



Genetic testing guides therapy in children with refractory cytopenias

by Yael Gernez, Bindu Sathi, Latha Rao, Joseph D. Hernandez, Bertil Glader, Alma-Martina Cepika, Elizabeth G. Hoyte, Kirsten Mouradian, Deepika Singh, Rosa Bacchetta, May Chien, David B. Lewis and Katja G. Weinacht

Received: August 8, 2025.

Accepted: December 24, 2025.

Citation: Yael Gernez, Bindu Sathi, Latha Rao, Joseph D. Hernandez, Bertil Glader, Alma-Martina Cepika, Elizabeth G. Hoyte, Kirsten Mouradian, Deepika Singh, Rosa Bacchetta, May Chien, David B. Lewis and Katja G. Weinacht. *Genetic testing guides therapy in children with refractory cytopenias.* Haematologica. 2026 Jan 8. doi: 10.3324/haematol.2025.288839 [Epub ahead of print]

Publisher's Disclaimer.

E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication.

E-publishing of this PDF file has been approved by the authors.

After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval; the final version of the manuscript will then appear in a regular issue of the journal.

All legal disclaimers that apply to the journal also pertain to this production process.

Genetic testing guides therapy in children with refractory cytopenias

Yael Gernez ¹; Bindu Sathi ²; Latha Rao ²; Joseph D. Hernandez ¹; Bertil Glader ³; Alma-Martina Cepika ³; Elisabeth G. Hoyte ¹; Kirsten Mouradian ³; Deepika Singh ⁴; Rosa Bacchetta ³; May Chien ³; David B. Lewis ¹ and, Katja G. Weinacht ³

Affiliations:

¹ Division of Allergy, Immunology and Rheumatology, Department of Pediatrics,
Stanford School of Medicine, CA, USA

² Division of Hematology, Department of Pediatrics, Valley Children Hospital,
Madera, CA, USA

³ Division of Hematology, Oncology, Stem Cell Transplantation and Regenerative
Medicine, Department of Pediatrics, Stanford School of Medicine, Stanford, CA,
USA

⁴ Division of Rheumatology, Department of Pediatrics, Valley Children Hospital,
Madera, CA, USA

Corresponding authors:

Yael Gernez, M.D., Ph.D.

Department of Pediatrics

Division of Allergy, Immunology, and Rheumatology

Stanford University School of Medicine

269 Campus Drive, CCSR Suite 3215, Stanford, CA 94305

Email: yaelg@stanford.edu

and

Katja G. Weinacht, M.D., Ph.D.

Department of Pediatrics

Division of Hematology, Oncology, Stem Cell Transplantation and Regenerative Medicine

Stanford University School of Medicine

Biomedical Innovations Building, 240 Pasteur Drive, Suite 2550, Stanford, CA 93405

Email: kgw1@stanford.edu

Key words: therapy-refractory immune cytopenia, genetic immune diseases, primary immune regulatory diseases, targeted therapy.

Contribution: YG and KGW saw and assessed patients, performed research and wrote manuscript. BS, LR, BG, DS, MC and DBL saw patients. AMC, JDH and RB revised manuscript.

Conflict of interested: The authors don't have any conflicts of interested.

Data sharing statement: The authors can make their original data and protocols available to other investigators without unreasonable restrictions except for deidentifying patient's information.

Immune cytopenias of childhood encompass a heterogeneous group of disorders characterized by immune-mediated destruction of red blood cells (autoimmune hemolytic anemia, AIHA), platelets (immune thrombocytopenia, ITP), neutrophils (autoimmune neutropenia, AIN), or multiple lineages (Evans syndrome). Most primary or “idiopathic” cases present acutely but respond to first- or second-line therapy (steroids, IVIG, \pm rituximab) without long-term sequelae¹. In contrast, cytopenias that fail to respond to repeated courses of multi-agent immunosuppressive therapy constitute a severe and often debilitating condition with substantial long-term morbidity². Such non-responsiveness should prompt evaluation for an underlying comorbid disorder, malignancy, infection, lymphoproliferation, rheumatoid disease, or an inborn error of immunity (IEI). Therapy-refractory cytopenias in children frequently conceal unrecognized IEIs or other hematopoietic defects that are amenable to targeted treatment. This study was approved by Stanford IRB in accordance with the Helsinki Declaration, and written informed consent was obtained.

We analyzed 24 pediatric and young adult patients with presumed immune cytopenias referred to our multidisciplinary clinic after failing multiple lines of immunosuppressive therapy (Figure 1). Comprehensive immune evaluation and genetic testing were performed in all cases. A contributory diagnosis was identified in 19 of 24 patients, directly guiding therapeutic decisions. Four patients initially thought to have autoimmune cytopenias were subsequently diagnosed with bone marrow failure syndromes: two carried pathogenic variants in *DNAJC21* or *SBDS* consistent with Shwachman-Diamond-like syndrome⁴, and one harbored a *FANCA* mutation

consistent with Fanconi anemia. All four underwent hematopoietic stem cell transplantation (HSCT) and were excluded from subsequent analyses.

