IPIG
International PNH Interest Group

PNH CLINICAL PRESENTATIONS

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- No Disclosures

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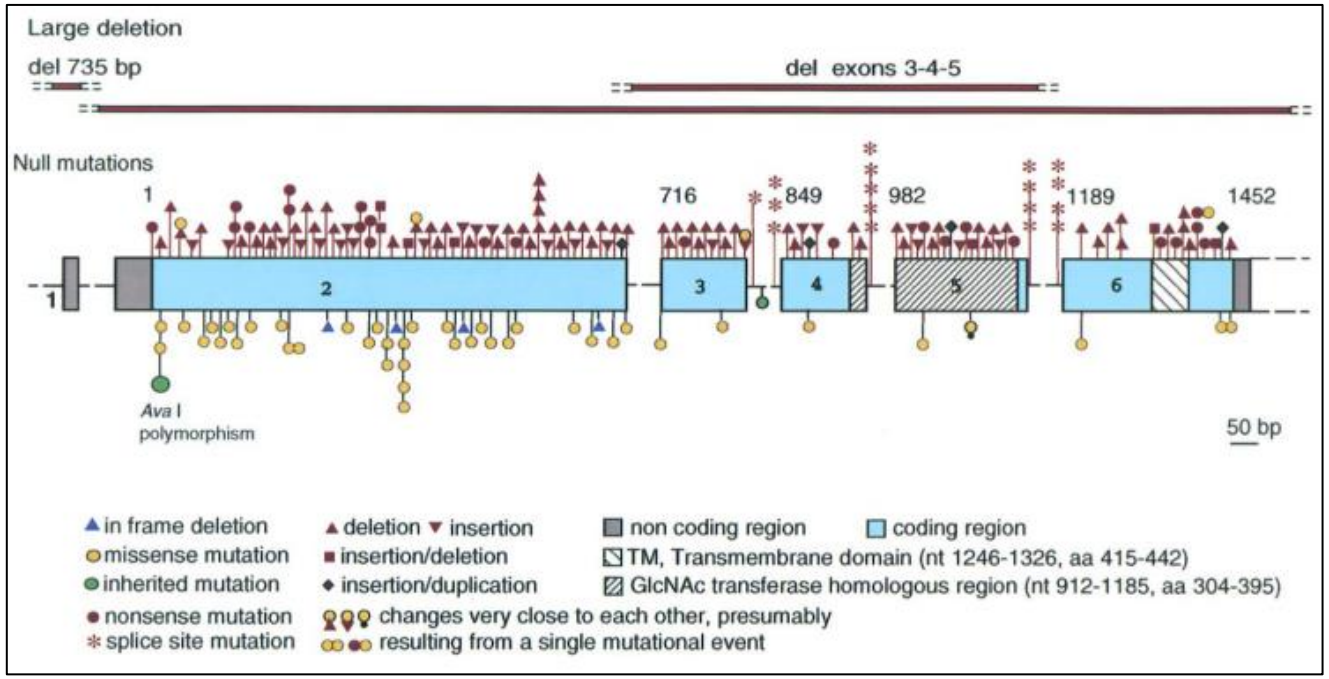
Please scan the QR code to participate in the Audience Response and Q&A

How many patients with PNH you have diagnosed and treated?

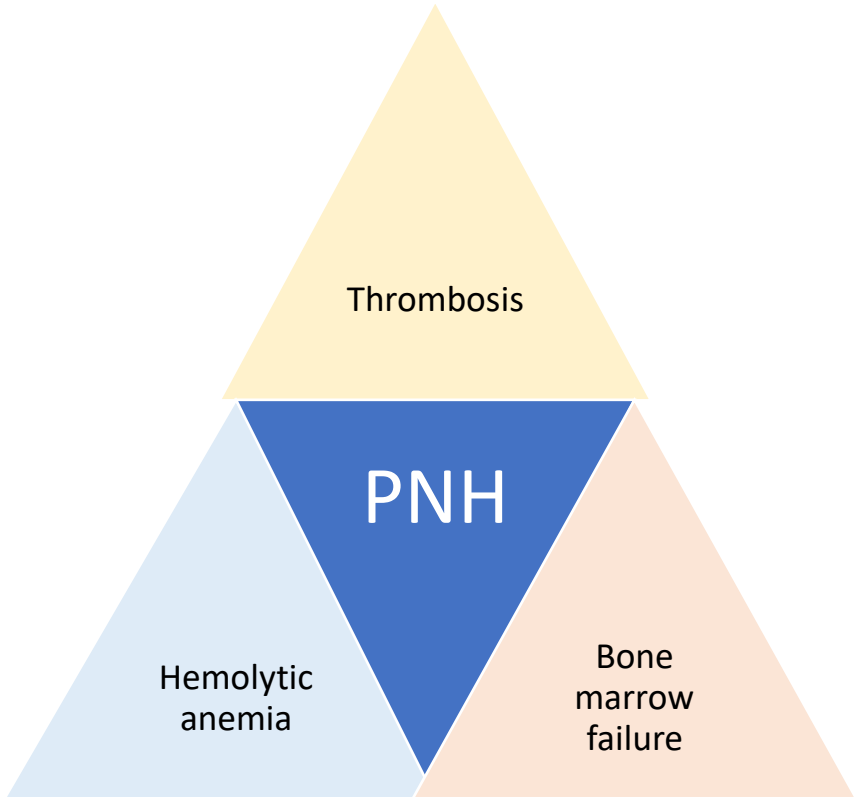
A. <5
B. 5-10
C. 11-20
D. >20

PNH – Acquired clonal non-malignant disease

Mutations throughout the coding region of the gene

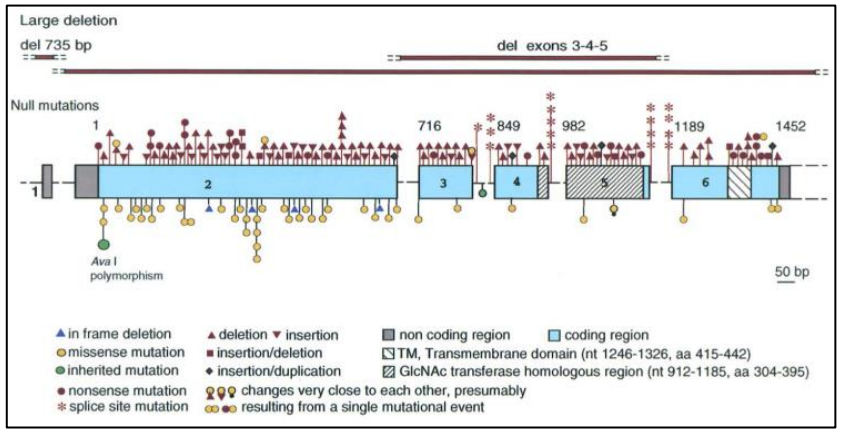


Luzzatto L. and Nafa K. 2000



Fatigue, abdominal pain, esophageal spasms, erectile dysfunction, pulmonary hypertension, and renal impairment

