Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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SECTION 1. ACKNOWLEDGEMENTS

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SECTION 2. RARE LUNG DISEASES CONSORTIUM ROSTER

National Institutes of Health Rare Lung Diseases Consortium

Sponsor: Office of Rare Disease Research: Director, Steve Groft, Pharm. D.
Administered by: National Center for Research Resources: Director, Barbara Alving, M.D., Elaine Collier, M.D., Carl Hunt, M.D.
Data Management and Monitoring Center: University of South Florida: Director, Jeffrey Krischer

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Co-director: Frank McCormack, M.D.
DSMB Chair: Robert Senior, M.D.

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Medical Director: Timothy P. Cripe, M.D., Ph.D.
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13. University Health Network/University of Toronto, Principal Investigator: Lianne Singer and G. Downey, M.D.; Personnel: M. Sichitu, J. Thenganatt
14. Data Management Coordinating Center, Pediatrics Epidemiology Center; University of South Florida, Principal Investigator: Jeffrey Krischer, Ph.D; Personnel: M. Abbondondolo, F. Badias, M. Colouris, D. Cuthbertson, K. Grant, H. Lee, K. Paulus
15. Data and Safety Monitoring Board, Chair: Robert M. Senior M.D.; Members: C. Redmond, J.P. Clancy, J. Ryu, K. Flaherty

SECTION 3. DETAILED CRITERIA FOR INCLUSION AND EXCLUSION

Inclusion Criteria

- Female, age 18 years or over
- Signed and dated informed consent
- Diagnosis of LAM as determined by compatible chest CT*
  and
  - Biopsy† (lung, abdominal mass, lymph node or kidney) or cytology (from thoracic or abdominal sources revealing HMB45 positive staining of spindled/epithelioid cells)
    or
  - Tuberous sclerosis, angiomyolipoma (diagnosed by CT, MRI by the site radiologist or biopsy) or chylous pleural effusion (verified by tap)
    or
  - Serum VEGF-D level ≥ 800 pg/ml ‡
- Post-bronchodilator forced expiratory volume in one second of ≤ 70% of predicted during baseline visit.

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* Chest CTs were reviewed by MILES radiologists

† In cases where the diagnosis of LAM was based on biopsy, review of the pathology specimens by pathologists experienced in reviewing LAM histopathology was obtained if not done so previously. Review was provided by MILES Core Pathologist, Dr. Thomas Colby, or site pathologist who were approved by study sponsor based on review of the curriculum vitae.

‡ See manuscript reference 21 regarding VEGF-D levels.

Exclusion Criteria

- History of myocardial infarction, angina or stroke related to atherosclerosis
- Pregnant, breast feeding, or plan to become pregnant within the next 2 years
- Inadequate contraception*****
- Significant hematologic or hepatic abnormality (i.e. transaminase levels > three times the upper limit of normal range, hematocrit < 30%, platelets < 80,000/cu mm, adjusted absolute neutrophil count < 1,000/cu mm, total white blood cell count < 3,000/cu mm)
- Intercurrent infection at initiation of study drug
- Recent surgery (involving entry into a body cavity or requiring 3 or more sutures) within eight weeks of initiation of study drug
- Use of an investigational drug within the 30 days prior to randomization
- Uncontrolled hyperlipidemia
- Previous lung transplantation or active on transplant list
- Inability to attend scheduled clinic visits
- Inability to give informed consent
- Inability to perform pulmonary function testing
- Creatinine > 2.5 mg/dl
- Chylous ascites sufficient to affect diaphragmatic function based on the opinion of the site investigator
- Pleural effusion sufficient to affect pulmonary function based on the opinion of the Site Investigator (generally > 500cc)
- Acute pneumothorax within the past two months†
- History of malignancy in the past two years, other than squamous cell or basal cell skin cancer or cervical cancer.
- Use of estrogen containing medications within the 30 days prior to randomization‡
- Known allergy to sirolimus

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* Sirolimus should not be taken during pregnancy. Participants who are fertile must maintain adequate contraception throughout the trial and for twelve weeks after stopping the drug. Acceptable contraceptive measures include prior hysterectomy, oophorectomy or tubal ligation, complete abstinence, double barrier methods which include both a cervical diaphragm and spermicidal jelly, intrauterine devices (IUD), progestin based contraceptives, or vasectomy in partner(s).GnRh agonists do not provide adequate contraception for the purposes of this trial. Pregnancy tests will be obtained from women of childbearing potential (defined as any fertility status other than natural or surgical menopause or post hysterectomy) at the baseline visit, each subsequent visit, and 12 weeks following the last time study drug is taken.

