ゾフルーザ錠 10mg，同 20mg

第 2 部 (モジュール 2) : CTD の概要 (サマリー)

2.6 非臨床試験の概要文及び概要表

2.6.3 薬理試験概要表

塩野義製薬株式会社

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略号及び用語定義一覧表

略号	用語定義
adjusted COD	coefficient of determination adjusted for degrees of freedom
APA	action potential amplitude
$APD_{30\ (90)}$	action potential duration at 30% (90%) repolarization
$APD_{30\text{-}90}$	a difference between APD_{90} and APD_{30}
$AUC_{0\text{-}12hr\ (0\text{-}24hr)}$	area under the plasma concentration-time curve from zero to 12 hours (zero to 24 hours) after dosing
$AUC_{0\text{-}inf}$	area under the plasma concentration-time curve extrapolated from 0 to infinity
BID	twice daily
BLQ	below the lower limit of quantification
Γ	slope factor
$C_{12\ (24)\ hr}$	plasma drug concentration at 12 (24) hours after dosing
CC_{50}	50% cytotoxicity concentration
CEN	cap-dependent endonuclease
CEN/RdRp	sequential reaction of CEN and RdRp
CI	combination Index
C_{max}	maximum plasma concentration
COD	coefficient of determination
CP	cyclophosphamide
CPE	cytopathic effect
C_{τ}	plasma concentration at the end of dosing interval after the first dose
DMSO	dimethyl sulfoxide
dpi	days post-infection
E_0	baseline effect
E199G	glutamic acid-to-glycine substitution at amino acid position 199
$EC_{50\ (90)}$	50% (90%) effective concentration
E_{max}	maximum effect
FBS	fetal bovine serum
FCCP	carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone
FOB	functional observational battery
GLP	Good Laboratory Practice
hERG	human ether-a-go-go related gene
HS	Human serum
I38I/T	mixture of no substitution and isoleucine-to-threonine substitution at amino acid position 38
I38T	isoleucine-to-threonine substitution at amino acid position 38
IC_{50}	50% inhibitory concentration
K362K/R	mixture of no substitution and lysine-to-arginine substitution at amino acid position 362
MC	methylcellulose aqueous solution
MMRM	mixed model repeated measures
MOI	multiplicity of infection
MRD	maximum rate of depolarization
NA	neuraminidase
N/A	not applicable
NA/H274Y	histidine-to-tyrosine substitution at amino acid position 274 in neuraminidase
N.C.	not calculated
PA	polymerase acidic protein
PA/E199G	glutamic acid-to-glycine substitution at amino acid position 199 in polymerase acidic protein

略号	用語定義
PA/I38I/T	mixture of no substitution and isoleucine-to-threonine substitution at amino acid position 38 in polymerase acidic protein
PA/I38T	isoleucine-to-threonine substitution at amino acid position 38 in polymerase acidic protein
PA/K362K/R	mixture of no substitution and lysine-to-arginine substitution at amino acid position 362 in polymerase acidic protein
PD	pharmacodynamics
PFU	plaque forming unit
PK	pharmacokinetics
PS	potency shift
QD	once daily
QTc	corrected QT interval
RdRp	RNA-dependent RNA polymerase
RMP	resting membrane potential
RNP	ribonucleoprotein
S.D.	standard deviation
S.E.	standard error
SI	selectivity index
$t_{1/2,z}$	elimination half-life
$T_{>2\ (>10,\ >50)}$	time which plasma concentration exceeds 2 (10, 50) ng/mL during 24 hours
$TCID_{50}$	50% tissue culture infectious dose
T_{max}	time to reach the maximum plasma concentration
WBP	whole body plethysmograph

2.6.3 薬理試験概要表

2.6.3.1 薬理試験： 一覧表

			Test Article: S-033188		
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**	**Location in CTD**
Primary Pharmacodynamics					
Inhibitory Effect of S-033447 on Cap-dependent Endonuclease Activity of Influenza Virus Laboratory Strains	Ribonucleoproteins extracted from influenza virus laboratory strains	In vitro	Shionogi & Co., Ltd.	S-033188-EB-201-N	4.2.1.1-01
Inhibitory Effect of RSC-033188 and RSC-033447 on in vitro Transcription Activity of Influenza A Virus	Recombinant 3P-TAP proteins derived from A/WSN/33 (H1N1) strain	In vitro	Shionogi & Co., Ltd.	R-033188-EB-082-N	4.2.1.1-02
Inhibitory Effect of S-033447 on Viral Replication in Cell Infected with Laboratory Strain	MDCK cells infected with influenza virus laboratory strains / Canine (Kidney)	In vitro	Shionogi & Co., Ltd.	S-033188-EB-163-N	4.2.1.1-03
Inhibitory Effect of RSC-033447 on Influenza Virus Replication in Cultured Cells Infected with Clinical Isolates	MDCK cells infected with influenza virus clinical isolates / Canine (Kidney).	In vitro	Shionogi & Co., Ltd.	R-033188-EB-069-N	4.2.1.1-04
Inhibitory Effect of S-033447 on Plaque Formation in Cultured Cells Infected with Laboratory Strains of Influenza Viruses	MDCK Cells infected with influenza virus laboratory strains / Canine (Kidney)	In vitro	Shionogi & Co., Ltd.	S-033188-EB-112-N	4.2.1.1-05
Inhibitory Effect of S-033447 on Plaque Formation in Cultured Cells Infected with Clinical Isolates of Influenza Viruses	MDCK cells infected with influenza virus clinical isolates / Canine (Kidney)	In vitro	Shionogi & Co., Ltd.	S-033188-EB-097-N	4.2.1.1-06
Inhibitory Effect of S-033447 on Plaque Formation in Cell Infected with Clinical Isolate (2)	MDCK cells infected with influenza virus clinical isolates / Canine (Kidney)	In vitro	Shionogi & Co., Ltd.	S-033188-EB-227-N	4.2.1.1-07
Inhibitory Effect of S-033447 on Plaque Formation in MDCK and MDCK-SIAT1 Cells Infected with Influenza Virus Vaccine Strains	MDCK cells infected with influenza virus clinical isolates / Canine (Kidney)	In vitro	Shionogi & Co., Ltd.	S-033188-EB-239-N	5.3.5.4-01
Inhibitory Effect of S-033447 on Viral Replication in Cell by Combination Treatment	MDCK cells infected with A/PR/8/34 (H1N1) strain / Canine (Kidney).	In vitro	Shionogi & Co., Ltd.	S-033188-EB-179-N	4.2.1.1-08
Isolation of Influenza A Virus Resistant to RSC-033447 in Cultured Cells	MDBK cells infected with A/WSN/33 (H1N1) strain / Bovine (Kidney)	In vitro	Shionogi & Co., Ltd. / [redacted]	R-033188-EB-063-N	4.2.1.1-09
Direct Sanger Sequencing Analysis of A/WSN/33 Virus Variants Isolated by Serial Passages in the Presence of S-033447	Sanger sequencing of A/WSN/33 (H1N1) virus variants without propagation of virus.	In vitro	Shionogi & Co., Ltd. / [redacted]	S-033188-EB-238-N	4.2.1.1-10

Replicative Capacity of Influenza A Virus Resistant to S-033447 in Cultured Cells	MDBK cells, MDCK cells, RPMI2650 cells or A549 cells infected with influenza A virus laboratory strains / Bovine (Kidney), Canine (Kidney), Human (Nasal Epithelial), Human (Lung).	In vitro	Shionogi & Co., Ltd.	S-033188-EB-116-N	4.2.1.1-11
Inhibitory Effect of S-033447 on Viral Replication in Cultured Cells Infected with Influenza A Virus Resistant to S-033447	MDCK cells infected with A/WSN/33 (H1N1) strain variants / Canine (Kidney)	In vitro	Shionogi & Co., Ltd.	S-033188-EB-123-N	4.2.1.1-12
Isolation of Influenza A Virus Resistant to S-033447 in Cultured Cells (2)	MDCK cells infected with A/Victoria/3/75 (H3N2) virus / Canine (Kidney)	In vitro	Shionogi & Co., Ltd. / [redacted]	S-033188-EB-208-N	4.2.1.1-13
Isolation of Influenza B Virus Resistant to S-033447 in Cultured Cells	MDCK cells infected with B/Maryland/1/59 strain / Canine (Kidney)	In vitro	Shionogi & Co., Ltd. / [redacted]	S-033188-EB-228-N	4.2.1.1-14
Inhibitory Effect of S-033447 on Replication of Highly Pathogenic Avian Influenza Virus (H5N1) in Cultured Cells	MDCK cells infected with A/Hong Kong/483/97 (H5N1) strain / Canine (Kidney)	In vitro	Shionogi & Co., Ltd.	S-033188-EB-140-N	4.2.1.1-15
Inhibitory Effect of S-033447 on Replication of Avian Influenza Virus (H7N9) in Cultured Cells	MDCK cells infected with A/Anhui/1/2013 (H7N9) strain / Canine (Kidney)	In vitro	Shionogi & Co., Ltd.	S-033188-EB-241-N	4.2.1.1-16
Inhibitory Effect of S-033447 on Replication of Animal Influenza A Viruses in Cultured Cells	MDCK cells infected with animal influenza viruses / Canine (Kidney)	In vitro	Shionogi & Co., Ltd.	S-033188-EB-240-N	4.2.1.1-17
Inhibitory Effect of S-033447 on Plaque Formation in Cultured Cells Infected with Influenza Viruses Used in Mouse Infection Model	MDCK cells infected with mouse-adapted influenza viruses / Canine (Kidney)	In vitro	Shionogi & Co., Ltd.	S-033188-EB-251-N	4.2.1.1-18
Inhibitory Effect of S-033188 and Oseltamivir Phosphate on Virus Replication in Lung of Mice Infected with A/WSN/33 Strain	Mice infected with A/WSN/33 (H1N1) strain were given test articles 5 days post-infection.	S-033188, Oseltamivir Phosphate: Oral, BID for 1 day	Shionogi & Co., Ltd.	R-033188-EB-056-N	4.2.1.1-19
Inhibitory Effect of S-033188 and Anti-influenza Virus Drugs on Virus Replication in Lung of Mice Infected with A/WSN/33 Strain	Mice infected with A/WSN/33 (H1N1) strain were given test articles 5 days post-infection.	S-033188, Favipiravir: Oral, BID for 1 day Zanamivir hydrate: intranasal, BID for 1 day Laninamivir octanoate: Intranasal, single	Shionogi & Co., Ltd.	R-033188-EB-056-N	4.2.1.1-19

PK Parameters of S-033447 after Single Oral Administration of S-033188 in Mice Infected with A/WSN/33 Strain	Mice infected with A/WSN/33 (H1N1) strain were given test articles 5 days post-infection.	S-033188: oral, single or BID for 1 day	Shionogi & Co., Ltd.	R-033188-EB-056-N	4.2.1.1-19
Inhibitory Effect of S-033188 on Virus Replication in the Lung of Mice Infected with B/Hong Kong/5/72	Mice infected with B/Hong Kong/5/72 strain were given test articles 5 days post-infection.	S-033188, Oseltamivir Phosphate: Oral, BID for 1 day	Shionogi & Co., Ltd.	S-033188-EB158-N	4.2.1.1-20
Inhibitory Effect of S-033188 on Virus Replication in the Lung of Mice Infected with A/Hong Kong/8/68 (H3N2)	Mice infected with A/Hong Kong/8/68 (H3N2) strain were given test articles 5 days post-infection.	S-033188, Oseltamivir Phosphate: Oral, BID for 1 day	Shionogi & Co., Ltd.	S-033188-EB158-N	4.2.1.1-20
Inhibitory Effect of S-033188 on Virus Replication in the Lung of Mice Infected with A/WSN/33-NA/H274Y (H1N1)	Mice infected with A/WSN/33-NA/H274Y (H1N1) strain were given test articles 5 days post-infection.	S-033188, Oseltamivir Phosphate: Oral, BID for 1 day	Shionogi & Co., Ltd.	S-033188-EB158-N	4.2.1.1-20
Time-Course Analysis of Inhibitory Effect of S-033188 on Influenza A Virus Replication in Mice	Mice infected with A/Osaka/129/2009 (H1N1) strain were given test articles 5 days post-infection.	S-033188: Oral, BID for 1 day or 3 days Oseltamivir Phosphate: oral, BID for 3 days	Shionogi & Co., Ltd.	R-033188-EB-067-N	4.2.1.1-21
Pharmacokinetic and Pharmacodynamic Analysis of S-033447 on Influenza A Virus Replication in Mice	Mice infected with A/WSN/33 (H1N1) strain were given test articles 5 days post-infection.	S-033447: Subcutaneous, once daily, twice daily, or four times a day for 1 day Oseltamivir Phosphate: Oral, BID for 1 day	Shionogi & Co., Ltd.	R-033188-EB-058-N	4.2.1.1-22
Inhibitory Effect of S-033447 on Viral mRNA Synthesis in Cells Infected with Influenza A Virus in the Presence of Serum	MDCK cells infected with A/WSN/33 (H1N1) strain / Canine (Kidney)	In vitro	Shionogi & Co., Ltd.	S-033188-EB-231-N	4.2.1.1-23
Protective Effect of S-033188 against Lethal Infection with Influenza A Virus in Mice	Mice infected with A/PR/8/34 (H1N1) strain were given test articles just after infection.	S-033188: Oral, BID for 1 day Oseltamivir Phosphate: oral, BID for 5 days	Shionogi & Co., Ltd.	S-033188-EB-124-N	4.2.1.1-24

Protective Effect of S-033188 against Lethal Infection with Influenza B Virus in Mice	Mice infected with B/Hong Kong/5/72 strain were given test articles just after infection.	S-033188: Oral, BID for 1 day Oseltamivir Phosphate: oral, BID for 5 days	Shionogi & Co., Ltd.	S-033188-EB-114-N	4.2.1.1-25
Protective Effect of Delayed Treatment with S-033188 against Lethal Infection with Influenza A Virus in Mice	Mice infected with A/PR/8/34 (H1N1) strain were given test articles 24, 48, 72,or 96 hours post-infection.	S-033188: Oral, BID for 5 days Oseltamivir Phosphate: oral, BID for 5 days	Shionogi & Co., Ltd.	S-033188-EB-188-N	4.2.1.1-26
Inhibitory Effect of Delayed Treatment with S-033188 on Virus Replication in Mice Lethally Infected with Influenza A Virus	Mice infected with A/PR/8/34 (H1N1) strain were given test articles 72 hours post-infection.	S-033188: Oral, BID for 1, 3 or 5 days Oseltamivir Phosphate: oral, BID for 1, 3 or 5 days	Shionogi & Co., Ltd.	S-033188-EB-233-N	4.2.1.1-27
Protective Effect of Delayed Treatment with S-033188 in Combination with Oseltamivir against Lethal Infection with Influenza A Virus in Mice	Mice infected with A/PR/8/34 (H1N1) strain were given test articles 96 hours post-infection.	S-033188: Oral, BID for 5 days Oseltamivir Phosphate:	Shionogi & Co., Ltd.	S-033188-EB-234-N	4.2.1.1-28
Therapeutic Effect of RSC-033188 on Influenza A Virus Infection in Ferrets	Ferrets infected with A/Kadoma/3/2006 (H1N1) strain were given test articles 24 hours post-infection.	S-033188: Oral, BID for 1 day Oseltamivir Phosphate: oral, BID for 2 days	Shionogi & Co., Ltd.	R-033188-EB-071-N	4.2.1.1-29
PK Parameters of S-033447 after Single Oral Administration of S-033188 in Ferrets	Non-infected ferrets	S-033188: Oral, Single	Shionogi & Co., Ltd.	R-033188-EB-071-N	4.2.1.1-29
Inhibitory Effect of S-033188 on Influenza Virus Replication in Immunocompromised Mice	Immunocompromised mice infected with A/PR/8/34 (H1N1) strain were given test articles 5 days post-infection.	S-033188: Oral, BID for 5 days Oseltamivir Phosphate: Oral, BID for 5 days	Shionogi & Co., Ltd.	S-033188-EB-194-N	4.2.1.1-30
Genotypic and Phenotypic Characterization of Influenza Viruses in Immunocompromised Mice Treated With S-033188	The lung homogenates of immunocompromised mice treated with S-033188	S-033188: Oral, BID for 5 days Oseltamivir Phosphate: Oral, BID for 5 days	Shionogi & Co., Ltd. [redacted]	S-033188-EB-252-N	4.2.1.1-31

Survival Effect of S-033188 on Highly Pathogenic Avian Influenza Virus (H5N1) Infection in Mice	Mice infected with A/Hong Kong/483/97 (H5N1) strain were given test articles just after infection.	S-033188: Oral, BID for 1 or 5 days Oseltamivir Phosphate: Oral, BID for 5 days	Shionogi & Co., Ltd.	S-033188-EB-110-N	4.2.1.1-32
Inhibitory Effect of S-033188 on Replication of Highly Pathogenic Avian Influenza Virus (H5N1) in Mice	Mice infected with A/Hong Kong/483/97 (H5N1) strain were given test articles just after infection.	S-033188, Oseltamivir Phosphate: Oral, BID for 1, 3 or 5 days	Shionogi & Co., Ltd.	S-033188-EB-110-N	4.2.1.1-32
Survival Effect of S-033188 on Avian Influenza Virus (H7N9) Infection in Mice	Mice infected with A/Anhui/1/2013 (H7N9) strain were given test articles just after infection.	S-033188: Oral, BID for 1 or 5 days Oseltamivir Phosphate: Oral, BID for 5 days	Shionogi & Co., Ltd.	S-033188-EB-226-N	4.2.1.1-33
Inhibitory Effect of S-033188 on Replication of Avian Influenza Virus (H7N9) in Mice	Mice infected with A/Anhui/1/2013 (H7N9) strain were given test articles just after infection.	S-033188, Oseltamivir Phosphate: Oral, BID for 1, 3 or 5 days	Shionogi & Co., Ltd.	S-033188-EB-226-N	4.2.1.1-33
Secondary Pharmacodynamics					
Effect of S-033447 on Cytotoxicity in Cultured Cells	MDCK, MDBK, RPMI2650, and A549 cell lines	In vitro	Shionogi & Co., Ltd.	S-033188-EB-117-N	4.2.1.2-01
Inhibitory Effect of S-033447 on Cytopathic Effect in Cultured Cells Infected with Influenza Virus	MDCK cells infected with influenza virus laboratory strains / Canine (Kidney)	In vitro	Shionogi & Co., Ltd.	S-033188-EB-209-N	4.2.1.2-02
Cytotoxicity Study of S-033447 in Cells from Multiple Human Tissues	BJ HEK-293, HepG2, HK2, HUVEC, Jurkat, MRC-5, SH-SY5Y, and SK-S-SH cell lines	In vitro	[redacted]	S-033188-EF-232-N	4.2.1.2-03
Mitochondrial Toxicity Test of S-033188 and S-033447 with Human Cells	HepG2 cell line	In vitro		S-033188-EF-197-N	4.2.1.2-04
In vitro selectivity study of S-033447 evaluated in various receptor, channel, and transporter binding and enzyme assays	Various receptors, channels, transporters and enzymes	In vitro		R-033188-EF-044-N	4.2.1.2-05
Safety Pharmacology					
Central nervous system					
Effects of S-033188 on central nervous system in rats	Rat/Sprague Dawley	Oral gavage	[redacted]	R-033188-SF-034-L	4.2.1.3-01
Cardiovascular system: In vitro					

Effects of S-033188 or S-033447 on myocardial action potential in isolated guinea pig papillary muscle	Guinea-pig papillary muscle	In vitro	[redacted]	R-033188-SF-038-L R-033188-SF-041-L	4.2.1.3-02, 03
Effects of S-033188 or S-033447 on potassium currents in cells expressing hERG channels	CHO cells expressing hERG channels	In vitro	[redacted]	R-033188-SF-039-L R-033188-SF-040-L	4.2.1.3-04, 05
Cardiovascular system: In vivo					
Effects of S-033188 on cardiovascular system in monkeys	Monkey/Cynomolgus	Oral gavage	[redacted]	R-033188-SF-035-L	4.2.1.3-06
Respiratory system					
Effects of S-033188 on respiratory system in rats	Rat/Sprague Dawley	Oral gavage	[redacted]	R-033188-SF-036-L	4.2.1.3-07

Abbreviations: BID, twice daily; hERG, human ether-à-go-go-related gene; NA, neuraminidase; NA/H274Y, histidine-to-tyrosine substitution at amino acid position 274 in neuraminidase.

2.6.3.2 効力を裏付ける試験

2.6.3.2.1 Inhibitory Effect of S-033447 on Cap-dependent Endonuclease Activity of Influenza Virus Laboratory Strains

Test Article: S-033447

Species/Biological material (Enzyme source)	RNPs extracted from influenza A viruses (A/WSN/33 [H1N1], A/PR/8/34 [H1N1], A/Victoria/3/75 [H3N2] and A/Hong Kong/8/68 [H3N2]) and influenza B viruses (B/Maryland/1/59, B/Hong Kong/5/72 and B/Lee/40)	
Final concentration (nmol/L)	0.39 to 25 (2-fold dilution)	
Reference article (Final concentration)	Suramin sodium salt (3.1 to 200 μmol/L [2-fold dilution])	
Vehicle	10% DMSO	
Substrate RNAs	5' - Cap - 8AA UA5 GCA UCA CUA GUA AGC UUU GCU CUA - 3'[a]	
Evaluation items	IC_{50} of CEN activity	
Evaluation method	Enzyme solution, test or reference article solution and substrate RNA solution were mixed and incubated at 37°C for 60 minutes, and then RNA fragment (enzymatic product) was defined and quantified with Applied Biosystems® genetic analyzer 3130xl and GeneMapper® v4.1 software.	
Results		
Strain	IC_{50} (nmol/L)	
	S-033447	Suramin sodium salt
A/WSN/33 [H1N1]	1.4 ± 1.0	22000 ± 2900
A/PR/8/34 [H1N1]	2.7 ± 0.12	70000 ± 4800
A/Victoria/3/75 [H3N2]	2.3 ± 0.47	96000 ± 4800
A/Hong Kong/8/68 [H3N2]	3.1 ± 1.1	150000 ± 17000
B/Maryland/1/59	8.9 ± 0.85	37000 ± 6100
B/Hong Kong/5/72	5.1 ± 1.1	34000 ± 4100
B/Lee/40	4.5 ± 0.51	33000 ± 9600
Abbreviations: CEN, cap-dependent endonuclease; DMSO, dimethyl sulfoxide; IC_{50}, 50% inhibitory concentration; S.D., standard deviation; RNPs, ribonucleoproteins. Each value represents the mean ± S.D. of more than 3 independent experiments. a 5' end Cap indicates 7-methyl-G linked to the adjacent base with 5' to 5' triphosphate bridge. Eight (8) indicates 2'-O-methyl-G. Five (5) indicates Cyanine 3-labeled U.		
Study number [Location in CTD]	S-033188-EB-201-N [4.2.1.1-01]	

2.6.3.2.2 Inhibitory Effect of RSC-033188 and RSC-033447 on in vitro Transcription Activity of Influenza A Virus

Test Article: S-033188, S-033447

Species/Biological material (Enzyme source)	Recombinant 3P-TAP proteins derived from A/WSN/33 (H1N1) virus, a source of CEN, RdRp, and CEN/RdRp activities		
Final concentration (nmol/L)	S-033188: 1.6 to 5000 (5-fold dilution) S-033447: 0.0128 to 40 (5-fold dilution)		
Reference article (Final concentration)	Suramin sodium salt (64 to 200000 nmol/L [5-fold dilution])		
Vehicle	10% DMSO		
Substrate RNAs	Sub1 (for CEN or CEN/RdRp assay): 5'-Cap-8AA UA5 GCA UCA CUA GUA AGC UUU GCU CUA-3'[a] Sub2 (for RdRp assay) : 5'-Cap-8AA UA5 GCA UCA-3'[a]		
5'- and 3'-terminal sequence of viral RNA	5'-vRNA: 5'-AGUAGAAACAAGGCC-3' 3'-vRNA: 5'-GGCCUGCUUUUGCU-3'		
Evaluation item	IC_{50} of CEN, RdRp, or CEN/RdRp activities		
Evaluation Method	Enzyme solution, test article solution or reference article solution, 5'- and 3'-terminal sequence of viral RNA and substrate RNA solution were mixed and incubated at 37°C for 60 minutes, and then RNA fragment (enzymatic product) was defined and quantified with Applied Biosystems® genetic analyzer 3130xl and GeneMapper® v4.1 software.		
Results			
Assay	IC_{50} (nmol/L)		
	S-033447	S-033188	Suramin sodium salt
CEN	2.5 ± 0.78	530 ± 110	11000 ± 2800
RdRp	>40	>5000	5700 ± 1200
CEN/RdRp	1.6 ± 0.17	340 ± 70	7400 ± 950
Abbreviations: RdRp, RNA-dependent RNA polymerase; CEN/RdRp, sequential reaction of CEN and RdRp. Each value represents the mean ± S.D. of 3 independent experiments. a 5' end Cap indicates 7-methyl-G linked to the adjacent base with 5' to 5' triphosphate bridge. Eight (8) indicates 2'-O-methyl-G. Five (5) indicates Cyanine 3-labeled U.			
Study Number [Location in CTD]	R-033188-EB-082-N [4.2.1.1-02]		

2.6.3.2.3 Inhibitory Effect of S-033447 on Viral Replication in Cell Infected with Laboratory Strains

Test Article: S-033447

Species/Biological material	MDCK cells infected with influenza virus laboratory strains {influenza A viruses (A/WSN/33 [H1N1], A/WSN/33-NA/H274Y [H1N1], A/PR/8/34 [H1N1], A/Victoria/3/75 [H3N2], A/Hong Kong/8/68 [H3N2]) and influenza B viruses (B/Maryland/1/59, B/Hong Kong/5/72, B/Lee/40)}.
Final concentration (nmol/L)	Influenza A viruses: 0.032, 0.16, 0.8, 4, and 20. Influenza B viruses: 0.16, 0.8, 4, 20, and 100
Reference substance (Final concentration)	Oseltamivir acid, Zanamivir hydrate and Laninamivir (Influenza A viruses: 0.64, 3.2, 16, 80, and 400 nmol/L. Influenza B viruses: 3.2, 16, 80, 400, and 2000 nmol/L) Favipiravir (Influenza A and B viruses: 160, 800, 4000, 20000, and 100000 nmol/L)
Vehicle	0.5% DMSO and 0.5% Water for Injection (DW)
Evaluation item	90% effective concentration (EC_{90}): Concentration achieving 90% of inhibition against the virus infection
Evaluation Method	The confluent monolayer MDCK cells in assay plates were infected with virus dilution at 100 $TCID_{50}$/well. After 1 hour incubation, the cells were washed and incubated with test article or reference article at 37°C in a CO_2 incubator for 24 hours for influenza A viruses or 30 hours for influenza B viruses. The culture fluid of each well was collected and stored until the viral titration is performed. The stored culture fluids was diluted and added into MDCK monolayers on 96-well plates. After 1 hour incubation, MDCK cells were washed and incubated at 37°C in a CO_2 incubator for 3 days. After incubation, virus-induced CPE was observed and virus titers were expressed as $log_{10}TCID_{50}$/mL.

