Report on the Deliberation Results

February 8, 2018

Pharmaceutical Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau
Ministry of Health, Labour and Welfare

Brand Name	Xofluza Tablets 10 mg
	Xofluza Tablets 20 mg
Non-proprietary Name	Baloxavir Marboxil (JAN*)
Applicant	Shionogi & Co., Ltd.
Date of Application	October 25, 2017

Results of Deliberation

In its meeting held on February 2, 2018, the Second Committee on New Drugs concluded that the product may be approved and that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The product is not classified as a biological product or a specified biological product. The re-examination period is 8 years. Neither the drug product nor its drug substance is classified as a poisonous drug or a powerful drug.

Condition of Approval

The applicant is required to develop and appropriately implement a risk management plan.

**Japanese Accepted Name (modified INN)*

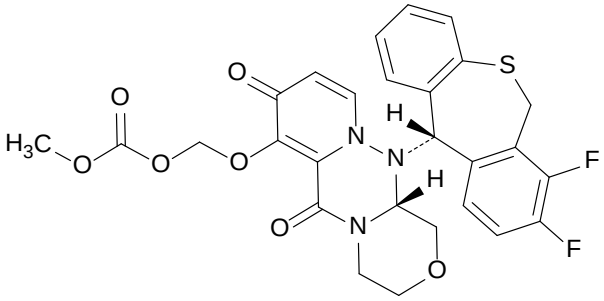
Review Report

January 17, 2018
Pharmaceuticals and Medical Devices Agency

The following are the results of the review of the following pharmaceutical product submitted for marketing approval conducted by the Pharmaceuticals and Medical Devices Agency (PMDA).

Brand Name	Xofluza Tablets 10 mg Xofluza Tablets 20 mg
Non-proprietary Name	Baloxavir Marboxil
Applicant	Shionogi & Co., Ltd.
Date of Application	October 25, 2017
Dosage Form/Strength	Scored uncoated tablets: Each tablet contains 10 mg of Baloxavir Marboxil. Film-coated tablets: Each tablet contains 20 mg of Baloxavir Marboxil.
Application Classification	Prescription drug, (1) Drug with a new active ingredient

Chemical structure



Molecular formula: $C_{27}H_{23}F_2N_3O_7S$
Molecular weight: 571.55
Chemical name:
({(12a*R*)-12-[(11*S*)-7,8-Difluoro-6,11-dihydrodibenzo[*b*,*e*]thiepin-11-yl]-6,8-dioxo-3,4,6,8,12,12a-hexahydro-1*H*-[1,4]oxazino[3,4-*c*]pyrido[2,1-*f*][1,2,4]triazin-7-yl}oxy)methyl methyl carbonate

Items Warranting Special Mention:

SAKIGAKE designation drug (SAKIGAKE Drug Designation No. 3 of 2015 [*27 yaku*], PSEHB/ELD Notification No. 1027-1 dated October 27, 2015, by the Evaluation and Licensing Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare [MHLW]). SAKIGAKE comprehensive evaluation consultation for drugs was conducted for the product.

Reviewing office Office of New Drug IV

Results of Review

On the basis of the data submitted, PMDA has concluded that the product has efficacy in the treatment of patients with influenza A or B virus infection, and that the product has acceptable safety in view of its benefits (see Attachment).

As a result of its review, PMDA has concluded that the product may be approved for the indication and dosage and administration shown below, with the following condition.

Indication

Influenza A or B virus infection

Dosage and Administration

1. The usual dosage for adults and adolescents aged ≥12 years is two 20-mg tablets (40 mg of baloxavir marboxil) administered orally as a single dose. In patients weighing ≥80 kg, four 20-mg tablets (80 mg of baloxavir marboxil) are administered orally as a single dose.

2. The usual dosage for children aged <12 years is the following, administered orally as a single dose.

Body weight	Dose
≥40 kg	Two 20-mg tablets (40 mg of baloxavir marboxil)
≥20 kg and <40 kg	One 20-mg tablet (20 mg of baloxavir marboxil)
≥10 kg and <20 kg	One 10-mg tablet (10 mg of baloxavir marboxil)

Condition of Approval

The applicant is required to develop and appropriately implement a risk management plan.

Attachment

Review Report (1)

December 13, 2017

The following is an outline of the data submitted by the applicant and content of the review conducted by the Pharmaceuticals and Medical Devices Agency.

Product Submitted for Approval

Brand Name	S-033188 Tablets 10 mg (at the time of marketing application) S-033188 Tablets 20 mg (at the time of marketing application)
Non-proprietary Name	Baloxavir Marboxil
Applicant	Shionogi & Co., Ltd.
Date of Application	October 25, 2017
Dosage Form/Strength	Scored uncoated tablets: Each tablet contains 10 mg of Baloxavir Marboxil. Film-coated tablets: Each tablet contains 20 mg of Baloxavir Marboxil.
Proposed Indication	Influenza A or B virus infection

Proposed Dosage and Administration

1. Adults and adolescents aged ≥12 years:
   The usual dosage is 40 mg of baloxavir marboxil administered orally as a single dose. In patients weighing ≥80 kg, 80 mg of baloxavir marboxil is administered orally as a single dose.
2. Children aged <12 years:
   The usual dosage of baloxavir marboxil is the following, administered orally as a single dose.

Body weight	Dose
≥40 kg	40 mg
≥20 kg and <40 kg	20 mg
≥10 kg and <20 kg	10 mg
≥5 kg and <10 kg	5 mg

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List of Abbreviations

See Appendix.

1. Origin or History of Discovery, Use in Foreign Countries, and Other Information

Baloxavir marboxil (hereinafter referred to as baloxavir, Figure 1) is an influenza antiviral drug discovered by Shionogi & Co., Ltd. It is rapidly hydrolyzed to the active form S-033447 (Figure 2) by arylacetamide deacetylase in the small intestine, blood, liver, etc. S-033447 inhibits the activity of endonuclease, which cleaves the cap structure of host pre-mRNA in cells infected with influenza virus (RNA virus). This results in the inhibition of transcription of influenza virus RNA in the infected cells, leading to suppression of viral replication in host cells.

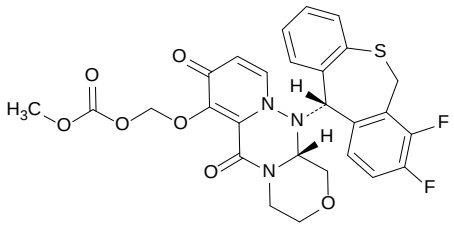

Figure 1. Structural formula for baloxavir marboxil

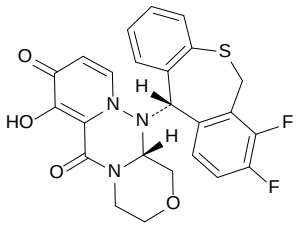

Figure 2. Structural formula for S-033447

Influenza A or B virus infection is an acute respiratory tract infection spread by airborne droplets. After a latent period of 1 to 4 days after infection, patients experience systemic symptoms such as pyrexia, chills, headache, myalgia, and inappetence. In recent years in Japan, an estimated 15 million people are infected with influenza virus each year, with about half of them being ≤14 years old (http://www.nih.go.jp/niid/images/idsc/disease/influ/fludoco1516.pdf [the most recent date of survey, December 2017]).

Currently in Japan, antiviral drugs approved for influenza virus infection are as follows: (1) Drugs that inhibit neuraminidase (NA) on the surface of influenza virus (oseltamivir phosphate, zanamivir hydrate, peramivir hydrate, and laninamivir octanoate hydrate), (2) a drug that inhibits M2 proton channel on the surface of influenza virus (amantadine hydrochloride), and (3) a drug that inhibits RNA-dependent RNA polymerase (RdRp) (favipiravir, a prodrug metabolized to favipiravir ribosyl triphosphate). However, amantadine hydrochloride is not approved for influenza B virus infection and, in Japan, its clinical use for influenza infection is limited in recent years. Favipiravir is indicated only for “novel or re-emerging pandemic influenza virus infection (limited to cases in which other influenza antiviral drugs are ineffective or not sufficiently effective)” because of the risk of teratogenicity. In addition, the condition for approval stipulates that “the applicant must not manufacture the product without the request of the Minister of Health, Labour and Welfare.” Other existing influenza antiviral drugs commonly used in clinical practice in Japan and other countries have similar mechanisms of action, posing a possible risk of spreading viruses cross-resistant to neuraminidase inhibitors in human society.

Baloxavir has a pharmacological action not shared by existing influenza antiviral drugs. The applicant has filed a marketing application for baloxavir, based on the results of clinical studies in patients with influenza virus infection (Studies T0821, T0831, and T0822), with the claim that baloxavir has been demonstrated to be effective and safe in patients with influenza A or B virus infection.

During the application review process, the brand name of baloxavir was changed from "S-033188 Tablets 10 mg, S-033188 Tablets 20 mg" to "Xofluza Tablets 10 mg, Xofluza Tablets 20 mg."

2. Data Relating to Quality and Outline of the Review Conducted by PMDA

2.1 Drug substance

2.1.1 Characterization

The drug substance is a white to light yellowish white powder. Its general properties (description, solubility, hygroscopicity, thermal analysis, pH, acid dissociation constant, partition coefficient, optical rotation, stereoisomers, and crystalline polymorphism) have been determined. Although the drug substance exists in 3 crystalline forms, it is manufactured only in one crystalline form in the commercial production, and the crystalline form has been demonstrated to be stable when stored at room temperature.

The drug substance has 2 asymmetric centers, and its chemical structure has been elucidated by elemental analysis, ultraviolet-visible spectrophotometry, infrared spectrophotometry, nuclear magnetic resonance spectrometry (^{1}H NMR and ^{13}C NMR), mass spectrometry, and X-ray crystallography.

2.1.2 Manufacturing process

The drug substance is synthesized using [redacted], [redacted], and [redacted] as the starting materials.

The quality control strategy was developed based on the following studies:

- Identification of [redacted] as critical quality attributes
- Identification of critical step parameters based on the quality risk assessment

The critical steps include the synthesis of intermediates [redacted],[1)] [redacted],[2)] and [redacted],[3)] and the synthesis and [redacted] of [redacted]. The critical intermediates are [redacted], [redacted], [redacted], and [redacted]. Process control parameters and control values are defined for each critical intermediate.

2.1.3 Control of drug substance

The proposed specifications for the drug substance include content, description, identification (ultraviolet-visible spectrophotometry and infrared spectrophotometry), purity (related substances [high-performance liquid chromatography], [redacted] [high-performance liquid chromatography], residual solvents [gas chromatography]), water content, residue on ignition, [redacted], and assay (high-performance liquid chromatography).

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1) [redacted]

2) [redacted]

3) [redacted]

2.1.4 Stability of drug substance

Table 1 shows the main stability studies for the drug substance. The photostability testing showed that the drug substance is photolabile.

Table 1. Stability studies for drug substance

Study	Primary batch	Temperature	Humidity	Storage form	Storage period
Long-term	3 pilot batches	30°C	65% RH	Low-density polyethylene bag (double-layered)	12 months
Accelerated	3 pilot batches	40°C	75% RH		6 months

Based on the above, a retest period of [redacted] months has been proposed for the drug substance when stored at room temperature protected from light in a double-layered polyethylene bag placed in a metallic can or a fiber drum, according to "Evaluation of Stability Data" (PFSB/ELD Notification No. 0603004 dated June 3, 2003, by the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW). The long-term testing of the drug substance will be continued for [redacted] months.

2.2 Drug product

2.2.1 Description and composition of drug product and formulation development

The drug product is scored uncoated tablets containing 10 mg of the drug substance per tablet and film-coated tablets containing 20 mg of the drug substance per tablet. Both 10- and 20-mg tablets contain excipients: lactose hydrate, croscarmellose sodium, povidone, microcrystalline cellulose, and sodium stearyl fumarate. The 20-mg tablets contain, in addition to the above, hypromellose, titanium oxide, and talc.

2.2.2 Manufacturing process

The drug product is manufactured through a process comprised of [redacted], tableting, [redacted], coating (20-mg tablets only), filling, labeling, packaging, testing, and storage. Among them, [redacted] were defined as critical steps. Process control parameters and control values were defined for [redacted].

Based on the following studies, the quality control strategy was constructed developed:

- Identification of [redacted] as critical quality attributes
- Identification of critical step parameters based on the quality risk assessment and on the assessment of the effect on the critical quality attributes

2.2.3 Control of drug product

The proposed specifications for the drug product include content, description, identification (high-performance liquid chromatography/ultraviolet-visible spectrophotometry), purity (related substances [high-performance liquid chromatography]), [redacted], uniformity of dosage units (content uniformity [high-performance liquid chromatography]), [redacted], dissolution (ultraviolet-visible spectrophotometry), and assay (high-performance liquid chromatography).

2.2.4 Stability of drug product

Table 2 shows the main stability studies for the drug product. The photostability testing showed that 10-mg tablets are photolabile while 20-mg tablets are photostable.

Table 2. Stability studies of drug product

Study	Primary batch	Temperature	Humidity	Storage form	Storage period
Long-term	10-mg tablets: 3 pilot batches	25°C	60% RH	Blister pack ([redacted]) with paper box packaging	12 months
	20-mg tablets: 3 commercial batches				
Accelerated	10-mg tablets: 3 pilot batches	40°C	75% RH		6 months
	20-mg tablets: 3 commercial batches				

Based on the above, a shelf-life of 24 months has been proposed for the drug product when stored at room temperature in a blister pack ([redacted]) with paper box packaging, according to "Evaluation of Stability Data" (PFSB/ELD Notification No. 0603004 dated June 3, 2003). The 10-mg tablets are to be stored protected from light. The long-term testing of the drug product will be continued for [redacted] months.

2.R Outline of the review conducted by PMDA

Based on the submitted data and on the results of the following reviews, PMDA has concluded that the quality of the drug substance and the drug product is controlled in an appropriate manner.

2.R.1 Quality of divided 10-mg tablets

The applicant's explanation about the quality of the scored 10-mg tablets after being divided into 2 halves:

The 10-mg tablets were cut into 2 halves and subjected to evaluation of uniformity of dosage units, dissolution, and stability. Thus, 5 each of 10-mg tablets from 3 product batches were cut, and the resultant 10 half-tablets were evaluated for uniformity of dosage units (content uniformity). Results showed the acceptance value of [redacted]% under uniformity of dosage units, demonstrating an excellent uniformity even after division. In order to compare dissolution before and after division, 2 half-tablets and 1 undivided 10-mg tablet, obtained from each of 3 batches of 10-mg tablets, were subjected to dissolution test. The dissolution rate [redacted] was [redacted]% for 2 half-tablets and [redacted]% for 1 undivided 10-mg tablet, showing similar dissolution behavior. In order to test the stability after division, divided tablets were stored for [redacted] months [redacted] at 25°C/60% RH protected from light. As a result, a slight [redacted] was observed, but the description, related substances in the purity test, dissolution, and content remained unchanged from initial data.

Based on the above, the applicant considers that the quality of 10-mg tablets is ensured even after division.

PMDA concluded that there are no particular problems in the explanation of the applicant.

3. Non-clinical Pharmacology and Outline of the Review Conducted by PMDA

The pharmacological action of baloxavir was investigated in primary pharmacodynamic studies, secondary pharmacodynamic studies, and safety pharmacology studies. In Section 3.1.4, 0.5% (w/v) aqueous methylcellulose solution was used as vehicle, unless otherwise specified.

3.1 Primary pharmacodynamics

3.1.1 Mechanism of action

3.1.1.1 Inhibitory effect of S-033447 against cap-dependent endonuclease activity of influenza virus (CTD 4.2.1.1-01)

Ribonucleoprotein complex extracted from influenza A (4 strains) and B (3 strains) viruses, was used to investigate the inhibitory effect of S-033447 and suramin sodium salt[4)] against cap-dependent endonuclease activity of influenza A and B viruses. The product of cap-dependent endonuclease activity (amount of RNA fragments) was used as an index of the inhibitory effect. Table 3 shows the results.

Table 3. Inhibitory effect of S-033447 and suramin sodium salt against cap-dependent endonuclease activity derived from influenza A and B viruses

Type/subtype	Strain	IC_{50} nmol/L	
		S-033447	Suramin sodium salt
A/H1N1	A/WSN/33	1.4	22.0×10^3
A/H1N1	A/PR/8/34	2.7	70.0×10^3
A/H3N2	A/Victoria/3/75	2.3	96.0×10^3
A/H3N2	A/Hong Kong/8/68	3.1	150×10^3
B	B/Maryland/1/59	8.9	37.0×10^3
B	B/Hong Kong/5/72	5.1	34.0×10^3
B	B/Lee/40	4.5	33.0×10^3

Mean

3.1.1.2 Inhibitory effect of baloxavir and S-033447 against transcription of genes of influenza A virus (CTD 4.2.1.1-02)

Recombinant RNA polymerase complex derived from an influenza A virus strain (A/WSN/33), was used to investigate the inhibitory effect of S-033447, baloxavir, and suramin sodium salt[4)] against transcription of genes of influenza virus. This study used 3 test systems to measure the following activities of RNA polymerase complex: (1) cap-dependent endonuclease activity, (2) RdRp activity, and (3) cap-dependent endonuclease/RdRp activity wherein cap-dependent endonuclease and RdRp react sequentially. The 50% inhibitory concentration (IC_{50}) of S-033447 against cap-dependent endonuclease activity, RdRp activity, and cap-dependent endonuclease/RdRp activity was 2.5, >40, and 1.6 nmol/L, respectively, whereas IC_{50} of baloxavir was 530, $>5.0 \times 10^3$, and 340 nmol/L, respectively. IC_{50} of suramin sodium salt was 11×10^3, 5.7×10^3, and 7.4×10^3 nmol/L, respectively.

The applicant's explanation based on the above results:

The inhibitory activity of baloxavir against cap-dependent endonuclease is lower than that of S-033447. This indicates that baloxavir inhibits transcription of influenza virus genes through inhibition of cap-dependent endonuclease activity by S-033447, the hydrolytic product of baloxavir.

4) A compound known to nonspecifically inhibit enzymes derived from various viruses (*PLoS Pathog* 2013;8:e58010, *Proc Natl Acad Sci USA* 1996;93:9742-7)

3.1.1.3 Inhibitory effect of S-033447 against transcription of viral genes in cells infected with influenza A virus (CTD 4.2.1.1-23)

Madin Darby canine kidney (MDCK) cells infected with influenza A virus (A/WSN/33) were cultured in the presence of S-033447 or favipiravir. The inhibitory effect of the agents against gene transcription was investigated by measuring intracellular viral mRNA levels at 6 hours after infection. The 90% effective concentration (EC_{90}) (the concentration required to reduce mRNA levels to one-tenth of those in cells cultured in the absence of test drug) was 5.27×10^3 nmol/L for S-033447 and 332×10^3 nmol/L for favipiravir. The effect of human serum on the S-033447- or favipiravir-induced inhibition of viral gene transcription was investigated. Viral mRNA levels did not change in proportion to human serum concentration, showing that human serum had little effect on the inhibitory effect of S-033447 or favipiravir.

3.1.2 *In vitro* antiviral activity

3.1.2.1 *In vitro* antiviral activity of S-033447 against influenza virus

3.1.2.1.1 Suppressive effect of S-033447 against release of influenza A and B viruses (CTD 4.2.1.1-03, 4.2.1.1-04)

MDCK cells infected with influenza A or B virus (laboratory strains and clinical isolates) were cultured in the presence of each of test drugs, and culture supernatants were collected after 24 hours (influenza A) or 30 hours (influenza B). The supernatants were added to MDCK cells and viral titer (50% tissue culture infectious dose [$TCID_{50}$]) was measured based on the cytopathic effect after 3 days of culture. EC_{90} was defined as the drug concentration required to decrease viral titer to one-tenth of that in the supernatants from cultures not containing any test drug. Table 4 shows the results.

Table 4. Suppressive effect of each test drug against release of influenza A and B viruses

Type/subtype	Strain	EC_{90} (nmol/L)				
		S-033447	Favipiravir	Active form of oseltamivir	Zanamivir hydrate	Laninamivir
Laboratory strains						
A/H1N1	A/WSN/33	0.79	3.78×10^3	161	233	17.7
A/H1N1	A/WSN/33-NA/H274Y[a)]	0.46	3.14×10^3	>400	198	19.1
A/H1N1	A/PR/8/34	0.79	3.94×10^3	181	170	9.03
A/H3N2	A/Victoria/3/75	0.98	4.81×10^3	64.6	202	45.0
A/H3N2	A/Hong Kong/8/68	0.64	2.94×10^3	44.2	98.1	23.2
B	B/Maryland/1/59	3.08	2.71×10^3	246	59.6	20.9
B	B/Hong Kong/5/72	2.21	1.80×10^3	538	178	40.5
B	B/Lee/40	3.40	3.73×10^3	372	249	46.0
Clinical isolates (isolated in Japan between 2006 and 2013)						
A/H1N1	A/Kadoma/3/2006	0.88	3.42×10^3	-	-	-
A/H1N1	A/Osaka/129/2009	0.86	4.18×10^3	-	-	-
A/H1N1	A/Osaka/180/2009[a)]	0.95	3.95×10^3	-	-	-
A/H3N2	A/Hokkaido/12H048/2013	0.63	3.34×10^3	-	-	-
A/H3N2	A/Niigata/12F392/2013	0.87	1.90×10^3	-	-	-
B	B/Hokkaido/11H011/2012	6.48	1.74×10^3	-	-	-
B	B/Gunma/12G045/2013	6.10	2.59×10^3	-	-	-

Mean

-, Not tested.

a) Strain with NA/H274Y, an amino acid substitution conferring resistance to NA inhibitors

3.1.2.1.2 Inhibitory effect of S-033447 against plaque formation by influenza A and B viruses (CTD 4.2.1.1-05, 4.2.1.1-06, 4.2.1.1-07, 5.3.5.2-01)

MDCK cells infected with influenza A or B virus (laboratory strains, clinical isolates, and vaccine strains) were cultured in the presence of S-033447 or favipiravir. The suppressive effect of the agents against viral replication was investigated by measuring the number of plaques formed after 3 days of culture. The 50% effective concentration (EC_{50}) was defined as the drug concentration required to reduce the number of plaques by 50% compared with that in the absence of any test drug. Table 5 shows the results.

Table 5. Inhibitory effect of S-033447 and favipiravir against plaque formation by influenza A and B viruses

Type/subtype	Strain	EC_{50} (nmol/L)	
		S-033447	Favipiravir
Laboratory strains			
A/H1N1	A/WSN/33	0.76	20.2×10^3
A/H1N1	A/WSN/33-NA/H274Y	0.49	19.7×10^3
A/H3N2	A/Victoria/3/75	0.76	10.6×10^3
A/H3N2	A/Hong Kong/8/68	0.35	5.06×10^3
B	B/Maryland/1/59	4.85	21.1×10^3
B	B/Hong Kong/5/72	4.33	15.8×10^3
Clinical isolates (isolated in Japan between 2006 and 2014)			
A/H1N1	A/Kadoma/3/2006	0.94	15.9×10^3
A/H1N1	A/Osaka/129/2009	0.26	6.00×10^3
A/H1N1	A/Osaka/180/2009[a)]	0.48	7.78×10^3
A/H1N1	A/Nagasaki/10N073/2011	0.20	7.11×10^3
A/H1N1	A/Kyoto/10K124/2011[a)]	0.35	7.52×10^3
A/H1N1	A/Kyoto/10K118/2011	0.80	9.29×10^3
A/H1N1	A/Hokkaido/13H020/2014	0.99	5.70×10^3
A/H1N1	A/Nagasaki/13N019/2014	0.52	5.21×10^3
A/H1N1	A/Nagasaki/13N059/2014[a)]	0.66	5.53×10^3
A/H1N1	A/Hokkaido/07H002/2008	1.55	12.4×10^3
A/H1N1	A/Nagasaki/07N020/2008[a)]	0.73	9.79×10^3
A/H3N2	A/Hyogo/10K051/2011	0.66	8.58×10^3
A/H3N2	A/Niigata/10F017/2011	0.43	10.9×10^3
A/H3N2	A/Niigata/11F027/2012	0.90	10.0×10^3
A/H3N2	A/Tokyo/11IM003/2012	0.49	8.61×10^3
A/H3N2	A/Hokkaido/12H048/2013	0.56	20.1×10^3
A/H3N2	A/Niigata/12F392/2013	0.68	11.0×10^3
A/H3N2	A/Kyoto/13SK042/2014	0.49	9.93×10^3
A/H3N2	A/Nagasaki/13N033/2014	0.42	6.25×10^3
A/H3N2	A/Niigata/05F067/2006	0.38	8.47×10^3
A/H3N2	A/Nagasaki/05N007/2006	0.80	5.60×10^3
A/H3N2	A/Kyoto/06K110/2007	0.55	7.40×10^3
B	B/Kyoto/10K131/2011	4.01	5.39×10^3
B	B/Hokkaido/11H011/2012	5.28	7.96×10^3
B	B/Gunma/12G045/2013	5.04	6.55×10^3
B	B/Gunma/13G004/2014	11.3	7.21×10^3
B	B/Niigata/06F075/2007	4.72	9.12×10^3
B	B/Gunma/06G040/2007	5.97	8.70×10^3
B	B/Kyoto/08K015/2009	5.04	5.06×10^3
B	B/Kyoto/11K272/2012	4.39	3.84×10^3
B	B/Nagasaki/13N013/2013	4.03	7.86×10^3
B	B/Niigata/13F044/2014	3.33	2.38×10^3
B	B/Kyoto/13K042/2014	5.96	8.53×10^3
Vaccine strains			
A/H1N1	A/Brisbane/59/2007	1.85	11.8×10^3
A/H1N1	A/California/7/2009	1.18	12.4×10^3
A/H3N2	A/Victoria/361/2011	1.87	11.4×10^3
A/H3N2	A/New York/39/2012	0.74	11.0×10^3
A/H3N2	A/Texas/50/2012	1.00	12.9×10^3

Type/subtype	Strain	EC_{50} (nmol/L)	
		S-033447	Favipiravir
A/H3N2	A/Switzerland/9715293/2013	1.04	10.4×10^3
B	B/Phuket/3073/2013	9.24	13.9×10^3
B	B/Malaysia/2506/2004	12.3	13.6×10^3
B	B/Brisbane/60/2008	10.6	9.60×10^3
B	B/Wisconsin/1/2010	13.0	9.45×10^3
B	B/Massachusetts/2/2012	9.53	9.40×10^3
B	B/Texas/2/2013	11.9	8.82×10^3

Mean

a) Strain with NA/H274Y, a substitution conferring resistance to NA inhibitors

3.1.2.1.3 Antiviral activity of S-033447 against clinical isolates of influenza virus in Japanese clinical studies (Studies T0821 and T0822) and global phase III study (Study T0831) (CTD 5.3.5.2-01, 5.3.5.4-05, 5.3.5.4-06, 5.3.5.4-17)

In a Japanese phase II study in adult patients (Study T0821), clinical virus strains isolated at baseline were replicated in MDCK-SIAT1 cells, to investigate antiviral activity of S-033447 against these baseline clinical isolates by plaque assay. Median EC_{50} against each type/subtype of virus isolated in Study T0821 was 1.40 nmol/L for subtype A/H1N1pdm, 1.05 nmol/L for subtype A/H3N2, and 6.90 nmol/L for type B. The median ratio of EC_{50} (the baseline clinical isolates/control virus strains) was 2.13 for subtype A/H1N1pdm, 1.32 for subtype A/H3N2, and 2.33 for type B (control virus strains: type A, A/Victoria/361/2011[H3N2]; type B, B/Wisconsin/1/2010). Among the samples with an EC_{50} ratio of >10 (the baseline clinical isolates/control virus strains), 2 samples with subtype A/H1N1pdm showed characteristic amino acid substitutions (A36V and I545T). The effect of these amino acid substitutions on the sensitivity to S-033447 was investigated using recombinant virus strains with amino acid substitution (A36V and I545T) produced by reverse genetics. The ratio of EC_{50} (the recombinant virus strains/control virus strains) was 3.59 for A36V and 0.73 for I545T (control virus: subtype rgA/WSN/33 [A/H1N1pdm]).

In a Japanese study in patients aged <12 years (Study T0822), clinical virus strains isolated at baseline were replicated in MDCK cells to investigate antiviral activity of S-033447 against these clinical isolates by [redacted] assay. Median EC_{50} against each type/subtype of virus was 17.96 nmol/L for subtype A/H1N1pdm, 4.48 nmol/L for subtype A/H3N2, and 18.67 nmol/L for type B. The median ratio of EC_{50} (the baseline clinical isolates/control virus strains) was 3.2 for subtype A/H1N1pdm, 0.9 for subtype A/H3N2, and 1.2 for type B (control virus strains: type A, A/Victoria/361/2011 [H3N2]; type B, B/Wisconsin/1/2010). There were no samples with an EC_{50} ratio of >10 (the baseline clinical isolates/control virus strains).

In a global phase III study in patients aged ≥12 years (Study T0831), clinical virus strains isolated at baseline were replicated in MDCK cells to investigate antiviral activity of S-033447 against these clinical isolates by [redacted] assay. Median EC_{50} against each type/subtype of the virus strains was 13.8 nmol/L for subtype A/H1N1pdm, 4.94 nmol/L for subtype A/H3N2, and 52.6 nmol/L for type B. The median ratio of EC_{50} (the baseline clinical isolates/control virus strains) was 2.4 for subtype A/H1N1pdm, 0.9 for subtype A/H3N2, and 1.2 for type B (control virus strains: type A, A/Victoria/361/2011 [H3N2]; type B, B/Wisconsin/1/2010). A sample obtained from 1 patient had an EC_{50} ratio of >10 (the baseline clinical isolates/control virus strains).

3.1.2.1.4 Effect of combination of S-033447 with NA inhibitor (CTD 4.2.1.1-08)

Using MDCK cells infected with influenza A virus strain (A/PR/8/34), the effect of combination of S-033447 with NA inhibitor was investigated by measuring the viral replication-induced cytopathic effect. Results are shown in Table 6. The applicant explained that S-033447 exerts a synergistic effect in suppressing viral replication when used in combination with NA inhibitor.

Table 6. Effect of combination of S-033447 with NA inhibitor

Type/subtype	Strain	Test drug	CI (combination index)[a)]	Combined effect[b)]
A/H1N1	A/PR/8/34	S-033447 + active form of oseltamivir	0.49	Synergistic effect
		S-033447 + zanamivir hydrate	0.52	Synergistic effect
		S-033447 + laninamivir	0.58	Synergistic effect
		S-033447 + peramivir hydrate	0.59	Synergistic effect

a) Calculated based on the inhibitory effect obtained by two drugs mixed at a ratio close to EC_{50} for each drug to allow equal contribution of both drugs to suppression of viral replication, according to the Chou-Talalay method (*Adv Enzyme Regul.* 1984;22:27-55).
b) The synergistic effect was defined as CI ≤0.8, the additive effect as CI >0.8 and ≤1.2, and the competitive effect as CI ≥1.2.

3.1.2.2 *In vitro* antiviral activity of S-033447 against non-seasonal influenza virus

3.1.2.2.1 Suppressive effect of S-033447 against release of highly pathogenic avian influenza virus A/H5N1 and avian influenza virus A/H7N9 (CTD 4.2.1.1-15, 4.2.1.1-16)

MDCK cells were infected with highly pathogenic influenza virus of subtype A/H5N1 (A/Hong Kong/483/97) isolated from a human, an avian influenza virus of subtype A/H7N9 (A/Anhui/1/2013), or a recombinant virus with NA/H274Y or NA/R292K substitution generated by reverse genetics (A/Hong Kong/483/97-NA/H274Y or A/Anhui/1/2013-NA/R292K). The infected MDCK cells were cultured for 24 hours in the presence of S-033447 or the active form of oseltamivir, and culture supernatants were collected. MDCK cells were inoculated with the supernatants and cultured for 3 days, after which viral titer ($TCID_{50}$) was determined based on the cytopathic effect. EC_{90} was defined as the drug concentration required to reduce viral titer to one-tenth of that in the supernatants from cultures not containing any test drug. Table 7 shows the results.

Table 7. Suppressive effect of S-033447 and the active form of oseltamivir against release of highly pathogenic avian influenza virus (A/H5N1) and avian influenza virus (A/H7N9)

Type/subtype	Strain	EC_{90} (nmol/L)	
		S-033447	Active form of oseltamivir
A/H5N1	A/Hong Kong/483/97	1.64	11.2
A/H5N1	A/Hong Kong/483/97-NA/H274Y	3.16	4.05×10^3
A/H7N9	A/Anhui/1/2013	0.80	15.4
A/H7N9	A/Anhui/1/2013-NA/R292K	1.12	142×10^3

Mean

3.1.2.2.2 Suppressive effect of S-033447 against release of animal-derived influenza virus (CTD 4.2.1.1-17)

MDCK cells were infected with 5 strains of influenza A virus isolated from animals (pig, chicken, and duck). The cells were cultured for 24 hours in the presence of S-033447 or favipiravir, and culture supernatants were collected. MDCK cells were spiked with the supernatants and cultured for 3 days, after which viral titer ($TCID_{50}$) was determined based on the cytopathic effect. EC_{90} was defined as the

drug concentration required to suppress viral titer to one-tenth of that in the supernatants from cultures not containing any test drug. Table 8 shows the results.

Table 8. Suppressive effect of S-033447 and favipiravir against release of animal-derived influenza virus strains[a)]

Type/subtype	Strain	EC_{90} (nmol/L)	
		S-033447	Favipiravir
A/H1N2	A/swine/Chiba/14/2012	1.20	25.3×10^3
A/H5N2	A/chicken/Taiwan/K703-1/2008	0.96	21.9×10^3
A/H5N6	A/duck/Vietnam/HU4-879/2015	0.73	20.5×10^3
A/H9N2	A/chicken/Vietnam/HU1-1050/2014	0.79	29.3×10^3
A/H9N2	A/duck/Vietnam/HU1-1512/2014	0.96	12.6×10^3

Mean

a) EC_{90} of S-033447 and favipiravir against control virus (seasonal influenza virus strain [A/PR/8/34]) was 0.66×10^3 nmol/L and 14.4×10^3 nmol/L, respectively.

