Advances in Paroxysmal Nocturnal Hemoglobinuria (PNH)

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

- Prevalence: 15.9 / million\(^1\)
- Diagnosed at all ages
  - Median age early 30s\(^{3,4}\)
- Progressive disease\(^{1-4}\)
  - Uncontrolled complement activation underlies the morbidities and mortality
- Despite best supportive care
  - 5 year mortality: 33\(^{3,4}\)

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

The expected survival of an age- and gender-matched control group is shown for comparison (Hillmen et al. 1995)

What is PNH?

- PNH is a disease of chronic complement-mediated hemolysis characterized by a somatic (acquired) mutation of the PIG-A gene in which blood cells lack key, naturally occurring terminal complement inhibitors (e.g., CD55 and CD59) on cell surfaces. This mutation results in a deletion of GPI anchors, rendering proteins unable to attach to the surface of the cell.
- PNH is a progressive and destructive disease that leads to:
  - Thrombosis
  - End-organ damage
  - Increased mortality

PNH: What it’s Not

- It is not paroxysmal
  - Even in the absence of symptoms, destructive progression of hemolysis is ongoing
- It is not nocturnal
  - Hemolysis in PNH is subtle and constant, 24 hours a day
- Hemoglobinuria is a less commonly seen complication
  - ¾ patients present without hemoglobinuria

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

References:
Autoreactive T Cells from Patients with PNH Specifically Recognize Glycosyl-Phosphatidyl-Inositol (GPI)

Abstract 647
Gargiulo et al

Paroxysmal Nocturnal Hemoglobinuria
Pathogenesis

Hematopoietic Stem Cells

PNH Triad

- Intravascular Hemolysis
  - Absence of complement regulators
- Thrombosis
  - Absence of complement regulators
- Bone Marrow Failure and cytopenia
  - Mechanism?
Evidence for a role of autoimmunity in PNH

- PNH is closely related to aplastic anemia – a T-cell-mediated autoimmune disorder: T cells are the likely agents suppressing normal hematopoiesis in PNH.
- The target of T cells could be either a GPI-linked protein or GPI itself.
- GPI has been found within the presentation groove of CD1d (Joyce et al. Science 1998; De Silva et al. J Immunol. 2002)
- Identical or quasi-identical TCRβ CDR3 sequences found in CD8+CD57+ T cells in a group of HLA-disparate PNH patients suggesting antigen not HLA-restricted (Gargiulo et al. Blood 2005)
- CD1d is expressed on human and murine hematopoietic stem and progenitor cells (Kotsianidis et al. Blood 2006; Broxmeyer et al. Blood 2012)

Hypothesis: GPI-specific, CD1d-restricted T cells responsible for selection of PNH cells

- T cell receptor
- GPI
- Karadimitris and Luzzatto, Leukemia 2001
Summary

- CD1d-restricted GPI-specific CD8+ T cells are present and expanded in PNH patients.

- T cells with a novel invariant TCRα chain have been discovered in some PNH patients.

- These T cells could be the “noxious agent” responsible for the pathogenesis of PNH.
GPI-specific, CD1d-restricted T cells are present in PNH patients and could be the responsible of the suppression of GPI+ HSC.

Future work: further structural and functional characterization

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 Role of Complement
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


Factors That Amplify Complement Activation

- Infection
- Surgery
- Autoimmune
- Pregnancy

Chronic Uncontrolled Complement Activation Leads to Devastating Consequences in PNH

C3 + H2O: always active (chronic) Amplification — C3b

- Anaphylaxis
- Inflammation
- Thrombosis

Consequences

C3b

- Hypersplenism
- Thrombocytopenia
- Neutropenia
- Anemia

Consequences

C5a

- Potent anaphylatoxin
- Chemotaxis
- Pro-inflammatory
- Leucocyte activation
- Endothelial activation
- Pro-thrombotic
Absence of CD59 Allows Terminal Complement Complex Formation

