Paroxismal Nocturnal Hemoglobinuria

Damian A. Laber, M.D., FACP

Senior Member and Chief
Section of Satellite Oncology at TGH
Moffitt Cancer Center

Professor of Medicine and Oncologic Sciences
Director Division of Hematology/Oncology
University of South Florida

PNH – What is it?

- Rare blood disease that causes red blood cells to break apart "hemolysis.“
- It happens because the surface of a person’s blood cells are missing a protein that protects them from the body's immune system.
- When red blood cells break apart, the hemoglobin inside is released causing damage to many organs

PNH Patient related care

PNH – Who gets it?

- PNH can appear at any age and in any race or gender but is diagnosed most often in people in their 30s and 40s.
- Estimate between 400 and 500 cases are diagnosed in the U.S. each year.
- Incidence of 1 to 10 cases per million

PROGNOSIS

- Pre-complement inhibitor era (1996)
  - 14.6 year median OS
  - 78, 65, and 48 percent at 5, 10, and 15 years after diagnosis, respectively
  - Eight-year rates of pancytopenia, thrombosis, and myelodysplastic syndrome were 15, 28, and 5 percent, respectively
- Pre-complement inhibitor era (2004)
  - OS for Japanese patients was 32 years, American patients 19 years
  - Japanese more likely to have aplastic anemia, and American patients were more likely to have thrombosis (38 versus 6 percent)
- Current survival with C5i or C3i similar to age-matched controls

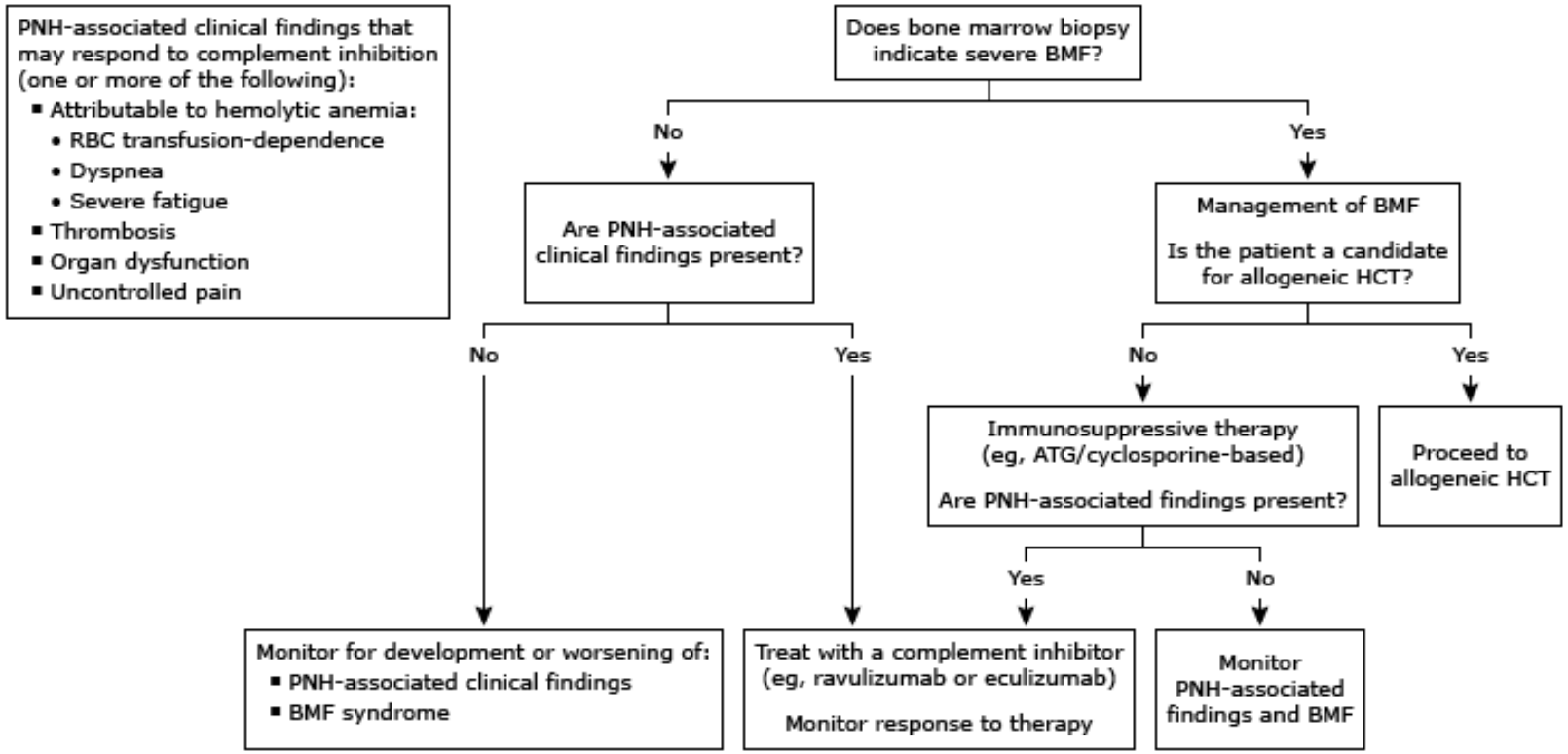
PNH Clinical manifestations

- Hemolysis-related
  - Fatigue, dyspnea, hemoglobinuria, and predisposition to venous thrombosis
- Bone marrow dysfunction
  - Bone marrow failure (BMF) may exacerbate hemolysis-associated cytopenias
- Thrombosis
  - The leading cause of death and often involve atypical sites, such as abdominal veins, cerebral veins, or dermal veins
- Smooth muscle dystonia
  - Depletion of intravascular nitric oxide (NO) due to free hemoglobin can cause smooth muscle dystonia with associated abdominal pain, erectile dysfunction, pulmonary hypertension, and/or renal insufficiency

PNH Evaluations

- History, examination, and laboratory studies to investigate complement-mediated hemolysis evaluate for thrombosis, organ dysfunction, and BMF
- Flow cytometry
  - A population of PNH-affected granulocytes and erythrocytes is demonstrated by loss of GPI-anchored proteins (eg, CD55, CD59)
- Bone marrow examination
  - Microscopy and cytogenetics to evaluate for BMF and MDS

Management of paroxysmal nocturnal hemoglobinuria



Diagnosis of PNH is based on absence or marked reduction of cell surface CD55/CD59 on two peripheral blood lineages by flow cytometry and FLAER. Refer to related UpToDate material for details of criteria for PNH diagnosis and severe BMF syndrome, PNH-associated clinical findings, supportive care, other aspects of PNH management, eligibility for allogeneic HCT, and management of BMF.

ATG: anti-thymocyte globulin; BMF: bone marrow failure; FLAER: fluorescent aerolysin; HCT: hematopoietic cell transplantation; PNH: paroxysmal nocturnal hemoglobinuria; RBC: red blood cells.

Management

- Treatment is guided by the type and severity of manifestations and complications of PNH

- Symptomatic hemolytic PNH without bone marrow failure (BMF)
  - C5 complement inhibitor
  - C3 complement inhibitor
- Subclinical PNH
  - No PNH-associated symptoms or BMF
  - Active surveillance
- PNH with BMF
  - Treat for aplastic anemia (sAA) or high-risk MDS
  - Consider adding C5 or C3 inhibitors

