(Regulatory) Challenges in the development of a CRISPR/Cas9 edited tacrolimus-resistant regulatory T cell product

Dr. Leila Amini

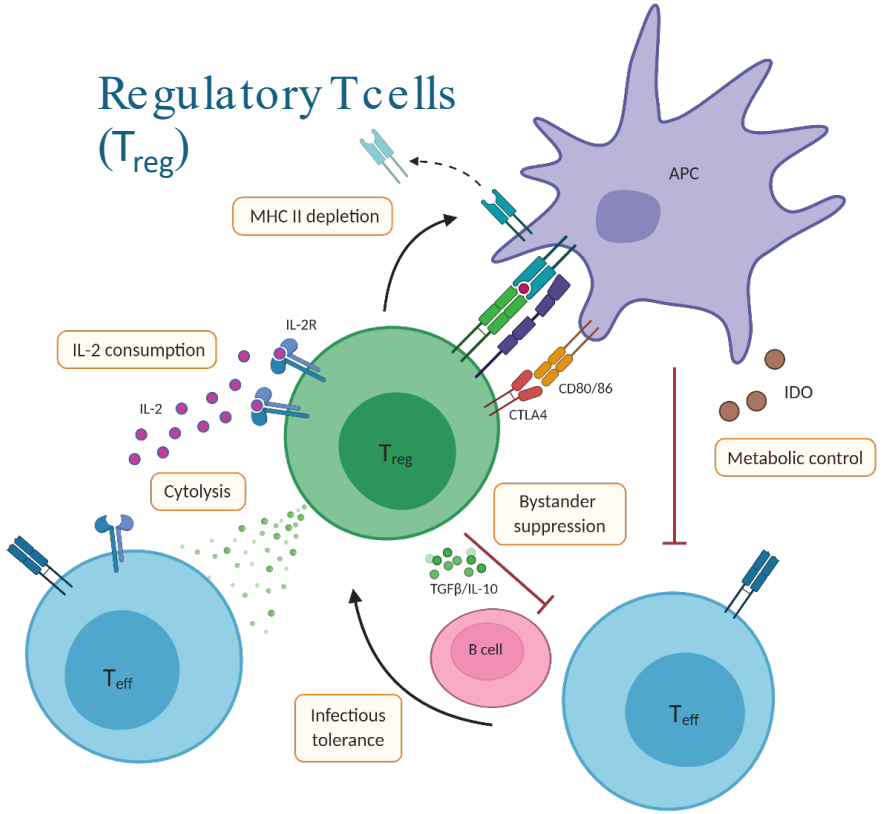
EU Innovation Network Meeting, November 15th, 2024

Solid organ transplantation

Lifelong immunosuppression

- Life saving (prevents allograft rejection)
- Adverse effects: malignancy, infection, toxicity
- Long-term outcome not improved recently

Adoptive Treg therapy



Wagner et al., Gene Ther, 2021

Adoptive Treg therapy

Research

Regulatory T cells for minimising immune suppression in kidney transplantation: phase I/IIa clinical trial

BMJ 2020 ; 371 doi: https://doi.org/10.1136/bmj.m3734 (Published 21 October 2020)
Cite this as: *BMJ* 2020;371:m3734

Clinical Observations	nTreg (n=11)	Cohort control (n=9)	nTreg (n=10)	Cohort control (n=9)
	60 weeks		9 years	
Immunosuppression				
Monotherapy	8	0	8	0
Dual Therapy	0	5	0	4
Triple therapy	3	4	3	5

ONE STUDY

Adoptive Treg therapy

Research

Regulatory T cells for minimising immune suppression in kidney transplantation: phase I/IIa clinical trial

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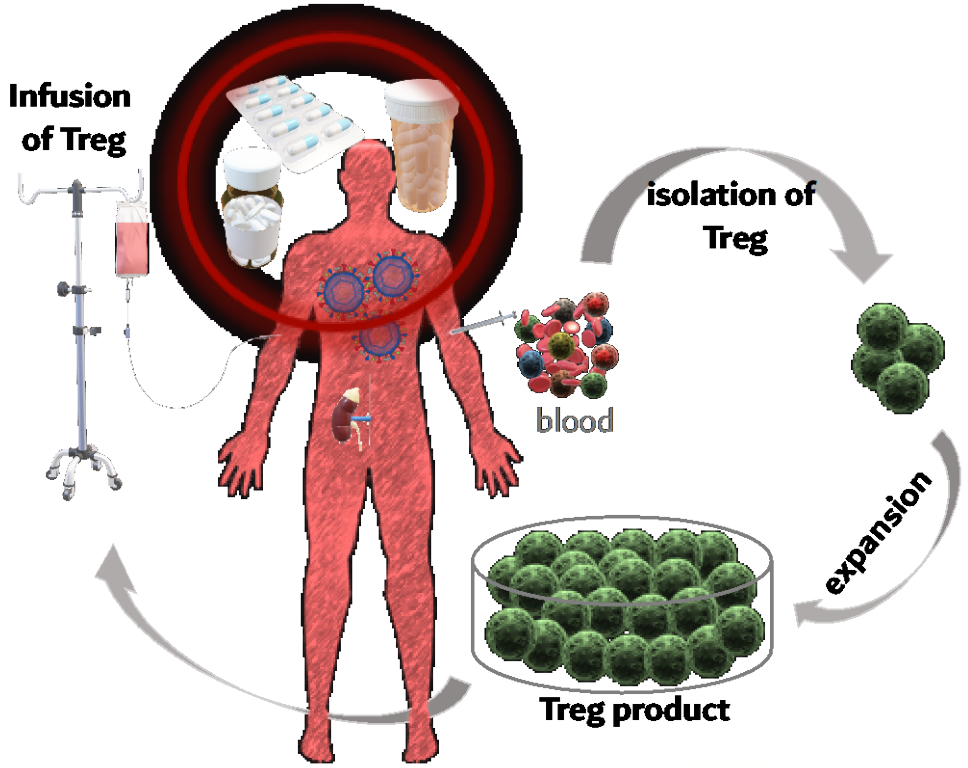
Clinical Observations	nTreg (n=11)	Cohort control (n=9)	nTreg (n=10)	Cohort control (n=9)
	60 weeks		2 years	
Immunosuppression				
Monotherapy	8	0	8	0
Dual Therapy	0	5	0	4
Triple therapy	3	4	3	5

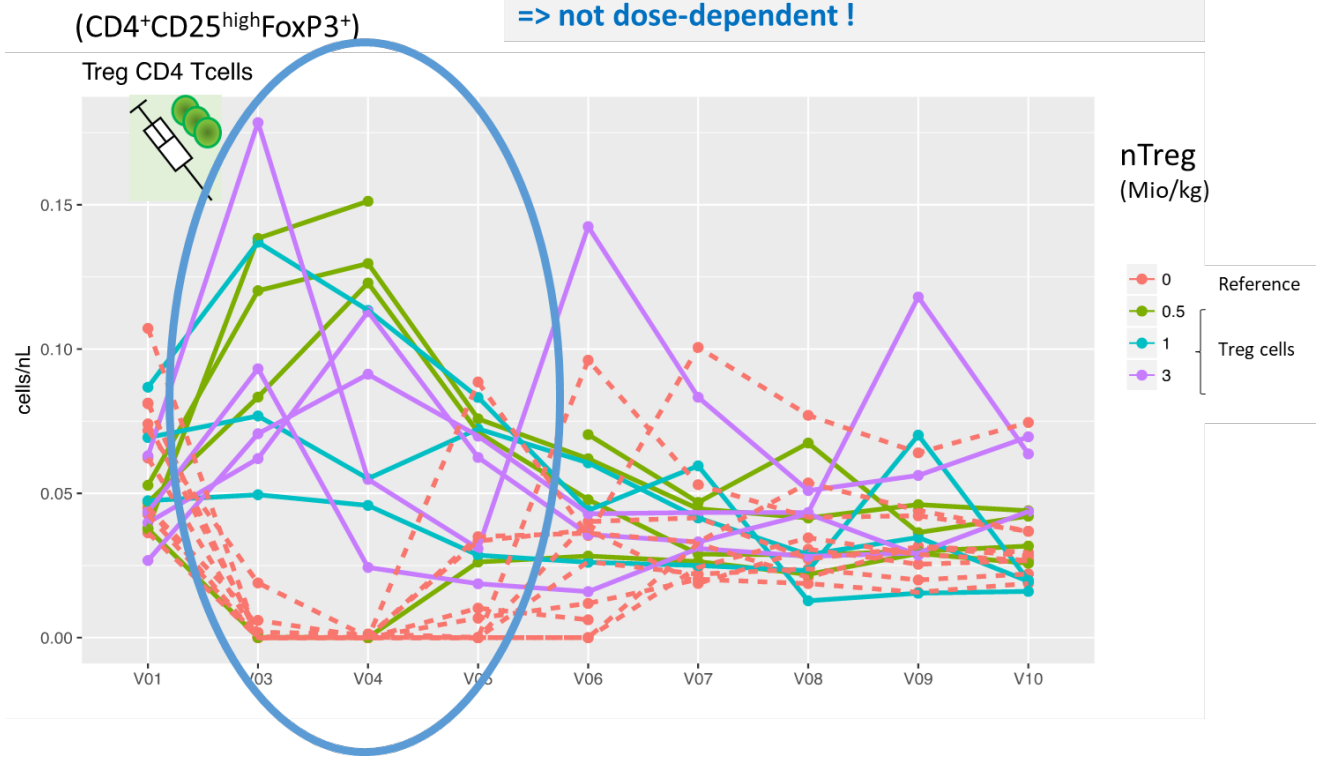
Clinical Observations	nTreg (n=10)	Cohort control (n=9)	nTreg (n=10)	Cohort control (n=9)
	5 years		8 years	
Immunosuppression				
Monotherapy	7	n/a	4	n/a
Dual Therapy	1	3	4	3
Triple therapy	2	6	2	6

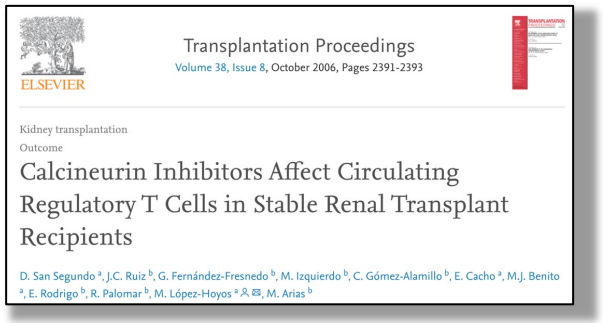
ONE STUDY

Adoptive Treg therapy - challenges



=> transient increase of nTreg counts (4-8 weeks)
=> not dose-dependent !





