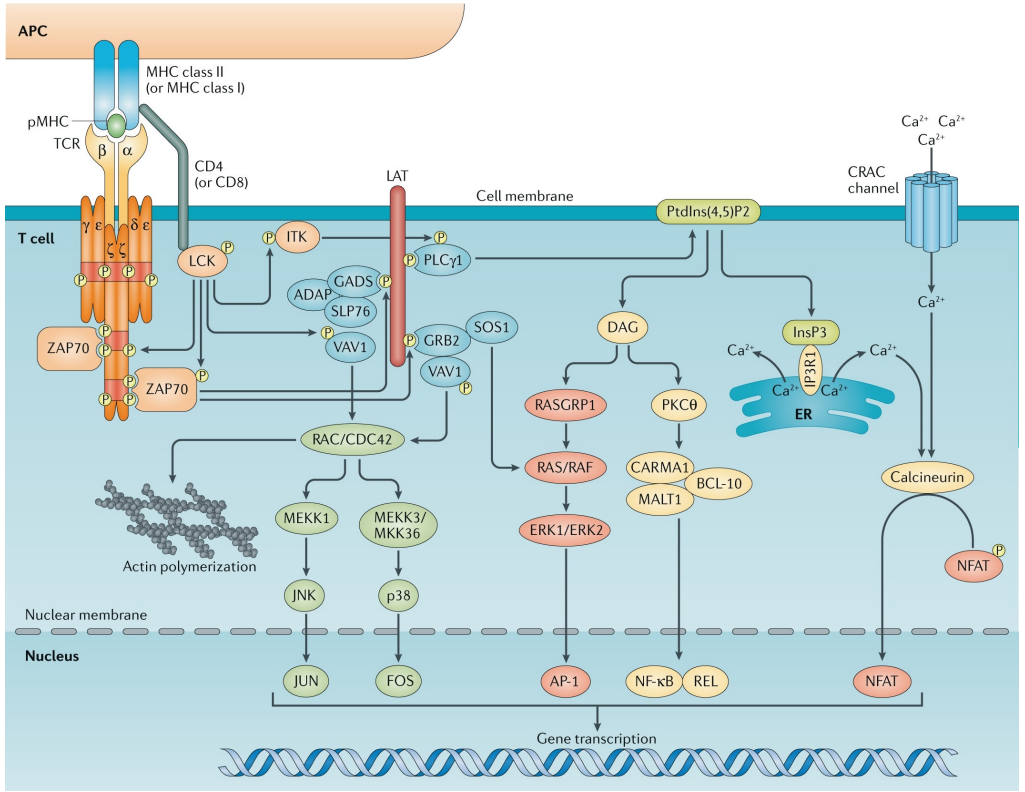
Infused Treg cell stability

	C012 (002-018)		C013 (002-019)			C014 (007-103)			C016 (002-022)		
Cell subset	Day 182	Day 365	Day 91	Day 182	Day 365	Day 91	Day 182	Day 365	Day 91	Day 182	Day 365
T_{regs}											
$CD4^+CD25^+CD127^{lo}$	2.0	1.0	2.5	2.0	1.6	1.0	0.8	0.6	2.4	1.5	0.9
Non-T_{regs}											
$CD45RO^+$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$CD45RO^+CD62L^{hi}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$CD45RO^+CD62L^{lo}$	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$CD45RO^{lo}CD62L^{hi}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

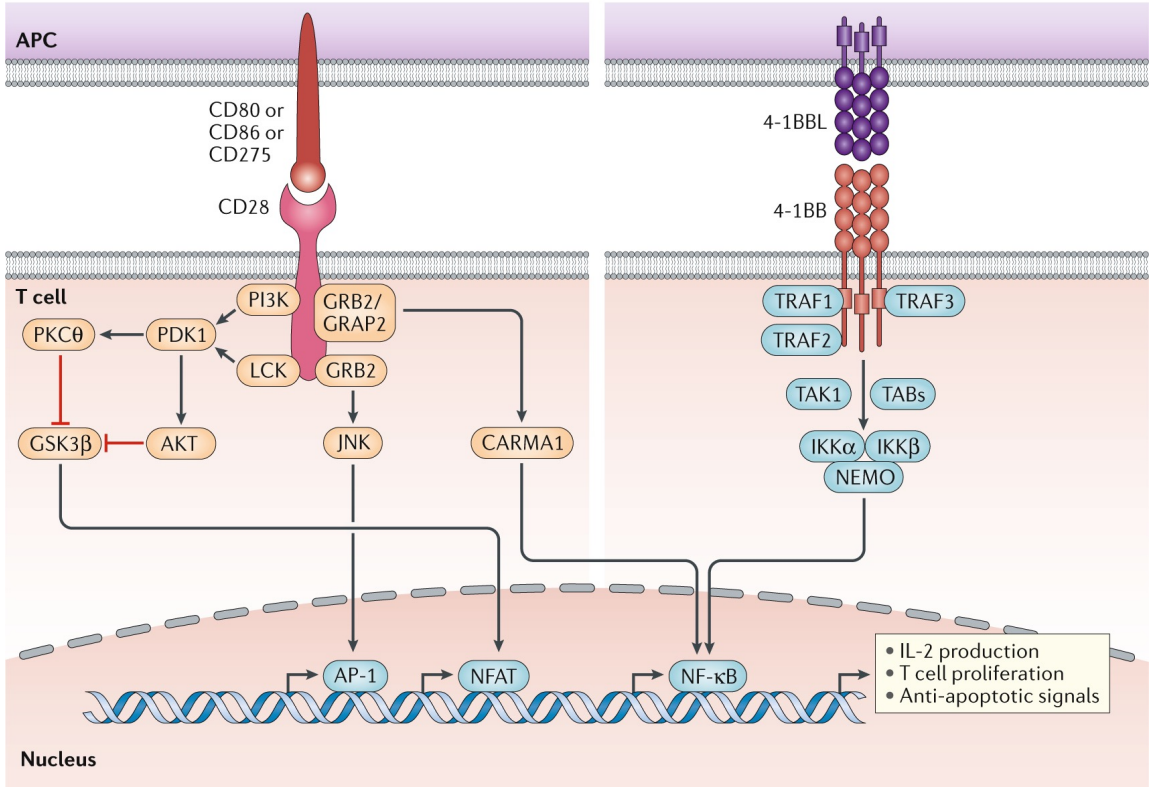
Infused Tregs labeled with deuterium (^2H)

T cell activation requires two signals

Signal 1 – T cell receptor



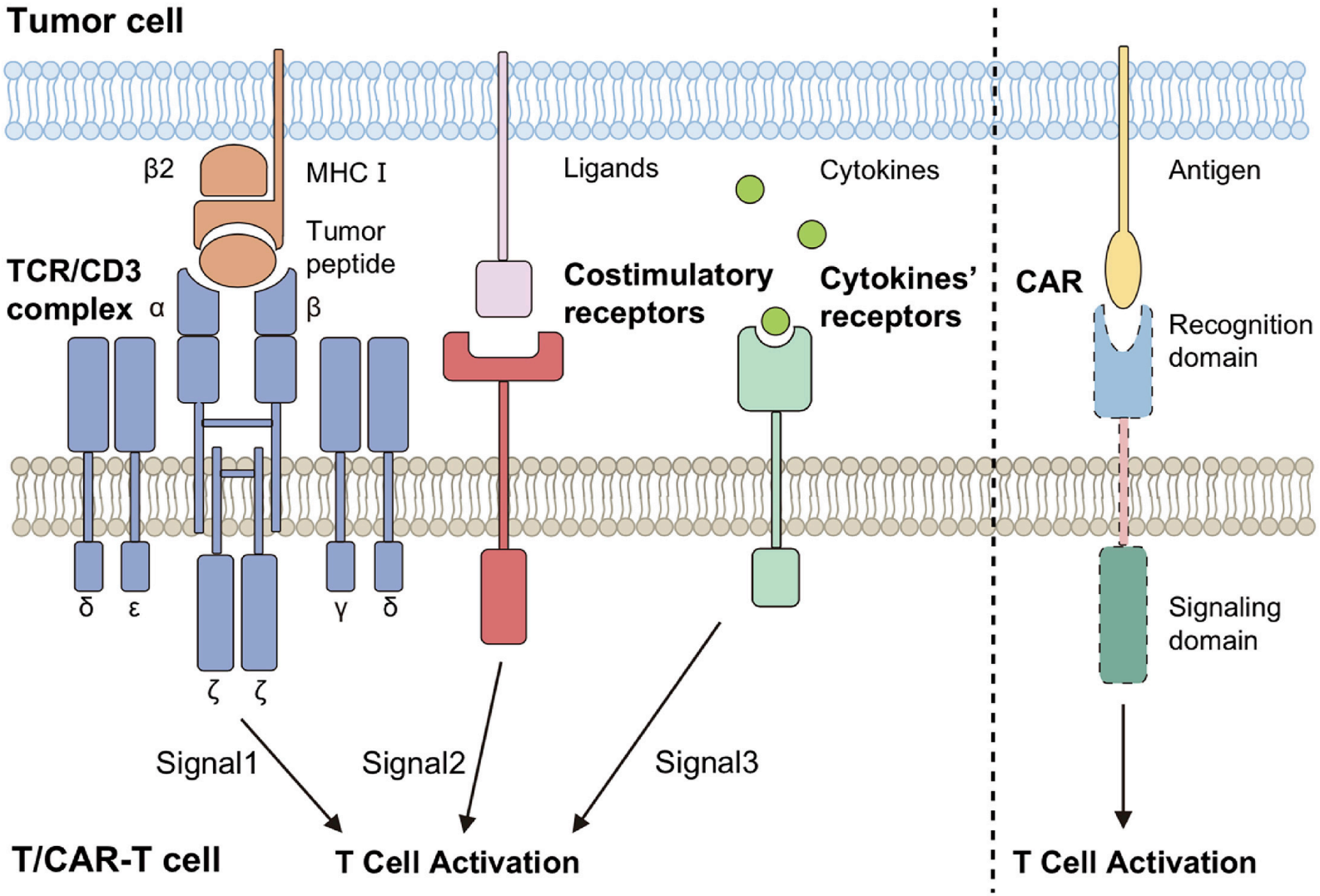
Signal 2 – Co-stimulation



Cappel & Kochenderfer, Nat Rev Clin Oncol 2021

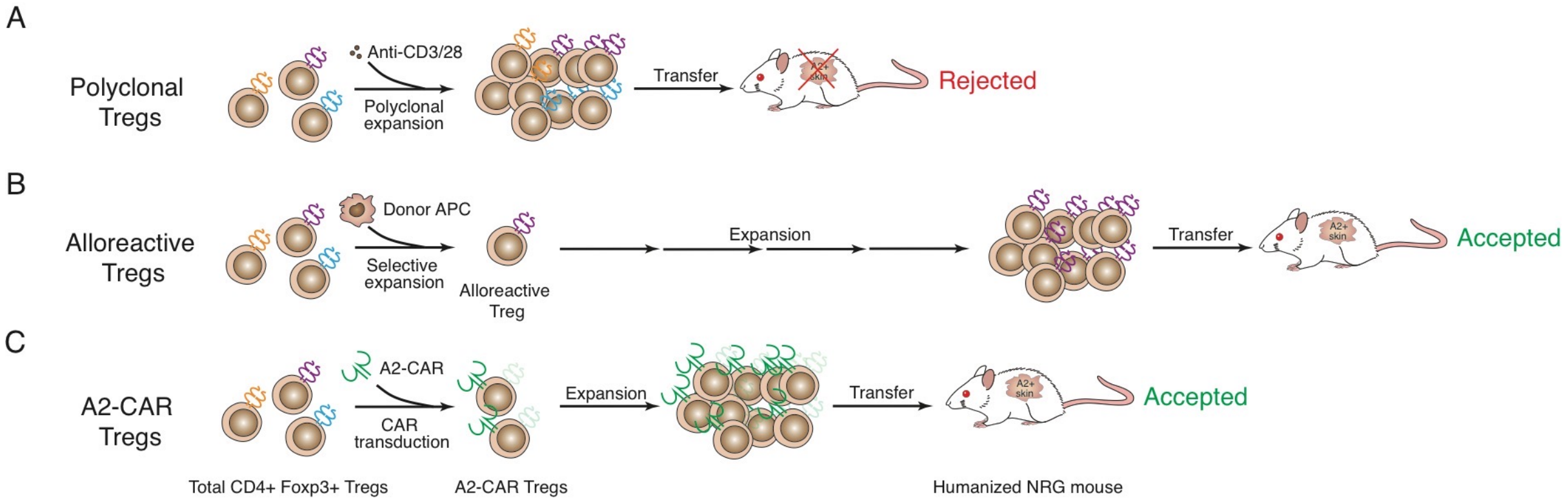
Gaud et al Nat Rev Immunol 2018

Chimeric antigen receptor (CAR)



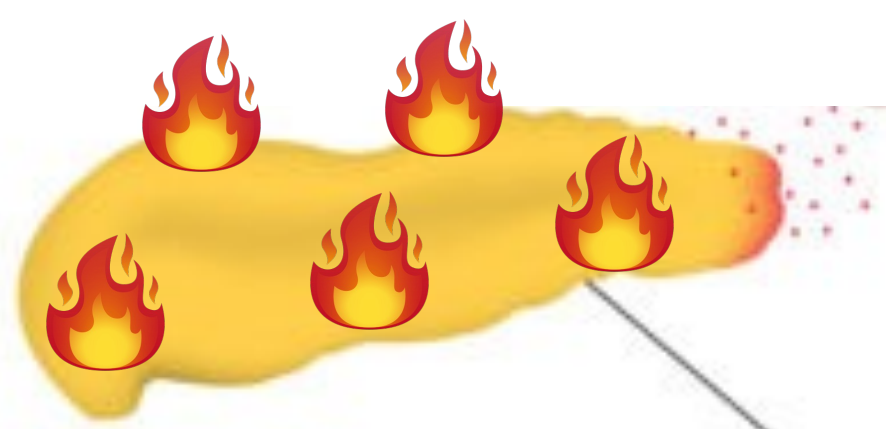
Si et al Synth Syst Biotech 2018

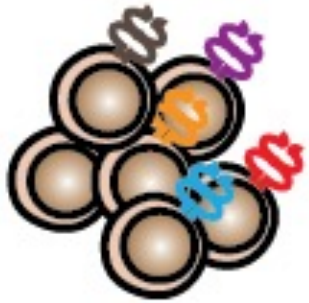
Can we put Treg therapy in the fast lane?



