ゾフルーザ顆粒 2%分包

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

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略号及び用語定義一覧表

略号	英語
EC_{50}	50% effective concentration
LLOQ	lower limit of quantification
NGS	next generation sequencing
PA	polymerase acidic protein
PA/I201T	isoleucine-to-threonine substitution at amino acid position 201 in polymerase acidic protein
PA/I38	isoleucine at amino acid position 38 in polymerase acidic protein
PA/I38F	isoleucine-to-phenylalanine substitution at amino acid position 38 in polymerase acidic protein
PA/I38M	isoleucine-to-methionine 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/I38X	isoleucine-to-amino acid substitution other than isoleucine (phenylalanine or methionine or threonine) at amino acid position 38 in polymerase acidic protein
PA/T20K	threonine-to-lysine substitution at amino acid position 20 in polymerase acidic protein
rg	reverse genetics
RT-PCR	reverse transcription-polymerase chain reaction
SD	standard deviation
$TCID_{50}$	50% tissue culture infectious dose
WT	wild type

2.6.3 薬理試験概要

2.6.3.1 薬理試験：一覧表

Type of Study	Test System	Method of Administration	Testing Facility	Study Number	Location in CTD
Primary Pharmacodynamics					
Competitive Fitness of Reverse Genetics-derived Influenza Viruses with Amino Acid Substitutions in Cultured Cells	MDCK cells [canine kidney-derived cells] infected with influenza A or B virus laboratory strains	In vitro	Shionogi & Co., Ltd.	S-033188-EB-337-N	4.2.1.1-01
Replicative Capacity of Influenza Virus Derived from Patients in S-033188 Clinical Study in Primary Human Nasal Epithelial Cells	MucilAir human nasal epithelial cells infected with influenza A(H3N2) viruses derived from swab samples in Phase 3 study	In vitro	Shionogi & Co., Ltd.	S-033188-EB-345-N	4.2.1.1-02
Replicative Capacity of Influenza Virus Derived from Patients in S-033188 Clinical Study in Primary Human Nasal Epithelial Cells	MucilAir human nasal epithelial cells infected with influenza A(H1N1) and B viruses derived from swab samples in Phase 2 or Phase 3 studies	In vitro	Shionogi & Co., Ltd.	S-033188-EB-361-N	4.2.1.1-03
Competitive Fitness of Influenza Virus Derived from Patients in S-033188 Clinical Study in Primary Human Nasal Epithelial Cells	MucilAir human nasal epithelial cells infected with influenza A(H3N2) viruses derived from swab samples in Phase 3 study	In vitro	Shionogi & Co., Ltd.	S-033188-EB-346-N	4.2.1.1-04
Competitive Fitness of Influenza Virus Derived from Patients in S-033188 Clinical Study in Primary Human Nasal Epithelial Cells	MucilAir human nasal epithelial cells infected with influenza A(H1N1), A(H3N2), and B viruses derived from swab samples in Phase 2 or Phase 3 studies	In vitro	Shionogi & Co., Ltd.	S-033188-EB-362-N	4.2.1.1-05

Replicative Capacity of Reverse Genetics-derived Influenza Viruses with Amino Acid Substitutions in Cultured Cells Effects of PA/I201T Substitution on Replicative Capacity of PA/I38 Substitution Virus	MDCK cells and RPMI2650 cells [human nasal squamous cell carcinoma-derived cells] infected with influenza A virus laboratory strains	In vitro	Shionogi & Co., Ltd.	S-033188-EB-339-N	4.2.1.1-06
Assessment of in vitro Drug Sensitivity of Influenza Viruses containing Mutations in the Polymerase Acidic Protein to Baloxavir Acid	MDCK cells infected with recombinant influenza B viruses	In vitro	[redacted]	[redacted]	4.2.1.1-07

PA = polymerase acidic protein; PA/I201T = isoleucine-to-threonine substitution at amino acid position 201 in polymerase acidic protein;
PA/I38 = isoleucine at amino acid position 38 in polymerase acidic protein

