

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

B. Paul Morgan¹, Jane Thanassi², Steven Podos², Guangwei Yang², Avinash Phadke², Venkat Gadhachanda², Godwin Pais², Akihiro Hashimoto², Qiuping Wang², Dawei Chen², Xiangzhu Wang², Atul Agarwal², Milind Deshpande², Yongqing Huang², Jason Wiles² and Mingjun Huang²

Abstract ID : 4817

¹Institute of Infection and Immunity, Cardiff University, Cardiff, UK

²Achillion Pharmaceuticals Inc., New Haven, CT, USA

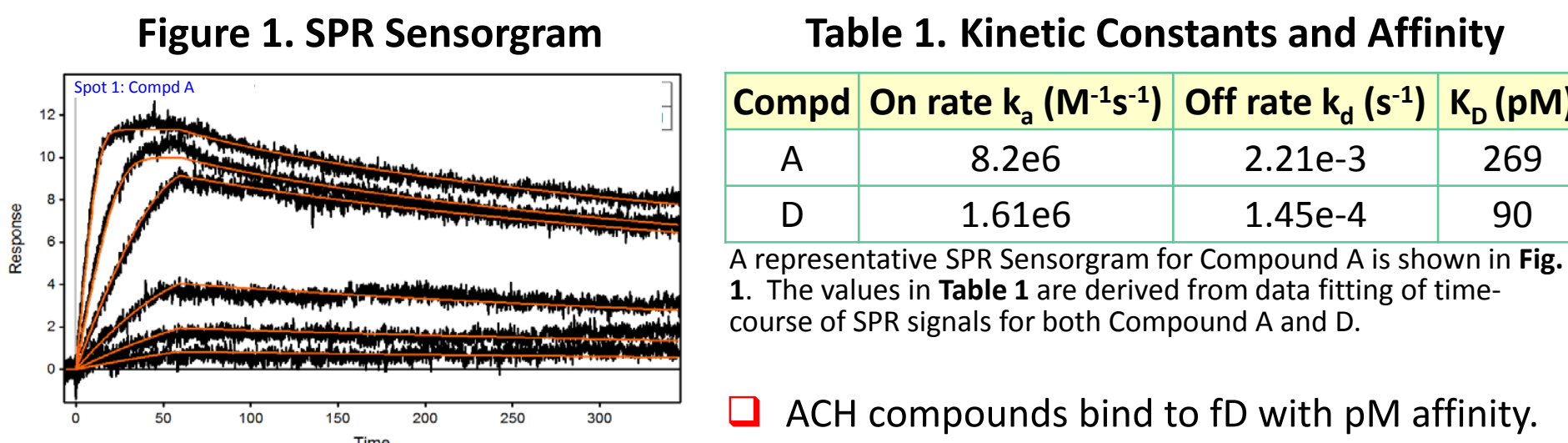
INTRODUCTION

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.