Ferreira & Tang, Am J Trans 2017

Type 1 diabetes



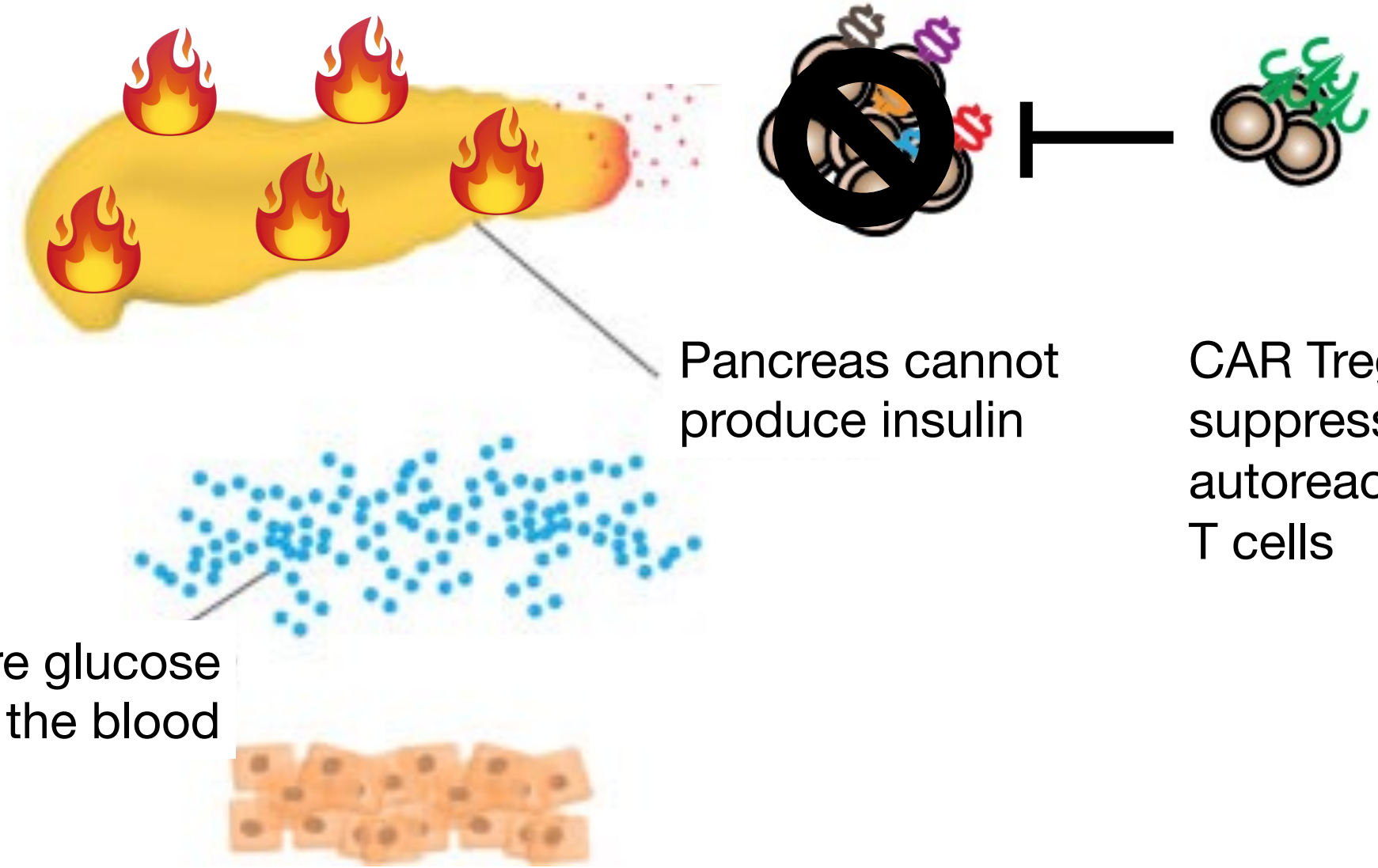


Autoreactive T cells destroy the pancreas

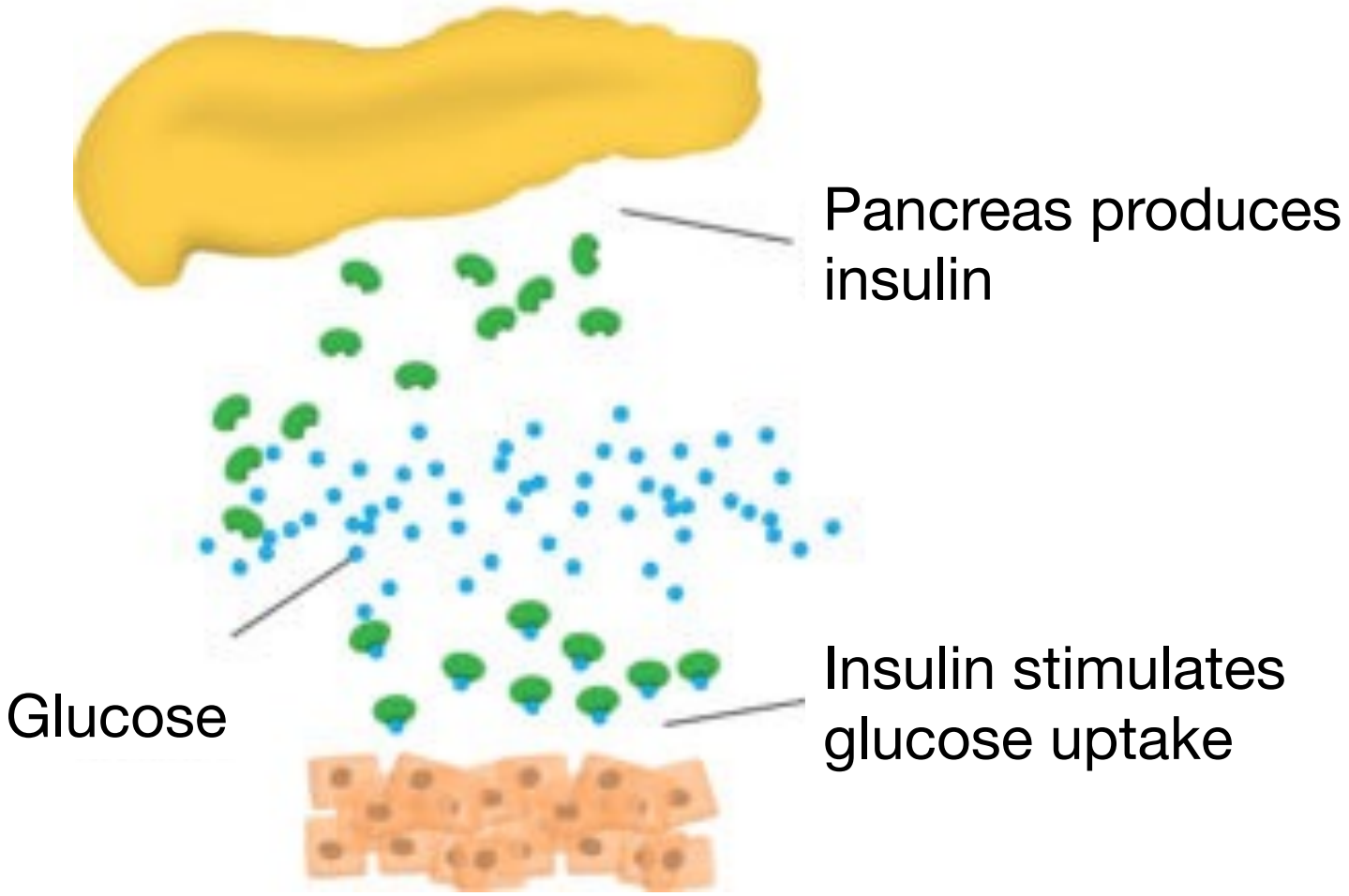
Pancreas cannot produce insulin

More glucose in the blood

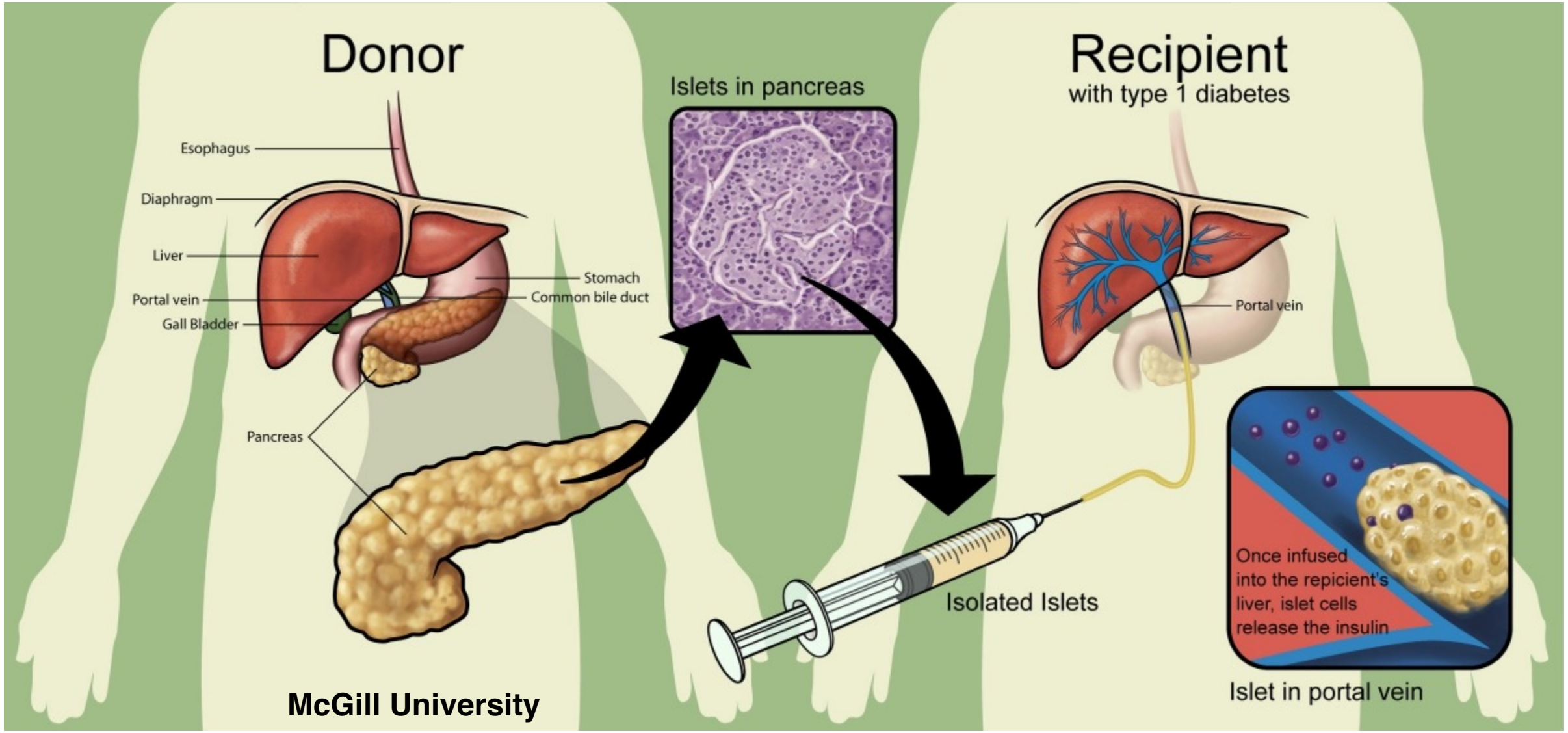
Treating type 1 diabetes using CAR Tregs



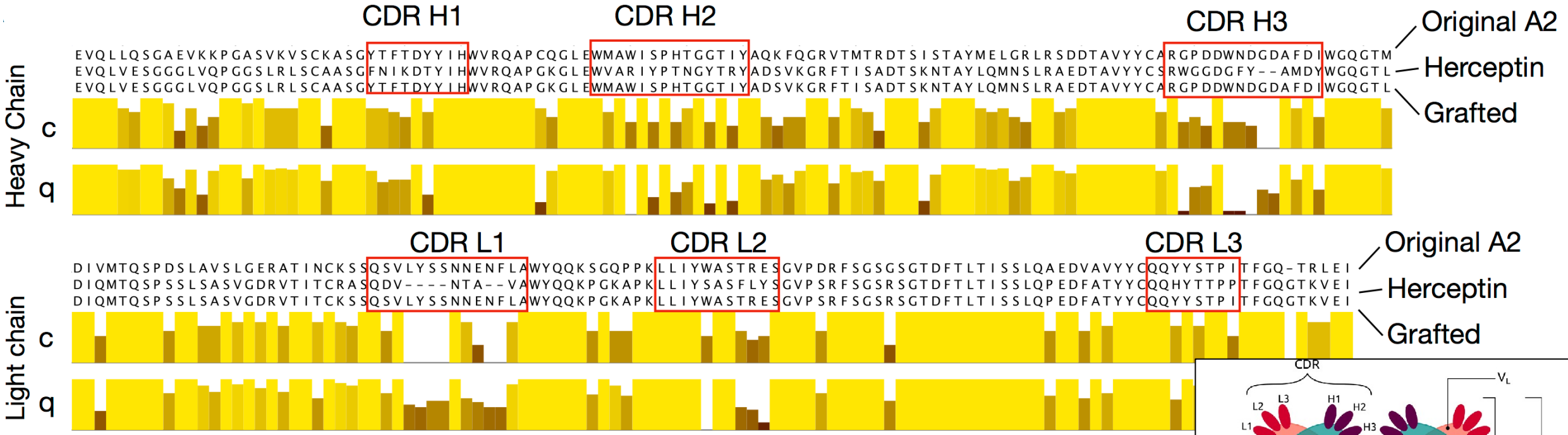
Treating type 1 diabetes using CAR Tregs

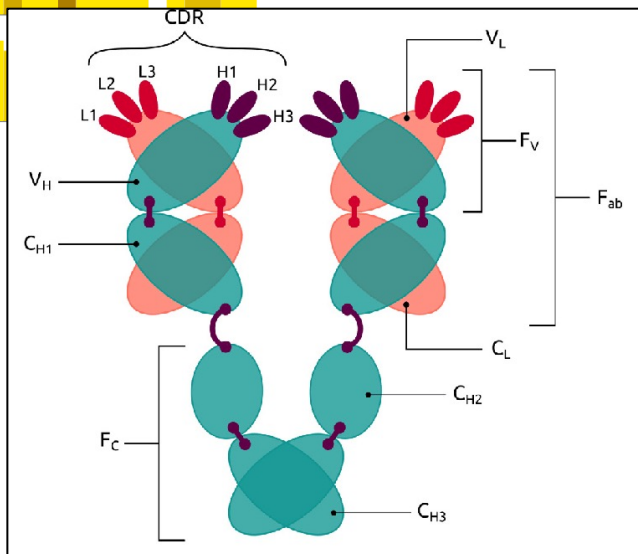


Islet transplant is a treatment for T1D



Grafting HLA-A2 CAR scFv specificity

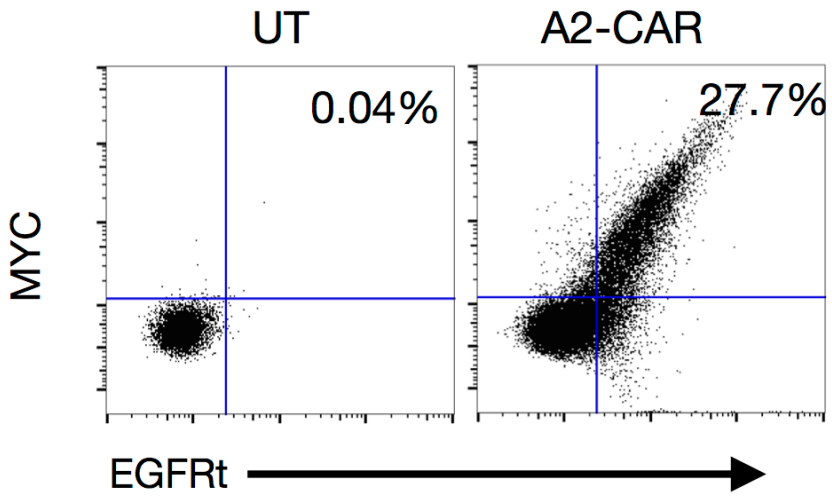




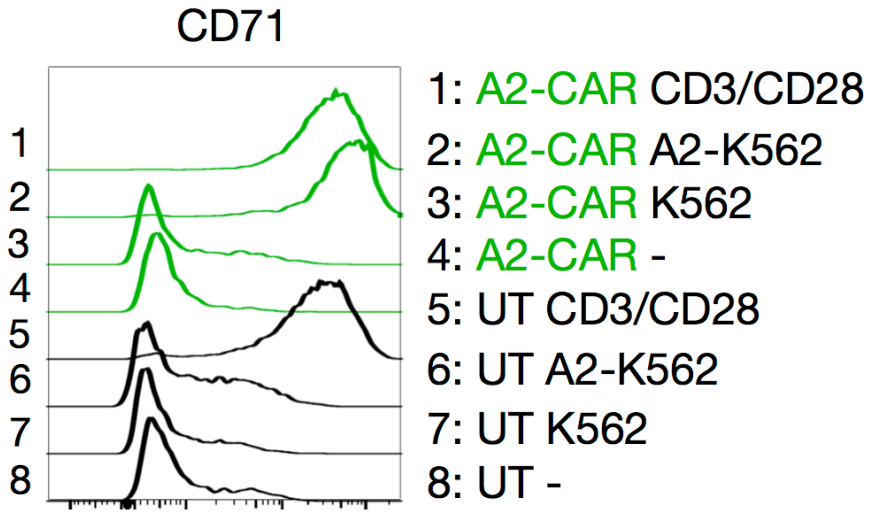
Muller, Ferreira et al, Front Immunol 2021