Transplantation Proceedings
Volume 38, Issue 8, October 2006, Pages 2391-2393

Kidney transplantation
Outcome

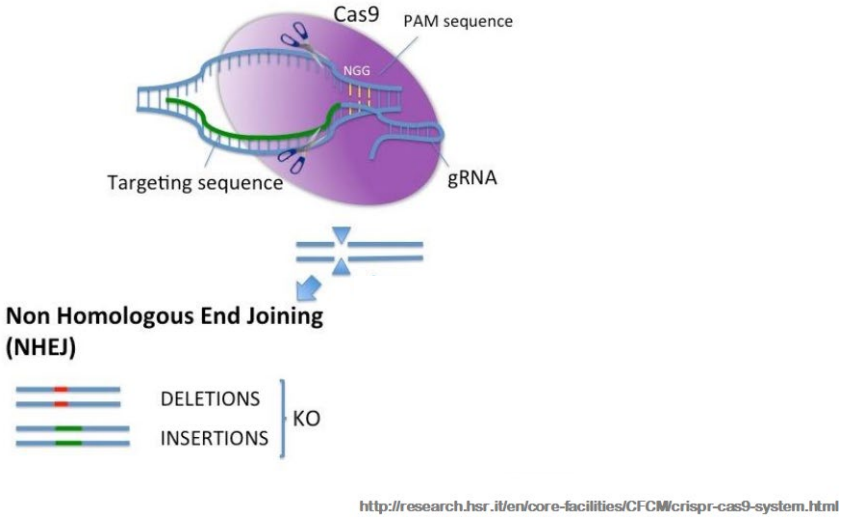
Calcineurin Inhibitors Affect Circulating Regulatory T Cells in Stable Renal Transplant Recipients

D. San Segundo [a], J.C. Ruiz [b], G. Fernández-Fresnedo [b], M. Izquierdo [b], C. Gómez-Alamillo [b], E. Cacho [a], M.J. Benito [a], E. Rodrigo [b], R. Palomar [b], M. López-Hoyos [a] ✉, M. Arias [b]

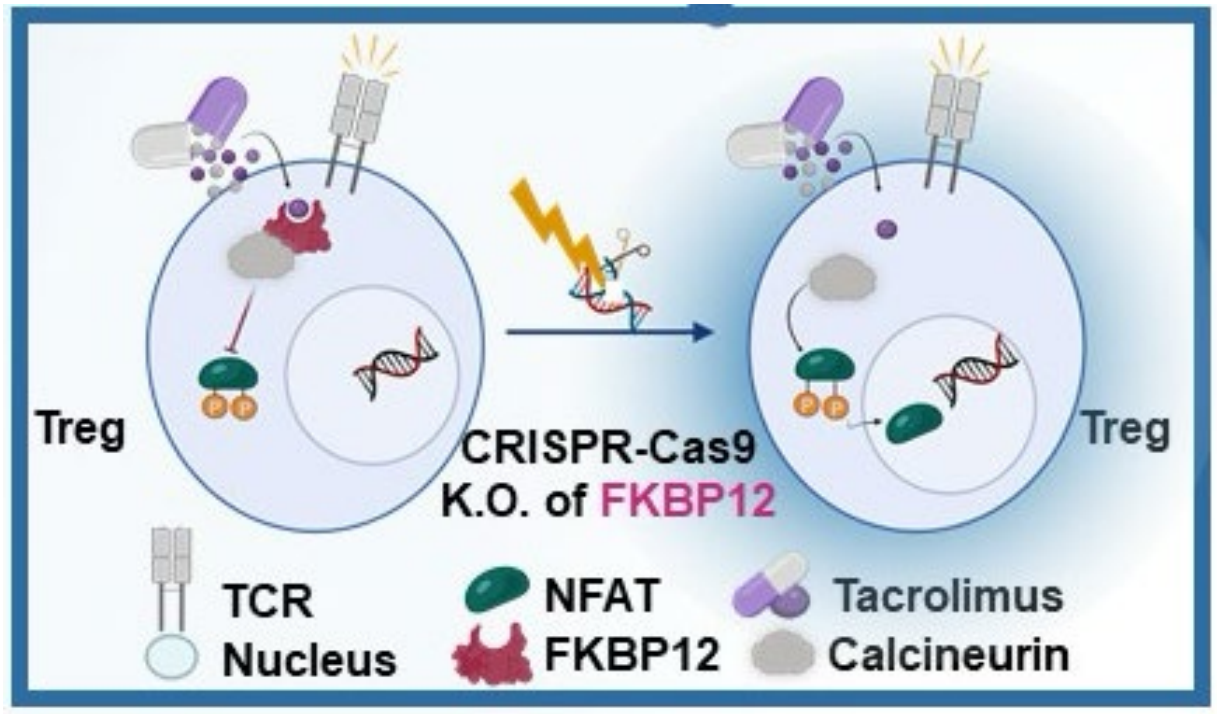
ONE STUDY

⇒ Only transient increase of Treg counts (4-8 weeks)
in the Treg group vs. Reference group
⇒ Not dose-dependent

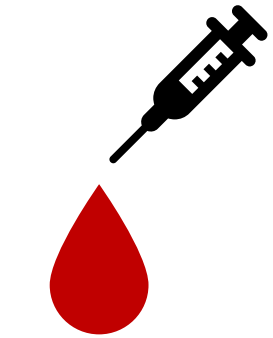
RNP based FKBP12^{KO} → TregTacRes



Gundry *et al.*, Cell Rep, 2016.



GMP compliant manufacturing of TregTacRes



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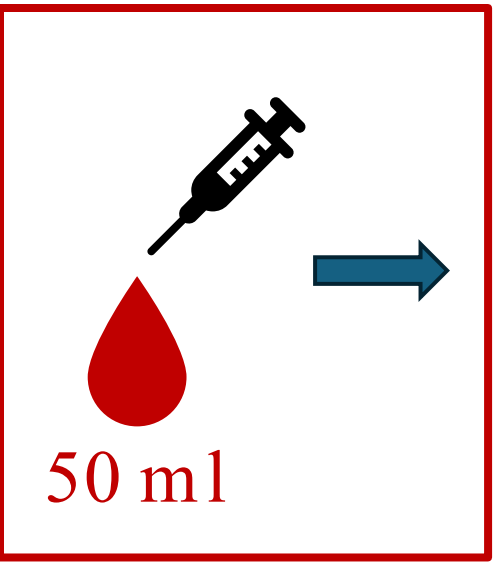


Challenge: Blood as starting material

ONE STUDY

Challenge: Blood as starting material

ONE STUDY

Local authority now applied:

- Haemotherapy Guideline
- Transfusion law

– Contaminants of animal origin

– CE certified for diagnostics only, not pharmaceutical grade

– Need for a certified collection site with manufacturing authorization to produce whole blood

Challenge: Blood as starting material

- Contaminants of animal origin
- CE certified for diagnostics only, not pharmaceutical grade

ONE STUDY

Stability of 48 h
Filled in the cleanroom

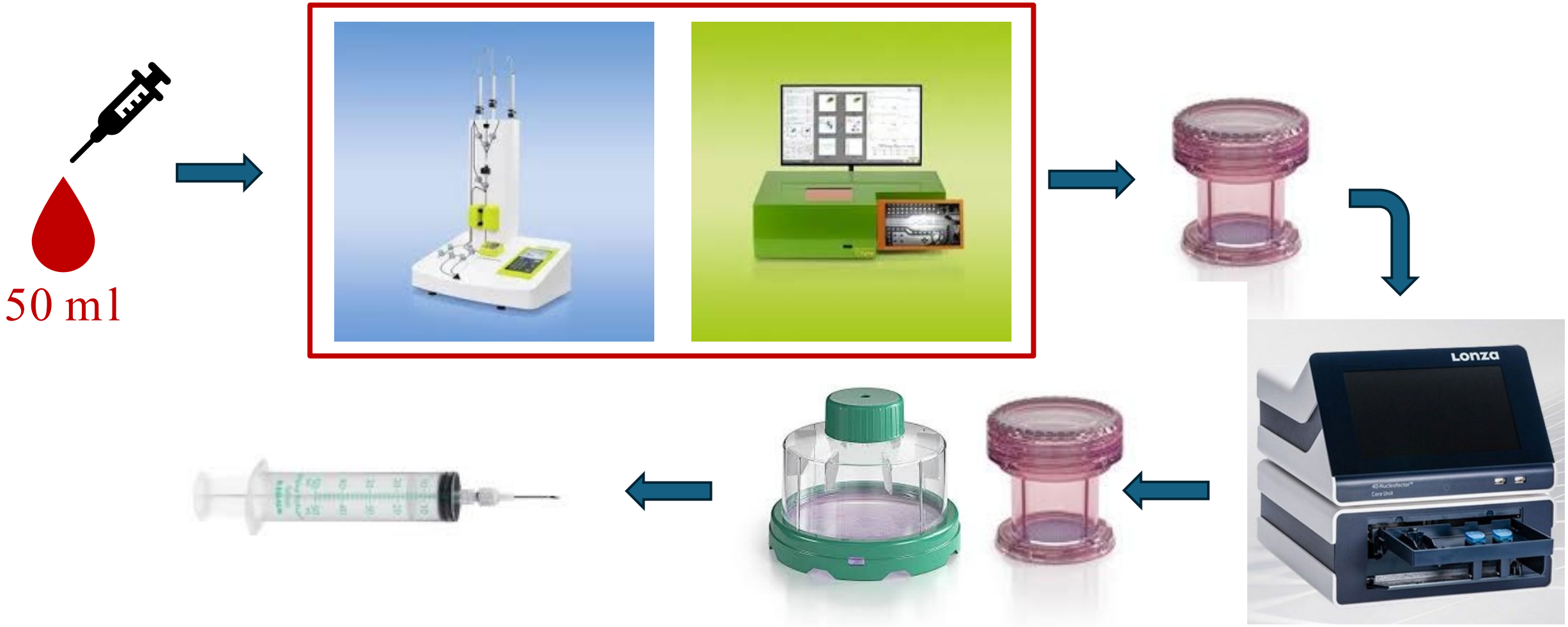
2024/1938 17.7.2024

REGULATION (EU) 2024/1938 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 13 June 2024

on standards of quality and safety for substances of human origin intended for human application and repealing Directives 2002/98/EC and 2004/23/EC

GMP compliant manufacturing of TregTacRes



Challenge: High purity isolation of Treg

1st generation:
CliniMACS
Two-step Strategy

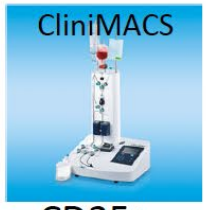
CD8-

CD25+

d7 of expansion: 55-70% Treg purity

Novel Cell Sorting Approach

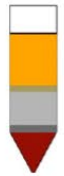
CD8-



CD25+

CD4+CD25+CD127-

Not feasible
(identical CD25 mAb clone)



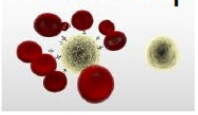
PBMC

two times

CD4+CD25+CD127-

>90% CD4+25+FoxP3+
Cons
Ficoll prep
Impurity CD8+ >0.1%

RosetteSep



Non-CD4 lin.neg.

