Report on the Deliberation Results

November 6, 2020
Pharmaceutical Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau
Ministry of Health, Labour and Welfare

Brand Name	Orladeyo Capsules 150 mg
Non-proprietary Name	Berotralstat Hydrochloride (JAN*)
Applicant	OrphanPacific, Inc.
Date of Application	January 31, 2020

Results of Deliberation

In its meeting held on October 30, 2020, the Second Committee on New Drugs concluded that the product may be approved and that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The product is not classified as a biological product or a specified biological product. The re-examination period is 10 years. The drug product is not classified as a poisonous drug or a powerful drug, and its drug substance is classified as a powerful drug.

Approval Conditions

1. The applicant is required to develop and appropriately implement a risk management plan.
2. Because of extremely limited number of patients participating in clinical studies in Japan, the applicant is required to conduct a post-marketing use-results survey covering all patients treated with the product, until data from a certain number of patients are collected, in order to identify the characteristics of patients treated with the product and obtain safety and efficacy data promptly, and thereby to take necessary measures to ensure the proper use of the product.

**Japanese Accepted Name (modified INN)*

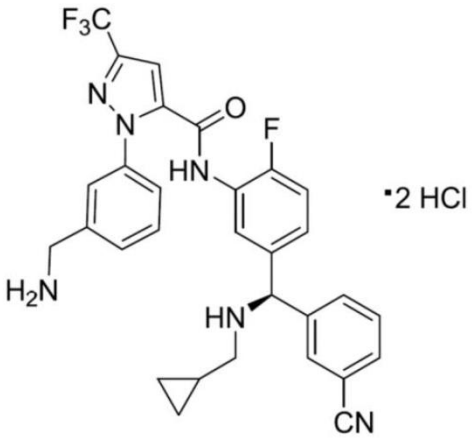
Review Report

October 21, 2020
Pharmaceuticals and Medical Devices Agency

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

Brand Name	Orladeyo Capsules 150 mg
Non-proprietary Name	Berotralstat Hydrochloride
Applicant	OrphanPacific, Inc.
Date of Application	January 31, 2020
Dosage Form/Strength	Hard capsules each containing 172.5 mg of Berotralstat Hydrochloride (equivalent to 150 mg berotralstat)
Application Classification	Prescription drug, (1) Drug with a new active ingredient

Chemical Structure



Molecular formula: $C_{30}H_{26}F_4N_6O \cdot 2HCl$

Molecular weight: 635.48

Chemical name:

1-[3-(Aminomethyl)phenyl]-*N*-(5-{(1*R*)-(3-cyanophenyl)[(cyclopropylmethyl)amino]methyl}-2-fluorophenyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxamide dihydrochloride

Items Warranting Special Mention

Orphan drug (Orphan Drug Designation No. 425 of 2018 [*30 yaku*]; PSEHB/PED Notification No. 0115-3 dated January 15, 2020, by the Pharmaceutical Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare)

SAKIGAKE designation drug (SAKIGAKE Drug Designation No. 4 of 2015 [*27 yaku*]; PSEHB/ELD Notification No. 1027-1 dated October 27, 2015, by the Evaluation and Licensing Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare)

Reviewing Office Office of New Drug IV

Results of Review

On the basis of the data submitted, PMDA has concluded that the product has efficacy in the prevention of acute attacks of hereditary angioedema, and that the product has acceptable safety in view of its benefits (see Attachment).

As a result of its review, PMDA has concluded that the product may be approved for the indication and dosage and administration shown below, with the following conditions. Hepatic dysfunction and QT interval prolongation need to be further investigated in the post-marketing setting.

Indication

Prevention of acute attacks of hereditary angioedema

Dosage and Administration

The usual dosage for adult and pediatric patients 12 years of age or older is berotralstat 150 mg (1 capsule) administered orally once daily.

Approval Conditions

1. The applicant is required to develop and appropriately implement a risk management plan.
2. Because of extremely limited number of patients participating in clinical studies in Japan, the applicant is required to conduct a post-marketing use-results survey covering all patients treated with the product, until data from a certain number of patients are collected, in order to identify the characteristics of patients treated with the product and obtain safety and efficacy data promptly, and thereby to take necessary measures to ensure the proper use of the product.

Attachment

Review Report (1)

October 7, 2020

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

Product Submitted for Approval

Brand Name	Orladeyo Capsules 150 mg
Non-proprietary Name	Berotralstat Hydrochloride Hydrate
Applicant	OrphanPacific, Inc.
Date of Application	January 31, 2020
Dosage Form/Strength	Hard capsules each containing 172.5 mg of Berotralstat Hydrochloride Hydrate (equivalent to 150 mg berotralstat)

Proposed Indication

Prevention of attacks of hereditary angioedema

Proposed Dosage and Administration

The usual dosage for adult and pediatric patients 12 years of age or older is berotralstat 150 mg (1 capsule) administered orally once daily.

Table of Contents

List of Abbreviations

See Appendix.

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

Berotralstat Hydrochloride[1)] (berotralstat), the active ingredient of Orladeyo Capsules 150 mg (the product), is a small molecule plasma kallikrein inhibitor discovered by BioCryst Pharmaceuticals (the US) (BioCryst).

Hereditary angioedema (HAE) is an autosomal dominant inherited disorder characterized by recurrent episodes of angioedema (HAE attacks) on various body sites, such as skin, pharynx, larynx, and gastrointestinal tract, which can be triggered by trauma, medical or dental procedures, emotional stress, changes in hormone levels, etc. (*N Engl J Med*. 1996; 334: 1666-7). HAE attacks typically persist for 1 to 5 days and can cause severe pain, disability, and disfigurement (*Lancet*. 2012; 379: 474-81). Untreated oropharyngeal or laryngeal attacks allow worsening of edema, resulting in death from asphyxiation in some cases (*J Allergy Clin Immunol*. 2012; 130: 692-7). In patients with HAE, mutations in the *SERPING-1* gene result in deficient or dysfunctional complement 1 esterase inhibitor (C1-INH), which usually inactivates plasma kallikrein (pKal) and contributes to the regulation of bradykinin production. Overproduced bradykinin induces excessive vasodilatation, increased vascular permeability, and smooth muscle contraction, leading to angioedema as clinical symptoms.

In Japan, approved drugs for the treatment of acute HAE attacks are lyophilized human C1-inactivator concentrate containing the active ingredient of human blood C1-INH and icatibant acetate, which is a synthetic peptide that functions as a bradykinin B2 receptor antagonist. However, there are no approved medications that prevent HAE attacks, and the development of new medication has been long awaited. Focusing on berotralstat's inhibitory effect on pKal, the applicant started to develop the product to prevent HAE attacks, and has recently filed a marketing application based on the results from clinical studies including a Japanese phase III study (Study 301).

The clinical development of berotralstat for HAE began in May 2015. An approval application was filed in the US in December 2019 and in the EU in March 2020, based mainly on the results from a phase III study conducted in the US, Canada, Czech Republic, etc. (Study 302). As of September 2020, the applications are under review.

Berotralstat was designated as a SAKIGAKE product (SAKIGAKE Drug Designation No. 4 of 2015 [*27 yaku*]) as of October 27, 2015 and as an orphan drug (Orphan Drug Designation No. 425 of 2018 [*30 yaku*]) as of December 27, 2018, with the intended indication of "the suppression of attacks of hereditary angioedema." Meanwhile, there was a delay in the review process, as described below.

(1) As a rule, SAKIGAKE designation drugs undergo SAKIGAKE comprehensive evaluation consultation aiming to expedite the process from submission to approval. The applicant applied for the SAKIGAKE evaluation consultation for berotralstat on December 11, 2019, followed by non-clinical data on December 18, 2019, quality data on January 15, 2020, and clinical data on January 16, 2020. However, the applicant then submitted a marketing application for berotralstat on January 31, 2020, shortly after the data submission for SAKIGAKE evaluation consultation. As a result, enough preparation time was not given to PMDA for the

[1)] Japanese accepted name at the time of regulatory submission was "Berotralstat Hydrochloride Hydrate," which was changed to "Berotralstat Hydrochloride" based on "Japanese accepted names for pharmaceuticals" (PSEHB/PED Notification No. 0414-3 dated April 14, 2020).

identification of problems in the quality, efficacy, and safety of berotralstat to make clear the issues to be focused before the acceptance of application.

(2) According to the applicant, the data on the long-term berotralstat treatment based on the only clinical study in Japanese patients with HAE (Study 301) was initially scheduled to be submitted in July 2020 at the earliest, due to the subject enrollment status. Because berotralstat is intended to prevent HAE attacks, PMDA had a view that the safety and efficacy of berotralstat be evaluated based on long-term treatment in Japanese patients with HAE with the study data mentioned. However, as mentioned earlier, the submission of marketing application was advanced to the end of January 2020, and PMDA was not able to complete the review of Study 301 data within 6 months, i.e., the target review period for SAKIGAKE designation drugs.

(3) According to the data initially submitted for the application, a rat carcinogenicity study revealed increased incidence of hemangiosarcoma in animals treated with berotralstat. After the submission, the applicant conducted a third party-pathology peer review on this histopathological finding and, as a result, hemangiosarcoma was corrected to hemangioma or angiomatous hyperplasia. There were no problems with the methodology for re-evaluation of the histopathological finding or the procedures for correction of the finding. Taking account of the applicant's discussion on the possible relationship between hemangioma or vascular endothelial hyperplasia and berotralstat treatment after the re-evaluation, PMDA accepted the applicant's revised conclusion that berotralstat has low carcinogenic potential. However, due to the need of the re-evaluation of carcinogenicity study data, the review period was inevitably extended [see Section 5.4.2].

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

2.1 Drug substance

2.1.1 Characterization

The drug substance is a white to pale yellowish white or pale grayish white powder. Its description, solubility, hygroscopicity, melting point, dissociation constant, pH, partition coefficient, optical rotation, thermal analysis, crystalline polymorphism, particle size, bulk density, and tap density were determined.

Its chemical structure was elucidated by elemental analysis, ultraviolet-visible spectrum, infrared absorption spectrum (IR), nuclear magnetic resonance spectrum (NMR) (^{1}H-, ^{13}C-, ^{19}F-NMR), mass spectrum (MS), X-ray powder diffraction, and single-crystal X-ray crystallography.

2.1.2 Manufacturing process

The drug substance is synthesized using [redacted] (Starting Material A), [redacted] (Starting Material B), and [redacted] (Starting Material C) as starting materials.

Quality by Design (QbD) approaches were used. The quality control strategy was established based on the following (Table 1):

● Identification of critical quality attributes (CQAs)

● Identification of critical process parameters (CPPs) through quality risk assessment and design of experiments

Table 1. Overview of drug substance control strategy

CQA	Method of control
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

Critical steps are Intermediate a synthesis, Intermediate b synthesis, and [redacted]. Controlled critical intermediates include [redacted] (Intermediate a), [redacted] (Intermediate b), and [redacted].

2.1.3 Control of drug substance

The proposed specifications for the drug substance consist of content, description, identification (IR), purity [related substances (high performance liquid chromatography [HPLC]), [redacted]], residual solvents (gas chromatography [GC]), water content, residue on ignition, chloride, and assay (HPLC).

2.1.4 Stability of drug substance

The primary stability studies on the drug substance are shown in Table 2. The studies demonstrated the stability of the drug substance. Photostability data showed that the drug substance is photosensitive.

Table 2. Stability studies on drug substance

Study	Primary batches	Temperature	Humidity	Storage package	Storage period
Long-term	3 production batches	25°C	60%RH	double polyethylene bags + high-density polyethylene drum	24 months
Accelerated	3 production batches	40°C	75%RH		6 months

Based on the above, a re-test period of 36 months was proposed for the drug substance when stored in double polyethylene bags within a high-density polyethylene drum to protect from light at room temperature, in accordance with "Guideline on Evaluation of Stability Data" (PMSB/ELD Notification No. 0603004 dated June 3, 2003). The long-term testing will be continued up to [redacted] months.

2.2 Drug product

2.2.1 Description and composition of drug product and formulation development

The drug product is an immediate release hard capsule. Each capsule contains 172.5 mg of berotralstat hydrochloride (equivalent to 150 mg berotralstat) and excipients including pregelatinized starch, crospovidone, colloidal silicon dioxide, and magnesium stearate.

2.2.2 Manufacturing process

The drug product is manufactured through a process comprising mixing, [redacted], [redacted], capsule filling, and packaging/labeling. Capsule filling is defined as a critical step, in which process control items and values are specified.

QbD approaches were used. A quality control strategy was established based on the following (Table 3):

- Identification of CQAs from the quality target product profile
- Identification of CPPs through quality risk assessment and design of experiments

Table 3. Overview of drug product control strategy

CQA	Method of control
[redacted]	[redacted]
[redacted]	[redacted]

2.2.3 Control of drug product

The proposed specifications for the drug product consist of strength, description (appearance), identification (HPLC, ultraviolet spectrum), purity [related substances (HPLC)], water content, uniformity of dosage units [content uniformity testing (HPLC)], dissolution (HPLC), microbial limits, and assay (HPLC).

2.2.4 Stability of drug product

The primary stability studies on the drug product are shown in Table 4, which demonstrated the stability of the drug product. Photostability data showed that the drug product is photostable.

Table 4. Stability studies on drug product

Study	Primary batches	Temperature	Humidity	Storage package	Storage period
Long term	3 production batches	25°C	60%RH	Blister packs	24 months
Accelerated	3 production batches	40°C	75%RH		6 months

Based on the above, a shelf-life of 36 months was proposed for the drug product when packaged in blister packs (films made from polyvinyl chloride and polychlorotrifluoroethylene/aluminum foils) and stored at room temperature, in accordance with "Guideline on Evaluation of Stability Data" (PMSB/ELD Notification No. 0603004 dated June 3, 2003). The long-term testing will be continued up to [redacted] months.

2.R Outline of the review conducted by PMDA

Based on the submitted data, PMDA concluded that the quality of the drug substance and the drug product is adequately controlled.

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

The applicant submitted the results from primary pharmacodynamic studies that evaluated the inhibition of pKal activity and bradykinin production by berotralstat and from secondary pharmacodynamic studies that evaluated the effect of berotralstat on the coagulation system. The data submitted also included the results from safety pharmacology studies evaluating the effect of berotralstat on the central nervous, respiratory, and

cardiovascular systems as well as the results from a follow-up study that evaluated the effect of berotralstat on the cardiovascular system. Unless otherwise specified, pharmacologic parameters are expressed as the mean.

3.1 Primary pharmacodynamics

3.1.1 Inhibition of human pKal (CTD 4.2.1.1-1 and 4.2.1.1-2)

The inhibition effect of berotralstat on human pKal was evaluated using a chromogenic substrate assay. The half maximal inhibitory concentration (IC_{50}) was 0.88 nmol/L, and the inhibition constant (*K*i) was 0.44 nmol/L. The IC_{50} values of berotralstat for pKal and 9 serine proteases are shown in Table 5.

Table 5. Inhibitory activity of berotralstat against pKal and other serine proteases

Serine protease	IC_{50} (nmol/L)
pKal	0.88
Tissue kallikrein	>30,000
Plasmin	3,967
Trypsin	11,000
Thrombin	>50,000
Tissue plasminogen activator (tPA)	>50,000
Activated protein C (APC)	>30,000
Complement C1s	>50,000
Activated coagulation factor X (FXa)	>50,000
Activated coagulation factor XII (FXIIa)	>50,000

3.1.2 Inhibition of pKal-dependent production of bradykinin (CTD 4.2.1.1-3)

Using human umbilical vein endothelial cells (HUVEC) coated with high-molecular-weight kininogen, the inhibitory effect of berotralstat against bradykinin generation through pKal-mediated cleavage of high-molecular-weight kininogen was evaluated. Bradykinin levels in the supernatant were determined by an enzyme-linked immunosorbent assay (ELISA). Berotralstat decreased the bradykinin level concentration-dependently with a half-maximal effective concentration (EC_{50}) of 5.56 nmol/L.

3.1.3 Inhibition of pKal activity in healthy subjects and patients with HAE (CTD 4.2.1.1-4 and 4.2.1.1-5)

The inhibitory effect of berotralstat against pKal activity in healthy subjects and patients with HAE was evaluated using a fluorogenic assay. The EC_{50} values of berotralstat for pKal activity in healthy subjects and patients with HAE were 5.4 nmol/L and 15.9 nmol/L, respectively.

3.2 Secondary pharmacodynamics

3.2.1 Effect on coagulation system (CTD 4.2.1.2-1 and 4.2.1.2-2)

Because pKal is an upstream trigger for the intrinsic coagulation pathway, the effect of berotralstat on the blood coagulation system was evaluated using human plasma. Berotralstat prolonged the prothrombin time (PT) and the activated partial thromboplastin time (APTT) concentration-dependently, and the berotralstat concentrations required to produce a doubling of PT and APTT in human plasma were >100 μmol/L (an estimate) and 73.4 μmol/L, respectively. Berotralstat did not affect PT at 10 μmol/L or APTT at 6 μmol/L. These concentrations were, respectively, 35- and 21-fold the mean C_{max} after multiple oral administration of berotralstat at the recommended clinical dose (150 mg/day) in humans, i.e. 163 ng/mL (289 nmol/L, see Section 6.2.4).

3.3 Safety pharmacology

3.3.1 Effect on central nervous system (CTD 4.2.1.3-1)

Following a single oral dose of berotralstat 25, 100, or 450 mg/kg to Wistar rats (10 males/group), the effect of berotralstat on the central nervous system was evaluated using the functional observation battery (FOB). There were no deaths or no treatment-related effects on clinical signs or neurobehavioral measurements at any dose level.

3.3.2 Effect on respiratory system (CTD 4.2.1.3-2)

Following a single oral dose of berotralstat 25, 100, or 450 mg/kg to Wistar rats (8 males/group), the effect of berotralstat on the respiratory system was evaluated. There were no deaths or no treatment-related effects on clinical signs or respiratory function (respiratory rate, tidal volume, minute ventilation) at any dose level.

3.3.3 Effect on cardiovascular system

3.3.3.1 *In vitro* studies

3.3.3.1.1 Effect on cardiac ion channels (CTD 4.2.1.3-9 to 4.2.1.3-12)

Using cells expressing different human cardiac ion channels, the effects of berotralstat on the ion channel currents were evaluated using the patch-clamp technique. Berotralstat inhibited all ion channels tested concentration-dependently. The IC_{50} values are shown in Table 6.

Table 6. Effects of berotralstat on cardiac ion channels

Cardiac ion channels	Test system	IC_{50} (μmol/L)	Attached document CTD
hERG (hERG potassium currents)	HEK293 cells expressing hERG potassium channels	0.2	4.2.1.3-11
hNav1.5 (Peak sodium currents)	HEK293 cells expressing hNav1.5	2.4	4.2.1.3-12
hNav1.5 (Late sodium currents)	HEK293 cells expressing hNav1.5	0.1	4.2.1.3-9
hCav1.2 (L-type calcium currents)	CHO cells expressing hCav1.2	2.8	4.2.1.3-10

3.3.3.1.2 Effect on action potentials in isolated rabbit cardiac Purkinje fibers (CTD 4.2.1.3-5)

The effect of berotralstat on the action potentials in isolated rabbit cardiac Purkinje fibers was evaluated. Berotralstat did not prolong the action potential duration (APD) as a surrogate for the QT interval on ECG, at the concentrations of 0.9 to 93.7 μmol/L tested. Other changes in action potentials include decreases in action potential amplitude and in the instant rate of voltage change. At ≥9.4 μmol/L, a depolarized resting membrane potential was observed.

3.3.3.1.3 Effect on electrophysiological changes in human iPS cell-derived cardiomyocytes (CTD 4.2.1.3-7)

Using autonomously beating human iPS cell-derived cardiomyocytes, the effect of berotralstat on the field potential of cardiomyocytes was evaluated. Berotralstat 0.3 μmol/L increased the field potential duration (FPD), a surrogate for the QT interval on ECG, and the corrected field potential duration (FPD_c).

3.3.3.1.4 Effects on various receptors (CTD 4.2.1.3-8)

The effects of berotralstat on 103 different receptors were evaluated using radioligand binding assays. The *K*i value was lower than the highest concentration tested of 3 μmol/L at the cannabinoid CB1 receptor, melanocortin MC5, and somatostatin SST1 receptors only, and the *K*i values were 1.56, 1.92, and 2.09 μmol/L, respectively.

The applicant's explanation:

Given that the cannabinoid CB1, melanocortin MC5, and somatostatin SST1 receptors occupancies[2)] (approximately 0.002%) at the estimated plasma free drug concentration[3)] (approximately 3.8 nmol/L) after multiple oral administration of berotralstat at the recommended clinical dose (150 mg/day) in humans, there are no clear effects of berotralstat on these receptors.

3.3.3.2 *In vivo* study (CTD 4.2.1.3-4)

Following a single oral dose of berotralstat 0 (vehicle: water), 15, 50, or 150 mg/kg to conscious, unrestrained cynomolgus monkeys (4 females), the effect of berotralstat on body temperature, blood pressure, heart rate, and ECG was evaluated. Berotralstat 50 mg/kg caused increases in the PR, QT/QTc intervals, and the QRS width on ECG, and berotralstat 150 mg/kg decreased blood pressure and heart rate and increased the RR, PR, QT/QTc intervals, and the QRS width on ECG.

3.R Outline of the review conducted by PMDA

3.R.1 Mechanism of action of berotralstat in preventing HAE attacks

The applicant's explanation about the action mechanism of berotralstat in preventing HAE attacks:

In patients with HAE, deficient or dysfunctional C1-INH, which inactivates pKal, etc., leads to the activation of the kinin-kallikrein pathway involving pKal (*J Allergy Clin Immunol*. 2010; 126: 918-25). In the kinin-kallikrein pathway, pKal cleaves high-molecular-weight kininogen and releases bradykinin. Free bradykinin causes vasodilatation, enhanced vascular permeability, smooth muscle contraction, etc., leading to clinical symptoms of HAE attacks such as angioedema (*J Surg Res*. 2011; 167: 70-7, *Mol Immunol*. 2010; 47: 2161-9, etc.).

In pharmacology studies, berotralstat inhibited pKal, pKal activity in *ex vivo* plasma from healthy subjects and from patients with HAE, and bradykinin production on endothelial cells. Thus, berotralstat is expected to prevent acute attacks of angioedema in patients with HAE by inhibiting pKal.

PMDA's conclusion:

The submitted data demonstrated the inhibition of pKal by berotralstat, and berotralstat has promising potential to prevent HAE attacks in humans.

[2)] Receptor occupancy (%) = (Berotralstat concentration/(Berotralstat concentration + *K*i)) × 100

[3)] Estimated value calculated from the mean berotralstat exposure after multiple oral administration of berotralstat at the recommended clinical dose (150 mg/day) in humans (C_{max}, 163 ng/mL [289 nmol/L], see Section 6.2.4) and the plasma protein binding of berotralstat (approximately 98.7%, see Section 4.2.2)

3.R.2 Effect of berotralstat on cardiovascular system

The applicant's explanation about the effect of berotralstat on the cardiovascular system:

Verapamil etc. are known to block multiple ion channels, as does berotralstat, and do not tend to induce Torsades de Pointes (*Cardiovasc Res*. 2003; 58: 32-45, *Am Heart J Circulation*. 2004: 110; 904-910) etc. The proarrhythmic risk of berotralstat is thus low in humans.

PMDA's view:

It is difficult to conclude that the proarrhythmic risk of berotralstat is low as that of verapamil based on berotralstat's multiple ion channel-blocking activity. While the evaluation using isolated rabbit cardiac Purkinje fibers did not suggest QT interval prolongation caused by berotralstat [see Section 3.3.3.1.2], findings from the evaluation of the extracellular potential of human iPS cell-derived cardiomyocytes were suggestive of QT interval prolonged by berotralstat [see Section 3.3.3.1.3]. Furthermore, ECG of cynomolgus monkeys showed a trend toward QT/QTc interval prolongation [see Section 3.3.3.2]. Given these findings, the potential risk of QT interval prolongation associated with berotralstat cannot be ruled out. Thus, the proarrhythmic risk of berotralstat in humans needs to be assessed comprehensively, taking account of the results of a thorough QT/QTc study and the occurrence of cardiovascular adverse events in the clinical studies in patients with HAE.

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

The applicant submitted the data on absorption, distribution, metabolism, excretion, and drug-drug interactions, in the form of the results from oral and intravenous administration studies in mice, rats, rabbits, and monkeys. Berotralstat or 2 types of ^{14}C-berotralstat (A or B)[4)] were used in the pharmacokinetic studies. Plasma berotralstat concentrations were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (lower limit of quantification, 1 ng/mL in mouse and rabbit plasma, 1 or 5 ng/mL in rat plasma, 5 ng/mL in monkey plasma), and radioactivity concentrations in samples were determined using liquid scintillation counter or quantitative autoradiography. Unless otherwise specified, pharmacokinetic parameters are expressed as the mean or the mean ± standard deviation (SD), and doses are expressed as free base.

4.1 Absorption

4.1.1 Single-dose studies (CTD 4.2.2.2-1 to 4.2.2.2-2)

Table 7 shows the pharmacokinetic parameters following a single oral or intravenous dose of berotralstat in rats or monkeys under fasting conditions. The absolute oral bioavailability of berotralstat was 33% in rats and 45% in monkeys.

4) ^{14}C was incorporated into [redacted] for ^{14}C-berotralstat A, and ^{14}C was incorporated into [redacted] for ^{14}C-berotralstat B. Radioactivity following administration of ^{14}C-berotralstat A is thought to be derived from unchanged drug and its associated metabolites, and radioactivity following administration of ^{14}C-berotralstat B is thought to be derived from unchanged drug, [redacted], and its associated metabolites.

Table 7. Pharmacokinetic parameters following a single dose of berotralstat

Species	Route of administration	Dose (mg/kg)	N	C_{max} (ng/mL)	AUC_{0-96} (ng·h/mL)	T_{max} (h)	CL (mL/min/kg)	$t_{1/2}$ (h)
Rat	IV	3	6M	773 ± 87	1,858 ± 221	—	27 ± 3.0	10.1 ± 0.5
	Oral	30	6M	475 ± 125	6,179 ± 498	2.0 ± 0.0	81 ± 6.7	18.5 ± 3.4
Monkey	IV	2.5	3M [a)]	673 ± 164	1,407 ± 180	—	30 ± 4.4	18.3 ± 3.8
	Oral	25	3M [a)]	342 ± 207	6,358 ± 2,762	4.7 ± 2.3	73 ± 26	22.7 ± 6.4

Mean ± SD; —, Not applicable
a) Administered in a crossover manner with a 2-week washout period.

