Preclinical Evaluation of Orally Bioavailable Small-Molecule Inhibitors of Complement Factor D as a Potential Treatment for Paroxysmal Nocturnal Hemoglobinuria

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INTRODUCTION

Complement factor D (fD), a serine protease, plays an essential role in the activation of the complement alternative pathway (AP) and provides important amplification of the classical and lectin complement pathways. Cleavage of factor B by fD generates C3 convertase that leads to opsonization of targeted surfaces with complement activation fragments and to the formation of the terminal complement complex (TCC); both events lead to cell lysis. Complement dysregulation underlies multiple hematological disorders including paroxysmal nocturnal hemoglobinuria (PNH), which is characterized by complement-mediated lysis of clonal populations of erythrocytes that lack glycophosphatidylinositol-anchored complement regulators. The current treatment for PNH is intravenous infusion of the anti-C5 monoclonal antibody eculizumab. Although eculizumab prevents intravascular hemolysis, it unmasks extravascular hemolysis occurring through C3 opsonization. In contrast, fD inhibitors are expected to inhibit both terminal complement pathway activation as well as opsonization and should, therefore, be well-positioned to potentially serve this unmet medical need. Herein, we present the preclinical evaluation of several of our small-molecule inhibitors of fD including potency, off-target activities, metabolism, and pharmacokinetic properties.

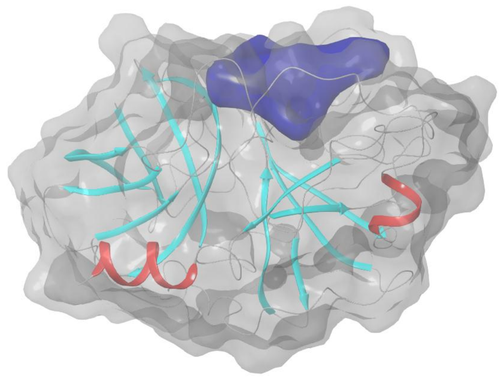
METHODS

- Recombinant fD was purified by serial chromatography and co-crystallized with compound A. Synchrotron X-ray diffraction data were collected and the structure was solved to 1.0 Å by molecular replacement using pdb entry 2XW9 as a search model.
- fD proteolyic activity was evaluated biochemically using the natural substrate consisting of C3b and complement factor B (fB). The cleavage product Bb was quantified with a commercial ELISA kit (Quidel).
- The AP hemolytic assay was conducted with unsensitized rabbit erythrocytes, 8% human serum, and 10 mM MgEGTA.
- Cell viability was assessed after 3–5 day compound treatment using either a CellTiter 96® AQueous One Solution kit or a CellTiter-Glo® Luminescent Cell Viability kit.
- Metabolic stability was determined by incubating compounds (0.1 μM) in liver microsomes (0.5 mg/mL) up to 2 h and quantifying by LC-MS/MS.
- Pharmacokinetic (PK) studies were performed following approved Institutional Animal Care and Use Committee (IACUC) protocols. Plasma concentrations of test compounds were measured by LC-MS/MS. For PK/PD analysis, AP activity was assessed in recovered serum samples by ELISA detection of TCC neoantigen generation following activation by LPS (Wieslab AP kit).

RESULTS

1 X-ray Structure of Inhibitor Bound to fD

Figure 1. Structure at 1.0 Å Resolution of Compound A Co-Crystallized with fD



- First X-ray structure of a reversible inhibitor (dark blue) bound to the single-chain serine protease fD.
- Inhibitor blocks entry to the active site (catalytic triad residues: Ser[183], His[41], and Asp[89]) and H-bonds with the oxyanion hole.
- An additional ~600 compounds have been synthesized and assessed for complement inhibitory activity.

2 Target Activity of Inhibitors

Table 1. Potent Inhibition of fD Proteolytic Activity and AP-Mediated Complement Activity

Compound	IC_{50} (nM)	
	Protease Assay	Hemolysis Assay
	fD-Mediated C3bB Cleavage	Alternative Pathway
A	35	17
B	34	23
C	9.3	8.0

- Compounds A–C demonstrated potent inhibitory effects on fD protease activity that translated to inhibition of cell lysis in a human AP hemolysis assay.
- Direct inhibition of fD was corroborated via a proteolytic assay using the non-specific synthetic substrate Z-Lys-SBzl (data not shown).

