OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA Number	210854
Link to EDR	[\\CDSESUB1\evsprod\NDA210854\210854.enx](\\CDSESUB1\evsprod\NDA210854\210854.enx)
Submission Date	04/24/2018
Submission Type	Priority Review
Brand Name	XOFLUZA
Generic Name	Baloxavir marboxil (S-033188)
Dosage Form and Strength	20 mg and 40 mg Tablets
Route of Administration	Oral
Proposed Indication	Treatment of influenza in patients 12 years of age and older who have been symptomatic for no more than 48 hours.
Applicant	Shionogi Inc./Genentech, Inc.
Associated IND	126653
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† Baloxavir marboxil, S-033188 and XOFLUZA are used interchangeably in this review.

‡ Baloxavir (S-033447) is the active metabolite of baloxavir marboxil. Baloxavir and S-033447 are used interchangeably in this review.

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1. EXECUTIVE SUMMARY

The Applicant is seeking the approval of baloxavir marboxil (S-033188), which is a prodrug that is rapidly metabolized to its active form, baloxavir (S-033447). Baloxavir is a first-in-class inhibitor of endonuclease activity of the polymerase acidic protein, which is necessary for replication of influenza viruses. The proposed indication of baloxavir marboxil is treatment of acute uncomplicated influenza in patients 12 years of age and older who have been symptomatic for no more than 48 hours.

The efficacy and safety of baloxavir marboxil at the proposed dosing regimen was evaluated in a Phase 3 trial (1601T0831); baloxavir marboxil was compared to both placebo and oseltamivir (active control). The median Time to Alleviation of Symptoms (TTAS) was 54 hours (95% CI of 50, 59) for subjects who received a 40 or 80 mg dose of baloxavir marboxil compared to 80 hours (95% CI of 73, 87) for placebo. There was no difference in the TTAS between subjects who received baloxavir marboxil (54 hours) and those who received oseltamivir (54 hours). For adolescents, the median TTAS was 54 hours (95% CI of 43, 81) for subjects who received baloxavir marboxil compared to 93 hours (95% CI of 64, 118) for subjects who received placebo. The to-be-marketed tablet strengths are 20 and 40 mg. The proposed dosing regimens are based on body weight; a single 40 mg PO tablet for patients weighing 40 kg to < 80 kg or a single 80 mg PO tablet for patients weighing ≥ 80 kg.

1.1 Recommendations

The Office of Clinical Pharmacology has reviewed the application and determined that this NDA is *approvable* from a clinical pharmacology perspective. The key review issues, specific recommendations, and comments are summarized below.

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	The primary evidence of effectiveness is provided by one Phase 3 trial (Study 1601T0831). The dose-range study (Study 1518T0821) provides supportive evidence.

General dosing instructions	A single dose of XOFLUZA should be taken orally within 48 hours of symptom onset without regard to food. The proposed body weight-based dosing regimen is acceptable:
	<table><tr><th>Patient Body Weight (kg)</th><th>Recommended Oral Dose</th></tr><tr><td>40 kg to < 80 kg</td><td>Single dose of 40 mg</td></tr><tr><td>≥ 80 kg</td><td>Single dose of 80 mg</td></tr></table>
Dosing in patient subgroups (intrinsic and extrinsic factors)	No dose adjustments are recommended based on intrinsic (other than body weight) or extrinsic factors.
Labeling	The labeling is generally acceptable. For specific contents and formatting recommendations, refer to Section 2.4.
Bridge between the to-be-marketed and clinical trial formulations	There are two to-be-marketed formulations; 20 mg and 40 mg tablets. No bridging is needed for the 20 mg to-be-marketed formulation since it is identical to the formulation used in the pivotal study. The 40 mg to-be-marketed formulation was not used in any clinical study. Therefore, a waiver of bioavailability evaluation between the to-be-marketed 20-mg formulation and the to-be-marketed 40-mg was requested and granted by the FDA on 07/31/2018.

1.2 Post-Marketing Requirements and Commitments

The review team is considering a PMC to determine the magnitude of interaction between baloxavir and calcium-rich food alone (e.g., dairy) to guide the dosing instruction.

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

Baloxavir marboxil is a prodrug that is rapidly metabolized to its active form, baloxavir. Baloxavir is an inhibitor of endonuclease of the polymerase acidic protein, which is necessary for replication of influenza viruses. The pharmacokinetics of baloxavir are summarized in Table 1

Table 1. Summary of Baloxavir Pharmacokinetics in Humans Following Oral Administration

Absorption	• T_{max}: 4 hours

	• Food (400 -500 Kcal) decreases C_{max} and AUC_{0-inf} by ~48% and ~36%, respectively.
	• Solubility/permeability are decreased in the presence of polyvalent cations.
	• Absolute bioavailability was not established.
Distribution	• The apparent volume of distribution(Vd/F) is 1180 (CV: 20.8%) Liter.
	• Protein binding ranges between 92.9% to 93.9%.
	• Blood-to-plasma ratio ranges between 48.5% to 54.4%.
Elimination	• The apparent oral clearance (CL/F) is 10.3 (CV: 22.5%) Liter/hour.
	• The elimination half-life is 79.1 (CV: 22.4%) hours.
	Metabolism
	• Baloxavir marboxil is rapidly hydrolyzed by esterases in the gastrointestinal lumen, intestinal epithelium, liver and blood into its active form baloxavir.
	• Baloxavir is metabolized by UGT1A3 with minor contribution from CYP3A4.
	Excretion
	• About 80% of the administered dose is excreted in the feces.
	• Urinary excretion is <15% of the administered dose.
Potential for drug interactions	• Co-administration of XOFLUZA with laxatives, antacids, and supplements containing polyvalent cations should be avoided as they could decrease its solubility and permeability and hence decrease its absorption.
	• No clinically significant changes in the pharmacokinetics of baloxavir marboxil and baloxavir were observed when co-administered with itraconazole (a strong CYP3A and P-gp inhibitor), probenecid (UGT inhibitor), or oseltamivir (antiviral agent).
	• No clinically significant changes in the pharmacokinetics of the following drugs were observed when co-administered with baloxavir marboxil: midazolam (CYP3A4 substrate), digoxin (P-gp substrate), rosuvastatin (BCRP substrate), or oseltamivir.

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

XOFLUZA is to be taken orally as a single dose with or without food within 48 hours of onset of influenza symptoms. The recommended dose of XOFLUZA in patients 12 years of age or older is a single weight-based dose as follows:

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Table 2. Recommended XOFLUZA Dosage in Adults and Adolescents 12 Years and Older

Patient Body Weight (kg)	Recommended Oral Dose
40 kg to less than 80 kg	Single Dose of 40 mg
At least 80 kg	Single Dose of 80 mg

2.2.2 Therapeutic individualization

Therapeutic individualization is necessary based on weight (*refer to Table 2*). Therapeutic individualization is not necessary based on sex, race, age, hepatic impairment, or renal impairment. Baloxavir marboxil and baloxavir exposure was not evaluated in subjects less than 12 years of age, less than 40 kg body weight, with severe hepatic impairment, or with severe renal impairment.

2.3 Outstanding Issues

None identified.

2.4 Summary of Labeling Recommendations

The Office of Clinical Pharmacology recommends the following labeling concepts to be included.

Section/heading	Comment
3. DOSAGE FORMS AND STRENGTHS	Include a statement to indicate that XOFLUZA should not be taken with dairy products or calcium-fortified beverages alone and supportive data for this statement in section 12.
7. DRUG INTERACTIONS	Retain clinically relevant drug interactions with clinical recommendations only. Other information (e.g., in vitro study results or interactions that are not clinically relevant) can be moved to Section 12.3. For drug interactions that are potentially clinically relevant (i.e., interactions with cation containing products), provide supportive data for the clinical comments in section 12.
12.2 Pharmacodynamics	Add language for the exposure-response relationship of baloxavir.
12.3 Pharmacokinetics	Streamline this section to follow the current labeling practice. Present PK data and ADME data in a table format. Include PK parameter estimates obtained from the phase 3 study; stratify by dose (i.e., for 40 and 80 mg doses).

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

- On 15 January 2016, an Investigational New Drug (IND) application, IND 126653, was submitted.
- On 17 August 2016, the applicant met with the IND 126653 review team to discuss the results of a phase 2 study (Study 1518T0821) and the design of a phase 3 study (Study 1601T0831).
- On 24 April 2018, this NDA was submitted.
- On 20 June 2018, a priority review for this application was granted.

3.2 General Pharmacology and Pharmacokinetic Characteristics

General Pharmacology

Mechanism of action

Baloxavir marboxil is a prodrug that is rapidly metabolized to its active form, baloxavir. Baloxavir is an inhibitor of endonuclease of the polymerase acidic protein, an influenza virus specific enzyme in the viral RNA polymerase complex required for replication of influenza viruses.

QT

No QT prolongation was observed at twice the observed exposures from the proposed dosing. Refer to QT-IRT review.

General Pharmacokinetic Characteristics

Absorption

Following a single oral administration of baloxavir marboxil, the time to achieve peak plasma concentration (T_{max}) of baloxavir was 4 hours in the fasted state. The absolute bioavailability of baloxavir marboxil has not been established.

Food effect

A food-effect study involving administration of baloxavir marboxil to healthy volunteers under a fasted state and with a meal (approximately 400 to 500 kcal including 150 kcal from fat) indicated that food decreased the C_{max} and AUC of baloxavir by 48% and 36%, respectively. T_{max} was unchanged in the presence of food. Solubility of baloxavir marboxil is independent of pH in the range of pH 1-9. Therefore, the pH change by food intake is not considered to affect the absorption of baloxavir marboxil. Baloxavir has a hydroxyl group and a ketone group adjacent to each other (Figure 1) which could form a chelate

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with metal ions contained in food. These chelates could lead to a decreased solubility and permeability of baloxavir.

Importantly, in clinical studies with influenza patients where baloxavir marboxil was administered with or without food, no clinically relevant differences in efficacy were observed. XOFLUZA is recommended to be administered without regard to food (*refer to subsection 3.3.4 for further details about this recommendation*). However, no study was conducted to determine baloxavir exposures when baloxavir is administered with dairy products or calcium-fortified beverages alone.

Distribution

In an *in vitro* study, the binding of baloxavir to human serum proteins, primarily albumin, is 92.9% to 93.9%. The apparent volume of distribution of baloxavir following a single oral administration (Vd/F) of baloxavir marboxil is approximately 1180 (CV: 20.8%) liters (N = 12 subjects). Blood-to-plasma ratio ranges between 48.5% to 54.4%.

Elimination

The apparent terminal elimination half-life ($t_{1/2,z}$) of baloxavir after a single oral administration of baloxavir marboxil is 79.1 (CV: 22.4%) hours (N = 12 subjects).

In vitro studies revealed that arylacetamide deacetylase (AADAC) in the small intestine and liver mainly contributes to the rapid conversion of baloxavir marboxil to baloxavir. The formation of baloxavir glucuronide is primarily mediated by UGT1A3, while the formation of two kinds of baloxavir sulfoxides is mediated by CYP3A4 (Figure 1).

Following single oral administration of [^{14}C]-baloxavir marboxil 40 mg to 6 healthy male adults in the fasted state, 80.1% and 14.7% of the administered radioactivity were excreted in feces and urine, respectively. The fraction of dose excreted in urine of the active form, baloxavir, was 3.3%, indicating a smaller contribution of renal excretion and a larger contribution of biliary excretion to the elimination of baloxavir. In plasma, baloxavir (accounting for 82.2% of total radioactivity in plasma) was primarily detected. Other detected metabolites in plasma were baloxavir glucuronide and baloxavir sulfoxide (accounting for 16.4% and 1.5%, respectively, of the total radioactivity in plasma). In urine, 8.9% of the administered radioactivity was detected as baloxavir glucuronide. Baloxavir and two kinds of baloxavir

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sulfoxides were also detected. In feces, baloxavir was primarily detected (accounting for 48.7% of the administered radioactivity) in addition to two kinds of baloxavir sulfoxides and baloxavir pyrrole.
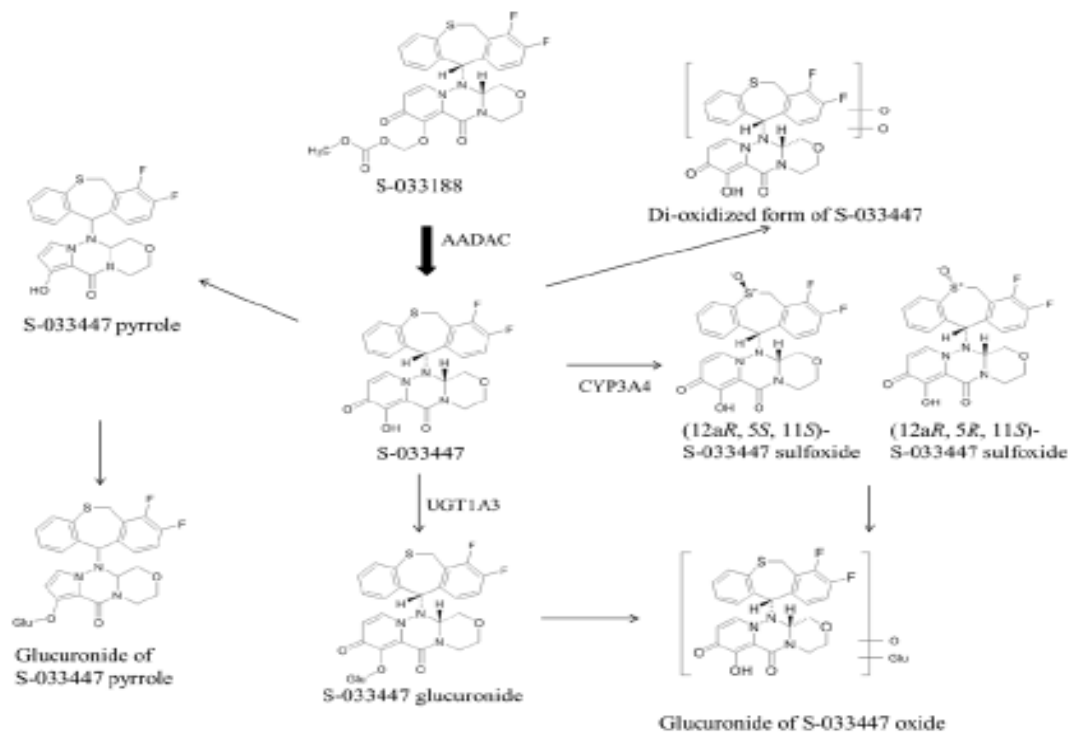
Figure 1. Estimated Metabolic Pathways of Baloxavir Marboxil in Humans

Dose proportionality

Dose proportionality assessment indicated that baloxavir exposure increases in a dose proportional manner over the proposed dose range of 40 – 80 mg (Table 3).

Table 3. Evaluation of Dose Proportionality of the C_{max} and AUC of baloxavir in the Dose Range of 40 to 80 mg (Study 1510T0811).

Dose (mg)	Parameter	Intercept	Slope	95% confidence interval for the slope	
				Lower limit	Upper limit
40, 60 and 80	C_{max} (ng/mL)	0.949	1.05	0.641	1.46
	AUC_{0-72} (ng·hr/mL)	4.59	0.965	0.616	1.31
	AUC_{0-inf} (ng·hr/mL)	5.70	0.839	0.462	1.22

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3.3 Clinical Pharmacology Review Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

The Phase 3 trial (1601T0831) provides the pivotal evidence of effectiveness and the available clinical pharmacology information provides supportive evidence of effectiveness. In Study 1601T0831 (active- and placebo-controlled study) baloxavir marboxil was studied in a total of 1,436 adult and adolescent subjects, 12 to 64 years of age, weighing at least 40 kg. Adults (20 to 64 years) received baloxavir marboxil or placebo as a single oral dose on Day 1 or oseltamivir (active control) twice a day for 5 days, while subjects 12 to less than 20 years of age received baloxavir marboxil or placebo as a single oral dose. Subjects weighing 40 to < 80 kg received baloxavir marboxil at a dose of 40 mg and subjects weighing 80 kg or more received an 80 mg dose. Baloxavir marboxil treatment resulted in a statistically significant shorter TTAS (primary end point) compared with placebo (Table 4). The drug exposure in adolescent patients was similar to that in adult patients, indicating that age alone has no or little effect on the pharmacokinetics of baloxavir (Table 5). The predominant influenza virus type in the Phase 3 trial was Type A (89%) and limited data are available for the efficacy with Type B. In the influenza B subset, median TTAS for the baloxavir marboxil treated group was numerically longer than the placebo group (93 hours vs. 77 hours), but the interpretation is limited due to the small number of subjects.

Table 4. Time to Alleviation of Symptoms in Study 1601T0831 (Median Hours)

	XOFLUZA (95% CI)	Placebo (95% CI)
Subjects (≥ 12 - ≤64 Years of Age)	54 hours (50, 59)	80 hours (73, 87)
Subjects (≥ 12 - < 18 Years of Age)	54 hours (43, 81)	93 hours (64, 118)
	XOFLUZA (95% CI)	**Oseltamivir (95% CI)**
Subjects (≥ 20 - ≤64 Years of Age)	54 hours (48, 59)	54 hours (50, 56)

CI: Confidence interval

The clinical pharmacology information was essential for the selection of the doses. Thorough understanding of these data enabled a series of simulation analyses of different dosing scenarios in different populations (e.g., Asians vs. non-Asians, subjects weighing > 80 kg vs. subjects weighing 40 – 80 kg). The outcome of these simulation analyses led to adopting a body weight-based dosing strategy in Study 1601T0831 which provided adequate exposure (Figure 2) and was demonstrated to be safe and effective.

Table 5. Comparison of $AUC_{0\text{-inf}}$, C_{max}, and C_{24} in Adolescent and Adult Patients (Study No. 1601T0831)

		40 mg				80 mg			
0	Race	N	Adolescent (≥ 12 - 18 years)	N	Adult (≥18 years)	N	Adolescent (≥12 - <18 years)	N	Adult (≥18 years)
$AUC_{0\text{-inf}}$ (ng·hr/mL)	Asian	34	7157 (2920 - 11680)	287	6486 (2186 - 14690)	0	---	37	9687 (4122 - 18330)
	Non-Asian	31	3687 (1100 - 9040)	97	3560 (1421 - 7094)	10	7160 (3365 - 10310)	88	5893 (2229 - 15600)
	All	65	5502 (1100 - 11680)	384	5747 (1421 - 14690)	10	7160 (3365 - 10310)	125	7016 (2229 - 18330)
C_{max} (ng/mL)	Asian	34	128 (30.6 - 226)	287	98.0 (23.9 - 244)	0	---	37	123 (33.3 - 243)
	Non-Asian	31	63.3 (14.0 - 142)	97	59.7 (16.2 - 138)	10	108 (63.5 - 177)	88	80.7 (25.6 - 211)
	All	65	96.9 (14.0 - 226)	384	88.3 (16.2 - 244)	10	108 (63.5 - 177)	125	93.3 (25.6 - 243)
C_{24} (ng/mL)	Asian	23	70.0 (21.7 - 112)	179	57.8 (5.81 - 158)	0	---	28	86.2 (39.3 - 142)
	Non-Asian	17	29.9 (7.35 - 55.6)	74	34.6 (0.322 - 81.4)	8	63.9 (24.7 - 97.4)	67	57.7 (17.5 - 209)
	All	40	52.9 (7.35 - 112)	253	51.1 (0.322 - 158)	8	63.9 (24.7 - 97.4)	95	66.1 (17.5 - 209)

Arithmetic mean (minimum-maximum)
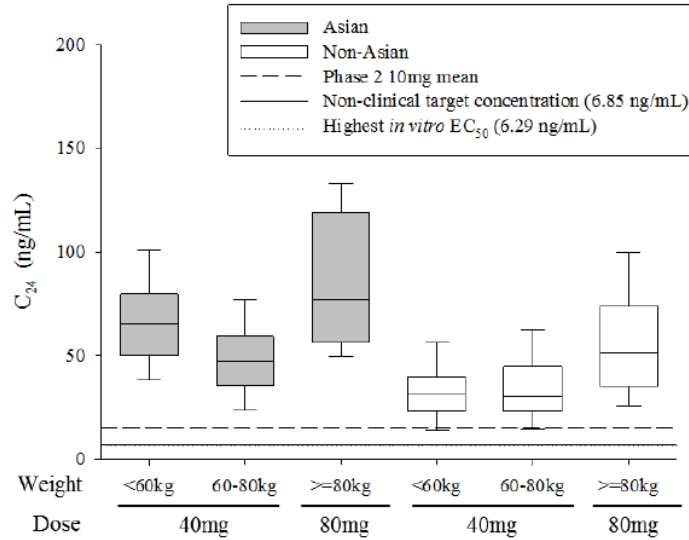
Figure 2. Correlations of Baloxavir plasma concentration at 24 hours post dose (C_{24}) with Body Weight stratified by race in the Phase 3 Study (Study 1601T0831)

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3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes. In Study 1601T0831 (outlined above), the proposed dosing regimen was appropriate (i.e., safe and effective) in influenza patients who are at least 12 years and weighing at least 40 kg.

Selection of Phase 3 dose

Dose selection of Baloxavir Marboxil for the Phase 3 trial was based on a dose-range study (Study 1518T0821T). Study 1518T0821 was a Phase 2, multicenter, randomized, double-blind, placebo-controlled, parallel-group, comparative study in Japanese patients aged ≥ 20 and < 65 years with influenza virus infection. Four hundred patients were randomized in a ratio of 1:1:1:1 to 1 of 4 treatment groups (10-, 20-, and 40-mg groups, and placebo group). The primary endpoint of this study, the TTAS (the median time) was 49.5 to 54.2 hours in the 3 S-033188 dose groups versus 77.7 hours in the placebo group (Table 6).

Table 6. Study 1518T0821: Time to Alleviation of Symptoms

	S-033188 10 mg	S-033188 20 mg	S-033188 40 mg	Placebo
Summary statistics				
- n	100	100	100	100
- Median (hrs)	54.2	51.0	49.5	77.7
- 95% confidence interval (hrs)	47.7, 66.8	44.5, 62.4	44.5, 64.4	67.6, 88.7
- Difference (vs placebo) (hrs)	-23.4	-26.6	-28.2	---
Cox proportional hazards model vs placebo [a]				
- Hazard ratio	0.758	0.810	0.817	---
- 95% confidence interval	0.571, 1.007	0.608, 1.078	0.614, 1.087	---
- P-value Adjusted by Hommel Method	0.1650	0.1650	0.1650	---
Stratified Generalized Wilcoxon test vs placebo [b]				
- P-value	0.0085	0.0182	0.0046	---

a   Covariates: smoking habit, composite symptom scores at baseline
None of the three dose groups versus placebo met the assumption of proportional hazards
b   Stratified factors: smoking habit, composite symptom scores at baseline
ITTI – intention to treat-infected population

The rationale for selection of Phase 3 dose is as follows:

- While there was no significant difference among the three evaluated doses in Study 1518T0821, suboptimal antiviral activity may occur in some subjects with Type B influenza virus infection at the 10 mg dose based on exposure-response relationship between C_{24} and changes in virus titer (Figures 3 and 4).

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- The Applicant initially proposed 40 mg as the dose to be evaluated in the Phase 3 trial. However, the Phase 2 study (Study 1518T0821) was conducted in Japan and two key covariates associated with U.S. populations (higher body weight and race) decrease baloxavir exposure. Therefore, a weight-based dosing regimen was recommended by the FDA and the Applicant accepted it.

