Sistema Socio Sanitario
Regione Lombardia
ASST Spedali Civili

UNIVERSITÀ DEGLI STUDI DI BRESCIA

Neutropenie congenite e immuni: come orientarsi nell'iter diagnostico Terapeutico

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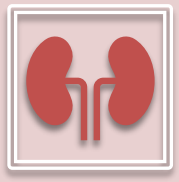
Neutropenie congenite e immuni

Come orientarsi nell'iter diagnostico-terapeutico



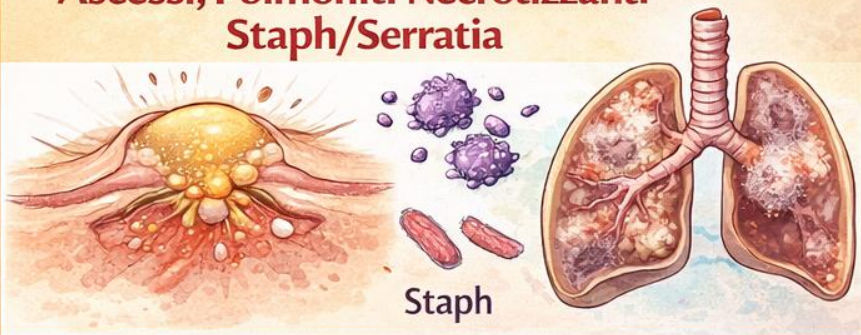
Variabili chiave: tipo di infezioni, gravità della neutropenia, età d'esordio

Le infezioni come e quando

1. **Tipo di infezioni**
2. **Gravità e pattern della neutropenia**
3. **Età d'esordio**

Tipologia di Infezioni Gravi e Tipo di IEI

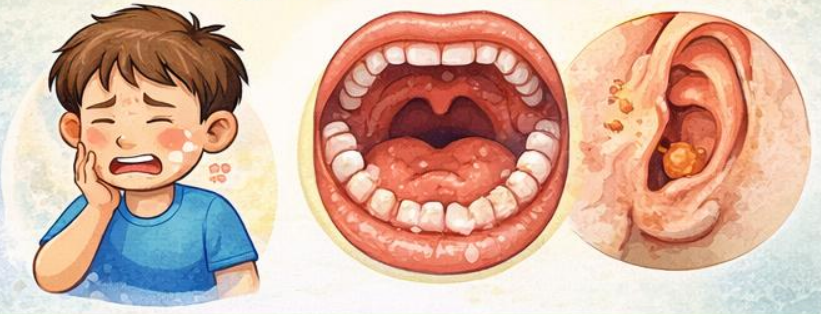
Ascessi, Polmoniti Necrotizzanti Staph/Serratia



DIFETTI FUNZIONALI

CGD · LAD · STAT3-HIES

Gengiviti, Stomatiti, Otiti



NEUTROPENI Autimmune / Congenite Moderate

Sepsi Neonatali, Omfaliti

NEUTROPENIE CONGENITE SEVERE

(ELANE, HAX1, G6PC3)

Infezioni Virali Severe

DIFETTI COMBINATI

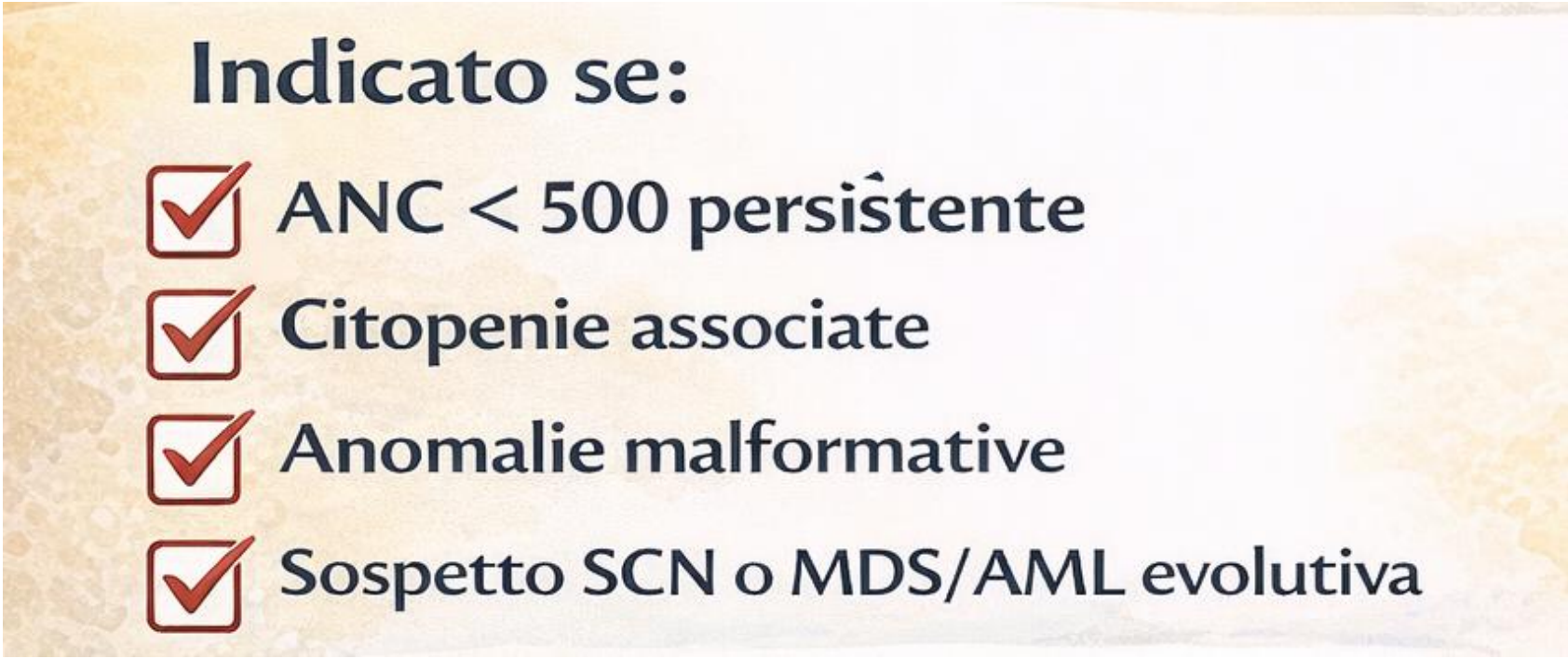
(WHIM, DOCK8)

Veronica, 13 mesi

Ricoverata in Reparto:

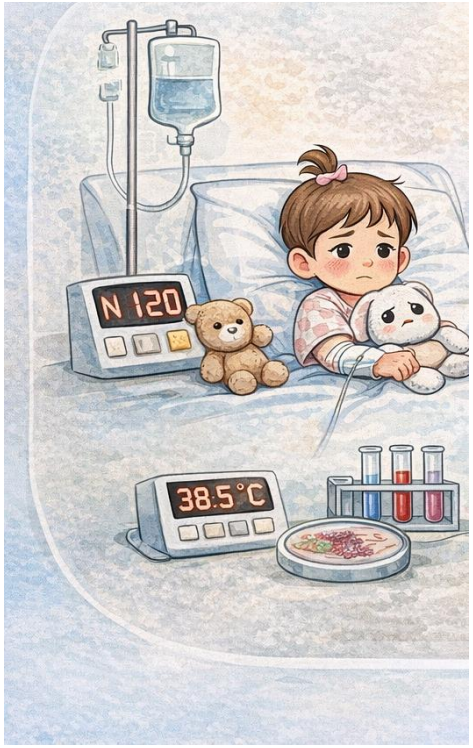
- ☑ Emocromo + formula: **GB 4400 cell/mmc, N 120 cell/mmc, L 4230 cell/mmc**, PLTs 210000/mmc.
- ☑ Esame morfologico striscio di sangue periferico: confermata la neutropenia severa, rappresentata da **neutrofili** morfologicamente **normali**.
- ☑ TORCH: negativo
- ✔ Sierologia Adenovirus: negativa
- ✔ Sierologia EBV: IgG EA 35,1, IgG VCA 58,1.
- ✔ Aspirato naso-faringeo, emocoltura su puntata, urocoltura, coprocoltura: negativi
- ✔ **Accertamenti per neutropenia:**
  → anticorpi anti-neutrofili

Midollo osseo



Infezioni batteriche/fungine invasive + ANC <500 + esordio precoce

→ Neutropenia congenita / difetti funzionali→ Step successivo: midollo osseo + geneticaTarget: ELANE, HAX1, G6PC3, (CGD, LAD)

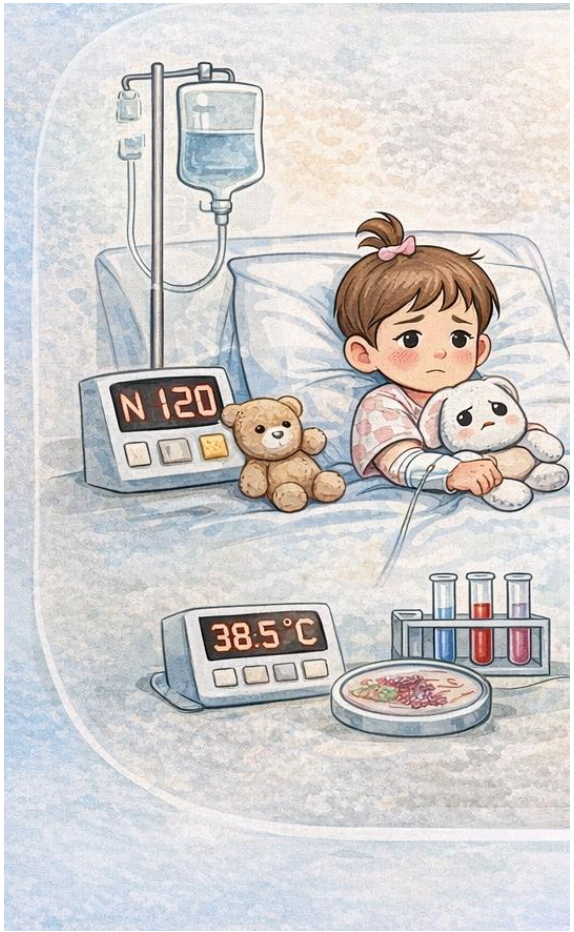


Adolescente + citopenie associate

→ **Sospetto mielodisplasia / predisposizione genetica**

→ tipo **GATA2 / SAMD9/SAMD9L**

→ Midollo + genetica obbligati

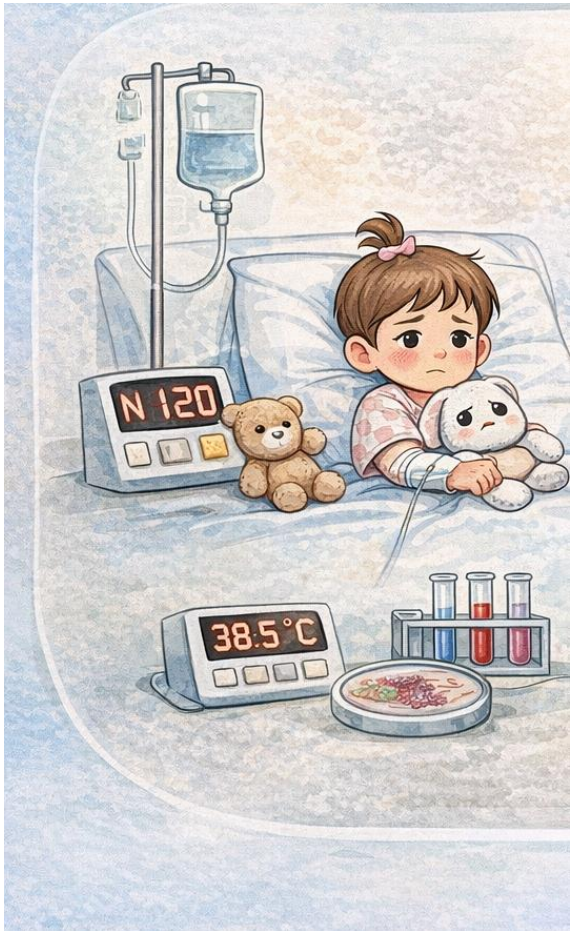


Infezioni lievi + ANC fluttuanti + età <2 anni

→ **Neutropenia autoimmune**

→ Follow-up clinico + anticorpi anti-neutrofili

→ Evitare eccesso di invasività



Berlin, Verein für Innere Medizin und Kinderheilkunde, 3. VII. 1922.

