IPEX Syndrome

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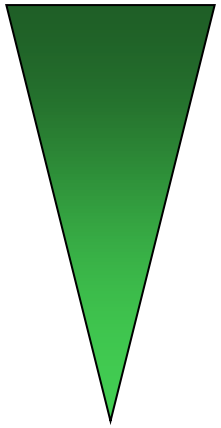
- **Nothing to disclose**

- Introduction on Primary Immune Regulatory Disorders
- IPEX syndrome natural history of the disease
- Immunological characterization > diagnostic hallmarks
- Novel mechanistic insights
- Standard treatments
- Gene therapy> present and future

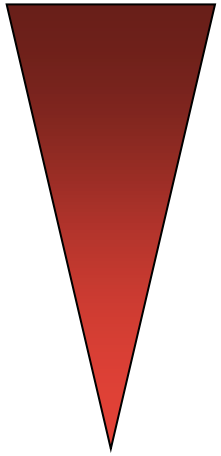
IMMUNE SYSTEM FUNCTIONS

EFFICIENTLY CLEAR NON SELF PATHOGENS



PRIMARY IMMUNODEFICIENCY

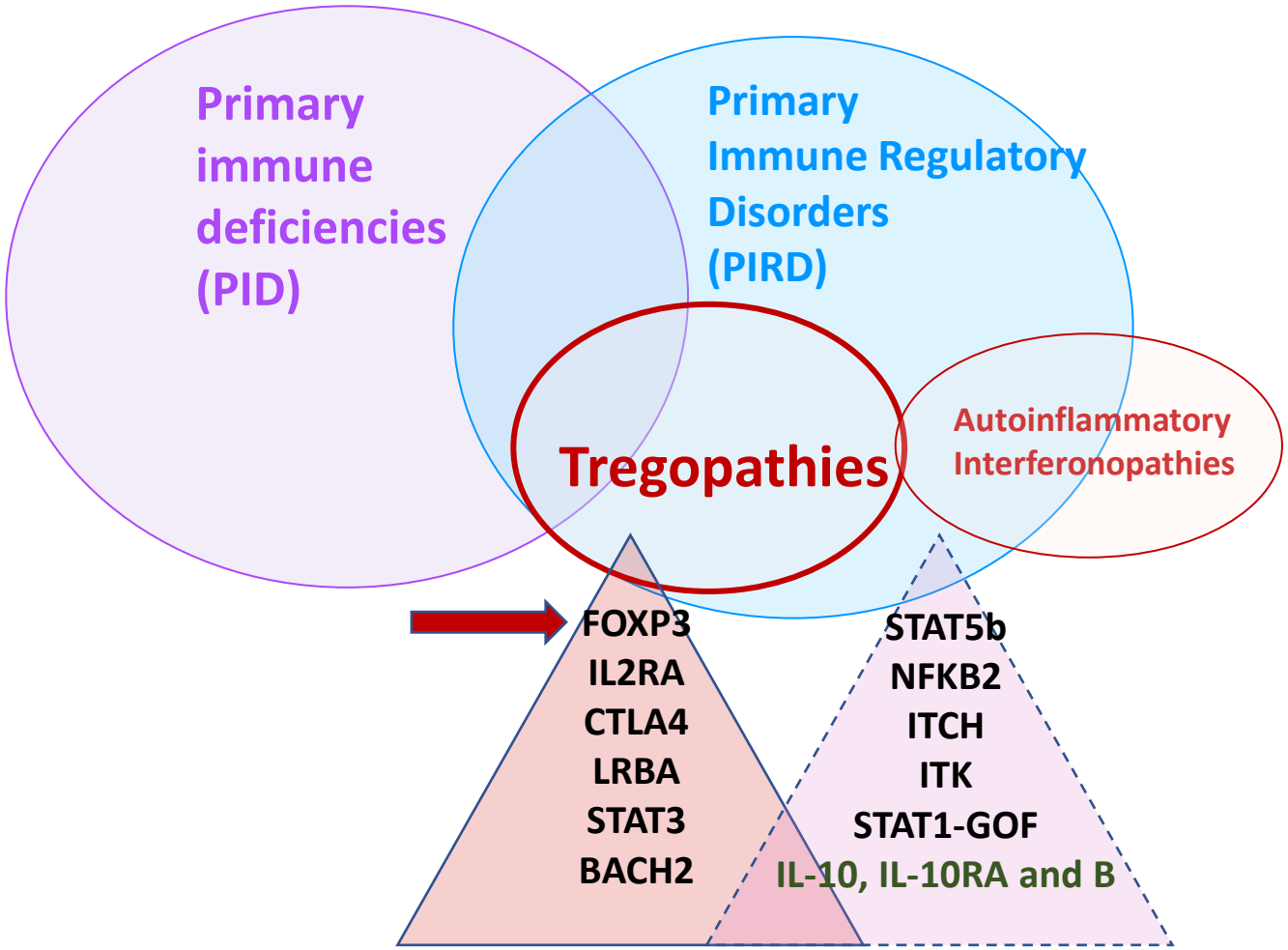
PREVENT AGGRESSION ON SELF-ANTIGENS

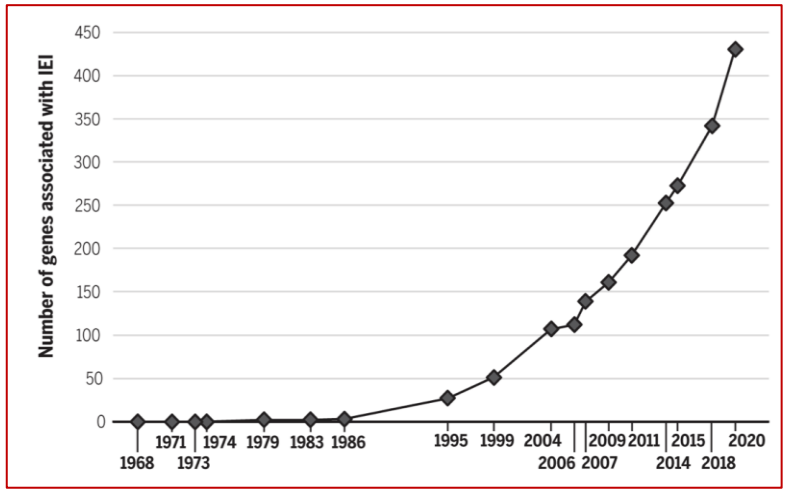


AUTOIMMUNITY

both can be caused by single gene mutations…

Inborn Error of immunity or Monogenic diseases of the immune system: an expanding medical need





Cepika AM et al Jaci 2019
Notarangelo et al. Science Immunology Rev, 2020

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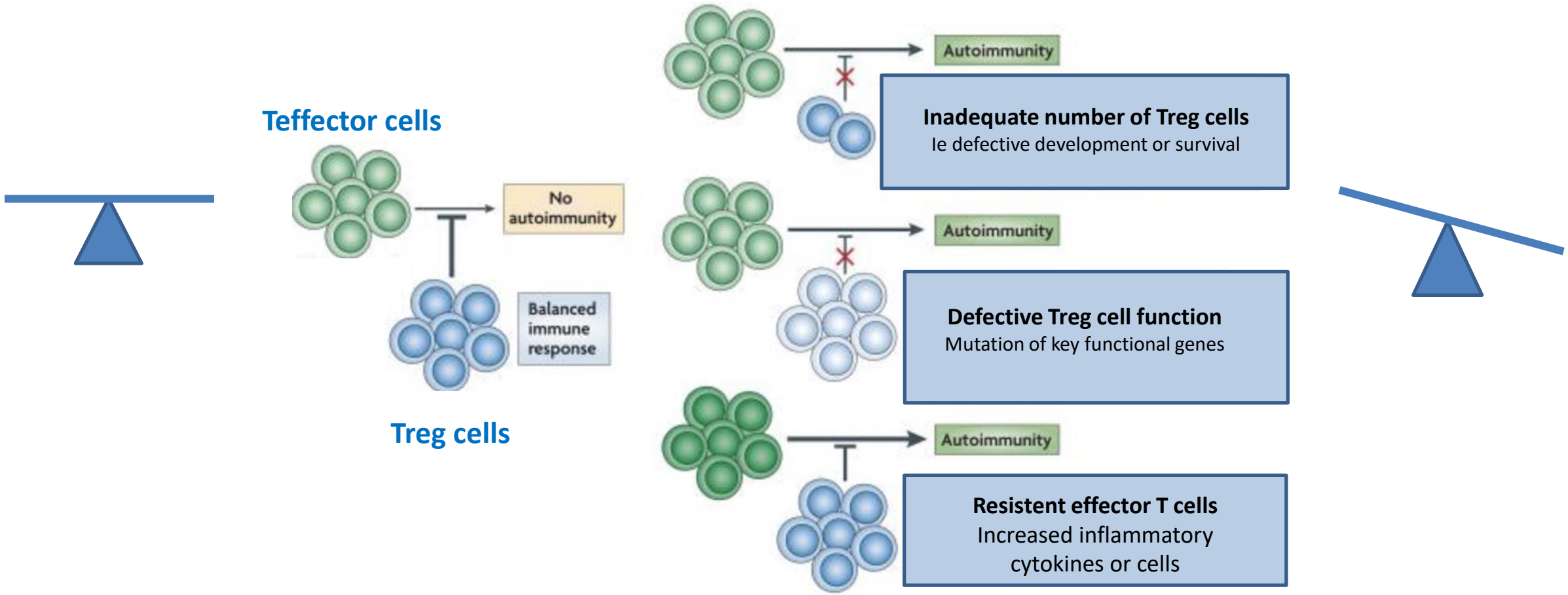
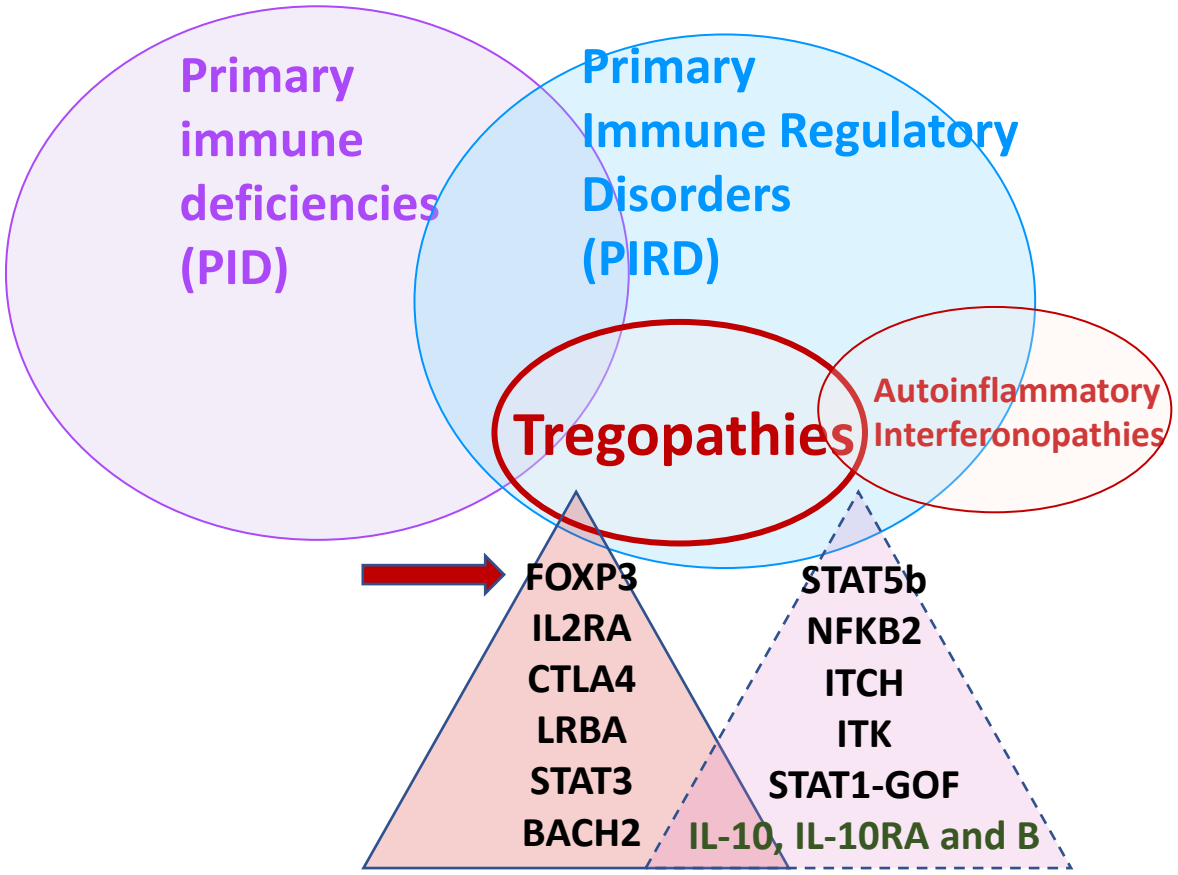
Monogenic disease with immune dysregulation: PIRD