Chronic Uncontrolled Complement Activation Leads to Tissue and End Organ Damage

Historically Viewed as a Hemolytic Anemia
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

Consequences of Nitric Oxide (NO) Depletion

Reduced Nitric Oxide Can Cause
- Smooth muscle dystonias
  - Vascular constriction – pulmonary and systemic hypertension, erectile dysfunction
  - Gastrointestinal contractions – dysphagia, abdominal pain
- Platelet activation and aggregation
  - Platelet hyperreactivity
  - Hypercoagulability

Chronic Uncontrolled Complement Activation Leads to Devastating Consequences

Thrombosis
Renal Failure
Pulmonary Hypertension
Abdominal Pain
Chest Pain
Dyspnea
Dysphagia
Fatigue
Hemoglobinuria
Erectile Dysfunction

LDH = lactate dehydrogenase.

**Historical Management of PNH**

Supportive care options do not impact progression and risk for severe morbidities and mortality\(^1\)
- Transfusions\(^1\) – risk of iron overload
- Anticoagulants\(^1\) – ineffective in many patients
- Red cell supplements\(^1\) – may expand clone and elevate hemolysis
- Steroids/androgen hormones\(^1\) – adverse events

Although BMT is the only potentially curative therapy for PNH, BMT is associated with significant morbidities and mortality\(^2,3\)
- In a study examining PNH patients (n=23)\(^2\)
  - 50% chronic GVHD, 42% acute GVHD\(^2\)
  - Transplant-related mortality was 43%\(^2\)
- BMT has a significant impact on quality of life (QoL) post-transplant\(^4,5\)


**Morbidities and Mortality in PNH**

Thrombosis Is the Leading Cause of Death in PNH\(^1\)
- Accounts for 40–67% of deaths\(^2\)
  - First thrombotic event (TE) can be fatal\(^1,2\)
  - First TE increases risk for death 5– to 10-fold\(^2\)
- Up to 44% of patients experience clinical thrombotic events\(^2\)
- Occurs in typical and atypical sites\(^3\)
- Is not adequately managed with anticoagulation\(^2\)
- All patients with PNH are at risk for thrombosis\(^2\)

Multifactorial Pathogenesis of Thrombosis in PNH

- Pathogenesis of thrombosis in PNH is a result of uncontrolled complement activation
- Activation of complement C5b-9
  - Hemolysis leads to reduced nitric oxide levels and vasoconstriction
  - Platelets undergo morphological changes, release microparticles, and aggregate
- Activation of complement C5a
  - Leukocytes release tissue factor and inflammatory cytokines (IL-6) to initiate coagulation
  - Leukocytes decrease expression of plasminogen activator receptor (PAR) leading to impaired fibrinolysis


Chronic Uncontrolled Complement Activation Leads to Vasoconstriction and Thrombosis

- Unimpaired regulation of smooth muscles
- Local vasoconstriction
- Pro-inflammatory effect on endothelial cells

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

Thrombosis is associated with risk of early mortality.

Thrombosis occurs in both typical and atypical sites.*
PNH Patients are at Risk of Thrombosis Despite Anticoagulation or Minimal Transfusion Requirements

![Thrombosis Rate Graph]

Thrombosis Can Occur Regardless of Clone Size

![Clone Size Thrombosis Graph]

Thrombosis in PNH Conclusions

- 40–67% mortality in PNH results from thrombosis
  - Thrombosis is the leading cause of death in PNH
  - First TE increases risk for death 5- to 10-fold
- LDH 1.5 X ULN at diagnosis is associated with TE and mortality
- DVT or PE most common clinical presentation
  - Arterial thromboses are also common
- Anticoagulant therapy may not be adequate to control thrombosis in PNH
- Clinical thrombosis evident in PNH patients:
  - No transfusion history
  - Smaller clone size
Kidney Pathology in PNH

- Complement-mediated 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.