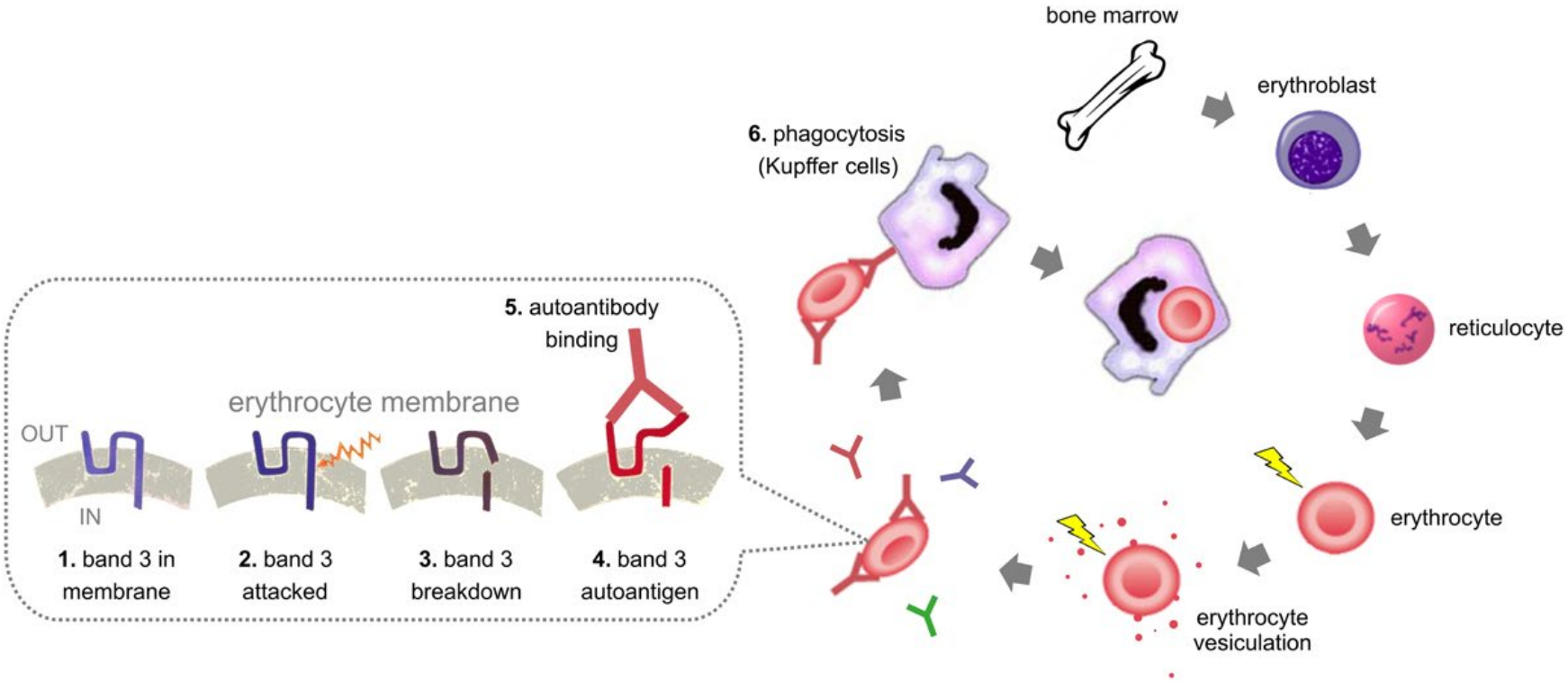
Management (Cont.)

- Thrombosis
  - C5i or C3i therapy

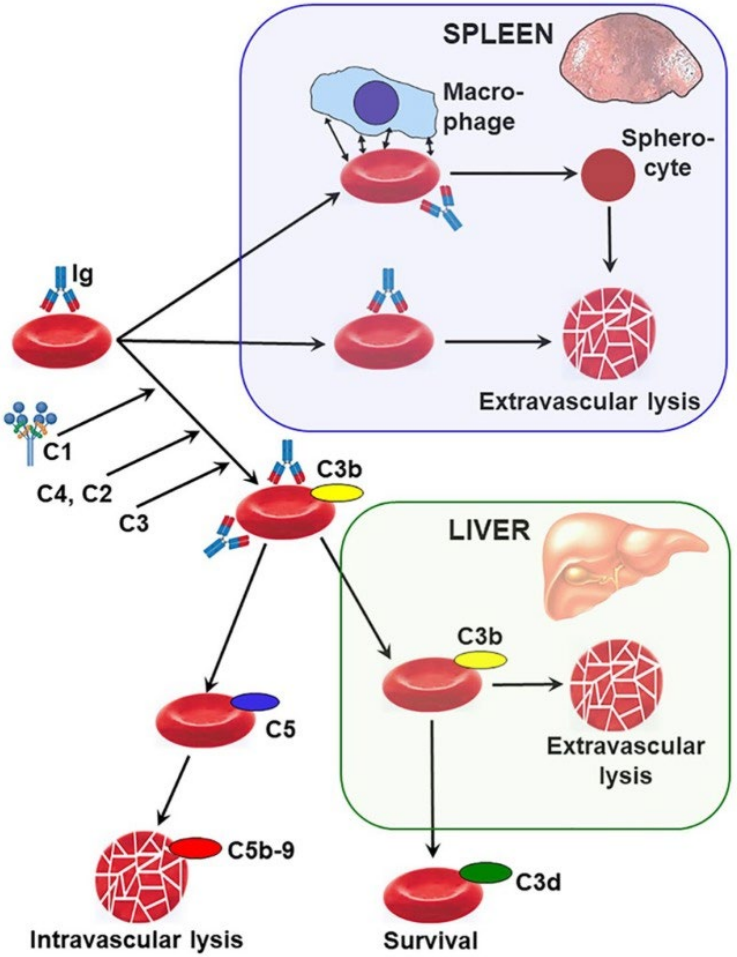
- Indications for allogeneic HCT in PNH include:
  - sAA or high-risk MDS on a case by case basis
  - Have a suitable donor.
  - Medically-fit for transplantation
    - No severe lung, heart, liver, or kidney disease
    - Adequate social supports

Red blood cells

Red blood cell physiology



Hemolysis (wAIHA)

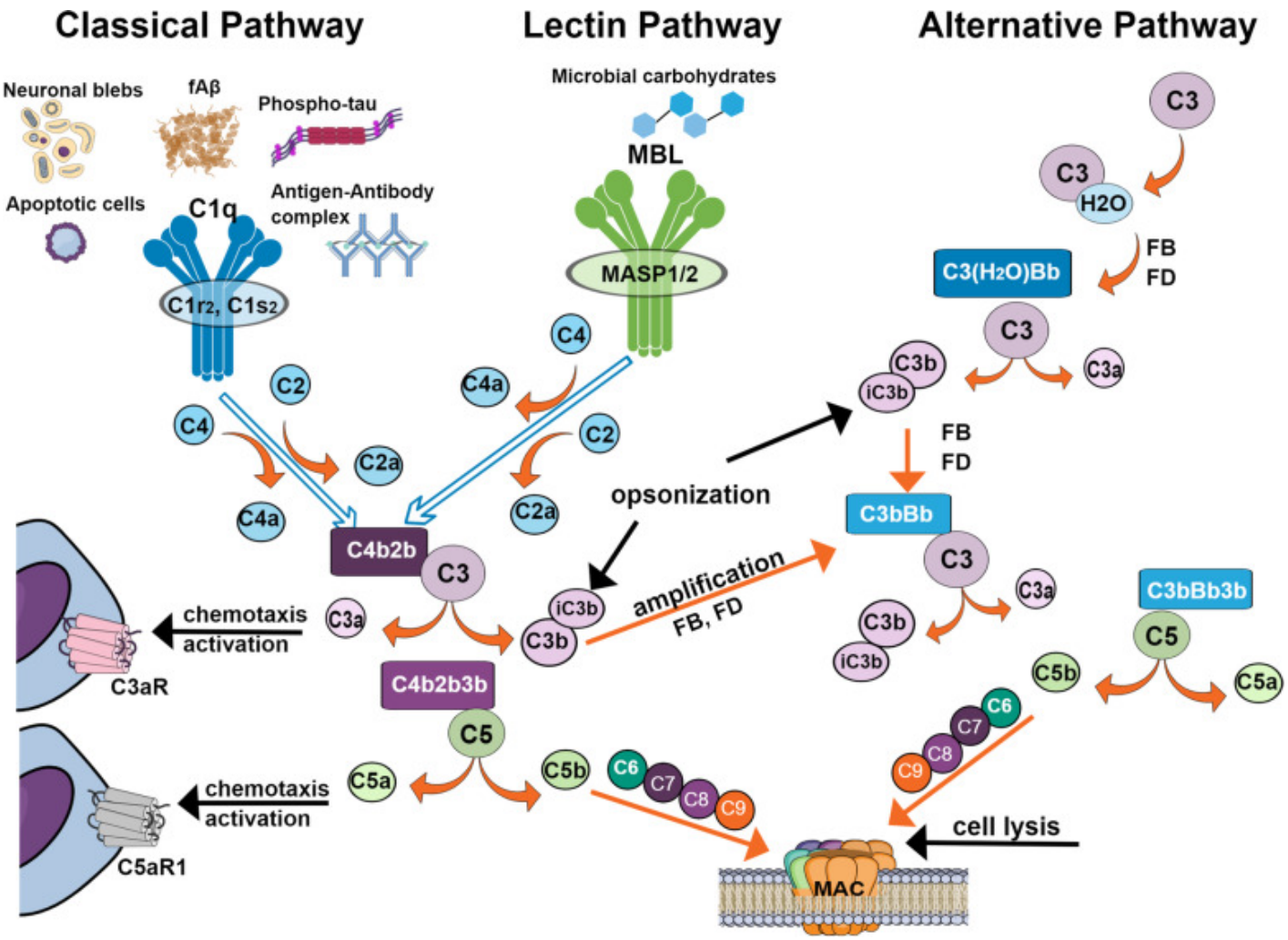


The Complement System

The complement system

- Is an ancient part of the innate immune system
- Comprises of more than thirty serum and membrane-bound proteins
- Complement activation leads to:
  - Opsonization of pathogens > removal by phagocytes
  - Cell lysis
- Inappropriate activation and complement deficiencies leads to diseases
- Three different activating pathways

