Treg Cell Therapy In Autoimmune Diseases And Allograft Rejection: Focus On CAR-Treg Cells

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OUTLINES

- Introduction
- Adoptive Treg cell Therapies
- Polyclonal /Antigen-Specific Treg Cells
- CAR-Treg Cells
- CAR-Treg Cells in Autoimmune Diseases And Transplantation
- Limitation Of CAR-Treg Cells

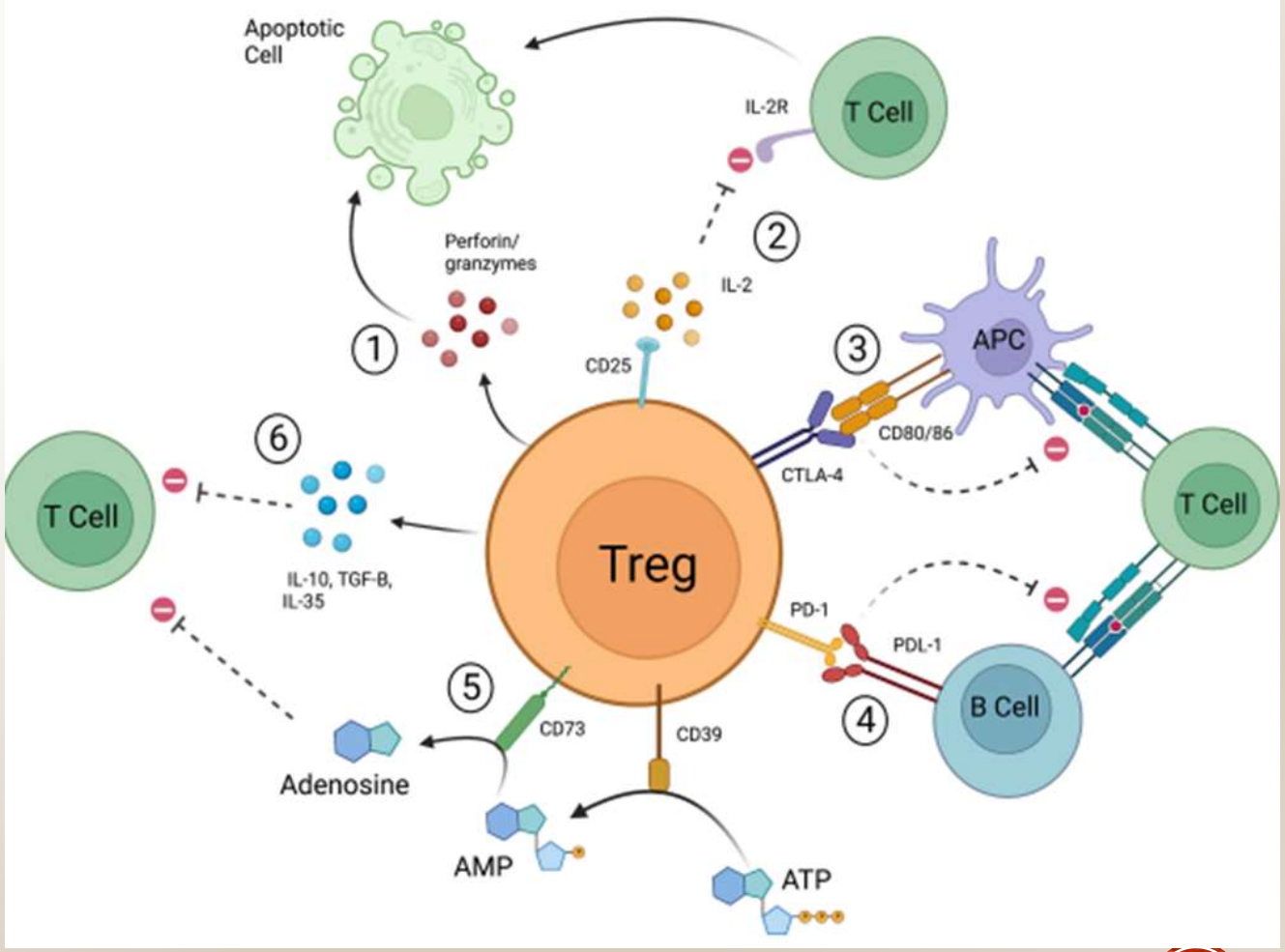
Introduction

- Autoimmune disease, Alloimmune response
- Approximately **8%** of **kidney** transplant
- Between **9%** and **50%** of patients, GVHD
- **Immunosuppressive drugs**
- Increased risk of infection, cardiovascular disease, and cancer
- Innovative therapeutic approaches
- Induce persistent **immune tolerance**

Requejo Cier et al, Frontiers in Molecular Biosciences, 2023

Introduction

- Treg, variety of mechanisms
- Treg suppress proliferation and function
- **Contact dependent mechanisms**
  - Granzymes and perforin
  - CTLA-4 and PD-1
- **Contact independent mechanisms**
  - Immunomodulatory cytokines

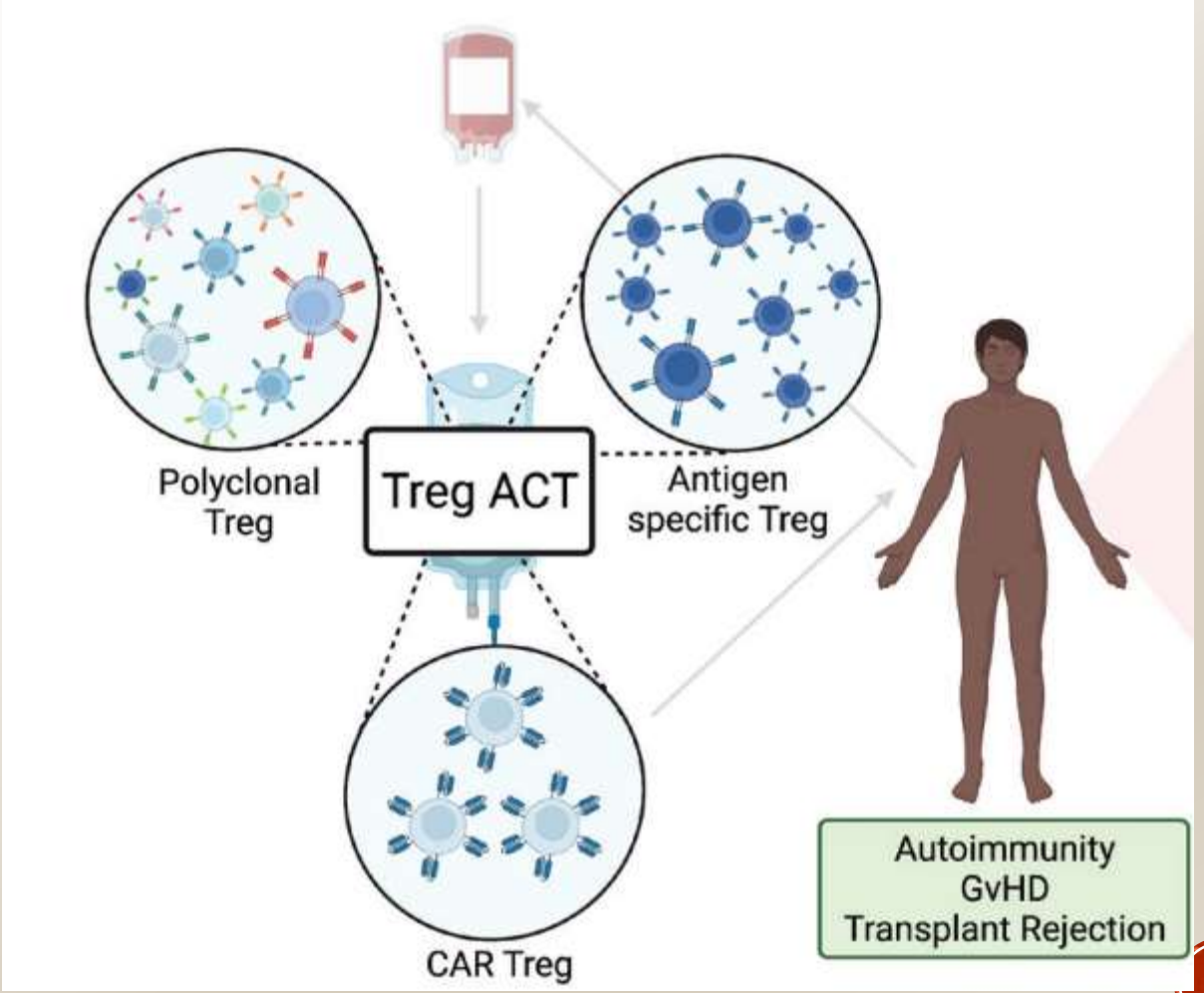


Sanders et al, Frontiers in Immunology, 2022

Adoptive Treg -Cell Therapies

- **Immunoregulatory functions**

- Tregs inhibit allograft rejection and autoimmunity
- **Animal models**
- **Clinical trials**

