LE NUOVE IMMUNODEFICIENZE: AI CONFINI DELL'ATOPIA

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SIAAIC, 8 aprile 2017
Firenze

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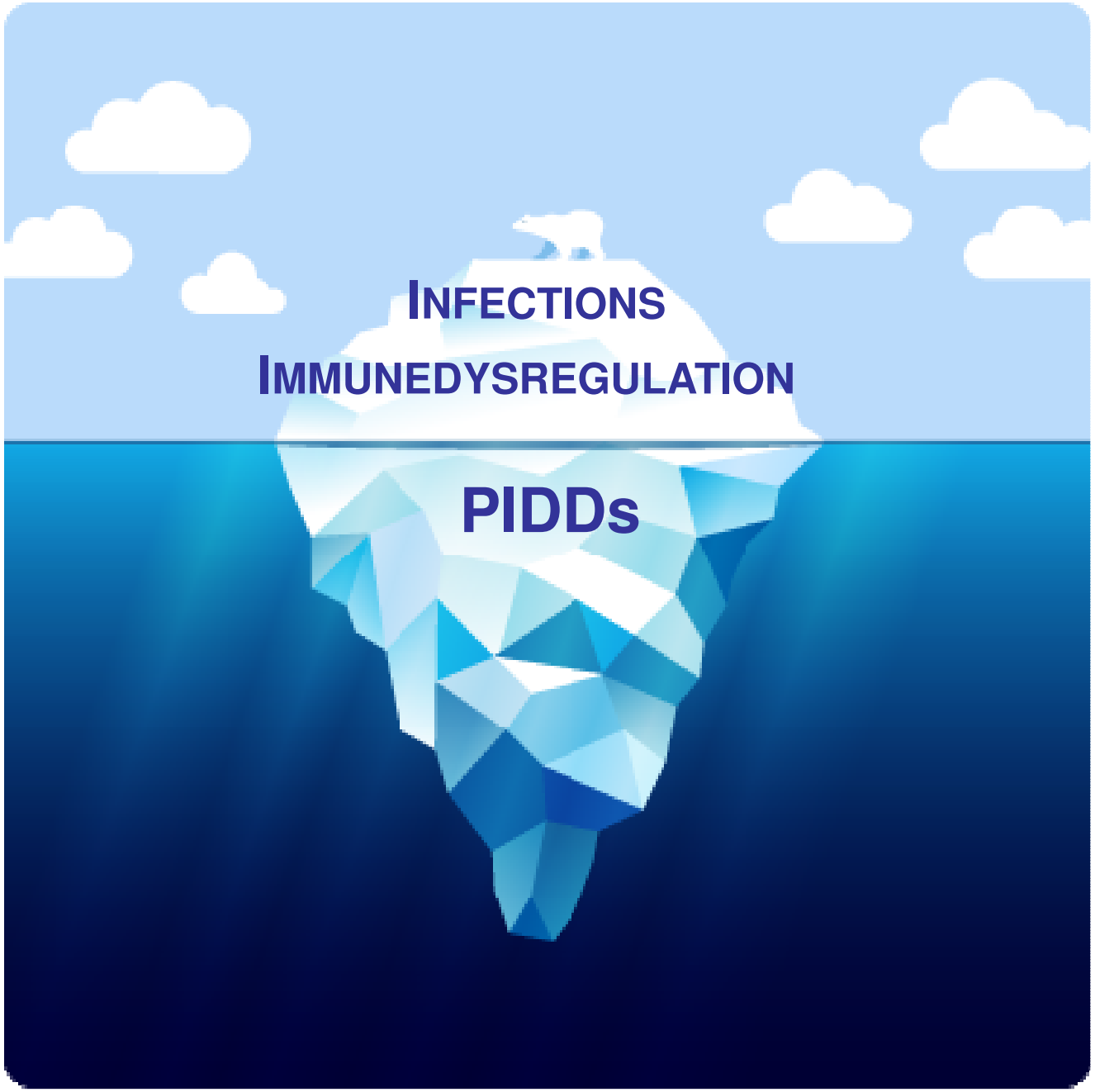
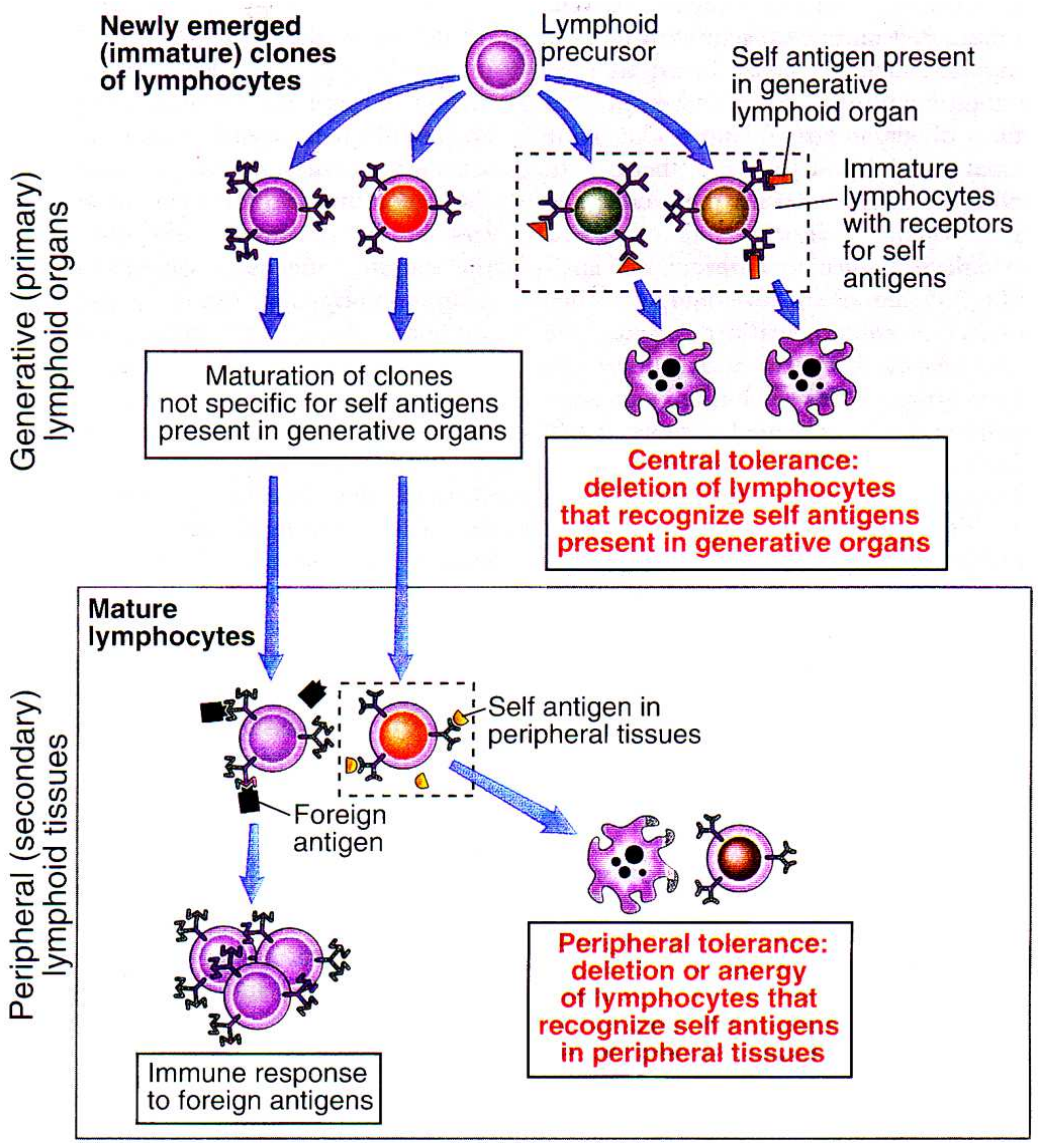