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.
  - Demonstrable by MRI even when no overt hemoglobinuria is seen.


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

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\(^4\)


Clone Size Does Not Correlate to Symptom Severity

<table>
<thead>
<tr>
<th>Clinical Symptoms</th>
<th>Gran Clone &lt;10%</th>
<th>Gran Clone 10–49%</th>
<th>Gran Clone ≥50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal Pain</td>
<td>42</td>
<td>49</td>
<td>51</td>
</tr>
<tr>
<td>Shortness of Breath</td>
<td>51</td>
<td>66</td>
<td>57</td>
</tr>
<tr>
<td>Chest Pain</td>
<td>33</td>
<td>27</td>
<td>33</td>
</tr>
<tr>
<td>Discolored Urine</td>
<td>68</td>
<td>74</td>
<td>74</td>
</tr>
<tr>
<td>Fatigue</td>
<td>63</td>
<td>75</td>
<td>78</td>
</tr>
</tbody>
</table>


Common PNH Symptoms are Associated With TE

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

Chronic Complement-Mediated Hemolysis in Combination With Clinical Symptoms Increase the Risk of Thrombosis

Data from South Korean National Registry.
Lee JW et al. Presented at the 54th Annual Meeting of the American Society of Hematology; December 8–11, 2012; Atlanta, GA. Abstract 1273.

- LDH ≥ 1.5 × ULN
- LDH and Abdominal Pain
- LDH and Chest Pain
- LDH and Dyspnea
- LDH and Hemoglobinuria

Odds Ratio for TE (Multivariate Analysis)

The risk of TE in patients with LDH ≥ 1.5 × ULN was 7.01 times greater than in patients with LDH < 1.5 × ULN (P=0.013).

Common Symptoms of PNH: Conclusions

- Common symptoms in PNH associated with TE should be considered as part of a comprehensive clinical assessment
  - Abdominal pain, chest pain, dyspnea, hemoglobinuria
- Abdominal pain and dyspnea are linked by underlying hemolysis and the threat of thrombosis
  - 66% of patients report shortness of breath
  - 59% of patients report abdominal pain
- 97% of patients report fatigue
  - Fatigue and severe dyspnea are prominent clinical features that can be associated with pulmonary hypertension and cardiac dysfunction
- 76% of patients with PNH have disruptions in daily activities
- Clone size does not correlate to symptom severity

Early Identification of Patients at High Risk for PNH
Advancements in Treatment Options WARRANT Early Diagnosis and Intervention

- Early diagnosis is essential for improved patient prognosis:
  - PNH
  - Bone marrow failure
- International PNH Registry
  - Provides evidence to inform clinical decision making
  - Over 2100 patients from 26 countries are currently enrolled in the PNH registry

Two Independent International Groups Recommend Testing High-Risk Patients for PNH

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

Michael J. Borowitz, MD, PhD; Fang-Lei Xing, MD; Eric A. Wu, MD; Michael A. Schiffer, MD; Jorge F. Martin, MD; Janet E. Heerema, MD; Robert Sullivan, MD; Carl T. Wittwer, PhD; Stephen J. Richards, PhD; on behalf of the International PNH Interest Group

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Suggestions for PNH Testing by ICCS PNH Guidelines

Clinical Indications for PNH Testing

- Intravascular hemolysis as evidenced by hemoglobinuria or elevated plasma hemoglobin
  - Evidence of intravascular hemolysis with accompanying:
    - Iron deficiency, or
    - Abnormal platelet or megakaryocytic, or
    - Thrombocytopenia (see below), or
    - Granulocyte/platelet/thrombocytopenia
  - Other acquired Coombs-negative, non-schistocytic, non-infectious hemolytic anemia

- Chaotic thrombosis with unusual features
  - Unusual sites:
    - Iatrogenic vein (Budd–Chiari syndrome)
    - Other intra-abdominal veins (portal, splenic, splanchnic)
    - Cerebral sinuses
    - Dermal veins
    - With signs of accompanying hemolytic anemia (see below)
    - With unexplained cytopenia

