Long-Term Effect of Sirolimus on Serum Vascular Endothelial Growth Factor D Levels in Patients With Lymphangioleiomyomatosis

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BACKGROUND: Sirolimus reduces serum levels of vascular endothelial growth factor D (VEGF-D); the size of chylous effusions, lymphangioleiomyomas, and angiomyolipomas; and stabilizes lung function in patients with lymphangioleiomyomatosis (LAM).

METHODS: To determine whether reductions in VEGF-D levels are sustained over time, as well as parallel changes in lung function and lymphatic disease, we evaluated 25 patients with LAM and measured VEGF-D levels, lung function, and extent of lymphatic disease before and during sirolimus therapy.

RESULTS: Treatment with sirolimus stabilized FEV₁ and diffusion capacity for carbon monoxide (DLCO) over a period of 4.5 ± 1.6 years, caused resolution of lymphatic disease, and reduced the size of angiomyolipomas and VEGF-D levels ($3,720 \pm 3,020$ pg/mL to 945 ± 591 pg/mL; $P < .0001$). Yearly changes in FEV₁ % predicted and DLCO % predicted were reduced from $-7.4\% \pm 1.4\%$ to $-0.3\% \pm 0.5\%$ ($P < .001$) and $-6.4\% \pm 0.9\%$ to $-0.4\% \pm 0.5\%$ ($P < .001$), respectively. Lower VEGF-D levels correlated with sirolimus therapy ($P < .001$), but no significant relationship was observed between reduction in VEGF-D levels and FEV₁ and DLCO during sirolimus therapy. The magnitude of VEGF-D decline was not related to the effect on lung function. Patients with lymphatic disease had higher serum VEGF-D levels, a greater reduction in VEGF-D levels, and better long-term sustained improvement in lung function during sirolimus therapy than did those without lymphatic disease.

CONCLUSIONS: Sirolimus therapy stabilizes lung function over many years of therapy while producing a sustained reduction in VEGF-D levels in patients with elevated levels preceding therapy. An association was not demonstrated between the magnitude of VEGF-D decline and the beneficial effect of sirolimus on lung function. Persistent improvement in lung function was observed in patients with lymphatic disease. CHEST 2018; 153(1):124-132

KEY WORDS: lung function; lymphangioleiomyomatosis; lymphatic disease; sirolimus; VEGF-D

ABBREVIATIONS: AML = angiomyolipoma; DLCO = diffusion capacity for carbon monoxide; LAM = lymphangioleiomyomatosis; MILES = Multicenter International Lymphangioleiomyomatosis Efficacy and Safety of Sirolimus; mTOR = mammalian target of rapamycin; NIH = National Institutes of Health; TSC = tuberous sclerosis complex; VEGF-D = vascular endothelial growth factor D

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Lymphangioleiomyomatosis (LAM) is a multisystem disease that primarily affects women and is associated with cystic lung destruction, lymphatic involvement (eg, lymphadenopathy, lymphangioleiomyomas, chylous pleural effusions, chylous ascites), and kidney tumors (eg, renal angiomyolipomas).^{1,2} LAM is caused by proliferation of neoplastic-like LAM cells that have features of smooth muscle cells and melanocytes.³ Mutations of tuberous sclerosis complex (*TSC*) genes 1 (*TSC1*) and 2 (*TSC2*) are recognized as the primary cause of this low-grade hamartomatous syndrome.⁴⁻⁶ These mutations lead to absence or loss of function of hamartin (encoded by the *TSC1* gene) or tuberin (encoded by the *TSC2* gene), which causes activation of the mammalian target of rapamycin (mTOR) signaling pathway and increased cell size and proliferation.^{3,7}

The Multicenter International Lymphangioleiomyomatosis Efficacy and Safety of Sirolimus (MILES) trial demonstrated that treatment with the mTOR inhibitor sirolimus prevented decline in lung function over a 12-month period.⁸ Discontinuation of therapy was associated with a renewed decline in lung function, as was observed in the placebo control group. Sirolimus was also shown to be effective in patients with LAM and lymphatic involvement, such as patients with lung infiltrates caused by the accumulation of chyle within the lung parenchyma.^{9,10}

Abnormalities in lymphangiogenesis have been recognized as a major factor in the pathogenesis of LAM,¹¹⁻¹⁴ and vascular endothelial growth factor-D