Results

Type/subtype	Strains	EC_{90} (nmol/L)				
		S-033447	Oseltamivir acid	Zanamivir hydrate	Laninamivir	Favipiravir
A/H1N1	A/WSN/33	0.79 ± 0.13	161.12 ± 186.18	233.25 ± 157.46	17.68 ± 8.63	3778.65 ± 1037.54
A/H1N1	A/WSN/33-NA/H274Y	0.46 ± 0.14	>400	197.64 ± 33.19	19.08 ± 9.51	3137.46 ± 859.44
A/H1N1	A/PR/8/34	0.79 ± 0.12	180.50 ± 73.86	169.77 ± 54.22	9.03 ± 7.92	3943.76 ± 1812.06
A/H3N2	A/Victoria/3/75	0.98 ± 0.20	64.61 ± 44.97	202.28 ± 162.43	45.02 ± 35.78	4808.71 ± 1039.81
A/H3N2	A/Hong Kong/8/68	0.64 ± 0.30	44.15 ± 25.68	98.12 ± 72.68	23.16 ± 18.95	2944.21 ± 1136.46
B	B/Maryland/1/59	3.08 ± 1.16	245.75 ± 165.74	59.55 ± 26.99	20.88 ± 4.09	2705.13 ± 547.94
B	B/Hong Kong/5/72	2.21 ± 0.65	537.50 ± 211.21	178.36 ± 66.88	40.53 ± 14.61	1800.32 ± 1043.52
B	B/Lee/40	3.40 ± 1.27	371.77 ± 152.75	249.27 ± 206.39	45.98 ± 14.41	3727.46 ± 478.15

Abbreviation: CPE, cytopathic effect; $TCID_{50}$, 50% tissue culture infectious dose.
Each value represents the mean ± S.D. of 3 independent experiments.

Study Number [Location in CTD]	S-033188-EB-163-N [4.2.1.1-03]

2.6.3.2.4 Inhibitory Effect of RSC-033447 on Influenza Virus Replication in Cultured Cells Infected with Clinical Isolates

Test Article: S-033447

Species/Biological material		MDCK cells infected with influenza virus clinical isolates {influenza A viruses (A/Kadoma/3/2006 [H1N1], A/Osaka/129/2009 [H1N1], A/Osaka/180/2009 [H1N1], A/Hokkaido/12H048/2013 [H3N2], A/Niigata/12F392/2013 [H3N2]) and influenza B viruses (B/Hokkaido/11H011/2012, B/Gunma/12G045/2013)}.	
Final concentration (nmol/L)		Influenza A viruses: 0.032, 0.16, 0.8, 4, and 20. Influenza B viruses: 0.16, 0.8, 4, 20, and 100	
Reference substance (Final concentration)		Favipiravir (Influenza A and B viruses: 160, 800, 4000, 20000, and 100000 nmol/L)	
Vehicle		0.5% DMSO	
Evaluation item		EC_{90}: Effective concentration achieving 90% of inhibition against the virus infection	
Evaluation Method		The confluent monolayer MDCK cells in assay plates were infected with virus dilution at 100 $TCID_{50}$/well. After 1 hour incubation, the cells were washed and incubated with test article or reference article at 37°C in a CO_2 incubator for 24 hours for influenza A viruses and 30 hours for influenza B viruses. The culture fluid of each well was collected and stored until the viral titration is performed. The stored culture fluids was diluted and added into MDCK monolayers on 96-well plates. After 1 hour incubation, MDCK cells were washed and incubated at 37°C in a CO_2 incubator for 3 days. After incubation, virus-induced CPE was observed and virus titers were expressed as $log_{10}TCID_{50}$/mL.	
Results			
Type/subtype	Strains	EC_{90} (nmol/L)	
		S-033447	Favipiravir
A/H1N1	A/Kadoma/3/2006	0.88 ± 0.49	3417.76 ± 1810.86
A/H1N1	A/Osaka/129/2009	0.86 ± 0.08	4183.69 ± 6.29
A/H1N1	A/Osaka/180/2009[a]	0.95 ± 0.41	3945.39 ± 1216.98
A/H3N2	A/Hokkaido/12H048/2013	0.63 ± 0.25	3335.60 ± 1102.13
A/H3N2	A/Niigata/12F392/2013	0.87 ± 0.52	1898.14 ± 806.10
B	B/Hokkaido/11H011/2012	6.48 ± 0.97	1735.19 ± 252.28
B	B/Gunma/12G045/2013	6.10 ± 0.33	2585.87 ± 1825.91
Each value represents the mean ± S.D. of 3 independent experiments. a NA/H274Y.			
Study Number [Location in CTD]		R-033188-EB-069-N [4.2.1.1-04]	

2.6.3.2.5 Inhibitory Effect of S-033447 on Plaque Formation in Cultured Cells Infected with Laboratory Strains of Influenza Viruses

Test Article: S-033447

Species/Biological material		MDCK cells infected with influenza virus laboratory strains {influenza A viruses (A/WSN/33 [H1N1], A/WSN/33-NA/H274Y [H1N1], A/Victoria/3/75 [H3N2], A/Hong Kong/8/68 [H3N2]) and influenza B viruses (B/Maryland/1/59, B/Hong Kong/5/72)}.	
Final concentration (nmol/L)		0.08, 0.4, 2, 10, and 50	
Reference article (Final concentration)		Favipiravir (0.4, 2, 10, 50, and 250 μmol/L)	
Vehicle		0.1% DMSO	
Evaluation items		EC_{50}: Effective concentration achieving 50% of inhibition of plaque number	
Evaluation method		MDCK cells seeded in 12-well plate were infected with virus dilution at approximately 50 PFU/well. The plates were incubated at 33°C in a CO_2 incubator for 1 hour. After incubation, the cells were washed twice to remove uninfected viruses and overlaid with 1 mL of plaque assay medium (including agar). The cells were incubated at 33°C in a CO_2 incubator for 3 days. After incubation, the cells were fixed with 0.5 mL of 5: 1 (v/v) mixture of methanol: acetic acid at room temperature for 30 minutes and the gel over the cells will be removed. The cells were stained with 300 μL of amido black solution and incubated at room temperature for 30 minutes. Finally, amido black solution was washed out with water and plaque number was counted under a microscopy.	
Results			
Type/subtype	Strains	EC_{50} (nmol/L)	
		S-033447	Favipiravir
A/H1N1	A/WSN/33	0.76 ± 0.36	20237.37 ± 7338.31
A/H1N1	A/WSN/33-NA/H274Y	0.49 ± 0.20	19708.66 ± 4792.24
A/H3N2	A/Victoria/3/75	0.76 ± 0.19	10622.91 ± 3284.25
A/H3N2	A/Hong Kong/8/68	0.35 ± 0.06	5061.25 ± 660.16
B	B/Maryland/1/59	4.85 ± 2.42	21061.74 ± 16213.15
B	B/Hong Kong/5/72	4.33 ± 2.69	15798.81 ± 3888.14
Abbreviation: PFU, plaque forming unit. Each value represents the mean ± S.D. of 3 independent experiments.			
Study number [Location in CTD]		S-033188-EB-112-N [4.2.1.1-05]	

2.6.3.2.6 Inhibitory Effect of S-033447 on Plaque Formation in Cultured Cells Infected with Clinical Isolates of Influenza Viruses

Test Article: S-033447

Species/Biological material	MDCK cells infected with influenza virus clinical isolates {influenza A viruses (A/Kadoma/3/2006 [H1N1], A/Osaka/129/2009 [H1N1], A/Osaka/180/2009 [H1N1], A/Nagasaki/10N073/2011 [H1N1], A/Kyoto/10K124/2011 [H1N1], A/Kyoto/10K118/2011 [H1N1] A/Hokkaido/13H020/2014 (H1N1), A/Nagasaki/13N019/2014 (H1N1), A/Nagasaki/13N059/2014 (H1N1), A/Hyogo/10K051/2011 [H3N2], A/Niigata/10F017/2011 [H3N2], A/Niigata/11F027/2012 [H3N2], A/Tokyo/11IM003/2012 [H3N2], A/Hokkaido/12H048/2013 [H3N2], A/Niigata/12F392/2013 [H3N2], A/Kyoto/13SK042/2014 [H3N2], A/Nagasaki/13N033/2014 [H3N2]) and influenza B viruses (B/Kyoto/10K131/2011, B/Hokkaido/11H011/2012, B/Gunma/12G045/2013, B/Gunma/13G004/2014)}.
Final concentration (nmol/L)	Influenza A viruses: 0.016, 0.08, 0.4, 2, and 10. Influenza B viruses: 0.08, 0.4, 2, 10, and 50.
Reference article (Final concentration)	Favipiravir (Influenza A and B viruses: 0.4, 2, 10, 50, and 250 μmol/L)
Vehicle	0.1% DMSO
Evaluation items	EC_{50}: Effective concentration achieving 50% of inhibition of plaque number
Evaluation method	MDCK cells seeded in 12-well plate were infected with virus dilution at approximately 50 PFU/well. The plates were incubated at 33°C in a CO_2 incubator for 1 hour. After incubation, the cells were washed twice to remove uninfected viruses and overlaid with 1 mL of plaque assay medium (including agar). The cells were incubated at 33°C in a CO_2 incubator for 3 days. After incubation, the cells were fixed with 0.5 mL of 5: 1 (v/v) mixture of methanol: acetic acid at room temperature for 30 minutes and the gel over the cells will be removed. The cells were stained with 300 μL of amido black solution and incubated at room temperature for 30 minutes. Finally, amido black solution was washed out with water and plaque numbers were counted under a microscopy.

Results

Type/subtype	Strains	EC_{50} (nmol/L)	
		S-033447	Favipiravir
A/H1N1	A/Kadoma/3/2006	0.94 ± 0.23	15921.25 ± 4777.96
A/H1N1	A/Osaka/129/2009	0.26 ± 0.03	5997.85 ± 1459.17
A/H1N1	A/Osaka/180/2009 [a]	0.48 ± 0.07	7781.85 ± 514.29
A/H1N1	A/Nagasaki/10N073/2011	0.20 ± 0.10	7107.27 ± 2961.82
A/H1N1	A/Kyoto/10K124/2011 [a]	0.35 ± 0.14	7519.05 ± 3467.41
A/H1N1	A/Kyoto/10K118/2011	0.80 ± 0.46	9285.86 ± 1037.96
A/H1N1	A/Hokkaido/13H020/2014	0.99 ± 0.17	5699.56 ± 3484.95
A/H1N1	A/Nagasaki/13N019/2014	0.52 ± 0.15	5213.67 ± 3691.01
A/H1N1	A/Nagasaki/13N059/2014 [a]	0.66 ± 0.12	5529.57 ± 3384.77
A/H3N2	A/Hyogo/10K051/2011	0.66 ± 0.34	8578.24 ± 4051.95
A/H3N2	A/Niigata/10F017/2011	0.43 ± 0.05	10898.58 ± 2215.27
A/H3N2	A/Niigata/11F027/2012	0.90 ± 0.35	9997.65 ± 2119.93
A/H3N2	A/Tokyo/11IM003/2012	0.49 ± 0.06	8608.13 ± 1821.01
A/H3N2	A/Hokkaido/12H048/2013	0.56 ± 0.02	20102.12 ±17491.38
A/H3N2	A/Niigata/12F392/2013	0.68 ± 0.36	10986.95 ±960.41
A/H3N2	A/Kyoto/13SK042/2014	0.49 ± 0.07	9930.30 ±780.08

A/H3N2	A/Nagasaki/13N033/2014	0.42 ± 0.06	6246.96 ± 3728.19
B	B/Kyoto/10K131/2011	4.01 ± 1.64	5388.38 ± 383.42
B	B/Hokkaido/11H011/2012	5.28 ± 0.26	7959.90 ± 369.61
B	B/Gunma/12G045/2013	5.04 ± 1.83	6547.81 ± 2294.34
B	B/Gunma/13G004/2014	11.26 ± 1.20	7206.97 ± 1363.49
Each value represents the mean ± S.D. of 3 independent experiments. a NA/H274Y.			
Study number [Location in CTD]		S-033188-EB-097-N [4.2.1.1-06]	

2.6.3.2.7 Inhibitory Effect of S-033447 on Plaque Formation in Cell Infected with Clinical Isolate (2)

Test Article: S-033447

Species/Biological material		MDCK cells infected with influenza virus clinical isolates {influenza A viruses (A/Hokkaido/07H002/2008 [H1N1], A/Nagasaki/07N020/2008 [H1N1], A/Niigata/05F067/2006 [H3N2], A/Nagasaki/05N007/2006 [H3N2] , A/Kyoto/06K110/2007 [H3N2]) and influenza B viruses (B/Niigata/06F075/2007, B/Gunma/06G040/2007, B/Kyoto/08K015/2009, B/Kyoto/11K272/2012, B/Nagasaki/13N013/2013, B/Niigata/13F044/2014, B/Kyoto/13K042/2014)}.	
Final concentration (nmol/L)		Influenza A viruses: 0.016, 0.08, 0.4, 2, and 10. Influenza B viruses: 0.08, 0.4, 2, 10, and 50.	
Reference article (Final concentration)		Favipiravir (Influenza A and B viruses: 0.4, 2, 10, 50, and 250 μmol/L)	
Vehicle		0.1% DMSO	
Evaluation items		EC_{50}: Effective concentration achieving 50% of inhibition of plaque number	
Evaluation method		MDCK cells seeded in 12-well plate were infected with virus dilution at approximately 50 PFU/well. The plates were incubated at 33°C in a CO_2 incubator for 1 hour. After incubation, the cells were washed twice to remove uninfected viruses and overlaid with 1 mL of plaque assay medium (including agar). The cells were incubated at 33°C in a CO_2 incubator for 3 days. After incubation, the cells were fixed with 0.5 mL of 5: 1 (v/v) mixture of methanol: acetic acid at room temperature for 30 minutes and the gel over the cells will be removed. The cells were stained with 300 μL of amido black solution and incubated at room temperature for 30 minutes. Finally, amido black solution was washed out with water and plaque numbers were counted under a microscopy.	
Results			
Type/subtype	Strains	EC_{50} (nmol/L)	
		S-033447	Favipiravir
A/H1N1	A/Hokkaido/07H002/2008	1.55 ± 0.78	12399.34 ± 1668.54
A/H1N1	A/Nagasaki/07N020/2008 [a]	0.73 ± 0.38	9792.83 ± 1135.66
A/H3N2	A/Niigata/05F067/2006	0.38 ± 0.14	8474.14 ± 1821.05
A/H3N2	A/Nagasaki/05N007/2006	0.80 ± 0.18	5596.88 ± 1176.08
A/H3N2	A/Kyoto/06K110/2007	0.55 ± 0.22	7399.62 ± 2797.93
B	B/Niigata/06F075/2007	4.72 ± 1.75	9117.34 ± 1354.79
B	B/Gunma/06G040/2007	5.97 ± 0.58	8695.90 ± 1787.95
B	B/Kyoto/08K015/2009	5.04 ± 1.52	5057.36 ± 2106.91
B	B/Kyoto/11K272/2012	4.39 ± 1.26	3839.75 ± 2194.88
B	B/Nagasaki/13N013/2013	4.03 ± 0.84	7860.73 ± 796.11
B	B/Niigata/13F044/2014	3.33 ± 0.59	2376.55 ± 286.16
B	B/Kyoto/13K042/2014	5.96 ± 2.96	8531.80 ± 1620.65
Each value represents the mean ± S.D. of 3 independent experiments. a NA/H274Y.			
Study number [Location in CTD]		S-033188-EB-227-N [4.2.1.1-07]	

2.6.3.2.8 Inhibitory Effect of S-033447 on Plaque Formation in MDCK Infected with Influenza Virus Vaccine Strains

Test Article: S-033447

Species/Biological material	MDCK cells infected with influenza virus clinical isolates {influenza A viruses (A/Brisbane/59/2007 (H1N1), A/California/7/2009 (H1N1), A/Victoria/361/2011 (H3N2), A/Switzerland/9715293/2013 (H3N2), A/New York/39/2012 (H3N2), A/Texas/50/2012 (H3N2), B/Phuket/3073/2013, B/Massachusetts/2/2012, B/Wisconsin/1/2010, B/Texas/2/2013, B/Brisbane/60/2008, B/Malaysia/2506/2004)}.
Final concentration (nmol/L)	Influenza A viruses: 0.016, 0.08, 0.4, 2, and 10. Influenza B viruses: 0.08, 0.4, 2, 10, and 50.
Reference article (Final concentration)	Favipiravir (Influenza A and B viruses: 0.4, 2, 10, 50, and 250 μmol/L)
Vehicle	0.1% DMSO
Evaluation items	EC_{50}: Effective concentration achieving 50% of inhibition of plaque number
Evaluation method	MDCK cells seeded in 12-well plate were infected with virus dilution at approximately 50 PFU/well. The plates were incubated at 33°C in a CO_2 incubator for 1 hour. After incubation, the cells were washed twice to remove uninfected viruses and overlaid with 1 mL of plaque assay medium (including agar). The cells were incubated at 33°C in a CO_2 incubator for 3 days. After incubation, the cells were fixed with 0.5 mL of 5: 1 (v/v) mixture of methanol: acetic acid at room temperature for 30 minutes and the gel over the cells will be removed. The cells were stained with 300 μL of amido black solution and incubated at room temperature for 30 minutes. Finally, amido black solution was washed out with water and plaque numbers were counted under a microscopy.

Results

Type/subtype	Strains	EC_{50} (nmol/L)	
		S-033447	Favipiravir
A/H1N1	A/Brisbane/59/2007	1.85 ± 0.20	11806.44 ± 1044.24
A/H1N1	A/California/7/2009	1.18 ± 0.13	12350.37 ± 1742.20
A/H3N2	A/Victoria/361/2011	1.87 ± 0.06	11431.86 ± 1389.60
A/H3N2	A/New York/39/2012	0.74 ± 0.28	11021.81 ± 3806.56
A/H3N2	A/Texas/50/2012	1.00 ± 0.14	12911.87 ± 1518.57
A/H3N2	A/Switzerland/9715293/2013	1.04 ± 0.57	10369.53 ± 1404.84
B	B/Phuket/3073/2013	9.24 ± 2.93	13919.59 ± 1987.55
B	B/Malaysia/2506/2004	12.26 ± 2.47	13628.90 ± 2105.20
B	B/Brisbane/60/2008	10.61 ± 3.20	9600.00 ± 3731.90
B	B/Wisconsin/1/2010	13.00 ± 1.68	9446.12 ± 2070.56
B	B/Massachusetts/2/2012	9.53 ± 2.49	9402.45 ± 1483.96
B	B/Texas/2/2013	11.91 ± 1.31	8821.58 ± 1066.49

Each value represents the mean ± S.D. of 3 independent experiments.

Study number [Location in CTD]	S-033188-EB-239-N [5.3.5.4-01]

2.6.3.2.9 Inhibitory Effect of S-033447 on Viral Replication in Cell by Combination Treatment

Test Article: S-033447

Species/Biological material	MDCK cells infected with influenza virus, A/PR/8/34 (H1N1)
Final concentration (nmol/L)	0.3125, 0.625, 1.25, 2.5, 5, 10, and 20
Reference article (Final concentration)	Oseltamivir acid (39.0625, 78.125, 156.25, 312.5, 625, 1250, 2500, 5000, 10000 nmol/L) Zanamivir hydrate (19.53125, 39.0625, 78.125, 156.25, 312.5, 625, 1250, 2500, 5000 nmol/L) Laninamivir (1.953125, 3.90625, 7.8125, 15.625, 31.25, 62.5, 125, 250, 500 nmol/L) Peramivir trihydrate (1.953125, 3.90625, 7.8125, 15.625, 31.25, 62.5, 125, 250, 500 nmol/L)
Vehicle	1% DMSO and 1% Water for Injection (DW)
Evaluation items	EC_{50}: Effective concentrations achieving 50% inhibition against influenza virus infection Combination Index values (CIs) under the condition that both substances were added at the closest ratio of each EC_{50} value 50% cytotoxicity concentration (CC_{50}): Concentrations achieving 50% of cytotoxicity
Evaluation method	The confluent monolayer MDCK cells in 96-well assay plates were initially infected with virus dilution at 200 $TCID_{50}$/well. After 1 hour incubation, the cells were washed with MEM to remove uninfected viruses. Fifty microliter of viral assay medium was added to each well and 25 µL of S-033447 and one of the reference substances serially diluted were added into each row or lane of 96-well plates. After 2 days incubation at 37°C in a CO_2 incubator, cell viability was assessed by using WST-8 kit. Cytotoxicity study: Cell culture fluid was removed from the confluent monolayer MDCK cells in 96-well assay plates. Fifty µL of viral assay medium and 25 µL of S-033447 and one of the reference substances were added same as antiviral study. After 2 days incubation at 37°C in a CO_2 incubator, cell viability was assessed by using WST-8 kit.

