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Chicago
1882: Strübing: First reported case.

1928: Enneking: “Paroxysmal Nocturnal Hemoglobinuria”.

1938: Ham: PNH RBCs lysis in an acidified serum by an antibody-independent complement-like factors.


1961: Dacie: PNH cells in BM failure /Link to somatic mutation: “abnormal cells must have as yet, not understood biological advantage”.

PNH: A Historical Perspective

- 1966: Rosse: PNH RBC’s were 25 times more sensitive to lysis by complement than normal RBC’s. C3 binding with complement activation.
- 1993: Kinoshita: Loss of GPI anchor is the result of a somatic mutation in PIG-A gene.

The Etiology of PNH:

- A Defective gene on chromosome x leads to inability to produce protein (GPI-anchor) on cell surface
- This loss of GPI-anchor leads to loss of complement-shielding molecules (CD-55/CD-59)
- Loss of CD55/CD59 results in cell destruction when complement activates (e.g. Hemolytic anemia)
- One mutation is required in either males or females to cause disease.
The Complement System

Lectin Pathway  Classical Pathway  Alternative Pathway

Proximal  Inflammation  C3  C3a  C3b  C5a  C5b  MAC  Intravascular Hemolysis
Extravascular Hemolysis

Terminal

C5b on RBC  Amplification loop

Immune cells
CD59 Deficiency and MAC* Formation

*C Membrane Attack Complex

C5b-8

CD59

C9

CD55

The Clinical Triad of PNH

Hemolysis/Hemoglobinuria

Thrombosis

Bone Marrow Failure*

Budd-Chiari Syndrome
Clinical Manifestations of PNH

- Hemolytic anemia
  - Anemia and fatigue
  - Hemoglobinuria
  - Kidney failure
  - Esophageal spasms
  - Abdominal pain
  - Headache
  - Erectile dysfunction

- Clotting Events
  - Blood clots in both arteries and veins
  - Intra-abdominal vein thrombosis
    - Budd-Chiari
    - Splenic/mesenteric/renal vein thrombosis
  - Cerebral vein thrombosis
  - Retinal and dermal vein thromboses
  - Arterial thromboses: MI/Strokes

Bone Marrow Failure
- Pancytopenia
- Propensity for infection
- Bleeding

Some patients can be asymptomatic.
Diagnostic Testing for PNH

- A blood sample should be sent for analysis by Flow cytometry of peripheral blood, **not** the bone marrow:

- Gold standard test for diagnosis of PNH
  - Looking for CD55 and CD59
  - FLAER (fluorescein-tagged pro-aerolysin variant) that binds a portion of GPI anchor

- More than one cell lineage should be evaluated
  - Granulocytes
  - Monocytes
  - Red Blood Cells (RBC)
Treatment options for PNH

- **Supportive care**
  - Transfusions.
  - Iron/folate.
  - Androgens.
  - Erythropoiesis stimulating agents.

- **Prednisone**
  - Dose required (20-30 mg/day).

- **Allogeneic Bone marrow transplantation.**

- **Complement Inhibition:**
  - Eculizumab (Soliris).
  - Ravulizumab (ultomiris)

- **Clinical trials/Novel agents.**

PNH Treatment Algorithm

Hemolysis
- Mild: Supportive care
- Severe: Anti-complement therapy
  - Response
  - No response

Thrombophilia
- No thrombosis: Anti-coagulants, ± thrombolysis
- Thrombosis: Supportive care

Aplastic anemia
- Moderate AA: Supportive care
- Severe AA
  - No sibling donor: No response
  - Sibling donor: ATG + CyA
    - No response
    - Response

BMT option contraindicated due to high transplant-related mortality
- Novel anti-complement therapies???
- BMT (MUD)
- BMT (sibling)
Eculizumab: Anti-C5 Antibody

Human Framework Regions
- No mutations
- Germline

Complementarity Determining Regions (murine origin)

Human IgG2 Heavy Chain
Constant Region 1 and Hinge

Human IgG4 Heavy Chain
Constant Regions 2 and 3

Rother R et al. Nat Biotech 2007;25:1256
Eculizumab Clinical Studies

**Pilot Study – NEJM. 2004**
N = 11
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 receiving minimal transfusions or with thrombocytopenia, N = 97

**Long-Term Extension Trial Hillmen Blood. 2007**
Evaluated long-term safety, efficacy and effect on thrombosis; Placebo patients switched to Eculizumab
N = 187
ALXN -1210: Ravulizumab

- A humanized monoclonal antibody to C5.
- Enhanced Fc receptor recycling.
- Half-life of 42 days (4x longer than Eculizumab).
- Administered IV q 8 weeks.
- Weight-based dosing.
- A sq. version is under investigation.
Ravulizumab VS. Eculizumab 2 Phase 3 Trials

- **PNH-301:**
  - Treatment-naïve PNH patients (n: 246).