Offizielles Protokoll.

Vorsitzender: His. Schriftführer: Magnus-Levy.

Leschke: **Vergleichende Blutdruckmessungen in verschiedenen Gefäßgebieten und histologische Befunde bei Aortenklappeninsuffizienz.** (Als Orig.-Art. in Nr. 40 erschienen.)

Besprechung. Arnoldi: Bei dem Vergleich des Blutdrucks des rechten und des linken Armes kann man Unterschiede antreffen, auch ohne daß besondere Veränderungen an den Gefäßen anzunehmen sind.

Werner Schultz: **Ueber eigenartige Halserkrankungen. a) Monozytenangina.** Es handelt sich um eine Gruppe von diphtherieähnlichen, aber diphtherienegativen, oberflächlich nekrotisierenden oder pseudomembranösen Anginen, welche junge, syphilisfrei befundene Individuen zwischen 12 und 27 Jahren betrafen. Neben Halsbefund und regionärer Drüsenschwellung hatten von sieben beobachteten Fällen drei generalisierte Lymphdrüsenschwellung mit Beteiligung seltener befallener Drüsen, wie Thorakal- und Trapeziusdrüsen. Stets war die Milz vergrößert palpabel, und sechsmal bestand ebenfalls palpable Leberschwellung, nie Ikterus. Alle fieberten anfangs hoch (39,2 bis 40,8°), und die Fieberdauer war lang (zwischen 13 und 34 Tagen). Die palpablen Milzschwellungen waren in einigen Fällen 3 bis 4 Wochen verfolgbar, in anderen 2 bis 3 Monate, in einem Fall etwa 2 Jahre. Im Blute war die Gesamtleukozytenzahl entweder normal oder nur leicht erhöht (bis 16700), bei erheblicher Vermehrung der Monozyten (Höchstzahlen zwischen 27% und 78%), teilweise nahmen auch die Lymphozyten an der Vermehrung teil, wobei vielfach die Abgrenzung monozytoider lymphoider Formen von sicheren Monozyten schwierig war. Die Oxydasereaktion von Monozyten fanden wir nach der Technik von N. Rosenthal entweder negativ oder positiv, aber im letzteren Falle oft feiner und spärlich und immer deutlich unterscheidbar von der positiven Oxydasereaktion der polynukleären Neutro- und Eosinophilen. Für den oben beschriebenen Symptomenkomplex haben wir im praktisch klinischen Gebrauch die Bezeichnung „Monozytenangina" bei uns eingeführt. Die Fälle zeigen eine gewisse Verwandtschaft zu denen amerikanischer Beobachtungen. Auch Beobachtungen von Türk und Marchand gehören hierher. **b) Gangränescierende Prozesse und Defekt des Granulozytensystems.** Sämtlich tödlich verlaufene Fälle betreffen normal ernährte Frauen von 38 bis 61 Jahren, ohne Syphilis, mit Ausnahme eines Falles (Aortitis).

Schultz W, ***DMW*** 1922

- a condition of the hematopoietic system characterized by severe leukopenia and the disappearance of granulocytes from the peripheral blood. This was accompanied by a **necrotic process of mucous surfaces**, principally of the mouth and pharynx, profound **sepsis**, and **death in from two to seven days**. The onset of the disease is sudden, with **prostration and a rapid rise in temperature**, which persists throughout the course.

THE JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION

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SATURDAY, APRIL 25, 1936

THE STATE BOARD STATISTICS

The annual report on the examination and licensure of physicians in the United States for the year 1935 appears in this issue. The statistical tables and the special summaries show conditions at present and indicate trends over many years. In 1935, 7,887 applicants were licensed; of these, 5,707 were licensed by examination and 2,180 by endorsement of credentials; 5,500 licentiates were added to the medical profession and some 4,000 died. The net gain was about 1,500.

Failures to pass examination seem to be increasing. In the past five years the total number of physicians examined per year has risen from 5,608 to 6,426. The number of failures rose from 348, or 6.2 per cent, to 585, or 9.1 per cent. The number that failed to pass in 1935 was larger than in any year since 1925.

The graduates of unapproved medical schools of recent and previous years were granted examination in several states, conspicuous among which were Illinois, which registered sixty-seven such graduates, Massachusetts twenty-six, and Ohio sixteen.

The requirement of a hospital internship by state boards began in 1914. At the present time nineteen boards have such a requirement. Because many graduates of European universities and medical schools are applying for internships in this country, the Council on Medical Education and Hospitals at a meeting in February voted that "when suitable graduates of class A schools of the United States and Canada are not available, hospitals approved for intern training

GRANULOCYTOPENIA, MALIGNANT NEUTROPENIA OR AGRANULOCYTOSIS

In 1922 Schultz[1] described a condition of the hematopoietic system characterized by severe leukopenia and the disappearance of granulocytes from the peripheral blood. This was accompanied by a necrotic process of mucous surfaces, principally of the mouth and pharynx, profound sepsis, and death in from two to seven days. The onset of the disease is sudden, with prostration and a rapid rise in temperature, which persists throughout the course. Redness and swelling of the pharyngeal mucous membrane is rapidly followed by deep ulcerations, which are covered with a dirty gray membrane. Only a moderate enlargement of the regional lymph nodes is associated. Examination of the blood reveals characteristic alterations in the leukocytes. Leukopenia and granulocytopenia progress in many cases to a total disappearance of granulocytes from the peripheral blood. The total number of lymphocytes and monocytes may remain normal, be increased or, more often, diminish because of the general diminution in the number of the white cells. Their percentage relationship, however, is always increased at the expense of the disappearing granulocytes. The bone marrow as obtained by puncture or at postmortem is pale. In histologic preparations of the marrow, diminution of morphologic elements is observed with a diminution or total absence of granulopoiesis. The surviving cells belong to the lymphoid type. The megakaryocytes and the erythropoietic cells appear normal. Postmortem studies likewise reveal numerous bacterial emboli obstructing the blood vessels, necrotic processes of the mucous surfaces of the mouth, pharynx, gastro-intestinal tract, liver and spleen, and the absence of suppurative processes. The pathologic alterations described are believed to be due to failing or arrested granulopoietic function of the bone marrow.

This disease has occurred with greatest frequency in the United States and in Germany, where it was first described. It has been seen predominantly in women (from 80 to 90 per cent) of middle age of the better social status and shows a peculiar predilection for members of the medical group.

Three hypotheses were promptly advanced as to the etiology of this condition. The first considered sepsis

Infezioni invasive

→ ANC < 500 + esordio precoce

⟶ Neutropenia congenita / difetti funzionali

➜ midollo + genetica

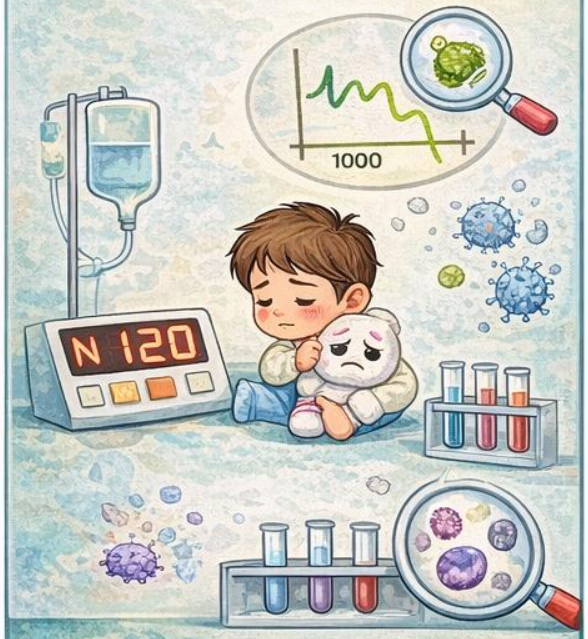
CGD, LAD, ELANE, HAX1, STAT3-HIES...

CGD, LAD, ELANE, HAX1, STAT3-HIES...

Infezioni lievi

→ ANC fluttuante + < 2 anni

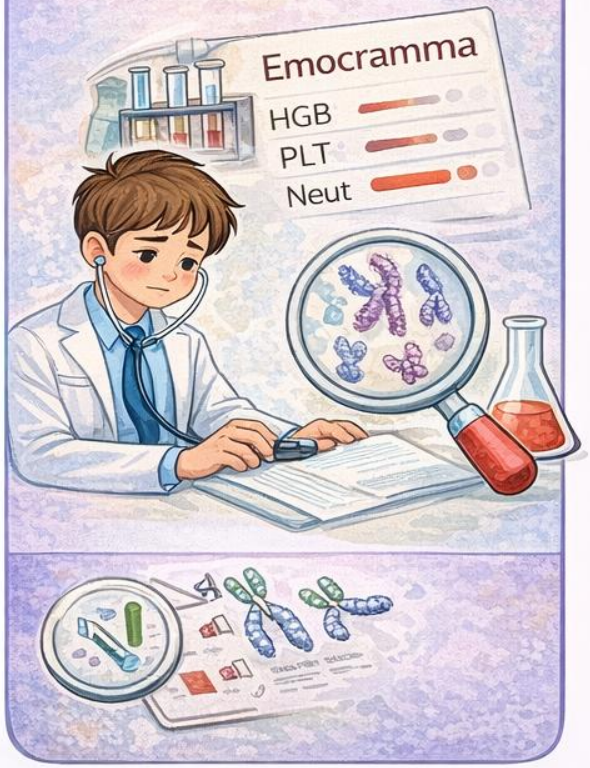
⟶ neutropenia autoimmune



Adolescente + citopenie associate

⟶ Mielodisplasie:

