PNH: Understanding Your Disease and Treatment Options

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The Defect in PNH

PNH clones are defined as PNH cells with a deficiency of proteins that require a GPI anchor for attachment to the cell membrane

CD59 (MIRL)
- Forms a defensive shield for red blood cells (RBCs) from complement-mediated lysis
- Inhibits the assembly of the membrane attack complex

CD55 (DAF)
- Prevents formation and augments instability of the C3 convertase, attenuating the complement cascade

CD59 (MIRL)
- GPI = glycerophosphatidylinositol; MIRL = membrane inhibitor of reactive lysis; DAF = decay accelerating factor.


Paroxysmal Nocturnal Hemoglobinuria (PNH): A Chronic, Systemic, and Life-Threatening Disease

- Prevalence: 15.9 / million
- Diagnosed at all ages
  - Median age early 30s
- Progressive disease
  - Uncontrolled complement activation underlies the morbidities and mortality
- Despite best supportive care
  - 5 year mortality: 35%

PNH Classification

- Classic PNH
  - Intravascular hemolysis
  - Reticulocytosis
  - Increased LDH
  - Increased indirect bilirubin
  - Low haptoglobin
- PNH + Bone Marrow Disorder
  - Intravascular hemolysis + AA/MDS/hypoplasia
- Subclinical PNH
  - No clinical/lab evidence of hemolysis
  - Detected by very sensitive flow cytometric analysis
  - In association with AA/MDS

PNH and Other BMF Syndromes

PNH overlaps with BMF syndromes, and the predominant clinical characteristics can evolve over time

AML, acute myelogenous leukaemia; AA, aplastic anaemia; DKC, dyskeratosis congenita; MDS, myelodysplastic syndrome; SDS, Shwachman-Diamond syndrome.

Expansion of the PNH Clone Is Necessary to Result in Clinical PNH

• Expansion may be due to another somatic mutation
• The need for both selection and expansion may explain the rarity of PNH


The Complement System: Always on, Strongly Amplified, Dependent on Natural Regulators

The complement system is a vital component of the natural (innate) protective immune system1
• Complement is activated by three mechanisms (classical, alternative, and lectin) which allow the system to respond to inflammatory, infectious, ischemic, necrotic, as well as foreign and self antigens
• Always ‘on’ to allow rapid immune response1
  – Rapid amplification leads to powerful and destructive immune reactions2
  – Natural inhibitors of complement keep amplification in check and prevent uncontrolled complement activation2

Chronic Uncontrolled Complement Activation Leads to Devastating Consequences in PNH

Absence of CD59 Allows Terminal Complement Complex Formation

Historically Viewed as a Hemolytic Anemia

Consequences of Nitric Oxide (NO) Depletion

Reduced Nitric Oxide (NO) Depletion

• Smooth muscle dystonias
  – Vascular constriction – pulmonary and systemic hypertension, erectile dysfunction
  – Gastrointestinal contractions – dysphagia, abdominal pain
• Platelet activation and aggregation
  – Platelet hyperactivity
  – Hypercoagulability

Hemolysis Leads to NO Consumption in PNH Patients

• LDH significantly correlates with free hemoglobin (Hgb)
  – Confirms LDH as a biomarker for hemolysis
  – LDH ≥1.5x at diagnosis had a 4.5-fold greater mortality
• Free Hgb significantly correlates with NO consumption
  – Hgb is in reduced state and reactive with NO

Elevated LDH Free Hemoglobin

Historical Management of PNH

Supportive care options do not impact progression and risk for severe morbidities and mortality:

- Transfusions – risk of iron overload
- Anticoagulants – ineffective in many patients
- Red cell supplements – may expand clone and elevate hemolysis
- Steroids/androgen hormones – adverse events

Although BMT is the only potentially curative therapy for PNH, BMT is associated with significant morbidities and mortality:

- In a study examining PNH patients (n=23):
  - 50% chronic GVHD; 42% acute GVHD
  - Transplant-related mortality was 42%

Although BMT is associated with significant morbidities and mortality, BMT has a significant impact on quality of life (QoL) post-transplant.


