Report on the Deliberation Results

May 26, 2020

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

Brand Name	Mayzent Tablets 0.25 mg Mayzent Tablets 2 mg
Non-proprietary Name	Siponimod Fumaric Acid (JAN*)
Applicant	Novartis Pharma K.K.
Date of Application	January 7, 2019

Results of Deliberation

In its meeting held on May 21, 2020, the First 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 and its drug substance are both classified as powerful drugs.

Approval Conditions

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

2. Because of the extremely limited number of subjects participating in Japanese clinical studies, the applicant is required to conduct a drug use-results survey involving all patients treated with the product after the market launch until data from a certain number of patients have been gathered, in order to understand the characteristics of patients using the product, and to promptly collect safety and efficacy data so that necessary measures are taken to ensure proper use of the product.

**Japanese Accepted Name (modified INN)*

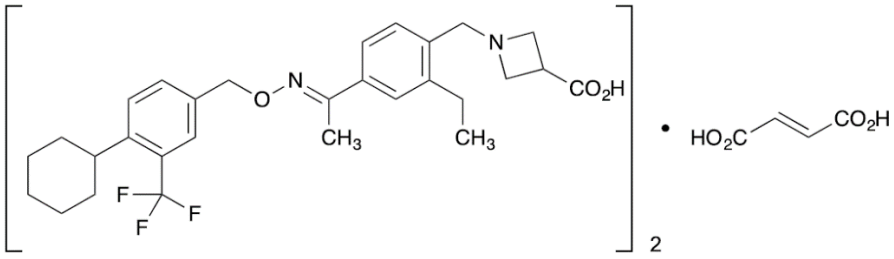
Review Report

April 15, 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	Mayzent Tablets 0.25 mg Mayzent Tablets 2 mg
Non-proprietary Name	Siponimod Fumaric Acid
Applicant	Novartis Pharma K.K.
Date of Application	January 7, 2019
Dosage Form/Strength	Each 0.25 mg tablet contains 0.278 mg of Siponimod Fumaric Acid (0.25 mg of Siponimod). Each 2 mg tablet contains 2.224 mg of Siponimod Fumaric Acid (2 mg of Siponimod).
Application Classification	Prescription drug, (1) Drug with a new active ingredient

Chemical Structure



Molecular formula: $(C_{29}H_{35}F_3N_2O_3)_2 \bullet C_4H_4O_4$

Molecular weight: 1149.26

Chemical name: 1-({4-[(1*E*)-1-({[4-Cyclohexyl-3-(trifluoromethyl)phenyl]methoxy}imino)ethyl]-2-ethylphenyl}methyl)azetidine-3-carboxylic acid hemifumaric acid

Items Warranting Special Mention

Orphan drug (Orphan Drug Designation No. 423 of 2018 [*30 yaku*]; PSEHB/PED Notification No. 1206-1 dated December 6, 2018, by the Pharmaceutical Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare)

Reviewing Office Office of New Drug III

Results of Review

On the basis of the data submitted, PMDA has concluded that the product has efficacy in preventing relapsing secondary progressive multiple sclerosis and in delaying the progression of associated physical disability, 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.

Indication Prevention of relapsing secondary progressive multiple sclerosis and delay in the progression of associated physical disability

Dosage and Administration The usual adult dosage of siponimod is 0.25 mg on Day 1 and Day 2, 0.5 mg on Day 3, 0.75 mg on Day 4, 1.25 mg on Day 5, 2 mg on Day 6, administered orally once daily every morning. From Day 7 onward, 2 mg, the maintenance dose, is administered once daily. The maintenance dose may be reduced according to the patient's condition.

Approval Conditions

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

2. Because of the extremely limited number of subjects participating in Japanese clinical studies, the applicant is required to conduct a drug use-results survey involving all patients treated with the product after the market launch until data from a certain number of patients have been gathered, in order to understand the characteristics of patients using the product, and to promptly collect safety and efficacy data so that necessary measures are taken to ensure proper use of the product.

Attachment

Review Report (1)

March 11, 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	Mayzent Tablets 0.25 mg Mayzent Tablets 2 mg
Non-proprietary Name	Siponimod Fumaric Acid
Applicant	Novartis Pharma K.K.
Date of Application	January 7, 2019
Dosage Form/Strength	Each 0.25 mg tablet contains 0.278 mg of Siponimod Fumaric Acid (0.25 mg of Siponimod). Each 2 mg tablet contains 2.224 mg of Siponimod Fumaric Acid (2 mg of Siponimod).
Proposed Indication	Prevention of relapsing secondary progressive multiple sclerosis and delay in the progression of associated physical disability

Proposed Dosage and Administration

The usual starting dosage for adults is 0.25 mg of siponimod administered orally once daily, followed by gradual dose increase up to the maintenance dose according to the table below (dose-escalation phase).

The maintenance dose is 2 mg administered orally once daily (maintenance phase).

Siponimod should be administered in the morning during the first 6 days of the dose-escalation.

	Dose-escalation phase					Maintenance phase
	Day 1	Day 2	Day 3	Day 4	Day 5	From Day 6 onward
Dose	0.25 mg	0.25 mg	0.5 mg	0.75 mg	1.25 mg	2 mg (maintenance dose)

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List of Abbreviations

See Appendix.

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

Multiple sclerosis (MS) is an inflammatory demyelinating disease with multiple lesions in the central nervous system including brain, optic nerves, and spinal cord. It is considered to be caused by autoimmune response targeted at the myelin sheath and oligodendrocytes. MS is classified into the following 3 forms depending on its clinical course: Relapsing-remitting multiple sclerosis (RRMS) which repeats acute aggravation (relapse) and remission, secondary progressive multiple sclerosis (SPMS) which follows after a certain period of an initial RRMS course and is characterized by gradual progression of disorders over time, regardless of relapses, and primary progressive multiple sclerosis (PPMS) which progresses from the onset without acute aggravation (relapse) (*Clinical Practice Guidelines for Multiple Sclerosis and Neuromyelitis Optica 2017* [in Japanese]). SPMS progresses gradually over 15 to 20 years from the onset of MS, with gait disturbance as the cardinal disorder observed, coupled with various disorders depending on the site of the demyelinating lesion including visual acuity/field disorder, sensory disturbance, dysuria, and dyschezia, causing devastating effects on the activities of daily living with irreversible physical disability (*Drugs*. 2017;77:885-910).

In Japan, MS is designated as an intractable disease, and the number of Recipient Certificates issued for Specific Disease Treatment was 19,389 in FY 2014 (https://www.nanbyou.or.jp/entry/1356). In the US and Europe, RRMS accounts for 80% to 90%, and PPMS 10% to 20%, among Caucasian patients with MS, whereas in Japan, PPMS accounts for approximately 5% of patients with MS and approximately half of patients with RRMS progress to SPMS 15 to 20 years after the onset (*Clinical Practice Guidelines for Multiple Sclerosis and Neuromyelitis Optica 2017*). Siponimod fumaric acid (hereinafter referred to as "siponimod") was designated as an orphan drug on December 6, 2018 with the intended indication of "Prevention of relapsing secondary progressive multiple sclerosis and delay in the progression of associated physical disability" (Orphan Drug Designation No. 423 of 2018 [*30 yaku*], PSEHB/PED Notification No. 1206-1 dated December 6, 2018).

Siponimod is a novel sphingosine 1 phosphate (S1P) receptor modulator discovered by Novartis. It is considered to exhibit a treatment effect by decreasing lymphocyte count in peripheral blood by acting on the S1P receptor on the surface of lymphocytes, thereby inhibiting the infiltration of autoimmune reaction-involved lymphocytes into the central nervous system.

In foreign countries, clinical studies on siponimod were initiated in October 2006 and, in the US, siponimod was approved in March 2019 for the indications of "relapsing forms of MS, to include clinically isolated syndrome (CIS), RRMS, and active SPMS, in adults." In Europe, siponimod was approved in January 2020 for the indication of "adult patients with SPMS with active disease evidenced by relapses or imaging features of inflammatory activity."

In Japan, clinical studies on the proposed indication were initiated in December 2012. Recently, the applicant submitted the marketing application for siponimod with the claim that the efficacy and safety of siponimod in the treatment of SPMS have been demonstrated.

In Japan, the following drugs are approved for indication for MS: Interferon beta-1a (genetical recombination), interferon beta-1b (genetical recombination), fingolimod hydrochloride, natalizumab

(genetical recombination), glatiramer acetate, and dimethyl fumarate. However, these drugs were approved mainly based on the results of clinical studies in patients with RRMS, and there are no therapeutic agents that are recommended for the treatment of SPMS in the Japanese clinical practice guideline (*Clinical Practice Guidelines for Multiple Sclerosis and Neuromyelitis Optica 2017*).

In the global phase III study (Common technical document [CTD] 5.3.5.1-1, Study A2304), it had been planned to construct 3 databases for ensuring blinding and to grant different database access rights depending on the tasks of study participants. However, because of a report of inadequate control of the access rights, PMDA conducted detailed reviews during the process of the document-based GLP/GCP inspections, which took time [see Sections 7.R.2 and 7.R.8] and resulted in the prolongation of the total review period.

The proposed Japanese brand name of Mayzent was *Meizento*. Since this name was confusingly similar to *Meiserin* (Meicelin for injection 1 g), an approved product, PMDA instructed the applicant to change the name from the point of view of risk management. In response, the applicant proposed to change the Japanese name to *Mehzento*, and PMDA accepted the proposal. This does not affect the English brand name.

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 powder. The following general properties of the drug substance have been determined: description, solubility, pH, differential scanning calorimetry, dissociation constant, distribution coefficient, and hygroscopicity. The drug substance exists in 10 different crystal forms (4 types of [redacted], 2 types of [redacted], and 4 types of [redacted]), but it is confirmed that anhydride (type A), the stable crystalline form, is obtained by the commercial manufacturing process. There is no optical isomer. [redacted]form is present as [redacted] isomer. [redacted] stable [redacted]form is isolated in the commercial manufacture process, and [redacted]form is controlled as [redacted] by tests for [redacted] and [redacted].

The chemical structure of the drug substance has been elucidated by elemental analysis, mass spectrometry, ultraviolet spectrum (UV), infrared absorption spectrum (IR), nuclear magnetic resonance spectrum (NMR) (^{1}H-NMR and ^{13}C-NMR), X-ray powder diffractometry, and single crystal X-ray diffractometry.

2.1.2 Manufacturing process

The drug substance is synthesized using [redacted], [redacted], and [redacted] as the starting materials. The process of [redacted] synthesis is defined as the critical step. [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], and [redacted] are controlled as critical intermediates.

2.1.3 Control of drug substance

The proposed specifications for the drug substance include content, description, identification (IR, X-ray powder diffractometry), purity ([redacted] [redacted]), related substances

[high performance liquid chromatography (HPLC)], residual solvents [gas chromatography (GC)], [redacted] and [redacted] [redacted], and [redacted]), water content, residue on ignition, microbial limit, [redacted], [redacted], and assay (HPLC).

[redacted] during the review process.

2.1.4 Stability of drug substance

Table 1 shows the main stability studies of the drug substance. Photostability testing showed that the drug substance was photostable.

Table 1. Stability studies of drug substance

Study	Primary batches	Temperature	Humidity	Storage package	Storage period
Long-term testing	3 pilot batches	30°C	75% RH	Polyethylene bag + [redacted]/[redacted]/aluminum/[redacted] bag	18 months
Accelerated testing	3 pilot batches	40°C	75% RH		6 months

Based on the above, a retest period of 30 months has been proposed for the drug substance when stored at room temperature in the polyethylene bag placed in the [redacted]/[redacted]/aluminum/[redacted] bag, according to Guideline on the Evaluation of Stability Data (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use [ICH] Q1E Guideline). Long-term testing will be continued for up to 60 months.

2.2 Drug product

2.2.1 Description and composition of drug product and formulation development

The drug product is immediate-release tablets, each containing 0.278 or 2.224 mg of the drug substance (0.25 or 2 mg of siponimod). The drug product contains excipients: lactose, cellulose, crospovidone, glyceryl behenate, anhydrous silicic acid, [redacted], [redacted], [redacted] (in 0.25 mg tablets only) and [redacted] (in 2 mg tablets only).

2.2.2 Manufacturing process

The drug product is manufactured through a process comprising mixing, sieving, final mixing, tableting, coating, packaging/labeling, and storage/inspection. [redacted] and [redacted] are specified as critical steps. In-process control have been established for [redacted] and [redacted].

2.2.3 Control of drug product

The proposed specifications for the drug product include content, description, identification (ultraviolet-visible spectrophotometry, HPLC), purity (related substances [HPLC]), water content, uniformity of dosage unit (content uniformity [HPLC]), dissolution, and assay (HPLC).

Identification (HPLC) and [redacted] during the review process.

2.2.4 Stability of drug product

Table 2 shows the main stability studies of the drug product. Photostability testing showed that the drug product is photostable.

Table 2. Stability studies of drug product

Study	Primary batches	Temperature	Humidity	Storage package	Storage period
Long-term testing	3 pilot batches	5°C	-	Blister-package consisting of polyamide/aluminum/polyvinyl chloride and aluminum foil	24 months
Accelerated testing	3 pilot batches	25°C	60% RH		24 months

Based on the above, the shelf life of 24 months has been proposed for the drug product when blister-packaged in polyamide/aluminum/polyvinyl chloride and aluminum foil and stored at 2°C to 8°C. Long-term testing will be continued for up to 36 months.

2.R Outline of the review conducted by PMDA

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

2.R.1 Strategy for controlling the content of related substances

Accelerated testing of the drug product (25°C/60% relative humidity [RH]) showed an increase in related substances (degradation products 026-09, 027-09, 010-10, and total) and a decrease in siponimod content. PMDA asked the applicant to explain the cause(s) of these changes and the effect of the changes on the quality of the drug product.

The applicant's explanation:

- 027-09 and 010-10 are produced when the temperature is increased in the presence of [redacted]. 026-09 is produced in the presence of [redacted]. Since [redacted] is used as an excipient of the drug product, [redacted] is present in the drug product. The increase in these related substances and the decrease in siponimod content tend to become evident when the drug product is stored for a long time under the storage conditions of [redacted]°C/[redacted]% RH, [redacted]°C/[redacted]% RH, and [redacted]°C/[redacted]% RH.

- The increase in the related substances and the decrease in siponimod content are considered to be caused by temperature and [redacted]. To ensure the quality of the drug product, acceptance criteria for [redacted] are included in the specifications of the drug product, and the drug product should be stored at 2°C to 8°C in an aluminum blister package,

PMDA accepted the above applicant's explanation.

2.R.2 Bioequivalence of drug product for clinical studies and the proposed commercial formulation

In the dissolution test[1)] to evaluate the equivalency of the formulation (final market image [FMI] formulation) used in the global phase III study (CTD 5.3.5.1-1, Study A2304) and the proposed commercial formulation, dissolution rate in the latter 6 vessels tended to be lower than that in the first 6 vessels of the total 12 vessels tested. PMDA therefore asked the applicant to explain the appropriateness of evaluating the results obtained from all 12 vessels collectively, and to explain the bioequivalence between FMI formulation and the proposed commercial formulation.

The applicant's explanation:

- The dissolution test was conducted in accordance with "Guideline for Bioequivalence Studies for Formulation Changes of Oral Solid Dosage Forms" (PMSB/ELD Notification No. 67 dated February 14, 2000, partially modified by PFSB/ELD Notification No. 0229-10 dated February 29, 2012) and "Guideline for Bioequivalence Studies of Generic Products for Different Strength of Oral Solid Dosage Forms" (PMSB/ELD Notification No. 64 dated February 14, 2000, partially revised by PFSB/ELD Notification No. 0229-10 dated February 29, 2012). For both of FMI formulation and the proposed commercial formulation, the drug substance [redacted] in the dissolution medium without polysorbate 80. When the same liquid chromatography system is used for measurement, it takes about [redacted] hours to measure all samples. This time lapse probably resulted in a relatively lower dissolution rate in the latter 6 vessels because [redacted] had progressed before the measurement. A dissolution test (6 vessels) was conducted on the proposed commercial formulation (2 mg) using (1) dissolution media without polysorbate 80 (pH 1.2, 4.0, and 6.8) or (2) water. The solutions were sampled at [redacted] minutes after the start of the test, and the samples were measured [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], and [redacted] hours after the sampling. The measured values tended to decrease with the increase in the waiting time. There was only a slight difference in the measured value between the first and latter half of vessels with the dissolution media [redacted], whereas a marked difference was observed in the measured value between the first and latter half with the dissolution media [redacted].

- With the dissolution media (pH 1.2, 4.0, and 6.8) containing the surfactant polysorbate 80, the solution after dissolution did not become [redacted], showing no difference in the measured value, irrespective of the order of measurement. FMI formulation and the proposed commercial formulation were similar in the dissolution profile.

- The determination that FMI formulation and the proposed commercial formulation are biologically equivalent remains unchanged, as evidenced by the following: (1) Both FMI formulation and the proposed commercial formulation were subjected to the dissolution test using the same testing method including the order of measurement, and the variations in measured values were observed to a similar extent, allowing the comparison of mean values, (2) large variations in individual values are considered to be worst cases, and (3) similar results were observed in dissolution tests using dissolution media with polysorbate 80.

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1) The test was conducted to compare dissolution between FMI formulations (0.25, 0.5, and 1 mg tablets) and the proposed commercial formulation (0.25 mg tablets) and between FMI formulation (2 mg) and the proposed commercial formulation (2 mg tablets).

PMDA's view:

- Since [redacted] in the vessels that took a longer time for measurement in the dissolution test, it is unclear whether the measurement was accurate. It is inappropriate to evaluate the measured values of all vessels collectively.

- However, the evaluation based on the results of the above test is acceptable, for the following reasons:
  (1) Given the characteristics of the drug substance, the applicant's explanation (i.e., in the dissolution media without polysorbate 80, [redacted], resulting in lower measured values of the latter half of the vessels) is understandable.
  (2) In the presence of polysorbate 80 (pH 1.2, 4.0, and 6.8), there was no difference in the measured value, irrespective of the order of measurement, and FMI formulation and the proposed commercial formulation showed similar dissolution profiles.

- The bioequivalence between the proposed commercial formulations with different strengths is discussed in Section 6.R.1.

2.R.3 New excipient

The drug product contains glyceryl behenate as a new excipient.

The same specifications and testing methods as specified by the Japan's Specifications and Standards for Food Additives (glycerin fatty acid ester) are applied for glyceryl behenate, based on which PMDA concluded that there is no particular problem in the specifications or the safety. PMDA also concluded that there is no safety problem related to the dose used in the drug product, judging from the data submitted.

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

The applicant submitted results of primary pharmacodynamics studies, secondary pharmacodynamic studies, and safety pharmacology studies as data of the nonclinical pharmacological studies. In some of the studies, the main metabolites of siponimod, M3 (glucuronate conjugate of hydroxylate form), M5 (hydroxylated form), and M17 (cholesterol ester form) were also subjected to investigation. Only the main study data are described below. Unless specified otherwise, the amount of siponimod is expressed in terms of free base, and values are expressed in mean or mean ± standard error.

3.1 Primary pharmacodynamics

3.1.1 *In vitro* studies

3.1.1.1 Agonist activity of siponimod and main metabolites for S1P receptor (CTD 4.2.1.1-1 to 4.2.1.1-3)

Using membrane preparations of Chinese hamster ovary (CHO) cells expressing various human S1P receptor subtypes ($S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$), the agonist activity of siponimod was investigated by guanosine ^{35}S-[gamma-thio] triphosphate (GTPγS[^{35}S]) binding test.[2)] Effective concentration, 50% (EC_{50}) for $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$ was 0.39 ± 0.07, >750, >750, 750 ± 487, and 0.98 ± 0.43

2) The binding test was conducted 7 times (3 times for $S1P_2$). The concentration of the test compounds was 1.0×10^{-4} to 1.0×10^{4} nmol/L.

nmol/L, respectively (an EC_{50} value exceeding 750 nmol/L could not be calculated, and is expressed as >750 nmol/L). EC_{50} of main metabolites M3, M5, and M17 for $S1P_1$ was >750, 470 ± 91, and 341 ± 86 nmol/L, respectively. EC_{50} of M17 for $S1P_5$ was 159 ± 9 nmol/L.

3.1.1.2 Activity to induce S1P receptor internalization

Using CHO cells engineered to express the human $S1P_1$ receptor, the number of cells expressing the $S1P_1$ receptor on the cell surface was counted by flow cytometry after treatment with siponimod, fingolimod phosphate, or S1P (all 1 μmol/L). The cell count decreased by 95%, 87%, and 56%, respectively, compared with the count in untreated cells (CTD 4.2.1.1-4).

Using CHO cells engineered to express the human $S1P_5$ receptor, the number of cells expressing the $S1P_5$ receptor on the cell surface was counted by flow cytometry after treatment with siponimod (1 μmol/L). The cell count in the treated cells was comparable to that in the untreated cells (CTD 4.3-3, *Neurology.* 2018;90 Suppl 15:P3.404).

3.1.2 *In vivo* studies

3.1.2.1 Activity to decrease lymphocyte count in blood

A single dose of siponimod (0.03, 0.1, 0.3, or 1.0 mg/kg) or fingolimod (0.01, 0.03, 0.1, 0.3, or 1.0 mg/kg) was administered orally to rats, and lymphocyte counts in blood were measured at 6 hours after administration. Both in the siponimod and fingolimod groups, lymphocyte count in blood decreased in a dose-dependent manner (effective dose, 50% [ED_{50}], 0.14 ± 0.03 mg/kg for siponimod, 0.097 ± 0.01 mg/kg for fingolimod). At 48 hours after administration, the lymphocyte count in blood returned to a level similar to that before administration in the siponimod group, whereas in the fingolimod group, the level was similar to that observed at 6 hours after administration (CTD 4.2.1.1-5).

A single dose of siponimod (0.03, 0.1, 0.3, 1.0, or 3.0 mg/kg) was administered orally to monkeys, and lymphocyte counts in blood were measured at 0.5, 1, 2, 4, 7, 24, 48, and 72 hours and at 7, 8, 9, and 10 days after administration. The lymphocyte count in blood decreased in a dose-dependent manner within 4 to 7 hours (approximately 50%-80% of baseline) and returned to baseline during the period from 72 hours to 7 days after administration. (CTD 4.2.2.2-5)

3.1.2.2 Effect on neurological symptoms and nerve lesions of experimental autoimmune encephalomyelitis (EAE) model

Rats were immunized by subcutaneous injection, into tail root, of cerebrospinal extract of syngeneic rats and bovine spinal cord together with complete Freund's adjuvant (CFA), and siponimod (0.03, 0.3, 3, or 10 mg/kg/day) or fingolimod (0.3 mg/kg/day) was administered orally once daily for 24 days starting from 11 days after the immunization, and the treatment effect of siponimod on chronic experimental autoimmune encephalomyelitis (EAE)[3)] was investigated. In both siponimod and fingolimod groups, the

[3)] Nerve lesions similar to those in SPMS, such as axon degeneration and microgliosis, are observed.

EAE score[4] had reached the maximum level (2.8 ± 0.2) within 11 days after immunization which was the starting day of administration. In the siponimod group, the EAE score started to decrease after administration in a dose-dependent manner and, in the ≥0.3 mg/kg groups, decreased continuously to a level similar to that observed in the fingolimod group (0.2 ± 0.1 at 25 days after immunization). Neither demyelination nor microglia infiltration/activation was observed in the spinal cord in the siponimod and fingolimod groups (CTD 4.2.1.1-7).

Mice were immunized by subcutaneous injection, into the back, of myelin oligodendrocyte glycoprotein (MOG) of syngeneic mice together with CFA, and siponimod (3 mg/kg/day) was administered orally once daily from the time point when the EAE score[4] reached 2.5 until the end of the study (Day 25-27 after the start of administration), and the treatment effect of siponimod on chronic EAE[3] was investigated. The EAE score decreased over time after administration of siponimod (1.0 ± 0.3 on Day 26 after the start of administration). After the end of the study, the spinal cord was subjected to (1) hematoxylin-eosin staining, (2) immunohistochemical staining by rabbit polyclonal antibody against microglia-specific calcium binding protein (staining of microglia), and (3) solochrome cyanine R staining (staining of myelin), and the lesion score was calculated. Results showed that siponimod decreased the lesion score[5] (CTD 4.2.1.1-9).

Siponimod (0.225, 0.45, or 4.5 μg/day) was administered into the cerebral ventricle of mice continuously for 4 weeks and, 7 days after the start of administration, MOG_{33-35} was administered subcutaneously together with CFA and *Mycobacterium tuberculosis* to induce EAE, and the nerve-protective effect of siponimod was investigated. In the siponimod 0.225 μg/day and 0.45 μg/day groups, the EAE score[4] increased from Day 13 after EAE induction, whereas in the siponimod 4.5 μg/day group, no increase in the EAE score was observed; instead, cluster of differentiation (CD)3-positive lymphocyte count decreased on Day 18 after EAE induction. Synapse current was measured in striatal slices isolated from the mice in the siponimod 0.45 μg/day group on Day 20 to 24 after EAE induction. Results showed increased frequency of GABAergic inhibitory synaptic current and decreased gamma-aminobutyric acid (GABA) neurons. Using the same mouse striatal preparations, the following measurements were performed: Astrocytes by glial fibrillary acidic protein (GFAP) staining, microglia by ionized calcium-binding adaptor molecule (IBA)1 staining, blood lymphocytes by CD3 staining, GFAP protein by Western blotting, and IBAa mRNA and CD3 mRNA expression levels by real-time PCR. Results showed decreased GFAP protein, decreased IBA1 mRNA expression level, and suppression of increase in CD3 mRNA expression level, which suggested suppression of astrogliosis and microgliosis together with reduced infiltration of peripheral lymphocytes (CTD 4.3-21, *J Neuroinflammation*. 2016;13:207).

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[4] Clinical symptoms of EAE were evaluated according to the following scores:
CTD 4.2.1.1-7 (6 grades): 0 = no abnormality, 1 = drooping tail, 2 = hindlimb weakness or gait ataxia, 3 = hindlimb paralysis, 4 = moribund state accompanied by complete paralysis of fore- and hindlimbs, and 5 = death
CTD 4.2.1.1-9 (10 grades): 0 = no abnormality, 0.5 = partial drooping of tail, 1 = complete drooping of tail, 1.5 = hindlimb weakness, 2 = partial paralysis of a unilateral hindlimb, 2.5 = partial paralysis of both hindlimbs, 3 = complete paralysis of both hindlimbs, 3.5 = weakness of forelimbs and complete paralysis of both hindlimbs, 4 = quadriplegia or moribund state, and 5 = death
CTD 4.3-21: *J Neuroinflammation*. 2016;13:207 (11 grades): 0 = no clinical signs, 1 = flaccid tail, 2 = hindlimb weakness, 3 = hindlimb paralysis, 4 = complete bilateral hindlimb paralysis, and 5 = death due to EAE; intermediate nerve signs were scored by adding 0.5.

[5] The extent of inflammatory cell infiltration, microglia infiltration and activation, and demyelinating changes on each lesion site was evaluated according to the following 5-point scale: 0 = no change, 1 = mild, 2 = moderate, 3 = severe, and 4 = severest

3.2 Secondary pharmacodynamics

3.2.1 Mixed lymphocyte reaction and bone marrow cell growth inhibition

Using CD4-positive T cells isolated from mononuclear cells in human peripheral blood, a lymphocyte reaction test[6)] was conducted to evaluate the cell growth-inhibitory effect of siponimod. The half maximal (50%) inhibitory concentration (IC_{50}) of siponimod and cyclosporin A, the positive control, was 1.42 μmol/L and 0.012 μmol/L, respectively. Siponimod at ≥30 μmol/L induced apoptosis (CTD 4.2.1.2-1).

A similar test was conducted using mouse splenic cells. IC_{50} of siponimod and cyclosporin A, the positive control, was 8.2 μmol/L and 0.017 μmol/L, respectively (CTD 4.2.1.2-2).

The effect of siponimod on the growth of mouse bone marrow cells was investigated. Siponimod did not inhibit the cell growth (IC_{50}, >10 μmol/L) (CTD 4.2.1.2-3).

3.2.2 Affinity to receptors, ion channels, and enzymes (CTD 4.2.1.2-4 to 4.2.1.2-7)

The binding affinity of siponimod (0.001-10 μmol/L) to 98 types of receptors, transporters, ion channels, and enzymes was investigated. Siponimod showed the highest affinity to the histamine H_2 receptor (IC_{50}, 1.5 μmol/L). Siponimod showed affinity also to the adenosine A_3 receptor (IC_{50}, 4.8 μmol/L), dopamine D_1 receptor (IC_{50}, 4.4 μmol/L), dopamine D_3 receptor (IC_{50}, 3.8 μmol/L), and norepinephrine transporter (IC_{50}, 4.4 μmol/L) (CTD 4.2.1.2-4). Similar tests were conducted on siponimod metabolites M3, M16 (methylester form), and M17 (10-30 μmol/L). M3 did not show affinity to any of the receptors, transporters, ion channels, or enzymes tested, and M16 and M17 showed affinity only to the serotonin 5-HT_{2A} receptor (IC_{50}, 7.4 and 9.6 μmol/L, respectively) (CTD 4.2.1.2-5-7).

3.2.3 Effect on primary and secondary immune responses

The effect of siponimod on immune response to keyhole limpet hemocyanin (KLH) administration[7)] with dinitrophenyl as the hapten was investigated in mice. Siponimod inhibited T-cell-dependent, antigen-specific production of immunoglobulin (Ig)M and IgG antibodies (CTD 4.2.1.2-8).

The effect of siponimod on IgG antibody production after the initial inoculation with tetanus vaccine was investigated in monkeys. Siponimod inhibited antibody production after the initial vaccination but did not affect antibody production after revaccination (CTD 4.2.1.2-9).

Siponimod (0.003, 0.03, 0.3, or 3 mg/kg) was administered orally to mice for 5 days, followed by administration of trinitrophenylated lipopolysaccharide (LPS),[8)] and immune response was investigated. T cell-independent, antigen-specific production of IgM and IgG antibody was inhibited in the 3 mg/kg group but not in lower dose groups (CTD 4.2.1.2-10).

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6) A 1:1 mixture of peripheral blood mononuclear cell samples isolated from 2 human donors was cultured, CD4-positive T cells were isolated using microbeads, cultured for 6 days in the presence of siponimod or cyclosporine (positive control, IC_{50}, 0.012 μmol/L), and ^{3}H-TdR uptake activity was measured. For the measurement of apoptosis, CD4-positive T cells were cultured for 16 hours in the presence of siponimod or fingolimod, and phosphatidylserine-expressing cells were counted by flow cytometry.

7) Induction of T cell-dependent response

8) Induction of T cell-independent response

3.2.4 Effect on vascular endothelial and epithelial barrier functions

A single dose of siponimod (0.03, 0.1, 0.3, 1, 3, 10, or 30 mg/kg) was administered to mice, followed by intravenous administration of Evans blue, and the effect of siponimod on the endothelial/epithelial barrier function was investigated using extravascular leakage of the pigment in the lung as the index. Siponimod caused dose-dependent extravascular leakage of the pigment (CTD 4.2.1.2-11).

Siponimod (30 mg/kg/day) was administered for 4 weeks to mice, followed by auricular transplantation of vascular endothelial growth factor (VEGF)-containing beads and, after 2 days, Evans blue was administered intravenously, and the effect of siponimod on the endothelial/epithelial barrier function was investigated using the extravascular leakage of the pigment in the auricle as the index. Siponimod inhibited VEGF-induced extravascular leakage of the pigment (CTD 4.2.1.2-12).

Siponimod (30 mg/kg/day) was administered orally to rats for 70 days, and the effect of siponimod on the endothelial/epithelial barrier function was investigated by magnetic resonance imaging (MRI) using the extravascular leakage of the contrast agent as the index. Siponimod did not affect the extravascular leakage of the contrast agent (CTD 4.2.1.2-13).

3.3 Safety pharmacology

Tables 3 to 5 show the outline of the results of safety pharmacology studies.

Table 3. Outline of the results of safety pharmacology study (central nervous system)

Test system	Evaluation item/method, etc.	Dose	Route of administration	Findings	CTD
Wistar rats (10 males/group)	Clinical signs	100, 200 mg/kg	p.o.	No effect	4.2.1.3-18

Table 4. Outline of the results of safety pharmacology studies (cardiovascular system)

Test system	Evaluation item/method, etc.	Dose	Route of administration	Findings	CTD
HEK293 cells (5 specimens for siponimod)	hERG current	Siponimod: 10, 25 μmol/L E-4031[a]: 1 μmol/L	*In vitro*	Inhibition rate: Siponimod 25 μmol/L 9%, E-4031 97.2%	4.2.1.3-2
Isolated rabbit heart (3 specimens)	Action current	0.1, 0.3, 1, 3, 10 μmol/L	*In vitro*	No effect	Reference 4.2.1.3-3
Isolated rabbit heart (6 specimens)	Cardiac cycle length, coronary blood flow volume	0.1, 0.3, 1, 3, 10 μmol/L	*In vitro*	≥3 μmol/L: Cardiac cycle length increased	Reference 4.2.1.3-4
Isolated rabbit blood vessels (3 specimens each of aorta and coronary artery)	Vascular contraction	0.5 μmol/L to 100 mmol/L	*In vitro*	No effect	Reference 4.2.1.3-5
Isolated guinea pig atrial myocytes (4 specimens)	GIRK current	0.1, 1, 3, 10 μmol/L	*In vitro*	Activation rate[b]: 3.2%, 22.9%, 56.4%, 61.7%	Reference 4.2.1.3-6
Isolated human myocytes (4 specimens)	GIRK current	0.01, 0.1, 1, 10, 100, 1000 nmol/L	*In vitro*	Activation rate[c]: 0.3%, 6.4%, 19.4%, 38.6%, 76.7%, 80.1%, EC_{50}: 15.8 nmol/L	Reference 4.2.1.3-8
Wistar rats (2 males/group)	Electrocardiogram	10 mg/kg	i.v.	10 mg/kg: Transient decrease in heart rate, isorhythmic atrioventricular dissociation, ventricular extrasystole	Reference 4.2.1.3-9
Anesthetized guinea pigs (2 or 4 males/group)	Blood pressure, electrocardiogram	0.0003, 0.03, 0.3, 1 mg/kg	i.v.	≥0.03 mg/kg: Decreased blood pressure (systolic, diastolic, mean arterial pressure), decreased heart rate, atrioventricular dissociation, atrioventricular block second degree	Reference 4.2.1.3-10

Test system	Evaluation item/method, etc.	Dose	Route of administration	Findings	CTD
Guinea pigs (4 males/group)	Blood pressure, electrocardiogram, body temperature	0.03, 0.3, 1.0 mg/kg	p.o.	≥0.03 mg/kg: Decreased blood pressure (systolic, diastolic, mean arterial pressure), pulse pressure decreased, heart rate decreased, PR interval prolonged, QTcB interval decreased, atrioventricular block second degree, RR interval prolonged	Reference 4.2.1.3-11
Guinea pigs (4 males/group)	Blood pressure, electrocardiogram	Siponimod 0.3 mg/kg and atropine[d)]	Siponimod: p.o. Atropine: s.c.	Atropine: Heart rate increased, PR interval decreased Siponimod: Decreased blood pressure (systolic, diastolic, mean arterial pressure), heart rate decreased, QTcB interval decreased, atrioventricular block second degree Atropine 60 minutes after administration of siponimod: No change in the effect of siponimod Atropine before and after administration of siponimod: Effect of siponimod on heart rate, QTcB interval, and atrioventricular block disappeared.	Reference 4.2.1.3-12
Guinea pigs (4 animals/group)	Blood pressure, electrocardiogram, body temperature	Siponimod 0.3 mg/kg/dose and atropine[e)]	Siponimod: p.o. Physiological saline and atropine: s.c.	Atropine: Heart rate increased, PR interval and QRS interval decreased Siponimod (first dose): Decreased blood pressure (systolic, diastolic, mean arterial pressure), heart rate decreased Atropine after administration of siponimod: No change in the effects of siponimod[f)]	Reference 4.2.1.3-14
NZW rabbits (2-4/sex/group)	Blood pressure, electrocardiogram	0.3, 1.0, 3.15[g)] mg/kg	i.v.	1.0 mg/kg: Heart rate decreased 3.15 mg/kg: Heart rate decreased (transient), arrhythmia, mean arterial pressure increased	Reference 4.2.1.3-15
Cynomolgus monkeys (1 or 2 males)	Electrocardiogram, body temperature	100 mg/kg on Day 2, 60 mg/kg on Day 8	p.o.	100 mg/kg: Heart rate decreased, QT interval prolonged, atrioventricular block second degree	Reference 4.2.1.3-16
Cynomolgus monkeys (4 males)	Blood pressure, electrocardiogram	Administered in the order of 50 and 150 mg/kg	p.o.	No effect	4.2.1.3-17

a) Human ether-a-go-go related gene (hERG) channel blocker
b) Activation rate is expressed as the percentage relative to G protein-coupled inwardly rectifying K+ channel (GIRK) current activation by acetylcholine (20 μmol/L).
c) Activation rate is expressed as the percentage relative to GIRK current activation by carbachol (20 μmol/L).
d) Three days after administration of atropine (0.1 mg/kg), siponimod and atropine (0.1 mg/kg at 60 minutes after administration of siponimod) were administered, followed by administration of siponimod and atropine (0.1 mg/kg/dose 15 minutes before and 30 minutes after administration of siponimod) after 4 days.
e) Atropine (0.5 mg/kg) on Day 1 or 2, atropine 0.5 mg/kg/dose (twice at 60-minute interval) on Day 8, siponimod on Day 11, siponimod and atropine 0.5 mg/kg/dose (90 and 150 minutes after administration of siponimod) on Day 15, and atropine 1.5 mg/kg/dose (3 times at 90-minute intervals) on Day 72.
f) One animal was added to substitute for an animal that showed aggravation of clinical signs before administration on Day 15. No marked change was observed in the heart rate of 3 animals receiving the second dose of siponimod on Day 15, whereas decreased heart rate was observed in 1 animal receiving the first dose of siponimod.
g) Cumulative administration of 0.45, 0.90, and 1.80 mg/kg at 45-minute intervals.

Table 5. Outline of the results of safety pharmacology studies (respiratory system)

Test system	Evaluation item/method, etc.	Dose	Route of administration	Findings	CTD
Wistar rats (6 males/group)	Tidal volume, respiratory rate, minute ventilation	100, 200 mg/kg	p.o.	No effect	4.2.1.3-19
Brown Norway rats (4 or 6 males/group)	Blood pressure, heart rate, airway resistance[a)]	30 mg/kg	p.o.	No effect	Reference 4.2.1.3-20
Wistar rats (7 or 8 males/group)	Airway resistance,[b)] histopathological examination of lung	30 mg/kg/day for 4 weeks	p.o.	Mild increase in airway resistance, methacholine-induced airway resistance increase enhanced	Reference 4.2.1.3-21

a) The airway was opened under anesthesia 3 hours after administration of siponimod, and airway resistance was measured after intravenous injection of serotonin, methacholine, and adenosine.
b) The airway was opened under anesthesia on Day 30 of the study, and airway resistance was measured after intravenous injection of serotonin, methacholine, and adenosine.

3.R Outline of the review conducted by PMDA

3.R.1 Mechanism of action of siponimod

PMDA asked the applicant to explain the mechanism of action of siponimod, based on the mechanism of development of MS.

The applicant's explanation:

- MS is an inflammatory demyelinating disease with multiple lesions in the central nervous system, and it is considered to be an autoimmune disease targeting the myelin sheath, etc. Patients with RRMS show infiltration of autoreactive lymphocytes into the central nervous system and accompanying inflammatory demyelination and breakdown of the blood-brain barrier, and progress to SPMS gradually over 15 to 20 years from the onset of RRMS. In the early phase of SPMS, the blood-brain has not been repaired and infiltration of autoreactive lymphocytes into the central nervous system and inflammatory demyelination are observed, whereas in advanced SPMS, the blood-brain barrier has been repaired and regained normal function but inflammation and nerve degeneration such as axonal degeneration and neurogliosis have advanced in the central nervous system (*Clinical Practice Guidelines for Multiple Sclerosis and Neuromyelitis Optica 2017*).

- Helper T cells 1 (Th1) and helper T cells 17 (Th17) play important roles in the formation of inflammatory demyelinating lesion observed in RRMS and in the early phase of SPMS. Autoreactive Th1/Th17 cells that have infiltrated in localized areas of the central nervous system are activated upon antigen presentation, mobilize microglia, macrophages, etc., by releasing inflammatory cytokines and chemokines, resulting in the formation of inflammatory demyelinating lesions (*Clinical Practice Guidelines for Multiple Sclerosis and Neuromyelitis Optica 2017*).

- The $S1P_1$ receptor is expressed mainly on lymphocytes and astrocytes. On lymphocytes, the $S1P_1$ receptor plays an important role in the emigration of lymphocytes from the secondary lymphoid tissue into the lymphatic vessels, and is thereby involved in the systemic circulation of lymphocytes. (*Science*. 2002;296:346-9, *Nature*. 2004;427:355-60, *Br J Pharmacol*. 2012;167:1035-47). In astrocytes, $S1P_1$ receptor stimulation is considered to inhibit cytokine secretion and activation (*J Neuroinflammation*. 2016;13:31). It is therefore expected that infiltration of autoreactive

lymphocytes into the central nervous system and resultant inflammation are suppressed by stimulation of the $S1P_1$ receptor on astrocytes.

- It has been shown that siponimod has the following activities: (1) To act as an agonist for the $S1P_1$ receptor and induce the internalization of the $S1P_1$ receptor [see Sections 3.1.1.1, 3.1.1.2], (2) to decrease blood lymphocyte count in rats and monkeys [see Section 3.1.2.1], and (3) following oral administration in chronic EAE models of rats and mice, to suppress the aggravation of neurological disorder, demyelination, and infiltration and activation of microglioma [see Section 3.1.2.2]. These findings suggest that siponimod, by its agonistic activity for the $S1P_1$ receptor on lymphocytes, internalizes the $S1P_1$ receptor and thereby acts as an antagonist functionally and, as a result, it inhibits the mobilization of autoreactive lymphocytes from lymph nodes and infiltration into the central nervous system, thereby suppressing the formation and progression of inflammatory demyelination lesions.