Among the remaining 20 patients, 14 had multilineage cytopenias, two had isolated autoimmune neutropenia (AIN; both with monoallelic pathogenic *NFKB1* variants), one had isolated autoimmune hemolytic anemia (AIHA; *CTLA4* haploinsufficiency), and three had isolated immune thrombocytopenia (ITP; one with common variable immunodeficiency [CVID], one with systemic lupus erythematosus [SLE], and one without an identifiable cause). Clinical and genetic features of these 20 patients are summarized in Table I.

An underlying genetic variant was identified in 75% (15/20) of patients, conferring a likely predisposition to autoimmunity or autoinflammation in 73% (11/15). Isolated cytopenias were observed in 25% (5/20) of genetically predisposed patients. Among the six patients without any identifiable genetic abnormality, two presented with isolated thrombocytopenia and four with combined anemia and thrombocytopenia (Tables I and II). Genetic diagnoses clustered into four mechanistic categories: (1) disorders driven by excessive type I interferon signaling, (2) inflammasome activation syndromes, (3) diseases characterized by imbalance between effector and regulatory T-cell (Treg) activity, and (4) other rare monogenic disorders. This framework provided a biologic rationale for targeted therapeutic approaches (Figure 1).

(1) Interferonopathy: Three patients carried pathogenic variants in *ACP5*, confirming the diagnosis of spondyloenchondrodysplasia with immune dysregulation (SPENCDI), a type I interferonopathy. Ruxolitinib and baricitinib, which inhibit JAK1 and JAK2 downstream of the type I interferon receptor, were used successfully in two patients. The third patient experienced

an acute SLE flare with pericarditis and responded to treatment with anifrolumab-fnia, a type I interferon receptor inhibitor approved for SLE⁵.

(2) Inflamasomopathy: An infant presented with life-threatening autoimmune hemolytic anemia (AIHA), severe jaundice, recurrent fevers, urticarial-like rash, severe pain, and markedly elevated IL-18 levels. Genetic testing identified a C-terminal *CDC42* variant associated with aberrant inflamasome activation. Treatment with the interleukin-1 receptor antagonist anakinra led to complete and durable resolution of symptoms⁶. Notably, the cytopenia in this patient was secondary to hypersplenism rather than immune-mediated destruction.

(3) Imbalance between effector and regulatory T-cell activation:

Seven patients had inborn errors of immunity (IEIs) associated with dysregulated effector and regulatory T-cell (Treg) balance. Three carried a 22q11.2 deletion, a defect in thymic development and function known to predispose to autoimmunity⁷. All were treated with rapamycin, which enhances Treg expansion and promotes immune tolerance⁸⁻¹⁰. Three sisters with variable immune cytopenias were found to have autosomal dominant *NFKB1* loss-of-function variants, a common monogenic cause of common variable immunodeficiency (CVID)¹¹. Two of the three responded to rapamycin, achieving normalization of neutrophil and platelet counts. The seventh patient harbored a *FOXP3* variant consistent with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome and presented with aplastic anemia and nephrotic syndrome. After initial therapy with steroids and tacrolimus, he was successfully transitioned to rapamycin following molecular diagnosis; both hematopoietic stem cell transplantation and *FOXP3* gene therapy were subsequently offered.

(4) Conditions with specific or directly targeted therapy:

A 28-year-old man with short stature, severe neutropenia, oral ulcers, and recurrent bacterial infections, previously treated with steroids and G-CSF for presumed autoimmune neutropenia and thrombocytopenia, was found to harbor a pathogenic variant in *G6PC3*, confirming *G6PC3* deficiency, a congenital neutropenia unresponsive to immunosuppression¹². *G6PC3* deficiency causes neutrophil dysfunction due to accumulation of 1,5-anhydroglucitol-6-phosphate (1,5-AG6P). Reducing 1,5-AG6P via inhibition of the sodium–glucose cotransporter 2 (SGLT2) restores neutrophil counts and function. Accordingly, treatment with the SGLT2 inhibitor empagliflozin led to complete normalization of neutrophil counts and resolution of symptoms.

A two-year-old boy presenting with ITP and anemia carried a *MAGT1* variant causing a magnesium transporter defect that disrupts intracellular magnesium homeostasis and immune function. Although magnesium supplementation alone was insufficient, his cytopenias stabilized on thrombopoietin mimetics. Another patient with *CTLA4* haploinsufficiency achieved sustained remission of autoimmune hemolytic anemia on CTLA-4-Ig (abatacept).

