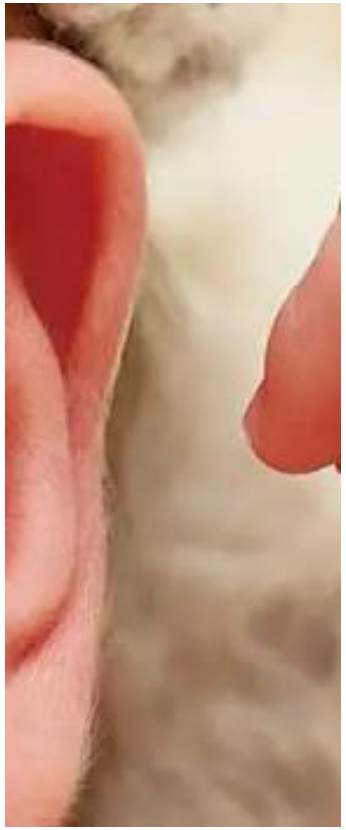
Sistema Socio Sanitario
Regione Lombardia
ASST Spedali Civili

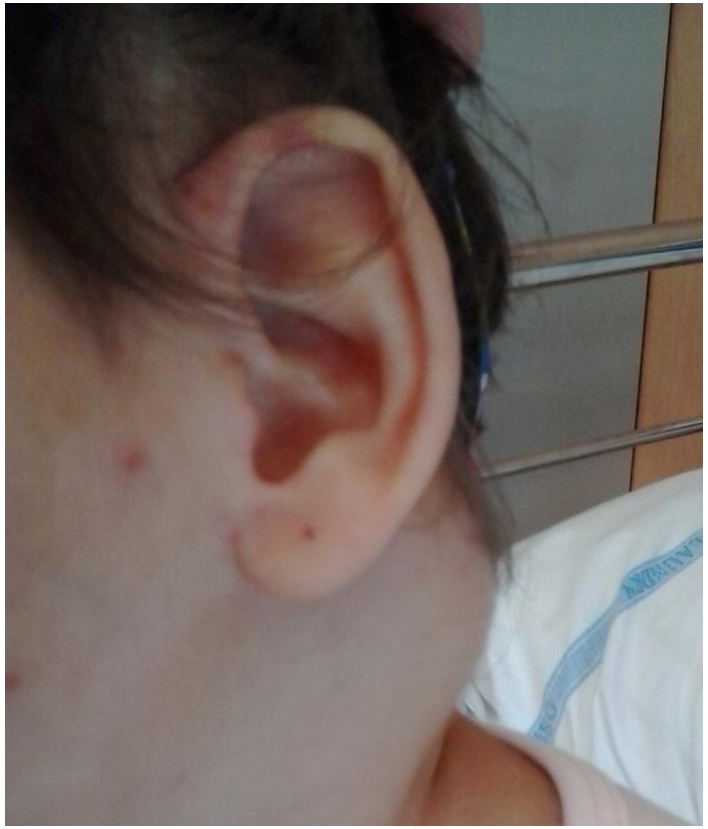
UNIVERSITÀ DEGLI STUDI DI BRESCIA

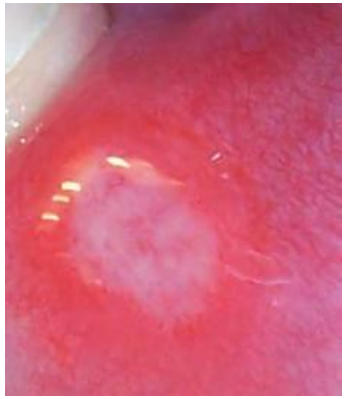
Le Citopenie immunomediate come manifestazione di immunodeficit congenito

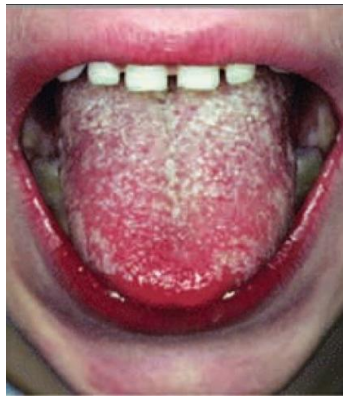
Raffaele Badolato Clinica Pediatrica, ASST Spedali Civili di Brescia, Università degli Studi di Brescia

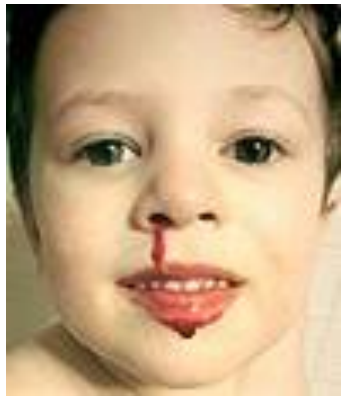


Manifestazioni di difetto del sistema immunitario









10 Segni di allarme di Immunodeficienza primitiva

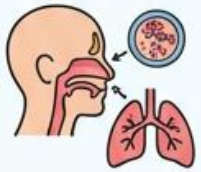
Jeffrey Modell Foundation | Curing PI. Worldwide.

- Anamnesi familiare di immunodeficienza o morte prematura inspiegabile (p.es., prima dei 30 anni)

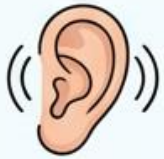
- Ritardo della crescita

- Necessità di antibiotici per via endovenosa e/o ricovero in ospedale per eliminare le infezioni

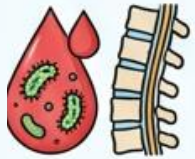
- 6 o più infezioni del tratto respiratorio in un anno



- 2 o più sinusiti o polmoniti gravi nell'arco di un anno



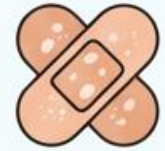
- 4 o più nuove infezioni dell'orecchio in un anno



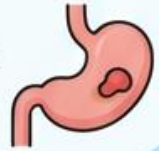
- 2 o più episodi di sepsi o meningite nel corso della vita

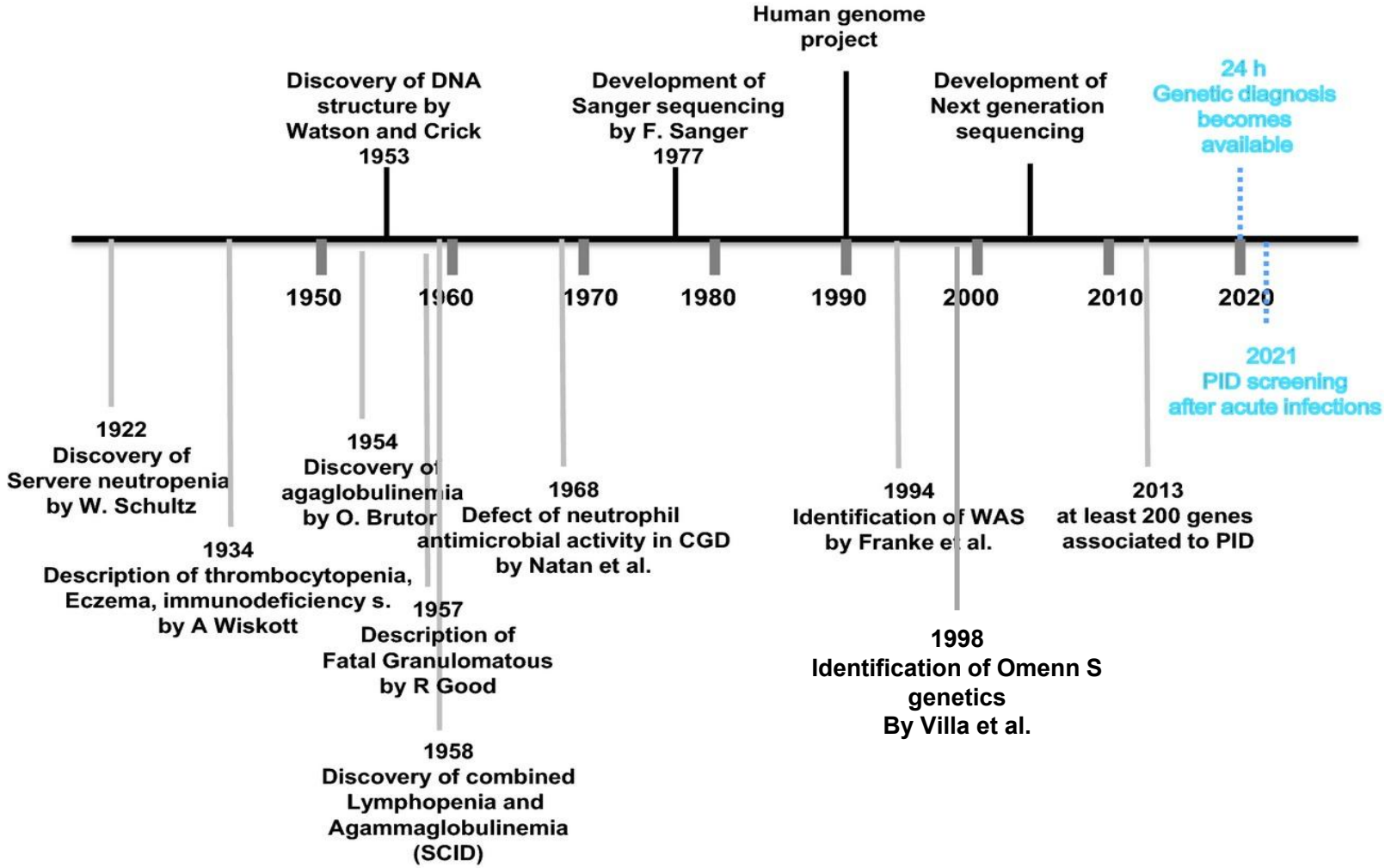
- 2 o più mesi di antibiotici con scarso effetto

- Candidosi orale o cutanea ricorrente o resistente



- Ascessi ricorrenti della pelle profonda o degli organi





Pediatric Allergy Immunology, Volume: 25, Issue: 1, Pages: 27-29, First published: 19 February 2014, DOI: (10.1111/pai.12192)

Cell, Vol. 78, 635–644, August 26, 1994, Copyright © 1994 by Cell Press

Isolation of a Novel Gene Mutated in Wiskott–Aldrich Syndrome

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Stanford, California 94305
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Summary

Wiskott–Aldrich syndrome (WAS) is an X-linked recessive immunodeficiency characterized by eczema, thrombocytopenia, and recurrent infections. Linkage studies have placed the gene at Xp11.22-p11.23. We have isolated from this interval a novel gene, *WASP*, which is expressed in lymphocytes, spleen, and thymus. The gene is not expressed in two unrelated WAS patients, one of whom has a single base deletion that produces a frame shift and premature termination of translation. Two additional patients have been identified with point mutations that change the same arginine residue to either a histidine or a leucine. *WASP* encodes a 501 amino acid proline-rich protein that is likely to be a key regulator of lymphocyte and platelet function.

Introduction

normal cell surface cytoarchitecture, seen as a paucity of microvillus surface projections on WAS T lymphocytes when compared with normal lymphocytes (Molina et al., 1992); abnormalities in patterns of O-glycosylation of cell surface proteins (Greer et al., 1989a; Piller et al., 1991; Higgins et al., 1991); defective transmembrane signaling (Simon et al., 1992); and a failure of WAS T cells to proliferate in response to certain antigenic stimuli (Molina et al., 1993).

Linkage analyses have localized the WAS gene to a >1 Mb region in Xp11.22-p11.23, bounded by the markers *DXS255* and *TIMP* (Peacocke and Siminovitch, 1987; Kwan et al., 1988, 1991; de Saint Basile et al., 1989; Greer et al., 1990, 1992). To isolate the WAS gene, we have used a positional cloning strategy that involved the construction of a clone contig in this interval and the subsequent isolation of cDNA sequences. Evaluation of several candidate cDNAs has led to the identification of a sequence whose expression is limited to lymphocytic and megakaryocytic cell lineages and which is altered in affected individuals. The identification of DNA mutations in four patients with classical WAS leads us to postulate that this gene is the WAS gene.

Results

Construction of a Clone Contig in Xp11.22-p11.23 Surrounding the WAS Locus

We have used yeast artificial chromosomes (YACs) and cosmid clones to build a clone contig from the hypervariable random sequence marker *DXS255* extending distally

1994 Wiskott Aldrich

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Professor of Pediatrics| Seattle, Washington

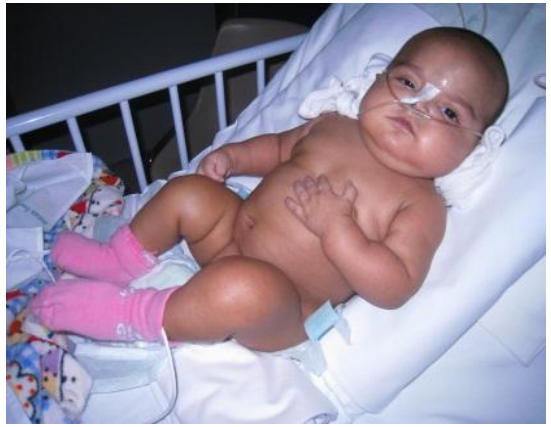
SCID = Severe combined immunodeficiency

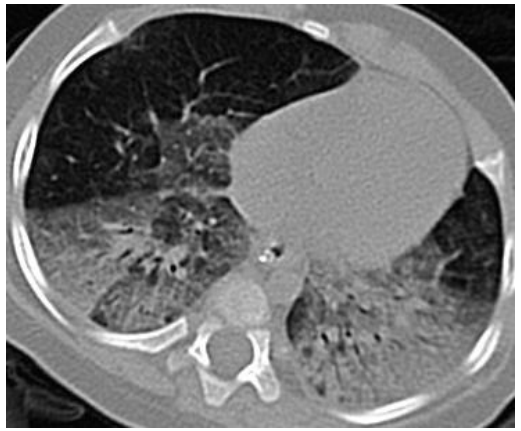
IL2RGC, JAK3, RAG1, RAG2, ADA, …..

Lymphopenia

X-linked or autosomal recessive inheritance

- Early onset in life
  - Bronchiolites
  - Interstitiale pneumonia → *P.jirovecii*
  - Otites, pneumonia
  - Sepsis (Pseudomonas, S.*aureus*)
  - Candidiasis
  - Diarrhea
  - Fungal infections (rare)
  - BCGites
- Failure to thrive, extreme distrophy
- Lethal if left untreated





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Partial V(D)J Recombination Activity Leads to Omenn Syndrome

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[1]Department of Human Genome
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via Fratelli Cervi 93
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1998 Omenn

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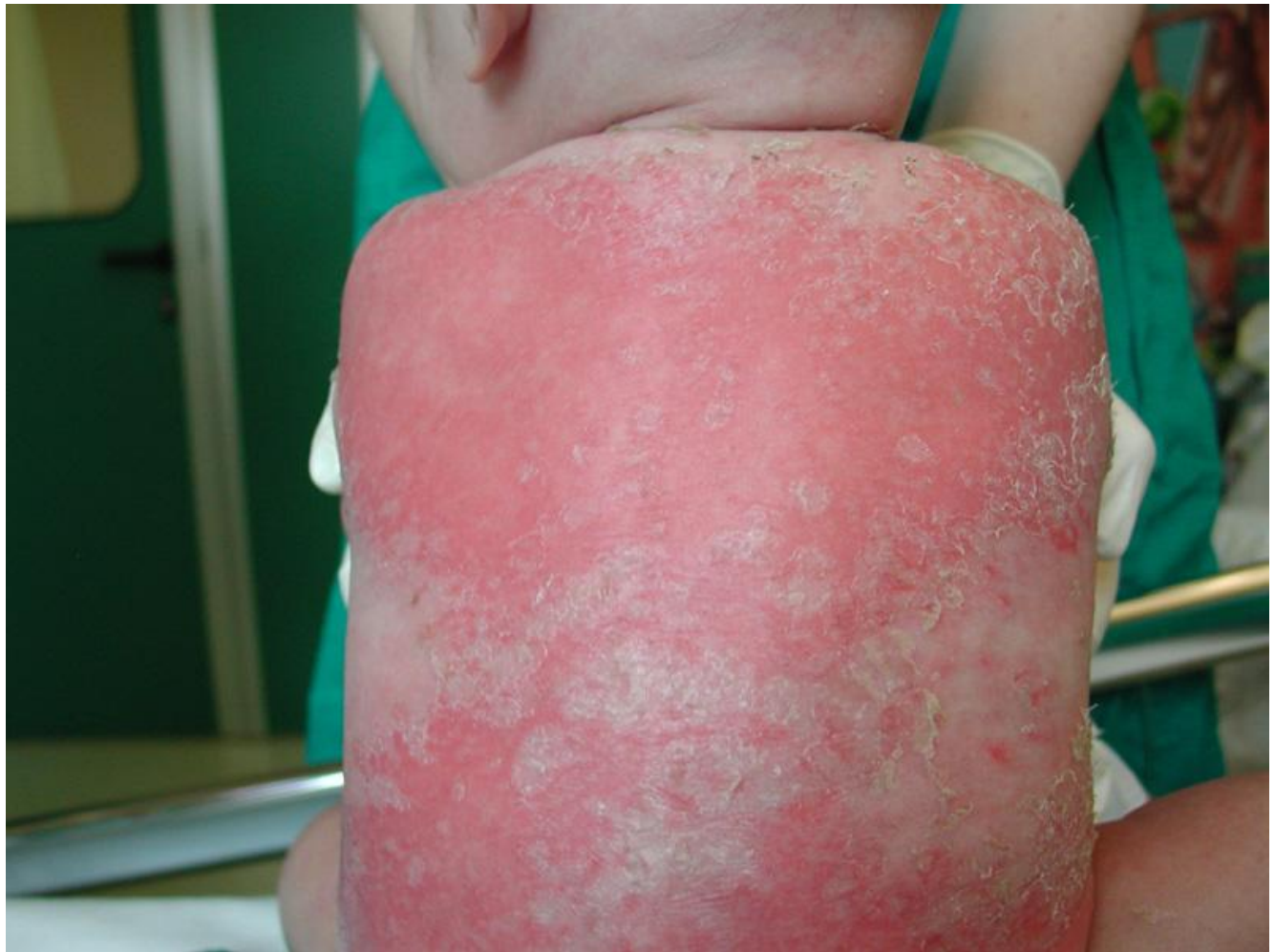
Professor of Pediatrics| Seattle, Washington

Omenn syndrome



Courtesy of A. Tommasini

The NEW ENGLAND JOURNAL of MEDICINE

Familial Reticuloendotheliosis with Eosinophilia

Gilbert S. Omenn, M.D.†

August 19, 1965
N Engl J Med 1965; 273:427-432

REPORTS of proliferative and infiltrative disorders of the reticuloendothelial system in children have documented a fascinating and frustrating category of disease. Most of these cases of multiorgan involvement and of unknown etiology have in common a

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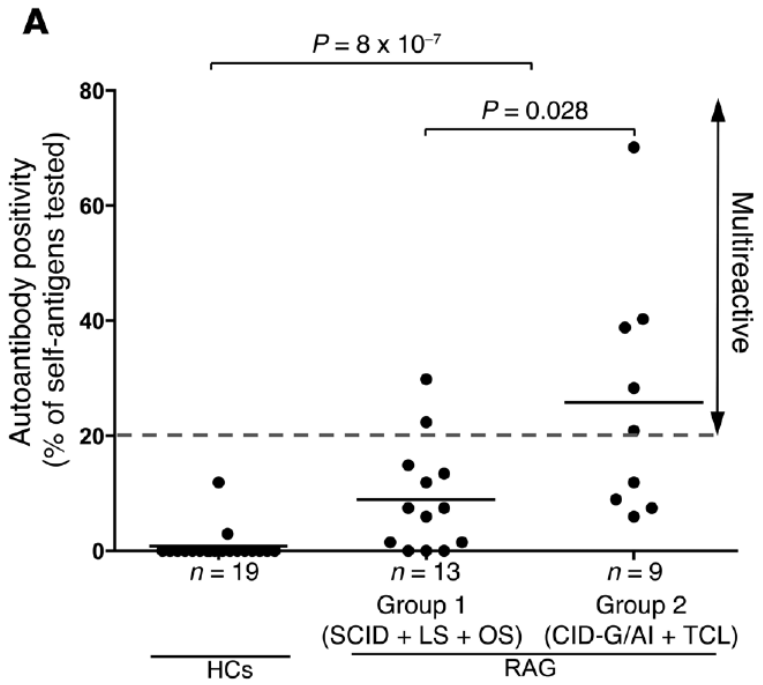
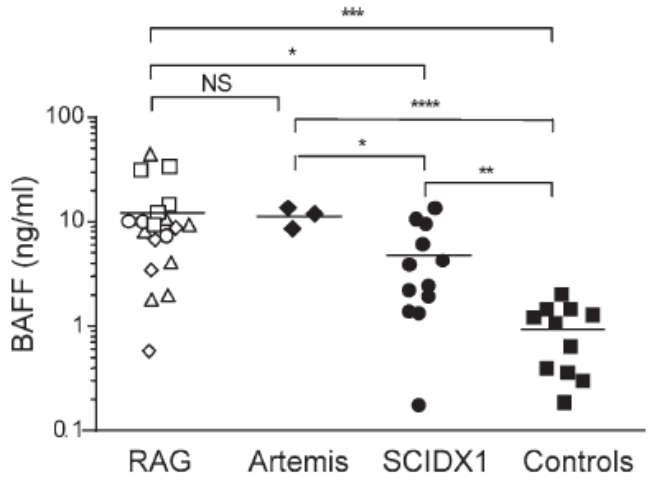
Autoimmune/granulomatouse and Autoantibodies in patients with RAG mutations



