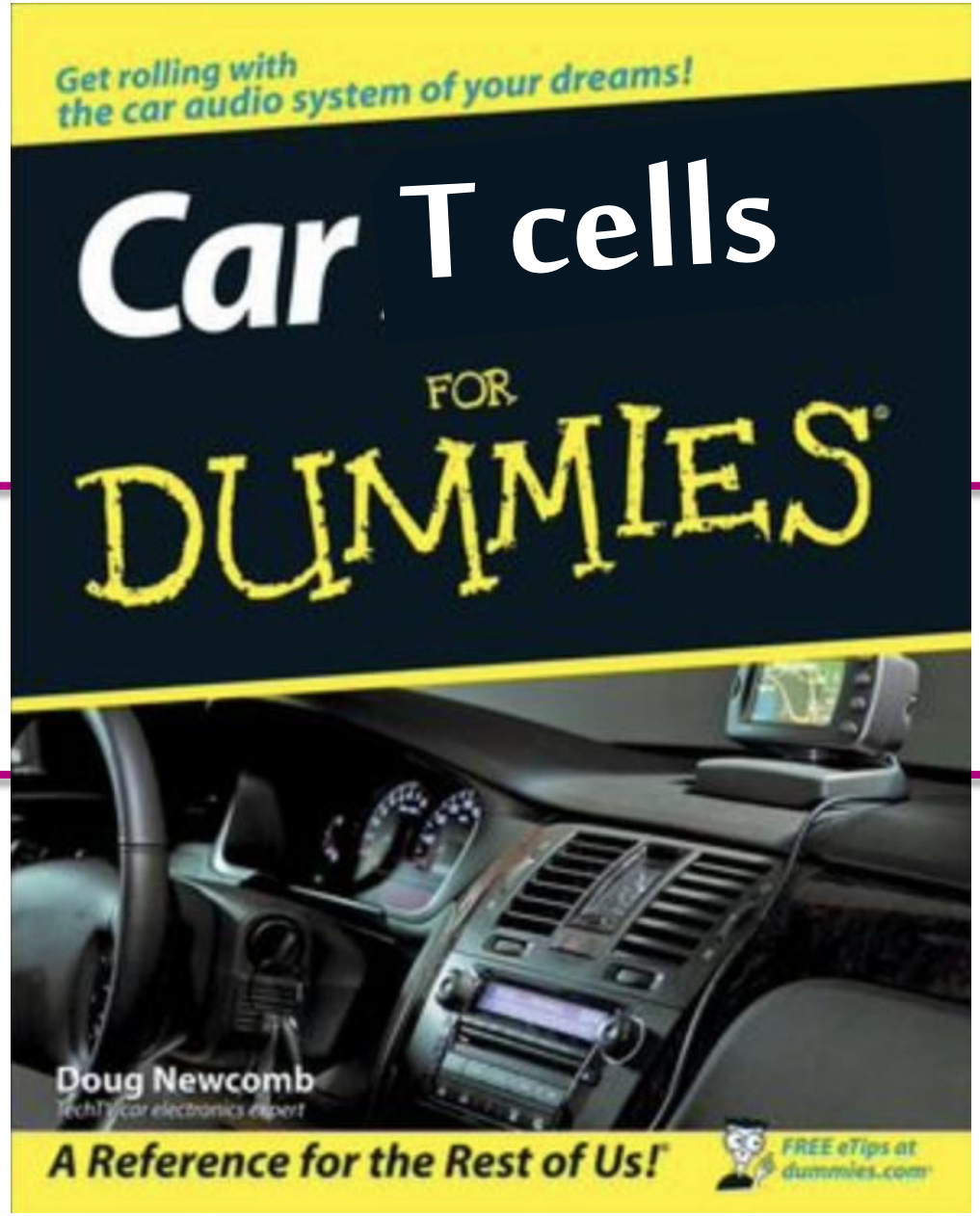
Université Paris Cité

imagine



Club d'ImmunoThérapie Cellulaire

CEnI Collège des Enseignants d'Immunologie

Université Paris Cité

imagine

for kids

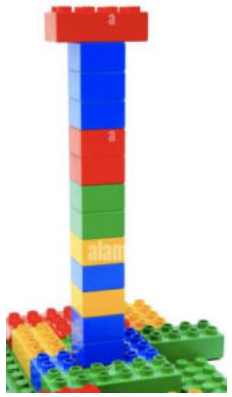
CAR T beyond cancer

Julien Zuber

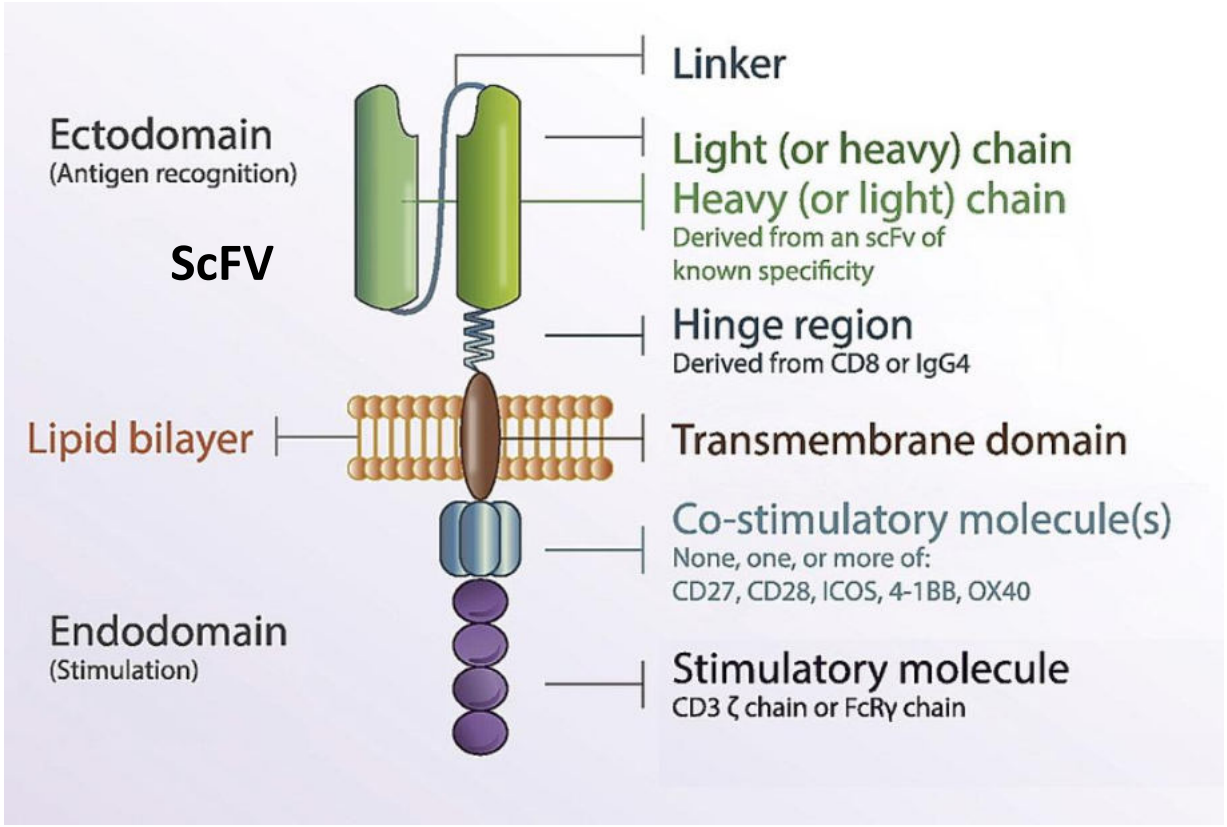
Lympho-Hematopoiesis Lab, Imagine Institute
Department of kidney and metabolic diseases, transplantation and clinical immunology, Necker Hospital

Club d'ImmunoThérapie Cellulaire

CEnI Collège des Enseignants d'Immunologie

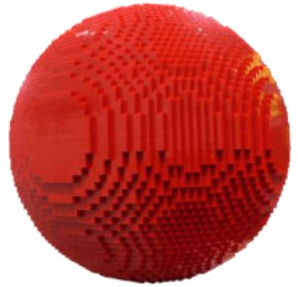


CAR design: draw and play



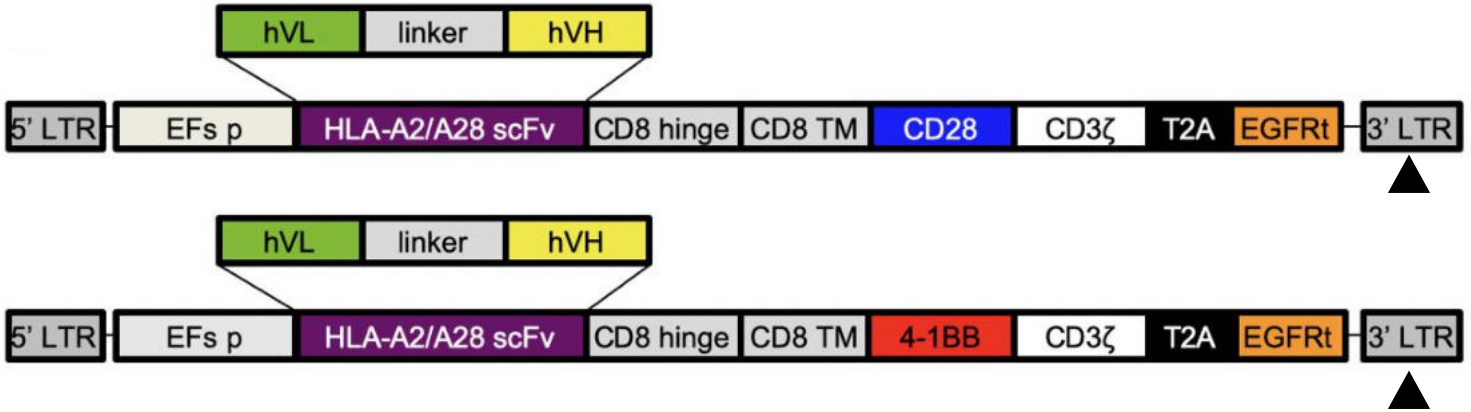
How to redirect T cells toward non tumor antigens?

Which cells should be engineered in non malignant diseases?

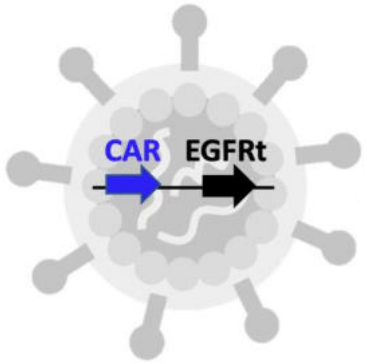


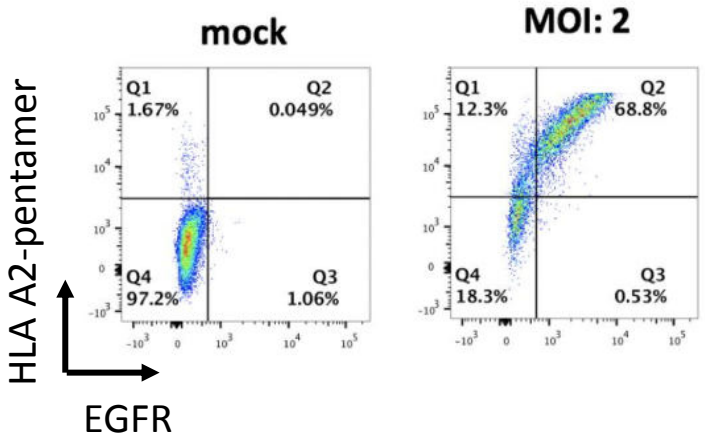
Self-inactivating lentiviral vectors

Soëli Charbonnier



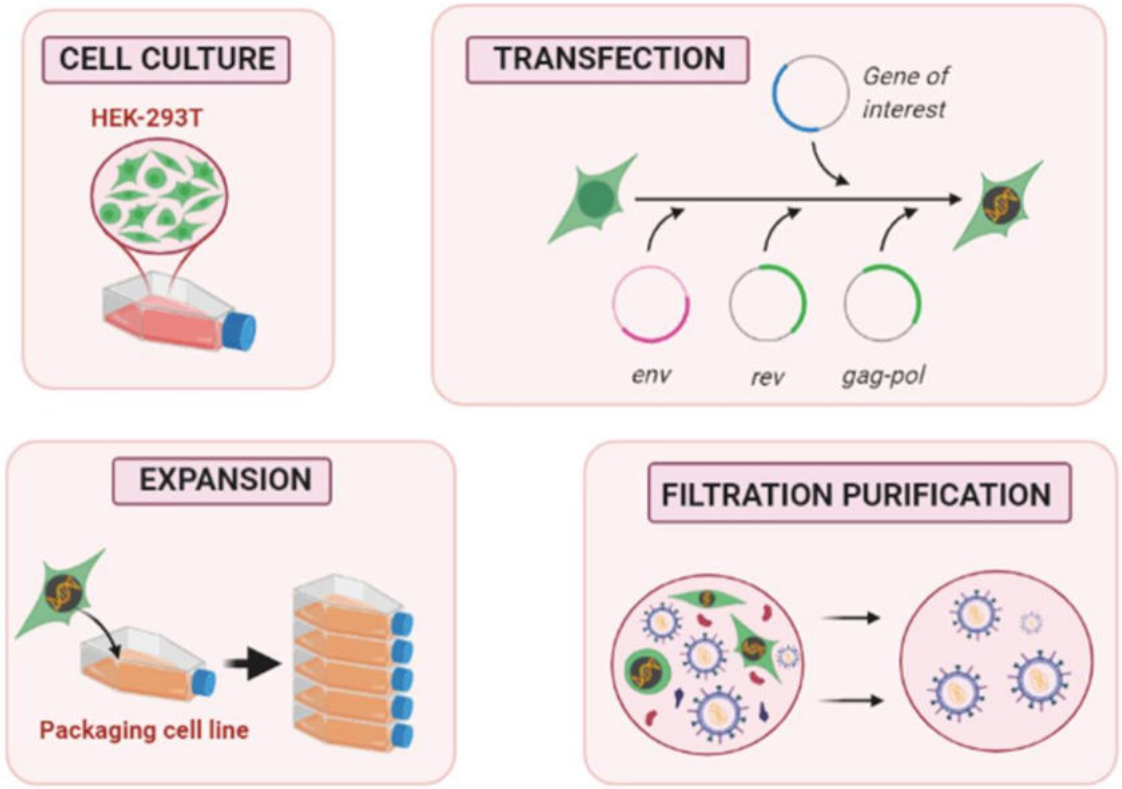
SIN LV vectors integrate genomic DNA, yet the promoter activity in the 3' LTR is abolished

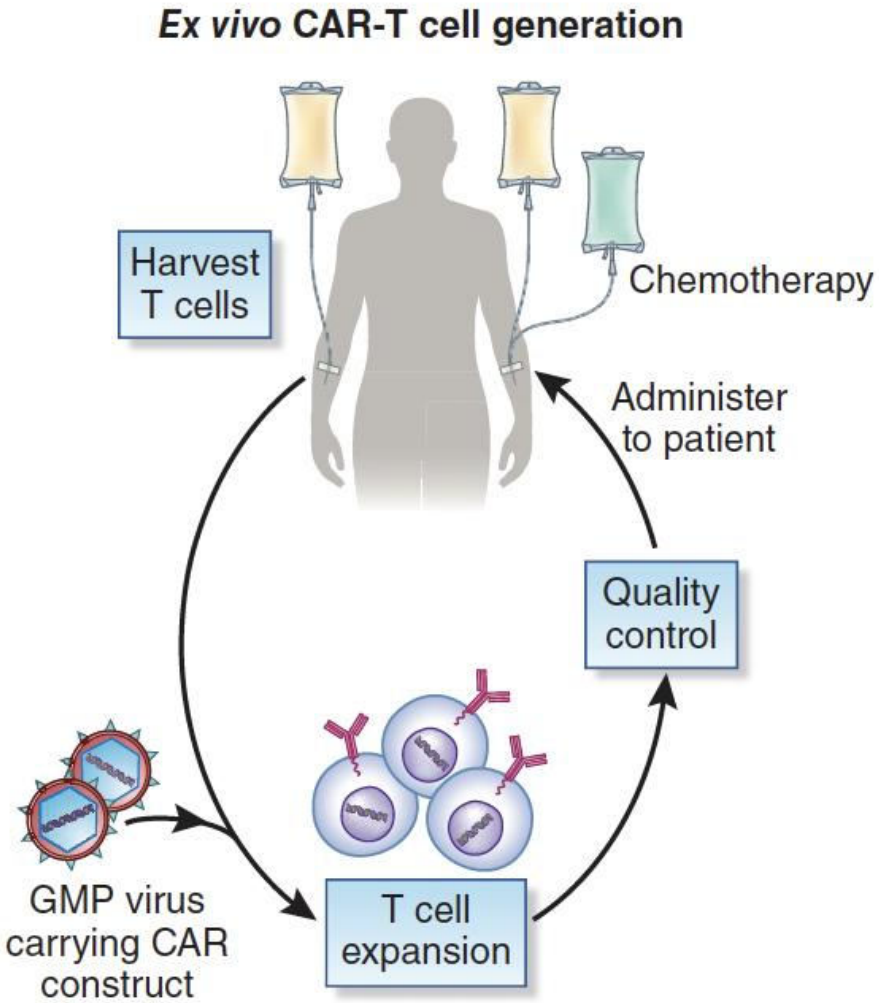




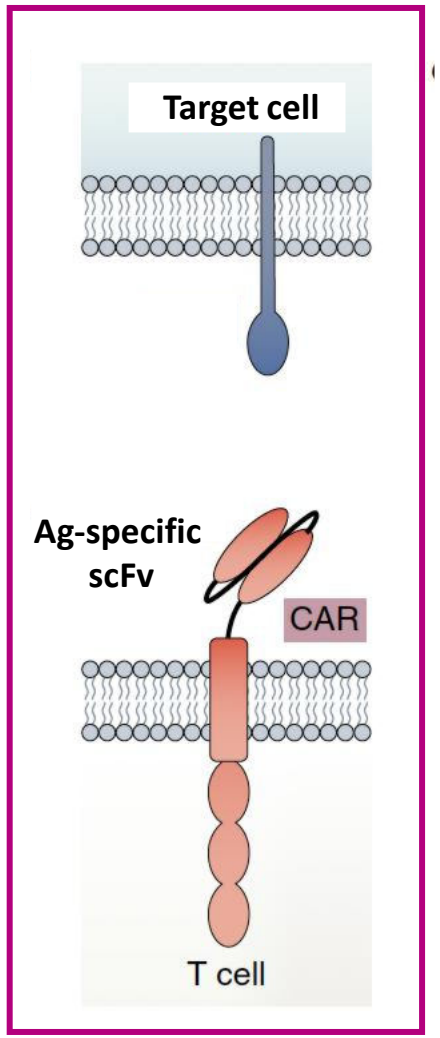
Non replicative lentiviral vectors – ex vivo manufacturing

