Clinical Indications for Terminal Complement Inhibition

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Paroxysmal Nocturnal Hemoglobinuria
Protean Manifestations

- Hemoglobinuria
- Anemia (macrocytic)
  - Fe deficiency
- Pancytopenia
- Thrombosis (venous)
- Abdominal pain
- CNS
  - Transient impotence
  - Esophageal spasm
PNH: Natural History

- Median survival: 10 - 15 years
  - Worse in pts with thrombosis, or severe pancytopenia
  - Frequent severe paroxysms

- Thrombosis: leading cause of death
  - Occurs in 1/3 of patients

- 5% to 10% evolve to MDS/AML

- 1/3 of new cases evolve out of AA, the rest are de novo
Paroxysmal Nocturnal Hemoglobinuria

- Acquired Clonal Multipotent Hematopoietic Stem Cell Disease
- PIG-A mutation
  - X(p22.1)
- PIG-A gene product necessary for 1st step in the biosynthesis of GPI anchors
- PNH cells have deficiency or absence of all GPI anchored proteins
**PIG-A Coding Region**

**PIGA Mutations**
- Frameshift – small insertions/deletions → Stop codon
- Nonsense → Stop codon
- Splice defect → Deleted exon
- Missence (substitution) → May have residual activity
GPI-AP Biosynthesis:
Involves 10 steps and > 20 genes
**PNH**

Pathogenesis of hemolytic anemia

- **CD59**
  - Membrane inhibitor of reactive lysis
  - Prevents incorporation of C9 into C5b-8; thus, MAC does not form

- **CD55**
  - Decay accelerating factor
  - Block C3 convertase

- Protect cells from complement-mediated destruction
Lectin Pathway

Classical Pathway

Alternate Pathway

MBL, MASP, C4 + C2

C1q, C1r, C1s C4 + C2

C3 Factor B + D

C3 convertases C4b2a, C3bBb

CD55 blocks C3 Convertases

C3b

C5 convertases C4b2a3b, C3bBb3b

CD59 prevents formation of MAC

C5a

C6, C7, C8,

C5b

Membrane attack complex (MAC)

CD59

C9

COAGULATION

INFLAMMATION
Effects of Terminal Complement on RBCs and Clinical Consequences in PNH

- Complement Activation

- Intact RBC

- Free hemoglobin in the blood from destroyed PNH RBC

- Free hemoglobin is a nitric oxide scavenger

- Anemia

- Fatigue

- Thrombosis ?

- Renal Failure

- Anemia/Transfusions

- esophageal spasm, abdominal pain, male ED
DIAGNOSIS
GPI-AP serve as Receptors for Proaerolysin

• Pore-forming protoxin secreted by *Aeromonas hydrophila*

• PNH cells uniquely resistant to aerolysin because they lack GPI-anchors

• FLAER - FLuoresceinated AERolysin variant that binds GPI-anchors but does not form channels.

Brodsky et. al., Blood 1999 93:1749
Mukhina et. al., Brit. J. Haematol. 2001 115: 482
Flow Cytometric Diagnosis of PNH

PNH

Aplastic anemia

Brodsky RA Blood 113:6522-27, 2009
Complement inhibition is a highly effective therapy for classical PNH

- **Allogeneic BMT**
  - Only curative therapy

- **Eculizumab**
  - Humanized monoclonal
  - Antibody to C5

![Diagram of complement inhibition]
Lectin Pathway
- MBL, MASP, C4 + C2

Classical Pathway
- C1q, C1r, C1s
  - C4 + C2

Alternate Pathway
- C3
  - Factor B + D

C3 convertases
- C4b2a, C3bBb

C3b

C5 convertases
- C4b2a3b, C3bBb3b

C5a

CD55

COAGULATION

CD59

Eculizumab (FDA approval 2007)

Membrane attack complex (MAC)
Effect of Eculizumab on Time to First Transfusion

Effect of Eculizumab on Time to First Transfusion

Effect of Eculizumab on Time to First Transfusion

Reduction in LDH During Eculizumab Treatment in TRIUMPH and SHEPHERD

Brodsky et al, Blood 2008, 111: 1840-47
Lessons from Eculizumab Trials (TRIUMPH, SHEPHERD, EXTENSION)

• Safe
  – Mild side-effects
  – Increased risk for Neisserial infections (~0.5% per year)

• Effective
  – Decreases intravascular hemolysis
  – Decreases (>90%) or eliminates (50%) need for PRBC
  – Improves quality of life
  – Reduces the risk for thrombosis by >90%
Lessons from Eculizumab Trials – cont.
(TRIUMPH, SHEPHERD, EXTENSION)

• **Drawbacks**
  - Lifelong therapy intravenous therapy
  - Cost (> 350K a year)