4.1.2 Repeated-dose studies (CTD 4.2.3.2-1, 4.2.3.2-3, 4.2.3.2-5, 4.2.3.2-7 to 4.2.3.2-8, 4.2.3.4.1-1 to 4.2.3.4.1-2, 4.2.3.4.2-1)

Table 8 shows the pharmacokinetic parameters following repeated oral doses of berotralstat in mice, rats, or monkeys. Berotralstat exposure increased in an approximately dose-proportional manner in mice and monkeys, and was supra proportional to dose in rats. While berotralstat exposure tended to be higher in female mice than in male mice, there were no consistent sex differences in rats and monkeys. No evident bioaccumulation of berotralstat occurred in mice, but berotralstat tended to accumulate in rats and at higher dose levels (≥55 mg/kg) in monkeys.

Table 8. Pharmacokinetic parameters following repeated oral doses of berotralstat

Species	N	Sampling day	Dose (mg/kg/day)	C_{max} (ng/mL)		AUC_{0-24} (ng·h/mL)		T_{max} (h)	
				Male	Female	Male	Female	Male	Female
Mouse	3/sex/time point	Day 1	8	231	433	2,100	3,280	1	2
			20	678	714	5,670	7,000	2	1
			50	1,880	2,430	16,100	19,000	2	1
		Day 182	8	164	535	2,280	4,690	1	2
			20	647	1,120	8,090	14,100	4	2
			50	2,280	2,970	23,100	31,800	1	1
Rat	3/sex/time point	Day 1	1	—	1.81	—	5.44	—	4
			2.5	6.68	16.7	68.4	133	4	2
			7.5	86.7	76.3	717	854	4	2
			20	312	289	2,990	3,390	2	2
		Day 182	1	4.44	4.65	52.3	61.5	4	2
			2.5	28.5	29.8	270	325	2	2
			7.5	182	158	2,020	2,030	1	2
			20	831	584	10,500	9,030	2	1
Monkey	7/sex	Day 1	30	531 ± 167	464 ± 130	7,980 ± 1,870	7,420 ± 1,780	8 [2, 8]	4 [4, 8]
	7/sex		55	485 ± 243	426 ± 176	8,180 ± 3,960	6,510 ± 3,500	8 [1, 24]	8 [2, 8]
	9/sex		80	620 ± 175	592 ± 221	10,600 ± 2,980	9,930 ± 4,480	8 [4, 8]	4 [2, 8]
	7/sex	Day 90	30	410 ± 108	427 ± 115	7,380 ± 1,370	7,930 ± 2,090	8 [1, 8]	8 [4, 8]
	7M6F		55	624 ± 254	508 ± 173	12,500 ± 4,950	10,100 ± 3,370	8 [2, 8]	8 [1, 8]
	8M9F		80	948 ± 419	1,030 ± 366	19,400 ± 9,180	20,600 ± 7,220	8 [4, 8]	8 [1, 8]
	4/sex	Day 270	30	222 ± 65.8	333 ± 311	4,100 ± 1,150	3,810 ± 1,470	8 [8, 8]	8 [4, 8]
	4/sex		55	395 ± 141	344 ± 131	8,300 ± 2,880	7,260 ± 2,670	8 [4, 8]	—
	6/sex		80	622 ± 226	761 ± 252	11,900 ± 4,220	15,100 ± 5,230	4 [2, 8]	4 [2, 8]

Mean or Mean ± SD; Median or Median [range] for T_{max}; —, Not applicable

4.2 Distribution

4.2.1 Tissue distribution (CTD 4.2.2.2-3, 4.2.2.2-5)

Following a single oral dose of ^{14}C-berotralstat A 30 mg/kg to albino and pigmented rats (1 male/time point[5)]), tissue distribution of radioactivity was determined by quantitative autoradiography. Radioactivity level peaked

[5)] Measured at 1, 4, 8, and 24 hours post-dose in albino rats and at 1, 4, 8, 24, 48, 72, 96, 168, 336, 672, 1,080, and 1,440 hours post-dose in pigmented rats.

at 8 hours post-dose in many tissues. High radioactivity was detected at 8 hours post-dose in the liver, renal medulla, spleen, adrenal gland, kidney, lung, pituitary gland, and renal cortex in albino rats, and in the liver, spleen, adrenal gland, renal medulla, lung, renal cortex, pituitary gland, and kidney in pigmented rats (in descending order). In pigmented rats, radioactivity was eliminated from most tissues by 1,440 hours post-dose, and residual radioactivity was highest in the uvea. Since the radioactivity level in the uvea peaked at 72 hours post-dose and then declined over time, berotralstat was considered to reversibly bind to melanin. A phototoxicity study also showed that berotralstat has little phototoxic potential [see Section 5.6.1].

Following a single oral dose of ^{14}C-berotralstat B 30 mg/kg to albino and pigmented rats (1 male/time point[6)]), tissue distribution of radioactivity was determined by quantitative autoradiography. Radioactivity level peaked at 48 hours post-dose in the brain, brown fat, eyeballs, fat, and uvea in pigmented rats, and at 8 hours post-dose in other tissues of pigmented rats and most tissues of albino rats. Because radioactivity levels in most tissues were higher than the blood radioactivity level at 48 hours post-dose, berotralstat was considered to be extensively distributed in tissues.

4.2.2 Plasma protein binding (CTD 4.2.2.3-2)

Berotralstat 3 μmol/L was added to the plasma from mouse, rat, rabbit,[7)] monkey,[7)] and human, and the plasma protein binding of berotralstat was determined using an equilibrium dialysis method. The plasma protein binding of berotralstat was 99.4 ± 0.03%, 98.9 ± 0.01%, 81.9 ± 0.37%, 74.1 ± 0.87%, and 98.7 ± 0.06%, respectively.

4.2.3 Distribution in blood cells (CTD 4.2.2.3-1)

When berotralstat 3 μmol/L was added to the whole blood from mouse, rat, rabbit,[7)] monkey,[7)] and human, the red blood cell to plasma ratios were 0.40, 1.08, 1.34, 0.33, and 1.74, respectively.

4.2.4 Placental transfer (CTD 4.2.3.5.2-3, 4.2.3.5.2-5)

Pregnant rats (8/group) were dosed with oral berotralstat 10, 25, or 75 mg/kg/day from gestation days 6 to 17, and maternal and fetal plasma concentrations of berotralstat were determined. Maternal C_{max} values on gestation day 17 in the berotralstat 10, 25, and 75 mg/kg/day groups were 191, 653, and 1,440 ng/mL, respectively. Fetal plasma concentrations of berotralstat at 4 hours after a maternal dose on gestation day 17 were 7.22, 46.3, and 73.7 ng/mL, respectively.

Pregnant rabbits (3/group) were dosed with oral berotralstat 20, 50, or 100 mg/kg/day from gestation days 7 to 20, and maternal and fetal plasma concentrations of berotralstat were determined. Maternal C_{max} values on gestation day 19 in the berotralstat 20, 50, and 100 mg/kg/day groups were 83.3, 266, and 367 ng/mL, respectively. Fetal plasma concentrations of berotralstat at 3 hours after a maternal dose on gestation day 20 were 5.81, 30.4, and 40.7 ng/mL, respectively.

[6)] Measured at 1, 4, 8, 24, 48, 72, 96, 120, 144, and 168 hours post-dose in albino rats and at 4, 8, 24, 48, and 96 hours post-dose in pigmented rats.
[7)] The results deserve careful interpretation because berotralstat may be unstable in rabbit and monkey plasma.

4.3 Metabolism

4.3.1 *In vitro* studies (CTD 4.2.2.4-1, 4.2.2.4-3, 4.2.2.4-4, 4.2.2.4-7, 4.2.2.4-8, 4.2.2.4-9)

Recombinant human CYP isoforms (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4) were incubated with berotralstat (2.7 μmol/L) for 1 hour in the presence or absence of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH). Incubation with CYP2D6 and CYP3A4 in the presence of NADPH resulted in 15.0% and 22.4% loss of berotralstat, respectively. Incubation with other CYP isoforms in the presence of NADPH or with CYP2D6 and CYP3A4 in the absence of NADPH resulted in <10% loss of berotralstat. These results suggested that berotralstat is predominantly metabolized by CYP2D6 and CYP3A4.

Recombinant human CYP isoforms (CYP2D6 and CYP3A4) were incubated with berotralstat (10 μmol/L). Unchanged berotralstat and 9 different metabolites including M3, M7, and M9 were detected.

Berotralstat (0.3 μmol/L) was added to human hepatocytes in the presence or absence of a CYP inhibitor, 1-aminobenzotriazole, and the metabolism of berotralstat was studied. The intrinsic clearance of berotralstat was 24.7 or 17.9 mL/min/kg, respectively, and the fraction of berotralstat metabolized by CYP was 0.275. Detected metabolites were M3 and M7.

When berotralstat (0.89-89 μmol/L) was added to human liver microsomes and incubated for 2 hours in the presence of uridine diphosphate glucuronic acid (UDPGA) 8 mmol/L, berotralstat was stable, suggesting that berotralstat does not undergo glucuronide conjugation.

Berotralstat (3 μmol/L) and cyclopropylcarboxylic acid (CPCA) (3 μmol/L) were added to rat or human hepatocytes and incubated for 24 hours. The carnitine and glycine conjugates of CPCA were identified. In rat hepatocytes, 82.0% of berotralstat remained intact, and predominant metabolites of M2, M3, M9, and M10 represented 3.76%, 4.56%, 2.63%, and 3.23%, respectively. In human hepatocytes, 92.3% of berotralstat remained intact, and predominant metabolites of M2, M3, M9, and M10 represented 2.18%, 0.52%, 0.02%, and 2.35%, respectively.

4.3.2 *In vivo* studies (CTD 4.2.2.2-3 to 4.2.2.2-6, 4.2.2.4-5, 4.2.2.4-10)

Metabolites in different samples following a single or repeated oral doses of berotralstat or ^{14}C-berotralstat A or B in mice, rats, or monkeys are shown in Table 9. In a mass balance study in humans, no human-specific metabolites were identified [see Section 6.2.1.2].

Table 9. Metabolite profiles in different species

Species	Dosing regimen	Test article	N	Plasma	Bile	Feces	Urine	Attached document CTD
Mouse	20 mg/kg/day Repeated oral doses	Berotralstat	3M	At 24 hours post-dose unchanged berotralstat, M3, M7, M9, M11, M12				4.2.2.4-10
Rat	30 mg/kg Single oral dose	^{14}C-A	3M /time point	Up to 48 hours post-dose unchanged berotralstat (60.9%[b]), M3, M9, M10		Up to 120 hours post-dose unchanged berotralstat (20.7%[b]), M3, M5, M7, M8, M9, M10, M23, M42, and uncharacterized metabolites	Up to 72 hours post-dose unchanged berotralstat (0.53%[b]), M3, M5, M11, M12, and uncharacterized metabolites	4.2.2.2-5
			3M[a] /time point		Up to 96 hours post-dose unchanged berotralstat (<0.3%), M3, M5, M7, M9, M23, M27, M29, M33, and uncharacterized metabolites	Up to 120 hours post-dose unchanged berotralstat (16.8[b]), M3, M5, M7, M8, M9, M10, M23, M42, and uncharacterized metabolites	Up to 72 hours post-dose unchanged berotralstat (1.33%[b]), M3, M5, M11, M12, M23, M27, and uncharacterized metabolites	
	30 mg/kg Single oral dose	^{14}C-B	3-5M /time point	Up to 8 hours post-dose unchanged berotralstat (10.8%), M1, M2	Up to 72 hours post-dose unchanged berotralstat (1.3%), M7, M10, and uncharacterized metabolites	Up to 48 hours post-dose unchanged berotralstat (18.1%), M7, M8, and uncharacterized metabolites	Up to 48 hours post-dose unchanged berotralstat (0.1%), M1	4.2.2.2-3
	20 mg/kg Repeated oral doses	Berotralstat	3M3F	At 8 and 48 hours post-dose unchanged berotralstat, M2, M3, M7, M8, M9, M10		Day after repeated dosing unchanged berotralstat, M3, M4, M5, M6, M7, M8, M9, M10	Up to 12 hours post-dose unchanged berotralstat, M1, M2, M3, M5,[c] M6, M7, M8, M9, M10	4.2.2.4-5
Monkey	30 mg/kg Single oral dose	^{14}C-A	3M	Up to 96 hours post-dose unchanged berotralstat, M5, M7, M8, M9, M10, M64, and uncharacterized metabolites		Up to 168 hours post-dose unchanged berotralstat (17.3%), M3, M5, M6, M7, M8, M9, M10, M61, M64, M65, M67, and uncharacterized metabolites	Up to 120 hours post-dose unchanged berotralstat (0.52%), M3, M5, M6, M7, M9, M10, M64, and uncharacterized metabolites	4.2.2.2-6
	30 mg/kg Single oral dose	^{14}C-B	3M /time point	Up to 12 hours post-dose unchanged berotralstat (7.2%), M1, M2, M7		Up to 72 hours post-dose unchanged berotralstat (15.6%), M7, M8, M9, M10	Up to 72 hours post-dose unchanged berotralstat (<0.05%), M1, M2, M7	4.2.2.2-4
	20 mg/kg/day Repeated oral doses	Berotralstat	6M6F	Up to 4 hours post-dose unchanged berotralstat, M2, M3, M5, M7, M8, M9, M10		Day after repeated dosing unchanged berotralstat, M3, M4, M5, M6, M7, M8, M9, M10	Day after repeated dosing unchanged berotralstat, M1, M2, M3, M5, M6, M7, M8, M9, M10	4.2.2.4-5

a) Bile duct-cannulated rats, b) Percentage of radioactivity derived from unchanged berotralstat relative to total radioactivity in samples up to the last time point
c) Detected in females only.

Based on the above, the hypothesized metabolic pathways of berotralstat are shown in Figure 1.

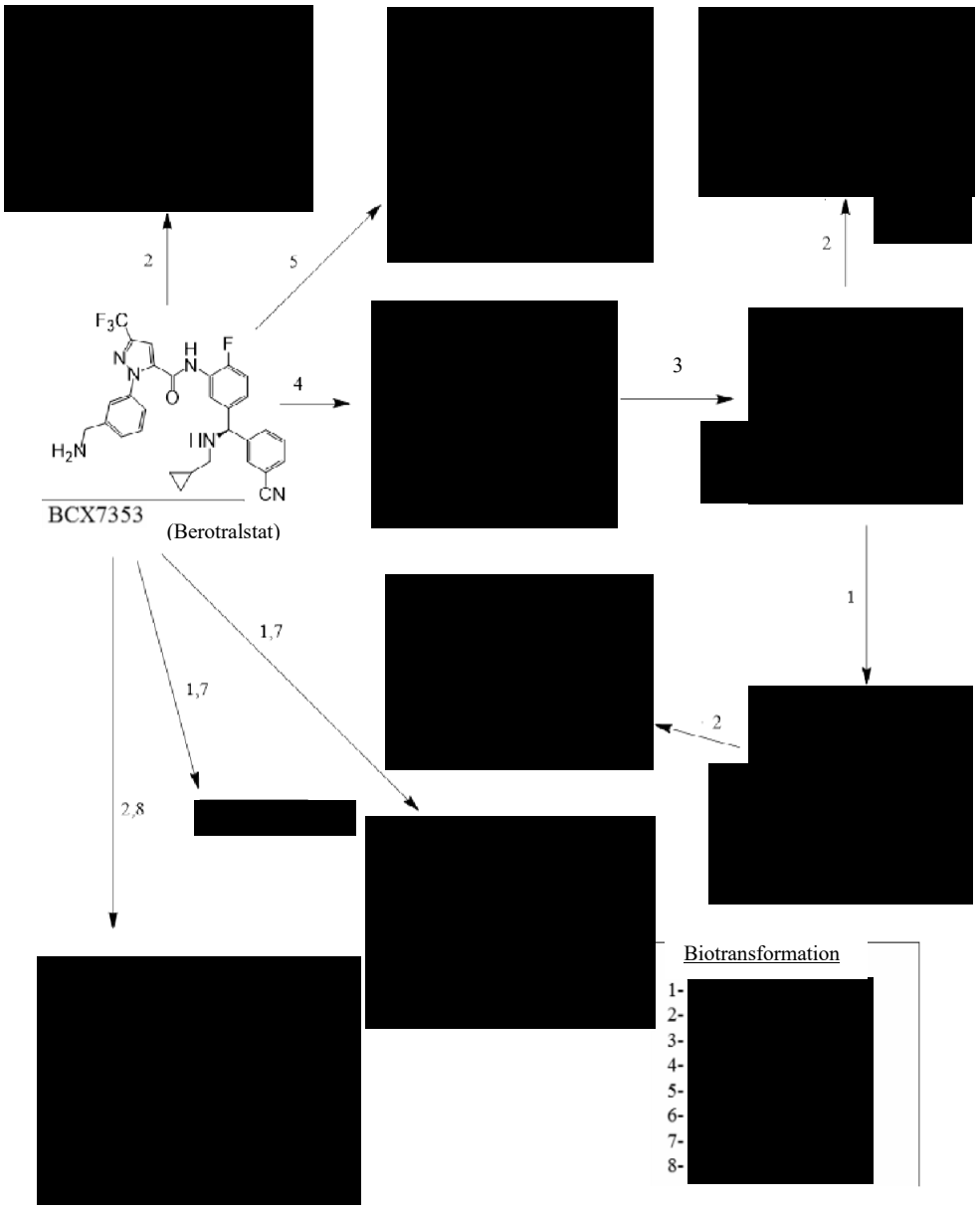


Figure 1. Hypothesized metabolic pathways of berotralstat

4.4 Excretion

4.4.1 Urinary and fecal excretion and biliary excretion (CTD 4.2.2.2-3 to 4.2.2.2-6)

Following the oral administration of ^{14}C-berotralstat A 30 mg/kg in albino rats (3 males), the mean total recovery of radioactivity up to 168 hours post-dose was 99.8%, and urinary and fecal recoveries of radioactivity were 2.47% and 94.0%, respectively. The predominant components in these samples (the percentage of total radioactivity administered) were M3 (1.35%) and unchanged berotralstat (0.531%) in the urine up to 72 hours post-dose and unchanged berotralstat (20.7%), M7 (6.59%), M5 (4.89%), and M3 (4.55%) in the feces up to 120 hours post-dose.

Following the oral administration of ^{14}C-berotralstat A 30 mg/kg in bile duct-cannulated rats (3 males), the mean total recovery of radioactivity up to 96 hours post-dose was 98.8%, and biliary, urinary, and fecal

recoveries of radioactivity were 22.6%, 6.59%, and 57.4%, respectively. The predominant components in these samples (the percentage of total radioactivity administered) were M5 (3.08%) and M3 (2.27%) in the bile up to 96 hours post-dose, M3 (2.35%) and unchanged berotralstat (1.33%) in the urine up to 72 hours post-dose, and unchanged berotralstat (16.8%) and M8 (4.4%) in the feces up to 96 hours post-dose.

Following the oral administration of ^{14}C-berotralstat A 30 mg/kg in cynomolgus monkeys (3 males), the mean total recovery of radioactivity up to 336 hours post-dose was 91.2%, and urinary and fecal recoveries of radioactivity were 4.23% and 85.3%, respectively. The predominant components in these samples (the percentage of total radioactivity administered) were M5 (1.37%), M3 (0.702%), and unchanged berotralstat (0.515%) in the urine up to 120 hours post-dose and unchanged berotralstat (17.3%), M5 (10.5%), and M7 (10.2%) in the feces up to 168 hours post-dose.

4.4.2 Excretion into milk (CTD 4.2.3.5.3-1)

Lactating rats (4/group) were dosed with oral berotralstat 10, 25, or 45 mg/kg/day from gestation day 6 to lactation day 14. The mean plasma berotralstat concentrations in pups on lactation day 14 at 10, 25, and 45 mg/kg/day of berotralstat were 1.11, 8.53, and 29.5 ng/mL, respectively, in male pups and 1.50, 9.98, and 30.0 ng/mL, respectively, in female pups, suggesting berotralstat excretion into milk. The plasma berotralstat concentrations in dams at 4 hours post-dose on lactation day 14 were 63.8, 360.3, and 688.3 ng/mL, respectively.

4.5 Pharmacokinetic interactions

4.5.1 Enzyme inhibition or induction (CTD 4.2.2.6-1 to 4.2.2.6-4)

Using human liver microsomes, the potential of berotralstat to inhibit CYP isoforms (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5) was assessed.[8)] The IC_{50} values for the CYP isoforms (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5) were 27, 14, 13, 18, 0.24, 0.36, 2.3, >100, and 2.5/2.2 μmol/L, respectively. Berotralstat was a time-dependent inhibitor of CYP3A4/5 only.

Using human hepatocytes, the potential of berotralstat 0.886 and 2.66 μmol/L to induce the activity and mRNA expression of CYP isoforms (CYP1A2, CYP2B6, CYP3A4/5) was assessed. Berotralstat induced 2.15- to 2.62-fold and 1.27- to 2.63-fold increases in CYP1A2 activity and mRNA expression, respectively, 1.61- to 2.92-fold and 1.77- to 2.93-fold increases in CYP2B6 activity and mRNA expression, respectively, and 0.85- to 1.37-fold and 6.04- to 40.6-fold[9)] increases in CYP3A4/5 activity and mRNA expression, respectively.

[8)] The following substrates of CYP isoforms were used: phenacetin for CYP1A2, coumarin for CYP2A6, bupropion for CYP2B6, amodiaquine for CYP2C8, diclofenac for CYP2C9, S-mephenytoin for CYP2C19, bufuralol for CYP2D6, chlorzoxazone for CYP2E1, midazolam and testosterone for CYP3A4/5

[9)] CYP3A4 mRNA expression

4.5.2 Evaluation of berotralstat as a substrate of drug transporters (CTD 4.2.2.6-5)

A study using MDCKII cells expressing human P-glycoprotein (P-gp) or breast cancer resistance protein (BCRP)[10] suggested that berotralstat is a substrate of P-gp and BCRP.

A study using human embryonic kidney 293 (HEK293) cells expressing human organic anion transporting polypeptide 1B1 (OATP1B1), OATP1B3, organic anion transporter 1 (OAT1), OAT3, organic cation transporter 2 (OCT2), multidrug and toxin extrusion 1 (MATE1), or MATE2-K[11] suggested that berotralstat is not a substrate of these transporters.

4.5.3 Evaluation of berotralstat as an inhibitor of drug transporters (CTD 4.2.2.6-5 to 4.2.2.6-7)

Using cells expressing various transporters, the potential of berotralstat to inhibit the transport of their substrates[12] was assessed. The results are shown in Table 10.

The applicant's explanation:
Given that the C_{max} following once daily oral dose of berotralstat 150 mg was 0.281 μmol/L, berotralstat may inhibit P-gp at the recommended clinical dose.

Table 10. Inhibition of drug transporters by berotralstat

Cells	Transporter	Berotralstat concentrations tested (μmol/L)	IC_{50} value (μmol/L)	Cells	Transporter	Berotralstat concentrations tested (μmol/L)	IC_{50} value (μmol/L)
Caco-2	P-gp	0.27 - 44	0.492	HEK293	OAT3	0.089 - 8.9	>8.9
MDCKII	BCRP	0.27 - 44	12.0			0.1 - 100	>30 [a)]
HEK293	OATP1B1	0.089 - 8.9	>8.9		OCT2	0.089 - 8.9	>8.9
		0.1 - 100	13.2			0.1 - 100	13.3
	OATP1B3	0.089 - 8.9	>8.9		MATE1	0.089 - 8.9	3.53
		0.1 - 100	>30 [a)]		MATE2-K	0.089 - 8.9	4.60
	OATP2B1	0.1 - 100	>30 [a)]	Vesicle	MRP2	0.1 - 100	>100
	OAT1	0.089 - 8.9	>8.9	Oocyte	OATP1A2	0.1 - 100	11.3
		0.1 - 100	>30 [a)]	Sf9	BSEP	0.01 – 10	>10

a) Because berotralstat ≥30 μmol/L was cytotoxic to HEK293 cells, the highest concentration tested was 30 μmol/L.

4.R Outline of the review conducted by PMDA

PMDA concluded that the submitted study results gave a grasp of the body's handling of berotralstat to a certain extent. The potential of berotralstat to cause relevant pharmacokinetic interactions in clinical use needs to be assessed, taking also account of the clinical study results [see Section 6.2.5].

5. Toxicity and Outline of the Review Conducted by PMDA

The toxicity studies of berotralstat included repeated-dose toxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, and other (a phototoxicity study and a study on impurities).

[10] The transporter inhibitors used: valspodar for P-gp, Ko143 for BCRP
[11] The transporter inhibitors used: rifampicin or cyclosporine for OATP1B1/3, probenecid or novobiocin for OAT1, probenecid or ibuprofen for OAT3, quinidine or cimetidine for OCT2, cimetidine for MATE1 and MATE2-K
[12] The substrates of transporters used: digoxin for P-gp, prazosin for BCRP, estradiol-17β-glucuronide for OATP1B1, OATP1B3, and MRP2, estrone-3-sulfate for OATP1A2, OATP2B1, and OAT3, *p*-aminohippuric acid for OAT1, metformin for OCT2, MATE1, and MATE2-K, taurocholic acid for BSEP

5.1 Single-dose toxicity

Single-dose toxicity studies of berotralstat were conducted in rats and cynomolgus monkeys (Table 11). In the rat study, mortality or moribund sacrifice was noted at 750 mg/kg, and the approximate lethal dose was determined to be 750 mg/kg. Major acute symptoms included vomiting/vomitus in cynomolgus monkeys.

Table 11. An overview of single-dose toxicity studies

Test system	Route of administration	Duration of dosing	Dose (mg/kg)	Major findings	Approximate lethal dose (mg/kg)	Attached document CTD
Male and female rats (Wister)	Oral gavage	Single dose	150, 250, 450, 750	750: deaths and moribund sacrifice (female); cecum dilation, liquid accumulation; limb hair loss; small spleen; reddish discoloration of the glandular mucosa of the stomach (a necropsied female rat) ≥450: pupillary reflex loss (male) 750: decreases in body weight, body weight gain, and food consumption (male and female); decreased activity; pupillary reflex loss; vocalization; hunched position; piloerection; cold skin (female) 450: decreased activity (male) 250: decreases in body weight and body weight gain (female)	750	4.2.3.2-1
Male and female cynomolgus monkeys	Oral gavage	Single dose	75, 150, 300, 500, 700	≥150: vomiting/vomitus (female) 500: decreased blood pressure (up to 4 hours post-dose, male and female) 300: vomiting/vomitus (male)	—	4.2.3.2-2

5.2 Repeated-dose toxicity

Repeated oral dose toxicity studies of berotralstat were conducted in rats (Table 12). The no-observed-adverse-effect level (NOAEL) in the rat 26-week repeated-dose toxicity study was determined to be 20 mg/kg. The $AUC_{0\text{-}24}$ at the NOAEL at Week 26 was 9,710 ng·h/mL, which was approximately 3.4-fold the human exposure[13] (AUC_{tau}, 2,850 ng·h/mL).

