Paroxysmal Nocturnal Hemoglobinuria:

Clinicopathologic Features, Treatment, & Outcomes

ADAM LYLE, DO

PATHOLOGY RESIDENT

UNIVERSITY OF UTAH

8/22/23

Objectives

- After attending this presentation, the attendee should be able to:
  - Explain the background of PNH including history, epidemiology, and pathophysiology.
  - Describe patient presentation, evaluation, and laboratory testing involved in diagnosing PNH.
  - Understand the classifications, treatment options, outcomes, and monitoring of PNH.

Paroxysmal Nocturnal Hemoglobinuria (PNH)

- An early described hematologic disorder dating back to 1866
  - Recognized by dark/tea colored urine at night/early morning
- Definitions
  - Paroxysm – a fit, attack, or sudden increase or recurrence of symptoms
    - In PNH the symptom is hemolysis releasing hemoglobin
  - Nocturnal – of, relating to, or occurring in the night
    - The hemolysis is ongoing occurring 24 hours a day
  - Hemoglobinuria – the presence of free hemoglobin in the urine
    - ~ 62% of patients experience hemoglobinuria[1]

Nakamura, N., et al. *J Med Case Reports* **5**, 550 (2011

https://www.merriam-webster.com/dictionary/

1. Schrezenmeier H, et al. *Haematologica*. 2014;99(5):922-929.

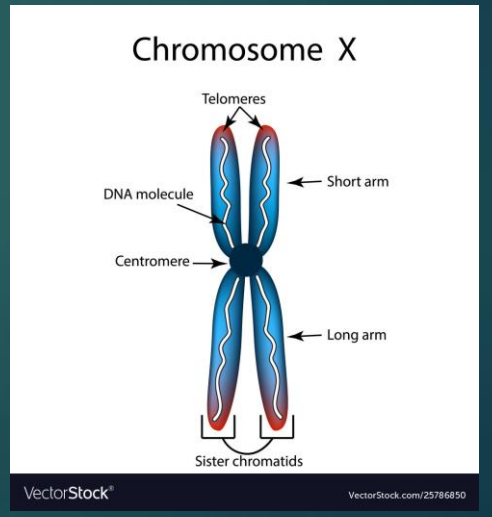
Epidemiology of PNH

- Worldwide with no racial, ethnic, or sex association
- Adults
  - Median age of diagnosis mid-30s
  - Childhood cases have been reported
- Rare
  - 1-13 cases per million[2]

2. Jalbert J, et al. *Blood* 2019; 134 (Supplement_1): 3407.

Pathophysiology of PNH

- Rare clonal acquired hematopoietic stem cell (HCS) disorder
- Arises de novo or in the setting of bone marrow failure
  - Aplastic anemia (AA), Myelodysplastic syndrome (MDS)
- Somatic mutation of **p**hosphatidyl**i**nositol **g**lycan class **A** (*PIG-A*) gene
  - Located on X chromosome (X-linked)
    - Males have one X chromosome
    - Females X chromosome inactivation (lyonization)
    - "Single hit" → PNH phenotype
  - Synthesis of **g**lycosyl**p**hosphatidyl**i**nositol (GPI) anchor



Glycosylphosphatidylinositol Anchor Proteins (GPI-APs)

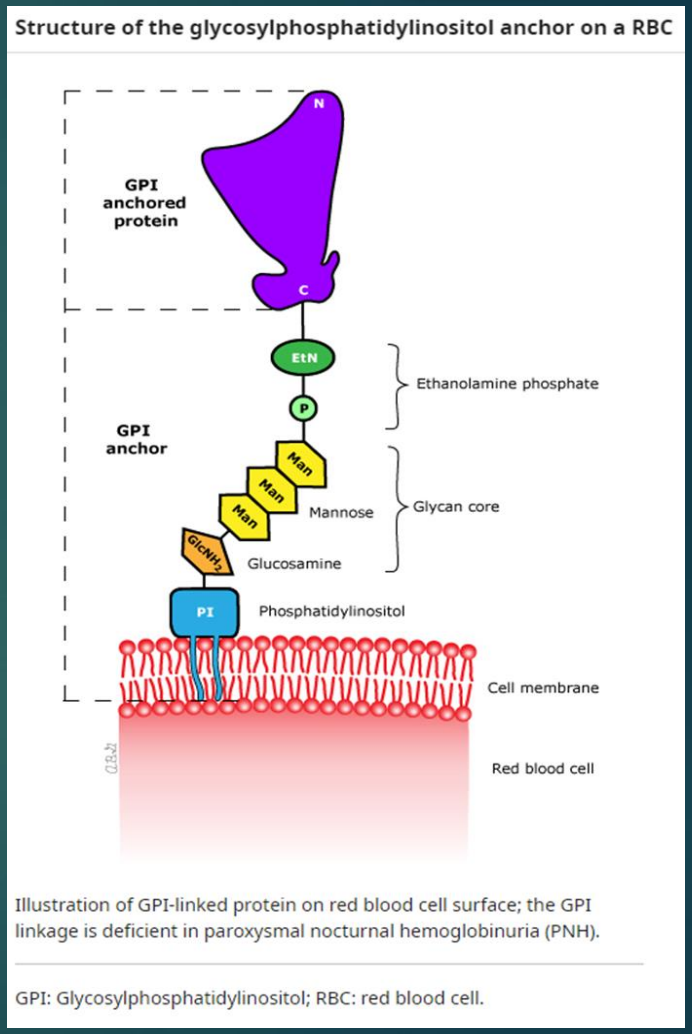
- Large glycolipid attached to C-terminus of a protein and anchored in the cellular plasma membrane
- GPI anchor links other proteins to the cell surface
- Absence of GPI anchor → Loss of surface proteins
  - Immune complement regulatory proteins

CD55

- Decay accelerating factor (DAF)
- Prevents formation of C3 Convertase

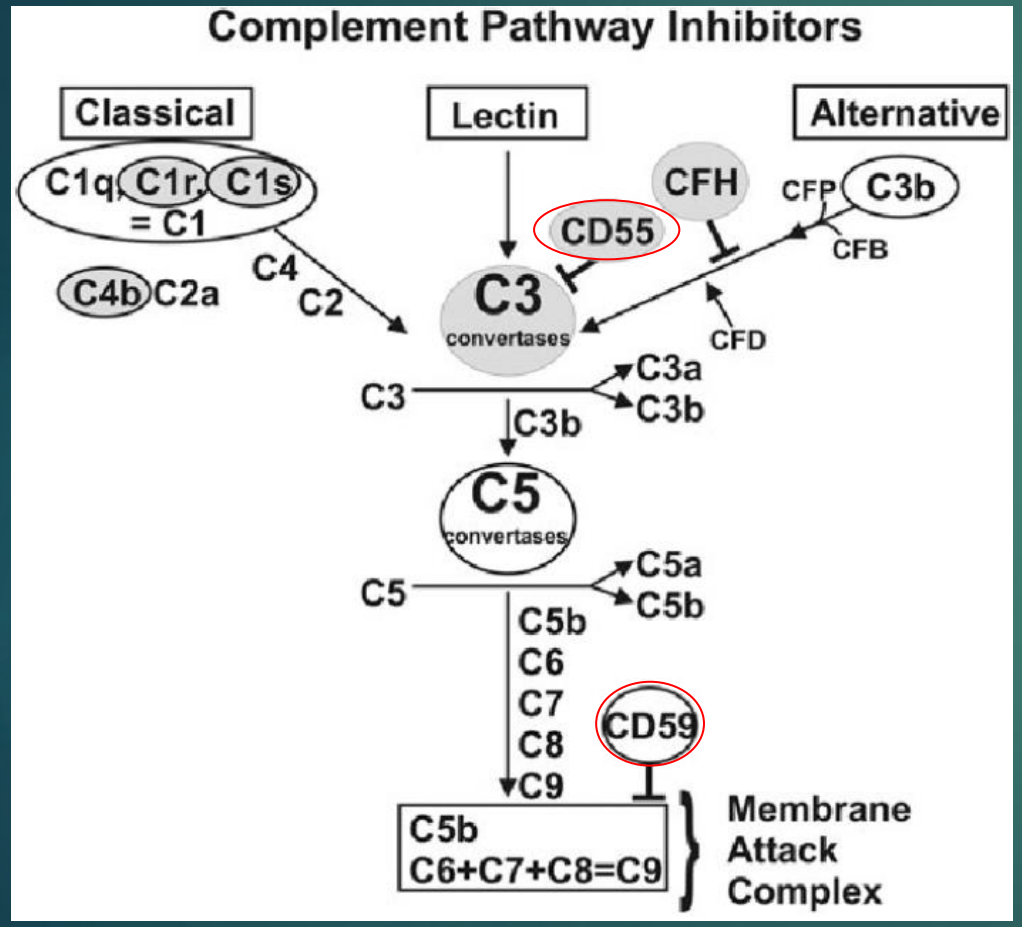
CD59

- Membrane inhibitor of reactive lysis (MIRL)
- Inhibits formation of the membrane attack complex (MAC)



