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Respiratory Investigation

journal homepage: www.elsevier.com/locate/resinv

Original article

The efficacy and safety of low-dose sirolimus for treatment of lymphangioleiomyomatosis

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ARTICLE INFO

Article history:
Received 30 November 2012
Received in revised form
18 February 2013
Accepted 22 March 2013

Keywords:
Chylous effusion
Lymphangioleiomyomatosis
mTOR inhibitor
Sirolimus

ABSTRACT

Background: Lymphangioleiomyomatosis (LAM) is a rare disease caused by dysregulated activation of the mammalian target of rapamycin (mTOR). Sirolimus, an inhibitor of mTOR, has been reported to decrease the size of angiomyolipomas and stabilize pulmonary function in patients with LAM. However, the optimal dose for the treatment of LAM remains unclear.

Methods: We conducted a retrospective, observational study of 15 patients with LAM who underwent sirolimus therapy for more than 6 months. The efficacy was evaluated by reviewing the patients' clinical courses, pulmonary function and chest radiologic findings before and after the initiation of sirolimus treatment.

Results: All patients had blood trough levels of sirolimus lower than 5 ng/mL. Sirolimus treatment improved the annual rates of change in FVC and FEV_1 in the 9 patients who were free from chylous effusion (FVC, −101.0 vs. +190.0 mL/y, $p=0.046$ and FEV_1, −115.4 vs. +127.8 mL/y, $p=0.015$). The remaining 7 patients had chylous effusion at the start of sirolimus treatment; the chylothorax resolved completely within 1–5 months of treatment in 6 of these cases. These results resembled those of previous studies in which blood trough levels of sirolimus ranged from 5 to 15 ng/mL.

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2212-5345/$ - see front matter
http://dx.doi.org/10.1016/j.resinv.2013.03.002

Please cite this article as: Ando K, et al. The efficacy and safety of low-dose sirolimus for treatment of lymphangioleiomyomatosis. Respiratory Investigation (2013), http://dx.doi.org/10.1016/j.resinv.2013.03.002

Conclusions: Low-dose sirolimus (trough level, 5 ng/mL or less) performed as well as the higher doses used previously for improving pulmonary function and decreasing chylous effusion in patients with LAM.

1. Introduction

Lymphangioleiomyomatosis (LAM), a rare disease seen primarily in women of childbearing age, is characterized by the proliferation of abnormal smooth muscle-like cells (LAM cells), which lead to cystic destruction of the lungs, chylous effusions, lymphangioleiomyomas, and, frequently, renal angiomyolipoma (AML) [1,2]. This condition can occur as a sporadic disease (sporadic LAM) or as a pulmonary manifestation of tuberous sclerosis complex (TSC) (TSC-associated LAM) [3–5]. The apparent cause of TSC-associated LAM is mutation of either of the tumor suppressor genes *TSC1* and *TSC2*, whereas sporadic LAM results mainly from mutation of *TSC2* [6–8]. Loss of *TSC* gene function allows constitutive activation of the mammalian target of rapamycin (mTOR) signaling pathway, which regulates multiple cellular functions such as growth, motility, and survival [9].

The mTOR inhibitors such as sirolimus and everolimus block mTOR-mediated activation of downstream kinases and restore homeostasis in cells with defective *TSC* gene function [9]. Administration of mTOR inhibitors has previously been shown to decrease the size of AML and stabilize lung function in patients with LAM [10,11]. The CAST (Cincinnati Angiomyolipoma Sirolimus Trial) was a phase 1–2 trial comprising 20 patients with TSC or sporadic LAM, and sirolimus reduced the size of renal AML in both groups [10]. In that study, the optimal dose for the reduction of AML was determined by first administering sirolimus at 0.25 mg per square meter of body-surface area to achieve a blood trough level between 1 and 5 ng/mL (1–5 ng/mL). If this did not decrease the size of the target AML by 10% of the baseline value within 2 months, the dose was increased to achieve a trough level of 5–10 ng/mL. Finally, if the lesion did not decrease in size by 10% of the baseline value within 4 months, the dose was increased to achieve a trough level of 10–15 ng/mL, and that dose was continued throughout the next 12 months. Under this regimen, 1 of the 20 patients remained at the initial target trough level of 1–5 ng/mL, whereas the other 19 patients were dosed to reach the highest target level (10–15 ng/mL).

The MILES (Multicenter International LAM Efficacy of Sirolimus) Trial was a double-blind, placebo-controlled trial in which sirolimus was shown to stabilize lung function and improve the quality of life in patients with LAM [11]. In that trial, sirolimus was administered to maintain blood trough level of 5–15 ng/mL, which was chosen on the basis of results of the CAST. However, it remains unclear whether this is the optimal level for the treatment of LAM, especially as other recent case reports have shown that a lower dose was also effective [12,13]. Therefore, to determine the efficacy and safety of sirolimus at a concentration lower than the currently recommended trough level, we conducted an observational study of Japanese patients with LAM who took sirolimus at doses that maintained blood trough levels lower than the currently recommended target of 5–15 ng/mL.