- Evidence of bone marrow failure
  - Suspected or proven aplastic or hypoplastic anemia
  - Refractory cytopenia with unilineage dysplasia
  - Other cytopenias of uncertain etiology after adequate workup

- Evidence of disseminated disease
  - Suspected or proven episodic or hystoplastic anemia
  - Refractory cytopenia with unilineage dysplasia
  - Other cytopenias of uncertain etiology after adequate workup

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Incidence of PNH Clones in High-Risk Patient Populations

<table>
<thead>
<tr>
<th>ICD-9 General Description</th>
<th>Incidence of PNH Clone</th>
<th>Patients (%) with PNH Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolytic anemia</td>
<td>14/647</td>
<td>2.2</td>
</tr>
<tr>
<td>Hemoglobinuria</td>
<td>14/74</td>
<td>18.9</td>
</tr>
<tr>
<td>AA</td>
<td>94/357</td>
<td>26.3</td>
</tr>
<tr>
<td>MDS</td>
<td>32/585</td>
<td>5.5</td>
</tr>
<tr>
<td>Unexplained cytopenia</td>
<td>13/230</td>
<td>5.7</td>
</tr>
<tr>
<td>Pancytopenia</td>
<td>63/1058</td>
<td>6.0</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>14/987</td>
<td>1.4</td>
</tr>
<tr>
<td>Anemia unspecified</td>
<td>40/1122</td>
<td>3.6</td>
</tr>
<tr>
<td>Hemolysis</td>
<td>18/227</td>
<td>7.9</td>
</tr>
<tr>
<td>Unspecified iron deficiency</td>
<td>7/278</td>
<td>2.5</td>
</tr>
</tbody>
</table>

ICD-9 General Description Incidence of PNH Clone

PNH Clonal Expansion in an AA Representative Population

- At the Start of Follow Up
  - PNH+ Patients: 7
- At the Last of Follow Up
  - Transitional pattern (Classic PNH) Expansion: 8 (11.1%), Persistent: 44 (59.3%), Newly developed: 5 (4.2%), Disappearance: 18 (24.4%)
Immunosuppressive Therapy (IST) Has Increased Efficacy in AA Patients With PNH Cells

The presence of PNH cells was the only significant predictor of response to IST in 140 AA patients (P=0.01) in multivariate analysis. Complete response (CR) was defined as hemoglobin normal for age, neutrophil count more than 1.5 × 10⁹/L, and platelet count more than 150 × 10⁹/L. Partial response (PR) was defined as transfusion independent and no longer meeting criteria for severe disease. Overall response = CR + PR.

Patients With Response

- Overall Response
- Complete Response

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Evidence of PNH Cells in RA-MDS

<table>
<thead>
<tr>
<th>EXPLORE TRIAL Patient Population Description</th>
<th>RA-MDS (n=1293)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNH cell/clone (Grans + RBC type III) &gt; 0.01%</td>
<td>17.16% (222 / 1293)</td>
</tr>
<tr>
<td>WBC PNH clone ≥ 1%</td>
<td>1.54% (20 / 1293)</td>
</tr>
<tr>
<td>Clone ≥ 1% and LDH &gt; ULN</td>
<td>40.0% (8 / 20)</td>
</tr>
<tr>
<td>WBC PNH Clone size ≥ 1%</td>
<td></td>
</tr>
<tr>
<td>Mean clone</td>
<td>32.19% (n=28)</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

Unexplained Cytopenias

- After thorough work-up

Cytopenias and Evidence of Hemolysis
- 5-LDH
- Haptoglobin
- Reticulocyte count (with or without anemia)

Cytopenia With Any of These Coexisting Findings
- Thrombosis
- Anemia
- Coombs-negative hemolytic anemia
- Bone marrow failure disorder
- Hemoglobinuria (dark-colored urine)

Test the Following Cytopenic Patients for PNH

93% of Patients With PNH Have Peripheral Blood Abnormalities

- Anemia
- Neutropenia
- Anemia and neutropenia
- Anemia and thrombocytopenia
- Pancytopenia
- No concomitant cytopenias

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

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

*Based on US population.
How Do You Test for PNH?