➜ GATA2 / SAMD9/9L



ELANE congenital neutropenia

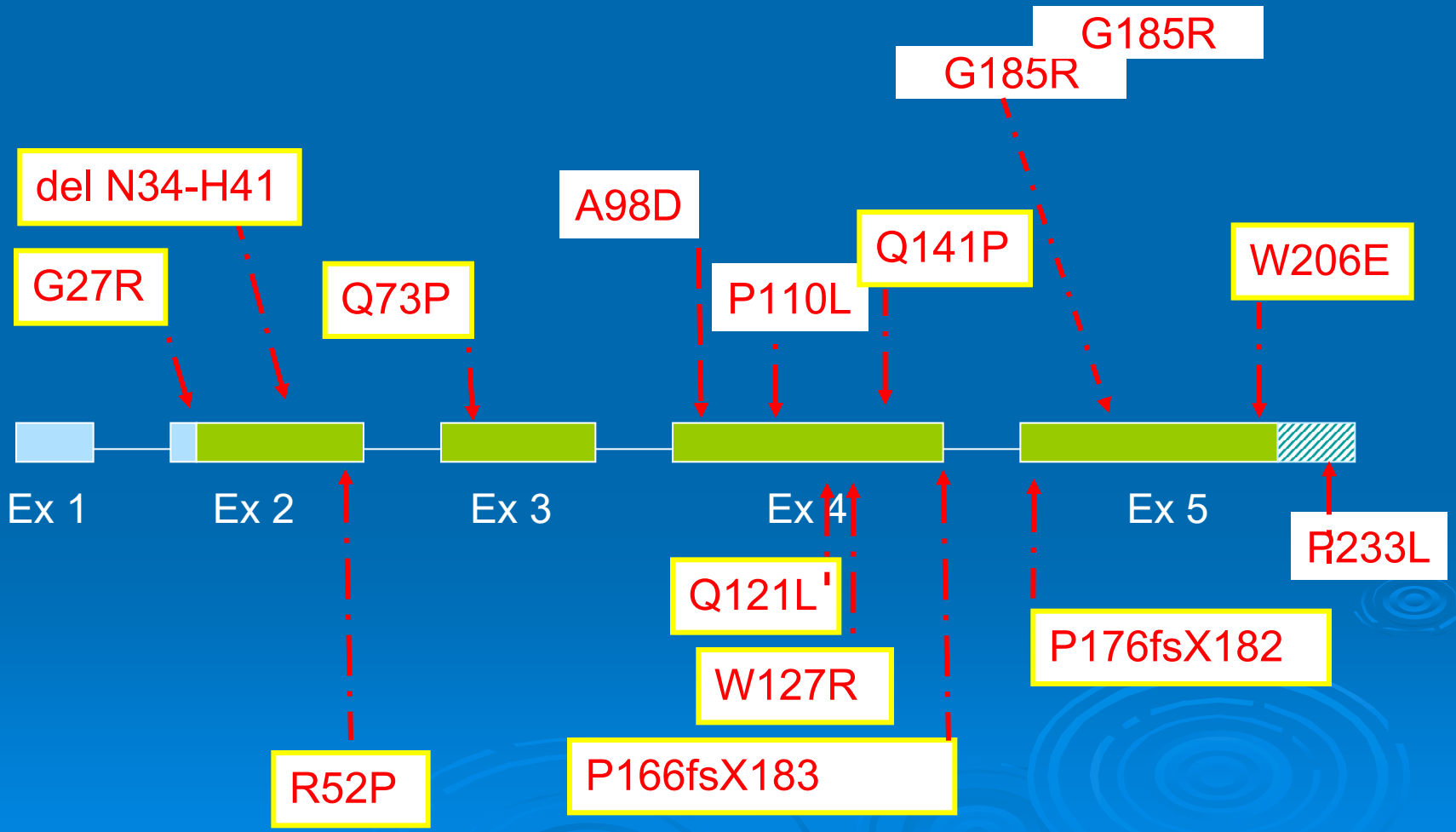
- **Clinical features**
- **Severe or recurrent infections**
- **Congenital neutropenia.** Recurrent fevers, sinusitis, gingivitis, and chronic and severe infections in the lung, liver, and soft tissues occurring at irregular intervals
- **Supportive laboratory findings**
  - At least three absolute neutrophil counts (ANCs) <500/µL obtained ≥3 months after birth supports the diagnosis.
  - ANCs are $<0.5x10^9$/L in most cases, and usually $<0.2x10^9$/L; in one series, the mean ANC was $0.112x10^9$/L.

ELANE congenital neutropenia

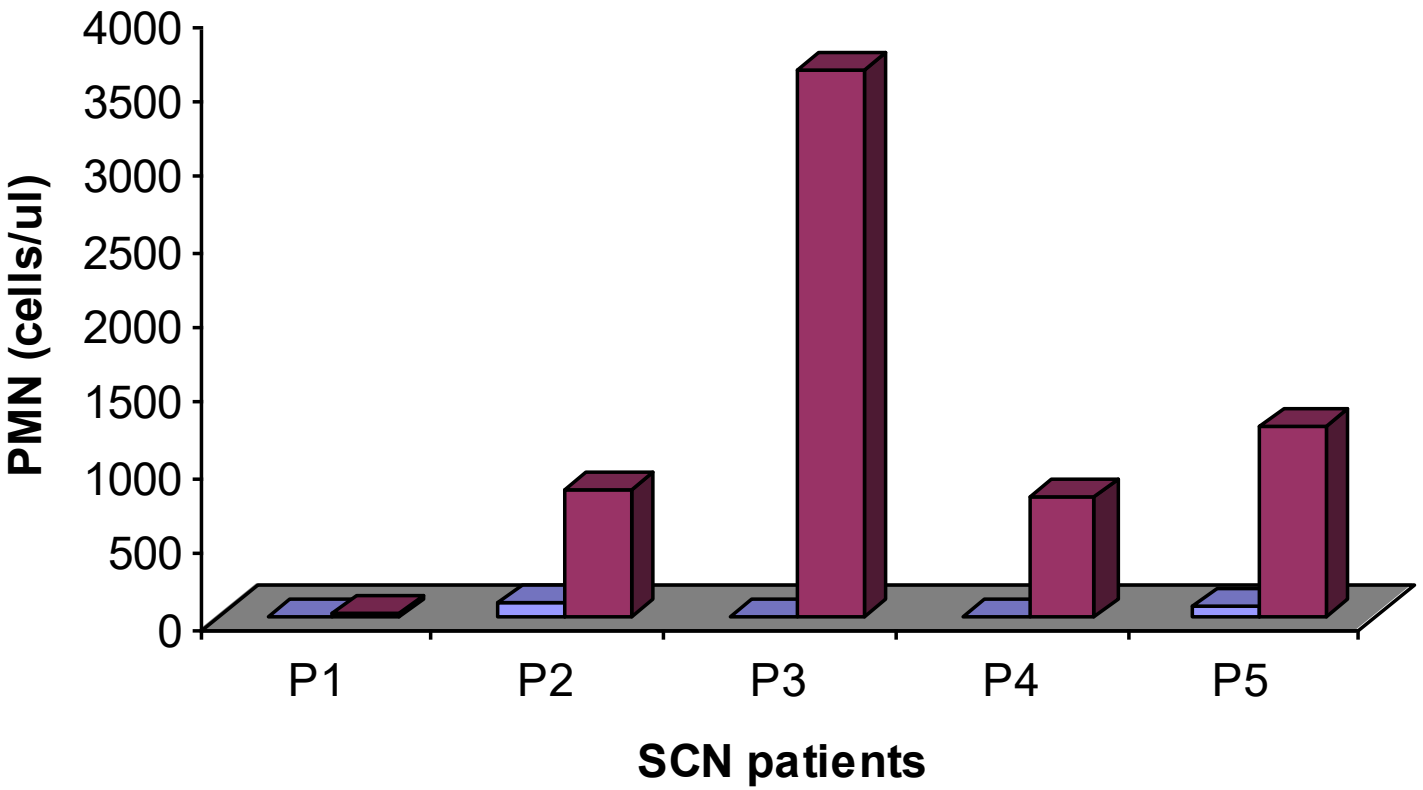
- Bone marrow aspirate typically shows "maturation arrest" at the promyelocyte or myelocyte stage of neutrophil formation. Increased bone marrow monocytes and eosinophils may be present.
- Cytogenetic analysis of bone marrow is normal.

Dale et al., Gene review 2002 updated 2018

ELANE mutations in SCN patients



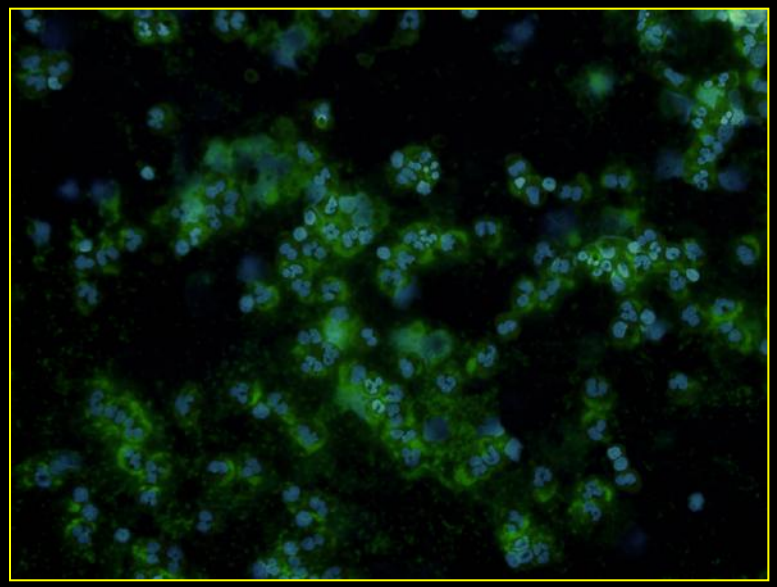
Response to G-CSF in SCN patients



G-CSF: 5-30 ug/kg/day

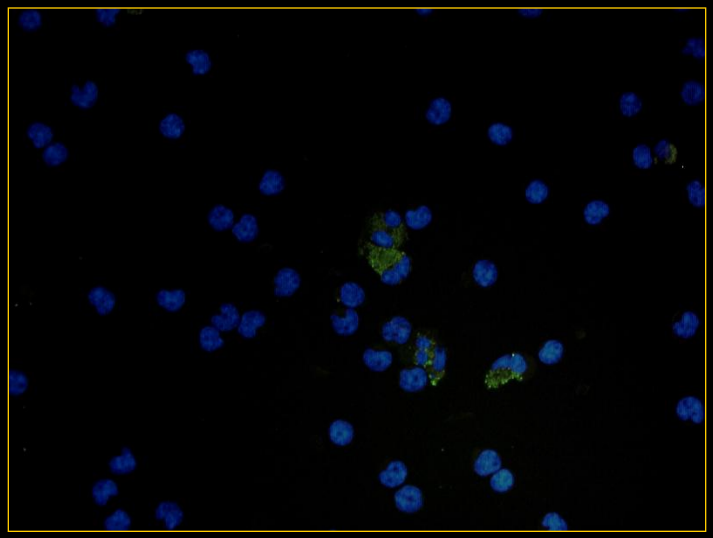
- **Cyclic neutropenia**
  - Mouth ulcers, pharyngitis, and fever recurring regularly at three-week intervals
  - Inflammation and infection of the sinuses, upper- and lower-respiratory tract, and skin including the perianal area
  - Abdominal pain and signs of an acute abdomen, suggesting sepsis and bacteremia from colonic ulcers