3.1.3 Selection of resistant virus

3.1.3.1 Selection of influenza A virus (H1N1) resistant to S-033447 (CTD 4.2.1.1-09, 4.2.1.1-10, 4.2.1.1-11, 4.2.1.1-12)

Madin Darby bovine kidney (MDBK) cells infected with influenza A virus A/WSN/33 (H1N1) strain were cultured in the presence of S-033447 (0.02, 0.1, 0.5, 2.5, or 12.5 nmol/L) (3 samples per concentration). The cells were serially passaged at a constant infectious titer (0.01 plaque forming unit/cell), with the drug concentration being doubled with each 3-day passage. The virus in the supernatants was replicated in MDBK cells in the absence of test drug; then, change in viral drug sensitivity was assessed by plaque suppression test, and polymerase acidic protein (PA) gene was analyzed by Sanger sequencing. Of 15 samples tested, 5 cultured at 2.5 or 12.5 nmol/L failed to survive the first passage and the remaining 10 failed to survive before the 10th passage (the final drug concentration $\leq$12.8 nmol/L). A 22.5- to 41.4-fold decrease in drug sensitivity was observed in the eighth and ninth passages of samples cultured at the initial drug concentration of 0.02 nmol/L and in the sixth and seventh passages of samples cultured at the initial drug concentration of 0.10 nmol/L; PA/I38T substitution[5)] was observed in all of these strains.

Various cells were infected with virus strains with PA/I38T substitution obtained in the resistant virus isolation test (A/WSN/33-P9-6-1 and A/WSN/33-P7-9-1), a recombinant virus strain with PA/I38T substitution generated by reverse genetics (rgA/WSN/33-PA/I38T), and their parent strains. The effect of PA/I38T substitution on viral replication was investigated by measuring the viral titer ($TCID_{50}$) in the culture supernatants. Table 9 shows the results.

5) In order to avoid missed detection, due to viral replication procedure, of genetic variants, genetic analysis by the Sanger method was repeated without replication of influenza virus contained in samples. As a result, PA/I38I/T substitution (i.e., mixture of no substitution and substitution) was detected in 1 of 4 samples, whereas all other variants had PA/I38T substitution. No other amino acid substitution was detected.

Table 9. Viral titer in cell culture supernatants obtained during the early stage of viral replication

Type/ subtype	Strain	Viral titer (log_{10} $TCID_{50}$/mL)[a)]			
		MDBK cells	MDCK cells	RPMI2650 cells	A549 cells
A/H1N1	A/WSN/33 (wild type)	4.67	6.72	4.48	2.91
	A/WSN/33-NA/H274Y	4.22	6.00	3.83	3.50
	A/WSN/33-P9-6-1	3.72	4.83	2.85	1.56
	A/WSN/33-P7-9-1	3.83	2.56	2.80	1.50
	rgA/WSN/33 (wild type)	4.00	5.06	4.33	4.72
	rgA/WSN/33-PA/I38T	3.28	3.72	3.06	3.59

Mean
A549 cells: Human lung adenocarcinoma-derived cells
a) Viral titer in the culture supernatants was measured after culture for 24 hours (MDBK cells, MDCK cells, and RPMI2650 cells) or 36 hours (A549 cells).

MDCK cells were infected with a virus strain with PA/I38T substitution obtained from the resistant virus isolation test (A/WSN/33-P9-6-1), a recombinant virus strain with PA/I38T substitution generated by reverse genetics (rgA/WSN/33-PA/I38T), or their parent strains. The cells were cultured for 24 hours in the presence of S-033447 or favipiravir, and culture supernatants were collected. MDCK cells were spiked with the supernatants and then cultured for 3 days, after which viral titer ($TCID_{50}$) was determined based on the cytopathic effect. EC_{90} was defined as the drug concentration required to decrease viral titer to one-tenth of that in the supernatants from cultures not containing any test drug. Table 10 shows the results.

Table 10. Suppressive effect of S-033447 and favipiravir against release of influenza virus strain A/WSN/33 with PA/I38T substitution

Type/subtype	Strain	EC_{90} (nmol/L)	
		S-033447	Favipiravir
A/H1N1	A/WSN/33 (wild type)	0.82	4.54×10^3
	A/WSN/33-P9-6-1	29.5	2.00×10^3
	rgA/WSN/33 (wild type)	0.47	3.75×10^3
	rgA/WSN/33-PA/I38T	15.6	4.23×10^3

Mean

3.1.3.2 Selection of influenza A virus (H3N2) resistant to S-033447 (CTD 4.2.1.1-13)

MDCK cells infected with influenza A virus A/Victoria/3/75 (H3N2) strain were cultured in the presence of S-033447 (0.02, 0.1, 0.5, 2.5, or 12.5 nmol/L) (3 samples per concentration). The cells were serially passaged at a constant infectious titer (0.01 plaque forming unit/cell), with the drug concentration being doubled with each 3-day passage. Without viral replication, change in viral drug sensitivity was assessed by plaque immunostaining[6)] and PA gene was analyzed by Sanger sequencing. Of 15 samples, 2 cultured at 12.5 nmol/L failed to survive the first passage and the remaining 13 failed to survive before the eighth passage (the final drug concentration ≤25 nmol/L). PA/K362K/R substitution was detected in 2 samples (one at the second to fourth passage; the other at the fifth passage) cultured at the initial drug concentration of 0.1 nmol/L. PA/I38I/T substitution was observed in 1 sample at the fourth passage cultured at the initial drug concentration of 0.5 nmol/L. PA/E199G substitution was observed in 1 sample at the first passage cultured at the initial drug concentration of 12.5 nmol/L. The sensitivity of these variants to S-033447 decreased 1- to 1.1-fold (PA/K362K/R), 34.9-fold (PA/I38I/T), and 3.2-fold (PA/E199G), compared with the parent strain. When samples containing amino acid substitutions PA/K362K/R or PA/I38I/T (both are mixture of no substitution and substitution) were cultured in the

6) When a virus strain with amino acid substitution was present among influenza virus particles in a test sample, the variant was isolated after replication in the presence of S-033447 and subjected to drug sensitivity test.

presence of S-033447, 5 strains with PA/I38T substitution were obtained. These variants showed a 31.8- to 105.6-fold decrease in sensitivity to S-033447 compared with the parent strain.

3.1.3.3 Selection of influenza B virus resistant to S-033447 (CTD 4.2.1.1-14)

MDCK cells infected with influenza B virus B/Maryland/1/59 strain were cultured in the presence of S-033447 (0.2, 1, 5, 25, or 125 nmol/L) (3 samples per concentration). The cells were serially passaged at a constant infectious titer (0.01 plaque forming unit/cell), with the drug concentration being doubled with each 3-day passage. PA gene was analyzed by Sanger sequencing without viral replication. Of 15 samples, 3 cultured at 125 nmol/L failed to survive the first passage and the remaining 12 failed to survive before the eighth passage (the final drug concentration ≤50 nmol/L). No amino acid substitution was detected in PA gene in any samples.

3.1.3.4 Emergence of S-033447-resistant strains in Japanese clinical studies (Studies T0821 and T0822) and global phase III study (Study T0831) (CTD 5.3.5.1-02, 5.3.5.2-01, 5.3.5.4-06, 5.3.5.4-08, 5.3.5.4-09, 5.3.5.4-10, 5.3.5.4-11, 5.3.5.4-12)

In a Japanese phase II study in adult patients (Study T0821), among patients receiving baloxavir, 182 were available for base sequence analysis of viral genome before and after administration of baloxavir. Samples collected from these patients were subjected to base sequence analysis of the PA region by Sanger sequencing. Amino acid substitution in the PA region was observed in 5 patients after administration of baloxavir (subtype A/H1N1pdm, I38T in 2 patients and I38F in 2 patients; type B, R548G [including R548R] in 1 patient). Among the 8 patients who showed an increase in viral titer after administration of baloxavir compared with the titer observed at the prior titration, 1 patient with subtype A/H1N1pdm showed amino acid substitution E23K. The effect of amino acid substitutions observed in these 6 patients on the sensitivity to S-033447 was investigated using recombinant viruses with amino acid substitution generated by reverse genetics. The ratio of EC_{50} of S-033447 (recombinant virus/control virus) was 27.24 for I38T (subtype A/H1N1pdm), 10.61 for I38F (subtype A/H1N1pdm), and 4.74 for E23K (subtype A/H1N1pdm), and 0.88 for R548G (type B) (control viruses: type A, rgA/WSN/33 [H1N1]; type B, rgB/Maryland/1/59). None of these amino acid substitutions affected the sensitivity to the active form of oseltamivir.

In a Japanese study in patients aged <12 years (Study T0822), 77 were available for viral base sequence analysis before and after administration of baloxavir. Using samples collected from these patients, the base sequence of the PA region was analyzed by Sanger sequencing. Amino acid substitution in the PA region was observed in 15 patients after administration of baloxavir (subtype A/H3N2, I38T in 12 patients [including 4 patients with mixture of I38T and I38I] and I38M in 3 patients [including 1 patient with mixture of I38M and I38I]). Among 25 patients who showed increased viral titer after administration of baloxavir compared with the titer observed at the prior titration, 3 with A/H3N2 subtype virus showed amino acid substitution I38T (including 1 patient with mixture of I38T and I38I). The effect of I38 amino acid substitution in subtype A/H3N2 on the sensitivity to S-033447 was investigated using recombinant viruses with amino acid substitution generated by reverse genetics. The ratio of EC_{50} of S-033447 (recombinant virus [subtype A/H3N2]/control virus type A [rgA/Victoria/3/75 (H3N2)]) was 56.59 for I38T and 13.77 for I38M. In contrast, these amino acid substitutions did not affect the sensitivity to the active form of oseltamivir.

In a global phase III study in patients aged ≥12 years (Study T0831), 370 patients were available for viral base sequence analysis before and after administration of baloxavir. Using samples collected from these patients, the base sequence of the PA region was analyzed by Sanger sequencing. As a result, I38 amino acid substitution was observed in 36 patients:

I38T substitution of subtype A/H3N2 in 35 patients (including 3 patients with mixture of I38T and I38I and 2 patients with mixture of I38T, I38T/T,[7] I38I, and I38M);

I38M substitution of subtype A/H3N2 in 1 patient (mixture of I38M and I38I); and

I38T substitution of type B in 1 patient (mixture of I38T and I38I; one of the 3 patients with mixture of I38T and I38I of subtype A/H3N2).

The effect of I38 amino acid substitution on viral replication was investigated in MDCK cells or human nasal septum carcinoma cells (RPMI2650 cells) by measuring viral titer at 24 or 48 hours after infection. Table 11 shows the results.

Table 11. Effect of amino acid substitution on viral replication

Strain	Viral titer (log $TCID_{50}$/mL)	
	MDCK cells	RPMI2650 cells
rgA/WSN/33 (H1N1)	5.56	7.06
rgA/WSN/33-PA/I38T (H1N1)	2.78	5.17
rgA/WSN/33-PA/I38F (H1N1)	3.28	5.19
rgA/WSN/33-PA/I38M (H1N1)	3.89	5.91
rgA/Victoria/3/75 (H3N2)	5.83	3.63
rgA/Victoria/3/75-PA/I38T (H3N2)	4.11	1.83
rgA/Victoria/3/75-PA/I38F (H3N2)	4.56	1.56
rgA/Victoria/3/75-PA/I38M (H3N2)	5.06	1.94
rgB/Maryland/1/59	4.06	3.91
rgB/Maryland/1/59-PA/I38T	3.94	3.61
rgB/Maryland/1/59-PA/I38F	3.33	2.44
rgB/Maryland/1/59-PA/I38M	4.50	4.35

Mean

In order to investigate the effect of I38 amino acid substitution observed in clinical studies on clinical symptoms (influenza virus symptoms and pyrexia),[8] clinical symptoms were evaluated in patients with and without I38 amino acid substitution. No correlation was observed between the presence/absence of I38 amino acid substitution and clinical symptoms [see Section 7.R.2.4].

3.1.4 *In vivo* antiviral activity

3.1.4.1 *In vivo* antiviral activity of S-033447 against influenza virus

3.1.4.1.1 Suppression of intrapulmonary viral replication in mouse infection model (CTD 4.2.1.1-19, 4.2.1.1-20, 4.2.1.1-21)

Female BALB/c mice were intranasally inoculated with influenza A virus A/WSN/33 (H1N1) strain (2.0 $\log_{10}$ [1.00×10^2] $TCID_{50}$ [non-lethal dose]). After 5 days, baloxavir (0 [vehicle], 0.5, 1.5, 5, 15, or 50 mg/kg BID) or oseltamivir phosphate (5 mg/kg BID [corresponding to the clinical dose] or 50 mg/kg BID) was administered orally for 1 day, and the intrapulmonary viral titer was measured at 24 hours after the first dose. The baloxavir 0.5 to 50 mg/kg BID groups showed a dose-dependent decrease in

[7] Different threonine codons

[8] "Influenza virus symptoms" were defined as moderate or severe cough and nasal congestion lasting for ≥21.5 hours on Day ≥5 or ≥6 after study drug administration. "Pyrexia" was defined as fever of ≥37.5°C lasting for ≥12 hours on Day ≥5 or ≥6.

intrapulmonary viral titer, and animals in the ≥1.5 mg/kg BID groups showed a greater decrease in intrapulmonary viral titer compared with those in the oseltamivir phosphate 5 and 50 mg/kg BID groups. The intrapulmonary viral titer in the baloxavir (1.5 mg/kg BID) 1 day group was lower by 0.99 $\log_{10}$ $TCID_{50}$/mL than that in the oseltamivir phosphate (5 mg/kg BID) 1 day group. The infection model animals generated by the same method described above received laninamivir octanoate hydrate (1 mg/kg QD [corresponding to the clinical dose] or 3 mg/kg QD) intranasally for 1 day, zanamivir hydrate (10 mg/kg BID) intranasally for 1 day, or favipiravir (50 or 150 mg/kg BID) orally for 1 day; the intrapulmonary viral titers at 24 hours were higher in these animals than in those receiving baloxavir 5 mg/kg BID.

Female BALB/c mice were intranasally inoculated with influenza A virus A/Hong Kong/8/68 (H3N2) strain (2.0 $\log_{10}$ [1.00×10^2] $TCID_{50}$ [non-lethal dose]) or influenza B virus B/Hong Kong/5/72 strain (2.6 $\log_{10}$ [4.00×10^2] $TCID_{50}$ [non-lethal dose]). After 5 days, baloxavir (0 [vehicle], 0.5, 1.5, 5, 15, or 50 mg/kg BID) or oseltamivir phosphate (5 mg/kg BID [corresponding to the clinical dose] or 50 mg/kg BID) was administered orally for 1 day, and the intrapulmonary viral titer was measured at 24 hours after the first dose. In both A/Hong Kong/8/68-infected mice and B/Hong Kong/5/72-infected mice, those receiving baloxavir 0.5 to 50 mg/kg BID showed a dose-dependent decrease in the intrapulmonary viral titer. A/Hong Kong/8/68-infected mice receiving ≥0.5 mg/kg BID and B/Hong Kong/5/72-infected mice receiving ≥5 mg/kg BID showed a greater decrease in the intrapulmonary viral titer compared with mice receiving oseltamivir phosphate 5 or 50 mg/kg BID. Among A/Hong Kong/8/68-infected mice, the intrapulmonary viral titer in the baloxavir (1.5 mg/kg BID) 1 day group was lower by 1.55 $\log_{10}$ $TCID_{50}$/mL than that in the oseltamivir phosphate (5 mg/kg BID) 1 day group. Among B/Hong Kong/5/72-infected mice, the intrapulmonary viral titer in the baloxavir (15 mg/kg BID) 1 day group was lower by 0.97 $\log_{10}$ $TCID_{50}$/mL than in the oseltamivir phosphate (5 mg/kg BID) 1 day group.

Female BALB/c mice were intranasally inoculated with influenza A virus A/Osaka/129/2009 (H1N1) strain (3.6 $\log_{10}$ [4.30×10^3] $TCID_{50}$ [non-lethal dose]). After 5 days, the animals received baloxavir (0.5 or 5 mg/kg BID) for 1 or 3 days or oseltamivir phosphate (5 mg/kg BID) for 3 days, and the intrapulmonary viral titer was measured during the 3 days after the first dose. All baloxavir groups showed a greater decrease in the intrapulmonary viral titer compared with the oseltamivir phosphate group. The number of animals showing a intrapulmonary viral titer below the lower limit of quantification (LLOQ) at 48 hours after the first dose was larger in the baloxavir 0.5 and 5 mg/kg BID 1 day groups (1 of 5 animals and 2 of 5 animals, respectively) and in the baloxavir 0.5 and 5 mg/kg BID 3 days groups (2 of 5 animals and 5 of 5 animals, respectively), than in the vehicle group (none) or the oseltamivir phosphate group (none).

3.1.4.1.2 Suppression of intrapulmonary viral replication in NA inhibitor-resistant virus infection mouse model (CTD 4.2.1.1-20)

Female BALB/c mice were intranasally inoculated with influenza A virus A/WSN/33-NA/H274Y (H1N1) strain with NA/H274Y substitution (2.0 $\log_{10}$ [1.00×10^2] $TCID_{50}$ [non-lethal dose]). After 5 days, baloxavir (0 [vehicle], 0.5, 1.5, 5, 15, or 50 mg/kg BID) or oseltamivir phosphate (5 mg/kg BID) was administered orally for 1 day, and the intrapulmonary viral titer was measured at 24 hours after the first dose. The intrapulmonary viral titer in the baloxavir 0.5 to 50 mg/kg BID groups was 1.60 to 3.86

$\log_{10}$ $TCID_{50}$/mL, showing a dose-dependent decrease in the intrapulmonary viral titer. Animals receiving baloxavir ≥0.5 mg/kg BID showed a greater decrease in the intrapulmonary viral titer than those receiving oseltamivir phosphate 5 mg/kg BID (4.54 $\log_{10}$ $TCID_{50}$/mL).

3.1.4.1.3 Suppression of intrapulmonary viral replication in immunosuppressed mouse infection model (CTD 4.2.1.1-30, 4.2.1.1-31)

Cyclophosphamide (CPA; 0.2 mg QD) was subcutaneously administered to female BALB/c mice for 11 days to induce immunosuppressive conditions. On the day after the last dose, the animals were intranasally inoculated with influenza A virus A/PR/8/34 (H1N1) strain (2.0 $\log_{10}$ [1.00×10^2] $TCID_{50}$ [non-lethal dose]). After 5 days, baloxavir (0 [vehicle], 1.5, 15, or 50 mg/kg BID) or oseltamivir phosphate (5 or 50 mg/kg BID) was administered orally for 5 days, and the intrapulmonary viral titer and rate of body weight decrease were measured over time. In CPA-treated mice, the intrapulmonary viral titer remained at ≥4 $\log_{10}$ $TCID_{50}$/mL from 5 to 10 days after viral inoculation, whereas in CPA-untreated mice, the intrapulmonary viral titer decreased to 2 $\log_{10}$ $TCID_{50}$/mL on Day 9 after viral inoculation. This demonstrates that CPA prolongs the viral infection period.

The baloxavir groups showed a dose-dependent decrease in intrapulmonary viral titer at 24 hours after the first dose of baloxavir. In the 15 and 50 mg/kg BID groups, the intrapulmonary viral titer reached a level below the LLOQ (1.5 $\log_{10}$ $TCID_{50}$/mL) on Day 3 after the first dose of baloxavir, showing a greater decrease than in the vehicle control and oseltamivir phosphate groups. The rate of body weight decrease was smaller in the baloxavir groups than in the vehicle control and oseltamivir phosphate groups.

In order to investigate the emergence of virus with low sensitivity to S-033447 in immunosuppressed mice receiving baloxavir orally for 5 days, PA gene was analyzed by Sanger sequencing using lung specimens collected 1, 3, and 5 days after the start of baloxavir (1.5-50 mg/kg BID) administration, without replication of influenza virus in the specimens. There was no virus with amino acid substitution in any of the specimens.

3.1.4.1.4 Suppression of intranasal virus replication in ferret infection model (CTD 4.2.1.1-29)

Ferrets were intranasally inoculated with influenza A virus A/Kadoma/3/2006 (H1N1) strain (3.0 $\log_{10}$ [1.00×10^3] $TCID_{50}$ [non-lethal dose]). After 24 hours, baloxavir (0 [vehicle], 10, or 30 mg/kg BID for 1 day) or oseltamivir phosphate (5 mg/kg BID for 2 days) was administered orally. Viral titer in nasal washes and change in body temperature were measured for 2 days after the first dose. In both baloxavir dose groups, the viral titer in nasal washes decreased below the LLOQ (0.5 $\log_{10}$ $TCID_{50}$/mL) at 1 day after the first dose. At 2 days after the first dose, the viral titer in nasal washes of animals receiving baloxavir increased above the LLOQ again, but was lower than the titers in the vehicle control and oseltamivir phosphate groups. The baloxavir groups had a smaller change in body temperature than the vehicle control and oseltamivir phosphate groups during the 2-day period after the first dose.

3.1.4.1.5 Pharmacokinetic/Pharmacodynamic (PK/PD) analysis (CTD 4.2.1.1-22)

BALB/c mice were intranasally inoculated with influenza A virus A/WSN/33 (H1N1) strain (2.0 $\log_{10}$ [1.00×10^2] $TCID_{50}$ [non-lethal dose]). After 5 days, S-033447 (0.0625-8 mg/kg) was subcutaneously administered QD, BID, or 4 times daily. PK/PD analysis was performed using the following parameters: $AUC_{0\text{-}24}$, C_{max}, C_{24}, C_τ, and the duration during which S-033447 exceeded 2, 10, or 50 ng/mL. Among the PK parameters, C_τ was most closely correlated with the PD parameter (intrapulmonary viral titer at 24 hours after the start of administration).

The efficacy target in the nonclinical infection mouse models was "to decrease the intrapulmonary viral titer after oral administration of S-033447 to one-tenth of the titer achieved in models receiving oseltamivir phosphate at a dose corresponding to the clinical dose." Based on (1) the results of studies on influenza A or B virus-infected mice [see Section 3.1.4.1.1] and (2) the difference in the sensitivity of influenza A and B viruses, the effective dose of baloxavir was determined to be 15 mg/kg BID orally for 1 day (C_τ, 6.85 ng/mL).

3.1.4.1.6 Treatment effect in mouse infection model (CTD 4.2.1.1-24, 4.2.1.1-25)

Female BALB/c mice were intranasally inoculated with influenza A virus A/PR/8/34 (H1N1) strain (3.1 $\log_{10}$ [1.38×10^3] $TCID_{50}$ or 4.6 $\log_{10}$ [4.42×10^4] $TCID_{50}$ [both lethal doses]). Immediately after inoculation, baloxavir (0 [vehicle], 0.05, 0.5, or 5 mg/kg BID for 1 day) or oseltamivir phosphate (5 or 50 mg/kg BID for 5 days) was administered orally, and the survival rate was evaluated at 21 days after inoculation. Table 12 shows the results.

Table 12. Survival rate at 21 days after inoculation in influenza A virus infection mouse model

Test drug	Dosage regimen	Survival rate at 21 days after inoculation	
		After inoculation at 1.38×10^3 $TCID_{50}$	After inoculation at 4.42×10^4 $TCID_{50}$
Vehicle	Vehicle BID for 1 day	0% (0/10)	0% (0/10)
Baloxavir	0.05 mg/kg BID for 1 day	30% (3/10)	0% (0/10)
	0.5 mg/kg BID for 1 day	100% (10/10)	100% (10/10)
	5 mg/kg BID for 1 day	100% (9/9)[a)]	100% (10/10)
Oseltamivir phosphate	5 mg/kg BID for 5 days	90% (9/10)	20% (2/10)
	50 mg/kg BID for 5 days	-	80% (8/10)

% (n/N)
-, Not tested.
a) One animal showed a marked decrease in body weight after influenza virus inoculation and died 2 days after the inoculation.

Female BALB/c mice were intranasally inoculated with influenza B virus B/Hong Kong/5/72 strain (5.5 $\log_{10}$ [3.3×10^5] $TCID_{50}$ or 6.3 $\log_{10}$ [1.98×10^6] $TCID_{50}$ [both lethal doses]). Immediately after the inoculation, baloxavir (0 [vehicle], 0.5, 5, or 50 mg/kg BID for 1 day) or oseltamivir phosphate (5 or 50 mg/kg BID for 5 days) was administered orally, and the survival rate was evaluated at 14 days after inoculation. Table 13 shows the results.

Table 13. Survival rate at 14 days after inoculation in influenza B virus infection model

Test drug	Dosage regimen	Survival rate at 14 days after inoculation	
		After inoculation at 3.3×10^5 $TCID_{50}$	After inoculation at 1.98×10^6 $TCID_{50}$
Vehicle	Vehicle BID for 1 day	0% (0/10)	0% (0/10)
Baloxavir	0.5 mg/kg BID for 1 day	20% (2/10)	0% (0/10)
	5 mg/kg BID for 1 day	100% (10/10)	100% (10/10)
	50 mg/kg BID for 1 day	100% (10/10)	100% (10/10)
Oseltamivir phosphate	5 mg/kg BID for 5 days	100% (10/10)	20% (2/10)
	50 mg/kg BID for 5 days	-	70% (7/10)

% (n/N)
-, Not tested.

3.1.4.1.7 Effect of the time of starting administration on therapeutic response in mouse infection model (CTD 4.2.1.1-26, 4.2.1.1-27)

Female BALB/c mice were intranasally inoculated with influenza A virus A/PR/8/34 (H1N1) strain (3.1 $\log_{10}$ [1.38×10^3] $TCID_{50}$ [lethal dose]). Starting at 24, 48, 72, or 96 hours after the inoculation, baloxavir (0 [vehicle], 1.5, or 15 mg/kg BID) or oseltamivir phosphate (5 mg/kg BID) was administered orally for 5 days, and the survival rate was evaluated at 28 days after inoculation. Table 14 shows the survival rate in each group. Viral replication-induced decrease in body weight was suppressed by baloxavir BID dose-dependently.

Table 14. Survival rate at 28 days after inoculation in mouse infection model, classified by the timing of starting treatment with baloxavir

Test drug	Dosage regimen	Survival rate at 28 days after inoculation			
		24 hours after inoculation	48 hours after inoculation	72 hours after inoculation	96 hours after inoculation
Vehicle	Vehicle BID for 5 days	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/10)
Baloxavir	1.5 mg/kg BID for 5 days	100% (10/10)	100% (10/10)	100% (10/10)	50% (5/10)
	15 mg/kg BID for 5 days	100% (10/10)	100% (10/10)	100% (10/10)	70% (7/10)
Oseltamivir phosphate	5 mg/kg BID for 5 days	90% (9/10)	70% (7/10)	10% (1/10)	10% (1/10)

% (n/N)

Female BALB/c mice were intranasally inoculated with influenza A virus A/PR/8/34 (H1N1) strain (3.1 $\log_{10}$ [1.38×10^3] $TCID_{50}$ [lethal dose]). At 72 hours after the inoculation, baloxavir (0 [vehicle], 1.5, or 15 mg/kg BID) or oseltamivir phosphate (5 mg/kg BID) was administered orally for 5 days, and the intrapulmonary viral titer was evaluated over time. The baloxavir 1.5 and 15 mg/kg BID groups showed a dose-dependent decrease in the intrapulmonary viral titer at 1 and 3 days after the first dose. The decrease in intrapulmonary viral titer was greater in the baloxavir groups than in the oseltamivir phosphate group.

3.1.4.1.8 Therapeutic effect of the combination of baloxavir and a NA inhibitor in mouse infection model (CTD 4.2.1.1-28)

Female BALB/c mice were intranasally inoculated with influenza A virus A/PR/8/34 (H1N1) strain (2.9 $\log_{10}$ [8.00×10^2] $TCID_{50}$ [lethal dose]). Starting at 96 hours after the inoculation, baloxavir (0 [vehicle], 0.5, 1.5, 15, or 50 mg/kg BID) or oseltamivir phosphate (10 or 50 mg/kg BID) was administered orally alone or in combination with oseltamivir phosphate for 5 days, and the survival rate was evaluated at 28 days after inoculation. Table 15 shows the survival rate in each group. Virus replication-induced decrease in body weight was suppressed by the combination of baloxavir and oseltamivir phosphate.

Table 15. Survival rate at 28 days after inoculation in mouse infection model receiving baloxavir and a NA inhibitor alone or in combination

Test drug and dosage regimen[a)]	Survival rate at 28 days after inoculation
Vehicle (5 days)	0% (0/10)
Oseltamivir phosphate 10 mg/kg BID (5 days)	10% (1/10)
Oseltamivir phosphate 50 mg/kg BID (5 days)	40% (4/10)
Baloxavir 0.5 mg/kg BID (5 days)	40% (4/10)
Baloxavir 1.5 mg/kg BID (5 days)	70% (7/10)
Baloxavir 15 mg/kg BID (5 days)	100% (10/10)
Baloxavir 50 mg/kg BID (5 days)	100% (10/10)
Baloxavir 0.5 mg/kg BID (5 days) + oseltamivir phosphate 10 mg/kg BID (5 days)	70% (7/10)
Baloxavir 0.5 mg/kg BID (5 days) + oseltamivir phosphate 50 mg/kg BID (5 days)	90% (9/10)
Baloxavir 1.5 mg/kg BID (5 days) + oseltamivir phosphate 10 mg/kg BID (5 days)	100% (10/10)
Baloxavir 1.5 mg/kg BID (5 days) + oseltamivir phosphate 50 mg/kg BID (5 days)	100% (10/10)

% (n/N)

a) As all animals receiving ≥15 mg/kg baloxavir alone survived, the effect of ≥15 mg/kg baloxavir + oseltamivir phosphate was not investigated.

3.1.4.2 *In vivo* antiviral activity of baloxavir against non-seasonal influenza virus

3.1.4.2.1 Therapeutic effect in highly pathogenic avian influenza virus (A/H5N1) infection mouse model (CTD 4.2.1.1-32)

Female BALB/c mice were intranasally inoculated with highly pathogenic avian influenza virus of subtype A/H5N1 isolated from a human (A/Hong Kong/483/97 strain [1.9 $\log_{10}$ (0.75×10^2) $TCID_{50}$ (lethal dose)]). Immediately after the inoculation, baloxavir (0 [vehicle], 0.5, 5, or 50 mg/kg BID) or oseltamivir phosphate (5 or 50 mg/kg BID) was administered orally for 1 or 5 days, to evaluate the survival rate at 14 days after inoculation and the intrapulmonary viral titer at 1, 3, and 5 days after the first dose of test drug. In all groups receiving baloxavir (5 or 50 mg/kg BID) for 1 or 5 days, the survival rate at 14 days after inoculation was 100% (10 of 10 animals), which was higher than the rate in the vehicle control group (0% [0 of 10 animals]) and in the oseltamivir phosphate groups (0 % [0 of 10] of animals receiving 5 mg/kg BID for 5 days, 70% [7 of 10] of animals receiving 50 mg/kg BID for 5 days). In animals receiving baloxavir (5 or 50 mg/kg BID) for 1 or 5 days, the intrapulmonary viral titer at 1, 3, and 5 days after the first dose was lower than the titers in the vehicle control and oseltamivir phosphate 5 mg/kg BID groups.

3.1.4.2.2 Therapeutic effect in avian influenza virus (A/H7N9) infection mouse model (CTD 4.2.1.1-33)

Female BALB/c mice were intranasally inoculated with avian influenza virus of subtype A/H7N9 isolated from a human (A/Anhui/1/2013 strain [5.6 $\log_{10}$ (4.00×10^5) $TCID_{50}$ (lethal dose)]). Immediately after the inoculation, baloxavir (0 [vehicle], 0.5, 5, or 50 mg/kg BID) or oseltamivir phosphate (5 or 50 mg/kg BID) was administered orally for 1 or 5 days, to evaluate the survival rate at 28 days after inoculation and the intrapulmonary viral titer at 1, 3, and 5 days after the first dose of test drug. In all groups receiving baloxavir (5 or 50 mg/kg BID) for 1 or 5 days, the survival rate at 28 days after inoculation was 100% (10 of 10 animals), which was higher than the rate in the vehicle control group (0% [0 of 10 animals]) and in the oseltamivir phosphate groups (30% [3 of 10] of animals receiving 5 mg/kg BID for 5 days, 50% [5 of 10] of animals receiving 50 mg/kg BID for 5 days). In animals receiving baloxavir (5 or 50 mg/kg BID) for 1 or 5 days, the intrapulmonary viral titer at 1, 3, and 5 days after the first dose was lower than the titers in the vehicle control and oseltamivir phosphate 5 mg/kg BID groups.

3.2 Secondary pharmacodynamics

3.2.1 Effect on enzymes and receptors (CTD 4.2.1.2-05)

The *in vitro* inhibitory effect of S-033447 on ligand binding to 66 types of receptors, ion channels, and transporters was investigated. S-033447 (10 μmol/L, approximately 39 times[9)] the clinical exposure [C_{max}]) did not induce a >50% inhibition of the binding of any of the ligands tested.

3.2.2 Cytotoxic effect against various types of cells

3.2.2.1 Cytotoxic effect against cultured cells (CTD 4.2.1.2-01, 4.2.1.2-03)

MDBK cells, MDCK cells, RPMI2650 cells, and human lung adenocarcinoma-derived A549 cells were cultured in a medium containing S-033447 (0.39-200 μmol/L) for 3 days. Then, the cytotoxic effect of S-033447 was investigated by measuring dehydrogenase activity. The 50% cytotoxicity concentration (CC_{50}) of S-033447 against each cell line was 47.5, 18.9, 22.8, and 17.3 μmol/L, respectively, for MDBK, MDCK, RPMI2650, and human lung adenocarcinoma-derived A549 cells.

Nine proliferating cell lines[10)] derived from various human tissues were cultured for 48 hours in a medium containing S-033447 (0.01-100 μmol/L). Then, the cytotoxic effect of S-033447 was investigated by measuring intracellular ATP level. IC_{50} of S-033447 against the proliferating cell lines was 2.2 to 50 μmol/L.

Five non-proliferating cell lines[11)] derived from human tissues were cultured for 48 hours in a medium containing S-033447 (0.01-100 μmol/L). Then, the cytotoxic effect of S-033447 was investigated by measuring intracellular ATP level. IC_{50} of S-033447 against the non-proliferating cell lines was >3.0 to >100 μmol/L.

3.2.2.2 Suppression of influenza virus-induced cell degeneration and cytotoxic effect (CTD 4.2.1.2-02)

MDCK cells infected with influenza A or B virus (2 strains each) were cultured in the presence of S-033447 (0.128-400 nmol/L), ribavirin (3.91-125 μmol/L), or favipiravir (0.977-31.3 μmol/L) for 6 days to investigate the suppressive effect of each test drug against viral replication-induced cell degeneration. Also, MDCK cells were cultured in the presence of S-033447 (16-50,000 nmol/L), ribavirin (62.5-2000 μmol/L), or favipiravir (31.3-1000 μmol/L) for 6 days, to investigate the cytotoxic effect of each test drug by measuring dehydrogenase activity. Table 16 shows the results.

