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Analysis of the MILES Cohort Reveals Determinants of Disease Progression and Treatment Response in Lymphangioleiomyomatosis

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Declaration of interests: LRY and FXM own a patent for performing the diagnostic assay to measure serum VEGF-D. All royalties from this patent are waived to the parent institution, the University of Cincinnati. All other authors have reported no conflict of interest.

Key words: LAM, Menopause, VEGF-D, Tuberous sclerosis complex, sirolimus

Take Home Message: Menopausal status and serum vascular endothelial growth factor-D levels are clinically useful variables that should be taken into consideration when making therapeutic decisions and designing clinical trials for patients with lymphangioleiomyomatosis.

Abstract

Rationale: The Multicenter International Lymphangioleiomyomatosis (LAM) Efficacy of Sirolimus (MILES) trial revealed that sirolimus stabilized lung function in patients with moderately-severe LAM. The purpose of this study was to further examine the MILES cohort for the effects of racial, demographic, clinical, and physiologic patient characteristics on disease progression and treatment response in LAM.

Methods: MILES subjects were stratified on the basis of menopausal status (premenopausal/postmenopausal), race (Asian/Caucasian), bronchodilator responsiveness (present/absent), initial FEV1 (51-70% versus ≤50%), and TSC-association (yes/no). Linear mixed effects model was used to compare slope differences, and non-parametric tests were used to compare medians and proportions between treatment groups in each stratum.

Results: In the MILES placebo group, premenopausal patients declined five-fold faster than postmenopausal patients (FEV1 slope -17±3 vs. -3±3 ml/month, p=0.003). Upon treatment with sirolimus, both premenopausal (-17±3 vs. -1±2 ml/month, p<0.0001), and postmenopausal patients (-3±3 vs. 6±3 ml/month, p=0.04) exhibited a beneficial response in mean FEV1 slope compared with the placebo group. Race, LAM subtype, bronchodilator responsiveness, or baseline FEV1 did not impact the rate of disease progression in the placebo group, or treatment response in the sirolimus group. Menopausal status and race had differential effects on the adverse event profile of

sirolimus. Baseline serum VEGF-D >600pg/ml identified subgroups of patients who were more likely to decline on placebo and respond to treatment with sirolimus.

Conclusions: In LAM patients, treatment with sirolimus is beneficial regardless of menopausal status, race, bronchodilator responsiveness, baseline FEV1, or TSC-association. Serum VEGF-D and menopausal status can help inform therapeutic decisions.

Introduction

Lymphangioleiomyomatosis (LAM) is a rare cystic lung disease that produces respiratory failure in women. LAM can occur sporadically (S-LAM), or in association with the heritable disease, tuberous sclerosis complex (TSC-LAM).[1] In both TSC-LAM and S-LAM, loss of function mutations in *TSC* genes result in constitutive activation of the mechanistic target of rapamycin (mTOR) signaling pathway, leading to inappropriate LAM cell growth, invasion, migration, lymphangiogenesis and destructive tissue remodeling.[2] The average age at diagnosis of LAM is about 35 years,[1] and the typical rate of decline in forced expiratory volume in one-second (FEV1) has been variably reported as 75-135mL per year.[3,4] In a recent randomized controlled trial, the Multicenter International LAM Efficacy of Sirolimus (MILES) trial, the mTOR inhibitor sirolimus was shown to stabilize lung function and to improve some measures of quality of life in patients with LAM.[4] Adverse events (AEs) were frequent and consistent with those known to be associated with mTOR inhibitor use, but serious adverse events (SAEs) were balanced between the placebo and sirolimus groups.[4]

Various retrospective reports have identified factors that may impact the natural history of LAM. For instance, postmenopausal women with LAM have a lower rate of decline in FEV1 compared to premenopausal women.[3,5,6] Race has been mentioned as a possible disease modifying factor, with reduced rates of decline in FEV1 reported among Asian women with LAM compared to Caucasian LAM patients.[6,7] The presence of bronchodilator responsiveness on spirometry has been linked to faster rate of decline in FEV1.[5,8] Patients with TSC-LAM are believed to have milder and less progressive disease compared to S-LAM.[9,10] The diagnostic and predictive value of serum vascular endothelial growth factor-D (VEGF-D) is well established,[11-13] but there exist conflicting reports with regards to the prognostic value of the biomarker.[5] The purpose of the current study was to exploit the prospective design of the MILES trial to investigate the impact of key demographic and clinical features on the natural history of lung function decline and response to treatment with sirolimus. Some of the results from this analysis have been previously published in abstract form.[14,15]

Methods

Background and study population

Our study population included the participants enrolled in the MILES trial (NCT00414648), a randomized, controlled trial where patients with a confirmed diagnosis of LAM and FEV1 $\leq$ 70% predicted were randomly assigned in a double-blind fashion to receive sirolimus or placebo for one year, followed by one year of observation. The initial dose of sirolimus was 2mg daily, which was dose-adjusted in a blinded

fashion to maintain a blood trough level of 5-15ng/ml. Pulmonary function tests (PFTs), serum VEGF-D levels, and patient-reported outcomes were gathered serially. Data from the second trial year, in which patients were observed off therapy, was not included in the current analysis.

Procedures

PFT methodology from the MILES trial has been previously reported.[4] All PFTs were performed in accordance with the American Thoracic Society (ATS)/European Respiratory Society (ERS) criteria,[16-18] with real time feedback to maintain quality of spirometry through the trial. The presence or absence of bronchodilator responsiveness was determined in all trial subjects at the baseline visit as per the ATS/ERS criteria.[16,19] Spirometry was performed at baseline and every three months in the first year.

Menopausal status, either natural (defined by the absence of menstrual flow for a period of at least 12 months) or surgical (defined as surgical removal of uterus with/without removal of the ovaries), was determined based on history at the enrolment visit. Serum VEGF-D concentrations were measured at baseline, 6, and 12 months. VEGF-D testing was done in a College of American Pathologists (CAP) and Clinical Laboratory Improvement Amendments (CLIA) accredited laboratory, by technicians masked to treatment assignment and clinical data. A modified form of the Quantikine Human VEGF-D Immunoassay (R&D Systems, Minneapolis, MN, USA) was used for the measurements.

Statistical analysis

For this study, the various analyses were stratified according to the condition of interest. A linear mixed-effects model was used to compare slope differences, and non-parametric tests were used to compare medians and proportions between treatment groups in each stratum. In the linear mixed-effects model analysis, we used the Kenward-Roger correction to adjust the degree of freedom to improve performance when data were missing.[20] For categorical outcomes, the data were compared with the use of Fisher's exact test. For continuous variables, the medians were compared with the use of the Wilcoxon rank-sum test. The 95% confidence interval for group differences was obtained from the mean estimates. With regards to AEs, the mean number of events per subject is reported in each group. In order to ascertain significance level of the difference between the various subgroups of interest, we compared the median subject specific proportions of AEs by using the Wilcoxon rank sum test. We deemed p values less than 0.05 to be significant. All reported p values are two-sided and unadjusted for multiple testing. All statistical analyses were performed using SAS, version 9.4, Cary NC.

Results

In the MILES trial, a total of 89 patients were enrolled across ten sites, including seven in the United States, two in Japan and one in Canada; 43 patients were randomized to the placebo arm and 46 to the sirolimus arm. At the end of the first year, there were 34 remaining patients in the placebo arm and 41 patients in the sirolimus arm. The average age of enrolled patients was 45.4±10.6 years. 30 (34%) patients in the MILES cohort were postmenopausal, including 16/43 (37%) patients in the placebo group and 14/46

(30%) patients in the sirolimus group. 59 patients (66%) in the MILES cohort were premenopausal, including 27/59 (46%) in the placebo group and 32/59 (54%) in the sirolimus group. At the end of the first year, in the postmenopausal group, there were 13 remaining patients in the placebo arm and 11 patients in the sirolimus arm. Similarly, in the premenopausal group, at the end of the first year, there were 21 remaining patients in the placebo arm and 30 patients in the sirolimus arm. There were 27 (30%) Asian patients: 12 in the placebo arm and 15 in the sirolimus arm. At the end of the first year, there were 10 remaining patients in the placebo arm and 13 in the sirolimus arm. There were 62 (70%) Caucasians: 31 each in the placebo and sirolimus arms. At the end of the first year, there were 24 remaining patients in the placebo arm and 28 in the sirolimus arm.