1- Self-inactivating lentivirus vector (Δ in the 3' LTR)
2- Non-replicative lentivirus vector (packaging cells)
3- Ex-vivo manufacturing





CAR-T beyond cancer: targeting non tumor antigens



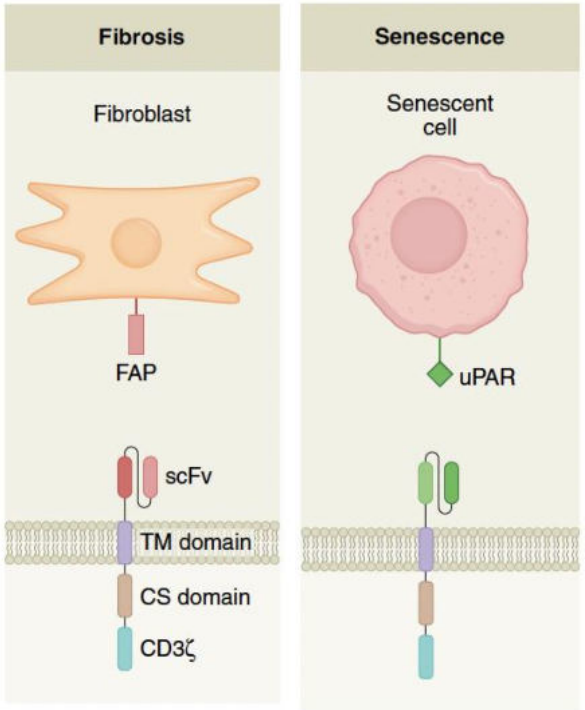
* CD19, BCMA
* FAP
* uPAR

→ Autoimmune diseases => E. Crickx

→ Sensitized patients awaiting kidney transplantation

Penn Medicine Leads $14m CAR T Immunotherapy Clinical Trial for High-Risk Kidney Transplantation Patients

Cytotoxic T cells

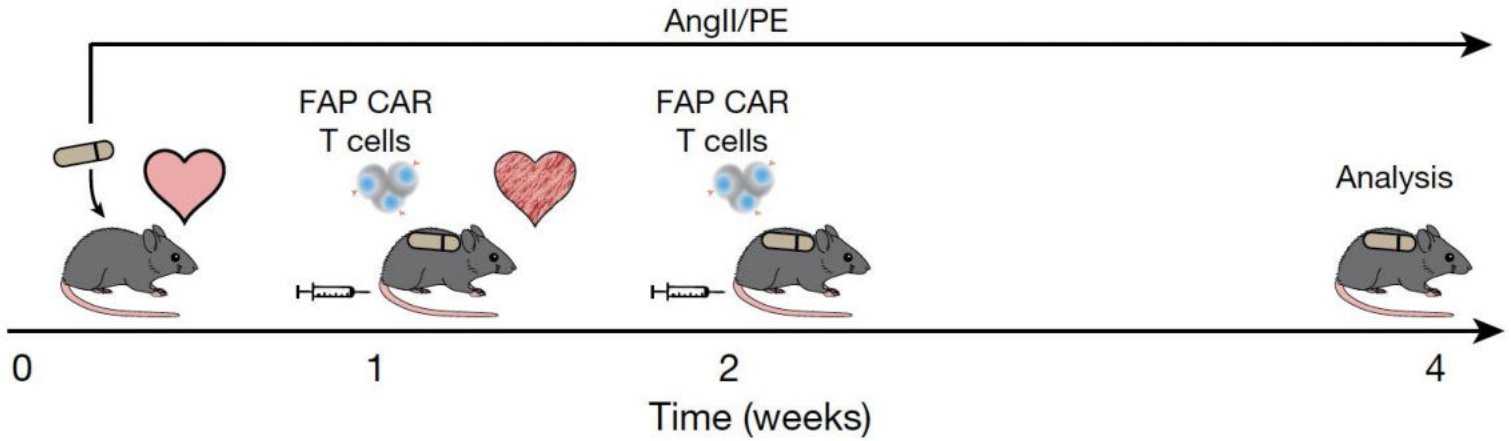


Adapted from

Boardman DA & Levings M Nature Biomedical Engineering 2019

FAP (Fibroblast Activation Protein)-specific CAR T reduce cardiac fibrosis

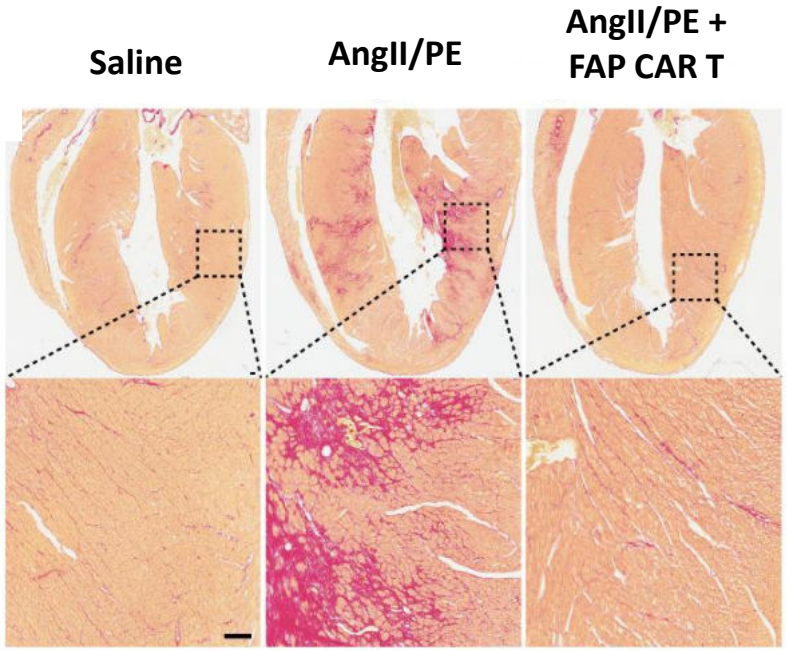
Cardiac fibrosis is induced by angiotensin II and phenylephrine

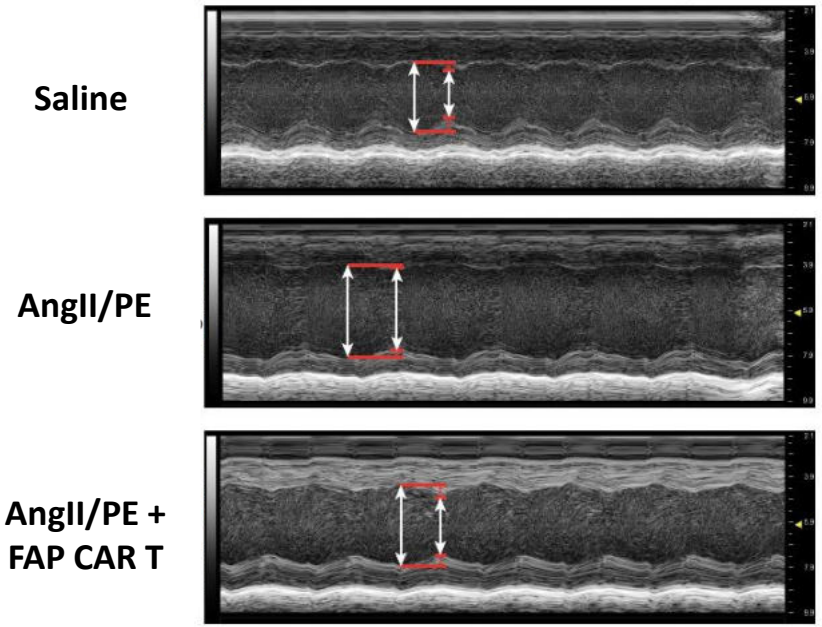


FAP-targeted CAR T

1- reduce fibrosis

2- restore cardiac function

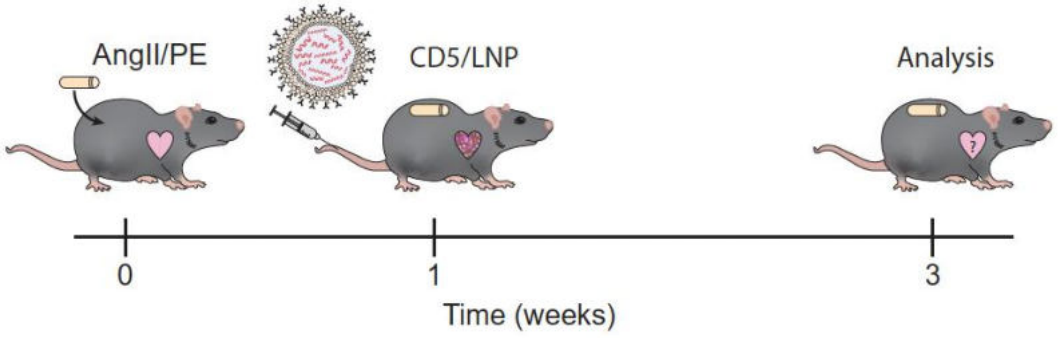


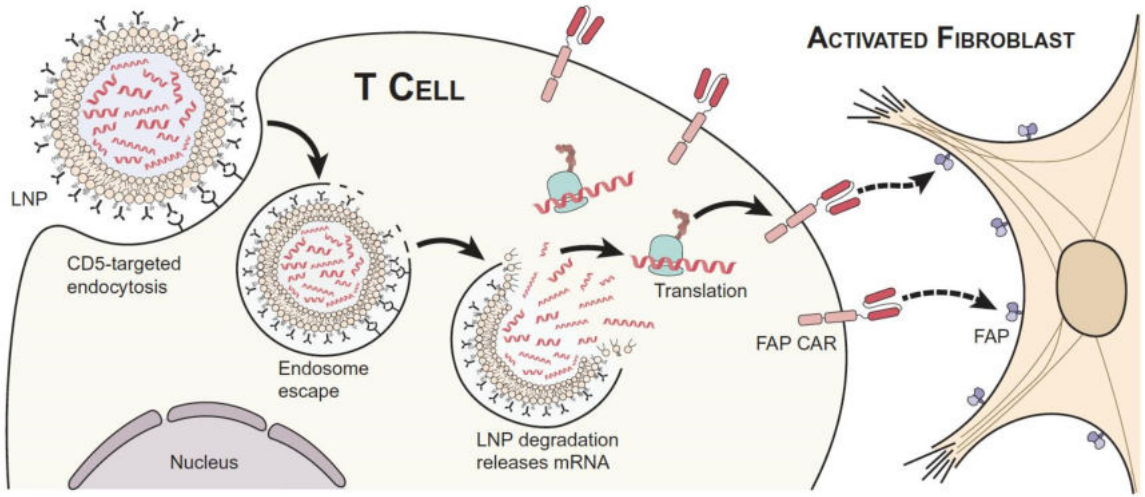


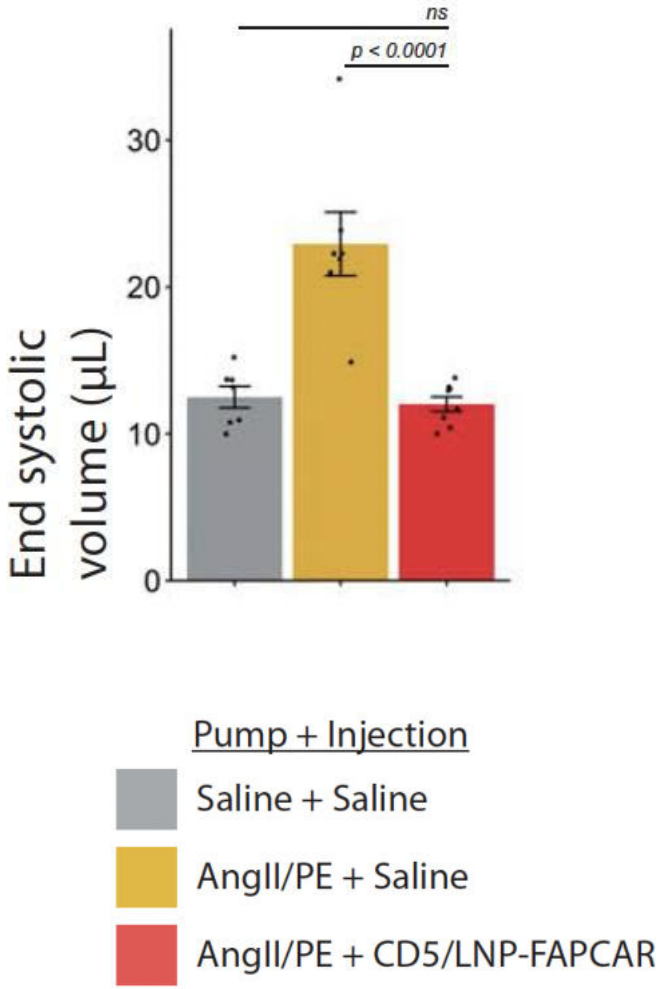
Aghajanian Nature 2019

FAP (Fibroblast Activation Protein)-specific CAR T reduce cardiac fibrosis

CD5-targeted lipidic nanoparticles







Rurik JG Science 2022

uPAR-targeted CAR T as senolytic agent

« When senescent cells linger, they can promote chronic inflammation resulting in age-related diseases ».

1- *PLAUR* (encoding the urokinase-type plasminogen activator receptor (uPAR)) was **the top gene** identified from 3 models of senescence in mice that fulfilled the two selection criteria:

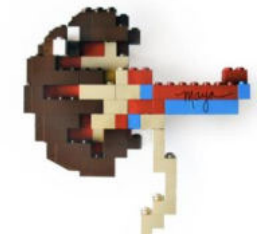
- **Greatest magnitude of upregulation (senescent / non senescent cells)**
- **Low expression in vital tissues**

2- *PLAUR* was also upregulated in public datasets of **human** senescent cells, and absent in many vital organs

3- suPAR is secreted by senescent cells as part of the senescence-associated secretory phenotype (**SASP**).

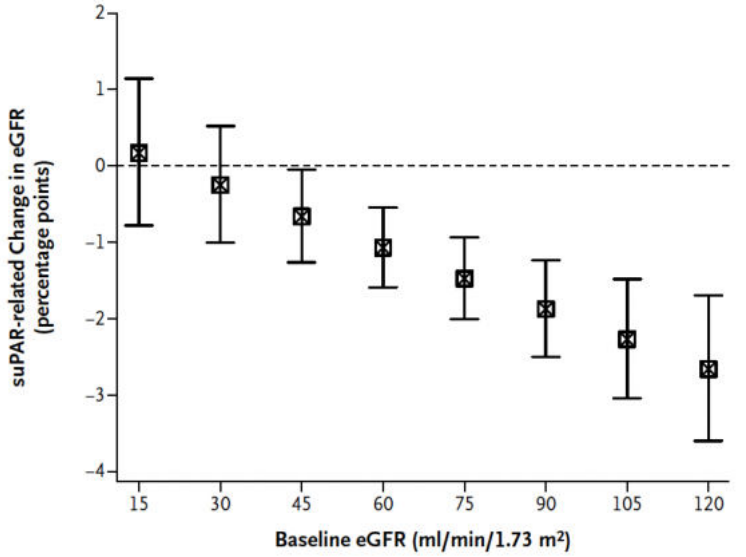
Mice that received senolytic uPAR-specific CAR T cells demonstrated improved outcome of liver fibrosis in animal models of non-alcoholic steatohepatitis

Amor C Nature 2020



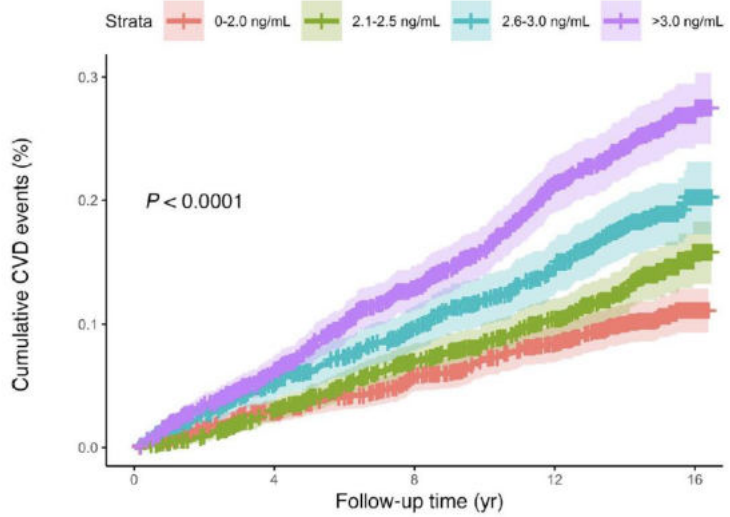
uPAR and kidney: something new out of the old?

