Diverse Clinical and Immunological Profiles in Patients with IPEX Syndrome: A Multicenter Analysis

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Abstract

Purpose: Immunodysregulation, Polyendocrinopathy, Enteropathy, and X-linked syndrome (IPEX), caused by *FOXP3* mutations, is a rare autoimmune disorder with diverse clinical features, including early-onset diabetes, eczema, and enteropathy. Atypical cases show milder symptoms and unique signs, requiring different treatments. Therefore, there are ambiguities in the accurate diagnosis and management of IPEX. We sought to present clinical, genetic, and immunological assessments of 12 IPEX patients with long-term follow-up to facilitate the diagnosis and management of the disease.

Methods: Clinical findings and treatment options of the patients were collected over time. Lymphocyte subpopulations, protein expressions, regulatory T (Treg) and circulating T follicular helper (cT_{FH}) cells, and T-cell proliferation were analyzed. **Results:** Predominant presentations included chronic diarrhea (75%), failure to thrive (66.7%), and eczema (58.3%). There were four classical and eight atypical IPEX individuals. Strikingly, the classical triad of IPEX was observed only in one patient. Allergic manifestations were more common in atypical patients. Notably, infections and chronic diarrhea demonstrated heightened severity compared to other manifestations. Four patients (33.3%) demonstrated eosinophilia, and nine (75%) showed high serum IgE levels. Most patients showed normal percentages of Treg cells with reduced CD25, FOXP3, and CTLA-4 expressions. Compared to healthy controls, the T_H2-like skewing accompanied by reduced T_H17-like responses was observed in cT_{FH} and Treg cells of patients. The impaired immune responses were corrected after hematopoietic stem cell transplantation (HSCT). Overall, nine patients (75%) received immunosuppressants (ISs), and six (50%) underwent HSCT, which was the only treatment revealing sustained control. Commonly used ISs included corticosteroids and sirolimus, but severe side effects led to therapy discontinuation in six patients.

Conclusions: This comprehensive analysis of clinical features and treatment responses contributes valuable insights for the improved diagnosis and management of IPEX syndrome, particularly emphasizing the atypical presentations and the efficacy of HSCT in achieving sustained control.

Introduction

Immunodysregulation, Polyendocrinopathy, Enteropathy, and X-linked syndrome (IPEX) is a rare, autoimmune, monogenic, and life-threatening disorder caused by X-linked forkhead box P3 (*FOXP3*) gene mutations (1–3). The global incidence of IPEX syndrome is less than one in a million, with over 300 patients documented to date, harboring more than 100 pathogenic mutations (4–7). Without treatments, IPEX patients mostly succumb within the early years of life due to sepsis, ketoacidosis, failure to thrive, or autoimmune complications (8). Hence, the outcome of patients is poor, and allogeneic hematopoietic stem cell transplantation (HSCT) emerges as the sole potentially curative therapy, complemented by immunosuppressants (IS) that serve as a crucial bridge for successful HSCT by alleviating symptoms (9).

The clinical recognition of IPEX dates back to 1982 when Powell et al. identified a family with 21 affected males, all of whom succumbed at an early life stage (10). Then, in 2000, genetic characterization of the disease caused by *FOXP3* mutations (initially termed *JM2*) was discovered by different groups (11, 12). Affected patients present with early-onset, insulin-dependent type 1 diabetes (T1DM), severe eczematous rashes, enteropathy with intractable diarrhea, and autoantibodies against target organs. These symptoms are accepted as a classical triad of the disease (7). Some IPEX cases have also described increased peripheral blood lymphocytes, eosinophils, and serum IgE levels (13). Notably, atypical cases exhibit a spectrum of phenotypes, ranging from mild presentations and late-onset symptoms to single-organ involvement or rare clinical signs, challenging the initially presumed homogeneity of IPEX (14). This heterogeneity of clinical presentation is being clarified by investigating the possible immunopathogenetic mechanisms. In this way, the location and type of mutations in the *FOXP3* gene and the number and function of regulatory T (Treg) cells are critical for the genotype-phenotype correlation (1, 15). However, measuring Treg-cell numbers or FOXP3 expression is not always a guide for diagnosing IPEX because the loss of FOXP3 is not the case in all patients, supporting the notion that FOXP3 is dispensable for thymic Treg development. In contrast, it is required for proper Treg function (16, 17).

FOXP3 gene is constituted of 12 exons, giving rise to protein containing a C-terminal forkhead (FKH) domain, a proline-rich N-terminal (PRR) or repressor domain, a C2H2 zinc finger (ZF), and a central leucine-zipper (LZ) domain. Mutations within the functional parts of FOXP3, those affecting the FKH, LZ domains, and polyadenylate region, are generally associated with more severe symptoms and poor prognosis. In contrast, missense mutations and mutations within the promoter and 5' untranslated region of *FOXP3* are associated with mild symptoms and better prognosis (7, 18–20). It has been observed that identical mutations may cause variable clinical presentations among individuals (21, 22). Considering these intricate facets, the heterogeneity of clinical and laboratory manifestations poses challenges to prompt diagnosis and identifying appropriate therapeutic approaches.

This study delves into the clinical and immunological findings of a well-defined cohort of 12 IPEX patients, offering insights into the broad spectrum of clinical phenotypes observed during long-term follow-up. Detailed genetic and immunological assessments presented herein not only aid in diagnosis and clinical management but also contribute novel perspectives on T-cell responses in IPEX syndrome over time.

Materials and Methods

This multicenter study involved 12 patients with *FOXP3* mutations. The genetic diagnosis was made by next-generation (targeted, whole exome) sequencing, confirmed by Sanger sequencing. Clinical and demographic features of the patients were retrieved from their medical records.

Clinical and laboratory evaluations. The demographic and clinical data include age at onset of symptoms, age at diagnosis, family history, immunodeficiency, autoimmunity findings, past infections, allergic manifestations, systems involved, and treatment options. We categorized the response rate in terms of the administered drugs as complete response (CR), partial response (PR), or nonresponsive (NR) (23).

Immunological assessments. Peripheral blood lymphocyte subset analyses were performed by flow cytometry, as described previously (24–29). Stained cells were acquired by Navios EX cytometer (Beckman Coulter) and analyzed by FlowJo software (TreeStar, Ashland, Ore). The details are presented in the

Supplementary file. The tertiary structure of the FOXP3 protein was retrieved from the AlphaFold Protein Structure Databank (30). The plausible effects of variants on 3D protein structure were investigated based on the predicted unfolding free energy change using PremPS web server (31, 32).

Statistical analysis. Comparisons between patient and control groups were made using the Mann-Whitney U test and Kruskal-Wallis with Dunn's post-test analysis, as indicated. The chi-square test was used to compare categorical values. Analysis of the probability of overall survival (OS) was done using the Kaplan–Meier method (log-rank test). Differences between values were considered significant at a p-value < 0.05. Statistical analysis was done using GraphPad Prism 9 (GraphPad Software Inc, San Diego, Calif).

Results

Clinical findings and comparisons between atypical and classical IPEX patients.

Twelve patients with *FOXP3* mutations were included in our study. Four patients (P1, P2, P3, P4) were reported previously in 2014 (33), but we provided detailed extended follow-up information regarding these patients. P8 and P9 were reported in research-based studies without clinical description (34, 35). The median age at the disease's onset was 4 (min-max: 0–95) months, which was within the neonatal period in 4 (33.3%), between 1 and 12 months in 5 (41.6%), late-onset beyond 12 months in 3 (25%) patients. The median current age was 10 (min-max: 0.25–26.5) years, with a diagnostic delay of 1.5 (0.08–17.3) years. There was 25% kinship between the parents. The demographic and clinical features are shown in Table 1.