2. Materials and methods

2.1. Study population

Records from 21 patients with LAM who were treated with sirolimus in the Department of Respiratory Medicine at Juntendo University Hospital between November 2009 and January 2012 were reviewed for this study. We excluded 6 patients for the following reasons: 2 patients discontinued sirolimus treatment within 2 months due to adverse events (drug-induced lung injury and skin rash/chest discomfort, respectively), 1 patient lacked serial data, 2 patients received sirolimus for too short a period to support analysis, and 1 patient underwent lung transplantation. The remaining 15 patients with LAM underwent sirolimus treatment for more than 6 months and had serial data available for retrospective analysis. To determine the efficacy of sirolimus, we retrospectively reviewed the patients' clinical courses, including adverse events, as documented in their medical records, and compared chest radiologic findings and pulmonary function before and after the initiation of sirolimus treatment. Patients' data were used under the comprehensive consent from the patients of its use and the approval of IRB (31 July 2007).

All patients were female, and their ages at the initiation of sirolimus therapy ranged from 27 to 56 years (mean, 40 y) (Table 1). Fourteen of the patients had been diagnosed with sporadic LAM and one with TSC-associated LAM. Seven patients had chylous effusion; all had chylothorax, and 3 of the 7 had chylous ascites. One patient had renal angiomyolipomas, 2 had pulmonary lymphedema, 2 had abdominal lymphadenopathy, 1 had pelvic lymphadenopathy, and 7 had both abdominal and pelvic lymphadenopathy. The diagnosis of LAM was established by histopathological examination ($n=13$), by cytological examination of chylous fluid [14] ($n=1$), or from a combination of characteristic high resolution computed tomography (HRCT) findings and an elevated serum VEGF-D level ($n=1$). Serum VEGF-D was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (R&D Systems, Inc., Minneapolis, MN, USA) [15].

Fourteen patients began the regimen with a dose of sirolimus of 1 mg/day. The dose was increased to 2 mg/day to improve the response in 4 cases (JUL46, JUL91, JUL248, and JUL316). Patient JUL97 had been treated for 1 year with everolimus, a similar mTOR inhibitor, at a dose of 1 mg/day before starting treatment with sirolimus. The trough level of sirolimus was measured within 1 month after the initiation

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Table 1 – Patients' characteristics at the initiation of sirolimus therapy.

	LAM ($n=15$)
Age—yr (range)	39.9±8.0 (27–56)
Sporadic/TSC-associated LAM	14/1
Clinical features–no. (%)	
Chylous effusion	7 (46.7)
Chylothorax only	4 (26.7)
Chylothorax with ascites	3 (20.0)
Pulmonary lymphedema	2 (13.3)
Angiomyolipoma	1 (6.7)
Abdominal lymphadenopathy	9 (60.0)
Pelvic lymphadenopathy	8 (53.3)
Home oxygen therapy	8 (53.3)
Diagnostic test–no. (%)	
TBLB	2 (13.3)
VATS	7 (46.7)
Abdominal lymph node biopsy	2 (13.3)
Pelvic tumor resection	2 (13.3)
Cytology of chylous fluid	1 (6.7)
Clinical diagnosis[a]	1 (6.7)
Serum VEGF-D (pg/mL) (range)	4074±1927 (1346–8281)

Plus–minus values are means±SD.
Abbreviations: TBLB, transbronchial lung biopsy; TSC, tuberous sclerosis complex and VATS, video-assisted thoracic surgery.
[a] Diagnosed on the basis of the combination of characteristic high resolution computed tomography (HRCT) findings and an elevated serum VEGF-D level.

of treatment or the increase in the dose. Blood sirolimus levels were assayed by Towa Environment Science Co., Ltd., (Osaka, Japan) using the method described by McCormack, et al. [11].

2.2. *The effect of sirolimus on LAM*

Our evaluation of sirolimus in patients with LAM included assessment of the changes in various parameters within the groups of patients with and without chylous effusion. In the 8 LAM patients without chylous effusion (Table 2), we used the Wilcoxon signed-rank test to compare the serial values of pulmonary function, forced vital capacity (FVC), and forced expiratory volume in 1 s (FEV_1) before and after the administration of sirolimus. The statistical analysis was conducted using SPSS version 20. For all statistical analyses, a p value less than 0.05 was considered significant.

For the 7 patients with chylous effusions, we reviewed the size or amount of effusion as assessed by radiologic or ultrasonographic examinations. We defined complete resolution (CR) as the absence of pleural effusion detectable by radiologic examination and a "stabilized" condition as effusion that persisted but in a smaller quantity than present at baseline. For chylous ascites, we defined "stabilized" as ascites that persisted but was no worse than that previously visible and "improved" as a quantity of ascites greater than physiologically normal but less than present at baseline. The patients' medical records indicated whether the radiologic findings accompanied changes in other parameters related to effusions, such as symptoms (dyspnea, heaviness in the chest, or a feeling of abdominal distention), abdominal circumference, and body weight.

3. Results

3.1. *Trough sirolimus levels in the blood*

Of the 15 patients treated with sirolimus who are reviewed here, all had trough blood levels lower than 5 ng/mL (mean, 2.16 ng/mL; range, 0.8–4.3 ng/mL). Accordingly, we arbitrarily designated the treatment regime "low-dose sirolimus treatment" because the trough levels of all patients were lower than those in previous studies [10,11,16,17].

