Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Elizabeth Griffiths, MD
Associate Professor
State University of New York
Buffalo, New York

Ilene C. Weitz, MD
Professor of Medicine
Keck School of Medicine
University of Southern California
Los Angeles, California

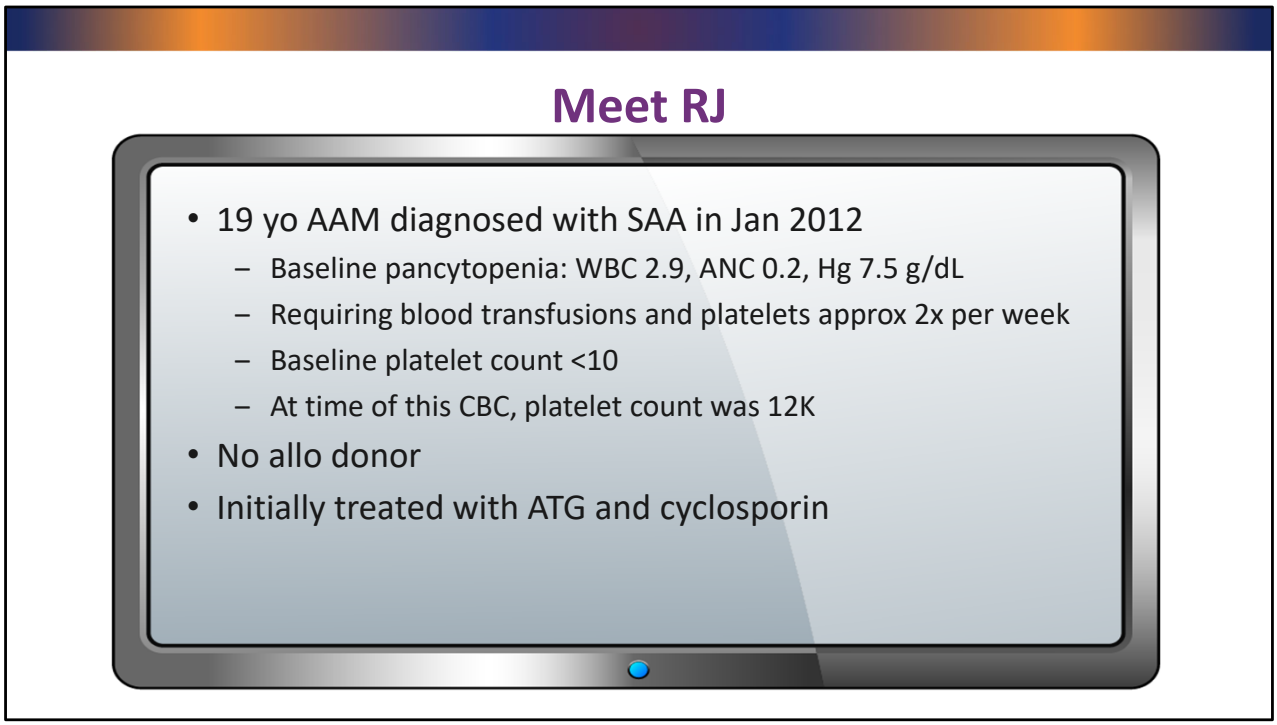
Dr. Griffiths: Hello. Today, we're going to talk to you about developments in the treatment of paroxysmal nocturnal hemoglobinuria. My name is Elizabeth Griffiths, and I practice at the Roswell Park Comprehensive Cancer Center in Buffalo, New York. My co-presenter is Dr. Ilene Weitz, who's a professor of clinical medicine at the Jane Anne Nohl Division of Hematology at the Keck School of Medicine at the University of Southern California.

Faculty Disclosures

- **Dr. Elizabeth Griffiths** has relevant financial relationships related to consulting from AbbVie Inc., Celgene Corporation – A Bristol-Myers Squibb Company, Genentech, Inc., Novartis AG, PicnicHealth, Taiho Pharmaceutical Co., Ltd, and Takeda Oncology, as well as advisory activities from Alexion and PicnicHealth. She has received research grants from Alexion, Apellis, Astex, Celgene Corporation – A Bristol-Myers Squibb Company, Genentech, and Novartis.
- **Dr. Ilene Weitz** has relevant financial relationships related to consulting from Alexion, Apellis, and BioCryst Pharmaceuticals, Inc. She is on the speakers' bureau for Alexion.

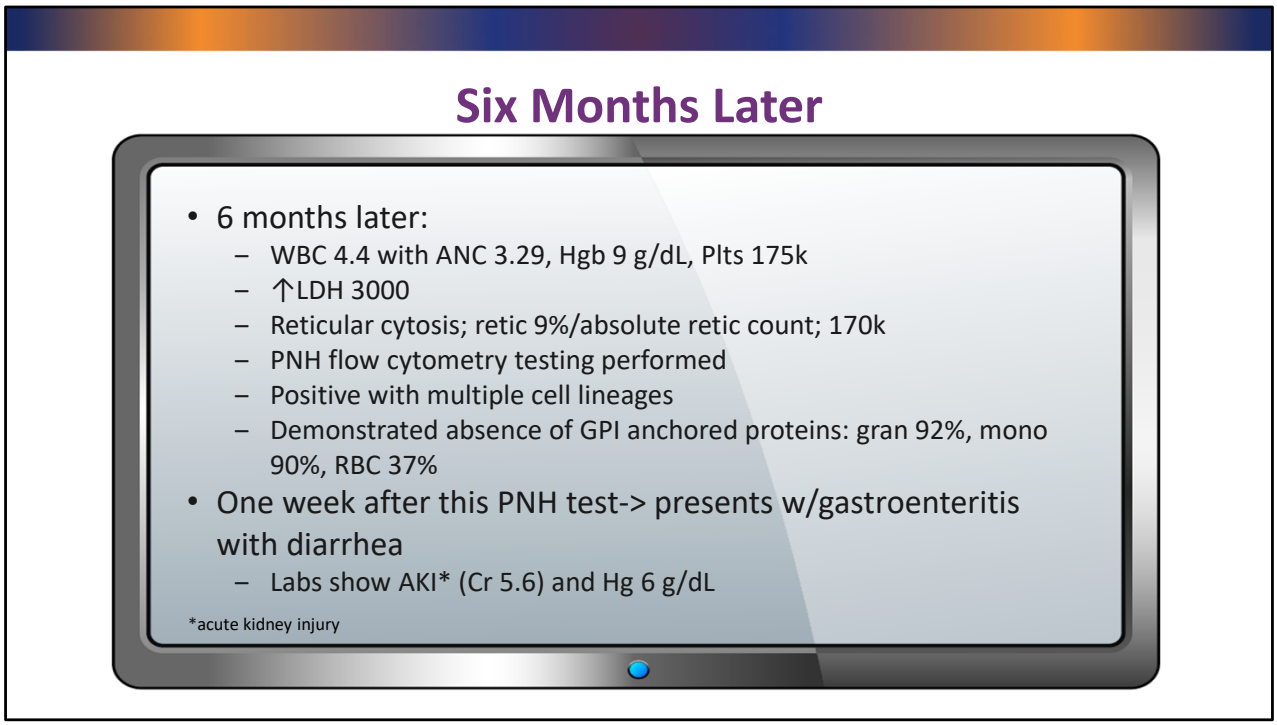
These are our disclosures.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria



We're going to begin this session with a clinical case of a patient of mine. R.J. was 19 years old at the time that he initially came to us, and he was diagnosed with severe aplastic anemia in January of 2012. He demonstrated baseline pancytopenia with a white blood cell count of 2.9, an ANC of 0.2, and a hemoglobin of 7.5 grams, requiring transfusions of blood and platelets about twice a week. His baseline platelet count was less than 10. At the time of this CBC, his platelet count was 12,000.

Unfortunately, no allogeneic bone marrow transplant donor was available for him. He received initial treatment with ATG and cyclosporin.



Six months later after receiving this treatment, the patient demonstrated relative normalization of the hemogram. His white count had improved to 4.4 with a neutrophil count of 3.29. His hemoglobin was around 9 grams, and his platelets had fully recovered to 175,000. At the time of follow-up, he was noted to have an increase in his LDH up to 3,000. He was noted also to have reticular cytosis with an absolute reticulocyte count of 170,000. Based on these findings, PNH flow cytometry testing was performed on the peripheral blood and subsequently was found to be positive with multiple cell lineages demonstrating absence of GPI-anchored proteins with the percentages you can see here.

Unfortunately, one week after his PNH test, which was not yet available to us, the patient presented with severe gastroenteritis with diarrhea, and labs showed an acute kidney injury with the creatinine rising up as high as 5.6 and a new profound anemia with a hemoglobin of 6 grams.

Polling Question #1

Why was this patient so sick? What caused his renal failure and hemoglobin drop?

A. Dehydration from the gastroenteritis
B. Intravascular hemolytic crisis triggered by a complement activating condition
C. Atypical hemolytic uremic syndrome
D. Minimal change disease
E. None of the above

Why was this patient so sick? What caused his renal failure and hemoglobinuria?

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

PNH: Pathophysiology

- Acquired clonal disorder of hematopoietic stem cells (HSCs)[1,2]
- PIG-A mutations-> decrease/loss of GPI-anchored proteins[1,2]
- PIG-A^{mut} HSC and progeny (PNH cells)
  - Lack surface complement inhibitors CD55 (DAF) and CD59 (MIRL)[1,2]
- PNH cells are lysed by complement in circulation
  - Causing chronic intravascular hemolysis/hemoglobinuria, thrombocytopenia, leukopenia, and thrombosis[1,2]
- Bone marrow dysfunction leads to leukopenia and thrombocytopenia[1,2]

PIG-A = phosphatidylinositol glycan-complementation class A.
1. Parker C, et al. *Blood.* 2005;106:3699-3709. 2. Brodsky RA. Paroxysmal nocturnal hemoglobinuria. In: Hoffman R, et al., eds. *Hematology-Basic Principles and Practices*. 4th ed. Philadelphia, PA: Churchill Livingstone Elsevier;2005:419-427.

