Inhibition of Complement Alternative Pathway by Orally Administered Inhibitors of Complement Factor D

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Complement Factor D (CFD)

- Druggable target for small molecules
  - Serine protease
  - High resolution 3-D structures available
  - Very low plasma concentration (~2 µg/mL)

- Well characterized function
  - Rate limiting enzyme in the complement alternative pathway (AP)
  - Highly specific protease: Complement Factor B (CFB) only known natural substrate

- Implications for diseases
  - Dysregulated AP involved in the pathogenesis of multiple disorders including PNH and C3G

- Deficiency in human: Healthy but increased risk of recurrent *Neisseria* infections
  - Protection elicited by vaccination (in vitro assessment)[1]
    - Preserved during CFD inhibition in most test samples
    - Blocked by C5 inhibition in all test samples

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Structural Biology

- 25 inhibitor–enzyme X-ray structures
  - Non-covalent binding to CFD

<table>
<thead>
<tr>
<th>Resolution of Inhibitor-FD Structure</th>
<th># of X-Ray Structures</th>
</tr>
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<tbody>
<tr>
<td>≤1.2 Å</td>
<td>13</td>
</tr>
<tr>
<td>1.3–2.0 Å</td>
<td>9</td>
</tr>
<tr>
<td>2.1–2.3 Å</td>
<td>3</td>
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</tbody>
</table>

- Firm understanding of the molecular recognition of inhibitor–enzyme complexes
- Enabled several key structure-guided designs to optimize potency
  - ~20% of compound collection demonstrate IC$_{50}$ <100 nM (screen AP hemolysis assay)
Distinct chemical series generate a wide range of drug-like properties

- Flexibility for different routes of administration
  - Oral systemic delivery
  - Topical use/Inhalation/GI-restricted exposure

>2800 SM CFD inhibitors
Small Molecule CFD Inhibitor: Delivery to Eye Posterior Segment

- Evaluated ACH-018 via a single injection into the vitreous (IVT) of the eye in rabbit.

![Graph showing concentration over time for ACH-018 0.33 mg/eye and ACH-018 1.0 mg/eye.]

- Retina/Choroid/RPE compound concentrations > IC\(_{90}\) value (AP hemolysis assay, APH) were maintained over three months after a single injection.

- High levels of compound remained in the drug depot (VH inferior) three months after the injection.

- Very low exposure in plasma.

- Well tolerated in rabbits based on ophthalmology and ocular histopathology.
Discovery of Small Molecule Inhibitors of CFD

Distinct chemical series generate a wide range of drug-like properties
- Flexibility for different routes of administration
- Oral administration
  - ACH-4471, first in Phase 2
  - Next-generation compounds have advanced into Phase 1 studies
Binding to CFD

- Binding properties were assessed with surface plasmon resonance (Biacore)
- ACH-4471 & 2\textsuperscript{nd} generation inhibitors bind to CFD
  - Reversibly with fast-on and variable off-rate kinetics
  - High affinity with $K_D = 0.08 - 0.44$ nM
Inhibition of CFB Cleavage

- Effect on proteolytic activity of CFD was assessed by biochemical assay
  - Natural substrate: CFB in complex with C3b
  - SDS/PAGE: Visualize cleavage process
  - Bb ELISA: Quantify reaction product

- ACH-4471 & 2nd generation compounds inhibit the cleavage of CFB (in complex with C3b) potently with $K_i$ values of 1.1 - 5.7 nM

* Adapted from [http://www.crystal.chem.uu.nl/~gros/research-complement.html](http://www.crystal.chem.uu.nl/~gros/research-complement.html) (Dr. Piet Gros Lab)
Effect on Classical, Lectin and Alternative Pathways

- Evaluated with pathway-specific functional assays: Hemolysis and/or Wieslab
  - No effect on CP and LP
  - Potent inhibition of AP

Full inhibition in AP hemolysis and Wieslab assays

Partial inhibition in AP hemolysis assay
Full inhibition in AP Wieslab assay
CFD or C5 Requirement in Two Standard AP Assays

- CFD or C5-depleted NHS reconstituted with purified CFD or C5 used to understand the difference

- Amount needed for 50% restoration of AP activity in hemolysis and Wieslab assays
  - CFD: Similar requirement
  - C5: ~1000-fold difference

![Graph showing CFD and C5 concentration vs. AP activity](attachment://graph.png)
In Vitro Study for PNH: Hemolysis

“AP” Conditions
GVB$^0$+MgEGTA, 20% Acidified NHS

“Physiologic” Conditions
GVB$^{++}$, 80% Acidified NHS

- Hemolysis of erythrocytes harvested from PNH patient
- “AP” conditions:
  - ACH-4471: Full inhibition
  - Eculizumab: Partial inhibition
- “Physiologic” conditions:
  - ACH-4471: Full inhibition
  - Eculizumab: Full inhibition
- Multiple Donors
  - ACH-4471: Findings similar
  - Eculizumab: Findings variable
- Reason(s)?