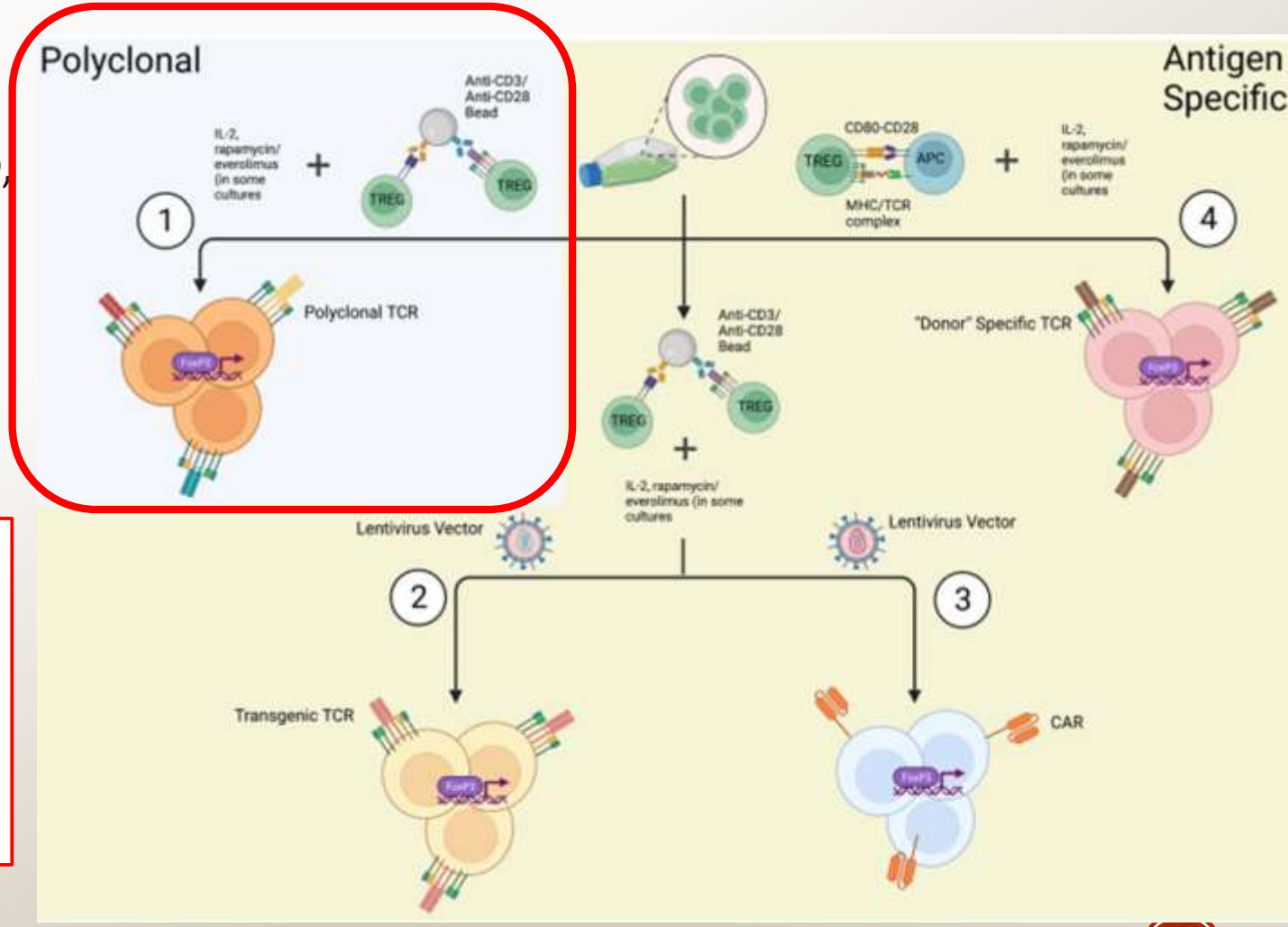


McCallion *et al*, *Clinical and Experimental Immunology*, 2023

Polyclonal Treg Cells

- Clinical phase 1 and 2 trials, T1D ,GVHD, crohn's disease, solid-organ transplantation
- Feasible, clinical benefits

- **Nonspecific immunosuppression**
- **Increase susceptibility to opportunistic infections or tumor development**
- **Viral reactivation**



Sanders et al, Frontiers in Immunology, 2022

Active Or Completed Treg Clinical Trials In Autoimmune Disease

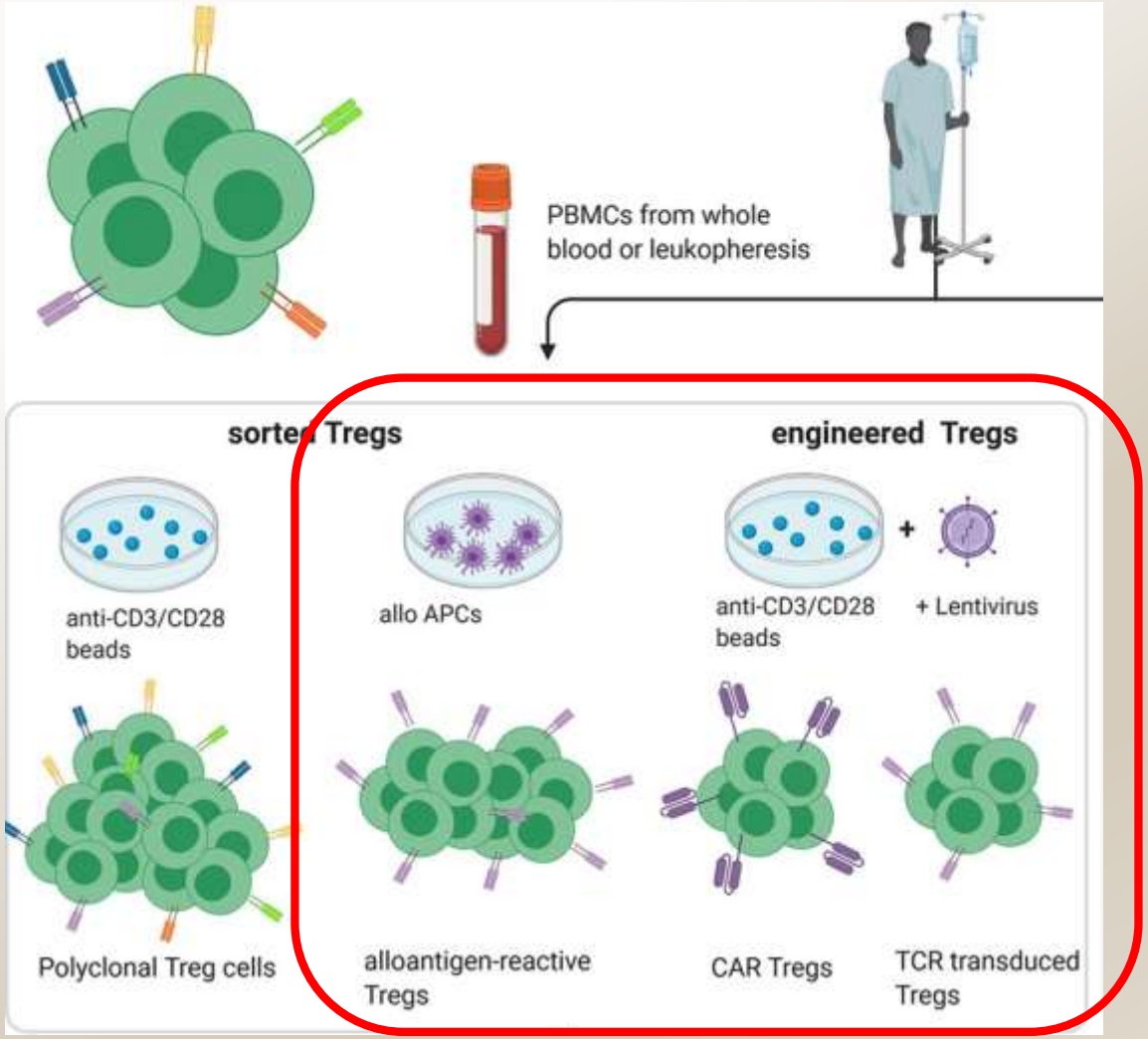
Autoimmune Disease NCT Number	Acronym	Phase	Disease	Cell Type
NCT05349591	cePolyTregs	Phase 1	Type 1 Diabetes	Autologous, polyclonal
NCT04820270 (161)		Phase 1	Type 1 Diabetes	Autologous, polyclonal
NCT04691232 (162)		Phase 1	Ulcerative Colitis	Autologous, polyclonal
NCT03444064		Phase 1	Type 1 Diabetes	Autologous, polyclonal
NCT03239470	PolyTregs	Phase 1	Pemphigus	Autologous, polyclonal
NCT03162237		Phase 1	Type 1 Diabetes	Autologous, polyclonal
NCT03011021		Phase 1|Phase 2	Type 1 Diabetes	Autologous, polyclonal
NCT02932826		Phase 1|Phase 2	Type 1 Diabetes	Autologous, polyclonal
NCT02772679 (163)	TILT	Phase 1	Type 1 Diabetes	Autologous, polyclonal
NCT01210664 (164)	Treg	Phase 1	Type 1 Diabetes	Autologous, polyclonal
Eudract 2006-004712-44 (165)		Phase 1|Phase 2	Crohn's Disease	Autologous, antigen-specific

Sanders et al, Frontiers in Immunology, 2022

Antigen-Specific Treg Cells

- Allogeneic or autologous DCs
- **Functionally superior** to polyclonal Tregs, **more potent at inhibiting T eff**

- **Contamination** with effector T cells
- Difficulty of selecting a **suitable target antigen**
- **Restricted to MHC**