Neutrophil elastase expression

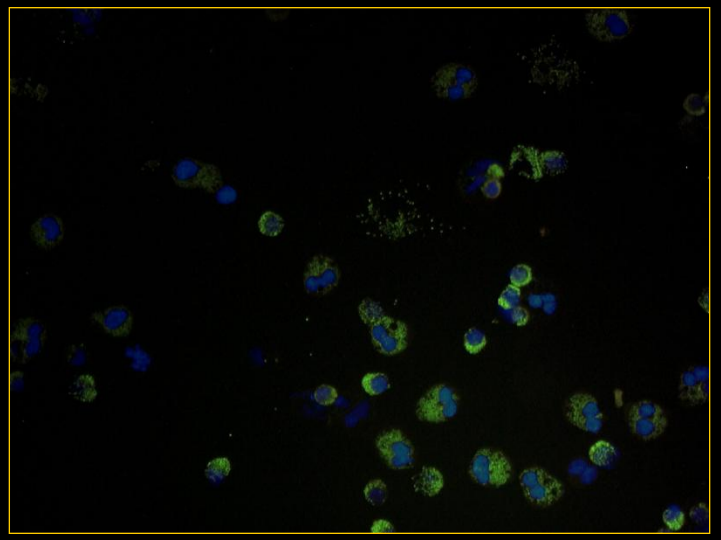


normal control

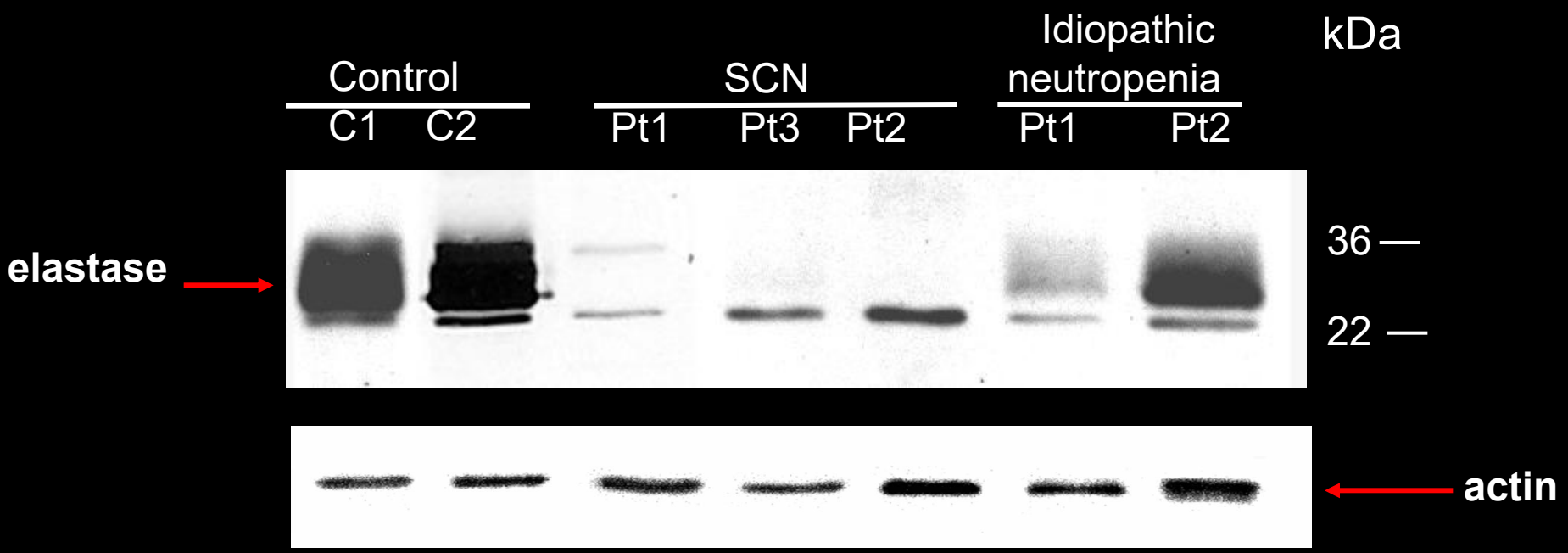
SCN patient



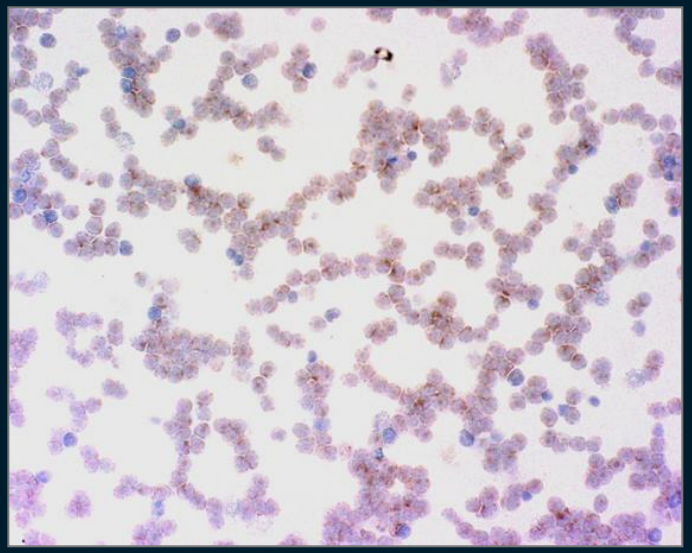
SCN patient



Western Blot analysis of Neutrophil Elastase

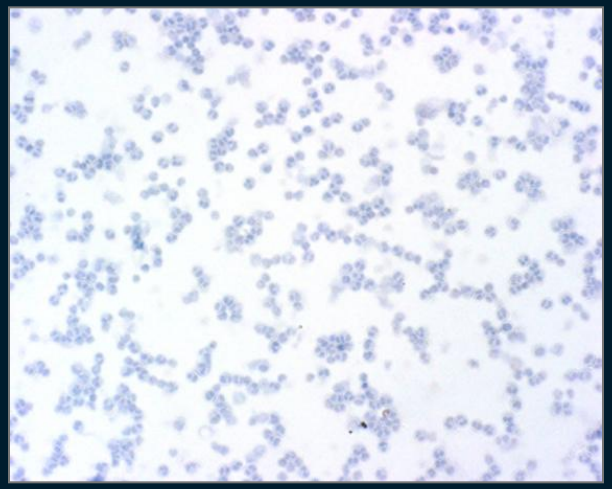


Myeloperoxidase staining

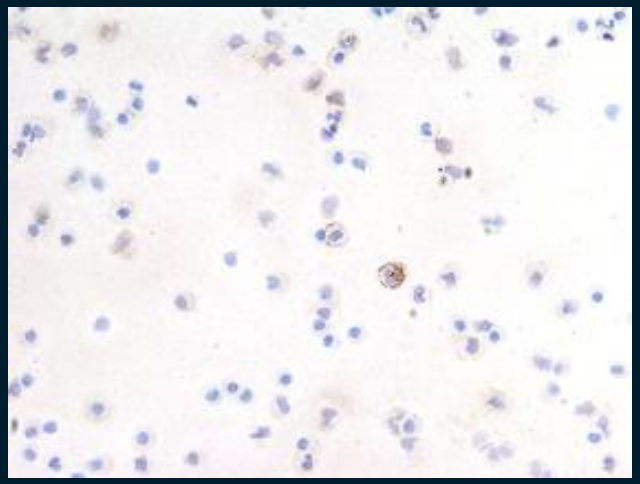


normal control

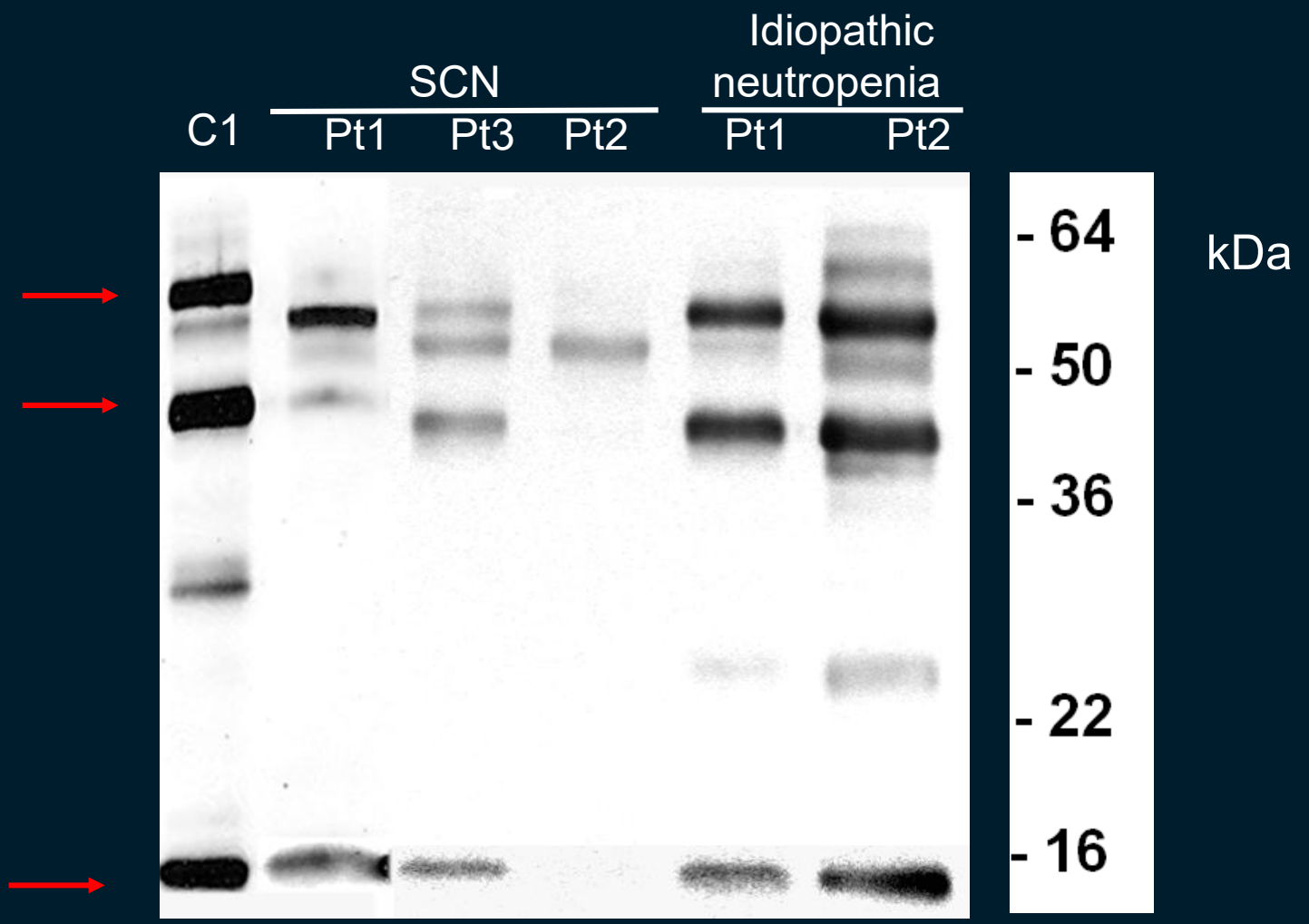
SCN Patient #1



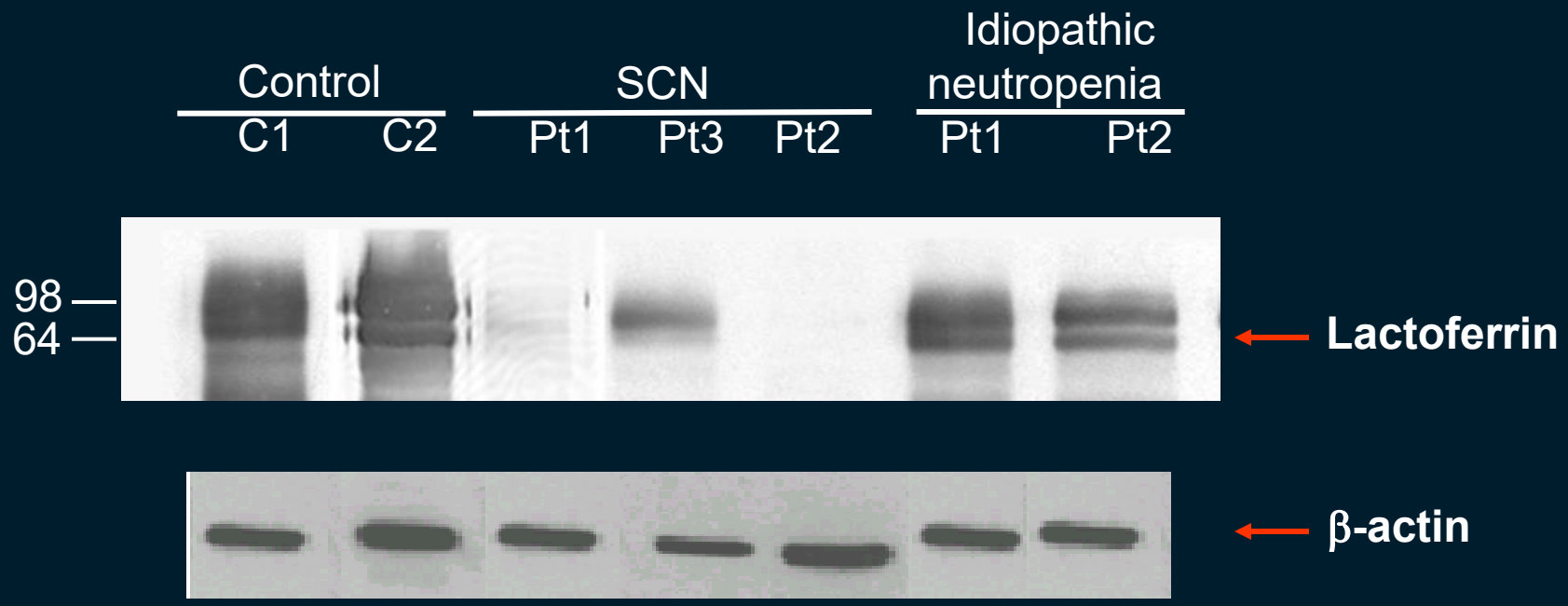
SCN Patient #3



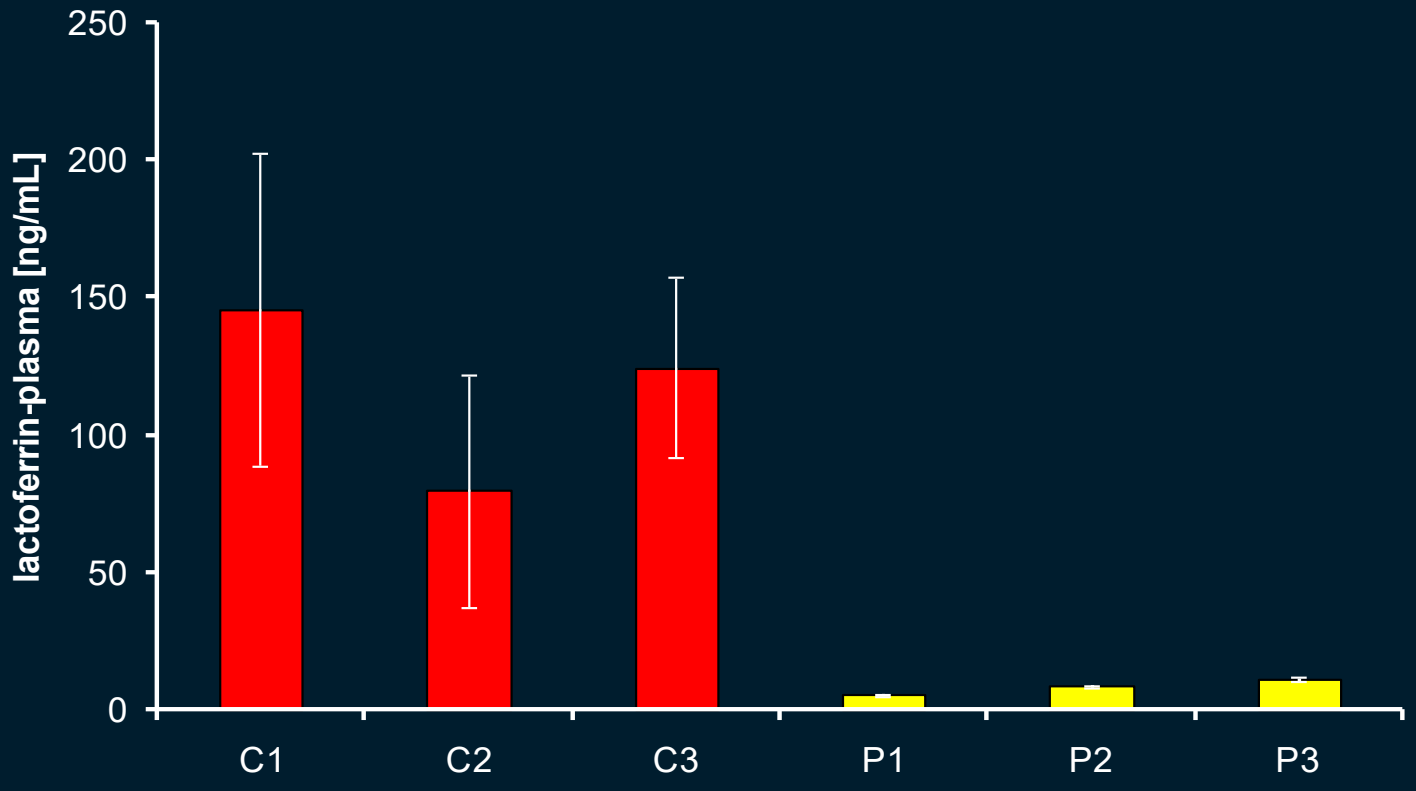
Myeloperoxidase expression



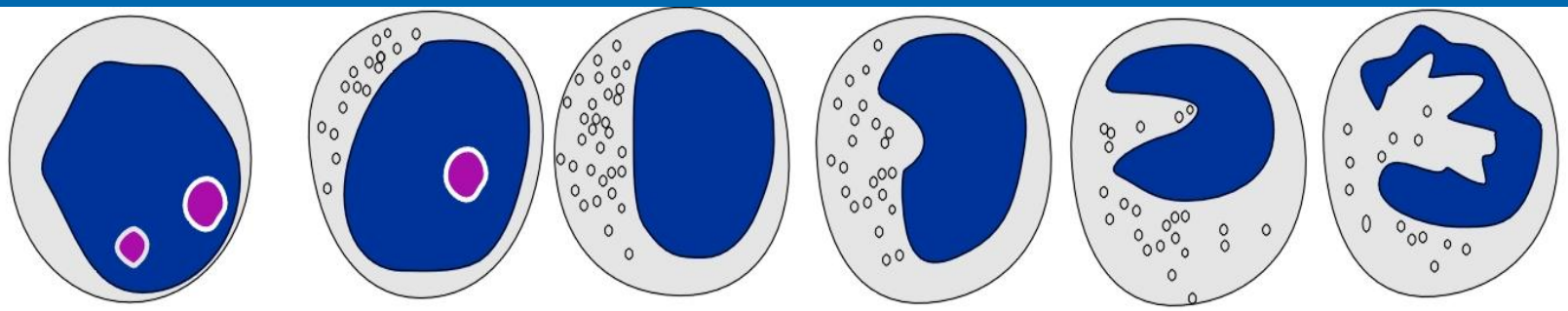
Western Blot analysis of lactoferrin expression in neutrophils



Low plasma lactoferrin levels in SCN



Neutrophils maturation stages



Myeloblaste | Promyelocyte | Myelocyte | Metamyelocyte | Band granulocyte | Segmented granulocyte

Azurophil granules →

Myeloperoxidase