Results

Substance A	Substance B	D_A (nmol/L)	D_B (nmol/L)	$D_{A/A+B}$ (nmol/L)	$D_{B/A+B}$ (nmol/L)	CI	Combination effect
S-033447	oseltamivir acid	4.51	3171.97	1.17	586.94	0.49	synergy
S-033447	zanamivir hydrate	4.49	1565.38	1.22	305.99	0.52	synergy
S-033447	laninamivir	4.52	212.74	1.12	56.02	0.58	synergy
S-033447	peramivir trihydrate	4.41	213.77	1.13	56.66	0.59	synergy

Abbreviation: CI, combination index.
D_A: the EC_{50} of substance A alone.
D_B: the EC_{50} of substance B alone.
$D_{A/A+B}$: the concentration of S-033447 giving 50% inhibition in combination with NA inhibitor at the closest ratio of each EC_{50} value
$D_{B/A+B}$: the concentration of NA inhibitor giving 50% inhibition in combination with S-033447 at the closest ratio of each EC_{50} value
$CI = (D_{A/A+B})/D_A + (D_{B/A+B})/D_B + (D_{A/A+B} \times D_{B/A+B})/(D_A \times D_B)$

Study number [Location in CTD]	S-033188-EB-179-N [4.2.1.1-08]

2.6.3.2.10 Isolation of Influenza A Virus Resistant to S-033447 in Cultured Cells

Test Article: S-033447

Species/Biological material	MDBK cells infected with influenza A virus (A/WSN/33 [H1N1]).
Final concentration (nmol/L)	Serial passage: 0.02, 0.1, 0.5, 2.5, and 12.5 (initial concentration) and the 2-fold increasing concentration for preceding passage. Inhibition of plaque formation: 0.08, 0.4, 2, 10, and 50
Reference article (Final concentration)	N/A
Vehicle	0.5% DMSO
Evaluation items	EC_{50}: Effective concentration achieving 50% of inhibition of plaque number, sequence analysis of polymerase acidic protein (PA) gene
Evaluation method	Viral infection and serial passage of virus: The confluent monolayer MDBK cells in 12-well plates were initially infected with virus dilution at a MOI of approximately 0.01 PFU/cell. After incubation at 4°C for 1 hour, the cells were washed to remove free viruses. The viral culture medium containing the test article was added into each well. The plates were incubated at 37°C in a CO_2 incubator for 3 days. The cultures will be performed in triplicate. The culture fluid of each well will be collected and the viral titration was performed. After measurement of the viral titer, the stored culture fluid from the preceding passage will be serially passaged to infect fresh MDBK cells at a MOI of approximately 0.01 PFU/cell in the presence of the 2-fold increasing concentration of the test article. This passage was repeated ten times. When the titer of the stored culture fluid was low for infection at a MOI of 0.01 PFU/cell, the passage was stopped (the maximum number of passage was 10). Measurement of viral titer: The stored culture fluid was diluted with viral assay medium. The dilutions were added into confluent monolayer MDBK cells in 12-well plates. After incubation at 37°C in a CO_2 incubator for 1 hour, the cells were washed to remove free viruses. The viral culture medium containing 1% agar was added into each well. The plates were incubated at 37°C in a CO_2 incubator for 3 days. The cells were overlaid with neutral red solution and incubated at 37°C for more than 2 hours. Finally, neutral red solution was removed and plaque number was counted from duplicate wells. Measurement of the susceptibility to S-033447: After the measurement of the viral titer of the last passage, the stored culture fluid was diluted with the viral assay medium to obtain an appropriate viral titer for infection. The virus dilutions were added into confluent monolayer MDBK cells in 12-well plates at approximately 25 PFU/well. After incubation at 37°C in a CO_2 incubator for 1 hour, the cells were washed to remove free viruses. The viral culture medium containing 1% agar and the test article were added into each well. The plates were incubated at 37°C in a CO_2 incubator for 3 days. The cells were overlaid with neutral red solution and incubated at 37°C for more than 2 hours. Finally, neutral red solution was removed and plaque number was counted from duplicate wells. When the virus from the last passage was resistant to S-033447, the measurement of the susceptibility and sequence analysis of the corresponding virus in the previous passage was conducted.

Results

Isolation of A/WSN/33 (H1N1) Virus Resistant to S-033447 and Amino Acid Substitutions in PA

Sample ID	Number of passage	Initial S-033447 concentration (nmol/L)	Last S-033447 concentration (nmol/L)	EC_{50} (nmol/L)	Fold change[a]	PA amino acid substitution[b]
A/WSN/33	0	N/A	N/A	0.29[c]	1.0	–
P10-1-1	10	0	0	0.33	1.1	–
P10-2-1	10	0	0	0.28	0.9	–
P10-3-1	10	0	0	0.25	0.8	–
P7-4-1	7	0.02	1.28	0.57	1.9	–
P7-5-1	7	0.02	1.28	0.52	1.8	–

P7-6-1[d]	7	0.02	1.28	0.40	1.4	−
P8-6-1[d]	8	0.02	2.56	6.62	22.5	I38T
P9-6-1	9	0.02	5.12	12.18	41.4	I38T
P5-7-1	5	0.1	1.6	0.27	0.9	−
P5-8-1	5	0.1	1.6	0.39	1.3	−
P5-9-1[d]	5	0.1	1.6	0.30	1.0	−
P6-9-1[d]	6	0.1	3.2	8.81	29.9	I38T
P7-9-1	7	0.1	6.4	11.66	39.6	I38T
P3-10-1	3	0.5	2	0.34	1.2	−
P3-11-1	3	0.5	2	0.30	1.0	−
P3-12-1	3	0.5	2	0.27	0.9	−
P1-13-1	1	2.5	2.5	0.39	1.3	−

Abbreviations: I38T, isoleucine-to-threonine substitution at amino acid position 38; MOI, multiplicity of infection; N/A, Not applicable.
−: No substitutions
a The fold change for isolated viruses was calculated as relative EC_{50} to parent virus.
b The amino acid substitutions were identified by the comparison to the sequences of parent virus.
c EC_{50} was calculated from 3 independent experiments.
d Course of passage were investigated for A/WSN/33(H1N1) virus resistant to S-033447.

Study number [Location in CTD]	R-033188-EB-063-N [4.2.1.1-09]

2.6.3.2.11 Direct Sanger Sequencing Analysis of A/WSN/33 Virus Variants Isolated by Serial Passages in the Presence of S-033447

Test Article: N/A

Species/Biological material	A/WSN/33 Virus Variants from the R-033188-EB-063-N study (P10-1, P10-2, P10-3, P7-4, P7-5, P7-6, P8-6, P9-6, P5-7, P5-8, P5-9, P6-9, P7-9, P3-10, P3-11, P3-12, P1-13)
Concentration (nmol/L)	N/A
Reference article (Concentration)	N/A
Vehicle	N/A
Evaluation items	Sequence analysis of PA gene
Evaluation method	Sequence analysis was conducted for the isolated viruses without propagation. Sequence analysis was performed by LSI Medience.

Results

Sample ID	Amino acid substitution[a]
P10-1	–
P10-2	–
P10-3	–
P7-4	–
P7-5	–
P7-6	–
P8-6	I38I/T
P9-6	I38T
P5-7	–
P5-8	–
P5-9	–
P6-9	I38T
P7-9	I38T
P3-10	–
P3-11	–
P3-12	–
P1-13	–

Abbreviation: I38I/T, mixture of no substitution and isoleucine-to-threonine substitution at amino acid position 38.
–: No substitutions
a The amino acid substitutions were identified by the comparison to the sequences of parent virus.

Study number [Location in CTD]	S-033188-EB-238-N [4.2.1.1-10]

2.6.3.2.12 Replicative Capacity of Influenza A Virus Resistant to S-033447 in Cultured Cells

Test Article: N/A

Species/Biological material	MDBK cells, MDCK cells, RPMI2650 cells or A549 cells infected with influenza A virus laboratory strains (A/WSN/33 [H1N1], rgA/WSN/33 [H1N1]), its resistant viruses to S-033447 (A/WSN/33-P9-6-1 [H1N1], A/WSN/33-P7-9-1 [H1N1] or rgA/WSN/33-PA/I38T [H1N1]) or its resistant viruses to NA inhibitors (A/WSN/33-NA/H274Y [H1N1]).			
Final Concentration (nmol/L)	N/A			
Reference article (Concentration)	N/A			
Vehicle	N/A			
Evaluation items	The virus titers ($TCID_{50}$/mL)			
Evaluation method	Viral infection: The confluent monolayer cells in 24-well plates were infected with viruses at 100 $TCID_{50}$/well. After 1 hour incubation, the cells were washed and incubated at 37°C in a CO_2 incubator. The culture fluid of each well was collected at 1, 8, 24, 36, 48, 72 hours after inoculation and stored until the viral titration was performed. Measurement of the Viral Titer: The stored culture fluids was diluted and added into MDCK monolayers on 96-well plates. After 1 hour incubation, MDCK cells were washed and incubated at 37°C in a CO_2 incubator for 3 days. After incubation, virus-induced CPE was observed and virus titers were expressed as $log_{10}TCID_{50}$/mL.			
Results				
Strains	$log_{10}TCID_{50}$/mL (Mean ± S.D.)			
	MDBK at 24 hours	MDCK at 24 hours	RPMI2650 at 24 hours	A549 at 36 hours
A/WSN/33	4.67 ± 0.33	6.72 ± 0.25	4.48 ± 0.30	2.91 ± 0.37
A/WSN/33-NA/H274Y	4.22 ± 0.38	6.00 ± 0.00	3.83 ± 0.29	3.50 ± 1.00
A/WSN/33-P9-6-1	3.72 ± 0.35	4.83 ± 0.44	2.85 ± 0.37	1.56 ± 0.10
A/WSN/33-P7-9-1	3.83 ± 0.44	2.56 ± 0.10	2.80 ± 0.64	1.50 ± 0.00
rgA/WSN/33	4.00 ± 0.33	5.06 ± 0.59	4.33 ± 0.17	4.72 ± 0.25
rgA/WSN/33-PA/I38T	3.28 ± 0.25	3.72 ± 0.25	3.06 ± 0.54	3.59 ± 0.16
Abbreviation: PA/I38T, isoleucine-to-threonine substitution at amino acid position 38 in polymerase acidic protein. Each value represents the mean ± S.D. of triplicate.				
Study number [Location in CTD]	S-033188-EB-116-N [4.2.1.1-11]			

2.6.3.2.13 Inhibitory Effect of S-033447 on Viral Replication in Cultured Cells Infected with Influenza A Virus Resistant to S-033447

Test Article: S-033447,

Species/Biological material	MDCK cells infected with influenza A virus laboratory strain (A/WSN/33 [H1N1], rgA/WSN/33 [H1N1]) or its resistant viruses to S-033447 (A/WSN/33-P9-6-1 [H1N1], rgA/WSN/33-PA/I38T [H1N1])			
Concentration (nmol/L)	A/WSN/33 (H1N1): 0.016, 0.08, 0.4, 2, and 10 Resistant viruses to S-033447: 0.4, 2, 10, 50, and 250			
Reference article (Concentration)	Favipiravir (160, 800, 4000, 20000, and 100000 nmol/L)			
Vehicle	0.5% DMSO			
Evaluation items	EC_{90}: Effective concentration achieving 90% of inhibition against the virus infection			
Evaluation method	Viral infection: The confluent monolayer MDCK cells in assay plates were infected with viruses at 100 $TCID_{50}$/well. After 1 hour incubation, the cells were washed and incubated with test or reference substances at 37°C in a CO_2 incubator for 24 hours. The culture fluid of each well was collected and stored until the viral titration is performed. Measurement of the Viral Titer: The stored culture fluids was diluted and added into MDCK monolayers on 96-well plates. After 1 hour incubation, MDCK cells were washed and incubated at 37°C in a CO_2 incubator for 3 days. After incubation, virus-induced CPE was observed and virus titers were expressed as $log_{10}TCID_{50}$/mL.			
Results				
Strain	EC_{90} (nmol/L)			
	S-033447		Favipiravir	
	Mean ± S.D.	Fold Change[a]	Mean ± S.D.	Fold Change[a]
A/WSN/33	0.82 ± 0.04	N/A	4540.49 ± 597.51	N/A
A/WSN/33-P9-6-1	29.52 ± 5.79	35.9	2002.75 ± 842.53	0.4
rgA/WSN/33	0.47 ± 0.03	N/A	3752.56 ± 999.69	N/A
rgA/WSN/33-PA/I38T	15.55 ± 0.98	33.0	4232.09 ± 1920.94	1.1
Each value of EC_{90} represents the mean ± S.D. of 3 independent experiments. a The fold change was calculated as relative EC_{90} to wild type.				
Study number [Location in CTD]	S-033188-EB-123-N [4.2.1.1-12]			

2.6.3.2.14 Isolation of Influenza A Virus Resistant to S-033447 in Cultured Cells (2)

Test Article: S-033447

Species/Biological material	MDCK cells infected with influenza A virus (A/Victoria/3/75 [H3N2]).
Final concentration (nmol/L)	Serial passage: 0.02, 0.1, 0.5, 2.5, and 12.5 (initial concentration) and the 2-fold increasing concentration for preceding passage.
Reference article (Final concentration)	N/A
Vehicle	0.5% DMSO
Evaluation items	EC_{50}: Effective concentration achieving 50% of inhibition of plaque number, sequence analysis of PA gene
Evaluation method	Viral infection and serial passage of virus: The confluent monolayer MDCK cells in 12-well plates were initially infected with virus dilution at a MOI of approximately 0.01 PFU/cell. After incubation at 33°C for 1 hour, the cells were washed to remove free viruses. The viral culture medium containing the test article was added into each well. The plates were incubated at 33°C in a CO_2 incubator for 3 days. The cultures were performed in triplicate. The culture fluid of each well was collected and the viral titration was performed. After measurement of the viral titer, the stored culture fluid from the preceding passage was serially passaged to infect fresh MDCK cells at a MOI of approximately 0.01 PFU/cell in the presence of the 2-fold increasing concentration of the test article. This passage was repeated eight times. When the titer of the stored culture fluid was low for infection at a MOI of 0.01 PFU/cell, the passage was stopped (the maximum number of passage was 8). Regarding samples with PA/K362K/R and PA/I38I/T which were the mixtures of amino acid substitution, isolation of virus was attempted by culturing MDCK cells in the presence of 10, 32, or 100 nmol/L S-033447. PA/I38T was isolated but PA/K362R was not isolated. Measurement of viral titer: The stored culture fluid was diluted with viral assay medium. The dilutions were added into confluent monolayer MDCK cells in 12-well plates. After incubation at 33°C in a CO_2 incubator for 1 hour, the cells were washed to remove free viruses. The viral culture medium containing 0.8% agar was added into each well. The plates were incubated at 33°C in a CO_2 incubator for 3 days. The cells were fixed for 30 minutes and overlaid with amido black solution and incubated at room temperature for 30 minutes. Finally, amido black solution was removed and plaque number was counted from single well. Sequence analysis: Sequence analysis was performed by Viroclinics Biosciences B.V.. Measurement of the susceptibility to S-033447: Sequence analysis was performed by Viroclinics Biosciences B.V..

Results

Isolation of A/Victoria/3/75 (H3N2) Virus Resistant to S-033447 and Amino Acid Substitutions in PA

Sample ID	Number of passage	Initial S-033447 concentration (nmol/L)	Last S-033447 concentration (nmol/L)	PA amino acid substitution[b]	EC_{50} (nmol/L)	Fold change[a]
A/Victoria/3/75	0	N/A	N/A	–	5.6	–
0 nM-1-P8	8	0	0	–	N/A	N/A
0 nM-2-P8	8	0	0	–	N/A	N/A
0 nM-3-P8	8	0	0	–	N/A	N/A
0.02 nM-1-P7	7	0.02	1.28	–	N/A	N/A
0.02 nM-2-P7	7	0.02	1.28	–	N/A	N/A
0.02 nM-3-P6	6	0.02	0.64	–	N/A	N/A
0.1 nM-1-P1	1	0.1	0.1	–	N/A	N/A
0.1 nM-1-P2	2	0.1	0.2	–	N/A	N/A
0.1 nM-1-P3	3	0.1	0.4	–	N/A	N/A

0.1 nM-1-P4	4	0.1	0.8	−	N/A	N/A
0.1 nM-1-P5	5	0.1	1.6	K362K/R	6.1	1.1
0.1 nM-2-P4	4	0.1	0.8	−	N/A	N/A
0.1 nM-3-P1	1	0.1	0.1	−	N/A	N/A
0.1 nM-3-P2	2	0.1	0.2	K362K/R	N/A	N/A
0.1 nM-3-P3	3	0.1	0.4	K362K/R	N/A	N/A
0.1 nM-3-P4	4	0.1	0.8	K362K/R	5.5	1.0
0.5 nM-1-P1	1	0.5	0.5	−	N/A	N/A
0.5 nM-1-P2	2	0.5	1	−	N/A	N/A
0.5 nM-1-P3	3	0.5	2	−	N/A	N/A
0.5 nM-1-P4	4	0.5	4	I38I/T	195.4	34.9
0.5 nM-2-P3	3	0.5	2	−	N/A	N/A
0.5 nM-3-P3	3	0.5	2	−	N/A	N/A
2.5 nM-1-P1	1	2.5	2.5	−	N/A	N/A
2.5 nM-2-P1	1	2.5	2.5	−	N/A	N/A
2.5 nM-3-P1	1	2.5	2.5	−	N/A	N/A
12.5 nM-1-P1	1	12.5	12.5	E199G	17.8	3.2

Drug Susceptibilities of Purified A/Victoria/3/75 Variant with I38T

Sample ID	Number of passage	S-033447 concentration for the purification (nmol/L)	PA amino acid substitution[b]	EC_{50} (nmol/L)	Fold Change relative to PA/ I38I/T variant[c]	Fold Change relative to parent virus[a]
0.5 nM-1-P4 PL19-B04	N/A	32	I38T	591.1	3.0	105.6
0.5 nM-1-P4 PL19-C04	N/A	10	I38T	584.9	3.0	104.4
0.5 nM-1-P4 PL20-G04	N/A	10	I38T	335.9	1.7	60.0
0.5 nM-1-P4 PL21-E03	N/A	100	I38T	178.0	0.9	31.8
0.5 nM-1-P4 PL21-G04	N/A	10	I38T	525.8	2.7	93.9

Abbreviations: E199G, glutamic acid-to-glycine substitution at amino acid position 199; K362K/R, mixture of no substitution and lysine-to-arginine substitution at amino acid position 362.
−: No substitutions
a The fold change for isolated viruses was calculated as relative EC_{50} to parent virus.
b The amino acid substitutions were identified by the comparison to the sequences of parent virus.
c The fold change for isolated viruses was calculated as relative EC_{50} to PA/ I38I/T variant.

Study number [Location in CTD]		S-033188-EB-208-N [4.2.1.1-13]

2.6.3.2.15 Isolation of Influenza B Virus Resistant to S-033447 in Cultured Cells

Test Article: S-033447

Species/Biological material	MDCK cells infected with influenza B virus (B/Maryland/1/59).
Final concentration (nmol/L)	Serial passage: 0.2, 1, 5, 25, and 125 (initial concentration) and the 2-fold increasing concentration for preceding passage.
Reference article (Final concentration)	N/A
Vehicle	0.5% DMSO
Evaluation items	sequence analysis of PA gene
Evaluation method	Viral infection and serial passage of virus: The confluent monolayer MDCK cells in 12-well plates were initially infected with virus dilution at a MOI of approximately 0.01 PFU/cell. After incubation at 33°C for 1 hour, the cells were washed to remove free viruses. The viral culture medium containing the test article was added into each well. The plates were incubated at 33°C in a CO_2 incubator for 3 days. The cultures were performed in triplicate. The culture fluid of each well was collected and the viral titration was performed. After measurement of the viral titer, the stored culture fluid from the preceding passage was serially passaged to infect fresh MDCK cells at a MOI of approximately 0.01 PFU/cell in the presence of the 2-fold increasing concentration of the test article. This passage was repeated eight times. When the titer of the stored culture fluid was low for infection at a MOI of 0.01 PFU/cell, the passage was stopped (the maximum number of passage was 8). Measurement of viral titer: The stored culture fluid was diluted with viral assay medium. The dilutions were added into confluent monolayer MDCK cells in 12-well plates. After incubation at 33°C in a CO_2 incubator for 1 hour, the cells were washed to remove free viruses. The viral culture medium containing 0.8% agar was added into each well. The plates were incubated at 33°C in a CO_2 incubator for 3 days. The cells were fixed for 30 minutes and overlaid with amido black solution and incubated at room temperature for 30 minutes. Finally, amido black solution was removed and plaque number was counted from single well. Sequence analysis: Sequence analysis was performed by LSI Medience.

Results

Isolation of B/Maryland/1/59 Virus Resistant to S-033447 and Amino Acid Substitutions in PA

Sample ID	Number of passage	Initial S-033447 concentration (nmol/L)	Last S-033447 concentration (nmol/L)	PA amino acid substitution[a]
B/Maryland/1/59	0	N/A	N/A	–
0 nM-1-P8	8	0	0	–
0 nM-2-P8	8	0	0	–
0 nM-3-P8	8	0	0	–
0.2 nM-1-P7	7	0.2	12.8	–
0.2 nM-2-P7	7	0.2	12.8	–
0.2 nM-3-P7	7	0.2	12.8	–
1 nM-1-P4	4	1	8	–
1 nM-2-P4	4	1	8	–
1 nM-3-P5	5	1	16	–
5 nM-1-P2	2	5	10	–
5 nM-2-P2	2	5	10	–
5 nM-3-P2	2	5	10	–

25 nM-1-P1	1	25	25	−
25 nM-2-P1	1	25	25	−
25 nM-3-P1	1	25	25	−
−: No substitutions a The amino acid substitutions were identified by the comparison to the sequences of parent virus.				
Study number [Location in CTD]	S-033188-EB-228-N [4.2.1.1-14]			

2.6.3.2.16 Inhibitory Effect of S-033447 on Replication of Highly Pathogenic Avian Influenza Virus (H5N1) in Cultured Cells

Test Article: S-033447

Species/Biological material	MDCK cells infected with highly pathogenic avian influenza virus (A/Hong Kong/483/97 [H5N1] or A/Hong Kong/483/97-NA/H274Y [H5N1])			
Concentration (nmol/L)	0.032, 0.16, 0.8, 4, 20 and 100 nmol/L			
Reference article (Concentration)	Oseltamivir acid (wild type virus: 0.64, 3.2, 16, 80, 400, and 2000 nmol/L, NA/H274Y mutant virus: 80, 400, 2000, 10000, 50000 and 250000 nmol/L)			
Vehicle	0.5% DMSO			
Evaluation items	EC_{90}: Effective concentration achieving 90% of inhibition against the virus infection			
Evaluation method	Viral infection: MDCK cells seeded on 96-well plates were inoculated with 100 $TCID_{50}$/well of virus suspensions. After incubation in 5% CO_2 at 35°C for 1 hour, the cells were washed and incubated in 5% CO_2 at 35°C for 24 hours with test or reference article solution. The culture supernatants were harvested and stored until use. Measurement of the viral titer: The culture supernatants were diluted serially and added into MDCK cells on 96-well plates. After incubation in 5% CO_2 at 35°C for 1 hour, the cells were washed and incubated medium. After 3 days, virus-induced CPE was observed and then virus titers were expressed as $\log_{10}$ $TCID_{50}$/mL.			
Results				
Strain	EC_{90} (nmol/L)			
	S-033447		Oseltamivir acid	
	Mean ± S.D.	Fold Change [a]	Mean ± S.D.	Fold Change [a]
A/Hong Kong/483/97	1.64 ± 1.59	N/A	11.16 ± 10.20	N/A
A/Hong Kong/483/97-NA/H274Y	3.16 ± 1.24	1.93	4054.91 ± 1295.66	363.34
Each value of EC_{90} represents the mean ± S.D. of 3 independent experiments. a Fold change was calculated as relative EC_{90} value to wild type (A/Hong Kong/483/97).				
Study number [Location in CTD]	S-033188-EB-140-N [4.2.1.1-15]			

2.6.3.2.17 Inhibitory Effect of S-033447 on Replication of Avian Influenza Virus (H7N9) in Cultured Cells

Test Article: S-033447

Species/Biological material	MDCK cells infected with avian influenza virus (A/Anhui/1/2013 [H7N9] or A/Anhui/1/2013-NA/R292K [H7N9])			
Concentration (nmol/L)	0.032, 0.16, 0.8, 4, 20 and 100 nmol/L			
Reference article (Concentration)	Oseltamivir acid (wild type virus: 0.64, 3.2, 16, 80, 400, and 2000 nmol/L, NA/R292K mutant virus: 80, 400, 2000, 10000, 50000 and 250000 nmol/L)			
Vehicle	0.5% DMSO			
Evaluation items	EC_{90}: Effective concentration achieving 90% of inhibition against the virus infection			
Evaluation method	Viral infection: MDCK cells seeded on 96-well plates were inoculated with 100 $TCID_{50}$/well of virus suspensions. After incubation in 5% CO_2 at 35°C for 1 hour, the cells were washed and incubated in 5% CO_2 at 35°C for 24 hours with test or reference article solution. The culture supernatants were harvested and stored until use. Measurement of the viral titer: The culture supernatants were diluted serially and added into MDCK cells on 96-well plates. After incubation in 5% CO_2 at 35°C for 1 hour, the cells were washed and incubated medium. After 3 days, virus-induced CPE was observed and then virus titers were expressed as $\log_{10}$ $TCID_{50}$/mL.			
Results				
Strain	EC_{90} (nmol/L)			
	S-033447		Oseltamivir acid	
	Mean ± S.D.	Fold Change [a]	Mean ± S.D.	Fold Change [a]
A/Anhui/1/2013	0.80 ± 0.36	**N/A**	15.41 ± 11.56	**N/A**
A/Anhui/1/2013-NA/R292K	1.12 ± 0.53	1.39	142389.79 ± 6601.02	9239.94
Each value of EC_{90} represents the mean ± S.D. of 3 independent experiments. a Fold change was calculated as relative EC_{90} value to wild type (A/Anhui/1/2013).				
Study number [Location in CTD]	S-033188-EB-241-N [4.2.1.1-16]			