Morbidities and Mortality in PNH

Thrombosis Is the Leading Cause of Death in PNH

- Accounts for 40–67% of deaths:
  - First thrombotic event (TE) can be fatal
  - First TE increases risk for death 5- to 10-fold
- Up to 44% of patients experience clinical thrombotic events
- Occurs in typical and atypical sites
- Is not adequately managed with anticoagulation
- All patients with PNH are at risk for thrombosis

The incidence of TE is increased in patients with elevated LDH at diagnosis:

- Univariate analysis showed that the incidence of TE was significantly increased in patients with LDH ≥1.5x ULN at diagnosis (43/171; 25.1%) compared with patients with LDH <1.5x ULN (2/53; 3.8%; OR 8.57; P<0.001)


Risk of First Ever Ischemic Stroke (FEIS) Elevated in PNH

- Age of FEIS in PNH patients is markedly less than in the general population


Chronic Uncontrolled Complement Activation Leads to Vasoconstriction and Thrombosis


Thrombosis

Thrombosis

Thrombosis

Thrombosis
Thrombosis is Associated With Risk of Early Mortality

- TE was an independent prognostic factor related to poor survival (HR 15.4; 95% CI 9.3–25.4; P<0.001) in a large cohort of French PNH patients.


**Hazard Ratio**

<table>
<thead>
<tr>
<th>French PNH Patients</th>
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<tbody>
<tr>
<td>15.40</td>
</tr>
</tbody>
</table>

(TE per 100 patient years)

Thrombosis Occurs in Both Typical and Atypical Sites

- Myocardial Infarction/Unstable angina
- Cerebrovascular accident/transient ischemic attack
- Pulmonary embolus
- Hepatic/portal vein thrombosis
- Mesenteric/splenic vein thrombosis

*124 events, 63 patients

Includes 18.5% lower extremity and 14.5% other (interior vena cava, bilateral lower extremity, pelvic, splanic, axillary, subclavian, and brachiocephalic veins).


PNH Patients are at Risk of Thrombosis Despite Anticoagulation or Minimal Transfusion Requirements

- Thrombosis Rate (TE per 100 patient years)

<table>
<thead>
<tr>
<th>Patients Treated With Anticoagulants (n=91)</th>
<th>Patients With 0–1 Transfusions Per Year (n=22)</th>
</tr>
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<tbody>
<tr>
<td>11.54</td>
<td>4.87</td>
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</table>

Thrombosis Can Occur Regardless of Clone Size

<table>
<thead>
<tr>
<th>Stage 3-5 CKD (n=40)</th>
<th>Stage 1-2 CKD (n=84)</th>
<th>No CKD (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.5</td>
<td>43.1</td>
<td>35.4</td>
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Chronic Kidney Disease: Morbidity and Mortality in PNH

- Kidney failure is the cause of 8–18% of PNH-related deaths
- 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


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

<table>
<thead>
<tr>
<th>Proportion of Patients (%)</th>
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<tr>
<td>Stage 3-5 CKD (n=40)</td>
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<tr>
<td>20.5</td>
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**Kidney Disease in PNH: Conclusions**

- Kidney failure is the cause of 8–18% of PNH-related deaths\(^1\).
- Kidney disease in PNH is caused by complement-mediated hemolysis\(^2,3\).
- 64% of patients with PNH exhibit chronic kidney disease at any one time\(^4\).
- Kidney disease is underappreciated in PNH\(^4\).


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**Clone Size Does Not Correlate to Symptom Severity**

<table>
<thead>
<tr>
<th>TE Category</th>
<th>Abdominal Pain</th>
<th>Chest Pain</th>
<th>Dyspnea</th>
</tr>
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<tbody>
<tr>
<td>≥ 10%</td>
<td>3.6</td>
<td>2.6</td>
<td>2.4</td>
</tr>
<tr>
<td>&lt; 10%</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Elevated LDH (≥1.5 × ULN) in combination with abdominal pain, chest pain, and dyspnea are associated with a higher risk of TE.

