PNH: A Review and an Update

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Paroxysmal Nocturnal Hemoglobinuria:
A Chronic Disabling and Life-Threatening Disease

- PNH is an acquired disorder of the hematopoietic stem cell
- Estimated 4,000 – 6,000 patients in U.S.1
- 5 year mortality: 35%2
- Diagnosed at all Ages – Median age early 30's3,4

The expected survival of an age- and sex-matched control group is shown for comparison (Hillmen et al 1995). In a patient population where ½ the patients have <30% clone, 1 in 7 patients died by 5 years.


Years After Diagnosis
Patients Surviving (%)

Age- and Gender-Matched Controls
Patients with PNH


The Defect in PNH
The Somatic Mutation of the X-chromosome PIG A Gene Prevents All GPI Anchored Proteins from Binding to Cell Surface

- CD55
  - Prevents formation and augments instability of the C3 convertases, attenuating the complement cascade
- CD59
  - Forms a defensive shield for EBCs from complement-mediated lysis
  - Inhibits the assembly of the membrane attack complex

Multimeric C9 Lesions on PNH Erythrocyte Membrane

Paroxysmal Nocturnal Hemoglobinuria
Clinical manifestations

- Hemolytic anemia
  - paroxysmal
  - Even in the absence of symptoms, destructive progression of hemolysis is ongoing
  - nocturnal
    - Hemolysis in PNH is subtle and constant, 24 hours a day
    - Hemoglobinuria is a less commonly seen complication
  - ¾ patients present without hemoglobinuria
- Bone marrow failure/pancytopenia
- Thrombophilia/Propensity for clots

Common Symptoms in Patients With PNH


41% Dysphagia
47% Pulmonary Hypertension
66% Dyspnea
57% Abdominal Pain
64% Chronic Renal Insufficiency
41% Erectile dysfunction
26% Hemoglobinuria
40% Thrombosis
89% Anemia
96% Fatigue, Impaired QoL
Classification of PNH

1. Classical PNH: Manifests with florid intravascular hemolysis and episodes of visible hemoglobinuria.

2. PNH in the setting of another BM failure state: This entity is characterized by mild hemolysis and a small clone size.

3. Subclinical PNH: With <1% clone, and no clinical or biochemical evidence of intravascular hemolysis.

PNH and Hemolysis

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.

Consequences of Chronic Hemolysis and Free Hemoglobin

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.

Thrombosis

Renal Failure

Pulmonary Hypertension

Abdominal Pain

Dyspnea

Dysphagia

Fatigue

Erectile Dysfunction

Hemoglobinuria

Fatigue

Hemoglobinuria

Thrombosis

Renal Failure

Pulmonary Hypertension

Abdominal Pain

Dyspnea

Dysphagia

Fatigue

Erectile Dysfunction

Hemoglobinuria
Chronic Hemolysis is the Underlying Cause of Progressive Morbidities and Mortality of PNH

Chronic Kidney Disease
- Renal insufficiency
- Dialysis
- Hypertension
- End Organ Damage
  - Brain
  - Liver
  - GI
- Anemia
  - Transfusions
  - Hemosiderosis
- Fatigue / Impaired Quality of Life
  - Abdominal pain
  - Dysphagia
  - Poor physical functioning
  - Erectile dysfunction

Pulmonary Hypertension
- Dyspnea
- Cardiac Dysfunction

Thrombosis
- Venous
  - Pulmonary
  - DVT
- Arterial
  - Cerebral
  - Dermal
  - Hepatic/Portal
  - Abdominal ischemia

Common Sites of Thrombosis Occur Frequently in PNH

<table>
<thead>
<tr>
<th>TE Type</th>
<th>Hillmen P et al. 1995 (N=80)</th>
<th>Hillmen P et al. 2007 (N=195)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DVT or PE</td>
<td>33%</td>
<td>48%</td>
</tr>
<tr>
<td>CVA/MI</td>
<td>16%</td>
<td>15%</td>
</tr>
<tr>
<td>Typical VTE most common VTE in PNH</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Atypical VTE more common in PNH than in the general population</td>
<td>√</td>
<td>√</td>
</tr>
</tbody>
</table>

Higher proportion of PE and/or DVT sites of thrombosis consistently found in PNH patients – Socie et al. 1996 (29%) and Nishimura and Rosse 2004 (27%).