The major systemic toxicities or abnormal findings included single cell necrosis and bile duct degeneration in the liver, increases in blood AST/ALT activity, bile duct hyperplasia/dilation, renal tubular degeneration/regeneration in the kidney, skeletal and cardiac muscle necrosis, and increases in blood amylase/lipase and the urinary/blood level of di-22:6 bis(monoacylglycerol)phosphate (BMP). Phospholipidosis-related changes included an increase in the urinary level of BMP as well as vacuolation/pigmentation in macrophages/histiocytes in the liver/bile duct and multiple organs/various tissues. Phospholipidosis-related vacuolation in macrophages/histiocytes etc. were observed also at the end of the recovery period. The abnormal values or changes noted at the NOAELs in these studies were considered of little toxicological significance, based on the presence or absence of correlative abnormalities, frequency, and the severity of the findings.

[13] Mean berotralstat exposure following multiple oral doses of berotralstat at the recommended clinical dose (150 mg/day) in humans (Study 106)

Table 12. An overview of repeated-dose toxicity studies in rats

Test system	Route of administration	Duration of dosing	Dose (mg/kg)	Major findings	NOAEL (mg/kg)	Attached document CTD
Male and female rats (Wister)	Oral gavage	7 days (once daily)	0,[a)] 15, 50, 150	≥50: salivation, audible breathing, struggling during dosing, increased blood fibrinogen (male and female), decreased blood albumin, increased blood globulin (female) 150: death (female), decreased activity, hunched position, decreases in body weight/body weight gain/food consumption, increases in white blood cell count/neutrophil count/monocyte count, decreased reticulocyte count, increases in blood AST/ALT/amylase/lipase, irregular surface of the forestomach/nodules, decreases in spleen/thymus weights, bile duct hyperplasia/bile duct inflammation/ centrilobular hepatocellular vacuolation in the liver, erosion or ulceration/squamous cell hyperplasia/subacute or chronic inflammation in the forestomach, vacuolation in the adrenal cortex, lymphoid depletion in the spleen, vacuolated macrophages, lymphoid depletion in the thymus (male and female), decreased blood albumin, increased blood globulin (male), piloerection, increases in red blood cell count/hemoglobin/hematocrit, increased blood cholesterol, brown foci in the forestomach, increased liver weight, decreased thyroid gland weight, single cell necrosis/periportal vacuolation in the liver (female)	50	Reference data 4.2.3.2-1
Male and female rats (Wister)	Oral gavage	4 weeks (once daily) + 2-week recovery period	0,[a)] 5, 10, 25, 75	Deaths 75: 5 males and 1 female, audible breathing, shallow breathing, dyspnea, reddish material around the nose, hunched position, decreased body weight, increases in white blood cell count/neutrophil count/monocyte count/platelet count, appearance of segmented neutrophils/basophilic neutrophils, increases in blood fibrinogen/globulin, decreases in red blood cell count/hemoglobin/hematocrit, increases in blood sodium/inorganic phosphate/urea nitrogen/triglycerides, decreased blood albumin, decreased lymphocyte count, increases in blood APTT/AST/ALT, inflammatory changes in the respiratory tissues, hepatocellular/skeletal muscle necrosis in the liver, lymphoid follicle atrophy in the thymus Surviving animals ≥5: vacuolated macrophages in the lung (male and female), bile duct hypertrophy in the liver (female) ≥10: bile duct hypertrophy in the liver (male), generalized rigidity, vacuolated macrophages in the liver (female) ≥25: bile duct degeneration/necrosis in the liver, vacuolated macrophages in the small intestine (jejunum or ileum) (male and female), vacuolated macrophages in the liver (male), increased reticulocyte count, vacuolation in the adrenal cortex, bile duct inflammatory changes in the liver, Kupffer cell hypertrophy/vacuolation in the liver, vacuolation in the pituitary gland, vacuolated macrophages in the duodenum, lymphoid depletion in the thymus (female) 75: audible breathing, piloerection, dyspnea, shallow breathing, reddish material around the nose, decreases in body weight/body weight gain/food consumption, decreased MCV, increased platelet count, increases in neutrophil count/monocyte count, increases in blood fibrinogen/blood lipase, decreased blood albumin, increased liver weight, decreased thymus weight, vacuoles in the choroid plexus in the brain, renal tubular regeneration/vacuolation in the kidney, vacuolated macrophages in the large intestine, myofiber degeneration or necrosis/regeneration in the larynx, vacuolated macrophages in the mandibular/mesenteric lymph nodes, hepatocellular vacuolation in the liver, vacuolation of acinar cells of the pancreas, vacuolation in the cells of the	10	4.2.3.2-3

Test system	Route of administration	Duration of dosing	Dose (mg/kg)	Major findings	NOAEL (mg/kg)	Attached document CTD
				salivary gland/parotid gland, myofiber degeneration/necrosis of skeletal muscles, vacuolated macrophages in the spleen, erosion/ulceration in the glandular stomach, erosion or necrosis of the laryngeal squamous epithelium/respiratory epithelium, erosion or necrosis/degeneration or regeneration/subacute or chronic inflammatory response of the laryngeal/tracheal respiratory epithelium, subacute or chronic inflammatory changes in the lung, neutrophil infiltration in the small intestine (male and female), rigidity, increased reticulocyte count, vacuolation in the adrenal cortex, bile duct inflammatory changes in the liver, Kupffer cell hypertrophy/vacuolation in the liver, acute inflammatory response in the lung, vacuolation in the pituitary gland, myofiber regeneration of skeletal muscles, vacuolated macrophages in the duodenum, lymphoid depletion in the spleen, lymphoid depletion in the thymus (male), decreased MCH, increases in blood AST/ALT, increased adrenal gland weight, myofiber degeneration or necrosis/regeneration in the esophagus, cardiac muscle degeneration or necrosis in the heart, bile duct hyperplasia in the liver, vacuolated macrophages in the ovary, single cell necrosis in the liver, vacuolated macrophages in the nonglandular stomach, myofiber necrosis in the tongue (female) These findings were reversible.		
Male and female rats (Wister)	Oral gavage	13 weeks (once daily) + 6-week recovery period	0,[a)] 2.5, 7.5, 20	≥7.5: increased urinary level of BMP, hypertrophy of the bile duct epithelium (male and female) 20: vacuolated macrophages in the bile duct, myelinosomes in Kupffer cells[1)] (male and female), increased liver weight, bile duct hyperplasia (female)	20	4.2.3.2-5
Male and female rats (Wister)	Oral gavage	26 weeks (once daily) + 13-week recovery period	0,[a)] 1, 2.5, 7.5, 20	≥2.5: wet fur on the lower jaw or around the mouth[2)] (male) 20: salivation[3)] (male and female), wet fur on the lower jaw or around the mouth [2)] (female) <u>At end of Week 13</u> ≥1: cytoplasmic vacuoles in lymphocytes[b)] (female) ≥2.5: cytoplasmic vacuoles in lymphocytes (male) ≥7.5: increased urinary level of BMP (male and female) 20: increased monocyte count (male and female), increased neutrophil count, increased urine volume, decreased urine specific gravity (male) <u>At end of Week 26</u> ≥2.5: bile duct vacuolation in the liver (female) ≥7.5: increased urinary level of BMP, portal foamy histiocytes or pigmentation in the liver, vacuolation in the mucosa of the bile duct (male and female), bile duct vacuolation/bile duct hyperplasia in the liver (male), foamy histiocytes or pigmentation in the bile duct/spleen/mesenteric lymph nodes (female) 20: cytoplasmic vacuoles in lymphocytes, bile duct dilation/hyperplasia, foamy histiocytes or pigmentation in the small intestine, degeneration of the olfactory epithelium of the nasal cavity (male and female), increased neutrophil count, increased urine volume, foamy histiocytes or pigmentation in the bile duct/spleen/mesenteric lymph nodes, nasopharyngeal degeneration or regeneration in the nasal cavity, exudate in the nasal cavity (male), bile duct hyperplasia in the liver, foamy histiocytes or pigmentation in the duodenum (female) These findings were reversible.	20	4.2.3.2-7

a) Vehicle: deionized water, b) Excluding the 2.5 mg/kg group
1) The finding obtained by electron microscopy, which was performed in the 0 and 20 mg/kg groups only.
2) The findings noted in males of the 20 mg/kg group only were considered adverse by the applicant.
3) The finding was considered associated with irritation caused by the low pH dosing solution refluxed from the stomach.

Repeated oral dose toxicity studies of berotralstat were conducted in cynomolgus monkeys (Table 13). The NOAEL in the 39-week repeated-dose toxicity study in cynomolgus monkeys was determined to be 30 mg/kg. The $AUC_{0\text{-}24}$ at the NOAEL at Week 39 was 3,950 ng·h/mL, which was approximately 1.4-fold the human exposure[13)] (AUC_{tau}, 2,850 ng·h/mL).

The major systemic toxicities or abnormal findings included single cell necrosis in the liver, increases in blood AST/ALT activity, renal tubular degeneration/hyperplasia in the kidney, myofiber necrosis of skeletal muscles, increased blood lipase activity, and ECG abnormalities (lengthening of the RR and QTc intervals and QRS duration). As phospholipidosis-related changes, increased urinary level of BMP, and vacuolation/pigmentation in macrophages/histiocytes in the liver/bile duct and multiple organs/various tissues were observed. Also at the end of the recovery period, phospholipidosis-related vacuolation in macrophages/histiocytes, etc. were observed. The abnormal values or changes noted at the NOAELs in these studies were considered of little toxicological significance, based on the presence or absence of correlative abnormalities, frequency, and the severity of the findings.

Table 13. An overview of repeated-dose toxicity studies in cynomolgus monkeys

Test system	Route of administration	Duration of dosing	Dose (mg/kg)	Major findings	NOAEL (mg/kg)	Attached document CTD
Male and female cynomolgus monkeys	Oral gavage	7 days	0,[a)] 10, 75, 200	≥10: decreased blood albumin (male and female) ≥75: vomiting, increases in blood AST/ALT/amylase/lipase (male and female), lymphoid depletion in the thymus (female) 200: decreased blood electrolytes/increases in total bilirubin/fibrinogen, decreased thymus weight (male and female), inappetence, centrilobular hepatocellular vacuolation in the liver, lymphoid depletion in the thymus (male), decreased blood cholesterol (female)	75	Reference data 4.2.3.2-2
Male and female cynomolgus monkeys	Oral gavage	4 weeks (once daily) + 3-week recovery period	0,[a)] 10, 30, 100	≥10: hyperplasia of oval cells in the liver, mononuclear infiltration in the kidney, myofiber regeneration of skeletal muscles,[c)] myofiber degeneration/regeneration in the tongue[c)] (male), increased hepatocellular glycogen in the liver, lymphocyte accumulation in the bone marrow, follicular lymphoid hyperplasia in the mesenteric lymph nodes, follicular lymphoid hyperplasia in the spleen (female) ≥30: watery or soft feces, increased blood lipase, histiocytic infiltration in the small intestine (jejunum or ileum) (male and female), vomiting or vomitus,[1)] renal tubular degeneration/regeneration in the kidney, subacute or chronic inflammation in the liver, follicular lymphoid hyperplasia in the spleen, thymic cortical atrophy (male), increased blood ALT, mononuclear infiltration in the kidney (female) 100: increases in blood AST/globulin/decreases in albumin/A/G ratio, increases in kidney/liver weights, histiocytosis in the mesenteric lymph nodes, histiocytic infiltration in the duodenum (male and female), increased blood ALT, increased urinary glucose, lymphocyte accumulation in the bone marrow, myofiber degeneration/regeneration in the larynx, diffused fatty changes/Kupffer cell hypertrophy or hyperplasia in the liver, diffuse hyperplasia in the mandibular lymph nodes/mesenteric lymph nodes (male), decreased heart rate, lengthening of the RR, QT, and QTc intervals and QRS duration, decreased blood inorganic phosphate, basophilic tubules/casts in the kidney, myofiber regeneration of skeletal muscles, thymic cortical atrophy (female)	10	4.2.3.2-4

Test system	Route of administration	Duration of dosing	Dose (mg/kg)	Major findings	NOAEL (mg/kg)	Attached document CTD
				30: single cell necrosis/pigmentation in Kupffer cells in the liver (male) These findings were reversible.		
Male and female cynomolgus monkeys	Oral gavage	13 weeks (once daily) + 6-week recovery period	0,[a] 2.5, 7.5, 20	≥2.5: increased urinary level of BMP (female) ≥7.5: increased urinary level of BMP (male) 20: increased blood ALT (male and female) These findings were reversible.	20	4.2.3.2-6
Male and female cynomolgus monkeys	Oral gavage	39 weeks (once daily) + 13-week recovery period	0,[b] 1, 2.5, 7.5, 20	≥2.5: increased urinary level of BMP (male and female) ≥7.5: salivation (male and female) 20: increased blood ALT (male and female), soft feces (male), single cell necrosis in the liver (female) These findings were reversible.	20	4.2.3.2-9
Male and female cynomolgus monkeys	Oral gavage	39 weeks (once daily) + 13-week recovery period	0,[a] 30, 55, 80	≥55: vomitus[1)] (male and female) 80: increased incidence of watery or soft feces, thin appearance (male and female), decreases in body weight/body weight gain (male), inappetence (female) <u>At end of Week 13</u> ≥30: increased blood ALT, increased urinary level of BMP (male and female), increased liver weight, renal tubular hyperplasia in the kidney, enlarged hepatocytes in the liver (female) ≥55: decreases in blood fibrinogen/albumin/increased AST, increased kidney weight, renal tubular degeneration[d)]/mononuclear infiltration in the kidney, foamy macrophages in the mesenteric lymph nodes/small intestine (jejunum or ileum), lymphoid depletion in the thymic cortex (male and female), decreases in red blood cell count/hemoglobin/hematocrit, increased red cell distribution width, increased monocyte count, increased liver weight, decreased thymus weight, brown discoloration in the kidney/liver, renal tubular hyperplasia in the kidney, hepatocellular hypertrophy in the liver (male), decreased uterine weight (female) 80: decreased blood inorganic phosphate, renal tubular vacuolation in the kidney (male and female), increased platelet count (male), decreases in red blood cell count/hemoglobin/hematocrit, increased red cell distribution width, increased monocyte count, decreased thymus weight, brown discoloration in the kidney/liver, uterine atrophy (female) <u>At end of Week 39</u> ≥30: increased blood ALT, increased urinary level of BMP, cytoplasmic vacuoles in the adrenal gland, pigmented foamy macrophages in the mesenteric lymph nodes/small intestine (jejunum or ileum) (male and female), increases in liver/spleen weights, mononuclear infiltration in the renal tubule (male), increased kidney weight/renal tubular degeneration, brown discoloration in the mesenteric lymph nodes, follicular lymphoid hyperplasia in the bone marrow (female) ≥55: decreases in blood fibrinogen/albumin/increased AST,[e)] brown discoloration or discoloration/tubular hyperplasia in the kidney, diffuse hepatocellular hypertrophy/increased Kupffer cells/pigmentation in the liver (male and female), increases in monocyte count/white blood cell count and other cell counts, increased kidney weight, renal hypertrophy/tubular degeneration, brown discoloration in the mesenteric lymph nodes (male), increases in liver/spleen weights, brown discoloration or discoloration in the liver, mononuclear infiltration in the renal tubule (female) 80: decreases in red blood cell count/hemoglobin/hematocrit, increased platelet count (male and female), decreased blood inorganic phosphate, decreased thymus weight, brown discoloration or discoloration in the liver, pigmented foamy macrophages in the lung	30	4.2.3.2-10

Test system	Route of administration	Duration of dosing	Dose (mg/kg)	Major findings	NOAEL (mg/kg)	Attached document CTD
				(male), increased red cell distribution width, increases in monocyte count/white blood cell count and other cell counts, focal lymphoid hyperplasia in the spleen (female) These findings were reversible.		

Vehicle: a) deionized water, b) ultrapure water,
c) Excluding the 30 mg/kg group, d) Excluding males in the 80 mg/kg group, e) Excluding females in the 55 mg/kg group
1) The finding was considered associated with the low pH and bitter taste of the dosing solution.

5.3 Genotoxicity

Berotralstat tested negative in the *in vitro* bacterial reverse mutation assay (Ames test), the *in vitro* chromosomal aberration assay in human peripheral blood lymphocytes, and the *in vivo* rat bone marrow micronucleus assay (Table 14).

Table 14. An overview of genotoxicity studies

Type of study		Test system	Metabolic activation (Treatment)	Concentration or Dose	Test result	Attached document CTD
In vitro	Ames test	*Salmonella typhimurium*: TA98, TA100, TA1535, TA1537 *Escherichia coli*: WP2*uvr*A	S9−/+	0,[a] 5.0, 15, 50, 150, 500, 1,500 µg/plate	Negative	4.2.3.3.1-1
	Chromosomal aberration assay in human peripheral blood lymphocytes	Human peripheral blood lymphocytes	S9− (20 hours)	0,[b] 2.5, 6, 12 µg/mL	Negative	4.2.3.3.1-2
			S9− (4 hours)	0,[b] 7.5, 25, 32.5 µg/mL		
			S9+ (4 hours)	0,[b] 15, 27.5, 35 µg/mL		
In vivo	Rat micronucleus assay	Male rat (SD) bone marrow		0,[c] 125, 250, 500 mg/kg/day (oral, single dose)	Negative	4.2.3.3.2-1

Vehicle: a) DMSO, b) sterile distilled water, c) deionized water

5.4 Carcinogenicity

5.4.1 Carcinogenicity study in Tg rasH2 transgenic mice

A 26-week carcinogenicity study of oral berotralstat was conducted in CByB6F1/Tg rasH2 (Tg rasH2) transgenic mice. No berotralstat-related neoplastic lesions were observed (Table 15).

The $AUC_{0\text{-}24}$ at the no-observed-effect level (NOEL) for carcinogenicity (50 mg/kg) (Day 182) was 27,450 ng·h/mL, which was approximately 9.6-fold the human exposure [13] (AUC_{tau}, 2,850 ng·h/mL).

Table 15. An overview of carcinogenicity study in Tg rasH2 transgenic mice

Test system	Route of administration	Duration of dosing	Major lesions	Sex	Dose levels (mg/kg/day)				NOEL for carcinogenicity (mg/kg)	Attached document CTD
					Vehicle	Berotralstat				
					0	8	20	50		
				N	25/sex	25/sex	25/sex	25/sex		
Male and female mice (Tg rasH2)	Oral gavage	26 weeks	Neoplastic lesions						50	4.2.3.4.1-2
			Malignant lymphoma	M	0	1	0	0		
				F	0	0	0	0		
			Whole body[a] /Hemangiosarcoma	M	2	2	1	2		
				F	0	3	0	3		
			Lung/Bronchioloalveolar adenoma or carcinoma	M	1	1	2	0		
				F	3	1	2	2		
			Stomach/Squamous cell papilloma or carcinoma	M	1	1	0	1		
				F	0	0	0	0		
			Skin/Squamous cell papilloma or carcinoma	M	0	0	0	1		
				F	1	1	0	0		
			Other findings							
			Survival rate (%)	M	96	96	96	88		
				F	100	96	88	92		
			≥8: neutrophilic inflammation in the nasal cavity (male and female) ≥20: decreases in body weight/body weight gain/food consumption (male and female) 50: erosion/ulceration of the nasal cavity epithelium (male)							

Vehicle: reverse osmosis deionized water
a) The sum of mice with hemangiosarcoma in any organ/tissue in the whole body

5.4.2 Carcinogenicity study in rats

A 104-week oral carcinogenicity study of berotralstat in rats was conducted. As major neoplastic lesions associated with berotralstat treatment, a significant increase in the incidence of hemangiosarcoma, mostly in the mesenteric lymph nodes, was observed in males in the 20 mg/kg group, according to the study data initially submitted (Table 16).

Table 16. Incidence of hemangiosarcoma/hemangioma/proliferative vascular lesions in rat carcinogenicity study in the initially submitted data

Test system	Route of administration	Duration of dosing	Major lesions	Sex	Dose levels (mg/kg/day)					NOEL for carcinogenicity (mg/kg)	Attached document CTD
					Vehicle	Berotralstat					
					0	3	8	20	60/40[b]		
				N	60/sex	60/sex	60/sex	60/sex	60/sex		
Male and female rats (Wister)	Oral gavage	104 weeks	Neoplastic lesions							8	4.2.3.4.1-1
			Whole body[a]/Hemangiosarcoma	M	2	2	4	9*	-		
				F	4	2	1	9	-		
			Whole body[a]/Hemangioma	M	1	0	0	0	-		
				F	0	0	0	0	-		
			Proliferative vascular lesions							—	
			Mesenteric lymph node /Angiomatous hyperplasia	M	0	0	0	0	-		
				F	0	0	0	0	-		

Vehicle: deionized water, *Considered related to berotralstat.
a) The sum of rats with hemangiosarcoma or hemangioma in any organ/tissue in the whole body
b) Dosed at 60 mg/kg from Weeks 1 to 17 and dosed at 40 mg/kg from Weeks 18 to 28.
Berotralstat was discontinued at Week 28 (Day 191).

After the application submission, the hemangiosarcoma findings were subjected to a pathology peer review, and they were re-classified into hemangioma or angiomatous hyperplasia and corrected in the final report accordingly. (Table 17). Based on the results of the re-evaluation, the applicant explained that berotralstat is unlikely to affect the occurrence of hemangiosarcoma, and that the trend toward increased incidence

of hemangioma or angiomatous hyperplasia was also an incidental change with an unlikely relationship to berotralstat.

Table 17. Re-evaluated incidences of hemangiosarcoma/hemangioma/proliferative vascular lesions in rat carcinogenicity study after the initial application submission

Test system	Route of administratio	Duration of dosing	Major lesions	Sex	Dose levels (mg/kg/day)					NOEL for carcinogenicity (mg/kg)	Attached document CTD
					Vehicle	Berotralstat					
					0	3	8	20	60/40[b)]		
				N	60/sex	60/sex	60/sex	60/sex	60/sex		
Male and female rats (Wister)	Oral gavage	104 weeks	Neoplastic lesions								
			Whole body[a)]/ Hemangiosarcoma	M	1	1	0	0	-	20	4.2.3.4.1-1
				F	0	1	0	2	-		
			Whole body[a)]/ Hemangioma	M	1	0	1	5	-		
				F	1	0	0	1	-		
			Proliferative vascular lesions							—	
			Mesenteric lymph node /Angiomatous hyperplasia	M	1	2	2	4	-		
				F	2	1	1	6	-		

Vehicle: deionized water
a) The sum of rats with hemangiosarcoma or hemangioma in any organ/tissue in the whole body
b) Dosed at 60 mg/kg from Weeks 1 to 17 and at 40 mg/kg from Weeks 18 to 28.
Berotralstat was discontinued at Week 28 (Day 191).

Table 18 shows abnormal findings other than hemangiosarcoma. Squamous cell carcinoma in the nasal cavity, which was not observed in the vehicle or historical controls, occurred in male and female rats in the 20 mg/kg group. Other neoplastic lesions that were not observed in the vehicle group or tended to be observed more frequently in animals dosed with berotralstat were endometrial stromal sarcoma in the uterus, undifferentiated sarcoma in the skin, malignant schwannoma in the whole body, and benign pheochromocytoma/malignant pheochromocytoma in the adrenal gland, which were considered unlikely related to berotralstat, based on the spontaneous background rate in the strain of rats used, a dose-response relationship, etc.

Major proliferative changes observed were increased incidences of adrenal cortical hyperplasia and bile duct hyperplasia in the liver, and increased histiocytes in the mesenteric lymph nodes. Major systemic toxicities or abnormal findings included hepatocellular necrosis and bile duct dilatation in the liver, skeletal muscle necrosis, and phospholipidosis-related vacuolation in macrophages/histiocytes in multiple organs/various tissues. Injuries in the nasal mucosa and associated respiratory disorder were noted, which was considered the primary cause of death in the 60/40 mg/kg group. The applicant explained that the injuries in the nasal mucosa were caused by the reflux of the low pH dosing solution after oral gavage (*Toxicol. Sci.* 2011; 39:337-347, *J. Appl. Toxicol.* 2011; 31:342-354).

Based on the above, the NOEL for carcinogenicity was determined to be 20 mg/kg, and the $AUC_{0\text{-}24}$ at the NOEL at Week 52 was 11,500 ng·h/mL, which was approximately 4.0-fold the human exposure[13)] (AUC_{tau}, 2,850 ng·h/mL).

Table 18. Overview of rat carcinogenicity study excluding vascular histopathological findings

Test system	Route of administratio	Duration of dosing	Major lesions	Sex	Dose levels (mg/kg/day)					NOEL for carcinogenicity (mg/kg)	Attached document CTD
					Vehicle	Berotralstat					
					0	3	8	20	60/40[b)]		
				N	60/sex	60/sex	60/sex	60/sex	60/sex		
Male and female rats (Wister)	Oral gavage	104 weeks	Neoplastic lesions							20	4.2.3.4.1-1
			Nasal cavity/Squamous cell carcinoma	M	0	0	0	1*	-		
				F	0	0	0	1*	-		
			Uterus/Endometrial stromal sarcoma	M	-	-	-	-	-		
				F	0	0	2	3	-		
			Skin/Undifferentiated sarcoma	M	0	1	2	3	-		
				F	1	1	0	0	-		
			Whole body[a)]/ Malignant schwannoma	M	2	3	1	5	-		
				F	4	0	4	1	-		
			Adrenal gland/Benign pheochromocytoma or malignant pheochromocytoma	M	2	3	5	1	-		
				F	0	0	1	1	-		
			Mammary gland/Fibroadenoma	M	0	0	2	0	-		
				F	13	17	10	4*	-		
			Mammary gland/ Adenocarcinoma	M	0	0	0	0	-		
				F	4	6	5	1*	-		
			Proliferative lesions							—	
			Adrenal gland/Focal hyperplasia in the cortex	M	5	12	5	14*	-		
				F	12	13	10	19	-		
			Liver/Bile duct hyperplasia	M	17	15	15	47*	-		
				F	25	24	27	55*	-		
			Mesenteric lymph node/ Increased histiocytes	M	1	10*	35*	59*	-		
				F	8	14	38*	58*	-		
			Survival rate								
			Survival rate (%)	M	72	65	70	55	85[c)]		
				F	58	60	62	47	68[c)]		
			Major lesions								
			Hepatocellular necrosis in the liver	M	1	0	0	0	0		
				F	0	1	0	2	4*		
			Hepatocellular focal necrosis in the liver	M	3	1	4	10*	1		
				F	0	2	0	4*	1		
			Centrilobular hepatocellular necrosis in the liver	M	0	0	1	1	0		
				F	0	0	1	0	0		
			Periportal hepatocellular necrosis in the liver	M	0	0	0	0	1		
				F	0	0	0	0	4		
			Mixed inflammation in the liver	M	1	0	5*	8*	3		
				F	0	0	1	18*	16*		
			Skeletal muscle degeneration/Necrosis	M	0	2	4	21*	5*		
				F	1	0	0	5*	6*		
			≥8: pigmented foamy macrophages in the adrenal gland, bile duct hypertrophy in the liver, exudate in the nasal cavity (male and female), extrahepatic bile duct dilatation[c)]/foamy macrophages, vacuolated macrophages in the liver, degeneration/necrosis of the nasal cavity epithelium, tracheal necrosis (male), worsening of chronic nephropathy in the kidney (female) ≥20: salivation, audible breathing, decreases in body weight/food consumption, increased incidence of vacuolation in the adrenal cortex, renal tubular vacuolation, bone proliferation in the nasal cavity, myofiber degeneration/necrosis in the pharynx, alveolar macrophage infiltration in the lung,[d)] foamy macrophages in the duodenum/small intestine (jejunum/ileum), vacuolated macrophages in the spleen, myofiber degeneration/necrosis in the tongue (male and female), thin appearance, vacuolation in the cerebral choroid plexus (male), salivation, extrahepatic bile duct dilatation[d)]/foamy macrophages, biliary cysts/vacuolated macrophages in the liver (female) 60/40: salivation, audible breathing (male and female), exudate/necrosis in the lung (male), thin appearance, luminal exudate in the pharynx, vacuolation in the cerebral choroid plexus, myofiber necrosis in the esophagus, degeneration/necrosis of the nasal cavity epithelium, tracheal necrosis (female) 20: fungal or yeast infection and subacute or chronic inflammation in the nasal cavity of animals with squamous cell carcinoma								

Vehicle: deionized water, *Considered related to berotralstat.
a) The sum of rats with malignant schwannoma in any organ/tissue in the whole body
b) Dosed at 60 mg/kg from Week 1 to Week 17 and dosed at 40 mg/kg from Week 18 to Week 28. Berotralstat was discontinued at Week 28 (Day 191).
c) Survival rate on Day 191; a significant decrease vs. the vehicle group in the same period
d) Excluding the 60/40 mg/kg group

5.5 Reproductive and developmental toxicity

An oral study of fertility and early embryonic development to implantation in male and female rats was conducted with berotralstat (Table 19). Berotralstat did not affect fertility in male or female rats. The $AUC_{0\text{-}24}$ values at the NOAEL (45 mg/kg) for male and female reproductive performance and early embryonic development estimated from a 4-week repeated-dose toxicity study in rats were 14,805 ng·h/mL in males and 20,115 ng·h/mL in females, which were approximately 5.2-fold (males) and approximately 7.1-fold (females) the human exposure[13] (AUC_{tau}, 2,850 ng·h/mL).