(VEGF-D), a growth factor that binds to VEGF receptor 3, is increased in the serum of patients with LAM, especially those with lymphatic involvement.^{12,13,15} Serum VEGF-D is of value as a diagnostic biomarker of LAM, and data from the MILES trial showed that increased serum levels of VEGF-D are associated with severity of lung disease, reduced exercise tolerance, and greater oxygen requirements.^{8,16} Moreover, serum VEGF-D levels were reduced by sirolimus therapy, whereas in the placebo group, levels remained unchanged.¹⁶ Further, a correlation between baseline serum VEGF-D levels before sirolimus therapy and changes in FEV₁ during sirolimus therapy was observed.^{8,16} Sixty-five percent of 23 patients who exhibited a reduction in baseline serum VEGF-D levels > 42% after 12 months of treatment with sirolimus experienced an improvement in FEV₁.¹⁶

In a group of patients with LAM undergoing sirolimus therapy,¹⁷ measurements of serum VEGF-D levels were obtained before and during treatment with sirolimus. Because in the MILES trial, serum VEGF-D levels were measured only at baseline, 6 months, and 12 months,⁸ we questioned whether sirolimus therapy is associated with a long-term sustained reduction in serum VEGF-D levels, paralleling stabilization of lung function and resolution of lymphatic disease. In addition, we asked whether the reduction in serum VEGF-D levels from pretreatment values was related to resolution of lymphatic disease and stabilization of lung function.

Methods

Patient Population

Twenty-five patients with LAM were the subject of this study. Patients were self-referred, referred by the LAM Foundation, the Tuberous Sclerosis Alliance, or their physicians to the National Institutes of Health (NIH) for participation in a LAM natural history and pathogenesis protocol (NHLBI Protocol 95-H-0186), which was approved by the National Heart, Lung, and Blood Institute Institutional Review Board. The decision to initiate treatment with sirolimus was based on disease severity or the presence of symptomatic lymphatic disease. Sirolimus was prescribed by the patients' physicians. Patients were seen at the

NIH Clinical Research Center one to two times per year. Drug dosage was adjusted by local physicians to keep serum levels between 5 and 15 ng/mL. Clinical and functional data were obtained at each visit. Data from two of the 25 subjects were reported previously. Lung volume, flow rate, and DLCO were measured as previously reported.^{17,18}

Serum VEGF-D Levels

Serum VEGF-D levels were measured using an enzyme-linked immunosorbent assay (Quantikine Human VEGF-D Immunoassay, R&D Systems) at the Translational Trials Development and Support Laboratory, Cincinnati Children's Hospital, and were expressed in pg/mL. Mean sirolimus blood levels were estimated for all patients based on values obtained at the time of each visit and number of visits.

Statistical Methods

We used mixed effects models to incorporate the within and between subject variability as well as the repeated measurements to compare the associations of VEGF-D levels with sirolimus treatment, age, presence of lymphatic disease, and pulmonary function tests. A log-transformed VEGF-D was used in all

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analyses to minimize the effect of the skewed to the right distribution. Physiological data from each subject were summarized by using the estimated yearly rate of change, as

previously reported.^{17,18} Rates of changes in FEV₁ and DLCO per year are expressed as means ($\pm$ SEM).¹⁷ All other data are presented as means ($\pm$ SD).

Results

Demographics

Age at diagnosis and age at first LAM-related symptoms were 38.3 ± 10.0 years (range 22-59 years) and 35.7 ± 9.4 years (range 20-54 years), respectively. All patients were women. Age at the time of the first visit to the NIH Clinical Research Center was 40.6 ± 9.4 years (range, 24-60 years). Twenty patients were white, two patients were Asian, two were Hispanic, and one was African American. Fifteen patients were diagnosed by tissue biopsy results, and the remaining patients were diagnoses by a combination of a characteristic chest CT scan and AML ($n = 2$), lymphatic involvement ($n = 3$), TSC ($n = 2$), and a blood VEGF-D level > 800 pg/mL ($n = 5$).¹⁹ Sixteen patients had evidence of lymphatic involvement, which was defined as the presence of lymphangioleiomyomas ($n = 16$), chylous effusions ($n = 8$), or lymphadenopathy ($n = 3$), or a combination. Seven patients had angiomyolipomas. Eleven of the 25 patients were menopausal and 14 were premenopausal throughout the duration of the study. The most frequent presenting symptoms were dyspnea ($n = 12$) and pneumothorax ($n = 8$). Twenty-two patients were receiving supplemental oxygen.