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)

Gating on GPA+ RBCs

Data Source: Dahl-Chase Diagnostic Services.
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

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
Global, observational, non-interventional study to collect real-world safety, effectiveness, and QoL data
- Open to all physicians treating patients with PNH regardless of therapy

Objectives
- Database for publications to enhance understanding of disease and improve outcomes
- Promote evidence-based medicine

Current enrollment
- Over 2000 patients enrolled
- Participation in 24 countries, including Argentina, Australia, Belgium, Canada, Denmark, Finland, France, Germany, The Netherlands, New Zealand, Russia, South Korea, Spain, Sweden, Switzerland, Taiwan, United Kingdom, United States

Enrollment information: www.pnhsource.com
Eculizumab Blocks Terminal Complement

**Complement Cascade**

- 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

**Therapeutic Indications and Usage**

Eculizumab is a Complement Inhibitor Indicated for:

- The treatment of patients with paroxysmal nocturnal hemoglobinuria (PNH) to reduce hemolysis
- The treatment of patients with atypical hemolytic uremic syndrome (aHUS) to inhibit complement mediated thrombotic microangiopathy

The effectiveness of Eculizumab in aHUS is based on the effects on thrombotic microangiopathy (TMA) and renal function. Prospective clinical trials in additional patients are ongoing to confirm the benefit of Eculizumab in patients with aHUS.

**WARNING: SERIOUS MENINGOCOCCAL INFECTIONS**

Eculizumab increases the patient’s susceptibility to meningococcal infection due to its mechanism of action.

- To reduce the risk of infection, a tetravalent vaccine against serotypes A, C, Y and W135 (preferably conjugate), is strongly recommended to vaccinate patients at least 14 days prior to receiving the first dose of eculizumab.
  - Patients less than 2 years of age or not vaccinated at least 14 days before starting treatment with eculizumab must receive treatment with appropriate prophylactic antibiotics until 14 days after vaccination. Revaccinate according to current medical guidelines for vaccine use.
- Vaccination may not be sufficient to prevent meningococcal infection and the use of antibacterial agents may need to be considered.
- Monitor patients for early signs of meningococcal infection; evaluate immediately if infection is suspected and treat with antibiotics if necessary.
What is the Long-Term Experience With Eculizumab?

- **Pilot Study – NEJM 2004**
  N=11
  Primary endpoint: reduction of hemolysis

- **TRIUMPH – NEJM 2004**
  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

Long-Term Extension Trial
Trillium Blood 2007
Evaluated long-term safety, efficacy, and effect on thrombosis, Placebo patients switched to eculizumab N=137

Eculizumab is a Chronic Treatment for a Chronic Disease

**Soliris PNH Dosing Schedule**

<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>1</td>
<td>2</td>
</tr>
<tr>
<td>Neisseria meningitidis vaccination</td>
<td>Soliris dose, mg</td>
<td>600</td>
</tr>
</tbody>
</table>

- In clinical trials all patients were vaccinated against Neisseria meningitidis
- Concomitant medications allowed:
  - Steroids, immunosuppressant drugs, anti-clotting agents and hematinics
- Eculizumab should be administered via IV infusion within 25-45 minutes every 7 days during induction and every 14 days during maintenance
- Eculizumab dose adjustment to every 12 days may be necessary for some patients to maintain LDH reduction

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

For full prescribing information, see the **Soliris® (eculizumab) Summary of Product Characteristics.** Alexion Europe SAS; 2012.
92% Reduction in Thrombotic Events