In the overall MILES cohort, the baseline lung function and VEGF-D levels were similar in the placebo and treatment arms.[4] In the current analysis, the Asian/Caucasian and premenopausal/postmenopausal subgroups of the placebo and sirolimus groups remained similarly matched (Supplemental Tables 1 and 2), except postmenopausal patients in the placebo arm had a higher baseline diffusion capacity of the lung for carbon monoxide (DLCO) compared to the sirolimus arm (12.09 vs. 9.42 ml/mmHg/min, p=0.05), and the baseline FEV1 was higher in the sirolimus subgroup than the placebo subgroup in the Asian cohort (1.43 vs. 1.11 litres, p=0.05).

Menopause

Pulmonary Function Tests

In the placebo group, premenopausal subjects exhibited a significantly faster mean rate of decline in FEV1 compared to the postmenopausal subjects (-17±3 vs. -3±3 ml/month, p=0.003). Similar to FEV1, premenopausal women in the placebo group had a faster rate of decline in forced vital capacity (FVC) as compared to the postmenopausal women (-17±4 vs. -3±4 ml/month, p=0.01). In the premenopausal placebo group, the FEV1 slope (-17±3 ml/month) was significantly less than zero (p=<0.0001), a finding that was consistent with declining lung function. The FEV1 slope in the sirolimus group (-1±2 ml/month) was not significantly different from zero (p=0.66); this was indicative of the stabilization of lung function during treatment. In the postmenopausal placebo group, the FEV1 slope (−3±3 ml/month) was not significantly different than zero (p=0.25), a finding that was consistent with stable lung function. The FEV1 slope in the postmenopausal sirolimus group (6±3 ml/month) although not statistically different than zero, had a trend towards significance (p=0.08), indicating potential improvement of lung function during treatment. When compared with placebo, sirolimus treatment resulted in a beneficial response in FEV1 and FVC slope in both the premenopausal and postmenopausal groups (Table 1, Figures 1A and 2A). The rate of decline of DLCO was not different in the placebo and sirolimus groups in the overall MILES cohort, or in the premenopausal patients (-0.07 vs. -0.02 ml/mmHg/min/month, p=0.4). However, in the postmenopausal patients, there was a significant between-group difference in DLCO slope, favoring the sirolimus group (-0.04 vs. 0.03 ml/mmHg/min/month, p=0.04).

VEGF-D response

The difference between placebo and sirolimus groups in VEGF-D slope was statistically significant in the premenopausal patients (-15±20 versus -99±18 pg/ml/month, p=0.003) and had a trend towards significance in the postmenopausal patients (22±27 versus -54±31 pg/ml/month, p=0.07) (Table 1, Figure 3A).

Adverse events

Premenopausal patients who were treated with sirolimus had more dermatologic AEs (3.1 vs. 1.7 events per subject, p=0.006) during the 12-month treatment period than premenopausal placebo patients. On the other hand, the sirolimus treated postmenopausal patients had more gastrointestinal AEs than the postmenopausal placebo patients (6.2 vs. 3.2 events per subject, p=0.015). Comparing the sirolimus groups between pre and postmenopausal subjects, there was no difference in the frequency or type of AEs or in the frequency of SAEs.

Race

Pulmonary Function Tests

After dividing the MILES cohort on the basis of race, the rate of decline in lung function (FEV1, FVC and DLCO) was similar in the Asian and Caucasian placebo groups (Table 2). Both racial subgroups had a beneficial response in their FEV1 and FVC slopes following treatment with sirolimus (Table 2, Figures 1B and 2B). There was no significant change in the rate of decline of DLCO after treatment with sirolimus in either the Asian and Caucasian cohorts (Table 2).

VEGF-D

Serum VEGF-D levels declined significantly following treatment with sirolimus as compared to placebo in both the Asian (-17±27 vs. -102±24pg/ml/month, p=0.03) and the Caucasian (3±22 vs. -79±22pg/ml/month, p=0.01) patients (Table 2, Figure 3B).

Adverse Events

In the race-stratified analysis, dermatologic AEs were more common in the sirolimus group than the placebo group in Asians (3.4 vs. 1.2 events per subject, p=0.03) but not in Caucasians. The frequency of SAEs did not differ between Asians and Caucasians. Comparing the sirolimus groups between Asians and Caucasians, there were no differences in the frequency or type of AEs.

TSC

There were 4 TSC-LAM patients each in the placebo and sirolimus subgroups. There was no difference in the rate of decline of FEV1 in the placebo group, or the magnitude of FEV1 response in the treatment group, after dividing the MILES cohort into TSC-LAM and S-LAM subgroups (Supplemental Tables E3 – E5, and Figure E1).

Bronchodilator responsiveness

Bronchodilator responsiveness was demonstrated at baseline in a total of 27 subjects (30.3%) enrolled in the MILES trial. The rate of decline in FEV1 in the placebo group was similar regardless of the presence or absence of bronchodilator responsiveness (-11·9

vs. -11·8 ml/month, p=0.98). Both subgroups exhibited a significant beneficial response to treatment with sirolimus (Supplemental Tables E6 and E7, and Figure E2).

Baseline FEV1

The MILES cohort was divided on the basis of baseline FEV1 into two groups: FEV1 51-70% and FEV1 ≤ 50%. The rate of decline in FEV1 in the placebo group was similar regardless of the baseline FEV1. Both subgroups exhibited a significant beneficial response to treatment with sirolimus (Supplemental Tables E8 and E9, and Figure E3).

Serum VEGF-D as a predictor of disease progression and treatment response

In a previous post-hoc analysis of the MILES trial, each 1-log increase in baseline VEGF-D was shown to be associated with a 134ml between-group difference in baseline to 12-month mean change in FEV1.[13] In the current analysis, we examined the utility of a VEGF-D cut-off as a biomarker of progression and treatment response in the MILES subjects. Above a VEGF-D serum level of 600pg/ml, patients in the placebo group were more likely to progress rapidly and to respond to treatment, whereas progression and treatment response were less robust in the group of patients with a serum VEGF-D level of less than 600pg/ml (Figure 4A). When percent baseline to 12-month change in VEGF-D was plotted as a function of percent baseline to 12-month change in FEV1 in the placebo and sirolimus groups, the placebo group remained normally distributed around the origin whereas the sirolimus group migrated toward the reduced VEGF-D/increased FEV1 quadrant (Figure 5).

Dose response and timing of adverse events

The mean serum trough level of sirolimus in the treatment group was 7.2 ± 3.4ng/ml. Sirolimus trough levels did not correlate with either FEV1 response or the incidence of AEs. The majority of AEs in both the sirolimus and the placebo groups occurred in the time period immediately following drug initiation, and decreased over time (Figure 6).

Discussion

The results of our analysis reveal that menopausal status has a significant effect on the natural history of disease progression in LAM, with premenopausal women exhibiting a faster rate of decline in FEV1 as compared to postmenopausal women. Although a reduced rate of decline in FEV1 among postmenopausal women with LAM has previously been reported in retrospective cohort analyses of LAM patients,[3,6] the magnitude of difference before and after the menopause transition was much greater in this prospective analysis (~5-fold compared to less than 2-fold). These data lend further credence to the role of sex steroids in the pathogenesis of LAM, and suggest that menopausal status should be taken into consideration when making management decisions for LAM patients.

Despite vastly different rates of decline of FEV1 among pre and postmenopausal LAM patients, both subgroups exhibited a beneficial response to treatment with sirolimus, although they varied by degree (Figure 4B). The premenopausal subgroup had stabilization of lung function decline after treatment with sirolimus while the postmenopausal group had a trend towards improvement. Interestingly, postmenopausal

patients treated with sirolimus also exhibited improvement in their rate of decline of DLCO, an effect that was not seen in either the overall MILES cohort or any of the other subgroup analyzed. The menopausal-status based differential responses to treatment with sirolimus remains unexplained, and because of the small numbers will require validation in future studies. If confirmed, the data could support consideration of treatment of postmenopausal women with the goal of improvement (rather than merely stabilization) of lung function.

In this study, race (Caucasian vs. Asian) did not have an effect on the rate of decline of FEV1 (placebo arm), or the treatment response (sirolimus arm). Previous cohort analyses conducted on Asian and Caucasian subjects with LAM have yielded varying rates of decline of FEV1, suggesting an effect of race on the natural history of disease progression. For example, a recent analysis of 89 LAM patients in Japan enrolled in the Japanese National Research Project revealed a rate of decline in FEV1 of 47ml/year compared to 75ml/year in 275 LAM patients enrolled in the National Heart, Lung, and Blood Institute (NHLBI) intramural program.[3,7] While racial and environmental differences may certainly play a role in the differences in these registry based studies, the results from our prospective analysis demonstrating similar rate of decline of FEV1 and magnitude of treatment response in Asians and Caucasians suggest that ascertainment bias and varying baseline disease severity are likely explanations for the race associated discrepancies in FEV1 decline estimates between the MILES cohort and previously reported retrospective cohorts.