Walker J et al., JCI 2015

Autoantibodies in Omenn patients

Serum BAFF



Autoantibodies

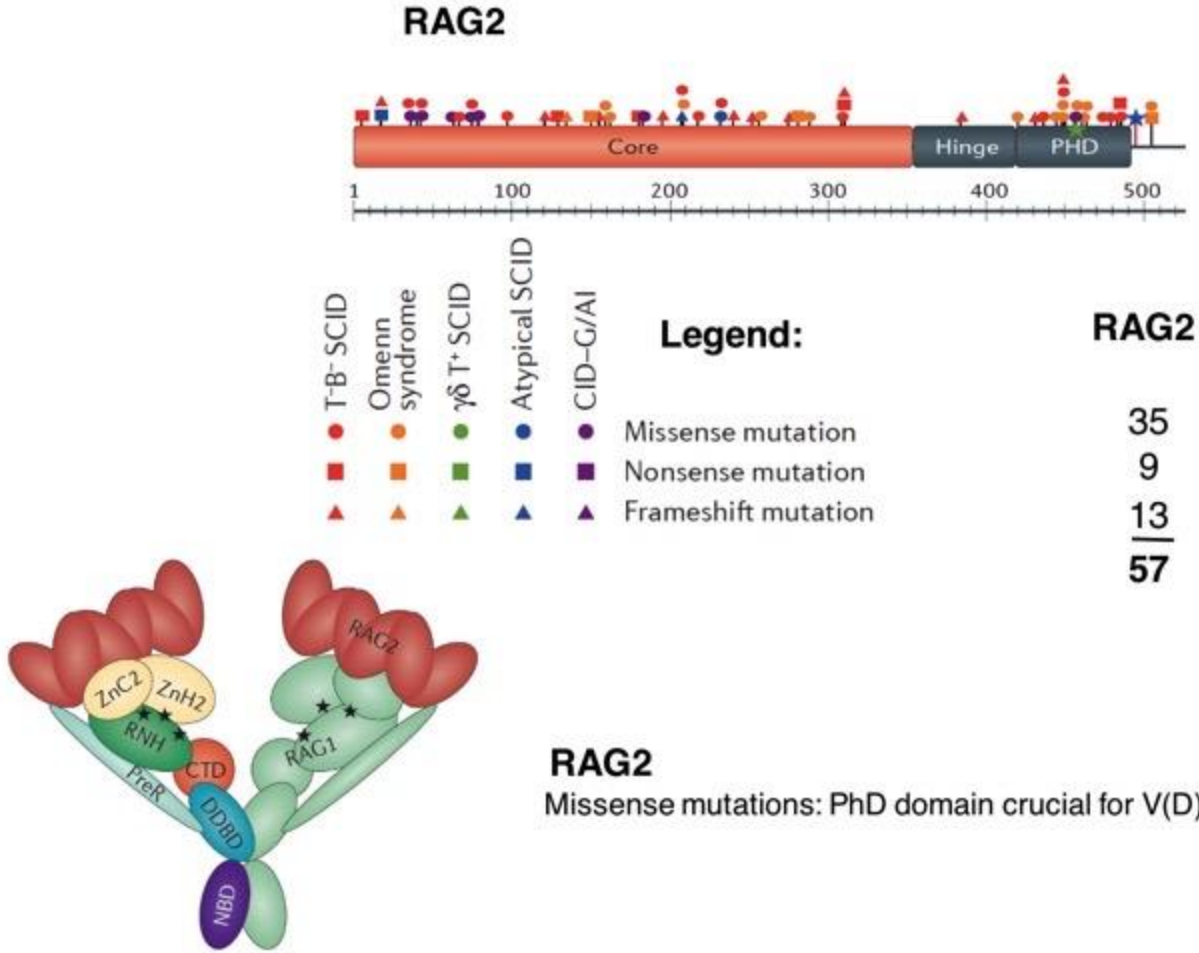
Table I. Autoantibodies in patients with hypomorphic *RAG* mutations

Patients	*n*	*n* (%) with autoantibodies	Specificity
***RAG* deficiency**			
OS/LS	14	9 (64.3)	Five ANA, four TPO, two pANCA, one TG, and one OmpC
T^- B^- SCID	5	0 (0)	
SCIDX1	14	0 (0)	
Controls	6	1 (16.7)	ANA

OS, Omenn syndrome; LS, leaky SCID; TPO, thyroid peroxidase; pANCA, perinuclear anti-neutrophil cytoplasmic antibody; TG, thyroglobulin; and OmpC, outer membrane porin protein C.

Walter J., JEM 2010

Spectrum of RAG2 Mutations



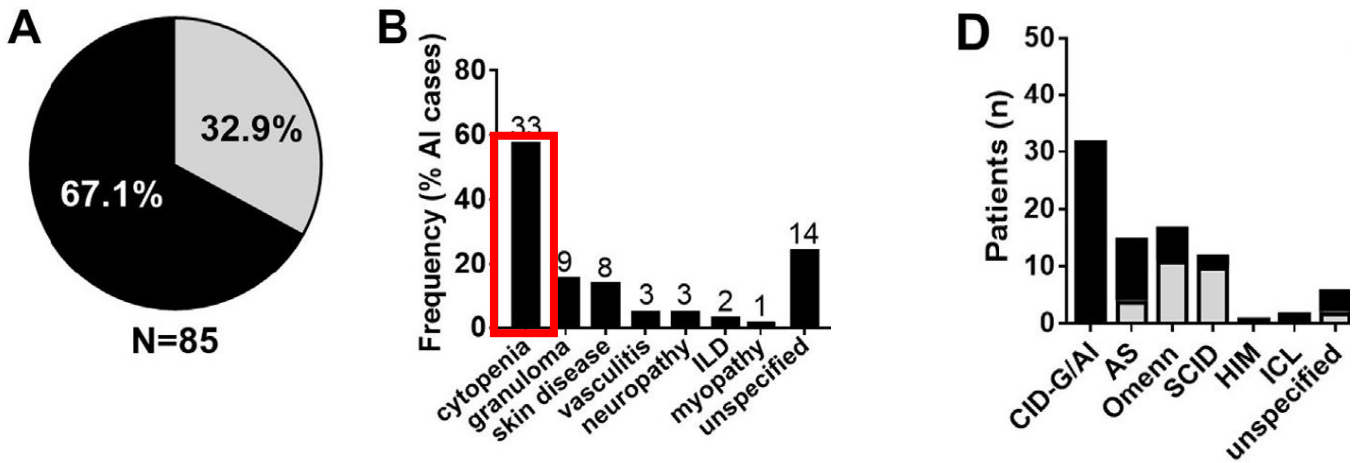
*catalytic residues

RAG2

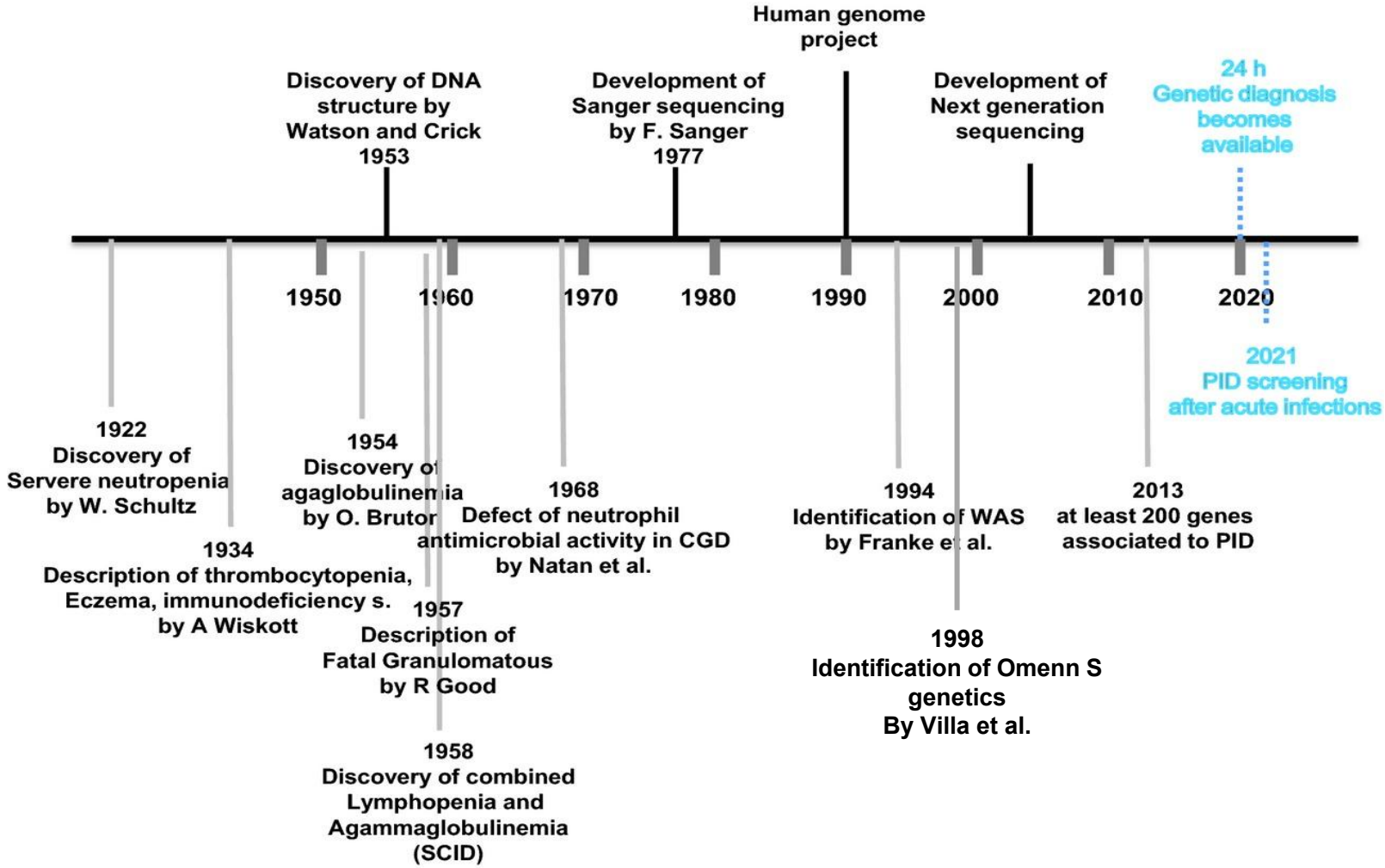
Missense mutations: PhD domain crucial for V(D)J recombination

Notarangelo, L.D., Nature Reviews | Immunology, 2016

Outcomes and Treatment Strategies for Autoimmunity and Hyperinflammation in Patients with RAG Deficiency



Farmer et al., JACI in Practise 2019



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2001 IPEX

The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of *FOXP3*

IPEX is a fatal disorder characterized by immune dysregulation, polyendocrinopathy, enteropathy and X-linked inheritance (MIM 304930). We present genetic evidence that different mutations of the human gene *FOXP3*, the ortholog of the gene mutated in scurfy mice (*Foxp3*), causes IPEX syndrome. Recent linkage analysis studies mapped the gene mutated in IPEX to an interval of 17–20-cM at Xp11.23–Xq13.3 (refs. 1,2).

1). The two affected males tested (V-2 and V-7; Fig. 1*a*) were hemizygous for the mutation. Although the mutant residue threonine at 384 is chemically similar to serine (the most common amino acid found at this residue for human FOX proteins[4]), we hypothesize that the loss of the uniquely hydrophobic alanine residue of the scurfin protein causes IPEX in this family. We used a combination of standard and single-base sequence analyses of an additional 500 control X chromosomes and did not detect the A384T mutation, excluding the possibility that this mutation represents a polymorphism. In family 2 (Fig. 1*b*), we identified a

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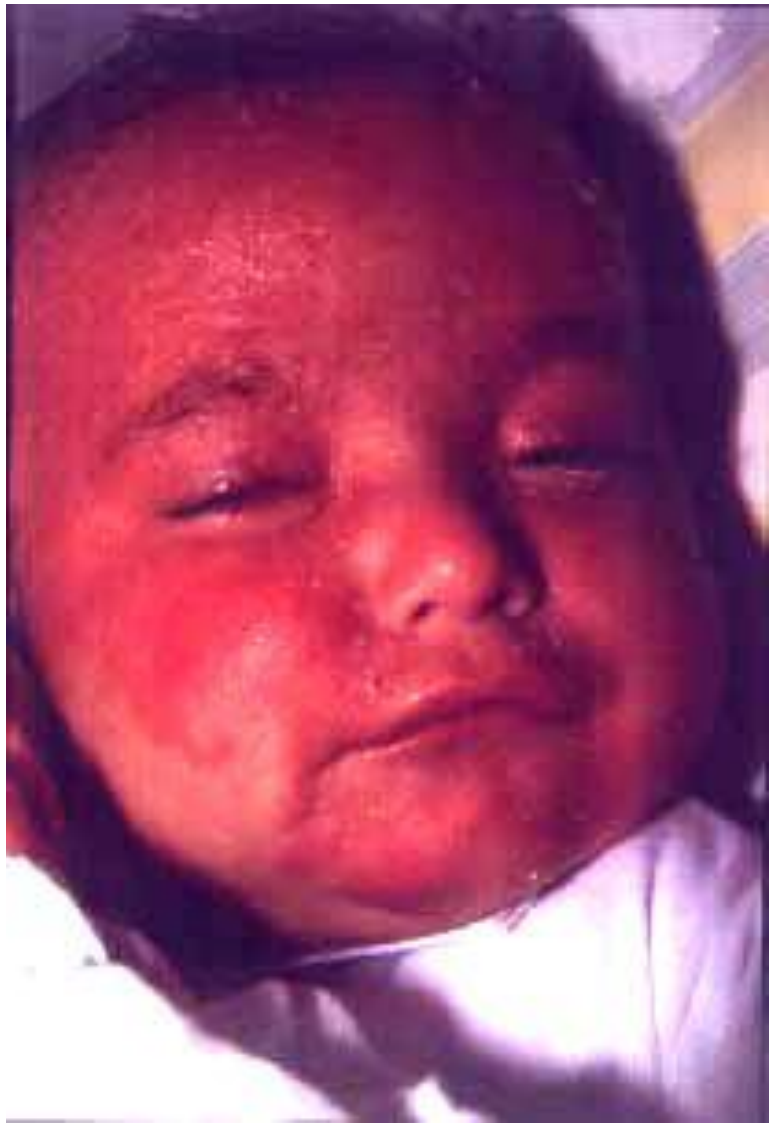
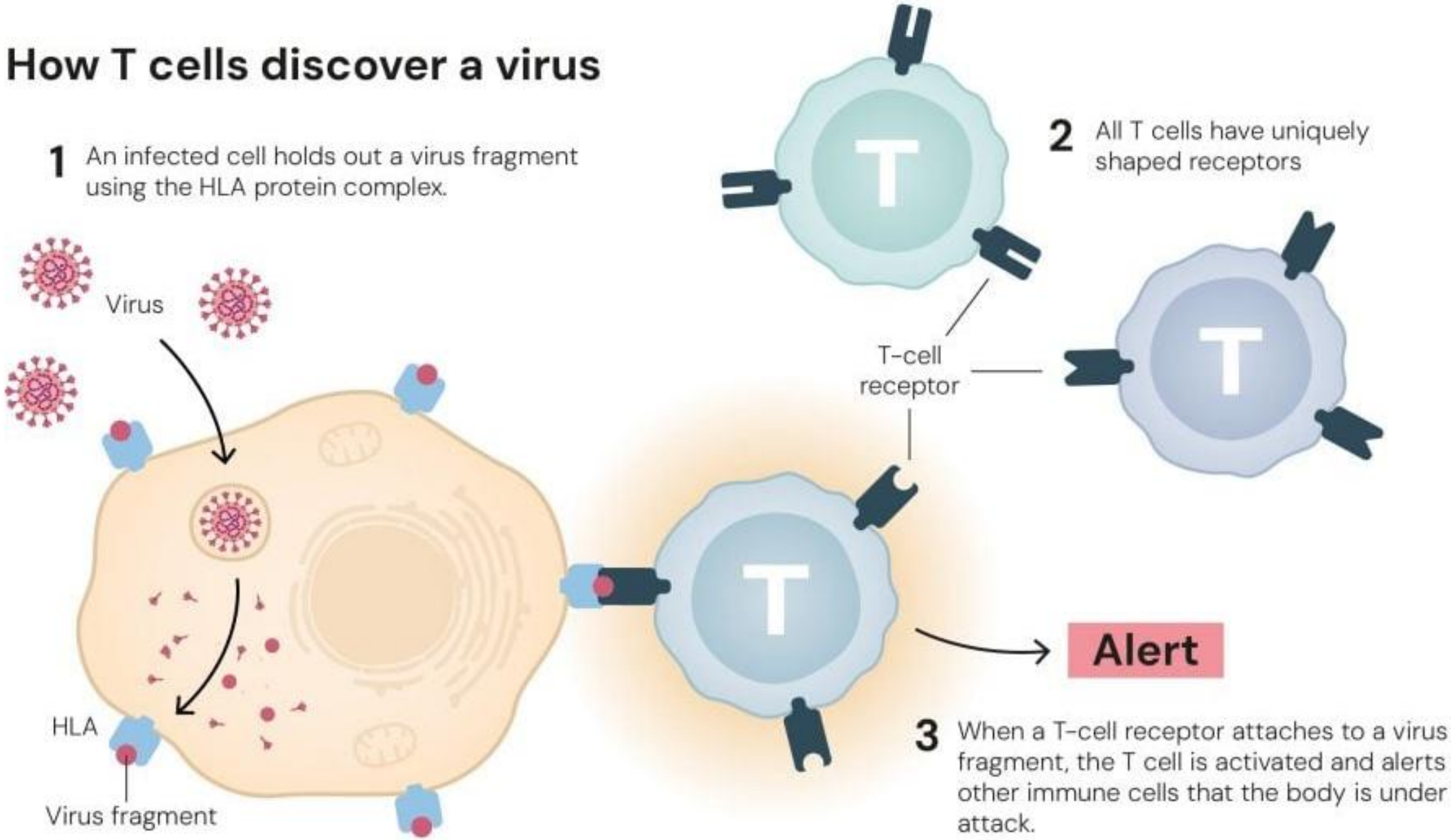
Professor of Pediatrics| Seattle, Washington

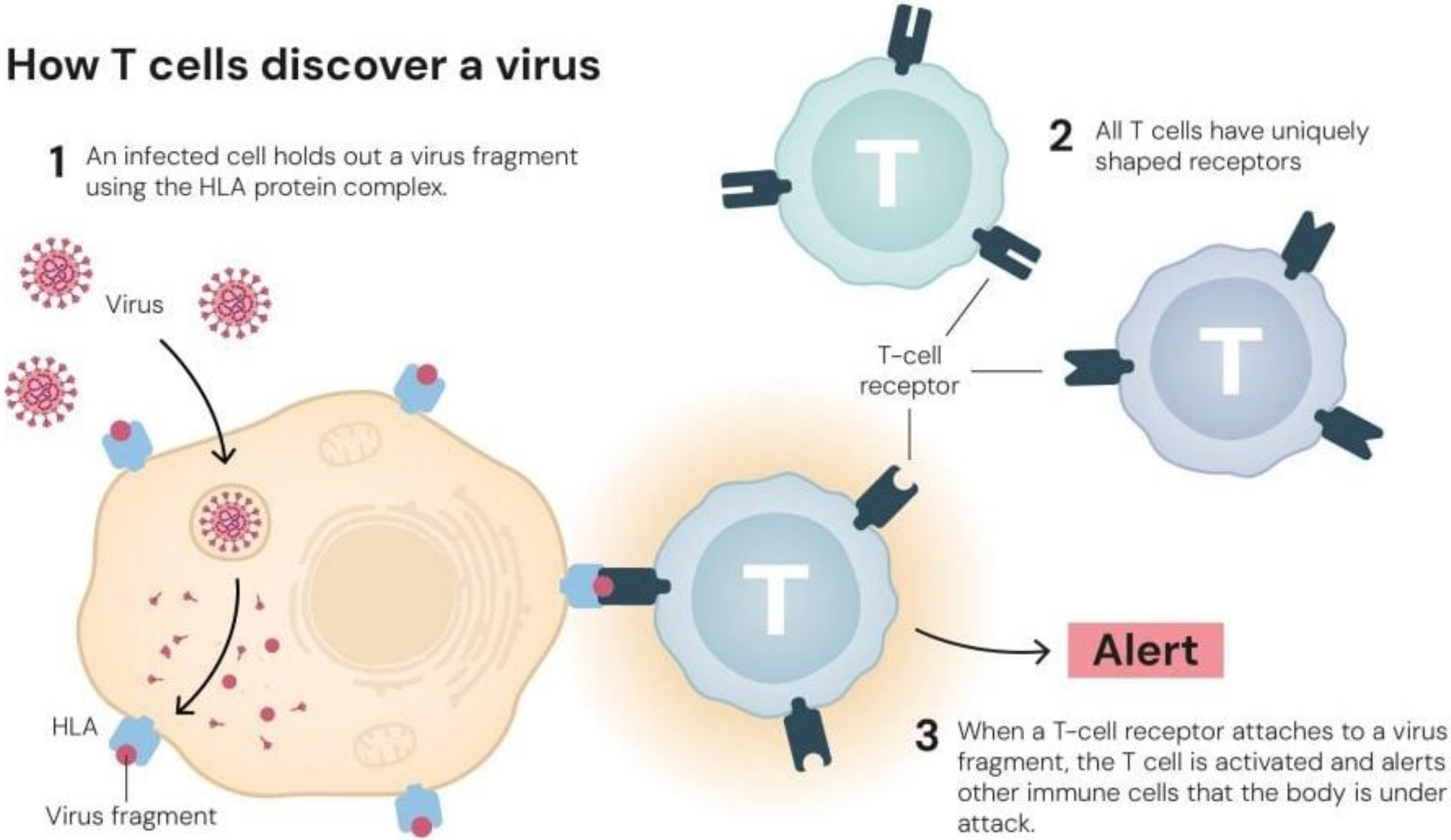
IPEX: clinical and laboratory features

- eczema
- enteropathy (diarrhea, villous atrophy)
- early-onset IDDM
- hypothyroidism
- hemolytic anemia, thrombocytopenia
- cholangitis
- lymphocytic infiltration of various organs
- lung consolidation, infiltrates
- elevated IgE
- increased expression of activation markers on
- CD4+ cells
- normal proliferation to mitogens
- usually lethal within first years