Clinical manifestations, pathophysiology and genetics



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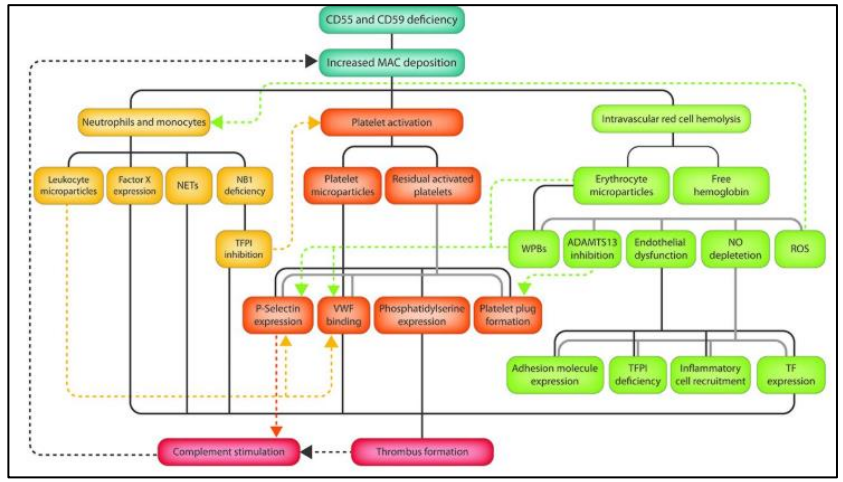
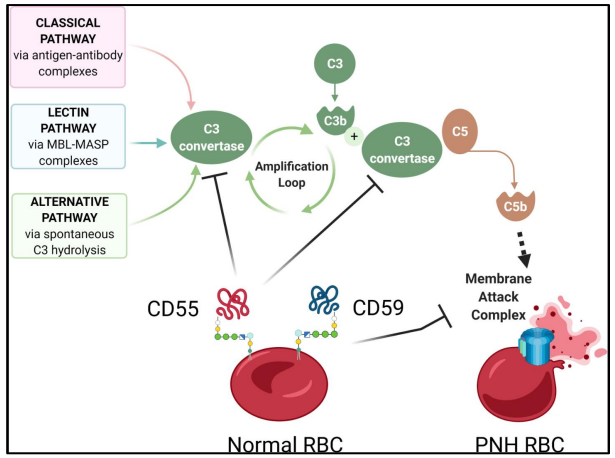
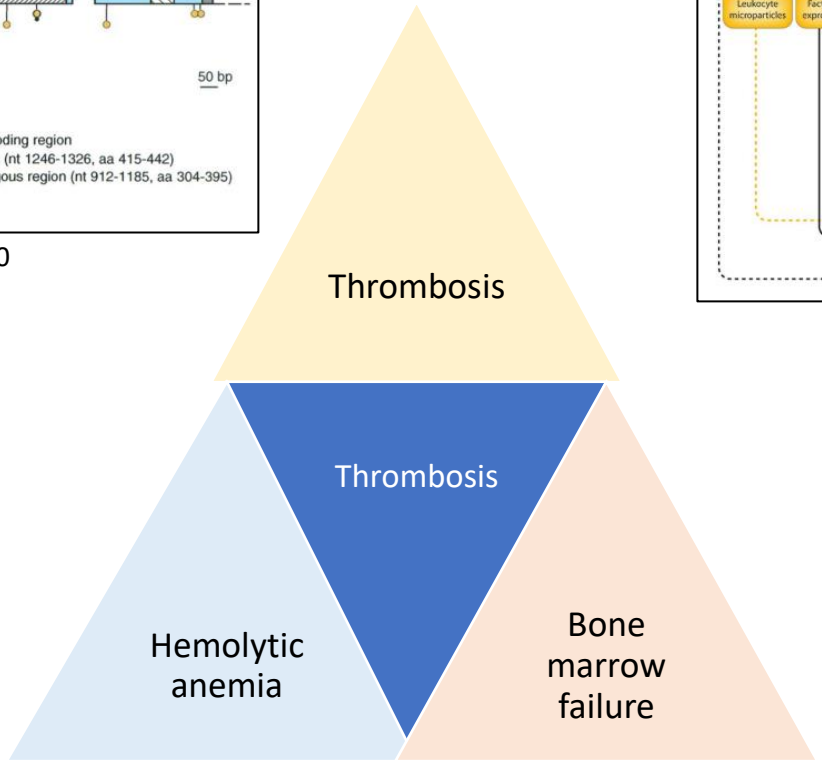
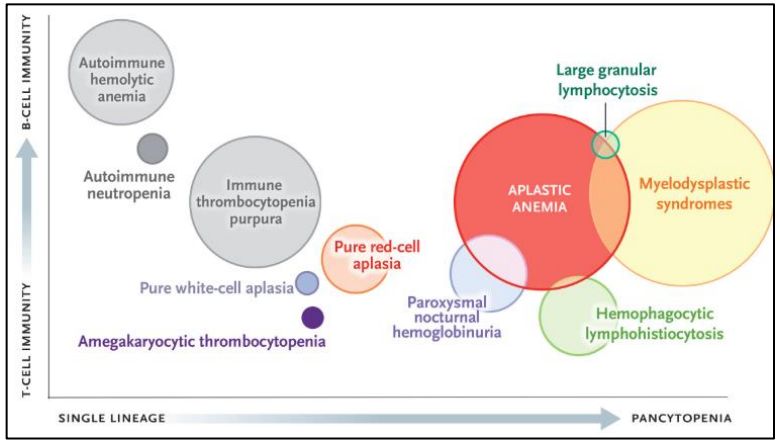


Hill A. et al. Blood. 2013, Szer J. Blood.2024



Colden MA. et al. Front.Immunol.2022





Young NS. N Eng J Med. 2018

Case #1

- 31 y.o female who recently delivered her first child presents to ER with left lower leg swelling for few days and progressive fatigue for 2 weeks
- **Laboratory** analysis showed severe anemia
- **Left lower leg Doppler** showed acute non-occlusive deep venous thromboembolism (DVT)

WBC (K/µL)	4.0
ANC (K/µL)	1.3
Hb (g/dL)	**6.5**
Platelets (K/µL)	187

Which additional tests should be performed for further work-up?

A. Coombs
B. PNH flow
C. Peripheral smear
D. PT/PTT/D-dimer
E. All of the above

Case #1

Further tests – D-dimer slightly elevated, PT/PTT normal, Coombs negative
Peripheral smear no spherocytes or schistocytes
PNH flow cytometry – GPI negative neutrophils 92.4%, GPI negative RBC 3.5%
Initial marrow showed variable cellularity (5-70%) with erythroid hyperplasia, no dysplasia

LDH (IU/L)	**700**
Total bilirubin	2.3
Direct bilirubin	1.1
Haptoglobi	<10
ARC (K/µL)	**175K/µL**

Diagnosis – Hemolytic PNH

When to suspect PNH?