Altered Immune Regulation: the Treg/Teff unbalance

Inborn Error of immunity:



Immune Dysregulation Polyendocrinopathy Enteropathy X-linked (IPEX) Syndrome

due to LOF FOXP3 gene mutation

FOXP3 is the essential gene for functional regulatory T cells

Prototype of Tregopathies

Prototype of genetic autoimmune disease

Cepika AM et al Jaci 2019
Notarangelo et al. Science Immunology Rev, 2020

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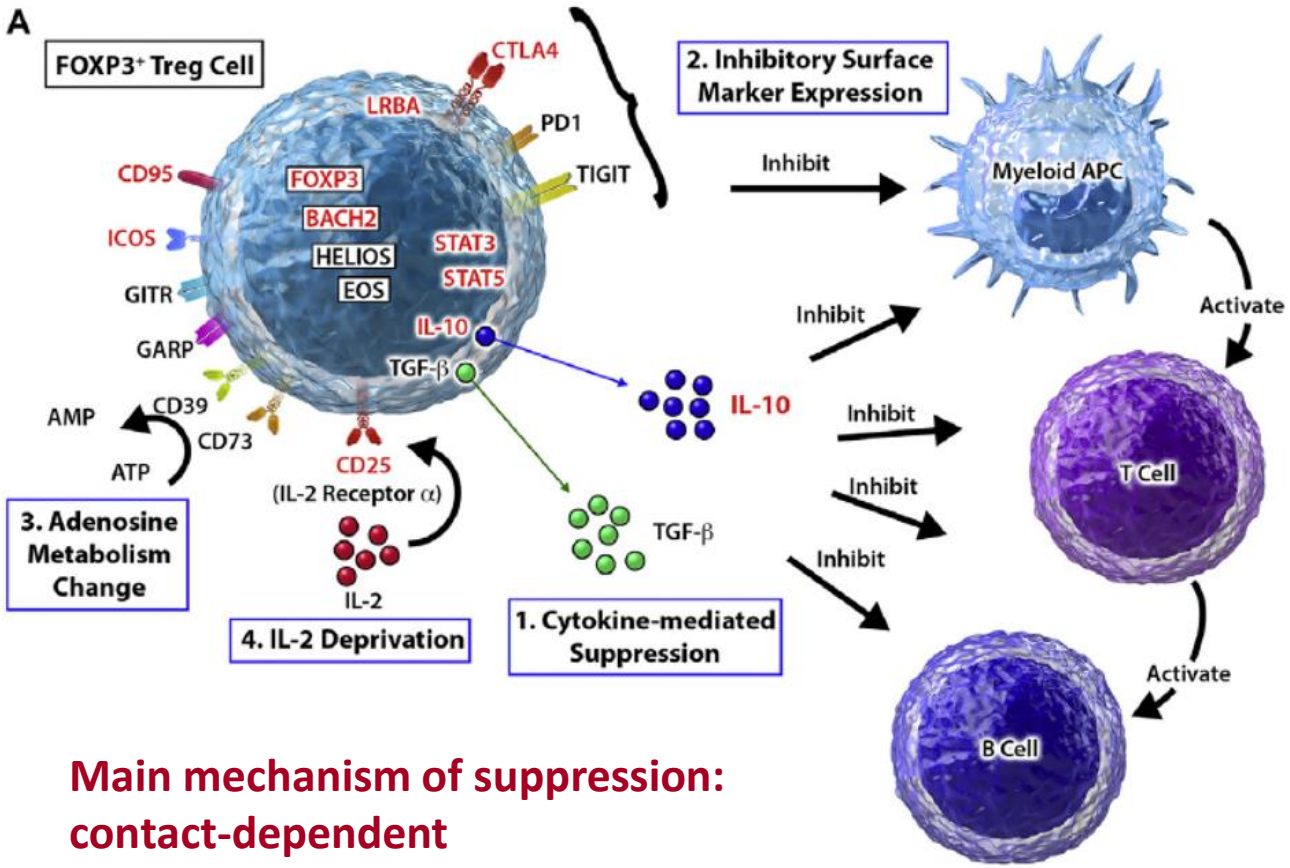
FOXP3+ CD4+ Regulatory T cells : key to immune homeostasis

FOXP3+ Treg cells
4-7% of CD4+ T cells

CD4+ FOXP3⁺ Tregs (thymic-derived)