2.6.3.2.18 Inhibitory Effect of S-033447 on Replication of Animal Influenza A Viruses in Cultured Cells

Test Article: S-033447

Species/Biological material	MDCK cells infected with avian influenza virus (A/swine/Chiba/14/2012 [H1N2], A/chicken/Taiwan/K703-1/2008 [H5N2], A/duck/Vietnam/HU4-879/2015 (H5N6), A/chicken/Vietnam/HU1-1050/2014 [H9N2], or A/duck/Vietnam/HU1-1512/2014 [H9N2])	
Concentration (nmol/L)	0.032, 0.16, 0.8, 4, 20 and 100 nmol/L	
Reference article (Concentration)	Favipiravir (32, 160, 800, 4000, 20000, 100000, and 500000 nmol/L [6 out of these 7 concentrations were selected.])	
Vehicle	0.5% DMSO	
Evaluation items	EC_{90}: Effective concentration achieving 90% of inhibition against the virus infection	
Evaluation method	Viral infection: MDCK cells seeded on 96-well plates were inoculated with 100 $TCID_{50}$/well of virus suspensions. After incubation in 5% CO_2 at 35°C for 1 hour, the cells were washed and incubated in 5% CO_2 at 35°C for 24 hours with test or reference article solution. The culture supernatants were harvested and stored until use. Measurement of the viral titer: The culture supernatants were diluted serially and added into MDCK cells on 96-well plates. After incubation in 5% CO_2 at 35°C for 1 hour, the cells were washed and incubated medium. After 3 days, virus-induced CPE was observed and then virus titers were expressed as $\log_{10}$ $TCID_{50}$/mL.	
Results		
Strain	EC_{90} (nmol/L)	
	S-033447	Favipiravir
A/swine/Chiba/14/2012	1.20 ± 0.51	25276.04 ± 26701.87
A/chicken/Taiwan/K703-1/2008	0.96 ± 0.55	21918.27 ± 25522.14
A/duck/Vietnam/HU4-879/2015	0.73 ± 0.53	20512.11 ± 20970.33
A/chicken/Vietnam/HU1-1050/2014	0.79 ± 0.55	29290.93 ± 28909.28
A/duck/Vietnam/HU1-1512/2014	0.96 ± 0.54	12605.88 ± 10328.79
Each value represents the mean ± S.D. of 3 independent experiments.		
Study number [Location in CTD]	S-033188-EB-240-N [4.2.1.1-17]	

2.6.3.2.19 Inhibitory Effect of S-033447 on Plaque Formation in Cultured Cells Infected with Influenza Viruses Used in Mouse Infection Model

Test Article: S-033447

Species/Biological material		MDCK cells infected with influenza virus used in mouse infection model (A/Osaka/129/2009 (mouse-adapted), A/Hong Kong/8/68 (mouse-adapted) and B/Hong Kong/5/72 (mouse-adapted)) or control strain (A/PR/8/34)	
Concentration (nmol/L)		0.016, 0.08, 0.4, 2, 10, and 50 nmol/L	
Reference article (Concentration)		Favipiravir (0.4, 2, 10, 50, and 250 μmol/L)	
Vehicle		0.1% DMSO	
Evaluation items		EC_{50}: Effective concentration achieving 50% of inhibition of plaque number	
Evaluation method		MDCK cells seeded in 12-well plate were washed and virus dilutions were added to the cells at 50 plaque forming unit (PFU)/well. After 1 hour incubation at 33°C in a CO_2 incubator, the cells were washed and overlaid with agar medium containing test or reference substances. After 3 days incubation at 33°C in a CO_2 incubator, the cells were fixed with fixative solution at room temperature for 30 minutes and then the gel over the cells was removed. The cells were stained with amido black solution and after 30 minutes incubation at room temperature, amido black solution was washed out and plaque number was counted under a microscopy.	
Results			
Type/subtype	Strain	EC_{50} (nmol/L)	
		S-033447	Favipiravir
A/H1N1	A/Osaka/129/2009 (mouse-adapted)	0.75 ± 0.38	9556.84 ± 2875.03
A/H3N2	A/Hong Kong/8/68 (mouse-adapted)	0.58 ± 0.13	12096.49 ± 1842.32
B	B/Hong Kong/5/72 (mouse-adapted)	8.37 ± 4.51	13230.20 ± 1075.35
Each value represents the mean ± S.D. of 3 independent experiments. EC_{50} value of S-033447 and Favipiravir against A/PR/8/34 (control strain) were 1.07 ± 0.09 and 13368.02 ± 1111.12 nmol/L, respectively.			
Study number [Location in CTD]		S-033188-EB-251-N [4.2.1.1-18]	

2.6.3.2.20 Inhibitory Effect of S-033188 on Virus Replication in Lung of Mice Infected with A/WSN/33 Strain

Test Article: S-033188

Species/Strain	Mouse/ BALB/cAnNCrlCrlj	
Gender and Number of animals	Female/10 group	
Virus	A/WSN/33 (H1N1)	
Vehicle/Formulation	0.5 w/v% methylcellulose (MC)	
Method of Administration	Oral administration twice daily (every 12 hours) for 1 day under non-anesthesia	
Dose (mg/kg BID)	0.5, 1.5, 5, 15, 50	
Reference article (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5 w/v% MC/oral administration twice daily (every 12 hours) for 1 day under non-anesthesia [Dose: 5 or 50 mg/kg as free base])	
Treatment	Mice were intranasally inoculated with 100 $TCID_{50}$ (100 µL/mouse) of the virus suspensions under anesthesia 5 days before the first administration.	
Evaluation items	Virus titers in lungs 24 hours after the first administration	
Evaluation method	Virus titers were measured by standard $TCID_{50}$ method using MDCK cells. Differences of the virus titers among the test article-treated groups, the reference article-treated groups and the vehicle-treated group were analyzed by the one-way analysis of variance followed by pairwise comparisons and the fixed sequence procedure.	
Results		
Article	Dose (mg/kg BID)	Log_{10} $TCID_{50}$/mL
Vehicle	–	5.10 ± 0.41
S-033188	0.5	$4.36 \pm 0.22^{*}$
	1.5	$3.57 \pm 0.36^{*\S\S}$
	5	$2.95 \pm 0.58^{**\dagger\dagger\S\S}$
	15	$1.93 \pm 0.41^{**\dagger\dagger\S\S}$
	50	$1.71 \pm 0.31^{**\dagger\dagger\S\S}$
Oseltamivir phosphate	5	$4.56 \pm 0.38^{*}$
	50	$4.06 \pm 0.55^{\ddagger}$
Each value represents the mean ± S.D. of 10 mice. The following P values were calculated by the one-way analysis of variance followed by pairwise comparisons and the fixed sequence procedure. * $P < 0.01$, ** $P < 0.0001$ vs vehicle. † $P < 0.01$, †† $P<0.0001$ vs oseltamivir phosphate 5 mg/kg. The following P values were calculated by the one-way analysis of variance followed by pairwise comparisons ‡ $P < 0.0001$ vs vehicle. § $P < 0.05$, §§ $P < 0.0001$ vs oseltamivir phosphate 50 mg/kg.		
Study number [Location in CTD]	R-033188-EB-056-N [4.2.1.1-19]	

2.6.3.2.21 Inhibitory Effect of S-033188 and Anti-influenza Virus Drugs on Virus Replication in Lung of Mice Infected with A/WSN/33 Strain

Test Article: S-033188

Species/Strain		Mouse/ BALB/cAnNCrlCrlj
Gender/ Number of Animals		Female/15 per group
Virus		A/WSN/33 (H1N1)
Vehicle/Formulation		0.5 w/v% MC
Method of Administration		Oral administration twice daily (every 12 hours) for 1 day under non-anesthesia
Dose (mg/kg BID)		5
Reference article (Vehicle/Formulation, Method of Administration)		Zanamivir hydrate (saline/intranasal administration twice daily (every 12 hours) for 1 day under anesthesia [Dose: 10 mg/kg BID as free base]) Laninamivir octanoate (saline/a single intranasal administration under anesthesia [Dose: 1 or 3 mg/kg as free base]) Favipiravir (0.4 w/v% carboxymethyl cellulose aqueous solution/oral administration twice daily (every 12 hours) for 1 day under non-anesthesia [Dose: 50 or 150 mg/kg BID])
Treatment		Mice were intranasally inoculated with 100 $TCID_{50}$ (100 µL/mouse) of the virus suspensions under anesthesia 5 days before the first administration.
Evaluation items		Virus titers in lungs 24 hours after the first administration
Evaluation method		Virus titers were measured by standard $TCID_{50}$ method using MDCK cells. Differences of the virus titers among the test article-treated groups, the reference article-treated groups and the vehicle-treated group were analyzed by the one-way analysis of variance followed by pairwise comparisons and the fixed sequence procedure.
Results		
Articles	Dose	Log_{10} $TCID_{50}$/mL
Vehicle	–	5.10 ± 0.28
S-033188	5 mg/kg BID	2.59 ± 0.53*†§
Zanamivir hydrate	10 mg/kg BID	4.40 ± 0.38*
Laninamivir octanoate	1 mg/kg QD	4.06 ± 0.38*
	3 mg/kg QD	3.82 ± 0.56‡
Favipiravir	50 mg/kg BID	4.33 ± 0.47*
	150 mg/kg BID	3.79 ± 0.39‡
Abbreviation: QD, once daily. Each value represents the mean ± S.D. of 15 mice. The following P values were calculated by the one-way analysis of variance followed by pairwise comparisons and the fixed sequence procedure. * $P < 0.0001$ vs vehicle. † $P < 0.0001$ vs zanamivir hydrate 10 mg/kg. laninamivir octanoate 1 mg/kg. favipiravir 50 mg/kg. The following P values were calculated by the one-way analysis of variance followed by pairwise comparisons. ‡ $P < 0.0001$ vs vehicle. § $P < 0.0001$ vs laninamivir octanoate 3 mg/kg. favipiravir 150 mg/kg.		
Study number [Location in CTD]		R-033188-EB-056-N [4.2.1.1-19]

2.6.3.2.22 PK Parameters of S-033447 after a Single Oral Administration of S-033188 in Mice Infected with A/WSN/33 Strain

Test Article: S-033188

Species/Strain	Mouse/ BALB/cAnNCrlCrlj					
Gender /Number of Animals	Female/3 per each sampling point					
Feed Condition	Non-fasting					
Virus	A/WSN/33 (H1N1)					
Vehicle/Formulation	0.5 w/v% MC aqueous solution					
Method of Administration	All the sampling groups except for the 24-hour sampling group: a single oral administration Twenty four-hour sampling group : oral administration twice daily (every 12 hours) for 1 day					
Dose (mg/kg)	0.5, 1.5, 5, 15, 50					
Treatment	Mice were intranasally inoculated with 100 $TCID_{50}$ (100 μL/mouse) of the virus suspensions under anesthesia 5 days before the first administration.					
Sample	Plasma					
Analyte	S-033188, S-033447					
Assay	LC/MS/MS					
Results						
	Pharmacokinetic Parameters	0.5 mg/kg	1.5 mg/kg	5 mg/kg	15 mg/kg [a]	50 mg/kg
S-033447	C_{max} (ng/mL)	5.05	14.3	45.7	175	284
	T_{max} (hr)	1.00	1.00	0.500	0.500	2.00
	$t_{1/2,z}$ (hr) [b]	2.24	2.56	2.45	2.26	3.14
	$AUC_{0\text{-}inf}$ (ng·hr/mL)	22.7	66.1	252	670	1690
	$AUC_{0\text{-}12hr}$ (ng·hr/mL)	22.0	62.6	242	648	1580
S-033188	The pharmacokinetic parameters of S-033188 are not determined because the concentration of S-033188 was BLQ in all plasma samples except for the 2-hour sampling point at 50 mg/kg (0.512 ng/mL).					
Abbreviations: BLQ, below the lower limit of quantification (< 0.500 ng/mL); C_{max}, maximum plasma concentration; T_{max}, time to reach the maximum plasma concentration; $t_{1/2,z}$, elimination half-life; $AUC_{0\text{-}inf}$, area under the plasma concentration-time curve extrapolated from 0 to infinity; $AUC_{0\text{-}12hr}$, area under the plasma concentration-time curve from 0 to 12 hours after dosing. a Plasma concentration of S-033447 12 hours after single oral administration of S-033188 (C_{12hr}) at the dose of 15 mg/kg was 6.85 ng/mL. b 0.5 mg/kg, $t_{1/2,(4\text{-}10hr)}$; from 1.5 to 50 mg/kg, $t_{1/2,(6\text{-}12\ hr)}$						
Study number [Location in CTD]	R-033188-EB-056-N [4.2.1.1-19]					

2.6.3.2.23 Inhibitory Effect of S-033188 on Virus Replication in the Lung of Mice Infected with B/Hong Kong/5/72

Test Article: S-033188

Species/Strain	Mouse/ BALB/cAnNCrlCrlj	
Gender/ Number of Animals	Female/15 per group	
Virus	B/Hong Kong/5/72 (mouse-adapted)	
Vehicle/Formulation	0.5 w/v% MC	
Method of Administration	Oral administration twice daily (every 12 hours) for 1 day under non-anesthesia	
Dose (mg/kg BID)	0.5, 1.5, 5, 15, 50	
Reference article (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5% MC/Oral administration BID (every 12 hours) for 1 day under non-anesthesia [Dose: 5 or 50 mg/kg as free base])	
Treatment	Mice were intranasally inoculated with 400 $TCID_{50}$ (100 µL/mouse) of the virus suspensions under anesthesia 5 days before the first administration.	
Evaluation items	Virus titers in lungs 24 hours after the first administration	
Evaluation method	Virus titers were measured by standard $TCID_{50}$ method using MDCK cells. Differences of the virus titers among the test article-treated groups, the reference article-treated groups, and the vehicle-treated group were analyzed by the one-way analysis of variance followed by pairwise comparison and the fixed-sequence procedure.	
Results		
Articles	Dose	Log_{10} $TCID_{50}$/mL
Vehicle	–	3.92 ± 0.35
S-033188	0.5 mg/kg BID	3.74 ± 0.50
	1.5 mg/kg BID	3.42 ± 0.37 *§
	5 mg/kg BID	2.87 ± 0.47 **†§§
	15 mg/kg BID	2.59 ± 0.41 **†§§
	50 mg/kg BID	2.30 ± 0.30 **†§§
Oseltamivir phosphate	5 mg/kg BID	3.56 ± 0.30 ‡
	50 mg/kg BID	3.89 ± 0.43
Each value represents the mean ± S.D. of 15 mice. The following P values were calculated by the one-way analysis of variance followed by pairwise comparison and the fixed-sequence procedure. * P<0.001, ** P<0.0001 vs vehicle. † P<0.0001 vs oseltamivir phosphate 5 mg/kg BID. The following P values were calculated by the one-way analysis of variance followed by pairwise comparison. ‡ P<0.01 vs vehicle. § P<0.005, §§ P<0.0001 vs oseltamivir phosphate 50 mg/kg BID.		
Study number [Location in CTD]	S-033188-EB-158-N [4.2.1.1-20]	

2.6.3.2.24 Inhibitory Effect of S-033188 on Virus Replication in the Lung of Mice Infected with A/Hong Kong/8/68 (H3N2)

Test Article: S-033188

Species/Strain	Mouse/ BALB/cAnNCrlCrlj	
Gender/Number of Animals	Female/10 per group	
Virus	A/Hong Kong/8/68 (H3N2) (mouse-adapted)	
Vehicle/Formulation	0.5 w/v% MC	
Method of Administration	Oral administration twice daily (every 12 hours) for 1 day under non-anesthesia	
Dose (mg/kg BID)	0.5, 1.5, 5, 15, 50	
Reference article (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5% MC/Oral administration BID (every 12 hours) for 1 day under non-anesthesia [Dose: 5 or 50 mg/kg as free base])	
Treatment	Mice were intranasally inoculated with 100 $TCID_{50}$ (100 μL/mouse) of the virus suspensions under anesthesia 5 days before the first administration.	
Evaluation items	Virus titers in lungs 24 hours after the first administration	
Evaluation method	Virus titers were measured by standard $TCID_{50}$ method using MDCK cells. Differences of the virus titers among the test article-treated groups, the referencearticle-treated groups, and the vehicle-treated group were analyzed by the one-way analysis of variance followed by pairwise comparison and the fixed-sequence procedure.	
Results		
Articles	Dose	Log_{10} $TCID_{50}$/mL
Vehicle	–	4.97 ± 0.38
S-033188	0.5 mg/kg BID	3.73 ± 0.48 *†§
	1.5 mg/kg BID	3.05 ± 0.57 *††§§
	5 mg/kg BID	3.08 ± 0.54 *††§§
	15 mg/kg BID	2.44 ± 0.62 *††§§§
	50 mg/kg BID	2.34 ± 0.51 *††§§§
Oseltamivir phosphate	5 mg/kg BID	4.60 ± 0.30 ‡
	50 mg/kg BID	4.27 ± 0.59 ‡‡
Each value represents the mean ± S.D. of 10 mice. The following P values were calculated by the one-way analysis of variance followed by pairwise comparison and the fixed-sequence procedure. * P<0.0001 vs vehicle. † P<0.0005, †† P<0.0001 vs oseltamivir phosphate 5 mg/kg. The following P values were calculated by the one-way analysis of variance followed by pairwise comparison. ‡ P<0.05, ‡‡ P<0.01 vs vehicle. § P<0.05, §§ P<0.0005, §§§ P<0.0001 vs oseltamivir phosphate 50 mg/kg.		
Study number [Location in CTD]	S-033188-EB-158-N [4.2.1.1-20]	

2.6.3.2.25 Inhibitory Effect of S-033188 on Virus Replication in the Lung of Mice Infected with A/WSN/33-NA/H274Y (H1N1)

Test Article: S-033188

Species/Strain	Mouse/ BALB/cAnNCrlCrlj	
Gender/Number of Animals	Female/10 per group	
Virus	A/WSN/33-NA/H274Y (H1N1)	
Vehicle/Formulation	0.5 w/v% MC	
Method of Administration	Oral administration twice daily (every 12 hours) for 1 day under non-anesthesia	
Dose (mg/kg BID)	0.5, 1.5, 5, 15, 50	
Reference article (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5% MC/Oral administration BID (every 12 hours) for 1 day under non-anesthesia [Dose: 5 mg/kg as free base])	
Treatment	Mice were intranasally inoculated with 100 $TCID_{50}$ (100 μL/mouse) of the virus suspensions under anesthesia 5 days before the first administration.	
Evaluation items	Virus titers in lungs 24 hours after the first administration	
Evaluation method	Virus titers were measured by standard $TCID_{50}$ method using MDCK cells. Differences of the virus titers among the test article-treated groups, the reference article-treated groups, and the vehicle-treated group were analyzed by the one-way analysis of variance followed by pairwise comparison and the fixed-sequence procedure.	
Results		
Articles	Dose	Log_{10} $TCID_{50}$/mL
Vehicle	–	4.95 ± 0.41
S-033188	0.5 mg/kg BID	3.86 ± 0.41 *†
	1.5 mg/kg BID	3.10 ± 0.47 *††
	5 mg/kg BID	2.49 ± 0.73 *††
	15 mg/kg BID	2.10 ± 0.48 *††
	50 mg/kg BID	1.60 ± 0.16 *††
Oseltamivir phosphate	5 mg/kg BID	4.54 ± 0.39 ‡
Each value represents the mean ± S.D. of 10 mice. The following P values were calculated by the one-way analysis of variance followed by pairwise comparison and the fixed-sequence procedure. * P<0.0001 vs vehicle. † P<0.005, †† P<0.0001 vs oseltamivir phosphate 5 mg/kg BID. The following P values were calculated by the one-way analysis of variance followed by pairwise comparison. ‡ P<0.05 vs vehicle.		
Study number [Location in CTD]	S-033188-EB-158-N [4.2.1.1-20]	

2.6.3.2.26 Time-Course Analysis of Inhibitory Effect of S-033188 on Influenza A Virus Replication in Mice

Test Article: S-033188

Species/Strain	Mouse/ BALB/cAnNCrlCrlj
Gender/Number of Animals	Female/15 per group (5/each time point)
Virus	A/Osaka/129/2009 (H1N1)
Vehicle/Formulation	0.5 w/v% MC
Method of Administration	Oral administration twice daily (every 12 hours) for 1 day or 3 days under non-anesthesia
Dose (mg/kg BID)	0.5, 5
Reference article (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5% MC/oral administration twice daily (every 12 hours) for 3 days under non-anesthesia [Dose: 5 mg/kg as free base])
Treatment	Mice were intranasally inoculated with 4.30×10^3 $TCID_{50}$ (100 µL/mouse) of the virus suspensions under anesthesia 5 days before the first administration.
Evaluation items	Virus titers in lungs 24, 48, or 72 hours after the first administration
Evaluation method	Virus titers were measured by standard $TCID_{50}$ method using MDCK cells. Differences of the virus titers for the overall time (72 hours) or at 24 and 48 hours among the test article-treated groups, the reference article -treated group, and the vehicle-treated group were analyzed by the two-way analysis of variance followed by pairwise comparisons and the fixed sequence procedure.

Articles	Dose (mg/kg)	Dosing schedule	Time point (hour)	Log_{10} $TCID_{50}$/mL
Non-treated	−	−	0	7.20 ± 0.40
Vehicle	−	BID for 1 day	24	5.41 ± 0.71
			48	3.30 ± 0.69
			72	1.57 ± 0.09
S-033188	0.5	BID for 1 day	24	3.62 ± 0.59 **††
			48	2.01 ± 0.46 *†
			72	1.50 ± 0.00
		BID for 3 days	48	1.60 ± 0.09 **††
			72	1.50 ± 0.00
	5	BID for 1 day	24	2.83 ± 0.44 **††
			48	1.71 ± 0.30 **††
			72	1.50 ± 0.00
		BID for 3 days	48	1.50 ± 0.00 **††
			72	1.50 ± 0.00
Oseltamivir phosphate	5	BID for 3 days	24	5.38 ± 0.44
			48	3.01 ± 0.51
			72	1.60 ± 0.22

Each value represents the mean ± S.D. of 5 mice.
The following P values at 24 and 48 hours were calculated by the two-way analysis of variance followed by pairwise comparisons and the fixed sequence procedure.
* $P < 0.001$, ** $P < 0.0001$ vs vehicle. † $P < 0.01$, †† $P < 0.001$, ††† $P < 0.0001$ vs oseltamivir phosphate 5 mg/kg.

Study number [Location in CTD]	R-033188-EB-067-N [4.2.1.1-21]

2.6.3.2.27 Pharmacokinetic and Pharmacodynamic Analysis of S-033447 on Influenza A Virus Replication in Mice

Test Article: S-033447

Species/Strain	Mouse/ BALB/cAnNCrlCrlj
Gender/Number of animals	Female/5 per group for pharmacodynamic (PD) study Female/3 per each sampling points for pharmacokinetic (PK) study
Virus	A/WSN/33 (H1N1)
Vehicle/Formulation	10 w/v% Tween 80 and 0.5 w/v% Vinylpyrrolidone-vinyl acetate copolymer (PVPVA) in sodium carbonate-sodium hydrogen carbonate buffer (pH 9)
Method of Administration	Subcutaneous administration once daily, twice daily (every 12 hours), or four times a day (every 6 hours) for 1 day under non-anesthesia
Dose (mg/kg)	0.0625, 0.125, 0.25, 0.5, 1, 2, 4, or 8
Reference article (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5% MC/Oral administration twice daily (every 12 hours) for 1 days under non-anesthesia [Dose: 5 mg/kg as free base])
Treatment	Mice were intranasally inoculated with 100 $TCID_{50}$ (100 µL/mouse) of the virus suspensions under anesthesia 5 days before the first administration.
Evaluation items for PD study	Virus titers in lungs 24 hours after the first administration
Evaluation method for PD study	Virus titers were measured by standard $TCID_{50}$ method using MDCK cells.
Analyte for PK study	S-033447
Assay for PK study	LC/MS/MS
Data analysis	The PK parameters were calculated based on the noncompartmental method and the PK parameters following multiple dosing were estimated using the corresponding single dose data by non-parametric superposition. The sigmoid maximum effect (E_{max}) model and the linear model were applied to virus titers at 24 hours after the first administration of S-033447 and the PK parameters ($AUC_{0\text{-}24hr}$, C_{max}, C_{24hr}, C_t, $T_{>2}$, $T_{>10}$, and $T_{>50}$). The goodness of fit for each PK parameter was evaluated by the coefficient of determination adjusted for degrees of freedom in these models.