- Siponimod has also been shown to pass through the blood-brain barrier [see Section 4.2.1] and to have an agonistic activity for the $S1P_5$ receptor expressed mainly on cells of the central nervous system [see Sections 3.1.1.1 and 3.1.1.2]. Since stimulation of the $S1P_5$ receptor promotes the differentiation and survival of oligodendrocytes and myelination (*Mol Cell Biol*. 2005;25:11113-21, *J Neurosci.* 2005;25:1459-69, *Stem Cells*. 2007;25:115-24), siponimod appears to directly exhibit a nerve-protective effect in the central nervous system via the $S1P_5$ receptor, given its nerve-protective effect upon intracranial administration at the dose not affecting the peripheral lymphocyte count [see Section 3.1.2.2]. Siponimod is thus expected to suppress the progression of nerve degeneration within the central nervous system observed in SPMS.

PMDA asked the applicant to compare the pharmacological profile between siponimod and fingolimod chloride, a functional antagonist of the S1P receptor like siponimod, and to explain the possible difference in safety and efficacy.

The applicant's explanation:

- Fingolimod chloride is a prodrug. Within the body, it is metabolized by sphingosine kinase (Sph kinase) into fingolimod phosphate, the active metabolite with pharmacological activity. The expression level of Sph kinase is 2 to 4 times higher in organs such as lung, kidney, and spleen than in the brain (*J Biol Chem.* 2003;278:47408-15), and the therapeutic effect of fingolimod phosphate depends on the distribution of Sph kinase in tissues. In contrast, metabolism by enzymes is unnecessary for siponimod to exhibit its activity.

- Fingolimod phosphate, the active metabolite of fingolimod chloride, acts on $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$ receptors among S1P receptor subtypes (*J Med Chem*. 2005;48:5373-7), whereas siponimod exhibits higher selectivity to $S1P_1$ and $S1P_5$ receptors and a weaker effect on $S1P_3$ and $S1P_4$ than does fingolimod phosphate.

- The $S1P_3$ receptor, upon stimulation by fingolimod phosphate, is internalized temporarily (*Br J Pharmacol.* 2014;171:4797-807). The $S1P_3$ receptor is involved in the enhancement of COX-2 and

IL-6 secretion from astrocytes (*J Neuroinflammation.* 2017;14:111) and in macrophage activation and the bactericidal effect (*Am J Respir Crit Care Med*. 2017;196:1559-70). Also, it is reported that fingolimod phosphate acts on the $S1P_4$ receptor. Although the function of the $S1P_4$ receptor has not been fully elucidated, the receptor may possibly regulate the differentiation and activation of plasmacytoid dendritic cells, migration of neutrophils, and activation of macrophages (reference CTD 4.3-9, *Cell Microbiol.* 2018;20:e12836, reference CTD 4.3-47, *Mediators Inflamm*. 2017;6059203, *J Lipid Res.* 2014;55:1596-608). Since siponimod acts neither on the $S1P_3$ receptor nor on the $S1P_4$ receptor, it does not affect the induction of immune reaction by astrocyte and macrophage activation, nor does it induce temporary internalization of the $S1P_3$ receptor. Siponimod is thus unlikely to affect immune response and host defense against microbial infection.

PMDA considers that a certain level of explanation on the mechanism of action of siponimod is provided based on the currently available evidence.

3.R.2 Safety of siponimod

PMDA asked the applicant to explain the possible safety problems related to the cardiovascular and respiratory systems in humans, taking account of the effects on these systems observed in safety pharmacology studies of siponimod [see Section 3.3].

The applicant's explanation about the effects on the cardiovascular systems:

- Transient decrease in heart rate was observed in all animal species tested (rats, guinea pigs, rabbits, and cynomolgus monkeys), and atrioventricular dissociation and atrioventricular block second degree were observed in rats and guinea pigs. Judging from the observation that, in the safety pharmacology study using atrial myocytes of humans and guinea pigs (reference CTD 4.2.1.3-6 to 4.2.1.3-8), siponimod activated G protein-coupled inwardly rectifying K+ channel (GIRK) current, heart rate decrease is considered to be induced by the agonistic effect on the $S1P_1$ receptor expressed on atrial myocytes and succeeding GIRK channel activation, and disappears when the cells are desensitized by $S1P_1$ receptor internalization, as is the case with decreased heart rate observed during the early phase after administration of fingolimod chloride (reference CTD 4.3-12, *Am Heart J*. 168:632-644).

- In the safety pharmacology study investigating the effect of siponimod orally administered on the cardiovascular system of monkeys (reference CTD 4.2.1.3-16), decreased heart rate and changes related to abnormal atrioventricular conduction were observed in the 100 mg/kg group. C_{max} and $AUC_{0\text{-}24h}$[9)] of siponimod in monkeys at the no observed adverse effect level (NOAEL) (60 mg/kg) were 724 and 710 times, respectively, of those[10)] in humans receiving the clinical dose (2 mg/day), fully satisfying the safety margin.

9) Estimated from C_{max} (11000 ng/mL) and $AUC_{0\text{-}24h}$ (198000 ng·h/mL) of siponimod in plasma following a single oral administration of siponimod (30 mg/kg) in the study in monkeys (reference CTD 4.2.1.3-16).

10) Maximum C_{max} and $AUC_{0\text{-}24h}$ of siponimod (30.4 ng/mL and 558 ng·h/mL, respectively) in plasma under steady state in multiple oral administration of siponimod (2 mg) in the study evaluating QT/QTc in non-Japanese healthy adults (CTD 5.3.4.1-1, Study A2118).

The applicant's explanation about the effect on the respiratory system.

- In the safety pharmacology study investigating the effect of siponimod orally administered for 4 weeks on the respiratory system in rats (reference CTD 4.2.1.3-21), a mild increase in airway resistance and an enhancement of the airway resistance-enhancing effect of methacholine were observed in the 30 mg/kg/day group. S1P induces smooth muscle contraction leading to enhanced airway hyperreactivity in human smooth muscle cells and in guinea pigs (*FASEB J.* 2003;17:1789-99, *J Pharmacol Exp Ther*. 2007;320:766-73), which suggests that the above findings are mediated by the $S1P_1$ receptor and may be relevant to humans.

- Table 6 shows the incidence of adverse events related to respiratory function[11)] during double-blind phase of the global phase III study (CTD 5.3.5.1-1, Study A2304). There was no tendency of higher incidence of adverse events in the siponimod group than in the placebo group. Respiratory function tests showed that the mean change in forced expiratory volume in one second (FEV_1) from baseline was -0.1 L in the siponimod group and 0.0 L in the placebo group, both at 3 and 6 months after the start of administration, and that the mean change in carbon monoxide diffusing capacity from baseline to 3 and 6 months after the start of administration was -0.9 and -1.5 mL/min/mmHg, respectively, in the siponimod group and 0.3 and 0.1 mL/min/mmHg, respectively, in the placebo group, showing a mild decrease in FEV_1 and carbon monoxide diffusing capacity, changes probably related to bronchoconstriction.

Table 6. Incidence of adverse events related to respiratory function (double-blind phase of Study A2304, safety analysis population)

	Placebo	Siponimod
No. of subjects evaluated	546	1099
Adverse events related to respiratory function	73 (13.4)	119 (10.8)
Serious adverse events	1 (0.2)	2 (0.2)
Adverse event leading to treatment discontinuation	2 (0.4)	3 (0.3)

- The above results suggest that siponimod is unlikely to cause clinically significant respiratory problems. However, the package insert should include the caution statement that siponimod caused decrease in FEV_1 and in carbon monoxide diffusing capacity.

PMDA's view:

- For effect on cardiorespiratory system, safety in humans and appropriateness of the precaution given in the package insert are discussed further in Section 7.R.4.3.

- For effect on respiratory system, given the decrease in FEV_1 and in carbon monoxide diffusing capacity observed in the clinical studies, precautions should be included in the package insert, such as a request for a respiratory function test during the treatment with siponimod.

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

The applicant submitted the data on absorption, distribution, metabolism, and excretion of siponimod in mice, rats, rabbits, dogs, and monkeys, as nonclinical pharmacokinetics studies.

11) Events classified in "Respiratory, thoracic and mediastinal disorders" in Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class (SOC).

The concentrations of unchanged siponimod and metabolites in biological samples were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (lower limit of quantitation, 1.00-50.0 ng/mL). Radioactivity concentration in biological samples in studies using ^{14}C-labeled siponimod was measured by a liquid scintillation counter (lower limit of quantitation, 1.8 times the background) or by quantitative whole-body autoradiography (lower limit of quantitation, 3 times the detection limit). Main study results are described below. Unless specified otherwise, the amount of siponimod is expressed in terms of free base, the pharmacological parameter t_{max} in median, and other pharmacological parameters in mean or mean ± standard deviation (SD).

4.1 Absorption

4.1.1 Single-dose studies

4.1.1.1 Single-dose study in mice

Following a single oral administration of siponimod (8 mg/kg) to male mice (n = 3/time point/group) under fed conditions, C_{max} and $AUC_{0\text{-}168h}$ of unchanged siponimod in plasma was 2300 ng/mL and 64060 ng•h/mL, respectively (reference CTD 4.2.2.2-1).

4.1.1.2 Single-dose study in rats

A single dose of siponimod was administered intravenously (1 mg/kg) or orally (3 mg/kg) to male rats (n = 3/group) under fed conditions, $AUC_{0\text{-}\infty}$ of unchanged siponimod in plasma following a single administration was 2090 ± 133 ng•h/mL, and C_{max} and $AUC_{0\text{-}96h}$ of unchanged siponimod in plasma following a single oral administration were 235 ± 32.3 ng/mL and 3470 ± 630 ng•h/mL, respectively (reference CTD 4.2.2.2-3).

Following a single intravenous administration of siponimod (1 mg/kg) to male and female rats (n = 3/sex/group) under fed conditions, $AUC_{0\text{-}24h}$ of unchanged siponimod in plasma was 6520 ± 655 and 1920 ± 220 ng•h/mL, respectively, in female rats and male rats, and $t_{1/2}$ was 28.8 ± 4.86 and 4.78 ± 0.459 hours, respectively (reference CTD 4.2.2.2-4).

4.1.1.3 Single-dose study in monkeys

A single dose of siponimod was administered orally (0.03, 0.1, 0.3, 1, or 3 mg/kg) or intravenously (1 mg/kg) to male and female monkeys (n = 4/sex/group) under fed conditions. Table 7 shows pharmacokinetic parameter values of unchanged siponimod in blood (reference CTD 4.2.2.2-5).

Table 7. Pharmacokinetic parameters of unchanged siponimod in blood following a single oral or intravenous administration of siponimod to male and female monkeys

Route of administration	Dose (mg/kg)	C_{max} (ng/mL)	t_{max}[a] (h)	$t_{1/2}$ (h)	$AUC_{0\text{-}\infty}$ (ng•h/mL)
i.v.	1			19 ± 0.5	10585 ± 1039
p.o.	0.03	9[b]	2[b]	14[b]	220[b]
	0.1	23[b]	3[b]	14[b]	576[b]
	0.3	99 ± 13	3 [2, 7]	15 ± 1.0	2650 ± 297
	1	354 ± 39	2 [2, 4]	18 ± 0.8	7561 ± 857
	3	731 ± 76	4 [4, 7]	20 ± 0.3	18864 ± 2654

Mean ± SD; Number of animals evaluated, 4/group.
a) Median [minimum, maximum], b) Calculated from the mean blood concentration.

4.1.2 Repeated-dose studies

Toxicokinetics was investigated in repeated oral dose toxicity studies in mice, rats, rabbits, and monkeys. Table 8 shows pharmacokinetic parameter values in each study (CTD 4.2.3.2-2, CTD 4.2.3.2-5, CTD 4.2.3.5.2-3, CTD 4.2.3.2-9).

Table 8. Pharmacokinetic parameters of unchanged siponimod in plasma following repeated oral administration of siponimod

Animal species	Measuring time point	Dose (mg/kg)	Sex (No. of animals)	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24h} (ng•h/mL)	CTD
Mice	Day 1	5	Female (2)	1600	3.00	25200	4.2.3.2-2
			Male (2)	2000	6.00	33400	
		15	Female (2)	4190	6.00	77600	
			Male (2)	5770	3.00	98900	
		35	Female (2)	10500	3.00	180000	
			Male (2)	15100	3.00	259000	
		80	Female (2)	23900	6.00	414000	
			Male (2)	30100	6.00	542000	
	Day 91	5	Female (2)	2570	3.00	40100	
			Male (2)	2730	3.00	44700	
		15	Female (2)	3590	3.00	62600	
			Male (2)	8860	6.00	155000	
		35	Female (2)	7940	3.00	139000	
			Male (2)	12200	3.00	195000	
		80	Female (2)	14600	3.00	229000	
			Male (2)	20300	6.00	355000	
Rats	Day 26	5	Female (3)	4140	6.00	70200	4.2.3.2-5
			Male (3)				
		15	Female (3)	7830	6.00	150000	
			Male (3)	2250	1.00	31400	
		50	Female (3)	17500	6.00	320000	
			Male (3)	6520	3.00	93600	
		150	Female (3)				
			Male (3)	13500	6.00	185000	
	Day 152	5	Female (3)	3820	6.00	77000	
			Male (3)				
		15	Female (3)	9680	6.00	191000	
			Male (3)	3330	6.00	48200	
		50	Female (3)	19400	6.00	372000	
			Male (3)	7060	6.00	106000	
		150	Female (3)				
			Male (3)	13300	6.00	198000	
Pregnant rabbits	Day 20	0.1	Female (4)	6.52 ± 0.755	3.00 [3.00, 7.00][a)]	-	4.2.3.5.2-3
		1	Female (5)	66.4 ± 10.3	3.00 [3.00, 7.00][a)]	958 ± 258	
		5	Female (5)	426 ± 48.8	3.00 [3.00, 3.00][a)]	5510 ± 646	
Monkeys	Day 1	10	Female (4)	3200 ± 685	4.50 [3.00, 6.00][a)]	51900 ± 20000	4.2.3.2-9
			Male (4)	4040 ± 628	4.50 [3.00, 6.00][a)]	67400 ± 9160	
		30	Female (4)	9380 ± 1810	4.50 [3.00, 6.00][a)]	157000 ± 32300	
			Male (4)	11000 ± 772	6.00 [3.00, 6.00][a)]	198000 ± 5450	
		100	Female (6)	16800 ± 4760	6.00 [6.00, 6.00][a)]	293000 ± 76800	
			Male (6)	19100 ± 5640	6.00 [6.00, 6.00][a)]	339000 ± 89200	
	Day 357	10	Female (4)	6620 ± 3170	3.00 [3.00, 6.00][a)]	124000 ± 80900	
			Male (4)	5440 ± 785	4.50 [3.00, 6.00][a)]	95400 ± 16600	
		30	Female (4)	12100 ± 3130	6.00 [6.00, 6.00][a)]	215000 ± 54200	
			Male (4)	18000 ± 2960	6.00 [3.00, 6.00][a)]	331000 ± 54000	
		100	Female (6)	31000 ± 8560	6.00 [6.00, 6.00][a)]	565000 ± 170000	
			Male (4)	46000 ± 20300	6.00 [6.00, 6.00][a)]	884000 ± 387000	

Mean or mean ± SD

a) Median [minimum, maximum]

4.2 Distribution

4.2.1 Tissue distribution

Following a single oral administration of ^{14}C-labeled siponimod (8 mg/kg) to male mice under fed conditions, the tissue radioactivity concentration reached the maximum level in almost all of the tissues at 24 hours after administration, after which the concentration decreased over time. High radioactivity

concentrations were observed in lacrimal gland, liver, kidney (cortex and corticomedullary junction), and lymph nodes. The radioactivity was detected in many tissues even at 336 hours after administration (CTD 4.2.2.2-1).

A single dose of ^{14}C-labeled siponimod was administered to male pigmented rats and male albino rats intravenously (1 mg/kg) or orally (3 mg/kg). Following the intravenous administration, radioactivity concentration in blood reached the maximum level at 0.25 hours after administration, then decreased below the lower limit of quantitation within 168 hours after administration. Following the oral administration, radioactivity concentration in blood reached the maximum level at 4 hours after administration, then decreased below the lower limit of quantitation within 168 hours after administration. The tissue distribution of the radioactivity in pigmented rats was similar to that observed in albino rats, indicating no accumulation of radioactivity in melanin-containing tissues (eye and skin) (reference CTD 4.2.2.2-3).

Following an once daily repeated oral administration of ^{14}C-labeled siponimod (3 mg/kg) to pigmented rats for 7 days, the ratio (tissue/blood) of $AUC_{0\text{-}168h}$ of siponimod in cerebellar white matter, callosum, and medulla oblongata was 15.1, 12.9, and 12.4, respectively (CTD 4.2.2.3-1).

4.2.2 Protein binding and distribution in blood cells

Following the addition of ^{14}C-siponimod (1-100 ng/mL) to plasma samples of mice, rats, rabbits, dogs, and monkeys, the fraction of the radioactivity unbound to plasma protein after gel filtration was 0.0110% to 0.0177%, 0.020% to 0.035%, 0.0231% to 0.0381%, 0.005% to 0.012%, and 0.024% to 0.031%, respectively (CTD 4.2.2.3-2, CTD 4.2.2.3-3).

Following the addition of ^{14}C-labeled M17 (cholesterol ester of siponimod; 2-200 ng/mL) to plasma samples of mice and rats, the fraction of the radioactivity unbound to plasma protein after gel filtration was 0.0144% to 0.0471% and 0.0219% to 0.0908%, respectively (CTD 4.2.2.3-4).

Following the addition of ^{14}C-siponimod (10-70000 ng/mL) to blood samples of mice, rats, rabbits, dogs, and monkeys, the mean blood/plasma concentration ratio of the radioactivity was 0.74 to 1.21 (CTD 4.2.2.3-2, CTD 4.2.2.3-3).

4.2.3 Placental transfer

Following a once daily oral administration of siponimod (0.1, 1, or 5 mg/kg) to pregnant rabbits from Gestation Day 7 to 20, plasma siponimod concentration in fetuses was below the lower limit of quantitation in the 0.1 mg/kg group but above the limit in 1 and 5 mg/kg groups, with the fetal/plasma concentration ratio being 2.6 and 1.6, respectively (CTD 4.2.3.5.2-3).

4.3 Metabolism

4.3.1 *In vitro* metabolism

^{14}C-labeled siponimod (5 μmol/L) was added to liver microsomes of mice, rats, and monkeys, and the mixtures were incubated at 37°C for 1 hour. As a result, M5 (hydroxylated form), M6, M7, and M3

(glucuronate conjugate of M5) were detected. Also, a minute amount of a reactive metabolite was detected in rats (CTD 4.2.2.4-2).

^{14}C-labeled siponimod (5 μmol/L) was added to hepatocytes of mice, rats, and monkeys, and the mixtures were incubated at 37°C for 0 to 3 hours. As a result, M3, M5, and M6 were detected as metabolites with mouse cells, and M3, M5, M6, M7, and M4 (sulfate conjugate of M5) with rat cells. Also, weakly to moderately reactive metabolites were detected in rats and monkeys (CTD 4.2.2.4-2).

4.3.2 *In vivo* metabolism

Following a single oral administration of ^{14}C-labeled siponimod (8 mg/kg) to male mice, the mainly detected compounds were unchanged siponimod and M17 in plasma; unchanged siponimod and M5 in feces; and M17 in urine (reference CTD 4.2.2.2-1).

Following a single oral administration of ^{14}C-labeled siponimod (3 mg/kg) to male rats, the mainly detected compounds were unchanged siponimod and M6 in plasma; M4, M6, and unchanged siponimod in feces; M1 (compound formed by cleavage of oxime ether bond) and M8 (reduced form of M1) in urine (reference CTD 4.2.2.2-2).

Following a single oral administration of ^{14}C-labeled siponimod (15 mg/kg) to male monkeys, unchanged siponimod and M3 were mainly detected in plasma. In feces, M5, and M4 (M4a [sulfate conjugate of M5], M4b [sulfate conjugate of M7], and M4c [sulfate conjugate of M6]) were mainly detected. Metabolites in urine accounted for ≤2% of total metabolites detected (reference CTD 4.2.2.2-6).

Following intravenous administration of ^{14}C-labeled siponimod (1 mg/kg) to bile duct-cannulated male rats, main metabolites detected in the bile were M4 (M4a, M4b, and M4c) (reference CTD 4.2.2.2-3).

4.4 Excretion

4.4.1 Urinary and fecal excretion

Following a single oral administration of ^{14}C-labeled siponimod (8 mg/kg) to male mice, 2.01% ± 0.612% and 74.9% ± 3.55%, respectively, of the total radioactivity administered were excreted in urine and feces within 168 hours after administration (reference CTD 4.2.2.2-1).

Following a single oral administration of ^{14}C-labeled siponimod (3 mg/kg) to male rats, 6.95% ± 1.26% and 92.0% ± 2.17%, respectively, of the total radioactivity administered were excreted in urine and feces within 96 hours after administration (reference CTD 4.2.2.2-2).

Following a single oral administration of ^{14}C-labeled siponimod (15 mg/kg) to male monkeys, 1.71% ± 0.52% and 98.2% ± 3.10%, respectively, of the total radioactivity administered were excreted in urine and feces within 336 hours after administration (reference CTD 4.2.2.2-6).

Following a single oral administration of ^{14}C-labeled siponimod (10 mg/kg) to bile duct-cannulated male rats, 1.35% ± 1.13%, 25.6% ± 9.59%, and 56.9% ± 13.9%, respectively, of the total radioactivity

administered were excreted in urine, feces, and bile within 48 hours after administration (reference CTD 4.2.2.2-3).

4.4.2 Excretion in milk

Following a single oral administration of ^{14}C-labeled siponimod (10 mg/kg) to lactating rats, radioactivity in plasma reached C_{max} (2310 ng Eq/mL) at 8 hours after administration. $AUC_{0-\infty}$ was 106000 ng Eq•h/mL. The radioactivity in milk reached C_{max} (1150 ng Eq/mL) at 8 hours after administration. $AUC_{0-\infty}$ was 63500 ng Eq•h/mL (CTD 4.2.2.3-5).

4.R Outline of the review conducted by PMDA

On the basis of the results of the nonclinical pharmacokinetic studies submitted, PMDA concludes that there are no particular problems.

5. Toxicity and Outline of the Review Conducted by PMDA

The applicant submitted the data of single-dose toxicity studies, repeated-dose toxicity studies, genotoxicity studies, carcinogenicity studies, reproductive and developmental toxicity studies, local tolerance studies, and other studies (immunotoxicity, mechanism of toxicity, phototoxicity, skin sensitization, dependence, and safety of impurities), as toxicology studies of siponimod. The dose of siponimod is expressed in terms of free base. Unless specified otherwise, 0.5% hydroxypropylcellulose solution was used as vehicle in *in vivo* studies.

5.1 Single-dose toxicity

The acute toxicity of siponimod was evaluated based on the results of single oral dose toxicity studies in mice and rats and a dose-titrated oral toxicity study in monkeys (Table 9). The approximate lethal dose of siponimod was determined to be >200 mg/kg in mice following intravenous administration, and >2000 mg/kg in rats following oral administration, and >60 mg/kg in monkeys following oral administration.

Table 9. Outline of the results of single-dose toxicity studies

Test system	Route of administration	Dose (mg/kg)	Main finding	Approximate lethal dose (mg/kg)	Attached document CTD
Male and female mice (CD1)	i.v.	0,[a] 50, 100, 150, 200	No toxic change	>200	4.2.3.1-1
Male and female rats (Wistar Hannover)	p.o.	0, 250, 500, 1000, 2000	2000 (female): Piloerection, decreased grooming, decreased body tension, abnormal gait, abnormal posture, decreased body weight	>2000	4.2.3.1-2
Male cynomolgus monkeys	p.o.	10,[b] 30, 60 (Titrated on Day 1, 3 and 5, respectively)	60: Decreased lymphocyte count, prolonged activated thromboplastin time (cytoplasmic inclusion bodies in hepatocytes after administration)	>60	Reference 4.2.3.1-3

a) Vehicle, 20% Cremophor/5% glucose solution
b) Vehicle, 0.5% Carboxymethylcellulose solution

5.2 Repeated-dose toxicity

Repeated-dose toxicity studies were conducted using mice (13 weeks), rats (4 and 26 weeks), and cynomolgus monkeys (4, 26, and 52 weeks) (Table 10). Main findings were effects on lymphoid tissues

(decreased peripheral lymphocyte count, atrophic changes in lymphoid tissues) and on the central nervous system (convulsion, tremor, etc.) in mice, rats, and cynomolgus monkeys; pulmonary findings (inflammation, fibrosis, deposits of fibrin/hyaline materials, etc.) in mice and rats; lacrimal findings (degeneration, atrophy) in mice; renal findings (hyaline droplets) in rats, and gastrointestinal findings (diarrhoea, inflammation, etc.), skeletal muscle findings (degeneration/regeneration), and skin findings (hair follicle shrinkage, dermatitis) in cynomolgus monkeys. The exposure ($AUC_{0\text{-}24h}$) to siponimod at the NOAEL was 106000 ng•h/mL and 191000 ng•h/mL, respectively, in male and female rats (26 weeks) and 95400 ng•h/mL and 124000 ng•h/mL, respectively, in male and female cynomolgus monkeys (52 weeks). These values were approximately 190 and 342 times, respectively, (male and female rats) and approximately 171 and 222 times, respectively, (male and female cynomolgus monkeys) greater than the exposure at the clinical dose (2 mg/day) ($AUC_{0\text{-}24h}$, 558 ng•h/mL).[10)] In mice (13 weeks), the NOAEL was not determined. The exposure to siponimod ($AUC_{0\text{-}24h}$, 44700 ng•h/mL [male] and 40100 ng•h/mL [female]) at the lowest dose (5 mg/kg) was approximately 80 times (males) and 72 times (females) greater than the exposure at the clinical dose.

Table 10. Outline of the results of repeated oral dose toxicity studies

Test system	Route of administration	Administration period	Dose (mg/kg)	Main findings	NOAEL (mg/kg/day)	Attached document CTD
Male and female mice (CD1)	p.o.	13 weeks (once daily)	0, 5, 15, 35, 80	≥5: Deposits of fibrin-hyaline materials/inflammation/fibrosis, increased foamy macrophages/intraalveolar macrophages in lung; atrophy of marginal zone lymphoid tissue, lymphoid hyperplasia/extramedullary hematopoiesis in PALS of spleen; lymphoid hyperplasia of thymic medulla, histiocytosis in mesenteric lymph node medulla ≥15: Centrilobular hypertrophy in liver, vacuolar degeneration in lacrimal gland ≥35: Hyperplasia of paracortical lymphoid tissue in mandibular lymph nodes (males), lacrimal gland atrophy (females) 80: Histiocytosis in liver, decreased eosinophilic property of cytoplasm in lacrimal acinar cells (males)	<5	4.2.3.2-2
Male and female rats (Wistar Hannover)	p.o.	4 weeks (once daily) + Withdrawal 4 weeks	0, 10, 50, 200[a)]	≥10: Decreased lymphocyte/basophil/LUC counts, decreased eosinophil count (male), decreased spleen/thyroid weight, increased liver/adrenal weight, increased thymic/cardiac weight (female), enlarged thymic medulla, lymphoid tissue atrophy in PALS of spleen, histiocytosis in bronchial lymph nodes/mandibular lymph nodes, histiocytosis in mesenteric lymph nodes, lymphocyte depletion in lymphatic sinuses, hyaline droplets in proximal renal tubules of kidney ≥50: Decreased feces, soiled perineal region, reduced body weight gain (female), decreased food consumption, centrilobular hepatocyte hypertrophy in liver, alveolitis/foamy macrophage accumulation in lung (female) 200→100 (female): Irritability, vocalization, dehydration, soiled fur, unkempt fur, decreased body weight, increased platelet count/reticulocyte count, increased blood potassium/inorganic phosphorus, decreased uterine weight, uterine atrophy, mucus production in vaginal epithelium	10[b)]	4.2.3.2-4

Test system	Route of administration	Administration period	Dose (mg/kg)	Main findings	NOAEL (mg/kg/day)	Attached document CTD
				200 (male); Decreased body weight/reduced body weight gain, increased platelet count, increased blood potassium/inorganic phosphorus, foamy macrophage accumulation/bronchiolitis in lung Reversibility: Reversible (except for hyaline droplets in kidney)		
Male and female rats (Wistar Hannover)	p.o.	26 weeks (once daily) + Withdrawal 8 weeks	0, 5 (female) 15, 50, 150 (male)	Death[c]:15 (1 female), 50 (1 female), 150 (1 male) ≥5: Decreased food consumption, decreased white blood cell count/lymphocyte count/monocyte count/eosinophil count/red blood cell count/hemoglobin/hematocrit, decreased total protein/albumin/globulin, decreased splenic weight, increased liver weight, reduced thymic medulla, lymphoid tissue atrophy in PALS/lymphoid tissue hyperplasia in marginal zone of spleen ≥15: Reduced body weight gain (female), increased blood inorganic phosphorus (female), hypertrophy of follicular epithelial cells in thyroid gland (male) ≥50: Decreased body weight, decreased food consumption (male), increased blood inorganic phosphorus (male), increased thymic/adrenal weight (female), decreased pituitary weight (female), centrilobular hepatocyte hypertrophy in liver, lymphoid tissue atrophy in bronchial lymph nodes/mesenteric lymph nodes, hypertrophy of follicular epithelial cells in thyroid gland (female) 150: Reduced body weight gain (male), increased thyroid weight Reversibility: Reversible	Male: 50[b] Female: 15[b]	4.2.3.2-5
Male and female cynomolgus monkeys	p.o.	4 weeks (once daily) + Withdrawal 4 weeks	0, 10, 50, 200→150[d]	Death: 200→150 (2 males)[e] ≥10: Decreases in white blood cell count and lymphocyte count ≥50: Soft faeces, diarrhoea, decreased food consumption 200→150: Ataxia, decrease in locomotor activity, clonic convulsion, tremor, eyelid ptosis, tarry stool, vomiting containing blood, decreased thymic weight, small thymus, lymphocyte depletion in thymus Reversibility: Reversible	10[b]	4.2.3.2-7
Male and female cynomolgus monkeys	p.o.	26 weeks (once daily) + Withdrawal 12 weeks	0, 10, 50, 100	Death: 50 (1 male), 100 (1 female)[e] ≥10: Decreased white blood cell count/lymphocyte count, decreased total protein/albumin, decreased T cell count/B cell count/helper T cell count/cytotoxic T cell count/NK cell count in peripheral blood, decreased anti-KLH IgM/IgG antibody titer, decreased thymic/splenic weight, lymphoid tissue atrophy in PALS of spleen, vascular disorders in multiple organs (stomach, duodenum, jejunum, cecum, colon, skin/subcutaneous tissue, kidney), inflammation accompanied by crypt hyperplasia/erosion/increased goblet cells in digestive tract (duodenum, jejunum, ileum, cecum, colon, rectum) (female)	Male: 10[b] Female: <10	4.2.3.2-8

Test system	Route of administration	Administration period	Dose (mg/kg)	Main findings	NOAEL (mg/kg/day)	Attached document CTD
				≥50: Increased liver weight, atrophy of paracortical lymphoid tissue in mesenteric lymph nodes, lymphoid tissue atrophy in thymus, inflammation of digestive tract accompanied by crypt hyperplasia/erosion/increased goblet cells (male) 100: Watery stool, soiled fur, backbone protrusion, abdominal distension, emaciation, hypersensitive reaction, tremor, decreased body weight (male), decreased red blood cell count/hemoglobin/hematocrit, increased platelet count/red cell distribution width/reticulocyte count (female), decreased ALP, hypertrophy of duodenal/jejunal/ileal walls, small thymus/spleen (female) Reversibility: Reversible		
Male and female cynomolgus monkeys	p.o.	52 weeks (once daily) + Withdrawal 8 weeks	0, 10, 30, 100	Death: 100 (3 males)[f)] ≥10: Decreased white blood cell count/lymphocyte count/basophil count/LUC count, increased RDW, decreased globulin/albumin/total protein, decreased ALP (male), decreased T cell/B cell/helper T cell/cytotoxic T cell counts in peripheral blood, decreased splenic weight, decreased cell density in thymic cortex/increased cell density in thymic medulla, decreased germinal centers in spleen, extramedullary hematopoiesis/decreased germinal centers/decreased paracortical cell density in mandibular lymph node, decreased germinal centers in gut-associated lymphoid tissue, hyperplasia of myeloid cells/lymphocyte accumulation in bone marrow, degeneration/regeneration of femoral biceps, hair follicle atrophy in skin ≥30: Pigmentation/keratosis of skin, scrotal abscess/skin cancer[g)] in scrotum 100: Tremor, spasm (male), watery stool, decreased food consumption, abdominal distension (male), vomiting, decreased body weight (male), decreased thymic weight (female), small thymus, dermatitis Reversibility: Reversible	10[b)]	4.2.3.2-9

a) The dose was decreased to 100 mg/kg on Day 13 in females only due to the aggravation of clinical signs and decreased body weight.
b) The findings observed in the group receiving the NOAEL are considered to be of little toxicological significance, judging from the relationship to the pharmacological effect, presence/absence of related histopathological findings, and their frequency and severity.
c) The cause of death is unknown, but the causal relationship to siponimod is unlikely, judging from the lack of dose correlation.
d) The dose was decreased to 150 mg/kg on Day 8 due to aggravation of clinical signs.
e) Moribund sacrificed because of aggravation of clinical signs.
f) One animal died, and 2 animals were moribund sacrificed because of aggravation of clinical signs.
g) Skin cancer did not show dose response. Skin cancer is observed spontaneously in cynomolgus monkeys. Therefore, the cancer is unlikely to be causally related to siponimod.

5.3 Genotoxicity

Bacterial reverse mutation assays, chromosomal aberration assays in mammalian cells, and bone marrow micronucleus assays in rodents were conducted. (Table 11). No genotoxicity was observed.

Table 11. Outline of the results of genotoxicity studies

Study type		Test system	Metabolic activation (treatment)	Concentration or dose	Results	Attached document CTD
In vitro	Bacterial reverse mutation assay	*Salmonella typhimurium*: TA97a, TA98, TA100, TA102, TA1535 (plating method)	S9–/+	0,[a] 8, 40, 200, 1000, 5000 (μg/plate)	Negative	4.2.3.3.1-1
		TA97a, TA98, TA100, TA102, TA1535 (preincubation method)	S9–/+	0,[a] 312.5,[b] 625, 1250, 2500,[c] 5000[d] (μg/plate)	Negative	
		TA100 (preincubation method)	S9–	0,[a] 2.344, 4.688, 9.375, 18.75, 37.5,[e] 75, 150, 300 (μg/plate)	Negative	
		TA100, TA102 (preincubation method)	S9–	0,[a] 19.531, 39.063, 78.125, 156.25,[e] 312.5 (μg/plate)	Negative	
	Chromosomal aberration assay in mammalian cells	Human lymphoblastoid TK6 cells	S9– (20 hours)	0,[a] 18.8, 37.5, 75, 150[d] (μg/mL)	Negative	4.2.3.3.1-2
			S9+ (3 hours)	0,[a] 37.5, 75, 150,[d] 300[d] (μg/mL)		
		Chromosomal aberration assay using human peripheral lymphocytes	S9– (20 hours)	0,[a] 32.5, 54.2, 90.4 (μg/mL)	Negative	4.2.3.3.1-3
			S9– (3 hours)	0[a], 49.3, 66.6, 90.0 (μg/mL)	Negative	
			S9+ (3 hours)	0,[a] 26.5, 43.1, 70.1 (μg/mL) 0,[a] 27.0, 49.3, 66.6 (μg/mL)	Negative	
In vivo	Bone marrow micronucleus assay in rodents	Male mice (CD1) Bone marrow		0, 125, 250, 500, 1000 (mg/kg) (p.o., twice)	Negative	4.2.3.3.2-1
		Male rats (Wistar Hannover) Bone marrow		0, 125, 395, 1250 (mg/kg) (p.o., twice)	Negative	4.2.3.3.2-2

a) Vehicle, Dimethyl sulfoxide (DMSO)
b) Growth of strains TA100 (S9–) and TA102 (S9–) was inhibited at this and higher concentrations.
c) Growth of strain TA98 (S9–) was inhibited at this and higher concentrations.
d) The test substance precipitated.
e) Growth was inhibited at this and higher concentrations.

5.4 Carcinogenicity

Long-term oral carcinogenicity studies were conducted in mice and rats (Tables 12-13). Increased incidences were observed in angiosarcoma/haemangioma in male and female mice, malignant lymphoma in female mice, renal tubule adenoma in male mice, and follicular epithelial cell adenoma/carcinoma in male rats as tumor lesions.

The angiosarcoma/haemangioma in male and female mice was probably caused by sustained induction of neovascularization. In an *in vitro* study, exposure to siponimod caused an increase in cell division and in placental growth factor 2 (PLGF2) concentration in mouse endothelial cells, whereas no such changes were observed in rat or human endothelial cells [see Section 5.7.2], which suggests that angiosarcoma is unlikely to develop in humans. As for the malignant lymphoma observed in female mice, female mice are known to develop spontaneous lymphoma at a high frequency, and it is likely that the incidence of spontaneous malignant lymphoma was increased by the immunomodulatory effect, pharmacological action of siponimod. The incidence of renal tubule adenoma in male mice was slightly higher (5.7%) in the 25 mg/kg group than the historical data of the study facility (5.0%). The finding is considered to be accidental, as judged by the following observations: (1) No nonneoplastic proliferative lesion was observed in the kidney of animals in any siponimod group, (2) there was no significant difference in the incidence between renal tubule adenoma and adenoma/carcinoma (combined), and (3) there was no

increase in the incidence of renal tubule adenocarcinoma. Follicular epithelial cell adenoma/carcinoma observed in male rats was caused by the stimulation of follicular epithelial cells of thyroid gland by enhanced thyroid stimulating hormone (TSH) secretion as a result of hepatocyte enzyme induction, and is unlikely to be relevant to humans.

Table 12. Outline of the results of carcinogenicity studies in mice

Test system	Route of administration	Administration period	Main lesions	Sex	Dose (mg/kg/day)				Noncarcinogenic dose (mg/kg/day)	Attached document CTD
					0	2	8	25		
					n = 70	n = 70	n = 70	n = 70		
Male and female mice (CD-1)	p.o.	104 weeks (once daily)	Malignant lymphoma[a)]	M	16	25	21	20	<2	4.2.3.4.1-1
				F	26	42	38	40		
			Angiosarcoma/ haemangioma[b)]	M	10	47	49	48		
				F	9	37	34	39		
			Renal tubule adenoma	M	1	1	1	4		
				F	0	0	0	0		
			Renal tubule adenocarcinoma	M	0	0	0	1		
				F	0	0	0	0		
			Nonneoplastic lesion	Vasodilatation in multiple tissues and organs (adipose tissue, femur, bone marrow, ovary, uterus, pancreas, subcutaneous tissue), haemangioma-like hyperplasia in heart, chronic inflammation accompanied by alveolar macrophages/eosinophilic substances within alveoli/fibrosis in lung, degeneration/atrophy of acinar cells in lacrimal gland, hepatocellular hypertrophy/pigmentation in liver, edema in subcutaneous tissue, hyperplasia/erosion/ulcer in glandular stomach, giant nuclei with intranuclear inclusions in renal tubules					

a) Number of animals with malignant lymphoma in reticuloendothelial system
b) Number of animals with angiosarcoma/haemangioma in any tissue (heart, liver, skeletal muscle, ovary, small intestine, spleen, subcutaneous tissue, uterus, etc.)

Table 13. Outline of the results of carcinogenicity study in rats

Test system	Route of administration	Administration period	Main lesion	Sex	Dose (mg/kg/day)					Noncarcinogenic dose (mg/kg/day)	Attached document CTD
					0	3	10	30	90		
					n = 50	n = 50	n = 50	n = 50	n = 50		
Male and female rats (Wistar Hannover)	p.o.	104 weeks (once daily)	Follicular epithelial cell adenoma of thyroid gland	M	2		6	7	16	Male: <10 Female: 90	4.2.3.4.1-2
				F	2	0	1	1			
			Follicular epithelial cell carcinoma of thyroid gland	M	1		2	5	5		
				F	1	0	0	0			
			Nonneoplastic lesion	Increased cell density in bone marrow, haemorrhage/vasculitis in brain, mineral deposition, corneal inflammation, vasculitis in heart, mononuclear cell infiltration in ileum, renal vasculitis/chronic progressive nephropathy, eosinophil nest/bile duct hyperplasia/centrilobular hepatocyte hypertrophy in liver, pleural fibrosis in lung, sinusoidal dilatation in mandibular lymph node, lymphocyte depletion in spleen, vasculitis/seminiferous tubule degeneration in testis, enlarged thymic medulla, thyroid epithelial cell hyperplasia, dilation/vascular tissue hypertrophy/hyperplasia/inflammation/ulcer/haemorrhage/endometrial hyperplasia/vasculitis in uterus						

5.5 Reproductive and developmental toxicity

A study of fertility and early embryonic development to implantation in rats, embryo-fetal development studies in rats and rabbits, and a study of effects on pre- and postnatal development, including maternal function in rats were conducted (Table 14). In the embryo-fetal development studies, teratogenic findings (abnormal limb rotation, cleft palate, cardiomegaly, abnormal morphology of clavicle, and generalized edema) were observed in rats, and an increased number of resorptions, decreased number of live fetuses, and skeletal anomalies in rats and rabbits. In rats, the NOAEL for embryo-fetal development was not determined, and the exposure ($AUC_{0\text{-}24h}$, 10300 ng•h/mL) to siponimod at the

lowest dose (1 mg/kg) was approximately 18 times greater than the exposure ($AUC_{0\text{-}24h}$, 558 ng•h/mL) following the administration of the clinical dose (2 mg/day).[10)] In rabbits, the exposure[12)] ($AUC_{0\text{-}24h}$, 96 ng•h/mL) to siponimod at the NOAEL (0.1 mg/kg) was approximately 0.2 times greater than the exposure following the administration of the clinical dose.

Siponimod is contraindicated in pregnant women or in women who may possible be pregnant, taking account of the findings that increased embryo-fetal mortality and teratogenicity were observed in rats and rabbits, and that babies with deformity were born to pregnant patients who had received fingolimod chloride, a functional S1P receptor antagonist similar to siponimod.