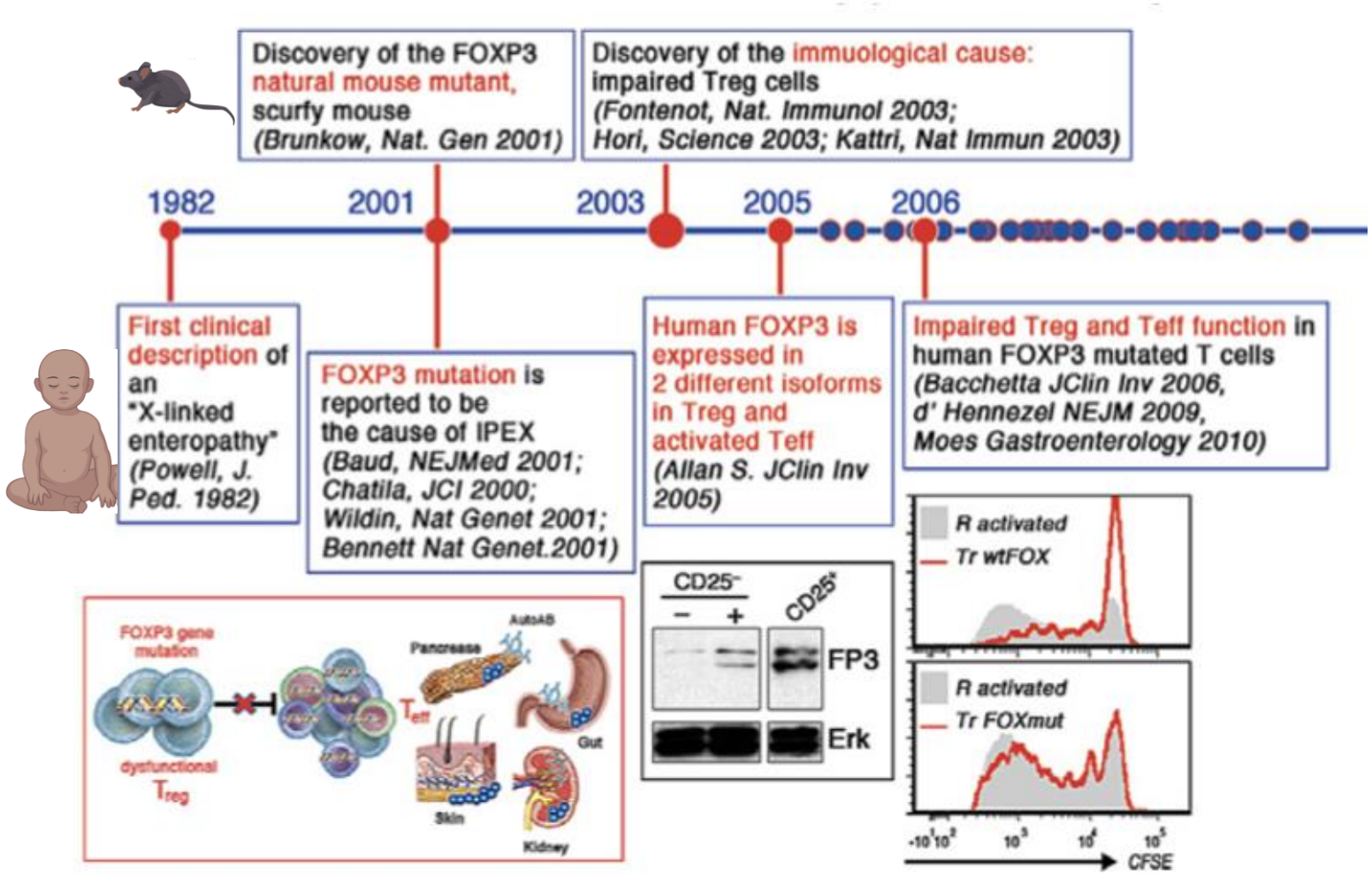


Main mechanism of suppression: contact-dependent

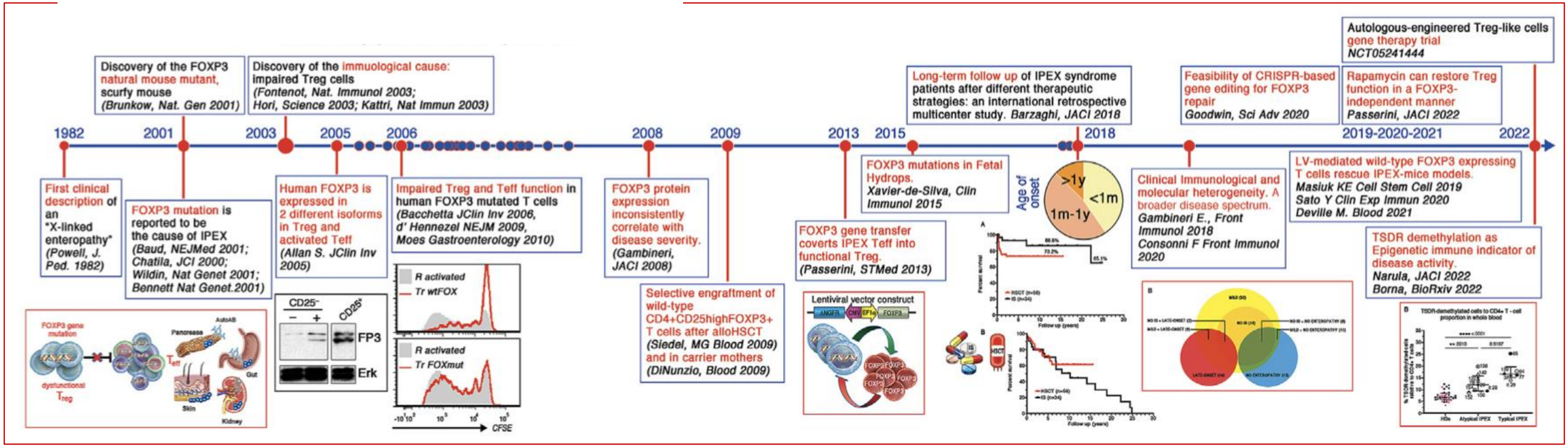
Altered Treg number or function result in autoimmunity and autoinflammation

Cepika AM et al, Journal of Allergy and Clinical Immunology 2018

DISCOVERY PATH OF IPEX Syndrome



DISCOVERY PATH OF IPEX Syndrome



Bacchetta&Roncarolo JACI Dec 2023

Number of patients described: about 600
Number of different mutations: 200
Overall mortality 40%

Real disease prevalence ?

Barzaghi F. JACI 2018; Gambineri E. Frontiers 2018; Duclaux Loras Gastroenterology, 2018; Park JH 2020; Jamee M. 2020

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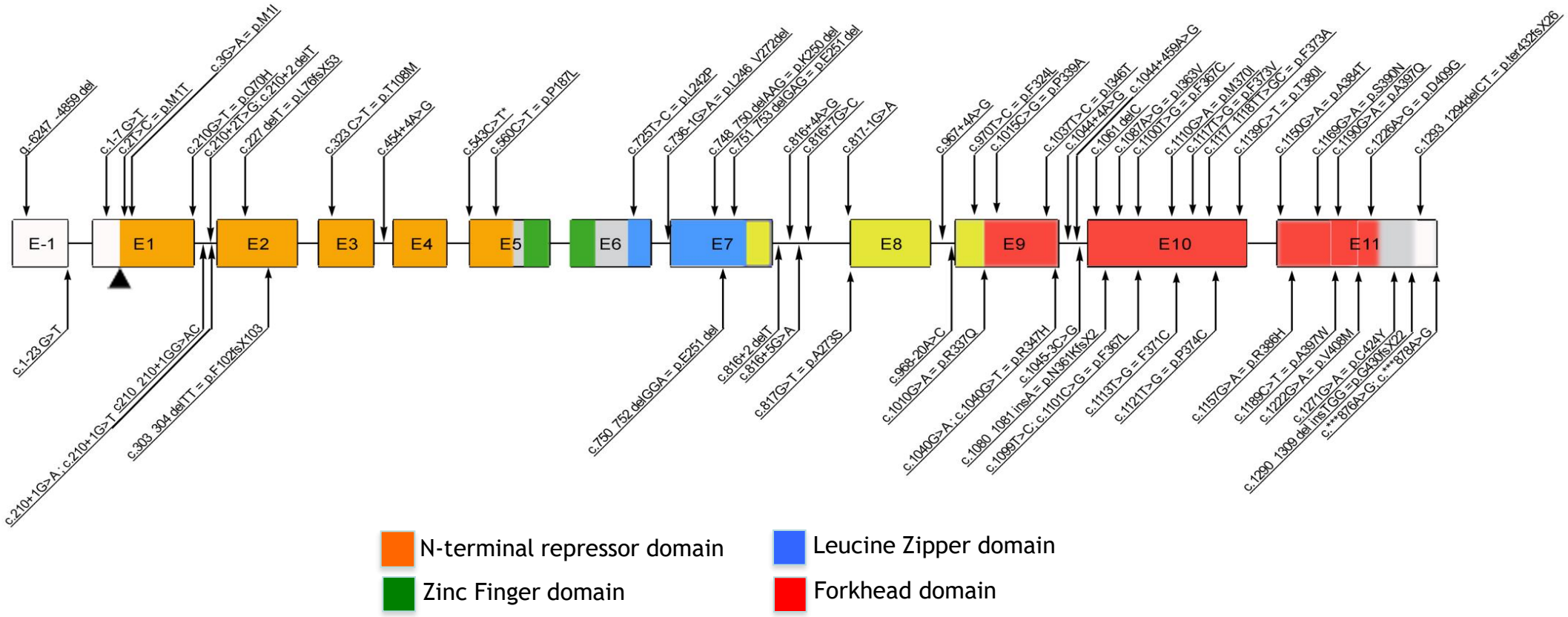
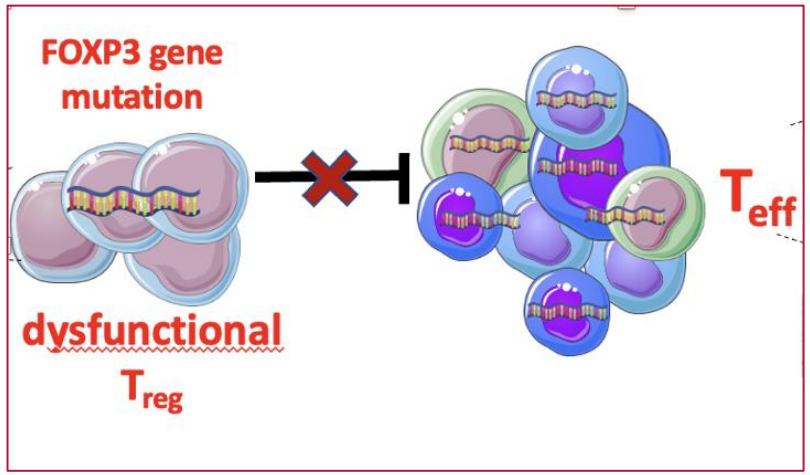


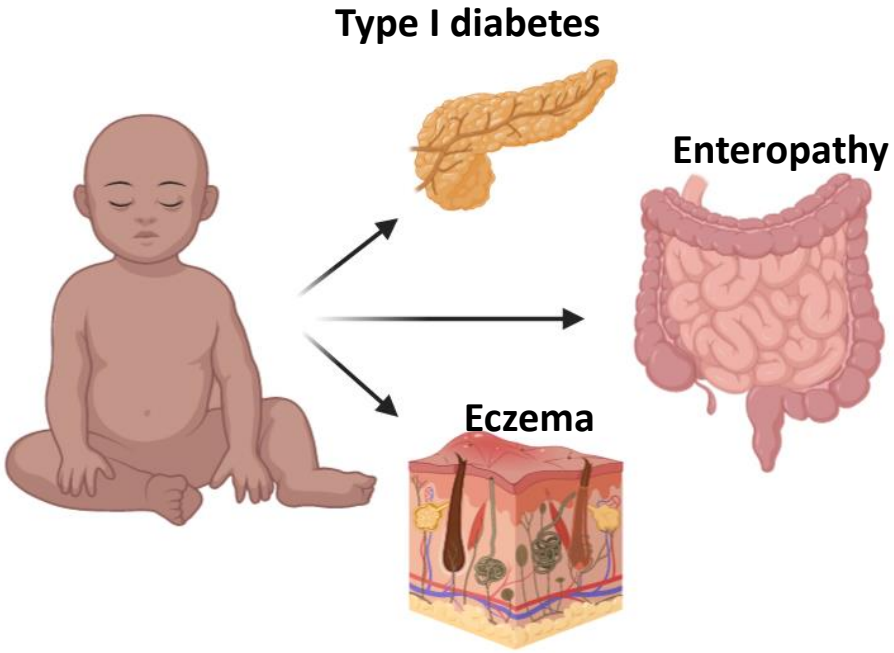
Mutations can be located at any site of the gene

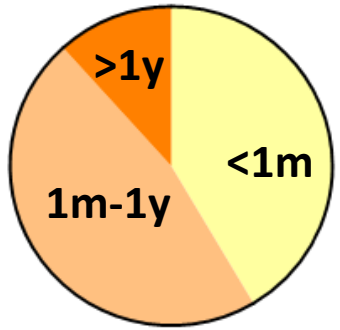
Barzaghi F., Passerini L., Bacchetta R., Frontiers in primary immunodeficiencies, 2012