Table 19. Overview of fertility and early embryonic development study

Type of study	Test system	Route of administration	Duration of dosing	Dose (mg/kg)	Major findings	NOAEL (mg/kg)	Attached document CTD
Fertility and early embryonic development to implantation	Male and female rats (SD)	Oral gavage	Females: from 14 days before mating to gestation day 7 (once daily) Males: from 28 days before mating to 63 days after mating (once daily)	0, 10, 25, 45	<u>Parental animals</u> Males ≥10: salivation[1] ≥25: audible breathing[2] 45: dyspnea, decreases in body weight/body weight gain/food consumption Females ≥25: audible breathing[2] 25: salivation[1] <u>Early embryonic development</u> None	Parental animals (General toxicity): 25 Parental animals (Reproductive performance): 45 Early embryonic development: 45	4.2.3.5.1-1

Vehicle: deionized water
1) The finding was related to the bitter taste of the dosing solution and irritation caused by low pH and was not considered systemic toxicity.
2) The finding was sporadic and was not considered adverse.

Embryo-fetal development studies of oral berotralstat were conducted in rats and New Zealand white (NZW) rabbits (Table 20). There were no effects on embryo-fetal development in rats or rabbits. The $AUC_{0\text{-}24}$ values of berotralstat at the NOAELs for embryo-fetal development (75 mg/kg/day in rats, 100 mg/kg/day in rabbits) were 24,600 ng·h/mL in rats and 4,410 ng·h/mL in rabbits, which were approximately 8.6-fold (rats) and approximately 1.5-fold (rabbits) the human exposure[13] (AUC_{tau}, 2,850 ng·h/mL).

Table 20. Overview of embryo-fetal development studies

Type of study	Test system	Route of administratio	Duration of dosing	Dose (mg/kg)	Major findings	NOAEL (mg/kg)	Attached document CTD
Embryo-fetal development	Female rats (SD)	Oral gavage	From gestation days 6 to 17 (once daily) Cesarean section: Gestation day 21	0, 10, 25, 75	Dams ≥25: decreased food consumption[1)] 75: salivation, wet fur on the lower jaw, abnormal breathing sounds, decreases in body weight/body weight gain Embryo-fetal development None	Dams (General toxicity): 25 Embryo-fetal development: 75	4.2.3.5.2-3
	Female rabbits (NZW)	Oral gavage	From gestation days 7 to 19 (once daily) Cesarean section: Gestation day 29	0, 20, 50, 100	Dams 100: clinical deterioration, decreases in body weight gain/food consumption, abortion associated with decreased food consumption Embryo-fetal development None	Dams (General toxicity): 50 Embryo-fetal development: 100	4.2.3.5.2-5

Vehicle: ultrapure water
1) The finding in the 25 mg/kg group was not associated with decreased body weight and was considered less adverse.

A study of oral berotralstat was conducted in rats to evaluate the effect of berotralstat on pre- and postnatal development, including maternal function (Table 21). The $AUC_{0\text{-}24}$ of berotralstat in dams at the NOAEL for F_1 pups (45 mg/kg/day) estimated from the embryo-fetal development study in rats was 22,320 ng·h/mL, which was approximately 7.8-fold the human exposure[13)] (AUC_{tau}, 2,850 ng·h/mL). The major toxicological findings included decreased pup body weight due to malnutrition associated with decreased maternal body weight, etc.

Table 21. Overview of study to evaluate the effect of berotralstat on pre- and postnatal development, including maternal function

Type of study	Test system	Route of administratio	Duration of dosing	Dose (mg/kg)	Major findings	NOAEL (mg/kg)	Attached document CTD
Pre- and postnatal development, including maternal function	Female rats (SD)	Oral gavage	Dams: from gestation day 6 to lactation day 20 (once daily)	0, 10, 25, 45	Dams ≥25: audible breathing,[1)] decreased food consumption[2)] 45: decreased body weight F_1 generation 45: decreased body weight (postnatal days 14-28) F_2 generation None	Dams: 25 F_1 pups: 45	4.2.3.5.3-1

Vehicle: deionized water
1) The finding was sporadic and not considered an adverse finding.
2) The finding in the 25 mg/kg group was not associated with decreased body weight and was considered less adverse.

5.6 Other toxicity studies

5.6.1 Phototoxicity

Because berotralstat absorbs light at wavelengths of 290 to 700 nm (CTD 3.2.S.3.1), the neutral red uptake phototoxicity assay of berotralstat in mouse fibroblasts was performed. Berotralstat was considered to have little phototoxic potential (Table 22).

Table 22. Overview of phototoxicity study

Type of study	Test system	Test method	Major findings	Attached document CTD
Phototoxicity	Mouse fibroblasts (BALB/c 3T3)	0, 1.00, 1.78, 3.16, 5.62, 10.0, 17.8, 31.6, 56.2 µg/mL Exposed to UVA 5 J/cm^2 and UVB 21-22 mJ/cm^2	Not phototoxic Assay 1: photo irritation factor, 1.176; mean photo effect, 0.006 Assay 2: photo irritation factor >1.165; mean photo effect, −0.030	4.2.3.7.7-1

Vehicle: PBS solution containing 1% DMSO

5.6.2 Toxicologic evaluation of impurities

Ames tests were performed on Impurities C, A, and D in the drug substance (CTD 4.2.3.3 to 4.2.3.5). Impurities C and A tested negative, and Impurity D tested positive. Impurity D was considered to be of little safety concern because human exposure to Impurity D is kept below the threshold of toxicological concern (TTC) for potential mutagenic impurities by controlling the manufacturing process.

Among the impurities in the drug substance, Impurities E, F, and B were considered nonmutagenic when assessed using Derek Nexus and Sarah Nexus. Although the proposed specification limits for these impurities in the drug substance are greater than the qualification threshold (0.15%),[14] there should be little safety concern, based on the results from a 13-week repeated-dose toxicity study with the drug substance containing these impurities in cynomolgus monkeys.

5.R Outline of the review conducted by PMDA

5.R.1 Systemic toxicity

5.R.1.1 Effects on liver

The applicant's explanation:

Increased blood liver enzyme activity was observed in rats and cynomolgus monkeys dosed with berotralstat. These are considered adaptive changes due to enhanced metabolism in the absence of tissue injury in the liver (*Toxicol Pathol*. 2012; 40: 971-94), and are less adverse.

PMDA's view on the effects of berotralstat on the liver:

Berotralstat is considered to induce tissue injury because hepatocellular necrosis was noted in rats and cynomolgus monkeys after repeated dosing of berotralstat. Increased blood ALT activity >2-fold the upper limit of normal was observed in cynomolgus monkeys at an exposure level comparable to the human exposure,[13] and berotralstat has low ability to induce metabolizing enzymes such as CYPs, which increases blood ALT activity [see Section 4.5.1]. Given these points, the above changes may have been due to hepatocellular injury (*Toxicol Pathol*. 2012; 40: 971-94). Thus, the safety of berotralstat with respect to its effect on the liver in humans requires careful discussion, taking also account of any abnormalities in blood biochemical parameters related to hepatic injury in the clinical studies [see Section 7.R.3.1].

5.R.1.2 Effects on kidney and pancreas

The applicant's explanation:

[14] Revision of the Guideline on Impurities in New Drug Substances (PMSB/ELD Notification No.1216001 dated December 16, 2002) (ICH Q3A guideline)

The abnormal findings, i.e., tubular degeneration observed in rats and cynomolgus monkeys and increased blood lipase activity observed in cynomolgus monkeys, following the administration of berotralstat are less adverse, because of no abnormal test values in association with tubular degeneration or histological changes in the pancreas in association with increased lipase.

PMDA's view on the effects of berotralstat on the kidney and pancreas:
Tubular degeneration and increased lipase activity in cynomolgus monkeys were observed at an exposure level similar to the human exposure.[13)] The safety of berotralstat in the renal tubule and pancreatic exocrine gland of humans needs to be assessed carefully, taking also account of the occurrence of adverse events in the clinical studies, etc. [see Section 7.R.3].

5.R.1.3 Effects on cardiac and skeletal muscles

The applicant's explanation:
Tissue injuries in the skeletal and cardiac muscles of rats and the skeletal muscle of cynomolgus monkeys after repeated dosing of berotralstat were not accompanied by abnormal findings or values in clinical observations or other test parameters. Berotralstat is unlikely to adversely affect the motor and tissue/organ functions.

PMDA's view on the effects of berotralstat on cardiac and skeletal muscles:
Berotralstat induces tissue injuries in cardiac and skeletal muscles, and skeletal muscle toxicity was observed at an exposure level lower than the human exposure[13)] in both rats and cynomolgus monkeys. Thus, the safety of berotralstat in the skeletal and cardiac muscles of humans needs to be assessed carefully, taking account of the incidence of adverse events in the clinical studies, etc. [see Section 7.R.3].

5.R.1.4 Phospholipidosis

The applicant's explanation about human safety with regard to vacuolated or foamy macrophages and histiocytes in multiple organs/various tissues observed in rats and cynomolgus monkeys following chronic repeated dosing of berotralstat.

- Vacuolated or foamy macrophages and histiocytes in multiple organs/various tissues in rats and cynomolgus monkeys are considered related to phospholipidosis, based on increased urinary levels of BMP and electronic microscopically identified myelinosomes in rat Kupffer cells.
- Phospholipidosis-related findings observed in the toxicity studies of berotralstat are considered adverse only when associated toxicological findings were noted in the organs/tissues. Otherwise, the findings are considered adaptive changes to the drug. Thus, phospholipidosis-related findings other than the above are not considered toxicities.

PMDA's view:
Although the applicant's explanation (the phospholipidosis-related changes in the toxicity studies of berotralstat were not associated with dysfunction or abnormal findings of safety concern) is understandable, based on the results from repeated-dose toxicity studies, complete recovery of phospholipidosis-related intravital changes

associated with berotralstat will require a long rest period. Given this point, the package insert should communicate the risk of phospholipidosis associated with berotralstat.

5.R.2 Carcinogenicity

5.R.2.1 Effect on hemangiosarcoma formation

According to the data submitted at the application, berotralstat significantly increased the incidence of hemangiosarcoma in male rats in a rat carcinogenicity study. Because the exposure at the NOEL for carcinogenicity (8 mg/kg) in this study was close to the human exposure,[13)] PMDA inquired about the development mechanism of hemangiosarcoma and its human relevance. The applicant explained that a third party pathology peer review had been conducted on the relevant histopathological findings during the regulatory review, based on which their conclusion would be corrected to "berotralstat is unlikely to be involved in hemangiosarcoma formation," for the following reasons.

- As a result of the pathology peer review, most of the histopathological findings of hemangiosarcoma in the data initially submitted were re-classified into hemangioma or angiomatous hyperplasia. After the re-classification, the incidence of hemangiosarcoma showed no difference between the vehicle and berotralstat groups.
- In the carcinogenicity study in Tg rasH2 transgenic mice, which show high spontaneous incidence of hemangiosarcoma, hemangiosarcoma was not observed at an exposure level that was approximately 10 times the human exposure.[13)]

According to the final report after re-evaluation, there was a tendency toward increased incidence of hemangioma in male rats and angiomatous hyperplasia in male and female rats in the berotralstat 20 mg/kg group. The applicant explained that the tendency was unlikely to be related to berotralstat and was incidental, for the following reasons.

- According to a statistical evaluation, the increase in the incidence of hemangioma in male rats of the berotralstat 20 mg/kg group was insignificant and observed within the range of the spontaneous background rate in the strain of rats used (*Charles River*. 2011; 61 In-house material, *Regul Toxicol Pharmacol*. 2012; 64: 435-41).
- pKal is involved in the production of bradykinin in the body. Bradykinin is an inflammatory mediator that promotes the production of pro-angiogenic factors (vascular endothelial growth factor [VEGF], fibroblast growth factor-2 [FGF-2]) and is involved in tumor growth and metastasis (*Biochem Pharmacol*. 2014; 87: 243-53, *Curr Pharm Des*. 2006; 12: 2599-607). However, berotralstat inhibits pKal and reduces the blood level of bradykinin, berotralstat is unlikely to be involved in vascular endothelial proliferation via the effect of bradykinin. Also, there has been no study report on the relationship between pKal inhibition and vascular endothelial cell proliferation to date.
- In a carcinogenicity study in Tg rasH2 transgenic mice, which have high spontaneous incidence of hemangiosarcoma, no proliferative vascular lesions were observed in mice dosed with berotralstat.

PMDA reviewed the scientific validity of the re-evaluated histopathological findings, and considered that the diagnoses of the findings were revised appropriately. Thus, PMDA concluded that the applicant's explanation is acceptable and that the trend toward increased incidences of hemangioma and angiomatous hyperplasia can be considered incidental.

5.R.2.2 Effect on squamous cell carcinoma in the nasal cavity

The applicant's explanation about the cause of squamous cell carcinoma in the nasal cavity (which is not observed in historical controls of the strain of rats used) occurring in the high dose group (male and female animals) of a rat carcinogenicity study of berotralstat:

Fungal or yeast infection and subacute or chronic inflammatory changes were observed in the nasal cavity with squamous cell carcinoma. Based on reported squamous cell carcinoma caused by foreign body-induced chronic inflammation in the nasal cavity (*Lab Anim*. 1989; 23: 241-7), squamous cell carcinoma in the rat carcinogenicity study of berotralstat was considered caused by fungal or yeast infection in the nasal cavity. Thus, squamous cell carcinoma mediated by systemic exposure to berotralstat is unlikely to occur.

PMDA accepted the applicant's explanation.

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

6.1 Summary of biopharmaceutic studies and associated analytical methods

The applicant submitted relative bioavailability studies etc. as biopharmaceutic studies.

In the clinical development of berotralstat, 2 types of formulations (Formulation 1 [capsules containing 5-100 mg of the active pharmaceutical ingredient only] and Formulation 2 [capsules containing 50-150 mg of the active pharmaceutical ingredient and excipients that share the same qualitative and quantitative composition]) were mainly used.[15)] Study 103 evaluated the relative bioavailability of Formulation 1 comparing with Formulation 2, and an *in vitro* dissolution testing demonstrated the comparability of dissolution profiles of these formulations. Formulation 2 (75-mg strength) was used as the phase III study formulation, and Formulation 2 (150-mg strength) was proposed for commercial use. An *in vitro* dissolution testing demonstrated the comparability of dissolution profiles of the phase III study formulation and the commercial formulation.

Plasma and urine concentrations of berotralstat were determined by LC-MS/MS (lower limit of quantification, 1.0 and 0.2 ng/mL in plasma and 5.0 ng/mL in urine). Unless otherwise specified, the data and pharmacokinetic parameters are expressed as the mean or the mean ± SD, and the doses of the formulations are expressed as free base.[16)]

[15)] Formulation 1; phase I studies (Studies 101, 102, 103, 105, and 109) and a phase II study (Study 203): Formulation 2; phase I studies (Studies 106, 107, 108, 112, and 113), phase III studies (Studies 301 and 302), and a long-term prophylactic study (Study 204)

[16)] Berotralstat free base 26 mg, 55 mg, 87 mg, 110 mg, 150 mg, 200 mg, 218 mg, 300 mg, 436 mg, and 871 mg are equivalent to approximately 30 mg, 62.5 mg, 100 mg, 125 mg, 175 mg, 230 mg, 250 mg, 350 mg, 500 mg, and 1,000 mg of berotralstat dihydrochloride, respectively.

6.1.1 Relative bioavailability study (CTD 5.3.1.2-1, Study 103 [February 2017 to May 2017])

A randomized, open-label, 3-treatment, 3-period, crossover study was conducted in 24 non-Japanese healthy adults. Relative bioavailability following a single oral dose of Formulation 1 or 2 at 300 mg under fasted conditions as well as the effect of food[17] after a single oral dose of Formulation 2 300 mg were investigated. Pharmacokinetic parameters are shown in Table 23.

Table 23. Pharmacokinetic parameters following a single oral dose of berotralstat 300 mg

Formulation (Dosing condition)	N	C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (ng·h/mL)	$AUC_{0\text{-inf}}$ (ng·h/mL)	T_{max} (h)	$t_{1/2}$ (h)	Geometric least-squares mean ratio [90% CI]		
							C_{max}	$AUC_{0\text{-last}}$	$AUC_{0\text{-inf}}$
Formulation 1 (fasted)	23	124 ± 34.4	2,380 ± 617	3,240 ± 899	3.00 [1.00, 6.00]	42.2 ± 10.9			
							Ratio vs. Formulation 1 (fasted)		
Formulation 2 (fasted)	22	133 ± 40.7	2,450 ± 690	3,390 ± 1,160	2.00 [1.00, 6.00]	42.6 ± 10.9	1.07 [0.99, 1.17]	1.05 [0.98, 1.13]	1.05 [0.97, 1.15]
							Ratio vs. Formulation 2 (fasted)		
Formulation 2 (fed)	22	148 ± 54.7	2,560 ± 788	3,430 ± 1,060	5.00 [1.00, 8.00]	42.5 ± 18.1	1.12 [1.03, 1.22]	1.03 [0.95, 1.11]	1.02 [0.93, 1.11]

Mean ± SD, Median [Min., Max.] for T_{max}

6.2 Clinical pharmacology

The applicant submitted clinical pharmacology data, in the form of the results from studies in healthy adults, patients with HAE, and subjects with hepatic or renal impairment, pharmacokinetic interaction studies, and population pharmacokinetic analysis, etc. Unless otherwise specified, the data and pharmacokinetic parameters are expressed as the mean or the mean ± SD, and the doses of the formulations are expressed as free base.[16]

6.2.1 Studies in healthy subjects

6.2.1.1 Phase I study (CTD 5.3.3.1-1, Study 101 [May 2015 to December 2015])

Following a single oral dose of berotralstat 26, 87, 218, 436, or 871 mg in healthy adults under fasted conditions (6 Japanese subjects per group, 4-6 non-Japanese subjects per group), the pharmacokinetics of berotralstat were evaluated. Pharmacokinetic parameters are shown in Table 24. The C_{max} and AUC of berotralstat showed a greater than dose-proportional increase in exposure over the dose range of 26 to 871 mg. The plasma concentration-time curve of berotralstat was biphasic, suggesting the possibility of enterohepatic recirculation.

Table 24. Pharmacokinetic parameters following a single dose of berotralstat

Dose (mg)		N	C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (ng·h/mL)	$AUC_{0\text{-inf}}$ (ng·h/mL)	T_{max} (h)	$t_{1/2}$ (h)	CL/F (L/h)	V_z/F (L)	CLr (L/h)
26	Non-Japanese	4	3.82 ± 0.84	19.3 ± 2.64	25.1 ± 3.66	2.00 [1.00, 3.00]	2.91 ± 1.25	1,210 ± 175	4,910 ± 1,490	4.83 ± 1.20
87	Non-Japanese	6	24.4 ± 8.22	375 ± 127	411 ± 181	5.00 [5.00, 5.00]	45.14 ± 3.67	269 ± 118	17,800 ± 9,130	2.18 ± 0.73
	Japanese	6	38.7 ± 8.49	565 ± 125	734 ± 150	4.99 [2.00, 5.00]	57.11 ± 15.17	142 ± 32.4	11,600 ± 3,570	2.51 ± 0.28
218	Non-Japanese	6	107 ± 26.6	1,580 ± 331	1,950 ± 321	2.00 [2.00, 5.00]	53.68 ± 17.09	131 ± 19.7	10,200 ± 3,640	2.30 ± 0.62
436	Non-Japanese	6	277 ± 155	4,800 ± 1,610	6,120 ± 1,980	2.50 [2.00, 5.00]	54.17 ± 9.30	90.4 ± 34.2	7,090 ± 2,960	2.66 ± 0.75
	Japanese	6	319 ± 80.5	5,900 ± 1,660	7,170 ± 2,150	4.99 [2.00, 5.18]	46.18 ± 6.50	74.1 ± 17.5	4,900 ± 1,120	2.84 ± 0.59
871	Non-Japanese	6	690 ± 120	13,300 ± 1,090	16,900 ± 2,230	5.00 [3.00, 6.00]	47.48 ± 9.03	60.1 ± 7.49	4,070 ± 661	2.13 ± 0.68

Mean ± SD, Median [Min., Max.] for T_{max}

[17] The effect of food was evaluated using a high-fat meal (approximately 50% fat/882 kcal).

Berotralstat 110, 218, 300, or 436 mg was administered orally once daily for 7 or 14 days to healthy adults (10 subjects each in the Japanese and non-Japanese groups), and the pharmacokinetics of berotralstat were evaluated. Pharmacokinetic parameters are shown in Table 25. Berotralstat accumulated with daily dosing, and was considered to reach a steady-state within 6 to 12 days.

Table 25. Pharmacokinetic parameters at steady state following multiple-dose administration of berotralstat

Dose (mg)		N	Sampling day	C_{max} (ng/mL)	AUC_{tau} (ng·h/mL)	T_{max} (h)	CLr (L/h)
110	Non-Japanese	10	Day 1	37.5 ± 15.7	346 ± 161	4.00 [1.00, 5.00]	1.75 ± 0.60
			Day 7	104 ± 47.5	1,740 ± 897	5.00 [2.00, 8.07]	2.77 ± 0.93
218	Non-Japanese	10	Day 1	79.3 ± 18.1	767 ± 173	2.00 [1.00, 6.02]	2.38 ± 0.74
			Day 7	222 ± 49.8	3,790 ± 822	6.00 [3.00, 12.00]	3.25 ± 1.31
	Japanese	10	Day 1	117 ± 24.1	1,080 ± 245	5.00 [1.00, 6.00]	2.71 ± 0.57
			Day 7	265 ± 49.0	4,250 ± 786	5.99 [2.00, 6.00]	3.84 ± 0.78
300	Non-Japanese	10	Day 1	164 ± 64.3	1,700 ± 687	5.00 [1.00, 6.02]	2.21 ± 0.73
			Day 14	384 ± 145	5,990 ± 1,800	4.00 [1.00, 8.02]	3.85 ± 1.45
436	Non-Japanese	10	Day 1	256 ± 84.4	2,740 ± 736	2.00 [1.00, 6.50]	2.25 ± 0.57
			Day 7	547 ± 193	8,620 ± 2,810	2.00 [1.00, 8.05]	2.30 ± 0.70

Mean ± SD, Median [Min., Max.] for T_{max}

6.2.1.2 Mass balance study (CTD 5.3.3.1-3, Study 104 [September 2017 to November 2017])

Following a single oral dose of ^{14}C-berotralstat A 300 mg to 7 non-Japanese healthy adults, the $t_{1/2}$ values of plasma radioactivity and plasma berotralstat were 101 and 91.1 hours, respectively, and the C_{max} values were 406 ng·eq/mL and 189 ng/mL, respectively. The whole blood to plasma ratio of total radioactivity up to 24 hours post-dose ranged from 0.96 to 1.03,[18)] suggesting that total radioactivity is mainly present in plasma, not in blood cells. There was a total recovery of radioactivity of 85.5 ± 5.9% up to 1,176 hours post-dose, and 8.1 ± 1.2% and 77.4 ± 4.9% of the administered radioactivity were recovered in the urine and feces, respectively. The metabolite profiles in the plasma, feces, and urine are shown in Table 26. None of the metabolites exceeded 10% of the total radioactivity exposure.

Table 26. Metabolite profiles in plasma, feces, and urine

Plasma	Feces	Urine
Up to 24 hours post-dose unchanged berotralstat (34%), M3, M5, M6, M7, M8, and uncharacterized metabolites	Up to 120 hours post-dose unchanged berotralstat (17.2%), M3, M5, M6, M7, M8, M9, M10, M42, M82, and uncharacterized metabolites	Up to 120 hours post-dose unchanged berotralstat (2.79%), M3, M5, M6, M42, and uncharacterized metabolites

6.2.2 Studies in patients with HAE

6.2.2.1 Foreign phase II study (CTD 5.3.5.1-2, Study 203 [August 2016 to August 2017])

Berotralstat 55, 110, 218, or 300 mg was administered orally once daily for 28 days to non-Japanese patients with HAE, and the pharmacokinetics of berotralstat were evaluated. Pharmacokinetic parameters are shown in Table 27.

[18)] The sampling time points up to 24 hours post-dose were at 1, 2, 4, 6, 8, 12, 16, and 24 hours.

