CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	210854
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Applicant's letter date:	April 24, 2018
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Product:	Baloxavir marboxil
Indication:	Treatment of acute uncomplicated influenza
Applicant:	Shionogi, Inc.
Review Division:	Division of Antiviral Products
Reviewer:	Deacqunita L. Diggs, Ph.D.
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1 Executive Summary

1.1 Introduction

Shionogi, Inc., has submitted a new drug application for baloxavir marboxil, a polymerase acidic (PA) endonuclease inhibitor, for the treatment of acute uncomplicated (b) (4) influenza (b) (4) in patients 12 years of age and older. Baloxavir marboxil (S-033188, RSC-033188) is a prodrug that gets converted to the active form, baloxavir (S-033447, RSC-033447), through metabolism. The proposed clinical regimen is a single oral dose of 40 mg for body weights ≤80 kg or 80 mg for body weights ≥ 80 kg.

1.2 Brief Discussion of Nonclinical Findings

Repeat-dose studies were conducted in Sprague-Dawley (SD) rats (20, 200, or 2000 mg/kg/day) and Cynomolgus monkeys (1, 10, 100 mg/kg/day) for one month with a one month recovery period. These studies were used to determine the toxicologic profile of baloxavir marboxil. Target organs of toxicity consisted of the liver and thyroid. Liver findings in the repeat-dose study in SD rats included increased weights; accentuated lobular pattern and liver enlargement at the high dose; and histopathology findings of minimal centrilobular hypertrophy, minimal to mild macrovesicular fatty change in periportal hepatocytes, and a mild increase in Kupffer cell phagocytosis. These effects resolved during recovery. There was an increase in hepatic enzymes (AST, ALT, ALP, GGT, and GLDH) in the repeat-dose study in monkeys. At the end of recovery there were no changes in hepatic enzymes.

Minimal diffuse follicular epithelial hyperplasia and minimal to mild decrease in colloid were observed in the thyroid at the mid and high doses in the repeat-dose study in SD rats at the end of dosing. These effects resolved during recovery. In the repeat-dose study in cynomolgus monkeys, increased thyroid weight in males at the high dose was observed at the end of dosing and remained through recovery. Thyroid histopathology findings in monkeys detected at the end of dosing included slight to moderate dilatation of follicles and follicular macrophages in all doses and controls which resolved during recovery. Exposure multiples at the NOAELs in rats and monkeys are 0.6 and 2.5 times the exposure at the recommended clinical dose, respectively.

Baloxavir marboxil-related effects observed in the embryo-fetal studies in SD rats and Kbl: NZW rabbits were a decrease in maternal body weights and food intake. Additional effects observed in Kbl: NZW rabbits included abortions and fetal skeletal variations (cervical rib and supernumerary ribs). Exposure multiples at the maternal and fetal NOAELs in SD rats and Kbl: NZW rabbits are 6 and 7 times the exposure at the recommended clinical dose, respectively.

All safety pharmacology (cardiovascular, neurological, respiratory, and skeletal muscles) and all genotoxicity studies were negative. Carcinogenicity studies were not conducted because the intended clinical use is a single dose. Baloxavir marboxil is not phototoxic to the skin or cause skin reactions in *in vivo* studies but showed phototoxic potential in *in vitro* assays.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data are sufficient to support approval.

1.3.2 Additional Nonclinical Recommendations

None.

1.3.3 Labeling

The label is under review.

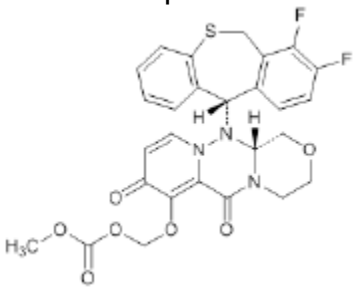
2 Drug Information

All figures and tables in this review were taken from the Sponsor's reports unless noted.

2.1 Drug

CAS Registry Number	1985606-14-1
Generic Name	Baloxavir marboxil
Code Name	S-033188
Chemical Name	Carbonic acid, [[(12aR)-12-[(11S)-7,8-difluoro-6,11 dihydrodibenzo[b,e]thiepin-11-yl]-3,4,6,8,12,12a-hexahydro- 6,8-dioxo-1H-[1,4]oxazino[3,4-c]pyrido[2,1-f][1,2,4]triazin- 7-yl]oxy]methyl ester (CAS) (USAN)
Molecular Formula/Molecular Weight	$C_{27}H_{23}F_2N_3O_7S$ / 571.55 g/mol

Structure or Biochemical Description



Pharmacologic Class	Polymerase acidic (PA) endonuclease inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 126653

2.3 Drug Formulation

Table 1: Baloxavir Marboxil Drug Formulation, 20 and 40 mg tablets

Component	Function	Quality Standard	Amount per Tablet (mg)	
			20 mg Tablet	40 mg Tablet
S-033188 Drug Substance [a]	Active ingredient	In-house standard	20	40
Lactose Monohydrate [a]	(b) (4)	NF/Ph.Eur./JP	(b) (4)	
Croscarmellose Sodium		NF/Ph.Eur./JP		
Povidone (b) (4)		USP/Ph.Eur./JP		
Microcrystalline Cellulose		NF/Ph.Eur./JP		
Sodium Stearyl Fumarate		NF/Ph.Eur./JPE		
(b) (4)		USP/Ph.Eur./JP		
		In-house standard		
		USP/Ph.Eur./JP		
Talc		USP/Ph.Eur./JP		
(b) (4)				
(b) (4)				

(b) (4)

2.4 Comments on Novel Excipients

There are no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

The impurities/degradants were reviewed by Dr. Zheng Li (detailed in Appendix 12.1). Four impurities were identified (b) (4) The

proposed specifications are considered acceptable. Other impurities identified were negative in the bacterial reverse mutation test.

2.6 Proposed Clinical Population and Dosing Regimen

A single oral dose of 40 mg for body weights ≤80 kg or 80 mg for body weights ≥ 80 kg in patients ≥ 12 years of age.

2.7 Regulatory Background

This NDA was submitted on April 24, 2018 and granted Priority Review. The Division reviewed this NDA under an Expedited Review process.

3 Studies Submitted

3.1 Studies Reviewed

Secondary Pharmacology
Study EB-117-N: Effect of S-033447 on Cytotoxicity in Cultured Cells
Study EB-209-N: Inhibitory Effect of S-033447 on Cytopathic Effect in Cultured Cells Infected with Influenza Virus
Study EF-044-N: Binding Activity of RSC-033447 on Various Receptors
Study EF-197-N: Mitochondrial Toxicity Test of S-033188 and S-033447 with Human
Study EF-232-N: Cytotoxicity Study of S-033447 in Cells from Multiple Human Tissues

Safety Pharmacology
Study No. R-033188-SF-035-L: Effects of RSC-033188 on Cardiovascular System in Monkeys
Study No. R-033188-TB-054-L: Determination of RSC-033188 and RSC-033447 in Monkey Plasma -Effects of RSC-033188 on Cardiovascular System in Monkeys
Study No. R-033188-SF-039-L: Effects of RSC-033188 on Potassium Current in hERG Transfected Cells
Study No. R-033188-SF-040-L: Effects of RSC-033447 on Potassium Current in hERG Transfected Cells
Study No. R-033188-SF-034-L: Effects of RSC-033188 on Central Nervous System in Rats
Study No. R-033188-SF-036-L: Effects of RSC-033188 on Respiratory System in Rats
Study No. R-033188-SF-038-L: Effects of RSC-033188 on Action Potential in Guinea Pig Papillary Muscles
Study No. R-033188-SF-041-L: Effects of RSC-033447 on Action Potential in Guinea Pig Papillary Muscles

Pharmacokinetics: Absorption
Study No. R-033188-PB-028-N: Dose-linearity of Concentration of RSC-033188 and RSC-033447 in Plasma after a Single Oral Administration of RSC-033188 in Monkeys

Study No. R-033188-PB-051-N:	Dose-linearity of Concentration of RSC-033188 and RSC-033447 in Plasma After a Single Oral Administration of RSC-033188 in Rats
Study No. R-033188-PB-052-N:	Determination of RSC-033188 and RSC-033447 in Monkey Plasma -Plasma Concentration of Radioactivity, RSC-033188, and RSC-033447 and Excretion of Radioactivity after a Single Oral Administration of [14C]-RSC-033188 in Monkeys.
Study No. R-033188-PB-057-N	Concentration of Radioactivity, RSC-033188, and RSC-033447 in Plasma after a Single Oral Administration of [14C]-RSC-033188 in Rats
Study No. S-033188-PB-244-N	Mineral Effect on Concentration of S-033447 in Plasma after a Single Oral Administration of S-033188 in Monkey
Study No. R- 033188-PF-026-N	Plasma Concentration of Radioactivity, RSC-033188, and RSC-033447 and Excretion of Radioactivity after a Single Oral Administration of [14C]-RSC-033188 in Monkeys
Study No. S-033188-PF-148-N	Concentrations of S-033188 and S-033447 in Plasma after a Single Oral Administration of S-033188 in Juvenile Rats
Study No. S-033188-PF-190-N	Concentration of S-033447 in Plasma after a Single Intravenous Administration of S-033447 in Juvenile Rats

Pharmacokinetics: Distribution

Study No. DMPK-2013-MTS-0576188A-03	Exploratory Study on Serum Protein Binding Ratio of MTS-0563447A in Mouse.
Study No. R-033188-PB-021-N	In Vitro Plasma/Blood Cell Partitioning and Protein Binding of [^{14}C]-RSC-033447
Study No. R-033188-PB-020-N	Quantitative Whole-Body Autoradiography After a Single Oral Administration of [^{14}C]-RSC-033188 in Pigmented Rats
Study No. S-033188-PF-177-L	Quantitative Whole-Body Autoradiography After a Single Oral Administration of [^{14}C]-S-033188 in Pregnant Rats

Pharmacokinetics: Metabolism

Study No. R-033188-PB-033-N	Metabolite Profiling and Identification of RSC-033188 after a Single Oral Administration of [14C]-RSC-033188 in Rats

Study No. R-033188-PB-055-N	Metabolite Profiling and Identification of RSC-033188 after a Single Oral Administration of [14C]-RSC-033188 in Monkeys
Study No. S-033188-CB-102-N	Metabolite Profiling and Identification of S-03188 After a Single, Oral Administration in Humans
Study No. PF-080-N	Effects of RSC-033188 on Hepatic Drug Metabolizing Enzymes in Two-Week Oral Toxicity Study in Rats

Pharmacokinetics: Excretion

Study No. R-033188-PB-013-N	Urinary, Fecal, and Biliary Excretion of Radioactivity After a Single Oral Administration of [^{14}C]-RSC-033188 in Rats
Study No. S-033188-PF-151-N	Enterohepatic circulation of Radioactivity After a Single Oral Administration of [^{14}C]-S-033188 in Rats
Study No. S-033188-PF-176-N	Excretion into Milk of Radioactivity After a Single Oral Administration of [^{14}C]-S-033188 in Nursing Rats

Toxicokinetics

Study No. R-033188-TB-077-L	Determination of RSC-033188 and RSC-033447 in rabbit plasma – Two-week oral toxicity study of RSC-033188 in non-pregnant rabbits
Study No. R-033188-TB-054-L	Determination of RSC-033188 and RSC-033447 in Monkey Plasma -Effects of RSC-033188 on Cardiovascular System in Monkeys

Repeat-Dose Toxicology

Study No. R-0299-TB-032-L	Two-Week Oral Toxicity Study of RSC-033188 in Rats
Study No. R-033188-TF-066-L	Two-week Oral Toxicity Study of RSC-033188 in Non-Pregnant Rabbits
Study No. R-033188-TF-043-L	Two-Week Oral Toxicity Study of RSC-033188 in Monkeys
Study No. R-033188-TF-083-L	Two-Week Oral Toxicity Study of RSC-033188 in Monkeys (Supplement)
Study No. S-033188-TB-131-L	One-month Oral Toxicity Study of S-033188 in Rats
Study No. S-033188-TF-132-L	One-month Oral Toxicity Study of S-033188 in Monkeys

Genotoxicology

Study No. R-033188-TB-064-L	Bacterial reverse mutation assay of RSC-033188
Study No. R-033188-TB-065-L	Bacterial reverse mutation assay of RSC-033447
Study No. R-033188-TF-059-L	Micronucleus Test of RSC-033447 with Cultured Mammalian Cells
Study No. R-033188-TF-060-L	Micronucleus test of RSC-033188 with Cultured Mammalian Cells
Study No. R-033188-TF-085-L	Micronucleus Test of RSC-033188 in Rats

Reproductive and Developmental Toxicology: Fertility and Early Embryonic Development

Study No. S-033188-TB-130-L	Oral Study for Effects of S-033188 of Fertility and Early Embryonic Development to Implantation in Rats

Reproductive and Developmental Toxicology: Embryo-fetal Development

Study No. R-033188-TB-070-L	Oral Study for Effects of RSC-033188 on Embryo-Fetal Development in Rats
Study No. R-033188-TF-087-L	Dose Range-Finding Oral Study for Effects of RSC-033188 on Embryo-Fetal Development in Rabbits
Study No. S-033188-TF-135-L	Oral Study for Effects of S-033188 on Embryo-Fetal in Rabbits

Reproductive and Developmental Toxicology: Pre-/postnatal Development

Study No. S-033188-TF-159-L	Oral Study for Effects of S-033188 on Pre- and Postnatal Development, Including Maternal Function, in Rats

Juvenile Toxicology

Study No. S-033188-TF-091-R	Preliminary Three-Week Oral Toxicity Study of S-033188 in Juvenile Rats
Study No. S-033188-TF-128-L	Oral Toxicity Study of S-033188 in Juvenile Rats

Special Toxicology

Study No. TOX-2013-MTS-056188A-04	Exploratory Photohemolysis Assay of MTS-056188A
Study No. TOX-2013-MTS-0563447A-01	Exploratory Photohemolysis Assay of MTS-0563447A
Study No. TOX-2013-MTS-0576188A-03	Exploratory Skin Phototoxicity Study of MTS-0576188A in Hairless Mice by Oral Dosing
Study No. S-033188-TB-144-R	Preliminary Single Intraperitoneal Toxicokinetics Study of S-033188 in Hairless Mice by Oral Dosing
Study no. S-033188-TB-149-L	Skin Phototoxicity Study of S-033188 in Hairless Mice by Oral and Intraperitoneal Dosing
Study no. R-033188-TB-007-R	Effect of Sterilized Food on RSC-033188-Induced PT and APTT Prolongation in Rats
Study no. R-033188-TB-047-L	Effect of Vitamin K on RSC-033188-Induced PT and APTT Prolongation in Rats

3.2 Studies Not Reviewed

Studies considered irrelevant for the nonclinical safety assessment were not reviewed.

3.3 Previous Reviews Referenced

Some studies were previously reviewed by Dr. Pritam S. Verma and Dr. David McMillan.

4 Pharmacology

4.1 Primary Pharmacology

S-033447 is a PA endonuclease inhibitor of influenza virus A and B replication. The IC_{50} of S-033447 was 1.4 to 3.1 nmol/L for influenza A and 4.5 to 8.9 nmol/L for influenza B. Additional complete details of the pharmacodynamics of S-033447 can be found in the clinical virology review.

4.2 Secondary Pharmacology

The cytotoxicity of S-033447 was tested in canine and bovine kidney cells and in human cells from lung and quasi-diploid tumor from nasal septum (study no. EB-117-N). Cytotoxicity (CC_{50}) of S-033447 in different cultured cells types ranged from 17.30 to 47.52 µmol/L. Under the conditions of this study, there was enough margin between cytotoxicity and an effective concentration of S-033447 to inhibit viral replication. The cytotoxicity of S-033447 was also tested in various human cell types (foreskin fibroblasts, embryonic kidney cells, hepatocellular carcinoma cells, renal glomeruli mesangial cells, vascular endothelial cells, leukemia cells, lung fibroblasts, and two different neuroblastoma cell lines) (study no. EF-232-N). With an IC_{50} ranging from 2.2 to 50 µmol/L and 3 to 100 µmol/L, S-033447 had significant cytotoxic effects on cells derived from human tissues in the proliferative and stationary phases, respectively. Additional complete details of the cytotoxicity of S-033447 can be found in the clinical virology review

To determine if S-033188 and S-033447 were toxic to mitochondria, the HepG2 cell line was used (study no. EF-197-N). There was no mitochondrial toxicity after 24 hour or 6-day exposure with S-033188 or S-033447 up to 200 µmol/L.

The inhibitory effects of S-033447 on cytopathic effect (CPE) induced by influenza virus was studied in the Madin-Darby canine kidney (MDCK) cell line (study no. EB-209-N). S-033447 induced inhibitory effects on CPE that were caused by influenza A and B.

The binding activity of RSC-033447 was tested in various receptors, ion channels, and transporters (study no. EF-044-N). The inhibitory ratios of RSC-033447 was less than 50%.