Activation pathways of the complement system



Complement deficiencies and associated clinical manifestations

Clinical manifestation	Complement deficiency
Atypical hemolytic syndrome	Factor H
Hereditary angioedema	C1-INH
Paroxysmal nocturnal hemoglobinuria	CD59 and DAF
Systemic lupus erythematosus	C1q, C1r, C1s, C2, C4
Susceptibility to *Neisseria meningitides* infections	C2, C3, C4, C5, C6, C7, C8, C9, properdin
Susceptibility to respiratory tract infections	MBL
Susceptibility to recurrent infections	MASP2, Factor I

PNH Science

PNH STEM CELL

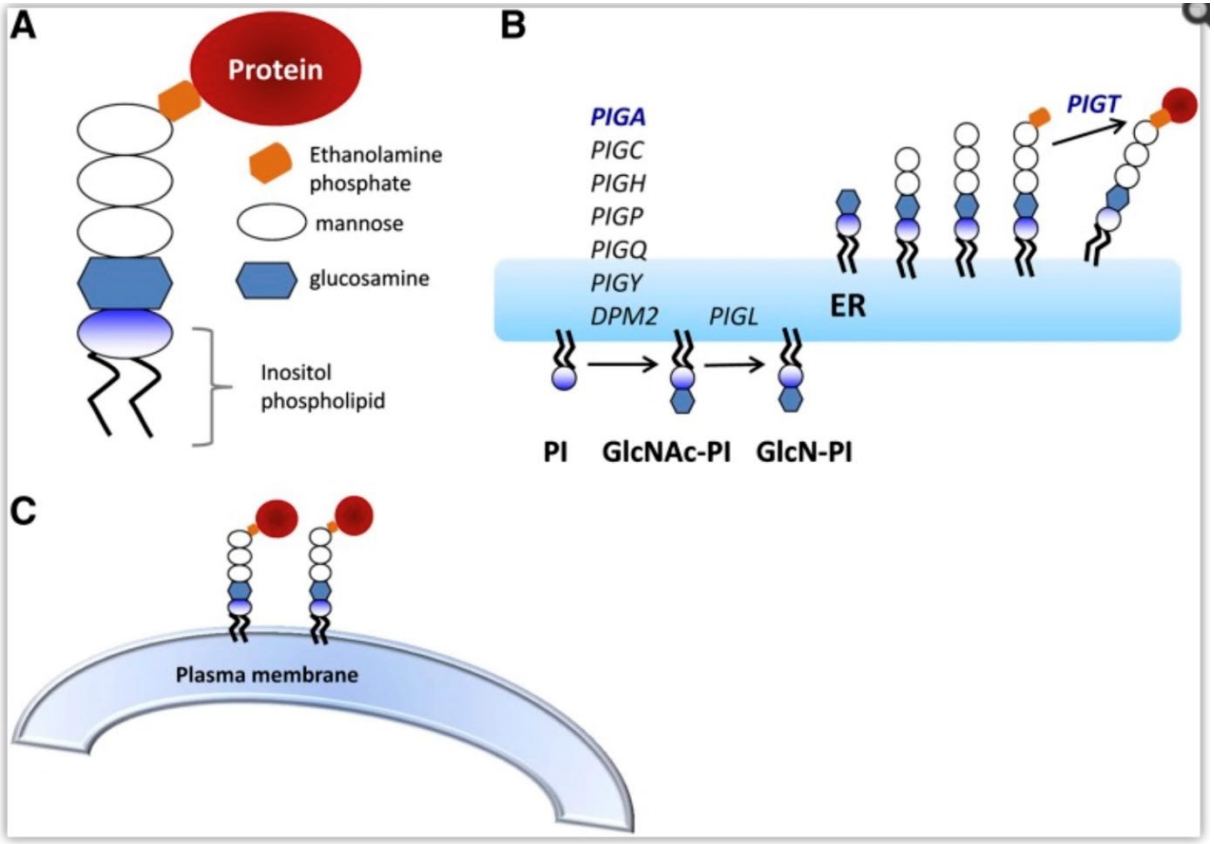
- PNH originates from an acquired genetic defect (mutation) in a
  - multipotent hematopoietic stem cell
  - hematopoietic progenitor cell that acquires stem cell properties and is able to survive, expand, and self-renew
- PNH can arise de novo or in the setting of an underlying bone marrow disorder such as aplastic anemia (AA), myelodysplastic syndrome (MDS), or primary myelofibrosis (PMF)

PIGA gene mutation

- The acquired mutation in PNH occurs in the PIGA gene
  - Phosphatidylinositol glycan anchor biosynthesis, class A
  - Responsible for the synthesis of the glycosylphosphatidylinositol (GPI) anchor that attaches a subset of proteins to the cell surface

- The PIGA gene is located on the X chromosome
  - Single "hit" (ie, a mutation in only one allele of the gene) will generate a PNH phenotype
  - males have only one X chromosome
  - females undergo X chromosome inactivation (lyonization) in every somatic cell, including hematopoietic stem cells

GPI anchor biosynthesis

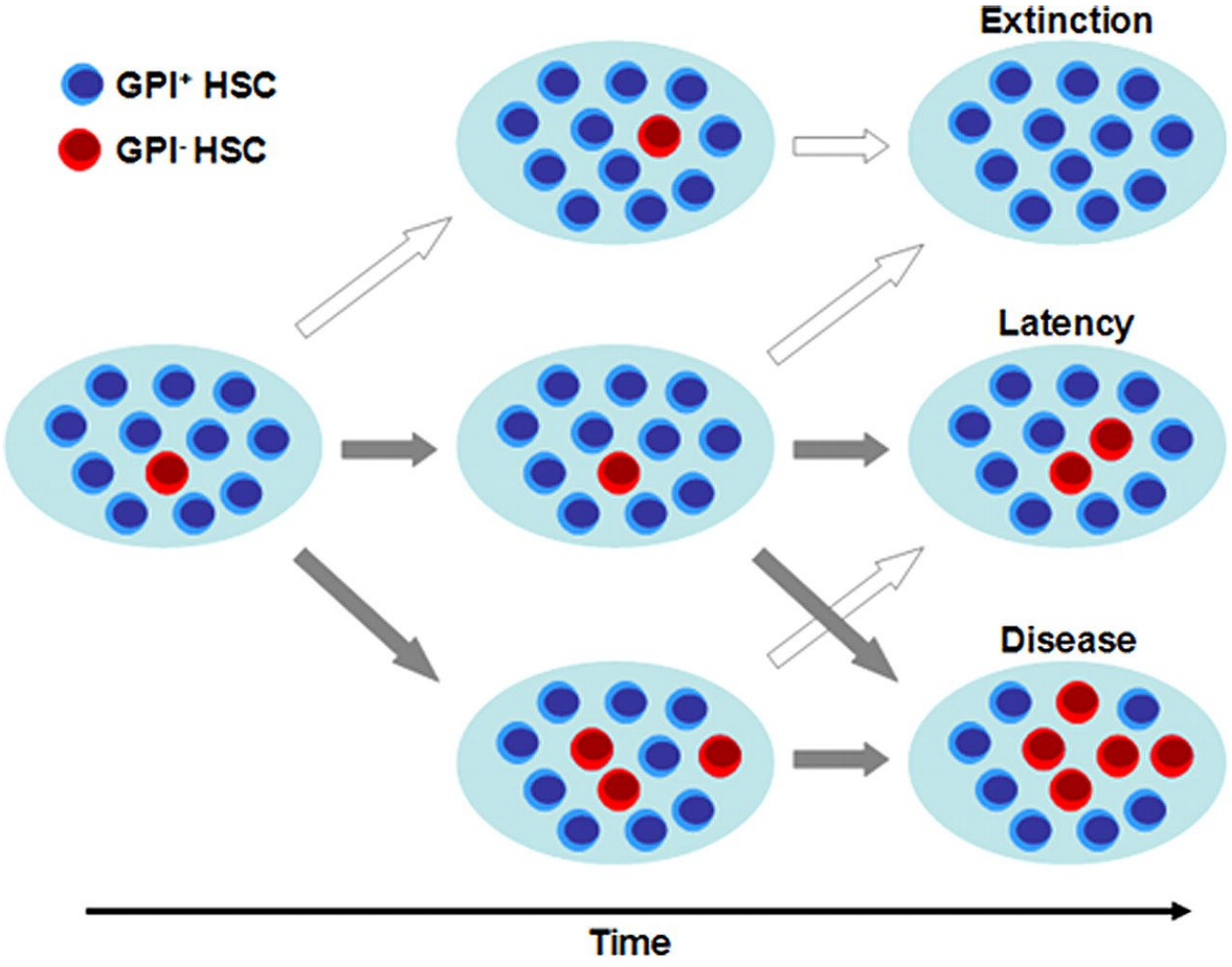


(A) Core structure of the GPI anchor. The inositol-phospholipid (PI) is anchors into the lipid bilayer of the plasma membrane. The glycan core consists of a molecule of N-glucosamine, 3 manose molecules (Man), and a molecule of ethanolamine phosphate. The protein is covalently attached through an amide bond to an ethanolamine on the terminal mannose. (B) GPI anchor biosynthesis takes place in the endoplasmic reticulum. PIGA is 1 of 7 subunits involved in the first step of GPI anchor biosynthesis. There are ≥10 additional steps and >25 genes involved. After the protein is attached to the GPI anchor, the mature GPI-anchored protein goes to the Golgi, where fatty acid remodeling occurs and (C) eventually the GPI anchored protein is transported to the plasma membrane.