INFECTIONS
IMMUNEDYSREGULATION
PIDDs

CENTRAL AND PERIPHERAL TOLERANCE

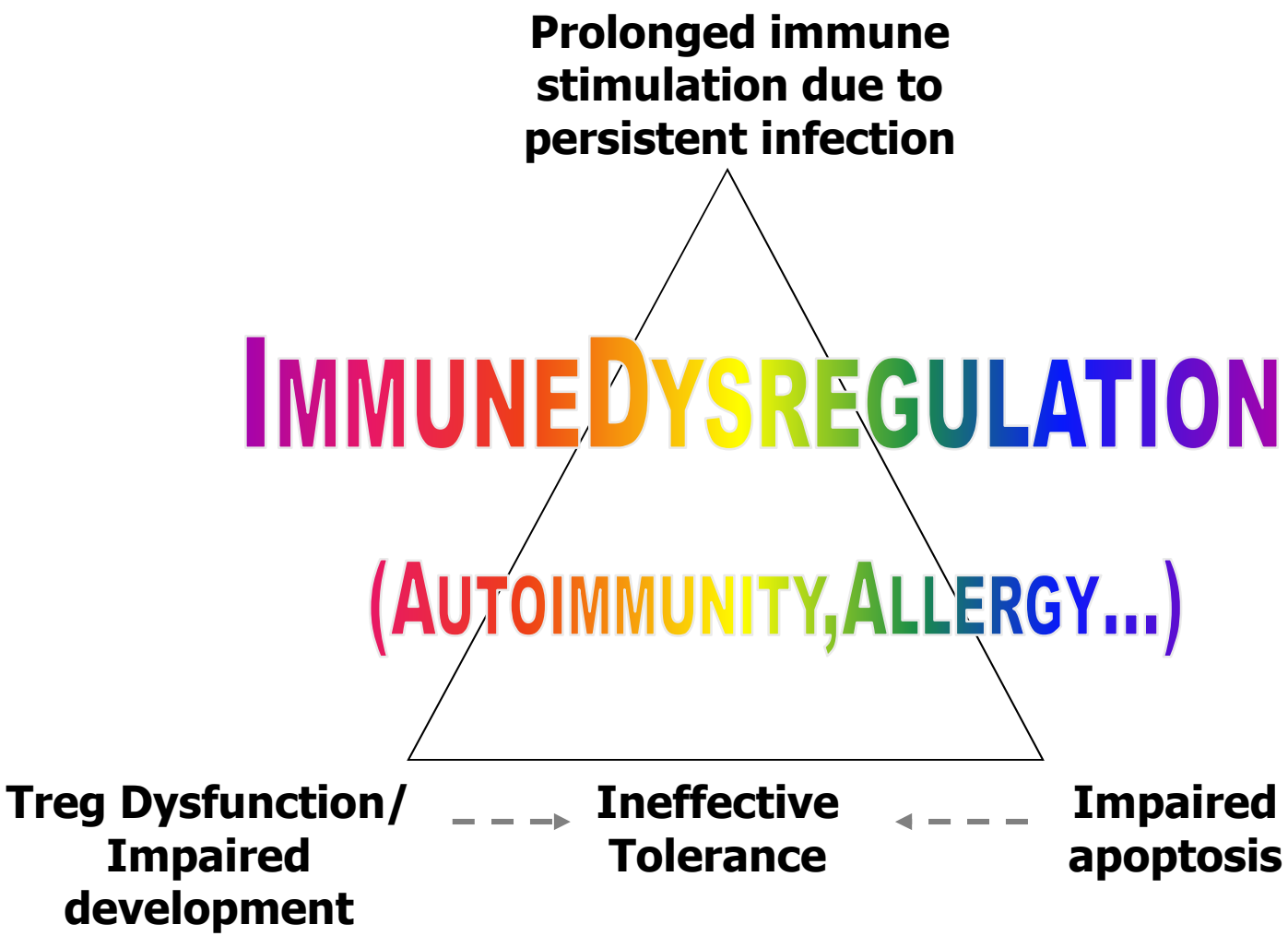


Central tolerance

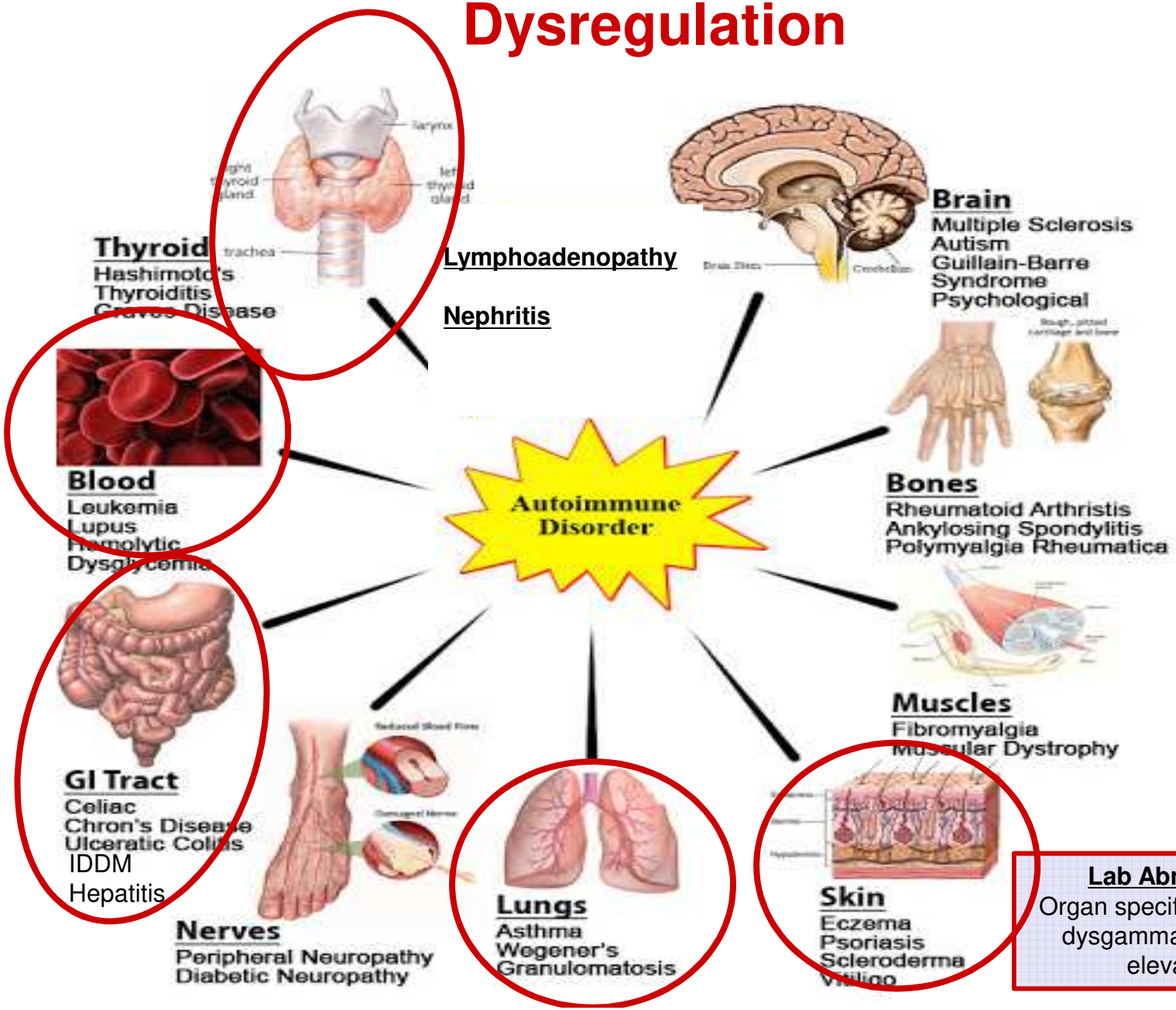
occurs during differentiation on immature B and T cells in primary lymphoid organs

Peripheral tolerance

occurs on mature B and T cells in secondary lymphoid organs


Prolonged immune stimulation due to persistent infection
IMMUNEDYSREGULATION
(AUTOIMMUNITY,ALLERGY...)
Treg Dysfunction/ Impaired development
Ineffective Tolerance
Impaired apoptosis

Clinical Features of Congenital Immune Dysregulation



Lab Abnormalities
Organ specific autoAb, DCT, dysgammaglobulinemia, elevated IgE

Prevalence of Food Allergy and AD among patients with PIDDs (Tuano et al. JACI 2015)

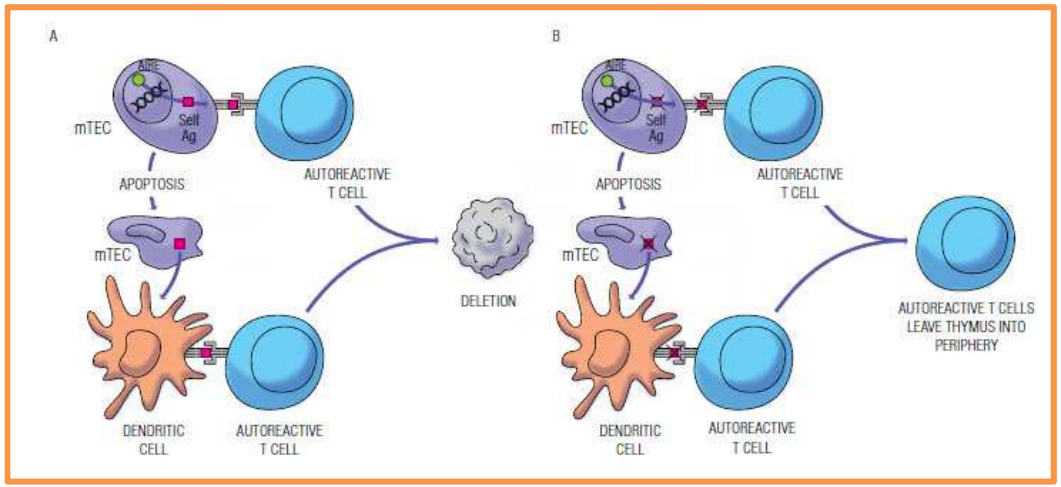
PIDDs (n = 2263)	Age (y), mean/median (range)	FA (n = 40)	AD (n = 136)
Agammaglobulinemia (n = 339)	37.5/42 (18-42)	0.6% (2)	1.5% (5)
X-linked (n = 332)		0.3% (1)	1.5% (5)
Unknown genetic cause (n = 7)		14.29% (1)	NR
CD40 ligand deficiency (n = 13)	NR	7.7% (1)	NR
Chronic granulomatous disease, X-linked (n = 283)	16.5/16.5 (13-20)	NR	0.7% (2)
CID (n = 3)	15	33.3% (1)	33.3% (1)
CSR defects and HIGM syndromes, unknown genetic cause (n = 112)	32	NR	0.9% (1)
CVID (n = 773)	36.6/33 (10-82)	3.1% (24)	4.4% (34)
DiGeorge syndrome (n = 362)	6.7/7 (6-7)	0.3% (1)	0.6% (2)
Chromosome 22q11.2 deletion (n = 314)		NR	0.3% (1)
Unknown genetic cause (n = 48)		2.1% (1)	2.1% (1)
HIES (n = 16)	22/18.5 (14-37)	6.3% (1)	25% (4)
STAT3 (n = 5)		20% (1)	60% (3)
Unknown genetic cause (n = 11)		NR	9.1% (1)
Selective IgA deficiency (n = 4)	8	25% (1)	NR
Other hypogammaglobulinemias (n = 28)	52/63.5 (10-71)	7.1% (2)	7.1% (2)
NEMO deficiency (n = 8)	11.2/10 (7-18)	NR	62.5% (5)
SCID, undefined (n = 131)	17	NR	0.8% (1)
Selective IgM deficiency (n = 3)	53	NR	33.3% (1)
WAS (n = 188)	31.7/30.5 (6-62)	3.7% (7)	41.5% (78)
Mutations in WASP (n = 14)		21.4% (3)	7.1% (1)
Unknown genetic cause (n = 173)		1.7% (3)	44.5% (77)
X-linked thrombocytopenia with mutations in WASP (n = 1)		100% (1)	NR
Total		1.77%	6.01%

CSR, Class-switch recombination; *HIGM*, hyper-IgM; *NEMO*, nuclear factor κB essential modulator; *NR*, not reported; *SCID*, severe combined immunodeficiency; *STAT3*, Signal transducer and activator of transcription 3; *WASP*, Wiskott-Aldrich syndrome protein.