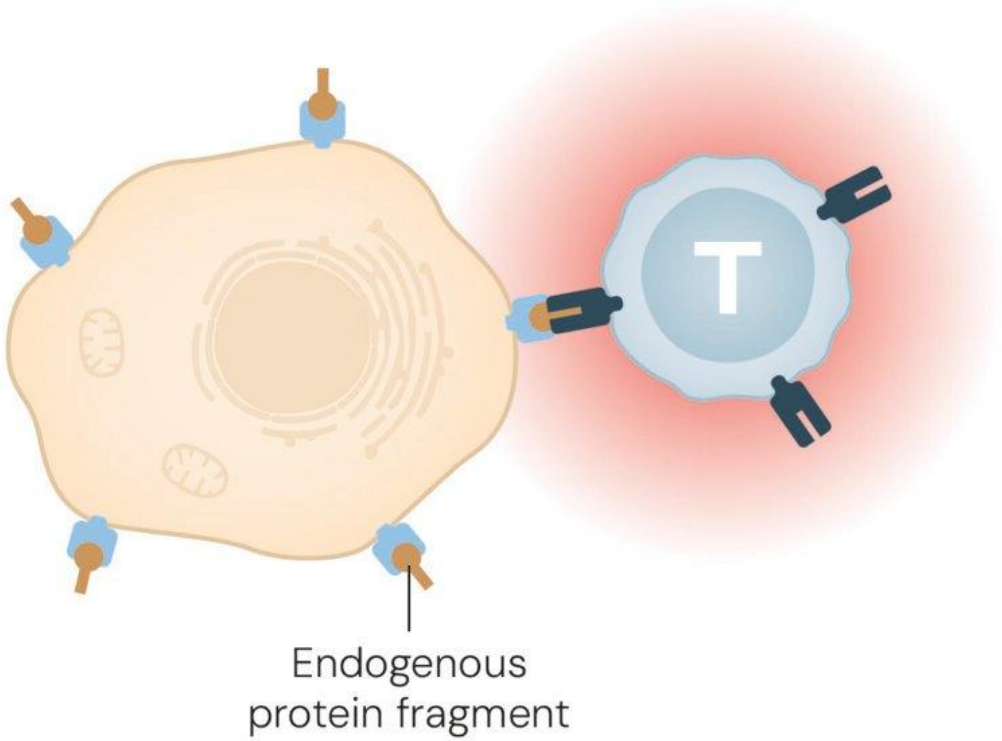
How T cells discover a virus © The Nobel Committee for Physiology or Medicine. Ill. Mattias Karlén



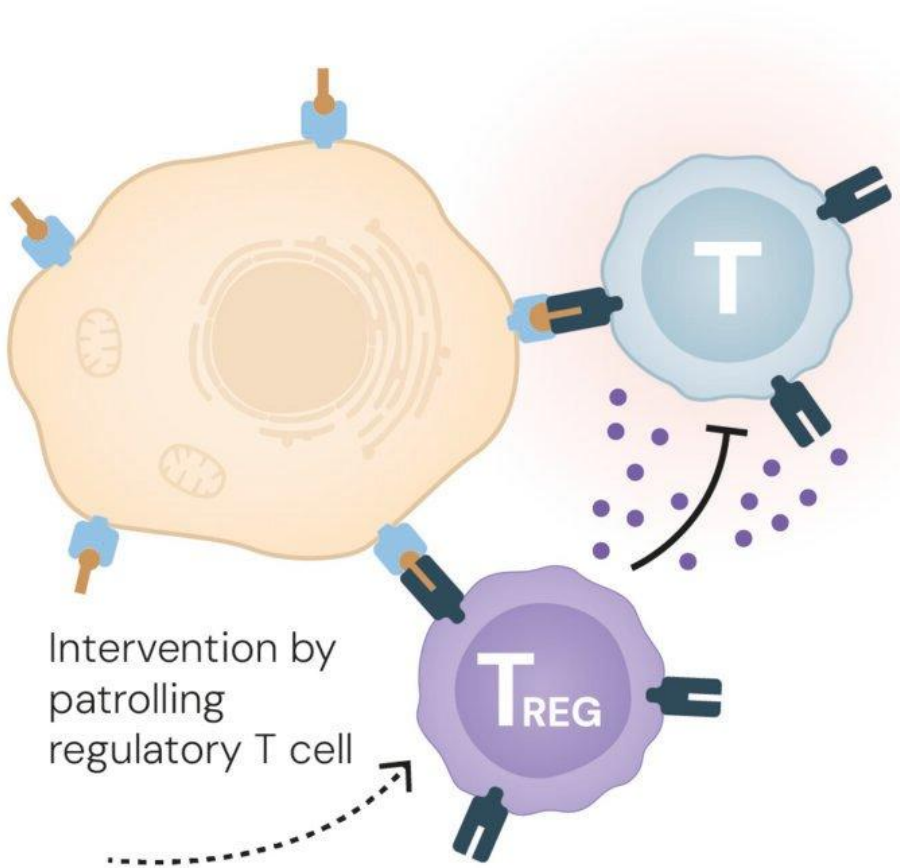
How T cells discover a virus © The Nobel Committee for Physiology or Medicine. Ill. Mattias Karlén

How regulatory T cells protect us

1 A T cell that has slipped through the test in the thymus reacts to a fragment from one of the body's proteins.



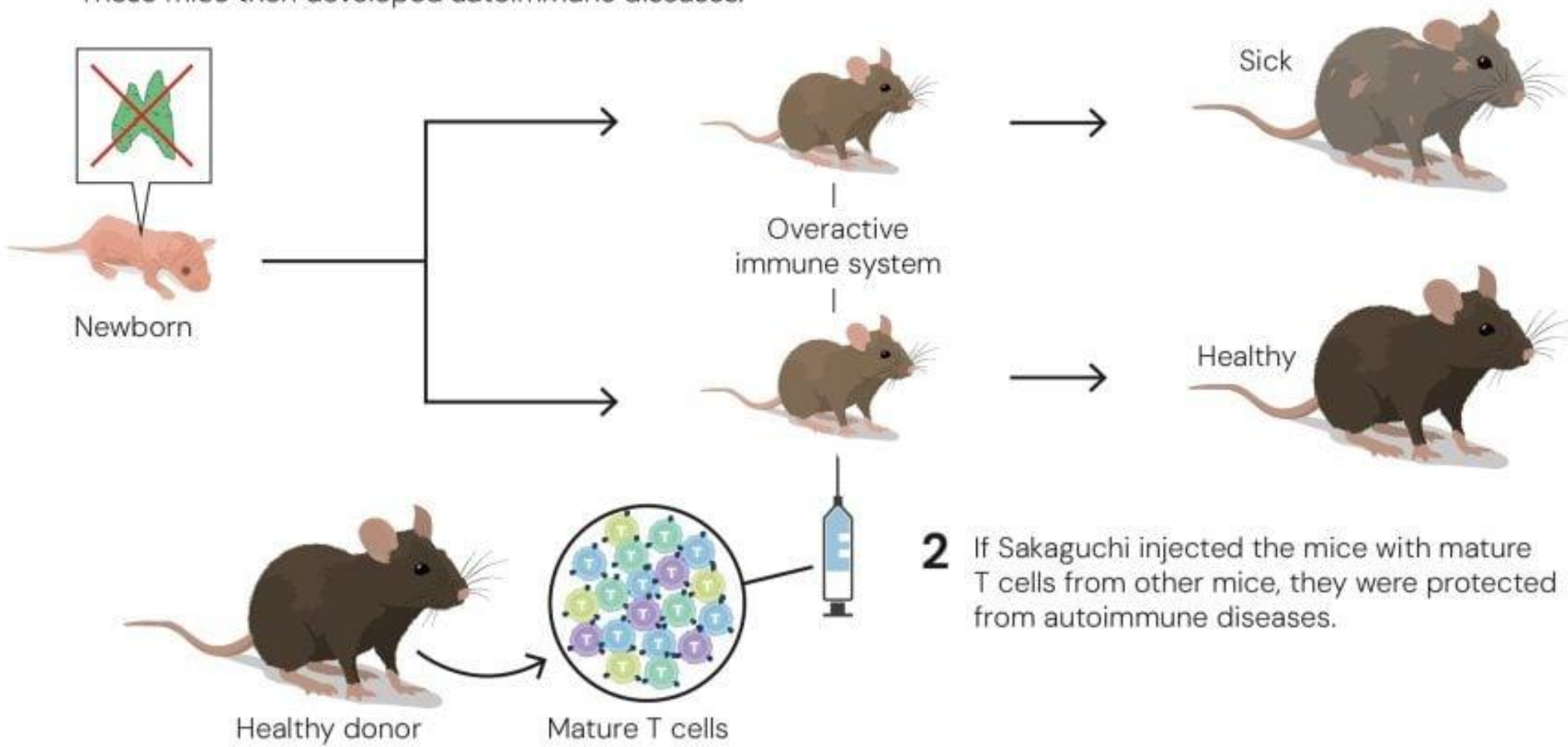
2 Regulatory T cells discover that the attack is a m calm it down. This prevents autoimmune disease



How regulatory T cells protect us © The Nobel Committee for Physiology or Medicine. Illustration: Mattias Karlén

The experiment that inspired Sakaguchi

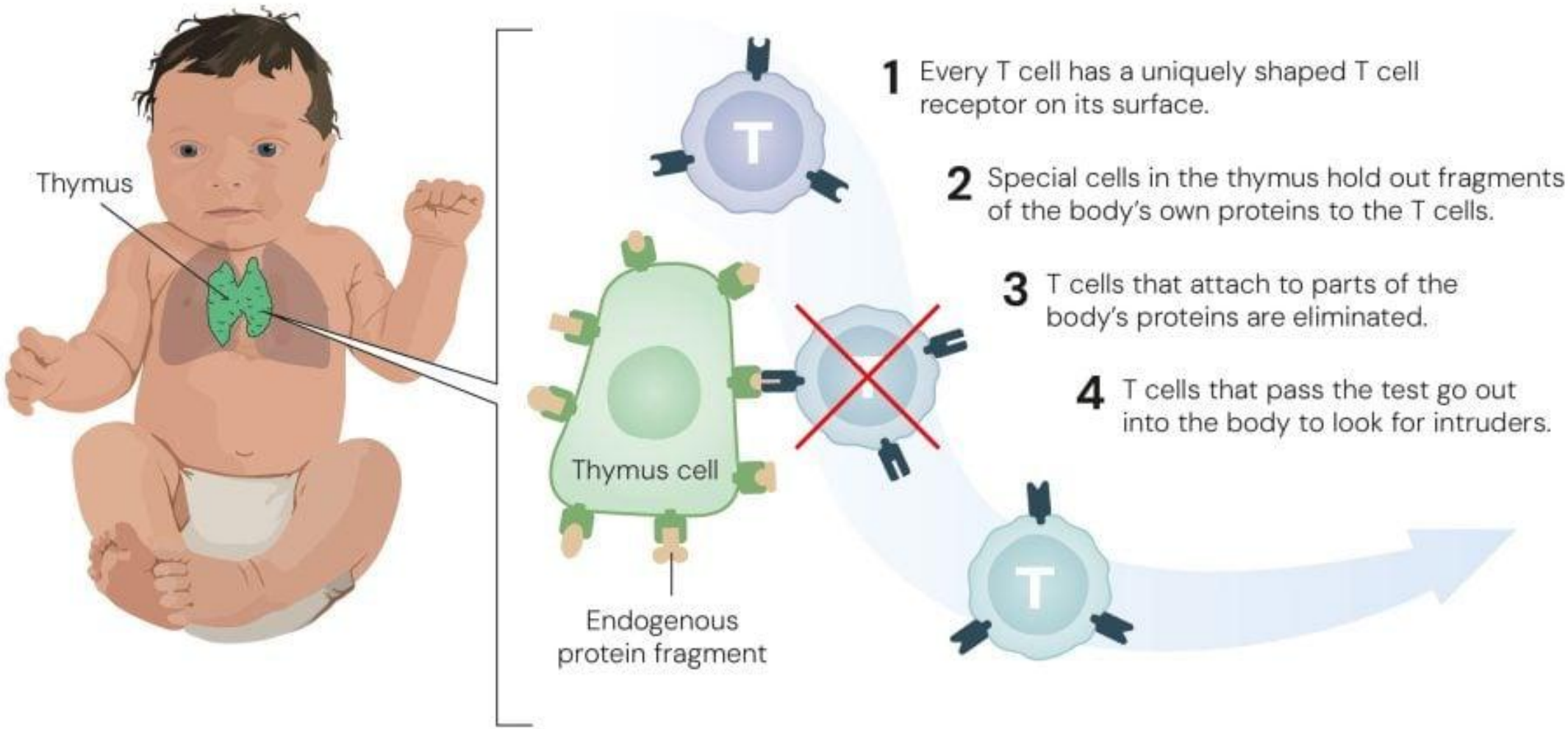
1 Sakaguchi removed the thymus from three-day-old mice. These mice then developed autoimmune diseases.

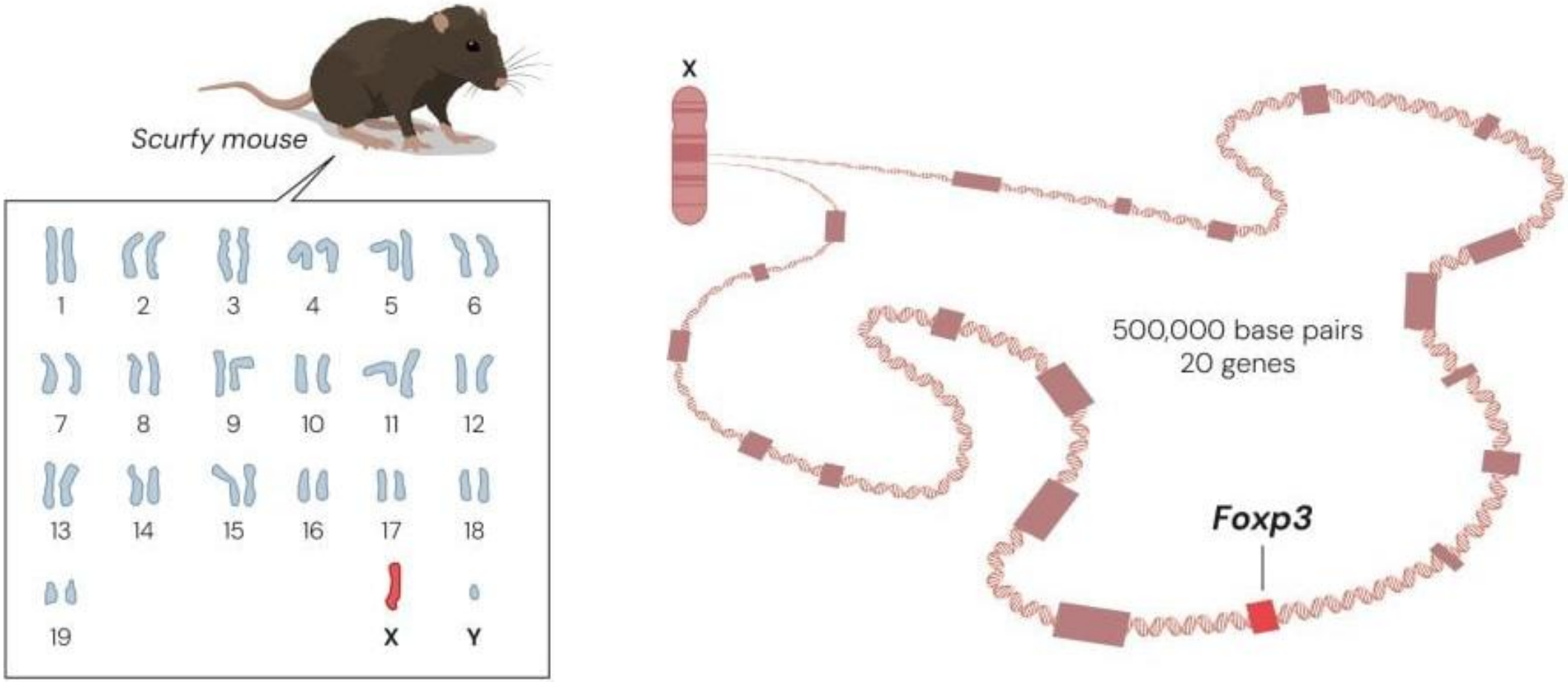


2 If Sakaguchi injected the mice with mature T cells from other mice, they were protected from autoimmune diseases.

How harmful T cells are eliminated

T cells mature in the thymus. The ones that recognise the body's own proteins are sorted and removed.
This process is called central tolerance.





Brunkow and Ramsdell find the scurfy mutation

The scurfy mutation causes a mutiny in the immune system. Brunkow and Ramsdell succeeded in narrowing down the area of the mutation and locating it in the *Foxp3* gene, which turned out to be decisive in the development of regulatory T cells.

Mary E. Brunkow

Institute for Systems Biology,
Seattle, USA

Fred Ramsdell

Sonoma Biotherapeutics,
San Francisco, USA

Shimon Sakaguchi

Osaka University,
Osaka, Japan

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Fabio Bozzi,[1] Silvia Giliani,[3]
Annalisa Frattini,[1] Luisa Imberti,[4]
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1998 Omenn

Introduction

Diversity of the immune repertoire is generated by somatic assembly of the antigen receptor variable gene segments, in a process termed V(D)J site-specific recombination (Tonegawa, 1983; reviewed by Lewis, 1994). This process is dependent on the activation of two key

2001 IPEX

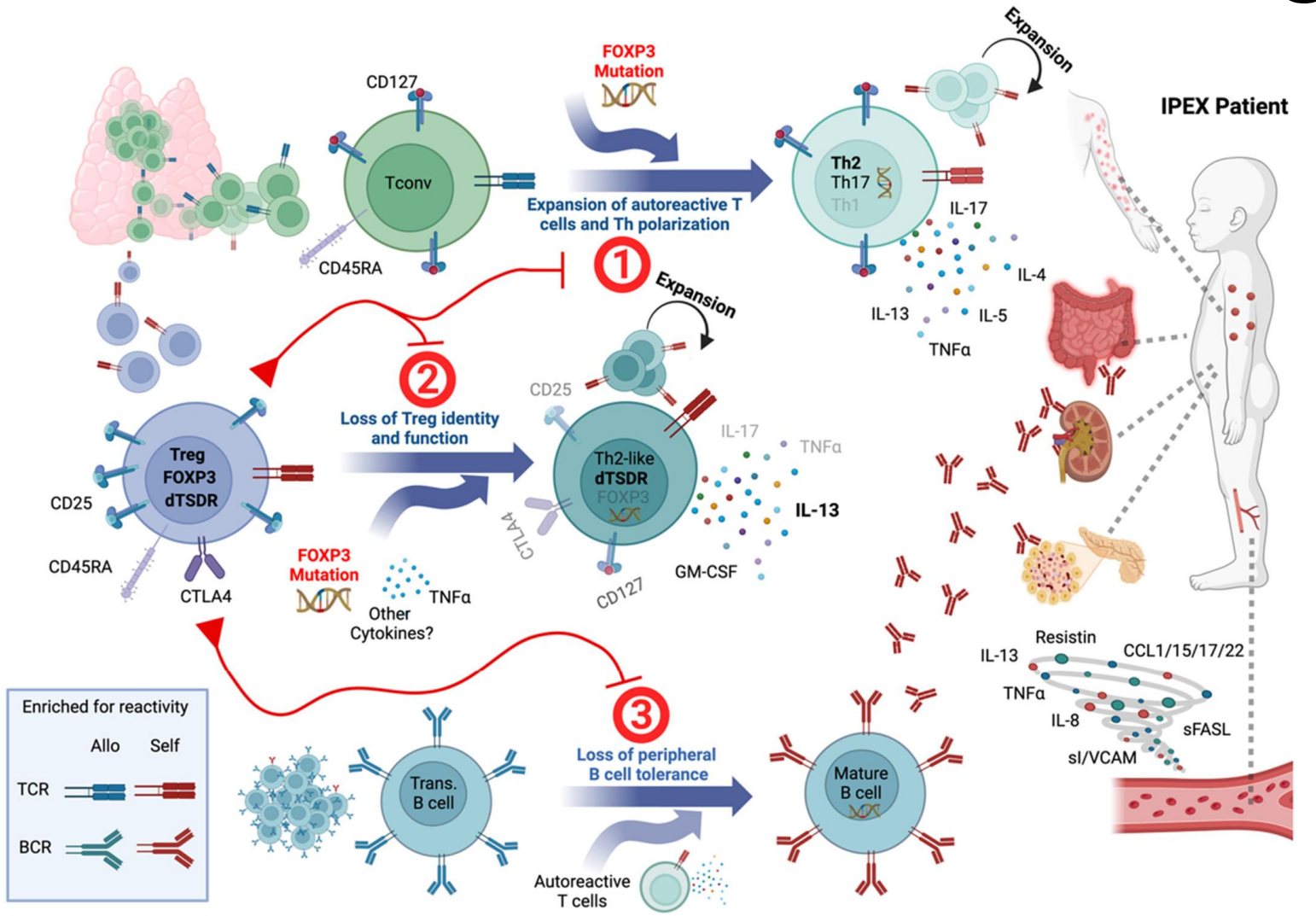
The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of *FOXP3*

IPEX is a fatal disorder characterized by immune dysregulation, polyendocrinopathy, enteropathy and X-linked inheritance (MIM 304930). We present genetic evidence that different mutations of the human gene *FOXP3*, the ortholog of the gene mutated in scurfy mice (*Foxp3*), causes IPEX syndrome. Recent linkage analysis studies mapped the gene mutated in IPEX to an interval of 17–20-cM at Xp11.23–Xq13.3 (refs. 1,2).