No pathogenic variants predisposing to immune dysregulation were identified in six of the 20 patients. Two of these fulfilled clinical criteria for systemic lupus erythematosus (SLE) and responded well to rapamycin, which suppresses the expansion of proinflammatory double-negative T cells in SLE. A third patient with an SLE-like phenotype harbored a heterozygous variant of uncertain significance (VUS) in *TNFRSF6B*, potentially predisposing to autoimmunity¹³. Another patient with severe ITP met clinical criteria for common variable immunodeficiency (CVID). The remaining two patients with ITP, one of whom was status post Fontan procedure, achieved sustained remission on rapamycin.

Distinguishing germline from somatic variants in patients with (1) a confirmed *de novo* pathogenic mutation (Pat#4) or (2) a pathogenic variant without available parental testing (Pat#3 and Pat#13) remains challenging without multi-tissue analysis. Age at presentation, clinical severity, and an allele frequency near 50% are insufficient to determine variant origin. Given the variable expressivity and incomplete penetrance often seen in inborn errors of immunity (IEI), phenotypic overlap between germline and somatic cases is common¹⁴. In the patient with a *de novo* *CDC42* mutation (Pat#3), low-level parental mosaicism cannot be excluded. In the patient carrying a *MAGT1* variant (Pat#13), an allele frequency of 50% in blood is consistent with a hemizygous germline change but does not exclude somatic mosaicism. Notably, somatic *MAGT1* variants have not been reported.

Genetic causes of immune dysregulation should be systematically considered in children with therapy-refractory cytopenias, as molecular diagnoses carry prognostic significance and directly inform treatment. Patients with identified monogenic disorders experienced substantial benefit from targeted therapies, which were generally well tolerated. Those with primary immune regulatory diseases (PIRD) involving effector-Treg imbalance or thymic dysfunction improved with mTOR inhibition; patients with interferonopathies responded to JAK inhibitors; and those with inflamasomopathies achieved remission with IL-1 blockade. In select ultra-rare conditions, such as *G6PC3* deficiency, repurposed therapies like SGLT2 inhibitors produced transformative outcomes.

Hadjadj et al. demonstrated that pediatric Evans syndrome is associated with a high frequency of potentially damaging variants in immune-related genes¹⁶⁻²⁰. In a national cohort of 203 children with early-onset Evans syndrome, systematic genetic testing of 80 consecutive cases revealed

that 65% carried pathogenic or likely pathogenic variants. These were identified in genes associated with autoimmune lymphoproliferative syndrome (ALPS; e.g., *FAS*), primary immunodeficiency (*CTLA4*, *LRBA*, *STAT3*), or genes not previously linked to autoimmunity (e.g., *KRAS*)¹⁸. Consistent with our findings, patients with such variants exhibited more severe disease, required more intensive therapy, and frequently presented with additional immunopathologic features compared to those without identified variants.

Long-term immunomodulation is often required to maintain remission, underscoring the need for coordinated multidisciplinary care among immunologists, hematologists, geneticists, and transplant specialists. As genomic testing becomes routine, the number of patients with genetic immune disorders identified in hematology clinics will continue to grow. Broad implementation of these tools will expand the hematologist's therapeutic repertoire. Currently available testing is aimed at identifying germ-line variants in IEI-defining genes¹⁵, however, detecting IEI-variants on a clonal level could become the next diagnostic frontier, rendering "idiopathic" immune cytopenias a diagnosis of the past.

References:

1. Ducassou S, Gourdonneau A, Fernandes H, et al. Second-line treatment trends and long-term outcomes of 392 children with chronic immune thrombocytopenic purpura: the French experience over the past 25 years. *Br J Haematol.* 2020;189(5):931-942.
2. Pincez T, Fernandes H, Leblanc T, et al. Long term follow-up of pediatric-onset Evans syndrome: broad immunopathological manifestations and high treatment burden. *Haematologica.* 2022;107(2):457-466.
3. Teachey DT, Lambert MP. Diagnosis and management of autoimmune cytopenias in childhood. *Pediatr Clin North Am.* 2013;60(6):1489-511.
4. Kawashima N, Oyarbide U, Cipolli M, Bezzetti V, Corey SJ. Shwachman-Diamond syndromes: clinical, genetic, and biochemical insights from the rare variants. *Haematologica.* 2023;108(10):2594-2605.
5. Kundnani NR, Levai MC, Popa MD, et al. Biologics in Systemic Lupus Erythematosus: Recent Evolutions and Benefits. *Pharmaceutics.* 2024;16(9):1176.
6. Gernez Y, de Jesus AA, Alsaleem H, et al. Severe autoinflammation in 4 patients with C-terminal variants in cell division control protein 42 homolog (CDC42) successfully treated with IL-1beta inhibition. *J Allergy Clin Immunol.* 2019;144(4):1122-1125.e6.
7. McDonald-McGinn DM, Sullivan KE, Marino B, et al. 22q11.2 deletion syndrome. *Nat Rev Dis Primers.* 2015;1:15071.
8. Bride KL, Vincent T, Smith-Whitley K, et al. Sirolimus is effective in relapsed/refractory autoimmune cytopenias: results of a prospective multi-institutional trial. *Blood.* 2016;127(1):17-28.