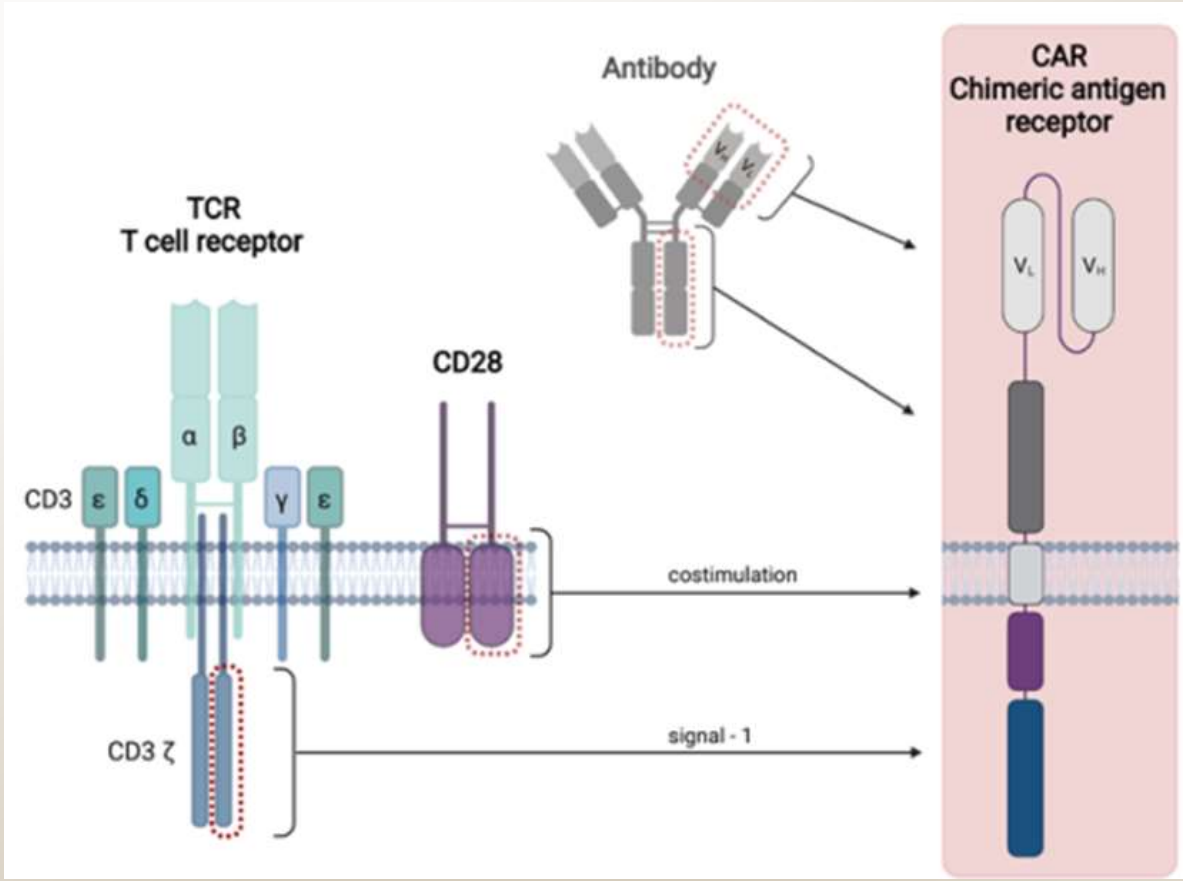


Pilat et al, Frontiers in Immunology. 2021

CAR-Treg Cells

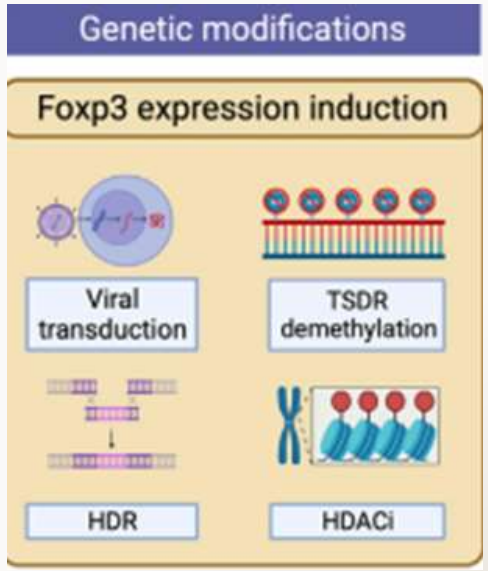
- Antigen-specific, **MHC independent**
- Whole proteins

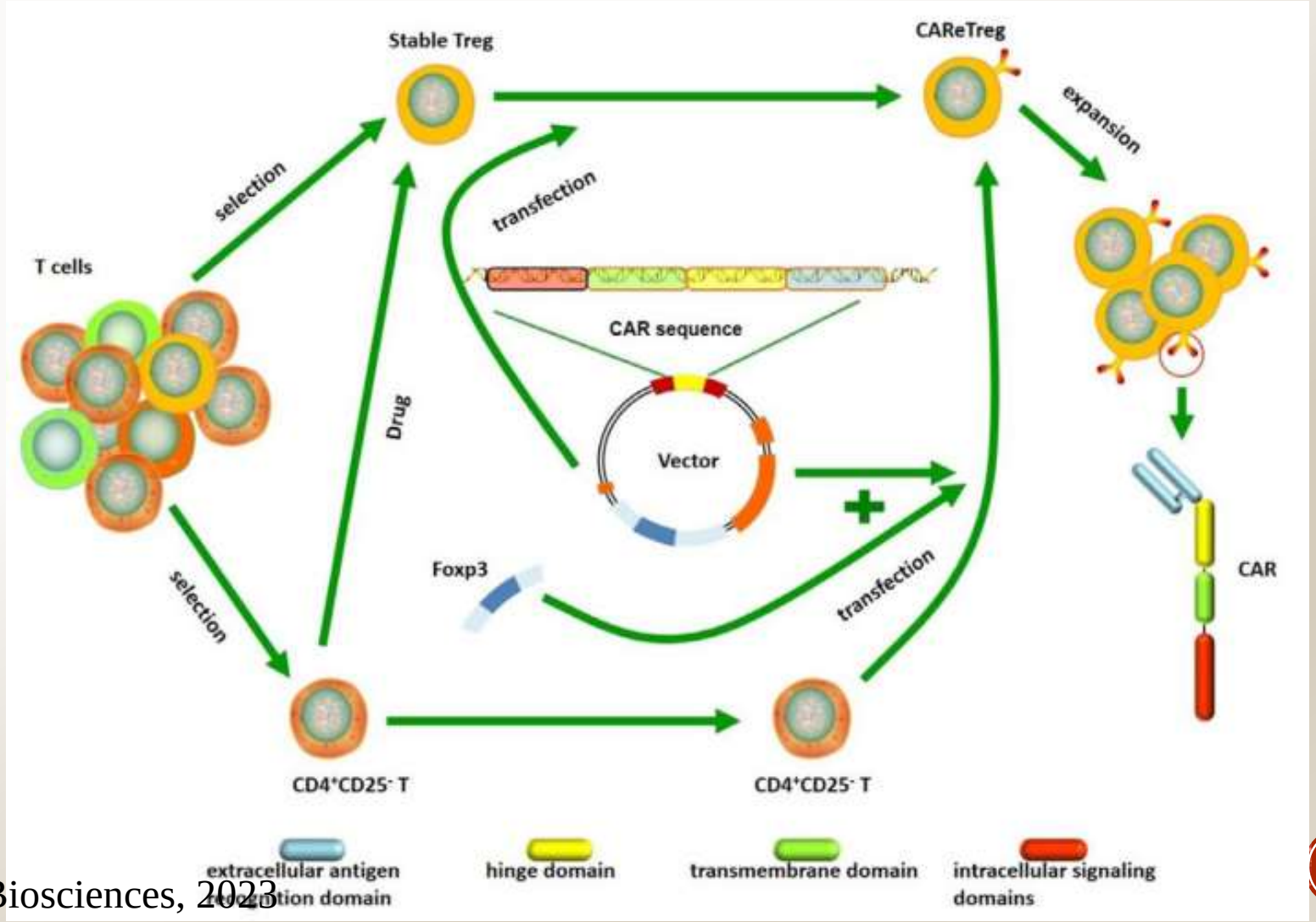
- Elinav and colleagues, **colitis** in mice
- **2,4,6-trinitrophenol (TNP)**
- **Improved colitis**
- Efficacy in treating autoimmune ,GVHD and solid organ transplantation