4.3 Safety Pharmacology

4.3.1 Cardiovascular

Study Title: Effects of RSC-033188 on Cardiovascular System in Monkeys (R-033188-SF-035-L)
Blood pressure (systolic, diastolic, and mean), heart rate, ECG parameters (PR, QRS, QT, and QTc intervals), and clinical signs were evaluated in telemeterized cynomolgus monkeys given a single, oral dose of 0, 200, and 400 mg/kg of RSC-033188. There were no significant effects on the cardiovascular parameters evaluated. The study was properly validated.

Study Title: Effects of RSC-033188 on Potassium Current in hERG Transfected Cells (R-033188-SF-039-L)
CHO cells transfected with hERG were used to evaluate the effect of 1, 3, or 10 µmol/L RSC-033188 on hERG potassium channel currents (the IC_{50} was not calculated). There were no significant effects on hERG currents. The assay was properly validated.

Study Title: Effects of RSC-033447 on Potassium Current in hERG Transfected Cells (R-033188-SF-040-L)
CHO cells transfected with hERG were used to evaluate the effects of 0.56, 2.06, or 8.44 µmol/L RSC-033447 on hERG potassium channel currents. There was no effect on hERG current at 0.56 or 2.06 µmol/L RSC-033447 compared to the negative control. However, a decrease in hERG currents was observed at 8.44 µmol/L (IC_{50} = 15.11 µmol/L) compared to the negative control. The assay was properly validated.

4.3.2 Neurological

Study Title: Effects of RSC-033188 on Central Nervous System in Rats (R-033188-SF-034-L)
General behavior and neurobehavioral functions in male SD rats were evaluated using a modified functional observation battery following a single, oral dose of RSC-033188 at 0, 200, 600, and 2000 mg/kg. At 600 and 2000 mg/kg there was a slight increase in rectal temperature and at 2000 mg/kg there was a decrease in urine quantity. The study was properly validated.

4.3.3 Respiratory

Study Title: Effects of RSC-033188 on Respiratory System in Rats (R-033188-SF-036-L)
Whole body plethysmography was used to measure respiratory rate, tidal volume, and minute volume in male SD rats given a single, oral dose of RSC-033188 at 0, 200, 600, or 2000 mg/kg. There were no significant effects on the respiratory parameters measured. The study was properly validated.

4.3.4 Skeletal muscle

Study Title: Effects of RSC-033188 on Action Potential in Guinea Pig Papillary Muscles (R-033188-SF-038-L)
The effects of RSC-033188 on action potential was tested in male guinea pig papillary muscles at 0.1, 0.3, or 1 µmol/L. There were no significant effects of RSC-033188 on action potential. The study was properly validated.

Study Title: Effects of RSC-033447 on Action Potential in Guinea Pig Papillary Muscles (R-033188-SF-041-L)
The effects of RSC-033447 on action potential was tested in male guinea pig papillary muscles at 0.082, 0.25, or 0.83 µmol/L. There were no significant effects of RSC-033447 on action potential. The study was properly validated.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

5.1.2 Absorption

Study Title: Dose-linearity of Concentration of RSC-033188 and RSC-033447 in Plasma After a Single Oral Administration of RSC-033188 in SD Rats (R-033188-PB-051-N)
Blood samples were collected 2, 5, 15, 30 minutes and 1, 2, 4, 6, 8, and 24 hours after IV (0.254 or 0.846 mg/kg RSC-033447) dosing and at 15 and 30 minutes and 1, 2, 4, 6, 8, 10, and 24 hours after oral (0.846 mg/kg RSC-033447, non-fasted; 0.3, 1, 3, and 10 mg/kg RSC-033188, non-fasted; or 1 mg/kg, fasted) dosing in SD rats. Pharmacokinetic parameters of RSC-033188 after oral or intravenous (IV) dosing are shown in Tables 3 and 4. The plasma concentration of RSC-033188 was below the limit of quantitation (BLQ).

Table 3: Pharmacokinetic Parameters of RSC-033447 in Plasma After a Single, Oral Dose of RSC-033188 at 0.3, 1, 3, and 10 mg/kg in Non-Fasted and 1 mg/kg in Fasted Rats

Pharmacokinetic parameter (Mean ± Standard Deviation):	Non-fasted rats				Fasted rats
	0.3 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	1 mg/kg
T_{max} (hr)	1.00 ± 0.00	1.25 ± 0.50	0.875 ± 0.250	0.875 ± 0.250	0.375 ± 0.144
C_{max} (ng/mL)	4.82 ± 0.85	17.1 ± 4.9	68.9 ± 5.8	169 ± 34	34.3 ± 13.8
AUC_{all} (ng·hr/mL)	34.7 ± 7.4	103 ± 20	468 ± 183	1110 ± 120	125 ± 11
AUC_{inf} (ng·hr/mL)	48.9 ± 21.2	101 ± 18	479 ± 195	1120 ± 120	122 ± 13
$t_{1/2,z}$ (hr)	10.4 ± 5.7	4.36 ± 0.57	4.02 ± 0.62	3.21 ± 0.10	3.68 ± 0.84
MRT_{inf} (hr)	14.1 ± 8.5	6.23 ± 0.82	5.99 ± 1.36	5.53 ± 0.67	4.74 ± 1.04
Bioavailability (%)	10.9 ± 2.3	9.77 ± 1.93	14.7 ± 5.8	10.5 ± 1.1	11.9 ± 1.0

Table 4: Pharmacokinetic Parameters of RSC-033447 in Plasma After a Single Oral (0.846 mg/kg) or IV (0.254 and 0846 mg/kg) Dose of RSC-033447 in SD Rats.

Pharmacokinetic parameter (Mean ± Standard Deviation):	Oral administration	Intravenous administration	
	0.846 mg/kg	0.254 mg/kg	0.846 mg/kg
T_{max} (hr)	5.00 ± 2.58		
C_{max} (ng/mL)	0.833 ± 0.064		
AUC_{all} (ng·hr/mL)	6.75 ± 2.70	318 ± 29	974 ± 98
AUC_{inf} (ng·hr/mL)	N.C.	314 ± 38	985 ± 97
$t_{1/2,z}$ (hr)	N.C.	3.74 ± 0.27	4.03 ± 0.15
MRT_{inf} (hr)	N.C.	4.53 ± 0.32	4.69 ± 0.31
CL_{tot} (mL/hr/kg)		819 ± 97	865 ± 83
Vd_{ss} (mL/kg)		3690 ± 270	4040 ± 290

N.C.: Not calculated

Study Title: Concentration of Radioactivity, RSC-033188, and RSC-033447 in Plasma after a Single Oral Administration of [^{14}C]-RSC-033188 in SD Rats (R-033188-PB-057-N).

The purpose of this study was to determine the concentration of radioactivity of RSC-033188 and RSC-033447 in plasma after a single oral dose of [^{14}C]-RSC-033188 (1 mg/1.94 MBq (52.4μCi)/2 mL/kg (nominal dosage)) in SD rats. The plasma concentration of RSC-033188 was BLQ. Almost 90% of plasma RSC-033447 was detected. After 24 hours, the concentration of radioactivity was low in blood cells. Pharmacokinetic parameters are presented in Table 5.

Table 5: Pharmacokinetic Parameters of Radioactivity and RSC-033447 in Plasma and Those of Radioactivity in Blood After a Single Oral Dose of [^{14}C]-RSC-033188 in Rats.

PK parameters:	Radioactivity in plasma	RSC-033447 in plasma	Radioactivity in blood
C_{max} (ng eq. of RSC-033447/mL or ng/mL)	20.6 ± 3.1	16.6 ± 3.1	20.8 ± 3.3
T_{max} (hr)	0.813 ± 0.375	1.06 ± 0.72	0.813 ± 0.375
$t_{1/2,z}$ (hr)	6.42 ± 3.31	4.93 ± 1.47	10.1 ± 5.5
AUC_{all} (ng eq. of RSC-033447·hr/mL or ng·hr/mL)	135 ± 14	121 ± 17	153 ± 16
AUC_{inf} (ng eq. of RSC-033447·hr/mL or ng·hr/mL)	149 ± 38	123 ± 22	200 ± 69
AUC_{all} ratio to radioactivity in plasma (%)	–	89.7 ± 7.2	–

Mean ± Standard deviation (n = 4)

–: Not applicable

Study Title: Dose-linearity of Concentration of RSC-033188 and RSC-033447 in Plasma after a Single Oral Administration of RSC-033188 in Cynomolgus Monkeys (R-033188-PB-028-N)

Pharmacokinetic parameters of RSC-033188 in cynomolgus monkeys after oral (0.3, 1, and 3 mg/kg RSC-033188, non-fasted; or 1 mg/kg RSC-033188, fasted) or IV (0.254 or 0.846 mg/kg RSC-033447) dosing are shown in Tables 6 and 7, respectively. The plasma concentration of RSC-033188 was BLQ.

Table 6: Pharmacokinetic Parameters of RSC-033447 in Plasma After a Single, Oral Dose of RSC-033188 at 0.3, 1, and 3 mg/kg in Non-Fasted and at 1 mg/kg in Fasted Monkeys.

Pharmacokinetic parameter (Mean ± Standard Deviation):	Non-fasted monkeys			Fasted monkeys
	0.3 mg/kg	1 mg/kg	3 mg/kg	1 mg/kg
T_{max} (hr)	2.17 ± 1.76	5.33 ± 3.06	3.33 ± 1.15	2.67 ± 1.15
C_{max} (ng/mL)	5.81 ± 1.26	19.6 ± 4.2	57.6 ± 5.4	108 ± 48
AUC_{all} (ng·hr/mL)	86.2 ± 11.8	309 ± 59	957 ± 118	1450 ± 610
AUC_{inf} (ng·hr/mL)	158 ± 86	438 ± 112	1340 ± 150	1770 ± 730
$t_{1/2,z}$ (hr)	19.6 ± 12.8	12.7 ± 2.0	12.5 ± 0.8	9.32 ± 0.32
MRT_{inf} (hr)	28.3 ± 18.1	19.0 ± 2.3	18.9 ± 1.2	14.3 ± 0.6
Bioavailability (%)	10.4 ± 1.4	11.1 ± 1.6	11.5 ± 1.3	50.6 ± 15.1

Table 7: Pharmacokinetic Parameters of RSC-033447 in Plasma After a Single Intravenous Dose of RSC-033447 at 0.254 and 0.846 mg/kg in Monkeys.

Pharmacokinetic parameter (Mean ± Standard Deviation):	0.254 mg/kg	0.846 mg/kg
AUC_{all} (ng·hr/mL)	836 ± 116	2810 ± 320
AUC_{inf} (ng·hr/mL)	1050 ± 150	3480 ± 410
$t_{1/2,z}$ (hr)	12.0 ± 0.8	11.3 ± 0.7
MRT_{inf} (hr)	13.9 ± 1.3	13.1 ± 0.6
CL_{tot} (mL/hr/kg)	245 ± 36	245 ± 27
Vd_{ss} (mL/kg)	3400 ± 550	3210 ± 370

Study Title: Determination of RSC-033188 and RSC-033447 in Monkey Plasma -Plasma Concentration of Radioactivity, RSC-033188, and RSC-033447 and Excretion of Radioactivity after a Single Oral Administration of [^{14}C]-RSC-033188 in Cynomolgus Monkeys (R-033188-PB-052-N).

Following a single dose of 3 mg/kg [^{14}C]-RSC-033188 in cynomolgus monkeys, the plasma concentrations of RSC-033447 were detected (see Table 8), while the plasma concentration of RSC-033188 was BLQ.

Table 8: Individual Plasma Concentrations of RSC-033447 in Monkeys.

Animal No.	Plasma Concentration (ng/mL)									
	0.25 hr	0.5 hr	1 hr	2 hr	4 hr	6 hr	8 hr	12 hr	24 hr	48 hr
01101	BLQ	3.65	15.6	23.3	43.6	39.7	37.0	28.6	21.0	7.39
01102	11.1	9.72	19.3	29.2	38.0	55.2	34.7	29.8	15.0	4.12
01103	19.9	55.1	80.1	180	130	95.6	96.2	57.8	34.4	16.2

Study Title: Plasma Concentration of Radioactivity, RSC-033188, and RSC-033447 and Excretion of Radioactivity after a Single Oral Administration of [14C]-RSC-033188 in Cynomolgus Monkeys (R- 033188-PF-026-N).

Pharmacokinetic parameters of radioactivity in blood and plasma and radioactivity in urine, feces, and bile were determined following a single oral dose of 3mg/kg [^{14}C]-RSC-

033188 in male cynomolgus monkeys. Approximately 80% of RSC-033447 in the plasma was accounted for (Table 9). However, detection of RSC-033188 was BLQ. The distribution ratios of radioactivity in the blood cells were 38 to 52% from 15 minutes to 48 hours after administration. The major excretion route was in the feces (Table 10).

Table 9: Pharmacokinetic Parameters of RSC-033447 in Plasma and Blood

PK parameters:	Radioactivity in plasma	RSC-033447 in plasma	Radioactivity in blood
C_{max} (ng eq. of RSC-033447/mL or ng/mL)	119 ± 98	92.9 ± 75.6	118 ± 92
t_{max} (h)	4.0 ± 2.0	4.0 ± 2.0	4.0 ± 2.0
$t_{1/2,z}$ (h)	17.8 ± 4.0	16.7 ± 3.7	17.3 ± 2.7
AUC_{all} (ng eq. of RSC-033447·h/mL or ng·h/mL)	1680 ± 820	1390 ± 800	1950 ± 1000
AUC_{inf} (ng eq. of RSC-033447·h/mL or ng·h/mL)	1990 ± 1020	1640 ± 1000	2270 ± 1150
AUC_{inf} ratio to radioactivity in plasma (%)	--	80.0 ± 7.5	--

Mean ± Standard deviation (n = 3)
--: Not applicable

Table 10: Cumulative Radioactive Excretion in Bile, Urine, and Feces After a Single Oral Dose of 3 mg/kg [14C]-RSC-033188 to Bile Duct-Cannulated Male Monkeys.

Time (h)	Cumulative radioactivity excretion (% of dose)				
	Bile	Urine	Feces	Cage washing	Total
0-6	2.3 ± 0.8	0.3 ± 0.1	--	0.1 ± 0.1	2.3 ± 0.8
-24	6.1 ± 0.5	0.8 ± 0.1	45.6 ± 25.1	0.2 ± 0.1	52.8 ± 25.5
-48	8.0 ± 0.5	1.1 ± 0.1	86.5 ± 3.7	0.3 ± 0.2	95.8 ± 3.5
-72	8.5 ± 0.6	1.2 ± 0.1	89.5 ± 2.3	0.3 ± 0.2	99.5 ± 2.0
Gastro-intestinal tract contents					0.2 ± 0.1
Total recovery					99.6 ± 2.0

Data are expressed as the mean ± S.D. of three animals.

--: Not determined

Study Title: Mineral Effects on the Concentration of S-033447 in Plasma after a Single Oral Administration of S-033188 in Cynomolgus Monkeys (S-033188-PB-244-N).

The effect of several minerals on the pharmacokinetic parameters of S-033447 was tested following a single oral dose of 0.1 mg/kg S-033188 in monkeys (Table 11). The exposure, AUC_{inf} and C_{max}, was decreased with co-administration of S-033447 and minerals.

Table 11: The Effect of Minerals on S-033447 in Plasma After a Single Oral Dose of S-033188 in Monkey.

Pharmacokinetic parameters	Condition 1 (Control)	Condition 2 (Ca)	Condition 3 (Mg + Al)	Condition 4 (Fe)	Condition 5 (Human food)
T_{max} (hr)	2.33 ± 1.53	2.00 ± 0.00	2.00 ± 0.00	1.33 ± 0.58	2.67 ± 1.15
C_{max} (ng/mL)	80.1 ± 46.3	50.1 ± 17.2	49.5 ± 20.1	38.3 ± 12.5	43.1 ± 18.6
AUC_{inf} (ng·hr/mL)	1220 ± 720	772 ± 247	755 ± 308	596 ± 217	757 ± 356

Data represent as the mean ± SD of 3 monkeys.

Study Title: Concentrations of S-033188 and S-033447 in Plasma after a Single Oral Administration of S-033188 in Juvenile SD Rats (S-033188-PF-148-N).
Following a single oral dose of S-033188 at 3, 10, and 30 mg/kg in male juvenile SD rats, the pharmacokinetic profile of S-033188 was only detected in rats at postnatal day (PND) 10 (Table 12). The Pharmacokinetic profile of S-0334477 could be determined at all ages and doses (Table 13).

Table 12: Pharmacokinetic Parameters of S-033188 After a Single Oral Dose of S-033188 in Male Juvenile Rats.