[12)] The exposure following administration of 0.1 mg/kg was estimated from $AUC_{0\text{-}24h}$ (958 ng·h/mL) observed in the repeated oral administration of siponimod (1 mg/kg) in the embryo-fetal development study in rabbits (CTD 4.2.3.5.2-3).

Table 14. Outline of the results of reproductive and developmental toxicity studies

Study type	Test system	Rout of administration	Administration period	Dose (mg/kg)	Main findings	NOAEL (mg/kg/day)	Attached document CTD
Study of fertility and early embryonic development to implantation	Male rats (Wistar Hannover)	p.o.	Male: From 28 days before mating to mating period (once daily)	0, 2, 20, 200	200: Decreased feces, decreased body weight, decreased epididymis weight No abnormality in a sperm test or fertility test	Parental animals (general toxicity): 20 Parental animals (fertility): 200	4.2.3.5.1-1
	Female rats (Wistar Hannover)	p.o.	Female: From 14 days before mating to Gestation Day 6 (once daily)	0, 0.1, 0.3, 1.0	0.3: Decreased food consumption No effect on fertility	Parental animals (general toxicity): 0.1 Parental animals (fertility): 1	4.2.3.5.1-2
Embryo-fetal development study	Female rats (Wistar Hannover)	p.o.	Gestation Day 6-17 (once daily)	0, 1, 5, 40	Parental animals: ≥1: Reduced body weight gain/body weight/food consumption, decreased uterine weight, total resorption 40: Decreased feces, increased locomotor activity, soiled perineal region Fetuses: ≥1: Increased early resorption/post-implantation embryonal mortality, decreased live fetuses, abnormal limb rotation, cleft palate, cardiomegaly, abnormal morphology of clavicle, generalized edema, skeletal anomalies (incomplete ossification between parietal bones, unossified phalanges of fore and hind limbs, sternebrae malformation and incomplete ossification, cervical ribs)	Parental animals (general toxicity): 5 Embryo-fetal development: <1	4.2.3.5.2-1
	Female rabbits (NZW)	p.o.	Gestation Day 7-20 (once daily)	0, 0.1, 1, 5	Parental animals: 5: Abortion, reduced body weight gain, decreased pregnant uterine weight Fetuses: 1: Increased resorptions, skeletal anomalies (incomplete ossification and curvature of hyoid bone, fusion and malformation of sternebrae) 5: Increased early and late resorptions, decreased live fetuses, small gallbladder, skeletal anomalies (incompletely ossified and unossified phalanges, delayed talus ossification, incompletely ossified and unossified phalanges of hind limbs)	Parental animals (general toxicity): 1 Embryo-fetal development: 0.1	4.2.3.5.2-3

Table 14. Outline of the results of reproductive and developmental toxicity studies

Study type	Test system	Rout of administration	Administration period	Dose (mg/kg)	Main findings	NOAEL (mg/kg/day)	Attached document CTD
Study of effects on pre- and postnatal development, including maternal function	Female rats (Wistar Hannover)	p.o.	Maternal animals: Gestation Day 6 to Postpartum Day 22 (once daily)	0, 0.05, 0.15, 0.5	Parental animals: ≥0.15: Reduced body weight gain (pregnant period), decreased food consumption (lactating period), prolonged gestation period 0.5: Reduced body weight gain (lactating period), decreased body weight (pregnant period) F1 offspring: ≥0.05: Incisor malocclusion, reduced body weight gain, change in splenic lymphocyte subsets (increases in total T cell count, helper T cell count, cytotoxic T cell count, and B cell count) ≥0.15: Decreased 4-day survival rate, decreased litter size, dehydration, hunger, emaciation, frailty, decreased locomotor activity, cold to touch, abnormally curved hind limbs, kinked tail, cranial flattening, early eyelid opening, uterine horn-like tissue (male), changes in peripheral lymphocyte subsets, (increases in total T cell count, helper T cell count, and cytotoxic T cell count, decreased natural killer cell count) 0.5: Decreased birth rate/live birth rate, increased stillbirths, decreased 7- and 14-day survival rate,[b] decreased weaning rate, decreased body weight/food consumption, decreased number of animals showing pupillary reflex, delayed auditory startle response, delayed vaginal opening and delayed preputial separation, malformed mandibular incisors, haemorrhage, necrosis of dental pulp cavity	Parental animals (general toxicity): 0.05 F1: Development of offspring: 0.05[a]	4.2.3.5.3-1

a) Reduced body weight gain and incisor malocclusion were mild or not accompanied by histopathological changes, and are therefore considered to be of little toxicological significance. Changes in splenic lymphocyte subsets were within the range of physiological variations, and are therefore considered to be accidental findings unrelated to siponimod.

b) External and visceral examinations of neonates that died 0 to 7 days after birth showed cleft palate, schistoglossia, truncus arteriosus, generalized edema, imperforate anus, remnant liver lobe, enlarged spleen, enlarged kidney, localized edema/hematoma, cranial swelling/softening, shortened distance between anus and genital tubercle, hypertrophy of caudal lobe of liver, and uterine horn-like tissue (in male animals together with testis).

5.6 Local tolerance

A local tolerance study was conducted in rabbits (Table 15). Siponimod did not have an irritating effect when administered subcutaneously, intravenously, intra-arterially, or periarterially, but had an irritating effect when administered ocularly.

Table 15. Outline of the results of local tolerance studies

Study type	Test system	Testing method	Main findings	Attached document CTD
Primary skin irritation test	Male rabbits (NZW)	Occluded application (0.5 g) to the back skin for 4.5 hours	None	4.2.3.6-1
Primary eye irritation test	Male rabbits (NZW)	A single-dose application (23.8 mg) into the conjunctival sac	Redness/edema/discharge in conjunctiva, and irritation (response to light) in iris were observed from 1 hour after application and resolved after 7 days.	4.2.3.6-2
Local irritation tests by intravenous, intra-arterial, and periarterial administration	Male rabbits (NZW)	A single intravenous administration (0.015 mg/kg)	None	4.2.3.6-3
		A single intra-arterial administration (0.00125 mg/kg)	None	
		A single periarterial administration (0.0005 mg)	None	

5.7 Other studies

5.7.1 Immunotoxicity

A 4-week oral immunotoxicity study was conducted in rats (Table 16). Siponimod caused atrophic change of lymphoid tissues, decreases in peripheral T and B cell counts, and decreased immune response to KLH. These changes were reversible after recovery period, from which the applicant considered that there was no severe immune suppression.

Table 16. Outline of the results of immunotoxicity study

Test system	Testing method	Main findings	Attached document CTD
Male and female rats (Wistar Hannover)	Siponimod (0.3 [female], 1.5 [male], 10, 50 mg/kg) was administered orally for 4 weeks, and the animals were immunized by KLH on Day 11 and 19. For the evaluation of reversibility, siponimod (50 mg/kg) was administered orally for 4 weeks, followed by a recovery period of 8 weeks. Animals were then immunized with KLH on Day 41 and 50.	≥0.3: Decreased body weight/body weight gain/food consumption, decreased lymphocyte count/white blood cell count/monocyte count/eosinophil count/basophil count/LUC count, decreased total T cell count/helper T cell count/cytotoxic T cell count/B cell count in peripheral blood, decreased lymphocyte count/total T cell count/helper T cell count/cytotoxic T cell count/B cell count in spleen, increased lymphocyte count/total T cell count/helper T cell count/cytotoxic T cell count in thymus, enlarged medulla with increased small lymphocytes in thymus, lymphocyte depletion in PALS of spleen, decreased anti-KLH IgM/IgG titers ≥1.5: Decreased splenic weight (male) 50: Decreased splenic weight (female), lymphocyte depletion in cortex/paracortex of mesenteric/mandibular lymph nodes Reversibility: Reversible	4.2.3.7.2-1

5.7.2 Studies on the mechanism of toxicity

In order to investigate the species specificity of siponimod-induced changes in vascular endothelial cells, studies on the mechanism of the toxicity were conducted *in vitro* (mouse, rat, and human vascular endothelial cells) and *in vivo* (mice and rats). Also, in order to investigate the risk of skin tumor due to immune suppression, a study on the effect of siponimod on dermal melanocytes was conducted in mice (Table 17). The applicant considered that, in mice, there were changes suggesting persisting activation of vascular endothelial cells and promotion of cell division, whereas no such changes were observed in vascular endothelial cells of rats or humans.

Table 17. Outline of the results of studies on the mechanism of toxicity

Study type	Testing method	Main findings	Attached document CTD
In vitro studies on the mechanism of toxicity using mouse, rat, and human vascular endothelial cells	Mouse-derived cells were treated with siponimod (0.001-30000 nM), rat- and human-derived cells with siponimod (0.001-10000 nM), and mouse, rat, and human-derived cells with the main metabolite M17 (0.0005-5000 nM), for 24 hours, and cell growth (measured by EdU uptake as the index) and PLGF2[a)] concentration were measured.	Results of the exposure to siponimod: Vascular endothelial cells derived from microvessels of mouse skeletal muscles: ≥1 nM: PLGF2 concentration increased, 10 nM to 1.25 μM: EdU uptake increased, >1.25 μM: EdU uptake decreased. Vascular endothelial cells derived from pulmonary microvessels or aorta of rats: No effect Vascular endothelial cells derived from human skin: ≥10 nM: EdU uptake decreased Vascular endothelial cells derived from human pulmonary microvessels: No effect M17 did not cause either cell growth or PLGF2 increase in any of cells.	Reference 4.2.3.7.3-1
Study on the mechanism of toxicity in mice	Siponimod (25, 75 mg/kg/days) was administered orally to male mice (CD1), and the animals were necropsied at various time points from Day 1 through 274. Genetic analysis using microarrays, measurement of angiogenic factor concentration in plasma, histopathological examination, drug concentration measurement (M16, M17), etc., were conducted.	Increase in PLGF2 (Day 1-274 of study) and decrease in endoglin[b)] concentration (Day 28-274 of study) in plasma, increased expression levels of *CD93•Prn•IcamI*[c)] (Day 1-274 of study), *Mki67•Cdc20•Hist1h2ab*,[d)] and *CD133*[e)] (Day 3-274 of study) genes in skeletal muscles, increase in expression level of CD93[e)] (Day 3-7 of study), and increase in Ki-positive cells (Day 3-274 of study) were observed.	Reference 4.2.3.7.3-2
Study on the mechanism of toxicity in rats	Siponimod (90 mg/kg/day) was administered orally to male rats (Wistar Hannover), and they were necropsied at various time points from Day 1 to 92. Genetic analysis using microarrays, measurement of angiogenic factor and thyroid hormone concentrations in plasma, histopathological examination, drug concentration measurement (M16, M17), etc., were conducted.	A transient increase in PLGF2 in plasma (Day 1-7 of study), increased expression levels of *CD93•Prn•IcamI* (Day 1-92 of study) and *Mki67•Ccna2•Cdk1*[d)] (Day 3 of study) genes in skeletal muscles, increases in expression level of CD133 (Day 7-92 of study), increase in Ki-positive cells (Day 3 of study), increase in TSH concentration (from Day 28 of administration), and increased in T4-UDP-GT in liver microsomes were observed.	Reference 4.2.3.7.2-3
Study on skin in mice	Siponimod (0.01, 0.3, 10 mg/kg/day) was administered orally to female mice (C57/Bl6), and they were necropsied on Day 14. Genetic analysis using microarrays, immunohistochemistry, and drug concentration measurement, etc., were conducted.	Siponimod had no effect on the expression of genes involved in division of melanocytes (siponimod concentration in skin was approximately 1/10 times the concentration in blood).	Reference 4.2.3.7.2-4

a) A factor involved in the growth of vascular endothelial cells and in neovascularization
b) Related to suppression of neovascularization
c) Related to activation of vascular endothelial cells
d) Related to cell division
e) A marker of (precursors of) vascular endothelial cells

5.7.3 Phototoxicity

A phototoxicity testing was conducted using 3T3 fibroblast cell line (Table 18). It was determined that siponimod is not phototoxic.

Table 18. Outline of the results of phototoxicity testing

Study type	Test system	Concentration (μg/mL)	Results	Attached document CTD
In vitro	Balb/c mouse 3T3 fibroblast cell line	0.316-1000 UV-A irradiation	Non-phototoxic (photo irritation factor 1.0)	4.2.3.7.7-1

5.7.4 Skin sensitization

A murine local lymph node assay was conducted (Table 19). Siponimod did not cause skin sensitization.

Table 19. Outline of the results of skin sensitization test

Study type	Test system	Testing method	Results	Attached document CTD
Local lymph node assay (LLNA)	Female mice (BALB/c)	Siponimod (0.5, 5, 50% solutions) was applied to both auricles for 3 days under non-occluded conditions.	Negative	4.2.3.7.7-2

5.7.5 Dependence

A physical dependence study, a drug discrimination study, and a self-administration study were conducted in rats (Table 20). Siponimod did not cause dependence.

Table 20. Outline of the results of dependence studies

Study type	Test system	Testing method	Results	Attached document CTD
Physical dependence test	Male and female rats (Wistar Hannover)	Siponimod was administered to male rats (at 0, 0.3, 1, 50, 150 mg/kg/day) and to female rats (at 0, 0.15, 0.5, 10, 50 mg/kg/day) orally for 4 weeks and, after 8-day withdrawal period, animals were observed for withdrawal symptoms.	No withdrawal symptoms were observed.	4.2.3.7.4-1
Drug discrimination test	Male and female rats (Lister Hooded)	Rats that had been trained to discriminate between the training drug (midazolam or amphetamine) and physiological saline were used. Siponimod was administered to male rats (at 0, 0.3, 1, 50, 150 mg/kg/day) and to female rats (at 0, 0.15, 0.5, 10, 50 mg/kg/day) orally, and their ability to discriminate the test drugs was evaluated.	No discriminative stimulus effect was observed.	4.2.3.7.4-2
Self-administration test	Male and female rats (Lister Hooded)	Rats that had been trained to self-inject cocaine intravenously were allowed to self-inject siponimod (0.01, 0.05, 0.4 mg/kg) intravenously, and the reinforcing effect was evaluated.	Administration of siponimod did not increase the number of self-administrations.	4.2.3.7.4-3

5.7.6 Safety evaluation of impurities

Among the impurities contained in the drug substance, Impurity 010-10, for which acceptance criteria are established exceeding the qualification threshold given in "Revision of the Guideline on Impurities in New Drug Substances" (PFSB/ELD Notification No. 1216001, dated December 16, 2002) (ICH Q3A Guideline), was subjected to safety evaluation by 4-week repeated oral dose toxicity study in rats and by a micronucleus test (Table 21). There was no safety concern. An *in silico* evaluation showed that Impurity 010-10 does not possess any structural alert for mutagenicity.

Table 21. Outline of the results of safety evaluation of impurity

Test system	Route of administration	Administration period	Dose (mg/kg)	Results	Attached document CTD
Male and female rats (Wistar Hannover)	p.o.	4 weeks (once/day)	Siponimod (0, 15, 50) spiked with the impurity[a)] Siponimod (50) not spiked with the impurity	The following findings were observed in both batch groups. No difference was observed in the toxicity profile. (Decreased body weight/food consumption, decreased red blood cell count/hemoglobin/hematocrit/white blood cell count/lymphocyte count/monocyte count/basophil count/LUC count, increased reticulocyte count, decreased blood total protein/albumin/globulin, increased bicarbonate/calcium, increased urine pH/specific gravity, urine protein, ketone bodies, hepatomegaly, decreased splenic/ovarian/uterine weight, increased liver weight, decreased lymphocyte count in PALS of spleen, extramedullary hematopoiesis, enlarged medulla/thin cortex in thymus, lymphocyte depletion/histiocytosis in lymphatic sinuses of mandibular and mesenteric lymph nodes, centrilobular hypertrophy in liver, hypertrophy of follicular epithelial cells in thyroid gland, eosinophilic cytoplasmic inclusion bodies in proximal renal tubular epithelial cells, abnormal estrous cycle in uterus, mucus production in vagina) Micronucleus induction: Negative	4.2.3.7.6-11

a) Batch spiked with the impurity (010-10 content, [redacted]%)

An *in silico* analysis detected structural alerts for mutagenicity in impurities [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], and [redacted] contained in siponimod. Therefore a reverse mutation test with *Salmonella typhimurium* was conducted for these impurities. Results showed mutagenic activity of [redacted], [redacted] and [redacted]. These impurities are controlled at levels below the acceptable intake level (1.5 μg/day) specified by "Guidelines for Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk" (ICH M7 Guideline).

5.R Outline of the review conducted by PMDA

5.R.1 Vascular findings

PMDA asked the applicant to explain the mechanism of development of vascular findings observed in repeated-dose toxicity studies and carcinogenicity studies (vasodilatation, haemangioma-like hyperplasia in heart, and angiosarcoma/haemangioma in mice; polyarteritis in rats; angiopathy in cynomolgus monkeys, etc.) and safety in humans.

The applicant's explanation:

- In the carcinogenicity study in mice, animals in the ≥2 mg/kg groups showed vasodilatation in multiple tissues and organs including adipose tissue, bone marrow, ovary, uterus, pancreas, and subcutaneous tissue [see Section 5.4]. In these tissues, the changes were not accompanied by proliferation of vascular endothelial cells, and the mechanism of vascular dilatation remains unclear. On the other hand, no vascular dilation was observed in studies in rats and cynomolgus monkeys.

During the double-blind phase of the global phase III study on siponimod (CTD 5.3.5.1-1, Study A2304), the incidence of adverse events related to angiopathy[13)] was 11.5 % (63 of 546) of subjects in the placebo group and 14.6% (161 of 1099) of subjects in the siponimod group, with a tendency of slightly higher level in the siponimod group. Most of them were hypertension (8.2% [45 of 546] of subjects in the placebo group, 10.6% [117 of 1099] of subjects in the siponimod group), and the incidence of other angiopathy-related events did not tend to be higher in the siponimod group. These results suggest that the above findings observed in mice are unlikely to be relevant to humans.

- In the carcinogenicity study in mice, animals in the ≥2 mg/kg groups showed increases in the incidence of haemangioma-like hyperplasia in heart and angiosarcoma/haemangioma. Vascular endothelial cells in mice showed changes suggestive of persisting activation of the cells and enhanced cell division, whereas no such changes were observed either in humans or rats [see Section 5.7.2]. It appears that endothelial cells of mice are more sensitive to siponimod than the cells in humans and rats, resulting in persistent enhancement of neovascularization associated with cell division and increased PLGF2 concentration. These changes are therefore unlikely to be relevant to humans.

- In the carcinogenicity study in rats, animals in the ≥3 mg/kg groups showed polyarteritis in various tissues and organs. In rats, the $S1P_1$ receptor is involved in the regulation of vascular permeability (*Am J Physiol Heart Circ Physiol*. 2009;296(1):H33-42), and the frequency of spontaneous polyarteritis increases with age (*Toxicologic Pathology.* 1st ed. Boca Raton, CRC press;p589-653). These findings suggest that, in rats, spontaneous polyarteritis was aggravated by the $S1P_1$ receptor-mediated effect of siponimod on vascular vessels, and that the changes are thus unlikely to be relevant to humans.

- In the 26-week repeated oral dose toxicity study in cynomolgus monkeys, animals in the ≥10 mg/kg groups showed mild angiopathy (growth of tunica media, neovascularization, mononuclear cell infiltration, etc.) in arteries in the kidney, gastrointestinal tract, and skin/subcutaneous tissue, etc., and 1 female in the 100 mg/kg group showed full-thickness necrosis and infiltration of neutrophils and mononuclear cells in duodenal vascular walls. Although the mechanism of development of these changes remains unclear, they are considered to be of little toxicological significance, judging from the following findings: (1) The changes were observed in only 1 or 2 animals in each group with no clear dose-dependency, (2) the changes had be reversible at the end of the recovery period, and (3) in the 52-week repeated-dose toxicity study in cynomolgus monkeys, no such changes were observed in animals with the same or higher levels of exposure to siponimod.

PMDA accepted the applicant's explanation. Monitoring for adverse events related to cardiovascular systems and appropriateness of raising caution are discussed further in Sections 7.R.4.2 and 7.R.4.3.

5.R.2 Degeneration and atrophy of lacrimal gland

PMDA asked the applicant to explain the mechanism of development of lacrimal degeneration and atrophy observed in mice and safety in humans.

13) Events included in "Vascular disorders" in MedDRA SOC or events containing the term "Vasculitis" or "Arteritis" in Preferred Term (PT).

The applicant's explanation:

- In the 13-week repeated-dose toxicity study in mice, animals in the ≥15 mg/kg groups showed lacrimal degeneration and atrophy. Although the mechanism of development of these changes is unknown, the possibility cannot be excluded that the findings are related to siponimod because of the distribution of siponimod in the lacrimal gland at a high concentration [see Section 4.2.1]. The exposure ($AUC_{0\text{-}24h}$) following the administration of the NOAEL in mice (5 mg/kg) was 44770 ng•h/mL (male) and 40100 ng•h/mL (female), which was approximately 80 times (male) and 72 times (female) greater than the exposure observed following the administration of the clinical dose (2 mg/kg/day)[10] ($AUC_{0\text{-}24h}$, 558 ng•h/mL).

- In the carcinogenicity study in mice, similar changes were observed in all dose groups including the lowest dose (2 mg/kg) group, precluding the determination of the NOAEL. The long-term treatment is likely to have caused aggravation of the symptoms. The findings in the lacrimal gland were observed neither in rats nor in cynomolgus monkeys, suggesting that the changes are unique to mice.

- During the double-blind phase of the global phase III study (CTD 5.3.5.1-1, Study A2304), the incidence of eye-related adverse events[14] was 9.9% (54 of 546) of subjects in the placebo group and 10.3% (113 of 1099) of subjects in the siponimod group. The incidence of eye-related adverse events did not increase except for macular oedema [see Section 7.R.4.6], showing no evidence suggestive of the effect of siponimod on the lacrimal gland. The changes observed in mice are therefore unlikely to be relevant to, and raise any safety concern, in humans.

PMDA accepted the above applicant's explanation. The safety in humans will be discussed further in Section 7.R.4.6, taking account of the incidence of eye-related adverse events in human subjects.

5.R.3 Tumor development

PMDA asked the applicant to explain the possibility of siponimod increasing the incidence of malignant lymphoma in humans, in a similar manner as observed in the 104-week carcinogenicity study in mice, taking account of the mechanism of the development of the disease.

The applicant's explanation:

- Drugs with an immunosuppressive effect are known to induce lymphoma by suppressing antitumor immunity (*Toxicol Pathol*. 2012;40:267-71). It is conceivable that siponimod increased the incidence of spontaneous lymphoma in mice by its immunomodulatory effect. Therefore, the possibility cannot be completely ruled out that siponimod increases the incidence of malignant lymphoma in humans as well.

- In a clinical study of fingolimod, a drug with a similar mechanism of action to that of siponimod, fingolimod chloride was administered to approximately 2300 patients with MS (total exposure period approximately 4000 patient-years) in Japan and foreign countries, and malignant lymphoma was reported in 1 patient, together with diffuse large B-cell lymphoma, lymphoproliferative disorder in lung, kidney, and thyroid, and cutaneous T-cell lymphoma. The post-marketing safety surveillance

[14] Events included in "Eye disorders" in MedDRA SOC.

of fingolimod chloride reported 29 events of malignant lymphoma in 27 patients during the exposure period of estimated approximately 17.5 billion patient-years, with the reported incidence of 0.24 per 1000 patient-years, showing no increase in the incidence reported after the marketing. The package insert of fingolimod chloride cautions against "malignant lymphoma" as a clinically significant adverse drug reaction.

- Following the administration of siponimod to patients with SPMS in the clinical studies of siponimod, central nervous system lymphoma (non-Hodgkin's lymphoma) was reported in 1 patient during the total exposure period of more than approximately 3700 patient-years.

- The spontaneous incidence of lymphoma in humans is 0.27 per 1000 patient-years, based on the number of patients diagnosed with malignant lymphoma in Japan in 2016 (Expedited Report of Cancer Incidence of Japan, 2016, Ministry of Heath, Labour, and Welfare). Currently there is no information suggestive of any increase in the incidence of lymphoma due to fingolimod hydrochloride, a drug with a mechanism of action similar to that of siponimod.

- Thus, there are no data that suggest any increase in the incidence of siponimod-induced malignant lymphoma in humans. Nevertheless, relevant precautions will be provided in the package insert, as is the case with fingolimod hydrochloride, and information will be collected continuously after the market launch.

PMDA asked the applicant to discuss the possibility that the increase in renal tubular adenoma observed in the 104-week carcinogenicity study in mice was caused by the immunosuppressive effect of siponimod.

The applicant's explanation:

- In rodents, induction of renal tubular tumor by chemical substances is reported to be caused by their genotoxic and non-genotoxic carcinogenicity, whereas increases in the incidence associated with immune suppression have not been reported (*Toxicol. Pathol.* 2018;48(8):956-69).

- In humans, skin and lymph tumors are known to increase with the administration of immunosuppressants (*Toxicol Pathol.* 2012;40(2):267-71), whereas there is no report of increase in renal tubular adenoma induced by immunosuppressants. However, given the immunosuppressive effect of siponimod, the risk of siponimod inducing tumor through suppression of antitumor immunity cannot be excluded completely.

- Thus, siponimod has a potential risk of inducing tumor by its immunosuppressive activity. Information on the risk of malignant tumor in a long-term treatment will be collected after the market launch.

PMDA accepted the above 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 reference data from a study of bioequivalence and the food effect in non-Japanese healthy adults (CTD 5.3.1.2-2, Study A2111) and from an absolute bioavailability (BA) study (reference CTD 5.3.1.1-1, Study A2126). Unchanged siponimod in plasma was measured by LC-MS/MS (lower limit of quantitation, 0.02-2.5 ng/mL). Unless specified otherwise, the amount of siponimod is expressed in terms of free base, and pharmacokinetic parameters are expressed in mean ± SD. The formulations for clinical studies (FMI formulation 0.25, 0.5, 1, and 2 mg tablets)[15] were used in the phase III study of siponimod. The bioequivalence between formulations for clinical studies (FMI formulation 0.25, 0.5, and 1 mg tablets) and the proposed commercial formulation (0.25 mg tablets) and between the formulation for clinical studies (FMI formulation, 2 mg tablets) and the proposed commercial formulation (2 mg tablets) was confirmed by dissolution tests.

6.1.1 Absolute BA (reference CTD 5.3.1.1-1, Study A2126)

Following a single oral administration of siponimod (FMI formulation 0.25 mg) to non-Japanese healthy adults (15 subjects included in the pharmacokinetics analysis), or following a single continuous intravenous administration (for 3 hours) of the injectable siponimod solution, C_{max} of unchanged siponimod in plasma was 1.75 ± 0.375 and 3.27 ± 0.535 ng/mL, respectively, and $AUC_{0-\infty}$ was 69.4 ± 17.2 and 82.4 ± 20.1 ng•h/mL, respectively. The geometric mean of the absolute BA in oral administration of siponimod (FMI formulation) relative to the BA in intravenous administration was 84%.

6.1.2 Bioequivalence (reference CTD 5.3.1.2-2, Study A2111)

A single dose of siponimod (Market Formulation [MF][16] [the formulation for clinical studies during the early development phase, 0.25 mg or 4 mg tablet] or FMI formulation [0.25 mg or 4 mg tablet]) was administered orally under fasting conditions to non-Japanese healthy adults (subjects included in the pharmacokinetics analysis, 31 per group), and the bioequivalence of the formulations was investigated by the cross-comparison method. The geometric mean ratio (MF formulation/FMI formulation) [90% confidence interval (CI)] of C_{max} and AUC_t of unchanged siponimod in plasma was 1.01 [0.97, 1.06] and 1.02 [1.00, 1.05], respectively, in 0.25 mg tablets and 1.00 [0.94, 1.06] and 0.98 [0.94, 1.02], respectively, in 4 mg tablets, demonstrating the bioequivalence between MF and FMI formulations for both 0.25 mg and 4 mg tablets.

6.1.3 Food effect (CTD 5.3.1.2-2, Study A2111)

A single dose of siponimod (FMI formulation, 0.25 or 4 mg) was administered orally to non-Japanese healthy adults (subjects included in the pharmacokinetics analysis, 31 per group) under fasting conditions or after a high-fat meal, and the effect of food on the pharmacokinetics of siponimod was investigated by the cross-comparison method. The geometric mean ratio (administration after meal/administration under fasting conditions) [90% CI] of C_{max} and $AUC_{0-\infty}$ was 1.00 [0.95, 1.04] and

[15] The formulation with the core identical to that of the proposed commercial formulation, coated with a film of a different color
[16] The formulation different from the proposed commercial formulation in the type and amount of lubricants, amounts of diluents and disintegrators, and color and amount of coating film.

1.00 [0.97, 1.02], respectively, in 0.25 mg tablets and 0.91 [0.86, 0.97] and 0.96 [0.92, 1.00], respectively, in 4 mg tablets, showing that food does not significantly affect the pharmacokinetics of siponimod either with 0.25 mg or 4 mg tablets.

6.2 Clinical pharmacology

The applicant submitted, as evaluation data, results of *in vitro* studies using human biomaterials,[17] a pharmacokinetic study in Japanese healthy adults (CTD 5.3.3.1-1, Study A1101), studies in special populations (CTD 5.3.3.3-1, Study A2122; CTD 5.3.3.3-2, Study A2128; CTD 5.3.3.3-3, Study A2129), a QT/corrected QT (QTc) study in non-Japanese healthy adults (CTD 5.3.4.1-1, Study A2118), a drug interaction study with itraconazole (CTD 5.3.3.4-1, Study A2124), and pharmacodynamics studies (CTD 5.3.4.1-2, Study A2110; CTD 5.3.4.1-3, Study A2107). The applicant also submitted, as reference data, results of pharmacokinetic studies in non-Japanese healthy adults[18] and drug-drug interaction studies.[19] Unless specified otherwise, the amount of siponimod is expressed in terms of free base, the pharmacological parameter t_{max} in median, and other pharmacological parameters in mean ± SD. Main pharmacokinetic study results are described below.

6.2.1 Study using human biomaterials

^{14}C-labeled siponimod (4-40 ng/mL) was added to human plasma, and the plasma protein binding rate was investigated by gel filtration. The fraction unbound was 0.013 to 0.022. The fraction of ^{14}C-labeled siponimod unbound to high-density lipoprotein, low-density lipoprotein, very-low-density lipoprotein, serum albumin, γ-globulin, and α1-acid glycoprotein was 0.04, 0.07, 0.11, 0.08 to 0.10, 9.07, and 0.21, respectively. ^{14}C-labeled siponimod (10-10000 ng/mL) was added to human blood, and distribution in blood cells was investigated. The distribution rate of siponimod was 32% (CTD 4.2.2.3-2, Study DMPK R0400881-01).

^{14}C-labeled siponimod (5-10 μmol/L) was added to human liver microsomes and hepatocytes, and the mixtures were incubated at 37°C for 1 to 3 hours. The main metabolites were hydroxylated forms of siponimod (M5, M6, and M7). The glucuronate conjugate of M5 (M3) and the sulfate conjugate of a hydroxylated form (M4) also were detected (CTD 4.2.2.4-2, Study R0400863-01).

Using microsomes expressing cytochrome P450 (CYP) isoforms,[20] metabolism of ^{14}C-labeled siponimod (10-40 μmol/L) was investigated. Siponimod was metabolized mainly by CYP2C9 and

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17) CTD 4.2.2.3-2, Study DMPK R0400881-01; CTD 5.3.2.1-1, Study DMPK R1300334, Study DMPK RCBAF312A2129-01; CTD 5.3.2.1-2, Study DMPK R1400021; CTD 4.2.2.3-4, Study DMPK R1500677-01; CTD 5.3.2.2.1, Study DMPK R0500432; CTD 5.3.2.2.2, Study DMPK R0500496; CTD 5.3.2.2-3, Study DMPK R0500497-01; CTD 5.3.2.2.4, Study DMPK R1200710; CTD 5.3.2.2.5, Study DMPK R1300188; CTD 5.3.2.2.6, Study DMPK R1300932; CTD 5.3.2.2.7, Study DMPK R1300933; CTD 5.3.2.2.8, Study DMPK R1500795; CTD 5.3.2.2.9, Study DMPK R1500796; CTD 5.3.2.2.10, Study DMPK R1600759-01; CTD 5.3.2.2.11, Study DMPK R1701078; CTD 5.3.2.3.1, Study DMPK R0800531; CTD 5.3.2.3.2, Study DMPK R1300921; CTD 5.3.2.3.3, Study DMPK R1200722; CTD 5.3.2.3.4, Study DMPK R1300847; CTD 5.3.2.3.6, Study DMPK R1300849; CTD 5.3.2.3.7, Study DMPK R1300852; CTD 5.3.2.3.8, Study DMPK R1300853; CTD 5.3.2.3.9, Study DMPK R1500825; CTD 5.3.2.3.10, DMPK Study R1500826; CTD 5.3.2.3.11, Study DMPK R1200723; CTD 5.3.2.3.12, Study DMPK R1200724; CTD 5.3.2.3.13, Study DMPK R1200725; CTD 5.3.2.3.15, Study DMPK R1300856; CTD 5.3.2.3.14, Study DMPK R1300855; CTD 5.3.2.3.16: Study DMPK R1300857, CTD 5.3.2.3.17, Study DMPK R1500827; CTD 5.3.2.3.18, Study DMPK R1500828; CTD 5.3.2.3.19, Study DMPK R1500829; CTD 5.3.2.3.5, Study DMPK R1300848; CTD 5.3.2.3.20, Study DMPK R1300854; CTD 5.3.2.3.21, Study DMPK R1500830

18) Reference CTD 5.3.3.1-2, Study A2101; reference CTD 5.3.3.1-3, Study A2102; reference CTD 5.3.3.1-4, Study A2105; reference CTD 5.3.3.1-5, Study A2104

19) Reference CTD 5.3.3.4-3, Study A2108; reference CTD 5.3.3.4-2, Study A2125; reference CTD 5.3.3.4-4, Study A2121; reference CTD 5.3.3.4-5, Study A2116

20) CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9*1, CYP2C18, CYP2C19, CYP2D6*1, CYP2E1, CYP2J2, CYP3A4, CYP3A5, CYP3A7, CYP4A11, CYP4F2, CYP4F3A, CYP4F3B, CYP4F12, and CYP19

CYP3A4. Using pooled human liver microsomes, metabolism of ^{14}C-labeled siponimod (1-300 μmol/L) was investigated. The apparent hepatic clearance of siponimod was 3.8 μL/mg/min. The intrinsic clearance, calculated from a study conducted using CYP2C9 and CYP3A4-expressing microsomes in a similar manner, was 75.2 μL/nmol/min and 8.3 μL/nmol/min, respectively. Using individual human liver microsome samples containing CYP2C9*1/*1, CYP2C9*2/*2, or CYP2C9*3/*3 obtained from different donors, metabolism of ^{14}C-labeled siponimod (10-40 μmol/L) was investigated. The metabolic rate of siponimod by CYP2C9*2/*2 and CYP2C9*3/*3 was 1/3 and 1/10 times of the rate by CYP2C9*1/*1, respectively (CTD 5.3.2.2-1, Study DMPK R0500432).

Using substrates[21)] specific to CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4/5, respectively, the inhibitory effect of siponimod (0.2-200 μmol/L), M3 (0.39-100 μmol/L), and M17 (0.05-12.5 μmol/L) against each CYP isoform in human liver microsomes was investigated. IC_{50} of siponimod against CYP2C9 and CYP3A4/5 was 230 and 100 μmol/L, respectively, and IC_{50} of M3 against CYP2B6 and CYP2C9 was 94 and 80 μmol/L, respectively. Siponimod, M3, and M17 did not inhibit other CYP isoforms within the concentration range tested. Siponimod inhibited CYP2C9 in a time-dependent manner with an inhibitory constant (Ki) and maximum inactivation rate constant (k_{inact}) of 54 ± 58 μmol/L and 0.006 ± 0.004 min^{-1}, respectively. However, the AUC ratio calculated by a static pharmacokinetics model and a physiological pharmacokinetics model was 1.09 and 1.01, respectively, from which the applicant considered that the time-dependent inhibition does not have any clinically significant effect. (CTD 5.3.2.2-3, Study DMPK R0500497-01; CTD 5.3.2.2-5, Study DMPK R1300188; CTD 5.3.2.2-6, Study DMPK R1300932; CTD 5.3.2.2-9, Study DMPK R1500796).

Siponimod (1-10 μmol/L), M3 (1-100 μmol/L), or M17 (1-10 μmol/L) was added to human hepatocytes, and the activities of siponimod, M3, and M17 to induce CYP1A2, CYP2B6, CYP2C9, and CYP3A4/5 was investigated. Neither siponimod nor metabolites M3 and M17 showed a clear induction on these metabolic enzymes (CTD 5.3.2.2-4, Study DMPK R1200710; CTD 5.3.2.2-7, Study DMPK R1300933; CTD 5.3.2.2-8, Study DMPK R1500795).

^{14}C-labeled siponimod (10 μmol/L) was added to human hepatocytes, and the mixture was incubated at 37°C for 0 to 3 hours. As a result, a reactive metabolite was detected, and its concentration (196 pmol/10^6 cells) was higher than that in rats and monkeys (47 pmol/10^6 cells in both animal species). However, taking account of the observation that C_{max} of unchanged siponimod under steady state following administration of siponimod (2 mg) was 0.6 nmol/L, the applicant considered that the reactive metabolite has only a minimal clinical effect (CTD 4.2.2.4-2, Study DMPK R0400863-01).

When siponimod (0.5-50 μmol/L) was added to a monolayer of Caco-2 cells, siponimod transport was not inhibited by specific inhibitors,[22)] which suggested that siponimod is not a substrate of P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), or multidrug resistance associated protein 2 (MRP2) (CTD 5.3.2.3-2, Study DMPK R1300921).

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21) CYP1A2, phenacetin; CYP2A6, coumarin; CYP2B6, bupropion; CYP2C8, paclitaxel; CYP2C9, diclofenac; CYP2C19, *S*-mephenytoin; CYP2D6, (±)-bufuralol; CYP2E1, chlorzoxazone; CYP3A4/5, midazolam, testosterone

22) P-gp, LY335979; BCRP, Ko143; MRP2, MK571

When siponimod (0.5-50 μmol/L) was added to human hepatocytes, siponimod transport was not inhibited by specific inhibitors,[23] which suggested that siponimod is not a substrate of organic anion transporting polypeptide (OATP), organic cation transporter (OCT), or organic anion transporter (OAT) (CTD 4.2.2.7-1, Study DMPK R0700963).

Siponimod (0.01-52.6 μmol/L) was added to MDCKII cells engineered to express BCRP, to LLC-PK1 cells engineered to express P-gp, or to Sf9 cells engineered to express bile salt export pump (BSEP) or MRP2. Siponimod did not affect the transporting activities of these drug transporters (CTD 5.3.2.3-3, Study DMPK R1200722; CTD 5.3.2.3-4, Study DMPK R1300847; CTD 5.3.2.3-6, Study DMPK R1300849).

M3 or M17 (0.07-400 μmol/L) was added to MDCKII cells engineered to express BCRP, to LLC-PK1 cells engineered to express P-gp, or to Sf9 cells engineered to express BSEP. Neither M3 nor M17 affected the transporting activity of these drug transporters. When M17 (0.07-1.35 μmol/L) was added to Sf9 cells engineered to express MRP2, M17 did not affect the transporting activity of MRP2 (CTD 5.3.2.3.7, Study DMPK R1300852; CTD 5.3.2.3.8, Study DMPK R1300853; CTD 5.3.2.3.9, Study DMPK R1500825; CTD 5.3.2.3.10, Study DMPK R1500826).

Siponimod (0.05-24.30 μmol/L) or M3 (0.1-400 μmol/L) was added to human embryonic kidney (HEK)293 cells engineered to express OATP1B1, OATP1B3, OAT1, OAT3, OCT1, or OCT2. IC_{50} of siponimod against OATP1B1 and OATP1B3 was 1.65 ± 1.59 and 2.88 ± 1.21 μmol/L, respectively, and IC_{50} of M3 was 3.7 ± 0.7 and 4.1 ± 0.3 μmol/L, respectively. Siponimod had no effect on the transporting activities of OAT1, OAT3, OCT1, and OCT2 (CTD 5.3.2.3-11, Study DMPK R1200723; CTD 5.3.2.3-12, Study DMPK R1200724; CTD 5.3.2.3-13, Study DMPK R1200725; CTD 5.3.2.3-14, Study DMPK R1300855; CTD 5.3.2.3-15, Study DMPK R1300856; CTD 5.3.2.3-16, Study DMPK R1300857).

When siponimod (0.20-40 μmol/L) was added to HEK293 cells engineered to express multidrug and toxic extrusion (MATE)1 or MATE2-K, siponimod did not affect the transporting activities of these drug transporters (CTD 5.3.2.3-5, Study DMPK R1300848).

6.2.2 Studies in healthy adults

A single oral administration of siponimod (0.5, 2.5, 5, or 10 mg) was administered to Japanese healthy adults (32 subjects included in the pharmacokinetics analysis). Table 22 shows the pharmacokinetic parameter values of unchanged siponimod in plasma (CTD 5.3.3.1-1, Study A1101).

[23] OATP, rifamycin SV; OCT, tetraethylammonium; OAT, p-aminohippuric acid

Table 22. Pharmacokinetic parameters of unchanged siponimod in plasma following a single oral administration of siponimod to Japanese healthy adults

Dose (mg)	No. of subjects evaluated	C_{max} (ng/mL)	t_{max} (h)[a)]	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng•h/mL)
0.5	8	4.55 ± 0712	4.00 [3.00, 6.00]	30.2 ± 9.23	162 ± 47.5
2.5	8	21.5 ± 2.73	4.00 [3.00, 4.00]	28.6 ± 2.20	686 ± 60.5
5	8	46.1 ± 9.96	3.50 [2.00, 12.0]	29.9 ± 7.16	1290 ± 262
10	8	102 ± 18.1	4.00 [3.00, 6.00]	42.2 ± 14.3	3330 ± 1000

Mean ± SD
a) Median [minimum, maximum]

A single dose of an oral siponimod solution (0.1, 0.3, 1, or 2.5 mg) or a siponimod capsule (2.5, 5, 10, 17.5, 25, or 75 mg) was administered to non-Japanese healthy adults (80 subjects included in the pharmacokinetics analysis). Table 23 shows the pharmacokinetic parameter values of unchanged siponimod in plasma (reference CTD 5.3.3.1-2, Study A2101).

