Everything You Ever Wanted to Know About PNH (but didn’t know to ask!)

Lawrence Rice, MD
Chief, Division of Hematology
Houston Methodist Hospital
Professor of Medicine
Weill Cornell Medical College
Houston, Texas

- 25 yo woman referred for anemia
- 3 year hx episodic red urine, every 2 months, lasting hours to days, usually beginning on awakening in the morning
- Associated Sx: substernal tightness and difficulty swallowing with episodes; chronic profound fatigue
- Prior w/u (all negative): urine cultures, IVP, cystoscopies
- Rx: antibiotics; oral iron

Hgb 9.5, WBC 4.1, plate 225K, MCV110
Further w/u:
  - Reticulocytes 7%; ferritin 38
  - Urine hgb and hemosiderin + (no RBC)
  - Ham’s Acidified Serum and Sucrose
  - Hemolysis tests strongly +
  - Dx: PNH
The year was 1976!

Machiafava – Micheli Disease

- Subsequent flow cytometries: clone size 80%
- Rx over the years: few days prednisone 3X/yr often aborts hemolytic episodes
- Hemoglobin levels 7.5 – 10.3
- 2004: Pulmonary Embolus
  - Coumadin ever since; also got first RBC Tx
- 2006: Most severe hemolytic episode; Tx 4 units; creatinine to 6.5, rapidly improved
- 2009: Last RBC Tx
- 2011: Rising creatinine (2.4)
What is PNH?
- An acquired intracorpuscular RBC defect arising from a stem cell PIG-A mutation(s)
- 1,500 – 5,000 affected in US (≥ 5 per million); more people study it than have it
- Illustrates important scientific insights can emerge from dissecting rare disorders
- Median age of diagnosis is early 30’s
- Diminished quality of life and shortened survival
  - Chronic Intravascular Hemolysis
  - Thrombosis occurs in about 40% of patients

GPI Anchor Defect in PNH

Stem Cells Carrying a Hereditary Gene Mutation

Acquired “Clonal” Mutation in Blood Stem Cell

The Beginning of PNH
- The mutation in the PIG-A gene in PNH stops the production of an anchor that ties many protein molecules to the outside of the cell (sometimes the stop is only partial and PNH II cells occur)
- About 25 molecules are not attached and lost, but only one plays a major role in causing PNH
  - This is CD59 (MIRL), which protects the cells from complement
Evolution of PNH in Marrow Stem Cell Produces All Blood Cell Lines

ABNORMAL CLONE

NORMAL CLONES

How Do These Abnormal Cells Take Over The Bone Marrow?

- Many normal people have blood stem cells with the abnormality characteristic of PNH in very small numbers (6 per million marrow cells)
- In PNH, something allows the abnormal cells to become a major population in the marrow and blood (anywhere from 1% to over 90%)
- This “something” may be “sick” normal stem cells, such as in aplastic anemia or MDS

Steps in Expansion of PNH Clone

What Does PNH Have To Do With Aplastic Anemia?

- Many PNH patients have aplastic anemia, MDS, or a history of aplastic anemia
- Many PNH patients show signs of inadequate blood cell formation, such as low white cell and platelet counts
- Therefore, whatever causes aplastic anemia (immune factors?) may allow PNH to develop
- Some recommend all AA and MDS patients be tested for PNH – even few such cells predict response to immunosuppression

Bone Marrow Dysfunction in PNH

- In the environment of bone marrow insult, PNH clones expand
- PNH is common in patients with bone marrow failure
  - Up to 60% of patients with aplastic anemia
  - Up to 25% of patients with MDS

The Complement System

Proteins that help fight infection
Contribute to disease when things go awry

PIG-A mutation

1,2

[References]

The Defect in PNH

The Somatic Mutation of the PIG-A gene in the hematopoietic stem cell prevents all GPI anchored proteins from binding to cell surface.

**CD59**
- Forms a defensive shield for RBCs from complement-mediated lysis
- Inhibits the assembly of the membrane attack complex

**CD55**
- Prevents formation and augments instability of the C3 convertases, attenuating the complement cascade

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Absence of CD59 Allows Terminal Complement Complex Formation

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Lysis Of Red Blood Cell By C5b-9

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What Happens in PNH When Complement Is Activated?

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

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Chronic Hemolysis is Central to the Morbidities and Mortality of PNH

Normal red blood cells are protected from complement attack by a shield of terminal complement inhibitors. Without this protective complement inhibitor shield, PNH red blood cells are destroyed.