HLA-A2 CAR Tregs recognize HLA-A2

CAR expression

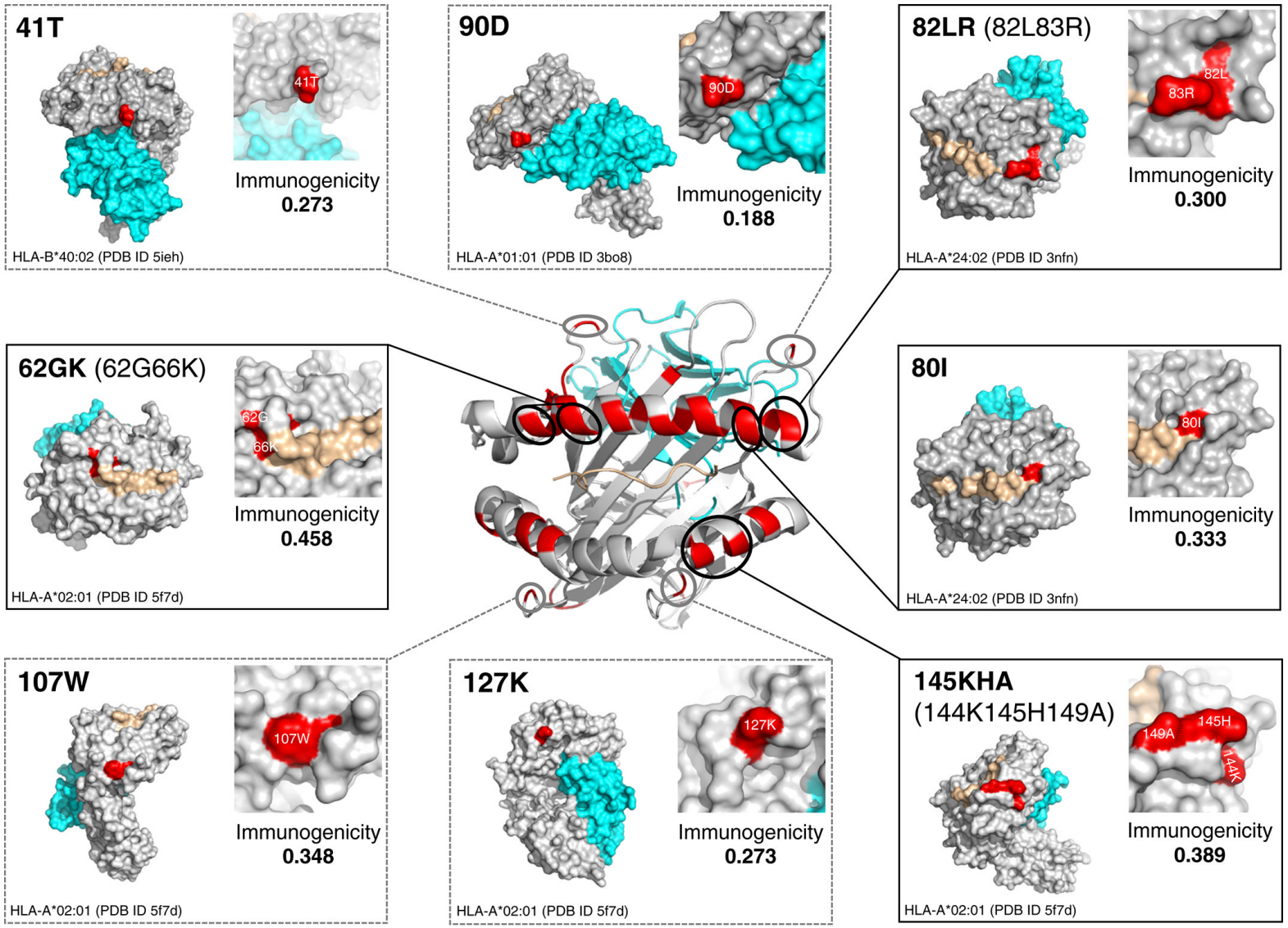


Treg activation



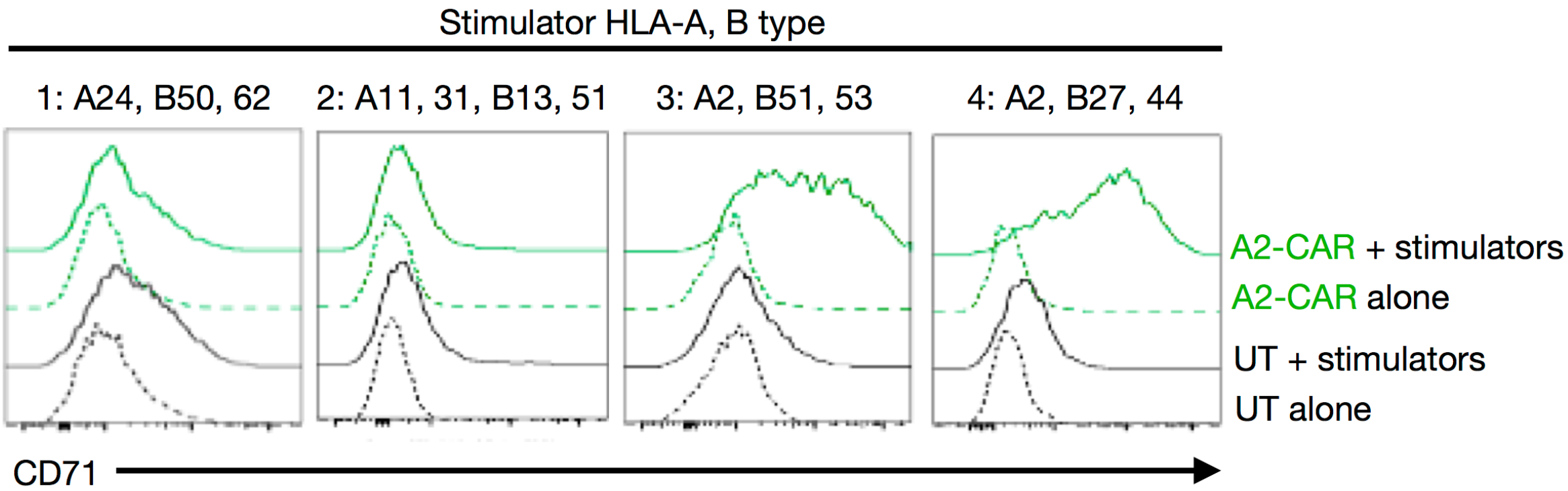
Muller, Ferreira et al, Front Immunol 2021

Immunogenic eplets in HLA-A2



Hönger et al HLA 2020

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

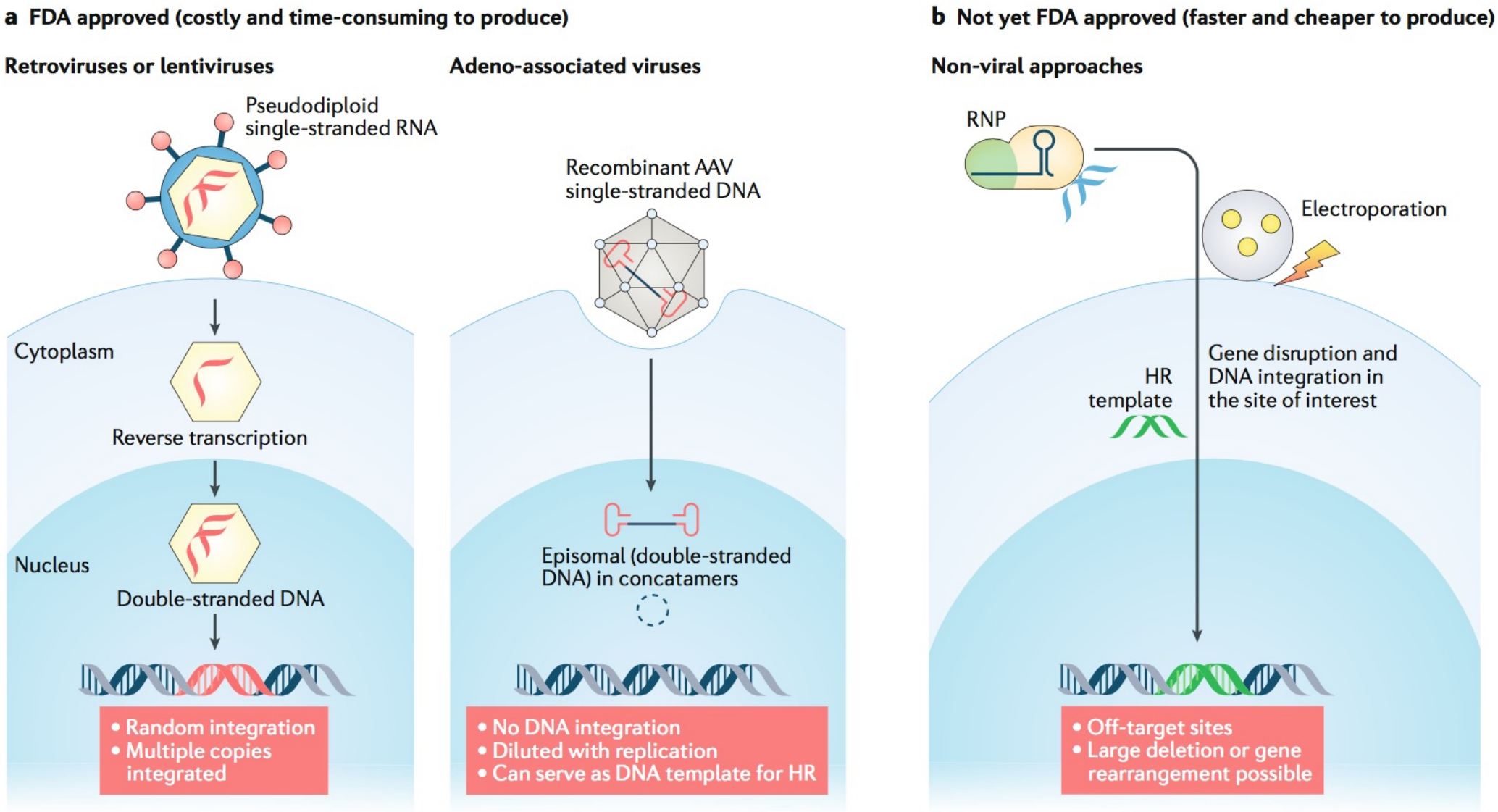


44RME: A11, A24, A31
105S: A24, A31
127K: A24

A2-CAR Tregs co-incubated with allogeneic human islets

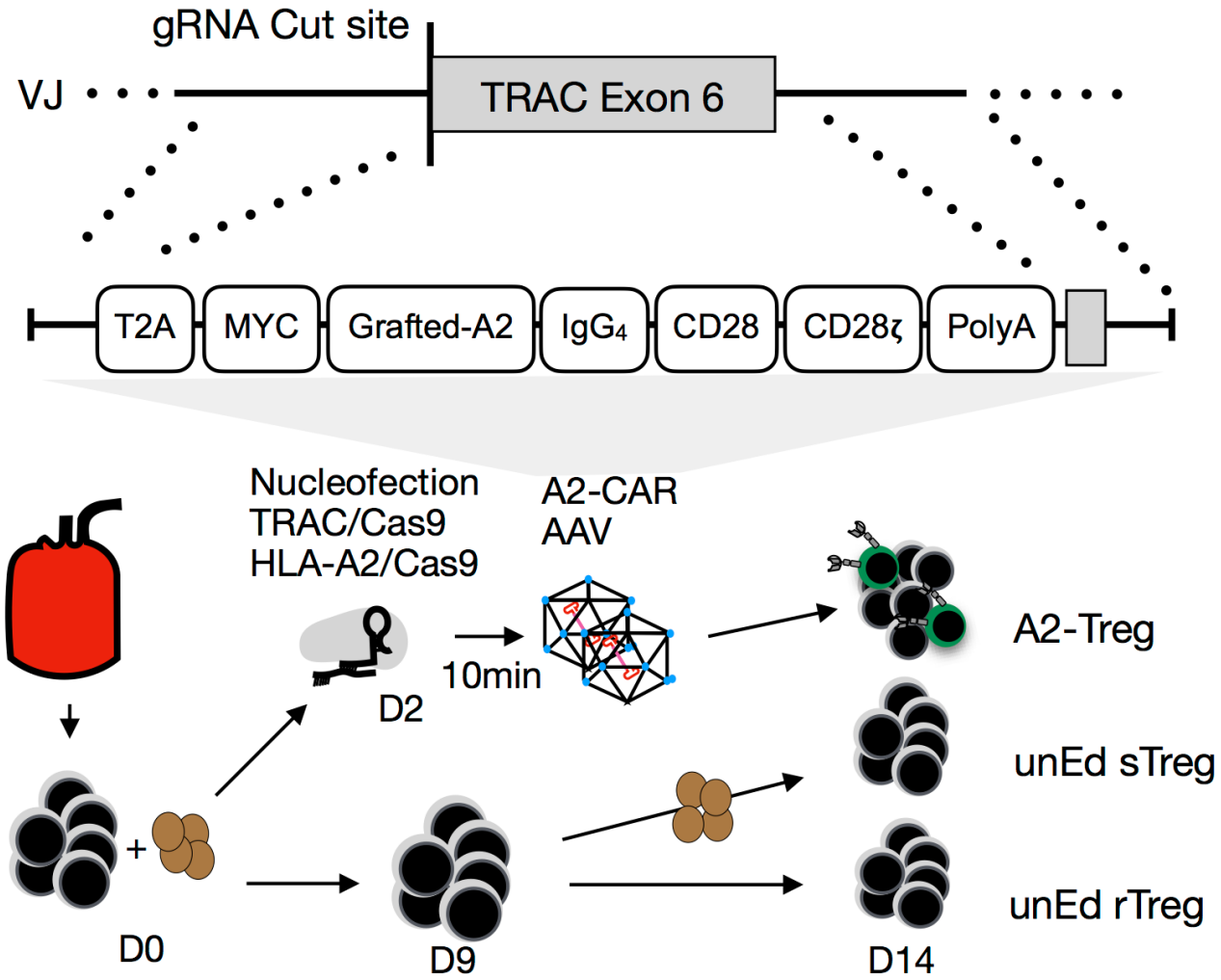
Muller, Ferreira et al, Front Immunol 2021

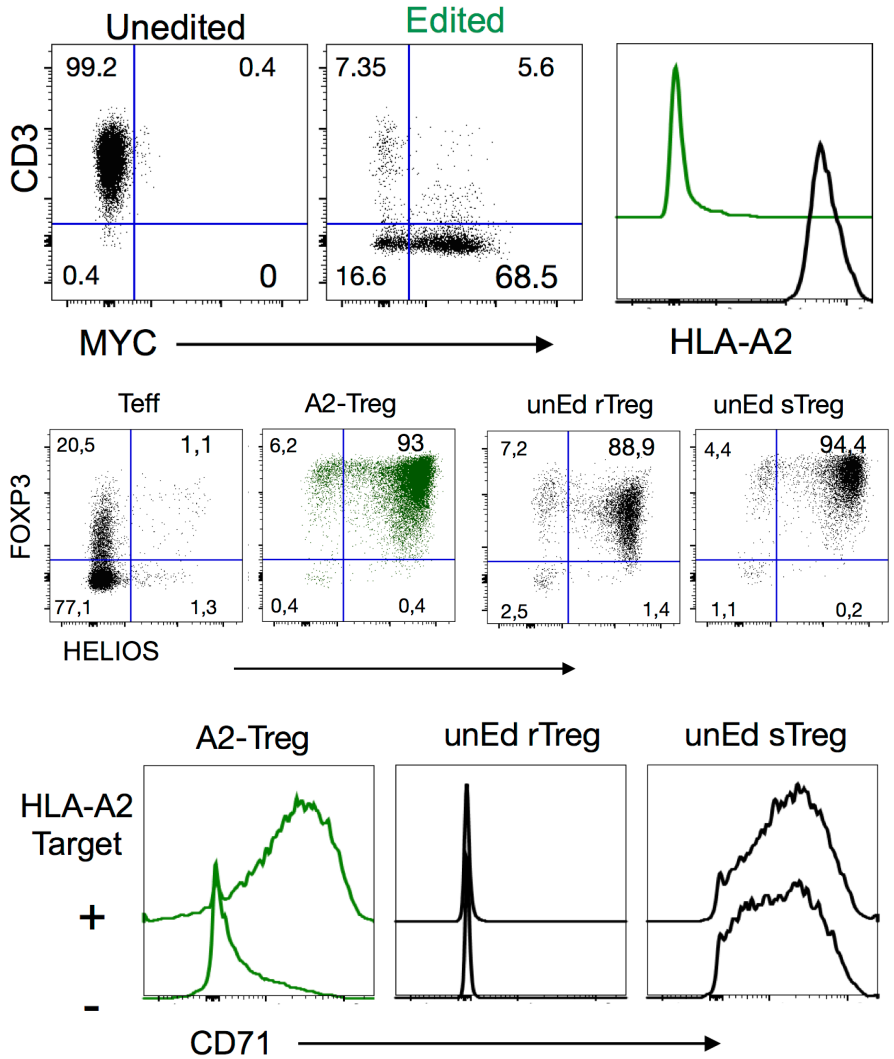
Precise genetic modification of human Tregs



