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

Amy E. DeZern, MD, MHS
AAMDS International Foundation
Saturday, March 28, 2015 9-10:30am

Disclosures

• No conflicts of interest to disclose for Dr. DeZern

March 24, 2015

Paroxysmal Nocturnal Hemoglobinuria

The clinical disease

• A rare, clonal, hematopoietic stem cell disorder
  – hemolytic anemia from uncontrolled complement activation
  – thrombosis
  – bone marrow failure
• Able to be treated and controlled

March 24, 2015

Natural History OF PNH

• Median survival: 10 - 15 years (this is changing)
  – Worse in pts with thrombosis, or severe pancytopenia
  – Frequent severe paroxysms
• Thrombosis: leading cause of M & M
  – Occurs in 1/3 of patients
• 5% to 10% evolve to MDS/AML
• 1/3 of new cases evolve out of AA, the rest are de novo

March 24, 2015

PNH Symptoms

• Hemoglobinuria
• Anemia (Macrocytic)
  – Iron deficiency
• Pancytopenia
• Thrombosis
  – Venous
  – Arterial
• Abdominal pain
• Transient impotence
• Esophageal Spasm
• Fatigue
• CNS changes

March 24, 2015
Mechanism and Pathophysiology

PNH Pathophysiology

- Acquired Clonal Multipotent Hematopoietic Stem Cell Disease
- PIG-A mutation – X(p22.1)
- PIG-A gene product necessary for 1st step in the biosynthesis of GPI anchors
- PNH cells have deficiency or absence of all GPI anchored proteins

March 24, 2015

PIG-A Coding Region

PIGA Mutations
- Frameshift – small insertions/deletions
- Nonsense
- Splice defect
- Missense (substitution)
  - May have residual activity

GPI-AP Biosynthesis:
Involves 10 steps and > 20 genes

PIH
Pathogenesis of hemolytic anemia

- CD59
  - Membrane inhibitor of reactive lysis
  - Prevents incorporation of C9 into C5b-8; thus, MAC does not form
- CD55
  - Decay accelerating factor
  - Block C3 convertase
- Protect cells from complement-mediated destruction

The Complement System

Microorganisms
- Lectin
- Classical
- Alternative

Antigen-Antibody

Constitutive/Microorganisms

C5
C5a
C5b
C5b-9

C3
C3a

Weak anaphylatoxin

Terminal Complement Complex (TCC)
- Cause of Hemolysis in PNH
- Lysis of Neisseria

Terminal Complement Complex (TCC)
- Cause of Hemolysis in PNH
- Lysis of Neisseria
Complement Activation in PNH

Complement successfully attacks the red cells and they break up (hemolysis)
- RBCs are destroyed, resulting in anemia
- Hemoglobin released from RBC into the plasma
- Free hemoglobin binds nitric oxide: esophageal spasm, abdominal pain, erectile dysfunction, fatigue
- Hemoglobin is cleared by the kidney, often resulting in red urine

Lectin Pathway
- MBL, MASP
- C4 + C2
Classical Pathway
- C1q, C1r, C1s
- C4 + C2
Alternate Pathway
- C3
- Factor B + D
- C3 convertases
  - C4b2a, C3bBb
- C3b
- C5 convertases
  - C4b2a3b, C3bBb3b
- C5
- C5a
- C6,7,8,9
  - Membrane attack complex (MAC)
- CD59 prevents formation of MAC
- CD55 blocks C3 Convertases

Effects of Terminal Complement on RBCs and Clinical Consequences in PNH

Free hemoglobin in the blood from destroyed PNH RBC
- DVT
- Liver
- Dermal
  - MI
- CV/TIA

Free hemoglobin is a nitric oxide scavenger

Smooth Muscle Dystonia
- Abdominal pain
- Dysphagia
- Erectile dysfunction

End Organ Damage
- Kidney
- GI
- Brain

Impaired QoL
- Disabling fatigue
- Poor physical functioning
- Pain
- Dyspnea

Anemia
- Transfusions
- Fatigue
- Dyspnea
- Angina

Thrombosis in PNH

- Thrombosis occurs in 40% of patients
  - Can be the presenting symptom in PNH
  - Contributes to end organ damage
  - Leading cause of death (40–67% of deaths)
- Multiple postulated mechanisms
- 25-33% of thrombotic events are DVT/PE
- 15-16% of thrombotic events are CVA/MI
- 27-28% unusual venous thromboses, especially hepatic veins (Budd-Chiari), splanchnic and cerebral veins