PNH is an acquired disorder of clonal hematopoietic stem cells. In patients like ours in the case, these GPI-anchored deficient stem cells can have a relative survival advantage in the context of an autoimmune-mediated attack on the bone marrow compartment, and after treatment, you can sometimes see expansion of such cells resulting in clinical PNH. PNH cells acquire mutations in a gene called PIG-A or phosphatidylinositol glycan A. These mutations result in a decreased presentation of protein on the cell surface as a result of loss of the GPI-anchored proteins. There are more than 100 GPI anchor proteins that are presented in this way. PIG-A mutant hematopoietic stem cells and their progeny lack surface complement inhibitors, including CD55, or decay-accelerating factor, and CD59, membrane inhibitor of reactive lysis. These complement deficient cells are lysed in circulation by complement. This lysis causes chronic intravascular hemolysis and hemoglobinuria, thrombocytopenia because all of the progeny of the mutant PNH cells lack complement inhibitory proteins, and leukopenia. This also increases the risk for thrombosis as a result of sequelae, which we'll describe in detail here. The bone marrow function results in leukopenia and thrombocytopenia, which can be seen in these patients.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

PNH: Pathophysiology

- Acquired clonal disorder of hematopoietic stem cells (HSCs)[1,2]
- PIG-A mutations-> decrease/loss of GPI-anchored proteins[1,2]
- PIG-A^{mut} HSC and progeny (PNH cells)
  - Lack surface complement inhibitors CD55 (DAF) and CD59 (MIRL)[1,2]

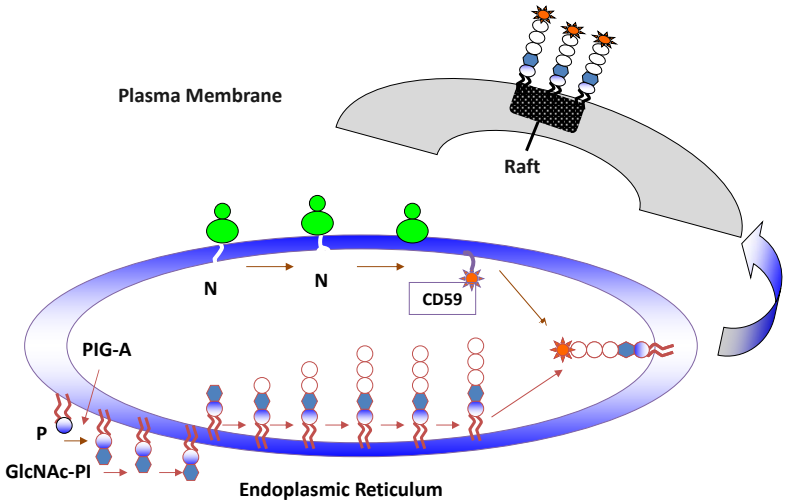
Knowledge Check:
Patients with PNH have substantial overlap in patients with acquired aplastic anemia

PIG-A = phosphatidylinositol glycan-complementation class A.
1. Parker C, et al. *Blood.* 2005;106:3699-3709. 2. Brodsky RA. Paroxysmal nocturnal hemoglobinuria. In: Hoffman R, et al., eds. *Hematology-Basic Principles and Practices*. 4th ed. Philadelphia, PA: Churchill Livingstone Elsevier;2005:419-427.

Again, as mentioned, these patients have substantial overlap with acquired aplastic anemia.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

GPI-AP Biosynthesis: Involves 10 Steps and >20 Genes

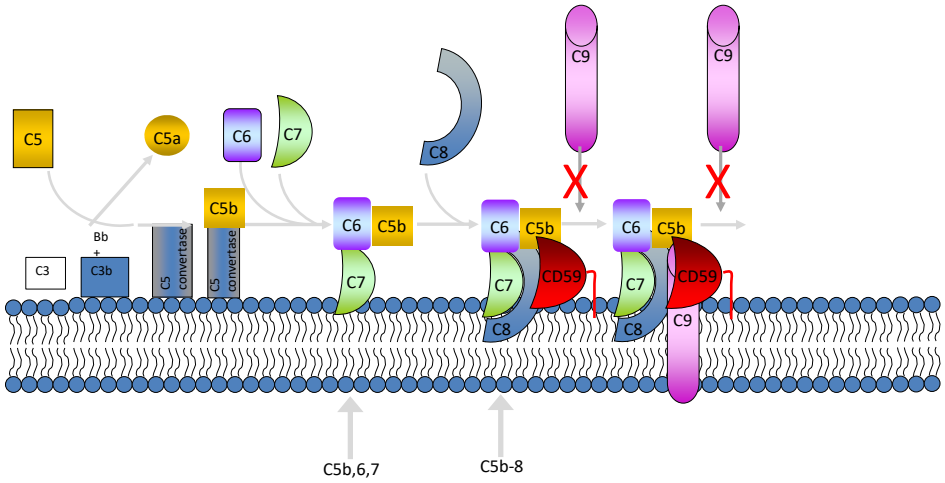


GlcNAc-PI = N-acetylglucosamine-phosphatidylinositol; GPI-AP = glycosylphosphatidylinositol anchored proteins; PI = phosphatidylinositol.
Brodsky RA. *Blood Rev.* 2008;22:65-74.

GPI-anchored biosynthesis is actually a very complicated process, which involves more than 10 steps and more than 20 genes. You can see here that phosphatidylinositol glycan A is initially, prepared in the Golgi, and then subsequently, loss of this anchor results in the loss of multiple types of different proteins, which are then no longer presented on the cell surface and can no longer inhibit complement.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Formation of the Terminal Complement Complex

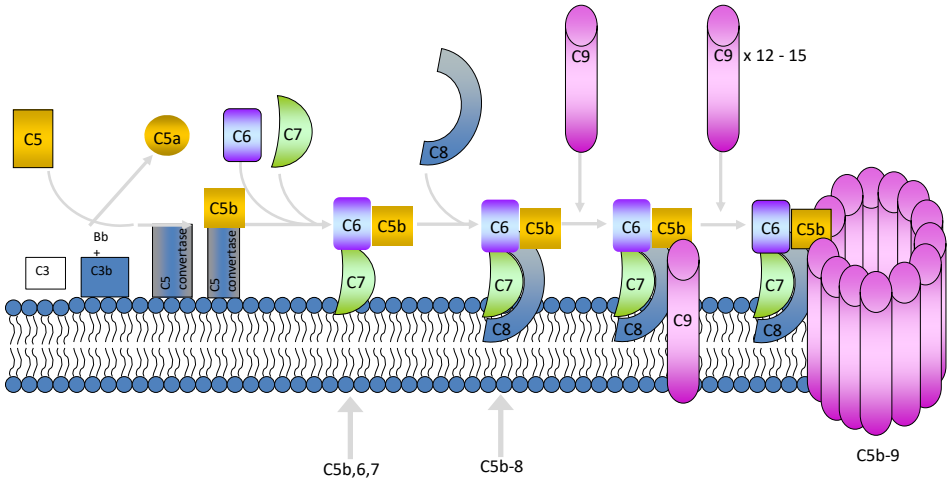


Adapted from: Abbas AK, et al. *Cellular and Molecular Immunology*. 6th ed. Philadelphia, PA: Saunders;2007:321-348.

Under normal circumstances, complement inhibitory proteins are present on the surface of all cells, and these complement inhibitory proteins prevent the complement system from attacking endogenous cells or parts of our own cells and instead allow complement to be targeted to bacterial invaders.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Formation of the Terminal Complement Complex



Adapted from: Abbas AK, et al. *Cellular and Molecular Immunology*. 6th ed. Philadelphia, PA: Saunders;2007:321-348.

You can see here that in the context of activated complement, the complement system results in formation of a membrane pore, which allows free flow of water intercellularly and causes cell lysis.

At this point, I will transition to Dr. Weitz who will talk to us about what is complement.

What is Complement?

- A system of >40 proteins in the blood and on cell surfaces[1]
- Provides immune surveillance to discriminate[2]:
  - Healthy host tissue
  - Cellular debris
  - Apoptotic cells
  - Foreign intruders
- Defense against microbial intruders
- Excessive activation -> immune, inflammatory, neurodegenerative, ischemic, and age-related diseases[2]

1. Merle NS, et al. *Front Immunol.* 2015;6:257. 2. Ricklin D, et al. *Nat Immunol.* 2010;11(9):785-797.

Dr. Weitz: Complement is a system of over 40 proteins in the blood and on cell surfaces that is part of our innate immune system. It helps us discriminate between abnormal tissues, cellular debris, apoptotic cells, or foreign intruders, such as viruses or bacteria. It's a really critical part of our defense against micro-bacterial intruders. Excess activation, whether it's due to an immune process or an infection, can lead to additional immune complications. Inflammation can contribute to neurodegeneration, ischemia, and other age-related diseases.

Complement and Disease

- Imbalance between activation and inhibition causes tissue damage[1,2]
- Congenital deficiencies can increase infection susceptibility[3]

C5 inhibition can affect meningococcal and Neisseria bacteria

C3 deficiencies can affect anything that's cleared through the spleen, including Haemophilus influenza, strep pneumonia, staph, etc.

- Altered complement function results in disease:
  - PNH, autoimmune diseases (APLS/SLE), TMAs (aHUS, TTP), transplant rejection, ischemic, neurodegenerative, age-associated diseases, scleroderma, and cancer[4-6]

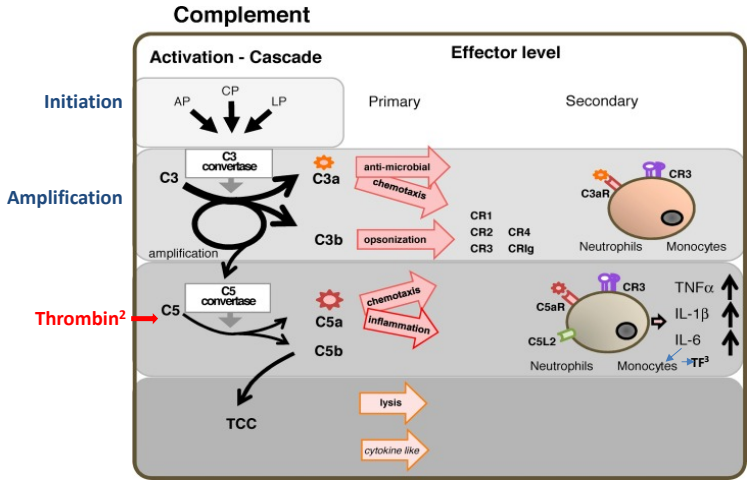
1. Murphy K. In: Murphy K, ed. *Janeway's Immunobiology*. 8th ed. New York, NY: Garland Science, Taylor & Francis Group LLC; 2012. 2. Bendapudi PK, et al. https://www.medrxiv.org/content/10.1101/2022.02.24.22271459v1.article-info. 3. Figueroa JE, et al. *Clin Microbiol Rev*. 1991;4(3):359-395. 4. Ricklin D, et al. *Nat Immunol.* 2010;11(9):785-797. 5. Merle NS, et al. *Front Immunol.* 2015;6:262. 6. Tüzün E, et al. *J Auto-immune*. 2011;37(2):136-143.