• **Not as effective in patients with AA/PNH**
  - Does not treat bone marrow failure
  - Does not treat extravascular hemolysis

• **Ideal PNH patient (classical PNH)**
  - Large PNH populations (>10% type III red cells, > 50% PNH granulocytes)
  - LDH > 3 x upper limit of normal
  - Retics > 3%
Response to Eculizumab

Patients continue to have compensated extravascular hemolysis
Intravascular and Extravascular Hemolysis in PNH

**Absence of CD55 & CD59**

→ intravascular hemolysis

MAC forms

= C3

**Extravascular hemolysis**

Absence of CD55 & CD59

PNH patient on eculizumab
C3 coating on RBCs by flow cytometry.


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Response to Eculizumab is Variable in PNH

• 30 PNH patients treated with Eculizumab at JHU
  – CR: nl hgb for age and sex; LDH < 1.5 ULN
  – GPR: decrease in transfusions, LDH < 1.5 ULN
  – Sub: unchanged transfusional requirements

• 4 CR (13%)
• 16 GPR (53%)
• 10 sub (33%)
CR Associated with Decrease in PNH Red Cell Clone

Factors: underlying BMF
size of PNH RBCs
underlying inflammatory conditions
aHUS: Overview of the Disease and Diagnosis

- Non-immune hemolytic anemia, thrombocytopenia and renal impairment

- Often heralded by bloody diarrhea

- Most cases, especially children, due to E. Coli 0157 (Shiga-like toxin) Strep pneumonia.
aHUS: Classification

- Familial
  - Poor prognosis
  - 50-80% $\rightarrow$ ESRD or death
  - Genetic abnormalities of complement system
- Sporadic
  - Triggers: HIV, Cancer, organ TX, pregnancy, drugs (csa, ticlopidine, clopididrel etc.)
  - Genetic abnormalities of complement system
- Genetic abnormalities
  - Factor H, MCP (CD46), Factor I, Factor B, C3
Genetic Loss of Natural Inhibitors Leads to Chronic Uncontrolled Complement Activation

**Consequences**

- Anaphylaxis
- Inflammation
- Thrombosis

**Natural Inhibitors:**
- Factor H, I, MCP, CD55
- CD59

**Lectin Pathway**
- Immune Complex Clearance
- Microbial Opsonization

**Classical Pathway**
- Amplification

**Alternative Pathway**
- C3 + H2O - ALWAYS ACTIVE (Chronic)

**C3**

**C5**

**C5a**
- Potent Anaphylatoxin
- Chemotaxis
- Proinflammatory
- Leukocyte Activation
- Endothelial Activation
- Prothrombotic

**C5b-9**
- Membrane Attack Complex
- Cell Lysis
- Proinflammatory
- Platelet Activation
- Leukocyte Activation
- Endothelial Activation
- Prothrombotic

**Consequences**

- Cell Destruction
- Inflammation
- Thrombosis

The Genetic Deficiency of Complement Regulators Leads to Life-long Risk of Systemic Complement-Mediated TMA

1. Normal platelets, endothelial cells, and leukocytes are protected from natural complement activity by complement regulatory factors.

   ![Cell surface and fluid phase complement Inhibitors](image)

2. Without regulatory factors, permanent uncontrolled and excessive complement activation causes chronic platelet, endothelial cell, leukocyte activation.

   ![Leads to inflammation and multiple thrombi with occlusion of small blood vessels throughout the body (systemic TMA)](image)

   - Sudden and progressive organ damage and failure

Complement-Mediated TMA Leads to the Morbidities and Mortality in aHUS

Complement-Mediated Thrombotic Microangiopathy

Cardiovascular2,3,4,6
- Myocardial infarction
- Thromboembolism
- Cardiomyopathy
- Diffuse vasculopathy

Renal7,8,9,11,12
- Elevated creatinine
- Edema
- Malignant hypertension
- Renal failure
- Dialysis
- Transplant

CNS1,2,3,4,5
- Confusion
- Seizures
- Stroke
- Encephalopathy
- Diffuse cerebral dysfunction

Gastrointestinal2,3,5,10,11,12
- Liver necrosis
- Pancreatitis
- Diabetes Mellitus
- Colitis
- Diarrhea
- Nausea/vomiting
- Abdominal pain