Every PNH Patient is Unique

<table>
<thead>
<tr>
<th>Clinical signs or symptoms</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombosis</td>
<td>40%</td>
</tr>
<tr>
<td>Anemia</td>
<td>89%</td>
</tr>
<tr>
<td>Bone marrow failure</td>
<td>10-45%</td>
</tr>
<tr>
<td>Fatigue, impaired QOL</td>
<td>96%</td>
</tr>
<tr>
<td>Hemoglobinuria</td>
<td>26%</td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>57%</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>41%</td>
</tr>
<tr>
<td>Erectile Dysfunction</td>
<td>47%</td>
</tr>
<tr>
<td>Chronic Renal Insufficiency</td>
<td>30%</td>
</tr>
</tbody>
</table>

IMPAIRED QoL
- Disabling fatigue
- Poor physical functioning
- Pain
- Dyspnea

Anemia in PNH

Extravascular Hemolysis
- Elevated LDH
- Reticulocytosis
- Schistocytes

Aplastic Anemia/ PNH

Production defect due to underlying marrow failure
- Reticulocytopenia
- Leukopenia
- Thrombocytopenia

Impaired Hemolysis
(if on eculizumab)
- Direct Coombs + with C3
- Spherocytes

Classical PNH

Destruction due to underlying hemolytic process
EXAM and LABORATORY FINDINGS:
How we make the DIAGNOSIS

PNH

- PNH continues to be primarily a clinical diagnosis, which can be confirmed by laboratory analyses
- Signs and symptoms are highly variable and may mimic other conditions
- The extent of clinical manifestations in patients with PNH may correlate with the level of hemolysis, the proportion of abnormal PNH RBCs (PNH RBC clone size), and the degree of complement activation
- Average diagnosis delay > 3 yrs; may be > 10 yrs

March 24, 2015

Which Patients Should Be Screened For PNH?

- Hemoglobinuria
- Hemolytic anemia
- Bone marrow dysfunction
  - Aplastic anemia (AA) or MDS screened periodically
- Coombs-negative intravascular hemolysis
  - Elevated serum LDH
- Unusual or unexplained venous thrombosis
  - Budd-Chiari syndrome
  - Mesenteric, portal, cerebral, or dermal veins
- Unexplained arterial thrombosis
- Episodic dysphagia or abdominal pain with evidence of chronic hemolysis

March 24, 2015

Abnormal lab findings

- CBC
  - Anemia
  - Pancytopenia
- Reticulocyte count
- Elevated LDH
- Low Haptoglobin
- Coombs negative
- Elevated bilirubin
- Urine for hemoglobin or hemosiderin

March 24, 2015

Flow Cytometry for PNH

- Perform on peripheral blood
- Test both granulocytes and erythrocytes
  - Erythrocytes alone are not sufficient due to hemolysis and the dilution effect of transfusions
- Use monoclonal antibodies against GPI-anchored proteins, such as CD59 or CD55
- Clone size = percent of cells missing GPI-anchored proteins
GPI-AP serve as Receptors for Proaerolysin

- Pore-forming protoxin secreted by *Aeromonas hydrophila*
- PNH cells uniquely resistant to aerolysin because they lack GPI-anchors
- FLAER - Fluoresceinated AERolysin variant that binds GPI-anchors but does not form channels.

Brodsky et al., Blood 1999 93:1749
Mikhailova et al., Brit. J. Haematol. 2001 115: 442

Types of PNH cells

- Type III cells: no GPI-AP
- Type II cells: partial GPI-AP Expression
- Type I cells: Normal GPI-AP

Classification of PNH

**IP Ig criteria**

- **Classical PNH**
  - hemolytic and thrombotic patients who have evidence of PNH in the absence of another bone marrow failure disorder
- **PNH in the context of other primary bone marrow disorders**
  - aplastic anemia or myelodysplastic syndrome
- **Subclinical PNH**
  - small PNH clones but no clinical or laboratory evidence of hemolysis or thrombosis

Parker et al. Blood 2005

TREATMENT

Historical (Read: old and outdated)

Management of PNH

Generally conservative and supportive

- Transfusions
- Anticoagulants
- Supplements
  - Folic acid; iron
- Steroids or androgen hormones
- Allogeneic bone marrow transplant
  - Curative in 50%, but high Rx-related mortality
  - 56% 2 yr survival with HLA-matched sib donor
  - Acute GVHD in 34%; chronic GVHD in 33%

