

# Inhibitory Effect of S-033188/S-033447, a novel inhibitor of influenza virus cap-dependent endonuclease, against highly pathogenic avian influenza virus A/H5N1

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## Introduction

Since 1997, human infections with A/H5N1 highly pathogenic avian influenza virus (HPAIV) have been reported. Although neuraminidase inhibitors (NAI) are used to treat HPAIV infection, NAI resistant viruses have been reported in some patients with high lethality after NAI treatment [de Jong MD et al., N Engl J Med. 2005]. Therefore, novel 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 randomized, double-blind, placebo-controlled, phase 2 study of S-033188 in otherwise healthy adult patients with influenza was completed in 2016 [OPTIONS IX, Oral presentation No.LBO-1] and global phase 3 studies are on-going. Here, we evaluated *in vitro* and *in vivo* efficacy of S-033188/S-033447 against HPAIV.

## Study Objective

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

## Methods

**In vitro study:** Madin-Darby canine kidney (MDCK) cells seeded on 96-well plates were inoculated with A/Hong Kong/483/97 (HK483, H5N1) or its NA/H274Y mutant strain at 100 tissue culture infectious dose 50 (TCID<sub>50</sub>) /well. After incubation in 5% CO<sub>2</sub> at 35°C for 1 hour, the cells were washed and incubated in 5% CO<sub>2</sub> at 35°C for 24 hours with S-033447 or oseltamivir acid. Virus titer in the culture supernatants was determined in MDCK cells and EC<sub>90</sub> was calculated.

**In vivo study:** Female BALB/c mice were intranasally inoculated with HK483 strain at 75 TCID<sub>50</sub>/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 day, vehicle (0.5 w/v% methylcellulose) or oseltamivir phosphate (OTV, 5 (clinically-equivalent dose [Ward P et al., J Antimicrob Chemother, 2005]) or 50 mg/kg/shot) twice a day for 5 days. Viral titer in the lung 1, 3, or 5 day(s) after the infection was determined in MDCK cells. Survival time and body weight change were then monitored through a 14-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 EC<sub>90</sub> values of S-033447 against HK483 and its NA/H274Y mutant strain was 1.64 and 3.16 nM, respectively. By contrast, the mean EC<sub>90</sub> values of oseltamivir acid against HK483 or its NA/H274Y mutant strain was 11.16 and 4054.91 nM, respectively. The fold change values of S-033447 and oseltamivir acid for this mutant virus were 1.93 and 363.34, respectively.

**In vivo study:** All mice survived by S-033188 (5 and 50 mg/kg) treatment while all vehicle-treated mice died within 7 days after infection. The survival time of S-033188-treated groups was compared with that of OTV-treated group. All groups treated with S-033188 showed significantly prolonged survival time as compared with group treated with 5 mg/kg of OTV (survival rate: 0%). S-033188 (5 and 50 mg/kg) also suppressed body weight loss due to virus infection. In contrast, OTV (5 or 50 mg/kg) had little or weak effect on body weight loss compared to S-033188 (5 and 50 mg/kg). Virus titers for the overall time in all S-033188-treated groups were significantly lower than those in vehicle-treated group. Notably, S-033188 (50 mg/kg) treatment strongly reduced virus titers (lower than limit of quantification (1.5 Log<sub>10</sub> TCID<sub>50</sub>/mL) until 5 days post-infection). In these conditions, there were significantly lower virus titers for S-033188 (5 or 50 mg/kg)-treated groups than OTV (5 or 50 mg/kg).

## Conclusions

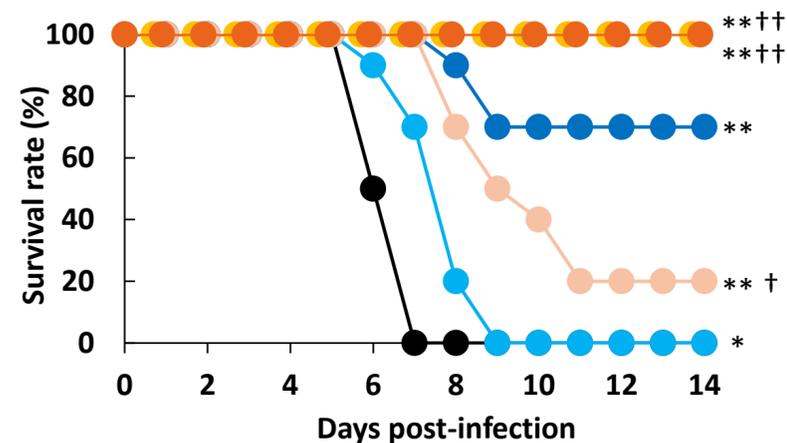
- S-033447 was a potent inhibitor against replication of A/Hong Kong/483/97 (H5N1) and its NA/H274Y mutant strain compared with oseltamivir acid.
- S-033447 exhibited no potency shift against NAI-resistant strains (NA/H274Y).
- One-day dosing of S-033188 (5 or 50 mg/kg BID) completely eliminated mortality accompanied by significant suppression of body weight loss and virus titers in the lung compared to those of oseltamivir phosphate.

