What’s new in aHUS?

aHUS Canada Annual Meeting 2014

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Christopher Patriquin MD FRCPC
Professional Disclosures

- Unrestricted Educational Grant
  - Alexion

- Advisory Board Honouria
  - Alexion
  - Celgene

- Travel Grants
  - Alexion
  - Amgen
  - Octapharma

GROUPE CANADIEN D'APHÉRÈSE
CANADIAN APHERESIS GROUP
Representing Apheresis Practitioners in Canada

CANADIAN PNH NETWORK
Partners in Care for PNH
Objectives

- **Background**
  - Overview of complement

- **Pathophysiology**

- **Diagnosis**
  - Differentiating from other TMA

- **Treatment**
  - Supportive
  - Eculizumab
  - Transplant
  - Upcoming treatments

- **Future Directions**
Overview of Complement
Overview of Complement

- Branch of the immune system
  - Highly conserved, prehistoric (dates back >1 billion years)
  - Traditionally associated with innate immunity

- First-line defense
  - Viruses, bacteria, foreign material, cancer, apoptosis
    - Recognition of conserved molecular patterns
  - Activation and amplification by cascade of serine proteases

- Tightly controlled
  - Over 40 proteins so far described
  - Multiple pathways of activation & interaction
  - Regulatory molecules circulating & cell-bound

Zhu Y et al. (2005) EMBO J
Complement Activation Pathways

- Classical
  - First pathway described
  - Exposure to microbes and/or Fc immunoglobulins

- Lectin
  - Triggered by exposure to microbial carbohydrates
  - Mannose-binding lectin (MBL) binds to MASP

- Alternative
  - No specific trigger, always “on” (tickover)
  - Can be amplified by proteins from any pathway

Image modified from: Noris M et al. (2012) Nat Rev Nephrol
Effector Functions of Complement

- Anaphylotoxin production
  - C3a, C5a
  - Potent proinflammatory molecules

- Opsonisation
  - C3b, iC3b, C3d bind target surfaces → mark for removal
  - Facilitate transport to RES (direct or via immune complexes)
    - Role of RBCs in the immune systems (CR1)

- Cytolysis
  - Binding of C5b to C6-9n
  - Direct transmembrane damage and apoptosis (cell death)

Botto M et al. (2009) Mol Immunol
# Regulators of Complement Activation

<table>
<thead>
<tr>
<th>Regulatory Molecule</th>
<th>Function(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C1 Inhibitor (C1INH)</strong></td>
<td>Binds to C1r and C1s, causing dissociation from C1q.</td>
</tr>
<tr>
<td><strong>C4 Binding Protein (C4BP)</strong></td>
<td>Binds to C4b, causing dissociation of C2a.</td>
</tr>
<tr>
<td></td>
<td>Associates as a cofactor with Complement Factor I (CFI) to cleave C4b.</td>
</tr>
<tr>
<td><strong>Complement Receptor 1 (CR1)</strong></td>
<td>Binds to C4b, causing dissociation of C2a.</td>
</tr>
<tr>
<td></td>
<td>Binds to C3b, causing dissociation of Bb.</td>
</tr>
<tr>
<td></td>
<td>Serves as a cofactor for CFI.</td>
</tr>
<tr>
<td><strong>Complement Factor H (CFH)</strong></td>
<td>Binds to C3b, causing dissociation of Bb.</td>
</tr>
<tr>
<td></td>
<td>Serves as a cofactor for CFI.</td>
</tr>
<tr>
<td><strong>Complement Factor I (CFI)</strong></td>
<td>Cleaves and inactivates C3b and/or C4b.</td>
</tr>
<tr>
<td><strong>Membrane Cofactor Protein (MCP or CD46)</strong></td>
<td>Promotes cleavage and inactivation of C3b and/or C4b by CFI.</td>
</tr>
<tr>
<td><strong>Decay Accelerating Factor (DAF or CD55)</strong></td>
<td>Causes dissociation of C3 convertases (i.e. C4b from C2a, C3b from Bb).</td>
</tr>
<tr>
<td><strong>Membrane Inhibitor of Reactive Lysis (MIRL/CD59)</strong></td>
<td>Binds to C8 and prevents association of C9, blocking MAC formation.</td>
</tr>
<tr>
<td><strong>Thrombomodulin (THBD)</strong></td>
<td>Inactivates C3b in the presence of CFH</td>
</tr>
<tr>
<td></td>
<td>Activates TAFI, which degrades active C3a and C5a</td>
</tr>
</tbody>
</table>

Table 1 The chromosomal location of genes encoding complement proteins, inherited deficiency and disease associations

<table>
<thead>
<tr>
<th>Component (or subunit)</th>
<th>Symbol of gene</th>
<th>Chromosomal location</th>
<th>Gene cluster</th>
<th>Disease association of the homozygote deficiency</th>
<th>Genetic basis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Activation components</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1q: A chain</td>
<td>C1QA</td>
<td>1p36.12</td>
<td></td>
<td>Immune complex diseases (ICD), SLE, SNP, recurrent infections (RI)</td>
<td></td>
</tr>
<tr>
<td>C1q: B chain</td>
<td>C1QB</td>
<td>1p36.12</td>
<td></td>
<td>ICD, SLE, RI</td>
<td>SNP</td>
</tr>
<tr>
<td>C1q: C chain</td>
<td>C1QC</td>
<td>1p36.11</td>
<td></td>
<td>ICD, SLE, RI</td>
<td>SNP</td>
</tr>
<tr>
<td>C1r</td>
<td>C1R</td>
<td>12q13</td>
<td></td>
<td>ICD, SLE, RI</td>
<td>–</td>
</tr>
<tr>
<td>C1s</td>
<td>C1S</td>
<td>12p13</td>
<td></td>
<td>ICD, SLE, RI</td>
<td>–</td>
</tr>
<tr>
<td>C2</td>
<td>C2</td>
<td>6p21.3</td>
<td>C-MHC III</td>
<td>SLE, Neisserial infections (NI), RI</td>
<td>SNR, gene partial deletion</td>
</tr>
<tr>
<td>Factor B</td>
<td>CFB</td>
<td>6p21.3</td>
<td>C-MHC III</td>
<td>Homozygote: vary rare (fatal), heterozygote: NI</td>
<td>–</td>
</tr>
<tr>
<td>C4A (isotype)</td>
<td>C4A</td>
<td>6p21.3</td>
<td>C-MHC III</td>
<td>ICD, RI, autoimmune disorders (e.g. SLE, type 1 diabetes mellitus, autoimmune hepatitis, scleroderma), RI, autoimmune disorders (e.g. primary biliary cirrhosis)</td>
<td>Gene deletion, gene conversion, non-expression due to insertion</td>
</tr>
<tr>
<td>C4B (isotype)</td>
<td>C4B</td>
<td>6p21.3</td>
<td>C-MHC III</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C3</td>
<td>C3</td>
<td>19p13.3-p13.2</td>
<td></td>
<td>Severe disseminated pyogenic infections, ICD</td>
<td>SNP, gene partial deletion</td>
</tr>
<tr>
<td><strong>Regulators and receptors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4 binding protein: α chain</td>
<td>C4BPA</td>
<td>1q32</td>
<td></td>
<td>RCA</td>
<td>–</td>
</tr>
<tr>
<td>C4 binding protein: β chain</td>
<td>C4BPB</td>
<td>1q32</td>
<td></td>
<td>RCA</td>
<td>–</td>
</tr>
<tr>
<td>CR1 (CD35)</td>
<td>CR1</td>
<td>1q32</td>
<td></td>
<td>RCA, C1q, glomerulonephritis</td>
<td>SNP</td>
</tr>
<tr>
<td>CR2 (CD21)</td>
<td>CR2</td>
<td>1q32</td>
<td></td>
<td>RCA</td>
<td>–</td>
</tr>
<tr>
<td>CD55 (decay accelerating factor, DAF)</td>
<td>CD55</td>
<td>1q32</td>
<td>RCA</td>
<td>Inhibitory blood group phenotype</td>
<td>Gene partial deletion</td>
</tr>
</tbody>
</table>