3.2. *Effect of sirolimus on pulmonary function*

The clinical characteristics of the 8 patients without chylous effusion are shown in Table 2. All of these patients had sporadic LAM, and their ages at the start of sirolimus treatment ranged from 33 to 56 years. The median follow-up time as of August 2012 was 17.5 months (SD, 5.9 months). The most frequent presenting feature was exertional dyspnea, which was noted in 6 of 8 patients (75.0%). Pneumothorax and retroperitoneal lymphadenopathy were each present in 1 patient. Two patients had pulmonary lymphedema.

Their mean (SE) annual changes in FVC and FEV_1 before sirolimus treatment were –101.0 mL (314.2 mL) and –115.4 mL (86.2 mL), respectively (Table 2 and Fig. 1). The annual change improved after the start of sirolimus treatment for both FVC and FEV_1 [FVC, +190.0 mL (246.1 mL), $p=0.046$ and FEV_1, +127.8 mL (289.6 mL), $p=0.015$]. Six of 8 patients had FEV_1 values at or above their baseline values after 1 year, whereas the remaining 2 patients showed slight decreases from baseline in FEV_1 [1.69–1.62 L (JUL123) and 0.85–0.78 L (JUL248)]. These results were similar to those of previous studies in which the blood trough level of sirolimus was maintained at 5–15 ng/mL [11,16,17].

We will briefly describe a clinical course representative of the patients without chylous effusion to depict the typical response to mTOR inhibition. JUL97 presented at 27 years of age with hemosputum and Polycystic Ovarian Syndrome (PCOS) (Fig. 2). Imaging studies demonstrated lymphatic congestion and edema (predominantly in the right lung). Cystic parenchymal destruction was evident as coalescing, irregular, bizarrely shaped, cavity-like, airspaces in the right upper lobe. Everolimus therapy was initiated 5 years later while she was on a waiting list for bilateral lung transplantation. She was receiving supplemental oxygen at 5 L/min for treatment of respiratory failure and her condition was complicated by bloody chylous sputum. Treatment with everolimus for 1 year resolved the bloody chylous sputum, substantially eased the pulmonary lymphedema, and counteracted the airflow limitation. The mTOR inhibitor was then changed from everolimus to sirolimus for financial reasons. Her condition continued to improve, and she no longer requires supplemental oxygen. Later, during the third year of mTOR inhibitor treatment, she developed an *Aspergillus* infection in her right upper lung lobe, in which severe parenchymal destruction had created a cavity.

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Table 2 – Patients without chylous effusion.

Registry number[a]	Age[b] (yr)	Presenting features	Duration[c] (m)	Trough level	Clinical characteristics	FVC[d]		FEV_1[d]		Serum VEGF-D[d]		Clinical course
						Pre	Post	Pre	Post	Pre	Post	
JUL46	44	Dyspnea	12	1.2	AML, abdominal lymphadenopathy	2.93	3.05	0.54	0.59	ND	1250	
JUL97	33	Dyspnea	20	1.2	Pulmonary lymphedema, abdominal and pelvic lymphadenopathy	2.07	2.54	1.56	2.13	8281	5277	(1) Pulmonary lymphedema decreased. (2) Developed an *Aspergillus* infection[e]
JUL117	55	Dyspnea	13	ND		2.42	2.32	0.47	0.47	3142	1706	
JUL123	37	PTX	23	1.8		2.88	3.00	1.69	1.62	1346	955	
JUL162	38	Dyspnea	27	2.1	Pulmonary lymphedema	2.7	2.7	1.82	1.99	6696	1897	Pulmonary lymphedema decreased
JUL197	36	Dyspnea	27	ND		1.05	1.34	0.42	0.44	3576	2184	The frequency of PTX decreased.
JUL210	56	Retroperitoneal lymphadenopathy	15	2.4	Abdominal and pelvic lymphadenopathy	3.34	2.81	1.83	1.96	3257	2195	
JUL248	41	Dyspnea	13	2.4		2.56	2.73	0.85	0.78	1354	569	Temporarily discontinued sirolimus due to a rash

All patients were diagnosed with sporadic LAM.

Abbreviations: AML, angiomyolipoma; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; ND, not determined and PTX, pneumothorax.

[a] Registry number: The registry number of each patient with LAM at Juntendo University Hospital. We use this to make it clear when cases have been duplicated in our manuscripts.

[b] Age at initiation of sirolimus therapy (years).

[c] The follow-up period after the initiation of sirolimus therapy (months).

[d] Values of FVC (L), FEV_1 (L), and serum VEGF-D (pg/mL) at baseline (pre) and 1 year after the initiation of sirolimus therapy (post).

[e] See also Fig. 2. JUL97 developed an *Aspergillus* infection 20 months after the initiation of sirolimus therapy.