Ferreira et al, Nat Rev Drug Discov 2019

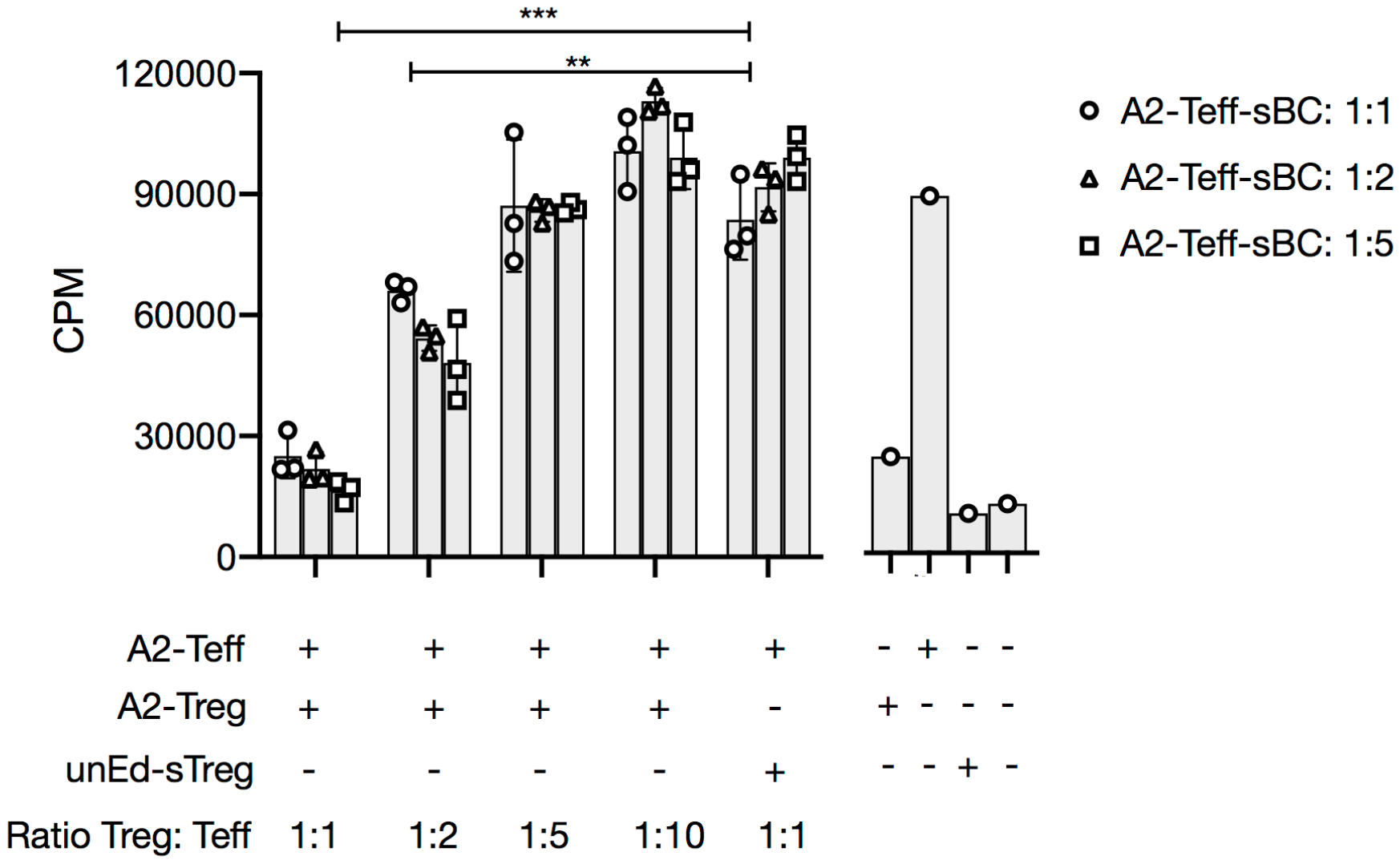
Reprogramming human Treg specificity





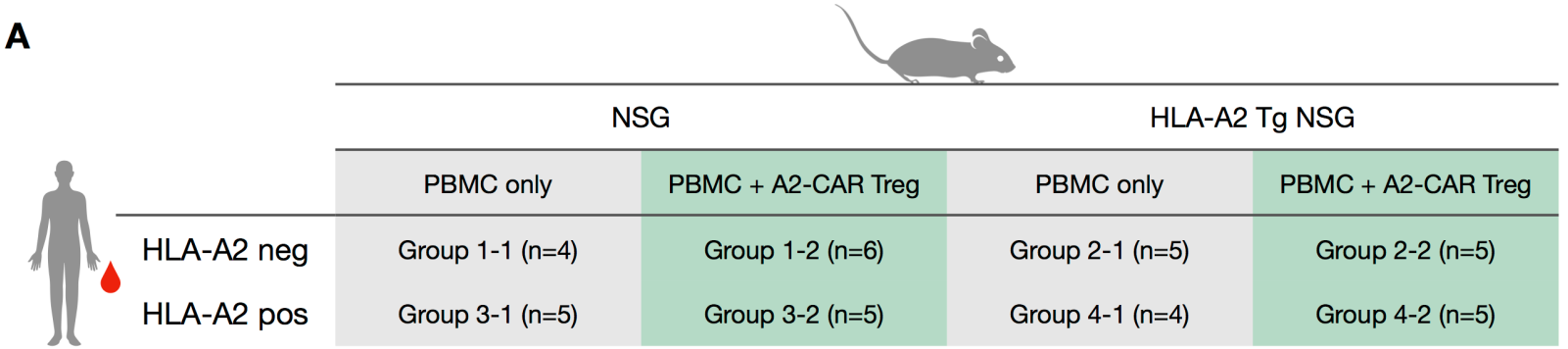
Muller, Ferreira et al Front Immunol 2021

HLA-A2 CAR Tregs suppress Teff cell proliferation

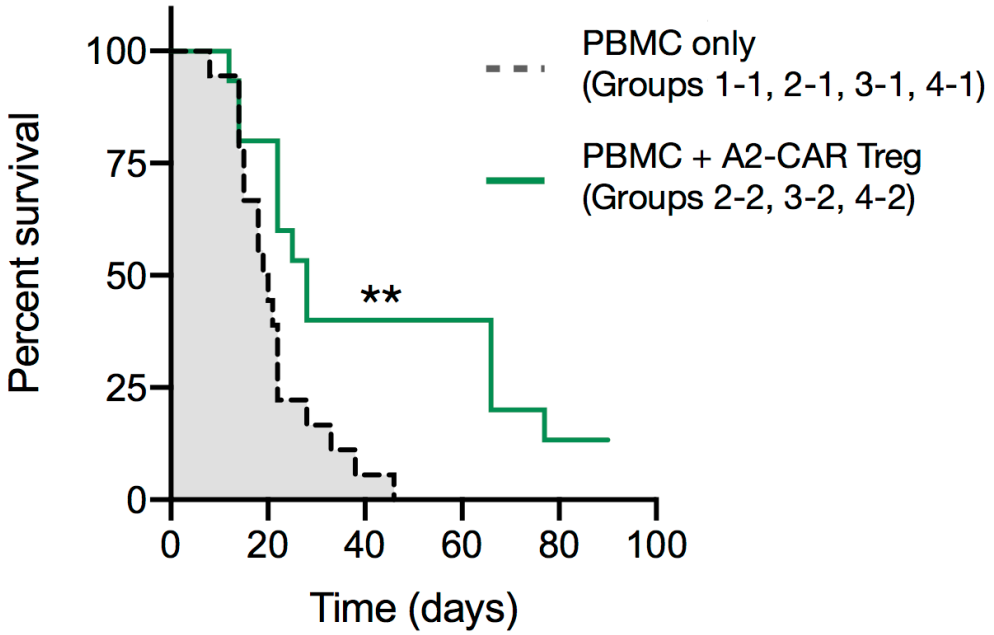


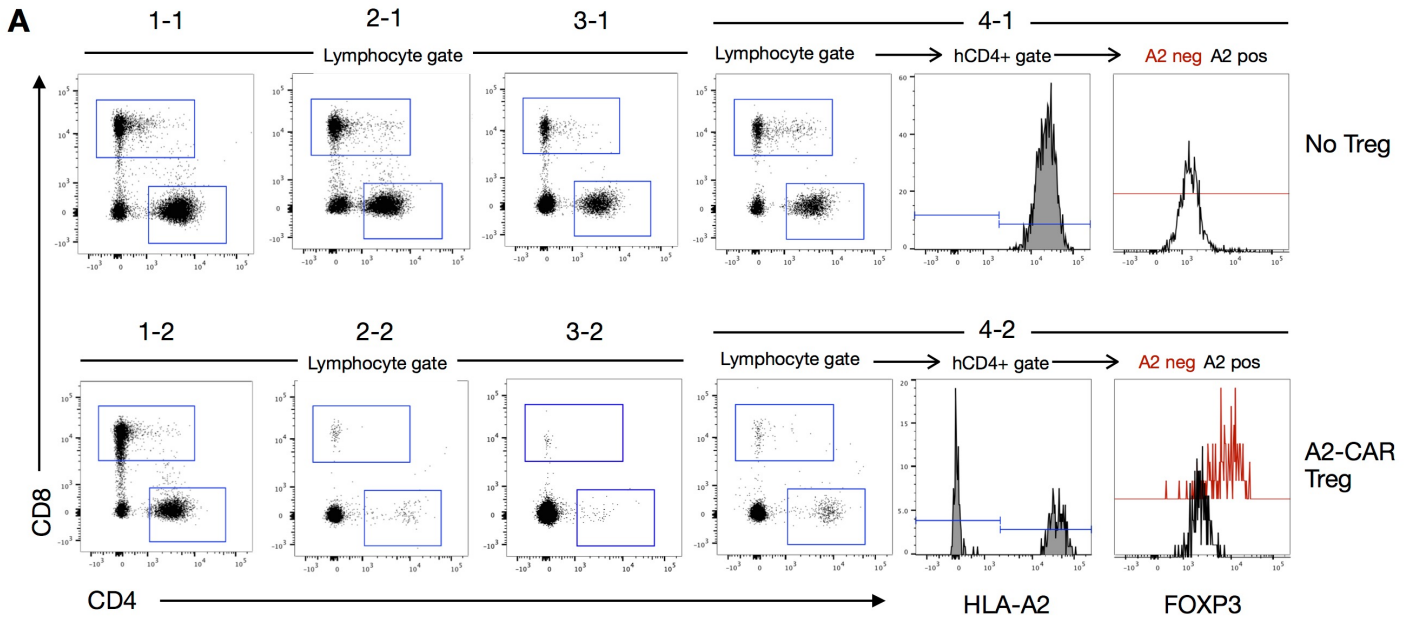
HLA-A2 CAR Tregs prevent graft-vs-host disease

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)

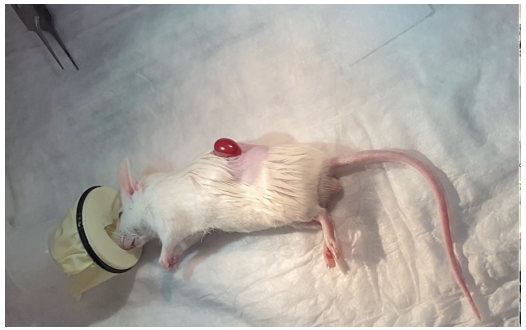




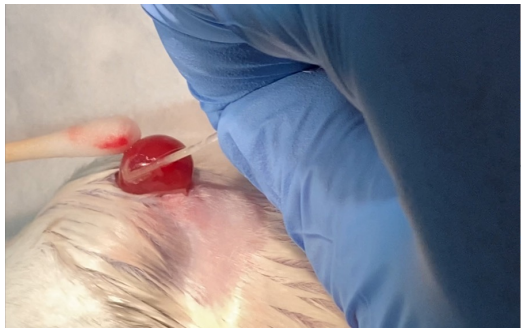
Muller, Ferreira et al Front Immunol 2021