Table 23. Pharmacokinetic parameters of unchanged siponimod in plasma following a single oral administration of siponimod to non-Japanese healthy adults

Formulation	Dose (mg)	No. of subjects evaluated	C_{max} (ng/mL)	t_{max} (h)[a)]	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng•h/mL)
Oral siponimod solution	0.1	11	0.661 ± 0.265	4.00 [3.00, 8.00]	34.1 ± 9.68	26.2 ± 9.73
	0.3	8	2.26 ± 0.141	5.00 [4.00, 8.00]	34.3 ± 9.43	90.8 ± 17.4
	1	8	8.02 ± 3.56	5.00 [4.00, 15.67]	46.9 ± 10.5	361 ± 98.0
	2.5	6	23.0 ± 7.70	4.00 [3.00, 8.00]	27.6 ± 6.17	803 ± 251
Siponimod capsule	2.5	7	19.6 ± 3.53	6.00 [4.00, 8.02]	29.7 ± 4.80	764 ± 181
	5	8	39.3 ± 8.60	3.00 [2.00, 8.02]	31.8 ± 5.77	1290 ± 257
	10	8	79.5 ± 21.4	5.00 [3.85, 16.00]	32.4 ± 5.29	2730 ± 350
	17.5	8	115 ± 29.8	4.00 [2.00, 8.00]	44.7 ± 15.2	4340 ± 968
	25	8	225 ± 63.6	3.50 [1.50, 12.00]	48.5 ± 9.31	8350 ± 1990
	75	8	542 ± 250	6.00 [2.00, 24.00]	57.1 ± 6.71	20800 ± 11700

Mean ± SD
a) Median [minimum, maximum]

Siponimod (0.3, 1, 2.5, 10, or 20 mg) was administered orally once daily for 28 days to non-Japanese healthy adults (34 subjects included in the pharmacokinetics analysis). Table 24 shows pharmacokinetic parameter values of unchanged siponimod in plasma on Day 28 (Reference CTD 5.3.3.1-4, Study A2105).

Table 24. Pharmacokinetic parameters of unchanged siponimod in plasma in multiple oral administration of siponimod to non-Japanese healthy adults

Formulation	Dose	No. of subjects evaluated	Day 1		Day 28	
			C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)	C_{max} (ng/mL)	AUC_{τ} (ng•h/mL)
Oral siponimod solution	0.3	6	2.14 ± 0.268	36.4 ± 3.54	5.36 ± 0.815	99.3 ± 17.6
	1	6	8.03 ± 0.443	136 ± 9.81	15.0 ± 1.45	285 ± 43.9
Siponimod capsule	2.5	5	20.5 ± 8.83	343 ± 145	40.1 ± 12.5	739 ± 266
	10	9	84.6 ± 9.67	1380 ± 153	151 ± 38.5	2640 ± 656
	20	8	165 ± 30.9	2760 ± 368	364 ± 59.3	6520 ± 1470

Mean ± SD

Following a single oral administration of ^{14}C-labeled siponimod (10 mg) to non-Japanese healthy adults (4 subjects included in the pharmacokinetics analysis), 3.70% and 86.7%, respectively, of the total radioactivity administered were excreted in urine and feces within 312 hours after administration. Analysis of the radioactivity in urine and feces collected within 192 hours after administration showed that the urine contained M3 (2.1% of the total radioactivity administered), M1 (compound formed by cleavage of oxime ether bond), M2 (hydroxylate form of oxime ether), and M8 (reduced form of M1)

(0.2%-0.5%) but not unchanged siponimod, and that the feces contained M5 (45.1% of the total radioactivity administered), M4 (12.6%), unchanged siponimod (9.2%), M7 (6.4%), and M6 (3.2%) (reference CTD 5.3.3.1-5, Study A2104).

6.2.3 Studies of intrinsic factors

6.2.3.1 Effect of hepatic function (CTD 5.3.3.3-1, Study A2122)

A single dose of siponimod (0.25 mg) was administered orally to non-Japanese subjects with normal hepatic function and to non-Japanese subjects with hepatic impairment (subjects included in the pharmacokinetics analysis, 14 subjects with normal hepatic function, 8 subjects with mild hepatic impairment [Child-Pugh score 5-6], 7 subjects with moderate hepatic impairment [Child-Pugh score 7-9], 8 subjects with severe hepatic impairment [Child-Pugh score 10-15]). Table 25 shows pharmacokinetic parameter values of unchanged siponimod in plasma. The geometric mean ratio (in percentage) [90% CI] of C_{max} and $AUC_{0-\infty}$ in subjects with hepatic impairment to C_{max} and $AUC_{0-\infty}$ in subjects with normal hepatic function was 116 [94.2, 142] and 105 [76.8, 143], respectively, in subjects with mild hepatic impairment, 86.8 [72.0, 105] and 89.5 [78.1, 103] in subjects with moderate hepatic impairment, and 83.7 [66.6, 105] and 115 [83.6, 159] in subjects with severe hepatic impairment. The fraction of siponimod unbound to plasma protein (equilibrium gel filtration method) was 0.0127% to 0.0142%, 0.0150%, 0.0154%, and 0.0186%, respectively, in subjects with normal hepatic function and subjects with mild, moderate, and severe hepatic impairment, showing no significant difference.

Table 25. Pharmacokinetic parameters of unchanged siponimod in plasma following a single oral administration of siponimod to non-Japanese subjects with normal hepatic function and non-Japanese subjects with hepatic impairment

	No. of subjects evaluated	C_{max} (ng/mL)	t_{max} (h)[a)]	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng•h/mL)
Subjects with normal hepatic function[b)]	8	1.74 ± 0.439	4.00 [3.00, 8.00]	27.6 ± 4.86	64.2 ± 20.8
Subjects with mild hepatic impairment	8	2.03 ± 0.532	4.00 [2.00, 8.00]	30.3 ± 15.1	68.3 ± 24.5
Subjects with normal hepatic function[b)]	8	1.80 ± 0.417	4.00 [3.00, 8.00]	27.8 ± 5.22	63.2 ± 18.6
Subjects with moderate hepatic impairment	7	1.54 ± 0.191	4.00 [3.00, 6.00]	27.0 ± 7.65	53.9 ± 7.57
Subjects with normal hepatic function[b)]	8	1.94 ± 0.603	4.00 [3.00, 8.00]	25.7 ± 4.55	64.9 ± 23.6
Subjects with severe hepatic impairment	8	1.58 ± 0.304	4.00 [3.00, 12.00]	40.1 ± 22.6	73.7 ± 25.4

Mean ± SD

a) Median [minimum, maximum]; b) Healthy adults matched for age, sex, and body mass index (BMI) with subjects with mild, moderate, or severe hepatic impairment (including duplicate counting)

6.2.3.2 Effect of renal function (CTD 5.3.3.3-3, Study A2129)

A single dose of siponimod (0.25 mg) was administered orally to non-Japanese subjects with normal renal function (eGFR ≥90 mL/min/1.73 m^2, 8 subjects included in the pharmacokinetics analysis) and to non-Japanese subjects with severe renal impairment (eGFR <30 mL/min/1.73 m^2, 8 subjects included in the pharmacokinetics analysis). Table 26 shows the pharmacokinetic parameter values of unchanged siponimod in plasma. The geometric mean ratio (in percentage) [90% CI] of C_{max} and $AUC_{0-\infty}$ in subjects with severe renal impairment to subjects with normal renal function was 92 [79, 108] and 124 [90, 172], respectively. The fraction of siponimod unbound to plasma protein (equilibrium gel filtration method)

was 0.0264% and 0.0291%, respectively, in subjects with normal renal function and subjects with severe renal impairment, showing no significant difference.

Table 26. Pharmacokinetic parameters of unchanged siponimod in plasma following a single oral administration of siponimod to non-Japanese subjects with normal renal function and non-Japanese subjects with renal impairment

	No. of subjects evaluated	C_{max} (ng/mL)	t_{max} (h)[a)]	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng•h/mL)
Subjects with normal renal function[b)]	8	2.27 ± 0.544	4.00 [3.00, 8.00]	26.3 ± 8.37	78.2 ± 22.0
Subjects with severe renal impairment	8	2.07 ± 0.360	6.00 [4.00, 8.00]	37.4 ± 11.0	102 ± 46.6

Mean ± SD

a) Median [minimum, maximum]; b) Healthy adults matched for age, sex, and BMI with subjects with severe renal impairment

6.2.3.3 Effect of CYP2C9 genotype (CTD 5.3.3.3-2, Study A2128)

A single dose of siponimod (0.25 mg) was administered orally to non-Japanese healthy adults with CYP2C9*1/*1, CYP2C9*2/*3, and CYP2C9*3/*3 (subjects included in the pharmacokinetics analysis, 12 subject with CYP2C9*1/*1, 6 subjects with CYP2C9*2/*3, 6 subjects with CYP2C9*3/*3). Table 27 shows the pharmacokinetic parameter values of unchanged siponimod in plasma and M3. In subjects with CYP2C9*2/*3 or CYP2C9*3/*3, C_{max} of unchanged siponimod in plasma was 1.21 and 1.16 times, respectively, greater than that in subjects with CYP2C9*1/*1, and $AUC_{0-\infty}$was 2.05 and 3.84 times greater. C_{max} of M3 in subjects with CYP2C9*2/*3 or CYP2C9*3/*3 decreased by 49% and 88%, respectively, and $AUC_{0-\infty}$ by 12% and 66%, respectively, from those observed in subjects with CYP2C9*1/*1.

Table 27. Pharmacokinetic parameters of unchanged siponimod and M3 in plasma following a single oral administration of siponimod to non-Japanese healthy adults

Genotype	No. of subjects evaluated	Unchanged siponimod				M3			
		C_{max} (ng/mL)	t_{max} (h)[a)]	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng•h/mL)	C_{max} (ng/mL)	t_{max} (h)[a)]	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng•h/mL)
*1/*1	12	2.06 ± 0.375	4.00 [2.00, 6.00]	28.6 ± 5.40	71.9 ± 15.8	0.717 ± 0.315	6.00 [6.00, 16.00]	33.4 ± 5.94	34.5 ± 11.4
*2/*3	6	2.47 ± 0.320	5.00 [4.00, 8.00]	53.0 ± 16.6	146 ± 22.1	0.358 ± 0.126	12.00 [8.00, 36.00]	55.7 ± 11.5	31.1 ± 13.3
*3/*3	6	2.43 ± 0.704	4.00 [4.00, 16.00]	127 ± 15.9	277 ± 66.9	0.0923 ± 0.0659	24.00 [12.00, 72.00]	100 ± 30.3	11.5 ± 3.21

Mean ± SD

a) Median [minimum, maximum]

6.2.4 Drug-drug interactions

Interactions of siponimod with the following drugs were investigated: Fluconazole, itraconazole, rifampicin, propranolol, ethinyl estradiol, and levonorgestrel. Table 28 shows the effect of concomitant drugs on the pharmacokinetics of siponimod, and Table 29 shows the effect of siponimod on the pharmacokinetics of the concomitant drugs.

Table 28. Effect of concomitant drugs on the pharmacokinetics of siponimod

Dosage regimen of siponimod	Concomitant drug (dosage regimen)	Analyte in plasma	No. of subjects evaluated	Geometric mean ratio[a] [90% CI]			CTD
				C_{max}	$AUC_{0-\infty}$	$t_{1/2}$	
4 mg single dose	Fluconazole (200 mg once daily)[b]	Unchanged siponimod	11[c]	1.10 [1.04, 1.16]	1.98 [1.87, 2.10]	1.51 [1.34, 1.71]	Reference 5.3.3.4-3, Study A2108
0.25 mg single dose	Itraconazole (100 mg twice daily)	Unchanged siponimod	16[d]	1.01 [0.96, 1.06]	0.90 [0.84, 0.96]	0.93 [0.88, 0.99]	5.3.3.4-1, Study A2124
0.25 mg single dose	Itraconazole (100 mg twice daily)	Unchanged siponimod	13[e]	0.94 [0.91, 0.97]	0.76 [0.69, 0.82]	0.78 [0.71, 0.86]	5.3.3.4-1, Study A2124
2 mg once daily	Rifampicin (600 mg once daily)	Unchanged siponimod	15[c]	0.55 [0.52, 0.58]	0.43 [0.41, 0.45][f]	-[g]	Reference 5.3.3.4-2, Study A2125
2 mg once daily	Propranolol (80 mg once daily)	Unchanged siponimod	18[h]	0.93 [0.84, 1.04]	0.93 [0.85 1.03][f]	0.86 [0.71, 1.04]	Reference 5.3.3.4-5, Study A2116

a) Concomitant use/siponimod alone, b) Twice a day on Day 1 only, c) CYP2C9*1/*1, d) CYP2C9*1/*2, e) CYP2C9*1/*3, f) AUC_{τ}, g) Not calculated, h) Except for CYP2C9*3/*3

Table 29. Effect of siponimod on pharmacokinetics of concomitant drugs

Dosage regimen of siponimod	Concomitant drug (dosage regimen)	Analyte in plasma	No. of subjects evaluated	Geometric mean ratio[a] [90% CI]			CTD
				C_{max}	AUC_{τ}	$t_{1/2}$	
2 mg once daily	Propranolol (80 mg once daily)	Propranolol	17[b]	0.85 [0.69, 1.04]	0.82 [0.66, 1.03]	0.86 [0.67, 1.11]	Reference 5.3.3.4-5, Study A2116
4 mg once daily	Ethinyl estradiol (30 µg once daily)	Ethinyl estradiol	23[c]	1.02 [0.96, 1.08]	1.00 [0.96, 1.05]	1.00 [0.90, 1.11]	Reference 5.3.3.4-4, Study A2121
4 mg once daily	Levonorgestrel (150 µg once daily)	Levonorgestrel	23[c]	1.18 [1.11, 1.26]	1.29 [1.24, 1.34]	0.97 [0.86, 1.09]	Reference 5.3.3.4-4, Study A2121

a) With siponimod/without siponimod, b) Except for CYP2C9*3/*3, c) CYP2C9*1/*1

6.2.5 Pharmacodynamics

6.2.5.1 Effect on QT/QTc interval (CTD 5.3.4.1-1, Study A2118)

In a parallel-group study, non-Japanese healthy adults (276 subjects included in the pharmacodynamics analysis) received placebo or siponimod (2-10 mg) orally once daily for 18 days, or received moxifloxacin (400 mg) orally once a day on Day 10 and 18, and the effect on QT/QTc interval was investigated. Subjects in the siponimod group received 0.25 mg on Day 1 to 2, 0.5 mg on Day 3, 0.75 mg on Day 4, 1.25 mg on Day 5, 2 mg on Day 6 to 10, 3 mg on Day 11, 5 mg on Day 12, 8 mg on Day 13, and 10 mg on Day 14 to 18. Table 30 shows the mean difference and 90% CI in ΔΔFridericia-corrected QT (QTcF) interval between the siponimod group and the placebo group measured at identical time points, on Day 10 and 18. In the siponimod group, the upper limit of 90% CI was less than 10 msec at all evaluation time points.[24]

[24] Geometric mean C_{max} of unchanged siponimod in plasma under steady state during the administration of 10 mg, the maximum dose for siponimod, was 152 ng/mL, which was comparable to the maximum C_{max} (165.6 ng/mL) expected in specific patient populations or in the presence of drug-drug interactions.

Table 30. ΔΔQTcF interval in non-Japanese healthy adults receiving siponimod or moxifloxacin

	Days after treatment	Drug	No. of subjects evaluated	Time after administration				
				0.5 hours	2 hours	4 hours	6 hours	12 hours
ΔΔQTcF interval	Day 10	Siponimod (2 mg)	92	4.34 [2.44, 6.24]	6.39 [4.20, 8.57]	6.58 [4.55, 8.61]	6.30 [4.22, 8.39]	4.19 [2.24, 6.13]
		Moxifloxacin (400 mg)	92	7.51 [5.27, 9.74]	11.16 [8.96, 13.36]	10.85 [8.74, 12.96]	8.17 [6.05, 10.28]	6.64 [4.47, 8.80]
	Day 18	Siponimod (10 mg)	92	3.74 [1.42, 6.05]	6.63 [4.28, 8.98]	5.80 [3.22, 8.39]	4.89 [2.30, 7.48]	3.29 [1.23, 5.35]
		Moxifloxacin (400 mg)	92	7.53 [5.13, 9.94]	11.49 [9.20, 13.78]	11.78 [9.36, 14.20]	9.94 [7.38, 12.50]	7.70 [5.46, 9.93]

Unit, msec; mean [90% CI]

6.2.5.2 Negative chronotropic effect at treatment resumption after washout period (CTD 5.3.4.1-2, Study A2110)

Placebo or siponimod (0.5, 1, 2, or 4 mg) was administered once daily for 10 days to non-Japanese healthy adults (117 subjects included in the pharmacodynamics analysis). After a washout period (48, 72, 96, 120, or 192 hours), a single dose of placebo or siponimod (0.5, 1, 2, or 4 mg) was administered orally, and the negative chronotropic effect of siponimod re-administered after the interruption of the multiple oral administration was evaluated.[25)] Table 31 shows the maximum decrease in heart rate ("mean heart rate before administration of study drug" – "mean heart rate after administration of study drug").

Table 31. Maximum decrease in heart rate following a single oral administration of siponimod after a washout period following multiple oral administration of siponimod to non-Japanese healthy adults

Dose	No. of subjects evaluated	C_{max} [a)] (ng/mL)	$AUC_{0\text{-}192h}$ [a)] (ng•h/mL)	Washout period				
				48 hours	72 hours	96 hours	120 hours	192 hours
Placebo	17	-	-	6.64[b)] [4.384, 8.894]	-	7.57 [5.377, 9.769]	-	10.26[b)] [8.008, 12.511]
0.5 mg	17	3.682 ± 1.0272	61.44 ± 17.263	10.81[c)] [8.469, 13.145]	14.22[b)] [11.962, 16.472]	12.79 [10.593, 14.985]	15.65[b)] [13.394, 17.904]	13.79 [11.594, 15.986]
1 mg	17	9.572 ± 2.4275	161.4 ± 40.832	11.86 [9.664, 14.056]	15.74[b)] [13.276, 18.197]	14.54 [12.345, 16.737]	17.39[b)] [15.137, 19.640]	19.15 [16.956, 21.348]
2 mg	16	16.14 ± 5.0816[e)]	272.1 ± 87.637	-	14.24 [11.987, 16.497]	17.53[c)] [15.188, 19.864]	17.69 [15.439, 19.950]	17.99[c)] [15.681, 20.306]
4 mg	16	39.04 ± 14.277[d)]	626.8 ± 227.37[d)]	-	11.77[c)] [9.436, 14.111]	14.71[c)] [12.394, 17.026]	16.31 [14.060, 18.563]	22.73[d)] [20.271, 25.191]

Unit, bpm; mean [90% CI]

a) Mean ± SD, b) n = 16, c) n = 15, d) n = 13, e) n = 17

6.2.5.3 Effect of dose-escalation on negative chronotropic effect (CTD 5.3.4.1-3, Study A2107)

Non-Japanese healthy adults (56 subjects included in the pharmacodynamics analysis) received (1) placebo or siponimod orally once daily for 12 days by a dose-escalation method according to Table 32,

25) Out of 25 combinations of the study drug (placebo, siponimod 0.5, 1, 2, or 4 mg) and the washout period (48, 72, 96, 120, or 192 hours), the following 21 combinations were evaluated: Placebo (48, 96, and 192 hours), siponimod 0.5 mg (48, 72, 96, 120, and 192 hours), siponimod 1 mg (48, 72, 96, 120, and 192 hours), siponimod 2 mg (72, 96, 120, and 192 hours), and siponimod 4 mg (72, 96, 120, and 192 hours).

or (2) siponimod 10 mg orally once daily for 12 days, and chronotropic action was compared. Table 33 shows the maximum decrease in heart rate from baseline in (1) subjects receiving siponimod once daily for 12 days by the dose-escalation method and in (2) subjects receiving siponimod 10 mg once daily for 12 days.

Table 32. Method for dose-escalation in Study A2107

Treatment group	Day 1-2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10-12
Dose-escalation 1	0.25 mg	0.25 mg	0.5 mg	1 mg	2 mg	4 mg	8 mg	10 mg	10 mg
Dose-escalation 2	0.25 mg	0.5 mg	0.75 mg	1.25 mg	2 mg	3 mg	5 mg	8 mg	10 mg
10 mg	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg

Table 33. Maximum decrease in heart rate from baseline in multiple administration of siponimod in non-Japanese healthy adults

Treatment group	No. of subjects evaluated	C_{max} (ng/mL)[a)]	AUC_t (ng•h/mL)[a)]	Day of evaluation				
				Day 2	Day 4	Day 6	Day 8	Day 12
Placebo	14	-	-	7.98 [4.96, 11.00]	7.02 [4.45, 9.59]	7.25 [4.06, 10.44]	8.39 [5.18, 11.59]	5.94 [2.86, 9.01]
Dose-escalation 1	13	159.0 ± 37.941	2924 ± 879.39	16.61 [13.59, 19.63]	15.80 [13.23, 18.36]	21.44 [18.26, 24.63]	14.30 [11.09, 17.50]	6.70 [3.63, 9.78]
Dose-escalation 2	12	170.5 ± 69.076	3250 ± 1555.1	14.29 [11.27, 17.31]	17.82 [15.35, 20.30]	18.70 [15.52, 21.89]	12.85 [9.64, 16.05]	6.22 [3.14, 9.29]
10 mg	12	181.9 ± 63.589	3439 ± 1447.1	16.33 [13.31, 19.36]	13.53 [10.96, 16.10]	15.72 [12.42, 19.03]	15.38 [11.91, 18.84]	12.08 [8.76, 15.40]

Unit, bpm; mean [95% CI]
a) Day 12 of administration, mean ± SD

6.2.6 PPK analysis

Population pharmacokinetics (PPK) analysis was conducted using the data of unchanged siponimod concentration in plasma (a total of 7858 measuring points in 190 healthy adults and 216 patients with RRMS) obtained from 4 phase I studies[26)] in healthy subjects and from a phase II study in patients with RRMS (CTD 5.3.5.1-2, Study A2201). The results showed that the pharmacokinetics of siponimod was described by a 2-compartment model including the first-order elimination process and a mixed zero- and first-order absorption process. Body weight was identified as the covariate for apparent total clearance (CL/F) and apparent central volume of distribution (V_c/F), and food consumption and dosage form (capsule, solution) as covariates for the zero-order administration period to the administration compartment (CTD 5.3.3.5-1, CBAF312A-PhaseI-II-PopPK analysis).

Separately, a PPK analysis was conducted using the data of unchanged siponimod concentration in plasma (a total of 3454 measuring points in 1045 patients with SPMS) obtained from a phase III study in patients with SPMS (CTD 5.3.5.1-1, Study A2304). Based on the final model constructed from the above PPK analyses, a basic model was constructed by incorporating the effect of body weight in CL/F and V_c/F as an allometric function. The results showed that a model was selected that estimated only the population means of CL/F and V_c/F, inter-individual variability, and residual sum of squares. Investigation of the effect of covariates on the constructed basic model identified CYP2C9 genotype as a covariate for CL/F (CTD 5.3.3.5-2, CBAF312A-Phase III-PopPK analysis).

[26)] Reference CTD 5.3.3.1-2, Study A2101; reference CTD 5.3.3.1-4, Study A2105; CTD 5.3.3.1-3, Study A2107; and CTD 5.3.3.1-1, Study A1101

6.R Outline of the review conducted by PMDA

6.R.1 Bioequivalence between formulations with different strengths

PMDA asked the applicant to explain the bioequivalence between 0.25 mg tablets and 2 mg tablets of the proposed commercial formulation.

The applicant's explanation:

- The interchangeable use of siponimod 0.25 mg tables and 2 mg tablets had not been expected. After the submission of the marketing application, the bioequivalence between 0.25 mg tablets and 2 mg tablets of the proposed commercial formulation was subjected to a dissolution test. When a pH 6.8 dissolution medium without polysorbate 80 was used, f2 function was determined to be 58, which was below the criteria of dissolution equivalence (≥61) specified by "Guideline for Bioequivalence Studies of Generic Products for Different Strengths of Oral Solid Dosage Forms" (PMSB/ELD Notification No. 64 dated February 14, 2000, partially revised by PFSB/ELD Notification No. 0229-10 dated February 29, 2012).

- However, in the dissolution medium without polysorbate 80, the drug substance [redacted] between sampling and measurement [see Section 2.R.2]. This may have caused the test samples to fail to meet the criteria. The intended amount of siponimod is released at the intended speed from both proposed commercial formulations, judging from the observations that they meet the criteria of the guideline in other dissolution media and that, even in the above dissolution medium, the 2 formulations showed similar dissolution behaviors, failing to meet the criteria by only a paper-thin margin. In the pH 6.8 dissolution medium without polysorbate 80, the mean dissolution rate was approximately ≤20%, precluding the determination of equivalence.

- Since siponimod 0.25 mg tablets and 2 mg tablets are not meant to be used interchangeably, it will be cautioned in the package insert that the bioequivalence of 0.25 mg tablets and 2 mg tablets is not demonstrated.

PMDA's view:

- The possibility that siponimod 0.25 mg tablets and 2 mg tablets are used interchangeably cannot be excluded completely. Given the medical environment in Japan, the bioequivalence of siponimod 0.25 mg tablets and 2 mg tablets should have been confirmed by a bioequivalence study in humans, etc., even if the above precautions are included in the package insert.

- However, taking account of the data of the dissolution test of siponimod 0.25 mg tablets and 2 mg tablets in other dissolution media, and of the experience of administering a higher dose as the maintenance dose [see Section 7.2], interchangeable use of the tablets is unlikely to cause any significant safety problem. There is little need to conduct an additional bioequivalence study.

- Precautions should be provided in the package insert that the bioequivalence between 0.25 mg tablets and 2 mg tablets has not been demonstrated, and that both formulations should not be used interchangeably.

6.R.2 Difference in pharmacokinetics between Japanese and non-Japanese subjects

PMDA asked the applicant to explain a difference in pharmacokinetics between Japanese and non-Japanese subjects.

The applicant's explanation:

Table 34 and Figure 1 show the pharmacokinetic parameters of unchanged siponimod in plasma following a single oral administration of siponimod (2.5, 5, or 10 mg) under fasting conditions in the Japanese phase I study (CTD 5.3.3.1-1, Study A1101) and in the foreign phase I study (reference CTD 5.3.3.1-2, Study A2101). C_{max} and $AUC_{0-\infty}$ in Japanese subjects were similar to those in non-Japanese subjects, with their individual values overlapping between Japanese and non-Japanese subjects. The PPK analyses (CTD 5.3.3.5-1, CBAF312A-PhaseI-II-PopPK analysis, CTD 5.3.3.5-2 CBAF312A-PhaseIII-PopPK analysis) did not detect any effect of race.

Table 34. Pharmacokinetic parameters of unchanged siponimod in plasma following a single oral administration of siponimod in Japanese and non-Japanese subjects

Dose (mg)	No. of subjects evaluated	Japanese subjects		Non-Japanese subjects	
		C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng•h/mL)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng•h/mL)
2.5	8	21.5 ± 2.73	686 ± 60.5	19.6 ± 3.53[a)]	764 ± 181[a)]
5	8	46.1 ± 9.96	1290 ± 262	39.3 ± 8.60	1290 ± 257
10	8	102 ± 18.1	3330 ± 1000	79.5 ± 21.4	2730 ± 350

Mean ± SD
a) n = 7

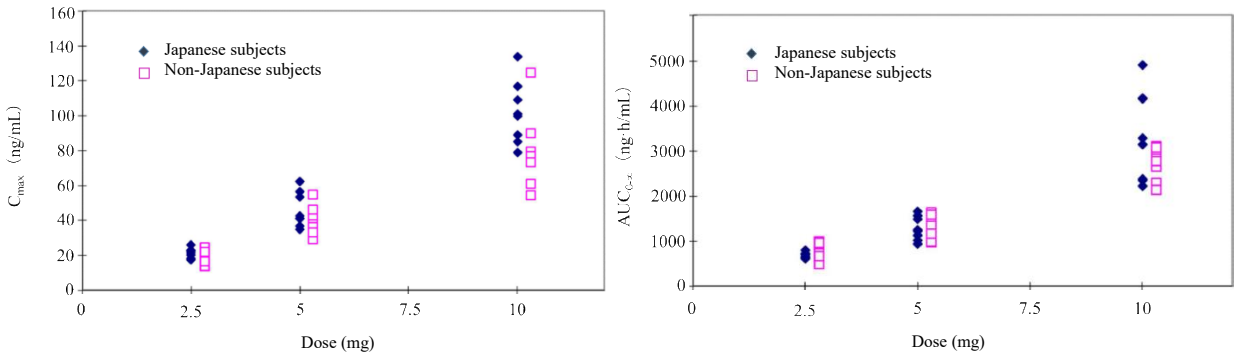

Figure 1. C_{max} and $AUC_{0-\infty}$ of unchanged siponimod in plasma following a single oral administration of siponimod to Japanese and non-Japanese healthy adults

These results demonstrate that there is no observable ethnic difference in the pharmacokinetics of siponimod.

PMDA accepted the above applicant's explanation.

6.R.3 Dose adjustment according to CYP2C9 genotype

PMDA asked the applicant to explain whether it is necessary to adjust the dose according to CYP2C9 genotype and to pay attention to CYP2C9 genotype, taking account of the observation that siponimod is metabolized mainly by CYP2C9 [see Section 6.2.1].

The applicant's explanation:

- Table 35 shows the percentage of each CYP2C9 genotype among different races and ethnicities (*Clin Pharmacol Ther*. 77:1-16, *Thromb Res*. 134:537-44, *Clin Pharmacol The.*; 77:1-16, *Pharmacogenetics.* 12:251-63).

Table 35. Percentage of each CYP2C9 genotype among different races

CYP2C9 genotype	Caucasians	Asians (Japanese)	Africans
*1/*1	62-65	85-97 (95-96)	75-87
*1/*2	20-24	0-5 (0)	5-9
*1/*3	9-12	4-7 (4-5)	4
*2/*2	1-2	0-0.1 (0)	0-0.1
*2/*3	1.4-1.7	0-0.2 (0)	0-0.1
*3/*3	0.3-0.4	0-0.2 (0.1)	0

Percentage reported (%)

- Following a single oral administration of siponimod (0.25 mg), $AUC_{0-\infty}$ in subjects with CYP2C9*2/*3 or CYP2C9*3/*3 was 2.05 and 3.84 times, respectively, higher than $AUC_{0-\infty}$ in subjects with CYP2C9*1/*1 (CTD 5.3.3.3-2, Study A2128). The population pharmacokinetic analysis (CTD 5.3.3.5-2, CBAF312A-PhaseIII-PopPK analysis) showed that AUC was 1.25, 1.61, and 1.91 times, respectively, higher in patients with CYP2C9*2/*2, CYP2C9*1/*3, or CYP2C9*2/*3 than in patients with CYP2C9*1/*1 or CYP2C9*1/*2.

- In patients with CYP2C9*3/*3, SPMS may possibly be treated with siponimod continuously over an extended period. Since there are no data of a long-term exposure to siponimod at the level approximately 4 times higher than the usual exposure, there is a safety concern, such as the effect on the immune system in particular, in administering siponimod over a long time period. In the global phase III study (CTD 5.3.5.1-1, Study A2304), patients with CYP2C9*3/*3 were excluded. Also, siponimod is contraindicated in patients with CYP2C9*3/*3 in the US and Europe. Based on the above, the precaution will be provided in the package insert that siponimod should not be used in patients with CYP2C9*3/*3, as in the US and Europe.

- As for administration in patients with CYP2C9*1/*3 or CYP2C9*2/*3, in whom the exposure to siponimod is 1.6 to 2 times higher than in patients with CYP2C9*1/*1, it will be desirable to reduce the maintenance dose to 1 mg/day, half the usual maintenance dose. When the maintenance dose of 1 mg/day is used, the dose of siponimod should be increased in a stepwise manner as in the case of the dose of 2 mg/day up to Day 4, and from Day 5, the maintenance dose of 1 mg/day should be administered.

- As for administration to patients with CYP2C9*1/*2 or CYP2C9*2/*2, no particular precaution will be necessary because the exposure to siponimod is only 1 to 1.25 times higher than in patients with CYP2C9*1/*1.

- Thus, a CYP2C9 genotype test is essential before administration of siponimod. The applicant will develop a system for conducting a CYP2C9 genotype test prior to the marketing of siponimod.

PMDA's view:

- Administration of siponimod to patients with CYP2C9*3/*3 should be avoided. It is therefore appropriate to contraindicate siponimod in this patient group.

- As for patients with CYP2C9*1/*3 or CYP2C9*2/*3, the maintenance dose should be decreased to 1 mg/day in these patient groups, given the unknown effect of a long-term treatment with siponimod at a high exposure on the immune system in patients with reduced CYP2C9 activity. When the maintenance dose of 1 mg/day is used, the dose of siponimod should be increased in a stepwise manner as in the case of the dose of 2 mg/day up to Day 4, and from Day 5, the maintenance dose of 1 mg/day should be administered, in a similar manner as in the dose-escalation method used in resuming administration in the global phase III study (CTD 5.3.5.1-1, Study A2304).

- No particular precaution is necessary in administering siponimod to patients with CYP2C9*1/*2 or CYP2C9*2/*2.

- Precautions should be provided in the package insert that a CYP2C9 genotype test should be performed before administration of siponimod.

6.R.4 Dose adjustment in co-administration with other drugs

Since siponimod is metabolized mainly by CYP2C9 and partly by CYP3A4 [see Section 6.2.1], PMDA asked the applicant about the necessity of giving precautions on the concomitant use of siponimod with an inhibitor or inducer of CYP2C9 or CYP3A4.

The applicant's explanation:

- Subjects with CYP2C9*1/*1 genotype received siponimod in combination with fluconazole (a moderate CYP2C9 and CYP3A4 inhibitor) in a clinical study. AUC and C_{max} were 1.98 and 1.1 times, respectively, higher than those observed following the administration of siponimod alone [see Section 6.2.4].

- Subjects with CYP2C9*1/*2 or CYP2C9*1/*3 genotypes received siponimod in combination with itraconazole (a potent CYP3A4 inhibitor) in a clinical study. AUC in subjects with CYP2C9*1/*2 genotype was not affected, whereas in subjects with CYP2C9*1/*3 genotype, AUC and C_{max} decreased by 24% and 6%, respectively, from the values obtained by the administration of siponimod alone [see Section 6.2.4].

- Subjects with CYP2C9*1/*1 received siponimod in combination with rifampicin (a moderate CYP2C9 inducer and potent CYP3A4 inducer) in a clinical study. AUC and C_{max} decreased by 57% and 45%, respectively, from the values obtained by the administration of siponimod alone [see Section 6.2.4].

- In order to investigate the effect of a typical inhibitors or inducer of CYP2C9 and CYP3A4 on the pharmacokinetics of siponimod in patient with each CYP2C9 genotype (CYP2C9*1/*1, CYP2C9*1/*2, CYP2C9*2/*2, CYP2C9*1/*3, CYP2C9*2/*3), a physiologically-based

pharmacokinetics (PBPK) model (CTD 5.3.2.2-10, 1600759-01 analysis) was constructed.[27)] PBPK model analysis was conducted using Simcyp version 16. Validation of the model was conducted using the data of the phase I studies (reference CTD 5.3.3.1-2, Study A2101 and reference CTD 5.3.3.1-4, Study A2105), the absolute BA study (reference CTD 5.3.1.1-1, Study A2126), the study on the effect of CYP2C9 genotype (CTD 5.3.3.3-2, Study A2128), and drug-drug interaction studies (CTD 5.3.3.4-1, Study A2124, reference CTD 5.3.3.4-3, Study A2108, and reference CTD 5.3.3.4-2, Study A2125). Tables 36 and 37 show the predicted and the measured values of C_{max} and AUC of unchanged siponimod following the administration of siponimod.

Table 36. Predicted and measured values of C_{max} and AUC of unchanged siponimod following the administration of siponimod (Studies A2101 and A2105)

Dose	Measured values		Predicted values	
	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng•h/mL)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng•h/mL)
Siponimod capsule (2.5 mg), a single oral administration[a)]	19.3	745	19.4	727
Siponimod capsule (10 mg), a single oral administration[a)]	77.3	2710	77.7	2908
Siponimod capsule (75 mg), a single oral administration[a)]	491	18600	582	21807
Siponimod capsule (2.5 mg), multiple once daily administration[b)]	38.3	692[d)]	40.0	719[d)]
Siponimod capsule (10 mg), multiple once daily administration[b)]	147	2580[d)]	160	2874[d)]
Siponimod capsule (20 mg), multiple once daily administration[b)]	359	6370[d)]	320	5749[d)]
Siponimod injection (0.25 mg), a single continuous intravenous infusion[c)]	3.22	80.1	3.02	82.1
Siponimod (0.25 mg FMI formulation), a single oral administration[c)]	1.71	67.4	1.88	69.7

Geometric mean
a) Study A2101, b) Day 28 in Study A2105, c) Study A2126, d) AUC_{0-24h}

Table 37. Predicted and measured values of C_{max} and $AUC_{0-\infty}$ of unchanged siponimod following administration of siponimod (Study A2128)

CYP2C9 genotype	Measured values		Predicted values	
	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng•h/mL)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng•h/mL)
*1/*1	2.03	70.5	1.90	70.6
*2/*3	2.45	144	2.02	142
*3/*3	2.35	271	2.10	348

Geometric mean

- Table 38 shows the results of the validation of the model by the measured values in studies on concomitant use of siponimod with itraconazole (potent CYP3A inhibitor), fluconazole (moderate CYP2C9 and CYP3A4 inhibitor) and rifampicin (moderate CYP2C9 and potent CYP3A4 inducer). For fluconazole and rifampicin, the ratios (combination with siponimod/siponimod alone) of C_{max} and $AUC_{0-\infty}$ predicted by the constructed PBPK model were almost identical to those observed.

[27)] A first-order absorption model was selected as the model for siponimod absorption, and a full PBPK model as the model for siponimod distribution. The contribution rate of CYP2C9 and CYP3A4 to siponimod metabolism was determined to be 79.3% and 18.5%, respectively, from the results of the mass balance study (reference CTD 5.3.3.1-5, Study A2104) and the results of *in vitro* metabolism [see Section 6.2.1]. CLint of CYP2C9 and CYP3A4 was estimated from a retrograde model. A renal clearance value was not assigned. For physiological parameters, the default values of SimCYP were used.

Table 38. Predicted and observed ratios (combination with siponimod/siponimod alone) of C_{max} and $AUC_{0-\infty}$ of unchanged siponimod following administration of siponimod

Concomitant drug	Dosage regimen of siponimod	CYP2C9 genotype	Ratio of $AUC_{0-\infty}$ (combination with siponimod/siponimod alone)		Ratio of C_{max} (combination with siponimod/siponimod alone)	
			Measured values	Predicted values	Measured values	Predicted values
Itraconazole	0.25 mg single dose	*1/*2	0.90	1.18	1.01	1.02
		*1/*3	0.76	1.30	0.94	1.02
Fluconazole	4 mg single dose	*1/*1	1.98	2.15	1.10	1.07
Rifampicin	2 mg once daily multiple dose	*1/*1	0.43[a)]	0.32[a)]	0.55	0.50

a) AUC_{0-24h}

- In patients with CYP2C9*1/*2 or CYP2C9*1/*3 receiving itraconazole and siponimod in combination, $AUC_{0-\infty}$ predicted by PBPK model was 1.18 to 1.30 times higher than that in patients with CYP2C9*1/*1, but measured values were 0.76 to 0.90 times those observed in patients with CYP2C9*1/*1. It was unclear why AUC decreased in patients receiving combination therapy with itraconazole, CYP3A inhibitor, in the clinical study. Thus, although PBPK model could not predict the unexplained decrease in AUC in patients receiving combination therapy with itraconazole in the clinical study, the model is considered to be appropriate because the predicted values were not significantly different from the measured values in patients receiving combination therapy with fluconazole or rifampicin.

- The above-constructed PBPK model was used to simulate the exposure to siponimod in patients with each CYP2C9 genotype (CYP2C9*1/*1, CYP2C9*1/*2, CYP2C9*2/*2, CYP2C9*1/*3, CYP2C9*2/*3) receiving combination therapy with siponimod at the dose for each genotype (maintenance dose, 2 mg for CYP2C9*1/*1, CYP2C9*1/*2, and CYP2C9*2/*2; 1 mg for CYP2C9*1/*3 and CYP2C9*2/*3) and fluconazole (moderate CYP2C9 and CYP3A4 inhibitor). The results showed that AUC was 2.15, 2.15, 2.73, 1.78, and 2.13, respectively, higher than that in patients with CYP2C9*1/*1 receiving siponimod alone at the maintenance dose of 2 mg.

- The PBPK model was used to simulate the exposure to siponimod in patients with each CYP2C9 genotype (CYP2C9*1/*1, CYP2C9*1/*2, CYP2C9*2/*2, CYP2C9*1/*3, CYP2C9*2/*3) receiving combination therapy with siponimod at the dose for each CYP2C9 genotype (maintenance dose, 2 mg for CYP2C9*1/*1, CYP2C9*1/*2 and CYP2C9*2/*2; 1 mg for CYP2C9*1/*3 and CYP2C9*2/*3) and rifampicin (a moderate CYP2C9 inducer and potent CYP3A4 inducer). Regardless of CYP2C9 genotype (CYP2C9*1/*1, CYP2C9*1/*2, CYP2C9*2/*2, CYP2C9*1/*3, or CYP2C9*2/*3), AUC decreased to 0.24 to 0.39 times the AUC in patients with CYP2C9*1/*1 receiving siponimod alone at the maintenance dose of 2 mg. In a similar manner, the model was used to simulate the exposure to siponimod in patients with each CYP2C9 genotype receiving combination therapy with siponimod at the dose for each CYP2C9 genotype and efavirenz (a moderate CYP3A4 inducer). AUC decreased to 0.70 to 0.81 times in patients with CYP2C9*1/*1, CYP2C9*1/*2 or CYP2C9*2/*2, and to 0.49 to 0.56 times in patients with CYP2C9*1/*3 or CYP2C9*2/*3, compared with the AUC in patients with CYP2C9*1/*1 receiving siponimod alone at the maintenance dose of 2 mg.