Kaljanac et al, Transplantation. 2023

Manufacture Of CAR-Treg Cells



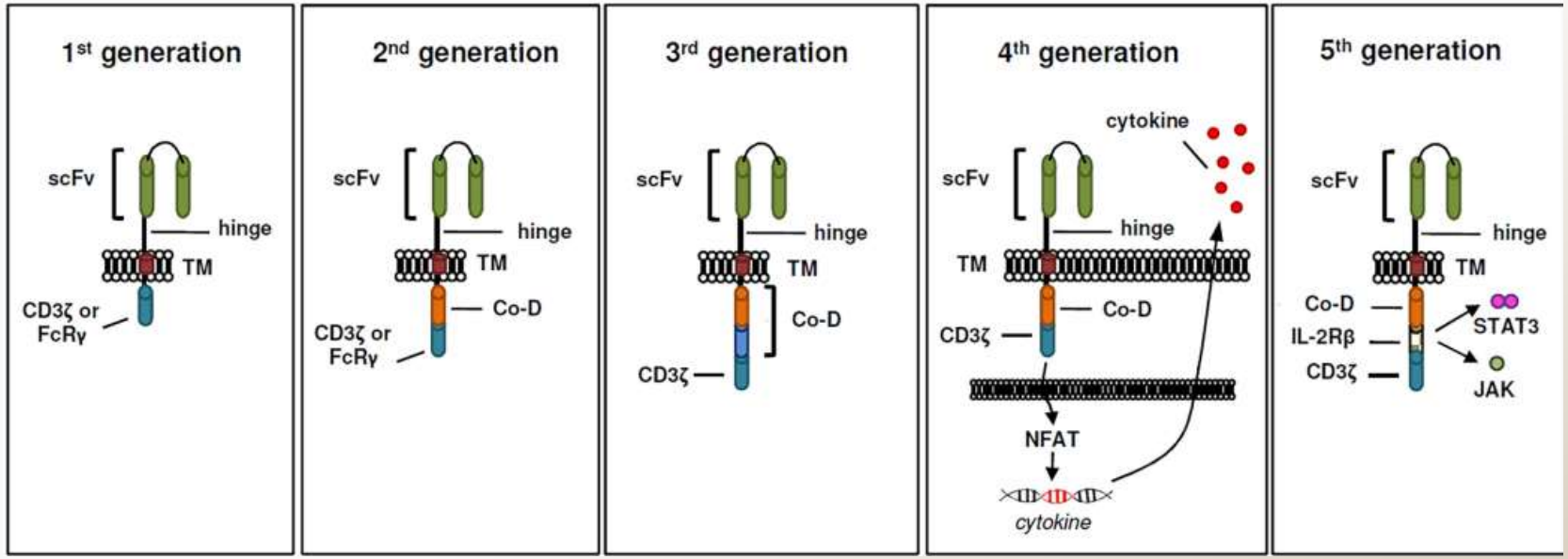


Yi LI et al, Cytotherapy. 2019

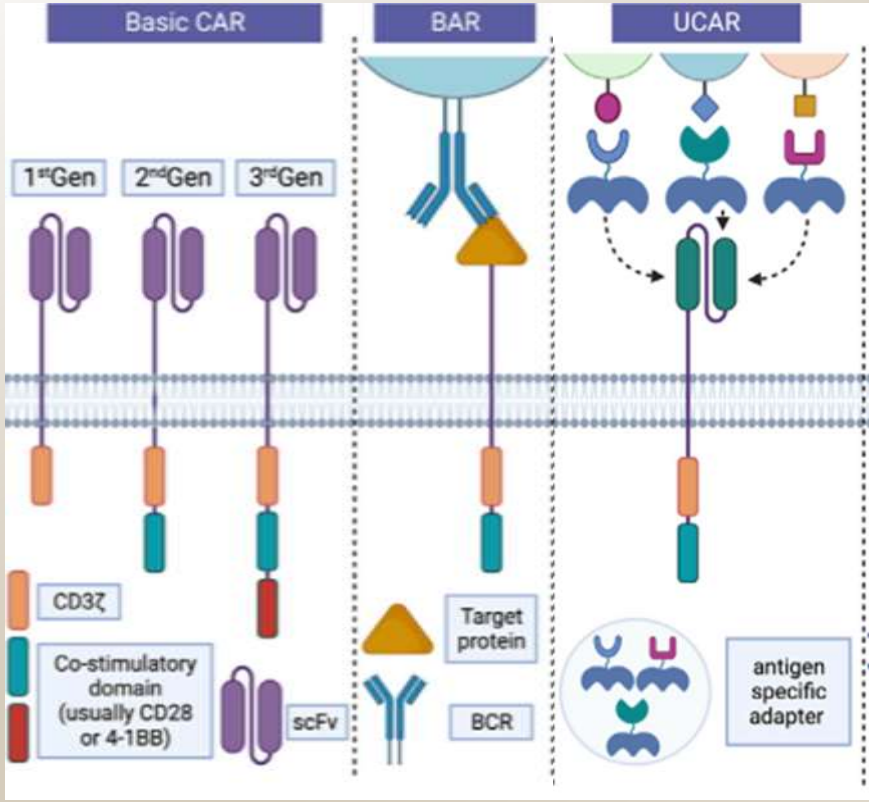
Requejo Cier et al, Frontiers in Molecular Biosciences, 2023

Different Generation Of CAR-Treg Cells

Based on signaling motifs



Based on specificity



Riet et al, Frontiers in Immunology. 2022
Requejo Cier et al, Frontiers in Molecular Biosciences, 2023

Preclinical Studies Using CAR-Tregs In Autoimmune Disease

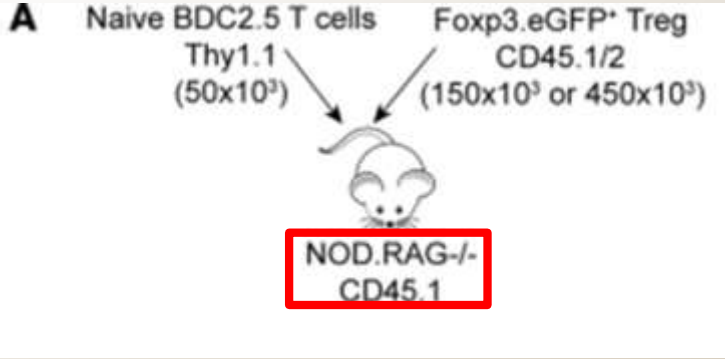
Autoimmune diseases			
Type 1 diabetes	Insulin B peptide-MHC class II	Mouse CAR-Tregs prevented adoptive transfer diabetes by BDC2.5 T cells in immunodeficient NOD mice and prevented spontaneous diabetes in wild type NOD mice	Spanier et al. (2023)
Type 1 diabetes	Insulin B peptide-MHC class II	Mouse InsB:R3-CAR-Tregs protected against spontaneous diabetes in NOD.CD28−/− mice	Obarorakpor et al. (2023)
Type 1 diabetes	Insulin B	Mouse effector T cells converted to Tregs using insulin-specific Foxp3+ CARs. Long-lived in diabetic mice	Tenspolde et al. (2019)
Colitis	TNP	Mouse TNP-CAR-Tregs improved colitis	Elinav et al. (2008) Elinav et al. (2009)
Colitis	CEA	Mouse CEA-CAR-Tregs improved colitis	Blat et al. (2014)
Colitis	Flagellin	Human FliC-CAR-Tregs promoted the establishment of colon-derived epithelial cell monolayers and had a preferential migration to the colon	Boardman et al. (2023)
Multiple sclerosis	MOG (myelin oligodendrocyte glycoprotein)	Engineered mouse MOG-CAR-Tregs suppressed autoimmune encephalomyelitis better than non-specific Tregs	Fransson et al. (2012)
Vitiligo	GD3 (ganglioside D3)	Mouse GD3-CAR Tregs delayed depigmentation compared to untransduced Tregs	Mukhatayev et al. (2020)

Requejo Cier et al, Frontiers in Molecular Biosciences, 2023

The Journal of Clinical Investigation RESEARCH ARTICLE

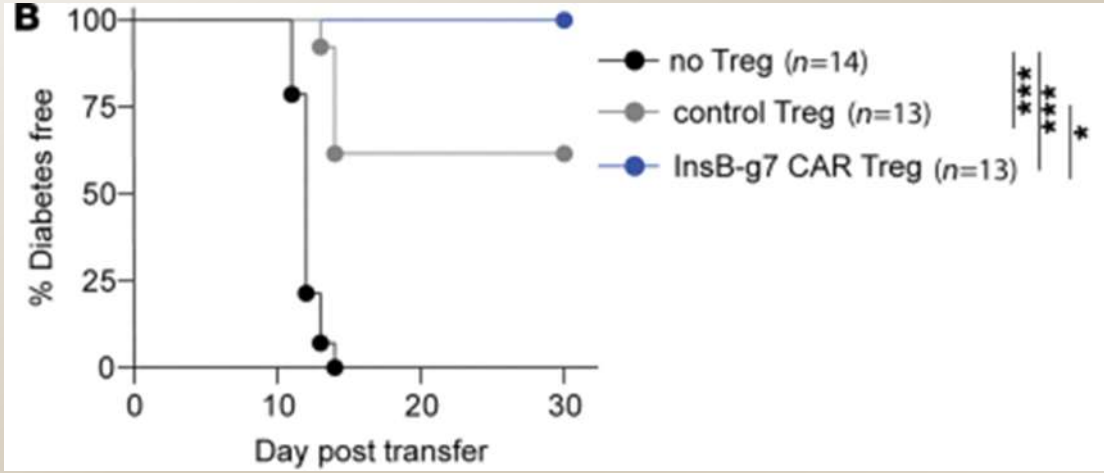
Tregs with an MHC class II peptide–specific chimeric antigen receptor prevent autoimmune diabetes in mice

Justin A. Spanier,[1,2,3] Vivian Fung,[4,5] Christine M. Wardell,[4,5] Mohannad H. Alkhatib,[1,3] Yixin Chen,[1,3] Linnea A. Swanson,[3] Alexander J. Dwyer,[1,3] Matthew E. Weno,[1,3] Nubia Silva,[1,3] Jason S. Mitchell,[1,2,6] Paul C. Orban,[4,5] Majid Mojibian,[4,5] C. Bruce Verchere,[4,5] Brian T. Fife,[1,2,3] and Megan K. Levings[4,5,7]



- In vivo function of InsB-g7 CAR Tregs

- BDC2.5 CD4+ T cells alone
- Untransduced control Tregs(3:1 or 9:1 ratio)
- InsB-g7 CAR Tregs (3:1 or 9:1 ratio)

