

## PNH: Current Thinking on the Disease, Diagnosis and Treatment

Jaroslaw Maciejewski, MD, PhD  
Cleveland Clinic

Carlos M. de Castro, MD  
Duke University Medical Center

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### What is PNH?

- **Paroxysmal** – sudden onset
- **Nocturnal** – occurring at night (or early in morning upon awakening)
- **Hemoglobinuria**



Despite the name, most patients do not present this way.

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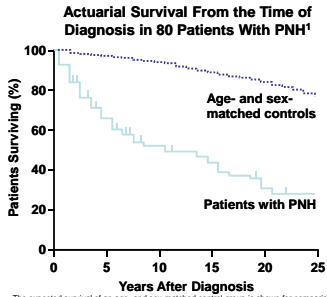
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### PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

- Estimated 4,000 – 6,000 patients in U.S.<sup>1</sup>
- 5 year mortality: 35%<sup>2</sup>
- Diagnosed at all Ages – Median age early 30's<sup>3,4</sup>
- Quality of life diminished<sup>5</sup>
- Progressive disease<sup>5</sup>

**Actuarial Survival From the Time of Diagnosis in 80 Patients With PNH<sup>1</sup>**



The expected survival of an age- and sex-matched control group is shown for comparison (Hilmen et al 1995). In a patient population where 1% the patients have <30% clone, 1 in 7 patients died by 5 years (de Latour et al. Blood. 2008; 112: 3099-3106)

1. Hill A et al. Blood 2006;108(11): 290a. Abstract 985 2. Hillmen et al. N Engl J Med. 1995;333:1253-1258.  
3. Hultmann II et al. Medicine 2004;83:150-507. 4. Scott R et al. J Intern Med 1996;240:573-77. 5. Hill A et al. Br J Haematol. 2007;137:181-87

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## CAUSES OF PNH

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**WHAT CAUSES PNH?**

- The mutation in the PIG-A gene in a hematopoietic stem cell leads to a defect in the production of an anchor protein that ties other proteins to the cell surface.