Multiple Graphs showing hemolysis curves for ACH-4471 and Eculizumab under different conditions.
Hypothetical Model: C5 Activation

A Model proposed by Dr. Schmidt [1]
- C5 conformational “priming” is required for proteolytic activation by a nearby convertase
- Eculizumab binding to C5 hinders conformational priming and blocks proteolytic activation
- However, under “AP” assay conditions (robust activation), high density “C3b clusters” compete for C5 binding, resulting in a residual C5 activation

Will ACH-4471 act synergistically with C5 inhibition because it blocks C3b deposition?

Figure adapted from [1]
In Vitro Study for PNH: C3 Fragment Deposition

- Erythrocytes harvested from PNH patient were evaluated under “physiologic” conditions by flow cytometry.
- Hemolysis: Inhibited by ACH-4471 and eculizumab.
- C3 fragment deposition: Blocked by ACH-4471 but not by eculizumab.
In Vitro Study for PNH: Combination with Eculizumab

- Combinatorial effects of ACH-4471 and eculizumab (in checkerboard pattern) were evaluated in hemolysis of PNH erythrocytes under AP conditions (20% NHS, GVB\(^0\) + MgEGTA)
- Analysis by Prichard and Shipman method: Volume of Synergy or Antagonism\(^1\)

<table>
<thead>
<tr>
<th>Volume ((\mu M \cdot \mu g/mL \cdot %) inhibition)</th>
<th>Interaction</th>
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<tbody>
<tr>
<td>Synergy</td>
<td></td>
</tr>
<tr>
<td>306 ± 57</td>
<td>Strongly Synergistic</td>
</tr>
<tr>
<td>Antagonism</td>
<td></td>
</tr>
<tr>
<td>-4 ± 6</td>
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</table>

Volume absolute values:
- <50 = additive interaction
- 50 to 100 = moderately synergistic or antagonistic interaction
- >100 = strongly synergistic or antagonistic interaction

ACH-4471 acts synergistically with eculizumab in preventing PNH erythrocytes from hemolysis in vitro

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In Vitro Evaluation for C3G: Fluid Phase Hyper-Activity

- In vitro model using CFH-depleted NHS in GVB⁺⁺ buffer
- In the absence of inhibitor, C3 was completely cleaved accompanied by release of large amount of C3a
- CFD inhibitors fully inhibited C3 consumption and C3a production

Western Blot with anti-C3 (alpha)

C3a ELISA

*+ 1 µM cmpd 3; Pre: No incubation
Phase 2 14-Day Clinical Trial in Patients with C3G or IC-MPGN

**CRITERIA**

Must have diagnosis of C3G or IC-MPGN based on central review of historical biopsy

Low C3 with normal/near-normal C4

**CLINICAL TRIAL DESIGN**

**GROUP 1**
2 patients received ACH-4471 100mg TID x 14 days followed by 7-day taper

**GROUP 2**
Up to 8 additional patients to receive ACH-4471 at doses up to 200mg TID x 14 days followed by 7-day taper

**Status:** Data available for 4 patients; recruitment ongoing

**OUTCOME MEASURES**

- Changes in systemic complement biomarkers
  - Ba production ex vivo
  - C3 concentration
  - C3 fragment concentration
  - Bb concentration
- Clinical manifestations
  - Albumin to creatinine ratio (ACR)
  - BP
  - eGFR
- Safety and tolerability
- Pharmacokinetic profile
- Exploratory studies in urinary complement biomarkers
  - Ba
  - sC5b-9

https://www.clinicaltrials.gov/ct2/show/NCT03124368
Key Baseline Patient Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Patient</th>
<th>Age (Y)</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Urine Dipstick for protein</th>
<th>ACR (0-2.5 mg/mmol) Day 1 Pre-dose</th>
<th>BP (mmHg)</th>
<th>Renal Biopsy Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>30</td>
<td>M</td>
<td>67</td>
<td>3+</td>
<td>259.3</td>
<td>126/72</td>
<td>C3GN</td>
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<tr>
<td></td>
<td>B</td>
<td>19</td>
<td>M</td>
<td>68</td>
<td>3+</td>
<td>580.3</td>
<td>123/80</td>
<td>IC-MPGN*</td>
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<tr>
<td>2</td>
<td>C</td>
<td>27</td>
<td>M</td>
<td>90</td>
<td>Trace</td>
<td>57.7</td>
<td>129/83</td>
<td>C3GN</td>
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<tr>
<td></td>
<td>D</td>
<td>22</td>
<td>M</td>
<td>39</td>
<td>3+</td>
<td>276.3</td>
<td>119/74</td>
<td>C3GN</td>
</tr>
</tbody>
</table>