9. Gallon L, Traitanon O, Yu Y, et al. Differential Effects of Calcineurin and Mammalian Target of Rapamycin Inhibitors on Alloreactive Th1, Th17, and Regulatory T Cells. *Transplantation*. 2015;99(9):1774-1784.

10. Kumar D, Nguyen TH, Bennett CM, et al. mTOR inhibition attenuates cTfh cell dysregulation and chronic T-cell activation in multilineage immune cytopenias. *Blood*. 2023;141(3):238-243.

11. Tuijnenburg P, Lango Allen H, Burns SO, et al. Loss-of-function nuclear factor kappaB subunit 1 (NFKB1) variants are the most common monogenic cause of common variable immunodeficiency in Europeans. *J Allergy Clin Immunol*. 2018;142(4):1285-1296.

12. Wortmann SB, Van Hove JLK, Derkx TGJ, et al. Treating neutropenia and neutrophil dysfunction in glycogen storage disease type Ib with an SGLT2 inhibitor. *Blood*. 2020;136(9):1033-1043.

13. Richard AC, Peters JE, Lee JC, et al. Targeted genomic analysis reveals widespread autoimmune disease association with regulatory variants in the TNF superfamily cytokine signalling network. *Genome Med*. 2016;8(1):76.

14. Chinn IK, Chan AY, Chen K, et al. Diagnostic interpretation of genetic studies in patients with primary immunodeficiency diseases: A working group report of the Primary Immunodeficiency Diseases Committee of the American Academy of Allergy, Asthma & Immunology. *J Allergy Clin Immunol*. 2020;145(1):46-69.

15. Poli CAI, Bousfiha AA, Cunningham-Rundles C, et al. Human inborn errors of immunity: 2024 update on the classification from the International Union of Immunological Societies Expert Committee. *J Hum Immun*. 2025;1(1):e20250003.

16. Besnard C, Levy E, Aladjidi N, et al. Pediatric-onset Evans syndrome: Heterogeneous presentation and high frequency of monogenic disorders including LRBA and CTLA4 mutations. *Clin Immunol.* 2018;188:52-57.
17. Grimes AB, Kim TO, Kirk SE, et al. Refractory autoimmune cytopenias in pediatric Evans syndrome with underlying systemic immune dysregulation. *Eur J Haematol.* 2021;106(6):783-787.
18. Hadjadj J, Aladjidi N, Fernandes H, et al. Pediatric Evans syndrome is associated with a high frequency of potentially damaging variants in immune genes. *Blood.* 2019;134(1):9-21.
19. Miano M, Guardo D, Grossi A, et al. Underlying Inborn Errors of Immunity in Patients With Evans Syndrome and Multilineage Cytopenias: A Single-Centre Analysis. *Front Immunol.* 2022;13:869033.
20. Novak W, Berner J, Svaton M, et al. Evans syndrome caused by a deleterious mutation affecting the adaptor protein SASH3. *Br J Haematol.* 2023;203(4):678-683.

Table I. Mechanism-based classification of genetic immune dysregulation underlying therapy-refractory cytopenias.

Patient / Age / Gender	Clinical presentation and laboratory findings	Genetic variants	Diagnosis	Targeted treatment and response to therapy
Interferonopathies				
Pat #1 (16 /M)	Severe thrombocytopenia and anemia; fatigue, short stature	Biallelic pathogenic variants in <i>ACP5</i> (compound heterozygous; WES trio)	AR SPENCDI – Evans syndrome (warm AIHA, ITP)	Remission on ruxolitinib
Pat #2 (4 /F)	Severe thrombocytopenia and anemia; fatigue, speech delay; SLE flare with pericarditis	Biallelic pathogenic variants in <i>ACP5</i> (compound heterozygous; PID panel)	AR SPENCDI – Evans syndrome (warm AIHA, ITP)	Remission on anifrolumab (Saphnelo)
Pat #3 (10 /F)	Severe thrombocytopenia and anemia; fatigue, short stature, speech delay	Biallelic homozygous pathogenic variant in <i>ACP5</i> (PID panel)	AR SPENCDI – Evans syndrome (warm AIHA, ITP)	No treatment rendered (posthumous diagnosis)
Inflamasomopathies				
Pat #4 (2 /F)	Bruising, failure to thrive, hepatosplenomegaly, short stature	De novo likely pathogenic variant in <i>CDC42</i> (WES trio)	AD LOF in <i>CDC42</i> – consumptive cytopenia due to hypersplenism	Long-term remission on anakinra
Cytopenias with monogenic disease benefiting from rapamycin				
Pat #5 (2 /F)	Severe neutropenia and thrombocytopenia; frequent infections	Monoallelic pathogenic variant in <i>NFKB1</i> (WES trio)	AD LOF in <i>NFKB1</i> – Evans syndrome (AIN, ITP)	Remission on rapamycin + monthly IVIG
Pat #6 (7 /F)	Severe neutropenia	Monoallelic pathogenic variant in <i>NFKB1</i> (WES trio)	AD LOF in <i>NFKB1</i> – severe neutropenia	Partial remission on rapamycin (improved ANC)
Pat #7 (11)	Intermittent mild neutropenia	Monoallelic pathogenic	AD LOF in <i>NFKB1</i> –	No treatment rendered