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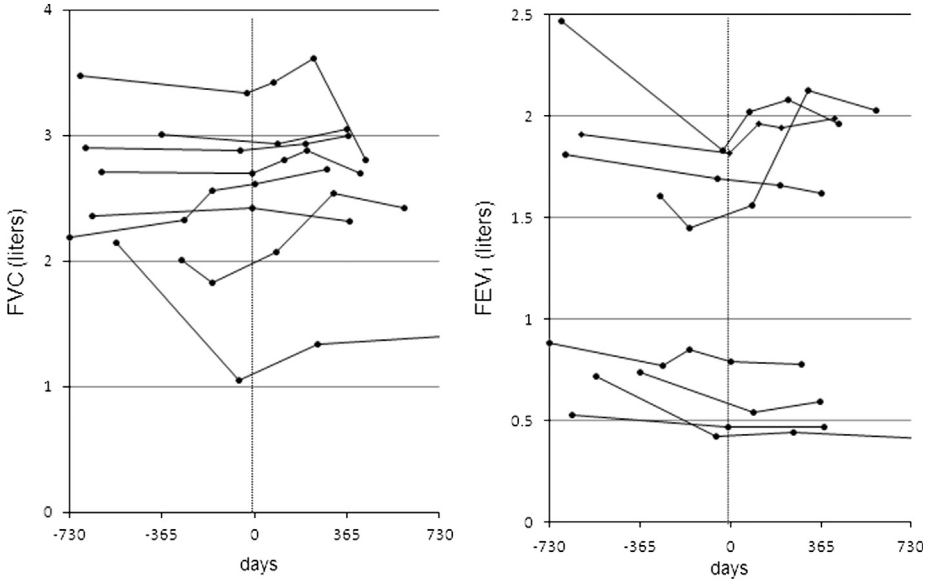

Fig. 1 – Serial changes in forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) before and after initiation of sirolimus treatment. Day 0 on the horizontal axis indicates the day on which sirolimus therapy was initiated; negative and positive numbers indicate days before and after the initiation of sirolimus administration, respectively.

3.3. *Effect of sirolimus on chylous effusions*

Seven patients had chylothorax, which was accompanied in 3 cases by chylous ascites (Table 3). Their median follow-up time as of August 2012 was 12.0 months (SD, 5.5). Four of the patients experienced complete resolution of chylothorax in 1 to 3 months after the initiation of sirolimus treatment. Of the 3 patients with both chylothorax and chylous ascites, 2 experienced complete resolution of the chylothorax and decreased amounts of ascites. Even the remaining patient (JUL79), in whom considerable pleural effusion and ascites persisted, had less fluid accumulation than before sirolimus therapy ("stabilized condition"). This situation may have resulted from her extremely low trough level (0.8 ng/mL).

We will describe a case TSC-associated LAM (JUL316) to illustrate how chylous effusion responded to low-dose sirolimus (Fig. 3). This 47-year-old woman had a moderate amount of right pleural effusion, dilatation of the thoracic duct, and lymphatic involvement in the mediastinum and upper abdomen (Fig. 3A and B). Lymphoscintigraphy revealed obstruction of axial lymphatic flow around the common iliac veins (Fig. 3C). She needed supplemental oxygen therapy to avoid hypoxemia. After taking sirolimus at 1 mg/day for 14 days (trough level, 2.2 ng/mL), she obtained symptomatic relief and amelioration of the lymphatic obstruction, as confirmed by lymphoscintigraphy (Fig. 3D). At her request, her dose of sirolimus was increased to 2 mg/day (trough level, 4.3 ng/mL), and her pleural effusion disappeared completely within 2 months (Fig. 3E). She eventually stopped needing supplemental oxygen.

We observed the effect of discontinuing sirolimus in 1 patient with chylous ascites (JUL137) (Fig. 4). This 43-year-old patient with sporadic LAM had received a peritoneovenous shunt 5 years prior to control intractable chylous ascites [18]. However, the small amount of chylous pleural effusion and moderate amount of chylous ascites that remained expanded her abdominal circumference to ~77–80 cm. Approximately 60 days after starting sirolimus treatment, her abdominal circumference had decreased to 72 cm, the sensation of abdominal distention disappeared, the chylothorax had completely resolved, and her body weight had decreased. However, she began to feel lower abdominal pain, probably because the lack of ascites allowed the tip of the shunt tube to irritate the inner surface of her abdomen. Accordingly, she discontinued the sirolimus therapy. Thereafter, her abdominal circumference gradually increased and returned to the baseline level within 153 days. After removal of the peritoneovenous shunt (data not shown) and reinstatement of sirolimus therapy, her chylous ascites diminished as before.

3.4. *The effect of sirolimus on the serum VEGF-D level*

Serum VEGF-D levels before and after sirolimus treatment were measured in 14 patients (all except JUL46) (Table 1). Sirolimus treatment decreased the serum VEGF-D level in all but 1 patient (JUL305) (Tables 2 and 3).

3.5. *Treatment-related adverse events*

The treatment-related adverse events are summarized in Table 4. The most common adverse events related to low-dose sirolimus were stomatitis (9 patients, 60.0%); gastrointestinal episodes (8 patients, 53.3%), including diarrhea ($n=6$) and stomach discomfort ($n=2$); and upper or lower respiratory infection (6 patients, 40.0%). Although these were not severe, i.e., usually grade 1 or 2, 1 patient (JUL316) temporarily discontinued sirolimus treatment due to grade 3 stomatitis. Hypercholesterolemia was not observed in any patient. One patient (JUL97) developed *Aspergillus* infection in her severely damaged lung parenchyma, where a cavity-like area was visible in the right upper lobe.