*To be continued*
<table>
<thead>
<tr>
<th>Component (or subunit)</th>
<th>Symbol of gene</th>
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<th>Genetic basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD46 (membrane cofactor protein, MCP) Factor H</td>
<td>CD46</td>
<td>1q32</td>
<td>RCA</td>
<td>Atypical hemolytic uremic syndrome (HUS), Nf, glomerulonephritis, HUS, thrombotic thrombocytopenic purpura, age-related macular degeneration (AMD)</td>
<td>SNP, gene partial deletion</td>
</tr>
<tr>
<td>Complement Factor H-Related 1</td>
<td>CFHR1</td>
<td>1q32</td>
<td>RCA</td>
<td>Atypical HUS; protective for AMD</td>
<td>Deletion</td>
</tr>
<tr>
<td>Complement Factor H-Related 2</td>
<td>CFHR2</td>
<td>1q31.3</td>
<td>RCA</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Complement Factor H-Related 3</td>
<td>CFHR3</td>
<td>1q32</td>
<td>RCA</td>
<td>Atypical HUS; protective for AMD</td>
<td>Deletion</td>
</tr>
<tr>
<td>Complement Factor H-Related 4</td>
<td>CFHR4</td>
<td>1q32</td>
<td>RCA</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Complement Factor H-Related 5</td>
<td>CFHR5</td>
<td>1q31.3</td>
<td>RCA</td>
<td>–</td>
<td>–</td>
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<tr>
<td>CSMD2 (CUB and Sushi multiple domains 2)</td>
<td>CSMD2</td>
<td>1p35.1-34.3</td>
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<tr>
<td>CSMD1 (CUB and Sushi multiple domains 1)</td>
<td>CSMD1</td>
<td>8p23.2</td>
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<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CSMD3 (CUB and Sushi multiple domains 3)</td>
<td>CSMD3</td>
<td>8p23.3</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CD59 (membrane inhibitor of reactive lysis, MIRL)</td>
<td>CD59</td>
<td>11p13</td>
<td>–</td>
<td>Paroxysmal nocturnal hemoglobinuria</td>
<td>–</td>
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<tr>
<td>Factor I</td>
<td>CFI</td>
<td>4q26</td>
<td>RI</td>
<td>–</td>
<td>SNP</td>
</tr>
<tr>
<td>C1 Inhibitor</td>
<td>SERPING1</td>
<td>11q12-q13.1</td>
<td>–</td>
<td>Angioedema</td>
<td>SNP, gene partial deletion</td>
</tr>
<tr>
<td>MASP-3</td>
<td>MASP1</td>
<td>3q27-q28</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MAP44</td>
<td>MASP1</td>
<td>3q27-q28</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MAP19</td>
<td>MASP2</td>
<td>1p36.3-p36.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CR3 (CD11b/CD18) α-chain (CR3A,CD11b) β-chain (β1-integrin (Leucocyte adhesion molecule, CD18))</td>
<td>ITGAM</td>
<td>16p11.2</td>
<td>–</td>
<td>Recurrent bacterial skin infections</td>
<td>–</td>
</tr>
<tr>
<td>CR4 (CD11c/CD18) α-chain (α1-integrin (CD11c))</td>
<td>ITGB2</td>
<td>21q22.3</td>
<td>Leucocyte adhesion deficiency</td>
<td>SNP, gene partial deletion</td>
<td></td>
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<tr>
<td>C5aR1 (C5a receptor 1)</td>
<td>C5AR1</td>
<td>19q13.3-q13.4</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C3aR1 (C3a receptor 1)</td>
<td>C3AR1</td>
<td>12p13.31</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>CD93</td>
<td>CD93</td>
<td>20p11.21</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Complement-Coagulation Interplay

- Similarities between the two systems
  - Innate defense mechanisms
    - Initiated by altered cell surfaces (localization)
  - Initiation, amplification, propagation

- Direct interactions
  - Thrombin-activated C5 cleavage $\rightarrow$ MAC formation
  - Anaphylotoxin release with exposure to IX, X, XI, XII
  - C3 & MAC enhance IIa-mediated platelet aggregation
  - Endothelial activation by C5a, MAC exposes TF

Reviewed in: Oikonomopoulou K et al. (2012) Semin Immunopathol
Complement Involved in Many Diseases

- Paroxysmal nocturnal hemoglobinuria
- Thrombotic microangiopathies (TMA)
  - TTP, STEC-HUS, aHUS
- Cold agglutinin disease (CAD)
- Hereditary angioedema
- Autoimmune disease (RA, SLE, SS)
- Anti-phospholipid syndrome (APS)
- MPGN Type II (DDD)
- Immune complex diseases (ICD)
- Multiple sclerosis
- Myasthenia gravis
- Age-related macular degeneration
- Recurrent fetal loss
- Recurrent infections (pyogenic, fungal)
- Ischemia-reperfusion injury
Renal Complementopathy by Pathway

- Classical
  - Lupus nephritis, PSGN, anti-GBM/Goodpastures

- Lectin
  - Ischemia-reperfusion injury

- Alternative
  - C3 nephritis/DDD, HUS (most forms), TMAs

- Combined
  - IgA, ANCA-astd, membranous nephritis, MPGN-I, TMAs

Hematologic Complementopathies

- Cold agglutinin disease
- Paroxysmal cold hemoglobinuria
- Thrombotic microangiopathies
  - TTP (1° and 2°)
  - Hemolytic uremic syndromes
    - aHUS
    - STEC-HUS, pneumococcal HUS
  - Others — drugs, malignancy, DIC/sepsis, HELLP, (C)APS
- Paroxysmal nocturnal hemoglobinuria
Thrombotic Microangiopathies

- Multiple causes
  - Final common pathway

- Similar (initial) presentation
  - Non-immune hemolysis
  - Thrombocytopenia (low platelets)
  - End-organ damage

Image from: TMA Forum 2014, Vienna AU
Licht C et al.
Blood.
2009
Noris M et al. NEJM. 2009
Renal Complementopathy - TMAs

Clinical consequences:
- Platelet consumption
- Mechanical haemolysis
- Blood clotting
- Vessel occlusion
- Inflammation
- Ischaemia

Systemic multi-organ complications

Image from: TMA Forum 2014, Vienna AU
Noris M et al. NEJM. 2009
Causes of TMA

- **Primary**
  - TTP
  - HUS

- **Secondary**
  - Sepsis/DIC
  - Malignancy – direct or paraneoplastic
  - Autoimmune – SRC, APS, SLE
  - Drugs – CsA, FK506, sirolimus, quinine, HIT
  - Vitamin B12 deficiency
  - Infections – HIV, pneumococcus, influenza
  - Pregnancy – HELLP, preeclampsia

Noris M et al. (2012) Nat Rev Nephrhol
Schmidtke J et al. (2013) AJKD
Thrombotic Thrombocytopenic Purpura
Thrombotic Thrombocytopenic Purpura

- Primary function of von Willebrand Factor (vWF)
  - Supports platelet adhesion at sites of injury
  - Binds GP1b/IX/V, IV-collagen
  - Unfolds under shear forces — cleaved by ADAMTS13

- Deficient or dysfunctional ADAMTS13
  - ULvWF multimers not cleaved, but unfolded
  - Increased platelet aggregation, activation
  - Thrombogenesis, platelet consumption