Table 27. Pharmacokinetic parameters at steady state following multiple-dose administration of berotralstat

Dose (mg)	N	C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (ng·h/mL)	T_{max} (h)	$t_{1/2}$ (h)	CL/F (L/h)	V_z/F (L)
55	7	33.7 ± 15.0	532 ± 329	3.8 [2.0, 7.9]	26.1 ± 8.58 [a)]	182 ± 98.4 [a)]	6,330 ± 2,840 [a)]
110	14	92.4 ± 42.0	1,420 ± 487	3.0 [1.1, 6.0]	27.8 ± 11.0 [b)]	97.4 ± 31.2	3,970 ± 1,870 [b)]
218	14	283 ± 133	4,180 ± 1,180	3.0 [1.9, 6.0]	27.5 ± 14.6	63.2 ± 14.5	2,370 ± 855
300	16	389 ± 184	5,980 ± 2,080	4.0 [2.0, 8.0]	28.0 ± 8.35 [c)]	67.3 ± 28.0	2,480 ± 1,100 [c)]

Mean ± SD, Median [Min., Max.] for T_{max}
a) N = 6, b) N = 12, c) N = 13

6.2.3 Intrinsic factor pharmacokinetic studies

6.2.3.1 Study in subjects with hepatic impairment (CTD 5.3.3.3-2, Study 108 [October 2018 to February 2019])

Following a single oral dose of berotralstat 150 mg to non-Japanese subjects with hepatic impairment (6 subjects each with Child-Pugh A, B, and C) and non-Japanese subjects with normal hepatic function (6 subjects), the pharmacokinetics of berotralstat were evaluated. Pharmacokinetic parameters are shown in Table 28. The percent of unbound berotralstat in plasma at 6 hours post-dose was 1.10% in subjects with normal hepatic function, 1.24% in subjects with mild hepatic impairment, 1.57% in subjects with moderate hepatic impairment, and 2.38% in subjects with severe hepatic impairment.

Table 28. Pharmacokinetic parameters following a single oral dose of berotralstat 150 mg

Degree of hepatic impairment	N	C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (ng·h/mL)	$t_{1/2}$ (h)	Geometric least-squares mean ratio [90% CI] (Hepatic impairment/Normal hepatic function)	
					C_{max}	$AUC_{0\text{-last}}$
Normal	6	55.1 ± 35	858 ± 428	96.3 ± 41.9		
Mild	6	56.3 ± 37.6	878 ± 367	102 ± 49.1	1.01 [0.54, 1.88]	1.04 [0.62, 1.77]
Moderate	6	90.1 ± 45	1,320 ± 442	125 ± 15.6	1.77 [0.95, 3.29]	1.70 [1.00, 2.87]
Severe	6	64.5 ± 36.3	730 ± 261	125 ± 41.8	1.27 [0.68, 2.37]	0.95 [0.56, 1.61]

Mean ± SD

6.2.3.2 Study in subjects with renal impairment (CTD 5.3.3.3-1, Study 107 [October 2017 to September 2018])

Following a single oral dose of berotralstat 200 mg to non-Japanese subjects with severe renal impairment (eGFR <30 mL/min/1.73 m^2) (7 subjects) and non-Japanese subjects with normal renal function (CL_{CR} ≥90 mL/min) (7 subjects), the pharmacokinetics of berotralstat were evaluated. Pharmacokinetic parameters are shown in Table 29.

Table 29. Pharmacokinetic parameters following a single oral dose of berotralstat 200 mg

Degree of renal impairment	N	C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (ng·h/mL)	$t_{1/2}$ (h)	Geometric least-squares mean ratio [90% CI] (Severe renal impairment/Normal renal function)	
					C_{max}	$AUC_{0\text{-last}}$
Normal	7	95.2 ± 44.5	1,730 ± 758	68.1 ± 18.2		
Severe	7	144 ± 96.7	1,970 ± 1,030	59.6 ± 7.29	1.39 [0.87, 2.20]	1.09 [0.69, 1.71]

Mean ± SD

6.2.4 Thorough QT/QTc study (CTD 5.3.4.1-1, Study 106 [March 2019 to April 2019])

Placebo or berotralstat 150 or 436 mg was administered orally once daily for 14 days to non-Japanese healthy adults (40 subjects), and the effect of berotralstat on QT interval was evaluated. The Day 1 and Day 14 data were pooled, and an exposure-response analysis was conducted to investigate the relationship between Fridericia's-corrected QT interval (QTcF) and plasma berotralstat concentrations using a regression equation

based on a linear mixed effects model. The C_{max} (geometric mean) on Day 14 and the difference in change from baseline in QTcF (least-squares mean) between berotralstat and placebo (ΔΔQTcF) [90% CI] at Day 14 were 158 ng/mL and 3.4 [0.0, 6.8] milliseconds, respectively, in the berotralstat 150 mg group and 577 ng/mL and 21.9 [14.4, 29.4] milliseconds, respectively, in the berotralstat 436 mg group.

6.2.5 Pharmacokinetic interaction studies (CTD 5.3.3.4-1, Study 102 [March 2016 to June 2016]; CTD 5.3.3.4-2, Study 105 [February 2017 to April 2017]; CTD 5.3.3.4-3, Study 112 [April 2018 to July 2018]; CTD 5.3.3.4-4, Study 115 [February 2019 to May 2019])

A total of 4 studies were conducted to assess the drug-drug interaction potential for berotralstat. The geometric least-squares mean ratios of pharmacokinetic parameters of berotralstat or concomitant drugs administered in combination to those administered alone and their 90% confidence intervals are shown in Table 30 and Table 31.

Table 30. Effect of concomitant drug on pharmacokinetic parameters of berotralstat

Dosing regimen		No. of subjects analyzed	Geometric least-squares mean ratio [90% CI]	
Test drug	Concomitant drug		C_{max}	$AUC_{0\text{-last}}$
Berotralstat 300 mg single dose	Cyclosporine 600 mg single dose	17	1.25 [1.05, 1.48]	1.55 [1.40, 1.72]

Table 31. Effect of berotralstat on pharmacokinetic parameters of concomitant drugs

Dosing regimen		No. of subjects analyzed	Geometric least-squares mean ratio [90% CI]	
Test drug (single dose)	Concomitant drug Berotralstat (once daily)		C_{max}	$AUC_{0\text{-last}}$
Digoxin 0.25 mg oral	300 mg	17	1.58 [1.20, 2.09]	1.48 [1.22, 1.79]
Rosuvastatin 10 mg oral	300 mg	17	0.76 [0.68, 0.85]	0.80 [0.73, 0.88]
Tolbutamide 500 mg oral	300 mg	18	1.15 [1.10, 1.21]	1.73 [1.55, 1.94]
	150 mg	21	1.19 [1.11, 1.27]	1.73 [1.63, 1.85]
Omeprazole 40 mg oral	300 mg	18	1.78 [1.46, 2.16]	2.07 [1.84, 2.33]
	150 mg	21	1.21 [1.00, 1.47]	1.24 [1.09, 1.40]
Dextromethorphan 30 mg oral	300 mg	18	6.64 [5.02, 8.79]	7.52 [5.71, 9.89]
	150 mg	21	2.96 [2.48, 3.55]	2.78 [2.33, 3.33]
Desipramine 50 mg oral	150 mg	18	1.64 [1.48, 1.82]	1.87 [1.67, 2.10]
Midazolam 1 mg IV	300 mg	18	1.04 [0.96, 1.13]	1.78 [1.64, 1.92]
Midazolam 2 mg oral	300 mg	18	2.06 [1.85, 2.30]	3.54 [3.16, 3.96]
Midazolam 4 mg oral	150 mg	21	1.45 [1.29, 1.63]	2.24 [2.05, 2.44]
Amlodipine 5 mg oral	150 mg	13	1.45 [1.27, 1.64]	1.77 [1.63, 1.93]
Danazol[19)] 200 mg oral	150 mg	18	0.76 [0.57, 1.01]	0.80 [0.61, 1.05]

[19)] An androgen formulation approved for the indication of the prevention of HAE attacks in the US/Europe etc.

6.3 Population pharmacokinetic analysis (CTD 5.3.3.5-1)

Using plasma berotralstat data obtained from 13 Japanese or foreign clinical studies in healthy subjects or patients with HAE[20] (10,437 PK observations from 771 subjects), a population pharmacokinetic analysis (NONMEM version 7.3.0) was performed.

The base model for berotralstat was a 3-compartment model with first-order absorption, an absorption lag time, and linear elimination. Based on the results of covariate analysis,[21] body weight for clearance and volume of distribution and dose for relative bioavailability were selected as covariates in the final model.

The final model-predicted pharmacokinetic parameters of berotralstat after reaching steady-state following once daily oral administration of berotralstat 150 mg are shown in Table 32.

Table 32. Final model-predicted pharmacokinetic parameters after administration of berotralstat 150 mg

	C_{max} (ng/mL)	AUC_{tau} (ng·h/mL)
12-18 years of age	153 (36.8)	2,515 (38.6)
Body weight (40-60 kg)	155 (33.9)	2,574 (36.7)
Body weight (60-80 kg)	129 (35.6)	2,211 (38.3)
Body weight (80-100 kg)	111 (34.3)	1,951 (37.0)
Body weight (100-120 kg)	101 (34.7)	1,818 (36.9)

Geometric mean (CV%)

6.4 Exposure-response analyses (CTD 5.3.5.1-2)

Exposure-response analyses were conducted to investigate the relationship between *ex vivo* pKal inhibition and plasma berotralstat concentrations using the data from Study 203. As shown in Figure 2, the relationship between *ex vivo* pKal inhibition activity and the plasma berotralstat concentration was well-described by a sigmoidal E_{max} model ($R^2 = 0.75$) with a predicted half-maximal effective concentration (EC_{50}) of 11 ng/mL.

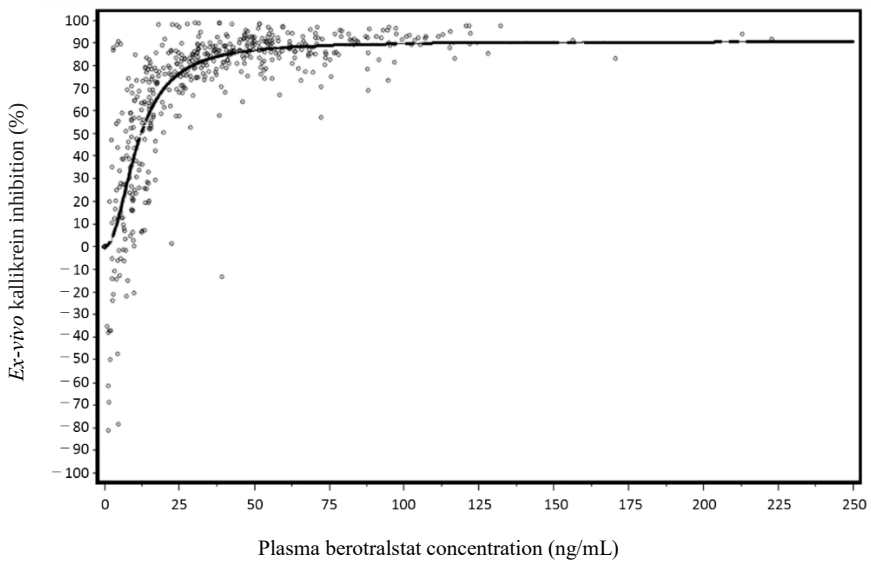


Figure 2. Exposure-response relationship for pKal inhibition in patients with HAE

[20] Phase I studies (Studies 101, 102, 103, 105, 106, 107, 108, 112, and 113), and phase II (Study 203), phase III (Studies 301 and 302), and long-term prophylactic (Study 204) studies in patients with HAE

[21] Age, body weight, CL_{CR}, eGFR, BMI, gender, race, HAE patient population, ethnicity, albumin, bilirubin, ALT, and AST were tested in the covariate analysis.

6.R Outline of the review conducted by PMDA

6.R.1 Ethnic differences in the pharmacokinetics of berotralstat

The applicant's explanation about ethnic differences in the pharmacokinetics of berotralstat:

In Study 101, the pharmacokinetic parameters (C_{max} and AUC_{tau}) following once daily administration of berotralstat 218 mg were approximately 20% and 12% higher, respectively, in Japanese subjects than in non-Japanese subjects (Table 25). However, the study revealed difference between Japanese and non-Japanese subjects in body weight (68.4 [61.2, 75.2] kg in Japanese subjects, 79.0 [55.6, 100] kg in non-Japanese subjects [mean (range)]). In the population pharmacokinetic analysis, body weight was the only significant covariate selected for the pharmacokinetic parameters of berotralstat, and race was not selected. Given these points, the observed pharmacokinetic differences between Japanese and non-Japanese subjects were considered attributable to the difference in body weight. Study 101 showed no marked differences in the safety of berotralstat between Japanese and non-Japanese subjects, and there were no marked differences also in the safety profile of berotralstat in the phase III study between Japanese and non-Japanese subjects.

Based on the above, the observed pharmacokinetic difference between Japanese and non-Japanese subjects were not considered clinically significant.

PMDA accepted the above explanation.

6.R.2 Pharmacokinetic interactions of berotralstat

The applicant's explanation:

Taking account of pharmacokinetics following the coadministration of berotralstat and cyclosporine/midazolam/dextromethorphan in the pharmacokinetic interaction studies [see Section 6.2.5], the package insert, etc. will provide precautions against interactions with cyclosporine (an inhibitor of P-gp, BCRP, and CYP3A4) and P-gp, CYP2D6, and CYP3A4 substrates.

PMDA accepted the above explanation and concluded that these precautions are appropriate.

6.R.3 Proarrhythmic risk of berotralstat

The applicant's explanation about the proarrhythmic risk of berotralstat:

Given that the upper bound of the two-sided 90% confidence interval for ΔΔQTcF was above the threshold of 10 milliseconds at a 436-mg dose of berotralstat in Study 106, an exposure-response model was developed to describe the relationship between plasma berotralstat concentrations and QTcF on the basis of data from this study. The model revealed that the upper bound of the two-sided 90% confidence interval for ΔΔQTcF would reach 10 milliseconds at the predicted plasma berotralstat concentration of 222 ng/mL. Accordingly, the proarrhythmic risk of berotralstat was assessed in the subgroups of patients in whom plasma berotralstat concentrations may exceed 222 ng/mL, as summarized below.

(a) Proarrhythmic risk in patients with hepatic impairment

In Study 108, berotralstat exposure was higher in subjects with moderate hepatic impairment than in subjects with severe hepatic impairment. However, given that there were no differences in the elimination half-life between the 2 groups and that the plasma unbound fraction of berotralstat increased with worsening of hepatic function, there is no evidence supporting differences in exposure according to the severity of hepatic impairment. The higher exposure in subjects with moderate hepatic impairment was likely attributable to random variation due to relatively small numbers of subjects in these groups. Thus, the data from subjects with moderate hepatic impairment and subjects with severe hepatic impairment were pooled for analysis. The steady-state C_{max} in subjects with moderate or severe hepatic impairment was predicted to be approximately 240 ng/mL (1.5-fold that in subjects with normal hepatic function), which exceeded 222 ng/mL. Thus, the package insert will advise that higher blood berotralstat concentrations may cause QT interval prolongation in patients with moderate or severe hepatic impairment.

(b) Proarrhythmic risk in low-body-weight patients

Body weight was the covariate selected in the population pharmacokinetic analysis, and berotralstat targets patients including children aged ≥12 years who are generally lighter than adults in weight. Therefore, empirical Bayes estimates were obtained from the model developed in the population pharmacokinetic analysis, using the average body weight of Japanese children aged 12 years (44.0 kg in boys, 43.7 kg in girls) according to a school health statistical survey (Analytical Research Planning Division, Education Policy Bureau, Ministry of Education, Culture, Sports, Science and Technology, 2019). The calculated C_{max} values in Japanese boys and girls aged 12 years with the average body weight of their age were 178.6 and 176.1 ng/mL, respectively, and, thus, were <222 ng/mL. Accordingly, the estimated increase in QTcF and the upper bound of the two-sided 90% confidence interval were also considered to be <10 milliseconds.

Among 76 subjects in Studies 302 and 204 weighing less than the median body weight (62.6 kg) of Japanese subjects in Study 301 (range, 40.1-62.6 kg), the maximum QTcF values in the berotralstat 110 mg, berotralstat 150 mg, and placebo groups were 472, 451, and 444 milliseconds, respectively, which were below the threshold associated with increasing risk for Torsades de Pointes[22)] (an absolute QTcF value of 500 milliseconds).

Based on these results, the administration of berotralstat is unlikely to lead to plasma berotralstat concentrations that may pose a proarrhythmic risk, over the likely body weight range of Japanese patients aged ≥12 years.

PMDA's view:

Because the upper bound of the two-sided 90% confidence interval for ΔΔQTcF was above the 10-millisecond threshold at a 436-mg dose of berotralstat in Study 106, and the exposure-response analysis showed QT interval prolonged with increasing berotralstat exposure, berotralstat has proarrhythmic potential.

Particularly in patients with moderate or severe hepatic impairment, as in Study 108, berotralstat exposure may be increased until the threshold concentration for proarrhythmic risk is reached. Given high plasma protein

[22)] *J Am Coll Cardiol.* 2010; 55: 934-47

binding of berotralstat, the possibility cannot be ruled out that the proarrhythmic risk is further increased in patients with moderate or severe hepatic impairment, who have generally decreased plasma protein concentrations.

As in the population pharmacokinetic analysis, low-body-weight patients tend to have higher exposure, the proarrhythmic risk of berotralstat may be increased in low-body-weight patients such as children, and caution should be used when administering berotralstat.

Based on the above, the appropriateness of the use of berotralstat needs to be determined carefully for patients with moderate or severe hepatic impairment or low-body-weight patients, taking account of the results from the clinical studies.

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

The applicant submitted the main efficacy and safety evaluation data, in the form of the results from 4 studies presented in Table 33.

Table 33. Listing of main efficacy and safety clinical studies

Geographical location	Study ID	Phase	Study population	No. of subjects enrolled	Dosing regimen	Main endpoints
Foreign	203 (APeX-1)	II	Patients with Type I or II HAE	(1) 7 (2) 14 (3) 15 (4) 18 (5) 23	Once daily oral administration (1) Berotralstat 55 mg (2) Berotralstat 110 mg (3) Berotralstat 218 mg (4) Berotralstat 300 mg (5) Placebo	Efficacy Safety PK
Foreign	204 (APeX-S)	II	Patients with Type I or II HAE	(1) 100 (2) 127	Once daily oral administration (1) Berotralstat 110 mg (2) Berotralstat 150 mg	Safety Efficacy
Japan	301 (APeX-J)	III	Patients with Type I or II HAE	(1) 6 (2) 7 (3) 6	Once daily oral administration (1) Berotralstat 110 mg (2) Berotralstat 150 mg (3) Placebo	Efficacy Safety
Foreign	302 (APeX-2)	III	Patients with Type I or II HAE	(1) 41 (2) 40 (3) 40	Once daily oral administration (1) Berotralstat 110 mg (2) Berotralstat 150 mg (3) Placebo	Efficacy Safety

The doses of the formulations are expressed as free base.
Berotralstat free base 55 mg, 110 mg, 150 mg, 218 mg, and 300 mg are equivalent to approximately 62.5 mg, 125 mg, 175 mg, 250 mg, and 350 mg of berotralstat dihydrochloride, respectively.

7.1 Phase II studies

7.1.1 Foreign phase II study (CTD 5.3.5.1-2, Study 203 [APeX-1] [August 2016 to August 2017])

A placebo-controlled, randomized, double-blind, parallel-group study was conducted in 11 countries or regions including Germany, the UK, and Macedonia to evaluate the dose response, efficacy, and safety of berotralstat in patients with Type I or II HAE[23)] (total target sample size, 70 subjects [6 in the 55 mg group, 12 in the 110 mg group, 12 in the 218 mg group, 18 in the 300 mg group, 22 in the placebo group]; target sample size for Part 1, 36 subjects [18 in the 300 mg group, 18 in the placebo group]; target sample size for Part 2, 14 subjects [6 in the 110 mg group, 6 in the 218 mg group, 2 in the placebo group]; target sample size for Part 3, 20 subjects [6 in the 55 mg group, 6 in the 110 mg group, 6 in the 218 mg group, 2 in the placebo group]).

[23)] Patients with HAE aged ≥18 and ≤70 years, with ≥2 documented HAE attacks per month for 3 consecutive months (93 days) within the 6 months before the screening visit

The study consisted of 3 parts, and berotralstat 55, 110, 218, or 300 mg, or placebo was administered orally once daily for 28 days. Parts 1, 2, and 3 were conducted in sequence. Parts 2 and 3 were planned to begin upon the completion of enrollment in the immediately preceding part. The study drug was administered to 36 subjects (18 in the 300 mg group, 18 in the placebo group) in Part 1, 15 subjects (7 in the 110 mg group, 6 in the 218 mg group, 2 in the placebo group) in Part 2, and 24 subjects (7 in the 55 mg group, 7 in the 110 mg group, 8 in the 218 mg group, 2 in the placebo group) in Part 3.

Of 77 randomized subjects (7 in the 55 mg group, 14 in the 110 mg group, 15 in the 218 mg group, 18 in the 300 mg group, 23 in the placebo group), 2 subjects who did not receive the study drug were excluded. The remaining 75 subjects (7 in the 55 mg group, 14 in the 110 mg group, 14 in the 218 mg group, 18 in the 300 mg group, 22 in the placebo group) were included in the full analysis set (FAS) and in the safety population. The FAS was used as the efficacy analysis population. Discontinuation occurred in 16.7% (3 of 18) of subjects in the 300 mg group, and the reasons for discontinuation were adverse events (3 subjects in the 300 mg group).

The primary efficacy endpoint of the rate of confirmed HAE attacks[24)] during the 28-day treatment period is shown in Table 34.

Table 34. HAE attack rate during the 28-day treatment period (FAS, OC)

	55 mg (N = 7)	110 mg (N = 14)	218 mg (N = 14)	300 mg (N = 18)	Placebo (N = 22)
HAE attack rate (No. of attacks/week)	0.93 ± 0.49	0.29 ± 0.28	0.47 ± 0.43	0.50 ± 0.51	0.90 ± 0.57

Mean ± SD

The incidences of adverse events were 57.1% (4 of 7 subjects) in the 55 mg group, 50.0% (7 of 14 subjects) in the 110 mg group, 78.6% (11 of 14 subjects) in the 218 mg group, 77.8% (14 of 18 subjects) in the 300 mg group, and 68.2% (15 of 22 subjects) in the placebo group. The main events are shown in Table 35.

No deaths were reported.

A serious adverse event was reported by 1 subject in the 218 mg group (gastrointestinal infection), but its causal relationship to the study drug was ruled out.

Adverse events leading to discontinuation occurred in 16.7% (3 of 18) of subjects in the 300 mg group.

The incidences of adverse drug reactions were 14.3% (1 of 7 subjects) in the 55 mg group, 21.4% (3 of 14 subjects) in the 110 mg group, 35.7% (5 of 14 subjects) in the 218 mg group, 50.0% (9 of 18 subjects) in the 300 mg group, and 9.1% (2 of 22 subjects) in the placebo group.

[24)] Subject-reported attacks adjudicated by an independent clinical endpoint adjudication panel

Table 35. Adverse events reported by ≥2 subjects in any group (Safety population)

Event term	55 mg (N = 7)	110 mg (N = 14)	218 mg (N = 14)	300 mg (N = 18)	Placebo (N = 22)
Headache	2 (28.6)	2 (14.3)	1 (7.1)	1 (5.6)	4 (18.2)
Nasopharyngitis	2 (28.6)	0	1 (7.1)	5 (27.8)	6 (27.3)
Fatigue	1 (14.3)	0	0	2 (11.1)	1 (4.5)
Abdominal pain	0	2 (14.3)	1 (7.1)	3 (16.7)	0
Nausea	0	0	3 (21.4)	3 (16.7)	0
Diarrhoea	0	0	2 (14.3)	4 (22.2)	2 (9.1)
Vomiting	0	0	0	2 (11.1)	0
Flatulence	0	0	0	2 (11.1)	0
ALT increased	0	0	0	2 (11.1)	0

n (%)

7.1.2 Foreign long-term prophylactic study (CTD 5.3.5.2-1, Study 204 [APeX-S] [ongoing since February 2018 (August 2019 Data Cutoff)])

An open-label, uncontrolled study was conducted to evaluate the long-term safety and efficacy of berotralstat in patients with Type I or II HAE aged ≥12 years[25] (target sample size, 475 subjects including those who participated in Study 202 or 203) in 22 countries or regions including Israel, Poland, and South Africa.

Berotralstat 110 or 150 mg was administered orally once daily for ≥48 weeks (up to Week 240).

All of 227 subjects who received the study drug (100 in the 110 mg group and 127 in the 150 mg group as of August 2019) were included in the safety population, which was also subjected to efficacy analyses. Among the 227 subjects, 103 subjects (30 in the 110 mg group, 73 in the 150 mg group) completed the Week-48 visit. The rates of discontinuations were 26.0% (26 of 100 subjects) in the 110 mg group and 26.0% (33 of 127 subjects) in the 150 mg group, and the main reasons for discontinuations were lack of efficacy (17.0% [17 of 100 subjects] in the 110 mg group, 8.7% [11 of 127 subjects] in the 150 mg group), adverse events (6.0% [6 of 100 subjects] in the 110 mg group, 10.2% [13 of 127 subjects] in the 150 mg group), etc.

The incidences of adverse events through the data cutoff date were 91.0% (91 of 100 subjects) in the 110 mg group and 90.6% (115 of 127 subjects) in the 150 mg group, and the main events are shown in Table 36.

No deaths were reported.

The incidences of serious adverse events were 18.0% (18 of 100 subjects) in the 110 mg group (hereditary angioedema [8], medical observation [2], and anal abscess, myocardial infarction, facial paralysis, lower limb fracture, gastroenteritis/hepatic enzyme increased, asthma/enteritis, abdominal pain/hereditary angioedema, and viral gastroenteritis/hereditary angioedema [1 each]), and 9.4% (12 of 127 subjects) in the 150 mg group (hereditary angioedema/medical observation, hereditary angioedema, chest pain, acute myelomonocytic

[25] Patients with HAE who had participated in Study 202 or 203, or patients with HAE defined as any of the following (a) to (c):
(a) A C1-INH functional level <50% and a C4 level below the LLN reference range
(b) In the absence of a low C4 level during the intercritical period, one of the following 1) to 3) was acceptable to confirm the diagnosis of HAE: 1) a *SERPING-1* gene mutation, 2) a confirmed family history of C1-INH deficiency, 3) a C4 redrawn and retested during an attack in the screening period with the results below the LLN reference range
(c) For patients with C1-INH function ≥50% and <74%, a *SERPING-1* gene mutation or a repeat C1-INH functional level <50%

leukaemia, pneumonia, diarrhoea/vomiting/anxiety, intervertebral disc protrusion, biliary colic, foot deformity, suicide attempt, liver function test abnormal, and pyelonephritis/splenic hamartoma [1 each]). A causal relationship to the study drug was ruled out for all those events, except for the events reported by 2 subjects in the 110 mg group (abdominal pain, and gastroenteritis/hepatic enzyme increased [1 each]) and 1 subject in the 150 mg group (liver function test abnormal).

The incidences of adverse events leading to discontinuation were 6.0% (6 of 100 subjects) in the 110 mg group and 10.2% (13 of 127 subjects) in the 150 mg group.

The incidences of adverse drug reactions were 56.0% (56 of 100 subjects) in the 110 mg group and 44.9% (57 of 127 subjects) in the 150 mg group.

