Report on the Deliberation Results

June 10, 2014

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

[Brand name]	Rapalimus Tablets 1 mg
[Non-proprietary name]	Sirolimus (JAN*)
[Applicant]	Nobelpharma Co., Ltd.
[Date of application]	October 21, 2013

[Results of deliberation]
In the meeting held on May 26, 2014, the Second Committee on New Drugs concluded that the product may be approved and that this result should be reported to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The re-examination period is 10 years, the drug substance and the drug product are both classified as powerful drugs, and the product is not classified as a biological product or a specified biological product.

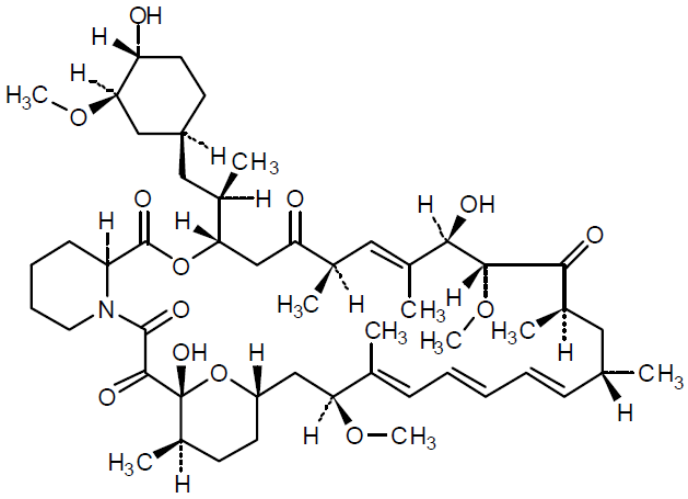
[Conditions for approval]
The applicant is required to conduct a drug use-results survey in all patients until data from a certain number of patients have been accumulated to understand the demographic information of patients treated with this product, because there are extremely few Japanese patients who have been treated with the drug. At the same time, safety and efficacy data on the product should be collected early and necessary measures should be taken to facilitate the proper use of the product.

**Japanese Accepted Name (modified INN)*

Review Report

May 15, 2014
Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following pharmaceutical product submitted for registration are as follows.

[Brand name]	Rapalimus Tablets 1 mg
[Non-proprietary name]	Sirolimus
[Applicant]	Nobelpharma Co., Ltd.
[Date of application]	October 21, 2013
[Dosage form/Strength]	A tablet containing 1 mg of sirolimus
[Application classification]	Prescription drug (1) Drug with a new active ingredient
[Chemical structure]	
Molecular formula:	$C_{51}H_{79}NO_{13}$
Molecular weight:	914.17
Chemical name:	(1*R*,9*S*,12*S*,15*R*,16*E*,18*R*,19*R*,21*R*,23*S*,24*E*,26*E*,28*E*,30*S*,32*S*,35*R*)-1,18-Dihydroxy-12-{(1*R*)-2-[(1*S*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylethyl}-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-azatricyclo[30.3.1.$0^{4,9}$]hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentaone
[Items warranting special mention]	Orphan drug (Designation No. [24 yaku] No. 286, Notification No. 0913-5 from the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, dated September 13, 2012)
[Reviewing office]	Office of New Drug IV

Review Results

May 15, 2014

[Brand name] Rapalimus Tablets 1 mg
[Non-proprietary name] Sirolimus
[Applicant] Nobelpharma Co., Ltd.
[Date of application] October 21, 2013
[Results of review]
Based on the submitted data, it is concluded that the efficacy of the product for lymphangioleiomyomatosis (LAM) has been demonstrated and the safety is acceptable in view of its observed benefits. Since the product was evaluated in an extremely limited number of patients with LAM in both Japanese and foreign clinical studies, and since serious adverse events including interstitial lung disease and serious infectious disease may occur, an all-case post-marketing surveillance should be conducted to further investigate the safety and efficacy of the product.

As a result of its regulatory review, the Pharmaceuticals and Medical Devices Agency has concluded that the product may be approved for the indication and dosage and administration as shown below, with the following conditions.

[Indication] Lymphangioleiomyomatosis

[Dosage and administration] The usual adult dosage is 2 mg of sirolimus orally administered once daily. The dose may be adjusted according to the patient's condition. The dose should not exceed 4 mg once daily.

[Conditions for approval] The applicant is required to conduct a drug use-results survey in all patients until data from a certain number of patients have been accumulated to understand the demographic information of patients treated with this product, because there are extremely few Japanese patients who have been treated with the drug. At the same time, safety and efficacy data on the product should be collected early and necessary measures should be taken to facilitate the proper use of the product.

Review Report (1)

April 17, 2014

I. Product Submitted for Registration

[Brand name]	Rapalimus Tablets 1 mg
[Non-proprietary name]	Sirolimus
[Applicant]	Nobelpharma Co., Ltd.
[Date of application]	October 21, 2013
[Dosage form/Strength]	A tablet containing 1 mg of sirolimus
[Proposed indication]	Lymphangioleiomyomatosis
[Proposed dosage and administration]	The usual adult dosage is 2 mg of sirolimus orally administered once daily. The dose may be adjusted according to the patient's condition.

II. Summary of the Submitted Data and Outline of Review by the Pharmaceuticals and Medical Devices Agency

The data submitted in this application and the outline of review by the Pharmaceuticals and Medical Devices Agency (PMDA) are as shown below.

1. Origin or history of discovery and usage conditions in foreign countries, etc.

Sirolimus, the active ingredient of the proposed product, is a drug that inhibits the activation of mammalian target of rapamycin (mTOR), a cell cycle regulatory protein, and was developed as an immunosuppressant by US Wyeth-Ayerst Research (now Pfizer Inc.). Sirolimus is approved for the indication for the prevention of renal transplant rejection in 89 countries as of September 2011. In Japan, sirolimus has not been approved as a drug, while a sirolimus-eluting stent, a medical device intended to treat patients with ischemic heart disease developed by Johnson & Johnson K.K., was approved in 2004.

Lymphangioleiomyomatosis (LAM) is a disease characterized by abnormal growth of smooth muscle-like cells (LAM cells) mainly in the lungs and lymph nodes in the trunk and by cyst formation in the lungs caused by tissue destruction. The disease develops mainly in women of child-bearing age and is accompanied by respiratory symptoms such as dyspnoea exertional and pneumothorax as well as by chylothorax and renal angiomyolipoma. In particular, progressive cystic lung disease has a poor prognosis; it causes respiratory failure in advanced stages requiring oxygen inhalation and, in more advanced stages, requires lung transplant. LAM is classified into sporadic LAM (S-LAM) and hereditary tuberous sclerosis complex (TSC)-associated LAM (TSC-LAM). Both are considered to be caused by a point mutation in the TSC1 or TSC2 gene, resulting in the loss of function of the tuberin-hamartin complex (complex of hamartin and tuberin encoded by TSC1 and TSC2, respectively), leading to constitutive activation of mTOR and thereby to the proliferation of LAM cells (Seyama K, *Respiration & Circulation*. 2010;58:1201-1210).

Treatments of LAM include hormone therapy (anti-estrogen therapy) and surgical ovariectomy, as well as administration of bronchodilators, home oxygen therapy, and lung transplant for respiratory symptoms, pleurodesis for pneumothorax or chylothorax, and embolization or surgical resection for angiomyolipoma (AML) (Hayashida M et al., *The Journal of the Japanese Respiratory Society*. 2008;46:428-431). However, there is no established treatment for LAM. Since involvement of estrogen in the pathology of LAM is suspected, hormone therapy (anti-

estrogen therapy) and surgical ovariectomy are carried out, but many researchers have a negative view on their efficacy (Johnson SR et al., *Am J Respir Crit Care Med.* 1999;160:628-633, Taveira-DaSilva AM et al., *Chest.* 2004;126:1867-1874, Banner AS et al., *N Engl J Med.* 1981;305:204-209, Svendsen TL et al., *Br J Dis Chest.* 1984;78:264-271, Tomasian A et al., *N Engl J Med.* 1982;306:745-746, de la Fuente J et al., *Eur J Med.* 1993;2:377-378, Rossi GA et al., *Am Rev Respir Dis.* 1991;143:174-176).

Since it became clear in 1990s that, in patients with LAM, mTOR is constitutively activated by the point mutation of the TSC1 or TSC2 gene, mTOR inhibitors have attracted attention as candidate therapeutic agents for LAM.

Against this background, a clinical study (Study CAST) was conducted in patients who were diagnosed with TSC or S-LAM and had AML. The results suggested that sirolimus therapy reduced AML and improved respiratory function (Bissler JJ et al., *N Engl J Med.* 2008;358:140-151), whereupon an investigator-initiated clinical development of sirolimus for LAM was undertaken. Thus, a clinical study was conducted from December 2006 in the US, Canada, and Japan (Study MILES[1]), and an investigator-initiated clinical study was conducted from August 2012 in Japan (Study MLSTS). In Japan, Nobelpharma Co., Ltd. obtained the rights for development and marketing in Japan from Pfizer Inc., US, and has now submitted a marketing application for sirolimus for the proposed indication of LAM, based on the results of these studies. At present, sirolimus has not been filed for the indication of LAM in other countries.

The estimated prevalence rate of LAM in Japan is 1.9 to 4.5 per 1,000,000 population (Kubo K et al., FY 2007 integrated/partial research report on the survey of respiratory failure supported by Health and Labour Sciences Research Grants for Research on Intractable Diseases. 2008; 37-41). The proposed product was designated as an orphan drug in September 2012 with the expected indication of "lymphangioleiomyomatosis (LAM)" (Designation [24 yaku] No. 286 [Notification No. 0913-5 from the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW, dated September 13, 2012]).

2. Data relating to quality

2.A Summary of the submitted data

2.A.(1) Drug substance

2.A.(1).1) Characterization

The drug substance is a white, crystalline powder and has been determined for description, solubility, distribution coefficient, optical activity, and crystalline polymorphism. The drug substance did not show crystalline polymorphism.

The chemical structure of the drug substance has been elucidated by elementary analysis, nuclear magnetic resonance spectrometry (^{1}H- NMR, ^{13}C-NMR), mass spectrometry, ultraviolet-visible spectrophotometry (UV), and infrared spectrophotometry (IR). The drug substance interconverts between isomers A, B, and C, but mainly consists of isomer B.

2.A.(1).2) Manufacturing process

[redacted].

[1] This study was conducted in compliance with ICH-GCP, while in Japan, it was conducted as clinical research.

2.A.(1).3) Control of drug substance

The proposed specifications for the drug substance include content[2] (calculated on the anhydrous basis), description, identification (IR), optical rotation, purity (heavy metals, related substances [high performance liquid chromatography (HPLC)], residual solvents [gas chromatography (GC)]), water content, residue on ignition, microbial limits, and assay (HPLC).

[redacted].

2.A.(1).4) Stability of drug substance

Table 1 shows the results of stability studies of the drug substance. Results of the photostability testing showed that the drug substance was unstable to light.

Table 1. Stability studies of the drug substance

Study	Primary batch	Temperature	Humidity	Storage container	Storage period
Long-term	4 commercial batches	5°C	-	[redacted]	36 months
Accelerated	4 commercial batches	25°C	60% RH		12 months

Based on the above, a retest period of 36 months has been proposed for the drug substance when stored in [redacted] polyethylene bags and placed in a metallic can at 2°C to 8°C protected from light.

2.A.(2) Drug product

2.A.(2).1) Description and composition of the drug product and formulation development

The drug product is a tablet containing 1 mg of the drug substance. The drug product contains, as excipients, lactose hydrate, microcrystalline cellulose, sucrose, magnesium stearate, talc, calcium sulfate, polyethylene glycol 20000 [redacted], polyethylene glycol 8000, povidone, glyceryl monooleate ([redacted]%), shellac solution, polyoxyethylene (160) polyoxypropylene (30) glycol, tocopherol [redacted], titanium oxide, and carnauba wax [redacted].

[redacted].

2.A.(2).2) Manufacturing process

[redacted].

[redacted].

[2] The content of the sum of isomers B and C and the content of isomer B are specified.

2.A.(2).3) Control of drug product
The proposed specifications for the drug product include content[3] (calculated on an anhydrous basis), description, identification (UV), purity (related substances [HPLC]), uniformity of dosage units (HPLC), dissolution (HPLC), and assay (HPLC).

2.A.(2).4) Stability of drug product
Table 2 shows the results of the stability studies of the drug product. Results of the photostability testing showed that the drug product was stable to light.

Table 2. Stability studies of the drug product

Study type	Primary batch	Temperature	Humidity	Storage container	Storage period
Long-term	3 commercial batches	25°C	60% RH	PTP packaging	24 months
Accelerated	3 commercial batches	40°C	75% RH		6 months

[redacted].

2.B Outline of the review by PMDA
PMDA reviewed the submitted data and concluded that the quality of the drug substance and the drug product was appropriately controlled.

2.*B*.(1) New excipient
The drug product contains a new excipient polyethylene glycol 8000 in an amount exceeding that of the previous uses for oral administration.

2.*B*.(1).1) Specifications and stability
PMDA reviewed the submitted data and concluded that there were no problems in the specifications or stability of polyethylene glycol 8000.

2.*B*.(1).2) Safety
PMDA reviewed the submitted data and concluded that there were no particular safety problems with polyethylene glycol 8000 at the level used in the proposed product.

3. Non-clinical data
3.(i) Summary of pharmacology studies
3.(i).A Summary of the submitted data
No primary pharmacodynamic studies were conducted for the present application; instead, publications (4.2.1.1-1 to 4.2.1.1-9) were submitted as reference data. In the secondary pharmacology studies, effects on various receptors, ion channels, enzymes, etc., were investigated. In safety pharmacology studies, effects on the central nervous system, respiratory system, cardiovascular system, gastrointestinal system, renal function, and bone metabolism were investigated. The results of these studies, except a part of studies that investigated the effect on the cardiovascular system (4.2.1.3-3), were submitted as reference data. No pharmacodynamic drug interaction studies were conducted.

[3] The content of the sum of isomers B and C is specified.

3.(i).A.(1) Primary pharmacodynamics

3.(i).A.(1).1) Studies on the mechanism of action

(a) Inhibition of ribosomal protein S6 phosphorylation (4.2.1.1-1, 4.2.1.1-3)

Using smooth muscle cells cultured from lung nodules of patients with LAM (LAM cells), uterine leiomyoma cells derived from TSC2 knockout Eker rats (ELT3 cells), or renal carcinoma cells (ERC15 cells), the effect of sirolimus on phosphorylation of p70S6K (a ribosomal protein S6 kinase), which is an index for mTOR activity, was investigated. In all of LAM, ELT3, and ERC15 cells, sirolimus (200 nM) inhibited p70S6K phosphorylation (Goncharova EA et al., *J Biol chem.* 2002;277:30958-30967).

ELT3 cells derived from TSC2 knockout Eker rats were subcutaneously transplanted to NCRNU-M athymic nude mice (n = 20 or 27/group), and the effect of sirolimus on S6 phosphorylation was investigated in these mice. Starting from the day when the tumor diameter reached 5 mm, sirolimus (1 mg/kg) was intraperitoneally administered 3 times a week for 20 days to these mice. As a result, S6 phosphorylation in the tumor tissue was suppressed (Goncharova EA et al., *Mol Cell Biol.* 2011;31:2484-2498).

(b) DNA synthesis inhibition, cell cycle inhibition, and apoptosis induction (4.2.1.1-7)

Using LAM patient-derived LAM cells, the effect of sirolimus (2-200 nM) on DNA synthesis was investigated with ^{3}H-thymidine uptake into DNA as an index. Sirolimus at ≥20 nM inhibited DNA synthesis in LAM cells in a concentration-dependent manner. Using the same cells, the effect of sirolimus on cell cycle was investigated with the intracellular uptake of 5-bromo-2'-deoxyuridine (BrdU) as an index for cell growth. In LAM cells stimulated with platelet-derived growth factor (PDGF, 10 ng/mL), the number of LAM cells in the G_0/G_1 phase increased and the number of cells in the S phase decreased after addition of sirolimus (200 nM). Furthermore, using LAM cells in serum-free medium or those stimulated with PDGF, the effect of sirolimus on apoptosis induction was investigated with the nuclei visualized with 4,6-diamidino-2-phenylindole as an index. Sirolimus (200 nM) enhanced apoptosis induction of LAM cells under both serum-free and PDGF-stimulated conditions (Goncharova EA et al., *Mol Pharmacol.* 2008;73:778-788).

(c) Inhibition of LAM cell proliferation (4.2.1.1-1)

Using LAM patient-derived LAM cells, the effect of sirolimus (0.02-200 nM) on the growth of LAM cells was investigated with the percentage of BrdU-positive cells in the entire cell population as an index. Sirolimus at ≥0.2 nM inhibited the percentage of BrdU-positive cell counts in a concentration-dependent manner (Goncharova EA et al., *J Biol chem.* 2002;277:30958-30967).

(d) Inhibition of vascular endothelial growth factor production (4.2.1.1-9)

Using TSC1 or TSC2-deficient fibroblasts, the effect of sirolimus on expression of vascular endothelial growth factor (VEGF) was investigated. Both in TSC1- and TSC2-deficient cells, intracellular VEGF content and extracellular production of VEGF increased compared with cells with intact TSC1 and TSC2 genes. In both gene-deficient cells, the intracellular content of VEGF decreased after addition of sirolimus (0.5-10 nM) in a concentration-dependent manner and the extracellular production of VEGF also decreased after addition of sirolimus (10 nM) (El-Hashemite N et al., *Cancer Res.* 2003;63:5173-5177).

(e) Inhibition of matrix metalloproteinase and of lung injury (4.2.1.1-8)

A mouse model of LAM was developed by tail vein injection of renal epithelial tumor cells derived from TSC2 knockout mice to NCRNU-M nude mice. Using this mouse model, the effect of sirolimus on the expression level of matrix metalloproteinase (MMP) in the bronchoalveolar lavage fluid and on lung injury was investigated. When sirolimus (1 mg/kg) was administered 3 times a week for 20 days starting from 3 to 10 days after tumor cell injection, MMP-2 expression

level in the bronchoalveolar lavage fluid decreased compared with the vehicle control group, and MMP-3 and MMP-9 expression levels tended to decrease compared with the vehicle control group. At 20 days after the start of injection, the vehicle control group showed a worsening of alveolar destruction and enlargement of alveolar airspace surrounding the lung parenchyma, whereas these changes were suppressed in the sirolimus group (Goncharova EA et al., *Sci Transl Med.* 2012;4:154ra134).

3.(i).A.(1).2) Studies in model animals

(a) Tumor-growth suppression and life prolongation in TSC2 knockout, tumor-bearing mice (4.2.1.1-2 to 4.2.1.1-3)

TSC2-deficient tumor cells were subcutaneously transplanted to CD-1nuBR nude mice (n = 5 or 6/group), and the effect of sirolimus on tumor growth and survival was investigated in these animals. Sirolimus (8 mg/kg) was intraperitoneally administered 5 times every week to each mouse, starting from the day when the tumor size reached 150 mm^3 (1 day after transplantation) until the size reached 3000 mm^3. As a result, the sirolimus group showed a decrease in tumor size on Day 16 after the beginning of administration and increased survival days, compared with the vehicle control group (Lee N et al., *BMC Pharmacol.* 2009;9:8doi:10,1186/1471-2210-9-8).

ELT3 cells derived from TSC2 knockout Eker rats were subcutaneously transplanted to NCRNU-M athymic nude mice (n = 20 or 27/group), and the effect of sirolimus on tumor growth and survival was investigated in these animals. Sirolimus (1 mg/kg) was intraperitoneally administered 3 times every week to each mouse for 50 days starting from the day when the tumor diameter reached 5 mm. As a result, the sirolimus group showed a decrease in tumor size on Days 10 to 41 after the beginning of administration and increased survival days, compared with the untreated group (Goncharova EA et al., *Mol Cell Biol.* 2011;31:2484-2498).

(b) Suppression of renal tumor growth in a rat model of tuberous sclerosis with TSC2 gene mutation (4.2.1.1-4)

Using Eker rats (n = 5) with TSC2 gene mutation, effect of sirolimus on the growth of renal tumor was investigated. Sirolimus was intraperitoneally administered at 0.1 mg/kg for 8 weeks or at 0.2 mg/kg for 2, 4, or 7 weeks. As a result, all sirolimus groups showed a decrease from baseline in the volume of renal tumor, measured by ultrasonic imaging (Kenerson H et al., *Pediatr Res.* 2005;57:67-75).

(c) Survival benefit in a mouse model of tuberous sclerosis with TSC1 gene deficiency (4.2.1.1-5)

Using $TSC1^{null\text{-}neuron}$ mice (n = 17 or 18/group) with neuron-specific TSC1 deficiency, the effect of sirolimus on survival rate was investigated. Sirolimus (6 mg/kg) was intraperitoneally administered on alternate days from 7 to 9 days after birth up to 30 or 100 days after birth. As a result, the survival rate increased in the group treated up to 100 days after birth compared with the group treated up to 30 days after birth and the untreated group (Meikle L et al., *J Neurosci.* 2008;28:5422-5432).

3.(i).A.(2) Secondary pharmacodynamics

3.(i).A.(2).1) Effects on receptors, ion channels, and enzymes (4.2.1.3-9)

The *in vitro* effect of sirolimus on various receptors was investigated. As a result, sirolimus over the concentration range from 1 to 10,000 nM did not have any significant effect on the receptor binding or second messenger binding in the sympathetic nervous system, parasympathetic nervous system, excitatory or inhibitory amino acids, Ca^{2+} channels, opioid system, or prostanoid system. Sirolimus inhibited histamine H_1 receptor but its IC_{50} was 100 to 500 nM, which was 20 to 100 times higher than the concentration that shows an immunosuppressive effect.

3.(i).A.(3) Safety pharmacology

3.(i).A.(3).1) Effect on the central nervous system (4.2.1.3-1)

Following a single intraperitoneal dose of sirolimus (0.5, 2.5 mg/kg) to male rats (n = 6/group), the effect on general symptoms, behavior, and locomotor activity was investigated. A slight decrease in locomotor activity was observed in the 0.5 mg/kg group, whereas no effect of sirolimus on general symptoms, behavior, or locomotor activity was observed in the 2.5 mg/kg group.

3.(i).A.(3).2) Effect on respiratory/cardiovascular system

(a) Effect on blood pressure and heart rate in spontaneously hypertensive rats (4.2.1.3-2)

Following a single oral dose of sirolimus (3 mg/kg) to spontaneously hypertensive rats (n = 7, 8/group), sirolimus had no effect on the mean arterial pressure or heart rate.

(b) Effect on electrocardiogram in monkeys (4.2.1.3-3)

Following repeated oral dose of sirolimus (0.5, 5, 10 mg/kg) to male and female cynomolgus monkeys (n = 6/group) for 3 months, sirolimus had no effect on electrocardiogram in any of the treated groups.

(c) Effect on pulmonary function in guinea pigs (4.2.1.3-4)

Following a single intraperitoneal dose of sirolimus (3 mg/kg) to male guinea pigs (n = 11/group), sirolimus had no effect on pulmonary vascular resistance, lung compliance, blood pressure, or heart rate up to 60 minutes after administration.

3.(i).A.(3).3) Effect on the gastrointestinal system (4.2.1.3-5)

Following a single oral dose of sirolimus (3 mg/kg) to male rats (n = 5-10/group), sirolimus had no effect on gastric acid secretion, gastric emptying, gastric mucosa, or small intestinal mucosa.

3.(i).A.(3).4) Effect on renal function (4.2.1.3-6, 4.2.1.3-7)

Following repeated oral dose of sirolimus (1, 10 mg/kg) for 14 days to male rats (n = 7 or 8/group), the effect on the renal function was investigated. In the 1 mg/kg group, sirolimus had no effect on body weight, urine output, plasma creatinine level, creatinine clearance, or renal weight, whereas the 10 mg/kg group showed weight loss and increased urine output. Based on the results of the pharmacokinetic study and the toxicity study (Reference data; 4.2.2.2-1, 4.2.3.2-6), maximum concentration (C_{max}) and area under the curve (AUC) of sirolimus following administration to rats at 10 mg/kg was estimated to be 43.4 to 47.9 ng/mL and 497.7 to 718.1 ng·hr/mL, respectively. The exposure ratio to the value following administration of the clinical dose[4] to humans was 9.64 to 10.64 for C_{max} and 11.06 to 15.96 for AUC.

Following a single oral dose of sirolimus (1 or 3 mg/kg) to male rats (n = 12/group) loaded with physiological saline, the effect on renal function was investigated. In the 1 mg/kg group, sirolimus had no effect on urine output, urinary Na^+ or K^+ excretion, urine osmolality, or urine pH, and the only change observed in the 3 mg/kg group was a slight decrease in urine pH. Based on the results of the pharmacokinetic study and the toxicity study, C_{max} and AUC of sirolimus following administration to rats at 3 mg/kg was estimated to be 9.3 to 13.8 ng/mL and 151.5 to 174.0 ng·hr/mL, respectively. The exposure ratio to the value following the administration of the clinical dose to humans was 2.07 to 3.07 for C_{max} and 3.37 to 3.87 for AUC.

3.(i).A.(3).5) Effect on bone metabolism (4.2.1.3-8)

Sirolimus (2.5 mg/kg), cyclosporine (15 mg/kg), or tacrolimus (5 mg/kg) was orally administered for 28 days to male rats (n = 8 or 9/group), and the effect on bone metabolism was investigated.

[4] The values were compared with C_{max} (4.5 ng/mL) and $AUC_{0-\infty}$ (120 ng·h/mL) observed in Study 186-UK in which 2 triangular sirolimus 1 mg tablets were administered orally at a single dose to healthy adult subjects.

In the sirolimus 2.5 mg/kg group, sirolimus had no effect on the percentage of trabeculae compared with the untreated group, whereas increased bone calcification rate and decreased remodeling period were observed, suggesting the effect on bone metabolism. The cyclosporine 15 mg/kg group showed an increased bone calcification rate, decreased remodeling period, and decreased percentage of trabeculae compared with the untreated group, and the tacrolimus 5 mg/kg group showed decreased percentage of trabeculae compared with the untreated group. Based on the results of the pharmacokinetic study and the toxicity study, C_{max} and AUC of sirolimus following administration to rats at 2.5 mg/kg was estimated to be 7.36 to 7.75 ng/mL and 126.3 to 145.0 ng·hr/mL, respectively. The exposure ratio to the value following the administration of the clinical dose to humans was 1.64 to 1.72 for C_{max} and 2.81 to 3.22 for AUC.

3.(i).B Outline of the review by PMDA

3.(i).*B*.(1) Mechanism of action of sirolimus

The applicant explained the mechanism of action of sirolimus against LAM as follows:
It is considered that both S-LAM and TSC-LAM develop according to the following mechanism. Point mutation of TSC1 or TSC2 gene results in the loss of function of tuberin-hamartin complex comprising TSC1-encoded hamartin and TSC2 gene-encoded tuberin, which in turn causes constitutive activation of mTOR, a cell cycle regulatory protein, leading to the proliferation of LAM cells (Seyama K, *Respiration & Circulation.* 2010;58:1201-1210). It is considered that proliferated LAM cells promote lymphatic vessel neogenesis by producing or expressing cytokines (VEGF-C, D) that act as lymphatic endothelial growth factors (Stacker SA et al., *Nat Rev Cancer*. 2002;2:573-583), leading to MMP production and thereby to lung tissue destruction and cyst formation (Matsui K et al., *Arch Pathol Lab Med*. 2000;124:267-275).

Publications show that sirolimus promotes the cell cycle from G1 to S phase in LAM cells by mTOR activation and suppresses LAM cell proliferation and, at the same time, enhances VEGF production and suppresses MMPs production and accompanying lung tissue destruction. It is considered that these actions lead to the suppression of LAM progression. As regards the immunosuppressive effect of sirolimus, it is considered that sirolimus exhibits the immunosuppressive effect by suppressing mTOR activation, thereby inhibiting T and B cell growth (Sehgal SN et al., *Med Res Rev*. 1994;14:1-22, Wood MA and Bierer BE, *Perspect Drug Discov Design.* 1994;2:163-184, Aagaard-Tillery KM and Jelinek DF, *Cell Immunol*. 1994;156:493-507, Kim HS et al., *Clin Exp Immunol*. 1994;96:508-512).

PMDA reviewed the submitted publications and concluded that the LAM cell growth-inhibitory effect of sirolimus was demonstrated and that the mechanism of the effect of sirolimus on LAM could be explained. Taking account of the fact that sirolimus acts against LAM and suppresses immune function by the same mechanism, i.e., by suppressing mTOR activity, and therefore that the proposed clinical dose regimen for LAM is not significantly different from that approved in foreign countries as an immunosuppressant, it is necessary to pay careful attention to the occurrence of adverse events caused by immunosuppression and inhibition of the growth of cells other than LAM cells in clinical use of sirolimus in patients with LAM.

3.(ii) Summary of pharmacokinetic studies

3.(ii).A Summary of the submitted data

For the data of absorption, distribution, metabolism, excretion, and drug interactions, the results from studies on oral and intravenous administration in mice, rats, rabbits, cynomolgus monkeys, and humans and publications were submitted as reference data. Pharmacokinetic studies were conducted using sirolimus and ^{14}C- or ^{3}H-labeled sirolimus. Concentrations of sirolimus and metabolites in blood, plasma, serum, and tissue were measured by liquid chromatography-tandem mass spectrometry (LC/MS/MS) (lower limit of quantitation; 0.05 or 0.1 ng/mL in blood, 0.1 ng/mL in plasma) or by high performance liquid chromatography (HPLC) (lower limit of

quantitation; 0.5 ng/mL in blood, 20 ng/mL in plasma, 5 ng/mL in serum). Radioactivity was measured in a liquid scintillation counter (lower limit of quantitation; 0.29 ng eq./mL in blood, 1.29 ng eq./mL in plasma, 0.46 ng eq./mL in amniotic fluid, 0.33 ng eq./g in placenta, 0.50 ng eq./g in fetuses, 0.26 or 0.30 ng eq./mL in blood and milk).

Measured values and pharmacokinetic parameters are expressed in mean or in mean ± standard deviation (SD) unless otherwise specified.