Tsai HM (2013) AJM
Pathophysiology of TTP

Image from: Tsai HM (2013) AJM
TTP – The Classic Pentad

- The classic TTP “pentad”
  - Microangiopathic hemolytic anemia
  - Thrombocytopenia
  - Renal failure
  - Fever
  - Neurological symptoms

- Initiate therapy before the pentad is achieved
  - Helpful before available testing
  - Full pentad predicts mortality
  - Non-specific: components seen in HUS and other TMAs

Furlan M et al. (1998) NEJM
Tsai HM et al. (1998) NEJM
Thrombotic Thrombocytopenic Purpura

- Causes of TTP
  - ADAMTS13 deficiency (inherited) – rare
  - ADAMTS13 inhibition via autoantibody – most common
- Consumptive thrombocytopenia, thrombosis does not occur when ADAMTS13 > 10%

Remuzzi G et al. (2002) Blood
Veyradier A et al. (2001) Blood
Sarode R et al. (2013) J Clin Apheresis
Cataland S & Wu HM (2014) Blood
ADAMTS13 Testing

- **Diagnostic**
  - Not necessary/recommended to wait for it
  - Most assays can detect <5%

- **Prognosis/monitoring**
  - ADAMTS13 deficiency correlates with relapse
  - Isolated deficiency does not require treatment (2\textsuperscript{nd} hit?)
    - Possible role for preventive therapy (rituximab)

- **Pre-analytic issues**
  - Hyperbilirubinemia and plasma-free Hb
  - EDTA, urea inhibit the enzyme

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Zheng XL et al. (2004) Blood
Kremer-Hovinga JA et al. (2010) Blood
Sarode R et al. (2013) J Clin Apheresis
Predicting ADAMTS13 Deficiency

- Time from TMA presentation to ADAMTS13 test results highly variable, centre-to-centre

- Prediction of ADAMTS13 deficiency using readily available laboratory tests
  - Platelet count <25-30 x 10⁹/L
  - Creatinine <150-200 µmol/L
  - ANA+
## Predicting ADAMTS13 Deficiency

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Adjusted Odds Ratio</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine level ≤200 μmol/L (2.26 mg/dL)</td>
<td>23.4</td>
<td>8.8–62.5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Platelet count ≤30×10⁹/L</td>
<td>9.1</td>
<td>3.4–24.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Positive ANA</td>
<td>2.8</td>
<td>1.0–8.0</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

### At Least 1 Positive Criterion vs All 3 Criteria Positive

<table>
<thead>
<tr>
<th></th>
<th>At Least 1 Positive Criterion</th>
<th>All 3 Criteria Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>98.8 (96.9–100)</td>
<td>46.9 (41.3–53.1)</td>
</tr>
<tr>
<td>Specificity</td>
<td>48.1 (38.9–59.3)</td>
<td>98.1 (94.4–100)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>85.0 (82.6–87.7)</td>
<td>98.7 (96.4–100)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>93.3 (85.2–100)</td>
<td>38.6 (35.8–41.9)</td>
</tr>
</tbody>
</table>

Data are provided as median percent with 95% confidence interval.
Treatment of TTP

- **Therapeutic plasma exchange (TPE)**
  - Initiate ASAP (mortality >90% → 10%)
    - Temporize for transfer
    - Earlier initiation improves outcome/survival
  - Exchange 1.5PV daily until day 2+ after plts normalize

- **Exchange fluid**
  - (F)FP – most readily available
  - CSP – lower [ULvWF], questionable added benefit
  - SDP – pooled from NAT- donors, S/D treatment
    - Fewer allergic reactions, pulmonary complications
    - ULvWF levels similar to CSP
    - Prion-reduction techniques

References:
Rock G et al. (1991) NEJM
Rock G et al. (1996) Br J Haematol
Rock G et al. (2005) Br J Haematol
Scully M et al. (2007) Vox Sang
Treatment of TTP

- **Corticosteroids**
  - No firm evidence – common to give IV methylpred
  - Higher doses may be better (10mg/kg vs 1mg/kg)

- **Rituximab (anti-CD20 monoclonal antibody)**
  - Relapses – monitor ADAMTS13 and clinical parameters
    - CAG study wrapping up
  - Initial presentation – fewer exchanges, shorter LOS, 80% relapse-free, prolonged TTNT (24 months)

- **Adjuncts**
  - ASA & VTE prophylaxis (when plts >50), folic acid

Scully M et al. (2011) Blood
McDonald V et al. (2010) JTH
Westwood JP et al. (2013) JTH
Hemolytic Uremic Syndromes
The Hemolytic Uremic Syndromes

- **Typical (STEC-HUS)**
  - Mediated by Shiga toxins (*E. coli, Shigella*)
  - More common in children (adults in outbreaks)

- **Pneumococcal**
  - Exposure of cryptic RBC T-antigen to natural anti-T IgM

- **Atypical HUS (aHUS)**
  - Dysfunctional AP function due to improper regulation

- **Diarrhea is not necessarily helpful**
  - 20-40% in aHUS
  - 10-15% in TTP
  - Moving away from the term diarrheal (D+ vs D-) HUS

George JN (2010) Blood
Atypical Hemolytic Uremic Syndrome
Atypical Hemolytic Uremic Syndrome

- Genetic/autoimmune-mediated AP dysfunction

- Chronic progressive course with premature mortality
  - 33-40% of patients die or progress to ESRD with the first clinical manifestation
  - 65% of all patients with and without identified mutations die, require dialysis, or have permanent renal damage within 1 year after diagnosis despite plasma exchange or plasma infusion
    - Same with/without mutations

Up to 70% of patients (with the most common mutation) die, require dialysis or have permanent renal damage within 1 year.

Sallee M et al. *Nephrol Dial Transplant.* 2010
Noris M et al. *CJASN.* 2010
Diagnosis aHUS

- RCA mutations – various techniques (time-intensive)
  - Anti-CFH antibodies

- ADAMTS13 normal (?)

- Readily available testing
  - Platelets > 30
  - Creatinine > 150-200
  - Time to response with TPE?

- Standard complement levels (low C3, normal C4)
  - Seen in only 50% of patients with mutations
  - Complement activity reported in other TMAs

References:
Fig 1. *Sheep erythrocyte haemolysis assay in plasma samples from patients with thrombotic microangiopathy.*
Variations in Complement “Footprint”

<table>
<thead>
<tr>
<th>Clinical Diagnosis</th>
<th>Complement Biomarkers (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Factor Bb (244.3~960.8)</td>
</tr>
<tr>
<td>Acquired TTP#7 (n=38)</td>
<td>2153 (343-5448)</td>
</tr>
<tr>
<td>aHUS# (n=19)</td>
<td>7386 (603-30610)</td>
</tr>
<tr>
<td>p-value*</td>
<td>0.063</td>
</tr>
</tbody>
</table>

Cataland S et al. (2014) Blood
Atypical Hemolytic Uremic Syndrome

- Most genetic causes found within the last 20 years
- Currently, 30-50% will not have an identified mutation

Rose KL et al. (2008) J Clin Invest
# Epidemiology of aHUS Subtypes

**Table 3 Main clinical characteristics of patients with atypical hemolytic uremic syndrome according to complement abnormality**