HLA-A2 CAR Tregs traffic to HLA-A2+ human islet grafts

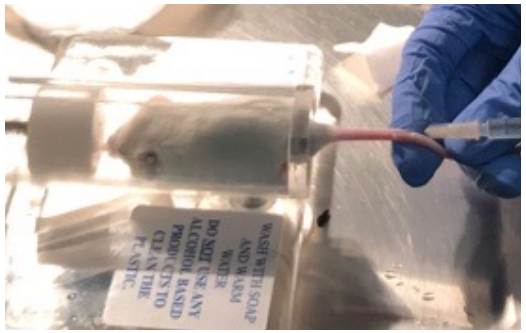
Kidney capsule exposure

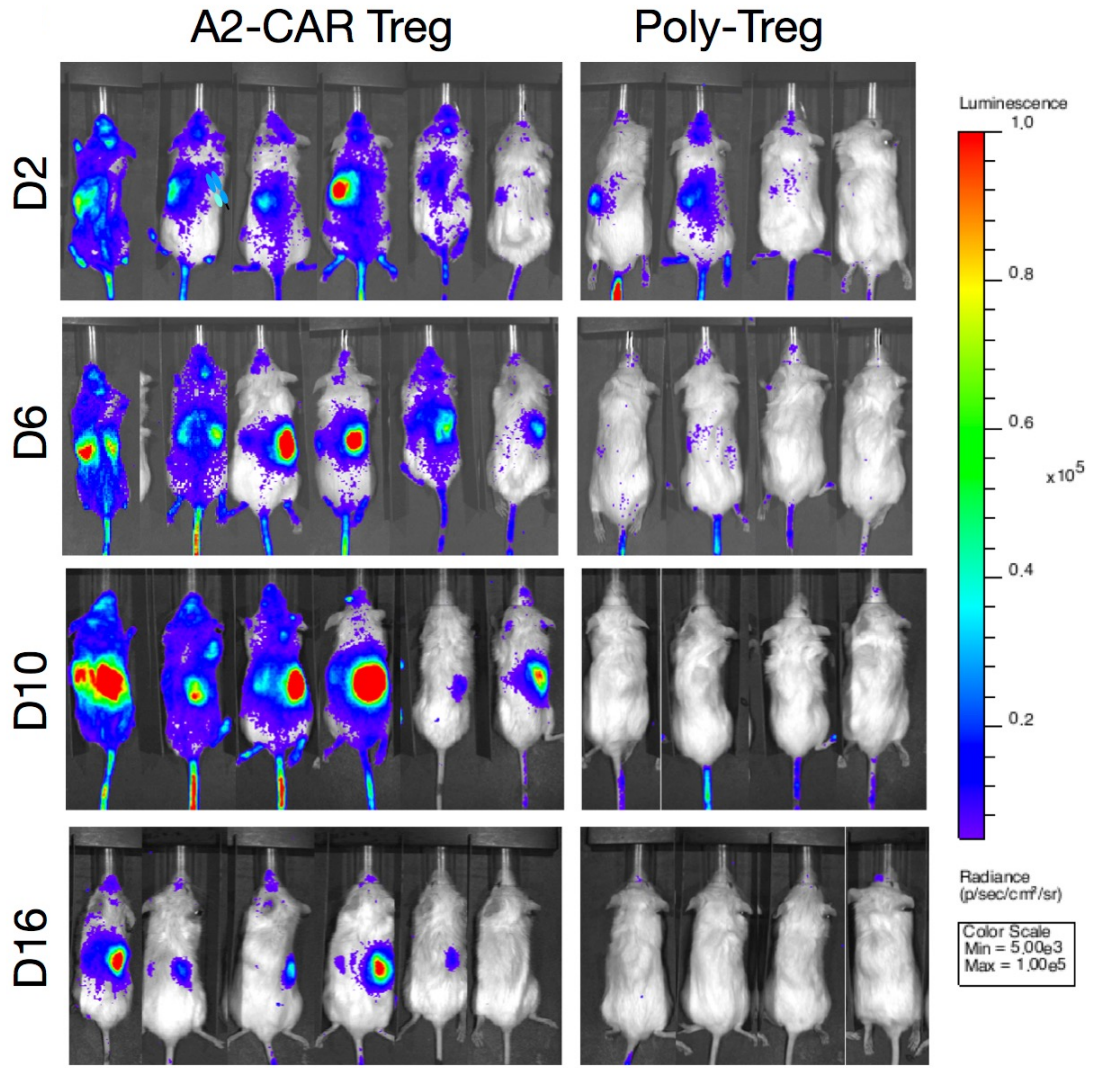


Human A2+ islet transplant



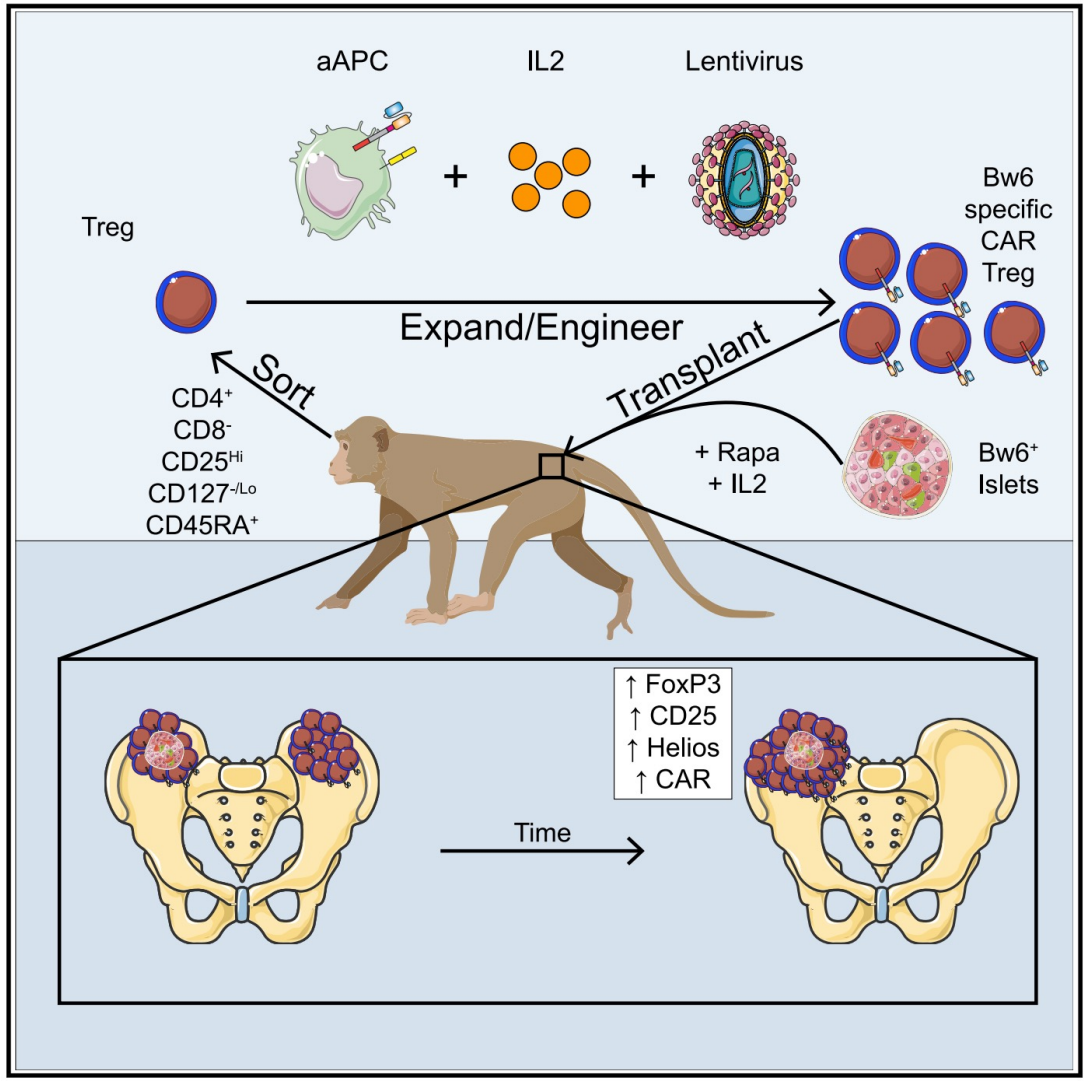
Luciferase+ A2-CAR Treg i.v. injection





Muller, Ferreira et al Front Immunol 2021

Non-human primate HLA allele-specific CAR Tregs for organ transplant tolerance



Day 0: αCD3.CD86 aAPC Stim.

↓

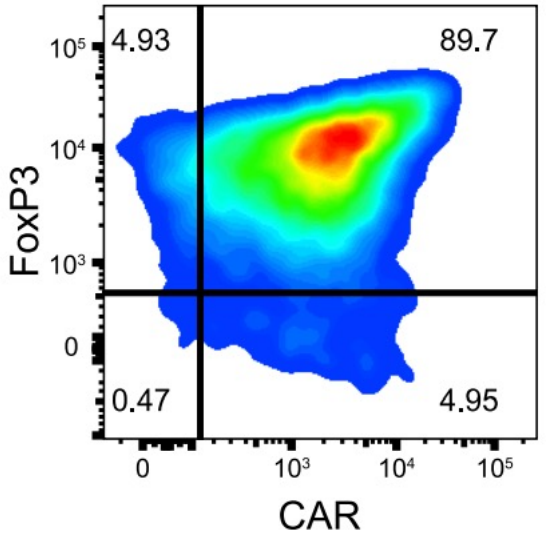
Day 7-9: Bw6.86 aAPC Stim.

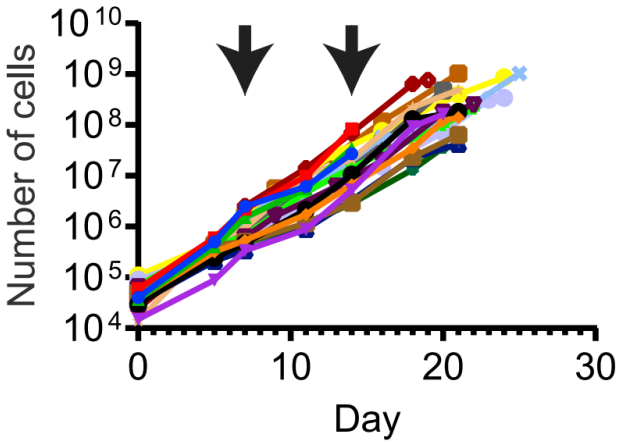
↓

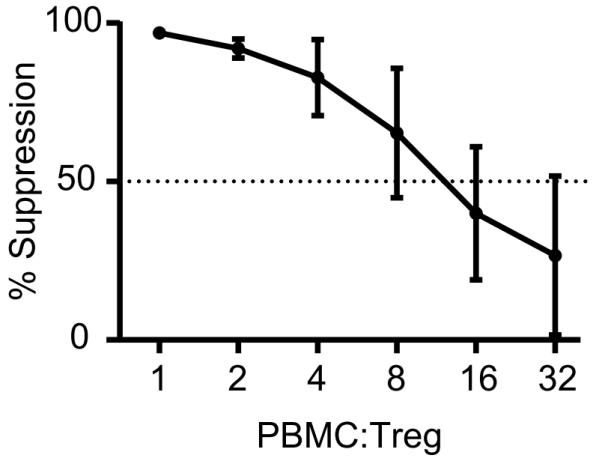
Day 14-16: Bw6.86 aAPC Stim.

↓

Day 20-23: Assay





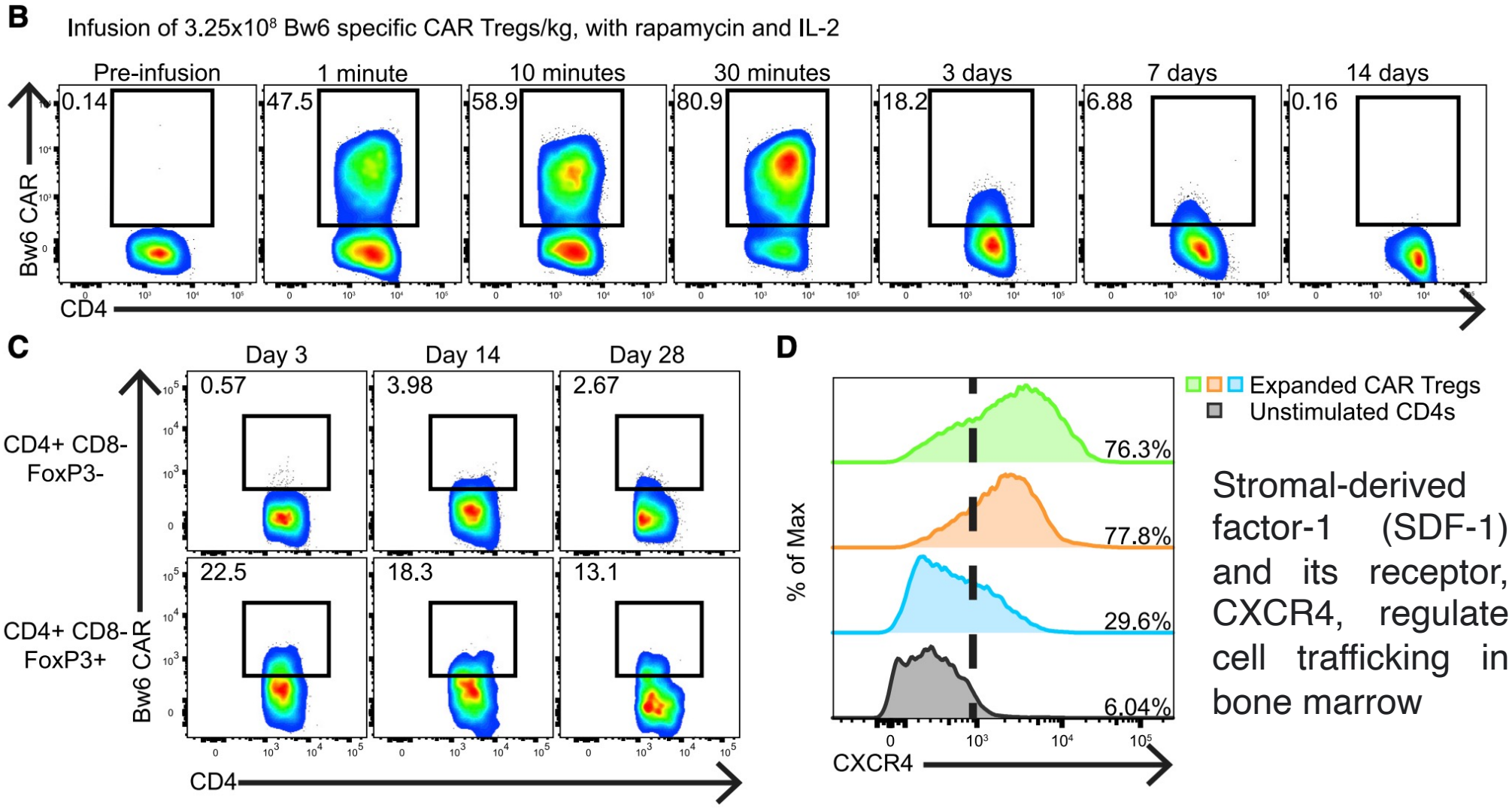


Ellis et al Cell Reports Med 2022

Macaque HLA-Bw7 CAR Tregs can be found in bone marrow, but not blood

Blood

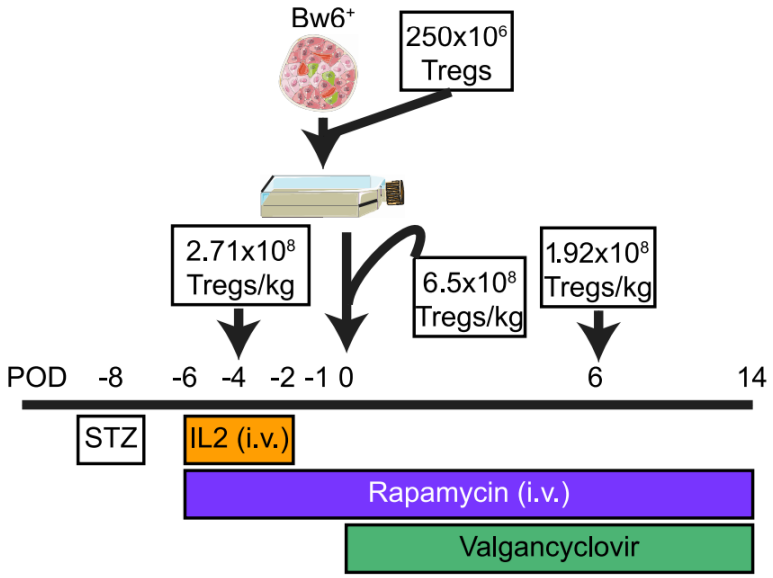
Bone marrow

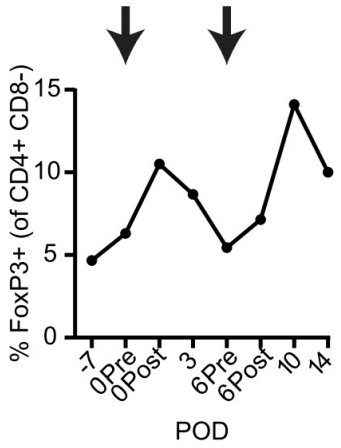


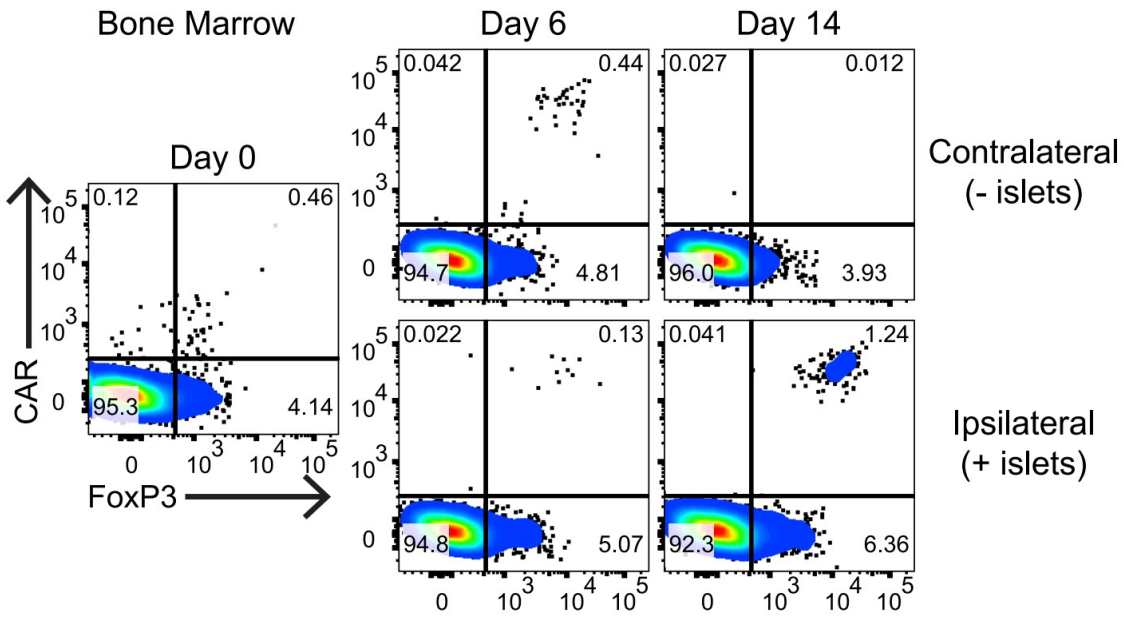
Stromal-derived factor-1 (SDF-1) and its receptor, CXCR4, regulate cell trafficking in bone marrow

Ellis et al Cell Reports Med 2022

Macaque HLA-Bw7 CAR Tregs traffic to HLA-Bw7+ pancreatic islet grafts and remain stable







STZ – streptozotocin, induces beta cell death
IL-2 – boosts Treg frequency
Rapamycin – selectively depletes Teff over Treg
Valganoclovir - antiviral