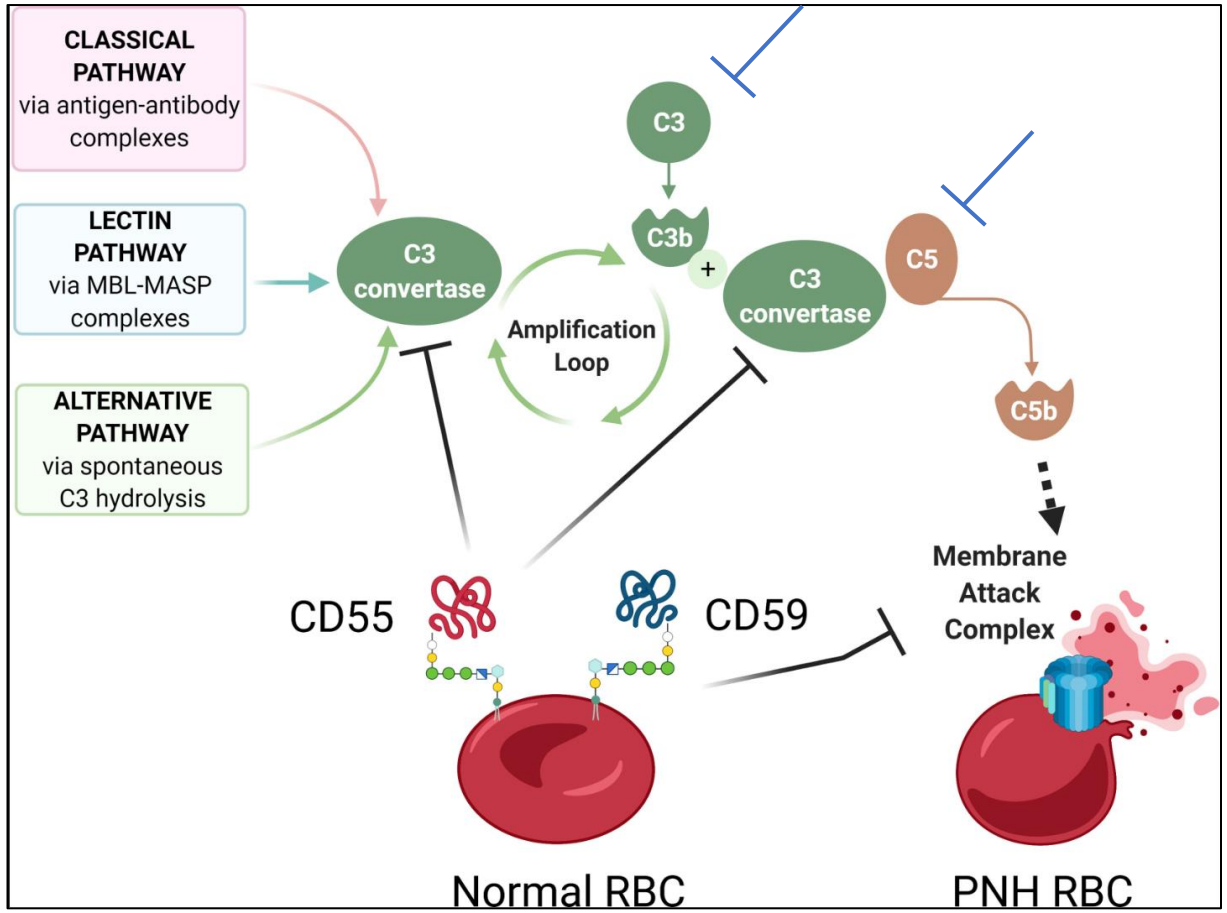
- Delay in diagnosis may occur due to rarity of disease, nonspecific and variable clinical presentations
  - Intravascular hemolytic anemia (Coombs negative)
  - Pancytopenia
  - Thrombosis, particularly in unusual sites or at young age
  - Vague smooth muscle dystonia (abdominal pain, dysphagia, erectile dysfunction)
- Other differential diagnosis to rule out: Thrombotic microangiopathy (TMAs), autoimmune hemolytic anemia, severe nutritional deficiencies, hereditary anemia (e.g. hemoglobinopathies), occult malignancy, other marrow failure syndromes (MDS, AA)
- PNH diagnosis straightforward with detection of peripheral blood cells deficient of GPI-anchored proteins, such as CD14, CD16, CD24 for white blood cells and CD55, CD59 and FLAIR for red blood cell using flow cytometry

Intravascular hemolytic anemia

Complement mediated intravascular hemolysis

Chronic hemolysis but hemolytic crisis can occur in stressful conditions (infections, trauma, and surgery)

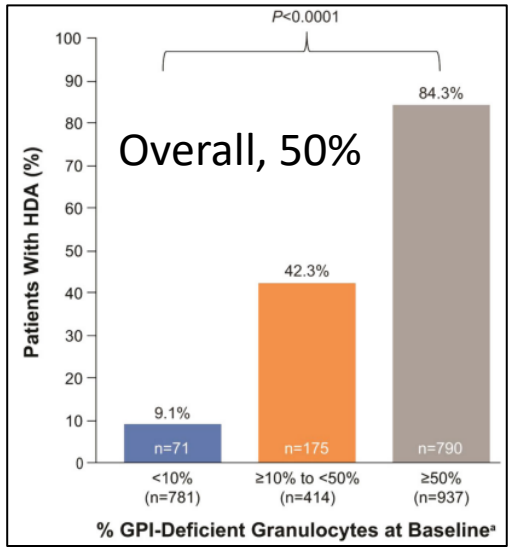
Therapeutic intervention blocking complement cascade (C5 inhibitor) → C3 fragment deposition on surviving PNH red cells → extravascular hemolysis



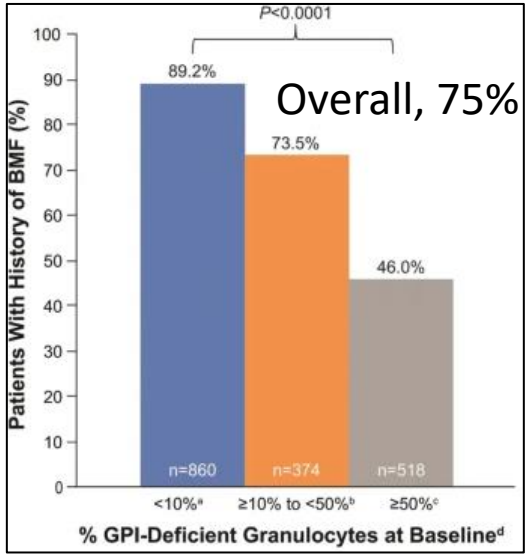
Colden MA. et al. Front.Immunol.2022, Kulasekararaj A, et al. Blood Advances. 2025

Clinical heterogeneity: PNH clone size and disease burden

- International PNH Registry Update
- Patients with a clinical diagnosis of PNH and/or detectable PNH clone in granulocytes and/or erythrocytes of ≥ 0.01%
- N=4439 (37% with clone size <10%, 20% between ≥ 10% to < 50%, and 43% had clone size ≥ 50%)



Intravascular hemolysis correlated with clone size



Marrow failure inversely correlated with clone size

Schrezenmeier H.et al. Ann Hematol.2020

Clinical heterogeneity: Erythrocyte phenotype

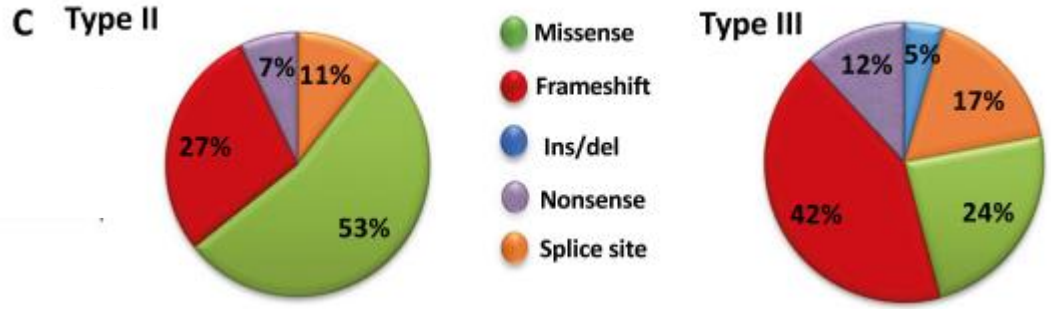
- Differences in degree of hemolysis and anemia even in patients with PNH granulocyte clone size >50% by flow
- Cases of “white PNH”
- Quantitative differences in complement sensitivity
- Result of molecular spectrum of PIGA mutations