<table>
<thead>
<tr>
<th>Gene or subgroup</th>
<th>Frequency in aHUS</th>
<th>Minimal age at onset</th>
<th>Risk of death or ESRD at 1&lt;sup&gt;st&lt;/sup&gt; episode or within &lt; 1 y</th>
<th>Risk of relapses</th>
<th>Risk of recurrence after renal transplantation</th>
<th>Plasma therapy indicated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CFH</strong></td>
<td>20-30%</td>
<td>Birth</td>
<td>50-70%</td>
<td>50%</td>
<td>75-90%</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>any age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CFI</strong></td>
<td>4 -10%</td>
<td>Birth</td>
<td>50%</td>
<td>10-30%</td>
<td>45-80%</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>any age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MCP</strong></td>
<td>5 -15%</td>
<td>&gt; 1 y</td>
<td>0-6%</td>
<td>70-90%</td>
<td>&lt; 20%</td>
<td>Questionable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>any age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C3</strong></td>
<td>2 -10%</td>
<td>7 m</td>
<td>60%</td>
<td>50%</td>
<td>40-70%</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>any age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CFB</strong></td>
<td>1-4%</td>
<td>1 m</td>
<td>50%</td>
<td>3/3 not in ESRD</td>
<td>100%</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>any age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>THBD</strong></td>
<td>3 -5%</td>
<td>6 m</td>
<td>50%</td>
<td>30%</td>
<td>1 patient</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rare</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-CFH Ab</td>
<td>6%</td>
<td>Mostly 7-11 y</td>
<td>30-40%</td>
<td>40-60%</td>
<td>Yes if high Ab titer</td>
<td>Yes (+ IS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CFH: factor H; CFI: factor I; MCP: membrane cofactor protein; CFB: factor B; THBD: thrombomodulin; Ab, antibodies; ESRD: end stage renal disease; IS: immunosuppressive treatment.

Loirat & Fremeaux-Bacchi (2011) Orphanet
Atypical Hemolytic Uremic Syndrome

- Types of complement dysfunction in aHUS
  - Loss-of-function (CFH, CFHR, CFI, MCP, THBD)
  - Gain-of-function (C3, CFB)
  - Autoantibodies (vs CFH)
  - Combined mutations also documented

Tsai HM (2013) AJM
Nishimura et al. (2014) NEJM
Lemaire M et al. (2013) Nat Genetics
Main Complement Mutations in aHUS

- Complement factor H (CFH) – 20-30%
  - Cofactor for CFI, enhances C3 dissociation/inactivation
  - Over 100 mutations identified – mostly qualitative
    - Most affect the C-terminus
  - Very similar to CFHR1-5 genes (risk of crossover)
  - Some mutations associated with anti-CFH autoantibodies
    - 5-10% total cases (25-50% of pediatric disease)

- Membrane cofactor protein (MCP, CD46) – 5-15%
  - Most mutations (75%) quantitative
  - Other mutations qualitative (impaired binding to C3, etc.)

References:
Caprioli J et al. (2006) Blood
Saunders RE et al. (2007) Hum Mutat
Joszi M et al. (2008) Blood
Warwicker P et al. (1998) Kidney Int
Noris M et al. (2003) Lancet
Main Complement Mutations in aHUS

- Complement factor I (CFI) – 4-10%
  - Quantitative dysfunction in 50% of cases

- Complement factor B (CFB) – 1-4%
  - Increased affinity for C3b
  - Hyperactive C3 convertase (AP), resists dissociation

- Complement protein 3 (C3) – 2-10%
  - Decreased binding of C3b to RCA

- Thrombomodulin (THBD) – 3-5%
  - Less efficient inactivation of cell-bound C3b

Goicoechea de Jorge E et al. (2007) PNAS
Fremeaux-Bacchi V et al. (2008) Blood
Delvaeye M et al. (2009) NEJM
Visceral Damage in aHUS

- Multiple pathophysiologic processes
  - MAC-induced endothelial injury \(\rightarrow\) thrombosis
  - Endothelial damage/inflammation \(\rightarrow\) edema/occlusion
  - Anaphylotoxin release \(\rightarrow\) chemotaxis, permeability

- Multiple reasons for organ dysfunction
  - Thrombosis (MAHA, TCP)
  - Non-thrombotic stenosis (MAHA, +/- TCP)
  - Vascular permeability (no/minimal MAHA, TCP)
    - Ex. Cerebral/pulmonary edema, PRES, effusions, anasarca
    - Common in aHUS (30-40%), rare in TTP

Tsai HM (2013) AJM
Nishimura et al. (2014) NEJM
Lemaire M et al. (2013) Nat Genetics
# Systemic Impact of aHUS

<table>
<thead>
<tr>
<th>System</th>
<th>Signs / symptoms</th>
<th>Number (%) of patients with complication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal</td>
<td>Kidney impairment</td>
<td>30 (100%)</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Thrombi (various locations), cardiac arrest, cardiomyopathy</td>
<td>11 (37%)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Diarrhoea, vomiting, pancreatitis, splenic vein occlusion</td>
<td>11 (37%)</td>
</tr>
<tr>
<td>Neurologic</td>
<td>Seizure, acute disseminated encephalomyelitis, stroke, transient ischaemic attacks, facial paralysis, headache</td>
<td>5 (17%)</td>
</tr>
<tr>
<td>aHUS complications in &gt;1 system</td>
<td></td>
<td>19 (63%)</td>
</tr>
</tbody>
</table>

Langman et al. (2012). EHA Annual Congress 2012 (Poster 0490)
Systemic Complications in aHUS

**Renal**
Up to 50% of patients progress to ESRD\(^1,2\)
- Elevated creatinine\(^3,4\)
- Proteinuria\(^3,4\)
- Oedema, malignant hypertension\(^3,4\)
- Decreased eGFR\(^3,4\)

**CNS**
Up to 48% of patients experience neurological symptoms\(^6\)
- Confusion\(^3,6\)
- Stroke\(^6\)
- Encephalopathy\(^4\)
- Seizure\(^3,5\)

**Blood**
- Thrombocytopenia\(^3\)
- Decreased haptoglobin\(^3\)
- Elevated LDH\(^3\)
- Decreased haemoglobin\(^3\)
- Schistocytes\(^3\)

**Cardiovascular**
Up to 43% of patients experience cardiovascular symptoms\(^5\)
- Myocardial infarction\(^7,8\)
- Hypertension\(^2,9\)
- Diffuse vasculopathy\(^8\)
- Peripheral gangrene\(^2,10\)
- Arterial stenosis\(^8\)

**Gastrointestinal**
Up to 30% of patients present with diarrhoea\(^11\)
- Colitis\(^6\)
- Nausea / vomiting\(^12\)
- Pancreatitis\(^12\)
- Abdominal pain\(^6,12\)
- Gastroenteritis\(^5,12\)
- Liver necrosis\(^5\)

**Pulmonary**
- Dyspnea\(^7\)
- Pulmonary haemorrhage\(^13\)
- Pulmonary oedema\(^7,8\)

**Visual**
- Ocular occlusion\(^14\)
Treatment of aHUS
Best Available Care for aHUS

⁻ Therapeutic plasma exchange (factor replacement)
  - Unpredictable end-organ damage (esp. extrarenal)
    - No controlled trials showing benefit in aHUS
    - Serious complications possible (55% pediatric, 15% adult)

⁻ Dialysis
  - Minimal impact on extrarenal disease

⁻ Transplant
  - Kidney, CLKT

⁻ Supportive care
  - Transfusions
  - Antihypertensives

## Best Available Care for aHUS

<table>
<thead>
<tr>
<th>Affected protein</th>
<th>Short-term outcome to PE / PI*</th>
<th>Long-term outcome to PE / PI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor H</td>
<td>60%</td>
<td>Death or ESRD: 70–80%</td>
</tr>
<tr>
<td>CFHR1, R3</td>
<td>70–80%</td>
<td>ESRD: 30–40%</td>
</tr>
<tr>
<td>MCP</td>
<td>No definitive indication for therapy</td>
<td>Death or ESRD: &lt;20%</td>
</tr>
<tr>
<td>Factor I</td>
<td>30–40%</td>
<td>Death or ESRD: 60–70%</td>
</tr>
<tr>
<td>Factor B</td>
<td>30%</td>
<td>Death or ESRD: 70%</td>
</tr>
<tr>
<td>C3</td>
<td>40–50%</td>
<td>Death or ESRD: 60%</td>
</tr>
<tr>
<td>THBD</td>
<td>60%</td>
<td>Death or ESRD: 60%</td>
</tr>
</tbody>
</table>