Clonal selection and expansion

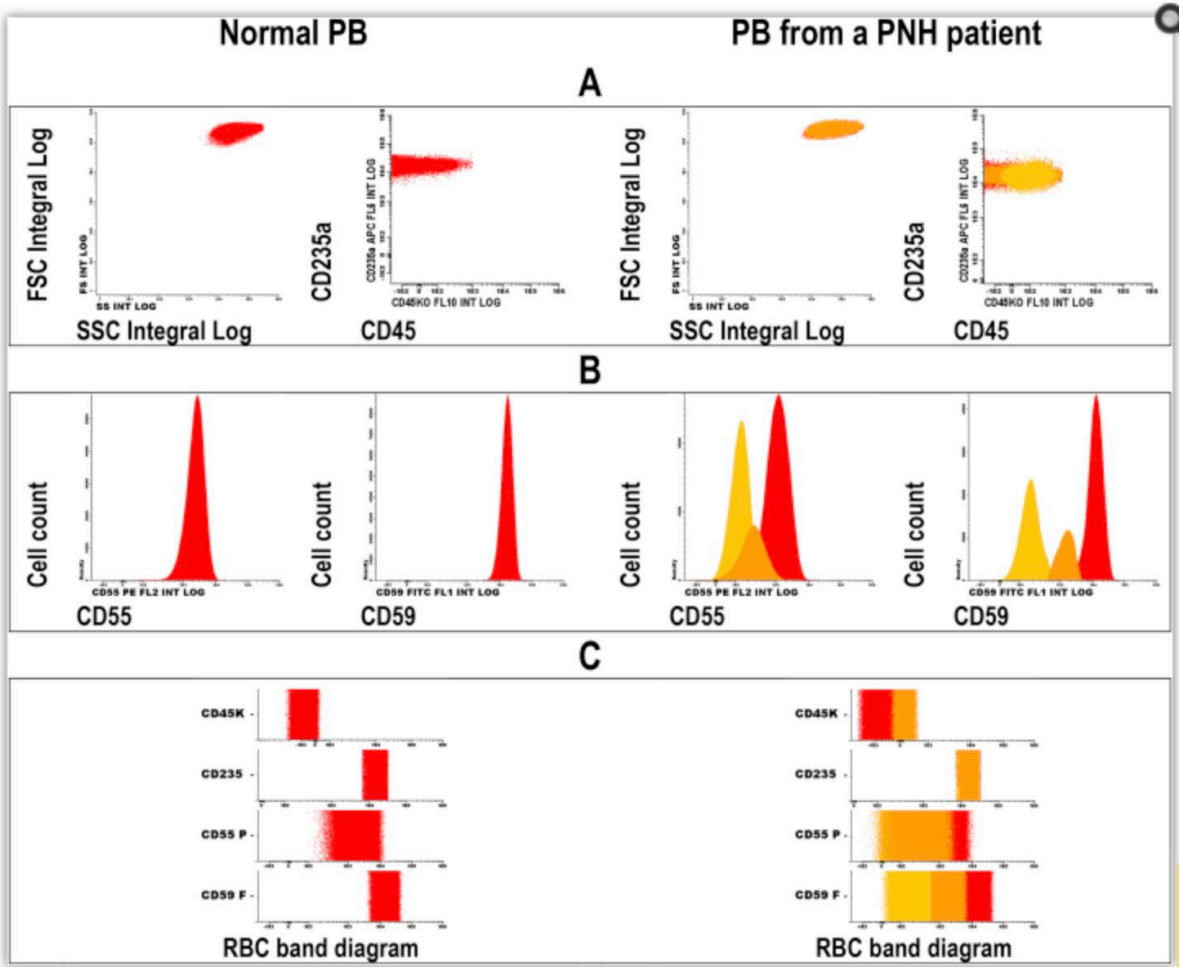
- For PNH to manifest clinically, the hematopoietic stem cell carrying a PIGA mutation must undergo clonal expansion
  - Immune escape
    - PIGA mutations protect cells from immune mediated destruction
  - Selective advantage for clonal expansion
    - Acquisition of additional mutation(s) or other modifications may provide a selective advantage
    - Accelerated proliferation or reduced apoptosis (programmed cell death); however, the mutation rate in PNH cells is similar to that of non-PNH cells [24-27].
  - Neutral evolution
    - PNH clones do not have a survival advantage, but one or more clones expand stochastically during periods of bone marrow regeneration.