- Taking account of the results of clinical drug interaction studies and of the simulation by PBPK model, the applicant considers that the following precautions for concomitant drugs should be included in the package insert:
  - It is desirable not to co-administer fluconazole with siponimod regardless of CYP2C9 genotype because AUC of siponimod is expected to increase 1.78- to 2.73- fold if siponimod is co-administered at the dose for each CYP2C9 genotype.
  - For CYP3A4 inhibitors, no precaution for concomitant use is necessary because the exposure to siponimod was comparable between subjects receiving combination therapy with the inhibitor and siponimod and subjects receiving siponimod alone in the drug interaction study with itraconazole (reference CTD 5.3.3.4-1, Study A2124).
  - As for inducers of CYP2C9 and CYP3A4, precautions are required because the efficacy of siponimod may be attenuated regardless of the patient's CYP2C9 genotype if co-administered with a drug with moderate CYP2C9 induction and potent CYP3A4 induction, as judged by the observation that the exposure to siponimod decreased when siponimod was co-administered with rifampicin in patients with CYP2C9*1/*1 in the drug-interaction study with rifampicin (reference CTD 5.3.3.4-2, Study A2125).

PMDA's view:

- Regarding the concomitant use with a drug with CYP2C9 induction and CYP3A4 induction, there is no problem in the applicant's opinion that the precaution will be provided that concomitant use with fluconazole should preferably be avoided regardless of CYP2C9 genotype. Physicians should be advised to avoid concomitant use with a moderate or potent CYP2C9 or CYP3A4 inhibitor (and use an alternative drug instead) because (1) there may be drugs with moderate or potent CYP2C9 induction and CYP3A4 induction, and (2) concomitant use with such a drug may possibly increase the exposure to siponimod to the same or greater extent than when fluconazole is concomitantly used.

- Regarding the concomitant use with a CYP2C9 inhibitor, concomitant use with a moderate or potent CYP2C9 inhibitor may possibly increase the exposure to siponimod to the same or greater extent than when fluconazole is concomitantly used, judging from the observations (1) that the contribution rate of CYP2C9 and CYP3A4 to the metabolism of siponimod is estimated to be 79.3% and 18.5%, respectively, from the results of the *in vitro* study [see Section 6.2.1] and (2) AUC increased approximately 2-fold in the presence of fluconazole with moderate CYP2C9 induction and CYP3A4 induction. Physicians should therefore be advised to avoid concomitant use with a moderate or potent CYP2C9 inhibitor (and use an alternative drug instead).

- Regarding concomitant use with a CYP3A4 inhibitor, there is no particular problem with the applicant's opinion that precaution for concomitant use is unnecessary, taking account of the results of the drug interaction study with itraconazole.

- Regarding the concomitant use with a drug with moderate CYP2C9 induction and potent CYP3A4 induction, there is no particular problem with the applicant's opinion to caution that efficacy of siponimod may be attenuated regardless of the CYP2C9 genotype, as judged from the results of the study on interaction with rifampicin.

- Regarding the concomitant use with a moderate or potent CYP3A4 inducer, the precaution should be provided that the efficacy of siponimod may be attenuated in patients with CYP2C9*1/*3 or *2/*3 because, in these patients, CYP2C9 activity is low, resulting in a higher contribution of CYP3A4 to the metabolism than in patients with CYP2C9*1/*1, and because the results of the simulation by PBPK model suggest the possibility that AUC may decrease to 0.49 to 0.56 times the level without the concomitant use.

- Based on the above, precautions for concomitant drugs should be specified as shown in Table 39. The conclusion on the appropriateness of these precautions will be finalized, taking account of comments from the Expert Discussion.

Table 39. Adjustment of the maintenance dose in the presence/absence of concomitant drug affecting the metabolism of siponimod, and precaution

<table>
<tr><td rowspan="2"></td><td colspan="6">CYP2C9 genotype</td></tr>
<tr><td>*1/*1</td><td>*1/*2</td><td>*1/*3</td><td>*2/*2</td><td>*2/*3</td><td>*3/*3</td></tr>
<tr><td>No concomitant drug affecting the metabolism of siponimod</td><td>2 mg/day</td><td>2 mg/day</td><td>1 mg/day</td><td>2 mg/day</td><td>1 mg/day</td><td rowspan="6">Contraindicated</td></tr>
<tr><td>Moderate or potent inhibitor of CYP2C9 and CYP3A4
(e.g., fluconazole)</td><td colspan="5">Precaution: Concomitant use should preferably be avoided.</td></tr>
<tr><td>Moderate or potent inhibitor of CYP2C9</td><td colspan="5">Precaution: Concomitant use should preferably be avoided.</td></tr>
<tr><td>CYP3A4 inhibitor</td><td colspan="5">Precaution unnecessary</td></tr>
<tr><td>Moderate CYP2C9 inducer and potent CYP3A4 inducer
(e.g., rifampicin)</td><td colspan="5">Precaution: Efficacy may possibly be attenuated.</td></tr>
<tr><td>Moderate or potent CYP3A4 inducer</td><td colspan="5">Precaution: Efficacy may possibly be attenuated in patients with CYP2C9*1/*3 or CYP2C9*2/*3</td></tr>
</table>

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

The applicant submitted efficacy and safety evaluation data, in the form of results data from clinical studies as shown in Table 40. The applicant also submitted the results of foreign clinical studies as reference data. Main study results are described below.

Table 40. List of clinical studies on efficacy and safety

Data category	Region	Study CTD	Phase	Study population	No. of enrollments	Dosage regimen	Major endpoints
Evaluation	Japan	Study A1101 5.3.3.1-1	I	Healthy adults	40	A single oral administration of siponimod (0.5 mg, 2.5 mg, 5 mg, 10 mg) or placebo	Safety Pharmacokinetics
	Foreign	Study A2201 5.3.5.1-2	II	Patients with relapsing-remitting multiple sclerosis	(1) 188 (2) 109	(1) Stage 1: Oral administration of siponimod (0.5 mg, 2 mg, 10 mg) or placebo once daily for 6 months (2) Stage 2: Oral administration of siponimod (0.25 mg, 1.25 mg) or placebo once daily for 3 months	Safety Efficacy
	Foreign	Study A2201E1 5.3.5.2-1	II	Patients with relapsing-remitting multiple sclerosis	184	Dose-blinded phase: Oral administration of siponimod (0.25 mg, 0.5 mg, 1.25 mg, 2 mg, 10 mg) or placebo orally once daily Unblinded phase: Oral administration of siponimod (2 mg) once daily	Safety Efficacy
	Global	Study A2304 5.3.5.1-1	III	Patients with secondary progressive multiple sclerosis	1651	Double-blind phase: Oral administration of siponimod (2 mg) or placebo once daily (for 37 months at the maximum) Extended administration phase: Oral administration of siponimod (2 mg) once daily for 7 years	Safety Efficacy

7.1 Japanese phase I study (CTD 5.3.3.1-1, Study A1101 [[redacted] to [redacted], 20[redacted]])

A placebo-controlled, randomized, double-blind study in Japanese healthy adult men (target sample size, 40 subjects; 10 per cohort [2 in the placebo group, 8 in the siponimod group]) was conducted to investigate the safety and pharmacokinetics of siponimod administered orally in a single dose [see Section 6.2.2 for pharmacokinetics].

A single dose of siponimod (0.5 mg in Cohort 1, 2.5 mg in Cohort 2, 5 mg in Cohort 3, or 10 mg in Cohort 4) or placebo was administered orally. All of the 40 randomized subjects were included in the safety analysis population. None of the subjects discontinued the study.

Adverse events (including laboratory abnormalities) were observed in 12.5% (1 of 8) of subjects in the placebo group, 12.5% (1 of 8) of subjects in the siponimod 0.5 mg group, 37.5% (3 of 8) of subjects in the 2.5 mg group, 37.5% (3 of 8) of subjects in the 5 mg group, and 87.5% (7 of 8) of subjects in the 10 mg group. No death or serious adverse event was observed. Adverse events for which a causal relationship to the study drug could not be ruled out were observed in 0% (0 of 8) of subjects in the placebo group, 12.5% (1 of 8) of subjects in the siponimod 0.5 mg group, 37.5% (3 of 8) of subjects in the 2.5 mg group, 37.5% (3 of 8) of subjects in the 5 mg group, and 87.5% (7 of 8) of subjects in the 10 mg group. Main events were headache (0 subject, 0 subject, 3 subjects, 2 subjects, 7 subjects) and atrioventricular block second degree (0 subject, 0 subject, 0 subject, 1 subject, 2 subjects). Clinically significant changes in vital signs[28)] observed were decreased pulse rate in 21 subjects (0 subject, 1 subject, 5 subjects, 7 subjects, 8 subjects), decreased diastolic blood pressure in 8 subjects (0 subject, 0 subject, 2 subjects, 2 subjects, 4 subjects), and decreased body temperature in 1 subject (siponimod 0.5 mg group). As for electrocardiogram, prolonged PR interval was observed in the siponimod 2.5 mg, 5 mg, and 10 mg groups, with the longest PR interval being observed at 6 hours after administration of

28) The following definitions were used: Increased systemic blood pressure, >160 mmHg; decreased systemic blood pressure, <90 mmHg; increased diastolic blood pressure, >90 mmHg; decreased diastolic blood pressure, <50 mmHg; increased pulse rate, >120 beats/minute; decreased pulse rate, <50 beats/minute; increased body temperature, >38.0°C; decreased body temperature, <36.0°C.

study drug. No clinically significant change was observed in QTc, QTcF, or QT interval corrected for heart rate using Bazett's method (QTcB interval).

7.2 Foreign phase II study (CTD 5.3.5.1-2, Study A2201 [March 2009 to May 2011])

A placebo-controlled, randomized, double-blind study in patients with RRMS[29] (target sample size, 275 subjects) was conducted to investigate the efficacy and safety of siponimod in 12 countries.[30]

The study consisted of Stage 1 and Stage 2. In Stage 1, patients were randomized to the placebo group, the siponimod 0.5 mg group, the 2 mg group, and the 10 mg group at a ratio of 1:1:1:1, and received the study drug once daily for 6 months. When a total of 181 patients completed the evaluation at Month 3, or discontinued the study, in Stage 1, an interim analysis[31] was conducted to determine the doses in Stage 2 and, as a result, 0.25 mg and 1.25 mg were selected as the doses of siponimod in Stage 2. In Stage 2, patients were randomized to the placebo group, the siponimod 0.25 mg group, and the 1.25 mg group at a ratio of 1:4:4, and received the study drug once daily for 3 months. In stage 2, the dose of siponimod was increased in a stepwise manner during the early stage of the administration as shown in Table 41.

Table 41. Dose-escalation during the early stage of administration in Stage 2 of Study A2201

Maintenance dose	Day 1	Day 2	Day 3	Day 4	Day 5
0.25 mg	0.25 mg	0.25 mg	0.25 mg	0.25 mg	0.25 mg
1.25 mg	0.25 mg	0.25 mg	0.5 mg	0.75 mg	1.25 mg

The study drug was administered to 187 of 188 patients who were randomized in Stage 1. All of the 187 patients receiving the study drug were included in the full analysis set (FAS) and the safety analysis population in Stage 1. Study discontinuation occurred in 30 patients (3 patients in the placebo group, 7 patients in the siponimod 0.5 mg group, 5 patients in the 2 mg group, 15 patients in the 10 mg group) from among patients in the safety analysis population. The main reasons for the discontinuation were adverse events (1 patient, 3 patients, 4 patients, 6 patients), consent withdrawal (0 patient, 2 patients, 0 patient, 3 patients), and problems in the control of the clinical study (1 patient, 0 patient, 1 patient, 2 patients).

In Stage 2, all of the 109 randomized patients received the study drug and were included in FAS and the safety analysis population of Stage 2. Among the patients in the safety analysis population, 3 patients discontinued the study (0 patient in the placebo group, 1 patient in the siponimod 0.25 mg group, 2 patients in the 1.25 mg group). The reasons for the discontinuation were adverse events in 2 patients (0 patient, 0 patient, 2 patients) and consent withdrawal (0 patient, 1 patient, 0 patient).

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[29] The main inclusion criteria were as follows: (a) Patients who have diagnosis of multiple sclerosis according to McDonald Diagnostic Criteria, 2005 revised edition, (b) One or more relapses within the past 1 year, 2 or more relapses within the past 2 years, or one more lesions detected by gadolinium contrast imaging at the screening (if the gadolinium-enhanced MRI imaging did not reveal lesion at the screening, the test could be performed after 1 month). (c) The Expanded Disability Status Scale (EDSS) score at the randomization was 0 to 5.0, and (d) Patients who are neurologically stable without relapse or treatment with a corticosteroid during 30 days before randomization.

[30] Canada, Finland, Germany, Hungary, Italy, Norway, Poland, Russia, Spain, Switzerland, Turkey, USs

[31] In the interim analysis, the data monitoring committee (DMC) evaluated (a) whether the study should be discontinued for futility or continued and, if the study was to be continued, (b) which 2 doses should be used in Stage 2 and (c) whether additional patients were necessary. As a result, it was determined that (a) the study should be continued, (b) 0.25 mg group and 1.25 mg group should be used in Stage 2, and (c) the number of patients should not be changed.

Using the reduction rate in the combined unique active lesion (CUAL) number[32] at 3 months of administration compared with placebo,[33] the primary endpoint, 5 candidate models of dose response were investigated by multiple comparison procedure -modeling (MCP-Mod) method. Table 42 shows the results of the primary analysis,[34] which showed that a significant dose response was obtained by the maximum effect (E_{max}) model and by the Hill-E_{max} model. The E_{max} model was selected as the more appropriate model based on Akaike Information Criterion (AIC). The CUAL number at 3 months of administration, estimated by E_{max} model, decreased by 50% compared with placebo at the dose of siponimod [95% CI] of 0.38 [0.02, ∞] mg.

Table 42. Candidate models of dose-response curve at 3 months of administration (FAS)

Model	t Statistic	One-sided *P* value[a]
Linear model	1.75	0.0696
E_{max} model (ED_{50} = 1 mg)	3.93	0.0001
Hill E_{max} model 1 (ED_{50} = 2 mg and Hill coefficient = 2)	2.53	0.0115
Hill E_{max} model 2 (ED_{50} = 3 mg and Hill coefficient = 3)	1.65	0.0858
Exponential function model (δ = 3.633)	1.20	0.1817

ED_{50}, The dose of siponimod giving half the asymptotic maximum change compared to placebo; δ, Rate of increase

a) A one-sided *P* value of <0.025 shows that the model is significantly different from the flat dose-response curve (the one without dose-response).

Annualized relapse rate (ARR)[35] [95% CI] within 6 months of administration in patients during Stage 1, the secondary endpoint, was 0.58 [0.337, 1.002] in the placebo group, 0.61 [0.351, 1.062] in the siponimod 0.5 mg group, 0.20 [0.081, 0.478] in the 2 mg group, and 0.30 [0.151, 0.613] in the 10 mg group, showing a tendency of lower values in the siponimod 2 mg and the 10 mg groups than in the placebo group.

Adverse events[36] were observed in 80.3% (49 of 61) of patients in the placebo group, 74.5% (38 of 51) of patients in the siponimod 0.25 mg group, 86.0% (37 of 43) of patients in the 0.5 mg group, 69.0% (29 of 42) of patients in the 1.25 mg group, 98.0% (48 of 49) of patients in the 2 mg group, and 96.0% (48 of 50) of patients in the 10 mg group. Death occurred in 1 patient[37] in the siponimod 1.25 mg group in Stage 2, and its causal relationship to the study drug could not be ruled out. Serious adverse events other than death were observed in 8 patients in the siponimod 0.5 mg group (multiple sclerosis relapse,

32) The number of active lesions on MRI imaging calculated by adding up, without redundancy, the number of new gadolinium-enhanced lesions and new or expanded T2 lesions.

33) The reduction rate in the placebo group was assumed to be 0%. Data obtained up to 3 months of administration in Stage 1 were used in the siponimod 10 mg group, the 2 mg group, and the 0.5 mg group. Data obtained in Stage 2 were used in the siponimod 1.25 and 0.25 mg groups. Data obtained up to 6 months of Stage 1 and those obtained in Stage 2 were combined and used in the placebo group.

34) Regarding the reduction rate of the CUAL number at 3 months of administration relative to the placebo group, the null hypothesis "the dose response is flat between 5 doses of siponimod (0.25, 0.5, 1.25, 2, and 10 mg) and the dose of placebo" was tested against the alternative hypothesis "the dose response curve shows a monotonic increase." The test statistic was calculated based on the linear combination of the optimum contrast coefficient for each of the 5 candidate models and, if the null hypothesis was rejected with one-sided significance level of 0.025, the dose-response relationship was assumed. If the null hypothesis is rejected in multiple models, the most suitable model was selected based on AIC, and the estimated dose (and 95% CI) that is expected to decrease the CUAL number by 50% relative to the placebo group was calculated.

35) ARR was calculated based on confirmed relapses. Relapses ([1] occurrence of neurologically abnormal findings and [2] aggravation, ≥30 days after the previous demyelinating event, of stable or improved neurological abnormal findings. Either of these should persist for ≥24 hours without pyrexia [≥37.5°C] and infection), were defined as "confirmed relapses" when an independent physician evaluated EDSS (consultation within 7 days after the occurrence of the symptom was recommended) and any of the following was observed: (a) EDSS aggravation by ≥0.5 points, (b) aggravation of 2 Functional System Scales by ≥1 point, and (c) aggravation of 1 Functional System Scale by ≥2 points (except for vesicorectal function and cerebral function).

36) Rigorous comparison is difficult because (1) the duration of administration differed between Stage 1 and Stage 2, (2) the dose escalation method was used only in Stage 2, and (3) the data of Stages 1 and 2 were combined in the placebo group. Thus, caution is necessary in interpreting the results.

37) Severe chest pain occurred on Day 29 of administration of study drug, resulting in treatment discontinuation on Day 52. The patient complained of fatigue 27 days after the discontinuation, and was found dead at home after approximately 8 hours. The medical examiner concluded that the cause of death was acute myocardial infarction.

optic neuritis, headache, myopathy, pyelonephritis acute/uterine leiomyoma, schizophreniform disorder, bradycardia, and basal cell carcinoma in 1 patient each), 1 patient in the 1.25 mg group (perineal abscess), 4 patients in the 2 mg group (atrioventricular block second degree in 3 patients, intentional overdose in 1 patient), and 3 patients in the 10 mg group (myocardial infarction, atrioventricular block second degree, benign intracranial hypertension in 1 patient each). A causal relationship to siponimod could not be ruled out for atrioventricular block second degree (4 patients) and myocardial infarction, perineal abscess, uterine leiomyoma, and bradycardia (1 patient each).

Adverse events for which a causal relationship to the study drug could not be ruled out were observed in 41.0% (25 of 61) of patients in the placebo group, 39.2% (20 of 51) of patients in the siponimod 0.25 mg group, 46.5% (20 of 43) of patients in the 0.5 mg group, 35.7% (15 of 42) of patients in the 1.25 mg group, 67.3% (33 of 49) of patients in the 2 mg group, 80.0% (40 of 50) of patients in the 10 mg group. Main events were headache (2 patients, 2 patients, 5 patients, 1 patient, 10 patients, 15 patients), bradycardia (2 patients, 2 patients, 2 patients, 0 patient, 3 patients, 14 patients), dizziness (2 patients, 0 patient, 3 patients, 1 patient, 3 patients, 11 patients), lymphopenia (0 patient, 0 patient, 0 patient, 0 patient, 2 patients, 5 patients), vertigo (1 patient, 1 patient, 1 patient, 2 patients, 6 patients, 2 patients), nausea (1 patient, 3 patients, 0 patient, 2 patients, 1 patient, 6 patients), and fatigue (4 patients, 0 patient, 1 patient, 4 patients, 0 patient, 6 patients).

Table 43 shows abnormal changes in vital signs (pulse rate and blood pressure) observed after the first dose.

Table 43. Abnormal changes in pulse rate and blood pressure in Study A2201 (safety analysis population)

Treatment group		Placebo	0.25 mg	0.5 mg	1.25 mg	2 mg	10 mg
No. of patients evaluated		61[a)]	51	43	42[b)]	49	50
Day 1							
Systolic blood pressure	≤90 mmHg	1 (1.7)	3 (5.9)	4 (9.3)	1 (2.4)	6 (12.2)	4 (8.0)
	Decreased from baseline by ≥20 mmHg	6 (10.0)	7 (13.7)	10 (23.3)	2 (4.9)	6 (12.2)	12 (24.0)
	≥160 mmHg	1 (1.7)	1 (2.0)	0	1 (2.4)	1 (2.0)	2 (4.0)
	Increased from baseline by ≥20 mmHg	8 (13.3)	10 (19.6)	2 (4.7)	4 (9.8)	6 (12.2)	3 (6.0)
Diastolic blood pressure	≤50 mmHg	2 (3.3)	1 (2.0)	0	2 (4.9)	0	3 (6.0)
	Decreased from baseline by ≥15 mmHg	9 (15.0)	8 (15.7)	18 (41.9)	8 (19.5)	10 (20.4)	22 (44.0)
	≥100 mmHg	2 (3.3)	3 (5.9)	0	0	2 (4.1)	1 (2.0)
	Increased from baseline by ≥15 mmHg	8 (13.3)	6 (11.8)	1 (2.3)	5 (12.2)	1 (2.0)	2 (4.0)
Pulse rate	<50 bpm	1 (1.7)	2 (3.9)	2 (4.7)	3 (7.1)	14 (28.6)	24 (48.0)
	Decreased from baseline by ≥15 bpm	11 (18.3)	22 (43.1)	17 (39.5)	16 (38.1)	32 (65.3)	42 (84.0)
	>120 bpm	0	0	0	0	0	0
	Increased from baseline by ≥15 bpm	10 (16.7)	1 (2.0)	2 (4.7)	3 (7.1)	3 (6.1)	1 (2.0)
Day ≥2							
Systolic blood pressure	≤90 mmHg	2 (3.3)	4 (7.8)	2 (4.7)	2 (4.8)	1 (2.1)	1 (2.0)
	Decreased from baseline by ≥20 mmHg	7 (11.5)	16 (31.4)	9 (20.9)	9 (21.4)	5 (10.6)	7 (14.3)
	≥160 mmHg	2 (3.3)	3 (5.9)	1 (2.3)	1 (2.4)	2 (4.3)	3 (6.1)
	Increased from baseline by ≥20 mmHg	11 (18.0)	15 (29.4)	4 (9.3)	7 (16.7)	9 (19.1)	4 (8.2)
Diastolic blood pressure	≤50 mmHg	1 (1.6)	3 (5.9)	0	4 (9.5)	1 (2.1)	0
	Decreased from baseline by ≥15 mmHg	12 (19.7)	20 (39.2)	12 (27.9)	16 (38.1)	6 (12.8)	8 (16.3)
	≥100 mmHg	1 (1.6)	3 (5.9)	2 (4.7)	1 (2.4)	2 (4.3)	4 (8.2)
	Increased from baseline by ≥15 mmHg	17 (27.9)	9 (17.6)	4 (9.3)	9 (21.4)	2 (4.3)	4 (8.2)
Pulse rate	<50 bpm	1 (1.6)	3 (5.9)	0	2 (4.8)	1 (2.1)	1 (2.0)
	Decreased from baseline by ≥15 bpm	14 (23.0)	22 (43.1)	11 (25.6)	25 (59.5)	7 (14.9)	5 (10.2)
	>120 bpm	0	0	0	1 (2.4)	0	0
	Increased from baseline by ≥15 bpm	28 (45.9)	11 (21.6)	11 (25.6)	8 (19.0)	16 (34.0)	12 (24.5)

Number of patients with events (incidence [%])

a) Data of systolic blood pressure, diastolic blood pressure, and pulse rate on Day 1 were available at each evaluation point before and after administration in 60 patients.

b) Data of systolic blood pressure and diastolic blood pressure on Day 1 were available at each evaluation point before and after administration in 41 patients.

Table 44 shows the findings on Holter electrocardiography (Holter ECG) at the first dose and the findings on mobile cardiac telemetry in Stage 2. Particularly notable abnormal findings on Holter ECG observed during treatment with siponimod were atrioventricular block second degree in 6.7% (3 of 45) of patients in the placebo group, 7.0% (3 of 43) of patients in the siponimod 0.5 mg group, 18.4% (9 of 49) of patients in the 2 mg group, and 14.0% (7 of 50) of patients in the 10 mg group in Stage 1; and in 18.8% (3 of 16) of patients in the placebo group and in none of the patients in the siponimod 0.25 mg and 1.25 mg groups in Stage 2.

Table 44 Findings on Holter ECG and mobile cardiac telemetry on Day 1 (Study A2201, safety analysis set)

	Stage 1				Stage 2		
	Placebo	0.5 mg	2 mg	10 mg	Placebo	0.25 mg	1.25 mg
No. of patients evaluated by Holter ECG	43	39	48	48	15	51	40
Sinus bradycardia	42 (97.7)	39 (100.0)	47 (97.9)	48 (100.0)	13 (86.7)	47 (92.2)	38 (95.0)
Sinus tachycardia	42 (97.7)	37 (94.9)	41 (85.4)	46 (95.8)	15 (100.0)	49 (96.1)	39 (97.5)
Premature atrial contraction	36 (83.7)	34 (87.2)	38 (79.2)	43 (89.6)	9 (60.0)	43 (84.3)	38 (95.0)
Premature ventricular contraction	22 (51.2)	19 (48.7)	26 (54.2)	24 (50.0)	5 (33.3)	31 (60.8)	23 (57.5)
Other arrhythmias	33 (76.7)	31 (79.5)	33 (68.8)	23 (47.9)	12 (80.0)	41 (80.4)	35 (87.5)
Mobitz type I atrioventricular block, second degree	1 (2.3)	2 (5.1)	6 (12.5)	5 (10.4)	2 (13.3)	0	0
Non-conducted premature atrial contraction	0	0	1 (2.1)	4 (8.3)	0	1 (2.0)	0
Supraventricular tachycardia	4 (9.3)	2 (5.1)	7 (14.6)	3 (6.3)	1 (6.7)	6 (11.8)	1 (2.5)
2:1 Atrioventricular block	0	0	2 (4.2)	3 (6.3)	0	0	0
Atrioventricular block first degree	0	0	2 (4.2)	2 (4.2)	2 (13.3)	2 (3.9)	0
No. of patients evaluated by mobile cardiac telemetry					11	30	24
Sinus tachycardia					9 (81.8)	16 (53.3)	16 (66.7)
Sinus bradycardia					1 (9.1)	4 (13.3)	7 (29.2)
Atrioventricular block first degree					0	1 (3.3)	0

No. of patients with events (incidence [%])

7.3 Global phase III study (CTD 5.3.5.1-1, Study A2304 [December 12 to data cut-off (the last observation day in double-blind phase) April 2016])

A placebo-controlled, randomized, double-blind, parallel-group study in patients with SPMS[38)] (target sample size, 1530 subjects[39)]; 510 in the placebo group, 1020 in the siponimod group) was conducted to investigate the efficacy and safety of siponimod in 31 countries.[40)]

The study consisted of a double-blind phase and an extended administration phase.[41)] The double-blind phase was approximately 3 years from the randomization of the first patient. The sum of the extended administration phase and the 3-year double-blind phase was to be maximally 10 years.

38) The main diagnostic criteria were (a) patients who have a history of relapsing-remitting multiple sclerosis according to McDonald Diagnostic Criteria (2010 revised edition), (b) patients with SPMS showing aggravation of a progressive disorder persisting ≥6 months, regardless of relapse, (c) patients with a disorder of the EDSS score ≥3.0 and ≤6.5 at screening, (d) patients with records of disorder progression based on EDSS during 2 years from enrollment in the study (≥1 point if the EDSS score at screening was <6.0, ≥0.5 point if the EDSS score at screening was ≥6.0), and (e) patients without relapse during 3 months before randomization and not being treated with corticosteroid.

39) The study was designed to allow detecting, with a 90% power of detection, 30% decrease in 3mCDP (hazard ratio 0.70) in the siponimod group compared with the placebo group. Assuming that, in the placebo group, the progression rate was 0.30 during 2 years, the drop-out rate was 20% in 2 years, and the monthly enrollment was 100 patients, the calculation showed that 1530 patients and 42 months of study period were necessary to evaluate at least 374 events.

40) Argentine, Australia, Austria., Belgium., Bulgaria, Canada., China, Czech Republic, Estonia, France, Germany, Greece, Hungary, Ireland, Israel, Italy, Japan, Latvia, Lithuania, the Netherlands, Poland, Portugal, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, Turkey, England, and US.

41) Patients showing aggravation of a progressive disorder persisting ≥6 months based on EDSS were to select either of the following options after giving a renewed informed consent: (a) Extended double-blind administration, (b) open-label siponimod administration, or (c) off-study drug (to switch to a drug for treating multiple sclerosis approved in the corresponding country or to no treatment, and continue the study with a simplified visit schedule). Patients who discontinued the treatment with siponimod and did not wish to continue participation in the study underwent a follow-up examination at 1 month after the last visit in the end of the study. During the extended administration phase, siponimod was administered under open-label conditions.

The placebo or siponimod was administered by the dose-escalation method according to Table 45 (maintenance dose 2 mg) and, from Day 6 on, the maintenance dose (placebo or siponimod 2 mg) was administered orally once daily. When lymphocyte count in blood was <200 cells/mm^3 in 2 successive tests, the dose reduction to 1 mg was allowed and the administration was continued at the reduced dose. When the patient could not take the study drug for ≥1 day during the dose-escalation phase, or could not take the study drug for ≥4 successive days during the maintenance administration phase, the patient was to take, at treatment resumption, the study drug with the dose-escalation method stipulated in Table 45, according to the maintenance dose before the interruption.

Table 45. Siponimod dose escalation in Study A2304

Maintenance dose	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1 mg	0.25 mg	0.25 mg	0.5 mg	0.75 mg	1 mg	1 mg
2 mg	0.25 mg	0.25 mg	0.5 mg	0.75 mg	1.25 mg	2 mg

Of 1651 patients randomized in the double-blind phase (546 in the placebo group, 1105 in the siponimod group), 1645 patients (546 patients, 1099 patients) were included in FAS and the safety analysis population. Among patients in the safety analysis population, 318 patients (122 patients, 196 patients) discontinued the study during the double-blind phase. The main reasons for the discontinuation were the decision by the patient or his/her legal guardian (77 patients, 95 patients), adverse events (18 patients, 45 patients), inadequate response (11 patients, 15 patients) and the decision of the physician (1 patient, 11 patients).

Table 46 and Figure 2 show time to "3-month confirmed disability progression based on Expanded Disability Status Scale (EDSS)" (3mCDP[42)]), the primary endpoint. A statistically significant difference was observed between the siponimod group and the placebo group.

Table 46. Evaluation of time to 3mCDP (double-blind phase in Study A2304, FAS)

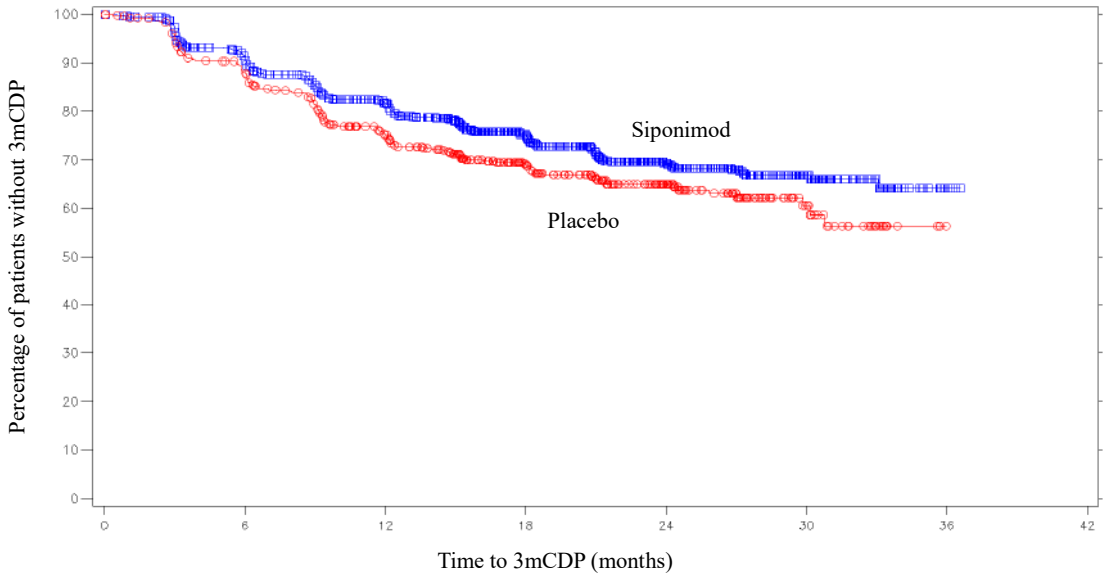
	Treatment group	Incidence of events (n/N)	Risk reduction rate[a)]	Hazard ratio[b)] [95% CI][b)]	*P* value[b)]
Entire population	Placebo	31.7% (173/545)	21.2%	0.79 [0.65, 0.95]	0.0134
	Siponimod	26.3% (288/1096)			

N: Number of patients without missing covariate data; n, Number of patients with events

a) (1 – hazard ratio) × 100

b) Based on the Cox proportional hazard model with covariates of treatment group, country or region, presence/absence of a relapse within 2 years before screening, and the baseline EDSS score.

[42)] In Study A2304, 3mCDP was defined as disability progression according to EDSS persisting for 3 months from the date of the confirmation of the start of disability progression after the start of the administration of study drug (increase in the EDSS score by ≥1 from baseline in patients with a baseline EDSS score of ≤5.0, and increase in the EDSS score by ≥0.5 from baseline in patients with a baseline EDSS score of ≥5.5).



At risk number								
Placebo	546	463	352	223	124	35	0	0
Siponimod	1099	947	781	499	289	101	4	0

Figure 2. Time to 3mCDP (double-blind phase of Study A2304, FAS)

Adverse events[43)] were observed in 81.7% (446 of 546) of patients in the placebo group and in 89.3% (981 of 1099) of patients in the siponimod group. Death occurred in 4 patients in the placebo group (haemorrhagic stroke, lung adenocarcinoma, gastric cancer, and death in 1 patient each) and in 4 patients in the siponimod group (completed suicide, urosepsis, septic shock, and malignant melanoma in 1 patient each). A causal relationship to the study drug could not be ruled out for lung adenocarcinoma in the placebo group and septic shock and malignant melanoma in the siponimod group. Serious adverse events other than death were observed in 14.8% (81 of 546) of patients in the placebo group and in 17.7% (194 of 1099) of patients in the siponimod group (Table 47).

43) During the cleaning of the long-term treatment data after locking the database of the double-blind phase, it was noticed that there were 108 adverse events that should be reported as those of the double-blind phase. Since the clinical study report was prepared based on the interim results of the double-blind phase obtained from the locked database, these events were not included in the clinical study report but are included in this review report.

Table 47. List of serious adverse events (double-blind phase of Study A2304, safety analysis population)

Placebo group 14.8% (81 of 546 patients)	Basal cell carcinoma* and multiple sclerosis relapse in 4 patients each, paraparesis in 3 patients, basal cell carcinoma, hip fracture, suicide attempt, and urinary tract infection in 2 patients each, ALT increased,* ALT increased/AST increased/non-alcoholic steatohepatitis, depression, depression/muscle spasticity, Campylobacter gastroenteritis, panic attack, gastroenteritis proteus/shock hypoglycaemic, Bowen's disease,* dysaesthesia, allodynia/anal fissure/multiple sclerosis relapse*/urinary retention, gastroenteritis,* lower limb fracture, meralgia paraesthetica, hypoaesthesia/uterine leiomyoma, liver function test abnormal,* basal cell carcinoma*/influenza, acute myocardial infarction/oesophagitis, pancreatitis acute, angina pectoris*/coronary artery disease,* musculoskeletal stiffness, amnestic disorder, actinic keratosis, cervical dysplasia, suicide attempt/walking disability, suicidal ideation, syncope, duodenal ulcer/multiple sclerosis relapse/prostate cancer, postoperative wound infection/testicular atrophy, circulatory collapse/multiple sclerosis relapse, small intestinal obstruction, supraventricular tachycardia, abdominal pain upper/metrorrhagia, glioma,* nephrolithiasis/urinary tract infection, pyelonephritis, hydronephrosis*/urinary retention*/urinary tract infection,* gastric ulcer perforation, prostate cancer/urinary tract infection,* wound infection,* femoral neck fracture, central nervous system lymphoma*/diplopia, intestinal obstruction, intervertebral disc protrusion, head injury, breast cancer/breast cancer,* urosepsis, cerebrovascular accident/transient ischaemic attack/transient ischaemic attack,* pneumonia*/pulmonary embolism,* pneumonia/urinary tract infection, cataract/retinal detachment/retinoschisis, meniscus injury/patellofemoral pain syndrome, putamen haemorrhage, anaemia,* anaemia/decubitus ulcer, constipation/diarrhoea, gait disturbance/vertigo, gait disturbance/tremor/urinary tract infection, gait disturbance/renal oncocytoma, oedema peripheral, tibia fracture, tendon rupture, and bladder cancer* in 1 patient each.
Siponimod group 17.7% (194 of 1099 patients)	ALT increased,*ALT increased*/AST increased,* and basal cell carcinoma* in 4 patients each, epilepsy, suicide attempt, urinary tract infection, and urinary tract infection* in 3 patients each, depression, basal cell carcinoma, basal cell carcinoma/syncope, menorrhagia, suicidal behaviour, femoral neck fracture, appendicitis, cerebrovascular accident, concussion, and seizure in 2 patients each, hepatitis E/radiculopathy, AST increased, ALT increased, ALT increased*/depression, influenza/pneumonia, depression/suicidal ideation, Columbia suicide severity rating scale abnormal/depression suicidal, Columbia suicide severity rating scale abnormal/concussion/dizziness*/laceration/orthostatic hypotension*/rib fracture, seminoma, seminoma,* epilepsy/hypertension,* panic attack, haemochromatosis, melanocytic naevus, gastroenteritis, transient ischaemic attack, carbon monoxide diffusing capacity decreased*/urge incontinence, ataxia/bursitis/walking distance test abnormal, macular oedema,* macular oedema/rash popular,* diarrhoea, hypersensitivity,* coronary artery disease, hepatic enzyme abnormal*/macular oedema,* hepatic enzyme increased,* hepatotoxicity, arthritis, arthralgia/immobile/laceration, eye contusion/injury corneal/laceration, intention tremor,* basal cell carcinoma/Bowen's disease/fall/musculoskeletal pain/splenic rupture/traumatic fracture/traumatic haemothorax/traumatic liver injury, basal cell carcinoma/basal cell carcinoma,* basal cell carcinoma/malignant melanoma in situ, single functional kidney, acute coronary syndrome/myocardial ischaemia, acute myocardial infarction/pulmonary embolism/trigeminal neuralgia/venous thrombosis limb, acute kidney injury,* cholecystitis acute, ischaemic stroke,*angina pectoris*/bradycardia,* muscular weakness/urinary retention, muscle spasticity/muscle spasticity*/pyrexia, muscle spasticity/seizure/suicidal ideation, bacteraemia/urinary tract infection, diverticulitis, cervical vertebral fracture, haematoma, thrombocytopenia,* blood bilirubin increased,* blood bilirubin increased*/fatigue/hemiparesis/muscular weakness/urinary tract infection/urinary tract infection*/urosepsis, blood glucose increased, hallucination/psychotic disorder, respiratory disorder,* oral viral infection, lip squamous cell carcinoma/squamous cell carcinoma, benign neoplasm of thyroid gland, lumbar spinal stenosis/synovial cyst, bundle branch block left/palpitations/sinus tachycardia, clavicle fracture, contusion/spinal deformity/subarachnoid haematoma/subdural haematoma/traumatic haemorrhage, trigeminal neuralgia, endometrial cancer,* suicidal behaviour/urinary retention, suicidal ideation/urethral stenosis, abortion spontaneous, aphasia/seizure, syncope, syncope,* blindness*/multiple sclerosis relapse,* bradycardia,* upper respiratory tract infection, upper respiratory tract infection/urinary tract infection, heart rate decreased, atrial fibrillation/epilepsy/muscular weakness/respiratory depression/urinary tract infection, deep vein thrombosis, neurogenic bladder, neurofibroma*/polyneuropathy, anxiety disorder due to a general medical condition/uterine leiomyoma, nephrolithiasis, hydronephrosis*/urosepsis,* hydronephrosis/septic shock/ureterolithiasis/urosepsis, spinal compression fracture, generalised tonic-clonic seizure,* ankle fracture/postoperative wound infection, ankle fracture/bladder dysfunction/urinary incontinence, foot deformity, multiple sclerosis, multiple sclerosis,* multiple sclerosis relapse/urinary tract infection, weight decreased, herpes zoster,* herpes zoster meningitis,* femoral neck fracture/gastritis erosive, Escherichia urinary tract infection/sepsis, atrioventricular block second degree,* atrioventricular block second degree*/bradycardia,* dehydration/urinary tract infection, cholelithiasis, biliary colic, cholecystitis, disturbance in attention/urinary incontinence, appendiceal abscess*/appendicitis,* intestinal obstruction, intervertebral disc protrusion, fracture displacement, intracranial aneurysm, head titubation, breast cancer*/trigeminal neuralgia, urinary retention, urinary tract infection/urinary tract infection*/urosepsis, thermal burn, brain stem infarction, concussion/fall/mental status changes, concussion/pulmonary hypertension, brain injury/clavicle fracture, back pain, pulmonary function test decreased,* lung adenocarcinoma,* leukopenia,* rash,* nasal abscess, malignant melanoma in situ,* anxiety, metrorrhagia, metrorrhagia,* hemiparesis/upper respiratory tract infection, hemiparesis/partial seizures, partial seizures, umbilical hernia, obstructive, osteoarthritis/urinary tract infection, status migrainosus, constipation, constipation/pyrexia, gait disturbance, cellulitis, cellulitis,* pyelonephritis chronic, chorioretinitis,* asthenia/dehydration/gastroenteritis/nausea/suicide attempt/vomiting, asthenia/urinary tract infection, depressed mood, depressive symptom/euphoric mood/mania,* ovarian disorder, laceration, incarcerated inguinal hernia, radius fracture, bladder dysfunction/labyrinthitis, calculus bladder, decubitus ulcer, and inguinal hernia in 1 patient each.