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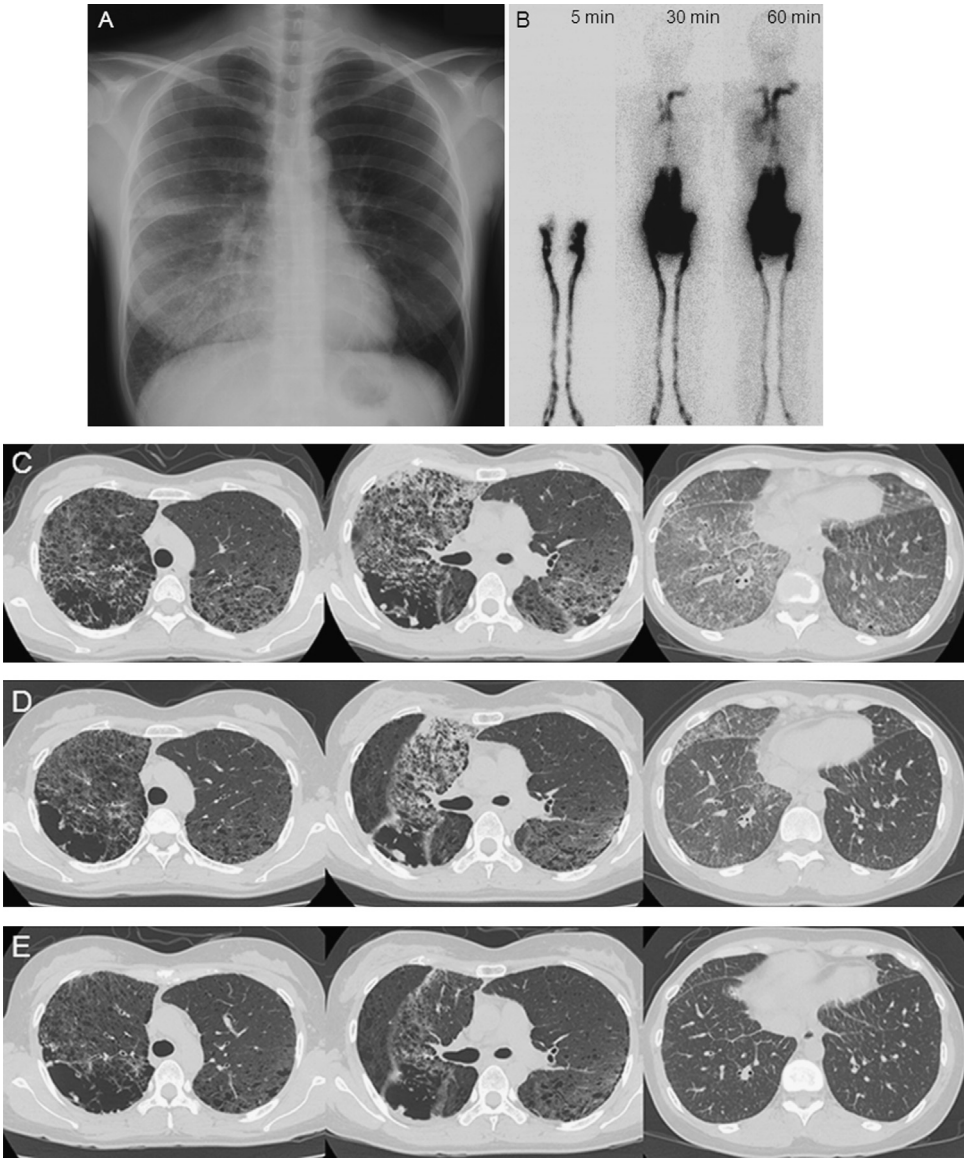

Fig. 2 – Radiologic findings for patient JUL97, whose pulmonary lymphedema resolved after administration of mTOR inhibitors. This patient's chest radiograph taken at presentation (January 2004) showed ground glass opacity in the right lower lung field (A). Neither pneumothorax nor pleural effusion was found. Lymphoscintigraphy utilizing 99mTc-labeled human serum albumin (HSA) revealed accumulation of radiolabeled HSA in the right lower lung field, as well as in the retroperitoneal and pelvic lymphangioleiomyomas, 60 min after subcutaneous injection of 99mTc-labeled HAS into the dorsal foot skin, indicating the presence of pulmonary lymphedema (B). Note the double thoracic ducts. Computed tomography (CT) images of the chest showed severe cystic destruction with irregular and bizarre shapes, especially in the right upper, right lower, and left lower lobes, as well as ground grass opacity and thickening of the interlobular septa, indicating pulmonary lymphedema (C, April 2009). Administration of everolimus at 0.5 mg/day began in April 2009, and the dose was increased to 1 mg/day from August 2009 through April 2010. CT images of the chest taken in April 2010 showed lessening of the pulmonary lymphedema (D). The mTOR inhibitor was then changed to sirolimus at 1 mg/day, and CT images of the chest in January 2011 showed almost complete resolution of the pulmonary lymphedema (E).

4. Discussion

This retrospective study documents the effectiveness of low-dose sirolimus for stabilizing pulmonary function and decreasing chylous effusion in patients with LAM. Our results resemble those of previous studies in which the blood trough level of sirolimus was maintained at 5–15 ng/mL [11,16,17]. Therefore, our data indicate that sirolimus was effective in Japanese patients even at a trough level less than 5 ng/mL. A particularly noteworthy patient was JUL97, a woman with atypical symptoms of severe lymphatic pulmonary edema and bloody chylous sputum who required continuous supplemental oxygen therapy. In contrast, Moua et al. recently described a patient with LAM in whom sirolimus therapy resolved chylous pulmonary congestion and respiratory

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Table 3 – Patients with chylous effusions.