Monogenic diseases of Immune regulation

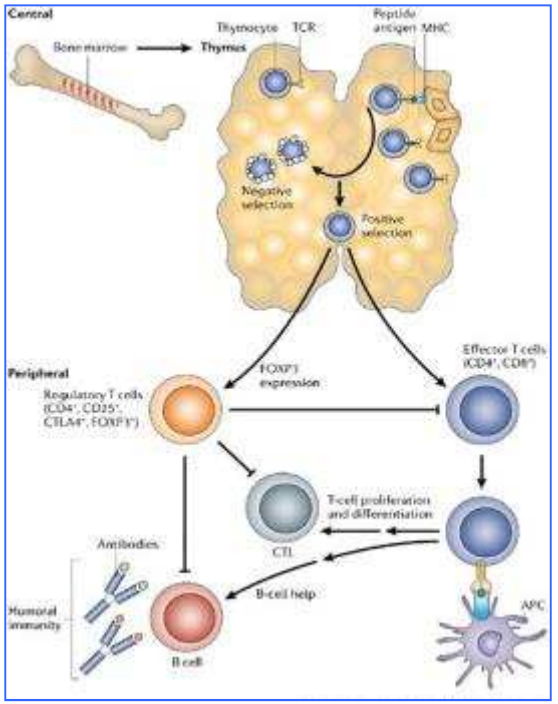
APECED
(*AIRE*)

Impairment in
thymic selection
(Central Tolerance)



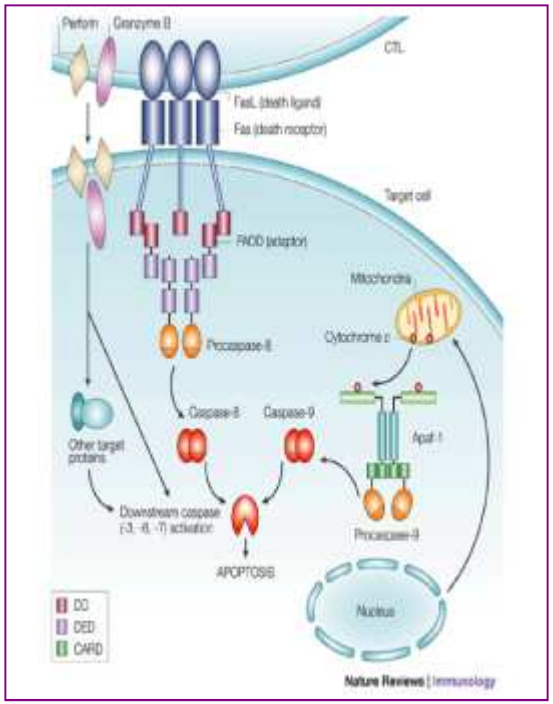
IPEX
(*FOXP3*)

Impairment in **Treg**
(Peripheral
Tolerance)



ALPS
(*FAS, FAS-L*)

Impairment in
Apoptosis
(Peripheral
Tolerance)



Immunedysregulation Polyendocrinopathy Enteropathy X-linked
IPEX

Rare genetic autoimmune disease due to mutation of *FOXP3* gene

Key molecular factor driving T cell tolerance

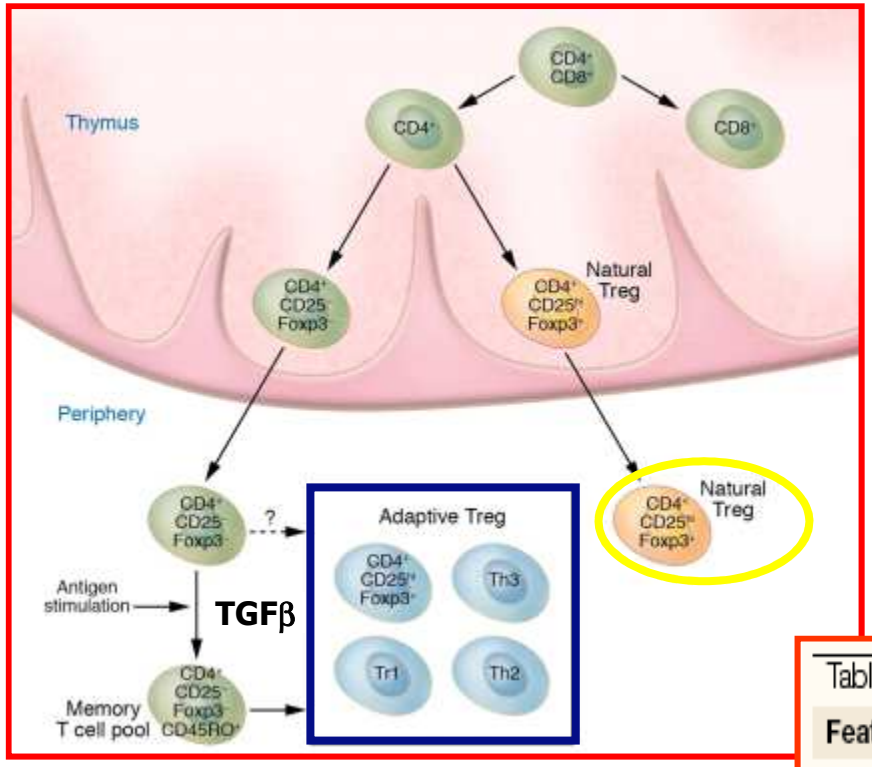
KEY MANIFESTATIONS:

- ✓ **Severe enteropathy**
- ✓ **Dermatitis** (mainly eczema)
- ✓ **Endocrinopathies** (IDDM, thyroid diseases)

OTHER MANIFESTATIONS:

- ✓ **Hyper-IgE, eosinophilia and autoantibodies** (in particular anti-enterocytes and anti-harmonin)
- ✓ **Autoimmune cytopenias**
- ✓ **Lymphoadenopathy, arthritis/vasculitis**
- ✓ **Alopecia, nephropathy, hepatitis**

Active suppression: Regulatory T cells



Make up **5-10%** of the normal **CD4+** T cell population

Characterized by expression of **CD4** and **CD25^{bright} , FOXP3, CTLA-4** and **GITR**

Require **activation** and **cell contact** to **repress proliferation** of other T cells but **do not appear to proliferate** themselves after activation

FOXP3 is a key factor for **develp** and **function** of **CD4+CD25+ Treg**

In humans FOXP3 expression can be induced upon TCR mediated stimulation (IL-2/TGFβ) in naive T cells

Baecher-Allan C et al J Imm 2001
Levings MK, et al J. Exp. Med. 2001
Walker MR, et al J. Clin. Invest. 2003
Wang J, et al Eur.J. Immunol

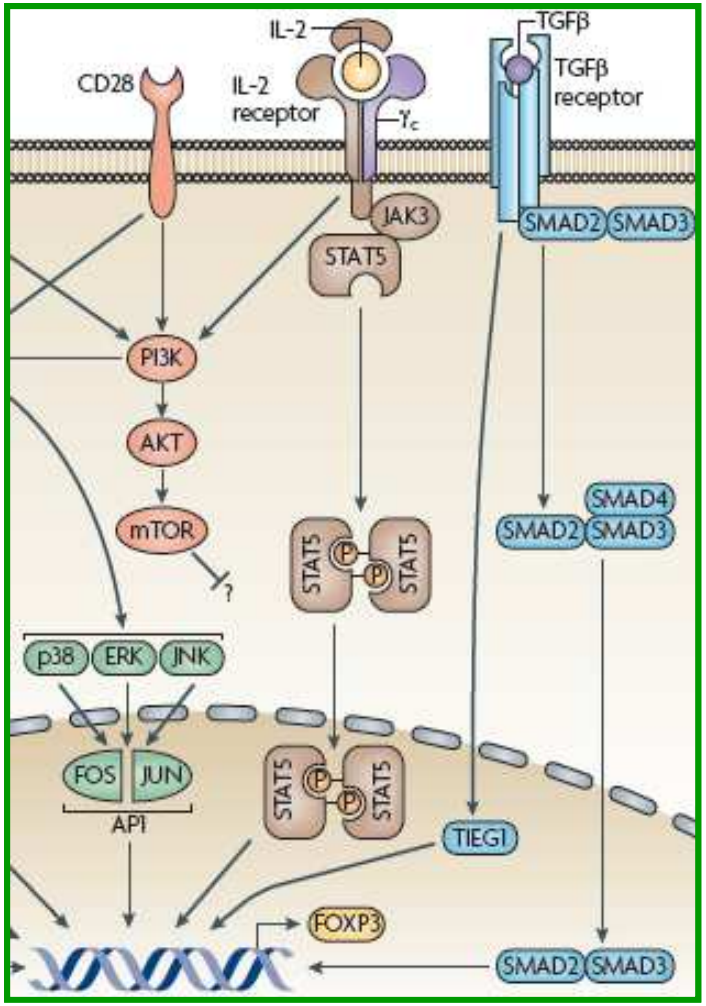
Table 1 | **A comparison of natural and adaptive regulatory T cells**

Feature	Natural T_{Reg} cells	Adaptive T_{Reg} cells
Site of induction	Thymus	Periphery
CD28–CD80/CD86 dependent	Yes	No
IL-2 dependent	Yes	Yes
CD25 expression	Yes (high)	Variable
Specificity	Self-antigens in thymus	Tissue-specific antigens and foreign antigens
Mechanism of effector-cell suppression	T-cell–T-cell/APC contact, cytokine independent	T-cell–T-cell/APC contact, cytokine dependent

APC, antigen-presenting cell; IL-2, interleukin-2; T_{Reg} cell, regulatory T cell.

