Introduction

Novel anti-influenza drugs that offer significant improvement over current therapy are urgently needed, because epidemic and pandemic influenza remain major public health concerns. S-033188, an active form of orally available prodrug S-033188, is a novel small molecule inhibitor of cap-dependent endonuclease (CEN) [1] of influenza A and B virus. CEN is an enzyme, located in the N-terminal domain of PA subunit of the influenza viral RNA polymerase complex, that is specific to influenza virus and essential for viral transcription and replication. Therefore, S-033188 represents a novel drug against a promising anti-influenza target. A Phase 3, Multicenter, Randomized, Double-blind Study of a Single Dose of S-033188 Compared with Placebo or Oseltamivir 75 mg Twice Daily for 5 Days in Otherwise Healthy Patients with Influenza was completed in 2017 [2].

Immunocompromised state seen in patient with a chronic disease leads to high risk for severe influenza infection, especially in children. A Phase 2, Randomized, Double-blind, Multicenter Study of S-033188 (0.75 mg/kg-3 mg/kg) Orally vs Placebo for the Induction of Immunocompromised State and Prolongation of the Period of Virus Infection was performed in Immunocompromised mice model. Female BALB/c mice were subcutaneously treated with 0.2 mg/mouse of cyclophosphamide (CP) from 1 day before to 9 days after the inoculation of A/Puerto Rico/8/34 (H1N1) virus for the induction of immunocompromised state and prolongation of the period of virus infection. CP-treated mice were intranasally inoculated with 100% 50% tissue culture infective dose (TCID_{50})/mouse of virus suspension and then orally given, from 5 days post-infection, 1.5 or 50 mg/kg of s-033188 (BID, for 5 days) or 5 mg/kg, clinically equivalent dose, or 50 mg/kg of oseltamivir phosphate (BID, for 5 days). For 5 days after the first administration, body weight change of mice was assessed and lung homogenates were prepared. Viral titer in the lung homogenates were quantified by standard TCID_{50} method. Sequence analysis of PA region: Sequence analysis of the PA gene of A/Puerto Rico/8/34 virus was performed by Sanger sequencing method. Sample RNA derived from the vehicle-treated group (sampling on 5 days post-infection (dpi)), the S-033188-treated groups (sampling on 6, 8, and 10 dpi), and the parent virus (A/Puerto Rico/8/34 virus) were analyzed.

Results

- The virus titer in the lung was maintained over 4 log_{10}TCID_{50}/mL from 5 to 10 days post-infection in CP-treated mice, whereas it decreased to 2 log_{10}TCID_{50}/mL by 9 days post-infection in CP-untreated mice, indicating a prolonged duration of virus infection by CP treatment (Figure 2).
- Body weight loss in oseltamivir phosphate-treated groups was slightly but not significantly suppressed, whereas that in S-033188-treated groups was significantly suppressed (Figure 1).
- In the S-033188 1.5, 15, and 50 mg/kg-treated groups, the virus titer in the lung decreased in a dose-dependent manner at 24 hours after the first dose, and then reached the lower limit of quantification (1.5 log_{10}TCID_{50}/mL) rapidly in a dose-dependent manner. The S-033188 1.5, 15, and 50 mg/kg-treated groups maintained a significantly lower virus titer in the lung than the vehicle-treated group and the oseltamivir phosphate 5 and 50 mg/kg-treated groups (Figure 2).
- No amino acid substitution in PA region was observed in all sequenced samples (vehicle-treated group [sampling on 5 dpi] and S-033188-treated groups [sampling on 6, 8, and 10 dpi] [5 mice/group]) compared to the parent virus (Table 1).

Conclusion

- Five days dosing of S-033188 significantly suppressed body weight loss compared to that of oseltamivir phosphate in immunocompromised mice model.
- Five days dosing of S-033188 suppressed virus replication more strongly and persistently than that of oseltamivir phosphate in immunocompromised mice model without emergence of PA amino acid substitution.
- S-033188 can be expected to inhibit virus replication more strongly and clearly the virus from the body more rapidly than oseltamivir phosphate in an immunocompromised high-risk group.

Reference


![Figure 1. Effect of S-033188 on body weight loss of A/Puerto Rico/8/34 virus-infected immunocompromised mice](image1)

![Figure 2. Reduction of viral titer by treatment with S-033188 or oseltamivir phosphate in A/Puerto Rico/8/34 virus-infected immunocompromised mice](image2)

<table>
<thead>
<tr>
<th>Dpi</th>
<th>Vehicle</th>
<th>S-033188</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5 mg/kg</td>
<td>15 mg/kg</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>ND</td>
<td>-</td>
</tr>
</tbody>
</table>

- ND, no amino acid substitution was observed in 5 mice, compared to the parent virus (A/Puerto Rico/8/34 virus); ND, not done

Table 1. Sequence analysis of the PA gene of A/Puerto Rico/8/34 virus derived from lung homogenates of S-033188-treated immunocompromised mice