Table 1
The Demographic and clinical features of IPEX patients

Patient	Total (n,%)	P1	P2	P3	P4	P5	P6	P7
Clinical phenotype	Atypical (58.3%)	Atypical	Classical	Classical	Atypical	Atypical	Classical	Atypical
Last documented age (mo)	-	120	168	3	56	22	30	124
Consanguinity	25%	-	-	-	-	-	-	+
AOO (mo)	Median: 4 (min-max: 0–95)	2	1	< 1	1	3	5	84
Insulin-dependent type 1 diabetes mellitus	16.7%	-	-	+	-	+	-	-
Respiratory tract infections (before IS or HSCT)	58.3%	Pneumonia	-	Pneumonia	-	-	-	URTI and Pneumonia
Bronchiectasis	-	-	-	-	-	NA	NA	-
Eczema	58.3%	-	+	+	-	-	+	+
Other skin features	8.3%	-	-	-	-	-	Severe diaper dermatitis	-
Allergic manifestation	50%	-	-	-	CMA	-	-	Asthma, allergic rhinitis
Documented bacterial infecitons	50%	*P.aeuriginosa* *M.gordonae*	*S.aureus* *S.epdermidis* *Stenotrophomonas maltophilia*	NA	*P.aeuriginosa*	-	*S.aureus* *Enterococcus* *Leuconostoc mesenteroides*	-
Documented fungal infections	50%	-	-	*C.albicans*	*C.albicans*	-	*C.albicans*	*C.albicans*
Documented viral infections	41.6%	CMV	-	-	CMV	-	COVID-19	CMV
Severe infections (before IS or HSCT)	16.6%	-	-	-	-	-	Sepsis	Enterocolitis Sepsis
Autoimmunity	91.6%	+	+	+	+	+	+	+
Chronic diarrhea	75%	+	+	+	+	-	+	+
Failure to thrive	66.7%	+	+	+	+	-	+	+
Organomegalies or lymphadenopathies	41.6%	HSM	Cervical and occipital LAPs	-	-	-	-	-
Other features	-	Nephrotic syndrome	Focal seizure	-	-	Grade 1 left renal hydronephrosis	-	-

Abbreviations: AOO: Age of onset, M.gordonae: *Mycobacterium gordonae*, P.aeruginosa: *Pseudomonas aeruginosa*, C.albicans: *Candida albicans*; S.aureus: *Cytomegalovirus*, COVID-19: Coronavirus-19, S.enterica: *Salmonella enterica*, URTI: Upper respiratory tract infection, SLE: Systemic lupus erythematosus, IgR Hepatosplenomegaly, CMA: Cow milk allergy, HT: hypertension, M: Male, F: Female, mo: Month, IS: Immunosuppressant, HSCT: Hematopoietic stem cell trar meningitis or osteomyelitis.

Within the median follow-up period of 2.6 (min-max: 0.25–13.5) years, the most common presentations were autoimmunity (91.6%), chronic diarrhea (CD) (75%), and failure to thrive (FTT) (66.7%), followed by eczema (58.3%) and recurrent infections (RIs) (58.3%). Specifically, patients with neonatal onset (P2, P3, P4, P10) showed CD (100%), T1DM (25%), and skin manifestations (50%). These findings proportionally were 80%, 20%, and 40% in patients with disease onset between 1 and 12 months (P1, P5, P6, P8, P9). The classical triad of IPEX was observed only in one patient (P3). Significantly, patients displayed a wide array of organ involvement (Fig. 1A), with RIs and CD presenting with the most severe levels in comparison to other clinical manifestations (Fig. 1B). One

patient (P12) had a narrower range of symptoms, characterized by recurrent wheezing and mild atopic dermatitis. Another case (P5) had only neonatal T1DM, controlled well with insulin infusion. Renal manifestations included nephrotic syndrome (P1) and hydronephrosis in two patients (P5, P8). The detailed course of the patients' clinical conditions over time is described in the **Supplementary file**.

The presentation of IPEX can be classified as classical or atypical based on clinical findings (14). The atypical form is characterized by a late-onset (i.e., > 1 year of age), mild disease course (i.e., long-term survival without IS or with first-line IS regimens), no enteropathy and/or unusual clinical features (i.e., infrequent manifestations that go beyond the classical triad and/or involve different organs). In this study, there were four patients with classical IPEX (P2, P3, P6, P8) and eight with atypical disease (P1, P4, P5, P7, P9-12) (Fig. 1B and **C**). Delay in diagnosis was higher in atypical cases (median: 72 vs. 4 months, p = 0.04). The distribution of clinical findings was similar between the atypical and the classical patients. However, allergic manifestations were more pronounced in atypical patients, while skin involvement, CD, and endocrinopathy tended to be frequent in classical IPEX (Fig. 1C).

Autoimmune and allergic findings of IPEX patients.

Autoimmune findings (n = 11, 91.6%) included enteropathy (n = 7, P1, P2, P4, P6, P7, P8, P9), T1DM (n = 2, P3, P5), autoimmune hepatitis (n = 2, P1, P11), autoimmune hemolytic anemia (AIHA) (n = 2, P2, P3), immune thrombocytopenia (ITP) (n = 2, P2, P4), and systemic lupus erythematosus (SLE) (n = 1, P10). Autoantibody testing was positive for coombs test (n = 4, P2, P3, P10, P11), anti-glutamic acid decarboxylase (n = 3, P5, P6, P7), anti-thyroid peroxidase (n = 2, P3, P5), anti-enterocyte antibody (n = 2, P2, P4), antinuclear antibody (n = 2, P10, P11), anti-insulin antibody (n = 1, P5), anti-partial antibody (n = 1, P9), lupus anticoagulant antibody (n = 1, P10), and anti-smooth muscle antibody (n = 1, P11).

Allergic manifestations included asthma (n = 4, P7, P9, P11, P12), rhinitis (n = 1, P7), and cow milk allergy (n = 2, P4, P8). Severe eczema complicated with skin infections was detected in 7 patients (P2, P3, P6, P7, P8, P11, P12) (Fig. 1D).

Endoscopic biopsies with histopathology revealed celiac-like disease in two patients (P6, P9), inflammatory bowel disease (IBD)-like features in two (P7, P8), villous atrophy in three (P2, P4, P6), subtotal villous atrophy in two (P7, P9), and pancolitis accompanied by cytomegalovirus (CMV) in two patients (P1, P8). P7 had chronic inflammation and cryptitis in the ileum (Fig. 1E). P8 also exhibited eosinophilic cryptitis and cow milk allergy. P10 had mucosal congestion in the duodenum and inactive chronic gastritis. P11 had esophageal varices.

Immunological findings of IPEX patients.

Table 2 summarizes laboratory findings of IPEX patients at the time of the diagnosis. Mild leukocytosis and mild lymphopenia were detected in four patients each (33.3% and 33%). Remarkably, four patients (33.3%) exhibited eosinophilia, and nine (75%) showed high serum IgE levels. Low IgG, IgM, and IgA levels were detected in 41.7%, 58.3%, and 50% of the patients, respectively, but vaccine responses were intact. Most patients' T, B, and NK cells were in the normal range. In contrast, some patients revealed lower percentages of T (16.7%), B (16.7%), and NK (16.7%) cells, probably associated with the use of ISs (Table 2). Treg cell quantification, characterized by the $CD4^{+}CD25^{+}FOXP3^{+}$ cells, revealed a reduction in percentage in only one subject (14.2%), P7, who harbored the R397Q mutation. Detailed immunological evaluations of the newly defined IPEX patients (P5-P12), including T-and B-cell subtypes, are represented in **Table S1**.