3.(ii).A.(1) Absorption

3.(ii).A.(1).1) Single-dose studies (4.2.2.2-1 to 4.2.2.2-4, 4.2.2.2-6 to 4.2.2.2-7, 4.2.2.2-9)

Following a single oral or intravenous dose of sirolimus to male rats, male rabbits, and male and female cynomolgus monkeys, pharmacokinetic parameters of sirolimus in the whole blood, plasma, and serum were as shown in Table 3. The absolute bioavailability following oral administration was 1.9% to 6.2%[5] in male rats and 3.7% in male cynomolgus monkeys, suggesting a high first-pass effect in the liver and the gastrointestinal tract. AUCs in rats and rabbits were non-linear. Following intravenous administration, clearance (CL) and the steady-state volume of distribution (V_{ss}) increased with dose increase in rabbits and decreased with dose increase in cynomolgus monkeys.

[5] Calculated from blood radioactivity concentration following a single intravenous dose of ^{3}H-labeled sirolimus (1 mg/kg) or a single oral dose of ^{3}H-labeled sirolimus (5 mg/kg) to male rats (n = 5/group) (4.2.2.2-3).

Table 3. Pharmacokinetic parameters following a single-dose administration of sirolimus to rats, rabbits, and cynomolgus monkeys

Species	Route of administration	Sample	Dose (mg/kg)	Sex (n)	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-48} (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)	$t_{1/2}$ (h)	CL (L/h/kg)	V_{ss} (L/kg)
Rat	p.o.	Whole blood	2	Male (5/time point)	12 ± 2.4	2	93 ± 5.0[a)]	103 ± 6.0[a)]	16 ± 1.8	-	-
		Plasma	2	Male (5/time point)	1.5 ± 0.5	2	27 ± 2.0[a)]	-	-	-	-
	p.o.	Whole blood	0.05	Male (5)	0.3 ± 0.1	3	-	3.9	7.2	-	-
			0.1	Male (5)	0.2 ± 0.1	6	-	4.3	13.7	-	-
			0.5	Male (5)	1.4 ± 0.2	8	-	34.1	18.4	-	-
	i.v.	Whole blood	0.25	Male (5)	-	-	-	999.5	30.8	0.3	10.8
	p.o.	Whole blood	0.25	Male (5)	1.2 ± 0.5	3	-	18.7	9.6	-	-
Rabbit	i.v.	Whole blood	0.05[b)]	Unknown (5)	-	-	-	-	12.8 ± 2.1 $t_{1/2\alpha}$: 0.1 ± 0.1 $t_{1/2\beta}$: 8.3 ± 1.8	1.0 ± 0.3[c)] 1.5 ± 0.4[c)]	1.1 ± 0.4 0.8 ± 0.2
			0.5[b)]	Unknown (5)	-	-	-	-	15.3 ± 1.2 $t_{1/2\alpha}$: 0.2 ± 0.1 $t_{1/2\beta}$: 15.0 ± 0.1	2.1 ± 0.2[c)] 2.1 ± 0.2[c)]	2.6 ± 0.2 2.8 ± 0.2
Cynomolgus monkey	i.v.	Serum	0.25	Male and female (4 in total)	-	-	-	22 ± 7	1.8 ± 0.5	11.9 ± 3.0	29.7 ± 5.6
			0.75	Male and female (4 in total)	-	-	-	53 ± 9	1.9 ± 0.4	14.3 ± 2.1	39.2 ± 11.3
			2.5	Male and female (3 in total)	-	-	-	971 ± 97	1.4 ± 0.1	2.6 ± 0.3	4.0 ± 0.8
	i.v.	Whole blood	0.25	Male (4)	-	-	-	1812.2 ± 412.8	14.3 ± 3.2	0.1 ± 0.0	2.8 ± 0.7
	p.o.	Whole blood	0.25	Male (4)	13.0 ± 4.5	1.0 ± 0.0	-	65.4 ± 5.8	5.6 ± 1.0	-	-

Mean or mean ± SD, -: No data
a) Mean ± standard error (SE),
b) Upper row: Model-independent analysis; lower row: 2-compartment model analysis, c) mL/min/kg
C_{max}: Maximum concentration, t_{max}: Time to maximum concentration, AUC: Area under the blood concentration-time curve, $t_{1/2}$: Elimination half-life, CL: Clearance, V_{ss}: Distribution volume under the steady state

3.(ii).A.(1).2) Repeat-dose studies (4.2.2.2-1, 4.2.2.2-8, 4.2.2.2-10 to 4.2.2.2-13)

Sirolimus was repeatedly administered orally or intravenously to male rats or male cynomolgus monkeys. As a result, pharmacokinetic parameters of sirolimus in the whole blood, plasma, and serum were as shown in Table 4. In all animal species, C_{max} and AUC increased in a dose-dependent manner. Regarding the increased AUC and decreased CL and V_{ss} observed in repeated intravenous administration in rats and cynomolgus monkeys, the applicant considered that, given the $t_{1/2}$ (4.3 hours in rats, 4.6 hours in monkeys) and the dosing interval (24 hours), the observed results were not caused by the accumulation of sirolimus, but caused by the saturated distribution of sirolimus in blood cells at the high dose, as judged from the high distribution rate in blood cells [see "3.(ii).A.(2).2) Distribution in blood cells"], which affected the apparent CL and V_{ss} values at a high serum sirolimus concentration. In contrast, oral administration in rats and cynomolgus monkeys did not show any significant difference in pharmacokinetic parameters between single- and repeat-dose administration.

Table 4. Pharmacokinetic parameters following repeat-dose administration of sirolimus to rats and cynomolgus monkeys

Species	Route of administration	Sample	Dose (mg/kg/day)	Sex (n)	Time point of measurement	C_{max} (ng/mL)	t_{max}	AUC_{0-t} (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)	$t_{1/2}$ (h)	CL (L/h/kg)	V_{ss} (L/kg)
Rats	i.v.	Serum	1.5	Male (5/time point)	Day 1	-	-	321[c)]	379	5.0	4.0	22.6
				Male (5/time point)	Day 17	-	-	444[c)]	506	4.3	3.0	15.3
	p.o.	Whole blood	0.2	Male (5/time point)	Day 14	1.2 ± 0.4	2	15 ± 1.4[a) b)]	-	-	-	-
		Plasma	0.2	Male (5/time point)		0.4 ± 0.1	15	6.7 ± 0.6[a) b)]	-	-	-	-
		Whole blood	2	Male (5/time point)	Day 14	9.2 ± 1.2	4	116 ± 45[a) b)]	-	-	-	-
		Plasma	2	Male (5/time point)		0.9 ± 0.2	2	13 ± 2.1[a) b)]	-	-	-	-
		Whole blood	6	Male (5/time point)	Day 14	26 ± 9.4	2	298 ± 19[a) b)]	-	-	-	-
		Plasma	6	Male (5/time point)		9.1 ± 6.9	2	53 ± 9.6[a) b)]	-	-	-	-
	p.o.	Whole blood	0.25	Male (5)	Day 1	0.7 ± 0.1	1	-	10 ± 0.5	14.6 ± 1.4	-	-
				Male (5)	Day 14	0.6 ± 0.1	8	10 ± 0.6[b)]	-	64.8 ± 31.8	-	-
Cynomolgus monkeys	i.v.	Serum	1.5	Male (4)	Day 1	-	-	163 ± 58[b)]	173 ± 59	2.8 ± 0.6	9.4 ± 2.9	24.9 ± 6.1
				Male (4)	Day 7	-	-	473 ± 194[b)]	490 ± 197	4.6 ± 1.3	3.6 ± 1.9	15.1 ± 1.7
	p.o.	Whole blood	0.1	Male (4)	Day 1	9.6 ± 4.5	1.3 ± 0.9	-	64.2 ± 29.1	11.2 ± 5.3	-	-
				Male (4)	Day 7	9.9 ± 2.0	1.1 ± 0.7	78.3 ± 26.0[b)]	-	29.4 ± 11.8	-	-
	p.o.	Whole blood	0.5	Male (4)	Day 1	25 ± 5.0	1.0 ± 0.0	136 ± 18[b)]	253 ± 14	32 ± 5	-	-
					Day 14	22 ± 7.8	0.8 ± 0.3	218 ± 12[b)]	-	-	-	-

Mean or mean ± SD, -: No data
a) Mean ± SE, b) t: 24 hours, c) t: 12 hours
C_{max}: Maximum concentration, t_{max}: Time to maximum concentration, AUC: Area under the blood concentration-time curve, $t_{1/2}$: Elimination half-life, CL: Clearance, V_{ss}: Distribution volume under the steady state

3.(ii).A.(1).3) Toxicokinetics (4.2.3.2-6, 4.2.3.2-9, 4.2.3.4-3, 4.2.3.4-5, 4.2.3.5.2-3, 4.2.3.5.2-6)

The pharmacokinetics of sirolimus was investigated in repeated oral dose toxicity studies in male and female mice for 104 weeks, in male and female rats for 52 and 104 weeks, in pregnant rats for 10 days, in pregnant rabbits for 12 days, and in cynomolgus monkeys for 4 weeks. The pharmacokinetic parameters of sirolimus in the whole blood are as shown in Table 5. In all animal species, both C_{max} and AUC_{0-24} increased in a dose-dependent manner, and no clear sex difference was observed in these parameters.

Table 5. Pharmacokinetic parameters following repeated oral dose of sirolimus to mice, rats, rabbits, and cynomolgus monkeys

Species	Treatment duration	Time point of measurement	Dose (mg/kg/day)	n	Male		Female	
					C_{max} (ng/mL)	AUC_{0-24} (ng·h/mL)	C_{max} (ng/mL)	AUC_{0-24} (ng·h/mL)
Mice	104 weeks	Week 52	1	2	214	797	315	951
			3	2	472	2018	1305	5814
			6	2	1803	6162	3530	10,174
Rats	52 weeks	Week 50	0.2	5	0.6	10.7	0.5	7.1
			0.65	5	1.5	22.2	1.4	16.7
			2.0	5	5.6	49.2	6.7	57.1
		Week 41	6.0	5	21.7	277	27.4	281
	104 weeks	Week 52	0.05	5	0.3[a)]	-	0.3[a)]	-
			0.1	5	0.5[a)]	-	0.5[a)]	-
			0.2	5	0.7[a)]	-	0.9[a)]	-
Pregnant rats	Gestation day 6 to 15	Gestation day 15	0.1	4	-	-	0.4	3.4
			0.5	4	-	-	2.6	16.5
Pregnant rabbits	Gestation day 6 to 18	Gestation day 18	0.025	4	-	-	3.4	45.8
Cynomolgus monkeys	4 weeks	Week 4	0.1	3	3.6[b)]	-	5.2[b)]	-
			0.25	3	13.6[b)]	-	13.1[b)]	-
			1	3	50.1[b)]	-	57.7[b)]	-

Mean
a) 2 hours after administration, b) 1 hour after administration
C_{max}: Maximum concentration, AUC: Area under blood concentration-time curve

3.(ii).A.(1).4) Pharmacokinetics of metabolites (4.2.2.2-14)

Following a single oral dose of sirolimus (9.5 mg/kg) to 4 male rats, the unchanged sirolimus and metabolites hydroxy sirolimus, seco-sirolimus, and 41-O-demethyl sirolimus were detected in plasma. C_{max} was 361, 113, 46 and 62 ng/mL, respectively, t_{max} was 0.5, 0.5, 1.0, and 1.0 hours, $AUC_{0-\infty}$ was 1325, 245, 241, and 227 ng·h/mL, and $t_{1/2}$ was 2.8, 2.7, 1.8, and 2.4 hours.

3.(ii).A.(2) Distribution

3.(ii).A.(2).1) Tissue distribution (4.2.2.3-1 to 4.2.2.3-2)

Following a single intravenous dose of ^{14}C-labeled sirolimus (0.5 mg/kg) to male rats (n = 50), the radioactivity was distributed throughout the body, reaching the maximum concentration at 6 or 12 hours after administration. The radioactivity concentration at 6 hours after administration was highest in the liver, followed by the lungs, salivary gland, pancreas, and lowest in the spleen. The radioactivity level at 12 hours after administration was highest in the lungs, followed by the liver, salivary gland, pituitary gland, and lowest in the pancreas. At 168 hours after administration, radioactivity was still detected in all tissues except the whole blood, plasma, platelet layer, red blood cells, and gastric content. The tissue/whole blood radioactivity concentration ratio was >1 in all tissues except plasma. $t_{1/2}$ of tissue radioactivity varied over the range from 25.4 hours (platelet layer) to 351 hours (testis) and was longer than $t_{1/2}$ in the whole blood (31.8 hours) except in the platelet layer.

Following a single oral dose of ^{14}C-labeled sirolimus (0.5 mg/kg) to male rats (n = 50), the radioactivity was distributed throughout the body, reaching the peak at 12 hours after administration. The radioactivity concentration at 12 hours after administration was highest in the large intestinal content, followed by the large intestine, adrenals, liver, and lowest in the small intestine. At 168 hours after administration, radioactivity was still detected in fat, kidney, liver, lung, lymph nodes, prostatic gland, salivary gland, spleen, thymus, and urinary bladder. Tissue/whole blood radioactivity concentration ratio was >1 in all tissues except in tissues where no radioactivity was detected, i.e., the brain, eyeballs, pituitary gland, testis, bone, and skeletal muscles. $t_{1/2}$ ranged from 8.26 (adrenals) to 112 hours (kidney) in all tissues in which the parameter could be calculated.

Following a single oral dose of ^{14}C-labeled sirolimus (2 mg/kg) to male pigmented rats (n = 5), radioactivity distribution in the uvea and skin, melanin-containing tissues, was similar to that observed in white male rats (n = 5).

3.(ii).A.(2).2) Distribution in blood cells (4.2.2.2-1, 4.2.2.2-5, 4.2.2.2-8, 4.2.2.2-10)
Following a single oral dose of ^{14}C-labeled sirolimus (20 mg/kg) to male mice (n = 10), whole blood/plasma radioactivity concentration ratio was 0.64 to 0.65.

Following a single intravenous dose of sirolimus (1.0 mg/kg) to male rats (n = 5), the red blood cell/plasma radioactivity concentration ratio was 4.91.

Following 14-day repeated oral dose of sirolimus (0.2, 2, 6 mg/kg/day) to male rats (9 per group), the whole blood/plasma radioactivity concentration ratio was 1 to 13.

Following a single or 14-day repeated oral dose of sirolimus (0.5 mg/kg/day) to male cynomolgus monkeys (n = 8 or 13), the whole blood/plasma radioactivity concentration ratio was 24 and 53, respectively.

These results showed that sirolimus was highly distributed in red blood cells regardless of the dose or the frequency of administration.

3.(ii).A.(2).3) Plasma protein binding (4.2.2.3-4)
Following the addition of ^{14}C-labeled sirolimus (200-300 ng/mL) to plasma samples of male mice, rats, cynomolgus monkeys, and humans, the plasma protein binding rate of sirolimus was 91.6% to 98.8%.

3.(ii).A.(2).4) Fetal transfer (4.2.2.3-3)
Following a single oral dose of ^{14}C-labeled sirolimus (0.5 mg/kg/day) to pregnant rats (3 at each time point) on gestation day 15, C_{max} of radioactivity in the whole blood, plasma, amniotic fluid, and placenta of maternal animals and in fetuses was 14.6, 11.2, 1.7 ng eq./mL, 21.3, and 6.84 ng eq./g, respectively; and $AUC_{0\text{-}t}$ was 103, 61.4, 46.7 ng eq.·h/mL, 492 and 158 ng eq.·h/g, respectively. These results showed that the unchanged sirolimus and/or metabolite(s) were transferred to fetuses.

3.(ii).A.(3) Metabolism
3.(ii).A.(3).1) Discovery of metabolites in the whole blood and plasma, and their structures (4.2.2.4-1 to 4.2.2.4-3)
Following a single oral dose of ^{14}C-labeled sirolimus (20 mg/kg) to male mice (n = 10/time point), the unchanged sirolimus and metabolites were detected in the whole blood and plasma. The metabolites were tentatively identified as didemethyl sirolimus (B'), 7-O-demethyl sirolimus (C), hydroxy sirolimus (D, F, G, J, D'), seco-sirolimus (E), and 41-O-demethyl sirolimus (H).

Following a single oral dose of ^{14}C-labeled sirolimus (6 mg/kg) to male rats (n = 12), the unchanged sirolimus and metabolites were detected in the whole blood and plasma. Main metabolites were tentatively identified as hydroxy sirolimus (A, B, C', D, E', F, G), 7-O-demethyl sirolimus (C), seco-sirolimus (E), 41-O-demethyl sirolimus (H), and 32-O-demethyl sirolimus (I). Metabolites tentatively identified as didemethyl sirolimus, hydroxy-demethyl sirolimus, and dihydroxy sirolimus were detected in very small amounts.

Following a single oral dose of ^{14}C-labeled sirolimus (5 mg/kg) to male cynomolgus monkeys (n = 3), the unchanged sirolimus and metabolites were detected in the whole blood and plasma. The metabolites were tentatively identified as hydroxy sirolimus (A, B, C'), hydroxy-demethyl

sirolimus (A'), didemethyl sirolimus (B'), 7-O-demethyl sirolimus (C), 41-O-demethyl sirolimus (H), and several types of dihydroxy sirolimus.

3.(ii).A.(3).2) Study of metabolites in non-Japanese healthy adult subjects (5.3.2.2-3, Study 129-US)

Following a single oral dose of ^{14}C-labeled sirolimus (40 mg) to non-Japanese healthy adult subjects (n = 6), the following metabolites were detected in blood by HPLC followed by radioactivity measurement: hydroxy/hydroxy-demethyl sirolimus (A/A', 2.7%-17.1%), hydroxy/didemethyl sirolimus (B/B', 4.9%-13.4%), hydroxy/7-O-demethyl sirolimus (C, 6.5%-16.9%), and 41-O-demethyl sirolimus (H, 5.9%-11.8%), together with the unchanged sirolimus (30.3%-65.4%). In a similar manner, the following metabolites were detected in blood by liquid chromatography-mass spectrometry (LC/MS) with selected ion monitoring (SIM): hydroxy sirolimus (A, 3.8%-11.3%), hydroxy-demethyl sirolimus (A', 5.6%-6.2%), hydroxy sirolimus (B, 8.3%-12.1%), didemethyl sirolimus (B', 1.1%-4.8%), hydroxy sirolimus (C, 3.4%-5.1%), 7-O-demethyl sirolimus (C, 4.6%-7.9%), and 41-O-demethyl sirolimus (H, 7.4%-11.7%), together with the unchanged sirolimus (44.6%-66.6%).

Based on the above results, the main metabolic pathway of sirolimus in mice, rats, cynomolgus monkeys, and humans is postulated as shown in Figure 1.

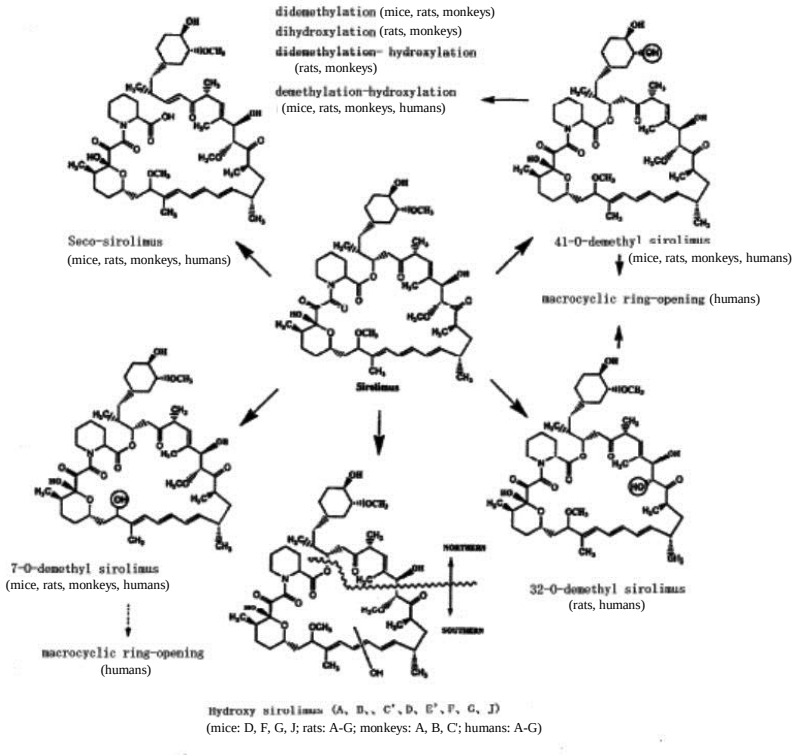


Figure 1. Possible metabolic pathways of sirolimus

3.(ii).A.(3).3) Discovery of metabolites in bile and their structures (4.2.2.4-4)

Following a single intravenous dose of sirolimus (1.3 mg/kg) to male rats (n = 12), the unchanged sirolimus and metabolites were detected in bile. The metabolites were tentatively identified as seco-sirolimus, dihydroxy sirolimus, didemethyl-hydroxy sirolimus, hydroxy sirolimus, and demethyl-hydroxy sirolimus.

3.(ii).A.(3).4) Metabolism by liver microsomes (4.2.2.4-5 to 4.2.2.4-8, 4.2.2.6-3)
Sirolimus (500 μM) was added to rat liver microsomes expressing dexamethasone-induced CYP3A, followed by incubation in the presence of NADPH. As a result, the unchanged sirolimus and metabolites were detected, and the metabolites were tentatively identified as didemethyl/hydroxy sirolimus, 7-O-demethyl/hydroxy sirolimus, hydroxy sirolimus, 11-hydroxy sirolimus, seco-sirolimus, and 41-O-demethyl sirolimus.

Sirolimus (1 mg/mL) was added to rat liver microsomes expressing dexamethasone-induced CYP3A, followed by incubation in the presence of NADPH. As a result, 3,4-dihydrodiol sirolimus and 5,6-dihydrodiol sirolimus were detected as metabolites (Nickmilder MJM et al., *Xenobiotica*. 1993;27:869-883.).

^{14}C-labeled sirolimus (50 μM) and one of the CYP3A inhibitors ketoconazole, cyclosporine, nicardipine, and methylprednisolone were added to rat liver microsomes expressing CYP3A induced by dexamethasone or pregnenolone-16α-carbonitrile, and the mixture was incubated. As a result, the metabolism of sirolimus was inhibited by ketoconazole, cyclosporine, and nicardipine.

From the above results, it was postulated that sirolimus was metabolized mainly by CYP3A in rat liver microsomes.

Sirolimus (50 μM) was added to the liver microsomes of dogs and cynomolgus monkeys, and the mixture was incubated in the presence of NADPH. As a result, sirolimus was metabolized both CYP-dependently and nonenzymatically. When the metabolites generated by nonenzymatic degradation from the liver microsomes of these animals were compared with those generated in rat liver microsomes, hydroxy sirolimus (A, D, G), 7-O-demethyl sirolimus (C), seco-sirolimus (E), and 41-O-demethyl sirolimus (H) were commonly detected in all animal species tested. In dog liver microsomes, hydroxy sirolimus (B), a metabolite generated by rat liver microsomes, was not detected; instead 2 new metabolites were detected. In the liver microsomes of cynomolgus monkeys, hydroxy sirolimus (F), which was generated by rat liver microsomes, was not detected.

3.(ii).A.(4) Excretion
3.(ii).A.(4).1) Excretion in urine, feces, and expired air (4.2.2.5-1 to 4.2.2.5-6)
Following a single oral dose of ^{14}C-labeled sirolimus (20 mg/kg) to male mice (n = 5/time point), the fecal and urinary excretion rate up to Day 7 after administration was 90.1% and 8.1%, respectively.

Following a single intravenous dose of ^{3}H-labeled sirolimus (1.1 mg/kg) to male rats (n = 5), the fecal and urinary excretion rate up to Day 7 after administration was 78.0% and 1.6%, respectively. Following a single oral dose of ^{3}H-labeled sirolimus (6.1 mg/kg) in a similar manner, the fecal and urinary excretion rate up to Day 7 after administration was 60.4% and 0.6%, respectively.

Following a single intravenous dose of ^{14}C-labeled sirolimus (0.5 mg/kg) to male rats (n = 6), the fecal and urinary excretion rate up to Day 14 after administration was 93.7% and 4.3%, respectively. Following a single oral dose of ^{14}C-labeled sirolimus (0.5 mg/kg) in a similar manner, the fecal and urinary excretion rate up to Day 14 after administration was 96.0% and 2.4%, respectively.

Following a single oral dose of ^{14}C-labeled sirolimus (5.0 mg/kg) to male rats (n = 2), the excretion rate in expired air up to 24 hours after administration was 0.015%, and the fecal and urinary excretion rate up to Day 7 after administration was 94.6% and 2.5%, respectively.

Following a single intravenous dose of ^{3}H-labeled sirolimus (0.85 mg/kg) to male cynomolgus monkeys (n = 4), the fecal and urinary excretion rate up to Day 19 after administration was 62.5% and 4.4%, respectively. Following a single oral dose of ^{3}H-labeled sirolimus (3.4 mg/kg) in a similar manner, the fecal and urinary excretion rate up to Day 7 after administration was 75.1% and 2.1%, respectively.

Following a 7-day repeated oral dose of ^{3}H-labeled sirolimus (7.2 mg/kg/day) to male cynomolgus monkeys (n = 4), the fecal and urinary excretion rate up to Day 37 after administration was 57.4% and 6.6%, respectively.

3.(ii).A.(4).2) Biliary excretion (4.2.2.5-7, 4.2.2.5-8)
Following a single intravenous dose of ^{3}H-labeled sirolimus (0.75 mg/kg) to bile duct-cannulated or non-cannulated male rats (n = 7/group), the biliary and fecal excretion rate in the cannulated rats up to 72 hours after administration was 45.1% and 6.0%, respectively, and the fecal excretion rate in the non-cannulated rats up to 72 hours was 58.3%.

Following a single oral dose of ^{14}C-labeled sirolimus (1 mg/kg) to bile duct-cannulated male rats (n = 8), the biliary and fecal excretion rate up to 72 hours after administration was 12.7% and 76.2%, respectively.

The above results showed that fecal excretion was mostly, but not totally, mediated by biliary excretion.

3.(ii).A.(4).3) Excretion in milk (4.2.2.5-9)
Following a single oral dose of ^{14}C-labled sirolimus (0.5 mg/kg) to lactating rats (n = 16) on day 10 postpartum, radioactivity concentrations in milk and the whole blood at 8 hours after administration were 7.9 and 4.2 ng eq./mL, respectively, showing that sirolimus was excreted into milk.

Following a single oral dose of ^{14}C-labeled sirolimus (0.5 mg/kg) to lactating rats (n = 9) on day 10 postpartum, radioactivity concentration in the whole blood of maternal animals at 24 hours after administration was 1.0 ng eq./mL, whereas radioactivity concentration in the whole blood of pups (n = 3/time point) on Day 10 after birth was below the lower limit of quantitation (0.30 ng eq./mL) at any time point, except in 1 pup at 4 hours after administration (0.7 ng eq./mL), suggesting that little or no sirolimus was transferred to pups via milk.

3.(ii).A.(5) Pharmacokinetic drug interactions
3.(ii).A.(5).1) Effect on drug-metabolizing enzymes in liver (4.2.2.6-1, 4.2.2.6-2)
Following a 7-day repeated intravenous dose of sirolimus (0, 0.025, 0.2, 1.5 mg/kg/day) to male rats (n = 4/group), total CYP concentration in liver microsomes was 0.8 to 1.2 nmol/mg protein, showing no significant difference between doses.

Following a 7-day repeated oral dose of sirolimus (0, 0.1, 0.5, 2.0 mg/kg/day) to male rats (n = 4/group), total CYP concentration in liver microsomes was 0.9 to 1.3 nmol/mg protein, showing no significant difference between doses, with no aminopyrine N-demethylase induced.

3.(ii).B Outline of the review by PMDA

3.(ii).B.(1) Tissue accumulation of sirolimus
In the single-dose studies in rats to investigate tissue distribution, many tissues showed higher radioactivity concentration and longer elimination half-life compared with the whole blood, but no repeat-dose study of tissue distribution was conducted. Therefore, PMDA asked the applicant to explain the safety in these tissues in long-term repeated administration of sirolimus, based on the results of toxicity studies, etc.

The applicant explained as follows:
In the oral-dose studies of tissue distribution in rats, the following tissues showed higher radioactivity concentration and longer elimination half-life than the whole blood: the adrenals, bone (femur), heart, kidneys, liver, lung, lymph nodes, pancreas, prostatic gland, skin, spleen, stomach, thymus, esophagus, fat (in the kidneys, abdomen, reproductive organs), large intestine, small intestine, salivary gland, thyroid gland, parathyroid gland, and urinary bladder.

In the 6- or 12-month repeat-dose oral toxicity study in rats, toxic findings were observed in the adrenals (cystic degeneration), bone (femur) (fracture, decreased bone mineral density and strength), heart (myocardial degeneration), kidneys (mineral deposition, hemosiderin deposition), liver (increased hematopoiesis), lungs (alveolar macrophage accumulation, hemosiderin deposition, perivasculitis), lymph nodes (atrophy, hemosiderin deposition), pancreas (vacuolar degeneration or atrophy of islet cells), prostatic gland (atrophy), skin (dermatitis), spleen (hemosiderin deposition, increased hematopoiesis), stomach (submucosal edema, acute gastritis), thymus (atrophy), and reproductive organ (atrophy or degeneration of seminiferous tubules, ovarian atrophy) [see "3.(iii) Summary of toxicology studies"]. Thus, all tissues examined, except testis, showed higher radioactivity concentration and longer elimination half-life than the whole blood in the tissue distribution studies in rats and a possible adverse impact on safety profiles cannot be excluded. Although distribution of radioactivity in the testis was not detected, the tissue showed the longest elimination half-life (351 hours) in the distribution study in rats following intravenous administration, which suggests that sirolimus is distributed only at a low level in the testis but remains for a long time period, and that the observed toxicity in the testis was partly caused by the long-term persistence of sirolimus in the tissue.

In the toxicity studies in rats and monkeys, in contrast, there were no new toxicity findings that occurred in a treatment duration-dependent manner. Events that occurred at an increasing frequency with increased treatment duration were effects on the intestinal tract such as diarrhoea, soft faeces, colitis, and cecitis and aggravated effects on lymphoid tissues such as atrophies of the spleen, thymus, and lymph nodes in monkeys; and, in rats, aggravated effects on the reproductive organs, bone, and pancreatic in addition to the above effects. However, given that most of the toxic findings in nonclinical studies are similar to those observed in other immunosuppressants, the applicant considered that they were changes secondary to the long-term immunosuppression.