Age (at drug administration)	Dose (mg/kg)	Pharmacokinetic parameters					
		C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{0\text{-}24\,h}$ (ng·h/mL)	AUC_{inf} (ng·h/mL)	MRT_{inf} (h)
10 days old	3	5.15	0.5	NC	2.91	NC	NC
	10	4.82	0.25	0.781	5.60	5.44	0.969
	30	14.8	0.25	1.17	17.6	17.3	1.60
20 days old	3	NC	NC	NC	NC	NC	NC
	10	NC	NC	NC	NC	NC	NC
	30	NC	NC	NC	NC	NC	NC
30 days old	3	NC	NC	NC	NC	NC	NC
	10	NC	NC	NC	NC	NC	NC
	30	NC	NC	NC	NC	NC	NC

NC: Not calculated

Table 13: Pharmacokinetic Parameters of S-033447 After a Single Oral Dose of S-033188 in Male Juvenile Rats.

Age (at drug administration)	Dose (mg/kg)	Pharmacokinetic parameters					
		C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{0\text{-}24\,h}$ (ng·h/mL)	AUC_{inf} (ng·h/mL)	MRT_{inf} (h)
10 days old	3	688	2	9.22	9420	11200	12.6
	10	1880	4	7.45	23800	26800	10.4
	30	4570	1	6.35	48100	51800	8.42
20 days old	3	202	1	4.29	1450	1470	6.15
	10	707	1	3.61	3490	3520	5.15
	30	962	0.5	2.85	7640	7670	5.00
30 days old	3	90.4	0.5	2.87	472	473	4.07
	10	313	1	2.86	1490	1490	3.73
	30	609	0.5	2.74	3150	3160	4.21

Study Title: Concentration of S-033447 in Plasma after a Single Intravenous Administration of S-033447 in Juvenile SD Rats (S-033188-PF-190-N).
Male juvenile SD rats were given a single IV dose of 0.254 mg/kg S-033447. Plasma concentrations were highest in the PND 10 (Table 14). The $t_{1/2}$ and exposure, $AUC_{0\text{-}24h}$ and AUC_{inf}, was longer in PND 10 (Table 15). Although bioavailability (BA) was detected at all ages, it was highest at PND 10 (Table 16).

Table 14: Plasma Concentration of S-033447 After a Single IV Dose of 0.254 mg/kg S-033447 in Male Juvenile Rats.

Time after administration (h)	Concentration (ng/mL)					
	10 days old		20 days old		30 days old	
	Individual	Mean ± SD	Individual	Mean ± SD	Individual	Mean ± SD
0.083	327 254 257	279 ± 41	158 124 125	136 ± 19	80.0 97.7 96.4	91.4 ± 9.9
0.25	239 191 210	213 ± 24	137 104 94.6	112 ± 22	53.6 53.0 59.2	55.3 ± 3.4
0.5	128 170 185	161 ± 30	94.0 65.5 67.7	75.7 ± 15.9	53.6 51.7 57.5	54.3 ± 3.0
1	123 112 142	126 ± 15	58.4 47.4 58.3	54.7 ± 6.3	32.7 38.2 33.9	34.9 ± 2.9
2	NA 89.7 NA	89.7*	45.3 32.0 50.6	42.6 ± 9.6	23.3 25.9 22.8	24.0 ± 1.7
4	95.1 103 93.3	97.1 ± 5.2	33.2 28.0 30.6	30.6 ± 2.6	12.0 16.0 11.1	13.0 ± 2.6
8	59.6 72.6 64.7	65.6 ± 6.6	12.6 14.4 12.1	13.0 ± 1.2	3.91 4.46 3.70	4.02 ± 0.39
24	18.2 12.0 18.1	16.1 ± 3.6	1.69 0.803 0.701	1.06 ± 0.54	BLQ BLQ BLQ	BLQ

BLQ: Below the lower limit of quantitation
NA: Not available
*: Individual value

Table 15: Pharmacokinetic Profile of S-033447 After a Single IV Dose of S-033447 in Male Juvenile Rats.

Pharmacokinetic parameters	Age (days old at drug administration)		
	10	20	30
$t_{1/2}$ (h)	7.77	4.15	2.33
$AUC_{0\text{-}24\ h}$ (ng·h/mL)	1460	410	190
AUC_{inf} (ng·h/mL)	1640	417	171
MRT_{inf} (h)	9.93	4.78	2.84
CL_{tot} (mL/h/kg)	155	610	1490
Vdss (mL/kg)	1540	2920	4230

Table 16: Bioavailability of S-033447 After a Single Oral Dose of S-033188 in Male Juvenile Rats.

Pharmacokinetic parameter	Dose (mg/kg as S-033188)	Age (days old at drug administration)		
		10	20	30
BA (%)	3	68.3	35.3	27.7
	10	49.0	25.3	26.1
	30	31.6	18.4	18.5

The BA was calculated from the AUC_{inf} of S-033447 after oral administration of S-033188 obtained in study 17-295[1)] and the AUC_{inf} of S-033447 after intravenous administration of S-033447.

5.1.3 Distribution

Study Title: Exploratory Study on Serum Protein Binding Ratio of MTS-0563447A in Mouse (DMPK-2013-MTS-0576188A-03).
The serum protein binding ratio of MTS-0563447 in 2000 ng/ml mouse serum was 99.2%.

Study Title: In Vitro Plasma/Blood Cell Partitioning and Protein Binding of [^{14}C]-RSC-033447 (R-033188-PB-021-N)
The *in vitro* protein biding ratios of [^{14}C]-RSC-033447 (50, 100, or 1000 ng/ml) in SD rat, cynomolgus monkey, and human sera under fasted conditions were 91.9 to 92.1%, 85 to 89.5%, and 92.9 to 93.9%, respectively. The protein binding ratios in 4% albumin, 0.08% α-1-acid glycoprotein, and 1% γ-globulin solutions were 91.2 to 92.1%, 52.2 to 59.3%, and 23.6 to 38.1%, respectively. Under fasted conditions, the concentration ratios of blood to plasma were 1.45 to 1.55, 1.15 to 1.23, and 1.14 to 1.22 in SD rat, cynomolgus monkey, and human sera, respectively. The distribution ratios in blood cells were 57.1 to 59.6%, 50.4 to 52.9%, and 48.5 to 54.4% in rat, monkey and human sera, respectively. There were no species differences or concentration-dependent changes in both the serum protein binding ratios and the distribution ratios in blood cells of [^{14}C]-RSC-033447.

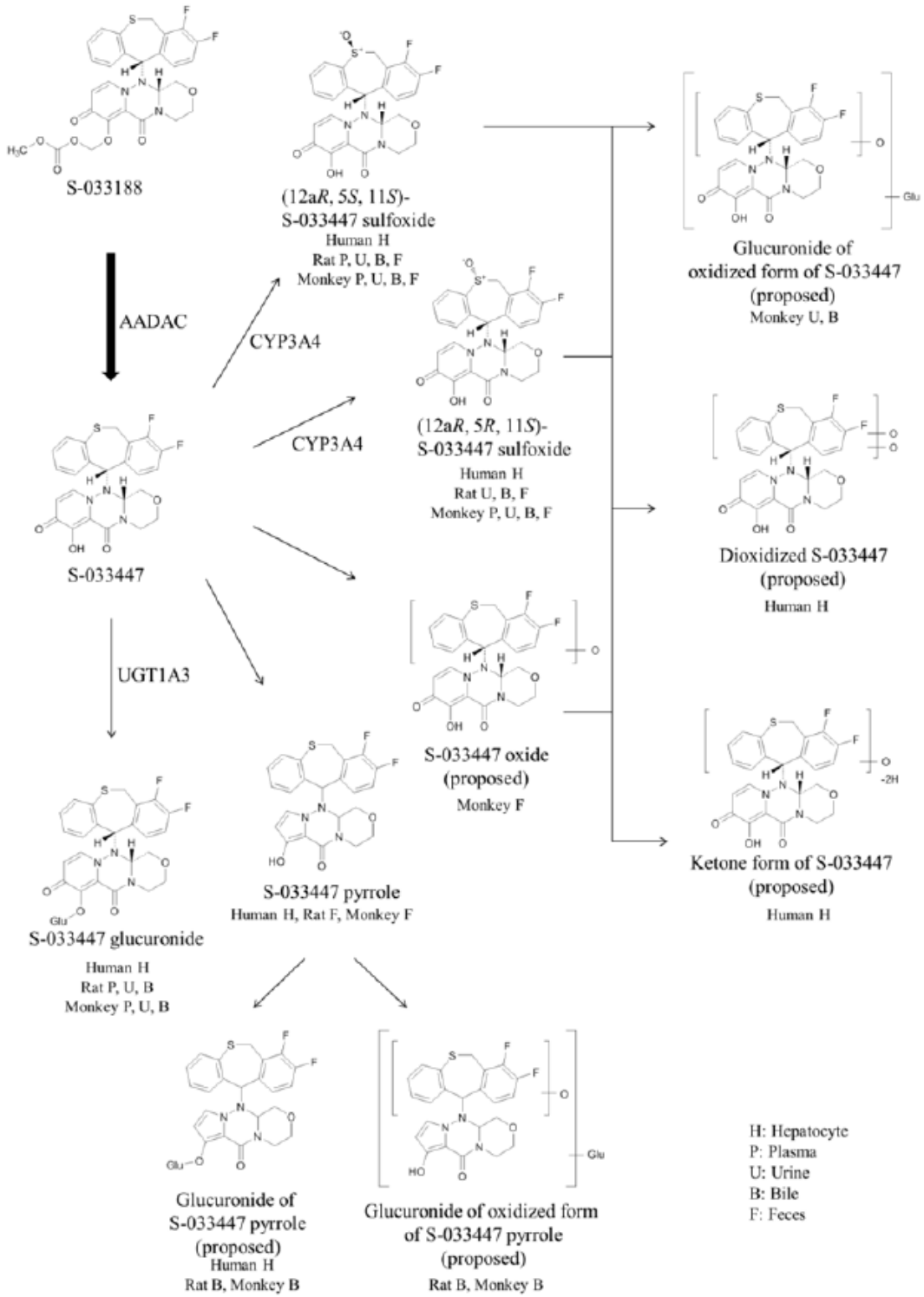
Study Title: Quantitative Whole-Body Autoradiography After a Single Oral Administration of [^{14}C]-RSC-033188 in Pigmented SD Rats (R-033188-PB-020-N).
Whole-body radiography was used to determine the distribution and retention of [^{14}C]-RSC-033447 in the tissues of pigmented SD rats after a single oral dose of 1 mg/kg [^{14}C]-RSC-033188. The radioactivity was mostly distributed to the intestinal mucosa and liver. The maximum levels of radioactivity were detected in most of the tissues between 1 and 2 hours post-dose. The radioactivity in the central nervous system was BLQ at all time points. At 24 hours post-dose, the radioactivity in most tissues was BLQ. The longest elimination half-life, $t_{1/2}$, was observed in the bone at 63 hours, in the liver at 13 hours, and in the bone marrow at about 10 hours. However, at 336 hours post-dose the radioactivity in these tissues were BLQ.

Study Title: Quantitative Whole-Body Autoradiography After a Single Oral Administration of [^{14}C]-S-033188 in Pregnant SD Rats (S-033188-PF-177-L).
Whole-body radiography was used to determine the distribution of radioactivity after a single oral dose of 1 mg/kg [^{14}C]-S-033188 in SD rats. Timed-pregnant dams received a single oral dose at gestation day 18. Peak concentration of radioactivity in the tissues of dams occurred at 8 hours post-dose. However, peak concentration of radioactivity in the liver occurred at one hour post-dose. The liver had the highest concentration of radioactivity compared to other tissues with the second highest being in the adrenal cortex, which had high concentrations of radioactivity up to 8 hours post-dose. Most tissue radioactivity was BLQ 24 hours post-dose. Low levels of radioactivity (less than twice the limit of quantification) were detected in fetal tissues. Elevated levels in fetal tissue were in the adrenal cortex and bones and could be detected up to 48 hours post dose. Elevated levels were also detected in the bones of the dams.

5.1.4 Metabolism

The metabolic pathway of RSC-033188 after a single, oral administration of [^{14}C]-RSC-033188 in SD rats (study no. R-033188-PB-033-N) and cynomolgus monkeys (R-033188-PB-055-N) and following a single, oral administration of S-033188 in humans (study no. S-033188-CB-102-N) is proposed in Figure 1.

Figure 1: Metabolic Pathway of Baloxavir Marboxil in Rats, Monkeys, and Humans.



S-033188 = baloxavir marboxil

Study Title: Effects of RSC-033188 on Hepatic Drug Metabolizing Enzymes in Two-Week Oral Toxicity Study in SD Rats (Study no. PF-080-N)
The purpose of this study was to determine the contents of microsomal proteins, total P450, and activities of drug-metabolizing enzymes (7-ethoxy-resorufin-O-deethylase (EROD), testosterone 6β-, 16α-, and 16β-hydroxylase, *p*-nitrophenol uridine 5'-diphospho-glucuronosyltransferase (UDPGT), T3 UDPGT, and T4 UDPGT) in frozen SD rat liver samples from the two-week oral toxicity study in SD rats (Study no. R-033188-TB-032-L). In female SD rats, there were statistically significant increases in microsomal proteins, total P450, testosterone 6β-, and 16β-hydroxylase, *p*-nitrophenol UDPGT, and T3 and T4 UDPGT in the 2000 m/kg/day group. There was no difference in EROD activity. In male rats, there were statistically significant increases in total P450, testosterone 6β-, and 16β-hydroxylase, and T3 and T4 UDPGT activities in the 2000 m/kg/day group. There was a statistically significant decrease in testosterone 16α-hydroxylase. There was no difference in microsomal protein content, EROD activity, or *p*-nitrophenol UDPGT activity.

5.1.5 Excretion

Study Title: Urinary, Fecal, and Biliary Excretion of Radioactivity After a Single Oral Administration of [^{14}C]-RSC-033188 in SD Rats (R-033188-PB-013-N)
The major excretory route of RSC-033188 and its metabolites was in the feces via the bile following a single oral dose of [^{14}C]-RSC-033188 (1 mg/1.94 MBq (52.4 µCi)/2 ml/kg) in rats. Urinary excretion was less than 1% of the dose.

Study Title: Enterohepatic circulation of Radioactivity After a Single Oral Administration of [^{14}C]-S-033188 in SD Rats (Study no. S-033188-PF-151-N)
The purpose of this study was to determine the enterohepatic circulation of S-033188 in male rats after a single oral dose of 1 mg/kg to donor rats. The bile from the donor rat was later removed and administered to the duodenum of a recipient rat via cannula. In the recipient rat, the ratio of reabsorption to the dose and to the amount of radioactivity recovered was 1.4% and 6.7%, respectively. The ratio of enterohepatic circulation to the dose was 0.9% and the amount of radioactivity recovered was 4.2%. Based on these results, S-033188 and its metabolites excreted into the bile were subjected to minor enterohepatic circulation.

Study Title: Excretion into Milk of Radioactivity After a Single Oral Administration of [^{14}C]-S-033188 in Nursing SD Rats (Study no. S-033188-PF-176-N)
The maximum radioactivity concentration of S-033447, 124 ng eq., in milk was reached 2 hours after a single, oral dose of 1 mg/kg [^{14}C]-S-033188 to nursing SD rats. The maximum radioactivity concentration in plasma of S-033447, 34.9 ng eq., was reached 1 hour after dosing. After 24 hours, the radioactivity concentration in plasma and milk was undetectable.

5.2 Toxicokinetics

Determination of RSC-033188 and RSC-033447 in Kbl: NZW Rabbit Plasma – Two-week Oral Toxicity Study of RSC-033188 in Non-pregnant Kbl: NZW Rabbits (Study no. R-033188-TB-077-L). Plasma samples were taken from the two-week oral toxicity study in non-pregnant Kbl: NZW rabbits (study no. R-033188-TF-066-L). All control samples tested were BLQ. Refer to Table 17 for the results.

Table 17: TK Parameters of RSC-033447 in Female Rabbits.

Day	Dosage Level (mg/kg/day)		$AUC_{0\text{-}24hr}$ (µg·hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	C_{24hr} (µg/mL)
1	100	Mean	13.7	1.36	1.3	0.209
		SD	3.33	0.388	0.6	0.0790
		N	3	3	3	3
	300	Mean	27.1	1.61	1.0	0.764
		SD	3.76	0.199	0.0	0.242
		N	3	3	3	3
	1000	Mean	36.6	1.65	13.3	1.60
		SD	2.88	0.108	9.2	0.107
		N	3	3	3	3
14	100	Mean	14.3	1.53	1.0	0.195
		SD	1.90	0.211	0.0	0.0655
		N	3	3	3	3
	300	Mean	20.5	1.80	1.0	0.371
		SD	3.34	0.174	0.0	0.0631
		N	3	3	3	3
	1000	Mean	28.1	1.69	4.7	0.742
		SD	5.43	0.119	1.2	0.242
		N	3	3	3	3

Study Title: Determination of RSC-033188 and RSC-033447 in Cynomolgus Monkey Plasma -Effects of RSC-033188 on Cardiovascular System in Monkeys (R-033188-TB-054-L). Male cynomolgus monkeys were given a single, oral dose of 0, 200, or 400 mg/kg RSC-033188 to measure plasma concentrations of RSC-033188 and plasma and evaluate its TK profile. RSC-033188 plasma samples were below the limit of quantitation. Plasma concentration and the TK profile of RSC-033447 are presented in Tables 18 and 19, respectively.