Patient / Age / Gender	Clinical presentation and laboratory findings	Genetic variants	Diagnosis	Targeted treatment and response to therapy
/F)		variant in <i>NFKB1</i> (WES trio)	moderate neutropenia	
Partial DiGeorge				
Pat #8 (0.2 /M)	Severe thrombocytopenia and anemia; cardiac defect	22q11.2 deletion (<i>TBX1</i> hemizygosity; FISH)	Partial DiGeorge – Evans syndrome (warm AIHA, ITP)	Remission on rapamycin
Pat #9 (16 /M)	Severe thrombocytopenia and anemia; speech delay, cardiac defect	22q11.2 deletion (<i>TBX1</i> haploinsufficiency; FISH)	Partial DiGeorge – Evans syndrome (warm AIHA, ITP)	Remission on rapamycin
Pat #10 (8 /M)	Severe thrombocytopenia and anemia; developmental delay	22q11.2 deletion (<i>TBX1</i> haploinsufficiency; FISH)	Partial DiGeorge – chronic ITP	Durable response on eltrombopag
Regulatory T-cell disorders				
Pat #11 (12 /M)	Severe thrombocytopenia and anemia; nephrotic syndrome (FSGS)	Monoallelic VUS in <i>FOXP3</i> (WES trio)	IPEX syndrome with aplastic anemia	Stable CBC on rapamycin; HSCT and LV- <i>FOXP3</i> gene therapy offered
Monogenic diseases predisposing to cytopenia with targeted therapy				
Pat #12 (21 /M)	Severe neutropenia and thrombocytopenia; recurrent infections	Biallelic pathogenic variants in <i>G6PC3</i> (WES trio)	AR <i>G6PC3</i> LOF – congenital neutropenia	Stable CBC on SGLT2 inhibitor (empagliflozin)
Pat #13 (1 /M)	Severe thrombocytopenia and anemia; hemorrhagic eye discharge	Monoallelic pathogenic variant in <i>MAGT1</i> (PID panel)	X-linked <i>MAGT1</i> deficiency – Evans syndrome	Remission on magnesium and eltrombopag
Pat #14 (12 /F)	Severe anemia; autoimmune enteropathy and Hashimoto thyroiditis	Monoallelic pathogenic variant in <i>CTLA4</i> (PID panel)	<i>CTLA4</i> haploinsufficiency – warm AIHA	Remission on CTLA-4-Ig (abatacept)
Others				
Pat #15	Severe thrombocytopenia and	Negative PID panel	SLE – Evans syndrome	Remission on rapamycin +

Patient / Age / Gender	Clinical presentation and laboratory findings	Genetic variants	Diagnosis	Targeted treatment and response to therapy
(14 /F)	anemia; arthralgia, hypothyroidism		(warm AIHA, ITP)	hydroxychloroquine
Pat #16 (15 /F)	Severe thrombocytopenia and anemia; oral ulcers	Monoallelic VUS in <i>TNFRSF6B</i> (PID panel)	SLE-like syndrome – chronic ITP	Remission on eltrombopag
Pat #17 (5 /M)	Severe thrombocytopenia; arthralgia, fatigue	Negative WES trio	SLE – Evans syndrome (warm AIHA, ITP)	Remission on rapamycin + hydroxychloroquine
Pat #18 (18 /M)	Thrombocytopenia; no other symptoms	Negative WES trio	CVID – chronic ITP	Remission on rituximab + IVIG
Pat #19 (17 /F)	Thrombocytopenia and anemia; post-Fontan status	Negative PID panel	Fontan syndrome – Evans syndrome (warm AIHA, ITP)	Remission on rapamycin
Pat #20 (17 /F)	Thrombocytopenia; depression	Negative WES trio	Chronic ITP	Remission on rapamycin

Comprehensive overview of the genetic and mechanistic categories uncovered in this study. Variants were grouped by dominant immune pathway disturbance—type I IFN signaling, inflammasome activation, effector–Treg imbalance, thymic dysfunction, effector-T-cell defect, and bone-marrow-failure syndromes. Each segment lists key representative genes and the corresponding targeted or repurposed therapy applied in affected patients, highlighting the translational value of pathway-guided treatment.

*Severe thrombocytopenia: platelet counts commonly in the range of $10\text{--}50 \times 10^9/\text{L}$ during acute cytopenic episodes. **Severe anemia: hemoglobin levels most often between 5 and 6 g/dL during acute cytopenic episodes.