In the combined data of 8 foreign clinical studies[6] in patients with organ transplant, the incidences of infectious diseases including respiratory tract infection and lower respiratory tract infection and cutaneous malignancies such as basal cell carcinoma, skin papilloma, and squamous cell carcinoma tended to gradually increase with time after the beginning of treatment, suggesting that the increased incidence was related to the long-term use of sirolimus. Other events, in contrast, occurred within 1 year after the beginning of treatment and did not show any tendency of increase in the incidence with long-term administration.

PMDA considers as follows:
In the tissue distribution study in rats, many tissues tended to show higher radioactivity concentration or longer elimination half-life (351 hours at the maximum) than the whole blood. In the repeat-dose toxicity study in rats, toxic findings were observed in many of these tissues at an exposure level lower than that achieved following the administration of the clinical dose to humans. These findings, together with the results of foreign clinical studies in patients with organ transplant, suggest that although the risk of adverse events is unlikely to substantially increase in a long-term administration associated with the accumulation of sirolimus, the incidence of infectious diseases and malignant tumor, diseases possibly associated with the

[6] Studies 217-US, 301-US, 302-GL, 309-GL, 310-GL, 313-GL, 316-GL, and 318-WW

immunosuppressive effect of sirolimus, tend to increase with long-term administration. Given the limited safety information in the long-term administration of sirolimus in clinical studies in patients with LAM, it is necessary to continue to closely investigate the safety of sirolimus in long-term administration in patients with LAM in the ongoing Study MLSTS, and the post-marketing surveillance, etc.

3.(iii) Summary of toxicology studies

3.(iii).A Summary of the submitted data

The following toxicology studies of sirolimus were conducted: single-dose toxicity, repeat-dose toxicity, genotoxicity, carcinogenicity, reproduction toxicity, and other toxicity (toxicity of impurities and degradation products, phototoxicity).

3.(iii).A.(1) Single-dose toxicity (4.2.3.1-1 to 4.2.3.1-4)

Oral and intravenous toxicity studies were conducted in mice and rats. The approximate lethal dose in mice was determined to be 500 mg/kg (male) or >800 mg/kg (female) in oral administration, and 250 mg/kg in intravenous administration. The approximate lethal dose in rats was determined to be >800 mg/kg (both males and females) in oral administration and 250 mg/kg (both males and females) in intravenous administration. Changes in clinical observations were eyelid ptosis, unkempt hair, and hypoactivity in mice receiving oral administration; and hypoactivity, eyelid ptosis, and excoriation and necrosis of the tail in mice receiving intravenous administration. Rats receiving oral administration did not show any sirolimus-related changes, whereas rats receiving intravenous administration showed quiescence, gait ataxia, tachypnea, hypoactivity, and discoloration (black) of the tail.

3.(iii).A.(2) Repeat-dose toxicity

Oral dose toxicity studies were conducted in rats (3, 6, 12 months) and in monkeys (3, 6 months). The main finding observed in both animal species was atrophy of lymphoid tissues caused by the immunosuppressive effect of sirolimus. Other findings observed were diabetes-like symptoms associated with vacuolization of pancreatic islet cells and atrophy of the reproductive organ in rats and colitis in monkeys. The no observed adverse effect level (NOAEL) in the 6-month repeat-dose oral toxicity study was determined to be 0.05 mg/kg/day both in rats and monkeys. Compared with the clinical dose (2 mg once daily) in humans,[7] the dose ratio was estimated to be 1.25 and the exposure ratio[8] was estimated to be <1 both for C_{max} and AUC. From the results of 3- and 6-month repeat-dose oral toxicity studies in monkeys, it was predicted that a longer-term toxicity study in monkeys would be difficult to continue because of the aggravation of clinical symptoms associated with colitis. In addition, the toxicity profile of sirolimus did not change by the prolonged treatment duration. Therefore, no repeat-dose toxicity study exceeding 6 months was conducted in monkeys.

3.(iii).A.(2).1) Three-month repeat-dose oral toxicity study in rats (4.2.3.2-3)

Sirolimus (0 [vehicle[9]], 0.5, 2, 5 mg/kg/day) was orally administered for 13 weeks to male and female Sprague Dawley (SD) rats. No death occurred. Sirolimus had no effects on clinical observations. Reduced body weight gain was observed in males in the ≥0.5 mg/kg/day groups and females in the 5 mg/kg/day group, while a tendency of increase in food consumption was observed in males in the ≥2 mg/kg/day groups. Ophthalmological examination showed cataract in males in the ≥2 mg/kg/day groups. Hematology showed increases in red blood cell count, hemoglobin, and hematocrit in females in the ≥0.5 mg/kg/day groups and in males in the ≥2

[7] 0.04 mg/kg/day by assuming human body weight to be 50 kg.

[8] The estimation was made by comparing C_{max} (4.5 ng/mL) and $AUC_{0-\infty}$ (120 ng·h/mL) in the single oral dose study (Study 186-UK) in healthy adult subjects receiving 2 triangular sirolimus 1 mg tablets with the exposure level in the 0.2 mg/kg/day group in the 12-month repeat-dose oral toxicity study in rats and with the exposure level in the 0.5 mg/kg/day group in the single oral dose toxicity study in monkeys.

[9] 2.5% dimethylacetamide solution

mg/kg/day groups. These findings are considered to be changes secondary to hemoconcentration associated with hyperglycemia. Increased relative neutrophil count and decreased relative lymphocyte count were observed in males in the ≥0.5 mg/kg/day groups and in females in the ≥2 mg/kg/day groups, and decreased platelet count was observed in the ≥2 mg/kg/day groups. These findings are considered to be changes related to immunosuppression or to accompanying secondary inflammation. Clinical chemistry showed decreases in total protein and albumin in the ≥0.5 mg/kg/day groups, increased glucose, decreases in uric acid and bilirubin in the ≥2 mg/kg/day groups, decreased globulin in the 5 mg/kg/day group, increased urea nitrogen, decreases in sodium, potassium, calcium, and chlorine in males of the ≥2 mg/kg/day groups, and increases in alanine aminotransferase (ALT), alkaline phosphatase, and creatinine, decreases in creatinine kinase and inorganic phosphorus in males of the 5 mg/kg/day group. These findings are considered to be changes suggestive of diabetes-like symptoms caused by hyperglycemia and were reversible or tended to be reversible after a recovery period. Decreased liver weight and decrease in the weight and size of testis were observed in males of the ≥2 mg/kg/day groups. The decreased testicular weight was not reversible even after a recovery period. Decreased uterine weight was observed in females of the ≥0.5 mg/kg/day groups. Histopathological examination showed submucosal oedema in the stomach in the ≥0.5 mg/kg/day groups and acute gastritis in the ≥2 mg/kg/day groups, both of which are considered to be due to the mucosa-irritating effect of sirolimus. Vacuolization of pancreatic islet cells and atrophy of seminiferous tubules accompanied by giant cells were observed in males in the ≥2 mg/kg/day groups, but these findings tended to be reversible after a recovery period. The above-mentioned diabetes-like symptoms such as hyperglycemia, increased food consumption accompanied by reduced body weight gain, and cataract are considered to be secondary changes caused by the vacuolization of pancreatic islet cells. Animals in the ≥0.5 mg/kg/day groups showed alveolar macrophage accumulation, myocardial degeneration, and mineral deposition in the kidneys supposedly due to decreased kidney function, with all of them being frequent and/or severe. They are considered to be sirolimus-induced aggravation of spontaneous lesions because similar findings were observed in the control group as well. The NOAEL was not determined.

3.(iii).A.(2).2) Six-month repeat-dose oral toxicity study in rats (4.2.3.2-4)

Sirolimus (0 [vehicle[10]], 0.05, 0.10, 0.50 mg/kg/day) was orally administered for 26 weeks to male and female SD rats. No death occurred. Sirolimus had no effect on clinical observations, food consumption, ophthalmological examination, or necropsy findings. Decreased body weight was observed in males of the 0.50 mg/kg/day group. Urinalysis detected glucose in males of the 0.50 mg/kg/day group. Hematology showed increased hemoglobin in males of the ≥0.50 mg/kg/day groups, increased red blood cell count in males of the ≥0.10 mg/kg/day groups, and increased fibrinogen in the 0.50 mg/kg/day group. These findings are considered to be secondary changes caused by hemoconcentration associated with hyperglycaemia. Clinical chemistry showed decreased triglycerides in males of the ≥0.05 mg/kg/day groups, increased ALT in males of the ≥0.10 mg/kg/day groups, and decreases in total protein and albumin, increased total cholesterol, and a tendency of increase in glucose in males of the 0.50 mg/kg/day group. All of these findings were reversible or tended to be reversible after a recovery period. Decreased kidney weight in males of the ≥0.05 mg/kg/day groups, decreased heart weight in males of the 0.50 mg/kg/day group, and decreased ovary weight and decreased uterine weight in females of the 0.50 mg/kg/day group were observed. None of these findings were related to histopathological findings, and they were reversible after a recovery period. Histopathology showed myocardial degeneration at a high frequency and/or in severe conditions in the ≥0.10 mg/kg/day groups. They are considered to be sirolimus-induced aggravation of spontaneous lesions because similar findings were observed in the control group as well. Based on the above, the NOAEL was determined to be 0.05 mg/kg/day.

[10] 0.2% dimethylacetamide solution

3.(iii).A.(2).3)Twelve-month repeat-dose oral toxicity study in rats (4.2.3.2-5 to 4.2.3.2-6)
Sirolimus (0 [vehicle[11]], 0.20, 0.65, 2.0, 6.0 mg/kg/day) was orally administered for 52 weeks to male and female SD rats. Death occurred in 17 of 250 animals, including 7 of 50 animals in the control group. Except for the death (caused by septic thrombus) in 1 of 25 males in the 0.65 mg/kg/day group, the death in other 16 animals was considered to be due to error in administration. All animals in the 6.0 mg/kg/day group were euthanized at 42 weeks of administration because of the aggravation of clinical symptoms including perioral ulcer, diarrhoea/soft feces, abdominal distension, salivation, and fracture. Gait disturbance supposedly due to decreased bone mass was observed in 15 of 250 animals, including 1 of 50 animals in the control group, and decreased body weight was observed in males of the ≥0.20 mg/kg/day groups and in females of the ≥2.0 mg/kg/day groups. Ophthalmological examination showed cataract in males of the ≥0.20 mg/kg/day groups and in females of the ≥2.0 mg/kg/day groups. Hematology showed increased red blood cell count in males of the ≥0.65 mg/kg/day groups and decreased platelet count in the 2.0 mg/kg/day group. Clinical chemistry showed increases in ALT and glucose and decreased chlorine in males of the ≥0.20 mg/kg/day groups, decreased potassium in females of the ≥0.20 mg/kg/day groups, decreases in sodium and potassium in males of the ≥0.65 mg/kg/day groups, increased triglycerides in females of the ≥0.65 mg/kg/day groups, and decreased calcium in the 2.0 mg/kg/day group. Hormone level measurement showed increased luteinizing hormone (LH) in females of the ≥0.65 mg/kg/day groups and decreased testosterone (TEST) and increased follicle-stimulating hormone (FSH) in males of the 2.0 mg/kg/day group. Bone morphometry showed decreased density and strength of the femur and decreased strength of the lumbar vertebrae in males of the ≥0.20 mg/kg/day groups, and shortening of the femur and decreased tibial bone mass in males of the ≥0.65 mg/kg/day groups. The decreased bone mass was considered to be caused by the decrease in estrogen due to the decreased TEST. Decreased liver and heart weight in males of the ≥0.20 mg/kg/day groups, increased adrenal weight in the 2.0 mg/kg/day group, and decreased ovarian weight in females of the same dose group were observed. Ocular opacity in males of the ≥0.20 mg/kg/day groups, discoloration of the lungs and hypertrophy of the adrenals in the ≥0.65 mg/kg/day groups, decrease in thymus size in the ≥2.0 mg/kg/day groups, and decrease in the size of testis, prostate gland, and seminal vesicle in males of the 6.0 mg/kg/day group were observed. Histopathological examination showed alveolar macrophage accumulation accompanied by phospholipidosis, perivasculitis, bronchiolitis/alveolitis, myocardial degeneration, lymph node atrophy, splenic hemosiderin deposition, and adrenal cystic degeneration in the ≥0.20 mg/kg/day groups; granuloma, cataract, pulmonary hemosiderin deposition, testicular interstitial cell hyperplasia, and prostate gland atrophy in males of the ≥0.20 mg/kg/day groups; lymph node hemosiderin deposition, hepatic and splenic hematopoiesis, and ovarian atrophy in females of the ≥0.20 mg/kg/day groups; renal hemosiderin deposition and pancreatic islet cell vacuolization in the ≥0.65 mg/kg/day groups; seminiferous tubule degeneration accompanied by gigantocellularis in males of the ≥0.65 mg/kg/day groups; thymic atrophy in the ≥2.0 mg/kg/day groups; and seminal vesicle atrophy in males of the ≥2.0 mg/kg/day groups. The NOAEL was not determined.

3.(iii).A.(2).4) Three-month repeat-dose oral toxicity study in monkeys (4.2.3.2-10)
Sirolimus (0 [vehicle[12]], 0.5, 5, 10 mg/kg/day) was orally administered for 13 weeks to male and female cynomolgus monkeys. Death occurred in 1 each of 3 females in the 5 and 10 mg/kg/day groups. One of 3 males in the 0.5 mg/kg/day group, 1 of 3 females in the 5 mg/kg/day group, and 1 of 3 each of males and females in the 10 mg/kg/day group were euthanized because of the aggravation of clinical observations caused by colitis-induced severe diarrhoea. The colitis was considered to be a secondary change caused by the enterotoxin of *E.coli* which became dominant in the intestine as a result of the immunosuppressive effect of sirolimus on intestinal bacterial flora. Diarrhoea/soft faeces were observed repeatedly in the ≥0.5 mg/kg/day groups and decreased

[11] 9.9% Phosal 50PG solution
[12] 8% dimethylacetamide solution

body weight or reduced body weight gain was observed in the ≥5 mg/kg/day groups. No effect was observed in ophthalmological examination, electrocardiogram, urinalysis, or organ weight. Hematology showed increased fibrinogen in females of the ≥0.5 mg/kg/day groups and in males of the 10 mg/kg/day group. Clinical chemistry showed increased creatine kinase in the ≥0.5 mg/kg/day groups and decreases in total protein and albumin in the ≥5 mg/kg/day groups. Histopathological examination showed colitis, cecitis, and atrophy of the spleen, thymus, and lymph nodes in the ≥0.5 mg/kg/day groups. The NOAEL was not determined.

3.(iii).A.(2).5) Six-month repeat-dose oral toxicity study in monkeys (4.2.3.2-11)

Sirolimus (0 [vehicle[13]], 0.05, 0.25, 0.50 mg/kg/day) was orally administered for 26 weeks to male and female cynomolgus monkeys. One of 6 each of males and females in the 0.50 mg/kg/day group was euthanized because of the aggravation of clinical symptoms due to chronic diarrhoea and soft faeces caused by colitis, decreased body weight, and decreased food consumption. Diarrhoea or soft faeces was observed in the ≥0.25 mg/kg/day groups. No effect of sirolimus was observed on body weight, food consumption, ophthalmological examination, electrocardiogram, clinical chemistry, urinalysis, organ weight, or necropsy. Hematology showed increased fibrinogen in the ≥0.25 mg/kg/day groups. The change was considered to be related to colitis. Histopathological examination showed colitis in the ≥0.25 mg/kg/day groups. Atrophy of thymus and lymph nodes were observed in the ≥0.25 mg/kg/day groups and atrophy of the spleen was observed in the 0.50 mg/kg/day group. These changes were considered to be known immunosuppressive effects. Based on the above, the NOAEL was determined to be 0.05 mg/kg/day.

3.(iii).A.(3) Genotoxicity (4.2.3.3.1-2 to 4.2.3.3.1-3, 4.2.3.3.2-1, 4.2.3.3.1-1)

Genotoxicity studies conducted include an *in vitro* bacterial reverse mutation assay (Ames test), mouse lymphoma tk assay, a chromosomal aberration assay with cultured mammalian cells, and *in vivo* mouse bone marrow micronucleus assay. As a result, the applicant considered that sirolimus has no genotoxic activity.

3.(iii).A.(4) Carcinogenicity

3.(iii).A.(4).1) Two-year repeat-dose oral carcinogenicity study in mice (4.2.3.4-2)

Sirolimus (0 [vehicle[14]], 1, 3, 6 mg/kg/day) was orally administered for 104 weeks to male and female CD-1 mice. Auricular erosion and ulcer were observed in the ≥3 mg/kg/day groups and, in the 6 mg/kg/day group, administration was terminated at Week 85 (males) or Week 97 (females) because of the progression of these skin lesions. The survival rate in the 6 mg/kg/day group was lower compared with that in the control group and other sirolimus groups. Decreased body weight was observed in the ≥1 mg/kg/day groups. Hematology showed increases in red blood cell count, hemoglobin, and hematocrit in females of the ≥1 mg/kg/day groups, and increases in reticulocyte count and neutrophil count, increasing tendency of monocyte count, and decreasing tendency of eosinophil count in males of the 6 mg/kg/day group. The changes in neutrophil, monocyte, and eosinophil counts were considered to be secondary to skin inflammation and/or infection. Neoplastic changes observed were hepatocellular adenoma and hepatocellular carcinoma in males of the ≥1 mg/kg/day groups and granulocytic leukemia in females of the ≥3 mg/kg/day groups. Lymphoma was observed in all treatment groups including the control group. Non-neoplastic changes observed were atrophy of the testis and uterus, atrophy of the thymus and lymph nodes which are considered to be known immunosuppressive effects, skin lesion supposedly caused by immunosuppression and bacterial infection, alveolar macrophage accumulation, brain and ocular inflammation, hepatic necrosis, and hyperplastic bone-marrow considered to be compensatory response to inflammation. The neoplastic lesions were not considered to be the results from the direct effect of sirolimus on DNA, based on the

[13] 1% dimethylacetamide solution
[14] 9.9% Phosal 50PG solution

lack of genotoxicity of sirolimus [see "3.(iii).A.(3) Genotoxicity"]. Hepatocellular adenoma and hepatocellular carcinoma were considered to be caused by cell division and repair associated with immunosuppression-related sepsis and localized necrosis of liver. Granulocytic leukemia and lymphoma were considered to be caused by the following process: inflammatory changes of immunosuppression-related skin and other organ stimulated the bone marrow hematopoiesis and lymphocyte production, which resulted in the enhancement of viral oncogene expression, leading to the increased occurrence of bone marrow tumor and lymphocytic tumor which are spontaneous lesions.

3.(iii).A.(4).2) Two-year repeat-dose oral carcinogenicity study in rats (4.2.3.4-4)

Sirolimus (0 [vehicle[15]], 0.05, 0.1, 0.2 mg/kg/day) was orally administered for 104 weeks to male and female CD rats. The survival rate was comparable between the sirolimus and control groups. Decreased body weight was observed in males of the ≥0.1 mg/kg/day groups. Gait disturbance, emaciation, and ocular opacity were observed in males of the ≥0.05 mg/kg/day groups. As a neoplastic change, testicular interstitial adenoma was observed in males of the ≥0.1 mg/kg/day groups. Non-neoplastic changes observed were atrophy of the spleen and cervical and mesenteric lymph nodes, and alveolar macrophage accumulation. In addition, cataract, hydronephrosis, urinary bladder inflammation, testicular interstitial cell hyperplasia were observed in males, and limb ulcer was observed in females. Testicular interstitial cell hyperplasia was considered to be a response to the change in the LH level associated with the decreased serum TEST level. Rats have approximately 14 times greater number of LH receptor than humans and are therefore highly sensitive to the change in LH level. Also, whereas testicular interstitial cells of rats have LH-releasing hormone (LHRH) receptor, those of humans do not (Prentice DE et al., *Human & Experimental Toxicology*. 1995;14:562-572, Hamada Y et al., *The Journal of Toxicological Sciences.* 1998;23:35-52). In rats, testicular interstitial adenoma is observed as a spontaneous lesion and the peak time of onset is widely different compared with humans. Based on these findings, the applicant considered that the neoplastic change observed in rats was not relevant to humans.

3.(iii).A.(5) Reproductive and developmental toxicity

Reproductive and developmental toxicity studies conducted include a study of fertility and early embryonic development to implantation in rats, studies for effects on embryo/fetal development in rats and rabbits, and a study for effects on pre- and postnatal development, including maternal function in rats.

3.(iii).A.(5).1) Study of fertility and early embryonic development to implantation (4.2.3.5.1-2)

Sirolimus (0 [vehicle[16]], 0.1, 0.5, 2 mg/kg/day) was orally administered to male SD rats from 11 weeks before mating until after mating with untreated female rats. No death occurred. Sirolimus had no effect on clinical symptoms, copulation, or fertility.[17] Decreased body weight and reduced body weight gain were observed in the ≥0.5 mg/kg/day groups and decreased food consumption was observed in the 2 mg/kg/day group. Decreased weight of testis, prostatic gland, seminal vesicle, and epididymis was observed in the ≥0.5 mg/kg/day groups. In addition, sirolimus (0 [vehicle], 0.05, 0.1, 0.5 mg/kg/day) was orally administered to female SD rats from 2 weeks before mating with untreated male rats until Gestation day 21. Neither death nor abortion occurred. Sirolimus had no effect on clinical symptoms, delivery, estrous cycle, days to copulation, copulation rate, pregnancy rate, or F_1 lactating behavior. Maternal animals showed prolonged pregnancy period in the ≥0.1 mg/kg/day groups and decreased food consumption, reduced body weight gain, and decreased pregnant uterine weight in the 0.5 mg/kg/day group. Examination of

[15] 9.9% Phosal 50PG solution
[16] 99% Phosal 50PG solution
[17] In the dose-finding study for fertility in rats (4.2.3.5.1-1), decrease in pregnancy rate was observed in the 4-week 2 and 5 mg/kg/day groups.

fetuses and F_1 pups showed decreased live fetal weight in the ≥0.1 mg/kg/day groups and decreased number of live fetuses, decreased number and body weight of surviving F_1 pups in the 0.5 mg/kg/day group, whereas sirolimus had no effect on sex ratio or physical development. Based on the above, the NOAEL was determined to be 0.1 mg/kg/day for general toxicity and reproductive function of parent animals and 0.05 mg/kg/day for embryos and fetuses.

3.(iii).A.(5).2) Embryo-fetal development

(a) Study of effects on embryo-fetal development in rats (4.2.3.5.2-2)

Sirolimus (0 [vehicle[18]], 0.1, 0.5, 1.0 mg/kg/day) was orally administered to pregnant SD rats from Gestation days 6 to 15. Neither death nor abortion occurred in maternal animals. Sirolimus had no effect on their clinical symptoms, behavior, or necropsy findings. Reduced body weight gain or tendency of reduced body weight gain, decreased pregnant uterine weight or tendency of decreased pregnant uterine weight in maternal animals, increased early resorption of embryos accompanied by decreased live fetuses or tendency of decreased live fetuses, and increased number of resorptions/dead fetuses were observed in the ≥0.5 mg/kg/day groups. Decreased weight of live fetuses, delayed vertebral ossification in fetuses, and increased vertebral variation were observed in the 1.0 mg/kg/day group. Based on the above, the NOAEL was determined to be 0.1 mg/kg/day both for general toxicity and reproductive function of maternal animals and for embryos and fetuses. The exposure ratio at the clinical dose in humans was 0.08 both for C_{max} and AUC.

(b) Study of effects on embryo-fetal development in rabbits (4.2.3.5.2-5)

Sirolimus (0 [vehicle[19]], 0.01, 0.025, 0.05 mg/kg/day) was orally administered to pregnant New Zealand White (NZW) rabbits from Gestation day 6 to 18. No death occurred in maternal animals. Sirolimus had no effect on clinical symptoms, behavior, pregnant uterine weight, or necropsy findings. Abortion was observed in 1 animal in the control group and in 2 each of animals in the 0.025 and 0.05 mg/kg/day groups, but the frequency was within the range of the variation of background data, from which the events were considered unrelated to sirolimus. Tendency of decreased body weight, tendency of reduced body weight gain, and decreased food consumption were observed in the 0.05 mg/kg/day group. Survival of fetuses, sex ratio, body weight, external surface, visceral organs, and skeleton was not affected. Based on the above, the NOAEL for general toxicity in maternal animals was determined to be 0.025 mg/kg/day, and the exposure ratio at the clinical dose in humans was 0.76 for C_{max} and 1.01 for AUC. The NOAEL in embryos and fetuses was determined to be 0.05 mg/kg/day, and the exposure ratio at the clinical dose in humans was 1.52 for C_{max} and 2.02 for AUC.

(c) Study of effects on pre- and postnatal development, including maternal function in rats (4.2.3.5.3-1)

Sirolimus (0 [vehicle[20]], 0.05, 0.1, 0.5 mg/kg/day) was orally administered to pregnant SD rats from Gestation day 6 to day 20 postpartum. Decreased food consumption and prolonged pregnancy period were observed in maternal animals of the 0.5 mg/kg/day group, while no effect was observed on clinical symptoms, necropsy findings, or nursing behavior. As regards pups, a decreased number of littermates and of surviving F_1 pups was observed in the 0.5 mg/kg/day group, while no effect was observed on sex ratio, body weight, reflex/physical development, righting reflex, sexual maturation, learning/memory function, or reproductive function. Based on the above, the NOAEL was determined to be 0.1 mg/kg/day for general toxicity and reproductive function of maternal animals as well as for F_1 pups.

[18] Solution containing 0.2% dimethylacetamide and 0.75% Phosal 50PG
[19] Solution containing 0.2% dimethylacetamide and 0.75% Phosal 50PG
[20] 99% Phosal 50PG solution

3.(iii).A.(6) Other toxicity studies

Repeat-dose toxicity studies were conducted on Impurity A, an impurity derived from the drug substance manufacturing process with the highest specification limit ([redacted]%), and Degradation Product A, a degradation product formed during the drug product-manufacturing process with the specification limit ([redacted]%) exceeding the qualification threshold. Also, a phototoxicity study of sirolimus was conducted in rabbits. Furthermore, since effects of sirolimus on the testis and bone as well as on alveolar macrophage accumulation were observed in oral toxicity studies in rats, a repeat-dose toxicity study was conducted to obtain more detailed information on the pertinent findings and investigate the reversibility of the findings.

3.(iii).A.(6).1) Twenty eight-day repeat-dose oral toxicity study of Impurity A in rats (4.2.3.7-1)

Sirolimus containing Impurity A at 0%, 3%, or 10% by weight was orally administered at the dose of 0.1, 1, or 5 mg/kg/day to male SD rats for 28 to 30 days. Presence of the impurity neither aggravated toxicity findings nor caused any new ones. In this study, the maximum dose of Impurity A was [redacted] µg//kg/day, and the dose ratio relative to the maximum dose of Impurity A ([redacted] µg/kg/day) at the clinical dose in humans was 576. Based on the above, the applicant determined that Impurity A does not pose any particular safety problem.

3.(iii).A.(6).2) Twenty eight-day repeat-dose oral toxicity study of Degradation Product A in rats (4.2.3.7-3)

Sirolimus containing Degradation Product A at 0%, 5%, or 12% by weight was orally administered at the dose of 0.1, 1, or 5 mg/kg/day to male SD rats for 28 to 30 days. Animals treated with 5 mg/kg/day of sirolimus containing Degradation Product A at 12% showed an increased frequency of cataract and a decreased frequency of callus formation during fracture healing. However, there were no other findings suggestive of interactions between the test drug and Degradation Product A, therefore, the applicant considered that the above findings were incidental changes. Presence of the degradation product neither aggravated the toxic findings nor caused any new toxicity findings. In this study, the maximum dose of Degradation Product A was [redacted] µg/kg/day, and the dose ratio to the maximum dose of Degradation Product A ([redacted] µg/kg/day) at the clinical dose in humans was 678. Based on the above, the applicant determined that Degradation Product A does not pose any particular safety problem.

3.(iii).A.(6).3) Phototoxicity study in rabbits (4.2.3.7-12)

Sirolimus (0 [vehicle[21]], 25 mg/kg) was orally administered in a single dose to male and female NZW rabbits. No skin reaction was observed at the light-irradiated site, therefore, the applicant determined that sirolimus does not have phototoxicity.

3.(iii).A.(6).4) Study on the reversibility of the effects of sirolimus on testis and bone (4.2.3.7-5)

Sirolimus (0 [vehicle[22]], 2, 6 mg/kg/day) was orally administered for 13 weeks to male SD rats. Atrophy of seminiferous tubules and decreased sperm count were observed in the 6 mg/kg/day group. These findings showed a tendency of reversibility with the progress of the recovery period to 1, 3, and 6 months, but were not completely reversible. Decreased bone mass was observed in the ≥2 mg/kg/day groups, and the thickness of the cortical bone was reversible after the recovery period of 1, 3, and 6 months, but the decreased bone mass was not completely reversible.

[21] Phosal 50PG solution
[22] 99.0% Phosal 50PG solution

3.(iii).A.(6).5) Study on the effect of sirolimus on alveolar macrophage accumulation (4.2.3.7-11)

Sirolimus (0 [vehicle[23]] 6 mg/kg/day) was orally administered for 13 weeks to male SD rats. Sirolimus and increased phospholipid content were observed in alveolar macrophages in the 6 mg/kg/day group. Electron microscopy confirmed a large amount of lamellar bodies, a characteristic feature of phospholipidosis, therefore, the alveolar macrophage accumulation was considered to be due to phospholipidosis. The contents of sirolimus and of lipids decreased after a 4-week withdrawal period. The applicant explained that since there is little or no report of phospholipid accumulation in humans, phospholipidosis in animals is unlikely to be relevant to humans (Reasor M.J, Cationic amphiphilic drugs, *Comprehensive Toxicology*, I.G. Sipes C.A. McQueen, and A.J. Gandolfi, Editors. Elsevier Science, Inc.: New York. 1997;555-566). The applicant also explained, as an opposite example, that although interstitial lung disease is known as an adverse drug reaction of mTOR inhibitors, fibrotic lesion of intralobular septum or subpleural tissue, the histopathological findings characteristic to interstitial lung disease, is not observed in rats.