Grade of GPI-Aps deficiency on RBCs

PNH type-I: normal expression of CD55 and CD59

PNH type-II: partial reduction

PNH type-III: complete absence of CD55 and CD59

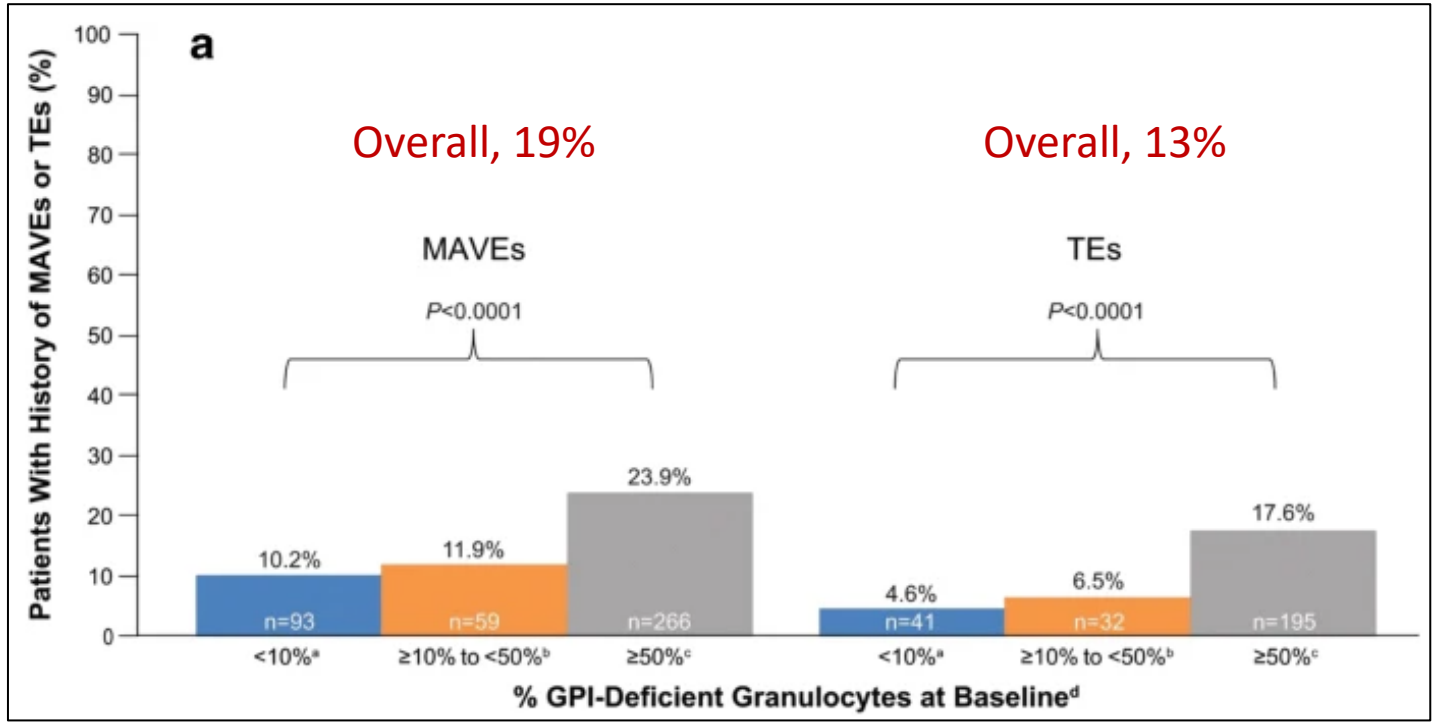


Gurnari C., Pagliuca S., et al. Leukemia.2021

Tombul Z, et al. Br J Haematol. 2024

Clinical heterogeneity: Thrombosis

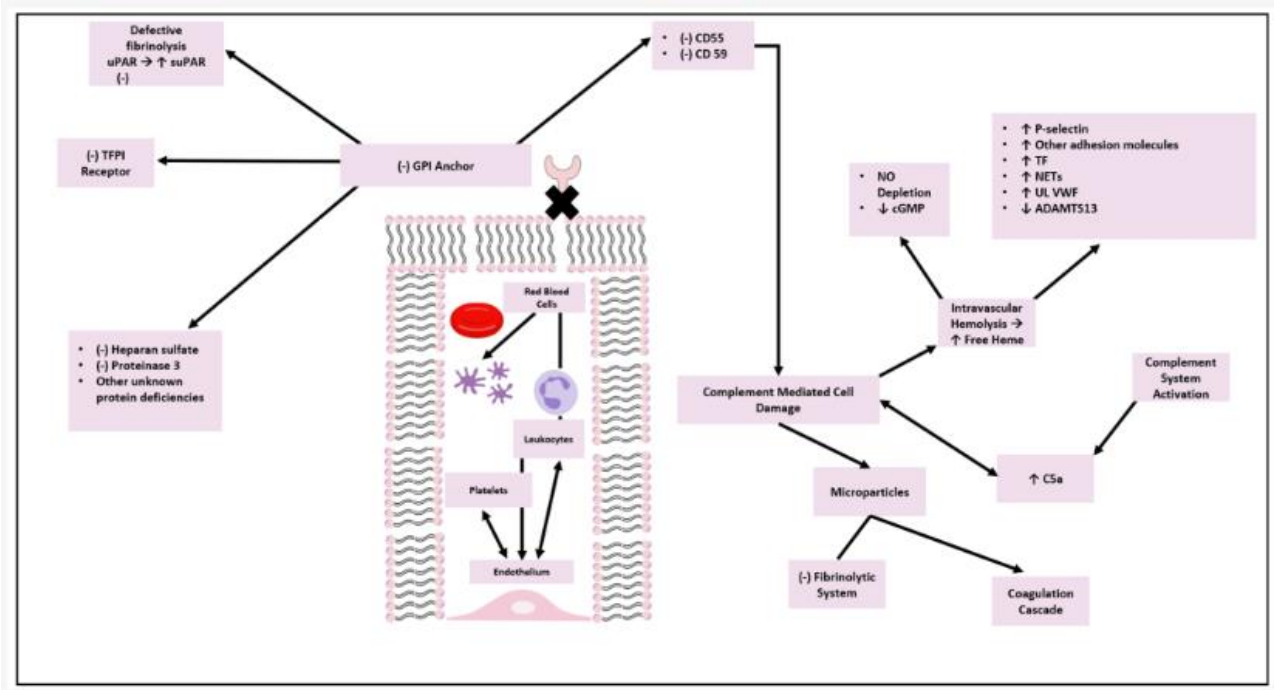
- International PNH Registry Update
- Patients with a clinical diagnosis of PNH and/or detectable PNH clone in granulocytes and/or erythrocytes of ≥ 0.01%
- N=4439 (37% with clone size <10%, 20% between ≥ 10% to < 50%, and 43% had clone size ≥ 50%)



Schrezenmeier H.et al. Ann Hematol.2020

Thrombosis in PNH

- High risk of thrombosis in untreated patients
- Most common cause of mortality
- Venous TE more common then arterial
  - VTE can occur in atypical location – hepatic, mesenteric, and cerebral veins
- Risk factors for TE: older age, high PNH clonal burden, presence of intravascular hemolysis, history of previous VTE



Complex pathophysiology – effect of complement activation and the cellular and biochemical consequences of intravascular hemolysis seem to play a big role

Kokoris S, et al., Int J Mol Sci. 2024
Babushok DV et al. *Hematology Am Soc Hematol Educ Program* 2021

Case #2

- 56 y.o male who presents with petechial rash and bruising x 1 week
- **Laboratory** analysis showed severe pancytopenia
- **Initial marrow** showed variable cellularity (5-50%) with mild erythroid hyperplasia, no dysplasia
- Due to elevated MCV and borderline Vitamin B12, patient started on supplements but remained pancytopenia
- **Repeat marrow** showed 5% cellularity, no dysplasia
- **Cytogenetics** 46, XY
- **NGS** showed DNMT3A p.Arg326His (5%)
- **PNH flow** – GPI negative neutrophils 10%, GPI negative RBC 9%

ANC (K/µL)	0.9
Hb (g/dL)	6.6
ARC (K/µL)	64K/µL
Platelets (K/µL)	7
LDH (IU/L)	**270**
Total bilirubin	1.7
Direct bilirubin	0.7
Haptoglobin	<10

What is the diagnosis?