Pulmonary1,6,14
- Dyspnea
- Pulmonary hemorrhage
- Pulmonary edema

Blood11
- Hemolysis
- Decreased platelets
- Fatigue
- Transfusions

Impaired Quality of Life13
- Fatigue
- Pain/anxiety
- Reduced mobility

**aHUS: Medical Evidence Challenges**

**Common Misperceptions**

**aHUS Patients Generally are Not Effectively and Safely Treated With Plasma Exchange/Infusion**

- 33-40% of patients die or progress to ESRD with the first clinical manifestation\(^1\),\(^2\)
- 65% of all patients die, require dialysis, or have permanent renal damage within the first year after diagnosis despite PE/PI\(^1\)
- No well-controlled clinical trials showing plasma exchange/infusion to be either safe or effective as aHUS therapy\(^3\),\(^4\)
- Frequent and severe complications in adults and in children\(^5\),\(^6\)
- Plasma exchange/infusion does not target the cause of aHUS: uncontrolled, excessive complement activation\(^2\)
  - Uncontrolled complement activation and resulting platelet activation demonstrated to persist during PE/PI\(^7\),\(^8\)

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Challenges in Diagnosing aHUS

- Clinical presentation can be similar to other systemic TMAs\(^1\)-\(^3\)
- Historical treatment did not require differential diagnosis between aHUS, TTP and STEC-HUS – historically grouped as TTP/HUS\(^4\),\(^5\)
- aHUS is a rare disease leading to lack of clinical suspicion
- Rarity of aHUS may impact accurate and rapid diagnosis
  - Perception that genetic mutation needs to be identified\(^6\)
  - Perception that aHUS is only a pediatric disease\(^6\)
  - Perception that aHUS is only a renal disease\(^6\)

aHUS: Diagnosis Does Not Require Identification of a Genetic Mutation

- Genetic mutation cannot be identified in 30%-50% of patients with aHUS\(^1\)
- Absence of identifiable genetic mutations does not rule out aHUS\(^1\)
- Results generally takes weeks to months – therefore does not impact initial clinical management
- Complement protein levels:\(^1\)
  - Serum C3 – normal in up to 80% of aHUS patients
  - Complement Factor H (CFH) protein levels – normal in 87% of aHUS patients with CFH mutation

aHUS:
Clinical Presentations Similar to Other Diseases of TMA
Clinical Presentation Alone Does Not Fully Differentiate aHUS from TTP

- **aHUS affects patients of all ages**
  - Perception: child ➤ “it’s aHUS”; adult ➤ “it’s TTP”
  - Medical evidence: 40% of aHUS patients are adults\(^1\)

- **aHUS patients frequently demonstrate CNS involvement**
  - Perception: patient has neurological symptoms ➤ “it’s TTP”
  - Medical evidence: up to 48% aHUS cases reported to have neurological dysfunction\(^2\)

- **ADAMTS13 activity differentiates between aHUS and TTP**
  - Perception: Clinical symptoms direct the differentiation between aHUS and TTP
  - Medical evidence: Severe ADAMTS13 activity separates TTP (<5%)\(^3\)

Clinical Presentation Alone Does Not Fully Differentiate aHUS and STEC-HUS

- Diarrhea is a common feature in aHUS
  - Perception: patients with a history of diarrhea ➤“it’s STEC-HUS”
  - Medical evidence: Diarrhea +/- blood reported in 30% of patients with aHUS

aHUS is a Clinical Diagnosis Supported by Appropriate Laboratory Exclusion of Other TMAs
Definition of aHUS

Signs and symptoms of complement-mediated TMA\(^1,2\)
- Decreased platelet count\(^1\)
- Evidence of microangiopathic hemolysis\(^1\)
- Evidence of organ impairment/damage (e.g., serum creatinine >ULN)\(^2,3\)

Differentiate from other TMA diseases\(^1,2\)
- ADAMTS13 Activity >5% → excludes severe ADAMTS13 deficiency (congenital or acquired TTP)\(^4,5,6,7\)
- Absence of positive STEC test → excludes STEC as sole cause of TMA\(^8\)

No requirement for identified complement gene mutation
- Genetic mutation cannot be identified in 30%-50% of patients with aHUS\(^5\)

**Differential Diagnosis for TMAs: aHUS, TTP and STEC-HUS**

**Thrombocytopenia**
- Platelet count <150,000 Or
- >25% Decrease from baseline

**Microangiopathic Hemolysis**
- Schistocytes and/or
- Elevated LDH and/or
- Decreased Haptoglobin and/or
- Decreased Hemoglobin

**Plus One or More of the Following:**

**Neurological Symptoms**
- Confusion and/or
- Seizures and/or
- Other Cerebral Abnormalities

**Renal Impairment**
- Elevated Creatinine and/or
- Decreased eGFR and/or
- Elevated Blood Pressure and/or
- Abnormal Urinalysis

**Gastrointestinal Symptoms**
- Diarrhea +/- Blood and/or
- Nausea/Vomiting and/or
- Abdominal Pain and/or
- Gastroenteritis