3.(iii).B Outline of the review by PMDA

3.(iii).B.(1) Effects on reproductive hormones and bone

The 12-month repeat-dose oral toxicity study in rats showed fracture and decreased bone mass supposedly caused by decreased estrogen associated with decreased TEST, and accompanying gait disturbance. The results of the safety pharmacology study also suggested the effect of sirolimus on bone metabolism parameters in rats [see "3.(i) Summary of pharmacology studies"]. Taking account of these findings, PMDA asked the applicant to explain the possibility of adverse events related to reproductive hormones and bone in the long-term administration of sirolimus at the clinical dose.

The applicant explained as follows:

In the safety pharmacology study, animals in the sirolimus groups showed increased bone calcification rate and decreased remodeling period, but did not show any decrease in the percentage of trabeculae. On the other hand, cyclosporine is known to decrease the bone mass (Goodman GR et al., *J Bone Miner Res*. 2001;16:72-78), and decreased percentage of trabeculae was observed in the cyclosporine and tacrolimus groups in safety pharmacology studies. This suggests that sirolimus has a smaller effect on bone than other immunosuppressants. However, considering decreased bone mineral density and decreased strength in male rats in the 12-month repeat-dose oral toxicity study in rats, supposedly caused by sirolimus-induced decrease in serum TEST, reproductive hormones and bone mineral content were measured in the investigator-initiated clinical trial (Study MLSTS) in Japanese patients with LAM. The results did not show any significant change from baseline in blood estrogen, blood progesterone, blood testosterone, or bone mineral content at Week 52 of administration (mean ± SD [number of subjects]: blood estrogen, -1.9 ± 97.2 [n = 49]; blood progesterone, -0.93 ± 4.93 [n = 49]; blood testosterone, -0.04 ± 0.74 [n = 48], bone mineral content, 0.01 ± 0.04 [n = 49]). Also, in the combined data of 8 foreign clinical studies[24] in patients with organ transplant, the most common adverse event related to reproductive hormones was blood testosterone decreased, but the incidence was not significantly different between the sirolimus group (0.8% [25 of 3272 patients]) and the placebo group (0.7% [2 of 284 patients]), and the incidences of other adverse events were all ≤0.1%. Similarly, the incidence of bone metabolism-related adverse events[25] was comparable between the sirolimus group (13.5% [441 of 3272 patients]) and the placebo group (12.3% [35 of 284 patients]).

[23] 9.9% Phosal 50PG solution
[24] Studies 217-US, 301-US, 302-GL, 309-GL, 310-GL, 313-GL, 316-GL, and 318-WW
[25] Osteoporosis, osteopenia, bone density decreased, fracture (all sites), skeletal injury, cartilage injury, and bone fissure

Based on the above, the applicant considers that although the effect of sirolimus on bone cannot be excluded given the results of nonclinical studies, long-term administration of sirolimus to patients with LAM is unlikely to have an effect on hormones or bone, taking account of the results of clinical studies.

PMDA considers as follows:
Although the relationship between sirolimus and reproductive hormone-related or bone metabolism-related adverse events is unclear, there is a concern that sirolimus may affect reproductive hormones and bone metabolism in patients with LAM, considering the following: (i) in the 12-month repeat-dose oral toxicity study in rats, the above adverse events are estimated to have occurred at an exposure level lower than that in humans; (ii) although the incidences of reproductive hormone-related adverse events and bone metabolism-related adverse events were similar between the sirolimus group and the placebo group in clinical studies in organ transplant patients, the guidelines of the European Respiratory Society (Johnson SR et al., *Eur Respir J.* 2010;35:14-26) state that bone mineral density decreases in patients with LAM. Because of the concern and limited experience in the long-term use of sirolimus, it is necessary to further investigate the effect of the long-term use of sirolimus on reproductive hormones and bone metabolism in the ongoing Study MLSTS and the post-marketing surveillance. In addition, toxicity studies in rats showed testicular toxicity supposedly caused by decreased TEST at an exposure level below that reached at the clinical dose in humans, and no reversibility was observed after a recovery period. Although LAM occurs mostly in women, it occurs in men as well, albeit rarely. Therefore, the package insert should include precautionary statements regarding testicular toxicity.

PMDA also considers as follows:
Repeat-dose toxicity studies showed, in addition to reproductive hormone- and bone-related findings described above, effects on lymphoid tissues, intestinal tract, and reproductive organs as well as findings related to infection and tumor possibly caused by the immunosuppressive effect of sirolimus, all occurring at an exposure level below that reached by the clinical dose in humans, suggesting that adverse events related to the above findings may occur in the clinical use. Therefore, the possible risk of these adverse events in administration of sirolimus should be carefully considered taking account of the results of clinical studies [see "4.(iii) Summary of clinical efficacy and safety"].

3.(iii).B.(2) Reproductive toxicity
PMDA considers that sirolimus should be contraindicated in pregnant women or in women who may be pregnant, as proposed by the applicant, because embryofetal toxicity (decreased fetal weight, fetal death) was observed in rats, although no teratogenicity was observed in the reproductive and developmental toxicity studies in rats and rabbits. Also, since LAM occurs often in women of child-bearing age, the clinical benefit should be carefully weighed against potential risks and the appropriateness of sirolimus therapy should be determined carefully before using sirolimus in these patients. Also, the package insert should include precautionary statements to the effect that the risk in fetuses/embryos should be thoroughly communicated to patients and that patients should take contraceptive measures during treatment with sirolimus.

4. Clinical data

4.(i) Summary of biopharmaceutic studies and associated analytical methods

4.(i).A Summary of the submitted data

The results of bioequivalence studies (Studies 165-US [5.3.1.2-1], 187-UK [5.3.1.2-2]) and of a food effect study (Study 172-US [5.3.3.1-3]), which were conducted in non-Japanese healthy adult subjects, were submitted as reference data. Pharmacokinetic studies were conducted using 1-, 2-, and 5-mg triangular tablets, 1- 2-, and 5-mg oval tablets, 1 and 5 mg/mL liquid preparations,

freeze-dried tablets, and radiolabeled sirolimus (^{14}C- and ^{3}H-labeled sirolimus).[26] Concentrations of sirolimus and metabolites in blood were measured by LC/MS/MS (lower limit of quantitation, 0.1 or 1 ng/mL), LC/MS, or HPLC (lower limit of quantitation, 7.6 ng/mL), and radioactivity was counted in a liquid scintillation counter.

Pharmacokinetic parameters are expressed as mean ± SD unless otherwise specified.

4.(i).A.(1) Bioequivalence study of oval tablets and liquid preparation in non-Japanese healthy adult subjects (5.3.1.2-1, Study 165-US [to 19])

The bioequivalence between oval tablets and a liquid preparation of sirolimus was investigated in a randomized, open-label, three-treatment, three-period cross-over study in non-Japanese healthy adult subjects (n = 23). Table 6 shows the pharmacokinetic parameters of sirolimus in blood following a single oral dose of oval 1- or 2-mg tablets (6 mg each in total) or 1 mg/mL liquid preparation (6 mg in total). The geometric least squares mean ratio [90% confidence interval (CI)] of C_{max} and AUC_{0-t} were 0.65 [0.56, 0.75] and 1.29 [1.14, 1.46], respectively, for 1-mg oval tablets versus 1 mg/mL liquid preparation, and 0.49 [0.43, 0.57] and 1.19 [1.05, 1.34], respectively, for 2-mg oval tablets versus 1 mg/mL liquid preparation. C_{max} decreased, whereas AUC_{0-t} increased, in 1- and 2-mg oval tablets compared with 1 mg/mL liquid preparation.

Table 6. Pharmacokinetic parameters following a single oral dose of oval tablets or liquid preparation to non-Japanese healthy adult subjects

	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-t} (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)	$t_{1/2}$ (h)	CL/F (L/h/kg)	V_{ss} (L/kg)
1-mg oval tablet × 6	15.5 ± 5.8	2.4 ± 1.7	327.2 ± 96.7	390.7 ± 113.4	67.4 ± 8.9	0.38 ± 0.16	27.2 ± 10.9
2-mg oval tablet × 3	11.5 ± 3.4	3.4 ± 1.7	301.9 ± 101.6	368.1 ± 119.6	70.0 ± 14.7	0.40 ± 0.15	31.6 ± 11.3
1 mg/mL liquid preparation × 6 mL	23.5 ± 7.6	1.1 ± 0.3	254.9 ± 87.6	311.6 ± 114.5	75.8 ± 19.0	0.48 ± 0.17	34.7 ± 12.1

Mean ± SD, n = 23

C_{max}: Maximum concentration, t_{max}: Time to maximum concentration, AUC: Area under blood concentration-time curve, $t_{1/2}$ Elimination half-life, CL/F: Clearance, V_{ss}: Distribution volume under steady state

4.(i).A.(2) Bioequivalence study of triangular tablets in non-Japanese healthy adult subjects (5.3.1.2-2, Study 187-UK [to 20])

The bioequivalence among triangular sirolimus tablets with different strengths was investigated by a randomized, open-label, three-treatment, three-period cross-over study in non-Japanese healthy adult subjects (n = 22). Table 7 shows the pharmacokinetic parameters of sirolimus in blood following a single oral dose of 1-, 2-, or 5-mg triangular tablets (10 mg each in total). The geometric least squares mean ratio [90% CI] of C_{max} and AUC_{0-t} were 0.93 [0.84, 1.02] and 1.05 [0.98, 1.12], respectively, for 2-mg triangular tablets versus 1-mg triangular tablets, and 0.88 [0.80, 0.96] and 1.13 [1.06, 1.20], respectively, for 5-mg triangular tablets versus 1-mg triangular tablets, showing similar C_{max} and AUC_{0-t} among different tablets.

Table 7. Pharmacokinetic parameters following a single oral dose of triangular tablets to non-Japanese healthy adult subjects

	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-t} (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)	$t_{1/2}$ (h)	CL/F (L/h/kg)	V_{ss} (L/kg)
1-mg triangular tablet × 10	23.6 ± 6.6	2.6 ± 1.8	629.3 ± 143.7	764.7 ± 191.1	66.6 ± 12.1	0.18 ± 0.04	16.7 ± 4.3
2-mg triangular tablet × 5	22.4 ± 7.4	2.8 ± 2.7	661.3 ± 174.8	791.6 ± 212.3	63.5 ± 9.1	0.17 ± 0.04	15.7 ± 5.0
5-mg triangular tablet × 2	20.8 ± 6.0	4.1 ± 2.8	711.3 ± 185.0	866.4 ± 241.2	65.7 ± 11.6	0.16 ± 0.04	14.6 ± 3.9

Mean ± SD, n = 22

C_{max}: Maximum concentration, t_{max}: Time to maximum concentration, AUC: Area under blood concentration-time curve, $t_{1/2}$: Elimination half-life, CL/F: Clearance, V_{ss}: Distribution volume under steady state

[26] Triangular 1-mg tablets are the product submitted for approval.

4.(i).A.(3) Food effect study (5.3.3.1-3, Study 172-US [[redacted] to [redacted] 19[redacted]])

The effect of meals (high fat diet) on the pharmacokinetics of sirolimus was investigated in a randomized, open-label, two-period cross-over study in non-Japanese healthy adult subjects (n = 24) in which 10 oval 1-mg tablets (10 mg in total) were orally administered at a single dose. The geometric least squares mean ratio [90% CI] of pharmacokinetic parameters of sirolimus in blood under fed conditions versus under fasted conditions was 1.65 [1.50, 1.82] for C_{max}, 1.23 [1.14, 1.33] for $AUC_{0-\infty}$, and 1.32 [1.04, 1.66] for t_{max}, showing increase in the exposure of sirolimus after a high fat diet.

4.(i).B Outline of the review by PMDA

Study 172-US showed that C_{max} and $AUC_{0-\infty}$ of sirolimus were increased by food intake. Therefore, PMDA considers it important to minimize intra-individual variation of blood sirolimus concentration caused by food intake, thereby to maintain stable blood concentration. Also, taking account of the fact that the efficacy of sirolimus was demonstrated in Study MILES, the confirmatory study of sirolimus, in which sirolimus was administered under the defined condition, either fasted or fed, [see "4.(iii) Summary of clinical efficacy and safety"], precautionary statements should be included in the Precautions for dosage and administration section of the package insert requiring administration under the defined condition, either fasted or fed, in accordance with the condition employed in Study MILES.

4.(ii) Summary of clinical pharmacology studies

4.(ii).A Summary of the submitted data

The results of an investigator-initiated clinical trial (Study MLSTS [5.3.5.2-2]) in Japanese patients with LAM were submitted as the evaluation data. The results of the following studies were submitted as reference data: a clinical study in Japanese and non-Japanese patients with LAM (Study MILES [5.4.3-2]), a pooled analysis (RPT-42893 [5.3.3.1-5]), studies in human biomaterials (4.2.2.3-4, 4.2.2.4-7 to 4.2.2.4-8, 4.2.2.6-3, 5.3.2.3-1, 5.3.2.3-3), a study on metabolites in non-Japanese healthy adult subjects (Study 129-US [5.3.2.2-3]), single oral dose studies (Study 166-EU [5.3.3.1-1], Study 186-UK [5.3.3.1-2]), a mass balance study (Study 129-US [5.3.3.1-4]), a study in patients with hepatic impairment (5.4.2-10 to 5.4.2-11), and studies on drug interactions (Study 135-EU [5.3.3.1-6], Study 183-US [5.3.3.1-7], Study 182-US [5.3.3.1-8], Study 136-US [5.3.3.1-9], Study 156-US [5.3.3.1-10], Study 168-US [5.3.3.1-11]).

Observed values and pharmacokinetic parameters are expressed in mean ± SD unless otherwise specified.

4.(ii).A.(1) Studies in human biomaterials

4.(ii).A.(1).1) Distribution in blood cells and plasma protein binding (4.2.2.3-4, 5.3.2.3-1, 5.3.2.3-3)

Following the addition of ^{3}H-labeled sirolimus (5-100 ng/mL) to the human whole blood samples, 94.5% ± 4.9% of sirolimus was distributed in red blood cells, 3.1% ± 2.5% in plasma, 1.0% ± 1.0% in lymphocytes, and 1.0% ± 0.9% in granulocytes. In the plasma, approximately 60% of sirolimus was bound to protein, 20.5% ± 5.9% to LDL, 19.5% ± 3.9% to HDL, and 1.2% ± 0.5% to VLDL, with 2.5% ± 0.2% remaining in unbounded form. The whole blood/plasma concentration ratio was 11.1. Similarly, following the addition of ^{14}C-labeled sirolimus (10-100 ng/mL) to human whole blood samples, the whole blood/plasma concentration ratio was 9.3 to 13.6.

After sirolimus (59-482 ng/mL) was added to human whole blood samples and the mixture was incubated at 0°C or 37°C, the whole blood/plasma concentration ratio was 7.1 to 15.9 at sirolimus concentration of up to 189 ng/mL and 1.1 to 4.0 at sirolimus concentration exceeding 189 ng/mL.

4.(ii).A.(1).2) Metabolism by human liver microsomes (4.2.2.4-7, 4.2.2.4-8, 4.2.2.6-3)
Using the probe substrates of CYP isoforms (CYP1A2, CYP2A6, CYP3A4, CYP2C18, CYP2C9/10, CYP2D6, CYP2E, CYP4A), CYP isoforms responsible for the metabolism of sirolimus in human liver microsomes were investigated. The results suggested that sirolimus was metabolized by CYP3A4.

^{14}C-labeled sirolimus (50 μM) and a CYP3A inhibitor ketoconazole, cyclosporine, nicardipine, and methylprednisolone were added separately to human liver microsomes, and the mixture was incubated in the presence of NADPH. As a result, ketoconazole, cyclosporine, and nicardipine inhibited the metabolism of sirolimus.

Sirolimus and CYP3A4 inhibitor triacetyl oleandomycin, gestodene, and anti-CYP3A4 antibody were added separately to human liver microsomes, and the mixture was incubated in the presence of NADPH. As a result, triacetyl oleandomycin and gestodene inhibited the formation of sirolimus metabolites 41-O-demethyl sirolimus and hydroxy sirolimus, and anti-CYP3A4 antibody inhibited the formation of 41-O-demethyl sirolimus (Sattler M et al., *Drug Metab Dispos.* 1992;20:753-761).

Sirolimus (50 μM) was added to human liver microsomes, and the mixture was incubated in the presence of NADPH. As a result, sirolimus was metabolized both in a CYP-dependent and independent manner. Comparison of metabolites generated by nonenzymatic degradation with those formed by rat liver microsomes showed that all metabolites generated by rat liver microsomes, i.e., hydroxy sirolimus (A, B, D, F, G), 7-O-demethyl sirolimus (C), seco-sirolimus (E), and 41-O-demethyl sirolimus (H), were detected.

These results suggested that sirolimus was metabolized in human liver microsomes mainly by CYP3A4.

4.(ii).A.(2) Studies in healthy adult subjects
4.(ii).A.(2).1) Single dose oral administration study of oval tablets in non-Japanese healthy adult subjects (5.3.3.1-1, Study 166-EU [[redacted] to [redacted], 19[redacted]])
The pharmacokinetics of sirolimus following a single oral dose of oval sirolimus tablets was investigated in a randomized, open-label, four-treatment, two-period cross-over study in non-Japanese healthy adult subjects (n = 18). Table 8 shows pharmacokinetic parameters of sirolimus in the whole blood following a single oral dose of 1 (5 mg), 2 (10 mg), 4 (20 mg), and 8 (40 mg) oval 5-mg sirolimus tablets.

Table 8. Pharmacokinetic parameters following a single oral dose of oval tablets to non-Japanese healthy adult subjects

Dose	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0\text{-}t}$ (ng·h/mL)	$AUC_{0\text{-}\infty}$ (ng·h/mL)	$t_{1/2}$ (h)	CL/F (L/h/kg)	V_{ss}/F (L/kg)
5 mg (5-mg oval tablet × 1)	6.2 ± 2.7	4.7 ± 2.9	293 ± 102	317 ± 105	91.0 ± 10.0	0.23 ± 0.08	24.0 ± 9.3
10 mg (5-mg oval tablet × 2)	11.0 ± 4.3	4.3 ± 3.4	547 ± 229	582 ± 240	86.3 ± 8.1	0.26 ± 0.09	24.6 ± 8.6
20 mg (5-mg oval tablet × 4)	18.9 ± 4.7	8.1 ± 7.0	1039 ± 211	1098 ± 228	83.2 ± 10.6	0.25 ± 0.07	22.6 ± 5.7
40 mg (5-mg oval tablet × 8)	35.5 ± 10.2	8.1 ± 5.7	2024 ± 491	2117 ± 505	78.8 ± 12.0	0.27 ± 0.07	22.7 ± 6.9

Mean ± SD, n = 18
C_{max}: Maximum concentration, t_{max}: Time to maximum concentration, AUC: Area under blood concentration-time curve, $t_{1/2}$: Elimination half-life, CL/F: Clearance, V_{ss}/F: Distribution volume under steady state

4.(ii).A.(2).2) Single oral dose study of triangular tablets in non-Japanese healthy adult subjects (5.3.3.1-2, Study 186-UK [[redacted] 20[redacted] to [redacted] 20[redacted]])

The pharmacokinetics of sirolimus following a single oral dose of triangular sirolimus tablets was investigated in a randomized, open-label, three-treatment, three-period cross-over study in non-Japanese healthy adult subjects (n = 27). Table 9 shows pharmacokinetic parameters of sirolimus in the whole blood following a single oral dose of 2 triangular 1-mg sirolimus tablets (2 mg), 2 triangular 2-mg tablets (4 mg), and 5 triangular 1-mg tablets (5 mg).

Table 9. Pharmacokinetic parameters following single oral dose of triangular tablets to non-Japanese healthy adult subjects

Dose	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-t} (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)	$t_{1/2}$ (h)	CL/F (L/h/kg)	V_{ss}/F (L/kg)
2 mg (1-mg triangular tablet × 2)	4.5 ± 1.0	1.9 ± 0.5	97 ± 23	120 ± 29	70.0 ± 15.5	0.24 ± 0.07	19.1 ± 6.2
4 mg (2-mg triangular tablet × 2)	8.2 ± 2.0	2.2 ± 0.6	201 ± 63	245 ± 79	66.9 ± 8.2	0.24 ± 0.08	18.4 ± 5.8
5 mg (1-mg triangular tablet × 5)	10.7 ± 2.6	2.0 ± 1.5	252 ± 68	306 ± 88	65.9 ± 9.9	0.24 ± 0.08	17.8 ± 5.1

Mean ± SD, n = 27

C_{max}: Maximum concentration, t_{max}: Time to maximum concentration, AUC: Area under blood concentration-time curve, $t_{1/2}$: Elimination half-life, CL/F: Clearance, V_{ss}/F: Distribution volume under steady state

4.(ii).A.(2).3) Mass balance study (5.3.3.1-4, Study 129-US [[redacted] to [redacted] 19[redacted]])

Mass balance and excretion were investigated in an open label study in non-Japanese healthy adult subjects (n = 6). Following a single oral dose of ^{14}C-labeled sirolimus solution (42 mg), fecal and urinary excretion rate up to Day 15 after administration was 91.0% ± 8.0% and 2.2% ± 0.9%, respectively.

4.(ii).A.(3) Studies in patients

4.(ii).A.(3).1) Clinical study (5.4.3-2, Study MILES [December 2006 to September 2010])

The pharmacokinetics of sirolimus following multiple oral dose of triangular sirolimus tablets (sirolimus) was investigated in a randomized, double-blind, placebo-controlled, parallel group comparative study in Japanese and non-Japanese patients with LAM (46 patients in total). Treatment was started with once daily oral administration[27] of 2 mg of sirolimus and, based on the measurement of trough whole blood sirolimus concentrations at 3 weeks and at 3, 6, 9, and 12 months after the beginning of treatment, the dose was to be adjusted so that the trough concentration of sirolimus was maintained within the range from 5 to 15 ng/mL. Table 10 shows the administration conditions during the 12-month treatment period, and Table 11 shows the time-course of trough whole blood sirolimus concentrations in 13 Japanese patients with LAM and 33 non-Japanese patients with LAM from 3 weeks to 12 months of treatment. The trough whole blood sirolimus concentration at 3 weeks of treatment was within the target range (5-15 ng/mL) in 52% (24 of 46 patients), and the mean dose ranged from 1.8 to 2.0 mg and the maximum dose up to 12 months of treatment was 4 mg.

[27] Sirolimus was to be administered either under fasting or under fed conditions, but the same administration method should be used throughout the study. Morning administration was recommended.

Table 10. Status of treatment

	Sirolimus group
All treated patients	46
Dose maintained at 2-mg[a)]	24 (52)
Dose increased (increased at least once during the treatment period)	14 (30)
Dose increased to 3 mg	12 (26)
Dose increased to 4 mg	2 (4)
Dose decreased to 1 mg (decreased the dose at least once during the treatment period)	10 (22)
Discontinuation/suspension	7 (15)
Other (e.g., dose not confirmed, irregular administration)	3 (7)

Number of patients (%)

a) 6 of them discontinued the treatment prematurely or suspended the treatment for the long-term.

Table 11. Distribution of trough whole blood sirolimus concentrations after multiple oral doses of sirolimus to Japanese and non-Japanese patients

		Dose (mg/day)	<5 ng/mL	≥5 ng/mL and <10 ng/mL	≥10 ng/mL and <15 ng/mL	≥15 ng/mL
Japanese patients (n = 13)	After 3 weeks	2.0 (2-2)	1 (9.1)	9 (81.8)	0	1 (9.1)
	After 3 months	2.0 (1-3)	1 (7.7)	10 (76.9)	2 (15.4)	0
	After 6 months	2.0 (1-3)	5 (38.5)	6 (46.2)	1 (7.7)	1 (7.7)
	After 9 months	1.8 (0-3)	2 (15.4)	9 (69.2)	2 (15.4)	0
	After 12 months	1.8 (0-3)	1 (7.7)	10 (76.9)	1 (7.7)	1 (7.7)
Non-Japanese patients (n = 33)	After 3 weeks	1.9 (0-2)	10 (38.5)	11 (42.3)	4 (15.4)	1 (3.8)
	After 3 months	1.9 (0-3)	10 (37.0)	13 (48.1)	4 (14.8)	0
	After 6 months	2.0 (0-4)	6 (27.3)	11 (50.0)	2 (9.1)	3 (13.6)
	After 9 months	1.9 (0-4)	6 (24.0)	14 (56.0)	3 (12.0)	2 (8.0)
	After 12 months	1.8 (0-4)	6 (25.0)	15 (62.5)	3 (12.5)	0

Number of patients (%), Dose is expressed in mean (range).

4.(ii).A.(3).2) Investigator-initiated clinical trial (Evaluation data 5.3.5.2-2, Study MLSTS [August 2012 to ongoing (cut-off date [redacted], 2013)])

The pharmacokinetics of sirolimus was investigated following the multiple oral doses of sirolimus in an open-label, uncontrolled study in Japanese patients with LAM (n = 63). Treatment was started with once daily oral administration of 2 mg sirolimus after breakfast and, based on the measurement of trough whole blood sirolimus concentrations at 1 and 3 weeks and at 3, 6, 9, 12, 15, 18, 21, and 24 months after the beginning of treatment, the dose was to be adjusted so that the trough concentration of sirolimus was maintained within the range from 5 to 15 ng/mL. Table 12 shows the administration conditions during the 12-month treatment period, and Table 13 shows the time-course of trough whole blood sirolimus concentrations from 1 week to 12 months of treatment. The trough whole blood sirolimus concentration at 1 week of treatment was within the target range (5-15 ng/mL) in 65% (41of 63 patients), and the mean dose ranged from 1.9 to 2.2 mg and the maximum dose up to 12 months of treatment was 4 mg.

Table 12. Status of treatment

All treated patients	63
Dose maintained at 2-mg	11 (17)
Dose increased	28 (44)
Dose increased to 3 mg	25 (40)
Dose increased to 4 mg	2 (3)
Dose increased, and decreased or treatment suspended due to adverse events	19 (30)
Dose decreased to 1 mg	13 (21)
Dose decreased due to adverse events	12 (19)
Treatment suspended due to adverse events	40 (63)

Number of patients (%)

Table 13. Distribution of trough whole blood sirolimus concentration after multiple oral doses of sirolimus to Japanese patients

	Dose (mg/day)	<5 ng/mL	≥5 ng/mL and <10 ng/mL	≥10 ng/mL and <15 ng/mL	≥15 ng/mL
After 1 week	1.9 (0-2)	22 (34.9)	35 (55.6)	6 (9.5)	0
After 3 weeks	2.0 (0-3)	17 (27.9)	37 (60.7)	7 (11.5)	0
After 3 months	2.2 (0-4)	14 (23.3)	32 (53.3)	12 (20.0)	2 (3.3)
After 6 months	2.1 (0-4)	15 (25.4)	37 (62.7)	7 (11.9)	0
After 9 months	2.0 (0-4)	12 (20.7)	39 (67.2)	7 (12.1)	0
After 12 months	2.0 (0-4)	13 (26.5)	34 (69.4)	2 (4.1)	0

Number of patients (%). Dose is expressed in mean (range).

The pharmacokinetics of sirolimus in the whole blood was investigated in 10 patients in whom the pharmacokinetics was considered to have reached the steady state after once daily administration of 2 mg sirolimus. Table 14 shows the pharmacokinetic parameters.

Table 14. Pharmacokinetic parameters of sirolimus in multiple oral doses of sirolimus to Japanese patients

C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24} (ng·h/mL)	$t_{1/2}$ (h)	CL/F (L/h/kg)	V_{ss}/F (L/kg)
22.4 ± 9.4	2.8 ± 0.7	276 ± 122	47.7 ± 41.0	0.16 ± 0.04	9.0 ± 6.5

Mean ± SD, n = 10

C_{max}: Maximum concentration, t_{max}: Time to maximum concentration, AUC: Area under blood concentration-time curve, $t_{1/2}$: Elimination half-life, CL/F: Clearance, V_{ss}: Distribution volume under steady state

4.(ii).A.(3).3) Pooled analysis (5.3.3.1-5, RPT-42893)

Effects of age, sex, and race on the pharmacokinetics of sirolimus were investigated using pharmacokinetic data obtained from 111 subjects (18-45 years old; 90 males, 21 females; 56 Caucasians, 36 Blacks, 18 Hispanics, 1 subject of other race) in clinical studies (155-US, 165-US, 166-EU, 168-US, 172-US) in which oval or freeze-dried sirolimus tablets (5-40 mg) were orally administered at a single dose to non-Japanese healthy adult subjects. The effect of age was also investigated using the pharmacokinetic data obtained from 36 subjects (22-68 years old; 24 males, 12 females; 24 Caucasians, 6 Blacks, 4 Hispanics, 2 subjects of other race) in multiple oral dose studies (306-US, 309-GL) in which oval 5 or 10 mg sirolimus tablets were administered to renal transplant patients, in addition to the above data. In the combined data of 5 single oral dose studies of oval tablets or freeze-dried sirolimus tablets, CL/F was 0.29 ± 0.11 L/h/kg and V_{ss}/F was 22.9 ± 8.8 L/kg. Sex and race were selected as covariates for CL/F, and race was selected as a covariate for V_{ss}/F. Female subjects showed a greater CL/F compared with male subjects (0.35 ± 0.18 L/h/kg in female subjects, 0.27 ± 0.09 L/h/kg in male subjects). Caucasians showed smaller CL/F and V_{ss}/F values (CL/F, 0.24 ± 0.07 L/h/kg; V_{ss}/F, 20.5 ± 7.64 L/kg) compared with Blacks (CL/F, 0.31 ± 0.09 L/h/kg; V_{ss}/F, 23.5 ± 6.13 L/kg) and Hispanics (CL/F, 0.37 ± 0.19 L/h/kg; V_{ss}/F, 27.3 ± 12.3 L/kg). Age had no effect on the pharmacokinetics of sirolimus.