Model of stochastic dynamics in the active hematopoietic SC pool



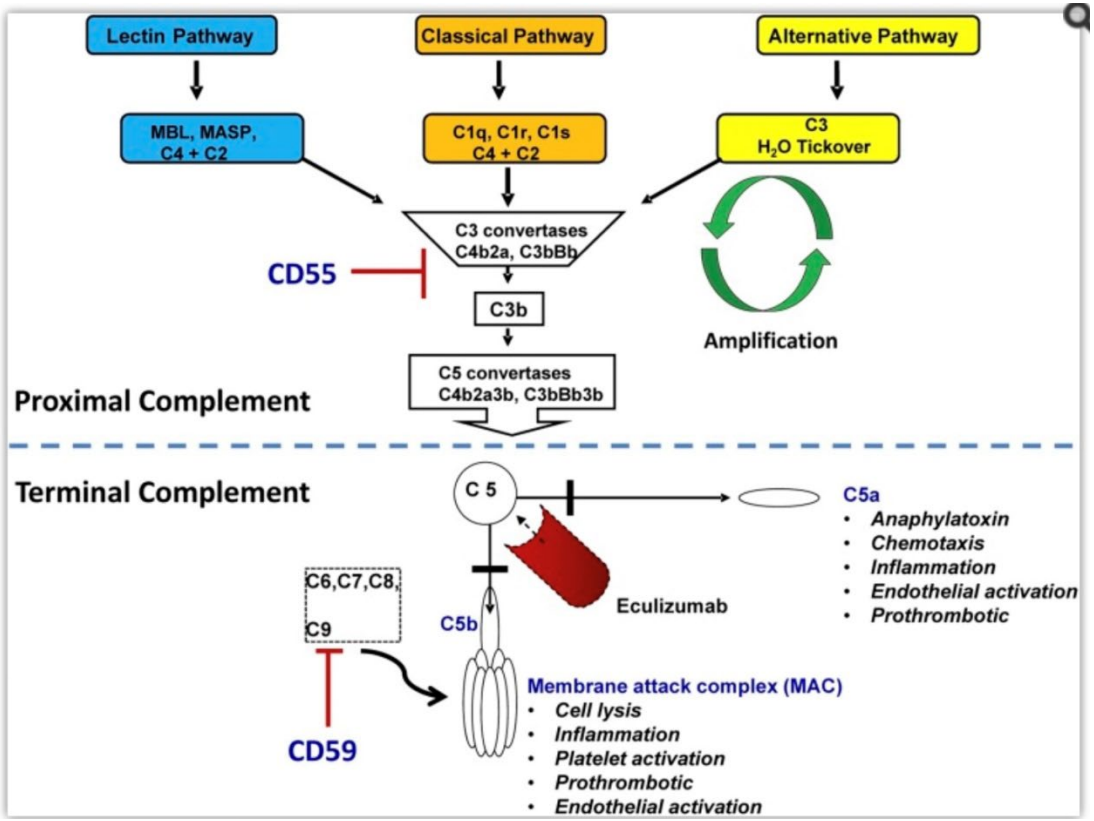
Flow cytometry PNH

Fig. 1



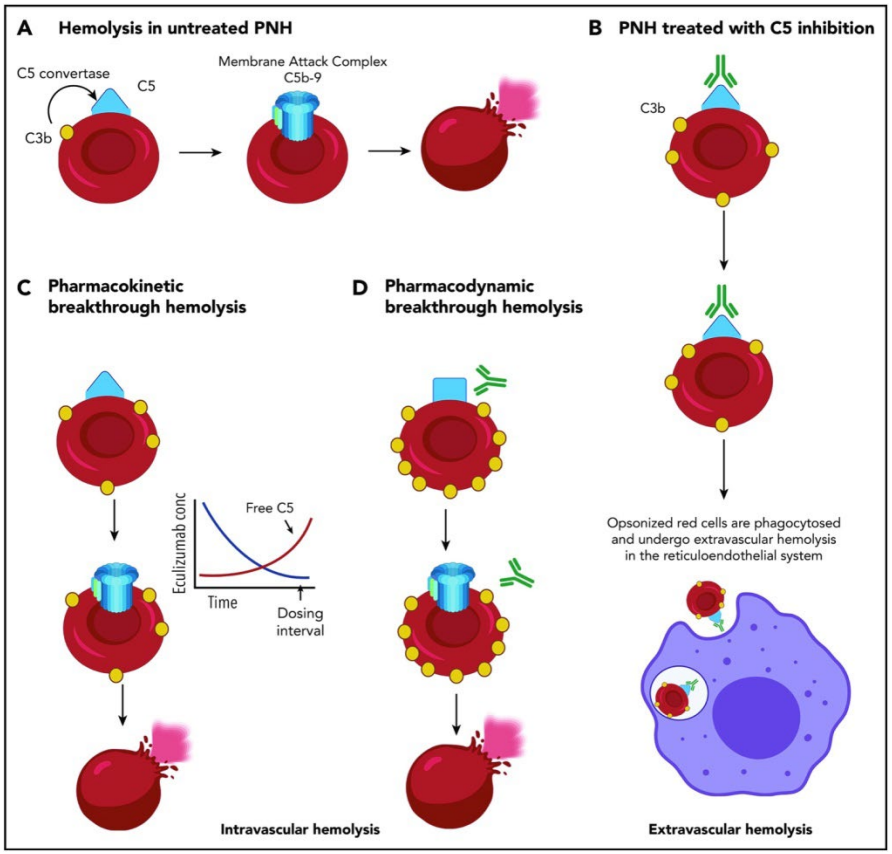
Abbreviations: APC, Allophycocyanin; FCM, Flow Cytometry; FITC, Fluorescein Isothiocyanate; FSC, Forward Scatter; GPI-AP, Glycosylphosphatidylinositol -anchored proteins; KO, Krome orange; PB, peripheral blood; PE, Phycoerythrin; PNH, Paroxysmal nocturnal hemoglobinuria; RBC, Red blood cells. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Complement regulation and C5i



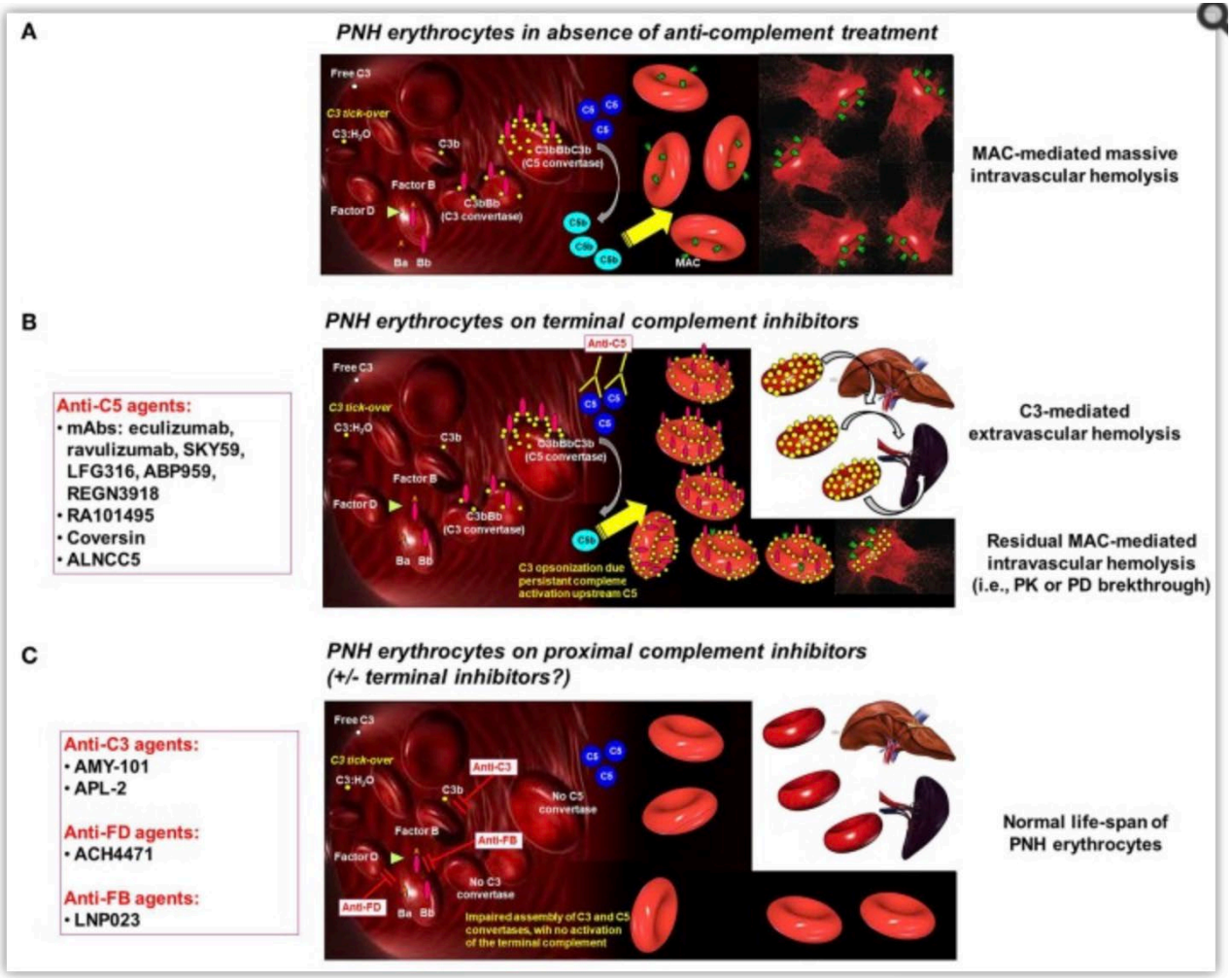
The lectin, classical, and alternative pathways converge at the point of C3 activation. In PNH, hemolysis is usually chronic because the alternative pathway is always in a low-level activation state through a process known as tick-over. Terminal complement begins with cleavage of C5 to C5a and C5b. C5b oligomerizes with C6, C7, C8, and multiple C9 molecules to form the MAC. CD55 inhibits proximal complement activation by blocking the formation of C3 convertases; CD59 inhibits terminal complement activation by preventing the incorporation of C9 into the MAC. The absence of CD55 and CD59 on PNH cells leads to hemolysis, inflammation, platelet activation, and thrombosis. Eculizumab inhibits terminal complement activation by binding to C5 and preventing generation of C5a and C5b

Mechanisms of hemolysis in PNH



(A) Loss of CD55 and CD59 on PNH red cells leaves them vulnerable to complement-mediated intravascular hemolysis. (B) PNH red cells from patients with PNH treated with C5 inhibition (eculizumab or ravulizumab) often become coated with C3 fragments that serve as opsonins and lead to extravascular hemolysis in the spleen and liver. C5 inhibition compensates for the loss of CD59 and prevents intravascular hemolysis; however, CD55, upstream to C5 is important for accelerating decay of the C3 convertase. The lack of CD55 from PNH red cells leads to the accumulation of C3b and its processed forms iC3b and C3dg. (C) Pharmacokinetic intravascular hemolysis caused by insufficient drug dosing allows free C5 levels to rise. (D) Pharmacodynamic intravascular hemolysis. Complement amplifying conditions (pregnancy, infection, major surgery) can result in excess C3b accumulation on PNH red cells that leads to a conformational change in C5 and decrease the binding of eculizumab or ravulizumab to C5, resulting in breakthrough hemolysis even in the absence of a rise in free C5.