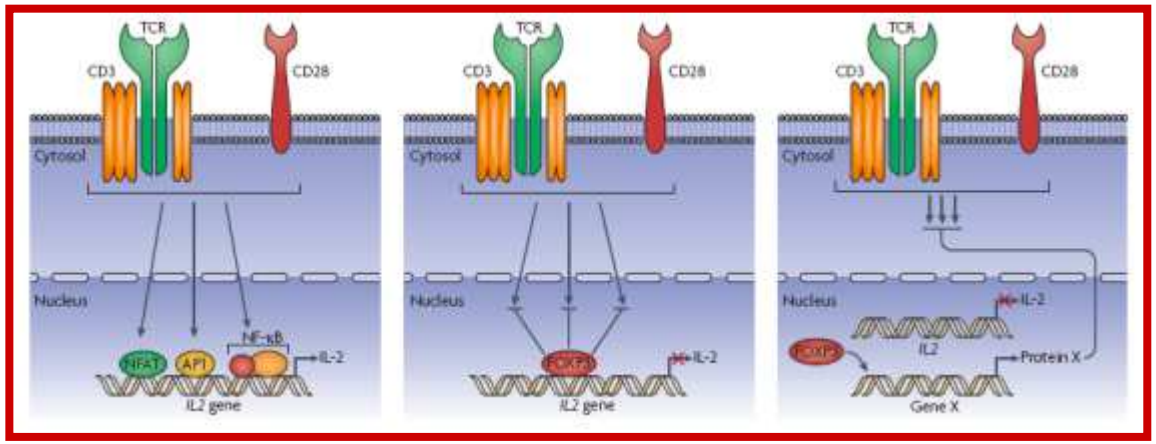
FOXP3

FOXP3 is a trascriptional factor and functions as a transcriptional repressor of cytokine promoters (in particular IL2) and inhibits NFAT function



Huehn J et al, Nature Reviews 2008

Schubert L, et al., J. Biol. Chem. 2001
Wu Y ,et al., Cell 2006
Bettelli E, et al. Proc. Natl Acad. Sci. 2005



Campbell DJ and Ziegler SF, Nature Reviews 2007

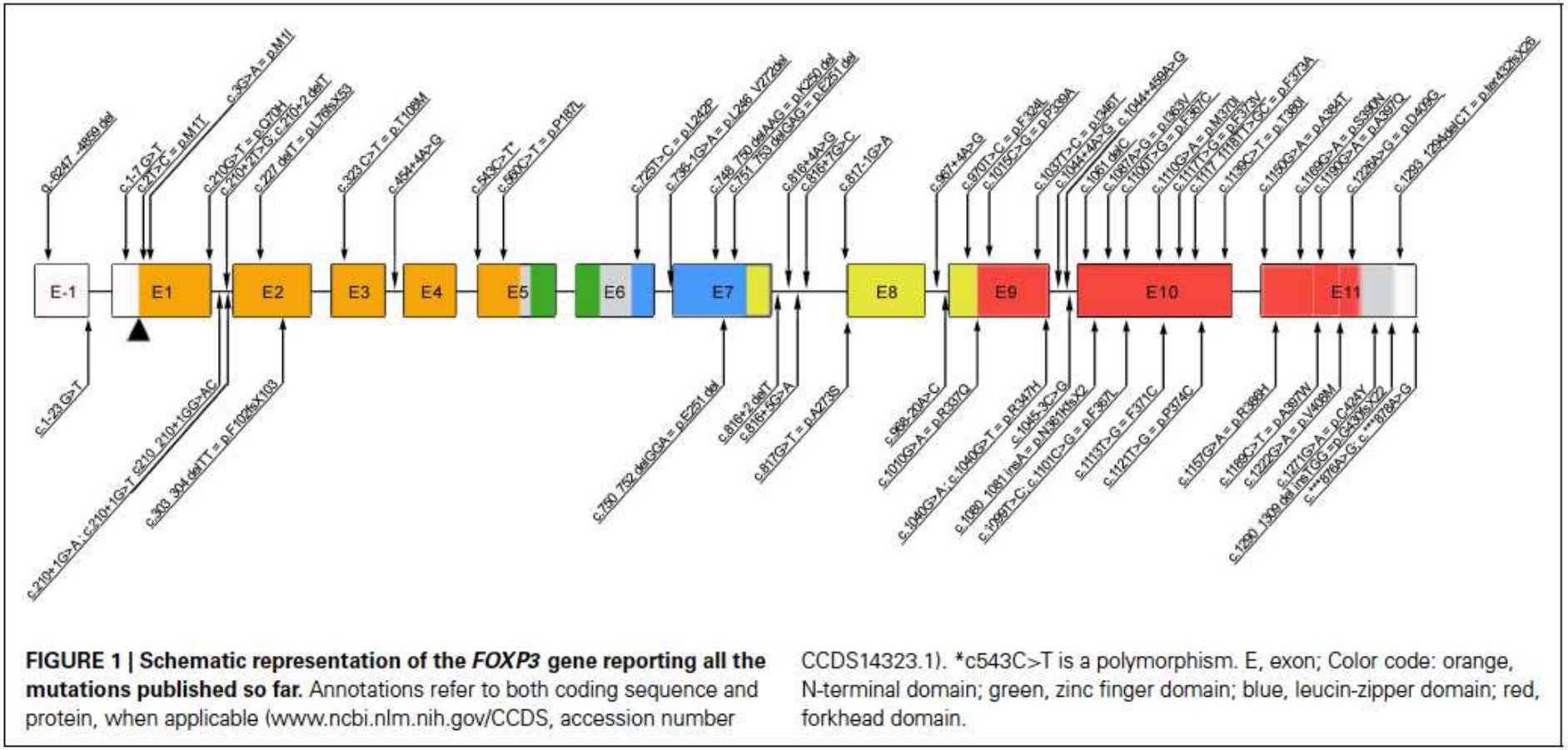
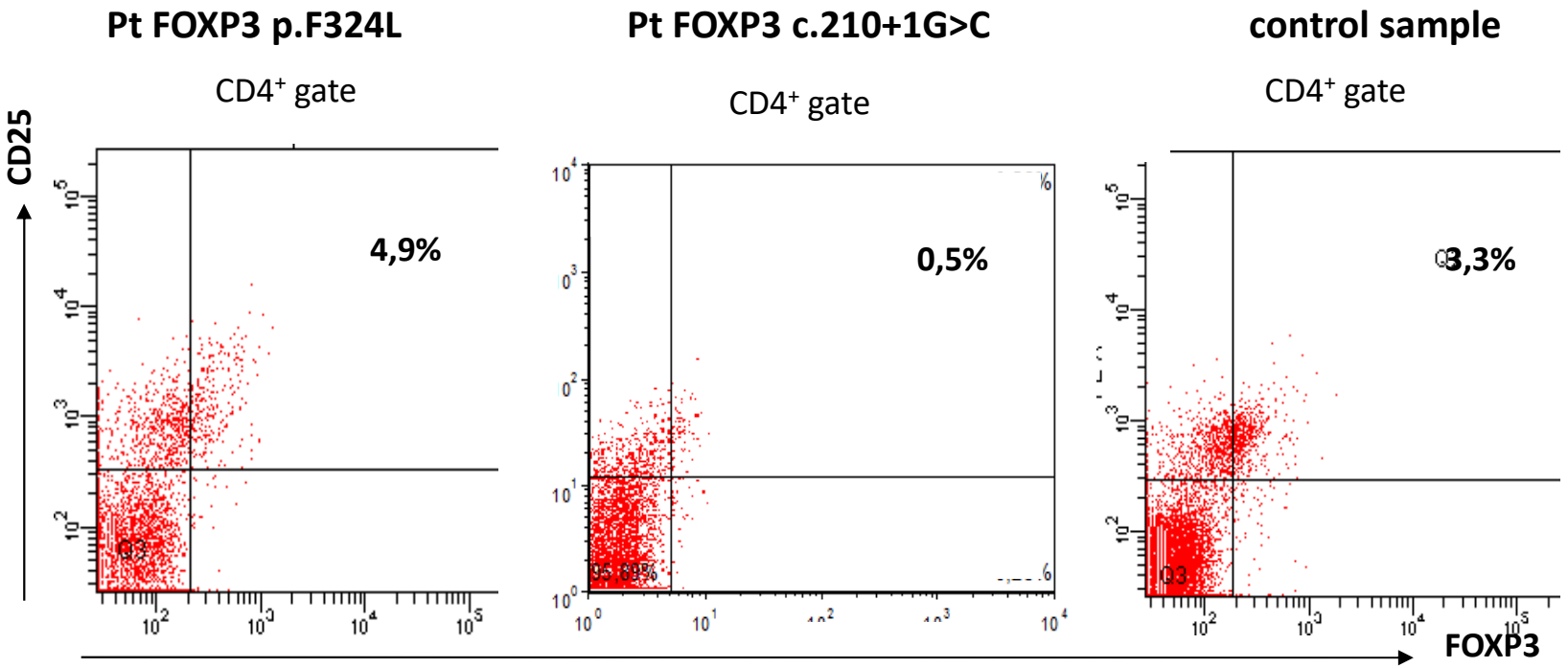


FIGURE 1 | Schematic representation of the *FOXP3* gene reporting all the mutations published so far. Annotations refer to both coding sequence and protein, when applicable (www.ncbi.nlm.nih.gov/CCDS, accession number CCDS14323.1). *c543C>T is a polymorphism. E, exon; Color code: orange, N-terminal domain; green, zinc finger domain; blue, leucin-zipper domain; red, forkhead domain.

