Novel Small-Molecule Inhibitors Targeting Complement Factor D for Therapy of Paroxysmal Nocturnal Hemoglobinuria

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INTRODUCTION

The complement system is a pivotal player in multiple hematological conditions that include paroxysmal nocturnal hemoglobinuria (PNH). The current standard of care for PNH is intravenous infusion of eculizumab, a humanized monoclonal antibody that targets the terminal complement protein C5 and thereby efficiently impairs intravascular hemolysis. However, an oral therapeutic would be highly desirable for chronic/life-long therapy of PNH. Furthermore, a significant fraction of PNH patients in clinical practice respond incompletely to eculizumab due to unmasking of extravascular hemolysis occurring through C3 opsonization. Additionally, a non-responsive sub-population has been identified with a rare genetic polymorphism in C5 that renders the variant incapable of binding eculizumab. Therefore, an unmet medical need remains for regimens that improve efficacy and that can be administered orally. To achieve this goal, we initiated a discovery program for small molecule inhibitors of complement factor D (fD), a serine protease that is the rate limiting enzyme of the alternative complement pathway. We have discovered novel inhibitors of fD that possess high potency and specificity as well as pharmacokinetic properties suitable for oral administration. Herein, we present a biochemical characterization of two ACH lead compounds (Compound A and D) and studies of their activities in various complement-mediated processes.

METHODS

- The binding affinity to fD was determined by surface plasmon resonance (Biacore) analysis.
- fD proteolytic activity was evaluated biochemically using both a nonspecific synthetic substrate and a natural substrate comprising C3b and complement factor B (fB). In the latter assay, the cleavage product Bb was quantified with a commercial ELISA kit (Quidel).
- The alternative pathway (AP) hemolysis assay was conducted with rabbit erythrocytes and 8% normal human serum (NHS) in the presence of 10 mM MgEGTA.
- AP-mediated terminal pathway activation in NHS was further assessed with the Wieslab AP ELISA assay that measures terminal complement complex (TCC) deposition after AP activation.
- C3 fragment deposition on the surface of rabbit erythrocytes was assessed by flow cytometry with a FITC-conjugated anti-C3c antibody after incubation with 20% C5-depleted NHS.
- Human PNH-like erythrocytes were prepared with anti-CD55 and CD59 monoclonal antibodies. AP-mediated hemolysis was assessed with normal or acidified (pH 6.4) AB donor serum in the presence of 8 mM EGTA and 2.5 mM $MgCl_2$; inhibition by compound was evaluated using 6.25% acidified serum (the final concentration after adding an equal volume of erythrocytes in buffer to 1:8 diluted serum).
- PK and PD studies were conducted in cynomolgus monkeys with Compound A. Compound plasma concentrations were measured by LC-MS/MS. Complement AP activity in serum was measured ex vivo by Wieslab AP ELISA assay.

1 Binding Affinity to Factor D

Figure 1. SPR Sensorgram

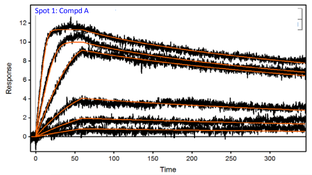


Table 1. Kinetic Constants and Affinity

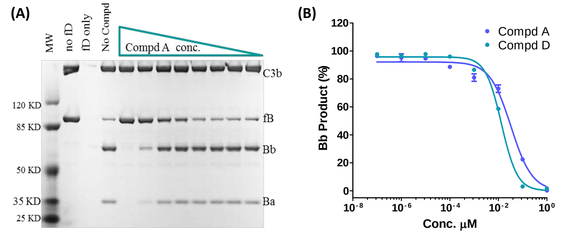
Compd	On rate k_a ($M^{-1}s^{-1}$)	Off rate k_d (s^{-1})	K_D (pM)
A	8.2e6	2.21e-3	269
D	1.61e6	1.45e-4	90

A representative SPR Sensorgram for Compound A is shown in **Fig. 1**. The values in **Table 1** are derived from data fitting of time-course of SPR signals for both Compound A and D.

- ACH compounds bind to fD with pM affinity.