Race as well as menopausal status had an impact on the frequency and subtype of AEs encountered in the placebo and sirolimus groups of MILES trial. Understanding subgroup dependent susceptibility to sirolimus associated AEs is useful for making treatment decisions, and warrants further investigation in longitudinal, prospective cohorts. We have also shown that the incidence of AEs is highest in the initial three months of sirolimus treatment. Declining rates of sirolimus-related AEs over time on treatment has also been shown by other recent reports.[21,22] Collectively, these results highlight the need for close monitoring of LAM patients at the beginning of sirolimus treatment.

We have also found that TSC versus S-LAM did not impact the rate of decline of FEV1 or treatment response to sirolimus. It has been suggested that patients with TSC-LAM have milder and less progressive disease compared to patients with S-LAM.[9,10] However, many investigators believe that lead-time bias may play a role in this conclusion, in that patients with TSC-LAM are often discovered earlier through screening. A recent analysis from the NHLBI Intramural program has shown that the rate of decline of FEV1 is similar in TSC-LAM and S-LAM patients matched for baseline disease severity.[23] In addition, we have recently reported that LAM is the second most common cause of death in TSC, and the most common cause of death in adult women with TSC.[24] Although the number of patients with TSC-LAM was small in the MILES trial, data presented here and recent reports from our group and others indicate that LAM can be as significant a health care burden and mortality risk for patients with TSC as it is for sporadic LAM patients, and requires close monitoring and appropriately aggressive and well-timed treatment interventions.

The presence of bronchodilator responsiveness on spirometry has been associated with a faster rate of decline in FEV1,[8,25] as well as increased risk of progression to death or lung transplantation.[5] These results were not replicated in the MILES cohort, in that both subgroups exhibited a similar rate of decline of FEV1 as well as treatment response to sirolimus. The exact reasons for these divergent results remains unknown, but may be partially explained by the differences between cohorts in disease severity based on baseline FEV1. For instance, the baseline FEV1 was similar in the patients with or without bronchodilator responsiveness in the MILES cohort, but the baseline FEV1 was significantly lower in the patients with a positive bronchodilator response compared to the patients without a bronchodilator response in the previous analyses.[5,25]

Baseline lung function at the time of trial enrolment did not have an impact on either the rate of decline in FEV1 (in the placebo group), or on treatment response (in the sirolimus group). These results are in keeping with a recent analysis of the NHLBI LAM Registry that showed that the rate of decline in FEV1 is remarkably consistent across all categories after dividing patients on the basis of initial FEV1.[5] Our results also highlight that treatment with sirolimus is beneficial even in patients with severe disease, and that a trial of sirolimus treatment is warranted even in the most advanced cases of LAM.

The identification of surrogate biomarkers that are associated with meaningful outcomes (e.g. lung function decline or survival) in LAM is an unmet need which would greatly accelerate trials and reduce the numbers of patients required for interventional studies.

Serum VEGF-D is a diagnostic biomarker for LAM, and is recommended for use in the diagnostic algorithm prior to undertaking invasive diagnostic procedures in patients with suspected LAM.[26] In a post-hoc analysis of the MILES trial, serum VEGF-D was also shown to have potential as a predictive biomarker of treatment response.[13] In this study, we found that a baseline serum VEGF-D cut-off level of 600pg/ml identified a subset of patients that was more likely to progress and more likely to respond to treatment. The choice of 600pg/ml as a cut-off was based on our previous report that this value represented the lower end of the range of VEGF-D levels that exhibited excellent diagnostic sensitivity and specificity for LAM among women with cystic lung disease.[12] The rate of decline in FEV1 in the high VEGF-D (>600pg/ml) group appeared to be fastest in the first three months of the study as compared to the remainder of the first year (Figure 4A). This divergence in the rate of decline across the study duration is likely driven by selective dropout of the most severe patients from the placebo group.

Although not conclusive by themselves, post-hoc analyses such as this one are important for formulating future research questions and priorities, as well as informing the design of clinical trials. For example, if the differential impact of menopausal status and serum VEGF-D levels had been clearly established prior to MILES, and recruitment had been restricted to premenopausal patients with an elevated serum VEGF-D >600pg/ml, the trial could have been powered with a sample size that was a fraction of the original enrolment target. Although one could argue that cohort stratification in this manner can limit the generalizability of results to the entire LAM population, in rare diseases with limited trial populations and finite resources, this strategy of targeted recruitment of

patients with the greatest potential for decline (based on clinical characteristics and biomarkers) could yield significant reductions in cost, time, and risk exposure and allow earlier access to treatment.

Strengths of this analysis included that the patients were enrolled prospectively, and were randomized in a 1:1 ratio to placebo versus sirolimus in a double-blind fashion. This design allows us to compare the various subgroups not only in terms of the natural history of disease progression (i.e. in the placebo arm), but also assess treatment response. The various study-related parameters such as PFTs were collected at pre-specified intervals in a rigorous manner with real-time quality control and feedback. Serum VEGF-D measurements were performed in a CAP/CLIA-approved laboratory by personnel who were blinded to the clinical information. The major limitations of our analysis include the relatively low number of subjects in each of the subgroups and the post-hoc approach. The randomized, placebo-controlled design likely introduced a selection bias leading to preferential recruitment of patients with more advanced and progressive disease, thus limiting the generalizability of these results to the entire LAM population. Lastly, these results represent observations from the first year of the MILES trial, and may not be representative of long-term outcomes, either in terms of natural history of disease progression or treatment response with sirolimus.

Conclusions

In LAM patients with moderately-severe disease enrolled in the MILES trial, the rate of decline in FEV1 in patients on placebo and the stabilizing effect of sirolimus on FEV1

were similar after stratifying patients on the basis of Asian versus Caucasian race, TSC versus S-LAM, baseline FEV1, and the presence or absence of bronchodilator responsiveness on spirometry. Serum VEGF-D is a clinically useful diagnostic, prognostic, and predictive biomarker for LAM. Menopausal status had a significant effect on the rate of decline of FEV1 in the placebo group, however both pre and postmenopausal women with LAM benefitted from treatment with sirolimus.

Table 1: Baseline to 12-month change in outcomes after dividing the MILES cohort into pre and postmenopausal subgroups

Variable	PreMP Placebo (n=27)	PreMP Sirolimus (n=32)	Between-group difference (95% CI)	p-value	PostMP Placebo (n=16)	PostMP Sirolimus (n=14)	Between-group difference (95% CI)	p-value
Rate of decline of FEV1 (ml/month) [estimate (se)]	-17 (3)	-1 (2)	(9.38, 22.28)	<0.0001	-3 (3)	6 (3)	(0.73, 17.45)	0.04
Rate of decline of FVC (ml/month) [estimate (se)]	-17 (4)	6 (4)	(11.93, 33.21)	0.0001	-3 (4)	16 (6)	(4.52, 33.28)	0.03
Rate of decline of DLCO (ml/mmHg/min/month) [estimate (se)]	-0.07 (0.04)	-0.02 (0.03)	(-0.048, 0.148)	0.4	-0.04 (0.02)	0.03 (0.03)	(-0.00, 0.14)	0.04
Serum VEGF-D slope (pg/ml/month) [estimate (se)]	-15 (20)	-99 (18)	(31.24, 136.56)	0.003	22 (27)	-54 (31)	(-3.93, 156.93)	0.07
Baseline-12month change in 6MWT distance, m [mean (se)]	31 (10)	24 (10)	(-21.73, 35.35)	0.63	18 (16)	23 (21)	(-46.72, 55.56)	0.69
Baseline-12month change in VAS [mean (se)]	-2.8 (3.8)	7 (3.2)	(0.04, 19.54)	0.07	-1.5 (3.4)	3.7 (4.6)	(-6.04, 16.58)	0.55
Baseline-12month change in FPI [mean (se)]	-0.04 (0.05)	0.10 (0.07)	(-0.03, 0.31)	0.26	-0.07 (0.06)	0.10 (0.11)	(-0.08, 0.42)	0.14

Abbreviations: 6MWT = six-minute walk test; CI = confidence interval; DLCO = Diffusion capacity of carbon monoxide; FEV1 = forced expiratory volume in one-second; FVC = forced vital capacity; FPI = functional performance inventory; m = meters, MILES = Multicenter International LAM Efficacy of Sirolimus; ml = milliliter; MP = menopausal; pg = picogram; se = standard error, VAS = visual analogue scale.