Exposure-Response Relationship for efficacy

Exposure-response analyses were conducted using the data obtained from the Phase 2 and Phase 3 trials. A flat relationship for efficacy was observed for the primary (TTAS) as well as secondary (viral shedding) endpoints for both Type A and Type B influenza after excluding placebo data. In addition, there was no clear relationship between baloxavir exposure and treatment-emergent substitutions.
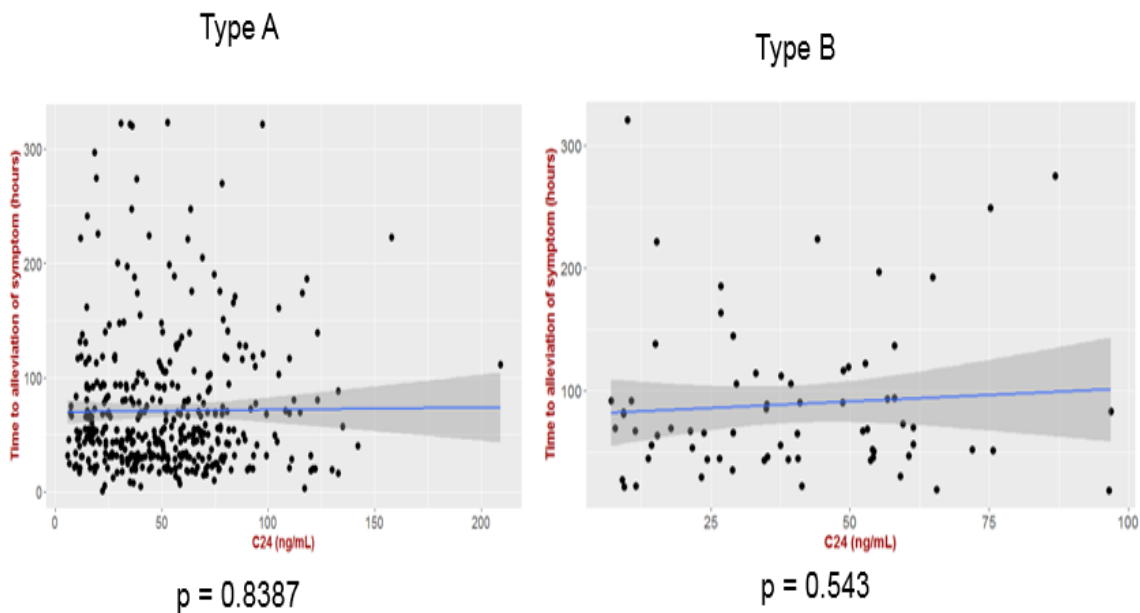
Figure 3. Exposure-response relationship for TTAS
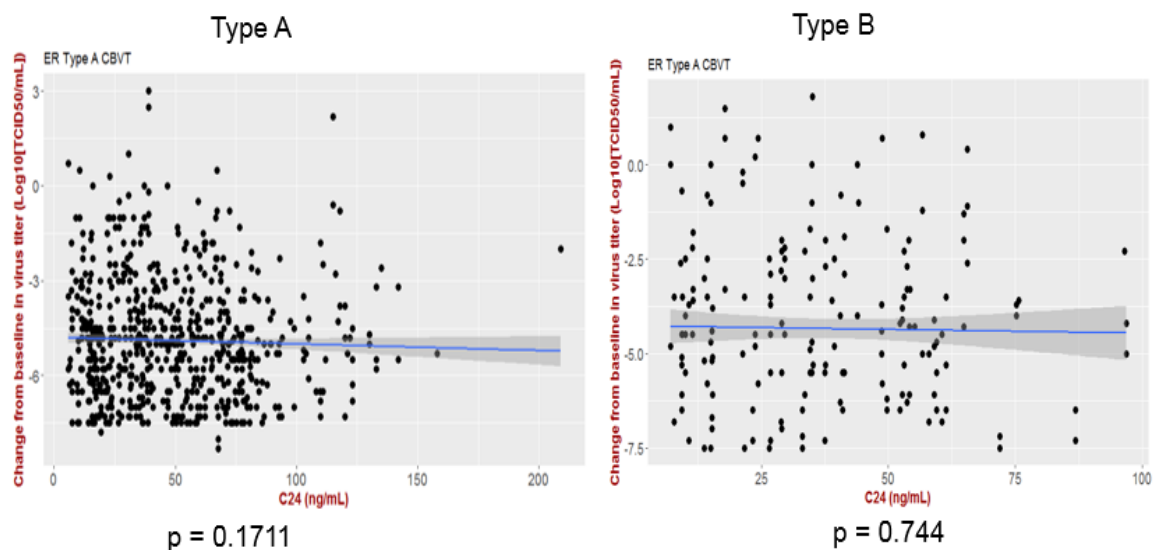
Figure 4. Exposure-response relationship for changes in virus titer on Day 2

Safety

Overall, XOFLUZA was well tolerated and the incidence of most frequent adverse events (> 2%, diarrhea, bronchitis, sinusitis, ALT increase) were similar between XOFLUZA and placebo groups. Therefore, no exposure-response relationship for safety was analyzed.

3.3.3 Is an alternative dosing regimen and/or management strategy required for subpopulations based on intrinsic factors?

There were no clinically significant differences in the pharmacokinetics of baloxavir based on age (adolescents as compared to adults), sex, or moderate (Child-Pugh class B) hepatic impairment. The effect of severe (Child-Pugh class C) hepatic impairment or renal impairment on the pharmacokinetics of baloxavir marboxil or its active metabolite, baloxavir, is unknown. Baloxavir marboxil exposure was not evaluated in subjects < 12 years of age, < 40 kg body weight, with severe hepatic impairment, or with renal impairment.

Body Weight

Body weight had a significant effect on the pharmacokinetics of baloxavir (as body weight increases, baloxavir exposure decreases). When patients were administered the proposed weight-based dosing in Phase 3 trial (40 mg for patients weighing less than 80 kg and 80 mg for patients weighing 80 kg

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and above), no clinically relevant exposure difference was observed between subjects with lower body weight and high body weight (Table 5). *(refer to Population PK Analyses, section 4.3).*

Race/Ethnicity

Based on a population pharmacokinetic analysis, race, in addition to body weight, are covariates on oral drug clearance (CL/F) of baloxavir. After accounting for the weight differences, AUC_{inf} values were approximately 34% lower in Non-Asians as compared to Asians (Figure 2).

No dose adjustment based on race is recommended since body weight-based dosing is considered sufficient to provide appropriate drug exposure levels, although it did not completely eliminate the effect of race (*refer to Figure 2 and Population PK Analyses, section 4.3).*

3.3.4 Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

Food-drug interactions

Food-effect studies conducted in healthy subjects indicated that food (approximately 400 to 500 kcal) decreased the C_{max} and AUC of baloxavir by 48% and 36%, respectively (*Study 1622T081F*). T_{max} was unchanged in the presence of food. The changes in C_{max} and AUC are not considered clinically relevant and baloxavir marboxil is recommended to be administered without regard to food Concomitant administration of baloxavir marboxil with dairy products or calcium-fortified beverages alone should be avoided since a further decrease in absorption is possible. The rationale behind this recommendation is as follows:

- In clinical studies with influenza patients (Studies 1518T0821 and 1601T0831, outlined above) where baloxavir marboxil was administered with or without food, there were no differences in baloxavir exposure (Table 7) and there were no clinically relevant differences in efficacy among patients who were administered baloxavir marboxil with and without food (Tables 8 and 9).

- Baloxavir plasma concentrations at 24 hours post dosing (C_{24}) would still be on the plateau of the exposure-response curve for efficacy after a ~ 50% decrease in exposures following the administration of 40 and 80 mg doses.

- Food containing polyvalent cations, such as dairy products, may decrease the solubility and permeability of baloxavir marboxil and hence decrease the exposure of baloxavir.

Table 7. Effect of Food Intake on C_{24} of S-033447 in the Phase 2 and Phase 3 studies (Study 1518T0821 = T0821 and Study 1601T0831 = T0831).

Study	Food Condition [a]	C_{24} (ng/mL)				Ratio of C_{24} to that after Dosing in the Fasted State			
		10 mg	20 mg	40 mg	80 mg	10 mg	20 mg	40 mg	80 mg
T0821	Fasted	16.0	38.7	61.7	-	-	-	-	-
	Intermediate	13.9	26.3	57.0	-	0.87	0.68	0.92	-
	Fed	13.0	23.2	56.3	-	0.81	0.60	0.91	-
T0831	Fasted)	-	-	46.3	58.0	-	-	-	
	Intermediate	-	-	46.9	62.5	-	-	1.01	1.08
	Fed	-	-	41.3	53.8	-	-	0.89	0.93

C_{24} = the observed plasma concentrations at 20 to 28 hours postdose

Geometric mean.

a   Fasted: Dosing > 4 hours before and > 4 hours after food intake; Intermediate: Dosing within 2 to 4 hours before or 2 to 4 hours after food intake; Fed: Dosing < 2 hours before or < 2 hours after food intake.

Table 8. TTAS by Timing of Food Intake and Dosing Group in the Phase 2 Study (T0821)

Meals Before and After Dosing	S-033188 10 mg	S-033188 20 mg	S-033188 40 mg	Placebo
Dosing > 4 hours before or > 4 hours after food intake				
n	31	32	25	30
Median (95% CI) (hours)	55.9 (43.1, 83.5)	51.8 (30.5, 104.8)	53.5 (37.6, 90.3)	91.3 (66.4, 105.8)
Difference (vs Placebo) (hours)	-35.4	-39.5	-37.8	---
P-value (G. Wilcoxon test) [a]	0.0813	0.0099	0.0069	---
Hazard ratio (95% CI) [b]	0.817 (0.487, 1.371)	0.746 (0.444, 1.252)	0.859 (0.485, 1.521)	---
P-value (Cox model) [b]	0.4435	0.2674	0.6022	---
Dosing ≥ 2 to ≤ 4 hours before or after food intake				
n	39	40	34	35
Median (95% CI) (hours)	51.3 (46.2, 71.0)	51.0 (44.2, 68.0)	45.7 (28.7, 69.0)	69.0 (53.3, 88.7)
Difference (vs Placebo) (hours)	-17.8	-18.0	-23.4	---
P-value (G. Wilcoxon test) [a]	0.0778	0.1961	0.0286	---
Hazard ratio (95% CI) [b]	0.668 (0.419, 1.064)	0.979 (0.610, 1.571)	0.682 (0.421, 1.106)	---
P-value (Cox model) [b]	0.0895	0.9295	0.1210	---
Dosing < 2 hours before or < 2 hours after food intake				
n	30	28	41	35
Median (95% CI) (hours)	55.4 (45.9, 73.2)	48.7 (31.2, 62.3)	52.5 (37.9, 68.9)	79.0 (63.4, 84.2)
Difference (vs Placebo) (hours)	-23.6	-30.3	-26.6	---
P-value (G. Wilcoxon test) [a]	0.4101	0.1722	0.4327	---
Hazard ratio (95% CI) [b]	0.797 (0.481, 1.322)	0.626 (0.372, 1.054)	0.806 (0.502, 1.294)	---
P-value (Cox model) [b]	0.3802	0.0780	0.3726	---

CI = confidence interval

a   Stratified Generalized Wilcoxon test vs placebo. Stratified factors: smoking habit, composite symptom score at baseline.

b   Cox proportional hazards model vs placebo. Covariates: smoking habit, composite symptom score at baseline.

Table 9. TTAS by Timing of Food Intake and Dosing Group in the Phase 3 Study (T0831)

Meals Before and After Dosing	S-033188	Placebo
Dosing > 4 hours before or > 4 hours after food intake		
- Summary Statistics		
- n	97	43
- Median (hours)	50.2	79.4
- 95% Confidence Interval (hours)	43.4, 66.3	62.4, 92.6
- Difference (vs Placebo) (hours)	-29.1	---
Stratified Generalized Wilcoxon Test vs Placebo [a]		
- P-value	0.0013	---
Dosing ≥ 2 to ≤ 4 hours before or after food intake		
- Summary Statistics		
- n	127	70
- Median (hours)	49.5	77.0
- 95% confidence interval (hours)	41.3, 54.9	62.7, 91.1
- Difference (vs Placebo) (hours)	-27.5	---
Stratified Generalized Wilcoxon test vs Placebo [a]		
- P-value	0.0224	---
Dosing < 2 hours before or < 2 hours after food intake		
- Summary Statistics		
- n	181	90
- Median (hours)	53.2	79.9
- 95% Confidence Interval (hours)	47.1, 63.2	69.0, 92.9
- Difference (vs Placebo) (hours)	-26.7	---
Stratified Generalized Wilcoxon Test vs Placebo [a]		
- P-value	< 0.0001	---

Drug-drug interaction

- Co-administration of baloxavir marboxil with laxatives, antacids, and supplements containing polyvalent cations is not recommended as they could decrease its absorption. It should be noted that this is based on baloxavir marboxil's chemical structure and no clinical studies have been conducted to confirm the interaction.

- No clinically significant changes in the pharmacokinetics of baloxavir marboxil and baloxavir were observed when co-administered with itraconazole (strong CYP3A and P-gp inhibitor), probenecid (UGT inhibitor), or oseltamivir (Figure 5).

- No clinically significant changes in the pharmacokinetics of the following drugs were observed when co-administered with baloxavir marboxil: midazolam (CYP3A4 substrate), digoxin (P-gp substrate), rosuvastatin (BCRP substrate), or oseltamivir (Figure 6).
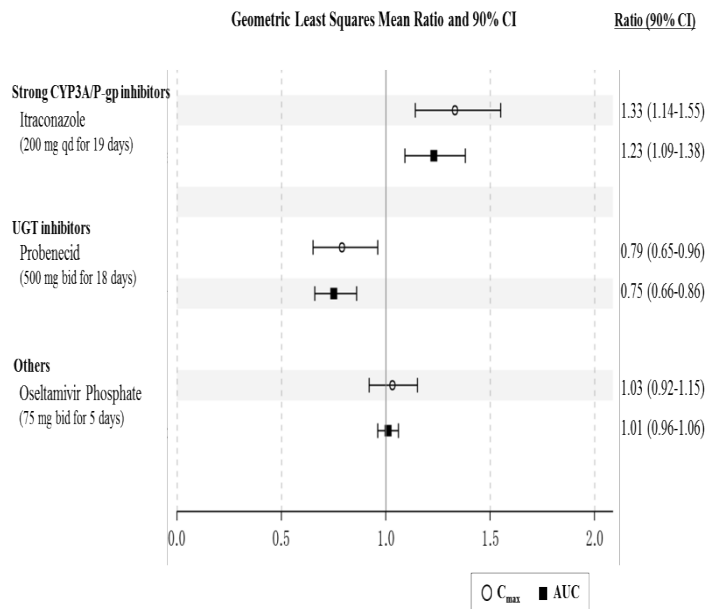
Figure 5. Effect of Co-Administered Drugs on the PK of Baloxavir Marboxil/Baloxavir
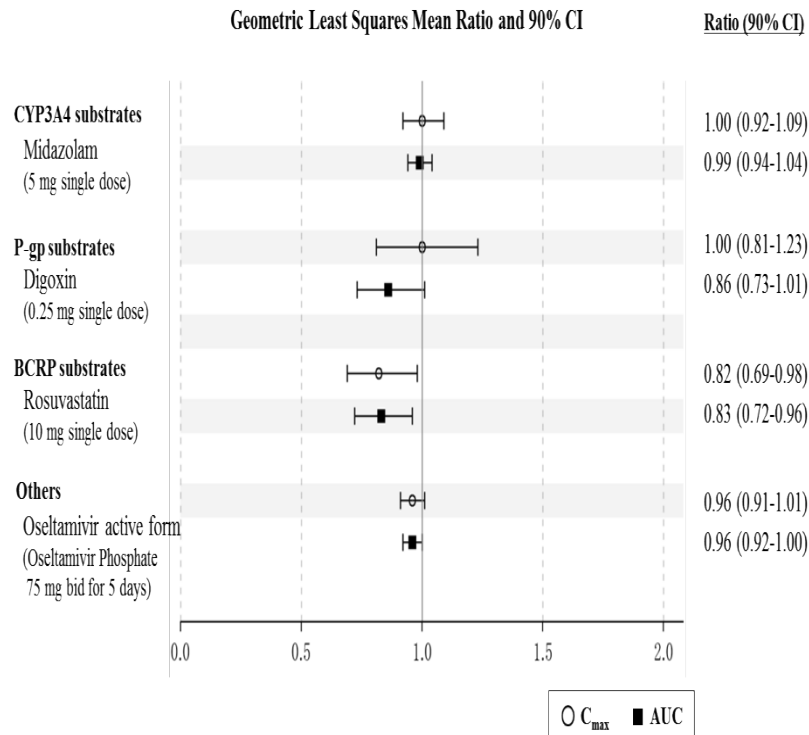
Figure 6. Effect of Baloxavir Marboxil/Baloxavir on the PK of Co-Administered Drugs

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In Vitro Studies Where Drug Interaction Potential Was Not Further Evaluated Clinically

Cytochrome P450 (CYP) Enzymes: Baloxavir marboxil and its active metabolite, baloxavir, did not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP2D6. Baloxavir marboxil and its active metabolite, baloxavir, did not induce CYP1A2, CYP2B6, or CYP3A4.

Uridine diphosphate (UDP)-glucuronosyl transferase (UGT) Enzymes: Baloxavir marboxil and its active metabolite, baloxavir, did not inhibit UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7, or UGT2B15

Transporter Systems: Both baloxavir marboxil and baloxavir are substrates of P-glycoprotein (P-gp). Baloxavir did not inhibit organic anion transporting polypeptides (OATP) 1B1, OATP1B3, organic cation transporter (OCT) 1, OCT2, organic anion transporter (OAT) 1, OAT3, multidrug and toxin extrusion (MATE) 1, or MATE2K.

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

The bioanalytical methods for quantification of baloxavir marboxil, baloxavir (S-033447, 3 methods), oseltamivir (and its metabolite oseltamivir carboxylate), midazolam, moxifloxacin, itraconazole, digoxin, rosuvastatin and probenecid in human plasma are summarized in Tables 10 – 19. All methods were adequately validated. The standard curves and QC data indicated that the assays were precise and accurate. All samples were stored and processed in the time frame supported by the stability data.

Table 10. Validation Data for Analytical Method with LC/MS/MS for Baloxavir Marboxil and S-033447 in Human Plasma		
Test Site	(b) (4)	
Matrix	**Plasma (Anticoagulant: heparin, Inhibitor for esterases: dichlorvos)**	
Study Number Supported	1622T081F, 1510T0813, 1510T0811, 1611T081B, 1519T0814, 1520T0815, 1612T081C, 1606T0818	
Analyte	Baloxavir marboxil	S-033447
Lower Limit of Quantification (ng/mL)	0.100	0.100
Quantification Range (ng/mL)	0.100 to 1000	0.100 to 1000
Calibration Curve Range (ng/mL)	0.1 to 300	0.1 to 300

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	LLOQ	Except for LLOQ	LLOQ	Except for LLOQ
QC Levels (ng/mL)	0.100	0.200, 10.0, 240	0.100	0.200, 10.0, 240
Within-run Precision (%CV)	5.5		5.7	
Within-run Accuracy (%Bias)	5.8	0.9 to 2.8	−4.6	0.6 to 3.3
Between-run Precision (%CV)	7.4	0.6 to 5.3	6.1	1.2 to 1.4
Between-run Accuracy (%Bias)	2.0	2.8 to 5.4	−1.5	2.4 to 5.9
		3.5 to 6.0		0.6 to 6.8
Dilution Factor	10		10	
Within-run Precision (%CV)	1.9		3.0	
Within-run Accuracy (%Bias)	0.3		0.7	
Stability in Matrix	Stable after 6 freeze (−20°C) –thaw (on ice) cycles, at least for 385 days at −20°C, 6 freeze (−80°C) –thaw (on ice) cycles, at least for 385 days at −80°C, at least for 6 hours on ice and at least for 2 hours at room temperature			
Processed Extract Stability	Stable at least for 72 hours at 15°C			

Source: P. 39, Summary of Biopharmaceutics Studies and Associated Analytical Methods.

Table 11. Validation Data for Analytical Method with LC/MS/MS for S-033447 in Human Plasma (site 1)		
Test Site	(b) (4)	
Matrix	Plasma (Anticoagulant: heparin)	
Study Number Supported	1527T0816, 1613T081D, 1518T0821	
Analyte	S-033447	
Lower Limit of Quantification (ng/mL)	0.100	
Quantification Range (ng/mL)	0.100 to 1000	
Calibration Curve Range (ng/mL)	0.1 to 300	
	LLOQ	Except for LLOQ

QC Levels (ng/mL)	0.100	0.200, 10.0, 240
Within-run Precision (%CV)	4.5	1.7 to 3.1
Within-run Accuracy (%Bias)	−13.2	−1.5 to 1.2
Between-run Precision (%CV)	7.2	2.3 to 4.8
Between-run Accuracy (%Bias)	−7.5	0.7 to 7.6
Dilution Factor	10	
Within-run Precision (%CV)	3.1	
Within-run Accuracy (%Bias)	−3.8	
Stability in Matrix	Stable after 6 freeze (−20°C) –thaw (on ice) cycles, at least for 367 days at −20°C, 6 freeze (−80°C) –thaw (on ice) cycles, at least for 367 days at −80°C, at least for 6 hours on ice and at least for 28 hours at 37°C	
Processed Extract Stability	Stable at least for 72 hours at 15°C	

Source: P. 40, Summary of Biopharmaceutics Studies and Associated Analytical Methods.

Table 12. Validation Data for Analytical Method with LC/MS/MS for S-033447 in Human Plasma (site 2)		
Test Site	(b) (4)	
Matrix	Plasma (Anticoagulant: heparin)	
Study Number Supported	1532T0817, 1601T0831	
Analyte	S-033447	
Lower Limit of Quantification (ng/mL)	0.100	
Quantification Range (ng/mL)	0.100 to 2250	
Calibration Curve Range (ng/mL)	0.1 to 300	
	LLOQ	Except for LLOQ
QC Levels (ng/mL)	0.100	0.300, 9.00, 225
Within-run Precision (%CV)	2.7 to 7.4	0.7 to 5.1
Within-run Accuracy (%RE)	−4.2 to 2.0	−4.9 to 4.0
Between-run Precision (%CV)	6.1	2.3 to 4.0

Between-run Accuracy (%RE)	−1.7	−3.1 to 1.0
Dilution Factor Within-run Precision (%CV) Within-run Accuracy (%RE)	10 2.2 −4.0	
Stability in Matrix	Stable at least for 26 hours at 4°C	
Processed Extract Stability	Stable at least for 24 hours at 4°C	

Source: P. 41, Summary of Biopharmaceutics Studies and Associated Analytical Methods.

Table 13. Validation Data for Analytical Method with LC/MS/MS for Oseltamivir and Oseltamivir Carboxylate in Human Plasma				
Test Site	(b) (4)			
Matrix	Plasma (Anticoagulant: EDTA-2K)			
Study Number Supported	1606T0818			
Analyte	Oseltamivir		Oseltamivir acid	
Lower Limit of Quantification (ng/mL)	1.00		10.0	
Quantification Range (ng/mL)	1.00 to 2000		10.0 to 20000	
Calibration Curve Range (ng/mL)	1 to 500		10 to 5000	
	LLOQ	Except for LLOQ	LLOQ	Except for LLOQ
QC Levels (ng/mL) Within-run Precision (%CV) Within-run Accuracy (%Bias) Between-run Precision (%CV) Between-run Accuracy (%Bias)	1.00 9.1 −4.9 10.0 1.5	3.00, 50.0, 400 3.7 to 6.9 −13.2 to −7.2 7.4 to 10.4 −7.8 to −3.8	10.0 4.0 −2.7 5.2 −0.9	30.0, 500, 4000 5.3 to 7.3 −4.8 to 8.8 5.1 to 7.7 −2.1 to 4.2
Dilution Factor Within-run Precision (%CV) Within-run Accuracy (%Bias)	5 4.7 −4.3		5 6.8 −1.9	

Stability in Matrix	Stable after 4 freeze (−20°C) –thaw (on ice) cycles, at least for 124 days at −20°Cb, 4 freeze (−70°C) –thaw (on ice) cycles, at least for 420 days at −70°Cb, and at least for 6 hours on ice	Stable after 4 freeze (−20°C) –thaw (on ice) cycles, at least for 420 days at −20°Cb, 4 freeze (−70°C) –thaw (on ice) cycles, at least for 420 days at −70°Cb, and at least for 6 hours on ice
Processed Extract Stability	Stable at least for 72 hours at 10°C	

Source: P. 42, Summary of Biopharmaceutics Studies and Associated Analytical Methods.

Table 14. Validation Data for Analytical Method with LC/MS/MS for Midazolam in Human Plasma		
Test Site	(b) (4)	
Matrix	Plasma (Anticoagulant: heparin)	
Study Number Supported	1519T0814	
Analyte	Midazolam	
Lower Limit of Quantification (ng/mL)	0.1000	
Quantification Range (ng/mL)	0.1000 to 1000	
Calibration Curve Range (ng/mL)	0.1 to 100	
	LLOQ	Except for LLOQ
QC Levels (ng/mL) Within-run Precision (%CV) Within-run Accuracy (%Bias) Between-run Precision (%CV) Between-run Accuracy (%Bias)	0.1000 5.7 −3.7 6.7 −5.8	0.2500, 5.000, 80.00 2.0 to 5.5 −5.1 to 5.1 3.6 to 5.1 −5.1 to 4.2
Dilution Factor Within-run Accuracy (%Bias)	10 −4.5	
Stability in Matrix	Stable after 6 freeze (−20°C) –thaw (room temperature) cycles, at least for 94 days at −20°C, and at least for 24 hours at room temperature	

Processed Extract Stability	Stable at least for 72 hours at 10°C

Source: P. 43, Summary of Biopharmaceutics Studies and Associated Analytical Methods.