CD4+CD25+CD127-

>90% CD4+25+FoxP3+
Impurity CD8+ <0.1%
Cons
Ficoll prep/RosetteSep

CD4+

CD4+CD25+CD127-

>90% CD4+25+FoxP3+
Impurity CD8+ <0.1%
Cons
None

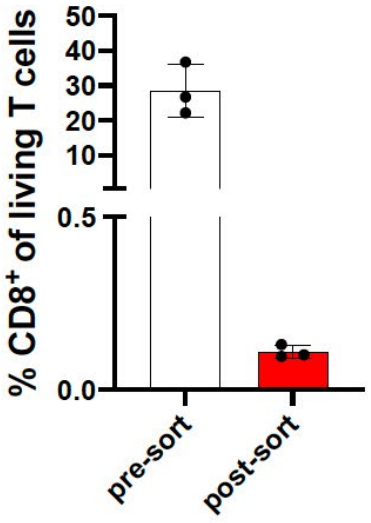
ONE STUDY

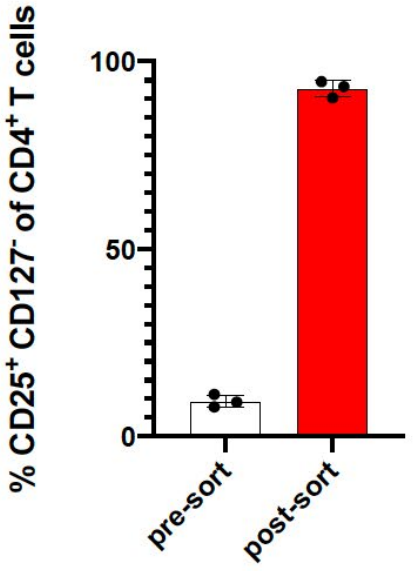
Challenge: High purity isolation of Treg

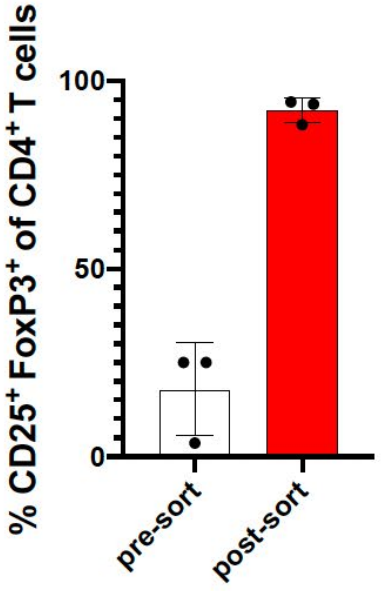
CD4+

CD4+CD25+CD127-

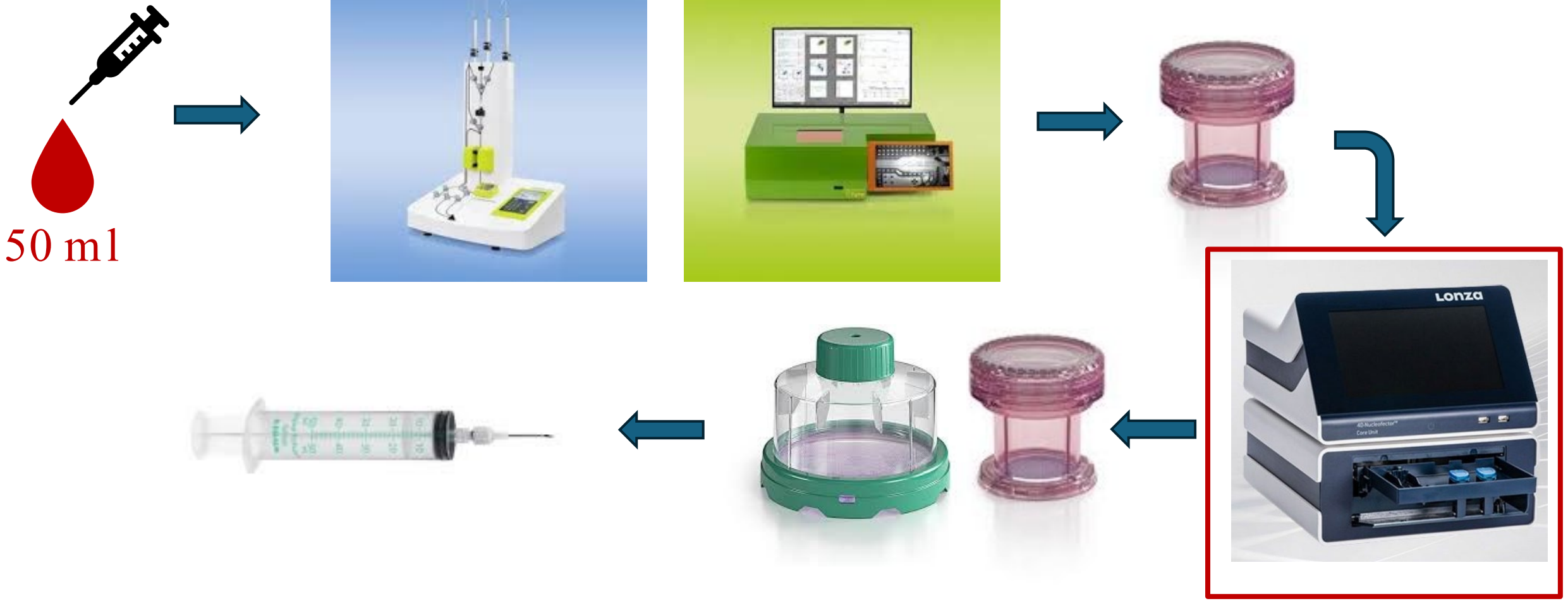
> 90% purity Treg < 0.1 % CD8+, NK, B



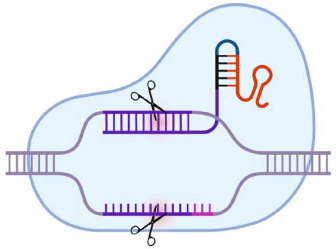


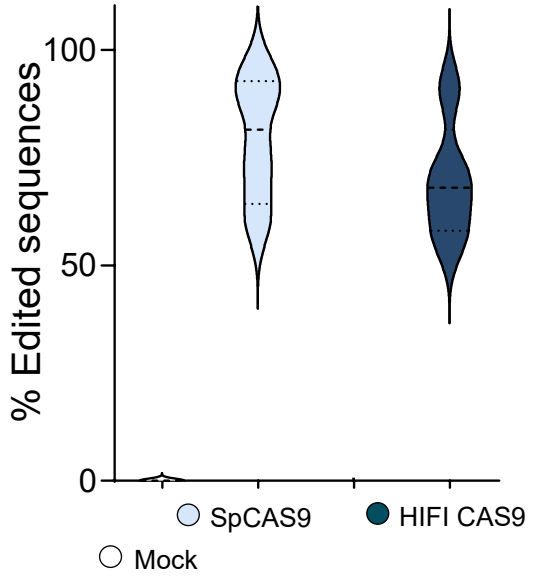


GMP compliant manufacturing of TregTacRes



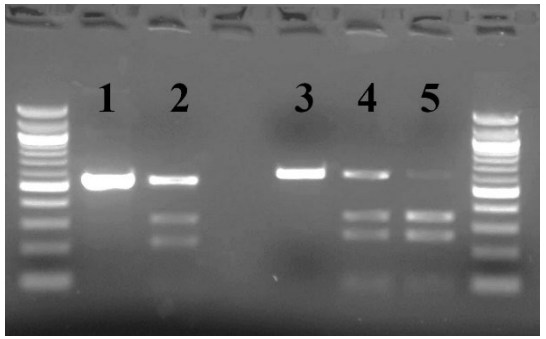
Challenge: Gene editing materials





→ Stability program for stored Cas9 and single guideRNA

Own design as no Pharm. Eu. Standard is established



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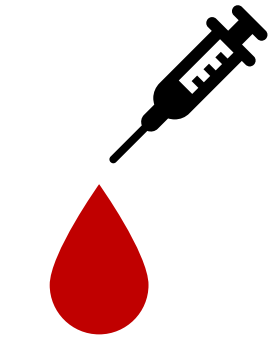
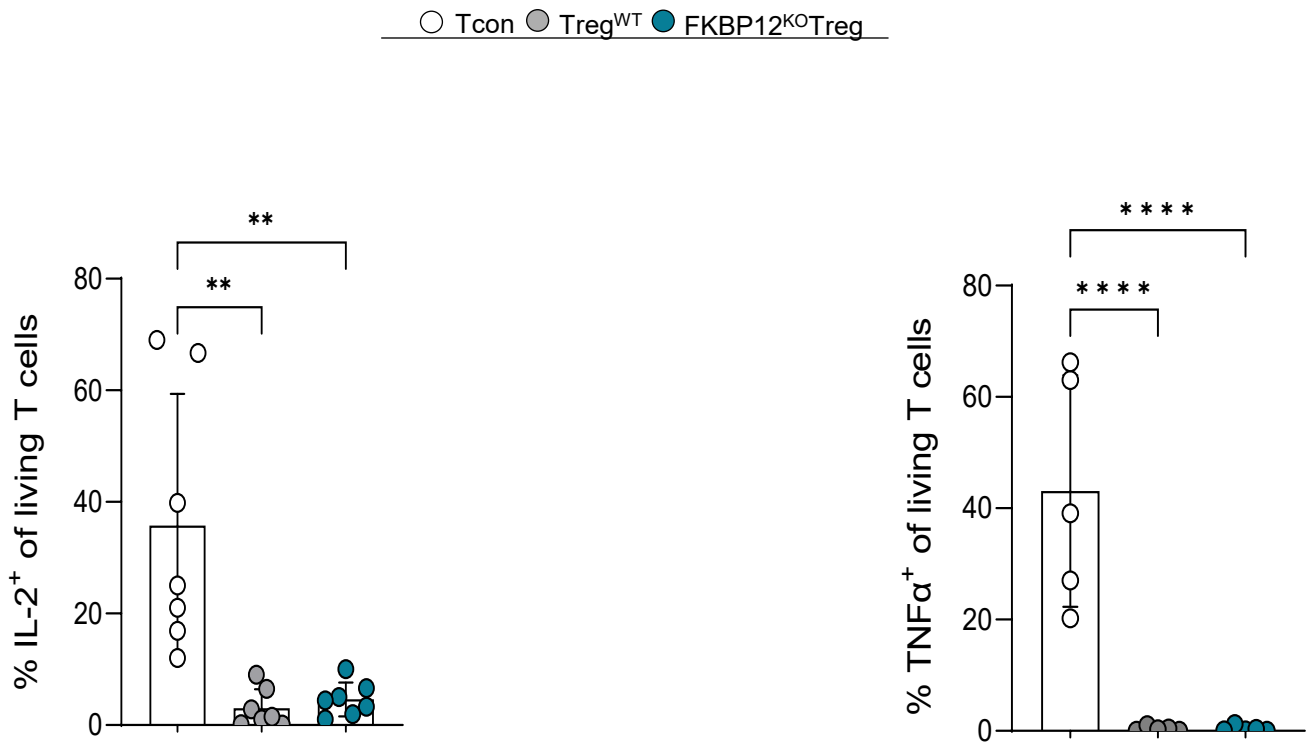
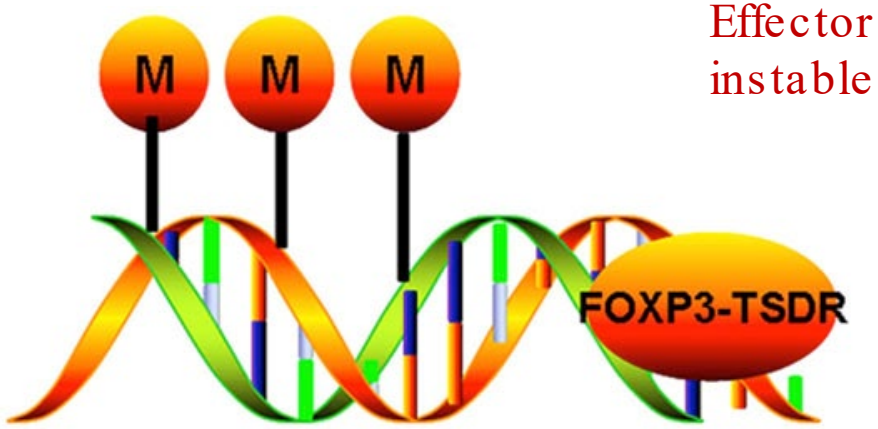