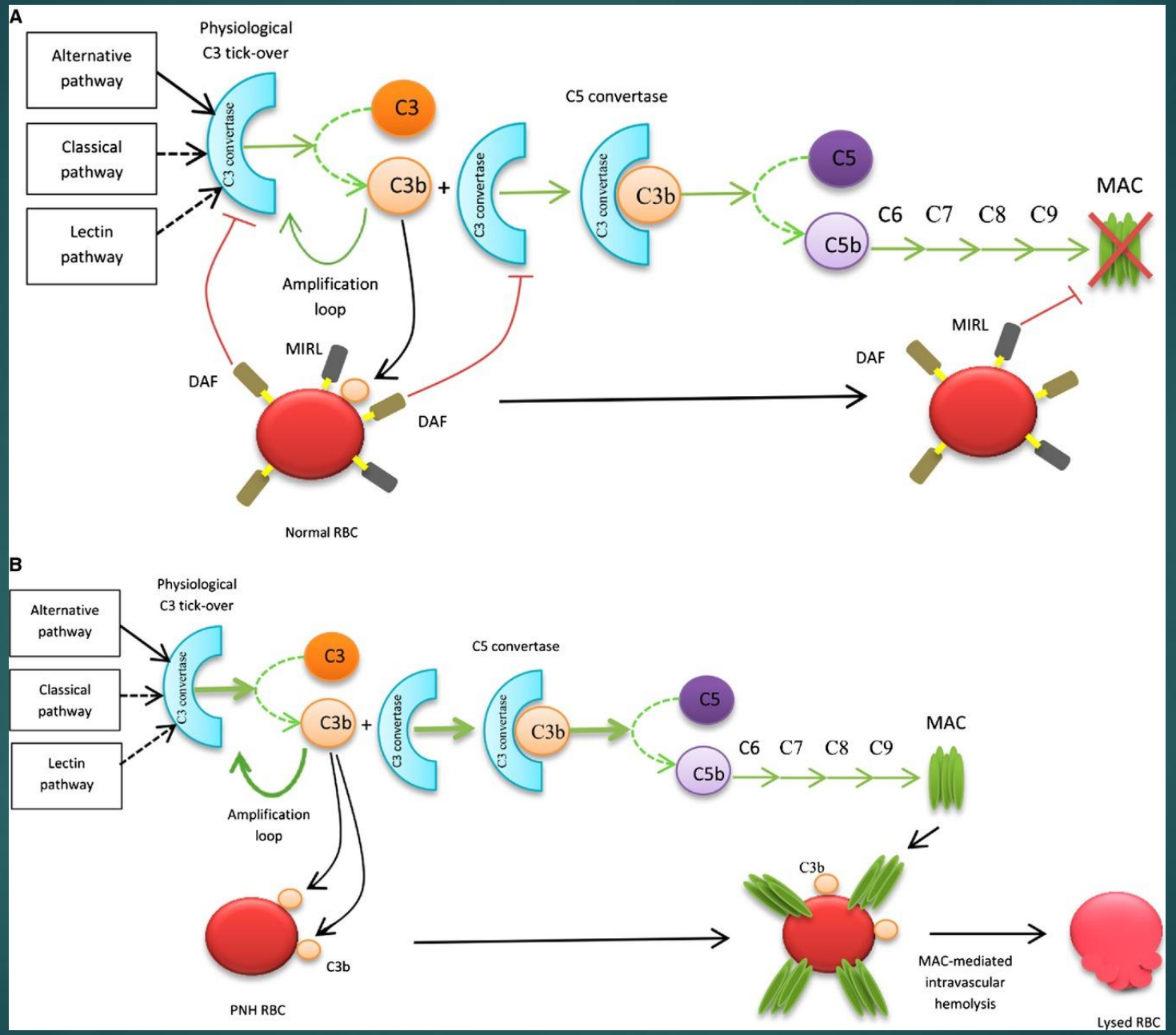
Courtesy of Robert A Brodsky, MD. UpToDate.com

Complement Pathway Inhibitors



Kenney MC, et al. *Hum Mol Genet*. 2014;23(13):3537-3551.

- Extravascular hemolysis
  - Reticuloendothelial macrophages in the liver and spleen
  - CD55 (DAF) prevents assembly of C3 and C5 convertases and opsonization upstream from MAC formation
- Intravascular hemolysis
  - Hemolysis in the circulation usually blocked by CD59 (MIRL)
  - CD59 (MIRL) prevents the final stage of complement assembly, production of the MAC that forms a pore in the target cell → Hemolysis



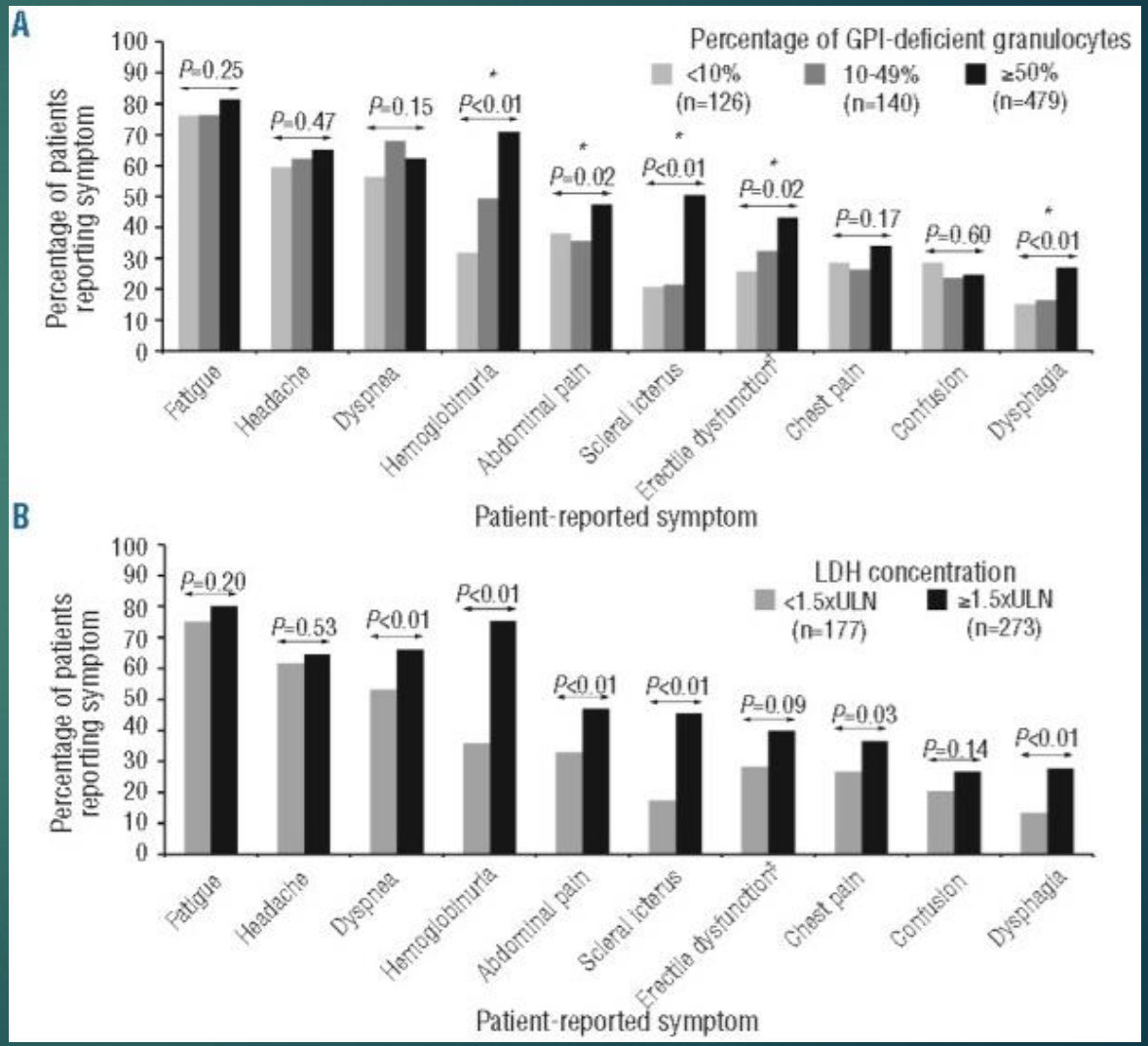
Devalet B, et al. *European Journal of Haematology*. 2015. *95*(3) 190-198.

Clonal Expansion

- Clinical manifestation requires expanded population of *PIG-A* cells
  - Deficiency may be complete or partial
  - Red blood cells (RBC) and white blood cells (WBC)
- Mechanisms:
  - Immune escape – *PIG-A* cells protected from immune mediated destruction
    - Aplastic anemia or other growth suppression of unaffected hematopoietic cells
  - Selective advantage
    - Additional mutations may provide selective advantage
      - Reduced apoptosis or accelerated proliferation
  - Neutral evolution
    - Random expansion during bone marrow regeneration
    - PNH mutations found in many unaffected blood donors

Clinical Manifestation of PNH

- >93% symptomatic
  - Poor quality of life
    - Hospitalization (23%)
    - Inability to work (17%)
- Symptoms related to:
  - % GPI-deficient granulocytes
  - [LDH]

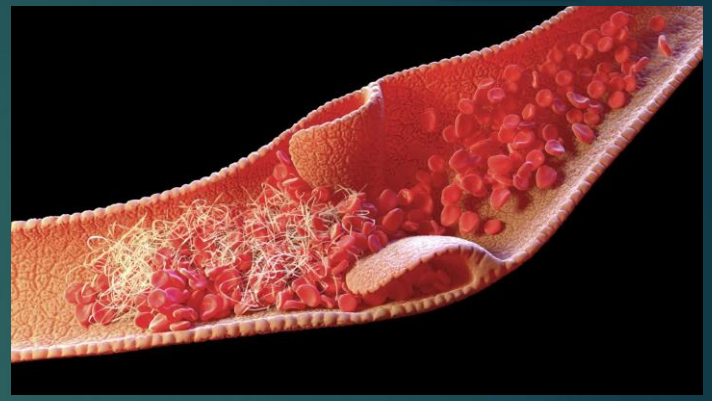


Schrezenmeier H, et al. *Haematologica*. 2014;99(5):922-929.