Table 15. Validation Data for Analytical Method with LC/MS/MS for Moxifloxacin in Human Plasma		
Test Site	(b) (4)	
Matrix	Plasma (Anticoagulant: heparin)	
Study Number Supported	1527T0816	
Analyte	Moxifloxacin	
Lower Limit of Quantification (ng/mL)	50.0	
Quantification Range (ng/mL)	50.0 to 30000	
Calibration Curve Range (ng/mL)	50 to 20000	
	LLOQ	Except for LLOQ
QC Levels (ng/mL)	50.0	100, 1000, 16000
Within-run Precision (%CV)	3.0	2.0 to 4.0
Within-run Accuracy (%Bias)	−12.8	−8.6 to −7.0
Between-run Precision (%CV)	5.2	4.1 to 6.9
Between-run Accuracy (%Bias)	−6.8	−11.0 to 0.3
Dilution Factor	10	
Within-run Precision (%CV)	1.6	
Within-run Accuracy (%Bias)	11.6	
Stability in Matrix	Stable after 3 freeze (−20°C) –thaw (room temperature) cycles, at least for 181 days at −20°C, 3 freeze (−80°C) –thaw (room temperature) cycles, at least for 181 days at −80°C, and at least for 24 hours at room temperature	
Processed Extract Stability	Stable at least for 72 hours at 4°C	

Source: P. 44, Summary of Biopharmaceutics Studies and Associated Analytical Methods.

Table 16. Validation Data for Analytical Method with LC/MS/MS for Itraconazole in Human Plasma	
Test Site	(b) (4)
Matrix	Plasma (Anticoagulant: heparin)

Study Number Supported	1520T0815	
Analyte	Itraconazole	
Lower Limit of Quantification (ng/mL)	0.100	
Quantification Range (ng/mL)	0.100 to 1600	
Calibration Curve Range (ng/mL)	0.1 to 100	
	LLOQ	Except for LLOQ
QC Levels (ng/mL) Within-run Precision (%CV) Within-run Accuracy (%Bias) Between-run Precision (%CV) Between-run Accuracy (%Bias)	0.100 1.6 1.3 3.9 −2.3	0.200, 5.00, 80.0 2.5 to 5.5 −0.6 to 1.1 2.1 to 5.2 0.8 to 2.1
Dilution Factor Within-run Precision (%CV) Within-run Accuracy (%Bias)	10 0.9 −2.6	20 3.5 −4.0
Stability in Matrix	Stable after 5 freeze (−20°C) –thaw (room temperature) cycles, at least for 97 days at −20°C, 5 freeze (−80°C) –thaw (room temperature) cycles, at least for 97 days at −80°C, and at least for 24 hours at room temperature	
Processed Extract Stability	Stable at least for 72 hours at 4°C	

Source: P. 45, Summary of Biopharmaceutics Studies and Associated Analytical Methods.

Table 17. Validation Data for Analytical Method with LC/MS/MS for Digoxin in Human Plasma	
Test Site	(b) (4)
Matrix	Plasma (Anticoagulant: heparin)
Study Number Supported	1613T081D
Analyte	Digoxin
Lower Limit of Quantification (ng/mL)	0.0100
Quantification Range (ng/mL)	0.0100 to 80.0

Calibration Curve Range (ng/mL)	0.01 to 10	
	LLOQ	Except for LLOQ
QC Levels (ng/mL)	0.0100	0.0200, 0.500, 8.00
Within-run Precision (%CV)	3.5	1.0 to 4.0
Within-run Accuracy (%Bias)	3.5	−8.0 to −0.8
Between-run Precision (%CV)	4.7	1.0 to 2.8
Between-run Accuracy (%Bias)	0.5	−5.9 to −0.4
Dilution Factor	10	
Within-run Precision (%CV)	0.6	
Within-run Accuracy (%Bias)	−12.1	
Stability in Matrix	Stable after 6 freeze (−20°C) –thaw (room temperature) cycles, at least for 98 days at −20°C, 6 freeze (−80°C) –thaw (room temperature) cycles, at least for 98 days at −80°C, and at least for 6 hours at room temperature	
Processed Extract Stability	Stable at least for 72 hours at 4°C	

Source: P. 46, Summary of Biopharmaceutics Studies and Associated Analytical Methods.

Table 18. Validation Data for Analytical Method with LC/MS/MS for Rosuvastatin in Human Plasma		
Test Site	(b) (4)	
Matrix	Plasma (Anticoagulant: heparin)	
Study Number Supported	1613T081D	
Analyte	Rosuvastatin	
Lower Limit of Quantification (ng/mL)	0.0100	
Quantification Range (ng/mL)	0.0100 to 80.0	
Calibration Curve Range (ng/mL)	0.01 to 10	
	LLOQ	Except for LLOQ
QC Levels (ng/mL)	0.0100	0.0200, 1.00, 8.00
Within-run Precision (%CV)	8.5	1.2 to 5.4

Within-run Accuracy (%)	107.3	97.0 to 101.1
Between-run Precision (%CV)	8.2	1.0 to 4.1
Between-run Accuracy (%)	99.9	97.5 to 101.5
Dilution Factor	10	
Within-run Precision (%CV)	0.6	
Within-run Accuracy (%)	90.4	
Stability in Matrix	Stable after 5 freeze (−20°C) –thaw (on ice) cycles, at least for 101 days at −20°C, 5 freeze (−80°C) –thaw (on ice) cycles, at least for 101 days at −80°C, and at least for 24 hours on ice	
Processed Extract Stability	Stable at least for 96 hours at 4°C	

Source: P. 47, Summary of Biopharmaceutics Studies and Associated Analytical Methods.

Table 19. Validation Data for Analytical Method with LC/MS/MS for Probenecid in Human Plasma		
Test Site	(b) (4)	
Matrix	Plasma (Anticoagulant: heparin)	
Study Number Supported	1612T081C	
Analyte	Probenecid	
Lower Limit of Quantification (µg/mL)	1.00	
Quantification Range (µg/mL)	1.00 to 4000	
Calibration Curve Range (µg/mL)	1 to 500	
	LLOQ	Except for LLOQ
QC Levels (µg/mL)	1.00	2.00, 20.0, 400
Within-run Precision (%CV)	4.9	2.1 to 3.4
Within-run Accuracy (%)	98.9	93.7 to 98.9
Between-run Precision (%CV)	4.0	2.0 to 2.5
Between-run Accuracy (%)	96.8	92.0 to 97.1
Dilution Factor	10	
Within-run Precision (%CV)	1.0	
Within-run Accuracy (%)	89.7	

Stability in Matrix	Stable after 5 freeze (−20°C) –thaw (room temperature) cycles, at least for 96 days at −20°C, 5 freeze (−80°C) –thaw (room temperature) cycles, at least for 96 days at −80°C, and at least for 24 hours at room temperature
Processed Extract Stability	Stable at least for 96 hours at 4°C

Source: P. 48, Summary of Biopharmaceutics Studies and Associated Analytical Methods.

4.2 Clinical Studies

The clinical development program of baloxavir marboxil includes 11 Phase 1 studies (Table 20), 1 phase 2 study and 2 phase 3 studies (Table 21).

Table 20. List of Phase 1 Studies			
Country /Region	**Study No.**	**Study Objectives**	**Dosage Regimen**
Japan	1622T081F	Evaluation of bioequivalence (BE) of baloxavir marboxil 10-mg and 20-mg tablets and food effect (FE).	BE part: baloxavir marboxil (10-mg tablet and 20-mg tablet): 20-mg, single oral dose. FE part: baloxavir marboxil (20-mg tablet): 40-mg, single oral dose (fasted/fed [moderate fat meal]). FE-10 mg part: baloxavir marboxil (10-mg tablet): 10- mg, single oral dose fasted/fed [moderate fat meal].
Japan	1510T0813	Evaluation of relative bioavailability (BA) of baloxavir marboxil 20-mg tablets and FE.	BA part: baloxavir marboxil (suspension and 20-mg tablet): 20-mg, single oral dose. FE part: baloxavir marboxil (20-mg tablet): 20- mg, single oral dose (fasted/before meal/fed [moderate fat meal]).
Japan	1510T0811	Evaluation of the pharmacokinetics and safety single ascending doses (SAD) of baloxavir marboxil and FE.	SAD part: Baloxavir marboxil (suspension): 6-, 20-, 40-, 60-, and 80-mg single dose. FE part: Baloxavir marboxil (suspension): 40 mg, single oral dose (fasted/fed [high-fat meal]).

US	1519T0814	Evaluation of drug-drug interaction with a CYP3A4 substrate	Baloxavir marboxil (tablet): 40 mg, single dose CYP3A4 substrate (midazolam): 5 mg, single dose
US	1520T0815	Evaluation of drug-drug interaction with a P-gp inhibitor	Baloxavir marboxil (tablet): 20 mg, single dose P-gp inhibitor (itraconazole): 200 mg once daily, multiple doses
Japan	1606T0818	Evaluation of drug-drug interaction with oseltamivir phosphate	Baloxavir marboxil (tablet): 40 mg, single dose Oseltamivir phosphate: 75 mg twice daily, multiple doses
US	1612T081C	Evaluation of drug-drug interaction with a UGT inhibitor	Baloxavir marboxil (tablet): 80 mg, single dose UGT inhibitor (probenecid): 500 mg twice daily, multiple doses
US	1613T081D	Evaluation of drug-drug interactions with a P-gp substrate and a BCRP substrate	Baloxavir marboxil (tablet): 80 mg, single dose P-gp substrate drug (digoxin): 0.25 mg, single dose BCRP substrate drug (rosuvastatin): 10 mg, single dose
UK	1532T0817	Evaluation of the absorption, distribution, metabolism, and excretion of baloxavir marboxil	[14C]-baloxavir marboxil (suspension): 40 mg, single dose
Japan	1527T0816	Evaluation of the effect on QTc interval and cardiovascular safety	Baloxavir marboxil (tablet): 40, 80 mg, single dose Moxifloxacin: 400 mg, single dose
US	1611T081B	Evaluation of the pharmacokinetics, tolerability, and safety in subjects with hepatic impairment	Baloxavir marboxil (tablet): 40 mg, single dose

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Table 21. List of Phase 2 and Phase 3 Studies

Country /Region	Study No.	Study Objectives	Study Population	Dosage Regimen
Controlled Study for the Proposed Indication				
Japan	1518T0821	Phase 2 Evaluation of the efficacy and safety in patients with influenza	Patients with influenza virus infection (≥20 years)	Baloxavir marboxil (tablet): 10, 20, 40 mg, single dose Placebo
Japan, US, Canada	1601T0831	Phase 3 Evaluation of the efficacy and safety in patients with influenza	Patients with influenza virus infection (≥12 years)	Baloxavir marboxil (tablet): Single dose according to body weight: Weight <80 kg: 40 mg Weight ≥80 kg: 80 mg Oseltamivir phosphate: 75 mg twice daily, multiple doses (5 days) Placebo
Open-label Study				
Japan	1618T0822 *(No label recommendation was based on this study)*	Phase 3 Evaluation of the efficacy and safety in patients with influenza	Patients with influenza virus infection (≥6 months and <12 years)	Baloxavir marboxil (tablet): Single dose according to body weight: Weight ≥5 and <10 kg: 5 mg Weight ≥10 and <20 kg: 10 mg Weight ≥20 and <40 kg: 20 mg Weight ≥40 kg: 40 mg

4.3 Population PK Analyses

The Applicant conducted a population PK analysis for baloxavir using data from ten Phase 1 studies, one Phase 2 study, and one Phase 3 study. The model described baloxavir plasma PK profiles following a

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single oral dose of baloxavir marboxil in healthy subjects and patients infected with influenza. A total of 8310 plasma concentrations were available for the analysis. The doses of baloxavir marboxil ranged from 6 to 80 mg.

Description of data used in population PK analysis by the Applicant

The subjects with at least one evaluable baloxavir concentration were included in the population PK analysis and their baseline characteristics are summarized in Table 22. Missing continuous covariate data were imputed with the median value of the population. Missing categorical covariate data were imputed with the population mode.

The following baseline characteristics were used as the candidate of covariates: age, body weight, body mass index (BMI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (Tbil), estimated glomerular filtration rate (eGFR), and creatinine clearance (CLcr) at baseline as continuous data, and gender (male, female), Child-Pugh category (normal hepatic function or moderate hepatic impairment [Child-Pugh score: 7 to 9]), race (Asian, White, or others), region (Japan/Asia or not Japan/Asia), health status (healthy subjects, otherwise healthy patients with influenza, or symptomatic patients without influenza) and food conditions (dosing ≥ 4 hours before and ≥ 4 hours after food intake [fasted], dosing within 2 to 4 hours before or 2 to 4 hours after food intake [intermediate], or dosing < 2 hours before or < 2 hours after food intake [fed]) as categorical data. The body-surface-area-adjusted eGFR (eGFRadj) was calculated as shown in Table 23 for patients aged ≥ 18 years and the Schwartz formula was used for patients aged < 18 years. The absolute eGFR (eGFRabs) was calculated using the body surface area (BSA) and the eGFRadj. CLcr was calculated by the Cockcroft-Gault equation for patients aged ≥ 18 years. CLcr for patients aged < 18 years was calculated by eGFRadj and BSA. The BSA was calculated using the following equation reported by Mostellar:

$BSA(m^2) = [height~(cm) \times body~weight~(kg)/3600]^{1/2}$

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Table 22. Summary of characteristics of subjects included in the population PK analysis (N=1109)

Study	Background characteristics	Mean (SD)	Median (range)
Overall	Age (years)	35.1 (12.4)	34 (12 - 70)
	Body weight (kg)	67.0 (15.3)	64.8 (36.0 - 131.0)
	BMI (kg/m^2)	23.9 (4.6)	22.7 (15.3 - 51.2)
	Aspartate aminotransferase (U/L)	23.0 (16.1)	20 (9 - 428)
	Alanine aminotransferase (U/L)	22.1 (17.6)	17 (6 - 320)
	Total bilirubin (mg/dL)	0.5 (0.3)	0.5 (0.2 - 2.7)
	eGFRadj (mL/min/1.73 m^2)	90.2 (24.9)	85.0 (45.0 - 238.5)
	eGFRabs (mL/min)	91.5 (28.6)	84.9 (42.6 - 271.8)
	CLcr (mL/min)	115.1 (31.6)	110.4 (51.8 - 337.1)
	Gender (Male : Female) [a]	660 (59.5%) : 449 (40.5%)	
	Child-Pugh category (Normal hepatic function : Moderate hepatic impairment) [a]	1101 (99.3%) : 8 (0.7%)	
	Race (Asian : White : others) [a]	799 (72.0%) : 254 (22.9%) : 56 (5.0%)	
	Region (Japan/Asia : not Japan/Asia) [a]	791 (71.3%) : 318 (28.7%)	
	Health status (Healthy subjects : Patients with influenza : Patients without influenza) [a]	223 (20.1%) : 748 (67.4%) : 138 (12.4%)	
	Food condition [b] (Fasted : Intermediate : Fed) [a]	511 (46.1%) : 275 (24.8%) : 323 (29.1%)	
	Adult (> 18 years old) : Adolescent (12 to 18 years old) [a]	1022 (92.2%) : 87 (7.8%)	
Phase 1 study	Age (years)	35.7 (11.7)	34 (20 - 70)
	Body weight (kg)	68.9 (13.7)	64.9 (46.0 - 118.9)
	BMI (kg/m^2)	23.6 (3.8)	22.5 (18.5 - 37.8)
	Aspartate aminotransferase (U/L)	19.7 (6.3)	18 (9 - 62)
	Alanine aminotransferase (U/L)	19.1 (9.8)	16 (6 - 74)
	Total bilirubin (mg/dL)	0.7 (0.3)	0.7 (0.2 - 2.4)
	eGFRadj (mL/min/1.73 m^2)	98.9 (26.1)	92.1 (58.9 - 238.5)
	eGFRabs (mL/min)	103.4 (32.0)	94.4 (60.0 - 227.2)
	CLcr (mL/min)	123.7 (29.2)	117.8 (77.7 - 237.2)
	Gender (Male : Female) [a]	187 (83.9%) : 36 (16.1%)	
	Child-Pugh category (Normal hepatic function : Moderate hepatic impairment) [a]	215 (96.4%) : 8 (3.6%)	
	Race (Asian : White : others) [a]	141 (63.2%) : 71 (31.8%) : 11 (4.9%)	
	Region (Japan/Asia : not Japan/Asia) [a]	141 (63.2%) : 82 (36.8%)	
	Health status (Healthy subjects : Patients with influenza : Patients without influenza) [a]	223 (100.0%) : 0 (0.0%) : 0 (0.0%)	
	Food condition [b] (Fasted : Intermediate : Fed) [a]	223 (100.0%) : 0 (0.0%) : 0 (0.0%)	
	Adult (> 18 years old) : Adolescent (12 to 18 years old) [a]	223 (100.0%) : 0 (0.0%)	

[a] Number of subjects (percentage of all subjects).

[b] Fasted: Dosing ≥ 4 hours before and ≥ 4 hours after food intake; Intermediate: Dosing within 2 to 4 hours before or 2 to 4 hours after food intake; Fed: Dosing < 2 hours before or < 2 hours after food intake.

Study	Background characteristics	Mean (SD)	Median (range)
Phase 2 study	Age (years)	37.7 (10.9)	37 (20 - 63)
	Body weight (kg)	63.5 (13.4)	62.6 (36.0 - 110.0)
	BMI (kg/m^2)	22.8 (3.8)	22.2 (16.7 - 36.6)
	Aspartate aminotransferase (U/L)	27.4 (26.5)	23 (13 - 428)
	Alanine aminotransferase (U/L)	26.9 (24.7)	20 (8 - 320)
	Total bilirubin (mg/dL)	0.6 (0.2)	0.5 (0.2 - 1.8)
	eGFRadj (mL/min/1.73 m^2)	81.7 (14.3)	80.2 (46.8 - 122.4)
	eGFRabs (mL/min)	80.3 (16.0)	78.8 (42.6 - 148.3)
	CLcr (mL/min)	108.0 (26.4)	104.8 (57.3 - 225.6)
	Gender (Male : Female) [a]	186 (62.0%) : 114 (38.0%)	
	Child-Pugh category (Normal hepatic function : Moderate hepatic impairment) [a]	300 (100.0%) : 0 (0.0%)	
	Race (Asian : White : others) [a]	299 (99.7%) : 0 (0.0%) : 1 (0.3%)	
	Region (Japan/Asia : not Japan/Asia) [a]	300 (100.0%) : 0 (0.0%)	
	Health status (Healthy subjects : Patients with influenza : Patients without influenza) [a]	0 (0.0%) : 300 (100.0%) : 0 (0.0%)	
	Food condition [b] (Fasted : Intermediate : Fed) [a]	89 (29.7%) : 112 (37.3%) : 99 (33.0%)	
	Adult (> 18 years old) : Adolescent (12 to 18 years old) [a]	300 (100.0%) : 0 (0.0%)	
Phase 3 study	Age (years)	33.5 (13.2)	32 (12 - 64)
	Body weight (kg)	68.1 (16.5)	65.6 (40.1 - 131.0)
	BMI (kg/m^2)	24.5 (5.1)	23.1 (15.3 - 51.2)
	Aspartate aminotransferase (U/L)	22.0 (10.1)	19 (10 - 137)
	Alanine aminotransferase (U/L)	20.8 (14.9)	16 (6 - 115)
	Total bilirubin (mg/dL)	0.4 (0.3)	0.4 (0.2 - 2.7)
	eGFRadj (mL/min/1.73 m^2)	91.2 (27.2)	84.5 (45.0 - 213.3)
	eGFRabs (mL/min)	92.8 (30.1)	85.1 (44.8 - 271.8)
	CLcr (mL/min)	115.4 (33.9)	110.7 (51.8 - 337.1)
	Gender (Male : Female) [a]	287 (49.0%) : 299 (51.0%)	
	Child-Pugh category (Normal hepatic function : Moderate hepatic impairment) [a]	586 (100.0%) : 0 (0.0%)	
	Race (Asian : White : others) [a]	359 (61.3%) : 183 (31.2%) : 44 (7.5%)	
	Region (Japan/Asia : not Japan/Asia) [a]	350 (59.7%) : 236 (40.3%)	
	Health status (Healthy subjects : Patients with influenza : Patients without influenza) [a]	0 (0.0%) : 448 (76.5%) : 138 (23.5%)	
	Food condition [b] (Fasted : Intermediate : Fed) [a]	199 (34.0%) : 163 (27.8%) : 224 (38.2%)	
	Adult (> 18 years old) : Adolescent (12 to 18 years old) [a]	499 (85.2%) : 87 (14.8%)	

[a] Number of subjects (percentage of all subjects).

[b] Fasted: Dosing ≥ 4 hours before and ≥ 4 hours after food intake; Intermediate: Dosing within 2 to 4 hours before or 2 to 4 hours after food intake; Fed: Dosing < 2 hours before or < 2 hours after food intake.

Source: Applicant's population PK report, Table 2, Page 31-32

Table 23. Equations used to calculate eGFRadj and CLcr for different age groups

Parameter	Age	Equation
eGFRadj (mL/min/1.73 m^2)	≥ 18 years	Asian: eGFRadj = $194 \times$ [age (years)]$^{-0.287} \times$ (Scr)$^{-1.094} \times$ (0.739 if female) Non-Asian: eGFRadj = $175 \times$ [age (years)]$^{-0.203} \times$ (Scr)$^{-1.154} \times$ (0.742 if female) $\times$ (1.212 if African American)
	13 to 17 years	Male: eGFRadj = $0.70 \times$ [height (cm)]/Scr Female: eGFRadj = $0.55 \times$ [height (cm)]/Scr
	12 years	eGFRadj = $0.55 \times$ [height (cm)]/Scr
CLcr (mL/min)	≥ 18 years	CLcr = [body weight (kg)] $\times$ (140 – age)/(72 $\times$ Scr) $\times$ (0.85 if female)
	< 18 years	CLcr = eGFR $\times$ BSA/1.73

BSA = body surface area (m^2); Scr = serum creatinine (mg/dL)

Methods

The population PK analysis was performed in NONMEM 7.3. A 2-compartment model with first-order absorption and absorption lag time was used as the structural model.

The evaluations included: the effect of body weight, BMI, gender, ALT, Tbil, eGFRadj, eGFRabs, CLcr, race (White or others), region, and health status (otherwise healthy patients with influenza or symptomatic patients without influenza) on CL/F; the effect of body weight, BMI, gender, race (White or others), region, and health status (otherwise healthy patients with influenza or otherwise healthy patients without influenza) on Vc/F; the effect of gender and health status (otherwise healthy patients with influenza) on Ka; the effect of food condition (Intermediate or Fed) on F; the effect of body weight on Q/F; and the effect of body weight, BMI, and Child-Pugh category (moderate hepatic impairment) on Vp/F. In addition, the effect of race (Non-Asian) on CL/F and Vc/F was also investigated.

Results

The final model included the effect of body weight on CL/F, Vc/F, Q/F, and Vp/F, the effect of race (Non-Asian) on CL/F and Vc/F, the effect of ALT on CL/F, the effect of gender on first-order rate of absorption (Ka), and the effect of food condition (Fed) on relative bioavailability (F). The final population PK parameter estimates and covariates effect are shown in Table 24. The ability of the final model to describe observations of baloxavir concentrations was evaluated with goodness-of-fit plots as shown in Figure 7. The model predicted and observed data are almost identical. The data were well distributed around the LOESS line (red) as shown in plots of CRWES versus population prediction or time after dose.