† A chronic stable loculated pneumothorax is not exclusionary.

‡ Prior or ongoing use of progestins, gonadotropic releasing hormone (GnRh) agonists or other, non-estrogen containing hormonal therapy are not a basis for exclusion. Participants will not be asked to modify their non-estrogen containing hormonal therapies.

SECTION 4. ADDITIONAL DETAIL FOR METHODS AND RESULTS

Study Operations and Oversight

The study was conducted by the National Institutes of Health supported Rare Lung Diseases Consortium (RLDC), which included a lead site at the University of Cincinnati and Cincinnati Children's Hospital Medicine Center (CCHMC) and 12 additional sites in the United States, Japan and Canada. The Translational Research Trials Office (TRTO) at CCHMC managed day-to-day operations of the trial, including coordination of care at the Cincinnati site and across all sites, management of adverse events, and reporting to the FDA, the DSMB and the DMCC. All efficacy and safety data in the database were monitored by the TRTO, and by contract research organizations in Japan and Canada.

Study Design and Endpoints

Measurement of postbronchodilator FEV1 and FVC on identical volume displacement spirometers distributed to all RLDC sites (OMI, Houston, TX) was performed according to 2005 American Thoracic Society (ATS)/European Respiratory Criteria on both days of the initial visit (including assessment of reversible airflow obstruction on the first day) and the data set with the highest postbronchodilator FEV1 was chosen as the baseline.

For safety, the following laboratory values were measured on every visit: white-cell and platelet counts and concentrations of hemoglobin, alanine and aspartate aminotransferases, alkaline phosphatase, bilirubin, albumin, creatinine, glucose, potassium, sodium, total cholesterol, and triglycerides and urine protein and albumin to creatinine ratio. Sirolimus serum level measurements for all sites were made by the Central Laboratory at CCHMC. Results were provided only the medical monitor at the Data Management and Coordinating Center. The serum VEGF-D measurements were made by technicians who were blinded to patient treatment assignment in a CAP/CLIA laboratory at CCHMC.

Statistical Analyses- Analysis Plan and Power Calculation

One hundred and twenty patients will be randomized to placebo or sirolimus groups, treated for one year and followed off of drug or placebo for one additional year. The primary endpoint will be FEV1 slope at 12 months year, the number of severity graded safety/toxicity incidences requiring dose interruption, modification or discontinuation, the number of qualifying events for the study termination. The secondary endpoints will include the following: FVC slope, DLCO slope, six minute walk distance, lung volume slope (residual volume, functional residual capacity or thoracic gas volume and total lung capacity), quality of life, dyspnea, fatigue, volumetric CT analysis of cyst size and mass of lung in the chest and biomarker analysis (e.g. VEGF-D) at 12 months. Up to 10 patients who participated in the first sirolimus study (CAST) in Cincinnati will also enroll in this study. To examine a potential selection bias, we will do a sensitivity analysis for those patients at the conclusion of the study.

Aim1: Determine the safety and efficacy of sirolimus in patients with LAM.