Thrombosis in PNH:
- 40% of patients experience clinical thrombotic events
- Leading cause of death – Accounts for 40-67% of deaths
- First thrombotic event can be fatal
- Median time to TE was 2.1-2.3 years from diagnosis
- First TE increases risk for death 5- to 10-fold

**39% of TE Events Occur at Arterial Sites**

*RLU = right upper lobe.*


**Clinical Symptoms Predictive of TE**


**Chronic Kidney Disease**

**Common Symptoms of PNH**

**Thrombosis**

**Potential Assessments to Identify Thrombosis Risk in PNH**

Baseline Platelet Count | Proportion with History of TE |
--- | --- |
Thrombocytopenic $<100,000 \times 10^9/L$ | 45% |
Non-Thrombocytopenic $\geq 100,000 \times 10^9/L$ | 27% |

- Patients with thrombocytopenia have elevated incidence of TE
  - Evidence of platelet consumption in PNH
  - Platelet consumption may result from microthrombi

- D-dimer and other markers of elevated inflammatory response
  - 61% and 82% of PNH patients in French and US studies demonstrated an elevated risk for TE as indicated by increased D-dimer levels
  - Chronic terminal complement activation leads to systemic inflammatory and hypercoagulable state in PNH

Chronic hemolysis and cell-free plasma hemoglobin lead to chronic kidney disease in PNH. Repetitive exposure of tissue to cell-free hemoglobin may lead to renal damage in PNH. 80% of PNH patients (median age of 31.5 years) had MRI evidence of significant renal hemosiderosis. Marked hemosiderin deposits in the proximal renal tubule are a common feature in all autopsy and biopsy reports dealing with PNH. Demonstrable by MRI even when no overt hemoglobinuria is seen. Autopsy and biopsy often show interstitial nephritis and fibrosis.

64% of Patients Exhibit Clinical Chronic Kidney Disease (CKD)

Impact of PNH on Quality of Life

~75% of Patients Reported Symptoms as Moderate to Very Severe

59% patients were transfusion-free for at least 12 mo or had never been transfused. 76% were forced to modify their daily activities to manage their PNH. 17% were unemployed due to PNH.
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.


Two Independent International Groups Recommend Testing High Risk Patient for PNH

Guidelines for the diagnosis and monitoring of paroxysmal nocturnal hemoglobinuria and related disorders by flow cytometry


Standard Diagnostic Test for PNH

- Flow cytometry performed on peripheral blood
- Granulocytes and at least one additional cell line should be evaluated
  - Red blood cells (RBCs)
  - Monocytes
- Quantitative results
  - Optimal-High sensitivity analysis: ≥0.01%
  - Routine analysis: ≥1%
- Easy to understand PNH reports
- Use more than one reagent against GPI-anchored proteins

Testing for PNH in Red Blood Cells

GPA = glycophorin A.

Data Source - Dahl-Chase Diagnostic Services.

Normal RBC's with normal CD59 expression (Type I cells)

PNH clone with complete CD59 deficiency (Type III cells)

PNH clone with complete CD59 deficiency (Type III cells) and partial CD59 deficiency (Type II cells)

Gating on GPA+ RBC's

PNH Patient With an 80% WBC Clone Size and 31% RBC Clone Size Indicating Hemolysis

Data Source - Dahl-Chase Diagnostic Services.

CD24- Granulocytes

FLAER- GPI Anchor Binding Marker

CD59 – GPI Anchored Protein

80.1 % of Granulocytes lack GPI proteins

31.4% RBCs are Type III PNH cells

Historical Management of PNH

Palliative Options Do Not Impact Progression and Risk for Severe Morbidities and Mortality

- Transfusions
  - Risk of iron overload
  - Transient treatment of anemia

- Anticoagulants
  - Risk of hemorrhage
  - Ineffective in many patients

- Red cell supplements
  - ESAs may expand clones and elevate hemolysis
  - Folic acid (or erythropoiesis-stimulating agents)

- Steroids/androgen hormones
  - No controlled clinical trials
  - AE’s

ESA = erythropoietin stimulating agents.