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9) The exposure ratio was calculated using plasma S-033447 concentration (C_{max}, 123 ng/mL) following a single dose of 40 mg baloxavir in Japanese healthy adult men under fasted conditions [see Section 6.2.1.1].

10) The following human tissue-derived cell lines were used:
Human foreskin-derived fibroblasts (BJ cells), human fetal kidney-derived cells (HEK293 cells), human hepatoma-derived cells (HepG2 cells), human proximal renal tubule-derived epithelial cells (HK2 cells), human umbilical vein-derived endothelial cells (HUVEC cells), human T cell leukemia-derived cells (Jurkat cells), human fetal lung-derived fibroblasts (MRC-5 cells), and human neuroblastoma-derived cells (SH-SY5Y cells and SK-N-SH cells).

11) The following human tissue-derived cell lines were used:
Human fetal kidney-derived cells (HEK293 cells), human proximal renal tubule-derived epithelial cells (HK2 cells), human T cell leukemia-derived cells (Jurkat cells), human fetal lung-derived fibroblasts (MRC-5 cells), and human neuroblastoma-derived cells (SH-SY5Y cells).

Table 16. Suppression of influenza virus-induced cell degeneration by S-033447, ribavirin, and favipiravir, and their cytotoxic effect

Strain	S-033447		Ribavirin		Favipiravir	
	EC_{50} (nmol/L)	SI[a)]	EC_{50} (nmol/L)	SI[a)]	EC_{50} (nmol/L)	SI[a)]
A/WSN/33 (H1N1)	1.23	2409	83.3×10^3	7.13	8.73×10^3	-
A/Victoria/3/75 (H3N2)	1.59	1857	64.7×10^3	9.18	7.47×10^3	-
B/Maryland/1/59	5.73	516.4	34.6×10^3	17.16	4.32×10^3	-
B/Hong Kong/5/72	2.02	1463	23.2×10^3	25.58	2.89×10^3	-

Mean

a) SI (selectivity index) was calculated as the ratio of CC_{50} of each test drug (S-033447, 2.96×10^3 nmol/L; ribavirin, 594×10^3 nmol/L) to its suppressive effect of viral replication-induced cell degeneration (EC_{50}). For favipiravir, SI was not calculated because CC_{50} could not be calculated.

3.2.3 Effect on mitochondria (CTD 4.2.1.2-04)

Human hepatoma-derived HepG2 cells were cultured in the presence of baloxavir or S-033447 in a medium containing glucose or galactose. The effect of test drug on human mitochondria was investigated by measuring intracellular ATP level after 24 hours or 6 days of culture. IC_{50} of baloxavir and S-033447 against intracellular ATP level after a 24-hour culture was >200 μmol/L under all culture conditions tested. After 6 days of culture containing glucose or galactose, IC_{50} of baloxavir was 20.5μmol/L (glucose) and 16 μmol/L (galactose), and IC_{50} of S-033447 was 11.1 μmol/L (glucose) and 8.79 μmol/L (galactose); thus, each drug had similar IC_{50} for both glucose-containing medium and galactose-containing medium. Based on these results, the applicant explained that neither baloxavir nor S-033447 has a selective effect on the function of mitochondria in HepG2 cells.

3.3 Safety pharmacology (CTD 4.2.1.3-01, 4.2.1.3-02, 4.2.1.3-03, 4.2.1.3-04, 4.2.1.3-05, 4.2.1.3-06, 4.2.1.3-07)

The effect of S-033447 on the central nervous system, respiratory system, and cardiovascular system was investigated (Table 17).

Table 17. Outline of safety pharmacology studies

Organ system	Test system	Parameters, evaluation methods, etc.	Dose	Route of administration	Findings
Central nervous	Male SD rats (n = 6/group)	FOB method	Baloxavir: 0 (vehicle), 200, 600, 2000 mg/kg	Oral	No effect on clinical signs up to 2000 mg/kg. Body temperature decreased in the 600 mg/kg group compared with the control group. Body temperature decreased and urine volume slightly decreased in the 2000 mg/kg group compared with the control group.
Cardiovascular	Chinese hamster ovary cells (n = 5/ concentration)	hERG current	Baloxavir: 0, 0.572, 1.71, 5.72 µg/mL (vehicle: 0.1% dimethyl sulfoxide [DMSO] solution) S-033447: 0, 0.300, 1.00, 4.08 µg/mL (vehicle: 0.1% DMSO solution)	*In vitro*	Baloxavir did not affect hERG current at any concentration tested. S-033447 inhibited hERG current by 0.24% (0.300 µg/mL), 2.72% (1.00 µg/mL), and 26% (4.08 µg/mL) (IC_{50} 7.31 µg/mL).
	Isolated guinea pig right ventricular papillary muscle (n = 5/ concentration)	Action potential, etc.	Baloxavir: 0, 0.0572, 0.171, 0.572 µg/mL (vehicle: 0.1% DMSO solution) S-033447: 0, 0.040, 0.12, 0.40 µg/mL (vehicle: 0.1% DMSO solution)	*In vitro*	No findings warranting special mention.
	Male cynomolgus monkeys (n = 4/group)	Telemetry	Baloxavir: 0 (vehicle), 200, 400 mg/kg	Oral	No effect on blood pressure, heart rate, or electrocardiogram parameters at any dose. Abnormal feces (soft feces, diarrhoea, or pale feces) in 2 animals receiving 200 mg/kg and 3 receiving 400 mg/kg. Decreased food consumption in 1 animal receiving 200 mg/kg and 3 receiving 400 mg/kg. Nausea/vomiting in 1 animal receiving 400 mg/kg.
Respiratory	Male SD rats (n = 8/group)	Respiratory rate, tidal volume, minute ventilation (WBP method)	Baloxavir: 0 (vehicle), 200, 600, 2000 mg/kg	Oral	No findings warranting special mention.

3.R Outline of the review conducted by PMDA

3.R.1 Antiviral activity of S-033447

(1) Antiviral activity of S-033447 against seasonal influenza virus

Based on the results described in Sections 3.1.2.1 and 3.1.4.1, the applicant explained that baloxavir suppresses replication of seasonal influenza A and B viruses more effectively than oseltamivir phosphate.

In the *in vitro* and *in vivo* studies, influenza B virus tended to be less sensitive to S-033447 than influenza A virus. PMDA asked the applicant to explain the reasons for the lower sensitivity of influenza B virus.

The applicant's explanation:

A docking model of PA and S-033447 was constructed based on the x-ray crystal structure of the N-terminal region of PA, the region containing the active center of cap-dependent endonuclease of

influenza virus. Results suggested the interaction of S-033447 with 17 amino acid residues.[12)] Using the database of the amino acid sequence of PA registered at US National Center for Biotechnology Information, conservation of the 17 amino acid residues was analyzed. The analysis showed that subtypes A/H1N1 and A/H3N2 had the 17 amino acid residues in common. Type A and type B viruses shared the amino acid residues at the active center supposed to interact with S-033447, whereas 3 of 5 amino acid residues that form the hydrophobic pocket differed between the two types (A20, Y24, and A37 in type A; T20, F24, and N37 in type B). Consequently, the hydrophobic pocket of type B virus is more hydrophilic than that of type A virus, affecting the hydrophobic interaction with the hydrophobic tricyclic structure of S-033447, thereby resulting in a difference in the antiviral activity of S-033447 to influenza A and B viruses.

PMDA's view:
In vitro and *in vivo* studies showed a tendency of weaker antiviral activity of S-033447 to influenza B virus than to influenza A virus. However, the *in vitro* studies evaluating the suppression of viral release showed that baloxavir has lower EC_{90} values against influenza A and B viruses than existing influenza antiviral drugs such as oseltamivir. Also, *in vivo* studies showed that both the intrapulmonary viral titer and mortality rate were lower in the baloxavir group than in the oseltamivir phosphate group. However, the efficacy of baloxavir against influenza A and B viruses should be evaluated based on the results of clinical studies [see Section 7.R.2].

(2) Antiviral activity of S-033447 against non-seasonal influenza virus
The applicant's explanation:
From the results described in Sections 3.1.2.2 and 3.1.4.2, S-033447 is expected to be effective against various subtypes of avian- or swine-origin influenza A viruses, as against seasonal influenza virus.

PMDA's view:
Although the submitted data suggest that S-033447 is effective against avian- or swine-origin influenza A viruses, the clinical effect is unknown currently.

3.R.2 S-033447-resistant virus

The applicant's explanation about the resistance to S-033447:
Studies to isolate resistant virus of A/H1N1 and A/H3N2 subtypes detected amino acid substitution of PA/I38T, whereas studies to isolate resistant type B virus did not detect any amino acid substitution. The effect of PA/I38T substitution on the sensitivity of virus to S-033447 and on viral replication was investigated using reverse genetics. Results showed that PA/I38T substitution decreased not only the sensitivity of influenza virus to S-033447 but also the replication capacity of the virus [see Sections 3.1.3.1 to 3.1.3.3]. PA/I38T is situated at the hydrophobic pocket of cap-dependent endonuclease and is thus considered to interact with the hydrophobic tricyclic structure of S-033447. The substitution from isoleucine (a hydrophobic amino acid residue) to threonine (a hydrophilic amino acid) probably attenuated the interaction between PA and S-033447, reducing the inhibitory effect of S-033447 on cap-dependent endonuclease activity.

[12)] The following 17 amino acid residues may contribute to the interaction between PA and S-033447: A20, M21, Y24, E26, K34, A37, I38, H41, E80, R84, L106, D108, E119, I120, G121, Y130, and K134 (written based on the amino acid sequence of subtype A/H1N1 pdm).

In the Japanese clinical studies (Studies T0821 and T0822) and the global phase III study (Study T0831), base sequence analysis was performed on the PA region of influenza virus isolated from baloxavir-treated patients available for base sequence analysis before and after administration of baloxavir. As a result, I38 amino acid substitution (I38F, I38M, or I38T) was observed in all studies, and a study by reverse genetics showed that these amino acid substitutions decreased the sensitivity of the virus to S-033447 [see Section 3.1.3.4].

There are no data supporting a decreased pathogenicity and transmissibility of low S-033447-sensitivity virus with PA/I38T substitution. However, several reports suggested that influenza viral replication capacity may be correlated with pathogenicity and transmissibility (*Science.* 2009;325:481-3, *Antimicrob Agent Chemother.* 2005;49:4075-84, *J Infect Dis.* 2004;190:1627-30), and PA/I38T variants tend to have a lower replication capacity than wild-type virus. Thus, PA/I38T variants are likely to have a decreased pathogenicity and transmissibility, and therefore are unlikely to result in serious symptoms or epidemic of low S-033447-sensitivity strains.

The effect of PA/I38 amino acid substitutions observed in clinical studies on the efficacy was investigated. Patients infected with influenza virus with I38 amino acid substitution did not show any characteristic influenza virus symptoms or persistence or flare-up of pyrexia, compared with patients infected with influenza virus without the substitution [see Section 3.1.3.4].

Thus, although the *in vitro* and clinical studies detected PA/I38 amino acid substitutions contributing to the decreased sensitivity to S-033447, these substitutions are unlikely to affect clinical symptoms.

PMDA's view:
PMDA confirmed that (a) in the *in vitro* studies to isolate resistant viruses, viruses with PA/I38T substitution were detected among influenza virus of A/H1N1 and A/H3N2 subtypes, and that (b) I38T substitution contributes to decreased sensitivity to S-033447 [see Sections 3.1.3.1 to 3.1.3.3]. PMDA also confirmed that, in the Japanese clinical studies (Studies T0821 and T0822) and the global phase III study (Study T0831), PA/I38 variants emerged at a certain frequency in the baloxavir groups [see Section 3.1.3.4]. Currently, there is no information available on the pathogenicity or transmissibility of the low S-033447-sensitivity strain with PA/I38T substitution or on the efficacy of baloxavir in patients who have influenza virus with I38 amino acid substitution before administration of baloxavir. Thus, it is unclear how these amino acid substitutions affect patients clinically. Information on acquisition of resistance to baloxavir obtained in clinical studies should be appropriately provided to healthcare professionals. Also, such information should be continuously collected after the market launch and provided appropriately to healthcare professionals. The effect of amino acid substitutions conferring resistance to baloxavir on the efficacy of baloxavir is described in Section 7.R.2.4.

4. Non-clinical Pharmacokinetics and Outline of the Review Conducted by PMDA

PK of baloxavir was investigated following the administration of baloxavir (^{14}C-labeled or unlabeled) to mice, rats, rabbits, monkeys, and ferrets. Also, studies on serum protein binding, drug metabolizing enzymes, and transporters were conducted using human or animal biomaterials.

Concentrations of baloxavir and its active metabolite S-033447 in biomaterials were measured by high-performance liquid chromatography/tandem mass spectrometry (LLOQ 0.500 or 5.00 ng/mL). Radioactivity of baloxavir and its metabolites in biomaterials was measured by liquid scintillation counting, and radioactivity in tissues was measured by quantitative whole-body autoradiography (LLOQ 8.51 ng eq./g). PK parameters are expressed in mean value unless specified otherwise.

4.1 Absorption

4.1.1 Single-dose studies (CTD 4.2.1.1-19, 4.2.1.1-29, 4.2.2.2-01, 4.2.2.2-02, 4.2.2.2-03, 4.2.2.2-04, 4.2.2.2-06, 4.2.2.2-07)

A single dose of ^{14}C-labeled or unlabeled baloxavir was administered intravenously or orally to infection model mice (A/WSN/33 [H1N1] strain [100 $TCID_{50}$] inoculated intranasally), rats (juvenile and mature), monkeys, and ferrets. Tables 18 and 19 show PK parameters of S-033447 and radioactivity observed. Table 20 shows PK parameters of S-033447 in plasma of rats and monkeys given a single intravenous or oral dose of S-033447.

In infection model mice, C_{max} and AUC_{inf} of S-033447 increased roughly dose-proportionally over the dose range of 0.5 to 15 mg/kg and less than dose-proportionally over the dose range of 15 to 50 mg/kg.

Following an oral dose of baloxavir to juvenile rats (10, 20, and 30 days old), C_{max} and AUC_{inf} of S-033447 were higher, and $t_{1/2}$ longer, in 10-day-old rats than in 20- and 30-day-old rats. Following the single oral dose of baloxavir, unchanged baloxavir was detected in plasma only in 10-day-old rats, and C_{max} and AUC_{0-24} of baloxavir were 0.75% and <0.04% of those of S-033447. Following an intravenous administration of S-033447 (0.254 mg/kg) to juvenile rats, AUC_{inf} of S-033447 was higher, and $t_{1/2}$ longer, in 10-day-old rats than in 20- and 30-day-old rats.

Following a single oral dose of baloxavir in mature rats and monkeys, C_{max} and AUC of S-033447 increased roughly dose-proportionally over the range tested, and were lower in fed animals than in fasted animals. The applicant provided the following explanation for the decreased C_{max} and AUC of S-033447 in fed animals receiving baloxavir, based on the combination therapy study of baloxavir and metal ions in monkeys [see Section 4.1.2]:

> In the fed animals, orally administered baloxavir was hydrolyzed in the digestive tract to S-033447, which was chelated with metal ions in the food, resulting in decreased solubility and decreased membrane permeability of S-033447. This led to the decreased C_{max} and AUC of S-033447.

Following a single oral dose of ^{14}C-labeled baloxavir in rats and monkeys at 1 and 3 mg/kg, respectively, AUC_{0-24} of S-033447 in plasma was 121 ng·h/mL in rats and 1390 ng·h/mL in monkeys, suggesting that approximately 90% (rats) and 80% (monkeys) of plasma radioactivity are present as S-033447.

After a single dose of baloxavir, the plasma concentration of unchanged baloxavir was below the LLOQ at all sampling time points in mature rats and monkeys, whereas in ferrets the concentration was measurable. In ferrets, C_{max} and AUC_{0-24} of unchanged baloxavir were 165 ng/mL and 715 ng·h/mL,

respectively, in the 10 mg/kg group; and 298 ng/mL and 1260 ng·h/mL, respectively, in the 30 mg/kg group.

Following an intravenous administration of S-033447 to rats and monkeys, $t_{1/2}$, CL, and $V_{d,ss}$ of S-033447 in plasma were almost constant regardless of the dose. In rats, C_{max} and AUC_{0-24} of S-033447 following a single oral dose of S-033447 (0.846 mg/kg) were 1/20.5 and 1/15.3 times, respectively, of the values following a single oral dose of baloxavir (1 mg/kg); based on this finding, the applicant explained that administration of the prodrug (baloxavir) of S-033447 increases the exposure to S-033447, compared with administration of S-033447.

Table 18. PK parameters of S-033447 following a single oral dose of baloxavir

Animal species		Dose (mg/kg)	N	Food consumption	C_{max} (ng/mL)	t_{max}[a] (h)	AUC_{0-24} (ng·h/mL)	AUC_{inf} (ng·h/mL)	$t_{1/2}$ (h)	BA (%)
Infection model mice		0.5	3 females/ time point	Fed	5.05	1.0	22.0[b]	22.7	2.24[c]	-
		1.5	3 females/ time point		14.3	1.0	62.6[b]	66.1	2.56[d]	-
		5	3 females/ time point		45.7	0.50	242[b]	252	2.45[d]	-
		15	3 females/ time point		175	0.50	648[b]	670	2.26[d]	-
		50	3 females/ time point		284	2.0	1580[b]	1690	3.14[d]	-
Rats (juvenile)	10 days old	3	3 males/ time point	Fed	688	2.0	9420	11,200	9.22	68.3
		10	3 males/ time point		1880	4.0	23,800	26,800	7.45	49.0
		30	3 males/ time point		4570	1.0	48,100	51,800	6.35	31.6
	20 days old	3	3 males/ time point		202	1.0	1450	1470	4.29	35.3
		10	3 males/ time point		707	1.0	3490	3520	3.61	25.3
		30	3 males/ time point		962	0.50	7640	7670	2.85	18.4
	30 days old	3	3 males/ time point		90.4	0.50	472	473	2.87	27.7
		10	3 males/ time point		313	1.0	1490	1490	2.86	26.1
		30	3 males/ time point		609	0.50	3150	3160	2.74	18.5
Rats (mature)		0.3	4 males	Fed	4.82 ± 0.85	1.0 [1.0-1.0]	34.7 ± 7.4	48.9 ± 21.2	10.4 ± 5.7	10.9 ± 2.3
		1	4 males	Fasted	34.3 ± 13.8	0.38 [0.25-0.50]	125 ± 11	122 ± 13	3.68 ± 0.84	11.9 ± 1.0
		1	4 males	Fed	17.1 ± 4.9	1.0 [1.0-2.0]	103 ± 20	101 ± 18	4.36 ± 0.57	9.77 ± 1.93
		3	4 males	Fed	68.9 ± 5.8	1.0 [0.50-1.0]	468 ± 183	479 ± 195	4.02 ± 0.62	14.7 ± 5.8
		10	4 males	Fed	169 ± 34	1.0 [0.50-1.0]	1110 ± 120	1120 ± 120	3.21 ± 0.10	10.5 ± 1.1
Monkeys		0.3	3 males	Fed	5.81 ± 1.26	2.0 [0.50-4.0]	86.2 ± 11.8	158 ± 86	19.6 ± 12.8	10.4 ± 1.4
		1	3 males	Fasted	108 ± 48	2.0 [2.0-4.0]	1450 ± 610	1770 ± 730	9.32 ± 0.32	50.6 ± 15.1
		1	3 males	Fed	19.6 ± 4.2	6.0 [2.0-8.0]	309 ± 59	438 ± 112	12.7 ± 2.0	11.1 ± 1.6
		3	3 males	Fed	57.6 ± 5.4	4.0 [2.0-4.0]	957 ± 118	1340 ± 150	12.5 ± 0.8	11.5 ± 1.3
Ferrets		10	4 females	Fasted	66.6 ± 14.6	1.5 [1.0-2.0]	421 ± 119	460 ± 130	6.91 ± 3.79	-
		30	4 females		365 ± 316	1.5 [1.0-4.0]	3240 ± 3240	3300 ± 3290	4.44 ± 0.67	-

Mean ± standard deviation (SD)
-, Not tested or not applicable.
a) Median [range]; b) AUC_{0-12}; c) $t_{1/2}$ from 4 to 10 hours after administration; d) $t_{1/2}$ from 6 to 12 hours after administration
Bioavailability (BA): The dose-adjusted ratio of "AUC_{0-24} (AUC_{inf} in juvenile rats) of S-033447 following a single oral dose of baloxavir" to "AUC_{0-24} (AUC_{inf} in juvenile rats) of S-033447 following a single intravenous dose of S-033447 (0.254 mg)."

Table 19. PK parameters of radioactivity following a single oral dose of ^{14}C-labeled baloxavir

Animal species	Dose (mg/kg)	N	Food consumption	Test sample	C_{max} (ng eq./mL)	t_{max}[a] (h)	AUC_{0-24} (ng eq.·h/mL)	AUC_{inf} (ng eq.·h/mL)	$t_{1/2}$ (h)
Rats	1	4 males	Fed	Blood	20.8 ± 3.3	1.0 [0.25-1.0]	153 ± 16	200 ± 69	10.1 ± 5.5
				Plasma	20.6 ± 3.1	1.0 [0.25-1.0]	135 ± 14	149 ± 38	6.42 ± 3.31
Monkeys	3	3 males	Fed	Blood	118 ± 92	4.0 [2.0-6.0]	1950 ± 1000	2270 ± 1150	17.3 ± 2.7
				Plasma	119 ± 98	4.0 [2.0-6.0]	1680 ± 820	1990 ± 1020	17.8 ± 4.0

Mean ± SD
a) Median [range]

Table 20. PK parameters of S-033447 following a single intravenous or oral dose of S-033447

Animal species	Route of administration	Dose (mg/kg)	N	Food consumption	C_{max} (ng/mL)	t_{max}[a)] (h)	AUC_{0-24} (ng·h/mL)	AUC_{inf} (ng·h/mL)	$t_{1/2}$ (h)	CL (mL/h/kg)	$V_{d,ss}$ (mL/kg)
Rats	Intravenous	0.254	4 males	Fed	-	-	318 ± 29	314 ± 38	3.74 ± 0.27	819 ± 97	3690 ± 270
		0.846	4 males	Fed	-	-	974 ± 98	985 ± 97	4.03 ± 0.15	865 ± 83	4040 ± 290
	Oral	0.846	4 males	Fed	0.833 ± 0.064	5.0 [2.0-8.0]	6.75 ± 2.70	-	-	-	-
Monkeys	Intravenous	0.254	3 males	Fed	-	-	836 ± 116	1050 ± 150	12.0 ± 0.8	245 ± 36	3400 ± 550
		0.846	3 males	Fed	-	-	2810 ± 320	3480 ± 410	11.3 ± 0.7	245 ± 27	3210 ± 370

Mean ± SD
-, Not tested or not applicable.
a) Median [range]

4.1.2 Single-dose study (coadministration of baloxavir and metal ion) (CTD 4.2.2.2-05)

A single oral dose of baloxavir (1 mg/kg) was administered alone or in combination with a metal ion(s) to fasted monkeys. Table 21 shows PK parameters of S-033447. C_{max} and AUC_{inf} of S-033447 were lower in monkeys receiving baloxavir and a metal ion(s) than in those receiving baloxavir alone, while the type of metal ion did not cause a marked difference either in C_{max} or in AUC_{inf}.

Table 21. PK parameters of S-033447 following a single oral dose of baloxavir

Coadministered compound(s)	None (baloxavir alone)	Calcium carbonate (41.7 mg/kg)	Magnesium hydroxide (26.7 mg/kg) + aluminum oxide (14.9 mg/kg)	Ferrous fumarate (4.5 mg/kg)	Mixed metallic compounds[a)]
No. of animals	3 males	3 males	3 males	3 males	3 males
C_{max} (ng/mL)	80.1 ± 46.3	50.1 ± 17.2	49.5 ± 20.1	38.3 ± 12.5	43.1 ± 18.6
t_{max}[b)] (h)	2.0 [1.0-4.0]	2.0 [2.0-2.0]	2.0 [2.0-2.0]	1.0 [1.0-2.0]	2.0 [2.0-4.0]
AUC_{inf} (ng·h/mL)	1220 ± 720	772 ± 247	755 ± 308	596 ± 217	757 ± 356

Mean ± SD
a) Mixture of calcium carbonate (40.6 mg/kg), magnesium hydroxide (2.7 mg/kg), and ferrous fumarate (0.16 mg/kg), b) median [range]

4.1.3 Repeated-dose studies (CTD 4.2.3.2-02, 4.2.3.2-05, 4.2.3.5-03, 4.2.3.5-05)

Baloxavir was orally administered once daily repeatedly to rats (juvenile and mature), pregnant rabbits (Gestation Day 7), and monkeys. Table 22 shows PK parameters of S-033447 observed. In rats and monkeys, no clear sex difference was observed in PK of S-033447, and C_{max} and AUC_{0-24} increased less than dose proportionally in the dose range tested.

In juvenile (10 days old) and mature rats, C_{max} and AUC_{0-24} of S-033447 decreased after repeated administration. In pregnant rabbits and monkeys, C_{max} and AUC_{0-24} of S-033447 did not decrease after repeated administration. In monkeys, C_{max} and AUC_{0-24} were similar at 14 and 28 days after administration.

Table 22. PK parameters of S-033447 in repeated oral doses of baloxavir

Animal species	Dose (mg/kg/day)	Day of sampling	Number of animals	C_{max} (ng/mL)		t_{max}[a] (h)		AUC_{0-24} (ng·h/mL)	
				Male	Female	Male	Female	Male	Female
Rats (juvenile)	20	1	3/sex/time point	2140	2290	1.00	1.00	26,100	27,800
		21	3/sex/time point	218	194	1.00	1.00	1230	844
		40	3/sex/time point	109	104	1.00	1.00	667	554
	200	1	3/sex/time point	7020	7090	1.00	4.00	87,100	103,000
		21	3/sex/time point	295	300	1.00	2.00	2420	2740
		40	3/sex/time point	295	382	1.00	2.00	2570	2760
	1000	1	3/sex/time point	8240	9690	1.00	1.00	133,000	147,000
		21	3/sex/time point	331	512	2.00	1.00	3590	5980
		40	3/sex/time point	248	282	1.00	2.00	2950	3050
Rats (mature)	20	1	4/sex	240 ± 61.8	189 ± 59.6	1.0 [1.0-1.0]	1.0 [1.0-1.0]	1340 ± 364	990 ± 111
		14	4/sex	137 ± 27.1	84.8 ± 19.5	1.0 [1.0-1.0]	1.0 [1.0-2.0]	807 ± 189	576 ± 89.7
		28	4/sex	124 ± 14.7	123 ± 37.4	1.0 [1.0-1.0]	1.0 [1.0-1.0]	817 ± 184	722 ± 179
	200	1	4/sex	713 ± 226	730 ± 166	2.0 [2.0-2.0]	2.0 [2.0-2.0]	5900 ± 1760	6180 ± 894
		14	4/sex	195 ± 46.3	229 ± 46.8	1.0 [1.0-2.0]	2.0 [1.0-2.0]	1740 ± 550	1830 ± 350
		28	4/sex	198 ± 42.6	204 ± 22.0	1.0 [1.0-1.0]	1.5 [1.0-2.0]	1660 ± 224	1720 ± 598
	2000	1	4/sex	1030 ± 124	1240 ± 185	1.5 [1.0-4.0]	2.0 [1.0-4.0]	11,100 ± 2550	17,300 ± 2920
		14	4/sex	307 ± 42.8	305 ± 86.5	1.5 [1.0-4.0]	2.0 [2.0-4.0]	3750 ± 818	3300 ± 505
		28	4/sex	275 ± 56.9	460 ± 70.0	4.0 [1.0-4.0]	2.0 [2.0-4.0]	3400 ± 855	4760 ± 763
Pregnant rabbits	30	1	5 females	-	586 ± 140	-	2.0 [1.0-4.0]	-	6290 ± 1760
		13	5 females	-	485 ± 163	-	1.0 [1.0-2.0]	-	4600 ± 1470
	100	1	4 females	-	1240 ± 219	-	2.5 [1.0-6.0]	-	14,400 ± 1290
		13	4 females	-	883 ± 155	-	1.0 [1.0-1.0]	-	9260 ± 1140
	1000	1	5 females	-	1830 ± 425	-	4.0 [1.0-8.0]	-	35,600 ± 7190
		13	5 females	-	1350 ± 416	-	4.0 [1.0-4.0]	-	20,900 ± 6360
Monkeys	1	1	3/sex	50.7 ± 8.96	52.3 ± 5.92	1.0 [1.0-2.0]	2.0 [2.0]	555 ± 135	589 ± 30.3
		14	3/sex	66.7 ± 13.1	69.4 ± 20.1	4.0 [2.0-4.0]	2.0 [1.0-2.0]	632 ± 78.9	740 ± 151
		28	3/sex	51.5 ± 4.88	52.7 ± 19.6	2.0 [1.0-2.0]	2.0 [2.0]	527 ± 109	456 ± 158
	10	1	3/sex	236 ± 40.9	189 ± 44.8	4.0 [2.0-4.0]	4.0 [2.0-6.0]	3430 ± 638	2660 ± 551
		14	3/sex	277 ± 72.0	285 ± 41.7	4.0 [2.0-4.0]	2.0 [2.0-8.0]	4140 ± 553	3690 ± 816
		28	3/sex	242 ± 10.4	229 ± 13.3	1.0 [1.0-2.0]	4.0 [1.0-4.0]	3050 ± 290	3140 ± 962
	100	1	5/sex	496 ± 225	449 ± 95.7	8.0 [4.0-8.0]	6.0 [2.0-8.0]	8960 ± 4520	7680 ± 768
		14	5/sex	876 ± 535	627 ± 109	4.0 [4.0-6.0]	4.0 [1.0-8.0]	14,800 ± 8040	10,200 ± 1460
		28	5/sex	714 ± 242	642 ± 169	6.0 [2.0-6.0]	8.0 [1.0-8.0]	11,600 ± 3000	9060 ± 2020

Mean ± SD
a) Median [range]

4.1.4 *In vitro* membrane permeability (CTD 4.2.2.7-01)

Membrane permeability of baloxavir and S-033447 was investigated using human colon cancer-derived Caco-2 cell lines. The apparent apical-to-basal permeability coefficient was 5.09×10^{-6} cm/s for baloxavir (2.8 μmol/L), 4.88×10^{-6} cm/s for minoxidil (a drug with high membrane permeability, 10 μmol/L), 0.809×10^{-6} cm/s for S-033447 (2.8 μmol/L), and 0.146×10^{-6} cm/s for atenolol (a drug with intermediate membrane permeability, 100 μmol/L).

4.2 Distribution

4.2.1 Tissue distribution (CTD 4.2.2.3-01)

A single dose of ^{14}C-labeled baloxavir was administered orally to pigmented rats (n = 1 male/time point), and tissue distribution of radioactivity was evaluated by whole body autoradiography. In most of the tissues, radioactivity concentration peaked at ≤2 hours after administration. The radioactivity concentration was high in small intestinal mucosa (3350 ng eq./g), the liver (879 ng eq./g), gastric mucosa (197 ng eq./g), and renal cortex (144 ng eq./g), and below the LLOQ (8.51 ng eq./g) in the cerebrum, cerebellum, and spinal cord at any sampling time point. The radioactivity concentration was below the LLOQ at ≤24 hours in most of the tissues, and at 336 hours in all tissues. The radioactivity concentration in melanin-containing uvea and pigmented skin was below the LLOQ at 24 hours after administration; this suggested that baloxavir and its metabolites do not accumulate in melanin-containing tissues.

4.2.2 Serum protein binding and distribution in blood cells (CTD 4.2.2.2-01, 4.2.2.2-03, 5.3.2.1-01)

The protein binding of ^{14}C-labeled S-033447 (50-1000 ng/mL) in the serum of rats, monkeys, and humans was 91.9% to 92.1%, 85.0% to 89.5%, and 92.9% to 93.9%, respectively. In human serum, the protein biding of ^{14}C-labeled S-033447 (50-1000 ng/mL) was 91.2% to 92.1% for serum albumin, 52.2% to 59.3% for α1 acid glycoprotein, and 23.6% to 38.1% for γ-globulin.

The distribution of S-033447 in blood cells in whole blood of rats, monkeys, and humans was 57.1% to 59.6%, 50.4% to 52.9%, and 48.5 to 54.4%, respectively.

Following a single oral dose of ^{14}C-labeled baloxavir (1 mg/kg) in rats and ^{14}C-labeled baloxavir (3 mg/kg) in monkeys, the distribution of the radioactivity in blood cells was 34.2% to 49.5% in rats (at 15 minutes to 24 hours after administration) and 38.4% to 52.3% in monkeys (at 15 minutes to 48 hours after administration).

4.2.3 Placental transfer (CTD 4.2.2.3-02)

Placental transfer of the radioactivity was investigated following a single oral dose of ^{14}C-labeled baloxavir (1 mg/kg) in rats of Gestation Day 18 (n = 1/time point). The radioactivity was distributed in fetal tissues; the radioactivity concentrations in fetal bones and adrenal glands were ≥4 times the maternal plasma concentration.

These results suggest that baloxavir and/or its metabolites cross the placenta to the fetus.

4.3 Metabolism[13)]

4.3.1 Possible metabolic pathways

Based on the results of the investigations in Sections 4.3.2 and 4.3.3, the metabolic pathway of baloxavir was proposed as shown in Figure 3.

