

IPEX syndrome from diagnosis to cure, learning along the way



Rosa Bacchetta, MD,^{a,b} and Maria Grazia Roncarolo, MD^{a,b,c}

Stanford, Calif

In the past 2 decades, a significant number of studies have been published describing the molecular and clinical aspects of immune dysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome. These studies have refined our knowledge of this rare yet prototypic genetic autoimmune disease, advancing the diagnosis, broadening the clinical spectrum, and improving our understanding of the underlying immunologic mechanisms. Despite these advances, Forkhead box P3 mutations have devastating consequences, and treating patients with IPEX syndrome remains a challenge, even with safer strategies for hematopoietic stem cell transplantation and gene therapy becoming a promising reality. The aim of this review was to highlight novel features of the disease to further advance awareness and improve the diagnosis and treatment of patients with IPEX syndrome. (*J Allergy Clin Immunol* 2024;153:595-605.)

Key words: *Regulatory T cells, FOXP3, TSDR, immune dysregulation, genetic autoimmunity, gene therapy*

In early 2003, 3 seminal studies demonstrated that (1) mutations in mouse Forkhead box protein 3 (*FoxP3*) caused CD4⁺ T-cell lymphoproliferation, scurfy hair, and wasting disease, leading to early death, and (2) *Foxp3* is required for regulatory T (Treg)-cell development and its overexpression impaired normal CD4⁺ T-cell development and increased the frequency of CD4⁺ and CD8⁺ with suppressive function.¹⁻³ These discoveries provided the primary mechanistic principle of the previously identified human disease, immune dysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome. IPEX syndrome is caused by pathogenic variants in *FOXP3*, designated as mutations throughout the text, and presents in affected infants with early-

Abbreviations used

auto-Ab: Autoantibody
 HSCT: Hematopoietic stem cell transplantation
 MN: Membranous nephropathy
 Teff: T effector
 Treg: Regulatory T
 TSDR: Treg-specific demethylation region

onset refractory diarrhea, diffuse eczema, and neonatal type 1 diabetes (T1D)⁴⁻⁶ (Fig 1).

IPEX syndrome was first described in 1982 in a single-family tree for a total of 19 male members⁷; by 2002, an additional 14 cases were reported.⁸ In the latter cohorts, multiorgan pathology beyond the gut, pancreas, and skin involvement became evident, including kidney, blood, and liver. Although a certain degree of clinical heterogeneity was already described in these initial reports, it is now well established that IPEX syndrome comprises a broad spectrum of clinical manifestations having an impact on different organs, with different degrees of severity and evolution.^{9,10} Despite the pathophysiologic importance of Treg-cell dysfunction in determining the disease, newly described immunologic abnormalities and complex epigenetic, transcriptional, and posttranslational modifications are emerging, which likely contribute to disease heterogeneity and evolution.^{11,12} Approaches to treatments have improved thanks to early diagnosis of IPEX syndrome and earlier intervention allowing for better responses to pharmacologic immunosuppression. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment for IPEX syndrome, but it has a low success rate with high disease recurrence.¹³

In this review, we summarize the most recent landscape of clinical, immunologic, and therapeutic understanding of IPEX syndrome, a disease that 20 years after its discovery remains life-threatening and imparts a significant loss of quality of life. Improved diagnostics with more accurate prediction of progression as well as targeted treatments, such as gene therapy, are urgently needed to better control the disease and improve the cure rate (Box 1).

CLINICAL MANIFESTATIONS: THE BROAD SPECTRUM OF THE DISEASE

Because of its recessive X-linked inheritance, IPEX syndrome presents exclusively in males; however, the same *FOXP3* mutation can manifest with different disease features and severity across patients. Between 2018 and 2020, 2 systematic reviews and 3 large independent cohort studies were published (Table I).¹³⁻¹⁷ These studies provided a comprehensive view of the

From ^athe Division of Hematology, Oncology, Stem Cell Transplantation and Regenerative Medicine, Department of Pediatrics, ^bthe Center for Definitive and Curative Medicine (CDCM), and ^cthe Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford.

Received for publication October 6, 2023; revised November 17, 2023; accepted for publication November 24, 2023.

Available online November 30, 2023.

Corresponding author: Rosa Bacchetta, MD, Division of Hematology, Oncology, Stem Cell Transplantation and Regenerative Medicine, Department of Pediatrics, Stanford University School of Medicine, 265 Campus Dr, Stanford, CA 94305. E-mail: rosab@stanford.edu.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749

© 2023 The Authors. Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

<https://doi.org/10.1016/j.jaci.2023.11.021>

Box 1. On the unknown of IPEX syndrome

Open questions	
Clinical	Real prevalence of the disease; definition of organ pathology beyond those included in the classical triad; extent and type of neurological and muscular manifestation; incidence and type of malignancies
Genotype-phenotype	Studies on a mutation-specific effect on protein function to understand downstream consequences of a mutation; investigate gene/epigenetic modifier; precisely quantify the isoforms in Treg and Teff cells; the role of a FOXP3 mutation in Treg cells/B cells interaction and auto-Ab formation
Pathophysiology	Prospective studies to understand the underlined immune dysregulation and monitor its evolution; how inflammation drives autoimmunity and vice versa
Diagnostics	Systematically use TSDR and precise measure of Treg-cell amount and their plasticity; precisely quantify Treg cells and their ratio with Teff cells; monitor the type of inflammation that develops overtime; monitor asymptomatic patients with proven FOXP3 mutation
Treatment	Choice of best immunomodulant alone or in combination; outcome of HSCT vs immunosuppression in prospective studies; outcome of Treg-cell replacement therapy; best hematopoietic stem cell gene therapy approach

clinical aspects of the disease, including some genotype-phenotype associations, and evaluation of current treatment outcomes.

The 3 most common organs involved: Intestine, pancreas, and skin

Multiple patients’ cohort studies confirmed that the clinical manifestations of IPEX syndrome are dominated by enteropathy characterized by diarrhea, mainly involving the small intestine, and showing villous atrophy, and lymphocyte and eosinophil infiltrates. The large intestine or stomach can also be affected, mostly in combination with the small bowel. About 50% of patients with IPEX syndrome manifest with insulin-dependent T1D with onset as early as the first month of life and preceding enteropathy. A recent retrospective study of 65 patients with FOXP3 mutations and T1D found that 77% presented with T1D, followed by enteropathy approximately 6 months later. Within this cohort, 95% had T1D onset within the first 6 months of life.¹⁸ The second most frequently reported endocrinopathy is thyroiditis. In addition, the presence of exocrine pancreas insufficiency may have been underestimated, especially in older patients. Skin pathology completes the well-known triad of IPEX syndrome symptoms, with eczema and dermatitis, but also psoriatic lesions, alopecia, and increased skin infections.

Less common targeted organs: Blood and kidneys

Blood cytopenia and especially autoimmune hemolytic anemia were observed with comparable frequency in the different patients’ cohorts. In recent years, kidney pathology has been detected more frequently, often reported as an isolated clinical manifestation in infancy, preceding other organ pathologies (Tables II and III).¹⁹⁻²⁴ Previously, kidney involvement was noted only in patients with IPEX syndrome with severe disease who had been treated with immunosuppression for several years. However, the interpretation of kidney damage as secondary to immunosuppression should be revised, because it is now clear that the kidney can be the target of the autoimmune damage in IPEX syndrome. Kidney pathology in IPEX syndrome is most often characterized by membranous nephropathy (MN), followed by tubulointerstitial nephritis, interstitial nephritis, minimal change nephrotic syndrome, and membranoproliferative glomerulonephritis.^{13,14,24}

MN results from an autoimmune process against podocyte antigens including M-type phospholipase A2 receptor (PLA2R), although not all the MN described were PLA2R⁺.²⁵ Although they are known as gut antigens, our own work found autoantibodies (auto-Abs) against harmonin and villin in the serum of patients with kidney pathology.²⁶ In multiple studies, MN was associated with high IgG4 and complement deposition. A recent review focusing on neonatal IPEX syndrome found kidney manifestations in 13 of 55 (23%) patients.²⁷ In our recent cohort, 7 of 40 (17.5%) patients had kidney involvement, with 2 patients developing kidney failure as their first manifestation of IPEX syndrome in adolescence.^{12,27}

Additional organ involvements

Additional organ involvements include (1) liver, associated with worsening of disease status, (2) lung, with occasionally reported interstitial lung disease, and (3) neuromuscular manifestations. The latter were reported in the 2 large cohorts described in 2018, with manifestations including seizures, ventriculomegaly, developmental delay and hypotonia, hemidiaphragmatic paralysis, peripheral neuropathy and muscular weakness, and fasciculation.^{13,14}

Infections can occur in IPEX syndrome, most likely related to the sequela of immunosuppression and/or organ damage (ie, gut and skin), although the relatively high incidence of Staphylococcus, Cytomegalovirus, and Candida infections could also be due to the presence of anticytokine antibodies. Anti-IFN type 1 and anti-IL-17 and anti-IL-12 antibodies are detected in the serum from many patients with IPEX syndrome, although at much lower titers when compared with patients with other genetic autoimmune diseases, such as autoimmune polyendocrinopathy candidiasis ectodermal dystrophy²⁹ (Bacchetta R, 2023, unpublished data).