suPAR as the circulating factor in FSGS



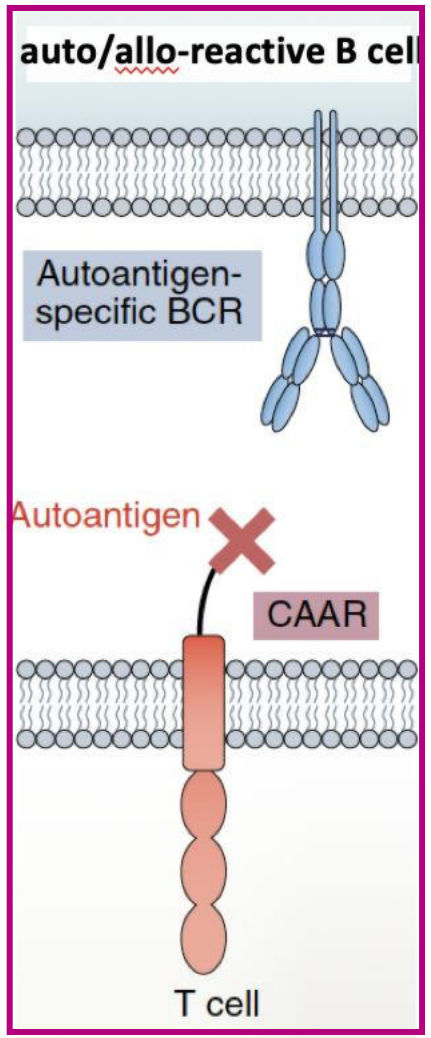
➡ **Elevated level of suPAR is independently associated with incident CKD and accelerated decline in the eGFR**

➡ **suPAR is predictive of cardiovascular events.**
suPAR promotes aorta infiltration by inflammatory macrophages



Quaglia, Musetti NEJM 2016, Hayek NEJM 2015, Hayek Nat Med 2017, Hindy JCI 2022

Chimeric Auto-Antigen Receptor (CAAR) to get rid of memory B cells



* **Desmoglein 3** → **Pemphigus vulgaris**
* **Musk** → **Myasthenia gravis**
* **PLA2R** → **Membranous nephropathy**

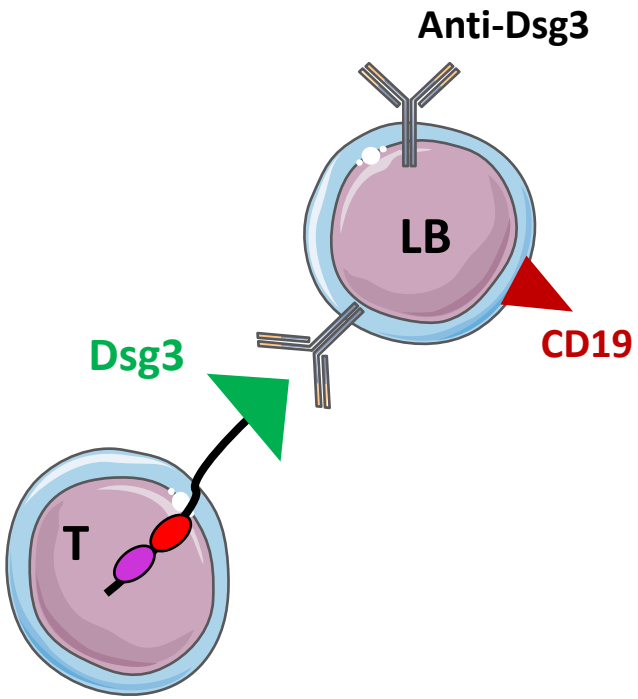
Cytotoxic T cells

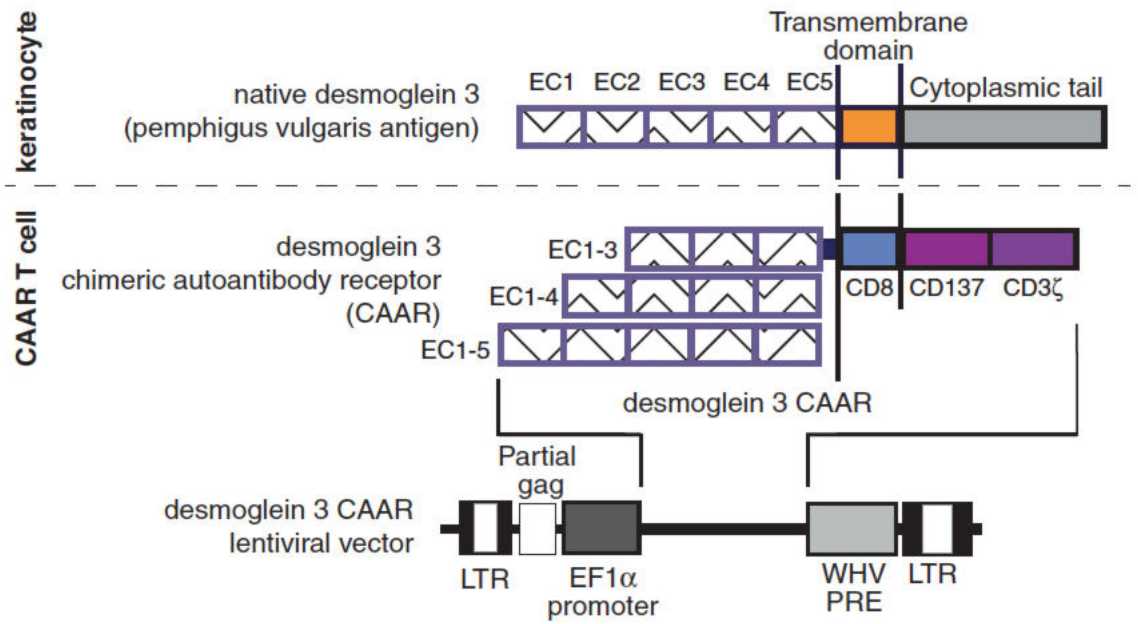
What's next ?

- Alloreactive HLA molecules
- ADAMTS13

CAAR: cytotoxic response redirected against self-reactive memory B cells

Antigen-specific targeting of memory B cells in a rodent model of pemphigus vulgaris

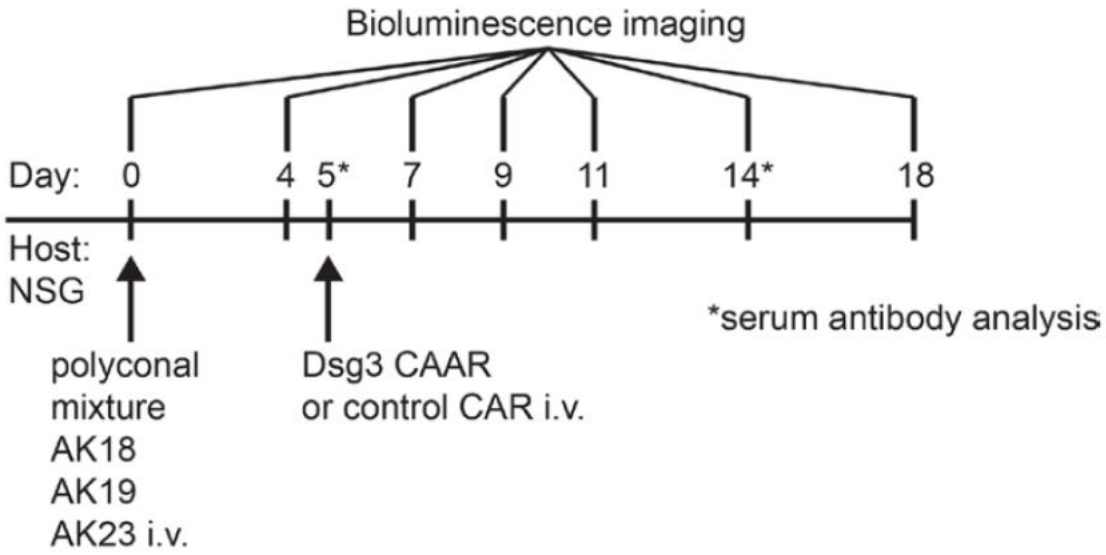




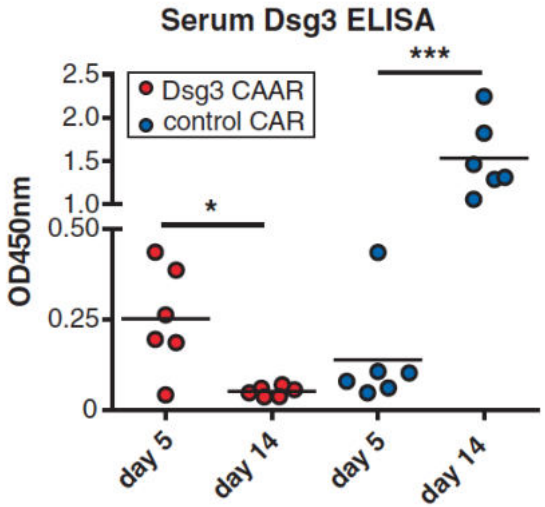
Ellebrecht Science 2016

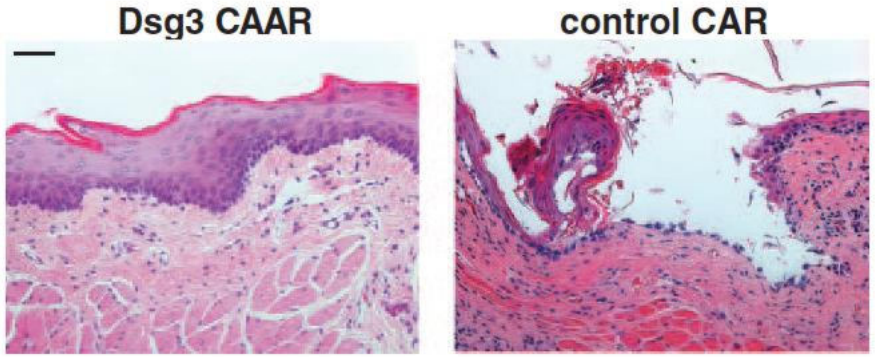
CAAR: cytotoxic response redirected against self-reactive memory B cells

Intravenous administration of a mix of 3 hybridomas that produce anti-Dsg3 antibodies



Dsg3 CAAR-T eliminate hybridoma cells, reduce anti-Dsg3 antibody levels, and prevent skin blistering

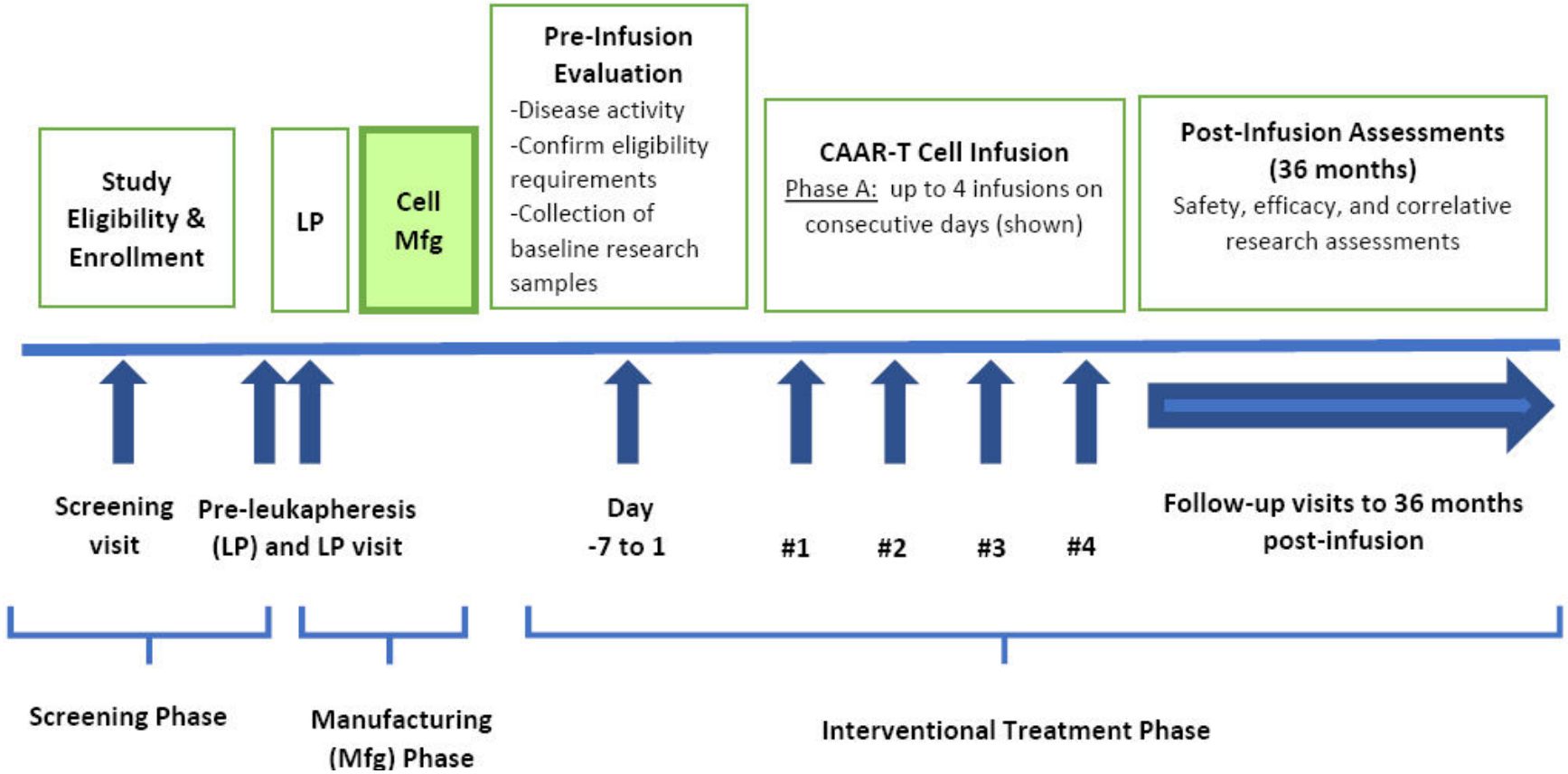




Ellebrecht Science 2016; Lee JCI 2020

Dsg3 CAAR already moved to the Clinic

Phase I: DSG3-CAAR-T cells

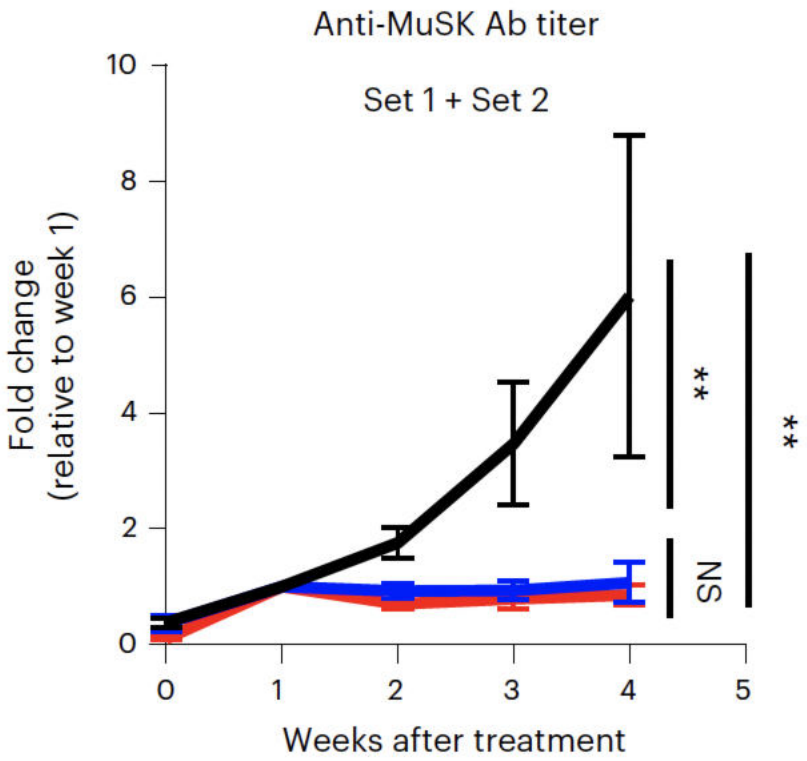


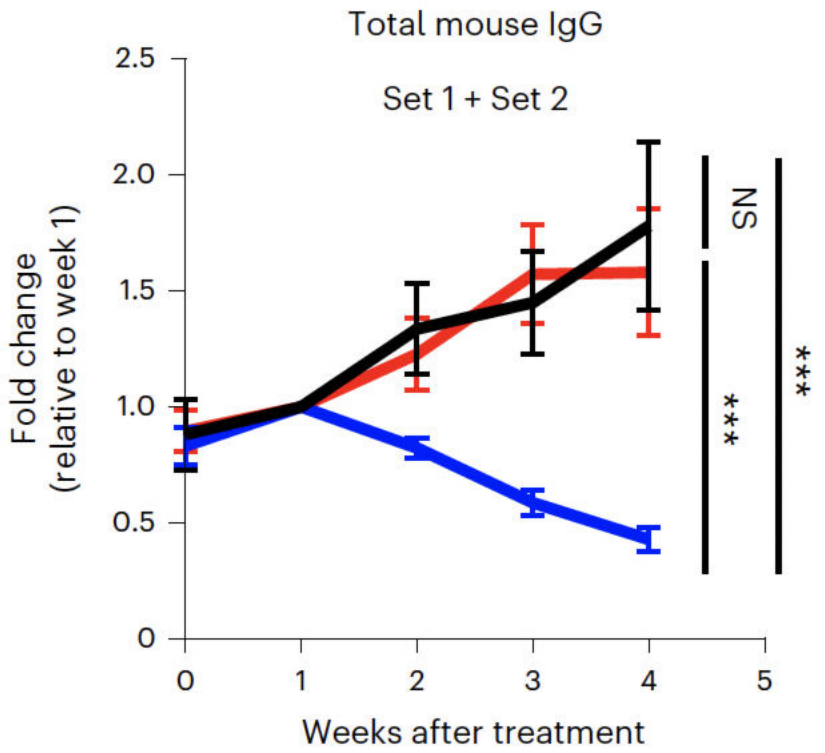
Recruiting	Open-label Study to Determine the Maximum Tolerated Dose of **DSG3**-CAART in Mucosal-dominant PV Patients (mPV)	• Mucosal -Dominant Pemphigus Vulgaris	• Biological: **DSG3**-CAART