Effect of Sirolimus on Lung Function and Blood VEGF-D Levels

Patients were treated with sirolimus for 4.5 ± 1.6 years. The mean sirolimus serum level over the treatment period was 9.4 ± 3.7 ng/mL. Seventeen of the 25 patients (68%) had 100% compliance with sirolimus treatment. Eight patients interrupted therapy an average of 11 ± 9 weeks (range, 3-28 weeks) because of elective surgical procedures or intercurrent infections requiring antibiotic therapy. Yearly changes in FEV₁ were $-7.4\% \pm 1.4\%$ before therapy and $-0.3\% \pm 0.5\%$ during sirolimus therapy, respectively ($P < .001$) (Table 1). The corresponding rates in change of DLCO per year were $-6.4\% \pm 0.9\%$ before therapy and $-0.4\% \pm 0.5\%$ predicted during sirolimus therapy, respectively ($P < .001$) (Table 1). During the treatment period, VEGF-D levels declined from $3,700 \pm 3,000$ pg/mL to 945 ± 591 pg/mL (Fig 1, Table 1). Log(VEGF-D) levels declined from 3.38 ± 0.44 to 2.88 ± 0.23 ($P < .0001$). High VEGF-D levels were associated with the presence of lymphatic disease. Lower VEGF-D levels were associated with sirolimus therapy ($P < .001$), older age ($P = .001$), and absence of lymphatic disease ($P = .016$). We found no relationship between reductions in presirrolimus VEGF-D levels that were produced by

TABLE 1 Changes in Serum VEGF-D Levels and Lung Function Before and During Sirolimus Therapy in 25 Patients With LAM^a

Variable	Before Sirolimus	After Sirolimus	P Value
Serum VEGF-D levels, pg/mL	3,720 $\pm$ 3,020	945 $\pm$ 591	< .001
Dose of sirolimus, mg	NA	2.6 $\pm$ 1	...
Serum sirolimus level, ng/mL	NA	9.4 $\pm$ 3.7	...
FEV ₁ , L	1.42 $\pm$ 0.53	1.44 $\pm$ 0.61	NS
FEV ₁ , % predicted	53.2 $\pm$ 18.9	56.2 $\pm$ 20.3	NS
DLCO, mL/min/mm Hg	8.6 $\pm$ 2.3	8.7 $\pm$ 3.2	NS
DLCO, % predicted	41.2 $\pm$ 11.5	42.8 $\pm$ 14.7	NS
Change in FEV ₁ , mL/y	-189 $\pm$ 28	-24 $\pm$ 15	< .001
Change in FEV ₁ , % predicted/y	-7.4 $\pm$ 1.4	-0.3 $\pm$ 0.5	< .001
Change in DLCO, mL/min/mm Hg/y	-2.7 $\pm$ 0.8	-0.7 $\pm$ 0.3	< .001
Change in DLCO, % predicted/y	-6.4 $\pm$ 0.9	-0.4 $\pm$ 0.5	< .001

DLCO = diffusion capacity for carbon monoxide; LAM = lymphangioleiomyomatosis; NA= not applicable; NS = not significant; VEGF-D = vascular endothelial growth factor D.

^aFEV₁ and DLCO values were measured before starting sirolimus therapy and after sirolimus therapy. Changes in FEV₁ and DLCO were estimated for the years before sirolimus treatment and during sirolimus therapy and are expressed as means $\pm$ SEM. All other values are expressed as means $\pm$ SD.

Figure 1 – Changes in blood VEGF-D levels and lung function in 25 patients with lymphangioleiomyomatosis (LAM) treated with sirolimus. A, Changes in VEGF-D levels for each patient before and during therapy with sirolimus. B, C, FEV₁ % predicted and DLCO % predicted values, respectively, for each patient before and during therapy with sirolimus. D, Plot of mean values of FEV₁ and DLCO over time before and during therapy with sirolimus. DLCO = diffusion capacity of carbon monoxide; VEGF-D = vascular endothelial growth factor D.

sirolimus therapy and FEV₁ % predicted or DLCO throughout the duration of therapy. Moreover, when we compared FEV₁ or DLCO in patients with a 42% or greater decline in VEGF-D levels¹⁶ with those with declines < 42%, we found no significant difference in the effect of sirolimus therapy on lung function ($P = .658$ for FEV₁ and $P = .113$ for DLCO).

The 11 menopausal patients were older (51.8 ± 7.6 years vs 38.4 ± 7.3 years) and had a lower rate of change in DLCO before sirolimus treatment ($-3.3\% \pm 0.6\%$ vs $-8.0\% \pm 1.8\%$ predicted; $P = .049$) than the 11 premenopausal patients. Six of the 11 menopausal patients and 10 of the 11 premenopausal patients had lymphatic disease. During sirolimus therapy, there was no significant difference between menopausal and premenopausal patients in rates of change in FEV₁ or DLCO.