4.(ii).A.(4) Studies in special populations

4.(ii).A.(4).1) Pharmacokinetics in non-Japanese subjects with hepatic impairment (5.4.2-10)

The pharmacokinetics of sirolimus was investigated in an open-label study in non-Japanese subjects with hepatic impairment (13 subjects with mild hepatic impairment [Child-Pugh score 5-6], 5 subjects with moderate hepatic impairment [Child-Pugh score 7-9]). Table 15 shows the pharmacokinetic parameters of sirolimus in the whole blood following a single oral dose of sirolimus solution (15 mg) in patients with mild or moderate hepatic impairment and healthy adult subjects matched for sex, age, body weight, and smoking habit (normal hepatic function group). In the mild hepatic impairment group, $AUC_{0-\infty}$ increased by 48%, CL/F decreased by 32%, and $t_{1/2}$ increased by 25%, compared with the normal hepatic function group. In the moderate hepatic impairment group, $AUC_{0-\infty}$ increased by 96%, CL/F decreased by 36%, and $t_{1/2}$ increased by 89%, compared with the normal hepatic function group (Zimmerman JJ et al., *J Clin Pharmacol.* 2005;45:1368-1372).

Table 15. Pharmacokinetic parameters following a single oral dose of 15 mg of liquid preparation to non-Japanese subjects with hepatic impairment and to healthy adult subjects

	N	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0-\infty}$ (ng·h/mL)	$t_{1/2}$ (h)	CL/F (L/h/kg)	V_{ss}/F (L/kg)
Normal hepatic function	18	78.2 ± 18.3	0.82 ± 0.17	970 ± 272	78.9 ± 12.1	0.22 ± 0.08	17.4 ± 5.9
Mild hepatic impairment	13	79.0 ± 25.2	0.87 ± 0.17	1439 ± 489	98.6 ± 22.1	0.15 ± 0.05	16.1 ± 5.3
Moderate hepatic impairment	5	75.0 ± 18.7	0.74 ± 0.15	1899 ± 840	149 ± 57	0.14 ± 0.09	21.2 ± 7.3

Mean ± SD

C_{max}: Maximum concentration, t_{max}: Time to maximum concentration, AUC: Area under blood concentration-time curve, $t_{1/2}$: Elimination half-life, CL/F: Clearance, V_{ss}/F: Distribution volume under steady state

4.(ii).A.(4).2) Pharmacokinetics in non-Japanese subjects with hepatic impairment (5.4.2-11)

The pharmacokinetics of sirolimus was investigated in an open-label study in non-Japanese subjects with hepatic impairment (9 subjects with severe impairment [Child-Pugh score 10-15]). Table 16 shows the pharmacokinetic parameters of sirolimus in the whole blood following a single oral dose of sirolimus solution (15 mg) in patients with severe hepatic impairment and healthy adult subjects matched for sex, age, body weight, and smoking habit (normal hepatic function group). In the severe hepatic impairment group, $AUC_{0-\infty}$ increased by 210%, CL/F decreased by 67%, and $t_{1/2}$ increased by 168%, compared with those in the normal hepatic function group (Zimmerman JJ et al., *J Clin Pharmacol*. 2008;48:285-292).

Table 16. Pharmacokinetic parameters following a single oral dose of 15 mg liquid preparation to non-Japanese subjects with hepatic impairment and to healthy adult subjects

	N	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0-\infty}$ (ng·h/mL)	$t_{1/2}$ (h)	CL/F (L/h/kg)	V_{ss}/F (L/kg)
Normal	9	72.3 ± 16.6	0.78 ± 0.16	838 ± 277	80.0 ± 5.4	0.30 ± 0.07	34.5 ± 7.2
Severe hepatic impairment	9	56.2 ± 23.1	0.82 ± 0.17	2597 ± 1092	214 ± 69	0.10 ± 0.04	29.1 ± 12.9

Mean ± SD

C_{max}: Maximum concentration, t_{max}: Time to maximum concentration, AUC: Area under blood concentration-time curve, $t_{1/2}$: Elimination half-life, CL/F: Clearance, V_{ss}/F: Distribution volume under steady state

The applicant explained that since the above results suggested that the exposure to sirolimus may increase in patients with hepatic impairment, the dose of sirolimus in these patients should be adjusted by monitoring sirolimus concentration in the whole blood, and it would be appropriate

to recommend to start the treatment at the half dose in patients with moderate or severe hepatic impairment. The applicant also explained that although no pharmacokinetic study was conducted in subjects with renal impairment, it would be unnecessary to adjust the dose of sirolimus based on the severity of renal impairment because the renal secretion rate of the unchanged sirolimus and metabolites is very low 2.2% [see "4.(ii).A.(2).3) Mass balance study].

4.(ii).A.(5) Drug interaction studies

4.(ii).A.(5).1) Diltiazem (5.3.3.1-6, Study 135-EU [[redacted] to [redacted] 19[redacted]])

Diltiazem (120 mg), a CYP3A4 inhibitor, was orally administered at a single dose to non-Japanese healthy adult subjects (n = 18), followed by a single oral dose of 2 mL sirolimus liquid preparation (5 mg/mL, 10 mg in total) at 1 hour after administration of diltiazem to investigate the pharmacokinetics of sirolimus and diltiazem. The geometric least squares mean ratio [90% CI] of pharmacokinetic parameters of sirolimus in the whole blood after concomitant use of sirolimus with diltiazem versus after administration of sirolimus alone was 1.43 [1.14, 1.81] for C_{max} and 1.69 [1.41, 2.02] for AUC_{0-t}. $t_{1/2}$ was 85.0 ± 42.3 hours after administration of sirolimus alone and 71.3 ± 30.6 hours after concomitant use with diltiazem. With diltiazem, the ratio was 1.02 [0.95, 1.09] for C_{max} and 1.02 [0.94, 1.10] for AUC_{0-t}, and $t_{1/2}$ was 4.11 ± 0.68 hours after administration of diltiazem alone and 3.67 ± 0.91 hours after concomitant use of diltiazem with sirolimus.

4.(ii).A.(5).2) Verapamil [5.3.3.1-7, Study 183-US [[redacted] to [redacted] 20[redacted]])

Verapamil (180 mg), a CYP3A4 inhibitor, was orally administered twice daily for 2 days to non-Japanese healthy adult subjects (n = 25), followed by once daily multiple oral dose of 2 mL sirolimus liquid preparation (1 mg/mL, 2 mg in total) for 8 days and concomitant use with verapamil (180 mg) twice daily for 2 days starting from Day 6 of administration of sirolimus, and the pharmacokinetics of sirolimus and verapamil was investigated. The geometric least squares mean ratio [90% CI] of pharmacokinetic parameters of sirolimus in the whole blood after concomitant use of sirolimus with verapamil versus after administration of sirolimus alone was 2.34 [2.14, 2.56] for C_{max} and 2.16 [2.00, 2.32] for AUC_{0-24}. With S-(-) verapamil, the ratio was 1.46 [1.30, 1.64] for C_{max} and 1.48 [1.34, 1.63] for AUC_{0-12}.

4.(ii).A.(5).3) Erythromycin (5.3.3.1-8, Study 182-US [[redacted] to [redacted] 20[redacted]])

Non-Japanese healthy adult subjects (24 subjects) received 2 mL of sirolimus liquid preparation (1 mg/mL, 2 mg in total), orally administered once daily for 7 days. On Days 6 and 7 of sirolimus administration (for 2 days), the patients also received erythromycin (800 mg), a CYP3A4 inhibitor, orally administered 3 times daily. The pharmacokinetics of sirolimus was then investigated. The geometric least squares mean ratio [90% CI] of pharmacokinetic parameters of sirolimus in the whole blood after concomitant use of sirolimus with erythromycin versus after administration of sirolimus alone was 4.43 [4.06, 4.83] for C_{max} and 4.24 [3.93, 4.57] for AUC_{0-24}. In a separate experiment, erythromycin (800 mg) was orally administered 3 times daily for 2 days and, on Day 2 of erythromycin administration, 2 mL sirolimus liquid preparation (1 mg/mL, 2 mg in total) was orally administered at a single dose, and the pharmacokinetics of erythromycin was investigated. As a result, the geometric least squares mean ratio [90% CI] of pharmacokinetic parameters of erythromycin after concomitant use of sirolimus with erythromycin versus after administration of erythromycin alone was 1.63 [1.40, 1.89] for C_{max} and 1.69 [1.46, 1.96] for AUC_{0-8}.

4.(ii).A.(5).4) Ketoconazole (5.3.3.1-9, Study 136-US [[redacted] to [redacted] 19[redacted]])

Ketoconazole (200 mg), a CYP3A4 inhibitor, was orally administered once daily for 10 days to non-Japanese healthy adult subjects (n = 23) and, on Day 5 of administration of ketoconazole, 1 mL sirolimus liquid preparation (5 mg/mL, 5 mg in total) was orally administered at a single dose, and the pharmacokinetics of sirolimus was investigated. The geometric least squares mean ratio [90% CI] of pharmacokinetic parameters of sirolimus in the whole blood after concomitant use of sirolimus with ketoconazole versus after administration of sirolimus alone was 4.42 [3.77,

5.17] for C_{max} and 10.27 [8.69, 12.1] for AUC_{0-t}. $t_{1/2}$ was 74.7 ± 14.2 hours after administration of sirolimus alone and 81.0 ± 20.1 hours after concomitant use with ketoconazole.

4.(ii).A.(5).5) Rifampicin (5.3.3.1-10, Study 156-US [[redacted] to [redacted] 19[redacted]])

Rifampicin (600 mg), a CYP3A4 inducer, was orally administered once daily for 14 days to non-Japanese healthy adult subjects (n = 14) and, on Day 9 of rifampicin administration, 4 mL of sirolimus liquid preparation (5 mg/mL, 20 mg in total) was orally administered at a single dose, and the pharmacokinetics of sirolimus was investigated. The geometric least squares mean ratio [90% CI] of pharmacokinetic parameters of sirolimus in the whole blood after concomitant use of sirolimus with rifampicin versus after administration of sirolimus alone was 0.29 [0.26, 0.32] for C_{max} and 0.19 [0.16, 0.22] for AUC_{0-t}. $t_{1/2}$ was 65.0 ± 10.6 hours after administration of sirolimus alone and 59.4 ± 8.0 hours after concomitant use with rifampicin.

4.(ii).A.(5).6) Cyclosporine (5.3.3.1-11, Study 168-US [[redacted] to [redacted] 19[redacted]])

To non-Japanese healthy adult subjects (n = 24), 10 sirolimus 1 mg oval tablets (10 mg in total) were orally administered at a single dose in combination with cyclosporine (300 mg), a CYP3A4 substrate, (simultaneous administration), or cyclosporine (300 mg) was orally administered at a single dose, followed by a single oral dose of 10 sirolimus 1 mg oval tablets (10 mg in total) at 4 hours after administration of cyclosporine (time lapse administration), and the pharmacokinetics of sirolimus and cyclosporine was investigated. The geometric least squares mean ratio [90% CI] of pharmacokinetic parameters of sirolimus in the whole blood after simultaneous administration of sirolimus and cyclosporine versus after administration of sirolimus alone was 6.12 [5.44, 6.89] for C_{max} and 2.59 [2.30, 2.67] for AUC_{0-t}, and the ratio after time lapse administration of sirolimus and cyclosporine versus after administration of sirolimus alone was 1.33 [1.19, 1.50] for C_{max} and 1.38 [1.28, 1.50] for AUC_{0-t}. $t_{1/2}$ was 58.2 ± 11.8 hours after administration of sirolimus alone, 55.0 ± 8.3 hours after simultaneous administration, and 52.5 ± 7.8 hours after time lapse administration. As for cyclosporine, the ratio after simultaneous administration of sirolimus and cyclosporine versus after administration of cyclosporine alone was 1.04 [0.97, 1.11] for C_{max} and 1.01 [0.95, 1.07] for AUC_{0-t}, and the ratio after time lapse administration of sirolimus and cyclosporine versus after administration of cyclosporine alone was 1.08 [1.01, 1.16] for C_{max} and 1.08 [1.02, 1.14] for AUC_{0-t}. $t_{1/2}$ was 13.2 ± 2.2 hours after administration of cyclosporine alone, 12.9 ± 1.8 hours after simultaneous administration, and 13.2 ± 2.2 hours after time lapse administration.

4.(ii).B Outline of the review by PMDA

4.(ii).*B*.(1) Pharmacokinetic drug interactions

The applicant explained the drug interactions of sirolimus with other drugs as follows:

Sirolimus is reported to be metabolized by CYP3A4 in the small intestine and liver (Lampen A et al., *J Pharmacol Exp Ther*. 1998;285:1104-1112, Arceci RJ et al., *Blood.* 1992;80:1528-1536), and to be countertransported by P-glycoprotein (P-gp), a drug-efflux pump, from small intestinal enterocytes (Crowe A et al., *Pharm Res.* 1998;15:1666-1672). This suggests the possibility that sirolimus may interact with drugs that inhibit or induce CYP3A4 or P-gp or those that serve as their substrates. Table 17 shows drugs that were investigated in drug interaction studies and reported to interact with sirolimus. The package insert and other materials will include precautionary statements to the effect that concomitant use with drugs that affect CYP3A4 or P-gp should be avoided whenever possible and that, when sirolimus is concomitantly administered with these drugs, the dose of sirolimus should be adjusted by monitoring sirolimus concentration in the whole blood.

Table 17. Drugs that may interact with sirolimus

Drugs that inhibit CYP3A4 or P-gp	Calcium channel blockers (diltiazem, nicardipine, verapamil) Prokinetic agent (metoclopramide) Antifungal agents (fluconazole, itraconazole, ketoconazole, voriconazole) Antibiotics (clarithromycin, erythromycin) Other drugs (bromocriptine, cimetidine, cyclosporine, danazol, protease inhibitors [e.g., anti-HIV and anti-hepatitis C agents containing ritonavir and indinavir]) Grapefruit juice
Drugs that induce CYP3A4 or P-gp	Anticonvulsant drugs (carbamazepine, phenobarbital, phenytoin) Antibiotics (rifabutin, rifampicin) Herbal preparation (St. John's wort)

The applicant explained the possibility of sirolimus inhibiting metabolic enzymes or transporters, as follows:
In a study on the inhibitory effect of sirolimus on metabolic enzymes and transporters using human liver microsomes, 50% inhibitory concentration (IC_{50}) of sirolimus against CYP3A4/5, CYP2D6, and CYP2C9 was 5, 2.9, and 8 μM, respectively (0.1 μM corresponds to approximately 100 ng/mL). In a separate experiment using a system with both competitive and noncompetitive inhibition, the inhibition constant (Ki) was investigated using testosterone, bufuralol (unapproved in Japan), and diclofenac as a specific probe for the activity of CYP3A4/5, CYP2D6, and CYP2C9, respectively. As a result, Ki was estimated to be 2, 5, and 20 μM, respectively. Furthermore, it is confirmed that sirolimus at ≥1 μg/mL inhibits the activity of P-gp on mononuclear cells in the peripheral blood of healthy adult subjects (Yacyshyn BR et al., *Scand J Immunol*. 1996;43:449-455) and that sirolimus at ≥500 ng/mL inhibits the binding of ^{125}I-iodoaryl azidoprazosin to P-gp (Arceci RJ et al., *Blood*. 1992;80:1528-1536), which suggests that sirolimus per se inhibits CYP3A4 and P-gp. However, taking account of the observations that the inhibitory effect of sirolimus on CYP3A4 and P-gp was observed only at concentrations exceeding C_{max} (≤100 ng/mL) reached after administration of the clinical dose to humans, and that no difference was observed in the activity of CYP3A4 and P-gp between organ transplant patients treated with 4 mg sirolimus and healthy adult subjects treated with 4 mg sirolimus (Lemahieu WP et al., *Am J Transplant*. 2004;4:1514-1522), sirolimus administered according to the clinical dosage regimen is unlikely to cause drug interactions by inhibiting CYP3A4 or P-gp.

PMDA considers as follows:
It has been shown that blood sirolimus concentration may be affected significantly when sirolimus is concomitantly administered with drugs that inhibit or induce CYP3A4 and P-gp, or serve as their substrates. Therefore, attention should be given to the prophylaxis of drug interactions with these drugs, as proposed by the applicant. In particular, patients with LAM are prone to respiratory infection because of the disease conditions, and macrolide antibiotics with CYP3A4-inhibitory activity, such as erythromycin and clarithromycin, are commonly used against respiratory infection. Therefore healthcare professionals should be strongly cautioned to avoid administration of these antibiotics to patients with LAM being treated with sirolimus, whenever possible.

4.(ii).B.(2) Racial differences in pharmacokinetics
The applicant explained the differences in pharmacokinetics between Japanese and non-Japanese populations as follows:
No study was conducted on the pharmacokinetics of sirolimus in multiple-dose administration in non-Japanese patients with LAM or in single-dose administration in Japanese patients with LAM, precluding accurate assessment of racial differences in pharmacokinetics of sirolimus in patients with LAM. However, a comparison of results from Study MLSTS (multiple doses of sirolimus administered to Japanese patients with LAM) and Study 309-GL (multiple doses of oval sirolimus tablets administered to non-Japanese renal transplant patients) (Table 18) suggested the possibility that the differences in dosage form (triangular tablets vs. oval tablets), timing of

administration (fed administration vs. administration at arbitrary timing), and concomitant drugs (in Study 309-GL, sirolimus was administered at 4 hours after cyclosporine administration) might have affected the pharmacokinetics, but there were no significant differences in the pharmacokinetic parameters between Japanese and non-Japanese patients, although the study results should be interpreted with care.

As regards the trough sirolimus concentration in the whole blood in Japanese and non-Japanese patients with LAM, the mean trough concentration of sirolimus in the whole blood over 12 months following the administration of 2 mg/day of sirolimus in Study MILES was 7.3 ± 1.5 ng/mL in Japanese patients (n = 13) and 7.0 ± 2.6 ng/mL in non-Japanese patients (n = 30). Therefore, the applicant considered that there was no significant difference in the trough concentration of sirolimus in the whole blood between Japanese and non-Japanese patients.

Table 18. Comparison of pharmacokinetic parameters between Japanese and non-Japanese patients

	N	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24} (ng·h/mL)	CL/F (L/h/kg)
Japanese patients (Study MLSTS [a)])	10	22.4 ± 9.4	2.8 ± 0.7	276 ± 122	0.16 ± 0.04
Non-Japanese patients (Study 309-GL [b)])	10	17.5 ± 6.0	4.6 ± 4.2	279 ± 89	0.13 ± 0.07

Mean ± SD

C_{max}: Maximum concentration, t_{max}: Time to maximum concentration, AUC: Area under blood concentration-time curve, CL/F: Clearance

a) Concentration in whole blood measured 13 or 26 weeks after fed administration of sirolimus (2 mg/day) in Japanese patients with LAM

b) Concentration in whole blood measured at 90 days after administration at arbitrary timing of oval sirolimus tablets 2 mg/day in non-Japanese renal transplant patients

PMDA considered as follows:

Pharmacokinetic parameters in multiple-dose administration in non-Japanese patients with LAM and in single-dose administration in Japanese patients with LAM are unknown, precluding accurate comparison of pharmacokinetics between Japanese and non-Japanese patients. However, pharmacokinetic parameters in Study MLSTS in Japanese patients with LAM were similar to those in non-Japanese renal transplant patients, and in Study MILES, the distribution of the trough concentration of sirolimus in the whole blood was similar between Japanese and non-Japanese patients. PMDA therefore concluded that there was no significant difference in the pharmacokinetics of sirolimus between Japanese and non-Japanese populations.

4.(iii) Summary of clinical efficacy and safety

4.(iii).A Summary of the submitted data

As the data of studies on the efficacy, safety, and pharmacokinetics of sirolimus, the results of a clinical study in patients with LAM (including Japanese patients) (Study MILES [5.4.3-2]) and an investigator-initiated clinical trial in Japanese patients with LAM (Study MLSTS [5.3.5.2-2]) were submitted [for pharmacokinetics, see "4.(ii) Summary of clinical pharmacology studies"].

4.(iii).A.(1) Clinical study (5.4.3-2, Study MILES [December 2006 to September 2010])

A randomized, double-blind, placebo-controlled, parallel group comparative study was conducted in Japan, the US, and Canada to investigate the efficacy and safety of sirolimus in Japanese and

non-Japanese patients with LAM[28] (target sample size, 120[29] [60 patients per group]).

Sirolimus or placebo was to be administered once daily[30] (starting dose of sirolimus, 2 mg/day). The dose of sirolimus was to be adjusted to maintain the trough concentration of sirolimus between 5 and 15 ng/mL by measuring the trough concentration in blood at 3 weeks and 3, 6, 9, and 12 months after the beginning of treatment.[31] The treatment duration was set at 12 months, followed by a 12-month post-treatment observation phase.

All of the 89 randomized patients were given the investigational products (46 patients in the sirolimus group, 43 patients in the placebo group) and were included in the intent-to-treat (ITT) population, safety analysis set, and efficacy analysis set. The percentage of discontinuation was 26.1% (12 of 46 patients) in the sirolimus group and 27.9% (12 of 43 patients) in the placebo group. The main reasons for discontinuation included off-study treatment with sirolimus (10.9% [5 of 46 patients] in the sirolimus group, 7.0% [3 of 43 patients] in the placebo group).

Of the total 89 treated patients, 24 (13 in the sirolimus group, 11 in the placebo group) were Japanese. In the Japanese subpopulation, the percentage of discontinuation was 7.7% (1 of 13 patients) in the sirolimus group and 9.1% (1 of 11 patients) in the placebo group. The reasons for the discontinuation were treatment completion (sirolimus group) and pneumothorax (placebo group).

Table 19 shows the slope (mL/month) of forced expiratory volume in 1 second (FEV_1,) up to 12 months after the beginning of treatment, the primary efficacy endpoint. Paired comparison between the sirolimus and placebo groups showed statistically significant difference, verifying the superiority of sirolimus to the placebo. Figure 3 shows the time course of FEV_1 (mL) from baseline up to 24 months after the beginning of treatment (post-treatment observation phase).

Table 19. Slope of FEV_1 (mL/month) up to 12 months after the beginning of treatment (ITT population, OC)

	Sirolimus group	Placebo group	Intergroup difference [95% CI] [b], *P* value [b]
Baseline [a] (mL)	1357 ± 400 (46)	1378 ± 446 (43)	
12 months after the beginning of treatment (mL)	1383 ± 394 (41)	1272 ± 414 (34)	
Change (mL)	19 ± 124 (41)	-134 ± 182 (34)	
Slope [b] (mL/month)	1.1 ± 2.0 (46)	-11.8 ± 2.0 (43)	12.9 [7.3, 18.5], $P < 0.0001$

Mean ± SD (number of patients). The slope is expressed in point estimate ± SE (number of patients).
a) The greater value of 2 measurements performed prior to treatment
b) Mixed-effects model with treatment group, time (month), and interaction between time and treatment group as the fixed effects, and subjects and time as the random effects

[28] Women aged ≥18 years for whom LAM were diagnosed with chest high-resolution computed tomography (HRCT), showed %FEV_1 of ≤70% after taking a bronchodilator, and met at least 1 of the criteria (a) to (c) below: (a) LAM detected by biopsy of lung, abdominal tumor mass, lymph nodes, or kidney; HMB45-positive cells detected by cytodiagnosis of spindle cells/epithelial cells taken from chest or abdomen; or LAM detected by chest CT, (b) TSC, AML, or chylothorax (confirmed by puncture) detected by CT, MRI, or biopsy, (c) Serum vascular endothelial growth factor-D (VEGF-D) level ≥800 pg/mL.

[29] At the start of the study, the target sample size had been set at 240, but changed to 120 after the start of the study in response to the results of Study CAST (Bissler N et al., *N Eng J Med.* 2008;358:140-151).

[30] The study drug was to be administered either under fasting or under fed conditions, but the same administration method should be used throughout the study. Morning administration was recommended.

[31] The data monitoring committee evaluated the trough concentration in blood and, if the concentration deviated from the range 5 to 15 ng/mL, indicated the recommended dose to the evaluator at the study site. The evaluator determined whether or not to accept the recommended dose within 48 hours after receiving the indication. If the recommended dose was accepted, trough concentration in blood was measured at 1 week (± 2 days) after the dose change, and subjected to re-examination at the data monitoring committee. This procedure was repeated until blood trough concentration became within the range from 5 to 15 ng/mL.

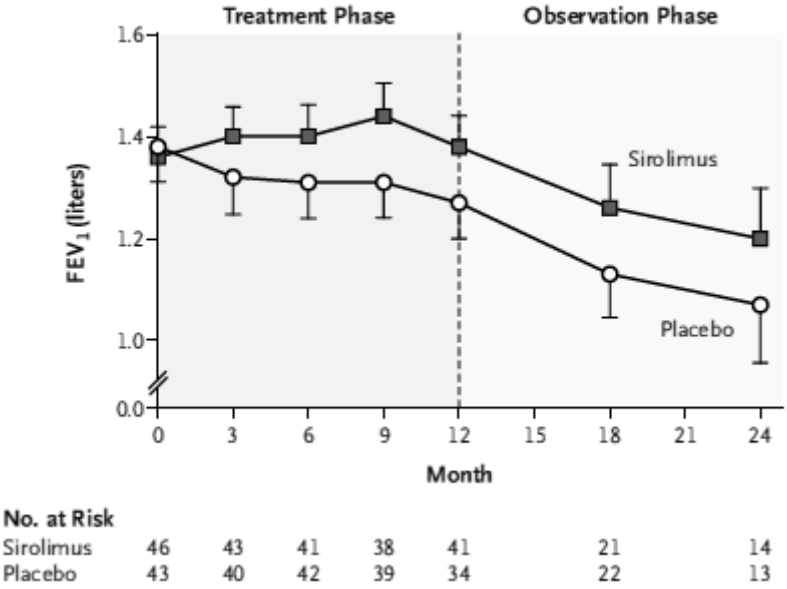


Figure 3. Time course of FEV_1 (mL) from baseline to 12 months (treatment phase) and 24 months (post-treatment observation phase) after the beginning of treatment (mean ± SD)

Table 20 shows the slope of FEV_1 (mL/month) up to 12 months after the beginning of treatment and the change (mL) in FEV_1 from baseline in the Japanese subgroup.

Table 20. Slope of FEV_1 (mL/month) up to 12 months after the beginning of treatment (Japanese subpopulation, OC)

	Sirolimus group	Placebo group	Between-group difference [95% CI] [b]
Baseline [a] (mL)	1400 ± 353 (13)	1096 ± 409 (11)	
12 months after the beginning of treatment (mL)	1400 ± 345 (13)	961 ± 365 (10)	
Change (mL)	0 ± 136 (13)	-171 ± 139 (10)	
Slope [b] (mL/months)	-1.2 ± 2.8	-13.3 ± 3.1	12.1 [3.5, 20.7]

Mean ± SD (number of patients). The slope is expressed in point estimate ± SE (number of patients).
a) The mean of the greater value of 2 measurements performed prior to treatment in each subject
b) Mixed-effects model with treatment group, time (month), and interaction between time and treatment group as the fixed effects, and subjects and time as the random effects

In this study, an interim analysis was planned[32] and conducted at the time point when 40 patients (20 patients in each group) completed the 12-month visit. As a result, the slope of FEV_1 over 1 year, the primary efficacy endpoint, showed statistically significant differences between the sirolimus and placebo groups (P = 0.0003, α = 0.002 based on O'Brien-Fleming significance level,[33] between-group difference in slope [95% CI][34] = 11.9 [5.8, 18.0]), meeting the withdrawal criteria. However, a total of 89 subjects had been randomized at this time point and all of them, except discontinued cases, had already been treated for ≥6 months. Therefore, according to the data monitoring committee's recommendation to continue the study until the 12-month data were obtained from all subjects, the study was continued under double-blind conditions.

[32] At the start of the study, an interim analysis was planned to be conducted at the time point when 100 patients (50 patients in each group) completed the 12-month visit. However, the time point of the interim analysis was changed pursuant to the change in the target sample size.
[33] The significance level in the entire study was set at 5% (one-sided).
[34] Mixed-effects model with treatment group, time (month), and interaction between time and treatment group as the fixed effects, and subjects and time as the random effects

Adverse events were observed in 46 of 46 patients (100%) in the sirolimus group and in 43 of 43 patients (100%) in the placebo group. Table 21 shows the main adverse events. Death occurred in 2 patients in the placebo group (haemorrhage intracranial in 1 patient, death due to fire accident in 1 patient), but a causal relationship with the investigational product was ruled out for both events. Serious adverse events were observed in 17.4% (8 of 46 patients) in the sirolimus group and 30.2% (13 of 43 patients) in the placebo group. Adverse events reported by ≥2 patients in either group were pain (6.5% [3 of 46 patients] in the sirolimus group, 2.3% [1 of 43 patients] in the placebo group), infection (4.3% [2 of 46 patients] in the sirolimus group, 7.0% [3 of 43 patients] in the placebo group), cardiac disorder (4.3% [2 of 46 patients] in the sirolimus group, 0 patient in the placebo group), pneumothorax (0 patient in the sirolimus group, 9.3% [4 of 43 patients] in the placebo group), and respiratory disorder (0 patient in the sirolimus group, 4.7% [2 of 43 patients] in the placebo group). Of these serious adverse events, a causal relationship with the investigational product could not be ruled out for those in 4 patients in the sirolimus group (infection in 2 patients, dyspnoea and pericardial effusion in 1 patient each) and 4 patients in the placebo group (vomiting/dyspnoea, pain, infection, and respiratory disorder in 1 patient each). Adverse events leading to discontinuation were observed in 4.3% (2 of 46 patients) in the sirolimus group (skin disorder and infection in 1 patient each) and 7.0% (3 of 43 patients) in the placebo group (pneumothorax in 2 patients, infection in 1 patient). A causal relationship with the investigational product could not be ruled out for adverse events in 1 patient in the sirolimus group (skin disorder) and 2 patients in the placebo group (pneumothorax and infection in 1 patient each). Adverse events for which a causal relationship with the investigational product could not be ruled out (adverse drug reactions) were observed in 97.8% (45 of 46 patients) in the sirolimus group and 95.3% (41 of 43 patients) in the placebo group.