NCT04422912

Muscle-specific tyrosine kinase myasthenia gravis

NTD-T
MuSK-CAART
CART-19

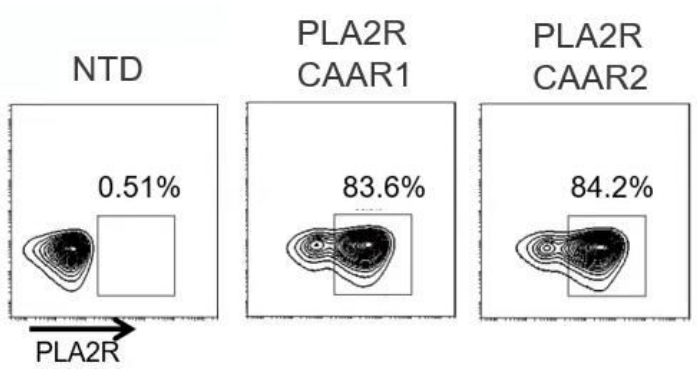




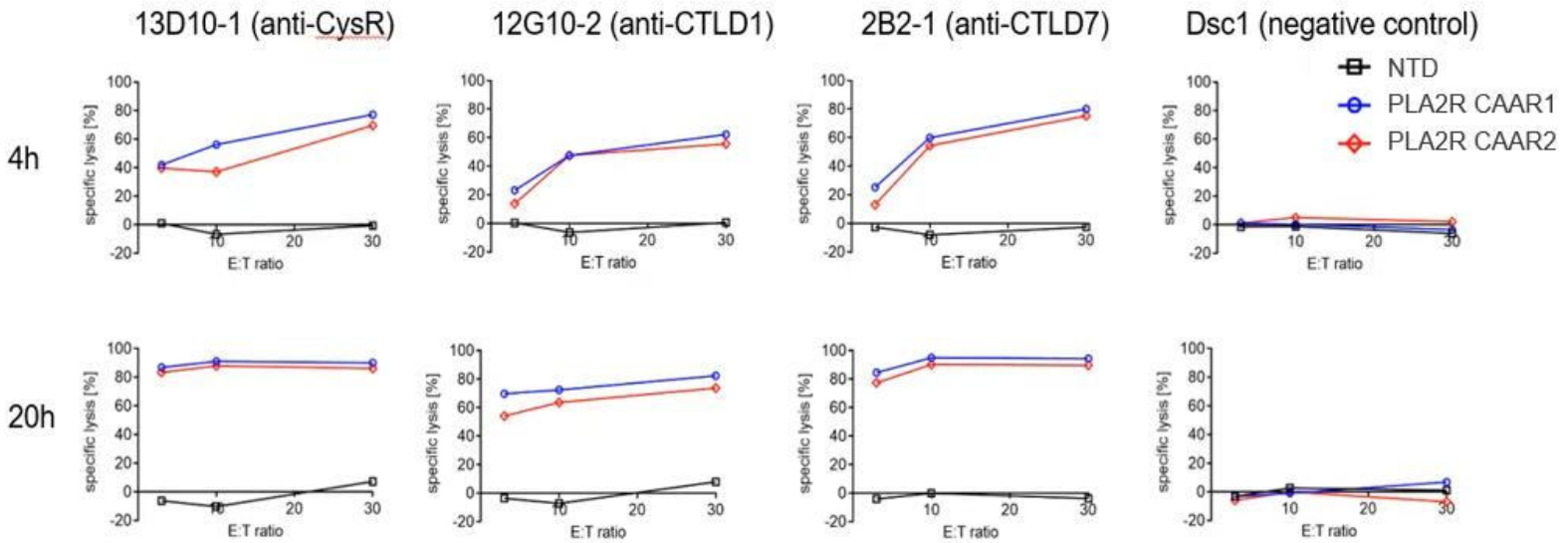
In an experimental model of autoimmune MG mouse model, MuSK-CAART reduced anti-MuSK igG without decreasing B cells or total IgG levels, reflecting **MuSK-specific B cell depletion**

Oh et al. Nat Biotech 2022

PLA2R CAAR in membranous nephropathy



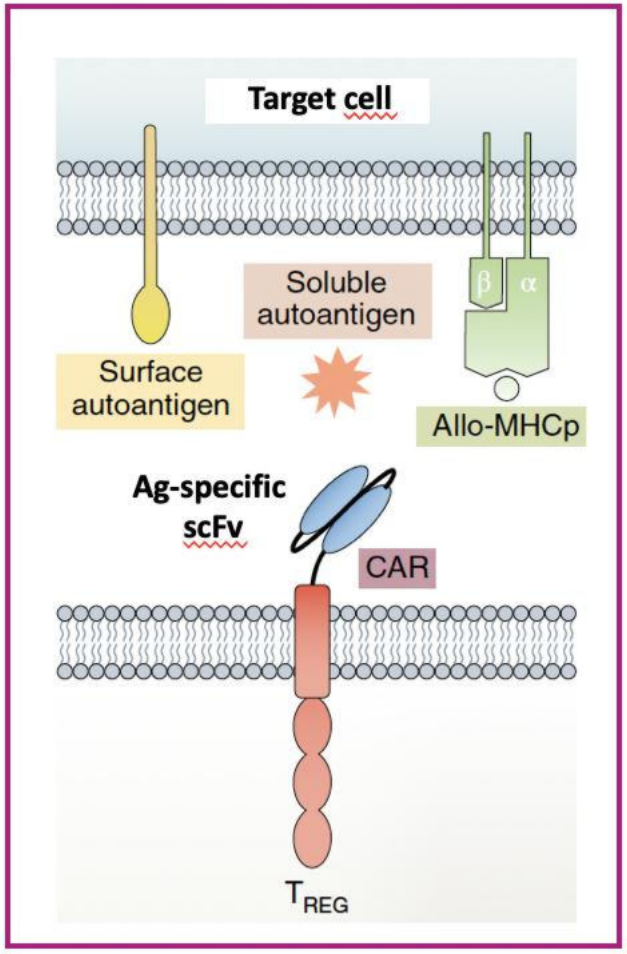
anti-PLA2R Ab-producing hybridomas



PLA2R CAARs efficiently kill hybridomas producing anti-PLA2R Ab in vitro

Payne et al., ASN Kidney Week 2021

Targeted regulatory cell therapy in AI diseases and transplantation



To successfully use **Tregs as living drugs**, 4 key properties are needed: summed up as the **four S's**

* **HLA A2** → **Transplantation**

* **Citrulinated protein** → **Rheumatoid arthritis**

Regulatory T cells

Ferreira Nature Reviews Drug Discovery 2019

HLA A2-targeted CAR Tregs

Megan Levings

THE UNIVERSITY OF BRITISH COLUMBIA

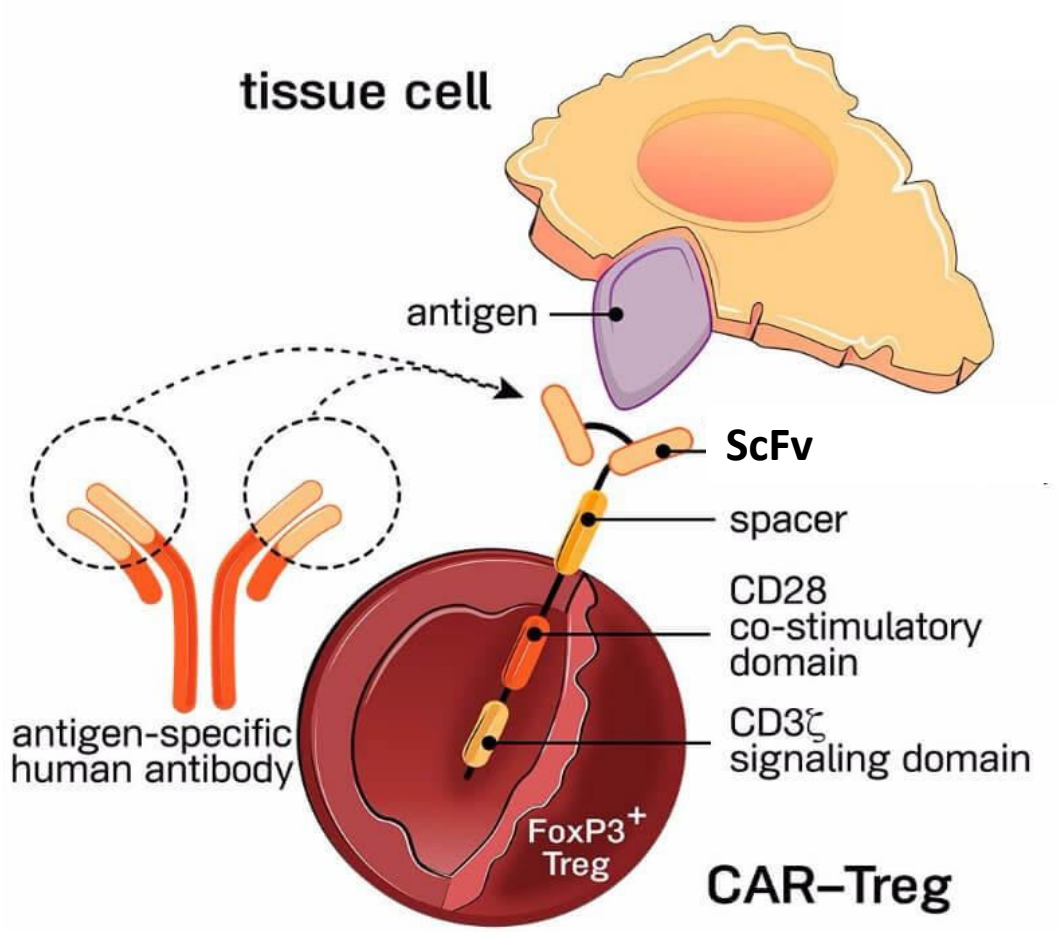
MacDonald, J Clin Invest , 2016
Dawson JCI Insight 2019
Dawson Sci Transl Med 2020
Lamarche PNAS 2023

Noyan Am J Transplant 2017

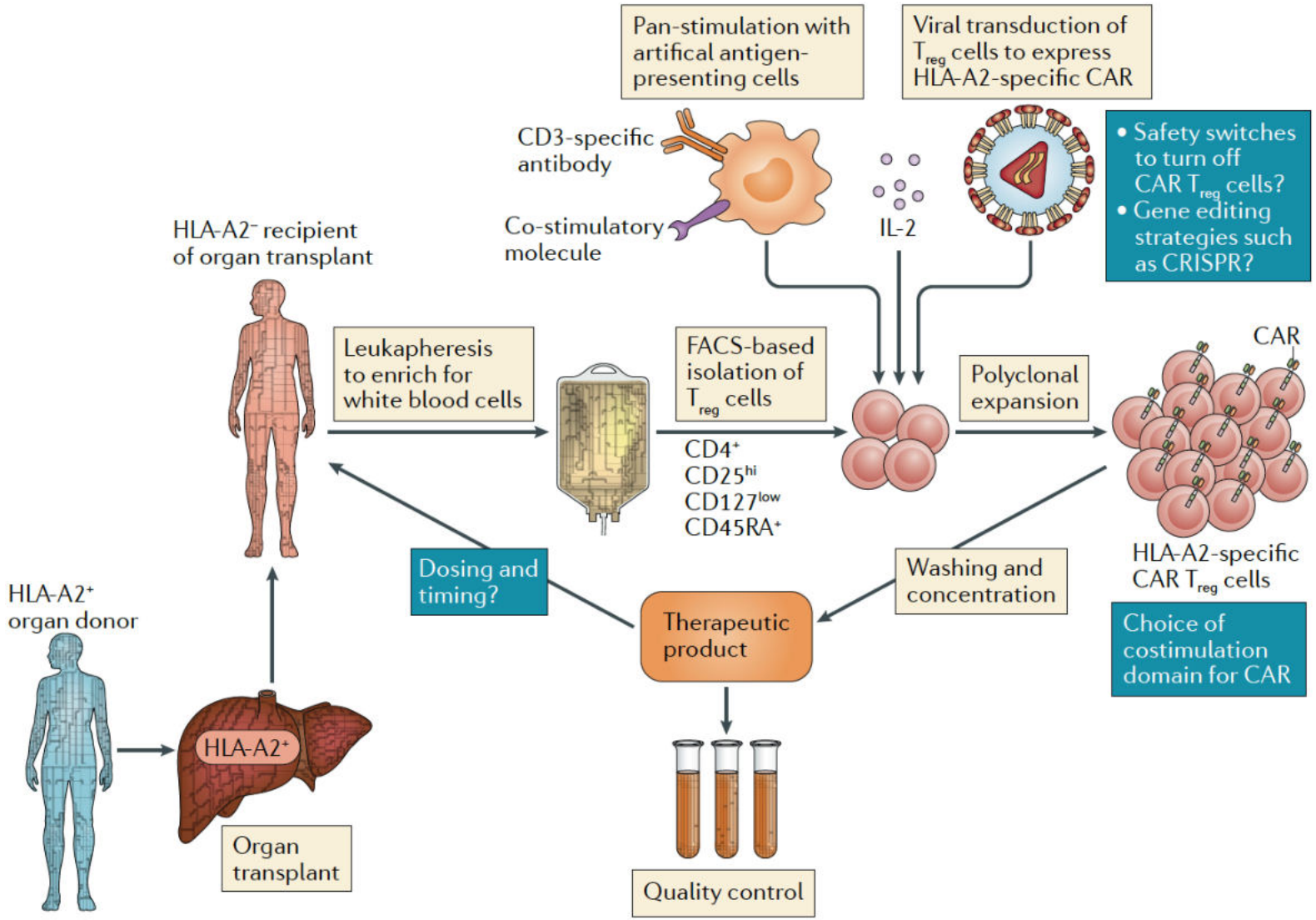
Boardman Am J Transplant 2017
Mohseni Eur J Immunol 2021