IPEX Syndrome, “Typical” clinical presentation



Typical IPEX: early onset





Age of onset
N=96

Enteropathy
Eczema
Type1 Diabetes

Retrospective study on 96 IPEX patients from 38 clinical centers

Barzaghi F. JACI 2018

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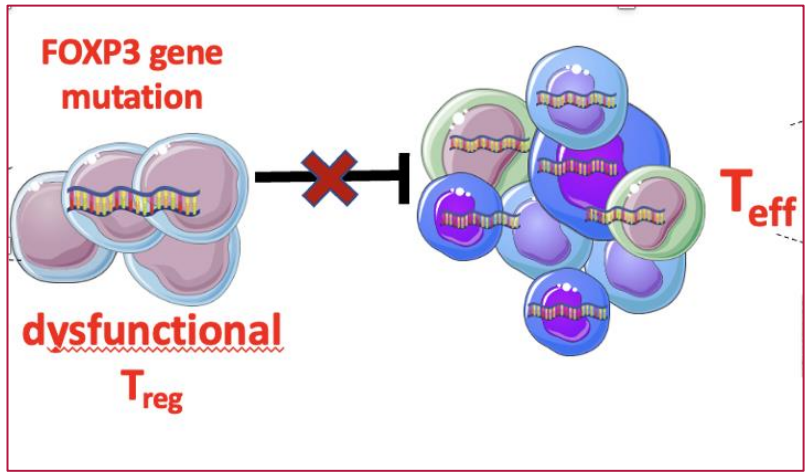
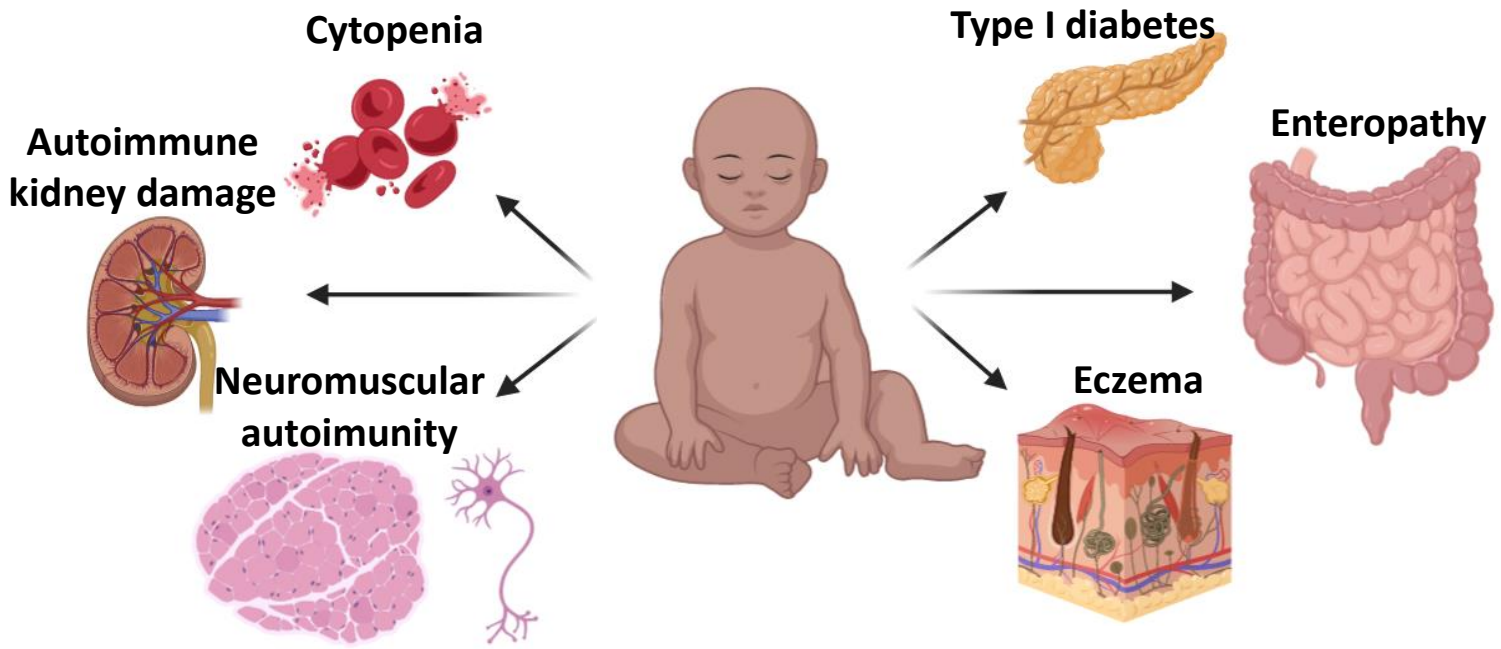
IPEX Syndrome, and “atypical” clinical presentation



Typical IPEX: early severe onset

Atypical IPEX: later onset, less severe but chronic



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Barzaghi F. JACI 2018;
Gambineri E. Frontiers 2018;
Duclaux Loras Gastroenterology, 2018;
Park JH 2020; Jamee M. 2020

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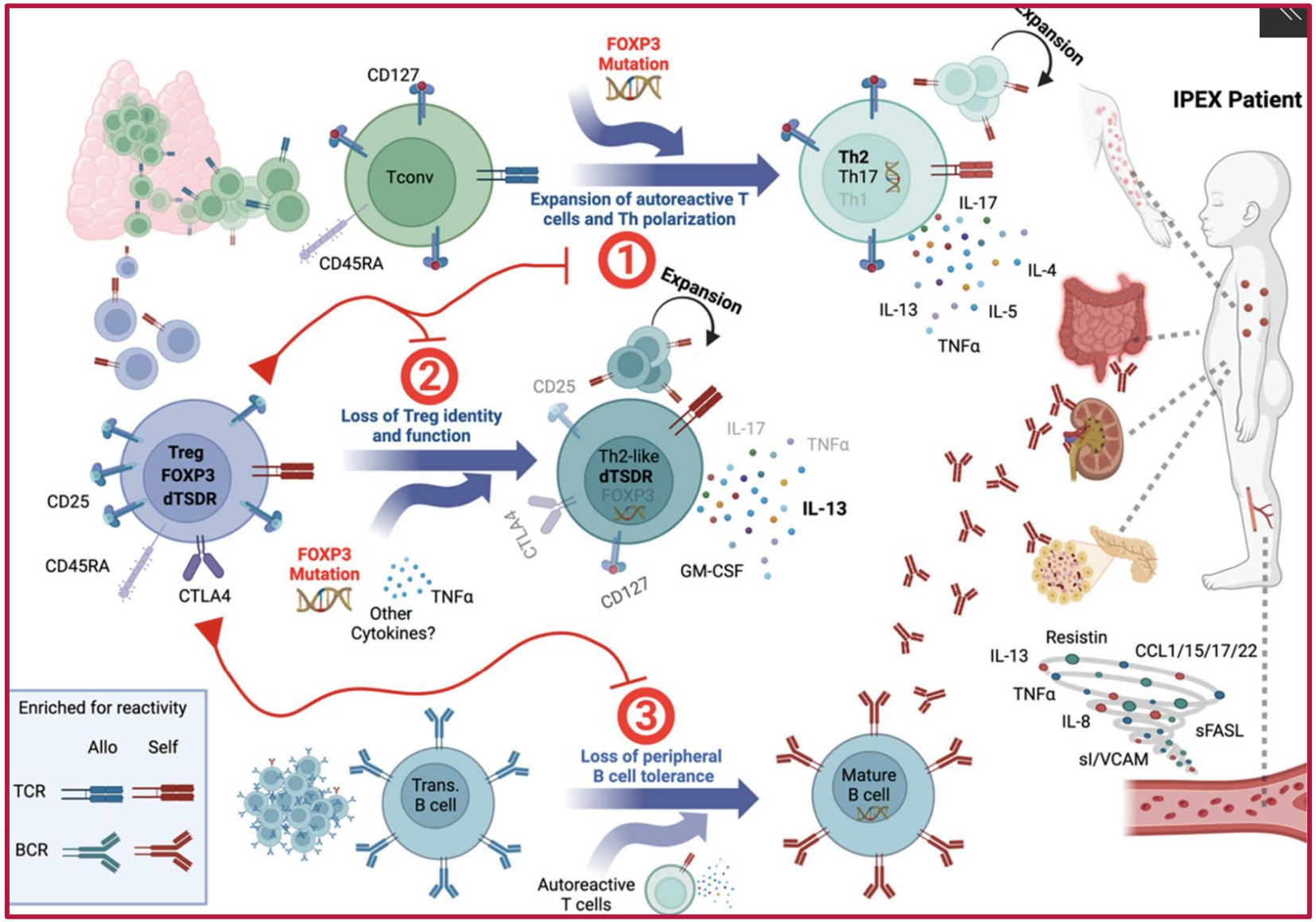
Pathogenesis of IPEX syndrome: multiple defects



1. Type 2 responses involved T cells and monocytes revealed by inflammatory markers that precede the clinical manifestations

2. Autoreactive T cells are detectable and expanded.
They comprise Teff cells and dysfunctional ”loss-of-identity Treg cells”