Registry number[a]	Age (yr)	Site[b]	Duration (months)	Trough level	Clinical characteristics	Response of chylothorax (time)[c]	Response of ascites	Serum VEGF-D	
								Pre	Post
Chylothorax only									
JUL235	39	R	19	2.0	Alveolar hemorrhage, abdominal lymphadenopathy	CR (1 month)		2791	976
JUL240	28	R	7	3.3	Abdominal and pelvic lymphadenopathy	CR (1 months)		5255	3024
JUL305	27	R	9	1.7	Abdominal and pelvic lymphadenopathy	CR (3 months)		2468	2570
JUL316[a]	47	R	12	4.3	Abdominal and pelvic lymphadenopathy	CR (2 months)		5143	3671
Chylothorax with ascites									
JUL79	38	L	12	0.8	Pelvic lymphadenopathy	SD	Stabilized	4931	2892
JUL91	36	B	19	2.7	Chylous vaginal discharge, abdominal and pelvic lymphadenopathy	CR (5 months)	Improved	5634	3394
JUL137	43	R	21	2.2	Peritoneovenous shunt had been placed. Abdominal and pelvic lymphadenopathy	CR (2 months)	Improved	3161	2308

Abbreviations: CR, complete response and SD, stable disease.
[a] JUL316 had TSC-associated LAM (All other patients had sporadic LAM.).
[b] Site of chylothorax: B, bilateral; L, left and R, right.
[c] Time elapsed before response (months).

failure so completely that a previously planned lung transplantation was no longer being considered [19]. We experienced a similar clinical outcome in patient JUL97 after treatment with low-dose everolimus and, subsequently, sirolimus. The response to low-dose everolimus in JUL97 clearly illustrates this regimen, like low-dose sirolimus treatment, effectively combats pulmonary lymphedema and improves lung function. We have also experienced a patient with LAM (33-year-old woman) who has been treated only with low-dose everolimus (0.5 mg/day for 42 months, blood trough level less than 2 ng/mL) and whose annual change in pulmonary function has stabilized (FVC, −110.4 vs. +84.6 mL/year and FEV_1, −171.2 vs. +14.1 mL/year). However, we excluded her from this analysis to focus on the effect of low-dose sirolimus treatment.

The clinical trials CAST and MILES demonstrated that the benefits of sirolimus treatments for 1 year disappeared after discontinuation of therapy, i.e., without the treatment, the size of AML and rate of decline in FEV_1 reverted to the baseline levels [10,11]. The effect of discontinuation on chylous effusion, however, had been unknown. Our study included 2 patients with chylous ascites who discontinued sirolimus treatment for brief periods. As represented by patient JUL137, cessation of therapy caused the abdominal circumference to revert gradually to the baseline level within 2–3 months, indicating that the beneficial effects of sirolimus on chylous effusions do not persist beyond 2 or 3 months.

The adverse events associated with sirolimus in our analysis were mostly low-grade and were consistent with the known toxicities. The incidence was similar to those previously reported despite the low-dose regimen [10,11]. There were 2 exceptions. One patient with possible sirolimus-related lung injury (2 mg/day; trough level not measured), which has never previously been reported in studies of patients with LAM, was excluded from this analysis. At baseline, this patient as receiving continuous supplemental oxygen due to respiratory failure; however, she was hospitalized and cured without specific treatment after discontinuation of sirolimus. The second was a patient with moderately limited airflow at baseline who developed mild chest tightness with slightly increased parenchymal opacity on chest radiographs after sirolimus treatment (1 mg/day; trough level, 3.7 ng/mL). These symptoms and findings resolved promptly after the discontinuation of sirolimus. *Aspergillus* infection developed in 1 patient (JUL97), indicating that particular caution is needed when prescribing mTOR inhibitors for any patient with LAM with an area of pulmonary airspace destruction. Meanwhile, hypercholesterolemia, one of the most common adverse events in earlier studies of LAM patients [10,11], was not observed in the present study. A subgroup analysis of the MILES trial recently showed that the types of adverse events differed between Japanese and American patients [20]. Accordingly, there may be racial differences in susceptibility to specific adverse events.

Our study had several limitations. First, the small number of patients included in this observational study could have biased the results. However, considering the rarity of LAM, our analysis of 15 patients is likely to be one of the larger such studies and thus warrant attention. Second, because our retrospective study was not controlled, it is possible that the pleural effusions might have resolved spontaneously. This possibility was raised in the previous study [16] but was deniable because we verified