* Events for which a causal relationship to the study drug could not be ruled out.

Among Japanese patients, adverse events were observed in 100% (8 of 8) of patients in the placebo group and in 93.3% (14 of 15) of patients in the siponimod group. No death occurred. A serious adverse event was observed in 1 patient in the placebo group (putamen haemorrhage), but its causal relationship to the study drug was ruled out.

Adverse events for which a causal relationship to the study drug could not be ruled out were observed in 36.3% (198 of 546) of patients in the placebo group and in 47.7% (524 of 1099) of patients in the siponimod group. Main events were headache (30 patients, 58 patients), hypertension (22 patients, 50 patients), bradycardia (14 patients, 49 patients), alanine aminotransferase (ALT) increased (3 patients, 42 patients), fatigue (19 patients, 36 patients), nausea (6 patients, 35 patients), and urinary tract infection (17 patients, 25 patients).

Table 48 shows the percentage of patients who showed abnormal changes in vital signs (pulse rate and blood pressure).

Table 48. Percentage of patients with abnormalities in pulse rate or blood pressure (Study A2304, safety analysis population)

		Double-blind phase					
		Day 1		Day 7		Day 8 onward	
Treatment group		Placebo	Siponimod	Placebo	Siponimod	Placebo	Siponimod
No. of patients in safety analysis population		546	1099	546	1099	546	1099
No. of patients evaluated		484	984	502	995	546	1089
Systolic blood pressure	≤90 mmHg	8 (1.7)	20 (2.0)	7 (1.4)	24 (2.4)	7 (1.3)	20 (1.8)
	≥20 mmHg decrease from baseline	43 (8.9)	99 (10.1)	67 (13.3)	143 (14.4)	103 (18.9)	152 (14.0)
	≥180 mmHg	4 (0.8)	6 (0.6)	4 (0.8)	6 (0.6)	2 (0.4)	8 (0.7)
	≥160 mmHg	18 (3.7)	32 (3.3)	22 (4.4)	33 (3.3)	24 (4.4)	71 (6.5)
	≥20 mmHg increase from baseline	47 (9.7)	76 (7.7)	36 (7.2)	77 (7.7)	104 (19.0)	311 (28.6)
Diastolic blood pressure	≤50 mmHg	1 (0.2)	15 (1.5)	4 (0.8)	19 (1.9)	3 (0.5)	12 (1.1)
	≥15 mmHg decrease from baseline	46 (9.5)	143 (14.5)	65 (12.9)	197 (19.8)	102 (18.7)	171 (15.7)
	≥110 mmHg	3 (0.6)	5 (0.5)	2 (0.4)	3 (0.3)	3 (0.5)	12 (1.1)
	≥100 mmHg	23 (4.8)	34 (3.5)	21 (4.2)	28 (2.8)	33 (6.0)	96 (8.8)
	≥15 mmHg increase from baseline	31 (6.4)	47 (4.8)	41 (8.2)	48 (4.8)	76 (13.9)	220 (20.2)
Pulse rate	<50 bpm	3 (0.6)	31 (3.2)	5 (1.0)	47 (4.7)	2 (0.4)	11 (1.0)
	≥15 bpm decrease from baseline	31 (6.4)	167 (17.0)	48 (9.6)	261 (26.2)	81 (14.8)	182 (16.7)
	>120 bpm	0	2 (0.2)	1 (0.2)	0	1 (0.2)	2 (0.2)
	≥15 bpm increase from baseline	54 (11.2)	28 (2.8)	63 (12.5)	26 (2.6)	140 (25.6)	247 (22.7)

No. of patients with events (incidence [%])

During the dose-escalation phase, QTcF increased to >450 msec (male) or to >470 msec (female) in 1.2% (6 of 483) of patients in the placebo group and in 1.1% (11 of 978) of patients in the siponimod group. QTcF increased to >500 msec in 0% (0 of 485) of patients in the placebo group and in 0.1% (1 of 982) of patients in the siponimod group. QTcF increased from baseline by >30 msec in 7.2% (35 of 484) of patients in the placebo group and in 11.1% (109 of 981) of patients in the siponimod group, and by >60 msec in 0% (0 of 484) of patients in the placebo group and in 0.3% (3 of 981) of patients in the siponimod group.

As for QT interval after the dose-escalation phase, QTcF newly increased to >450 msec (male) or to >470 msec (female) in 0.9% (5 of 531) of patients in the placebo group and in 0.3% (3 of 1057) of patients in the siponimod group. QTcF increased to >500 msec in none of the patients. QTcF increased from baseline by >30 msec in 5.8% (31 of 533) of patients in the placebo group and in 10.2% (108 of 1061) of patients in the siponimod group, and by >60 msec in 0% (0 of 533) of patients in the placebo group and in 0.3% (3 of 1061) of patients in the siponimod group.

7.R Outline of the review conducted by PMDA

7.R.1 Clinical positioning

PMDA asked the applicant to explain the clinical positioning of siponimod.

The applicant's explanation:

- MS is an inflammatory demyelinating disease with multiple lesions in the brain, optic nerves, and spinal cord. It is classified into the following 3 disease types: Relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS), and primary progressive multiple sclerosis (PPMS). SPMS is defined as the disease type showing a history of gradual worsening after an initial relapsing disease course (RRMS), with or without exacerbations (relapses) during the progressive course (*Neurology*. 2014;83:287-86).

- The pathology of MS is considered to be caused by autoimmune response targeted at central nerves. In RRMS, active demyelinating foci are formed in the white matter of the brain and the spinal cord by acute inflammation. When RRMS progresses to SPMS, the active demyelination becomes localized, and axon degeneration progresses in the white matter that appears normal by MRI. Thus, in SPMS, the disability progression is shown by the pathology characterized mainly by the axon degeneration, whereas in other types of MS, the disability often progresses as a result of frequent relapses accompanied by inflammatory reactions or as a result of incomplete recovery. Therefore, in the Japanese clinical practice guideline, the progression in SPMS is expressed a "worsening" to differentiate from progressions in other types of MS (*Clinical Practice Guidelines Multiple Sclerosis and Neuromyelitis Optica 2017*).

- RRMS is considered to transition to SPMS within 15 to 20 years in approximately half of the patients. However, there are no clear imaging or pathological criteria for determining the time point of transition to SPMS. Instead, diagnosis of SPMS is made when the disease conditions have gradually progressed without clear relapses (*Clinical Practice Guidelines for Multiple Sclerosis and Neuromyelitis Optica 2017*).

- According to the diagnostic criteria of the Ministry of Health, Labour and Welfare, SPMS is defined as the disease type that "shows gradual progression of the disease conditions without any clear relapse after repeating relapses and remissions (relapsing and remitting type) for a certain period."[44] In the global phase III study (CTD 5.3.5.1-1, Study A2304) in patients with SPMS, the patients had to be experiencing disease progression for ≥6 months with or without relapse. However, the diagnosis of SPMS[38] was made according to the criteria similar to those of the Guideline and of the Ministry of Health, Labour and Welfare.

- Multiple therapeutic agents are approved for the treatment of MS in Japan, and most of them have shown efficacy in clinical studies in mainly patients with RRMS. Although interferon beta-1b (genetical recombination) is approved for indications including SPMS with relapses in Japan and overseas countries, a systematic review on the efficacy of interferon beta formulations (interferon beta-1a [genetical recombination] and interferon beta-1b [genetical recombination]) in patients with SPMS reported that the disease progression of SPMS could not be suppressed by these treatments (*J Neurol Neurosurg Psychiatry.* 2013;84:420-6). The Japanese clinical practice guideline states merely that interferon beta may possibly be effective in progressive MS (*Clinical Practice Guidelines for*

[44] https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000062437.html (the last confirmed date, March 11, 2020)

Multiple Sclerosis and Neuromyelitis Optica 2017). Thus, there is no drug with proven efficacy in patients with progressive MS including SPMS.

- Siponimod has been shown to be effective in suppressing the progression of physical disability in the global phase III study (CTD 5.3.5.1-1, Study A2304) [see Section 7.R.3.5]. As for the safety, although siponimod is not free from the risk of adverse events observed with S1P receptor modulators, such as infection, hepatic dysfunction, macular oedema, hypertension, and decreased heart rate and arrhythmia during the early stage of treatment, they can be managed by appropriate precautions [see Section 7.R.4]. Siponimod is thus considered to provide a novel treatment option for SPMS.

PMDA's view:

- Conventional therapeutic agents for MS have been shown to be effective in clinical studies in patients with RRMS, whereas siponimod has proven effective in patients with SPMS in clinical studies [see Section 7.R.3]. Thus, siponimod provides a novel treatment option in patients with SPMS.

- The indication of siponimod is discussed further in Section 7.R.5.

7.R.2 Effect of inappropriate control of access rights to database in global phase III study (CTD 5.3.5.1-1, Study A2304)

In the global phase III study (CTD 5.3.5.1-1, Study A2304), the database-accessing right that was restricted for maintaining the blinding was inappropriately controlled. PMDA asked the applicant to explain the details and the effect on efficacy and safety evaluation.

The applicant's explanation:

- In confirmatory studies on patients with MS which use an EDSS-related primary endpoint, usually EDSS evaluation and other evaluations (except for MRI evaluation) are performed by separate investigators (*Ann Neurol.* 2001;49:290-7, *N Engl J Med.* 2006;354:899-910). In Study A2304, a person responsible for the first dose was additionally included because decrease in blood pressure and pulse rate is observed at the start and resumption of treatment with siponimod. Information possibly affecting the blinding was controlled as independent databases, as shown in Table 49.

Table 49. Outline of databases in Study A2304

Database	Accessible users	Main data items
EDSS database	EDSS evaluator	EDSS score
Main database	Investigator and supporting staff	The following data after the dose-escalation phase[a]: • Vital signs, adverse events • Other safety data (pulmonary function test, skin test, ophthalmological test, etc.) • Secondary or exploratory efficacy endpoints (other than EDSS and MRI)
First dose database	Person responsible for the first dose and supporting staff	The following data during the dose-escalation phase[a]: • Vital signs, adverse events

a) Dose-escalation phase, 7 days from the start of administration of study drug or from the resumption after treatment interruption

- However, due to inappropriate control of the restricted database access rights, access rights to an inappropriate database were given to some evaluators, possibly resulting in the broken blinding in

some patients. Table 50 shows the reasons for the breakage and the number of patients affected by each type of breakage.

Table 50. Reasons for potentially unblinding and the number of patients potentially unblinded for each reason (Study A2304)

Original access right	Reason for potentially unblinding	No. of patients (including duplicate counting)
EDSS DB	C1: EDSS evaluator may have accessed the initial dose DB.	7
	C2: Main DB user evaluated EDSS.	3
	C3: EDSS evaluator may have accessed the main DB.	52
	C4: EDSS evaluator inputted data into, or corrected, main DB.	5
Main DB	C5: Main DB user may have accessed the initial dose DB.	92
	C6: Main DB user inputted data into, or corrected, the initial dose DB.	10
	C7: Violations related to the blinding to the main DB evaluator[a)]	50
	C8: Adverse events or serious adverse events	63
Initial dose DB	C9: The initial dose DB user may have accessed the main DB.	133
	C10: The initial dose DB user inputted data into, or corrected, the main DB.	9

DB, database

a) The following cases among the protocol violations: (1) Leaking the information possibly allowing the main DB user to predict treatment group assignment, and (2) indirectly obtaining the information that is contained in the first dose DB (e.g., "the main DB user [investigator] supported the initial dose DB user in evaluating the Holter ECG during the dose-escalation phase").

- The following populations were identified as populations potentially affected by blinding regarding secondary endpoint ARR[45)] and safety during the period until the observation of 3mCDP, the primary endpoint:
  (a) The population in whom the blinding may have been broken due to the access of the EDSS evaluator to forbidden databases (C1, C3, and C4) (22 patients in the placebo group, 40 patients in the siponimod group; excluding duplicate counting).
  (b) (1) The EDSS evaluator or the investigator may have accessed the forbidden databases, being patients potentially unblinded (C1-C6), (2) a case of protocol violation in which the investigator may have obtained the information that could allow predicting the treatment group assignment (C7), and (3) the person responsible for the first dose accessed and corrected the forbidden main data (C10) (74 patients in the placebo group, 143 patients in the siponimod group; excluding duplicate counting).
  (c) The blinding to the EDSS evaluator and to the investigator may have been broken (C1, C3, C4, C5, and C6; 57 patients in the placebo group, 105 patients in the siponimod group).
  (d) The blinding to the investigator may have been broken (C5, C6, and C10; 38 patients in the placebo group, 72 patients in the siponimod group).

- As for the time to 3mCDP, the primary endpoint, since 3mCDP was evaluated by EDSS, results of the populations mainly related to EDSS evaluation (populations [a] and [b]) and each of other populations were compared. Table 51 shows the results of the comparison. In both of the populations (a) and (b), the incidence of the events tended to be lower in the siponimod group than in the placebo group, albeit comparison based on a limited number of patients.

45) ARR was calculated based on confirmed relapses. Relapses (aggravation, ≥30 days after the previous demyelinating event, of stable or improved neurological abnormal findings, persisting for ≥24 hours without pyrexia [≥37.5°C] and infection), were defined as "confirmed relapses" when an independent physician evaluated EDSS and any of the following was observed: (1) EDSS aggravation by ≥0.5 points, or (2) aggravation of ≥1 Functional System Scales by ≥1 point.

Table 51. Comparison of the evaluation of time to 3mCDP in patients potentially unblinded (Study A2304)

Population	Treatment group	Incidence (n/N)	Risk reduction rate[a)]	Hazard ratio[b)] [95% CI]
FAS	Placebo	31.7% (173/545)	21.2%	0.79 [0.65, 0.95]
	Siponimod	26.3% (288/1096)		
(a) Populations C1, C3, and C4	Placebo	27.3% (6/22)	45.6%	0.54 [0.18, 1.60]
	Siponimod	20.0% (8/40)		
Populations other than (a)	Placebo	31.9% (167/523)	20.7%	0.79 [0.65, 0.96]
	Siponimod	26.5% (280/1056)		
(b) Populations C1 to C7 and C10	Placebo	40.5% (30/74)	50.6%	0.49 [0.30, 0.81]
	Siponimod	23.8% (34/143)		
Populations other than (b)	Placebo	30.4% (143/471)	15.3%	0.85 [0.69, 1.04]
	Siponimod	26.7% (254/953)		

N, No. of patients without missing covariate data; n, No. of patients with events
a) (1 – hazard ratio) × 100
b) Based on the Cox proportional hazard model with treatment group, country or region, presence/absence of relapse within 2 years before screening, and the baseline EDSS score as covariates.

- As for the secondary endpoint ARR, since a confirmed relapse was evaluated based on EDSS evaluation by the EDSS evaluator on patients in whom a relapse was suspected by the attending physician, ARR was evaluated among population (c), population (b), and other populations. As shown in Table 52, ARR did not tend to be significantly different in any population from ARR observed in FAS, albeit based on a limited number of patients in populations (c) and (b).

Table 52. Comparison of ARR among patients potentially unblinded (Study A2304)

Population	Treatment group	No. of patients evaluated	ARR [95% CI]	ARR reduction rate	ARR ratio [95% CI]
FAS	Placebo	546	0.160 [0.123, 0.207]	55.5%	0.445 [0.337, 0.587]
	Siponimod	1099	0.071 [0.055, 0.092]		
(c) Populations C1, C3, C4, C5, and C6	Placebo	57	0.234 [0.146, 0.374]	32.6%	0.674 [0.366, 1.239]
	Siponimod	105	0.158 [0.107, 0.233]		
Populations other than (c)	Placebo	489	0.146 [0.116, 0.182]	58.6%	0.414 [0.303, 0.566]
	Siponimod	994	0.060 [0.049, 0.075]		
(b) Populations C1 to C7 and C10	Placebo	74	0.251 [0.158, 0.398]	40.4%	0.596 [0.330, 1.076]
	Siponimod	143	0.150 [0.103, 0.217]		
Other than (b)	Placebo	472	0.141 [0.112, 0.176]	59.0%	0.410 [0.299, 0.563]
	Siponimod	956	0.058 [0.046, 0.072]		

Analysis conducted using a negative binomial regression model. Treatment group, country or region, presence/absence of a relapse within 2 years before screening, the baseline EDSS score, and the baseline number of gadolinium enhanced lesions were used as covariates, and natural logarithm of the length (in years) of participation in the double-blind phase was included in the model as the offset variable.

- Regarding safety, Table 53 shows the incidence of adverse events, showing no tendency of any significant difference between each population and the safety analysis population.

Table 53. Comparison of the incidence of adverse events in patients potentially unblinded (Study A2304)

Population	Safety analysis population		(d) Population C5, C6, and C10		Population other than (d)	
Treatment group	Placebo	Siponimod	Placebo	Siponimod	Placebo	Siponimod
No. of patients evaluated	546	1099	38	72	508	1027
All adverse events	81.7 (446)	89.3 (981)	71.1 (27)	90.3 (65)	82.5 (419)	89.2 (916)
Serious adverse events	13.6 (74)	17.2 (189)	13.2 (5)	18.1 (13)	14.4 (73)	17.5 (180)
Adverse events leading to treatment discontinuation	5.1 (28)	7.8 (86)	2.6 (1)	11.1 (8)	5.3 (27)	7.6 (78)
Main adverse events						
Headache	13.0 (71)	14.4 (158)	15.8 (6)	33.3 (24)	12.8 (66)	13.0 (134)
Nasopharyngitis	14.5 (79)	13.6 (149)	15.8 (6)	19.4 (14)	14.4 (73)	13.1 (135)
Urinary tract infection	14.8 (81)	12.1 (133)	10.5 (4)	12.5 (9)	15.2 (77)	12.1 (124)
Fall	11.2 (61)	11.6 (128)	15.8 (6)	8.3 (6)	10.8 (55)	11.9 (122)
Hypertension	7.9 (43)	10.9 (120)	0	12.5 (9)	7.7 (39)	10.8 (111)
Fatigue	9.3 (51)	9.2 (101)	13.2 (5)	13.9 (10)	9.1 (46)	8.9 (91)
Upper respiratory tract infection	7.5 (41)	8.3 (91)	10.5 (4)	9.7 (7)	7.3 (37)	8.2 (84)
Nausea	3.5 (19)	6.8 (75)	0	12.5 (9)	3.7 (19)	6.4 (66)
Dizziness	4.8 (26)	6.8 (75)	5.3 (2)	6.9 (5)	4.7 (24)	6.8 (70)
Influenza	7.3 (40)	6.7 (74)	2.6 (1)	6.9 (5)	7.7 (39)	6.7 (69)
Diarrhoea	4.2 (23)	6.4 (70)	5.3 (2)	11.1 (8)	4.1 (21)	6.0 (62)
Back pain	8.1 (44)	6.2 (68)	15.8 (6)	5.6 (4)	7.5 (38)	6.2 (64)
ALT increased	1.5 (8)	5.9 (65)	0	6.9 (5)	1.6 (8)	5.8 (60)
Pain in extremity	3.8 (21)	5.6 (61)	7.9 (3)	6.9 (5)	3.5 (18)	5.5 (56)
Arthralgia	6.4 (35)	4.7 (52)	10.5 (4)	5.6 (59	6.1 (31)	4.7 (48)
Depression	5.5 (30)	4.6 (51)	7.9 (3)	1.4 (1)	5.3 (27)	4.9 (50)

Incidence (%) (No. of patients with events)

- Sensitivity analyses of the above and other parameters were conducted on data, excluding those of patients possibly affected by inappropriate access right provision. Results did not show any tendency of major differences from those obtained from the entire population. These results suggest that it is appropriate to evaluate the efficacy and safety of siponimod based on the data obtained from the entire population of Study A2304.

PMDA's view:

- In the "population other than (b)," the population from which patients potentially unblinded were excluded most conservatively, the hazard ratio of time to 3mCDP, the primary endpoint, between the siponimod group and the placebo group was greater than in FAS. This result suggests the possibility that the inappropriate access right provision affected efficacy evaluation.

- However, taking into account that (1) all of the populations listed in Tables 51 and 52 showed a tendency of improvement in each efficacy endpoint in the siponimod group than in the placebo group and the incidence of adverse events did not show any clear tendency of difference among populations, and (2), currently in Japan, there is no therapeutic agents with proven efficacy in patients with SPMS in clinical studies, with the resulting high medical need for siponimod, there is no choice but to evaluate the efficacy and safety of siponimod based on the results of Study A2304.

- Taking account of the above, it is not inappropriate to evaluate the results of Study A2304 including potentially unblinded data. In the following discussions, the results of the analysis of the entire population, including the patients potentially unblinded, are described, upon evaluating the effect of inappropriate access to the database on the main results of analyses.

- In the US and Europe, the main evaluation in the entire population was conducted using Study A2304 as the confirmatory study, and siponimod has been approved for the indication including patients with SPMS.

- The above conclusion will be finalized, taking account of comments raised in the Expert Discussion.

7.R.3 Efficacy

7.R.3.1 Intrinsic and extrinsic ethnic factors in global phase III study (CTD 5.3.5.1-1, Study A2304)

Since the global phase III study (CTD 5.3.5.1-1, Study A2304) was conducted as an international joint study with participants from many countries, PMDA asked the applicant to explain the effect of intrinsic and extrinsic ethnic factors on the efficacy and safety of siponimod.

The applicant's explanation:

- The pharmacokinetics study in Japanese and non-Japanese healthy adults showed no significant difference in the pharmacokinetics between the ethnic groups, and the population pharmacokinetic study did not detect any effect of race or ethnicity [see Section 6.R.2]. These results suggest that there is no significant difference in the pharmacokinetics of siponimod between ethnicities.

- During the early stage of the clinical study, McDonald Diagnostic Criteria (*Ann Neurol.* 2011;69:292-302) were used internationally. In Japan, the diagnostic criteria adjusted for Japanese patients with MS, worked out by the Neuroimmunological Disease Research Committee of the Ministry of Health, Labour and Welfare Japan, were used mainly. Both sets of diagnostic criteria use dissemination in time (repeated relapse and remission over time) and dissemination in space (development of lesions in distinct anatomical locations within the CNS) as the main diagnostic criteria, indicating that there is no significant difference in the diagnostic criteria for MS in Japan and overseas countries.

- In RRMS, acute inflammation caused by autoimmune response disrupts the blood-brain barrier, resulting in active demyelination. When RRMS has progressed to SPMS, dysfunction and death of nerve cells are induced by cytokines produced by activated microglia and oxidative stress (*Clinical Practice Guidelines for Multiple Sclerosis and Neuromyelitis Optica 2017*). There are no reports of an ethnic difference in the pathogenesis of SPMS. In clinical studies of fingolimod, a drug with the mechanism of action similar to that of siponimod, in patients with RRMS, fingolimod showed similar effects on suppression of inflammatory foci in the brain and on prevention of a clinical relapse in Japan and overseas countries. The above results suggest that there is no significant difference in the pathology of patients with MS and their response to siponimod between Japanese and Westerners.

- At the time when Study A2304 was started, there was no established treatment method for SPMS globally (*Neurology.* 2008;70:1134-40). In the US and in Europe, mitoxantrone hydrochloride and interferon beta-1b (genetical recombination), respectively, was approved for the treatment of MS including SPMS and, in Japan, interferon beta-1a (genetical recombination), interferon beta-1b (genetical recombination), and fingolimod were approved for the treatment of MS at the start of the clinical study. The main drugs that had been used for the treatment of MS in patients enrolled in study

A2304 were interferon beta-1b (genetical recombination) and interferon beta-1a (genetical recombination) both in the entire population and in the Japanese population, showing no significant difference in the prior treatment received between Japanese and non-Japanese patients.

- Based on the above, it is considered appropriate that Study A2304 was conducted as a global study.

PMDA accepted the applicant's explanation.

7.R.3.2 Justification for setting the target patients and efficacy endpoints

PMDA asked the applicant to provide the justification for the study patients and the efficacy endpoints in the global phase III study (CTD 5.3.5.1-1, Study A2304).

The applicant's explanation:

- In Study A2304, the inclusion criteria[38)] were patients with a history of RRMS showing disease progression persisting for ≥6 months with or without relapses, by referring to the globally used definition of SPMS, i.e., "a type of MS that shows gradual worsening after an initial relapsing and remitting disease course, with or without relapses, mild remissions, and sojourn periods during the progressive course" (*Neurology.* 1996;46:907-11). In order to exclude patients with RRMS, the eligible patients had to meet the following criteria: (1) The EDSS score was 3.0 to 6.5, (2) disease progression assessed by EDSS was observed during the 2 years before the start of the study, and (3) there was no relapse within 3 months before randomization. Although there are no criteria that clearly identify the transition of RRMS to SPMS [see Section 7.R.1], in the observation of spontaneous courses, patients with an EDSS score of ≤3.0 were already in the process of transiting to SPMS (*Brain.* 2006;129:584-94).

- As for efficacy endpoints in Study A2304, in order to appropriately evaluate the disease progression in SPMS, the time to 3mCDP,[42)] the parameter evaluating disease progression, was selected as the primary endpoint. Although EDSS is a standard index for the comprehensive evaluation of the physical disorders of patients with MS based on neurological tests, the relationship between the extent in the increase in the score and the extent of the clinical progression of the disease differs depending on the baseline EDSS score, suggesting that it is inappropriate to use the change in EDSS as the parameter for the between-group comparison of SPMS progression.[46)] Therefore, by also referring to the guidance[46)] for European Medicines Agency (EMA) at the start of the study, the following "disease progression based on EDSS" was defined: (1) Increase in the EDSS score by ≥1 from baseline in patients with a baseline EDSS score of ≤5.0, and (2) increase in the EDSS score by ≥0.5 from baseline in patients with a baseline EDSS score of ≥5.5, and the percentage of patients showing disease progression of the groups was compared.

- Since Study A2304 was a clinical study in patients with SPMS, who included patients with a relapse, ARR, the parameter on a relapse commonly used for the evaluation of inflammatory disease activity in clinical studies on patients with RRMS, was used as the secondary endpoint.

46) Guideline on clinical investigation of medicinal products for the treatment of multiple sclerosis, CHMP/EWP/561/98 Rev. 1: 2007

PMDA's view on the patients included in Study A2304 and the efficacy endpoints:

- There are no problems either in the patients included in the study, or in the primary endpoint (time to 3mCDP) and the secondary endpoint (ARR) used in the study.

- The efficacy of siponimod against a relapse and against the progression of a physical disorder is discussed further in Sections 7.R.3.4 and 7.R.3.5, and indication in Section 7.R.5.

7.R.3.3 Efficacy evaluation in the global phase III study (Study A2304)

PMDA asked the applicant to explain the patient characteristics possibly affecting the efficacy of siponimod and to explain the appropriateness of evaluating the efficacy in Japanese patients based on the results of the entire population in the global phase III study (CTD 5.3.5.1-1, Study A2304).

The applicant's explanation:

- Table 54 shows the time to 3mCDP during the double-blind phase in Study A2304, classified by patient characteristics. In the population with high baseline EDSS and in the population with a prior treatment with interferon beta-1b (genetical recombination), the intergroup difference between the siponimod group and the placebo group tended to be small. In any populations with different characteristics, however, time to 3mCDP tended to shorter in the siponimod group than in the placebo group.

Table 54. Evaluation of time to 3mCDP in populations with different characteristics (double-blind phase of Study A2304, FAS)

		Placebo	Siponimod	Hazard ratio [95% CI]
Baseline EDSS score	<4.0	33.3 (17/51)	28.2 (31/110)	0.75 [0.41, 1.36]
	4.0-4.5	32.3 (32/99)	20.9 (43/206)	0.62 [0.39, 0.98]
	5.0-6.0	37.8 (104/275)	34.2 (166/486)	0.89 [0.69, 1.13]
	>6.0	16.7 (20/120)	16.3 (48/294)	0.91 [0.54, 1.54]
Sex	Male	33.6 (75/223)	29.7 (129/435)	0.81 [0.60, 1.07]
	Female	30.3 (98/323)	23.9 (159/664)	0.77 [0.60, 1.00]
Prior treatment with interferon beta-1b (genetical recombination)	Yes	28.6 (44/154)	25.9 (89/344)	0.90 [0.62, 1.29]
	No	32.9 (129/392)	26.4 (199/755)	0.75 [0.60, 0.94]
No. of gadolinium enhanced T1 lesions	≥1	30.8 (128/415)	26.4 (219/828)	0.64 [0.42, 0.95]
	0	35.1 (40/114)	25.8 (61/236)	0.82 [0.66, 1.02]
Patients in early progression[c)]	Yes	41.4 (60/145)	31.1 (82/264)	0.65 [0.46, 0.91]
	No	28.2 (113/401)	24.7 (206/835)	0.86 [0.69, 1.09]
Moderate or severe MS[d)]	Yes	30.7 (141/459)	25.7 (232/904)	0.80 [0.65, 0.99]
	No	36.8 (32/87)	28.7 (56/195)	0.73 [0.47, 1.13]

Incidence (%) (No. of patients with events/No. of patients evaluated)

c) Defined as patients showing increase in the EDSS score by 1.5 points from 2 years before the start of the study. Progression of dysfunction during this period was not assessed.

d) Defined as ≥4 points in the Multiple Sclerosis Severity Score.

- Table 55 shows the time to 3mCDP, the primary endpoint, in the entire population and in the Japanese population. In the siponimod group of the Japanese population, the time to 3mCDP did not tend to become shorter than in the placebo group. This may be possibly due to the effect of the baseline characteristics, as judged by the following: (1) The percentage of patients with baseline EDSS ≥6.0 was 55.6% (54.2% in the placebo group, 56.3% in the siponimod group) in the entire population and 65.2% (62.5% in the placebo group, 66.7% in the siponimod group) in the Japanese population, and (2) the between-group difference in the time to 3mCDP tends to be shorter in the population with a high baseline EDSS (Table 54).

Table 55. Evaluation of time to 3mCDP in the entire population and in the Japanese population (double-blind phase of Study A2304, FAS)

Population	Treatment group	Incidence of event (n/N)	Risk reduction rate[a)]	Hazard ratio [95% CI][b)]
Entire population	Placebo	31.7% (173/545)	21.2%	0.79 [0.65, 0.95]
	Siponimod	26.3% (288/1096)		
Japanese population	Placebo	12.5% (1/8)	-133.4%	2.33 [0.26, 20.91]
	Siponimod	26.7% (4/15)		

N, No. of patients without missing covariate data; n, No. of patients with events

a) (1 – hazard ratio) × 100

b) Results of the analysis of the entire population are based on the Cox proportional hazard model with treatment group, country or region, presence/absence of a relapse within 2 years before screening, and the baseline EDSS score as covariates. Results of the analysis of the Japanese population are based on the Cox proportional hazard model with treatment group, country or region, presence/absence of a relapse within 2 years before screening, the baseline EDSS score, and interaction between treatment group and country/region as covariates.

From the number of patients in the entire population and the number of patients in the Japanese population enrolled in Study A2304, the probability of obtaining the consistent results[47)] between the entire population and the Japanese population was calculated to be 58.6%. The estimated global prevalence of SPMS, the target disease, is 33 per 100,000 people, and that the higher the latitude, the higher the prevalence.[48)] On the other hand, in Japan, the prevalence is estimated to be 7.7 per 100,000 people (*Mult Scler.* 2009;15:159-73), precluding the enrollment of Japanese patients in a number sufficiently large to fully ensure the consistency in the results of comparison between the entire population and the Japanese population.

- As for the secondary endpoint ARR, the relapse rate tended to decrease more in the siponimod group than in the placebo group, both in the entire population and in the Japanese population (Table 56).

Table 56. ARR in the entire population and in the Japanese population (double-blind phase in Study A2304, FAS)

Population	Treatment group	ARR [95% CI]	ARR reduction rate	ARR ratio [95% CI]
Entire population	Placebo (546 patients)	0.160 [0.123, 0.207]	55.5%	0.445 [0.337, 0.587]
	Siponimod (1099 patients)	0.071 [0.055, 0.092]		
Japanese population	Placebo (8 patients)	0.460 [0.103, 2.053]	100%	0.000 [0.000, -]
	Siponimod (15 patients)	0 [0.000, -]		

Analysis conducted using a negative binomial regression model. In the entire population, treatment group, country or region, presence/absence of a relapse within 2 years before screening, the baseline EDSS score, and the baseline number of gadolinium enhanced lesions were used as covariates, and natural logarithm of the length (in years) of participation in the double-blind phase was included in the model as the offset variable. In the Japanese population, treatment group, country or region, presence/absence of a relapse within 2 years before screening, the baseline EDSS score, the baseline number of gadolinium enhanced lesions, and interaction between treatment group and country/region were used as covariates, and natural logarithm of the length (in years) of participation in the double-blind phase was included in the model as the offset variable.

- Thus, although there are limitations to the interpretation of the results because of the limited number of patients in the Japanese population, it is considered appropriate to evaluate the efficacy in Japanese patients with SPMS based on the results of the entire population, which suggests the efficacy of siponimod in Japanese patients as well.

PMDA's view:

- The efficacy of siponimod was demonstrated in the entire population of Study A2304.

47) By assuming the hazard ratio of 3mCDP in the entire population and in the Japanese population to be HR_{all} and HR_{JP}, respectively, if the risk reduction rate in the entire population (1 - HR_{all}) was greater than 0.5 times that in the Japanese population (1 - HR_{JP}), the consistency was assumed.

48) https://www.msif.org/wp-content/uploads/2014/09/Atlas-of-MS.pdf (the last confirmed date, March 11, 2020)

- Taking account of the extremely limited number of patients with SPMS in Japan, it is acceptable to evaluate the efficacy and safety in Japanese patients based on the results obtained from the entire population of Study A2304, as proposed by the applicant.

7.R.3.4 Efficacy against relapse

PMDA asked the applicant to explain the efficacy of siponimod in preventing a relapse.

The applicant's explanation:

- The global phase III study (CTD 5.3.5.1-1, Study A2304) enrolled patients who showed progressive disease aggravation lasting for ≥6 months with or without a relapse, had not shown a relapse within 3 months before randomization, and were not receiving corticosteroid. The percentage of patients without a relapse within 2 years before the screening was 62.8% (343 of 546) of patients in the placebo group and 64.4% (712 of 1105) of patients in the siponimod group.

- Table 57 shows ARR during the double-blind phase in Study A2304, which revealed a decrease in the relapse rate in the siponimod group than in the placebo group. A relapse was observed even in patients without a relapse during the 2 years before the study, and the relapse rate decreased more in the siponimod group than in the placebo group regardless of a relapse during the 2 years before the study.

- These results demonstrate the efficacy of siponimod against a relapse.

Table 57. ARR in Study A2304 (double-blind phase, FAS)

Characteristics factor	Treatment group	No. of patients evaluated	ARR [95% CI]	ARR ratio [95% CI]
Entire population	Placebo	546	0.160 [0.123, 0.207]	0.445 [0.337, 0.587]
	Siponimod	1099	0.071 [0.055, 0.092]	
Patient with relapse within past 2 years[a)]	Placebo	202	0.240 [0.180, 0.319]	0.579 [0.399, 0.839]
	Siponimod	388	0.139 [0.108, 0.178]	
Patient without relapse within past 2 years[a)]	Placebo	343	0.134 [0.097, 0.184]	0.347 [0.229, 0.525]
	Siponimod	708	0.046 [0.033, 0.064]	

Analysis conducted using a negative binomial regression model. In the entire population, treatment group, country or region, presence/absence of a relapse within 2 years before screening, the baseline EDSS score, and the baseline number of gadolinium enhanced lesions were used as covariates, and natural logarithm of the length (in years) of participation in the double-blind phase was included in the model as the offset variable. In the subpopulations classified by the presence/absence of a relapse within the past 2 years, treatment group, the baseline EDSS score, and the baseline number of gadolinium enhanced lesions were used as covariates, and natural logarithm of the length (in years) of participation in the double-blind phase was included in the model as the offset variable.

a) Counting backwards from the time of screening

PMDA's view:

Study A2304 showed a decrease in ARR in the siponimod 2 mg group than in the placebo group. Although ARR is the second endpoint, the results suggest the relapse-suppressive effect of siponimod in patients with SPMS. The indication of siponimod is discussed further in Section 7.R.5.

7.R.3.5 Efficacy against progression of physical disability

PMDA asked the applicant to explain the efficacy of siponimod against progression of physical disability.

The applicant's explanation:

- The global phase III study (CTD 5.3.5.1-1, Study A2304) demonstrated the superiority of siponimod to placebo in the time to 3mCDP, the primary endpoint [see Section 7.3].

- By taking account of the possible interaction between a relapse during the study period and siponimod's disease progression-delaying effect, the suppressive effect against disease progression was investigated in patients without a relapse within 2 years before screening. Table 58 shows the results. Although the hazard ratio of 3mCDP (siponimod group/placebo group) tended to be larger in the group without a relapse within the past 2 years than in those with a relapse, 3mCDP tended to be more suppressed in the siponimod group than in the placebo group in both populations.

Table 58. Evaluation of time to 3mCDP in patients with or without relapse within past 2 years (Study A2304, FAS)

	Placebo	Siponimod	Hazard ratio [95% CI]
Patients without relapse within past 2 years[a)]	29.4 (101/343)	26.8 (190/708)	0.87 [0.68, 1.11]
Patients with relapse within past 2 years[a)]	35.6 (72/202)	25.3 (98/388)	0.67 [0.49, 0.91]

a) Counting backwards from the time of screening

- A relapse was observed during the double-blind phase even in patients without a relapse within 2 years before screening, and siponimod suppressed the relapse (Table 57), suggesting the possibility that the relapse-suppressive effect contributed to the effect of siponimod to delay disease progression. Whichever the case, siponimod tended to improve the symptoms both in the population with high disease activity (patients with a relapse in the past) and in the population with low disease activity (patients without a relapse in the past).

- Thus, siponimod is expected to be effective against the progression of physical disability.

PMDA's view:

- Patients with SPMS show disease progression by frequent relapses and incomplete recovery (worsening) and the pathology characterized mainly by axon degeneration (progression). Study A2304 demonstrated the superiority of siponimod to placebo in the time to 3mCDP, the primary endpoint. On the other hand, there were some patients who had relapses during the study period, and siponimod suppressed the relapse [see Section 7.R.3.4]. Thus, the study did not clearly demonstrate the efficacy of siponimod against the relapse-independent disease progression.

- Nevertheless, judging from the observation that 3mCDP was suppressed more in the siponimod group than in the placebo group even in the patient population who did not show a relapse within the past 2 years, i.e., the population supposed to have lower disease activity, siponimod can be expected to have some efficacy against relapse-independent disease progression.

- The indication for siponimod is discussed further in Section 7.R.5.

7.R.4 Safety

7.R.4.1 Safety of siponimod

PMDA asked the applicant to explain the appropriateness of evaluating the safety in Japanese population based on the results in the entire population in the global phase III study (CTD 5.3.5.1-1, Study A2304).

The applicant's explanation:

Table 59 shows the incidence of adverse events in the entire population and in the Japanese population during the double-blind phase of Study A2304. Mainly observed events were headache, nasopharyngitis, etc., and there were no events with a particularly higher incidence in Japanese population, albeit based on limited data.

Table 59. Incidence of adverse events in the Japanese population and in the entire population (double-blind phase of Study A2304, safety analysis population)

	Entire population		Japanese population	
Treatment group	Placebo (n = 546)	Siponimod (n = 1099)	Placebo (n = 8)	Siponimod (n = 15)
All adverse events	446 (81.7)	981 (89.3)	8 (100.0)	14 (93.3)
Death	4 (0.7)	4 (0.4)	0	0
Serious adverse events	74 (13.6)	189 (17.2)	1 (12.5)	0
Adverse events leading to treatment discontinuation	28 (5.1)	86 (7.8)	0	2 (13.3)
Main events				
Headache	71 (13.0)	158 (14.4)	1 (12.5)	2 (13.3)
Nasopharyngitis	79 (14.5)	149 (13.6)	2 (25.0)	4 (26.7)
Urinary tract infection	81 (14.8)	133 (12.1)	1 (12.5)	0
Fall	61 (11.2)	128 (11.6)	0	2 (13.3)
Hypertension	43 (7.9)	120 (10.9)	1 (12.5)	1 (6.7)
Fatigue	51 (9.3)	101 (9.2)	0	0
Upper respiratory tract infection	41 (7.5)	91 (8.3)	0	0
Nausea	19 (3.5)	75 (6.8)	0	0
Dizziness	26 (4.8)	75 (6.8)	0	2 (13.3)
Influenza	40 (7.3)	74 (6.7)	0	0
Diarrhoea	23 (4.2)	70 (6.4)	0	2 (13.3)
Back pain	44 (8.1)	68 (6.2)	1 (12.5)	0
ALT increased	8 (1.5)	65 (5.9)	0	0
Pain in extremity	21 (3.8)	61 (5.6)	0	0
Arthralgia	35 (6.4)	52 (4.7)	0	0
Depression	30 (5.5)	51 (4.6)	0	0

No. of patients with events (incidence [%])

PMDA accepted the above applicant's explanation.

Taking account of the above safety profile of siponimod, PMDA reviewed the following events: Adverse events related to decreased heart rate and bradyarrhythmia during the early stage of administration, cardiovascular adverse events, decreased lymphocyte count, etc., and infection, hepatic dysfunction, macular oedema, and convulsive seizure.

7.R.4.2 Adverse events related to decreased heart rate and bradyarrhythmia during the early stage of administration

Adverse events related to decreased heart rate, bradyarrhythmia, etc. were observed during the early stage of administration of siponimod in clinical studies on siponimod. PMDA asked the applicant to explain the incidence of adverse events related to vital signs and bradyarrhythmia in clinical studies.

The applicant's explanation about the safety monitoring during the early stage of administration in clinical studies:

- Since siponimod activates the $S1P_1$ receptor-mediated GIRK channel in the membrane of atrial myocytes, heart rate decreases in the early phase of administration of siponimod. However, during the repeated administrations, the $S1P_1$ receptor is desensitized by internalization, gradually resulting

in loss of the heart rate-reducing effect. This has been confirmed in nonclinical studies [see Section 3.R.2].

- In the clinical pharmacology study in healthy subjects (CTD 5.3.4.1.3, Study A2107), the dose-escalation resulted in a decrease in the incidence of bradyarrhythmia during the early stage of administration (Table 60). In all dose-escalation groups, the heart rate returned to the level similar to that observed in the placebo group within 10 days. In the global phase III study (CTD 5.3.5.1-1, Study A2304), therefore, the dose was increased in a stepwise manner (Table 45) up to the maintenance dose.