Table 18: Mean Plasma Concentrations of RSC-033447 in Monkeys.

Dose Level (mg/kg)		Plasma Concentration (µg/mL)					
		2 hr	4 hr	6 hr	8 hr	10 hr	24 hr
200	Mean	0.614	0.849	0.944	0.952	0.796	0.436
	SD	0.0647	0.0752	0.134	0.191	0.210	0.160
	N	4	4	4	4	4	4
400	Mean	0.621	0.852	1.19	1.09	1.27	1.21
	SD	0.0808	0.0889	0.359	0.329	0.426	1.06
	N	4	4	4	4	4	4

Table 19: TK Parameters of RSC-033447 in Monkeys.

Dose Level (mg/kg)		$AUC_{0\text{-}24hr}$ (µg·hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	C_{24hr} (µg/mL)
200	Mean	16.1	1.02	7.5	0.436
	SD	2.77	0.132	1.0	0.160
	N	4	4	4	4
400	Mean	26.2	1.64	11.5	1.21
	SD	11.3	0.823	8.5	1.06
	N	4	4	4	4

6 General Toxicology

6.1 Single-Dose Toxicity

There were no single-dose toxicity studies conducted. Acute toxicity was examined in the 2-week repeat dose toxicity studies in SD rats, Kbl: NZW rabbits, and cynomolgus monkeys.

6.2 Repeat-Dose Toxicity

Two-week Oral Toxicity Study of RSC-033188 in Rats (Study No. R-0 299-TB-032-L). Mortality, clinical observations, body weight, food consumption, ophthalmoscopy, clinical pathology, organ weights, gross pathology, histopathology, and toxicokinetic parameters were evaluated in SD rats following daily oral doses of vehicle (0.5 w/v% methylcellulose (MC) aqueous solution) or drug (20, 200, 2000 mg/kg/day; 10/sex/main group and 10/sex/TK group) for 2 weeks. There were no drug-related mortalities. Prolongation of prothrombin time PT and activated partial thromboplastin time (APTT) were noted at the mid and high doses. Increased liver weights due to hepatocyte hypertrophy noted at the mid and high doses correlated with fatty change of periportal hepatocytes (at all doses). Thyroid hyperplasia of the follicular epithelium was noted at the mid and high doses and a decrease in colloid was noted in all doses.

Two-week Oral Toxicity Study of RSC-033188 in Non-Pregnant Rabbits (Study No. R-033188-TF-066-L). Mortality, clinical observations, body weight, food consumption, ophthalmoscopy, clinical pathology, gross pathology at necropsy, and toxicokinetic parameters were evaluated in non-pregnant Kbl: NZW rabbits following daily oral doses of vehicle (0.5 w/v% MC aqueous solution) or drug (100, 300, and 1000 mg/kg/day; 3/sex/group) for 2 weeks. In the high dose, there was a significant decrease in body weight noted on days 6 and 7 of dosing. On day 4 there was a decrease in food consumption noted in the high dose group. Abnormal stool color was noted in the high dose group towards the end of the dosing period. There were no gross findings detected at necropsy.

Two-week Oral Toxicity Study of RSC-033188 in Monkeys (Study No. R-033188-TF-043-L). Mortality, clinical observations, body weight, food consumption, ophthalmoscopy, ECG, clinical pathology, organ weights, gross pathology, histopathology, and toxicokinetic parameters were evaluated in cynomolgus monkeys following daily oral doses of vehicle (0.5 w/v% MC aqueous solution) or drug (0, 20, 60, and 200 mg/kg; 3/sex/main group; 2/sex/main group saved for the recovery period) for 2 weeks. There were no drug-related mortalities. Sporadic vomiting occurred in 4/5 males and 3/5 females at the high dose 1 or 4 hours after dosing from dose day 4 until the end of the dosing period. There were statistically significant increases of hepatic enzymes (ALT, GLDH, LAP, and GGT) at all doses on dosing days 7 and 14. Although there were no changes in liver weights or histopathology to correlate with the increase in liver enzymes. The Sponsor repeated the two-week oral toxicity study in monkeys at lower doses (see below study no. R-033188-TF-083-L).

Two-week Oral Toxicity Study of RSC-033188 in Monkeys (Supplement) (R-033188-TF-083-L). Mortality, clinical observations, body weight, food consumption, blood chemistry and toxicokinetic parameters were evaluated in cynomolgus monkeys following daily oral doses of vehicle (0.5%w/v% methylcellulose aqueous solution) or drug (0, 3, and 10 mg/kg; 3/sex/main group) for 2 weeks. There were no drug-related mortalities. There were significant increases in AST, ALT, and GLDH noted in 1/3 females at the mid dose. There were no findings changes at the high dose.

Study title: One-Month Oral Toxicity Study of S-033188 in Rats

Study no.:	S-033188-TB-131-L
Study report location:	EDR 4.2.3.2
Conducting laboratory and location:	Shionogi Pharmaceutical Research Center Shionogi and Co., Ltd. 3-1-1, Futaba-cho, Toyonaka, Osaka 561-0825, Japan
Date of study initiation:	October 29, 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	S-033188, lot#A580012, 98.2% pure

Key Study Findings

- NOAEL = 20 mg/kg/day (Day 28: $AUC_{0\text{-}24hr}$ = 0.770 µg•hr/ml, C_{max} = 0.123 µg/ml).
- The NOAEL was based on the following findings:
  - Findings in liver of increased weights with correlative gross pathology, histopathology, and serum chemistry findings at the mid and high doses.
  - Changes in platelet levels, clotting factors, and histopathology in the thyroid at the mid and high doses.
    - Changes in clotting factors were due to the 16-hour fast prior to necropsy. Special toxicology studies (study no. R-033188-TB-007-R and R-033188-TB-047-L) determined minor or no PT and/or APTT prolongation when animals were not fasted prior to necropsy or were administered vitamin K injections under fasting conditions prior to necropsy.

Methods

Doses:	0, 20, 200, and 2000 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Oral
Dose volume:	10 ml/kg
Formulation/Vehicle:	0.5 w/v% aqueous MC solution
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	10/sex/main study 5/sex/recovery
Age:	6 weeks
Weight:	196.3 to 250.9 g (males) 142.1 to 190.6 g (females)
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that affected data interpretation.

Observations and Results

Mortality

Rats were observed once daily for mortality and morbidity. All animals survived until their scheduled sacrifice.

Clinical Signs

Clinical examinations were conducted once daily. Partially white feces were noted at Day 29 in 7 high dose males and 5 high dose females.

Body Weights

Rats were weighed once every 3 to 4 days until the end of recovery. There were no drug-related effects on body weights.

Feed Consumption

Food intake was measured once every 3 to 4 days until the end of recovery. Decrease food intake in high dose males occurred from days 1 to 4 and in high dose females from days 12 to 16. There was an increase in food intake in high dose females from days 16 to 20.

Ophthalmoscopy

Ophthalmic exams were conducted at pretreatment and on days 22 and 54/55. During pre-dose examinations, animals used as control or dosed groups had ocular findings of punctate opacity in the cornea, lens nuclear opacity, lens anterior subcapsular opacity, persistent pupillary membrane and fundus hemorrhage. These ocular findings detected prior to dosing were see in historical control (Clinton and Lange, 1991) rats of this strain at this facility and SD rats are reported to develop spontaneous ocular lens and/or hemorrhage (Durand, et al. 2001). There were no drug-related findings.

Hematology

Blood was collected for hematology and clinical chemistry on Days 29 and 56/57. The following findings were observed:

- Increased platelets (14%) in high dose males.
- Increased PT (19-79%) and APTT (32-34%) in high dose males and females. Increased APTT (24%) in mid dose females.
- Increased fibrinogen (19%) in high dosed females.

Separate studies (study no. R-033188-TB-007-R and R-033188-TB-047-L) examining the food and vitamin K1 effects revealed minor or no prolongation of PT and/or APTT when animals were not fasted prior to necropsy, when animals were administered vitamin K1 injections under fasted conditions, or when animals were fed.
There were no drug-related changes observed at the end of the recovery.

Clinical Chemistry

The following changes were observed at day 29:

- A statistically significant increased total protein (5%) in high dose males and decreased A/G ratio (13%) in mid and (23%) high dose females.
- Decreased total bilirubin (25-50%) in all dosed animals.
- Decreased triglycerides (21%) in the mid and (40%) in the high dose males.
- Increased cholesterol (36%) and glucose (10%) in high dose females.
- Increase amylase (17-37%) in all dosed females.

There was also an increase in calcium in the high dose males and females at Day 29 but its significance is unclear.

The following changes observed at the end of recovery (Days 56/57) were not statistically significant:

- Decreased AST (17%) in high dose males.
- Increased amylase (25%) in high dose females.

Urinalysis

Urine was collected at Days 26 and 55/56. There were no drug-related findings.

Gross Pathology

Gross pathology was evaluated at necropsy at days 29 and 55/56. The following findings were observed at day 29:

- Accentuated lobular pattern in the liver in 3 males and 3 females at the high dose.
- Liver enlargement in 2 high dose males.
- Discoloration in the left liver lobe in 1 control female and 1 high dose male.

There were no findings observed at the end of recovery.

Organ Weights

All tissues were weighed. The following changes were observed at necropsy on day 29:

- Increased liver weight (7%) in the mid and (36%) high dose females.

There were no findings noted at the end of the recovery period.

Histopathology

Adequate Battery Yes

Peer Review No

Histological Findings

Histopathology was evaluated at necropsy on days 29 and 56/57. Liver, thyroid, and pituitary gland tissues from all animals were evaluated. All other tissues were only

evaluated from control and high dose groups. The following changes in liver and thyroid are detailed in Tables 20 and 21, respectively.

Table 20: Drug-related Changes in the liver at the End of Dosing.

sex		Male				Female			
Dose (mg/kg/day)		0	20	200	2000	0	20	200	2000
Number of examined		10	10	10	10	10	10	10	10
Hypertrophy of hepatocyte, centrilobular	minimal	0	0	0	3	0	0	0	3
Macrovesicular fatty change of hepatocyte, periportal	minimal	0	1	1	1	1	4	2	3
	mild	0	0	0	1	0	0	1	1

Table 21: Drug-related changes in the Thyroid at the End of Dosing.

sex		Male				Female			
Dose (mg/kg/day)		0	20	200	2000	0	20	200	2000
Number of examined		10	10	10	10	10	10	10	10
Decrease of colloid	minimal	2	2	4	4	0	0	3	4
	mild	0	0	0	1	0	0	0	2
Hyperplasia of follicular epithelium, diffuse	minimal	0	0	1	2	0	0	0	2

Toxicokinetics

Blood was collected for TK analysis at predose and on days 14 and 28 at 1, 2, 4, 8, and 24 hours post dose. Plasma concentrations of S-033188 were BLQ at 24 hours post dose. Toxicokinetic parameters for S-033447 are represented in Table 22.

Table 22: TK Parameters of S-033447 in Rats.

TK Parameters of S-033447		Dose of S-033188 (mg/kg/day)					
		20		200		2000	
		Male	Female	Male	Female	Male	Female
C_{max} (µg/mL)	Day 1	0.240	0.189	0.713	0.730	1.03	1.24
	Day 14	0.137	0.0848	0.195	0.229	0.307	0.305
	Day 28	0.124	0.123	0.198	0.204	0.275	0.460
$AUC_{0\text{-}24hr}$ (µg·hr/mL)	Day 1	1.34	0.990	5.90	6.18	11.1	17.3
	Day 14	0.807	0.576	1.74	1.83	3.75	3.30
	Day 28	0.817	0.722	1.66	1.72	3.40	4.76

Mean value (n = 4).

Dosing Solution Analysis
Acceptance criteria were met for homogeneity and concentration. There was no drug detected in control formulations.

Study title: One-month Oral Toxicity Study of S-033188 in Monkeys

Study no.:	S-033188-TF-132-L
Study report location:	EDR 4 2 3 2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 27, 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	S-033188, lot#A580013, 98% pure

Key Study Findings

- NOAEL = 10 mg/kg (Day 28: $AUC_{0\text{-}24hr}$ = 3.1 µg•hr/ml, C_{max} = 0.13 µg/ml).
- The NOAEL was based on the following findings:
  - Statistically significant increases in AST, ALT, GLDH in high dose males and females that correlated to increased liver weights.
  - Histopathology findings in the thyroid and testes at the mid and high doses.
    - Testicular findings are not likely due to baloxavir marboxil because there was no clear dose response; the sponsor provided historical control data for this species of monkey with the same findings; testicular findings were not observed at the end of recovery in the one-month study; and testicular findings were not observed in the 2-week repeat dose study in monkey at higher doses.

Methods

Doses:	0, 1, 10, and 100 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral
Dose volume:	5 ml/kg
Formulation/Vehicle:	0.5 %w/v aqueous MC solution
Species/Strain:	Cynomolgus monkey (China)
Number/Sex/Group:	3/sex/main study group (all doses) 2/sex/recovery group (control and high dose)
Age:	4 to 6 years
Weight:	4.2 to 5.89 kg (male); 2.81 to 3.75 kg (female)
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that would affect data interpretation.

Observations and Results

Mortality

Monkeys were observed once daily for mortality and morbidity. All animals survived until their scheduled sacrifice.

Clinical Signs

Clinical examinations were conducted once daily. There were no drug-related findings.

Body Weights

Monkeys were weighed at pretreatment and once every 4 days until the end of recovery (Day 56). There were no drug-related effects on body weights.

Feed Consumption

Food intake was measured daily. There were no drug-related effects on consumption.

Ophthalmoscopy

Ophthalmic exams were conducted at pretreatment and during recovery (day 50). There were no drug-related findings.

ECG

ECG's were conducted at pretreatment, on dose day 22, and during recovery (Day 50). There were no drug-related changes.

Hematology

Blood was collected at pretreatment; on dosing days 14 and 27; and during recovery (Days 42 and 56). There were no drug-related findings.

Clinical Chemistry

Blood was collected at pretreatment; on dosing days 7, 14, 21, and 27; and on recovery days 35, 42, and 56. The following findings were observed:

- Statistically significant increased AST (1.5- to 2.8-fold), ALP (1.2- to 1.9-fold), ALT (1.7- to 6.3-fold) in high dose males and females during dosing.
- Increased ALP (1.8-fold) in high dose females only at recovery.
- Increased GGT (1.2- to 2.1-fold) in high dose males during dosing.
- Increased GLDH in high dose males (1.6- to 23.12-fold) and females (3- to 12.5-fold) during dosing and remained increased in males only during recovery.

There were no findings detected at the end of recovery.

Urinalysis

Urine was collected at pretreatment; on dosing day 26; and on recovery day 56. There were no drug-related findings.

Gross Pathology

Gross pathology was evaluated at necropsy at the end of dosing on day 29 and at the end of recovery on day 57. One male animal at the mid dose had an adhesion on the right testis/epididymis noted on day 29. This same animal had marked fibrosis and seminiferous tubule degeneration/necrosis on the right testis. Testicular findings are not likely drug-related because the sponsor provided historical control data for this species of monkey with the same findings; they were not seen at the end of the recovery period; and they were not observed in the 2-week repeat-dose study in monkey up to 200 mg/kg.

Organ Weights

The following findings were observed at the end of recovery.

- Increased bilateral thyroid weight (over 100%) in high dose males.
- Decrease prostate weight (35%) in high dose males.
- Decreased liver (11%), kidney (16%), pancreas (22%), and pituitary (17%) weights in high dose males. These changes were not significant and may be incidental and not drug-related.

Histopathology

Adequate Battery Yes

Peer Review No

Histological Findings
Histopathology was evaluated at necropsy at the end of dosing on day 29 and at the end of recovery on day 57. Table 23 summarizes the testicular findings observed at day

29 that are not drug-related because they were not seen at the end of the recovery period, they were not observed in the 2-week repeat-dose study in monkey up to 200 mg/kg, and similar findings were seen in historical control animals.