Disease forms and evolution

Overall, the recent clinical studies confirmed the hallmark disease manifestations of IPEX syndrome, but also revealed that a broader spectrum of organs can be involved. Furthermore, there is significant diversity in time of onset and disease severity, both of which determine disease progression and prognosis. In our international retrospective study, data were collected from 2001

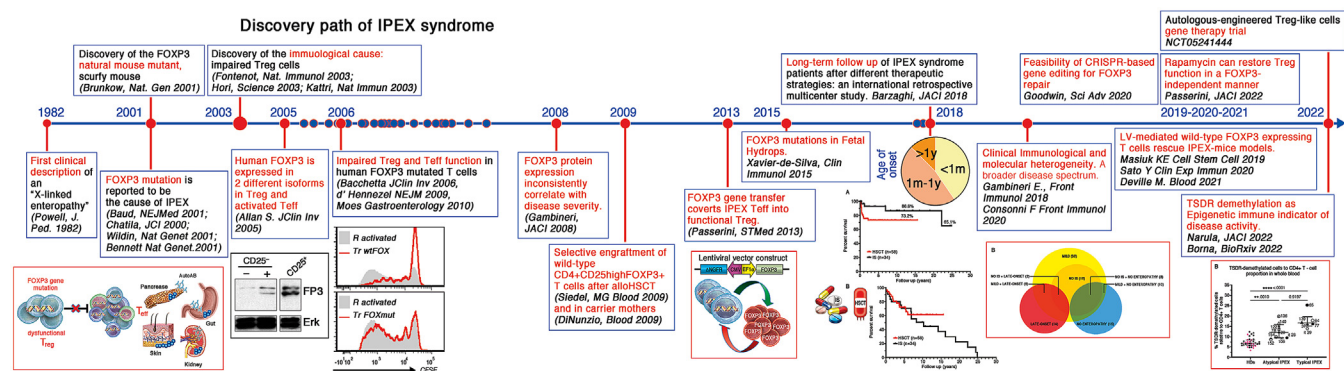


FIG 1. Discovery path for IPEX syndrome. Main steps toward the understanding of the disease from the mutated gene, the clinical manifestations, and the immune dysregulation to guide diagnoses and treatments.

to 2015 on 96 patients with IPEX syndrome from multiple centers, including the Primary Immune Deficiency Treatment Consortium and the European Society of Immune Deficiencies Inborn Errors Working Party¹³; 95% of these patients presented with disease onset during the first year of life, with 50% manifesting in the first month of life. Patients who experience disease onset after the first year are usually less severe or present with atypical manifestations. It is also becoming clear that a distinct, more moderate, but chronic form of IPEX syndrome exists, occurring because of partially controlled initially acute disease or because starting during childhood with progressively new disease manifestations over time. On the basis of all these different facets of the disease, a useful classification has been recently proposed by Consonni et al³⁰ whereby all patients with IPEX syndrome who do not experience early onset of the classical triad of symptoms should be defined as *atypical*. Thus, atypical IPEX syndrome may include 1 or more of the following: (1) late onset, (2) mild disease, (3) lacking severe enteropathy or sparing gut manifestations completely, and (4) presenting with clinical pathologies beyond the triad, such as those described earlier. In such patients, enteropathy can be recurrent instead of manifested with acute and intractable diarrhea, alternate to constipation, or manifest as predominant gastritis. In addition, it can be mistaken for food intolerance resulting in long-term malnutrition, with impaired life quality and failure to thrive. The long-term prognosis of patients with atypical IPEX syndrome is not clear, and it is challenging to determine the best treatment option, specifically whether to proceed with HSCT, weighing the potential benefits and risks.³⁰ It is notable that severe acute early-onset typical IPEX syndrome is generally diagnosed faster than in the past, but diagnosis of late moderate atypical IPEX syndrome should also not be delayed because it can have a deep impact on the patient's quality of life. Awareness of atypical IPEX syndrome is critical for physicians to implement genetic diagnosis without delay.

The actual incidence and prevalence of IPEX syndrome are difficult to measure because of the high mortality rate for early-onset severe disease (ie, can precede a diagnosis) and the heterogeneous atypical phenotypes in many patients.^{13,30} From a literature search in 2020, we gathered 383 patients diagnosed with IPEX syndrome worldwide, with an age of diagnosis of younger than 18 years and a mortality rate of 40.44%. In systematic reviews, Park et al¹⁶ reported a 53% death rate for untreated patients and Jamee et al¹⁷ an overall death rate of 37% (Table I). The mortality rate was only 20% in our international retrospective

study because all symptomatic patients were treated with either immunosuppression or HSCT or both.

GENOTYPE AND PHENOTYPE HETEROGENEITY

Effects of pathogenic variants in different domains

Pathogenic *FOXP3* mutations have been found across the entire locus. Although the precise frequencies of mutations affecting the different functional domains vary depending on the report, there is consensus that 40% are within the sequence encoding the C-terminal FKH DNA-binding domain, 23% are in the N-terminal PRR domain, 14% in the leucine zipper (LZ)-FKH loop, 9% in the LZ domain, and 6% in either the 5' untranslated region upstream of the initiating ATG or the C-terminal end of the ORF¹⁰; 12% are located in the *FOXP3* polyadenylation signal, a region that may not be included in clinical diagnostic assays.¹⁴ The most frequently found mutations across different studies are provided in Table I. Mutations that abrogate protein expression by introducing early stop codons or those located in the FKH domain (required for *FOXP3* DNA-binding functions) or in the polyadenylation region generally lead to severe typical IPEX syndrome. Studies in mice and patients confirmed that the absence of *FOXP3* protein causes a very severe phenotype with early onset or even death *in utero*.³¹ However, the effects of mutations in each domain of the *FOXP3* protein vary, and, to date, it has been difficult to identify a clear genotype-phenotype correlation also because of the difference between human *FOXP3* and mouse *foxP3* and the limitations of reproducing in the mouse the same effect of a human mutation.³² A recent in-depth study used CRISPR to insert known human mutations in mouse *foxP3*. Despite the interesting single-cell molecular data reported in mutated Treg and T effector (Teff) cells, mice developed a clear phenotype only with *foxP3* knockout or a single pathogenic FKH domain mutation. No disease was observed when other human *FOXP3* disease-causative mutations from the N-terminal, zinc-finger, or between the leucine zipper and the FKH were inserted, suggesting that other genetic or environmental factors contribute to determining human heterogeneity. Failure to consistently identify or predict a phenotype from a genotype is clearly demonstrated by the presence of different manifestations and severity in siblings with the same *FOXP3* mutations, including those with FKH domain mutations¹² (Table III). Overall, 2 possible interpretations emerge. The first is that other genes have an impact on the phenotype for better or worse. The other possibility is that

TABLE I. Overview of key genetic and clinical features of IPEX syndrome from most recent large cohort studies