Complement activation on PNH erythrocytes



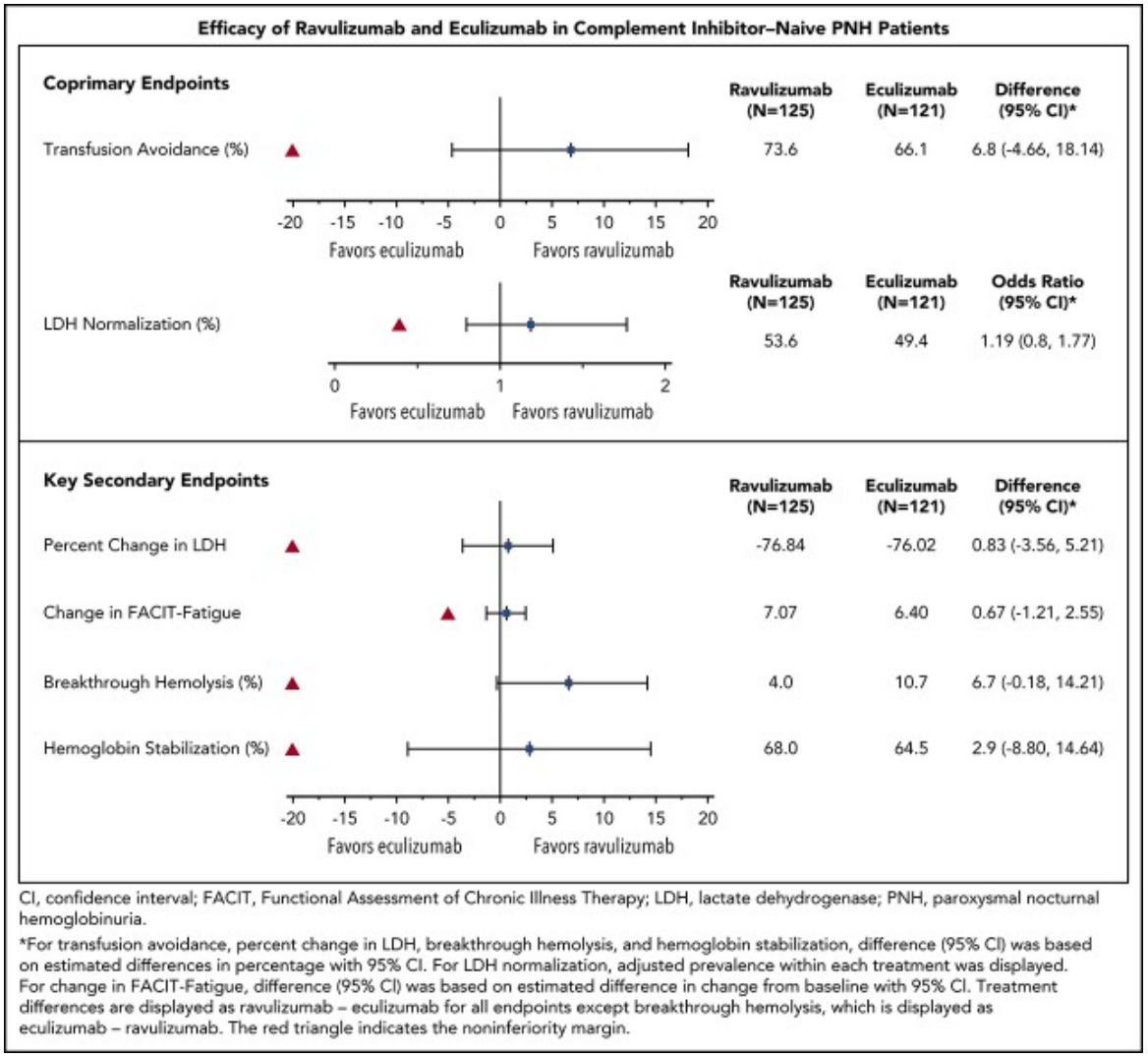
(A) PNH erythrocytes in absence of anti-complement treatment. (B) PNH erythrocytes on terminal complement inhibitors.
(C) PNH erythrocytes on proximal complement inhibitors(± terminal complement inhibitors).

PNH Treatments

Eculizumab - N Engl J Med 2006

- METHODS
  - Double-blind, randomized, placebo-controlled, multicenter, phase 3 trial
  - Placebo or eculizumab (600 mg weekly for 4 weeks, followed 1 week later by a 900-mg dose and then 900 mg every other week through week 26)
- End points:
  - Stabilization of hemoglobin and number of units of PRBC transfused
  - Biochemical indicators of intravascular hemolysis
  - Quality of life
- RESULTS
  - Eighty-seven patients
  - Stabilization of hemoglobin levels in the absence of transfusions
    - 49% (21 of 43) eculizumab and none (0 of 44) placebo
    - 0 units PRBC eculizumab vs 10 units in the placebo during 26 weeks of study
    - Eculizumab reduced intravascular hemolysis
    - Improved the quality of life

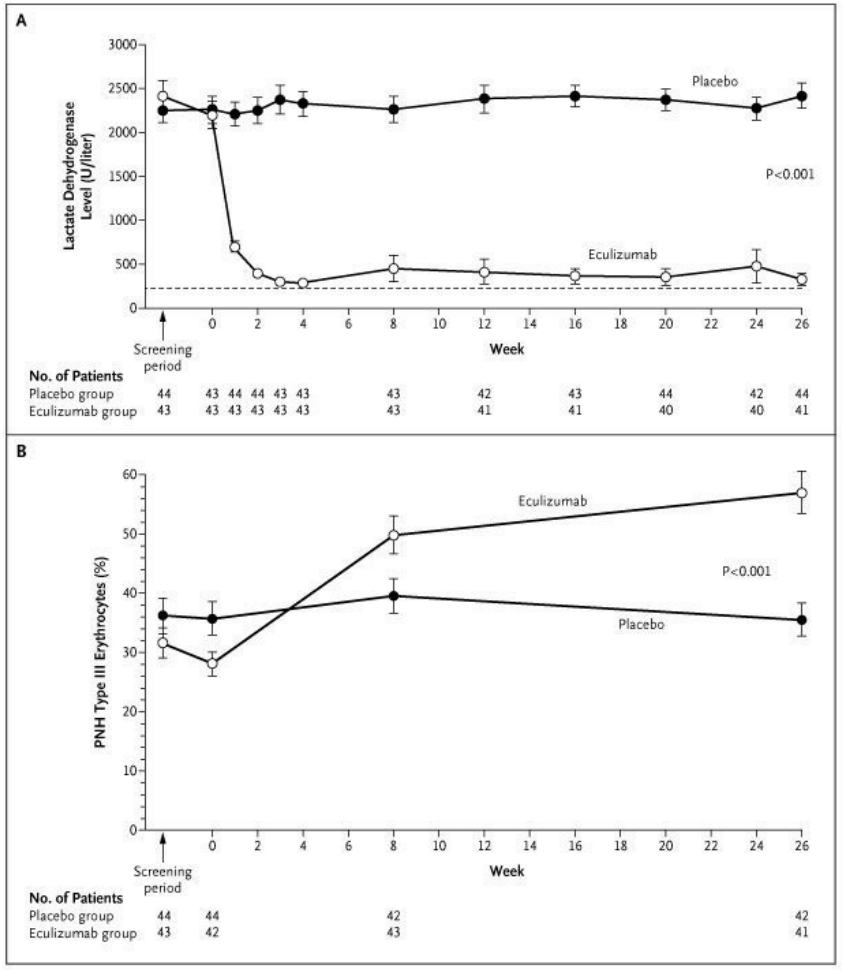
Ravulizumab vs eculizumab (naive) Study 301- Blood 2019



CI, confidence interval; FACIT, Functional Assessment of Chronic Illness Therapy; LDH, lactate dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria.