Table 24. Parameter estimates of the final population PK model for baloxavir

		Final model					
					Bootstrap estimates		
Pharmacokinetic parameters	Units	Estimate	%RSE	Median	95% CI (lower - upper)		
CL/F	(L/hr)	5.40	1.5	5.38	5.23	-	5.56
Vc/F	(L)	333	2.7	332	314	-	353
Q/F	(L/hr)	6.27	4.5	6.28	5.69	-	6.79
Vp/F	(L)	212	2.3	212	203	-	222
Ka	(1/hr)	1.10	6.5	1.10	0.964	-	1.31
ALAG	(hr)	0.32	3.6	0.32	0.29	-	0.34
Effect of food condition (Fed) on F		0.869	2.7	0.869	0.823	-	0.922
Effect of body weight on CL/F		1.04	Fixed	1.04	Fixed		
Effect of race (Non-Asian) on CL/F		1.72	3.2	1.72	1.63	-	1.85
Effect of ALT on CL/F		-0.115	11.6	-0.115	-0.137	-	-0.0859
Effect of body weight on Vc/F		1.76	Fixed	1.76	Fixed		
Effect of race (Non-Asian) on Vc/F		1.36	4.6	1.36	1.24	-	1.49
Effect of body weight on Q/F		0.473	Fixed	0.473	Fixed		
Effect of body weight on Vp/F		0.642	Fixed	0.642	Fixed		
Effect of gender on Ka		0.613	10.0	0.607	0.479	-	0.723
Inter-individual variability							
CL/F	%	38.7	5.5	38.8	36.4	-	41.2
Covariance between CL/F and Vc/F		0.177	6.3	0.176	0.153	-	0.197
Vc/F	%	54.8	6.2	54.6	50.8	-	58.0
Vp/F	%	22.2	13.8	22.3	18.9	-	25.4
Ka	%	111.8	7.7	112.1	103.7	-	121.3
Intra-individual variability							
Proportional residual error	%	20.3	2.5	20.3	19.2	-	21.4
Additive residual error	(ng/mL)	0.134	30.1	0.132	0.0640	-	0.217
Shrinkage							
sh_ηp (CL/F)	%	4.5	-	-	-		
sh_ηp (Vc/F)	%	10.4	-	-	-		
sh_ηp (Vp/F)	%	47.7	-	-	-		
sh_ηp (Ka)	%	29.7	-	-	-		
sh_ε	%	15.3	-	-	-		

CI = confidence interval; sh_ηp = shrinkage in the standard deviation of inter-individual variability parameters η;
sh_ε = shrinkage in the standard deviation of intra-individual variability parameters ε; %RSE = relative standard error in percent

$CL/F = 5.40 * (body\ weight/64.8)^{1.04} * (1.72\ for\ Non\text{-}Asian) * (ALT/17)^{-0.115}$

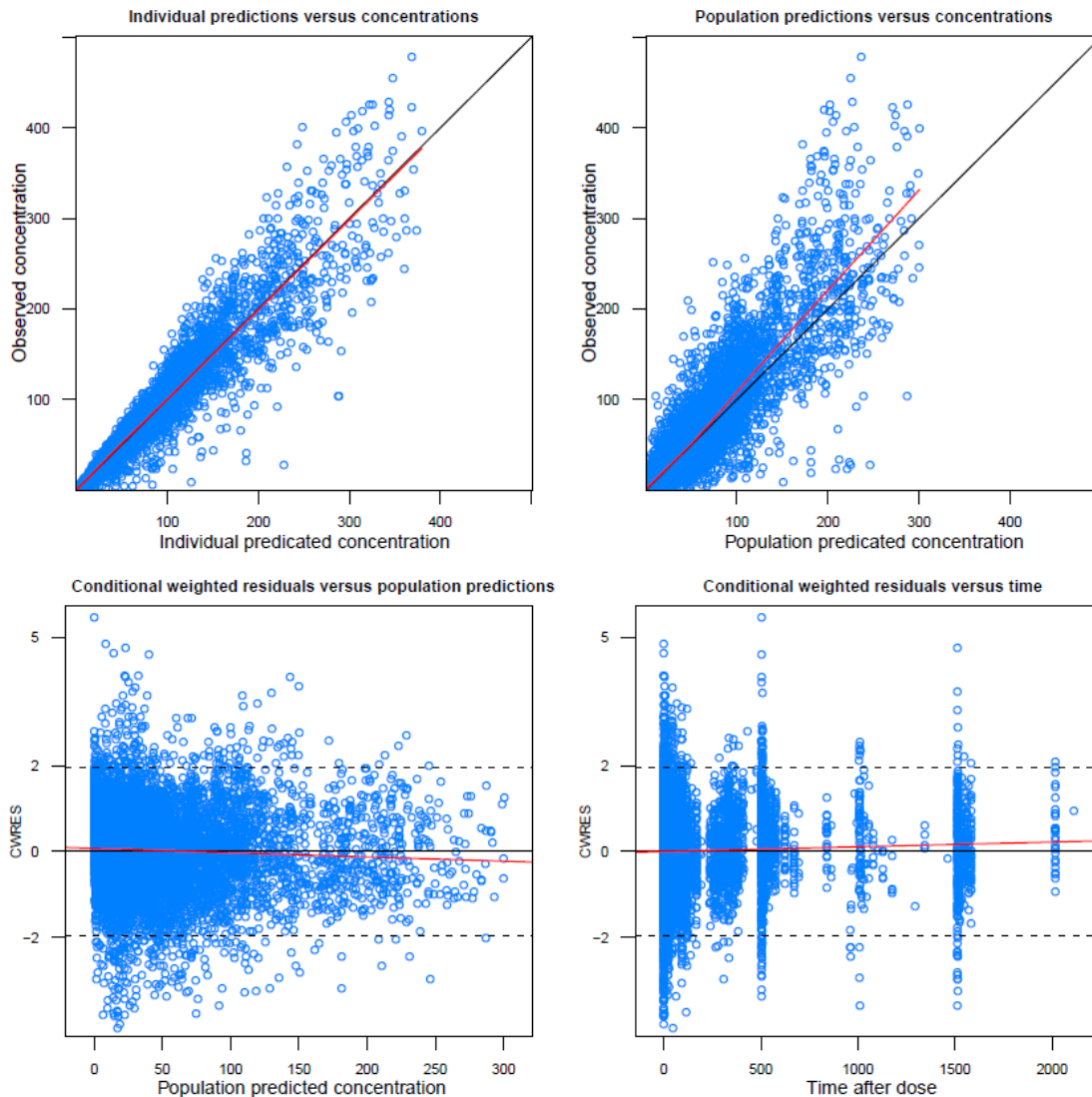$Vc/F = 333 * (body\ weight/64.8)^{1.76} * (1.36\ for\ Non\text{-}Asian)$

$Q/F = 6.27 * (body\ weight/64.8)^{0.473}$

$Vp/F = 212 * (body\ weight/64.8)^{0.642}$

$Ka = 1.10 * (0.613\ for\ female)$

Source: Applicant's population PK report, Table 5, Page 44

Figure 7. Goodness-of-fit plots for baloxavir. The blue open circles represent observed data, red solid line is the LOESS line, and solid black line is the line of identity

Source: FDA analysis

The effect of body weight on CL/F, Vc/F, Q/F, and Vp/F was assessed over a relevant range of body weights (36.0 to 131.0 kg). The results showed the values of CL/F, Vc/F, Q/F, and Vp/F for patients with a weight range of 36.0 to 131.0 kg, were estimated as 0.543 to 2.08, 0.355 to 3.45, 0.757 to 1.40, and 0.686 to 1.57-fold compared to those for patients with a body weight of 64.8 kg, respectively. Figure 8 shows the comparison of C24 and CL/F in subjects with different body weight groups. The effect of ALT on CL/F was assessed over a relevant range of ALT (6 to 320 U/L). The results indicated the effect of ALT on CL/F was estimated as 1.13 to 0.714-fold compared to that in patients with ALT of 17 U/L.

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(1) Correlation of C$_{24}$ with Body Weight
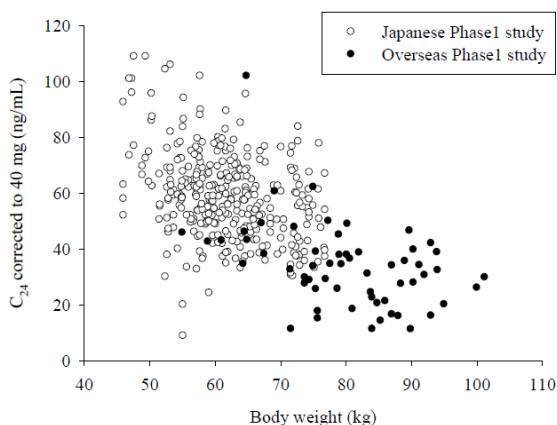
(2) Correlation of CL/F with Body Weight
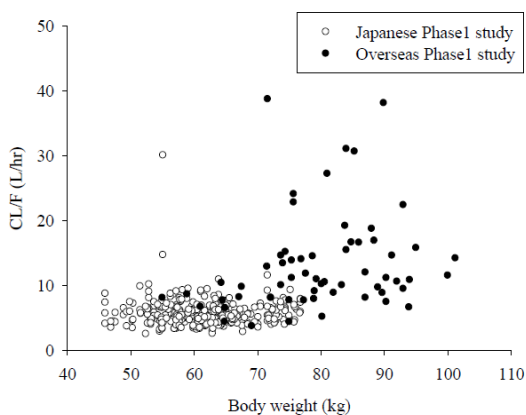
Figure 8. Correlations of C24 and CL/F with body weight in healthy subjects

Source: Applicant's Summary of Clinical Pharmacology, Figure 2.7.2-29, page 77.

The effect of taking food <2 hours before or <2 hours after dosing resulted in a 13.1% decrease in the exposure of baloxavir in plasma compared to taking food ≥2 hours before or ≥2 hours after dosing (including taking food 2-4 hours before or 2-4 hours after dosing and taking no food). Non-Asians had a 72% and 36% higher CL/F and Vc/F, respectively, than Asians. Furthermore, females had a 38.7% lower Ka compared with males.

Applicant's population PK analysis conclusions

- 2-compartment model with first-order absorption and absorption lag time adequately described the population PK of baloxavir
- The exposures decrease as body weight increases
- The exposures for Non-Asians are lower than those for Asians

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- The exposures are similar between adults and adolescents

Reviewer's comment: The applicant's population PK analysis reasonably described the PK of baloxavir as shown in goodness-of-fit plots. The final population PK model is reproducible, and this FDA reviewer agrees with the identified covariates, which supports the applicant's labeling claims of body weight based-dosing (40 mg for patients weighing 40 kg to less than 80 kg; 80 mg for patients weighing greater than or equal to 80 kg). No dose adjustment is necessary based on gender, based on race, and in adolescents. The reviewer agrees with the applicant that Non-Asians had lower exposure as shown in Table 25.

Table 25. Summary of PK parameters for all Phase 3 subjects following 40 mg for < 80 kg or 80 mg for ≥ 80 kg. Data are expressed as geometric mean (%CV)				
Region	AUC24h (ng·hr/mL) (%CV)	AUCinf (ng·hr/mL) (%CV)	C24 (mg/mL) (%CV)	Cmax (ng/mL) (%CV)
All patients	1260 (55.2)	5130 (50.9)	49.5 (50.1)	75.4 (58.6)
Asians	1425 (50.8)	6042 (40.3)	56.5 (41.4)	87.8 (50.8)
Non-Asians	1034 (54.6)	3960 (53.0)	39.9 (54.2)	59.0 (59.8)

Source: FDA analysis

It is worth noting that based on population PK model, the food effect was estimated to result in 13% lower exposure in patients under fed condition in comparison to patients under fasted or intermediate fed conditions. The Applicant pooled the patients under fasted or intermediate fed conditions together. An independent analysis was conducted by the Reviewer, and the result showed that food effect was estimated to result in 17% lower exposure in patients under fed condition in comparison to patients under fasted condition. The estimated food effect was lower than that observed in the dedicated food effect study.

4.4 Exposure-Response Analyses

The exposure-response analyses for efficacy were conducted using the data obtained from the Phase 2 study in adult patients and the Phase 3 study in both adult and adolescent patients. The efficacy endpoints were TTAS (primary endpoint) and change in virus titer (secondary). The applicant tested a linear model and the Emax model. The C24 (the observed concentrations at 20 to 28 hours postdose) of baloxavir was used as an exposure index in exposure-response analyses; Cmax and AUC were generally correlated with C24 thus no additional analyses were conducted using Cmax or AUC.

The applicant included placebo data in the exposure-response analyses. Inclusion of placebo data is likely to confound the analysis that a non-existence trend can be falsely obtained. The FDA reviewer conducted an exposure-response analysis after the placebo data were excluded.

The TTAS was evaluated using a linear model for both Type A and Type B. As shown in Figure 9, no clear exposure-response relationships were identified for both Type A and Type B virus.
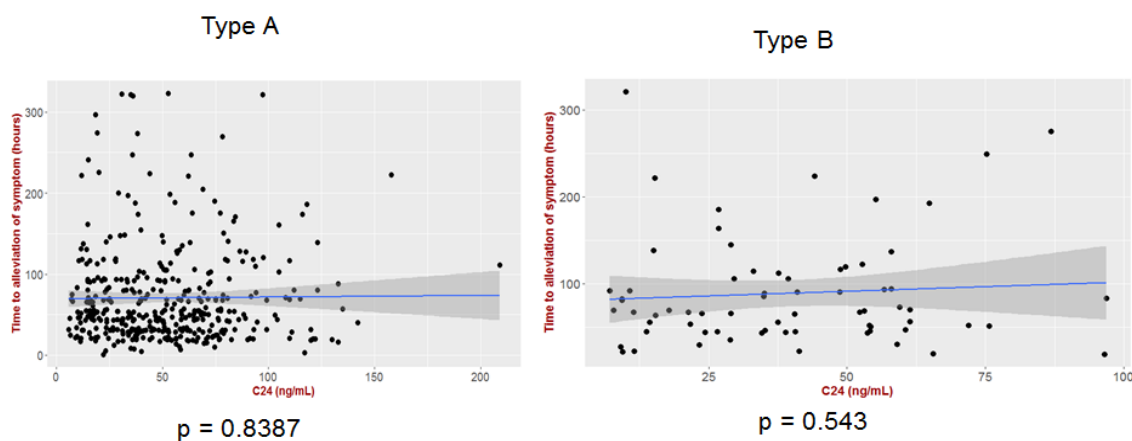
Figure 9. Correlation between C24 and Time to Alleviation of Symptoms by viral type
Source: FDA analysis

The correlation between the baloxavir C24 and changes in virus titer (Day 2) was analyzed using a linear model after excluding placebo data. Changes in virus titer were analyzed separately by virus subtypes (type A and type B). No clear exposure-response relationships were identified for both Type A and Type B virus. The correlations between changes in virus titer on Day 2 (24 hours post-dosing) and C24 are shown in Figure 10.

Reference ID: 4323329
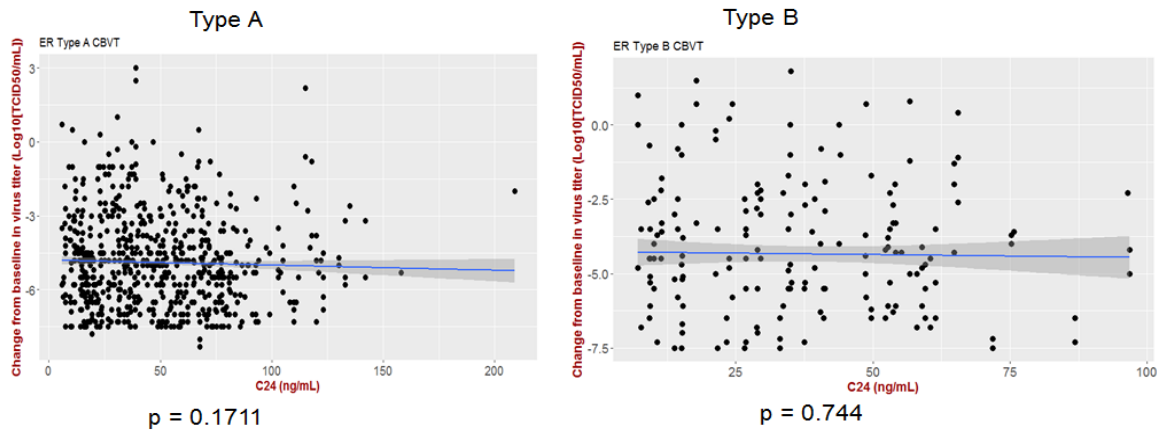
Figure 10. Correlation between C24 and Changes from Baseline in Virus Titer on Day 2
Source: FDA analysis

The FDA reviewer conduced further exposure-response analyses for the primary endpoint stratified by virus type and ethnicity as shown in Figure 11 (Type A) and Figure 12 (Type B). No clear exposure-response relationships were identified. However, the interpretation is limited due to low sample size.
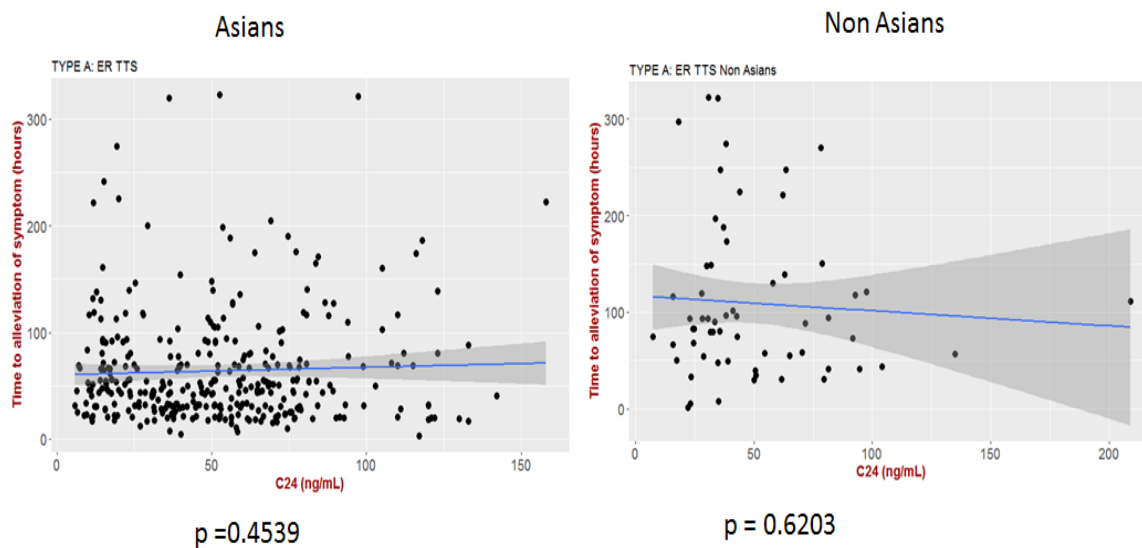
Figure 11. Correlation between C24 and Time to Alleviation of Symptoms for Type A stratified by ethnicity
Source: FDA analysis
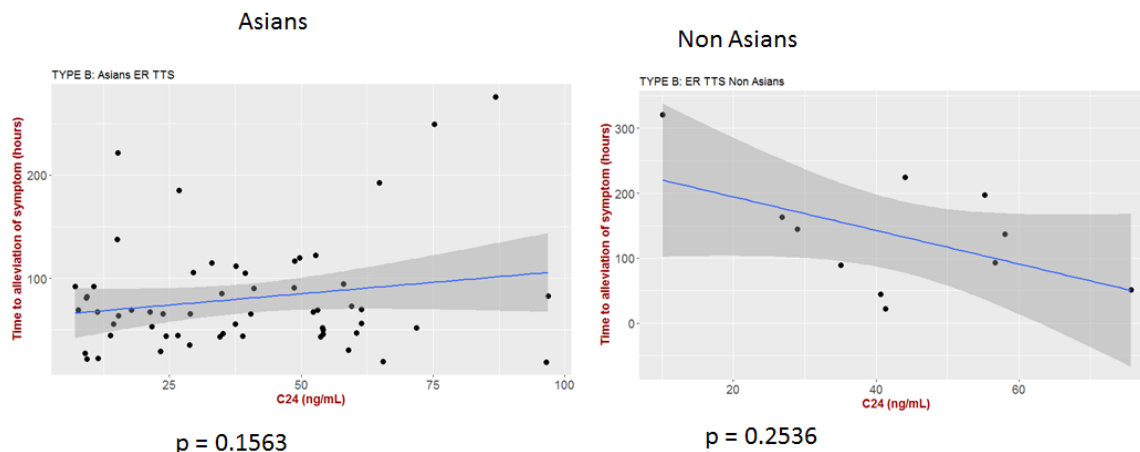
Figure 12. Correlation between C24 and Time to Alleviation of Symptoms for Type B stratified by ethnicity
Source: FDA analysis

Reviewer's comments: The FDA reviewer concluded that there was no clear exposure-response relationship for the primary endpoint, which is TTAS, and the secondary endpoint, which is changes in baseline virus titer on day 2. Similar results were seen in both the Asian population and the Non-Asian population for Type A virus. For Type B, there was trend than Non-Asian population has less time to alleviation as the C24 increases. The sample size for non-Asians infected with Type B virus was very small and the results were not statistically significant.

Exposure-resistance analysis

The dataset used for exposure-resistance analysis was obtained from the FDA Virology Review Team. The cut-off of 1.5 $\log_{10}$ virus titer was used for treatment-emergent resistance (TER). The TER status of a subject is defined as having a treatment-emergent substitution at any time post baseline. Treatment-emergent substitutions were typically observed between days 3 and 6 post treatment initiation, often coincident with virus rebound. For exposure-resistance analysis, logistic regression models were used to evaluate the relationship between C24 and TER for virus Type A and Type B (Figure 13). The results showed there was a trend of a positive relationship for Type A. These results suggested that low exposures may not increase the likelihood of resistance, and increased concentration (C24) may not reduce incidence of resistance within the studied exposure range. No clear exposure-resistant relationship was identified for Type B.

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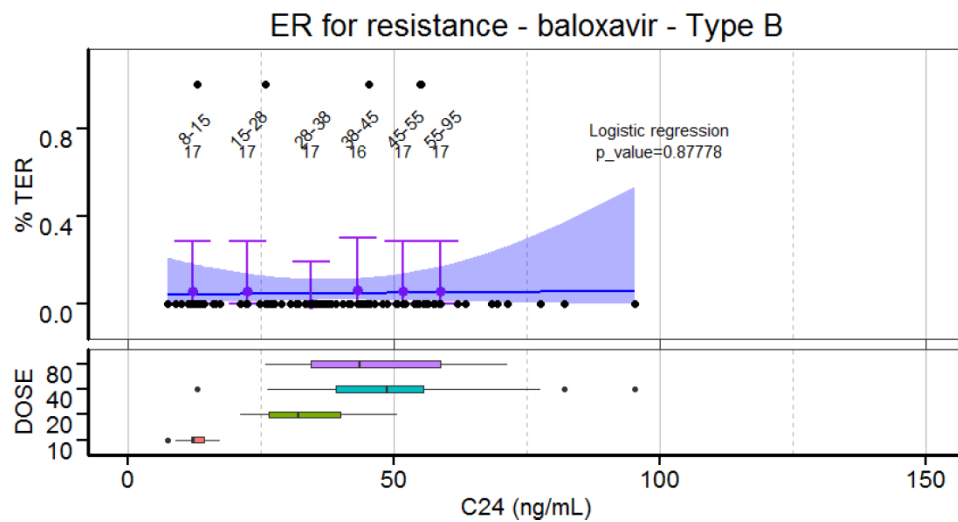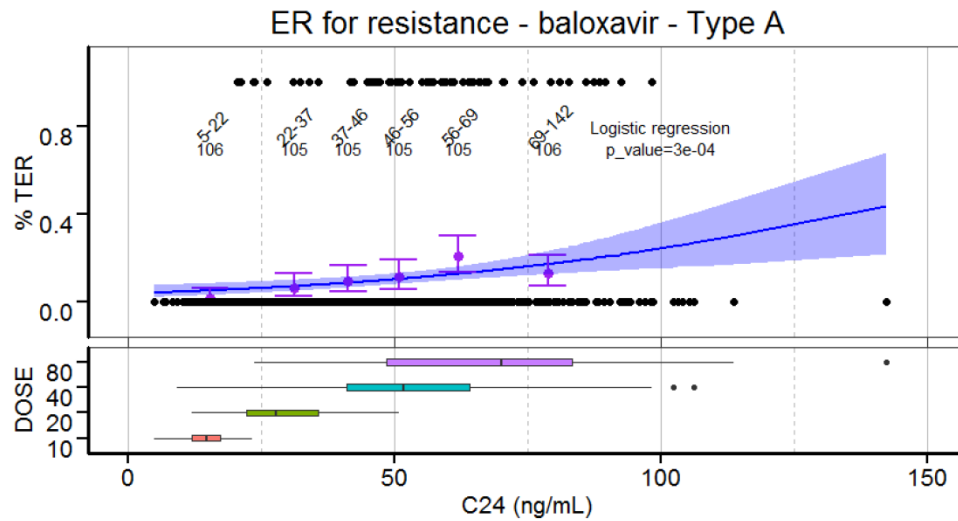
Figure 13. Relationship between C24 and TER for virus Type A and Type B.
Source: FDA analysis

Reviewer's comments: The results indicated that increasing the dose may not reduce incidences of resistance for Type A, while no exposure-resistant relationship was identified for Type B virus.