Key features of patients with IPEX syndrome	Barzaghi F et al, 2018 ¹³	Gambineri E et al, 2018 ¹⁴	Duclaux-Loras R et al, 2018 ¹⁵	Park JH et al, 2020 ¹⁶	Jamee M et al, 2020 ¹⁷
Type of study	38 centers; retrospective data collection	2 centers; retrospective data collection	4 French centers; retrospective review	Systematic review*	Systematic review*
No. of patients with <i>FOXP3</i> mutation	96	88	30	195	312
No. of independent mutations	54 (21 new)	44 (9 new)	19 (5 new)	149	111
Most frequent mutations	c.1040 G>A	c.1150 G>A	c.751_753del	c.1150 G>A	c.1150 G>A
	c.816+5G>A	c.1157 G>A		c.751_753del	c.1040 G>A
	c.1150 G>A	c.1040 G>A			c.751_753del
	c.751_753del	c.751_753del			
Enteropathy (%)	90	97	100	97.9 (mostly diarrhea)	70 (biopsy-proven)
Endocrinopathy (%)	65 (T1D, 42)	65 (T1D, 49)	50 (T1D)	53.3 (mostly T1D)	51 (T1D, 39)
Skin (%)	73 (eczema-dermatitis)	89 (eczema-dermatitis)	85	62.1 (eczema-dermatitis)	66
Blood (%)	45 (mostly AIHA)	42 (mostly AIHA)	29 (mostly AIHA)	38.5 (mostly AIHA)	34
Kidney (%)	34	28	25	16.4 (membranous KD)	20
Liver-lung (%)	19-NA	20-23	14-NA	5.6-NA	11-14
Neuromuscular (%)	16	24	NA	NA	NA
Infections (%)	NA	47	NA	40; mostly sepsis and pneumonia	28; sepsis, 19; pneumonia, 11
Received IS (%)	34	NA	90	NA	36 (6% disease control)
Received HSCT (%)	58	45	23	NA	28
Death (%)	20 overall 11.7 post-IS (24 y) 27.5 post-HSCT (15 y)	22.5 post-HSCT	40 overall	36.4 post-IS 16.7 post-HSCT 6.1 post both 53 untreated	37 overall 19.5 post-HSCT
Overall survival (%)	86.8 post-IS (15 y) and 65 (24 y) 73.2 post-HSCT (15 y)	65 (at 10 y) 52 (at 30 y) 72.8 post-HSCT (10 y) 57.3 no HSCT	43 overall (10 y) 52 post-IS (10 y) 42 post-HSCT (10 y)	61.1 post-IS 29.5 post-HSCT 16.8 post both 16.8 untreated	60 post-HSCT
Disease-free survival (%)	37 post-IS (15 y) 61 post-HSCT (15 y)	NA	NA	NA	NA
WBC	Normal	NA	NA	NA	Normal L in 71%; low L in 28%; high Eo in 95%
Lymphocyte subsets	Mostly normal	Mostly normal	NA	NA	Mostly normal
Frequency of Treg cells (<i>FOXP3</i> expression)	Normal	Low CD25 ⁺ <i>FOXP3</i> ⁺	Low in 6/10; Normal in 4/10	NA	Low in 68% (variable)
Immunoglobulin	NA	Normal IgG, IgA, IgM High IgE (92)	High IgE (35% food allergy)	NA	Normal IgG, IgA, IgM High IgE

AIHA, Autoimmune hemolytic anemia; Eo, eosinophils; IS, immunosuppression; KD, kidney disease; L, lymphocytes; NA, not available; WBC, whole blood cell.

*The systematic reviews were published in 2020 but did not include the 3 retrospective studies published in 2018.

external triggers (different pathogens or nonpathogenic antigens) may induce a response differently in different individuals. Consonni et al³⁰ attempted to group patients with atypical IPEX syndrome by *FOXP3* mutation and phenotype, which may be useful for initial consultation and treatment plans. In Table III, we provide an additional list of *FOXP3* mutations and phenotype for patients referred to us and updated as compared with patients included in the study by Narula et al.¹² Remarkably, we found that the mutation c.1222 G>A was associated with muscular manifestations in 4 of 6 patients.

FOXP3 isoforms

There is another set of mutations that affects the expression of the *FOXP3* protein isoforms that are generated by pre-mRNA splicing.³³ As first shown in 2005 in human T cells,³⁴ *FOXP3* is expressed as 2 predominant isoforms, the full-length isoform *FOXP3FL* and an alternatively spliced isoform *FOXP3Δ2* that

lacks exon 2. Notably, exon 2 is not alternatively spliced in mouse Treg cells, which therefore expresses only the FL. Two other isoforms, *FOXP3Δ2Δ7* and *FOXP3Δ7*, have also been described in human lymphocytes and epithelial cells, but unlike *FOXP3FL* and *FOXP3Δ2*, which are both necessary and sufficient for Treg-cell-suppressive activity, neither of these isoforms appears to play a role in Treg-cell development and suppressive activity. Exon 7 is more relevant in activated T cells in which *FOXP3Δ7* promotes T_H17 differentiation.³⁵ In Treg cells from healthy human subjects, *FOXP3FL* and *FOXP3Δ2* isoforms are equally expressed, whereas Treg cells with an increased ratio of Δ2:FL have been described in several autoimmune and inflammatory diseases.^{33,36,37} A systematic study of the ratio of Δ2:FL in patients with IPEX syndrome has not been done. However, patients with exon 2 or exon 7 mutations have been described in multiple studies.^{14,30,33} A recent article reported a family with a *FOXP3* mutation (c.305delT: p.Phe102fs) that led to the loss of *FOXP3FL* but maintained the expression of *FOXP3Δ2*. Affected male

TABLE II. Characteristics of reported examples of kidney disease in patients with IPEX syndrome

Reported by	FOXP3 mutation	Age at kidney disease diagnosis	Immunopathology	Treatment/kidney disease outcome
Moudgil et al ¹⁹	303–304 del TT	6 mo	GN and TBM IC	2 HSCT; CR after second HSCT
Chuva et al ²¹	c.1010 G>A	3 mo	MN PLA2R ⁺ /SEMA3B ⁺ IgG4>>	Unknown
Sheikine et al ^{20**}	c.-23 G>A	1 mo	PGN, IN, MN PLA2R ⁺ ; IgG4>>	HSCT at 6 mo; CR
	c.1010 G>A	<3 y	PGN, MN IC	IS; expired at 18 y because of infection
	c.751_753delCAG	7 mo	GN and TBM IC	IS and HSCT at 7 y; CR
Tan et al ²²	c.766A > G	5 y	MN; PLA2R ⁺ THSD7A ⁺	IS; CR
Gentile et al ²³	c.779T>A	16 y	MN; PLA2R ⁺ THSD7A ⁺	Sirolimus and steroids; CR
	c.1087A>G	2 y	MN; PLA2R ⁺ THSD7A ⁺ IgG4 IC	HSCT from affected donor: no benefit; renal transplant and IS
Miller et al ²⁴	Not reported	1.5 y	MN PLA2R ⁺ IgG4>>	HSCT at 7 y; PR

CR, Complete remission; GN, glomerulonephritis; IC, immunocomplex deposition; IN, interstitial nephritis; IS, immunosuppression; PGN, primary glomerular nephropathy; PLA2R, phospholipase A2 receptor; PR, partial remission; SEMA3B, semaphorin 3b; TBM, tubular basement membrane; THSD7A, thrombospondin type I domain-containing 7A.

members reached adulthood, suggesting a level of immune functionality, but they did develop late autoimmune manifestations including T1D, inflammatory bowel disease, and psoriatic arthritis.³⁸ Phenotypic Treg cells were present in these and other patients we have investigated with exon 2 mutations (Bacchetta R, 2023, unpublished data). These findings indicate that Treg cells can differentiate with FOXP3Δ2, and that they can protect from severe IPEX syndrome, but expression of only FOXP3Δ2 may over time lead to impaired suppressive function, demonstrating a requirement for FOXP3FL. This hypothesis is supported by recent data in a new mouse model in which exon 2 was deleted and therefore only foxP3Δ2 was expressed.³⁹ Although mice do not ordinarily express foxP3Δ2, the authors report that Treg cells with preserved Treg-cell-specific demethylation region (TSDR) demethylation (an epigenetic marker for Treg cells; see later for more details) and suppressive function developed in this mouse model. However, the mice develop various autoimmune/inflammatory manifestations and also show a downregulation of genes essential for stable Treg-cell identity. This foxP3Δ2-induced Treg-cell instability could relate to the late-onset evolution of autoimmunity observed in patients. Collectively, these current data hint that both FOXP3FL and FOXP3Δ2 isoforms are necessary for Treg cells to maintain stable long-term immune homeostasis.

Overall, there is more to understand about FOXP3 isoform expression and function in Treg-cell and Teff-cell lineages in healthy donors and in patients with IPEX syndrome (Box 1). Gene modification tools and *in vitro* thymic organ culture or *in vivo* humanized mice models hold promise to better understand their roles in human T cells and how mutations in each isoform affect FOXP3 functions.