**Evaluate ADAMTS13 Activity and Shiga-toxin/EHEC* Test**

- ≤5% ADAMTS13 Activity
- >5% ADAMTS13 Activity
- Shiga-toxin/EHEC Positive

**TTP**

**aHUS**

**STEC-HUS**
Soliris Multinational, Multi-Center Clinical Program in aHUS (N=67)

Clinical Diagnosis of aHUS With:
- TMA (measured by platelet count, hemolysis)
- Organ Damage (serum creatinine ≥ ULN)
- ADAMTS13 > 5%; no positive STEC test
- No requirement for identified genetic mutation

Prospective
- Study C08-002 (N=17)
  Adult/Adolescent
- Study C08-003 (N=20)
  Adult/Adolescent

Retrospective
- Study C09-001 (N=30)
  19 Pediatric Patients

Long-term Extension Studies
86% (32/37) of patients continued Soliris treatment in extension studies
Study C08–003: Patient Population at Baseline

- Long duration since aHUS diagnosis and substantial organ damage despite chronic PE/PI
  - 48 months median duration (range, 0.66-286 mo) from aHUS diagnosis to screening
  - Patients had PE/PI for a median duration of 10 months (range, 2.4-47 mo) prior to Soliris treatment
  - 90% of patients with CKD Stage 3-5

- 40% of patients with 1 or more prior kidney transplants (1-4 per patient)

- 30% of patients with no identified genetic mutations
Renal Function Maintained During Soliris Treatment: Treatment Initiated 48 Months* After aHUS Diagnosis

- Mean change in eGFR during Soliris treatment: +6.1 mL/min/1.73 m²
- Median (range) change in eGFR during Soliris treatment: +5.0 (-1, 20) mL/min/1.73 m²
- No patient required new dialysis

*Median (range, 0.66-286 mo).
Study C08–002: Patient Population at Baseline

- aHUS patients with progressing clinical TMA complications despite intensive PE/PI

- Shorter duration since aHUS diagnosis compared with Study C08-003
  - 10 months median duration (range, 0.26-236 mo) from aHUS diagnosis

- 41% of patients received prior kidney transplant

- 24% of patients with no identified genetic mutations
Inhibition of Complement-Mediated TMA as Measured by Platelet Increase During Soliris Treatment

95% CI: 40-105 x10⁹/L

- LS-mean platelet increase between baseline and end of study: 73 x10⁹/L
- Significant increase in platelet count as early as Day 7
- Platelet increase sustained through end of study period

Least-Squares Mean (95% CI) Change in Platelets (x10⁹/L)

Days on Soliris Treatment
## Patient History and Outcomes Across Soliris Clinical Program in aHUS

<table>
<thead>
<tr>
<th>Study</th>
<th>Median (Range) Duration Since aHUS Diagnosis</th>
<th>Uncontrolled Complement Activation Inhibited</th>
<th>Complement-Mediated TMA Inhibited</th>
<th>Burden of Interventions Reduced</th>
<th>Renal Function</th>
<th>Requirement for Dialysis</th>
<th>Safety During Study Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>C08-003 Adolescent/Adult Prospective Study (N=20)</td>
<td>48 months (0.66-286 mo)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Maintained</td>
<td>No New Dialysis</td>
<td>Well tolerated*</td>
</tr>
<tr>
<td>C08-002 Adolescent/Adult Prospective Study (N=17)</td>
<td>10 months (0.26-236 mo)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Improved</td>
<td>Eliminated in 80%</td>
<td>Well tolerated*</td>
</tr>
<tr>
<td>C09-001 Retrospective Pediatric Study (N=19)</td>
<td>19 months (0.39-177 mo)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Improved</td>
<td>Eliminated in 50%, no new dialysis</td>
<td>Well tolerated†</td>
</tr>
</tbody>
</table>

*All patients alive; no meningococcal infections.
†1 patient died (not drug related); 1 meningococcal infection (post-study follow-up period).
Soliris Outcomes in Patients With aHUS

- Soliris inhibited uncontrolled complement activation and complement-mediated TMA during the study period.
- Soliris significantly reduced or eliminated the need for PE/PI and new dialysis.
- In patients with Soliris initiation 48 months* (range, 0.66-286 mo) after aHUS diagnosis, Soliris maintained renal function during the study period.
- In patients with Soliris initiation 10 months* (range, 0.26-236 mo) after aHUS diagnosis, Soliris resulted in sustained improvement in renal function and 80% of patients eliminated dialysis.
- Soliris was well tolerated in prospective studies.
- Prospective studies showed similar response to Soliris between patients with and without identified genetic mutation.
- Efficacy and safety in pediatric patients consistent with prospective studies of adolescent and adult patients.

*Median.