Table 2: Baseline to 12-month change in outcomes after dividing the MILES cohort on the basis of race into Asian and Caucasian categories

Variable	Asians Placebo (n = 12)	Asians Sirolimus (n = 15)	Between-group difference (95% CI)	p-value	Caucasians Placebo (n = 31)	Caucasians Sirolimus (n = 31)	Between-group difference (95% CI)	p-value
Rate of decline of FEV1 (ml/month) [estimate (se)]	-12 (3)	-1 (3)	(3.16, 19.22)	0.01	-12 (3)	2 (3)	(6.61, 21.39)	0.0005
Rate of decline of FVC (ml/month) [estimate (se)]	-13 (5)	4 (6)	(1.59, 32.21)	0.06	-11 (3)	11 (4)	(11.6, 31.2)	0.0002
Rate of decline of DLCO (ml/mmHg/min/month) [estimate (se)]	-0.07 (0.04)	-0.04 (0.03)	(-0.07, 0.13)	0.47	-0.05 (0.04)	0.01 (0.03)	(-0.04, 0.16)	0.26
Serum VEGF-D slope (pg/ml/month) [estimate (se)]	-17 (27)	-102 (24)	(14.2, 155.8)	0.03	3 (22)	-79 (22)	(20.58, 143.94)	0.01
Baseline-12month change in 6MWT distance, m [mean (se)]	29 (11)	13 (11)	(-14.2, 45.6)	0.44	21 (15)	42 (17)	(-22.0, 65.0)	0.36
Baseline-12month change in VAS [mean (se)]	-1.7 (3.1)	5.4 (3.2)	(-1.6, 15.8)	0.18	-3.8 (5.2)	7.3 (4.8)	(-2.8, 25.0)	0.20
Baseline-12month change in FPI [mean (se)]	-0.01 (0.05)	0.02 (0.05)	(-0.1, 0.2)	0.82	-0.14 (0.06)	0.24 (0.13)	(0.1, 0.7)	0.02

Abbreviations: Same as Table 1.

Figure Legend:

Figure 1: Effect of menopause and race on FEV1 response. 1A) FEV1 response by menopausal status: In the overall cohort, sirolimus stabilized FEV1 decline. In the placebo group, premenopausal women exhibited a significantly faster rate of decline of FEV1 compared to postmenopausal women. Both pre and postmenopausal women exhibited a beneficial response to treatment with sirolimus, with premenopausal women experiencing a slowing in the rate of decline and postmenopausal women trending towards improvement. **1B) FEV1 response by race:** In the overall cohort, sirolimus stabilized FEV1 decline. There was no difference in the rate of decline of FEV1 in the Asian and Caucasian patients enrolled in MILES and both subgroups responded to treatment with sirolimus.

Figure 2: Effect of menopause and race on FVC response. 2A) FVC response by menopausal status: In the overall cohort, sirolimus stabilized FVC decline. In the placebo group, premenopausal women had a significantly faster rate of decline in FVC compared to postmenopausal women. Both pre and postmenopausal women had a beneficial response to treatment with sirolimus. **2B) FVC response by race**: The rate of decline in FVC was similar in the placebo groups of both the Asian and Caucasian patients. The difference between placebo and sirolimus groups in FVC slope was statistically significant in the Caucasian patients, and trended towards significance in the Asian patients.

Figure 3: Effect of menopause and race on serum VEGF-D response. 3A) Change in serum VEGF-D levels among pre and postmenopausal patients: In the overall cohort, serum VEGF-D levels were stable over time in the placebo group and declined in the sirolimus group. This was also true in the premenopausal patients, but the between group difference in postmenopausal patients did not achieve significance, likely due to the small patient numbers. **3B) Change in serum VEGF-D levels among Asian and Caucasian patients**: Both Asian and Caucasian subjects exhibited a significant decline in serum VEGF-D levels following treatment with sirolimus.

Figure 4: Baseline to 12 month FEV1 response in the MILES Trial placebo and sirolimus groups, stratified by VEGF-D levels and menopausal status. 4A) Baseline VEGF-D > or ≤ 600pg/ml: Lung function in patients with baseline serum VEGF-D ≤ 600pg/ml remained relatively stable throughout the 12-month monitoring period in both placebo (open circle) and sirolimus (open square) subgroups. Patients with a serum VEGF-D > 600pg/ml exhibited a faster decline in the placebo subgroup (closed circle) and improved treatment response to sirolimus (closed square). **4B) Pre versus postmenopausal patients:** Premenopausal patients on placebo (open circle) had the highest rate of decline of lung function, and had an overall stabilization of FEV1 decline on sirolimus (open square). Postmenopausal patients on placebo (closed circle) remained relatively stable during the 12-month monitoring period and exhibited an overall improvement in their FEV1 following treatment with sirolimus (closed square).

Figure 5: Percent change from baseline in serum VEGF-D levels and FEV1 over time. The placebo group (open circle) exhibited progressive decline in FEV1 with relatively stable VEGF-D levels, whereas the sirolimus group (solid squares) tended to have stable FEV1 with declining serum VEGF-D values during the 12-month treatment period.

Figure 6: Time course of adverse events during the first 12 months of the MILES trial. The total numbers of adverse events (AEs) are shown for both sirolimus and placebo arms. In general, there were more AEs in the sirolimus arm as compared to the placebo arm, and there was a decrease in the frequency of AEs over the course of the trial in both arms.

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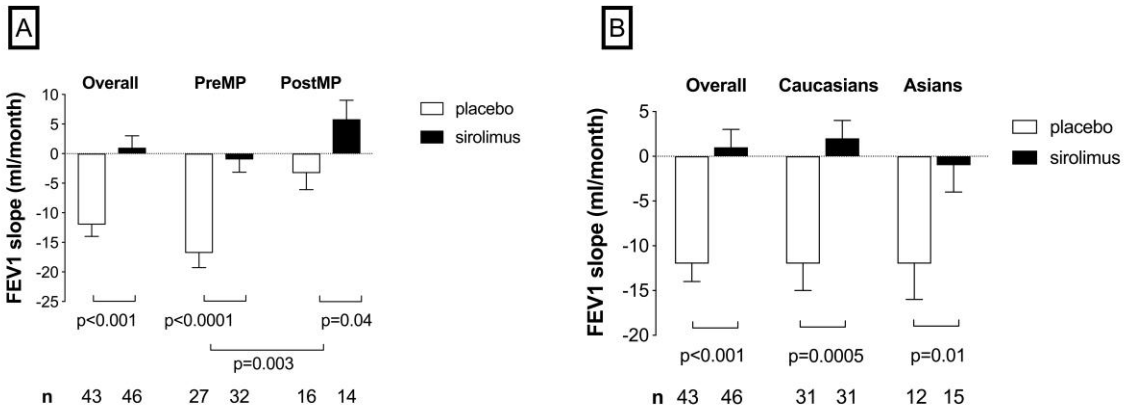
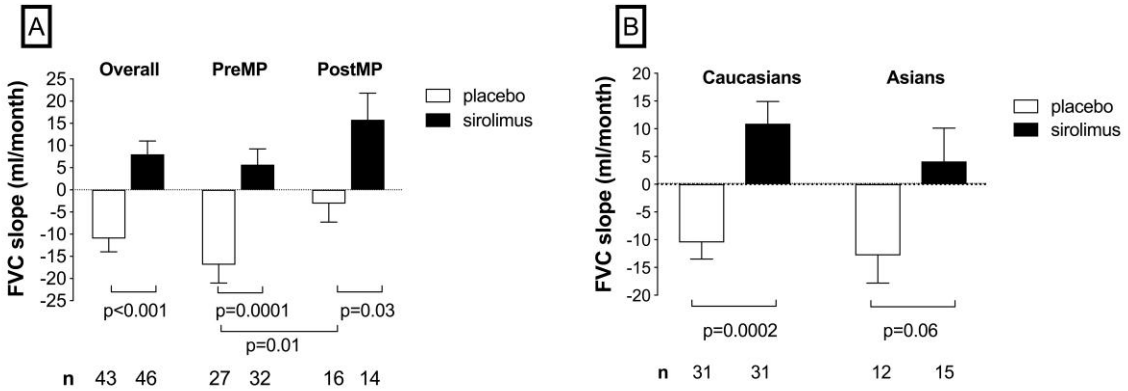