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

Eculizumab Reduced Thrombosis in Patients Treated with Anticoagulants

94% reduction in event rate with Eculizumab

Effect of Eculizumab on Chronic Kidney Disease in PNH

Please see full prescribing information for Soliris® (eculizumab).
64% of Patients Exhibit Chronic Kidney Disease (CKD)\(^1\)

<table>
<thead>
<tr>
<th>Population</th>
<th>Proportion of Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 3-5 CKD</td>
<td>20.5</td>
</tr>
<tr>
<td>Stage 1-2 CKD</td>
<td>43.1</td>
</tr>
<tr>
<td>No CKD</td>
<td>36.4</td>
</tr>
</tbody>
</table>

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

Renal Function With Eculizumab in Different Baseline PNH Populations – 6 Months\(^1\)

<table>
<thead>
<tr>
<th>Segment of PNH Population</th>
<th>Proportion of Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (n=189)</td>
<td>60.3 (P&lt;0.001)</td>
</tr>
<tr>
<td>Stage 1–2 (n=81)</td>
<td>71.4 (P=0.05)</td>
</tr>
<tr>
<td>Stage 3–5 (n=40)</td>
<td>75.0 (P&lt;0.001)</td>
</tr>
</tbody>
</table>

Renal Function With Eculizumab in Different Baseline PNH Populations – 18 Months\(^1\)

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<th>Segment of PNH Population</th>
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<tr>
<td>Overall (n=189)</td>
<td>67.2 (P&lt;0.001)</td>
</tr>
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<tr>
<td>Stage 3–5 (n=40)</td>
<td>74.2 (P&lt;0.001)</td>
</tr>
</tbody>
</table>

Chronic Kidney Disease in PNH: Summary

- Early intervention with eculizumab has shown a time-dependent improvement in renal function in protecting against progression of renal damage\(^1\)


Is the Primary Cause of Fatigue in PNH Anemia or Hemolysis?

TRIUMPH Demonstrated That Improvement in Fatigue Occurred Independent of Hemoglobin Response

- In SHEPHERD, 78% of patients reported a significant improvement in fatigue\(^1\)

1 FACIT = Functional Assessment of Chronic Illness Therapy.


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

- 73% of Ecu patients achieved transfusion independence vs 3% of patients not on Ecu
- Patients with concomitant bone marrow dysfunction may continue to require transfusions

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

- Dyspnea: 0 to 1.2
- EORTC Fatigue: 0 to 1.13
- FACIT Fatigue: 0 to 1.12
- Pain: 0 to 1.0

Eculizumab Reduces Hemolysis and Improves Fatigue in IST-Treated Patients

- IST: Severe hemolysis
- Ecu: Significant and sustained reduction in hemolysis
- IST: Severe fatigue
- Ecu: Significant and sustained reduction in fatigue
- Demonstrates effectiveness of treatment with Eculizumab to reduce hemolysis and fatigue in AA patients despite concomitant IST treatment
How Does Eculizumab Impact Pulmonary Hypertension and Dyspnea in PNH?

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

What is the Long-Term Experience With Eculizumab?
What is the Clinical Data on the Long-term Outcome of Treatment with Eculizumab in Patients with PNH?

Eculizumab in PNH: 10-Year Multicenter Experience

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N=153</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, n (%)</td>
<td>77 (50.3)</td>
</tr>
<tr>
<td>Median age at diagnosis, years (range)</td>
<td>34 (12-80)</td>
</tr>
<tr>
<td>Median age at commencement of Eculizumab, years (range)</td>
<td>42 (14-84)</td>
</tr>
<tr>
<td>Concomitant immunosuppression, n (%)</td>
<td>25 (16.3)</td>
</tr>
<tr>
<td>Concomitant anticoagulation, n (%)</td>
<td>93 (60.8)</td>
</tr>
<tr>
<td>LDH level, IU/L (range)</td>
<td>2302 (151–10,300)*</td>
</tr>
</tbody>
</table>
Long-term use of eculizumab demonstrates the impact on quality of life and reduction in complications, thereby improving long-term outcomes for patients with PNH.
Treatment Expectations: What is the Benefit of Eculizumab Therapy in PNH Patients?