Table 60. Heart rate change and incidence of sinus arrest in Study A2107 (safety analysis population)

	Placebo (n = 14)		Dose-escalation 1 (n = 14)		Dose-escalation 2 (n = 14)		Without dose-escalation (n = 14)	
	Heart rate change (bpm)[a)]	No. of subjects with sinus arrest[b)]	Heart rate change (bpm)[a)]	No. of subjects with sinus arrest[b)]	Heart rate change (bpm)[a)]	No. of subjects with sinus arrest[b)]	Heart rate change (bpm)[a)]	No. of subjects with sinus arrest[b)]
Baseline	59.5 ± 6.73	0	59.9 ± 8.23	1 (7.1)[c)]	60.7 ± 8.63	0	59.9 ± 7.35	0
Day 1	-10.5 ± 6.60	0	-14.3 ± 5.58	2 (14.3)[d)]	-14.6 ± 4.13	0	-26.1 ± 6.40	4 (28.6)[e)]
Day 2	-8.0 ± 6.39	0	-16.6 ± 5.98	2 (14.3)[c)]	-14.2 ± 5.48	0	-16.4 ± 5.39	0
Day 3	-7.8 ± 5.83	0	-16.3 ± 8.04	2 (14.3)[f)]	-16.4 ± 4.36	0	-15.6 ± 6.71	0
Day 4	-7.2 ± 4.78	0	-15.8 ± 6.56	1 (7.7)[c)]	-17.6 ± 5.81	0	-13.6 ± 5.45	0
Day 5	-7.6 ± 4.99	0	-19.6 ± 6.11	1 (7.1)[g)]	-18.6 ± 5.05	0	-16.0 ± 6.30	0
Day 6	-7.3 ± 6.07	0	-21.5 ± 9.34	2 (14.3)[h)]	-18.5 ± 6.11	1 (7.1)[i)]	-15.8 ± 6.58	0
Day 7	-6.2 ± 6.24	0	-17.2 ± 8.40	1 (7.1)[j)]	-15.8 ± 5.71	0	-15.8 ± 6.24	0
Day 8	-8.5 ± 5.56	0	-14.4 ± 9.15	1 (7.1)[k)]	-12.8 ± 6.10	0	-15.2 ± 4.49	0
Day 9	-6.7 ± 5.99	0	-9.1 ± 6.06	0	-9.2 ± 5.21	0	-16.4 ± 8.07	0
Day 10	-7.3 ± 4.29	0	-9.9 ± 6.99	0	-7.5 ± 4.16	0	-16.3 ± 6.92	0
Day 11	-7.4 ± 4.47	0	-7.8 ± 6.34	1 (7.1)[c)]	-5.7 ± 3.87	0	-15.6 ± 5.43	0
Day 12	-6.1 ± 6.43	0	-6.9 ± 8.47	0	-6.1 ± 4.05	0	-11.8 ± 7.06	0

In the group without dose-escalation, 10 mg was administered from Day 1.

a) Mean ± SD (heart rate at baseline, change in the trough mean heart rate over 1 hour from baseline on Day 1 and onward)

b) No. of subjects showing sinus arrest for >2 seconds on Holter ECG (percentage)
The number of times of sinus arrests in the subject showing the maximum number of sinus arrests during the period from baseline to Day 12.

c) 1, d) 5, e) 834, f) 2, g) 22, h) 142, i) 6, j) 450, k) 3

- In study A2304, patients with a high cardiovascular risk[49] were excluded, and all patients underwent a wide range of safety monitoring[50] at the start of the study, as shown in Table 61. When appropriately half of the target number of patients were enrolled in the study, data monitoring committee (DMC) evaluated the data of the safety monitoring. As a result, in the latter half of the study, patients who met the criteria shown in Table 62 continuously underwent a wide range of safety monitoring, while subjects who did not meet the criteria underwent the usual safety monitoring in Table 61.

- In Study A2304, patients attended visits on Day 1, 7, 28, and on Month 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, and underwent 12-lead electrocardiogram (12-lead ECG) on Day 1 and 7, and on Month 3, 12, 24, and 36.

- In subjects who underwent a wide range of safety monitoring, at 6 hours after administration on Day 1 and 7, if patients met the criteria ([a] heart rate returned to ≥50 bpm or heart rate decrease before baseline was <10 bpm (met at least one of the [a] or [b]), [b] heart rate was not the lowest among the values observed during the follow-up period, [c] there were no symptoms associated with heart rate decrease, and [d] electrocardiography after 6 hours did not show a new serious electrocardiogram abnormal [excluding sinus bradycardia] not observed in electrocardiography before administration), they were discharged. If they failed to meet the criteria, they underwent continued monitoring.

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[49] The following patients were excluded from the study as patients with a high cardiovascular risk:
- Patient with a past or current history of serious cardiac disease including cardiac failure (New York Heart Association [NYHA] class II-IV), myocarditis, cardiomyopathy, angina pectoris or myocardial infarction (within past 6 months), angina unstable (within past 6 months), stroke (within past 6 months), transient ischemic attacks (TIA) (within past 6 months), decompensated heart failure necessitating hospitalization (within past 6 months), or poorly controlled arterial hypertension.
- Patients who have any of the following disorders if without the help of an effective pacemaker: Complete left bundle branch block, sinus arrest or sinoatrial block, symptomatic bradycardia, sick sinus syndrome, cardiac conduction or rhythm disorder including Mobitz type II second degree atrioventricular block or second or higher degree atrioventricular block (past history or observed at screening)
- Patients with arrhythmia requiring treatment or past history of cardiac syncope
- Patients being treated with class Ia or III antiarrhythmic agent
- Patients requiring treatment with drugs that may cause atrioventricular block or suppress atrioventricular conduction other than β blockers (e.g., carbamazepine, non-dihydropyridine calcium channel blocker, cardiotonic glycoside)
- Patients being treated with calcium channel blocker with a heart rate-decreasing effect or other drugs with a potential heart rate-decreasing effect (e.g., digoxin, anticholinesterase, pilocarpine) at the time of randomization (the start of administration)
- Patients with PR interval exceeding 230 msec
- Patients showing long QT syndrome or QTcF prolongation by >450 msec (men) or >470 msec (women) on the electrocardiogram test at screening
- Patients with severe autonomic dysfunction
- Patients with heart conditions requiring catheter ablation
- Patients whose safety may possibly be seriously affected by treatment with siponimod, as judged by the investigator

[50] Taking account of the observations that symptomatic atrioventricular block was observed only on the first day of dosing in clinical studies prior to Study A2304, and that no clinically significant symptomatic bradyarrhythmia was observed during the dose-escalation phase including the first day of dosing in studies that employed the dose-escalation method (Study A2107, the second phase of Study A2201, and Study A2201E1), safety monitoring was conducted focused on the first day of dosing.

Table 61. Usual and wide-range safety monitoring up to Day 7 (Study A2304)

Monitoring		Usual safety monitoring	Wide-range safety monitoring
At screening	Vital signs	Measured	Measured
	12-lead ECG	Measured	Measured
	Mobile cardiac telemetry	Measured for ≥3 successive days	Measured for ≥3 successive days
	Holter ECG[a)]	Measured for 24 hours	Measured for 24 hours
Day 1	Vital signs	Measured before the first dose	Measured before the first dose and at 1-hour intervals up to 6 hours after administration
	12-lead ECG	Measured before the first dose	Measured before the first dose, and at 3 and 6 hours after administration
	Mobile cardiac telemetry	Measured for 6 days from Day 1 (findings at interim evaluation on Day 4 were notified to the subject)	Measured for 6 days from Day 1 (findings at interim evaluation on Day 4 were notified to the subject)
	Holter ECG[a)]	Measured for 24 hours, on Day 1 and 4 each	Measured for 24 hours, on Day 1 and 4 each
Day 7	Vital signs	Measured before administration	Measured before the administration and at 1-hour intervals up to 6 hours after administration
	12-lead ECG	Measured before administration	Measured before the administration, and at 3 and 6 hours after administration
	Holter ECG[a)]	Measured up to 6 hours after administration	Measured up to 6 hours after administration

a) Only if mobile cardiac telemetry could not be used, Holter ECG was used.

Table 62. Criteria for the conditions of cardiovascular system requiring wide-range safety monitoring

1. Heart rate at screening was <55 bpm.
2. Conduction disorder such as incomplete left bundle branch block and Mobitz type I atrioventricular block, second degree (past history or observation at screening)
3. Electrocardiography at screening: PR interval >200 msec and ≤230 msec, QRS interval ≥120 msec, QTcF >430 msec and ≤450 msec (men), QTcF >450 msec and ≤470 msec (women)
4. Past or current cardiac disorder such as cardiac failure of NYHA class I, history of myocardial infarction before enrollment in the study
5. Patients being treated with a β-blocker
6. Other disease conditions suggesting possible suppressed atrioventricular conduction, as judged by the investigator, or other risk factors necessitating wide-range safety monitoring
7. When patients were diagnosed with right bundle branch block at the screening before the double-blind phase in all patients or during the study period, they underwent a wide-range safety monitoring before entering the extended administration phase.

In addition, the applicant's explanation about the effect on cardiac function in the clinical study:

- Table 63 shows the mean change in pulse rate from before administration up to 6 hours after administration on Day 1 and 7 in Study A2304. The pulse rate decreased within 1 hour after the administration, reached the lowest level after approximately 4 hours, and tended to return to the original level within 6 hours after administration.

Table 63. Mean change in pulse rate from before administration on Day 1 and 7 (Study A2304, safety analysis population)

Pulse rate (bpm)		Placebo			Siponimod		
		N[a)]	Measured value	Change from before administration	N[a)]	Measured value	Change from before administration
Day 1 (the first day of dosing)	Before administration	546	73.27 ± 10.789		1099	72.85 ± 10.307	
	1 hour after administration	483	72.26 ± 10.671	-1.16 ± 6.736	981	70.96 ± 10.335	-1.67 ± 7.145
	2 hours after administration	481	72.63 ± 10.927	-0.79 ± 7.707	976	69.05 ± 9.782	-3.56 ± 7.497
	3 hours after administration	481	73.19 ± 10.345	-0.23 ± 7.576	978	67.41 ± 9.639	-5.22 ± 7.786
	4 hours after administration	480	74.18 ± 10.463	0.76 ± 7.677	977	67.27 ± 9.311	-5.30 ± 7.666
	5 hours after administration	479	73.98 ± 10.074	0.54 ± 7.612	973	67.46 ± 9.277	-5.12 ± 7.721
	6 hours after administration	477	73.82 ± 10.299	0.43 ± 7.338	968	67.77 ± 9.402	-4.81 ± 7.256
Day 7	Before administration	541	74.05 ± 10.360		1078	68.37 ± 9.675	
	1 hour after administration	473	72.66 ± 9.910	-1.76 ± 6.586	954	66.54 ± 9.389	-1.60 ± 5.550
	2 hours after administration	470	72.44 ± 10.052	-1.95 ± 7.635	953	65.15 ± 9.063	-2.97 ± 6.410
	3 hours after administration	472	72.39 ± 10.185	-2.00 ± 7.649	953	65.03 ± 9.335	-3.08 ± 6.853
	4 hours after administration	472	74.06 ± 10.188	-0.32 ± 7.390	950	65.80 ± 8.940	-2.26 ± 6.683
	5 hours after administration	471	74.04 ± 9.685	-0.35 ± 7.532	947	66.34 ± 9.129	-1.75 ± 6.556
	6 hours after administration	468	74.22 ± 10.117	-0.17 ± 7.595	945	67.20 ± 9.351	-0.90 ± 6.323

Mean ± SD

a) Patients with data both before and after administration at each measuring time point

- Table 64 shows the trend of the daily mean (hourly averaged) trough heart rate during the dose-escalation phase, measured by mobile cardiac telemetry in separate safety monitoring groups (wide-range safety monitoring population, patients who met the criteria of Table 62; usual safety monitoring population, patients who did not meet the criteria of Table 62). In the siponimod group, the daily trough heart rate tended to decrease up to Day 5. The extent of the decrease in heart rate during the dose-escalation phase was the largest on Day 1.

Table 64. Daily mean trough heart rate in safety monitoring groups (Study A2304, safety analysis population)

Heart rate (bpm)	Wide-range safety monitoring population		Usual safety monitoring population	
	Placebo	Siponimod	Placebo	Siponimod
Baseline	60.44 ± 9.314 (123)	61.76 ± 8.653 (249)	63.82 ± 7.954 (314)	64.33 ± 8.034 (616)
Day 1 (the first day of dosing)	60.71 ± 8.810 (121)	57.48 ± 8.393 (247)	63.86 ± 8.208 (314)	60.07 ± 6.894 (622)
Day 2	60.52 ± 9.084 (121)	57.00 ± 8.692 (244)	64.13 ± 8.551 (312)	59.10 ± 7.309 (621)
Day 3	61.13 ± 9.185 (124)	55.39 ± 8.755 (244)	63.65 ± 8.527 (310)	57.91 ± 7.242 (621)
Day 4	61.14 ± 9.709 (123)	54.68 ± 8.509 (242)	63.63 ± 8.381 (302)	57.35 ± 8.073 (613)
Day 5	60.89 ± 8.838 (121)	54.20 ± 8.372 (238)	63.82 ± 8.357 (299)	56.86 ± 7.818 (604)
Day 6	62.20 ± 9.496 (119)	56.83 ± 10.550 (234)	64.29 ± 8.215 (291)	57.94 ± 8.054 (587)

Mean ± SD (No. of patients evaluated)

- Table 65 shows the percentage of patients with daily mean trough heart rate (hourly averaged) of <40 bpm (measured by mobile cardiac telemetry) and the percentage of patients who had sinus arrest of ≥3 seconds in each safety monitoring group. Sinus arrest was observed only in the siponimod group; the timing of the first onset was widespread from Day 1 to 6. The 8 patients in the siponimod

group who experienced sinus arrest of ≥3 seconds did not show any tendency of the occurrence of sinus arrest at any specific timing, neither did bradycardia tend to occur more frequently before sinus arrest.

Table 65. Day of occurrence of hourly-averaged trough heart rate <40 bpm and sinus arrest for ≥3 seconds in each safety monitoring group (double-blind phase of Study A2304, measured by mobile cardiac telemetry)

	Trough heart rate <40 bpm				Sinus arrest for ≥3 seconds			
	Wide-range safety monitoring population		Usual safety monitoring population		Wide-range safety monitoring population		Usual safety monitoring population	
	Placebo	Siponimod 2 mg	Placebo	Siponimod 2 mg	Placebo	Siponimod 2 mg	Placebo	Siponimod 2 mg
Before administration	0	0	0	0	0	0	0	0
Newly observed after administration	0	7.0 (17/244)	0	1.4 (9/624)	0	0.8 (2/247)	0	1.0 (6/624)
Day 1	0	0.8 (2/247)	0	0.2 (1/622)	0	0	0	0
Day 2	0	0.4 (1/244)	0	0.5 (3/621)	0	0	0	0.2 (1/621)
Day 3	0	1.6 (4/244)	0	1.0 (6/621)	0	0	0	0
Day 4	0	2.9 (7/242)	0	0.8 (5/613)	0	0.4 (1/242)	0	0.3 (2/613)
Day 5	0	4.2 (10/238)	0	1.0 (6/604)	0	0	0	0.3 (2/604)
Day 6	0	3.0 (7/234)	0	1.0 (6/587)	0	0.4 (1/234)	0	0.3 (2/587)

Incidence (%) (No. of patients with events/No. of patients evaluated

- The daily mean trough heart rate (hourly averaged) of <50 bpm (measured by mobile cardiac telemetry) was observed in 17.5% (17 of 97) of patients in the placebo group and in 40.8% (78 of 191) of patients in the siponimod group in the wide-range safety monitoring population, and in 7.4% (20 of 272) of patients in the placebo group and in 29.7% (668 of 1166) of patients in the siponimod group in the usual safety monitoring population. In both populations, the incidence tended to be higher in the siponimod group than in the placebo group.

- Among the patients who underwent monitoring of >6 hours due to abnormal electrocardiogram on the first day of dosing, 4 patients reported related adverse events (atrioventricular block first degree in 2 patients, electrocardiogram QT prolonged, and atrioventricular block second degree/bradycardia in 1 patient each), all of which except for atrioventricular block second degree/bradycardia were mild requiring no intervening treatment, and lasted for 3 days at the maximum.

- Decreased heart rate (<40 bpm) on 12-lead ECG was observed in 2 patients in the siponimod group only. One patient showed heart rate of 35 bpm at 5 hours after administration and atrioventricular block second degree on Holter ECG. The atrioventricular block second degree was asymptomatic but considered as a serious adverse event, but resolved within 3 days after the discontinuation of administration of siponimod. The other patient showed heart rate of 39 bpm at 2 hours after administration on Day 7 and was diagnosed with bradycardia (adverse event), but resolved on the same day without any intervening treatment, and the patient continued receiving the study drug.

- Sinus bradycardia (<40 bpm) and sinus arrest were observed by mobile cardiac telemetry in 3 patients in the siponimod group only. A pacemaker was implanted in one of them. This patient experienced asymptomatic sinus bradycardia (non-serious) on Day 1 of administration of the study drug. The

trough heart rate, measured by the mobile cardiac telemetry, was 40, 36, and 33 bpm, respectively, on Day 1, 2, and 3. On Day 4, the patient had sinus arrest of >2 seconds and showed heart rate of 20 bpm at 2 different time points, whereupon the study drug was discontinued on the same day. On Day 5 (the next day of discontinuation of the study drug) and succeeding days, heart rate of 20 bpm and sinus arrest of >2 seconds were observed, whereupon a pacemaker was implanted on Day 24 to treat idiopathic sinus bradycardia. The sinus bradycardia was assessed to be causally related to the study drug.

- During the dose-escalation phase in which patient conditions were evaluated by mobile cardiac telemetry, pre-defined noteworthy findings[51)] were observed in 12 patients, but the trough heart rate on Day 1 was observed 1 to 5 hours after administration, and resolved eventually.

The applicant's explanation about the incidence of bradyarrhythmia-related adverse events in the clinical study:

- Table 66 shows the incidence of bradyarrhythmia-related adverse events[52)] during the early stage of Study A2304. Although adverse events (including serious adverse events) occurred at a higher incidence in the siponimod group than in the placebo group, there was no clear difference between safety monitoring groups, and no particular tendency was observed in the comparison of the incidence between subgroups classified by other characteristics. Also, analysis of subgroups classified by the extent of heart rate decrease on Day 1 did not show any significant tendency in the occurrence of bradyarrhythmia-related adverse events from Day 2.

51) Subjects who met any of the following criteria: (a) Patients with abnormal electrocardiogram requiring treatment, (b) patients who were once discharged on Day 1 but made a return visit on Day 2 for additional monitoring because of the occurrence of a symptomatic event, (c) patients with low heart rate (<40 bpm), and (d) patients with symptomatic bradycardia confirmed by 12-lead ECG, mobile cardiac telemetry, or Holter ECG.

52) Events classified in "Bradyarrhythmias (incl conduction defects and disorders of sinus node function)" Standardized MedDRA Query (SMQ) or in the following PTs in MedDRA:
Bradycardia, heart rate decreased, syncope, blood pressure decreased, diastolic blood pressure decreased, blood pressure fluctuation, systolic blood pressure decreased, depressed level of consciousness, dizziness, dizziness exertional, dizziness postural, hypotension, loss of consciousness, orthostatic hypotension, presyncope, and malaise.

Table 66. Incidence of bradyarrhythmia-related adverse events up to Day 14 in each safety monitoring group (double-blind phase of Study A2304, safety analysis population)

	Wide-range safety monitoring population		Usual safety monitoring population	
	Placebo (n = 174)	Siponimod (n = 358)	Placebo (n = 372)	Siponimod (n = 741)
All adverse events	9 (5.2)	46 (12.8)	23 (6.2)	82 (11.1)
Serious adverse events	0	4 (1.1)	0	3 (0.4)
Adverse events leading to treatment discontinuation	0	4 (1.1)	0	7 (0.9)
Main events				
Bradycardia	6 (3.4)	20 (5.6)	8 (2.2)	28 (3.8)
Dizziness	1 (0.6)	8 (2.2)	13 (3.5)	32 (4.3)
Sinus bradycardia	1 (0.6)	6 (1.7)	0	8 (1.1)
Atrioventricular block first degree	0	3 (0.8)	1 (0.3)	7 (0.9)
Atrioventricular block second degree	0	2 (0.6)	0	3 (0.4)
Heart rate decreased	0	2 (0.6)	0	2 (0.3)
Hypotension	1 (0.6)	1 (0.3)	0	3 (0.4)
Electrocardiogram QT prolonged	0	3 (0.8)	0	0
Syncope	0	1 (0.3)	0	2 (0.3)

No. of patients with events (incidence (%))

PMDA asked the applicant to explain the appropriateness of the method of monitoring cardiac conditions at the start of the administration of siponimod, based on the results of clinical studies.

The applicant's explanation:
Based on the proposal of the DMC, the clinical study protocol of Study A2304 was revised. According to the revised protocol, patients who did not meet the criteria of Table 62 underwent the usual safety monitoring shown in Table 61. As a result, 11.2% (61 of 546) of patients in the placebo group and 10.3% (113 of 1099) of patients in the siponimod group underwent the usual safety monitoring. During the dose-escalation phase of Study A2304, there were no bradyarrhythmia-related adverse events requiring prompt treatment, which suggests that the safety management in Study A2304 was appropriate.

Based on the above, it is considered appropriate to include, in the package insert, the following safety management procedures and precautions at the start of administration:

- In medical practice, it is recommended to monitor the following patients for (1) clinical signs for 6 hours after the first dose and (2) cardiac conditions through 12-lead ECG before, and for 6 hours after, the first dose, according to the criteria for requiring the wide range safety monitoring conducted in Study A2304 (Table 62): Patients with sinus bradycardia (heart rate <55 bpm), with Mobitz type I atrioventricular block, first or second degree, or with a past history of myocardial infarction or cardiac failure. The above monitoring method and the precautions provided in the package insert are in line with those of the US and Europe.

- Treatment with siponimod should preferably be avoided in patients with severe bradyarrhythmia. In case of necessity, the most appropriate safety monitoring method should be consulted with a cardiologist. The above precautions will be provided in the package insert.

- Since siponimod has a transient bradycardic effect during the early phase of administration, it is contraindicated in patients with a particularly high risk among patients who meet the exclusion criteria of Study A2304[49)] (patients who had myocardial infarction, angina unstable, or cardiac failure

of New York Heart Association [NYHA] class III or IV within 6 months before treatment with siponimod).

- During the dose-escalation phase of administration of siponimod, the daily-averaged mean trough heart rate tended to decrease up to Day 5 in the siponimod group (Table 64). All patients receiving siponimod, with or without cardiovascular risk, should pay attention to symptoms during the dose-escalation phase such as decreased heart rate, dizziness, and staggering gait and, upon noticing any abnormality, should report promptly to the physician. The information material containing the emergency contact number should be provided.

PMDA's view:

- Regarding cardiovascular adverse events such as bradyarrhythmia that occur mainly during the early phase of administration of siponimod, the trough heart rate in the siponimod group tended to decrease up to Day 5 during the dose-escalation phase (Table 64). Also, the time of the first onset of decreased heart rate (<40 bpm) and sinus arrest (≥3 seconds) was widely distributed over the dose-escalation phase even in the usual monitoring population (Table 65).

- These results suggest the necessity of paying attention to bradyarrhythmia-related events and findings during the dose-escalation phase, and this information should be adequately provided to healthcare professionals and to patients. Regardless of the cardiovascular risks, heart rate and electrocardiogram should be measured before administration of siponimod on the first day of dosing (or on the day of resuming the administration after interruption of ≥4 days). After the administration, patients should be monitored for heart rate and electrocardiogram until it is confirmed that the post-administration decrease in heart rate shows a tendency to resolve and, depending on the conditions of patients, whether to continue monitoring should be decided. Monitoring of pulse rate and symptoms should be performed on each administration day during the subsequent remaining dose-escalation phase.

- Siponimod should be contraindicated in patients with a high cardiovascular risk, a patient group excluded from Study A2304.

- Precautions in the package insert and the appropriateness of the safety monitoring will be finalized, also taking account of comments from the Expert Discussion.

7.R.4.3 Cardiovascular adverse events

PMDA asked the applicant to explain the incidence of cardiovascular adverse events associated with siponimod.

The applicant's explanation:

- Table 67 shows the incidence of cardiovascular adverse events[53)] in the global phase III study (CTD 5.3.5.1-1, Study A2304). The incidence was higher in the siponimod group than in the placebo group.

[53)] Events included in "Cardiac disorders" or "Vascular disorders" in MedDRA SOC, or in "Cardiac and vascular investigations (excl enzyme tests)" in high level group terms (HLGT).

Bradycardia and sinus bradycardia were observed most frequently during the early stage of administration, whereas other adverse events such as hypertension were observed even after Day 14. The incidence tended to be higher in the wide-range safety monitoring population.

Table 67. Incidence of cardiovascular adverse events (double-blind phase of Study A2304, safety analysis population)

	Placebo (n = 546)						Siponimod (n = 1099)					
All adverse events	116 (21.2)						282 (25.7)					
Serious adverse events	4 (0.7)						14 (1.3)					
Adverse events leading to treatment discontinuation	1 (0.2)						13 (1.2)					
Events by the time of first onset												
Time of first onset	Day 1-7	Day 8-14	Day 15-28	Month 0-3	Month 0-6	Month 0-12	Day 1-7	Day 8-14	Day 15-28	Month 0-3	Month 0-6	Month 0-12
All adverse events	36 (6.6)	3 (0.5)	10 (1.8)	20 (3.7)	12 (2.2)	19 (3.5)	115 (10.5)	12 (1.1)	17 (1.5)	40 (3.6)	42 (3.8)	52 (4.7)
Main evens												
Bradycardia	14 (2.6)	0	0	0	0	0	48 (4.4)	0	0	0	1 (0.1)	0
Sinus bradycardia	1 (0.2)	0	0	1 (0.2)	0	0	14 (1.3)	0	0	0	0	0
Atrioventricular block first degree	1 (0.2)	0	0	0	1 (0.2)	0	10 (0.9)	0	1 (0.1)	0	2 (0.2)	0
Hypertension	10 (1.8)	0	3 (0.5)	8 (1.5)	7 (1.3)	5 (0.9)	10 (0.9)	1 (0.1)	8 (0.7)	21 (1.9)	21 (1.9)	29 (2.6)
Palpitations	1 (0.2)	2 (0.4)	2 (0.4)	3 (0.5)	0	2 (0.4)	7 (0.6)	3 (0.3)	3 (0.3)	0	1 (0.1)	3 (0.3)
Atrioventricular block second degree	0	0	0	0	0	0	5 (0.5)	1 (0.1)	0	1 (0.1)	0	0
Blood pressure increased	0	0	1 (0.2)	0	0	2 (0.4)	2 (0.2)	1 (0.1)	0	5 (0.5)	3 (0.3)	6 (0.5)
Haematoma	0	0	1 (0.2)	0	0	0	1 (0.1)	0	1 (0.1)	1 (0.1)	5 (0.5)	3 (0.3)

No. of patients with events (incidence [%])

- The baseline mean systolic blood pressure and diastolic blood pressure in Study A2304 were 123.3 ± 15.66 mmHg and 77.7 ± 10.63 mmHg, respectively, in the placebo group and 122.9 ± 14.73 mmHg and 78.2 ± 10.13 mmHg in the siponimod group. Blood pressure after administration of the study drug was greater by approximately 2 mmHg in the siponimod group than in the placebo group, both for systemic and diastolic blood pressures.

- Hypertension-related adverse events[54)] were observed in 10.1% (55 of 546) of patients in the placebo group and in 13.0% (143 of 1099) of patients in the siponimod group. The main observed events were hypertension (45 patients in the placebo group, 117 patients in the siponimod group) and blood pressure increased (8 patients, 20 patients). There were no adverse events leading to treatment discontinuation. A serious adverse event was observed in 1 patient in the siponimod group only, and its causal relationship to the study drug could not be ruled out, but the treatment with study drug was continued. Although the mechanism of siponimod-induced hypertension is unclear, it is known that S1P acts on both contraction and dilatation of blood vessels through smooth muscle cells and endothelial cells in order to maintain vascular homeostasis. Taking into account that hypertension is observed with fingolimod, a drug with a mechanism of action similar to that of siponimod, precaution against hypertension should be included in the package insert as one of the adverse events.

- Although posterior reversible encephalopathy syndrome, an adverse event observed in clinical studies of fingolimod and considered to be associated with vascular endothelial disorder, was not

54) Events included in "Hypertension (narrow)" in MedDRA SMQ

observed in clinical studies of siponimod, a precautionary statement against the disorder should be provided in the package insert, taking account of the seriousness of the event and the mechanism of action of siponimod.

- As for QT intervals, in the study on the effect of siponimod on QT/QTc intervals in healthy adults (CTD 5.3.4.1-1, Study A2118), the upper limit of the 90% CI of ΔΔQTcF interval in the siponimod group was below 10 msec [see Section 6.2.5.1] whereas, during the double-blind phase of Study A2304, the percentage of patients who showed QT interval >500 msec or longer than the baseline value by >30 or 60 msec tended to be higher in the siponimod group than in the placebo group [see Section 7.3]. Table 68 shows adverse events related to QT interval prolongation.[55)] Although the incidence tended to be slightly higher in the siponimod group than in the placebo group for all of the adverse events observed, the incidence of serious adverse events or adverse events leading to treatment discontinuation did not tend to be higher in the siponimod group than in the placebo group. The incidence did not show any tendency of increase with the prolongation of the administration period during the extended administration phase.

Table 68. Incidence of adverse events related to QT interval prolongation (double-blind phase of Study A2304, safety analysis population)

	Placebo (n = 546)	Siponimod (n = 1099)
All adverse events	8 (1.5)	28 (2.5)
Serious adverse events	1 (0.2)	6 (0.5)
Adverse events leading to treatment discontinuation	0	0
Main events observed		
Syncope	8 (1.5)	15 (1.4)
Seizure	0	7 (0.6)
Electrocardiogram QT prolonged	0	3 (0.3)
Loss of consciousness	0	3 (0.3)
Ventricular tachycardia	0	1 (0.1)
Sudden cardiac death	0	0

No. of patients with events (incidence [%])

Thus, siponimod is unlikely to have a risk of causing QT interval prolongation. However, taking account of the possibility that siponimod may prolong QT intervals indirectly by decreasing heart rate, precautions against QT interval prolongation will be included in the package insert.

Taking account of the observation that peripheral arterial occlusive disease was observed in patients treated with fingolimod, a drug with a mechanism of action similar to that of siponimod, PMDA asked the applicant to explain the incidence of adverse events related to thromboembolism.

The applicant's explanation:
The incidence of adverse events related to thromboembolism[56)] in Study A2304 was 3.3% (18 of 546) of patients in the placebo group and 3.6% (40 of 1099) of patients in the siponimod group. The incidence of serious adverse events was 1.6% (9 of 546 patients) of patients in the placebo group (paraparesis in 3 patients, cerebrovascular accident/transient ischaemic attack, coronary artery disease/angina pectoris, acute myocardial infarction, pulmonary embolism, haemorrhagic stroke, and putamen haemorrhage in

55) Events classified in "Torsade de pointes/QT prolongation" in MedDRA SMQ or in "Seizure" in PT
56) Events included in "Haemorrhagic central nervous system vascular conditions (broad)," "Ischaemic central nervous system vascular conditions (broad)," "Embolic and thrombotic events," or "Ischaemic heart disease (broad)" in MedDRA SMQ

1 patient each); and in 1.5% (17 of 1099) of patients in the siponimod group (hemiparesis in 3 patients, cerebrovascular accident in 2 patients, acute coronary syndrome/myocardial ischaemia, acute myocardial infarction/pulmonary embolism/venous thrombosis limb, angina pectoris, aphasia, brain injury, brain stem infarction, coronary artery disease, deep vein thrombosis, intracranial aneurysm, ischaemic stroke, subarachnoid haematoma/subdural haematoma, and transient ischaemic attack in 1 patient each). Among the serious adverse events observed in the siponimod group, a causal relationship to siponimod could not be ruled out for ischaemic stroke and angina pectoris in 1 patient each.

Although peripheral arterial occlusive disease was observed in patients treated with fingolimod, a drug with a mechanism of action similar to that of siponimod, the mechanism of causing the disease is unknown. In addition, the incidence of thromboembolism did not differ significantly between the placebo group and the siponimod group in clinical studies. It is therefore unnecessary to include the precaution in the package insert.

PMDA's view:

- Except for bradycardia which occurs frequently during the early stage of administration, hypertension tends to occur at a high incidence among cardiovascular adverse events associated with siponimod. The incidence of hypertension is high with fingolimod, drug in the same class, as well. Therefore, it is appropriate to include precaution against hypertension in the package insert.

- In study A2304, QT prolongation and adverse events related to QT prolongation were observed frequently in the siponimod group. Precautions against the risk of QT prolongation should be included in the package insert.

- There is a high incidence of serious adverse events related to thromboembolism. They are observed also with fingolimod, a drug with a similar mechanism of action, and precautions against these adverse events are included in the package insert of fingolimod. Therefore, similar precautions should be included in the package insert of siponimod as well.

- Information on the incidences of cardiovascular adverse events, including decreased heart rate, should be collected continuously after the market launch.

- The above conclusion by the PMDA will be finalized, taking account of comments raised in the Expert Discussion.

7.R.4.4 Decreased lymphocyte count, etc., and infection

PMDA asked the applicant to explain the incidences of decreased counts of lymphocytes, etc., and infection caused by siponimod, and the necessity of blood cell monitoring after administration of siponimod and of providing precaution.

The applicant's explanation:

- It has been shown that siponimod decreases lymphocyte count in blood by acting on the $S1P_1$ receptor. In the global phase III study (CTD 5.3.5.1-1, Study A2304), the maintenance dose of siponimod

(2 mg/day) was to be decreased to the half dose (1 mg/day) if blood lymphocyte count was <200 cells/mm^3 in successive measurements, and administration of siponimod was to be discontinued if blood lymphocyte count was <200 cells/mm^3 in successive measurements even after the dose reduction [see Section 7.3]. In 5 patients who showed blood lymphocyte count of <200 cells/mm^3 in successive measurements, the maintenance dose was reduced to 1 mg, upon which lymphocyte count returned to ≥200 cells/mm^3 in all of them. Infection-related adverse events observed in these patients while being treated with 2 mg/day of siponimod were nasal herpes, nasopharyngitis, onychomycosis, and urinary tract infection. No serious event was observed.

- Table 69 shows the changes over time in white blood cell count, lymphocyte count, and neutrophil count in Study A2304, and Table 70 shows the percentage of patients who showed abnormal decrease in blood cell count. Blood lymphocyte count decreased rapidly after administration of siponimod. After administration of siponimod, the minimum blood lymphocyte count decreased to <500 cells/mm^3 in 75.3% (819 of 1088) of patients.

Table 69. Changes over time in white blood cell count, lymphocyte count, and neutrophil count (double-blind phase of Study A2304, safety analysis population)

	White blood cells (×10^9 cells/L)		Lymphocytes (cells/mm^3)		Neutrophils (×10^9 cells/L)	
	Placebo	Siponimod	Placebo	Siponimod	Placebo	Siponimod
Baseline	6.7 ± 1.99 (546)	6.6 ± 1.91 (1099)	1799 ± 569.8 (546)	1784 ± 572.3 (1099)	4.3 ± 1.73 (546)	4.3 ± 1.57 (1099)
Day 28	6.7 ± 2.02 (541)	5.0 ± 1.64 (1073)	1839 ± 593.3 (536)	567 ± 282.5 (1066)	4.3 ± 1.70 (536)	3.9 ± 1.45 (1066)
Month 3	6.8 ± 2.00 (530)	4.9 ± 1.66 (1048)	1864 ± 576.8 (527)	517 ± 280.8 (1046)	4.4 ± 1.74 (527)	3.8 ± 1.49 (1046)
Month 6	6.9 ± 1.92 (515)	4.9 ± 1.62 (1001)	1881 ± 576.6 (512)	521 ± 265.0 (992)	4.4 ± 1.64 (512)	3.9 ± 1.46 (992)
Month 9	6.9 ± 1.83 (488)	4.9 ± 1.58 (956)	1862 ± 555.3 (487)	513 ± 264.4 (947)	4.5 ± 1.54 (487)	3.9 ± 1.42 (947)
Month 12	7.0 ± 2.12 (439)	4.9 ± 1.55 (898)	1892 ± 610.7 (438)	523 ± 267.2 (892)	4.5 ± 1.82 (438)	3.8 ± 1.37 (891)
Month 18	7.0 ± 1.94 (285)	5.0 ± 1.79 (614)	1899 ± 604.0 (284)	520 ± 267.1 (609)	4.5 ± 1.58 (284)	4.0 ± 1.63 (609)
Month 24	6.7 ± 2.36 (161)	4.8 ± 1.62 (379)	1788 ± 586.2 (161)	505 ± 304.8 (378)	4.4 ± 1.94 (161)	3.8 ± 1.43 (378)
Month 30	7.0 ± 1.82 (51)	4.8 ± 1.45 (143)	1987 ± 716.2 (51)	515 ± 274.8 (142)	4.4 ± 1.45 (51)	3.8 ± 1.28 (142)
Month 36	6.5 ± 2.39 (5)	6.0 ± 2.68 (14)	1500 ± 216.0 (4)	500 ± 130.1 (14)	3.5 ± 0.63 (4)	4.8 ± 2.51 (14)

Mean ± SD

Table 70. Percentage of patients showing abnormal white blood cell count, lymphocyte count, or neutrophil count (double-blind phase of Study A2304, safety analysis population)

		Placebo (n = 546)	Siponimod (n = 1099)
White blood cells	<2×10^9 cells/L	0	15 (1.4)
Lymphocytes	<200 cells/mm^3	1 (0.2)	29 (2.7)
Neutrophils	<1×10^9 cells/L	0	2 (0.2)

No. of patients with events (incidence [%])

- Table 71 shows the incidence of infection-related adverse events[57)] in Study A2304. There was no tendency of increase in the incidence of infection-related adverse events in the siponimod group than in the placebo group. During the double-blind phase, death occurred in none of the patients in the placebo group and in 1 patient in the siponimod group (urosepsis). A causal relationship of the death

57) Events classified as "Infections and infestations" in MedDRA SOC, "Fungal infectious disorders" in HLGT, "Lower respiratory tract and lung infections" in High Level Term (HLT), or as any of the following events in PT:
Herpes zoster meningitis, hepatitis E, hepatitis E antibody positive, hepatitis E antigen positive, viral hepatitis carrier, herpes genital, herpes simplex, herpes virus infection, oral herpes, nasal herpes, post herpetic neuralgia, trigeminal neuralgia, herpes zoster, herpes zoster oticus, herpes zoster ophthalmicus, and varicella-zoster virus infection.

to siponimod in this patient was ruled out. The incidence of serious adverse events tended to be higher in the siponimod group than in the placebo group. During the extended administration phase, death occurred in 2 patients in the siponimod group (septic shock and pneumonia in 1 patient each), and a causal relationship of the deaths to siponimod could not be ruled out.

Table 71. Incidence of infection-related adverse events (double-blind phase of Study A2304, safety analysis population)

	Placebo (n = 546)	Siponimod (n = 1099)
All adverse events	277 (50.7)	533 (50.3)
Serious adverse events	16 (2.9)	39 (3.5)
Adverse events leading to treatment discontinuation	4 (0.7)	3 (0.3)
Main events observed		
Nasopharyngitis	82 (15.0)	152 (13.8)
Urinary tract infection	80 (14.7)	136 (12.4)
Upper respiratory tract infection	41 (7.5)	95 (8.6)
Influenza	43 (7.9)	75 (6.8)
Bronchitis	18 (3.3)	39 (3.5)
Cystitis	13 (2.4)	29 (2.6)
Herpes zoster	4 (0.7)	25 (2.3)
Pharyngitis	11 (2.0)	20 (1.8)
Rhinitis	11 (2.0)	15 (1.4)

No. of patients with events (incidence [%])

- Among infection-related adverse events observed, meningitis cryptococcal was observed as a noteworthy event in 1 patient in the siponimod group during the extended administration phase. The patient was a 62-year-old woman who showed speech disorder, confusion, and temporal disorientation from Day 1576, and was diagnosed with meningitis cryptococcal, whereupon administration of siponimod was discontinued. The patient underwent treatment with antibiotics and antifungal agents, and recovered after 57 days. This event was handled as a serious adverse event, and its causal relationship to the study drug could not be ruled out. Progressive multifocal leukoencephalopathy, an adverse event observed with approved S1P receptor modulators, was not observed.

- The siponimod-induced reduction in blood lymphocyte count is considered to occur not as a result of removal of lymphocytes from the body, but as a result of suppression of emigration of lymphocytes into circulating blood from the secondary lymphoid tissues such as lymph nodes through action on the $S1P_1$ receptor on lymphocytes. In nonclinical studies using mice and monkeys, siponimod suppressed the T cell-dependent primary immune response but did not affect the secondary immune response or immunological memory [see Section 3.2.3]. In clinical studies, no clear relationship was observed between blood lymphocyte count and the incidence of infection-related adverse events.

- These results suggest that siponimod-induced lymphocyte count reduction does not significantly affect immunological memory or immune surveillance.

Based on the above results of clinical studies, the applicant's explanation about the necessity of blood cell monitoring after administration of siponimod and of providing precautions:

- In Study A2304, the dose of siponimod was decreased to 1 mg/day if blood lymphocyte count decreased to <200 cells/mm^3 in successive measurements, and the administration of siponimod was discontinued if blood lymphocyte count decreased to <200 cells/mm^3 in 2 successive measurements

even after the dose reduction. Also, in Study A2304, if blood lymphocyte count returned to ≥600 cells/mm^3 after treatment interruption, the maintenance dose could be increased to 2 mg again, according to the criteria for treatment resumption.

- Therefore, blood lymphocyte count should be monitored periodically during administration of siponimod, and the dose reduction or treatment discontinuation should be performed if lymphocyte count has decreased, and the administration may be resumed when the count has recovered to the acceptable level, as stipulated in the protocol of Study A2304. During the process of Study A2304, blood lymphocyte count decreased to <200 cells/mm^3 in 5 patients, but returned to ≥200 cells/mm^3 after a dose reduction, which suggests that administration of siponimod may be continued even after the dose reduction.