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4.5 Individual Clinical Pharmacology Report Reviews

Food Effect Evaluation for S-033188 10-mg and 20-mg Tablets

Title (Study #1622T081F)*

A Phase 1 Study to Evaluate the Bioequivalence of S-033188 10-mg and 20-mg Tablets and Effect of Food on the Pharmacokinetics in Healthy Adults

*Note: only the food effect portion of the study is reviewed below

Information Regarding the Clinical Trial Site and Duration of the Trial

The study was conducted at Medical Corporation Heishinkai OPHAC Hospital (Osaka, Japan) between January 18, 2017 and July 11, 2017.

Critical Trial Information

This study was a randomized, 2-sequence 2-period crossover study that consisted of a bioequivalence (BE) part to assess the bioequivalence between 2 x 10-mg tablets and 1 x 20-mg tablet, and a food effect (FE) part to assess the effect of food on the 10-mg and 20-mg tablets. A total of 28 subjects were enrolled in the food effect evaluation portion of the study. See tables below for the dosing sequences of the FE portion of the study. For administration in the fasted state, subjects fasted at least 10 hours after the previous meal prior to dosing. For administration in the fed state, subjects initiated breakfast 30 minutes prior to dosing. All inclusion and exclusion criteria, including prohibited concomitant medications appeared standard for a healthy volunteer study and are acceptable.

Group	Number of subjects	Dosing sequence	
		Period 1	Period 2
FE-1	7	Two S-033188 20-mg tablets Fasted [Treatment C]	Two S-033188 20-mg tablets Fed [Treatment D]
FE-2	7	Two S-033188 20-mg tablets Fed [Treatment D]	Two S-033188 20-mg tablets Fasted [Treatment C]

Group	Number of subjects	Dosing sequence	
		Period 1	Period 2
FE-3	7	One S-033188 10-mg tablet Fasted [Treatment E]	One S-033188 10-mg tablet Fed [Treatment F]
FE-4	7	One S-033188 10-mg tablet Fed [Treatment F]	One S-033188 10-mg tablet Fasted [Treatment E]

Washout period between periods: 21 days

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<u>Type of meal for fed groups</u>: the exact contents of the meal were not provided; however, the total caloric content of the breakfast was approximately 400 to 500 kcal; fat accounting for approximately 150 kcal of the total.

Sample Collection and Bioanalysis

Sample Collection

Plasma samples were collected at the following time points for analysis of S-033188 and S-033447 (active, measurable form): predose, and 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 72, 120, 168, 336 and 504 hours postdose in each period.

Bioanalytical method

Acceptable precision and accuracy for all standard curve and QC runs. All samples were analyzed within the long-term storage stability duration of 385 days at a storage temperature range of -20 to -80° C. Incurred sample reanalysis (ISR) was also performed for this study and the results were within acceptable limits.

Results

- No major protocol deviations were reported. All 28 enrolled subjects completed the study.
- The presence of food decreased the C_{max} and AUC of S-033447 either when S-033188 was administered as 2 x 20-mg tablets or as 1 x 10-mg tablet; however, the effect was greater when 2 x 20-mg tablets were administered (36-48% lower) as compared with 1 x 10-mg tablet (18-31% lower).

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Statistical Analyses of S-033447 Following Single Dose Administration of Two S-033188 20-mg Tablets in Fasted and Fed States

Parameter	Plasma S-033447		
	Geometric Least Squares Mean [a]		Geometric Least Squares Mean Ratio [a] (90% CI: lower, upper)
	Fasted (N = 14)	Fed (N = 14)	Fed / Fasted
C_{max} (ng/mL)	130	67.6	0.52 (0.45, 0.61)
AUC_{0-last} (ng·hr/mL)	6932	4406	0.64 (0.57, 0.71)
AUC_{0-inf} (ng·hr/mL)	7086	4540	0.64 (0.57, 0.72)
$t_{1/2,z}$ (hr)	93.9	97.5	1.04 (1.00, 1.08)
MRT (hr)	106	119	1.13 (1.09, 1.17)

a   The analysis was based on the linear mixed effects model: ln (Parameter) = Treatment + Group + Period + Subject + Random error, where treatment, group, and period were fixed effects, and subject was a random effect. Results were exponentiated to present geometric least squares mean and ratio.

Source: p.68 of Clinical Study Report

Statistical Analyses of S-033447 Following Single Dose Administration of One S-033188 10-mg Tablet in Fasted and Fed States

Parameter	Plasma S-033447		
	Geometric Least Squares Mean [a]		Geometric Least Squares Mean Ratio [a] (90% CI: lower, upper)
	Fasted (10-mg tablet) (N = 14)	Fed (10-mg tablet) (N = 14)	Fed (10-mg tablet) / Fasted (10-mg tablet)
C_{max} (ng/mL)	15.2	12.5	0.82 (0.75, 0.90)
AUC_{0-last} (ng·hr/mL)	1653	1143	0.69 (0.56, 0.86)
AUC_{0-inf} (ng·hr/mL)	1726	1206	0.70 (0.57, 0.86)
$t_{1/2,z}$ (hr)	108	112	1.04 (0.98, 1.10)
MRT (hr)	150	157	1.05 (0.99, 1.10)

a   The analysis was based on the linear mixed effects model: ln (Parameter) = Treatment + Group + Period + Subject + Random error, where treatment, group, and period were fixed effects, and subject was a random effect. Results were exponentiated to present geometric least squares mean and ratio.

Source: p.69 of Clinical Study Report

Conclusions

- Food lowers the bioavailability of the active form, S-033447, when S-033188 is given either as a 10-mg tablet or as 20-mg tablets.

- The effect is less pronounced with the 10-mg tablet as compared with the 20-mg tablet.

- Data for the 20-mg tablet in this study is consistent with the results from study 1510T0813, as well as data from study 1510T0811 using the suspension formulation.

- Of note, when the 20-mg tablet was used in phase 3 trials, it was administered without regard to food.

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Relative BA and Food Effect Evaluation for S-033188 20-mg Tablets

Title (Study # 1510T0813)

A phase 1 study to evaluate the relative bioavailability of S-033188 20-mg tablets and suspension and the effect of food on the pharmacokinetics in healthy adult subjects

Information Regarding the Clinical Trial Site and Duration of the Trial

The study was conducted at Medical Corporation Heishinkai OPHAC Hospital (Osaka, Japan) between August 11, 2015 and November 9, 2015.

Critical Trial Information

This was a randomized, two-sequence two-period or three-sequence three-period crossover study. The study consisted of the following 2 parts: the BA part to compare the PK of S-033188 tablets versus suspension and the FE part to assess the effects of food on the PK of S-033188 tablets. A total of 29 healthy subjects (14 for the BA part and 15 for the FE part) were enrolled. The 20-mg tablet formulation of S-033188 was used in both parts of the study. For the relative BA portion of the study, all doses were administered in the fasted state. For the FE portion of the study, the 3 separate prandial conditions are described underneath the table below. All inclusion and exclusion criteria, including prohibited concomitant medications appeared standard for a healthy volunteer study and are acceptable.

Dosing scheme for FE part of study

Dosing sequence group	Period 1	Period 2	Period 3	Number of subjects
FE-1	Fasted state	Fed state	Before meal	5
FE-2	Fed state	Before meal	Fasted state	5
FE-3	Before meal	Fasted state	Fed state	5

Administration in the fasted state:	No food was allowed for at least 10 hours pre-dose and at least 4 hours post-dose.
Administration in the fed state:	Subjects ingested a meal 30 minutes before the initiation of administration, and no food was allowed for at least 4 hours post-dose.
Administration before meal:	No food was allowed for at least 10 hours pre-dose, and subjects ingested a meal 1 hour post-dose.

Washout period between periods: 21 days

Type of meal for fed groups: the exact contents of the meal were not provided; however, the total caloric content of the breakfast was approximately 400 to 500 kcal; fat accounting for approximately 150 kcal of the total (the sponsor refers to this as a "moderate meal").

Sample Collection and Bioanalysis

Sample Collection

Plasma samples were collected for analysis of S-033188 and S-033447 (active, measurable form) for up to 504 hours postdose for both study parts.

Bioanalytical method

Acceptable precision and accuracy for all standard curve and QC runs. All samples were analyzed within the long-term storage stability duration of 94 days at a storage temperature range of -20 to -80° C.

Results

- No major protocol deviations were reported. Of the 29 subjects who enrolled, 25 completed the study. All dropped subjects were withdrawn by the investigator due to TEAE's (common cold, tonsillitis, mononucleosis, and mild rash).

- The 20-mg tablet and suspension formulation (containing 20 mg S-033188) exhibited very similar bioavailability. The 90% CI of the GMR for all exposures parameters (C_{max}, AUC_t, and AUC_{inf}) are contained within the "no effect" bounds of 80-125%.

- When food is ingested approximately 30 mins (or less) prior to single dose administration of a 20-mg tablet (labeled as "Fed" in table below), C_{max} and AUC were 37-47% lower as compared to fasting. Similarly, when food is ingested 1 hour <u>after</u> drug administration (labeled as "Before meal" in table below), C_{max} and AUC were 40-48% lower as compared to fasting.

Statistical Analysis for the Effect of Food on the PK Parameters of Plasma S-033447 after a Single Oral Dose of S-033188 20-mg Tablet in the Fasted State, Fed State, and Before Meal

	Plasma S-033447				
	Geometric Least Squares Mean [a]			Geometric Least Squares Mean Ratio [a] (90% CI: lower, upper)	
Parameter	Fasted (N = 15)	Fed (N = 12)	Before meal (N = 12)	Fed / Fasted	Before meal / Fasted
C_{max} (ng/mL)	49.1	26.1	25.7	0.5311 (0.4321, 0.6527)	0.5245 (0.4267, 0.6446)
AUC_{0-last} (ng·hr/mL)	3697	2319	2231	0.6273 (0.5596, 0.7033)	0.6034 (0.5382, 0.6764)
AUC_{0-inf} (ng·hr/mL)	3867	2452	2345	0.6341 (0.5671, 0.7090)	0.6066 (0.5425, 0.6783)

Abbreviation: CI, confidence interval.

a   The analysis was based on the linear mixed effects model: ln (Parameter) = Treatment + Group + Period + Subject + Random error, where treatment, group, and period were fixed effects and subject was a random effect. Results were exponentiated to present geometric least squares mean and ratio.

Conclusions

- The tablet formulation (20-mg strength) has comparable bioavailability to the suspension formulation.

- Food lowers the bioavailability of the active form, S-033447, either when a meal is begun just prior to S-033188 (30 mins before) being administered as a single 20-mg tablet or the meal is eaten 1 hour after drug administration, as compared with fasting.

- Data for the 20-mg tablet in this study is consistent with the results from study 1622T081F as well as study 1510T0811 using the suspension formulation.

- Of note, when the 20-mg tablet was used in phase 3 trials, it was administered without regard to food.

SAD Evaluation for S-033188 (6 to 80 mg)

Title (Study # 1510T0811)

A phase 1, randomized, placebo-controlled, single- and multiple-dose* study of S-033188 in healthy adult subjects

*Note: After obtaining the results from the SAD portion of the study, the MAD portion was cancelled by the sponsor.

Information Regarding the Clinical Trial Site and Duration of the Trial

The study was conducted at Medical Corporation Heishinkai OPHAC Hospital (Osaka, Japan) between April 7, 2015 and June 16, 2015.

Critical Trial Information

This study was a first-in-human, randomized, double-blind, placebo-controlled study to assess the safety, tolerability and PK of single and multiple doses (as originally planned) of S-033188 in healthy adult subjects under fasting conditions. Of note, this study originally planned to evaluate doses up to 600 mg, but ended up not exceeding 80 mg. Subjects were randomized 6:2 (active:placebo) in one of 5 cohorts: A.) 6 mg, B.) 20 mg, C.) 40 mg, D.) 60 mg, and E.) 80 mg. A total of 40 subjects were enrolled. Cohort C included 3 periods: fasting, fed, and in-between meal (defined as any time between 2 hours after last meal and 30 mins before next meal). All inclusion and exclusion criteria, including prohibited concomitant medications appeared standard for a healthy volunteer study and are acceptable.

Formulation used in all cohorts: oral suspension

Washout period between periods: 14 days (between periods in Cohort C only)

Type of meal for fed groups: the exact contents of meals were not provided; however, for administration of study drug in the fed state, subjects were given a high-fat meal 30 minutes before the dose. For the between-meal treatment, subjects were given a light meal 2 hours before drug administration and a high-fat meal 30 minutes after drug administration.

Sample Collection and Bioanalysis

Sample Collection

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Plasma samples were collected for analysis of S-033188 and S-033447 (active, measurable form) for up to 336 hours postdose for all cohorts. Urine samples were collected prior to dosing (-12 to 0 hr) and 0-12 hrs, 12-24 hrs, 24-48 hrs, and 48-72 hrs post-dose for measurement of S-033447.

Bioanalytical method

Acceptable precision and accuracy for all standard curve and QC runs. All samples were analyzed within the long-term storage stability duration of 94 days at a storage temperature range of -20 to -80° C. Incurred sample reanalysis (ISR) was also performed for this study and the results were within acceptable limits.

Results

- No major protocol deviations were reported. Of the 40 subjects who enrolled, 37 completed the study. All dropped subjects were withdrawn by the investigator due to TEAE's (elevated liver function tests, sore throat, and increased white blood cell count).

- Plasma concentrations of S-033188 were below the quantification limit (BQL) (< 0.100 ng/mL) in all subjects in the 6 mg, 20 mg, and 40 mg (between meals) groups. S-033188 is eliminated from the plasma within 2 hours after (minimal) detection and concentrations were BQL after 12 hours post-dose in all subjects.

- Using power model analysis, the estimates of slopes (95% CI) for C_{max}, AUC_{0-72}, and AUC_{0-inf} of S-033447 were 1.25 (1.14 to 1.35), 1.09 (1.01 to 1.17), and 0.983 (0.873 to 1.09), respectively. These results suggest that S-033447 exposures (C_{max} and AUC) increase in a near dose-proportional manner in the dose range from 6 to 80 mg.

- No S-033188 was detected in any urine samples. Feu_{0-72}(%) ranged from 1.7 to 2.3 across the entire range of doses used in the study.

- After a single dose of S-033188 in the fed state, the C_{max}, AUC_{0-72}, AUC_{0-last}, and AUC_{0-inf} of S-033447 decreased by 67%, 49%, 42%, and 41%, respectively, compared with those in the fasted state. After single dose of S-033188 between meals, the C_{max}, AUC_{0-72}, AUC_{0-last}, and AUC_{0-inf} of S-033447 decreased by 57%, 44%, 36%, and 34%, respectively, compared with those in the fasted state.

Reviewer comment:

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Although the slope of AUC between 6 and 80 mg would generally indicate dose proportionality, when comparing exposures between 6 and 40 mg, the relationship appears to be greater than dose proportional.

Summary of Pharmacokinetic Parameters of Plasma S-033447 after Single Oral Dose of S-033188 in the Fasted State

Parameters	Plasma S-033447 Geometric Mean (CV% Geometric Mean)				
	6 mg	20 mg	40 mg	60 mg	80 mg
N	6	6	6	6	6
C_{max} (ng/mL)	11.0 (22.3)	40.2 (32.5)	123 (31.0)	193 (15.7)	253 (23.9)
T_{max}^{a} (hr)	2.00 (1.00, 2.50)	3.50 (1.50, 4.00)	3.50 (3.50, 5.00)	3.25 (2.50, 4.00)	3.50 (2.50, 4.00)
AUC_{0-24} (ng·hr/mL)	172.0 (23.5)	673.7 (24.9)	1710 (24.4)	2565 (12.6)	3425 (24.9)
AUC_{0-72} (ng·hr/mL)	417.4 (22.1)	1484 (21.5)	3475 (22.5)	5073 (11.8)	6795 (25.5)
AUC_{0-last}^{b} (ng·hr/mL)	417.4 (22.1)	1484 (21.5)	6285 (20.9)	8767 (15.7)	11490 (27.0)
AUC_{0-inf} (ng·hr/mL)	1018 (35.7)	2419 (24.8)	6669 (20.8)	9141 (17.5)	11970 (27.8)
$t_{1/2,z}$ (hr)	90.9 (55.7)	48.9 (30.1)	85.9 (8.2)	75.2 (15.3)	75.9 (11.1)
λ_z (1/hr)	0.0076 (55.7)	0.0142 (30.1)	0.0081 (8.2)	0.0092 (15.3)	0.0091 (11.1)
MRT (hr)	133 (53.7)	72.9 (28.4)	108 (9.2)	94.6 (18.0)	93.7 (13.5)
CL/F (L/hr)	4.99 (35.7)	6.99 (24.8)	5.07 (20.8)	5.55 (17.5)	5.65 (27.8)
V_z/F (L)	655 (33.0)	494 (28.4)	629 (22.3)	603 (10.3)	619 (23.3)
C_{24} (ng/mL)	6.92 (22.1)	24.4 (22.5)	57.6 (20.1)	84.4 (12.7)	112 (27.2)

a   Median (Min, Max)

b   Calculated based on the plasma concentration data from 0 to 72 hours post dose for 6 and 20 mg, and from 0 to 336 hours post dose for 40 to 80 mg.

Source: p.69 of Clinical Study Report Amendment 1

Conclusions

- Following single dose administration, exposures following doses between 6 and 40 mg appear to be greater than dose proportional, while doses between 40 and 80 mg are dose proportional.

- Food lowers the bioavailability of S-033447 whether a meal is ingested 30 mins before drug administration or drug is administered in between meals (between 2 hours after last meal and 30 mins before next meal). The results are consistent with two other subsequent food effect evaluations (Studies 1622T081F and 1510T0813) that assessed food effect on tablet formulations. The effect of food appears to be independent of the specific oral dosage form.

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Title (Study # 1532T0817)

- A phase 1 study to investigate the absorption, distribution, metabolism, and excretion of [^{14}C]-S-033188 following oral dose administration in healthy adult male subjects

Information Regarding the Clinical Trial Site and Duration of the Trial

- The study was conducted at Quotient Clinical, Mere Way, Ruddington Fields, Ruddington, Nottingham NG11 6JS, UK between August 30, 2016 and September 29, 2016

Objective

- The main objective of the study was to assess the absorption, distribution, metabolism, and excretion of S-033188 and its metabolite, S-033447, after administration of a single 40-mg dose of [^{14}C]-S-033188 as an oral suspension in the fasted state.

Critical Trial Information

- This was an open-label, single center, non-randomized, single dose study in 6 healthy adult male subjects

Formulation, Dose and Mode of Administration

S-033188: 40 mg [^{14}C]-S-033188 suspension for oral administration (20 mL) containing not more than 3.77 MBq (102 μCi) of ^{14}C administered under fasted condition.

Sample Collection and Bioanalysis

Sample Collection

Total Radioactivity and S-033447

- Plasma samples (n=18) were collected prior to S-033188 administration and up to 384 hours post dose.
- Urine samples (n=20) were collected prior to S-033188 administration and up to 432 hours post dose.
- Fecal samples (n=19) were collected prior to S-033188 administration and up to 432 hours post dose.

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Bioanalytical method

- S-033447 was quantified in plasma and urine samples using validated LC-MS/MS methods. Precision and accuracy values for all standard curves and QC runs were acceptable. All samples were analyzed within the long-term storage stability duration of 367 days and 45 days for plasma and urine samples, respectively. Incurred sample reanalysis of plasma and urine samples was performed and the results were within acceptable limits.

- Total radioactivity was quantified using liquid scintillation counting.

Results

- PK parameters of S-033188 were not estimated since it undergoes rapid conversion (i.e., within few hours post administration) to S-033447.

- The AUC_{0-72} values for S-033447 and total radioactivity were 2185 and 2574 ng.hr/mL, respectively.

- Other detected metabolites in plasma were S-033447 glucuronide and S-033447 sulfoxide (accounting for 16.4% and 1.5%, respectively, of the total radioactivity in plasma).

- In feces, S-033447 was primarily detected (accounting for 48.7% of administered radioactivity) while S-033188 was minimally detected, and 2 kinds of S-033447 sulfoxides and S-033447 pyrrole were detected.

- In urine, 8.9% of the administered radioactivity was detected as S-033447 glucuronide. S-033447 and 2 kinds of S-033447 sulfoxides were also detected.

- The fraction of dose excreted in urine of the active form, S-033447, was 3.3 administered radioactivity% f.

Table 1. Mean Cumulative Excretion of Total Radioactivity in Urine and Feces Following a Single 40-mg Dose of [^{14}C]-S-033188 as an Oral Suspension

Collection Interval (hr)	Urine CumFe (%) (N = 6)	Feces CumFe (%) (N = 6)	Urine and Feces CumFe (%) (N = 6)
0 to 12	2.847	-	-
0 to 24	4.528	0.870	5.400
0 to 48	7.015	11.637	18.652
0 to 72	8.793	26.678	35.475
0 to 96	10.022	40.832	50.853
0 to 120	11.092	51.715	62.807
0 to 144	11.855	58.960	70.813
0 to 168	12.450	64.855	77.310
0 to 192	12.918	67.258	80.177
0 to 216	13.302	70.460	83.763
0 to 240	13.620	72.013	85.632
0 to 264	13.860	73.988	87.852
0 to 288	14.085	75.392	89.475
0 to 312	14.258	76.745	91.002
0 to 336	14.403	77.593	91.997
0 to 360	14.522	78.633	93.155
0 to 384	14.620	79.180	93.800
0 to 408	14.708	79.862	94.570
0 to 432	14.708	80.125	94.835

Source: p.52 of Clinical Study Report

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Reviewer's comments:

- *The majority of total radioactivity (80%) was recovered in feces indicating fecal excretion was the main route of elimination. About 12 % of the total radioactivity was recovered in feces within 24 hours post dosing indicating that most of the administered dose was absorbed and reached the systemic circulation.*

- *About 15 % of the total radioactivity was recovered in the urine within 432 hours post dosing indicating minimal elimination of S-033447 through the renal route.*

- *Evaluating the exposures of S-033188 and S-033447 in hepatically impaired subjects is necessary since hepatic elimination is the major route of elimination of baloxavir.*

- *Evaluating the exposures of S-033188 and S-033447 in renally impaired subjects may not be necessary because:*

  - *Renal elimination is not a major route of elimination of baloxavir marboxil.*
  - *Baloxavir marboxil is intended for single-dose administration and there are no major clinical concerns with single dose administration.*

Conclusions

- Fecal excretion is the main route of elimination.
- $[^{14}C]$-S-033447 accounts for ~85% of the circulating total radioactivity (based on AUC_{0-72} values) and hence it is considered the major metabolite of $[^{14}C]$-S-033188.
- The study is acceptable.

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Title (Study # 1611T081B)

- A 2-Part, Open-label, Sequential Study to Assess the Pharmacokinetics and Safety of S-033188 in Patients with Mild or Moderate Hepatic Impairment and Healthy Matched Control Subjects

Information Regarding the Clinical Trial Site and Duration of the Trial

- The study was conducted at Clinical Pharmacology of Miami, Inc., 550 W. 84th Street, Hialeah, FL 33014 between October 11, 2016 and December 27, 2016

Rationale

- In vitro studies indicated that S-033188 is rapidly metabolized to its active form, S-033447, in the liver, serum and small intestine by arylacetamide deacetylase. S-033447 is predominantly metabolized via UGT1A3. It was anticipated that hepatic metabolism accounts for a substantial portion (> 20%) of the elimination of S-033447. As such, the objective of this study was to evaluate the PK of S-033188 and S-033447 in subjects with impaired hepatic function.

Critical Trial Information

- This was a 2-part sequential, open-label, single-dose study to evaluate the PK, safety and tolerability of S-033188 (and S-033447) in subjects with moderate or mild hepatic impairment.
  - Part 1: PK assessment in subjects with *moderate* hepatic impairment (n=8) and healthy subjects (n=8).
  - Part 2 (*optional, based on data from Part 1*): PK assessment in subjects with *mild* hepatic impairment (n=8) and healthy subjects (n=8).