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**PNH Clonal Expansion in an AA Representative Population**

<table>
<thead>
<tr>
<th>Follow Up</th>
<th>Transitional pattern</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At the Start of Follow Up</td>
<td>Classic PNH</td>
<td>80 (11%)</td>
</tr>
<tr>
<td></td>
<td>Expansion</td>
<td>13 (17%)</td>
</tr>
<tr>
<td></td>
<td>Persistent</td>
<td>44 (59%)</td>
</tr>
<tr>
<td></td>
<td>Newly developed</td>
<td>5 (4%)</td>
</tr>
<tr>
<td></td>
<td>Disappearance</td>
<td>16 (24%)</td>
</tr>
</tbody>
</table>

At the Last of Follow Up:

- n=114
- n=75

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**Immunosuppressive Therapy (IST) Has Increased Efficacy in AA Patients With PNH Cells**

<table>
<thead>
<tr>
<th>Response</th>
<th>PNH+ vs PNH-</th>
</tr>
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<tbody>
<tr>
<td>Overall</td>
<td>82%</td>
</tr>
<tr>
<td>Complete</td>
<td>46%</td>
</tr>
<tr>
<td>5%</td>
<td></td>
</tr>
</tbody>
</table>

The presence of PNH cells was the only significant predictor of response to IST in 140 AA patients (\(P<0.01\)) in multivariate analysis\(^2\).


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**Management of Patients With Overlapping Signs and Symptoms**

- Some patients may present with elements of both PNH and BMF, with the clinical picture evolving over time\(^1\).
- Targeted treatments should address both haemolysis and BMF\(^1,2\).


**Treatment Algorithm**

- Eculizumab + IST or HSCT

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**Notes:**

Evidence of PNH Cells in RA-MDS

**EXPLORE TRIAL**
**Patient Population Description**

<table>
<thead>
<tr>
<th>RA-MDS (n=1293)</th>
<th>PNH cells/clone (Grans + RBC type III) &gt; 0.01%</th>
<th>17.16% (222 / 1293)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC PNH clone ≥ 1%</td>
<td>1.54% (20 / 1293)</td>
<td></td>
</tr>
<tr>
<td>Clone ≥1% and LDH &gt; ULN</td>
<td>40.0% (8 / 20)</td>
<td></td>
</tr>
<tr>
<td>WBC PNH Clone size ≥ 1%</td>
<td>Mean clone 32.19% (n=20)</td>
<td></td>
</tr>
</tbody>
</table>

- Interim Results from EXPLORE, a Multi-center Prevalence Study of PNH Clone Size in Patients with AA, MDS, and other BMF

Patients With Unexplained Cytopenias are at High Risk for PNH

Test the Following Cytopenic Patients for PNH
- After thorough work-up

Cytopenia and Evidence of Hemolysis
- LDH
- Haptoglobin
- Reticulocyte count (with or without anemia)
- Thrombocytopenia
- Anemia
- Coombs-negative hemolytic anemia
- Bone marrow failure
- Hemoglobinuria
- Dark-colored urine

Unexplained Cytopenias

Standard Diagnostic Test for PNH
- Flow cytometry performed on peripheral blood
- Granulocytes and at least one additional cell line should be evaluated
  - 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 RBCs

Patient 1:
- Normal RBCs with normal CD59 expression (Type I cells)

Patient 2:
- PNH clone with complete CD59 deficiency (Type III cells)

Patient 3:
- PNH clone with complete CD59 deficiency (Type III cells) and partial CD59 deficiency (Type II cells)

Why Look Beyond RBCs for PNH?
- Granulocytes provide more accurate representation of PNH clone size
- Percentages of PNH RBCs may be affected by:
  - Hemolysis
  - Blood transfusion

PNH reports should provide quantitative results expressing clone size on both granulocytes and RBCs
PNH Patient With an 80% WBC Clone Size and 31% RBC Clone Size Indicating Hemolysis

- 80.1% of Granulocytes lack GPI proteins
- 31.4% RBCs are Type III PNH cells

Data Source: Dahl-Chase Diagnostic Services.

ICCS Recommendations for Follow-Up Testing of Patients With an Identified PNH Clone
- Annual monitoring:
  - Stable patients
  - Patients with aplastic anemia and small PNH clone
  - Patients with refractory cytopenia with unilineage dysplasia (RCUD) and small PNH clone
- More frequent monitoring to evaluate for expanding clones:
  - Patients with changing symptoms or lab values
  - Patients in early stages of treatment

Eculizumab (soliris) Humanized First in Class Anti-C5 Antibody
- Human Framework Regions
  - No mutations
  - Germline
- Complementarity Determining Regions (murine origin)
- Human IgG, Heavy Chain
  - Constant Region 1 and Hinge
  - (Eliminates Fc receptor binding)
- Human IgG, Heavy Chain
  - Constant Regions 2 and 3
  - (Eliminates complement activation)