- The incidence of infection-related adverse events did not differ significantly between the siponimod group and the placebo group. However, taking into account that death due to serious infection, cryptococcal meningitis, etc., were observed in the siponimod group, it will be appropriate to include the following precautions in the package insert: (1) If a serious infection has occurred during administration of siponimod, administration of siponimod should be interrupted and appropriate measures should be taken, and (2) after recovery, whether to resume administration should be determined upon careful evaluation of therapeutic benefits and possible risks.

- The pharmacodynamic effects of siponimod such as lymphocyte count decrease may last 3 to 4 weeks at the maximum after treatment discontinuation. It will therefore be appropriate to advise to continuously pay attention to infection during this period.

PMDA accepted the applicant's explanation. Information on the incidence of infection-related adverse events should be collected continuously after the market launch.

7.R.4.5 Hepatic dysfunction

Taking into account that abnormal liver function test values are observed with fingolimod, a drug with a mechanism of action similar to that of siponimod, PMDA asked the applicant to explain the incidence of adverse events related to hepatic dysfunction.

The applicant's explanation:

- In the global phase III study (CTD 5.3.5.1-1, Study A2304), it was specified that administration of siponimod should be discontinued if hepatic enzyme levels exceeded 3 times the upper limit of normal, accompanied by symptoms related to decreased hepatic function. Table 72 shows the percentage of patients who showed abnormal liver function test values in Study A2304. The percentage of patients showing increased hepatic enzyme levels was higher in the siponimod group than in the placebo group.

Table 72. Percentage of patients who showed abnormal liver function test values (double-blind phase of Study A2304, safety analysis population)

	Placebo (n = 546)	Siponimod (n = 1088)
ALT or AST >3 ULN	8 (1.5)	61 (5.6)
ALT or AST >5 ULN	3 (0.5)	15 (1.4)
ALT or AST >10 ULN	0	2 (0.2)
ALT or AST >20 ULN	0	0
ALT or AST >3 ULN, total bilirubin >2 ULN, and ALP <2 ULN	0	0

No. of patients with events (incidence [%])

- Of the 2 patients with ALT or aspartate aminotransferase (AST) >10 ULN, one was a 59-year-old Caucasian woman. Her baseline ALT and AST were within the normal range, but serious hemochromatosis occurred on Day 169 and 260, and administration of siponimod was discontinued on Day 274. On Day 316, ALT increased to Grade 4 (901 U/L), and AST to Grade 3 (751 U/L), but both ALT and AST returned within the normal range on Day 385. A causal relationship of hemochromatosis to the study drug was ruled out. The other patient was a 45-year-old Caucasian man whose baseline ALT and AST were within the normal range, and he was negative for IgG and IgM antibodies against hepatitis E virus at the screening. On Day 52, he experienced serious hepatitis E, and administration of siponimod was interrupted. AST increased to Grade 3 (577 U/L) on the same day and ALT increased to Grade 3 (365 U/L) on Day 58, but both ALT and AST returned within normal range on Day 85. A causal relationship of hepatitis E to the study drug was ruled out. The administration of study drug was resumed on Day 190. ALT increased to Grade 1 (48-54 U/L) on Day 457 to 664 after the resumption of the administration, but no hepatic dysfunction-related adverse events were observed.

- In the pooled data of the long-term treatment study (1737 patients in the siponimod [2-10 mg] group), there were no patients who met the criteria of ALT or AST>3 ULN, total bilirubin >2 ULN, and alkaline phosphatase (ALP) <2 ULN.

- In the pooled data of the placebo controlled studies, both the mean ALT and AST values increased (ALT, 30.632 U/L; AST, 23.209 U/L) within 1 month after the start of administration of the study drug (the first evaluation time point), and remained at higher levels in the siponimod group than in the placebo group throughout the study period, but the mean ALT did not exceed 40 U/L, and the mean AST did not exceed 30 U/L. ALT and AST returned to the baseline levels within approximately 1 to 3 months after discontinuation of siponimod.

- Table 73 shows the incidence of hepatic dysfunction-related adverse events.[58)] The incidence was higher in the siponimod group than in the placebo group, whereas the incidences of serious adverse events and adverse events leading to treatment discontinuation were low both in the siponimod group and in the placebo group.

[58)] Events included in “Cholestasis and jaundice of hepatic origin (broad),” “Drug related hepatic disorders - severe events only (broad),” or “Liver related investigations, signs and symptoms (broad)” in MedDRA SMQ.

Table 73. Incidence of adverse events related to hepatic dysfunction (double-blind phase of Study A2304, safety analysis population)

	Placebo (n = 546)	Siponimod (n = 1099)
All adverse events	24 (4.4)	147 (13.4)
Serious adverse events	3 (0.5)	15 (1.4)
Adverse events leading to treatment discontinuation	0	8 (0.7)
Main adverse events observed		
ALT increased	9 (1.6)	66 (6.0)
Gamma-glutamyltransferase increased	7 (1.3)	44 (4.0)
Hepatic enzyme increased	2 (0.4)	16 (1.5)
AST increased	4 (0.7)	15 (1.4)
Blood ALP increased	0	12 (1.1)
Blood bilirubin increased	2 (0.4)	12 (1.1)

No. of patients with events (incidence [%])

- Thus, taking into account that the incidence of adverse events related to hepatic dysfunction is high in the siponimod group, it is considered appropriate to provide precautions against hepatic dysfunction in the package insert.

PMDA accepted the above applicant's explanation. Information on hepatic dysfunction induced by siponimod should be collected continuously after the market launch.

7.R.4.6 Macular oedema

Macular oedema was reported in patients receiving fingolimod, a drug with a mechanism of action similar to that of siponimod. PMDA asked the applicant to explain the incidence of siponimod-associated adverse events related to macular oedema.

The applicant's explanation:

- It is known that $S1P_1$ receptor modulators may cause macular oedema as a result of enhanced vascular permeability due to decreased $S1P_1$ receptor expression on vascular endothelial cells. By referring to the method specified for an ophthalmological examination for patients receiving fingolimod, an $S1P_1$ receptor modulator like siponimod, a detailed ophthalmological examination (taking history of ophthalmological diseases, measurement of best-corrected visual acuity, ophthalmoscopy, optical coherence tomography) was performed periodically (at Month 3 and 12, then at every 12 months) throughout the treatment period in the global phase III study of siponimod (CTD 5.3.5.1-1, Study A2304). In high-risk patients (patients with well-controlled diabetes mellitus, patients with a past history of macular oedema or uveitis, patients complicated with uveitis), more frequent and detailed tests were conducted for a wider range of ophthalmological monitoring (additional ophthalmological examinations on Day 28 and at Month 6 and, if uveitis was detected, fluorescein fundus angiography). Administration of the study drug had to be interrupted if macular oedema occurred, but could be resumed upon complete recovery of the edema.

- During the double-blind phase of Study A2304, adverse events related to macular oedema[59)] were observed in 0.4% (2 of 546) of patients in the placebo group and in 1.8% (20 of 1099) of patients in the siponimod group. Serious adverse events were observed in 3 patients (macular oedema) in the siponimod group only. In the pooled data of Study A2304 and the foreign phase II study (CTD

[59)] Events classified as macular oedema, cystoid macular oedema, or retinal oedema in MedDRA PT.

5.3.5.1.2, Study A2201), the time of the first onset of macular oedema-related adverse events was within 3 months in 9 patients, Month 3 to 6 in 5 patients, Month 6 to 12 in 3 patients, and after Month 12 in 3 patients, with not a few of macular oedema-related adverse events being observed within 3 months after the start of administration. In high-risk patients who underwent a wide range of ophthalmological monitoring during the double-blind phase of Study A2304, the incidence of macular oedema was 9.3% (4 of 43) of patients in the siponimod group, which was higher than the incidence in the entire siponimod group (1.8%).

- Thus, taking into account the occurrence of serious macular oedema, it is considered appropriate to provide the following precautions: (1) Siponimod should be administered in cooperation with an ophthalmologist, (2) before the start of administration, an ophthalmological examination should be performed on patients with a high risk of macular oedema, (3) an ophthalmological examination should be performed at 3 to 4 months after the start of administration of siponimod, and (4) the patient should be advised to promptly contact the healthcare professional if a visual disorder is noticed.

PMDA's view:

- Precautions should be provided in the package insert to administer siponimod in cooperation with an ophthalmologist, and to perform an ophthalmological examination on patients with a high risk of macular oedema, before the start of administration of siponimod.

- Because macular oedema may be asymptomatic during the early stage of the disease, physicians should be advised to perform an ophthalmological examination in patients with a high risk of macular oedema not only at 3 to 4 months after the start of administration, but also periodically while siponimod is being administered, and to perform an appropriate ophthalmological examination if the patient presents with eye symptoms.

- Information on siponimod-induced macular oedema should be collected continuously after the market launch.

7.R.4.7 Convulsive seizure

PMDA asked the applicant to explain adverse events related to convulsive seizure associated with siponimod.

The applicant's explanation:

- Taking into account the report that patients with MS are prone to experience epilepsy, the incidence of adverse events related to convulsive seizure[60)] was investigated. During the double-blind phase of the global phase III study (CTD 5.3.5.1-1, Study A2304), convulsive seizure-related adverse events were observed in 0.5% (3 of 546) of patients in the placebo group and in 1.9% (21 of 1099) of patients in the siponimod group. No death occurred. Serious adverse events were observed in none of the patients in the placebo group and in 12 patients in the siponimod group (epilepsy in 5 patients, seizure in 4 patients, partial seizures in 2 patients, generalised tonic-clonic seizure in 1 patient). A causal relationship to siponimod could not be ruled out for generalised tonic-clonic seizure (1 patient).

60) Events included in "Convulsions (broad)" in MedDRA SMQ

Generalised tonic-clonic seizure occurred in a 52-year-old woman, who experienced serious generalised tonic-clonic seizure at 111 days after the start of administration. The administration of study drug was discontinued and oral levetiracetam was administered, which led to recovery from the symptom. During the combined double-blind and extended administration phase of Study A2304, convulsive seizure-related adverse events were observed in 2.5% (38 of 1517) of patients, and serious adverse events in 25 patients. The adverse events occurred roughly evenly over time without correlation to the siponimod administration phase.

- The incidence of convulsive seizure-related adverse events in the pooled data of placebo-control studies (0.5% [3 of 607] of patients in the placebo group, 1.7% [19 of 1148] of patients in the siponimod group) and in the pooled data of the long-term treatment studies (2.4% [42 of 1737] of patients in the siponimod 2-10 mg group) did not exceed the incidence (3.5%) in the epidemiologic data on patients with multiple sclerosis (*Neurology.* 2017;89:2462-8). Also, nonclinical studies did not show changes suggestive of the seizure-inducing effect of siponimod.

- Thus, the risk of siponimod inducing convulsive seizure is considered to be low. Nevertheless, convulsive seizure will be included in the Other adverse reactions section in the package insert.

PMDA's view:
Taking account of the observations that, in study A2304, the incidence of convulsive seizure was higher in the siponimod group than in the placebo group, and that serious adverse events and events for which a causal relationship to the study drug could not be ruled out were observed only in the siponimod group, precautions against convulsive seizure should be included in the package insert.

7.R.5 Indication

PMDA asked the applicant to explain the appropriateness of the proposed indication for siponimod, by also referring to the difference from the indication in overseas countries.

The applicant's explanation about the approved indications in foreign countries:

- In the US, the new drug application was filed with the proposed indication for SPMS based on the results of the global phase III study (CTD 5.3.5.1-1, Study A2304). However, the Food and Drug Administration (FDA) concluded that there is insufficient evidence of clinical benefits in non-relapsing SPMS with low disease activity, for the following reason: The hazard ratio on 3mCDP in the siponimod group tended to be higher in patients without a relapse within 2 years from the start of the study than in patients with a relapse (Table 58). On the other hand, siponimod not only showed a relapse-preventing effect in Study A2304 [see Section 7.R.3.4], but also decreased the number of active lesions on MRI images in the foreign phase II study in patients with RRMS (CTD 5.3.5.1-2, Study A2201). Based on the above, the approved indication was "Relapsing forms of MS, to include CIS, RRMS, and active SPMS, in adult." Pursuant to the approval of siponimod, the FDA instructed to revise the indication of other drugs indicated for relapsing form of MS to the same indication as that of siponimod.

- In Europe, the guideline[61)] for clinical evaluation of MS requires that, in order to indicate a drug for SPMS regardless of a relapse (disease activity), it is necessary to demonstrate the efficacy against the progression of physical disability, regardless of an MS relapse. During the review process of siponimod, the European regulatory agency concluded that there was no sufficient evidence for the efficacy against disease progression independent of an MS relapse. Based on the above, the approved indication is "adult patients with SPMS with active disease evidenced by relapses or imaging features of inflammatory activity."

- In Australia, siponimod is approved with the indication for SPMS.

The applicant's explanation about the appropriateness of the proposed indication in Japan:

- In Study A2304, patients who had a past history of RRMS, showed disease progression lasting for ≥6 months with or without a relapse [see Section 7.R.3.5], and showed EDSS of 3.0 to 6.5 were enrolled as subjects. Patients with disease progression for ≥6 months was used as one of the inclusion criteria in order to specify a uniform criterion for the duration of "disease progression with or without a relapse," the definition used internationally as a definition of SPMS (*Neurology.* 1996;46:907-11). Another inclusion criterion "EDSS 3.0 to 6.5" with the lower limit for EDSS was included in order to exclude RRMS. Since transition from RRMS to SPMS is usually evaluated retrospectively, it is difficult to clearly judge the transition to SPMS during the transition period (*Neurology.* 2014;83:278-86). However, the secondary progression phase starts when EDSS exceeds 3.0 (*Brain.* 2006;129:584-94), based on which the above lower limit for EDSS was 3.0.

- Siponimod was superior to placebo in time to 3mCDP, the primary endpoint in Study A2304. The subpopulation analysis, classified by presence/absence of a relapse within the past 2 years (Table 58) and other characteristics (Table 54) also showed a tendency of improvement in the siponimod group than in the placebo group.

- Siponimod showed a statistically significant effect also on ARR, the secondary endpoint [see Section 7.R.3.4]. In Study A2304, approximately 80% of patients did not show gadolinium enhanced lesions, MRI findings indicative of inflammatory disease activity, before the start of the study. Siponimod decreased the number of gadolinium enhanced lesions by 86.3% and the number of new or expanded T2 lesions by 80.6% over 24-month administration, compared with placebo. Judging from the observation that the efficacy endpoints demonstrating inflammatory disease activity improved consistently in the siponimod group, siponimod is expected to be effective in preventing a relapse in patients with SPMS.

- Based on the above, it is considered appropriate to indicate siponimod for "prevention of relapsing secondary progressive multiple sclerosis and delay in the progression of associated physical disability" in patients with SPMS.

- In order to allow neurologists not specializing in MS to evaluate patients treatable with siponimod, it will be appropriate to include the following precautions in the package insert: (1) The inclusion

[61)] Guideline on clinical investigation of medicinal products for the treatment of Multiple Sclerosis

criteria for Study A2304 should be referred to, (2) patients with relapsing MS may also be diagnosed with SPMS, depending on the patient conditions, and (3) transition to SPMS is suspected if patients show dysfunction in gait or cognitive function, commonly observed in SPMS.

PMDA's view on the indication:
The final conclusion will be made, taking account of comments raised in the Expert Discussion.

- In the US and Europe, patients with non-relapsing SPMS are excluded from the target population for treatment with siponimod because the efficacy against the relapse-independent progression of physical disability is considered unclear. However, it is acceptable to indicate siponimod for SPMS as a whole, including non-relapsing SPMS, for the following reasons: (1) Available data suggest that siponimod tends to suppress disease progression, regardless of the relapse within the past 2 years (Table 58), (2) siponimod is expected to be effective even in patients with advanced disease with fewer relapses, (3) there is no other drug that demonstrated efficacy in patients with SPMS in clinical studies, and (4) other therapeutic agents for MS approved in Japan are indicated for multiple sclerosis including SPMS and PPMS, based on the results of clinical studies in mainly patients with RRMS, with the description in the package insert that the efficacy and safety in progressive multiple sclerosis have not been established, with consideration of medical need.

- For the proposed indication "prevention of relapsing secondary progressive multiple sclerosis and delay in the progression of associated physical disability," in Study A2304, a relapse-preventing effect of siponimod was consistently observed, as evaluated by ARR and by MRI findings, albeit not the primary endpoint. In addition, ARR and MRI findings were used as the primary efficacy endpoints in clinical studies on patients with RRMS. It is therefore acceptable to indicate siponimod for prevention of a relapse. Regarding delaying the progression of physical disability, time to 3mCDP, the primary endpoint, tended to improve more in the siponimod group than in the placebo group in the subpopulation analysis classified by presence or absence of a relapse within the past 2 years, with the improvement being observed in both subpopulations. Among patients without a relapse within 2 years before the start of the study, a certain percentage of patients showed a relapse during the study period. Thus, some of the patients with SPMS experience a relapse during the early phase, as is the case with RRMS. It is of clinical significance to suppress the progression of irreversible physical disability by preventing a relapse. Given these observations, it is acceptable to indicate siponimod for suppression of physical disability.

- In the US, siponimod is approved with the indication including RRMS. However, since there are no clinical studies that investigated the efficacy and safety of siponimod in Japanese patients with RRMS, the indication should be limited to SPMS. However, it is desirable to consider development of siponimod for patients with RRMS because (1) siponimod is suggested to be effective against a relapse in patients with MS, and (2) it is desirable that patients be treated with the same drug even if RRMS has progressed to SPMS.

7.R.6 Dosage and administration

7.R.6.1 Dose-escalation

PMDA asked the applicant to explain the justification and appropriateness of the method of escalation in the dose of siponimod.

The applicant's explanation:

- In the study (CTD 5.3.4.1.3, Study A2107) on dose-escalation involving healthy adults, conducted with consideration given to the effect on cardiac conduction, 56 subjects were randomized to 4 groups (see Table 32 for the schedule of dose increase in the placebo group, dose-escalation group 1, dose-escalation group 2, and no dose-escalation group) and received the study drug for 12 days under blinded conditions. As a result, adverse events were observed in 0% (0 of 14) of subjects in the placebo group, 21.4% (3 of 14) of subjects in dose-escalation group 1, 28.6% (4 of 14) of subjects in dose-escalation group 2, and 64.3% (9 of 14) of subjects in no dose-escalation group. No serious adverse events were observed. In both dose-escalation groups, the incidences of heart rate decrease, atrioventricular block, and sinus arrest, which were observed on Day 1 in the no dose-escalation group, decreased (Table 60).

- Based on these results, in the global phase III study (CTD 5.3.5.1-1, Study A2304), siponimod was administered according to the method for dose escalation shown in Table 45 at the start of administration, and subjects were monitored for safety against cardiovascular adverse events during the early stage of administration [see Section 7.R.4.2].

- In Study A2304, the clinical study protocol recommended to take the study drug at an identical time in the morning everyday regardless of the meal. As it turned out, approximately 90% (86.8%-94.8%) of the subjects took the study drug in the morning. Results showed that, during the dose-escalation phase (from the first day of dosing up to Day 6), the heart rate measured by mobile cardiac telemetry over 24 hours after administration of study drug remained at a level similar to baseline on all days in the placebo group, whereas in the siponimod group, the heart rate decreased to the lowest level within 6 hours after administration, then showed a tendency of returning to the baseline level. Also, regardless of whether the study drug was administered, the heart rate was the lowest during midnight (1-3 a.m.).

- Based on the above, it will be advised in the package insert to take siponimod in the morning in order to avoid the overlapping of the peak of the siponimod-induced heart rate reduction (within 6 hours after administration) and the heart rate decrease due to diurnal variation (1-3 am at midnight).

7.R.6.2 Maintenance dose

PMDA asked the applicant to explain the justification for the maintenance dose (2 mg/day) used in the global phase III study (CTD 5.3.5.1-1, Study A2304), and to explain the appropriateness of setting the maintenance dose of siponimod at 2 mg/day.

The applicant's explanation:

- Patients with SPMS, upon progression from RRMS, show gradual progress in disorders with or without relapses. There are no established MRI findings or biomarkers for the disease progression of SPMS. In Study A2304, the maintenance dose was based on the results of the analysis of the dose-response relationship of siponimod relative to placebo in the foreign phase II study (CTD 5.3.5.1-2, Study A2201) in patients with RRMS [see Section 7.2]. Thus, investigation of the dose-response relationship in patients with RRMS after 3 months of administration based on the number of CUALs showed that the suppressive effect of siponimod was comparable between 2 mg and 10 mg, indicating that the maximum effect is almost achieved at 2 mg. Siponimod at 2 mg tended to prevent a relapse, as suggested not only by suppression of increase in the number of CUALs but also by the decrease in ARR [see Section 7.R.3.4]. In Study A2304, the same dosage regimen was used in Japanese and non-Japanese patients because there were no clear ethnic differences in the pharmacokinetics between Japanese and non-Japanese subjects, as suggested by the Japanese phase I study (CTD 5.3.3.1-1, Study A1101) and the foreign phase I study (reference CTD 5.3.3.1-2, Study A2101) [see Section 6.R.2].

- It is considered appropriate to select the maintenance dose of siponimod in patients with SPMS at 2 mg, taking into account that, in Study A2304, a significant suppression of disease progression was observed in the siponimod 2 mg group than in the placebo group, as judged from the evaluation of time to 3mCDP, the primary endpoint, and that ARR was lower in the siponimod group than in the placebo group, albeit the secondary endpoint [see Section 7.R.3.4].

7.R.6.3 Dosage regimen for treatment resumption after interruption

PMDA asked the applicant to explain the dosage regimen in resuming administration of siponimod after treatment interruption.

The applicant's explanation:

In a clinical study (CTD 5.3.4.1-2, Study A2110) which evaluated the negative chronotropic effect after resumption of administration of siponimod in non-Japanese subjects, the maximum decrease in heart rate exceeded 30 bpm in some subjects after 120-hour interruption following administration of siponimod 1 mg, after 120-hour interruption following administration of siponimod 2 mg, and after 192-hour interruption following administration of siponimod 2 mg. Atrioventricular block second degree was observed in 3 subjects (after administration of placebo, after 120-hour interruption following administration of siponimod 1 mg, and after 72-hour interruption following administration of siponimod 2 mg in 1 subject each), but all of them were asymptomatic. Sinus arrest of >2 seconds occurred after administration of placebo, after 48-hour interruption following administration of siponimod 0.5 mg, after 96-hour interruption following administration of siponimod 1 mg, and after 96-hour interruption following administration of siponimod 4 mg, in 1 subject each. Most of sinus arrest occurred at night when the vagus nerve is dominant, and was asymptomatic in all subjects. The sinus arrest lasted for 2.26 seconds maximally, and did not cause any clinically significant problems.

Taking into account that, in Study A2110, heart rate decrease of >30 bpm was observed in patients after 120 hours of interruption period although the relationship among the length of the interruption,

atrioventricular block, and sinus arrest is unclear, it was specified in Study A2304 that, if siponimod is interrupted for ≥4 days during the administration at the maintenance dose, the administration should be resumed by the dose-escalation method. Interruption of ≥4 days occurred in 2.4% (13 of 546) of subjects in the placebo group and in 6.0% (66 of 1099) of subjects in the siponimod group. Among them, 3 subjects in the placebo group and 17 subjects in the siponimod group did not receive siponimod by the dose-escalation method, but cardiovascular adverse events were not observed within 2 weeks after the treatment resumption in any of these subjects in the siponimod group.

Results of Study A2110 suggest that, if administration of siponimod is interrupted for ≥4 days during administration at the maintenance dose, the treatment should be resumed by the dose-escalation method (Table 45). This precaution will be included in the package insert.

PMDA's view on Sections 7.R.6.1 through 7.R.6.3:
These conclusions will be finalized, taking account of comments raised in the Expert Discussion.

- Compared with initiating the administration without titration, the dose-escalation attenuates the effect on cardiac conduction during the early stage of administration. It is therefore appropriate to administer siponimod by the dose-escalation method based on the results of Studies A2107 and A2304. It is also appropriate to specify that siponimod be administered in the morning for 6 days from the start of the dose-escalation in order to mitigate the effect on cardiac conduction.

- Although there are limitations to determine the maintenance dose for preventing disease progression in SPMS based on the efficacy against MRI lesion that indicates inflammatory disease activity in RRMS, a significant difference was observed in time to 3mCDP, the primary endpoint in Study A2304, between the siponimod 2 mg/day group and the placebo group. Also, the secondary endpoint ARR was lower in the siponimod 2 mg/day group than the placebo group, demonstrating the relapse-suppressive effect. In addition, the safety profile was acceptable. Based on these results, there is no problem in using siponimod at the maintenance dose of 2 mg/day.

- As for the dosage regimen for resuming administration after interruption, it is appropriate to administer siponimod by the dose-escalation method after interruption for ≥4 days.

7.R.7 Proper use of siponimod

Siponimod may cause infection, cardiovascular adverse events, hepatic dysfunction, eye disorders such as macular oedema, possibly necessitating cooperation with other departments. PMDA asked the applicant to explain the measures for the proper use of siponimod in order to ensure the safety of patients.

The applicant's explanation:
Similar adverse events are observed with fingolimod, a drug that acts on the $S1P_1$ receptor as is the case with siponimod, with some of them being serious. In order to address these adverse events appropriately, the following measures are taken for fingolimod. Also, a card is prepared for each patient receiving the prescription and, after confirmation of the identity of the medical institution entered in the prescription, fingolimod is prescribed to the patient.

- The prescribing physician, upon understanding the method for the proper use of fingolimod (including the requirements for the institution, confirmation of the information on cooperation with specialists in other departments, etc.), signs the proper use acknowledgment letter, which is then delivered to the marketing authorization holder.

- The marketing authorization holder explains the proper use of fingolimod and obtains the agreement on the cooperation system with the ophthalmological department, and the cooperating ophthalmologist signs the ophthalmological cooperation acknowledgment letter, which is then delivered to the marketing authorization holder.

- The prescribing physician attends a lecture on the characteristics and proper use of fingolimod by E-learning, and the marketing authorization holder confirms that the physician has understood the method for the proper use of fingolimod.

Since siponimod may also cause macular oedema, a significant adverse drug reaction requiring cooperation with an ophthalmologist, the same measures for facilitating the proper use similar to those for fingolimod will be taken. Also, a patient card will be prepared in a similar manner as in the case of fingolimod, and siponimod will be prescribed upon confirmation of the identity of the medical institution entered in the prescription.

PMDA accepted the applicant's explanation.

7.R.8 Post-marketing investigations

The applicant's explanation:
In order to collect information on the safety and efficacy of siponimod in clinical use, the applicant plans to conduct a use-results survey as an additional safety pharmacovigilance activity, covering all patients with SPMS receiving siponimod with the target number of 330 patients and with 12-month follow-up period for each patient.

PMDA's view:
The main survey items in the all-case surveillance will be finalized, taking account of comments raised in 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 Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics. As a result, it was found out that the control of access rights to 3 independent databases, which were constructed to ensure the blinding in CTD 5.3.5.1-1, was inadequate, possibly affecting the evaluation of study results. PMDA therefore concluded that the submitted

application data should be reviewed upon confirmation of the effect of the above inadvertence on the evaluation of efficacy and safety.

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

The new drug application data (CTD 5.3.5.1-1) were subjected to an on-site GCP inspection, in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics. PMDA concluded that the clinical studies overall were conducted in compliance with GCP and that there were no obstacles to conducting its review based on the application documents submitted. The inspection revealed the following findings requiring corrective action at some of the study sites and by the applicant, although they had no significant impact on the review of the overall clinical studies. PMDA notified the head of the study sites and the sponsor of the problems.

Corrective actions required

Study sites

- Deviations from the protocol (noncompliance to the rules for prohibited concomitant drugs)

Sponsor

- Inappropriate access rights granted to some users of the electronic data transfer system

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

On the basis of the data submitted, PMDA has concluded that siponimod has efficacy in preventing relapsing secondary progressive multiple sclerosis and in delaying the progression of associated physical disability, and that siponimod has acceptable safety in view of its benefits. Siponimod provides a novel treatment option for patients with secondary progressive multiple sclerosis, and thus has a clinical significance. Bradycardia observed during the early phase of the treatment, serious infection, and macular oedema require appropriate monitoring and cooperation with other departments. The safety and indication of siponimod, as well as the post-marketing investigations require further deliberations.

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

Review Report (2)

April 13, 2020

Product Submitted for Approval

Brand Name	Mayzent Tablets 0.25 mg Mayzent Tablets 2 mg
Non-proprietary Name	Siponimod Fumaric Acid
Applicant	Novartis Pharma K.K.
Date of Application	January 7, 2019

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).

At the Expert Discussion, the expert advisors supported PMDA's conclusions described in the Review Report (1) and in Section "6.R.4 Dose adjustment in co-administration with other drugs."

PMDA conducted additional reviews on the following points and took appropriate measures.

1.1 Effect of inadequately granted database access rights in global phase III study (CTD 5.3.5.1-1, Study A2304)

PMDA's conclusion:
The inappropriate control of database access rights may have affected the efficacy evaluation in the global phase III study (CTD 5.3.5.1-1, Study A2304). However, since Tables 51 and 52 showed the tendency of improvement in the efficacy endpoint in the siponimod group compared to the placebo group, PMDA considered that the efficacy can be evaluated based on the results of the entire population of Study A2304 [see Section 7.R.2 in the Review Report (1)].

The above conclusion of PMDA was supported by the expert advisors.

Also, the following comments were raised from the expert advisors:

(1) The applicant should appropriately inform healthcare professionals that part of the study personnel may have not been blinded to some cases because of the inadequate control of data access rights in Study A2304.

(2) The results of analysis excluding these cases should also be provided to healthcare professionals in an appropriate manner.

Based on the above, PMDA instructed the applicant to appropriately provide the above information to healthcare professionals, and the applicant took appropriate actions.

1.2 Decreased heart rate, adverse events related to bradyarrhythmia, and other cardiovascular adverse events during the early stage of treatment

PMDA's view:

During the escalation of the dose of siponimod, it is necessary to pay attention to events or findings related to bradyarrhythmia, and this information should be provided appropriately to healthcare professionals and to patients. Regarding the monitoring for, and precautions against, heart rate reduction at the start of treatment with siponimod, it is necessary to measure heart rate and electrocardiogram on the first day of treatment with siponimod (or on the day of treatment resumption after interruption for ≥4 days), with or without cardiovascular risk factors. Patients should be monitored for heart rate and electrocardiogram until the siponimod-induced heart rate reduction shows a tendency of recovery, and whether to continue monitoring should be determined based on the patient's conditions. Also, on each dosing day during the dose-escalation, patients should be monitored for pulse rate and clinical signs. Siponimod should be contraindicated in patients with a high risk of major cardiovascular events (This patient group was excluded from Study A2304 [Section 7.R.4.2 in the Review Report (1)]). In addition, since QT prolongation and adverse events related to QT prolongation occurred frequently in the siponimod group of Study A2304, the package insert should contain precautions against the risk of QT prolongation [Section 7.R.4.3 in the Review Report (1)].

The above conclusions of PMDA were supported by the expert advisors.

The following comments were raised from the expert advisors:

- The package insert should clearly provide the following information: (1) The timing of monitoring heart rate and electrocardiogram after administration of siponimod on the first day of treatment and (2) the period during which patients should be monitored for pulse rate and clinical symptoms in the dose-escalation phase.

- The information leaflet for patients should contain the specific criteria for pulse rate and subjective symptoms that should be notified to the physician during the dose-escalation phase.

- Precautions should be given regarding the following contraindications: (1) Patients with QT prolongation and (2) co-administration with drugs with a QT-prolonging effect.

Based on the above, PMDA instructed the applicant to take the following actions. The applicant responded appropriately:

- The Warning section of the package insert should contain the following description: Siponimod decreases heart rate during the period of dose-escalation (6 days). Administration of siponimod should be started under management that enables appropriate actions, such as collaboration with a cardiology specialist.

- Siponimod should be contraindicated in patients with marked QT prolongation.

- The Important precautions section of the package insert should contain the following descriptions:
  - Patients should be monitored continuously for up to 6 hours after the first dose. During the period of the dose-escalation (6 days), continuous electrocardiogram monitoring should be considered, depending on the clinical course after the first dose.
  - Heart rate decreases during the period of dose-escalation. The patient or his/her family member should be instructed to notify the attending physician if symptoms such as syncope, dizziness, and breathlessness occur.
  - Patients should measure pulse rate at home at least until Day 7 and, if pulse rate is below 50 bpm, should notify the attending physician.

- Information materials for healthcare professionals and for patients should be prepared. Each material should describe the monitoring method in an easy-to-understand manner.

1.3 Indication

PMDA's conclusion [Section 7.R.5 in the Review Report (1)]:

There is no particular problem in the indication of "Prevention of relapsing secondary progressive multiple sclerosis and delay in the progression of associated physical disability." The development of siponimod for RRMS should also be actively pursued.

The above conclusion of PMDA was supported by the expert advisors.

The applicant explained that it would consider developing siponimod for RRMS, taking account of actual treatment given to patients with RRMS and the needs of hospitals and clinics after the marketing of siponimod.

1.4 Dosage and administration

The expert advisors supported the following conclusions of PMDA [Section 7.R.6 in the Review Report (1)]:

(1) The dose-escalation method should be the same as that used in the global phase III study (CTD 5.3.5.1-1, Study A2304).
(2) Siponimod should be administered in the morning during the first 6 days of the dose-escalation
(3) The maintenance dose should be 2 mg/day.

PMDA instructed the applicant to modify the dosage and administration as below. The applicant responded appropriately.

Dosage and Administration

The usual adult dosage of siponimod is 0.25 mg on Day 1 and Day 2, 0.5 mg on Day 3, 0.75 mg on Day 4, 1.25 mg on Day 5, 2 mg on Day 6, administered orally once daily every morning. From Day 7 onward, 2 mg, the maintenance dose, is administered once daily. The maintenance dose may be reduced according to the patient's condition.

1.5 Risk management plan (draft)

In view of the discussions presented in Section "7.R.8 Post-marketing investigations" in the Review Report (1) and comments from the expert advisors at the Expert Discussion, PMDA has concluded that the risk management plan (draft) for siponimod should include the safety and efficacy specifications presented in Table 74, and that the applicant should conduct additional pharmacovigilance activities and additional risk minimization activities presented in Tables 75.

Table 74. Safety and efficacy specifications in risk management plan (draft)

Safety specifications		
Important identified risks	Important potential risks	Important missing information
• Lymphocyte count decreased, infection • Bradyarrhythmia at the start of administration (including conduction disorder) • QT prolongation • Macular oedema • Malignant tumor • Posterior reversible encephalopathy syndrome • Thromboembolism	• Reproductive and developmental toxicity	• Safety and efficacy after switching from other disease modifying drugs
Efficacy specifications		
None		

Table 75. Summary of additional pharmacovigilance activities and additional risk minimization activities included under the risk management plan (draft)

Additional pharmacovigilance activities	Additional risk minimization activities
• Early post-marketing phase vigilance • Specified drug use-results survey (all-case surveillance) • Post-marketing clinical study[a)]	• Information provision based on the early post-marketing phase vigilance • Preparation and distribution of materials for healthcare professionals • Preparation and distribution of materials for patients • Establishment of the conditions for the use of siponimod • Publication and provision of information on incidences of adverse reactions to siponimod by the company website, etc.

a) The ongoing Study A2304 will be continued as a post-marketing clinical study after approval.

Based on the above, PMDA instructed the applicant to conduct post-marketing surveillance to investigate these items. The applicant explained the plan for a specified use-results survey in patients with SPMS, as shown in Table 76.

Table 76. Outline of the specified use-results survey (draft)

Objective	To investigate the safety and efficacy of siponimod in clinical use
Survey method	All-case surveillance
Population	All patients with SPMS who receive siponimod after the market launch
Observation period	24 months
Planned sample size	330 patients
Main survey items	Patient characteristics (age, sex, concurrent illness, results of genetic testing for CYP2C9, etc.) Use of siponimod Prior therapy, concomitant drugs Incidences of adverse events, laboratory values, electrocardiography EDSS Presence or absence of relapse

PMDA accepted the applicant's explanation. Results obtained from this surveillance should be provided to healthcare professionals without delay.

2. Overall Evaluation

As a result of the above review, PMDA has concluded that the product may be approved for the indication and dosage and administration as shown below, with the following approval conditions. Since the product is an orphan drug, the re-examination period is 10 years. The drug product is not classified as a biological product or a specified biological product. The drug product and its drug substance are both classified as powerful drugs.

Indication

Prevention of relapsing secondary progressive multiple sclerosis and delay in the progression of associated physical disability

Dosage and Administration

The usual adult dosage of siponimod is 0.25 mg on Day 1 and Day 2, 0.5 mg on Day 3, 0.75 mg on Day 4, 1.25 mg on Day 5, 2 mg on Day 6, administered orally once daily every morning. From Day 7 onward, 2 mg, the maintenance dose, is administered once daily. The maintenance dose may be reduced according to the patient's condition.

Approval Conditions

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

2. Because of the extremely limited number of subjects participating in Japanese clinical studies, the applicant is required to conduct a drug use-results survey involving all patients treated with the product after the market launch until data from a certain number of patients have been gathered, in order to understand the characteristics of patients using the product, and to promptly collect safety and efficacy data so that necessary measures are taken to ensure proper use of the product.

Appendix

List of Abbreviations

3mCDP	3-month confirmed disability progression based on EDSS
6mCDP	6-month confirmed disability progression based on EDSS
AIC	Akaike Information Criterion
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ARR	Annualized Relapse Rate
AST	Aspartate aminotransferase
AUC	Area Under Concentration-time Curve
BA	Bioavailability
BCRP	Breast Cancer Resistance Protein
BMI	Body mass index
BSEP	Bile Salt Export Pump
CD	Cluster of Differentiation
CFA	Complete Freund's Adjuvant
CHO	Chinese Hamster Ovary
CI	Confidence Interval
CIS	Clinically Isolated Syndrome
CL/F	Apparent Total Clearance
C_{max}	Maximum Serum (Plasma) Concentration
CTD	Common Technical Document
CUAL	Combined Unique Active Lesion
CYP	Cytochrome P450
[redacted]	[redacted]
DMC	Data Monitoring Committee
DMSO	Dimethyl Sulfoxide
EAE	Experimental Autoimmune Encephalomyelitis
EC_{50}	Effective Concentration, 50%
ED_{50}	Effective Dose, 50%
EDSS	Expanded Disability Status Scale
EdU	5-ethynyl-2-deoxyuridine
EMA	European Medicines Agency
E_{max}	Maximum Effect
FAS	Full Analysis Set
FDA	Food and Drug Administration
FEV_1	Forced Expiratory Volume in one second
FMI	Final Market Image Formulation
GABA	Gamma-aminobutyric Acid
GC	Gas Chromatography
GCP	Good Clinical Practice
GFAP	Glial Fibrillary Acidic Protein
GIRK	G protein-coupled Inwardly Rectifying K+ channel
GTPγS[^{35}S]	Guanosine ^{35}S-[gamma-thio] Triphosphate
HEK	Human Embryonic Kidney
hERG	Human Ether-a-go-go Related Gene
HLGT	High Level Group Terms
HLT	High Level Term
HPLC	High Performance Liquid Chromatography
IBA	Ionized calcium-Binding Adaptor molecule

IC_{50}	Half Maximal (50%) Inhibitory Concentration
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICH M7 Guideline	"Guidelines for Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk" "PSEHB/ELD Notification No. 1110-3 dated November 10, 2015)
ICH Q1E Guideline	"Guideline on the Evaluation of Stability Data" (PFSB/ELD Notification No. 0603004 dated June 3, 2003).
ICH Q3A Guideline	"Revision of the Guideline on Impurities in New Drug Substances" (PFSB/ELD Notification No. 1216001, dated December 16, 2002)
Ig	Immunoglobulin
IR	Infrared Absorption Spectrum
Ki	Inhibitory Constant
k_{inact}	Maximum Inactivation Rate Constant
KLH	Keyhole Limpet Hemocyanin
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LPS	Lipopolysaccharide
LUC	Large Unstained Cell
[redacted]	[redacted]
MATE	Multidrug and Toxic Extrusion
[redacted]	[redacted]
[redacted]	[redacted]
MCP-Mod	Multiple Comparison Procedure -modeling
[redacted]	[redacted]
[redacted]	[redacted]
MedDRA	Medical Dictionary for Regulatory Activities
MF	Market Formulation
MOG	Myelin Oligodendrocyte Glycoprotein
MRI	Magnetic Resonance Imaging
MRP2	Multidrug Resistance Associated Protein 2
MS	Multiple Sclerosis
NMR	Nuclear Magnetic Resonance Spectrum
NYHA	New York Heart Association
NZW	New Zealand White
OAT	Organic Anion Transporter
OATP	Organic Anion Transporting Polypeptide
OCT	Organic Cation Transporter
PALS	Periarteriolar Lymphoid Sheath
PBPK	Physiologically-based Pharmacokinetics
P-gp	P-glycoprotein
PLGF2	Placental Growth Factor 2
PMDA	Pharmaceuticals and Medical Devices Agency
PPK	Population Pharmacokinetics
PPMS	Primary Progressive Multiple Sclerosis
PT	Preferred Term
QTc	Corrected QT
QTcB interval	QT Interval Corrected for Heart Rate Using Bazett's Method
QTcF	Fridericia-corrected QT
RDW	Red Cell Distribution Width
RH	Relative Humidity
RRMS	Relapsing-Remitting Multiple Sclerosis
S1P	Sphingosine 1 phosphate
Siponimod	Siponimod fumaric acid
SMQ	Standardized MedDRA Query

SOC	System Organ Class
Sph kinase	Sphingosine kinase
SPMS	Secondary Progressive Multiple Sclerosis
$t_{1/2}$	Elimination Half-life
T4-UDP-GT	T4-Thyroxine-UDP-Glucuronosyl transferase
Th1	Helper T cell 1
Th17	Helper T cell 17
Mayzent	Mayzent Tablets 0.25 mg, Mayzent Tablets 2 mg
TIA	Transient Ischemic Attacks
t_{max}	Time to Reach Maximum Concentration
TSH	Thyroid Stimulating Hormone
UV	Ultraviolet Spectrum
Vc/F	Apparent Central Volume of Distribution
VEGF	Vascular Endothelial Growth Factor
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]