Challenge: Safety of a gene-edited Treg product



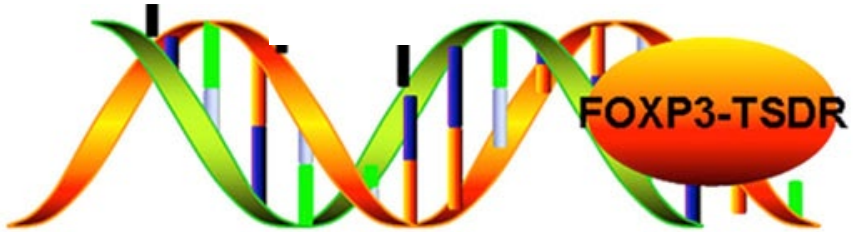
Absence of effector cytokine production

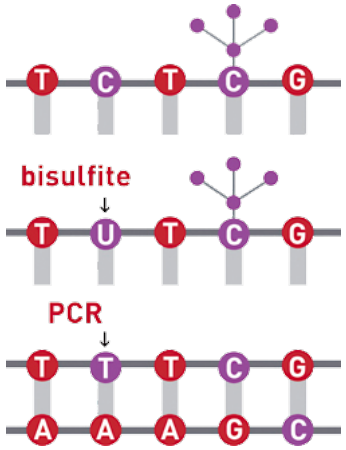
TregTacRes are stable Treg – TSDR demethylation

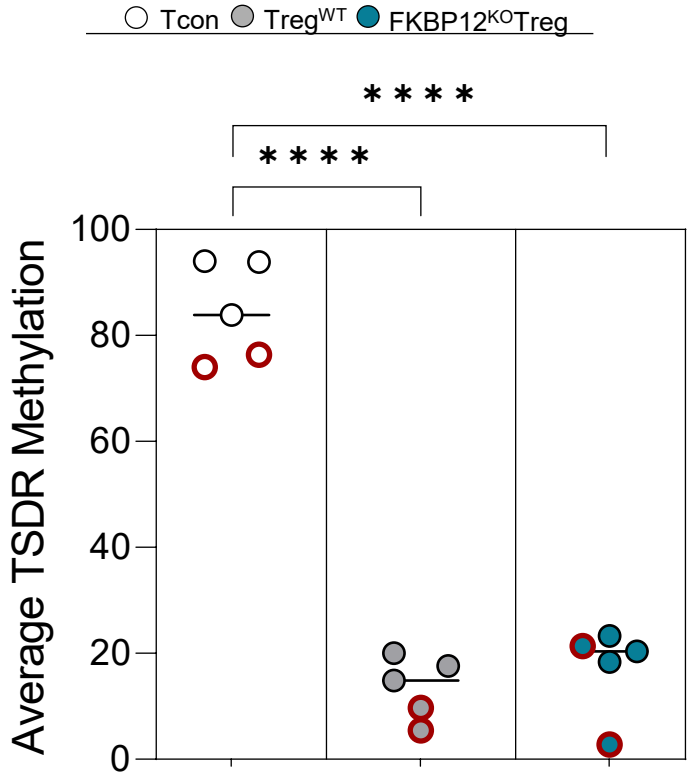


Effector Tcell/
instable Treg

Stable Treg



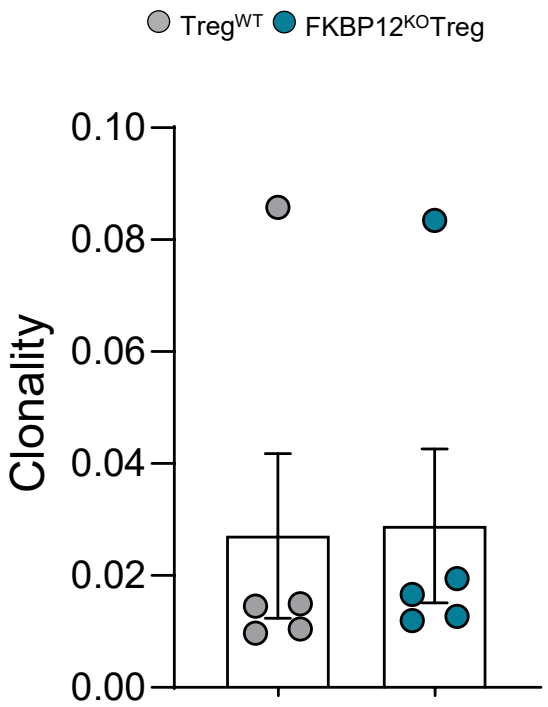




Adapted from
https://www.ahajournals.org/doi/full/10.1161/HYPERTENSIONAHA.119.12852

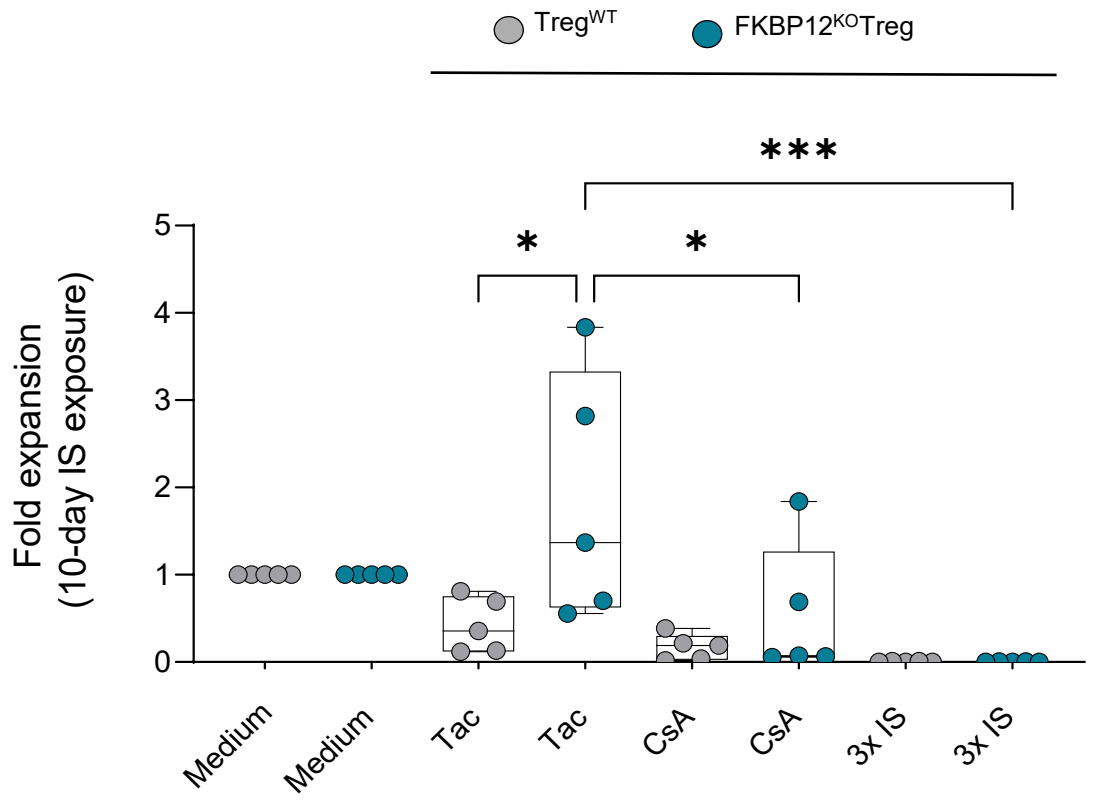
https://www.diagenode.com/en/applications/dna-bisulfite-conversion