Articles	Dosing schedule	Dose (mg/kg)	Virus titers ($logTCID_{50}$/ml)	$AUC_{0\text{-}24hr}$ (ng·hr/mL)	C_{max} (ng/mL)	C_{24hr} (ng/mL)	C_t (ng/mL)	$T_{>2}$ (hr)	$T_{>10}$ (hr)	$T_{>50}$ (hr)
Vehicle	QID	–	5.36 ± 0.14	–	–	–	–	–	–	–
S-033447	QD	0.25	3.90 ± 0.32	84.91	17.4	0.0818	0.0818	9.40	3.33	0
		0.5	4.12 ± 0.44	173.1	34.3	0.222	0.222	13.17	5.62	0
		1	4.11 ± 0.51	344.7	56.5	0.751	0.751	18.55	9.63	1.68
		2	3.51 ± 0.18	687.9	101	1.81	1.81	23.40	14.10	5.87
		4	3.71 ± 0.30	1322	167	4.38	4.38	23.98	19.12	9.55
		8	2.90 ± 0.47	2589	299	9.52	9.52	23.98	23.67	14.07

Articles	Dosing schedule	Dose (mg/kg)	Virus titers ($logTCID_{50}$/ml)	$AUC_{0\text{-}24hr}$ (ng·hr/mL)	C_{max} (ng/mL)	C_{24hr} (ng/mL)	C_{τ} (ng/mL)	$T_{>2}$ (hr)	$T_{>10}$ (hr)	$T_{>50}$ (hr)
S-033447	BID	0.125	3.90 ± 0.40	79.78	9.27	0.405	0.394	12.03	0	0
		0.25	3.37 ± 0.36	164.8	18.4	1.20	1.12	19.12	6.85	0
		0.5	3.29 ± 0.27	334.8	36.6	2.78	2.56	23.97	11.52	0
		1	2.39 ± 0.63	657.6	62.1	7.29	6.54	23.98	19.90	3.90
		2	2.11 ± 0.39	1303	113	16.3	14.5	23.98	23.97	12.20
		4	2.42 ± 0.57	2473	192	38.0	33.6	23.98	23.97	19.93
	QID	0.0625	3.91 ± 0.24	76.48	5.50	1.23	0.995	17.82	0	0
		0.125	3.68 ± 0.47	153.0	11.0	2.46	1.99	23.92	3.00	0
		0.25	3.29 ± 0.21	314.4	22.5	5.87	4.38	23.97	15.82	0
		0.5	2.67 ± 0.41	637.3	45.4	12.7	9.15	23.97	23.60	0
		1	2.80 ± 0.27	1233	82.3	32.5	23.0	23.98	23.95	12.18
		2	2.50 ± 0.85	2425	156	72.2	50.7	23.98	23.97	23.82
Oseltamivir phosphate	BID	5	5.15 ± 0.43	–	–	–	–	–	–	–

PK parameters	Model parameter	Estimate	S.E.	95% confidence interval	P value	COD [adjusted COD]
Sigmoid E_{max} model						
$AUC_{0\text{-}24hr}$ (ng·hr/mL)	E_0	3.821	0.116	(3.590, 4.052)	< 0.0001	0.415 [0.395]
	E_{max}	1.091	0.170	(0.752, 1.429)	< 0.0001	
	EC_{50}	431.596	81.757	(269.068, 594.124)	< 0.0001	
	γ	4.170	2.291	(−0.385, 8.725)	0.0722	
C_{max} (ng/mL)	E_0	3.806	0.208	(3.391, 4.220)	< 0.0001	0.261 [0.236]
	E_{max}	1.022	0.333	(0.360, 1.683)	0.0029	
	EC_{50}	42.528	14.034	(14.629, 70.427)	0.0032	
	γ	2.421	1.972	(−1.500, 6.342)	0.2230	
C_{24hr} (ng/mL)	E_0	3.928	0.116	(3.697, 4.159)	< 0.0001	0.595 [0.581]
	E_{max}	1.499	0.206	(1.090, 1.908)	< 0.0001	
	EC_{50}	4.767	1.047	(2.687, 6.848)	< 0.0001	
	γ	1.843	0.649	(0.554, 3.133)	0.0056	
C_{τ} (ng/mL)	E_0	3.958	0.126	(3.707, 4.210)	< 0.0001	0.595 [0.581]
	E_{max}	1.554	0.233	(1.091, 2.016)	< 0.0001	
	EC_{50}	4.108	0.961	(2.199, 6.018)	< 0.0001	
	γ	1.697	0.604	(0.498, 2.897)	0.0061	
$T_{>50}$ (hr)	E_0	3.588	0.094	(3.403, 3.774)	< 0.0001	0.309 [0.285]
	E_{max}	1.913	2.859	(−3.770, 7.596)	0.5051	
	EC_{50}	16.534	38.174	(−59.353, 92.421)	0.6660	
	γ	1.324	1.438	(−1.534, 4.183)	0.3597	

PK parameters	Model parameter	Estimate	S.E.	95% confidence interval	P value	COD [adjusted COD]
linear model						
$AUC_{0\text{-}24hr}$ (ng·hr/mL)	E_0	5.705	0.326	(5.056, 6.354)	<0.0001	0.399
	β	0.400	0.052	(0.296, 0.504)	<0.0001	[0.392]
C_{max} (ng/mL)	E_0	4.542	0.248	(4.050, 5.034)	<0.0001	0.249
	β	0.335	0.062	(0.212, 0.458)	<0.0001	[0.241]
C_{24hr} (ng/mL)	E_0	3.624	0.066	(3.493, 3.756)	<0.0001	0.527
	β	0.303	0.031	(0.242, 0.364)	<0.0001	[0.522]
C_τ (ng/mL)	E_0	3.603	0.064	(3.475, 3.731)	<0.0001	0.532
	β	0.318	0.032	(0.255, 0.381)	<0.0001	[0.527]
$T_{>2}$ (hr)	E_0	5.141	0.297	(4.550, 5.732)	<0.0001	0.324
	β	0.090	0.014	(0.062, 0.117)	<0.0001	[0.317]
$T_{>10}$ (hr)	E_0	4.095	0.103	(3.890, 4.301)	<0.0001	0.513
	β	0.060	0.006	(0.048, 0.072)	<0.0001	[0.508]
$T_{>50}$ (hr)	E_0	3.569	0.083	(3.403, 3.734)	<0.0001	0.303
	β	0.055	0.009	(0.037, 0.072)	<0.0001	[0.295]

Abbreviations: QID, four times a day; $AUC_{0\text{-}24hr}$, area under the plasma concentration-time curve from zero to 24 hours after dosing; C_{24hr}, plasma drug concentration at 24 hours after dosing; Cτ, plasma concentration at the time point of the dosage interval (τ) after the first dosing; $T_{>2}$, time which plasma concentration exceeds 2 ng/mL; $T_{>10}$, time which plasma concentration exceeds 10 ng/mL; $T_{>50}$, time which plasma concentration exceeds 50 ng/mL; S.E., standard error; COD, coefficient of determination; adjusted COD, coefficient of determination adjusted for degrees of freedom; E_0, baseline effect; E_{max}, maximum effect; γ, slope factor.
Virus titers represent the mean ± S.D. of 5 mice.

Study number [Location in CTD]	R-033188-EB-058-N [4.2.1.1-22]

2.6.3.2.28 Inhibitory Effect of S-033447 on Viral mRNA Synthesis in Cells Infected with Influenza A Virus in the Presence of Serum

Test Article: S-033447

Species/Biological material	MDCK cells infected with influenza virus (A/WSN/33 [H1N1])
Final concentration (nmol/L)	0.072, 0.36, 1.8, 9.0, and 45 nmol/L
Reference article (Final concentration)	Favipiravir (4000, 20000, 100000, 500000 nmol/L)
Vehicle	DMSO
Evaluation items	The EC_{90} against viral mRNA synthesis and potency shift in the presence of each concentration of human serum (HS).
Evaluation method	S-033447 or favipiravir was incubated in the medium including 0%, 12.5%, 25%, and 50% HS at room temperature for 1.5 hours. Confluent monolayer of MDCK cells in 96-well plates were infected with the A/WSN/33 viruses at a multiplicity of infection of 5, and incubated on ice for 1 hour. The medium was changed to S-033447 or favipiravir solution including HS, and the cells were incubated for 6 hours at 37°C in a 5% CO_2 humidified incubator, followed by total RNA extraction from the cells. The first strand DNAs complementary to influenza viral mRNA of NP segment were synthesized using the reverse transcriptase with the specific primer, and the amount of viral mRNA was measured by quantitative PCR. The amount of canine 18S rRNA was measured by quantitative RT-PCR, and the viral mRNA levels were normalized by the canine 18s rRNA levels. The EC_{90} value was determined by software XLfit. The potency shift was calculated by dividing EC_{90} value in the presence of each concentration of HS by EC_{90} value in the presence of 0% of HS.

Results

	S-033447		Favipiravir	
HS concentration (%)	EC_{90} (nmol/L)	PS (fold)	EC_{90} (nmol/L)	PS (fold)
0%	5.27 ± 2.43	1.00 ± 0.00	332209.91 ± 55553.44	1.00 ± 0.00
12.5%	6.59 ± 1.22	1.65 ± 1.31	162725.18 ± 31085.47	0.50 ± 0.13
25%	6.98 ± 1.01	1.65 ± 1.05	254384.73 ± 110051.44	0.76 ± 0.30
50%	10.84 ± 3.74	2.86 ± 2.68	305690.41 ± 136403.40	0.91 ± 0.37

Abbreviations: HS, human serum; PS, potency shift
Each value represents the mean ± S.D. of 3 independent experiments.
The potency shift (PS) was calculated as a relative value to 0% human serum.

Study number [Location in CTD]	S-033188-EB-231-N [4.2.1.1-23]

2.6.3.2.29 Protective Effect of S-033188 against Lethal Infection with Influenza A Virus in Mice

Test Article: S-033188

Species/Strain	Mouse/ BALB/cAnNCrlCrlj
Gender/Number of Animals	Female/10 or 9 per group
Virus	A/PR/8/34 (H1N1)
Vehicle/Formulation	0.5 w/v% MC
Method of Administration	Oral administration twice daily (every 12 hours) for 1 day under non-anesthesia
Dose (mg/kg BID)	0.05, 0.5, 5
Reference article (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5 w/v% MC/Oral administration twice daily [every 12 hours] for 5 days under non-anesthesia [Dose: 5 or 50 mg/kg as free base])
Treatment	Mice were intranasally inoculated with 1.38×10^3 $TCID_{50}$ or 4.42×10^4 $TCID_{50}$ (100 µL/mouse) of the virus suspensions under anesthesia immediately before the first administration.
Evaluation items	Survival time
Evaluation method	Mice were examined daily for death through 21 days after infection. Mice were euthanized and regarded as dead if their body weights in the morning were lower than 70% of the initial body weights (in the morning on the day of virus infection) according to humane endpoints.

Results						
Dose (mg/kg BID)		0	0.05	0.5	5	5
Articles		Vehicle	S-033188			Oseltamivir phosphate
Administration		1 day				5 days
Inoculation		1.38×10^3 $TCID_{50}$/mouse				
Animal number (survived / total)	Day 0	10/10	10/10	10/10	9/9	10/10
	Day 1	10/10	10/10	10/10	9/9	10/10
	Day 2	10/10	10/10	10/10	9/9	10/10
	Day 3	10/10	10/10	10/10	9/9	10/10
	Day 4	10/10	10/10	10/10	9/9	10/10
	Day 5	10/10	10/10	10/10	9/9	10/10
	Day 6	9/10	10/10	10/10	9/9	9/10
	Day 7	3/10	8/10	10/10	9/9	9/10
	Day 8	0/10	3/10	10/10	9/9	9/10
	Day 9	0/10	3/10	10/10	9/9	9/10
	Day 10	0/10	3/10	10/10	9/9	9/10
	Day 11	0/10	3/10	10/10	9/9	9/10
	Day 12	0/10	3/10	10/10	9/9	9/10
	Day 13	0/10	3/10	10/10	9/9	9/10
	Day 14	0/10	3/10	10/10	9/9	9/10
	Day 15	0/10	3/10	10/10	9/9	9/10
	Day 16	0/10	3/10	10/10	9/9	9/10
	Day 17	0/10	3/10	10/10	9/9	9/10
	Day 18	0/10	3/10	10/10	9/9	9/10
	Day 19	0/10	3/10	10/10	9/9	9/10
	Day 20	0/10	3/10	10/10	9/9	9/10
	Day 21	0/10	3/10 *	10/10 **	9/9 **	9/10 **

Articles		Vehicle	S-033188			Oseltamivir phosphate	
Dose (mg/kg BID)		0	0.05	0.5	5	5	50
Administration		1 day				5 days	
Inoculation		4.42×10^4 $TCID_{50}$/mouse					
Animal number (survived/total)	Day 0	10/10	10/10	10/10	10/10	10/10	10/10
	Day 1	10/10	10/10	10/10	10/10	10/10	10/10
	Day 2	10/10	10/10	10/10	10/10	10/10	10/10
	Day 3	10/10	10/10	10/10	10/10	10/10	10/10
	Day 4	10/10	10/10	10/10	10/10	10/10	10/10
	Day 5	10/10	10/10	10/10	10/10	10/10	10/10
	Day 6	1/10	9/10	10/10	10/10	10/10	10/10
	Day 7	1/10	3/10	10/10	10/10	7/10	10/10
	Day 8	0/10	1/10	10/10	10/10	3/10	10/10
	Day 9	0/10	0/10	10/10	10/10	2/10	8/10
	Day 10	0/10	0/10	10/10	10/10	2/10	8/10
	Day 11	0/10	0/10	10/10	10/10	2/10	8/10
	Day 12	0/10	0/10	10/10	10/10	2/10	8/10
	Day 13	0/10	0/10	10/10	10/10	2/10	8/10
	Day 14	0/10	0/10	10/10	10/10	2/10	8/10
	Day 15	0/10	0/10	10/10	10/10	2/10	8/10
	Day 16	0/10	0/10	10/10	10/10	2/10	8/10
	Day 17	0/10	0/10	10/10	10/10	2/10	8/10
	Day 18	0/10	0/10	10/10	10/10	2/10	8/10
	Day 19	0/10	0/10	10/10	10/10	2/10	8/10
	Day 20	0/10	0/10	10/10	10/10	2/10	8/10
	Day 21	0/10	0/10	10/10 ***†	10/10 ***†	2/10 **	8/10 **
The following P values were calculated by the log-rank test and the fixed-sequence procedure. * P<0.05, ** P<0.001, *** P<0.0001 vs vehicle. † P<0.0005 vs oseltamivir phosphate 5 mg/kg.							
Study number [Location in CTD]		S-033188-EB-124-N [4.2.1.1-24]					

2.6.3.2.30 Protective Effect of S-033188 against Lethal Infection with Influenza B Virus in Mice

Test Article: S-033188

Species/Strain		Mouse/ BALB/cAnNCrlCrlj				
Gender and Number of animals		Female/10 per group				
Virus		B/Hong Kong/5/72 (mouse-adapted)				
Vehicle/Formulation		0.5 w/v% MC				
Method of Administration		Oral administration twice daily (every 12 hours) for 1 day under non-anesthesia				
Dose (mg/kg BID)		0.5, 5, 50				
Reference article (Vehicle/Formulation, Method of Administration)		Oseltamivir phosphate (0.5 w/v% MC/Oral administration twice daily [every 12 hours] for 5 days under non-anesthesia [Dose: 5 or 50 mg/kg as free base])				
Treatment		Mice were intranasally inoculated with 3.30×10^5 $TCID_{50}$ or 1.98×10^6 $TCID_{50}$ (100 μL/mouse) of the virus suspensions under anesthesia immediately before the first administration.				
Evaluation items		Survival time				
Evaluation method		Mice were examined daily for death through 14 days after infection. Mice were euthanized and regarded as dead if their body weights in the morning were lower than 70% of the initial body weights (in the morning on the day of virus infection) according to humane endpoints.				
Results						
Articles		Vehicle	S-033188			Oseltamivir phosphate
Dose (mg/kg BID)		0	0.5	5	50	5
Administration		1 day				5 days
Inoculation		3.30×10^5 $TCID_{50}$/mouse				
Animal number (survived/total)	Day 0	10/10	10/10	10/10	10/10	10/10
	Day 1	10/10	10/10	10/10	10/10	10/10
	Day 2	10/10	10/10	10/10	10/10	10/10
	Day 3	10/10	10/10	10/10	10/10	10/10
	Day 4	10/10	10/10	10/10	10/10	10/10
	Day 5	10/10	10/10	10/10	10/10	10/10
	Day 6	4/10	10/10	10/10	10/10	10/10
	Day 7	0/10	8/10	10/10	10/10	10/10
	Day 8	0/10	4/10	10/10	10/10	10/10
	Day 9	0/10	2/10	10/10	10/10	10/10
	Day 10	0/10	2/10	10/10	10/10	10/10
	Day 11	0/10	2/10	10/10	10/10	10/10
	Day 12	0/10	2/10	10/10	10/10	10/10
	Day 13	0/10	2/10	10/10	10/10	10/10
	Day 14	0/10	2/10 **	10/10 ***	10/10 ***	10/10 ***

Articles		Vehicle	S-033188			Oseltamivir phosphate	
Dose (mg/kg BID)		0	0.5	5	50	5	50
Administration		1 day				5 days	
Inoculation		1.98×10^6 $TCID_{50}$/mouse					
Animal number (survived / total)	Day 0	10/10	10/10	10/10	10/10	10/10	10/10
	Day 1	10/10	10/10	10/10	10/10	10/10	10/10
	Day 2	10/10	10/10	10/10	10/10	10/10	10/10
	Day 3	10/10	10/10	10/10	10/10	10/10	10/10
	Day 4	9/10	9/10	10/10	10/10	10/10	10/10
	Day 5	1/10	7/10	10/10	10/10	8/10	10/10
	Day 6	0/10	2/10	10/10	10/10	2/10	8/10
	Day 7	0/10	1/10	10/10	10/10	2/10	7/10
	Day 8	0/10	0/10	10/10	10/10	2/10	7/10
	Day 9	0/10	0/10	10/10	10/10	2/10	7/10
	Day 10	0/10	0/10	10/10	10/10	2/10	7/10
	Day 11	0/10	0/10	10/10	10/10	2/10	7/10
	Day 12	0/10	0/10	10/10	10/10	2/10	7/10
	Day 13	0/10	0/10	10/10	10/10	2/10	7/10
	Day 14	0/10	0/10	10/10 ***†	10/10 ***†	2/10 *	7/10 **
The following P values were calculated by the log-rank test and the fixed-sequence procedure. * P<0.05, ** P<0.001, *** P<0.0001 vs vehicle. † P<0.0005 vs oseltamivir phosphate 5 mg/kg.							
Study number [Location in CTD]		S-033188-EB-114-N [4.2.1.1-25]					

2.6.3.2.31 Protective Effect of Delayed Treatment with S-033188 against Lethal Infection with Influenza A Virus in Mice (Survival Rate)

Test Article: S-033188

Species/Strain	Mouse/ BALB/cAnNCrlCrlj
Gender/Number of Animals	Female/10 per group
Virus	A/PR/8/34 (H1N1)
Vehicle/Formulation	0.5 w/v% MC
Method of Administration	Oral administration twice daily (every 12 hours) for 5 days under non-anesthesia.
Dose (mg/kg BID)	1.5, 15
Reference article (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5 w/v% MC/Oral administration twice daily [every 12 hours] for 5 days under non-anesthesia [Dose: 5 mg/kg as free base])
Treatment	Mice were intranasally inoculated with 1.38×10^3 $TCID_{50}$ (100 μL/mouse) of the virus suspensions under anesthesia. The initial administration of each article or vehicle was performed 24, 48, 72 or 96 hours after virus infection.
Evaluation items	Survival time
Evaluation method	Mice were examined daily for death through 28 days after infection. Mice were euthanized and regarded as dead if their body weights in the morning were lower than 70% of the initial body weights (in the morning on the day of virus infection) according to humane endpoints.

Results					
Articles		Vehicle	S-033188		Oseltamivir phosphate
Dose (mg/kg BID)		0	1.5	15	5
Administration		5 days			
Timing of Initial Administration		24 hours post-infection			
Animal number (survived/total)	Day 0	10/10	10/10	10/10	10/10
	Day 1	10/10	10/10	10/10	10/10
	Day 2	10/10	10/10	10/10	10/10
	Day 3	10/10	10/10	10/10	10/10
	Day 4	10/10	10/10	10/10	10/10
	Day 5	10/10	10/10	10/10	10/10
	Day 6	10/10	10/10	10/10	10/10
	Day 7	3/10	10/10	10/10	10/10
	Day 8	1/10	10/10	10/10	9/10
	Day 9	0/10	10/10	10/10	9/10
	Day 10	0/10	10/10	10/10	9/10
	Day 11	0/10	10/10	10/10	9/10
	Day 12	0/10	10/10	10/10	9/10
	Day 13	0/10	10/10	10/10	9/10
	Day 14	0/10	10/10	10/10	9/10
	Day 15	0/10	10/10	10/10	9/10
	Day 16	0/10	10/10	10/10	9/10
	Day 17	0/10	10/10	10/10	9/10
	Day 18	0/10	10/10	10/10	9/10
	Day 19	0/10	10/10	10/10	9/10
	Day 20	0/10	10/10	10/10	9/10
	Day 21	0/10	10/10	10/10	9/10
	Day 22	0/10	10/10	10/10	9/10
	Day 23	0/10	10/10	10/10	9/10
	Day 24	0/10	10/10	10/10	9/10
	Day 25	0/10	10/10	10/10	9/10
	Day 26	0/10	10/10	10/10	9/10
	Day 27	0/10	10/10	10/10	9/10
	Day 28	0/10	10/10 ***	10/10 ***	9/10 ***

Articles		Vehicle	S-033188		Oseltamivir phosphate
Dose (mg/kg BID)		0	1.5	15	5
Administration		5 days			
Timing of Initial Administration		48 hours post-infection			
Animal number (survived/total)	Day 0	10/10	10/10	10/10	10/10
	Day 1	10/10	10/10	10/10	10/10
	Day 2	10/10	10/10	10/10	10/10
	Day 3	10/10	10/10	10/10	10/10
	Day 4	10/10	10/10	10/10	10/10
	Day 5	10/10	10/10	10/10	10/10
	Day 6	10/10	10/10	10/10	10/10
	Day 7	5/10	10/10	10/10	10/10
	Day 8	1/10	10/10	10/10	9/10
	Day 9	0/10	10/10	10/10	8/10
	Day 10	0/10	10/10	10/10	7/10
	Day 11	0/10	10/10	10/10	7/10
	Day 12	0/10	10/10	10/10	7/10
	Day 13	0/10	10/10	10/10	7/10
	Day 14	0/10	10/10	10/10	7/10
	Day 15	0/10	10/10	10/10	7/10
	Day 16	0/10	10/10	10/10	7/10
	Day 17	0/10	10/10	10/10	7/10
	Day 18	0/10	10/10	10/10	7/10
	Day 19	0/10	10/10	10/10	7/10
	Day 20	0/10	10/10	10/10	7/10
	Day 21	0/10	10/10	10/10	7/10
	Day 22	0/10	10/10	10/10	7/10
	Day 23	0/10	10/10	10/10	7/10
	Day 24	0/10	10/10	10/10	7/10
	Day 25	0/10	10/10	10/10	7/10
	Day 26	0/10	10/10	10/10	7/10
	Day 27	0/10	10/10	10/10	7/10
	Day 28	0/10	10/10 ***	10/10 ***	7/10 ***