3 Off-Target Activity of Inhibitors

Table 2. Weak Activity Against Other Human Proteases

Human Protease	% Inhibition of Control Specific Activity		
	Inhibitors (Test Concentration 10 μM)		
	A	B	C
Thrombin	7	11	3
Urokinase	–14	6	–8
Chymase	–6	2	28
Elastase	3	16	–3
Cathepsin G	–10	0	8
DPP-IV[a]	–2	–6	–2
HNE[a]	11	40	–5
Kallikrein	–3	7	3
Tryptase	4	8	–4
Trypsin	0	–5	–6

a. Abbreviations: DPP-IV, Dipeptidyl peptidase IV; HNE, Human neutrophil elastase.

- Compounds A–C were highly selective toward fD and displayed no significant inhibitory effect on 10 other human serine proteases (Cerep, Table 2).
- Compounds A–C showed minimal cellular toxicity (CC_{50} >30 μM) in Huh, MT4, HepG2, and Hep2 cell lines; Compound A showed no mitochondrial toxicity as indicated by Crabtree assay (no decrease in CC_{50} when MT4 cells were cultured in galactose medium; compounds B and C not tested).
- No significant inhibition of the hERG potassium ion channel (<3–12% for compounds A–C at 3 μM).
- No significant inhibition of the major drug-metabolizing cytochrome P450 isozymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 (IC_{50} >30 μM for compounds A and C; <40% inhibition at 10 μM for compound B).

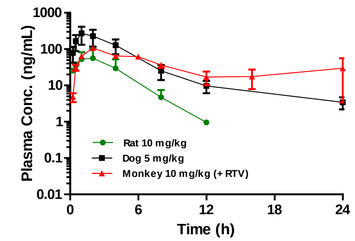
4 Metabolism and Pharmacokinetics of Inhibitors in Animal Species

Table 3. Good In Vitro Metabolic Stability of Inhibitors in Human

Compound	In Vitro Clearance Liver Microsomes (%Qh)[a]			
	Rat	Dog	Monkey	Human
A	<9	<15	16.3	<17
B	<9	<15	23.3	<17
C	<9	<15	<10	<17

a. In vitro clearance was scaled from the rate of compound metabolism and expressed as percent of hepatic blood flow.

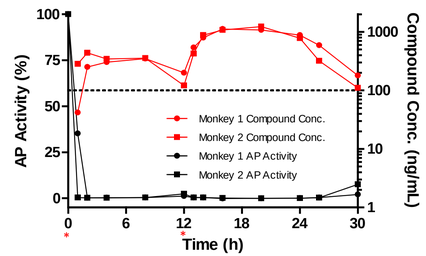
Figure 2. Significant Plasma Exposure after Single Oral Doses of Compound A



- Compounds A (Figure 2) and B (not shown) demonstrated oral bioavailability in rat, dog, and monkey.
  - Due to low metabolic stability in monkey, Compound A was co-dosed orally with ritonavir, a metabolic booster. In human, metabolic booster may not be needed due to high metabolic stability in microsomes.
- Compound A was selected for PK/PD evaluation based on potency and oral bioavailability (up to 22%).

5 Pharmacokinetics/Pharmacodynamics of Inhibitors in Non-Human Primates

Figure 3. Complete Inhibition of Complement AP Activity for 24 h following Oral Administration of Compound A in Cynomolgus Monkeys



- Oral dosing of compound A (100 mg/kg q12h, asterisks denote dosing times) with ritonavir (PK enhancer) demonstrated complete inhibition (>99%) of AP activity for 24 h. Three monkeys were dosed orally with Compound A. 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.
- In a separate dosing experiment in monkeys, serum analysis (ELISA) revealed no increase in fD concentration through 24 h. In contrast, the serum fD concentration increased up to 10-fold following single-dose IV administration of lampalizumab (an anti-fD antibody) in cynomolgus monkeys.[1]
- Preliminary human dose projection, from allometric scaling of preclinical animal PK parameters and assuming a 100 ng/mL C_{trough} value (see Poster 4817), suggests that complete suppression of AP activity may be achieved with 340 mg PO bid dosing without ritonavir.

CONCLUSIONS

- We have discovered highly selective small-molecule inhibitors of fD that demonstrate (1) strong inhibition of AP-mediated complement terminal pathway activation, (2) low potential for off-target effects, and (3) good ADME characteristics that includes oral bioavailability in animal species.
- Compound A possesses a desirable PK profile in preclinical species that enables complete inhibition of AP activity for 24 h in non-human primates after oral dosing.
- These results support the therapeutic potential of this class of inhibitors as promising development candidates for the oral treatment of complement-mediated diseases.
- This platform could play a role in addressing the needs of PNH patients, including those who fail to respond or respond sub-optimally to eculizumab.

Reference: 1. Loyet, K. M., et al., J Pharmacol Exp Ther 2014, 351(3): 527–537.

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