- ✧ Mainly localized within FKD
- ✧ Usually disease course:
  - **...severe** disease if mutation abrogate protein expression (i.e. promoter)
  - **...variable** if mutation is on a splice site
  - **...unpredictable** if mutation affect the FKD
- ✧ No clear genotype-phenotype correlation!

FOXP3 expression

Levels of FOXP3 expression in $CD4^+$ T cells are variable both in patients with *FOXP3* mutation and in those with wild type *FOXP3* sequence.



IPEX Studies

(collaborators: University of Washington, Seattle, USA & HSR, Tiget, Milano)

- ✓ FOXP3 expression is not necessarily abrogated in IPEX patients and peripheral analysis of FOXP3 protein expression cannot always predict whether genetic alterations in FOXP3 are present (Gambineri et al JACI 2008)

- ✓ Defective suppressive activity of Treg and defective function ot T effectors (Bacchetta, Gambineri et al. JCI 2006)

- ✓ Mutations in FOXP3 that cause IPEX have diverse abilities to reprogram T cells into T regulatory cells (McMurchy, Bacchetta, Gambineri et al JACI 2010)

- ✓ Functional type 1 regulatory T cells develop regardless of FOXP3 mutations in patients with IPEX syndrome (Passerini, Gambineri, Bacchetta et al Eur. J. Immunol. 2011)

- ✓ Peripheral B-cell tolerance is defective in IPEX patients, suggesting that Tregs are involved in the maintenance of B-cell tolerance (Blood 2013)

TREATMENT

IMMUNOSUPPRESSION

- ✓ only partial efficacy (**steroids, cyclosporine, tacrolimus**)
- ✓ more promising results with **Rapamycin** *Battaglia, Blood 2005*
  Bindl, J.Pediatrics 2005
  Sullivan, J. Clin Immun. 2008

BONE MARROW TRANSPLANTATION

- ✓ IPEX old data are not in favor
- ✓ presently, patients alive and well when treated **very early**
  (Mazzolari E, et al 2005; Rao A et al, 2007; Lucas KG et al 2007; Zhan et al, 2008; Dorsey MJ, et al 2009; Burroughs LM, et al 2010; Horino S, et al 2014; Nademi Z, et al 2014)
  Overall…complete remission:
  - MUD (10/10), MSD, Haplo
  - Different conditioning regimens have been used, but usually RIC succesful
  - The majority have been performed early (first 5 years)
  - Follow up from 6 mo to 15 years
  - Peripheral donor *chimerism ranged 15-100%*
  - Preferential expansion of Treg

CELL/ GENE THERAPY?

…If you have a patient presenting with :

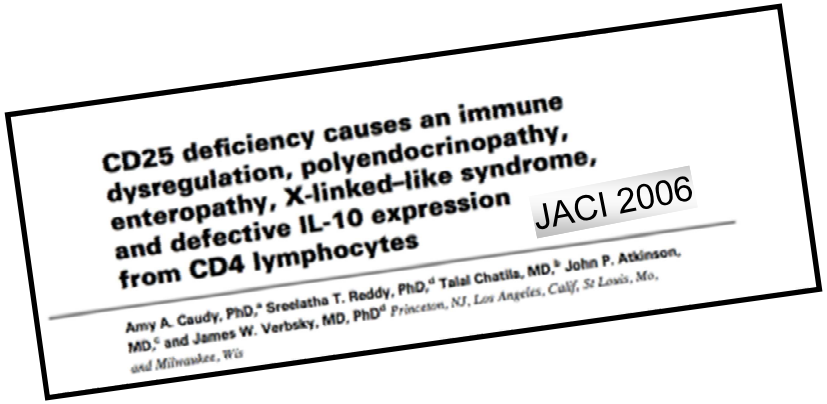
➔ Severe Autoimmune Entheropathy

➔ 1+ other autoimmune manifestations

But….

No FOXP3 mutation!

Congenital IPEX-like Immune Dysregulation



CD25 deficiency causes an immune dysregulation, polyendocrinopathy, enteropathy, X-linked–like syndrome, and defective IL-10 expression from CD4 lymphocytes

JACI 2006

Amy A. Caudy, PhD,[a] Sreelatha T. Reddy, PhD,[a] Talal Chatila, MD,[b] John P. Atkinson, MD,[c] and James W. Verbsky, MD, PhD[a] *Princeton, NJ, Los Angeles, Calif, St Louis, Mo, and Milwaukee, Wis*

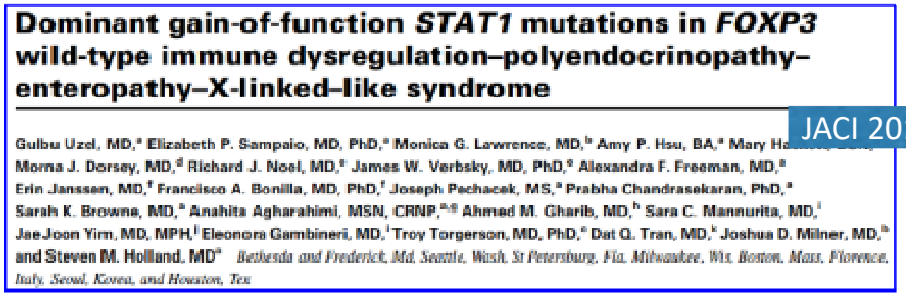
JOURNAL OF IMMUNOLOGY

CUTTING EDGE

Cutting Edge: Decreased Accumulation and Regulatory Function of CD4$^+$CD25^{high} T Cells in Human STAT5b Deficiency[1]

Aileen C. Cohen, Kari C. Nadeau,* Wenwei Tu,* Vivian Hwa,† Kira Dionis,* Liliana Bezrodnik,‡ Alejandro Teper,‡ Maria Gaillard,‡ Juan Heinrich,‡ Alan M. Krensky,* Ron G. Rosenfeld,*† and David B. Lewis*†*

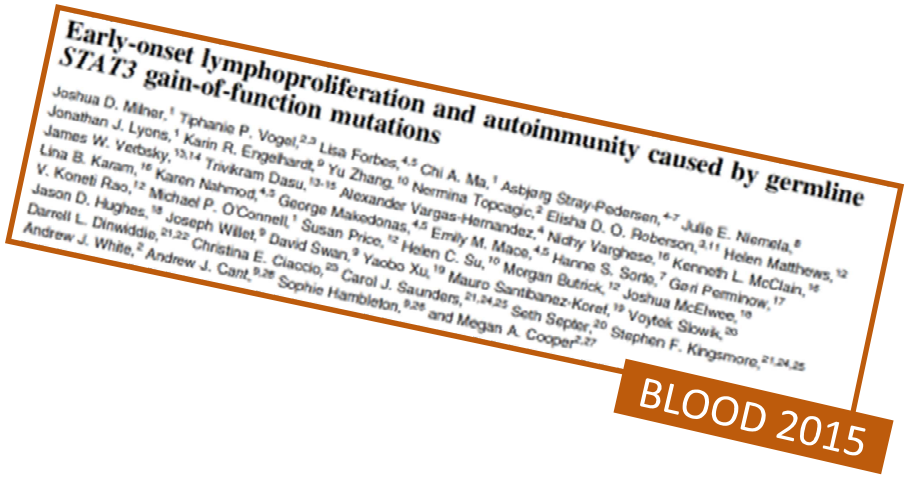
J. Immunol. 2006



Dominant gain-of-function *STAT1* mutations in *FOXP3* wild-type immune dysregulation–polyendocrinopathy–enteropathy–X-linked–like syndrome

JACI 2013

Gulbu Uzel, MD,[a] Elizabeth P. Sampaio, MD, PhD,[a] Monica G. Lawrence, MD,[b] Amy P. Hsu, BA,[a] Mary Ha… Morna J. Dorsey, MD,[d] Richard J. Nosl, MD,[e] James W. Verbsky, MD, PhD,[e] Alexandra F. Freeman, MD,[a] Erin Janssen, MD,[a] Francisco A. Bonilla, MD, PhD,[f] Joseph Pechacek, MS,[a] Prabha Chandrasekaran, PhD,[a] Sarah K. Browne, MD,[a] Anahita Agharahimi, MSN, CRNP,[a,g] Ahmad M. Gharib, MD,[h] Sara C. Mannurita, MD,[i] Jae Joon Yim, MD, MPH,[i] Eleonora Gambineri, MD,[j] Troy Torgerson, MD, PhD,[c] Dat Q. Tran, MD,[k] Joshua D. Milner, MD,[b] and Steven M. Holland, MD[a] *Bethesda and Frederick, Md, Seattle, Wash, St Petersburg, Fla, Milwaukee, Wis, Boston, Mass, Florence, Italy, Seoul, Korea, and Houston, Tex*



Early-onset lymphoproliferation and autoimmunity caused by germline *STAT3* gain-of-function mutations