2.6.3.2 効力を裏付ける試験

2.6.3.2.1 PA/I38 変異ウイルスの野生型ウイルス共存下における競合増殖性

Species/Biological material	MDCK cells infected with influenza A virus laboratory strains (rgA/WSN/33 [H1N1], rgA/WSN/33-PA/I38T [H1N1], rgA/WSN/33-PA/I38F [H1N1], rgA/Victoria/3/75 [H3N2], rgA/Victoria/3/75-PA/I38T [H3N2], and rgA/Victoria/3/75-PA/I38M [H3N2]) or influenza B viruses (rgB/Maryland/1/59 and rgB/Maryland/1/59-PA/I38T)
Evaluation items	Sequencing chromatograms at the position 38 in the PA subunit
Evaluation method	Viral infection: The confluent monolayer of MDCK cells in 24-well plates were coinfected with WT virus and the virus harboring I38 substitution at a ratio of 1 to 1 based on viral titers at 200 $TCID_{50}$/well. After 1-hour incubation, the cells were washed and incubated at 37°C in a CO_2 incubator. The culture supernatant of MDCK cells was collected at 48 hours post infection. Fresh MDCK cells were infected with the viruses in culture supernatant from the previous passage. Passage of the viruses in cell culture was repeated three times. Sequence analysis: Each culture supernatant of the infected cells was subjected to RT-PCR for amplification of the PA segment with specific primers. Sanger sequencing of the RT-PCR products was conducted.
Results: For all tested virus pair, the population of PA/I38X was greater than or comparable level to that of WT at Passage 0, whereas the population of PA/I38X decreased to the basal level and WT increased to a dominant population at Passage 3.	
PA/I38F = isoleucine-to-phenylalanine substitution at amino acid position 38 in polymerase acidic protein; PA/I38M = isoleucine-to-methionine 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/I38X = isoleucine-to-amino acid substitution other than isoleucine (phenylalanine or methionine or threonine) at amino acid position 38 in polymerase acidic protein; RT-PCR = reverse transcription-polymerase chain reaction; $TCID_{50}$ = 50% tissue culture infectious dose; WT = wild type, rg = reverse genetics The assay was performed in duplicate.	
Study number [Location in CTD]	S-033188-EB-337-N [4.2.1.1-01]

2.6.3.2.2 臨床で分離された PA/I38 変異ウイルスの増殖性

Biological material	MucilAir human nasal epithelial cells infected with A(H3N2) influenza viruses isolated from the nasopharyngeal/pharyngeal swab samples in phase 3 (T0831) (Strains: T0831_[redacted]_D1[H3N2], T0831_[redacted]_D9-PA/I38T [H3N2], T0831_[redacted]_D1 [H3N2], T0831_[redacted]_D5-PA/I38T [H3N2], T0831_[redacted]_D1 [H3N2], T0831_[redacted]_D5-PA/I38T [H3N2])				
Evaluation items	The virus titers ($TCID_{50}$/mL)				
Evaluation method	Viral infection: The confluent monolayer MucilAir human nasal epithelial cells in 24-well plates were infected with the clinical isolates at 500 $TCID_{50}$/well. After 1-hour incubation, the cells were washed and incubated at 37°C in a CO_2 incubator. The culture fluids were collected at 0, 24, 48 and 72 hours after inoculation and stored until the viral titration was performed. Measurement of the viral titers: The stored culture fluids were diluted and added into MDCK-SIAT1 monolayers on 96-well plates and the cells were absorbed by centrifuging at 1000 rpm for 30 minutes at room temperature. After removal of the inoculum, MDCK-SIAT1 cells were washed and incubated at 33°C in a CO_2 incubator for 3 days. After incubation, virus-induced cytopathic effect was observed, and virus titers were expressed as $\log_{10}$ $TCID_{50}$/mL.				
Results					
Strains	Virus titer ($\log_{10}$ $TCID_{50}$/mL)				
	MucilAir human nasal epithelial cells				
	0 hours	24 hours	48 hours	72 hours	
T0831_[redacted]_D1 (H3N2)(WT)	1.50 ± 0.00	5.33 ± 0.30	8.20 ± 0.00	7.59 ± 0.14	
T0831_[redacted]_D9-PA/I38T (H3N2)	1.50 ± 0.00	5.21 ± 0.79	7.81 ± 0.39	7.81 ± 0.34	
T0831_[redacted]_D1 (H3N2) (WT)	1.50 ± 0.00	4.63 ± 0.05	7.71 ± 0.08	7.60 ± 0.05	
T0831_[redacted]_D5-PA/I38T (H3N2)	1.50 ± 0.00	4.47 ± 0.78	7.71 ± 0.08	7.83 ± 0.44	
T0831_[redacted]_D1 (H3N2) (WT)	1.50 ± 0.00	5.55 ± 0.15	8.14 ± 0.32	7.55 ± 0.04	
T0831_[redacted]_D5-PA/I38T (H3N2)	1.50 ± 0.00	4.41 ± 0.68	7.70 ± 0.29	7.58 ± 0.08	
SD = standard deviation Each value represents the mean ± SD of triplicate.					
Study number [Location in CTD]	S-033188-EB-345-N [4.2.1.1-02]				