Eculizumab Blocks Terminal Complement

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

Clinical Trials With Eculizumab

- Pilot Study – NEJM 2004
  - Primary endpoint: reduction of hemolysis
- TRIUMPH – NEJM 2006
  - Pivotal Phase II, Double-Blind, Placebo-Controlled Trial, N=87
- SHEPHERD – Blood 2008
  - Broader patient population, including those receiving minimal transfusions or with thrombocytopenia, N=97
- Long Term Extension Trial
  - Hildeman Blood 2007
  - Evaluated long-term safety, efficacy and effect on thrombosis; Placebo patients switched to eculizumab N=187
Eculizumab is a Chronic Treatment for a Chronic Disease

<table>
<thead>
<tr>
<th>Soliris PNH Dosing Schedule</th>
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<tbody>
<tr>
<td>Pretreatment</td>
</tr>
<tr>
<td>400 mg/kg intravenous</td>
</tr>
<tr>
<td>+2 weeks before induction</td>
</tr>
<tr>
<td>~2 weeks after induction</td>
</tr>
<tr>
<td>Induction Phase</td>
</tr>
<tr>
<td>500 mg/kg every 7 days</td>
</tr>
<tr>
<td>Maintenance Phase</td>
</tr>
<tr>
<td>300 mg/kg every 7 days</td>
</tr>
</tbody>
</table>

- In clinical trials all patients were vaccinated against Neisseria meningitidis<sup>1</sup>
- Concomitant medications allowed:
  - Steroids, immunosuppressants drugs, antiplatelet agents and hematinics<sup>2</sup>
  - Eculizumab should be administered via IV infusion within 25—45 minutes every 7 days during induction and every 14 days during maintenance<sup>3</sup>
- Eculizumab dose adjustment to every 12 days may be necessary for some patients to maintain LDH reduction<sup>4</sup>

86% Reduction in LDH: TRIUMPH and SHEPHERD

- TRIUMPH placebo patients switched to Eculizumab after Week 26
- All TRIUMPH patients entered the long-term extension study

92% Reduction in Thrombotic Events

- 63% of patients received concomitant anticoagulants<sup>1</sup>
- The effect of anticoagulant withdrawal was not studied<sup>2</sup>
- Events observed in both venous and arterial sites<sup>3</sup>
- There were fewer thrombotic events with Eculizumab treatment than during the same period of time prior to treatment<sup>2</sup>

Eculizumab Reduced Thrombosis in Patients Treated with Anticoagulants<sup>3</sup>

- Excludes patients on antiplatelet agents

64% of Patients Exhibit Chronic Kidney Disease (CKD)<sup>1</sup>

- 59% of patients with minimal (0—1) transfusion history had CKD (n=22)

Renal Function With Eculizumab in Different Baseline PNH Populations – 18 Months<sup>1</sup>

- Excludes patients on antiplatelet agents

Eculizumab Treatment Results in Large and Clinically Meaningful Improvements in Patient-Reported Outcomes

- FACIT-Fatigue: Significantly improved after 26 weeks of treatment compared to baseline.
- EORTC Fatigue: Significant improvement at Week 26.
- Dyspnea: Reduction observed, though not statistically significant.
- Pain: Small clinical impact noted.

Reduction of Pulmonary Hypertension With Eculizumab as Measured by NT-ProBNP

- NT-ProBNP levels decreased significantly with Eculizumab treatment compared to placebo.
- 50% reduction observed in the treatment group.

Recommendations for Monitoring PNH Patients on Eculizumab

- Monthly
  - Complete Blood Count
  - Reticulocyte count
  - Serum LDH
- Yearly
  - PNH Flow Cytometry
  - If Evidence of Extravascular Hemolysis (anemia and increased retic)
    - Direct Antiglobulin Test

Summary of Clinical Efficacy

- 86% sustained reduction in hemolysis as measured by LDH
- 92% reduction in thrombotic events
- 73% reduction in transfusion requirements across all patient populations
- 78% clinically meaningful improvement in fatigue
- Sustained improvement in overall quality of life
- Patients treated with eculizumab experienced improvement in CKD and pulmonary hypertension

*Please see full prescribing information for Soliris® (eculizumab) Summary of Product Characteristics. Alexion Europe SAS; 2012.
**Summary of Clinical Efficacy and Safety**

1–5

In a multicenter analysis, eculizumab showed a major impact on survival in PNH; survival is comparable to age- and gender-matched controls.