- **Anemia**
- **Fatigue**
- **Hemoglobinuria**
- **Thrombosis**
- **Pulmonary Hypertension**
- **Smooth Muscle Dysfunctions including**
  - **Dysphagia, Abdominal Pain, and Male ED**
- **Renal Failure**
- **Significant Impact on Quality of Life**
- **Significant Impact on Survival**

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Proposed Classification of PNH

- **Classic PNH**: Clinical evidence of intravascular hemolysis, no evidence of another bone marrow disorder.
- **PNH in the setting of another specified bone marrow disorder**: Same as classical PNH but concurrent bone marrow disorder such as aplastic anemia, refractory anemia/myelodysplastic syndromes, myelodysplastic/myelofibrotic diseases.
- **PNH subclinical (PNH-sc) in the setting of another specified bone marrow disorder**: No clinical evidence of intravascular hemolysis, small populations of PNH cells, concurrent bone marrow disorder.

Diagnosis of PNH

- **Anemias**
  - Decrease in Red Cell Number
  - Common symptoms: fatigue, exertional intolerance, worsening of other diseases
  - Three mechanisms can underly anemia:
    + Decreased RBC production – such as iron, B12 deficiencies; aplasia
    + Bleeding
    + Hemolytic Anemias
  - Initial Evaluation of Anemia
    + History and Physical
    + Blood smear and Reticulocyte Count

Hemolytic Anemias

- Can be Hereditary (such as sickle cell anemia) or Acquired
- Can be Intravascular; usually Extravascular
- Can be Inacropuscular or Extracorpuscular
- PNH is an Acquired, Inacropuscular defect leading to Intravascular Hemolysis
Screening Tests for Hemolysis

- Blood Smear
- Reticulocyte Count: 99% sensitive outpts, > 90% sensitive inpatients, confounders usually obvious (eg. B12 shot in B12 deficient patient)
- Bilirubin, Haptoglobin, LDH are NOT screening tests for hemolysis each has substantial issues with sensitivity and specificity

Congenital Causes of Hemolysis

- Membrane abnormalities
  - Hereditary spherocytosis
  - Hereditary elliptocytosis
  - Others
- Enzymopathies
  - Glucose-6-phosphate dehydrogenase deficiency
  - Pyruvate kinase deficiency
  - Others
- Hemoglobinopathies
  - Sickle cell anemia, SC disease, hemoglobin C disease
  - Thalassemias
  - Unstable hemoglobin

Acquired Causes of Hemolysis

- Immunologic - warm antibody, cold agglutinin, allo-immune
- Fragmentation - TTP, HUS, DIC, valve hemolysis
- Infections - malaria, Clostridia, babesiosis
- Venoms, toxins, chemicals, drugs - oxidants, lead, copper, snakes, spiders
- Metabolic - hypophosphatemia, Wilson's disease, fresh water drowning
- Membrane abnormalities - paroxysmal nocturnal hemoglobinuria, spur cell anemia
- Hypersplenism

Intravascular Hemolysis

Free Hemoglobin Binds NO

Free Hemoglobin

Smooth Muscle Relaxation

NO

Smooth Muscle Contraction
Which Patients Should Be Screened For PNH?
- Hemoglobinuria
- Hemolytic anemia
- Bone marrow dysfunction
  - Aplastic anemia (AA) or MDS screened annually
  - 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

Who Is Smarter Here?
Red Urine? Me Do Cystoscopy
Joe Urologist

Chronic Hemolysis Is Central to the Symptoms and Complications of PNH
- Thrombosis
  - Intraabdominal
  - Mesenteric
  - Portal
  - Cerebral
  - Dermal
- Impaired QoL
  - disabling fatigue
  - Poor physical functioning
  - Pain
  - Dyspnea
- Anemia
  - Transfusions
  - Fatigue
  - Dyspnea
  - Angina
- End organ damage
  - Kidney
  - GI
  - Liver
  - Brain

PNH Can Be Disabling and Life-Threatening
- An estimated 5,000 affected in US
- Median age of diagnosis is early 30’s
- Diminished quality of life
  - Anemia, dyspnea, pain, and disabling fatigue
- Life-threatening
  - Thrombosis occurs in about 40% of patients

Significant Mortality in PNH
Hillmen, NEJM, 1995
- 5 year mortality: 35%
- Median time from Dx to death: 10 yrs

Mortality rates in patients with PNH

80 patients with PNH treated between 1940 and 1970

454 French patients with PNH treated between 1950 and 2005

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
- 27–29% unusual venous thromboses, especially hepatic veins (Budd-Chiari), splanchic and cerebral veins

Incidence of VTE and Relative Risk in PNH vs Inherited Hypercoagulable States

PNH is a less common disease than inherited hypercoagulable states
PNH patients have a higher risk for VTE than inherited hypercoagulable state patients

PNH in France
de Latour, Blood, Oct 2008

- Survey 58 Hematology Centers 1950-2005
- 460 PNH patients:
  - 113 Classic PNH
  - 93 Intermediate
  - 224 AA-PNH Syndrome
- Median survival 23 yrs; 5 yr survival 75%
- better survival Dx after 1986
- classic PNH slightly better prognosis
- Thrombosis major cause of death in all groups