Joshua D. Milner,[1] Tiphanie P. Vogel,[2,3] Lisa Forbes,[4,5] Chi A. Ma,[1] Asbjørg Stray-Pedersen,[4-7] Julie E. Niemela,[8] Jonathan J. Lyons,[1] Karin R. Engelhardt,[9] Yu Zhang,[10] Nermina Topcagic,[2] Elisha D. O. Roberson,[2,11] Helen Matthews,[12] James W. Verbsky,[13,14] Trivikram Dasu,[13-15] Alexander Vargas-Hernandez,[4] Nidhy Varghese,[16] Kenneth L. McClain,[16] Lina B. Karam,[16] Karen Nahmod,[4,5] George Makedonas,[4,5] Emily M. Mace,[4,5] Hanne S. Sorte,[7] Geri Perminow,[17] V. Koneti Rao,[12] Michael P. O'Connell,[1] Susan Price,[12] Helen C. Su,[10] Morgan Butrick,[12] Joshua McElwee,[18] Jason D. Hughes,[18] Joseph Willet,[9] David Swan,[9] Yaobo Xu,[19] Mauro Santibanez-Koref,[19] Voytek Slowik,[20] Darrell L. Dinwiddie,[21,22] Christina E. Ciaccio,[23] Carol J. Saunders,[21,24,25] Seth Septer,[20] Stephen F. Kingsmore,[21,24,25] Andrew J. White,[2] Andrew J. Cant,[9,26] Sophie Hambleton,[9,26] and Megan A. Cooper[2,27]

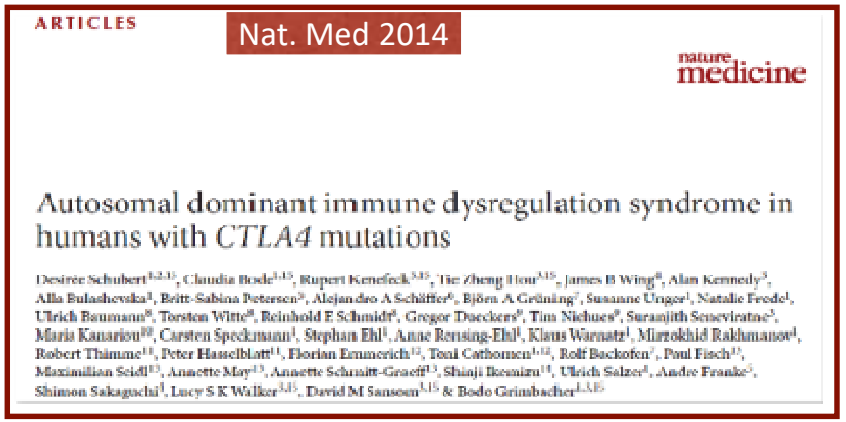
BLOOD 2015

ARTICLE

Deleterious Mutations in *LRBA* Are Associated with a Syndrome of Immune Deficiency and Autoimmunity

Gabriela Lopez-Herrera,[1,2] Giacomo Tampella,[2,19] Qiang Pan-Hammarström,[4,19] Peer Herholz,[5,19] Claudia M. Trujillo-Vargas,[1,6,19] Kanchan Phadwal,[7] Anna Katharina Simon,[7,8] Michel Moutschen,[9] Amos Etzioni,[10] Adi Mory,[10] Izhak Srugo,[10] Doron Melamed,[10] Kjell Hultenby,[4] Chonghai Liu,[4,11] Manuela Baronio,[3] Massimiliano Vitali,[3] Pierre Philippet,[12] Vinciane Dideberg,[13] Asghar Aghamohammadi,[14] Nima Rezaei,[15] Victoria Enright,[1] Likun Du,[4] Ulrich Salzer,[5] Hermann Eibel,[5] Dietmar Pfeifer,[16] Hendrik Veelken,[17] Hans Stauss,[1] Vassilios Lougaris,[3] Alessandro Plebani,[3] E. Michael Gertz,[18] Alejandro A. Schäffer,[18] Lennart Hammarström,[4] and Bodo Grimbacher[1,5,*]

Am. J. Hum. Genet 2012



ARTICLES

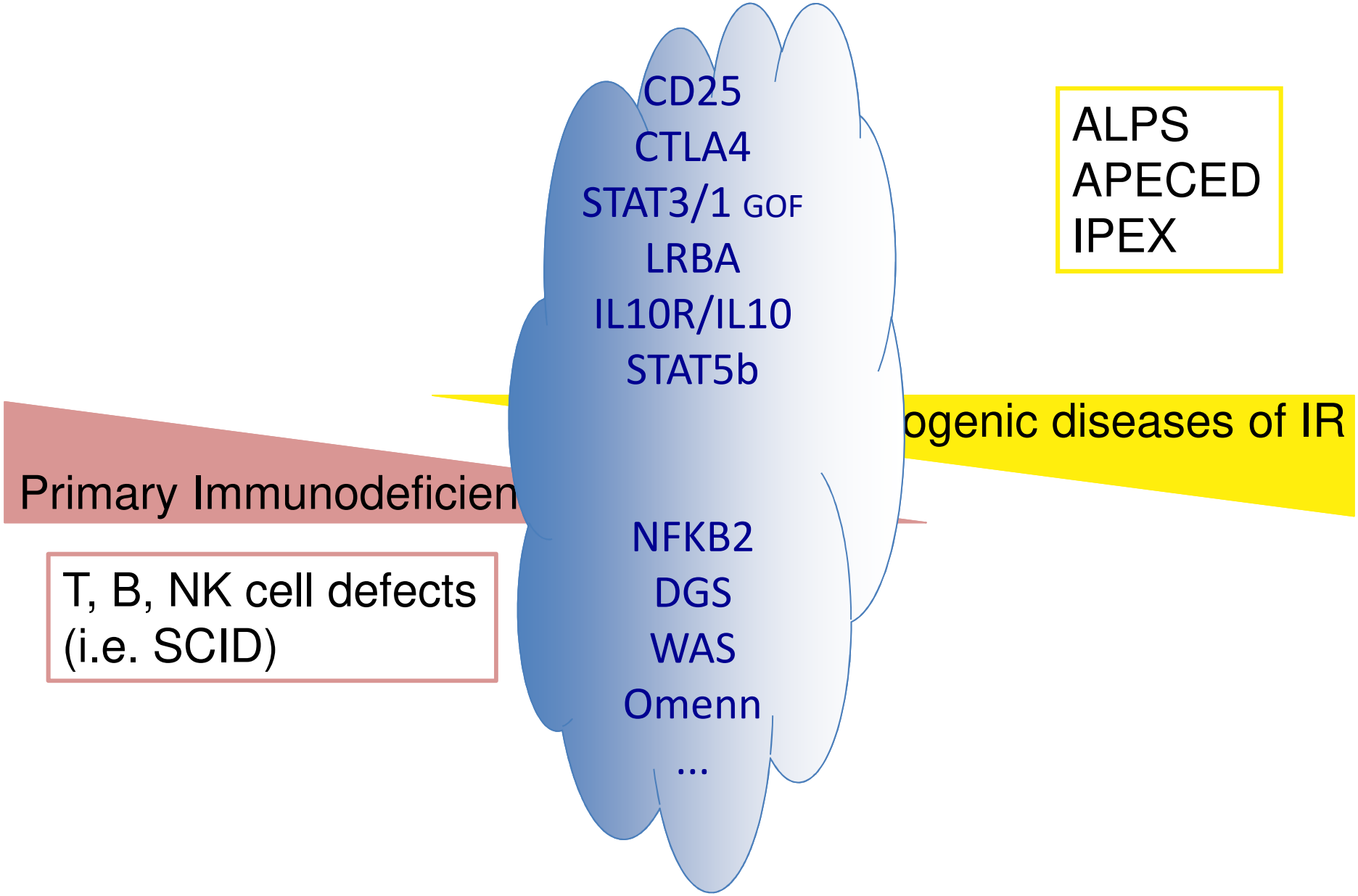
Nat. Med 2014

nature medicine

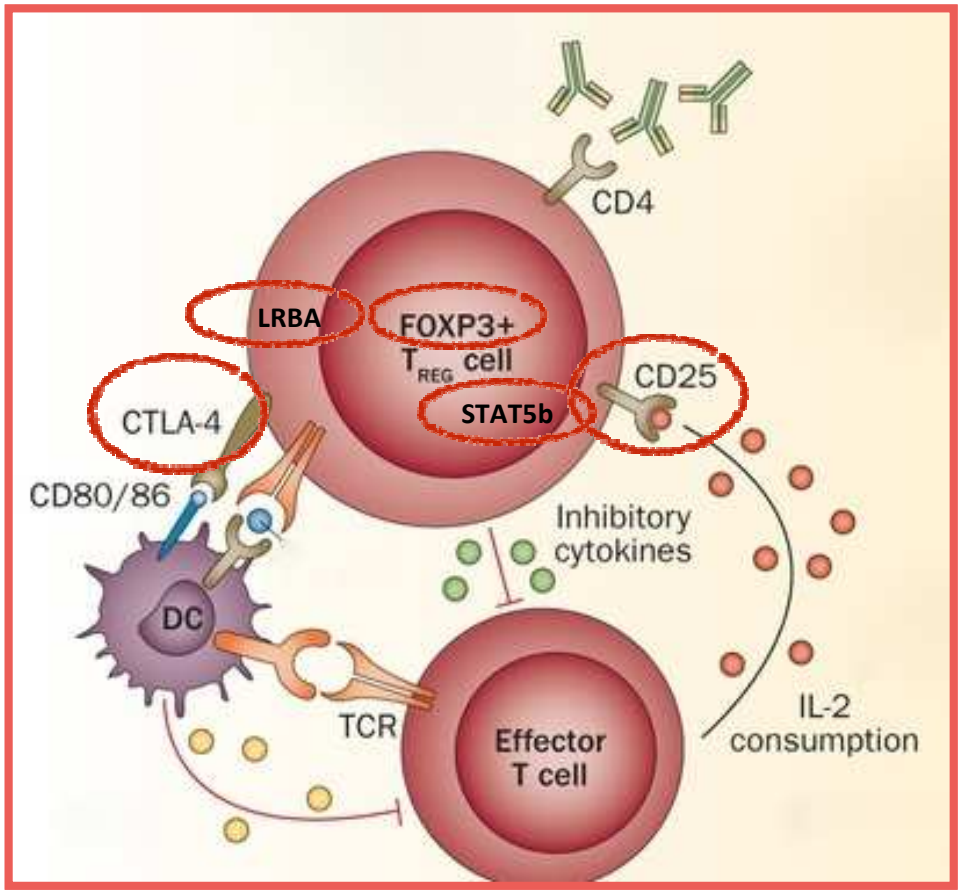
Autosomal dominant immune dysregulation syndrome in humans with *CTLA4* mutations