Formulation, Dose and Mode of Administration

S-033188: Oral tablet, 40 mg single dose (2 × 20 mg tablet) administered under fasted condition.

Sample Collection and Bioanalysis

Sample Collection

S-033188 and S-033447

- Plasma samples (n=19) were collected prior to S-033188 administration and up to 504 hours post dose.

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Bioanalytical method

- S-033188 and S-033447 samples were analyzed using validated LC-MS/MS quantification methods. Precision and accuracy values for all standard curves and QC runs, for all quantification methods, were acceptable. All samples were analyzed within the long-term storage stability duration of 385 days. Incurred sample reanalysis was performed and the results were within acceptable limits.

Results

Table 1. PK Parameter Estimates of S-033447 After Administration of a Single 40-mg Dose of S-033188 in Subjects with Moderate Hepatic Impairment and Healthy Subjects with Normal Hepatic Function

Parameter	N	Geometric LS Mean[a]	Ratio Geometric LS Mean Ratio[a]	Ratio 90% CI[a,b]
C_{max} (ng/mL)				
Moderate Hepatic Impairment	8	76.7	0.7991	0.4980, 1.2824
Healthy Subjects	8	95.9		
AUC_{0-last} (ng·hr/mL)				
Moderate Hepatic Impairment	8	4596	1.1000	0.7650, 1.5819
Healthy Subjects	8	4178		
AUC_{0-inf} (ng·hr/mL)				
Moderate Hepatic Impairment	8	4739	1.1188	0.7780, 1.6088
Healthy Subjects	8	4236		

Source: p.8 of Clinical Study Report

- Part 2 was not conducted because no clinically meaningful effect was observed in Part 1 (Table 1).

Reviewer's comments:

- *The 90% confidence intervals of the geometric mean ratio values of C_{max}, AUC_{0-inf} and AUC_{0-last} of S-033447 were outside the pre-determined no effect limit, 80 -125% (Table 1). However, this is not considered to impose any clinically relevant effect based on safety profile of baloxavir and no clear exposure-response relationship for safety. In addition, at twice the exposure from recommended dosing, there was no new or concerning safety issue identified (refer to the QTc study review).*

Conclusions

- No dose adjustment is needed for subjects with moderate or mild hepatic impairment.
- The study is acceptable.

Title (Study # 1519T0814)

- A Study to Assess the Effects of S-033188 on the Pharmacokinetics of Midazolam in Healthy Adult Subjects

Information Regarding the Clinical Trial Site and Duration of the Trial

- The study was conducted at WCCT Global, LLC, 5630 Cerritos Avenue, Cypress, CA 90630 between March 01, 2016 and April 04, 2016.

Rational

- In vitro studies indicated that S-033188 and its metabolite, S-033447, inhibited CYP3A4. The objective of this study was to evaluate the effect of administration of a single 40-mg dose of S-033188 on the PK of a single 5 mg dose of midazolam, a CYP3A4 substrate.

Critical Trial Information

- This was an open-label, non-randomized, single-sequence, 2-period single-dose study conducted in 12 healthy adult subjects.
    - Period 1: midazolam alone (Day 1)
    - Period 2: midazolam and S-033188 (Day 3)

Formulation, Dose and Mode of Administration

Midazolam: Oral syrup (2 mg/ml), 5-mg single dose

S-033188: Oral tablet, 40 mg single dose (2 × 20 mg tablet)

Study drugs were administered under fasted condition.

Sample Collection and Bioanalysis

Sample Collection

Midazolam

- Plasma samples (n=12) were collected prior to dose administration and up to 24 hours post dose.

S-033188 and S-033447

- Plasma samples (n=15) were collected prior to S-033188 administration and up to 72 hours post dose. Note: S-033188 and S-33227 data re not presented in this review as they did not contribute to main conclusion.

Bioanalytical method

- Midazolam, S-033188 and S-033447 samples were analyzed using validated LC-MS/MS quantification methods. Precision and accuracy values for all standard curves and QC runs, for all quantification methods, were acceptable. All samples were analyzed within the long-term storage stability duration of 94 days (midazolam) and 192 days (S-033188 and S-033447). Incurred sample reanalysis was performed and the results were within acceptable limits.

Results

Table 1. PK Parameter Estimates of Midazolam After Administration of a Single 5-mg Dose of Midazolam Alone and After Co-Administration with a Single 40-mg Dose of S-033188

PK Parameter of Midazolam	Geometric LS Mean Midazolam Alone[a]	Geometric LS Mean Co-Administration (Midazolam + S-033188)[a]	Ratio of Geometric LS Mean ([Midazolam + S-033188]/Midazolam Alone) (90% CI)[a]
C_{max} (ng/mL)	23.8	23.8	1.0014 (0.9170, 1.0936)
AUC_{0-inf} (ng·hr/mL)	61.55	60.97	0.9906 (0.9423, 1.0414)
AUC_{0-last} (ng·hr/mL)	59.32	58.92	0.9933 (0.9444, 1.0448)

a   Geometric least squares means and confidence intervals obtained from a mixed model with a fixed effect for treatment and a random effect for subject.

Source: p.52 of Clinical Study Report

Reviewer's comments:

- *Exposure of S-033447, the active metabolite of S-033188, in non-Asian subjects enrolled in this study was lower (by > 50%) than that observed in Asian subjects enrolled in Study 1510T0811 who were administered the same dose. This suggests that race could be a significant covariate that affects the exposure of S-033447. Refer to population pharmacokinetic analysis for further information.*

Conclusions

- Midazolam exposure was not affected when an oral dose of 40 mg S-033188 was co-administered with 5 mg oral dose of midazolam in healthy subjects.
- The study is acceptable.

Title (Study # 1520T0815)

- A Study to Assess the Effect of Itraconazole on the Pharmacokinetics of S-033188 in Healthy Adult Subjects

Information Regarding the Clinical Trial Site and Duration of the Trial

- The study was conducted at Clinical Pharmacology of Miami, Miami, FL 33014 between May 04, 2016 and June 15, 2016.

Rational

- In vitro studies indicated that S-033188 and its metabolite, S-033447, are P-glycoprotein (P-gp) substrates. The objective of this study was to evaluate the effect of multiple administration of 200 mg itraconazole (potent P-gp inhibitor) for 19 days on the PK of S-033188 and S-033447.

Critical Trial Information

- This was an open-label, non-randomized, 2-period crossover study conducted in 12 subjects
  - Period 1: S-033188 alone (Day 1)
  - Period 2: Itraconazole (Days 1-19) and S-033188 (Day 5)

Formulation, Dose and Mode of Administration

Itraconazole: Oral tablet, 200-mg tablet dose given b.i.d. on Day 1 and q.d. on Days 2 - 19

S-033188: Oral tablet, 20 mg single dose (1 × 20 mg tablet)

Itraconazole and S-033188 doses were administered under fed and fasted conditions, respectively. However, on Day 5 of Period 2, both drugs were administered under fasted condition.

Sample Collection and Bioanalysis

Sample Collection

Itraconazole

- Plasma samples (n=11) were collected on Day 5 prior to itraconazole administration and up to 24 hours post dose. *Note: Itraconazole PK data are not presented in this review as they did not contribute to the conclusion.*

S-033188 and S-033447

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- Plasma samples (n=18) were collected for quantification of S-033188 and S-033447 prior to S-033188 administration and up to 336 hours post dose.

Bioanalytical method

- Itraconazole, S-033188 and S-033447 samples were analyzed using validated LC-MS/MS quantification methods. Precision and accuracy values for all standard curves and QC runs, for all quantification methods, were acceptable. All samples were analyzed within the long-term storage stability duration of 97 days (itraconazole) and 385 days (S-033188 and S-033447). Incurred sample reanalysis was performed and the results were within acceptable limits.

Results

- PK parameters of S-033188 were not estimated since plasma concentrations were below the quantification limit (< 0.100 ng/mL) at most time points. *Note: S-033188* undergoe*s* rapid conversion (i.e., within few hours post administration) to S-033447.

Table 1. PK Parameter Estimates of S-033447 After Administration of a Single 20 mg Dose of S-033188 Alone and After Co-administration with Itraconazole on Day 5 of a 19-day Regimen.

Parameter	N	Geometric LSMean[a]	Ratio GLSMR[a] (S-033188+ Itraconazole)/ (S-033188 Alone)	90% CI[a]
C_{max} **(ng/mL)**				
Co-administration (S-033188 with Itraconazole)	12	40.3	1.3271	1.1373, 1.5485
S-033188 Alone	12	30.3		
$AUC_{0\text{-}last}$ **(ng·hr/mL)**				
Co-administration (S-033188 with Itraconazole)	12	2723	1.2219	1.0793, 1.3833
S-033188 Alone	12	2228		
$AUC_{0\text{-}inf}$ **(ng·hr/mL)**				
Co-administration (S-033188 with Itraconazole)	12	2859	1.2251	1.0873, 1.3804
S-033188 Alone	12	2333		

a From an ANOVA model for the log transformed parameter results with fixed effect of treatment and random effect of subject.

Source: p.56 of Clinical Study Report

Reviewer's comments:

- *S-033188 and S-033447 are substrates of P-gp and CYP3A which could both be inhibited by itraconazole. In vitro data suggest that CYP3A involvement in metabolism of S-033188 and S-033447*

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is minimal. Therefore, the study results are attributed mainly to the inhibitory effect of itraconazole on P-gp.

- *Itraconazole dose (200 mg) used in this study is a clinically relevant dose and is acceptable.*

- *The 90% confidence intervals of the geometric mean ratio values of C_{max}, AUC_{0-inf} and AUC_{0-last} of S-033447 were outside the pre-determined no effect limit of 80 -125% (Table 1). However, this is not considered to impose any clinically relevant. However, this is not considered to impose any clinically relevant effect based on the safety profile of baloxavir and no clear exposure-response relationship for safety. In addition, at twice the exposure from recommended dosing, there was no new or concerning safety issue identified (refer to the QTc study review).*

- *The dose used in this study (S-033188 20 mg) is lower than the proposed doses. This is deemed acceptable since this dose is within the dose proportional range (6-80 mg). Dose proportionality was assessed in Study 1510T0811.*

Conclusions

- Itraconazole increased the exposure of S-033447. However, this increase is minimal and is not expected to impose any clinically relevant effect.

- The study is acceptable.

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Title (Study # 1612T081C)

- A Study to Assess the Effect of Probenecid on the Pharmacokinetics of S-033188 in Healthy Adult Subjects

Information Regarding the Clinical Trial Site and Duration of the Trial

- The study was conducted at Clinical Pharmacology of Miami, Inc., 550 W. 84th Street, Hialeah, FL 33014 between Sep 28, 2016 and Dec 12, 2016.

Rationale

- In vitro studies indicated that S-033447 (active major metabolite of S-033188) is metabolized by UGT1A3. The objective of this study was to evaluate the effect of probenecid, UGT inhibitor, on the PK of S-033188 and S-033447

Critical Trial Information

- This was an open-label, non-randomized, 2-period crossover study conducted in 12 healthy adult subjects.
  - Period 1: S-033188 alone (Day 1)
  - Period 2: Probenecid (Days 1-18) and S-033188 (Day 4)

Formulation, Dose and Mode of Administration

Probenecid: 500-mg oral tablet was administered b.i.d. on Days 1 – 17 and once on Day 18

S-033188: Oral tablet, 80 mg single dose (4 × 20 mg tablet)

Probenecid doses were administered without regard to food except on study Day 4 when it was co-administered with S-033188 under fasted condition. S-033188 dose was administered under fasted condition.

Sample Collection and Bioanalysis

Sample Collection

Probenecid

- Plasma samples (n=10) were collected prior to probenecid administration and up to 12 hours post dose on study Day 4 of treatment period 2. *Note: collected data did not contribute to the conclusion.*

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S-033188 and S-033447

- Plasma samples (n=18) were collected for quantification of S-033188 and S-033447 prior to S-033188 administration and up to 336 hours post dose.

Bioanalytical method

- Probenecid, S-033188 and S-033447 samples were analyzed using validated LC-MS/MS quantification methods. Precision and accuracy values for all standard curves and QC runs, for all quantification methods, were acceptable. All samples were analyzed within the long-term storage stability duration of 96 days (probenecid) and 385 days (S-033188 and S-033447) at a storage temperature range of -20 to -80° C. Incurred sample reanalysis was performed and the results were within acceptable limits.

Results

- PK parameters of S-033188 were not estimated since plasma concentrations were below the quantification limit (BQL) (< 0.100 ng/mL) with few exceptions. Note: S-033188 undergoes rapid conversion (i.e., within few hours post administration) to S-033447

Table 1. PK Parameter Estimates of S-033447 After Administration of a Single 80 mg Dose of S-033188 Alone and After Co-administration with Probenecid on Day 4 of an 18-day Regimen.

Parameter	N	Geometric LS Mean[a]	Ratio Geometric LS Mean Ratio[a]	Ratio 90% CI[a,b]
C_{max} (ng/mL)				
Co-Administration (S-033188 + Probenecid)	12	114	0.7882	0.6453, 0.9627
S-033188 Alone	12	145		
AUC_{0-last} (ng·hr/mL)				
Co-Administration (S-033188 + Probenecid)	12	4802	0.7616	0.6651, 0.8722
S-033188 Alone	12	6305		
AUC_{0-inf} (ng·hr/mL)				
Co-Administration (S-033188 + Probenecid)	12	4906	0.7489	0.6555, 0.8556
S-033188 Alone	12	6551		

a: From an ANOVA model for the log transformed parameter results with fixed effect treatment and random effect subject.
b: In case of unbalanced data, the Kenward-Roger method was used to compute the denominator degrees of freedom used to obtain the critical value for calculation of the confidence interval.

Source: p.6 of Clinical Study Report

Reviewer's comments:

- *Co-administration of S-033188 with probenecid was expected to increase, rather than decrease, S-033447 (UGT1A3 substrate) exposure due to the inhibitory effect of probenecid on UGT1A3.*

- *The observed reduction of S-033447 exposure in the presence of probenecid (Table 1) might be due to inhibitory effect of probenecid on arylacetamide deacetylase, the enzyme responsible for the conversion of S-033188 to S-033447. Note: there was ~ 2.3 fold increase in S-033188 exposure when it was co-administered with probenecid as compared to when it was administered alone (source: Table 14.2.1.1 and Fig 14.2.1.2, clinical study report).*

- *The 90% confidence intervals of the geometric mean ratio values of C_{max}, AUC_{0-inf} and AUC_{0-last} of S-033447 were outside the pre-determined no effect limit, 80 -125% (Table 1) however, this is not considered to impose any clinically relevant effect based on presence of no clear exposure-response relationship for efficacy (refer to Section 4.4).*

Conclusions

- Probenecid decreased the exposure of S-033447. However, this decrease is minimal and is not expected to impose any clinically relevant effect.

- The study is acceptable.

Title (Study # 1613T081D)

- A Study to Assess the Effects of S-033188 on the Pharmacokinetics of Digoxin and Rosuvastatin in Healthy Adult Subjects

Information Regarding the Clinical Trial Site and Duration of the Trial

- The study was conducted at Clinical Pharmacology of Miami, Inc., Hialeah, FL 33014 between Oct 12, 2016 and Nov 18, 2016.

Rationale

- In vitro studies indicated that S-033188 inhibited P-gp while its metabolite, S-033447, inhibited both P-g and BCRP. The objective of this study was to evaluate the effect of administration of a single 80-mg dose of S-033188 on the PK of digoxin (P-gp substrate) and rosuvastatin (BCRP substrate).

Critical Trial Information

- This was a 2-part study; each part consists of an open-label, non-randomized, 2-period crossover design conducted in 24 subjects.
  - Part 1
    - Period 1: a single dose of digoxin (Day 1)
    - Period 2: co-administration of a single dose of digoxin and a single dose of S-033188 (Day 1)
  - Part 2
    - Period 1: a single dose of rosuvastatin (Day 1)
    - Period 2: co-administration of a single dose of rosuvastatin and a single dose of S-033188 (Day 1)

Formulation, Dose and Mode of Administration

Digoxin: Oral tablet, 0.25 mg single dose (1 × 0.25 mg tablet)

Rosuvastatin: Oral tablet, 10 mg single dose (1 × 10 mg tablet)

S-033188: Oral tablet, 80 mg single dose (4 × 20 mg tablet)

Study drugs were administered under fasted condition.

Sample Collection and Bioanalysis

Sample Collection

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Digoxin

- Plasma samples (n=17) were collected prior to digoxin administration and up to 168 hours post dose.

Rosuvastatin

- Plasma samples (n=14) were collected prior to rosuvastatin administration and up to 72 hours post dose.

S-033188 and S-033447

- Plasma samples (n=14) were collected for quantification of S-033188 and S-033447 prior to S-033188 administration and up to 72 hours post dose. *Note: S-033188 and S-033447 data are not presented in this review as they did not contribute to the conclusion.*

Bioanalytical method

- Digoxin, rosuvastatin, S-033188 and S-033447 samples were analyzed using validated LC-MS/MS quantification methods. Precision and accuracy values for all standard curves and QC runs, for all quantification methods, were acceptable. All samples were analyzed within the long-term storage stability duration of 98 days (digoxin), 101 days (rosuvastatin) and 367 days (S-033188 and S-033447). Incurred sample reanalysis was performed and the results were within acceptable limits.

Results

Table 1. PK Parameter Estimates of Digoxin alone and After Co-administration with a Single 80-mg Dose of S-033188

			Ratio	
Parameter	N	Geometric LS Mean[a]	Geometric LS Mean Ratio[a]	90% CI[a,b]
C_{max} (ng/mL)				
Co-Administration (Digoxin + S-033188)	12	0.998	0.9973	0.8061, 1.2339
Digoxin Alone	12	1.00		
AUC_{0-last} (ng·hr/mL)				
Co-Administration (Digoxin + S-033188)	12	12.30	0.8450	0.6970, 1.0245
Digoxin Alone	12	14.56		
AUC_{0-inf} (ng·hr/mL)				
Co-Administration (Digoxin + S-033188)	12	13.54	0.8593	0.7320, 1.0087
Digoxin Alone	12	15.75		
AUC_{0-168} (ng·hr/mL)				
Co-Administration (Digoxin + S-033188)	12	12.55	0.8549	0.7204, 1.0145
Digoxin Alone	12	14.68		

a: From an ANOVA model for the log transformed parameter results with fixed effect treatment and random effect subject.
b: In case of unbalanced data, the Kenward-Roger method was used to compute the denominator degrees of freedom to obtain the critical value for calculation of the confidence interval.

Table 2. PK Parameter Estimates of Rosuvastatin alone and After Co-administration with a Single 80-mg Dose of S-033188

Parameter	N	Geometric LSMean[a]	Ratio Geometric LSMean Ratio[a]	90% CI[a,b]
C_{max} (ng/mL)				
Co-Administration (Rosuvastatin + S-033188)	12	3.91	0.8205	0.6886, 0.9778
Rosuvastatin Alone	12	4.77		
AUC_{0-last} (ng·hr/mL)				
Co-Administration (Rosuvastatin + S-033188)	12	45.51	0.8523	0.7413, 0.9799
Rosuvastatin Alone	12	53.40		
AUC_{0-inf} (ng·hr/mL)				
Co-Administration (Rosuvastatin + S-033188)	12	47.37	0.8303	0.7172, 0.9613
Rosuvastatin Alone	12	57.05		

a: From an ANOVA model for the log transformed parameter results with fixed effect treatment and random effect subject.
b: In case of unbalanced data, the Kenward-Roger method was used to compute the denominator degrees of freedom used to obtain the critical value for calculation of the confidence interval.

Reviewer's comments:

- *Digoxin and rosuvastatin exposures were expected to increase, rather than decrease, when either drug is co-administered with S-033188.*

- *The 90% confidence intervals of the geometric mean ratio values of AUC_{0-inf} and AUC_{0-last} of digoxin and rosuvastatin were outside the pre-determined no effect limit, 80 -125% (Tables 1 and 2). This is not expected to impose any clinically relevant effect since digoxin and rosuvastatin are administered chronically while baloxavir marboxil is to be administered as a single dose.*

Conclusions

- The exposures of digoxin and rosuvastatin were decreased by the co-administration of S-033188. This is not expected to impose a clinically relevant effect.
- The study is acceptable.

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Title (Study # 1606T0818)

- A Study to Assess the Effect of Oseltamivir on the Pharmacokinetics of S-033188 and the Effect of S-033188 on the Pharmacokinetics of Oseltamivir in Healthy Adult Subjects

Information Regarding the Clinical Trial Site and Duration of the Trial

- The study was conducted at Souseikai Hakata Clinic, Random square, 6-18, Tenyamachi, Hakata-ku, Fukuoka 812-0025, Japan, between June 23, 2016 and August 30, 2016

Rationale

- Oseltamivir phosphate (Tamiflu®) is anticipated to be co-administered with S-033188 for treatment of influenza. The objective of this study was to evaluate the effect of concurrent use of S-033188 and Oseltamivir phosphate on the PK and safety profiles of both compounds.

Critical Trial Information

- This was a single-center, open-label, randomized, 6-sequence, 3-period, 3-treatment crossover study conducted in 18 subjects (Table 1).

Table 1:   Cohorts and Dosing Sequences

Group	Number of subjects	Dosing sequence		
		Period 1	Period 2	Period 3
1	3	S-033188 40 mg single dose [Treatment A]	Tamiflu® 75 mg BID for 5 days [Treatment B]	S-033188 40 mg single dose + Tamiflu® 75 mg BID for 5 days [Treatment C]
2	3	S-033188 40 mg single dose [Treatment A]	S-033188 40 mg single dose + Tamiflu® 75 mg BID for 5 days [Treatment C]	Tamiflu® 75 mg BID for 5 days [Treatment B]
3	3	S-033188 40 mg single dose + Tamiflu® 75 mg BID for 5 days [Treatment C]	S-033188 40 mg single dose [Treatment A]	Tamiflu® 75 mg BID for 5 days [Treatment B]
4	3	S-033188 40 mg single dose + Tamiflu® 75 mg BID for 5 days [Treatment C]	Tamiflu® 75 mg BID for 5 days [Treatment B]	S-033188 40 mg single dose [Treatment A]
5	3	Tamiflu® 75 mg BID for 5 days [Treatment B]	S-033188 40 mg single dose + Tamiflu® 75 mg BID for 5 days [Treatment C]	S-033188 40 mg single dose [Treatment A]
6	3	Tamiflu® 75 mg BID for 5 days [Treatment B]	S-033188 40 mg single dose [Treatment A]	S-033188 40 mg single dose + Tamiflu® 75 mg BID for 5 days [Treatment C]

BID = twice daily.
S-033188 40 mg: S-033188 20-mg tablet × 2.
Tamiflu® 75 mg: Tamiflu® 75-mg capsule × 1.

Source: p.23, Clinical Study Report

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Formulation, Dose and Mode of Administration

Oseltamivir phosphate: Oral capsules (1 × 75 mg capsule), 75 mg BID for 5 days

S-033188: Oral tablet, single 40 mg dose (2 × 20 mg tablet)

S-033188 doses were administered under fasted condition. Oseltamivir phosphate doses were

administered under fed condition with the exception when co-administered with *S-033188.*

Sample Collection and Bioanalysis

Sample Collection

Oseltamivir and Oseltamivir carboxylate

Plasma samples (n=22) were collected prior to the first oseltamivir phosphate dose administration and

up to 108 hours post first dose.

S-033188 and S-033447

- Plasma samples (n=18) were collected prior to S-033188 administration and up to 504 hours post

  dose*.*

Bioanalytical method

- Oseltamivir, oseltamivir carboxylate (active metabolite), S-033188 and S-033447 samples were

  analyzed using validated LC-MS/MS quantification methods. Precision and accuracy values for all

  standard curves and QC runs, for all quantification methods, were acceptable. All samples were

  analyzed within the long-term storage stability duration of 420 days (oseltamivir and oseltamivir

  carboxylate) and 385 days (S-033188 and S-033447). Incurred sample reanalysis was performed and

  the results were within acceptable limits.

Results

- PK parameters of S-033188 were not estimated since plasma concentrations were below the

  quantification limit (< 0.100 ng/mL) at most time points. Note: S-033188 undergoes rapid conversion

  (i.e., within few hours post administration) to S-033447.