Elastase

Specific granules ↔

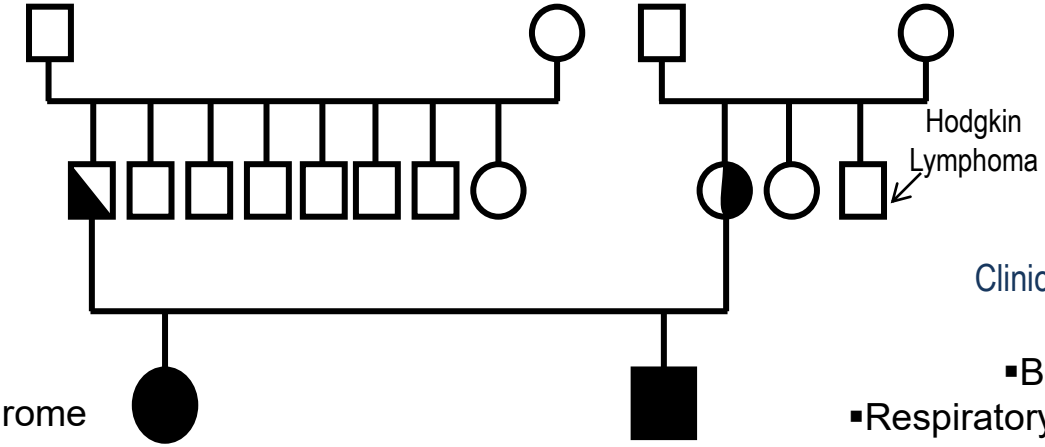
Lactoferrin

Gelatinase granules

SEVERE CONGENITAL NEUTROPENIA
CYCLIC NEUTROPENIA

↔ Gelatinase

SPECIFIC GRANULES DEFICIENCY



Clinical Presentation @Onset

- Born "early term"
- Respiratory Distress Syndrome
- Neonatal sepsis
- Poor growth
- Dismorphisms
- Neurodevelopmental delay
- Heart ventricular hypertrophia

On examination

- ✓Light skin
- ✓Light blue eyes
- ✓Clear hair
- ✓Nistagmus
- ✓Strabism
- ✓Hypotonia
- ✓Mild hepato-spleno-megaly

Immunological work-up

-**FBC**: WBC 8800/mmc, **N 167**/mmc, L 79%, M 9%, Hb 9.4 g/dl, PLTs 217000/mmc
- **Lymphocyte subsets**: CD3+ 3476/mmc (50%), CD4+ 2085/mmc (30%), CD8+ 1460/mmc (21%), CD19+ 2711/mmc (39%), CD16+ 556/mmc (8%)
-Partial IgA deficiency
-Normal lymphoproliferative response to mitogens
-**BM**: granulopoiesis dysplasia; hypocellularity