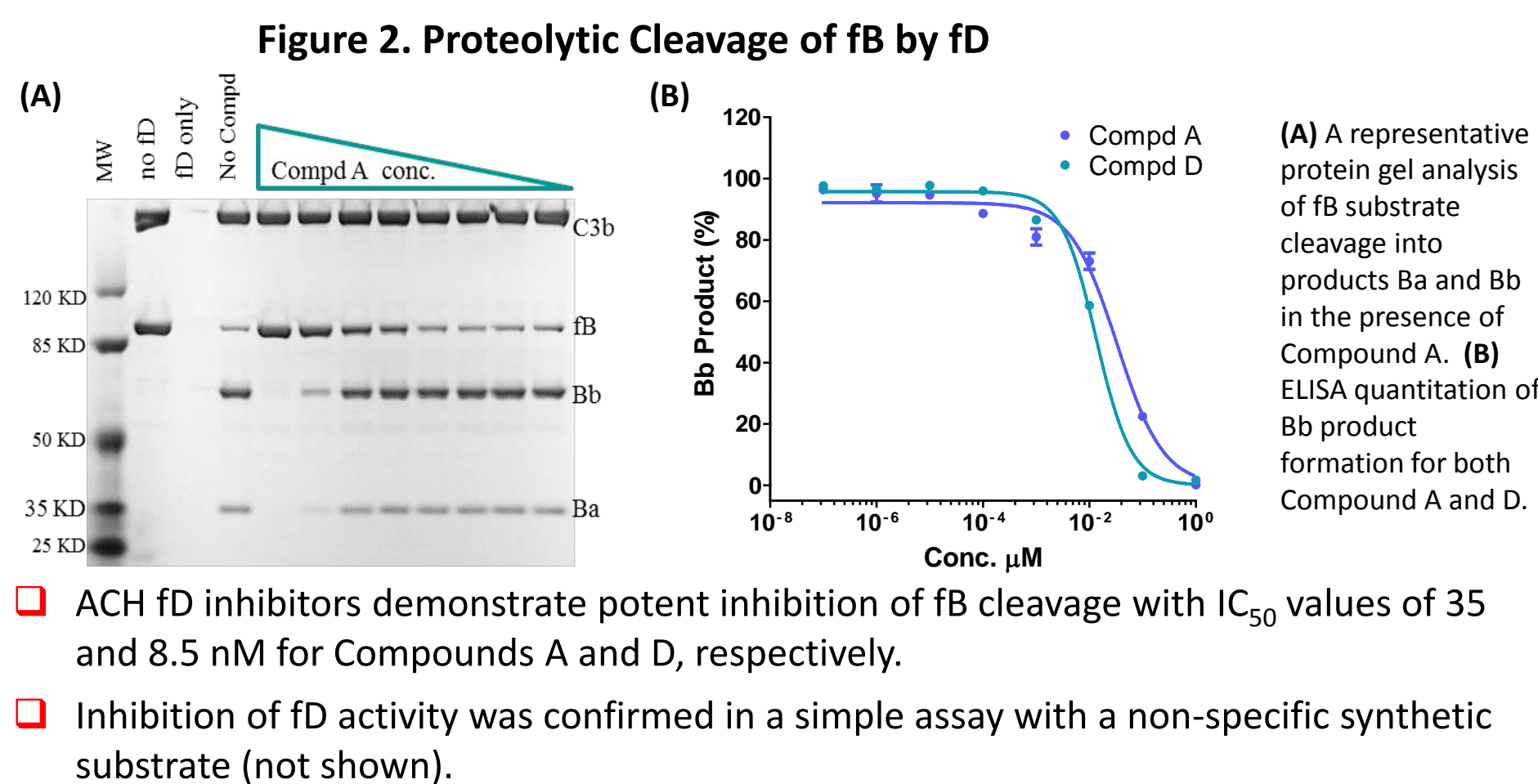
METHODS

- 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 MgCl₂; 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