DIAGNOSTICS AND FOLLOW-UP: THE NEED TO GO BEYOND GENE SEQUENCING

Treg cells in IPEX syndrome

Another confounding complexity in the diagnosis of patients with IPEX syndrome is that most FOXP3 mutations do not alter FOXP3 protein expression or significantly have an impact on the number of Treg cells within the CD4⁺ T cells, as commonly detected by flow-cytometry staining using CD25 and CD127, combined with FOXP3 intracellular staining in certain laboratories.^{14,15,28,40–42} We recently reported that in patients with

IPEX syndrome (n = 15), the frequency of cells expressing FOXP3 within the gated CD4⁺CD25⁺CD127⁺ cells was only slightly lower than in healthy subjects, with significant variability among patients (ie, from 2.4% to 93% as compared with >85% in healthy subjects).¹² However, the intensity of FOXP3 expression was lower in most of the patients tested. Overall, the presence of the FOXP3 protein *per se* does not shed light on disease severity, because it can be expressed even in typical IPEX syndrome, although its complete absence is certainly related to negative clinical outcomes.^{12,40} In addition, the frequency of Treg cells can be altered by the ongoing immunosuppression, making this test less reliable.⁴⁰ The overall blood cell counts and main immune cell subsets are remarkably within normal ranges, with the exception of a possible increase in CD4⁺ T cells, mostly with a memory phenotype even in infants, and increased eosinophils and hyper-IgE, as seen in other immunodeficiencies with immune dysregulation. Although present in normal numbers, FOXP3-mutated Treg cells fail to suppress proliferation or cytokine production by activated Teff cells *in vitro*.^{41,43–45} This impaired suppressive activity has been reported by several groups, but it has not been tested consistently for different disease-causing FOXP3 mutations. Our laboratory has been the most consistent in performing suppression assays from patients with IPEX syndrome.^{41,43–45} The suppression assay has several technical limitations: (1) Treg-cell sorting can decrease cell viability and increase functional variability, especially after sample shipment from different centers; (2) large numbers of cells are needed and very often *bonafide* Treg cells are not detectable or are scarce in patients with IPEX syndrome; (3) the Treg-cell-suppressive activity and Teff-cell proliferation are both affected by ongoing immunosuppression (eg, patients on rapamycin normalize their suppressive capacity,⁴⁵ whereas patients on calcineurin inhibitors or steroids have reduction of both functions); (4) even normal donors show variability in Treg-cell-suppressive function ranging from 30% to 90%.⁴⁶ Thus, the suppression assay may not reliably clarify residual function for a given FOXP3 mutation.¹²

The epigenetic marker of Treg cells in IPEX syndrome

Our group has previously shown that patients with IPEX syndrome have a higher frequency of T cells in the peripheral blood that are fully demethylated at the FOXP3 TSDR, as

TABLE III. Mutations and organ involvement of current patients referred to Stanford research protocol

FOXP3 mutation	Protein Domains	Gut Pathology*	Pancreatic involvement	Skin Pathology†	Arthritis/muscle weakness	Renal disorder‡	Autoimmune hepatitis	Autoimmune cytopenia	Other
c.200C >T§ (p.Ser67Leu) c.1042T >G (p.Trp348Gly)	N-terminal	X		X	X	X			
c.244T >G (p.Ser82Ala)								AIN	
c.340C >T§ (p.Arg114Trp)			T1D e.o.						
c.434C >T (p.Ala145Val)		X	T1D e.o.	X		X		AIHA	
c.454+4A>G (miss-spliced p.)		X e.o.				X			
c.694T >G§ (p.Cys232Gly)	Zinc-Finger		T1D e.o.	X e.o.	X				
			AutoAb+						
c.725C>T (p. Leu242Pro)		X	AutoAb+	X				X	
c.737 T>C¶ (p. Leu246Pro)	Intron 7-Leucine Zipper						X		Burkitt lymphoma
		X e.o.							
c. 751_753del (p.Glu251del)		X e.o.	AutoAb+	X					
c. 782_807delIns TGAGCC§ (p. Ala261Val+S*36)	Leucine Zipper-Forkhead Loop		AutoAb+		X				Growth, cardiac and pulmonary birth defect
c.994 A>G§ (p.Lys332Glu)		X	T1D e.o.	X	X				HypoIgG
c.1036A>G§ (p.Ile346Val)	Forkhead	X	T1D						HypoIgG
c.1040A>G (p.Arg347His)		X	T1D						HypoIgG
c.1087A>G§ (p.Ile363Val)				X					Hyper-IgE Lympho-adenopathy
c.1091A>G (p.Tyr364Cys)		X	T1D e.o.						
c.1106G>A (p. Arg369His)		X	AutoAb+						HypoIgG
c.1118T >C (p.Phe373Ser)		X		X				X	
c.1129C >G§ (p.His377 Asp)		X e.o.	T1D	X					
		X			X				
c.1150G >A§ (p.Ala384Thr)		X e.o.	T1D	X					
		X		X	X				
c. 1190G>A (p.Arg397Gln)		X	AutoAb+	X				AIN	
			AutoAb+						
		X					X	X	
			AutoAb+						
		X							
c.1200C >A§ (p.Ser400Arg)		X	T1D	X	X	X		ITP	Lupus-like symptoms
c.1222G>A (p.Val408Met)			T1D		X				
		X#	T1D		X				
		X#	T1D		X				
			AutoAb+			X			
					X	X		AIHA	Lupus-like symptoms
			AutoAb+						

(Continued)

TABLE III. (Continued)

<i>FOXP3</i> mutation	Protein Domains	Gut Pathology*	Pancreatic involvement	Skin Pathology†	Arthritis/muscle weakness	Renal disorder‡	Autoimmune hepatitis	Autoimmune cytopenia	Other
c.1270_1272delinsC§ (p.Cys424Leufs)	PolyA sequence/3'UTR	X e.o.		X					
c.1271G >A (p.Cys424Tyr)		X e.o.		X			X		
c.1280C >G§ (p.Pro427Arg)		X	AutoAb +			X		AA	

AA, Aplastic anemia; AIHA, autoimmune hemolytic anemia; AIN, autoimmune neutropenia; e.o., early onset (<1 y old); ITP, immune thrombocytopenic purpura; p., protein prediction.

*Enteropathy, colitis, or gastropathy.

†Eczema or alopecia.

‡Nephrotic syndrome or glomerulopathy.

§Patients included in the study by Narula et al¹² and/or Borna et al.²⁸

||Sibling pairs.

¶Deceased.

#Twin brothers.

compared with IPEX-like patients or healthy subjects.^{12,28,41} The TSDR is a defined CpG island in CNS2 of the *FOXP3* loci; its demethylation is a specific lineage marker of *FOXP3*-expressing Treg cells established during thymic development.⁴⁷⁻⁴⁹ Because of its specificity, the TSDR distinguishes Treg cells from Teff cells that also express *FOXP3* on activation, but transiently and at a lower level. Healthy donor thymic-derived Treg cells are fully demethylated at the TSDR, in contrast to all nontarget cells, including all major leukocyte subpopulations.⁴⁷ Thus, TSDR quantification allows precise enumeration of Treg cells independent of their phenotypic markers and independent of *FOXP3* expression. This specific epigenetic signature of Treg cells has been broadly demonstrated in mice and humans.^{9,47,50-52} Treg-cell quantification by analysis of the cell methylation state of the TSDR can be precisely performed using a few drops of blood in different pathologic settings including other primary immune deficiencies or cancer.⁴⁸

We have used TSDR measurement in several patients with IPEX syndrome and have recently reported that the frequency of TSDR demethylated cells within the CD3⁺ or the CD4⁺ T cells is significantly higher in patients with typical and atypical IPEX syndrome and this frequency correlates with disease activity or level of dysregulation.^{12,28} Blood from patients with a “null” *FOXP3* mutation, in whom TSDR demethylation is normal at birth, displays rapid elevation of demethylation shortly after birth, along with the early severe onset of the disease. Interestingly, a higher frequency of TSDR demethylated T cells is also present in asymptomatic affected brothers who have a cytokine plasma profile common to IPEX syndrome. Moreover, the presence of wild-type Treg cells in patients with mixed chimerism after HSCT, for example, can normalize this abnormal value.²⁸ Therefore, we believe that quantification of the TSDR demethylated cells is a very useful method that, complementary to gene sequencing, can provide information on the underlined dysregulation caused by *FOXP3* deficiency. In a new ongoing study, we have shown that the discrepancy between high TSDR demethylated cells and the normal/low phenotypic Treg cells is due to the presence of T cells that have the epigenetic hallmark of Treg cells but have lost the expression of CD25, and therefore are not identified by immune phenotype. These cells could acquire Teff-cell function.²⁸

The identification of reliable assays that faithfully report the disease is important, especially to physicians evaluating patients with *FOXP3* “variants of unknown significance” as was reported for patients presenting with early-onset T1D.¹⁸ In this study, our collaborator studied TSDR demethylation in 41 patients with confirmed IPEX syndrome, 17 with *FOXP3* variants of unknown significance, 13 with IPEX-like monogenic autoimmunity, and 29 healthy controls. The data showed that *FOXP3* mutation was concordant with increased TSDR demethylation when compared with those with other monogenic forms of infancy-onset diabetes or controls (ROC-AUC 0.085). Therefore, detection of increased TSDR demethylation in the presence of *FOXP3* mutation could support the early diagnosis of IPEX syndrome and help with the correct and precise interpretation of novel variants of unknown significance.