**Table 1. EC<sub>90</sub> (nM) values of S-033447 and Oseltamivir acid against highly pathogenic avian influenza viruses in virus yield reduction assay**

Strain	S-033447			Oseltamivir acid		
	Mean	±	SD	Mean	±	SD
A/Hong Kong/483/97 (H5N1)	1.64	±	1.59	11.16	±	10.20
A/Hong Kong/483/97-NA/H274Y <sup>a</sup>	3.16	±	1.24	4054.91	±	1295.66

The mean and SD were calculated from 3 independent experiments.  
<sup>a</sup> H274Y substitution in the neuraminidase

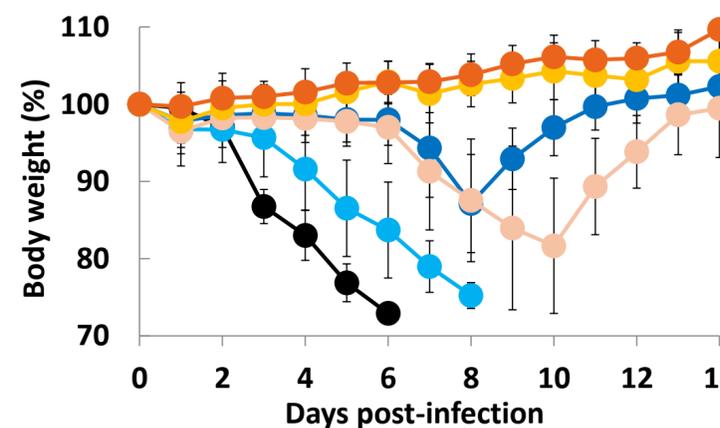
**Figure 1. Effect of S-033188 on mortality due to infection with HK483 strain in mice**



\* P<0.005 vs Vehicle, \*\* P<0.0001 vs Vehicle, † P<0.005 vs OTV 5 mg/kg, †† P<0.0001 vs OTV 5 mg/kg (log-rank test)

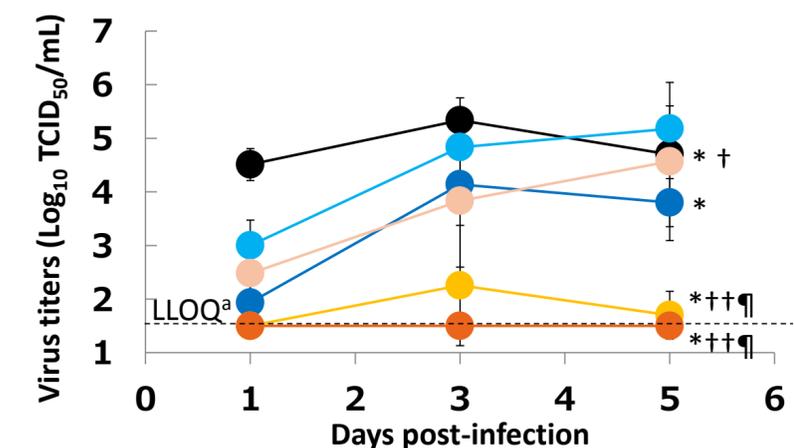
● Vehicle      ● OTV, 5 mg/kg, BID, 5 days      ● OTV, 50 mg/kg, BID, 5 days  
 ● S-033188, 0.5 mg/kg, BID, 1 day      ● S-033188, 5 mg/kg, BID, 1 day      ● S-033188, 50 mg/kg, BID, 1 day

**Figure 2. Effect of S-033188 on body weight loss due to infection with HK483 strain in mice**



Values are mean body weight change ± SD from the living mice at each day.

**Figure 3. Effect of S-033188 on virus titers in lungs at 1, 3, and 5 days post-infection in HK483 strain-infected mice**



<sup>a</sup> LLOQ: Lower limit of quantification (1.5 Log<sub>10</sub> TCID<sub>50</sub>/mL)  
 \* P<0.0001 vs Vehicle, † P<0.005 vs OTV 5 mg/kg, †† P<0.0001 vs OTV 5 mg/kg, ¶ P<0.0001 vs OTV 50 mg/kg (two-way analysis of variance)