Great intraindividual variability in presirolium serum VEGF-D levels was observed when the test was repeated in the same subject before sirolimus therapy was begun. In 17 patients in whom VEGF-D levels were tested at least twice before the initiation of sirolimus therapy (Fig 2A), the mean difference in VEGF-D values between two measurements was $-175 \text{ pg/mL} \pm 156 \text{ pg/mL}$, with values ranging from $-4,680$ to $+2,480 \text{ ng/mL}$. When this analysis was confined to eight patients retested within 6 months before initiation of therapy, the mean difference in VEGF-D values between the two measurements was $66 \pm 1,390 \text{ pg/mL}$ (5%), with values ranging from $-2,440$ to $+2,010 \text{ ng/mL}$, -41% to 36% of the first test. The variability in subjects with levels < 800 pg/mL was less. There was no relationship between the magnitude of the first VEGF-D test and the change in value of the second test. Blood VEGF-D levels were more uniform during sirolimus therapy (Fig 2B).

Figure 2 – Blood VEGF-D levels before and during treatment with sirolimus in 17 patients with lymphangioleiomyomatosis (LAM) who underwent multiple measurements of serum VEGF-D levels prior to and during sirolimus therapy. A, VEGF-D levels for each patient over an average of 2.9 ± 1.1 years before initiation of sirolimus therapy. B, Serum VEGF-D levels during an average of 3.7 ± 1.4 years of sirolimus therapy. Occasional large fluctuations in serum VEGF-D levels were seen before initiation of sirolimus therapy. During long-term sirolimus therapy, VEGF-D levels were reduced. See Figure 1 legend for expansion of abbreviations.

Serum VEGF-D Levels, Lung Function, Lymphatic Disease, and Angiomyolipomas During Sirolimus Therapy

Not all patients experienced a decline in VEGF-D levels during sirolimus therapy (Table 2). In three patients in whom baseline VEGF-D levels were 500 pg/mL or less, VEGF-D levels did not decline during sirolimus therapy. A reduction in VEGF-D levels was not always associated with stabilization or improvement in lung function. In three patients, VEGF-D levels decreased from $5,780 \pm 7,290$ pg/mL to $1,170 \pm 1,200$ pg/mL, a $70\% \pm 21\%$ reduction; however, FEV₁ and DLCO continued to decline. Of two patients who experienced a reduction in VEGF-D levels, one experienced a decline in FEV₁, and the other had a decline in DLCO. In another patient, FEV₁ and DLCO remained stable while receiving sirolimus therapy, even though VEGF-D levels were not reduced. In an additional patient, an increase in VEGF-D levels from 830 to 1,630 pg/mL during sirolimus therapy was associated with declines in FEV₁ and DLCO (see Table 2).

In 16 of the 25 patients with lymphatic involvement, complete resolution of the lymphatic pathologic conditions was observed during sirolimus therapy. In seven patients with AML, a consistent reduction in the volume of the tumors was observed.

Changes in VEGF-D Levels and Lung Function in Patients With and Those Without Lymphatic Involvement

A statistically significant ($P < .001$) relationship was observed between sirolimus therapy and lymphatic disease. The effect of sirolimus therapy on the VEGF-D

levels was different in patients with and those without lymphatic disease. In 16 of the 25 patients who had evidence of lymphatic involvement, treatment with sirolimus induced complete resolution of the lymphangioleiomyomas and chyloous effusions and reduced serum VEGF-D levels from $5,130 \pm 3,380$ pg/mL to $1,170 \pm 770$ pg/mL ($P = .0003$). In the nine patients without lymphatic involvement, VEGF-D levels decreased from $1,840 \pm 1,210$ pg/mL to 840 ± 510 pg/mL ($P = .006$) (Figs 3A, 3B). We estimated the rate of change of log(VEGF-D) levels per year before and during sirolimus therapy. In patients with lymphatic disease, the rate of change in log(VEGF-D) levels was -0.174 before treatment and -0.196 after treatment. In patients without lymphatic disease, presirolimus and postsirolimus log(VEGF-D) level changes were -0.213 and -0.090 , respectively, that is, in the subjects with lymphatic disease, the rate of decline of serum VEGF-D levels increased during sirolimus therapy, but in those without lymphatic disease, the rate of decline of VEGF-D levels did not increase during sirolimus therapy.