2.6.3.2.3　臨床で分離された PA/I38 変異ウイルスの増殖性 (2)

Biological material	MucilAir human nasal epithelial cells infected with A/H1N1pdm09 and type-B influenza viruses isolated from the nasopharyngeal/pharyngeal swab samples in phase 2 (T0821) or phase 3 (T0831) (Strains: T0821_[redacted]_D1 [H1N1pdm], T0821_[redacted]_D6 -PA/I38T [H1N1pdm], T0821_[redacted]_D1 [H1N1pdm], T0821_[redacted]_D9 -PA/I38T [H1N1pdm], T0831_[redacted]_D6-8-6 [B], T0831_[redacted]_D6-9-PA/I38T [B])				
Evaluation items	The virus titers ($TCID_{50}$/mL)				
Evaluation method	Viral infection: The confluent monolayer MucilAir human nasal epithelial cells in 24-well plates were infected with the clinical isolates at 500 $TCID_{50}$/well for influenza A viruses and at 5000 $TCID_{50}$/well for influenza B viruses, respectively. After 1-hour incubation, the cells were washed and incubated at 37°C in a CO_2 incubator. The culture fluids were collected at 0, 24, 48 and 72 hours after inoculation and stored until the viral titration was performed. Measurement of the viral titers: The stored culture fluids were diluted and added into MDCK-SIAT1 monolayers on 96-well plates and the cells were absorbed by centrifuging at 1000 rpm for 30 minutes at room temperature. After removal of the inoculum, MDCK-SIAT1 cells were washed and incubated at 33°C in a CO_2 incubator for 3 days. After incubation, virus-induced cytopathic effect was observed, and virus titers were expressed as log_{10} $TCID_{50}$/mL.				
Results					
Strains	Virus titer (log_{10} $TCID_{50}$/mL)				
	MucilAir human nasal epithelial cells				
	0 hours	24 hours	48 hours	72 hours	
T0821_[redacted]_D1 (H1N1pdm) (WT)	1.50 ± 0.00	4.09 ± 0.64	7.43 ± 0.37	7.09 ± 0.47	
T0821_[redacted]_D6 -PA/I38T (H1N1pdm)	1.50 ± 0.00	3.56 ± 0.10	6.23 ± 0.75	5.63 ± 0.34*	
T0821_[redacted]_D1 (H1N1pdm) (WT)	1.50 ± 0.00	6.03 ± 0.84	7.58 ± 0.08	6.07 ± 0.44	
T0821_[redacted]_D9 -PA/I38T (H1N1pdm)	1.50 ± 0.00	5.67 ± 0.33	6.36 ± 0.41*	6.10 ± 1.39	
T0831_[redacted]_D6-8-6 (B) (WT)	1.50 ± 0.00	5.50 ± 0.17	5.42 ± 0.08	6.20 ± 0.67	
T0831_[redacted]_D6-9-PA/I38T (B)	1.50 ± 0.00	1.50 ± 0.00*	2.29 ± 0.16*	4.66 ± 0.15	
Each value represents the mean ± SD of triplicate. * $p < 0.05$ to WT by Welch's *t*-test					
Study number [Location in CTD]	S-033188-EB-361-N [4.2.1.1-03]				