Clinical Manifestation of PNH

- Hemolysis
  - Anemia related symptoms – fatigue, weakness, jaundice, hemoglobinuria
- Chronic kidney disease
  - Hemoglobin and iron deposition and acute toxicity from free hemoglobin
- Depleted nitric oxide (NO), scavenged by free hemoglobin
  - NO relaxes smooth muscle, when depleted muscles contract
  - Smooth muscle dystonia and vasospasm
    - Esophageal spasm and abdominal pain
    - Pulmonary hypertension
    - Renal insufficiency

Clinical Manifestation of PNH



gentside.co.uk

- Thrombosis – Hypercoagulable state
  - Venous involving unusual site or early age
    - Abdominal, hepatic, IVC, portal, splenic, cerebral, dermal
  - Nitric oxide depletion from circulating free hemoglobin
  - Disrupted fibrinolysis due to deficient GPI anchored anticoagulants
  - Disrupted tissue factor inhibitor pathway
  - Procoagulants released from platelets
  - Increased complement components
    - Proinflammatory and prothrombotic cytokines
- Bone marrow dysfunction
  - Associated with aplastic anemia (AA) or myelodysplastic syndrome (MDS)
    - Other cytopenias including neutropenia and/or thrombocytopenia

Clinical Evaluation

- History
  - Symptoms related to clinical manifestations
- Physical exam
  - Anemia
    - Pallor, tachycardia, tachypnea
  - Excessive bleeding/bruising or infection
  - Evidence of thrombosis
    - Redness or swelling of extremity
    - Splenomegaly

fastmed.com

Lab Findings

- Hematology
  - CBC with differential count
  - Reticulocyte count
    - Bone marrow response to anemia, generally elevated but lower than expected based on severity of anemia
  - Blood smear for RBC morphology
  - Direct antiglobulin (Coombs) testing (DAT)
    - Coombs-negative to rule out other causes of anemia (autoimmune)
  - Prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, D-dimer
- Urine
  - Hemoglobin and hemosiderin

Lab Findings

- Serum chemistries
  - Electrolytes, blood urea nitrogen (BUN), creatinine, and liver function tests
  - Lactate dehydrogenase (LDH), indirect and direct bilirubin
    - Elevated
  - Serum haptoglobin
    - Low
  - Free hemoglobin
  - Iron, transferrin saturation, and ferritin

Finding	Change in hemolytic anemia
Anemia*	Decreased hemoglobin Decreased hematocrit
Bone marrow response/recovery	Increased reticulocyte count Underestimation of HbA1C
Release of RBC contents	Increased LDH Increased indirect bilirubin Decreased haptoglobin Hemoglobinemia in intravascular hemolysis¶ Hemoglobinuria in intravascular hemolysis¶
RBC morphology changes$^{\Delta}$	Spherocytes or microspherocytes in immune hemolysis Schistocytes in microangiopathic hemolysis Blister or bite cells in oxidant injury Sickle cells in sickle cell disease Target cells and teardrop cells in thalassemia

UpToDate.com

Hemolytic Anemias Testing

ARUP Consult

Click here for topics associated with this algorithm

Abbreviations

aHUS	Atypical hemolytic uremic syndrome
DIC	Disseminated intravascular coagulation
EIA	Enzyme immunoassay
G6PD	Glucose-6-phosphate dehydrogenase
HELLP	Hemolysis, elevated liver enzymes, low platelet count
HHA	Hereditary hemolytic anemia
HUS	Hemolytic uremic syndrome
IgG	Immunoglobulin G
PCH	Paroxysmal cold hemoglobinuria
PNH	Paroxysmal nocturnal hemoglobinuria
RBC	Red blood cell
TMA	Thrombotic microangiopathy
TTP	Thrombotic thrombocytopenic purpura
WBC	White blood cell

INDICATIONS FOR TESTING

Signs of anemia hemolysis:
- Elevated reticulocyte count, lactate dehydrogenase, and bilirubin
- Low haptoglobin

ORDER Direct Coombs (Anti-Human Globulin)

- C3 positive → Cold agglutinin disease or PCH → Confirm PCH with Donath Landsteiner testing
- Negative → **PERFORM** Peripheral smear
- IgG positive
  - Yes → Autoimmune hemolytic anemia (consider drug induced, hemolytic disease of the newborn, autoimmune disease)
  - No → Recluse spider venom, clostridium sepsis

PERFORM Peripheral smear → Consider Hereditary Hemolytic Anemia Cascade if HHA is suscpected based on family history and/or peripheral smear

Agglutination → **CONSIDER** Cold agglutinins disease

Schistocytes, thrombocytopenia → Microangiopathic RBC destruction
CONSIDER DIC, TTP, HELLP, HUS, aHUS, mechanical cardiac valve, vasculitis, malignant hypertension
→ **ORDER** D-Dimer
- Pregnant → **CONSIDER** HELLP
- Normal AND Clinical presentation consistent with TMA → **ORDER** ADAMTS13 Reflex Panel or ADAMTS13 Activity **OR** *E. coli* Shiga-like Toxin by EIA (depending on presentation)
  - ADAMTS13 activity <10% → TTP
  - Normal → aHUS
  - Positive Shiga toxin and history of diarrheal illness → Classical HUS
- Increased → DIC

Sickle cells → Consider sickle cell disease (diverse genotypes): SS, SC, SE, Sβ thalassemia, S Lepore → Hemoglobin evaluation

Basophilic stippling → Acquired
- No → Congenital 5'nucleotidase deficiency → Consider 5'Nucleotidase testing
- Yes → Consider lead poisoning → Consider Lead, Blood (Venous)

Polychromasia only with or without platelet decrease → **CONSIDER** PNH → **ORDER** PNH, High Sensitivity, RBC and WBC

Polychromasia or no significant morphologic abnormality → **CONSIDER** RBC enzyme testing (eg, G6PD, pyruvate kinase, etc.) AND Hemoglobinopathies and thalassemias
- Negative → Suspect congenital dyserythropoietic anemia

Spherocytes, pyropoikilocytes, or acanthocytes → **CONSIDER** RBC membrane disorder (hereditary spherocytosis, hereditary elliptocytosis, autoimmune hemolysis) → **CONSIDER** RBC Band 3 Protein Reduction AND Osmotic Fragility, Erythrocyte
- Positive → May consider molecular testing (Hereditary Hemolytic Anemia Panel Sequencing)

Content reviewed: October 2022 Last updated: October 2022

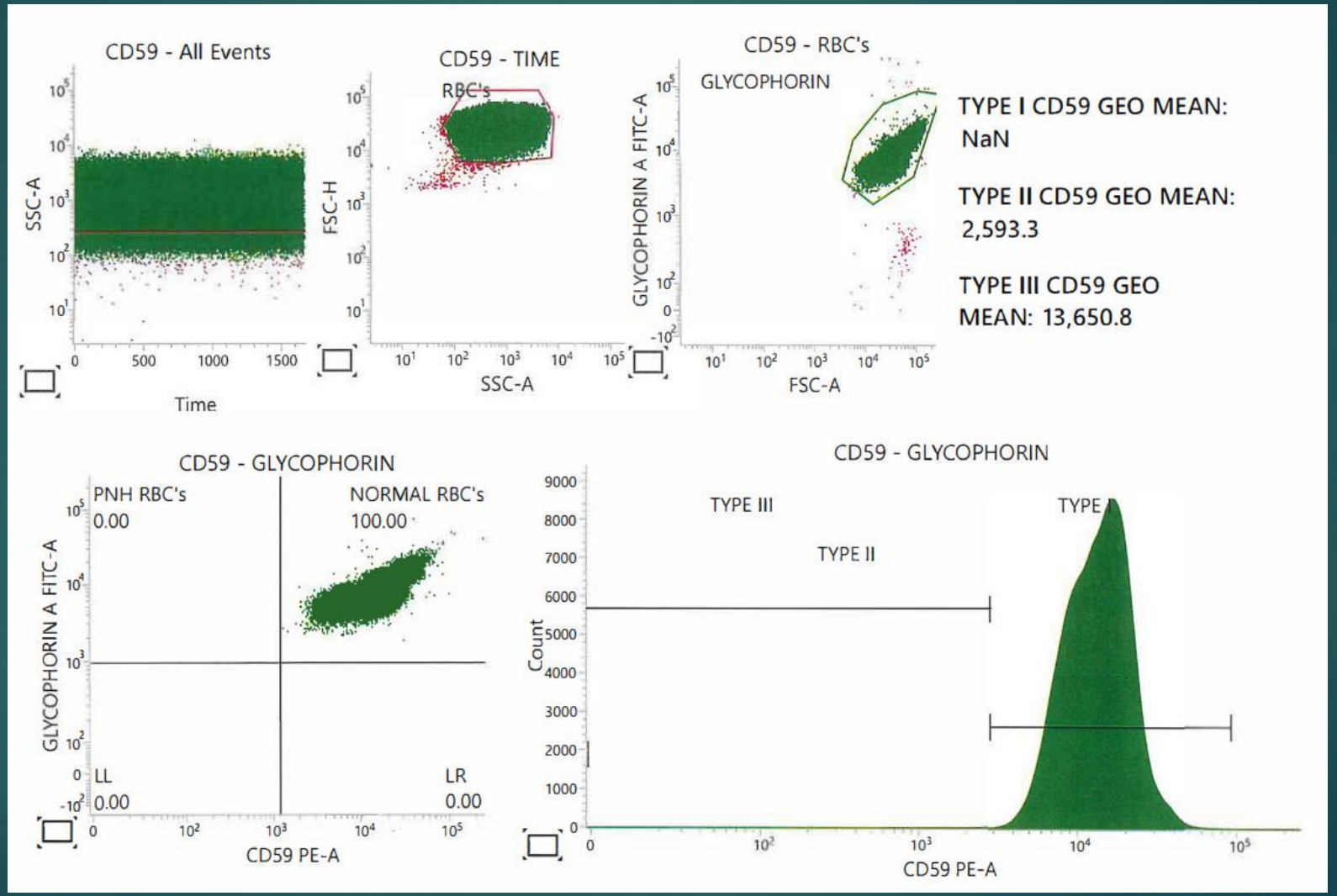
Diagnosis of PNH

- Flow cytometry
  - Reduction or loss of GPI anchored proteins on blood cells
    - Red blood cells (RBCs) and white blood cells (WBCs)
  - PNH clone size
    - Percentage of blood cells that partially or entirely lack GPI linked antigens compared to normal cells
- Multiple specific GPI linked reagents
  - Fluorescently labeled monoclonal antibodies that bind to GPI anchored proteins
    - CD55 and CD59
  - RBCs: CD235a (Glycophorin A)
  - WBCs:
    - **Fl**uorescent **Aer**olysin (FLAER)
      - Derived from bacterial toxin aerolysin that directly binds the GPI anchor
    - CD157 – GPI lined marker
    - CD15 and CD64 – lineage specific markers for granulocytes and monocytes