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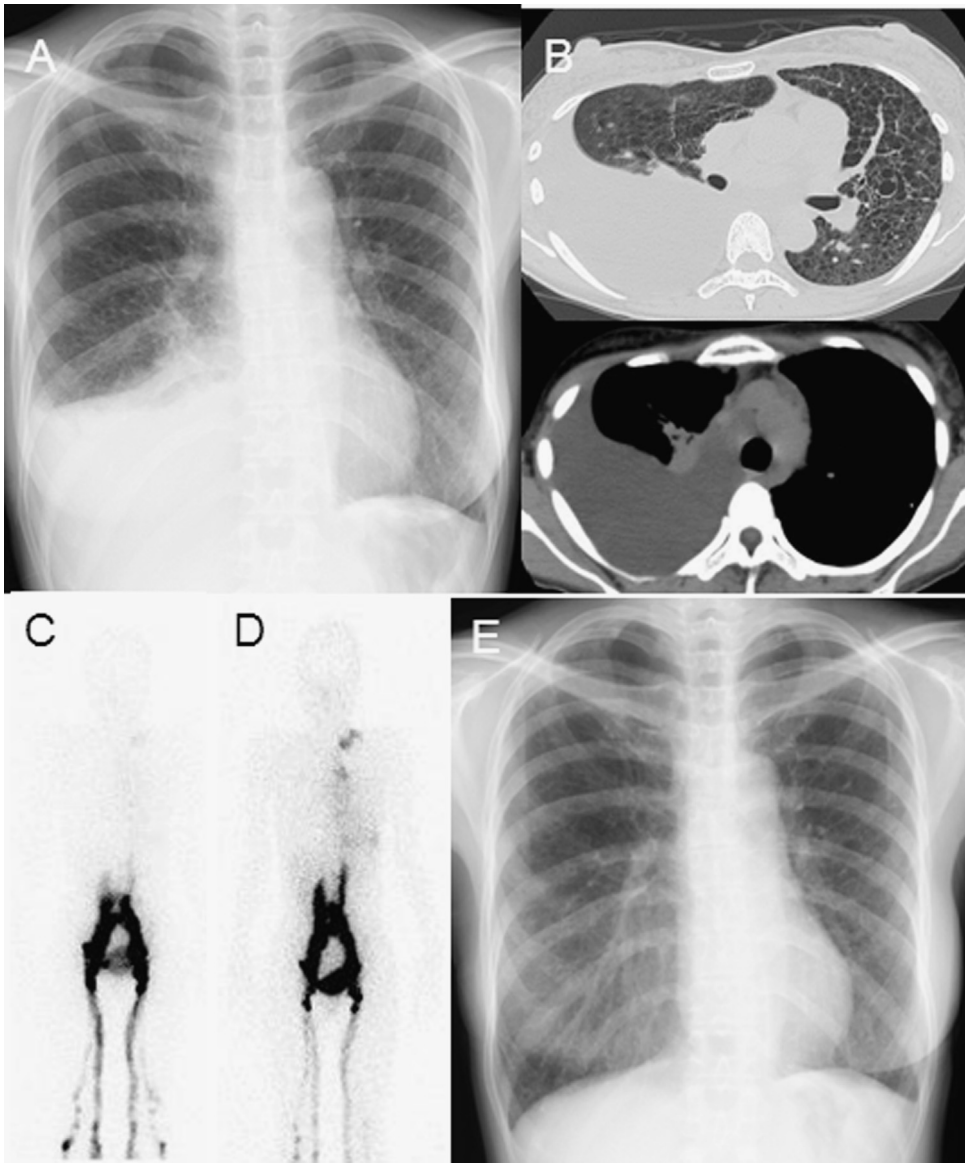

Fig. 3 – Radiologic findings for patient JUL316, whose chylothorax resolved after the administration of sirolimus. This patient's chest radiograph (A) and computed tomography (CT) images (B) at presentation showed right pleural effusion, moderate numbers of thin-walled cysts scattered throughout both lungs, and swelling of multiple mediastinal lymph nodes. Lymphoscintigraphy revealed accumulation of radiolabeled human serum albumin (HSA) in her pelvis 90 min after subcutaneous injection of 99mTc-labeled HSA into the dorsal foot skin, suggesting obstruction of axial lymphatic flow around the common iliac veins (C). Fourteen days after the initiation of sirolimus treatment, repeat lymphoscintigraphy showed the left venous angle 90 min after the injection (D), indicating the amelioration of lymphatic obstruction. Two months after the initiation of sirolimus therapy, no pleural effusion was observed (E).

that long-term GnRH therapy and a low-fat diet did not resolve the chylous effusions in our patients before initiating sirolimus treatment.

In conclusion, careful review of Japanese patients with LAM who were treated with low doses of sirolimus provided clear evidence of improved or stabilized pulmonary function and decreased chylous effusions. Even at a blood trough level of less than 5 ng/mL, sirolimus appears to be clinically beneficial in terms of effectiveness, cost, and safety. The results of our retrospective study warrant a prospective study to compare the effects of low (< 5 ng/mL) and conventional (5–15 ng/mL) doses of sirolimus on the clinical course of LAM.

Source of support

This study was supported by a grant to the Respiratory Research Group from the Ministry of Health, Labour, and Welfare, Japan; in part by the High Technology Research Center Grant from the Ministry of Education, Culture, Sports,

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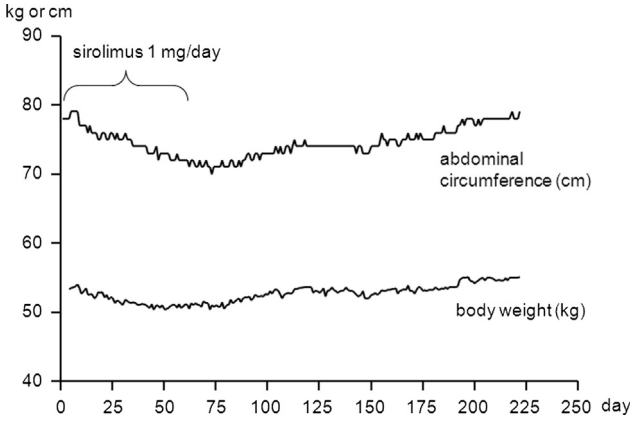

Fig. 4 – Serial changes in abdominal circumference and body weight in patient JUL137. The administration of sirolimus (1 mg/day) for 53 days decreased the patient's abdominal circumference (from 78 to 72 cm) and body weight. After discontinuation of sirolimus, the abdominal circumference gradually returned to the higher baseline level within 153 days.