Abbreviations: ACP5: acid phosphatase 5, tartrate resistant, AD: autosomal dominant; AIHA: autoimmune hemolytic anemia; AR: autosomal recessive; CDC42: cell division cycle 42; *CTLA4*: cytotoxic T-lymphocyte–associated protein 4; CVID: common variable immunodeficiency; *G6PC3*: glucose-6-phosphatase catalytic subunit 3; F: female; FSGC: focal segmental glomerulosclerosis, Hb: hemoglobin; IPEX: immune dysregulation, polyendocrinopathy, enteropathy, X-linked; ITP: immune thrombocytopenia; IVIG: intravenous immunoglobulin replacement therapy; HSCT: hematopoietic stem cell transplantation; LV-*FOXP3*: lentiviral *FOXP3*; M: male; *MAGT1*: magnesium transporter 1; MMF: mycophenolate mofetil; *NFKB1*: nuclear factor NF-κB subunit 1; PID panel: primary immunodeficiency genetic panel (Invitae, San Francisco, CA, USA); SPENCDI: spondyloenchondrodysplasia with immune dysregulation; SGLT2: sodium-glucose cotransporter 2; Plt: platelet; SLE: systemic lupus erythematosus; *TBX1*: T-box transcription factor 1; TNFRSF6B: tumor necrosis factor receptor superfamily member 6B; VUS: variant of uncertain significance; yo: year old; WES: whole-exome sequencing; WES trio: WES from patient and both biological parents.

Table II. Clinical, genetic, and therapeutic characteristics of the patient cohort before precision-guided treatment.

Patient / Age / Gender	Clinical features and comorbidities	Other laboratory findings / bone- marrow biopsy (BMB)	Genetic variants	Diagnosis	Previous therapies
Interferonopathies					
Pat #1 (16 /M)	Fatigue, short stature	No BMB performed	Biallelic pathogenic variants in <i>ACP5</i> (WES trio)	AR SPENCDI	Steroids, rituximab x4, MMF, rapamycin, bortezomib, TPO- RA
Pat #2 (4 /F)	Fatigue, short stature, speech delay; acute SLE flare with pericarditis	Normocellular marrow with trilineage hematopoiesis	Biallelic pathogenic variants in <i>ACP5</i> (PID panel)	AR SPENCDI	Steroids + azathioprine for SLE
Pat #3 (10 /F)	Fatigue, short stature, speech delay	Hypercellular marrow with erythroid hyperplasia	Biallelic pathogenic variant in <i>ACP5</i> (PID panel)	AR SPENCDI	Steroids
Inflamasomopathies					
Pat #4 (2 /F)	Bruising, failure to thrive, hepatosplenomegaly, developmental delay	Normocellular marrow with maturing trilineage hematopoiesis	De novo likely pathogenic variant in <i>CDC42</i> (WES trio)	AD LOF in <i>CDC42</i>	High-dose steroids
Cytopenias with monogenic disease benefiting from rapamycin					
Pat #5 (2 /F)	Frequent infections, easy bruising, fatigue	Normocellular marrow with trilineage hematopoiesis	Monoallelic pathogenic variant in <i>NFKB1</i> (WES trio)	AD LOF in <i>NFKB1</i>	–
Pat #6 (7 /F)	No clinical symptoms	No BMB performed	Monoallelic pathogenic variant in <i>NFKB1</i> (WES trio)	AD LOF in <i>NFKB1</i>	–

Patient / Age / Gender	Clinical features and comorbidities	Other laboratory findings / bone-marrow biopsy (BMB)	Genetic variants	Diagnosis	Previous therapies
Pat #7 (11 /F)	No clinical symptoms	No BMB performed	Monoallelic pathogenic variant in <i>NFKB1</i> (WES trio)	AD LOF in <i>NFKB1</i>	No treatment rendered
Partial DiGeorge					
Pat #8 (0.2 /M)	Bleeding, fatigue, cardiac defect	Normocellular marrow with myeloid left shift and ↑ hematogones	22q11.2 deletion (<i>TBX1</i> hemizygosity; FISH)	Partial DiGeorge	Steroids, IVIG
Pat #9 (16 /M)	Bleeding, fatigue, cardiac defect, speech delay	No BMB performed	22q11.2 deletion (<i>TBX1</i> haploinsufficiency; FISH)	Partial DiGeorge	Steroids, IVIG
Pat #10 (8 /M)	Bleeding, developmental delay, learning differences	No BMB performed	22q11.2 deletion (<i>TBX1</i> haploinsufficiency; FISH)	Partial DiGeorge	Steroids, IVIG (DLT with rapamycin oral ulcers)
Regulatory T-cell disorders					
Pat #11 (12 /M)	Bruising, fatigue, nephrotic syndrome (FSGS)	Markedly hypocellular marrow	Monoallelic VUS in <i>FOXP3</i> (WES trio)	IPEX syndrome	IVIG, steroids, eltrombopag
Monogenic diseases predisposing to cytopenia					
Pat #12 (21 /M)	Recurrent infections, short stature, mouth ulcers	Normocellular marrow with relative myeloid hyperplasia (G-CSF)	Biallelic pathogenic variants in <i>G6PC3</i> (WES trio)	AR <i>G6PC3</i> LOF	Steroids, G-CSF
Pat #13 (1 /M)	Bleeding, hemorrhagic eye discharge	Normocellular marrow with trilineage hematopoiesis	Monoallelic pathogenic variant in <i>MAGT1</i> (PID panel)	X-linked <i>MAGT1</i> deficiency	—
Pat #14 (12 /F)	Hashimoto thyroiditis, autoimmune enteropathy, short stature	No BMB performed	Monoallelic pathogenic variant in <i>CTLA4</i> (PID panel)	<i>CTLA4</i> haploinsufficiency	—