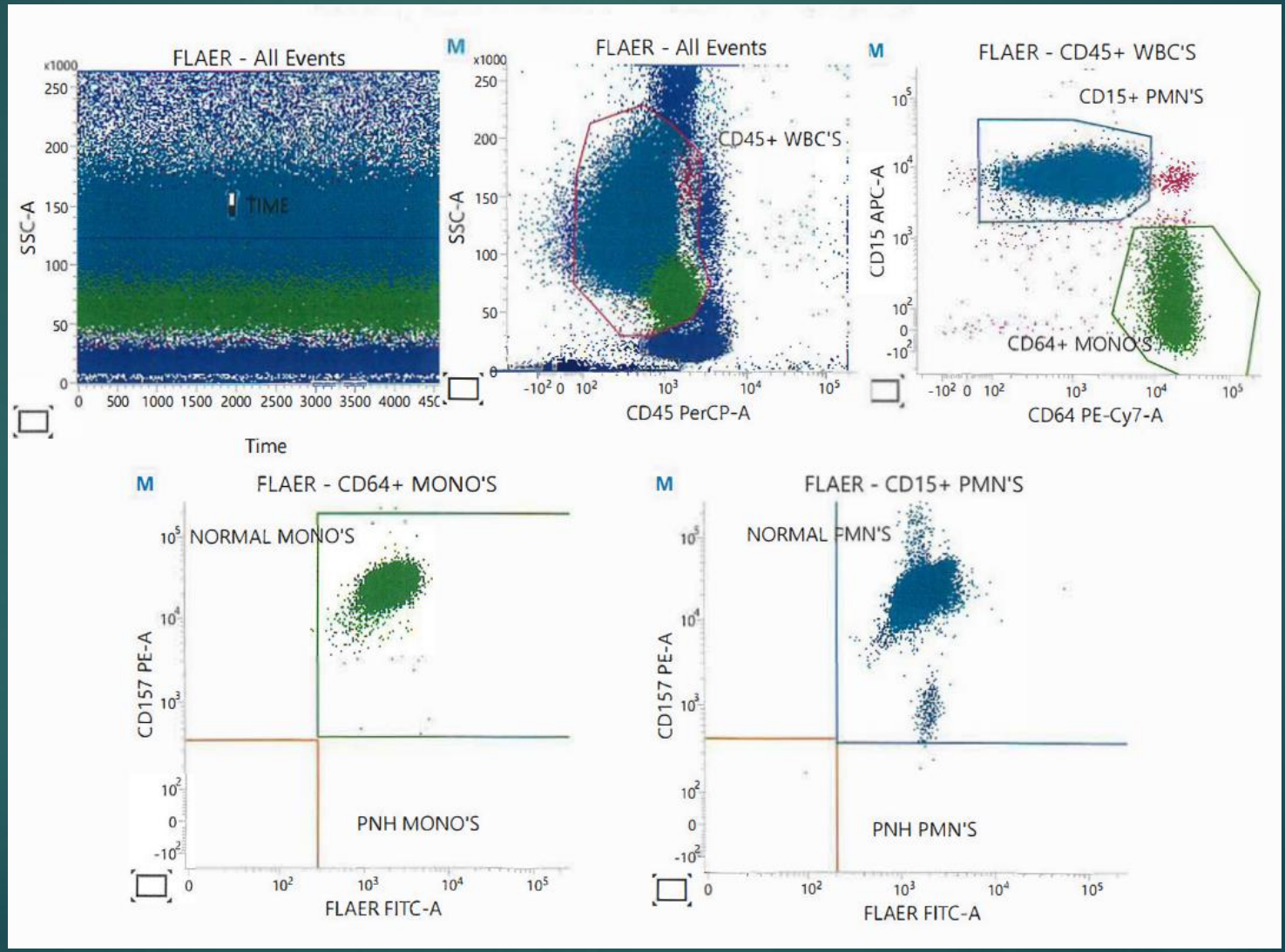
Two Cell Lineages

- Red blood cells (RBCs)
  - Testing of RBCs alone may underrepresent the size of the PNH clonal population
    - Extravascular and intravascular hemolysis
    - Dilution from transfused blood of unaffected donors
- Granulocytes/PMNs
  - Better representation of clonal population
- Report a percentage of PNH cell types
  - Type I – normal expression of GPI anchored proteins
  - Type II – partial expression
  - Type III – absent expression