For safety, we will present the descriptive statistics of severity graded adverse events, and number and severity of chylous effusions, pneumothoraces, hemorrhagic renal episodes, and all cause mortality within placebo or sirolimus group. With continuously

measured outcomes, we will use a two sample t-test, and with categorized outcomes, $\chi 2$ test will be used to see if there is any difference between placebo group and sirolimus group. For efficacy, we will compare the rate of change over the first year of each outcome in sirolimus group with that in placebo group. The primary outcome is FEV1 response, while the secondary outcomes are responses in FVC, diffusing capacity for carbon monoxide, lung volume measurements, distance walked in six minutes, volumetric CT estimate of lung cyst size and mass of tissue in the chest, and biomarker analyses such as VEGF-D. Taking into account within subject correlation of each outcome, we will implement a linear mixed effects model to test whether the slope of each outcome in the sirolimus group is the same as that in the placebo group. Repeated measurements will be used as a vector outcome, and as an indicator of the sirolimus group as a fixed effects covariate including time, and as an indicator of the sirolimus group and the time interaction term. We will use two types of random effects models: one is a random intercept and the other is an intercept and random slope for each subject. Maximum likelihood estimates (MLE) will be obtained for regression coefficients for fixed effects and restricted MLE for the variance component. A linear predictor will be obtained for the random intercept and random slope to identify individuals with steeper change.

Aim2: Determine the relationship between changes in lung function and some of the secondary endpoints (e.g., quality of life, dyspnea and fatigue) within the placebo or sirolimus group.

In each arm, as described for Aim 1, we will use a linear mixed effects model for lung function measurement (FEV1 or FVC) as a vector outcome, considering the within-subject correlation of each outcome. Each secondary endpoint will be adjusted as a covariate in the model. We will first use the dichotomized measurement based on the median at the baseline as a fixed effects covariate. In addition, we will adjust for all repeated measures of each secondary endpoint as a time dependent covariate in the model. For efficacy in Aim 1, the power to test the hypothesis that there is no difference between two groups in changes of outcomes over time is calculated at a significance level α of 0.05. Assuming compound symmetry for the covariance structure of repeated measures from the same subject, we used the following simplified formula (Diggle et al. (2003)):$Z1-\beta=[(N*m*sx2*d2)/(2*\sigma2*(1-\rho))]0.5-Z1-\alpha/2$, where $sx2=\Sigma(xj-mean(x))2/m$; n: the number of subjects in each group; m: the number of repeated measures; d: the difference between two slopes; $\sigma2$: the common variance of outcome; ρ: the correlation between outcomes from the same subject; β: the type II error rate. For the first year, FEV1 and other lung function will be measured at the baseline, 3month, 6month and 12 months, while some secondary endpoints (e.g., quality of life) will be measured at the baseline, 6 months and 12 months. From the previous study of 11 subjects with three repeated measures, we find that the within-subject correlation of each outcome distributes from 0.80 to 0.98. For example, the variance of FEV1 is 0.62 and within-subject correlation of FEV1 is approximately 0.98 for the assumed covariance structure. For Aim 2, the hypothesis to test would be that there is no slope difference between two groups (e.g., QOL>50% vs. QOL<=50%). Since this analysis is planned within each arm, we expect to have a half of the samples in the analysis. The power was calculated assuming unit standard deviation and within-subject correlations from 0.7-0.9. The statistical powers will be less than 60% when the slope difference between two groups is 0.03 and within subject correlation smaller than 0.9.

Missing Data

If a participant drops out of the study, the coordinator at the site will try to ascertain the reason for the person not continuing. If a person misses one of the clinic visits, a telephone call will be made to determine if the participant will come in for the next clinic visit. As the study progresses, missing data will be monitored to ensure that there is not one data point that, for some reason, is routinely not being captured. If data are missing, the analyses will be performed in several ways: We will first analyze the data assuming missing data occurred completely at random. However, when the data that is missing depends on the outcome, the parameter estimation will be most likely biased. As a secondary analysis, we will investigate the missing data mechanism given observed outcomes. When the missing data depends on the set of observed outcomes, a correctly specified covariance structure can accommodate the situation. But if the missing data is due to a specific outcome value that should have been obtained at the time, we will do a sensitivity analysis under various plausible assumptions concerning the missing data process. Dropout is also considered in a monotone missing data pattern. When the dropout is completely at random or unrelated to all future outcome values, an imputation will be incorporated to fill out the missing data. However, when the dropout depends on current and future unobserved outcomes, there will be no standard approach to accommodate this situation.