The imbalance between activation and inhibition can lead to accelerated terminal complement, which can then cause additional tissue damage. We know that complement deficiencies can increase the susceptibility to infection. That's particularly true for C5 inhibition, which can affect meningococcal and neisseria bacteria. C3 deficiencies can affect anything that's cleared through the spleen, through the splenic macrophage, including Haemophilus influenza, strep pneumonia, staph, etc. Alterations in complement function can then result in diseases such as PNH, autoimmune diseases such as APLS, systemic lupus, thrombotic microangiopathies, transplant rejection, ischemia, neurodegenerative disorders, and age-related diseases, scleroderma, and cancer.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Human Complement Activation and Amplification[1]

- Complement can be activated through three pathways: classical, lectin, and alternative

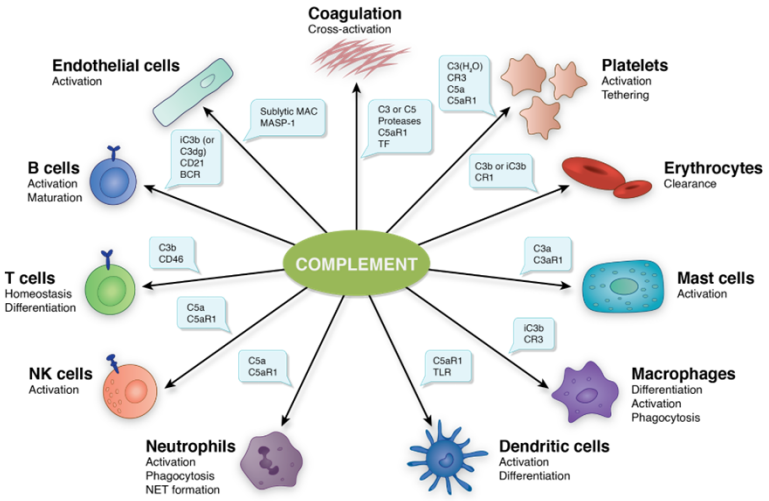


1. Zipfel PF, et al. *Mol Immunol.* 2013;56(3):152-160. 2. Krisinger MJ, et al. *Blood.* 2012;120(8):1717-1725. 3. Hill A, et al. *Blood.* 2013;121:4985-4996.

Looking at the complement system, you can see that it's activated by three pathways: the classical pathway, antigen-antibody; the lectin pathway through galactomannose; and the alternative pathway that acts as a bit of a priming system to allow the system to always be ready for upregulation if there's a need. They all converge on the generation of C3 convertase, which then converts C3, complement protein 3, to C3B and C3A. C3B is very important because there are receptors for C3B. C3A is an inflammatory protein and also contributes to the activation of other cells. Two C3Bs and a BB fraction also act as the amplification arm of complement. Once you activate C3 to C3B, you generate bundles of C3B, which then serve as an enzyme that can activate C5. C5 is then cleaved to C5A and C5B. C5B fixes the terminal complement proteins that accomplishes the next function of complement, which is to punch a hole in the membrane. C5A is a very potent inflammatory protein. It's also involved in chemotaxis. It activates their receptors on platelets, monocytes, and granulocytes, leading to activation of all these cells, contributing to thrombogenicity. It also increases the inflammatory response by increasing TNF-alpha, IL-1 beta, and IL-6, which then can activate monocytes to generate more tissue factor. The terminal proteins then are activated and cause cell lysis.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Roles of Complement

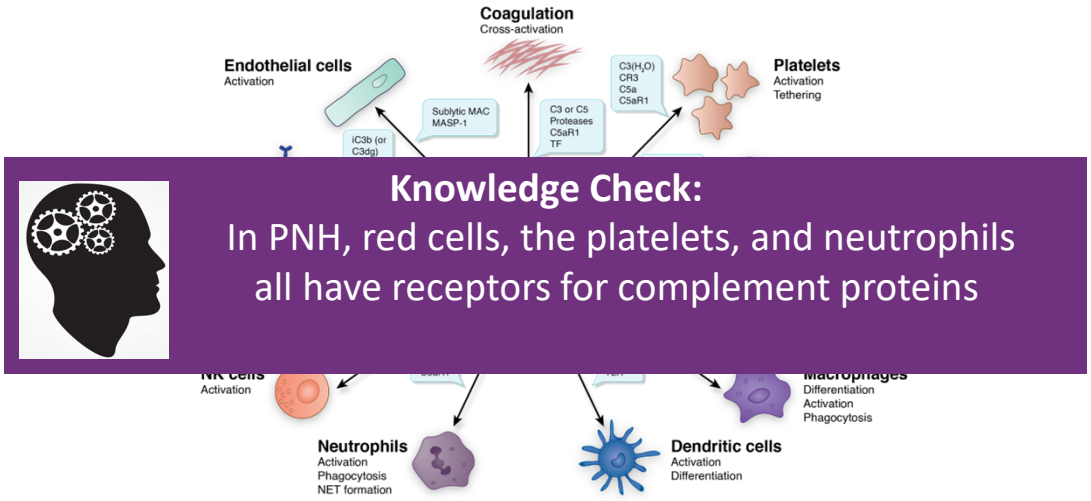


1. Ehrnthaller C, et al. *Mol Med.* 2011;17(3-4):317-329. 2. Murphy K. In: Murphy K, ed. *Janeway's Immunobiology*. 8th ed. New York, NY: Garland Science, Taylor & Francis Group LLC; 2012.

As you can see, complement affects multiple cells, including endothelial cells, B and T cells, and K cells.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Roles of Complement



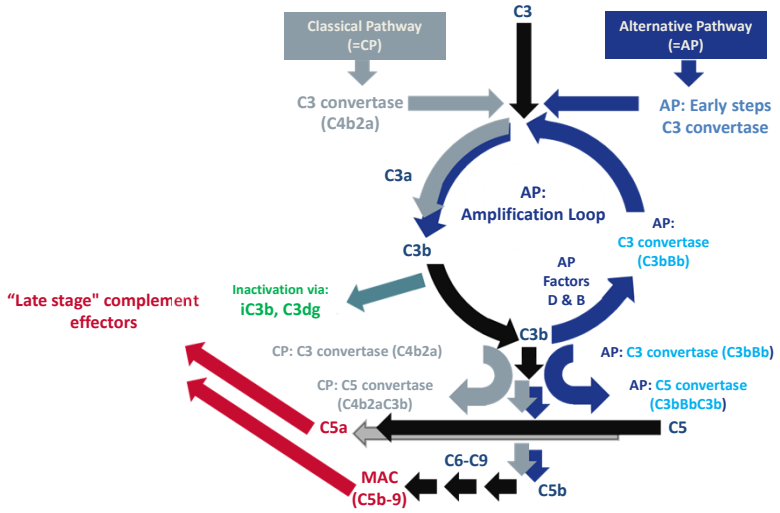
Knowledge Check:
In PNH, red cells, the platelets, and neutrophils all have receptors for complement proteins

1. Ehrnthaller C, et al. *Mol Med.* 2011;17(3-4):317-329. 2. Murphy K. In: Murphy K, ed. *Janeway's Immunobiology*. 8th ed. New York, NY: Garland Science, Taylor & Francis Group LLC; 2012.

But most important for PNH are the red cells, the platelets, and the neutrophils, which all have receptors for complement proteins.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Regulation of the Complement System[1,2]

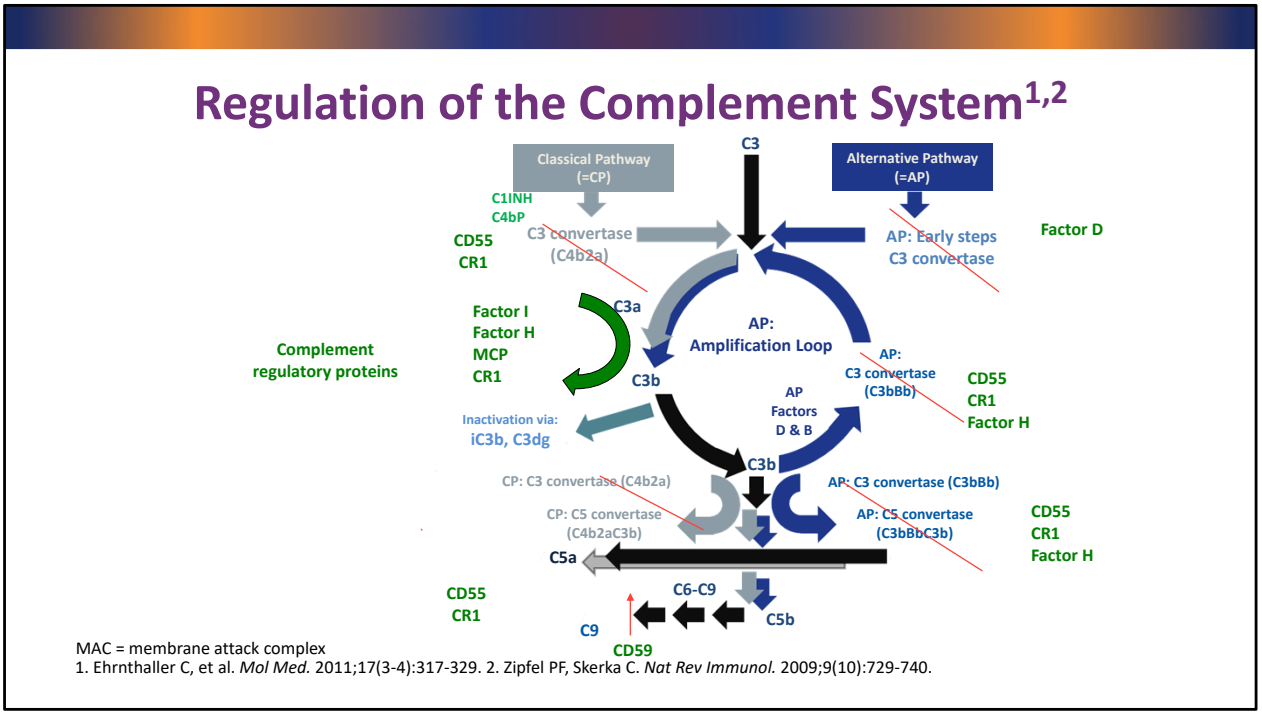


MAC = membrane attack complex
1. Ehrnthaller C, et al. *Mol Med.* 2011;17(3-4):317-329. 2. Zipfel PF, Skerka C. *Nat Rev Immunol.* 2009;9(10):729-740.