Articles		Vehicle	S-033188		Oseltamivir phosphate
Dose (mg/kg BID)		0	1.5	15	5
Administration		5 days			
Timing of Initial Administration		72 hours post-infection			
Animal number (survived/total)	Day 0	10/10	10/10	10/10	10/10
	Day 1	10/10	10/10	10/10	10/10
	Day 2	10/10	10/10	10/10	10/10
	Day 3	10/10	10/10	10/10	10/10
	Day 4	10/10	10/10	10/10	10/10
	Day 5	10/10	10/10	10/10	10/10
	Day 6	10/10	10/10	10/10	10/10
	Day 7	8/10	10/10	10/10	8/10
	Day 8	1/10	10/10	10/10	3/10
	Day 9	0/10	10/10	10/10	1/10
	Day 10	0/10	10/10	10/10	1/10
	Day 11	0/10	10/10	10/10	1/10
	Day 12	0/10	10/10	10/10	1/10
	Day 13	0/10	10/10	10/10	1/10
	Day 14	0/10	10/10	10/10	1/10
	Day 15	0/10	10/10	10/10	1/10
	Day 16	0/10	10/10	10/10	1/10
	Day 17	0/10	10/10	10/10	1/10
	Day 18	0/10	10/10	10/10	1/10
	Day 19	0/10	10/10	10/10	1/10
	Day 20	0/10	10/10	10/10	1/10
	Day 21	0/10	10/10	10/10	1/10
	Day 22	0/10	10/10	10/10	1/10
	Day 23	0/10	10/10	10/10	1/10
	Day 24	0/10	10/10	10/10	1/10
	Day 25	0/10	10/10	10/10	1/10
	Day 26	0/10	10/10	10/10	1/10
	Day 27	0/10	10/10	10/10	1/10
	Day 28	0/10	10/10 ***††	10/10 ***††	1/10

Articles		Vehicle	S-033188		Oseltamivir phosphate
Dose (mg/kg BID)		0	1.5	15	5
Administration		5 days			
Timing of Initial Administration		96 hours post-infection			
Animal number (survived/total)	Day 0	10/10	10/10	10/10	10/10
	Day 1	10/10	10/10	10/10	10/10
	Day 2	10/10	10/10	10/10	10/10
	Day 3	10/10	10/10	10/10	10/10
	Day 4	10/10	10/10	10/10	10/10
	Day 5	10/10	10/10	10/10	10/10
	Day 6	10/10	10/10	10/10	10/10
	Day 7	6/10	10/10	10/10	8/10
	Day 8	2/10	7/10	10/10	2/10
	Day 9	0/10	5/10	9/10	1/10
	Day 10	0/10	5/10	8/10	1/10
	Day 11	0/10	5/10	7/10	1/10
	Day 12	0/10	5/10	7/10	1/10
	Day 13	0/10	5/10	7/10	1/10
	Day 14	0/10	5/10	7/10	1/10
	Day 15	0/10	5/10	7/10	1/10
	Day 16	0/10	5/10	7/10	1/10
	Day 17	0/10	5/10	7/10	1/10
	Day 18	0/10	5/10	7/10	1/10
	Day 19	0/10	5/10	7/10	1/10
	Day 20	0/10	5/10	7/10	1/10
	Day 21	0/10	5/10	7/10	1/10
	Day 22	0/10	5/10	7/10	1/10
	Day 23	0/10	5/10	7/10	1/10
	Day 24	0/10	5/10	7/10	1/10
	Day 25	0/10	5/10	7/10	1/10
	Day 26	0/10	5/10	7/10	1/10
	Day 27	0/10	5/10	7/10	1/10
	Day 28	0/10	5/10 **†	7/10 ***††	1/10
The following P values were calculated by the log-rank test and the fixed-sequence procedure. ** P<0.01, *** P<0.0001 vs vehicle. † P<0.05, †† P<0.01, ††† P<0.0001 vs oseltamivir phosphate 5 mg/kg.					
Study number [Location in CTD]		S-033188-EB-188-N [4.2.1.1-26]			

2.6.3.2.32 Protective Effect of Delayed Treatment with S-033188 against Lethal Infection with Influenza A Virus in Mice (Body Weight)

Test Article: S-033188

Species/Strain	Mouse/ BALB/cAnNCrlCrlj
Gender/Number of Animals	Female/10 per group
Virus	A/PR/8/34 (H1N1)
Vehicle/Formulation	0.5 w/v% MC
Method of Administration	Oral administration twice daily (every 12 hours) for 5 days under non-anesthesia.
Dose (mg/kg BID)	1.5, 15
Reference article (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5 w/v% MC/Oral administration twice daily [every 12 hours] for 5 days under non-anesthesia [Dose: 5 mg/kg as free base])
Treatment	Mice were intranasally inoculated with 1.38×10^3 $TCID_{50}$ (100 μL/mouse) of the virus suspensions under anesthesia. The initial administration of each article or vehicle was performed 24, 48, 72 or 96 hours after virus infection.
Evaluation items	Body weight change
Evaluation method	Mice were examined daily for body weights during treatment. Mice were euthanized and regarded as dead if their body weights in the morning were lower than 70% of the initial body weights (in the morning on the day of virus infection) according to humane endpoints. If the mouse died before evaluated time point, the proportion of body weight of the mouse at all time point after the time point was regarded as 70%. Initial body weight means the body weight of mouse in the morning on the day of virus infection.

Articles		Vehicle	S-033188		Oseltamivir phosphate
Dose (mg/kg BID)		–	1.5	15	5
Administration		5 days			
		Body weight change (% of initial body weight)			
24 hours post infection	Day 2	−2.0 ± 1.9	−1.5 ± 1.8	−0.9 ± 3.2	−0.5 ± 2.5
	Day 3	−11.5 ± 2.1	−5.5 ± 1.8 ***††	−2.1 ± 2.5 ***††	−8.5 ± 1.7 **
	Day 4	−16.2 ± 2.8	−6.5 ± 1.5 ***††	−1.5 ± 2.3 ***††	−12.6 ± 1.9 **
	Day 5	−20.1 ± 3.9	−6.5 ± 1.5 ***††	−2.2 ± 2.0 ***††	−14.4 ± 2.6 **
	Day 6	−24.9 ± 3.2	−6.1 ± 2.1 ***††	−3.9 ± 2.6 ***††	−19.5 ± 2.2 **
48 hours post infection	Day 3	−10.6 ± 2.2	−8.1 ± 2.7 **††	−5.5 ± 3.2 **††	−11.1 ± 1.4
	Day 4	−16.1 ± 3.3	−8.2 ± 2.9 ***††	−4.3 ± 2.1 ***††	−14.4 ± 2.5
	Day 5	−20.5 ± 3.0	−6.9 ± 3.2 ***††	−3.4 ± 1.9 ***††	−15.4 ± 2.6 **
	Day 6	−24.7 ± 2.7	−6.6 ± 3.4 ***††	−2.4 ± 1.9 ***††	−20.6 ± 2.5 **
	Day 7	−28.5 ± 2.5	−7.1 ± 3.8 ***††	−2.3 ± 2.7 ***††	−25.5 ± 2.0 **
72 hours post infection	Day 4	−16.5 ± 2.2	−14.6 ± 1.6	−14.7 ± 2.9	−16.8 ± 1.9
	Day 5	−20.9 ± 2.1	−15.9 ± 3.0 **††	−14.2 ± 3.3 **††	−20.5 ± 2.7
	Day 6	−25.1 ± 2.2	−18.0 ± 4.0 **††	−13.8 ± 3.6 ***††	−24.2 ± 2.5
	Day 7	−28.4 ± 1.9	−18.9 ± 4.0 ***††	−14.3 ± 4.3 ***††	−27.8 ± 1.5
	Day 8	−29.8 ± 0.8	−17.0 ± 5.3 ***††	−10.8 ± 3.9 ***††	−29.6 ± 0.7

Articles		Vehicle	S-033188		Oseltamivir phosphate
Dose (mg/kg BID)		–	1.5	15	5
Administration		5 days			
96 hours post infection	Day 5	−20.8 ± 2.1	−17.9 ± 2.5 †	−16.7 ± 2.3 **††	−20.7 ± 1.9
	Day 6	−25.0 ± 1.5	−21.5 ± 2.9 **††	−19.7 ± 2.8 ***†††	−24.8 ± 1.5
	Day 7	−28.7 ± 1.4	−25.1 ± 2.7 **††	−22.6 ± 3.6 ***††	−28.5 ± 1.3
	Day 8	−29.7 ± 0.8	−27.8 ± 2.9	−23.9 ± 5.7 **††	−29.8 ± 0.7
	Day 9	−30.0 ± 0.0	−26.4 ± 5.0 ††	−21.6 ± 7.6 **††	−29.9 ± 0.4

Each value represents the mean ± S.D. of the living mice at each day (AM).
If the mouse died before evaluated time point, the body weight change of the mouse was regarded as −30%.
The following P values were calculated by the log-rank test and the fixed-sequence procedure.
* P<0.05, ** P<0.01, *** P<0.0001 vs vehicle. † P<0.05, †† P<0.01, ††† P<0.0001 vs oseltamivir phosphate 5 mg/kg.

Study number [Location in CTD]	S-033188-EB-188-N [4.2.1.1-26]

2.6.3.2.33 Inhibitory Effect of Delayed Treatment with S-033188 on Virus Replication in Mice Lethally Infected with Influenza A Virus

Test Article: S-033188

Species/Strain	Mouse/ BALB/cAnNCrlCrlj
Gender/Number of Animals	Female/8 per group
Virus	A/PR/8/34 (H1N1)
Vehicle/Formulation	0.5 w/v% MC
Method of Administration	Oral administration twice daily (every 12 hours) for 5 days under non-anesthesia
Dose (mg/kg BID)	1.5, 15
Reference article (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5 w/v% MC/Oral administration twice daily [every 12 hours] for 1, 3 or 5 days under non-anesthesia [Dose: 5 mg/kg as free base])
Treatment	Mice were intranasally inoculated with 1.38×10^{-3} $TCID_{50}$ (100 μL/mouse) of the virus suspensions under anesthesia. The initial administration of each article or vehicle was performed 72 hours after virus infection.
Evaluation items	Virus titers in lungs 1, 3, 4, 6, 8 or 10 days after infection.
Evaluation method	Virus titers were measured by standard $TCID_{50}$ method using MDCK cells. For the comparison of the virus titers in lung tissues among the test article-treated group, the vehicle-treated group or the reference article-treated group at each analysis time point, Day 4 and Day 6, the one-way analysis of variance model and contrast method by the analysis time points was applied.

Results

Sampling time point of lungs (days post-infection)	Articles	Dose (mg/kg BID)	Number of mice	Virus titers (Log_{10} $TCID_{50}$/mL)
1	Untreated	–	8	4.21 ± 0.57
3	Untreated	–	8	5.92 ± 0.38
4	Vehicle	–	8	5.87 ± 0.28
	Oseltamivir phosphate	5	8	5.42 ± 0.20 **
	S-033188	1.5	8	3.69 ± 0.41 ***,†††
		15	8	2.90 ± 0.50 ***,†††
6	Vehicle	–	7	4.71 ± 0.36
	Oseltamivir phosphate	5	6	4.89 ± 0.39
	S-033188	1.5	8	3.08 ± 0.37 ***,†††
		15	8	2.07 ± 0.62 ***,†††
8	Vehicle	–	0	–
	Oseltamivir phosphate	5	1	1.50
	S-033188	1.5	8	1.50 ± 0.00
		15	8	1.50 ± 0.00

Sampling time point of lungs (days post-infection)	Articles	Dose (mg/kg BID)	Number of mice	Virus titers (Log_{10} $TCID_{50}$/mL)
10	Vehicle	–	0	–
	Oseltamivir phosphate	5	1	1.50
	S-033188	1.5	7	1.50 ± 0.00
		15	8	1.50 ± 0.00
Each value represents the mean ± S.D. of 6 to 8 mice except for n=1. The following P values were calculated by the one-way analysis of variance followed by pairwise comparison and the fixed-sequence procedure: ** P<0.01, *** P<0.0001 vs vehicle, ††† P<0.0001 vs oseltamivir phosphate 5 mg/kg.				
Study number [Location in CTD]	S-033188-EB-233-N [4.2.1.1-27]			

2.6.3.2.34 Protective Effect of Delayed Treatment with S-033188 in Combination with Oseltamivir against Lethal Infection with Influenza A Virus in Mice (Survival rate)

Test Article: S-033188

Species/Strain	Mouse/ BALB/cAnNCrlCrlj
Gender/Number of Animals	Female/10 per group
Virus	A/PR/8/34 (H1N1)
Vehicle/Formulation	0.5 w/v% MC
Method of Administration	Oral administration twice daily (every 12 hours) for 5 days under non-anesthesia
Dose (mg/kg BID)	0.5, 1.5, 15, 50
Reference article (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5 w/v% MC/Oral administration twice daily [every 12 hours] for 5 days under non-anesthesia [Dose: 10, 50 mg/kg as free base])
Treatment	Mice were intranasally inoculated with 8.00×10^2 $TCID_{50}$ (100 µL/mouse) of the virus suspensions under anesthesia. Mice were administrated with each article, vehicle or test article in combination with reference article. The initial administration of each article or vehicle was performed 96 hours after virus infection.
Evaluation items	Survival time
Evaluation method	Mice were examined daily for death through 28 days after infection. Mice were euthanized and regarded as dead if their body weights in the morning were lower than 70% of the initial body weights (in the morning on the day of virus infection) according to humane endpoints.

Results								
Articles		Vehicle	S-033188				Oseltamivir phosphate	
Dose (mg/kg BID)		0	0.5	1.5	15	50	10	50
Administration		5 days						
Animal number (survived/ total)	Day 0	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Day 1	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Day 2	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Day 3	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Day 4	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Day 5	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Day 6	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Day 7	8/10	10/10	10/10	10/10	10/10	10/10	10/10
	Day 8	3/10	7/10	8/10	10/10	10/10	3/10	9/10
	Day 9	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 10	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 11	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 12	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 13	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 14	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 15	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 16	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 17	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 18	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 19	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 20	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 21	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 22	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 23	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 24	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 25	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 26	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 27	0/10	4/10	7/10	10/10	10/10	1/10	4/10
	Day 28	0/10	4/10*	7/10** ††	10/10 *** ††† ¶¶	10/10 *** ††† ¶¶	1/10	4/10**

Results							
Articles (Dose)		S-033188 (0.5 mg/kg BID) + Oseltamivir phosphate (10 mg/kg BID)	S-033188 (0.5 mg/kg BID) + Oseltamivir phosphate (50 mg/kg BID)	S-033188 (1.5 mg/kg BID) + Oseltamivir phosphate (10 mg/kg BID)	S-033188 (1.5 mg/kg BID) + Oseltamivir phosphate (50 mg/kg BID)	S-033188 (15 mg/kg BID) + Oseltamivir phosphate (10 mg/kg BID)	S-033188 (15 mg/kg BID) + Oseltamivir phosphate (50 mg/kg BID)
Administration		5 days					
Animal number (survived/ total)	Day 0	10/10	10/10	10/10	10/10	10/10	10/10
	Day 1	10/10	10/10	10/10	10/10	10/10	10/10
	Day 2	10/10	10/10	10/10	10/10	10/10	10/10
	Day 3	10/10	10/10	10/10	10/10	10/10	10/10
	Day 4	10/10	10/10	10/10	10/10	10/10	10/10
	Day 5	10/10	10/10	10/10	10/10	10/10	10/10
	Day 6	10/10	10/10	10/10	10/10	10/10	10/10
	Day 7	10/10	10/10	10/10	10/10	10/10	10/10
	Day 8	8/10	9/10	10/10	10/10	10/10	10/10
	Day 9	8/10	9/10	10/10	10/10	10/10	10/10
	Day 10	7/10	9/10	10/10	10/10	10/10	10/10
	Day 11	7/10	9/10	10/10	10/10	10/10	10/10
	Day 12	7/10	9/10	10/10	10/10	10/10	10/10
	Day 13	7/10	9/10	10/10	10/10	10/10	10/10
	Day 14	7/10	9/10	10/10	10/10	10/10	10/10
	Day 15	7/10	9/10	10/10	10/10	10/10	10/10
	Day 16	7/10	9/10	10/10	10/10	10/10	10/10
	Day 17	7/10	9/10	10/10	10/10	10/10	10/10
	Day 18	7/10	9/10	10/10	10/10	10/10	10/10
	Day 19	7/10	9/10	10/10	10/10	10/10	10/10
	Day 20	7/10	9/10	10/10	10/10	10/10	10/10
	Day 21	7/10	9/10	10/10	10/10	10/10	10/10
	Day 22	7/10	9/10	10/10	10/10	10/10	10/10
	Day 23	7/10	9/10	10/10	10/10	10/10	10/10
	Day 24	7/10	9/10	10/10	10/10	10/10	10/10
	Day 25	7/10	9/10	10/10	10/10	10/10	10/10
	Day 26	7/10	9/10	10/10	10/10	10/10	10/10
	Day 27	7/10	9/10	10/10	10/10	10/10	10/10
	Day 28	7/10 ¶¶	9/10 ‡¶	10/10 †††	10/10 ¶¶	10/10 †††	10/10 ¶¶
The following P values were calculated by log-rank test and the fixed-sequence procedure: * P<0.05, **P<0.01, *** P<0.0001 vs vehicle, ¶¶ P<0.01, ††† P<0.0001 vs oseltamivir phosphate 10 mg/kg, ¶ P<0.05, ¶¶ P<0.01 vs oseltamivir phosphate 50 mg/kg, ‡ P<0.05 vs S-033188 alone.							
Study number [Location in CTD]		S-033188-EB-234-N [4.2.1.1-28]					

2.6.3.2.35 Protective Effect of Delayed Treatment with S-033188 in Combination with Oseltamivir against Lethal Infection with Influenza A Virus in Mice (Body weight)

Test Article: S-033188

Species/Strain	Mouse/ BALB/cAnNCrlCrlj
Gender/Number of Animals	Female/10 per group
Virus	A/PR/8/34 (H1N1)
Vehicle/Formulation	0.5 w/v% MC
Method of Administration	Oral administration twice daily (every 12 hours) for 5 days under non-anesthesia
Dose (mg/kg BID)	0.5, 1.5, 15, 50
Reference article (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5 w/v% MC/Oral administration twice daily [every 12 hours] for 5 days under non-anesthesia [Dose: 10, 50 mg/kg as free base])
Treatment	Mice were intranasally inoculated with 8.00×10^2 $TCID_{50}$ (100 μL/mouse) of the virus suspensions under anesthesia. Mice were administrated with each article, test article in combination with reference article or vehicle. The initial administration of each article or vehicle was performed 96 hours after virus infection.
Evaluation items	Body weight change
Evaluation method	Mice were examined daily for body weight during treatment. Mice were euthanized and regarded as dead if their body weights in the morning were lower than 70% of the initial body weights (in the morning on the day of virus infection) according to humane endpoints.

Articles	Vehicle	S-033188				Oseltamivir phosphate	
Dose (mg/kg BID)	–	0.5	1.5	15	50	10	50
Administration	5 days						
	Body weight change (% of initial body weight)						
Day 5	−17.5 ± 2.5	−16.1 ± 2.7†	−14.7 ± 3.4†	−16.3 ± 3.2†	−12.8 ± 2.4 **††	−19.1 ± 1.7	−14.2 ± 3.1 **
Day 6	−23.1 ± 2.4	−20.8 ± 2.4	−19.5 ± 4.2 *†	−18.6 ± 3.7 **††	−12.8 ± 3.4 ***††¶¶	−23.4 ± 2.4	−18.4 ± 3.5 **
Day 7	−27.3 ± 2.5	−25.0 ± 2.2	−22.1 ± 5.2 **†	−21.5 ± 5.0 **††	−14.2 ± 3.9 ***††¶¶	−26.7 ± 2.2	−22.9 ± 2.3 **
Day 8	−29.5 ± 0.9	−28.5 ± 2.0	−22.0 ± 7.8 **††	−20.0 ± 6.3 **†¶	−11.7 ± 4.6 ***††¶¶	−29.0 ± 1.7	−26.4 ± 3.8 **
Day 9	−30.0 ± 0.0	−27.7 ± 3.1	−20.4 ± 8.8 **†¶	−16.4 ± 6.9 ***††¶¶	−9.0 ± 4.4 ***††¶¶	−29.4 ± 1.9	−26.7 ± 4.6 *

Articles (Dose)	S-033188 (0.5 mg/kg BID) + Oseltamivir phosphate (10 mg/kg BID)	S-033188 (0.5 mg/kg BID) + Oseltamivir phosphate (50 mg/kg BID)	S-033188 (1.5 mg/kg BID) + Oseltamivir phosphate (10 mg/kg BID)	S-033188 (1.5 mg/kg BID) + Oseltamivir phosphate (50 mg/kg BID)	S-033188 (15 mg/kg BID) + Oseltamivir phosphate (10 mg/kg BID)	S-033188 (15 mg/kg BID) + Oseltamivir phosphate (50 mg/kg BID)
Administration	5 days					
	Body weight change (% of initial body weight)					
Day 5	−16.0 ± 3.3†	−14.6 ± 3.4	−12.7 ± 2.6†††	−14.7 ± 3.7	−10.6 ± 4.6†††‡‡	−11.5 ± 3.1‡‡
Day 6	−18.7 ± 3.4†	−17.7 ± 4.3	−14.3 ± 2.9†††‡	−15.9 ± 4.4‡	−11.5 ± 6.2†††‡‡	−11.4 ± 3.2¶¶‡‡
Day 7	−22.1 ± 4.3	−19.9 ± 5.5‡	−15.8 ± 4.7†††‡	−16.6 ± 5.7¶¶‡	−12.4 ± 8.1†††‡‡	−11.7 ± 3.7¶¶¶‡‡
Day 8	−22.4 ± 6.9†‡	−19.2 ± 6.6¶¶‡‡	−12.4 ± 5.9†††‡	−13.6 ± 7.6¶¶‡	−10.7 ± 8.8†††‡‡	−8.5 ± 3.5¶¶¶‡‡
Day 9	−21.1 ± 9.2†	−16.4 ± 8.6¶¶‡‡	−7.9 ± 5.2†††‡	−11.7 ± 8.1¶¶‡	−8.6 ± 8.7†††‡	−5.1 ± 2.9¶¶¶‡‡

Each value represents the mean ± S.D. of the living mice at each day (AM).
If the mouse died before evaluated time point, the body weight change of the mouse was regarded as −30%.
The following P values were calculated by the one-way analysis of variance followed by pairwise comparisons and the fixed sequence procedure: * P<0.05, ** P<0.01, *** P<0.0001 vs vehicle,
† P<0.05, †† P<0.01, ††† P<0.0001 vs oseltamivir phosphate 10 mg/kg, ¶ P<0.05, ¶¶ P<0.01, ¶¶¶ P<0.0001 vs oseltamivir phosphate 50 mg/kg, ‡ P<0.05, ‡‡ P<0.01 vs S-033188 alone.

Study number [Location in CTD]	S-033188-EB-234-N [4.2.1.1-28]

2.6.3.2.36 Therapeutic Effect of S-033188 on Influenza A Virus Infection in Ferrets

Test Article: S-033188

Species/Strain	Ferret
Gender/Number of Animals	Female/4 per group
Virus	A/Kadoma/3/2006 (H1N1)
Vehicle/Formulation	Solution in 5% (w/v) sodium dodecyl sulfate (SDS) and 10% (w/w) polyoxyethylene (20) sorbitan monooleate (Tween 80) in water (5% SDS/10% Tween 80/distilled water)
Method of Administration	Oral administration twice daily for 1 day under anesthesia
Dose (mg/kg BID)	10, 30
Reference substance (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5 w/v% MC/Oral administration twice daily for 2 day under anesthesia [Dose: 5 mg/kg as free base])
Treatment	Ferrets were intranasally inoculated with1000 $TCID_{50}$ (200 μL/ferret) of the virus suspensions under anesthesia. One day after viral inoculation, vehicle or S-033188 was administered twice daily (every 12 hours) for 1 day or oseltamivir phosphate was administered twice daily (every 12 hours) for 2 days.
Evaluation items	Virus titers in nasal wash fluids and body temperature.
Evaluation method	Virus titers were measured by the standard $TCID_{50}$/mL method using MDCK cells. Differences of the virus titers over time among the test article-treated groups, the reference article-treated group, and the vehicle-treated group were analyzed by the two-way analysis of variance followed by pairwise comparison and the fixed-sequence procedure. The comparison of change from baseline in body temperature was performed in a similar fashion to the comparison of the virus titers.