1. Severe aplastic anemia (SAA)
2. PNH
3. SAA with PNH clone
4. Hypoplastic MDS
5. Fanconi anemia

PNH pathogenesis

- Very small populations of GPI negative PNH granulocytes can be detected in most healthy people

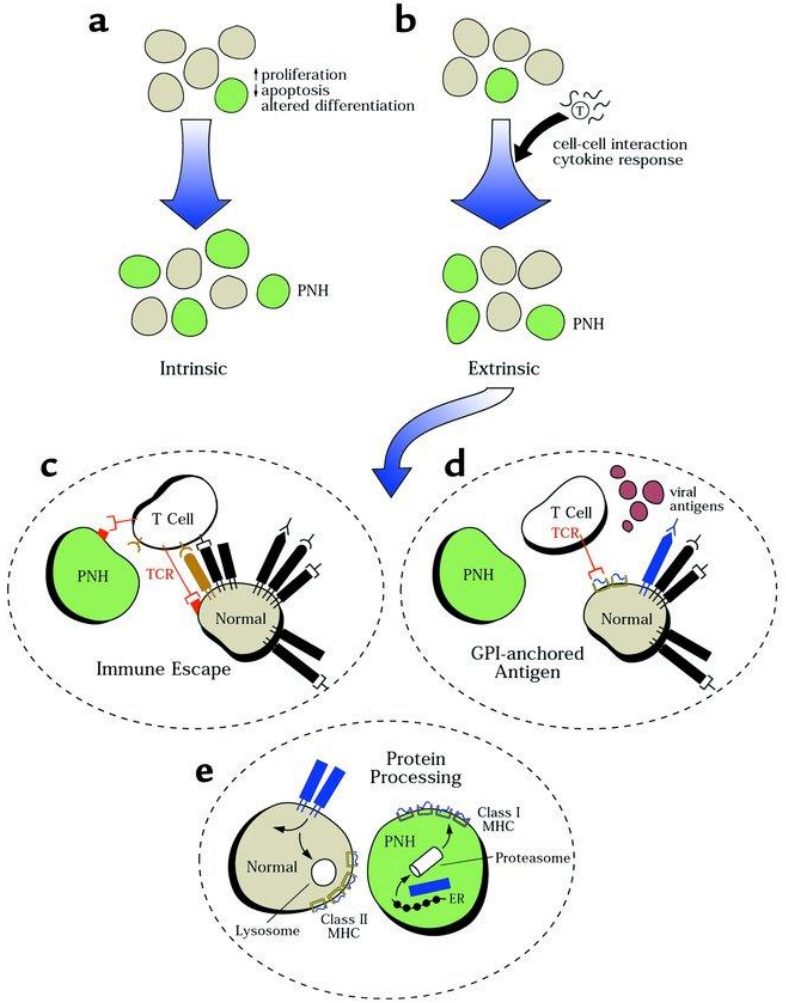
- *In vitro* and murine studies of PNH indicate
  - PNH clones have no intrinsic growth advantage
  - Do not seem to expand under normal conditions
  - Are not resistant to apoptosis

- Many patients with PNH present with features of marrow failure and ~30-60% of patients with aplastic anemia and 15% of patients with low-risk MDS have detectable PNH granulocytes and erythrocytes

Babushok DV et al. *Hematology Am Soc Hematol Educ Program* 2021, Luzzatto L., and Nakao S., Blood. 2025, Rotoli B., and Luzzatto L., Luzzatto L et al. Cell.1997

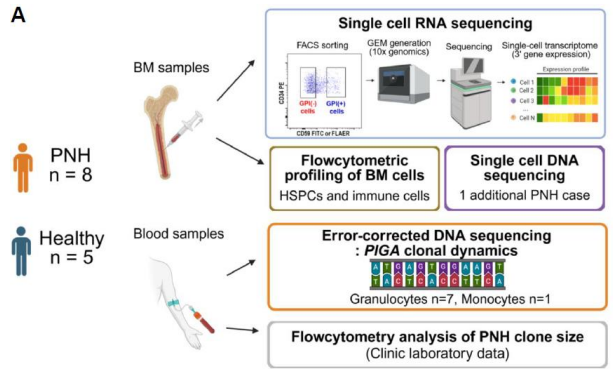
Mechanism of clonal expansion: Immune evasion

- Conditional growth advantage (hypothesis initially made in 1980s by Dr. Rotoli and Dr. Luzzatto)
  - GPI-anchor deficiency might produce a global deficit in immune recognition
  - Target involves a GPI molecule – CD1d-restricted GPI-specific T cells found in PNH and AA patients
  - Altered protein processing due to absent anchor structures change the surface peptides
- Multiple PIGA mutations in same patient indicating similar selection pressure

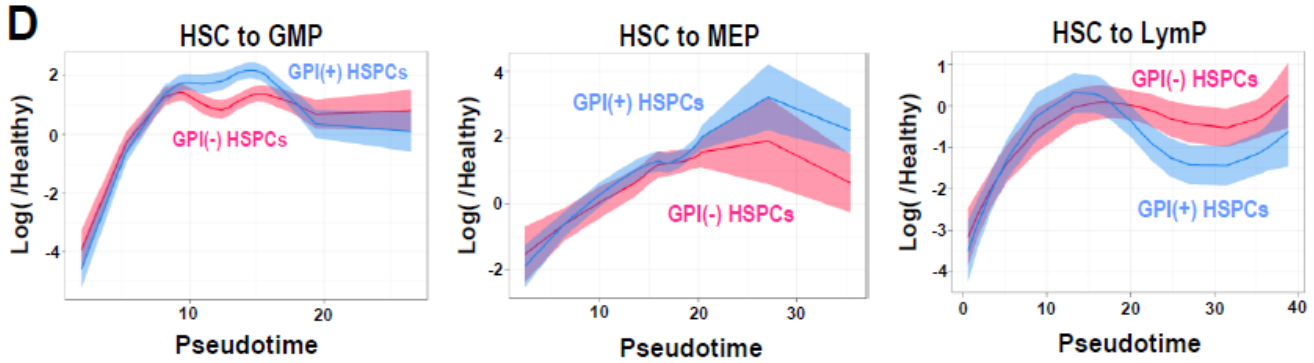


Luzzatto et al.Cell.1997, Young NS and Maciejewski J. JCI.

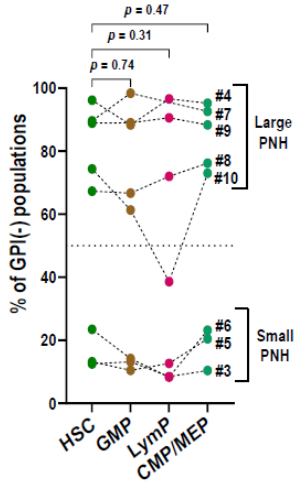
Single cell RNA-seq in PNH patients (Unpublished)