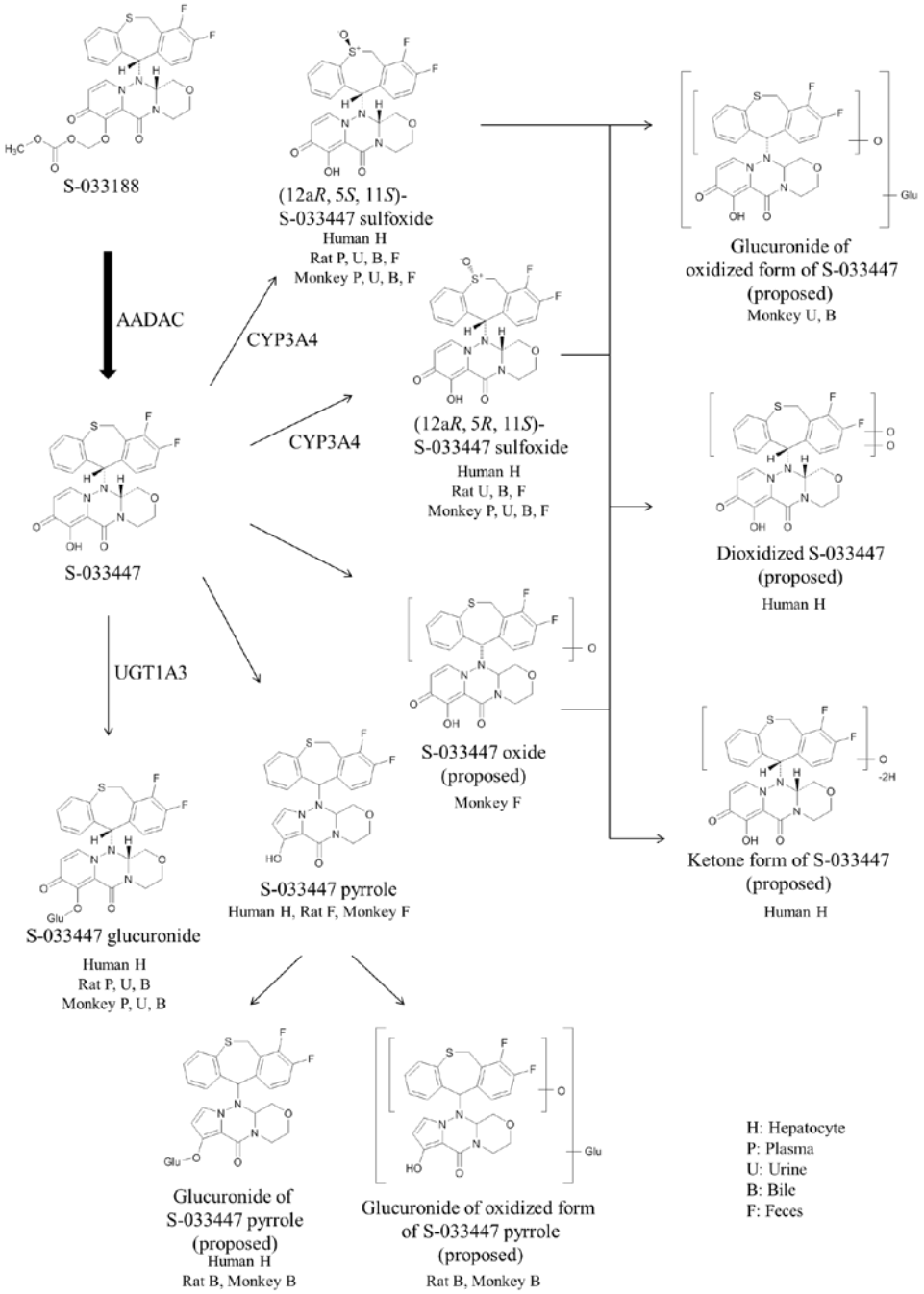

Figure 3. Possible metabolic pathways of baloxavir (cited from CTD 2.6.4 Figure 2.6.4.5-1)

AADAC, arylacetamide deacetylase

[13)] Metabolites described in this section are as follows:
M1, (12a*R*,5*S*,11*S*)-S-033447 sulfoxide; M2, (12a*R*,5*R*,11*S*)-S-033447 sulfoxide; M3, dioxidized S-033447 (proposed); M4, S-033447 glucuronide; M5, glucuronide of S-033447 pyrrole (M7) (proposed); M6, ketone form of S-033447 (proposed); M7, S-033447 pyrrole; M8 and M12, glucuronides of oxidized form of S-033447 pyrrole (M7) (proposed); M9, glucuronide of oxidized form of S-033447 (proposed); M10 and M11, S-033447 oxides (proposed)

4.3.2 *In vitro* metabolism (CTD 5.3.2.2-01, 5.3.2.2-02, 5.3.2.2-03, 5.3.2.2-04, 5.3.2.2-05)

Human serum, liver S9 fraction, and small intestine S9 fraction were incubated with ^{14}C-labeled baloxavir (10 or 100 μmol/L) for 1 hour, to investigate metabolism of baloxavir. The rate of S-033447 formation was 71.6% (serum), 100% (the liver), and 81.8% (the small intestine), suggesting that baloxavir is hydrolyzed to S-033447 in serum, the liver, and the small intestine. Human liver S9 fraction and small intestine S9 fraction were incubated with ^{14}C-labeled baloxavir in the presence of inhibitor of various enzymes. Baloxavir hydrolysis was not inhibited by an inhibitor of carboxyesterase 1 (digitonin), but inhibited by 9.0% to 13.2% by an inhibitor of carboxyesterase 2 (telmisartan) and by 45.9% to 75.6% by the inhibitor of carboxyesterase 2 and arylacetamide deacetylase (vinblastine). The applicant explained that these results suggest that arylacetamide deacetylase is mainly involved in the hydrolysis of baloxavir to S-033447.

Human liver cells were incubated with ^{14}C-labeled baloxavir or S-033447 (5 or 50 μmol/L) for 4 hours, to investigate metabolites formed. The main metabolite observed was S-033447 (accounting for 86.1% to 86.4% [baloxavir 5 μmol/L] and 85.3% to 89.0% [S-033447 5 μmol/L]). Other metabolites detected were M1, M2, M3, M4, M5, M6, and M7.

In order to identify enzymes involved in the oxidation and glucuronidation of S-033447 in humans, the following studies were conducted. Results suggested that S-033447 is oxidized mainly by CYP3A and glucuronidated mainly by uridine diphosphate glucuronosyltransferase (UGT) 1A3.

- Human liver microsomes were incubated with ^{14}C-labeled S-033447 in the presence of an inhibitor of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 or CYP3A.[14)] In the presence of the inhibitor of CYP3A, formation of M1, M2, and M7 was inhibited by 74.2%, 77.3%, and 75.4%, respectively. Inhibitors of the other CYP isoforms did not markedly inhibit the formation of M1 or M2.
- Recombinant human CYP isoforms (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) were incubated with ^{14}C-labeled S-033447. Results showed that, in the CYP3A4 expression system, M1 and M2 were produced in a greater amount than in other CYP isoform-expression systems.
- Recombinant human UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7, and UGT2B15) were incubated with ^{14}C-labeled S-033447. As a result, M4 was formed only in the UGT1A3-expression system.

4.3.3 *In vivo* metabolism (CTD 4.2.2.4-01, 4.2.2.4-02)

A single oral dose of ^{14}C-labeled baloxavir (5 mg/kg) was administered to bile duct-cannulated and non-cannulated rats (2 and 8 males, respectively), to identify metabolites of baloxavir in plasma, bile, urine, and feces. In non-cannulated rats, S-033447 was the main metabolite observed in plasma at 4 hours after administration (90.5% of plasma radioactivity), and M1 and M4 were also detected as metabolites (6.5% and 3.1%, respectively). In urine samples collected up to 48 hours after administration, a trace amount of S-033447, M1, M2, M4, and unidentified metabolites was detected. In feces collected up to 48 hours

[14)] The following compounds were used as inhibitors of CYP isoforms:
CYP1A2, α-naphthoflavone; CYP2B6, thiotepa; CYP2C8, montelukast; CYP2C9, sulphaphenazole; CYP2C19, benzylnirvanol; CYP2D6, quinidine; CYP3A, ketoconazole

after administration, mainly S-033447 was detected (69.3% of the administered radioactivity) together with M1, M2, M7, and unidentified metabolites. In bile duct-cannulated rats, 13.3% of the administered radioactivity was excreted into bile by 48 hours after administration, and S-033447, M1, M2, M4, M5, and M8 were detected in the bile.

A single oral dose of ^{14}C-labeled baloxavir (3 mg/kg) was administered to bile duct-cannulated and non-cannulated monkeys (3 males each), to identify metabolites of baloxavir in plasma, bile, urine, and feces. In non-cannulated monkeys, S-033447 was the main metabolite detected in plasma at 24 hours after administration (83.0% of plasma radioactivity), and M1, M2, M4, and unidentified metabolites were detected as metabolites within 24 hours after administration. In urine samples collected up to 48 hours after administration, a trace amount of S-033447, M1, M2, M4, and M9 was detected. In feces collected up to 96 hours after administration, mainly S-033447 was detected (60.0% of the administered radioactivity) together with unchanged baloxavir, M1, M2, M7, M10, and M11. In bile duct-cannulated rats, S-033447, M1, M2, M4, M5, M8, M9, and M12 were detected in the bile within 72 hours after administration.

4.4 Excretion

4.4.1 Biliary, urinary, and fecal excretion (CTD 4.2.2.2-03, 4.2.2.5-01, 4.2.2.5-02)

The applicant explained that the following results suggest that most of baloxavir is excreted into feces without being absorbed into the body, and that the absorbed baloxavir is excreted mainly into feces via bile:

- Following a single oral dose of ^{14}C-labeled baloxavir (1 mg/kg) in 4 male rats, the urinary and fecal excretion rates of radioactivity within 168 hours after administration were 0.4% and 96.6%, respectively.
- Following a single oral dose of ^{14}C-labeled baloxavir (1 mg/kg) in 5 bile duct-cannulated male rats, the urinary, biliary, and fecal excretion rates of radioactivity within 48 hours after administration were 0.6%, 18.5%, and 55.7%, respectively.
- Following a single oral dose of ^{14}C-labeled baloxavir (3 mg/kg) in 3 male monkeys, the urinary and fecal excretion rates of radioactivity within 168 hours after administration were 1.0% and 94.4%, respectively.
- Following a single oral dose of ^{14}C-labeled baloxavir (3 mg/kg) in 3 bile duct-cannulated male monkeys, the urinary, biliary, and fecal excretion rates of radioactivity within 72 hours after administration were 1.2%, 8.5%, and 89.5%, respectively.

Bile duct-cannulated rats (4 each of donors and recipients) were connected in such a way that the bile of the donor rat flowed into the duodenal lumen of the recipient rat, and a single oral dose of ^{14}C-labeled baloxavir (1 mg/kg) was administered to donor rats. As a result, 1.4% of the administered radioactivity was resorbed by the recipient rats via bile of donor rats. The enterohepatic circulation rate was 4.2% of the radioactivity transferred to recipient rats (21.2% of the administered radioactivity was transferred to recipient rats) and 0.9% of the administered radioactivity.

4.4.2 Excretion in milk (CTD 4.2.2.3-03)

Following a single oral dose of ^{14}C-labeled baloxavir (1 mg/kg) in lactating rats at 11 days post-partum (n = 5/time point), radioactivity in milk peaked (124 ng eq./mL) at 2 hours after administration. The milk-to-plasma ratio of radioactivity at 2, 4, and 8 hours after administration was 5.1, 6.9, and 7.4, respectively. The above results showed that baloxavir and/or its metabolites were excreted in milk.

4.5 Pharmacokinetic interactions

4.5.1 Enzyme inhibition and induction (CTD 4.2.2.4-03, 5.3.2.2-06, 5.3.2.2-07, 5.3.2.2-08)

The inhibitory effect of baloxavir and S-033447 (both at 0.1-100 μmol/L) against metabolism of substrates of CYP isoforms[15)] (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A) was investigated using human liver microsomes. Baloxavir inhibited the activity of CYP2B6 (IC_{50} 46.0 μmol/L), CYP2C8 (IC_{50} 63.2 μmol/L), and CYP3A (IC_{50}, 50.2 μmol/L [substrate, testosterone] or 23.2 μmol/L [substrate, midazolam]). S-033447 inhibited the activity of CYP2B6 (IC_{50} 29.3 μmol/L) and CYP3A (IC_{50} 43.2 μmol/L [substrate, midazolam]). Neither baloxavir nor S-033447 showed a clear inhibitory effect against other CYP isoforms. Both drugs did not inhibit any CYP isoforms in a time-dependent manner.

The inhibitory effect of baloxavir (0.1-100 μmol/L) and S-033447 (0.03-30 μmol/L) against metabolism of the substrates of UGT isoforms[16)] (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7, and UGT2B15) was investigated using human liver microsomes. Baloxavir inhibited the activity of UGT2B15 (IC_{50} 94 μmol/L) but did not inhibit the activity of the other UGT isoforms. S-033447 did not show a clear inhibitory effect against any UGT isoforms tested.

Based on the above results and on the C_{max} (0.25 μmol/L) of S-033447 in subjects receiving 40 mg baloxavir[17)] in the Japanese phase I study (Study T0811) [see Section 6.2.1.1], the applicant explained that neither baloxavir nor S-033447, at the clinical dose, is likely to inhibit the metabolizing enzymes.

Using human liver cells, the inducing effect of baloxavir (0.1-3 μmol/L) and S-033447 (0.1-30 μmol/L) on CYP isoforms (CYP1A2, CYP2B6, and CYP3A4) was investigated by measuring mRNA expression level and enzyme activity. Baloxavir did not induce mRNA expression or enzymatic activity of any of the CYP isoforms tested. In contrast, S-033447 increased mRNA expression level of CYP1A2, CYP2B6, and CYP3A4 in a concentration-dependent manner (1.48-2.51, 0.901-2.22, and 1.62-2.53 times, respectively, that of the vehicle control), but its inducing effect was <20% of that of positive controls.[18)] S-033447 did not induce the enzyme activity of any of the CYP isoforms tested.

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15) CYP1A2, phenacetin; CYP2B6, bupropion; CYP2C8, paclitaxel; CYP2C9, tolbutamide; CYP2C19, *S*-mephenytoin; CYP2D6, dextromethorphan; CYP3A, testosterone and midazolam

16) UGT1A1, 17β-estradiol; UGT1A3, chenodeoxycholic acid; UGT1A4, trifluoperazine; UGT1A6, 1-naphthol; UGT1A9, propofol; UGT2B7, morphine; UGT2B15, oxazepam

17) The applicant's explanation: The proposed dosage and administration of baloxavir is "40 mg/day in adults weighing <80 kg and 80 mg/day in adults weighing ≥80 kg." However, C_{max} and AUC_{inf} estimated using population pharmacokinetics parameters did not show clear difference between patients weighing 40 to 80 kg and patients weighing ≥80 kg. Therefore, the possibility of drug interactions was investigated using C_{max} observed following administration of 40 mg baloxavir in the Japanese phase I study (Study T0811) in which all subjects in the 40 mg group weighed <80 kg.

18) CYP1A2, omeprazole; CYP2B6, phenobarbital; CYP3A4, rifampicin

Based on the above results and on the C_{max} (0.25 μmol/L) of S-033447 in subjects receiving 40 mg baloxavir in the Japanese phase I study (Study T0811) [see Section 6.2.1.1], the applicant explained that neither baloxavir nor S-033447, at the clinical dose, is likely to induce the metabolizing enzymes.

Baloxavir (2000 mg/kg/day) was orally administered for 2 weeks to male and female rats, to investigate the effect on hepatic drug-metabolizing enzymes. Baloxavir increased protein content in liver microsomes (mg/g liver) 1.1-fold in males and 1.2-fold in females, and CYP content (nmol/mg protein) 1.8-fold in males and 1.6-fold in females. Baloxavir increased CYP2B activity per mg protein 3.3-fold in males and 4.6-fold in females, and CYP3A activity 3.3-fold in males and 31-fold in females, but did not markedly affect CYP1A or CYP2C activity. Baloxavir increased UGT activity per mg protein 1.1- to 4.4-fold in males and 1.4- to 5.5-fold in females.

4.5.2 Substrate activity for drug transporters (CTD 5.3.2.2-09)

According to the applicant, the following studies suggested that baloxavir and S-033447 serve as substrates for P-glycoprotein (P-gp):

- Transport of baloxavir (2 or 10 μmol/L) mediated by each transporter was investigated using Caco-2 cells. In the absence of P-gp and breast cancer resistance protein (BCRP) inhibitors, the efflux ratio (basal-to-apical/apical-to-basal) of baloxavir was 6.2 (2 μmol/L) and 4.4 (10 μmol/L). In the presence of a P-gp inhibitor (verapamil), the efflux ratio decreased to 1.2 at both baloxavir concentrations. In the presence of a BCRP inhibitor (Ko143), the efflux ratio was 6.8 (2 μmol/L) and 3.8 (10 μmol/L), showing no clear change.
- Transport of S-033447 (2 or 10 μmol/L) mediated by each transporter was investigated using Caco-2 cells. In the absence of P-gp and BCRP inhibitors, the efflux ratio of S-033447 was 6.3 at both S-033447 concentrations. In the presence of a P-gp inhibitor (verapamil), the efflux ratio decreased to 2.4 (2 μmol/L) and 2.0 (10 μmol/L). In the presence of a BCRP inhibitor (Ko143), the efflux ratio was 4.7 (2 μmol/L) and 6.2 (10 μmol/L), showing no clear change.
- Transport of S-033447 (1 or 5 μmol/L) mediated by each transporter was investigated using human fetal kidney-derived HEK293 cell lines expressing organic anion transporting polypeptides (OATP)1B1 and OATP1B3. No clear difference was observed in the intracellular uptake of S-033447 between OATP1B1- or OATP1B3-expressing cells and non-expressing cells.

4.5.3 Inhibition of drug transporters (CTD 5.3.2.2-10, 5.3.2.2-11)

The inhibitory effect of baloxavir (0.994-78.1 μmol/L) and S-033447 (0.282-20.9 μmol/L) against the transport of the substrate of P-gp or BCRP (^{3}H-labeled digoxin or estrone sulfate) was investigated using Caco-2 cells. Baloxavir inhibited P-gp (IC_{50} 8.75 μmol/L) and S-033447 inhibited BCRP (IC_{50} 7.10 μmol/L).

The inhibitory effect of S-033447 against the transport of transporter substrates[19)] was investigated using HEK293 cells expressing OATP1B1, OATP1B3, organic cation transporter (OCT)1, OCT2, organic

[19)] OATP1B1 and OATP1B3, estradiol 17β-D-glucuronide; OAT1, para-aminohippuric acid; OAT3, estrone sulfate; OCT1, OCT2, MATE1, and MAE2-K, metformin

anion transporter (OAT)1, OAT3, multidrug and toxin extrusion protein (MATE)1, or MATE2-K.[20] S-033447 inhibited the transport of the substrates of OATP1B1, OCT1, MATE1, and MATE2-K (IC_{50}, 6.81, 6.52, 11.2, and 1.91 μmol/L, respectively).

The inhibitory effect of S-033447 (0.472-78.0 μmol/L) against the transport of the substrate of bile salt export pump (BSEP) (taurocholic acid) was investigated using membrane vesicles prepared from insect ovary-derived Sf9 cells. S-033447 did not show a clear inhibitory effect against the transport of the substrate of BSEP.

The applicant's explanation:
The above results, together with the C_{max} (0.25 μmol/L) of S-033447 in subjects receiving 40 mg baloxavir in the Japanese phase I study (Study T0811) [see Section 6.2.1.1] and the estimated gastrointestinal concentration[21] (280 μmol/L), suggested the possibility of drug-drug interactions due to the inhibition of gastrointestinal P-gp and BCRP, respectively, by baloxavir and S-033447 in humans receiving the clinical dose of baloxavir. Therefore, pharmacokinetic interactions were investigated in clinical studies.

4.5.4 Pharmacokinetic interactions mediated by protein binding (CTD 5.3.2.1-02)

An *in vitro* study suggested that serum albumin was the main S-033447-binding protein in human serum [see Section 4.2.2]. Therefore, protein binding-mediated pharmacokinetic interactions between S-033447 and warfarin, diazepam, or digoxin were investigated because these drugs bind to human serum albumin at different sites. Human serum albumin was reacted with ^{14}C-labeled S-033447 (0.1 μg/L) and then spiked with warfarin (1-100 μg/L), diazepam (1-100 μg/L), or digoxin (0.001-0.1 μg/L). In drug-spiked samples, the protein binding of ^{14}C-labeled S-033447 was 0.99 to 1.10 times (warfarin), 0.87 to 1.07 times (diazepam), and 0.98 to 1.01 times (digoxin) that in unspiked samples, showing no clear difference between the spiked and unspiked samples. Human serum albumin was reacted with radio-labeled warfarin, diazepam, or digoxin, and then spiked with S-033447 (0.1-10 μg/L). In S-033447-spiked samples, the protein binding of the radio-labeled drugs was 0.99 to 1.00 times (warfarin), 0.97 to 0.98 times (diazepam), and 0.99 to 1.00 times (digoxin) that in unspiked samples, showing no clear difference between the spiked and unspiked samples.

Based on the above results and on the C_{max} (0.123 μg/L) of S-033447 in subjects receiving 40 mg baloxavir in the Japanese phase I study (Study T0811) [see Section 6.2.1.1], the applicant explained that baloxavir is unlikely to cause protein binding-mediated pharmacokinetic interactions with warfarin, diazepam, or digoxin.

4.R Outline of the review conducted by PMDA

PMDA concluded that there are no particular problems regarding the submitted data on PK obtained from nonclinical studies on baloxavir.

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[20] The following S-033447 concentrations were used to test interaction with each transporter:
OATP1B1, OATP1B3, OAT3, and OCT2, 0.398 to 20.4 μmol/L; OAT1, 0.439 to 20.4 μmol/L; OCT1, 0.294 to 19.8 μmol/L; MATE1 and MATE2-K, 0.193 to 22.7 μmol/L.
[21] Calculated from the dose (40 mg)/250 mL.

5. Toxicity and Outline of the Review Conducted by PMDA

The applicant submitted the toxicity study data of baloxavir: the results from repeated-dose toxicity studies, genotoxicity studies, reproductive and developmental toxicity studies, and other toxicity studies (e.g., mechanistic study, skin phototoxicity study). Unless specified otherwise, 0.5% (w/v) methylcellulose solution was used as vehicle.

5.1 Single-dose toxicity

No single-dose toxicity study was conducted. No death or acute symptoms occurred in male and female rats receiving a 2-week repeated oral administration of baloxavir 2000 mg/kg/day (CTD 4.2.3.2-01); the approximate lethal dose was determined to be >2000 mg/kg in both male and female rats. Soft feces, diarrhoea, and queasy/vomiting were observed in male cynomolgus monkeys receiving a single oral dose of baloxavir 400 mg/kg (CTD 4.2.1.3-06); the approximate lethal dose was determined to be >400 mg/kg in male cynomolgus monkeys. No death or acute symptoms occurred in male and female cynomolgus monkeys receiving a 2-week repeated oral administration of baloxavir 200 mg/kg/day (CTD 4.2.3.2-03).

5.2 Repeat-dose toxicity

Repeated oral dose toxicity studies were conducted in rats and monkeys (2- and 4-week studies in both animal species). The no observed adverse effect level (NOAEL) in 4-week repeated oral dose toxicity studies was 2000 mg/kg/day in both male and female rats, and 10 mg/kg/day in both male and female monkeys. $AUC_{0\text{-}24}$ of S-033447 was 3.40 μg·h/mL in male rats, 4.76 μg·h/mL in female rats, 3.05 μg·h/mL in male monkeys, and 3.14 μg·h/mL in female monkeys. These values were 2.0, 2.8, 1.8, and 1.8 times (male rats, female rats, male monkeys, and female monkeys, respectively) the plasma exposure ($AUC_{0\text{-}24}$, 1.71 μg·h/mL)[22)] in humans receiving the oral clinical dose.

Main toxicity findings observed were prolongation of prothrombin time (PT) and activated partial thromboplastin time (APTT) in rats, and toxicity findings suggesting hepatic dysfunction and cholestasis in cynomolgus monkeys.

5.2.1 Four-week repeated oral dose toxicity study with a 4-week recovery period in rats (CTD 4.2.3.2-02)

Baloxavir (0 [vehicle], 20, 200, or 2000 mg/kg/day) was orally administered for 4 weeks to rats (n = 10/sex/group). Animals in the vehicle and 2000 mg/kg/day groups (n = 5/sex/group) were allowed to undergo a 4-week recovery period. The 2000 mg/kg/day group showed pale feces suggesting unabsorbed baloxavir. The ≥200 mg/kg/day groups showed PT and APTT prolongation. The ≥200 mg/kg/day groups also showed changes in the inflammation-related parameters of haematology/clinical chemistry (increases in fibrinogen, total protein, and platelet count, and decreased albumin/globulin ratio [A/G ratio]); these changes were considered toxicologically insignificant because histopathological examination did not show related abnormal findings. The ≥200 mg/kg/day groups showed increased liver weight, hyperplasia of thyroid follicular epithelial cells, decreased thyroid colloid, and hypertrophy of pituitary basophils; male and female animals in the 2000 mg/kg/day group showed hypertrophy of

22) $AUC_{0\text{-}24}$ estimated from AUC_{inf} following a single dose of 40 mg baloxavir in Japanese healthy adults [see Section 6.2.1.1].

hepatocytes; and females in the 2000 mg/kg/day group showed increased cholesterol. These abnormal levels and findings in the liver, thyroid, and pituitary gland are unlikely to be relevant to humans, because they are probably rat-specific compensatory changes caused by increased blood thyroid hormone clearance due to the increased activity of CYP2B, CYP3A, and UGT associated with the baloxavir-induced increase in hepatic cytochrome P450 content. Female animals in the ≥200 mg/kg/day groups showed increased amylase activity; this finding was considered toxicologically significant because no related pathological findings were observed in the pancreas or other tissues, and because the amylase activity level was within the range of the historical data of the study facility.

Based on the above results, the NOAEL was determined to be 2000 mg/kg/day.

5.2.2 Two-week repeated oral dose toxicity study in monkeys (CTD 4.2.3.2-03, 4.2.3.2-04)

Baloxavir (0 [vehicle], 20, 60, or 200 mg/kg/day) was orally administered for 2 weeks to cynomolgus monkeys (n = 3/sex/group). The ≥20 mg/kg/day groups showed increases in alanine aminotransferase (ALT), glutamate dehydrogenase (GLDH), leucine aminopeptidase, and γ-glutamyl transferase (γ-GTP). The ≥60 mg/kg/day group showed increases in aspartate aminotransferase (AST) and total bile acids. The 200 mg/kg/day group showed vomiting and increases in alkaline phosphatase, total bilirubin, direct bilirubin, phospholipids, total cholesterol, and triglycerides, which were considered to be toxicity findings suggestive of hepatic dysfunction and cholestasis.

Baloxavir (0 [vehicle], 3, or 10 mg/kg/day) was orally administered for 2 weeks to cynomolgus monkeys (n = 3/sex/group). Increases in AST, ALT, and GLDH were observed in 1 female animal in the 3 mg/kg/day group but not in the 10 mg/kg/day group, showing no dose-correlation. Therefore, these findings were considered toxicologically insignificant.

Based on the above, the NOAEL was determined to be 10 mg/kg/day.

5.2.3 Four-week repeated oral dose toxicity study with a 4-week recovery period in monkeys (CTD 4.2.3.2-05)

Baloxavir (0 [vehicle], 1, 10, or 100 mg/kg/day) was orally administered for 4 weeks to cynomolgus monkeys (n = 3/sex/group). Animals in the vehicle and 100 mg/kg/day groups (n = 2/sex/group) were allowed to undergo a 4-week recovery period. The 100 mg/kg/day group showed increases in AST, ALT, GLDH, leucine aminopeptidase, and γ-GTP, which were considered toxicological findings suggestive of hepatic dysfunction and cholestasis. These toxicity findings were reversible during the 4-week recovery period.

Based on the above, the NOAEL was determined to be 10 mg/kg/day.

5.3 Genotoxicity (CTD 4.2.3.3-01, 4.2.3.3-02, 4.2.3.3-03, 4.2.3.3-04, 4.2.3.3-05)

The applicant conducted *in vitro* studies (Ames test and micronucleus assay using human lymphoblastoma cell-derived TK6 cells) and an *in vivo* study (rat bone marrow micronucleus assay). Baloxavir was shown to be non-genotoxic by both *in vitro* and *in vivo* studies, and S-033447 by *in vitro* studies.

5.4 Carcinogenicity

Baloxavir is expected to be administered as a single dose clinically, and results of genotoxicity studies suggested that neither baloxavir nor S-033447 is likely to be genotoxic. Therefore, no carcinogenicity study was conducted.

5.5 Reproductive and developmental toxicity

The applicant conducted a study of fertility and early embryonic development to implantation in male and female rats; studies of embryo-fetal development in rats and rabbits; a study for effects on pre- and postnatal development, including maternal function in rats; and a repeated-dose toxicity study in juvenile rats. Rats showed no abnormality. Fetal rabbits showed an increased rate of short extra cervical ribs.

$AUC_{0\text{-}24}$ of S-033447 in rats (7.17 μg·h/mL) and rabbits (9.26 μg·h/mL) treated with oral baloxavir at the NOAEL for embryo-fetal development (1000 mg/kg/day in rats, 100 mg/kg/day in rabbits) was 4.2 times (rats) and 5.4 times (rabbits) the plasma exposure in humans ($AUC_{0\text{-}24}$, 1.71 μg·h/mL).[23)] Skeletal variations in fetal rabbits were observed at $AUC_{0\text{-}24}$ 12.2 times that in humans receiving the clinical dose.

In rats, baloxavir and/or its metabolites crossed the placenta and were excreted in milk [see Sections 4.2.3 and 4.4.2].

5.5.1 Fertility and early embryonic development to implantation in rats (CTD 4.2.3.5-01)

Baloxavir (0 [vehicle], 20, 200, or 1000 mg/kg/day) was orally administered to rats (n = 20/sex/group) from 4 weeks prior to mating through the mating period until 1 day before necropsy (50 days in total) in males and from 14 days prior to mating until Gestation Day 7 (22-35 days in total) in females. Male and female parents in the 1000 mg/kg/day group showed pale feces suggesting unabsorbed baloxavir. Females in the ≥200 mg/kg/day groups showed a transient decrease in food consumption, but this finding was considered toxicologically insignificant. No baloxavir-related toxicity findings were observed either in the reproductive function of males and females or in the early embryonic development.

Based on the above, NOAEL was determined to be 1000 mg/kg/day for general toxicity, reproductive toxicity, and early embryonic developmental toxicity in male and female parents.

5.5.2 Reproductive and developmental toxicity

5.5.2.1 Reproductive and developmental toxicity in rats (CTD 4.2.3.5-02)

Baloxavir (0 [vehicle], 20, 200, or 1000 mg/kg/day) was orally administered to pregnant rats (n = 20/group) from Gestation Days 6 to 17 (12 days in total). Maternal animals in the ≥200 mg/kg/day groups showed reduced body weight gain and decreased food consumption during the early stage of treatment. These findings were considered toxicologically insignificant because they were transient changes and, at the lase dose, their body weight was similar to that of animals receiving vehicle. The 1000 mg/kg/day group showed pale feces suggesting the unabsorbed baloxavir. No baloxavir-related toxicity findings were observed in maternal animals or in embryos/fetuses.

[23)] $AUC_{0\text{-}24}$ estimated from AUC_{inf} following a single dose of 40 mg baloxavir in Japanese healthy adults [see Section 6.2.1.1].

Based on the above, the NOAEL was determined to be 1000 mg/kg/day for general toxicity, reproductive toxicity, and embryo-fetal developmental toxicity in maternal animals.

5.5.2.2 Reproductive and developmental toxicity in rabbits (CTD 4.2.3.5-03)

Baloxavir (0 [vehicle], 30, 100, or 1000 mg/kg/day) was orally administered to pregnant rabbits (n = 18-20/group) from Gestation Days 7 to 19. Abortion was observed in 2 maternal animals in the 1000 mg/kg/group. These maternal animals showed a marked decrease in food consumption and body weight; the abortions were therefore considered secondary effects associated with baloxavir-induced suppression of food consumption. Maternal animals in the 1000 mg/kg/day group also showed grayish brown feces suggesting the unabsorbed baloxavir and, during the early stage of treatment, reduced body weight gain associated with decreased food consumption. Fetuses in the 1000 mg/kg/day group had an increased incidence of short extra cervical ribs.

Based on the above, the NOAEL was determined to be 100 mg/kg/day for general toxicity, reproductive toxicity, and embryo-fetal developmental toxicity in maternal animals.

5.5.3 Effects on pre- and postnatal development, including maternal function in rats (CTD 4.2.3.5-04)

Baloxavir (0 [vehicle], 20, 200, or 1000 mg/kg/day) was orally administered to pregnant rabbits (n = 20 or 22/group) from Gestation Day 6 to Post-partum Day 20. Offspring in the ≥200 mg/kg/day groups showed swelling and dark reddening of one eyeball. Ophthalmological examination of these abnormal offspring showed bleeding into the anterior chamber of the eye, corneal opacity, bleeding from the iris, etc., and histopathological examination showed bleeding from the iris, blood accumulation in the eyeball, vacuolation and necrosis of the lens, adhesion of iris to cornea or lens, etc. In offspring with no abnormal clinical signs in the eyes, no abnormal findings were found by ophthalmologic and histopathological examinations. The abnormal findings of the eyeball were probably due to the bleeding into the anterior chamber of the eye and considered unrelated to baloxavir, for the following reasons: (a) The findings were observed in one eye. (b) The percentage of offspring with the findings was 1.1% in the 200 mg/kg/day group and 2.4% in the 1000 mg/kg/day group; these values were below the historical percentage (7.4%) of offspring with findings suggestive of bleeding into the anterior chamber of the eye.

Based on the above, the NOAEL in maternal animals and in the development of the offspring was determined to be 1000 mg/kg/day.

5.5.4 Repeated oral dose toxicity study in juvenile rats (CTD 4.2.3.5-05)

Baloxavir (0 [vehicle], 20, 200, or 1000 mg/kg/day) was orally administered to rats (n = 12/sex/group) for 40 days starting from 10 days after birth. Animals in the vehicle, 20, 200, and 1000 mg/kg/day groups (n = 10/sex/group) were allowed to undergo a 4-week recovery period. The 1000 mg/kg/day group showed abdominal distension on Days 2 and 3 of administration (in both males and females) and a transient decrease in body weight and in body weight gain on Days 3 to 15 (in males) and on Day 4 to 7 (in females), but necropsy did not show any abnormality in the abdomen. Males and females in the ≥200 mg/kg/day groups showed increased red cell distribution width and prolonged APTT, males in the

1000 mg/kg group showed a decreased A/G ratio, and females in the 1000 mg/kg/day group showed an increased platelet count. These changes were considered toxicologically insignificant because they were mild compared with those observed in the vehicle group, and because no abnormal findings were revealed by histopathological examination. The ≥200 mg/kg/day groups showed enlarged thyroid follicular cells, and male animals in the 1000 mg/kg/day group showed increased thyroid weight. These findings were considered compensatory changes due to the enhanced metabolism of thyroid hormone, as in normal animals treated repeatedly, and unlikely to be relevant to humans.