3. Loss of peripheral B cell tolerance, AutoAb production

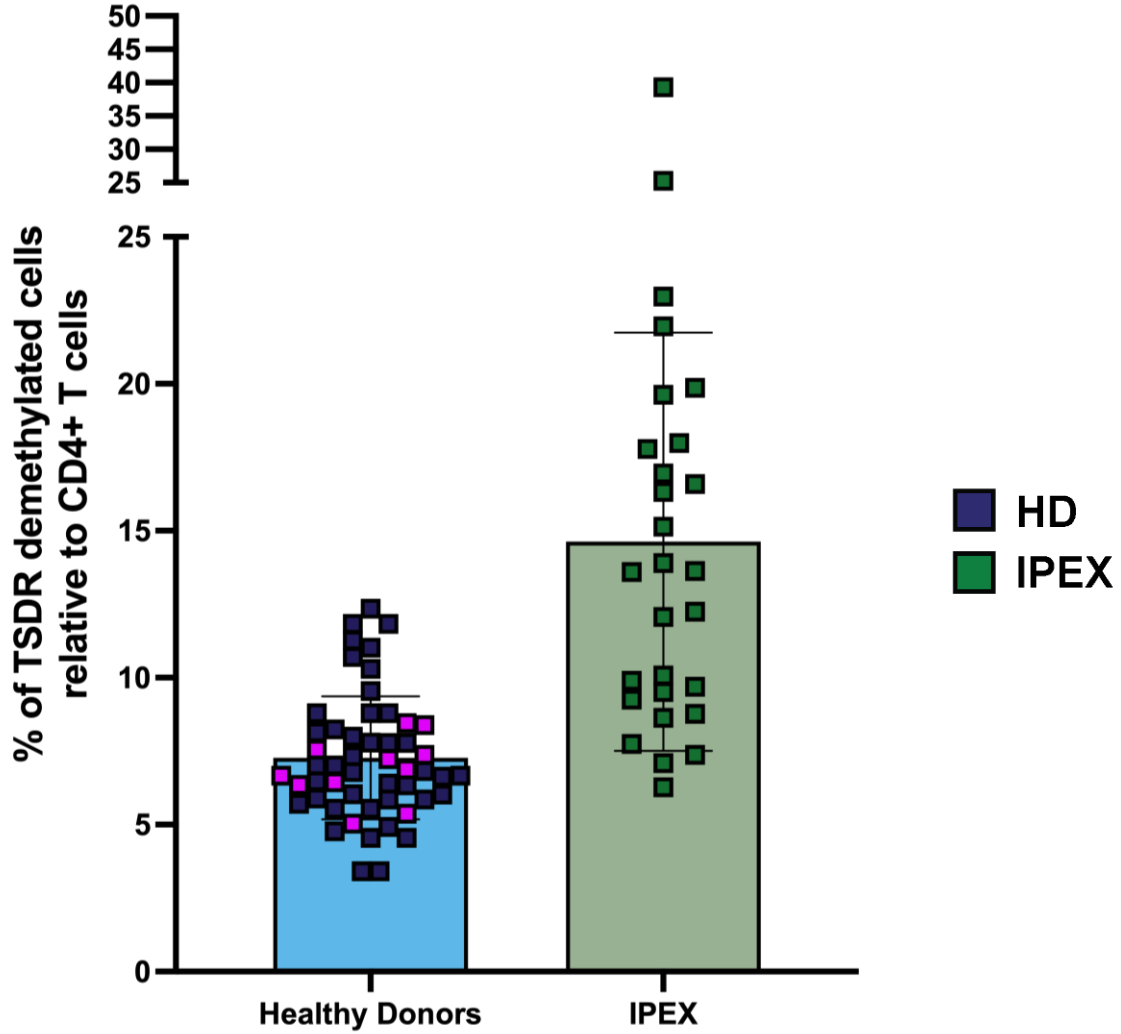
Clinical diagnosis> genetic diagnosis> immunological evaluation

- CD4 > CD8, normal B and NK cell number
- Eosinophils counts are increased
- IgG are normal, IgA are normal or increased
- IgE are elevated
- presence of specific autoAb
- Elevated proinflammatory, Th2 cytokines and macrophage derived chemokine

FOXP3 protein expression is variable
Treg cells, and peripheral CD4
CD25hiCD127lo cells, are detectable
(Gambineri JACI 2008, Bacchetta NYAS 2016
Narula M JACI 2022)

Barzaghi F, Frontiers Immun, 2012
Bacchetta R, NYAS 2016

Demethylation of Treg specific epigenetic marker is consistently elevated in IPEX



Borna S. et al, Sci Transl Med 2023

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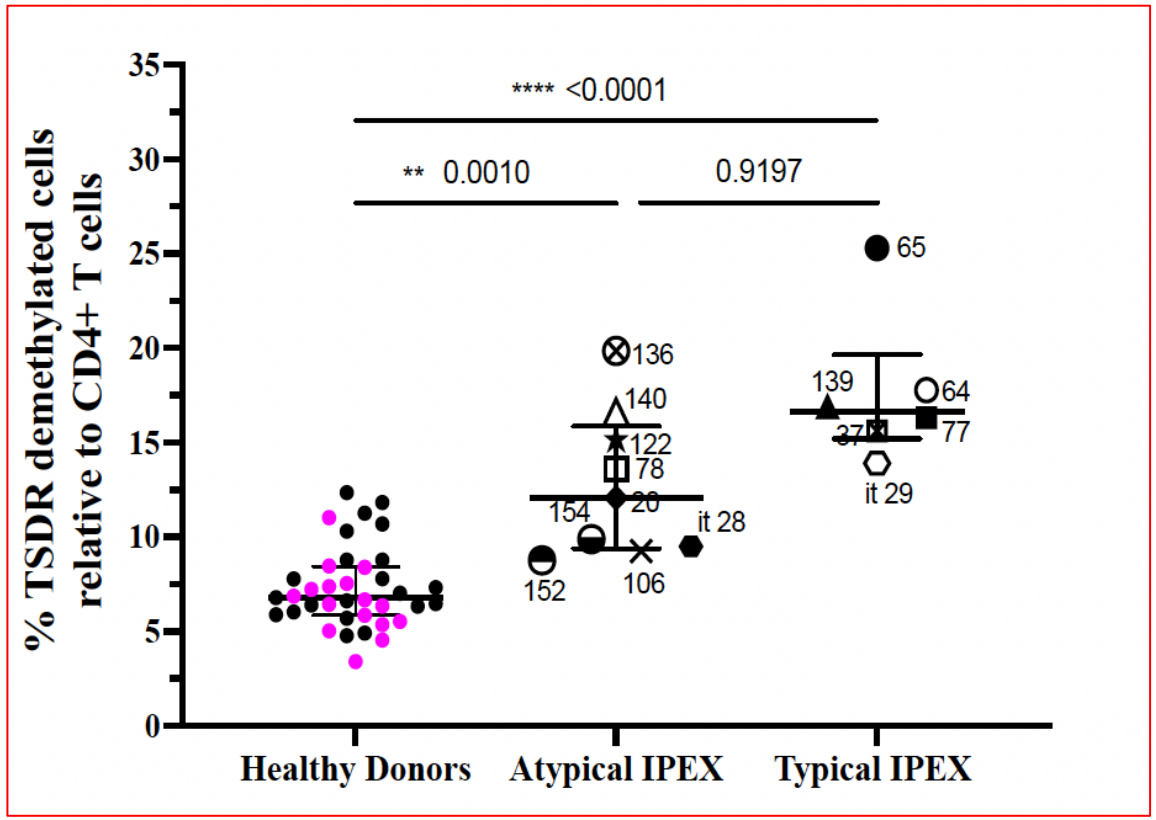
Elevated frequency of TSDR Demethylated cells to CD4+T cell ratio in whole blood is associated with active disease)



Narula M. et al, JACI Sept 2022

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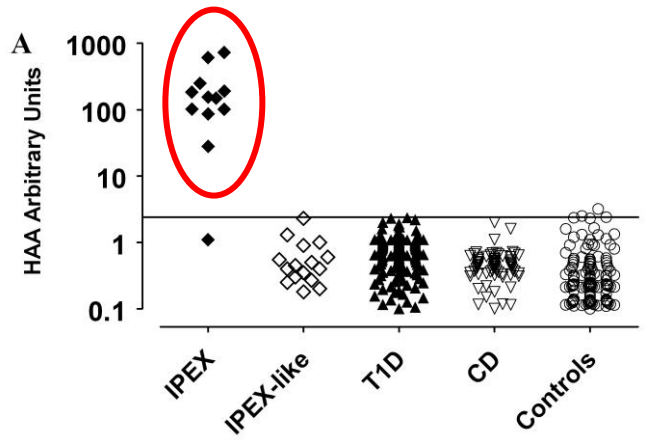
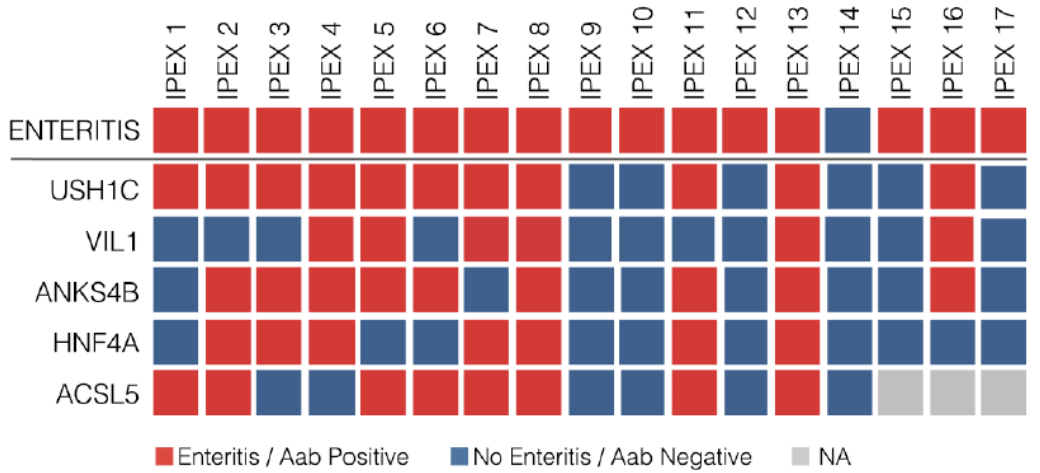
Presence of specific autoAb in the patients' serum

Anti-enterocytes (harmonin) Abs are present and highly specific





Specific autoAb against gut epithelial Ags correlated to active enteritis

(Lampasona PlosOne 2013, Eriksson JACI 2019)

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Poor genotype-phenotype correlation even between siblings