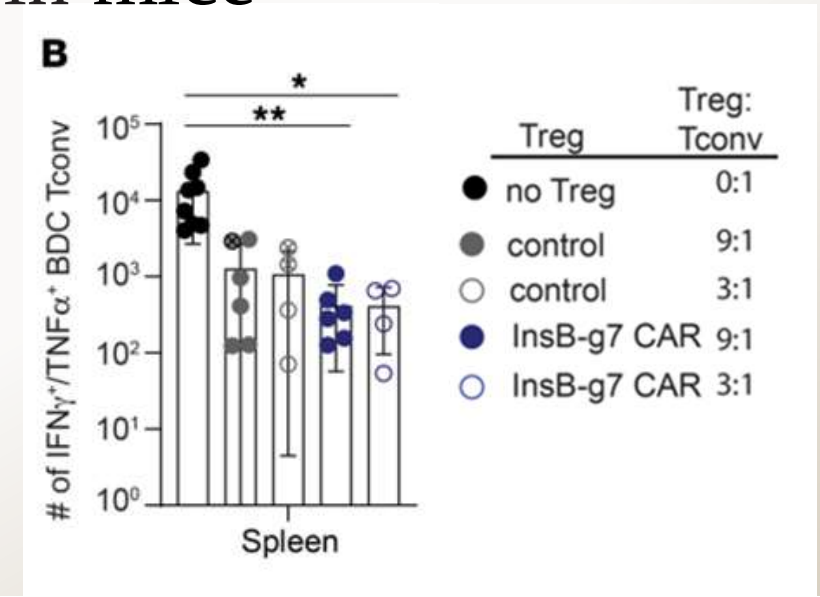


Spanier et al, The Journal of clinical investigation. 2023

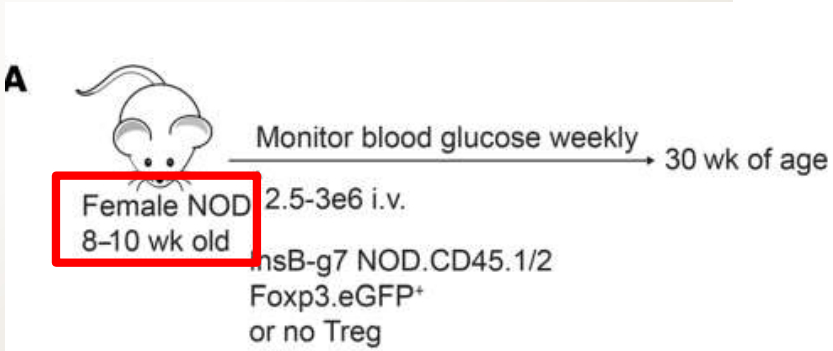
Tregs with an MHC class II peptide–specific chimeric antigen receptor prevent autoimmune diabetes in mice

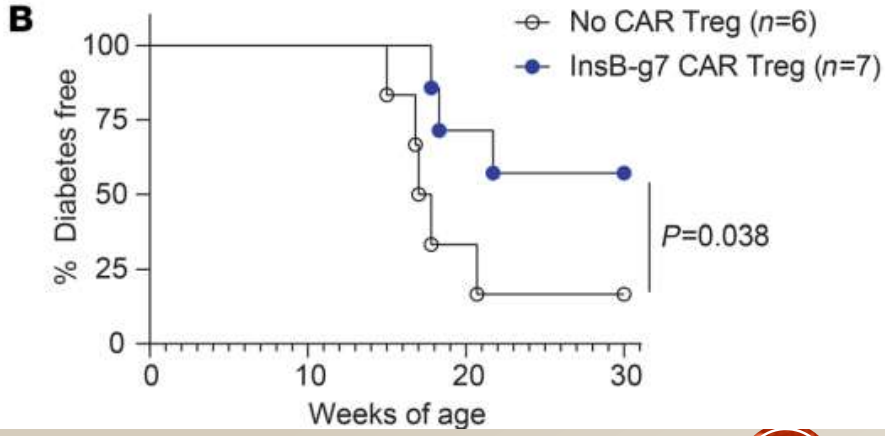
- Effects of CAR Tregs on BDC2.5 T cell

- BDC2.5 CD4+ T cells
- Untransduced control Tregs(3:1 or 9:1)
- InsB-g7 CAR Tregs (3:1 or 9:1)



- Effects on prevention of diabetes





Spanier et al, The Journal of clinical investigation. 2023

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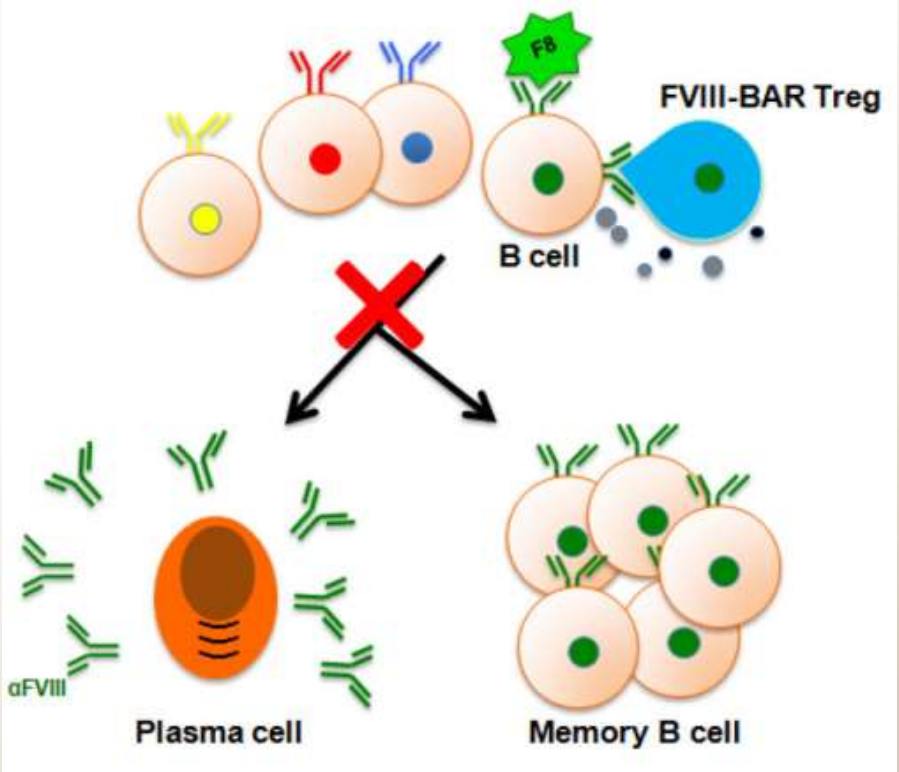
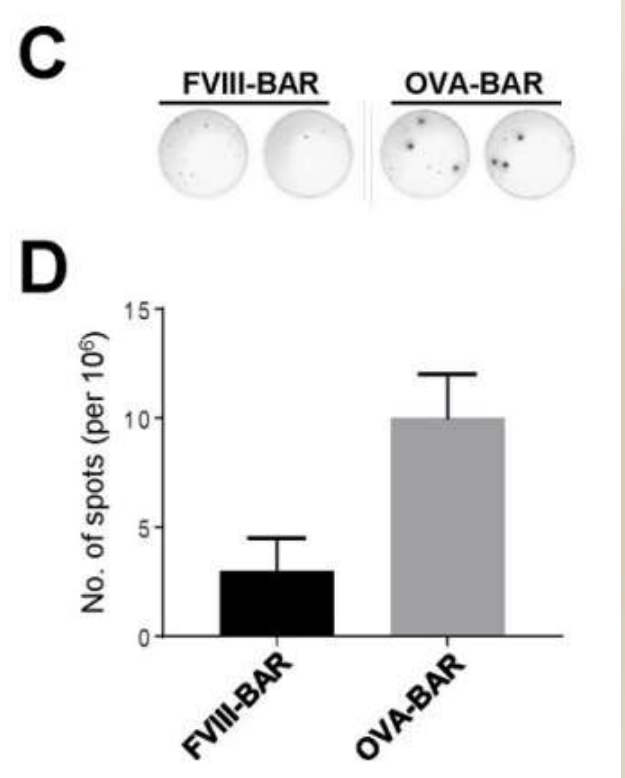
Targeting antigen-specific B cells using antigen-expressing transduced regulatory T cells

Ai-Hong Zhang[*], **Jeongheon Yoon**[*], **Yong Chan Kim**[*], and **David W. Scott**[¶]
Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