Table 21. Adverse events reported by ≥10% of patients in either group (safety analysis population)

Events	Sirolimus group (N = 46)	Placebo group (N = 43)
Pain	32 (69.6)	33 (76.7)
Infection	32 (69.6)	29 (67.4)
Stomatitis	31 (67.4)	28 (65.1)
Diarrhoea	29 (63.0)	14 (32.6)
Respiratory disorder	27 (58.7)	26 (60.5)
Musculoskeletal disorder	20 (43.5)	12 (27.9)
Acne	20 (43.5)	5 (11.6)
Skin disorder	19 (41.3)	8 (18.6)
Cough	18 (39.1)	16 (37.2)
Nausea	18 (39.1)	11(25.6)
Fatigue	14 (30.4)	13 (30.2)
Gastrointestinal disorder	13 (28.3)	13 (30.2)
Laboratory test abnormal	13 (28.3)	2 (4.7)
Dyspnoea	12 (26.1)	17 (39.5)
Oedema peripheral	11 (23.9)	7 (16.3)
Dizziness	9 (19.6)	9 (20.9)
Hypercholesterolaemia	9 (19.6)	6 (14.0)
Mood altered	8 (17.4)	6 (14.0)
Insomnia	7 (15.2)	5 (11.6)
Exfoliative rash	6 (13.0)	7 (16.3)
Pyrexia	6 (13.0)	4 (9.3)
Aspartate aminotransferase increased	6 (13.0)	3 (7.0)
Pneumothorax	5 (10.9)	6 (14.0)
Vomiting	5 (10.9)	5 (11.6)
Dyspepsia	5 (10.9)	4 (9.3)
Pulmonary haemorrhage	5 (10.9)	4 (9.3)
Contusion	5 (10.9)	4 (9.3)
Eye disorder	5 (10.9)	3 (7.0)
Haemorrhage	5 (10.9)	3 (7.0)
Rhinitis allergic	5 (10.9)	3 (7.0)
Nervous system disorder	5 (10.9)	3 (7.0)
Urogenital disorder	5 (10.9)	3 (7.0)
Hypoxia	5 (10.9)	2 (4.7)
Weight decreased	5 (10.9)	2 (4.7)
Hypertension	4 (8.7)	5 (11.6)
Bronchospasm	3 (6.5)	5 (11.6)
Pruritus	3 (6.5)	5 (11.6)

Number of patients (%)

In the Japanese subpopulation, adverse events were observed in 100% (13 of 13 patients) in the sirolimus group and 100% (11 of 11 patients) in the placebo group. Table 22 shows main adverse events. No death occurred. Serious adverse events were observed in 1 patient in the sirolimus group (infection) and 3 patients in the placebo group (infection/cholecystitis, infection, and pneumothorax in 1 patient each). A causal relationship with the investigational product could not be ruled out in 1 each patient in the sirolimus group and the placebo group (infection in both cases). An adverse event leading to discontinuation was observed in 1 patient in the placebo group (pneumothorax), but its causal relationship with the investigational product was ruled out. Adverse drug reactions were observed in 100% (13 of 13 patients) in the sirolimus group and 81.8% (9 of 11 patients) in the placebo group.

Table 22. Adverse events reported by ≥10% of patients in either group (Japanese subpopulation)

Adverse events	Sirolimus group (N = 13)	Placebo group (N = 11)
Infection	10 (76.9)	9 (81.8)
Acne	10 (76.9)	2 (18.2)
Stomatitis	9 (69.2)	7 (63.6)
Respiratory disorder	9 (69.2)	7 (63.6)
Pain	8 (61.5)	6 (54.5)
Diarrhoea	6 (46.2)	4 (36.4)
Gastrointestinal disorder	4 (30.8)	1 (9.1)
Fatigue	3 (23.1)	3 (27.3)
Pneumothorax	3 (23.1)	2 (18.2)
Skin disorder	3 (23.1)	1 (9.1)
Musculoskeletal disorder	2 (15.4)	3 (27.3)
Eye disorder	2 (15.4)	2 (18.2)
Immune system disorder	2 (15.4)	1 (9.1)
Urogenital disorder	2 (15.4)	1 (9.1)
Dry eye	2 (15.4)	0
Nervous system disorder	2 (15.4)	0
Mood altered	2 (15.4)	0
Haemorrhage	1 (7.7)	2 (18.2)
Cough	1 (7.7)	2 (18.2)
Dyspnoea	1 (7.7)	2 (18.2)
Dizziness	0	3 (27.3)
Petechiae	0	2 (18.2)

Number of patients (%)

4.(iii).A.(2) Investigator-initiated clinical trial (5.3.5.2-2, Study MLSTS [August 2012 to ongoing (data cut-off date, [redacted], 2013)])

An open-label, uncontrolled study was conducted to evaluate the safety and efficacy of sirolimus in Japanese patients with LAM[35] (target sample size, 65).

Sirolimus was to be orally administered once daily after breakfast (starting dose, 2 mg/day). The dose of sirolimus was to be adjusted to maintain the trough concentration of sirolimus between 5 and 15 ng/mL by measuring the blood trough concentration at 1 and 3 weeks and 3, 6, 9, 12, 15, 18, 21, and 24 months after the beginning of treatment.[36] Treatment duration was set at 24 months.

Of 71 registered patients, all of the 63 treated patients were included in the ITT population, the safety analysis population, and the efficacy analysis population. Study discontinuation within 12 months after the beginning of treatment occurred in 9.5% (6 of 63 patients). The main reasons for study discontinuation included subjects' request for discontinuation (6.3% [4 of 63 patients]).

Adverse events were observed in 100.0% (63 of 63 patients) within 12 months after the beginning of treatment. Table 23 shows main adverse events. No death occurred. Serious adverse events were observed in 23.8% (15 of 63 patients). Adverse events reported by ≥2 patients were dyspnoea (3.2% [2 of 63 patients]) and lung disorder (3.2% [2 of 63 patients]). Of the serious adverse events, a causal relationship with the investigational product could not be ruled out for

[35] Women aged ≥18 years who showed cystic lesion consistent with LAM on HRCT and met at least 1 of the criteria (a) to (d) below: (a) LAM detected by biopsy, (b) LAM cell clusters detected by cytodiagnosis of chylous fluid, (c) VEGF-D ≥800 pg/mL, and (d) Clinical findings characteristic of LAM (tuberous sclerosis, complication with renal angiomyolipoma, complication with chylothorax or milky ascites, swelling of retroperitoneal lymph node or pelvic cavity lymph node) (whether or not the patient met criterion [d] was assessed by the coordinating committee).

[36] The dose was to be decreased from 2 mg/day to 1 mg/day if blood trough concentration was ≥15 ng/mL, and to be increased from 2 mg/day to 3 mg/day if blood trough concentration was <5 ng/mL. When the dose was changed, blood trough concentration was to be measured at 1 week (± 2 days) after the change to confirm that the trough concentration was within the range from 5 to 15 ng/mL. If blood trough concentration was <5 ng/mL after dose increase to 3 mg/day, the dose was to be increased until the trough concentration reached the range between 5 and 15 ng/mL.

dyspnoea and lung disorder in 2 patients each, small intestinal obstruction, enterocolitis, abdominal pain, chest pain, pyrexia, abortion induced, bronchitis, herpes zoster, pneumonia, anaemia, acute respiratory failure, menorrhagia, ovarian cyst, and decreased appetite in 1 patient each. An adverse event leading to discontinuation (lung disorder) was observed in 1.6% (1 of 63 patients), and a causal relationship with the investigational product could not be ruled out for the event. Adverse drug reactions were observed in 100.0% (63 of 63 patients).

Table 23. Adverse events reported by ≥10% of patients (up to 12 months after the beginning of treatment, safety analysis population)

Adverse event	Patients treated with sirolimus (N = 63)
Stomatitis	56 (88.9)
Nasopharyngitis	35 (55.6)
Upper respiratory tract inflammation	30 (47.6)
Headache	29 (46.0)
Diarrhoea	22 (34.9)
Rash	19 (30.2)
Dermatitis acneiform	18 (28.6)
Menstruation irregular	14 (22.2)
Bronchitis	12 (19.0)
Acne	11 (17.5)
Cheilitis	9 (14.3)
Constipation	8 (12.7)
Nausea	8 (12.7)
Abdominal pain upper	8 (12.7)
Gastroenteritis	8 (12.7)
Back pain	8 (12.7)
Abdominal pain	7 (11.1)
Vomiting	7 (11.1)
Chest pain	7 (11.1)
White blood cell count decreased	7 (11.1)

Number of patients (%)

Table 24 shows the time course of the slope of FEV_1 (measured within 2 hours after administration of bronchodilator, mL/month) up to 12 months after the beginning of treatment, the primary efficacy endpoint, and the time course of change from baseline (mL).

Table 24. Time course of slope of FEV_1 up to 12 months after the beginning of treatment (mL/month) and of the change from baseline (mL, within 2 hours after administration of bronchodilator) (ITT population, OC)

	Patients treated with sirolimus	Change
Baseline [a)] (mL)	1806 ± 687 (56)	
3 months after the beginning of treatment (mL)	1823 ± 644 (51)	17 ± 162 (50)
6 months after the beginning of treatment (mL)	1821 ± 678 (50)	21 ± 213 (49)
9 months after the beginning of treatment (mL)	1844 ± 683 (51)	30 ± 220 (50)
12 months after the beginning of treatment (mL)	1935 ± 639 (42)	56 ± 240 (42)
Slope (mL/month)	3.0 ± 1.9 (57)	

Mean ± SD (number of patients). The slope is expressed in point estimate ± SE (number of patients).
a) Mean value immediately before administration

4.(iii).B Outline of the review by PMDA

4.(iii).B.(1) Efficacy

PMDA considers as follows:

In Study MILES, the confirmatory study on sirolimus conducted in the US, Canada, and Japan, the slope of FEV_1 (mL/month) up to 12 months after the beginning of treatment was analyzed as the primary efficacy endpoint. This parameter has not been established as an endpoint and its clinical significance is unclear. However, the change (mean ± SD) in FEV_1 from baseline up to 12 months after the beginning of treatment, the secondary efficacy endpoint, was 19 ± 124 mL (n = 41) in the sirolimus group and -134 ± 182 mL (n = 34) in the placebo group, demonstrating the efficacy of sirolimus for the treatment LAM. In addition, there are no significant differences in the disease condition, diagnostic criteria, treatment strategy, etc., for LAM between Japan and other countries, according to the ERS Guidelines and the Japanese guidelines by the study group on respiratory failure (Hayashida M et al., *The Journal of the Japanese Respiratory Society*. 2008;46:425-427). There are no significant differences in the pharmacokinetics of sirolimus between Japanese and non-Japanese patients [see "4.(ii) Summary of clinical pharmacology studies"]. Similar tendencies were observed between the Japanese population and the entire study population in the difference between the sirolimus group and the placebo group regarding the slope of FEV_1 up to 12 months after the beginning of treatment and the change in FEV_1 from baseline [see "4.(iii).A *Summary of the submitted data*"]. Sirolimus showed a similar tendency toward efficacy in the investigator-initiated clinical trial (Study MLSTS). On the basis of the above findings and the results of Study MILES, PMDA concluded that the efficacy of sirolimus in Japanese patients with LAM has been demonstrated.

PMDA asked the applicant to explain the disease stage of patients with LAM eligible for sirolimus therapy and treatment duration required for such patients.

The applicant explained as follows:

There is no clear definition for the disease stage of LAM. In Study MILES, of patients with definitive diagnosis of LAM, those with baseline $\%FEV_1 \leq 70\%$ were enrolled in the study, and the superiority of sirolimus to the placebo was demonstrated in this patient group. In Study MLSTS, all patients with definitive diagnosis of LAM were enrolled. Analysis of subgroups stratified by baseline $\%FEV_1$ showed the slope of FEV_1 (mean ± SE) in patients with baseline $\%FEV_1 < 70\%$ (n = 31) to be 4.7 ± 2.7 mL/month, suggesting an increasing tendency. Meanwhile, the slope in patients with baseline $\%FEV_1 \geq 70\%$ (n = 25) was 0.9 ± 2.7 mL/month, showing no increase. Based on the above results, sirolimus is recommended for patients with LAM with $\%FEV_1 < 70\%$, while the necessity of sirolimus therapy in patients with $\%FEV_1 \geq 70\%$ cannot be determined at present. However, given the irreversible nature of the lung structure destruction in LAM, it is considered appropriate to start treatment with sirolimus at an early stage after diagnosis. Therefore, even in patients with $\%FEV_1 \geq 70\%$, administration of sirolimus should be considered if they show any of LAM-related progressive disease conditions including exertional dyspnoea, pulmonary cystic disease, renal angiomyoma, abdominal lymphangioleiomyomatosis, chylothorax, and ascites. Table 25 shows the results of subgroup analysis in Study MILES stratified by baseline $\%FEV_1$,[37] LAM duration, and presence/absence of concomitant hormone therapy. In all of the subgroups analyzed, the slope of FEV_1 and the change from baseline in the sirolimus group tended to be greater compared with the placebo group. Therefore, the applicant considers that the use of sirolimus should be considered regardless of the baseline $\%FEV_1$, disease duration, and presence or absence of concomitant hormone therapy. As regards the duration of treatment with sirolimus, decreased respiratory function was observed after interruption of administration of sirolimus in the 1-year observation period succeeding to the 1-year study treatment period in Study MILES, which suggests that sirolimus should be continuously administered to maintain the respiratory function. The applicant considers that

[37] Baseline $\%FEV_1$ (after bronchodilator administration) of ≤70% was selected as an inclusion criterion.

respiratory function should be tested periodically and administration of sirolimus should be continued while carefully evaluating the safety.

Table 25. Subgroup analysis of the slope of FEV_1 (mL/month) at 12 months after the beginning of treatment in Study MILES (ITT population, OC)

			Sirolimus group	Placebo group
Baseline $\%FEV_1$	<50%	Baseline [a)]	1100 ± 248 (27)	1020 ± 294 (21)
		12 months after the beginning of treatment	1125 ± 262 (23)	973 ± 270 (17)
		Change	18 ± 114 (23)	-118 ± 107 (17)
		Slope [b)]	0.1 ± 2.0 (27)	-10.0 ± 2.2 (21)
	≥50%	Baseline [a)]	1723 ± 267 (19)	1719 ± 255 (22)
		12 months after the beginning of treatment	1713 ± 266 (18)	1572 ± 299 (17)
		Change	20 ± 139 (18)	-151 ± 238 (17)
		Slope [b)]	2.3 ± 3.5 (19)	-12.7 ± 3.3 (22)
Disease duration	<5 years	Baseline [a)]	1379 ± 364 (15)	1459 ± 366 (14)
		12 months after the beginning of treatment	1506 ± 321 (12)	1218 ± 342 (12)
		Change	43 ± 124 (12)	-187 ± 248 (12)
		Slope [b)]	3.9 ± 4.1 (15)	-15.5 ± 3.9 (14)
	≥5 years	Baseline [a)]	1345 ± 438 (28)	1335 ± 496 (27)
		12 months after the beginning of treatment	1337 ± 432 (26)	1285 ± 463 (20)
		Change	18 ± 129 (26)	-126 ± 116 (20)
		Slope [b)]	0.9 ± 2.2 (28)	-10.8 ± 2.3 (27)
Hormone therapy	Yes	Baseline [a)]	1399 ± 375 (14)	1221 ± 550 (12)
		12 months after the beginning of treatment	1388 ± 354 (14)	1202 ± 473 (10)
		Change	-11 ± 141 (14)	-145 ± 113 (10)
		Slope [b)]	-1.0 ± 2.5 (14)	-10.9 ± 2.9 (12)
	No	Baseline [a)]	1339 ± 415 (32)	1439 ± 392 (31)
		12 months after the beginning of treatment	1380 ± 420 (27)	1302 ± 394 (24)
		Change	34 ± 114 (27)	-130 ± 207 (24)
		Slope [b)]	2.2 ± 2.6 (32)	-11.6 ± 2.6 (31)

Mean ± SD (number of patients). The slope is expressed in point estimate ± SE (number of patients).
a) The greater value of 2 measurements performed prior to treatment
b) Mixed-effects model with treatment group, time (month), and interaction between time and treatment group as the fixed effects, and subjects and time as the random effects

PMDA considers as follows:
Given the irreversible nature of the lung structure destruction in LAM and the unavailability of other drug therapy for LAM at present, it is understandable that sirolimus may be indicated for patients with relatively mild disease conditions. However, sirolimus therapy should be started after the patient has been fully informed of the risk of sirolimus, because (i) sirolimus has to be administered continuously, (ii) sirolimus may induce serious infectious diseases and interstitial pneumonia, and (iii) effects of sirolimus on fetuses were suggested in toxicity studies [see "3.(iii) Summary of toxicology studies"]. Particularly in patients with child-bearing potential who have mild LAM, the appropriateness of sirolimus therapy should be carefully evaluated by weighing the clinical benefit against potential risks.

4.(iii).B.(2) Safety
The applicant explained the safety of sirolimus in patients with LAM as follows, based on the results of Studies MILES and MLSTS in patients with LAM, together with the pooled data of 8 foreign clinical studies in organ transplant patients conducted for approval for indication for the

prophylaxis of post-renal transplant rejection (Study 217-US,[38] Study 301-US[39], Study 302-GL[40], Study 309-GL[41], Study 310-GL[42], Study 313-GL[43], Study 316-GL[44], Study 318-WW[45], hereinafter "the studies in organ transplant patients"). Table 26 summarizes the adverse events observed in these clinical studies.

Table 26. Summary of adverse events

	Study MILES		Study MLSTS	Studies in organ transplant patients		
	Sirolimus group (N = 46)	Placebo group (N = 43)	Patients treated with sirolimus (N = 63)	Sirolimus group [a] (N = 3272)	Placebo group [a] (N = 284)	Comparator group [b] (N = 668)
Adverse events	46 (100.0)	43 (100.0)	63 (100.0)	3260 (99.6)	283 (99.6)	663 (99.3)
Serious adverse events	8 (17.4)	13 (30.2)	15 (23.8)	1098 (33.6)	23 (8.1)	329 (49.3)
Adverse events for which a causal relationship with the investigational product could not be ruled out	45 (97.8)	41 (95.3)	63 (100.0)	3104 (94.9)	245 (86.3)	464 (69.5)
Death	0	2 (4.7)	0	214 (6.5)	15 (5.3)	19 (2.8)

Number of patients (%)
a) Azathioprine, cyclosporine, corticosteroid, etc., were concomitantly administered both in the sirolimus group and the placebo group.
b) Cyclosporine, cyclosporine + tacrolimus, mycophenolate mofetil, and azathioprine

In Studies MILES and MLSTS, 2 patients in the placebo group (haemorrhage intracranial and fire accident in 1 patient each) died, but none in the sirolimus group. In studies in organ transplant patients, mortality was 6.5% (214 of 3272 patients) in the sirolimus group, 5.3% (15 of 284 patients) in the placebo group, and 2.8% (19 of 668 patients) in the comparator group. The main cause of death included unknown cause (0.9% [30 of 3272 patients] in the sirolimus group, 0.4% [1 of 284 patients] in the placebo group, 1.5% [10 of 668 patients] in the comparator group), sepsis (0.5% [16 of 3272 patients] in the sirolimus group, 0.4% [1 of 284 patients] in the placebo group), cardiac arrest (0.4% [12 of 3272 patients] in the sirolimus group, 0.7% [2 of 284 patients] in the placebo group), and myocardial infarction (0.2% [7 of 3272 patients] in the sirolimus group). A causal relationship with the investigational product could not be ruled out for 1 of 7 deaths caused by myocardial infarction in patients in the sirolimus group.

In Study MILES, adverse events that occurred with ≥10% higher incidence in the sirolimus group than that in the placebo group included diarrhoea (63.0% [29 of 46 patients] in the sirolimus group, 32.6% [14 of 43 patients] in the placebo group), acne (43.5% [20 of 46 patients] in the sirolimus

[38] Study in pediatric patients with renal transplantation. 64 patients in the sirolimus liquid preparation group (starting dose 3 mg/m^2/day, target trough concentration 5-15 ng/mL), 35 patients in the comparator group. Treatment duration, 3 years
[39] Study in renal transplant patients. 281 patients in the sirolimus liquid preparation 2 mg group (starting dose 6 mg/day, maintenance dose 2 mg/day), 269 patients in the sirolimus 5 mg group (starting dose 15 mg/day, maintenance dose 5 mg/day), 160 patients in the placebo group. Treatment duration, 2 years
[40] Study in renal transplant patients. 218 patients in the sirolimus liquid preparation 2 mg group (starting dose 6 mg/day, maintenance dose 2 mg/day), 208 patients in the sirolimus 5 mg group (starting dose 15 mg/day, maintenance dose 5 mg/day), 124 patients in the placebo group. Treatment duration, 3 years
[41] Study in renal transplant patients. 457 patients in the sirolimus liquid preparation or sirolimus tablet 2 mg group (starting dose 6 mg/day, maintenance dose 2 mg/day). Treatment duration, 1 year
[42] Study in renal transplant patients. 215 patients in the sirolimus liquid preparation or sirolimus tablet 2 mg group (starting dose 6 mg/day, maintenance dose 2 mg/day), 215 patients in the sirolimus group with trough concentration adjustment (starting dose 6 mg/day, maintenance dose 2-5 mg [target trough concentration 5-15 ng/mL]), 95 patients without group assignment (discontinued the study after administration of sirolimus [2 mg/day] until group assignment). Treatment duration, 5 years
[43] Study in hepatic transplant patients. 389 patients in the sirolimus liquid preparation or sirolimus tablet group (starting dose 10-15 mg/day, maintenance dose 3-5 mg/day [target trough concentration 8-16 ng/mL]), 210 patients in the comparator group. Treatment duration, 6 years
[44] Study in renal transplant patients. 551 patients in the sirolimus liquid preparation or sirolimus tablet group (starting dose 12-20 mg/day, target trough concentration 8-20 ng/mL), 273 patients in the comparator group. Treatment duration, 4 years
[45] Study in renal transplant patients. 310 patients in the sirolimus liquid preparation or sirolimus tablet group (starting dose 15 mg/day, target trough concentration 8-15 ng/mL), 161 patients in the comparator group. Treatment duration, 26 months

group, 11.6% [5 of 43 patients] in the placebo group), laboratory test abnormal (28.3% [13 of 46 patients] in the sirolimus group, 4.7% [2 of 43 patients] in the placebo group), skin disorder (41.3% [19 of 46 patients] in the sirolimus group, 18.6% [8 of 43 patients] in the placebo group), musculoskeletal disorder (43.5% [20 of 46 patients] in the sirolimus group, 27.9% [12 of 43 patients] in the placebo group), and nausea (39.1% [18 of 46 patients] in the sirolimus group, 25.6% [11 of 43 patients] in the placebo group).

In studies in organ transplant patients, adverse events that occurred with ≥10% higher incidence in the sirolimus group than that in the placebo group included hypercholesterolaemia (32.9% [1077 of 3272 patients] in the sirolimus group, 21.8% [62 of 284 patients] in the placebo group) and hypertriglyceridaemia (23.0% [752 of 3272 patients] in the sirolimus group, 7.0% [20of 284 patients] in the placebo group). Diarrhoea was also found with a nearly 10% higher incidence in the sirolimus group than that in the placebo group (38.9% [1274 of 3272 patients] in the sirolimus group, 29.6% [84 of 284 patients] in the placebo group).

PMDA reviewed adverse event data focusing on the following events, taking account of the tendency of adverse events observed in patients with LAM and organ transplant patients and the pharmacological action of sirolimus.

4.(iii).B.(2).1) Interstitial lung disease

mTOR inhibitors everolimus and temsirolimus are known to cause interstitial lung disease at a high frequency (White DA et al., *Am J Respir Crit Care Med.* 2010;182:396-403). The applicant explained the occurrence of interstitial lung disease and other related lung diseases associated with the administration of sirolimus, as follows:

Table 27 shows the incidences of interstitial lung disease and other related lung disease in Study MILES, Study MLSTS, and studies in organ transplant patients. In Study MILES, pneumonitis occurred in 1 patient in the sirolimus group. A causal relationship with the investigational product could not be ruled out for the disease, however, it developed more than 6 months after the completion of 12-month treatment with sirolimus, and eventually improved. In Study MLSTS, lung disorder (drug-induced lung disorder) was observed in 2 patients (3.2%) as of the cut-off date ([redacted], 2013). Both patients recovered from the disorder after treatment discontinuation or suspension. Treatment with sirolimus was resumed in one of them and, as of 23 weeks after the treatment resumption, no significant clinical symptoms or findings including relapse were observed. In studies in organ transplant patients, interstitial lung disease was observed in 0.7% (22 of 3272 patients) in the sirolimus group and 0.4% (1 of 284 patients) in the placebo group. A causal relationship with sirolimus could not be ruled out in 15 of 22 patients in the sirolimus group. Two patients in the sirolimus group died of interstitial lung disease; a causal relationship between death and sirolimus could not be ruled out in 1 patient.

Table 27. Incidences of interstitial lung disease and other related lung diseases

Adverse event	Study MILES		Study MLSTS	Studies in organ transplant patients		
	Sirolimus group (N = 46)	Placebo group (N = 43)	Patients treated with sirolimus (N = 63)	Sirolimus group [a)] (N = 3272)	Placebo group [a)] (N = 284)	Comparator group [b)] (N = 668)
Pneumonia	0	0	3 (4.8)	342 (10.5)	17 (6.0)	32 (4.8)
Lung disorder	0	0	2 (3.2)	66 (2.0)	5 (1.8)	4 (0.6)
Interstitial lung disease	0	0	0	22 (0.7)	1 (0.4)	0
Pneumonitis	1 (2.2)	0	0	19 (0.6)	0	0
Pneumonitis chemical	0	0	0	1 (<0.1)	0	0

Number of patients (%)

a) Azathioprine, cyclosporine, corticosteroid, etc., were concomitantly administered both in the sirolimus group and in the placebo group.

b) Cyclosporine, cyclosporine + tacrolimus, mycophenolate mofetil, and azathioprine

Of Japanese patients with LAM, a total of 5 patients experienced interstitial lung disease or other related lung disease: 2 patients with drug-induced lung disorder in Study MLSTS and 3 patients with confirmed or suspected interstitial lung disease who had been using the privately imported sirolimus. Table 28 shows the details of these cases.

Table 28. Summary of cases of interstitial lung disease observed in Japan

	Sex	Age (yrs)	Sirolimus dose (mg/day)	Diagnosis	Seriousness	Days from the beginning of treatment to onset	Disease duration (days)	Causal relationship with sirolimus	Outcome	Handling of sirolimus administration	Treatment given
Study MLSTS	F	[redacted]	2 mg	Drug-induced lung disorder	Serious	Day 49	7	Related	Recovered	Discontinued on Day 49	Oxygen inhalation, corticosteroid
	F	[redacted]	2 mg	Drug-induced lung disorder	Serious	Day 189	35	Related	Recovered	Temporarily discontinued on Day 196 Resumed on Day 289 at 1 mg/day upon improvement of drug-induced lung disorder	Untreated
Private import	F	[redacted]	2 mg	Drug-induced lung disorder	Serious	Day 51	-	Related	Recovered	Discontinued on Day 48	Antimicrobial agents
	F	[redacted]	2 mg	Organising pneumonia	Non-serious	Day 212	35	Related	Recovered	Dose reduced to 1 mg/day on Day 212 with the suspected drug-induced pneumonia Temporarily discontinued on Day 247 because of common cold Resumed on Day 254 at 1 mg/day upon improvement of drug-induced pneumonia	Antimicrobial agents
	F	[redacted]	1 mg	Right pulmonary infiltrative shadow	Death	Day 38	Unknown	Related	Death[a)]	Unknown	Unknown

a) A causal relationship between death and sirolimus was ruled out.

In the combined data of Studies 301-US and 302-GL conducted in organ transplant patients overseas, the mean trough whole blood sirolimus concentration in 7 patients with interstitial lung disease was 17.8 ± 7.6 ng/mL, which tended to be higher than the mean trough whole blood sirolimus concentration observed in 896 patients without interstitial lung disease (13.4 ± 7.6 ng/mL). Also, the incidence of interstitial lung disease observed in the combined data was 0.23% (1 of 437 subjects) in the sirolimus 2 mg/day group and 1.29% (6 of 466 subjects) in the sirolimus 5 mg/day group. These results suggested the possibility that the risk for sirolimus-induced interstitial lung disease may increase with the increase in trough concentration and dose.

The applicant plans to provide the information on the following safety measures against interstitial lung disease in the package insert and other information materials, to adequately inform healthcare professionals of the measures.

- Sirolimus should be used under the supervision of a physician who has sufficient knowledge of the product and of lymphangioleiomyomatosis.
- Before the start of treatment with sirolimus, the patient or his/her family member should be thoroughly informed of the characteristic features of interstitial lung disease, precautions to be taken during treatment with the product, and the fact that the treatment may cause death in some patients, by referring to the manuals for management of serious adverse drug reactions issued by MHLW.
- Chest X-ray, chest CT, and other necessary tests should be performed periodically.
- The risk of interstitial lung disease may increase in a manner dependent on the trough whole blood sirolimus concentration and the dose. If the disease occurred, sirolimus should be suspended or discontinued, depending on the symptoms and severity. Sirolimus should be administered with caution to patients with factors generally known as risks for interstitial lung disease, i.e., advanced age, smoking habit, interstitial lung disease and pulmonary fibrosis, post lung operation, decreased respiratory function, high-concentration oxygen inhalation, and pulmonary radiation.

PMDA considers as follows:
Because of the limited number of patients with LAM who have been treated with sirolimus, the relationship between the administration of sirolimus and the occurrence of interstitial lung disease is unclear. However, the combined data of 8 clinical studies in organ transplant patients showed that the incidences of interstitial lung disease and other related lung diseases tended to be greater in the sirolimus group than in the placebo group, and that the risk of interstitial lung disease tended to increase in a manner dependent on the trough whole blood sirolimus concentration or the dose. In addition, interstitial lung disease is known as an adverse drug reaction common to mTOR inhibitors. Taking account of these facts, due caution is necessary in using sirolimus in patients with LAM as well. In patients with LAM, in particular, interstitial lung disease may not be differentiated from the primary disease. Therefore, in order to avoid delayed diagnosis of interstitial lung disease resulting in fatal outcome, healthcare professionals should be strongly cautioned to closely monitor clinical symptoms including cough, pyrexia, and dyspnoea, and to perform imaging test periodically. Sirolimus therapy may be resumed in patients who have recovered from mild interstitial lung disease, because patients (although extremely limited in number) in clinical studies were able to resume and continue sirolimus therapy only when their conditions were closely monitored, and because other drug therapies are unavailable for LAM at present. It is necessary to provide detailed information on the conditions for the treatment resumption and to provide precautions so that appropriateness of resuming sirolimus be evaluated by carefully weighing the benefits against the risks. Also, the post-marketing surveillance should be performed to collect sufficient safety information related to interstitial lung disease, including the safety after resumption of administration after occurrence of interstitial lung disease, and thereby safety measures should be upgraded continuously.