2.6.3.2.4 臨床で分離された PA/I38 変異ウイルスの野生型ウイルス共存下における競合増殖性

Species/Biological material	MucilAir human nasal epithelial cells infected with A(H3N2) influenza viruses isolated from the nasopharyngeal/pharyngeal swab samples in phase 3 study (T0831) (Strains: T0831_[redacted]_D1 [H3N2], T0831_[redacted]_D9-PA/I38T [H3N2], T0831_[redacted]_D1 [H3N2], T0831_[redacted]_D5-PA/I38T [H3N2], T0831_[redacted]_D1 [H3N2], T0831_[redacted]_D5-PA/I38T [H3N2])
Evaluation items	Proportion of amino acid at position 38 in the PA subunit
Evaluation method	Viral infection: The WT virus and the virus harboring I38T substitution isolated from patients in S-033188 clinical study were premixed at 1 to 1 ratio based on viral titers calculated as $TCID_{50}$/mL. The confluent monolayer of MucilAir human nasal epithelial cells in transwell-24 plates were infected with the premixed viruses at 500 $TCID_{50}$/well each. After 1-hour incubation, the cells were washed and incubated at 37°C in a CO_2 incubator. The culture supernatant of MucilAir human nasal epithelial cells were collected at 48 hours post infection. The collected samples were measured the titers using MDCK-SIAT1 cells. Fresh MucilAir human nasal epithelial cells were infected with the viruses in culture supernatant from the previous passage. Passage of the viruses in cell culture was repeated three times. NGS analysis: Each culture supernatant of the infected cells was subjected to NGS analysis with Illumina MiSeq system.

Results

Strains		Passage 0	Passage 1	Passage 2	Passage 3
T0831_[redacted]_D1 (H3N2) + T0831_[redacted]_D9-PA/I38T (H3N2)	Average of PA/I38T mean ± range (%)	35.9 ± 4.79	18.6 ± 11.12	15.6 ± 16.01	1.4 ± 1.95
	Proportion of PA/I38T (%)	32.54	26.48	26.95	2.76
		39.31	10.76	4.31	-
T0831_[redacted]_D1 (H3N2) + T0831_[redacted]_D5-PA/I38T (H3N2)	Average of PA/I38T mean ± range (%)	40.6 ± 0.04	56.2 ± 10.69	25.5 ± 0.88	1.6 ± 2.31
	Proportion of PA/I38T (%)	40.58	48.68	26.10	3.26
		40.64	63.80	24.86	-
T0831_[redacted]_D1 (H3N2) + T0831_[redacted]_D5-PA/I38T (H3N2)	Average of PA/I38T mean ± range (%)	45.1 ± 0.46	11.5 ± 14.57	6.1 ± 8.59	0.8 ± 1.12
	Proportion of PA/I38T (%)	44.75	1.22	-	-
		45.40	21.82	12.15	1.59

LLOQ = lower limit of quantification, NGS = next generation sequencing
Each value represents the mean ± range of duplicate.
The LLOQ for the proportion of PA/I38T was 1% and " - " indicated less than 1%.
The averages and ranges were calculated with samples below LLOQ set to "0".