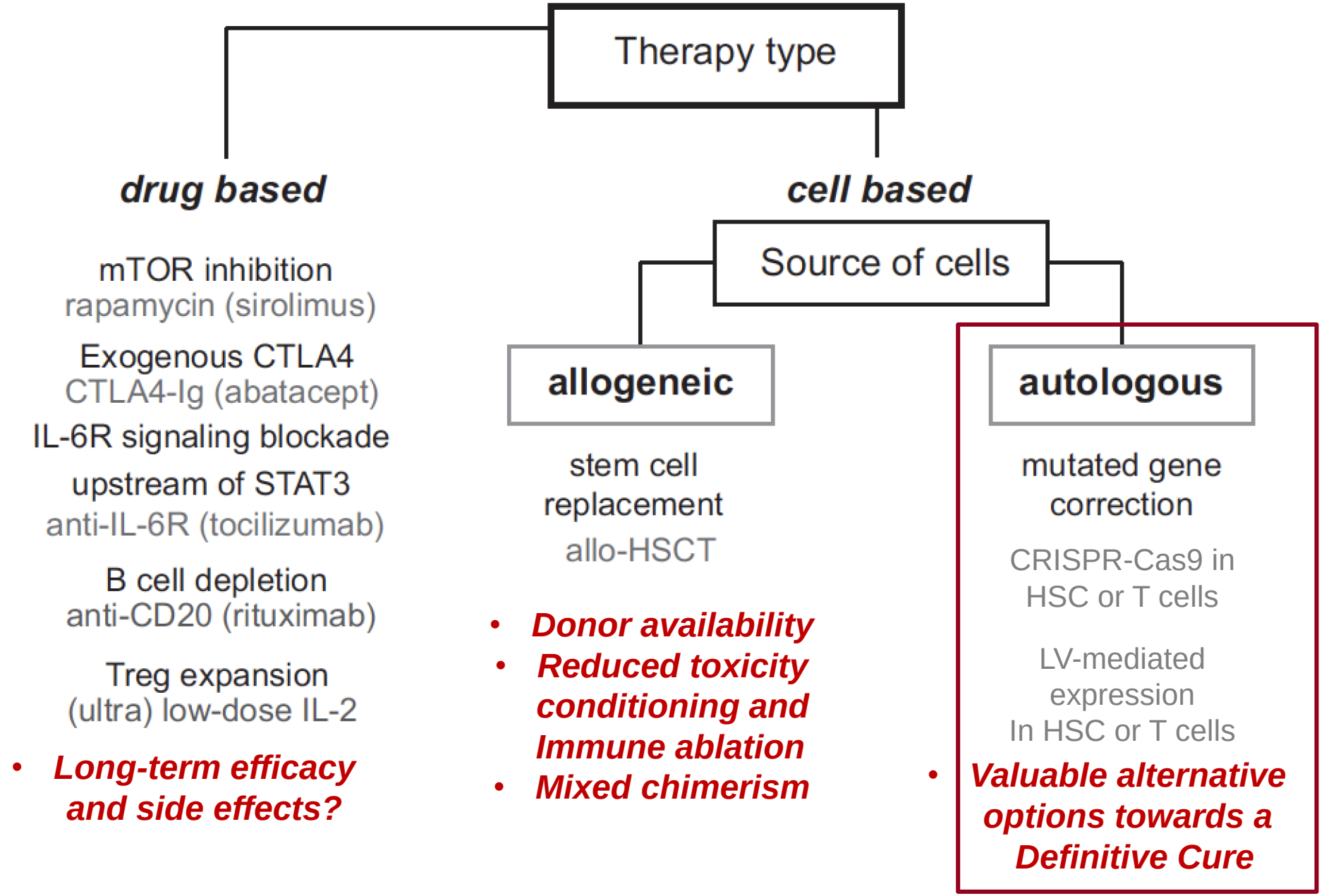
FOXP3 mutation	Sibling pair	Patient ID	[1]Gut Pathology	Pancreatic involvement	[2]Skin Pathology	Arthritis/ Muscle weakness	[3]Renal disorder	Auto-immune hepatitis	[4]Autoimmune cytopenia	Other
c.694T >G (p.Cys232Gly)	1	139		TID e.o.	✓ e.o.	✓				
		140		AutoAb+						
c.737 T>C (p. Leu246Pro)	2	*Unenrolled #7						✓		Burkitt lymphoma
		*Unenrolled #?	✓ e.o.							
c.1129C >G (p.His377 Asp)	3	77	✓ e.o.	TID	✓					
		78	✓			✓				
c. 1190G>A (p.Arg397Gln)	4	217	✓	AutoAb	✓				AIN	
		232		AutoAb						
	5	it 28		AutoAb						
		it 29	✓							
c.1222G>A (p.Val408Met)	6	Unenrolled #28	✓	TID		✓				
		Unenrolled #29	✓	TID		✓				
	7	152				✓	✓		AIHA	Lupus-like symptoms
		154		AutoAb						
c.1270_1272del (p.Cys424Leufs)	8	64	✓ e.o.		✓					
		65	✓ e.o.		✓			✓		

[1]Enteropathy, colitis or gastropathy; [2]Eczema or alopecia; [3]Nephrotic syndrome or glomerulopathy; [4]AIN (autoimmune neutropenia), AIHA (autoimmune hemolytic anemia), ITP (Immune thrombocytopenic purpura), AA (Aplastic Anemia); *Deceased; e.o., early onset (<1 y old)

Role of gene modifiers? Environmental factors?

Bacchetta, JACI, 2024

Treatment options and challenges in PIRD/Tregopathies



Cepika AM et al, JACI 2018

Current Treatment options and challenges in IPEX/Tregopathies

Drug-based treatments

A. Small molecules

mTOR inhibitor
Rapamycin, Sirolimus
JAKs and PI3K inhibitor

B. Proteins

Exogenous CTLA4
CTLA4-Ig, Abatacept

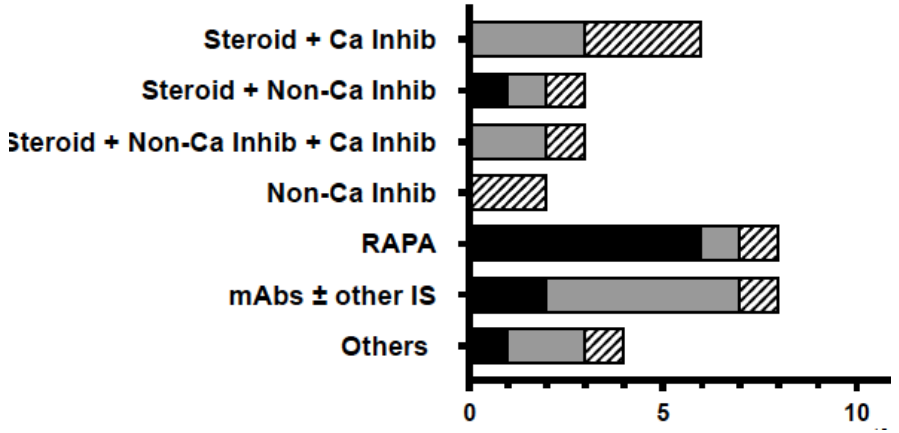
Treg expansion
Low dose IL2/mutated IL2

C. Antibodies

IL-6R-STAT3 signaling blockade
Anti-IL6R, Tocilizumab

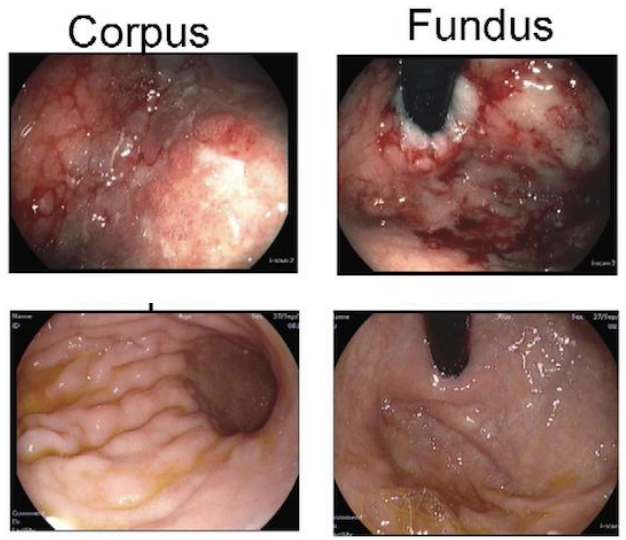
B cell deletion
Anti-CD20,Rituximab

Long-term efficacy and side effects?



Rapamycin increases suppressive function of Treg cells in a FOXP3 independent manner.

Charbonnier LM, Nat Immun 2019
Passerini Jaci 2020



Pre RAPA

Post RAPA

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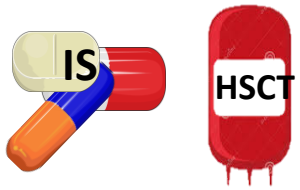
Current Treatment options and challenges in IPEX/Tregopathies

Cell-based treatments

I. Allogeneic cells HSC replacement

Allo-HSCT and Haplo-HSCT

- ***Donor availability***
- ***Toxicity after conditioning and immune ablation***
- ***Mixed chimerism***
- **HSCT rejection or Failure 7-17%**
- **persistence of symptoms 24%**

