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

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 hematologic disorders including paroxysmal nocturnal hemoglobinuria (PNH), which is characterized by complement-mediated lysis of clonal populations of erythrocytes that lack glycoporphatidylinositol-anchored complement regulators. The current treatment for PNH is intravenous infusion of the anti-C5 metabolic booster. In human, metabolic booster may not be needed due to high metabolic stability in micromolars.

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 resolution. In contrast, FD inhibitors are expected to inhibit both terminal AP and 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.

RESULTS

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (nM)</th>
<th>Protease Assay</th>
<th>Hemolytic Assay</th>
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<tbody>
<tr>
<td>A</td>
<td>35</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>34</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>C</td>
<td>9.3</td>
<td>17</td>
<td>20</td>
</tr>
</tbody>
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| Target Activity of Inhibitors

- 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: Ser183, His41, and Asp89) and H-bonds with the oxygen hole.
- An additional ~600 compounds have been synthesized and assessed for complement inhibitory activity.
- In a separate dosing experiment in monkeys, serum analysis (ELISA) revealed no increase in FD concentration through 24 h. In one monkey (not shown), there was slight rebound of AP activity (~30%) only at 24 h. In all monkeys, AP activity was completely suppressed at 24 h. All monkeys showed complete AP inhibition at 24 h. Administration of Compound A in cynomolgus monkeys showed no significant inhibition of the major drug-metabolizing cytochrome P450 isozymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, and 3A5 in Huh, MT4, HepG2, and Hep2 cell lines; Compound A showed no metachronal toxicity as indicated by Crable assay (no decrease in C5 activation). In all monkeys, plasma and plasma C and B not tested.

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 human species.


Disclosure: All authors are employees and share holders of Achillion Pharmaceuticals, Inc.