So how can we modulate the system? We can modulate the system by having a series of inhibitors that act as a lid so you don't generate too much complement and damage your own cells.

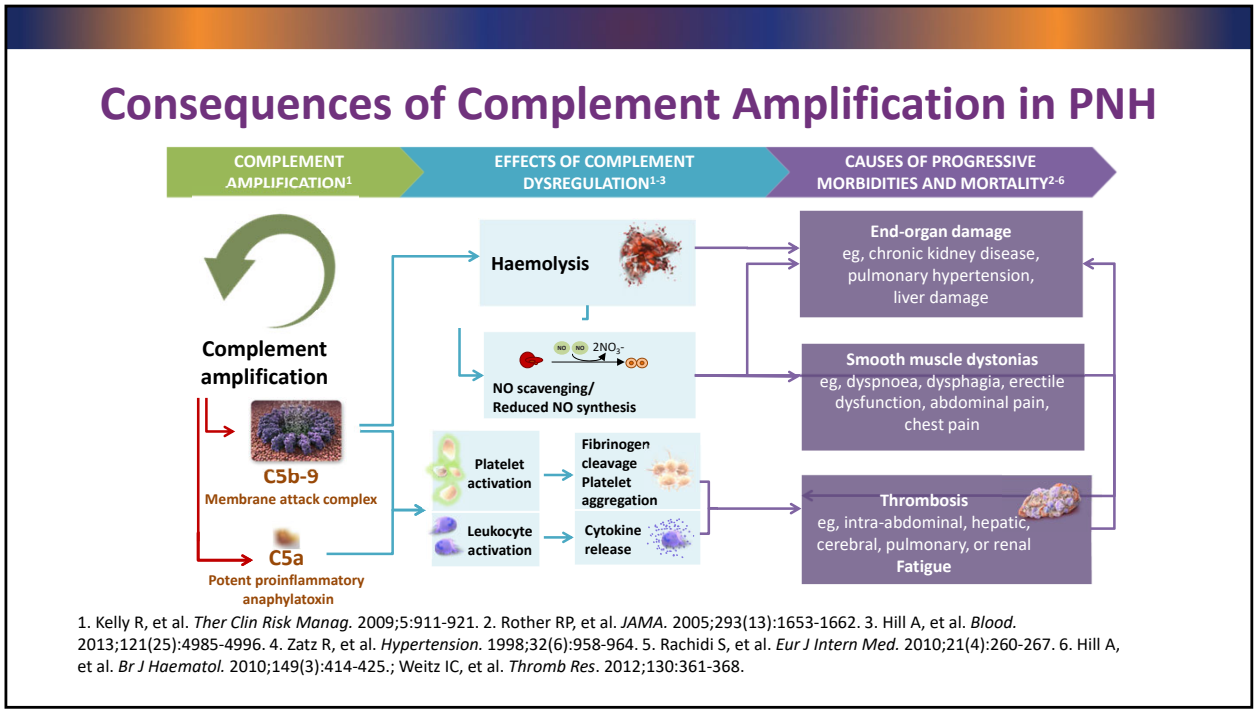
How can we modulate this system? We can modulate the system by having a series of inhibitors that act as a lid so you don't generate too much complement and damage your own cells.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria



Those include the membrane-bound inhibitors that we've talked about: CD55 as well as CD59. CD59 blocking the terminal complement complex insertion into the membrane, and CD55 affecting the generation of C3B in activating C3B so that you can't then proceed on to C5 cleavage. There are circulating inhibitors, which are important in AHUS, for example, factor I, its cofactor, factor H, and the membrane cofactor, as well as complement receptor 1 that modulates the amount of C3B on the cell.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria



1. Kelly R, et al. *Ther Clin Risk Manag.* 2009;5:911-921. 2. Rother RP, et al. *JAMA.* 2005;293(13):1653-1662. 3. Hill A, et al. *Blood.* 2013;121(25):4985-4996. 4. Zatz R, et al. *Hypertension.* 1998;32(6):958-964. 5. Rachidi S, et al. *Eur J Intern Med.* 2010;21(4):260-267. 6. Hill A, et al. *Br J Haematol.* 2010;149(3):414-425.; Weitz IC, et al. *Thromb Res*. 2012;130:361-368.

Dr. Griffiths: In PNH, the consequence of complement activation can have disparate effects on multiple organ systems. Inappropriate complement activation in circulation results in amplification of complement and generation of the membrane attack complex, which actually causes direct lysis of red cells intravascularly. This releases free hemoglobin into circulation, which scavenges nitric oxide and produces vascular hyperactivity. It also causes release of free hemoglobin, which is then filtered directly at the kidney and can cause renal hemosiderosis. The scavenged nitric oxide can cause pulmonary hypertension, and the iron release can cause liver damage. Additionally, one can see clinical evidence of nitric oxide scavenging as a result of demonstration of smooth muscle dystonias. These can present as symptoms of dyspnea, dysphasia, and a majority of men with PNH will demonstrate erectile dysfunction.

Patients can also present with idiosyncratic abdominal pain and chest pain. Activation of the membrane attack complex at the surface of platelets and the potent inflammatory effects of C5A can directly activate platelets and cause platelet membrane blebbing as well as leukocyte activation, which can directly activate the final common pathways of coagulation. In the concert of increased cytokine release from neutrophils and activated leukocytes, one can see a very marked increased risk for thrombosis, and indeed, thrombotic events are a common feature of this disease process. A majority of patients with PNH will also complain of fatigue, and this is for myriad factors, but also due to the free release of hemoglobin and circulation and the scavenged nitric oxide.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Effects of Complement C5 Blockade in PNH

- Reduction of hemolysis, decreased anemia, RBC transfusions[1,2]
- Improved platelet counts in some[2]
- Decreased markers of thrombosis[3,4]
- Decreased markers of thrombosis[3,4]
- Improved pregnancy outcomes[5]
- No worsening of side effects over time[6]

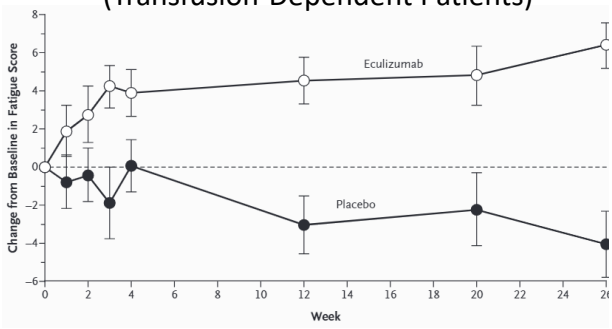
1. Hillmen P, et al. *Blood*. 2007;110(12):4123-4128. 2. Brodsky RA, et al. *Blood*. 2008;111(4):1840-1847. 3. Hillmen P, et al. *Br J Haematol*. 2013;162(1):62-73. 4. Weitz IC, et al. *Thromb Research*. 2012;130:361-368. 5. Kelly R, et al. *N Engl J Med*. 2015;373(11):1032-1039. 6. Socié G, et al. *Br J Haematol.* 2019;185(2):297-310.

Treatment of PNH has largely focused on inhibition of C5, the late activation of complement, as described so elegantly by Dr. Weitz. C5 inhibition in PNH has been demonstrated to reduce hemolysis, decrease anemia, and decrease red cell transfusion dependence. We can see improvement of platelets in some patients because, indeed, the platelets are no longer being targeted by terminal complement activation. One can see decreased markers of thrombosis, improved pregnancy outcome, and indeed no worsening of side effects over time.

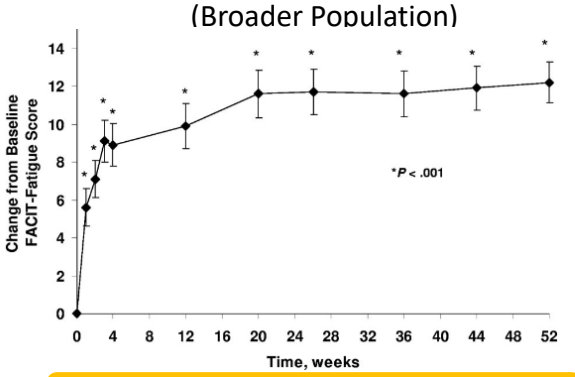
Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Eculizumab: Improvements in Fatigue with C5 Inhibition

TRIUMPH Study[a]
(Transfusion-Dependent Patients)



SHEPHERD Study[b]
(Broader Population)



Patients reported significant improvements in fatigue regardless of baseline hemolysis

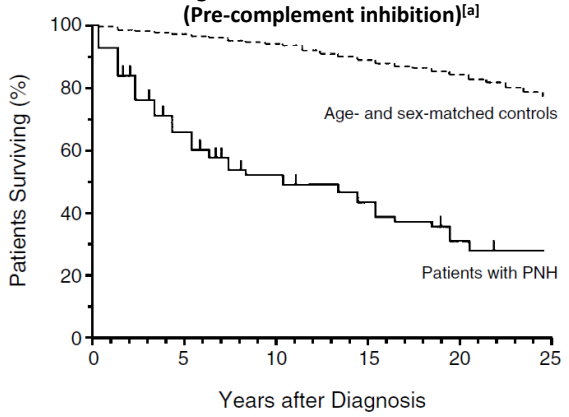
a. Hillmen P, et al. *N Engl J Med.* 2006;355:1233-1243;
b. Brodsky RA, et al. *Blood.* 2008;111:1840-1847.