Results

Articles	Dose (mg/kg BID)	Sampling point (Day)	Virus titers (Log_{10} $TCID_{50}$/mL)[a]	Mean of virus titer from day 2 to 3 post-viral inoculation
Vehicle	–	1	2.29 ± 0.55	–
		2	6.96 ± 0.48	5.24
		3	3.53 ± 0.75	
S-033188	10	1	2.46 ± 0.42	–
		2	0.50 ± 0.00	1.98
		3	3.46 ± 0.42 **†	
	30	1	2.48 ± 0.51	–
		2	0.50 ± 0.00	1.90
		3	3.29 ± 0.42 **†	
Oseltamivir phosphate	5	1	2.17 ± 0.43	–
		2	5.38 ± 0.48	4.54
		3	3.71 ± 0.32 *	

Articles	Dose (mg/kg BID)	Sampling point (Day)	Body temperature change (°C)	Mean of body temperature change from day 1.33 to 3 post-viral inoculation
Vehicle	–	1	0.00 ± 0.00	–
		1.33	−0.45 ± 0.15	0.36
		1.67	−0.41 ± 0.21	
		2	−0.08 ± 0.48	
		2.33	1.36 ± 0.23	
		2.67	1.22 ± 0.35	
		3	0.52 ± 0.31	
S-033188	10	1	0.00 ± 0.00	–
		1.33	−0.82 ± 0.27	−0.60
		1.67	−0.86 ± 0.32	
		2	−0.85 ± 0.40	
		2.33	−0.58 ± 0.29	
		2.67	−0.52 ± 0.51	
		3	0.05 ± 0.54 *†	
	30	1	0.00 ± 0.00	–
		1.33	−0.60 ± 0.49	−0.34
		1.67	−0.59 ± 0.32	
		2	−0.41 ± 0.37	
		2.33	−0.56 ± 0.21	
		2.67	−0.26 ± 0.27	
		3	0.40 ± 0.45 *†	
Oseltamivir phosphate	5	1	0.00 ± 0.00	–
		1.33	−0.28 ± 0.33	0.22
		1.67	−0.16 ± 0.19	
		2	−0.28 ± 0.42	
		2.33	0.57 ± 0.76	
		2.67	1.00 ± 0.24	
		3	0.45 ± 0.32	

Each value represents the mean ± S.D. from 4 ferrets.
The following P values of virus titers for the overall time (from 2 to 3 days post-infection [dpi]) were calculated by the two-way analysis of variance followed by pairwise comparisons and the fixed-sequence procedure.
* P<0.01, ** P<0.0001 vs vehicle. † P<0.0001 vs oseltamivir phosphate 5 mg/kg.
The following P values of body temperature change were calculated by the two-way analysis of variance followed by pairwise comparison and the fixed-sequence procedure.
** P<0.0001 vs vehicle. † P<0.0001 vs oseltamivir phosphate 5 mg/kg.

Study number [Location in CTD]	R-033188-EB-071-N [4.2.1.1-29]

2.6.3.2.37 PK Parameters of S-033447 after a Single Oral Administration of S-033188 in Ferrets

Test Article: S-033188

Pharmacokinetic analysis				
Species/Strain	Ferret			
Gender /Number of sampling	Female/4 per group			
Feeding Condition	Fasting			
Vehicle/Formulation	Solution in 5% SDS/10% Tween 80/distilled water			
Method of Administration	Single oral administration			
Dose (mg/kg)	10, 30			
Sample	The plasma at 0.5, 1, 2, 4, 6, 8, and 24 hours after dosing			
Analyte	S-033188 and S-033447			
Assay	LC/MS/MS			
Results				
	S-033447		S-033188	
Dose (mg/kg)	10	30	10	30
C_{max} (ng/mL)	66.6 ± 14.6	365 ± 316	165 ± 179	298 ± 88
T_{max} (hr)	1.50 ± 0.58	2.00 ± 1.41	1.13 ± 0.63	0.875 ± 0.250
$t_{1/2,z}$ (hr) [a]	6.91 ± 3.79	4.44 ± 0.67	N.C.[b]	N.C.[b]
$AUC_{0\text{-}24hr}$ (ng·hr/mL)	421 ± 119	3240 ± 3240	715 ± 758	1260 ± 150
$AUC_{0\text{-}inf}$ (ng·hr/mL)	460 ± 130	3300 ± 3290	N.C.[b]	N.C.[b]
Each value represents the mean ± S.D. of 4 ferrets. Abbreviations: $AUC_{0\text{-}24hr}$, area under the plasma concentration-time curve from zero to 24 hours after dosing; N.C., not calculated. a $t_{1/2,(6\text{-}24\ hr)}$. b The values of $t_{1/2,z}$, and $AUC_{0\text{-}inf}$ of S-033188 were not calculated in all ferrets because elimination rate was not accurately evaluated from the plasma concentration-time profile.				
Study number [Location in CTD]	R-033188-EB-071-N [4.2.1.1-29]			

2.6.3.2.38 Inhibitory Effect of S-033188 on Influenza Virus Replication in Immunocompromised Mice (Virus Titers in lung)

Test Article: S-033188

Species/Strain	Mouse/ BALB/cAnNCrlCrlj
Gender/Number of Animals	Female/5 per group
Virus	A/PR/8/34 (H1N1)
Vehicle/Formulation	0.5 w/v% MC
Method of Administration	Oral administration twice daily (every 12 hours) for 5 days under non-anesthesia
Dose (mg/kg BID)	1.5, 15, 50
Reference article (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5 w/v% MC/Oral administration twice daily [every 12 hours] for 5 days under non-anesthesia [Dose: 5 or 50 mg/kg as free base])
Treatment	Mice were subcutaneously treated with 0.2 mL of 1 mg/mL cyclophosphamide (CP) solution (0.2 mg/mouse, approximately 10 mg/kg) once a day for 11 days starting from 1 day prior to infection. CP-untreated group was subcutaneously treated with 0.2 mL of saline in the same manner. Mice were intranasally inoculated with 100 $TCID_{50}$ (0.1 mL/mouse) of the virus suspensions under anesthesia.
Evaluation items	Virus titers in lungs 5, 6, 7, 8, 9 or 10 days after infection
Evaluation method	Virus titers were measured by standard $TCID_{50}$ method using MDCK cells. For the virus titers in lungs for the overall time (from 5 to 10 days after infection), each dose group of the test article was compared to the vehicle-treated group or the reference article-treated groups using the linear model with heterogeneous variances including group and time point as fixed effects and group-by-time point interaction. In the fixed-sequence procedure applied to these comparisons, only if current testing hypothesis was rejected, the test for successive hypothesis was conducted.

Cyclophosphamide	Articles	Dose (mg/kg BID)	Post infection day (days)	Log_{10} $TCID_{50}$/mL Mean ± S.D.
Untreated	Vehicle	0	5	5.35 ± 0.23
Treated				5.43 ± 0.25
Treated	Vehicle	0	6	5.49 ± 0.50
	S-033188	1.5		3.17 ± 0.67
		15		2.40 ± 0.25
		50		1.70 ± 0.22
	Oseltamivir phosphate	5		4.59 ± 0.12
		50		4.91 ± 0.24
Untreated	Vehicle	0	7	3.73 ± 0.40
Treated				4.40 ± 0.33
Treated	S-033188	1.5		2.93 ± 0.65
		15		1.63 ± 0.22
		50		1.53 ± 0.07
	Oseltamivir phosphate	5		4.42 ± 0.31
		50		4.67 ± 0.20

Cyclophosphamide	Articles	Dose (mg/kg BID)	Post infection day (days)	Log_{10} $TCID_{50}$/mL Mean ± S.D.
Treated	Vehicle	0	8	4.37 ± 0.22
	S-033188	1.5		2.13 ± 0.68
		15		1.50 ± 0
		50		1.50 ± 0
	Oseltamivir phosphate	5		4.73 ± 0.28
		50		4.37 ± 0.41
Untreated	Vehicle	0	9	2.00 ± 0.45
Treated				4.30 ± 0.45
Treated	S-033188	1.5		1.73 ± 0.35
		15		1.50 ± 0
		50		1.50 ± 0
	Oseltamivir phosphate	5		4.50 ± 0.35
		50		3.45 ± 0.30
Treated	Vehicle	0	10	4.50 ± 0.51
	S-033188	1.5		1.57 ± 0.15*†‡
		15		1.50 ± 0*†‡
		50		1.50 ± 0*†‡
	Oseltamivir phosphate	5		3.90 ± 1.06
		50		2.33 ± 0.79

Each value represents the mean ± S.D. of 5 mice.
The following P values for the overall time (from 5 to 10 dpi) were calculated by the linear model with heteroscedastic errors and the fixed-sequence procedure.
* P<0.0001 vs vehicle. † P<0.0001 vs oseltamivir phosphate 5 mg/kg.
The following P value for the overall time (from 5 to 10 dpi) was calculated by the linear model with heteroscedastic errors.
‡ P<0.0001 vs oseltamivir phosphate 50 mg/kg.

Study number [Location in CTD]	S-033188-EB-194-N [4.2.1.1-30]

2.6.3.2.39 Inhibitory Effect of S-033188 on Influenza Virus Replication in Immunocompromised Mice (Body Weight)

Test Article: S-033188

Species/Strain		Mouse/ BALB/cAnNCrlCrlj					
Gender/Number of Animals		Female/5 per group					
Virus		A/PR/8/34 (H1N1)					
Vehicle/Formulation		0.5 w/v% MC					
Method of Administration		Oral administration twice daily (every 12 hours) for 5 days under non-anesthesia					
Dose (mg/kg BID)		1.5, 15, 50					
Reference article (Vehicle/Formulation, Method of Administration)		Oseltamivir phosphate (0.5 w/v% MC/Oral administration twice daily [every 12 hours] for 5 days under non-anesthesia [Dose: 5 or 50 mg/kg as free base])					
Treatment		Mice were subcutaneously treated with 0.2 mL of 1 mg/mL CP solution (0.2 mg/mouse, approximately 10 mg/kg) once a day for 11 days starting from 1 day prior to infection. CP-untreated group was subcutaneously treated with 0.2 mL of saline in the same manner. Mice were intranasally inoculated with 100 $TCID_{50}$ (0.1 mL/mouse) of the virus suspensions under anesthesia.					
Evaluation items		Body weight change after initial treatment					
Evaluation method		Virus-infected mice euthanized on 10 dpi were examined daily in the morning for body weight. In this analysis, baseline value was defined as the value at the initial day of administration (5 days after infection). For the proportion of body weight to the infected day, each dose group of the test article was compared to the vehicle-treated group or the reference aricle-treated groups using the mixed model repeated measures (MMRM) including group and time point as fixed effects and group-by-time point interaction. Covariate in this model was baseline value of body weight, and the structure of covariance matrix was unstructured. Comparisons using MMRM were conducted in the same manner as that in the primary analysis for virus titers.					
Results							
		Body weight change (% of body weight on 5 days after infection)					
Test or Reference article		Vehicle	Oseltamivir phosphate		S-033188		
Dosing Schedule							
Dose (mg/kg BID)		0	5	50	1.5	15	50
Days Post-Infection	Day 6	95.26 ± 1.98	95.35 ± 2.36	96.07 ± 2.31	97.95 ± 1.39	100.72 ± 2.32	101.04 ± 3.48
	Day 7	90.85 ± 3.15	91.64 ± 1.70	92.78 ± 2.46	97.32 ± 2.51	101.84 ± 2.48	100.31 ± 3.16
	Day 8	85.95 ± 3.75	90.43 ± 3.28	90.25 ± 2.06	97.90 ± 3.39	103.91 ± 3.76	102.97 ± 4.86
	Day 9	82.45 ± 4.20	86.58 ± 3.36	89.47 ± 3.21	99.93 ± 3.30	106.02 ± 3.10	105.22 ± 6.01
	Day 10	79.50 ± 5.21	85.45 ± 6.47	93.02 ± 3.05	104.50 ± 2.96*†‡	110.50 ± 2.16*†††‡‡	109.10 ± 5.72*†††‡‡

Each value represents the mean ± S.D. of 5 mice.
The following P value for the overall time (from 5 to 10 dpi) by the mixed model repeated measures and the fixed-sequence procedure.
* P<0.0001 vs vehicle. † P<0.0005, †† P<0.0001 vs oseltamivir phosphate 5 mg/kg.
The following P value for the overall time (from 5 to 10 dpi) by the mixed model repeated measures.
‡ P<0.005, ‡‡ P<0.0001 vs oseltamivir phosphate 50 mg/kg.

Study number [Location in CTD]	S-033188-EB-194-N [4.2.1.1-30]

2.6.3.2.40 Resistance Analysis in Immunocompromised Mouse Treated with S-033188

Test Article: S-033188

Species/Biological materials (Viral RNA source)	Lung homogenates (n = 5) of A/PR/8/34 strain-infected immunocompromised mice treated with S-033188 1.5, 15, or 50 mg/kg on 6, 8, or 10 days after infection					
Evaluation item	Quantification of viral RNA, sequence analysis of PA gene					
Evaluation method	Viral RNA extracted from sample homogenate was quantified by qPCR assay. Sequence analysis of PA gene was performed by sangar sequencing method. Quantification of viral RNA and sequence analysis of PA gene were performed by Viroclinics Biosciences B.V..					
Results						
Post infection day (days)	Viral RNA (Log_{10} VP/mL)			PA amino acid substitution		
	1.5 mg/kg BID	15 mg/kg BID	50 mg/kg BID	1.5 mg/kg BID	15 mg/kg BID	50 mg/kg BID
6	8.33 ± 0.10	8.32 ± 0.10	8.19 ± 0.09	–	–	–
8	7.92 ± 0.11	7.72 ± 0.19	7.52 ± 0.29	–	–	–
10	6.39 ± 0.31	6.32 ± 0.19	6.26 ± 0.27	–	–	–
Abbreviation: qPCR, quantitative polymerase chain reaction. VP, viral particles. –: No amino acid change was observed in 5 mice, compared to the parent virus (A/Puerto Rico/8/34 strain). Each value of viral RNA represents the mean ± S.D. of 5 mice.						
Study number [Location in CTD]	S-033188-EB-252-N [4.2.1.1-31]					

2.6.3.2.41 Survival Effect of S-033188 on Highly Pathogenic Avian Influenza Virus (H5N1) Infection in Mice

Test Article: S-033188

Species/Strain	Mouse/ BALB/cCr Slc
Gender/Number of Animals	Female/10 per group
Virus	A/Hong Kong/483/97 (H5N1),
Vehicle/Formulation	0.5 w/v% MC
Method of Administration	Oral administration twice daily (every 12 hours) for 1 day or 5 days under non-anesthesia
Dose (mg/kg BID)	0.5, 5, 50
Reference substance (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5 w/v% MC/Oral administration twice daily [every 12 hours] for 5 day under non-anesthesia [Dose: 5 or 50 mg/kg as free base])
Treatment	Mice were intranasally inoculated with 75 $TCID_{50}$ (50 μL/mouse) of the virus suspensions under anesthesia immediately before the first administration.
Evaluation items	Survival time
Evaluation method	Mice were examined daily for death through 14 days after infection. Mice were euthanized and regarded as dead if their body weights in the morning were lower than 30% of the initial body weights (in the morning on the day of virus infection) according to humane endpoints. Differences of the survival time between each group of the test article and the reference article-treated group or the vehicle-treated group were analyzed by the log-rank test and the fixed sequence procedure.

Results

Articles		Vehicle	S-033188						Oseltamivir phosphate	
Dose (mg/kg BID)		0	0.5	5	50	0.5	5	50	5	50
Administration		5 days				1 day			5 days	
Animal number (survived/total)	Day 0	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Day 1	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Day 2	10/10	10/10	10/10	9/9 [a]	10/10	10/10	10/10	10/10	10/10
	Day 3	10/10	10/10	10/10	9/9	10/10	10/10	10/10	10/10	10/10
	Day 4	10/10	10/10	10/10	9/9	10/10	10/10	10/10	10/10	10/10
	Day 5	10/10	10/10	10/10	9/9	10/10	10/10	10/10	10/10	10/10
	Day 6	5/10	10/10	10/10	9/9	10/10	10/10	10/10	9/10	10/10
	Day 7	0/10	10/10	10/10	9/9	10/10	10/10	10/10	7/10	10/10
	Day 8	0/10	9/10	10/10	9/9	7/10	10/10	10/10	2/10	9/10
	Day 9	0/10	9/10	10/10	9/9	5/10	10/10	10/10	0/10	7/10
	Day 10	0/10	7/10	10/10	9/9	4/10	10/10	10/10	0/10	7/10
	Day 11	0/10	7/10	10/10	9/9	2/10	10/10	10/10	0/10	7/10
	Day 12	0/10	7/10	10/10	9/9	2/10	10/10	10/10	0/10	7/10
	Day 13	0/10	7/10	10/10	9/9	2/10	10/10	10/10	0/10	7/10
	Day 14	0/10	6/10 **††	10/10 **††	9/9 **††	2/10 **†	10/10 **††	10/10 **††	0/10 *	7/10 ‡

a On 2 days post-infection, 1 out of 10 mice was died because of an obvious oral administration error. The following P values were calculated by the log-rank test and the fixed-sequence procedure. * $P < 0.005$, ** $P < 0.0001$ vs vehicle. † $P < 0.005$, †† $P < 0.0001$ vs oseltamivir phosphate 5 mg/kg. The following P values were calculated by the log-rank test. ‡ $P < 0.0001$ vs vehicle.	
Study number [Location in CTD]	S-033188-EB-110-N [4.2.1.1-32]

2.6.3.2.42 Inhibitory Effect of S-033188 on Replication of Highly Pathogenic Avian Influenza Virus (H5N1) in Mice

Test Article: S-033188

Species/Strain	Mouse/ BALB/cCr Slc
Gender/Number of Animals	Female/5 per group
Virus	A/Hong Kong/483/97 (H5N1),
Vehicle/Formulation	0.5 w/v% MC
Method of Administration	Oral administration twice daily (every 12 hours) for 1, 3 and 5 days under non-anesthesia
Dose (mg/kg BID)	0.5, 5, 50
Reference article (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5 w/v% MC/Oral administration twice daily [every 12 hours] for 1, 3 and 5 days under non-anesthesia [Dose: 5 or 50 mg/kg as free base])
Treatment	Mice were intranasally inoculated with 75 $TCID_{50}$ (50 μL/mouse) of the virus suspensions under anesthesia immediately before the first administration.
Evaluation items	Virus titers in lungs 1, 3, or 5 days after the first administration
Evaluation method	Virus titers were measured by standard $TCID_{50}$ method using MDCK cells. For the virus titers in lungs for the overall time (from 5 to 10 days after infection), each dose group of the test article was compared to the vehicle-treated group or the reference article-treated groups by the two-way analysis of variance model followed by pairwise comparisons and the fixed sequence procedure.

Articles	Dose (mg/kg)	Dosing schedule	Time point (day)	Log_{10} $TCID_{50}$/mL
Vehicle	–	BID for 1 day	1	4.51 ± 0.30
		BID for 3 days	3	5.33 ± 0.42
		BID for 5 days	5	4.70 ± 0.07
S-033188	0.5	BID for 1 day	1	2.48 ± 0.67
			3	3.83 ± 1.24
			5	4.57 ± 1.47 ** †
		BID for 3 days	3	3.31 ± 0.39
		BID for 5 days	5	3.00 ± 0.87 ** ††
	5	BID for 1 day	1	1.50 ± 0.00
			3	2.25 ± 1.12
			5	1.70 ± 0.45 ** †† §
		BID for 3 days	3	1.53 ± 0.07
		BID for 5 days	5	1.50 ± 0.00 ** †† §
	50	BID for 1 day	1	1.50 ± 0.00
			3	1.50 ± 0.00
			5	1.50 ± 0.00 ** †† §
		BID for 3 days	3	1.50 ± 0.00
		BID for 5 days	5	1.50 ± 0.00 ** †† §

Articles	Dose (mg/kg)	Dosing schedule	Time point (day)	Log_{10} $TCID_{50}$/mL
Oseltamivir phosphate	5	BID for 1 day	1	3.00 ± 0.47
		BID for 3 days	3	4.83 ± 0.46
		BID for 5 days	5	5.18 ± 0.43 *
	50	BID for 1 day	1	1.93 ± 0.45
		BID for 3 days	3	4.14 ± 0.46
		BID for 5 days	5	3.80 ± 0.45 ‡

Each value represents the mean ± S.D. of 5 mice.
The following P values were calculated by the two-way analysis of variance followed by pairwise comparisons and the fixed-sequence procedure.
* $P < 0.05$, ** $P < 0.0001$ vs vehicle. † $P < 0.005$, †† $P < 0.0001$ vs oseltamivir phosphate 5 mg/kg.
The following P values were calculated by the two-way analysis of variance followed by pairwise comparisons.
‡ $P < 0.0001$ vs vehicle. § $P < 0.0001$ vs oseltamivir phosphate 50 mg/kg.

Study number [Location in CTD]	S-033188-EB-110-N [4.2.1.1-32]

2.6.3.2.43 Survival Effect of S-033188 on Avian Influenza Virus (H7N9) Infection in Mice

Test Article: S-033188

Species/Strain	Mouse/ BALB/cCr Slc
Gender/Number of Animals	Female/10 per group
Virus	A/Anhui/1/2013 (H7N9)
Vehicle/Formulation	0.5 w/v% MC
Method of Administration	Oral administration twice daily (every 12 hours) for 1 day or 5 days under non-anesthesia
Dose (mg/kg BID)	0.5, 5, 50
Reference substance (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5 w/v% MC/Oral administration twice daily [every 12 hours] for 5 days under non-anesthesia [Dose: 5 or 50 mg/kg as free base])
Treatment	Mice were intranasally inoculated with 4×10^5 $TCID_{50}$ (50 µL/mouse) of the virus suspensions under anesthesia immediately before the first administration.
Evaluation items	Survival time
Evaluation method	Mice were examined daily for death through 28 days after infection. Mice were euthanized and regarded as dead if their body weights in the morning were lower than 30% of the initial body weights (in the morning on the day of virus infection) according to humane endpoints. Differences of the survival time between each group of the test article and the reference article-treated group or the vehicle-treated group were analyzed by the log-rank test and the fixed sequence procedure.

Results

Articles		Vehicle	S-033188						Oseltamivir phosphate	
Dose (mg/kg BID)		0	0.5	5	50	0.5	5	50	5	50
Administration		5 days				1 day			5 days	
Animal number (survived/total)	Day 0	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Day 1	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Day 2	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Day 3	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Day 4	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Day 5	7/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Day 6	3/10	10/10	10/10	10/10	10/10	10/10	10/10	6/10	9/10
	Day 7	0/10	10/10	10/10	10/10	10/10	10/10	10/10	4/10	5/10
	Day 8	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 9	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 10	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 11	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 12	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 13	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 14	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 15	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 16	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 17	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10

	Day 18	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 19	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 20	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 21	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 22	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 23	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 24	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 25	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 26	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 27	0/10	10/10	10/10	10/10	9/10	10/10	10/10	3/10	5/10
	Day 28	0/10	10/10 *†§	10/10 *†§	10/10 *†§	9/10 *†§	10/10 *†§	10/10 *†§	3/10 ‡	5/10 ‡‡

The following P values were calculated by the log-rank test and the fixed-sequence procedure.
* $P < 0.0001$ vs vehicle. † $P < 0.005$ vs oseltamivir phosphate 5 mg/kg.
The following P values were calculated by the log-rank test.
‡ $P < 0.05$, ‡‡ $P < 0.001$ vs vehicle. § $P < 0.05$ vs oseltamivir phosphate 50 mg/kg.

Study number [Location in CTD]	S-033188-EB-226-N [4.2.1.1-33]

2.6.3.2.44 Inhibitory Effect of S-033188 on Replication of Avian Influenza Virus (H7N9) in Mice

Test Article: S-033188

Species/Strain	Mouse/ BALB/cCr Slc
Gender/Number of Animals	Female/5 per group
Virus	A/Anhui/1/2013 (H7N9)
Vehicle/Formulation	0.5 w/v% MC
Method of Administration	Oral administration twice daily (every 12 hours) for 1, 3 and 5 days under non-anesthesia
Dose (mg/kg BID)	0.5, 5, 50
Reference article (Vehicle/Formulation, Method of Administration)	Oseltamivir phosphate (0.5 w/v% MC/Oral administration twice daily [every 12 hours] for 1, 3 and 5 days under non-anesthesia [Dose: 5 or 50 mg/kg as free base])
Treatment	Mice were intranasally inoculated with 4×10^5 $TCID_{50}$ (50 µL/mouse) of the virus suspensions under anesthesia immediately before the first administration.
Evaluation items	Virus titers in lungs 1, 3, or 5 days after the first administration
Evaluation method	Virus titers were measured by standard $TCID_{50}$ method using MDCK cells. For the virus titers in lungs for the overall time (from 5 to 10 days after infection), each dose group of the test article was compared to the vehicle-treated group or the reference article-treated groups by the two-way analysis of variance model followed by pairwise comparisons and the fixed sequence procedure.