*Short-term outcome was defined as the rate response to short-term plasma therapy; long-term outcome was defined as the outcome 5–10 years after onset.
Anti-complement therapy: eculizumab

a. Human germline framework regions

b. Eculizumab epitope

C5 convertase

C5 convertase

C5a

C5b

C5a

C5b

C7, C6, C5b

Membrane attack complex C5b-9

Zuber J et al. (2012) Nat Rev Nephrol
Terminal Complement Inhibitor Eculizumab in Atypical Hemolytic–Uremic Syndrome


ABSTRACT
# C08-002/003: Baseline Demographics

<table>
<thead>
<tr>
<th></th>
<th>Patients with long duration of aHUS and CKD (C08-003)</th>
<th>aHUS patients with progressing TMA (C08-002)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Onset</strong></td>
<td>48 months’ median duration from aHUS diagnosis to screening (range 0.66–286)</td>
<td>10 months’ median duration from aHUS diagnosis to screening (range 0.26–236)</td>
</tr>
<tr>
<td><strong>PE / PI</strong></td>
<td>Long-term PE / PI</td>
<td>Intensive PE / PI</td>
</tr>
<tr>
<td></td>
<td>• 10 months’ median duration on PE / PI (range 2.4-47)</td>
<td>• 0.7 months median duration on PE / PI</td>
</tr>
<tr>
<td></td>
<td>• Median 1.5 PE / PI sessions in the week before eculizumab treatment</td>
<td>• 6 median PE / PI sessions in the week before eculizumab treatment</td>
</tr>
<tr>
<td><strong>Renal damage</strong></td>
<td>50% of patients with CKD Stage 4–5</td>
<td>70% of patients with CKD Stage 4-5</td>
</tr>
<tr>
<td></td>
<td>2 patients on chronic dialysis for ESRD</td>
<td>5 patients on dialysis</td>
</tr>
<tr>
<td><strong>Transplant</strong></td>
<td>40% of patients received ≥1 prior kidney transplant (1–4 per patient)</td>
<td>41% of patients received prior kidney transplant</td>
</tr>
<tr>
<td><strong>Genetic mutations</strong></td>
<td>30% of patients had no identified genetic mutation</td>
<td>24% of patients had no identified genetic mutation</td>
</tr>
</tbody>
</table>
### C08-002/003: Study Designs

<table>
<thead>
<tr>
<th></th>
<th>Patients with long duration of aHUS and CKD (n=20)</th>
<th>aHUS patients with progressing TMA (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction in TMA (change in platelet count)</td>
<td>✓</td>
<td>Primary</td>
</tr>
<tr>
<td>TMA event-free status&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Primary</td>
<td>✓</td>
</tr>
<tr>
<td>Haematological normalisation&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>Co-primary</td>
<td>Co-primary</td>
</tr>
<tr>
<td>Reduction in PE / PI or new dialysis (TMA intervention rate)</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Change in renal function (eGFR, CKD stage)</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>HRQoL measures</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

- All primary analyses were performed in the intent-to-treat population

---

Legendre et al. (2013) NEJM
# Eculizumab Dosing for aHUS

<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>Week → 1 2 3 4</td>
<td>5 6 7 8 9 and every 2 weeks thereafter</td>
</tr>
<tr>
<td><strong>Neisseria meningitidis vaccination</strong></td>
<td>Eculizumab dose, mg → 900 900 900 900 1200 X 1200 X 1200</td>
<td></td>
</tr>
</tbody>
</table>

- **Administration:** IV infusion over 35 min every 7 days during induction and every 14 days during maintenance\(^1\)
  - Dose adjustment to every 12 days may be necessary

- **Meningococcal prophylaxis:** patients received meningococcal vaccination prior to receipt of eculizumab or received prophylaxis treatment with appropriate antibiotics until 2 weeks after vaccination\(^1\)

- **Severe TMA complications:** observed in aHUS patients deviating from recommended dosing schedule\(^1\)
  - 5 / 18 patients experienced TMA complications following missed dose
  - Eculizumab was re-initiated in 4 / 5 patients

---

Eculizumab Product Monograph(2013)
Gruppo RA et al. ISTH World Congress 2011
C08-002: Effect on Dialysis

- Patients eliminated dialysis within 14 days following initiation of eculizumab
- The benefit was sustained through the entire study period (median eculizumab 2 years)
- Long-term eculizumab treatment improved renal function and prevented progression of renal disease
C08-002: Effect on Plasma Therapy

- In every patient:
  - Reduction of PE / PI
- Reduction from:
  - Median 6 interventions per patient per week before eculizumab treatment, to...
  - Median 0 interventions during study period
- Sustained improvements (for all 13 patients who entered the extension study) for the entire study period (median eculizumab 64 weeks)
C08-003: Effect on Plasma Therapy

Legendre et al. (2013) NEJM

- In every patient:
  - Elimination of PE / PI
  - No new dialysis

- Reduction from:
  - Approximately median (range) 1.5 (0.4–7.6) interventions per patient per week before eculizumab treatment, to...
  - 0 interventions in all 20 patients (p<0.0001) during the entire study period

Median (range) TMA intervention rate (per patient per day)

Before eculizumab  (n=20)

With eculizumab  (n=20)

0.23 (0.05–1.09)  
P<0.0001
C08-002: Effect on HRQoL

Legendre et al. (2013) NEJM

Clinically meaningful threshold = 0.06

*p<0.0001
†p<0.001
‡p<0.05
C08-003: Effect on HRQoL

Legendre et al. (2013) NEJM
Eculizumab appears to work well in patients without an identified mutation.

<table>
<thead>
<tr>
<th>Haematological normalisation and TMA event free</th>
<th>Patients with long duration of aHUS and CKD [C08-003]</th>
<th>aHUS patients with progressing TMA [C08-002]</th>
</tr>
</thead>
<tbody>
<tr>
<td>With known identified mutations</td>
<td>13 / 14 (93%)</td>
<td>12 / 13 (92%)</td>
</tr>
<tr>
<td>Without known complement mutation</td>
<td>5 / 6 (83%)</td>
<td>3 / 4 (75%)</td>
</tr>
</tbody>
</table>

Legendre et al. (2013) NEJM
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
- No specification for PE/PI prior to enrollment*

Prospective

First Prospective Trial of Pediatric Patients with aHUS (N=22) <18 years old

Largest Prospective Trial of Adult Patients with aHUS (N=41) ≥18 years old

Ongoing Long-Term Extension Study

Ongoing Long-Term Extension Study

*In prospective pediatric trial, no more than 5 weeks of prior PE/PI.
C10-003:
OPEN-LABEL, MULTI-CENTER CLINICAL TRIAL OF ECULIZUMAB IN PEDIATRIC PATIENTS WITH ATYPICAL HEMOLYTIC-UREMIC SYNDROME