Other immune abnormalities

In patients with IPEX syndrome, the presence of activated macrophage-derived inflammatory cytokines in the serum and an increased proportion of T_H2 cells, and in some patients also T_H17 cells,^{12,53,54} have been observed.

As mentioned earlier, other correlative assays that can complement *FOXP3* mutational analyses in IPEX syndrome include the detection of well-known organ-specific auto-Abs and a broad range of newly identified auto-Abs, especially those specific for the gut tissue, and anticytokines such as anti-IFN antibodies.^{13,26,29,55,56} The presence of these latter antibodies in the serum has gained attention because of their ability to neutralize the effect of the interferons and the association with negative outcomes of coronavirus disease 2019 infection. Patients with IPEX syndrome have levels of these antibodies that are higher than those in the general population but lower than those present in patients with autoimmune polyendocrinopathy candidiasis ectodermal dystrophy, for example, in whom they have a pathogenic role²⁹ (Bacchetta R, 2023, unpublished data). In addition, there are no reports that severe coronavirus disease 2019 has occurred in patients with IPEX syndrome, and therefore the anticytokine antibodies in IPEX syndrome, although important indicators of the immune dysregulation, remain of unclear clinical significance.

MECHANISTIC: WHAT IS STILL PUZZLING IN THE IPEX IMMUNE RESPONSE

Ordinarily, Treg cells maintain immunologic self-tolerance and immune homeostasis, and FOXP3 is the key transcription factor for the function of Treg cells.⁵⁷ Thus, it is a little surprising that IPEX syndrome remains the ultimate demonstration of the importance of the function of FOXP3-expressing Treg cells.^{43,44,58} The occurrence of severe autoimmunity, allergy, and immunopathology in humans and rodents with mutated *FOXP3* demonstrates that deficiency or dysfunction of thymic-derived CD4⁺CD25⁺FOXP3⁺ Treg cells alone is sufficient to break self-tolerance.¹⁰ Indeed, the specific loss of suppressive function toward autoreactive Teff cells can cause severe acute IPEX syndrome and is a major failure in immune regulation.

The increasing number of patients with IPEX syndrome diagnosed has allowed more in-depth immunologic studies, and together with studies in new mouse models testing the effect of human disease causing *FOXP3* mutations, it has led to the understanding that multiple direct and indirect causes contribute to the disease. We and others have shown the presence of aberrant Treg cells in patients with IPEX syndrome, which more closely resemble Teff cells.^{28,52} Bonafide Teff cells can also be affected by lower/impaired FOXP3 expression because FOXP3 is transiently upregulated on activation in the Teff-cell compartment, where it plays a role in regulating proliferation and expansion, although it does not confer suppressive function.^{59,60}

In addition to the multiple ways in which IPEX T cells can cause pathology, inflammation remains an important yet ill-defined contributing factor. Patients with IPEX syndrome have increased macrophage activation–derived cytokines and chemokines in plasma even before clinical manifestations of the disease.¹² Macrophage hyperactivation can be detrimental, and mutant Treg cells may have heightened susceptibility leading to loss of residual function.

Another mechanistic aspect is the type 2 immune response that is predominant in patients with IPEX syndrome and may contribute early to auto-Ab formation by a B-cell repertoire that has increased autoreactive B cells.⁶¹ The effect of FOXP3 (wild-type and disease-causing mutations) and Treg cells in B-cell development remains to be investigated. For example, it is not clear why some patients have hypogammaglobulinemia (Table III) even though they have never been exposed to B-cell–depleting agents.

Mechanisms that can counteract the immune dysregulation in IPEX syndrome or compensate for the lack of FOXP3⁺ Treg-cell–dependent regulation are poorly understood and warrant further investigation. The evidence of such mechanisms derives from the fact that several patients who have early severe onset show improvement and disease stabilization in later years. One possibility is that other regulatory mechanisms come into play to compensate. In the study by Narula et al,¹² it was demonstrated that the frequency of peripheral IL-10–producing type 1 regulatory cells is increased in long-term patients with IPEX syndrome, especially in atypical cases in which patients are less likely to be exposed to strong immunosuppressants.^{12,62} In addition, as discussed earlier, having brothers with IPEX syndrome carrying the same *FOXP3* mutation but manifesting with different degrees of disease severity suggests that other genes may contribute, perhaps also in disease prevention. These factors are important when

considering combined treatments, such as drugs that could increase FOXP3⁺ Treg-cell function or other Treg-cell subsets or drugs that do not block TCR-mediated stimulation.

ADVANCEMENTS IN CONVENTIONAL TREATMENTS

Pharmacologic immunosuppression

Without treatment, IPEX syndrome in its classical typical form is rapidly fatal. Most patients with IPEX syndrome need prompt therapeutic intervention at onset, which is usually within the first year of life.¹³ In addition to nutritional support, control of dehydration and electrolyte unbalance, and replacement therapy for endocrine organ failure, the treatment of IPEX syndrome currently relies on pharmacologic immunosuppression and allo-HSCT. Both therapeutic approaches have specific indications, advantages, and limitations. Immunosuppression, which is always used as a first-line therapeutic, has been administered with variable benefits often requiring a combination of drugs.¹³ Steroids have usually been the first immunosuppression of choice followed by the addition of calcineurin inhibitors, especially tacrolimus, or alternatively the mTOR inhibitor rapamycin. Tacrolimus may be preferred during the acute phase in severe IPEX syndrome, because of its rapid pharmacokinetics; however, rapamycin has considerable advantages over calcineurin inhibitors.

We demonstrated that rapamycin suppresses Teff-cell functions but does not have an impact on Treg cells. Indeed, rapamycin can increase Treg-cell function in patients with T1D who express wild-type FOXP3 but have functionally defective Treg cells.^{63,64} Furthermore, we showed that rapamycin can increase the suppressive function of Treg cells isolated from patients with IPEX syndrome, each with different *FOXP3* mutations, and activate FOXP3-independent pathways such as those mediated by GITR, IL-35, and CD39.⁴⁵ These human studies are in line with the work of Charbonnier et al⁶⁵ in mice, showing that mTORC inhibitors such as rapamycin enforce a metabolic switch that suppresses Teff-cell reprogramming in *foxP3*-deficient Treg cells, partially restoring regulatory function and attenuating the disease.⁶⁵ In our retrospective outcome study, rapamycin (alone or combined with steroids) is the only treatment that has shown benefit in controlling acute disease manifestations in most of the patients (67%). Thanks to these studies, rapamycin is increasingly being administered alone or initially combined with steroids, with many patients with IPEX syndrome remaining on rapamycin long-term.^{30,45}

Patients with IPEX syndrome, especially those with moderate gut manifestations, also respond well to anti-TNF- α by blocking this proinflammatory cytokine that has been detected at high levels in patient plasma. Moreover, Janus kinase inhibitors, CTLA4-Ig abatacept, mycophenolate, methotrexate, and azathioprine have been used, although generally in combination with other drugs, making it difficult to dissect the benefit of each single agent.¹³ Overall, the prolonged use of immunosuppression has significant negative side effects including increased infections, viral reactivations, and organ function deterioration. Our survey, the largest available on IPEX syndrome treatment outcomes with a follow-up of 25 years, showed that the disease-free survival of patients with IPEX syndrome receiving immunosuppression ($n = 31$) progressively declines.¹³

Therefore, immunosuppression does not prevent disease progression despite its chronic administration and has significant side effects, increasing toxicity and decreasing quality of life.

Now that we are aware of patients with *FOXP3* mutations but not yet showing clinical manifestations, it is important to determine whether immunosuppression could delay disease onset. This could be achieved by monitoring the degree of immune dysregulation, including the frequency of TSDR demethylated T cells and serum proinflammatory cytokines, which are more sensitive indicators than levels of auto-Abs.