- Reduction in hemolysis (as measured by LDH)*
- Reduction in fatigue*
- Improvement in quality of life
- Improvement in dyspnea
- Reduction in frequency of transfusions
- Stabilization of hemoglobin level
- Continuous improvement in quality of life
- Continuous improvement in dyspnea
- Continuous improvement in fatigue
- Continuous reduction in frequency of transfusions
- Continuous stabilization of hemoglobin level
- Continuous improvement in fatigue

Between 2 and 6 Months
- Reduction in hemolysis (as measured by LDH)
- Reduction in fatigue
- Stabilization of hemoglobin level
- Continuous improvement in quality of life
- Continuous improvement in dyspnea
- Continuous improvement in fatigue
- Continuous reduction in frequency of transfusions
- Continuous stabilization of hemoglobin level
- Continuous improvement in fatigue

Between 6 Months and Up to 10 Years
- Reduction in hemolysis (as measured by LDH)
- Reduction in fatigue
- Stabilization of hemoglobin level
- Continuous improvement in quality of life
- Continuous improvement in dyspnea
- Continuous improvement in fatigue
- Continuous reduction in frequency of transfusions
- Continuous stabilization of hemoglobin level
- Continuous improvement in fatigue

Adverse Reactions Reported in ≥ 5% of Eculizumab 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 (0)</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>

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

Soliris® SmPC: Soliris® (eculizumab) summary of product characteristics. Alexion Europe SAS 2012.
Summary of Clinical Efficacy1–5

• 86% sustained reduction in hemolysis as measured by LDH
  – Maintained over a 36 month treatment period1–4
• 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
  – Eculizumab provided a rapid and durable effect on dyspnea, a key marker of hemolysis-induced PHT

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


Summary of Clinical Efficacy and Safety1–5

• 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).


Conclusions

• Chronic complement-mediated hemolysis is the underlying cause of progressive morbidities and mortality in PNH
• Thrombosis is the leading cause of death in PNH1
• Renal failure has been identified as the cause of death in approximately 8–18% of PNH patients2–3
• PNH may be more common than you think
  – Delays in diagnosis range from 1 to more than 10 years4
  – High sensitivity flow cytometry, performed on peripheral blood, is the gold standard test
  – Advancements in treatment options warrant early diagnosis and intervention

Long Term Experience with Soliris in PNH

What is the Impact of Long-term Soliris Treatment on Clinical Outcomes and Survival?

Uncontrolled Complement Activation

Hemolysis

Complications Associated With Elevated Hemolysis (LDH)

End Organ Damage

- TE
- Renal
- Gastrointestinal
- Pulmonary
- Cardiac
- Hepatic

Decreased Mortality?

Long-term Treatment With Soliris in PNH: Sustained Efficacy and Improved Survival

- 153 consecutive patients with PNH treated with Soliris
  - May 2002 – April 2012
  - Duration mean of treatment 3.5 years (range <1.0 - 9.9 years)
- Mortality and disease symptoms were evaluated
Patient Characteristics (n=153)

<table>
<thead>
<tr>
<th>Presenting Features</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>76 (49.7%)</td>
</tr>
<tr>
<td>Age at diagnosis (median)</td>
<td>34 yrs (12 - 80)</td>
</tr>
<tr>
<td>Age at initiation of Soliris</td>
<td>42 yrs (14 – 84)</td>
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<td>LDH level (normal &lt;430IU/L)</td>
<td>2302 (151 – 10,300)</td>
</tr>
</tbody>
</table>

74 Thrombotic Events in 50 Patients Prior to Soliris Treatment

- 28 thrombotic episodes in 15 patients anticoagulated prior to initiation of Soliris therapy

