INTRODUCTION

Cidara Therapeutic’s novel Cloudbreak antiviral Fc-conjugates (AVCs) comprise stable conjugates of potent, surface-acting antiviral agents with the Fc domain of human IgG1. Human IgG1 Fc provides potent immune activation via Fcg receptor (FcgR) engagement as well as long half-life. AVCs demonstrate a multimodal mechanism of action – direct antiviral activity and immune-mediated clearance. A novel AVC, CB-012, is under development for the prevention and treatment of influenza. CB-012 has demonstrated potent, broad-spectrum activity against influenza and efficacy in multiple influenza infection models (immunocompetent and immune-compromised).1,4 Herein, we evaluated the contribution of immune-mediated effector functions to CB-012 activity in functional cell-based assays in vitro and in a lethal mouse model of influenza infection.

METHODS

FcgR interaction was determined by binding of AVCs to immobilized FcgRIIA by ELISA. Antibody-dependent cellular cytotoxicity (ADCC) was determined using a reporter cell assay according to the manufacturer’s protocol (Promega). BALB/c mice were challenged intranasally with 3E2 PFU (3x LD50) of mouse-adapted influenza A/Puerto Rico/8/1934 (H1N1) and treated 2 h post-challenge intravenously with AVCs at doses ranging from 0.1 – 1 mg/kg. Body weight was recorded daily and body weight loss of > 20% was recorded as mortality.

To determine the immune contribution to the activity of CB-012, we designed an immune-silent analog of CB-012, CB-012a, which uses the mutant Fc, N297A, that abrogates FcgR binding instead of a WT Fc. First, we determined the intrinsic activity in a cell-based cytotoxic effect (CPE) assay for CB-012 and CB-012a.

To determine the immune contribution to the activity of CB-012 in a mouse model of influenza infection, we compared the efficacy of CB-012 to CB-012a.

RESULTS

CB-012 and CB-012a demonstrated comparable EC50 in a CPE assay of 1.01 nM and 1.28 nM against influenza A, respectively. Next, we determined the binding to a representative FcgR, FcgRIIIA, in an ELISA format and confirmed that CB-012a has reduced binding to FcgRIIIA receptor, a receptor expressed on monocytes, macrophages and NK cells.

<table>
<thead>
<tr>
<th>Fcg receptor interaction [AUC]</th>
<th>IgG1 Fc (WT)</th>
<th>CB-012</th>
<th>IgG1 Fc (N297A)</th>
<th>CB-012a</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcgRIIA</td>
<td>1.79</td>
<td>2.54</td>
<td>0.68</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Fc-FcgRIIIA engagement results in immune effector cell-mediated viral clearance mechanisms, including antibody-dependent cellular cytotoxicity (ADCC), CB-012 showed higher ADCC than CB-012a.

<table>
<thead>
<tr>
<th>Effector function [AUC]</th>
<th>IgG1 Fc (WT)</th>
<th>CB-012</th>
<th>IgG1 Fc (N297A)</th>
<th>CB-012a</th>
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<tbody>
<tr>
<td>ADCC</td>
<td>9.74</td>
<td>18.54</td>
<td>N.D.</td>
<td>15.96</td>
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</tbody>
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CB-012 was fully protective at 0.3 mg/kg. In contrast, CB-012a was fully protective only at 1 mg/kg. The inability of CB-012a to bind to FcgR reduced protection by > 3-fold. These data clearly demonstrate that immune cell engagement contributes to the efficacy of CB-012 in a lethal mouse model of influenza infection.

CONCLUSIONS

CB-012 demonstrated exquisite potency in mouse models of prevention and treatment against influenza. Immune engagement significantly enhanced antiviral activity of CB-012 in a lethal mouse efficacy model of influenza infection by >3-fold. These results support further development of the AVC CB-012 for treatment and prophylaxis of influenza infection.

REFERENCES