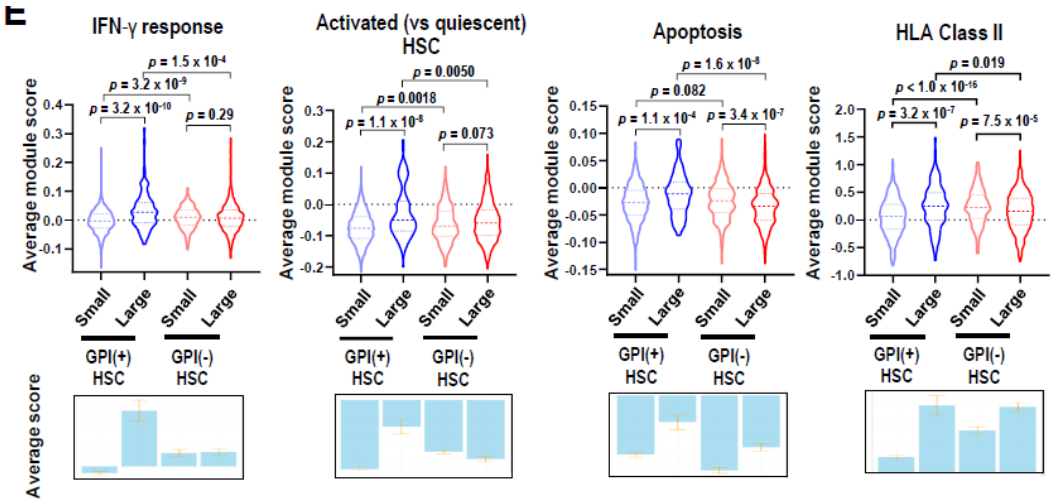


Similar differentiation potential



Large clones: GPI+ and GPI- HSCs upregulated immune response/apoptosis and downregulated cell-cycling pathways
Small clones: GPI+ HSCs show downregulated immune response/apoptosis and upregulated cell-cycling pathways





Mizumaki H. et al. In Revision

Mechanism of clonal expansion: Intrinsic drivers

- Cases of JAK2, CALR mutations in patients with co-existing PNH
- No additional somatic mutations conclusively identified in most patients

Shen W, et al. J Clin Invest. 2014

PRESENTATION ID 25
Hyatt - Plaza Int'l HIJK
Saturday, December 6
09:30 AM - 11:00 AM EST

Clonal architecture and dynamics of somatic evolution in aplastic anemia and paroxysmal nocturnal hemoglobinuria

Michael Spencer Chapman, MBBS, PhD, FRCPath

Single cell WGS to infer the timing of the mutation and clonal expansion

PRESENTATION ID 28
Hyatt - Plaza Int'l HIJK
Saturday, December 6
09:30 AM - 11:00 AM EST

Elucidating gene alterations driving hematopoietic dysfunction in PNH via patient-derived ips cell modeling and whole-genome sequencing

Jiyuan Liao

Matched PNH- and N-iPSC lines from four patients and modeled hematopoietic abnormalities in vitro

Case #3

26-year-old male presented with easy bruising and gum bleeding

Labs were significant for severe pancytopenia

Marrow examination – hypocellular marrow (10%), no dysplasia

Cytogenetics – 46, XY

PNH flow – GPI negative neutrophils (7.2%)

Other labs – LDH 220, HIV negative, EBV/CMV negative, B12 and folate normal

PMHx of hereditary spherocytosis (mild)

ANC (K/µL)	**0.38**
Hb (g/dL)	5
ARC (K/µL)	6.9K/µL
Platelets (K/µL)	7

Case #3

Diagnosed with SAA

No matched sibling donor, treated with hATG, CSA, and EPAG

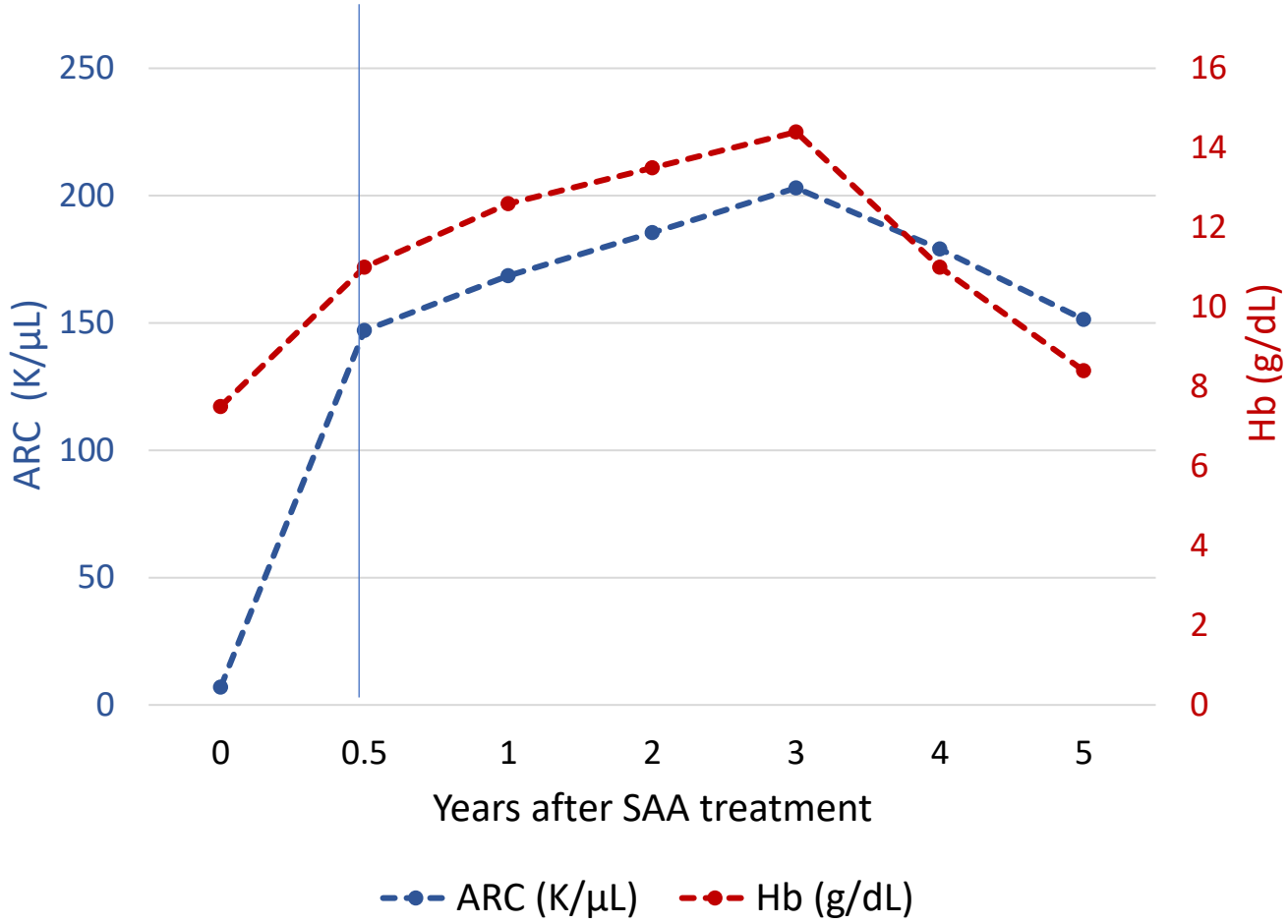
Achieved a partial response at 3 months and complete response (ANC >1, Hb >10, Platelets >100) at 6 months, discontinued high-dose CSA and EPAG.