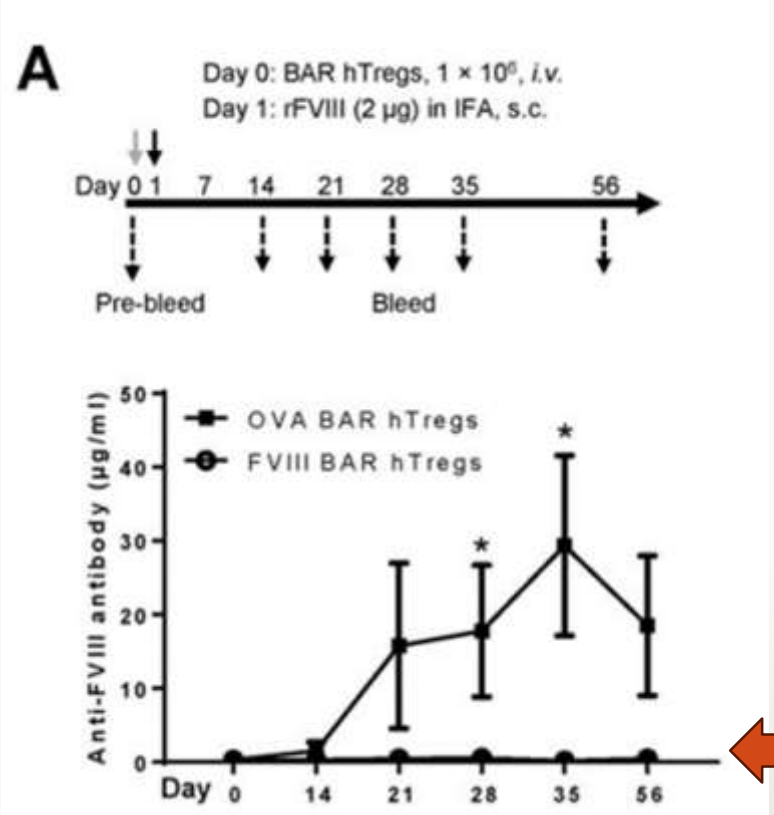
- Hemophilia A
- ***In vitro***
- Anti-F8 B cell ELISPOT assay



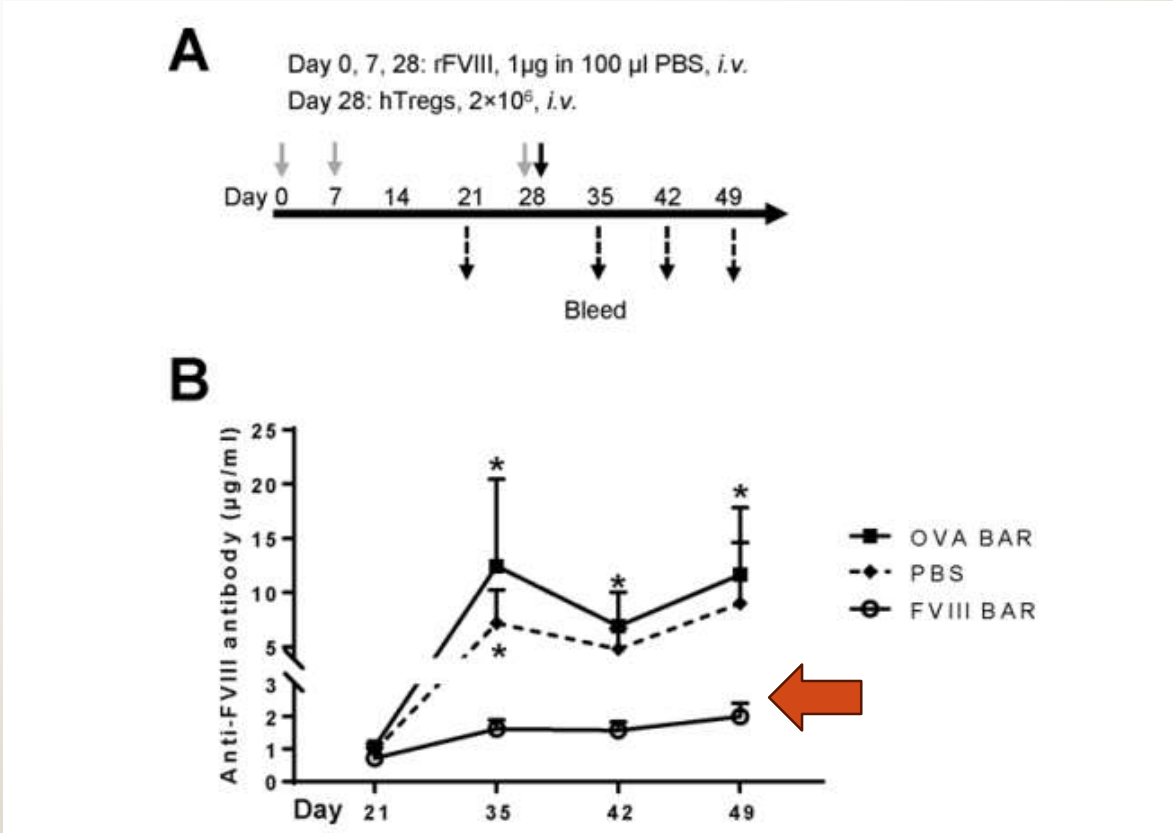


Zhang el al, The Journal of Immunology. 2018

Targeting antigen-specific B cells using antigen-expressing transduced regulatory T cells



- Preventive
- FVIII-BAR Tregs suppress the formation of antibodies



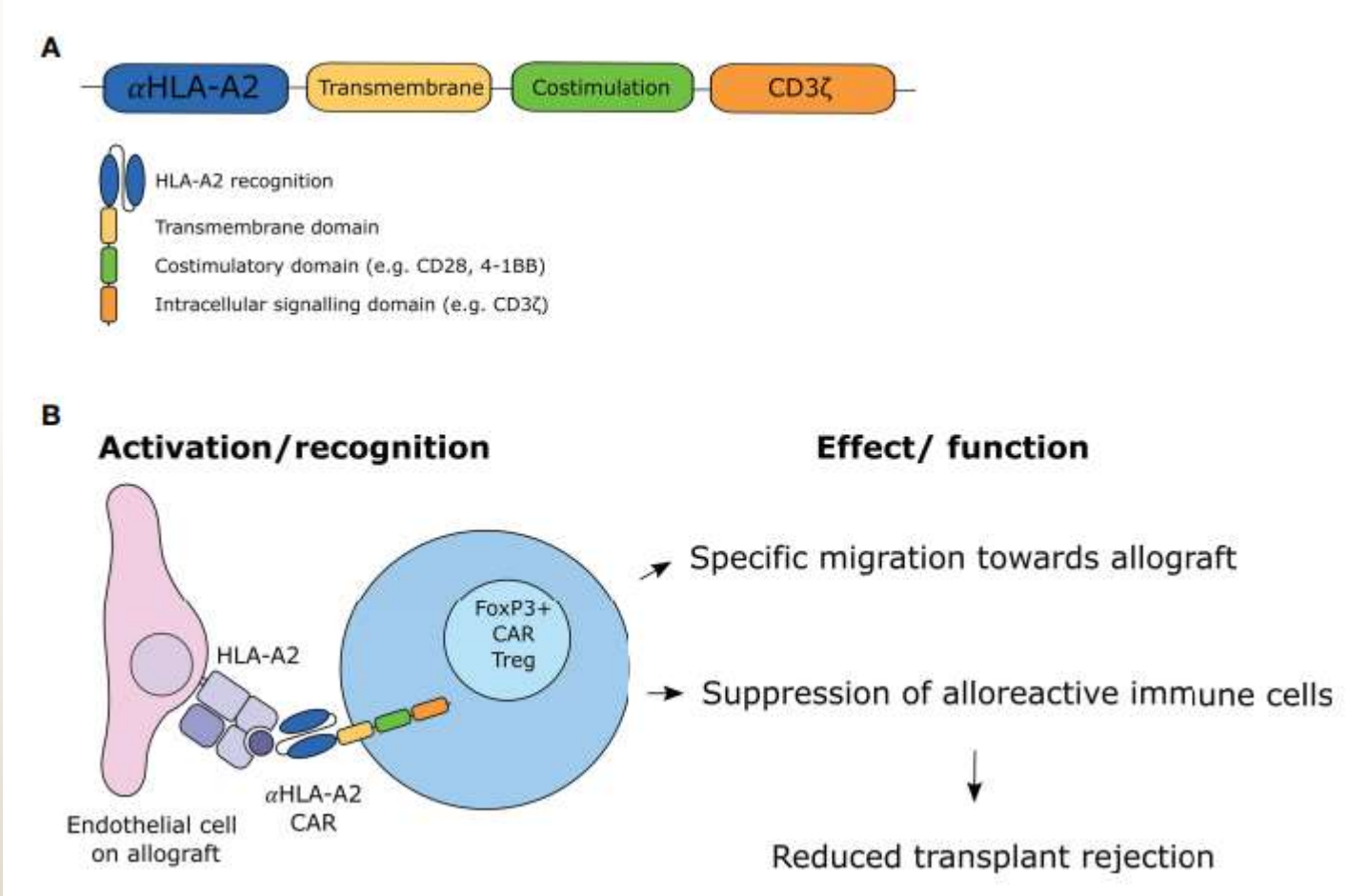
- Therapeutic effect of FVIII-BAR Tregs on mice with preexisting anti-FVIII antibodies

Zhang el al, The Journal of Immunology. 2018

CAR-Treg In Transplantation

- **HLA mismatch**
- MHC class I
- Nearly all transplanted cells

- **HLA-A2 is highly prevalent (>40%) in white donors**
- **25% HLA-A2 mismatch**
- Poor outcomes