Muller Front Immunol 2021

Lamarthée Nat Commun 2021



CAR-Tregs in transplantation

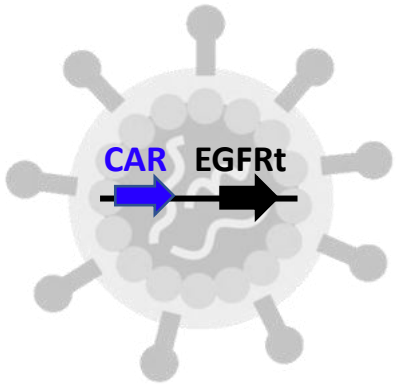


Maldini Nat Rev Immunol 2018

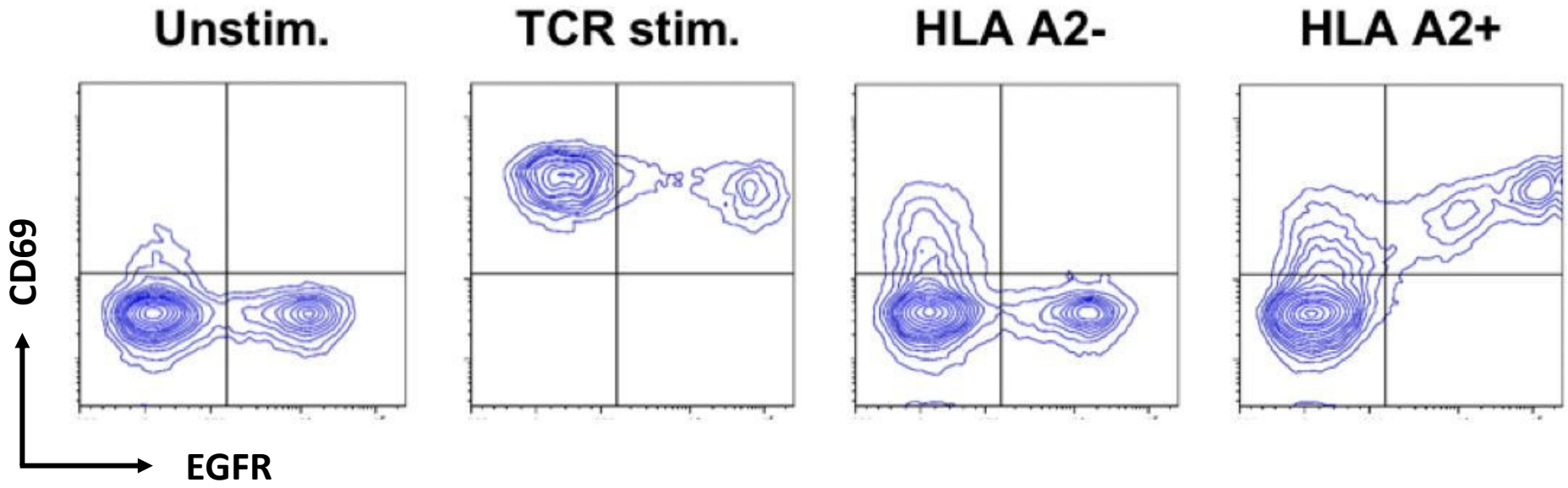
HLA A2-specific activation

Baptiste Lamarthée

Soëli Charbonnier



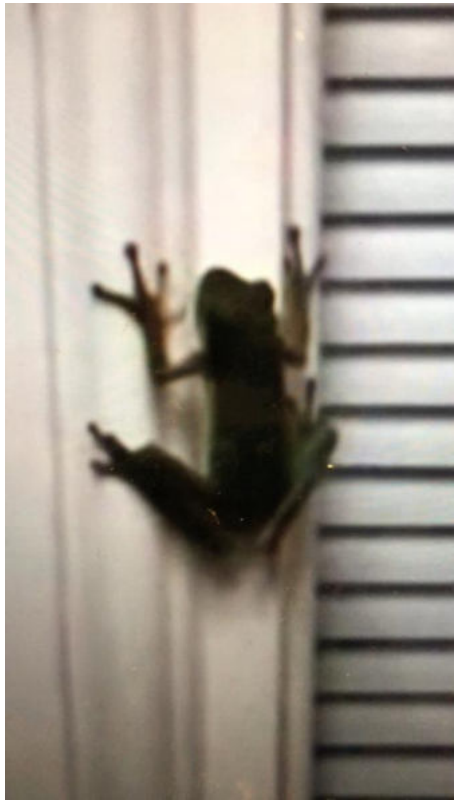
Specificity



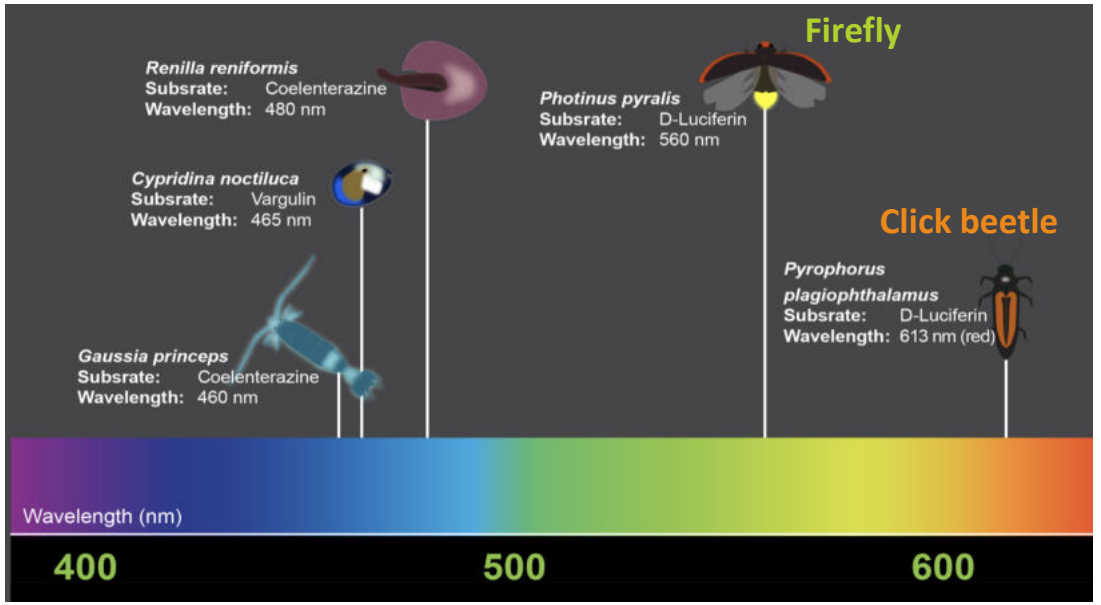
Lamarthée et al. Nat Commun 2021

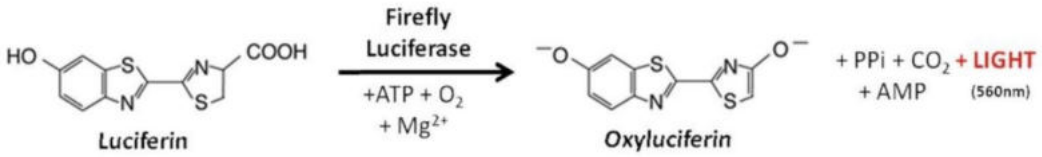
In vivo cell tracking with bioluminescence

T. Blein

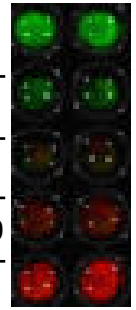


Firefly





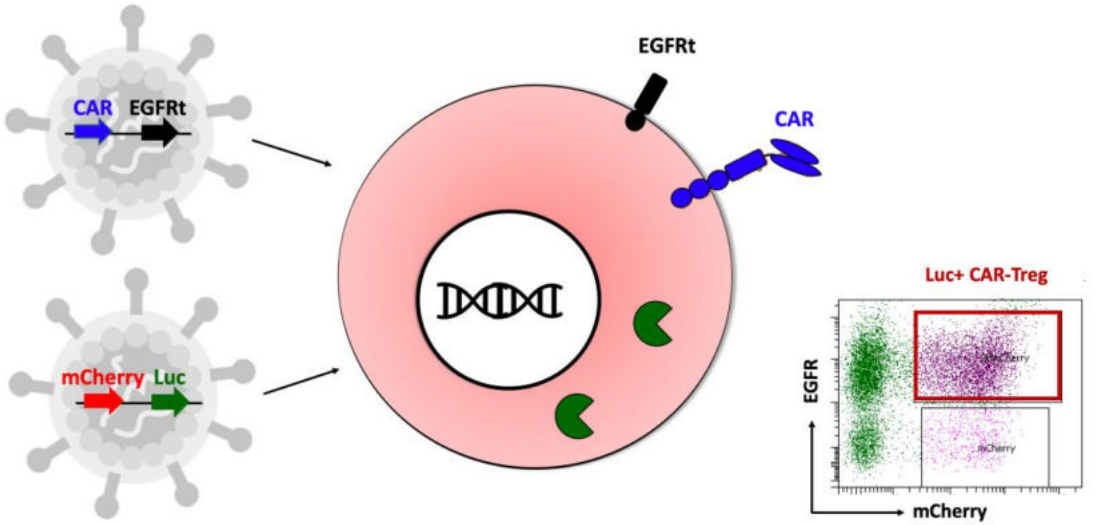
Number of cells	
CBG99+	**Fluc+**
100 000	0
50 000	12 500
25 000	25 000
12 500	50 000
0	100 000



Antigen-specific homing

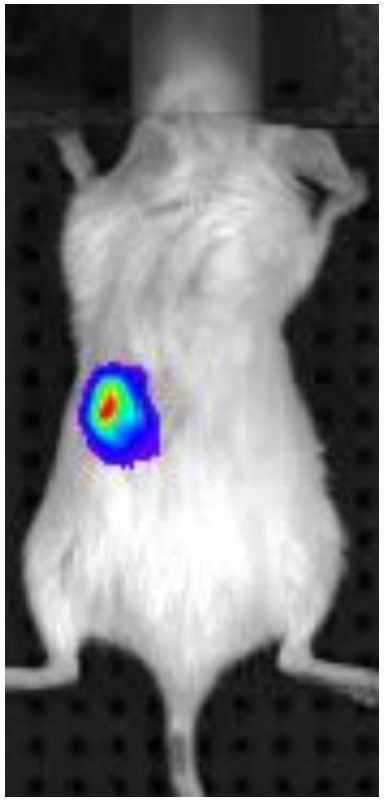
F. Valette

T. Blein



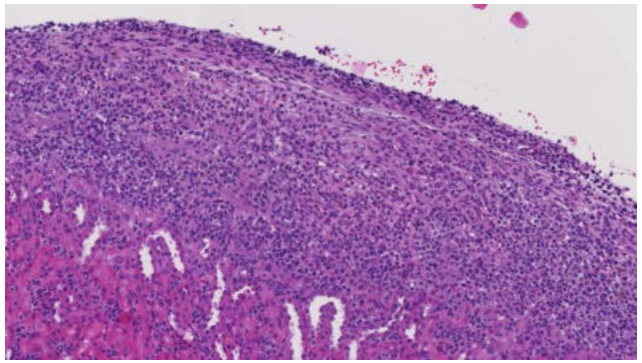
Specificity

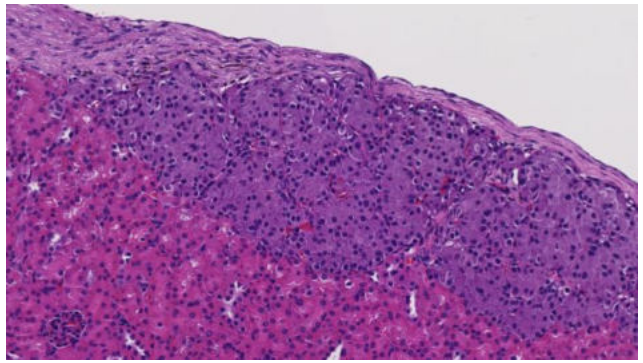
A2-CAR-Luc **mock-Luc**



HLA A2+ pancreatic Islet Tx

A2-CAR-Luc **mock-Luc**





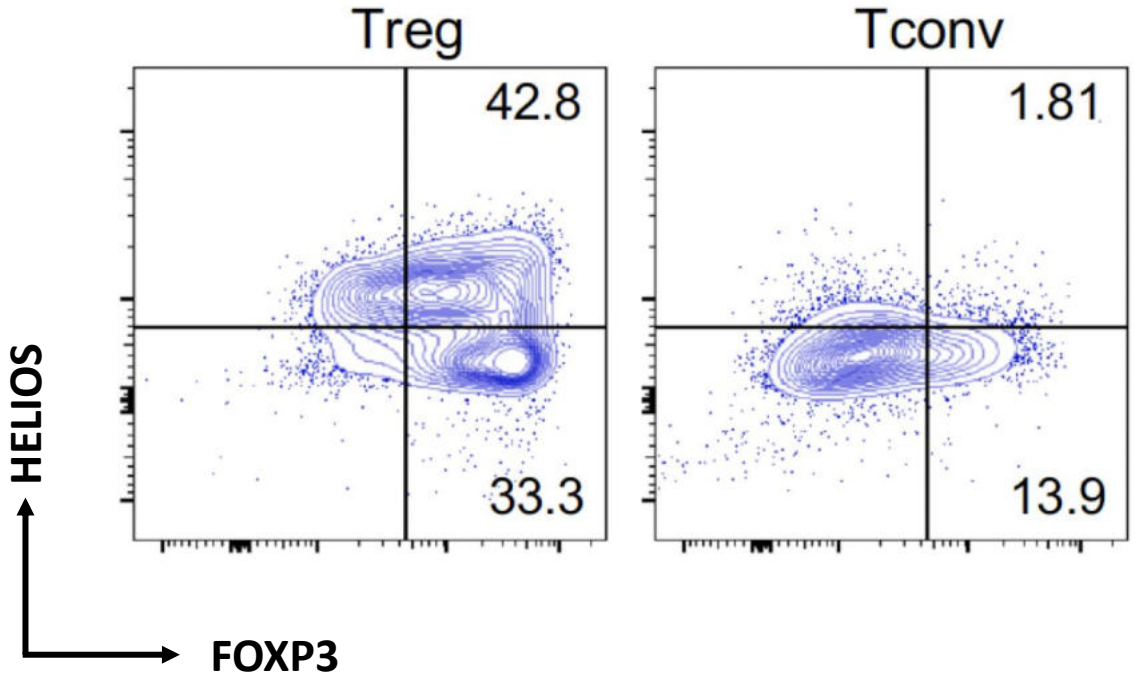
Unpublished results

CAR-Tregs remain stable over a 3-wk ex-vivo expansion

Soëli Charbonnier

Armance Marchal

Stability



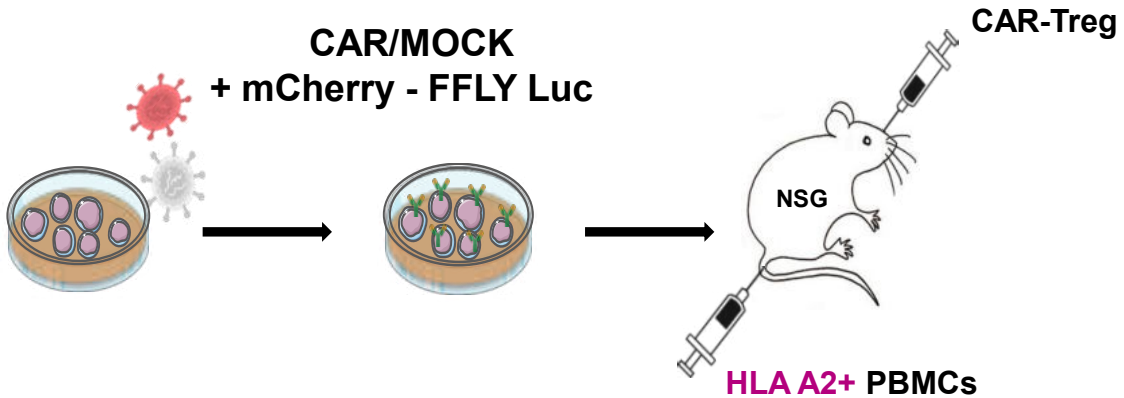
Lamarthée et al. Nat Commun 2021

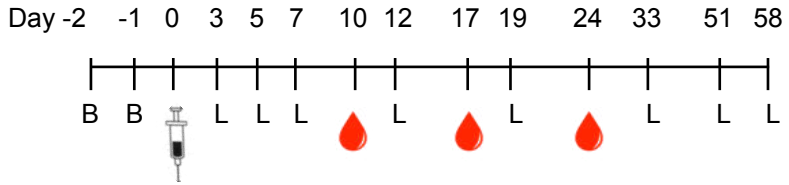
CAR-Tregs outperform polyclonal Tregs at suppressing GVHD

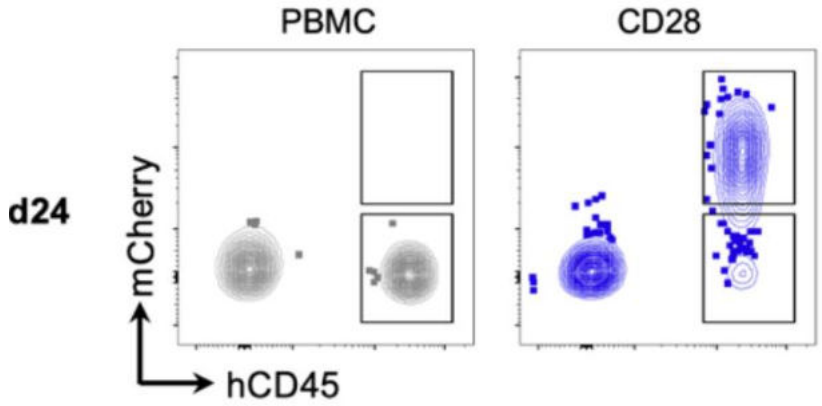
Baptiste Lamarthée

Tifanie Blein

Suppressive







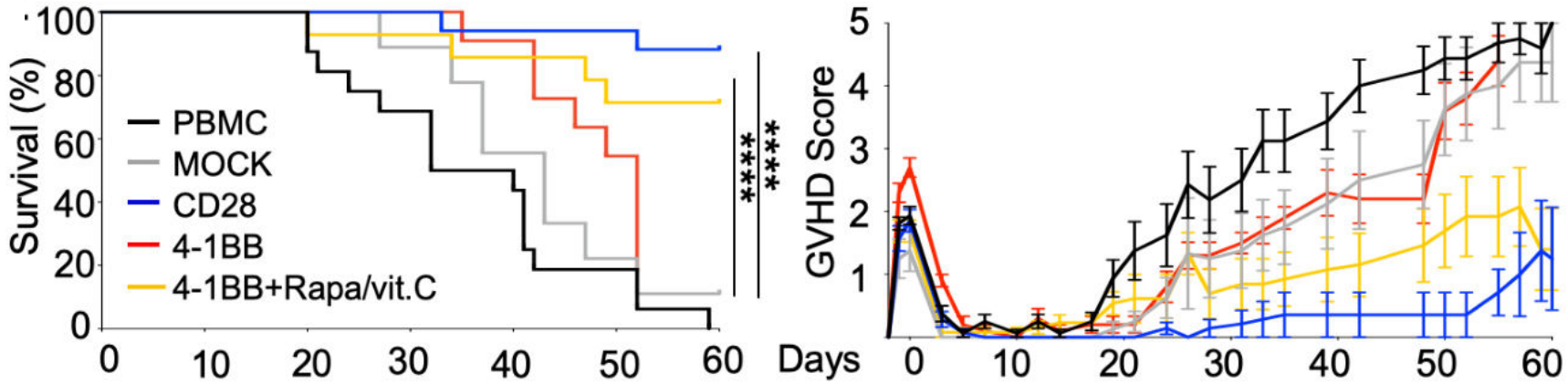
Lamarthée et al. Nat Commun 2021

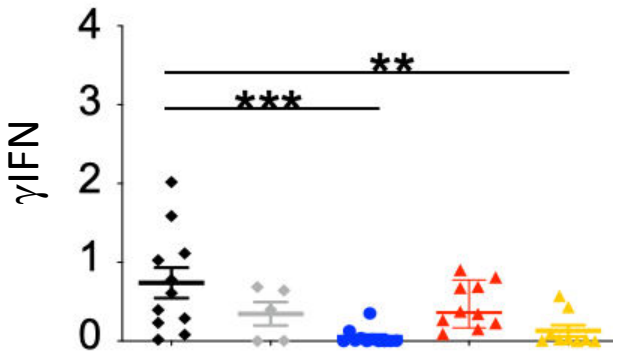
CD28 CAR-Treg outperform 4-1BB CAR / polyclonal Tregs