1). The two affected males tested (V-2 and V-7; Fig. 1*a*) were hemizygous for the mutation. Although the mutant residue threonine at 384 is chemically similar to serine (the most common amino acid found at this residue for human FOX proteins[4]), we hypothesize that the loss of the uniquely hydrophobic alanine residue of the scurfin protein causes IPEX in this family. We used a combination of standard and single-base sequence analyses of an additional 500 control X chromosomes and did not detect the A384T mutation, excluding the possibility that this mutation represents a polymorphism. In family 2 (Fig. 1*b*), we identified a

Hans D. Ochs
Professor of Pediatrics| Seattle, Washington

IPEX: mechanisms of immunedisregulation

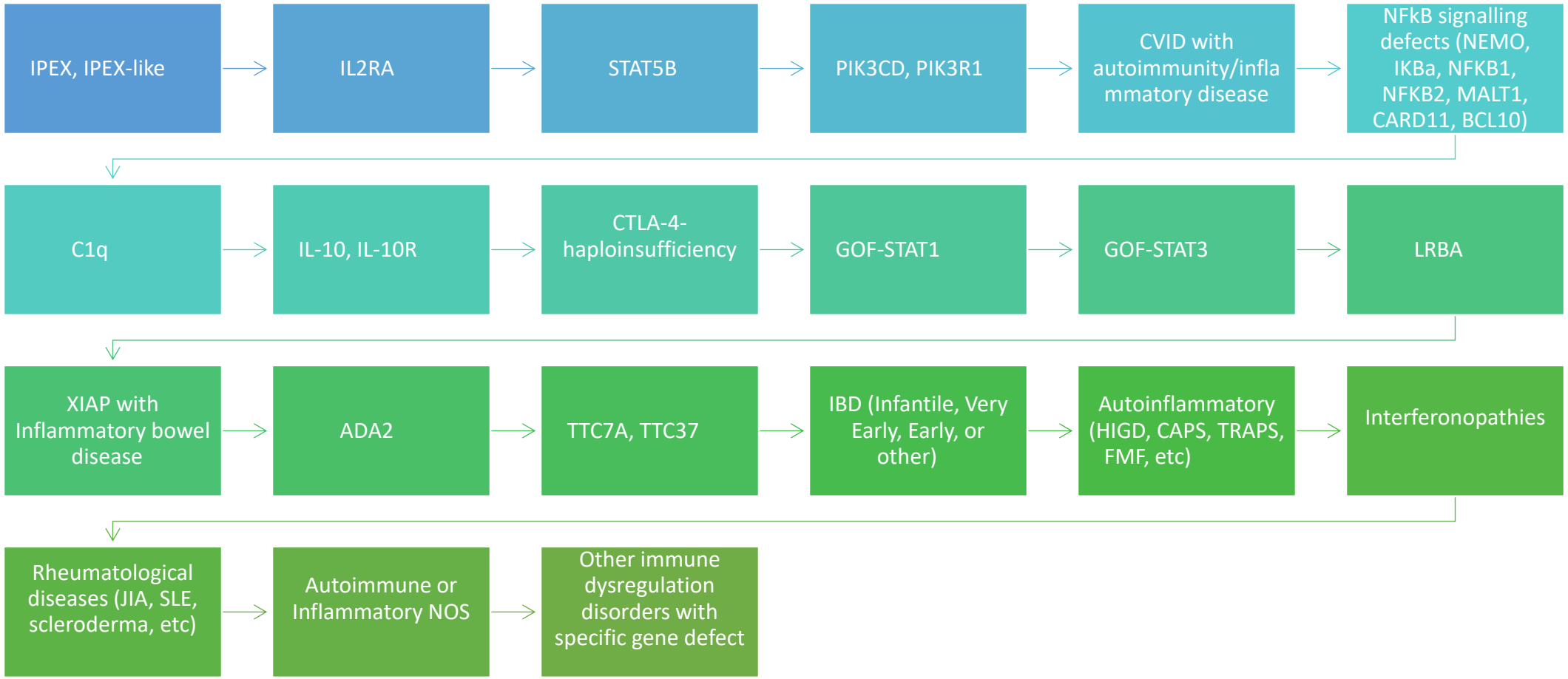


Regulatory T-lymphocyte defect (absent/reduced Tregs and/or functional Treg defect)

IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked)	*FOXP3*	XL	Autoimmune enteropathy, early onset type 1 diabetes mellitus, eczema, thyroiditis, hemolytic anemia, thrombocytopenia, elevated IgE
CTLA4 haploinsufficiency	*CTLA4*	AD	Autoimmunity particularly cytopenias, enteropathy, type 1 diabetes, lymphoproliferation, interstitial lung disease, recurrent infections, hypogammaglobulinemia
LRBA deficiency	*LRBA*	AR	Autoimmunity particularly cytopenias, lymphoproliferation, recurrent infections, enteropathy
STAT3 GOF	*STAT3*	AD	Autoimmunity, lymphoproliferation, infections, short stature
CD25 deficiency	*IL2RA*	AR	Autoimmunity, lymphoproliferation

Flinn AM, et al. Orph J Rare Dis. 2022

List of diseases



Chan A et al., Front Immunol 2020

Errori congeniti dell'immunità

Immunodeficienze

- Infezioni opportunistiche
- Infezioni invasive
- Linfoadenopatia (infettiva)
- Infezioni che richiedono terapia prolungata
- Polmonite
- Ritardo di crescita
- Diarrea

Mal. immunodisregolatorie

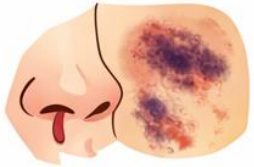
- Linfadenopatia/linfomi
- Citopenie autoimmuni
- Splenomegalia
- Enteropatia
- Endocrinopatia
- LES, autoanticorpi
- Febbri periodiche

***malattie monogeniche**

AIC
Citopenie Immunomediate

ITP
Porpora Trombocitopenica Immune

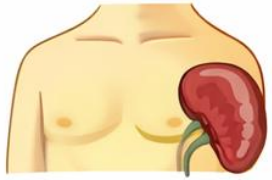
Piastrine Basse



Sanguinamenti ed Ematomi

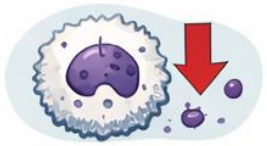
AIHA
Anemia Emolitica Autoimmune

Emolisi con Coombs+



Ittero e Splenomegalia

AIN
Neutropenia Immune



Neutrofili Bassi

Febbri e Infezioni

Sindrome di Evans

Citopenie Combinata

Manifestazioni Autoimmuni

Autoanticorpi Patogeni

Meccanismo Immunitario

CVID = Common Variable Immunodeficiency

NFKB1, CD19, CD20, CD21, BAFFR, TACI, CD81, PIK3CD, PIK3R1, NFKB2 or unknown genes (poligenic-CVID)

Features

1. **Sinopulmonary infections**
2. **Splenomegaly**
3. **Malignancies**
4. **Autoimmune diseases**
   - Autoimmune cytopenia

Autoimmune cytopenia	31.4 %
Gastrointestinal disorders	24.4
Skin	14.1
Rheumatologic disorders	12.8
Endocrine disorders	8.1
Lung	3.5
Eye	1.7
Kidney	1.3
Vasculitis	1.0
Neurologic disorders	1.0
Urologic disorders	0.4
Other	0.3

Fischer et al., JACI 2017

Tuijnenburg et al., JACI, 2018

CLINICAL TRIALS AND OBSERVATIONS

Pediatric Evans syndrome is associated with a high frequency of potentially damaging variants in immune genes

Jérôme Hadjadj,[1,2,*] Nathalie Aladjidi,[3,4,*] Helder Fernandes,[3,4] Guy Leverger,[5] Aude Magérus-Chatinet,[1,2] Fabienne Mazerolles,[1,2] Marie-Claude Stolzenberg,[1,2] Sidonie Jacques,[1,2] Capucine Picard,[2,6] Jérémie Rosain,[2,6] Cécile Fourrage,[7,8] Sylvain Hanein,[9] Mohammed Zarhrate,[8,10] Marlène Pasquet,[11] Wadih Abou Chahla,[12] Vincent Barlogis,[13] Yves Bertrand,[14] Isabelle Pellier,[15] Elodie Colomb Bottollier,[16] Fanny Fouyssac,[17] Pascale Blouin,[18] Caroline Thomas,[19] Nathalie Cheikh,[20] Eric Dore,[21] Corinne Pondarre,[22,23] Dominique Plantaz,[24] Eric Jeziorski,[25] Frédéric Millot,[26] Nicolas Garcelon,[2,27] Stéphane Ducassou,[3,4] Yves Perel,[3,4] Thierry Leblanc,[28] Bénédicte Neven,[1,2,29] Alain Fischer,[2,29-31] and Frédéric Rieux-Laucat[1,2] on behalf of members of the French Reference Center for Pediatric Autoimmune Cytopenia (CEREVANCE)

Gene (no. of patients)	Mutation type and consequences
Pathogenic mutations, n = 32, 40%	
TNFRSF6 (6)	Heterozygous/LOF
CTLA4 (8)	Heterozygous/LOF
STAT3 (6)	Heterozygous/GOF
PIK3CD (1)	Hetezozygous/GOF
CBL (1)	Heterozygous/LOF
ADAR1 (1)	Heterozygous/LOF
LRBA (4)	Homozygous/LOF
RAG 1 (2)	Compound heterozygous/LOF
TNFRSF6 somatic (1)	Heterozygous/LOF
KRAS somatic (2)	Heterozygous/GOF

Gene (no. of patients)	Mutation type and consequences
Probably pathogenic mutations, n = 20, 25%	
Immune cell receptors	
IFNAR1 (1)	Homozygous/likely LOF
TNFR2 (1)	Heterozygous/likely GOF
TGFBR2 (1)	Heterozygous/likely LOF
Intracellular signaling	
JAK1 (2)	Heterozygous/likely GOF
JAK2 (1)	Heterozygous/likely GOF
PLCG2 (1)	Heterozygous/likely GOF
TRAF3 (1)	Heterozygous/likely GOF
CARD11 (1)	Heterozygous/likely GOF
ARHGEF4 (1)	Heterozygous/likely GOF
PTPN11 (1)	Heterozygous/likely GOF
PARP4 (1)	Compound heterozygous/likely LOF
Apoptosis regulation	
RIPK2 (2)	Heterozygous/likely LOF
APAF1 (1)	Heterozygous/likely GOF
Transcription factors	
IKZF1 (2)	Heterozygous/likely GOF
NFATC1 (2)	Heterozygous/likely GOF
IKZF2 (1)	Heterozygous/likely LOF

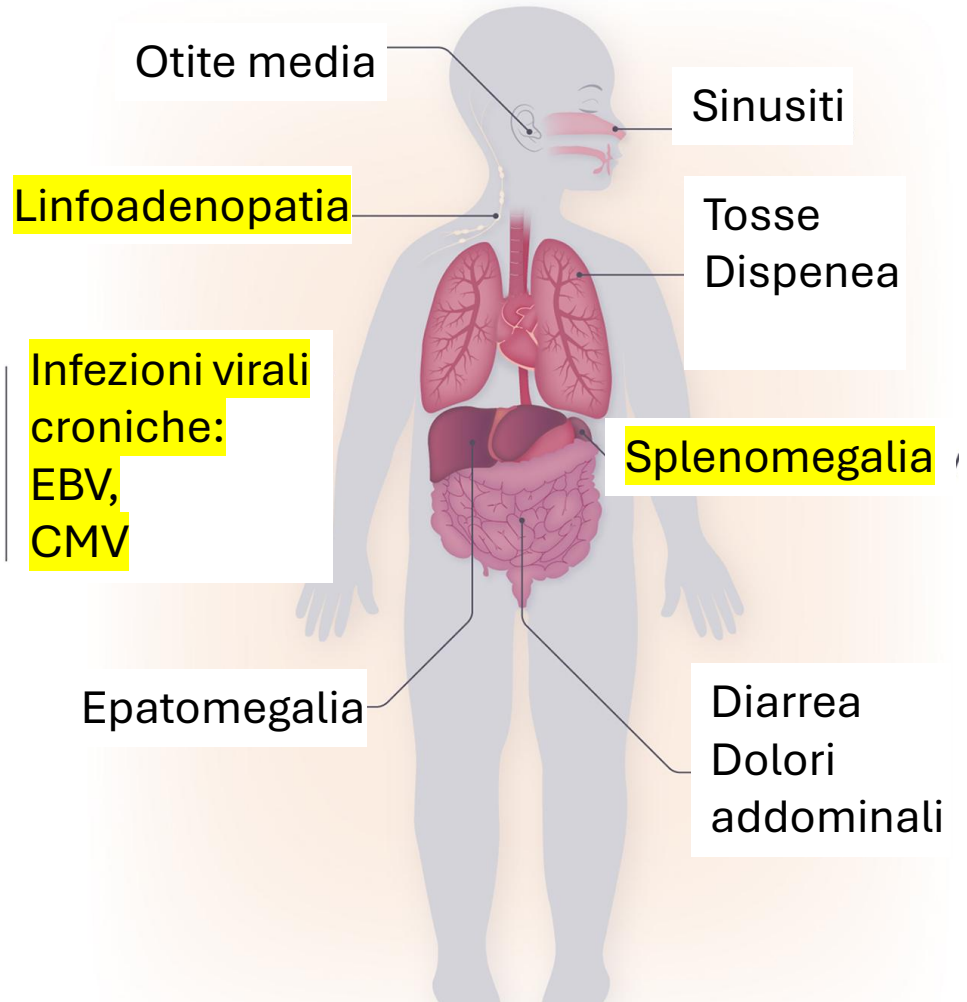
Blood, 2019

Target Therapies nelle Immunodeficienze Primitive

Anno	Immunodeficienza / Condizione	Bersaglio molecolare	Farmaco mirato
2000	IPEX (FOXP3 mutato), disregolazione Treg	mTOR	Sirolimus
2007	Malattia granulomatosa cronica (CGD), difetti inflammasoma	TNF, IL-1, IL-6	Anti-TNF / Anakinra / Canakinumab / Tocilizumab
2011	Deficit di CTLA-4 o LRBA	CTLA-4 pathway	Abatacept (CTLA-4-Ig)
2017	STAT1/STAT3 gain-of-function	JAK-STAT pathway	Ruxolitinib, Baricitinib
2018	HLH genetiche (difetti perforina/esocitosi)	IFN-γ	Emapalumab
2022	APDS (Activated PI3Kδ Syndrome)	PI3Kδ	Leniolisib

APDS

- Infezioni respiratorie
- Bronchiettasie
- **Linfoadenopatia cronica**
- **Infezioni da virus herpetici: EBV, CMV**
- **Citopenia autoimmune**
- Manifestazioni gastrointestinali
- Rischio di linfoma
- Lieve ritardo dello sviluppo neurologico (APDS2)
- Genetica: autosomica dominante

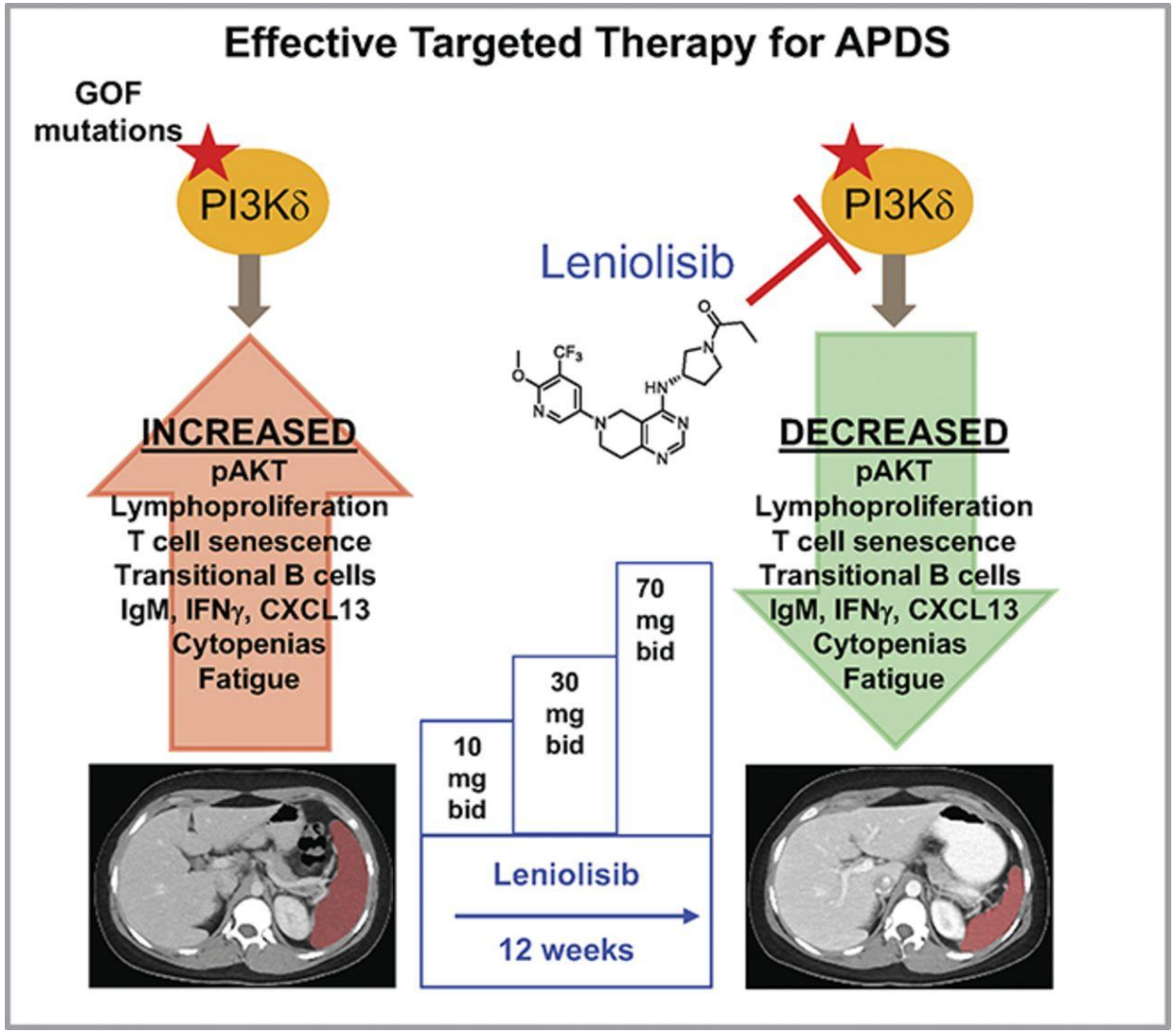


APDS1: PIK3CD: p110-delta

APDS2: PIK3R1: p85

modificato da Newmann et al., Blood 2023

Effective “activated PI3Kδ syndrome”–targeted therapy with the PI3Kδ inhibitor leniolisib



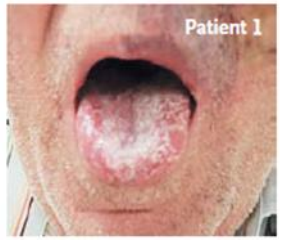
V. Koneti Rao, et al., Blood, 2017

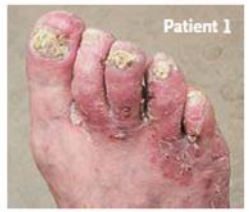
STAT1-CMC: Candidiasis associated with STAT1 mutations

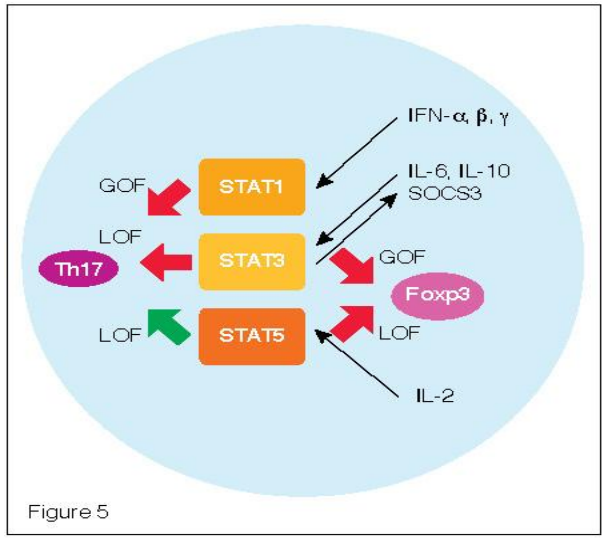
Genes: STAT1 gain of function:

increased STAT1 phosphorylation

Autosomal dominant inheritance



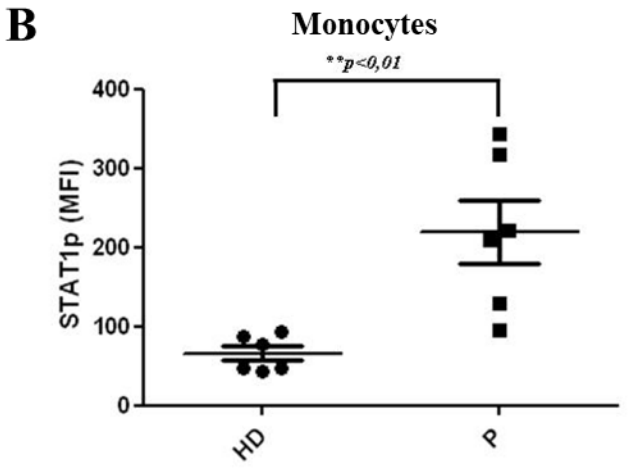




Features

1. **Candidiasis**
2. **Respiratory infections**
3. **Autoimmunity**

   Thyroidites, vitiligo
4. **Malignancies**

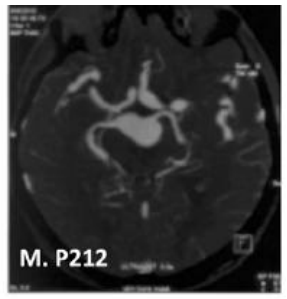


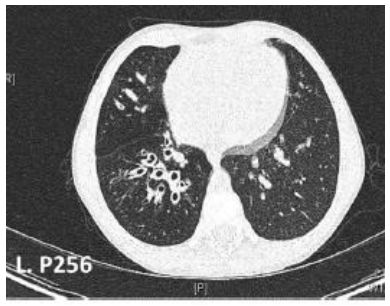
Dotta et al., J Clin Immunol 2016;36:73-84