Significant Mortality in PNH

*Hillmen, NEJM, 1995*

- 5 year mortality: 35%
- Median time from Dx to death: 10 yrs

**Patients Surviving (%)**

<table>
<thead>
<tr>
<th>Years After Diagnosis</th>
<th>Patients With PNH</th>
<th>Age- and Sex-Matched Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>85</td>
<td>80</td>
</tr>
<tr>
<td>10</td>
<td>70</td>
<td>65</td>
</tr>
<tr>
<td>15</td>
<td>55</td>
<td>50</td>
</tr>
<tr>
<td>20</td>
<td>40</td>
<td>35</td>
</tr>
<tr>
<td>25</td>
<td>30</td>
<td>25</td>
</tr>
</tbody>
</table>

**Patients Surviving (%)**

- 80 patients
Complement inhibition is a highly effective therapy for classical PNH

**Eculizumab**
- Humanized monoclonal
- Antibody binds to C5
- Terminal complement activity is blocked
- Proximal functions of complement remain intact
  - Weak anaphylatoxin
  - Immune complex clearance
  - Microbial opsonization

**Eculizumab Studies in PNH**

- **Pilot Study – NEJM 2004**
  - N = 11

- **TRIUMPH – NEJM 2006**
  - Pivotal Phase III, Double-Blind, Placebo-Controlled Trial, N = 87

- **SHEPHERD – Blood 2008**
  - Broader patient population, including those receiving minimal transfusions or with thrombocytopenia, N = 97

**Dosing Schedule Used for Eculizumab**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Induction Phase</th>
<th>Maintenance Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 2 weeks before induction</td>
<td>Week</td>
<td>1</td>
</tr>
<tr>
<td>Neisseria meningitidis vaccination</td>
<td>SOLIRIS® dose, mg</td>
<td>600</td>
</tr>
</tbody>
</table>

- SOLIRIS® should be administered via IV infusion over 35 minutes every 7 days during induction and every 14 days during maintenance
- SOLIRIS® dose adjustment to every 12 days may be necessary for some patients to maintain LDH reduction

**Effect of Eculizumab on Time to First Transfusion**


- 51% efficacy


- 0% efficacy
Effect of Eculizumab on Time to First Transfusion

- Eculizumab: 44% reduction in PRBC units transfused
- Placebo: 0%

Study Week
0 2 4 6 8 10 12 14 16 18 20 22 24 26

Patients Avoiding Transfusion (%)


Reduction in LDH During Eculizumab Treatment in TRIUMPH and SHEPHERD

- TRIUMPH: Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Soliris</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH levels at end of study, median (U/L)</td>
<td>2,167</td>
<td>239*</td>
</tr>
<tr>
<td>Packed RBC units Txed per pt, median (range)</td>
<td>10 (2-21)</td>
<td>0* (0-16)</td>
</tr>
<tr>
<td>Transfusion avoidance, %</td>
<td>0</td>
<td>51*</td>
</tr>
<tr>
<td>Patients with stabilized hemoglobin</td>
<td>0</td>
<td>49*</td>
</tr>
<tr>
<td>Free hemoglobin at end, median</td>
<td>62</td>
<td>5*</td>
</tr>
</tbody>
</table>

*P < 0.001

Clots in Patients With and Without Eculizumab

- Pre-Soliris® Treatment: 39 clots
- SOLIRIS® Treatment: 3 clots

P = 0.0001

Adverse Reactions Reported in ≥ 5% of SOLIRIS®-Treated Patients

<table>
<thead>
<tr>
<th>Reaction</th>
<th>SOLIRIS® (43)</th>
<th>Placebo (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>19 (44)</td>
<td>12 (27)</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>10 (23)</td>
<td>8 (18)</td>
</tr>
<tr>
<td>Back pain</td>
<td>8 (19)</td>
<td>4 (9)</td>
</tr>
<tr>
<td>Nausea</td>
<td>7 (16)</td>
<td>5 (11)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>5 (12)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Cough</td>
<td>5 (12)</td>
<td>4 (9)</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>3 (7)</td>
<td>0</td>
</tr>
<tr>
<td>Sinusitis</td>
<td>3 (7)</td>
<td>0</td>
</tr>
<tr>
<td>Respiratory tract infection</td>
<td>3 (7)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Constipation</td>
<td>3 (7)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>3 (7)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Pain in extremity</td>
<td>3 (7)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Influenza-like illness</td>
<td>2 (5)</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>


92% Fewer thrombotic events with SOLIRIS treatment
7.37 clots/100 pt yrs vs 1.07 clots/100 pt yrs Most patients (63%) received concomitant anticoagulants The effect of anticoagulant withdrawal was not studied
Lessons from Eculizumab Trials (TRIUMPH, SHEPHERD, EXTENSION)

• Safe
  – Mild side-effects
  – Increased risk for Neisserial infections (~0.5% per year)