Table 2
The immunological and genetic findings with outcomes of IPEX patients

Patient	Total (n,%)	P1	P2	P3	P4	P5	P6	P7	P8
Leukocytosis (mild)	33.3%	-	-	-	-	+	+	-	+
Lymphopenia (mild)	33.3%	+	-	-	+	-	-	-	-
Eosinophilia	33.3%	-	+	+	+	+	-	-	-
Low IgG	41.7%	+	-	-	-	-	-	+	+
Low IgM	58.3%	+	-	-	-	-	+	+	+
Low IgA	50%	+	-	+	-	-	+	-	+
High IgE	75%	-	-	+	+	+	+	+	+
Low $CD3^+$ T cells (%)	16.7%	+	-	-	+	-	-	-	-
Low $CD4^+$ T cells (%)	33.3%	+	-	-	+	-	-	-	+
Low $CD8^+$ T cells (%)	16.7%	+	-	-	+	-	-	-	-
Low $CD19^+$ B cells (%)	16.7%	+	+	-	-	-	-	-	-
Low $CD16^+/56^+$ NK cells (%)	16.7%	+	-	-	-	-	-	-	+
Low $CD4^+CD25^+FOXP3^+$ Tregs (%)	14.2%	-	-	NA	NA	-	-	+	-
Impaired vaccine responses	None	-	-	NA	-	-	-	-	-
Impaired proliferation	33.3%	+	+	-	+	-	-	-	+
Mutation	-	IVS8, c.816 + 5G > A	IVS8, c.816 + 5G > A	IVS8, c.816 + 5G > A	c.751-753del, E251del	c.1117_1118delTTinsGC F373A	IVS8, c.816 + 5G > A	c.1190G > A R397Q	c.598_600del K200del
Anti-microbial prophylaxis	100%	+	+	+	+	+	+	+	+
IgRT	75%	+	+	-	-	+	+	+	+
Systemic immunosuppressants	75%	+	+	+	+	-	+	+	+
HSCT	50%	-	+	+	+	-	+	+	+
Outcome	Alive (58.3%)	Dead	Alive	Dead	Dead	Alive	Alive	Dead	Alive
Reason of death	-	Respiratory failure		Respiratory failure, intracranial hemorrhage	Respiratory failure	-	-	GvHD, sepsis	-

Abbreviations: NA: Not Available; GvHD: Graft versus host disease; HSCT: Haploidentical stem cell transplantation; IgRT: Immunoglobulin replacement thera
NM_014009.4.

Evaluation of T-cell responses and changes with treatment.

Before HSCT, we investigated $CD4^+$ T-cell subtypes in three patients (P5, P7, P9). These included circulating follicular helper T cells (cT_{FH}, $CD4^+CXCR5^+PD\text{-}1^+$ or $CD4^+CXCR5^+CD45RA^-$), Treg cells ($CD4^+CD25^{hi}FOXP3^+$ and $CD4^+CD25^{hi}CD127^{lo}$), circulating follicular regulatory T cells (cT_{FR}, $CD4^+CXCR5^+CD45RA^-CD25^{hi}CD127^{lo}$). The gating strategies of these analyses are presented in **Fig.S1** and **Fig.S2**. The cT_{FH} cell ($CD4^+CXCR5^+PD\text{-}1^+$) percentages were similar between the patients and the healthy controls; however, their PD-1 expression was significantly higher in the patients, indicating their activation status. Interestingly, the PD-1 expression was diminished following HSCT (Fig. 2A-C).

A T_H2 ($CXCR3^-CCR6^-$) skewing and reduced T_H17 ($CXCR3^-CCR6^+$) responses were observed in cT_{FH} ($CD4^+CXCR5^+CD45RA^-$) and Treg ($CD4^+CD25^{hi}CD127^{lo}$) cells without significant differences between the pre-HSCT samples vs. healthy controls. These abnormal responses were corrected after HSCT (Fig. 2D-G).

We further quantified the percentage of natural Treg cells ($CD4^+CD25^{hi}FOXP3^+$) and found comparable results between the patients (P2, P5, P6, P7, P8, P9) and healthy controls. However, the canonical markers of Tregs, including CD25 (IL-2RA), FOXP3, and CTLA-4, were lower in the patients pre-HSCT than in

healthy controls and increased from baseline after successful transplantation (Fig. 3A-C). Furthermore, we also evaluated the cT_{FR} cell population within the cT_{FH} cell. The frequency of these cells before and after HSCT was similar to that of healthy control subjects (Fig. 3D). T-cell activation and proliferation responses were similar in the tested patients to healthy controls (**Fig.S3**).

Genetic variants of IPEX patients.

Twelve patients in the study harbored seven previously reported mutations (33, 36–44). There were missense (n = 4; P5, P7, P9, P10, P11, P12), inframe deletions (n = 2; P4, P8), and splice site (n = 1; P1, P2, P3, P6) mutations (Table 2). Overall, three mutations were located at the FKH domain (P5, P7, P11, P12), one at the N-terminal (P9, P10), one (P8) at the ZF domain, one at the LZ domain (P4), and one (P1, P2, P3, P6) between the LZ and FKH domains, close to the LZ domain. The overall structure of FOXP3 and localization of variants with atypical and classical presentations is depicted in Fig. 4A. The K200del variant results in a 53% loss of FOXP3 protein, while the E251del variant leads to a 43% loss. Deletion-causing variants can significantly reduce protein levels and contribute to folding defects, impacting protein function. The potential effects of missense variants (C169Y, R347H, F373A, and R397Q) on protein structure were investigated based on the predicted unfolding free energy change (31). As shown in **Table S2**, all of the detected missense variants destabilize the FOXP3, supporting the potential pathogenicity of the variants. The C169Y variant can interfere with the physical interaction between the FOXP3 protein and IKZF4 and ZFP90 proteins (Fig. 4A and **B**). Following the C169Y variant, a new intramolecular hydrophobic interaction occurs with the F171 amino acid (Fig. 4C, **Table S3**). The R347H, F373A, and R397Q variants are located in the FKH at the protein's C-terminal, crucial for DNA interaction (Fig. 4B). These variants destabilize the protein structure by leading to the loss of intramolecular interactions (Fig. 4C, **Table S3**) and may disrupt nuclear localization and hinder the formation of the necessary head-to-head dimer structure for DNA binding (45). All the described mutations were conserved among the species (**Fig.S4**).

Interestingly, P1, P2, P3, and P6 were from the same family and had a mutation that caused a defective splice site, leading to exon 8 skipping (33). While P1 showed atypical presentation characterized by colitis and autoimmune hepatitis, P2, P3, and P6 presented with early onset classical phenotype. Mutations associated with IPEX phenotypes (classical or atypical) in our cohort and comparison with previously reported patients with the same mutations displayed divergent presentations (**Table S4**), and the clinical and immunological comparisons between all our variant types and involved domains did not reveal any differences and were not associated with survival. Therefore, we concluded that no strong genotype–phenotype relationship governed the manifestations of IPEX syndrome.

Treatment and disease course during the follow-up of IPEX patients.

All patients received antimicrobial prophylaxis (100%), nine patients (75%) received IS, and six patients (50%) underwent HSCT (Fig. 5A, Table 2, **Table S1**). At the end of the study, seven (58.3%) patients are still surviving. P1, P3, and P4 died due to respiratory failure, and P7 because of CMV sepsis in the 4th month of HSCT. P10 died from intracranial hemorrhage secondary to trauma while receiving only immunoglobulin replacement therapy without IS or HSCT.