Clinical Presentation @Onset

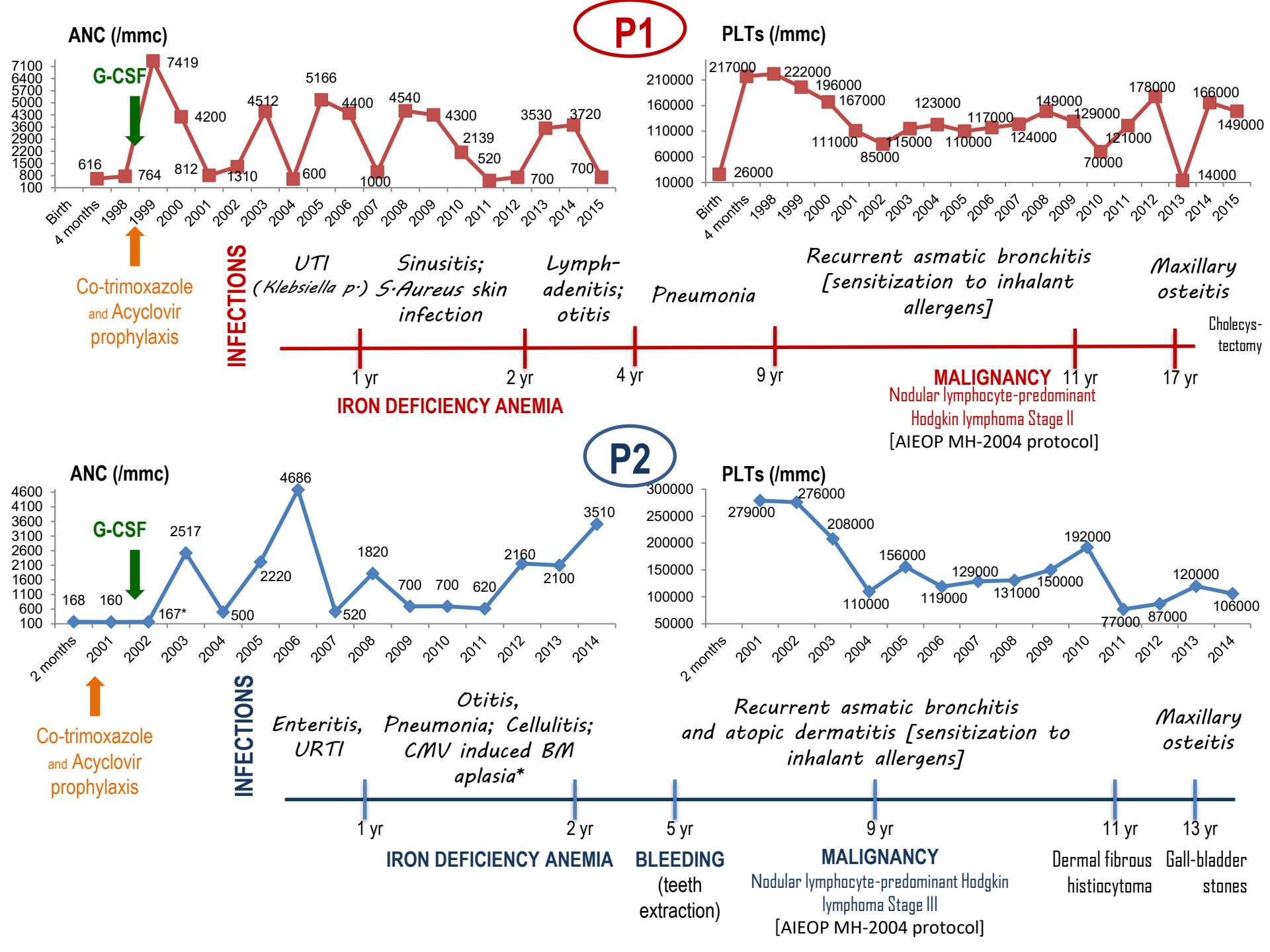
- Born at term
- Respiratory Distress Syndrome
- Poor weight gain
- Dismorphisms
- *Heart system*: ventricular hypertrophia; subaortic stenosis; mild aortic insufficiency

On examination

- ✓Light blue eyes
- ✓Reduction in pupillary diameter
- ✓Iris and retinal hypopigmentation
- ✓Nistagmus
- ✓Strabism
- ✓Light skin
- ✓Clear hair

Immunological work-up

-**FBC**: WBC 6900/mmc, **N 880/mmc**, L75%, M 11%, Hb 9.2 g/dl, PLT 279000/mmc
- **Lymphocyte subsets**: CD3+ 3674/mmc (71%), CD4+ 2950/mmc (57 %), CD8+ 776/mmc (15%), CD19+ 1345/mmc (26%), CD16+ 52/mmc (1%)
-Normal Immunoglobulins
-Normal lymphoproliferative response to mitogens
-**BM**: granulopoiesis dysplasia


P1
ANC (/mmc)
G-CSF
7100 6400 5700 5000 4300 3600 2900 2200 1500 800 100
616 764 812 1310 600 1000 4512 7419 4200 5166 4400 4540 4300 2139 520 3530 700 3720 700
Birth 4 months 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015
Co-trimoxazole and Acyclovir prophylaxis
PLTs (/mmc)
210000 160000 110000 60000 10000
217000 26000 222000 196000 167000 111000 85000 115000 123000 110000 117000 124000 149000 129000 70000 121000 178000 14000 166000 149000
Birth 4 months 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015
INFECTIONS
UTI (Klebsiella p.)
Sinusitis; S.Aureus skin infection
Lymph-adenitis; otitis
Pneumonia
Recurrent asmatic bronchitis [sensitization to inhalant allergens]
Maxillary osteitis
Cholecys-tectomy
1 yr 2 yr 4 yr 9 yr 11 yr 17 yr
IRON DEFICIENCY ANEMIA
MALIGNANCY
Nodular lymphocyte-predominant Hodgkin lymphoma Stage II
[AIEOP MH-2004 protocol]
P2
ANC (/mmc)
G-CSF
4600 4100 3600 3100 2600 2100 1600 1100 600 100
168 160 167* 2517 500 2220 4686 520 1820 700 700 620 2160 2100 3510
2 months 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014
Co-trimoxazole and Acyclovir prophylaxis
PLTs (/mmc)
300000 250000 200000 150000 100000 50000
279000 276000 208000 110000 156000 119000 129000 131000 150000 192000 77000 87000 120000 106000
2 months 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014
INFECTIONS
Enteritis, URTI
Otitis, Pneumonia; Cellulitis; CMV induced BM aplasia*
Recurrent asmatic bronchitis and atopic dermatitis [sensitization to inhalant allergens]
Maxillary osteitis
1 yr 2 yr 5 yr 9 yr 11 yr 13 yr
IRON DEFICIENCY ANEMIA
BLEEDING (teeth extraction)
MALIGNANCY
Nodular lymphocyte-predominant Hodgkin lymphoma Stage III
[AIEOP MH-2004 protocol]
Dermal fibrous histiocytoma
Gall-bladder stones

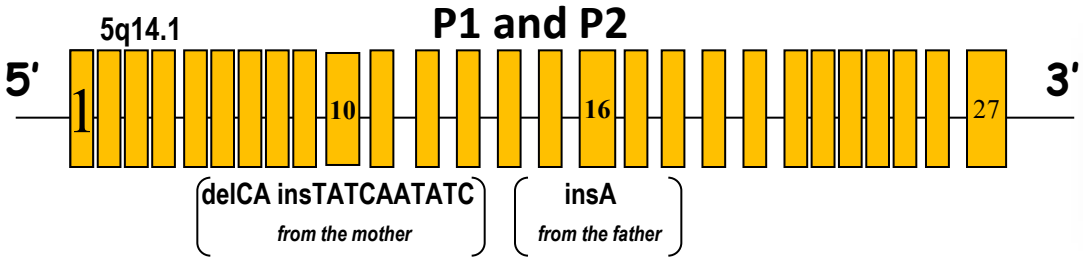
Immunodeficiency and albinism

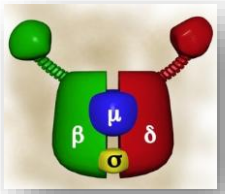
- ✓**INFECTIONS**
- ✓**PARTIAL OCULO-CUTANEOUS ALBINISM**
  - Strabism
  - Orizontal Nistagmus

with

- ✓**BLEEDING, defective PLTs aggregation**
- ✓**NEUTROPENIA, responding to G-CSF**