4.(iii).B.(2).2) Infectious diseases
The immunosuppressive effect of sirolimus may increase the risk of causing or aggravating infectious diseases induced by bacteria, fungi, viruses, and protozoan, and opportunistic infections due to activation of latent viral infection. The applicant explained the occurrence of infectious diseases as follows:
Table 29 shows the incidences of infectious diseases in Studies MILES, MLSTS, and studies in organ transplant patients. The incidence of infectious diseases in Study MILES was 69.6% (32 of 46 subjects) in the sirolimus group and 67.4% (29 of 43 subjects) in the placebo group, showing similar results in both groups. In Study MLSTS, the main adverse events observed were

nasopharyngitis in 55.6% (35 of 63 subjects) and upper respiratory tract inflammation in 47.6% (30 of 63 subjects). In studies in organ transplant patients, influenza, oral herpes, and wound infection were observed in the sirolimus group more frequently than in the placebo group.

Table 29. Incidence of infectious diseases

Adverse event	Study MILES		Study MLSTS	Studies in organ transplant patients		
	Sirolimus group (N = 46)	Placebo group (N = 43)	Patients treated with sirolimus (N = 63)	Sirolimus group [a)] (N = 3272)	Placebo group [a)] (N = 284)	Comparator group [b)] (N = 668)
Urinary tract infection	0	0	0	956 (29.2)	91 (32.0)	133 (19.9)
Upper respiratory tract infection	0	0	0	712 (21.8)	60 (21.1)	106 (15.9)
Nasopharyngitis	0	0	35 (55.6)	324 (9.9)	22 (7.7)	70 (10.5)
Bronchitis	0	0	12 (19.0)	229 (7.0)	9 (3.2)	36 (5.4)
Influenza	0	0	2 (3.2)	235 (7.2)	16 (5.6)	58 (8.7)
Oral herpes	0	0	3 (4.8)	173 (5.3)	4 (1.4)	13 (1.9)
Wound infection	0	0	0	173 (5.3)	12 (4.2)	12 (1.8)
Postoperative wound infection	0	0	0	125 (3.8)	13 (4.6)	4 (0.6)
Cytomegalovirus infection	0	0	0	110 (3.4)	12 (4.2)	25 (3.7)
Viral infection	0	0	0	92 (2.8)	5 (1.8)	9 (1.3)
Infection[c)]	32 (69.6)	29 (67.4)	0	84 (2.6)	3 (1.1)	19 (2.8)
Device related infection	0	0	0	74 (2.3)	6 (2.1)	8 (1.2)
Upper respiratory tract inflammation	0	0	30 (47.6)	1 (0.0)	0	0

Number of patients (%)
a) Azathioprine, cyclosporine, corticosteroid, etc., were concomitantly administered both in the sirolimus group and the placebo group.
b) Cyclosporine, cyclosporine + tacrolimus, mycophenolate mofetil, and azathioprine
c) In Study MILES, various infections were tabulated as "infection."

Table 30 shows the incidences of serious infectious diseases[46] in Study MILES, Study MLSTS, and studies in organ transplant patients. The incidence of serious infectious diseases in Study MILES was 4.3% (2 of 46 subjects) in the sirolimus group and 7.0% (3 of 43 subjects) in the placebo group, showing similar results in both groups, but a causal relationship between disease and the investigational product could not be ruled out in 2 patients in the sirolimus group. In Study MLSTS, bronchitis, pyelonephritis acute, herpes zoster, and pneumonia were observed in 1.6% (1 of 63 subjects), respectively. In studies in organ transplant patients, the incidence of serious infectious diseases in the sirolimus group was higher than that in the placebo group but similar to that in the comparator group.

The Warnings and Precautions sections (Careful Administration, Important Precautions, Clinically significant adverse reactions) of the package insert will include precautionary statements regarding infectious diseases, including the risks for opportunistic infections such as reactivation of hepatitis B virus, BK virus-associated nephropathy, and progressive multifocal leukoencephalopathy (PML).

[46] Among serious adverse events, those classified as "Infections and infestations" in SOC

Table 30. Incidence of serious infectious diseases (those reported by ≥10 patients in any group)

Adverse events	Study MILES		Study MLSTS	Studies in organ transplant patients		
	Sirolimus group (N = 46)	Placebo group (N = 43)	Patients treated with sirolimus (N = 63)	Sirolimus group [a)] (N = 3272)	Placebo group [a)] (N = 284)	Comparator group [b)] (N = 668)
Cytomegalovirus infection	0	0	0	10 (0.3)	0	13 (1.9)
Gastroenteritis	0	0	0	40 (1.2)	0	12 (1.8)
Infection[c)]	2 (4.3)	3 (7.0)	0	16 (0.5)	0	2 (0.3)
Bronchopneumonia	0	0	0	10 (0.3)	0	3 (0.4)
Respiratory tract infection	0	0	0	10 (0.3)	0	2 (0.3)
Pyelonephritis acute	0	0	1 (1.6)	11 (0.3)	0	5 (0.7)
Upper respiratory tract infection	0	0	0	10 (0.3)	0	4 (0.6)
Pneumonia	0	0	1 (1.6)	96 (2.9)	1 (0.4)	21 (3.1)
Pyelonephritis	0	0	0	25 (0.8)	0	7 (1.0)
Urinary tract infection	0	0	0	72 (2.2)	0	25 (3.7)
Sepsis	0	0	0	51 (1.6)	0	9 (1.3)
Cellulitis	0	0	0	22 (0.7)	0	8 (1.2)

Number of patients (%)
a) Azathioprine, cyclosporine, corticosteroid, etc., were concomitantly administered both in the sirolimus group and the placebo group.
b) Cyclosporine, cyclosporine + tacrolimus, mycophenolate mofetil, and azathioprine
c) In Study MILES, various infections were tabulated as "infection."

PMDA considers as follows:
Study MILES in patients with LAM did not show any significant difference in the incidence of infectious diseases or serious infectious diseases between the sirolimus group and the placebo group. However, since sirolimus has an immunosuppressive effect, due caution should be exercised against the occurrence of infectious diseases. In fact, the incidence of serious infectious diseases in studies in organ transplant patients tended to be higher in the sirolimus group than in the placebo group. In addition, patients with LAM are considered to be prone to respiratory tract infection because of the disease conditions. Therefore, patients should be closely monitored for the occurrence of infectious diseases during treatment with sirolimus. Thus, special precautionary statements regarding this risk should be included in the package insert and other materials, as is the case with other immunosuppressants. Also, the occurrence of infectious diseases during treatment with sirolimus should be continuously investigated in the post-marketing surveillance.

4.(iii).B.(2).3) Fluid retention and poor wound healing
mTOR inhibitors are known to inhibit the production of some growth factors that may affect the vascular permeability, neovascularisation, and fibroblast growth. The inhibitory effect of sirolimus on VEGF production was shown in the pharmacological studies of sirolimus as well [see "3.(i) Summary of pharmacology studies"]. The applicant explained the occurrence of fluid retention and poor wound healing, adverse events possibly associated with these pharmacological effects of sirolimus, as follows:
Table 31 shows the incidences of fluid retention and poor wound healing in Study MILES, Study MLSTS, and studies in organ transplant patients. The incidences of oedema peripheral and pericardial effusion tended to be higher in the sirolimus group than in the placebo group in Study MILES, while the incidences were similar between the sirolimus and placebo groups in studies in organ transplant patients. In Study MLSTS, oedema peripheral (6.3% [4 of 63 subjects]) and pleural effusion (6.3% [4 of 63 subjects]) were observed as the main adverse events.

Poor healing was observed in 3.2% (2 of 63 subjects) in Study MLSTS, and in studies in organ transplant patients, the incidence of poor healing tended to be higher in the sirolimus group than

in the placebo group.

Precautionary statements regarding pericardial effusion will be provided in the package insert. Meanwhile, lung transplant is indicated for patients with LAM with advanced symptoms, and poor wound healing is suggested as an adverse event associated with sirolimus, with reported cases of fatal bronchial anastomotic dehiscence in non-Japanese lung transplant patients treated with sirolimus. Therefore the applicant plans to provide precautionary statements in the package insert to raise awareness about poor wound healing and to ensure a sufficiently long washout period for sirolimus before lung transplant in patients with LAM.

Table 31. Incidences of fluid retention and poor wound healing

Adverse event	Study MILES		Study MLSTS	Studies in organ transplant patients		
	Sirolimus group (N = 46)	Placebo group (N = 43)	Patients treated with sirolimus (N = 63)	Sirolimus group [a] (N = 3272)	Placebo group [a] (N = 284)	Comparator group [b] (N = 668)
Oedema peripheral	11 (23.9)	7 (16.3)	4 (6.3)	1670 (51.0)	177 (62.3)	135 (20.2)
Pleural effusion	2 (4.3)	0	4 (6.3)	127 (3.9)	23 (8.1)	9 (1.3)
Poor healing	0	0	2 (3.2)	116 (3.5)	4 (1.4)	11 (1.6)
Ascites	0	0	3 (4.8)	68 (2.1)	5 (1.8)	3 (0.4)
Localised oedema	1 (2.2)	0	0	61 (1.9)	3 (1.1)	1 (0.1)
Eyelid oedema	0	0	2 (3.2)	42 (1.3)	1 (0.4)	2 (0.3)
Fluid retention	0	0	0	41 (1.3)	5 (1.8)	0
Pericardial effusion	3 (6.5)	2 (4.7)	0	28 (0.9)	0	0
Lymphoedema	0	0	0	18 (0.6)	0	0

Number of patients (%)
a) Azathioprine, cyclosporine, corticosteroid, etc., were concomitantly administered both in the sirolimus group and the placebo group.
b) Cyclosporine, cyclosporine + tacrolimus, mycophenolate mofetil, and azathioprine

PMDA considers as follows:
Fluid retention-related adverse events including oedema peripheral are predicted from the pharmacological action of sirolimus and are actually observed relatively frequently after administration of sirolimus. Therefore, close attention should be continuously paid to such adverse events in the post-marketing surveillance, etc. Poor wound healing is also an adverse event possibly related to the pharmacological action of sirolimus, and poor wound healing after lung transplant may be fatal. Therefore, healthcare professionals should be strongly cautioned to carefully consider the necessity of sirolimus washout before lung transplant, as proposed by the applicant.

4.(iii).B.(2).4) Dyslipidaemia
Studies in organ transplant patients suggest that increased serum cholesterol and increased serum triglycerides requiring treatment are related to administration of sirolimus. The applicant explained the occurrence of dyslipidaemia as follows:
Table 32 shows the incidence of dyslipidemia in Study MILES, Study MLSTS, and studies in organ transplant patients. In Study MILES, the incidences of hypercholesterolaemia and hypertriglyceridaemia tended to be higher in the sirolimus group than in the placebo group and, also in Study MLSTS, hypercholesterolaemia was observed in 3.2% (2 of 63 subjects) and hypertriglyceridaemia in 1.6% (1 of 63 subjects). In studies in organ transplant patients, the incidences of hypercholesterolaemia, hypertriglyceridaemia, and hyperlipidaemia tended to be higher in the sirolimus group than in the placebo group and the comparator group.

Table 32. Incidence of dyslipidaemia

Adverse event	Study MILES		Study MLSTS	Studies in organ transplant patients		
	Sirolimus group (N = 46)	Placebo group (N = 43)	Patients treated with sirolimus (N = 63)	Sirolimus group [a] (N = 3272)	Placebo group [a] (N = 284)	Comparator group [b] (N = 668)
Hypercholesterolaemia	9 (19.6)	6 (14.0)	2 (3.2)	1077 (32.9)	62 (21.8)	91 (13.6)
Hypertriglyceridaemia	4 (8.7)	2 (4.7)	1 (1.6)	752 (23.0)	20 (7.0)	96 (14.4)
Hyperlipidaemia	0	0	2 (3.2)	539 (16.5)	36 (12.7)	44 (6.6)
Blood cholesterol increased	0	0	3 (4.8)	419 (12.8)	35 (12.3)	13 (1.9)
Dyslipidaemia	0	0	4 (6.3)	105 (3.2)	2 (0.7)	21 (3.1)
Type II hyperlipidaemia	0	0	0	2 (0.1)	0	0
Lipids abnormal	0	0	1 (1.6)	0	0	0

Number of patients (%)
a) Azathioprine, cyclosporine, corticosteroid, etc., were concomitantly administered both in the sirolimus group and the placebo group.
b) Cyclosporine, cyclosporine + tacrolimus, mycophenolate mofetil, and azathioprine

In Study MILES, the change (mean ± SD) in total cholesterol level at 12 months after the beginning of treatment from baseline was 12.5 ± 22.5 mg/dL (n = 41) in the sirolimus group and 1.5 ± 26.9 mg/dL (n = 35) in the placebo group, and the change in triglycerides from baseline was 27.3 ± 42.5 mg/dL (n = 41) in the sirolimus group and -5.6 ± 31.8 mg/dL (n = 35) in the placebo group, showing increased total cholesterol and triglyceride levels in the sirolimus group compared with those in the placebo group.

The applicant plans to provide precautionary statements in Precautions (Important Precautions, Clinically significant adverse reactions) of the package insert regarding the monitoring of lipid-related test values and as-needed administration of lipid-lowering drugs.

PMDA considers as follows:
Dyslipidaemia was observed more frequently in the sirolimus group than in the placebo group and, in studies in organ transplant patients, some patients required treatment with lipid-lowering drugs. Therefore, healthcare professionals should be cautioned to periodically monitor lipid-related laboratory values and to take appropriate measures such as administration of lipid-lowering drugs as necessary, as proposed by the applicant. At present, the relationship between sirolimus and cardiovascular adverse events is unclear. However, since sirolimus is expected to be administered to patients with LAM over a long-term period, the possibility cannot be excluded that abnormalities in lipid-related laboratory values caused by long-term sirolimus therapy may increase the risk of cardiovascular events. In fact, in studies in organ transplant patients, 1 patient died of myocardial infarction for which a causal relationship with sirolimus could not be ruled out. Therefore, detailed information about cardiovascular events reported to in the post-marketing surveillance should be collected, including the time-course change of lipid-related laboratory values during sirolimus therapy.

4.(iii).B.(2).5) Lymphoma and other malignant tumors
In carcinogenicity studies in mice, neoplastic changes and lymphoma, etc., suspected to be associated with the immunosuppressive effect of sirolimus, were observed [see "3.(iii) Summary of toxicology studies"]. The applicant explained the occurrence of lymphoma and other malignant tumors as follows:
Table 33 shows the incidences of lymphoma and other malignant tumors in Study MILES, Study MLSTS, and studies in organ transplant patients. In Studies MILES and MLSTS, neither lymphoma nor any other malignant tumors were observed. In studies in organ transplant patients, malignant skin tumors such as basal cell carcinoma, squamous cell carcinoma, and squamous cell carcinoma of skin, as well as lymphoma were observed. The incidences of these tumors were

similar to those in the placebo group and the comparator group, showing no tendency of increase in the incidence of malignant tumors in the sirolimus group.

The applicant plans to provide precautionary statements in the package insert, etc., regarding malignant lymphoma and malignant tumors.

Table 33. Incidences of lymphoma and other malignant tumors

Adverse event	Study MILES		Study MLSTS	Studies in organ transplant patients		
	Sirolimus group (N = 46)	Placebo group (N = 43)	Patients treated with sirolimus (N = 63)	Sirolimus group [a] (N = 3272)	Placebo group [a] (N = 284)	Comparator group [b] (n = 668)
Basal cell carcinoma	0	0	0	80 (2.4)	9 (3.2)	23 (3.4)
Squamous cell carcinoma	0	0	0	48 (1.5)	5 (1.8)	29 (4.3)
Squamous cell carcinoma of skin	0	0	0	25 (0.8)	3 (1.1)	7 (1.0)
Skin cancer	0	0	0	13 (0.4)	2 (0.7)	5 (0.7)
Lymphoma	0	0	0	10 (0.3)	1 (0.4)	0
Prostate cancer	0	0	0	10 (0.3)	0	3 (0.4)
Renal cell carcinoma	0	0	0	8 (0.2)	0	0
Pseudolymphoma	0	0	0	6 (0.2)	0	0
Basosquamous carcinoma	0	0	0	4 (0.1)	0	3 (0.4)
Colon cancer	0	0	0	4 (0.1)	0	2 (0.3)

Number of patients (%)
a) Azathioprine, cyclosporine, corticosteroid, etc., were concomitantly administered both in the sirolimus group and the placebo group.
b) Cyclosporine, cyclosporine + tacrolimus, mycophenolate mofetil, and azathioprine

PMDA considers as follows:
The risk of sirolimus-induced malignant tumors, such as lymphoma, cannot be excluded given the drug's immunosuppressive effect, and sirolimus is expected to be administered to patients with LAM over a long-term period. Therefore the trend of the occurrence of lymphoma and other malignant tumors should be continuously monitored in the post-marketing surveillance, etc.

4.(iii).B.(2).6) Gastrointestinal disorders
Toxicity studies in monkeys showed gastrointestinal disorders including diarrhoea and vomiting that were considered to be related to the immunosuppressive effect of sirolimus [see "3.(iii) Summary of toxicology studies"]. The applicant explained the occurrence of gastrointestinal disorders as follows:
Table 34 shows the incidences of gastrointestinal disorders in Study MILES, Study MLSTS, and studies in organ transplant patients. Colitis was not reported in any of the clinical studies, whereas the incidences of diarrhoea, constipation, and nausea were high in all of the studies. The incidence of serious gastrointestinal disorders was 2.2% (1of 46 subjects) in the sirolimus group and 2.3% (1 of 43 subjects) in the placebo group in Study MILES; 4.8% (3 of 63 subjects) in Study MLSTS; and 6.1% (199 of 3272 subjects) in the sirolimus group, 0% (0 of 284 subjects) in the placebo group, and 9.4% (63 of 668 subjects) in the comparator group in studies in organ transplant patients. Thus, in studies in organ transplant patients, the incidence in the sirolimus group was higher than in the placebo group but similar to that in the comparator group.

The applicant plans to provide precautionary statements in the package insert, etc., regarding gastrointestinal disorders frequently observed during treatment with sirolimus including stomatitis, nausea, diarrhoea, and vomiting.

Table 34. Incidences of gastrointestinal disorders (those reported by ≥10% of patients in any group)

Adverse events	Study MILES		Study MLSTS	Studies in organ transplant patients		
	Sirolimus group (N = 46)	Placebo group (N = 43)	Patients treated with sirolimus (N = 63)	Sirolimus group [a] (N = 3272)	Placebo group [a] (N = 284)	Comparator group [b] (N = 668)
Nausea	18 (39.1)	11 (25.6)	8 (12.7)	861 (26.3)	130 (45.8)	83 (12.4)
Gastrointestinal disorder	13 (28.3)	13 (30.2)	0	30 (0.9)	4 (1.4)	5 (0.7)
Diarrhoea	29 (63.0)	14 (32.6)	22 (34.9)	1274 (38.9)	84 (29.6)	162 (24.3)
Cheilitis	1 (2.2)	0	9 (14.3)	21 (0.6)	1 (0.4)	1 (0.1)
Stomatitis	31 (67.4)	28 (65.1)	56 (88.9)	220 (6.7)	6 (2.1)	19 (2.8)
Dyspepsia	5 (10.9)	4 (9.3)	1 (1.6)	354 (10.8)	63 (22.2)	33 (4.9)
Abdominal pain	0	0	7 (11.1)	543 (16.6)	59 (20.8)	68 (10.2)
Vomiting	5 (10.9)	5 (11.6)	7 (11.1)	705 (21.5)	97 (34.2)	96 (14.4)
Abdominal pain upper	0	0	8 (12.7)	202 (6.2)	32 (11.3)	40 (6.0)
Abdominal distension	1 (2.2)	1 (2.3)	1 (1.6)	359 (11.0)	57 (20.1)	20 (3.0)
Constipation	3 (6.5)	1 (2.3)	8 (12.7)	698 (21.3)	106 (37.3)	66 (9.9)
Gastroenteritis	0	0	8 (12.7)	211 (6.4)	14 (4.9)	39 (5.8)

Number of patients (%)
a) Azathioprine, cyclosporine, corticosteroid, etc., were concomitantly administered both in the sirolimus group and the placebo group.
b) Cyclosporine, cyclosporine + tacrolimus, mycophenolate mofetil, and azathioprine

PMDA considers as follows:
In Study MILES in patients with LAM, adverse events of gastrointestinal system, such as diarrhoea and nausea, were observed more frequently in the sirolimus group than in the placebo group. Since these events are likely to affect the medication compliance of patients, the trend of the occurrence of gastrointestinal events should be closely monitored in the post-marketing surveillance, etc.

4.(iii).B.(2).7) Hyperglycaemia

Toxicology studies in rats showed findings suggestive of hyperglycaemia and accompanying diabetes-like symptoms [see "3.(iii) Summary of toxicology studies"]. mTOR inhibitors everolimus and temsirolimus are also known to cause hyperglycaemia in patients treated with these drugs. The applicant explained the occurrence of glucose tolerance-related adverse events including hyperglycaemia as follows:
Table 35 shows the incidences of glucose tolerance-related adverse events in Study MILES, Study MLSTS, and studies in organ transplant patients. In Study MILES, the incidence of glucose tolerance-related adverse events was similar between the sirolimus group and the placebo group. In Study MLSTS, no such adverse events were observed. In studies in organ transplant patients, the incidences of hyperglycaemia and blood glucose increased, etc., in the sirolimus group tended to be higher than those in the compactor group, but similar to those in the placebo group.

Table 35. Incidences of glucose tolerance-related adverse events (those reported by ≥2% of patients in any group)

Adverse events	Study MILES		Study MLSTS	Studies in organ transplant patients		
	Sirolimus group (N = 46)	Placebo group (N = 43)	Patients treated with sirolimus (N = 63)	Sirolimus group [a)] (N = 3272)	Placebo group [a)] (N = 284)	Comparator group [b)] (N = 668)
Hyperglycaemia	2 (4.3)	2 (4.7)	0	486 (14.9)	53 (18.7)	55 (8.2)
Hypoglycaemia	2 (4.3)	0	0	124 (3.8)	11 (3.9)	11 (1.6)
Glucose tolerance impaired	0	1 (2.3)	0	24 (0.7)	2 (0.7)	1 (0.1)
Diabetes mellitus	0	0	0	257 (7.9)	21 (7.4)	29 (4.3)
Blood glucose increased	0	0	0	159 (4.9)	12 (4.2)	17 (2.5)
Cataract	0	0	0	136 (4.2)	13 (4.6)	11 (1.6)
Glycosuria	0	0	0	56 (1.7)	8 (2.8)	0

Number of patients (%)
a) Azathioprine, cyclosporine, corticosteroid, etc., were concomitantly administered both in the sirolimus group and the placebo group.
b) Cyclosporine, cyclosporine + tacrolimus, mycophenolate mofetil, and azathioprine

In Study MILES, the change (mean ± SD) in blood glucose at 12 months after the beginning of treatment from baseline was -2.3 ± 12.8 mg/dL (n = 41) in the sirolimus group and 1.1 ± 7.9 mg/dL (n = 35) in the placebo group.

PMDA considers as follows:
Currently available data do not suggest the necessity of providing precautionary statements in the package insert, etc., regarding the monitoring of blood glucose level. However, since hyperglycaemia was observed in non-clinical studies and with a drug of the same class, data on blood glucose level should be collected in the post-marketing surveillance, thereby to further investigate the risk of sirolimus-induced hyperglycaemia.

4.(iii).B.(2).8) Renal disorder
mTOR inhibitors everolimus and temsirolimus are known to increase blood creatinine level. The applicant explained the occurrence of kidney-related adverse events as follows:
Table 36 shows the incidences of kidney-related adverse events in Study MILES, Study MLSTS, and studies in organ transplant patients. In Study MILES, the incidence of kidney-related adverse events was 21.7% (10 of 46 subjects) in the sirolimus group and 11.6% (5 of 43 subjects) in the placebo group, showing a higher tendency in the sirolimus group, whereas in Study MLSTS, the incidence (12.7% [8 of 63 subjects]) was similar to that in the placebo group of Study MILES. In studies in organ transplant patients, the incidence of kidney-related adverse events was 63.2% (2068 of 3272 subjects) in the sirolimus group, 66.5% (189 of 284 subjects) in the placebo group, and 46.6% (311 of 668 subjects) in the comparator group, showing a higher tendency in the sirolimus group compared with the comparator group, whereas the incidence was similar between the sirolimus group and the placebo group.

Table 36. Incidence of kidney-related adverse events (those reported by ≥2% of patients in any group)

Adverse events	Study MILES		Study MLSTS	Studies in organ transplant patients		
	Sirolimus group (N = 46)	Placebo group (N = 43)	Patients receiving sirolimus (N = 63)	Sirolimus group [a] (N = 3272)	Placebo group [a] (N = 284)	Comparator group [b] (N = 668)
Urogenital disorder	5 (10.9)	3 (7.0)	0	0	0	0
Proteinuria	3 (6.5)	1 (2.3)	2 (3.2)	487 (14.9)	26 (9.2)	68 (10.2)
Urogenital haemorrhage	3 (6.5)	0	0	0	0	0
Blood creatinine increased	0	1 (2.3)	0	1056 (32.3)	104 (36.6)	121 (18.1)
Asymptomatic bacteriuria	0	0	2 (3.2)	2 (0.1)	0	2 (0.3)
Protein urine	0	0	2 (3.2)	1 (<0.1)	0	0
Renal tubular necrosis	0	0	0	446 (13.6)	43 (15.1)	8 (1.2)
Haematuria	0	0	0	427 (13.1)	47 (16.5)	33 (4.9)
Complications of transplanted kidney	0	0	0	367 (11.2)	27 (9.5)	38 (5.7)
Hydronephrosis	0	0	0	123 (3.8)	12 (4.2)	6 (0.9)
Renal impairment	0	0	0	114 (3.5)	7 (2.5)	45 (6.7)
Blood urea increased	0	0	0	112 (3.4)	17 (6.0)	26 (3.9)
Renal failure acute	0	0	0	106 (3.2)	2 (0.7)	13 (1.9)
Pyelonephritis	0	0	0	78 (2.4)	7 (2.5)	9 (1.3)
Renal failure	0	0	0	75 (2.3)	4 (1.4)	19 (2.8)
Renal pain	0	0	0	48 (1.5)	10 (3.5)	3 (0.4)
Renal graft loss	0	0	0	40 (1.2)	2 (0.7)	22 (3.3)

Number of patients (%)
a) Azathioprine, cyclosporine, corticosteroid, etc., were concomitantly administered both in the sirolimus group and the placebo group.
b) Cyclosporine, cyclosporine + tacrolimus, mycophenolate mofetil, and azathioprine

In Study MILES, the change (mean ± SD) in blood creatinine level at 12 months after the start of treatment from baseline was -0.05 ± 0.16 mg/dL (n = 41) in the sirolimus group and -0.02 ± 0.08 mg/dL (n = 35) in the placebo group.

The applicant plans to provide precautionary statements in the package insert to take appropriate measures including discontinuation of administering sirolimus in case renal disorder is observed.

PMDA considers as follows:
The results of clinical studies did not suggest any increase in blood creatinine induced by administration of sirolimus. However, the incidence of urine protein tended to be higher in the sirolimus group than in the placebo group in both Study MILES in patients with LAM and the studies in organ transplant patients. Furthermore, Study 316-GL, one of the studies in organ transplant patients, showed a higher incidence of nephrotic syndrome, including new-onset cases, in the sirolimus group (2.2% [11 of 493 subjects]) than in the comparator group treated with an immunosuppressive drug (0.8% [2 of 244 subjects]). Therefore healthcare professionals should be cautioned, through the package insert, etc., to periodically monitor protein urine, and the risk of sirolimus-induced nephritis, etc. should be investigated in the post-marketing surveillance, etc.

4.(iii).B.(2).9) Skin disorder and hypersensitivity reaction
Studies in organ transplant patients suggest that anaphylaxis/anaphylactic reaction, angioedema, dermatitis exfoliative, hypersensitivity vasculitis, etc., may be associated with administration of sirolimus. The applicant explained the occurrence of skin disorder and hypersensitivity reaction as follows:
Table 37 shows the incidences of skin disorders and hypersensitivity reactions in Study MILES, Study MLSTS, and studies in organ transplant patients. In Study MILES, the incidences of acne and skin disorder tended to be higher in the sirolimus group than in the placebo group. In Study

MLSTS, rash (30.2% [19 of 63 subjects]), dermatitis acneiform (28.6% [18 of 63 subjects]), and acne (17.5% [11 of 63 subjects]) were observed as the main adverse events. In studies in organ transplant patients, the incidence of rash tended to be higher in the sirolimus group than in the placebo group.

The applicant plans to provide precautionary statements in the package insert, etc. regarding anaphylaxis and skin disorders including acne and rash, which are often observed during treatment with sirolimus.