FIRST PROSPECTIVE TRIAL OF PEDIATRIC PATIENTS WITH AHUS
# C10-003: Trial Overview

<table>
<thead>
<tr>
<th><strong>Design</strong></th>
<th>Open-label, single-arm, multi-center, multinational, 26-week study evaluation period (N=22)</th>
</tr>
</thead>
</table>
| **Patients** | Patients 1 month to <18 years of age and body weight ≥ 5 kg, with aHUS:  
  • Platelet count <150 x 10^9/L  
  • LDH ≥1.5 x ULN  
  • Hemoglobin ≤ LLN  
  • Serum Creatinine (SCr) ≥97 percentile for age at screening  
  • ADAMTS13 >5%  
  • No positive Shiga toxin test  
  • No requirement for identified complement mutation or antibody  
  • No requirement for PE/PI prior to enrollment (but no more than 5 weeks of prior PE/PI) |
| **Dosing** | Administered by i.v. depending on body weight |
| **Endpoints** |  
  • Complete TMA response  
  • Safety and tolerability  
  • Additional efficacy measures:  
    - Hematologic normalization  
    - Platelet count normalization  
    - % Discontinued PE/PI  
    - Renal function measures  
    - % Discontinued Dialysis |
| **Geography** | US, Canada, Australia, EU (Belgium, France, Germany, Italy, Netherlands, UK) |
| **Evaluation Period** | 26-week study evaluation period |

C10-003: Efficacy Endpoints

- **Complete TMA Response (primary efficacy endpoint):**
  - Platelet count normalization: Platelet count $\geq 150 \times 10^9$/L
  - Normalization of lactate dehydrogenase (LDH < ULN)
  - $\geq 25\%$ improvement in serum creatinine (SCr) from baseline

- **Hematologic normalization: Platelets $\geq 150$ and LDH $\leq$ ULN**
  - Platelet count improvement/normalization

- **Renal function measures**
  - eGFR improvement: Increase from baseline $\geq 15$ mL/min/1.73m$^2$
  - eGFR change from baseline
  - Serum creatinine $\geq 25\%$ decrease from baseline
  - Dialysis

# Patient Enrollment

<table>
<thead>
<tr>
<th>ITT Population</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screened</td>
<td>27</td>
</tr>
<tr>
<td>Treated</td>
<td>22</td>
</tr>
<tr>
<td>Completed Initial Study Evaluation Period (26 weeks)</td>
<td>19 (86)</td>
</tr>
<tr>
<td>Withdrew from Initial 26-week Study Evaluation Period</td>
<td>3 (14)</td>
</tr>
<tr>
<td><strong>Reason for study withdrawal</strong></td>
<td></td>
</tr>
<tr>
<td>• Other: positive Shiga toxin result received via local lab</td>
<td>1 (5)</td>
</tr>
<tr>
<td>• Other: withdrew after SAE (agitation)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>• Family requested to withdraw patient (travel back home overseas)</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

## Baseline Demographics

<table>
<thead>
<tr>
<th>Age at first infusion (years)</th>
<th>Median (min; max)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.5 (0.0; 17.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age Category</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month to &lt;23 months</td>
<td>5 (23)</td>
</tr>
<tr>
<td>≥23 months to &lt;5 years</td>
<td>5 (23)</td>
</tr>
<tr>
<td>≥5 to &lt;12 years</td>
<td>8 (36)</td>
</tr>
<tr>
<td>≥12 to &lt;18 years</td>
<td>4 (18)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Female Gender, n (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 (46)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Race, n (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Asian</td>
<td>2 (9)</td>
</tr>
<tr>
<td>Black or African American</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>18 (82)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient-reported family history of aHUS, n (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 (27)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Identified complement regulatory protein mutation or autoantibody, n (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 (45)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patients with CFHR1/3 polymorphism, n (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

C10-003: Patient Characteristics

- 73% (16/22) of patients were newly diagnosed
- 55% (12/22) received eculizumab without TPE
  - Median of 6 days from current TMA manifestation to first dose of eculizumab (0.20 months [0.03-4.3])
- 50% (11/22) of patients on dialysis at baseline
- 9% (2/22) of patients had prior kidney transplant
- 55% (12/22) had no identified genetic mutation

### C10-003: Baseline Laboratory Values

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Platelet count (x 10^9/L)</strong></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>87.5 (42.3)</td>
</tr>
<tr>
<td>Platelet count &lt; 150 x 10^9/L, n (%)</td>
<td>22 (100)</td>
</tr>
<tr>
<td><strong>LDH (U/L)</strong></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>1943.7 (1824.4)</td>
</tr>
<tr>
<td>LDH &gt; ULN, n (%)</td>
<td>19 (86)</td>
</tr>
<tr>
<td><strong>Hemoglobin (g/L)</strong></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>80.2 (15.3)</td>
</tr>
<tr>
<td><strong>Creatinine (umol/L)</strong></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>154.5 (116.4)</td>
</tr>
<tr>
<td><strong>eGFR (mL/min/1.73m^2)</strong></td>
<td></td>
</tr>
<tr>
<td>– Mean (SD)</td>
<td>32.7 (30.4)</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m^2), n (%)</td>
<td></td>
</tr>
<tr>
<td>&lt; 15</td>
<td>10 (46)</td>
</tr>
<tr>
<td>15-29</td>
<td>4 (18)</td>
</tr>
<tr>
<td>30-44</td>
<td>2 (9)</td>
</tr>
<tr>
<td>45-59</td>
<td>2 (9)</td>
</tr>
<tr>
<td>60-89</td>
<td>2 (9)</td>
</tr>
<tr>
<td>≥ 90</td>
<td>2 (9)</td>
</tr>
<tr>
<td>eGFR &lt; 90 mL/min/1.73m^2, n (%)</td>
<td>20 (91)</td>
</tr>
</tbody>
</table>

Complete TMA Response in 64%

**Complete TMA Response:**

- Normalization of platelet count and LDH and
- ≥25% improvement in SCr from baseline
- Two consecutive measurements obtained at least 4 weeks (28 days) apart

**Median Time to Complete TMA Response - Days (Min-Max):**

60.0 (7.0-153.0)

Platelet Count Normalization in 95%

Platelet Count Normalization:

- \( \geq 150 \times 10^9/L \)
- Two consecutive measurements obtained at least 4 weeks (28 days) apart

Of the 10 patients on PE/PI at baseline, all patients (100%) discontinued PE/PI by the end of the study evaluation period.

Median Time to Platelet Count Normalization - Days (Min-Max): 7.0 (1.0-80.0)

Rapid/Sustained Improvement in Platelets with Ongoing Eculizumab

164 x 10⁹/L: Mean Change from Baseline in Platelets at Week 27

Mean (SD) platelet count (x10⁹/L) at baseline: 87.5 (42.34); Mean (SD) platelet count at Week 27: 262.4 (66.24).

Hematologic Normalization in 82%

Hematologic Normalization:
- Normalization of platelet count and LDH
- Two consecutive measurements obtained at least 4 weeks (28 days) apart

Median Time to Hematologic Normalization:
- Days (Min-Max): 55.0 (1.0-153.0)

Continued eGFR Improvement with Ongoing Eculizumab Treatment

64 mL/min/1.73m²: Mean Change from Baseline in eGFR at Week 27

†Mean (SD) eGFR at baseline: 32.7 (30.37); Mean (SD) eGFR at Week 27: 97.2 (51.78).

Dialysis Eliminated in 82%

Patients on Dialysis at Baseline

Patients Not on Dialysis at Baseline:
- Of the 11 patients not on dialysis at baseline, all patients (100%) remained dialysis-free through the end of the study evaluation period.

*One patient discontinued dialysis during baseline window and before first dose of eculizumab.