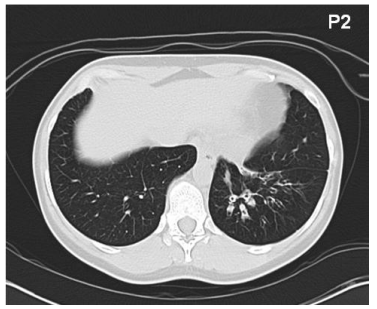
- ✓ **Tiroidite**
- ✓ **Malattia infiammatoria cronica intestinale**
- ✓ **Diabete Mellito Tipo 1**
- ✓ **Lupus Eritematoso Sistemico**
- ✓ **Citopenia autoimmune**
- ✓ **Anemia aplastica**
- ✓ **Epatite autoimmune**

- ✓ **Tumori**

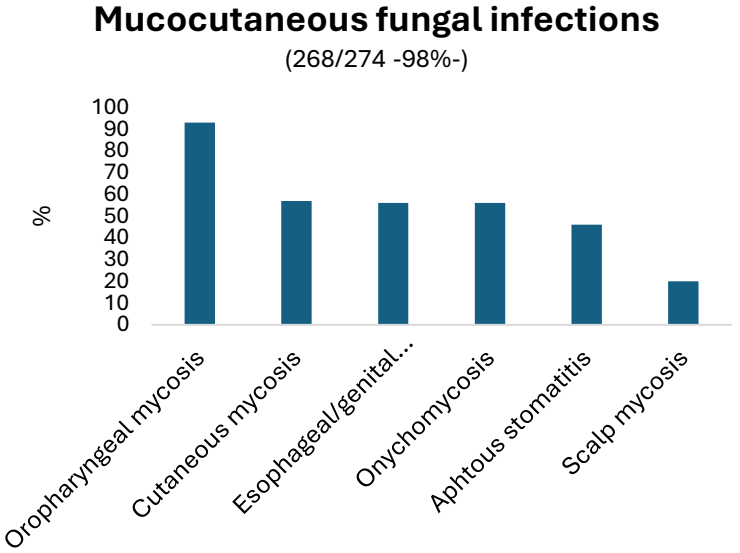
- ✓ **Neuroinfiammazione**
  - ✓ **Vasculopatie**
  - ✓ **Aneurismi**
  - ✓ **Deficit sviluppo**







- ✓ **Candidiasi mucocutanea cronica**



- ✓ **Infezioni batteriche e virali**
  - Otiti, polmoniti ricorrenti, ascessi cutanei, sepsi (*S. pneumoniae, P.aeruginosa, H.influenzae, S.aureus, micobatteri*)
  - Herpes, Zoster, Varicella complicata, CMV/EBV, HPV (verruche), mollusco contagioso
- ✓ **Altre infezioni fungine e parassitarie**
  - Dermatofiti
  - Infezioni invasive (*Aspergillus spp., Criptococcus spp., Histoplasma spp., Coccidioides spp., Trichosporon;* giardiasi, leishmaniosi, mucormicosi)
- ✓ **Malattia polmonare cronica**

Sindrome STAT1-GOF e tumori

Over-espressione STAT1:

- Induzione molecole inibitorie (es.PD-L1) → immunoevasione, perdita di efficacia di chekpoint inhibitors
- Ridotta sensibilità a farmaci citotossici e radiazioni

Trattamento con JAK inibitori (i.e. ruxolitinib):

- Riduzione della sorveglianza mediata da IFNs, NK/T citotossici
- Rischio tumori cutanei non melanocitari, linfomi (EBV), tumori solidi

Table 4. Other clinical features and outcome of patients with *STAT1* GOF mutations

	Patients (%)
Noninfectious phenotypes	**n = 274**
Autoimmunity/inflammatory disease	101 (37)
Thyroid disease	61 (22)
Other endocrine disease*	12 (4)
Skin disease†	28 (10)
Gastrointestinal disease‡	11 (4)
Autoimmune hepatitis	6 (2)
Autoimmune cytopenia§	11 (4)
Others‖	3 (1)
Aneurysm	17 (6)
Cerebral	14 (5)
Extracerebral	3 (1)
Tumor	17 (6)
Benign	2 (0.7)
Squamous cell carcinoma	11 (4)
Gastrointestinal carcinoma	2 (0.7)
Others¶	3 (1)
Other clinical features	
Asthma/eczema	54 (20)
Bone fragility	5 (2)
Clinical outcome	
Failure to thrive	33 (12)
Dysphagia/esophageal stenosis	31 (11)
Bronchiectasis	57 (21)
Death	34 (12)

*Diabetes mellitus, Addison's disease, growth hormone deficiency.
†Systemic lupus erythematosus (SLE), vitiligo, psoriasis, alopecia, scleroderma.
‡Biermer anemia, celiac disease, colitis.
§Immunological anemia or thrombocytopenia.
‖Multiple sclerosis, ankylosing spondylitis.
¶Melanoma, basal cell carcinoma, acute lymphoblastic leukemia.

CTLA4 deficit

Egg et al., JACI 2022

123 pazienti

77

pazienti sintomatici

62% della coorte totale; 32/77 erano già in IgRT prima della comparsa della citopenia autoimmune.

Messaggio chiave: la IgRT non previene questa complicanza in modo affidabile.

45

ITP

manifestazione più frequente tra i pazienti sintomatici

43

Evans syndrome

quasi sovrapponibile a ITP per frequenza

Spettro delle citopenie autoimmuni

Risposta ai trattamenti

- **Corticosteroidi:** 50 trattati, 43 responder transitori (89%).
- **Rituximab:** 30 trattati, 25 responder (83%); 3 pazienti hanno risposto anche a un secondo ciclo.
- **Immunoglobuline EV:** 6 trattati, 5 responder (83%).
- **Splenectomia:** 16 pazienti, età mediana 20 anni, risposta sostenuta solo in 4 (25%).
- **Abatacept:** beneficio riportato in 3 casi cronici, uno con ITP, uno con AIHA e uno con PRCA.

LRBA deficit

PARAMETRO	G0	G1	G2	G3	G4
A. Autoimmune cytopenia	24	9	10	23	2
B. Enteropathy / IBD	13	5	10	31	9
C. Lymphoproliferation / spleno-hepatomegaly	13	14	23	17	1
D. Parenchymal lung disease / GLILD / LIP	28	4	7	23	6
E. Skin or eye manifestations	43	4	11	9	1
F. Endocrinopathy	50	5	1	10	2
G. Arthritis / musculoskeletal	50	4	4	9	1
H. Autoimmune hepatitis / cholangitis / pancreatitis	55	8	2	3	0
I. Renal involvement	56	2	5	2	3
J. Neurologic manifestations	53	1	2	6	7
K. Failure to thrive / malabsorption / wasting	19	11	13	22	3
L. Severe / opportunistic infections	18	6	22	15	7

INTERPRETAZIONE DEL GRADING

- **Grade 0**: assente / inattivo
- **Grade 1**: lieve, transitorio, senza terapia
- **Grade 2**: moderato, terapia intermittente
- **Grade 3**: severo, terapia continuativa
- **Grade 4**: life-threatening / refrattario / irreversibile