Gille et al, Frontiers in Immunology, 2022

Preclinical Studies Using CAR-Tregs In Transplantation

Disease	Antigen specificity	Main results	References
Transplantation			
GvHD	HLA-A2	Human A2-CAR-Tregs were superior to irrelevant CAR-Tregs to prevent GvHD.	MacDonald et al. (2016)
GvHD and skin transplant	HLA-A2	Human A2-CAR-Tregs prevented A2-expressing human skin graft rejection and prevented GvHD	Noyan et al. (2017)
Skin transplant	HLA-A2	Human A2-CAR-Tregs prevented A2-expressing human skin graft rejection	Boardman et al. (2017)
GvHD and skin transplant	HLA-A2	Human A2-CAR-Tregs prevented A2-expressing human skin graft rejection and prevented GvHD	Dawson et al. (2019)
Skin transplant	HLA-A2	Mouse A2-CAR-Tregs delayed skin rejection, decreased donor-specific antibodies formation and A2-specific B cells formation, but only in unsensitized mice	Sicard et al. (2020)
Heart transplant	HLA-A2	Mouse A2-CAR-Tregs prolonged the survival of heterotopic heart transplants	Wagner et al. (2022)
GvHD, islet and skin	Universal CAR	Mouse mAb-CAR-Tregs could prevent GvHD, prolong survival of islet allografts and secondary skin allografts	Pierini et al. (2017)
GvHD	HLA-A2	Human A2-CAR-Tregs modified to overexpress Foxp3 were stable under proinflammatory conditions and had a survival advantage under IL-2 deprived conditions and could prevent human A2+ PBMC engraftment in humanized mouse model	Henschel et al. (2023)

Requejo Cier et al, Frontiers in Molecular Biosciences, 2023

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Anti-HLA-A2-CAR Tregs prolong vascularized mouse heterotopic heart allograft survival

Johanna C. Wagner[1], **Emilie Ronin**[1], **Patrick Ho**[1], **Yani Peng**[1], **Qizhi Tang**[1,2,3,*]

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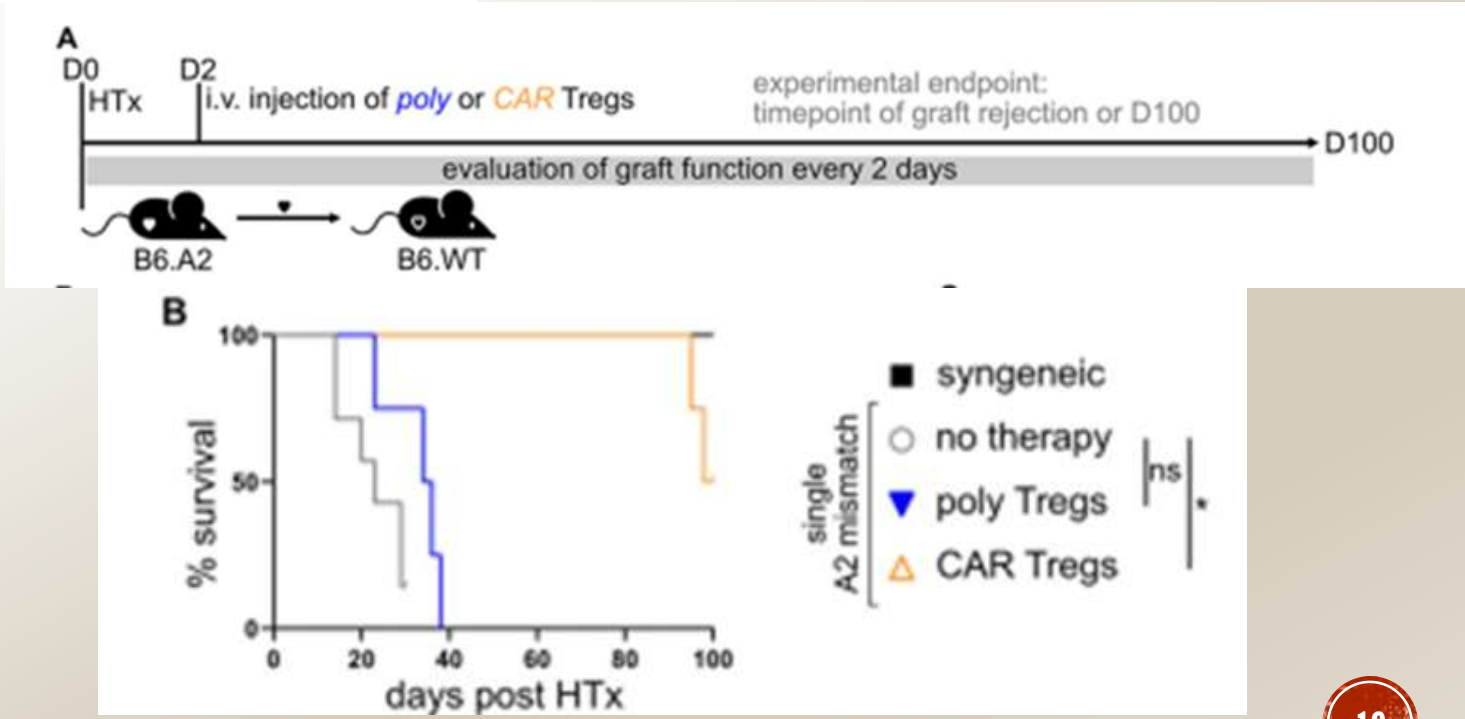
- Single A2-mismatch
- In vivo function of A2-CAR Tregs

- No therapy
- Polyclonal Treg
- CAR-Treg

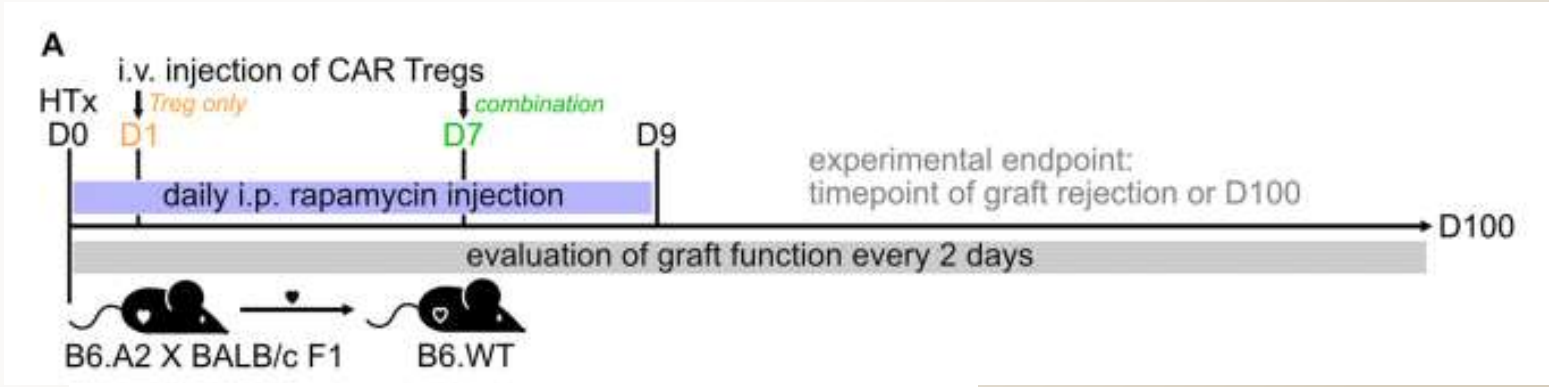


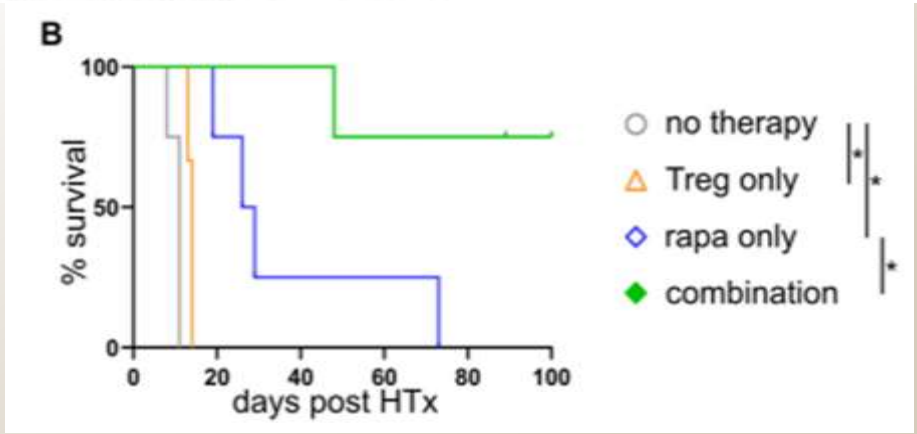
Wagner et al, American Journal of Transplantation, 2022

Anti-HLA-A2-CAR Tregs prolong vascularized mouse heterotopic heart allograft survival

- Haplo-mismatch
- Immunosuppressive drug

- CAR Treg
- Rapamycin
- combination





Wagner et al, American Journal of Transplantation, 2022

Clinical Trial Using CAR-Treg Cells

Trial name	Population	CAR characteristics	Clincaltrial.gov ID
STeadfast	Living donor renal transplant recipients	HLA-A2 CAR-Tregs, autologous	NCT04817774 (protocol in Schreeb et al. (2022), commented in Lamarche and Maltzman (2022))
LIBERATE	Liver transplant recipients	HLA-A2 CAR-Tregs, autologous	NCT05234190
Allogeneic CD6-CAR Tregs for the treatment of patients with chronic graft versus host disease after allogeneic hematopoietic cell transplantation	Allogenieic hematopoietic cell transplantation recipients, for the treatment of chronic graft-versus-host disease	CD6-CAR-Tregs, allogeneic (donor-derived)	NCT05993611
CAR19 Regulatory T Cells (CAR19-tTreg) in Adults With Relapsed/Refractory $CD19^+$ B Acute Lymphocytic Leukemia	Adults with relapsed/refractory (R/R) $CD19^+$ B Acute Lymphocytic Leukemia (B-ALL)	CAR19-Treg, allogeneic	NCT05114837

Requejo Cier et al, Frontiers in Molecular Biosciences, 2023

KI REPORTS
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Chimeric Antigen Receptor Regulatory T Cell in Transplantation: The Future of Cell Therapy?