- Eculizumab significantly reduced hemolysis, the underlying cause of morbidity and mortality in PNH.
- Significant reductions in AEs were observed, suggesting good tolerability and a favorable risk/benefit ratio over the long term.

Please see full prescribing information for Soliris® (eculizumab).


**Paroxysmal Nocturnal Hemoglobinuria: Compelling Long Term Clinical Benefits in PNH Patients**

**Role of Complement 3 in Continued Extravascular Hemolysis**

**Role of Splenectomy**

**Hemoglobin Normalization after Splenectomy**
Novel Complement Inhibitors

- ALXN 1210—C5 antibody prolonged half life administered every 8 weeks.
- AMY 101/APL-2-compstatin analog. Synthetic peptide that inhibits C3
- Coversin-small molecule protein derived from a tick that inhibits C5, daily subq dosing
- ACH-4471—oral agent. Small molecule inhibits factor D. C3 proactivator convertase. Early phase 1 trials. Active independent of C5 inhibitor
- OMS-721—complement inhibitor lectin pathway, IV, monoclonal antibody targets MASP-2
- ALN-CCS-RNAi C5 inhibitor

Transplantation for PNH

Complete Attack in PNH

Complement Cascade

Effect of C3 inhibitors on hemolysis and C3 fragment deposition on PNH erythrocytes.
HCT for PNH Long Term Results

Santarone et al. Haematologica 2010;95:983-988

N=22 MRD 65%
N=26 overall 57%
1988-2006
MA 15
RIC 11
BM 20
PBPC 6

De Lataur Haematologica 2012;97:1666-1673

HCT for PNH

1978-2007

SAŠO ET AL.
Br J Haematol. 1999:104;392-396

N=22 MRD 65%
N=26 overall 57%
1988-2006
MA 15
RIC 11
BM 20
PBPC 6

N=48 MRD 56%
1978-2007

MA 15
RIC 11
BM 20
PBPC 6

Farah et al. ASH 2011 abstract 2047

RIC HCT for PNH

Meningitis Vaccine in PNH

Farah et al. ASH 2011 abstract 2047

15/19 NED

15 patients with PNH
Flu 90 mg/m² + TBI 2-4 Gy

Probability Survived

Months after HCT

Meningitis Vaccines

• Meningitis A, C, Y, W-135 (Quadrivalent Vaccines)
  – MenHibrix (Hib-Men CY-TT) BIVALENT children 6 weeks-18 mos
  – Menveo (Men ACWY-CRM) 2 months-55 years of age
  – Menactra (Men ACWWY-D) 9 months-55 years of age
  – Menomune (MPSV4) polysaccharide
    • allergic reactions
    • Older than 55
    • No mucosal immunity
    • Duration of immunity less than 3 years—no memory T cells

Current ACIP Recommendations for Complement Deficiencies

ACIP=Advisory Committee on Immunization Practices

5/21/2018
Distribution of Meningitis Serotype in Norway

Meningitis Vaccines

• Meningitis A, C, Y, W-135 (Quadrivalent Vaccines)
  – MenHbrix (Hib-Men CY-TT) BIVALENT children 6 weeks-18 mos
  – Menveo (Men ACWY-CRM) 2 months-55 years of age
  – Menactra (Men ACWY-D) 9 months-55 years of age
• Meningococcal (MPSV4) polysaccharide
  – allergic reactions
  – Older than 55
  – No mucosal immunity
  – Duration of immunity less than 3 years—no memory T cells
• Meningitis B
  – Bexsero (Novartis) 10-25 years of age
    – 2 dose series (0 and 1-6 months)
  – Trumenba (Pfizer) 10-25 years of age
    – 3 dose series (0,2, and 6 months)

Meningitis X

  – North America, Europe, Australia and West Africa
  – No commercially available vaccine

Laboratory Analysis in PNH

Chemistry panel in PNH

Hematology panel in PNH
PNH Flow Testing

LDH in PNH

78.2%  67.1%  35.3+0.86=36.1