Ellis et al Cell Reports Med 2022

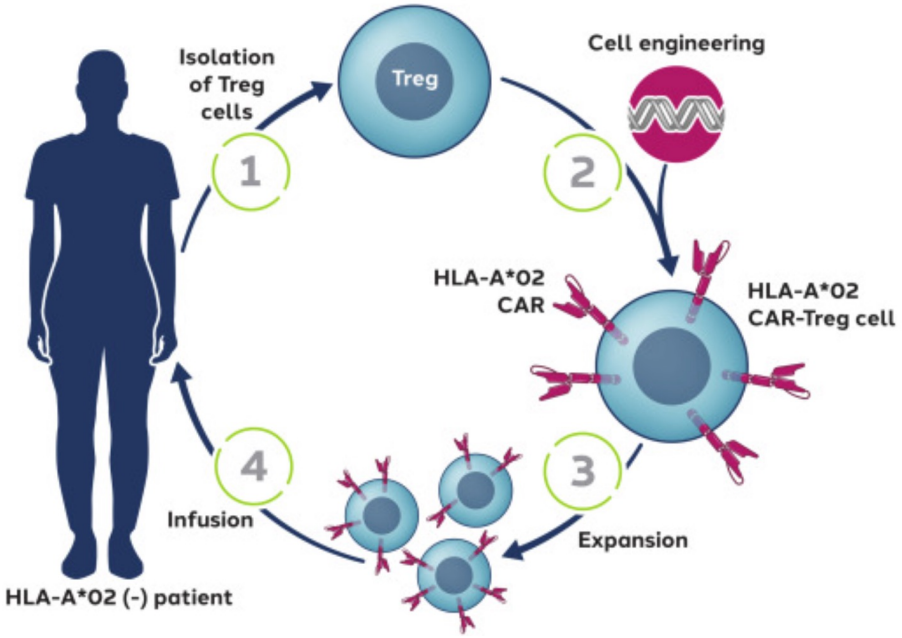
Clinical translation of CAR Tregs

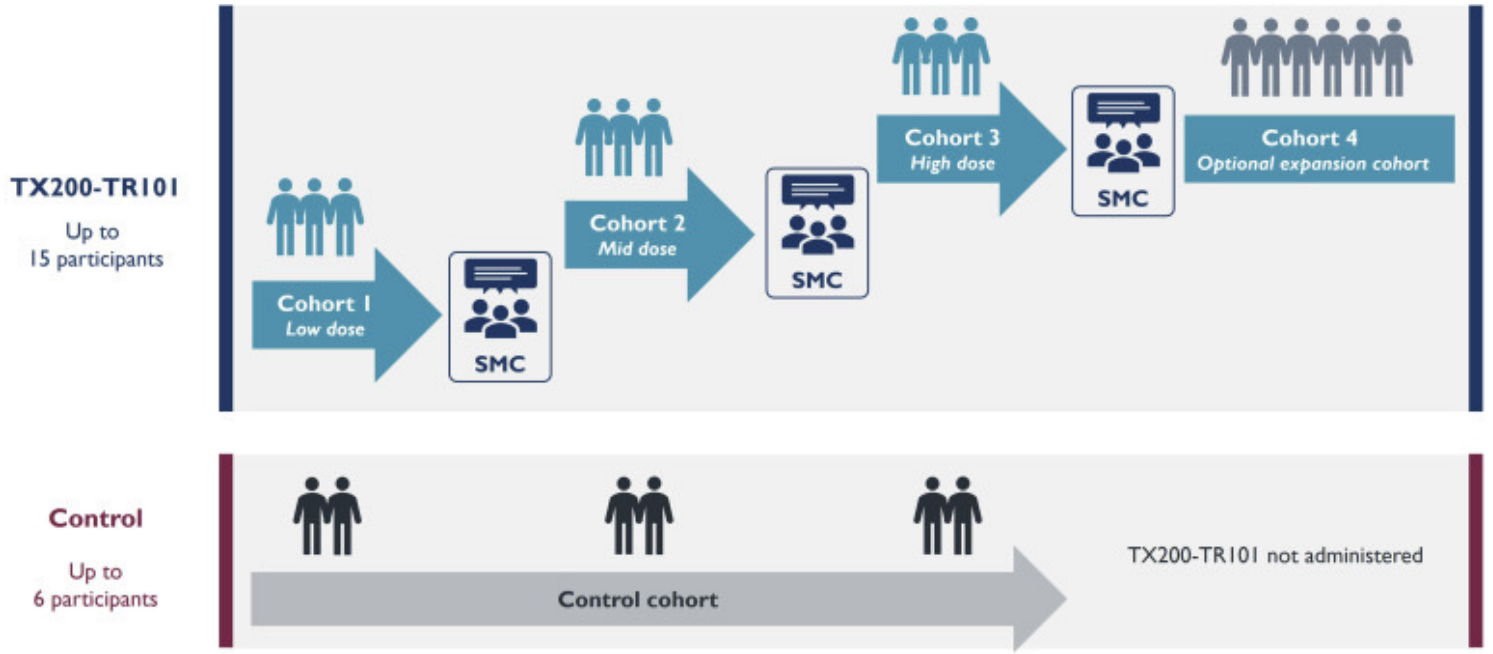
QEL-001: liver transplantation
LIBERATE TRIAL
https://clinicaltrials.gov/ct2/show/NCT05234190

TX200: kidney transplantation
STEADFAST TRIAL
https://clinicaltrials.gov/ct2/show/NCT04817774

SBT-77-7200: Rheumatoid arthritis

HLA-A2 CAR Treg to protect HLA-A2+ kidney transplants from rejection





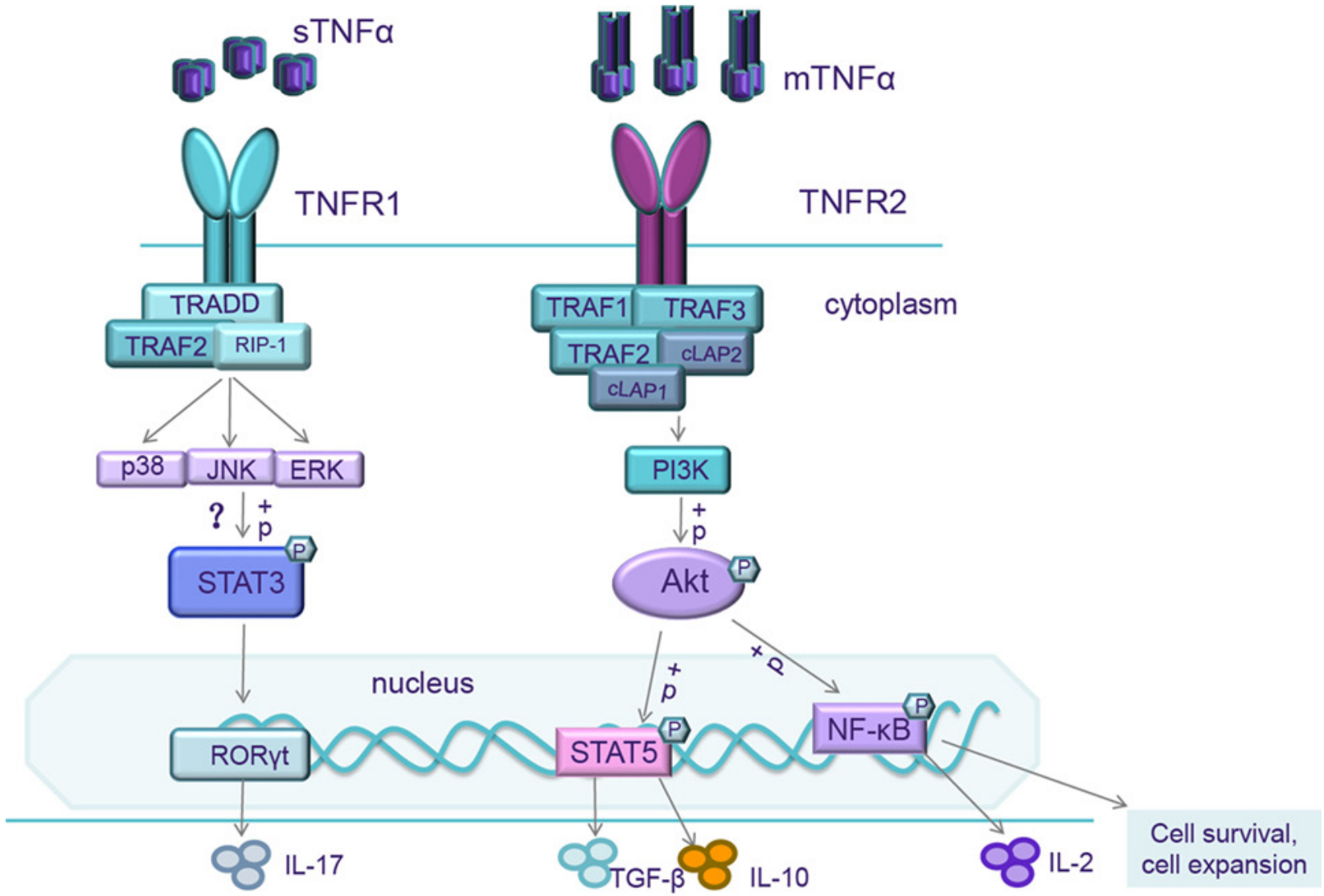
10^4 to 10^9 cells/kg body weight
An adult has 5 x 10^7 Tregs
(5 liters of blood x 10^9 PBMC/liter x 1% Treg/PBMC)

Schreeb et al, Kidney International Reports 2022

Ongoing research in Treg cell therapy

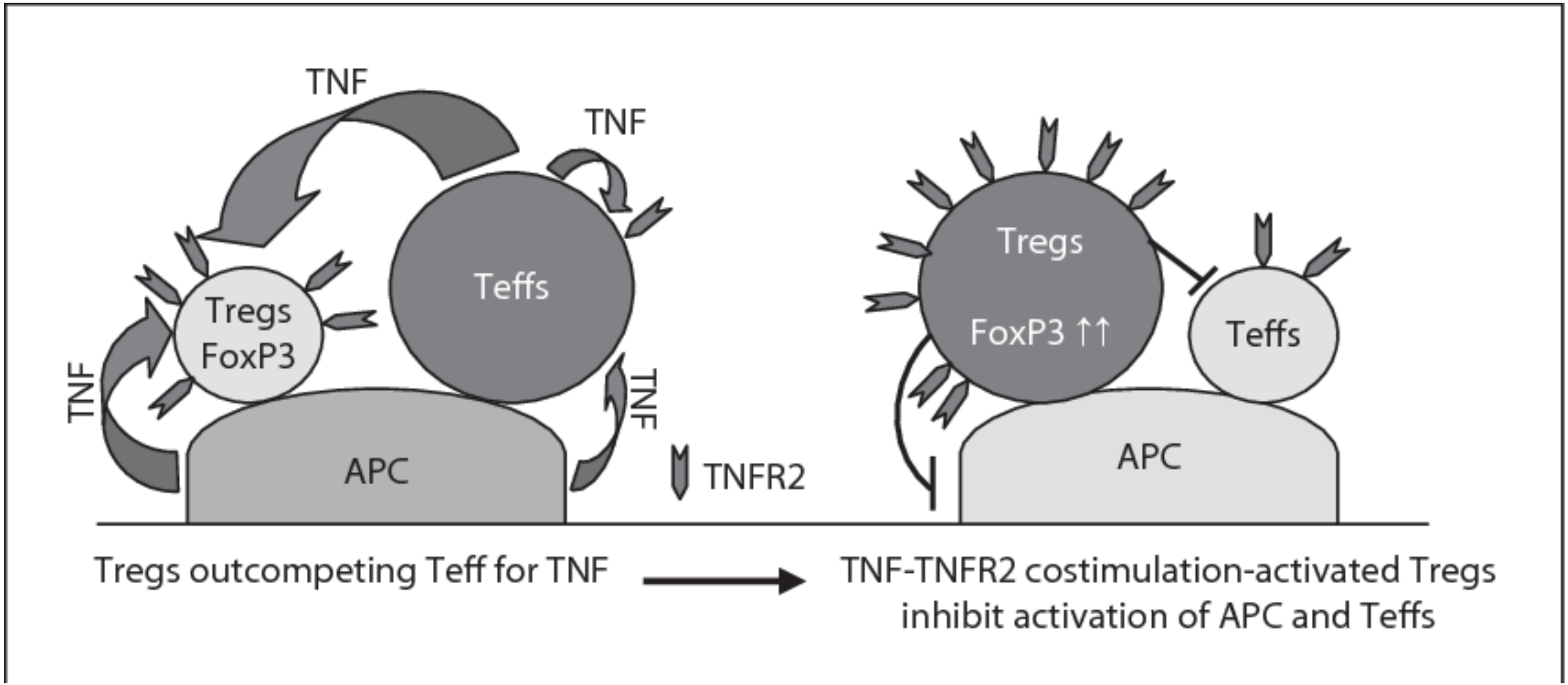
Maximizing Treg expansion

The dichotomous roles of TNF-a



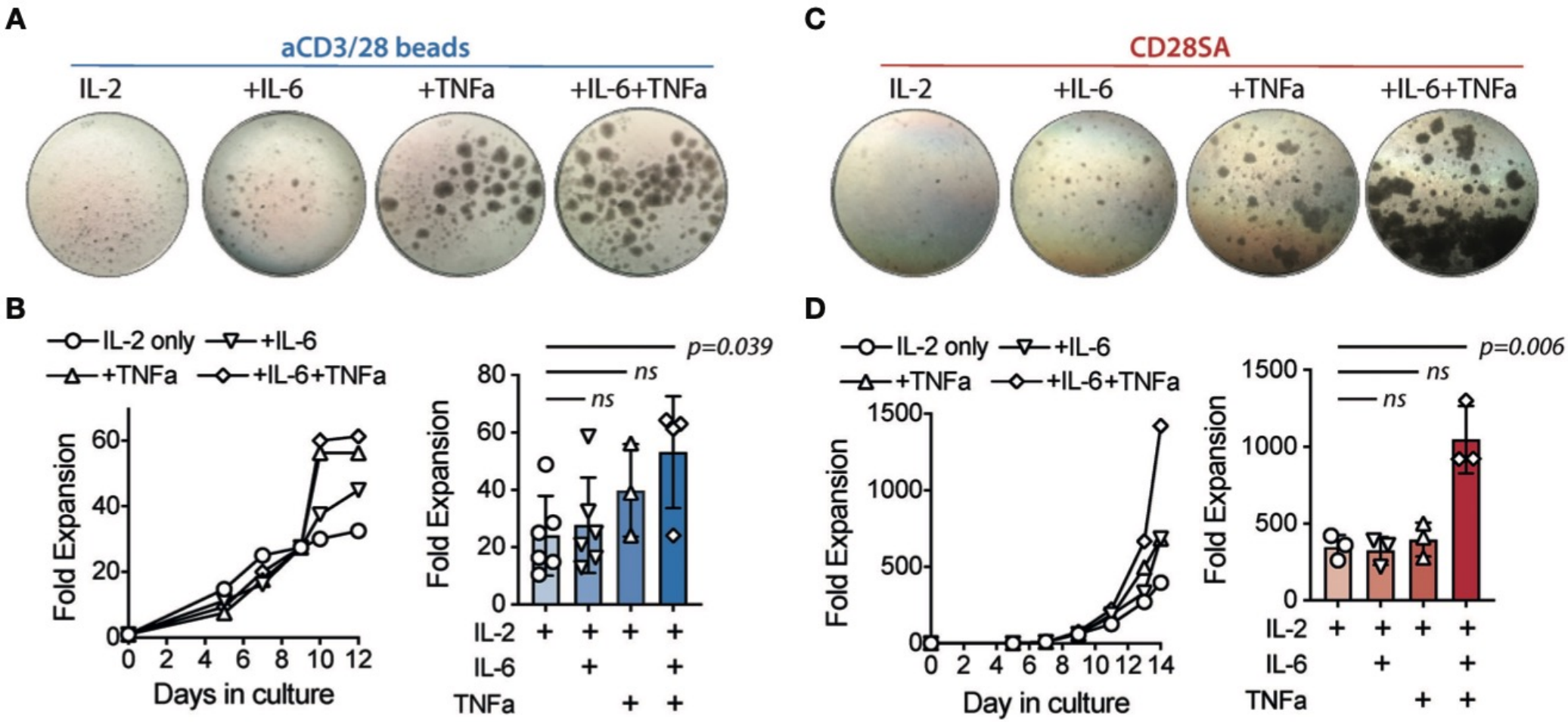
Yang et al, Front Immunol 2018

The dichotomous roles of TNF-a



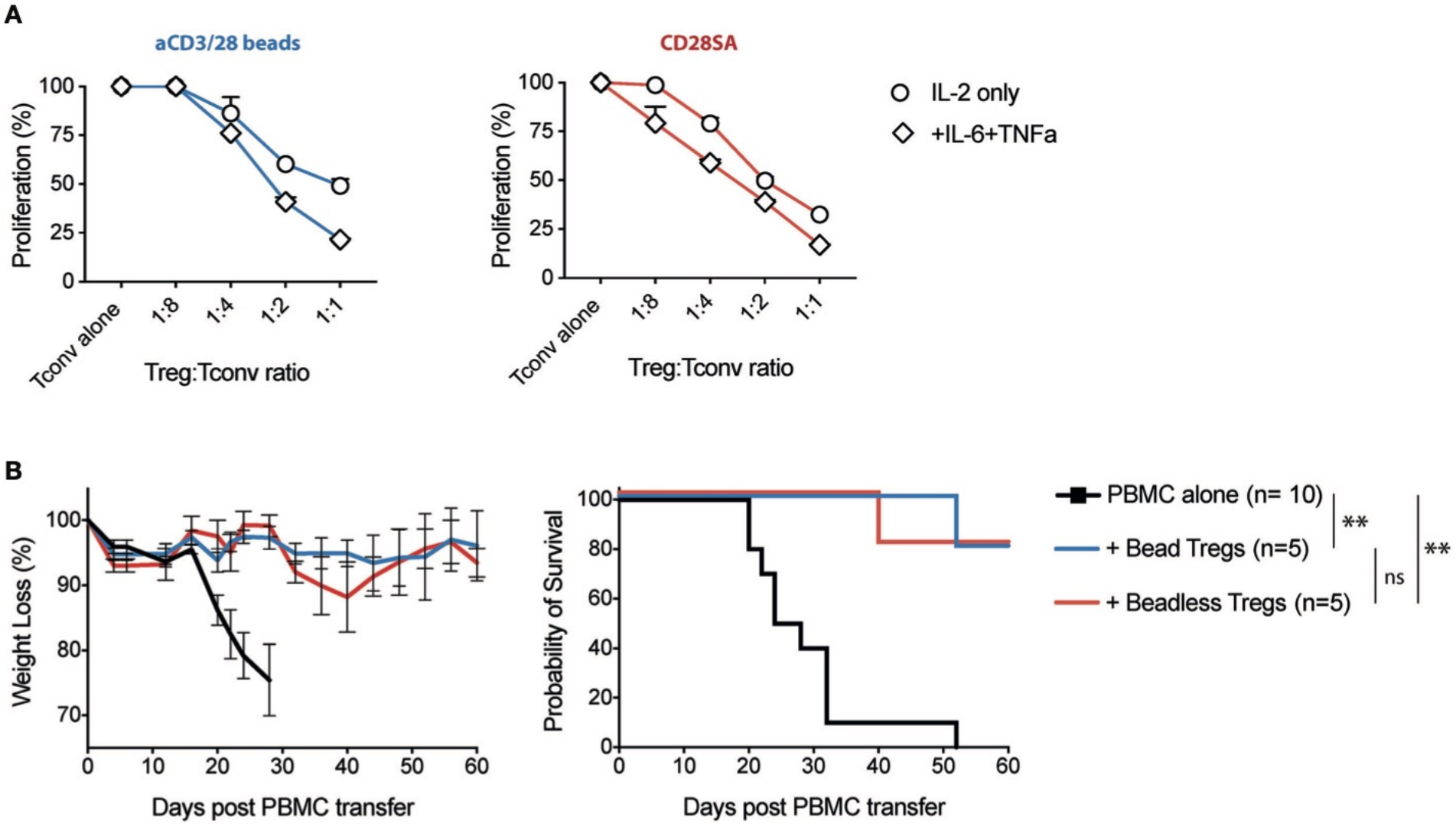
Chen & Oppenheim, Curr Dir Autoimmun 2010