Study number [Location in CTD]	S-033188-EB-346-N [4.2.1.1-04]

2.6.3.2.5　臨床で分離された PA/I38 変異ウイルスの野生型ウイルス共存下における競合増殖性 (2)

Species/Biological material	MucilAir human nasal epithelial cells infected with A(H1N1)pdm09, A(H3N2), and type-B influenza viruses isolated from the nasopharyngeal/pharyngeal swab samples in phase 2 (T0821) or phase 3 study (T0831) (Strains: T0821_[redacted]_D1 [H1N1pdm], T0821_[redacted]_D6 -PA/I38T [H1N1pdm], T0821_[redacted]_D1 [H1N1pdm], T0821_[redacted]_D9 -PA/I38T [H1N1pdm], T0831_[redacted]_D1 [H3N2], T0831_[redacted]_D5-PA/I38T [H3N2], T0831_[redacted]_D1 [H3N2], T0831_[redacted]_D5-PA/I38T [H3N2], T0831_[redacted]_D6-8-6 [B], T0831_[redacted]_D6-9-PA/I38T [B])
Evaluation items	Proportion of amino acid at position 38 in the PA subunit
Evaluation method	Viral infection: The WT virus and the virus harboring I38T substitution isolated from patients in S-033188 clinical studies were premixed at 1 to 1 or 2 to 8 ratio based on viral titers calculated as $TCID_{50}$/mL. The confluent monolayer of MucilAir human nasal epithelial cells in transwell-24 plates were infected with the premixed viruses at 1000 $TCID_{50}$/well for influenza A viruses and at 10000 $TCID_{50}$/well for influenza B viruses, respectively. After 1-hour incubation, the cells were washed and incubated at 37°C in a CO_2 incubator. The culture supernatant of MucilAir human nasal epithelial cells were collected at 48 hours post infection. The collected samples were measured the titers using MDCK-SIAT1 cells. Fresh MucilAir human nasal epithelial cells were infected with the viruses in culture supernatant from the previous passage at 500 $TCID_{50}$/well for influenza A viruses and at 5000 $TCID_{50}$/well for influenza B viruses, respectively. Passage of the viruses in cell culture was repeated three times. NGS analysis: Each culture supernatant of the infected cells was subjected to NGS analysis with Illumina MiSeq system.

Results

Strains	Initial ratio (WT: I38T)		Passage 0		Passage 1		Passage 2		Passage 3	
T0821_[redacted]_D1 (H1N1pdm) + T0821_[redacted]_D6 -PA/I38T (H1N1pdm)	1:1	Average of PA/I38T (%)	97.0		43.3		21.2		8.2	
		Proportion of PA/I38T (%)	96.9	97.0	66.3	20.3	35.3	7.2	13.5	3.0
T0821_[redacted]_D1 (H1N1pdm) + T0821_[redacted]_D6 -PA/I38T (H1N1pdm)	2:8	Average of PA/I38T (%)	98.8		68.1		53.8		42.0	
		Proportion of PA/I38T (%)	98.8	98.8	97.0	39.3	94.0	13.6	84.0	-
T0821_[redacted]_D1 (H1N1pdm) + T0821_[redacted]_D9 -PA/I38T (H1N1pdm)	1:1	Average of PA/I38T (%)	98.3		78.2		44.8		23.8	
		Proportion of PA/I38T (%)	98.4	98.3	86.2	70.3	54.7	34.8	41.1	6.5
T0821_[redacted]_D1 (H1N1pdm) + T0821_[redacted]_D9 -PA/I38T (H1N1pdm)	2:8	Average of PA/I38T (%)	99.3		97.2		83.0		78.8	
		Proportion of PA/I38T (%)	99.3	99.3	95.3	99.0	81.6	84.4*	74.1	83.4*
T0831_[redacted]_D1 (H3N2) + T0831_[redacted]_D5-PA/I38T (H3N2)	2:8	Average of PA/I38T (%)	88.4		75.0		63.2		51.4	
		Proportion of PA/I38T (%)	88.2	88.7	66.4	83.6	46.3	80.0	28.7	74.2
T0831_[redacted]_D1 (H3N2) + T0831_[redacted]_D5-PA/I38T (H3N2)	2:8	Average of PA/I38T (%)	76.5		44.4		31.1		21.4	
		Proportion of PA/I38T (%)	75.9	77.0	33.6	55.2	18.4	43.8	11.3	31.4
T0831_[redacted]_D6-8-6 (B) +	1:1	Average of PA/I38T (%)	87.5		76.6		62.2		45.3	