A
Overall
PreMP
PostMP
FEV1 slope (ml/month)
10
5
0
-5
-10
-15
-20
-25
placebo
sirolimus
p<0.001
p<0.0001
p=0.04
p=0.003
n 43 46 27 32 16 14
B
Overall
Caucasians
Asians
FEV1 slope (ml/month)
5
0
-5
-10
-15
-20
placebo
sirolimus
p<0.001
p=0.0005
p=0.01
n 43 46 31 31 12 15


A
Overall
PreMP
PostMP
FVC slope (ml/month)
25
20
15
10
5
0
-5
-10
-15
-20
-25
placebo
sirolimus
p<0.001
p=0.0001
p=0.03
p=0.01
n 43 46 27 32 16 14
B
Caucasians
Asians
FVC slope (ml/month)
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10
5
0
-5
-10
-15
-20
placebo
sirolimus
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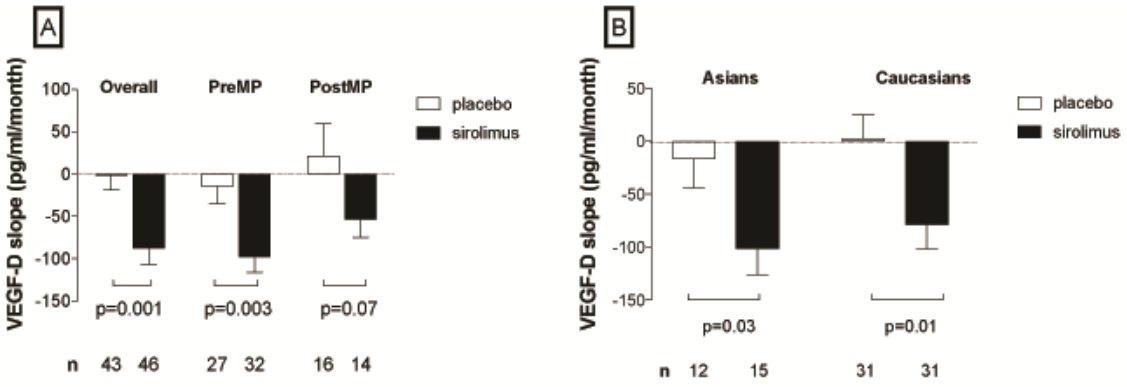
n 31 31 12 15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VEGF-D slope (pg/ml/month)
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50
0
-50
-100
-150
Overall
PreMP
PostMP
placebo
sirolimus
p=0.001
p=0.003
p=0.07
n 43 46 27 32 16 14
B
VEGF-D slope (pg/ml/month)
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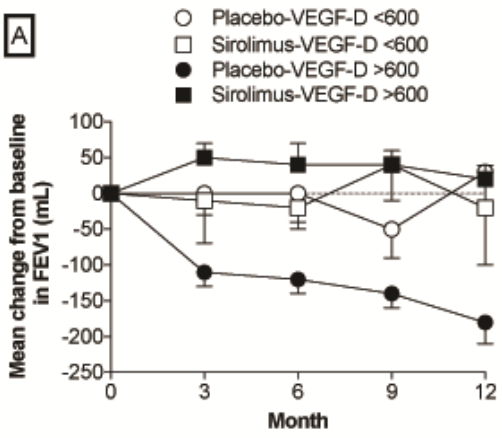
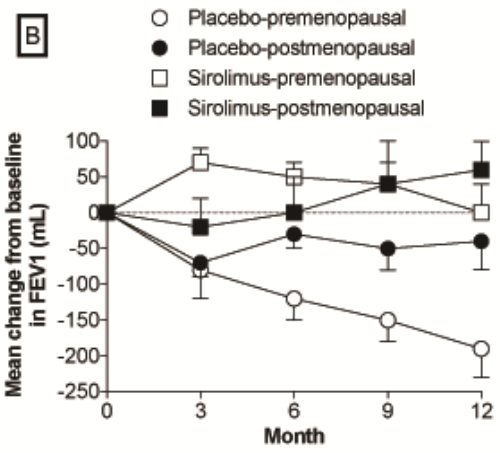
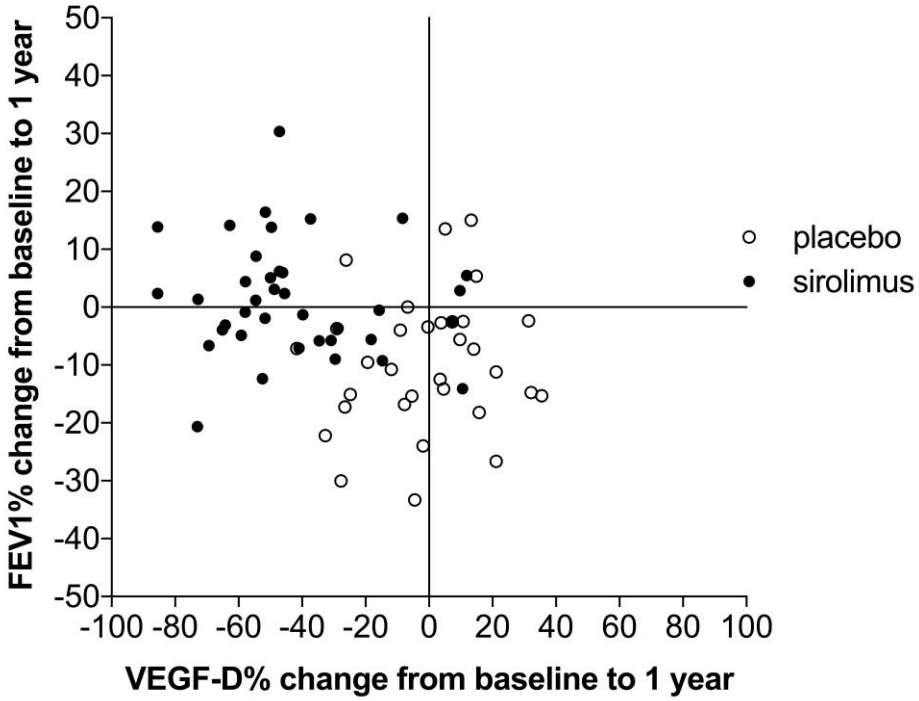
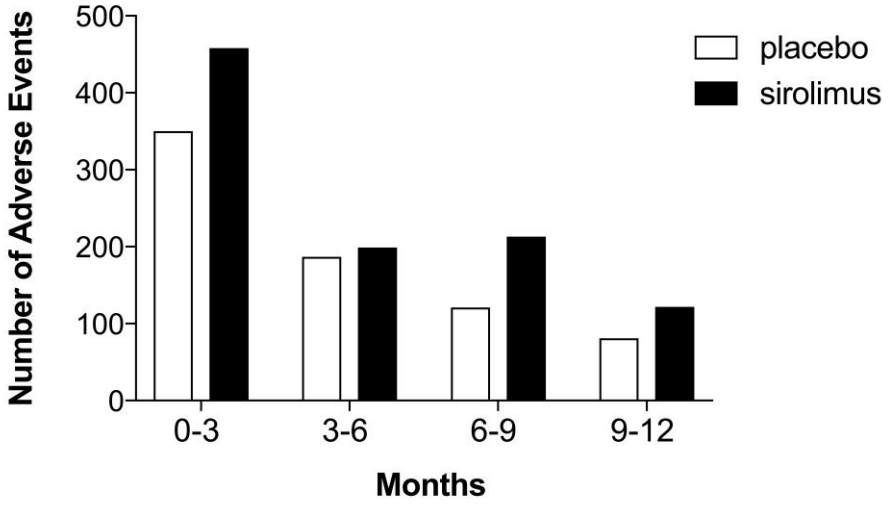
0
-50
-100
-150
Asians
Caucasians
placebo
sirolimus
p=0.03
p=0.01
n 12 15 31 31


A
Placebo-VEGF-D <600
Sirolimus-VEGF-D <600
Placebo-VEGF-D >600
Sirolimus-VEGF-D >600
Mean change from baseline in FEV1 (mL)
100
50
0
-50
-100
-150
-200
-250
0
3
6
9
12
Month


B
Placebo-premenopausal
Placebo-postmenopausal
Sirolimus-premenopausal
Sirolimus-postmenopausal
Mean change from baseline in FEV1 (mL)
100
50
0
-50
-100
-150
-200
-250
0
3
6
9
12
Month