Adverse Events Consistent with Other Eculizumab Studies

<table>
<thead>
<tr>
<th>C10-003 (N=22) AEs occurring in ≥20%</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrexia</td>
<td>11 (50)</td>
</tr>
<tr>
<td>Cough</td>
<td>8 (36)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>7 (32)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>7 (32)</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>7 (32)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>6 (27)</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>6 (27)</td>
</tr>
</tbody>
</table>

- Most AEs were mild or moderate
- No meningococcal infections
- One patient was noted to have low positive values for neutralizing antibodies to eculizumab
  - Overall, there had been no observed correlation of antibody development to clinical response or adverse events
- No new reports of hepatotoxicity during eculizumab treatment
- No new safety concerns
- No deaths

C10-003: Key Findings

- Majority received eculizumab without prior use of PE/PI
- 64% (14/22) achieved complete TMA response
- 95% (21/22) achieved platelet count normalization
- 100% of patients on PE/PI at baseline discontinued
- 82% (18/22) discontinued dialysis
- 86% (19/22) achieved an increase in eGFR of ≥15 mL/min/1.73m²
  - Mean eGFR increase was 64 mL/min/1.73m²
- Most AEs were mild/moderate
C10-004: A PHASE 2, OPEN-LABEL, MULTI-CENTER CLINICAL TRIAL OF ECULIZUMAB IN ADULT PATIENTS WITH ATYPICAL HEMOLYTIC-UREMIC SYNDROME

LARGEST PROSPECTIVE STUDY IN AHUS TO DATE
# C10-004: Study Design

<table>
<thead>
<tr>
<th>Design</th>
<th>Open-label, single-arm, multi-center, multinational, 26-week study evaluation period (N=41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>Adult Patients ≥18 years with aHUS:</td>
</tr>
<tr>
<td></td>
<td>• Platelet count &lt; 150x10⁹/L</td>
</tr>
<tr>
<td></td>
<td>• Hemoglobin ≤ LLN</td>
</tr>
<tr>
<td></td>
<td>• LDH ≥1.5 x ULN</td>
</tr>
<tr>
<td></td>
<td>• SCr ≥ULN at screening</td>
</tr>
<tr>
<td></td>
<td>• ADAMTS13 &gt;5%</td>
</tr>
<tr>
<td></td>
<td>- No positive Shiga toxin test</td>
</tr>
<tr>
<td></td>
<td>- No requirement for identified complement mutation or antibody</td>
</tr>
<tr>
<td></td>
<td>- No specification for PE/PI prior to enrollment</td>
</tr>
<tr>
<td>Dosing</td>
<td>Eculizumab 900 mg i.v. once a week for 4 weeks, 1200 mg at Week 5, then 1200 mg every two weeks</td>
</tr>
<tr>
<td>Endpoints</td>
<td>▪ Complete TMA response (primary)</td>
</tr>
<tr>
<td></td>
<td>▪ Safety and tolerability</td>
</tr>
<tr>
<td></td>
<td>▪ Additional efficacy measures:</td>
</tr>
<tr>
<td></td>
<td>• Hematologic normalization</td>
</tr>
<tr>
<td></td>
<td>• Platelet count normalization</td>
</tr>
<tr>
<td></td>
<td>• % Discontinued PE/PI</td>
</tr>
<tr>
<td></td>
<td>• Renal function measures</td>
</tr>
<tr>
<td></td>
<td>• % Discontinued Dialysis</td>
</tr>
<tr>
<td>Geography</td>
<td>US, EU (Belgium, France, Germany, Italy, Spain, UK)</td>
</tr>
<tr>
<td>Evaluation Period</td>
<td>26-week study evaluation period</td>
</tr>
</tbody>
</table>

C10-004: Study Endpoints

- Complete TMA response (primary efficacy endpoint):
  - Platelet and LDH normalization
  - Preservation of renal function (<25% increase in sCr from baseline)

- Modified complete TMA response
  - Normalization of platelet count and LDH and
  - ≥25% decrease in SCr from baseline

- Hematologic normalization: Platelet count ≥150 and LDH < ULN
  - Platelet count improvement/normalization

- Renal function measures
  - eGFR improvement: Increase from baseline ≥15 mL/min/1.73 m²
  - eGFR change from baseline
  - Dialysis

## Patient Enrollment

<table>
<thead>
<tr>
<th>ITT Population</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screened</td>
<td>44</td>
</tr>
<tr>
<td>Treated</td>
<td>41</td>
</tr>
<tr>
<td>Completed Initial Study Evaluation Period (26 weeks)</td>
<td>38 (93)</td>
</tr>
<tr>
<td>Withdrew from Initial 26-week Study Evaluation Period</td>
<td>3</td>
</tr>
<tr>
<td>Reason for withdraw</td>
<td></td>
</tr>
<tr>
<td>SAE (meningococcal meningitis)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Lack of efficacy</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

## Baseline Demographics

<table>
<thead>
<tr>
<th>Age at first infusion (years)</th>
<th>Median (min, max)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35 (18, 80)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Female gender, n (%)</th>
<th>28 (68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race, n (%)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Black or African American</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>38 (93)</td>
</tr>
</tbody>
</table>

| Patient-reported family history of aHUS, n (%) | 6 (15) |
| Identified complement regulatory protein mutation or autoantibody, n (%) | 20 (49) |
| CFHR 1/3 polymorphism, n (%)                  | 1 (2)  |
C10-004: Patient Characteristics

- 73% (30/41) were newly diagnosed
  - Median duration of 2 wks from current TMA manifestation to eculizumab (0.50 mos [0.0-19.2])
- 59% (24/41) of patients on dialysis at baseline
- 15% (6/41) received eculizumab without prior PT
- 22% (9/41) of patients had prior kidney transplant
- 51% (21/41) had no identified genetic mutation

Baseline Laboratory Values

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count (x 10⁹/L)</td>
<td>119.1 (66.1)</td>
</tr>
<tr>
<td>Platelet count &lt;150x 10⁹/L, n (%)</td>
<td>27 (66)</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>492.9 (500.9)</td>
</tr>
<tr>
<td>LDH &gt; ULN, n (%)</td>
<td>32 (78)</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>86.7 (21.3)</td>
</tr>
<tr>
<td>Creatinine (umol/L)</td>
<td>411.0 (264.6)</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>17.3 (12.1)</td>
</tr>
<tr>
<td>eGFR &lt;60 mL/min/1.73m², n (%)</td>
<td>41 (100)</td>
</tr>
</tbody>
</table>

Complete TMA Response in 73%

Complete TMA Response:
- Normalization of platelet count and LDH and
- <25% increase in Scr from baseline
- Two consecutive measurements obtained at least 4 weeks (28 days) apart

Modified Complete TMA Response Achieved: 23/41 (56%)
- Normalization of platelet count and LDH and
- ≥25% decrease in Scr from baseline

Median Time to Complete TMA Response - Days (Min-Max): 56.0 (2.0-147.0)

Platelet Normalization (26 wks) in 98%

Platelet Count Normalization:
- ≥150 x 10^9/L
- Two consecutive measurements obtained at least 4 weeks (28 days) apart

Median Time to Platelet Count Normalization - Days (Min-Max): 8.0 (0.0-84.0)

Rapid/Sustained Improvement in Platelets With Ongoing Eculizumab

135 x 10⁹/L: Mean Change from Baseline in Platelets at Week 26

†Mean (SD) platelet count (×10⁹/L) at baseline: 119.1 (66.09); Mean (SD) platelet count at Week 26: 252.5 (70.38).

Hematologic Normalization in 88%

- Normalization of platelet count and LDH
- Two consecutive measurements obtained at least 4 weeks (28 days) apart

Median Time to Hematologic Normalization - Days (Min-Max)
55.0 (2.0-146.0)

Significant/Continued eGFR Improvement with Ongoing Eculizumab

**29.3 mL/min/1.73m²: Mean Change from Baseline in eGFR at Week 26**

Mean (SD) eGFR at baseline: 17.3 (12.05); Mean (SD) eGFR at Week 25: 47.0 (24.35).

Before Soliris, 100% of patients had eGFR <60 mL/min/1.73m²

Dialysis Eliminated in 83%

Patients on Dialysis at Baseline

- Of the 17 patients not on dialysis at baseline, 15 patients (88%) remained dialysis-free through the study evaluation period.