Patient / Age / Gender	Clinical features and comorbidities	Other laboratory findings / bone-marrow biopsy (BMB)	Genetic variants	Diagnosis	Previous therapies
Others					
Pat #15 (14 /F)	Arthralgia, hypothyroidism	ANA positive; no BMB	Negative PID panel	SLE	Steroids, IVIG
Pat #16 (5 /F)	Fatigue, oral ulcers	ANA positive; no BMB	Monoallelic VUS in <i>TNFRSF6B</i> (PID panel)	SLE-like syndrome	Steroids, IVIG
Pat #17 (5 /M)	Arthralgia, fatigue	ANA positive; no BMB	Negative WES trio	SLE	Steroids, IVIG, rituximab ×4
Pat #18 (18 /M)	No clinical symptoms	No BMB performed	Negative WES trio	CVID	Steroids
Pat #19 (17 /F)	Depression; post-Fontan status	Normocellular marrow with trilineage hematopoiesis	Negative PID panel	Fontan syndrome	Steroids
Pat #20 (17 /F)	Depression	No BMB performed	Negative WES trio	Chronic ITP	Steroids, IVIG, rituximab ×4, MMF, eltrombopag

Comprehensive overview of the diagnostic evaluation of 20 children with therapy-refractory cytopenias. For each case, age, clinical presentation, laboratory findings, bone-marrow morphology, and identified genetic variants are shown, together with relevant comorbidities and prior therapies. Variants were identified by PID panel or WES and classified according to ACMG guidelines. The table highlights the diagnostic heterogeneity of cytopenias and underscores how genetic testing clarified disease mechanism and therapeutic direction.

Abbreviations:

ACP5: acid phosphatase 5, tartrate resistant; AD: autosomal dominant; AIHA: autoimmune hemolytic anemia; ANA: antinuclear antibody; AR: autosomal recessive; BMB: bone marrow biopsy; CBC: complete blood count; CDC42: cell division cycle 42; *CTLA4*: cytotoxic T-lymphocyte-associated protein 4; CVID: common variable immunodeficiency; DAT: direct antiglobulin test; DLT: dose-limiting toxicity; G-CSF: granulocyte colony-stimulating factor; *G6PC3*: glucose-6-phosphatase catalytic subunit 3; F: female; Hb: hemoglobin; IPEX: immune dysregulation, polyendocrinopathy, enteropathy, X-linked; ITP: immune thrombocytopenia; IVIG: intravenous immunoglobulin replacement therapy; HSCT: hematopoietic stem cell transplantation; LV-*FOXP3*: lentiviral *FOXP3*; M: male; *MAGT1*: magnesium transporter 1; MDS: myelodysplastic syndrome; MMF: mycophenolate mofetil; *NFKB1*: nuclear factor NF-κB subunit 1; PID panel: primary immunodeficiency genetic panel (Invitae, San Francisco, CA, USA); SPENCDI: spondyloenchondrodysplasia with immune dysregulation; SGLT2: sodium-glucose cotransporter 2; Plt: platelet; SLE: systemic lupus erythematosus; *TBX1*: T-box transcription factor 1; TNFRSF6B: tumor necrosis factor receptor superfamily member 6B; VUS: variant of uncertain significance; yo: year old; WES: whole-exome sequencing; WES trio: WES from patient and both biological parents.

Figure 1. Genetic immune diseases in the hematologist's waiting room.

Breakdown of genetic variants identified in our patient cohort according to disease mechanism and corresponding targeted therapies. Comprehensive overview of the diagnostic evaluation of 20 children with therapy-refractory cytopenias. For each case, age, clinical presentation, laboratory findings, bone-marrow morphology, and identified genetic variants are shown, together with relevant comorbidities and prior therapies. Variants were identified by Primary Immunodeficiency Panel (PID) or Whole Exome Sequencing (WES) and classified according to ACMG guidelines. The table highlights the diagnostic heterogeneity of cytopenias and underscores how genetic testing clarified disease mechanism and therapeutic direction.