• Effective
  – Decreases intravascular hemolysis
  – Decreases (>90%) or eliminates (50%) need for PRBC
  – Improves quality of life
  – Reduces the risk for thrombosis by >90%

Lessons from Eculizumab Trials – cont. (TRIUMPH, SHEPHERD, EXTENSION)

• Drawbacks
  – Lifelong therapy intravenous therapy
  – Cost (> 350K a year)

• Not as effective in patients with AA/PNH
  – Does not treat bone marrow failure
  – Does not treat extravascular hemolysis

• Ideal PNH patient (classical PNH)
  – Large PNH populations (>10% type III red cells, > 50% PNH granulocytes)
  – LDH > 3 x upper limit of normal
  – Retics > 3%
  – Bone marrow: normal to hypercellular

What Eculizumab Does?

• Quickly and markedly reduces INTRAvascular hemolysis
  – Improves anemia (may not be normal)
  – Markedly reduces transfusion needs

• Reduces symptoms
  – fatigue, esophageal spasm, abdominal pain, erectile dysfunction

• Reduce thrombosis
  – changes role of blood thinners

What Eculizumab Does NOT Do?

• Does not improve genetic defect
• Does not treat extravascular hemolysis
• Does not improve impaired hematopoiesis (bone marrow dysfunction)
  – Low white count or low platelet count persist

To consider with Eculizumab

• Susceptibility meningococcal sepsis/ meningitis
  – All patients must be vaccinated
  – All patients must know to seek medical help at once when fever happens
  – All patients should carry info describing this complication

• Inconvenience
  – Must be given intravenously every 12-14 days

• Cost

Intravascular and Extravascular Hemolysis in PNH

- Absence of CD55 & CD59
- MAC = C3
- INTRAvascular hemolysis
- EXTRAvascular hemolysis
Extravascular Hemolysis

Complement Cascade and PNH

**Legend:**
- Classic
- Alternative
- Lectin
- Complement Cascade
- PNH
- Eculizumab
- C3 convertase
- MAC
- Extravascular Hemolysis
- B
- D
- C3b, Bb
- C4a, 2b
- C3
- C5
- C1r/C1s
- C4a
- C2b
- C4a, 2b
- C3
- C5
- C1q/C1s
- MBL
- C1q
- C3b
- C4a

**PKN Summary**

- Understood down to genetic and molecular levels
  - The PIGA gene product is required for the biosynthesis of GPI anchored proteins.
  - The absence of GPI-anchored proteins leads to complement-mediated intravascular hemolysis since two important complement regulatory proteins (CD55 and CD59) are missing from PNH cells.
- Hemolysis in PNH occurs intravascularly
  - Leads to the release of free hemoglobin, a potent nitric oxide scavenger
  - Depletion of nitric oxide at the tissue level contributes to the symptoms
- Eculizumab decreases hemolysis in PNH by binding to C5 and blocking the terminal portion of the complement cascade
  - Extravascular hemolysis
  - Bone marrow failure may persist
- Transplant is only cure

**Questions?**

Many thanks to
- Our patients
- Our researchers
- Rob Brodsky, MD
- Donna Dorr, RN

---

**Cure**

- Allogeneic bone marrow transplant remains only cure for PNH
- Can be done with less toxicity now with lesser intensity conditioning regimens
  - Myeloablation is NOT required to eradicate the PNH clone
- Usually only considered in patients without response to eculizumab

**Clinical care of PNH patients**

<table>
<thead>
<tr>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection of PNH clone by flow cytometry and FLAER assay</td>
</tr>
<tr>
<td>Complete blood count (CBC)</td>
</tr>
<tr>
<td>Lactate dehydrogenase (LDH) measurement</td>
</tr>
<tr>
<td>Reticulocyte Count</td>
</tr>
<tr>
<td>Bone marrow biopsy if marrow failure suggested by CBC</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eculizumab intravenously</td>
</tr>
<tr>
<td>Loading: 600 mg weekly x four weeks</td>
</tr>
<tr>
<td>Maintenance (followed one week later): 900 mg every two weeks thereafter</td>
</tr>
<tr>
<td>Consideration of HSCT in suboptimal responders</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Monitoring while on Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least monthly</td>
</tr>
<tr>
<td>CBC, LDH, Reticulocyte Count, chemistries</td>
</tr>
<tr>
<td>At least yearly</td>
</tr>
<tr>
<td>PNH clone measurement</td>
</tr>
<tr>
<td>If concern for extravascular hemolysis</td>
</tr>
<tr>
<td>Direct antiglobulin test</td>
</tr>
</tbody>
</table>