We then focused on whether there were differences in the effects of sirolimus on lung function between patients with and those without lymphatic disease. In patients with lymphatic disease, rates of FEV₁ decline were $7.8\% \pm 2.0\%$ per year before therapy and $-0.4\% \pm 0.7\%$ predicted per year during sirolimus therapy, respectively ($P < .001$) (Fig 3C). The corresponding rates of change of DLCO were $-7.4\% \pm 1.5\%$ per year before therapy and $-0.1\% \pm 0.2\%$ predicted per year during sirolimus therapy, respectively ($P < .001$) (Fig 3C). The rates of

TABLE 2] Changes in VEGF-D Levels, FEV₁ and DLCO in 25 Patients With LAM Treated With Sirolimus^a

Patient No.	Baseline VEGF-D	Final VEGF-D	Baseline FEV ₁ (%)	Final FEV ₁ (%)	Baseline DLCO (%)	Final DLCO (%)	Sirolimus (Years of Treatment)
1	2,602	604	17	20	34	32	3.2
2	2,217	831	71	72	33	49	2.1
3	3,369	1,390	31	36	30	28	5.4
4	2,668	864	76	82	46	38	4.8
5	501	765	67	66	50	47	3.4
6	2,116	394	91	68	65	52	5.1
7	1,050	568	68	51	38	33	3.2
8	1,581	918	49	50	39	36	8.9
9	9,511	2,572	47	48	34	34	2.6
10	5,972	772	35	42	29	29	7.2
11	4,162	655	76	94	46	63	6.4
12	311	396	42	43	67	59	2.9
13	6,449	1,703	78	85	47	59	5.3
14	14,176	2,554	53	37	40	23	3.6
15	335	289	42	37	24	17	5.5
16	6,486	792	23	27	29	31	5.3
17	992	894	57	53	32	37	3.9
18	5,146	1,311	46	40	52	56	2.9
19	3,303	1,104	67	81	25	26	6.1
20	4,396	928	48	90	51	75	5.5
21	1,485	2,205	29	47	31	42	4.4
22	3,625	726	38	44	45	47	3.2
23	3,204	963	63	70	49	64	5.5
24	8,306	467	46	53	50	49	3.6
25	2,166	708	70	71	45	51	2.9

See Table 1 legend for expansion of abbreviations.

^aSerum VEGF-D levels are expressed in pg/mL.

change of FEV₁ % predicted for patients without lymphatic disease were $-5.4\% \pm 1.6\%$ per year before therapy and $-0.7\% \pm 0.8\%$ predicted per year during sirolimus therapy, respectively ($P < .001$) (Fig 3D). The corresponding rates of change of DLCO were $-4.3\% \pm 1.0\%$ per year before therapy and $-1.2\% \pm 0.9\%$ predicted per year after therapy ($P < .001$) (Fig 3D). In our study, we did not find a relationship between reduction in VEGF-D and lung function response. We looked at changes in VEGF-D within 6 months of therapy and changes in FEV₁ % predicted and DLCO thereafter. We found no relationship between the magnitude of VEGF-D decline and yearly rates of change of FEV₁ or DLCO.

Discussion

Our study shows that treatment with sirolimus caused a sustained reduction in serum VEGF-D levels that

persisted over years of therapy. Along with a reduction in VEGF-D levels, there was stabilization of lung function, resolution of lymphangioleiomyomas and chylous effusions, and reduction in size of AMLs. A relationship between reduction in VEGF-D levels and stabilization in lung function could not be established. Indeed, no statistically significant relationship between the effect of sirolimus on VEGF-D levels and FEV₁ or DLCO was observed, that is, there were patients in whom sirolimus reduced VEGF-D levels but failed to prevent decline in lung function, whereas in other patients, lower initial VEGF-D values did not preclude stabilization of lung function.