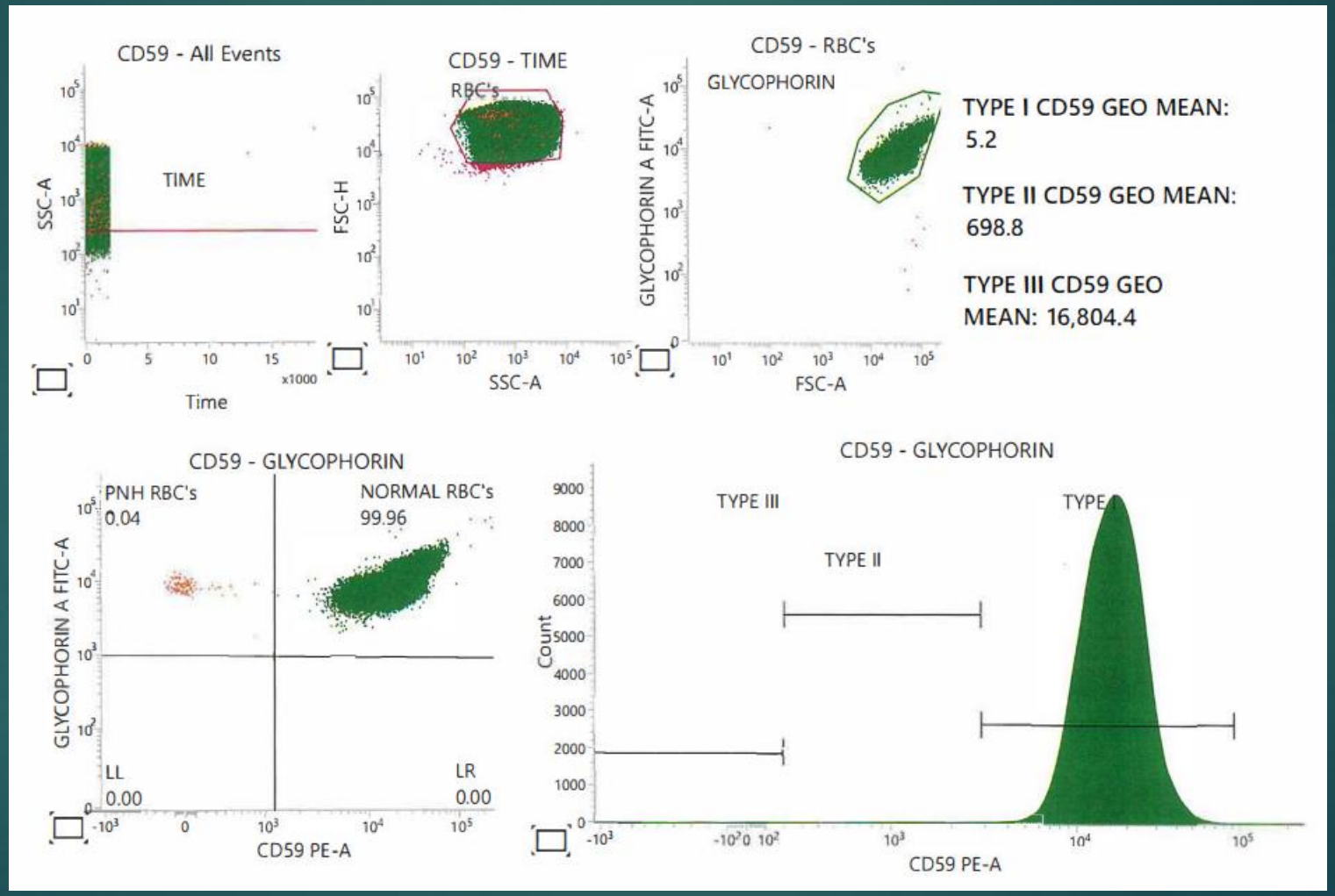
Flow Results - Normal



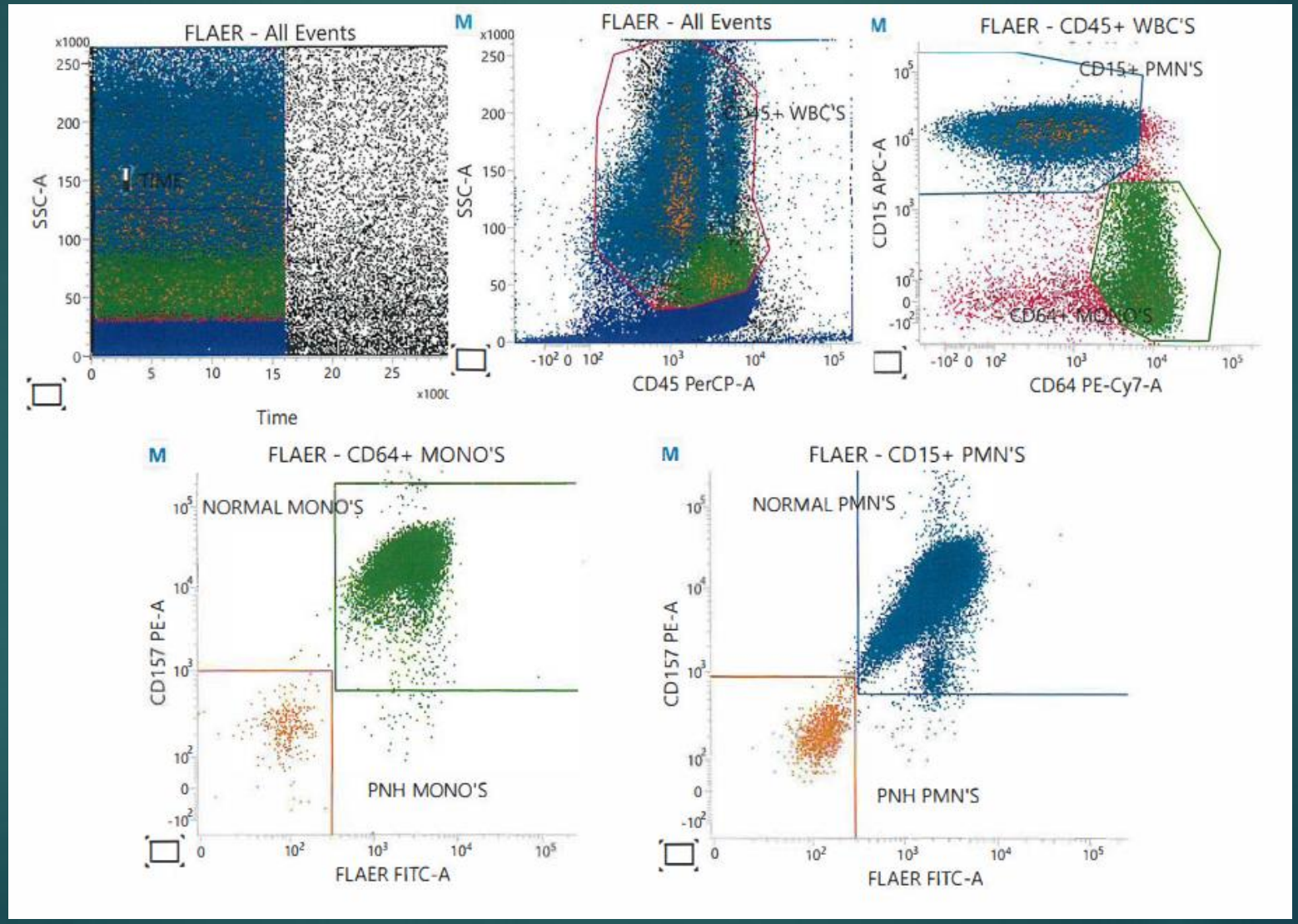
Flow Results - Normal



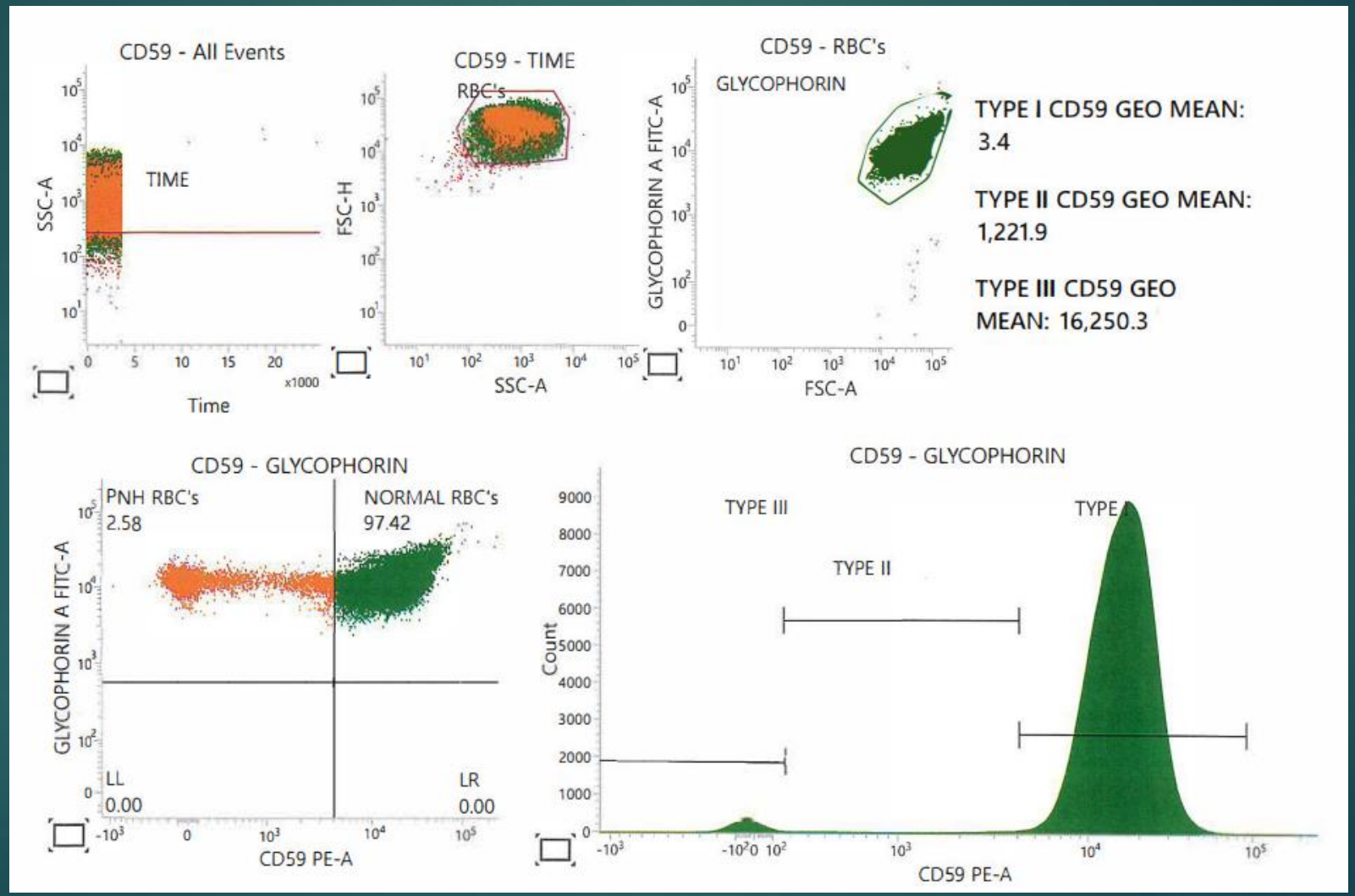
Flow Results - Subclinical



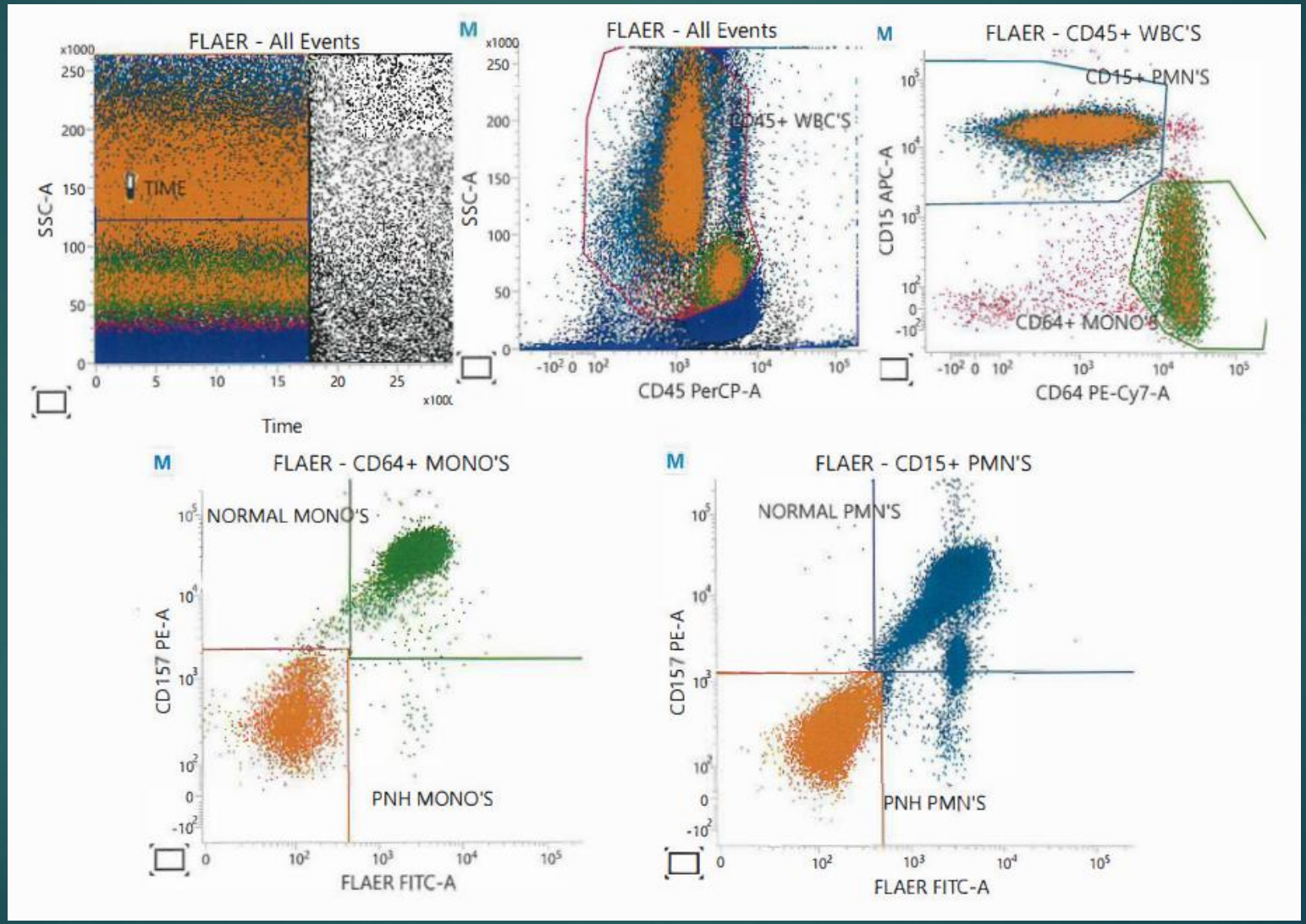
Flow Results - Subclinical



Flow Results - Clinical



Flow Results - Clinical



Bone Marrow Examination

- Not required for diagnosis
- Required for classification of PNH
  - Primary bone marrow disorders
- Patients with leukopenia and or thrombocytopenia
  - Evaluate aplastic anemia or myelodysplastic syndrome
- If bone marrow transplantation is being considered

Classification of PNH/Clinical Categories

- Based on presentation → treatment plan

Classification of PNH			
Classification	**Presentation**	**Markers**	**PNH Clone Size**
Classic PNH	Hemolysis and/or thrombosis	Reticulocytosis, high LDH, high bilirubin, low haptoglobin, normal leukocyte and platelet counts	Large (>50%)
PNH in the context of another disorder	Primary bone marrow disorder (eg, aplastic anemia, myelodysplastic syndrome)	Reticulocytosis, variable LDH, high bilirubin, low haptoglobin, low leukocyte and platelet counts	Varies, but generally small (<50%)
Subclinical PNH	No hemolysis or thrombosis	Normal or near normal LDH, bilirubin, and haptoglobin	Small (<10%)

Sources: Parker, 2016[1]; Borowitz, 2010[2]; Parker, 2005[3]