✓ Hermansky-Pudlak Syndrome type 2

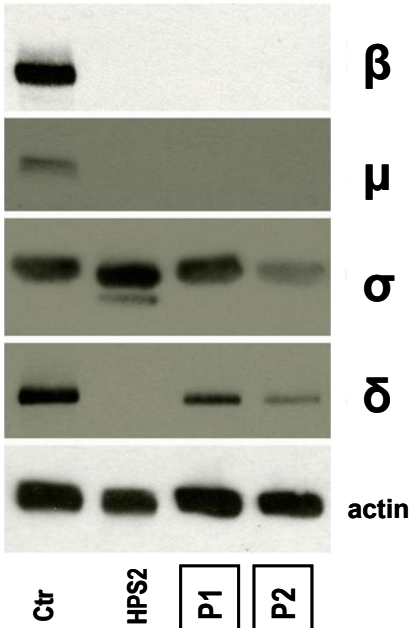


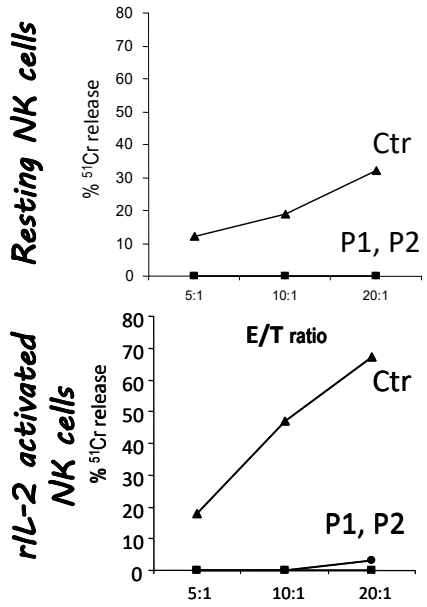


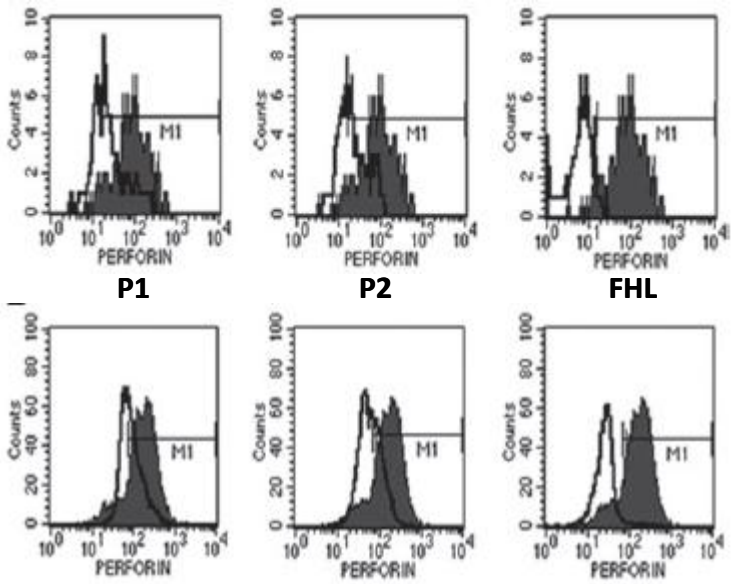
AP3B1
β3A subunit of AP3 complex

⇒ *Defective NK cytolytic activity*

⇒ *Reduced perforin content*





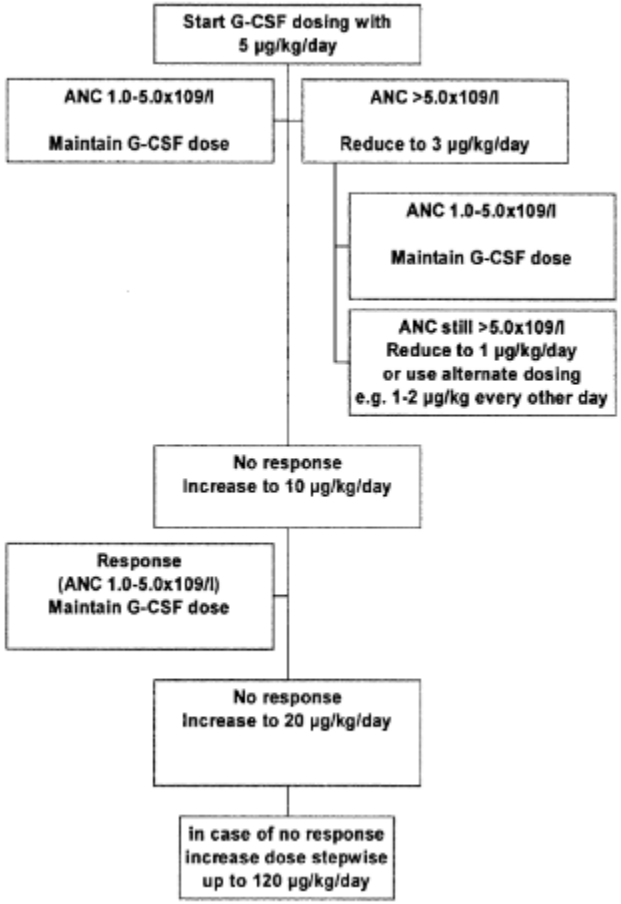


Albinism and immunodeficiency: clinical and immunological features

	Locus	Oculocutaneous albinism	Bleeding disorders	Short stature	Neurological symptoms	HLH	Neutropenia	NKs defects	CTLs defects	Giant granules
CHS	CHS1	+	+	-	+	+	+/-*	+	+	+
GS2	RAB27A	+	-	-	-	+	+/-*	+	+	-
HPS2	ADTB3A	+	+	-	-	+	+	+	+	-
HPS9	PLDN	+	-	-	-	-	-	+	N/A	-
MAPBPIP deficiency	LAMTOR2	+	-	+	-	-	+	+	+	-
HPS10	AP3D1									

modified from Dotta et al., Orph. J Rare Dis. 2013

G-CSF in SCN



Diagnosis	*n*	Mean (µg/kg/d)	Median (µg/kg/d)	Range (µg/kg/d)
Congenital	241	12·8	6·0	0·3–240
Cyclic	101	2·5	2·2	0·5–11
Idiopathic	153	2·4	1·1	0·3–55

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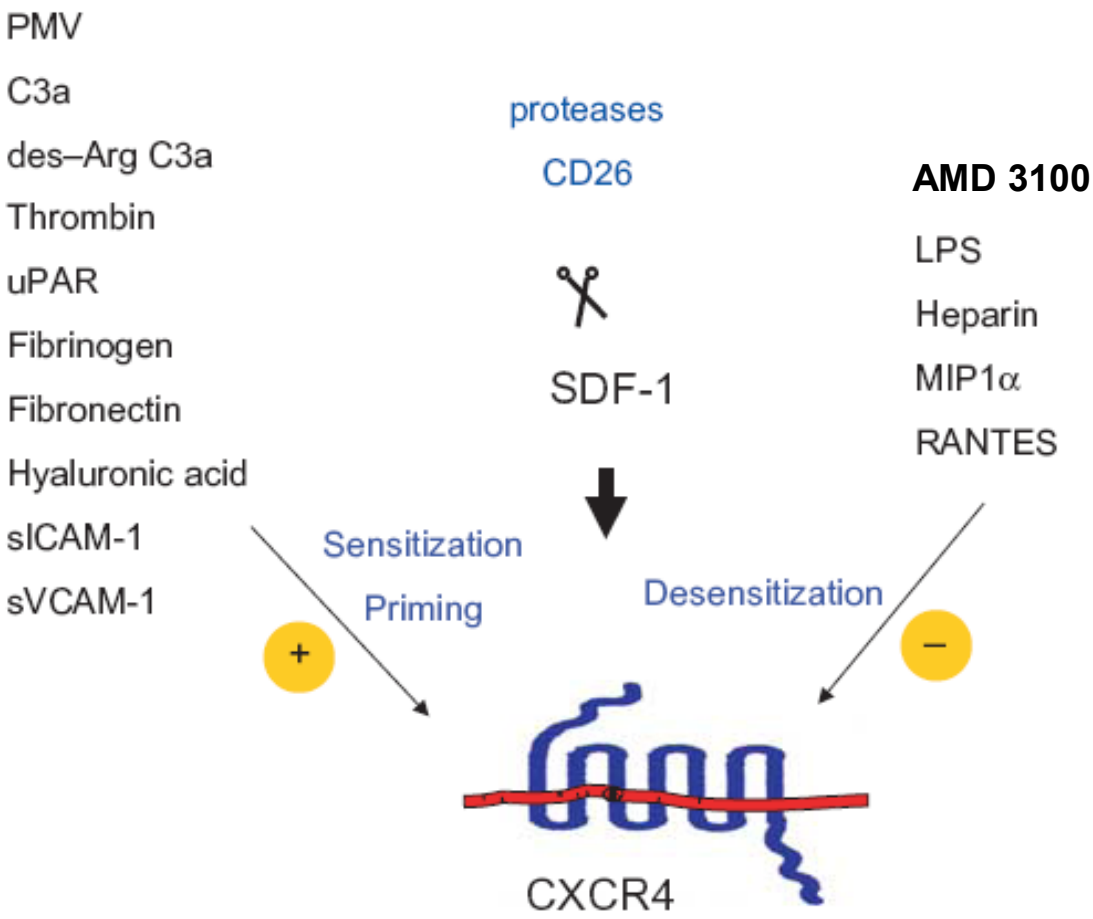
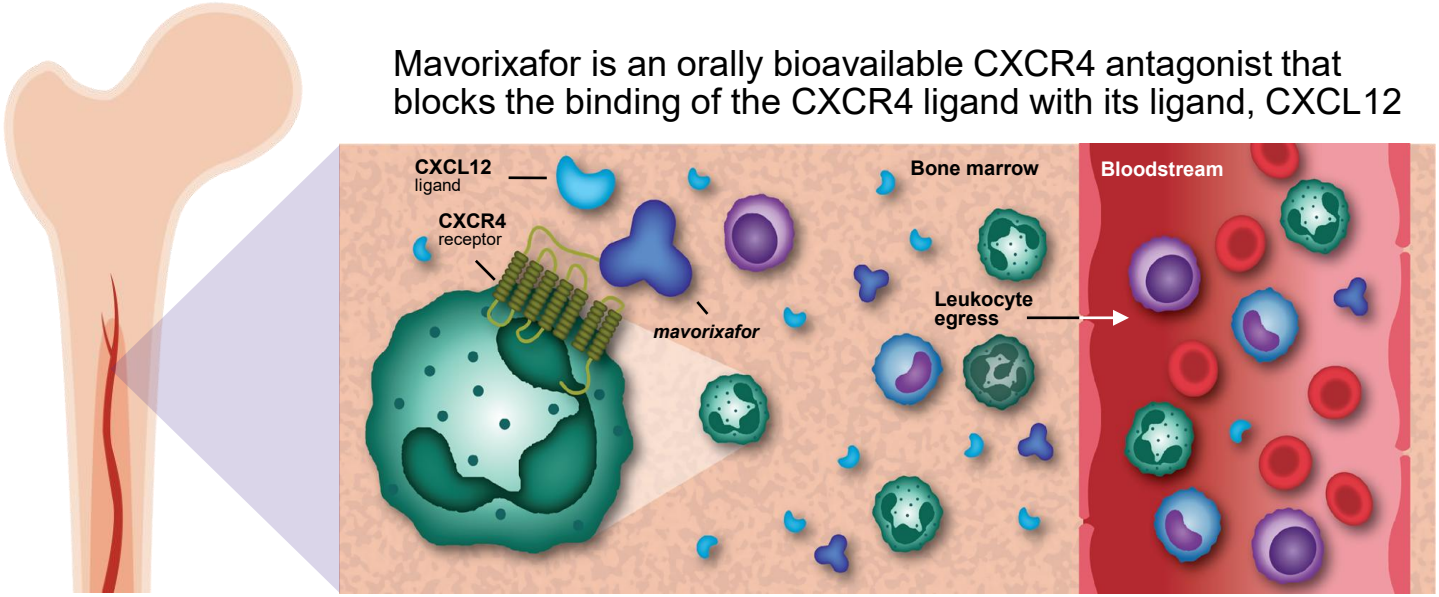

PMV
C3a
des–Arg C3a
Thrombin
uPAR
Fibrinogen
Fibronectin
Hyaluronic acid
sICAM-1
sVCAM-1
proteases
CD26
SDF-1
AMD 3100
LPS
Heparin
MIP1α
RANTES
Sensitization
Priming
Desensitization
+
–
CXCR4

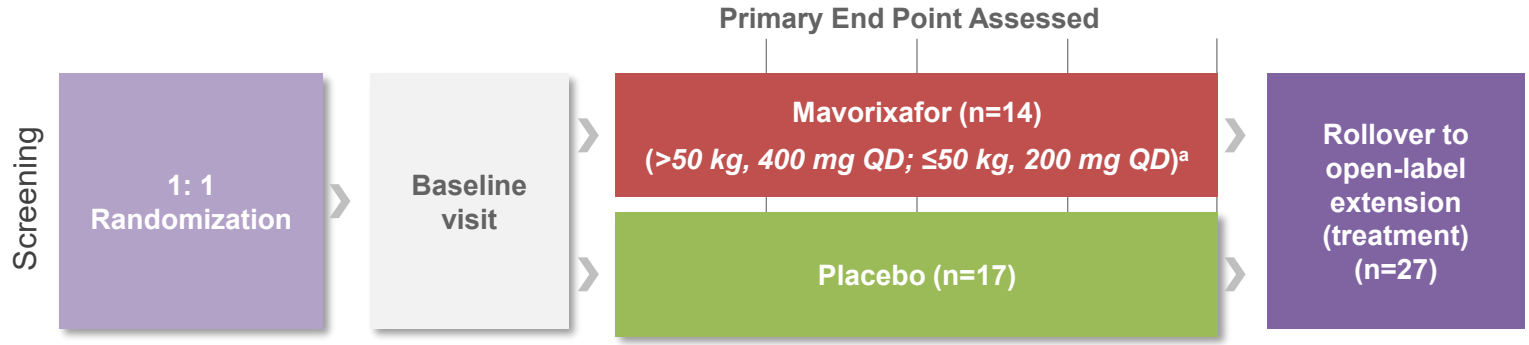
Mavorixafor is an oral CXCR4 antagonist



Mavorixafor is an orally bioavailable CXCR4 antagonist that blocks the binding of the CXCR4 ligand with its ligand, CXCL12

Mavorixafor has been show to increase mobilization of neutrophils and lymphocytes from the bone marrow into peripheral circulation

4WHIM Phase 3 Trial Design (WHIM 108)



Primary end point

- Mean TAT_{ANC} – mean of the 13, 26, 39, and 52-week assessments[b]

First key secondary end point[c]

- Mean TAT_{ALC} – mean of the 13, 26, 39, and 52-week assessments[d]

Other secondary end points[e]

- Infection-related end points
- Wart-related end points
- Safety and tolerability across 52 weeks

ALC, absolute lymphocyte count; ANC, absolute neutrophil count; QD, once daily; TAT, time above threshold.
[a]Adults and adolescents (aged 12-17 years) weighing >50 kg received 400 mg mavorixafor QD; adolescents aged 12-17 years weighing ≤50 kg received 200 mg QD. [b]TAT_{ANC} is defined as time (in hours) above threshold ANC ≥500 cells/μL over a 24-hour period, assessed every 3 months for 52 weeks. [c]Secondary end points were analyzed per a hierarchical approach prespecified in the trial protocol; not all key secondary end points included in the hierarchical sequence are shown. [d]TAT_{ALC} is defined as time (in hours) above threshold ALC ≥1000 cells/μL over a 24-hour period, assessed every 3 months for 52 weeks. [e]Not all other secondary end points are shown.