- **PNH-302:**
  - Previously–treated adult patients (n: 197).
  - At least 6 months of eculizumab therapy, LDH < 1.5 X ULN
  - Primary efficacy endpoint was hemolysis /percentage change in LDH level from baseline to day 183.

- Ravulizumab (ALXN 1210) reported in both trials to be noninferior to eculizumab.
Percentage of patients achieving LDH normalization overtime in the ravulizumab and eculizumab treatment groups.
PNH: Ongoing Dilemmas Despite anti-complement Therapy

- C5 polymorphism/lack of response to Eculizumab
- Ongoing anemia as a result of
  - Breakthrough hemolysis
  - Concomitant bone marrow failure
  - Extravascular hemolysis (+ DAT/C3 deposits)
- Increased risk for meningococcal infection. ? Prophylactic antibiotics
- Need for IV infusions (q 2 with eculizumab, or 8 week with ravulizumab).
- Worsening bone marrow failure / thrombocytopenia and neutropenia (causing bleeding /infections respectively)
Table 1. Patient demographics and baseline characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P-Abx Use (n = 501)</th>
<th>No P-Abx Use (n = 730)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>236 (47.1)</td>
<td>339 (46.4)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>265 (52.9)</td>
<td>391 (53.6)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black or African descent</td>
<td>26 (5.2)</td>
<td>30 (4.1)</td>
</tr>
<tr>
<td>Asian</td>
<td>28 (5.6)</td>
<td>135 (18.5)</td>
</tr>
<tr>
<td>Native/Aboriginal</td>
<td>0</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>Pacific Islander</td>
<td>0</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>White/Caucasian</td>
<td>428 (86.3)</td>
<td>543 (74.6)</td>
</tr>
<tr>
<td>Other</td>
<td>14 (2.8)</td>
<td>18 (2.5)</td>
</tr>
<tr>
<td>Missing</td>
<td>5 (1.0)</td>
<td>2 (0.3)</td>
</tr>
<tr>
<td>Geographic region, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>North America</td>
<td>63 (12.6)</td>
<td>163 (22.3)</td>
</tr>
<tr>
<td>Europe</td>
<td>384 (76.6)</td>
<td>420 (57.5)</td>
</tr>
<tr>
<td>Rest of the world</td>
<td>54 (10.8)</td>
<td>147 (20.1)</td>
</tr>
<tr>
<td>Age of PNH onset, mean (SD), y</td>
<td>37.3 (18.09)</td>
<td>36.8 (16.86)</td>
</tr>
<tr>
<td>Age at eculizumab initiation, mean (SD), y</td>
<td>44.4 (17.66)</td>
<td>43.1 (16.76)</td>
</tr>
</tbody>
</table>

P-Abx, prophylactic antibiotics; PNH, paroxysmal nocturnal hemoglobinuria; SD, standard deviation.  
* Australia/New Zealand (n = 33); Asia (n = 29); Central/South America (n = 1).  
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* Data missing for 1 patient.

Table 2. Rate of meningococcal infections in patients treated with eculizumab with or without P-Abx

<table>
<thead>
<tr>
<th>Meningococcal Infections</th>
<th>P-Abx Use (n = 501)</th>
<th>No P-Abx Use (n = 730)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with events PY</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Estimated rate per 100 PY</td>
<td>2,134.3</td>
<td>2,808.5</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>0.0-0.4</td>
<td>0.1</td>
<td>0.1-0.4</td>
</tr>
</tbody>
</table>

Cl, confidence interval; P-Abx, prophylactic antibiotics; PY, patient-years.
CASE Study

- A young woman with no prior medical history presented to PCP with fatigue and exercise intolerance.
- A CBC was performed:
  - Severe anemia with Hemoglobin 4.5 g/dl, (lower limit of normal: 12 gm/dl)
  - High LDH > 3000
  - Extensive workup for causes of hemolytic anemia was negative.
- A bone marrow biopsy was performed
- Bone marrow biopsy: Normocellular marrow
- No dysplasia present.
CASE 1: Results and Treatment

- Flow cytometry on a peripheral blood sample showed a large PNH clone:
  - Type I RBC 69.29%
  - Type II RBC 20.26%
  - Type III RBC 10.45%
- Granulocytes FLAER/CD24 deficiency: 80%
- Monocytes FLAER/CD14 deficiency: 85%
- Treated with eculizumab
- Continued to be transfusion-dependent
CASE 1: Hgb

Eculizumab

Stem Cell Transplantation
CASE 1: Platelets
CASE 2

- A 50 year old man diagnosed with aplastic anemia in 2000.
- Treated with immunosuppressive therapy.