*2 patients not on dialysis at BL initiated and remained on dialysis through 26 weeks.

Adverse Event Consistent with Other Eculizumab Studies

- Most AEs were mild or moderate
- Two cases of meningococcal infection
  - One patient recovered and discontinued from the study due to meningococcal infection
  - The second patient recovered, and stayed on eculizumab treatment
- No new safety concerns
- No deaths

<table>
<thead>
<tr>
<th>C10-004 (N=41) AEs occurring in ≥15%</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>15 (37)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>13 (32)</td>
</tr>
<tr>
<td>Edema (peripheral)</td>
<td>9 (22)</td>
</tr>
<tr>
<td>Cough</td>
<td>8 (20)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>7 (17)</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>7 (17)</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>7 (17)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>7 (17)</td>
</tr>
<tr>
<td>Anemia</td>
<td>7 (17)</td>
</tr>
</tbody>
</table>

C10-004: Main Findings

- Confirms the results of previous prospective eculizumab trials (C08-002/003)

- Sustained improvements in hematologic/renal parameters:
  - 73% (30/41) achieved complete TMA response
  - 98% (40/41) achieved platelet count normalization
    - Mean increase in platelet count from baseline was 135
  - 83% (20/24) discontinued dialysis
  - Mean eGFR increase from baseline was 29.3 mL/min/1.73m²

- Most of AEs were mild/moderate in severity
  - Two cases of meningococcal meningitis – one patient recovered/stayed on drug, one recovered but discontinued
Pre-treatment ADAMTS13 Activity

Suspected TMA
Initiate PEX Therapy
- Platelet count
- Serum creatinine

PEX Therapy (4-5 days)

Response
Continue PEX Until Platelets and LDH Normal
Obtain Complement Mutation Studies if ADAMTS13 >10%

Poor Response

ADAMTS13 Activity <10%
Continue Daily PEX
Intensify Immune Suppression

ADAMTS13 Activity >10%
Consider aHUS as Diagnosis

Initiate Eculizumab Therapy
Obtain Complement Mutation Studies

Vaccinate ASAP
Transplant in aHUS

- Graft failure in 60-80% depending on subtype
  - CFH has the highest risk of recurrence/graft failure
  - MCP single mutation has the lowest risk

- Transplant protocols
  - No clear risk with CsA, but increased risk with MTORi
  - Risk-adapted strategies — TPE, eculizumab, ritux, etc.
    - Eculizumab for high-risk patients, pre- and post-transplant
  - Caution with living-related donors

Zuber et al. (2012) Nat Rev Nephrol
<table>
<thead>
<tr>
<th>Complement abnormality</th>
<th>Frequency (%)</th>
<th>Number of transplanted patients</th>
<th>Recurrences (% of patients)</th>
<th>Number of grafts</th>
<th>Recurrences (% of grafts)</th>
<th>Graft failure for recurrence (% of all recurrences)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CFH mutations and CFH/CFHR1 hybrid gene</strong></td>
<td>20–30</td>
<td>60</td>
<td>68% (41/60)</td>
<td>76</td>
<td>64% (49/76)</td>
<td>82% (40/49)</td>
</tr>
<tr>
<td>CFH autoantibodies</td>
<td>5–10</td>
<td>12</td>
<td>33% (4/12)</td>
<td>17</td>
<td>29% (5/17)</td>
<td>80% (4/5)</td>
</tr>
<tr>
<td>CFI mutations</td>
<td>4–10</td>
<td>23</td>
<td>78% (18/23)</td>
<td>26</td>
<td>73% (19/26)</td>
<td>95% (18/19)</td>
</tr>
<tr>
<td>MCP mutations</td>
<td>10–15</td>
<td>15</td>
<td>20% (3/15)</td>
<td>17</td>
<td>18% (3/17)</td>
<td>66% (2/3)</td>
</tr>
<tr>
<td>C3 mutations</td>
<td>8–10</td>
<td>18</td>
<td>78% (14/18)</td>
<td>30</td>
<td>53% (16/30)</td>
<td>75% (12/16)</td>
</tr>
<tr>
<td>CFB mutations</td>
<td>1–2</td>
<td>3</td>
<td>100% (3/3)</td>
<td>4</td>
<td>100% (4/4)</td>
<td>100% (4/4)</td>
</tr>
<tr>
<td>THBD mutations</td>
<td>3–5</td>
<td>1</td>
<td>1/1</td>
<td>1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>DGKE mutations</td>
<td>n.d.</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

CFH, complement factor H; CFI, complement factor I (degrades C3b); MCP, membrane cofactor protein.
Nonresponders to Eculizumab

- Different diagnosis
  - Important to review/reconsider if not responding
  - Other TMAs may be complement-mediated (in part)

- DGKE
  - Intracellular enzyme expressed in kidney/elsewhere
    - Not related to complement activation
    - Loss leads to increased inflammatory signals (PKC)
  - Microangiopathy, endothelial injury, HTN, proteinuria
    - All reports describe onset in the first 12 months of life

- Drug levels/response
  - Distribution, clearance, anti-eculizumab antibodies
  - Complement component polymorphisms

LeMaire M et al. (2013) Nat Genet
Nishimura J et al. (2014) NEJM
Genetic Variants in C5 and Poor Response to Eculizumab

Jun-ichi Nishimura, M.D., Ph.D., Masaki Yamamoto, M.D., Shin Hayashi, M.D., Ph.D., Kazuma Ohyashiki, M.D., Ph.D., Kiyoshi Ando, M.D., Ph.D., Andres L. Brodsky, M.D., Ph.D., Hideyoshi Noji, M.D., Kunio Kitamura, M.D., Ph.D., Tetsuya Eto, M.D., Toru Takahashi, M.D., Masayoshi Masuko, M.D., Ph.D., Takuro Matsumoto, M.D., Yuji Wano, M.D., Tsutomu Shichishima, M.D., Ph.D., Hirohiko Shibayama, M.D., Ph.D., Masakazu Hase, Ph.D., Lan Li, M.D., Krista Johnson, M.Sc., Alberto Lazarowski, Ph.D., Paul Tamburini, Ph.D., Johji Inazawa, M.D., Ph.D., Taro Kinoshita, Ph.D., and Yuzuru Kanakura, M.D., Ph.D.
Future Therapeutic Options

- MASP-2
- pCFH
- Properdin
- TT30

Omeros Press Release (10 March 2014)
Heinen S et al. (2013) Molecular Immunology
Risitano et al. (2012) Blood
Untying the TMA Knot

- Limited by our current case definitions
  - Diagnostic testing is slow, variable, specialized
    - Rapid and specific functional tests lacking
  - Reliance on clinical decision trees

- Clinical and health-economic impacts
  - Continue with TPE, start eculizumab, start rituximab?

- Non-responders to eculizumab
  - Wrong disease
    - Wrong subtype (DGKE mutations)
  - Drug dose/clearance
  - Polymorphisms (e.g. C5 binding)

Tsai HM (2013) AJM
Nishimura et al. (2014) NEJM
Lemaire M et al. (2013) Nat Genetics
Future Directions

☐ National organizations to best study rare, serious diseases
  ☐ Research – registry, cases reports, etc.
  ☐ Education – colleagues, patients
  ☐ Clinical Excellence – guidelines

☐ Research questions
  ☐ Natural history of TMA – national online registry is coming
    ▬ Different from the international aHUS registry (more inclusive)
    ▬ Improved classification – mechanistic, pathologic, etc.
  ☐ Refine current strategies (e.g. pheresis, rituximab, eculizumab)
    ▬ Long-term surveillance of various treatments
  ☐ New therapies/approaches (e.g. TT30, CFH, RT protocols, etc.)
Thank you!

Questions? Comments?