TregTacRes do not show hyperproliferation



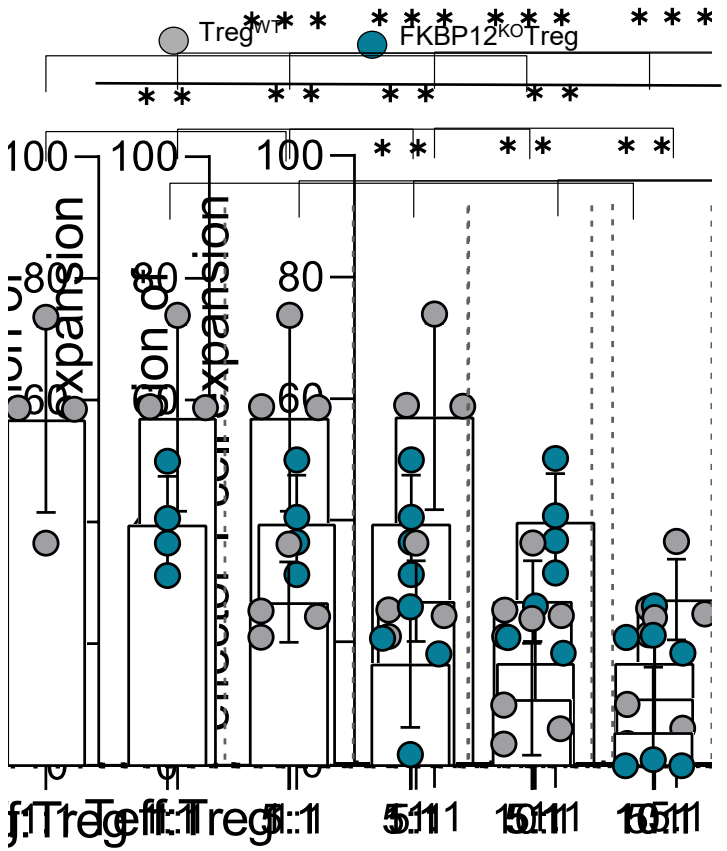
TCRseq data

Challenge: Potency assays



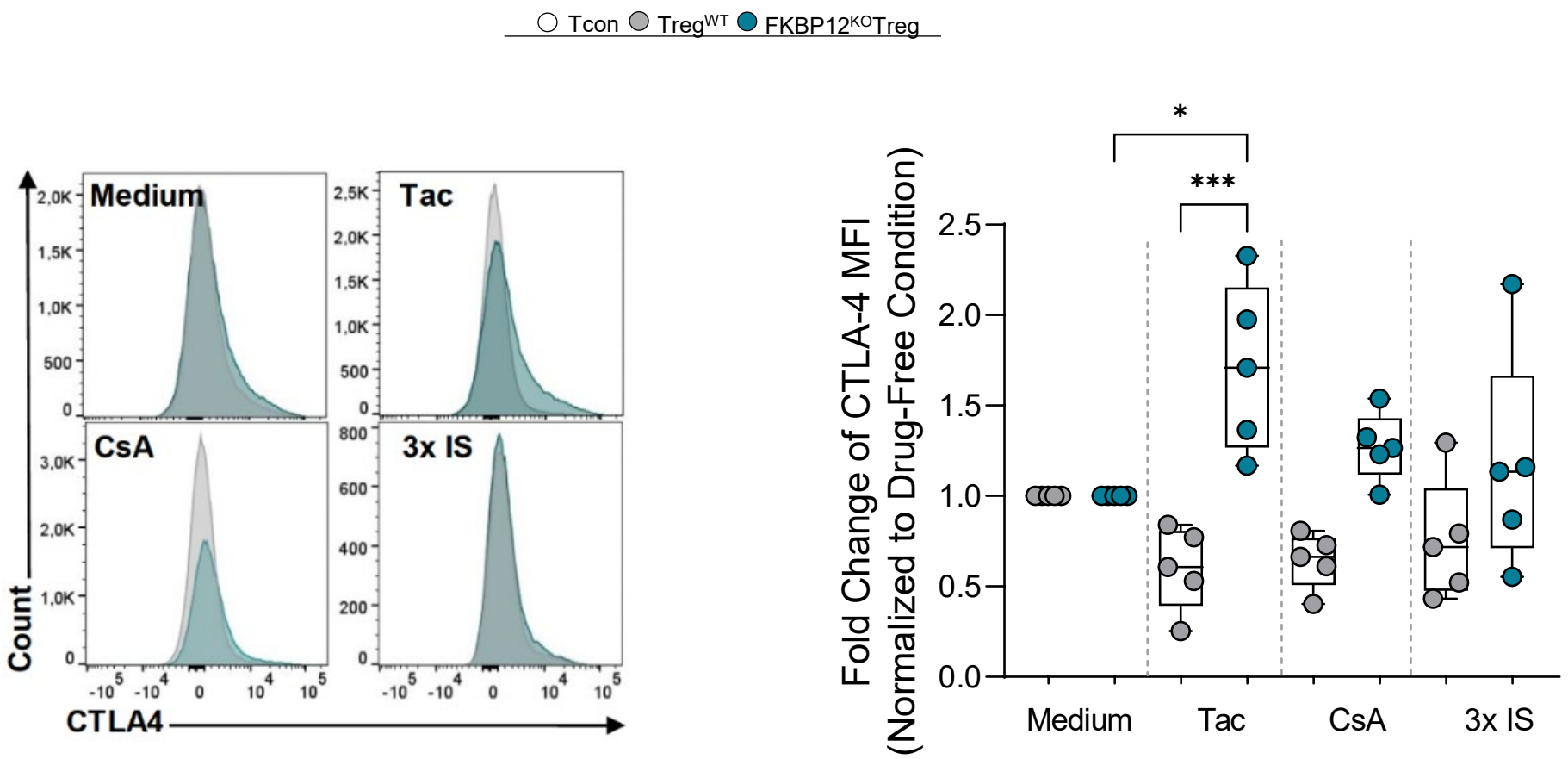
→ TregTacRes expand in presence of tacrolimus

Challenge: Potency assays

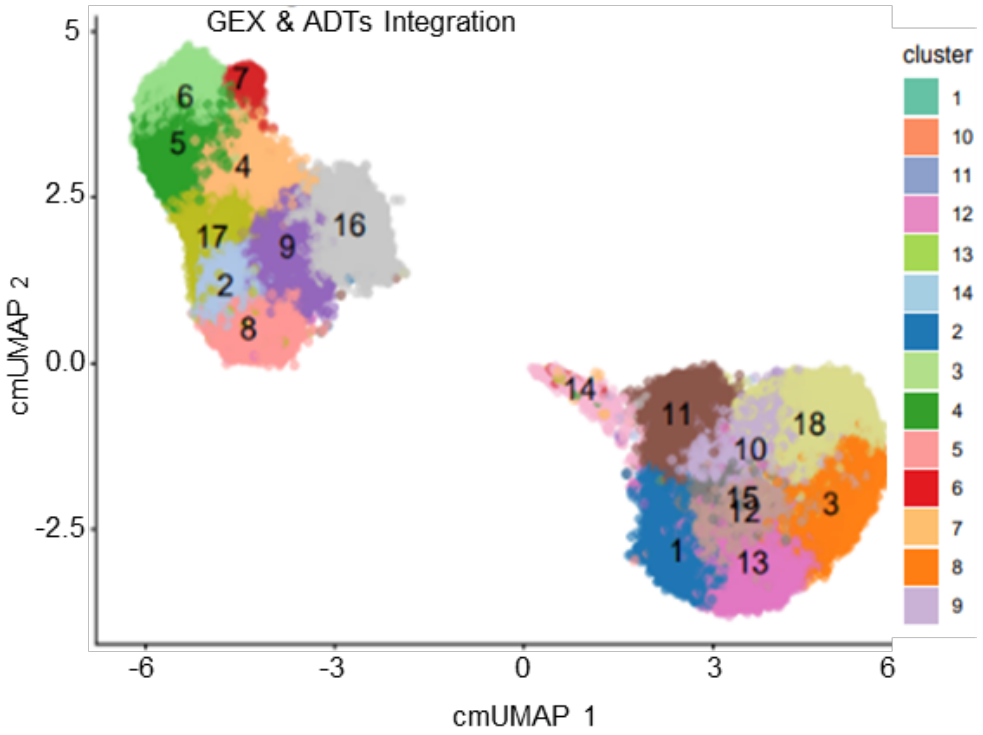


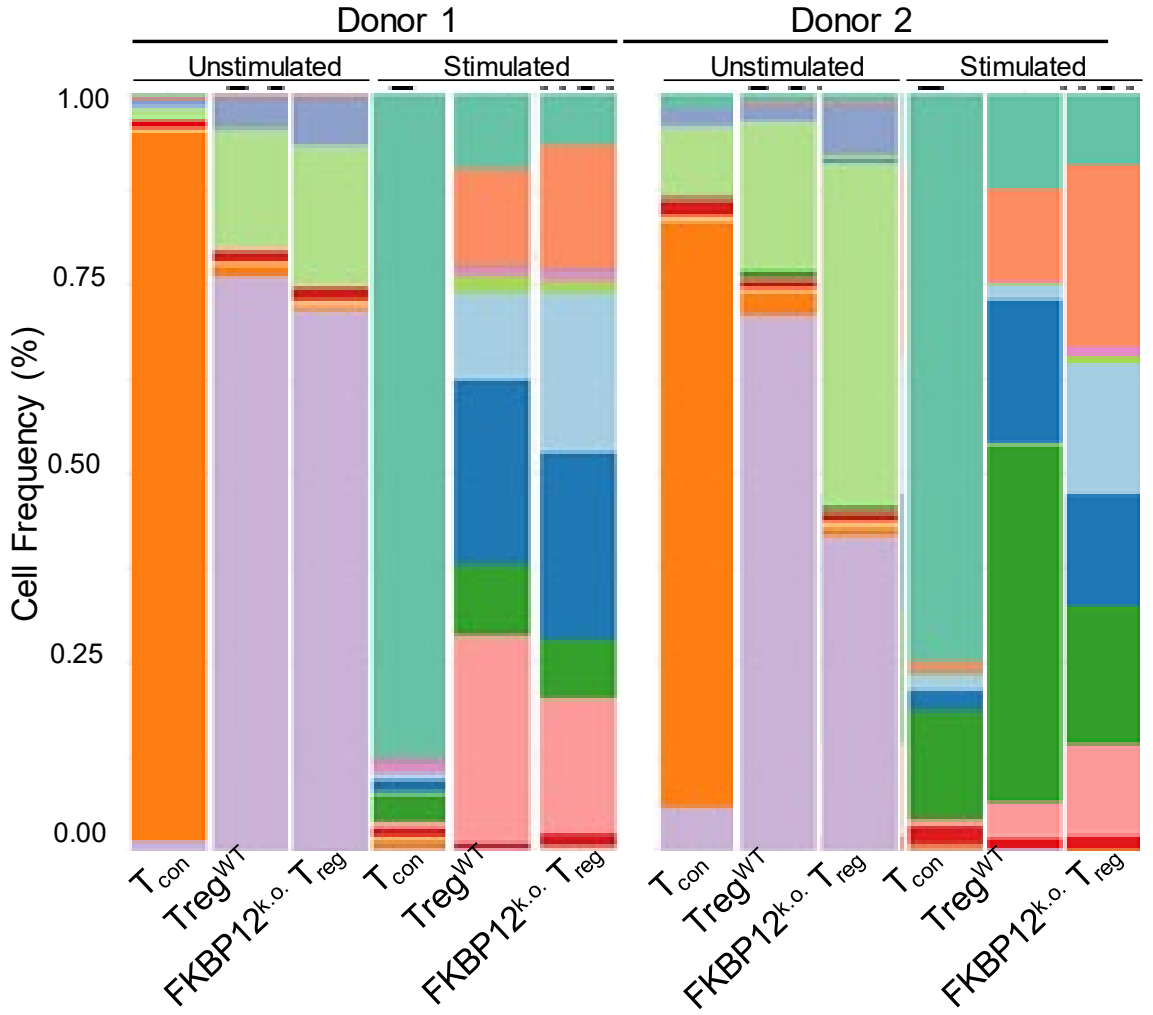
→ Adequate potency assays to test multiple modes of action are missing