The canonical C5 inhibitor is eculizumab. This drug was initially developed and tested in the TRIUMPH and SHEPHERD studies. You can see here treatment with eculizumab resulted in improvements in fatigue and improvement in hemoglobin.

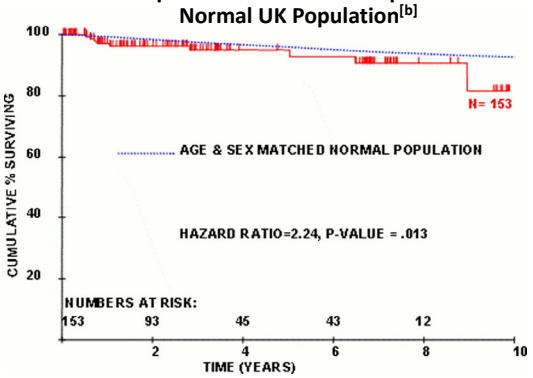
Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Eculizumab: Long-Term Clinical Benefits of C5 Inhibition for PNH

Actuarial Survival From the Time of Diagnosis in 80 Patients With PNH (Pre-complement inhibition)[a]



Survival of Patients With PNH Treated With Complement Inhibition Compared With the Normal UK Population[b]



a. Hillmen P, et al. *N Engl J Med.* 1995;333:1253-1258. b. Hill A, et al. *Blood.* 2012;120:Abstract 3472.

Long-term treatment with eculizumab has resulted, as you can see here, in a substantial change in life expectancy for patients with PNH. On the left side, you can see actuarial survival from the time of PNH diagnosis in 80 patients in the pre-eculizumab era. You can see here that such patients often had substantially decreased overall survival. On the right, you can see a later follow-up of a separate cohort of patients compared with age and sex-matched healthy controls. What you can see here is, in the post-eculizumab era, relative normalization of survival within this population, suggesting the profound importance of C5 inhibition or of complement inhibition in patients with PNH.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Breakthrough Hemolysis with Eculizumab

- Types of breakthrough
  - Intrinsic resistance (RARE C5 variants, <1%)
  - Insufficient dosing, higher clearance: clue D8 LDH nl, increases by D15
  - Increased chronic complement activation: clue high CH50 trough ~D15
  - Complement trigger events (infection, surgery, pregnancy, etc)
  - Extravascular hemolysis: see next slides
- Management strategies:
  - Increase dose frequency or extra doses (for trigger event)
  - Increase dose (1200 mg q 14 days)
  - Alternative therapy (for C5 variant)

Despite the efficacy of C5 inhibition with eculizumab, there were issues with this drug, particularly, issues of breakthrough hemolysis. These can result from a variety of different causes, including intrinsic resistance, which was described as a rare C5 variant occurring predominantly in an Asian population. We can also see insufficient dosing. Eculizumab is dosed with flat dosing, irrespective of weight and size. Some people had an intrinsically higher clearance of eculizumab, and some people were underdosed relative to size. This insufficient dosing could be identified in patients who had normal Day 8 LDH levels, but then LDH levels increased by Day 15, suggesting breakthrough hemolysis at the end of the dosing interval. One could also see increased chronic complement activation, and this can be identified through evidence of high CH50 troughs. CH50 should be completely blocked in patients on effective complement inhibitor therapy. Some patients have increased chronic complement due to chronic infections or occult complement-activating conditions. We can also see breakthrough hemolysis in the context of triggering events, things like surgery, infection, and pregnancy, which can increase the baseline production of complement. We can see extravascular hemolysis, which we'll touch on in a moment. Management strategies that have been heretofore effective for patients with evidence of breakthrough hemolysis include things like increasing the dosing frequency, which is described in the package insert for use of eculizumab, decreasing the interval to 10 or 12 days, or increasing the dose, which is somewhat more convenient, giving it every 14 days. The alternative is to use an alternative therapy for C5 variants or for those with other forms of breakthrough.

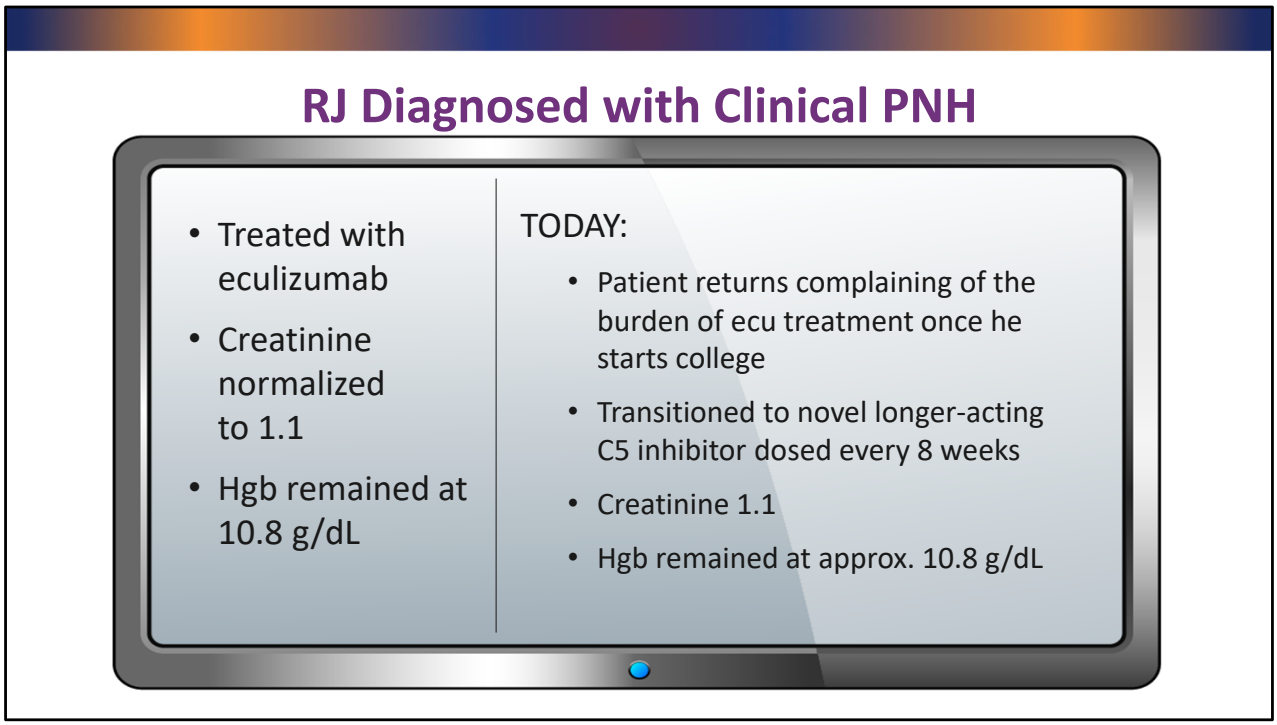
At this point, I'll transition over to Dr. Weitz.

New Complement Inhibitors for PNH

- Ravulizumab – C5 inhibitor with extended half-life (Q 8wk) FDA-approved
  - Weekly SC dosing trial underway
- C5 - Biosimilars (several in development)
- C3 inhibitor (APL-2/pegcetacoplan) approved
- Small molecule C5R blockade
- Factor D inhibitors
  - Danicopan oral phase 3 trial overlapping with C5 inhibitor
  - BCX9930 stand alone in trials
- Factor B inhibitor – iptacopan

Dr. Weitz: As you've heard, ravulizumab was approved for patients with PNH and does have an extended half-life, can be given every 7 to 8 weeks per the package insert, and does seem to be non-inferior to eculizumab. There are a host of biosimilars that are still in development, C5 biosimilars. Then there are several other agents that affect the alternative pathway, including the C3 inhibitor, pegcetacoplan, which was recently approved; small molecules, C5 receptor blockade; and then factor D and factor B inhibitors, which we'll talk about in a minute.

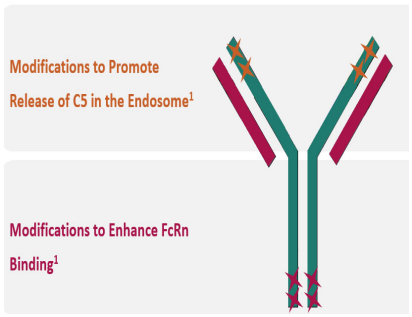
Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria



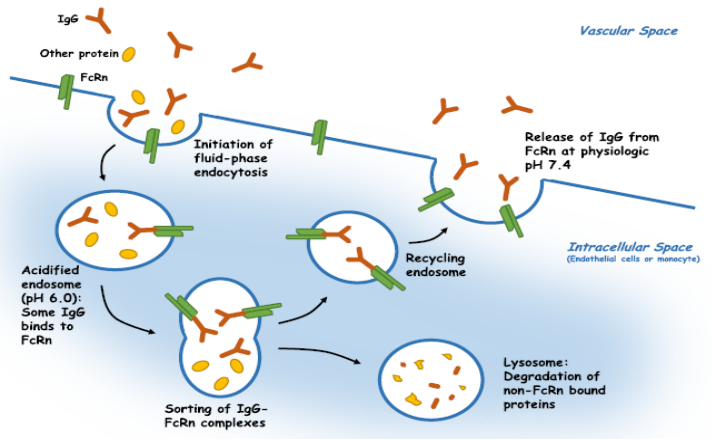
Dr. Griffiths: Let's go back to our case. Our patient was diagnosed with clinical PNH. He was vaccinated, and on eculizumab therapy his creatinine normalized to 1.1. His hemoglobin remained at about 10.8. He returns to see us complaining that eculizumab represents a substantial burden on him because he has to come every 2 weeks, and he's planning to start college. At this point, the decision is made to transition him to a longer-acting C5 inhibitor, which is now dosed every 8 weeks. He becomes very happy on this, and his creatinine remains normal, and his hemoglobin remains in the 10 to 11-gram range.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Ravulizumab



FcRn Salvage Pathway[1]