- Concomitant medication doses stable for > one month prior to the first dose of study drug
  - mycophenolate mofetil (n=2), prednisone (n=2), ACE/ARB (n=4), atorvastatin (n=2), and spironolactone (n = 3)
- eGFR > 60 mL/min/1.73 m² in all patients

* Final review by central pathologist confirmed that the historical biopsy met criteria for IC-MPGN
Changes in Systemic Complement Proteins with 14-Day ACH-4471 Treatment

**Serum Ba Production** (Mean, N=4)

Ex vivo Functional Assay

**Serum C3** (Mean, N=4)
Normal Range 0.78 – 1.82

**Plasma Bb** (Mean, N=4)
Normal Range 0.49 – 1.42

**% Plasma Fragment C3** (Mean, N=4)
Normal Range 1.45 – 5.41

- Ex vivo Ba production, a functional biomarker for AP C3 convertase formation, was inhibited
- Bb, an in vivo biomarker for C3 AP convertase level, was reduced
- C3 was increased accompanied with reduced % C3 fragments, indicating lower C3 consumption in vivo
Changes in Systemic Complement Proteins with 14-Day ACH-4471 Treatment

- Ex vivo Ba production, a functional biomarker for AP C3 convertase formation, was inhibited.
- Bb, an in vivo biomarker for AP C3 convertase level, was reduced.
- C3 was increased accompanied with reduced % C3 fragments, indicating lower C3 consumption in vivo.
- In parallel, C4 and CFD levels were unchanged.
- Overall trend indicates that ACH-4471 tempered the AP hyperactivity in these patients.
Changes in Urinary Complement Proteins with 14-Day ACH-4471 Treatment

- Urinary Ba and sC5b-9 concentrations were elevated above normal at baseline
- Urinary Ba and sC5b-9 levels were reduced during treatment
- Urinary Ba and sC5b-9 levels normalized to urinary creatinine were reduced during treatment
- The changes were similar when normalized to urinary albumin

*The geometric mean was used due to the widely different values among 4 patients*
Reduction in Proteinuria with 14-Day ACH-4471 Treatment

- ~ 50% reduction in ACR observed in 3 out of 4 patients
- Highly variable ACR values seen in 1 patient (Patient D, BMI = 12.9)
  - 24-hour urinary protein levels were evaluated on days 14 and 17
    - Day 14=0.7 g/day
    - Day 17=2.44 g/day
- Stable eGFR and blood pressure observed in all patients through the treatment period
Interim Data Summary:
Phase 2 14-Day Clinical Trial in Patients with C3G or IC-MPGN

- Serum and plasma complement biomarkers showed evidence of inhibition of AP activity
- Observed changes in urinary complement biomarkers further indicated a tempering of AP hyperactivation
- In parallel, two-week treatment with ACH-4471 was associated with proteinuria reduction
- Generally well tolerated to date
Conclusions: Complement Factor D Inhibitors

- We have discovered small molecule inhibitors against complement factor D
  - Display potent and specific inhibition of human AP activity
  - Inhibit hemolysis of PNH erythrocytes & prevent C3 fragment deposition on PNH erythrocytes
  - Block high fluid-phase C3 consumption in an *in vitro* C3G model
  - Achieve robust exposures by different routes of administration based on PK studies in animals

- Several compounds for oral systemic administration have been advanced to various stages of clinical development

- ACH-4471: first-in-class orally administered CFD inhibitor in phase 2 clinical investigation for PNH and C3G
Complement Factor D Portfolio

**PROGRAM | INDICATION**

<table>
<thead>
<tr>
<th>ACH-4471</th>
<th>Factor D Inhibitor</th>
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<tbody>
<tr>
<td><strong>DELIVERY</strong></td>
<td>Oral</td>
</tr>
<tr>
<td><strong>PRECLINICAL</strong></td>
<td></td>
</tr>
<tr>
<td><strong>CLINICAL</strong></td>
<td>Phase 1 Phase 2 Phase 3</td>
</tr>
<tr>
<td>PNH: Monotherapy proof-of-concept (POC) open-label untreated PNH</td>
<td></td>
</tr>
<tr>
<td>PNH: POC add-on to eculizumab sub-optimal PNH responders</td>
<td></td>
</tr>
<tr>
<td>C3G: 14-day proof-of-mechanism (POM)</td>
<td></td>
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<tr>
<td>C3G: 12-month POC open label</td>
<td></td>
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<tr>
<td>C3G: 6-month POC randomized, placebo-controlled</td>
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*Next-Generation Factor D Inhibitors*

| AP-mediated diseases | Oral |
Acknowledgments

ACHILLION DISCOVERY TEAM

Chemistry
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