31
massimo G3: enteropatia/IBD

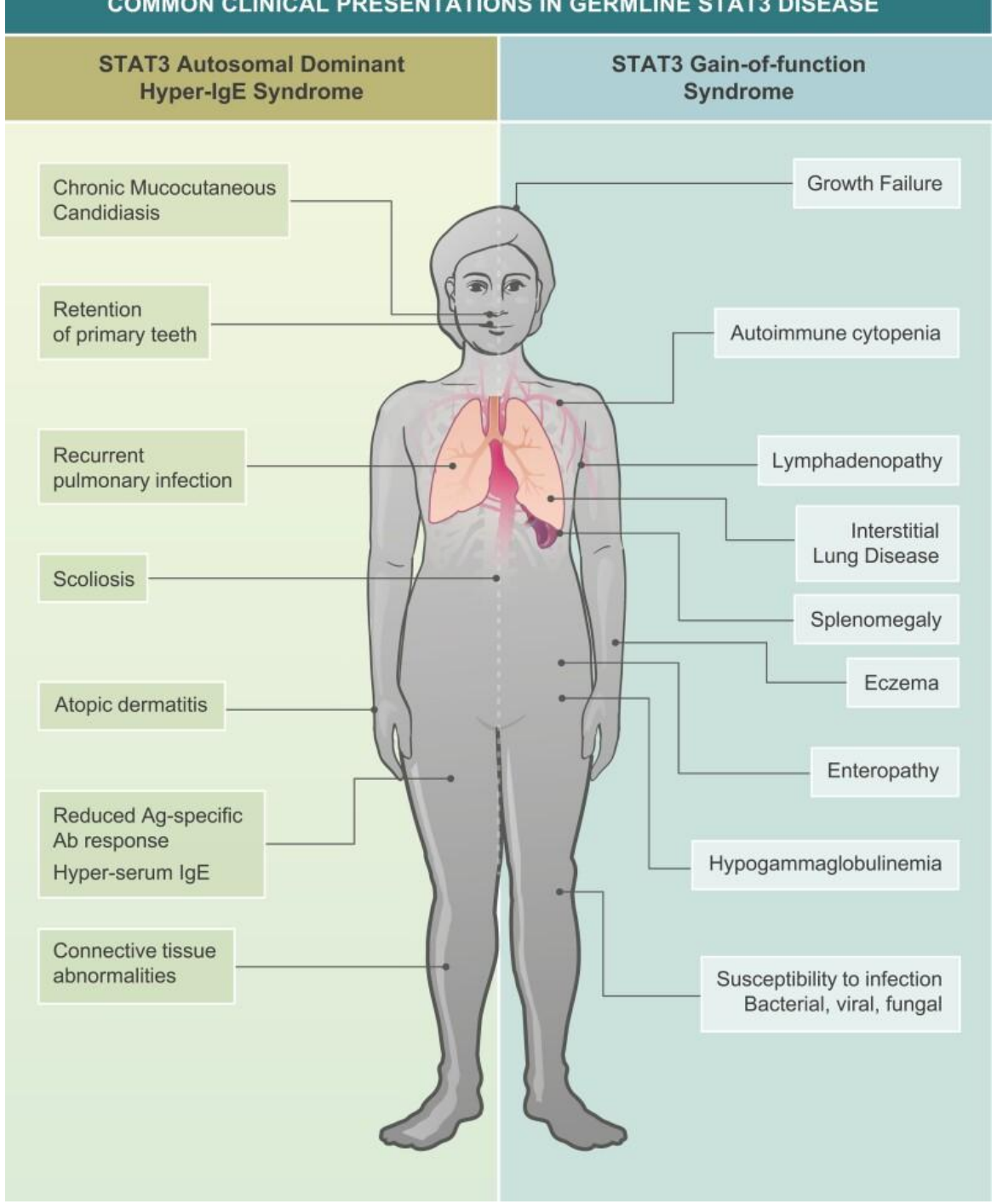
9
massimo G4: enteropatia/IBD

23
G3 anche in citopenia autoimmune

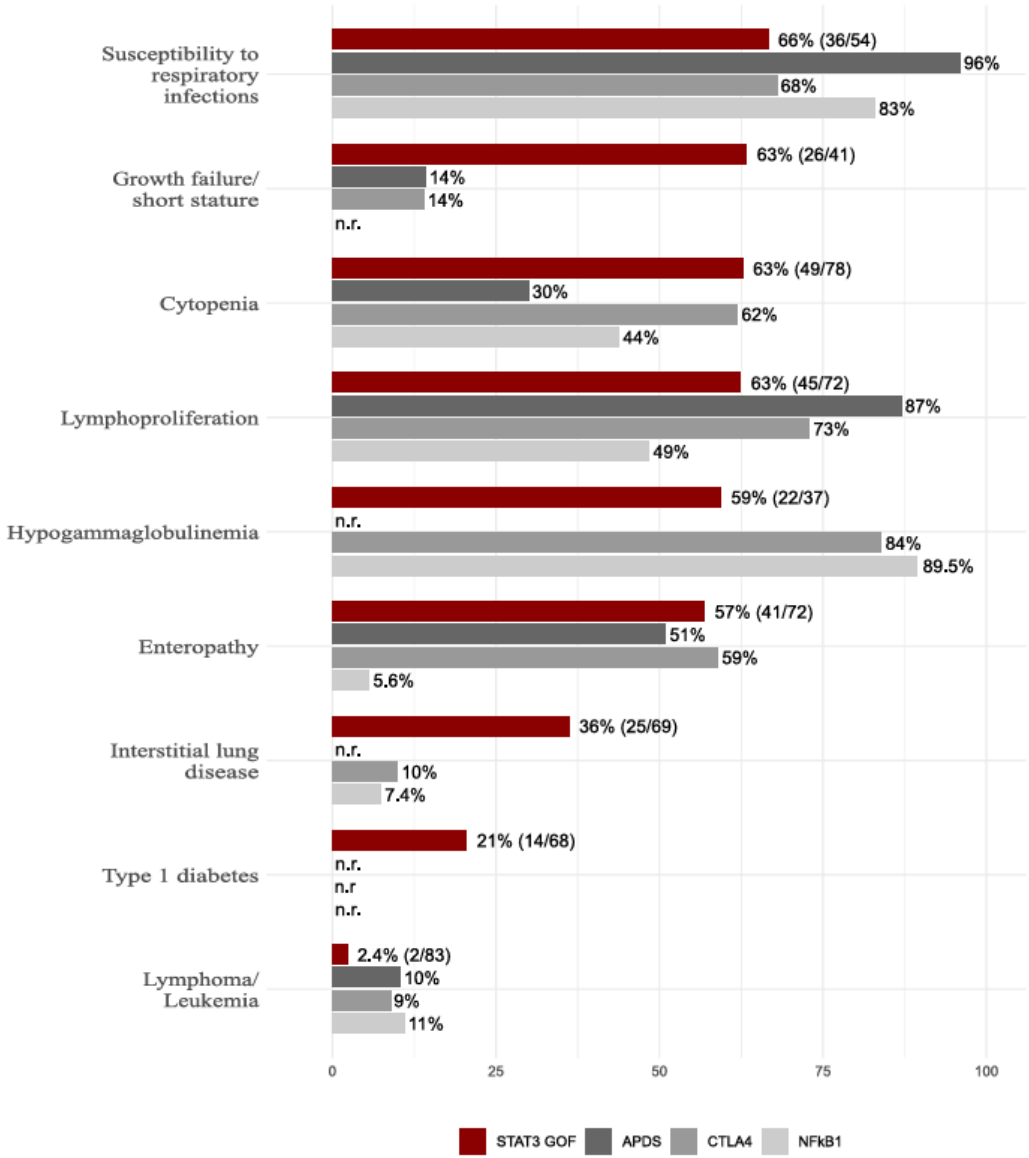
7
G4 neurologico e infezioni severe

Tesch et al., JACI 2020

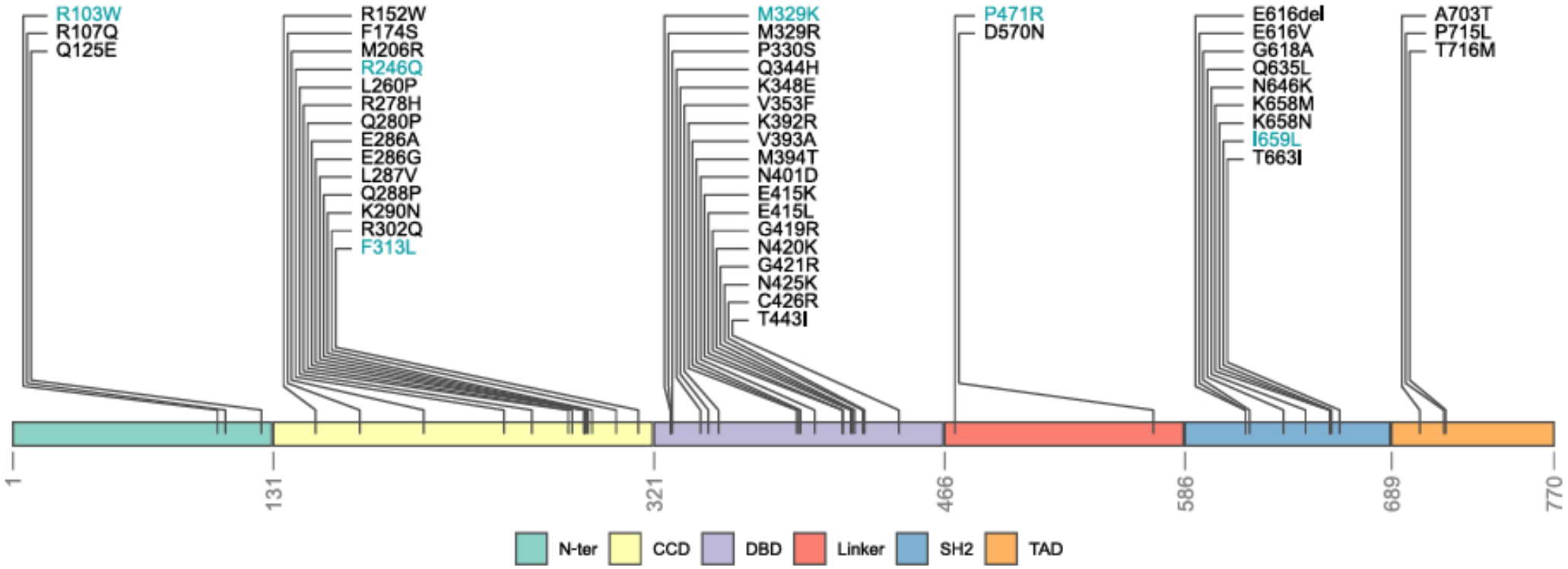
. 2023



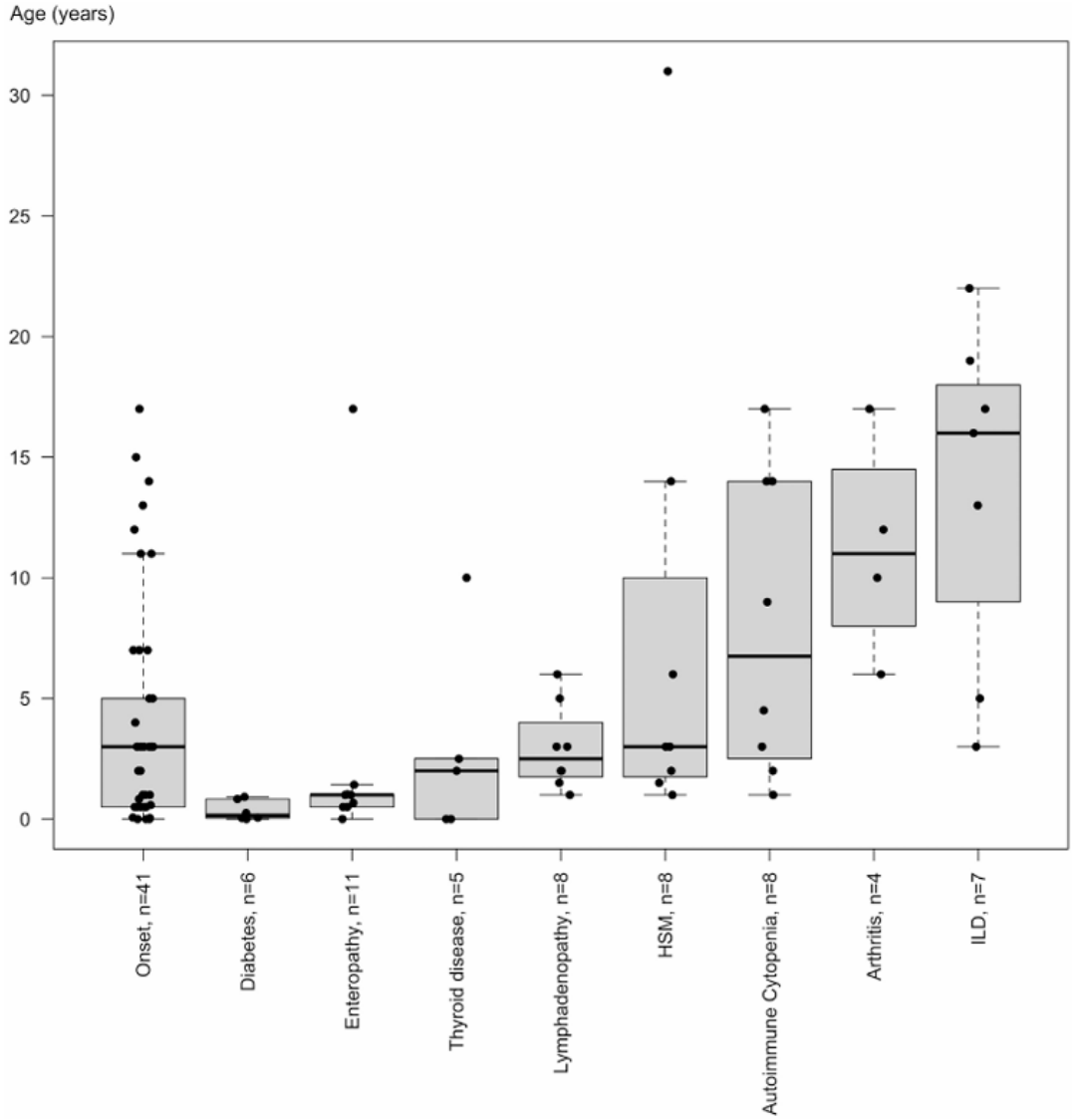
Mackie eta l., Clin Exp Immunol 2023



Faletti et al., Biomedical journal 2021



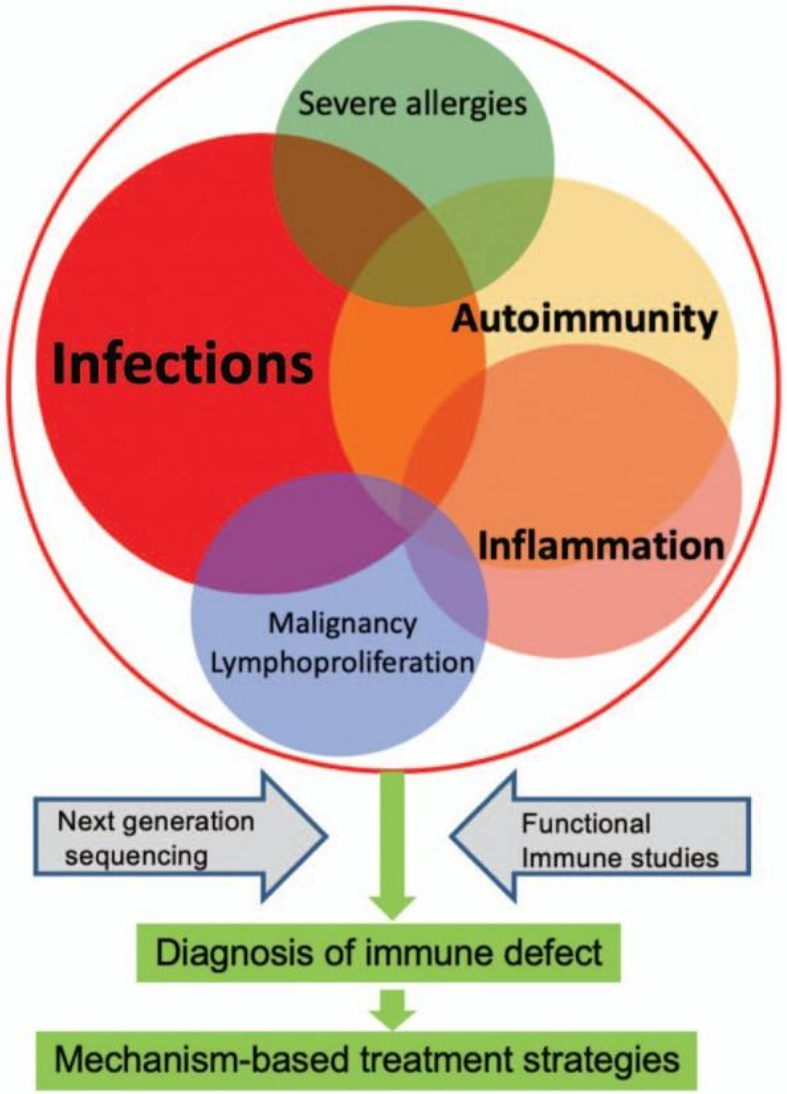
Faletti et al., Biomedical journal 2021

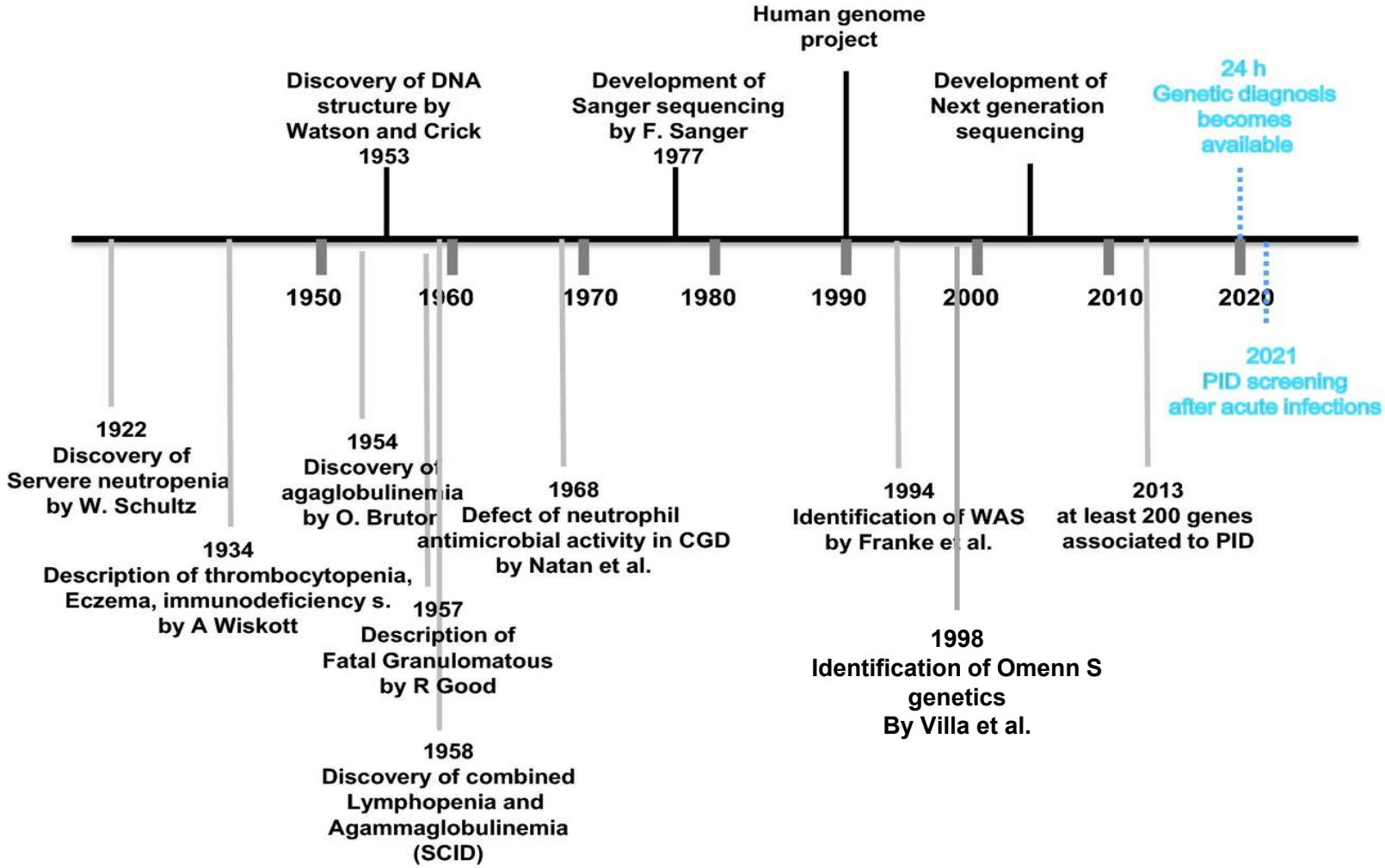


Fabre et., JACI in practise 2019

Flowchart Diagnosis of IEI

- Walter J et al., Curr Opin Pediatr. 2019





Pediatric Allergy Immunology, Volume: 25, Issue: 1, Pages: 27-29, First published: 19 February 2014, DOI: (10.1111/pai.12192)

2 anni, infezione herpes zoster

- 👦 **Bambino**
- Nato a termine, 38w – PN 3400 g
- Ritardo psicomotorio
- Vaccinazioni complete (MPR + VZV, 12/2017)
- ⬇️
- 🦠 **ZOSTER PRECOCE (01/2018)**
- Localizzazione lombare/glutea
- Post-vaccino
- Terapia: Aciclovir

- ⬇️
- 📉 **LINFOPENIA**
- Linfociti: **460/mm^3**
- ⬇️

- 🩸 **ANEMIA SEVERA (07/2018)**
- Hb: **3,9 g/dl**
- Reticolociti: **35.200/mm³ (↓)**
- **WORK-UP NEGATIVO**
- Coombs, G6PD, assetto marziale, B12, folati
- Sierologie infettive negative
- 🧪 **IMMUNOGLOBULINE**
- IgG: **635 mg/dl**
- IgA: **70 mg/dl**
- IgM: **58 mg/dl**

➡️ **Nella norma per età**

- 🧬 **MIDOLLO OSSEO**
- Ipocellulare
- Eritroblasti ↓
- Megacariociti ↓ (piccoli, ipolobati)
- Granulociti ↓
- ➡️ Diagnosi iniziale: **Eritroblastopenia transitoria**

- ⚠️ **IMMUNODEFICIENZA SEVERA**
- **T linfociti ↓↓↓**
- CD3: **3–16% (3–10/mm³)**
- CD4: **0–2/mm³**
- CD8: **1–2/mm³**
- **B linfociti assenti**
- CD19: **0% (0/mm³)**
- **NK ↑**
- CD16: **96% (104/mm³)**
- ➡️ **Pattern: T– B– NK+**

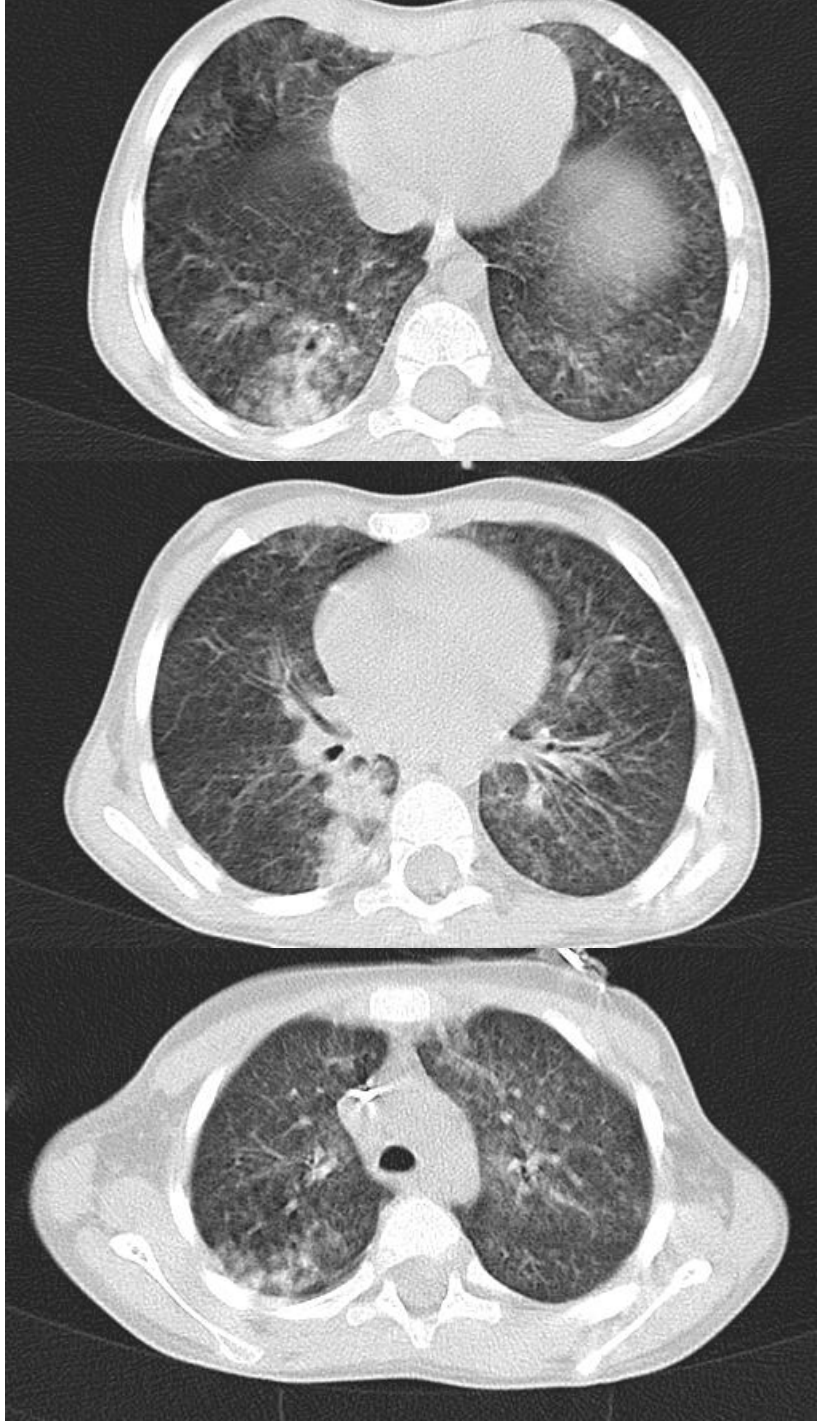
- ⬇️
- 🧬 **DIAGNOSI**
- PNP: **0 U/gHb**
- Acido urico: **indosabile**
- Genetica:
  - c.51_52delTC
  - c.314T>C
- ➡️ **Deficit di PNP**

3 anni

1. Peggioramento respiratorio con aumento degli indici infiammatori (PCR fino a 17,8 mg/L),

2. Trattamento inizialeAvvio di terapia antibiotica con Amikacina e somministrazione di immunoglobuline EV

3. ImagingTC torace con evidenza di interessamento interstiziale bilaterale, aree ground-glass e consolidamenti parenchimali a destra, con possibile eziologia fungina.



2019

- Alla luce del progressivo peggioramento respiratorio con necessità di supporto con HFNC, nonostante la terapia antibiotica ad ampio spettro, la terapia antifungina e la terapia anti-Pneumocystis, e alla luce del risultato dello studio HLA familiare (il paziente, la sorella e il fratello risultavano genotipicamente identici) il paziente veniva sottoposto a **trapianto di cellule staminali da fratello HLA identico,** senza condizionamento.

Quando la citopenia immunomediata può essere espressione di un Errore Congenito dell'Immunitá?

Età di insorgenza <1 anno

Recidiva dopo terapia

Refrattaria alla terapia

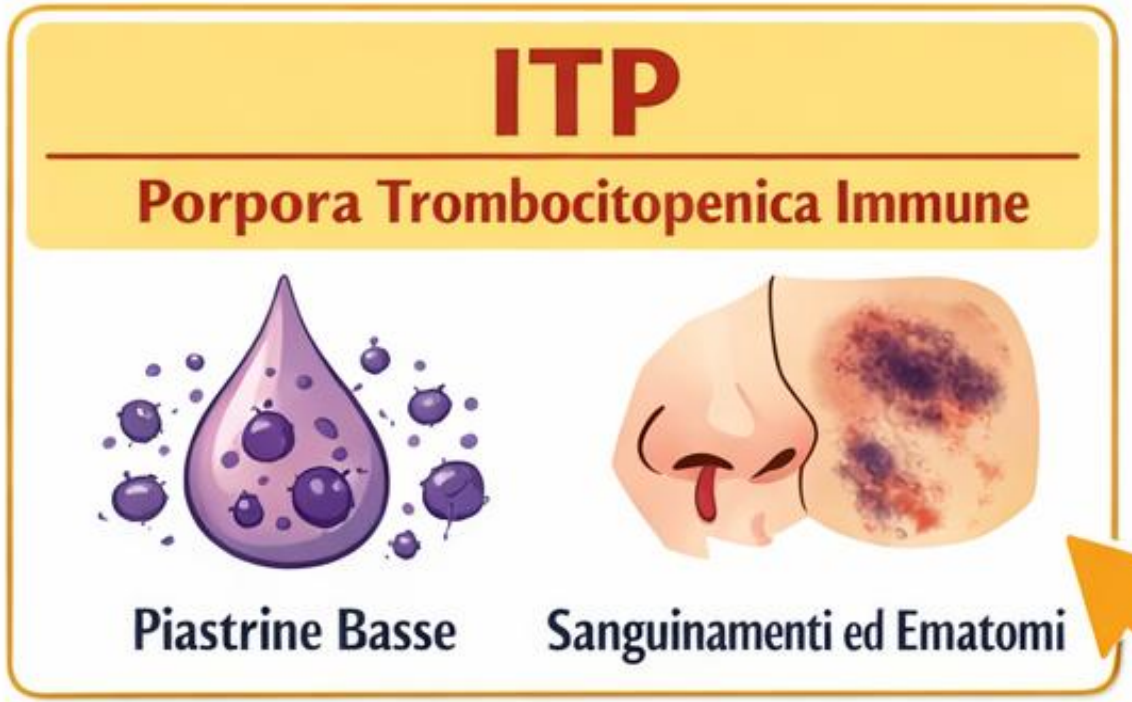
Multilineare/Evans

Associata ad infezioni invasive

Esordio precoce (<12 mesi)

AIHA, ITP o AIN

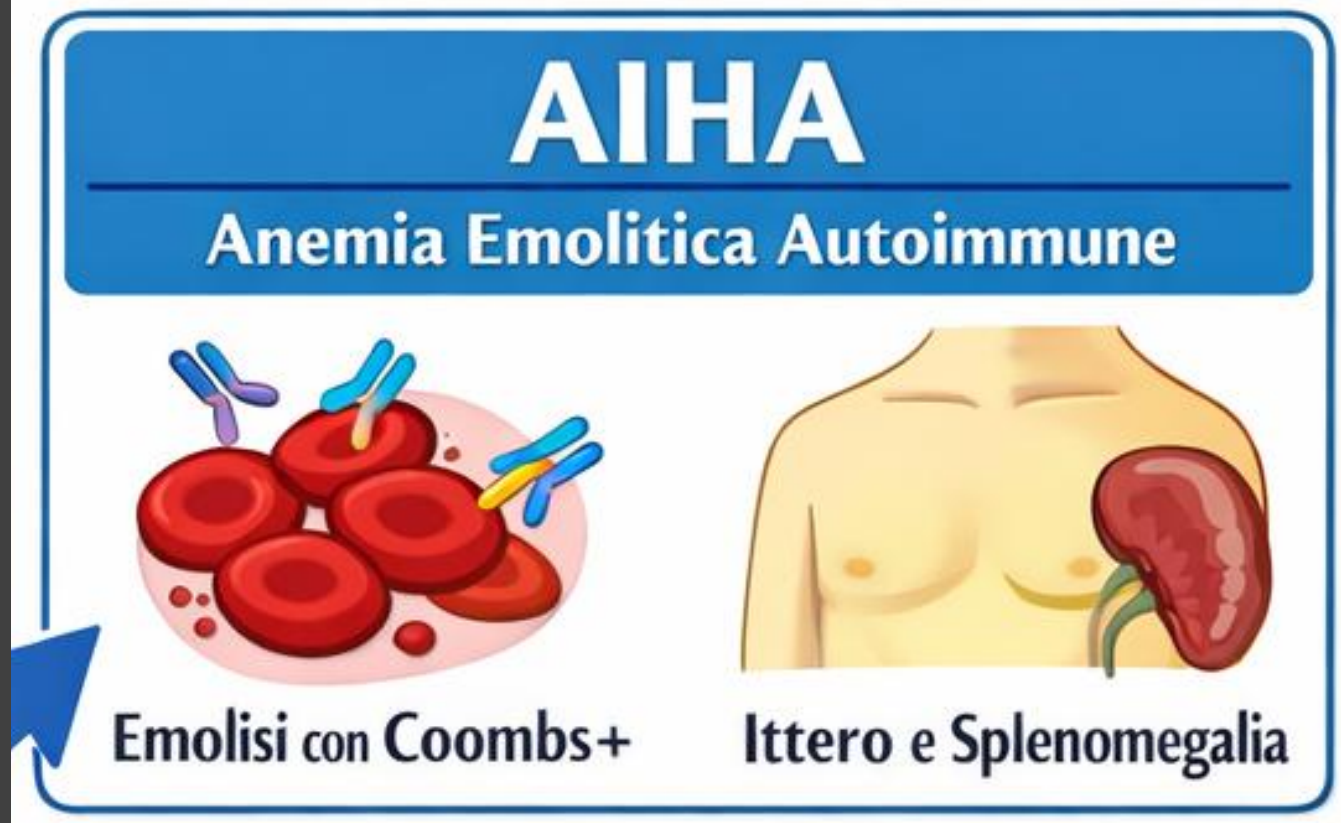
IEI fino a prova contraria



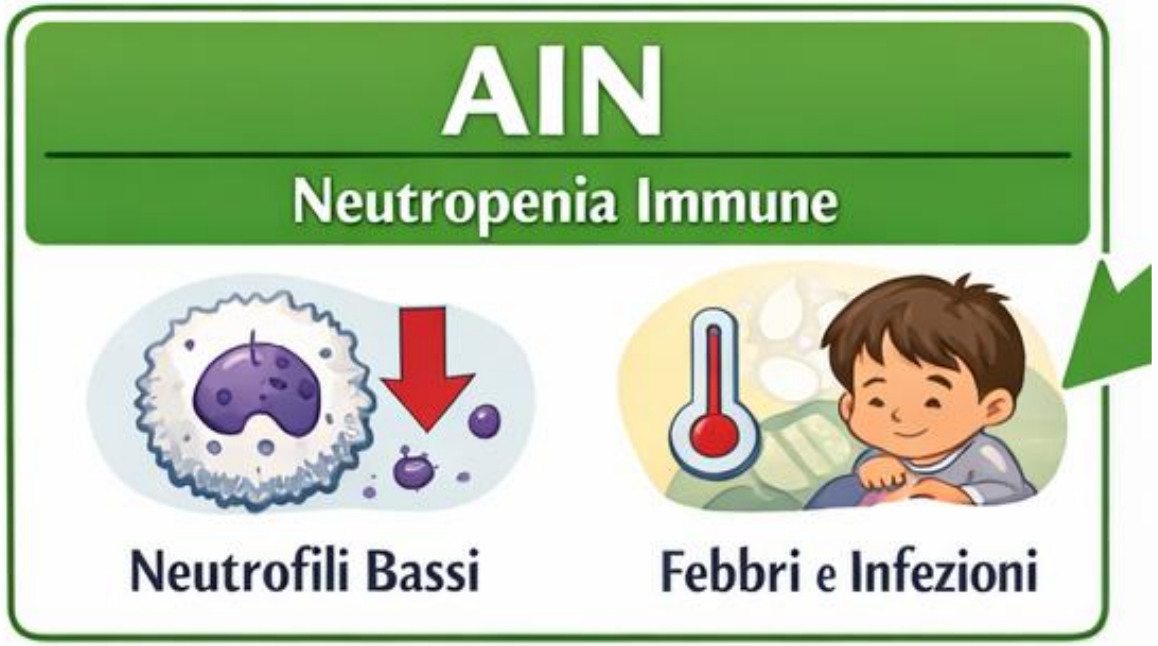
Forma pediatrica “classica” idiopatica: 2–7 anni, spesso post-infettiva, acuta, autolimitante.