34 Thrombotic events in 21 patients prior to eculizumab treatment

First Ever Ischemic Stroke Incidence in PNH vs General

- FEIS risk is elevated in patients with PNH
- Age of FEIS in PNH patients is markedly less than in the general population
Mechanisms of Thrombosis

- Platelet hyperreactivity
- Diminished NO availability
- Thromboplastic RBC membranes
- Endothelial perturbations
- Impaired fibrinolysis

Risk Factors for Thrombosis

- Clinical Symptoms
  - Abdominal pains; dysphagia
  - Overt hemoglobinuria
- Lab Indices
  - LDH (an index of hemolysis)
  - D-dimer (an index of clotting)
- Clone size

PNH Clone Size and Thrombosis
(excluding warfarin prophylaxis patients)

Incidence of Thrombosis is Highest in Patients With a Large PNH Clone

Every PNH Patient is Unique

Average diagnosis delay > 3 yrs; may be > 10 yrs

Clinical signs or symptoms | Incidence (%) |
--- | --- |
Thrombosis | 40% |
Anemia | 89% |
Bone marrow failure | 10-45% |
Fatigue, impaired QOL | 96% |
Hemoglobinuria | 26% |
Abdominal Pain | 57% |
Dysphagia | 41% |
Erectile Dysfunction | 47% |
Chronic Renal Insufficiency (GFR<60/ml/min) | 30%

Flow Cytometry: Diagnostic Test 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

Pain is a Common Symptom in PNH Patients

Almost 3 out of 5 (58%) patients reported significant pain. 47% of patients with pain required medical intervention.
Historical 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%

SOLIRIS® (Eculizumab) Blocks Terminal Complement

- SOLIRIS is a monoclonal AB binding tightly to C5
- Terminal complement activity is blocked
- Proximal functions of complement remain intact
- Weak anaphylatoxin
- Immune complex clearance
- Microbial opsonization

Dosing Schedule
Used Throughout Clinical Development

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>SOLIRIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 44)</td>
<td>(n = 43)</td>
<td></td>
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</tbody>
</table>

- 8 weeks before induction
- Week 0
- Week 1
- Week 2
- Week 3
- Week 4
- Week 8

Eculizumab Studies in PNH

TRIUMPH:
- Results
- 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

Reduction in LDH During Eculizumab Treatment in TRIUMPH and SHEPHERD

- TRIUMPH – Placebo/extension
- TRIUMPH – SOLIRIS/extension
- SHEPHERD – SOLIRIS

TRIUMPH: Results

<table>
<thead>
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<th>Placebo</th>
<th>SOLIRIS</th>
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<tbody>
<tr>
<td>(n = 44)</td>
<td>(n = 43)</td>
<td></td>
</tr>
<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; 1 denotes co-primary endpoints
Effect of Soliris® on Transfusion

Transfused Units/Patient (median)

Placebo Soliris®

P < 0.0000001


Clots in Patients With and Without Eculizumab

- 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

Comparison of eculizumab treatment versus pre-treatment; signed rank test.

Mortality in 79 PNH patients on eculizumab

Comparison of eculizumab treatment versus age and sex matched control averages obtained using 2001 UK census data from the UK Office of National Statistics

Survival curves were derived using the Kaplan-Meier method using the log-rank test to analyze the difference between the groups

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

<table>
<thead>
<tr>
<th>Reaction</th>
<th>SOLIRIS® (n=43)</th>
<th>Placebo (n=44)</th>
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</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>

Mortality in different age groups

χ² = 7.49

P = .0062
**What Does Soliris® Do?**
- Quickly and markedly reduces hemolysis
- Improves anemia (may not be normal)
- Markedly reduces transfusion needs
- Reduces symptoms assoc. with hemolysis
  - fatigue, esophageal spasm,
  - abdominal pain, erectile dysfxn
- Appears to reduce thrombosis
  - May change role of blood thinners

**What is Eculizumab NOT expected to Do?**
- Does not improve genetic defect
- Does not improve impaired hematopoiesis
  - (bone marrow dysfunction)
- Low white count or low platelet count persist

**Some shortcomings of Eculizumab**
- Does not address genetic defect
- Does not address underlying marrow dysfunction
- Extravascular hemolysis (C3-mediated) may continue
- Risk of Hemolytic Crisis with sudden withdrawal
- Susceptibility to meningococcemia/ meningitis
- More rapid metabolism in some patients
- Very rare genetic resistance (C5 mutation)
- Cost
- Inconvenience (IV infusion every 12-14 days)

**PNH Summary**
- A rare but fascinating blood disorder
- Incredibly well-understood down to genetic and molecular levels
- Myriad manifestations, most importantly:
  - intravascular hemolytic anemia
  - clotting tendency, including unusual sites
- Scientific understanding increasing rapidly
- Targeted therapy now available; more options on the horizon

*Wonderful…Just Wonderful! So much for instilling them with a sense of awe…*