- A few years later, he presented with worsening anemia and LDH: >4000 (upper limit of normal: 240).
- Initially treated with steroids and later with eculizumab.
CASE 2: Results of Diagnostic Testing

- **Bone marrow biopsy:**
  - Normocellular bone marrow, so this is not relapse of aplastic anemia
  - Erythroid hyperplasia, meaning that the bone marrow is functioning.
  - No overt dysplasia seen, so this is not MDS.
  - Absent storage iron. Why?

- **Flow cytometry results:**
  - RBC Type I: Normal CD59 level. 74.81%
  - RBC Type II: Partial CD59 deficiency 1.13%
  - RBC Type III: Complete CD59 deficiency 24.06%
  - Granulocytes FLAER/CD24 deficiency 76.06%
  - Monocytes FLAER/CD14 deficiency 83.65%
CASE 2: LDH

Eculizumab
CASE 2: Hemoglobin

Anemia in PNH has multiple causes:
- Breakthrough hemolysis
- Extravascular hemolysis
- Bone marrow failure
- Vitamin / mineral deficiencies
Novel Agents

**C5 Inhibitors:**
- REGN3918: Administered SQ. Ongoing trials in PNH
- Eculizumab biosimilars
  - A Randomized, Double-Blind, Active-Controlled Phase 3 Study Evaluating the Efficacy and Safety of ABP 959 Compared With Eculizumab in Adult Subjects With PNH
- Coversin
  - Phase III Safety and Efficacy in Three-Part, Two-Arm, Randomised Open Label Evaluation in Patients With PNH
  - CONSERVE: rVA576 (Coversin) Long Term Safety and Efficacy Surveillance Study
- SKY59/RO711268/Crovalimab
  - Crovalimab, a sequential monoclonal antibody against C5 invented with recycling technology antibody
  - Engineered for extended self-administered subcutaneous dosing of small volumes once every 4 weeks.

**C3 Inhibitors:**
- AMY-101: A C3 inhibitor Compstatin analog
- APL-2: Phase 3, Randomized, Multicenter, Open-Label, Controlled Study to Evaluate the Efficacy and Safety of APL-2 in Patients With PNH

**Selective inhibitors of the alternative pathway**
- ACH-4471: Anti complement factor D (FD)
  - ACH-4471 is small oral FD inhibitor developed by Achillion which showed inhibitory activity of hemolysis in PNH in vitro
  - ACH-4471 was combined with eculizumab at three different doses (100, 150, or 200 mg thrice a day) to assess change in hemoglobin levels
- LNP023: Anti complement factor B (FB)
  - LNP023 is a novel oral small molecular weight compound, that inhibits alternative complement pathway (AP)
  - Blockade of the AP with oral LNP023 has the potential to prevent both intra- and extravascular hemolysis.

APE-2 PEGASUS Phase III Trial design

**Population**
PNH patients on eculizumab who continue to be anemic (Hb < 10.5 g/dL at screening), elevated retic counts

**Primary Endpoint**
Week 16 change from baseline in hemoglobin level

**Design**
Randomized 1:1 APL-2:eculizumab. 1 Month Run In; 4 Month Randomization; 6 Month Open Label; 1 Month Follow Up

**Dosing**
Dose 1080 mg 2/week, SC infusion via injection pump

**Sample size**
70 patients (35/group) randomized 1:1
Key Takeaways

- PNH is a rare disease with features of hemolysis, bone marrow failure and propensity for clotting events.
- Diagnosis can be delayed due to disease rarity and non-specific presentation/symptomatology.
- Outcomes for this disease have improved significantly with the availability of effective agents/complement inhibitors.
- Two C5 inhibitors are currently approved for the treatment of this disease with comparable efficacy and safety profile.
- Anemia / need for transfusion represent a diagnostic and therapeutic challenge.
- Anemia has multiple etiologies and has to be investigated accordingly.
- SCT is reserved for patients with PNH/ severe bone marrow failure, or those who do not respond to complement inhibition.
- Multiple novel agents explored in single and in combination trials are currently ongoing.
- Participation in ongoing clinical trials is highly encouraged.