Increased CTLA-4 in TregTacRes



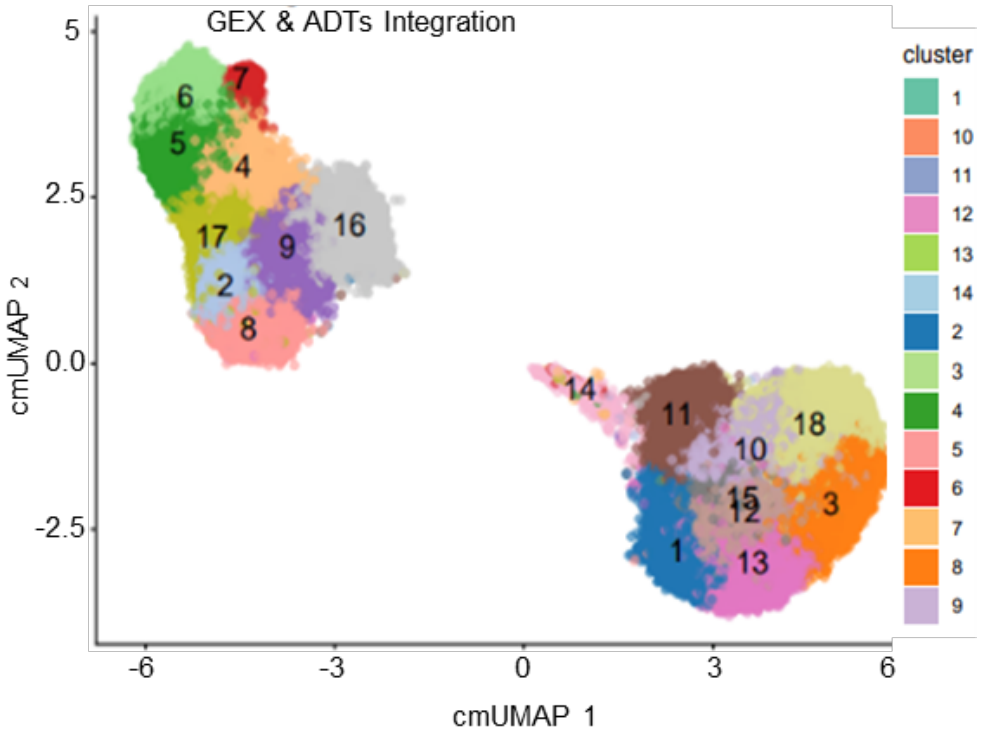
TregTacRes are Treg-like

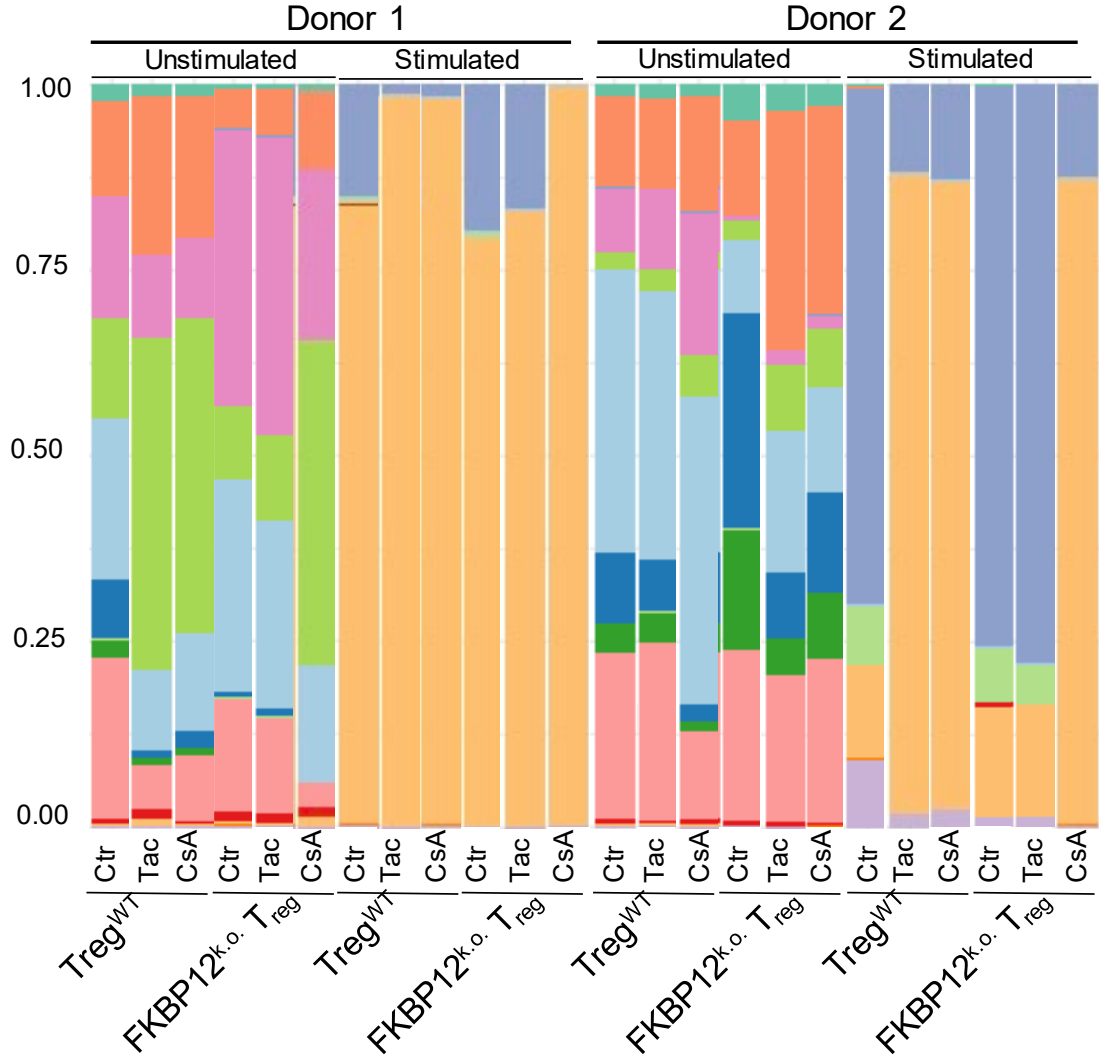




CITEseq data

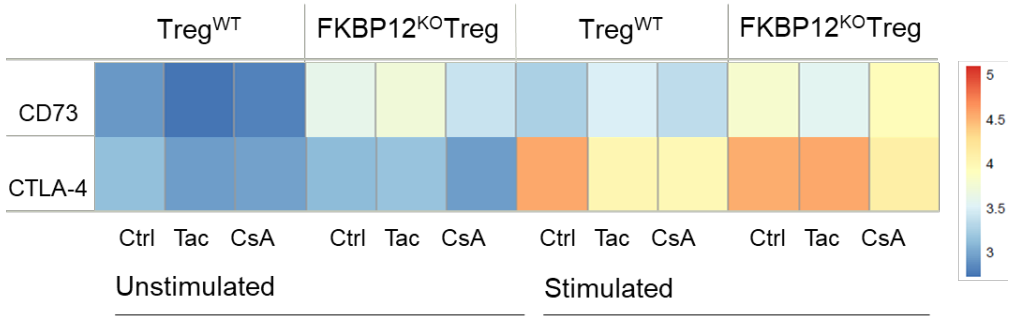
TregTacRes' tacrolimus resistance

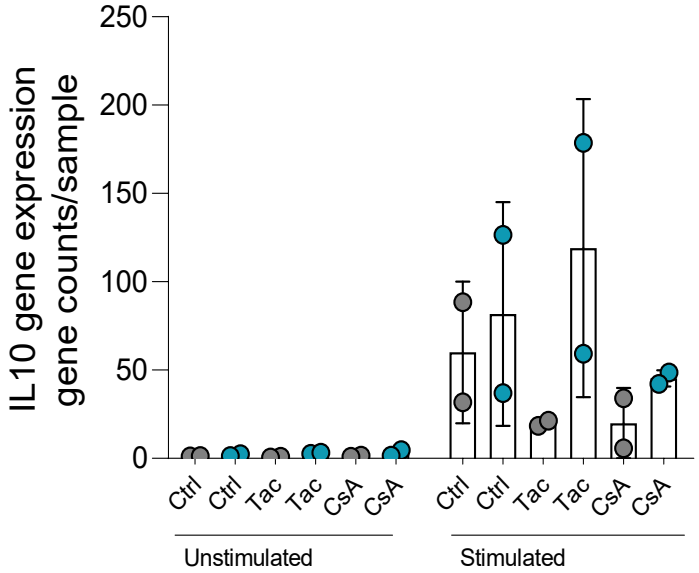




CITEseq data

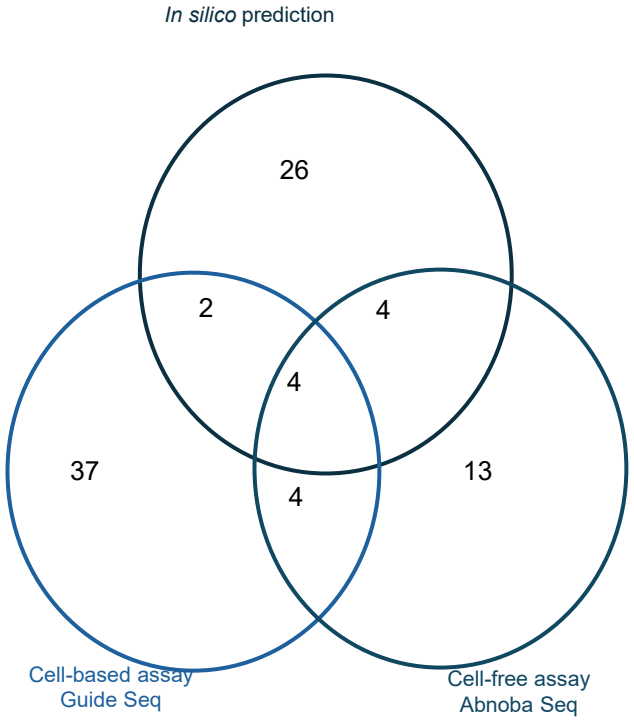
Hints for functional superiority of TregTacRes in presence of Tac