Table 23: Testicular Histopathology Findings (Day 29)

Findings	Group	0.5% MC			S-033188			S-033188			S-033188		
	Dose (mg/kg/day)				1			10			100		
	Animal No.	3	4	5	11	12	13	17	18	19	25	26	27
Testis (left)													
Cellular infiltration, mononuclear cell		-	-	-	-	-	+	-	-	-	-	-	-
Degeneration, seminiferous tubule		-	-	-	-	-	+	-	-	-	-	+	-
Dilatation, segmental		-	-	±	-	-	2+	-	±	-	-	2+	-
Spermatocele		-	-	-	-	-	2+	-	-	-	-	±	-
Testis (right)													
Cellular infiltration, mononuclear cell		-	-	-	-	-	±	-	-	+	+	-	-
Degeneration, seminiferous tubule		-	-	-	-	-	±	-	-	-	2+	+	-
Dilatation, segmental		-	-	±	-	±	2+	-	±	-	2+	+	-
Fibrosis		-	-	-	-	-	-	-	-	3+	-	-	-
Necrosis/degeneration, seminiferous tubule		-	-	-	-	-	-	-	-	3+	-	-	-
Spermatocele		-	-	-	-	-	+	-	-	-	2+	-	-

Notes) - : No abnormal changes ± : Very slight + : Slight 2+: Moderate 3+: Marked
P : Non-graded change NE: Not examined NA: Not applicable

- Slight to moderate cystic dilatation of follicles in the thyroid were observed in the following animals: 2 control females; 2 males and 1 female at the low dose; 2 males and 1 female at the mid dose; 1 high dose male.
    - Follicular macrophages in thyroid in 1 low dose, 2 mid dose, and 1 high dose males and 1 high dose female.
- Squamous mesothelial metaplasia in the left atrium/ventricle of the heart in 1 high dose male. The significance of this finding is unclear.

Special Evaluation: Electron Microscopy

Electron microscopy (supplementary report # S-033188-TF-189-N) was performed on the left lobe of the livers in control and high dose animals at day 29. There were no drug-related findings.

Toxicokinetics

Blood was collected at predose and 1, 2, 4, 6, 8, and 24 post dose on days 1, 14, and 28. Plasma concentrations of S-033188 were below the level of detection. Toxicokinetic parameters for S-033447 are presented in Table 24.

Tables 24: TK Parameters in Monkeys.

TK Parameters of S-033447		Dose of S-033188 (mg/kg/day)					
		1		10		100	
		Male	Female	Male	Female	Male	Female
C_{max} (µg/mL)	Day 1	0.0507	0.0523	0.236	0.189	0.496	0.449
	Day 14	0.0667	0.0694	0.277	0.285	0.876	0.627
	Day 28	0.0515	0.0527	0.242	0.229	0.714	0.642
$AUC_{0\text{-}24hr}$ (µg·hr/mL)	Day 1	0.555	0.589	3.43	2.66	8.96	7.68
	Day 14	0.632	0.740	4.14	3.69	14.8	10.2
	Day 28	0.527	0.456	3.05	3.14	11.6	9.06

Mean value (n = 3 [1 and 10 mg/kg/day] or 5 [100 mg/kg/day]).

Dosing Solution Analysis

Acceptance criteria were met for homogeneity and concentration. There was no drug detected in control formulations.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Bacterial Reverse Mutation Assay of RSC-033188

Study no.:	S-033188-TB-064-L
Study report location:	EDR 4.2.3.3.1
Conducting laboratory and location:	Shionogi Pharmaceutical Research Center Shionogi and Co., Ltd. 3-1-1, Futaba-cho, Toyonaka, Osaka, Japan
Date of study initiation:	September 14, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	S-033188, lot#A47001, 97.5% pure

Key Study Findings: RSC-033188 was considered negative with no potential to induce gene mutations under the conditions of this study.

Methods

Strains:	Salmonella typhimurium (TA98, TA100, TA1535, and TA1537) and Escherichia coli (E. coli) strain WP2uvrA.
Concentrations in definitive study:	Without S9 mix: 156, 313, 625, 1250, 2500, and 5000 µg/plate for all strains. With S9 mix: 9.77, 19.5, 39.1, 78.1, 156, and 313 µg/plate mix for TA98; and 2.44, 4.88, 9.77, 19.5, 39.1, and 78.1 µg/plate for TA100, TA1535, TA1537, and WP2uvrA.
Basis of concentration selection:	Results from the dose range finding study.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2); Sodium azide (NaN3); 9-Aminoacridine hydrochloride (9AA); 2-Aminoanthracene (2AA)
Formulation/Vehicle:	Dimethyl sulfoxide (DMSO)
Incubation and sampling time:	The plates were incubated at 37°C for 48 hours with or without S9-induced metabolic activation.

Study Validity **All validity criteria were met.**

Study title: Bacterial Reverse Mutation Assay of RSC-033447

Study no.:	R-033188-TB-065-L
Study report location:	EDR 4.2.3.3.1
Conducting laboratory and location:	Shionogi Pharmaceutical Research Center, Shionogi and Co., Ltd. 3-1-1, Futaba-cho, Toyonaka, Osaka 561-0825, Japan
Date of study initiation:	September 8, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RSC-033447, lot#A46001, 99.4% pure

Key Study Findings:

Under the conditions of this study, RSC-033447 was negative in the bacterial reverse mutation test and had no potential to induce gene mutations.

Methods

Strains:	Salmonella typhimurium (TA98, TA100, TA1535, and TA1537) and Escherichia coli (E. coli) strain WP2uvrA.
Concentrations in definitive study:	Without S9 mix: 0.61, 1.22, 2.44, 4.88, 9.77, and 19.5 µg/plate for TA100, TA1535, and TA 1537. 0.61, 2.44, 4.88, 9.77, 19.5, 39.1, and 78.1 µg/plate for WP2*uvrA* and TA 98. With S9 mix: 0.61, 1.22, 2.44, 4.88, 9.77, and 19.5 µg/plate for TA 1535 and TA 1537. 2.44, 4.88, 9.77, 19.5, 39.1, and 78.1 µg/plate for TA 100 and WP2*uvrA*. 9.77, 19.5, 39.1,78.1, 156, and 313 µg/plate for TA 98.
Basis of concentration selection:	Results from the dose range finding study.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2); Sodium azide (NaN3); 9-Aminoacridine hydrochloride (9AA); 2-Aminoanthracene (2AA)
Formulation/Vehicle:	Dimethyl sulfoxide (DMSO)
Incubation and sampling time:	The plates were incubated at 37°C for 48 hours with or without S9-induced metabolic activation.

Study Validity **All validity criteria were met.**

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Micronucleus Test of RSC-033447 with Cultured Mammalian Cells

Study no.:	R-033188-TF-059-L
Study report location:	EDR 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 2, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RSC-033447, lot#A46001, 99.4% pure

Key Study Findings

Under the conditions of this study, RSC-033447 did not induce micronuclei in the cultured human lymphoblast cell line (TK6).

Methods

Cell line:	A human lymphoblast cell (TK6).
Concentrations in definitive study:	Without S9 mix: 12.5, 25.0, 50.0, 80.0, 100 and 160 µg/mL for With S9 mix: 12.5, 25.0, 50.0, 80.0, 100, 160 and 200 µg/ml. 24-hour assay: 0.781, 1.56, 3.13, 6.25, 12.5 and 25.0 µg/ml. Micronucleus analysis was conducted at 50.0, 80.0 and 160 µg/mL in the -S9 and +S9 assays, and at 1.56, 3.13 and 6.25 µg/mL in the 24-hour assay.
Basis of concentration selection:	Results from the dose range finding study.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	Cyclophosphamide and mitomycin C
Formulation/Vehicle:	Dimethyl sulfoxide (DMSO)
Incubation and sampling time:	The TK6 cells were treated with the drug or control substance for 3 hours in the absence (-S9 assay) or presence (+S9 assay) of the S9 mix followed by a 24-hour recovery or for 24 hours in the absence of the S9 mix (24 hour assay).

Study Validity **All validity criteria were met.**

Study title: Micronucleus Test of RSC-033188 with Cultured Mammalian Cells.

Study no.:	R-033188-TF-060-L
Study report location:	EDR 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 2, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RSC-033188, lot#A47001, 97.5% pure

Key Study Findings

Under the conditions of this study in the human lymphoblast cell line, TK6, RSC-033188 did not induce micronuclei.

Methods

Cell line:	A human lymphoblast cell line (TK6).

Concentrations in definitive study:	Cytotoxicity: Short-term -S9 (15.6, 25.9, 43.2, 72, 120, and 200 µg/ml); short-term +S9 (9.33, 15.6, 25.9, 43.2, 72, and 120 µg/ml); continuous (0.728, 1.21, 2.02, 3.37, 5.62, 9.36, and 15.6 µg/ml). Micronucleus: Short-term -S9 (43.2, 72, and 120 µg/ml); short-term +S9(25.9, 43.2, and 72 µg/ml); continuous (2.02, 5.62, and 15.6 µg/ml).
Basis of concentration selection:	Dose range finding study
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	Colchicine, cyclophosphamide and mitomycin C
Formulation/Vehicle:	Dimethyl sulfoxide (DMSO)
Incubation and sampling time:	The TK6 cells were treated with the drug or control substance for 3 hours in the absence (-S9 assay) or presence (+S9 assay) of the S9 mix followed by a 24-hour recovery or for 24 hours in the absence of the S9 mix (24 hour assay).

Study Validity

All validity criteria were met**.**

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Micronucleus Test of RSC-033188 in Rats

Study no:	R-033188-TF-085-L
Study report location:	EDR 4.2.3.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 27, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RSC-033188, lot#A47001, 97.5% pure

Key Study Findings

Under the conditions of this study in rat bone marrow cells, RSC-033188 did not induce micronucleated erythrocytes.

Methods

Doses in definitive study:	500, 1000, and 2000 mg/kg/day
Frequency of dosing:	Once daily for 2 consecutive days.
Route of administration:	Oral
Dose volume:	50, 100, and 1000 mg/ml, respective to dose.
Formulation/Vehicle:	0.5 w/v% aqueous MC solution
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	10 males/group
Satellite groups:	None
Basis of dose selection:	Dose range-finding study
Negative control:	0.5 w/v% aqueous MC solution
Positive control:	Mitomycin C

Study Validity

All validity criteria were met**.**

7.4 Other Genetic Toxicity Studies

Refer to Appendix 12.1 for evaluation of impurities.

8 Carcinogenicity

Carcinogenicity studies are not required because the intended clinical use is a single-dose.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Oral Study for Effects of S-033188 of Fertility and Early Embryonic Development to Implantation in SD Rats.

Study no.:	S-033188-TB-130-L
Study report location:	EDR 4.2.3.5.1
Conducting laboratory and location:	Shionogi Pharmaceutical Research Center (SPRC), Shionogi and Co., Ltd. 3-1-1, Futaba-cho, Toyonaka, Osaka 561-0825, Japan
Date of study initiation:	October 16, 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	S-033188, lot#A580012, 98.2% pure

Key Study Findings

- Female NOAEL = 1000 mg/kg/day.
- Exposure from the embryo-fetal development study (study no. R-033188-TB-070-L) in SD rats (Gestation Day (GD) 17) was used to calculate plasma exposure of the drug: $AUC_{0\text{-}24hr}$ = 7.17 µg•hr/ml and C_{max} = 0.621 µg/ml.
- Male NOAEL = 1000 mg/kg/day.

Methods

Doses:	0, 20, 200, 1000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 ml/kg
Route of administration:	oral
Formulation/Vehicle:	0.5 w/v% aqueous MC solution
Species/Strain:	Crl:CD(SD) rat
Number/Sex/Group:	20/sex/group
Satellite groups:	None
Study design:	Males were dosed with vehicle or drug for 4 weeks prior to mating, throughout the mating period, and necropsy was conducted at 14 weeks old. Females were dosed for more than 2 weeks prior to the mating period and throughout the mating period until gestation day 13.
Deviation from study protocol:	None that affected data interpretation.

Observations and Results

Mortality

Rats were observed three times daily during week days and twice daily on the weekends and holidays for mortality and morbidity. All animals survived until their scheduled sacrifice. One male animal dosed with 200 mg/kg was found dead due to the cage being filled with water (possibly leak in the water feeder). There were no other accidental or drug-related deaths.

Clinical Signs

Clinical observations were conducted three times daily during week days; twice daily on the weekends and holidays; and once daily during gestation days 8 to 13 in copulated females. Several male and female dosed at 1000 mg/kg had abnormal colored feces due to undigested drug.

Body Weight

Body weights were taken prior to dosing every 4 days for males (until day 50) and females (until day 15) and on gestation days (GD) 0 through 8, 11, and 13 in copulated females. There were no drug-related effects on body weights.

Feed Consumption

Food intake was measured daily every 4 days in males and females and on days 1,4, 8, 11, 15, 18, 22, 25, and 29 for males and on days 1, 4, 8, 11, and 15 for females and on GD 0, 4, 8, 11, and 13 in copulated females. There were no drug-related effects on food intake.

Toxicokinetics

Toxicokinetic analysis was not conducted in this study.

Dosing Solution Analysis

Dosing formulations analysis for concentration and homogeneity met acceptance criteria. There was no test article detected in the control formulation.

Necropsy

Males 14 weeks old and females on gestation day 13 were euthanized by exsanguination under anesthesia with isoflurane inhalation. The following changes were observed in males:

- Dilated pelvic cavity in the 1000 mg/kg group in one male.
- One male dosed with 1000 mg/kg group had a small left testis.
- There was a 9% decrease in sperm count in the 1000 mg/kg group relative to testis weight (not statistically significant).

Organ Weights

There were no drug-related effects on organ weights.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)
Females were cesarean sectioned on GD 13. There were no drug-related effects.

9.2 Embryonic Fetal Development

Study title: Oral Study for Effects of RSC-033188 on Embryo-Fetal Development in Rats.

Study no.:	R-033188-TB-070-L
Study report location:	EDR 4.2.3.5.2
Conducting laboratory and location:	Shionogi Pharmaceutical Research Center, Shionogi and Co., Ltd. Osaka 561-0825, Japan
Date of study initiation:	October 30, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RSC-033188, lot#A48005, 97.4% pure

Key Study Findings

- NOAEL was the highest dose tested (1000 mg/kg/day) for maternal reproduction and embryo-fetal development.
  - GD 17: $AUC_{0\text{-}24hr}$ = 7.17 µg•hr/ml and C_{max} = 0.621 µg/ml.
- Maternal findings included a slight decrease in body weight gain and food consumption at the mid and high doses during early dosing periods only.

Methods

Doses:	0, 20, 200, 1000 mg/kg
Frequency of dosing:	Once daily
Dose volume:	10 ml/kg
Route of administration:	Oral
Formulation/Vehicle:	0.5 w/v% aqueous MC solution
Species/Strain:	Crl:CD (SD) rats
Number/Sex/Group:	20/group
Satellite groups:	5/group for TK parameters
Study design:	Pregnant female SD rats were assigned to four groups. RSC-033188 at 0, 20, 200, or 1000 mg/kg/day by once daily oral gavage on GD 6 through GD 17 at a dose volume of 10 mL/kg.
Deviation from study protocol:	None

Observations and Results

All animals were observed for mortality and any clinical signs three times a day (before dosing, after dosing in the morning and afternoon) on weekdays or twice daily (before dosing and after dosing in the morning) on weekends and holidays during the dosing period (GD 6 to 17) and once daily (a.m.) after the completion of dosing period (GD 18 to 21 in the main study and Gestation Day 18 in the TK study).

Mortality

There were no drug-related deaths.

Clinical Signs

There were no drug-related effects observed.

Body Weight

There was a 38 and 26% decrease in body gain on GD 8 and 9, respectively, in females in the 200 mg/kg group. Females in the 1000 mg/kg group had a statistically significant decrease (73%) in body weight gain on GD 7. These drug-related changes correlated to a decrease in food intake.

Feed Consumption

There were significant decreases in food intake between GD 6 to 10 by 8 to 13% and 11 to 19% at 200 and 1000 mg/kg, respectively.

Toxicokinetics

Plasma concentrations of RSC-033188 in all dose groups were BLQ (< 0.00500 µg/mL). For RSC-033447, toxicokinetic parameters are presented in Table 25.

Table 25: TK Parameters in Female Rats.

Gestation Day	Dosage of RSC-033188 (mg/kg/day)		$AUC_{0\text{-}24hr}$ (µg·hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	C_{24hr} (µg/mL)
6	20	Mean	1.26	0.214	1.0	0.00275
		SD	0.0726	0.0359	0.0	0.00550
		N	4	4	4	4
	200	Mean	6.49	0.739	1.4	0.0218
		SD	2.19	0.228	0.5	0.00368
		N	5	5	5	5
	1000	Mean	15.3	1.25	2.3	0.0973
		SD	2.74	0.206	1.3	0.0411
		N	4	4	4	4
17	20	Mean	1.88	0.324	1.0	0.00673
		SD	0.208	0.0279	0.0	0.00147
		N	4	4	4	4
	200	Mean	6.18	0.622	1.8	0.0281
		SD	1.61	0.175	0.4	0.0164
		N	5	5	5	5
	1000	Mean	7.17	0.621	3.3	0.0643
		SD	1.22	0.127	1.5	0.0329
		N	4	4	4	4

Dosing Solution Analysis

Acceptance criteria were met for homogeneity and concentration. There was no drug detected in control formulations.

Necropsy

There were no drug-related macroscopic observations in any animals at necropsy.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

There were no drug-related effects on any parameter of the cesarean section, external and placental morphologies of live fetuses.