2 Inhibition of Factor D Proteolytic Activity

Figure 2. Proteolytic Cleavage of fB by fD



(A) A representative protein gel analysis of fB substrate cleavage into products Ba and Bb in the presence of Compound A. **(B)** ELISA quantitation of Bb product formation for both Compound A and D.

- ACH fD inhibitors demonstrate potent inhibition of fB cleavage with IC_{50} values of 35 and 8.5 nM for Compounds A and D, respectively.
- Inhibition of fD activity was confirmed in a simple assay with a non-specific synthetic substrate (not shown).

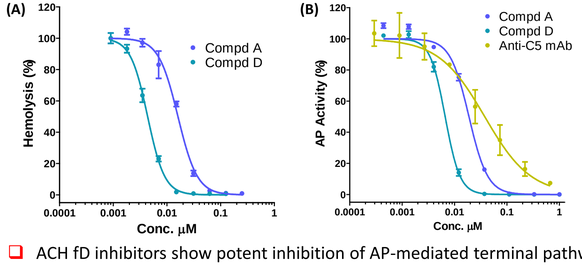
3 Inhibition of AP-Mediated Terminal Pathway Activation

Table 2. Potency of Inhibitors in Various Assays

Inhibitor	AP Hemolysis (Human Serum) IC_{50} (nM)	AP Hemolysis (Human Serum) IC_{90} (nM)	AP Wieslab ELISA (Human Serum) IC_{50} (nM)	AP Wieslab ELISA (Human Serum) IC_{90} (nM)	AP Hemolysis (Rat Serum) IC_{50} (nM)
Compd A	17	45	17	48	>100,000
Compd D	6.4	14	6.0	13	>100,000
Anti-C5 mAb[a]	-	-	38	346	-
FUT-175	12,000	32,000	-	-	12,000

a. Anti-C5 monoclonal antibody from Quidel (Cat#A217) ; "-" indicates not done or not shown.

Figure 3. Compound Dose-Response



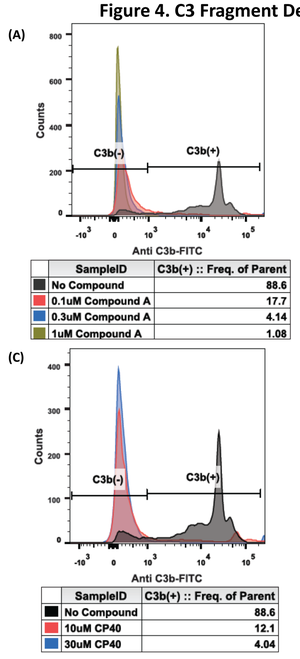
Inhibition of AP-mediated terminal pathway activation determined with AP hemolysis assay (**A**) or Wieslab AP ELISA assay (**B**).

- ACH fD inhibitors show potent inhibition of AP-mediated terminal pathway activation as assessed by multiple endpoints.
  - The steeper dose-response of ACH fD inhibitors results in a 7- to 27-fold greater potency than anti-C5 mAb as measured by IC_{90} (Table 2 & Fig. 3B).
- ACH fD inhibitors are highly specific. Unlike the broad spectrum serine protease inhibitor FUT-175, ACH fD inhibitors showed no effect in AP hemolysis assay with rat serum (Table 2). ACH fD inhibitors also did not inhibit a panel of human serine proteases (see Poster 4819).

RESULTS

4 Blockage of Opsonization

Figure 4. C3 Fragment Deposition on Erythrocytes



SampleID	C3b(+) :: Freq. of Parent
No Compound	88.6
0.1uM Compound A	17.7
0.3uM Compound A	4.14
1uM Compound A	1.08

SampleID	C3b(+) :: Freq. of Parent
No Compound	88.6
0.1uM Compound D	6.47
0.3uM Compound D	1.90
1uM Compound D	0.27

SampleID	C3b(+) :: Freq. of Parent
No Compound	88.6
10uM CP40	12.1
30uM CP40	4.04

Flow cytometry analysis of the rabbit erythrocytes that stained positive for C3b after incubation with 20% C5-depleted normal human serum in the absence or presence of Compound A (**A**) or Compound D (**B**) at 0.1, 0.3 and 1 µM, or Compstatin (CP-40) at 10 and 30 µM (**C**).