Table 2 **Effect of Tamiflu® on the Pharmacokinetics of S-033447**

| | Plasma S-033447 | | |
| | Geometric Least Squares Mean[a] | | Geometric Least Squares Mean Ratio[a] (90% CI: lower, upper) |
Parameter	S-033188 alone [N = 17]	Co-administration [N = 18]	Co-administration / S-033188 alone
C_{max} (ng/mL)	137	141	1.0288 (0.9190, 1.1517)
AUC_{0-last} (ng·hr/mL)	6506	6562[b]	1.0085 (0.9608, 1.0585)
AUC_{0-inf} (ng·hr/mL)	6692	6730[b]	1.0057 (0.9575, 1.0563)

Source: p.51, Clinical Study Report

Table 3 **Effect of S-033188 on the Pharmacokinetics of Oseltamivir**

(a) Day 1

| | Plasma Oseltamivir | | |
| | Geometric Least Squares Mean[a] | | Geometric Least Squares Mean Ratio[a] (90% CI: lower, upper) |
Parameter	Oseltamivir phosphate alone (Day 1) [N = 17]	Co-administration (Day 1) [N = 18]	Co-administration (Day 1) / Oseltamivir phosphate alone (Day 1)
C_{max} (ng/mL)	83.5	80.6	0.9664 (0.8199, 1.1392)
AUC_{0-t} (ng·hr/mL)	143.0	175.6	1.2278 (1.1366, 1.3264)

CI = confidence interval.

[a] The analysis was based on the analysis of variance model: ln (Parameter) = Treatment + Group + Period + Subject + Random error, where treatment, group, and period were fixed effects and subject was a random effect. Results were exponentiated to present geometric least squares mean and ratio (90% CI).

(b) Day 5

| | Plasma Oseltamivir | | |
| | Geometric Least Squares Mean[a] | | Geometric Least Squares Mean Ratio[a] (90% CI: lower, upper) |
Parameter	Oseltamivir phosphate alone (Day 5) [N = 17]	Co-administration (Day 5) [N = 17]	Co-administration (Day 5) / Oseltamivir phosphate alone (Day 5)
C_{max} (ng/mL)	74.3	71.0	0.9559 (0.8263, 1.1058)
AUC_{0-t} (ng·hr/mL)	176.4	188.3	1.0672 (0.9937, 1.1462)

Source: p.52, Clinical Study Report

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Table 4 **Effect of S-033188 on the Pharmacokinetics of Oseltamivir Carboxylate**

(a) Day 1

	Plasma Oseltamivir Carboxylate		
	Geometric Least Squares Mean[a]		Geometric Least Squares Mean Ratio[a] (90% CI: lower, upper)
Parameter	Oseltamivir phosphate alone (Day 1) [N = 17]	Co-administration (Day 1) [N = 18]	Co-administration (Day 1) / Oseltamivir phosphate alone (Day 1)
C_{max} (ng/mL)	403	386	0.9594 (0.9074, 1.0145)
$AUC_{0-\tau}$ (ng·hr/mL)	3056	2931	0.9591 (0.9179, 1.0021)

CI = confidence interval.

[a] The analysis was based on the analysis of variance model: ln (Parameter) = Treatment + Group + Period + Subject + Random error, where treatment, group, and period were fixed effects and subject was a random effect. Results were exponentiated to present geometric least squares mean and ratio (90% CI).

(b) Day 5

	Plasma Oseltamivir Carboxylate		
	Geometric Least Squares Mean[a]		Geometric Least Squares Mean Ratio[a] (90% CI: lower, upper)
Parameter	Oseltamivir phosphate alone (Day 5) [N = 17]	Co-administration (Day 5) [N = 17]	Co-administration (Day 5) / Oseltamivir phosphate alone (Day 5)
C_{max} (ng/mL)	493	476	0.9648 (0.9299, 1.0010)
$AUC_{0-\tau}$ (ng·hr/mL)	4279	4224	0.9873 (0.9630, 1.0122)

Source: p.53, Clinical Study Report

Reviewer's comments:

- *S-033188 increased the exposure of oseltamivir on the first day of co-administration only. The 90% confidence interval of the geometric mean ratio value of $AUC_{0-\tau}$ was outside the pre-determined no effect limit, 80 -125% on Day 1[Table3(a)]. This increase did not result in subsequent increase in exposure of oseltamivir carboxylate [Table4] and as a result, is not expected to impose any clinically relevant effect.*

Conclusions

- Oseltamivir did not alter the PK of S-033447

- S-033188 increased the exposure of oseltamivir on the first day of co-administration only. This increase is not expected to impose any clinically relevant effect.

- S-033188 did not alter the PK of oseltamivir carboxylate.

- The study is acceptable.

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4.6 In Vitro Study Reviews

Transport Studies

Title: Transcellular Transport of S-033188 and S-033447 Mediated by P-gp and BCRP Across Caco-2 Cell								
Study # S-033188-PF-111-N (EDR Link)								
Cells	Caco-2 cells							
Incubation time	2 h							
Analyte	$[^{14}C]$-S-033188, $[^{14}C]$-S-033447, $[^{3}H]$Digoxin (P-gp substrate) and $[^{3}H]$ES (BCRP substrate)							
Typical inhibitor	Verapamil (P-gp) and Ko143 (BCRP)							
Test Substance, Model Substrate	Inhibitor		P_{app} (×10^{-6} cm/sec)				P_{app} ratio	% of control
	Compound	Concentration	Apical to basal			Basal to apical		
$[^{14}C]$-S-033188 (2 µmol/L)	-	-	4.75 ± 0.36		29.4 ± 1.4		6.2	100.0
	Verapamil	30 µmol/L	17.1 ± 0.7		20.4 ± 0.2		1.2	3.8
	Ko143	1 µmol/L	4.52 ± 0.12		30.8 ± 3.3		6.8	111.5
$[^{14}C]$-S-033188 (10 µmol/L)	-	-	6.34 ± 0.14		27.9 ± 0.4		4.4	100.0
	Verapamil	30 µmol/L	17.2 ± 0.6		20.5 ± 0.4		1.2	5.9
	Ko143	1 µmol/L	7.30 ± 0.12		27.5 ± 2.1		3.8	82.4
$[^{14}C]$-S-033447 (2 µmol/L)	-	-	0.503 ± 0.022		3.15 ± 0.59		6.3	100.0
	Verapamil	30 µmol/L	0.428 ± 0.370		1.04 ± 0.18		2.4	26.4
	Ko143	1 µmol/L	0.650 ± 0.071		3.03 ± 0.44		4.7	69.8
$[^{14}C]$-S-033447 (10 µmol/L)	-	-	0.495 ± 0.010		3.12 ± 0.18		6.3	100.0
	Verapamil	30 µmol/L	0.634 ± 0.037		1.25 ± 0.09		2.0	18.9
	Ko143	1 µmol/L	0.488 ± 0.014		3.03 ± 0.14		6.2	98.1
$[^{3}H]$Digoxin (1 µmol/L)	-	-	0.737 ± 0.018		15.0 ± 0.5		20.4	100.0
	Verapamil	30 µmol/L	4.18 ± 0.11		5.45 ± 0.35		1.3	1.5
$[^{3}H]$ES (0.1 µmol/L)	-	-	0.871 ± 0.054		37.7 ± 2.2		43.3	100.0
	Ko143	1 µmol/L	4.22 ± 0.11		5.34 ± 0.46		1.3	0.7
Conclusion	The Papp ratios of S-033188 and S-033447 were more than two at 2 and 10 µmol/L, and decreased by verapamil but not Ko143, indicating that S-033188 and S-033447 are substrates of P-gp and not substrates of BCRP.							

Papp value represents mean ± SD of triplicate determinations.

Title: Uptake of S-033447 Mediated by OATP1B1 and OATP1B3 in Transporter-expressing HEK293 Cells				Study # S-033188-PF-111-N (EDR Link)						
Cells	HEK293 cells									
Incubation time	2, 5 and 10 min									
Analyte	$[^{14}C]$-S-033447 and $[^{3}H]E_2 17\beta G$ (OATP1B1 and OATP1B3 substrate)									
Typical inhibitor	Rifampicin (OATP1B1 and OATP1B3)									
Test Substance, Model	Inhibitor		Incubation time	Cleared volume (µL/mg protein)						
Substrate	Compound	Concentration	(min)	Control cells			OATP1B1-expressing cells		OATP1B3-expressing cells	
$[^{14}C]$-S-033447 (1 µmol/L)	-	-	2	148	±	4	155	± 2	152	± 6
			5	245	±	4	241	± 9	267	± 12
			10	348	±	17	397	± 23	399	± 25
	Rifampicin	10 µmol/L	2	144	±	10	134	± 6	139	± 9
			5	263	±	11	237	± 20	249	± 11
			10	365	±	4	436	± 22	431	± 31
$[^{14}C]$-S-033447 (5 µmol/L)	-	-	2	141	±	8	133	± 4	136	± 5
			5	255	±	9	225	± 4	236	± 7
			10	367	±	17	405	± 42	433	± 23
	Rifampicin	10 µmol/L	2	126	±	17	92.4	± 4.4	111	± 16
			5	232	±	21	174	± 16	191	± 20
			10	325	±	31	379	± 21	491	± 6
$[^{3}H]E_2 17\beta G$ (0.05 µmol/L)	-	-	2	0.267	±	0.061	130	± 8	18.7	± 0.7
	Rifampicin	10 µmol/L	2	0.225	±	0.029	22.5	± 1.5	1.39	± 0.07
Conclusion	The cleared volume of S-033447 into OATP1B1- or OATP1B3-expressing cells was similar to that into the control cells at 1 and 5 µmol/L, indicating that S-033447 is not a substrate of OATP1B1 and OATP1B3									

Cleared volume represents mean ± SD of triplicate determinations.

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Title: Inhibitory Effects of S-033188 on the Typical Substrate Transport Mediated by P-gp								Study # S-033188-PF-113-N (EDR Link)	
Cells	Caco-2 cells								
Incubation time	2 hours								
Analyte	$[^3H]$Digoxin (P-gp substrate)								
Model Substrate	Test Substance, Inhibitor			P_{app} (×10^{-6} cm/sec)				P_{app} ratio	% of control
	Compound	Prepared concentratio	Corrected concentration [a]	Apical to basal		Basal to apical			
$[^3H]$Digoxin (1 µmol/L)	None	-	-	0.795 ± 0.099		13.6 ± 1.1		17.1	100.0
	S-033188	1 µmol/L	0.994 µmol/L	0.954 ± 0.065		14.1 ± 1.4		14.8	85.7
	S-033188	2 µmol/L	2.00 µmol/L	0.872 ± 0.048		14.5 ± 0.7		16.6	96.9
	S-033188	5 µmol/L	5.00 µmol/L	1.16 ± 0.02		13.1 ± 0.5		11.3	64.0
	S-033188	10 µmol/L	10.0 µmol/L	1.40 ± 0.11		11.7 ± 0.3		8.4	46.0
	S-033188	25 µmol/L	25.0 µmol/L	1.92 ± 0.25		9.06 ± 1.02		4.7	23.0
	S-033188	50 µmol/L	50.0 µmol/L	2.83 ± 0.12		7.06 ± 0.17		2.5	9.3
	S-033188	100 µmol/L	78.1 µmol/L	3.94 ± 0.18		5.85 ± 0.33		1.5	3.1
	Verapamil	30 µmol/L	-	3.60 ± 0.37		4.71 ± 0.58		1.3	1.9
Conclusion	S-033188 showed an inhibitory effect on P-gp-mediated transport of $[^3H]$digoxin with an IC_{50} value of 8.75 µmol/L.								

Papp value represents the mean ± SD of three samples. Papp ratio was calculated by using the mean Papp value of three samples.

[a] The value represents the concentration corrected with the adhesion ratio.

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Title: Inhibitory Effects of S-033447 on the Typical Substrate Transport Mediated by P-gp									Study # S-033188-PF-113-N (EDR Link)	
Cells	Caco-2 cells									
Incubation time	2 hours									
Analyte	[^{3}H]Digoxin (P-gp substrate)									
Model Substrate	Test Substance, Inhibitor			P_{app} (×10^{-6} cm/sec)						
	Compound	Prepared concentratio	Corrected concentration a)	Apical to basal			Basal to apical		P_{app} ratio	% of control
[^{3}H]Digoxin (1 µmol/L)	None	-	-	0.795	±	0.099	13.6	±	1.1	17.1	100.0
	S-033447	0.5 µmol/L	0.282 µmol/L	0.889	±	0.150	13.3	±	1.2	15.0	87.0
	S-033447	3 µmol/L	0.637 µmol/L	0.795	±	0.091	13.1	±	1.7	16.5	96.3
	S-033447	4 µmol/L	0.778 µmol/L	0.852	±	0.012	12.9	±	0.4	15.1	87.6
	S-033447	5 µmol/L	3.14 µmol/L	0.895	±	0.075	12.7	±	0.4	14.2	82.0
	S-033447	10 µmol/L	7.01 µmol/L	1.20	±	0.02	12.4	±	0.5	10.3	57.8
	S-033447	20 µmol/L	9.54 µmol/L	1.11	±	0.06	13.4	±	0.8	12.1	68.9
	S-033447	30 µmol/L	20.9 µmol/L	1.19	±	0.13	13.2	±	0.4	11.1	62.7
	Verapamil	30 µmol/L	-	3.60	±	0.37	4.71	±	0.58	1.3	1.9
Conclusion	S-033447 showed an inhibitory effect on P-gp-mediated transport of [^{3}H]digoxin with an IC_{50} value of >20.9 µmol/L.									

P_{app} value represents the mean ± SD of three samples. P_{app} ratio was calculated by using the mean P_{app} value of three samples.
a) The value represents the concentration corrected with the adhesion ratio.

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Title: Inhibitory Effects of S-033188 on the Typical Substrate Transport Mediated by BCRP								Study # S-033188-PF-113-N (EDR Link)	
Cells	Caco-2 cells								
Incubation time	2 hours								
Analyte	Estrone sulfate, ammonium salt, [6,7-^{3}H(N)]- ([^{3}H]ES, BCRP substrate)								
Model Substrate				P_{app} (×10^{-6} cm/sec)				P_{app} ratio	% of control
	Compound	Prepared concentratio	Corrected concentration $^{a)}$	Apical to basal		Basal to apical			
[^{3}H]ES (0.1 µmol/L)	None	-	-	0.768 ± 0.028		44.6 ± 2.0		58.1	100.0
	S-033188	1 µmol/L	0.994 µmol/L	0.667 ± 0.041		36.7 ± 0.5		55.0	94.6
	S-033188	2 µmol/L	2.00 µmol/L	0.623 ± 0.047		36.5 ± 1.6		58.6	100.9
	S-033188	5 µmol/L	5.00 µmol/L	0.642 ± 0.003		38.9 ± 4.1		60.6	104.4
	S-033188	10 µmol/L	10.0 µmol/L	0.599 ± 0.091		32.8 ± 0.6		54.8	94.2
	S-033188	25 µmol/L	25.0 µmol/L	0.703 ± 0.143		33.5 ± 2.2		47.7	81.8
	S-033188	50 µmol/L	50.0 µmol/L	0.821 ± 0.134		34.4 ± 1.7		41.9	71.6
	S-033188	100 µmol/L	78.1 µmol/L	0.840 ± 0.092		25.6 ± 1.6		30.5	51.7
	Ko143	1 µmol/L	-	2.75 ± 0.24		5.04 ± 0.33		1.8	1.4
Conclusion	S-033188 showed an inhibitory effect on BCRP-mediated transport of [^{3}H]ES with an IC$_{50}$ value of >78.1 µmol/L.								

P_{app} value represents the mean ± SD of three samples. P_{app} ratio was calculated by using the mean P_{app} value of three samples.

$^{a)}$ The value represents the concentration corrected with the adhesion ratio.

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Title: Inhibitory Effects of S-033447 on the Typical Substrate Transport Mediated by BCRP									Study # S-033188-PF-113-N (EDR Link)	
Cells	Caco-2 cells									
Incubation time	2 h									
Analyte	[3H]ES (BCRP substrate)									
Model Substrate	Test Substance, Inhibitor			P_{app} ($\times 10^{-6}$ cm/sec)						
	Compound	Prepared concentratio	Corrected concentration [a]	Apical to basal			Basal to apical		P_{app} ratio	% of control
[3H]ES (0.1 µmol/L)	None	-	-	0.768	±	0.028	44.6	±	2.0	58.1	100.0
	S-033447	0.5 µmol/L	0.282 µmol/L	0.976	±	0.098	38.6	±	4.8	39.5	67.4
	S-033447	5 µmol/L	0.637 µmol/L	0.994	±	0.055	37.2	±	1.3	37.4	63.7
	S-033447	6 µmol/L	0.778 µmol/L	1.08	±	0.09	37.5	±	0.7	34.7	59.0
	S-033447	5 µmol/L	3.14 µmol/L	0.852	±	0.012	30.8	±	0.8	36.2	61.6
	S-033447	10 µmol/L	7.01 µmol/L	0.987	±	0.136	33.4	±	1.6	33.8	57.4
	S-033447	20 µmol/L	9.54 µmol/L	0.801	±	0.106	24.8	±	1.0	31.0	52.5
	S-033447	30 µmol/L	20.9 µmol/L	0.740	±	0.031	19.1	±	0.2	25.8	43.4
	Ko143	1 µmol/L	-	2.75	±	0.24	5.04	±	0.33	1.8	1.4
Conclusion	S-033447 showed an inhibitory effect on BCRP-mediated transport of [3H]ES with an IC50 value of 7.10 µmol/L.									

P_{app} value represents the mean ± SD of three samples. P_{app} ratio was calculated by using the mean P_{app} value of three samples.
[a] The value represents the concentration corrected with the adhesion ratio.

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Title: Inhibitory Effects of S-033447 on the Typical Substrate Transport Mediated by OATP1B1 and OATP1B3														Study # S-033188-PF-113-N (EDR Link)	
Cells	HEK293 cells														
Incubation time	2 min														
Analyte	Estradiol 17β- D-glucuronide, [estradiol-6,7-^{3}H(N)]- ([^{3}H]E$_2$17βG, OATP1B1 and OATP1B3 substrate)														
Model Substrate	Test Substance, Inhibitor			Cleared volume (µL/mg protein)								% of control			
	Compound	Prepared concentratio	Corrected concentration ^{a}	Control cells			OATP1B1-expressing cells			OATP1B3-expressing cells			OATP1B1-expressing cells	OATP1B3-expressing cells
[^{3}H]E$_2$17βG (0.05 µmol/L)	None	-	-	0.769	±	0.169	166	±	13	21.5	±	0.6	100.0	100.0
	S-033447	0.5 µmol/L	0.398 µmol/L	0.629	±	0.117	161	±	5	21.9	±	1.7	97.1	102.6
	S-033447	7 µmol/L	0.789 µmol/L	0.721	±	0.150	155	±	11	21.2	±	1.0	93.4	98.8
	S-033447	8 µmol/L	1.30 µmol/L	0.641	±	0.089	108	±	7	18.0	±	1.3	65.0	83.7
	S-033447	5 µmol/L	3.46 µmol/L	0.722	±	0.134	94.0	±	3.9	21.2	±	1.5	56.5	98.8
	S-033447	10 µmol/L	7.98 µmol/L	1.13	±	0.31	84.4	±	10.1	18.7	±	0.4	50.4	84.8
	S-033447	20 µmol/L	11.3 µmol/L	2.11	±	0.59	72.7	±	13.7	19.3	±	0.6	42.7	82.9
	S-033447	30 µmol/L	20.4 µmol/L	3.59	±	0.47	62.3	±	5.4	17.6	±	2.3	35.5	67.6
	Rifampicin	10 µmol/L	-	0.509	±	0.108	36.5	±	2.2	2.41	±	0.18	21.8	9.2
Conclusion	S-033447 showed an inhibitory effect on OATP1B1-mediated uptake of [^{3}H]E$_2$17βG with an IC$_{50}$ value of 6.81 µmol/L and OATP1B3-mediated uptake of [^{3}H]E$_2$17βG with an IC$_{50}$ value of >20.4 µmol/L.														

Cleared volume represents the mean ± SD of three samples. % of control was calculated using the mean value of cleared volume.
^{a} The value represents the concentration corrected with the adhesion ratio.

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Title: Inhibitory Effects of S-033447 on the Typical Substrate Transport Mediated by OAT1				Study # S-033188-PF-113-N (EDR Link)		
Cells	HEK293 cells					
Analyte	Aminohippuric acid, p-[glycyl-2-^{3}H] ([^{3}H]PAH, OAT1 substrate)					
Model Substrate	Test Substance, Inhibitor			Cleared volume (µL/mg protein)		% of control
	Compound	Prepared concentration	Corrected concentration ^{a}	Control cells	OAT1-expressing cells	OAT1-expressing cells
[^{3}H]PAH	None	-	-	0.419 ±	18.4 ± 1.3	100.0
(1 µmol/L)	S-033447	0.5 µmol/L	0.439 µmol/L	0.480 ± 0.199	17.9 ± 2.3	96.9
	S-033447	1 µmol/L	0.813 µmol/L		17.9 ± 1.7	95.7
	S-033447	2 µmol/L	1.07 µmol/L	0.697 ± 0.498	15.3 ± 1.3	83.1
	S-033447	5 µmol/L	3.56 µmol/L		18.8 ± 1.5	100.3
	S-033447	10 µmol/L	7.33 µmol/L	0.359 ± 0.030	19.6 ± 1.4	105.6
	S-033447	20 µmol/L	11.0 µmol/L		18.1 ± 4.5	97.1
	S-033447	30 µmol/L	20.4 µmol/L	0.770 ± 0.074	18.0 ± 1.3	95.6
	Probenecid	100 µmol/L	-	0.237 ±	3.28 ± 0.39	16.9
Conclusion:	S-033447 showed no inhibitory effect on OAT1-mediated uptake of [3H]PAH with an IC50 value of >20.4 µmol/L.					

Cleared volume represents the mean ± SD of three samples. % of control was calculated using the mean value of cleared volume.
^{a} The value represents the concentration corrected with the adhesion ratio.

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Title: Inhibitory Effects of S-033447 on the Typical Substrate Transport Mediated by OAT3									Study # S-033188-PF-113-N ([EDR Link](#))
Cells	HEK293 cells								
Incubation time	2 min								
Analyte	$[^3H]ES$ (OAT3 substrate)								
Model Substrate	Test Substance, Inhibitor			Cleared volume (µL/mg protein)					% of control
	Compound	Prepared concentratio	Corrected concentration [a]	Control cells			OAT3- expressing cells		OAT3- expressing cells
$[^3H]ES$ (0.05 µmol/L)	None	-	-	1.34	±	0.18	93.5 ±	6.1	100.0
	S-033447	0.5 µmol/L	0.398 µmol/L	1.24	±	0.22	91.5 ±	0.2	97.9
	S-033447	1 µmol/L	0.789 µmol/L	1.49	±	0.31	82.9 ±	8.2	88.3
	S-033447	2 µmol/L	1.30 µmol/L	1.37	±	0.37	83.8 ±	8.5	89.4
	S-033447	5 µmol/L	3.46 µmol/L	1.80	±	0.62	72.4 ±	7.5	76.6
	S-033447	10 µmol/L	7.98 µmol/L	1.87	±	0.94	79.7 ±	7.6	84.5
	S-033447	20 µmol/L	11.3 µmol/L	1.64	±	0.63	72.9 ±	9.2	77.3
	S-033447	30 µmol/L	20.4 µmol/L	1.33	±	0.26	79.4 ±	0.5	84.7
	Probenecid	100 µmol/L	-	0.708	±	0.069	9.01 ±	1.48	9.0
Conclusion	S-033447 showed no inhibitory effect on OAT3-mediated uptake of $[^3H]ES$ with an IC_{50} value of >20.4 µmol/L.								

Cleared volume represents the mean ± SD of three samples. % of control was calculated using the mean value of cleared volume.
[a] The value represents the concentration corrected with the adhesion ratio.