Offspring (Malformations, Variations, etc.)

There were no drug-related effects on fetal visceral or skeletal alterations (malformation and degree of ossified bone.

Study title: Dose Range-Finding Oral Study for Effects of RSC-033188 on Embryo-Fetal Development in Rabbits

Study no.:	R-033188-TF-087-L
Study report location:	EDR 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 23, 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RSC-033188, lot#A48005, 97.4% pure

Key Study Findings

- NOAEL = 100 mg/kg.
- The NOAEL was based on the following findings:
  - Post-implantation loss rates in the mid and high dose.
  - Embryo-fetal deaths at the high dose tended to be slightly higher than controls.
  - Decrease in food intake and body weight at the high dose.
  - For skeletal examination, the incidence of cervical rib was 24.1% at the high dose (noted in 16 fetuses from 3 dams) was higher than those in the controls and the historical controls.

Methods

Doses:	0, 100, 300, or 1000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 ml/kg
Route of administration:	Oral

Formulation/Vehicle:	0.5 w/v% aqueous MC solution
Species/Strain:	Kbl: NZW rabbits
Number/Sex/Group:	7-8/group
Satellite groups:	None
Study design:	Pregnant rabbits were dosed daily via oral gavage on GD 7 to 19. Dams were euthanized on GD 28 and underwent a cesarean section.
Deviation from study protocol:	None

Observations and Results

Animals were observed daily for clinical signs, and body weight and food consumption were measured on Days 0 and GD 6 to 28, and GD 6 to 27, respectively. They were euthanized on Days 28 of gestation and underwent a caesarian section to examine the following parameters: numbers of corpora lutea and implantations, numbers of embryo-fetal deaths and live fetuses, sex, fetal body weight, placental weight, gross findings of placentae and external, visceral and skeletal morphologies of live fetuses.

Mortality

All dams survived until their scheduled sacrifice.

Clinical Signs

In the 1000 mg/kg group, abnormal stool color (greyish brown) was observed in 3/8 dams between gestation days 15 and 20.

Body Weight and Feed Consumption

In the 1000 mg/kg group, decreases in food consumption and body weight gain were noted during the dosing period. In the 300 mg/kg group, food consumption and body weight gain tended to be low from the latter half of the dosing period until several days after the dosing period.

Toxicokinetics

Not conducted.

Dosing Solution Analysis

Acceptance criteria were met for homogeneity and concentration. There was no drug detected in control formulations.

Necropsy

There were no gross pathological abnormalities noted in any animal.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Post-implantation loss rates, mainly observed as placental remnant, in the 300 and 1000 mg/kg groups (10.7% and 13.9%, respectively) were higher than those in the controls (5.5%) and historical controls (0.0% to 9.6%). The number of embryo-fetal deaths in the 1000 mg/kg group (1.1 deaths) were slightly higher than the controls (0.5 deaths) but comparable to the historical controls (0.0 to 1.0 deaths). The number of live fetuses in the 1000 mg/kg group (6.3 fetuses) were lower than the controls (8.0 fetuses).

Offspring (Malformations, Variations, etc.)

A fused rib and absent lumbar vertebra were each observed in 1 fetus in the 1000 mg/kg group.

Study title: Oral Study for Effects of S-033188 on Embryo-Fetal Development in Rabbits.

Study no.:	S0.033188-TF-135-L
Study report location:	EDR 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 8, 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	S-033188, lot#A580013, 98% pure

Key Study Findings

- NOAEL = 100 mg/kg/day (Day GD 19: $AUC_{0\text{-}24hr}$ = 9.26 µg•hr/ml and C_{max} = 0.883 µg/ml
- The NOAEL was based on the following findings:
  - Decrease in body weight and food intake at the high dose.
  - Abortions at the high dose
  - Fetal skeletal variations in the high dose

Methods

Doses:	0, 30, 100, 1000 mg/kg/day
Frequency of dosing:	Once daily from gestation day 7 to 19
Dose volume:	5 ml/kg/day
Route of administration:	Oral
Formulation/Vehicle:	0.5 w/v% aqueous MC solution (0.5 w/v%)
Species/Strain:	Kbl: NZW rabbits

Number/Sex/Group:	0 mg/kg (n=20); 30 mg/kg (n=18); n=19 for 100 and 1000 mg/kg.
Satellite groups:	5 animals in each group had blood drawn on gestation days 7 and 19 for TK analysis.
Study design:	Pregnant rabbits were dosed daily via oral gavage on GD 7 to 19 to determine any adverse effects of S-033188 on pregnant dams and embryo-fetal development. Dams were euthanized on GD 28 and cesarean sectioned (C-section).
Deviation from study protocol:	None that would affect interpretation of the data.

Observations and Results

Mortality

Animals were observed three times daily during the dosing period and once daily during the non-dosing period for mortality and morbidity. All animals survived until their scheduled sacrifice

Clinical Signs

Animals were observed for clinical signs three times daily during the dosing period and once daily during the non-dosing period. One control dam aborted her litter on GD 26 and 2 dams dosed with 1000 mg/kg group aborted their litters on GD 25 and 26. There was also a decrease in stool volume, no stool, or abnormal stool color in 3 dams dosed with 1000 mg/kg group.

Body Weight

Animals were weighed once daily on GD 0 and 6 to 28. There was statistically significant decrease in body weight gain in dams from gestation day 7 to 11 in the 1000 mg/kg group. Body weight gain of dams dosed with 1000 mg/kg remained below that of controls until GD 25.

Feed Consumption

Food intake was measured daily from GD 6 to 27. A statistically significant decrease in food intake by 29% on GD 8 and 15% on GD 9 occurred in the 1000 mg/kg group.

Toxicokinetics

Toxicokinetic parameters for S-033447 following repeat dosing of S-033188 in rabbits are presented in Table 26.

Table 26: Toxicokinetic Parameters for S-033447 in Rabbits.

TK Parameters of S-033447		Dose of S-033188 (mg/kg/day)		
		30	100	1000
		Female	Female	Female
C_{max} (µg/mL)	G7	0.586	1.24	1.83
	G19	0.485	0.883	1.35
$AUC_{0\text{-}24hr}$ (µg·hr/mL)	G7	6.29	14.4	35.6
	G19	4.60	9.26	20.9

Mean value (n = 5 [30 and 1000 mg/kg/day] or 4 [100 mg/kg/day]).

Dosing Solution Analysis

Acceptance criteria were met for homogeneity and concentration. There was no drug detected in control formulations.

Necropsy

Animals were euthanized by exsanguination under anesthesia with an injection of sodium pentobarbital on GD 28. There were no drug-related macroscopic observations in any animals.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

There were no drug-related effects on each parameter of the cesarean section.

Offspring (Malformations, Variations, etc.)

There was a statistically significant high incident of cervical rib and low incident of full supernumerary rib in the 1000 mg/kg group.

9.3 Prenatal and Postnatal Development

Study title: Oral Study for Effects of S-033188 on Pre- and Postnatal Development, Including Maternal Function, in Rats.

Study no.:	S-033188-TF-159-L
Study report location:	EDR 4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 4, 2016
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	S-033188, lot#A580012, 98.2% pure

Key Study Findings

- NOAEL = 1000 mg/kg/day for general maternal toxicity and reproduction and for pre- and post-natal development in F1 rats.
- Exposure from the embryo-fetal development study (study no. R-033188-TB-070-L) in SD rats on GD 17 was used to calculate plasma exposure of the drug: $AUC_{0\text{-}24hr}$ = 7.17 µg•hr/ml and C_{max} = 0.621 µg/ml.

Methods

Doses:	0, 20, 200, 1000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 ml/kg/day
Route of administration:	Oral
Formulation/Vehicle:	0.5 w/v% aqueous MC solution
Species/Strain:	Rat, Crl: CD(SD)
Number/Sex/Group:	0 mg/kg (n=22); 20 mg/kg (n=20); 200 and 1000 mg/kg (n=22).
Satellite groups:	All pups were euthanized on PND 21 except a subset that were used to evaluate behavior (1 male and 1 female per litter) or reproductive (1 male and 1 female per litter) function. These animals were necropsied 15 to 17 weeks after birth.
Study design:	Pregnant rats were dosed daily from GD 6 to postnatal day 20 to test the adverse effects of S-033188 on pregnant and lactating female rats and on the conceptus development and pups. Dams were necropsied at weaning (Day 21 after F_1 birth).
Deviation from study protocol:	None.

Observations and Results

F_0 Dams

Survival:	All dams survived to their scheduled necropsy.
Clinical signs:	There were no drug-related observations.
Body weight:	There were no drug-related effects on body weight or body weight gain.
Feed consumption:	There was a statistically significant decrease (11 to 15%) in food intake in the 1000 mg/kg group from GD 7 to 8.
Uterine content:	There were no effects on implantation or delivery of pups.
Necropsy observation:	The drug did not result in gross pathological findings.
Toxicokinetics:	Toxicokinetic parameters were not analyzed in this study.
Dosing Solution Analysis:	The concentration and homogeneity of the dosing solutions met the acceptance criteria. The drug was not detected in the control formulation.
Other:	No other studies were conducted.

F_1 Generation – Ocular Findings

Observations in the eyes were noted in 2 and 4 pups in the 200 and 1000 mg/kg groups, respectively (Tables 27 to 30). Due to their low numbers, their relation to the historical control data, and there having been seen during ocular development in rats (Inagaki, et al. 2014), these effects are unlikely related to baloxavir marboxil.

Table 27: Slit-lamp Examinations in F_1 Male Pups

Group	Control	S-033188		
Dose (mg/kg/day)	0	20	200	1000
Slit-lamp examination				
Days 21 to 24				
No. of animals	20	19	22	22
No abnormal changes	19	18	18	20
Lens opacity	1	1	3	1
Anterior chamber hyphema	0	0	1	1
Days 71 to 74				
No. of animals	20	19	22	22
No abnormal changes	19	18	19	20
Lens opacity	1	1	2	2
Anterior chamber hyphema	0	0	1	0
Corneal opacity	0	0	1	1
Corneal vascularization	0	0	0	1
Iris mass	0	0	0	1
Abnormal iris blood flow	0	0	0	1

Day: Day after birth

Table 28: Funduscopic Examination in F_1 Male Pups

Group	Control	S-033188		
Dose (mg/kg/day)	0	20	200	1000
Funduscopic examination				
Days 21 to 24				
No. of animals	20	19	22	22
No abnormal changes	20	19	22	22
Days 71 to 74				
No. of animals	20	19	22	22
No abnormal changes	20	19	20	20
Hyperreflective fundus	0	0	2	1
Fundus hemorrhage	0	0	0	1

Day: Day after birth

Table 29: Slit-lamp Examinations in F_1 Female Pups

Group	Control	S-033188		
Dose (mg/kg/day)	0	20	200	1000
Slit-lamp examination				
Days 21 to 24				
No. of animals	20	19	22	21
No abnormal changes	18	19	18	18
Lens opacity	2	0	3	1
Anterior chamber hyphema	0	0	1	2
Proterior synechia	0	0	0	1
Anterior chamber opacity	0	0	0	1
Enlargement of anterior chamber	0	0	0	1
Days 71 to 74				
No. of animals	20	19	22	21
No abnormal changes	18	19	19	18
Lens opacity	2	0	2	2
Anterior chamber hyphema	0	0	0	1
Anterior chamber opacity	0	0	0	1
Corneal mass	0	0	1	0
Corneal opacity	0	0	0	2
Corneal vascularization	0	0	0	1
Iris protrude	0	0	0	1
Iris hyphema	0	0	0	1
Abnormal sclera color	0	0	0	1
Enlargement of eyeball	0	0	0	1

Day: Day after birth

Table 30: Funduscopic Examination in F_1 Female Pups

Group	Control	S-033188		
Dose (mg/kg/day)	0	20	200	1000
Funduscopic examination				
Days 21 to 24				
No. of animals	20	19	22	21
No abnormal changes	20	19	22	21
Days 71 to 74				
No. of animals	20	19	22	21
No abnormal changes	20	19	22	21

Day: Day after birth

9.4 Juvenile Toxicology

Study title: Preliminary Three-week Oral Toxicity Study of S-033188 in Juvenile Rats.

Study no.:	S-033188-TF-091-R
Study report location:	EDR 4 2 3 5 4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 23, 2015.
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	S-033188, lot#A4X006, 98.8% pure

Key Study Findings

- NOAEL = 1000 mg/kg/day (Day 20: $AUC_{0\text{-}24hr}$ = 5.6 µg•hr/ml and C_{max} = 0.6 µg/ml).
- The NOAEL was based on the following findings:
  - Histopathology findings in the liver were detected at all doses. Increased liver weights correlated to histopathology findings of hepatocyte hypertrophy.

Results

One female SD rat in the 1000 mg/kg group was found dead prior to being administered the second dose. There were no other deaths in this dosing group; no indication of pain or distress prior to death; no gross changes observed at necropsy; and no deaths in the pivotal 1-month study in juvenile SD rats. Therefore, the death was not due to the drug. Twenty-four to 48 hours after the- first dose, the abdomen of animals dosed with 1000 mg/kg were swollen for 4 days. Animals dosed with 1000 mg/kg had a statistically significant decrease in body weight (5 to 8%) and body weight gain (37%) from PND 13 to 21.

There were slight decreases in mean cell hemoglobin concentration (MCHC), eosinophils, and mean cell volume (MCV) at 1000 mg/kg. Slight increase in total protein was noted in animals dosed with 1000 mg/kg and a slight decrease in the albumin/globulin (A/G) ratio was noted in animals dosed with 300 or 1000 mg/kg. Mean adjusted and relative (to body weight) liver weights in males dosed 300 or 1000 mg/kg were 16% and 25% higher, respectively. In females dosed with 300 and 1000 mg/kg they were increased by 6% and 13%, respectively. Relative spleen weights were 28 and 20% higher in males dosed with 300 and 1000 mg/kg, respectively. They were significantly higher across all doses in females, but not in a dose responsive manner. Liver histopathology results are presented in Table 31.

Table 31: Histopathology of Liver Findings in Juvenile Rats

Group/sex Dose (mg/kg/day)	1M 0	2M 100	3M 300	4M 1000	1F 0	2F 100	3F 300	4F 1000
Extramedullary Haemopoiesis								
Minimal	4	10	8	8	4	9	6	10
Total	4	10	8	8	4	9	6	10
Prominent Increase in Mitotic Activity								
Minimal	2	3	1	7	3	3	3	9
Total	2	3	1	7	3	3	3	9
Hepatocyte Hypertrophy, Generalised								
Minimal	1	0	0	9	2	0	0	5
Total	1	0	0	9	2	0	0	5
Number of tissues examined	10	10	10	10	10	10	10	10

Exposure, C_{max} and $AUC_{0\text{-}24hr}$, of S-033188 could be detected on day 1 only (Table 32).

Table 32: Toxicokinetic Parameters of S-033188 in Juvenile Rats

Dose (mg/kg)	Day	C_{max} (µg/mL)		$AUC_{0\text{-}24hr}$ (µg·hr/mL)	
		M	F	M	F
100	1	0.018	0.0201	0.0294	0.0275
	10	N.C.	N.C.	N.C.	N.C.
	20	N.C.	N.C.	N.C.	N.C.
300	1	0.0437	0.04	0.114	0.157
	10	N.C.	N.C.	N.C.	N.C.
	20	N.C.	N.C.	N.C.	N.C.
1000	1	0.196	0.0922	0.478	0.275
	10	N.C.	N.C.	N.C.	N.C.
	20	N.C.	N.C.	N.C.	N.C.

Exposure of the active form, S-033447, were detected at all time points (Table 33). The exposure, AUC, increased with an increase in dose but there was no clear trend in C_{max}.

Table 33: Toxicokinetic Parameters of S-033447 in Juvenile Rats

S-033447					
Dose (mg/kg)	Day	C_{max} (µg/mL)		AUC_{0-24hr} (µg·hr/mL)	
		M	F	M	F
100	1	6.75	7.06	79.3	84.9
	10	1.3	1.03	7.56	7.17
	20	0.367	0.282	1.71	2.13
300	1	6.22	6.83	84.9	96.7
	10	1.67	1.68	14.3	11.4
	20	0.456	0.497	3.40	3.67
1000	1	8.28	7.05	91.1	76.4
	10	1.17	1.27	17	17.6
	20	0.475	0.705	4.13	7.05

Study title: Oral Toxicity Study of S-033188 in Juvenile Rats.

Study no.: S-033188-TF-128-L
Study report location: 4.2.3.5.4
Conducting laboratory and location: (b) (4) [redacted]

Date of study initiation: October 22, 2015
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: S-033188, lot#A580012, 98.1% pure

Key Study Findings

- NOAEL = 1000 mg/kg/day (Day 40: AUC_{0-24hr} = 3 µg•hr/ml and C_{max} = 0.27 µg/ml).
- There were minor changes noted in organ weights, hematology, and blood chemistry after dosing. They were either not detected; showed signs of recovery; or within historical control at the end of recovery.