1. Sheridan D, et al. *PLoS One.* 2018;13(4):e0195909. 2. Roth A, et al. *Blood Adv.* 2018;2:2176-2185.

Ravulizumab is, like eculizumab, a humanized mouse monoclonal antibody that targets C5. Ravulizumab is distinct from eculizumab in that the FCR region has been modified to result in increased recycling back to the cell surface, so it has less high affinity to the binding within the clathrin-coated pit and is so recycled to the cell's surface, allowing longer half-life for this drug. The business end of the molecule that binds C5 is unchanged from the parent drug eculizumab.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Ravulizumab Noninferior to Eculizumab

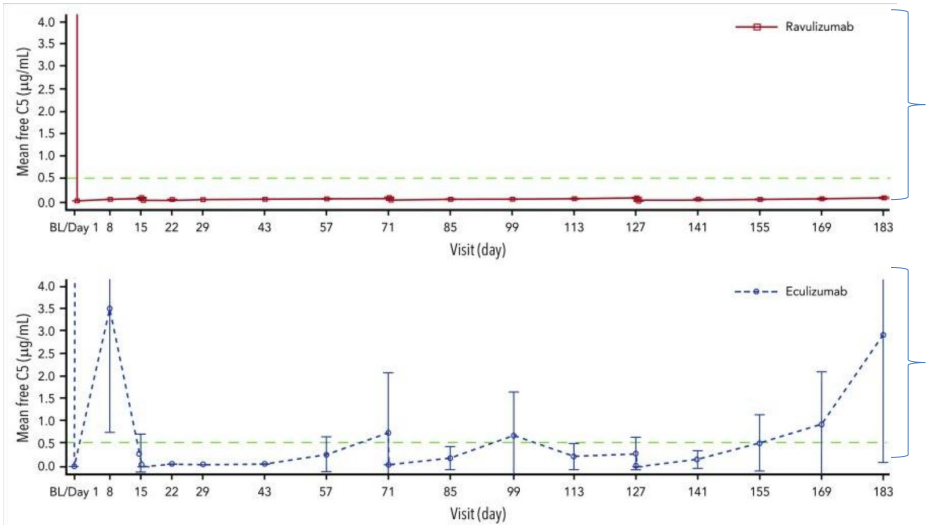
Day 183 endpoint	Ravulizumab (n = 125)	Eculizumab (n = 121)	Treatment effect (95% CI)
Transfusion avoidance rate, %	73.6	66.1	6.8 (-4.66, 18.14)
LDH normalization, %	53.6	49.4	1.19 (0.80, 1.77)
< Sq mean % change in LDH, %	-76.84	-76.02	-0.83 (-5.21, 3.56)
< Sq mean change in FACIT-fatigue score, %	7.07	6.40	0.67 (-1.21, 2.55)
Breakthrough hemolysis rate, %	4.0	10.7	-6.7 (-14.21, 0.18)
Hemoglobin stabilization rate, %	68.0	64.5	2.9 (-8.80, 14.64)

Lee JW, et al. *Blood.* 2019;133:530-539.

Clinical studies using ravulizumab demonstrated non-inferiority to eculizumab. You can see here that in terms of transfusion avoidance, LDH normalization, FACIT-fatigue score, and breakthrough hemolysis, ravulizumab is essentially identical to eculizumab and non-inferior in this study, thus many people have transitioned their patients from eculizumab, the parent drug dosed every 2 weeks, to ravulizumab, the second-generation drug, which is dosed every 8 weeks.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

C5 Suppression with Ravulizumab vs Eculizumab



Ravulizumab - Inhibition by end of first infusion, sustained

Eculizumab - More frequent breakthrough events

Lee JW, et al. *Blood*. 2019;133:530-539.

Studies have demonstrated superior suppression of C5 levels using ravulizumab compared to eculizumab, and you can see here in this longitudinal measurement study in which C5 levels were measured over time in patients on Rav versus Ecu, that those patients on eculizumab appeared to have more breakthrough events associated with increased levels of C5, presumably due to complement-activating events or dosing breakthrough.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

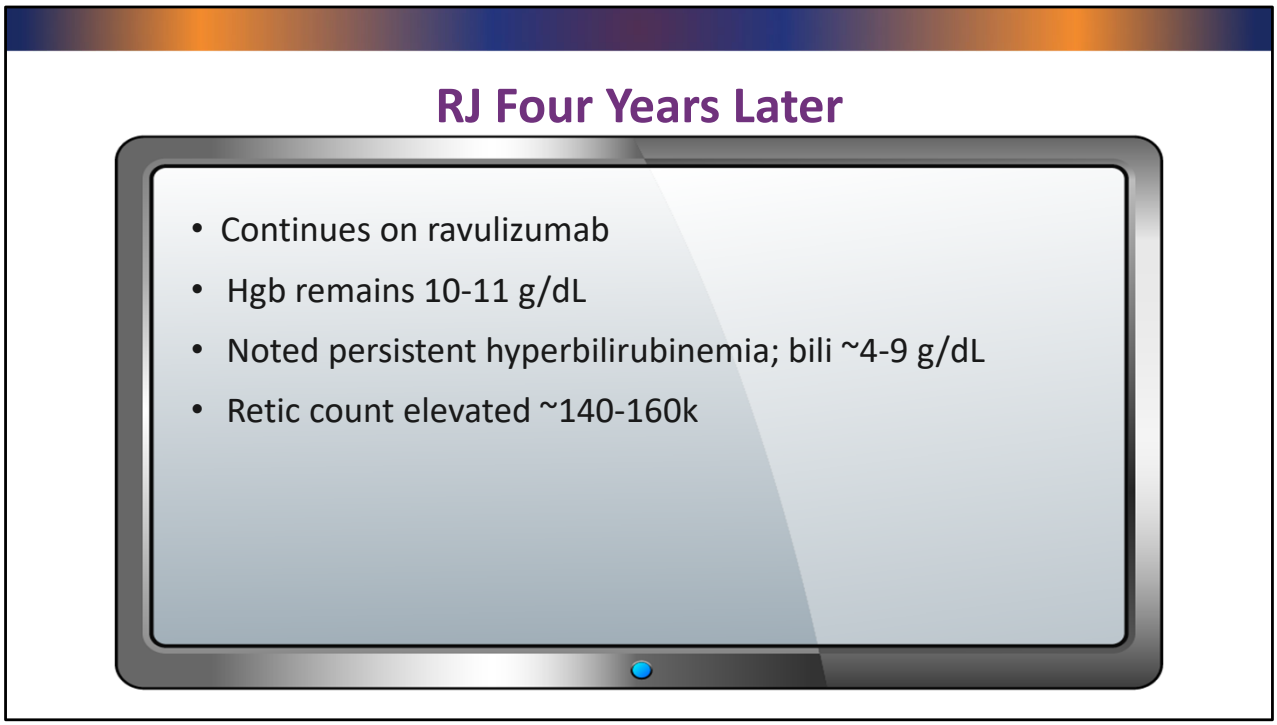
Breakthrough Hemolysis: Ravulizumab vs Eculizumab

Parameter	Study 301 (Ecu Naive Pts)[1]		Study 302 (Pts Stable on Ecu)[2]	
	Rav (n = 125)	Ecu (n = 121)	Rav (n = 97)	Ecu (n = 98)
Pts w/ breakthrough hemolysis, n (%)	5 (4.0)	13 (10.7)	0 (0.0)	5 (5.1)
Breakthrough hemolysis events, n	5	15	0	7
W/ free C5 ≥0.5 µg/mL	0	7	0	4
W/ infection (with no free C5 elevation)	4	4	0	2
Unrelated to elevated free C5 or infection	1	4	0	1

1. Lee JW, et al. *Blood.* 2019;133:530-539; 2. Kulasekararaj AG, et al. *Blood.* 2019;133:540-549.

Rates of breakthrough were higher clinically with Ecu versus ravulizumab here although rates of breakthrough are low with both drugs.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria



Here's our patient 4 years later. He remains on ravulizumab. His hemoglobin is between 10 and 11 grams, but he's noted to have persistent hyperbilirubinemia with bilirubins ranging between 4 and 9 and retic counts that remain elevated in the 140 to 160 range. The remainder of his counts are normal.

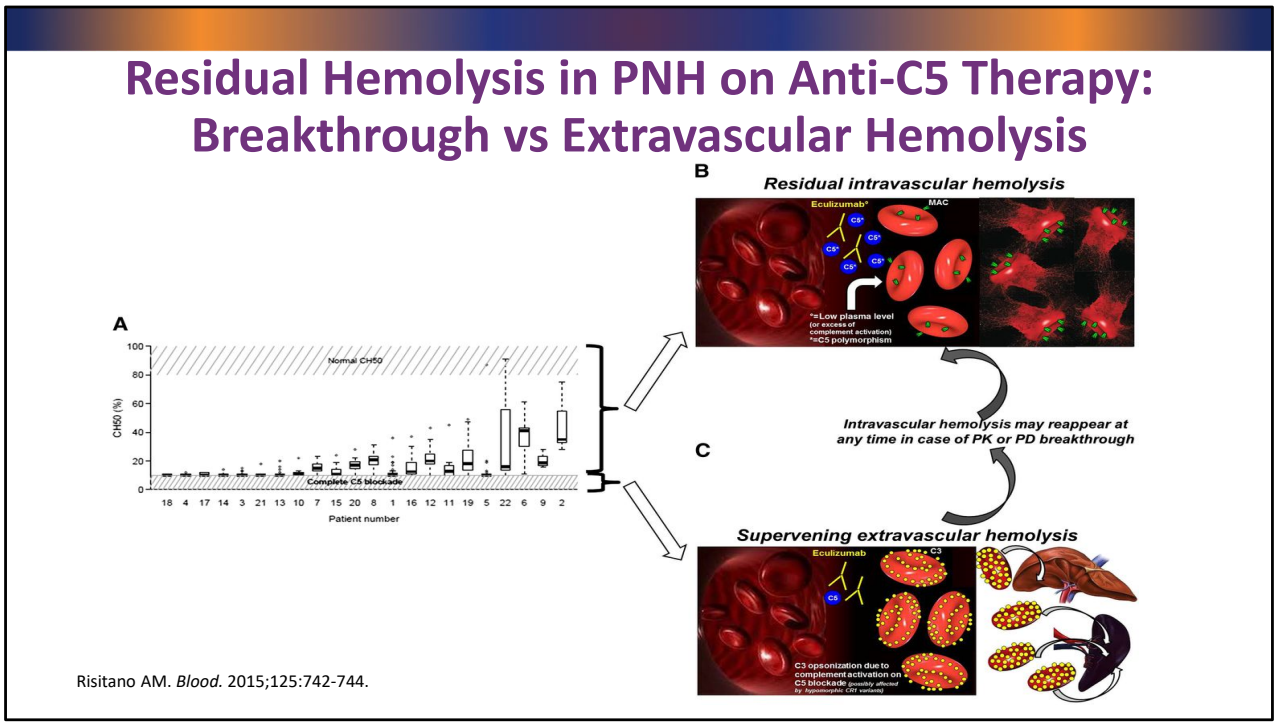
Polling Question #2

Why does the patient have ongoing hemolysis?