Table 36. Adverse events reported by ≥5% of subjects in either group (through data cutoff date, Safety population)

Event term	110 mg (N = 100)	150 mg (N = 127)	Even term	110 mg (N = 100)	150 mg (N = 127)
Nasopharyngitis	27 (27.0)	43 (33.9)	Sinusitis	6 (6.0)	6 (4.7)
Headache	21 (21.0)	19 (15.0)	ALT increased	5 (5.0)	9 (7.1)
Abdominal pain	14 (14.0)	16 (12.6)	Gastroenteritis	5 (5.0)	7 (5.5)
Diarrhoea	12 (12.0)	18 (14.2)	AST increased	5 (5.0)	6 (4.7)
Abdominal pain upper	10 (10.0)	7 (5.5)	Abdominal discomfort	5 (5.0)	5 (3.9)
Hereditary angioedema	10 (10.0)	2 (1.6)	Influenza	4 (4.0)	9 (7.1)
Nausea	8 (8.0)	10 (7.9)	Oropharyngeal pain	4 (4.0)	8 (6.3)
Gastrooesophageal reflux disease	8 (8.0)	2 (1.6)	Bronchitis	4 (4.0)	7 (5.5)
Constipation	7 (7.0)	4 (3.1)	Vomiting	3 (3.0)	11 (8.7)
Rash	7 (7.0)	2 (1.6)	Flatulence	2 (2.0)	8 (6.3)
Upper respiratory tract infection	6 (6.0)	13 (10.2)	Back pain	1 (1.0)	7 (5.5)
Urinary tract infection	6 (6.0)	11 (8.7)	Arthralgia	0	12 (9.4)
Abdominal distension	6 (6.0)	8 (6.3)			

n (%)

The efficacy endpoint of the rate of HAE attacks[26] during the 48-week treatment period is shown in Table 37.

Table 37. HAE attack rate during the 48-week treatment period (Safety population)

		110 mg (N = 100)	150 mg (N = 127)
HAE attack rate (No. of attacks/28 days)		1.16 ± 1.13	1.36 ± 1.51
HAE attack rate by anatomic location (No. of attacks/28 days)			
	Abdominal-only	0.24 ± 0.45	0.29 ± 0.57
	Peripheral-only	0.49 ± 0.74	0.56 ± 0.80
	Mixed-location	0.44 ± 0.67	0.51 ± 0.91

Mean ± SD

[26] (a) Presence of ≥1 symptom of swelling, (b) no factors (allergic reaction, viral cold, etc.) other than an angioedema attack accountable for the symptom, (c) an attack that did not begin within 24 hours of the end of a previous attack, and (d) if untreated, an attack lasted ≥24 hours.

7.2 Phase III studies

7.2.1 Japanese phase III study (CTD 5.3.5.1-3, 5.3.5.1-5, 5.3.5.1-6, Study 301 [APeX-J] [ongoing since December 2018 (May 2020 Data Cutoff)])

A placebo-controlled, randomized, double-blind, parallel-group study was conducted to evaluate the efficacy and safety of berotralstat in patients with Type I or II HAE aged ≥12 years[27] (target sample size, 24 subjects [8 per group]).

Berotralstat 110 or 150 mg or placebo was administered orally once daily through Week 24. Subjects randomized to berotralstat 110 or 150 mg continued to receive the same berotralstat dose through Week 52. Subjects in the placebo group underwent the second randomization at Week 24 to receive either 110 or 150 mg of berotralstat orally once daily through Week 52. After Week 52, all subjects received open-label berotralstat 150 mg orally once daily.

All of 19 subjects experiencing ≥2 HAE attacks[28] during the run-in period of 56 days from the screening visit who were randomized[29] (6 in the 110 mg group, 7 in the 150 mg group, 6 in the placebo group) received the study drug, and were included in the intent-to-treat (ITT) population and in the safety population. The ITT population was used as the efficacy analysis population.

Through Week 24, 16.7% (1 of 6) of subjects in the placebo group discontinued, and the reason for discontinuation was an adverse event (urticaria).

Table 38 shows the primary efficacy endpoint of the rate of HAE attacks[30] during the 24-week treatment period. Pairwise comparisons showed a statistically significant difference between the berotralstat 150 mg and placebo groups.

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[27] Patients with HAE defined as any of the following (a) to (c):
(a) A C1-INH functional level <50% and a C4 level below the LLN reference range
(b) In the absence of a low C4 level during the intercritical period, one of the following 1) to 3) was acceptable to confirm the diagnosis of HAE: 1) a *SERPING-1* gene mutation, 2) a confirmed family history of C1-INH deficiency, 3) a C4 redrawn and retested during an attack in the screening period with the results below the LLN reference range
(c) For patients with C1-INH function of ≥50% and <74%, a *SERPING-1* gene mutation

[28] HAE attacks that met all of the following criteria: (a) The attacks included symptoms of swelling. Symptoms of swelling, in addition to visible swelling, may include symptoms in the oropharyngeal or abdominal regions. (b) The attacks had either been treated, required medical attention, or been documented to cause functional impairment (restricted in his or her daily activities) based on the subject's entry in the electronic diary (c) an attack that did not begin within 48 hours of the end of a previous attack. (d) The attacks were confirmed by the investigator to be HAE attacks.

[29] Stratified by monthly HAE attack rate during the run-in period of 56 days from the screening visit (<2 vs. ≥2 attacks per month).

[30] Independent expert-confirmed HAE attacks based on the subject's entry in the electronic diary and the information collected by the investigator. All HAE attacks required symptoms of swelling (in addition to visible swelling, may include symptoms in the oropharyngeal or abdominal regions).

Table 38. HAE attack rate during the 24-week treatment period (ITT population, OC)

	110 mg (N = 6)	150 mg (N = 7)	Placebo (N = 6)
HAE attack rate (No. of attacks/28 days)	1.96 ± 1.30	1.09 ± 0.92	2.73 ± 1.64
Active vs. placebo[a] (%) [95% CI] *P*-value[a), b)]	−24.6 [−50.1, 14.0] 0.181	−49.1 [−67.5, −20.4] 0.003	

Mean ± SD

a) Statistical analysis based on a negative binomial regression model with treatment group and baseline attack rate as covariates, and the logarithm of observation duration as an offset variable

b) Two-sided alpha of 5%; adjusted for multiplicity in hypothesis testing by the Hochberg step-up procedure

The incidences of adverse events through Week 24 were 100% (6 of 6 subjects) in the 110 mg group, 100% (7 of 7 subjects) in the 150 mg group, and 100% (6 of 6 subjects) in the placebo group. The main events are shown in Table 39.

No deaths were reported.

A serious adverse event occurred in 16.7% (1 of 6) of subjects in the 110 mg group (pneumonia), but its causal relationship to the study drug was ruled out.

An adverse event leading to discontinuation occurred in 16.7% (1 of 6) of subjects in the placebo group.

The incidences of adverse drug reactions were 33.3% (2 of 6 subjects) in the 110 mg group, 28.6% (2 of 7 subjects) in the 150 mg group, and 33.3% (2 of 6 subjects) in the placebo group.

Table 39. Adverse events reported by ≥2 subjects (through Week 24, Safety population)

Event term	110 mg (N = 6)	150 mg (N = 7)	Placebo (N = 6)
Nasopharyngitis	2 (33.3)	2 (28.6)	4 (66.7)
Cough	2 (33.3)	0	0
Abdominal pain	1 (16.7)	1 (14.3)	0
Diarrhoea	1 (16.7)	1 (14.3)	0
Pyrexia	1 (16.7)	1 (14.3)	0

n (%)

The incidences of adverse events from Week 24 through Week 52 were 83.3% (5 of 6 subjects) in the 110 mg group, 42.9% (3 of 7 subjects) in the 150 mg group, 50% (1 of 2 subjects) in the placebo→110 mg group, and 100% (2 of 2 subjects) in the placebo→150 mg group. The main events were influenza (2 subjects in the 110 mg group), etc.

No deaths were reported.

A serious adverse event occurred in 16.7% (1 of 6) of subjects in the 110 mg group (hereditary angioedema), but its causal relationship to the study drug was ruled out.

An adverse event leading to discontinuation occurred in 14.3% (1 of 7) of subjects in the 150 mg group.

An adverse drug reaction occurred in 14.3% (1 of 7) of subjects in the 150 mg group.

7.2.2 Foreign phase III study (CTD 5.3.5.1-4, Study 302 [APeX-2] [ongoing since March 2018 (August 2019 Data Cutoff)])

A placebo-controlled, randomized, double-blind, parallel-group study was conducted to evaluate the efficacy and safety of berotralstat in patients with Type I or II HAE aged ≥12 years[31] (target sample size, 96 subjects [32 per group]) in 11 countries or regions including the US, Canada, and the Czech Republic.

Berotralstat 110 or 150 mg, or placebo was administered orally once daily through Week 24. Subjects randomized to berotralstat 110 or 150 mg continued to receive the same berotralstat dose through Week 52. Subjects randomized to placebo underwent the second randomization at Week 24 to receive either 110 or 150 mg of berotralstat orally once daily through Week 52. After Week 52, all subjects received open-label berotralstat 150 mg orally once daily.

All of 121 subjects experiencing ≥2 HAE attacks[28] during the run-in period of 56 days from the screening visit who were randomized[29] (41 in the 110 mg group, 40 in the 150 mg group, 40 in the placebo group) were included in the ITT population, and 120 subjects who received the study drug (41 in the 110 mg group, 40 in the 150 mg group, 39 in the placebo group) were included in the safety population. The ITT population was used as the efficacy analysis population.

The rates of discontinuation through Week 24 were 9.8% (4 of 41 subjects) in the 110 mg group, 7.5% (3 of 40 subjects) in the 150 mg group, and 12.5% (5 of 40 subjects) in the placebo group. The main reasons for discontinuations were adverse events (7.3% [3 of 41 subjects] in the 110 mg group, 2.5% [1 of 40 subjects] in the 150 mg group, 2.5% [1 of 40 subjects] in the placebo group), lack of efficacy (2.4% [1 of 41 subjects] in the 110 mg group, 2.5% [1 of 40 subjects] in the 150 mg group, 5.0% [2 of 40 subjects] in the placebo group), etc.

Table 40 summarizes the primary efficacy endpoint of the rate of HAE attacks[32] during the 24-week treatment period. Pairwise comparisons showed statistically significant differences between 110 or 150 mg of berotralstat and placebo, demonstrating the superiority of berotralstat over placebo.

[31] Patients with HAE defined as any of the following (a) to (c):
(a) A C1-INH functional level <50% and a C4 level below the LLN reference range
(b) In the absence of a low C4 level during the intercritical period, one of the following 1) to 3) was acceptable to confirm the diagnosis of HAE: 1) a *SERPING-1* gene mutation, 2) a confirmed family history of C1-INH deficiency, 3) a C4 redrawn and retested during an attack in the screening period with the results below the LLN reference range
(c) For patients with C1-INH function ≥50% and <74%, a *SERPING-1* gene mutation or a repeat C1-INH functional level <50%

[32] Investigator-confirmed HAE attacks based on the subject's entry in the electronic diary and the information collected by the investigator. All HAE attacks required symptoms of swelling (in addition to visible swelling, may include symptoms in the oropharyngeal or abdominal regions).

Table 40. HAE attack rate during the 24-week treatment period (ITT population, OC)

	110 mg (N = 41)	150 mg (N = 40)	Placebo (N = 40)
HAE attack rate (No. of attacks/28 days)	1.90 ± 1.71	1.63 ± 1.66	2.47 ± 1.60
Active vs. placebo[a)] (%) [95% CI] *P*-value[a), b)]	−30.0 [−48.7, −4.6] 0.024	−44.2 [−59.5, −23.0] < 0.001	

Mean ± SD

a) Statistical analysis based on a negative binomial regression model with treatment group and baseline attack rate as covariates, and logarithm of duration of observation as an offset variable

b) A two-sided alpha of 5%; adjust for multiplicity in hypothesis testing by the Hochberg step-up procedure

The incidences of adverse events through Week 24 were 82.9% (34 of 41 subjects) in the 110 mg group, 85.0% (34 of 40 subjects) in the 150 mg group, and 76.9% (30 of 39 subjects) in the placebo group. The main events are shown in Table 41.

No deaths were reported.

The incidences of serious adverse events were 2.4% (1 of 41 subjects) in the 110 mg group (plasma cell myeloma) and 7.7% (3 of 39 subjects) in the placebo group (uterine leiomyoma; pneumonia; and diverticulum intestinal haemorrhagic/transient ischaemic attack [1 subject each]). A causal relationship to the study drug was ruled out for all those events.

The incidences of adverse events leading to discontinuation were 7.3% (3 of 41 subjects) in the 110 mg group, 2.5% (1 of 40 subjects) in the 150 mg group, and 2.6% (1 of 39 subjects) in the placebo group.

The incidences of adverse drug reactions were 41.5% (17 of 41 subjects) in the 110 mg group, 37.5% (15 of 40 subjects) in the 150 mg group, and 33.3% (13 of 39 subjects) in the placebo group.

Table 41. Adverse events reported by ≥5% of subjects in any group (through Week 24, Safety population)

Event term	110 mg (N = 41)	150 mg (N = 40)	Placebo (N = 39)
Nasopharyngitis	6 (14.6)	9 (22.5)	9 (23.1)
Nausea	6 (14.6)	6 (15.0)	7 (17.9)
Upper respiratory tract infection	6 (14.6)	3 (7.5)	1 (2.6)
Vomiting	4 (9.8)	6 (15.0)	1 (2.6)
Diarrhoea	4 (9.8)	5 (12.5)	0
Dyspepsia	4 (9.8)	3 (7.5)	3 (7.7)
Gastrooesophageal reflux disease	4 (9.8)	2 (5.0)	0
Headache	3 (7.3)	4 (10.0)	2 (5.1)
Fatigue	3 (7.3)	2 (5.0)	1 (2.6)
Oropharyngeal pain	3 (7.3)	0	3 (7.7)
Abdominal pain	2 (4.9)	4 (10.0)	2 (5.1)
Flatulence	2 (4.9)	3 (7.5)	1 (2.6)
Back pain	1 (2.4)	4 (10.0)	1 (2.6)
Abdominal discomfort	1 (2.4)	3 (7.5)	3 (7.7)
Abdominal pain upper	1 (2.4)	2 (5.0)	1 (2.6)
Gastroenteritis	0	2 (5.0)	2 (5.1)
Oral herpes	0	2 (5.0)	1 (2.6)
Hordeolum	0	2 (5.0)	0
Polymenorrhoea	0	2 (5.0)	0

n (%)

The incidences of adverse events from Week 24 through Week 52 were 59.5% (22 of 37 subjects) in the 110 mg group, 73.0% (27 of 37 subjects) in the 150 mg group, 76.5% (13 of 17 subjects) in the placebo→110 mg group, and 70.6% (12 of 17 subjects) in the placebo→150 mg group. The main events are shown in Table 42.

No deaths were reported.

Serious adverse events occurred in 2.7% (1 of 37) of subjects in the 150 mg group (medical observation) and 5.9% (1 of 17) of subjects in the placebo→150 mg group (uterine leiomyoma), but a causal relationship to the study drug was ruled out for both events.

Adverse events leading to discontinuation occurred in 2.7% (1 of 37) of subjects in the 110 mg group, 5.4% (2 of 37) of subjects in the 150 mg group, and 5.9% (1 of 17) of subjects in the placebo→150 mg group.

The incidences of adverse drug reactions were 10.8% (4 of 37 subjects) in the 110 mg group, 18.9% (7 of 37 subjects) in the 150 mg group, 23.5% (4 of 17 subjects) in the placebo→110 mg group, and 41.2% (7 of 17 subjects) in the placebo→150 mg group.

Table 42. Adverse events reported by ≥2 subjects in any group (through Week 52, Safety population)

Event term	110 mg (N = 37)	150 mg (N = 37)	Placebo→110 mg (N = 17)	Placebo→150 mg (N = 17)
Nasopharyngitis	4 (10.8)	10 (27.0)	3 (17.6)	1 (5.9)
Upper respiratory tract infection	3 (8.1)	4 (10.8)	1 (5.9)	1 (5.9)
Nausea	2 (5.4)	3 (8.1)	1 (5.9)	3 (17.6)
Urinary tract infection	2 (5.4)	1 (2.7)	2 (11.8)	0
Gastroenteritis viral	2 (5.4)	1 (2.7)	0	0
Iron deficiency	2 (5.4)	0	1 (5.9)	0
Chest discomfort	2 (5.4)	0	0	1 (5.9)
Migraine	2 (5.4)	0	0	0
Sinusitis	1 (2.7)	3 (8.1)	1 (5.9)	0
Abdominal pain	1 (2.7)	2 (5.4)	1 (5.9)	2 (11.8)
Flatulence	1 (2.7)	0	0	2 (11.8)
Vomiting	0	3 (8.1)	0	2 (11.8)
Dyspepsia	0	2 (5.4)	1 (5.9)	2 (11.8)
Abdominal discomfort	0	2 (5.4)	1 (5.9)	0
Gastrooesophageal reflux disease	0	0	0	2 (11.8)

n (%)

7.R Outline of the review conducted by PMDA

7.R.1 Development plan

The applicant's explanation about the development plan of berotralstat:

For the purpose to evaluate the efficacy and safety of berotralstat in Japanese patients with HAE, inclusion of Japanese patients with HAE in Study 302, which was in the planning stage at that time, was considered. However, it was decided that Study 301 be conducted separately for Japanese subjects out of concern over the likely delay in the start of enrollment in Study 302 in Japan as compared to foreign countries/regions, due to necessary preparation of the clinical trial system. Yet, extremely limited number of HAE patients in Japan precluded a placebo-controlled, parallel-group study with a sample size adequately powered to evaluate the

efficacy of berotralstat. Given no critical differences in the genetic and clinical characteristics of HAE between Japanese and non-Japanese populations or no clear ethnic differences in the pharmacokinetics of berotralstat [see Section 6.2.1.1], Study 301 was consequently designed almost identically to Study 302, but with a sample size determined by non-statistical hypothesis testing, to evaluate efficacy and safety in Japanese patients with HAE, taking account of data comparison between Studies 301 and 302 as well.

The target sample size was 24 at the designing stage of Study 301. After the new drug application for berotralstat was filed in the US in early December of 2019 based mainly on the results from Study 302, it was decided in Japan that the filing of a new drug application be advanced to the earliest possible date or by early February 2020. Accordingly, and partly due to its sluggishness, subject enrollment in Study 301 was stopped after 19 patients had been enrolled.

- Dosing regimen selected for phase III studies
  The following are the reasons for the selection of 2 doses of berotralstat, 110 mg and 150 mg, for Studies 301 and 302.
  - As described in Section 7.1.1, in the foreign phase II study (Study 203), berotralstat 110 mg showed the highest efficacy without safety concerns. In contrast, in the berotralstat 218 mg and 300 mg groups, the incidences of gastrointestinal adverse events and the rate of HAE attacks were higher than in the berotralstat 110 mg group. The applicant therefore concluded that 218 mg and 300 mg should not be recommended for clinical use, in view of the risk-benefit balance.
  - Based on a report on the relationship between the dose of a subcutaneous human plasma-derived C1-INH, which is marketed as a prophylactic HAE medication overseas, and reduced rate of HAE attacks (*N Engl J Med*. 2017; 376: 1131-40), which of these dosing regimen would show potential efficacy was simulated. The calculation revealed that clinically significant reduction in HAE attack rate would be achieved by a concentration 5.3- to 6.3-fold the EC_{50} for plasma kallikrein inhibition. According to exposure-response analyses using the data from Study 203, the predicted EC_{50} of berotralstat for plasma kallikrein inhibition was 11 ng/mL [see Section 6.4]. In the berotralstat 110 mg group of Study 203, the proportion of subjects with a berotralstat plasma trough concentration >4-fold the EC_{50} was 64%, and that of those with a berotralstat plasma trough concentration >6-fold the EC_{50} was 43%. Simulations based on the results from Studies 203 and 101 predicted that a 150-mg dose provides a berotralstat plasma trough concentration >4-fold the EC_{50} in 93% of patients and >6-fold the EC_{50} in 80% of patients. Thus, the 150-mg dose of berotralstat was expected to have enhanced efficacy.

PMDA's view:
The participation of Japanese patients in Study 302 should have been planned rigorously at the beginning of development. The enrollment of Japanese patients in Study 301 should have been completed, instead of discontinuing for the reason of early submission of a Japanese new drug application, etc. However, given that HAE is a rare disease, the efficacy and safety of berotralstat in Japanese patients with HAE can be evaluated based on the results from Study 301 and the foreign clinical studies.

7.R.2 Efficacy

The applicant's explanation about the efficacy of berotralstat:

As shown in Tables 36 and 38, respectively, in Studies 301 and 302, the primary endpoint of the rate of HAE attacks during the 24-week treatment period showed a statistically significant difference between the 150 mg and placebo groups by pairwise comparisons. The trend favored berotralstat as compared to placebo also in the secondary and other efficacy endpoints (Table 43), and the efficacy of berotralstat was maintained throughout the treatment period (Figure 3, Figure 4).

Table 43. Results of efficacy endpoints (Studies 301 and 302, through Week 24, ITT population)

Endpoint		301			302		
		110 mg (N = 6)	150 mg (N = 7)	Placebo (N = 6)	110 mg (N = 41)	150 mg (N = 40)	Placebo (N = 40)
Number of days with HAE symptoms (Days)		45.2 ± 34.0	16.6 ± 15.9	41.5 ± 26.5	20.8 ± 19.2	19.4 ± 21.5	29.2 ± 24.3
HAE attack rate by anatomic location[a)]							
	Abdominal-only	0.14 ± 0.22	0.02 ± 0.06	0.04 ± 0.10	0.32 ± 0.50	0.19 ± 0.34	0.40 ± 0.68
	Peripheral-only	0.91 ± 0.72	0.57 ± 0.59	1.33 ± 0.93	0.99 ± 1.27	0.67 ± 0.91	1.32 ± 1.14
	Mixed location	0.91 ± 0.58	0.50 ± 0.52	1.34 ± 1.79	0.59 ± 0.66	0.77 ± 0.98	0.75 ± 0.94
	Laryngeal	0.14 ± 0.19	0.14 ± 0.15	0.19 ± 0.12	0.10 ± 0.21	0.08 ± 0.17	0.18 ± 0.29
Rate of HAE attacks treated with rescue medication[a)]		1.74 ± 1.26	0.81 ± 0.75	2.43 ± 1.76	1.64 ± 1.69	1.44 ± 1.69	2.21 ± 1.71
Rate of moderate or severe HAE attacks[a)]		0.66 ± 0.67	0.73 ± 0.69	0.73 ± 0.34	1.16 ± 1.27	0.82 ± 0.78	1.24 ± 1.20
Reduction from baseline in HAE attack rate (%)		23.2 ± 36.9	52.5 ± 25.9	−5.51 ± 25.9	44.3 ± 38.7	51.2 ± 43.6	23.5 ± 42.9
Proportion of subjects with a reduction from baseline in HAE attack rate							
	≥50% reduction	33.3 (2/6)	57.1 (4/7)	0	51.2 (21/41)	57.5 (23/40)	25.0 (10/40)
	≥70% reduction	0	28.6 (2/7)	0	26.8 (11/41)	50.0 (20/40)	15.0 (6/40)
	≥90% reduction	0	0	0	9.8 (4/41)	22.5 (9/40)	7.5 (3/40)
Change from baseline in angioedema QOL total score[b)]		−9.5 ± 6.9	−15.8 ± 6.4	3.2 ± 6.8	−12.5 ± 2.5	−14.6 ± 2.6	−9.7 ± 2.6

Mean ± SD or % (n)
a) Number of HAE attacks per 28 days (expert-confirmed HAE attacks in Study 301, investigator-confirmed HAE attacks in Study 302)
b) At Week 24, Least-squares mean ± SE

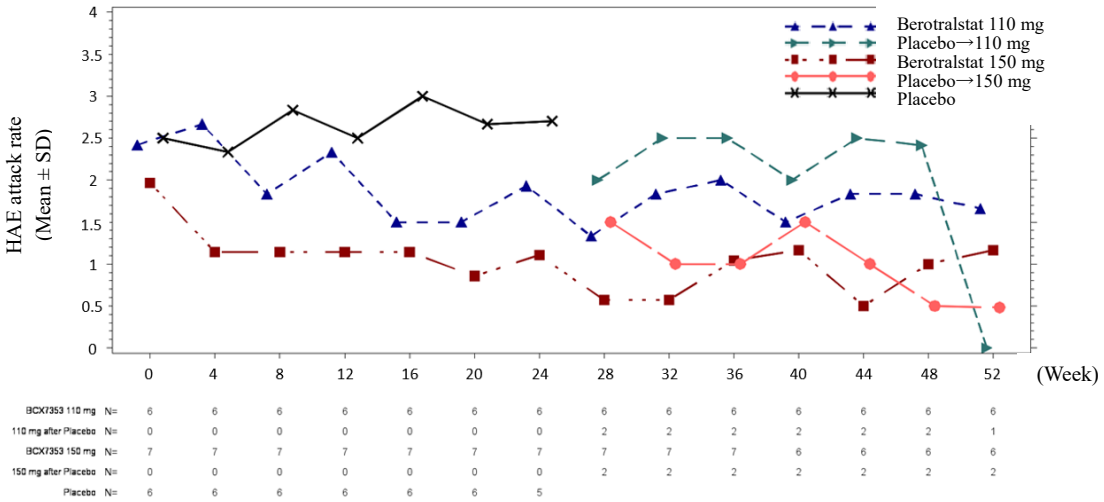

Figure 3. Mean monthly HAE attack rate through Week 52 (Study 301, ITT population)

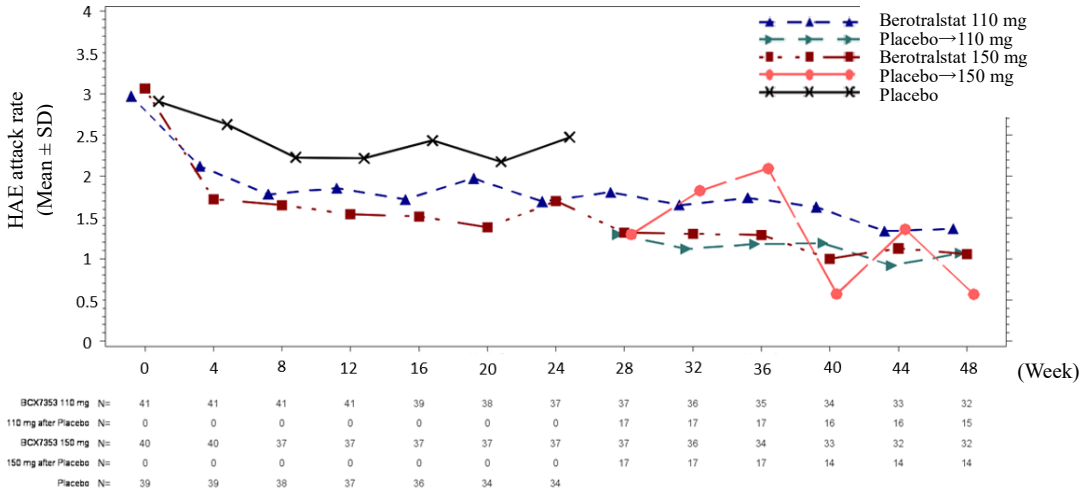

Figure 4. Mean monthly HAE attack rate through Week 48 (Study 302, ITT population)

The results of subgroup analyses by patient characteristics in the pooled Studies 301 and 302 are shown in Table 44. Although the results deserve careful interpretation because of the extremely limited number of subjects in the subgroups, there were no clear differences in the efficacy of berotralstat 150 mg across the subgroups.