Methods

Doses: 0, 100, 300, or 1000 mg/kg/day
Frequency of dosing: daily
Dose volume: 5 ml/kg
Route of administration: Oral
Formulation/Vehicle: 0.5 w/v% aqueous MC solution
Species/Strain: Crl:CD(SD) rats
Number/Sex/Group: 10/sex/ main study
Satellite groups: 3-8/sex/recovery group
Study design: Juvenile rats were dosed daily for 40 days (PND 10 to 49). Following the dosing period a satellite group of animals were not dosed for 4-weeks.
Deviation from study protocol: None

Observations and Results

Mortality
There were no drug-related deaths.

Clinical Signs
The abdomen of animals in the 1000 mg/kg groups were swollen after dosing in half the animals on PND 11 and 12. This observation did not occur at any other time or in any other group during the treatment period.

Body Weight
In males, there was a decrease in mean body weight by 3 and 20% on days 12 and 14, respectively in the 200 mg/kg group. In males dosed with 1000 mg/kg, there was a 5 to 8% decrease from days 12 to 24 and a 31% decrease from days 10 to 14.
In females, there was a 6 to 7% decrease in mean body from days 13 to 16 in the 1000 mg/kg group.

Food Consumption
In males, food intake was decreased by 11% from days 21 to 27 and 66 to 69 in the 1000 mg/kg group. There were no effects on food intake in females.

Hematology
The following hematology differences were observed at the end of dosing:

- White blood cell counts were 19% lower in males dosed with 1000 mg/kg which correlated with low lymphocyte and basophil counts.
- Increased red blood cell distribution width (RDW) in 200 and 1000 mg/kg males (4% at both doses) and females (6 and 13%, respectively)
- There was a statistically significant increase, 11%, in platelets in females dosed with 1000 mg/kg.
- Statistically significant decreases in prothrombin time (PT) of 10% in males treated with 200 mg/kg and at 8.6% and 11% in males and females, respectively, dosed with 1000 mg/kg. There were no significant effects on activated prothrombin time (APTT) in females. In males, APTT was increased by 12.6 and 22% in the 200 and 1000 mg/kg groups, respectively.

At the end of the recovery period, all hematology effects had resolved except effects seen in RDW in males.

Clinical Chemistry
The following effects in blood chemistry were observed at the end of dosing were statistically significant:

- Non-dose dependent decrease (21 to 26%) in creatine kinase in females.
- Increase in amylase (9.5 and 34%) in 200 and 1000 mg/kg females, respectively.
- Decrease in bilirubin (100%) in 200 and 1000 mg/kg females. However, the differences were within the range of the historical control animals.

- Increase in cholesterol (17 and 32%) in 200 and 1000 mg/kg females, respectively.
- Total protein was increased (5 and 3%) in 1000 mg/kg males and females, respectively.
- A/G ratio was decreased (8 and 13%) in 1000 m/kg males and females, respectively.

At the end of the recovery period the only blood chemistry change that remained statistically significant was the decreased in A/G ratios.

Gross Pathology

During gross pathology observations at the end of dosing, a dark area in the eye was observed in 1 of 12 males in the 200 mg/kg group and enlargement and opacity of the eye in 1 of 12 females in the 200 mg/kg group.

Organ Weights

At the end of dosing, the relative (to body weight) brain weight was 4% lower in 1000 mg/kg males and females. In both males and females dosed with 1000 mg/kg, relative liver weights were increased by 14 and 31%, respectively, and increased by 19% in 200 mg/kg females. The relative thyroid and parathyroid weights were increased by 16% in 1000 mg/kg males. In females dosed with 200 or 1000 mg/kg, the relative pituitary weights decreased by 19 and 11%, respectively.

At the end of the recovery period, there was a 36 and 53% increase in relative spleen weights in 200 and 1000 mg/kg males, respectively. In females dosed with 200 or 1000 mg/kg, there was an 8.5% decrease in relative brain weight. The relative liver weights were increased by 9% in the 1000 mg/kg females.

Histopathology

Adequate Battery: Yes
Peer Review: Yes

Histological Findings

At the end of dosing, histopathology assessment in the liver revealed minimal follicular cell hypertrophy in the thyroid in males and females dosed with 200 or 1000 mg/kg (Table 34). There were no findings detected at the end of the recovery period.

Table 34: Findings in the Thyroid in Juvenile Rats Dosed with Baloxavir Marboxil.

Group/sex Dose (mg/kg/day)	1M 0	2M 20	3M 200	4M 1000	1F 0	2F 20	3F 200	4F 1000
Follicular Cell Hypertrophy								
Minimal	0	0	4	6	0	0	3	8
Total	0	0	4	6	0	0	3	8
Number of tissues examined	12	12	12	12	12	12	12	12

Special Evaluation – Growth, Neurobehavioral Examinations, Sexual Maturation
Ulna length was used to assess growth. Animals dosed with 1000 mg/kg had shorter ulna length on day 14 but it was only statistically significant on day 28.
During neurobehavioral examinations on motor activity, the 1000 mg/kg males had a statistically significant lower number of high beam break scores in the 6, 12, and 18-minute time interval. In females, there were non-dose dependent low number of beam breaks in all dose groups at the 30 and 54-minute interval. There were no effects on motor activity in dosed animals at the end of recovery.
For assessment of sexual maturation, males from the 1000 mg/kg group had a statistically significant later age (1.3 days later) of balano-preputial separation.

Toxicokinetics
Systemic exposure, $AUC_{0\text{-}24hr}$ and C_{max}, of baloxavir marboxil increased with an increase in dose on day 1 only (Table 35).

Table 35: Systemic Exposure of Baloxavir Marboxil in Juvenile Rats.

Dose level (mg/kg/day)	C_{max} (ng/mL)					
	Day 1		Day 21		Day 40	
	Males	Females	Males	Females	Males	Females
20	7.74	9.83	-[a]	-[a]	-[a]	-[a]
200	28.4	75.6	-[a]	-[a]	-[a]	-[a]
1000	999	5400	-[a]	-[a]	-[a]	-[a]

Dose level (mg/kg/day)	$AUC_{0\text{-}24h}$ (ng.h/mL)					
	Day 1		Day 21		Day 40	
	Males	Females	Males	Females	Males	Females
20	-[b]	-[b]	-[a]	-[a]	-[a]	-[a]
200	154	450	-[a]	-[a]	-[a]	-[a]
1000	2080	5650	-[a]	-[a]	-[a]	-[a]

[a] Mean plasma concentrations were not available at any sampling time
[b] Mean plasma concentrations were available at fewer than three sampling times; $AUC_{0\text{-}24h}$ not calculated

The systemic exposure of baloxavir increased with an increase in dose on each day tested (Table 36). However, exposure decreased with age.

Table 36: Systemic Exposure of Baloxavir in Juvenile Rats.

Dose level (mg/kg/day)	C_{max} (ng/mL)					
	Day 1		Day 21		Day 40	
	Males	Females	Males	Females	Males	Females
20	2140	2290	218	194	109	104
200	7020	7090	295	300	295	382
1000	8240	9690	331	512	248	282

Dose level (mg/kg/day)	AUC_{0-24h} (ng.h/mL)					
	Day 1		Day 21		Day 40	
	Males	Females	Males	Females	Males	Females
20	26100	27800	1230	844	667	544
200	87100	103000	2420	2740	2570	2760
1000	133000	147000	3590	5980	2950	3050

Dosing Solution Analysis

Acceptance criteria were met for homogeneity and concentration. There was no drug detected in control formulations.

10 Special Toxicology Studies

Study Title: Exploratory Photohemolysis Assay of MTS-056188A (Study no. TOX-2013-MTS-056188A-04).

The phototoxicity of the prodrug, baloxavir marboxil, was evaluated using sheep red blood cells (0.1, 0.02, 0.004, or 0.0008 w/v%) by ultraviolet radiation. S-033188 tested positive for phototoxicity at 0.1 and 0.02 (w/v%) and negative at 0.004 and 0.0008 w/v%. At each concentration of baloxavir marboxil, the methemoglobin was within the allowance (less than 0.05). Under the study conditions, baloxavir marboxil has phototoxic potential.

Study Title: Exploratory Photohemolysis Assay of MTS-0563447A (Study no. TOX-2013-MTS-0563447A-01).

The phototoxicity of the active form of baloxavir marboxil, baloxavir, at 0.1, 0.02, 0.004, or 0.0008 w/v% was evaluated using sheep red blood cells ultraviolet radiation. Baloxavir tested negative for phototoxicity at each concentration and the methemoglobin was within the allowance (less than 0.05). Under the study conditions, baloxavir does not have phototoxic potential.

Study Title: Exploratory Skin Phototoxicity Study of MTS-0576188A in Hairless Mice by Oral Dosing (Study no. TOX-2013-MTS-0576188A-03)

The skin phototoxicity of baloxavir marboxil was evaluated in Hos:HR-1 female hairless mice following a single oral dose with vehicle (0.5 w/v% MC), 200 or 1000 mg/kg baloxavir marboxil (MTS-0576188A) and ultraviolet radiation for 1 hour 45 minutes, 1 hour after dosing. Animals were observed for mortality, body temperature and thickness of the skin, posture, behavior, respirator status, condition of visible mucous membrane,

egesta and secretion. There were no drug-related skin reactions. Under the study conditions, baloxavir marboxil is not phototoxic to the skin.

Study Title: Preliminary Single Intraperitoneal Toxicokinetics Study of S-033188 in Hairless Mice (Study no. S-033188-TB-144-R)
The intraperitoneal (IP) tolerance of baloxavir marboxil following a single IP injection with vehicle (0.5 w/v% MC), 1, 10, or 100 mg/kg baloxavir marboxil was evaluated in female Hos:HR-1 hairless mice. Animals were observed for mortality and clinical observations. Baloxavir marboxil did not cause any adverse effects when injected into the peritoneum.

Study Title: Skin Phototoxicity Study of S-033188 in Hairless Mice by Oral and Intraperitoneal Dosing (Study no. S-033188-TB-149-L)
The skin phototoxicity of baloxavir marboxil following a single oral dose (1000 mg/kg) or IP injection at 10, 30, or 100 mg/kg baloxavir marboxil was evaluated in female Hos:HR-1 hairless mice. Orally dosed mice were exposed to ultra violet radiation 1 hour post-dose and intraperitoneally dosed mice were exposed 0.5 hours post-dose. There were no drug related skin reactions in either the oral or IP dosed groups. Under the study conditions, baloxavir marboxil is not phototoxic to the skin.

Study Title: Effect of Sterilized Food on RSC-033188-Induced PT and APTT Prolongation in Rats (Study no. R-033188-TB-007-R)
The effect of food sterilization on PT and APTT prolongation and activities of Vitamin K depended factors following oral dosing of control (0.5% w/v MC) and baloxavir marboxil (RSC-033188) at 600 mg/kg/day for 7 days was evaluated in SD male rats (Table 37) given sterilized or non-sterilized food under fasting or non-fasting conditions 17 hours prior to necropsy.

Table 37: RSC-033188 dose and food groups in male rats.

Group No.	Treatment	Dose* (mg/kg/day)	Dosing Formulation (mg/mL)	Food Sterilization	Fasting
1	Control	0	0.5% MC	Non-sterilized	−
2	Control	0	0.5% MC		+
3	RSC-033188	600	60		−
4	RSC-033188	600	60		+
5	Control	0	0.5% MC	Autoclaved	−
6	Control	0	0.5% MC		+
7	RSC-033188	600	60		−
8	RSC-033188	600	60		+
9	Control	0	0.5% MC	Radiation-sterilized	−
10	Control	0	0.5% MC		+
11	RSC-033188	600	60		−
12	RSC-033188	600	60		+

* Dose volume: 10 mL/kg, −: non-fasting before dissection, +: fasting before dissection

Animals that were dosed and fasted prior to necropsy had prolonged PT and/or APTT and a decrease in coagulation factors II, VII, IX, and XI. This effect was not seen when they were fed non-sterilized food. Prolonged PT and/or APTT and decreases in factors II, VII, IX, and X occurred in most fasted compared to fed animals. PT and/or APTT were not prolonged in dosed animals fed non-sterilized food compared to controls. PT and/or APTT were prolonged in controls fed non-sterilized food and fasting compared with those fed non-sterilized food. The amount of total vitamin K in non-sterilized food was higher than in sterilized food but comparable to the amount in autoclaved and radiation-sterilized food.
Baloxavir marboxil will likely decrease vitamin K coagulation factors II, VII, IX, and X and result in prolonged PT and APTT. These effects are not seen when animals were fed non-sterilized food and were not fasted.

Study Title: Effect of Vitamin K on RSC-033188-Induced PT and APTT Prolongation in Rats (Study no. R-033188-TB-047-L)
The effect of feeding or feeding and vitamin K_1 administration on PT and APTT prolongation was examined in SD rats after orally administration of control (0.5 w/v% MC) and doses of baloxavir marboxil (2000 mg/kg/day; 5 days). Vitamin K_1 was administered subcutaneously (0.3 mg/kg/day) for 2 weeks within 1 hours after dosing with baloxavir marboxil under fasted conditions. The effect of food was examined by fasting 16 hours prior to necropsy. There were no deaths or moribund animals observed during the study. Dosed animals that were fasted or fed and baloxavir marboxil /vitamin

K_1 had white-colored feces throughout dosing. There were no changes noted during clinical observations or changes in body weight. There was a 20, 23, and 16% decrease in food intake (days 1 to 2) in the dosed and fasted, dosed and non-fasted, and dosed/Vitamin K_1 and fast groups, respectively.
Prolonged PT and/or APTT was observed in baloxavir marboxil dosed animals under fasted and fed conditions compared to control animals under fed and fasted conditions. Prolongations were minor between fed control and dosed groups compared to fasted controls and dosed, suggesting prolongations were less when animals were fed before necropsy. When subcutaneous injections of Vitamin K_1 were administered 1 hour after dosing, the baloxavir marboxil induced prolongations of PT and/or APTT recovered.
The toxicokinetic parameters of baloxavir did not reveal any significant differences between exposure, $AUC_{0\text{-}24hr}$ and C_{max}, when dosed with baloxavir marboxil or baloxavir marboxil /Vitamin K_1 (Table 38).

Table 38: Toxicokinetic Parameters of baloxavir After Oral Doses of Baloxavir Marboxil (2000 mg/kg/day) and Vitamin K_1 (0.3 mg/kg/day)

Day	RSC-033188 Dosage (mg/kg/day)	Vitamin K_1 Dosage (mg/kg/day)		$AUC_{0\text{-}24hr}$ (µg·hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	C_{24hr} (µg/mL)
1	2000	0	Mean	10.4	0.897	1.7	0.0812
			SD	0.516	0.0533	0.6	0.0129
			N	3	3	3	3
	2000	0.3	Mean	10.4	0.878	1.7	0.161
			SD	1.54	0.0927	0.6	0.120
			N	3	3	3	3
14	2000	0	Mean	3.59	0.295	2.0	0.0213
			SD	0.346	0.0275	0.0	0.00397
			N	3	3	3	3
	2000	0.3	Mean	3.77	0.263	3.0	0.0308
			SD	0.392	0.0163	1.7	0.0170
			N	3	3	3	3

The TK parameters ($AUC_{0\text{-}24hr}$, C_{max}, T_{max}, and C_{24hr}) in the control group were not calculated.

11 Integrated Summary and Safety Evaluation

The new drug application 210854 was submitted in support of baloxavir marboxil, a PA endonuclease inhibitor, for the treatment of acute, uncomplicated influenza (b) (4) in patients 12 years of age and older. Baloxavir marboxil is a prodrug that is rapidly converted to its active form, baloxavir, through metabolism. Baloxavir marboxil is a new molecular entity and will be marketed as an oral formulation. The proposed clinical regimen is a single oral dose of 40 mg for body weights ≤ 80 kg or 80 mg for body weights ≥ 80 kg. The nonclinical findings for baloxavir marboxil are summarized in the following sections.

Pharmacokinetics

Due to the rapid metabolism of the prodrug baloxavir marboxil to the active form, baloxavir, the plasma concentration of baloxavir marboxil was BLQ in adult SD rats and cynomolgus monkeys as early as 0.25 hour after oral dosing.

Increasing doses of baloxavir marboxil resulted in a less than dose-proportional increases in exposure (AUC and C_{max}) of baloxavir in both SD rats and cynomolgus monkeys under fed conditions. Exposure of baloxavir was greater in both animal species under fasted conditions. In cynomolgus monkeys, concomitant dosing with minerals (calcium, magnesium and aluminum, and iron) and baloxavir marboxil resulted in a decrease in exposure of baloxavir. In juvenile rats, exposure of baloxavir marboxil was detect at postnatal day (PND) 10 only but baloxavir exposure was detected at all ages following a single oral dose.