T0831_[redacted]_D6-9-PA/I38T (B)		Proportion of PA/I38T (%)	87.0	87.9	74.1	79.1	59.4	64.9	43.2	47.5
T0831_[redacted]_D6-8-6 (B) + T0831_[redacted]_D6-9-PA/I38T (B)	2:8	Average of PA/I38T (%)	96.4		93.3		87.3		78.4	
		Proportion of PA/I38T (%)	96.6	96.1	93.9	92.7	88.3	86.3	82.9	73.8
NGS = next generation sequencing. Each value represents the mean of duplicate. The lower limit of quantification (LLOQ) for the proportion of PA/I38T was 1% and " - " indicated less than 1%. The averages and ranges were calculated with samples below LLOQ set to "0". * In addition to I38T, a small population of I38A virus was transiently observed at 7.7% at passege2 and 3.0% at passege3 in this lineage.										
Study number [Location in CTD]	S-033188-EB-362-N [4.2.1.1-05]									

2.6.3.2.6 PA/I201T が PA/I38 変異ウイルスの増殖性に与える影響

Species/Biological material	MDCK cells and RPMI2650 cells infected with influenza A virus laboratory strains (rgA/Victoria/3/75 [H3N2], rgA/Victoria/3/75-PA/I38T [H3N2], rgA/Victoria/3/75-PA/I38M [H3N2], rgA/Victoria/3/75-PA/I201T [H3N2], rgA/Victoria/3/75-PA/I38T+I201T [H3N2], rgA/Victoria/3/75-PA/I38M+I201T [H3N2])
Evaluation items	The virus titers ($TCID_{50}$/mL)
Evaluation method	Viral infection: The confluent monolayer cells in 24-well plates were infected with the recombinant viruses at 10 $TCID_{50}$/well for MDCK cells and 100 $TCID_{50}$/well for RPMI2650 cells, respectively. After 1-hour incubation, the cells were washed and incubated at 37°C in a CO_2 incubator. The culture fluids of MDCK cells were collected at 6, 24, 48, and 72 hours, or those of RPMI2650 cells were collected at 6, 24, 48, 72, and 96 hours after inoculation and stored until the viral titration was performed. Measurement of the Viral Titer: The stored culture fluids were 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 cytopathic effect was observed, and virus titers were expressed as $\log_{10}$ $TCID_{50}$/mL.