Based on the above, the NOAEL was determined to be 1000 mg/kg/day.

5.6 Other toxicity studies

5.6.1 Effect of vitamin K on PT and APTT prolongation in rats (CTD 4.2.3.7-01)

Baloxavir (0 [vehicle] or 2000 mg/kg/day) alone or baloxavir (2000 mg/kg/day) in combination with vitamin K (0.3 mg/kg/day) was orally administered to male rats (n = 5/group) for 14 days. Animals in the baloxavir + vitamin K group were necropsied under fasted conditions. Animals in the baloxavir alone group were divided into the fasted and non-fasted subgroups, to evaluate the effect of fasting before necropsy. The animals receiving baloxavir and vitamin K did not show PT or APTT prolongation compared with those receiving vehicle, whereas both fasted and non-fasted animals receiving baloxavir alone showed PT and APTT prolongation. These results suggested that PT and APTT prolongation in baloxavir-treated animals was caused by vitamin K deficiency.

5.6.2 Skin phototoxicity study (CTD 4.2.3.7-02)

A single oral dose of baloxavir (0 [vehicle] or 1000 mg/kg) or a single intraperitoneal dose of baloxavir (10, 30, or 100 mg/kg) was administered to female mice (n = 15/group). At 1 hour after oral administration or 0.5 hours after intraperitoneal administration, ultraviolet light (10 J/cm^2) was irradiated for 100 minutes. At 2, 24, and 48 hours after the ultraviolet irradiation, skin reaction was monitored to evaluate phototoxicity. Neither the oral nor intraperitoneal administration group showed abnormality of the back skin, suggesting that baloxavir is unlikely to induce phototoxicity.

5.R Outline of the review conducted by PMDA

5.R.1 Effect of vitamin K-deficiency on coagulation system

Following the repeated oral administration of baloxavir to rats, PT and APTT prolongation occurred under the condition of insufficient vitamin K intake. Therefore, PMDA asked the applicant to explain the possibility of increased bleeding tendency in neonates and infants who are considered to be prone to insufficient vitamin K intake.

The applicant's explanation:

Rats require a greater amount of vitamin K (50 μg/kg/day) than human neonates and infants (1.5-4.0 μg/kg/day), and humans including neonates and infants are able to intake a sufficient amount of vitamin K from meals. Therefore, the vitamin K deficiency-induced events observed in rats are unlikely to be relevant to humans.

PMDA's view:

The applicant's explanation that the risk of baloxavir-induced bleeding is low in neonates and infants who intake a sufficient amount of vitamin K is acceptable. However, since there is no experience of baloxavir administration to neonates or infants in clinical studies so far conducted, vitamin K in combination with baloxavir should be considered if it is difficult to intake sufficient amount of vitamin K from meals due to aggravation of disease conditions, etc.

5.R.2 Effect on the liver

Cynomolgus monkeys receiving repeated oral administration of baloxavir showed toxicity findings suggesting hepatic dysfunction and cholestasis. Therefore, PMDA asked the applicant to explain the possibility that metabolites of baloxavir are involved in these findings.

The applicant's explanation:

Following a single oral dose of ^{14}C-labeled baloxavir in monkeys and rats, metabolites in plasma were investigated. No metabolites unique to cynomolgus monkeys were detected, suggesting that the hepatotoxicity was probably unrelated to monkey-specific metabolites. In healthy adults receiving ^{14}C-labeled baloxavir, the metabolites observed in rats and monkeys were detected, but no human-specific metabolites were detected. The hepatotoxicity observed in cynomolgus monkeys is unlikely to pose safety concerns in humans, for the following reasons: (a) In cynomolgus monkeys, hepatotoxicity findings observed after repeated oral administration of baloxavir were reversible. (b) No abnormalities were revealed by histopathological examination of the liver, including the fine structure, in cynomolgus monkeys. (c) Baloxavir is intended to be administered as a single dose to humans in clinical practice.

PMDA's view:

The applicant's explanation regarding the effect on the liver observed in toxicity studies is acceptable. Given that no serious hepatic dysfunction was observed in clinical studies, baloxavir is unlikely to pose toxicological concern about its effect on the liver. Liver dysfunction-related adverse events observed in clinical studies are discussed in Section 7.R.3.1.2.

6. Summary of Biopharmaceutic Studies and Associated Analytical Methods, Clinical Pharmacology, and Outline of the Review Conducted by PMDA

6.1 Summary of biopharmaceutic studies and associated analytical methods

In the clinical development of baloxavir, the following 5 formulations were used: Formulation 1 ([redacted]), Formulation 2 ([redacted] containing 10 mg baloxavir), Formulation 3 (uncoated tablets containing 10 mg baloxavir), Formulation 4 ([redacted] containing 20 mg baloxavir), and Formulation 5 ([redacted] film-coated tablets containing 20 mg baloxavir).[24] Formulations 3 and 5 are proposed for marketing in Japan. The comparability of Formulation 4 and Formulation 5 has been demonstrated by a dissolution test.[25] This section describes the results of a study

[24] These formulations were used mainly in the following clinical studies:
Formulation 1, phase I study (Study T0811); Formulation 2, phase II study (Study T0821); Formulation 3, phase I study (Study T081F) and phase III study (Study T0822); Formulation 4, phase I studies (Studies T0814, T0815, T0816, and T0818) and phase II study (Study T0821); Formulation 5, phase I studies (Studies T081B, T081C, T081D, and T081F) and phase III studies (Studies T0822 and T0831)

[25] Dissolution test was performed by the paddle method (pH [redacted] test medium, [redacted] rpm; [redacted], [redacted] rpm) using test media (pH [redacted], [redacted], [redacted], and [redacted]) at 37°C.

conducted to evaluate the bioequivalence and food effect for Formulations 3 and 5, the to-be-marketed formulations. Concentrations of baloxavir in human plasma and urine and concentrations of S-033447 in human plasma, urine, and plasma equilibrium dialysate were measured by high-performance liquid chromatography/tandem mass spectrometry (LLOQ, 0.100 ng/mL).

Study of bioequivalence and food effect (CTD 5.3.1.2-2, Study T081F [[redacted] 20[redacted] to [redacted] 20[redacted]])

The bioequivalence between Formulations 3 and 5 was evaluated using PK parameters of S-033447 following a single oral dose of 20 mg baloxavir (2 tablets of Formulation 3 or 1 tablet of Formulation 5) in Japanese healthy subjects (78 subjects included in PK analysis) under fasted conditions.[26)] The effect of food on PK of baloxavir and S-033447 was investigated following a single oral dose of 40 mg baloxavir (2 tablets of Formulation 5) or 10 mg baloxavir (1 tablet of Formulation 3) under fasted conditions or after a meal (administered at 30 minutes after starting to eat an ordinary diet of 400-500 kcal containing approximately 150 kcal fat).[26)] Table 23 shows the results. The least square geometric mean ratio [90% confidence interval (CI)] (Formulation 3/Formulation 5) of C_{max} and AUC_{last} of S-033447 were 0.76 [0.71, 0.81] and 0.87 [0.84, 0.91], respectively; the ratio of C_{max} did not fall within the pre-specified acceptable range of bioequivalence (0.80-1.25). The least square geometric mean ratio [90% CI] (fed/fasted) of C_{max} and AUC_{last} of S-033447 was 0.82 [0.75, 0.90] and 0.69 [0.56, 0.86], respectively, for Formulation 3, and 0.52 [0.45, 0.61] and 0.64 [0.57, 0.71], respectively, for Formulation 5. C_{max} and AUC_{last} of S-033447 under fed conditions tended to be lower than those under fasted condition for both formulations.

Table 23. PK parameters of S-033447 following administration of Formulation 3 or 5 under fasted or fed conditions

	N	C_{max} (ng/mL)	t_{max}[a)] (h)	AUC_{last} (ng·h/mL)	AUC_{inf} (ng·h/mL)	$t_{1/2}$ (h)	CL/F (L/h)	V_z/F (L)
Baloxavir 20 mg (bioequivalence)								
Formulation 3 (2 tablets)	50	35.1 (34.4)	4.00 [1.00-24.0]	2877 (25.1)	2974 (26.4)	96.3 (20.2)	5.69 (26.4)	790 (24.3)
Formulation 5 (1 tablet)	49	46.4 (28.5)	4.00 [2.00-6.00]	3288 (24.0)	3382 (24.9)	96.7 (19.6)	5.00 (24.9)	698 (20.3)
Baloxavir 40 mg (2 tablets of Formulation 5) (food effect)								
Fasted	14	130 (24.1)	4.00 [3.00-5.00]	6932 (19.2)	7086 (19.6)	93.9 (21.6)	4.78 (19.6)	647 (19.1)
Fed	14	67.6 (40.0)	4.00 [0.50-5.00]	4406 (38.8)	4540 (39.1)	97.5 (22.8)	7.45 (39.1)	1050 (35.6)
Baloxavir 10 mg (1 tablet of Formulation 3) (food effect)								
Fasted	14	15.2 (34.1)	3.50 [2.00-24.0]	1653 (23.6)	1726 (24.0)	108 (15.7)	4.90 (24.0)	764 (25.0)
Fed	14	12.5 (26.7)	2.50 [0.50-5.00]	1143 (50.2)	1206 (49.7)	112 (17.5)	7.02 (49.7)	1130 (46.1)

Geometric mean (coefficient of variation [CV]%)
a) Median [range]

6.2 Clinical pharmacology

The applicant submitted results of Japanese and foreign phase I studies (PK study in healthy subjects, PK study in subjects with hepatic impairment, pharmacokinetic interaction study, etc.), results of Japanese and foreign clinical studies in patients with influenza virus infection, and results of population pharmacokinetic (PPK) analysis for the present application. *In vitro* studies using human biomaterials are described in the sections of nonclinical pharmacokinetics [see Sections 4.2.2, 4.3.2, and 4.5].

[26)] This study was a two-treatment, two-period, crossover study

PK parameter values are expressed in geometric means unless specified otherwise.

6.2.1 Studies in healthy subjects

6.2.1.1 Study in Japanese subjects

Phase I study (CTD 5.3.3.1-01, Study T0811 [[redacted] to [redacted]])

Among subjects enrolled in Study T0811, 30 healthy subjects were evaluated for the plasma PK of S-033447 and F_u of baloxavir and S-033447 following a single oral dose of baloxavir under fasted conditions. As shown in Table 24 (the results of plasma PK analysis of S-033447), C_{max} and AUC_{inf} of S-033447 in plasma were generally dose-proportional over the dose range of 6 to 80 mg. Baloxavir was not detected in the plasma of subjects receiving 6 or 20 mg, but detected at several time points in several subjects receiving 40 to 80 mg. At 12 hours after administration, plasma baloxavir level was below the LLOQ in all subjects. F_u up to 72 hours after administration was <0.05% for baloxavir, and 1.7% to 2.3% for S-033447.

Table 24. PK parameters of S-033447 in plasma following a single oral dose of baloxavir in Japanese subjects

Dose (mg)	N	C_{max} (ng/mL)	t_{max}[a)] (h)	AUC_{last} (ng·h/mL)	AUC_{inf} (ng·h/mL)	$t_{1/2}$ (h)	CL/F (L/h)	Vz/F (L)
6	6	11.0 (22.3)	2.00 [1.00-2.50]	417.4 (22.1)	1018 (35.7)	90.9 (55.7)	4.99 (35.7)	655 (33.0)
20	6	40.2 (32.5)	3.50 [1.50-4.00]	1484 (21.5)	2419 (24.8)	48.9 (30.1)	6.99 (24.8)	494 (28.4)
40	6	123 (31.0)	3.50 [3.50-5.00]	6285 (20.9)	6669 (20.8)	85.9 (8.2)	5.07 (20.8)	629 (22.3)
60	6	193 (15.7)	3.25 [2.50-4.00]	8767 (15.7)	9141 (17.5)	75.2 (15.3)	5.55 (17.5)	603 (10.3)
80	6	253 (23.9)	3.50 [2.50-4.00]	11,490 (27.0)	11,970 (27.8)	75.9 (11.1)	5.65 (27.8)	619 (23.3)

Geometric mean (CV%)
a) Median [range]

6.2.1.2 Study in non-Japanese subjects

Mass balance study (CTD 5.3.2.2-17 and 5.3.3.1-02, Study T0817 [[redacted] to [redacted]])

Mass balance and metabolites in plasma, urine, and feces were investigated following a single oral dose of ^{14}C-labeled baloxavir 40 mg in healthy subjects under fasted conditions (6 subjects included in PK analysis). Of the administered radioactivity, 5% was recovered within 24 hours after administration and 95% within 432 hours. Of the administered radioactivity, 12% was recovered in feces within 48 hours and 80% within 432 hours, suggesting that most of the radioactivity recovered in feces was excreted via the bile. F_u up to 408 hours after administration was 14.7% for the radioactivity and 3.28% for S-033447, suggesting only a minor contribution of renal excretion. C_{max}, AUC_{last}, and AUC_{0-72} of radioactivity after administration of baloxavir were 75.9 ng eq./mL (C_{max}), 2106 ng eq.·h/mL (AUC_{last}), and 2420 ng eq.·h/mL (AUC_{0-72}) in blood, and 82.6 ng eq./mL (C_{max}), 2215 ng eq.·h/mL (AUC_{last}), and 2574 ng eq.·h/mL (AUC_{0-72}) in plasma. These values in blood and plasma were similar, suggesting no selective distribution in blood cell components.

Metabolites of baloxavir detected in plasma from 48 to 72 hours after administration were S-033447 (82.18% of plasma radioactivity), S-033447 glucuronide (M4) (16.37%), and (12a*R*, 5*R*, 11*S*)-S-033447 sulfoxide (M2) (1.45%). Metabolites of baloxavir detected in urine from 216 to 264 hours after

administration were M4 (8.91% of the administered radioactivity), S-033447 (2.03%), M2 (1.79%), and (12a*R*, 5*S*, 11*S*)-S-033447 sulfoxide (M1) (1.38%). Metabolites of baloxavir detected in feces from 0 to 48 hours up to 240 to 312 hours after administration were S-033447 (48.69%), M1 (3.34%), M2 (2.79%), and S-033447 pyrrole (M7) (1.44%).

6.2.2 Studies in patients

6.2.2.1 Japanese phase II study (CTD 5.3.5.1-01, Study T0821 [December 2015 to April 2016])

A single oral dose of baloxavir (10, 20, or 40 mg) was administered to 202 patients with influenza virus infection. Mean C_{24} [range] was generally dose-proportional: 15.1 [6.38, 29.0] ng/mL for 10 mg, 32.9 [7.48, 75.2] ng/mL for 20 mg, and 61.5 [27.9, 118] ng/mL for 40 mg.

6.2.2.2 Global phase III study in patients aged ≥12 years (CTD 5.3.5.1-02, Study T0831 [October 2016 to June 2017])

Among subjects enrolled in Study T0831, 396 with influenza virus infection received a single oral dose of baloxavir (40 or 80 mg).[27)] Mean C_{24} [range] of baloxavir in plasma was 51.3 [0.322, 158] for 40 mg, and 65.9 [17.5, 209] ng/mL for 80 mg.

6.2.2.3 Japanese study in patients aged <12 years (CTD 5.3.5.2-01, Study T0822 [November 2016 to April 2017])

A single oral dose of baloxavir (5, 10, 20, or 40 mg)[28)] was administered to 84 patients aged <12 years who had influenza virus infection. Mean C_{24} of plasma S-033447 [range] was 11.2 ng/mL for 5 mg, 45.5 [16.8, 146] ng/mL for 10 mg, 59.0 [16.5, 103] ng/mL for 20 mg, and 86.4 [51.0, 136] ng/mL for 40 mg. These values were within the range of C_{24} ([0.322, 209]) in patients aged ≥12 years enrolled in a phase III study (Study T0831).

6.2.3 Studies of intrinsic factors

6.2.3.1 Foreign study in subjects with hepatic impairment (CTD 5.3.3.3-01, Study T081B [[redacted] to [redacted]])

PK of baloxavir and S-033447 was investigated following a single oral dose of baloxavir 40 mg in 8 subjects with moderate hepatic impairment (Child-Pugh class B) and 8 subjects with normal hepatic function. Table 25 shows the results. C_{max} of S-033447 tended to be lower, and AUC_{inf} higher, in subjects with moderate hepatic impairment than in subjects with normal hepatic function, but the applicant explained that the differences were not clinically significant. The plasma protein binding of S-033447 did not differ markedly between subjects with and without hepatic impairment: 94.0% (at 4 hours after administration) and 94.2% (at 24 hours) in subjects with normal hepatic function; and 95.3% (at 4 hours) and 95.7% (at 24 hours) in subjects with moderate hepatic impairment.

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27) Baloxavir was administered at the following doses:
40 mg in subjects weighing ≥40 kg and <80 kg, 80 mg in subjects weighing ≥80 kg

28) Baloxavir was administered at the following doses:
5 mg in subjects weighing ≥5 kg and <10 kg; 10 mg in subjects weighing ≥10 kg and <20 kg; 20 mg in subjects weighing ≥20 kg and <40 kg; 40 mg in subjects weighing ≥40 kg

Table 25. PK parameters of S-033447 following a single dose of baloxavir in subjects with hepatic impairment and subjects with normal hepatic function

Severity of hepatic impairment	N	C_{max} (ng/mL)	t_{max}[a)] (h)	AUC_{inf} (ng·h/mL)	$t_{1/2}$ (h)	F_u (Day 1) (%)	Least square geometric mean ratio [90% CI] (hepatic impairment/normal hepatic function)	
							C_{max}	AUC_{inf}
Moderate	8	76.7 (69.9)	2.50 [1.00-4.00]	4739 (37.8)	94.8 (27.4)	0.0455 (24.1)	0.799 [0.498-1.28]	1.12 [0.778-1.61]
Normal	8	95.9 (44.3)	3.00 [2.00-4.00]	4236 (47.9)	73.5 (52.3)	0.0536 (69.4)	-	-

Geometrical mean (CV%)
-, Not applicable
a) Median [range]

6.2.3.2 PK in subjects with renal impairment

No clinical study was conducted to investigate PK of baloxavir and S-033447 in subjects with renal impairment.

The applicant's explanation:
In the mass balance study (Study T0817), F_u up to 408 hours after administration of ^{14}C-labeled baloxavir 40 mg was 14.7% for radioactivity and 3.28% for S-033447, suggesting an only minor contribution of renal excretion [see Section 6.2.1.2]. Also, creatinine clearance was not selected as a covariate for CL/F from the results of PPK analysis in adults [see Section 6.2.6.1]. These results suggest that renal excretion makes only a minor contribution to the elimination of orally administered baloxavir.

6.2.4 Studies of pharmacokinetic interactions[29)]

Five studies were conducted to investigate the interaction between baloxavir and concomitant drugs. Tables 26 and 27 show the least square geometric mean ratios [90% CI] (dual therapy/monotherapy) of PK parameters of S-033447 and concomitant drugs.

Table 26. Effect of concomitant drug on PK parameters of S-033447

Concomitant drug	Dosage regimen		N	Least square geometric mean ratio [90% CI]	
	Concomitant drug	Baloxavir		C_{max}	AUC_{inf}
Itraconazole	200 mg QD	20 mg single dose	12	1.33 [1.14, 1.55]	1.23 [1.09, 1.38]
Oseltamivir	75 mg BID	40 mg single dose	18[a)]	1.03 [0.92, 1.15]	1.01 [0.96, 1.06]
Probenecid	500 mg BID	80 mg single dose	12	0.79 [0.65, 0.96]	0.75 [0.66, 0.86]

a) N = 17 for C_{max} and AUC_{inf} in subjects receiving baloxavir alone, and AUC_{inf} in subjects receiving baloxavir and oseltamivir.

Table 27. Effect of baloxavir on PK parameters of concomitant drug

Drug	Dosage regimen		N	Least square geometric mean ratio [90% CI]	
	Concomitant drug	Baloxavir		C_{max}	AUC_{inf}
Midazolam	5 mg single dose	40 mg single dose	12	1.00 [0.92, 1.09]	0.99 [0.94, 1.04]
Oseltamivir	75 mg BID	40 mg single dose	Day 1, 18[a)]	0.97 [0.82, 1.14]	1.23 [1.14, 1.33][b)]
			Day 5, 18[a)]	0.96 [0.83, 1.11]	1.07 [0.99, 1.15][b)]
Digoxin	0.25 mg single dose	80 mg single dose	12	1.00 [0.81, 1.23]	0.86 [0.73, 1.01]
Rosuvastatin	10 mg single dose	80 mg single dose	12	0.82 [0.69, 0.98]	0.83 [0.72, 0.96]

a) N = 17 for subjects receiving baloxavir and oseltamivir, b) AUC_{tau} (AUC in each dosing interval)

[29)] 5.3.2.2-12, Study T0814 [[redacted]]; 5.3.2.2-13, Study T0815 [[redacted]]; 5.3.2.2-14, Study T0818 [[redacted]]; 5.3.2.2-15, Study T081C [[redacted]]; 5.3.2.2-16., Study T081D [[redacted]]

6.2.5 QT/QTc study (CTD 5.3.4.1-01, Study T0816 [[redacted] 20[redacted] to [redacted] 20[redacted]])

The effect of a single oral dose of baloxavir (40 or 80 mg) on QT/QTc interval was investigated in 64 Japanese healthy subjects using moxifloxacin (400 mg single oral dose) as the positive control.[30] At 3 hours after administration, the moxifloxacin group showed the maximum difference from the placebo group in "the change from baseline in QT interval corrected for the heart rate by Fridericia's formula." The between-group difference (versus placebo) in the least squares mean [90% CI] was 14.07 [12.33, 15.80] ms. The baloxavir 40 and 80 mg groups showed the maximum difference from the placebo group in "the change from baseline in QT interval corrected for the heart rate by Fridericia's formula " at 8 hours (40 mg) and 6 hours (80 mg) after administration. The between-group difference (versus placebo) in the least squares mean [90% CI] was 0.60 [−1.13, 2.33] for 40 mg and 2.18 [0.45, 3.90] ms for 80 mg, with the upper limit of the 90% CI being less than 10 ms. From these results, the applicant explained that baloxavir up to 80 mg does not prolong QTc interval. Following the administration of baloxavir 40 mg or 80 mg, C_{max} was 157 and 291 ng/mL, respectively, and AUC_{last} was 4249 and 7384 ng·h/mL, respectively.

6.2.6 PPK analysis and exposure-response analysis

6.2.6.1 PPK analysis in subjects aged ≥12 years (CTD 5.3.3.5-01)

PPK analysis (NONMEM version 7.3) was conducted using PK data of S-033447 obtained from healthy subjects, subjects with hepatic impairment, and patients with influenza virus infection in 12 Japanese and foreign clinical studies[31] (8310 sampling time points in 1109 subjects). The final model was described as a 2-compartment model with first-order absorption and lag time. The following parameters were selected as covariates: Sex for absorption rate constant (k_a); presence/absence of food consumption for bioavailability; body weight, ethnicity (Asian, non-Asian), and ALT for CL/F; body weight and ethnicity (Asian, non-Asian) for V_c/F; and body weight for Q/F and V_p/F.[32] Table 28 shows PK parameter values, estimated using the final model, following a single oral dose of baloxavir 40 mg (body weight ≥40 kg and <80 kg) or 80 mg (body weight ≥80 kg) in ≥12-year-old Asian patients with influenza virus infection in the global phase III study (Study T0831).

Table 28. PK parameters of S-033447 in Asians (estimated by the final model)

Dose (body weight)	N	C_{max} (ng/mL)	AUC_{inf} (ng·h/mL)
40 mg (<80 kg)	309	102 [23.9-244]	6598 [2186-14,690]
80 mg (≥80 kg)	34	126 [33.3-243]	9949 [4122-18,330]

Mean [range]

6.2.6.2 PPK analysis in subjects aged <12 years (CTD 5.3.3.5-02)

A PPK analysis (NONMEM version 7.3) was conducted using PK data of S-033447 (328 sampling time points in 107 subjects) obtained from a Japanese study in patients aged <12 years with influenza virus infection (Study T0822). The final model was described as a 2-compartment model with first-order

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30) This study was a four-treatment, four-period, crossover study. A ≥21 day-washout period was allowed between treatment periods.

31) Phase I studies (Studies T0811, T0813, T0814, T0815, T0816, T0817, T0818, T081B, T081C, and T081D), Phase II study (Study T0821), and phase III study (Study T0831)

32) The following possible covariates were examined: (1) Body weight, age, BMI, sex, AST, ALT, total bilirubin, eGFR, CL_{cr}, presence/absence of moderate hepatic impairment, ethnicity (Asian, White, others; or Asians vs. non-Asians), region (Japan/Asia, other), and health status (healthy subjects, patients with influenza virus infection, patients without infection) for CL/F; (2) body weight, age, BMI, sex, presence/absence of moderate hepatic impairment, ethnicity (Asian, White, others; or Asians vs. non-Asians), region (Japan/Asia, others) and health status (healthy subjects, patients with influenza virus infection, patients without infection) for V_c/F; (3) age, sex, health status (healthy subjects, patients with influenza virus infection, patients without infection), and food intake (fasted, fed) for k_a; (4) food intake (fasted, fed) for bioavailability; and (5) body weight for V_p/F and Q/F.

absorption and lag time. In subjects aged <2 years, maturation factor[33] was incorporated in CL/F in order to simulate PK, because PK parameters in the age group cannot be explained by body weight alone (*Paediatr Anaesth.* 2011;21:222-37). Body weight was selected as the covariate for CL/F, V_c/F, Q/F, and V_p/F.[34] Table 29 shows PK parameters, estimated using the final model, following a single oral dose of baloxavir in patients with influenza virus infection at a dose of 5 mg (body weight ≥5 kg and <10 kg), 10 mg (body weight ≥10 kg and <20 kg), 20 mg (body weight ≥20 kg and <40 kg), or 40 mg (body weight ≥40 kg).

Table 29. PK parameters of S-033447 (estimated using the final model)

Dose (body weight)	N	C_{max} (ng/mL)	AUC_{inf} (ng·h/mL)
5 mg (≥5 kg and <10 kg)	2	40.3, 52.7	1628, 2356
10 mg (≥10 kg and <20 kg)	31	76.9 [43.2-109]	3408 [2170-5344]
20 mg (≥20 kg and <40 kg)	66	100 [37.6-150]	5081 [2316-9115]
40 mg (≥40 kg)	8	115 [58.8-145]	7236 [6014-10,160]

Mean [range]

6.2.6.3 Exposure-response analysis (CTD 5.3.5.1-2)

In the Japanese phase II study (Study T0821) and the global phase III study (Study T0831), the relationship between C_{24} of plasma S-033447 and efficacy following a single oral dose of baloxavir (10-80 mg) in patients with influenza virus infection was investigated using a linear model and an E_{max} model. In the study with the linear model, C_{24} of S-033447 was correlated with "duration of influenza illness," "time to resolution of symptoms,"[35] "time to return to normal temperature," and "change in viral titer." On the other hand, C_{24} of S-033447 was not correlated with "time to resolution of coughing" or "time to resolution of sore throat." In the study with the E_{max} model, viral titer on Day 2 after baloxavir administration decreased progressively with increase in C_{24} of S-033447; 50% effective concentration (i.e., C_{24} required to achieve half the maximum effect in reducing viral titer) was 6.27 ng/mL for all viral subtypes combined, 4.10 ng/mL for type A virus, and 20.2 ng/mL[36] for type B virus.

The relationship between safety and C_{max} or AUC_{inf} of S-033447 was investigated using (a) the combined data from the Japanese phase II study in adults (Study T0821) and the global phase III study in patients aged ≥12 years (Study T0831); and (b) data from the Japanese study in patients aged <12 years (Study T0822). In both data (a) and (b), C_{max} and AUC_{inf} of S-033447 were unrelated to the incidence of all adverse events combined or of individual adverse events.

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33) Maturation factor = (postconceptional weeks)$^{\gamma}$/ ([postconceptional weeks]$^{\gamma}$ + [postconceptional weeks up to 50% maturation])$^{\gamma}$; γ, Hill coefficient
As postconceptional weeks up to 50% maturation and Hill coefficient, those of morphine (54.2 weeks and 3.92, respectively), a compound with metabolic and elimination processes most closely resembling those of S-033447 among compounds for which the values of these parameters are published, were used.

34) The following possible covariates were examined: (1) Body weight, age, BMI, sex, AST, ALT, total bilirubin, eGFR, CL_{cr}, health status (patients with influenza virus infection, patients without infection) for CL/F; (2) body weight, age, BMI, sex, and health status (patients with influenza virus infection, patients without infection) for V_c/F; (3) age, sex, health status (patients with influenza virus infection, patients without infection), and food intake (fasted, fed) for k_a; (4) food intake (fasted, fed) for bioavailability; and (5) body weight for V_p/F and Q/F.

35) Headache, nasal congestion, feverishness or chills, myalgia or arthralgia, and fatigue.

36) The applicant explained that, because of a small number of patients with influenza B virus infection, there was no clear leveling-off observed in the relationship between C_{24} and change in viral titer, resulting in a low reliability in the estimated value for influenza B virus.

6.R Outline of the review conducted by PMDA

6.R.1 Ethnic differences in the PK of S-033447

The applicant explained the ethnic differences in PK of S-033447 observed in the global phase III study (Study T0831).

The applicant's explanation:

In the global phase III study (Study T0831), a single oral dose of baloxavir was administered at 40 mg (patients weighing <80 kg) or 80 mg (patients weighing ≥80 kg), taking account of the effect of body weight on plasma S-033447 exposure. Table 30 shows PK parameters of S-033447 in Asian and non-Asian patients after administration of baloxavir. C_{max}, $AUC_{0\text{-}inf}$, and C_{24} of S-033447 tended to be higher in Asian patients than in non-Asian patients. Of 358 Asian patients studied, 348 were from Japan.

Table 30. PK parameters of S-033447 in Asian and non-Asian patients

	Asian (N = 358)	Non-Asian (N = 226)
C_{max} (ng/mL)[a)]	103 [23.9-244]	70.5 [14.0-211]
AUC_{inf} (ng·h/mL)[a)]	6881 [2186-18,330]	4645 [1100-15,600]
C_{24} (ng/mL)	62.5 [5.81-158][b)]	44.9 [0.322-209][c)]

Mean [range]
a) Bayesian estimate, b) N = 230, c) N = 166

PMDA has confirmed that there were ethnic differences in PK of S-033447 following administration of baloxavir in the global phase III study (Study T0831). The effect of the ethnic differences in PK of S-033447 on the efficacy and safety of baloxavir is discussed in Sections 7.R.2.1 and 7.R.3.1.

6.R.2 Food effect on PK of S-033447 and the timing of dosing in relation to meals

The applicant explained the food effect on PK of S-033447 and the timing of dosing in relation to meals in the proposed dosage and administration of baloxavir.

The applicant's explanation:

In the Japanese phase I study (Study T081F), C_{max} and AUC_{last} of S-033447 following administration of baloxavir after a meal were lower than those following the administration under fasted conditions [see Section 6.1]. The effect of a meal on PK of S-033447 was presumably caused by chelation of S-033447 and divalent metal ions.

In the Japanese phase I study (Study T0813), C_{24} of S-033447 was 29.2 ng/mL in subjects receiving 20 mg baloxavir (Formulation 4) under fasted conditions. The nonclinical studies suggested that the effective C_τ of S-033447 was 6.85 ng/mL (see Section 3.1.4.1.5). These C_{24} and C_τ values suggest that baloxavir 10 to 40 mg would be effective even if S-033447 exposure is reduced by half due to administration under fed condition. Therefore, the Japanese phase II study (Study T0821) was conducted without instructions regarding meal timing relative to dose. As a result, there were no events of safety concerns with baloxavir 10 to 40 mg, and the duration of influenza illness did not differ by meal timing.[37)] The global phase III study (Study T0831) was therefore conducted without instructions on meal timing, and its results demonstrated the efficacy and safety of baloxavir.

[37)] Baloxavir was administered (a) at ≥4 hours before or after a meal (administration under fasted condition), (b) within 2 to 4 hours before or after a meal, or (c) within 0 and 2 hours before or after a meal

The food effect was observed in the Japanese phase I study (Study T081F), but the global phase III study (Study T0831) showed the efficacy and safety of baloxavir without any restriction on meal timing. Therefore, no restriction on the timing of a meal was included in the proposed dosage and administration. Meal timing[37)] did not affect the duration of influenza illness in the global phase III study (Study T0831).

PMDA accepted the explanation of the applicant and considers it acceptable not to set any rule on a meal in the dosage regimen of baloxavir.

6.R.3 Rationale for dosage regimen in global phase III study (Study T0831) and Japanese study (Study T0822)

The applicant explained the rationale for the dosage regimen in the global phase III study in patients aged ≥12 years (Study T0831) and in the Japanese study in patients aged <12 years (Study T0822).

The applicant's explanation:
In the global phase III study in patients aged ≥12 years (Study T0831), baloxavir was administered orally as a single dose at 40 mg (in patients weighing ≥40 kg and <80 kg) or 80 mg (in patients weighing ≥80 kg), for the following reasons:

- The Japanese phase II study in patients with influenza virus infection (Study T0821) evaluated the efficacy and safety of a single dose of baloxavir 10-40 mg. The results showed no differences in the efficacy or safety between the different doses, and median C_{24} was 14.8, 29.6, and 57.1 ng/mL, respectively, following treatment with 10, 20, and 40 mg baloxavir.
- PPK analysis was performed using PK data from the Japanese and foreign phase I studies (Studies T0813, T0814, and T0815) and the phase II study (Study T0821). The results showed the effect of body weight on C_{24} of S-033447, suggesting that C_{24} of S-033447 in American patients was lower by 30% to 40% than that in Japanese patients.
- Simulation of C_{24} of S-033447, classified by body weight, showed that C_{24} of S-033447 following a single oral dose at 40 mg (patients weighing ≥40 kg and <80 kg) or 80 mg (patients weighing ≥80 kg) exceeded 14.8 ng/mL, the level shown to be effective in the Japanese phase II study (Study T0821) even if the effect of ethnicity, body weight, and a meal was taken into account.
- The safety of baloxavir up to 80 mg was demonstrated in the Japanese phase I studies (Studies T0811 and T0816).