Interim analysis

In addition, an interim analysis for efficacy will be performed when 40 participants reach the primary endpoint, which is the slope of the FEV1 response at one year. Although the target accrual is 120, the protocol aims to analyze 100 patients after considering a projected 15-20% attrition rate. Thus, employing the Obrien-Fleming type spending function with the information fraction 0.4(=40/100), Obrien-Fleming type boundary is derived as 2.888 (nominal significance level=0.002) with one-sided 0.05 level of significance. A protocol driven sensitivity analysis comprised of patients who had 12 month FEV1 data available, but which excluded data from the 5 patients with persistently sub-therapeutic sirolimus levels, those who were identified as outliers in the fitted model, or those who entered the trial through the VEGF-D eligibility option, was also performed.

SECTION 5. CONDUCT OF THE TRIAL FOLLOWING THE INTERIM ANALYSIS

Factors Limiting the Duration of Enrollment Period in the MILES Trial

MILES enrollment closed with 89 patients randomized, which was 31 patients short of the goal of 120. Multiple factors contributed to the investigator's decision to terminate enrollment before the target was reached, but chief among them was expiration of the study drug, which became irreplaceable over the course of the protracted enrollment period. A more complete explanation follows.

The original enrollment target was 240 patients. This estimate was based on a power analysis using data that was available at the time the initial protocol was drafted, but was decreased to 120 patients when the results of the Cincinnati Angiomyolipoma Sirolimus Trial became available in January 2008. These data indicated that MILES would be adequately powered with half as many patients as originally planned.

Regulatory and contracting hurdles delayed the opening of sites across the network and slowed patient accrual considerably, requiring an extension of the enrollment period from 12 to 30 months. The initial supply of sirolimus for MILES therefore expired well before enrollment was complete. Production of the study drug had required interrupting the normal production schedule of commercial sirolimus tablets to manufacture unlabeled tablets, which resulted in considerable expense for Wyeth (now Pfizer). There was therefore reluctance on the part of the company to provide a second batch of the drug when the first lot expired. After prolonged negotiations, Wyeth agreed to produce a second lot of the study drug for the MILES trial but clearly stipulated it would be the last.

The expiration date of the second lot of study drug provided was August 31, 2010. Since the period of use of the drug had to end by this date, termination of enrollment had to occur by August, 2009. Therefore, the last patient was randomized in August 2009 and the last clinic visit occurred in August 2010.

Factors Impacting the Decision to Truncate the Observation Period of the MILES Trial

In January 2010, prior to the planned MILES interim analysis, trial investigators sent a memo to the DSMB requesting permission to truncate the observation period. The primary reason for this request was that delays in opening of sites and in patient accrual had extended the trial beyond the funding period of the primary grant that supported the trial. There was also a desire on the part of the investigators to accelerate the reporting of trial outcomes to participants, consistent with the commitments made in the written informed consent and feedback we had received from the DSMB.

Unbeknownst to trial investigators, the interim analysis conducted in February 2010 indicated that an efficacy-based stopping criterion had been reached and reviewed by the DSMB. Notwithstanding, the DSMB recommended in a March 15, 2010 memo to: (1) continue the study until all enrolled patients had completed the treatment period to optimize the data set related to treatment, and (2) perform the final analysis approximately 8 months after the interim analysis (thus effectively truncating the observation period). This decision was influenced by several factors including (1) a common perception among DSMB members of the potential for a small effect size at the primary endpoint, (2) interest in maximizing the collection of drug safety data in the LAM patient population, and (3)

concern that further extension of the study would violate the commitment to patients for timely reporting of outcome data as specified in the written informed consent document.

In this context, upon receiving the trial investigators request and consideration of the various factors, the DSMB agreed to endorse the investigator-initiated proposal to truncate the observation period of the study and recommended doing so after all primary endpoint data had been collected.