Nine patients (75%) received immunoglobulin replacement therapy (IgRT) for 20 months (min-max:3–48 months). The ISs included prednisolone (n = 8, P1, P2, P3, P4, P7, P8, P10, P11), sirolimus (n = 6, P2, P3, P4, P6, P10, P11), azathioprine (n = 5, P1, P7, P8, P10, P11), cyclosporine A (n = 2, P2, P3), mycophenolate mofetil (MMF) (n = 2, P2, P11), infliximab (n = 1, P8), hydroxychloroquine (n = 1, P10), and cyclophosphamide (n = 1, P10) (Fig. 5B, **Table S1**). Three patients were receiving quadruple, 2 patients were receiving triple, and 3 patients were receiving dual ISs to control disease activity (Fig. 5C). Severe side effects of ISs were observed during the follow-up, leading to caseation of therapies (Fig. 5D). In a more comprehensive breakdown, P1 exhibited myeloid aplasia and bicytopenia, P2 presented with Perthes disease and genu valgum, P9 experienced an allergic skin reaction, P10 demonstrated proteinuria, and P11 grappled with severe aphthous stomatitis alongside nasal bleeding. Overall, there was only PR (n = 5, 55%) or NR (n = 4, 45%) with ISs, but 33.3% of the patients showed CR to HSCT (Fig. 5E). The detailed clinical courses and treatment options are provided in the **Supplementary file**; however, essential therapeutic steps are mentioned as follows:

P1 exhibited complex findings, including nephrotic syndrome, autoimmune hepatitis, and enteropathy. Despite receiving azathioprine and prednisolone, there was only a partial response. At age 9, he developed neutropenia, potentially linked to prolonged azathioprine use, partially improved upon discontinuation of the drug and use of high-dose corticosteroids and colony-stimulating factor. Unfortunately, he succumbed to severe respiratory distress at age 9. P2 presented at 6 months with eczematous skin rash, diarrhea, vomiting, and protein-losing enteropathy. Corticosteroids and cyclosporine improved symptoms until HSCT at 8 months. Following a period of stability, he developed ITP at 3 years and responded to prednisolone, but later experienced AIHA attacks treated with sirolimus for 5 years. However, he showed difficulty walking and hip pain; he developed avascular necrosis and was diagnosed with Perthes disease and genu valgum, possibly related to a side effect of sirolimus, leading to drug withdrawal. Subsequently, he started MMF to control his hemolysis. P3, who had neonatal T1DM, dermatitis, and respiratory distress syndrome right after birth, needed ventilatory support and developed AIHA during follow-up. He was treated with surfactant, antibiotics, corticosteroids, and cyclosporine A with no significant improvement. On day 43, sirolimus was initiated, which led to successful weaning off mechanical ventilation five days later. P4, diagnosed with IPEX at 48 months due to early-onset diarrhea without other autoimmunities, showed no response to corticosteroids and sirolimus. P5 had T1DM without other symptoms, waiting for HSCT. P6 received sirolimus for diarrhea and eczematous lesions, resulting in a successful control. P7, who had late-onset CD, showed partial improvement to prednisolone. Therefore, azathioprine was added for 5 months without responsiveness.

P8 presented atopic dermatitis and CD before 12 months of age and was diagnosed with CMV colitis and IBD at 13 months of age. He received ganciclovir, intermittent prednisolone, and azathioprine treatments for approximately 1.5 years. Additionally, he received infliximab treatment once a month, 4 times in total. He did not benefit from any therapy until HSCT. P9 started IgRT but discontinued due to an allergic reaction. P10 was diagnosed with SLE at 11 years and experienced splenomegaly and thrombocytopenia despite azathioprine, hydroxychloroquine, and prednisolone treatments. After IPEX diagnosis, sirolimus was started, which improved lymphoproliferation but led to proteinuria, ultimately resulting in drug discontinuation. He died due to intracranial hemorrhage while awaiting HSCT. P11 was diagnosed with autoimmune hepatitis at the age of 6 and was first treated with systemic corticosteroids and then

sequentially with azathioprine and MMF for approximately 3 years. After diagnosis with IPEX, he started to receive sirolimus. However, within 3 months, his symptoms did not regress. He experienced severe aphthous stomatitis and fatigue, leading to drug discontinuation. His family objects to HSCT, and he is followed in poor condition. P12, a twin of P11, only had asthma and mild atopic dermatitis and never received IS therapy.

There were 6 out of 12 patients who underwent HSCT. The overall survey after transplantation was 50% (Table 2). P2, now 14 years old, was transplanted from his HLA-identical sister at the age of 8 months. He engrafted well and showed an almost full donor cell chimerism 4 weeks after HSCT. He had ITP at the age of 3 years and recurrent AIHA attacks between the age of 4.5-6 years. In the current situation, his last chimerism was 88%, and he has received IgRT and MMF to control his hemolysis. P3 and P4 were transplanted from HLA-identical umbilical cord blood. However, they died early due to the ARDS after the procedure, and P4 chimerism was only 6% and failed to engraft (33). P6 and P8 were successfully transplanted. Their chimerism levels were 100% in the 2nd and 3rd months of HSCT. P6 is doing well within 2 years of transplantation, with 100% chimerism and free of eczema and diarrhea. P8 had received bone marrow stem cells from a 10/10 HLA-compatible sibling donor. His symptoms improved, and he has been followed up without medication for the last 5 years post-HSCT. P7 had received peripheric blood stem cells from an unrelated 10/10 HLA-compatible donor. While chimerism was 99% in the 1st and 2nd months of HSCT, he developed steroid-resistant grade 4 gastrointestinal GvHD. He received tacrolimus, MMF, corticosteroids, and ruxolitinib for 4 months. Extracorporeal photopheresis was applied, and mesenchymal stem cells were given 2 times due to resistant GvHD. The patient developed BK virus hemorrhagic cystitis and CMV reactivation, complicated with pneumonia, resistant to ganciclovir and foscarnet treatments. He showed respiratory failure and died due to CMV sepsis at the age of 10 years, 4 months after HSCT. In total, 3 patients' families (P9, P11, P12) objected to the HSCT. The details of the indication and course of HSCT in IPEX patients are presented in Table 3.

Table 3
Indication and course of hematopoietic stem cell transplantation in IPEX patients

Patient's code	Indication of transplantation	Age of transplantation (years)	Graft source	HLA-match	Conditioning regimens	GvHD prophylaxis	Post-HSCT infections	Post-HSCT complications	GvHD (grade)	Post-HSCT IgRT	I
P2	Uncontrolled CD, eczema	0.8	BM	MSD-10/10	TREO/FLU/ATG	CsA, MTX	-	-	-	-	8
P3	Severe respiratory distress, neonatal DM, eczema, AIHA	0.1	UCBSC	MUD-10/10	BU/FLU/ATG	CsA, MTX	Candida	Intracranial hemorrhage, respiratory failure	-	No	-
P4	Uncontrolled CD	4	UCBSC	MUD-10/10	BU/FLU/ATG	CsA, MTX	CMV	Failed to engraft	-	No	6
P6	Uncontrolled CD, eczema	1.3	PBSC	MUD-10/10	TREO/FLU/Cy	CsA, MTX	-	-	-	No	1
P7	Uncontrolled CD	10	PBSC	MUD-10/10	TREO/FLU/THIO	CsA, MTX	CMV, BK virus	GI GvHD, hemorrhagic cystitis	IV	No	9
P8	Uncontrolled CD, eczema	2.5	BM	MSD-10/10	BU/FLU/Cy	CsA, ATG	-	-	-	No	1

Abbreviations: AIHA: Autoimmune hemolytic anemia; ATG: Anti-thymoglobulin; ARDS: Acute respiratory distress syndrome; BM: Bone marrow; Bu: Busulfan; Cyclosporine A; Cy: cyclophosphamide; DM: Diabetes Mellitus; Flu: Fludarabine; GvHD: Graft versus host disease; ITP: Immune thrombocytopenia; MUD: Mat sibling donor; MTX: Methotrexate; THIO: Thiotepa; UCBSC: Umbilical cord blood stem cell; PBSC: Peripheral blood stem cell.

There were no significant differences in the probability of OS between patients who received or did not receive transplantation. However, the estimated OS post-HSCT exhibited a favorable trend compared to patients treated with ISs, as depicted in **Fig.S5A** and **B**. Furthermore, individuals with autoimmunities, CD, FTT, LRTI, and skin manifestations demonstrated a better probability of OS. However, statistical significance was not achieved, likely attributed to the limited number of patients in these subgroups (**Fig.S5C** and **D**, **FigS6A-C**).