PNH
Bone Marrow Transplant
- BMT is the only potentially curative therapy for PNH1.
- Indications for transplant include
  1. uncontrollable hemolysis
  2. thrombosis
  3. Bone marrow failure state
- There is considerable morbidity and mortality associated with BMT for PNH.
- Patient selection and timing of transplant are important variables in making the decision.


In a recent retrospective study in France examining PNH patients2 – 54% had GVHD
In another study examining PNH patients (n=23)1 – 50% chronic GVHD; 42% acute GVHD
BMT has a significant impact on quality of life post transplant3,4
Allogeneic BMT recommended for PNH patients with life-threatening cytopenias or possibly the rare patient with disabling hemolysis or thrombosis not controlled with existing therapy5


BMT In PNH

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Pub (N)</th>
<th>Age</th>
<th>Study Population</th>
<th>Mortality</th>
<th>GVHD</th>
<th>Cause of Death</th>
<th>Risk of Death or GVHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Santarone S et al. H</td>
<td>2008</td>
<td>26 33 (20-59)</td>
<td>42% overall</td>
<td>34% at 6 mo. (from abstract)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>De Latour et al. E</td>
<td>2009</td>
<td>185 30 (23-38)</td>
<td>54%</td>
<td>BMF; N=69 (45%) PNH</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ruggeri et al. E</td>
<td>2009</td>
<td>58 12 51</td>
<td>7 PNH</td>
<td>53% 2 years (projected)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>de Latour et al. B</td>
<td>2008</td>
<td>52</td>
<td>42% (death)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>de Latour et al. A</td>
<td>2008</td>
<td>141</td>
<td>30 (23-36)</td>
<td>30% at 5 yrs vs. 32.2% at 10 years for controls (n=401; 1950-2005)</td>
<td></td>
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</tr>
<tr>
<td>Witherspoon et al 2007</td>
<td>2007</td>
<td>14</td>
<td>32 (19-42)</td>
<td>43% &lt; 6 months 50%</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Parker 2005</td>
<td>2005</td>
<td>121</td>
<td>PNH Patients</td>
<td>44% (10 yr) NA NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hegenbart et al.</td>
<td>2003</td>
<td>7</td>
<td>34 (25-49)</td>
<td>Hemolytic PNH; 2 BMF</td>
<td></td>
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</tr>
<tr>
<td>Saso et al.</td>
<td>1999</td>
<td>57</td>
<td>28 (10-47)</td>
<td>32% SAA</td>
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</table>

At least 43%
Eculizumab (Soliris)

**Complement Cascade**

- SOLIRIS® binds with high affinity to C5
- Terminal complement - C5a and C5b-9 activity blocked
- Proximal functions of complement remain intact:
  - Weak anaphylatoxin
  - Immune complex clearance
  - Microbial opsonization

**Eculizumab Clinical Studies in PNH**

**Pilot Study – NEJM 2004**
- Primary endpoint: reduction of hemolysis

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

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

**Long-Term Extension Trial (HERMES)**
- Evaluates long-term safety, efficacy and effect on transplantation, FACTJEM: Eculizumab, N = 107
In clinical trials all patients received a meningococcal vaccination

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

Concomitant medications allowed

- Steroids, immunosuppressant drugs, anti-clotting agents and hematinics

TRIUMPH and SHEPHERD: Response

100% response after the first dose

TRIUMPH placebo patients switched to SOLIRIS® after week 26.
All TRIUMPH patients entered the long-term extension study.