SECTION 6. MILES STUDY VISIT CALENDAR, SCREENING PROCEDURES, BASELINE MEASUREMENTS, AND SAFETY MEASURES

A. Study Visit Calendar[a]

Event	Baseline1	Baseline 2*	3 wks**	3 mo***	6 mo***	9 mo***	12 mo***	18 mo***	24 mo***
	Day 1	Day 2-Day 7	Week 3	Week 13	Week 26	Week 39	Week 52	Week 78	Week 104
Visit number	0	1	2	3	4	5	6[f]	7 [k]	8 [k]
Telephone call**** from study coordinator at weeks 1, 6, 19, 32, 45, 65, 91									
All study visit dates after baseline are calculated from the date the subject starts the study drug (week 0)[j].									
Informed consent	X								
History[b] and physical	X		X	X	X	X	X	X	X
Liver, renal profiles, glucose	X[f]		X	X	X	X	X	X	X
Urinalysis and albumin/creatinine ratio	X[f]		X	X	X	X	X	X	X
Urine pregnancy test[h]	X[f]		X	X	X	X	X	X	X
Fasting lipid profile	X[f]		X	X	X	X	X	X	X
CBC, diff	X[f]		X	X	X	X	X	X	X
Two view chest X-ray	X[f]								X
Volumetric high resolution CT scan[i]	X[f]						X		X
Sirolimus level[c]			X	X	X	X	X	X	X
Six min walk with oximetry[e]	X	X		X	X		X	X	X
Full pulmonary function tests (spirometry, lung volumes, DLCO)[d, e]	X	X		X	X		X	X	X
Spirometry only						X			
Dispense study article and drug diary		X	X	X	X	X			
Collect study article and drug diary			X	X	X	X	X		
St. George's Respiratory Questionnaire	X[f]				X		X		X
SF 36	X[f]				X		X		X
F.P.I/G.W.B	X[f]			X	X	X	X	X	X
Dyspnea, Fatigue and EuroQOL scale	X[f]		X	X	X	X	X	X	X
Saved serum and plasma	X[f]				X		X		X
Randomization		X							

a. Abbreviations: CBC, complete blood count; CT, computed tomography; DLCO, diffusing capacity for carbon monoxide; SF 36, Multiple Outcome Survey Short-Form 36; F.P.I, Functional Performance Inventory; G.W.B, General Well Being Questionnaire
b. Interval history only at visit 2-8.
c. Additional sirolimus levels were obtained with each dose adjustment that is originated by the DMCC. Dose adjustments (other than to zero) which were originated by the site (for toxicities or other reasons) were followed by a sirolimus level if duration of the dose change was more than 2 weeks . Levels were obtained within one week (+/- two days) of final adjustment.

d. The visit 0 spirometry will be pre- and post-bronchodilator, all subsequent spirometry was post-bronchodilator. Plethysmography is the preferred method for measuring lung volumes.
e. Supplemental oxygen titrated on Visit 0 as necessary to maintain sat > 90%, if possible.
f. These tests were performed at either baseline visit.
g. Subjects who are withdrawn from the study prior to the 12 month time point had these observations performed at the final study visit.
h. Urine pregnancy test was performed at month 15, or if early withdrawal occurs, 12 weeks after study drug is last taken. A pregnancy test was obtained prior to any radiology procedure and also 12 weeks after study drug was stopped.
i. A subset of sites obtained volumetric CTs. Participants received the CT scan immediately following the 6MWT. If the CT was not completed within 2 hours of the bronchodilator, another dose of bronchodilator was administered.
j. Pregnancy tests were only required for women of childbearing potential (defined as any fertility status other than natural or surgical menopause or post hysterectomy).
k. Participants were required to commence taking study article within 30 calendar days of randomization.

* Window for Visit 1: up to one week after Visit 0
** Window for Visit 2: +/- one week
*** Window for Visit 3 – Visit 8: +/- two weeks
**** Window for Telephone calls: +/- two business days