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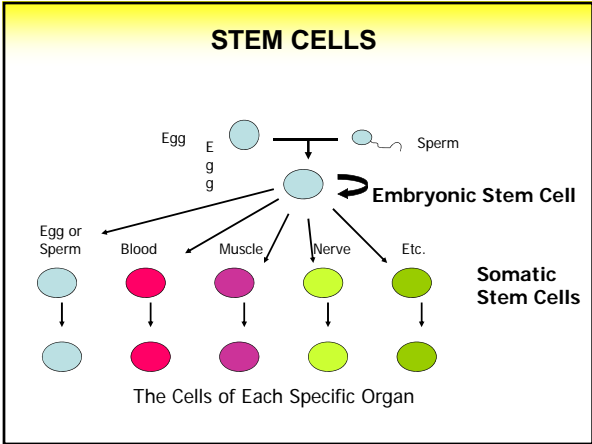
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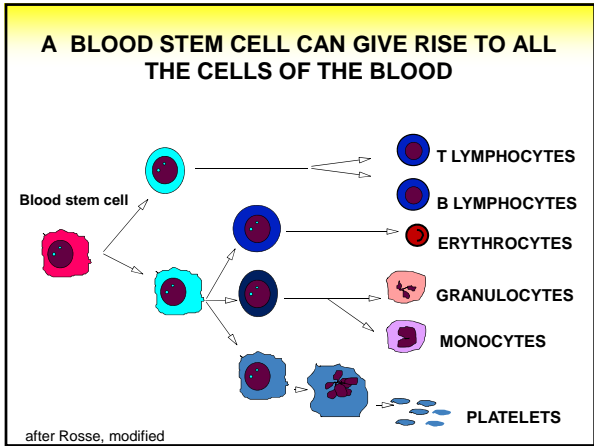
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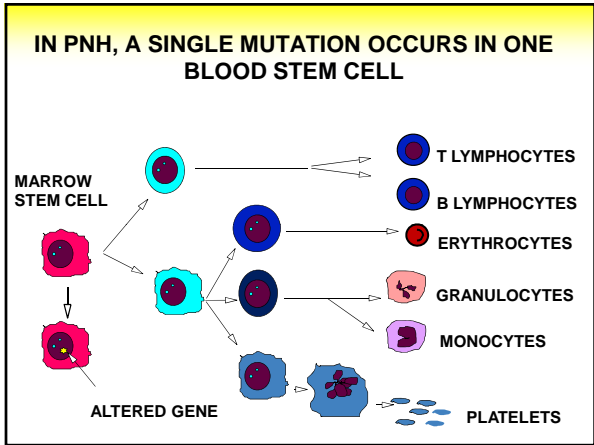
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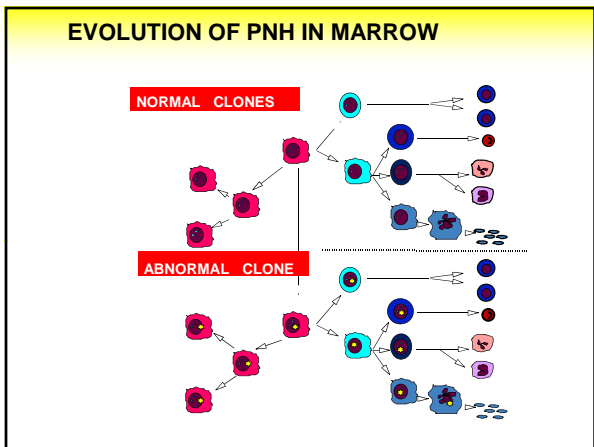
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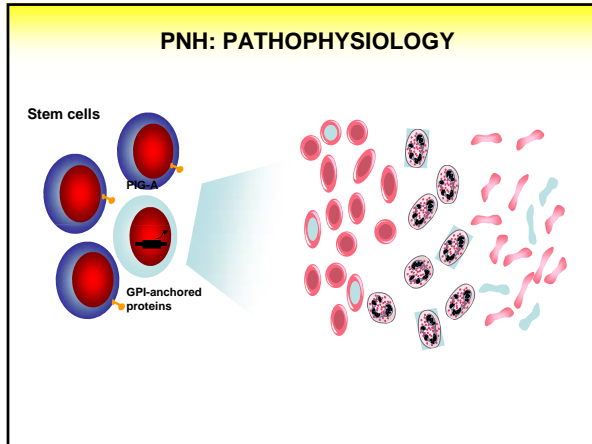
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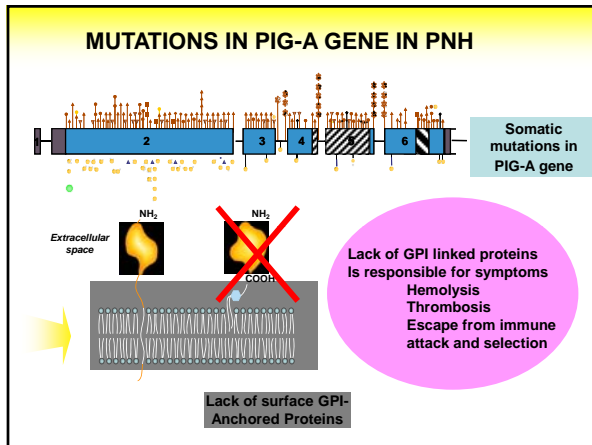
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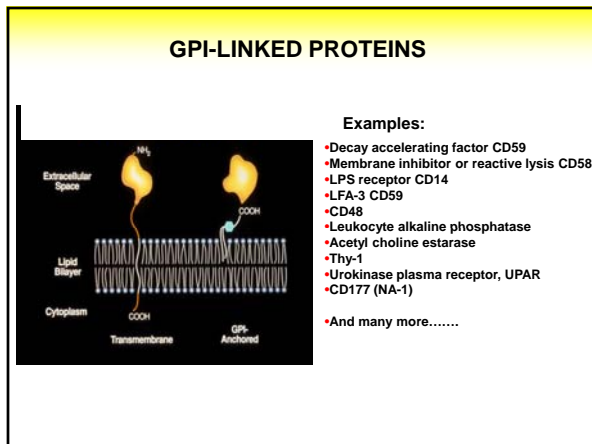
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**WHAT HAPPENS WHEN RED CELLS  
HEMALYZE?**

- The red cells are destroyed - anemia
- Hemoglobin is released into the plasma (the fluid part of blood)
- Some of the hemoglobin passes through the kidneys and into the urine leading to the dark color of the urine
  - Loss of iron
  - May lead to kidney damage in the long run
- Free hemoglobin binds nitric oxide
  - What is nitric oxide?