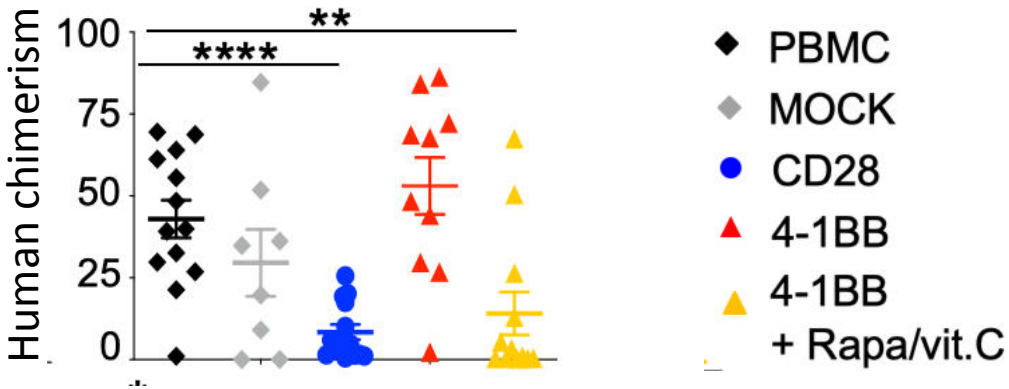
Baptiste Lamarthée

Tifanie Blein

Suppressive





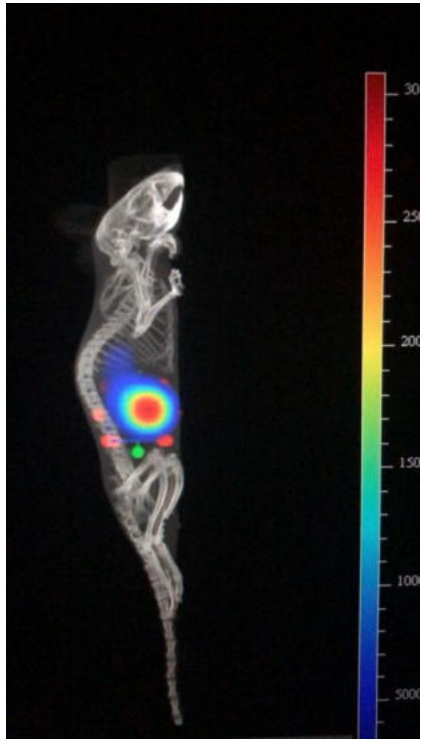


Lamarthée et al. Nat Commun 2021

Longer persistence of circulating CD28-CAR Tregs than their 4-1BB counterparts

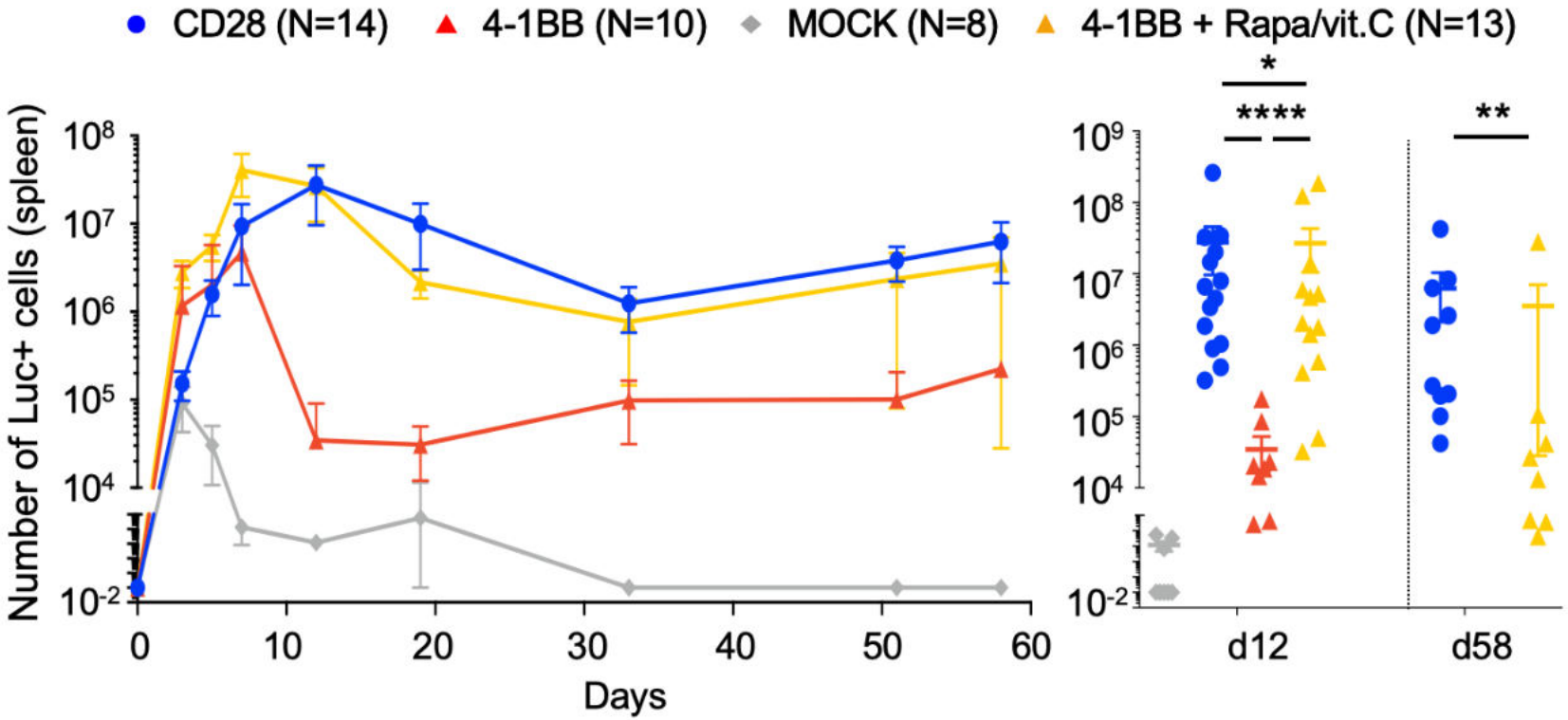
Baptiste Lamarthée

Tifanie Blein



dim Thérapie Génique ileDeFrance

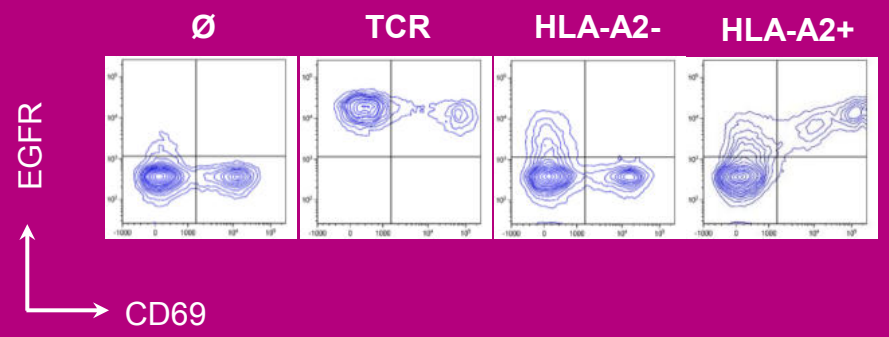
Survival



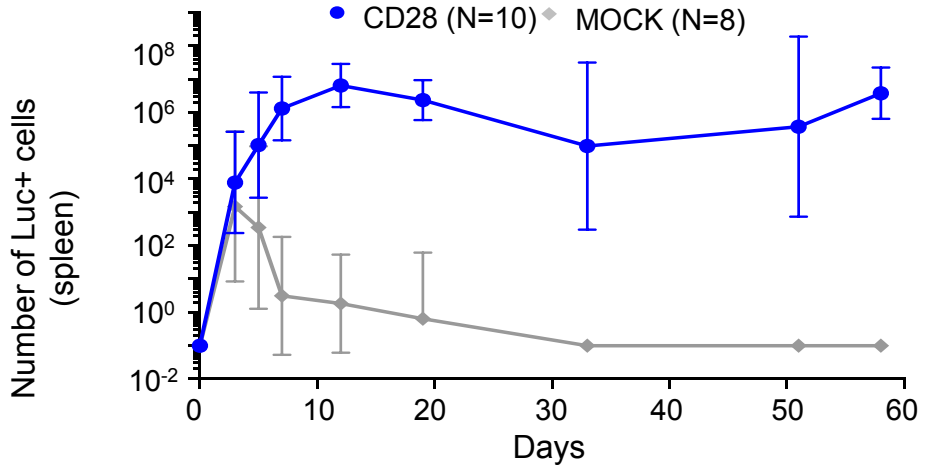
Lamarthée et al. Nat Commun 2021

CD28 CAR-Tregs meet the requirements

Specificity



Survival



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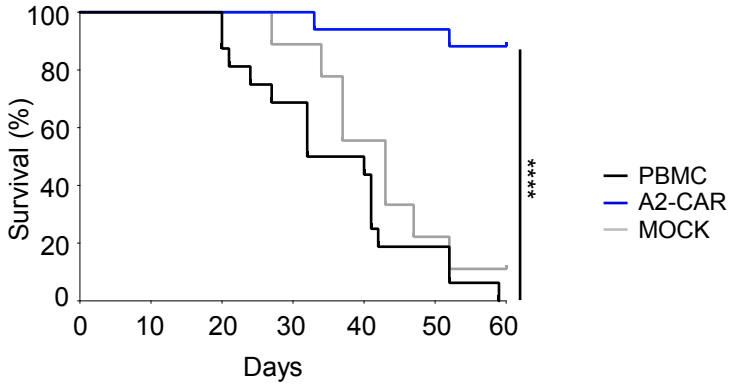
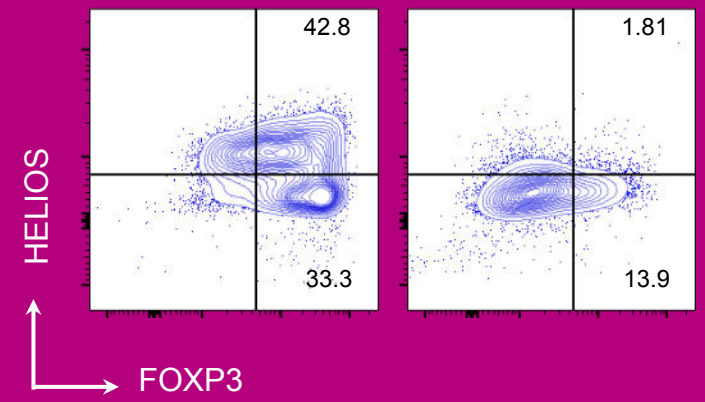
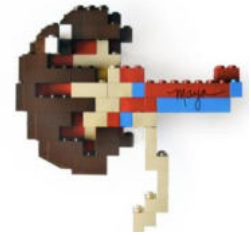


Stability

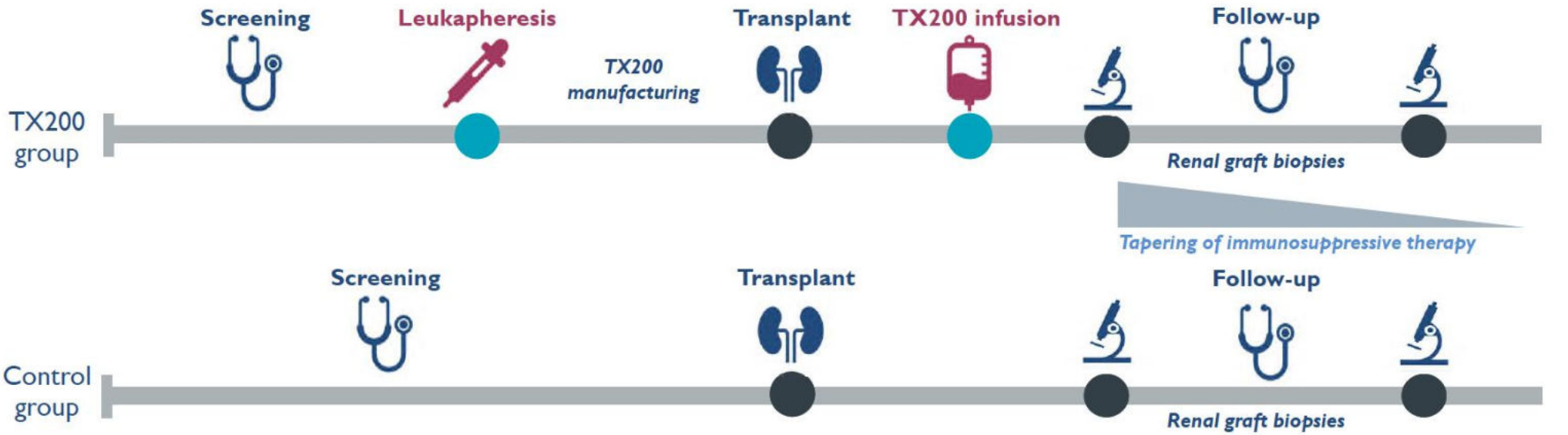


Lamarthée et al. Nat Commun 2021

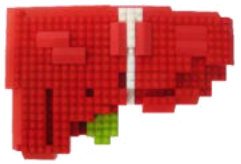


Ongoing clinical trial

STEADFAST Phase I/IIa Study: Multicentre, open-label, single ascending dose, dose-ranging (NCT04817774)



1	☐	Recruiting	Safety & Tolerability Study of Chimeric Antigen Receptor T-Reg Cell Therapy in Living Donor Renal Transplant Recipients



Ongoing clinical trial

QEL-001: Highly engineered modular Treg product

Targeting	**Anti-HLA A2 CAR** Drive local Treg suppression
Phenotype lock	**FOXP3 technology** Stabilize Treg phenotype
Safety	**Proprietary suicide switch**

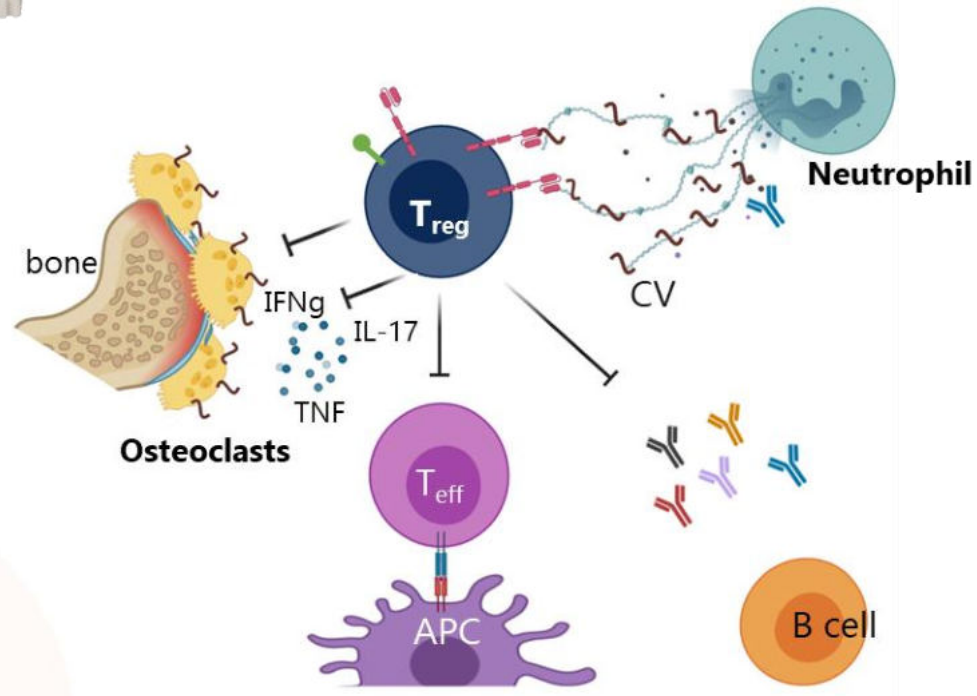
Single-arm, open-label, multicentre phase I/II study evaluating the safety and clinical activity of QEL-101 in **HLA A2- patients that have received an HLA A2pos liver transplant from 1 to 5 years before inclusion**

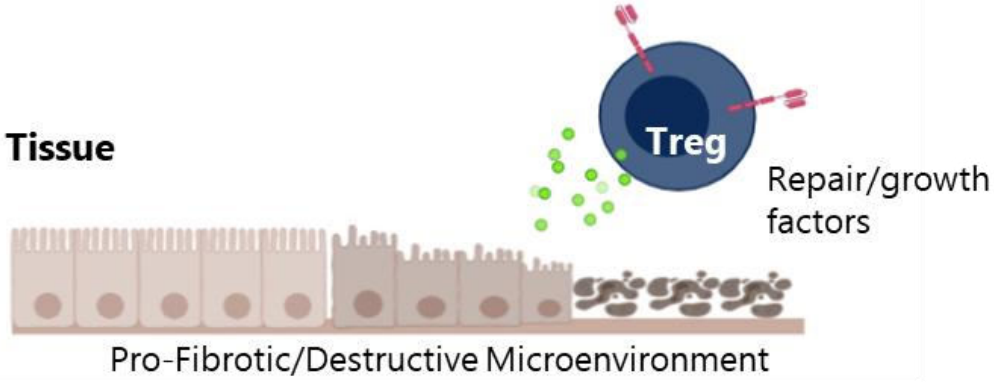
1 ☐ Recruiting Safety and Clinical Activity of **QEL-001** in A2-mismatch Liver Transplant Patients