Allogeneic HSCT

Allo-HSCT has proven curative, and haploidentical donor transplants with graft manipulation that depletes α/β T cells and B cells have been successfully used for patients with IPEX syndrome, thus broadening donor availability^{66,67} (Bacchetta R, 2023, manuscript in preparation). Data from our survey showed that overall survival of patients with IPEX syndrome after allo-HSCT is 73% ($n = 58$), with the greatest number of deaths occurring early after transplantation (Fig 1).¹³ Two other independent studies on overall survival after allo-HSCT in patients with IPEX syndrome and other primary immune regulatory disorders showed similar results.^{14,68} Our study found that the incidence of graft-versus-host disease in patients with IPEX syndrome after allo-HSCT is comparable with that of other similar primary immune deficiencies. In contrast, we found that engraftment failure (7%) or disease recurrence (33%) was unexpectedly high post-allo-HSCT, reducing the overall disease survival to 60%. These data are probably an underestimate because many unsuccessful transplants with loss of engraftment may not have been reported. Furthermore, they highlight that immune ablation is an important part of the conditioning regimen in patients with IPEX syndrome. Whether partial chimerism is sufficient to overcome the disease remains to be determined. In our retrospective study, partial chimerism does not correlate with either bad or good outcomes.¹³ However, when Treg-cell lineage chimerism was specifically assessed, donor Treg cells do have an advantage over Treg cells carrying *FOXP3* mutations. Therefore, even if the rest of the cell lineages have low donor chimerism, Treg cells can be fully donor-derived.^{28,69}

These data suggest that even a low number of donor-derived Treg cells may be sufficient to prevent recurrence, but how many and for how long remain unclear. Similar to the selective advantage of donor Treg cells after allo-HSCT, a selective advantage of Treg cells expressing wild-type *FOXP3* has been observed in mothers of patients with IPEX syndrome who carry *FOXP3* mutations but display nonrandom X inactivation whereby cells with wild-type *FOXP3* predominate.⁷⁰ Interestingly, a case has been reported about a carrier mother successfully used as HSCT donor for her affected son.⁷¹

The degree of organ impairment at the time of allo-HSCT was the most important variable predicting outcome, independent of age, donor source, or conditioning regimen. This finding strongly supports the importance of stabilizing the patient's clinical condition before allo-HSCT.

In summary, there is substantial evidence that the presently available treatments are only partially successful and that both immunosuppression and allo-HSCT as a standard of care for IPEX syndrome lead to suboptimal results.

THE PROMISE OF GENE THERAPY

FOXP3 gene transfer in autologous T cells

IPEX syndrome is a good candidate for gene therapy approaches. However, compared with other monogenic blood diseases, restoration of *FOXP3* in IPEX syndrome presents several challenges.^{72,73} *FOXP3* is a transcription factor with multiple intracellular interactions that regulate other genes. It is itself differentially regulated in different cell types (ie, transiently expressed on activation in T_H17 cells and constantly highly expressed in Treg cells) and exists as several alternatively spliced isoforms. We have developed a strategy to produce Treg-like cells by lentiviral vector-mediated expression of a wild-type cDNA encoding full-length *FOXP3* in CD4⁺ conventional T cells (CD4^{LVFOXP3}) isolated from patients with IPEX syndrome. The engineered autologous CD4^{LVFOXP3} cells are stable and preserve the appropriate self-antigen specificity acquired *in vivo*.^{74,75} Also, the CD4^{LVFOXP3} cells have cellular markers, anergic phenotype, cytokine production, and suppressive function that are superimposable to that of naturally occurring thymic-derived Treg cells. These features make CD4^{LVFOXP3} an ideal functional replacement therapy for dysfunctional Treg cells, thus addressing a significant unmet medical need. A phase 1 clinical trial (NCT05241444) is now open at Stanford for patients with IPEX syndrome of different ages, from childhood to age 35 years, with a history of (1) progressive disease manifestations and immunosuppressive treatment, (2) acute refractory disease, or (3) unsuccessful allo-HSCT transplant with disease recurrence. As a first-of-its-kind clinical trial, the main end points are (1) feasibility of preparing the Treg-cell product at clinical grade and at large scale from patients with IPEX syndrome CD4⁺ T cells and (2) the safety of the infusion, together with assessment of the persistence of CD4^{LVFOXP3} cells at different doses. The expression of a surface marker gene on the CD4^{LVFOXP3} allows us to track the cells and to determine how many are required to achieve physiologic Treg-cell levels in the peripheral blood. In parallel, a broad set of clinical and laboratory evaluations will assess the impact of the treatment on disease manifestations and immune dysregulation. The study, although currently single-center, aims to enroll patients from all over the world, working closely with local physicians for the follow-up assessments. Phase 1 to 2 cell therapy trials with Treg cells, either allogeneic or autologous, have been completed or are currently ongoing in various clinical settings including graft-versus-host disease, organ rejection, or autoimmune diseases such as T1D and inflammatory bowel disease.⁷⁶ These Treg-cell products were mostly derived from peripheral Treg cells purified and expanded *in vitro*, and they have been shown to be safe. Considering the proven safety of Treg-cell product infusion tested in other clinical settings for different indications, our approach in patients with IPEX syndrome should be safe, but it remains to be determined whether the cells persist and continue to be functional long-term.

FOXP3 gene transfer in hematopoietic stem cells

A second gene therapy approach appropriate for monogenic diseases of the immune system is to gene-modify patient hematopoietic stem cells.⁷² We and others are investigating this therapeutic strategy that would guarantee long-term persistence of gene-modified cells and long-term restoration of Treg-cell function.^{46,77} Masiuk et al⁷⁷ have proposed lentiviral vector-

mediated random insertion of a full-length cDNA encoding *FOXP3* together with the endogenous promoter and regulatory regions. Similarly, we have shown the feasibility of restoring *FOXP3* expression by site-specific insertion of the cDNA of full-length wild-type *FOXP3* by CRISPR/Cas9 gene editing and AAV6 gene delivery.⁴⁶ When tested in humanized mice, this approach does not induce any toxicity on engraftment or undesired expression in other cell types; however, the frequency of Treg cells expressing the inserted *FOXP3* is suboptimal. Thus, although promising, we are developing strategies to optimize this approach.

Conclusions

The current view of IPEX syndrome displays a multiplicity of clinical presentations and severity with inconsistent prediction when based only on genotype but similar underlying signs of immune dysregulation (Box 1). Early diagnosis remains key to limiting organ impairment, preventing complications and choosing the best available treatment. Multicenter collaborations are long awaited to gain further perspective insights into best treatment responses. Further mechanistic studies are warranted to refine novel treatments including gene therapy.

DISCLOSURE STATEMENT

R.B. is an Anne T. and Robert M. Bass Faculty Scholar in Pediatric Cancer and Blood Diseases, Stanford University School of Medicine. The study was supported by grants from the Stanford Maternal and Child Health Research Institute (to R.B.), the Center of Genetic Immune Diseases and the Center for Definitive and Curative Medicine at Stanford University (to M.G.R.), the RTW Foundation (to R.B.), and the California Institute for Regenerative Medicine (to R.B.).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

We thank the patients and families who made all the studies on IPEX syndrome possible over the years by kindly donating their blood. In addition to all the colleagues and physicians who contributed to our previous publications with their patients' information, we gratefully acknowledge the following: Dr Eyal Grunebaum (The Hospital for Sick Children, Toronto, ON, Canada), Dr Jie Zheng (Beijing Children's Hospital), Dr Costea George-Claudiu (Fundeni Clinical Institute, Bucharest, Romania), Dr Catherine Biggs (UBC Children's Hospital Research Institute, Vancouver, BC, Canada), Dr Sharat Chandra (Cincinnati Children's Hospital, Cincinnati, Ohio), Dr Allen Chen and Dr Sarah W. Peters (Johns Hopkins Hospital, Baltimore, Md), Dr Andriani Vazeou (Kyriakou Children's Hospital, Athens, Greece), Dr Magdalena Schellotto (Hospital Británico, Montevideo, Uruguay), Dr Leonardo Oliveira Mendonca (Hospital 9 de Julho, Sao Paulo, Brazil), Dr Isabelle Meyts (UZ Leuven, Leuven, Belgium), Dr Abigail T. Lang (Lurie Children's Hospital of Chicago, Chicago, Ill), and Dr Leif Gunnar Hanitsch (Charité—Universitätsmedizin Berlin, Berlin, Germany). We also kindly acknowledge Rhonda Perriman for thoughtful feedback and revision of the manuscript and Yu-Hsuan Kuo for helping with the patient data. We gratefully acknowledge the funding support for the studies on IPEX syndrome.