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**WHAT IS NITRIC ACID?**

- A gas produced by the body to regulate smooth muscle cells.
- An increase in free nitric oxide causes smooth muscle cells to relax. A decrease causes smooth cells to contract.
- Smooth muscle cells are in many tissue
  - Blood vessel walls: ischemia, impotence
  - Esophagus and GI tract: esophageal spasm, reflux, abdominal pain

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**SYMPTOMS OF PNH**

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**CARDINAL SYMPTOMS**

- Hemolysis
- Propensity for blood clots (thrombosis)
- Decreased production of blood cells

65% of patients lived longer than 10 years and 48% longer than 15 years

Predictors of decreased survival:

- Development of low counts (relative risk of 5.5)
- Occurrence of thrombosis (relative risk 10)
- Development of MDS (relative risk of 19)

In 50% of patients PNH does not affect their survival

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**SIGNS AND SYMPTOMS OF PNH**

Clinical Signs or Symptoms	Incidence Rate (%)
Thrombosis	40%
Dyspnea	66%
Chronic Renal Disease stage 1 – 5	65%
Abdominal Pain	57%
Anemia	Up to 100%
Fatigue, impaired QOL	96%
Hemoglobinuria	26%
Dysphagia	41%
Erectile Dysfunction	47%

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**PNH - Thrombosis**

- 10% of PNH patients present with thrombosis (blood clots)
- Up to 40% will develop clots during the course of their disease.
- While approximately 1/3 of these clots are typical deep vein thrombosis , another 1/3 occur in the GI system (liver, spleen), and the last third are in unusual locations (CNS, dermal veins, arterial sites).
- Once a clot develops, there is a strong tendency towards developing further clots, even with anticoagulation.
- Once a thrombosis develops, the prognosis is grim.

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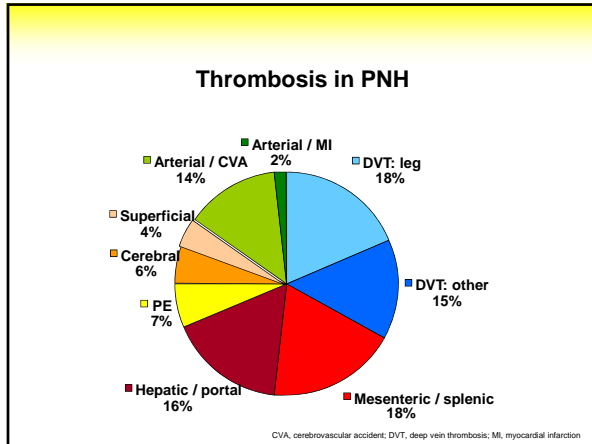
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- ### PNH - Thrombosis
- The cause of blood clots in PNH is still not clear.
  - The role of prophylactic anticoagulation (warfarin) is controversial
  - Treatment of an acute thrombotic event may include “clot busters” – lytic agents
  - Anticoagulation is standard (warfarin, heparin, enoxaparin, etc.)
  - In the past, bone marrow transplantation was strongly considered for a PNH patient if they developed a clot.
  - Eculizumab appears to markedly reduce the incidence of blood clots

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## DIAGNOSING PNH

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**DIAGNOSIS OF PNH**

- Clinical symptoms/suspicion
  - History of aplastic anemia
  - Fatigue – anemia
  - Hemolysis
  - Sudden blood clots
  - Dark urine
- Laboratory testing
  - Low blood counts
  - Hemolysis
    - LDH high
    - Haptoglobin low
    - Often low iron
    - Flow cytometry for PNH cells
    - Often high reticulocytes
    - Bone marrow exam

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**CAUSES OF HEMOLYTIC ANEMIA**

<p><b>EXTRINSIC CAUSES</b></p> <ul style="list-style-type: none"> <li>• Immune mediated           <ul style="list-style-type: none"> <li>- AIHA</li> <li>- Drug induced</li> <li>- Delayed transfusion rx</li> <li>- PCH</li> <li>- Cold agglutinin dz</li> </ul> </li> <li>• Microangiopathic           <ul style="list-style-type: none"> <li>- DIC</li> <li>- TTP/HUS</li> <li>- Valve hemolysis</li> <li>- Other trauma (March, burns)</li> </ul> </li> <li>• Other causes           <ul style="list-style-type: none"> <li>- Snake bites</li> <li>- Toxins</li> <li>- Infusion of hypotonic sol'n</li> </ul> </li> </ul>	<p><b>INTRINSIC CAUSES</b></p> <ul style="list-style-type: none"> <li>• Enzyme deficiencies           <ul style="list-style-type: none"> <li>- G6PD</li> <li>- Pyruvate kinase</li> <li>- Others</li> </ul> </li> <li>• Hemoglobinopathies           <ul style="list-style-type: none"> <li>- Sickle cell dz</li> <li>- Thalassemia</li> <li>- Unstable Hgb</li> </ul> </li> <li>• Membrane defects           <ul style="list-style-type: none"> <li>- Hereditary spherocytosis</li> <li>- Hereditary elliptocytosis</li> </ul> </li> <li>• Paroxysmal Nocturnal Hemoglobinuria (PNH)</li> </ul>
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**METHODS OF HISTORICAL INTEREST**