Table 44. HAE attack rate (Number of attacks/28 days) by patient characteristics
(Pooled Studies 301 and 302, through Week 24, Pooled ITT population)

Patient characteristics		110 mg (N = 47)	150 mg (N = 47)	Placebo (N = 46)
Sex	Male	1.30 ± 1.03 (12)	1.25 ± 1.53 (18)	2.65 ± 1.00 (14)
	Female	2.12 ± 1.78 (35)	1.74 ± 1.61 (29)	2.44 ± 1.80 (31)
Age	12-17 years	0.66 ± 0.94 (2)	0.08 ± 0.12 (2)	0.08 ± 0.12 (2)
	18-64 years	2.05 ± 1.69 (39)	1.63 ± 1.60 (44)	2.70 ± 1.54 (40)
	≥65 years	1.42 ± 1.44 (6)	0.83 (1)	1.44 ± 0.85 (3)
Geographical location	North America	1.79 ± 1.53 (32)	1.59 ± 1.82 (27)	2.43 ± 1.79 (27)
	Europe	2.29 ± 2.30 (9)	1.72 ± 1.36 (13)	2.54 ± 1.13 (12)
	Japan	1.96 ± 1.30 (6)	1.09 ± 0.92 (7)	2.73 ± 1.64 (6)
Body weight	<76.20 kg	1.80 ± 1.20 (27)	1.45 ± 1.30 (20)	2.65 ± 1.49 (22)
	≥76.20 kg	2.06 ± 2.14 (20)	1.62 ± 1.78 (27)	2.36 ± 1.69 (23)
BMI	18.5 - 24.9 kg/m^2	1.75 ± 1.22 (21)	1.43 ± 1.31 (14)	2.49 ± 1.20 (14)
	25.0 - 29.9 kg/m^2	2.29 ± 1.98 (12)	1.85 ± 1.55 (16)	2.27 ± 1.65 (16)
	> 30 kg/m^2	1.82 ± 1.96 (14)	1.38 ± 1.84 (17)	2.75 ± 1.88 (15)
Baseline attack rate	≥2 attacks/month	2.35 ± 1.82 (30)	1.96 ± 1.66 (34)	2.96 ± 1.61 (30)
	<2 attacks/month	1.13 ± 0.92 (17)	0.48 ± 0.48 (13)	1.58 ± 1.10 (15)
Prior androgen use	Yes	1.58 ± 1.24 (19)	1.87 ± 1.86 (24)	2.75 ± 1.66 (26)
	No	2.13 ± 1.87 (28)	1.22 ± 1.18 (23)	2.16 ± 1.45 (19)

Mean ± SD (n), Observed value for n = 1

The data from subjects dosed for ≥12 months in a foreign long-term prophylactic study (Study 204) are available, although limited in number. The mean monthly HAE attack rate was kept <1 throughout the treatment period. The results of other efficacy endpoints also suggested no decreased efficacy with long-term dosing (Table 45).

Table 45. Results of efficacy endpoints (Study 204, through data cutoff date, Safety population)

Endpoint		110 mg	150 mg
Number of days with angioedema symptoms (Days)			
	Month 1 (Days 1-28)	3.4 ± 4.2 (100)	3.4 ± 4.1 (127)
	Month 3 (Days 57-84)	2.7 ± 3.3 (82)	3.1 ± 4.0 (112)
	Month 6 (Days 141-168)	2.5 ± 3.1 (78)	2.7 ± 4.0 (105)
	Month 9 (Days 225-252)	3.0 ± 4.6 (63)	1.8 ± 2.5 (97)
	Month 12 (Days 309-336)	1.1 ± 2.6 (47)	1.6 ± 2.2 (83)
	Month 15 (Days 393-420)	0.3 ± 0.8 (15)	1.8 ± 2.5 (30)
	Month 18 (Days 477-504)	－ (0)	0.7 ± 1.2 (12)
Proportion of HAE attack-free subjects (%)			
	Weeks 0-24	8.0 (8/100)	11.2 (14/125)
	Weeks 0-48	3.6 (2/56)	5.8 (6/104)
Change from baseline in angioedema QOL total score			
	Week 4	−8.9 ± 19.0 (81)	−11.2 ± 17.2 (114)
	Week 48	−12.8 ± 16.0 (23)	−14.7 ± 17.8 (73)

Mean ± SD (n) or % (n); －, Not applicable

Given the following points, berotralstat has promising efficacy in Japanese adolescent (12-17 years of age) patients with HAE, as in adult patients, though no adolescent subjects were enrolled in Study 301 including patients with HAE aged ≥12 years.

- The pathophysiology of HAE (Mutations in the *SERPING-1* gene result in deficiency or dysfunction of C1-INH, a plasma protein that inhibits bradykinin activation, leading to vascular permeability and edema [*Clin Immunol*. 2005; 114: 3-9]) is the same in adolescent and adult patients.
- In the overall populations of Studies 301 and 302, multiple efficacy endpoints showed similarity between Japanese and non-Japanese patients with HAE in the efficacy of berotralstat (Table 38, Table 40, Table 43).

Due to the limited number of adolescent subjects in Study 302 and the low HAE attack rate in the placebo group of adolescent subjects, there were no differences in the HAE attack rate between the berotralstat and placebo groups (Table 44). However, the study seems to have failed to clearly show the efficacy of berotralstat in adolescent subjects, given that HAE attacks can be triggered by changes in hormone levels or emotional stress etc., and the frequency also varies between individuals.

Based on the above, the efficacy of berotralstat in Japanese patients with HAE was demonstrated.

PMDA's view:

Although Study 301 had limited number of Japanese patients with HAE, there was a statistically significant difference in the primary endpoint of the HAE attack rate during the 24-week treatment period between the berotralstat 150 mg and placebo groups in Study 301 as in Study 302, and the results of other endpoints also indicated the efficacy of berotralstat. Thus, the efficacy of berotralstat in Japanese adult patients with HAE was demonstrated. Even though no Japanese adolescent patients with HAE received berotralstat in Study 301 while Study 302 also failed to show evident efficacy in adolescent patients, given the same pathology of HAE in adult and adolescent patients and the dosing regimen of berotralstat that can be used for both adult and adolescent patients based on its action mechanism and pharmacokinetics, the berotralstat has promising efficacy in adolescent patients with HAE, as in adult patients with HAE. Because the investigation of the efficacy of berotralstat was insufficient in Study 301, e.g., the lack of data from adolescent patients with HAE,

it is necessary to collect information via post-marketing surveillance etc. and provide obtained information to healthcare professionals as appropriate.

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

7.R.3 Safety

The applicant's explanation about the safety of berotralstat, based on the data from Study 301 in Japanese patients with HAE and the pooled data from a foreign long-term prophylactic study (Study 204) and a foreign phase III study (Study 302) (hereinafter referred to as "the pooled foreign studies"):

The summary of safety data from Study 301 and the pooled foreign studies and the main adverse events are shown in Table 46 and Table 47, respectively. There were no clear differences in the types or rates of adverse events between Japanese and foreign clinical studies.

Although the toxicity studies revealed abnormal values or findings in the kidney, pancreas, and cardiac and skeletal muscles following the administration of berotralstat, no relevant adverse events were reported in the clinical studies.

Table 46. Summary of safety data from Study 301 and pooled foreign studies (Safety population)

	Study 301				Pooled foreign studies (Pooled Studies 204 and 302)			
	110 mg (N = 6)	150 mg (N = 7)	All active (N = 17)	Placebo (N = 6)	110 mg (N = 141)	150 mg (N = 206)	All active (N = 381)	Placebo (N = 39)
Person-years of exposure	6.6	7.7	16.7	2.6	107.4	152.8	289.0	16.5
Adverse events	6 (100.0) 1950.4	7 (100.0) 313.5	16 (94.1) 959.1	6 (100.0) 497.2	130 (92.2) 721.5	170 (82.5) 660.2	327 (85.8) 657.4	30 (76.9) 750.1
Serious adverse events	2 (33.3) 30.5	0	2 (11.8) 12.0	0	22 (15.6) 49.3	16 (7.8) 13.1	40 (10.5) 26.0	2 (5.1) 18.1
Death	0	0	0	0	0	0	0	0
Adverse events leading to discontinuation	0	1 (14.3) 13.1	1 (5.9) 6.0	1 (16.7) 38.2	11 (7.8) 15.8	20 (9.7) 19.0	32 (8.4) 16.6	1 (2.6) 6.0
Adverse drug reactions	2 (33.3) 167.6	3 (42.9) 65.3	5 (29.4) 95.9	2 (33.3) 76.5	77 (54.6) 162.0	88 (42.7) 151.8	177 (46.5) 147.7	13 (33.3) 145.2

Upper row: n (%), Lower row: Number of events per 100 person-years

Table 47. Main adverse events in Study 301 and pooled foreign studies (Safety population)

	Study 301				Pooled foreign studies (Pooled Studies 204 and 302)			
	110 mg (N = 6)	150 mg (N = 7)	All active (N = 17)	Placebo (N = 6)	110 mg (N = 141)	150 mg (N = 206)	All active (N = 381)	Placebo (N = 39)
Person-years of exposure	6.6	7.7	16.7	2.6	107.4	152.8	289.0	16.5
Adverse events reported by ≥6% of subjects in any group of the pooled foreign studies or ≥2 subjects in any group of Study 301								
Nasopharyngitis	2 (33.3) 30.5	2 (28.6) 26.1	6 (35.3) 36.0	4 (66.7) 191.2	40 (28.4) 78.2	61 (29.6) 85.7	108 (28.3) 78.5	9 (23.1) 90.7
Headache	1 (16.7) 15.2	0	1 (5.9) 6.0	1 (16.7) 38.2	26 (18.4) 47.5	29 (14.1) 26.8	55 (14.4) 31.8	2 (5.1) 12.1
Diarrhoea	1 (16.7) 121.9	1 (14.3) 13.1	2 (11.8) 54.0	0	19 (13.5) 20.5	31 (15.0) 24.2	51 (13.4) 21.1	0
Abdominal pain	1 (16.7) 61.0	1 (14.3) 13.1	4 (23.5) 42.0	0	16 (11.3) 41.0	26 (12.6) 24.9	45 (11.8) 29.4	2 (5.1) 12.1
Nausea	1 (16.7) 15.2	0	1 (5.9) 6.0	0	17 (12.1) 18.6	23 (11.2) 19.0	44 (11.5) 18.3	7 (17.9) 48.4
Upper respiratory tract infection	0	0	0	0	12 (8.5) 20.5	24 (11.7) 17.0	38 (10.0) 17.3	1 (2.6) 6.0
Vomiting	0	0	0	0	7 (5.0) 8.4	19 (9.2) 15.7	29 (7.6) 12.5	1 (2.6) 12.1
Urinary tract infection	1 (16.7) 15.2	0	1 (5.9) 6.0	0	11 (7.8) 13.0	14 (6.8) 12.4	27 (7.1) 12.5	0
Gastrooesophageal reflux disease	0	0	0	0	13 (9.2) 12.1	10 (4.9) 7.2	25 (6.6) 9.3	0
Upper abdominal pain	0	1 (14.3) 13.1	1 (5.9) 6.0	0	11 (7.8) 13.0	12 (5.8) 12.4	24 (6.3) 11.8	1 (2.6) 6.0
Flatulence	1 (16.7) 15.2	0	1 (5.9) 6.0	0	7 (5.0) 7.4	13 (6.3) 9.2	23 (6.0) 8.7	1 (2.6) 6.0
Dyspepsia	0	0	0	0	7 (5.0) 6.5	11 (5.3) 8.5	21 (5.5) 8.7	3 (7.7) 36.3
Influenza	2 (33.3) 30.5	0	2 (11.8) 12.0	0	8 (5.7) 7.4	12 (5.8) 9.8	20 (5.2) 8.0	0
Abdominal discomfort	1 (16.7) 15.2	0	1 (5.9) 6.0	1 (16.7) 38.2	7 (5.0) 7.4	10 (4.9) 8.5	19 (5.0) 9.3	3 (7.7) 18.1
Back pain	0	2 (28.6) 26.1	2 (11.8) 12.0	0	4 (2.8) 3.7	13 (6.3) 11.1	18 (4.7) 7.6	1 (2.6) 6.0
Arthralgia	0	0	0	0	1 (0.7) 0.9	15 (7.3) 9.8	18 (4.7) 6.2	1 (2.6) 6.0
Constipation	0	0	0	0	9 (6.4) 10.2	6 (2.9) 3.9	15 (3.9) 5.9	1 (2.6) 6.0
Hereditary angioedema	1 (16.7) 15.2	0	1 (5.9) 6.0	0	12 (8.5) 34.4	2 (1.0) 1.3	14 (3.7) 13.5	0
Cough	2 (33.3) 30.5	0	2 (11.8) 12.0	0	5 (3.5) 4.7	5 (2.4) 3.3	11 (2.9) 3.8	0
Anxiety	0	0	0	0	4 (2.8) 5.6	4 (1.9) 3.3	9 (2.4) 4.2	3 (7.7) 18.1
Viral gastroenteritis	0	0	0	0	5 (3.5) 4.7	2 (1.0) 1.3	8 (2.1) 2.8	3 (7.7) 18.1
Pain in extremity	0	0	0	0	2 (1.4) 1.9	4 (1.9) 2.6	7 (1.8) 2.4	3 (7.7) 18.1
Pyrexia	1 (16.7) 15.2	1 (14.3) 13.1	2 (11.8) 12.0	0	2 (1.4) 1.9	2 (1.0) 1.3	4 (1.0) 1.4	0
Pruritus	0	1 (14.3) 13.1	2 (11.8) 12.0	0	1 (0.7) 0.9	2 (1.0) 1.3	3 (0.8) 1.0	0

Upper row: n (%), Lower row: Number of events per 100 person-years
MedDRA V19.1
In Studies 301 and 302, events were counted by treatment group assigned until Week 24. Events occurring beyond Week 24 in subjects switched from placebo to berotralstat at Week 24 were included in the all active group.

No deaths were reported in the Japanese and foreign clinical studies.

Serious adverse events occurred in 11.8% (2 of 17) of berotralstat-treated subjects in Study 301 and 10.5% (40 of 381) of berotralstat-treated subjects in the pooled foreign studies. The main events were hereditary angioedema (1 subject in Study 301, 12 subjects in the pooled foreign studies), medical observation (4 subjects in the pooled foreign studies), etc. Their causal relationship to the study drug was ruled out.

As a result of the safety evaluation in Japanese adolescent (12-17 years of age) patients with HAE, berotralstat is considered tolerable for the following reasons, although no adolescent subjects were enrolled in Study 301 targeting patients with HAE aged ≥12 years.

- Among adolescent subjects in the pooled foreign studies, the incidences of adverse events were 81.3% (13 of 16 subjects) in the berotralstat group and 100% (2 of 2 subjects) in the placebo group. Serious adverse events occurred in 12.5% (2 of 16) of subjects in the berotralstat group (facial paralysis; and hereditary angioedema and medical observation [1 subject each]), and an adverse event leading to discontinuation occurred in 6.3% (1 of 16) of subjects in the berotralstat group (upper abdominal pain) only. Although limited in number, adolescent subjects showed no clear differences in the types or rates of adverse events as compared with the overall population, or reported no adverse events of particular concern.
- As shown in Table 46 and Table 47, there were no clear differences in the types or rates of adverse events between Japanese and foreign clinical studies, and no adverse events of particular concern were reported by Japanese subjects.

Taking account of the pharmacological effects of berotralstat, disease characteristics of patients with HAE, etc., PMDA's safety review focused on the following adverse events.

7.R.3.1 Hepatic dysfunction

The applicant's explanation about the effect of berotralstat on the liver function:

Single cell necrosis and bile duct degeneration in the liver and increases in blood AST/ALT activity were observed in the repeated-dose toxicity studies of berotralstat in cynomolgus monkeys. Single cell necrosis and bile duct degeneration in the liver, increases in blood AST/ALT activity, and bile duct hyperplasia/dilation were noted in the repeated-dose toxicity studies in rats [see Section 5.2].

The occurrence of adverse events related to hepatic dysfunction in Study 301 and the pooled foreign studies are shown in Table 48.

Table 48. Occurrence of adverse events related to hepatic dysfunction in Study 301 and pooled foreign studies (Safety population)

	Study 301				Pooled foreign studies (Pooled Studies 204 and 302)			
	110 mg (N = 6)	150 mg (N = 7)	All active (N = 17)	Placebo (N = 6)	110 mg (N = 141)	150 mg (N = 206)	All active (N = 381)	Placebo (N = 39)
Person-years of exposure	6.6	7.7	16.7	2.6	107.4	152.8	289.0	16.5
Adverse events related to hepatic dysfunction								
Adverse events	0 0	1 (14.3) 13.1	1 (5.9) 6.0	0	11 (7.8) 16.8	14 (6.8) 22.2	25 (6.6) 18.0	1 (2.6) 6.0
Serious adverse events	0 0	0 0	0 0	0	1 (0.7) 0.9	1 (0.5) 0.7	2 (0.5) 0.7	0
Adverse events leading to discontinuation	0 0	1 (14.3) 13.1	1 (5.9) 6.0	0	3 (2.1) 3.7	6 (2.9) 4.6	9 (2.4) 3.8	0
Main events (PTs)								
ALT increased	0 0	0 0	0 0	0	6 (4.3) 6.5	9 (4.4) 11.8	15 (3.9) 8.7	0
AST increased	0 0	0 0	0 0	0	5 (3.5) 5.6	6 (2.9) 4.6	11 (2.9) 4.5	0
GGT increased	0 0	0 0	0 0	0	1 (0.7) 0.9	4 (1.9) 2.6	5 (1.3) 1.7	1 (2.6) 6.0
Hepatic enzyme increased	0 0	1 (14.3) 13.1	1 (5.9) 6.0	0	1 (0.7) 0.9	1 (0.5) 0.7	2 (0.5) 0.7	0
Liver function test increased								
ALT								
≥3 × ULN	0	1 (14.3)	1 (5.9)	0	9 (6.4)	8 (3.9)	17 (4.5)	0
≥5 × ULN	0	1 (14.3)	1 (5.9)	0	3 (2.1)	5 (2.4)	8 (2.1)	0
≥10 × ULN	0	1 (14.3)	1 (5.9)	0	0	2 (1.0)	2 (0.5)	0
AST								
≥3 × ULN	0	1 (14.3)	1 (5.9)	0	1 (0.7)	4 (1.9)	5 (1.3)	0
≥5 × ULN	0	1 (14.3)	1 (5.9)	0	0	1 (0.5)	1 (0.3)	0
≥10 × ULN	0	0	0	0	0	0	0	0
Total bilirubin								
≥1.5 ×	0	0	0	0	1 (0.7)	0	1 (0.3)	2 (5.1)
≥2 ×	0	0	0	0	0	0	0	0

Upper row: n (%), Lower row: Number of events per 100 person-years
In Studies 301 and 302, events were counted by treatment group assigned until Week 24. Events occurring beyond Week 24 in subjects switched from placebo to berotralstat at Week 24 were included in the all active group.

No serious adverse events related to hepatic dysfunction were reported in Study 301. In the pooled foreign studies, serious adverse events related to hepatic dysfunction occurred in 1 subject in the 110 mg group (hepatic enzyme increased) and 1 subject in the 150 mg group (liver function test abnormal), and a causal relationship to the study drug could not be ruled out for both events. Both events had an outcome of "resolved."

Hepatic dysfunction-related adverse events leading to discontinuation occurred in 1 subject in the 150 mg group of Study 301 (hepatic enzyme increased) and 3 subjects in the 110 mg group (AST increased/ALT increased; liver function test abnormal; and hepatic enzyme increased [1 each]) and 6 subjects in the 150 mg group (ALT increased; and liver function test abnormal [2 each]; and ALT increased/AST increased; and hepatic enzyme increased [1 each]) in the pooled foreign studies.

In any of the clinical studies in which berotralstat was administered, hepatic disorder defined as hepatic synthetic dysfunction did not occur, and the reported hepatic disorder events were predominantly asymptomatic increase in hepatic enzyme. All of subjects with ALT elevations >3 times the upper limit of normal in the pooled foreign studies (17 subjects) had a history of androgen use for the prevention of HAE attacks in the US and Europe, etc. In Study 204, patients on prior androgens were required to discontinue their use ≥7 days prior to

the initiation of berotralstat, and 82.4% (14 of 17) of subjects with ALT elevations >3 times the upper limit of normal had discontinued androgens within 2 weeks prior to the initiation of berotralstat, suggesting the potential relationship between increased hepatic enzyme observed in the clinical studies and their prior androgen use. In response to these results, Studies 301 and 302 required patients to discontinue androgens ≥28 days prior to screening, and the Study 204 protocol was also amended accordingly. As a result, no ALT elevations were reported in Studies 301 and 302 or from subjects who were enrolled in Study 204 after the protocol amendment.

Based on the above, all events of hepatic dysfunction observed in the clinical studies resolved, and their relationship to prior androgen use was suggested. Thus, the relationship between berotralstat and the risk of hepatic dysfunction is unclear.

PMDA's view:
With regard to hepatic dysfunction, the berotralstat non-clinical studies showed hepatic single cell and bile duct toxicities across different animal species, and the clinical studies revealed serious adverse events for which a causal relationship to study drug could not be ruled out. Although androgens are unapproved for the prevention of HAE attacks in Japan, the Japanese guideline ("Guideline for hereditary angioedema, Revised Version, 2014" [*Journal of Japanese Association for Complement Research*. 2014; 51: 22-3]) describes about the long-term prophylaxis of HAE attacks by androgens. The foreign clinical studies suggested the relationship between the use of androgen plus berotralstat and hepatic dysfunction. Thus, the package insert should provide cautionary advice about hepatic dysfunction including the effect of androgen. In addition, the occurrence of hepatic dysfunction warrants close attention in Japan and overseas further in the post-marketing setting, and obtained information should be provided to healthcare professionals as appropriate.

7.R.3.2 QT interval prolongation

The applicant's explanation about the effect of berotralstat on QT interval prolongation:
Non-clinical safety pharmacology data suggested the pro-arrhythmic risk of berotralstat [see Section 3.R]. In a thorough QT study (Study 106), the upper bound of the two-sided 90% confidence interval for ΔΔQTcF at a 436-mg dose of berotralstat (exposures were 4-fold higher than achieved at the clinical dose) was estimated as 29.4 milliseconds, which deviated from the normal range (the 10 millisecond threshold) [see Section 6.2.4].

The occurrence of QT interval prolongation in Study 301 and the pooled foreign studies are shown in Table 49.

Table 49. Occurrence of QT interval prolongation in Study 301 and pooled foreign studies

	Study 301				Pooled foreign studies (Pooled Studies 204 and 302)			
	110 mg (N = 6)	150 mg (N = 7)	All active (N = 17)	Placebo (N = 6)	110 mg (N = 141)	150 mg (N = 206)	All active (N = 381)	Placebo (N = 39)
Number of events assessed	73	84	177	43	1,031	1,297	2,511	259
QTcF value								
>400 msec and ≤450 msec	5 (83.3)	7 (100.0)	16 (94.1)	6 (100.0)	140 (99.3)	184 (89.3)	358 (94.0)	39 (100.0)
	61 (83.6)	84 (100.0)	165 (93.2)	41 (95.3)	1,011 (98.1)	1,256 (96.8)	2,438 (97.1)	247 (95.4)
>450 msec and ≤480 msec	2 (33.3)	0	2 (11.8)	1 (16.7)	13 (9.2)	19 (9.2)	37 (9.7)	6 (15.4)
	12 (16.4)	0	12 (6.8)	2 (4.7)	20 (1.9)	39 (3.0)	71 (2.8)	12 (4.6)
>480 msec and ≤500 msec	0	0	0	0	0	2 (1.0)	2 (0.5)	0
	0	0	0	0	0	2 (0.2)	2 (<0.1)	0
>500 msec	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
Change from baseline								
≤30 msec	6 (100.0)	7 (100.0)	17 (100.0)	6 (100.0)	140 (99.3)	184 (89.3)	358 (94.0)	39 (100.0)
	72 (98.6)	83 (98.8)	175 (98.9)	43 (100.0)	997 (96.7)	1,239 (95.5)	2,418 (96.3)	252 (97.3)
>30 msec and ≤60 msec	1 (16.7)	1 (14.3)	2 (11.8)	0	22 (15.6)	33 (16.0)	56 (14.7)	4 (10.3)
	1 (1.4)	1 (1.2)	2 (1.1)	0	34 (3.3)	55 (4.2)	90 (3.6)	7 (2.7)
>60 msec	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0

Upper row: n (%), Lower row: Number of events (%)
In Studies 301 and 302, events were counted by treatment group assigned until Week 24. Events occurring beyond Week 24 in subjects switched from placebo to berotralstat at Week 24 were included in the all active group.

In Study 301 and the pooled foreign studies, there were no clear differences in the occurrence of QT interval prolongation between the placebo and berotralstat groups. Adverse events of arrhythmia or other ECG changes etc. occurred in 5 subjects in the 110 mg group (chest discomfort [3]; and atrioventricular block first degree; and myocardial infarction [1 each]) and 8 subjects in the 150 mg group (chest pain [4]; and palpitations; atrioventricular block first degree; palpitations/tachycardia; and chest discomfort [1 each]). A causal relationship to the study drug was ruled out for all those events except for atrioventricular block first degree (1 subject) in the 110 mg group and palpitations/tachycardia (1 subject) in the 150 mg group. Chest pain reported by 2 subjects in the 150 mg group led to study drug interruption.

Based on the above, although the results from Study 301 and the foreign clinical studies (Studies 302 and 204) indicated that the risk of QT interval prolongation or arrhythmia is not high at the proposed dose, berotralstat has the potential to cause QT interval prolongation as described in Sections 3.R.2 and 6.R.3, QT interval prolongation will be specified as an identified risk of berotralstat, and a relevant precaution will be given in the package insert, etc.

PMDA's view:
Although QT interval prolongation or serious arrhythmia has not been reported with the proposed dosing regimen of berotralstat at present, berotralstat has the potential risk of QT interval prolongation as mentioned in Sections 3.R.2 and 6.R.3. Based on the following considerations, a decision on the use of berotralstat in patients with moderate or severe hepatic impairment should be made carefully. Berotralstat exposure increases in low-body-weight patients as mentioned in Section 6.3, and thus patients with a body weight of ≤40 kg (no berotralstat treatment experience in Japanese or foreign phase III studies) should be fully informed of and understand the risk of berotralstat prior to the treatment. It is also necessary to continue to collect information

on the occurrence etc. of events via post-marketing surveillance etc. and provide obtained information to healthcare professionals as appropriate.