Thrombotic Events in Equivalent Time Periods

- 12 months prior to initiating Soliris
  - 36 thrombotic episodes in 22 (14.3%) patients
- In the most recent 12 months on therapy
  - 3 thromboses in 3 (2.0%) patients
    - 1 Budd-Chiari during complement blockade breakthrough caused by infection
    - 1 CVA during reversal of warfarin oversatcoagulation
    - 1 TIA/lacunar infarct thought to be due to diabetic small vessel disease

Hill A. ASH 2012.
Discontinuing Anticoagulation

• Primary prophylaxis with warfarin has been safely stopped in 43/50 (86%) patients
• Secondary prophylaxis discontinued in 4 (<10%) due to haemorrhages, risk of bleeding from varices and/or thrombocytopenia
• With no thrombotic sequelae

Reduction in Intravascular Haemolysis

All patients had a rapid reduction in LDH

Median Transfusion Requirements in the 12 Months Prior to Soliris and Most Recent 12 Months on Soliris

66% of transfused patients become transfusion independent
Reasons for Transfusion in the 25 Patients in Leeds Not Transfusion Independent

25 patients still requiring transfusions:
The mean number of transfusions fell significantly from 24.6 units (range 4-44) to 11.4 units (range 2-45), P=0.0002

Outcomes (n=153 patients)
137 patients on treatment as of April 2012
- 7 discontinued eculizumab therapy
  - 1 predominant AA
  - 2 spontaneous remissions of PNH clone
  - 3 treated for indication of pregnancy alone
  - 1 successfully transplanted for VSAA
- 9 patients died

Outcomes
9 patients died
- 3 due to progression of their underlying bone marrow failure to MDS/AML
- One died immediately after BMT (veno-occlusive disease)
  - 27 yrs old
  - 2000 AA
  - 2009 PNH
  - Mar 2011 AML
  - May 2011 Ecu
  - Oct 2011 transplant; VOD
- Remaining 5 not directly related to PNH
Causes of Death

- MDHAML
- Myelodysplastic syndromes
- Premalignancy
- Hepatocellular carcinoma
- Congestive heart failure
- GI bleed/CVA

Mortality With Best Supportive Care

Actuarial survival from the time of diagnosis in 80 patients with PNH.

Patients surviving, %

5 year mortality of 35% recently confirmed.

Pre-Soliris from time of diagnosis in 80 patients with PNH.

Survival of PNH patients treated with Soliris compared with the normal UK population.

Despite best supportive care - 5 year mortality 35%.

Paroxysmal Nocturnal Hemoglobinuria: Compelling Long Term Clinical Benefits in PNH Patients
Sustained Complement Inhibition Leads to Reduced Hemolysis, Thrombosis and Improvements in Survival

- 1. Reduction in LDH (P<0.001)
  - 100% response rate in pivotal clinical trial programs (As measured by reduction in LDH)
  - 92% (P<0.0001) reduction in TE
  - Significant reduction in abdominal pain
  - Improvement in dyspnea, dysphagia, fatigue, hemoglobinuria
  - 4 fold improvement in CKD over placebo (P<0.04)
  - Soliris appears to normalize survival in patients with PNH

Soliris appears to normalize survival in patients with PNH.

Global, observational, non-interventional study to collect real world safety, effectiveness and QoL data

- Open to all physicians treating patients with PNH regardless of therapy

Objectives

- Database for publications to enhance understanding of disease and improve outcomes
- Promote evidence-based medicine

Current enrollment

- Over 2000 patients enrolled
- Participation in 24 countries, including Argentina, Australia, Belgium, Canada, Denmark, Finland, France, Germany, Japan, Luxembourg, Netherlands, New Zealand, Norway, Russia, South Korea, Spain, Sweden, Switzerland, Taiwan, United Kingdom, United States

Enrollment information: www.pnhsource.com

Discussion

- How does this data impact your motivation to more closely monitor patients with PNH?