Desirée Schubert[1,2,12], Claudia Bode[3,12], Rupert Kenefeck[3,12], Tie Zheng Hou[3,12], James B Wing[4], Alan Kennedy[3], Alla Bulashevska[5], Britt-Sabina Petersen[6], Alejandro A Schäffer[6], Björn A Grüning[7], Susanne Unger[1], Natalie Frede[1], Ulrich Baumann[8], Torsten Witte[8], Reinhold E Schmidt[8], Gregor Dueckers[9], Tim Niehues[9], Suranjith Seneviratne[3], Maria Kanariou[10], Carsten Speckmann[1], Stephan Ehl[1], Anne Rensing-Ehl[1], Klaus Warnatz[1], Mirzokhid Rakhmanov[1], Robert Thimme[11], Peter Hasselblatt[11], Florian Emmerich[12], Toni Cathomen[11,12], Rolf Backofen[7], Paul Fisch[13], Maximilian Seidl[13], Annette May[13], Annette Schmitt-Graeff[13], Shinji Ikemizu[14], Ulrich Salzer[5], Andre Franke[5], Shimon Sakaguchi[4], Lucy S K Walker[3,15], David M Sansom[3,15] & Bodo Grimbacher[1,3,12]

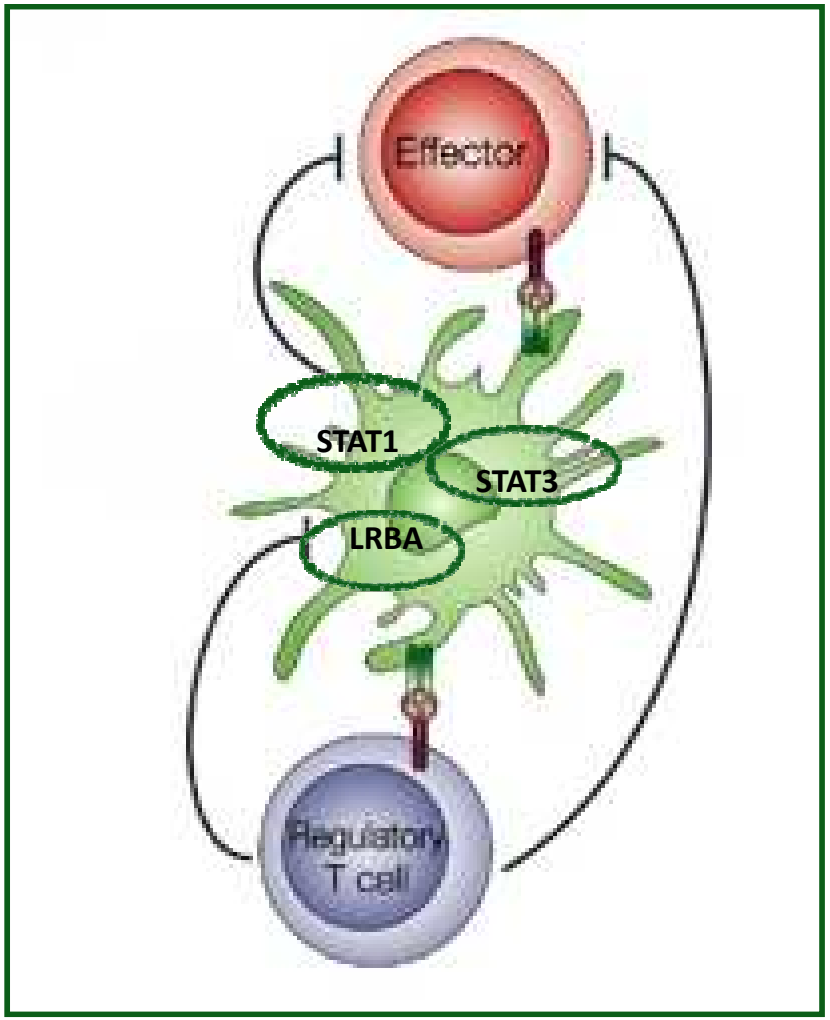
Immune dysregulation in PID



Other genes involved in immune dysregulation



Treg related mechanisms



Treg unrelated mechanisms

Treg intrinsic

CD25	STAT5b	IL10/IL10R	CTLA4
AR, Early onset	AR, Progressive onset	AR, Early onset (<1y)	AD, Progressive onset
Severe diarrhea/enteropathy	Chronic diarrhea	Colitis/IBD involving the colon and to a lesser extend the small intestine	Chronic Diarrhea
IDDM	Autoimmune Cytopenias	-	Autoimmune cytopenias/thyroiditis/Arthritis
Eczema	Eczema	Persistent folliculitis	Psoriasis
Infections (Candida, CMV)	Pulmonary Infections/Herpes viruses infections	Recurrent upper and lower respiratory tract infections	Respiratory Infections/ILD
Hepatosplenomegaly	Poor postnatal growth Low IGF-1, IGFBP-3 with normal GH	Poor response to standard immunesuppression	Organ infiltration
Impaired lymphocyte counts/subsets/function	Low CD4 and CD8 T cells, NK/T memory phenotype and activated cells	Unremarkable immunological indices	Low B cells
Autoantibodies	Autoantibodies and hypergammaglobulinemia	-	Hypogammaglobulinemia
Normal/Increased IgE levels	Normal/Increased IgE levels	Normal/Increased IgE levels	Normal/Increased IgE levels
Normal FOXP3/Absent CD25 expression	Low Treg	-	Low Treg

Treg extrinsic

LRBA	STAT1 GOF	STAT3 GOF
AR, Progressive onset	AD, Early onset	AD, Early onset
Enteropathy	Enteropathy	Early onset (<1 y) colitis/IBD involving the colon and to a lesser extend the small intestine
IDDM/Autoimmune Cytopenias/Thyroiditis/Uveitis	IDDM/Autoimmune Cytopenias/Thyroiditis	IDDM/Autoimmune Cytopenias/Thyroiditis/Arthritis
Eczema/Alopecia	Eczema	Eczema/Alopecia
Recurrent upper and lower respiratory tract infections/ILD	Pulmonary Infections/Herpes viruses infections/Candida infections	Recurrent upper and lower respiratory tract infections/ILD
Hepatosplenomegaly/Lymphoade nopathy/Organ infiltration	Hepatosplenomegaly/Vascular abnormalities/Osteopenia	Hepatosplenomegaly/Lymphoade nopathy/Organ infiltration
Hypogammaglobulinemia	Unremarkable immunological indices	Low B cells/Hypogammaglobulinemia
Autoantibodies	Autoantibodies	Autoantibodies
Normal/Increased IgE levels	Normal/Increased IgE levels	Normal/Increased IgE levels
Low Treg	Low Treg	Low Treg

TREATMENT

IMMUNOSUPPRESSION

- ✓ steroids, cyclosporine, tacrolimus, rapamycin, MMF, azathioprine
- ✓ Monoclonal antibodies (anti CD20-Rituximab, CTLA4-Ig, Abatacept)