73% Reduction in Mean Units Transfused Across all Subgroups: TRIUMPH

- 51% of SOLIRIS patients achieved transfusion independence vs 0% of patients not on SOLIRIS
- Patients with concomitant bone marrow dysfunction may continue to require minimal transfusions
Patients Report Rapid and Sustained Improvement Across Broad Range of Measures

<table>
<thead>
<tr>
<th>Measure</th>
<th>P Value 1</th>
<th>P Value 2</th>
</tr>
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<tbody>
<tr>
<td>EORTC Functioning</td>
<td></td>
<td></td>
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<tr>
<td>EORTC Symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FACIT-Fatigue†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EORTC Fatigue†</td>
<td></td>
<td></td>
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<tr>
<td>Global Health†</td>
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<tr>
<td>Physical†</td>
<td></td>
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<tr>
<td>Role†</td>
<td></td>
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<tr>
<td>Cognitive*</td>
<td></td>
<td></td>
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<tr>
<td>Dyspnea†</td>
<td></td>
<td></td>
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<tr>
<td>Pain*</td>
<td></td>
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<tr>
<td>Insomnia*</td>
<td></td>
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<tr>
<td>Constipation</td>
<td></td>
<td></td>
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<tr>
<td>Nausea</td>
<td></td>
<td></td>
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<tr>
<td>Diarrhea</td>
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</table>

92% Reduction in Thrombotic Events

- 63% of patients received concomitant anticoagulants\(^1\)
- The effect of anticoagulant withdrawal was not studied\(^2\)
- Events observed in both venous and arterial sites\(^3\)

Renal Function with SOLIRIS\(\superscript{\text{\textregistered}}\) in Different Baseline Populations – 6 Months

<table>
<thead>
<tr>
<th>Segment of PNH Population</th>
<th>Overall (n=189)</th>
<th>Stage 1 – 2 (n=81)</th>
<th>Stage 3 – 5 (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of Patients (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>60.3</td>
<td>24.7</td>
<td>75.0</td>
<td>31.7</td>
</tr>
</tbody>
</table>

\(^1\) There were fewer thrombotic events with SOLIRIS treatment than during the same period of time prior to treatment.
\(^2\) SOLIRIS® (eculizumab) [package insert]. Alexion Pharmaceuticals; 2009.
Eculizumab has a Major Impact on Survival in PNH
Survival is comparable to age and sex matched control population

- 96% (76/79) patient survival.
- There was no difference in mortality between patients on eculizumab and the normal population ($P=0.46$).
- 2 patients over 70 years of age had worse survival ($P=0.0042$). No patients under the age of 50 years died.


Warning
WARNING: SERIOUS MENINGOCOCCAL INFECTION

- SOLIRIS® increases the risk of meningococcal infections. Meningococcal infection may become rapidly life-threatening or fatal if not recognized and treated early.
  - Vaccinate patients with a meningococcal vaccine at least 2 weeks prior to receiving the first dose of SOLIRIS®
  - Revaccinate according to current medical guidelines for vaccine use
  - Monitor patients for early signs of meningococcal infections, evaluate immediately if infection is suspected, and treat with antibiotics if necessary

Safety: Warnings and Precautions (cont.)

- The effect of withdrawal of anticoagulant therapy during SOLIRIS® treatment has not been established. Therefore, treatment with SOLIRIS should not alter anticoagulant management
- Patients who discontinue SOLIRIS must be monitored closely for signs of serious hemolysis
  - If serious hemolysis occurs after SOLIRIS discontinuation, consider the following procedures/treatments: blood transfusion (packed RBCs), or exchange transfusion if the PNH RBCs are >50% of the total RBCs by flow cytometry; anticoagulation; corticosteroids; or reinstitution of SOLIRIS
  - In clinical trials, 16 of 196 PNH patients discontinued SOLIRIS treatment; no serious hemolysis was observed
Safety: Warnings and Precautions (cont.)

- LDH levels may be used to monitor hemolysis
  - SOLIRIS® dose adjustment to every 12 days may be necessary for some patients to maintain LDH reduction
- Infusion reactions may occur
  - In clinical trials, no patients experienced infusion reactions that required discontinuation
  - SOLIRIS treatment should be interrupted in all patients experiencing severe infusion reactions and appropriate medical therapy administered

SOLIRIS® (eculizumab) [package insert]. Alexion Pharmaceuticals; 2009.

