**Introduction**

Human infection with avian influenza A (H7N9) virus first emerged in China in 2013 and 2016/2017 season saw the largest epidemic to date [1]. Although neuraminidase inhibitors (NAI) are used to treat influenza A (H7N9) infection, NAI-resistant viruses have been reported in some patients [2]. Therefore, novel anti-influenza drugs that improve over current therapy are urgently needed. S-033447, an active form of orally available prodrug S-033188, is a novel small molecule inhibitor of cap-dependent endonuclease that is essential for influenza virus gene transcription and replication. 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 [3]. Here, in vitro and in vivo efficacy against avian influenza virus A/Anhui/1/2013 (H7N9) strain was evaluated.

**Study Objective**

- To investigate the cell culture antiviral activity of S-033447 against influenza A (H7N9) and its NAI-resistant mutant virus.
- To investigate the efficacy of S-033188 in mice infected with influenza A (H7N9).

**Material and Method**

**In vitro study**: Madin-Darby canine kidney (MDCK) cells seeded on 96-well plates were inoculated with A/Anhui/1/2013 (H7N9) or its NA/R292K mutant strain at 100 tissue culture infectious dose 50 (TCID₅₀)/well. After incubation in 5% CO₂ at 35°C for 1 hour, the cells were washed and incubated in 5% CO₂ at 35°C for 24 hours with S-033447 or oseltamivir acid. Virus titer in the culture supernatants was determined in MDCK cells and EC₅₀ was calculated.

**In vivo study**: Female BALB/c mice were intranasally inoculated with A/Anhui/1/2013 strain at 4.0 × 10⁶ TCID₅₀/mouse. Immediately after the infection, mice were orally treated with S-033188 (0.5, 5, or 50 mg/kg/shot) twice a day (12 hours interval between each dosing) for 1 or 5 days, vehicle (0.5 w/v% methylcellulose) or oseltamivir acid phosphate (OTV, 5 clinically-equivalent dose 4) or methylcellulose (vehicle) twice a day for 5 days. Virus titers in the lungs 1, 3, or 5 day(s) after the infection was determined in MDCK cells. Survival time and body weight change were monitored through a 28-day period after the infection. Mice were euthanized and regarded as dead if their body weights were lower than 70% of the initial body weights according to humane endpoints.

**Results**

**In vitro study**: The mean EC₅₀ values of S-033447 against A/Anhui/1/2013 and its NA/R292K mutant strain was 0.80 and 1.12 nM, respectively. By contrast, the mean EC₅₀ values of oseltamivir acid against A/Anhui/1/2013 and its NA/R292K mutant strain was 15.41 and 142389.79 nM, respectively. The fold change values of S-033447 and oseltamivir acid for this mutant virus were 1.39 and 9239.94, respectively.

**In vivo study**: All mice survived by 5-day dosing of S-033188 while vehicle-treated mice died within 7 days after infection. One-day dosing of S-033188 was also strongly effective: 90% (0.5 mg/kg) or 100% (5 or 50 mg/kg) of mice survived, respectively (data not shown). The survival time of S-033188-treated groups was compared with that of OTV-treated groups. All groups treated with S-033188 significantly prolonged the survival time as compared with the group treated with OTV 5 mg/kg (survival rate: 30%). Five-day dosing of S-033188 suppressed body weight loss due to virus infection. In contrast, OTV had little or weak effect on body weight loss compared to S-033188. Virus titers for the overall time in all groups treated with S-033188 were significantly lower than those in vehicle-treated group. Notably, virus titers of almost all of mice, 5-day treatment with S-033188 at dose of 50 mg/kg, showed LLOQ. The virus titers in S-033188-treated groups were compared with those in OTV-treated groups. There was significantly less virus titers for the overall time in S-033188-treated groups than those in OTV 5 or 50 mg/kg-treated group.

**Conclusion**

- S-033447 was a potent inhibitor against replication of A/Anhui/1/2013 (H7N9) and its NA/R292K mutant strain compared with oseltamivir acid.
- S-033447 exhibited no potency shift against NAI-resistant strains (NA/R292K).
- S-033188 (5 or 50 mg/kg BID) completely improved mortality accompanied by suppression of body weight loss and virus titers in the lung compared to those of oseltamivir phosphate.

**Reference**

3. Study Protocol Number: 1601T0831

**Table 1.** EC₅₀ (nM) values of S-033447 and Oseltamivir acid against A/Anhui/1/2013 and its NA/R292K mutant strain in virus yield reduction assay

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>A/Anhui/1/2013 (H7N9)</td>
<td>0.80 ± 0.36</td>
<td>15.41 ± 11.56</td>
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<tr>
<td>A/Anhui/1/2013-NA/R292K* (H7N9)</td>
<td>1.12 ± 0.53</td>
<td>142389.79 ± 6601.02</td>
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* R292K substitution in the neuraminidase

**Figure 1.** Effect of S-033188 on mortality due to infection with A/Anhui/1/2013 strain in mice

**Figure 2.** Effect of S-033188 on body weight loss due to infection with A/Anhui/1/2013 strain in mice

**Figure 3.** Effect of S-033188 on virus titers in lungs at 1, 3, and 5 days post-infection in A/Anhui/1/2013 strain-infected mice