B. Screening Procedures

- Complete history and physical examination.
- Screening spirometry will be repeated on both day 1 and day 2 of the baseline visit just prior to randomization; the FEV1 is required to agree within 10% of the screening value. FEV1 ≤ 70% predicted is required for enrollment. Percent predicted values for FEV1 and FVC will be calculated according to the formula of Hankinson. For Pacific Rim Asians, a correction factor of .94 will be used.
- HRCT will be performed by a subset of sites. These data will be reported in a future manuscript. The HRCT studies will be conducted in the prone position at suspended end-inspiration [total lung capacity]; the HRCT will be examined for exclusions [e.g., masses, pneumothorax] by the participating radiologist at each site. For subjects who enroll by VEGF-D criteria, the HRCT will also be viewed by the MILES core thoracic radiologist (Dr. Meyer) prior to randomization.
- Pathology-Biopsies of lung, lymph node or abdominal tumor that were previously reviewed and confirmed to be LAM by an expert lung pathologist will be considered acceptable for eligibility purpose. For all other cases involving biopsy, slides will be obtained and reviewed by MILES core pathologist (T.V.C.) or designated site pathologists who were approved (based on review of their CV by study sponsor, F.X.M., and MILES Core Pathologist, T.V.C.) to read LAM pathology for MILES (based on review of their CV by study sponsor, F.X.M., and MILES Core Pathologist, T.V.C.).
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C. Baseline Measures Prior to Initiating Therapy

- Spirometry on OMI volume spirometer distributed to all sites* [1-4]
- Whole-body plethysmographic lung volumes [5]
- Diffusing capacity for carbon monoxide[6]
- Volumetric high resolution computed tomography (HRCT)
- Modified six minute walk test‡[7]
- The 36-item Medical Outcomes Survey (MOS-SF36)[8]
- St. George's Respiratory Questionnaire[9]
- The Functional Performance Inventory[10]
- The General Well Being Scale[11]
- EuroQOL/Dyspnea/Fatigue VAS scales[12]

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*All pulmonary function technicians were trained on the OMI spirometer and performance of spirometry was monitored by the pulmonary function quality assessment core. Feedback was provided to sites about test performance throughout the trial. All lung function testing was performed in accordance with American Thoracic Society standards. For standardization and consistency, volume spirometers (OMI) were distributed to all sites. Reference values for calculation of percent predicted FEV1 and FVC were from Hankinson.[13] Reference values for calculation of percent predicted TLC, RV and FRC were those of Crapo.[14] Reference values for calculation of percent predicted DLCO were those of Gulsvik.[15]

‡Six minute walk testing was performed according to ATS criteria, with modifications related to oxygen use. The first test performed at Visit 0 in the study table above was a

'practice and titration study', which included an assessment of the liter flow rate of supplemental oxygen required to maintain saturation > 90% throughout the walk. The supplemental oxygen flow rate determined during the first walk was used for every subsequent six minute walk throughout the study. A second six minute walk study was done on the second day of the baseline visit, and the longest distance walked between the Visit 0 and Visit 1 studies was recorded as the baseline value. Six minute walk testing was terminated if saturation fell below 80% and the distance walked at that point was determined.

D. Safety Measures and Management of Intercurrent Events

Metabolic derangements

Levels of electrolytes, blood urea nitrogen, creatinine, glucose, hepatic enzymes, urine protein and albumin to creatinine ratio, bilirubin, serum lipids, and sirolimus were performed at every visit.

Mouth Ulcers

If minor mouth pain symptoms occur, mouthwash preparations will be prescribed per protocol, and at the discretion of the investigator, the sirolimus or placebo dose will be decreased up to 100% or withheld for a few days to a few weeks. Sirolimus will be withheld if mouth pain is severe or interferes significantly with the ability to eat or drink, and restarted at 50% - 100% of the prior dose when symptoms resolve, at the discretion of the investigator. Re-escalation of the sirolimus or placebo will be permitted as the toxicity resolves.

Proteinuria

The urine albumin to creatinine ratio will be obtained at every visit to monitor for microscopic proteinuria as a marker for the development of renal toxicity, which has been reported in some renal transplant patients.

Hyperlipidemia

Hypercholesterolemia will be treated in accordance with the National Cholesterol Education Program ATP III guidelines, which is based on risk factors and LDL cholesterol levels (www.nhlbi.nih.gov/guidelines/cholesterol/index.htm). A protocol for making decisions regarding diet or pharmacologic treatment will be provided to all centers. Triglycerides will be treated (most often with niacin or gemfibrozil) for levels that exceed 400 mg/dl, but lesser levels may be observed because they tend to return to normal with continued treatment. If cholesterol and triglyceride levels are not reduced to acceptable levels (per site PI) with lipid lowering drugs, then the sirolimus or placebo dose will be reduced by 50% until levels fall below the acceptable threshold. Re-escalation of the sirolimus or placebo dose will be permitted if the toxicity resolves.