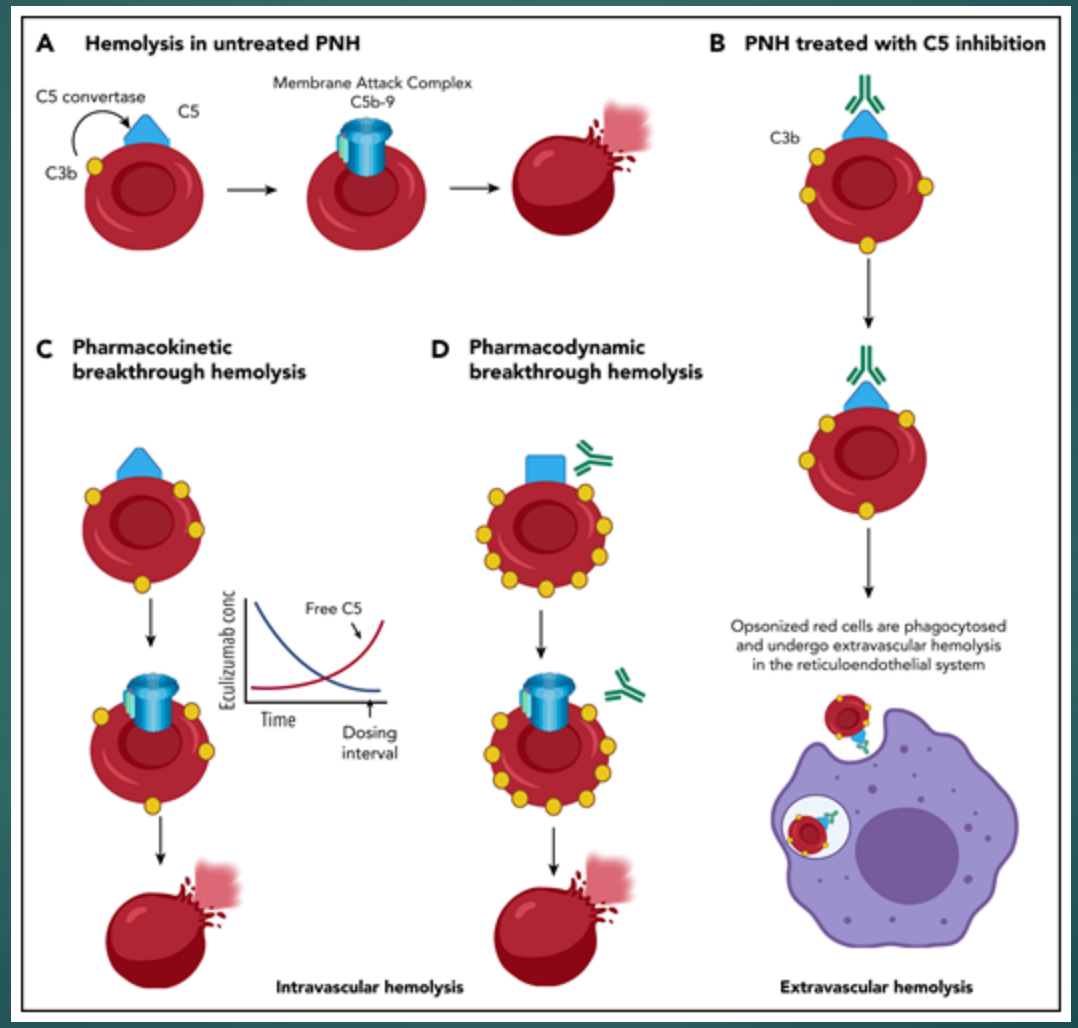
Treatment

- Symptomatic Hemolytic PNH
  - Complement inhibitor
    - Effective symptom relief
    - Prevention of thromboses
    - Modest toxicity
  - Supportive care alone
    - Alleviate pain and anemia relates symptoms
    - Transfusion related iron overload
    - Alloimunization
    - Thrombotic events
      - Difficult to predict and may recur/progress despite anti-thrombotic therapy

Complement Inhibitors

- C5 complement inhibitors (C5i):
  - Eculizumab
    - TRIUMPH trial
      - Reduced transfusion-dependence and improved quality of life (QoL)
      - No deaths or serious adverse events, higher survival rates
  - Ravulizumab
    - Similar rates of transfusion independence, normalization of serum LDH, and toxicity
    - Initial treatment with Ravulizumab
      - Lower expense, greater convenience, longer half life, and fewer episodes of pharmacokinetic breakthrough hemolysis
  - Increased risk of infection
    - Meningococcal (*Neisseria meningitidis)* or other encapsulated bacteria
  - Limited reports for other complement inhibitor treatments
  - Treatment continued indefinitely
- Do not treat PNH-associated bone marrow failure

Break Through Hemolysis

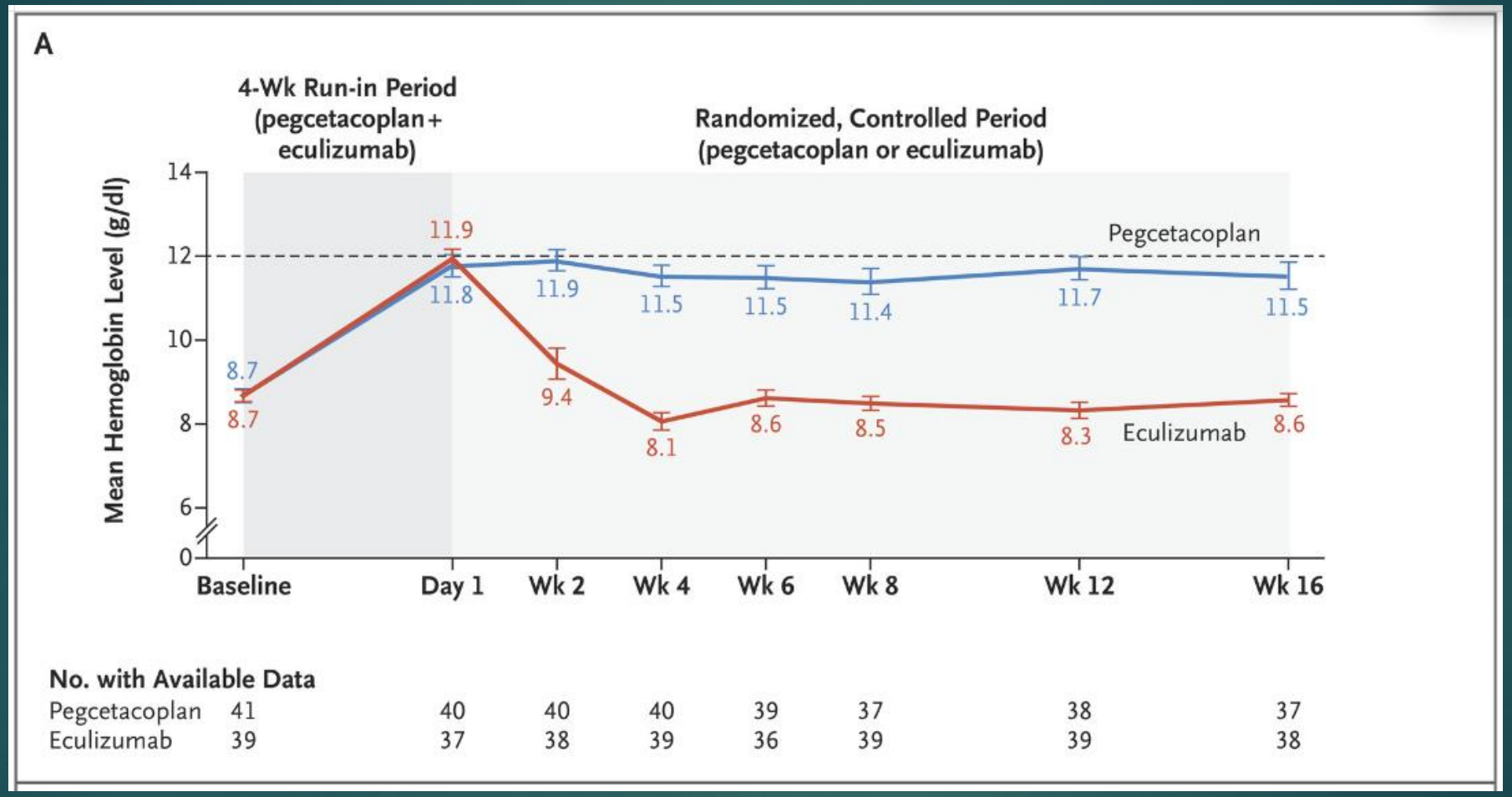


Brodsky RA. *Blood* 2021; 137 (10): 1304–1309.