Discussion

This report presents the clinical manifestations, genetic, and laboratory characteristics of 12 IPEX patients from seven unrelated families. Most IPEX patients showed incomplete triad and atypical phenotypes. Our cohort revealed that IPEX patients can present with manifestations characterized by broad autoimmune features, increased infection rates, high IgE levels, and eosinophilia, but usually with normal lymphocyte subset distribution. These findings support the blended phenotype of the disease, which should be involved in the differential diagnosis of IPEX-like disorders (2). Our study provides long-term follow-up results by comparing typical and atypical cases and unveils the detailed consequences of different therapeutic modalities in IPEX syndrome.

We found autoimmune manifestations, predominantly with GI involvement, similar to reported patients, while the rate of T1DM was relatively lower in our patients compared to the literature (1, 9, 20, 21, 46). Other autoimmune manifestations in our patients included AIHA, ITP, and autoimmune hepatitis, demonstrating that autoimmune beyond T1DM should also be reminded of IPEX disease. Interestingly, in late-onset IPEX form, enteropathy and nephropathy can be the first presenting symptoms (20, 47, 48). This pattern of presentation was also observed in our atypical patients (P1, P7, and P9). On the other hand,

in our patients, the RI rate was 58.3%, and the frequency of lower RTI was relatively high (50%) when compared to the reported patients (46). Additionally, we observed CMV (33.3%) and Candida (50%) infections frequently. These distributions were similar to other previously identified organisms (9, 13, 14, 46). Overall, the severity of RIs and CD symptoms in our cohort revealed the essence of these findings in facilitating early diagnosis.

Intronic *FOXP3* mutations generally presented with the classical triad and survived successfully after HSCT (9, 13). In our cohort, P2 and P6 received transplantation, and P6 showed complete disease control, but P2 experienced recurrent AIHA after HSCT. Although IPEX is associated with both missense and nonsense mutations of *FOXP3*, most patients had the former group of mutations, affecting all domains with the predominance of the FKH DNA binding side (9). Mutations in the FKH and LZ domains in mouse models and humans have been associated with severe clinical manifestations (13, 43). However, some patients with atypical and mild clinical course have reported mutations on FKH (37, 42, 48–50). Generally, IPEX disease reveals heterogeneous presentations beyond the actual mutations, which could combine other genetic and environmental perturbations and intra-familial variations (9, 15, 35). Therefore, clinical symptoms and severity vary between patients with the same affected domain of the *FOXP3* gene and even within the identical mutation, as demonstrated in our cohort in P1-P4, belong to the same family, and between the P11-P12 siblings.

In this study, four patients had mutations on the FKH domain (P5, P7, P11, and P12). P5 (F373A) had only T1DM and high IgE levels. Interestingly, this mutation was previously reported to cause severe enteropathy, eczema, and T1DM, requiring an early HSCT with a successful outcome (43). The mutation of R397Q in P7 is generally associated with an early severe presentation and needed HSCT (43). However, similar to our P7, one case was reported as late-onset (72 months), presented with first symptoms as IBD, T1DM, AIHA, and FTT, controlled by HSCT (37). But, our P7 died due to severe GvHD after HSCT. Overall, patients with F373A and R397Q were usually presented with classical IPEX, and HSCT seems to be the best treatment option (9, 48–50).

The other two patients (P11, P12) with the missense mutation (R347H) on FKH showed late-onset and atypical symptoms. Most reported patients with R347H mutations were presented atypically in the literature (42, 48, 50, 51). An identical mutation was reported, having controlled T1DM and alive without further complaints (51). The symptoms in R347H can be controlled only with IS treatment, as in the literature, one patient presented with recurrent ear infections, severe chronic gastritis, hyper-IgE, FTT, and another patient with hyperglycemia, diarrhea, and hepatitis received only IS treatment. They were alive at 10 and 19 years, respectively (43).

LZ domain mutations are generally associated with poor prognosis, like FKH domain mutations (13, 52). In the French cohort, patients with E251del mutations showed age at onset as 4 and 6 weeks, respectively. One of them demonstrated agranulocytosis and hepatitis, treated only with IS treatment, and died at 8 months due to the *pseudomonas* infection. The other one presented with hypothyroidism, interstitial nephritis, and AIHA and underwent HSCT, and he was alive at the age of 17 years (20). However, HSCT may not always be curative for these patients, as we presented in our P4, who suffered from severe protein-losing enteropathy without autoimmune manifestations and died due to engraftment failure and ARDS after HLA-identical HSCT (33).

The C169Y variant is located in the repressor domain, and functional studies revealed that this variant causes decreased FOXP3 expression with defective Treg suppression and decreased DNA binding capacity (34, 35). Our detailed analysis also revealed reduced Treg markers for this variant. Both of our patients demonstrated atypical manifestations characterized by FTT, late-onset enteropathy, autoimmune gastritis, and SLE. Intriguingly, while one patient (P9) showed mild phenotype and followed without treatment, the other (P10) required multiple ISs to control disease activity. Mutations in the repressor domain can show atypical presentation and are linked to increased RIs and autoimmune hepatitis compared to other domain mutations (1, 21).

HSCT has been linked to an increased survival rate in individuals with IPEX syndrome. The essential factor determining the outcome is the extent of organ impairment at the time of HSCT, surpassing the influence of age, donor source, or conditioning regimen (9). Therefore, optimal outcomes appear to be associated with early HSCT, particularly when implemented prior to the onset of significant organ damage (20). We detected complete disease control only after HSCT compared to patients with ISs who showed partial or no responses. The estimated OS after HSCT (50%) was higher than the reported largest cohorts (29.5% and 42%) (20, 21); however, lower than others (73.2% and 72.8%) (1, 9). It is worth mentioning that HSCT in IPEX generally revealed a lower success rate with further disease relapse, especially in case of inadequate donor-derived Treg cell reconstitution (7). This data would support the need for a higher donor chimerism for better disease control than other IEI diseases, such as severe combined immunodeficiencies, where 5–10% donor chimerism would be enough to control the disease manifestations (53).

While the HSCT is more affordable for the classical form of disease, treatment of atypical cases with single-organ involvement or late-onset disease is still ambiguous, and there have been many reported patients on ISs for long-term periods (14, 48). However, the prolonged use of ISs has significant adverse side effects, and generally, ISs do not prevent disease progression, as only 29% of patients can show complete remission (7, 9). Additionally, the side effects of ISs negatively impact survival (9). Similarly, in our cohort, we observed severe adverse effects regarding used ISs, delignating the potentially harmful consequences of these types of therapies in IPEX syndrome. Recent studies have focused on the reprogramming function of *FOXP3* mutation on Treg cells and the capability to reverse their function using dual mTOR inhibitors (34). Additionally, gene therapy in IPEX disease can provide favorable outcomes in the near future when compared to insufficient results of HSCT or ISs (7, 54).

Despite the normal percentage of Treg cells in most IPEX patients (except P7), key canonical Treg markers such as CD25 (IL2RA), FOXP3, and CTLA-4 were found to be decreased, supporting ineffective Treg cells functioning (6, 55, 56). It should be noted that FOXP3 expression in Treg cells may not always serve as a definitive diagnostic marker, though significant loss in its expression could be associated with disease severity, as reported in patients with in-frame, frameshift deletions, or polyadenylation site mutations (1, 15, 43, 57). However, this relation was not observed in our tested patients. In our study, following HSCT, the expression of these canonical markers was corrected, suggesting a therapeutic effect on pre-HSCT Treg cells. Our study revealed elevated PD-1 levels in cT_{FH} cells, signifying their activation, probably due to defective Treg suppression (55). Furthermore, our patients exhibited an elevated T_H2-like deviation in cT_{FH} and Treg cells, normalized after transplantation, supporting the enhanced T_H2 polarity within Tregs (15, 58, 59). Overall, the skewed T_H2 cell responses contribute to clinical manifestations such as eczema, infections, and autoimmunity (8, 60).