LIBERATE **study**

CV-targeted CAR-Tregs in rheumatoid arthritis

CV: **citrullinated vimentin**





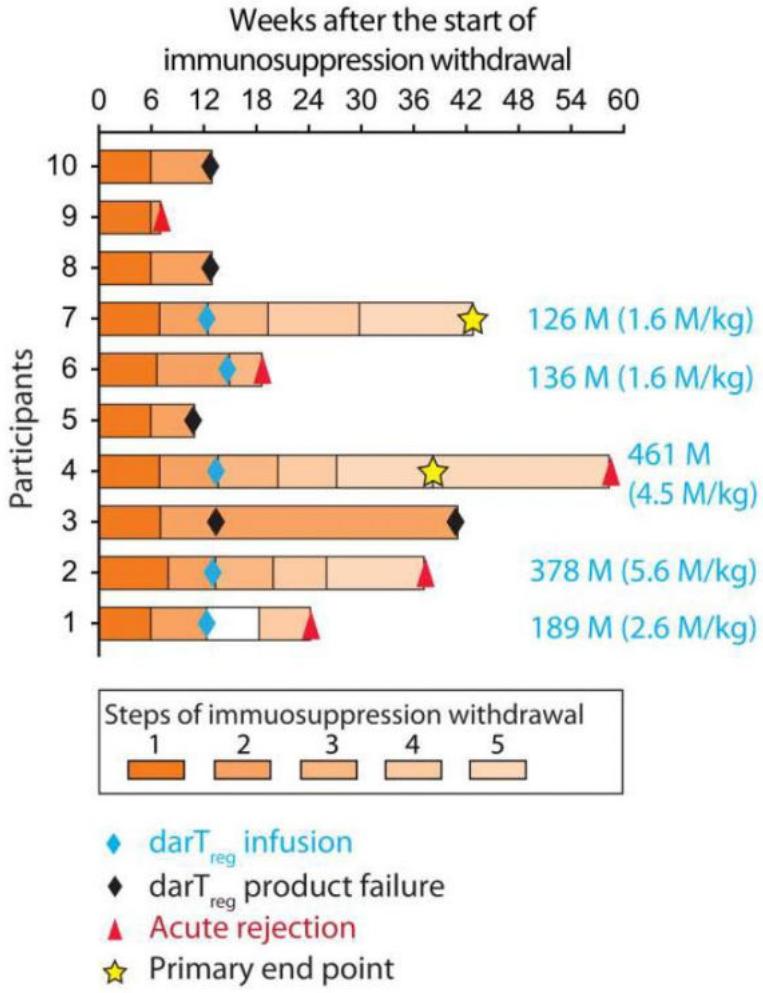
Fast-moving field of investigations

Company	Approach			Indication								Status	Comments
				Autoimmune					Neuro	Trans-plant	Other		
	Natural T_{reg} Cell Therapy	Converted T_{reg} Cell Therapy	Targeting/ specificity	RA	IBD	T1D	MS	Other					
SONOMA BIOTHERAPEUTICS	✓		CAR/ TCR	✓	✓				✓			Preclinical/ clinical	• T_{regs} derived from natural CD4+ T_{reg} cells and edited to CP-specific CAR • T_{eff} depleting agent to combine with T_{reg} therapy
gentibio		✓	TCR/ CAR			✓	✓			✓		Preclinical	• T_{regs} derived from CD4+ T cells and edited to express FOXP3 • "Immune evasive tech" to enable durable engraftment of allogeneic cells
QuellTX	✓		CAR/ TCR			✓			✓	✓		Ph 1 ready (liver transplant)	• Primary indication and expertise in liver transplant, anticipate entering clinic in Q1 2022, granted CTA approval (Oct 2021) • T1D and ALS in target discovery and validation
abata THERAPEUTICS	✓		TCR			✓	✓	✓				Preclinical	• MS (lead), T1D, Inclusion body myositis (IBM) • Expect to be in the clinic in 2023
Sangamo THERAPEUTICS	✓	✓	CAR		✓		✓		✓	✓	✓	Ph 1/2 (kidney transplant)	• Autologous therapies for hemoglobinopathies derived from edited CD34+ stem cells • Autologous therapies for immunological diseases derived from T_{regs} • First patient dosed in Ph1/2 renal transplant (March 2022)
xCR		✓	Not disclosed					✓			✓	Preclinical/ Stanford trials in IPEX	• Transducing FOXP3 in T cells from IPEX patients • ATC using Tr1 cells • Universal cell therapy
TeraImmune	✓		TCR						✓		✓	IND filed	• Platform consists of both natural T_{regs} isolated from patients and induced T_{regs} converted from a patient's T_{eff} cells • Lead program is FVIII TCR for Hemophilia A
COYA THERAPEUTICS	✓		Not modified					✓	✓			ALS	• Expansion of autologous T_{regs} administered to patients • Used in combination with IL-2

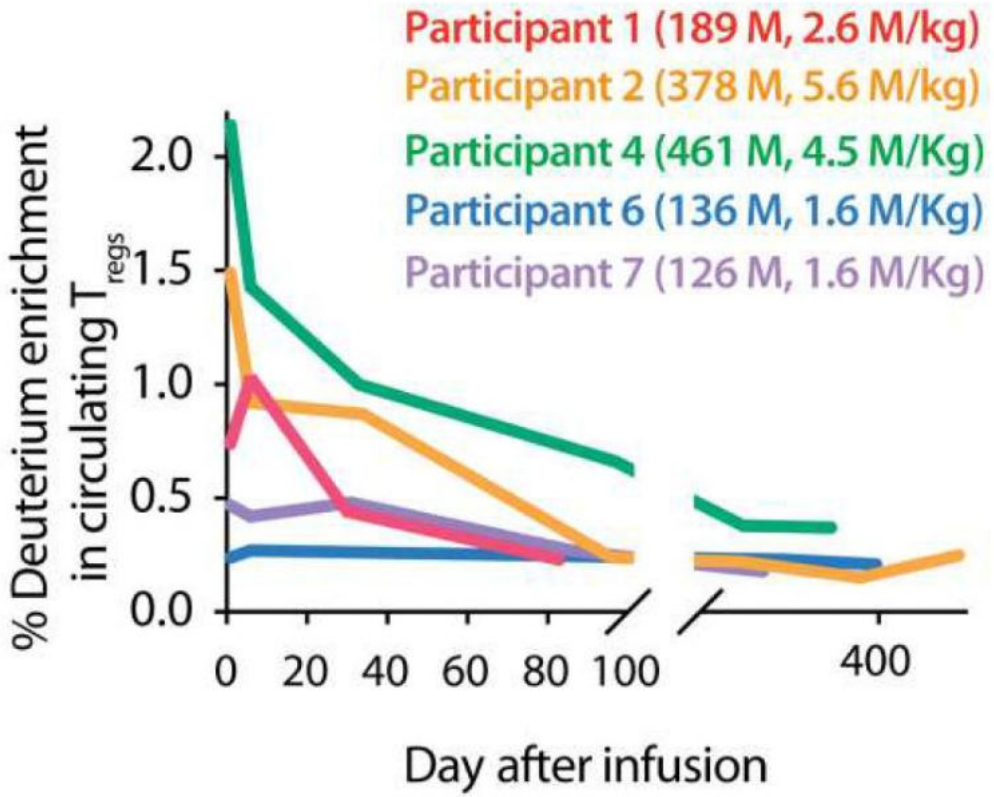
Fast decrease of donor-reactive-Tregs after LTx

Qizhi Tang

UCSF



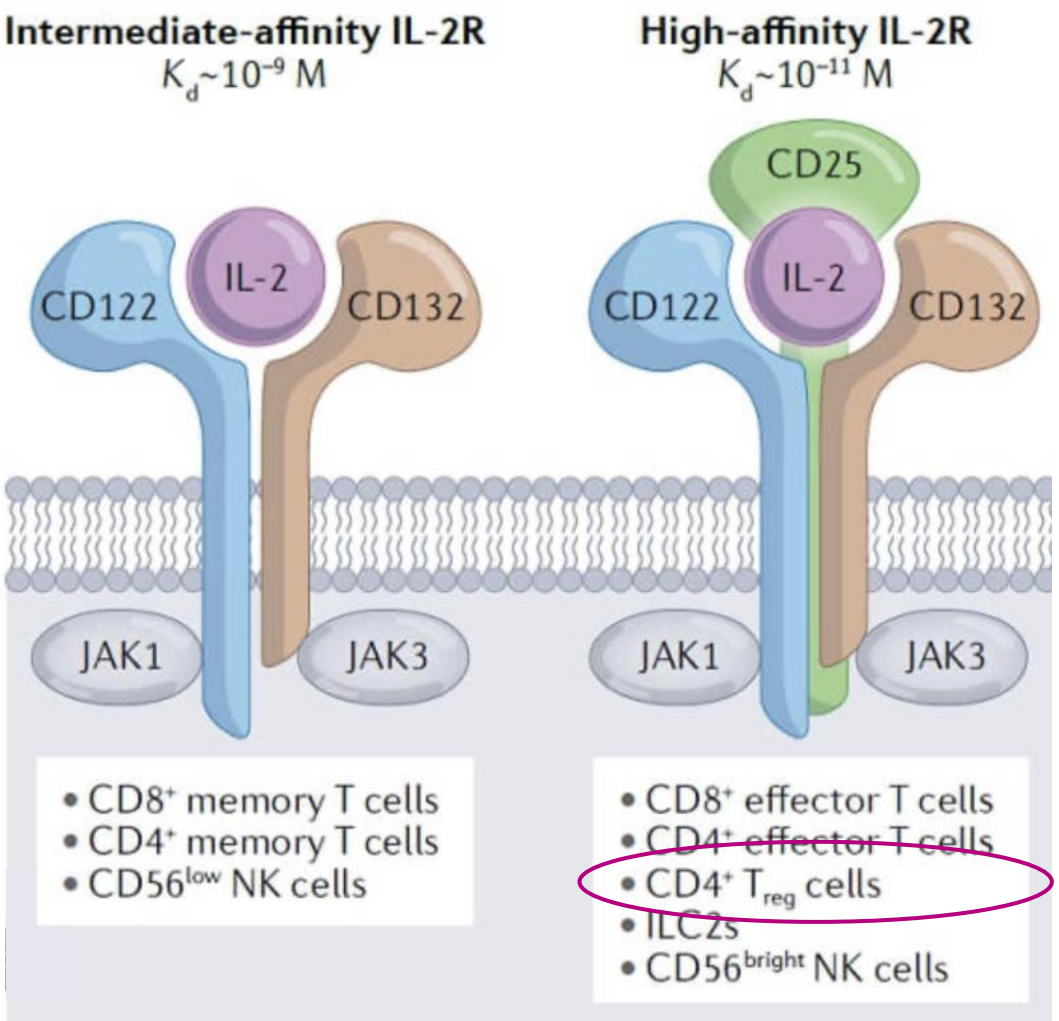
Disappointing effectiveness



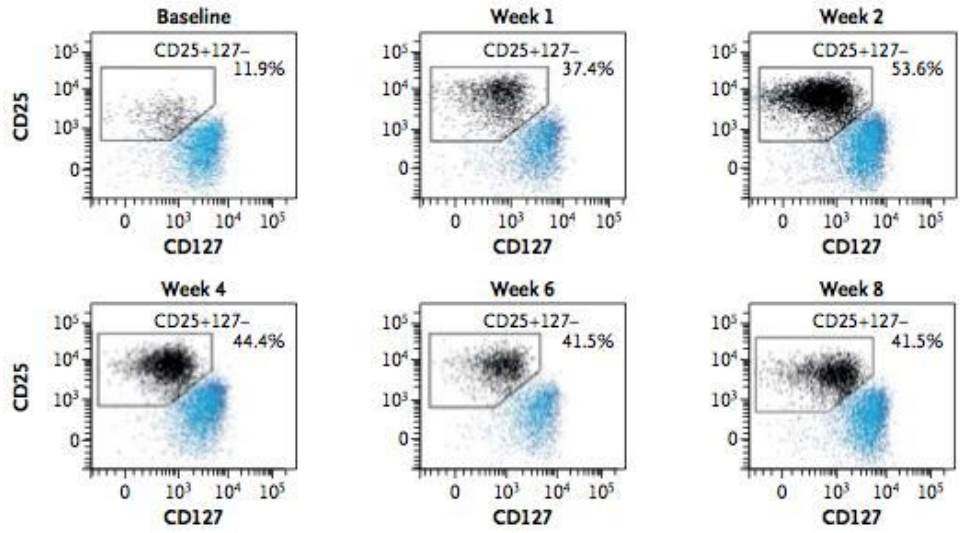
Accelerated reduction of infused donor-reactive Tregs

Tang et al. Sci Transl Med 2022

Low-dose IL-2 to expand selectively Tregs

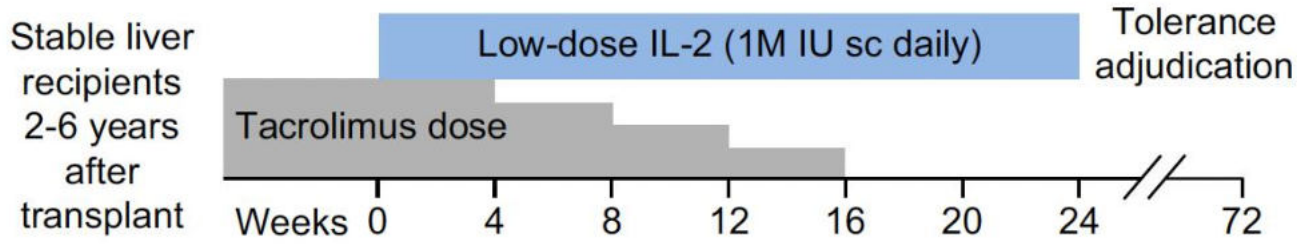


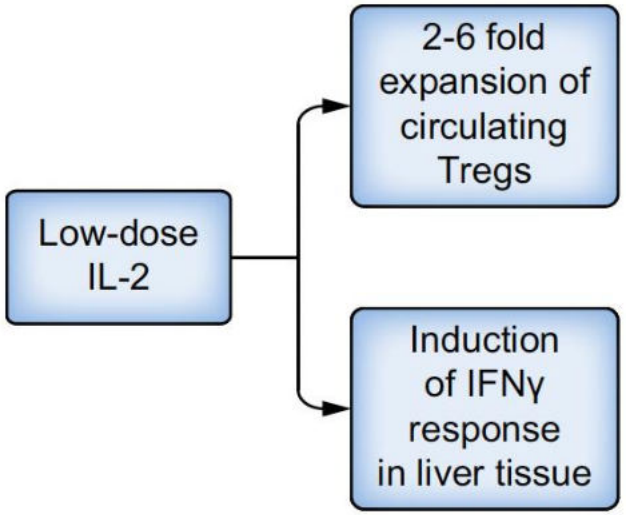
cGVHD

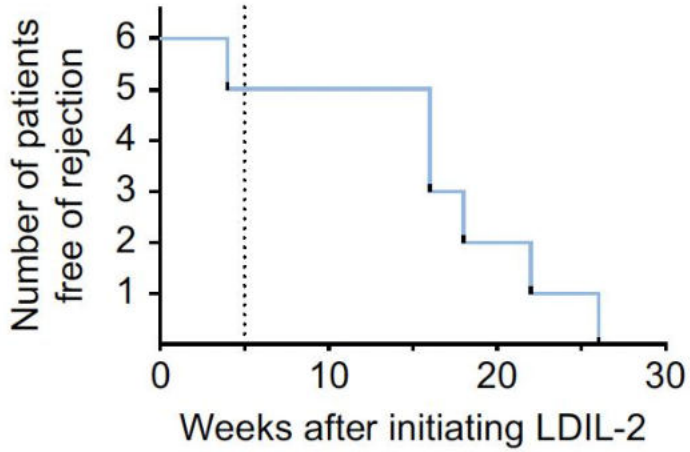


Koreth NEJM 2011

But low-dose IL-2 / CNI taper down precipitated rejection after LTx





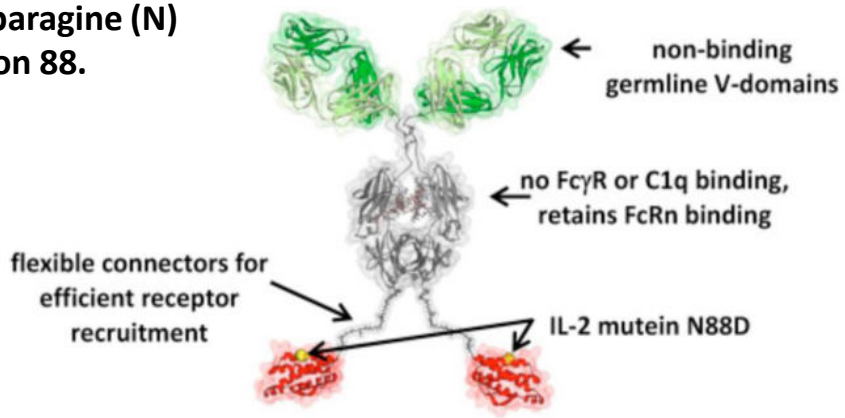


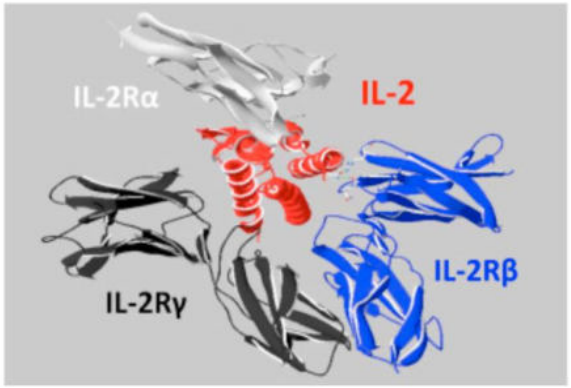
Lim et al J Hepatol 2023

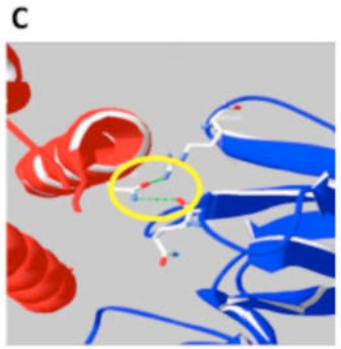
Strategies to overcome the lack of specificity of low-dose IL-2

IL-2 muteins

Substitution of aspartic acid (D) for asparagine (N) at position 88.