IL-6 and TNF-α drive extensive proliferation of human Tregs

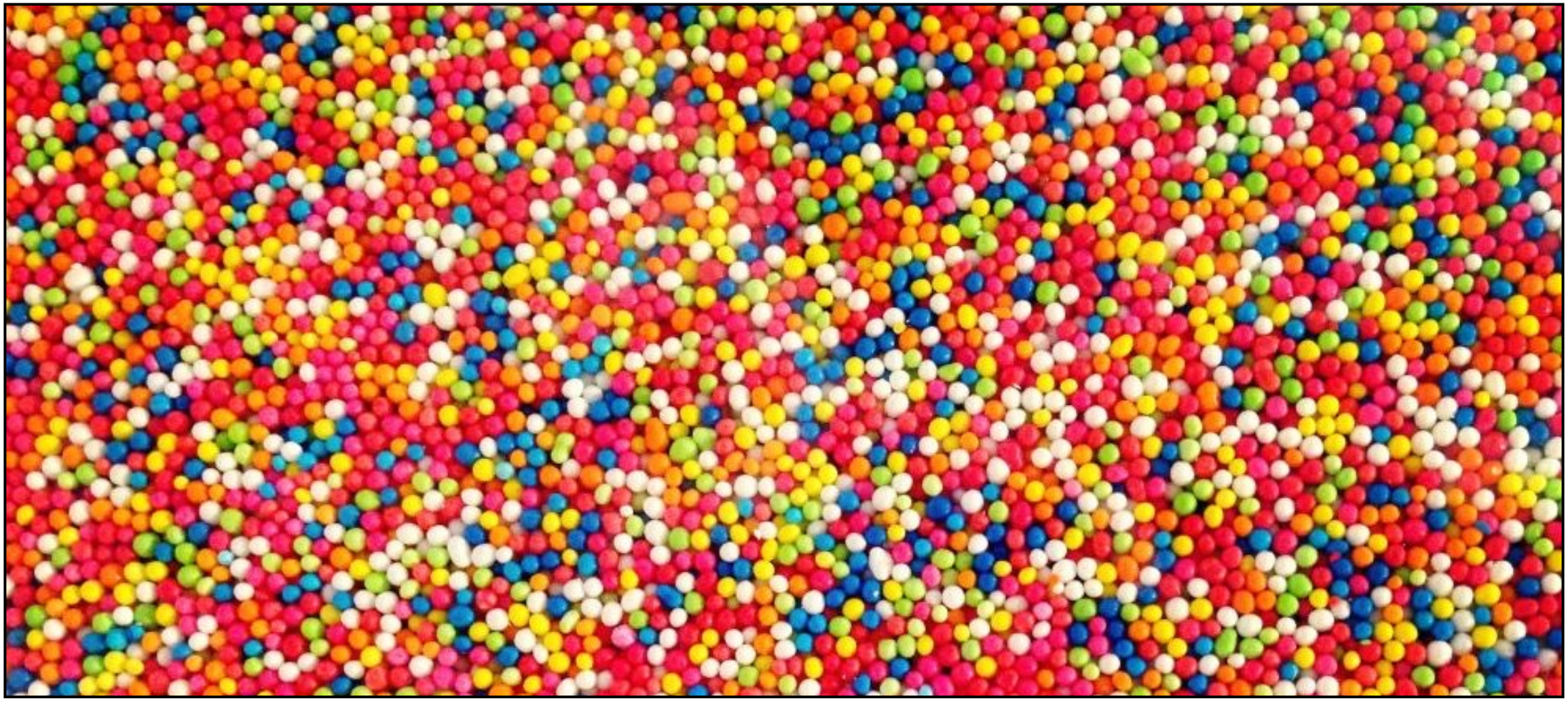


Skartsis et al, Front Immunol 2021

IL-6 and TNF-α do not compromise human Treg function

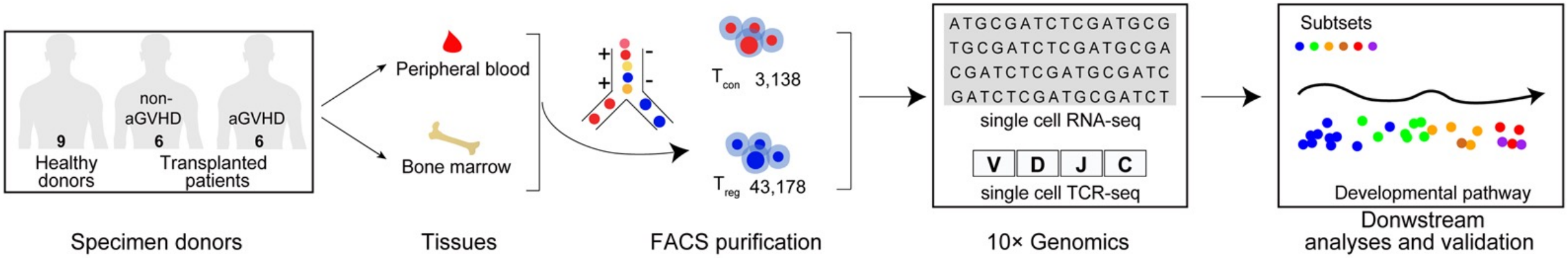


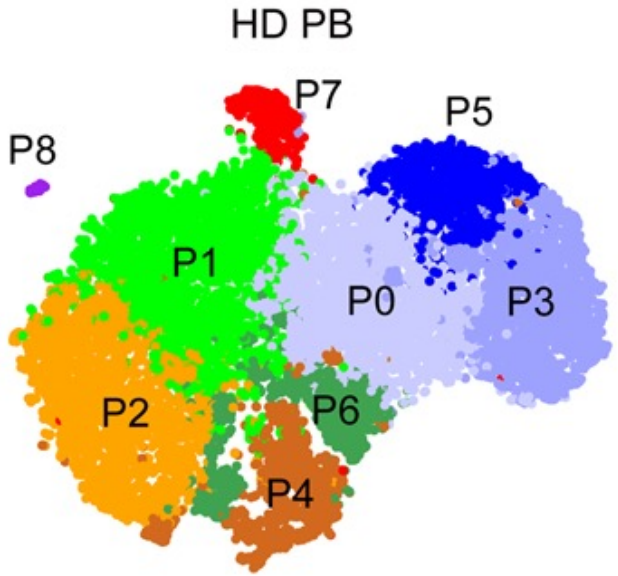
Skartsis et al, Front Immunol 2021

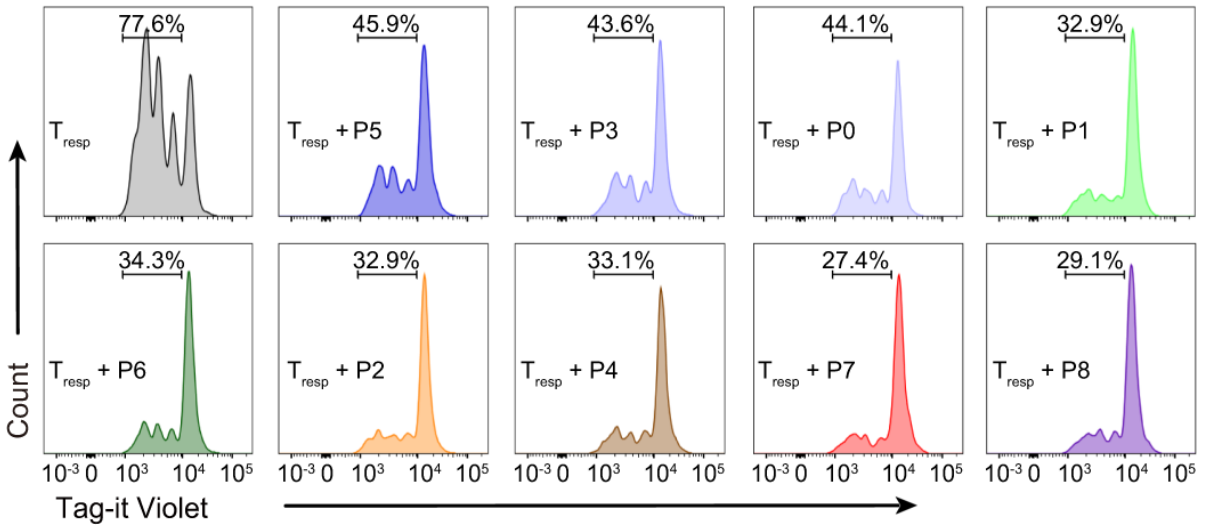


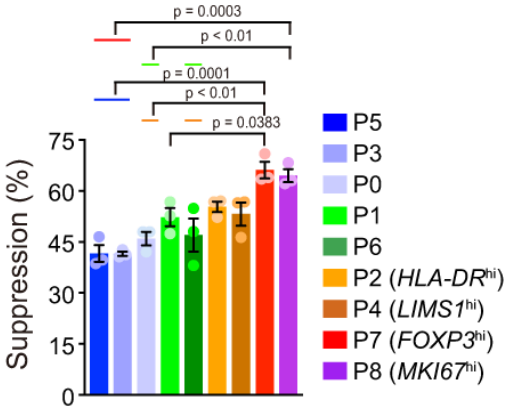
How heterogenous is the Treg compartment?