2 Inhibition of Factor D Proteolytic Activity

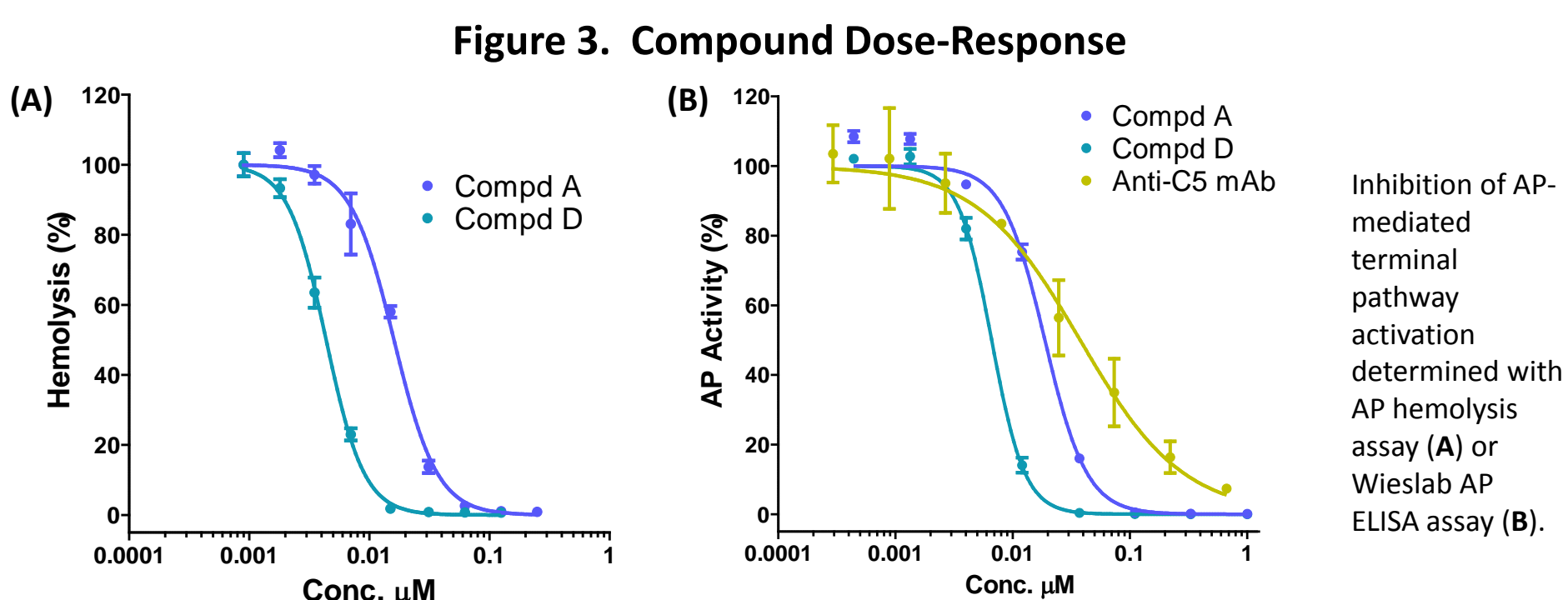


3 Inhibition of AP-Mediated Terminal Pathway Activation

Table 2. Potency of Inhibitors in Various Assays

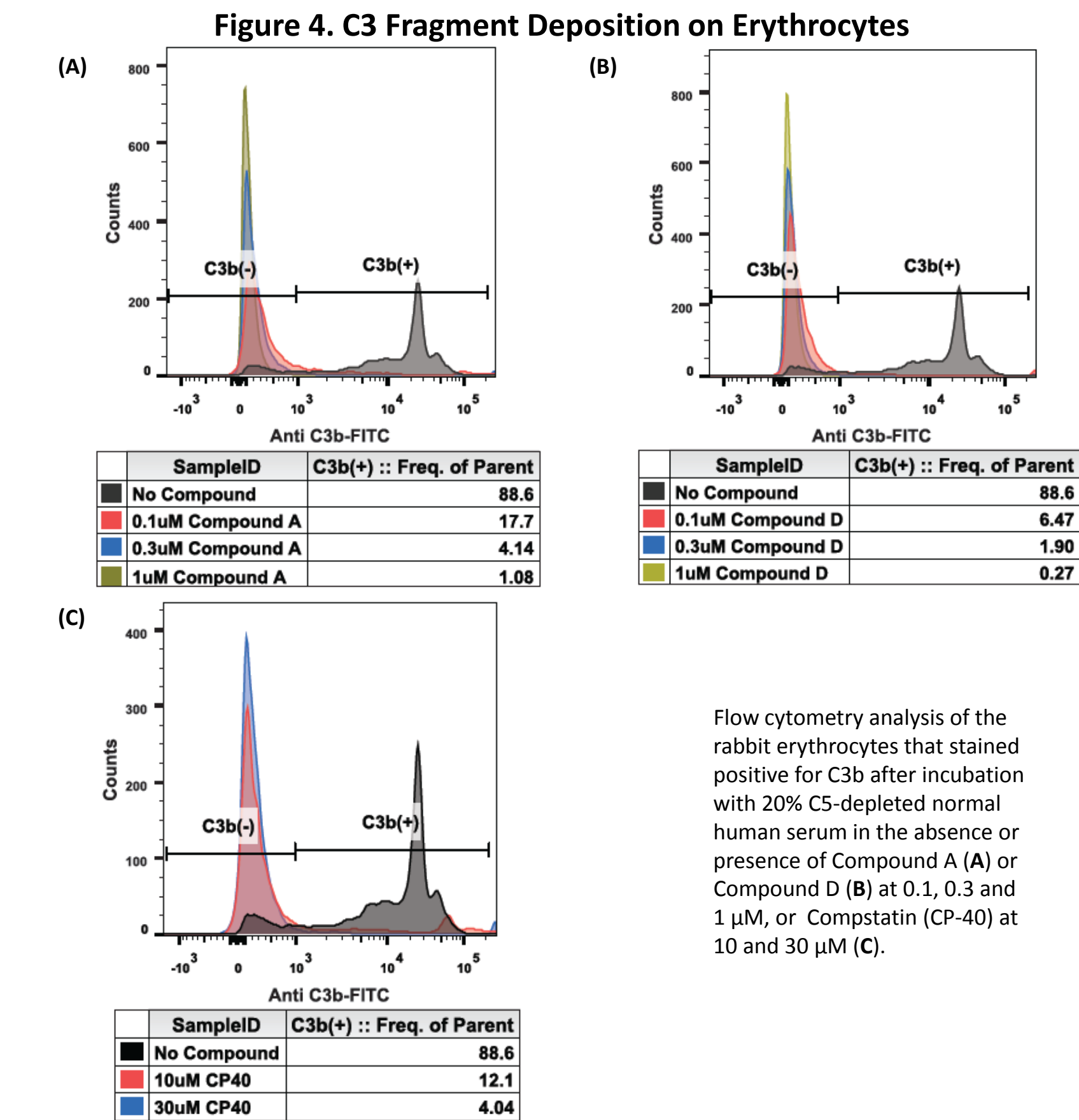
Inhibitor	AP Hemolysis (Human Serum)		AP Wieslab ELISA (Human Serum)		AP Hemolysis (Rat Serum)
	IC ₅₀ (nM)	IC ₉₀ (nM)	IC ₅₀ (nM)	IC ₉₀ (nM)	IC ₅₀ (nM)
Compd A	17	45	17	48	>100,000
Compd D	6.4	14	6.0	13	>100,000
Anti-C5 mAb ^a	-	-	38	346	-
FUT-175	12,000	32,000	-	-	12,000