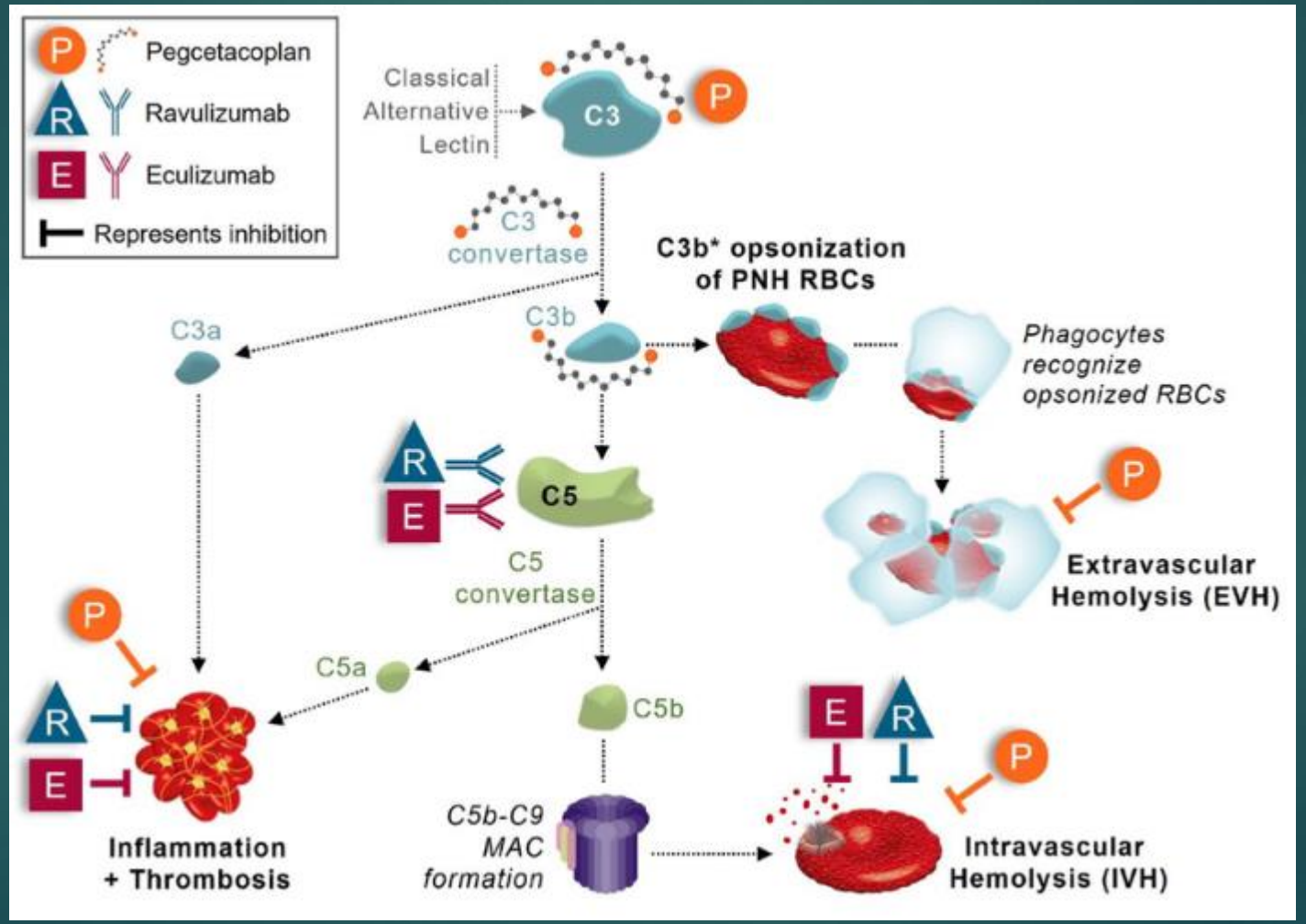
Pegcetacoplan

- Pegylated peptide targeting proximal complement protein C3
  - Inhibits both intravascular and extravascular hemolysis (c5i)
- Superior to eculizumab, PEGASUS trial
  - Change in hemoglobin level
    - No longer needing transfusion
    - Fewer breakthrough hemolysis, headache, and fatigue
- Adverse events
  - Injection site reactions, diarrhea
- Not for use in pregnant patients

Pegcetacoplan vs Eculizumab



Complement Inhibitor Mechanism



Wong RSM. *Therapeutic Advances in Hematology*. 2022;13.

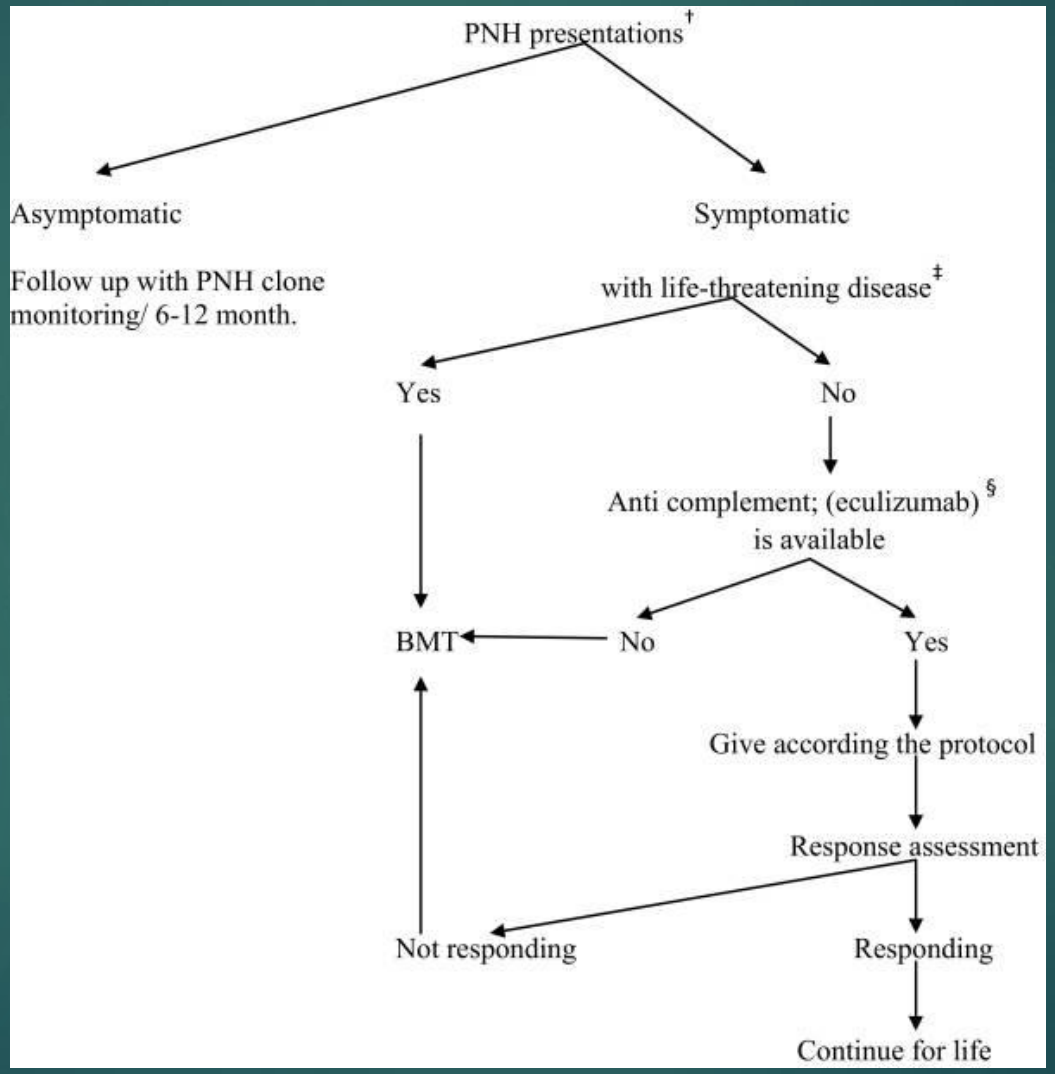
Treatment Continued

- PNH with thrombosis
  - Anticoagulation and C5i
    - PNH controlled: [LDH] <1.5 ULN with no other thrombosis predisposing factors
      - 3-6 months
    - New thrombotic event on C5i
      - Anticoagulate indefinitely
    - Additional risk factors
      - Individualized therapy
- Subclinical PNH
  - Watchful waiting
    - Avoids expense and potential toxicities
  - Monitored regularly

