What is PNH?

- A rare and unusual acquired hematologic disorder characterized by:
  - Intravascular hemolysis (breaking apart of red cells in the blood vessels)
  - Bone marrow failure (cytopenias= low blood counts)
  - Thrombosis (Blood clots)
- There is an incredible amount of clinical heterogeneity amongst patients with PNH.

1st published case report of PNH - 1866

Gull WW. Guy’s Hospital Reports 12:381-392, 1866.
What causes PNH?

- **PNH requires “two-hits”**
  1. A mutation must occur in a hematopoietic stem cell.
     - Partial or complete deficiency of the GPI anchor
  2. PNH is due to a condition that allows this mutated cell to become the dominant cell in the bone marrow.

The Missing Proteins in PNH

- Complement defense proteins
  - CD55 (decay accelerating factor, DAF)
  - CD59 (membrane inhibitor of reactive lysis)
- Enzymes
  - Acetylcholinesterase
  - Alkaline phosphatase
- Immune system ligands
  - Adhesion molecules
    - NCAM
    - Fibronectin receptor
- Growth Factors and receptors
- Differentiation antigens
  - CD45 (monocytes)
  - CD19 (B cells)
- Anti-procoagulant proteins
  - uPAR (CD87)
### Complement Activation

- **Classical Pathway**
- **Lectin Pathway**
- **Alternative Pathway**

**Complement Activation Consequences**
- Membrane Attack Complex (MAC) formation leads to:
  - Prothrombotic
  - Potent anaphylatoxin
  - Chemotaxis
  - Cell Activation
  - Proinflammatory

**Membrane Attack Complex**
- 

**Complement Sensitivity of Normal and PNH Red Cells**

- Normal cells are specifically sensitive, while PNH cells are insensitive.

**Models of pathogenesis**

- Normal Marrow
- Aplastic Anemia
- PNH
- MDS

**Bone Marrow Failure Syndromes and PNH**

- Normal
- PNH w/AA or MDS
- MDS (occasional PNH clone)

**Glycosylphosphatidylinositol-specific, CD16-restricted T cells in paroxysmal nocturnal hemoglobinuria**

- Key Points:
  - The mechanism of bone marrow failure (BMF) in PNH is not known.
  - Novel CD16-restricted GPI-specific T cells are present in PNH patients and might be responsible for BMF.
Clonal selection – T cell mediated process
Clonal dominance – ?
Clonal expansion – ?

Deep sequencing reveals stepwise mutation acquisition
in paroxysmal nocturnal hemoglobinuria

The clinical picture of PNH

- Hemolysis due to complement activation
  - Anemia and fatigue
  - Hemoglobinuria, kidney damage
  - Nitric oxide trapping >> Esophageal spasm, abdominal pain, pulmonary hypertension, impotence, fatigue

- Thrombosis – Cause of blood clots is still unknown
  - Unusual sites of blood clots
- Bone marrow failure
  - Decreased blood counts (cytopenias)

Fatigue in PNH is significant

- Rosse book chapter (Hoffman-Hematology)¹
  - “Many patients note a feeling of fatigue that may be disabling during periods of hemoglobinuria.”
  - This is not related to hemoglobin level (anemia), as it disappears when the hemoglobinuria stops.”

- Brodsky book chapter (Hoffman-Hematology)²
  - “PNH patients frequently complain of disabling fatigue that is often out of proportion to the degree of anemia.”


Clinical Aspects of PNH

Significant Mortality in PNH

- 5 year mortality: 35%
- Diagnosed at all ages - median time from diagnosis to death: 10-15 yrs

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Clone size

Clone size refers to how many of the bone marrow stem cells have the mutation. In PNH, since the PNH red cells are being destroyed, the % of red cells that are CD59 – (PNH cells) does not give an accurate estimate of clone size. The white cells (granulocytes or monocytes) are not destroyed. Therefore the % of abnormal granulocytes is a more accurate estimate of the percentage of abnormal stem cells in the bone marrow.

What about thrombosis (blood clots) in PNH?

- Blood clots are a presenting sign in 10-20% of patients with PNH.
- Can occur in up to 40% of patients with PNH.
- Occur in unusual locations – veins of the liver (Budd-Chiari syndrome), spleen, brain, and skin.
- Associated with a very bad prognosis
- Cause of these blood clots is unknown – possibly related to complement activation.

Chronic Renal Insufficiency in PNH

- Associated with hemolysis and/or microvascular thrombosis
- Insidious and progressive chronic renal insufficiency (CRI, GFR <60/ml/min) in up to ~30% of patients
- May be acute renal failure, which is frequently reversible
- Renal failure reported as cause of death in ~8% of US PNH patients

Diagnosis of PNH

Average delay to diagnosis exceeds 3 years; may be greater than 10 years

- PNH continues to be primarily a clinical diagnosis, which can be confirmed by laboratory analyses
- Signs and symptoms are highly variable and may mirror other conditions
- Most common symptoms at presentation are not unique to PNH
  - Hemolytic anemia, often requiring transfusions
  - Fatigue
  - Dyspnea
  - Abdominal pain or dysphagia

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
- PNH blood cells (PNH clone) are cells missing GPI-anchored proteins
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

Who Should Be Screened For PNH?

- Patients with:
  - 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

LDH = lactate dehydrogenase; MDS = myelodysplastic syndrome.

What happens to PNH patients?

PNH - What do patients die from?

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Duke</th>
<th>Japan</th>
</tr>
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<tbody>
<tr>
<td>Thrombosis</td>
<td>16 (42%)</td>
<td>3 (8%)</td>
</tr>
<tr>
<td>Abd site</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Other site</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Arterial</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>4 (10.5%)</td>
<td>9 (24%)</td>
</tr>
<tr>
<td>Severe Infection</td>
<td>14 (36.5%)</td>
<td>14 (36.8%)</td>
</tr>
<tr>
<td>MDS/AML</td>
<td>3 (8%)</td>
<td>6 (16%)</td>
</tr>
<tr>
<td>Renal failure</td>
<td>3 (8%)</td>
<td>7 (18%)</td>
</tr>
<tr>
<td>Other malignancy</td>
<td>2 (5%)</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (5%)</td>
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</tr>
</tbody>
</table>


Possible long term effects of Eculizumab

- Improve kidney function
- Prevent pulmonary hypertension
- Increase survival

PNH Survival - Pre-eculizumab

Actuarial Survival From the Time of Diagnosis in 80 Patients With PNH

- Improve kidney function
- Prevent pulmonary hypertension
- Increase survival
Eculizumab Has a Major Impact on Survival in PNH

- 96% (76/79) patient survival
- There was no difference in mortality between patients on eculizumab and the normal population (P=0.46)

Survival is comparable to age and gender-matched control population out to 8 years