Supplementary file 1

Table A — Clinical characteristics of the six patients (P1–P6) in the CNE3i-CoBEII IPEX cohort, organized into infections, autoimmune manifestations, allergic manifestations, and outcome. Presence of each feature is indicated by "x" and absence by "–"; the Total row shows the number of patients affected (n). ART, arthritis; ENTP, enteropathy; TBP, thrombocytopenia; HA, haemolytic anaemia; T1D, type 1 diabetes; ALFW, alive at last follow-up.

	Infections	**Autoimmunity**						*Allergy*					Outcome
	Sinusitis	*ART*	*ENTP*	*Vitiligo*	*TBP*	*HA*	*T1D*	*Rhinitis*	*Conjunctivitis*	*Asthma*	*Eczema*	*Food Allergy*	ALFW
P1	x	x	x	-	x	-	-	x	x	x	x	-	*x*
P2	x	-	x	-	-	-	x	x	-	-	x	x	-
P3	x	x	x	x	-	x	-	x	-	x	x	-	x
P4	x	-	-	-	-	-	x	x	x	x	x	x	x
P5	-	-	x	-	-	-	-	x	-	-	x	x	-
P6	-	x	x	-	-	x	-	x	-	x	-	-	x
Total	n=4	n=3	n=5	n=1	n=1	n=2	n=2	n=6	n=2	n=4	n=5	n=3	n=4

Table B — Molecular characterization of FOXP3 variants in the six patients of the CNE3i-CoBEII IPEX cohort. FOXP3 variants in the CNE3i-CoBEII IPEX cohort segregate into two mechanistic classes. showing the geographic origin in Brazil (GLB), coding DNA change (c.DNA), exonic location, predicted protein-level effect, and the affected functional domain of FOXP3. GLB, geographic localization in Brazil; FKH, forkhead domain; ZFM, zinc-finger motif; PRD, proline-rich domain

	GLB	c.DNA	Exonic location	Protein	FOXP3 domain
P1	MG	c.1150G>A	Exon 11	p.Ala384Thr	FKH
P2	GO	c.1117_1118delinsGC	Exon 11	p.Phe373Ala	FKH
P3	SP	c.1150G>A	Exon 11	p.Ala384Thr	FKH
P4	PE	c.434C>T	Exon 4	p.Ala145Val	PRD
P5	DF	c.1139C>T	Exon 11	p.Thr380Ile	FKH
P6	ES	c.61G>C	Exon 2	p.Gly21Arg	PRD

Table C — Serum cytokine concentrations (pg/mL) measured by Cytometric Bead Array (CBA) in sirolimus-treated CNE3i-CoBEII IPEX patients with active disease (P1–P6) and in controls (CTRL1–CTRL10). Values are shown for each of the seven cytokines assayed; dashes indicate samples not available (P4, P6). P, patient; CTRL, control.

	IL-17A	IFN-γ	TNF-α	IL-10	IL-6	IL-4	IL-2
P1	18.8	3.6	3.7	4.4	2.3	4.8	2.5
P2	18.8	3.6	3.7	4.4	2.67	4.8	2.5
P3	34.05	3.6	3.7	4.4	2.3	4.8	2.5
P4	-	-	-	-	-	-	-
P5	23.50	3.6	3.7	4.77	7.73	4.8	2.5
P6	-	-	-	-	-	-	-
CTRL1	18,9	3,6	3,8	4,5	2,4	4,8	2,5
CTRL2	18,9	3,6	3,8	4,5	2,4	4,8	2,5
CTRL3	18,9	3,6	3,8	4,5	2,4	4,8	2,5
CTRL4	18,9	3,6	3,8	4,5	3,79	4,8	2,5
CTRL5	18,9	3,6	3,8	4,5	2,4	4,8	2,5
CTRL6	18,9	3,6	3,8	4,5	2,4	4,8	2,5
CTRL7	18,9	3,6	3,8	4,5	2,54	4,8	2,5
CTRL8	18,9	3,6	3,8	4,5	2,4	4,8	2,5
CTRL9	18,9	3,6	3,8	4,5	3,07	4,8	2,5
CTRL10	18,9	3,6	3,8	4,5	2,4	4,8	2,5

Table D — Baseline serum immunoglobulin concentrations and double-negative T-cell frequencies in the six patients of the CNE3i-CoBEII IPEX cohort. Immunoglobulins are reported as IgG, IgA, and IgM (mg/dL) and IgE (IU/mL); αβ double-negative ($CD3^+CD4^-CD8^-$) T cells are expressed both as a percentage of $CD3^+$ T cells and as a percentage of total lymphocytes. Values marked "high" exceeded the age-matched reference range. NA, not available. IgG/IgA/IgM/IgE, immunoglobulin G/A/M/E.