In the Japanese study in patients aged <12 years (Study T0822), baloxavir was administered orally as a single dose of 5 mg (in patients weighing ≥5 kg and <10 kg), 10 mg (≥10 kg and <20 kg), 20 mg (≥20 kg and <40 kg), or 40 mg (≥40 kg).

C_{max}, C_{24}, and AUC_{inf} in children weighing 10 to 40 kg and in infants aged ≥6 months and <1 year were calculated by simulation, using the model obtained by PPK analysis based on PK data from Japanese phase I studies (Studies T0813, T0814, and T0815) and the phase II study (Study T0821). C_{24} and AUC_{inf} following the administration of baloxavir 5 mg to infants aged <1 year, 10 mg to children weighing ≥10 and <20 kg, and 20 mg to children weighing ≥20 and <40 kg, were not significantly different from those in patients receiving baloxavir 40 mg in the Japanese phase II study (Study T0821; this study suggested

the efficacy of baloxavir), and were lower than C_{max} and AUC_{inf} in patients receiving baloxavir 80 mg (≤80 mg doses were shown to be safe) in the Japanese phase I study (Study T0811).

In the Japanese study in patients aged <12 years (Study T0822), AUC_{inf} in those aged <2 years or weighing <20 kg was slightly lower than that in the global phase III study (Study T0831),[38)] but their C_{max} and C_{24} were generally within the range observed in Study T0831.

PMDA accepted the applicant's rationale for the dosage regimen in the global phase III study in patients aged ≥12 years (Study T0831) and in the Japanese study in patients aged <12 years (Study T0822).

6.R.4 Choice between 10-mg tablets (Formulation 3) and 20-mg tablets (Formulation 5)

The applicant's explanation about the choice between the 2 to-be-marketed formulations: 10-mg tablets (Formulation 3) and 20-mg tablets (Formulation 5):
The 20-mg tablets (Formulation 5) should be administered to patients receiving ≥20 mg, and 10-mg tablets (Formulation 3) to those receiving ≤10 mg, because bioequivalence of these 2 formulations was not demonstrated in the Japanese phase I study (Study T081F) [see Section 6.1].

PMDA accepted the applicant's explanation regarding the choice between 10-mg tablets (Formulation 3) and 20-mg tablets (Formulation 5), the to-be-marketed formulations.

7. Clinical Efficacy and Safety and Outline of the Review Conducted by PMDA

The applicant submitted efficacy and safety evaluation data of baloxavir (the results from 3 clinical studies) for the present application. Table 31 shows the outline of the studies.

Table 31. Outline of major clinical studies on the efficacy and safety of baloxavir

Study (phase)	Subjects	Dosage regimen	Number of patients analyzed	Main endpoints
Study T0821 (Japanese phase II study) [evaluation data]	Patients aged ≥20 years with influenza virus infection	(a) Baloxavir 10 mg single dose (b) Baloxavir 20 mg single dose (c) Baloxavir 40 mg single dose (d) Placebo single dose	400 (N = 100/group)	Efficacy Safety Pharmacokinetics
Study T0831 (global phase III study) [evaluation data]	Patients aged ≥12 years with influenza virus infection	(a) Baloxavir 40 mg (body weight <80 kg) or 80 mg (body weight ≥80 kg) single dose (b) Oseltamivir (75 mg) twice daily for 5 days [a)] (c) Placebo single dose or twice daily for 5 days	(a) 456 (b) 377 (c) 231	Efficacy Safety Pharmacokinetics
Study T0822 (Japanese phase III study) [evaluation data]	Patients aged <12 years with influenza virus infection	(a) Single dose of baloxavir 5 mg (body weight ≥5 and <10 kg), 10 mg (≥10 and <20 kg), 20 mg (≥20 and <40 kg), or 40 mg (≥40 kg)	(a) 104	Efficacy Safety Pharmacokinetics

a) Patients aged ≥20 years only

38) The applicant's explanation:
Since S-033447 has a long $t_{1/2}$, the AUC during the early post-dose period has only a minor contribution to AUC_{inf}. This suggests that regardless of age or weight, patients achieve similar exposure to S-033447 in the early post-dose period, during which baloxavir is expected to achieve efficacy.

7.1 Japanese phase I study (CTD 5.3.3.1-01, Study T0811 [[redacted] 20[redacted] to [redacted] 20[redacted]])

A placebo-controlled, randomized, double-blind, parallel-group study was conducted to investigate the safety and tolerability of baloxavir in healthy adults (target sample size, 68 subjects at the maximum [8 in each cohort during the single-dose phase, 10 in each cohort during the multiple-dose phase]) in a study site in Japan.

A single dose of baloxavir (6, 20, 40, 60, or 80 mg) or placebo was administered orally under fasted conditions. The study also included cohorts receiving baloxavir 40 mg orally after a meal or between meals.

Safety results:

Among 40 subjects, adverse events (including abnormal changes in laboratory tests) were observed in 1 subject in the placebo group (liver function test abnormal), 1 subject in the baloxavir 6 mg group (headache), and 4 subjects in the 40 mg group (ALT increased, blood bilirubin increased, nasopharyngitis, and sinus arrhythmia in 1 subject each). The outcome was "resolved" in all of them. Of these adverse events, liver function test abnormal, headache, and ALT increased were assessed as causally related to the study drug. As for abnormal liver function-related adverse events, liver function test abnormal in the placebo group occurred on Day 29 after study drug administration, ALT increased and blood bilirubin increased in the baloxavir 40 mg group occurred on Day 20 and 43, respectively, after study drug administration.

7.2 Japanese phase II study (CTD 5.3.5.1-01, Study T0821 [December 2015 to April 2016])

A placebo-controlled, randomized, double-blind, parallel-group study[39)] was conducted to investigate the efficacy and safety of baloxavir in patients aged ≥20 years with influenza virus infection (target sample size, 400 subjects [100 per group]) in 72 study sites in Japan.

Baloxavir (10, 20, or 40 mg) or placebo was administered orally in a single dose.

A total of 400 patients (100 per group) who were randomized and treated with at least 1 dose of the study drug were included in the safety analysis population and intention-to-treat infected (ITTI) population,[40)] and the ITTI population was subjected to efficacy analysis.

Efficacy results:

Table 32 and Figure 4 show the results of the primary efficacy endpoint (i.e., duration of influenza illness). The duration of influenza illness was defined as the period between the start of study treatment and the time when all of the influenza symptoms (cough, sore throat, headache, nasal congestion, feverishness or chills, myalgia or arthralgia, and fatigue) were recorded as "0, none" or "1, mild" in the patient diary.[41)]

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39) Patients were assigned to treatment groups by minimization for the following allocation factors: the composite score of 7 influenza symptoms (≤11 points, ≥12 points) and smoking habit (yes/no) at enrollment.

40) Patients who received at least 1 dose of the study drug and tested positive for influenza virus by the rapid test with nasal or throat swab

41) Each influenza symptom was scored on a 4-point scale (0 [none], 1 [mild], 2 [moderate], and 3 [severe]) and recorded in the patient diary. Influenza illness was considered to have resolved when the scores of all symptoms remained "0 (none)" or "1 (mild)" for at least 21.5 hours.

Table 32. Duration of influenza illness (ITTI population)

	10 mg (N = 100)	20 mg (N = 100)	40 mg (N = 100)	Placebo (N = 100)
Median [95% CI] (hours)	54.2 [47.7, 66.8]	51.0 [44.5, 62.4]	49.5 [44.5, 64.4]	77.7 [67.6, 88.7]
Hazard ratio to placebo [95% CI][a)]	0.758 [0.571, 1.007]	0.810 [0.608, 1.078]	0.817 [0.614, 1.087]	-
Adjusted *P* value[a) b)]	0.1650	0.1650	0.1650	-

-, Not applicable

a) Cox proportional hazard model with the following covariates: treatment group; smoking habit (yes/no) at enrollment; and the composite score of 7 influenza symptoms at baseline.

b) Two-sided significance level of 5%. Multiplicity of test was adjusted by the Hommel method.

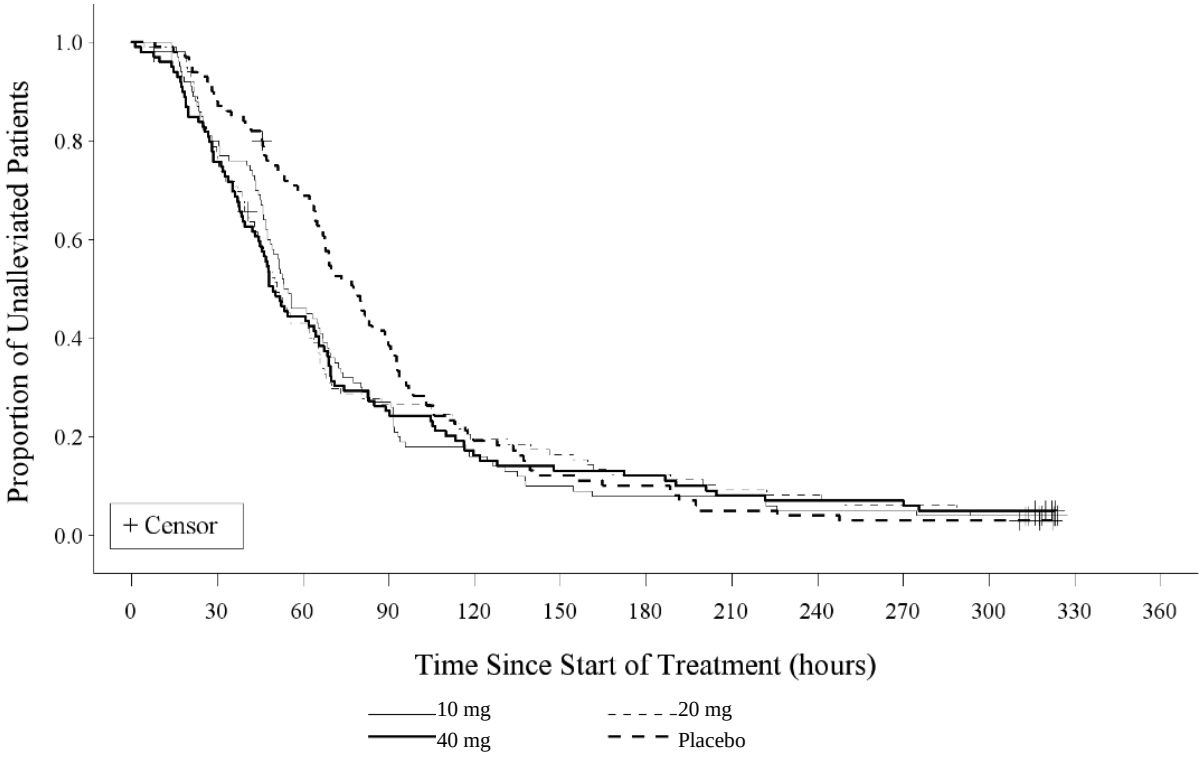

Figure 4. Kaplan-Meier plots of duration of influenza illness (ITTI population) (cited from CTD 2.7.6 Figure 2.7.6.13-2)

Safety results:

Adverse events (including abnormal changes in laboratory tests) were observed in 27.0% (27 of 100) of patients in the 10 mg group, 23.0% (23 of 100) of patients in the 20 mg group, 26.0% (26 of 100) of patients in the 40 mg group, and 29.0% (29 of 100) of patients in the placebo group. Adverse drug reactions[42)] were observed in 9.0% (9 of 100) of patients in the 10 mg group, 7.0% (7 of 100) of patients in the 20 mg group, 6.0% (6 of 100) of patients in the 40 mg group, and 10.0% (10 of 100) of patients in the placebo group. Adverse events and adverse drug reactions reported by ≥2% of patients in any group are shown in Table 33. There were no deaths, serious adverse events, or adverse events leading to discontinuation.

[42)] Study drug related-adverse events for which a causal relationship to study drug cannot be ruled out.

Table 33. Adverse events and adverse drug reactions reported by ≥2% of patients in any group (safety analysis population)

Events	Adverse events				Adverse drug reactions			
	40 mg (N = 100)	20 mg (N = 100)	10 mg (N = 100)	Placebo (N = 100)	40 mg (N = 100)	20 mg (N = 100)	10 mg (N = 100)	Placebo (N = 100)
All events	26 (26.0)	23 (23.0)	27 (27.0)	29 (29.0)	6 (6.0)	7 (7.0)	9 (9.0)	10 (10.0)
Headache	4 (4.0)	1 (1.0)	3 (3.0)	3 (3.0)	0	0	0	1 (1.0)
Vertigo	2 (2.0)	0	0	0	0	0	0	0
Diarrhoea	2 (2.0)	3 (3.0)	0	5 (5.0)	0	1 (1.0)	0	2 (2.0)
ALT increased	2 (2.0)	0	3 (3.0)	3 (3.0)	2 (2.0)	0	2 (2.0)	2 (2.0)
Seasonal allergy	0	2 (2.0)	0	0	0	0	0	0
Gastritis	0	2 (2.0)	0	0	0	0	0	0
Liver function test abnormal	0	2 (2.0)	1 (1.0)	0	0	2 (2.0)	1 (1.0)	0
Nasopharyngitis	1 (1.0)	0	2 (2.0)	2 (2.0)	0	0	0	0
Stomatitis	0	1 (1.0)	2 (2.0)	0	0	1 (1.0)	1 (1.0)	0
AST increased	1 (1.0)	0	3 (3.0)	1 (1.0)	1 (1.0)	0	2 (2.0)	1 (1.0)
White blood cell count decreased	0	1 (1.0)	3 (3.0)	0	0	0	3 (3.0)	0
Blood bilirubin increased	0	1 (1.0)	2 (2.0)	1 (1.0)	0	1 (1.0)	1 (1.0)	0
Thrombocytosis	1 (1.0)	1 (1.0)	0	2 (2.0)	1 (1.0)	1 (1.0)	0	2 (2.0)
Rash	0	0	0	2 (2.0)	0	0	0	2 (2.0)
γ-GTP increased	0	0	0	2 (2.0)	0	0	0	2 (2.0)

n (%)

7.3 Phase III studies

7.3.1 Global study (CTD 5.3.5.1-02, Study T0831 [October 2016 to June 2017])

A placebo- and oseltamivir phosphate-controlled, randomized, double-blind, parallel-group study[43)] was conducted to investigate the efficacy and safety of baloxavir 20-mg tablets in patients aged ≥12 years with influenza virus infection (target sample size, 1494 subjects [636 in the baloxavir group, 540 in the oseltamivir group, 318 in the placebo group]) in 297 study sites in Japan, US, and Canada.[44)] The Japanese package insert for oseltamivir phosphate states: "There have been reports of abnormal behavior leading to an accident such as fall after taking oseltamivir in pediatric patients aged ≥10 years. As a rule, oseltamivir should not be used in patients of this age group unless they are considered high-risk patients according to current or past medical conditions, etc." Of the patients enrolled in this study, those aged <20 years were assigned to the baloxavir or placebo group, and those aged ≥20 years to the baloxavir, oseltamivir, or placebo group.

Patients received (a) a single oral dose of baloxavir 40 mg (body weight <80 kg) or 80 mg (body weight ≥80 kg), (b) oseltamivir (75 mg) twice daily for 5 days (patients ≥20 years only), or (c) placebo.

Of 1436 randomized patients, 1432 who received at least 1 dose of the study drug were included in the safety analysis population. Of the 1432 patients, 1064 (456 in the baloxavir group, 377 in the oseltamivir group, 231 in the placebo group) were included in the ITTI population,[45)] excluding patients without evidence of influenza virus infection. The ITTI population was subjected to efficacy analysis.

Efficacy results:

43) Patients were stratified by age (≥12 and <20 years, ≥20 years), and composite score of 7 influenza symptoms (≤11 points, ≥12 points) and region (Japan/Asia, other country/region) were used as allocation factors.
44) Subjects were actually enrolled in the study only in Japan and US.
45) Patients who received at least 1 dose of the study drug and tested positive for influenza virus by reverse transcription-polymerase chain reaction.

Table 34 and Figure 5 show the results of the primary efficacy endpoint (i.e., duration of influenza illness). The duration of influenza illness was defined as the period between the start of study treatment and the time when all of the influenza symptoms (cough, sore throat, headache, nasal congestion, feverishness or chills, myalgia or arthralgia, and fatigue) were recorded as "0, none" or "1, mild" in the patient diary.[41)] Paired comparison between the baloxavir and placebo groups showed a statistically significant difference, demonstrating the superiority of baloxavir to placebo. In patients aged ≥20 years (the subpopulation that included the oseltamivir group), the median duration of influenza illness [95% CI] was 53.5 [48.0, 58.5] hours in the baloxavir group, 53.8 [50.2, 56.4] hours in the oseltamivir group, and 77.8 [68.8, 85.0] hours in the placebo group.

Table 34. Duration of influenza illness (ITTI population [a)])

	Baloxavir (N = 455)	Placebo (N = 230)
Median [95% CI] (hours)	53.7 [49.5, 58.5]	80.2 [72.6, 87.1]
P value[b)]	<0.0001	-

a) Subjects without data of duration of influenza illness (1 each in the baloxavir and placebo groups) were excluded from ITTI population.
b) Two-sided significance level of 5%. Generalized Wilcoxon test stratified by the composite score of 7 influenza symptoms (≤11 points, ≥12 points) and region (Japan/Asia, other country/region).

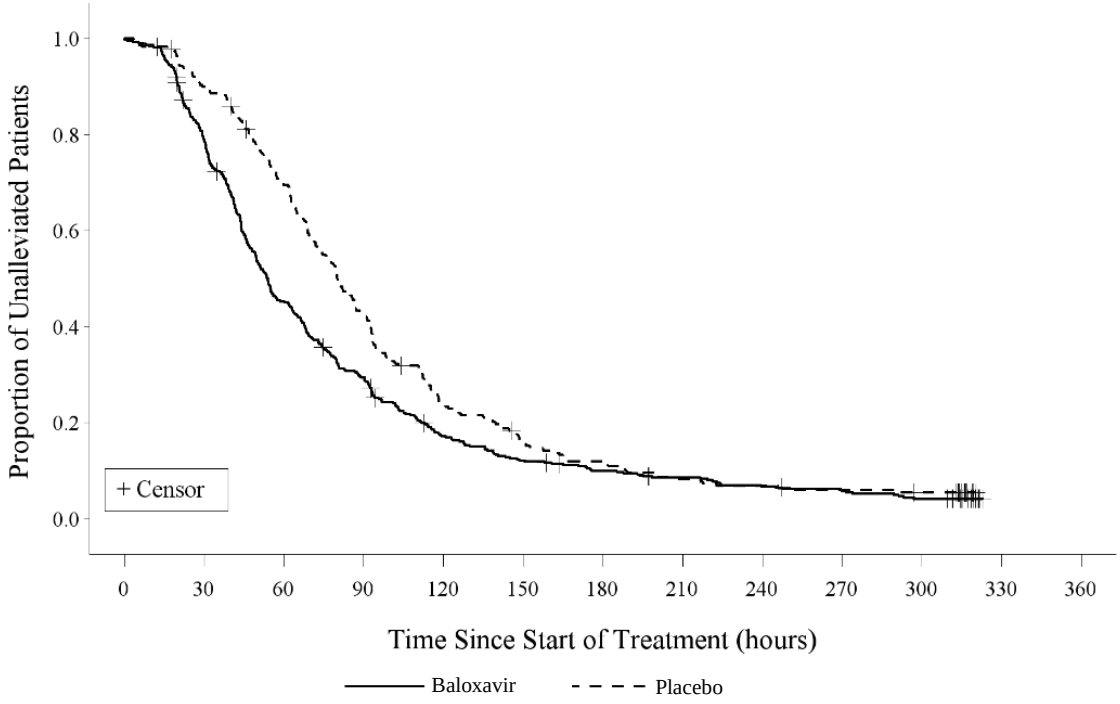

Figure 5. Kaplan-Meier plots of duration of influenza illness (ITTI population)
(cited from CTD 2.7.6 Figure 2.7.6.14-2)

In the Japanese subpopulation (342 in the baloxavir group, 174 in the placebo group), the median duration of influenza illness [95% CI] was 46.4 [43.8, 52.1] hours in the baloxavir group and 77.7 [68.8, 86.5] hours in the placebo group. Figure 6 shows Kaplan-Meier plots. In Japanese patients aged ≥20 years (the subpopulation that included oseltamivir group [293 in the baloxavir group, 303 in the oseltamivir group, 150 in the placebo group]), the median duration of influenza illness [95% CI] was 46.8 [43.8, 53.4] hours in the baloxavir group, 51.1 [47.2, 54.6] hours in the oseltamivir group, and 74.0 [68.3, 84.7] hours in the placebo group.

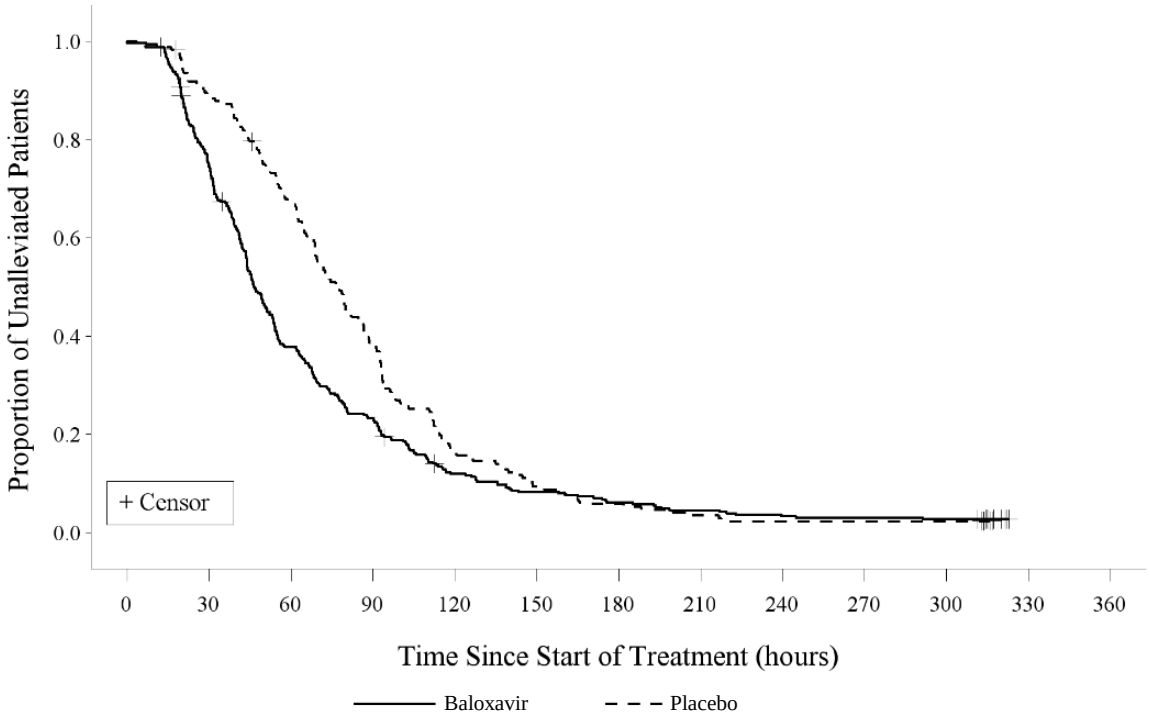

Figure 6. Kaplan-Meier plots of duration of influenza illness (ITTI population [Japanese patients]) (cited from CTD 2.7.6 Figure 2.7.6.14-3)

Safety results:

Adverse events (including abnormal changes in laboratory tests) were observed in 20.7 % (126 of 610) of patients in the baloxavir group, 24.6% (76 of 309) of patients in the placebo group, and 24.8% (127 of 513) of patients in the oseltamivir group. Adverse drug reactions[42)] were observed in 4.4% (27 of 610) of patients in the baloxavir group, 3.9% (12 of 309) of patients in the placebo group, and 8.4% (43 of 513) of patients in the oseltamivir group. Adverse events and adverse drug reactions reported by ≥1% of patients in any group are shown in Table 35.

Table 35. Adverse events and adverse drug reactions reported by ≥1% of patients in any group (safety analysis population)

Events	Adverse events			Adverse drug reactions		
	Baloxavir (N = 610)	Placebo (N = 309)	Oseltamivir [a)] (N = 513)	Baloxavir (N = 610)	Placebo (N = 309)	Oseltamivir[a)] (N = 513)
All events	126 (20.7)	76 (24.6)	127 (24.8)	27 (4.4)	12 (3.9)	43 (8.4)
Diarrhoea	18 (3.0)	14 (4.5)	11 (2.1)	11 (1.8)	4 (1.3)	7 (1.4)
Bronchitis	16 (2.6)	17 (5.5)	18 (3.5)	0	0	0
Nasopharyngitis	9 (1.5)	2 (0.6)	4 (0.8)	0	0	0
Nausea	8 (1.3)	4 (1.3)	16 (3.1)	2 (0.3)	2 (0.6)	8 (1.6)
Sinusitis	7 (1.1)	8 (2.6)	5 (1.0)	0	0	0
ALT increased	6 (1.0)	4 (1.3)	7 (1.4)	4 (0.7)	1 (0.3)	4 (0.8)
Dizziness	3 (0.5)	4 (1.3)	1 (0.2)	0	0	0
Leukopenia	0	3 (1.0)	1 (0.2)	0	1 (0.3)	1 (0.2)
Headache	5 (0.8)	3 (1.0)	4 (0.8)	1 (0.2)	1 (0.3)	1 (0.2)
Constipation	0	3 (1.0)	0	0	0	0
Vomiting	5 (0.8)	2 (0.6)	6 (1.2)	2 (0.3)	1 (0.3)	4 (0.8)

n (%)

a) Subjects aged ≥20 years

No death occurred.

Serious adverse events were observed in 2 patients in the baloxavir group (meningitis viral and incarcerated inguinal hernia in 1 patient each), but their causal relationship to the study drug was ruled out and the outcome was “resolved.”

Adverse events leading to discontinuation were observed in 2 patients in the baloxavir group (bronchitis in 2 patients, pneumonia in 1 patient [including duplicate counting]), 1 patient in the placebo group (nausea, arthralgia, back pain, pain in jaw in 1 patient each [including duplicate counting]), and 2 patients in the oseltamivir group (nausea and pneumonia in 1 patient each). Nausea, arthralgia, back pain, and pain in jaw in the placebo group and nausea in the oseltamivir group were assessed as causally related to the study drug, while other events were assessed as causally unrelated to the study drug. The outcome was “not resolved” for nausea in the oseltamivir group and “resolved” for all the other events.

In the Japanese population, the incidence of adverse events (including abnormal changes in laboratory tests) was 23.9% (85 of 355) of patients in the baloxavir group, 26.1% (47 of 180) of patients in the placebo group, and 26.5% (82 of 310) of patients in the oseltamivir group, and the incidence of adverse drug reactions was 5.1% (18 of 355) of patients in the baloxavir group, 3.9% (7 of 180) of patients in the placebo group, and 8.4% (26 of 310) of patients in the oseltamivir group.

No death or serious adverse event was observed.

Adverse events leading to discontinuation were observed in 1 patient in the baloxavir group (bronchitis and pneumonia in 1 patient). Their causal relationship was assessed as unrelated to the study drug, and their outcome was “resolved.”

7.3.2 Japanese study (CTD 5.3.5.2-01, Study T0822 [November 2016 to April 2017])

An uncontrolled, open-label study was conducted to investigate the efficacy and safety of baloxavir 10- and 20-mg tablets in patients aged <12 years with influenza virus infection (target sample size, 100 subjects) in 41 study sites in Japan. A single oral dose of baloxavir was administered at 5 mg (a half 10-mg tablet) to patients weighing ≥5 and <10 kg; at 10 mg (one 10-mg tablet) to those weighing ≥10 and <20 kg); at 20 mg (two 10-mg tablets or one 20-mg tablet) to those weighing ≥20 and <40 kg; or at 40 mg (two 20-mg tablets) to those weighing ≥40 kg.

A total of 107 patients (105 patients aged ≥2 and <12 years, 2 patients aged ≥6 months and <2 years) receiving at least 1 dose of the study drug were included in the safety analysis population. Among them, 104 patients (103 patients aged ≥2 and <12 years, 1 patient aged 6 months and <2 years) were included in ITTI population,[45)] excluding patients without evidence of influenza virus infection. The ITTI population was subjected to the primary efficacy analysis.

Efficacy results:

The duration of influenza illness, the primary efficacy endpoint, was defined as the period between the start of study treatment and the resolution of influenza symptoms (i.e., “0, none” or “1, mild” for both

"cough" and "runny nose/nasal congestion" and axillary temperature <37.5°C, based on patient diary entries). The median value [95% CI] of duration of influenza illness was 44.6 [38.9, 62.5] hours. Figure 7 shows Kaplan-Meier plotting.

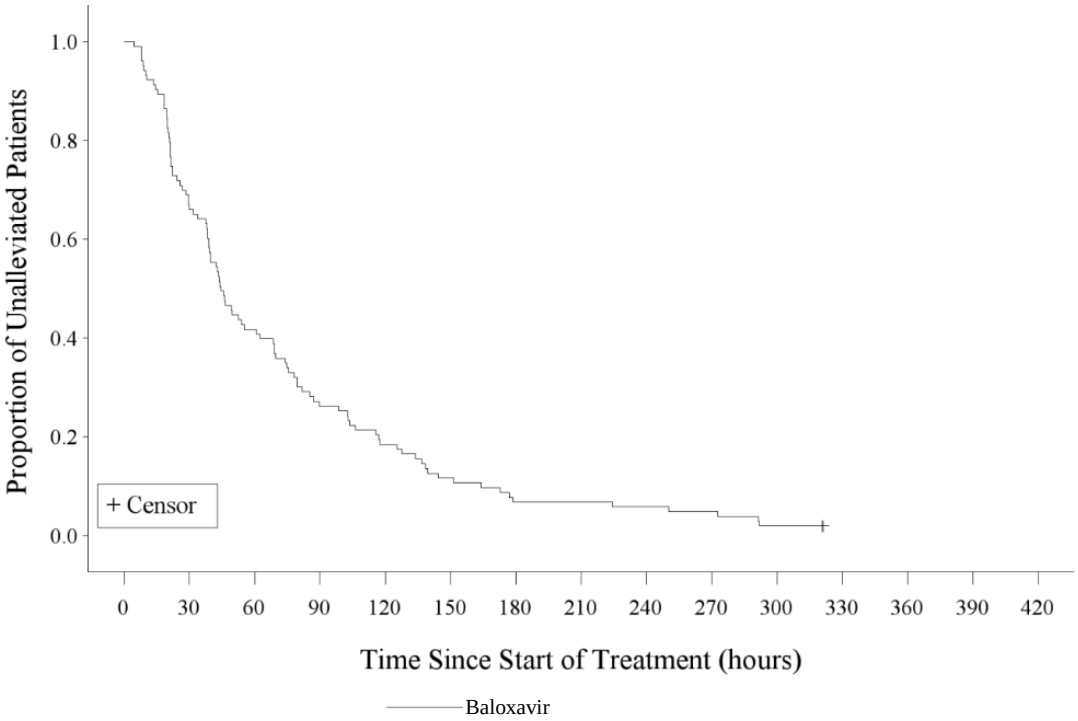

Figure 7. Kaplan-Meier estimate of duration of influenza illness (ITTI population) (cited from CTD 2.7.6 Figure 2.7.6.15-1)

Safety results:

Adverse events (including abnormal changes in laboratory tests) were observed in 34.6% (37 of 107) of patients, and adverse drug reactions[42)] in 3.7% (4 of 107) of patients. Table 36 shows adverse events and adverse drug reactions reported by ≥1% of patients. There was no death, serious adverse event, or adverse event leading to discontinuation.

Table 36. Adverse events and adverse drug reactions reported by ≥1% of patients (safety analysis population)

Events	Adverse events (N = 107)	Adverse drug reactions (N = 107)
All events	37 (34.6)	4 (3.7)
Vomiting	8 (7.5)	0
Pharyngitis	3 (2.8)	0
Diarrhoea	3 (2.8)	2 (1.9)
Bronchitis	2 (1.9)	0
Sinusitis	2 (1.9)	0
Oral herpes	2 (1.9)	0
Headache	2 (1.9)	0
Upper respiratory tract inflammation	2 (1.9)	0
Constipation	2 (1.9)	0
Ligament sprain	2 (1.9)	0

n (%)

7.R Outline of the review conducted by PMDA

7.R.1 Study plan

The applicant's explanation about the justification for planning and conducting the phase III study (Study T0831) as a global study:

- The pathology and the clinical course after influenza virus infection are similar regardless of ethnicity, etc.
- The type/subtype of influenza virus epidemic during the same season may be different depending on the country/region. In recent years, however, subtype A/H1N1pdm or A/H3N2, or type B is prevalent in all countries/regions. In nonclinical studies, baloxavir has been shown to be pharmacologically active against a wide range of types/subtypes of influenza virus [see Sections 3.1.2 and 3.1.4], and the Japanese phase II study (Study T0821) demonstrated the efficacy of baloxavir regardless of the type/subtype of influenza virus. These findings suggest that the difference in the type/subtype of influenza virus epidemic in different counties/regions, if any, would only minimally affect the efficacy of baloxavir.
- The duration of influenza illness, the primary endpoint, is the endpoint commonly used in Japan and other countries. This endpoint was considered appropriate for a global clinical study because it can be used across countries.
- There are ethnic differences in PK of S-033447, the active metabolite of baloxavir. Nevertheless, baloxavir 40 mg allows the maintenance of plasma S-033447 concentration at a level with sufficient efficacy and acceptable safety in almost all patients [see Sections 6.R.1 and 6.R.3].

The applicant's explanation about the significance of using placebo and oseltamivir as controls in the global phase III study (Study T0831):

The primary objective of the global phase III study (Study T0831) was to demonstrate the superiority of baloxavir to placebo in patients aged ≥12 years with influenza virus infection. The secondary objective of the study was to clarify the clinical positioning of baloxavir by evaluating the efficacy and safety of baloxavir versus oseltamivir phosphate (control). Oseltamivir phosphate was used as the control, because it has abundant evidence, collected through wide use in Japan and other countries, for the treatment of adult patients with influenza virus infection.

PMDA's view:

Conducting the phase III study (Study T0831) as a global study was appropriate. The control groups used in the study (placebo and oseltamivir) were also appropriate.