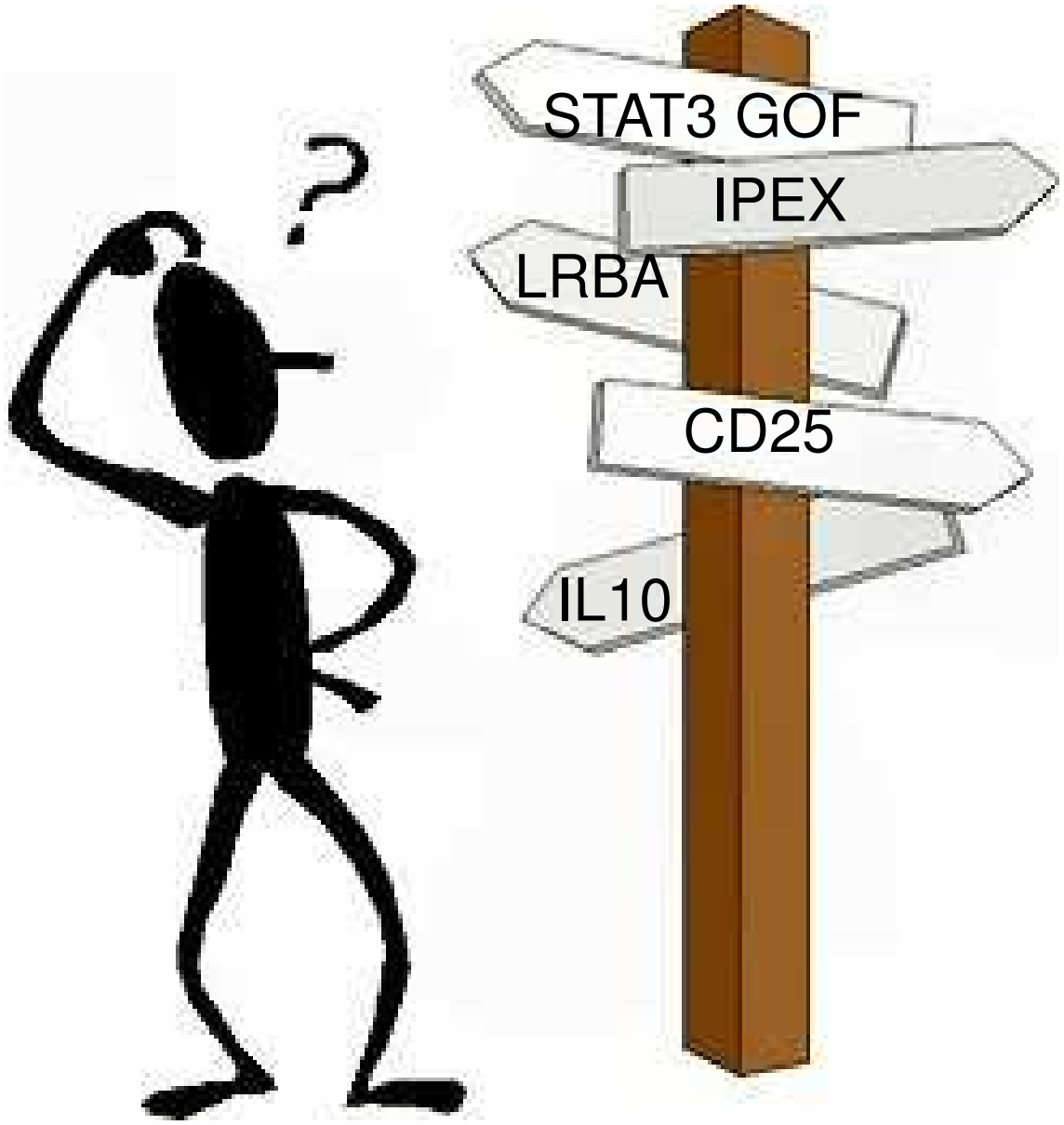
SUPPORTIVE CARE

DISEASE-SPECIFIC IMMUNE MODULATION

- ✓ JAK3 inhibitors (STAT1 and STAT3 GOF)
- ✓ Anti-IL6 (STAT3 GOF)
- ✓ CTLA4-Ig (CTLA4)

BONE MARROW TRANSPLANTATION

- ✓ No much experience on newly discovered diseases but only personal communications (Slatter MA, et al 2016)


STAT3 GOF
IPEX
LRBA
CD25
IL10

Patients and Origin

Italy 76

UK 17

Turkey 12

Belgium 4

Thailand 3

Australia 2

Greece 2

Portugal 2

Serbia 1

India 1

Romania 1

Sweden 1

Czech Rep1

Slovenia 1

Switzerland 1

Germany 1

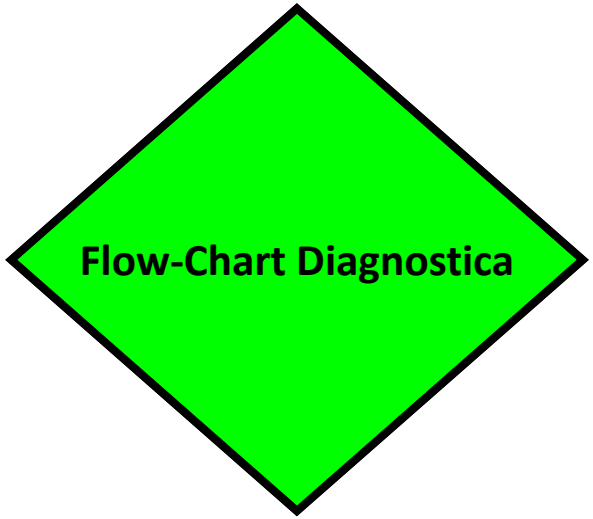
Egypt 1

- ✓ **From 2003 to date: 127 patients and 70 family members from all over the world**
- ✓ **From 2014: the only center of expertise for IPEX disease in Italy**
- ✓ **Cellular and molecular studies provided on research bases**

18/90 patients with FOXP3 mutation

6/90 patients with mutations in other genes correlated with immune dysregulation

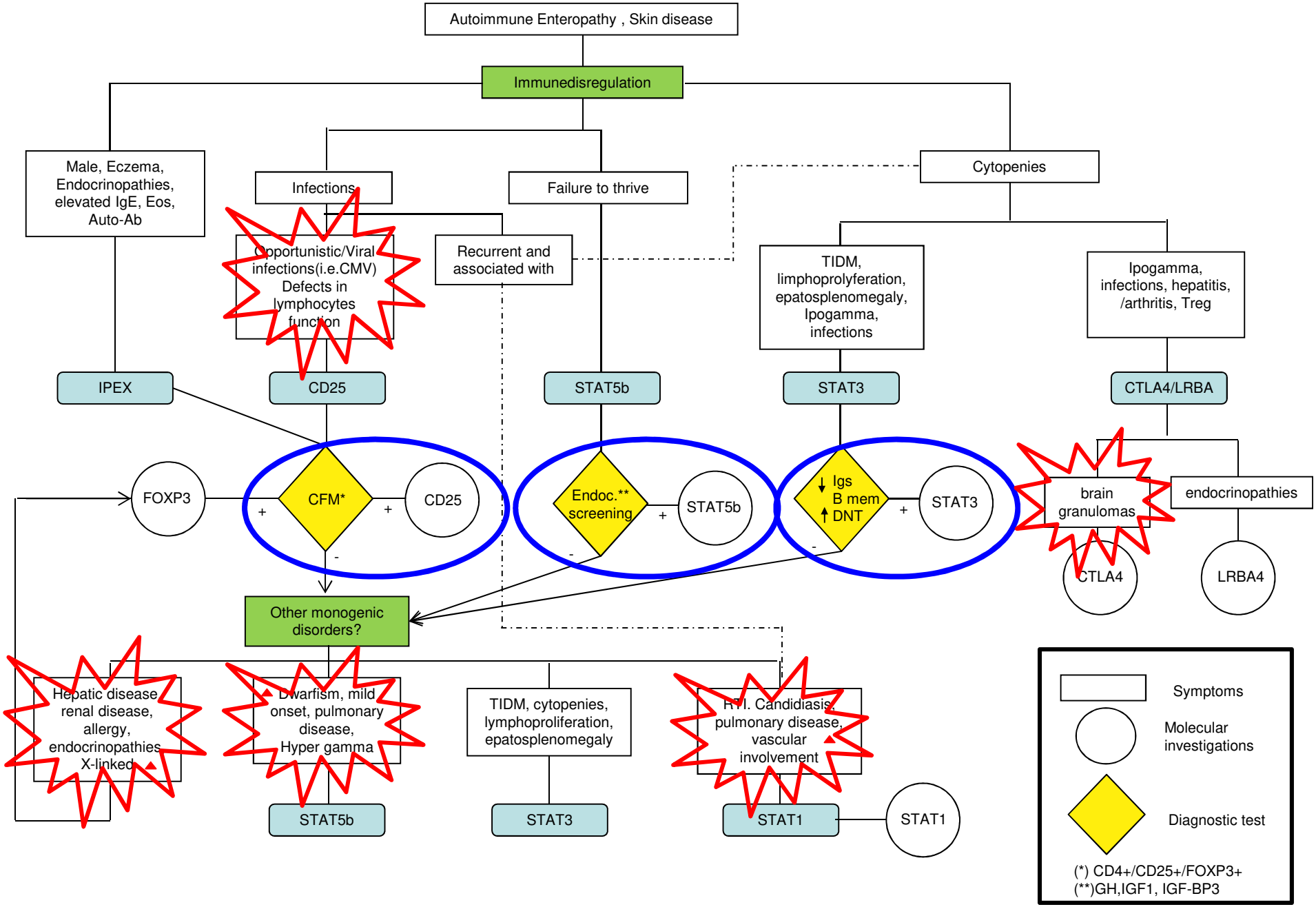
10/90 patients with NGS ongoing



FOXP3 WT patients

Cluster CD25	Opportunistic/viral infections+ Impaired lympho fx + enteropathy →	**20 pts: 11 tested, 3 CD25 mut**
Cluster STAT5b	Dwarfism, pumonary disease, low Treg, hypergamma →	**14 pts: 7 tested, 1 STAT5b mut**
Cluster STAT1 GOF	Candida infections+ enteropathy +vascular diseases →	**15 pts: 8 tested, 1 STAT1 GOF mut**
Cluster STAT3 GOF	AI cytopenias+IDDM+ Lymphoproliferation →	**13 pts: 1 tested, 1 STAT3 GOF mut**
Cluster CTLA4	AI cytopenias+lymphoproliferation+ Enteropathy+ dysgamma →	**19 pts: 5 tested, 0 mut**
Cluster LRBA	Enteropathy+ AI cytopenias+ Organomegaly+Hypogamma →	**16 pts: seq ongoing, 2 LRBA mut (NGS)**

Diagnostic Flow Chart Proposal



Messages to take home

- → PID (especially monogenic diseases as IPEX) help to understand the mechanism of autoimmunity
- → **Diversity** and **Plasticity** of Immune Tolerance…if one mechanism is lacking another is supporting (lack of strong genotype-phenotype correlation). **ANY** compartment of the immune system can be involved!
- → Role for **other mechanisms** contributing in immune balance/dysregulation: too much or too little signal from master receptors leads to alterations at tolerance checkpoints and autoimmunity
- → Given the rapid discovery of new conditions a **clinical/lab flow-chart** can be a useful tool for improving diagnosis

Have any patients?

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Patients and their families