Human peripheral blood Tregs are heterogeneous



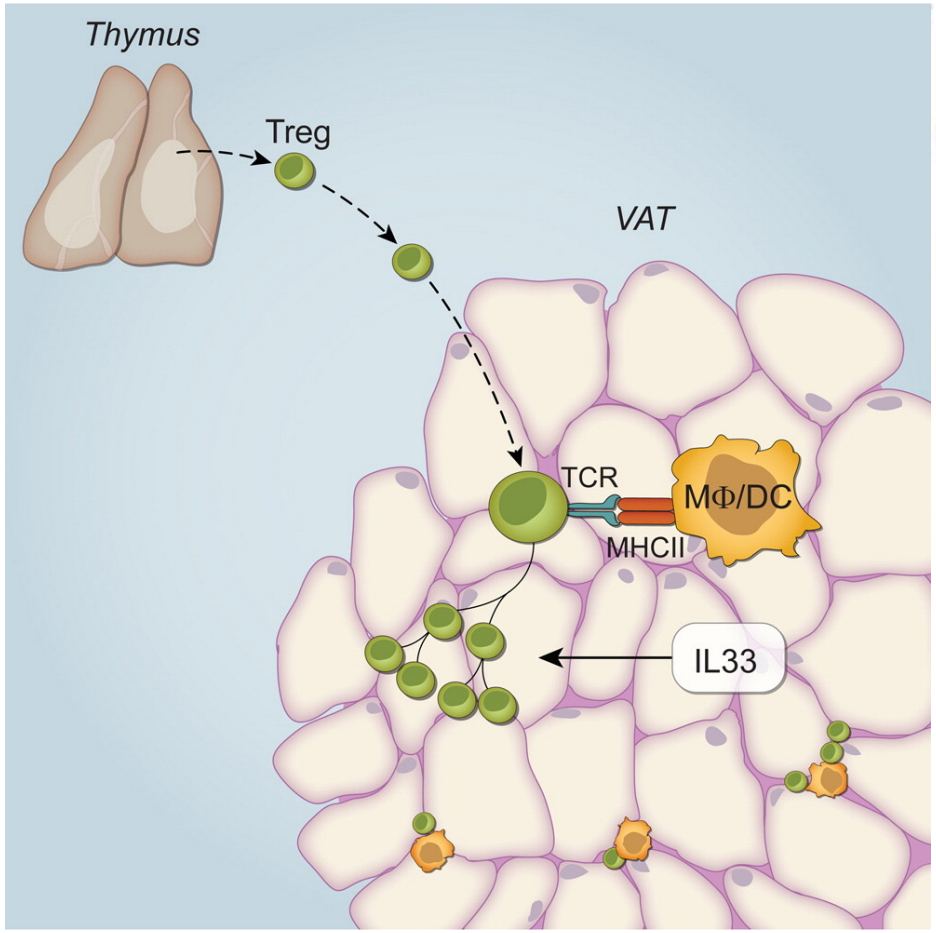


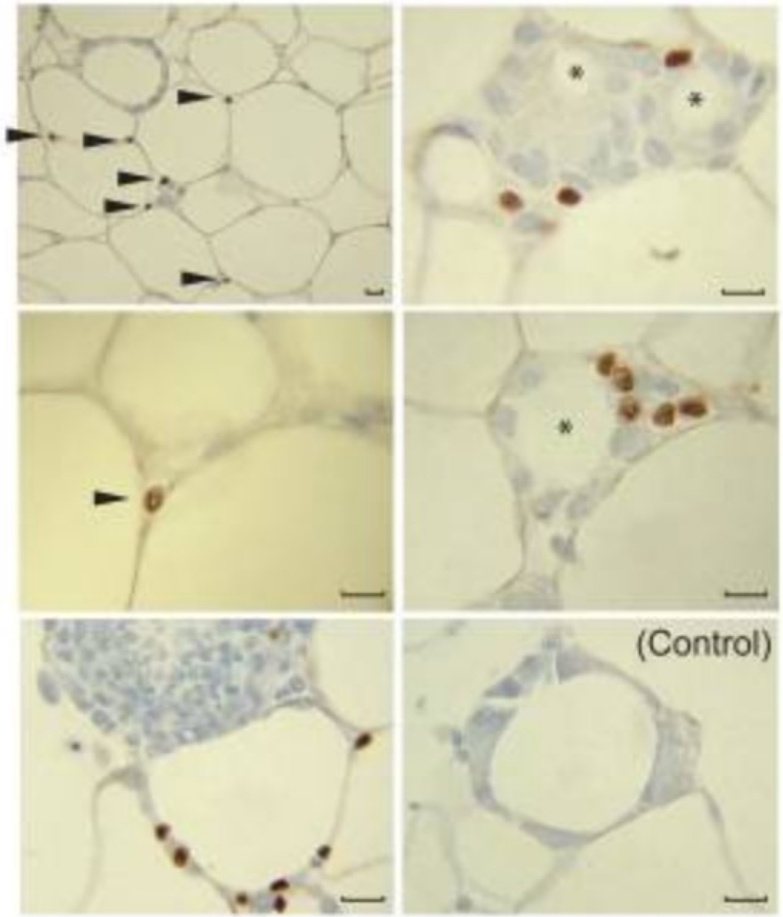


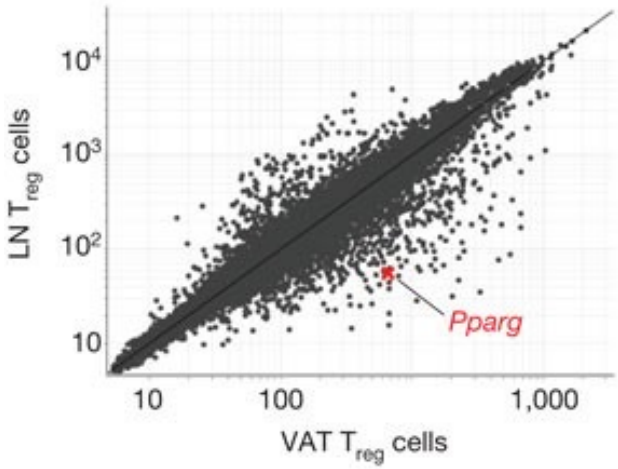


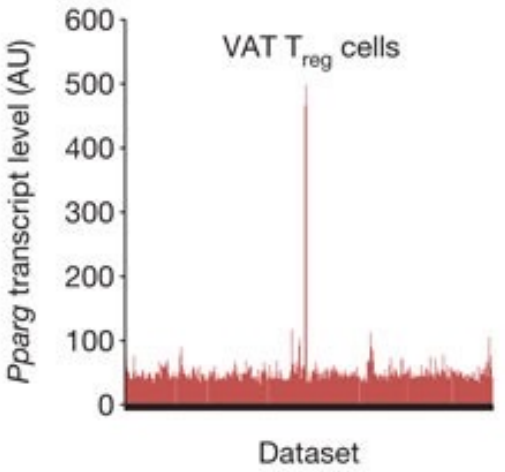
Luo et al Nat Comm 2021

The prototypical tissue-specific Treg: Adipose tissue Treg







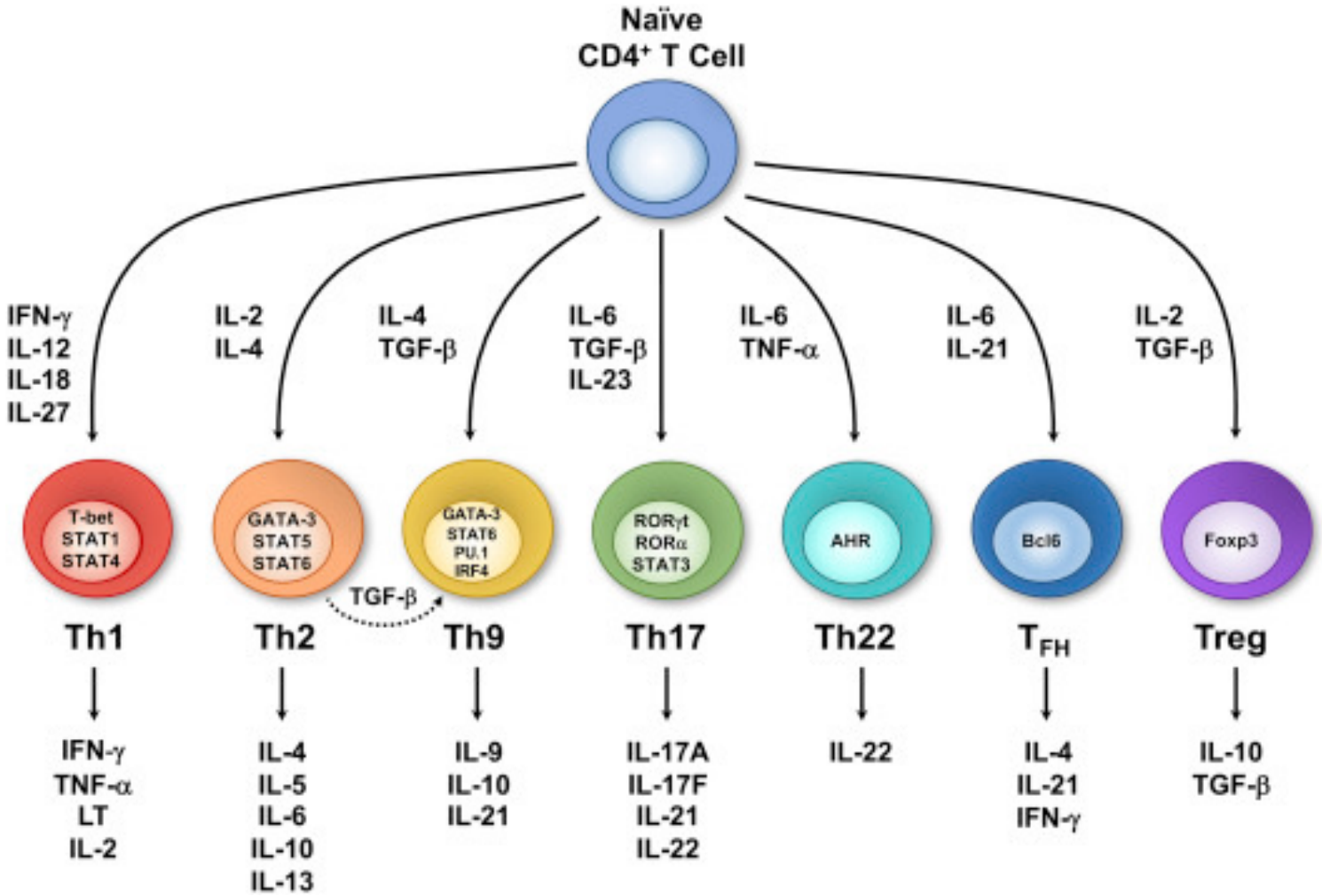


Kolodin et al Cell Metab 2015

Feuerer et al Nat Med 2009

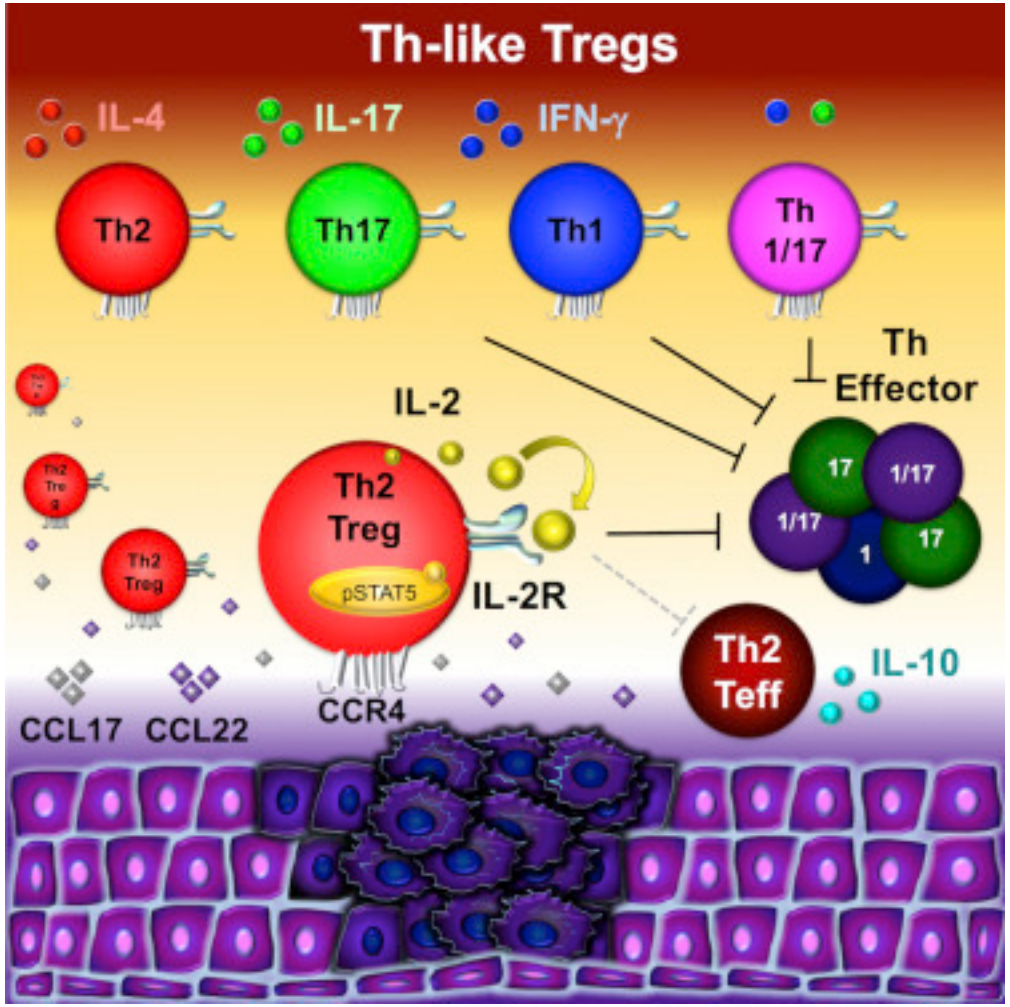
Cipolletta et al Nature 2012

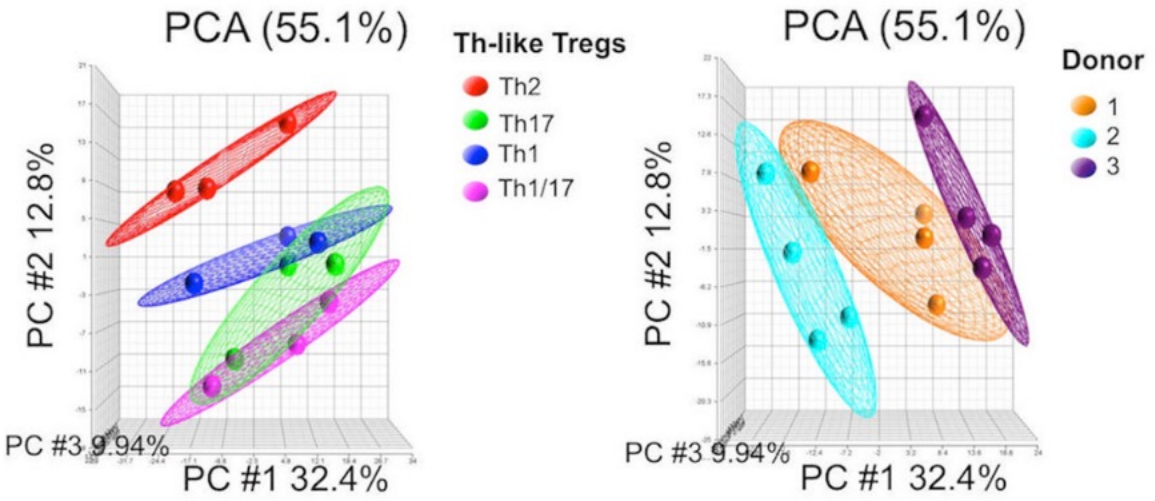
Naïve T helper cell polarization

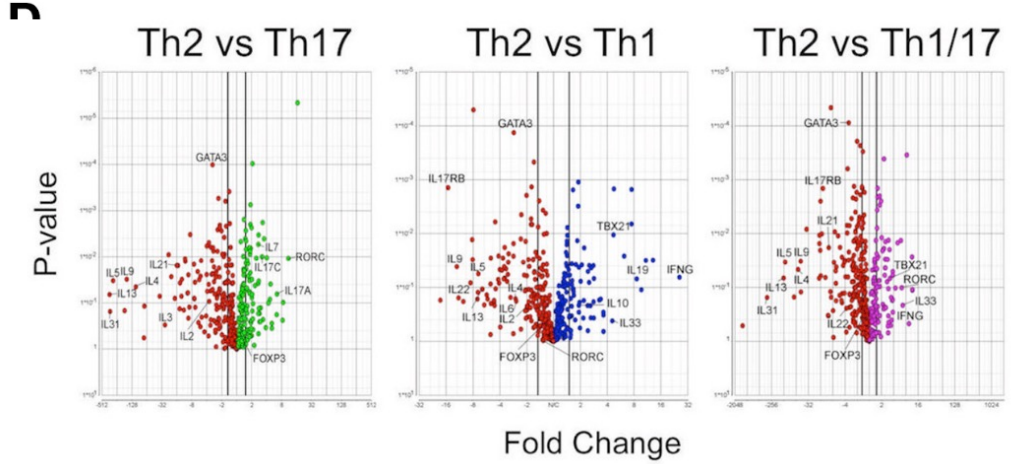


Liudahl et al Cell Rep 2017

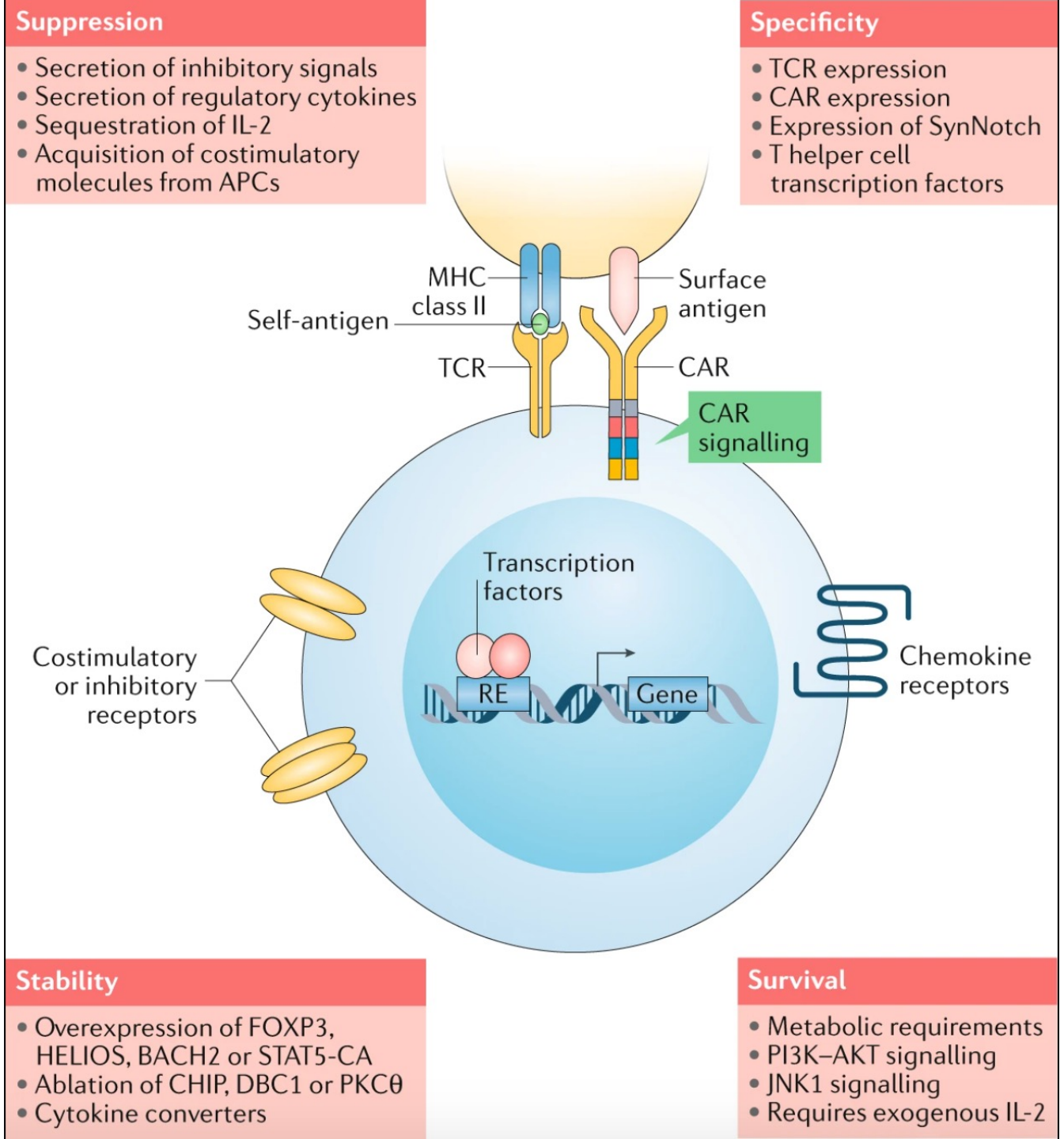
Th2-like Tregs are preferentially found in tissues







Halim et al Cell Rep 2017



Ferreira et al, Nat Rev Drug Discov 2019

Outstanding questions and future directions

- How does TCR ablation impact CAR Treg survival and function?
- What unique challenges do CAR Tregs face?
- Can CAR Tregs prevent disease in humans? Reverse it?
- What are the best targets and CAR designs for Tregs?
- What is the best source and manufacture protocol for Tregs?

MUSC
Ferreira Laboratory of Designer Immunology

Ferreira Lab

Designing and developing engineered immune cell therapies

for autoimmune disease, cancer, and aging

Contact us

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