Articles	Dose (mg/kg)	Dosing schedule	Time point (day)	Log_{10} $TCID_{50}$/mL
Vehicle	–	BID for 1 day	1	5.63 ± 0.25
		BID for 3 days	3	5.45 ± 0.21
		BID for 5 days	5	4.91 ± 0.24
S-033188	0.5	BID for 1 day	1	4.73 ± 0.19
			3	5.39 ± 0.55
			5	4.86 ± 0.51 *
		BID for 3 days	3	4.21 ± 0.22
		BID for 5 days	5	4.03 ± 0.18 **†§
	5	BID for 1 day	1	1.63 ± 0.07
			3	4.73 ± 0.28
			5	4.74 ± 0.28 **†§
		BID for 3 days	3	2.35 ± 0.88
		BID for 5 days	5	2.69 ± 0.43 **†§
	50	BID for 1 day	1	1.50 ± 0.00
			3	3.63 ± 0.63
			5	4.03 ± 0.34 **†§
		BID for 3 days	3	1.50 ± 0.00
		BID for 5 days	5	1.53 ± 0.07 **†§

Oseltamivir phosphate	5	BID for 1 day	1	5.35 ± 0.23
		BID for 3 days	3	5.40 ± 0.32
		BID for 5 days	5	4.60 ± 0.15 ‡
	50	BID for 1 day	1	5.10 ± 0.47
		BID for 3 days	3	5.35 ± 0.10
		BID for 5 days	5	5.10 ± 0.37
Each value represents the mean ± S.D. of 5 mice. The following P values were calculated by the two-way analysis of variance followed by pairwise comparisons and the fixed-sequence procedure. * P<0.01, ** P<0.0001 vs vehicle. † P<0.0001 vs oseltamivir 5 mg/kg. The following P values were calculated by the two-way analysis of variance followed by pairwise comparisons. ‡ P<0.05 vs vehicle, § P<0.0001 vs oseltamivir 50 mg/kg.				
Study number [Location in CTD]		S-033188-EB-226-N [4.2.1.1-33]		

2.6.3.3 副次的薬理試験

2.6.3.3.1 Effect of S-033447 on Cytotoxicity in Cultured Cells

Test Article: S-033447

Species/Biological material	MDCK, MDBK, RPMI2650, and A549 cell lines
Evaluation item	CC_{50}: Concentration achieving 50% of cytotoxicity
Concentration (μmol/L)	0.39, 0.78, 1.6, 3.1, 6.3, 13, 25, 50, 100, and 200 (final concentration)
Reference substance	N/A
Vehicle	0.5% DMSO
Evaluation Method	The confluent monolayer cells cultured in cell assay medium were prepared in assay plates. The culture fluid in each well was replaced by 100 μL of the cell assay medium containing concentrated S-033447. The cultures were performed in triplicate. The assay plates were incubated at 37°C in a CO_2 incubator for 3 days.
Results	
Cell lines	CC_{50} (μmol/L)
MDBK	47.52 ± 1.18
MDCK	18.94 ± 4.36
RPMI2650	22.79 ± 3.23
A549	17.30 ± 6.57
Each value represents the mean ±S.D. of 3 independent experiments.	
Study Number [Location in CTD]	S-033188-EB-117-N [4.2.1.2-01]

2.6.3.3.2 Inhibitory Effect of S-033447 on Cytopathic Effect in Cultured Cells Infected with Influenza Virus

Test Article: S-033447

Species/Biological material	MDCK cells infected with influenza A viruses (A/WSN/33 [H1N1] and A/Victoria/3/75 [H3N2]) and. influenza B viruses (B/Maryland/1/59 and B/Hong Kong/5/72)				
Final concentration (nmol/L)	Antiviral Study: 0.128, 0.64, 3.2, 16, 80, and 400 Cytotoxicity study: 16, 80, 400, 2000, 10000, and 50000				
Reference article (Final concentration)	Ribavirin (Antiviral Study: 3.90625, 7.8125, 15.625, 31.25, 62.5, and 125 µmol/L, Cytotoxicity study: 62.5, 125, 250, 500, 1000, and 2000 µmol/L) Favipiravir (Antiviral Study: 0.9765625, 1.953125, 3.90625, 7.8125, 15.625, and 31.25 µmol/L, Cytotoxicity study: 31.25, 62.5, 125, 250, 500, and 1000 µmol/L)				
Vehicle	0.5% DMSO				
Evaluation items	EC_{50}: Effective concentrations achieving 50% inhibition of CPE induced by influenza virus CC_{50}: Concentrations achieving 50% of cytotoxicity against MDCK cells Selectivity index (SI)				
Evaluation Method	Antivral Study: The subconfluent monolayer MDCK cells in 96-well plates were infected with virus dilution at 50 $TCID_{50}$/well. After 1 hour incubation, the cells were washed with MEM to remove uninfected viruses. One hundred microliter of the viral assay medium and the substance dilutions were added to each well. After 6 days incubation at 37°C in a CO_2 incubator, cell viability was assessed by using WST-8 kit. Cytotoxicity study: Cell culture fluid was removed from the subconfluent monolayer MDCK cells in 96-well plates. One hundred microliter of the viral assay medium and the substance dilutions were added to each well. After 6 days incubation at 37°C in a CO_2 incubator, cell viability was assessed by using WST-8 kit.				
Results					
Strain	S-033447		Ribavirin		Favipiravir
	EC_{50} (nmol/L)	SI[a]	EC_{50} (nmol/L)	SI[a]	EC_{50} (nmol/L)
A/WSN/33 (H1N1)	1.23 ± 0.83	2409.07	83336.77 ± 24987.93	7.13	8731.48 ± 366.66
A/Victoria/3/75 (H3N2)	1.59 ± 1.09	1856.61	64730.41 ± 8546.75	9.18	7473.68 ± 1015.2
B/Maryland/1/59	5.73 ± 3.48	516.35	34642.81 ± 3421.6	17.16	4322.54 ± 482.38
B/Hong Kong/5/72	2.02 ± 1.31	1463.26	23239.97 ± 9205.56	25.58	2885.77 ± 1015.42
Each value represents the mean ± S.D. of 3 independent experiments. a The mean value of CC_{50} (S-033447: 2960.98 ± 1324.78 nmol/L, ribavirin: 594415.82 ± 279626.10 nmol/L) was divided with the mean values of EC_{50}. The SI of favipiravir was not calculated because the CC_{50} of favipiravir could not be calculated.					
Study number [Location in CTD]	S-033188-EB-209-N [4.2.1.2-02]				

2.6.3.3.3 Cytotoxicity Study of S-033447 in Cells from Multiple Human Tissues

Test Article: S-033447

Species/Biological material	BJ human foreskin fibroblasts, HEK-293 human embryonic kidney cells, HepG2 human hepatocellular carcinoma cells, HK2 human renal glomeruli mesangial cells, HUVEC human vascular endothelial cells, Jurkat human T cells leukemia, MRC-5 human lung fibroblasts, SH-SY5Y human neuroblastoma cells, SK-N-SH human neuroblastoma cells	
Evaluation item	The concentration of S-033447 achieving 50% of inhibition cellular ATP amount (IC_{50})	
Concentration (μmol/L)	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 3.125, 6.25, 12.5, 25, 50 and 100	
Reference substance	Positive control: Tamoxifen	
Vehicle	0.3% DMSO	
Evaluation Method	The proliferating or non-proliferating (sub-confluent) cells cultured in cell assay medium were prepared in assay plates. The cultures were performed in duplicate. The assay plates were incubated at 37°C in a CO_2 incubator for 48 hours. The cellular ATP amounts were measured to evaluate the cytotoxicity.	
Results		
Test Item: S-033447		
Cellular condition	Proliferating condition	Non-proliferating condition
Cell lines	IC_{50} (μmol/L)	IC_{50} (μmol/L)
BJ	28	N/A
HEK-293	50	>100
HepG2	44	N/A
HK2	7.9	14
HUVEC	37	N/A
Jurkat	2.2	>3.0
MRC-5	22	48
SH-SY5Y	8.9	19
SK-N-SH	18	N/A
Test Item: Tamoxifen		
Cellular condition	Proliferating condition	Non-proliferating condition
Cell lines	IC_{50} (μmol/L)	IC_{50} (μmol/L)
BJ	13	N/A
HEK-293	14	26
HepG2	16	N/A
HK2	12	12
HUVEC	10	N/A
Jurkat	11	13
MRC-5	12	11
SH-SY5Y	13	17
SK-N-SH	13	N/A
Study Number [Location in CTD]	S-033188-EB-232-N [4.2.1.2-03]	

2.6.3.3.4 Mitochondrial Toxicity of S-033188 and S-033447 with Human Cells

Test Article: S-033188, S-033447

Species/Biological material	HepG2 human hepatocellular carcinoma cells
Evaluation item	The concentration of S-033188 and S-033447 achieving 50% of inhibition cellular ATP amount (IC_{50})
Concentration (μmol/L)	0, 0.390625, 0.78125, 1.5625, 3.125, 6.25, 12.5, 25, 50, 100 and 200
Reference substance	Positive controls: Rotenone, Antimycin, FCCP, Oligomycin, and Chloramphenicol
Vehicle	DMSO
Evaluation Method	The cells were incubated with each test substance for 24 hours or 6 days under the separate conditions, glucose or galactose-containing mediums. The differences in cellular ATP amount were measured to evaluate the mitochondrial toxicity.

Results

Test Article	Exposure time	FBS-containing	IC_{50} (μmol/L)	
			Glucose-containing medium	Galactose-containing medium
S-033188	24 hours	With-FBS	>200	>200
		Without-FBS	>200	>200
	6 days	With-FBS	20.5	16.0
S-033447	24 hours	With-FBS	>200	>200
		Without-FBS	>200	>200
	6 days	With-FBS	11.1	8.79
Rotenone	24 hours	With-FBS	>0.200	0.0531
		Without-FBS	>0.200	0.0117
Antimycin	24 hours	With-FBS	>0.0200	0.0160
		Without-FBS	>0.0200	0.00494
FCCP	24 hours	With-FBS	171	10.9
		Without-FBS	121	5.49
Oligomycin	24 hours	With-FBS	>0.200	0.00818
		Without-FBS	>0.200	0.00845
Chloramphenicol	6 days	With-FBS	606	180

Abbreviations: ATP, adenosine triphosphate; FBS, fetal bovine serum; FCCP, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone.

Study Number [Location in CTD]	S-033188-EF-197-N [4.2.1.2-04]

2.6.3.3.5 In Vitro Selectivity Study of S-033447 Evaluated in Various Receptor, Channel, and Transporter Binding and Enzyme Assays

Test Article: S-033447

Receptors, channels, transporters, and enzymes	Listed in the table of results.
Concentration (μmol/L)	10
Vehicle	DMSO
Positive controls (Concentration)	Listed in the table of Results
Evaluation item	Inhibition effect (% inhibition) on radioligand binding to various receptors, channels, and transporters and on enzymes
Evaluation method for receptor, channel, and transporter	The inhibition ratio of S-033447 or positive control on the binding of tracer to each receptor, channel, or transporter was calculated to assess the binding potency. The assay solution was prepared by diluting the membrane fractions prepared from each clone or tissue with the appropriate assay buffer. Each replacement substance solution was added to tube for calculation of non-specific binding, and 10% DMSO was added to tube for calculation of total binding. The solution of S-033447 or positive control was added to tube for calculation of inhibition ratio. After addition of the assay buffer and tracer solution, the assay solution was added to each tube. The mixture was incubated under the appropriate conditions. The incubated mixture was filtered using a cell harvester or treated using dextran charcoal. The radioactivity of sample was counted using a liquid scintillation counter. In the assay system using ^{125}I tracer, the radioactivity was counted using a gamma counter.
Evaluation method for enzyme	The inhibition ratio of S-033447 or positive control on each enzyme reaction was calculated to assess the inhibitory potency. The enzyme solution was prepared from each tissue or clone. Assay medium or 10% DMSO was added to tube or well for measurement of background value and 100% activity. The solution of S-033447 or positive control was added to the tube or well for calculation of inhibition ratio. The enzyme mixture was incubated under the appropriate conditions for each enzyme and the enzyme activity was measured by the appropriate method.
Calculation of inhibition ratio	Inhibition ratio = [1 - (B - N) / (B0 - N)] × 100 (%). B, Bound radioactivity in the presence of the test substance (individual value). B0, Total bound radioactivity in the absence of the test substance (mean value). N: Non-specific bound radioactivity (mean value).
Results	

Assay Name	S-033447 (1×10^{-5} mol/L)	Positive control	
Adenosine A1 (Human)	8.00	96.82	(DPCPX, 1×10^{-5} mol/L)
Adenosine A2a (Human)	17.27	100.00	(CGS21680, 1×10^{-5} mol/L)
Adenosine A2b (Human)	2.47	100.00	(NECA, 1×10^{-5} mol/L)
α1A-Adrenergic (Human)	0.00	100.00	(HEAT, 1×10^{-5} mol/L)
α1B-Adrenergic (Human)	1.02	99.99	(Prazosin, 1×10^{-5} mol/L)
α2A-Adrenergic (Human)	4.43	97.76	(Rauwolscine, 1×10^{-5} mol/L)
α2B-Adrenergic (Human)	12.21	100.00	(Rauwolscine, 1×10^{-5} mol/L)
α2C-Adrenergic (Human)	0.00	99.24	(Rauwolscine, 1×10^{-5} mol/L)
β1-Adrenergic (Human)	2.19	100.00	((±)-Propranolol, 1×10^{-5} mol/L)
β2-Adrenergic (Human)	2.63	100.00	((±)-Propranolol, 1×10^{-5} mol/L)
β3-Adrenergic (Human)	6.83	98.10	((±)-Propranolol, 1×10^{-5} mol/L)
Benzodiazepine (Bovine)	2.84	100.00	(Diazepam, 1×10^{-5} mol/L)

Assay Name	S-033447 (1×10^{-5} mol/L)	Positive control	
Ca Channel (Type L, Benzothiazepine)	43.27	97.69	((+)-*cis*-Diltiazem, 1×10^{-5} mol/L)
Ca Channel (Type L, Dihydropyridine)	14.36	100.00	(Nitrendipine, 1×10^{-5} mol/L)
Ca Channel (Type L, Phenylalkylamine)	37.97	98.77	((±)-Methoxyverapamil, 1×10^{-5} mol/L)
Ca Channel (Type N)	0.00	100.00	(ω-Conotoxin GVIA, 1×10^{-5} mol/L)
Cannabinoid CB1 (Human)	1.22	99.25	((*R*)-(+)-WIN55212-2, 1×10^{-5} mol/L)
CCK A (Human)	0.00	100.00	(CCK-8, 1×10^{-5} mol/L)
CCK B (Human)	2.51	99.61	(CCK-8, 1×10^{-5} mol/L)
Dopamine D2	12.26	96.39	((+)-Butaclamol, 1×10^{-5} mol/L)
Dopamine D1 (Human)	20.69	100.00	(*R*(+)-SCH-23390, 1×10^{-5} mol/L)
Dopamine D2 Short (Human)	0.00	100.00	((+)-Butaclamol, 1×10^{-5} mol/L)
Dopamine D2 Long (Human)	0.52	99.84	((+)-Butaclamol, 1×10^{-5} mol/L)
Dopamine Transporter (Human)	20.33	100.00	(GBR12909, 1×10^{-5} mol/L)
GABA A (Agonist Site)	0.00	100.00	(Muscimol, 1×10^{-5} mol/L)
GABA A (BZ Central)	0.98	100.00	(Diazepam, 1×10^{-5} mol/L)
GABA A (Chloride Channel)	36.32	99.40	(Picrotoxin, 1×10^{-5} mol/L)
GABA Transporter	5.24	100.00	(GABA, 1×10^{-5} mol/L)
GABA B1a (Human)	0.00	99.76	(CGP54626, 1×10^{-5} mol/L)
GABA B1b (Human)	0.00	98.51	(CGP54626, 1×10^{-5} mol/L)
GABA B1a+R2 (Human)	0.00	92.91	(CGP54626, 1×10^{-5} mol/L)
GABA B1b+R2 (Human)	0.00	97.96	(CGP54626, 1×10^{-5} mol/L)
Glutamate (Non-selective)	3.91	99.04	(L-Glutamic acid, 1×10^{-5} mol/L)
Glutamate (AMPA)	8.04	100.00	((*S*)-AMPA, 1×10^{-5} mol/L)
Glutamate (Kainate)	1.80	100.00	(Kainic acid, 1×10^{-5} mol/L)
Glutamate (NMDA Agonist Site)	3.69	100.00	(L-Glutamic acid, 1×10^{-5} mol/L)
Glutamate (NMDA Glycine Site)	0.00	100.00	(MDL105,519, 1×10^{-5} mol/L)
Glutamate (NMDA Phencyclidine Site)	0.10	100.00	((+)-MK-801, 1×10^{-5} mol/L)
Glutamate (NMDA Polyamine Site)	0.00	99.83	(Ifenprodil, 1×10^{-5} mol/L)
Histamine H1 (Human)	0.00	100.00	(Pyrilamine, 1×10^{-5} mol/L)
Histamine H2 (Human)	6.76	100.00	(Cimetidine, 1×10^{-5} mol/L)
K Channel KA	8.49	100.00	(α-Dendrotoxin, 1×10^{-6} mol/L)
K Channel KATP	5.75	93.56	(Glybenclamide, 1×10^{-5} mol/L)
K Channel Kv	1.21	99.65	(Charybdotoxin, 1×10^{-6} mol/L)
K Channel SKCa	3.57	95.79	(Apamin, 1×10^{-5} mol/L)
K Channel hERG (Human)	0.00	99.71	(Astemizole, 1×10^{-5} mol/L)
Monoamine Transporter	0.00	97.62	(Ketanserin, 1×10^{-5} mol/L)
Muscarinic (Non-selective)	5.87	100.00	(Atropine, 1×10^{-5} mol/L)
Muscarinic M1	1.15	99.12	(Atropine, 1×10^{-5} mol/L)
Muscarinic M2	0.00	100.00	(Atropine, 1×10^{-5} mol/L)

Assay Name	S-033447 (1×10^{-5} mol/L)	Positive control	
Neuropeptide Y1 (Human)	5.46	99.44	(Neuropeptide Y, 1×10^{-6} mol/L)
Neuropeptide Y2 (Human)	2.33	99.52	(Neuropeptide Y, 1×10^{-6} mol/L)
Opiate (Non-selective)	29.33	100.00	(Naloxone, 1×10^{-5} mol/L)
Opiate δ (Human)	14.42	100.00	(Naltriben, 1×10^{-5} mol/L)
Opiate κ (Human)	39.09	99.80	(U-69593, 1×10^{-5} mol/L)
Opiate μ (Human)	2.73	99.95	(DAMGO, 1×10^{-5} mol/L)
Opiate ORL1 (Human)	0.00	90.19	(Nociceptin, 1×10^{-5} mol/L)
Serotonin 5HT1A (Human)	0.00	100.00	(Serotonin, 1×10^{-5} mol/L)
Serotonin 5HT1B (Human)	2.68	100.00	(GR127935, 1×10^{-5} mol/L)
Serotonin 5HT2A (Human)	0.00	100.00	(Ketanserin, 1×10^{-5} mol/L)
Serotonin 5HT2B (Human)	6.09	100.00	(Serotonin, 1×10^{-5} mol/L)
Serotonin 5HT2C (Human)	4.80	99.78	(Mianserin, 1×10^{-5} mol/L)
Serotonin Transporter (Human)	0.00	99.90	(Imipramine, 1×10^{-5} mol/L)
Sigma (Non-selective)	2.83	100.00	(Haloperidol, 1×10^{-5} mol/L)
Sigma σ1	3.66	100.00	((+)-Pentazocine, 1×10^{-5} mol/L)
Vasopressin V1	15.02	99.12	([Arg^8]-Vasopressin, 1×10^{-5} mol/L)
Each value represents the mean values of duplicate samples.			
Study Number [Location in CTD]	R-033188-EF-044-N [4.2.1.2-05]		

2.6.3.4 安全性薬理試験

2.6.3.4.1 中枢神経系

2.6.3.4.1.1 *In Vivo* 試験

Test Article: S-033188

Organ Systems Evaluated	Species/ Strain	Method of Admin. (Vehicle/ Formulation)	Doses (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	GLP Com-pliance	Study Number/ Location in CTD
Central nervous system (modified FOB method)	Rat/ Crl:CD(SD)	Oral gavage (0.5 w/v% MC/Suspension)	S-033188: 200, 600, 2000	6M	No effect on general behavior at up to 2000 mg/kg. 600 mg/kg: Decrease in rectal temperature[a] 2000 mg/kg: Decrease in rectal temperature [a] Slight decrease in urine quantity[b]	Yes	R-033188-SF-034-L/ 4.2.1.3-01

Abbreviations: FOB, functional observational battery; GLP, Good Laboratory Practice.
a At 1 and 2 hours after dosing.
b Between 0 and 1 hours, 1 and 2 hours, and between 0 and 8 hours after dosing

2.6.3.4.2 心血管系

2.6.3.4.2.1 *In Vitro* 試験

Test Article: S-033188, S-033447

Organ Systems Evaluated	Test System	Method of Administration	Concentration (μmol/L)	No. per Group	Noteworthy Findings	GLP Compliance	Study Number/ Location in CTD
Cardiovascular system	Guinea-pig papillary muscle	In vitro	S-033188: 0.1, 0.3, 1	5	No effects on RMP, APA, MRD, APD_{90}, APD_{30} or APD_{30-90})	Yes	R-033188-SF-038-L/ 4.2.1.3-02
			S-033447: 0.082, 0.25, 0.83	5		Yes	R-033188-SF-041-L/ 4.2.1.3-03
Cardiovascular system	hERG transfected CHO cells	In vitro	S-033188: 1, 3, 10	5	>10 μmol/L: Inhibited potassium currents (hERG currents) by 2.18%. The IC_{50} value was estimated to be 10 more than μmol/L (5.72 μg/mL).	Yes	R-033188-SF-039-L/ 4.2.1.3-04
			S-033447: 0.56, 2.06, 8.44	5	>8.44 μmol/L: Inhibited potassium currents (hERG currents) by 26.00%. The IC_{50} value was estimated to be 15.11 μmol/L (7.31 μg/mL).	Yes	R-033188-SF-040-L/ 4.2.1.3-05

Abbreviations: APD_{90}, action potential duration at 90% of repolarization; APD_{30}, action potential duration at 30% of repolarization; APD_{30-90}, difference in action potential duration between 90% and 30% repolarization; RMP, resting membrane potential; APA, action potential amplitude; MRD; maximum rate of depolarization

2.6.3.4.2.2 *In Vivo* 試験

Test Article: S-033188

Organ Systems Evaluated	Species/ Strain	Method of Admin. (Vehicle/ Formulation)	Doses (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	GLP Com-pliance	Study Number/ Location in CTD
Cardio-vascular system (telemetry)	Cynomolgus monkey	Oral gavage (0.5 w/v% MC/Suspension)	S-033188: 200, 400	4M [a]	No effects on blood pressure, heart rate, or electrocardiogram (PR interval, QRS duration, QT interval, and QTc [Bazett]). Arrhythmia was not induced at any dose. Toxicokinetics	Yes	R-033188-SF-035-L / 4.2.1.3-06

Dose (mg/kg)		200	400
C_{max} (μg/mL	S-033447	1.02	1.64
	S-033188	ND [b]	
$AUC_{0\text{-}24hr}$ (μg·hr/mL)	S-033447	16.1	26.2
	S-033188	ND [b]	

Abbreviation: QTc, corrected QT interval.
a The same animals were dosed repeatedly at 7-day intervals using an escalating dose design.
b The all analyzed samples were almost the same as LLOQ (0.00500 μg/mL) or lower.

2.6.3.4.3 呼吸器系

2.6.3.4.3.1 *In Vivo* 試験

Test Article: S-033188

Organ Systems Evaluated	Species/ Strain	Method of Admin. (Vehicle/ Formulation)	Doses (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	GLP Com-pliance	Study Number/ Location in CTD
Respiratory system (WBP method)	Rat/ Crl:CD(SD)	Oral gavage (0.5 w/v% MC/Suspension)	S-033188: 200, 600, 2000	8M	No effects on respiratory rate, tidal volume, or minute volume.	Yes	R-033188-SF-036-L/ 4.2.1.3-07

Abbreviation: WBP, whole body plethysmograph.

2.6.3.5 薬力学的薬物相互作用試験

該当する試験なし.