7.R.2 Efficacy

Based on the following review, PMDA concluded that baloxavir is expected to be effective in patients with influenza A or B virus infection. However, newly available information should be appropriately provided to healthcare professionals, for the following reasons: (a) Patients aged ≥65 years were excluded from the Japanese phase II study (Study T0821) and the global phase III study (Study T0831). (b) As of November 2017, a clinical study is ongoing in patients with influenza virus infection who have high risk factors, including advanced age (Study T0832). (c) Only limited information is currently available on the effect of baloxavir in patients aged <12 years with influenza virus infection.

The above conclusion of PMDA will be discussed at the Expert Discussion.

7.R.2.1 Efficacy in patients aged ≥12 years

The applicant's explanation about the efficacy of baloxavir in patients aged ≥12 years with influenza virus infection:

The elimination half-life after a single dose of baloxavir is 48.9 to 90.9 hours [see Section 6.2.1.1]. Given its pharmacokinetic profile, the proposed single-dose regimen of baloxavir is appropriate. The effective C_{24} of S-033447 in non-clinical models was determined to be 6.85 ng/mL [see Section 3.1.4.1.5], and the phase I study (Study T0811) suggested that baloxavir 6 mg would achieve C_{24} of 6.92 ng/mL, a level exceeding 6.85 ng/mL. This suggests that baloxavir >20 mg will achieve C_{24} of 25.2 ng/mL, a level expected to have therapeutic efficacy. Also, no particular problem was observed in the safety or tolerability in single administration of baloxavir up to 80 mg. Based on the above, the dose-finding Japanese phase II study (Study T0821) was planned and conducted to evaluate a single oral dose of baloxavir 10, 20, and 40 mg. In this study, the duration of influenza illness (median) [95% CI], the primary endpoint, was 54.2 [47.7, 66.8] hours in the 10 mg group, 51.0 [44.5, 62.4] hours in the 20 mg group, 49.5 [44.5, 64.4] hours in the 40 mg group, and 77.7 [67.6, 88.7] hours in the placebo group. The duration of influenza illness thus tended to be shorter in all baloxavir groups than in the placebo group.

In the Japanese phase II study (Study T0821), plasma S-033447 concentration (C_{24}) following administration of baloxavir 40 mg exceeded the plasma level required to achieve clinical efficacy, and S-033447 exposure varied depending on body weight. Because of these findings, in the global phase III study (Study T0831), baloxavir was administered orally in a single dose at 40 mg (in patients weighing <80 kg) or at 80 mg (in patients weighing ≥80 kg) [see Section 6.R.3]. In Study T0831, the median duration of influenza illness [95% CI], the primary endpoint, was 53.7 [49.5, 58.5] hours in the baloxavir group and 80.2 [72.6, 87.1] hours in the placebo group. The paired comparison between the baloxavir and placebo groups showed a statistically significant difference (generalized Wilcoxon test stratified by the composite score of 7 influenza symptoms [≤11 points, ≥12 points] and region [Japan/Asia, other countries/regions]), demonstrating the superiority of baloxavir to placebo [see Section 7.3.1].

In the subgroup of patients aged ≥20 years (192 in the placebo group, 375 in the baloxavir group, 377 in the oseltamivir group) in the global phase III study (Study T0831), the median duration of influenza illness was 77.8 hours in the placebo group, 53.5 hours in the baloxavir group, and 53.8 hours in the oseltamivir group, with the difference [95% CI] between the baloxavir and oseltamivir groups being −0.3 [−6.6, 6.6] hours (Figure 8). In the subgroup of patients aged ≥12 and <20 years (38 in the placebo group, 80 in the baloxavir group), the median duration of influenza illness was 103.8 hours in the placebo group and 54.6 hours in the baloxavir group (Figure 9).

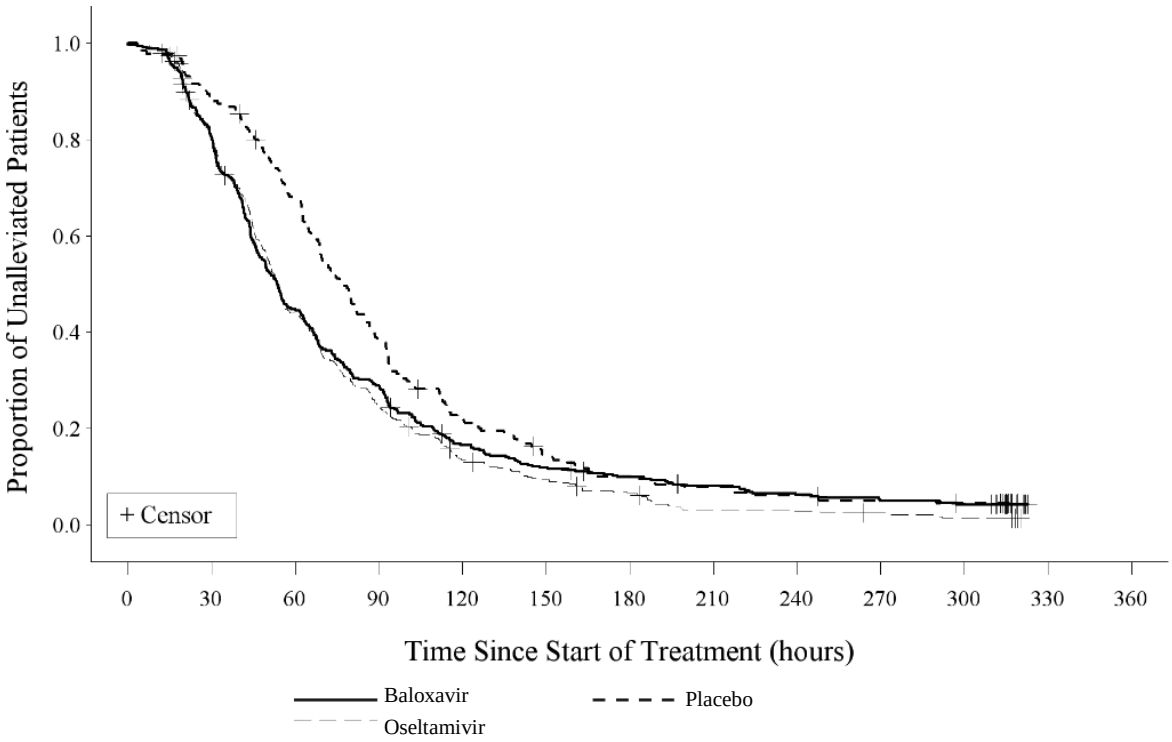

Figure 8. Kaplan-Meier plots of duration of influenza illness (ITTI population, patients aged ≥20 years) (cited from CTD 2.7.6 Figure 2.7.6.14-2)

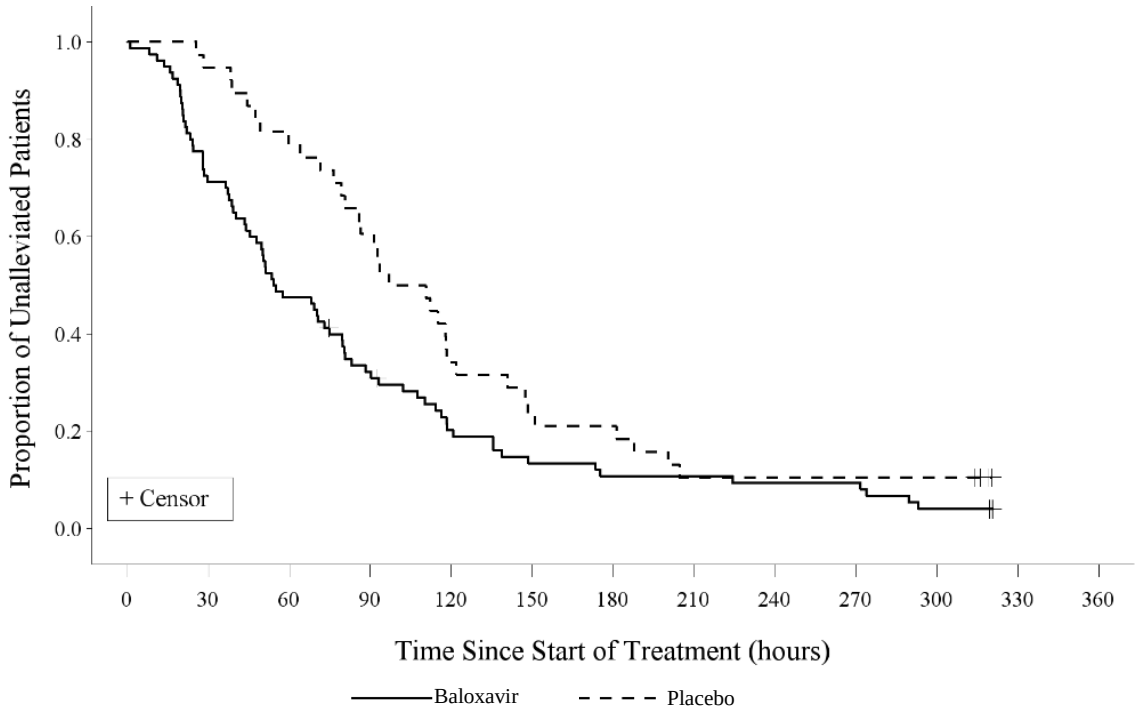

Figure 9. Kaplan-Meier plots of duration of influenza illness (ITTI population, patients aged ≥12 and <20 years) (cited from CTD 2.7.6 Figure 2.7.6.14-4)

Table 37 shows the duration of influenza illness in subpopulations classified by age and region (Japan and US). The duration of influenza illness was shorter in the baloxavir group than in the placebo group in all age groups both in Japan and the US.

Table 37. Duration of influenza illness, classified by region (ITTI population)

		Japan		US	
		N	Median [95% CI] (hours)	N	Median [95% CI] (hours)
≥12 years	Baloxavir	342	46.4 [43.8, 52.1]	113	87.3 [72.9, 96.8]
	Placebo	174	77.7 [68.8, 86.5]	56	117.9 [80.2, 148.5]
≥12 and <20 years	Baloxavir	49	45.5 [36.5, 53.4]	31	107.5 [72.9, 120.8]
	Placebo	24	86.1 [59.8, 97.0]	14	166.1 [117.9, -]
≥20 years	Baloxavir	293	46.8 [43.8, 53.4]	82	80.0 [66.2, 94.0]
	Oseltamivir	303	51.1 [47.2, 54.6]	74	85.4 [57.1, 104.4]
	Placebo	150	74.0 [68.3, 84.7]	42	82.1 [62.7, 125.8]

-, Not calculable

The median duration of influenza illness classified by body weight was 51.3 hours in the baloxavir group and 79.3 hours in the placebo group among patients weighing <80 kg, and 66.8 hours in the baloxavir group and 85.4 hours in the placebo group among patients weighing ≥80 kg. Thus, the duration of influenza illness tended to be shorter in the baloxavir group than in the placebo group regardless of body weight (<80 kg or ≥80 kg).

PMDA's view:
The global phase III study (Study T0831) demonstrated (a) the superiority of baloxavir to placebo and (b) a tendency to shorter duration of influenza illness in the baloxavir group than in the placebo group among patients aged ≥12 and <20 years. These results demonstrate the efficacy of baloxavir in patients aged ≥12 years with influenza virus infection. In addition, the analysis of the Japanese subpopulation has suggested the efficacy of baloxavir in Japanese patients aged ≥12 years. Baloxavir exhibited a higher suppressive effect than oseltamivir phosphate against replication of seasonal influenza A and B viruses [see Section 3.R.1]. However, the global phase III study (Study T0831) did not show a clear difference in the duration of influenza illness between the oseltamivir and baloxavir groups, suggesting similar efficacy of baloxavir and oseltamivir phosphate.

7.R.2.2 Efficacy in patients aged <12 years

The applicant's explanation about the efficacy of baloxavir in patients aged <12 years with influenza infection:
The median duration of influenza illness in the baloxavir group was 44.6 hours [see Section 7.3.2] in patients aged <12 years (the Japanese study [Study T0822]), and 53.7 hours in patients aged ≥12 years (the global phase III study [Study T0831]), showing similar results in both age groups. The median duration classified by dose (body weight) was 60.9 hours at 40 mg (≥40 kg, 8 patients), 45.6 hours at 20 mg (≥20 kg and <40 kg, 65 patients), 39.1 hours at 10 mg (≥10 kg and <20 kg, 29 patients), and 139.4 hours at 5 mg (≥5 kg and <10 kg, 1 patient).

The above results demonstrate the efficacy of baloxavir in patients aged <12 years with influenza virus infection.

PMDA's view:
Baloxavir is expected to be effective in patients aged <12 years with influenza virus infection, for the reasons listed below. Since the experience with baloxavir in patients aged <2 years is extremely limited (with only 1 patient weighing ≥5 and <10 kg treated so far), relevant information should be collected

after the market launch and when new findings become available, the information should be appropriately provided to healthcare professionals.

- The efficacy of baloxavir was demonstrated by the global phase III study in patients aged ≥12 years (Study T0831).
- The exposure to the active metabolite S-033447 following administration of baloxavir was similar in the global phase III study (Study T0831) and the Japanese study (Study T0822) [see Section 6.R.3].
- The efficacy of baloxavir was similar in the global phase III study (Study T0831) and the Japanese study (Study T0822).

7.R.2.3 Efficacy by type/subtype of virus

The applicant's explanation about the efficacy of baloxavir against different types/subtypes of influenza virus:

Table 38 shows the duration of influenza illness, classified by type/subtype of virus, in the Japanese phase II study (Study T0821) and the global phase III study (Study T0831).

Table 38. Duration of influenza illness, classified by type/subtype (ITTI population)

	Study T0821				Study T0831				
	10 mg	20 mg	40 mg	Placebo	Entire population		≥20 years		
					Baloxavir	Placebo	Baloxavir	Oseltamivir	Placebo
Subtype A/H1N1pdm									
N	66	71	61	69	7	7	7	2	6
Median [95% CI] (hours)	52.9 [45.9, 65.6]	47.1 [39.4, 55.3]	48.2 [35.2, 65.5]	70.6 [64.9, 89.9]	43.7 [22.0, 109.1]	141.0 [82.1, -]	43.7 [22.0, 109.1]	65.9 [23.0, 108.8]	129.6 [82.1, -]
Subtype A/H3N2									
N	13	5	12	6	392	195	320	332	163
Median [95% CI] (hours)	66.0 [28.1, 83.5]	65.8 [21.3, 188.5]	45.4 [23.5, 113.4]	100.0 [18.9, 113.1]	52.2 [47.0, 56.8]	79.5 [69.5, 86.8]	52.1 [46.1, 56.0]	51.8 [48.1, 54.7]	74.7 [66.2, 84.7]
Type B									
N	21	23	24	23	38	20	33	34	16
Median [95% CI] (hours)	63.3 [44.5, 82.3]	65.4 [46.4, 73.2]	63.3 [43.3, 69.8]	83.1 [58.1, 92.8]	93.0 [53.4, 135.4]	77.1 [46.8, 189.0]	111.8 [56.0, 136.6]	87.6 [57.1, 112.4]	77.1 [52.0, 199.7]

-, Not calculable

In the Japanese phase II study (Study T0821), the median duration of influenza illness tended to be shorter in all baloxavir groups than in the placebo group, regardless of type/subtype of virus. In the global phase III study (Study T0831), the duration of influenza illness of subtypes A/H1N1pdm and A/H3N2 viruses was shorter in patients receiving baloxavir than in those receiving placebo, but the duration of influenza illness of type B virus tended to be longer in patients receiving baloxavir than in those receiving placebo. The longer illness duration of type B virus may have been due to the small number of patients infected with influenza B virus.

PMDA's view:

The efficacy in patients with influenza A virus infection:

The Japanese phase II study (Study T0821) and the global phase III study (Study T0831) were conducted in different years. Because of the uneven distribution of the epidemic strains from year to year and from region to region, it is necessary to carefully assess the efficacy of baloxavir against different types and

subtypes of influenza virus. However, given the results obtained, baloxavir is expected to be effective in patients with influenza A virus infection.

The efficacy in patients with influenza B virus infection:
Only a limited number of patients were evaluated in the Japanese phase II study (Study T0821) and the global phase III study (Study T0831), and the results of these studies did not always have similar trends. Nevertheless, in the Japanese phase II study (Study T0821), the duration of influenza illness tended to decrease in all baloxavir dose groups, and in non-clinical studies, baloxavir suppressed the replication of influenza B virus and improved the mortality associated with viral infection. Therefore baloxavir may be expected to offer a certain level of efficacy against influenza B virus infection. Information on the efficacy of baloxavir against influenza B virus infection should be continuously collected after the market launch and the findings obtained should be appropriately provided to healthcare professionals.

7.R.2.4 Resistance to baloxavir

The applicant's explanation about how amino acid substitutions conferring resistance against baloxavir affect the efficacy of baloxavir:
Figure 10 and Table 39 show changes over time in viral titer, with and without PA/I38 amino acid substitution, in the baloxavir group of the global phase III study (Study T0831). In the baloxavir group, viral titer rapidly decreased on the next day of treatment with baloxavir regardless of PA/I38 amino acid substitution, decreasing to a level close to the LLOQ on Day 2 or 3 after administration of baloxavir. However, a transient increase in viral titer was observed in patients with I38 amino acid substitution on Day 5 or 6, the time point when PA/I38 amino acid substitution was detected in many patients. This tendency was also observed in the Japanese phase II study (Study T0821) and in the Japanese study (Study T0822). A similar viral titer increase was reported with an existing drug (oseltamivir phosphate) as well (*Lancet.* 2004;364:759-65). However, the PA/I38 amino acid substitution was not detected in samples collected from patients before administration of baloxavir, and *in vitro* studies showed decreased replication capacity of PA/I38 variants. These findings suggest that, in the natural environment, the variants are unlikely to become more dominant than the wild type.

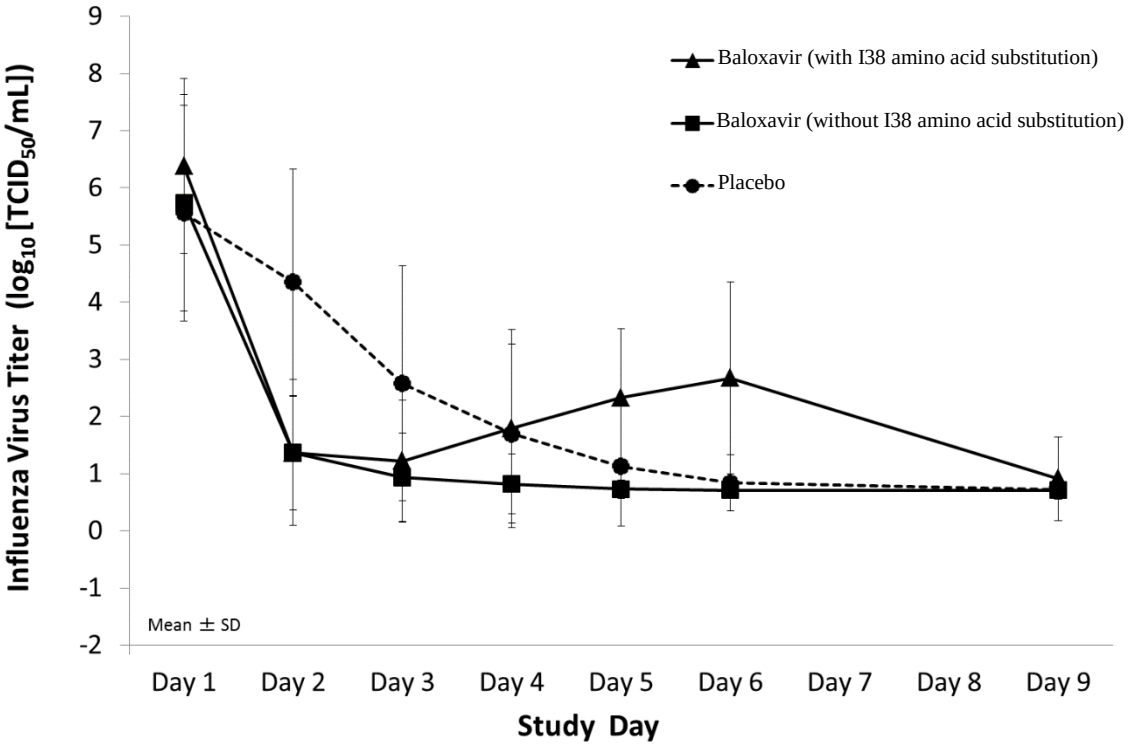

Figure 10. Changes over time in viral titer with and without PA/I38 amino acid substitution in the global phase III study (Study T0831) (mean ± SD) (cited from CTD 2.7.2 Figure 2.7.2.4-3)

Table 39. Changes over time in viral titer in the global phase III study (Study T0831) (log_{10} [$TCID_{50}$/mL]) (ITTI population)

	Placebo		Baloxavir			
			Without PA/I38 substitution		With PA/I38 substitution	
	No. of patients	Viral titer	No. of patients	Viral titer	No. of patients	Viral titer
Day 1[a)]	210	5.56 ± 1.89	393	5.74 ± 1.89	34	6.38 ± 1.53
Day 2	201	4.35 ± 1.98	380	1.37 ± 1.28	34	1.36 ± 0.99
Day 3	193	2.58 ± 2.05	372	0.93 ± 0.78	34	1.22 ± 1.06
Day 4	57	1.70 ± 1.56	107	0.82 ± 0.52	7	1.79 ± 1.73
Day 5	192	1.13 ± 1.05	376	0.73 ± 0.16	30	2.33 ± 1.20
Day 6	48	0.84 ± 0.49	91	0.71 ± 0.05	6	2.67 ± 1.68
Day 9	197	0.72 ± 0.11	374	0.71 ± 0.16	34	0.91 ± 0.73

Mean ± SD

a) The day of study drug administration was defined as Day 1.

Table 40 shows the effect of PA/I38 amino acid substitution on the efficacy of baloxavir in patients receiving baloxavir in the global phase III study (Study T0831) and the Japanese study (Study T0822).

Table 40. Outline of the efficacy with and without amino acid substitution (ITTI population)

	Study T0831		Study T0822	
	With PA/I38 substitution	Without PA/I38 substitution	With PA/I38 substitution	Without PA/I38 substitution
Number of patients	36	419	17	86
Median duration of influenza illness [95% CI] (hours)	63.1 [52.2, 87.7]	52.4 [47.8, 56.8]	79.6 [39.8, 116.9]	43.0 [31.8, 52.4]
No influenza symptom[b)] on Day ≥5 or ≥6[a)] after administration of baloxavir	83.3% (30/36)	83.8% (347/414)	82.4% (14/17)	87.2% (75/86)
No pyrexia[c)] on Day ≥5 or ≥6[a)] after administration of baloxavir	91.7% (33/36)	91.2% (374/410)	100% (17/17)	98.8% (85/86)

a) Day ≥5 in Study T0831, Day ≥6 in Study T0822.
b) Definitions of influenza symptoms: at least 1 moderate or severe influenza symptom lasting for ≥21.5 hours in Study T0831; moderate or severe respiratory symptoms (cough or nasal congestion [including runny nose]) lasting for ≥21.5 hours in Study T0822.
c) Definitions of pyrexia: fever of ≥37.0°C lasting for ≥12 hours in Study T0831; fever of ≥37.5°C lasting for ≥12 hours in Study T0822.

Thus, the clinical studies have detected I38 amino acid substitution in PA, the region affecting the sensitivity to S-033447, but this amino acid substitution is unlikely to affect the clinical symptoms.

PMDA's view:
PMDA confirmed the following findings: (a) Both in the Japanese clinical studies (Studies T0821 and T0822) and in the global phase III study (Study T0831), PA/I38 amino acid substitution occurred at a certain frequency in patients receiving baloxavir [see Section 3.1.3.4]. (b) In patients infected with influenza virus that underwent I38 amino acid substitution during treatment with baloxavir, viral titers increased again from Day 3 after administration of baloxavir and, from Day 4, tended to increase to levels higher than those in patients receiving placebo. Currently, there is no information available on the pathogenicity or transmissibility of the low S-033447-sensitivity strain with PA/I38T substitution or on the efficacy of baloxavir in patients infected with influenza virus with I38 amino acid substitution at baseline. Therefore, the clinical effect of I38 amino acid substitutions is unclear. However, attention should be paid, as a public health concern, to the occurrences of I38 amino acid substitution observed in clinical studies and to the rebound of viral titer in patients infected with influenza virus that underwent I38 amino acid substitution.

The applicant should appropriately inform healthcare professionals about the virus resistance to baloxavir observed in clinical studies, etc. After the market launch, the applicant should continue to survey the long-term (over years) trends of drug resistance of each type/subtype of influenza virus, and should provide information obtained to healthcare professionals.

7.R.3 Safety

Based on the following review, PMDA concluded that a single dose of baloxavir has acceptable safety in patients with influenza A or B virus infection.

After the market launch, the applicant should continue to collect information on (a) the safety in children aged <2 years (because of limited experience with baloxavir in this age group) and (b) baloxavir-induced hepatic dysfunction, and should provide information obtained to healthcare professionals. No abnormal behavior was observed in clinical studies of baloxavir. However, there are reports of abnormal behavior after administration of approved influenza antiviral drugs, although the causal relationship to the drug

is unknown. Patients and healthcare professionals who use baloxavir should be alerted to this risk in the same manner as those using other influenza antiviral drugs.

The above conclusion of PMDA will be discussed at the Expert Discussion.

7.R.3.1 Safety of baloxavir

The applicant's explanation about the safety of baloxavir in patients with influenza virus infection:
Table 41 shows the outline of the safety of baloxavir in the Japanese phase II study (Study T0821), the global phase III study (Study T0831), and the Japanese study (Study T0822).

In the combined data of the Japanese phase II study (Study T0821) and the global phase III study (Study T0831), the incidence of adverse events was 22.2% (202 of 910) of patients in the baloxavir group, 25.7% (105 of 409) of patients in the placebo group, and 24.8% (127 of 513) of patients in the oseltamivir group; the incidence of adverse drug reactions was 5.4% (49 of 910) of patients in the baloxavir group, 5.4% (22 of 409) of patients in the placebo group, and 8.4% (43 of 513) of patients in the oseltamivir group. The baloxavir and placebo groups thus had similar incidences of adverse events and adverse drug reactions. The baloxavir had no adverse events with an incidence of ≥5%. The incidence of Grade ≥3[46)] adverse events was 0.7% (6 of 910) of patients in the baloxavir group (nausea/vomiting, polydipsia/diarrhoea, meningitis viral, otitis media, incarcerated inguinal hernia, and headache in 1 patient each), 1.0% (4 of 409) of patients in the placebo group (sinusitis, bronchitis, diarrhoea, and nausea/arthralgia/back pain/pain in jaw in 1 patient each), and 0.2% (1 of 513) of patients in the oseltamivir group (otitis media). Among them, only otitis media in the baloxavir group was a Grade 4 adverse event. Table 42 shows adverse events and adverse drug reactions reported by ≥2% of patients in any group.

Table 41. Outline of safety (Studies T0821/T0831 combined and Study T0822)

	Studies T0821/T0831 combined		Study T0822	
	Baloxavir (N = 910)	Placebo (N = 409)	Oseltamivir[a)] (N = 513)	Baloxavir (N = 107)
Adverse events	202 (22.2)	105 (25.7)	127 (24.8)	37 (34.6)
Adverse drug reactions	49 (5.4)	22 (5.4)	43 (8.4)	4 (3.7)
Serious adverse events	2 (0.2)	0	0	0
Adverse events leading to discontinuation	2 (0.2)	1 (0.2)	2 (0.4)	0
Adverse events resulting in death	0	0	0	0

n (%)
a) Only patients aged ≥20 years in Study T0831 were assigned to the oseltamivir group.

Table 42. Adverse events and adverse drug reactions reported by ≥2% of patients in any group (Studies T0821/T0831 combined)

	Adverse events			Adverse drug reactions		
	Baloxavir (N = 910)	Placebo (N = 409)	Oseltamivir[a)] (N = 513)	Baloxavir (N = 910)	Placebo (N = 409)	Oseltamivir[a)] (N = 513)
Diarrhoea	23 (2.5)	19 (4.6)	11 (2.1)	12 (1.3)	6 (1.5)	7 (1.4)
Bronchitis	17 (1.9)	17 (4.2)	18 (3.5)	0	0	0
Nausea	9 (1.0)	5 (1.2)	16 (3.1)	2 (0.2)	2 (0.5)	8 (1.6)
Sinusitis	7 (0.8)	8 (2.0)	5 (1.0)	0	0	0

n (%)
a) Only patients aged ≥20 years in Study T0831 were assigned to the oseltamivir group.

[46)] CTCAE v4.0

Tables 43 and 44 show the outline of safety by region and adverse events reported by ≥2% of patients in any group. The incidence of adverse events did not significantly differ between the baloxavir and placebo groups in Japan, and the incidence of individual adverse events did not significantly differ between Japan and US.

Table 43. Outline of safety by region (Studies T0821/T0831 combined)

	Baloxavir		Placebo		Oseltamivir	
	Japan (N = 655)	US (N = 255)	Japan (N = 280)	US (N = 129)	Japan (N = 310)	US (N = 203)
Adverse events	161 (24.6)	41 (16.1)	76 (27.1)	29 (22.5)	82 (26.5)	45 (22.2)
Adverse drug reactions	40 (6.1)	9 (3.5)	17 (6.1)	5 (3.9)	26 (8.4)	17 (8.4)
Serious adverse events	0	2 (0.8)	0	0	0	0
Adverse events leading to discontinuation	1 (0.2)	1 (0.4)	0	1 (0.8)	0	2 (1.0)
Adverse events resulting in death	0	0	0	0	0	0

n (%)

Table 44. Adverse events reported by ≥2% of patients in any group by region (Studies T0821/T0831 combined)

	Baloxavir		Placebo		Oseltamivir	
	Japan (N = 655)	US (N = 255)	Japan (N = 280)	US (N = 129)	Japan (N = 310)	US (N = 203)
Diarrhoea	14 (2.1)	9 (3.5)	16 (5.7)	3 (2.3)	10 (3.2)	1 (0.5)
Bronchitis	11 (1.7)	6 (2.4)	13 (4.6)	4 (3.1)	9 (2.9)	9 (4.4)
Nausea	7 (1.1)	2 (0.8)	2 (0.7)	3 (2.3)	4 (1.3)	12 (5.9)
Sinusitis	5 (0.8)	2 (0.8)	4 (1.4)	4 (3.1)	2 (0.6)	3 (1.5)

n (%)

PMDA concluded that baloxavir has acceptable safety in patients with influenza virus infection, based on its review (see Sections 7.R.3.1.1 to 7.R.3.1.3) and on the incidences of adverse events in subjects receiving baloxavir in the Japanese phase II study (Study T0821), the global phase III study (Study T0831), and the Japanese study (Study T0822).

7.R.3.1.1 Safety of baloxavir by age

The applicant's explanation about the safety of baloxavir by age:

Table 45 summarizes the safety of baloxavir by age group. The incidence of adverse events was 34.6% (37 of 107) of patients aged <12 years in the baloxavir group; 16.3% (17 of 104) of patients aged ≥12 and <20 years in the baloxavir group; 25.9% (14 of 54) of patients aged ≥12 and <20 years in the placebo group; 23.0% (185 of 806) of patients aged ≥20 years in the baloxavir group; and 25.6% (91 of 355) of patients aged ≥20 years in the placebo group. The incidence did not significantly differ between the baloxavir and placebo groups among patients aged ≥20 years. Two patients aged ≥20 years who received baloxavir experienced serious adverse events (meningitis viral in 1 patient and incarcerated inguinal hernia in the other), but both events were unrelated to the study drug.

Safety in patients aged <12 years who received baloxavir:

No death, serious adverse event, or adverse event leading to discontinuation was observed. The only adverse event with an incidence of ≥5% was vomiting (7.5%), and all adverse events were Grade 1 or 2.[46)] The outcome of all adverse events was "recovered," except for 3 events (dry skin, dental caries, and ligament sprain), thus posing no clinically significant problems. No abnormal behavior was observed in patients receiving baloxavir in clinical studies.

Table 45. Outline of safety by age

	Study T0822	Studies T0821/T0831 combined				
	<12 years	≥12 and <20 years		≥20 years		
	Baloxavir (N = 107)	Baloxavir (N = 104)	Placebo (N = 54)	Baloxavir (N = 806)	Placebo (N = 355)	Oseltamivir (N = 513)
Adverse events	37 (34.6)	17 (16.3)	14 (25.9)	185 (23.0)	91 (25.6)	127 (24.8)
Adverse drug reactions	4 (3.7)	4 (3.8)	4 (7.4)	45 (5.6)	18 (5.1)	43 (8.4)
Serious adverse events	0	0	0	2 (0.2)	0	0
Adverse events leading to discontinuation	0	0	0	2 (0.2)	1 (0.3)	2 (0.4)
Adverse events resulting in death	0	0	0	0	0	0

n (%)

PMDA's view on the safety by age group:

PMDA confirmed that although the incidence of adverse event tended to be high in patients aged <12 years in the baloxavir group, no clinically significant problem was observed. No baloxavir-induced abnormal behavior was observed in clinical studies so far conducted, but patients and healthcare professionals who use baloxavir should be alerted to this risk in the same manner as those using other influenza antiviral drugs.

7.R.3.1.2 Safety of baloxavir by body weight

The applicant's explanation about the safety of baloxavir by body weight:

Tables 46 and 47 show adverse events with an incidence of ≥2% in any group, classified by body weight, in the global phase III study (Study T0831) and the Japanese study (Study T0822). There were no significant differences either in the incidence or the type of adverse events between subgroups classified by body weight.

Table 46. Adverse events with an incidence of ≥2% in any group, by body weight (Study T0831)

Events	Baloxavir[a)]		Placebo		Oseltamivir	
	<80 kg (N = 469)	≥80 kg (N = 141)	<80 kg (N = 236)	≥80 kg (N = 73)	<80 kg (N = 382)	≥80 kg (N = 131)
All adverse events	99 (21.1)	27 (19.1)	58 (24.6)	18 (24.7)	100 (26.2)	27 (20.6)
Bronchitis	12 (2.6)	4 (2.8)	14 (5.9)	3 (4.1)	12 (3.1)	6 (4.6)
Sinusitis	5 (1.1)	2 (1.4)	7 (3.0)	1 (1.4)	4 (1.0)	1 (0.8)
Diarrhoea	13 (2.8)	5 (3.5)	11 (4.7)	3 (4.1)	10 (2.6)	1 (0.8)
Nausea	7 (1.5)	1 (0.7)	2 (0.8)	2 (2.7)	13 (3.4)	3 (2.3)

n (%)

a) Baloxavir 40 mg in subjects weighing <80 kg and 80 mg in subjects weighing ≥80 kg.