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



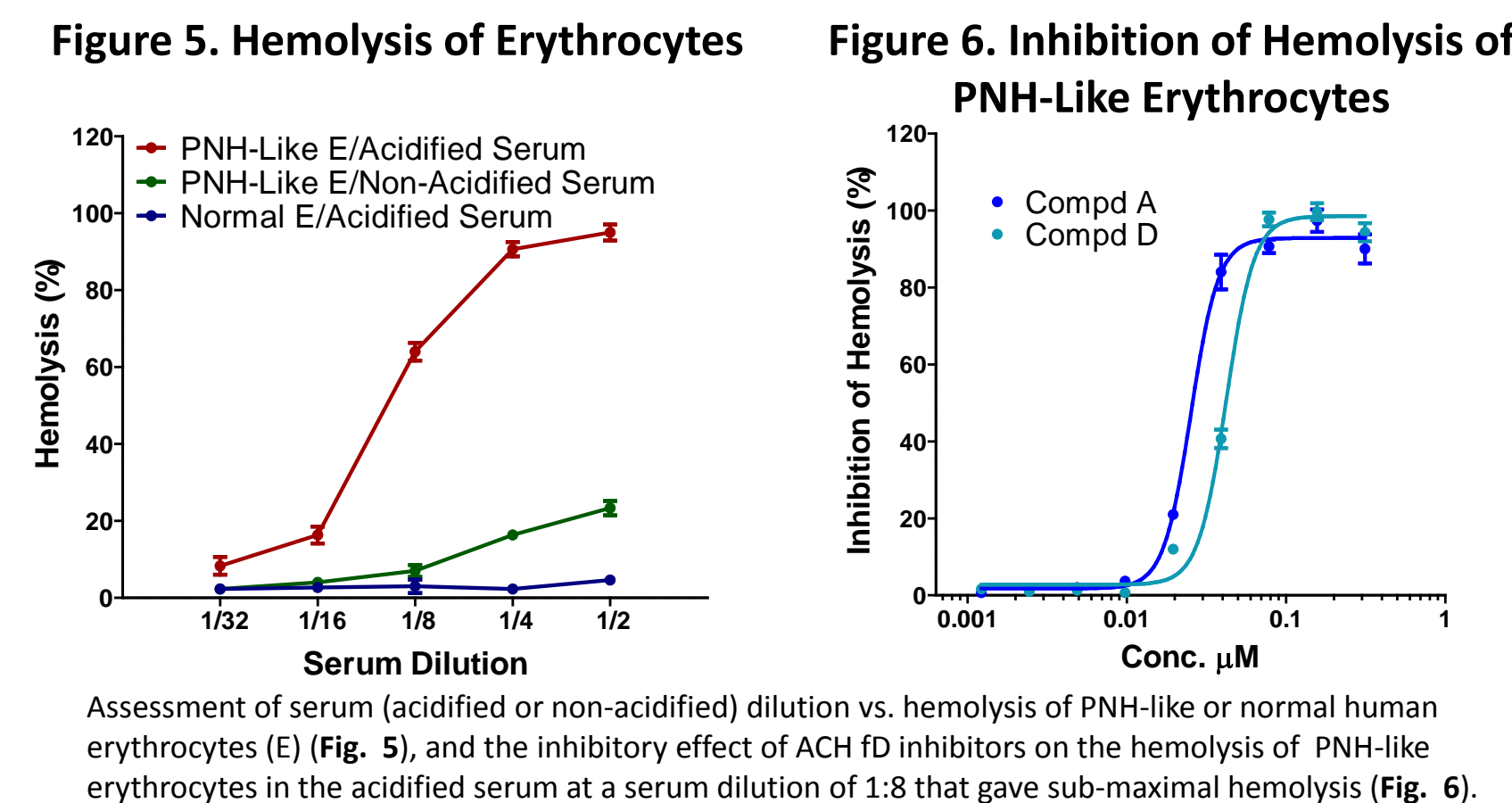
RESULTS

4 Blockage of Opsonization

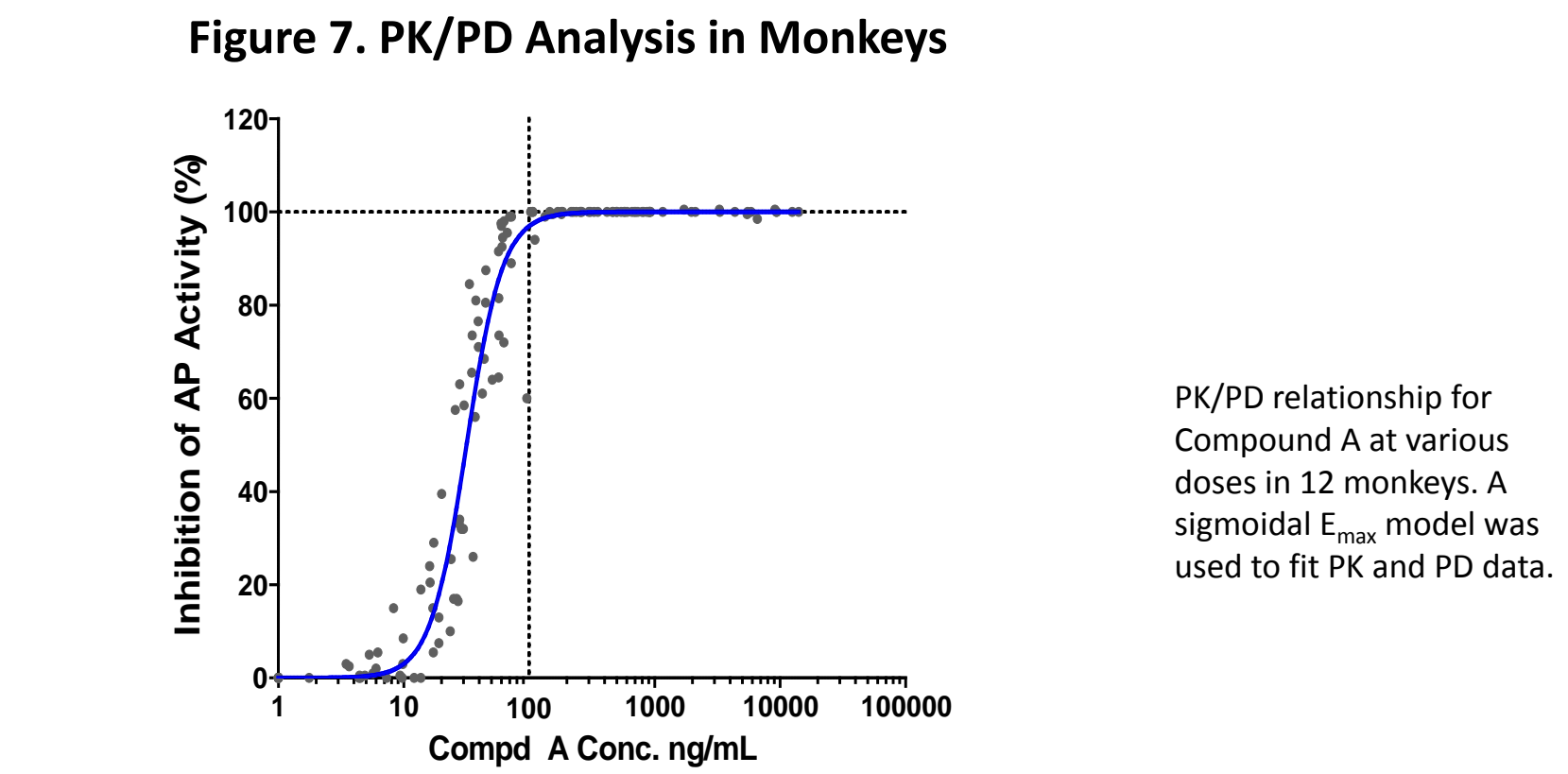


- ACH fD inhibitors blocked C3 fragment deposition effectively (< 5% of cells stained positive at 0.3 μM) (Fig. 4A and B).