FEV1% change from baseline to 1 year
50
40
30
20
10
0
-10
-20
-30
-40
-50
-100 -80 -60 -40 -20 0 20 40 60 80 100
VEGF-D% change from baseline to 1 year
placebo
sirolimus


Number of Adverse Events
500
400
300
200
100
0
0-3
3-6
6-9
9-12
Months
placebo
sirolimus

Online Data Supplement

Supplemental Table E1: Baseline characteristics of the study subjects after dividing the MILES patients into pre and postmenopausal subgroups

Variable	PreMP Placebo (n=27)	PreMP Sirolimus (n=32)	PostMP Placebo (n=16)	PostMP Sirolimus (n=14)
FEV1 (liters) [mean (sd)]	1.36 (0.48)	1.42 (0.40)	1.41 (0.39)	1.21 (0.37)
FEV1 % predicted [mean (sd)]	45 (15)	51 (14)	52 (12)	45 (12)
FVC (liters) [mean (sd)]	3.03 (0.75)	2.72 (0.60)	2.71 (0.73)	2.60 (0.68)
FVC % predicted [mean (sd)]	82 (18)	80 (16)	79 (18)	75 (16)
DLCO (ml/mmHg/min) [mean (sd)]	9.46 (5.22)	10.33 (5.02)	12.09 (3.60)	9.42 (2.90)
DLCO % predicted [mean (sd)]	38 (21)	44 (20)	54 (16)	42 (13)
Serum VEGF-D (pg/ml) [mean (sd)]	2,237 (2217)	1,976 (1690)	2,199 (4044)	1,530 (937)
Supplemental oxygen use [n (%)]	15 (56)	18 (56)	8 (50)	11 (79)
H/o pneumothorax [n (%)]	17 (63)	20 (63)	12 (75)	4 (29)
Angiomyolipoma [n (%)]	11 (41)	14 (44)	11 (69)	8 (57)
6MWT distance, metres [mean (sd)]	378 (128)	408 (98)	434 (84)	404 (96)
Visual Analogue Scale [mean (sd)]	64.7 (21.4)	67.5 (19.8)	71.1 (17.7)	70.9 (15.9)
Functional Performance Inventory [mean (sd)]	2.3 (0.5)	2.3 (0.5)	2.5 (0.4)	2.2 (0.5)

Abbreviations: DLCO = Diffusion capacity of carbon monoxide; FEV1 = forced expiratory volume in one-second; FVC = forced vital capacity; MILES = Multicenter International LAM Efficacy of Sirolimus; ml = milliliter; MP = menopausal; n = number of subjects; pg/ml = picogram per milliliter; sd = standard deviation; VEGF-D = vascular endothelial growth factor-D.

Supplemental Table E2: Baseline characteristics of the study subjects after dividing the MILES cohort on the basis of race into Asian and Caucasian categories

Variable	Asians Placebo (n = 12)	Asians Sirolimus (n = 15)	Caucasians Placebo (n = 31)	Caucasians Sirolimus (n = 31)
FEV1 (liters) [mean (sd)]	1.11 (0.39)	1.43 (0.34)	1.48 (0.43)	1.32 (0.43)
FEV1 % predicted [mean (sd)]	42 (15)	55 (11)	50 (14)	47 (14)
FVC (liters) [mean (sd)]	2.64 (0.61)	2.60 (0.63)	3.01 (0.78)	2.72 (0.62)
FVC % predicted [mean (sd)]	81 (13)	83 (16)	81 (19)	77 (16)
DLCO (ml/mmHg/min) [mean (sd)]	7.47 (4.33)	8.54 (4.41)	11.65 (4.53)	10.78 (4.38)
DLCO % predicted [mean (sd)]	33 (21)	38 (20)	48 (19)	46 (16)
Serum VEGF-D (pg/ml) [mean (sd)]	2,165 (2197)	2,048 (1709)	2,246 (3296)	1,748 (1428)
Supplemental oxygen use [n (%)]	7 (58)	10 (67)	16 (52)	19 (61)
H/o pneumothorax [n (%)]	6 (50)	9 (60)	23 (74)	15 (48)
Angiomyolipoma [n (%)]	5 (42)	2 (13)	17 (55)	20 (65)
6MWT distance, metres [mean (sd)]	306 (85)	346 (66)	435 (106)	436 (96)
Visual Analogue Scale [mean (sd)]	61.3 (22.4)	63.3 (23.7)	69.3 (19.1)	71.0 (15.4)
Functional Performance Inventory [mean (sd)]	2.2 (0.7)	2.2 (0.6)	2.4 (0.4)	2.3 (0.5)

Abbreviations: Same as Supplemental Table E1.

Supplemental Table E3: Baseline demographic and clinical characteristics of the TSC and sporadic LAM patients enrolled in the MILES trial

Characteristic	**TSC-LAM (N = 8)**	**Sporadic LAM (N = 81)**
Randomized to sirolimus group (n, %)	4 (50)	42 (52)
Age at enrollment (years), Mean ± S.D.	47.4 ± 12.1	45.2 ± 10.5
Clinical Features		
Postmenopausal, n (%)	4 (50)	26 (32)
History of angiomyolipoma, n (%)	7 (88)	37 (46)
History of pneumothorax, n (%)	5 (63)	48 (59)
History of chylothorax	0 (0)	9 (11)
Oxygen therapy requirement	3 (38)	49 (60)
Pulmonary Function Testing		
FEV1 (mL), Mean ± S.D.	1,163 ± 498	1,387 ± 410
FVC (mL), Mean ± S.D.	2,443 ± 823	2,826 ± 674
TLC (mL), Mean ± S.D.	5,071 ± 699	5,054 ± 1289
FRC (mL), Mean ± S.D.	3,160 ± 1121	2,989 ± 896
RV (mL), Mean ± S.D.	2,721 ± 833	2,227 ± 879
DLCO (ml/mmHg/min), Mean ± S.D.	11.81 ± 5.37	10.06 ± 4.54
6 minute walk distance (m), Mean ± S.D.	422 ± 95	401 ± 107
Visual Analogue Scale Quality of life, Mean ± S.D.	68.63 ± 16.80	67.74 ± 19.57
Functional Performance Inventory, Mean ± S.D.	2.44±0.37	2.28±0.51
General Well Being Questionnaire, Mean ± S.D.	61.13±3.98	62.86±4.76
Serum VEGF-D concentration (pg/ml), Mean ± S.D.	4,208 ± 5595	1,808 ± 1645

Abbreviations: Same as Supplemental Table E1 plus, FRC = functional residual capacity; LAM = lymphangioleiomyomatosis; RV = residual volume; TLC = total lung capacity; TSC = tuberous sclerosis complex,

Supplemental Table E4: Baseline characteristics of the subjects after dividing the MILES cohort on the basis of presence or absence of tuberous sclerosis complex.

Variable	TSC Placebo (n = 4)	TSC Sirolimus (n = 4)	S-LAM Placebo (n = 39)	S-LAM Sirolimus (n = 42)
FEV1 (liters) [mean (sd)]	1.16 (0.59)	1.16 (0.47)	1.40 (0.43)	1.38 (0.39)
FEV1 % predicted [mean (sd)]	44 (18)	39 (13)	48 (14)	50 (13)
FVC (liters) [mean (sd)]	2.25 (0.59)	2.64 (1.06)	2.98 (0.74)	2.69 (0.58)
FVC % predicted [mean (sd)]	69 (13)	72 (28)	82 (18)	79 (15)
DLCO (ml/mmHg/min) [mean (sd)]	13.28 (6.12)	10.35 (4.91)	10.11 (4.65)	10.02 (4.49)
DLCO % predicted [mean (sd)]	59 (24)	43 (18)	42 (20)	43 (18)
Serum VEGF-D (pg/ml) [mean (sd)]	6,544 (7605)	1,873 (818)	1,768 (1741)	1,845 (1572)
Supplemental oxygen use [n (%)]	2 (50)	1 (25)	21 (54)	28 (67)
H/o pneumothorax [n (%)]	3 (75)	2 (50)	26 (67)	22 (52)
Angiomyolipoma [n (%)]	3 (75)	4 (100)	19 (49)	18 (43)
6MWT distance, metres [mean (sd)]	366 (92)	479 (62)	402 (118)	400 (97)
Visual Analogue Scale [mean (sd)]	66.3 (20.6)	71.0 (14.9)	67.2 (20.3)	68.3 (19.1)
Functional Performance Inventory [mean (sd)]	2.6 (0.3)	2.3 (0.4)	2.3 (0.5)	2.2 (0.5)

Abbreviations: Same as Supplemental Tables E1 and E3.