- Ham Test – acidified serum lysis test
  - Specific but not sensitive
- Sugar Water Test – serum in isotonic sucrose solution
  - Sensitive but not specific
- Complement lysis sensitivity test – lysis by antibody and limiting complement
  - Defined PNH II (moderately abnormal) and PNH III (markedly abnormal red blood cells)

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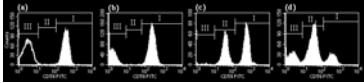
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**Flow Cytometry:  
Diagnostic Test for PNH**

- Perform on peripheral blood
- Use monoclonal antibodies against GPI-anchored proteins, such as CD59 or CD55<sup>1,2</sup>
- PNH blood cells (PNH clone) are cells missing GPI-anchored proteins



<sup>1</sup>Parker, et al. Blood. 2005;106:3699-3709.  
<sup>2</sup>Hall & Rosse. Blood. 1996;87:5332-5340.

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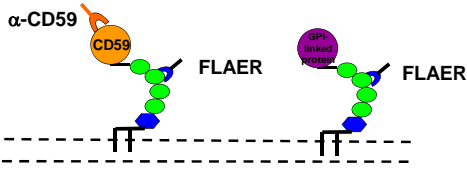
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**Fluorescent AERolysin (FLAER)**

- FLAER binds to the GPI-anchor itself, rather than to a single protein such as CD55 or CD59
- FLAER provides much greater signal noise and better accuracy than an antibody against a single target



The diagram shows two GPI-anchored proteins on a cell membrane. The left one has an orange  $\alpha$ -CD59 antibody bound to its extracellular domain. The right one has a purple FLAER molecule bound to its GPI anchor. Labels include  $\alpha$ -CD59, CD59, GPI linked protein, and FLAER.

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Types of PNH

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### TYPES OF PNH

Primary hemolytic PNH	AA/PNH syndrome
<ul style="list-style-type: none"> <li>No evidence for marrow failure</li> <li>Can evolve from AA</li> <li>Often primary disease</li> <li>Anemia solely due to hemolysis</li> <li>No benefit of immunosuppression</li> </ul>	<ul style="list-style-type: none"> <li>Counts often depressed</li> <li>Marrow hypocellular</li> <li>May benefit from immunosuppression</li> <li>Usually a late complication of AA</li> </ul>

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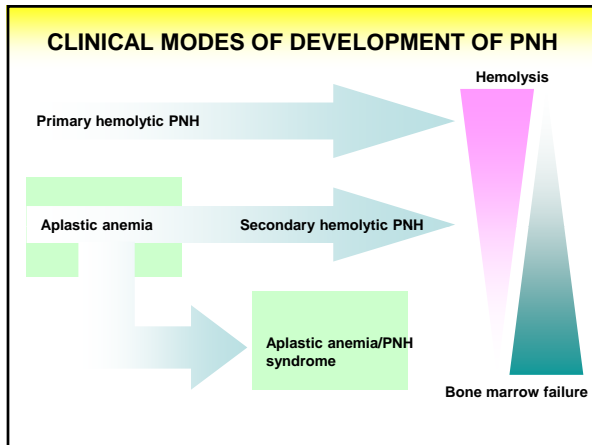
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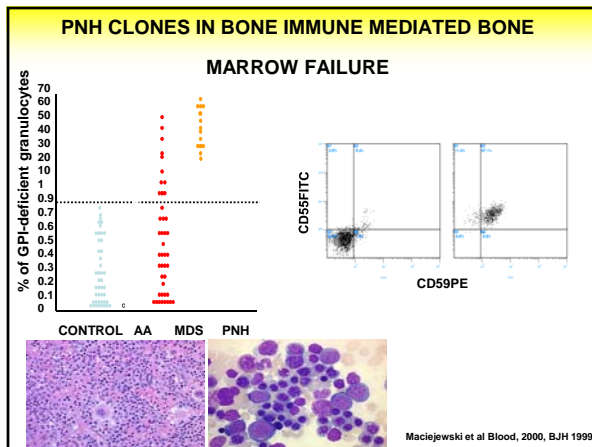
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