Pneumothorax

In the event of a pneumothorax while on study, subjects will be taken off study article for at least 30 days beyond documentation of complete resolution of the

pneumothorax. Patients are permitted to continue in the trial, but pulmonary function tests are not allowed for six weeks after the pneumothorax or the last intervention.

Possible Sirolimus Pneumonitis

If minor but new and unambiguous pulmonary infiltrates are found on a PA chest film, the investigator will either hold the drug or reduce the dose of sirolimus or placebo by 50%. If infiltrates do not steadily resolve, or if FEV1 or FVC falls by more than 30% from baseline, or if shortness of breath, troublesome cough or other significant pulmonary toxicities occur, the drug will be held until the participant returns to baseline and then the sirolimus dose may be restarted at 50% of the prior dose, at the discretion of the investigator. Re-escalation of sirolimus or placebo is permitted if the toxicity resolves.

Criteria for Termination of the Study

Enrollment and treatment will be suspended and the DSMB will review the data within 14 days if the medical review officer ascertains that any of the following are occurring in greater percentage in the treatment arm:

- Death: greater than 5% increase over control group
- New pulmonary infiltrates developing in association with a need for hospitalization greater than 20% increase over control group. The trial data will be analyzed quarterly to see whether these study stopping rules have been met

SECTION 7. DOSE ADJUSTMENTS

Sirolimus levels will be measured at the Children's Hospital Medical Center laboratory. When the sirolimus level fall outside the range of 5-15 ng/ml, the local research team and PI will be contacted by the medical monitor at the DMCC, and a dose recommendation will be made. The site investigator will accept or decline the proposed dose modification from the DMCC with 48 hours after receiving the dose modification request. When accepted, the dose will be adjusted within 48 hrs by the site personnel per protocol, and the sirolimus level will be redrawn one week (+/- two days) later. The site team was blinded with respect to treatment assignment at all times. This process was repeated until the sirolimus level falls within the target range. For each participant in the treatment group who undergo dose adjustment, a participant in the placebo group will be contacted and the same procedure will be followed.

Reasons for withholding the study article or changing the dose of the study article include:

- New pulmonary infiltrates
- Fall in FEV1 or FVC by 30% or more
- New shortness of breath, troublesome cough, or other significant symptoms
- Infections (upper respiratory tract, urinary tract, etc) requiring antibiotics
- Mouth ulcers
- Pneumothorax
- Hemorrhage into an angiomyolipoma
- Nonelective surgery
- Pericarditis
- Drug fever