In conclusion, we describe detailed clinical findings and abnormal immune responses in IPEX patients. Our results indicate that appropriate IS therapies may be associated with survival outcomes similar to HSCT, especially in patients with mild phenotypes. However, ISs bring together other potential side effects impacting patient outcomes. Conversely, only HSCT can perpetuate complete control and should be implemented in patients with severe phenotypes or who display resistance to ISs. Awareness of atypical forms of IPEX would yield earlier diagnostic capability and thus provide more favorable outcomes.

Declarations

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Figures

Figure 1

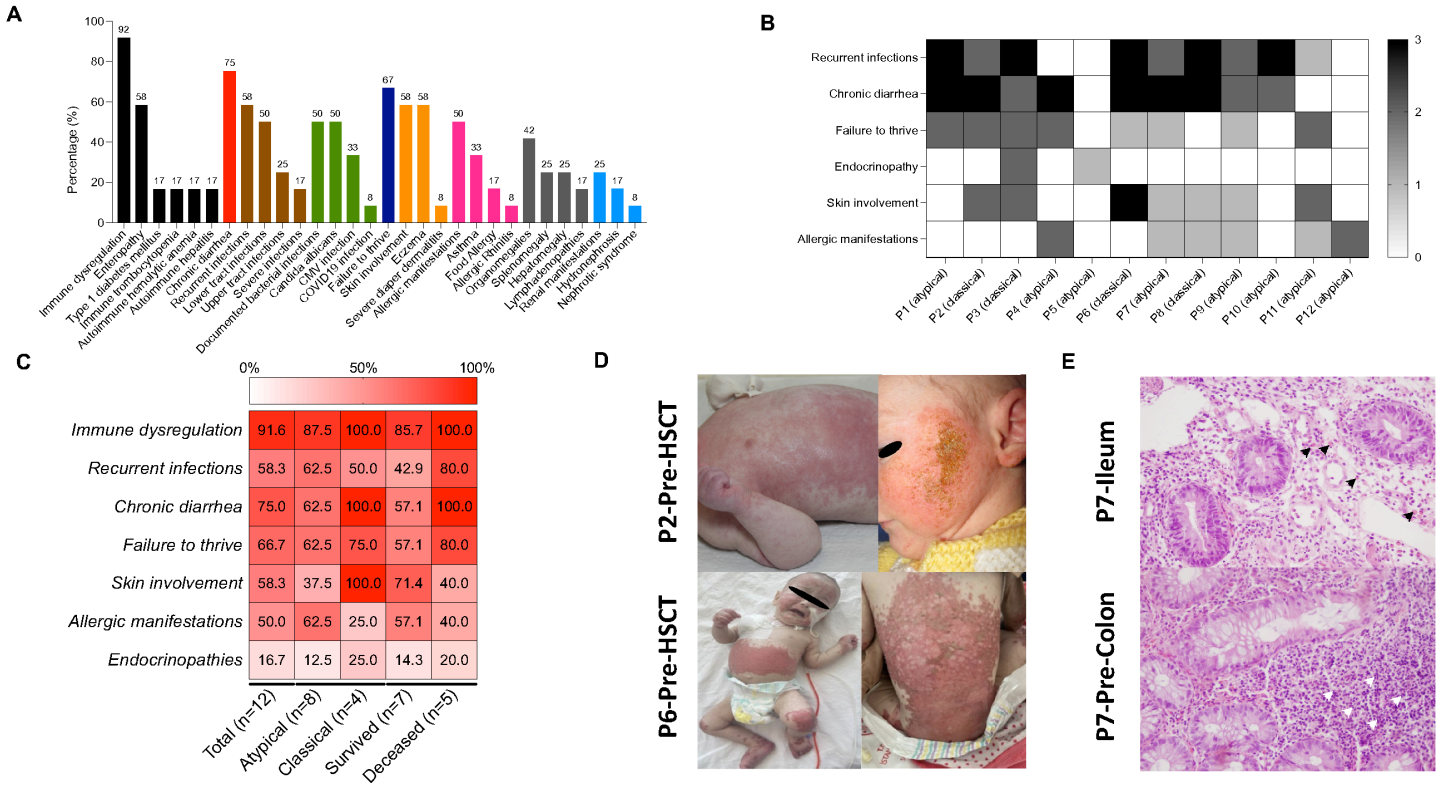

Figure 1

Clinical findings and outcome of IPEX patients. **(A)** Clinical features of IPEX patients. **(B)**Severity of clinical features of IPEX patients. Manifestations are evaluated on a scale ranging from 0 to 3. A score of 0 signifies the absence of symptoms, 1 indicates mild severity, 2 denotes moderate severity, and a score of 3 corresponds to severe manifestations. **(C)** A heatmap display of key clinical features, in comparison to the type of presentation (atypical vs. classical) and the disease outcome (survived vs. deceased), with the numbers in each cell indicating the percentage of each feature in a given column. **(D)**Skin findings depicting erythroderma (top left and bottom left), severe eczema (top right), and impetigo-like skin infection (bottom right). **(E)** histopathological sections from the ileum and descending colon of P7, showing numerous eosinophils (black arrows) and polymorphonuclear cells (white arrows), aligning with chronic inflammation and cryptitis, respectively. Hematoxylin & Eosin, original magnification: X400.