Table 4 – Adverse events related to sirolimus therapy.

	Number of patients, *n* (%)		
	Total	Grade 1–2	Grade 3
Infection	6 (40.0)	5 (33.3)	1 (6.7)
Upper respiratory infection	5	5	0
Fungal (*Aspergillus*) infection	1	0	1
Hypercholesterolemia	0	0	0
Gastrointestinal event	8 (53.3)	8 (53.3)	0
Diarrhea	6	6	0
Stomach discomfort	2	2	0
Stomatitis	9 (60.0)	8 (53.3)	1 (6.7)

Science, and Technology, Japan; and in part by the Institute for Environmental and Gender-Specific Medicine, Juntendo University, Graduate School of Medicine.

Conflict of interest

The authors have reported to *Respiratory Investigation* that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in the article.

Acknowledgment

We thank Phyllis Minick for excellent assistance with English usage.

REFERENCES

[1] Corrin B, Liebow AA, Friedman PJ, et al., 1975. Pulmonary lymphangiomyomatosis. A review. Am J Pathol 1975;79:348–82.

[2] Carrington CB, Cugell DW, Gaensler EA, et al. Lymphangioleiomyomatosis. Physiologic—pathologic—radiologic correlations. Am Rev Respir Dis 1977;116:977–95.

[3] Astrinidis A, Khare L, Carsillo T, et al. Mutational analysis of the tuberous sclerosis gene TSC2 in patients with pulmonary lymphangioleiomyomatosis. J Med Genet 2000;37:55–7.

[4] Carsillo T, Astrinidis A, Henske E, et al., 2000. Mutations in the tuberous sclerosis complex gene TSC2 are a cause of sporadic pulmonary lymphangioleiomyomatosis. Proc Natl Acad Sci USA 2000;97:6085–90.

[5] Sato T, Seyama K, Fujii H, et al. Mutation analysis of the TSC1 and TSC2 genes in Japanese patients with pulmonary lymphangioleiomyomatosis. J Hum Genet 2002;47:20–8.

[6] Van Slegtenhorst M, de Hoogt R, Hermans C, et al. Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. Science 1997;277:805–8.

[7] Consortium TECTS, et al., 1993. Identification and characterization of the tuberous sclerosis gene on chromosome 16. Cell 1993;75:1305–15.

[8] Povey S, Burley MW, Attwood J, et al. Two loci for tuberous sclerosis: one on 9q34 and one on 16p13. Ann Hum Genet 1994;58:107–27.

[9] Sengupta S, Peterson TR, Sabatini DM, et al., 2010. Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. Mol Cell 2010;40:310–22.

[10] Bissler JJ, McCormack FX, Young LR, et al. Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangioleiomyomatosis. N Engl J Med 2008;358:140–51.

[11] McCormack FX, Inoue Y, Moss J, et al. Efficacy and safety of sirolimus in lymphangioleiomyomatosis. N Engl J Med 2011;364:1595–606.

[12] Ohara T, Oto T, Miyoshi K, et al. Sirolimus ameliorated post lung transplant chylothorax in lymphangioleiomyomatosis. Ann Thorac Surg 2008;86:e7–8.

[13] Peces R, Cuesta-López E, Peces C, et al. Giant bilateral renal angiomyolipomas and lymphangioleiomyomatosis presenting after two successive pregnancies successfully treated with surgery and rapamycin. Sci World J 2011;11:2115–23.

[14] Hayashida M, Seyama K, Inoue Y, et al. Criteria for designation of lymphangioleiomyomatosis in the Specified Disease Treatment Research Program. Nihon Kokyuki Gakkai Zasshi 2011;49:67–74 [article in Japanese].

[15] Seyama K, Kumasaka T, Souma S, et al. Vascular endothelial growth factor-D is increased in serum of patients with lymphangioleiomyomatosis. Lymphat Res Biol 2006;4:143–52.

[16] Taveira-DaSilva AM, Hathaway O, Stylianou M, et al. Changes in lung function and chylous effusions in patients with lymphangioleiomyomatosis treated with sirolimus. Ann Intern Med 2011;154:797–805.

[17] Davies DM, de Vries PJ, Johnson SR, et al. Sirolimus therapy for angiomyolipoma in tuberous sclerosis and sporadic lymphangioleiomyomatosis: A phase 2 trial. Clin Cancer Res 2011;17:4071–81.

[18] Makino Y, Shimanuki Y, Fujiwara N, et al. Peritoneovenous shunting for intractable chylous ascites complicated with lymphangioleiomyomatosis. Internal Med 2008;47:281–5.

[19] Moua T, Olson St EJ, Jean HC, et al. Resolution of chylous pulmonary congestion and respiratory failure in LAM with sirolimus therapy. Am J Respir Crit Care Med 2012 [Epub ahead of print].

[20] Inoue Y, McCormack FX, Lee HS, et al. The MILES Trial: the effect of Asian race on outcomes in patients with lymphangioleiomyomatosis. Am J Respir Crit Care Med 2012;185:A4444.

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