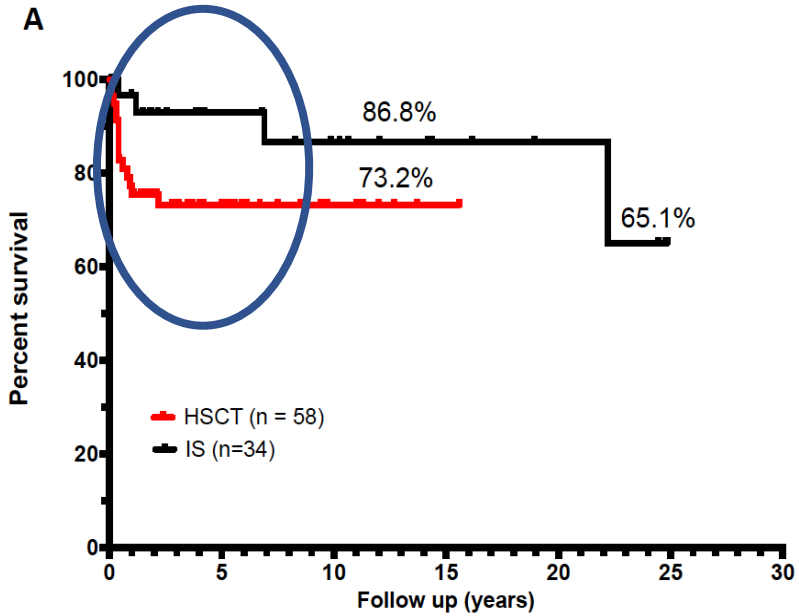


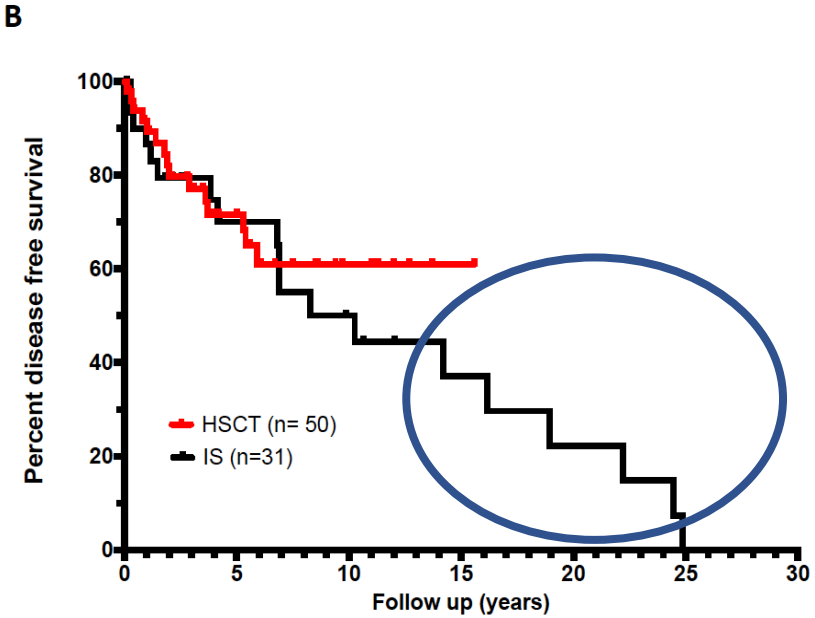
Kucuk et al, JACI 2016

Barzaghi et al. JACI 2018

Gambineri E. et al Frontiers Immun 2018

Chan A., Frontiers Immun 2020





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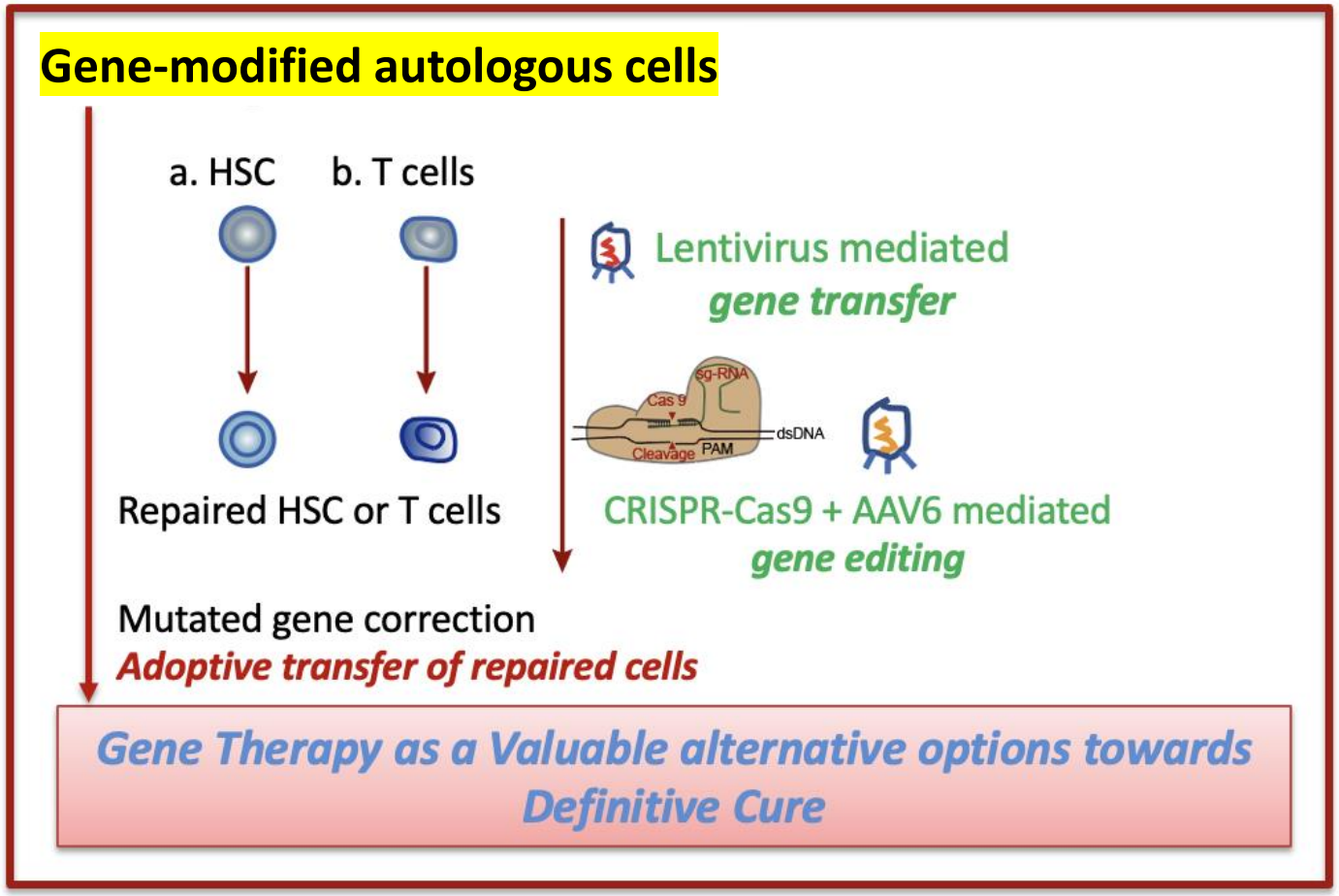
Novel therapeutic strategies:
IPEX syndrome is an optimal disease candidate for cell/gene therapy



The present

And

The Future……

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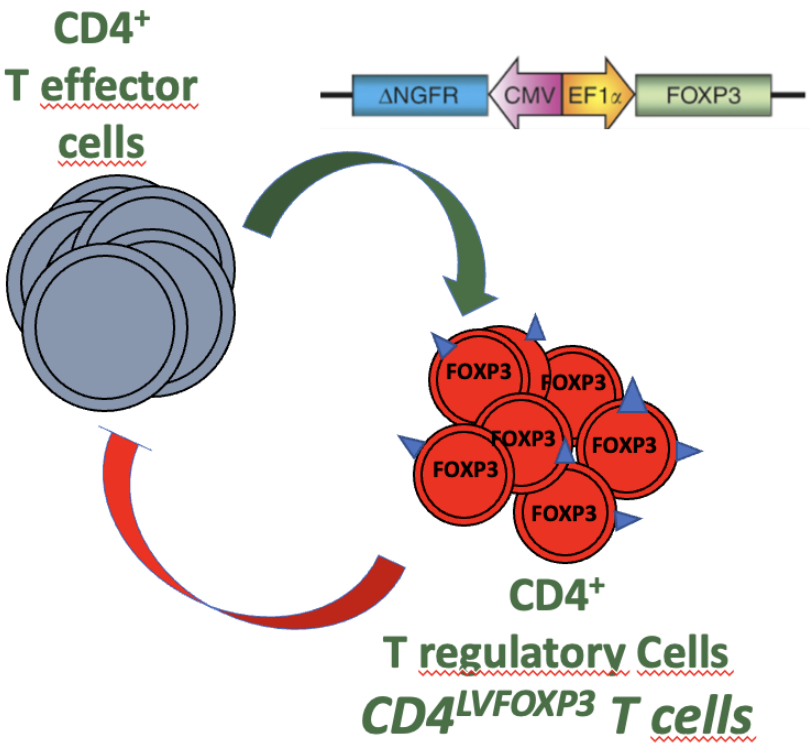
CD4+ T cells conversion into engineered Treg-like cells using lentiviral delivery of the *FOXP3* gene.



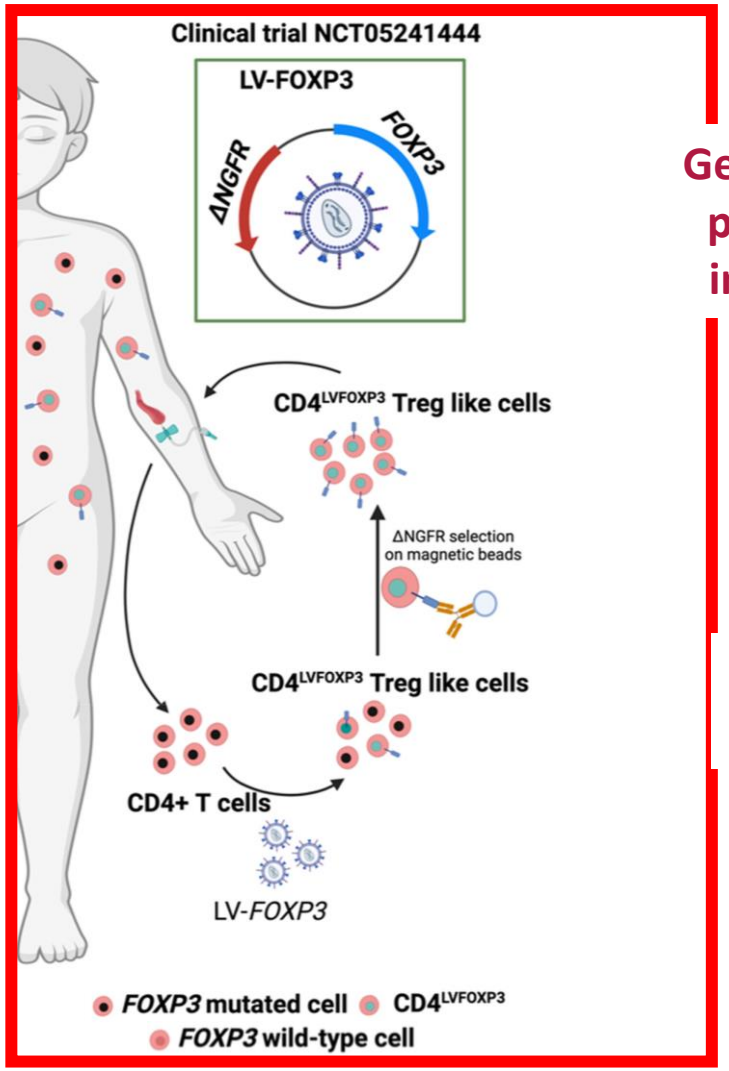
Science Translational Medicine

CD4+ T Cells from IPEX Patients Convert into Functional and Stable Regulatory T Cells by *FOXP3* Gene Transfer