Figure 2

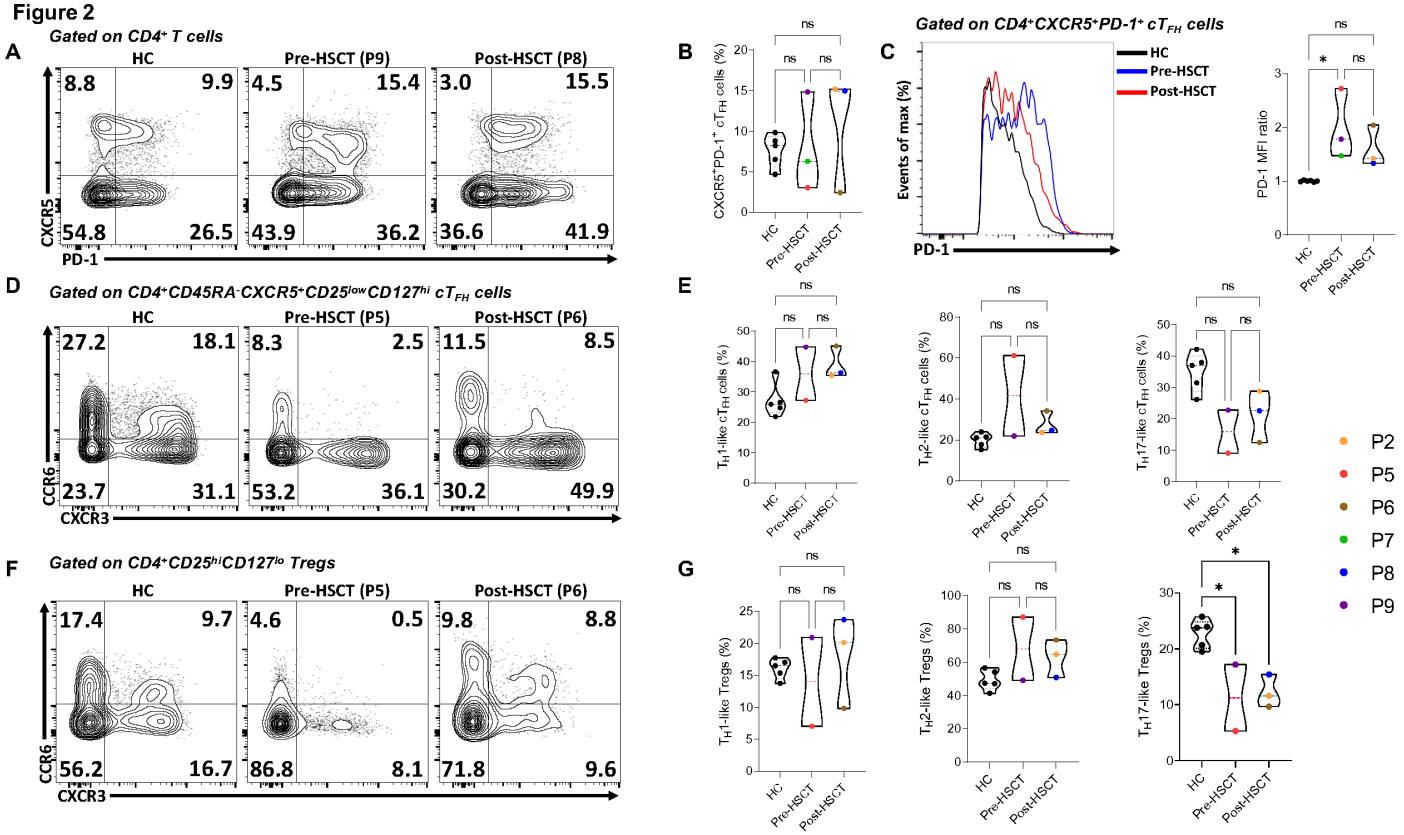

Figure 2

Abnormal T-cell subtype responses in IPEX patients. **(A)** Representative plots of $CXCR5^{+}PD\text{-}1^{+}$ cT_{FH} cells. **(B)** The percentages of subtypes of the cT_{FH} cells compared to healthy controls. **(C)** Mean fluorescence intensity of PD-1 expression in the patients' and healthy controls' $CD4^{+}CXCR5^{+}PD\text{-}1^{+}$ cT_{FH} cells (Black line: Healthy control, Blue line: Pre-HSCT, Red line: Post-HSCT). **(D)** Representative plots of subtypes of the cT_{FH} cells. **(E)** The percentages of subtypes of the cT_{FH} cells compared to healthy controls. **(F)** Representative plots and **(G)** percentages of subtypes of Treg cells in the patients compared to healthy controls. Pre-HSCT: pre-hematopoietic stem cell transplantation, Post-HSCT: post-hematopoietic stem cell transplantation, HC: healthy controls. *p<.05, ns: non-significant. Kruskal-Wallis with Dunn's post-test.

Figure 3

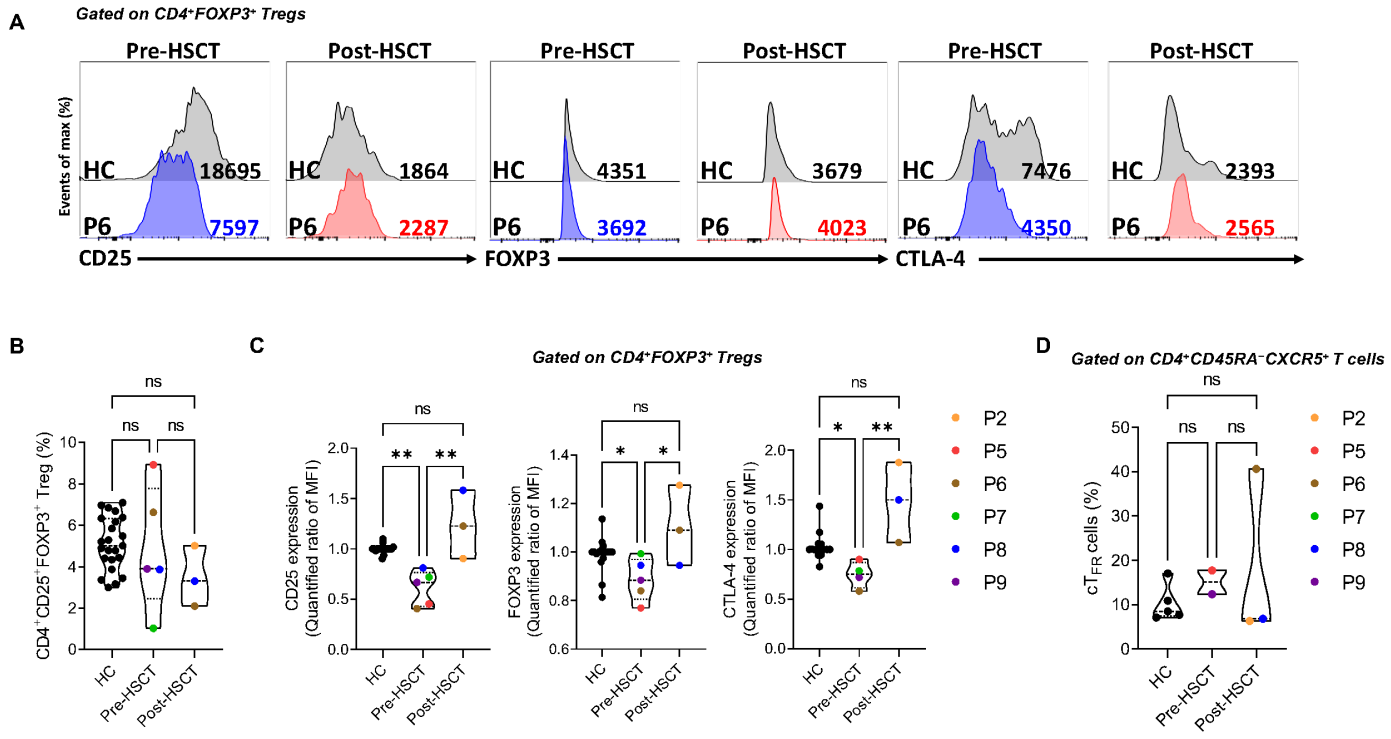

Figure 3

Assessment of Treg markers in IPEX patients before and after HSCT. **(A)** Representative histograms of FOXP3, CD25, and CTLA-4 expressions in the P6 and a healthy control, gated on $CD4^+FOXP3^+$ Tregs (Grey filled: healthy control, Blue filled: pre-HSCT, Red filled: post-HSCT). **(B)** Percentages of $CD4^+CD25^+FOXP3^+$ Tregs in the patients and healthy controls. **(C)** Quantified mean fluorescence intensity ratio according to healthy controls of FOXP3, CD25, and CTLA-4 expressions in patients' $CD4^+FOXP3^+$ Tregs. **(D)** Percentages of cT_{FR} cells in the patients and healthy controls. Pre-HSCT: pre-hematopoietic stem cell transplantation, Post-HSCT: post-hematopoietic stem cell transplantation HC: healthy controls, MFI: mean Fluorescence Intensity, Treg: regulatory T cells, cT_{FR}: circulating T follicular regulatory cell. **p<.01, *p<.05, ns: non-significant. Kruskal-Wallis with Dunn's post-test.