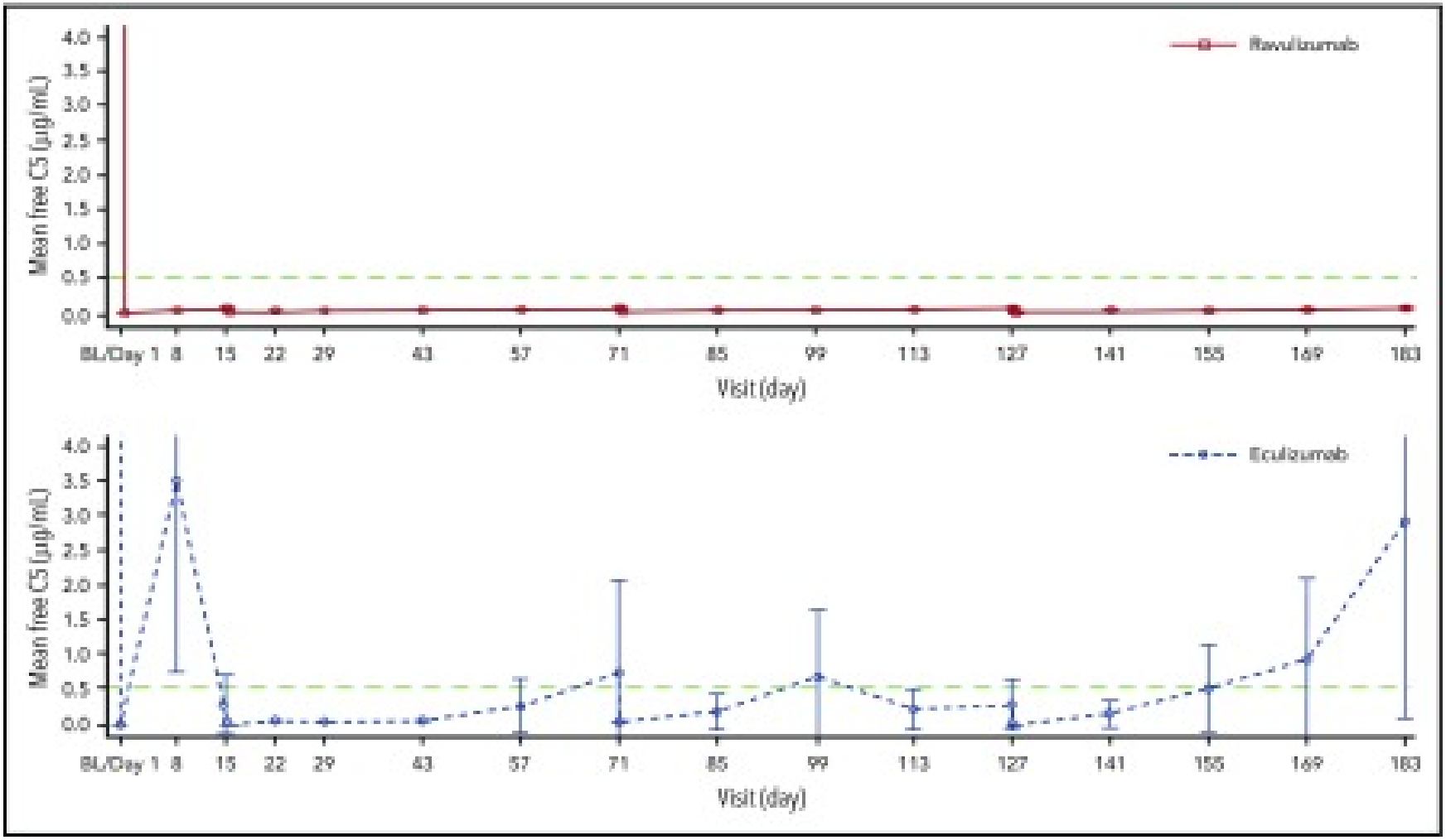
*For transfusion avoidance, percent change in LDH, breakthrough hemolysis, and hemoglobin stabilization, difference (95% CI) was based on estimated differences in percentage with 95% CI. For LDH normalization, adjusted prevalence within each treatment was displayed. For change in FACIT-Fatigue, difference (95% CI) was based on estimated difference in change from baseline with 95% CI. Treatment differences are displayed as ravulizumab – eculizumab for all endpoints except breakthrough hemolysis, which is displayed as eculizumab – ravulizumab. The red triangle indicates the noninferiority margin.

Levels of Lactate Dehydrogenase and PNH Type III Erythrocytes during Treatment with Eculizumab.

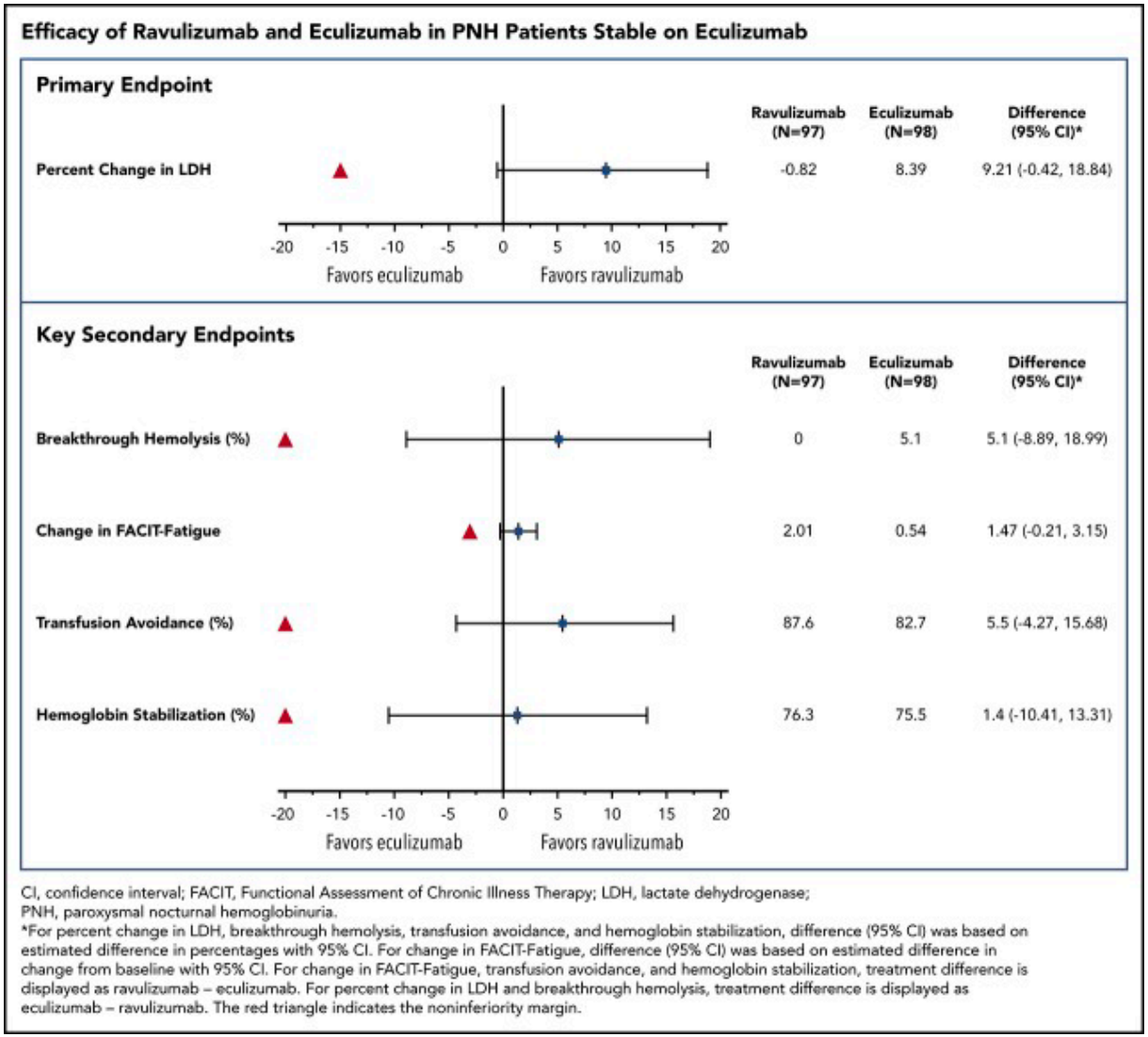


- A) degree of intravascular hemolysis according to the mean levels of lactate dehydrogenase from baseline (week 0) to week 26 in the two study groups. The dashed line indicates the upper limit of the normal range for lactate dehydrogenase
- B) mean proportion of PNH type III erythrocytes in patients in the two groups

Mean free C5 concentrations



Ravulizumab vs eculizumab (switch) Study 302- Blood 2019



CI, confidence interval; FACIT, Functional Assessment of Chronic Illness Therapy; LDH, lactate dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria.

*For percent change in LDH, breakthrough hemolysis, transfusion avoidance, and hemoglobin stabilization, difference (95% CI) was based on estimated difference in percentages with 95% CI. For change in FACIT-Fatigue, difference (95% CI) was based on estimated difference in change from baseline with 95% CI. For change in FACIT-Fatigue, transfusion avoidance, and hemoglobin stabilization, treatment difference is displayed as ravulizumab – eculizumab. For percent change in LDH and breakthrough hemolysis, treatment difference is displayed as eculizumab – ravulizumab. The red triangle indicates the noninferiority margin.