Forma cronica / atipica / IEI-correlata: infanzia tardiva → adolescenza, ma può comparire **anche <2 anni**.

Esordio <12 mesi → fortemente suggestivo di IEI (ALPS-like, CTLA4, LRBA, STAT3 GOF, CVID-like precoce).



- **Esordio idiopatico pediatrico:** tipicamente **<4 anni**, spesso post-infettivo.
- **AIHA ricorrente o cronica:** più frequente in **età scolare e adolescenza**.
- **Esordio nel primo anno di vita** → considerare IEI (deficit di regolazione, difetti del complemento, sindromi linfoproliferative).



- **Forma “benigna” idiopatica: 6–24 mesi**, con risoluzione spontanea entro 3–5 anni.
- **AIN persistente / severa / associata ad altre autoimmunità:** esordio **oltre i 3 anni** → sospetto IEI (CVID-like, ALPS-like, CTLA4/LRBA, STAT3 GOF).

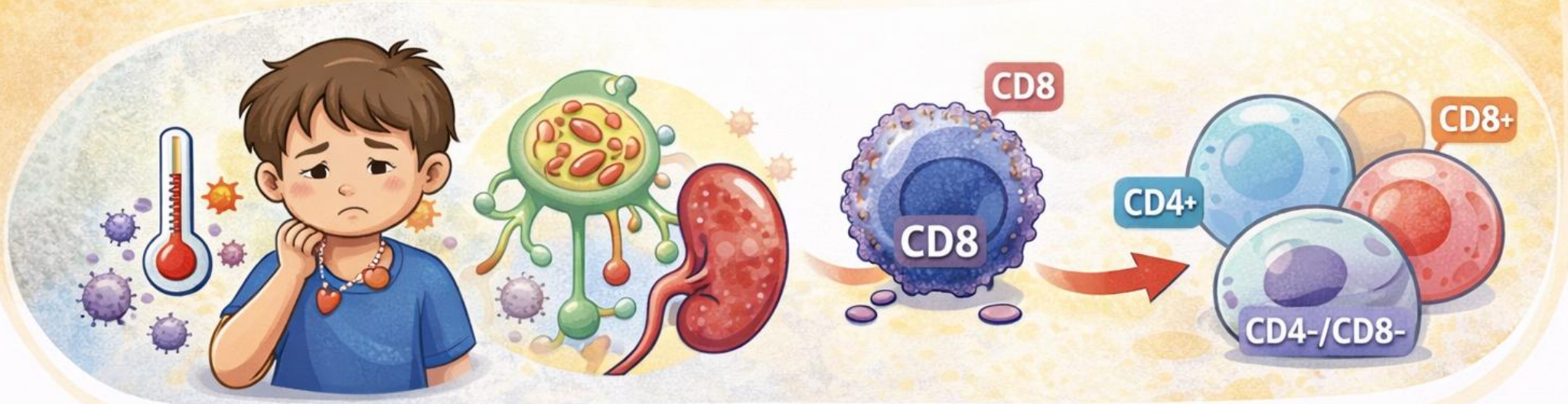
- **Picco pediatrico: 2–12 anni**, spesso con andamento recidivante.
- **Esordio molto precoce (<2 anni)** o **adolescenziale** → fortemente associato a IEI.
- **>60%** dei bambini con Evans ha un IEI sottostante.

Rischio IEI se Decorso recidivante o refrattario

- Risposta incompleta o dipendenza da steroidi
- Necessità di più linee terapeutiche

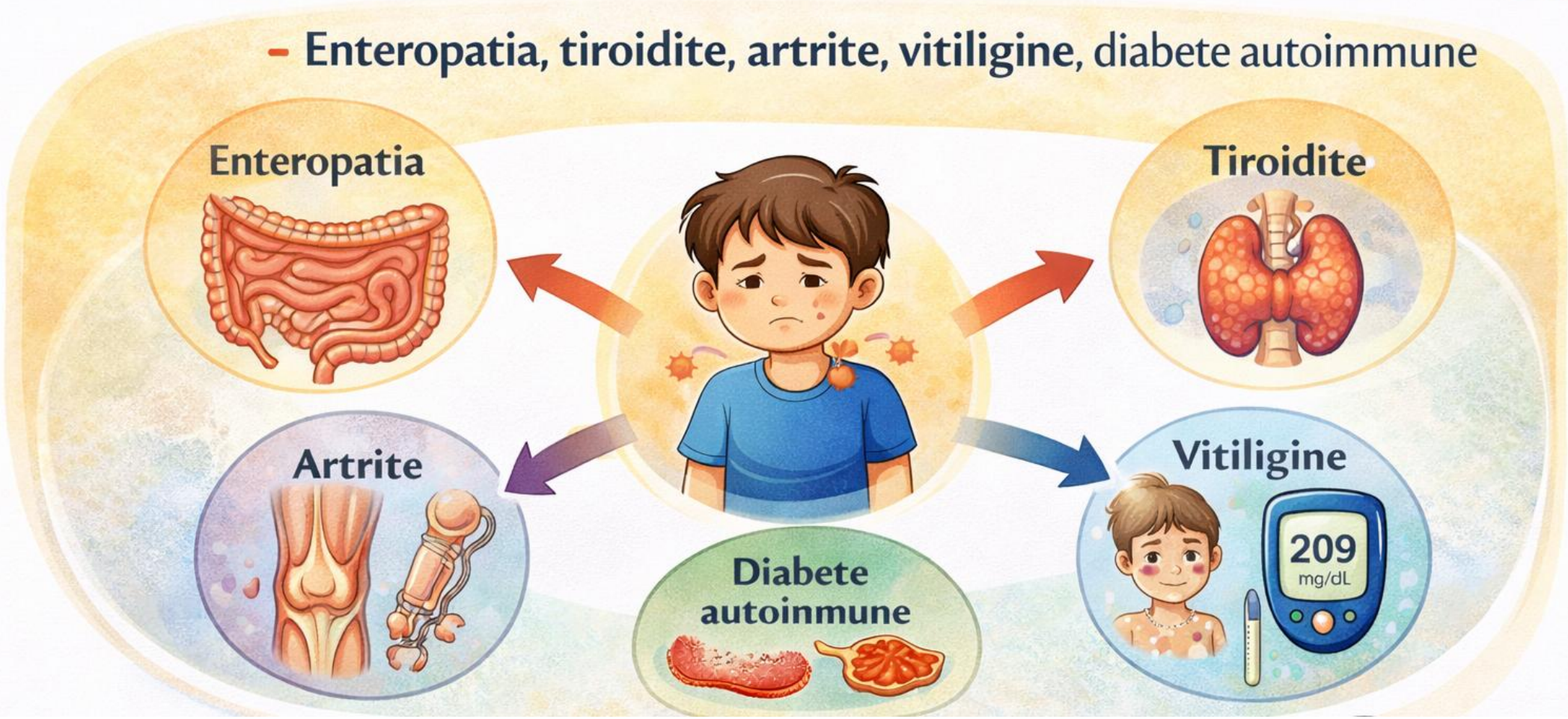
Rischio IEI se Linfoproliferazione persistente

- Linfoadenopatie, splenomegalia
  - espansione CD8
    - linfociti T doppi negativi (CD4-/CD8-)



Rischio IEI se Associazione con altre autoimmunità

- Enteropatia, tiroidite, artrite, vitiligine, diabete autoimmune



Livello 1: esami immunologici

- Infezioni opportunistiche
- Infezioni invasive
- Linfoadenopatia (infettiva)
- Infezioni che richiedono terapia prolungata
- Polmonite
- Ritardo di crescita
- Diarrea

Esami immunologici: cosa cercare?

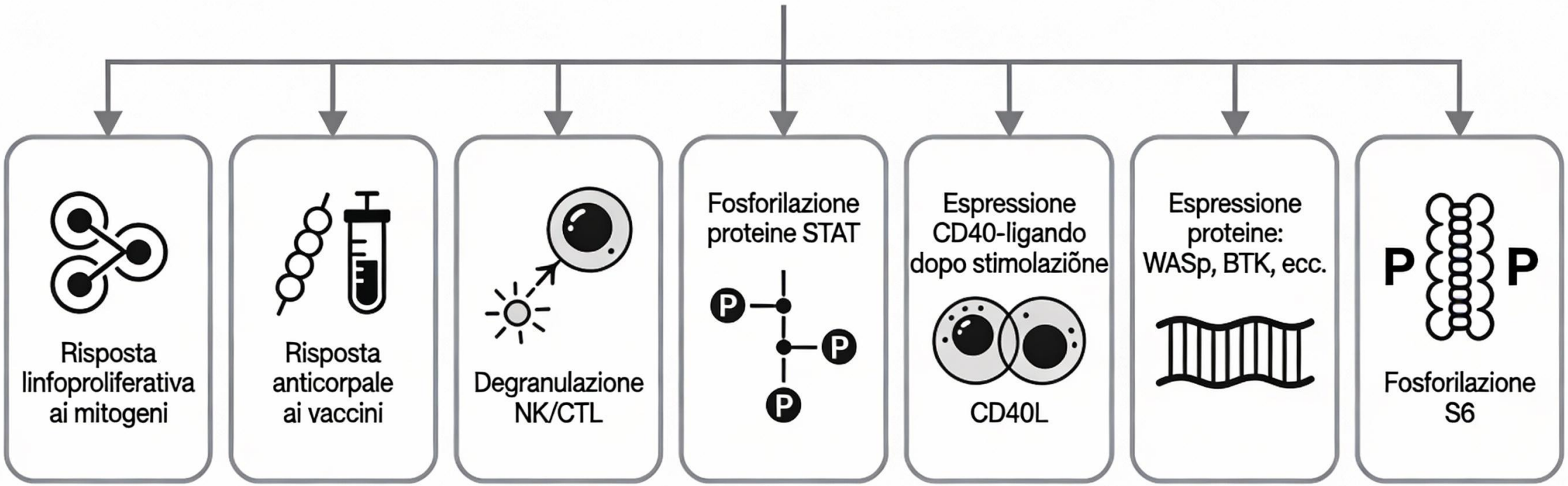
Alterazioni B

- Ig basse o disomogenee
- Riduzione switched memory B,
- Aumento linfociti B CD21^low

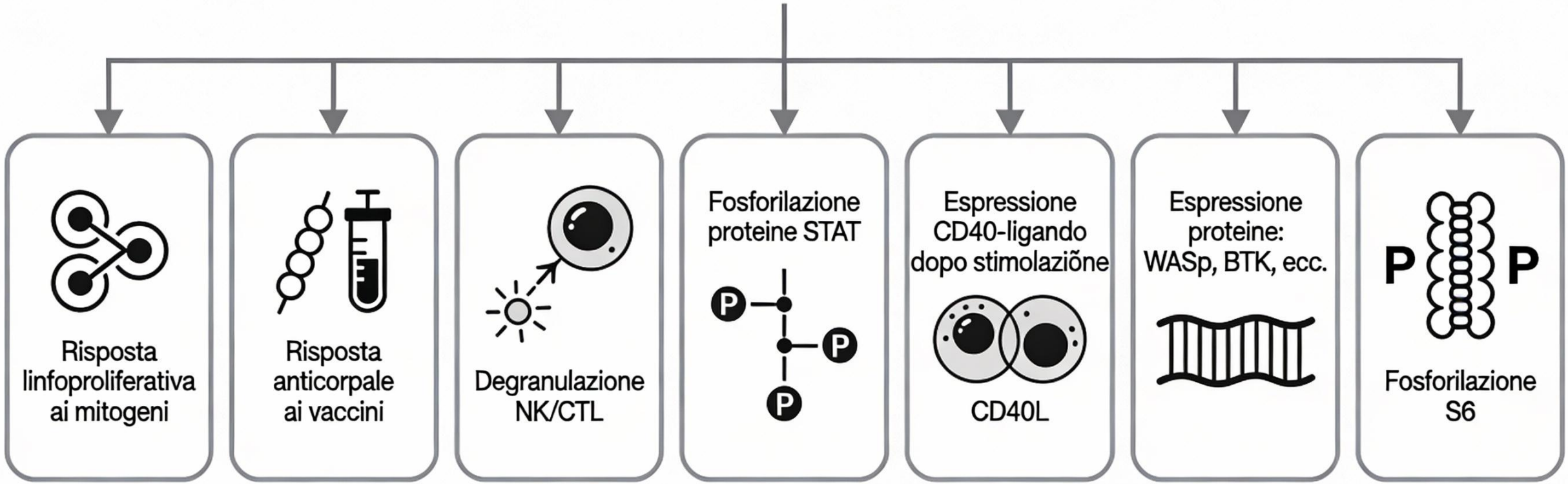
Alterazioni T

- Linfopenia T
- Inversione CD4/CD8
- Aumento dei Linfociti T doppi negativi
- Riduzione linfociti T naïve
- Proliferazione linfocitaria ai mitogeni ridotta

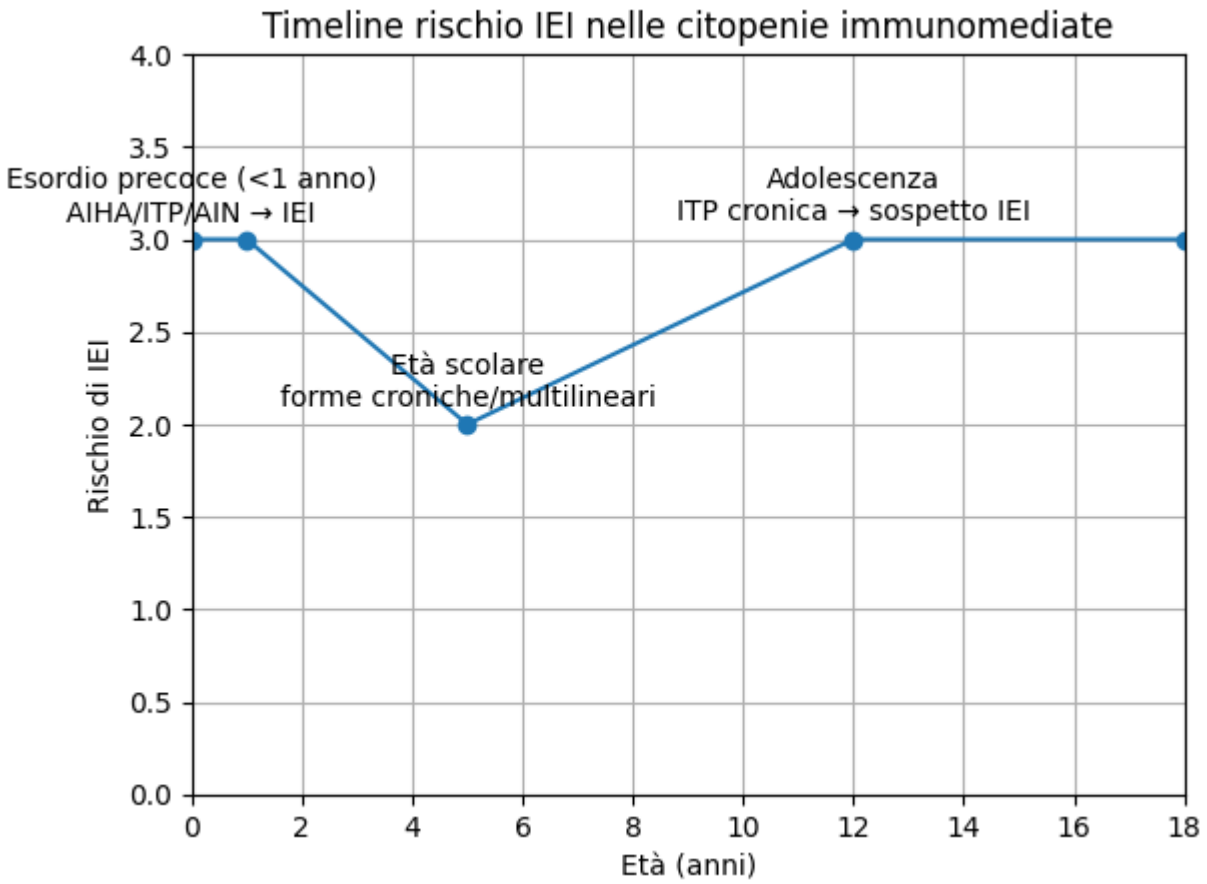
Livello immunologico 2: Esami funzionali/espressione della proteina