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Title: Inhibitory Effects of S-033447 on the Typical Substrate Transport Mediated by OCT1									Study # S-033188-PF-113-N (EDR Link)	
Cells	HEK293 cells										
Incubation time	5 min										
Analyte	[14C]Metformin (OCT1 substrate)										
Model Substrate	Test Substance, Inhibitor			Cleared volume (µL/mg protein)						% of control	
	Compound	Prepared concentratio	Corrected concentration [a]	Control cells			OCT1-expressing cells			OCT1-expressing cells	
[14C]Metformin (10 µmol/L)	None	-	-	1.35	±	0.01	28.9	±	0.3	100.0	
	S-033447	0.5 µmol/L	0.294 µmol/L	1.52	±	0.08	28.1	±	0.7	96.5	
	S-033447	1 µmol/L	0.686 µmol/L	1.52	±	0.16	27.9	±	1.2	95.8	
	S-033447	2 µmol/L	1.16 µmol/L	1.40	±	0.06	23.5	±	0.6	80.2	
	S-033447	5 µmol/L	2.95 µmol/L	1.24	±	0.17	15.4	±	1.0	51.4	
	S-033447	10 µmol/L	7.65 µmol/L	1.39	±	0.12	14.7	±	0.9	48.3	
	S-033447	20 µmol/L	9.80 µmol/L	1.30	±	0.02	12.8	±	0.5	41.7	
	S-033447	30 µmol/L	19.8 µmol/L	1.25	±	0.13	12.3	±	0.4	40.1	
	Quinidine	100 µmol/L	-	0.408	±	0.071	1.00	±	0.02	2.1	
Conclusion	S-033447 showed an inhibitory effect on OCT1-mediated uptake of [14C]metformin with an IC_{50} value of 6.52 µmol/L.										

Cleared volume represents the mean ± SD of three samples. % of control was calculated using the mean value of cleared volume.
[a] The value represents the concentration corrected with the adhesion ratio.

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Title: Inhibitory Effects of S-033447 on the Typical Substrate Transport Mediated by OCT2									Study # S-033188-PF-113-N ([EDR Link](#))

Cells	HEK293 cells								
Incubation time	2 min								
Analyte	$[^{14}C]$Metformin (OCT2 substrate)								

Model Substrate	Test Substance, Inhibitor			Cleared volume (µL/mg protein)					% of control
	Compound	Prepared concentratio	Corrected concentration [a]	Control cells			OCT2- expressing cells		OCT2- expressing cells
$[^{14}C]$Metformin (10 µmol/L)	None	-	-	0.86	±	0.051	67.5	±	3.5	100.0
	S-033447	0.5 µmol/L	0.398 µmol/L	0.98	±	0.096	66.3	±	0.7	98.0
	S-033447	1 µmol/L	0.789 µmol/L	1.03	±	0.05	63.3	±	2.3	93.5
	S-033447	2 µmol/L	1.30 µmol/L	0.82	±	0.037	63.8	±	5.3	94.5
	S-033447	5 µmol/L	3.46 µmol/L	0.75	±	0.068	57.4	±	3.2	85.0
	S-033447	10 µmol/L	7.98 µmol/L	0.72	±	0.085	50.2	±	4.4	74.3
	S-033447	20 µmol/L	11.3 µmol/L	0.71	±	0.045	50.5	±	6.0	74.7
	S-033447	30 µmol/L	20.4 µmol/L	0.64	±	0.078	49.3	±	2.4	73.0
	Quinidine	300 µmol/L	-	0.26	±	0.012	1.31	±	0.42	1.6

Conclusion	S-033447 showed an inhibitory effect on OCT2-mediated uptake of $[^{14}C]$metformin with an IC_{50} value of >20.4 µmol/L.

Cleared volume represents the mean ± SD of three samples. % of control was calculated using the mean value of cleared volume.
[a] The value represents the concentration corrected with the adhesion ratio.

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Title: Inhibitory Effects of S-033447 on the Typical Substrate Transport Mediated by MATE1						Study # S-033188-PF-113-N ([EDR Link](#))	
Cells	HEK293 cells						
Incubation time	5 min						
Analyte	$[^{14}C]$Metformin (MATE1 substrate)						
Model Substrate	Test Substance, Inhibitor			Cleared volume (µL/mg protein)			% of control
	Compound	Prepared concentratio	Corrected concentration a)	Control cells		MATE1- expressing cells	MATE1- expressing cells
$[^{14}C]$Metformin (10 µmol/L)	None	-	-	1.12 ± 0.06		21.7 ± 1.9	100.0
	S-033447	0.5 µmol/L	0.193 µmol/L	0.97 ± 0.109		23.3 ± 1.6	108.5
	S-033447	1 µmol/L	0.554 µmol/L	1.13 ± 0.21		22.4 ± 1.5	103.4
	S-033447	2 µmol/L	0.822 µmol/L	1.09 ± 0.08		19.7 ± 0.6	90.4
	S-033447	5 µmol/L	2.01 µmol/L	0.93 ± 0.122		15.3 ± 1.8	69.8
	S-033447	10 µmol/L	5.99 µmol/L	0.96 ± 0.007		13.0 ± 1.7	58.5
	S-033447	20 µmol/L	13.0 µmol/L	1.58 ± 0.23		11.4 ± 0.6	47.7
	S-033447	30 µmol/L	22.7 µmol/L	0.92 ± 0.114		10.2 ± 0.7	45.1
	Cimetidine	10 µmol/L	-	0.49 ± 0.013		2.09 ± 0.09	7.8
Conclusion	S-033447 showed an inhibitory effect on MATE1-mediated uptake of $[^{14}C]$metformin with an IC_{50} value of 11.2 µmol/L.						

Cleared volume represents the mean ± SD of three samples. % of control was calculated using the mean value of cleared volume.
a) The value represents the concentration corrected with the adhesion ratio.

Title: Inhibitory Effects of S-033447 on the Typical Substrate Transport Mediated by MATE2-K									Study # S-033188-PF-113-N ([EDR Link](#))	
Cells	HEK293 cells									
Incubation time	5 min									
Analyte	[^{14}C]Metformin (MATE2-K substrate)									
Model Substrate	Test Substance, Inhibitor			Cleared volume (µL/mg protein)						% of control
	Compound	Prepared concentratio	Corrected concentration ^{a}	Control cells			MATE2-K- expressing cells			MATE2-K- expressing cells
[^{14}C]Metformin (10 µmol/L)	None	-	-	1.38	±	0.60	19.7	±	3.0	100.0
	S-033447	0.5 µmol/L	0.193 µmol/L	1.28	±	0.33	21.5	±	4.2	110.4
	S-033447	1 µmol/L	0.554 µmol/L	1.56	±	0.48	19.8	±	1.6	99.6
	S-033447	2 µmol/L	0.822 µmol/L	1.92	±	1.12	10.9	±	1.8	49.0
	S-033447	5 µmol/L	2.01 µmol/L	1.38	±	0.27	7.80	±	1.58	35.0
	S-033447	10 µmol/L	5.99 µmol/L	1.16	±	0.25	6.87	±	1.70	31.2
	S-033447	20 µmol/L	13.0 µmol/L	1.47	±	0.54	4.43	±	0.36	16.2
	S-033447	30 µmol/L	22.7 µmol/L	0.93	±	0.181	4.11	±	0.94	17.3
	Cimetidine	100 µmol/L	-	0.59	±	0.003	3.23	±	0.22	14.4
Conclusion	S-033447 showed an inhibitory effect on MATE2-K-mediated uptake of [^{14}C]metformin with an IC$_{50}$ value of 1.91 µmol/L.									

Cleared volume represents the mean ± SD of three samples. % of control was calculated using the mean value of cleared volume.
^{a} The value represents the concentration corrected with the adhesion ratio.

Title: Inhibitor Assessment of S-033447 for BSEP						Study # S-033188-PF-168-N ([EDR Link])				
Vesicles	BSEP Expressing Vesicles									
Incubation time	5 min									
Analyte	[³H]taurocholic acid (TCA, 1 µmol/L)									
Typical substrate	Test substance or Representative inhibitor	Prepared concentration	Adhesion ratio	Corrected concentration	Cleared volume (µL/mg protein)					% of control
		(µmol/L)	(%)	(µmol/L)	AMP			ATP		
[³H]TCA	-	0	-	-	29.1 ± 2.8			209 ± 18		100.0
(1 µmol/L)	S-033447	1	52.8	0.472	28.9 ± 7.2			204 ± 5		97.2
		2	58.8	0.824	29.8 ± 0.7			257 ± 6		126.1
		5	57.9	2.11	29.8 ± 2.4			245 ± 25		119.4
		15	50.9	7.37	32.6 ± 3.0			253 ± 4		122.2
		30	42.7	17.2	32.4 ± 0.8			248 ± 16		120.0
		60	24.5	45.3	32.4 ± 0.5			238 ± 7		114.4
		100	22.0	78.0	28.4 ± 0.9			218 ± 9		105.6
	Cyclosporin A	10	-	-	30.4 ± 1.3			55.2 ± 1.1		13.8
Conclusion	S-033447 at 78.0 µmol/L showed no inhibition of BSEP. The IC50 value was estimated to be >78.0 µmol/L.									

Cleared volume represents the mean ± SD of three samples. % of control was calculated using the mean value of cleared volume.

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Metabolism Studies

Title: CYP Induction by S-033188 and S-033447 in Cultured Human Hepatocytes (CYP1A2)					Study # S-033188-PF-105-N (EDR Link)		
Study system	Primary culture of cryopreserved human hepatocytes						
Donor	3 Donors (Lot Numbers: Hu8160, Hu1389, and RSF)						
Exposure	72 hours						
Concentration of test article	0.1, 1, 3, 10, 30, and 60 µmol/L (S-033188 and S-033447)						
Enzyme / reaction	CYP1A2 / Phenacetin O-dealkylation						
Compound	Conc. (µmol/L)	mRNA level (individual data)			Enzyme activity (individual data)		
		Fold induction [a]			Fold induction [a]		
S-033188	0.1	0.681	0.680	0.990	1.020	0.765	0.971
	1	0.752	0.791	1.067	0.866	0.716	0.987
	3	0.717	0.802	0.972	0.782	0.697	0.806
	10[b]	1.057	1.075	1.175	0.647	0.603	0.717
	30[b]	1.034	2.345	1.557	0.204	0.134	NC
	60[b]	NC	NC	NC	NC	NC	NC
S-033447	0.1	0.895	0.930	1.035	0.993	0.888	1.031
	1	1.576	1.721	1.159	1.244	1.338	1.272
	3	1.464	1.500	1.090	1.613	1.458	1.637
	10	1.382	1.503	1.422	1.155	1.170	1.612
	30	1.761	2.510	1.482	0.518	1.176	0.854
	60[b]	1.861	2.665	1.249	0.351	0.781	0.330
Prototype inducer (Omeprazole)	50	61.560	65.682	32.547	13.023	17.571	13.655
Conclusion	S-033188 and S-033447 had weak potential to induce CYP1A2 at the tested concentration range						

a: Relative to the corresponding control
b: Data were shown as reference for cytotoxicity. Cytotoxicity could have affected the data at this concentration.
NC: Not calculated

Title: CYP Induction by S-033188 and S-033447 in Cultured Human Hepatocytes (CYP2B6)						Study # S-033188-PF-105-N (EDR Link)		
Study system	Primary culture of cryopreserved human hepatocytes							
Donor	3 Donors (Lot Numbers: Hu8160, Hu1389, and RSF)							
Exposure	72 hours							
Concentration of test article	0.1, 1, 3, 10, 30, and 60 µmol/L (S-033188 and S-033447)							
Enzyme / reaction	CYP2B6 / Bupropion hydroxylation							
Compound	Conc. (µmol/L)	mRNA level (individual data)			Enzyme activity (individual data)			
		Fold induction [a]			Fold induction [a]			
S-033188	0.1	0.825	1.172	0.936	1.052	0.979	0.851	
	1	1.135	1.236	1.023	1.092	1.007	0.730	
	3	0.969	1.462	1.151	0.981	1.020	0.757	
	10 [b]	1.631	1.667	1.255	0.804	0.964	0.749	
	30 [b]	1.029	1.988	3.085	NC	NC	NC	
	60 [b]	NC	8.418	7.900	NC	NC	NC	
S-033447	0.1	1.099	1.065	0.903	1.007	0.970	1.117	
	1	1.188	1.105	1.024	1.039	0.939	1.115	
	3	1.425	1.412	1.170	1.054	0.963	1.031	
	10	1.128	1.577	1.498	1.040	0.945	1.175	
	30	0.901	2.224	1.785	0.531	0.957	0.664	
	60 [b]	0.880	2.182	1.978	0.259	0.488	0.414	
Prototype inducer (Phenobarbital)	1000	7.410	9.621	6.740	9.261	5.084	6.346	
Conclusion	S-033188 and S-033447 had weak potential to induce CYP2B6 at the tested concentration range.							

a: Relative to the corresponding control

b: Data were shown as reference for cytotoxicity. Cytotoxicity could have affected the data at this concentration.

NC: Not calculated

Title: CYP Induction by S-033188 and S-033447 in Cultured Human Hepatocytes (CYP3A)							Study # S-033188-PF-105-N (EDR Link)	
Study system	Primary culture of cryopreserved human hepatocytes							
Donor	3 Donors (Lot Numbers: Hu8160, Hu1389, and RSF)							
Exposure	72 hours							
Concentration of test article	0.1, 1, 3, 10, 30, and 60 µmol/L (S-033188 and S-033447)							
Enzyme / reaction	CYP3A / Midazolam 1'-hydroxylation							
Compound	Conc. (µmol/L)	mRNA level (individual data)			Enzyme activity (individual data)			
		Fold induction [a]			Fold induction [a]			
S-033188	0.1	1.116	1.510	1.051	0.931	1.052	1.172	
	1	1.032	1.228	0.950	0.887	1.117	1.175	
	3	0.857	1.021	1.237	0.964	1.095	1.074	
	10 [b]	0.711	0.752	0.284	0.112	0.154	0.166	
	30 [b]	NC	NC	NC	NC	NC	NC	
	60 [b]	NC	NC	NC	NC	NC	NC	
S-033447	0.1	0.743	0.870	1.029	0.963	1.026	1.075	
	1	0.818	1.043	1.045	0.969	1.135	1.233	
	3	1.239	1.605	1.044	0.997	1.152	1.210	
	10	1.492	1.916	1.361	0.953	1.250	1.108	
	30	2.090	2.533	1.619	0.909	0.795	0.994	
	60 [b]	1.388	1.621	1.536	0.509	0.718	0.796	
Prototype inducer (Rifampicin)	10	11.369	14.226	19.998	8.943	8.334	21.051	
Conclusion	S-033188 and S-033447 had weak potential to induce CYP3A at the tested concentration range.							

a: Relative to the corresponding control

b: Data were shown as reference for cytotoxicity. Cytotoxicity could have affected the data at this concentration.

 NC: Not calculated

Title: Evaluation of S-033188 as an Inhibitor of Human CYP Enzymes		Study # S-033188-PB-090-N (EDR Link)

System	Human Liver Microsomes
Test substance	S-033188 (0.1 – 100 µmol/L)

Results

Enzyme	Substrate	Direct inhibition		Time-dependent inhibition		
		Preincubation 0 min		Preincubation 30 min		Potential for time-dependent inhibition [b]
		IC_{50} (µmol/L) [a]	% of control at 100 µmol/L (%)	IC_{50} (µmol/L) [a]	% of control at 100 µmol/L (%)	
CYP1A2	Phenacetin	> 100	88.6	> 100	110	None
CYP2B6	Bupropion	46.0	32.8	44.2	33.3	None
CYP2C8	Paclitaxel	63.2	42.6	> 100	68.6	None
CYP2C9	Tolbutamide	> 100	72.1	> 100	77.8	None
CYP2C19	S-Mephenytoin	> 100	82.2	> 100	83.0	None
CYP2D6	Dextromethorphan	> 100	63.5	> 100	72.4	None
CYP3A4	Testosterone	50.2	32.5	77.4	40.9	None
CYP3A4	Midazolam	23.2	16.5	31.7	16.0	None

a: Average data (i.e., percent of control activity) obtained from duplicate samples for S-033188 concentration were used to calculate IC_{50} values.

b: When IC_{50} values were calculated, time-dependent inhibition was determined by comparison of IC_{50} values for preincubation 0 min and preincubation 30 min. If the observed inhibition was insufficient to calculate IC_{50} values, % of control values for each concentration were compared.

Conclusion	S-033188 inhibited CYP2B6, CYP2C8, and CYP3A4. Time-dependent inhibitory effect of S-033188 was not observed for any CYP enzyme.

Title: Evaluation of S-033447 as an Inhibitor of Human CYP Enzymes		Study # S-033188-PB-090-N (EDR Link)
System	Human Liver Microsomes	
Test substance	S-033447 (0.1 – 100 μmol/L)	

Results

Enzyme	Substrate	Direct inhibition		Time-dependent inhibition		Potential for time-dependent inhibition [b]
		Preincubation 0 min		Preincubation 30 min		
		IC_{50} (μmol/L) [a]	% of control at 100 μmol/L (%)	IC_{50} (μmol/L) [a]	% of control at 100 μmol/L (%)	
CYP1A2	Phenacetin	> 100	91.9	> 100	102	None
CYP2B6	Bupropion	29.3	26.9	39.7	32.8	None
CYP2C8	Paclitaxel	> 100	64.4	> 100	69.8	None
CYP2C9	Tolbutamide	> 100	80.3	> 100	82.0	None
CYP2C19	S-Mephenytoin	> 100	73.1	> 100	90.9	None
CYP2D6	Dextromethorphan	> 100	66.6	> 100	77.9	None
CYP3A4	Testosterone	> 100	51.8	95.8	49.7	None
CYP3A4	Midazolam	43.2	26.8	31.7	20.2	None

a: Average data (i.e., percent of control activity) obtained from duplicate samples for S-033447 concentration were used to calculate IC_{50} values.

b: When IC_{50} values were calculated, time-dependent inhibition was determined by comparison of IC_{50} values for preincubation 0 min and preincubation 30 min. If the observed inhibition was insufficient to calculate IC_{50} values, % of control values for each concentration were compared.

Conclusion	S-033447 inhibited CYP2B6 and CYP3A4. Time dependent inhibitory effect of S-033447 was not observed for any CYP enzyme.

Title: Hydrolysis of [^{14}C]-S-033188	Study # R-033188-PB-030-N (EDR Link)
Cell System Used	Human sera Human liver and intestinal S9
Investigational Drug (concentration range)	[^{14}C]-S-033188: 10 µmol/L (sera), 10 and 100 µmol/L (liver and intestinal S9)
Incubation Time	0.5 or 1 hour
Results	• Hydrolysis ratios of [^{14}C]-S-033188 at 10 µmol/L for 1 hour incubation with human sera was 71.6%. • [^{14}C]-S-033188 was completely hydrolyzed to S-033447 after incubation with human liver S9 at 10 and 100 µmol/L for 1 hour. • Hydrolysis ratios of [^{14}C]-S-033188 at 10 and 100 µmol/L for 1 hour incubation with human intestinal S9 were 93.2% and 81.8 %, respectively.
Conclusion	S-033188 was hydrolyzed to S-033447 in human intestinal S9, liver S9 and sera.

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Title: Metabolite Profiling and Identification of [14C]-S-033188 and [14C]-S-033447 in Cryopreserved Human Hepatocytes	Study # R-033188-PB-050-N (EDR Link)
Cells	Human hepatocytes
Investigational Drug Conc.	[14C]-S-033188 or [14C]-S-033447 at 5 and 50 µmol/L
Samples Timepoints	0, 2, and 4 hours
Results	The *in vitro* prominent metabolic pathways of S-033188 and S-033447 in cryopreserved human hepatocytes [14C]-RSC-033188 and [14C]-RSC-033447 are [14C]-S-033188 or [14C]-S-033447, respectively
Conclusion	S-033447 was the main detected metabolite of S-033188. Other minor metabolites were identified.

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Reference ID: 4323329

Title: Identification of the Responsible Enzymes for Metabolism of [14C]-S-033188	Study # S-033188-PB-101-N (EDR Link)

System	Intestinal and liver S9 from human (protein concentration: 1 mg/mL[intestine] and 0.5 mg/mL[liver])
Investigational Drug Conc.	[14C]-S-033188 (100 µmol/L)
Incubation with inhibitors	1 hour
Esterase inhibitors	

List of inhibitor and inhibited enzymes

Inhibitor	Inhibited Enzyme(s) Family	Inhibited Enzyme(s) Member
Paraoxon		
Diisopropyl fluorophosphates (DFP)		
Phenylmethylsulfonyl fluoride (PMSF)		Non-specific
Eserine		
Bis(4-nitrophenyl) phosphate (BNPP)	Serine esterase	Carboxylesterase (CES) and arylamide deacetylase (AADAC)
Tetraisopropyl pyrophosphoramide (iso-OMPA)		Acetylcholinesterase and butyrylcholinesterase
Digitonin		Carboxylesterase1 (CES1)
Telmisartan		Carboxylesterase2 (CES2)
Vinblastine		Carboxylesterase2 (CES2) and arylacetamide deacetylase (AADAC)
EDTA 2Na	Ca-dependent A-esterase	Non-specific
5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB)	Arylesterase	Non-specific

Results

Table 1 Inhibitory effect of chemical hydrolysis enzyme(s) inhibitors on the hydrolysis of [14C]-S-033188 in human liver S9

Inhibitor	Inhibited Enzyme (s) Family	Inhibited Enzyme (s) Member	Hydrolysis ratio (%)	% inhibition (%)
Eserine			3.2	96.4
Paraoxon		Non-specific	0.0	100.0
DFP			0.0	100.0
PMSF	Serine esterase		31.3	65.0
BNPP		CES and AADAC	6.4	92.9
iso-OMPA		Acetylcholinesterase and butyrylcholinesterase	88.8	0.9
EDTA 2Na	Ca-dependent A-esterase	Non-specific	83.9	9.0
DTNB	Arylesterase	Non-specific	87.9	1.9

Table 2 Inhibitory effects of chemical hydrolysis enzyme(s) inhibitors on the hydrolysis of [14C]-S-033188 in human intestinal S9

Inhibitor	Inhibited Enzyme (s) Family	Inhibited Enzyme (s) Member	Hydrolysis ratio (%)	% inhibition (%)
Eserine			2.9	97.1
Paraoxon		Non-specific	0.4	99.6
DFP	Serine esterase		0.9	99.1
PMSF			49.1	49.9
BNPP		CES and AADAC	16.4	83.3
iso-OMPA		Acetylcholinesterase and butyrylcholinesterase	93.6	4.5
EDTA 2Na	Ca-dependent A-esterase	Non-specific	93.8	5.0
DTNB	Arylesterase	Non-specific	97.5	0.5

Table 3 Inhibitory effect of Specific Inhibitors on the hydrolysis of [14C]-S-033188 in human liver S9

Inhibitor	Inhibited Enzyme (s)	Hydrolysis ratio (%)	% inhibition (%)
Digitonin	CES1	91.8	0.5
Telmisartan	CES2	77.7	13.2
Vinblastine	CES2 and AADAC	22.5	75.6

Conclusion	Serine esterases, especially AADAC, mainly contributed to S-033188 hydrolysis in intestinal and liver S9.

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Title: Identification of the Responsible Enzymes for Metabolism of [^{14}C]-S-033447	Study # S-033188-PB-109-N (EDR Link)
System	Human liver microsomes - Recombinant human cDNA-expressed UDP-glucuronosyl transferase enzymes (rhUGT)
Investigational Drug	[^{14}C]-S-033447 (5 and 50 µmol/L)
rhUGT	[^{14}C]-S-033447 was incubated with 7 kinds of rhUGT including UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7, and UGT2B15.
Results	At concentrations of 5 and 50 µmol/L of [^{14}C]-S-033447, only UGT1A3 produced S-033447 glucuronide with formation rates of 11.3 and 36.5 pmol/min/mg protein, respectively.
Conclusion	UGT1A3 is the responsible enzyme for the glucuronidation of [^{14}C]-S-033447.

Plasma/Blood Cell Partitioning and Protein Binding Studies

Title: In vitro Plasma/Blood Cell Partitioning and Protein Binding of [^{14}C]-S-033447		Study # R-033188-PB-021-N (EDR Link)	
Sample	Human serum, purified serum human proteins (albumin, α1-acid glycoprotein, γ-globulin)		
Evaluation method	Equilibrium dialysis method		
Incubation	37°C, 24 hours		
	Binding ratio (%)		
Concentration of [^{14}C]-S-033447	50 ng/mL	100 ng/mL	1000 ng/mL
Human serum	92.9 ± 0.6	93.0 ± 0.4	93.9 ± 0.4
Purified human serum protein			
4% Albumin	91.4 ± 0.3	91.2 ± 0.1	92.1 ± 0.1
0.08% α1-Acid glycoprotein	59.3 ± 2.1	59.0 ± 0.5	52.2 ± 0.6
1% γ-Globulin	24.5 ± 2.2	23.6 ± 1.1	38.1 ± 0.7
	Distribution ratio in blood cells (%)		
Human Blood	54.4 ± 1.8	53.0 ± 1.7	48.5 ± 1.4
Conclusion	S-033447 binds highly to serum proteins, primarily to albumin.		

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