Serious Adverse Events: Clinical Trial Experience

- Meningococcal infections are the most important adverse events that may be experienced by patients receiving SOLIRIS®
- In clinical studies, 2 out of 196 patients developed serious meningococcal infections while receiving treatment with SOLIRIS
  - Both patients had been vaccinated
- In clinical studies among non-PNH patients, meningococcal meningitis occurred in one patient, who was unvaccinated
- In post-marketing experience, cases of serious or fatal meningococcal infections have been reported

SOLIRIS® (eculizumab) [package insert]. Alexion Pharmaceuticals; 2009.

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

<table>
<thead>
<tr>
<th>Reaction</th>
<th>SOLIRIS® (n=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 infections</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>

SOLIRIS® (eculizumab) [package insert]. Alexion Pharmaceuticals; 2009.
Patient Counseling
Prior to treatment, patients should be informed and fully understand:
- The risks and benefits of SOLIRIS®, in particular the risk of meningococcal infection
- Meningococcal vaccine does not prevent all meningococcal infections
- They are required to receive a meningococcal vaccination at least 2 weeks prior to receiving the first dose of SOLIRIS®, if they have not previously been vaccinated
- There is a potential for serious hemolysis when SOLIRIS® is discontinued and that they will be monitored by their healthcare professional for at least 8 weeks following SOLIRIS® discontinuation

Patient Safety Card
- Patients should be informed that they will be provided with a Patient Safety Card
- Patients should carry the card with them at all times
- The card describes symptoms, which if experienced, should prompt the patient to seek immediate medical attention
- Instruct patients to show the card to all health care providers involved in their care

PNH Registry Overview
The PNH Registry is an ongoing global, observational, non-interventional study collecting safety, effectiveness, clinical characteristic and quality of life data on patients with PNH irrespective of clone size or treatment.

The PNH Registry has been established in order to describe the real world outcomes of PNH, capturing a wide range of patients from all over the world.
PNH Registry Objectives

- Enhance the understanding of PNH demographics and real world outcomes
- Provide real world data characterizing clinical and subject-reported outcomes associated with PNH treatment regimens
- Raise disease awareness in the medical community and PNH patient population

Patient and Physician Participation

- All patients with a diagnosis of PNH, regardless of treatment, are eligible to enroll
  - Patients can be newly or previously diagnosed
  - The diagnosis of PNH can include any patient with evidence of a PNH clone
  - Patients followed for at least 5 years
    - Patients may discontinue participation at any time without penalty
- All Physicians treating PNH Patients are eligible to participate

PNH Registry: A Global Study

- The PNH Registry is the largest, most comprehensive database on PNH
  - First patient enrolled in January 2005
  - As of December 2010
    - 5 continents
    - 19 countries
    - 182 enrolling sites
    - 1000 patients
PNH Registry Data Collection

- Physician-Reported Data
  - Data collected at study enrollment and every six months thereafter
  - Data entry minimally includes: demographics, medical history, PNH diagnosis, flow cytometry results, symptoms, and clinical outcomes
  - All necessary information can be gathered from patient medical records

- Patient Reported Outcomes
  - Patients complete questionnaires at study enrollment and every six months thereafter
    - EORTC QLQ-C30
    - FACT-F-fatigue scale
    - Overall health status
    - Symptom frequency and bother
    - Healthcare utilization
    - Work Status
  *validated quality-of-life instruments in other disease states

PNH Registry: Future Research Topics

- PNH and Thrombotic Events
- PNH and Renal Dysfunction
- PNH in the Pediatric Setting
- Association of Clinical & Patient Characteristics with PNH Treatment
- Evolution of PNH Clones
- Survival / Mortality
- Correlation of PNH with Laboratory Markers

PNH: Conclusions

- PNH is a rare and life threatening disease
- Delays in diagnosis range from 1 to more than 10 years
- high-risk patients should be identified and tested for PNH
- Reliable testing and reporting procedures matter
  - Granulocyte analysis in all cases
  - PNH testing on RBCs alone is not adequate
  - Adding quantitative results to report forms is essential
- With the advent of treatment options for PNH, there is a compelling reason to identify patients

The commercial availability of Eculizumab has certainly made a positive impact on patients quality of life, and survival.

Bone marrow transplantation is the only curative modality for bone marrow failure states.

Other therapeutic options for PNH are being currently evaluated in clinical trials.