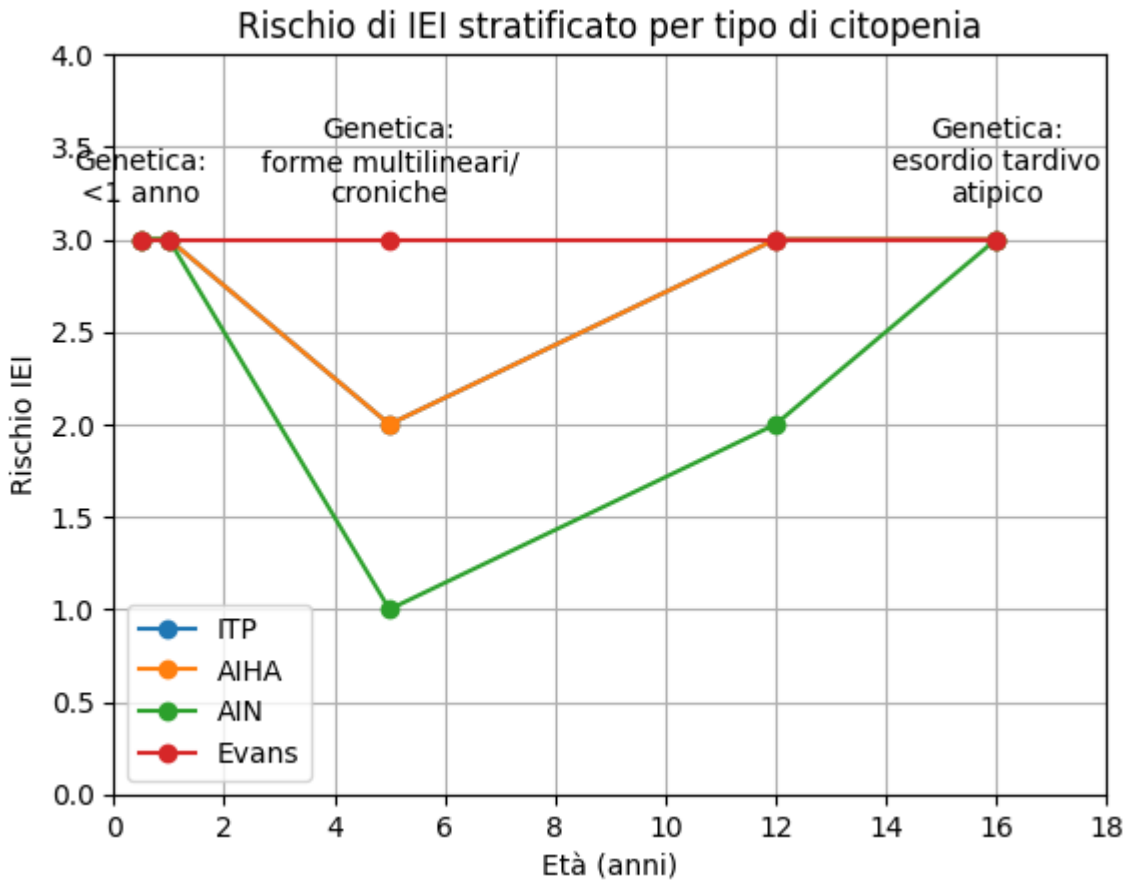


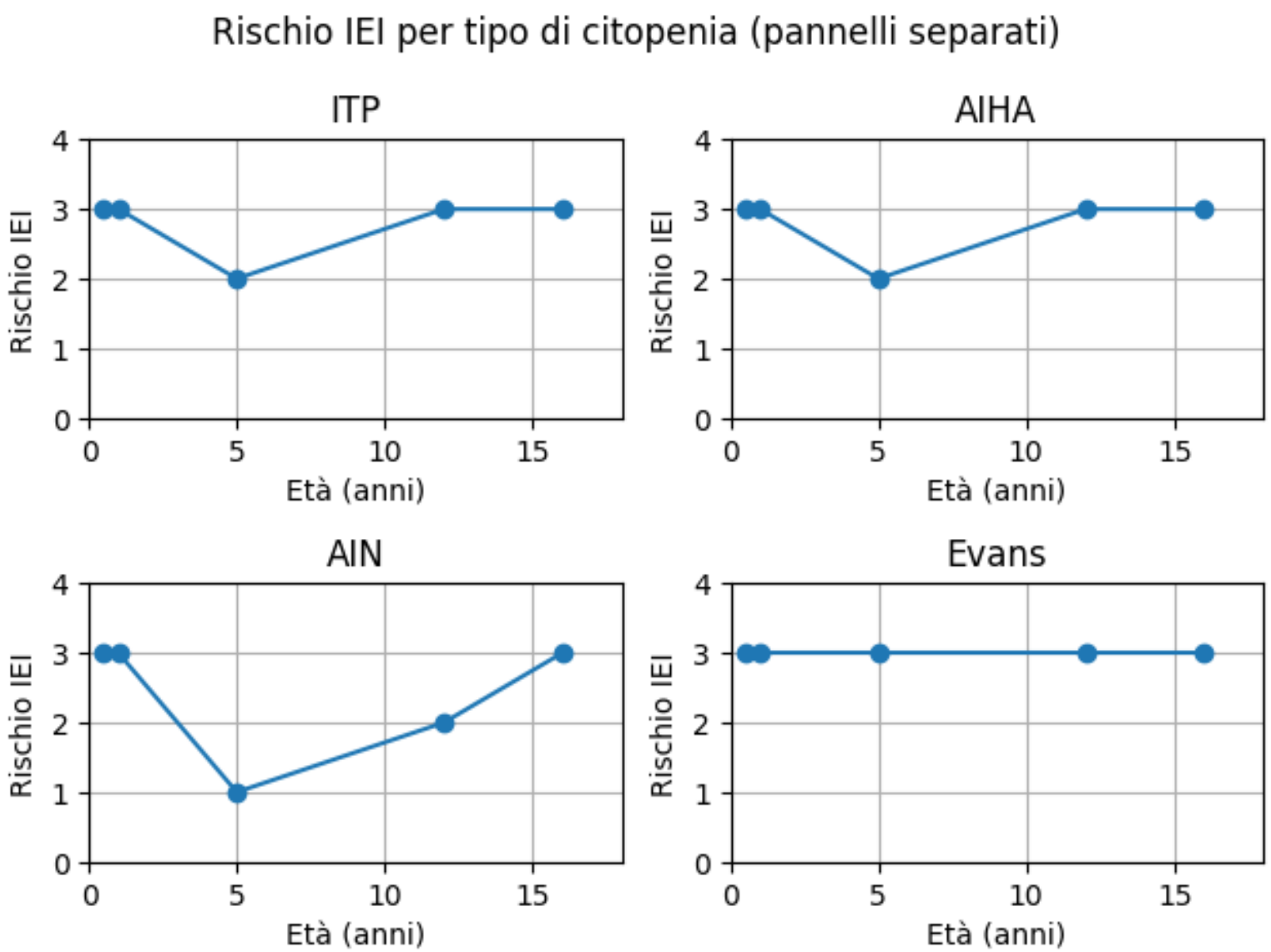
Livello immunologico 2: Esami funzionali/espressione della proteina



Rischio di IEI in citopenie atoimmuni






Rischio IEI per tipo di citopenia (pannelli separati)
ITP
AIHA
AIN
Evans
Rischio IEI
Età (anni)
0
1
2
3
4
0
5
10
15

Uso della genetica nella diagnosi delle immunodeficienze

Approccio 1: clinico

Storia clinica + fenotipo

↓

Ipotesi di diagnosi

↓

Ricerca gene malattia mirata

Approccio 2: genomico

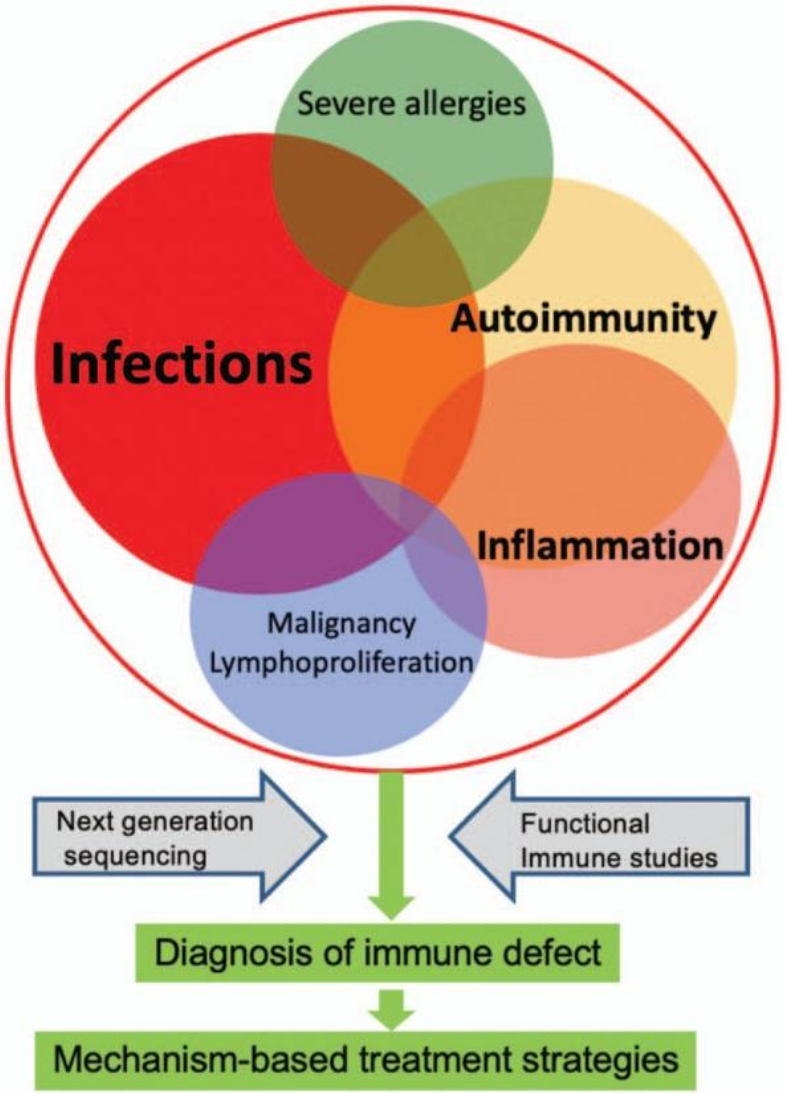
Esoma / Whole genome

Lista geni candidati

Validazióne con test funzionali

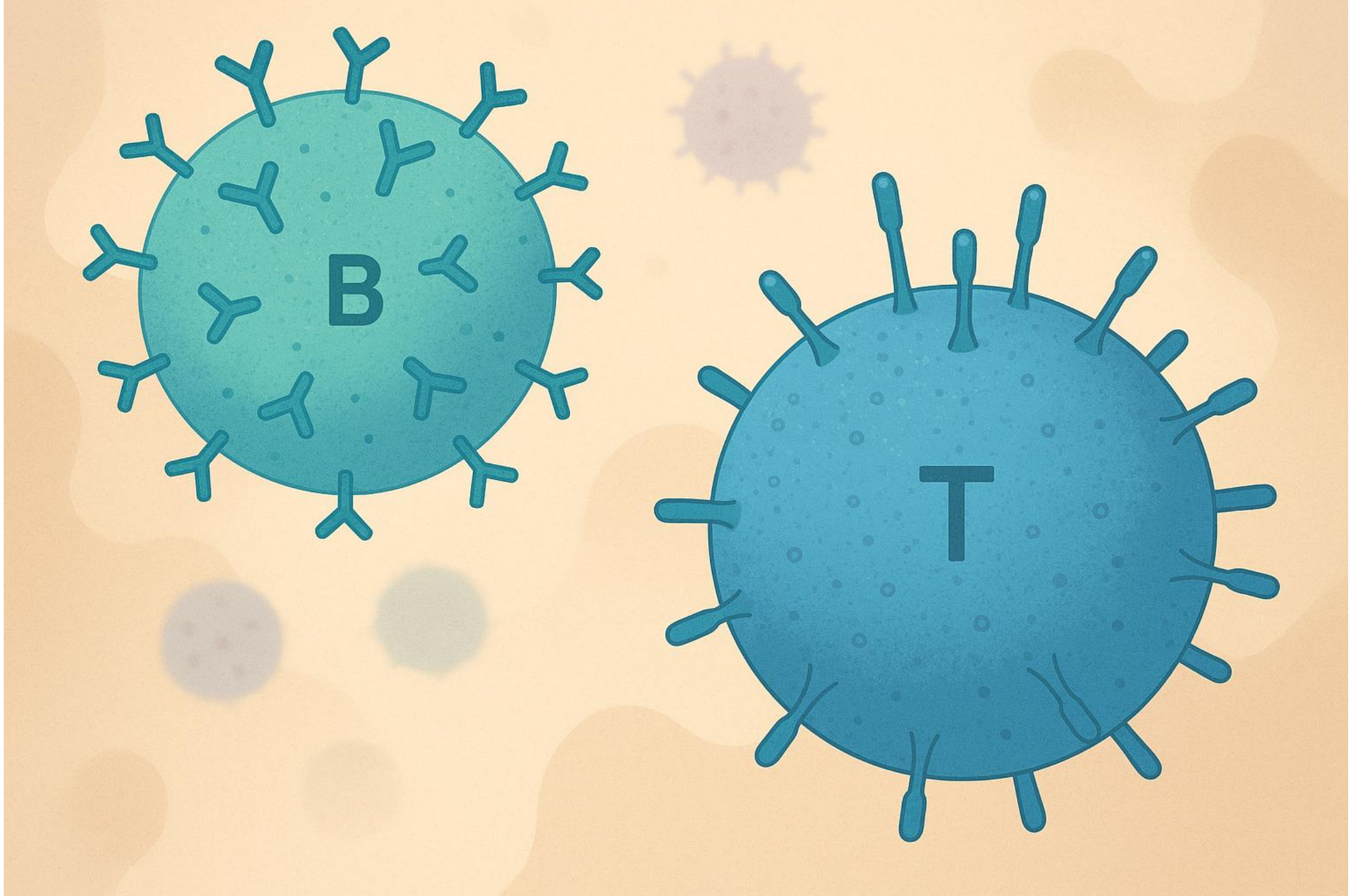
Flowchart Diagnosis of IEI

- Walter J et al., Curr Opin Pediatr. 2019



Conclusioni

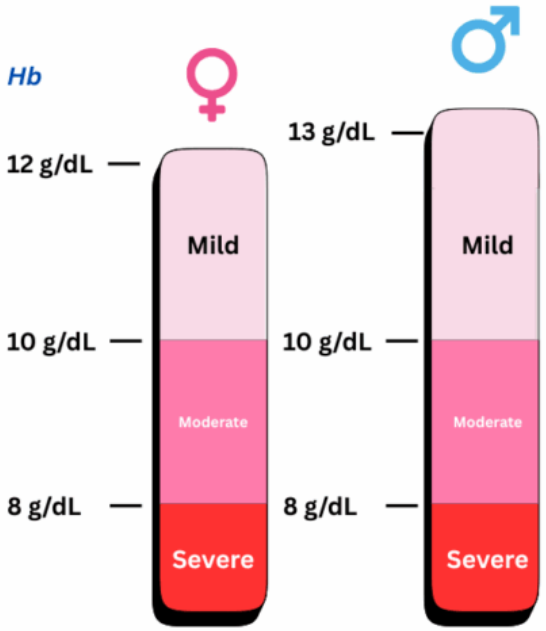
- Gli errori congeniti dell'immunità non sono più definiti dalle sole infezioni:
  - autoimmunità, come citopenie o esordio precoce;
  - le malattie infiammatorie intestinali e le malattie reumatologiche possono essere i primi segni clinici.
- Ogni paziente con citopenia autoimmune deve essere sottoposto a **screening immunologico**
- Citopenie associate ad altre malattie autoimmuni o a mancata risposta alla terapia richiedono **screening genetico**


B
T

Sintomo	Sindrome di Wiskott-Aldrich	XLT (Piastrinopenia X-recessiva)
Emorragia	Grave e precoce	Presente
Eczema	Quasi sempre presente	Assente
Infezioni ricorrenti	Molto frequenti	Poco frequenti
Autoimmunità	Presente	Assente

Standard Definitions of Cytopenia Severity

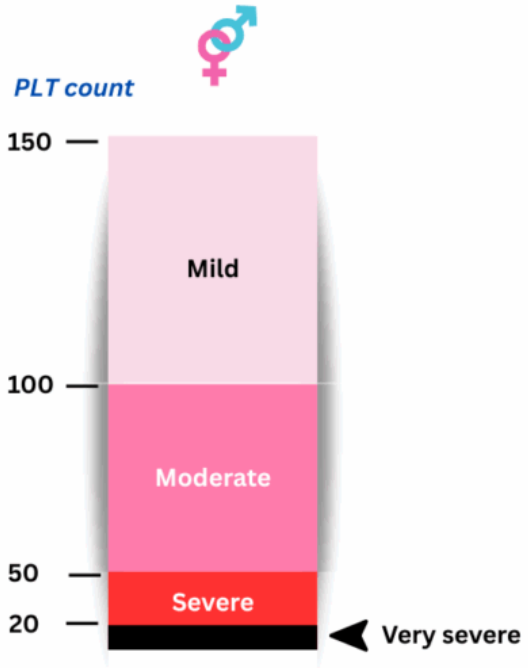
Anemia



WHO, 2024

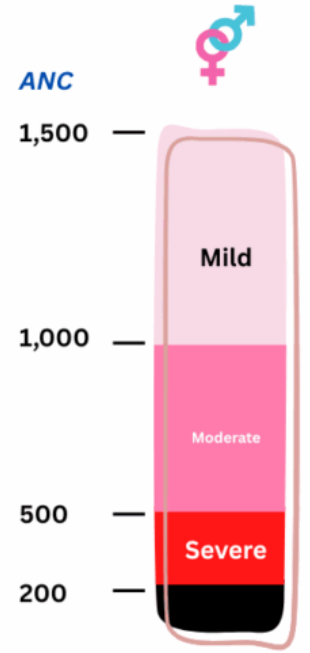
- *Platelet count and ANC are shown as x 10^9 /L*
- *ANC, absolute neutrophil count*

Thrombocytopenia



These categories are reflected in clinical literature and are useful for risk stratification and management decisions, but they are not codified in official guidelines

Neutropenia



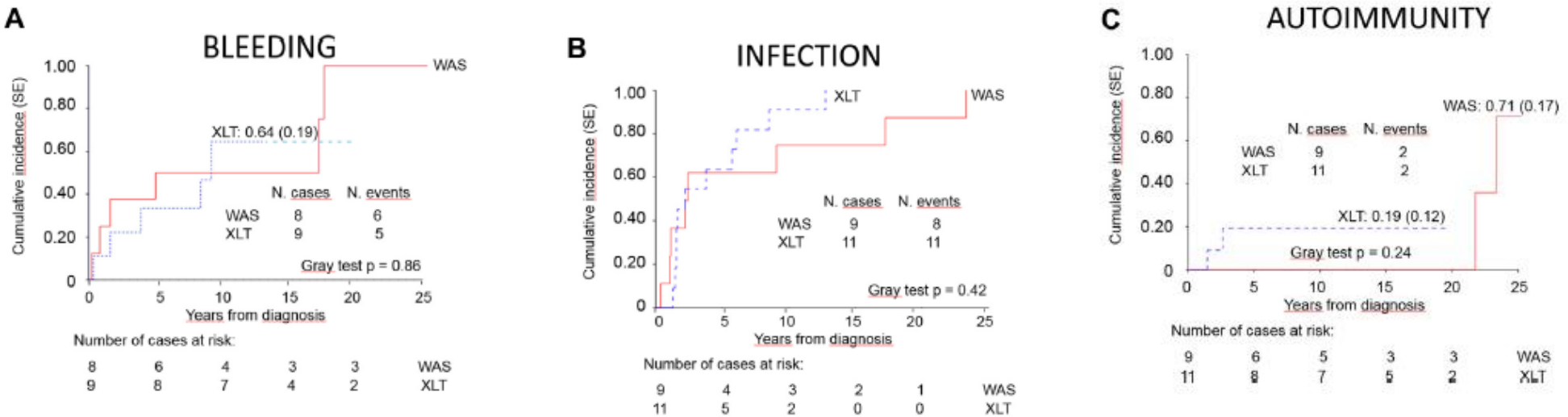
Some guidelines define ANC < 100 as "profound neutropenia"

*Note: **there is no consensus on definitions for leukopenia severity** - makes sense since it matters what cell subtype is reduced!*

https://www.thebloodproject.com/

Long-term outcome in Wiskott-Aldrich syndrome and X-linked thrombocytopenia patients: an observational -prospective multi-center study of the Italian Primary Immune Deficiency Network (IPINET)

Annarosa Soresina,[a] Roberto Rondelli,[b,x] Lucia Dora Notarangelo,[c] Franco Locatelli,[d] Alessandro Aiuti,[e,f] Alessandra Biffi,[g] Marco Rabusin,[h] Claudio Pignata,[i] Giuseppe Menna,[j] Arcangelo Prete,[k] Maura Faraci,[l] Attilio Rovelli,[m] Francesca Conti,[b] Patrizia Bertolini,[n] Chiara Azzari,[o] Caterina Cancrini,[p] Marco Zecca,[q] Francesca Ferrua,[e] Maria Pia Cicalese,[e,f] Francesco Cecere,[r] Laura Dotta,[a] Baldassarre Martire,[s] Silvia Giliani,[t] Daniele Moratto,[t] Cinzia Mazza,[u] Alessandro Plebani,[t] Luigi D. Notarangelo,[v] Andrea Pession,[b,x] Raffaele Badolato,[a,t,y,*] and Fulvio Porta[w,y]



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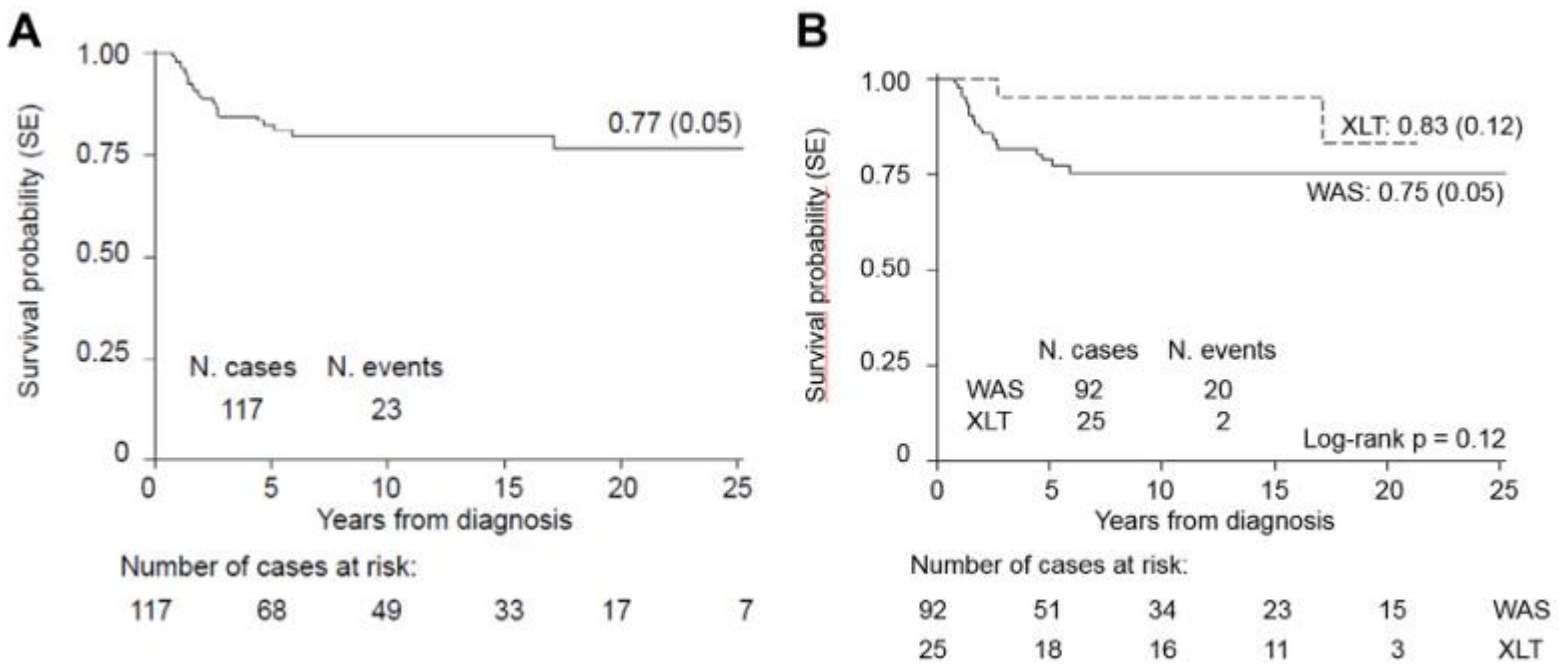
2025;84: 103271



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