The bioavailability of baloxavir was approximately 11.5% in rats and 11% in monkeys under fed conditions and 11.9% in SD rats and 51% in cynomolgus monkeys under fasted conditions. The half-life under fed and fasted conditions ranged between 0.36 and 1.25 hours in SD rats and 2.17 and 5.33 hours in cynomolgus monkeys. In juvenile SD rats dosed with baloxavir marboxil, the bioavailability of baloxavir decreased as the dose increased. The half-life in juvenile SD rats was between 2 and 8 hours.

Protein binding of baloxavir was approximately 92% in rats, 85 to 89.5% in monkeys, and 92.9 to 93.9% in humans. Baloxavir primarily binds to human serum albumin (HSA). The distribution ratios of baloxavir in blood cells were 57.1 to 59.6% in rats, 50.4 to 52.9% in monkeys, and 48.5 to 54.4% in humans. Baloxavir was widely distributed (but mainly in the intestinal mucosa and liver) between 1 and 2 hours post-dose in adult SD rats. Drug detection in tissues was BLQ at 24 hours post-dose. In pregnant rats, peak baloxavir distribution occurred 8 hours post-dose. The liver had the highest distribution followed by the adrenal cortex. Low levels of baloxavir were detected in fetal tissues with detection up to 48 hours in adrenal cortex and bones.

Baloxavir marboxil metabolites were primarily products of glucuronidation and oxidation of baloxavir in SD rats and cynomolgus monkeys. In male and female SD rat liver samples, there were statistically significant increases in total P450, testosterone 6β-, and 16β-hydroxylase, ant T3 and T4 UDPGT enzyme activities. The radioactivity was primarily excreted in the feces via the bile following a single oral administration of [^{14}C]-baloxavir marboxil in rats and monkeys. Baloxavir and its related metabolites was detected in milk from lactating SD rats. Maximum milk concentration of baloxavir was reached 2 hours post dosing and maximum plasma concentration was reach 1 hour post dosing. After 24 hours, baloxavir was undetectable in plasma and milk.

Safety Pharmacology

Cardiovascular parameters were evaluated in cynomolgus monkeys and in a hERG assay using baloxavir marboxil or baloxavir. Neurological and respiratory parameters were evaluated in rats. Skeletal muscles were evaluated in the guinea pig. There were no adverse effects of baloxavir marboxil or baloxavir observed in these studies.

Repeat-Dose Toxicology

Repeat-dose studies were conducted in Sprague-Dawley (SD) rats (20, 200, or 2000 mg/kg/day) and Cynomolgus monkeys (1, 10, 100 mg/kg/day) for one month with a one month recovery period. These studies were used to determine the toxicologic profile of baloxavir marboxil. The liver and thyroid were the target organs of toxicity.

In SD rats receiving oral baloxavir marboxil, liver findings included increased liver weight (7% at the mid and 36% at the high dose); gross pathology findings (accentuated lobular pattern and liver enlargement at the high dose) which were not present at the end of recovery; and histopathology findings (minimal centrilobular hypertrophy, minimal to mild macrovesicular fatty change in periportal hepatocytes, and a mild increase in Kupffer cell phagocytosis). Macrovesicular fatty change remained present in 2 high dose males at the end of recovery. Thyroid histopathology (minimal diffuse follicular epithelial hyperplasia at the mid and high doses; and an increased number of animals at the mid and high doses had minimal to mild decrease in thyroid colloid) findings at the end of dosing were not detected at the end of recovery.

Clinical chemistry findings in SD rats (decreased A/G ratio by 13% at the mid and 23% at the high dose females; decreased total bilirubin between 25 to 50 % at all doses; decreased triglycerides by 21% at the mid and 40% at the high dose; increased cholesterol (36%) and glucose (10%) in high dose females; and a 17 to 37% increase in amylase in all dosed females) detected at the end of dosing were not detected the end of recovery except the elevated amylase in high dose female rats. Prolongations in PT and/or APTT were detected at the mid and high doses at the end of dosing but were not seen at the end of the recovery period. Separate studies (study no. R-033188-TB-007-R and R-033188-TB-047-L) examining the food and vitamin K_1 effects revealed minor or no prolongation of PT and/or APTT when animals were not fasted prior to necropsy or animals were administered vitamin K_1 injections under fasted conditions. Specials studies conducted in rats showed baloxavir marboxil may have the potential to decrease vitamin K coagulation factors resulting in prolonged PT and/or APTT. These affects were due to reduction in vitamin K from fasting 16 hours prior to necropsy and are not necessarily applicable to humans.

Predose ocular findings (punctate opacity in the cornea, lens nuclear opacity, lens anterior subcapsular opacity, persistent pupillary membrane, and fundus hemorrhage) in controls and dosed rats remained detectable at the end of recovery. These ocular findings detected prior to dosing were seen in historical control rats of this strain at this facility and SD rats are reported to develop spontaneous ocular lens and/or hemorrhage (Durand et al.,2001).

The exposure multiple at the NOAEL (20 mg/kg/day) in the pivotal one-month oral study in rats was 0.6-times the exposure at the recommended clinical dose. Although the safety margin is <1, the findings observed at the mid and high doses were minor and resolved during recovery. In addition, the highest dose used in the SD rat study was the limit dose which reached plasma exposures of baloxavir approximately 2- to 3-fold of that at the intended clinical dose.

In cynomolgus monkeys receiving oral baloxavir marboxil, there were increases in hepatic liver enzymes (AST up to 2.8-fold, ALP up to 1.9-fold, ALT up to 6.3-fold, ALP up to 1.8-fold, GGT up to 2.1-fold, and GLDH up to 23-fold in males and 12.5-fold in

females) were detected in either males (all doses) or females (high dose). These changes were not detected at the end of recovery. Electron microscopy analysis (study no. S-033188-TF-189-N) using liver specimens from the pivotal one-month cynomolgus monkey study did not reveal any changes in hepatocytes due to dosing with baloxavir marboxil.

Thyroid histopathology findings (increased weight in males only at high dose at the end of dosing remained until the end of the recovery period; slight to moderate dilatation of follicles at all doses and controls, and follicular macrophages in all doses and controls) observed at the end of dosing were not observed at the end of recovery in cynomolgus monkeys.

Testicular findings (an adhesion on the right testis/epididymis of 1 high dose male that had marked fibrosis and seminiferous tubule degeneration/necrosis; decrease prostate weight at the high dose; fibrosis and necrosis/degeneration in the seminiferous tubule in 1 mid dose male) in cynomolgus monkeys were not attributed to baloxavir marboxil due to the following reasons: there was no clear dose response; the sponsor provided historical control data for this species of monkey with the same findings; testicular findings were not observed at the end of recovery in the one-month study; and testicular findings were not observed in the 2-week repeat dose study in cynomolgus monkey at higher doses

The exposure multiple at the NOAEL (10 mg/kg/day) in the pivotal one-month oral study in cynomolgus monkeys was 2.5 times the exposure at the recommended clinical dose. Adverse events related to liver function (increases in ALT, AST, GGT and abnormal hepatic function) observed in clinical trials (study 1601T0831) with baloxavir marboxil was 1.5%.

Genotoxicology and Carcinogenicity

Baloxavir marboxil and baloxavir were negative for mutagenesis determined by the Ames assay; did not induce micronuclei; and tested negative for clastogenesis *in vitro* and *in vivo* micronucleus tests. Carcinogenicity studies with baloxavir marboxil have not been conducted because the intended clinical use is a single dose.

Reproductive and Developmental Toxicology

Assessment of fertility and early embryonic development was conducted in rats. There were no effects on female or male fertility parameters observed up to the highest dose tested. Plasma exposure of baloxavir was based on the embryo-fetal development study in rats because plasma exposure was not measured in this study. Exposure multiple at the NOAEL (1000 mg/kg/day) in the rats was 6-times the exposure at the recommended clinical dose.

Embryo-fetal development studies were conducted in SD rats and Kbl: NZW rabbits. In the SD rat study, decreases in body weight by 38 and 26 % occurred on GD 8 and 9, respectively, at the mid-dose. There was a 73% decrease in body weight at the high dose on GD 7. These decreases only occurred on 1 or 2 days only. There were significant decreases in food intake on GD 7 to 10 and 21 at the mid and high doses.

Exposure multiples at maternal and fetal NOAELs (1000 mg/kg/day) in the rat were 6-times the exposure at the recommended clinical dose.

In the Kbl: NZW rabbit embryo-fetal development study, findings included abortions in 2 high-dose females; decrease stool volume, no stool, or abnormal stool color in 3 high-dose females; decrease body weight and body weight gain in high-dose females; and decreased food intake in high-dose females. There was a high incidence of cervical rib and low incident of full supernumerary rib in offspring from the high-dosed offspring.

The exposure multiple at the maternal and fetal NOAEL (100 mg/kg/day) in the rabbit embryo-fetal study was 7 times the exposure at the recommended clinical dose.

In the pre-/postnatal development study conducted in SD rats. There were no findings in F_0 animals. Ocular findings in 2 F_1 pups at the mid dose and 4 F_1 pups at the high dose (unilateral dark red discoloration of the eyeball observed; opacity and bulge; histopathology observations of inflammation in the cornea and iris, lens necrosis, and iris lens adhesion; and ophthalmology using slit lamp revealed anterior chamber hyphema, opacity and enlargement, and fundus hemorrhage) were not attributed to baloxavir marboxil. Historical control data of spontaneous lesions observed in SD rats from Charles River Laboratories was provided by the Sponsor. The Sponsor also included literature reference reporting spontaneous intraocular hemorrhage in SD rats during postnatal ocular development (Inagaki, et al. 2014).

Plasma exposure of S-033447 was calculated using the embryo-fetal development study in rats because plasma exposure was not measured in this study. Exposure multiples at the NOAEL (1000 mg/kg/day) in the rats was 6-times the exposure at the recommended clinical dose.

A juvenile toxicology study was conducts in SD rats. Minimal changes in mean body weights, hematology parameters, blood chemistry, organ weights (mainly liver and thyroid), and histopathology (liver and thyroid) observed after dosing were not seen at the end of recovery.

Systemic exposure of baloxavir marboxil could only be determined for day 10 only. However, systemic exposure of the active form, baloxavir, was determined at all sampling times. The exposure multiple at the NOAEL (1000 mg/kg/day) in the juvenile toxicity study in SD rats is 2 times the exposure at the recommended clinical dose.

Special Toxicology Studies

Baloxavir marboxil is not phototoxic to the skin and did not cause skin reactions in mice but considered to have phototoxic potential in an *in vitro* test using sheep red blood cells.

Exposure margins

The exposure margins for baloxavir marboxil are presented in Table 39.

Table 39: Baloxavir marboxil exposure margins

Study	NOAEL (mg/kg/dose)	Toxicities Observed	Nonclinical AUC (µg•hr/mL)	Exposure Multiple
Repeat-Dose				
One month – Rat	20[b]	Liver, thyroid	0.8	0.6

One month – Monkey	10[b]	Liver (minor)	3.1	2.5
Reproductive Toxicology				
Fertility/Early Embryonic Development Rat	1000	None	7.2[c]	5.7
Embryo-fetal Development				
Rat	1000[d]	↓ Body weight gain, ↓ food intake.	7.2 9.3	5.7 7.3
Rabbit	100[e]	↓Food intake, ↓ body weight gain, abortions, skeletal variations.		
Pre-/postnatal Development				
Rat	1000	None	7.2[c]	5.7
Juvenile				
Rat	1000[f]	Liver (minor)	3	2.4

[a] Based on exposures in Phase 3 trial (all patients) at the clinical dose (40 mg for < 80 kg or 80 mg for > 80 kg): $AUC_{0\text{-}24hr}$ = 1.26 µg•hr/mL, C_{max} = 0.075 µg/ml.

[b] Day 28 data

[c] In reproductive and developmental toxicology studies, plasma exposure of S-033447 in rats was only calculated in the embryo-fetal development study.

[d] Gestation Day 17 data

[e] Gestation Day 19 data

[f] Post-natal Day 49 data

12 Appendix/Attachments

12.1 Comments on Impurities/Degradants of Concern

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: NDA210854

Supporting document/s:

Supporting Document	Sponsor Submission Date	CDER Received Date
1	04/24/18	04/24/18

Product: XOFLUZA™ (baloxavir marboxil)

Indication:	Treatment of acute uncomplicated influenza in patients 12 years of age and older who have been symptomatic for no more than 48 hours
Applicant:	Shionogi Inc.
Review Division:	Division of Antiviral Products
Reviewer:	Zheng Li, Ph.D.
Supervisor/Team Leader:	Hanan Ghantous, Ph.D., DABT
Division Director:	Debra B. Birnkrant, M.D.
Project Manager:	Victoria Tyson, B.S.

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1 Executive Summary

Shionogi Inc. has submitted an NDA to support the XOFLUZA™ (baloxavir marboxil) 20 mg and 40 mg for treating of acute uncomplicated influenza in patients 12 years of age and older who have been symptomatic for no more than 48 hours.

This review focuses on qualification of organic impurities, (b) (4) elemental impurities, and degradants. Regulatory decisions utilize recommendations from ICH M7 "Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk", ICH Q3A(R2) "Impurities in New Drug Substances", ICH Q3B(R2) "Impurities in New Drug Products", ICH Q3C(R5) "Impurities: Guideline for Residual Solvents", and ICH Q3D "Guideline for Elemental Impurities".

Overall, proposed specifications (or lack of specifications) are considered acceptable from a pharmacology/toxicology perspective.

2 Qualification of XOFLUZA™ Drug Substance

2.1 Specified Impurities

2.1.1 Organic Impurities

The qualification of specified organic impurities in the drug substance is based on results from general toxicology studies, experimental genotoxicity data, and/or assessments of mutagenic potential using (quantitative) structure-activity relationship [(Q)SAR]. Acceptance criteria of stereoisomers impurities (b) (4) are below the qualification threshold.

General Toxicology – Specified impurities were present in the drug lots used in 1-month rats and monkey studies, reproductive/developmental studies in rats and rabbits, and rat juvenile study (S-033188-TF-132-L And S-033188-TF-189-N, S-033188-TB-131-L, R-033188-TF-130-L, R-033188-TB-135-L, S-033188-TF-159-L, and S-033188-TF-128-L reviewed by Dr. Deacqunita Diggs). Using NOAELs established in these studies, qualified impurity levels are adequate to support the proposed specifications (Table 1).

(b) (4)

(b) (4)

Genotoxicity – Recommended testing for impurities with exposures exceeding the ICH Q3A qualification threshold (b) (4) include a genetic toxicology evaluation consisting of Ames and *in vitro* mammalian cell assays. In contrast, ICH M7 indicates that initial genetic toxicology qualification can be limited to (Q)SAR predictions of mutagenic potential for impurities with exposures (b) (4) mg/day. Because proposed specifications for any individual impurity do not exceed (b) (4) mg/day, (Q)SAR predictions of mutagenic potential are considered sufficient for qualification.

A review of available data and computational toxicology assessment were conducted by the sponsor to evaluate potential mutagenicity of the impurities. Database searched included Ministry of Health, Labor and Welfare (MHLW), Chemical Effects in Biological System (CEBS), European Chemicals Agency (ECHA), Chemical Risk Information Platform (CHRIP), Toxicology Data Network (TOXNET), Carcinogenic Potency Database (CPDB) and CASE Ultra. (Q)SAR evaluation included Derek Nexus (v5.0.1 and v2.1.0) and CASE Ultra (v1.6.2.1 and 1.6.2.3). Evaluation summary are presented in Table 2.

Table 2. Genotoxicity summary for specified XOFLUZA™ drug substance organic impurities

(b) (4)

data

(b) (4)

2.1.2 (b) (4)

The (b) (4) in the drug substance was (b) (4). The potential residual solvents in the drug substance were (b) (4) in the XOFLUZA™ drug substance are listed in the ICH Q3C(R5) guideline. The proposed specifications for all listed (b) (4) (Table 3). There is no toxicological concern.

Table 3. XOFLUZA™ drug substance (b) (4) specifications

(b) (4)

2.1.3 Elemental Impurities

None

2.2 Unspecified Impurities

None

3 Qualification of the XOFLUZA™ Drug Product

3.1 Degradants

There are 4 identified process impurities carried into the XOFLUZA™ drug products by the drug substance, [redacted]. Among them, [redacted] The proposed specification for [redacted] in drug product is considered acceptable. Please see Section 2.1.1 for the derivation of the qualified level and mutagenicity evaluation for [redacted]

12.2 References

[redacted]

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Inagaki K, Koga H, Inoue K, Suzuki K and Suzuki H. Spontaneous Intraocular Hemorrhage in Rats during Postnatal Ocular Development. Comparative Medicine 2014; 64 (1): 34-43.

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/s/

DEACQUNITA L DIGGS
09/21/2018

HANAN N GHANTOUS
09/21/2018
I concur with Dr. Diggs conclusion that the nonclinical data are sufficient to support approval of Baloxavir marboxil.