	Baseline IgG	Baseline IgA	Baseline IgM	Baseline IgE	CD3 Lymphocyte Double Negative T cells	Total Lymphocyte Double Negative T cells
P1	1057	67,1	82,5	4,9	1,20%	1,38%
P2	1048	85,2	108	13704	1,71% (high)	3% (high)
P3	3334	5	189	15,7	1,62% (high)	1,98%
P4	414	18,2	16,2	3094	1,20%	1,60%
P5	75	<10	140	<1	NA	NA
P6	944	56	104	15,7	0,90%	1,30%

Table E — Therapeutic interventions and outcomes in the six patients of the CNE3i-CoBEII IPEX cohort, showing treatment with sirolimus, additional immunobiologic agents and their main indication, use of bone marrow transplantation (BMT) and gene therapy (GT), and vital status (death). Drugs: ADMBB, adalimumab; ABTCPT, abatacept; INFX, infliximab; USTK, ustekinumab. Indications: JIA, juvenile idiopathic arthritis; IBD, inflammatory bowel disease. NA, not applicable.

	Sirolimus	Other Immunobiologics	Main Reason of changing	Main Indication of Biologics	BMT	GT	Death
P1	Yes	NA	NA	NA	yes	no	no
P2	Yes	NA	NA	NA	yes	no	yes
P3	Yes	ADMBB (JIA)	not changed - disease controlled	JIA	no	yes	no
P4	Yes	NA	NA	NA	yes	no	no
P5	Yes	INFX; ABTCPT; ADMBB	severe inflammatory bowel disease with gastrointestinal bleeding	IBD	yes	no	yes
P6	Yes	USTK ; INFX; ABTCPT	severe inflammatory bowel disease with gastrointestinal bleeding	IBD	no	yes	no

Table F — Regulatory T-cell frequency and FOXP3 expression in the CNE3i-CoBEll IPEX cohort. Cryopreserved PBMCs (isolated by Ficoll-Hypaque density-gradient centrifugation) were thawed and surface-stained with anti-CD3, anti-CD4, anti-CD127, and anti-CD25 monoclonal antibodies, with viability assessed by Fixable Viability Stain 780; cells were then fixed, permeabilized, and stained intracellularly for FOXP3, with fluorescence-minus-one (FMO) controls used to set $FOXP3^+$ gates. Regulatory T cells were defined by sequential gating on viable $CD3^+CD4^+CD25^+CD127^-FOXP3^+$ events (FACSCanto II; FlowJo v10). For each patient, the table shows the frequency of $CD4^+CD25^+CD127^-$ cells (%), the proportion of $FOXP3^+$ cells within this gate (%), and intracellular FOXP3 mean fluorescence intensity (MFI, arbitrary units).

	%Céls CD127-/ CD4+/CD25+	% Céls FOXP3+	MFI FOXP3
P1	4,46	71,6	1171
P2	7,14	79,8	1073
P3	6,84	72,9	814
P4	6,49	15,2	827
P5	4,79	10,8	1033
P6	5,73	70,1	1290

Figure A - **Intrinsic disorder profile of human FOXP3.** Per-residue intrinsic disorder propensity was predicted using IUPred2A with the human FOXP3 protein sequence. The horizontal line at a score of 0.5 represents the threshold for predicted intrinsic disorder. The thin vertical lines indicate the positions **of Gly21 and Ala145**, corresponding to the variants p.Gly21Arg and p.Ala145Val, respectively. Both residues are located within the predicted intrinsically disordered N-terminal region of FOXP3.