A. Extravascular destruction of C3 coated GPI deficient RBC
B. RBC membrane defect
C. B12 deficiency
D. Warm autoimmune hemolytic anemia
E. None of the above

Why does this patient have ongoing hemolysis, although clinically relatively insignificant?

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria



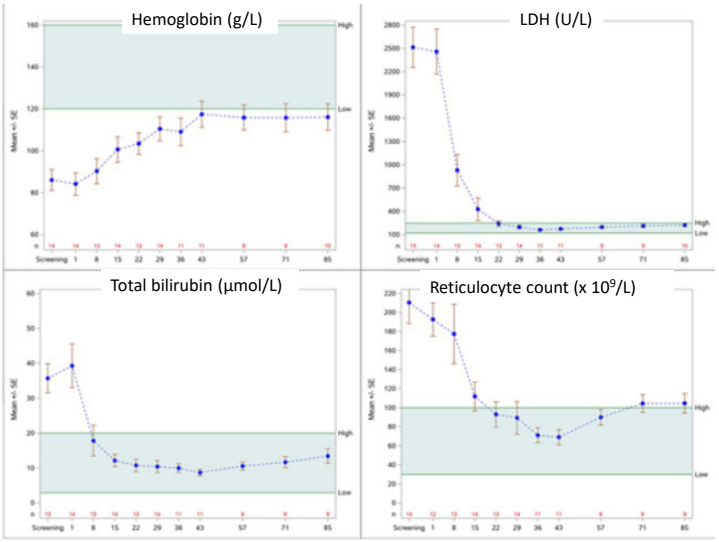
Residual hemolysis in PNH patients on anti-C5 therapy can be due to either breakthrough events or extravascular hemolysis due to deposition of activated C5 on the surface of red cells. Intravascular hemolysis events usually occur in the context of some sort of complement-activating condition. If the patient has gastroenteritis or an infection, or they get sick, or they have a surgical procedure, breakthrough homolysis events can happen. By contrast, like in our patient, R.J., one can have evidence of chronic ongoing extravascular hemolysis, and this is usually mediated by a C3 deposition on the surface of red cells, as delineated here in this slide.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Pegcetacoplan: Targeting C3, Phase 1/2 Trials

- **PADDOCK**: phase 1b dose-escalation study of APL-2 daily SC in complement inhibitor-naïve patients
- **PHAROAH:** phase 1b study is evaluating APL-2 add-on to eculizumab in severely anemic and transfusion-dependent patients
- **PRINCE:** naïve PNH patients
- **PEGASYS:** phase 3 - inadequate response to eculizumab
- **FDA approved** pegcetacoplan in April 2021

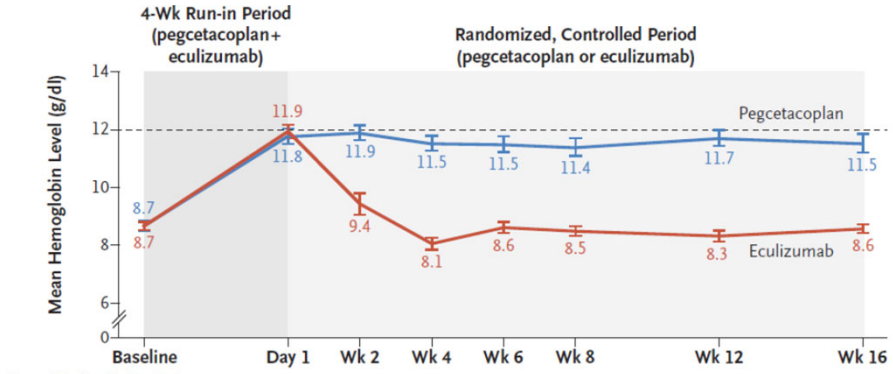
ClinicalTrials.gov. NCT02264639.;
Wong RS, et al. *Blood.* 2018;132(Suppl 1):2314.



Hemolysis mediated by C3 deposition can be overcome by targeting C3. Indeed, recently, we have had the approval of the drug pegcetacoplan. There were several studies, initially, the phase 1A and B studies, which demonstrated that if you take patients who are on chronic C5 inhibitor therapy and you switch them to C3 inhibitor therapy, you can see improvements in hemoglobin, decreases in bilirubin, decreases in LDH, and decreases in reticulocyte count. Based on these kinds of data, the FDA approved pegcetacoplan as a terminal complement inhibitor in April of 2021.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

PEGASUS (P3) Study Design: Open-Label to Week 48



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

	PEG to PEG			ECU to PEG		
	Base	Wk 16	Wk 48	Base	Wk 16	Wk 48
Hb g/dL NI (12-18)	8.7 (1.1)	11.5 (2.0)	11.3 (1.8)	8.7 (0.9)	8.6 (1.0)	11.6 (2.2)

Hillmen P, et al. *N Engl J Med*. 2021;384:1028-1037.; de Latour RP, et al. Presented at: EHA 2021 Virtual Congress. Abstract S174.

The PEGASUS phase 3 study allowed patients who were on eculizumab at the time to receive a run-in period where they received both drugs together, and all the patients who were receiving both drugs were noted to have an increase in their hemoglobin. Then patients were randomized to either remain on pegcetacoplan or to transition back to single-agent eculizumab. What you can see here is the improvement in hemoglobin associated with treatment with the C3 inhibitor compared with the C5 inhibitor, and this was sustained.

PEGASUS: Safety

- Breakthrough hemolysis occurred in 13 patients
  - Nine treated with ecu
  - Four treated with peg
- Three patients in peg group d/c'd due to TEAE of hemolysis
  - Included one serious adverse events
- Injection site reactions, mostly mild
  - None led to change in dose or study discontinuation
- Diarrhea, mostly mild (one moderate)
  - No dose changes due to diarrhea

Hillmen P, et al. *N Engl J Med*. 2021;384:1028-1037.

This PEGASUS study demonstrated the safety of terminal complement inhibition with C3 inhibitors. Breakthrough hemolysis events did occur in 13 patients, 9 on the Ecu arm and 4 on the pegcetacoplan arm. Three patients in the pegcetacoplan arm discontinued treatment due to treatment-emergent adverse events of hemolysis and one serious adverse event. Injection site reactions with pegcetacoplan, which is parenthetically dosed subcutaneously twice a week as a relatively large-volume injection, usually 20 mills, were the most common reaction. Most of these were relatively mild, and they did not lead to changes in dose or study discontinuation.
There was some diarrhea reported on this study, one episode of moderate diarrhea.

PEGASUS: Conclusions C3 Inhibition (pegC)

- PNH patients with persistent anemia on ecu, pegC **superior to ecu:**
  - Improved hg, decreased tx
- Adverse effects mainly injection site irritation and diarrhea
- In patients on ecu with persistent anemia, proximal complement inhibition with pegC improves **disease control vs ecu**
- No overt thrombosis but evaluation of effect on thrombotic risk is ongoing

Hillmen P, et al. *N Engl J Med*. 2021;384:1028-1037.

PNH patients with persistent anemia on eculizumab can be transitioned to pegcetacoplan, and pegcetacoplan was deemed superior to eculizumab in terms of improvements in hemoglobin and decreased transfusion requirements. Adverse events were predominantly related to the injection site reaction and diarrhea. Patients on eculizumab who have persistent anemia, particularly those who are symptomatic or have more proximal complement inhibition with pegcetacoplan, can improve disease control versus eculizumab. There have been no overt thrombotic events on this agent as yet. I will parenthetically note that there are some pretty robust breakthrough hemolytic events that I have seen in my patients in the context of complement-activating conditions. In my experience, this has been associated with patients who have occult inflammatory conditions. In one case, a patient with substantial chronic urinary infection in the context of a BK virus infection that was unrecognized.

Dr. Weitz will now talk about additional complement inhibitors.

Additional Complement Inhibitors

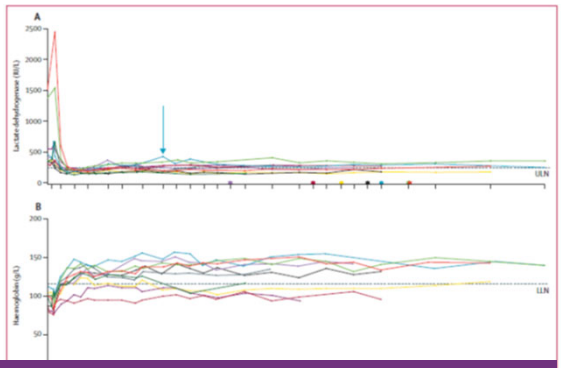
- Iptacopan
  - Oral factor B inhibitor (in phase 3 clinical trial)
- Danicopan
  - Oral factor D inhibitor (in phase 3 clinical trial)
- BCX9930
  - Oral factor D inhibitor
- Crovalimab (R7112689)
  - Anti C5 monoclonal antibody
- Pozelimab
  - Anti C5 monoclonal antibody
- Zilucoplan (RA101495)
  - Peptide binds and blocks C5- devel program suspended for PNH, approval likely for MG

Dr. Weitz: Pegcetacoplan was the first new or novel drug available for the treatment of PNH, but there are a host of other agents that are in clinical trials. These include other alternative pathway inhibitors, including an oral factor B inhibitor, iptacopan, danicopan, which is an oral factor D inhibitor, and BCX9930, another oral factor D inhibitor. There's also another monoclonal-to-C5, crovalimab, pozelimab, which is an additional C5 monoclonal antibody, and zilucoplan, which is a suppressing mRNA.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Iptacopan (LNP023): Oral Factor B Inhibitor