Phase 2 Clinical Trial in Chronic Neutropenia: Goals and Design

Main Phase 2 Study Goals

- ✓ Confirm durability of positive Phase 1b results
- ✓ Explore whether physicians will reduce G-CSF
- ✓ Assess long-term safety and tolerability
- ✓ Inform design of and derisk Phase 3 pivotal trial

Phase 2 Study: Assessing Safety, Durability of ANC Levels over 6-Month Period[1]

1. The neutrophil life-cycle is 10-14 days (https://doi.org/10.3389/fimmu.2021.766620); Phase 2 study's measurements of ANC over 6 months (at 30-day intervals) assess bone marrow status and durability of neutrophil production. 2. Modifications to G-CSF dosing allowed after Month 2 at physician's discretion.

Phase 2 Clinical Study in Chronic Neutropenia: Participant Disposition

Study group representative of typical CN population

Phase 2 Study Enrolled a Total of 23 Participants

Participant Disposition (n=23)	
Type of CN	
Idiopathic	15
Congenital[1]	6
Cyclic	2
Sex	
Male	10
Female	13
Mean Age	34

Mavorixafor Monotherapy	
	Baseline
Total	10

Mavorixafor + G-CSF	
	Baseline
Stable G-CSF Total	4
Adjusted G-CSF[2] Total	9

Neutrophil Functionality Sub-Study	
	Assessed
Total Evaluable Population[3]	9

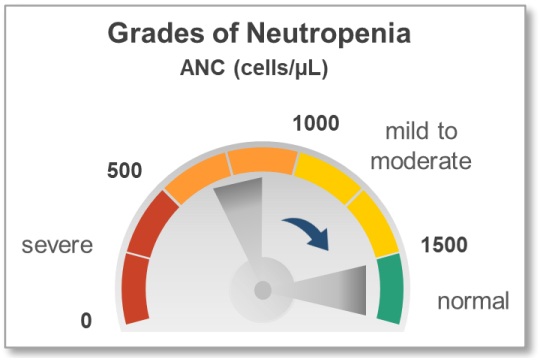
1. Congenital CN participants included those with *ELANE* variant (n=2), VPS13B variant (Cohen syndrome), G6PC3 variant/ deficiency, SRP54 variant (SDS-like syndrome), WASp variant (Wiskott-Aldrich syndrome).

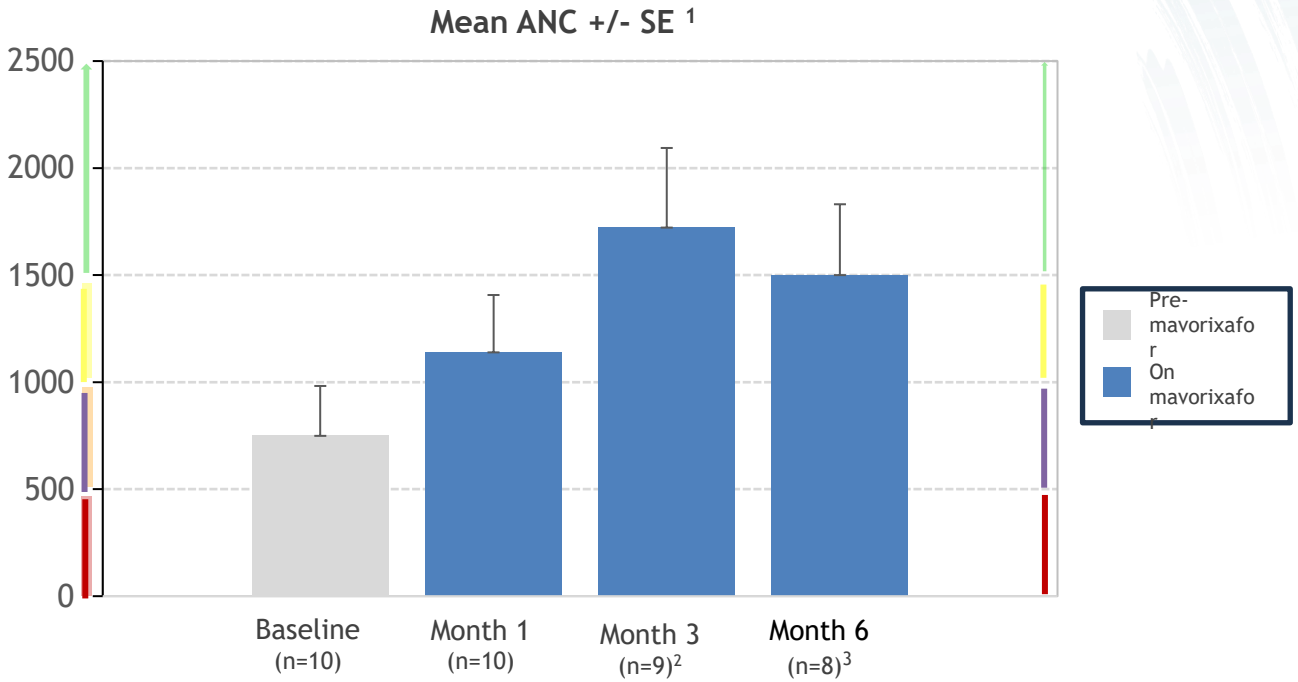
2. Modifications to G-CSF dosing allowed after Month 2 visit

3. Samples assessed for neutrophil functionality were limited by proximity to validated testing facility – complete data were available for 9 of the 23 enrolled participants.

Results increase confidence in successful Phase 3 trial outcome

- Mean ANC reached normal levels (ANC ≥ 1,500 cells/µL) at 3 and 6 months of treatment



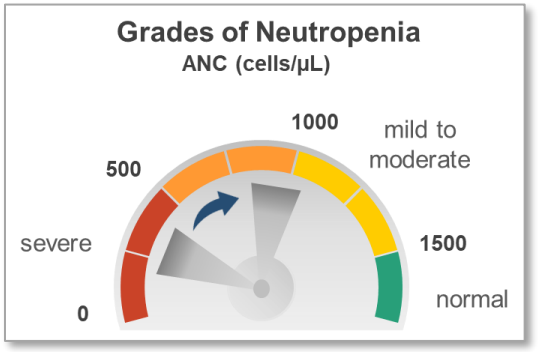


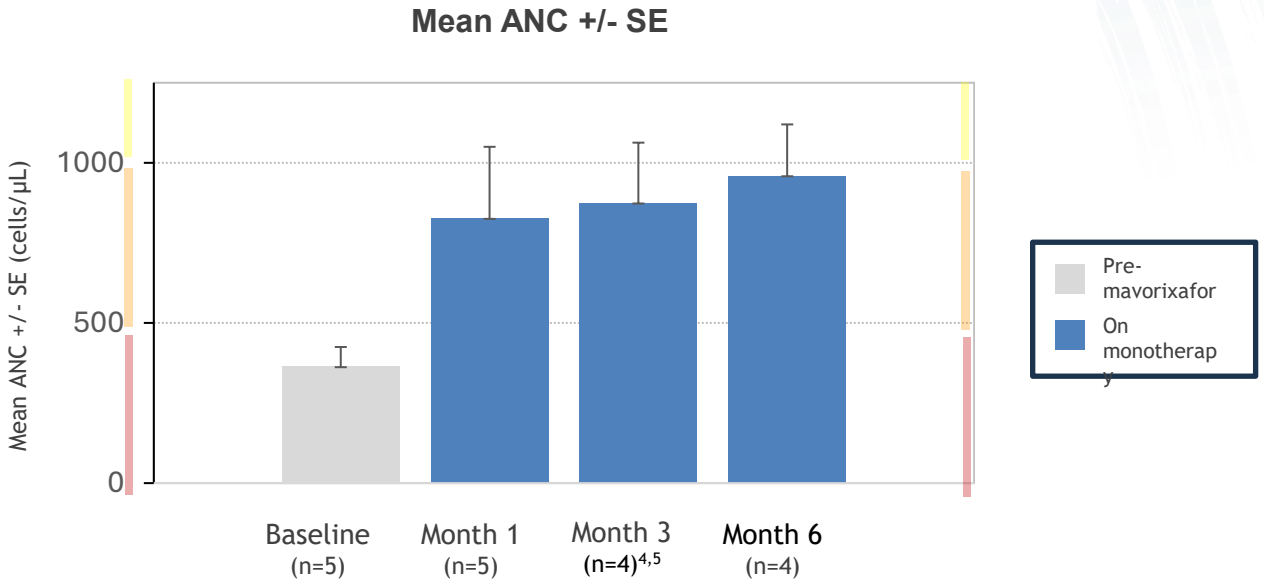
1. Data set contains two LOCF (last observation carried forward) values: one value missing at M3 assessment, one value missing at M6. 2. One patient discontinued prior to Month 3 assessment (no change from data set presented on June 27, 2024). 3. One patient discontinued prior to Month 6 assessment (no change from data set presented on June 27, 2024).

Mavorixafor Monotherapy Durably and Meaningfully Increased Mean ANC in Severe CN

Results increase confidence in successful Phase 3 trial outcome

- Physicians typically target ANC between 800 and 1,000 cells/µL in severe CN patients[1,2,3]
- Those with severe CN achieved >2x Baseline mean ANC through Month 6





1. Platzbecker, U, et al. *Blood*. 2019 Mar;133(10):1020-1030. 2. Donadieu J, et al. *Expert Rev Hematol*. 2021 Oct;14(10):945-960. 3. Newburger PE, et al. *Seminars in Hematology* 2013 Jul;50(3):198-206. 4. Data set contains one LOCF (last observation carried forward) value, due to missing ANC at M3. 5. One patient discontinued prior to Month 3 assessment (no change from data set presented on June 27, 2024)

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