LAURA PASSERINI, EVA ROSSI MEL, CLAUDIA SARTIRANA, GEORGIA FOUSTERI, ATTILIO BONDANZA, LUIGI NALDINI, MARIA GRAZIA RONCAROLO, AND ROSA BACCHETTA

Authors Info & Affiliations

SCIENCE TRANSLATIONAL MEDICINE · 11 Dec 2013 · Vol 5, Issue 215 · p. 215ra174 · DOI: 10.1126/scitranslmed.3007320



Gene addition under EF1a-promoter for activation-independent expression

Treg-replacement therapy

Clinical & Translational Immunology

Original Article | Open Access

Human-engineered Treg-like cells suppress FOXP3-deficient T cells but preserve adaptive immune responses *in vivo*

Yohei Sato, Laura Passerini, Brian D Piening, Molly Javier Uyeda, Marianne Goodwin, Silvia Gregori, Michael P Snyder, Alice Bertaina, Maria-Grazia Roncarolo, Rosa Bacchetta

First published: 25 November 2020 | https://doi.org/10.1002/cti2.1214 | Citations: 27

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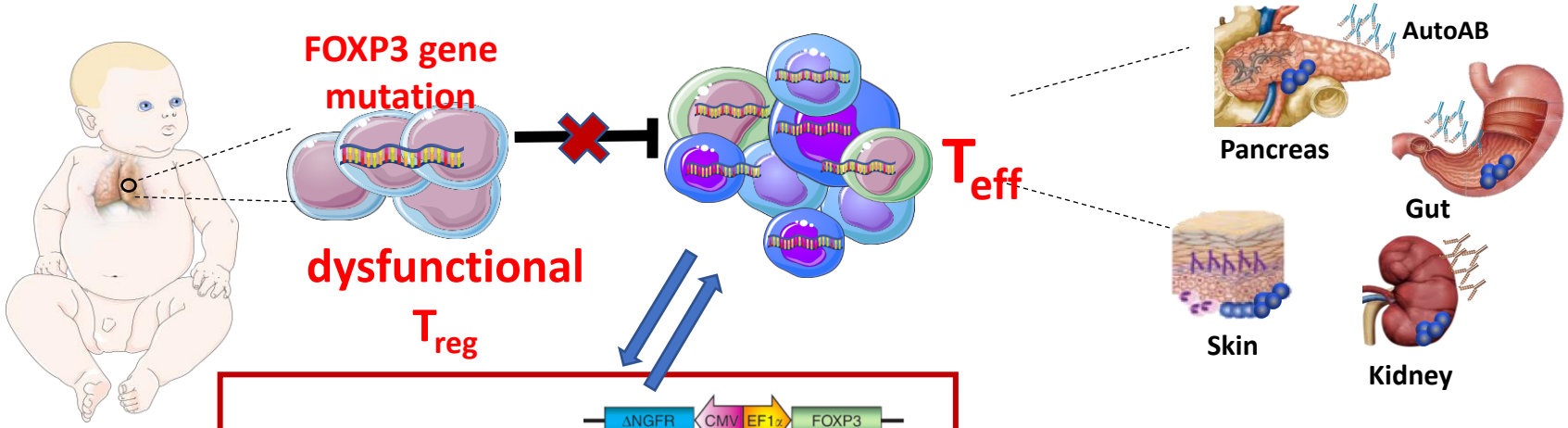
Engineered CD4^{LVFOXP3} Treg-like in the clinic for IPEX patients

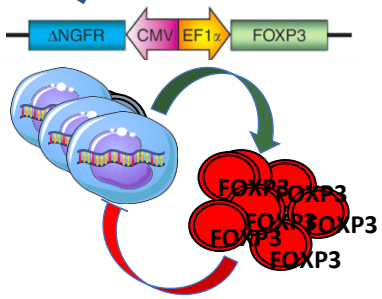
The trial is now open at Stanford (*NCT05241444*)

- *CIRM CLIN2 and FDA/NIH Funding*



Proposed new treatment: ODD and PRD designations by FDA

Autologous CD4^{+} Converted into Treg Cells: *CD4^{LVFOXP3} T cells*



~~Severe diarrhea~~
~~Eczema~~
Diabetes

Immune system back in control will not cause organ damage

- Age up to 35 yr old; with detected FOXP3 mutation
- In the presence of clinical history or IPEX with/out ongoing immunosuppression
- Refractory to treatment or who had unsuccessful HSCT

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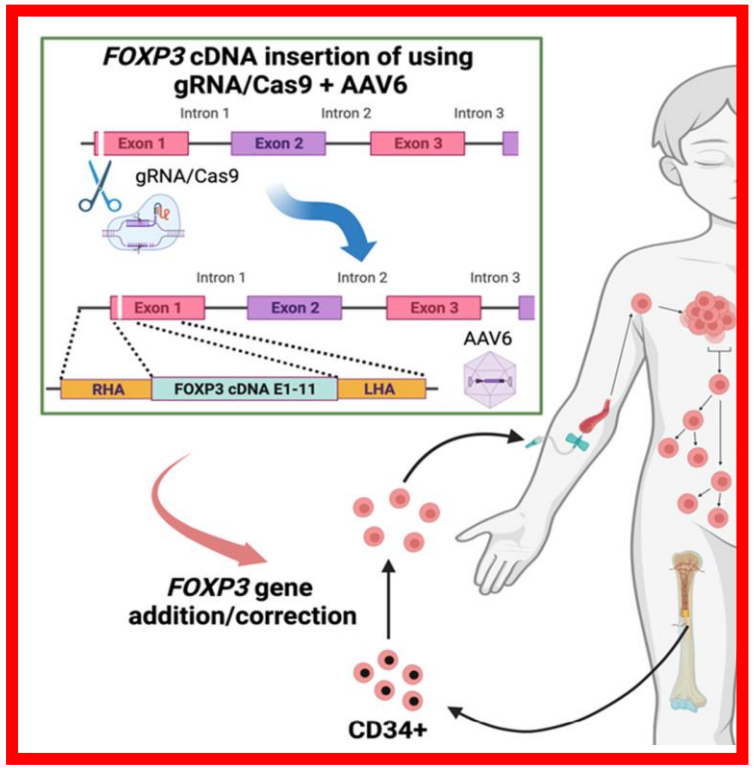
FOXP3 gene ”correction” using CRISPR/CAS9 followed by AAV6-mediated delivery of therapeutic gene in blood stem cells



Novel CRISPR/Cas9 editing strategy:

- Preserves endogenous regulation
- Allows Physiological gene splicing
- Still covers 85% of the patients’ FOXP3 mutations

CRISPR-based gene editing enables *FOXP3* gene repair in IPEX patient cells

M. GOODWIN, E. LEE, U. LAKSHMANAN, S. SHIPP, L. FROESSL, F. BARZAGHI, L. PASSERINI, M. NARULA, A. SHEIKALI, [...], AND R. BACCHETTA

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SCIENCE ADVANCES · 6 May 2020 · Vol 6, Issue 19 · DOI: 10.1126/sciadv.aaz0571

Currently under preclinical development

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Clinical Trials Office

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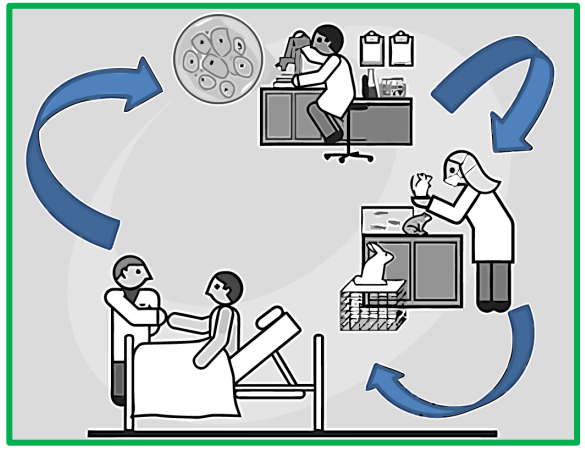

Funding Agencies

CIRM CALIFORNIA'S STEM CELL AGENCY

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CDCM Center for Definitive and Curative Medicine

Lucile Packard Foundation for Children's Health

Lucile Packard Children's Hospital Stanford

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- *CIRM Quest, CLIN1*
- *GSK- Stanford Alliance*
- *SPARK Funding*
- *MCHRI Postdoc Award*
- *JSPS Foundation*

www.IPEXfoundation.org

Regulatory T Cells: the Many Faces of Foxp3

Peter Georgiev [1,2,3] · Louis-Marie Charbonnier [1,2] · Talal A. Chatila [1,2]

Received: 12 June 2019 / Accepted: 23 August 2019 / Published online: 2 September 2019

Annual Review of Immunology

Regulatory T Cells and Human Disease

Shimon Sakaguchi,[1,2] Norihisa Mikami,[1] James B. Wing,[1] Atsushi Tanaka,[1] Kenji Ichiyama,[1] and Naganari Ohkura[1]

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Emerging Functions of Regulatory T Cells in Tissue Homeostasis

*Amit Sharma[1,2] and Dipayan Rudra[1,2]**

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ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
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REVIEW

From IPEX syndrome to *FOXP3* mutation: a lesson on immune dysregulation

Rosa Bacchetta,[1] Federica Barzaghi,[2] and Maria-Grazia Roncarolo[1]

Towards gene therapy for IPEX syndrome

Simon Borna, [1] Esmond Lee, [1,2] Yohei Sato, [1] and Rosa Bacchetta [1,3]

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FOXP3 deficiency, from the mechanisms of the disease to curative strategies

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Affiliations + expand

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REVIEW

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Genetic engineering of regulatory T cells for treatment of autoimmune disorders including type 1 diabetes

Karoliina Tuomela[1,2] · Megan K. Levings[1,2,3]

2023

T_{reg} cell-based therapies: challenges and perspectives

*Caroline Raffin[1,2], Linda T. Vo[1,2] and Jeffrey A. Bluestone[1]**

Abstract | Cellular therapies using regulatory T (T_{reg}) cells are currently undergoing clinical trials for the treatment of autoimmune diseases, transplant rejection and graft-versus-host disease. In this Review, we discuss the biology of T_{reg} cells and describe new efforts in T_{reg} cell engineering to enhance specificity, stability, functional activity and delivery. Finally, we envision that the success of T_{reg} cell therapy in autoimmunity and transplantation will encourage the clinical use of adoptive T_{reg} cell therapy for non-immune diseases, such as neurological disorders and tissue repair.

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