Supplemental Table E5: Baseline to 12-month change in outcomes after dividing the MILES cohort on the basis of presence or absence of tuberous sclerosis complex.

Variable	TSC Placebo (n = 4)	TSC Sirolimus (n = 4)	Between-group difference (95% CI)	p-value	S-LAM Placebo (n = 39)	S-LAM Sirolimus (n = 42)	Between-group difference (95% CI)	p-value
Rate of decline of FEV1 (ml/month) [estimate (se)]	-7 (5)	13 (5)	(5.53, 34.6)	0.04	-12 (2)	0.3 (2)	(6.9, 18.6)	<0.0001
Rate of decline of FVC (ml/month) [estimate (se)]	-7 (8)	29 (9)	(12.9, 59.0)	0.03	-12 (4)	7 (3)	(9.0, 27.7)	0.0003
Rate of decline of DLCO (ml/mmHg/min/month) [estimate (se)]	-0.03 (0.07)	0.13 (0.08)	(-0.05, 0.37)	0.19	-0.06 (0.03)	-0.02 (0.03)	(-0.04, 0.12)	0.35
Serum VEGF-D slope (pg/ml/month) [estimate (se)]	92 (99)	-105 (110)	(-93.6, 487)	0.23	-9 (15)	-85 (14)	(35.9, 117.5)	0.0004
Baseline-12month change in 6MWT distance, m [mean (se)]	57 (29)	38 (9)	(-41.0, 78.6)	1	23 (9)	23 (10)	(-25.8, 27.0)	0.99
Baseline-12month change in VAS [mean (se)]	4.8 (8.7)	5.0 (7.6)	(-22.4, 22.8)	1	-3.3 (2.8)	6.2 (2.8)	(1.74, 17.3)	0.05
Baseline-12month change in FPI [mean (se)]	-0.23 (0.13)	0.13 (0.19)	(-0.09, 0.81)	0.25	-0.03 (0.04)	0.10 (0.06)	(-0.01, 0.27)	0.14

Abbreviations: Same as Supplemental Tables E1 and E3 plus, CI = confidence interval, FPI = functional performance inventory, m = meters, se = standard error, VAS = visual analogue scale.

Supplemental Table E6: Baseline characteristics of the subjects after dividing the MILES cohort on the basis of presence or absence of bronchodilator responsiveness.

Variable	BR+ Placebo (n = 13)	BR+ Sirolimus (n = 14)	No BR Placebo (n = 30)	No BR Sirolimus (n = 32)
FEV1 (liters) [mean (sd)]	1.32 (0.51)	1.30 (0.45)	1.40 (0.42)	1.38 (0.38)
FEV1 % predicted [mean (sd)]	43 (15)	46 (16)	50 (14)	51 (12)
FVC (liters) [mean (sd)]	3.20 (0.81)	3.06 (0.69)	2.78 (0.70)	2.52 (0.52)
FVC % predicted [mean (sd)]	86 (18)	87 (18)	78 (17)	75 (13)
DLCO (ml/mmHg/min) [mean (sd)]	9.92 (4.35)	9.91 (3.43)	10.66 (5.08)	10.11 (4.91)
DLCO % predicted [mean (sd)]	40 (16)	42 (13)	46 (22)	44 (20)
Serum VEGF-D (pg/ml) [mean (sd)]	2,931 (2,778)	1,807 (866)	1,905 (3,083)	1,866 (1,743)
Supplemental oxygen use [n (%)]	8 (62)	9 (64)	15 (50)	20 (62)
H/o pneumothorax [n (%)]	8 (62)	2 (14)	21 (70)	22 (69)
Angiomyolipoma [n (%)]	8 (62)	6 (43)	14 (47)	16 (50)
6MWT distance, metres [mean (sd)]	390 (149)	438 (98)	403 (101)	393 (94)
Visual Analogue Scale [mean (sd)]	55.5 (21.6)	67.5 (17.9)	72.1 (17.5)	68.9 (19.2)
Functional Performance Inventory [mean (sd)]	2.1 (0.6)	2.2 (0.5)	2.4 (0.4)	2.3 (0.5)

Abbreviations: Same as Supplemental Table E1, plus BR = Bronchodilator responsiveness

Supplemental Table E7: Baseline to 12-month change in outcomes after dividing the MILES cohort on the basis of presence or absence of bronchodilator responsiveness.

Variable	BR+ Placebo (n = 13)	BR+ Sirolimus (n = 14)	Between-group difference (95% CI)	p-value	No BR Placebo (n = 30)	No BR Sirolimus (n = 32)	Between-group difference (95% CI)	p-value
Rate of decline of FEV1 (ml/month) [estimate (se)]	-12 (4)	2 (4)	(3.28, 23.8)	0.016	-12 (3)	1 (2)	(6.08, 19.6)	0.0005
Rate of decline of FVC (ml/month) [estimate (se)]	-11 (7)	17 (7)	(9.09, 47.7)	0.008	-11 (4)	5 (4)	(6.37, 26.1)	0.002
Rate of decline of DLCO (ml/mmHg/min/month) [estimate (se)]	-0.04 (0.03)	-0.07 (0.03)	(-0.05, 0.11)	0.39	-0.07 (0.03)	0.02 (0.03)	(0.01, 0.17)	0.09
Serum VEGF-D slope (pg/ml/month) [estimate (se)]	-25 (22)	-72 (21)	(-13.4, 107.8)	0.14	12 (22)	-94 (22)	(44.6, 166.8)	0.001
Baseline-12month change in 6MWT distance, m [mean (se)]	41 (17)	19 (17)	(-24.9, 68.3)	0.51	21 (10)	26 (11)	(-24.9, 34.5)	0.71
Baseline-12month change in VAS [mean (se)]	2.4 (5.0)	2.1 (4.4)	(-14.3, 14.9)	0.95	-4.2 (3.1)	7.8 (3.3)	(3.11, 20.9)	0.03
Baseline-12month change in FPI [mean (se)]	0.08 (0.08)	0.008 (0.102)	(-0.18, 0.33)	0.87	-0.1 (0.04)	0.14 (0.07)	(0.08, 0.40)	0.02

Abbreviations: Same as Supplemental Tables E1 and E5

Supplemental Table E8: Baseline characteristics of the subjects after dividing the MILES cohort on the basis of starting FEV1 (51 – 70% vs. ≤ 50%).

Variable	FEV1 51-70% Placebo (n = 22)	FEV1 51-70% Sirolimus (n = 18)	FEV1 ≤ 50% Placebo (n = 21)	FEV1 ≤ 50% Sirolimus (n = 28)
FEV1 (liters) [mean (sd)]	1.72 (0.26)	1.74 (0.27)	1.02 (0.29)	1.11 (0.25)
FEV1 % predicted [mean (sd)]	59 (5)	63 (4)	36 (10)	40 (7)
FVC (liters) [mean (sd)]	3.05 (0.61)	2.84 (0.67)	2.76 (0.86)	2.57 (0.59)
FVC % predicted [mean (sd)]	85 (14)	84 (15)	77 (20)	75 (15)
DLCO (ml/mmHg/min) [mean (sd)]	11.73 (3.98)	11.79 (5.35)	9.05 (5.32)	9.05 (3.65)
DLCO % predicted [mean (sd)]	49 (17)	50 (21)	38 (22)	39 (15)
Serum VEGF-D (pg/ml) [mean (sd)]	1693 (2028)	2020 (1921)	2806 (3782)	1711 (1244)
Supplemental oxygen use [n (%)]	9 (41)	8 (47)	14 (67)	21 (75)
H/o pneumothorax [n (%)]	16 (73)	9 (53)	13 (62)	15 (54)
Angiomyolipoma [n (%)]	13 (59)	6 (35)	9 (43)	16 (57)
6MWT distance (metres)	420 (111)	412 (104)	376 (118)	398 (89)
Visual Analogue Scale [mean (sd)]	71.4 (17.3)	75.2 (19.1)	62.6 (22.2)	63.9 (17.3)
Functional Performance Inventory [mean (sd)]	2.51 (0.35)	2.43 (0.46)	2.17 (0.55)	2.11 (0.50)

Abbreviations: Same as Supplemental Table E1

Supplemental Table E9: Baseline to 12-month change in outcomes after dividing the MILES cohort on the basis of starting FEV1 (51 – 70% vs. ≤ 50%).