Treatment Continued

- PNH with Bone Marrow Failure (BMF)
  - Severe AA (sAA)
    - Absolute neutrophic count (ANC) <500/microL
    - Platelet count <20,000/micorL
    - Reticulocyte count <60,000/microL
  - Higher-risk MDS
    - Revised international prognostic scoring system (IPSS-R)
      - ≥4.0 points
    - Original IPPS
      - ≥1.5 points
  - Indications for transplantation
    - Medically fit, allogenic hematopoietic cell transplantation preferred (HST)
  - Only curative treatment option

PNH Treatment Algorithm

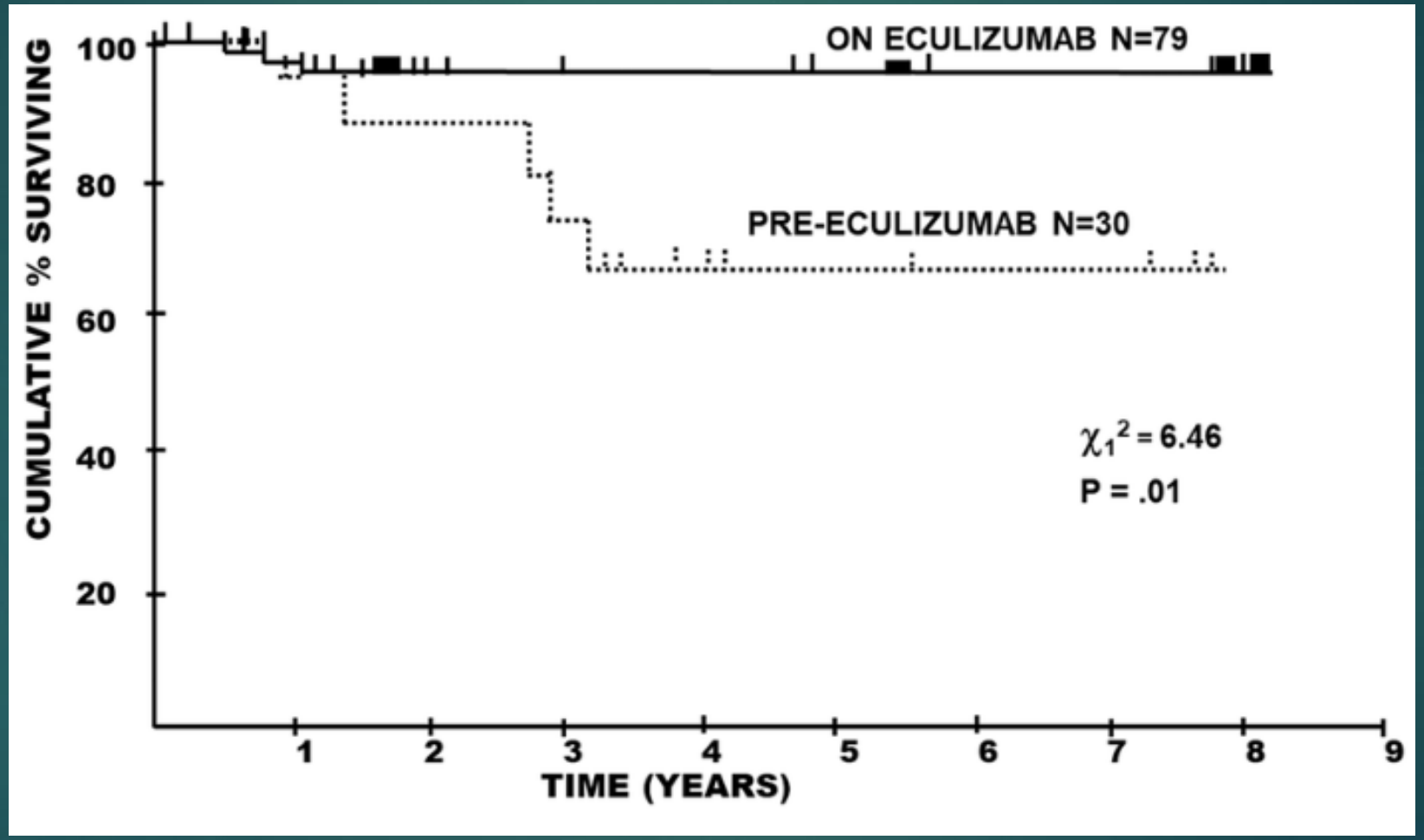


Mohammed A, et al. *Indian J Hematol Blood Transfus*. 2016 Dec; 32(4): 383–391.

PNH Prognosis

- Chronic disease with significant morbidity and mortality
  - C5i mortality improved
    - 5-year survival 95.5%
    - Pre complement inhibitor (1996) 66.8%
    - Adverse prognostic factors included thrombosis, evolution to pancytopenia, myelodysplastic syndrome, or acute leukemia, and age >55 years at disease onset
  - Differences between American and Japanese patients (2004)
    - Japanese overall survival 32 years compared to 19 years for American
      - Japanese more likely to develop AA
      - Americans more likely to develop thrombosis

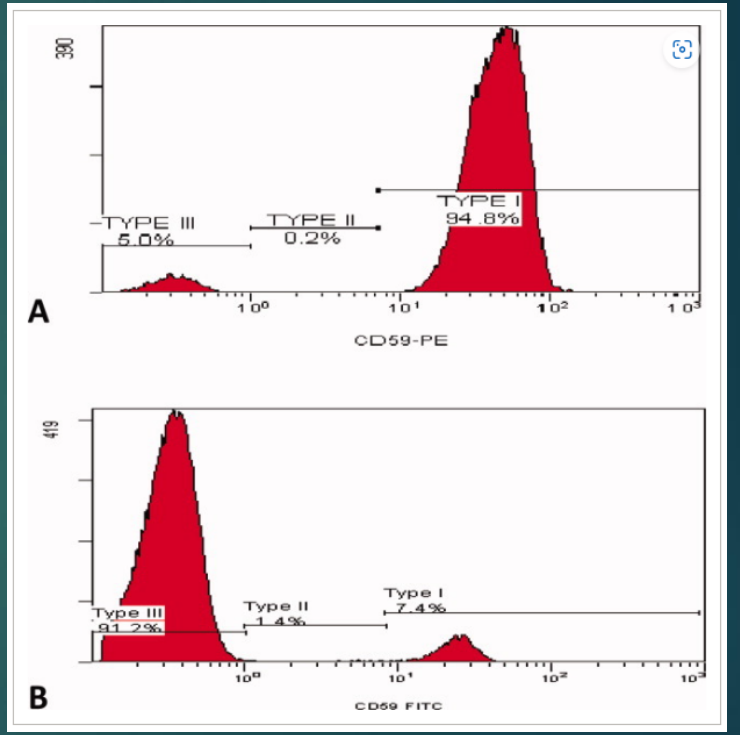
Survival



Kelly RJ, et al. *Blood* 2011; 117 (25): 6786–6792.

Monitoring

- Flow cytometry
  - Clone size to assess disease progression
- Frequency:
  - Annual monitoring recommended if stable clone size
  - More frequent if clone size is changing
- Therapeutic response and to determine transfusion needs
  - If patient is receiving eculizumab
- RBCs
  - Subclinical PNH and response to eculizumab
  - Quantification of cells partially deficient
- WBCs
  - Clone size



Borowitz MJ, et al. *Cytometry B Clin Cytom*. 2010;78(4):211-230.

Monitoring Continued

- LDH
  - Near normal levels when treated with eculizumab
- Anemia and hemolysis
- Iron and erythropoietin
  - Additional treatment to facilitate erythropoiesis

Questions

bluelock.com

References

- Merriam-Webster. (n.d.). Paroxysm, Nocturnal, Hemoglobinuria. In *Merriam-Webster.com dictionary*. Retrieved August 3, 2023, from https://www.merriam-webster.com/dictionary/
- Schrezenmeier H, Muus P, Socié G, et al. Baseline characteristics and disease burden in patients in the International Paroxysmal Nocturnal Hemoglobinuria Registry. *Haematologica*. 2014;99(5):922-929. doi:10.3324/haematol.2013.093161
- Jalbert J, Chaudhari U, Zhang H, Weyne J, Shammo J. Epidemiology of PNH and Real-World Treatment Patterns Following an Incident PNH Diagnosis in the US. *Blood* 2019; 134 (Supplement_1): 3407. doi: https://doi.org/10.1182/blood-2019-125867
- Kenney MC, Chwa M, Atilano SR, et al. Inherited mitochondrial DNA variants can affect complement, inflammation and apoptosis pathways: insights into mitochondrial-nuclear interactions. *Hum Mol Genet*. 2014;23(13):3537-3551. doi:10.1093/hmg/ddu065
- Devalet B, Mullier F, Chatelain B, et al. Pathophysiology, diagnosis, and treatment of paroxysmal nocturnal hemoglobinuria: a review. *European Journal of Haematology*. 2015. *95*(3) 190-198.
- Wong RSM. Safety and efficacy of pegcetacoplan in paroxysmal nocturnal hemoglobinuria. *Therapeutic Advances in Hematology*. 2022;13. doi:10.1177/20406207221114673
- Kelly RJ, et al. Long-term treatment with eculizumab in paroxysmal nocturnal hemoglobinuria: sustained efficacy and improved survival. *Blood* 2011; 117 (25): 6786–6792. doi: https://doi.org/10.1182/blood-2011-02-333997
- Brodsky RA. How I treat paroxysmal nocturnal hemoglobinuria. *Blood* 2021; 137 (10): 1304–1309. doi: https://doi.org/10.1182/blood.2019003812
- Hillmen P, et al. Pegcetacoplan versus Eculizumab in Paroxysmal Nocturnal Hemoglobinuria. *N Engl J Med*. 2021; 384:1028-1037.