REFERENCES

- Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003;299:1057-61.
- Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat Immunol* 2003;4:337-42.
- Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 2003;4:330-6.
- Chatila TA, Blaese F, Ho N, Lederman HM, Voulgaropoulos C, Helms C, et al. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic dysregulation syndrome. *J Clin Invest* 2000;106:R75-81.
- Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* 2001;27:20-1.
- Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet* 2001;27:18-20.
- Powell BR, Buist NR, Stenzel P. An X-linked syndrome of diarrhea, polyendocrinopathy, and fatal infection in infancy. *J Pediatr* 1982;100:731-7.
- Wildin RS, Smyk-Pearson S, Filipovich AH. Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. *J Med Genet* 2002;39:537-45.
- Barzaghi F, Passerini L, Bacchetta R. Immune dysregulation, polyendocrinopathy, enteropathy, x-linked syndrome: a paradigm of immunodeficiency with autoimmunity. *Front Immunol* 2012;3:211.
- Bacchetta R, Barzaghi F, Roncarolo MG. From IPEX syndrome to FOXP3 mutation: a lesson on immune dysregulation. *Ann N Y Acad Sci* 2018;1417:5-22.
- Grover P, Goel PN, Piccirillo CA, Greene MI. FOXP3 and Tip60 structural interactions relevant to IPEX development lead to potential therapeutics to increase FOXP3 dependent suppressor T cell functions. *Front Pediatr* 2021;9:607292.
- Narula M, Lakshmanan U, Borna S, Schulze JJ, Holmes TH, Harre N, et al. Epigenetic and immunological indicators of IPEX disease in subjects with FOXP3 gene mutation. *J Allergy Clin Immunol* 2023;151:233-46.e10.
- Barzaghi F, Amaya Hernandez LC, Neven B, Ricci S, Kucuk ZY, Bleesing JJ, et al. Long-term follow-up of IPEX syndrome patients after different therapeutic strategies: an international multicenter retrospective study. *J Allergy Clin Immunol* 2018;141:1036-49.e5.
- Gambineri E, Ciullini Mannurita S, Hagin D, Vignoli M, Anover-Sombke S, De-Boer S, et al. Clinical, immunological, and molecular heterogeneity of 173 patients with the phenotype of immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. *Front Immunol* 2018;9:2411.
- Duclaux-Loras R, Charbit-Henrion F, Neven B, Nowak J, Collardeau-Franchon S, Marcus C, et al. Clinical heterogeneity of immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome: a French multicenter retrospective study. *Clin Transl Gastroenterol* 2018;9:201.
- Park JH, Lee KH, Jeon B, Ochs HD, Lee JS, Gee HY, et al. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome: a systematic review. *Autoimmun Rev* 2020;19:102526.
- Jamee M, Zaki-Dizaji M, Lo B, Abolhassani H, Aghamahi F, Mosavian M, et al. Clinical, immunological, and genetic features in patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) and IPEX-like syndrome. *J Allergy Clin Immunol Pract* 2020;8:2747-60.e7.
- Wyatt RC, Olek S, De Franco E, Samans B, Patel K, Houghton J, et al. FOXP3 TSDR measurement could assist variant classification and diagnosis of IPEX syndrome. *J Clin Immunol* 2023;43:662-9.
- Moudgil A, Perriello P, Loechelt B, Przygodzki R, Fitzgerald W, Kamani N. Immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome: an unusual cause of proteinuria in infancy. *Pediatr Nephrol* 2007;22:1799-802.
- Sheikine Y, Woda CB, Lee PY, Chatila TA, Keles S, Charbonnier LM, et al. Renal involvement in the immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) disorder. *Pediatr Nephrol* 2015;30:1197-202.
- Chuva T, Pfister F, Beringer O, Felgentreff K, Buttner-Herold M, Amann K. PLA2R-positive (primary) membranous nephropathy in a child with IPEX syndrome. *Pediatr Nephrol* 2017;32:1621-4.
- Tan L, An Y, Yang Q, Yang H, Zhang G, Li Q, et al. A novel FOXP3 mutation in a Chinese child with IPEX-associated membranous nephropathy. *Mol Genet Genomic Med* 2022;10:e1945.
- Gentile M, Miano M, Terranova P, Giardino S, Faraci M, Pierri F, et al. Case report: atypical manifestations associated with FOXP3 mutations. The "Fil Rouge" of Treg between IPEX features and other clinical entities? *Front Immunol* 2022;13:854749.
- Miller P, Lei L, Charu V, Higgins J, Troxell M, Kambham N. Clinicopathologic features of non-lupus membranous nephropathy in a pediatric population. *Pediatr Nephrol* 2022;37:3127-37.
- Huang G, Liu F, Yu L, Wang J, Chen J, Mao J. Pediatric membranous nephropathy: in the novel antigens era. *Front Immunol* 2022;13:962502.
- Lampasona V, Passerini L, Barzaghi F, Lombardoni C, Bazzigaluppi E, Brigatti C, et al. Autoantibodies to harmonin and villin are diagnostic markers in children with IPEX syndrome. *PLoS One* 2013;8:e78664.
- Hou AN, Wang Y, Pan YQ. A case report of IPEX syndrome with neonatal diabetes mellitus and congenital hypothyroidism as the initial presentation, and a systematic review of neonatal IPEX. *J Clin Immunol* 2023;43:979-88.