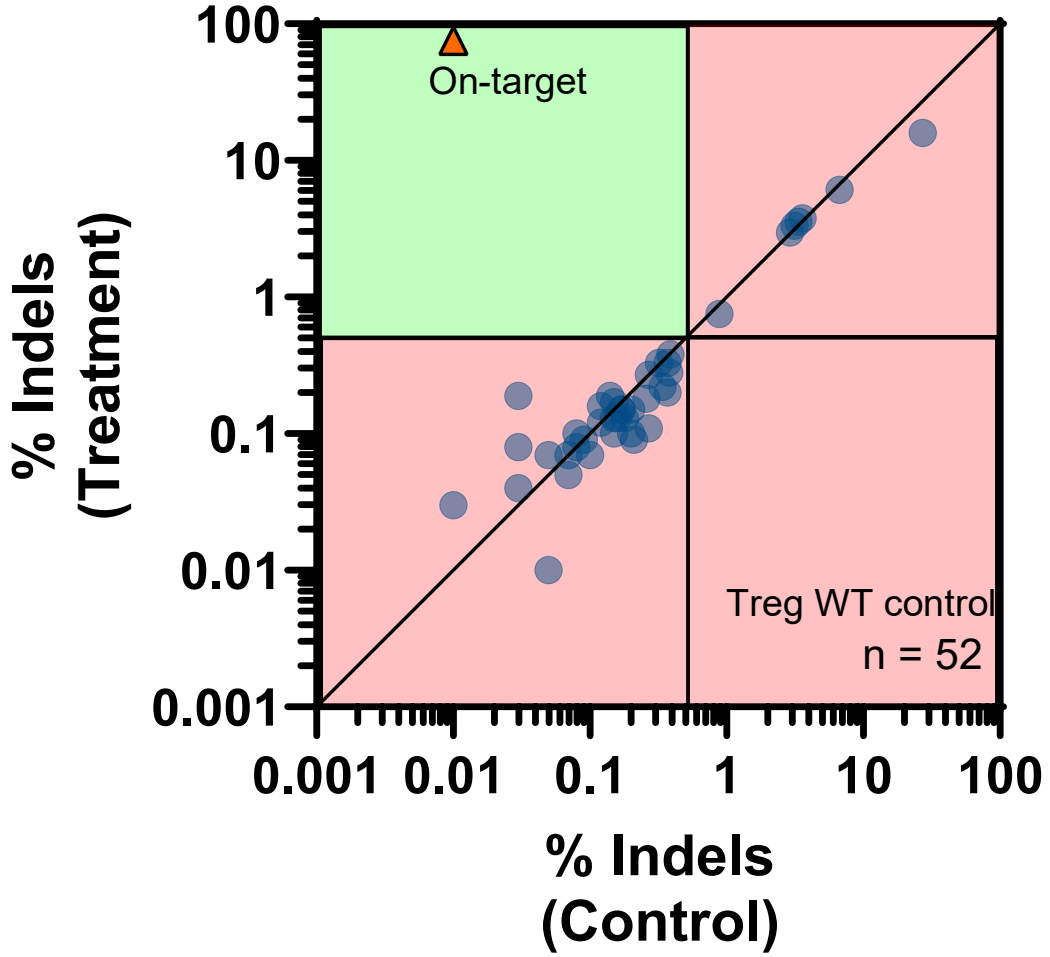


Challenge: Off-target analysis

Off-target prediction



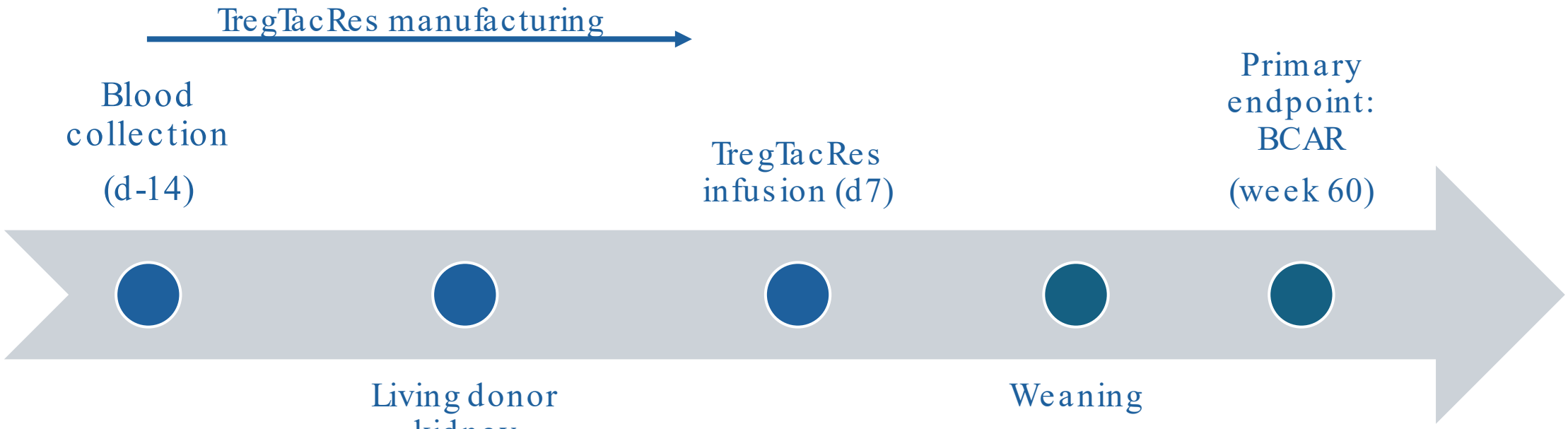
Off-target validation

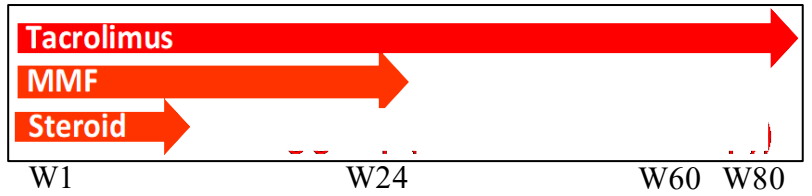


Regulatory achievements

- Authorization to “produce“ starting material (whole blood)
- Qualification of BeCAT GMP laboratories for gene technologies
- Validation runs for TregTacRes
- Application for Manufacturing authorization (12/2022)
- Inspection by the local authority (07/2023)
- Manufacturing authorization (05/2024)
- Submission of Clinical Trial Application (11/2024)

FIH Clinical trial TregTacRes





Sponsor representative
Prof. Petra Reinke

PI: Prof. Robert Öllinger, surgery

n = 12 - 24

Weaning from triple immunosuppression to tacrolimus monotherapy

TregTacRes basket trial – ATMP pilot EMA

Single investigational treatment: TregTacRes to control undesired memory/ effector (allo)T-cell responses in combination with approved Tac application

⇒ enhanced Treg survival/function

⇒ weaning standard IS to Tacrolimus monotherapy

basket trial

0.25×10^6
0.5×10^6
1×10^6
3×10^6

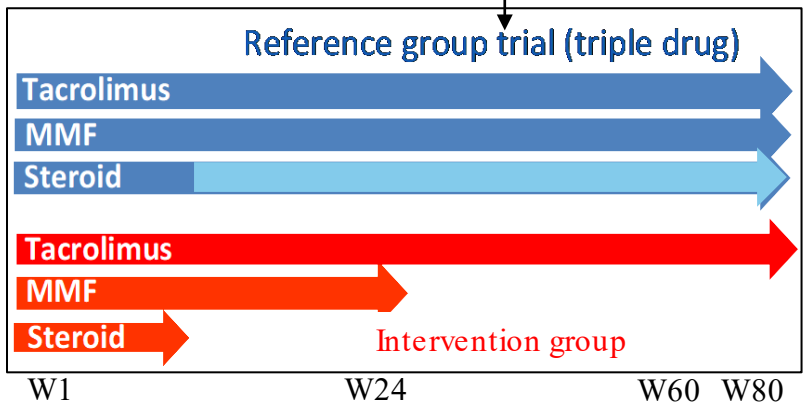
TregTacRes

2-3 weeks after SOT

+

Triple IS medication:
Tacrolimus
Mycophenolat-Mofetil (MMF)
Methylprednisolone

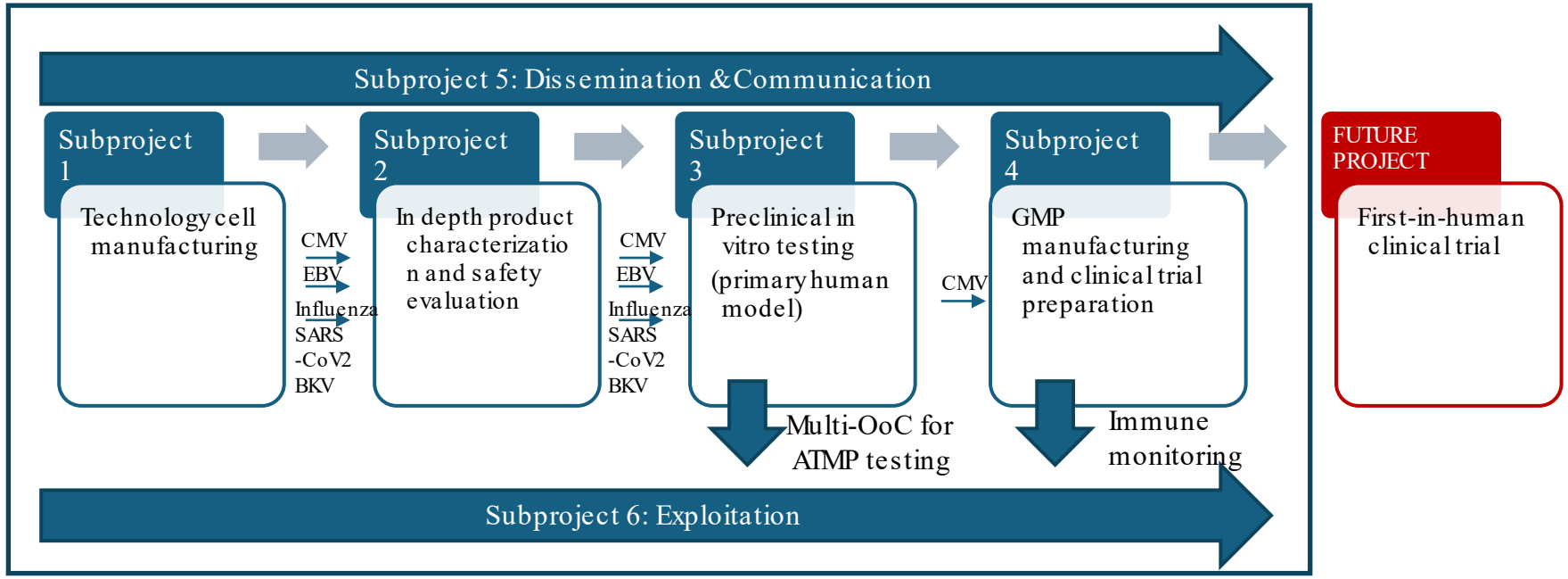
D1: kidney
D2: liver
D3: heart
D4: lung
D5: pancreas
D6: XY
D7: XY