- Compastatin (CP-40) achieved the same level of effectiveness at 30 μM (Fig. 4C).

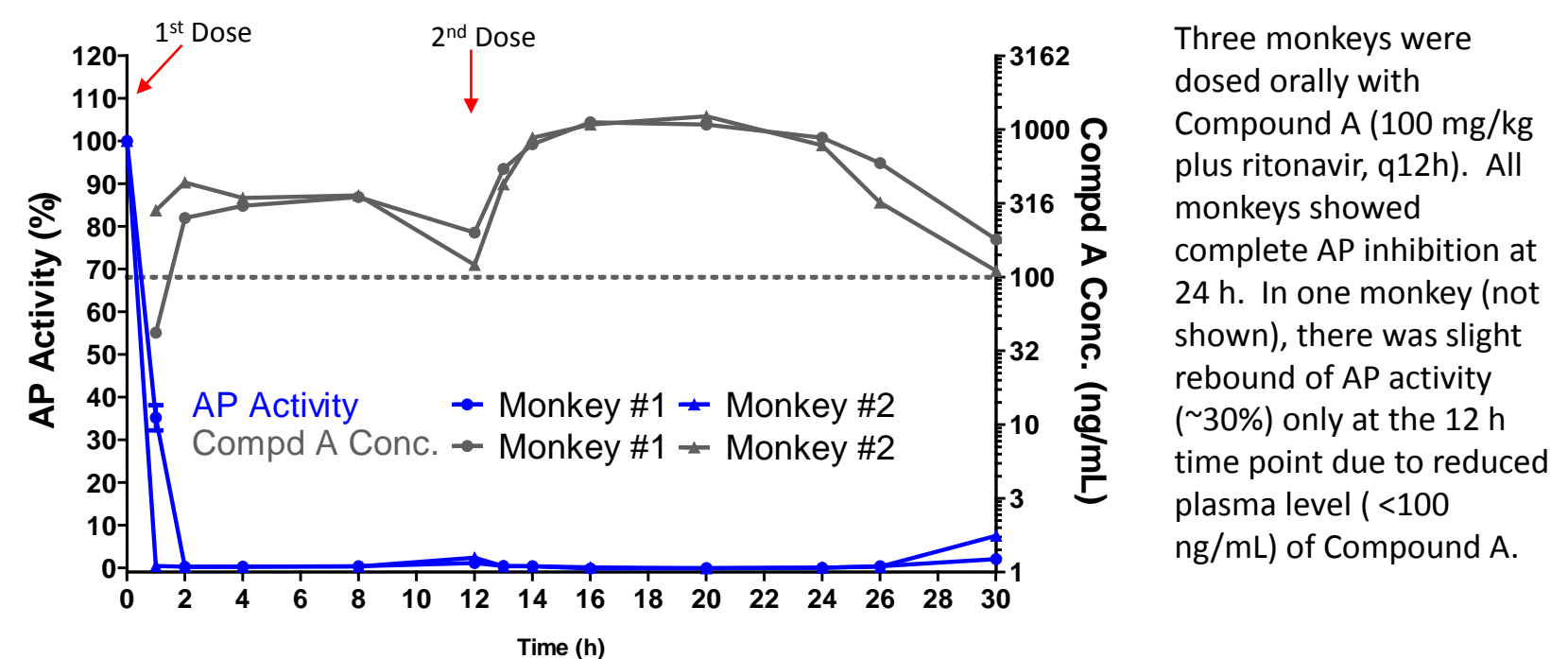
5 Effect on PNH-Like Erythrocytes



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



8 Complement Inhibition in Monkeys after Oral Dosing



- 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.