Caroline Lamarche[1] and Jonathan S. Maltzman[2,3]

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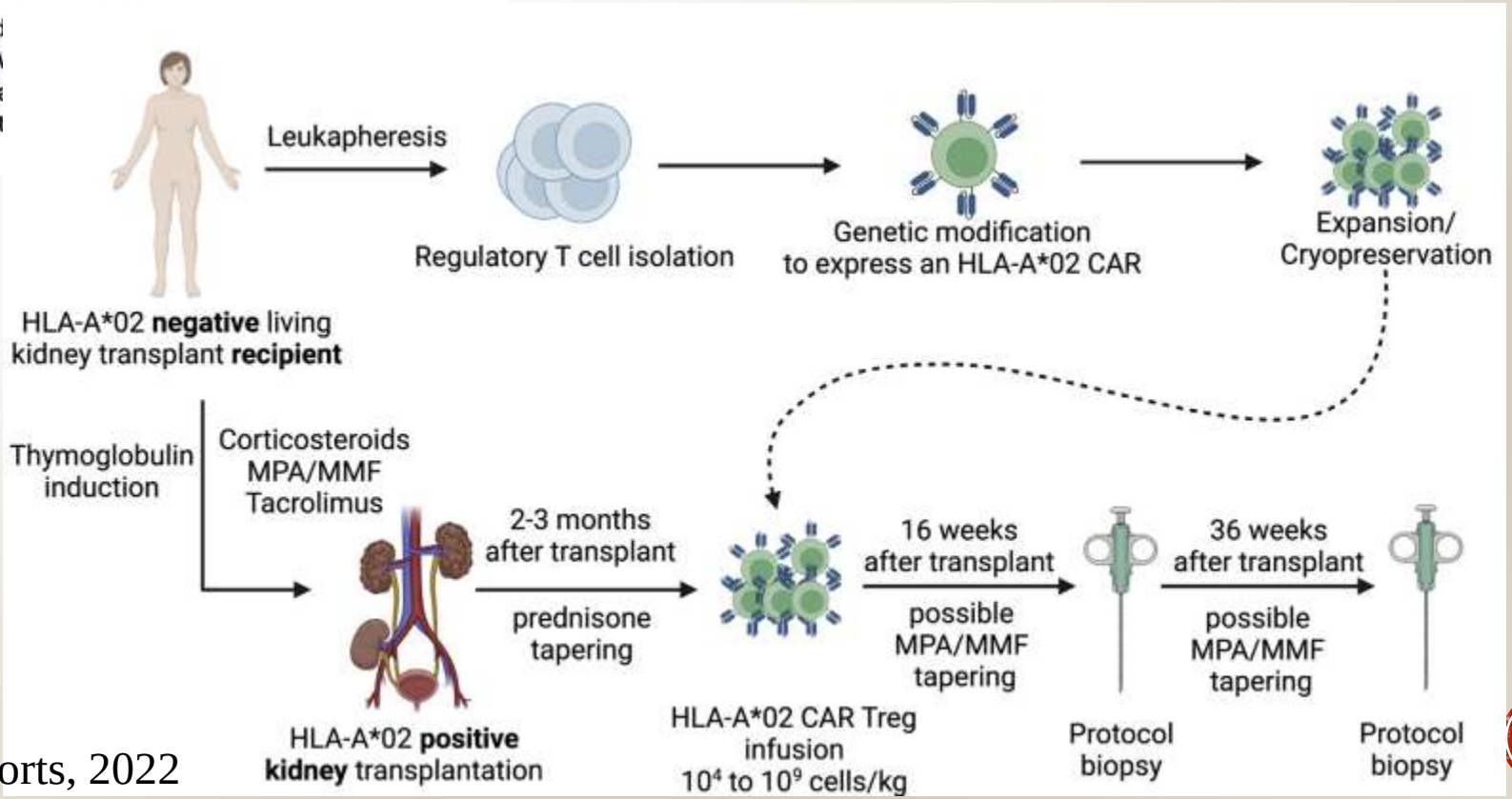
[2]Division of Nephrology, Department of Medicine, Sta
and [3]Geriatric Research Education and Clinical Cent
Care System, Palo Alto, CA, USA



Lamarche et al, Kidney International Reports, 2022

Gene Therapy www.nature.com/gt

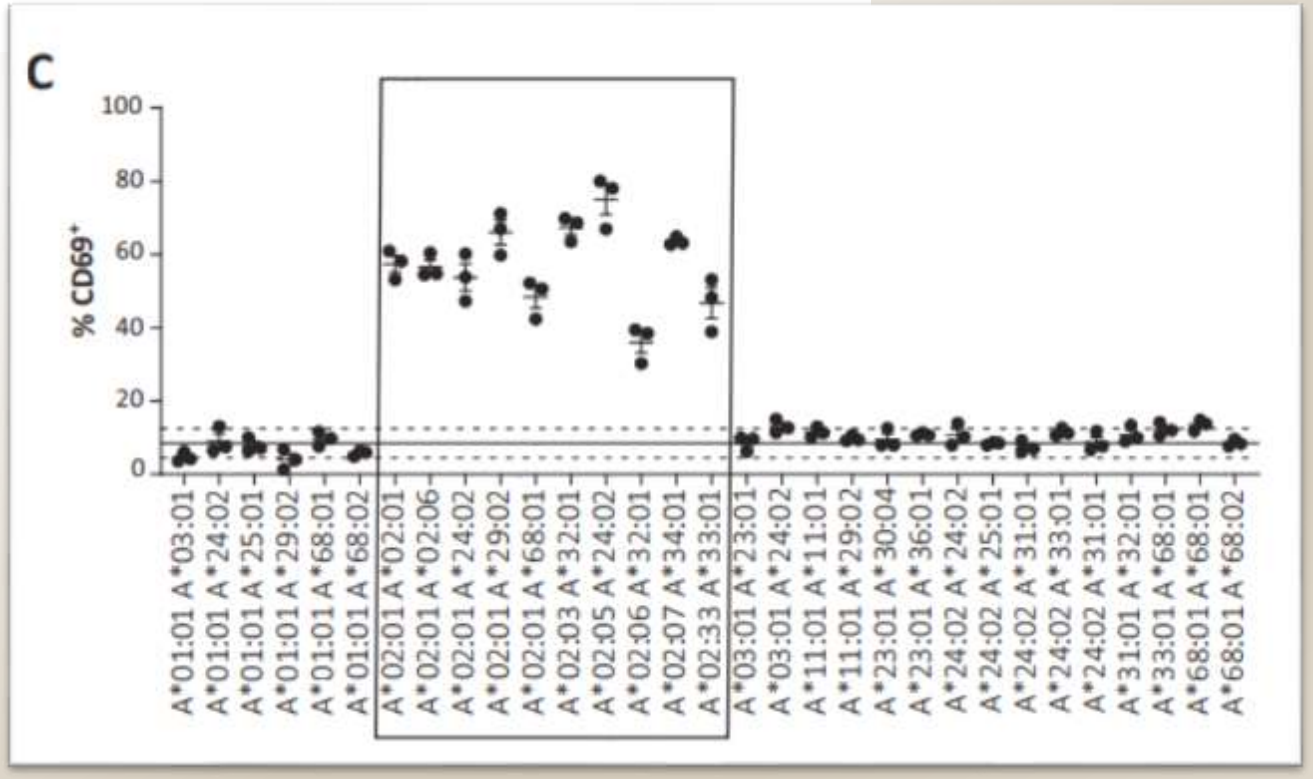
ARTICLE OPEN

Check for updates

Preclinical assessment of antigen-specific chimeric antigen receptor regulatory T cells for use in solid organ transplantation

Emma Proics[1,5], Marion David[1,5], Majid Mojibian[2,3], Madeline Speck[2,3], Nadia Lounnas-Mourey[1], Adeline Govehovitch[1], Wissam Baghdadi[1], Justine Desnouveaux[1], Hervé Bastian[1], Laura Freschi[1], Geoffrey Privat[1], Cédric Pouzet[1], Mauro Grossi[1], Pierre Heimendinger[1], Tobias Abel[1], David Fenard[1], Megan K. Levings[2,3,4], François Meyer[1] and Céline Dumont[1]✉

- Specificity of A2-CAR-Tregs
- PBMCs with various HLA-A alleles



Proics et al, Gene Therapy, 2023

Limitation Of CAR-Treg Cells

- Amounts of cells needed for infusions
- **High costs** for *in vitro* Treg expansion
- **Viral vectors, oncogenic genetic changes**
- **Selection of antigens** targeted by CARs are difficult in some disease models
- **Contamination** of Treg products with conventional T cells, which could exacerbate autoimmune diseases
- **Cross-reactivity**

Conclusion

- Adoptive Treg cell therapy
- **Safety and efficacy** of Treg adoptive cell technology in human disease
- Strategies to improve the **in vivo stability** and **suppressive function** of Tregs
- Best dosing regimen, what is the required frequency of Treg infusion, what immunosuppressive protocol
- Development of advanced generations of CARs
- Combinations of different costimulatory domains
- Enhancements of homing abilities through chemokine receptors

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