Remained on CSA maintenance (2mg/kg daily) for additional 18 months

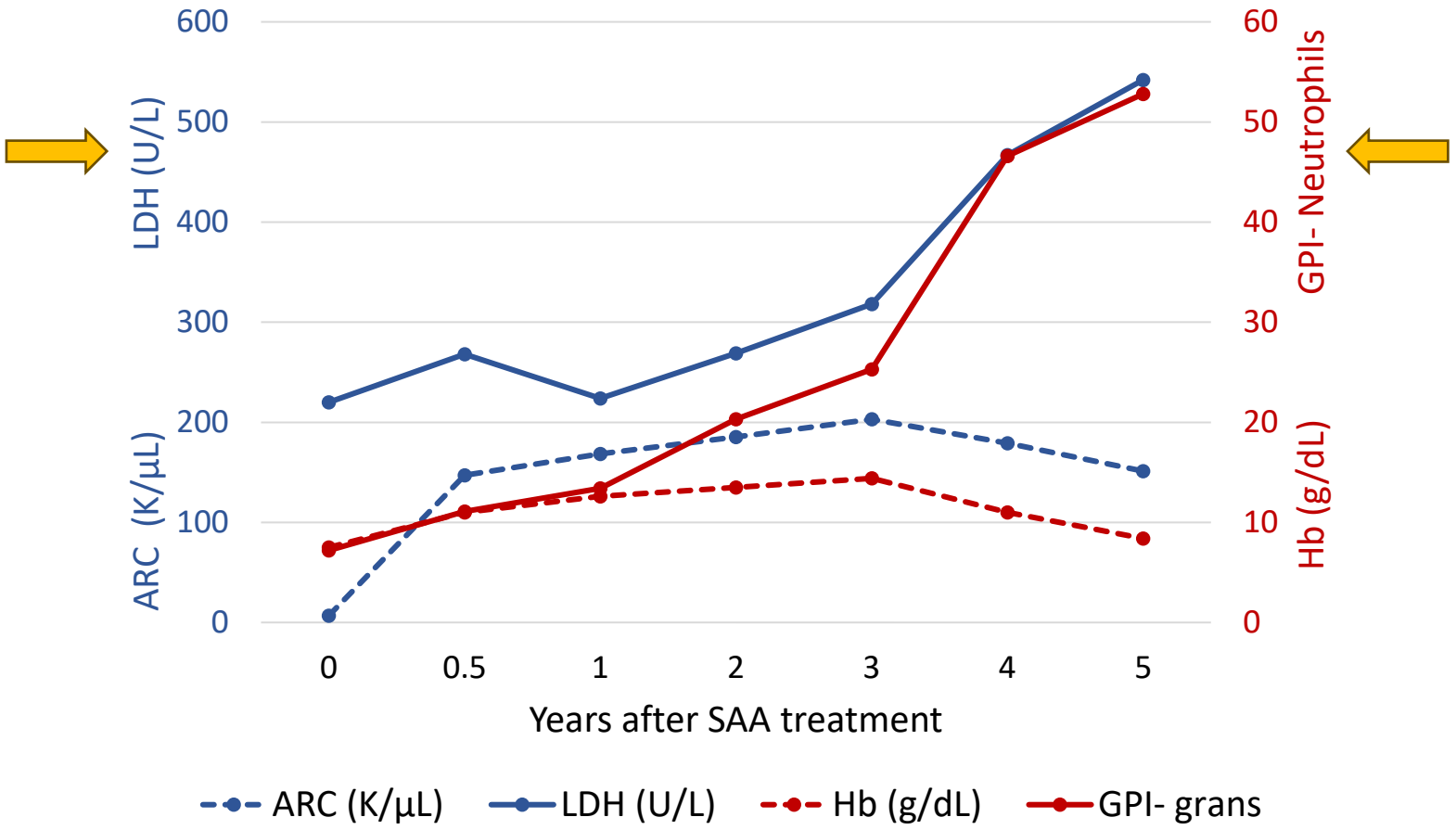
Blood counts remained stable until year 4 after treatment when hemoglobin started trending down

	Baseline	3-month	6-month
ANC (K/μL)	0.38	1.0	1.2
Hb (g/dL)	5.0	9.0	11.0
ARC (K/μL)	6.9	144	147
Platelets (K/μL)	7	59	110

Case #3


250
200
150
100
50
0
ARC (K/µL)
16
14
12
10
8
6
4
2
0
Hb (g/dL)
0
0.5
1
2
3
4
5
Years after SAA treatment
ARC (K/µL)
Hb (g/dL)

Case #3



Other blood counts

ANC 1.5

Platelets 167

Bone marrow examination

Variably hypercellular (75%), M:E ratio 1:1, trilineage hematopoiesis with mild erythroid hyperplasia