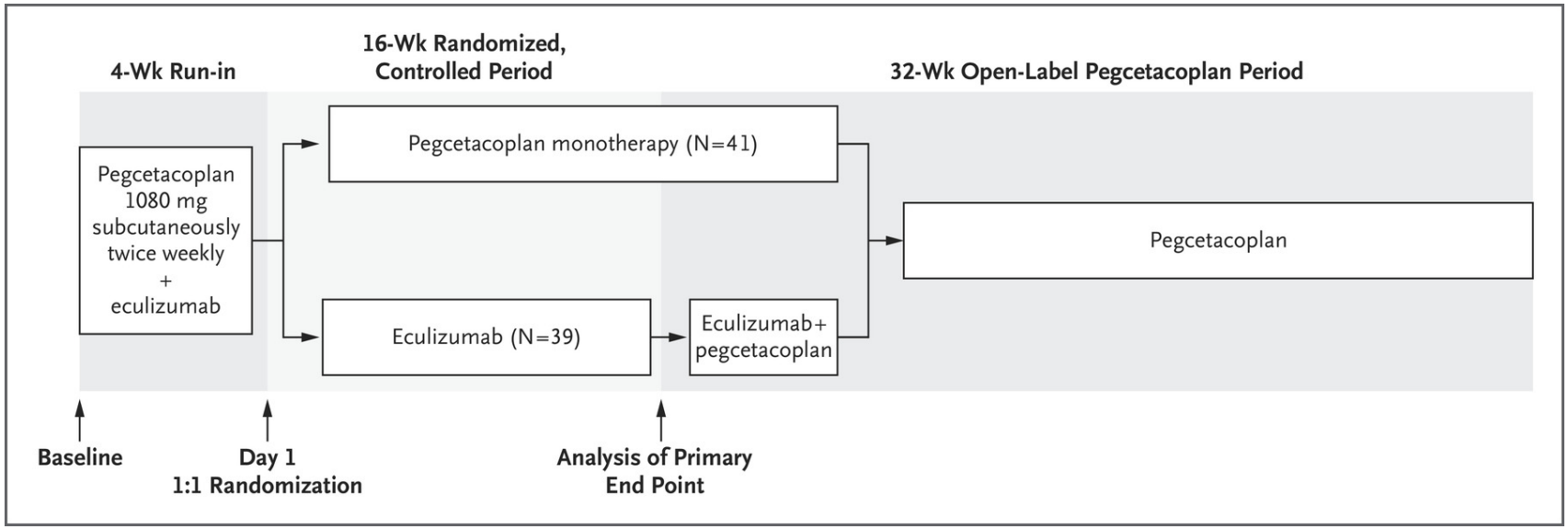
Pegcetacoplan versus Eculizumab NEJM 2021

- METHODS
- We conducted a phase 3 open-label, controlled trial to assess the efficacy and safety of pegcetacoplan as compared with eculizumab in adults with PNH and hemoglobin levels lower than 10.5 g per deciliter despite eculizumab therapy. After a 4-week run-in phase in which all patients received pegcetacoplan plus eculizumab, we randomly assigned patients to subcutaneous pegcetacoplan monotherapy (41 patients) or intravenous eculizumab (39 patients). The primary end point was the mean change in hemoglobin level from baseline to week 16. Additional clinical and hematologic markers of hemolysis and safety were assessed.

- RESULTS
- Pegcetacoplan was superior to eculizumab with respect to the change in hemoglobin level from baseline to week 16, with an adjusted (least squares) mean difference of 3.84 g per deciliter ($P<0.001$). A total of 35 patients (85%) receiving pegcetacoplan as compared with 6 patients (15%) receiving eculizumab no longer required transfusions. Noninferiority of pegcetacoplan to eculizumab was shown for the change in absolute reticulocyte count but not for the change in lactate dehydrogenase level. Functional Assessment of Chronic Illness Therapy–Fatigue scores improved from baseline in the pegcetacoplan group. The most common adverse events that occurred during treatment in the pegcetacoplan and eculizumab groups were injection site reactions (37% vs. 3%), diarrhea (22% vs. 3%), breakthrough hemolysis (10% vs. 23%), headache (7% vs. 23%), and fatigue (5% vs. 15%). There were no cases of meningitis in either group.

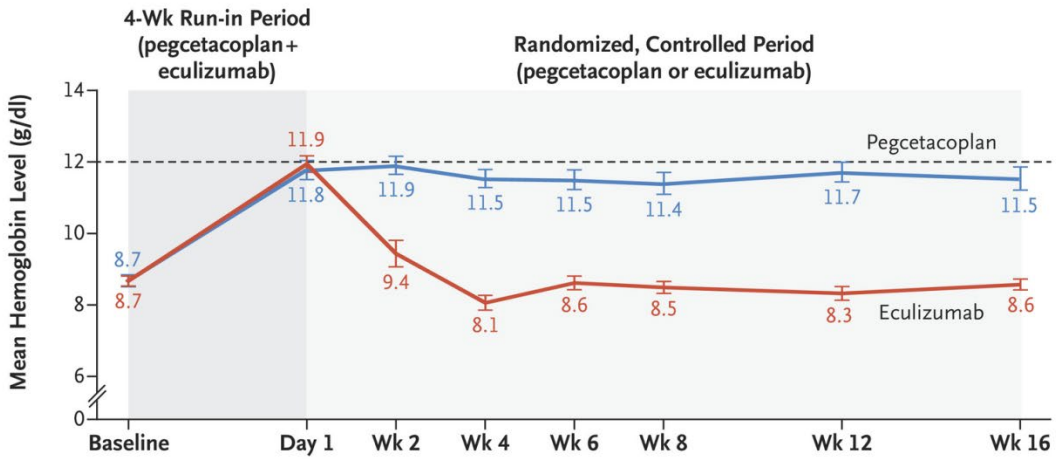
- CONCLUSIONS
- Pegcetacoplan was superior to eculizumab in improving hemoglobin and clinical and hematologic outcomes in patients with PNH by providing broad hemolysis control, including control of intravascular and extravascular hemolysis.

Pegcetacoplan versus Eculizumab NEJM 2021



Pegcetacoplan versus Eculizumab NEJM 2021

A



No. with Available Data								
Pegcetacoplan	41	40	40	40	39	37	38	37
Eculizumab	39	37	38	39	36	39	39	38

B

Subgroup	No. of Patients		Adjusted Change in LS Mean Hemoglobin		Difference (95% CI)
	Pegcetacoplan	Eculizumab	Pegcetacoplan	Eculizumab	
			g/dl±SE		
Censored for transfusion events, all values after intercurrent events set to missing	41	39	2.37±0.36	−1.47±0.67	3.84 (2.33–5.34)
All available data, regardless of tranfusion events	41	39	2.66±0.25	−0.03±0.26	2.69 (1.99–3.38)
No. of transfusions in previous 12 mo					
<4	20	16	2.97±0.36	−0.01±0.49	2.98 (1.73–4.23)
≥4	21	23	2.11±0.60	−4.02±2.40	6.13 (0.79–11.48)

TABLE 6 Novel therapeutic agents at less advanced stages of research

Agent	Mechanism of action	Phase of clinical trials	Safety	Treatment outcomes
Crovalimab	C5 inhibitor	III	Subcutaneous, self--administration, small volumes, every 4 weeks	Sustained LDH response
Nomacopan	C5 inhibitor	III	Self-administration, safe, well-tolerated for long-term treatment	Transfusion independence
Tesidolumab	C5 inhibitor	II	Favorable safety profile	Decreased transfusion dependency, reduction of LDH concentrations
Pozelimab	C5 inhibitor	II	Well-tolerated	Normalization of LDH in normal and variant C5 carriers
Zilucoplan	C5 inhibitor	II	Well-tolerated	Under study
Cemdisiran	siRNA targeting C5 mRNA, C5 inhibitor	II	Tolerated	Under study
Pegcetacoplan	Proximal complement pathway inhibitor, C3 inhibitor	approved for PNH treatment	Well-tolerated	Prevents extravascular hemolysis, may affect residual intravascular hemolysis
Danikopan	Proximal complement pathway inhibitor, factor D inhibitor	II/III	Oral, well-tolerated	Prevents extravascular hemolysis, may affect residual intravascular hemolysis
Iptacopan	Proximal complement pathway inhibitor, factor B inhibitor	II/III	Oral, well-tolerated	Prevents extravascular hemolysis, may affect residual intravascular hemolysis

Abbreviations: mRNA, messenger RNA; siRNA, small interfering RNA; others, see TABLE 4

Questions?

“Thank you”

Damian A. Laber, M.D., FACP

Senior Member
Head Section of Medical Oncology at TGH
Moffitt Cancer Center

Professor of Medicine
Director Division of Hematology/Oncology
University of South Florida