All variants were classified according to ACMG guidelines.

Abbreviations:

ACP5: acid phosphatase 5, tartrate resistant, CAPS: cryopyrin-associated periodic syndrome; CDC42: cell division cycle 42; *CTLA4*: cytotoxic T-lymphocyte-associated protein 4; *G6PC3*: glucose-6-phosphatase catalytic subunit 3; F: female; IPEX: immune dysregulation, polyendocrinopathy, enteropathy, X-linked; *MAGT1*: magnesium transporter 1; NLRP12-AID: NLRP12 associated autoinflammatory disease; *NFKB1*: nuclear factor NF-κB subunit 1; SAVI: STING-associated vasculopathy with onset in infancy; SPENCDI: spondyloenchondrodysplasia with immune dysregulation; *SGLT2*: sodium-glucose cotransporter 2; SLE: systemic lupus erythematosus; *TBX1*: T-box transcription factor 1.

BONE MARROW FAILURE SYNDROMES/CONGENITAL NEUTROPENIA

Disease	Gene	Therapy
Fanconi Anemia	<i>FANCA</i>	Hematopoietic Stem Cell Transplantation, Gene Therapy
Shwachman-Diamond-like syndrome	<i>DNAJC21</i>	Hematopoietic Stem Cell Transplantation

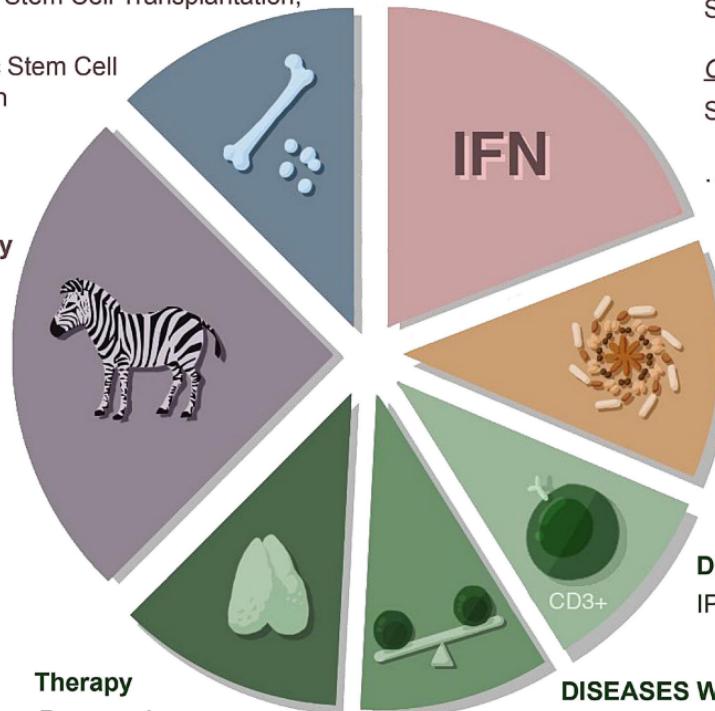
SPECIFIC DEFECTS

Disease	Gene	Targeted Therapy
CTLA-4 haploinsufficiency	<i>CTLA4</i>	Abatacept, Rapamycin
PI3Kdelta activation syndrome	<i>PIK3CD</i> or <i>PIK3R1</i>	Leniolisib
G6PC3 deficiency	<i>G6PC3</i>	SGLT2 inhibitor
MAGT1 deficiency	<i>MAGT1</i>	TPO mimetic
...And others		

THYMIC DEFECTS

Disease	Gene	Therapy
22q11 Deletion Syndrome.	<i>delChr22q</i>	Rapamycin
TBX1	<i>TBX1</i>	Rapamycin

... And others



INTERFERONOPATHIES (type I IFN pathway)

Disease	Gene	Targeted Therapy
SPENCDI	<i>ACP5</i>	JAK inhibitors, Anifrolumab-fnia
<u>Other examples:</u>		
SAVI	<i>TMEM173</i>	JAK inhibitors, Anifrolumab-fnia
... others		

INFLAMMASOMOPATHIES (IL-1/IL-18 pathway)

Disease	Gene	Targeted Therapy
CDC42 deficiency	<i>CDC24</i>	IL-1/IL-18 inhibition
<u>Other examples:</u>		
CAPS	<i>NLRP3</i>	IL-1/IL-18 inhibition
NLRP12-AID	<i>NLRP12</i>	IL-1/IL-18 inhibition
...		

REGULATORY T CELL DEFECT

Disease	Gene	Targeted Therapy
IPEX	<i>FOXP3</i>	Rapamycin, Gene Therapy Hematopoietic Stem Cell Transplantation

DISEASES WITH EFFECTOR T CELL DYSFUNCTION

Disease	Gene	Therapy
NFKB1 Deficiency	<i>NFKB1</i>	Rapamycin
... And others		