- Because of unknown causes of variability in berotralstat exposure in patients with hepatic impairment, patients with severe hepatic impairment may potentially be exposed to berotralstat at a similar level to those in patients with moderate hepatic impairment (1.77-fold the exposure in subjects with normal hepatic function), reaching the threshold concentration for a proarrhythmic risk [see Section 6.R.3].
- Based on the results of a population pharmacokinetic analysis showing higher berotralstat exposure in low-body-weight patients [see Section 6.3] and a tendency toward lower body weight in Japanese patients than in non-Japanese patients (68.4 [61.2, 75.2] kg in Japanese subjects; 79.0 [55.6, 100] kg in non-Japanese subjects [mean (range)]) [see Section 6.R.1], berotralstat exposure may be higher in Japanese patients.
- Based on the results from Studies 108 and 106, patients with AST or ALT ≥3 times ULN at screening and patients with clinically significant abnormal ECG,[33)] etc.[34)] were excluded from Japanese and foreign phase III studies, i.e. Studies 301 and 302. Thus, there is limited clinical experience with berotralstat in HAE patients with such characteristics at present.
- Berotralstat is an oral drug intended for long-term treatment for the prevention of HAE attacks. It is difficult to keep monitoring the pro-arrhythmic effect of berotralstat throughout the treatment period.

7.R.3.3 Gastrointestinal symptoms

The applicant's explanation about the incidence of gastrointestinal symptoms following the administration of berotralstat:

In both berotralstat-treated healthy subjects and patients with HAE, diarrhoea, nausea, vomiting, flatulence, and abdominal pain occurred in a dose-response manner and more frequently after multiple dosing than after single dosing and at 218 or 300 mg [see Section 7.1.1].

The occurrence of gastrointestinal symptoms in Study 301 and the pooled foreign studies are shown in Table 50, and there were no clear differences in the incidences of gastrointestinal symptoms between Japanese and non-Japanese patients with HAE.

[33)] a QTcF ≥470 milliseconds for women or a QTcF ≥450 milliseconds for men, and a PR interval ≥220 milliseconds (both sexes)

[34)] Patients with a clinically significant history of angina pectoris, myocardial infarction, syncope, arrhythmia, left ventricular hypertrophy, cardiomyopathy, or any other clinically significant cardiovascular abnormality such as poorly controlled hypertension, or known family history of sudden cardiac death, or history of or current implanted defibrillator or pacemaker

Table 50. Occurrence of gastrointestinal symptoms and the main adverse events in Study 301 and pooled foreign studies (Adverse events reported by ≥5% of subjects in the pooled foreign studies or ≥2 subjects in Study 301) (Safety population)

	Study 301				Pooled foreign studies (Pooled Studies 204 and 302)			
	110 mg (N = 6)	150 mg (N = 7)	All active (N = 17)	Placebo (N = 6)	110 mg (N = 141)	150 mg (N = 206)	All active (N = 381)	Placebo (N = 39)
Person-years of exposure	6.6	7.7	16.7	2.6	107.4	152.8	289.0	16.5
Incidence of gastrointestinal symptoms[a)]								
Adverse events	3 (50.0) 228.6	3 (42.9) 52.3	8 (47.1) 125.9	1 (16.7) 38.2	73 (51.8) 162.0	88 (42.7) 143.9	177 (46.5) 146.7	14 (35.9) 157.3
Serious adverse events	0	0	0	0	1 (0.7) 1.9	1 (0.5) 1.3	2 (0.5) 1.4	0
Adverse events leading to discontinuation	0	0	0	0	4 (2.8) 5.6	7 (3.4) 5.9	12 (3.1) 5.9	0
Main events (PTs)								
Diarrhoea	1 (16.7) 121.9	1 (14.3) 13.1	2 (11.8) 54.0	0	19 (13.5) 20.5	31 (15.0) 24.2	51 (13.4) 21.1	0
Abdominal pain	1 (16.7) 61.0	1 (14.3) 13.1	4 (23.5) 42.0	0	16 (11.3) 41.0	26 (12.6) 24.9	45 (11.8) 29.4	2 (5.1) 12.1
Nausea	1 (16.7) 15.2	0	1 (5.9) 6.0	0	17 (12.1) 18.6	23 (11.2) 19.0	44 (11.5) 18.3	7 (17.9) 48.4
Vomiting	0	0	0	0	7 (5.0) 8.4	19 (9.2) 15.7	29 (7.6) 12.5	1 (2.6) 12.1
Upper abdominal pain	0	1 (14.3) 13.1	1 (5.9) 6.0	0	11 (7.8) 13.0	12 (5.8) 12.4	24 (6.3) 11.8	1 (2.6) 6.0
Flatulence	1 (16.7) 15.2	0	1 (5.9) 6.0	0	7 (5.0) 7.4	13 (6.3) 9.2	23 (6.0) 8.7	1 (2.6) 6.0
Gastrooesophageal reflux disease	0	0	0	0	13 (9.2) 12.1	10 (4.9) 7.2	25 (6.6) 9.3	0
Dyspepsia	0	0	0	0	7 (5.0) 6.5	11 (5.3) 8.5	21 (5.5) 8.7	3 (7.7) 36.3
Abdominal discomfort	1 (16.7) 15.2	0	1 (5.9) 6.0	1 (16.7) 38.2	7 (5.0) 7.4	10 (4.9) 8.5	19 (5.0) 9.3	3 (7.7) 18.1
Abdominal distension	0	0	0	0	8 (5.7) 8.4	9 (4.4) 5.9	17 (4.5) 6.2	2 (5.1) 12.1
Constipation	0	0	0	0	9 (6.4) 10.2	6 (2.9) 3.9	15 (3.9) 5.9	1 (2.6) 6.0

Upper row: n (%), Lower row: Number of events per 100 person-years
MedDRA V19.1
In Studies 301 and 302, events were counted by treatment group assigned until Week 24. Events occurring beyond Week 24 in subjects switched from placebo to berotralstat at Week 24 were included in the all active group.
a) PTs in gastrointestinal signs and symptoms (HLGT) and gastrointestinal motility and defaecation conditions (HLGT)

No serious gastrointestinal symptoms were reported in Study 301. In the pooled foreign studies, serious gastrointestinal symptoms occurred in 1 subject in the 110 mg group (abdominal pain) and 1 subject in the 150 mg group (diarrhoea/vomiting). Their causal relationship to the study drug could not be ruled out, and all those events had an outcome of "resolved."

No gastrointestinal symptoms leading to discontinuation were reported in Study 301. In the pooled foreign studies, gastrointestinal symptoms leading to discontinuation occurred in 4 subjects in the 110 mg group (upper abdominal pain [2]; nausea/vomiting/dyspepsia; and nausea [1 each]) and 8 subjects in the 150 mg group (diarrhoea; abdominal pain; abdominal pain/diarrhoea; upper abdominal pain; nausea/vomiting; vomiting; anal incontinence; and diarrhoea/gastrooesophageal reflux disease [1 each]).

Gastrointestinal symptoms associated with berotralstat tended to occur frequently during early dosing (Table 51).

Table 51. Proportion of subjects with gastrointestinal symptom[a)]-related events over time (Pooled foreign studies)

	110 mg	150 mg	Placebo
Weeks 1-12	41.8 (66/158)	33.7 (62/184)	33.3 (13/39)
Weeks 13-24	11.3 (16/142)	12.7 (21/166)	8.1 (3/37)
Weeks 25-36	9.9 (13/131)	12.2 (19/156)	0
Weeks 37-48	3.6 (4/112)	6.5 (9/139)	0

a) PTs in gastrointestinal signs and symptoms (HLGT) and gastrointestinal motility and defaecation conditions (HLGT)

Based on the above, most of gastrointestinal symptoms such as diarrhoea and abdominal pain were mild or moderate in severity, and serious events were limited. Although gastrointestinal symptoms occurred frequently during early dosing, new events decreased with prolonged dosing, and the symptoms resolved without berotralstat discontinuation in many subjects. Thus, gastrointestinal symptoms were not considered clinically relevant events.

The gastrointestinal tract is the critical anatomic site of HAE. HAE attacks involving the gastrointestinal tract cause partial or complete duct obstruction, leading to abdominal colic, nausea, vomiting, or diarrhoea. The major symptoms are mostly crampy or colicky pain, the mean duration of abdominal pain in severe symptoms has been reported to be 3.3 days (*Am J Gastroenterol.* 2006; 101: 619-27), which can be shortened with medication for HAE attacks. On the other hand, abdominal pain may occur as an adverse event following administration of berotralstat, but the symptom was mild in severity. Many patients with nausea or diarrhoea did not have abdominal pain, and the event resolved without the use of drugs. The duration of abdominal pain was not consistent with the natural history of abdominal symptoms of HAE attacks, ranging from 1 day to ≥10 days.

PMDA's view:
Although severe or serious events were limited, the rates of gastrointestinal symptoms was higher in berotralstat-treated subjects than in placebo-treated subjects. The package insert should provide cautionary advice on the occurrence of this event. Given the limited number of patients evaluated at present, it is necessary to investigate the occurrence, etc. of gastrointestinal symptoms via post-marketing surveillance, etc., and provide obtained information to healthcare professionals as appropriate.

The above conclusions by PMDA presented in Section 7.R.3 will be discussed at the Expert Discussion.

7.R.4 Clinical positioning

The applicant's explanation about the clinical positioning of berotralstat in the treatment of HAE:
In Japan, an approved prophylactic HAE medication is lyophilized human C1-inactivator concentrate for short-term prophylaxis, which prevents acute HAE attacks triggered by invasive dental or surgical procedures. However, no medications for long-term prophylaxis of HAE attacks have been approved. The Japanese guideline ("Guideline for hereditary angioedema, Revised Version, 2014" [*Journal of Japanese Association for Complement Research*. 2014; 51: 22-3]) advises that "consider long-term prophylaxis if patients have ≥1 attack per month, ≥5 days of disability per month, or a history of laryngeal edema" and mentions off-label drugs of tranexamic acid and danazol.[19)]

Study 302 confirmed the efficacy of the proposed dosing regimen of berotralstat 150 mg orally once daily and raised no particular safety concerns, and Study 301 yielded similar results to Study 302. Thus, berotralstat is a promising first-line drug for long-term prophylaxis of HAE attacks as the first drug that demonstrated efficacy in preventing acute HAE attacks.

In Japan, approved medications for the treatment of HAE attacks include lyophilized human C1-inactivator concentrate and icatibant acetate. The medications used to treat acute attacks in Study 301 are shown in Table 52. In Studies 301 and 302, there were no particular safety concerns with on-demand medication use in patients on berotralstat.

Table 52. Medications used to treat HAE attacks (Study 301, through Week 24)

	110 mg (N = 6)	150 mg (N = 7)	Placebo (N = 6)
Total number of HAE attacks	71	46	94
Lyophilized human C1-inactivator concentrate	42 (59.2)	34 (73.9)	80 (85.1)
Icatibant acetate	27 (38.0)	17 (37.0)	10 (10.6)
Others[a)]	42 (59.2)	21 (45.7)	80 (85.1)

No. of attacks (%), In some cases, ≥1 medication was used to treat 1 HAE attack.
a) human plasma-derived C1-INH and recombinant C1-INH unapproved in Japan, and fresh frozen plasma

In Studies 301 and 302, berotralstat was not used in combination with tranexamic acid, androgens, or prophylactic HAE medications such as C1-INH, and the safety and efficacy of berotralstat used in combination with those medications are unclear at present.

PMDA's view:
Given the efficacy and safety profiles of berotralstat currently available, berotralstat can be used chronically for the prevention of HAE attacks. Although, to date, there have been no particular safety concerns with the use of on-demand medication to treat HAE attacks in patients on berotralstat, the investigation on Japanese patients with HAE is insufficient. Berotralstat safety information including the safety of on-demand medication use in patients on berotralstat should be further collected in the post-marketing setting, and obtained information should be provided to healthcare professionals as appropriate.

Acute attacks can occur even during prophylaxis with berotralstat. It is important to be prepared for appropriately treating acute attacks with medications for HAE attacks.

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

7.R.5 Indication

Based on the discussions in Sections 7.R.2 and 7.R.3, PMDA considers that berotralstat has promising efficacy in the prevention of HAE attacks, and that berotralstat has acceptable safety. Thus, taking account of the positioning of berotralstat, PMDA has concluded that the proposed indication may be modified to "the prevention of acute attacks of hereditary angioedema."

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

7.R.6 Dosage and administration

The applicant's explanation about the proposed dosage and administration, based on the results of clinical studies submitted as evaluation data:

As mentioned in Section 7.R.2, Studies 301 and 302, both phase III studies, showed a statistically significant difference in the primary endpoint of the HAE attack rate during the 24-week treatment period between berotralstat 150 mg and placebo. While Study 302 showed a statistically significant difference between berotralstat 110 mg and placebo, there was a consistent trend toward higher efficacy with 150 mg than 110 mg in the primary and other endpoints for Studies 301 and 302 (Table 43). Furthermore, as mentioned in Section 7.R.3, the currently available data showed the acceptable safety profile of berotralstat 150 mg once daily regimen in patients with HAE, and suggested no clinically relevant obvious risks.

As explained in Section 7.R.4, oropharyngeal or laryngeal HAE attacks, etc. can cause asphyxiation resulting in death. Berotralstat should preferably be administered at a dose that can maximize the prevention of HAE attacks within the tolerable range. Thus, the dosing regimen of berotralstat 150 mg orally once daily is appropriate.

PMDA concluded that the proposed dosing regimen of berotralstat 150 mg orally once daily is acceptable based on the currently available clinical study data.

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

7.R.7 Post-marketing investigations and safety measures

The applicant is planning to conduct post-marketing surveillance to assess the safety, etc. of berotralstat (including its long-term safety) in all HAE patients treated with berotralstat in the post-marketing setting. The package insert will advise of the risk of hepatic dysfunction and QT interval prolongation associated with berotralstat.

PMDA's view:

As mentioned in Section 7.R.3, the safety of berotralstat is largely acceptable based on clinical study data. However, a use-results survey should be conducted because of limited clinical experience with berotralstat in Japanese patients and undeniable possibility to cause hepatic dysfunction, QT interval prolongation, etc. based on the data including non-clinical and clinical study results. The survey should cover all patients treated with berotralstat to further collect information, aiming to understand the safety profile of berotralstat, including the occurrence of unknown adverse events, and to continue to assess the safety etc. of berotralstat carefully.

The above conclusion by PMDA and the need for further safety measures will be discussed at the Expert Discussion.

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

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

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

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

The new drug application data (CTD 5.3.5.1-3) were subjected to an on-site GCP inspection, in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices. On the basis of the inspection, PMDA concluded that since the clinical study as a whole was performed in compliance with GCP, there were no obstacles to conducting its review based on the application documents submitted. The inspection revealed the following finding at the sponsor, although which did not affect the overall assessment of the study significantly. The sponsor (clinical trial in-country representative) was notified of this matter and asked for a corrective action.

Finding requiring corrective action

Sponsor (Clinical trial in-country representative)

· There were no written procedures for protocol development at the time of initiating the clinical study.

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

On the basis of the data submitted, PMDA has concluded that berotralstat has efficacy in the prevention of acute attacks of HAE, and that berotralstat has acceptable safety in view of its benefits, as long as adequate safety measures are taken against the risk of hepatic dysfunction and QT interval prolongation. Berotralstat is clinically meaningful because it offers a new treatment option for the prophylaxis of acute HAE attacks. Because the number of participants in the clinical studies of berotralstat was extremely limited, the safety, etc. of berotralstat should be further investigated in the post-marketing setting.

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

10. Others

The definition of the endpoint in the clinical studies is shown below.

Endpoint	Definition
Angioedema QOL score	Subject-assessed, angioedema-specific QOL scores, of which lower total scores for 4 domains (functioning, fatigue, nutrition, fears/shame) indicate better control (Scores range from 0 to 100.)

Review Report (2)

October 21, 2020

Product Submitted for Approval

Brand Name	Orladeyo Capsules 150 mg
Non-proprietary Name	Berotralstat Hydrochloride
Applicant	OrphanPacific, Inc.
Date of Application	January 31, 2020

List of Abbreviations

See Appendix.

1. Content of the Review

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

1.1 Efficacy, clinical positioning, and indication

At the Expert Discussion, the expert advisors supported PMDA's conclusions on the efficacy, clinical positioning, and indication of berotralstat presented in the Review Report (1), and made the following comment.

- The risk of QT interval prolongation associated with berotralstat increases with increasing exposure as explained in Section 7.R.3.2 in the Review Report (1). It is important to advise physicians not to administer additional doses of berotralstat to treat an acute attack of HAE.

1.2 Dosage and administration

At the Expert Discussion, the expert advisors largely supported PMDA's conclusion on the dosage and administration of berotralstat described in the Review Report (1), and made the following comment.

- Berotralstat has associated risk of hepatic dysfunction and QT interval prolongation [see Section 7.R.3 in the Review Report (1)]. Given higher berotralstat exposure in Japanese patients than in non-Japanese patients [see Section 6.R.1 in the Review Report (1)], dosage and administration needs to be defined carefully.

PMDA's view:

The Japanese and foreign clinical studies showed no clear differences between Japanese and non-Japanese subjects in the incidence of adverse events following once daily dosing of berotralstat 150 mg, and berotralstat safety profile is also considered tolerable. Berotralstat 150 mg once daily is acceptable dosing regimen for Japanese patients with HAE. At the same time, as discussed in Section 1.3, higher exposure in Japanese patients than in non-Japanese patients is a concern, and the risk of QT interval prolongation increases with increasing exposure to berotralstat. Thus, it is important that particularly patients with risk factors be adequately reminded of the risk and that safety measures be taken prior to the use of berotralstat in this patient population. Because of the extremely limited number of Japanese patients with HAE studied at present, the occurrence of adverse events, etc. needs to be further investigated via post-marketing surveillance etc., and obtained information should be provided to healthcare professionals as appropriate.

1.3 Safety, post-marketing investigations, and risk management plan (draft)

At the Expert Discussion, the expert advisors supported PMDA's conclusions on the safety of berotralstat including carcinogenicity assessment and post-marketing safety measures presented in the Review Report (1), and made the following comments on the risk of QT interval prolongation.

- The consistency observed among the evaluation of the effect of berotralstat on cardiac ion channels, a study using human iPS cell-derived cardiomyocytes, ECG evaluation in cynomolgus monkeys, and a thorough QT study indicates berotralstat’s potential pro-arrhythmic risk. Multiple channel blockers like berotralstat may further enhance the pro-arrhythmic risk.
- Berotralstat administered as per the proposed dosing regimen is unlikely to pose a high risk of QT interval prolongation to patients without factors of increasing berotralstat exposure. Nevertheless, patients with higher exposure such as those with moderate or severe hepatic impairment and low-body-weight patients are more likely to have QT interval prolongation. The package insert, etc. should give adequate cautionary advice.
- In the clinical studies, berotralstat was not administered to patients with a risk of QT interval prolongation such as arrhythmia, ischemic heart disease, or hypokalemia, and medication that are known to cause QT interval prolongation. If berotralstat exposure is increased in these patients because of their hepatic impairment or low body weight, etc., the risk of induction or worsening of QT interval prolongation is even higher. Thus, special caution should be used.
- Prior to and during treatment with berotralstat, it is important to pay attention to the cardiovascular status, e.g., through ECG as needed, and closely monitor the patient's condition.
- Data on the occurrence of QT interval prolongation-related adverse events should be further collected in the post-marketing setting, and it is important that available information on serious arrhythmia or marked QT interval prolongation be provided to healthcare professionals.

Taking account of the considerations in Section 7.R.3 in the Review Report (1) and the comments from the expert advisors at the Expert Discussion, PMDA concluded that the package insert should present the following cautionary statements regarding the risk of QT interval prolongation associated with berotralstat, and that it

is necessary to investigate the occurrence, etc. of QT interval prolongation-related adverse events in the clinical setting also via post-marketing surveillance, etc. The applicant was instructed to conduct a use-results survey.

- QT interval prolongation may occur or worsen in patients with QT interval prolongation or a history of QT interval prolongation and those with a risk of QT interval prolongation (patients with arrhythmia, ischemic heart disease, or hypokalemia, etc.). Caution should be exercised in the concomitant use of a drug that is known to cause QT interval prolongation.
- Patients with moderate or severe hepatic impairment, low-body-weight patients, etc. are more likely to have QT interval prolongation due to increased blood concentrations of berotralstat.
- Prior to and during treatment with berotralstat, the patient's condition should be closely monitored, such as by ECG.
- Prior to the use of berotralstat, patients or their families should be fully informed of and understand safety concerns of berotralstat, including the risk of QT interval prolongation.

Based on the discussion in Section 7.R.7 in the Review Report (1) and the comments from the expert advisors at the Expert Discussion, PMDA has concluded that the risk management plan (draft) for berotralstat should include the safety specification presented in Table 53, and that the applicant should conduct additional pharmacovigilance activities and risk minimization activities presented in Table 54.

Table 53. Safety and efficacy specifications in the risk management plan (draft)

Safety specification		
Important identified risks	Important potential risks	Important missing information
· Hepatic dysfunction · QT prolongation	None	None
Efficacy specification		
None		

Table 54. Summary of additional pharmacovigilance activities, efficacy survey and studies, and additional risk minimization activities included under the risk management plan (draft)

Additional pharmacovigilance activities	Efficacy survey and studies	Additional risk minimization activities
· Early post-marketing phase vigilance · General use-results survey (all-case surveillance) · Post-marketing clinical study[a)]	None	· Develop information materials to be distributed to patients · Disseminate data gathered during early post-marketing phase vigilance

a) After approval of berotralstat, the ongoing Study 301 will be reclassified as a post-marketing clinical study and continued until the delivery of berotralstat to medical institutions.

The applicant accepted PMDA's conclusion and explained as follows:

The package insert will advise of the risk of QT interval prolongation associated with berotralstat, and safety measures will be taken. A general use-results survey will be conducted covering all patients treated with berotralstat, until data from a certain number of patients (target sample size, 80 patients) are collected, in order to assess the safety of berotralstat in clinical use, as outlined in Table 55.

Table 55. Outline of general use-results survey (draft)

Objective	To collect berotralstat safety and efficacy data in clinical use.
Survey method	All-case surveillance
Population	Patients with HAE
Observation period	1 year
Planned sample size	80 patients (for the safety analysis population)
Main survey items	· Safety specification: hepatic dysfunction, QT prolongation · Patient characteristics (age, sex, body weight, disease duration, prior therapies, complications/medical history, etc.) · Use of berotralstat · Concomitant medications · HAE attack events · Clinical laboratory values · Adverse events · Efficacy

2. Overall Evaluation

As a result of the above review, PMDA has concluded that the product may be approved for the proposed indication modified as below, with the following conditions. As the product has been designated as an orphan drug, the re-examination period is 10 years. The product is not classified as a biological product or a specified biological product. The drug product is not classified as a poisonous drug or a powerful drug, and its drug substance is classified as a powerful drug.

Indication

Prevention of acute attacks of hereditary angioedema

(Underline denotes an addition to the proposed indication.)

Dosage and Administration

The usual dosage for adult and pediatric patients 12 years of age or older is 150 mg berotralstat (1 capsule) taken orally once daily.

Approval Conditions

1. The applicant is required to develop and appropriately implement a risk management plan.
2. Because of extremely limited number of patients participating in clinical studies in Japan, the applicant is required to conduct a post-marketing use-results survey covering all patients treated with the product, until data from a certain number of patients are collected, in order to identify the characteristics of patients treated with the product and obtain safety and efficacy data promptly, and thereby to take necessary measures to ensure the proper use of the product.

List of Abbreviations

A/G ratio	Albumin/globulin ratio
ALT	Alanine aminotransferase
APD	Action potential duration
APTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
AUC_{tau}	Area under the concentration vs. time curve from time 0 to the end of the dosing interval
$AUC_{0\text{-}inf}$	Area under the concentration vs. time curve from time 0 extrapolated to infinite time
$AUC_{0\text{-}last}$	Area under the concentration vs. time curve from time 0 to the last measurable time point
$AUC_{0\text{-}t}$	Area under the concentration vs. time curve from time 0 to t hours
BCRP	Breast cancer resistance protein
berotralstat	Berotralstat Hydrochloride
BMI	Body mass index
BMP	Di 22:6 bis(monoacylglycerol) phosphate
BSEP	Bile salt export pump
CHO	Chinese hamster ovary
CI	Confidence interval
CL	Clearance
CL/F	Apparent clearance
CL_{CR}	Creatinine clearance
CLr	Renal clearance
C_{max}	Maximum observed concentration
CPCA	Cyclopropylcarboxylic acid
CYP	Cytochrome P-450
C1-INH	Complement 1 esterase inhibitor
DMSO	Dimethyl sulfoxide
EC_{50}	Half-maximal effect dose
eGFR	Estimated glomerular filtration rate
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
E_{max}	Maximum efficacy
FAS	Full analysis set
FGF-2	Fibroblast growth factor-2
GC	Gas chromatography
GGT	Gamma-glutamyl transferase
HAE	Hereditary angioedema
hCav1.2	Cloned human L-type calcium channel 1.2 expressed in CHO cells
HEK	Human embryonic kidney
hERG	Human ether-à-go-go related gene
HLGT	High level group terms
hNav1.5	Cloned human sodium channel 1.5 expressed in CHO cells
HPLC	High performance liquid chromatography
IC_{50}	Half maximal inhibitory concentration
iPS	Induced pluripotent stem cell
IR	Infrared absorption spectrum
ITT	Intent-to-treat
*K*i	Inhibition constant
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LLN	Lower limit of normal

MATE	Multidrug and toxin extrusion
MCH	Mean corpuscular hemoglobin
MCV	Mean corpuscular volume
MRP	multi-drug resistance protein
MS	Mass spectrum
NADPH	Nicotinamide adenine dinucleotide phosphate-oxidase
NMR	Nuclear magnetic resonance spectrum
OAT	Organic anion transporter
OATP	Organic anion transporting polypeptide
OC	Observed case
OCT	Organic cation transporter
P-gp	P-glycoprotein
pKal	Plasma kallikrein
PMDA	Pharmaceuticals and Medical Devices Agency
PT	Prothrombin time
PTP	Press Through Packaging
QbD	Quality by Design
QOL	Quality of life
QTc	corrected QT interval
QTcF	QT interval corrected using Fridericia's method
RH	Relative humidity
SD	Sprague Dawley
Study XXX	Study BCX7353-XXX
T_{max}	Time to C_{max}
$t_{1/2}$	Half-life
the product	Orladeyo Capsules 150 mg
ULN	Upper limit of normal
VEGF	Vascular endothelial growth factor
V_z/F	Apparent volume of distribution of the drug