- Multicenter, open-label, sequential, 2-cohort trial
  - Patients (N = 10) aged 24 to 78 years with PNH with active hemolysis despite eculizumab treatment
  - Treatment: iptacopan twice daily with concomitant eculizumab
  - Meaningful improvements in LDH and hemoglobin; normalization in 8/10 cases



Please Note:
Phase 3 trials are in the process of being completed and analyzed

The data from iptacopan, the phase 3 trials, are in the process of being completed and analyzed.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Iptacopan (LNP023): Oral Factor B Inhibitor

- Multicenter, open-label, sequential, 2-cohort trial
  - Patients (N = 10) aged 24 to 78 years with PNH with active hemolysis despite eculizumab treatment
  - Treatment: iptacopan twice daily with concomitant eculizumab
  - Meaningful improvements in LDH and hemoglobin; normalization in 8/10 cases
  - Seven patients discontinued eculizumab and continued iptacopan as monotherapy
  - No treatment discontinuations or treatment-related serious AEs

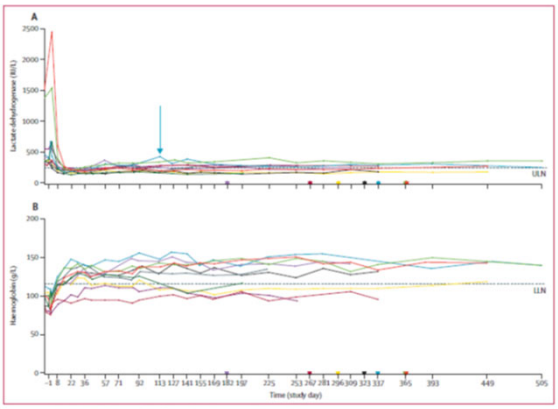


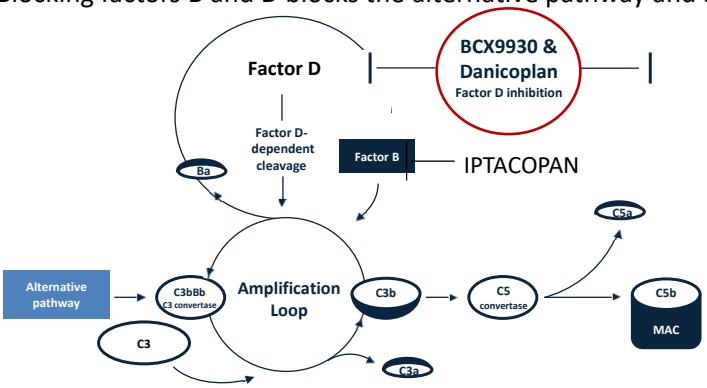
Figure 1: Overlaying individual time profiles of lactate dehydrogenase and haemoglobin
Coloured lines represent individuals. Asterisks show the day of last infusion of eculizumab for patients who switched from combination treatment to monotherapy. Coloured crosses show the last dose of eculizumab. (A) Overlaying individual time profiles of lactate dehydrogenase. Blue arrow highlights one participant who had a bleeding event due to low platelets and warfarin therapy and showed a reactive reticulocytosis. (B) Overlaying individual time profiles of haemoglobin concentration; the two patients who did not reach haemoglobin normalisation had a reticulocyte count that was too low as compared with the degree of anaemia (or with concentration of haemoglobin), suggesting subclinical underlying bone marrow failure. LLN=lower limit of normal. ULN=upper limit of normal.

This is from previous studies. You can see that oral factor B inhibitor is very effective at reducing the LDH and improving the hemoglobin. The iptacopan is dosed twice daily. Initially, it was overlapped, and there is some current evidence that the eculizumab can be discontinued and the iptacopan can be given as monotherapy.
There were no treatment discontinuations or serious adverse events noted.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Alternative Pathway Inhibitors

- Factors B and D is essential to initiate the alternative pathway
- Blocking factors B and D blocks the alternative pathway and all downstream products



Spectrum of Alternative Pathway Dysregulation Diseases

HEMATOLOGY

Paroxysmal nocturnal hemoglobinuria

Atypical hemolytic uremic syndrome

RHEUMATOLOGY

Antineutrophil cytoplasmic antibody-associated vasculitis

Lupus nephritis

IgAN vasculitis

NEPHROLOGY

Complement component 3 glomerulonephapthy

Primary membranous nephropathy

IgAN

IgA = immunoglobulin A; IgAN = immunoglobulin A nephropathy.
Weitz I, et al. *Front Immunol.* 2021. doi: 10.3389/fimmu.2021.712572 [Epub ahead of print].

Other alternative pathway inhibitors include factor D inhibitors, including danicopan, as I mentioned, and BCX9930.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

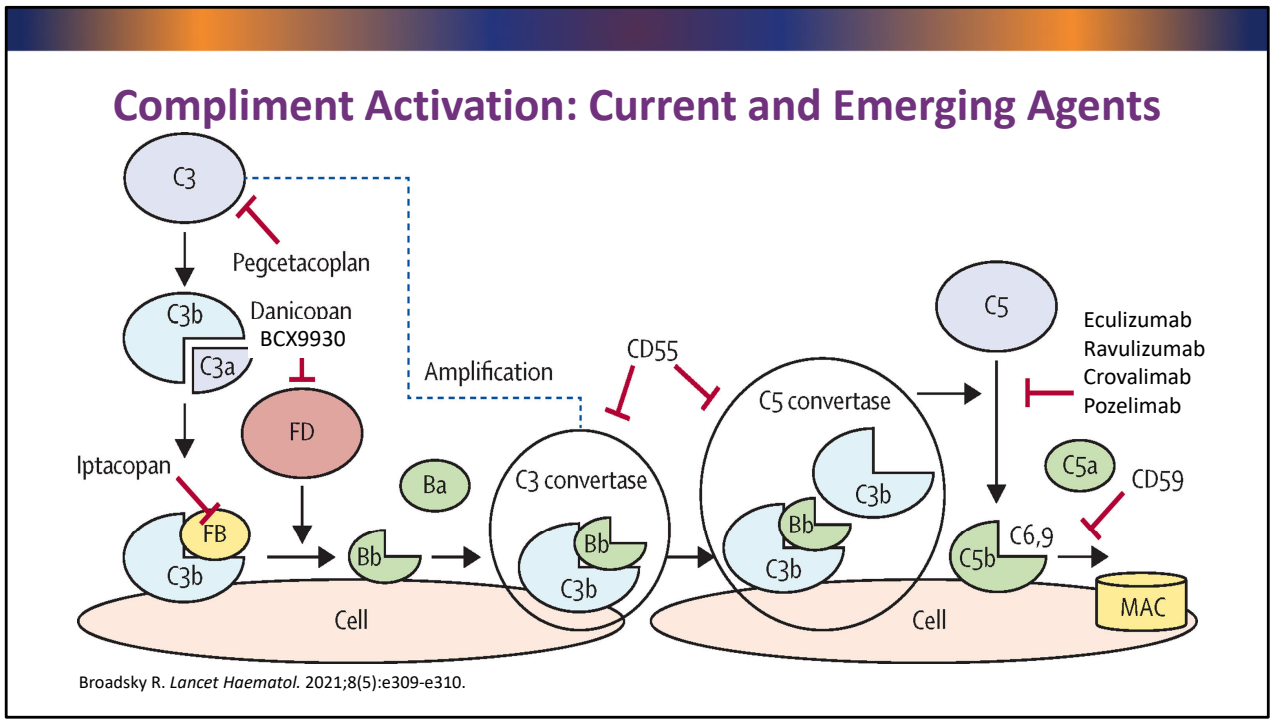
Phase 2 Study of Eculizumab vs Eculizumab + Factor Di

	Eculizumab Monotherapy	Danicopan + Eculizumab (interim results*)	
Hemolysis Marker	**Day 1 (n=11)**	**12 Weeks (n=8)**	**24 Weeks (n=4)**
Hemoglobin (g/dL)	7.9**	9.2	10.8
Reticulocytes (xULN)	2.3	1.7	0.9
PNH RBC Clone Size (%)	54	78	76
Indirect Bilirubin (xULN)	1.6	1.1	0.8
Direct Bilirubin (xULN)	1.6	1.1	0.7
LDH (xULN)	1.1	1.1	1.0
QoL Measure	**Day 1 (n=11)**	**12 Weeks (n=8)**	**24 Weeks (n=4)**
FACIT-FATIGUE† (Max Score 52)	34	42	42

The New Era in Aplastic Anemia & PNH Meeting. 2019. Napoli, Italy.

In the phase 2 study of eculizumab versus eculizumab plus danicopan, you can see that in the danicopan arm, there was a substantial increase in the hemoglobin at 12 weeks, which continued to improve at 24 weeks. The fatigue scores also improved. The LDHs remained stable. The bilirubins also improved, indirect bilirubin improved, and there was no change in the PNH clone size. Reticulocyte counts also decreased as the hemoglobin increased.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria



There are additional alternative pathway inhibitors, including BCX9930, which has been used as monotherapy in its phase 2 trials. It is entering phase 3 trials now, although there is no data at this point. All of these represent opportunities for the patients. BCX9930 and danicopan are oral factor D inhibitors. Iptacopan, the oral factor B inhibitor, all appear to be effective in decreasing intravascular hemolysis in these patients. Their effect on other aspects of PNH remains to be determined, including thrombosis and markers of hemostatic activation.

Developments in the Treatment of Paroxysmal Nocturnal Hemoglobinuria

Summary

- Complement inhibitors of C5 and C3 are available in clinical practice
- Long-term proximal (C5 inhibition) improves survival, limits end organ damage in PNH
- Patients with breakthrough extravascular hemolysis can benefit from a switch to C3 inhibitor therapy with improved hemoglobin and decreased transfusion dependence
- Novel inhibitors of complement are in development including inhibitors of complement factors B and D for PNH and other diseases

Complement inhibitors of C5 and C3 are available in clinical practice now. Long-term proximal C5 inhibition improves survival and limits organ damage. Patients with breakthrough hemolysis can benefit from a switch to C3 inhibitor therapy with improved hemoglobin and decreased transfusion dependence. The novel inhibitors of complement through the alternative pathway are in development. Those include inhibitors of factor B, factor D, which appear to have efficacy in PNH.