Results

Strains	Cells	Virus titer ($\log_{10}$ $TCID_{50}$/mL)				
		6 hours	24 hours	48 hours	72 hours	96 hours
rgA/Victoria/3/75 (H3N2)	MDCK	1.50 ± 0.00	5.57 ± 0.21	7.42 ± 0.14	6.76 ± 0.08	N/T
rgA/Victoria/3/75-PA/I38T (H3N2)	MDCK	1.50 ± 0.00	4.51 ± 0.09	7.02 ± 0.24	6.65 ± 0.03	N/T
rgA/Victoria/3/75-PA/I38M (H3N2)	MDCK	1.50 ± 0.00	5.27 ± 0.21	6.81 ± 0.31	6.54 ± 0.37	N/T
rgA/Victoria/3/75-PA/I201T (H3N2)	MDCK	1.52 ± 0.04	5.60 ± 0.17	7.41 ± 0.20	6.85 ± 0.21	N/T
rgA/Victoria/3/75-PA/I38T+I201T (H3N2)	MDCK	1.50 ± 0.00	4.61 ± 0.25	6.64 ± 0.20	6.53 ± 0.10	N/T
rgA/Victoria/3/75-PA/I38M+I201T (H3N2)	MDCK	1.50 ± 0.00	5.01 ± 0.61	6.82 ± 0.17	6.53 ± 0.17	N/T
rgA/Victoria/3/75 (H3N2)	RPMI2650	1.50 ± 0.00	1.58 ± 0.08	3.99 ± 0.29	6.02 ± 0.24	5.97 ± 0.28
rgA/Victoria/3/75-PA/I38T (H3N2)	RPMI2650	1.50 ± 0.00	1.50 ± 0.00	2.33 ± 0.30	4.24 ± 0.58	4.68 ± 0.19
rgA/Victoria/3/75-PA/I38M (H3N2)	RPMI2650	1.50 ± 0.00	1.50 ± 0.00	2.96 ± 0.56	4.13 ± 0.43	4.47 ± 0.55
rgA/Victoria/3/75-PA/I201T (H3N2)	RPMI2650	1.50 ± 0.00	1.56 ± 0.10	3.78 ± 0.28	5.68 ± 0.11	5.89 ± 0.28
rgA/Victoria/3/75-PA/I38T+I201T (H3N2)	RPMI2650	1.50 ± 0.00	1.55 ± 0.04	2.53 ± 0.09	3.70 ± 0.12	4.77 ± 0.12
rgA/Victoria/3/75-PA/I38M+I201T (H3N2)	RPMI2650	1.50 ± 0.00	1.50 ± 0.00	2.97 ± 0.30	4.23 ± 0.62	5.36 ± 0.39

N/T; not tested
Each value represents the mean ± SD of triplicate.

Study number [Location in CTD]	S-033188-EB-339-N [4.2.1.1-06]

2.6.3.2.7　臨床試験で認められたポリメラーゼタンパク上の変異のバロキサビル マルボキシルに対する感受性評価

Test Article: S-033447

Species/Biological material		MDCK cells infected with influenza B virus generated by reverse genetics. (Strains: rgB/Washington/02/2019 [B], rgB/Washington/02/2019-PA/T20K [B])	
Final concentration (nmol/L)		S-033447 (0.037, 0.15, 0.59, 2.3, 9.4, 37.5, 150, and 600 nmol/L)	
Reference substance (Final concentration)		Favipiravir (229, 686, 2058, 6173, 18159, 55556, 166667, and 500000 nmol/L)	
Vehicle		0.5% DMSO	
Evaluation item		EC_{50}: Effective concentration achieving 50% reduction of influenza virus nucleoprotein-expressing infected cells	
Evaluation Method		The MDCK cells in assay plates were infected with virus dilution at 500 Dot Forming Unit (DFU)/well. Immediately after addition of the virus, add test article or reference article at 34°C in a CO_2 incubator for 20 to 24 hours. Following incubation, the culture medium was removed, and then the cells were fixed, stained, and scanned. The EC_{50} value, which was presented as EC_{50} in in inhibition dose-response curve fitting by the 4-parameter logistic regression model, was determined using GraphPad Prism 9.1.2.	
Results			
Type/subtype	Strains	EC_{50} (nmol/L) (Fold Change)	
		S-033447	Favipiravir
B	rgB/Washington/02/2019	3.48 ± 0.24	62010 ± 9270
B	rgB/Washington/02/2019-PA/T20K	23.92 ± 2.94 (6.88 ± 0.90)	47140 ± 12080 (0.75 ± 0.08)
EC_{50} = 50% effective concentration; PA/T20K = threonine-to-lysine substitution at amino acid position 20 in polymerase acidic protein, Each value represents the mean ± SD of 3 independent experiments.			
Study Number [Location in CTD]		[redacted] [4.2.1.1-07]	

2.6.3.3 副次的薬理試験

本申請に際して新たな試験は実施していない.

2.6.3.4 安全性薬理試験

本申請に際して新たな試験は実施していない.

2.6.3.5 薬力学的薬物相互作用

本申請に際して新たな試験は実施していない.