Table 37. Incidences of skin disorder and hypersensitivity reaction

Adverse event	Study MILES		Study MLSTS	Studies in organ transplant patients		
	Sirolimus group (N = 46)	Placebo group (N = 43)	Patients treated with sirolimus (N = 63)	Sirolimus group [a)] (N = 3272)	Placebo group [a)] (N = 284)	Comparator group [b)] (N = 668)
Acne	20 (43.5)	5 (11.6)	11 (17.5)	631 (19.3)	51 (18.0)	32 (4.8)
Dermatitis acneiform	0	0	18 (28.6)	18 (0.6)	1 (0.4)	2 (0.3)
Pruritus	3 (6.5)	5 (11.6)	5 (7.9)	238 (7.3)	21 (7.4)	29 (4.3)
Exfoliative rash	6 (13.0)	7 (16.3)	1 (1.6)	2 (0.1)	0	0
Skin disorder	19 (41.3)	8 (18.6)	0	31 (0.9)	5 (1.8)	5 (0.7)
Rash	0	0	19 (30.2)	360 (11.0)	11 (3.9)	33 (4.9)
Urticaria	1 (2.2)	0	0	27 (0.8)	2 (0.7)	1 (0.1)
Hypersensitivity	0	2 (4.7)	0	26 (0.8)	1 (0.4)	0
Drug hypersensitivity	0	0	0	19 (0.6)	1 (0.4)	3 (0.4)
Angioedema	0	0	0	17 (0.5)	0	0
Dermatitis exfoliative	0	0	0	7 (0.2)	0	0
Anaphylactic reaction	0	0	0	4 (0.1)	0	0
Anaphylactic shock	0	0	0	1 (<0.1)	0	0
Leukocytoclastic vasculitis	0	0	0	1 (<0.1)	0	0

Number of patients (%)
a) Azathioprine, cyclosporine, corticosteroid, etc., were concomitantly administered in both the sirolimus group and the placebo group.
b) Cyclosporine, cyclosporine + tacrolimus, mycophenolate mofetil, and azathioprine

PMDA considers as follows:
In clinical studies, skin disorders including acne and rash tended to occur more frequently in the sirolimus group than in the placebo group. In Study MILES, the incidence of acne tended to be higher in the Japanese subpopulation than in the entire patient population [see "4.(iii).A *Summary of the submitted data*"], which suggests the possibility that the risk of skin disorder may be higher in Japanese patients with LAM. Therefore, the trend of the occurrence of skin disorder should be monitored continuously in the post-marketing surveillance.

4.(iii).B.(3) Dosage and administration
The applicant explained the justification for the dosage and administration employed in Studies MILES and MLSTS and for the proposed dosage and administration, as follows:
The dosage and administration of sirolimus in Studies MILES and MLSTS were determined based on the dosage and administration used in Study CAST in patients with AML and a diagnosis of TSC or S-LAM, and on the results of the investigation of the therapeutic concentration range in Study 301-US, one of the studies in organ transplant patients.

In Study CAST, the dosage and administration were determined by referring to the dosage and administration approved for the prophylaxis of renal transplant rejection in other countries. Thus, treatment started at 0.25 mg/m^2/day (corresponding to 0.5-1 mg of sirolimus, target trough concentration 1-5 ng/mL). If the longest coronal plane dimension of AML did not decrease by ≥10% at 2 months after the beginning of treatment from baseline, the dose was increased to 0.5 mg/m^2/day (corresponding to 1-2 mg of sirolimus, target trough concentration 5-10 ng/mL). If the

above decrease was not achieved even at 4 months after the beginning of treatment, the dose was further increased to 1 to 3 mg/m^2/day (corresponding to 2-6 mg of sirolimus, target trough concentration 10-15 ng/mL). The treatment duration was set at 12 months. The longest coronal plane dimension of AML at 12 months after the beginning of treatment, expressed as a percentage relative to baseline, was 53.2% ± 26.6% (n = 20). In patients with LAM who underwent evaluation for respiratory function (n = 11), the change in FEV_1 at 12 months after the beginning of treatment from baseline was 0.12 ± 0.33 L, and the change in FVC from baseline was 0.39 ± 0.57 L. In 19 of 20 patients in this study, the dose was increased until the trough whole blood sirolimus concentration reached 10 to 15 ng/mL (corresponding to 2-6 mg of sirolimus). No treatment discontinuation due to adverse events occurred within the trough whole blood sirolimus concentration range of 5 to 15 ng/mL (corresponding to 1-6 mg of sirolimus), except 1 case of treatment discontinuation due to diarrhoea.

In Study 301-US, sirolimus (2 or 5 mg/day) or azathioprine [47] was orally administered concomitantly with cyclosporine or corticosteroid for 24 months. The results of this study suggested that the risk of acute rejection increased ≥4-fold if the trough whole blood concentration of sirolimus was <2.70 ng/mL, indicating that the trough whole blood concentration of sirolimus should be maintained at 5 to 10 ng/mL in order to suppress the incidence of acute rejection to approximately 15%. In the sirolimus 5 mg/day group, the incidences of adverse events including herpes simplex infection, thrombocytopenia, and serum lipid increased were higher compared with those in the sirolimus 2 mg/day group and the azathioprine group. In the sirolimus 5 mg/day group, the rate of treatment discontinuation due to adverse events and other reasons was 21.2% (57 of 269 subjects) and 15.6% (42 of 269 subjects), respectively, which was higher than that in the sirolimus 2 mg/day group (11.7% [33 of 281 subjects] and 9.3% [26 of 281 subjects], respectively) and the azathioprine group (13.1% [21 of 160 subjects] and 7.5% [12 of 160 subjects] , respectively). The mean blood trough concentration of sirolimus (mean ± SD) was 9.1 ± 3.9 ng/mL in the sirolimus 2 mg/day group and 18.4 ± 7.3 ng/mL in the sirolimus 5 mg/day group. From the above results, it was considered appropriate to set the upper limit of the therapeutic concentration range at 15 ng/mL.

Based on the above, for the dosage and administration of sirolimus in Studies MILES and MLSTS, treatment was to be started at 2 mg once daily, and the dose was to be adjusted to maintain the trough whole blood sirolimus concentration within the range from 5 to 15 ng/mL.

It was considered appropriate to set the proposed dosage and administration according to the dosage and administration used in Studies MILES and MLSTS. Therapeutic drug monitoring (TDM) need not be mandatory based on the results of the following investigation.

The distribution of blood concentration classified by daily dose of sirolimus in Studies MILES and MLSTS is shown in Figure 4; the trough whole blood sirolimus concentration following administration of sirolimus at 2 mg/day was thus expected to be within the therapeutic range (5-15 ng/mL) in most of the patients treated.

[47] Starting dose 5 mg/kg, maintenance dose 2 to 3 mg/day

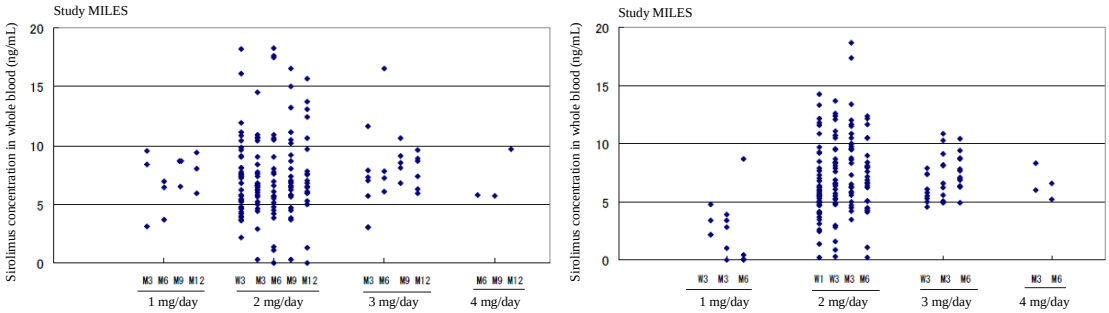


Figure 4. Distribution of trough whole blood sirolimus concentration by daily dose of sirolimus (left, Study MILES; right, Study MLSTS)
(W3, 3 weeks after the beginning of treatment; M3, 3 months after the beginning of treatment; M6, 6 months after the beginning of treatment; M9, 9 months after the beginning of treatment; M12, 12 months after the beginning of treatment)

As regards the relationship between trough whole blood sirolimus concentration and safety, adverse events by trough whole blood sirolimus concentration in Studies MILES and MLSTS are shown in Table 38. The incidence of all adverse events tended to be lower in the population with mean trough whole blood sirolimus concentration of <5 ng/mL than in other populations. In the population with trough whole blood concentration of ≥5 ng/mL, the incidence of all adverse events did not tend to significantly increase with the increase in trough whole blood concentration. Similarly, in Study 301-US in organ transplant patients, the incidence of adverse events was similar regardless of the trough whole blood concentration. Oedema peripheral and blood creatinine increased were mainly observed in the population with trough whole blood concentration of ≥15 ng/mL, but the incidences of these adverse events did not tend to increase with the increase in the trough concentration (oedema peripheral, 76.5% [13 of 17 subjects] at <5 ng/mL, 69.0% [100 of 145 subjects] at ≥5 ng/mL and <10 ng/mL, 69.4% [100 of 144 subjects] at ≥10 ng/mL and <15 ng/mL, 73.6% [131 of 178 subjects] at ≥15 ng/mL; blood creatinine increased, 70.6% [12 of 17 subjects] at <5 ng/mL, 37.2% [54 of 145 subjects] at ≥5 ng/mL and <10 ng/mL, 44.4% [64 of 144 subjects] at ≥10 ng/mL and <15 ng/mL, 45.5% [81 of 178 subjects] at ≥15 ng/mL). The above results suggested that trough concentrations exceeding the upper limit of the therapeutic concentration range (15 ng/mL) were unlikely to significantly change the safety profile observed within the therapeutic concentration range.

Table 38. Adverse events by trough whole blood sirolimus concentration [a)] (those reported by ≥20% of patients in any population)

Adverse event	Study MILES					Study MLSTS			
	Sirolimus group				Placebo group (N = 43)	Patients treated with sirolimus			
	<5 ng/mL (N = 36)	≥5 ng/mL and <10 ng/mL (N = 40)	≥10 ng/mL and <15 ng/mL (N = 18)	≥15 ng/mL (N = 9)		<5 ng/mL (N = 46)	≥5 ng/mL and <10 ng/mL (N = 62)	≥10 ng/mL and <15 ng/mL (N = 20)	≥15 ng/mL (N = 3)
All adverse events	29 (80.6)	40 (100.0)	16 (88.9)	9 (100.0)	43 (100)	36 (78.3)	60 (96.8)	18 (90.0)	3 (100.0)
Nausea	6 (16.7)	6 (15.0)	3 (16.7)	0	11(25.6)	2 (4.3)	4 (6.5)	1 (5.0)	0
Gastrointestinal disorder	5 (13.9)	9 (22.5)	1 (5.6)	0	13 (30.2)	0	0	0	0
Diarrhoea	7 (19.4)	17 (42.5)	5 (27.8)	1 (11.1)	14 (32.6)	7 (15.2)	12 (19.4)	3 (15.0)	0
Stomatitis	8 (22.2)	23 (57.5)	6 (33.3)	3 (33.3)	28 (65.1)	22 (47.8)	42 (67.7)	8 (40.0)	2 (66.7)
Abdominal pain upper	0	0	0	0	0	2 (4.3)	4 (6.5)	3 (15.0)	1 (33.3)
Fatigue	4 (11.1)	5 (12.5)	2 (11.1)	0	13 (30.2)	0	0	0	0
Oedema peripheral	2 (5.6)	6 (15.0)	2 (11.1)	2 (22.2)	7 (16.3)	1 (2.2)	2 (3.2)	1 (5.0)	0
Pain	13 (36.1)	19 (47.5)	6 (33.3)	0	33 (76.7)	0	0	0	0
Infection	12 (33.3)	22 (55.0)	4 (22.2)	2 (22.2)	29 (67.4)	0	0	0	0
Nasopharyngitis	0	0	0	0	0	13 (28.3)	27 (43.5)	4 (20.0)	0
Musculoskeletal disorder	7 (19.4)	11 (27.5)	3 (16.7)	1 (11.1)	12 (27.9)	0	0	0	0
Cough	5 (13.9)	12 (30.0)	4 (22.2)	0	16 (37.2)	1 (2.2)	1 (1.6)	0	0
Dyspnoea	2 (5.6)	8 (20.0)	2 (11.1)	0	17 (39.5)	1 (2.2)	1 (1.6)	1 (5.0)	0
Respiratory disorder	9 (25.0)	18 (45.0)	3 (16.7)	4 (44.4)	26 (60.5)	0	0	0	0
Upper respiratory tract inflammation	0	0	0	0	0	8 (17.4)	21 (33.9)	5 (25.0)	1 (33.3)
Headache	0	0	0	0	0	7 (15.2)	25 (40.3)	3 (15.0)	0
Dizziness	2 (5.6)	5 (12.5)	1 (5.6)	1 (11.1)	9 (20.9)	1 (2.2)	1 (1.6)	1 (5.0)	0
Blood cholesterol increased	0	0	0	0	0	1 (2.2)	2 (3.2)	1 (5.0)	1 (33.3)
Hypertriglyceridaemia	1 (2.8)	1 (2.5)	1 (5.6)	1 (11.1)	2 (4.7)	0	1 (1.6)	0	1 (33.3)
Acne	4 (11.1)	10 (25.0)	2 (11.1)	2 (22.2)	5 (11.6)	1 (2.2)	5 (8.1)	2 (10.0)	1 (33.3)
Rash	0	0	0	0	0	7 (15.2)	13 (21.0)	1 (5.0)	1 (33.3)
Skin disorder	3 (8.3)	15 (37.5)	3 (16.7)	1 (11.1)	8 (18.6)	0	0	0	0
Aspartate aminotransferase increased	1 (2.8)	4 (10.0)	0	2 (22.2)	3 (7.0)	0	0	0	0
Laboratory test abnormal	5 (13.9)	10 (25.0)	1 (5.6)	1 (11.1)	2 (4.7)	0	0	0	0

Number of patients (%)
a) Value obtained by dividing AUC from Week 1 to Week 26 by time

Table 39 shows the trough whole blood sirolimus concentrations at the onset of adverse events in Studies MILES and MLSTS. The trough whole blood sirolimus concentrations were similar in patients who discontinued, suspended, or reduced sirolimus doses because of adverse events and in patients who continued sirolimus therapy at unchanged doses after adverse events.

The above results indicate that TDM need not be mandatory in patients with LAM who receive sirolimus therapy at the proposed dosage and administration. Thus, it is considered appropriate to administer sirolimus 2 mg once daily as the usual dose, and to adjust the dose depending on the patient condition, as appropriate. The applicant considered that TDM usually need not be performed in patients treated with sirolimus. However, if an adverse drug reaction is suspected, if a drug affecting CYP3A4 has to be used in combination with sirolimus for a long-term period, or if sirolimus is used in patients with hepatic impairment, blood concentration should be measured. Therefore, a system for blood concentration measurement has been developed to allow TDM as necessary.

Table 39. Trough whole blood sirolimus concentration immediately before and after the occurrence of adverse events in Studies MILES and MLSTS

	Study MILES		Study MLSTS	
	Entire population Discontinuation/suspension/ dose reduction	Entire population No change in administration	Japanese population Discontinuation/suspension/ dose reduction	Japanese population No change in administration
Number of patients with adverse events	20	43	42	63
Number of adverse events	119	820	84	822
Mean ± SD	7.9 ± 6.1	6.4 ± 3.3	6.5 ± 2.5	6.9 ± 3.1
Minimum	0	0	1.1	0
Maximum	19.1	18.2	13.7	27.5

PMDA considers as follows:
Because of the rarity of LAM, the dosage and administration of sirolimus has not been sufficiently investigated. However, Study MILES showed that sirolimus was effective without any significant safety problem when administered at 2 mg once daily with dose adjustment within the range between 1 to 4 mg. Therefore the following usual dosage and administration of sirolimus proposed by the applicant is acceptable: "The usual adult dosage is 2 mg if sirolimus is administered orally once daily." As regards the necessity of TDM, trough whole blood sirolimus concentrations exceeding 15 ng/mL are unlikely to substantially increase safety concerns compared with concentrations below 15 ng/mL, the upper limit in Studies MILES and MLSTS, for the following reasons: (i) in Studies MILES and MLSTS, most patients who received sirolimus 2 mg/day showed trough whole blood sirolimus concentrations within the therapeutic concentration range (5-15 ng/mL), and the safety profile did not significantly differ among trough concentrations up to 4 mg/day (dose) or 19.1 ng/mL (trough whole blood sirolimus concentration), although only a limited number of patients showed a trough whole blood concentration of ≥15 ng/mL, and (ii) in Study 301-US in organ transplant patients, which was conducted using the higher daily dose (starting dose 6 or 15 mg/day, maintenance dose 2 or 5 mg/day) than that in Studies MILES and MLSTS, no significant difference in the safety profile was observed. In the studies of organ transplant patients, the lower limit (5 ng/mL) of the therapeutic concentration range was defined to avoid fatal outcome caused by acute rejection. In patients with LAM, however, there is no need to strictly control the whole blood concentration of sirolimus at ≥5 ng/mL. Therefore, there is no need to perform periodical TDM in routine clinical usage of sirolimus in patients with LAM. However, healthcare professionals should be cautioned, through the package insert etc., to measure blood sirolimus concentration in cases when the concentration is expected to be affected, such as when an adverse drug reaction is suspected after dose increase, a drug affecting CYP3A4 has to be co-administered for a long-term period, or when patients have hepatic impairment. Also, information on the system for drug concentration measurement should be provided to medical institutions. The maximum dose permitted should be 4 mg, because it is the maximum dose used in Studies MILES and MLSTS and is unlikely to result in trough whole blood sirolimus concentrations far exceeding 15 ng/mL.

Based on the above, the description of dosage and administration should be revised as follows. A final decision will be made, taking account of comments from the Expert Discussion.

[Dosage and administration] The usual adult dosage is 2 mg of sirolimus orally administered once daily. The dose may be adjusted according to the patient's condition. <u>The dose should not exceed 4 mg once daily.</u>
(The underlined part has been added to the proposed indication)

4.(iii).B.(4) Indication

PMDA concluded that there is no particular problem with the indication of lymphangioleiomyomatosis (LAM) as proposed by the applicant.

4.(iii).B.(5) Physicians who use sirolimus

PMDA asked the applicant to explain whether non-specialist physicians may have to use sirolimus in patients with LAM living in rural areas and, if such is the case, to explain the currently planned system that allows non-specialist physicians to consult specialists who have experience in diagnosis and treatment of LAM.

The applicant explained as follows:

Because of very few specialists knowledgeable about the diagnosis and treatment of LAM, routine diagnosis and treatment of LAM is mostly carried out by non-specialist physicians. To allow non-specialist physicians to properly use sirolimus in collaboration with sentinel medical institutions staffed with specialists, the following measures have been planned.

- Medical institutions requesting a supply of sirolimus will be informed of a sentinel medical institution staffed with specialists and will be provided with a patient diary to record the name of the sentinel medical institution, contact address of the specialist, information on the prescription of sirolimus, patient findings, items to be monitored, etc.

- Patients will be provided with a patient diary by their attending physician and will be instructed to show the diary to the physician or a specialist at visits.

- Patients will be instructed to periodically visit the sentinel medical institution for examination performed by a specialist at least once every 3 to 6 months during the first year of treatment and at intervals determined by the specialist from the second year of treatment. Patients are to be checked for progression of LAM, chest CT findings, adverse drug reactions, etc. The specialist is to disclose the important findings to the attending physician and instruct the physician to follow up patient conditions as necessary.

- Scientific meetings and the company website will be used to provide physicians (including non-specialist physicians who may prescribe sirolimus) and patients with information on sentinel medical institutions and of periodic adverse drug reaction reports from all-case surveillance, particularly noteworthy symptoms and imaging findings regarding interstitial lung disease. A system that allows specialists to answer inquiries from attending physicians and other healthcare professionals will be developed.

- Periodical seminars will be held at scientific meetings and workshops to educate non-specialist physicians who may prescribe sirolimus on the diagnosis and treatment of LAM and on the points to consider when using sirolimus.

PMDA considers as follows:

LAM is a rare disease and there are very few specialists knowledgeable about the diagnosis and treatment of the disease, and frequent visits to a distant specialist office may become difficult when LAM becomes aggravated leading to respiratory failure. Therefore, it is acceptable for a local physician to use sirolimus in patients with LAM living in a rural area without a specialist, only after a diagnosis of LAM has been given by a specialist. However, LAM is a potentially fatal, serious disease, and sirolimus has a risk of serious adverse events such as interstitial lung disease and serious infectious diseases. Therefore non-specialist physicians should, before using sirolimus, receive detailed instructions to gain sufficient knowledge about treatment of LAM and the risk of sirolimus. Furthermore, a system should be constructed that allows close cooperation with the specialist, as proposed by the applicant.

4.(iii).B.(6) Safety measures after the market launch

PMDA considers as follows:

Sirolimus were evaluated in only an extremely limited number of patients with LAM in both Japanese and foreign clinical studies. Also, the results of clinical studies suggest the possibility that serious adverse events including interstitial lung disease and serious infectious diseases may occur, as discussed in section "4.(iii).B.(2) Safety". Taking account of the above, an all-case surveillance should be conducted after the market launch to collect further information on the safety and efficacy of sirolimus. Investigation items for the surveillance should include "interstitial lung disease" and "serious infectious diseases," as discussed in section "4.(iii).B.(2) Safety". Also, since main target patients for treatment with sirolimus are women of childbearing age, sirolimus not only has the risk of serious adverse events in patients themselves but also has a potential effect on fetuses, as suggested by toxicological studies. Therefore, sirolimus should be used properly only after carefully weighing the benefits against the risks. For this purpose, it is appropriate to limit the use of sirolimus to physicians with sufficient knowledge about the treatment of LAM and the risk of sirolimus. In order to facilitate the proper use of sirolimus, it is essential to supply detailed materials to physicians and other healthcare professionals, to provide instruction manuals for patients to explain the risk and benefit of the treatment in an appropriate and easy-to-understand manner, and publish information obtained after the market launch in a timely manner, thereby providing information to healthcare professionals and patients appropriately and promptly.

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

The assessment is currently ongoing, and the results and the conclusion by PMDA will be reported in Review Report (2).

IV. Overall Evaluation

Based on the submitted data, it is concluded that the efficacy of sirolimus for LAM has been demonstrated, and that the safety is acceptable in view of their observed benefits. Sirolimus provides a drug therapy option for patients with LAM, a disease with no established drug therapy at present, and is thus of clinical significance. All-case post-marketing surveillance should be conducted to collect further information on the safety and efficacy of sirolimus, because sirolimus was evaluated in an extremely limited number of patients with LAM in both Japanese and foreign clinical studies, and because sirolimus therapy may result in serious adverse events including interstitial lung disease and serious infectious diseases.

PMDA considers that sirolimus may be approved if the drug is not considered to have any particular problems based on the Expert Discussion.

Review Report (2)

May 14, 2014

I. Product Submitted for Registration

[Brand name]	Rapalimus Tablets 1 mg
[Non-proprietary name]	Sirolimus
[Applicant]	Nobelpharma Co., Ltd.
[Date of application]	October 21, 2013

II. Content of the Review

The outline of the comments from the Expert Discussion and the subsequent review by the Pharmaceuticals and Medical Devices Agency (PMDA) are described in the following sections. The expert advisors for the Expert Discussion were nominated based on their declarations, etc., concerning the product submitted for registration, in accordance with the provisions of the "Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency" (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

The conclusion of PMDA described in Review Report (1) was supported at Expert Discussion. Also, the expert advisors raised the following comments: (i) precautionary statements should be provided in the package insert, etc., on the possibility of interstitial lung disease induced by sirolimus, and occurrence of the disease should be carefully monitored continuously after the market launch, (ii) because of extremely limited experience of sirolimus therapy in Japanese patients with LAM, attention should also be given to adverse drug reactions that were not observed in clinical studies of sirolimus but reported with other mTOR inhibitors, and (iii) an effective cooperative system with a specialist should be constructed when sirolimus is used by a physician not specialized in LAM treatment. Taking account of these comments, PMDA took the following measures.

(1) Risk management plan (draft)

PMDA concluded that the current risk management plan (draft) should include the safety and efficacy specifications shown in Table 40 and the additional pharmacovigilance activities and risk minimization activities shown in Table 41. This conclusion is based on the review described in the Review Report (1) "4.(iii).B.(6) Safety measures after the market launch" and on the comments from the expert advisors at the Expert Discussion. Also, PMDA concluded that the following events should be set as important potential risks because they are observed with other mTOR inhibitors and for reasons described below.

- Pancytopenia, thrombocytopenia, neutropenia, anaemia, etc.: In clinical studies in patients with LAM, leukopenia was observed in 3.7% (4 of 109 subjects), lymphopenia in 2.8% (3 of 109 subjects), blood disorder in 1.8% (2 of 109 subjects), and anaemia in 0.9% (1 of 109 subjects), although they were non-serious except for 1 subject with anaemia. In the studies in organ transplant patients, the following serious adverse events occurred: pancytopenia (0.2% [5 of 3272 subjects]), thrombocytopenia (0.2% [7 of 3272 subjects]), neutropenia (0.1% [2 of 3272 subjects]), leukopenia (0.4% [12 of 3272 subjects]), and anaemia (1.4% [47 of 3272 subjects]).

- Venous thromboembolism (e.g., pulmonary embolism, deep vein thrombosis): In Study MILES, serious thrombosis was observed in 2.2% (1 of 46 subjects) although a causal relationship with sirolimus was ruled out.

- Thrombotic microangiopathy: Thrombotic microangiopathy was not observed in clinical studies in patients with LAM, but serious adverse events of thrombotic microangiopathy occurred in 0.1% (3 of 3272 subjects) in clinical studies in organ transplant patients.

- Alveolar proteinosis: Alveolar proteinosis was not observed in clinical studies in patients with LAM, but it was observed in <0.1% (1 of 3272 subjects) in studies in organ transplant patients, and toxicity studies revealed increased amounts of sirolimus and phospholipids in alveolar macrophages.

Table 40. Safety and efficacy specifications in risk management plan (draft)

Safety specifications		
Important identified risks	Important potential risks	Important missing information
• Interstitial lung disease • Serious infectious diseases • Anaphylaxis • Fluid retention (pericardial effusion, oedema peripheral, pleural effusion, ascites) • Dyslipidaemia • Poor wound healing • Renal disorder • Gastrointestinal disorders • Skin disorder • Drug interaction related to CYP3A and P-glycoprotein	• Malignant lymphoma and malignant tumor • Adverse events related to reproductive hormone and bone metabolism • Pancytopenia, thrombocytopenia, neutropenia, anaemia, etc. • Venous thromboembolism (e.g., pulmonary embolism, deep vein thrombosis) • Thrombotic microangiopathy • Alveolar proteinosis • Hyperglycaemia	• None
Efficacy specifications		
• Efficacy under routine use of sirolimus		

Table 41. Outline of additional pharmacovigilance activities and risk minimization activities in risk management plan (draft)

Additional pharmacovigilance activities	Additional risk minimization activities
• Early post-marketing phase vigilance • Use-results survey (all-case surveillance) • Study MLSTS (ongoing) [a)]	• Early post-marketing phase vigilance • Preparation and provision of materials for healthcare professionals • Preparation and provision of notebooks for patients • Information provision by the product website

a) To be conducted up to 24 months after the beginning of treatment

PMDA instructed the applicant to conduct an all-case post-marketing surveillance until a certain amount of data are accumulated from patients treated with sirolimus in order to carry out the above-described investigations.

The applicant responded as follows:
As shown in Table 42, a use-result survey with a 1-year observation period will be conducted in all treated patients until data are collected from 300 patients (target sample size). In this survey, safety of sirolimus under routine use will be investigated with the following events as the priority items: interstitial lung disease, serious infectious diseases, skin disorder and hypersensitivity reaction, fluid retention, dyslipidaemia, poor wound healing, renal disorder, gastrointestinal disorder, malignant tumor, adverse events related to reproductive hormones and bone metabolism, and hyperglycaemia. Safety data on interstitial lung disease, including data following re-administration after the occurrence of the disease, will also be collected. The analysis will be performed when data are collected from 300 patients, but the survey will be continued until the final evaluation by the regulatory agency is completed. The survey will include the confirmation that non-specialist physicians have a certain level of knowledge about the treatment of LAM and the proper use of sirolimus and that they have a cooperative relationship with a LAM specialist.

Table 42. Outline of use-results survey plan (draft)

Objective	To evaluate the safety and efficacy of sirolimus used in routine clinical practice
Survey method	All-case surveillance
Patients studied	Patients with LAM
Observation period	1 year
Target sample size	300 (all patients registered within the defined period)
Priority survey items	Interstitial lung disease Serious infectious diseases Skin disorder and hypersensitivity reaction Fluid retention Dyslipidaemia Poor wound healing Renal disorder Gastrointestinal disorder Malignant tumor Adverse events related to reproductive hormones or bone metabolism Hyperglycaemia
Main survey items	Patient demographics (e.g., disease type, severity classification, disease duration, age, prior treatment, complications) Sirolimus medication compliance Concomitant drugs/therapies Efficacy evaluation Adverse events

PMDA considers that the survey should be conducted promptly and information obtained from the survey should be provided to healthcare professionals promptly and appropriately.

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

1. PMDA's conclusion on the results of document-based GLP/GCP inspections and data integrity assessment

A document-based inspection and data integrity assessment were conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application. As a result, PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted product application documents.

2. PMDA's conclusion on the results of GCP on-site inspection

The GCP on-site inspection took place in accordance with the provisions of the Pharmaceutical Affairs Act for the data to be submitted in the new drug application (5.3.5.2-1). The inspection revealed the following irregularities: (i) the person who intended to be an investigator had not submitted the audit plan to the head of the medical institution prior to conducting the study; (ii) the head of the medical institution had not submitted the audit plan to the institutional review board in advance; (iii) the institutional review board reviewed the conduct of the study without a submitted audit plan; and (iv) there were protocol deviations (noncompliance with the rules for some tests). Thus, there were matters requiring improvements. However, they were handled appropriately, and the clinical studies as a whole were conducted in compliance with GCP. PMDA therefore concluded that there should be no problem in conducting a regulatory review based on the submitted product application documents.

IV. Overall Evaluation

As a result of the above review, PMDA concludes that the product may be approved after

modifying the indication and the dosage and administration as shown below, with the following conditions. Since the product is an orphan drug, the re-examination period is 10 years. Both the drug substance and the drug product are classified as powerful drugs, and the product is not classified as a biological product or a specified biological product.

[Indication]	Lymphangioleiomyomatosis
[Dosage and administration]	The usual adult dosage is 2 mg of sirolimus orally administered once daily. The dose may be adjusted according to the patient's condition. The dose should not exceed 4 mg once daily.
[Conditions for approval]	The applicant is required to conduct a drug use-results survey in all patients until data from a certain number of patients have been accumulated to understand the demographic information of patients treated with this product, because there are extremely few Japanese patients who have been treated with the drug. At the same time, safety and efficacy data on the product should be collected early and necessary measures should be taken to facilitate the proper use of the product.