Figure 4

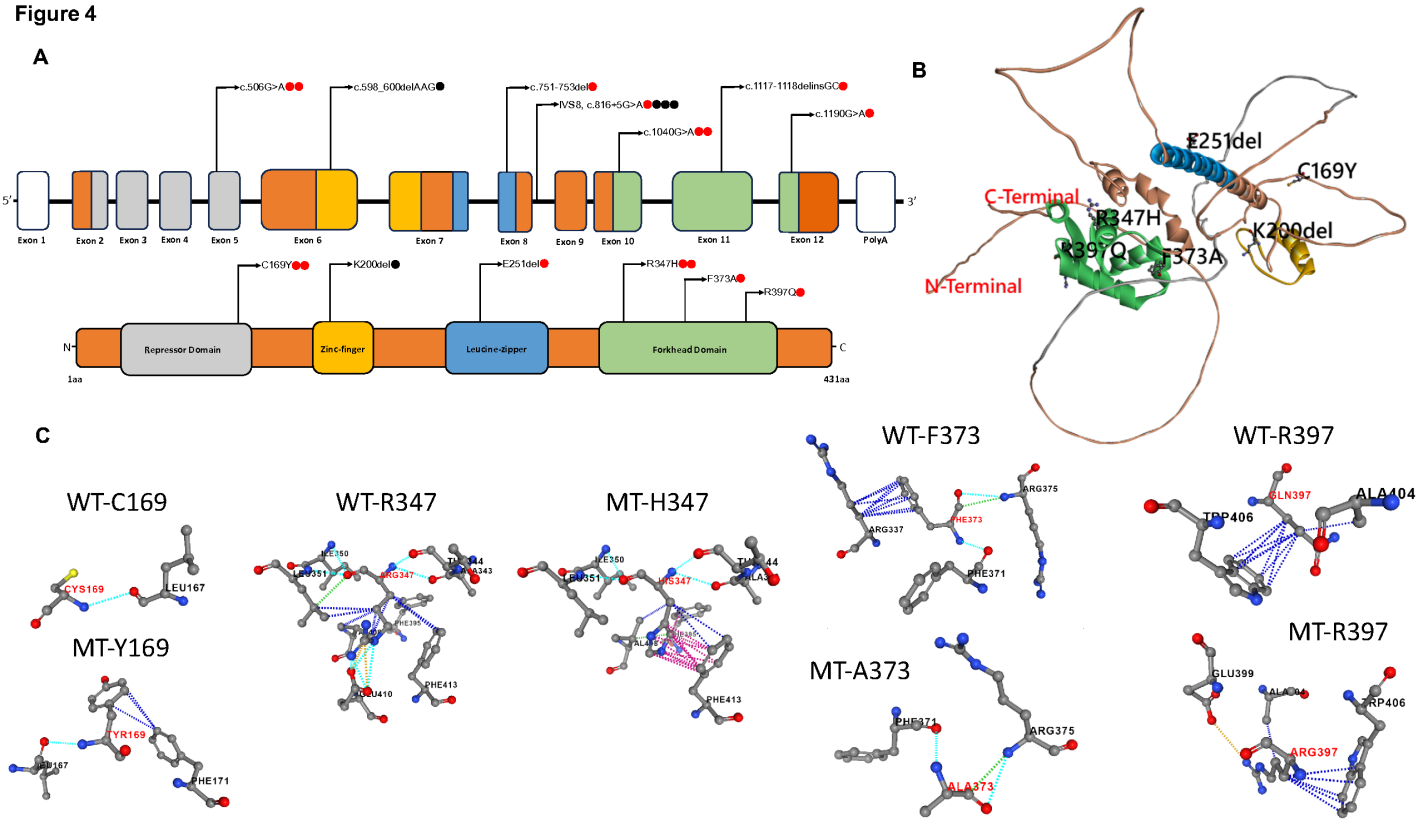

Figure 4

FOXP3 mutations in the IPEX patients described in this cohort and in previous reports. **(A)** Depiction of the structure of the *FOXP3*gene, highlighting the locations of reported mutations relative to the gene's exons and functional domains. Red dots: atypical patients, Black dots: classical patients. **(B)** The positions of amino acid changes caused by variants in the FOXP3 protein were obtained from the AlphaFold Protein Structure Database (AF- Q9BZS1-F1). R347H, F373A, R397Q, K200del, and E251del variants are localized on alpha helices, while the C169Y variant is localized on the coiled-coil region. Orange regions indicate linker domains, grey regions represent repressor domains, yellow areas denote zinc finger domains, blue regions signify leucine zipper domains, and green regions correspond to forkhead domains. **(C)** Representation of intramolecular interactions resulting from amino acid alterations arising from variants in the *FOXP3* gene, comparing wild-type (WT) and mutated (MT) configurations. The classification of interactions based on colors is as follows: Blue: hydrophobic interactions, Orange: carbonyl interactions, Plum: clashes, Cyan: polar interactions, Yellow: ionic interactions, Green: van der Waals interactions, Violet: aromatic interactions, and Purple: hydrogen bonds.

Figure 5

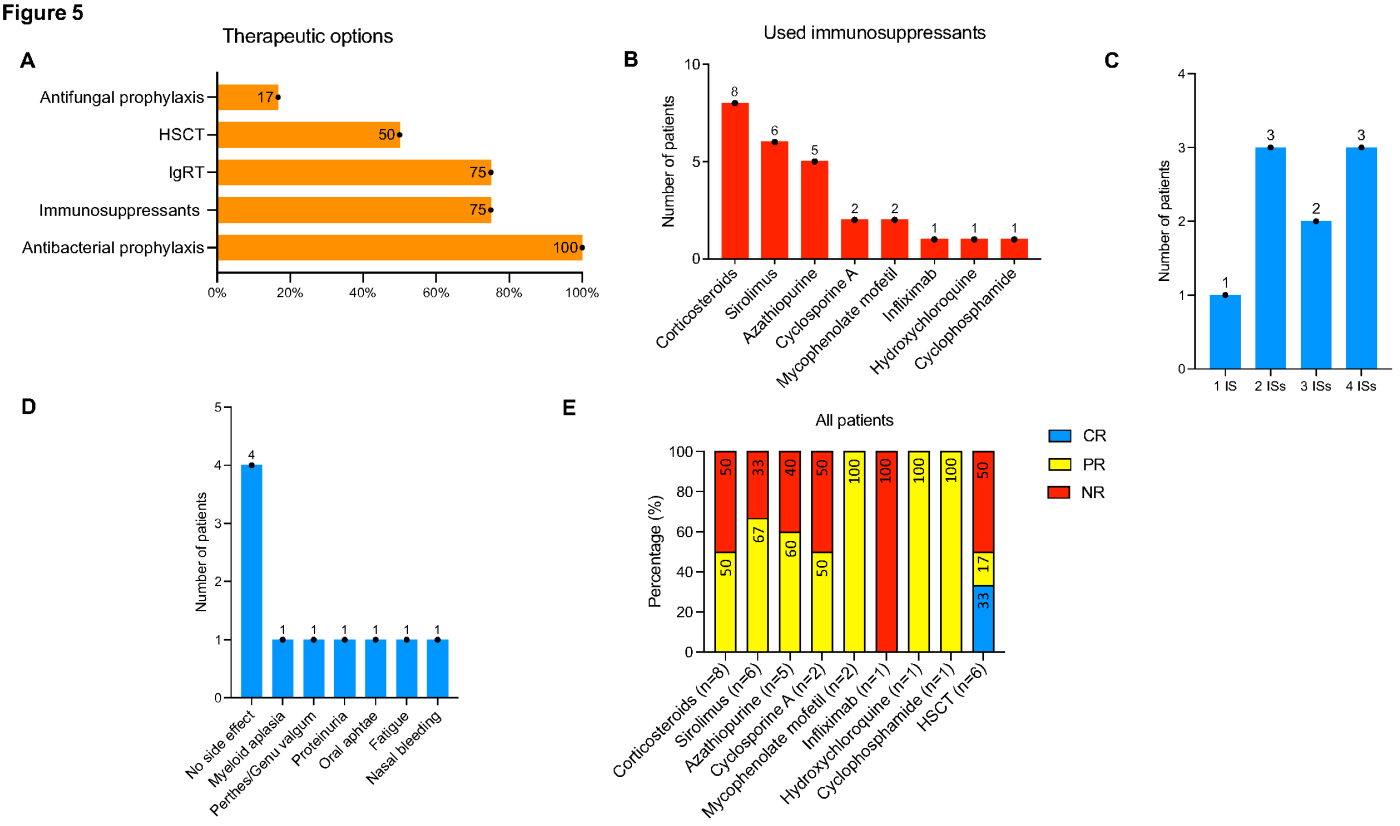

Figure 5

Therapeutic modalities, adverse events, and response rates in IPEX patients. **(A)** Broad classes of therapeutics received by IPEX patients. **(B)** Immunosuppressive medications used in IPEX patients to control various autoimmune manifestations. **(C)** Distribution of patients receiving one or more immunosuppressive agents. **(D)** Distribution of adverse events attributed to immunosuppressive medication use. **(E)** Physician-rated control levels of patients in response to different therapeutics. IS: immunosuppressive, CR: complete response, PR: partial response, NR: nonresponsive.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.pdf
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- FigureS3.pdf
- FigureS4.pdf
- FigureS5.pdf
- FigureS6.pdf
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