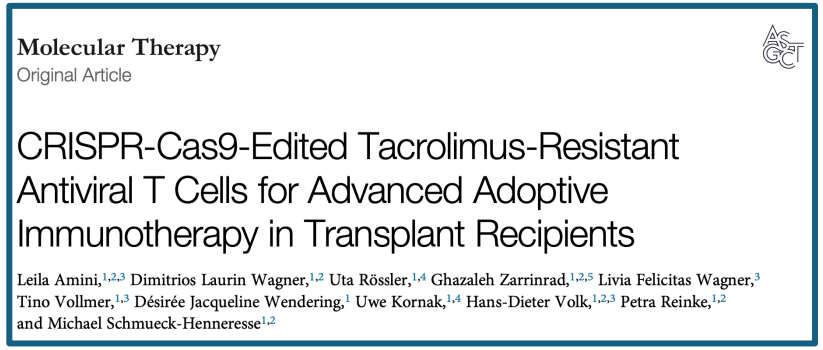
Project lead: Prof. Petra Reinke

Platform technology: Tacrolimus-resistant antiviral T cell products (TReAT)

TReAT PROJECT



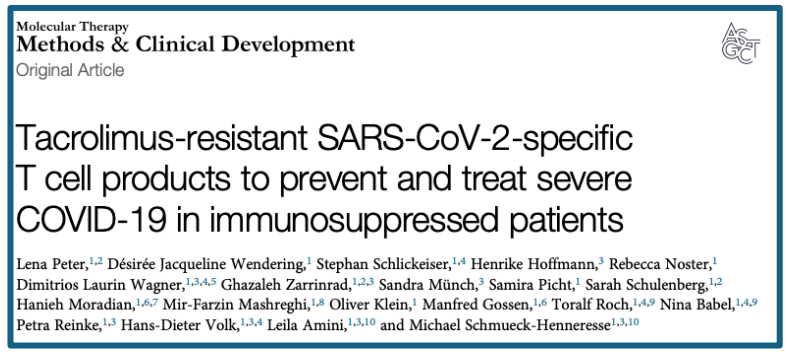
BMBF



Molecular Therapy
Original Article

CRISPR-Cas9-Edited Tacrolimus-Resistant Antiviral T Cells for Advanced Adoptive Immunotherapy in Transplant Recipients

Leila Amini,[1,2,3] Dimitrios Laurin Wagner,[1,2] Uta Rössler,[1,4] Ghazaleh Zarrinrad,[1,2,5] Livia Felicitas Wagner,[3] Tino Vollmer,[1,3] Désirée Jacqueline Wendering,[1] Uwe Kornak,[1,4] Hans-Dieter Volk,[1,2,3] Petra Reinke,[1,2] and Michael Schmueck-Henneresse[1,2]



Molecular Therapy
Methods & Clinical Development
Original Article

Tacrolimus-resistant SARS-CoV-2-specific T cell products to prevent and treat severe COVID-19 in immunosuppressed patients

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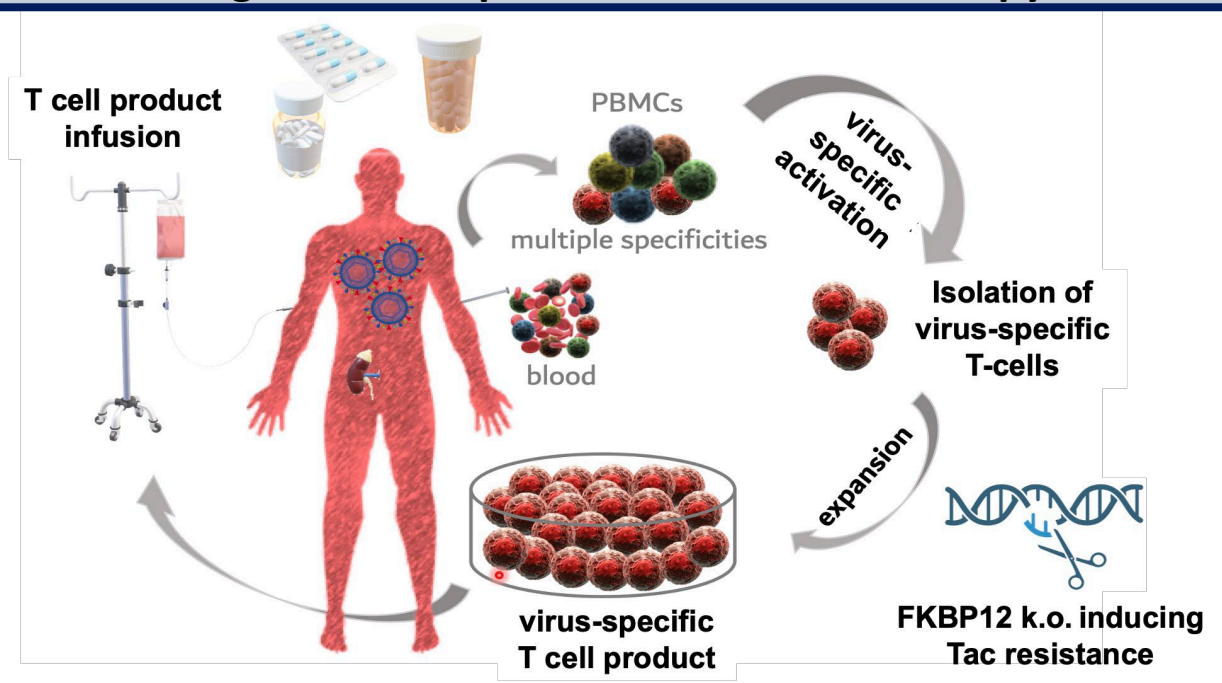
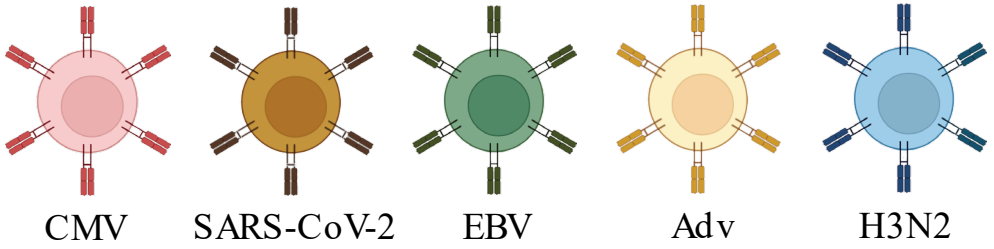
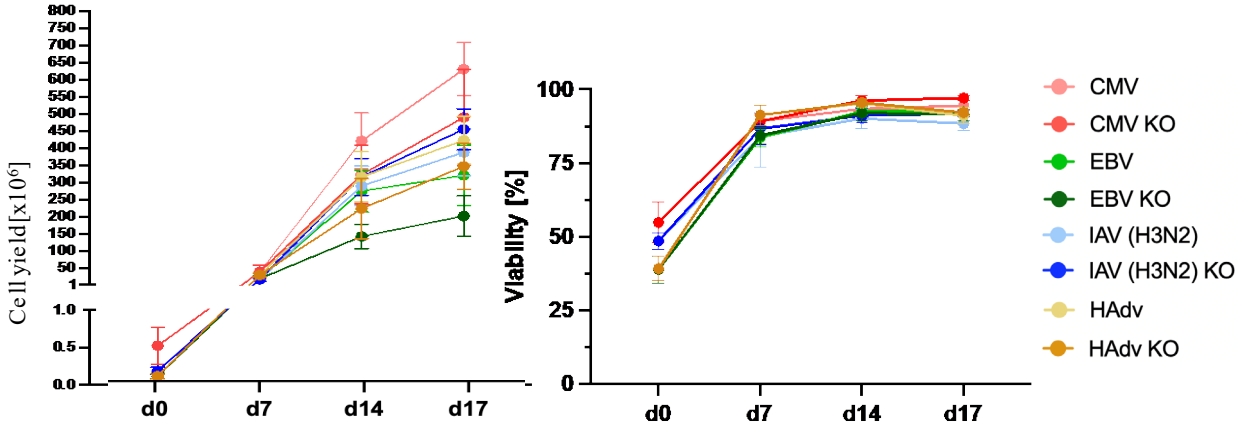
Platform technology: Tacrolimus-resistant antiviral T cell products (TReAT)

Background: Adoptive Antiviral T-cell Therapy



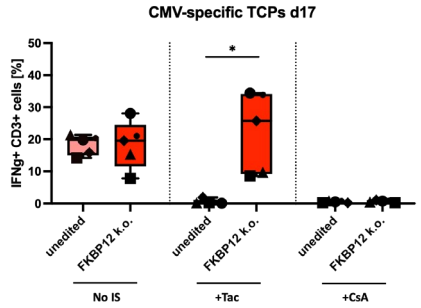


GMP-compliant Production

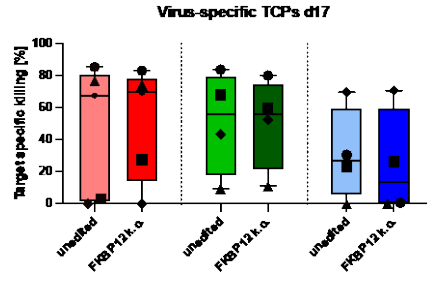


Deep Characterization

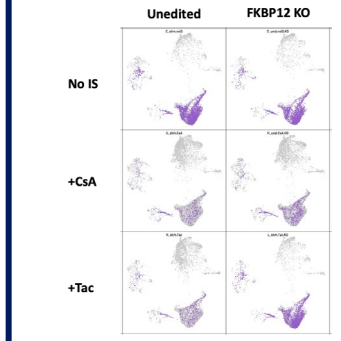
Effector cytokine production



Killing efficiency



CITE-Sequencing



Summary: challenges on the translational path

- Starting materials and certification of collection site &system
- Appropriate process design for products with advanced requirements (gene editing)
- Establishing novel standards for specific materials included in novel processes
- Find appropriate techniques to demonstrate safety and address concerns about novel tools
- Appropriate potency assays?
- Find alternative clinical trial designs appropriate for novel products
- How to handle platform technologies

Thank you!!!

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EINSTEIN CENTER Regenerative Therapies

ReSHAPE

BIH Regeneration
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TReAT PROJECT

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Deutsches Rheuma-Forschungszentrum
Ein Institut der Leibniz-Gemeinschaft

BeCAT
BERLIN CENTER FOR ADVANCED THERAPIES