Table 47. Adverse events with an incidence of ≥2% in any group, by body weight (Study T0822)

Events	≥5 and <10 kg (N = 2)	≥10 and <20 kg (N = 31)	≥20 and <40 kg (N = 66)	≥40 kg (N = 8)
All adverse events	1 (50.0)	12 (38.7)	20 (30.3)	4 (50.0)
Pharyngitis	0	1 (3.2)	2 (3.0)	0
Vomiting	0	2 (6.5)	5 (7.6)	1 (12.5)
Diarrhoea	0	0	2 (3.0)	1 (12.5)

n (%)

PMDA confirmed that there were no significant differences either in the incidence or the type of adverse events between subgroups classified by body weight in the global phase III study (Study T0831) and the Japanese study (Study T0822).

7.R.3.1.3 Hepatic dysfunction

The applicant's explanation about hepatic dysfunction in patients receiving baloxavir:
Non-clinical toxicity studies showed increases in blood chemistry parameters suggesting hepatotoxicity [see Section 5.2]. In the Japanese phase I study in healthy adults (Study T0811), mild abnormality in liver function test (ALT increased and blood bilirubin increased in 1 subject each) was observed [see Section 7.1]. ALT increased occurred on Day 20 after the study drug administration and blood bilirubin increased on Day 43.

Table 48 shows the incidence of adverse events and adverse drug reactions related to hepatic dysfunction[47)] in the combined data of the Japanese phase II study (Study T0821) and the global phase III study (Study T0831). There was no adverse event reported by ≥2% of patients in any group, with no significant difference in the incidence of each adverse event between the baloxavir and placebo groups. Adverse events occurring on Day ≥15 were ALT increased (3 patients in the baloxavir group, 2 in the placebo group, 3 in the oseltamivir group), AST increased (2 patients in the baloxavir group, 1 in the oseltamivir group), blood bilirubin increased (2 patients in the baloxavir group, 2 in the placebo group), hepatic function abnormal (1 patient in the baloxavir group), liver function test abnormal (1 patient in the placebo group), urobilinogen urine increased (1 patient in the baloxavir group), and blood bilirubin unconjugated increased (1 patient in the placebo group).

Table 48. Incidence of adverse events and adverse drug reactions related to hepatic dysfunction (Studies T0821/T0831 combined)

	Adverse events			Adverse drug reactions		
	Baloxavir (N = 910)	Placebo (N = 409)	Oseltamivir (N = 513)	Baloxavir (N = 910)	Placebo (N = 409)	Oseltamivir (N = 513)
All adverse events	24 (2.6)	12 (2.9)	13 (2.5)	17 (1.9)	5 (1.2)	10 (1.9)
ALT increased	11 (1.2)	7 (1.7)	7 (1.4)	8 (0.9)	3 (0.7)	4 (0.8)
AST increased	6 (0.7)	1 (0.2)	3 (0.6)	4 (0.4)	1 (0.2)	1 (0.2)
Blood bilirubin increased	3 (0.3)	2 (0.5)	1 (0.2)	2 (0.2)	0	1 (0.2)
Hepatic function abnormal	3 (0.3)	0	2 (0.4)	3 (0.3)	0	2 (0.4)
Liver function test abnormal	3 (0.3)	1 (0.2)	1 (0.2)	3 (0.3)	0	1 (0.2)
γ-GTP increased	2 (0.2)	2 (0.5)	2 (0.4)	1 (0.1)	2 (0.5)	2 (0.4)
Urobilinogen urine increased	1 (0.1)	0	0	0	0	0
Blood bilirubin unconjugated increased	0	1 (0.2)	0	0	0	0
ALP increased	0	1 (0.2)	0	0	1 (0.2)	0
Liver function test increased	0	0	2 (0.4)	0	0	2 (0.4)

n (%)

In the Japanese study (Study T0822), the incidence of adverse events related to hepatic dysfunction[47)] was 1.9% (2 of 107) of patients. AST increased and ALT increased occurred in 1 patient (0.9%) each, and only ALT increased was assessed as causally related to the study drug. Both were mild and resolved. Neither of these events occurred on or after Day 15.

In the foreign phase I study in subjects with hepatic impairment (Study T081B), adverse events occurring in subjects with moderate hepatic impairment (Child-Pugh class B) were all mild, as those in subjects with normal hepatic function (3 events in 3 of 8 subjects with moderate hepatic impairment; 2 events in 2 of 8 subjects with normal hepatic function). Thus, there were no hepatic impairment-associated increase in the incidence of adverse events.

47) Events categorized under "Drug related hepatic disorders - comprehensive search" in Standardised MedDRA Queries (SMQ)

PMDA's view:

In the Japanese phase II study (Study T0821) and the global phase III study (Study T0831), the AST increased and ALT increased occurring in patients receiving baloxavir were all Grade ≤2,[46] except for the event in 1 patient assessed as unrelated to the study drug. In addition, baloxavir did not pose any particular safety problem in subjects with moderate hepatic impairment compared with subjects with normal hepatic function. However, given the following findings, information on baloxavir-induced hepatic dysfunction should be continuously collected after the market launch, and the information thus obtained should be appropriately provided to healthcare professionals:

- In nonclinical studies and clinical studies, hepatic dysfunction-related events were observed.
- In the Japanese phase I study (Study T0811), hepatic dysfunction observed in subjects receiving baloxavir 40 mg (ALT increased and bilirubin increased) were late-onset events [see Section 7.1].
- Hepatic dysfunction-related events[47] of late onset were observed in the Japanese phase II study (Study T0821), the global phase III study (Study T0831), and the Japanese study (Study T0822) as well.

7.R.4 Clinical positioning

The applicant's explanation about the clinical positioning of baloxavir in the treatment of patients with influenza A or B virus infection:

Baloxavir is hydrolyzed to the active metabolite S-033447 by arylacetamide deacetylase, etc., in the small intestine, blood, liver, and other tissues. S-033447 thus formed inhibits activity of cap-dependent endonuclease, the enzyme unique to influenza virus, thereby inhibiting the transcription of viral RNA, resulting in suppression of viral replication. Baloxavir is thus an influenza antiviral drug with a novel mechanism of action not shared by approved drugs. The efficacy of a single oral dose of baloxavir against influenza virus infection was demonstrated in the Japanese phase II study in adult patients (Study T0821), the global phase III study in patients aged ≥12 years (Study T0831), and the Japanese study in patients aged <12 years (Study T0822) [see Sections 7.2 and 7.3]. In patients aged ≥20 years in the global phase III study (Study T0831), the viral titer in those receiving baloxavir rapidly decreased on Day 2 after administration, compared with that in those receiving oseltamivir. (Figure 11).

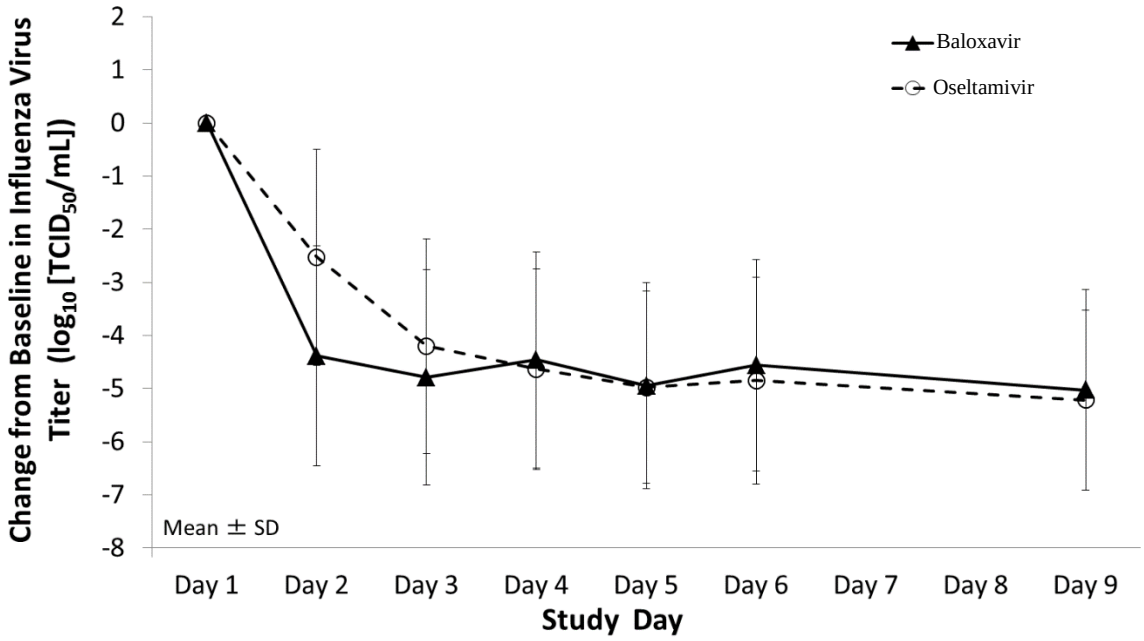

Figure 11. Changes over time in viral titer in global phase III study (Study T0831) (mean ± SD) (cited from CTD 2.5.6 Figure 2.5.6.1-3)

Since baloxavir has a mechanism of action not shared by existing drugs such as NA inhibitors, it is expected to be effective against influenza virus resistant to existing drugs and against avian influenza virus (H5N1 and H7N9) [see Sections 3.1.2.2.1, 3.1.4.1.2, 3.1.4.2.1, and 3.1.4.2.2].

The applicant's explanation about the efficacy and safety of baloxavir in patients with high risk factors:
The efficacy and safety of baloxavir in patient populations with high risk factors[48] were investigated using the combined data of Japanese phase II study (Study T0821) and the global phase III study (Study T0831), and data of the Japanese study (Study T0822).

Efficacy results:
In the combined data of the Japanese phase II study (Study T0821) and the global phase III study (Study T0831) (12 patients in the baloxavir group, 4 patients in the placebo group), the median duration [95% CI] of influenza infection was 60.5 [20.8, 109.1] hours in the baloxavir group and 92.6 [77.1, -[49]] hours in the placebo group; the duration thus tended to be shorter in the baloxavir group than in the placebo group. The duration in the Japanese study (Study T0822, 14 patients) was 56.2 [21.1, 136.7] hours, a value similar to that in Studies T0821 and T0831 combined, the definition of the duration of influenza illness differed between Study T0822 and Studies T0821/T0831 [see Sections 7.2 and 7.3].

Safety results:
In the combined data of the Japanese phase II study (Study T0821), the global phase III study (Study T0831), and the Japanese study (Study T0822), the incidence of adverse events (including abnormal

[48] Patients who met any of the following criteria: (1) Patients with hepatic impairment (baseline ALT >3 times the upper limit of the reference range established by the study site, baseline AST >3 times the upper limit of the reference range established by the study site, baseline total bilirubin >1.5 times the upper limit of the reference range established by the study site), (2) patients with renal impairment at baseline (CL_{cr} ≥30 mL/min and <60 mL/min), or (3) patients aged <5 years.
[49] The upper limit of 95% CI was incalculable.

changes in laboratory tests) was similar in the baloxavir group (33.3%, 10 of 30 patients) and the placebo group (40.0%, 2 of 5 patients), and no adverse events tended to occur more frequently in the baloxavir group than in the placebo group. A global phase III study in high-risk patients with influenza virus infection (Study T0832)[50] is ongoing since October 2016. As of October 3, 2017, 737 patients[51] have been enrolled in the study (target sample size, 2157 subjects [719 each in the baloxavir group, the placebo group, and the oseltamivir group]). As of October 3, 2017, 10 serious adverse events have been reported in 8 patients (1 event each of hypotension, liver function test increased, cholecystitis acute, bile duct stone, cholelithiasis, acute myocardial infarction, pneumonia, cerebrovascular accident, headache, and hyperglycaemia). Of these, only liver function test increased was assessed as related to the study drug, and the blind was broken for this patient with an emergency. The patient was found to be in the oseltamivir group.

Thus, baloxavir can be administered easily as a single oral dose, with a low risk of noncompliance, to patients with influenza virus infection, including high-risk patients. It will offer a new treatment option substituting for existing drugs against influenza virus infection.

PMDA's view:
PMDA understands that there is a concern about possible spread of virus with cross resistance to approved drugs with NA inhibitory effects. However, in clinical studies, baloxavir did not show a clearly higher efficacy than approved drugs in improving clinical symptoms associated with influenza virus infection, and there is currently no spread of virus strains with cross-resistance to NA inhibitors. This means that, as with other anti-influenza drugs, baloxavir should be considered as an option among other therapies, although it has novel mechanism of action. Meanwhile, amino acid substitution (PA/I38) contributing to the decrease in sensitivity to baloxavir was detected after administration of baloxavir [see Section 3.R.2]. It is therefore essential to facilitate the proper use of baloxavir taking account of new findings to be obtained in the future, with consideration given to the drug resistance of prevailing influenza virus.

The clinical efficacy of baloxavir for avian influenza virus (e.g., subtype A/H5N1 and A/H7N9) is unknown because baloxavir has not been administered to patients infected with such virus. Healthcare professionals should be appropriately informed of the results to be obtained from the clinical study in high-risk patients with influenza virus infection.

The above conclusion of PMDA will be discussed at the Expert Discussion.

7.R.5 Indication

Based on the results of reviews in Sections 7.R.2 and 7.R.3, PMDA concluded that the proposed indication of baloxavir, "Influenza A or B virus infection," was appropriate.

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[50] Patients with influenza A or B virus infection who met at least one of the inclusion criteria established by the applicant according to the definition of high-risk patients by the US Centers for Disease Control (CDC).

[51] [redacted] patients in US, [redacted] in Japan, [redacted] in South Africa, [redacted] in New Zealand, [redacted] in Philippines, and [redacted] in Taiwan

Information on clinical efficacy against influenza B virus should be actively and continuously collected after the market launch, and new information obtained should be provided to healthcare professionals.

The above conclusion of PMDA will be discussed at the Expert Discussion.

7.R.6 Dosage and administration

Based on the results of reviews in Sections 6.R, 7.R.2, and 7.R.3 and on the following review, PMDA concluded that the following dosage and administration of baloxavir were appropriate:

1. The usual dosage for adults and adolescents aged ≥12 years is two 20-mg tablets (40 mg of baloxavir marboxil) administered orally as a single dose. In patients weighing ≥80 kg, four 20-mg tablets (80 mg of baloxavir marboxil) are administered orally as a single dose.
2. The usual dosage for children aged <12 years is the following, administered orally as a single dose.

Body weight	Dose
≥40 kg	Two 20-mg tablets (40 mg of baloxavir marboxil)
≥20 kg and <40 kg	One 20-mg tablet (20 mg of baloxavir marboxil)
≥10 kg and <20 kg	One 10-mg tablet (10 mg of baloxavir marboxil)
≥5 kg and <10 kg	A half 10-mg tablet (5 mg of baloxavir marboxil)

The above conclusion of PMDA will be discussed at the Expert Discussion.

7.R.6.1 Dosage and administration in patients aged ≥12 years

The applicant's explanation about the dosage and administration in patients aged ≥12 years:
In the global phase III study in patients aged ≥12 years with influenza virus infection (Study T0831), baloxavir 40 mg was administered to patients weighing <80 kg and baloxavir 80 mg to patients weighing ≥80 kg. The baloxavir group showed improvement of influenza symptoms and antiviral effect, compared with the placebo group; this suggests that baloxavir has acceptable safety and tolerability within the dosage range used in the study.

There is limited experience with baloxavir in adult and adolescent patients weighing <40 kg. In the Japanese phase II study (Study T0821), baloxavir was administered to 4 patients weighing <40 kg (1 in the 10 mg group, 2 in the 20 mg group, 1 in the 40 mg group). AUC following administration of 40 mg in each subject, estimated by the Bayesian method, was 8453 ng·h/mL (body weight 36.0 kg), 10,580 ng·h/mL (body weight 36.4 kg), 6412 ng·h/mL (body weight 39.0 kg), and 11,970 ng·h/mL (body weight 39.4 kg). These values were not significantly different from the mean AUC [range] observed in the global phase III study (6016 [1100, 18,330] ng·h/mL).

Based on the above, the applicant concluded that the following dosage is appropriate for patients aged ≥12 years with influenza virus infection: a single oral dose of 40 mg (in patients weighing <80 kg) or 80 mg (in patients weighing ≥80 kg).

PMDA's view:
The following dosage and administration of baloxavir are appropriate for patients aged ≥12 years with influenza virus infection: "The usual dosage is 40 mg of baloxavir marboxil (two 20-mg tablets)

administered orally as a single dose. In patients weighing ≥80 kg, 80 mg of baloxavir marboxil (four 20-mg tablets) is administered orally as a single dose."

7.R.6.2 Dosage and administration in patients aged <12 years

The applicant's explanation about the dosage and administration in patients aged <12 years:
In the Japanese study in patients aged <12 years with influenza virus infection (Study T0822), the patients received a single oral dose of baloxavir at a dose determined by body weight. The treatment provided improvement of influenza symptoms and the antiviral effect to the same extent as observed in patients aged ≥12 years; this suggests that baloxavir has acceptable safety and tolerability in patients aged <12 years within the dosage range used in the study. The exposure to S-033447 following administration of baloxavir in the Japanese study (Study T0822) was similar to that in the global phase III study (Study T0831) [see Section 6.R.3].

Based on the above, the applicant concluded that the following dosage is appropriate for patients aged <12 years with influenza virus infection: 5 mg in patients weighing ≥5 and <10 kg, 10 mg in patients weighing ≥10 and <20 kg, 20 mg in patients weighing ≥20 and <40 kg, and 40 mg in patients weighing ≥40 kg.

PMDA's view based on the above explanation of the applicant and on the review in Section 6.R.4:
The following dosage and administration of baloxavir are appropriate for patients aged <12 years with influenza virus infection: A single dose of baloxavir marboxil at 5 mg (a half 10-mg tablet) in patients weighing ≥5 and <10 kg; 10 mg (one 10-mg tablet) in patients weighing ≥10 and <20 kg; 20 mg (one 20-mg tablet) in patients weighing ≥20 and <40 kg; and 40 mg (two 20-mg tablets) in patients weighing ≥40 kg.

7.R.7 Post-marketing investigations

The applicant plans to conduct the following post-marketing surveillance of baloxavir.

Use-results survey
- Objective: To investigate the safety and efficacy of baloxavir in clinical use.
- Sample size: 3000 patients
- Observation period: 7 days after administration of baloxavir
- Survey period: 13 months after the market launch

Specified use-results survey (sensitivity survey)
This survey is designed to collect information on a possible decrease in sensitivity to baloxavir and tendency of drug resistance acquisition in clinical isolates after the market launch.

PMDA considers that information on the following should also be collected after the market launch:
- Efficacy in influenza B virus infection
- Safety in patients aged <2 years

The target sample size in the use results surveys should be determined based on the specific safety items to be investigated in the surveys.

The above conclusion of PMDA will be discussed at the Expert Discussion.

8. Results of Compliance Assessment Concerning the New Drug Application Data and Conclusion Reached by PMDA

8.1 PMDA's conclusion concerning the results of document-based GLP/GCP inspections and data integrity assessment

The inspection is currently ongoing. Results and the conclusion of PMDA will be reported in the Review Report (2).

8.2 PMDA's conclusion concerning the results of the on-site GCP inspection

The inspection is currently ongoing. Results and the conclusion of PMDA will be reported in the Review Report (2).

9. Overall Evaluation during Preparation of the Review Report (1)

On the basis of the data submitted, PMDA has concluded that baloxavir has efficacy in the treatment of influenza A or B virus infection, and that baloxavir has acceptable safety in view of its benefits. Baloxavir is clinically meaningful because it offers a new treatment option for patients with influenza A or B virus infection.

PMDA has concluded that baloxavir may be approved if baloxavir is not considered to have any particular problems based on comments from the Expert Discussion.

Review Report (2)

January 16, 2018

Product Submitted for Approval

Brand Name	S-033188 Tablets 10 mg (at the time of marketing application)
	S-033188 Tablets 20 mg (at the time of marketing application)
Non-proprietary Name	Baloxavir Marboxil
Applicant	Shionogi & Co., Ltd.
Date of Application	October 25, 2017

List of abbreviations
See Appendix.

1. Content of the Review

Comments made during the Expert Discussion and the subsequent review conducted by the Pharmaceuticals and Medical Devices Agency (PMDA) are summarized in the following. The expert advisors present during the Expert Discussion were nominated based on their declarations etc. concerning the product submitted for marketing approval, in accordance with the provisions of the Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

The expert advisors at the Expert Discussion supported the PMDA's conclusion on issues presented in the Review Report (1) [Sections "7.R.4 Clinical positioning" and "7.R.5 Indication"].

PMDA also discussed the following points and took action as necessary.

1.1 Treatment with baloxavir in infants and young children (infants weighing ≥5 and <10 kg, in particular)

The expert advisors at the Expert Discussion supported the PMDA's conclusion on the efficacy, safety, and dosage regimen of baloxavir described in the Review Report (1) [Sections "7.R.2 Efficacy," "7.R.3 Safety," and "7.R.6 Dosage and administration"]. Also, the following comments were raised regarding treatment with baloxavir in infants and other children:

- Administration of tablets to infants and very young children poses the risk of aspiration of residual drug in the mouth. In general, tablets should not be administered to infants and young children.
- A 10-mg tablet has a diameter of 5.0 mm. According to the proposed dosage and administration, infants weighing ≥5 and <10 kg have to take a half of the tablet. Taking the half tablet is difficult for infants with poor systemic conditions due to influenza virus infection.

- It is a welcome step that the applicant is planning to develop baloxavir granules. The dosage and administration for infants and young children should be defined for the dosage form appropriate for children.
- There is only limited experience with baloxavir (5 mg of baloxavir marboxil) in infants weighing ≥5 and <10 kg (2 safety-evaluable patients).

Based on the comments raised in the Expert Discussion, PMDA concluded that another regulatory review should be conducted to evaluate baloxavir therapy in children weighing ≥5 and <10 kg after new information has become available from the population. PMDA instructed the applicant to specify the dosage and administration of baloxavir in children aged <12 years as shown below. The applicant agreed.

1. The usual dosage for adults and adolescents aged ≥12 years is two 20-mg tablets (40 mg of baloxavir marboxil) administered orally as a single dose. In patients weighing ≥80 kg, four 20-mg tablets (80 mg of baloxavir marboxil) are administered orally as a single dose.
2. The usual dosage for children aged <12 years is the following, administered orally as a single dose.

Body weight	Dose
≥40 kg	Two 20-mg tablets (40 mg of baloxavir marboxil)
≥20 kg and <40 kg	One 20-mg tablet (20 mg of baloxavir marboxil)
≥10 kg and <20 kg	One 10-mg tablet (10 mg of baloxavir marboxil)

The "Pediatric Use" section of the package insert should contain a statement to the effect that baloxavir should be administered only to children who can properly take the tablet orally. PMDA instructed the applicant to appropriately inform healthcare professionals about the risk of administering tablets to young children (e.g., aspiration of residual drug in the mouth) by disseminating information materials. The applicant agreed.

The applicant explained that a Japanese clinical study is ongoing to evaluate a granule formulation for pediatric use.

1.2 Resistance to baloxavir

The expert advisors at the Expert Discussion supported the PMDA's conclusion on the resistance to baloxavir in the Review Report (1) [Section "7.R.2.4 Resistance to baloxavir"]. Also, the following comments were raised from the expert advisors:

- *In vitro* studies showed that influenza virus with PA/I38 amino acid substitution has a decreased replication capacity. However, it should be noted that, under conditions where the replication capacity of baloxavir-sensitive wild-type influenza virus is suppressed, even the slow-replicating influenza virus with PA/I38 amino acid substitution may emerge as the predominant population. In the clinical studies so far conducted, influenza virus with PA/I38 amino acid substitution was not detected in samples collected before administration of baloxavir, and observed only after administration of baloxavir. These findings strongly suggest the involvement of the above mechanism.
- In the Japanese study in patients aged <12 years (Study T0822), the median duration [95% CI] of influenza illness tended to be longer in those infected with influenza virus with PA/I38 amino acid

substitution (79.6 [39.8, 116.9] hours) than in those infected with influenza virus without the substitution (43.0 [31.8, 52.4] hours). In clinical studies so far conducted, gene analysis was performed by Sanger sequencing. However, if gene analysis is performed using a next-generation sequencer with higher detection sensitivity, PA/I38 amino acid substitution may be detected in samples collected at earlier stages after administration of baloxavir, indicating the presence of PA/I38 amino acid substitution from the early stage. Therefore, the possibility cannot be excluded that a transient increase in the viral titer affected the clinical symptoms.

- Infants and young children with underdeveloped basic immune function and patients with immunodeficiency are considered to have a high risk of emergence of drug-resistant variant viruses. However, the clinical effect of PA/I38 amino acid substitution in these populations is currently unknown. Also, there is a report that a virus that has a NA inhibitor-resistant mutation in the NA region with reduced replication capacity and transmissibility has gained a high replication capacity and transmissibility upon acquiring a new mutation. Given the above findings, the possibility cannot be excluded that the influenza virus with PA/I38 amino acid substitution, which is considered to have lower replication capacity and transmissibility than the wild-type virus, may become highly replicative and transmissible by acquiring a new mutation. Therefore, emergence of the influenza virus with PA/I38 amino acid substitution should be recognized as a public health concern.
- Sensitivity of influenza virus to baloxavir should be continuously surveyed over years after the market launch. RNA polymerase complex is composed of 3 subunits: PA, polymerase basic protein 1 (PB1), and polymerase basic protein 2 (PB2). This means that amino acid substitution in PB1 or PB2 also may affect the sensitivity of the virus to baloxavir. Therefore, the effect of amino acid substitution in PB1 or PB2 on the sensitivity of the virus to baloxavir should be investigated after the market launch, and information, obtained from the post-marketing study or from other sources, should be appropriately provided to healthcare professionals.
- In clinical studies, the duration of influenza illness after administration of baloxavir was similar to that after administration of existing NA inhibitors. However, viral titer and RNA level disappeared earlier in patients receiving baloxavir than in those receiving an existing drug. This suggests that the frequency of the emergence of drug-resistant virus may be lower with baloxavir than with existing drugs in infants with underdeveloped basic immune function.

PMDA informed the applicant of the comments on the resistance to baloxavir raised at the Expert Discussion. The applicant agreed to take the following actions:

- To appropriately provide healthcare professionals with information on the emergence of resistance to baloxavir, obtained from clinical studies and elsewhere. To continuously conduct a survey of the trend of emergence of resistance by type/subtype of influenza virus over years after the market launch, and to appropriately provide the information thus obtained to healthcare professionals.
- To investigate the effect of amino acid substitution of PB1 or PB2 on the sensitivity to baloxavir after the market launch, to collect relevant information from other sources as well, and to appropriately provide the information thus obtained to healthcare professionals.

1.3 Risk management plan (draft)

In view of the discussions presented in Section "7.R.7 Post-marketing investigations" in the Review Report (1) and comments raised from the expert advisers at the Expert Discussion, PMDA considers that the post-marketing surveillance should also cover the following issues:

- Efficacy against influenza B virus infection
- Safety and efficacy in children aged <12 years.

PMDA requested that the applicant investigate these issues during post-marketing surveillance. The applicant agreed to take such action.

Also, PMDA instructed the applicant to determine the target sample size in the use-results survey taking account of the patient populations with the specific characteristics, because the survey should collect information especially from such populations.

The applicant's response:

The target sample size in the use-results survey is 3000 patients in total. This number includes 480 children aged <12 years (including approximately 120 children aged <6 years) and 150 elderly patients, because the survey should collect information especially from patients in both age groups.

In view of the discussion above, PMDA has concluded that the risk management plan (draft) for baloxavir should include the safety and efficacy specifications presented in Table 49, and that the applicant should conduct additional pharmacovigilance activities and risk minimization activities presented in Table 50. PMDA approved the outlines of use-results survey (draft) and specified use-results survey (draft) shown in Tables 51 and 52, respectively.

Table 49. Safety and efficacy specifications in the risk management plan (draft)

Safety specification		
Important identified risks	Important potential risks	Important missing information
None	• Neuropsychiatric symptoms • Hepatic dysfunction	None
Efficacy specification		
• Change in sensitivity to baloxavir		

Table 50. Summary of additional pharmacovigilance activities and risk minimization activities included under the risk management plan (draft)

Additional pharmacovigilance activities	Additional risk minimization activities
• Early post-marketing phase vigilance • Use-results survey • Specified use-results survey (sensitivity survey)	• Disseminate data gathered during early post-marketing phase vigilance. • Organize and disseminate the following information for patients and their guardians: "Information for guardians of patients prescribed Xofluza tablets."

Table 51. Outline of use-results survey (draft)

Objective	To collect information on the safety and efficacy of baloxavir in routine clinical use
Survey method	Survey of consecutive patients
Study population	Patients with influenza A or B virus infection
Observation period	7 days after the administration of baloxavir
Planned sample size	3000 (including 480 children aged <12 years [including approximately 120 children aged <6 years] and 150 elderly patients)
Main survey items	Hepatic dysfunction, the safety and efficacy in patients aged <12 years, the efficacy against influenza B virus infection

Table 52. Outline of specified use-results survey (draft)

Objective	To collect information on the sensitivity of clinical isolates to baloxavir and on the change in resistant mutation over years
Target number of clinical isolates	100 strains per year
Planned duration of survey	From 2018/2019 season until the end of the re-examination period

2. Results of Compliance Assessment Concerning the New Drug Application Data and Conclusion Reached by PMDA

2.1 PMDA's conclusion concerning the results of document-based GLP/GCP inspections and data integrity assessment

The new drug application data were subjected to a document-based compliance inspection and a data integrity assessment in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics. On the basis of the inspection and assessment, PMDA concluded that there were no obstacles to conducting its review based on the application documents submitted.

2.2 PMDA's conclusion concerning the results of the on-site GCP inspection

The new drug application data (CTD 5.3.5.1-01, 5.3.5.1-02, and 5.3.5.2-01) were subjected to an on-site GCP inspection, in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics. As a result, PMDA concluded that the clinical studies as a whole were conducted in compliance with GCP and that there were no obstacles to conducting its review based on the application documents submitted. The following finding was noted in the work carried out by a study site although it did not significantly affect the overall evaluation of the study. The finding was notified to the head of the pertinent study site for improvement.

Matters to be improved

Study site

- Flaws in obtaining renewed informed consent (using revised informed consent materials) from some subjects

3. Overall Evaluation

As a result of the above review, PMDA has concluded that the product may be approved after modifying the proposed indication and dosage and administration as shown below, with the following condition of approval. Since the product is a drug with a new active ingredient, the re-examination period is 8 years. The product is not classified as a biological product or a specified biological product. Neither the drug product nor its drug substance is classified as a poisonous drug or a powerful drug.

Indication

Influenza A or B virus infection

Dosage and Administration

1. The usual dosage for ~~A~~adults and adolescents aged ≥12 years~~: The usual dosage~~ is two 20-mg tablets (40 mg of baloxavir marboxil) administered orally as a single dose. In patients weighing ≥80 kg, four 20-mg tablets (80 mg of baloxavir marboxil) are administered orally as a single dose.

2. The usual dosage for ~~C~~children aged <12 years~~: of baloxavir marboxil~~ is the following, administered orally as a single dose.

Body weight	Dose
≥40 kg	Two 20-mg tablets (40 mg of baloxavir marboxil)
≥20 kg and <40 kg	One 20-mg tablet (20 mg of baloxavir marboxil)
≥10 kg and <20 kg	One 10-mg tablet (10 mg of baloxavir marboxil)
~~≥5 kg and <10 kg~~	~~5 mg~~

(The underline denotes text added to the proposed application. Strike-through denotes deletion from the proposed application.)

Condition of approval

The applicant is required to develop and appropriately implement a risk management plan.

List of Abbreviations

A/G ratio	Albumin/globulin ratio
ALT	Alanine aminotransferase
APTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
AUC	Area under the concentration versus time curve
AUC_{inf}	Area under the concentration versus time curve extrapolated to infinite time
AUC_{last}	Area under the concentration versus time curve from time of dosing to the time of the last quantifiable concentration
BCRP	Breast cancer resistance protein
BID	bis in die
BSEP	Bile salt export pump
C_{24}	Plasma concentration at 20-28 hours post-dose
CC_{50}	50% cytotoxicity concentration
CL	Total clearance
CL/F	Apparent total body clearance
CL_{cr}	Creatinine clearance
C_{max}	Maximum plasma concentration
CPA	Cyclophosphamide
C_{τ}	Plasma concentration at the end of dosing interval after the first dose
DMSO	Dimethyl sulfoxide
$EC_{50\ (90)}$	50% (90%) effective concentration
Efflux ratio	Basal-to-apical versus apical-to-basal ratio
eGFR	Estimated glomerular filtration rate
F_u	Fraction of dose excreted in urine
GLDH	Glutamate dehydrogenase
IC_{50}	50% inhibitory concentration
ITTI	Intention-to-treat infected
k_a	Absorption rate constant
LAP	Leucyl aminopeptidase
MATE	Multidrug and toxin extrusion protein
MDBK cell	Madin Darby bovine kidney cell
MDCK cell	Madin Darby canine kidney cell
NA	Neuraminidase
OAT	Organic anion transporter
OATP	Organic anion transporting polypeptide
OCT	Organic cation transporter
PA	Polymerase acidic protein
PB1	Polymerase basic protein 1
PB2	Polymerase basic protein 2
PD	Pharmacodynamics
P-gp	P-glycoprotein
PK	Pharmacokinetics
PMDA	Pharmaceuticals and Medical Devices Agency
PPK	Population pharmacokinetics
PT	Prothrombin time
Q/F	Apparent inter-compartmental clearance
QD	quaque die
RdRp	RNA-dependent RNA polymerase
RPMI2650 cell	Human nasal septum carcinoma cell
$t_{1/2}$	Estimate of the terminal elimination half-life
$TCID_{50}$	50% tissue culture infectious dose

t_{max}	Time to maximum concentration
UGT	Uridine diphosphate glucuronosyltransferase
V_c/F	Apparent volume of central compartment
V_d	Volume of distribution
$V_{d,ss}$	Volume of distribution at steady state
V_p/F	Apparent volume of peripheral compartment
V_z/F	Apparent volume of distribution based on the terminal phase
γ-GTP	γ-glutamyl transferase