Variable	FEV1 51-70% Placebo (n = 22)	FEV1 51-70% Sirolimus (n =18)	Between-group difference (95% CI)	p-value	FEV1 ≤ 50% Placebo (n = 21)	FEV1 ≤ 50% Sirolimus (n = 28)	Between-group difference (95% CI)	p-value
Rate of decline of FEV1 (ml/month) [estimate (se)]	-13 (4)	2 (4)	(4.73, 25.3)	0.007	-10 (2)	0.3 (2)	(4.75, 16.4)	0.001
Rate of decline of FVC (ml/month) [estimate (se)]	-9 (4)	11 (5)	(7.95, 32.7)	0.003	-14 (5)	6 (5)	(6.52, 33.5)	0.006
Rate of decline of DLCO (ml/mmHg/min/month) [estimate (se)]	-0.07 (0.03)	0.01 (0.03)	(-0.003, 0.163)	0.084	-0.05 (0.05)	-0.015 (0.038)	(-0.09, 0.16)	0.585
Serum VEGF-D slope (pg/ml/month) [estimate (se)]	-10 (22)	-106 (24)	(33.23, 159.5)	0.005	9 (26)	-71 (23)	(12.04, 147)	0.026
Baseline-12month change in 6MWT distance, m [mean (se)]	29 (14)	34 (13)	(-32.6, 42.6)	0.76	23 (11)	18 (13)	(-28.8, 37.8)	0.91
Baseline-12month change in VAS [mean (se)]	-3.9 (3.2)	3.5 (3.8)	(-2.42, 17.2)	0.19	-0.7 (4.4)	7.9 (3.8)	(-2.71, 19.9)	0.20
Baseline-12month change in FPI [mean (se)]	-0.02 (0.04)	0.15 (0.07)	(0.01, 0.33)	0.15	-0.08 (0.07)	0.08 (0.09)	(-0.06, 0.38)	0.19

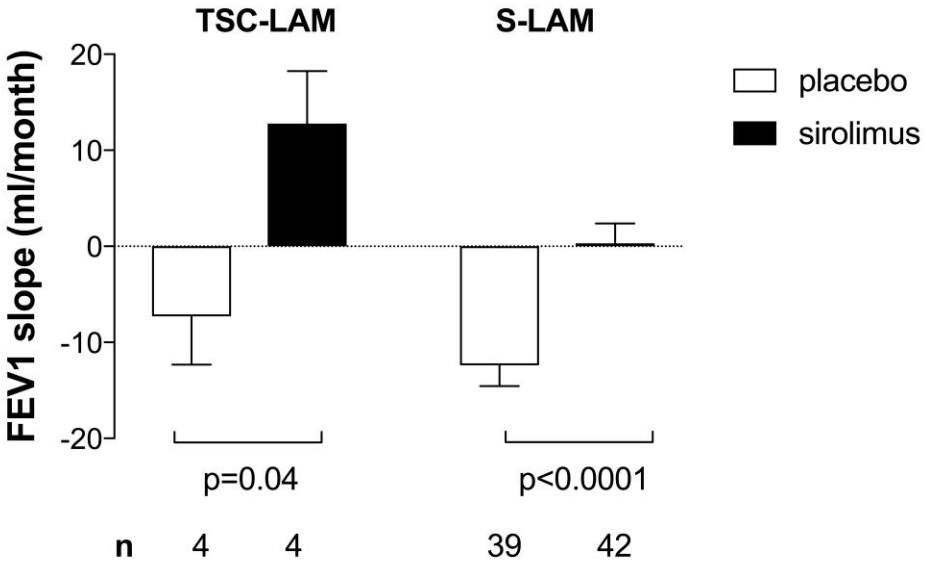
Abbreviations: Same as Supplemental Tables E1 and E5

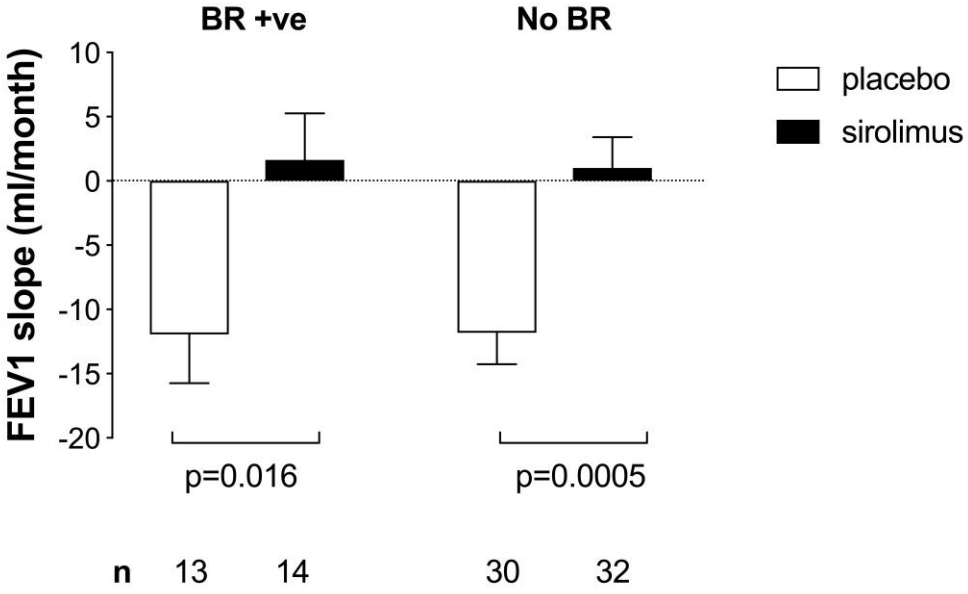
Supplemental Figure Legend:

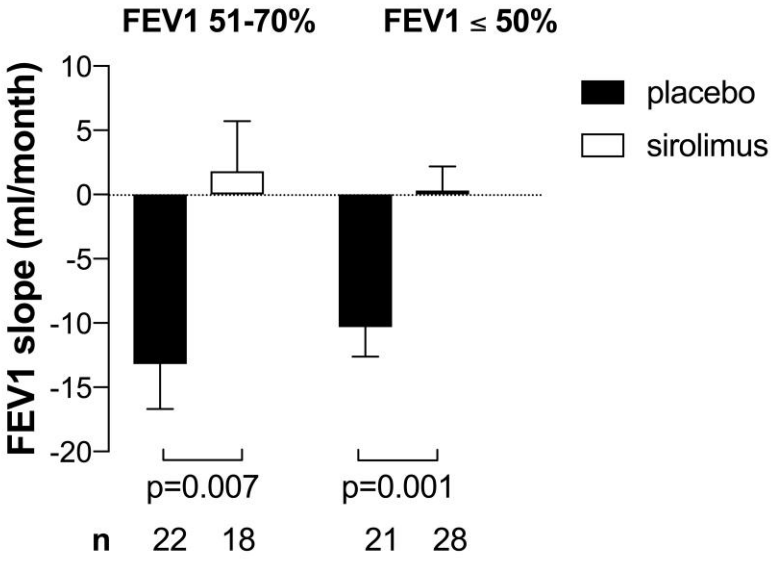
Supplemental Figure E1: Effect of LAM subtype (TSC-LAM vs. sporadic LAM) on the rate of change in FEV1 (FEV1 slope): The FEV1 slope was similar in the placebo group in both TSC and sporadic LAM patients. Both groups exhibited a significant beneficial response to treatment with sirolimus.

Supplemental Figure E2: Rate of change in FEV1 (FEV1 slope) after dividing the MILES cohort on the basis of bronchodilator responsiveness at baseline: The presence or absence of bronchodilator responsiveness had no impact on the FEV1 slope in the placebo patients. Both subgroups exhibited a significant beneficial response to treatment with sirolimus.

Supplemental Figure E3: Rate of change in FEV1 (FEV1 slope) after dividing the MILES cohort on the basis of baseline FEV1 (51 – 70% vs. ≤ 50%): Baseline FEV1 did not impact subsequent rate of disease progression in the placebo group. Both subgroups exhibited a beneficial response to treatment with sirolimus.


TSC-LAM
S-LAM
FEV1 slope (ml/month)
20
10
0
-10
-20
placebo
sirolimus
p=0.04
p<0.0001
n
4
4
39
42


BR +ve
No BR
FEV1 slope (ml/month)
10
5
0
-5
-10
-15
-20
placebo
sirolimus
p=0.016
p=0.0005
n
13
14
30
32


FEV1 51-70%
FEV1 ≤ 50%
FEV1 slope (ml/month)
10
5
0
-5
-10
-15
-20
placebo
sirolimus
p=0.007
p=0.001
n
22
18
21
28