28. Borna S, Lee E, Nideffer J, Ramachandran A, Wang B, Baker J, et al. Identification of unstable regulatory and autoreactive effector T cells that are expanded in patients with *FOXP3* mutations. *Sci Transl Med* 2023;15:eadg6822.
29. Rosenberg JM, Maccari ME, Barzaghi F, Allenspach EJ, Pignata C, Weber G, et al. Neutralizing anti-cytokine autoantibodies against interferon-alpha in immunodysregulation polyendocrinopathy enteropathy X-linked. *Front Immunol* 2018;9:544.
30. Consonni F, Ciullini Mannurita S, Gambineri E. Atypical presentations of IPEX: expect the unexpected. *Front Pediatr* 2021;9:643094.
31. Carneiro-Sampaio M, Moreira-Filho CA, Bando SY, Demengeot J, Coutinho A. Intrauterine IPEX. *Front Pediatr* 2020;8:599283.
32. Leon J, Chowdhary K, Zhang W, Ramirez RN, Andre I, Hur S, et al. Mutations from patients with IPEX ported to mice reveal different patterns of FoxP3 and Treg dysfunction. *Cell Rep* 2023;42:113018.
33. Mailer RK. IPEX as a consequence of alternatively spliced FOXP3. *Front Pediatr* 2020;8:594375.
34. Allan SE, Passerini L, Bacchetta R, Crellin N, Dai M, Orban PC, et al. The role of 2 FOXP3 isoforms in the generation of human CD4+ Tregs. *J Clin Invest* 2005;115:3276-84.
35. Mailer RK, Joly AL, Liu S, Elias S, Tegner J, Andersson J. IL-1beta promotes Th17 differentiation by inducing alternative splicing of FOXP3. *Sci Rep* 2015;5:14674.
36. Magg T, Mannert J, Ellwart JW, Schmid L, Albert MH. Subcellular localization of FOXP3 in human regulatory and nonregulatory T cells. *Eur J Immunol* 2012;42:1627-38.
37. Joly AL, Seitz C, Liu S, Kuznetsov NV, Gertow K, Westerberg LS, et al. Alternative splicing of FOXP3 controls regulatory T cell effector functions and is associated with human atherosclerotic plaque stability. *Circ Res* 2018;122:1385-94.
38. Frith K, Joly AL, Ma CS, Tangye SG, Lohse Z, Seitz C, et al. The FOXP3Delta2 isoform supports Treg cell development and protects against severe IPEX syndrome. *J Allergy Clin Immunol* 2019;144:317-20.e8.
39. Du J, Wang Q, Yang S, Chen S, Fu Y, Spath S, et al. FOXP3 exon 2 controls T(reg) stability and autoimmunity. *Sci Immunol* 2022;7:eabo5407.
40. Gambineri E, Perroni L, Passerini L, Bianchi L, Doglioni C, Meschi F, et al. Clinical and molecular profile of a new series of patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome: inconsistent correlation between forkhead box protein 3 expression and disease severity. *J Allergy Clin Immunol* 2008;122:1105-12.e1.
41. Barzaghi F, Passerini L, Gambineri E, Ciullini Mannurita S, Cornu T, Kang ES, et al. Demethylation analysis of the FOXP3 locus shows quantitative defects of regulatory T cells in IPEX-like syndrome. *J Autoimmun* 2012;38:49-58.
42. Zemmour D, Charbonnier LM, Leon J, Six E, Keles S, Delville M, et al. Single-cell analysis of FOXP3 deficiencies in humans and mice unmasks intrinsic and extrinsic CD4(+) T cell perturbations. *Nat Immunol* 2021;22:607-19.
43. Bacchetta R, Passerini L, Gambineri E, Dai M, Allan SE, Perroni L, et al. Defective regulatory and effector T cell functions in patients with FOXP3 mutations. *J Clin Invest* 2006;116:1713-22.
44. d'Hennezel E, Ben-Shoshan M, Ochs HD, Torgerson TR, Russell LJ, Lejtenyi C, et al. FOXP3 forkhead domain mutation and regulatory T cells in the IPEX syndrome. *N Engl J Med* 2009;361:1710-3.
45. Passerini L, Barzaghi F, Curto R, Sartirana C, Barera G, Tucci F, et al. Treatment with rapamycin can restore regulatory T-cell function in IPEX patients. *J Allergy Clin Immunol* 2020;145:1262-71.e13.
46. Goodwin M, Lee E, Lakshmanan U, Shipp S, Froessl L, Barzaghi F, et al. CRISPR-based gene editing enables FOXP3 gene repair in IPEX patient cells. *Sci Adv* 2020;6:eaz0571.
47. Baron U, Floess S, Wiczorek G, Baumann K, Grutzkau A, Dong J, et al. DNA demethylation in the human FOXP3 locus discriminates regulatory T cells from activated FOXP3(+) conventional T cells. *Eur J Immunol* 2007;37:2378-89.
48. Baron U, Werner J, Schildknecht K, Schulze JJ, Mulu A, Liebert UG, et al. Epigenetic immune cell counting in human blood samples for immunodiagnostics. *Sci Transl Med* 2018;10:eaan3508.
49. Herppich S, Toker A, Pietzsch B, Kitagawa Y, Ohkura N, Miyao T, et al. Dynamic imprinting of the Treg cell-specific epigenetic signature in developing thymic regulatory T cells. *Front Immunol* 2019;10:2382.
50. Polansky JK, Kretschmer K, Freyer J, Floess S, Garbe A, Baron U, et al. DNA methylation controls Foxp3 gene expression. *Eur J Immunol* 2008;38:1654-63.
51. Toker A, Engelbert D, Garg G, Polansky JK, Floess S, Miyao T, et al. Active demethylation of the Foxp3 locus leads to the generation of stable regulatory T cells within the thymus. *J Immunol* 2013;190:3180-8.
52. Zemmour D, Zilionis R, Kiner E, Klein AM, Mathis D, Benoist C. Single-cell gene expression reveals a landscape of regulatory T cell phenotypes shaped by the TCR. *Nat Immunol* 2018;19:291-301.
53. Passerini L, Olek S, Di Nunzio S, Barzaghi F, Hambleton S, Abinun M, et al. Forkhead box protein 3 (FOXP3) mutations lead to increased TH17 cell numbers and regulatory T-cell instability. *J Allergy Clin Immunol* 2011;128:1376-9.e1.
54. Van Gool F, Nguyen MLT, Mumbach MR, Satpathy AT, Rosenthal WL, Giacometti S, et al. A mutation in the transcription factor Foxp3 drives T helper 2 effector function in regulatory T cells. *Immunity* 2019;50:362-77.e6.
55. Eriksson D, Bacchetta R, Gunnarsson HI, Chan A, Barzaghi F, Ehl S, et al. The autoimmune targets in IPEX are dominated by gut epithelial proteins. *J Allergy Clin Immunol* 2019;144:327-30.e8.
56. Vazquez SE, Mann SA, Bodansky A, Kung AF, Quandt Z, Ferre EMN, et al. Autoantibody discovery across monogenic, acquired, and COVID-19-associated autoimmunity with scalable PhIP-seq. *Elife* 2022;11:e78550.
57. Gavin MA, Rasmussen JP, Fontenot JD, Vasta V, Manganiello VC, Beavo JA, et al. Foxp3-dependent programme of regulatory T-cell differentiation. *Nature* 2007;445:771-5.
58. d'Hennezel E, Yurchenko E, Sgouroudis E, Hay V, Piccirillo CA. Single-cell analysis of the human T regulatory population uncovers functional heterogeneity and instability within FOXP3+ cells. *J Immunol* 2011;186:6788-97.
59. Allan SE, Crome SQ, Crellin NK, Passerini L, Steiner TS, Bacchetta R, et al. Activation-induced FOXP3 in human T effector cells does not suppress proliferation or cytokine production. *Int Immunol* 2007;19:345-54.
60. McMurchy AN, Gillies J, Gizzi MC, Riba M, Garcia-Manteiga JM, Cittaro D, et al. A novel function for FOXP3 in humans: intrinsic regulation of conventional T cells. *Blood* 2013;121:1265-75.
61. Kinnunen T, Chamberlain N, Morbach H, Choi J, Kim S, Craft J, et al. Accumulation of peripheral autoreactive B cells in the absence of functional human regulatory T cells. *Blood* 2013;121:1595-603.
62. Passerini L, Di Nunzio S, Gregori S, Gambineri E, Cecconi M, Seidel MG, et al. Functional type 1 regulatory T cells develop regardless of FOXP3 mutations in patients with IPEX syndrome. *Eur J Immunol* 2011;41:1120-31.
63. Battaglia M, Stabellini A, Roncarolo MG. Rapamycin selectively expands CD4+CD25+FoxP3+ regulatory T cells. *Blood* 2005;105:4743-8.
64. Monti P, Scirpoli M, Maffi P, Piemonti L, Secchi A, Bonifacio E, et al. Rapamycin monotherapy in patients with type 1 diabetes modifies CD4+CD25+FOXP3+ regulatory T-cells. *Diabetes* 2008;57:2341-7.
65. Charbonnier LM, Cui Y, Stephen-Victor E, Harb H, Lopez D, Bleesing JJ, et al. Functional reprogramming of regulatory T cells in the absence of Foxp3. *Nat Immunol* 2019;20:1208-19.
66. Shah RM, Elfeky R, Nademi Z, Qasim W, Amrolia P, Chiesa R, et al. T-cell receptor alphabeta(+) and CD19(+) cell-depleted haploidentical and mismatched hematopoietic stem cell transplantation in primary immune deficiency. *J Allergy Clin Immunol* 2018;141:1417-26.e1.
67. Merli P, Pagliara D, Galaverna F, Li Pira G, Andreani M, Leone G, et al. TCRalphabeta/CD19 depleted HSCT from an HLA-haploidentical relative to treat children with different nonmalignant disorders. *Blood Adv* 2022;6:281-92.
68. Chan AY, Leiding JW, Liu X, Logan BR, Burroughs LM, Allenspach EJ, et al. Hematopoietic cell transplantation in patients with primary immune regulatory disorders (PIRD): a Primary Immune Deficiency Treatment Consortium (PIDTC) survey. *Front Immunol* 2020;11:239.
69. Seidel MG, Fritsch G, Lion T, Jurgens B, Heitger A, Bacchetta R, et al. Selective engraftment of donor CD4+25high FOXP3-positive T cells in IPEX syndrome after nonmyeloablative hematopoietic stem cell transplantation. *Blood* 2009;113:5689-91.
70. Di Nunzio S, Cecconi M, Passerini L, McMurchy AN, Baron U, Turbachova I, et al. Wild-type FOXP3 is selectively active in CD4+CD25(hi) regulatory T cells of healthy female carriers of different FOXP3 mutations. *Blood* 2009;114:4138-41.
71. Otsubo K, Kanegane H, Kamachi Y, Kobayashi I, Tsuge I, Imaizumi M, et al. Identification of FOXP3-negative regulatory T-like (CD4(+)CD25(+)CD127(low)) cells in patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. *Clin Immunol* 2011;141:111-20.
72. Borna S, Lee E, Sato Y, Bacchetta R. Towards gene therapy for IPEX syndrome. *Eur J Immunol* 2022;52:705-16.
73. Pavel-Dinu M, Borna S, Bacchetta R. Rare immune diseases paving the road for genome editing-based precision medicine. *Front Genome Ed* 2023;5:1114996.
74. Passerini L, Rossi Mel E, Sartirana C, Foustieri G, Bondanza A, Naldini L, et al. CD4(+) T cells from IPEX patients convert into functional and stable regulatory T cells by FOXP3 gene transfer. *Sci Transl Med* 2013;5:215ra174.
75. Sato Y, Passerini L, Piening BD, Uyeda MJ, Goodwin M, Gregori S, et al. Human-engineered Treg-like cells suppress FOXP3-deficient T cells but preserve adaptive immune responses in vivo. *Clin Transl Immunol* 2020;9:e1214.
76. Bluestone JA, McKenzie BS, Beilke J, Ramsdell F. Opportunities for Treg cell therapy for the treatment of human disease. *Front Immunol* 2023;14:1166135.
77. Masiuk KE, Laborada J, Roncarolo MG, Hollis RP, Kohn DB. Lentiviral gene therapy in HSCs restores lineage-specific Foxp3 expression and suppresses autoimmunity in a mouse model of IPEX syndrome. *Cell Stem Cell* 2019;24:309-17.e7.