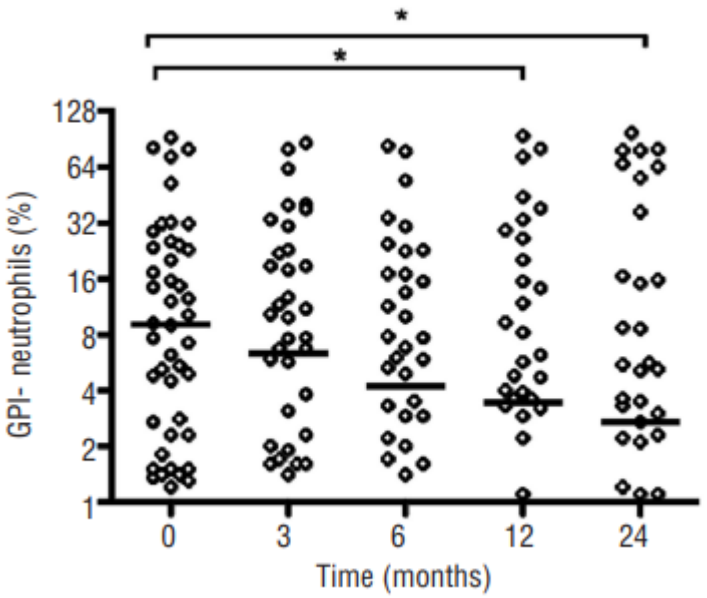
PNH Diagnosis

Clone size >50%

Hemolytic anemia

Evolution of PNH clones in SAA patients treated with IST

Pre-treatment clone ≥ 1%



hATG + CSA treated patients

Scheinberg P et al. Hematologica.2010

Evolution of PNH clones in SAA patients treated with IST

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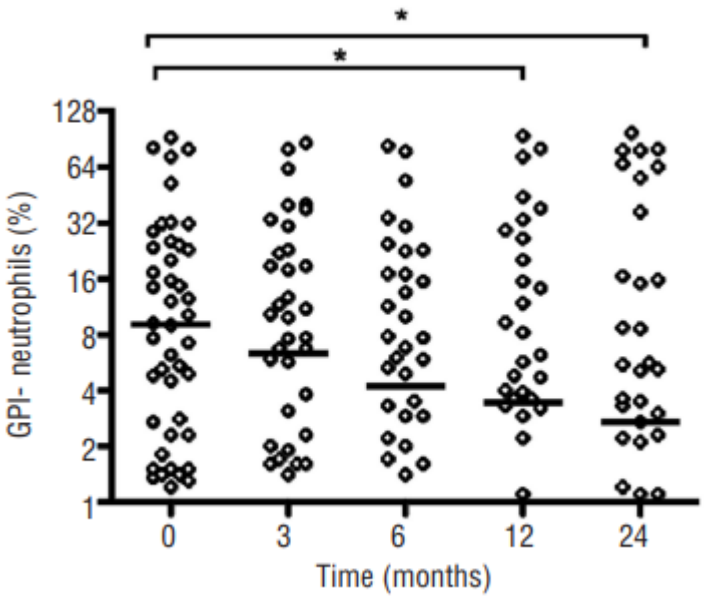
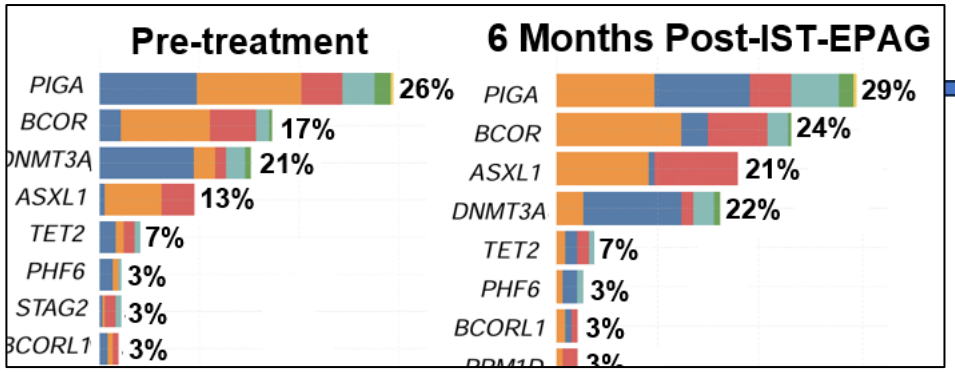
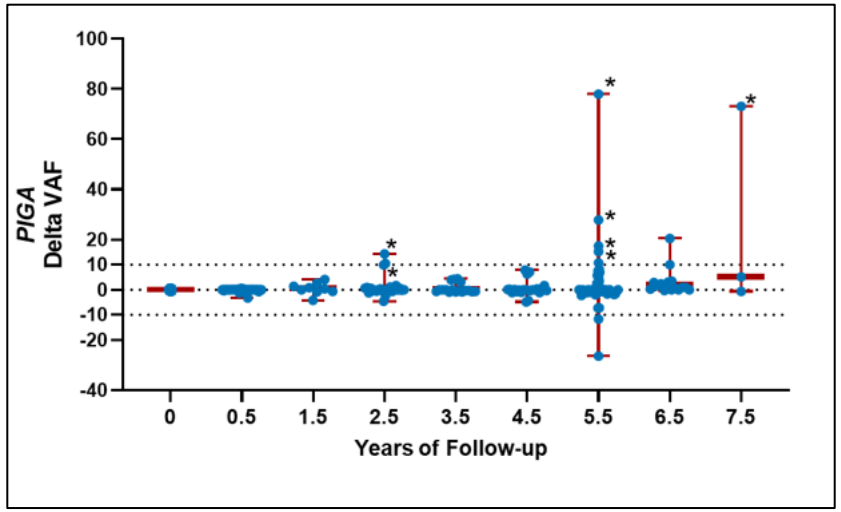


hATG + CSA treated patients

Scheinberg P et al. Hematologica.2010



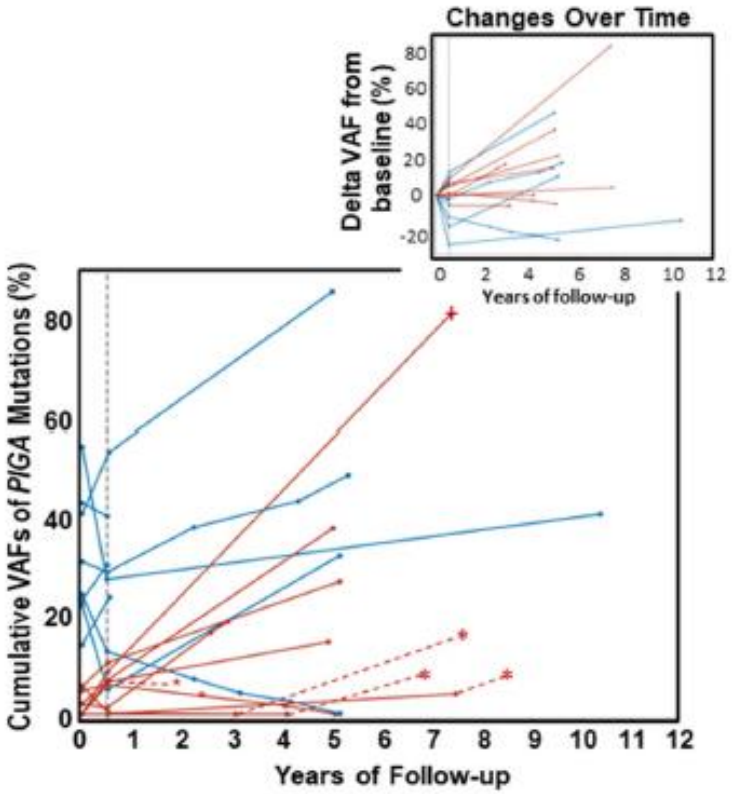
PNH clones ≥ 1% in 35% by flow

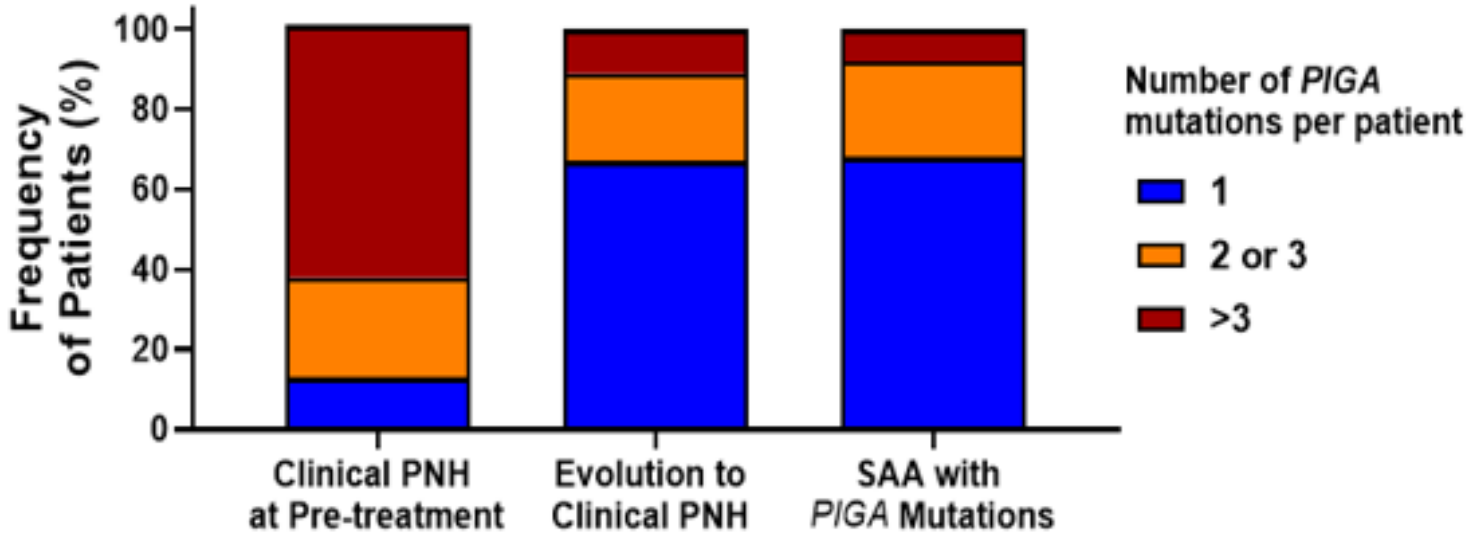


IST + EPAG treated patients

Unpublished. Do not post please

Evolution to PNH after IST + EPAG





5% of SAA patients had evolution to classical PNH 4-5 years after their IST+ EPAG treatment

Unpublished. Do not post please

Practical Guidance

- All patients with AA diagnosis should be screened for PNH at diagnosis
  - Positive PNH supports immune mediated AA
  - Depending on the clone size and clinical manifestations, may require treatment for both diseases

- Monitoring for clonal change post IST can be considered for SAA patients with PNH clone pre-treatment
  - if any change in bloodcounts to indicate hemolysis
  - diagnosis of thrombosis
  - increasing clone size

Thank you!

Special thanks to IPIG Committee members, Dr. Antonio Risitano and Dr. Neal Young

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Lemlem Alemu
Diego Quinones Raffo

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International PNH Interest Group

THANK YOU!