Data from the MILES trial suggested that patients with a $> 42\%$ reduction from baseline presirrolimus VEGF-D levels were more likely to have improvement in lung function.¹⁶ The results of our study extend those data and show differences with long-term treatment. First, we

Figure 3 – Changes in serum VEGF-D serum levels and lung function in 16 patients with and nine patients without lymphatic involvement during treatment with sirolimus. A, B, Changes in VEGF-D levels in patients with (A) and patients without (B) lymphatic involvement. C, Yearly rates of change in FEV₁ % predicted and DLCO % predicted in 16 patients with lymphatic disease, before (red columns) and during (blue columns) sirolimus therapy. During sirolimus therapy, there was a sustained reduction in rates of decline in FEV₁ and DLCO. D, Yearly rates of change in FEV₁ % predicted and DLCO in nine patients without lymphatic disease before (red columns) and during (blue columns) sirolimus therapy. In patients without lymphatic disease, FEV₁ and DLCO still decreased but at lower rates than prior to sirolimus therapy. See Figure 1 legend for expansion of abbreviations.

found a large intraindividual variability in presirrolimus VEGF-D values (Fig 2A), with differences between two VEGF-D levels measurements ranging from –41% to +59%. Second, we found that after adjusting for age and initial lung function, there was no statistically significant association between a decline in VEGF-D levels and FEV₁ % predicted and DLCO % predicted values over the duration of treatment with sirolimus. In a different study involving patients with lymphangioleiomyomas in whom VEGF-D levels were measured on successive days at the same time of the day and in either a fasting or nonfasting state, we found differences between the two measurements in individual patients ranging from –24% to 59%.²⁰ These data, along with those of another study,²¹ strongly suggest that individual patients with LAM may experience large spontaneous fluctuations in serum VEGF-D levels.

Several studies have described an association between serum VEGF-D levels and severity of lung disease assessed by measurements of flow rates and DLCO,¹² CT

scan grading,¹³ and oxygen requirements.¹⁶ However, others have reported²² that although patients with LAM with lymphatic involvement tended to have more severe disease and more rapid disease progression than those without it, only DLCO was significantly correlated with serum VEGF-D levels. This could be due to the fact that higher VEGF-D levels are observed most frequently in patients with lymphatic disease, and these patients may have chylous pulmonary infiltrates.¹⁰

Regarding the observation that there was a positive correlation between log(VEGF-D) percent and changes in FEV₁ and that a large reduction in serum VEGF-D from pretreatment levels was associated with lung function improvement,¹⁴ we suggest that these patients may have composed a population of patients who had subclinical lung lymphatic disease, for example, parenchymal chylous congestion. Patients with LAM and lymphatic involvement may respond to sirolimus therapy with a greater improvement in lung function than those without lymphatic disease.^{9,10} Indeed, we

found that the effect of sirolimus on VEGF-D was especially marked in patients with LAM and lymphatic involvement and was associated with resolution of chylous effusions and lymphangioleiomyomas. In this subgroup of patients, the beneficial effects of sirolimus on lung function seemed to be more sustained, whereas in patients without lymphatic involvement, DLCO tended to continue to decline (Fig 3). We hypothesized that sirolimus causes a more sustained improvement in lung function, especially diffusion capacity, in those patients with lymphatic disease, because a large component of the stabilization or improvement in lung function results from resolution of lymphatic infiltrates and perhaps a reduction in the number of lymphatic vessels infiltrating the lung parenchyma.^{9,10,16} Another mechanism by which sirolimus therapy could induce a sustained improvement in lung diffusion capacity would be by either reducing the number or volume of the lung cysts (an unlikely possibility) or by improving ventilation to lung units upstream from obstructed airways that have low ventilation/perfusion ratios. We suggest resolution of pulmonary parenchymal chylous congestion as the most likely mechanism. Therefore, it would not be unexpected that patients with lymphatic involvement would have a greater improvement in lung function than those without lymphatic disease. In patients without lymphatic disease, stabilization of lung function is

thought to result exclusively from inhibition of proliferation of LAM cells and a reduction in LAM cell burden or volume in airways and cyst walls.²³ Indeed, there is no clear evidence yet that sirolimus reduces the size or number of lung cysts.¹⁷

We conclude that treatment with sirolimus is effective in producing a sustained reduction in serum VEGF-D levels in patients with LAM with high pretherapy VEGF-D levels, which is associated with stabilization of lung function. Because of spontaneous variations in baseline, pretherapy serum VEGF-D levels caused by factors yet to be understood,²¹ and the stabilization of lung function observed in patients with LAM and low baseline serum VEGF-D levels, an association between the magnitude of VEGF-D decline and a beneficial effect on lung function during sirolimus therapy could not be demonstrated. Our data suggest that coupling of a reduction in VEGF-D levels associated with sirolimus therapy and improvement or stabilization of lung function may be seen primarily in those patients with lymphatic disease involving the lung parenchyma, which on resolution during sirolimus therapy would result in sustained improvement in lung function. Our study indicates that lung function may continue to decline during sirolimus therapy, but this appears to be less likely to occur in patients with lymphatic disease.

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