- ACH fD inhibitors blocked C3 fragment deposition effectively (< 5% of cells stained positive at 0.3 µM) (Fig. 4A and B).
- Compastatin (CP-40) achieved the same level of effectiveness at 30 µM (Fig. 4C).

5 Effect on PNH-Like Erythrocytes

Figure 5. Hemolysis of Erythrocytes

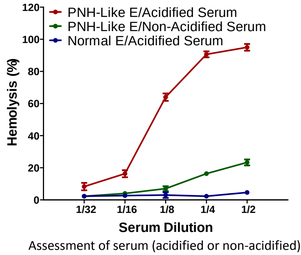
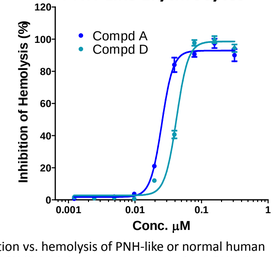


Figure 6. Inhibition of Hemolysis of PNH-Like Erythrocytes

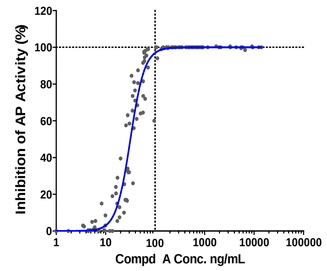


Assessment of serum (acidified or non-acidified) dilution vs. hemolysis of PNH-like or normal human erythrocytes (E) (**Fig. 5**), and the inhibitory effect of ACH fD inhibitors on the hemolysis of PNH-like erythrocytes in the acidified serum at a serum dilution of 1:8 that gave sub-maximal hemolysis (**Fig. 6**).

- Antibody block of CD55 and CD59 renders erythrocytes susceptible to AP-mediated hemolysis (Fig. 5).
  - At pH 6.4 (acidified serum), hemolysis of PNH-like erythrocytes was near-complete at serum dilution of 1:2 and 1:4 and between 63 - 85% at 1:8.
- Compound A and D effectively inhibited AP-mediated hemolysis of PNH-like erythrocytes (Fig. 6), with respective IC_{50} values of 25 nM and 42 nM and respective IC_{90} values of 39 nM and 66 nM.

6 Proof-of-Concept Study in Non-Human Primates

Figure 7. PK/PD Analysis in Monkeys



PK/PD relationship for Compound A at various doses in 12 monkeys. A sigmoidal E_{max} model was used to fit PK and PD data.

Figure 8. Complement Inhibition in Monkeys after Oral Dosing

Three monkeys were dosed orally with Compound A (100 mg/kg plus ritonavir, q12h). All monkeys showed complete AP inhibition at 24 h. In one monkey (not shown), there was slight rebound of AP activity (~30%) only at the 12 h time point due to reduced plasma level (<100 ng/mL) of Compound A.

- Compound A exposure of 100 ng/mL is sufficient for nearly complete inhibition of complement AP activity in monkeys (Fig. 7).
- Sustained suppression of complement AP activity for 24 h was achieved in monkeys when Compound A plasma concentration was maintained above 100 ng/mL after q12h oral dosing (Fig. 8).
- No change in plasma fD levels was seen in response to Compound A administration (see Poster 4819).

CONCLUSIONS

- Orally bioavailable, potent, and specific complement factor D inhibitors (ACH compounds) have been discovered.
- ACH compounds bind factor D with high affinity and inhibit factor D proteolytic activity with high potency, resulting in complete blockage of AP-mediated complement terminal pathway activation.
- ACH compounds effectively block C3 fragment deposition on cells, in contrast to C5-targeted therapies.
- ACH compounds demonstrate complete suppression of complement AP activity after oral dosing to non-human primates.
- ACH fD inhibitors represent a promising oral therapy for PNH patients, particularly for those refractory to eculizumab due to extravascular hemolysis or due to the genetic polymorphism in C5.

Disclosures

BPM is Achillion Advisory Board member and received Achillion funding for researching Achillion compounds. All others are employees and share holders of Achillion.

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