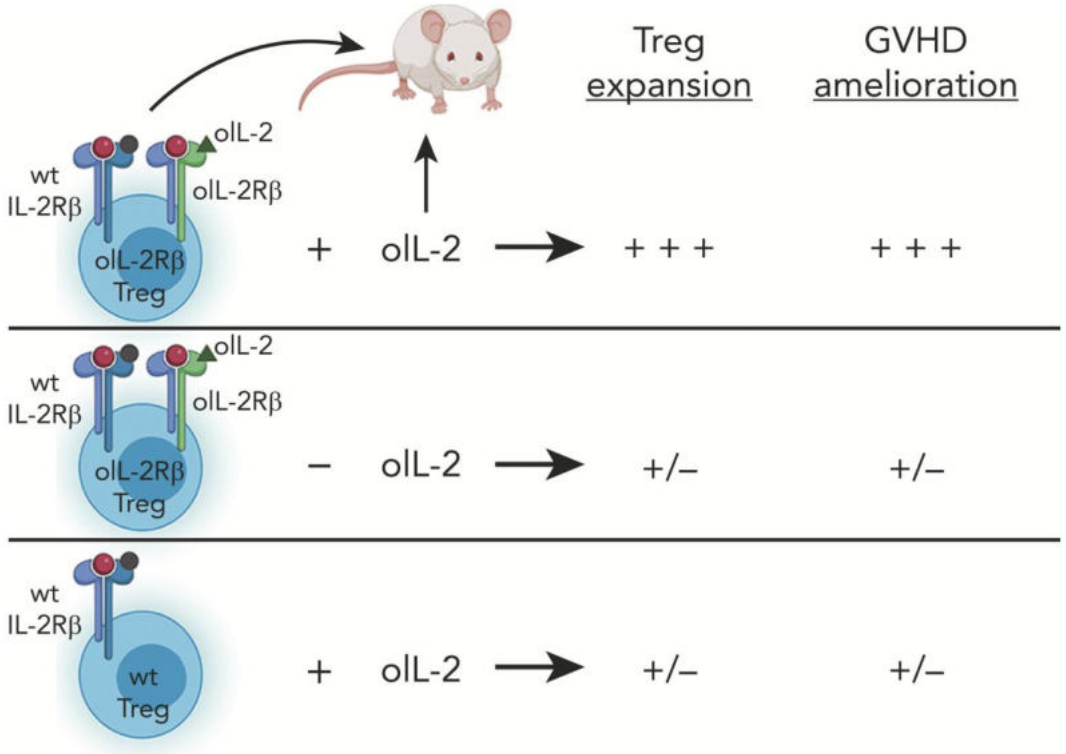






Peterson J Autoim 2018; Khoryati Sci Immunol 2020

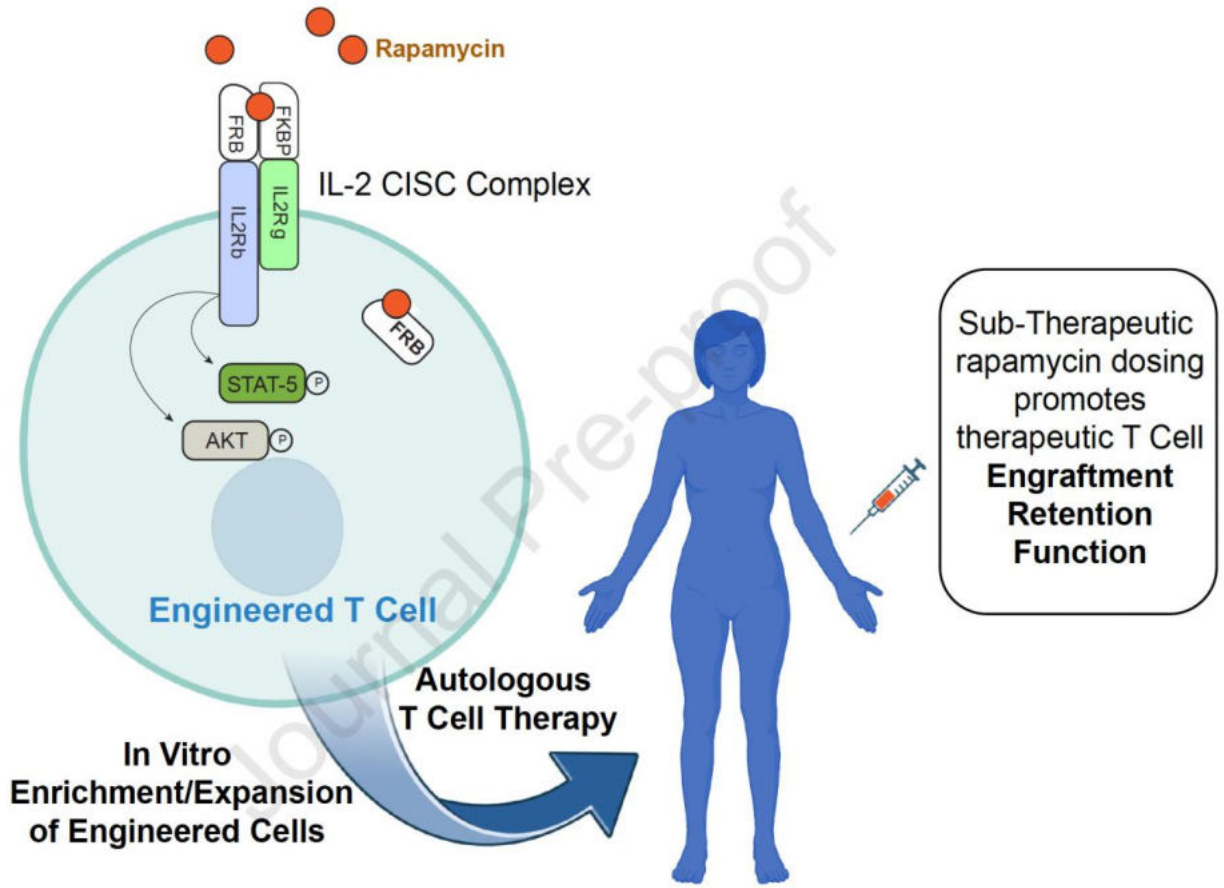
Orthogonal IL-2 / IL2R

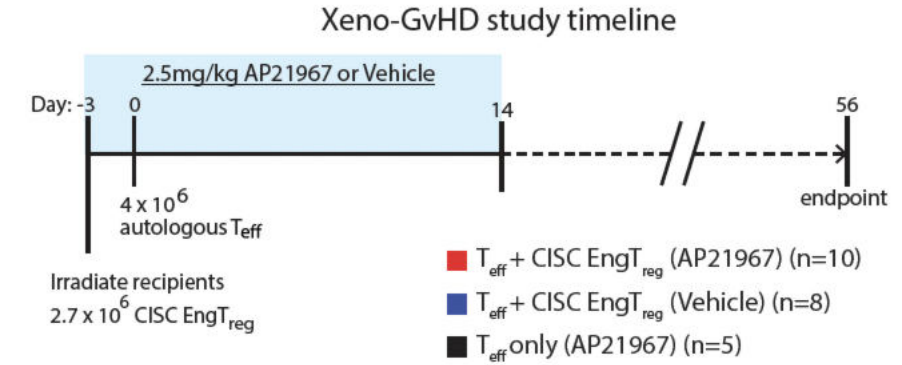


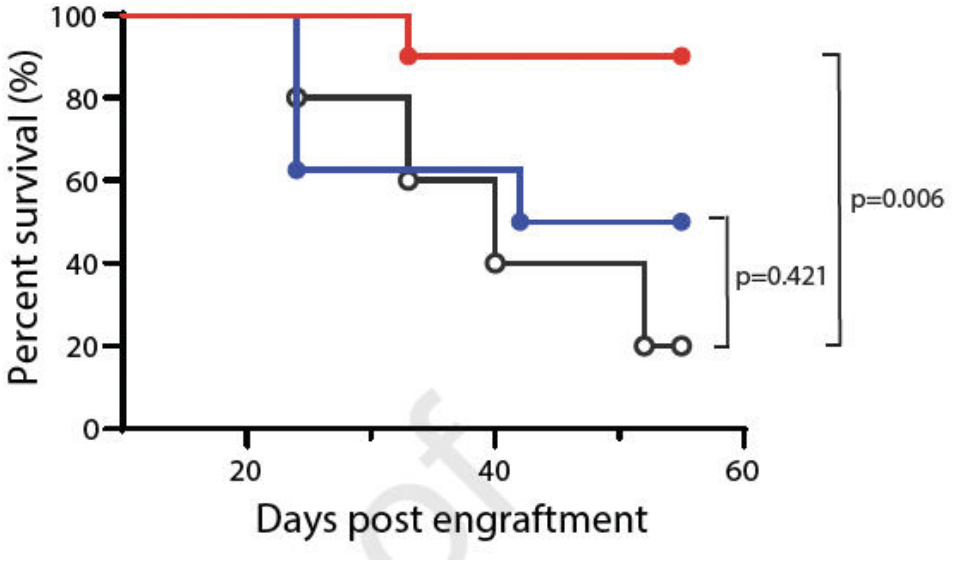
Sockolosky Science 2018, Hirai J Clin Invest 2021, Ramos TL Blood 2023

Chemically Inducible IL-2 Receptor Signaling Complex (CISC)

CISC





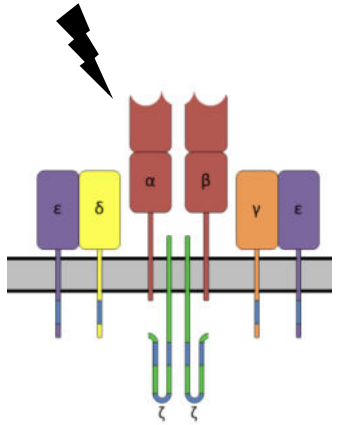


Cook Mol Therap 2023

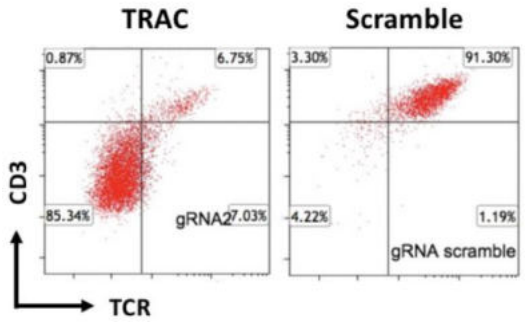
Tip the balance toward “shielded CAR-Tregs”

Tifanie Blein

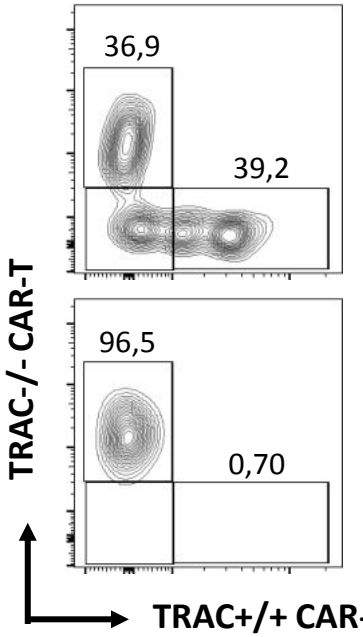
CRISPR/Cas9 targeting the *TRAC* locus



TRAC deletion disrupts the whole TCR/CD3 complex



Dramatic enrichment in *TRAC*-deficient CAR-T upon anti-CD3 Ab



Saline solution

Anti-hCD3

Unpublished data

A. Scheffold

H. Isambert

S. Fillatreau C. Boitard

Identification and sequencing of insulin-specific TCR from Tregs

Multi-parameter machine learning approach to identify the best TCR candidates

Validation of in silico selected TCRs through in vitro and in vivo studies in mice

J. Beltrand

HLA DQ8+ children with juvenile T1D

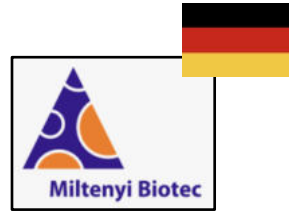
ARTiDE

P. Van Endert

D. Klatzmann

Empowering of TCR-Treg therapy (tethered Ortho IL-2 /IL2R and nanoparticles)

GMP-grade production of LV encoding the selected TCR



J. Zuber

Functional and toxicity assessment of GMP-grade TCR-Treg in humanized mice

Finetuning of GMP-grade manufacturing

P. Trzonkowski

High manufacturing cost

Dramatic cost cutting is expected with *in-house* academic manufacturing

J. Larghero

MEARY CENTER
Medical Engineering and Research in cell & gene therapy

ASTRAVEUS

J. Laurent

Miniaturizing cellular therapies and their manufacturing

Conclusions

1- Clinical use of B cell / plasma cell-targeted CAR T cells in non malignant diseases.

- Anti-CD19 in severe SLE
- Anti-CD19 / BCMA in highly sensitized patients on the waiting list

2- New target and new technologies (CAAR) in preclinical & clinical development

- Anti-FAP CAR T to prevent / mitigate the development of fibrosis
- CAAR T to target antigen-specific memory B cells

3- CAR-Tregs : the first clinical trials are underway

- **Sangamo** Kidney transplantation
- **Quell$_{TX}$** Liver transplantation
- **Sonoma$_{biotherapeutics}$** Rheumatoid arthritis

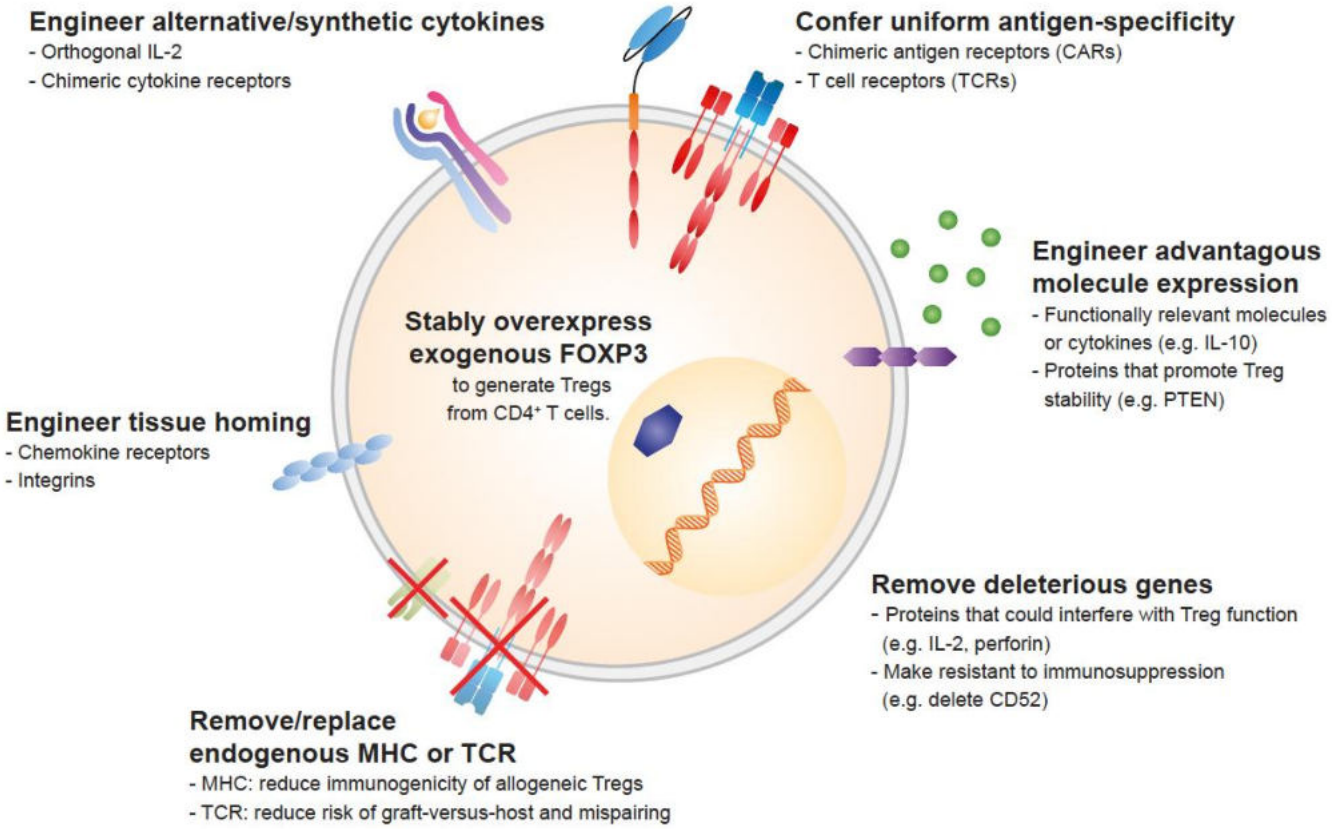
STEADFAST

Quell Therapeutics Raises $156 million

Jeito

Sonoma Biotherapeutics Raises $265 Million in an Oversubscribed Series B

Your limitation is only your imagination



Inexhaustible possibilities

Boardman DA JACI 2022

Acknowledgements

CAR Treg team

Armance Marchal
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Soeli Charbonnier
Tifanie Blein
Lucas Rabaux
Akshay Joshi
Nicolas Ayas

imagine
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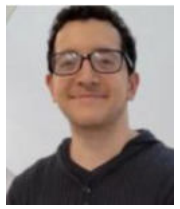
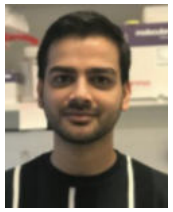
Emmanuelle Six
Marina Cavazzana
Isabelle André
Matthias Titeux
Emmanuel Martin
Sylvain Latour





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