SECTION 8. BASELINE CHARACTERISTICS

TABLE A. Demographics and clinical characteristics of patients with Lymphangioleiomyomatosis(LAM)*				
Characteristic	**All patients (n = 89)**	**Placebo Group (n = 43)**	**Sirolimus Group (n = 46)**	**P Value**
Age (years)				
Mean ± S.D.	45.4 ± 10.6	45.9 ± 10.3	45.0 ± 10.9	0.736†
Median (Min, Max)	45 (23,65)	45 (25,65)	44.5 (23,63)	
Race				
Caucasian, n (%)	59 (66)	30 (71)	29 (63)	0.581¶
Asian, n (%)	27 (30)	12 (28)	15 (33)	
Black, n (%)	2 (2)	1 (2)	1 (2)	
Other, n (%)	1 (1)	0 (0)	1 (2)	
Clinical Features				
Tuberous sclerosis complex, n (%)	8 (9)	4 (9)	4 (9)	1.000§
Post-menopause, n (%)	30 (34)	16 (37)	14 (30)	0.499¶
History of angiomyolipoma, n (%)	44 (49)	22 (51)	22 (48)	0.753¶
History of pneumothorax, n (%)	53 (60)	29 (67)	24 (52)	0.143¶
Pneumothoraces (mean no. per patient)	4.1	3.6	4.7	
History of chylothorax	9 (10)	5 (12)	4 (5)	0.734¶
Oxygen therapy requirement				
Continuous use, n (%)	28 (32)	14 (33)	14 (30)	0.829¶
Intermittent use, n (%)	52 (58)	23 (54)	29 (63)	0.361¶
Qualifying inclusion criteria				
Biopsy, n (%)	54 (61%)	25 (58%)	29 (63%)	0.887¶
Clinical context, n (%)	23 (26%)	12 (28%)	11 (24%)	
VEGF-D, n (%)	12 (13%)	6 (14%)	6 (13%)	
Pulmonary Function Testing†				
FEV1 (ml)	1367 ± 420	1378 ± 446	1357 ± 400	0.691‡
FEV1 (% predicted)	48.54 ± 13.77	47.73 ± 14.37	49.29 ± 13.31	0.771‡
FVC (ml)	2791 ± 692	2909 ± 749	2682 ± 622	0.143‡
FVC (% predicted)	79.71 ± 16.60	80.77 ± 17.62	78.73 ± 15.70	0.546‡
FEV1/FVC	0.50 ± .15	0.48 ± .15	0.52 ± .16	0.354‡
TLC (ml)	5056 ± 1249	5270 ± 1463	4856 ± 986	0.182‡
TLC (% predicted)	105.21 ± 25.63	106.70 ± 29.45	103.83 ± 21.71	0.607‡
FRC (ml)	3000 ± 905	3175 ± 1059	2838 ± 710	0.204‡
FRC (% predicted)	112.49 ± 31.32	116.61 ± 38.29	108.67 ± 22.97	0.429
RV (ml)	2266 ± 881	2409 ± 1029	2133 ± 703	0.333‡
RV (% predicted)	141.42 ± 59.22	147.48 ± 69.25	135.78 ± 48.15	0.802‡
DLCO (ml/mmHg/min)	10.23 ± 4.61	10.42 ± 4.82	10.05 ± 4.47	0.524‡
DLCO (% predicted)	43.43 ±18.97	43.77 ±20.56	43.12 ±17.66	0.699‡
6 minute walk distance (feet)	403 ± 105	399 ± 115	407 ± 96	0..78‡
Health Related Symptom Scores†				
SGRQ ‖	46.41 ± 15.17	45.79 ± 15.58	46.99 ± 14.92	0.691‡
SF-36**				
Mental Component	48.65 ± 11.04	48.53±10.74	48.76 ± 11.44	0.802‡
Physical Component	37.66 ± 9.17	38.01 ± 9.60	37.33 ± 8.85	0.873‡
Visual Analogue Scale††				
Fatigue	49.87 ± 24.64	49.51 ± 24.20	50.2 ± 25.31	0.961‡
Dyspnea	44.25 ± 23.63	39.72 ± 22.06	48.48 ± 24.49	0.081‡
Quality of life	67.82 ± 19.25	67.09 ± 20.11	68.5 ± 18.61	0.833‡

Functional Performance Inventory‡‡	2.29 ± 0.50	2.35 ± 0.49	2.25 ± 0.51	0.354‡
General Well Being Questionnaire§§	62.71 ± 4.71	62.63 ± 4.24	62.78 ± 5.15	0.559‡
Biomarker Analysis				
Serum VEGF-D concentration (pg/ml)	2029 ± 2343	2223 ± 2997	1848 ± 1514	0.566‡

*Details of patient enrollment, randomization, and evaluation are provided in the Methods and Supplemental Appendix.

† Data are expressed as the mean ± standard deviation (SD) except as indicated.

‡ Comparison of placebo and sirolimus groups using the Wilcoxon rank sum test.

§ Comparison of placebo and sirolimus groups using the Fisher's exact test.

¶ Comparison of placebo and sirolimus groups using the Chi- square test.

‖ The St. Georges Respiratory Questionnaire (SGRQ) total score is shown. SGRQ scores are based on a scale of 0-100, with lower scores indicating better functioning.

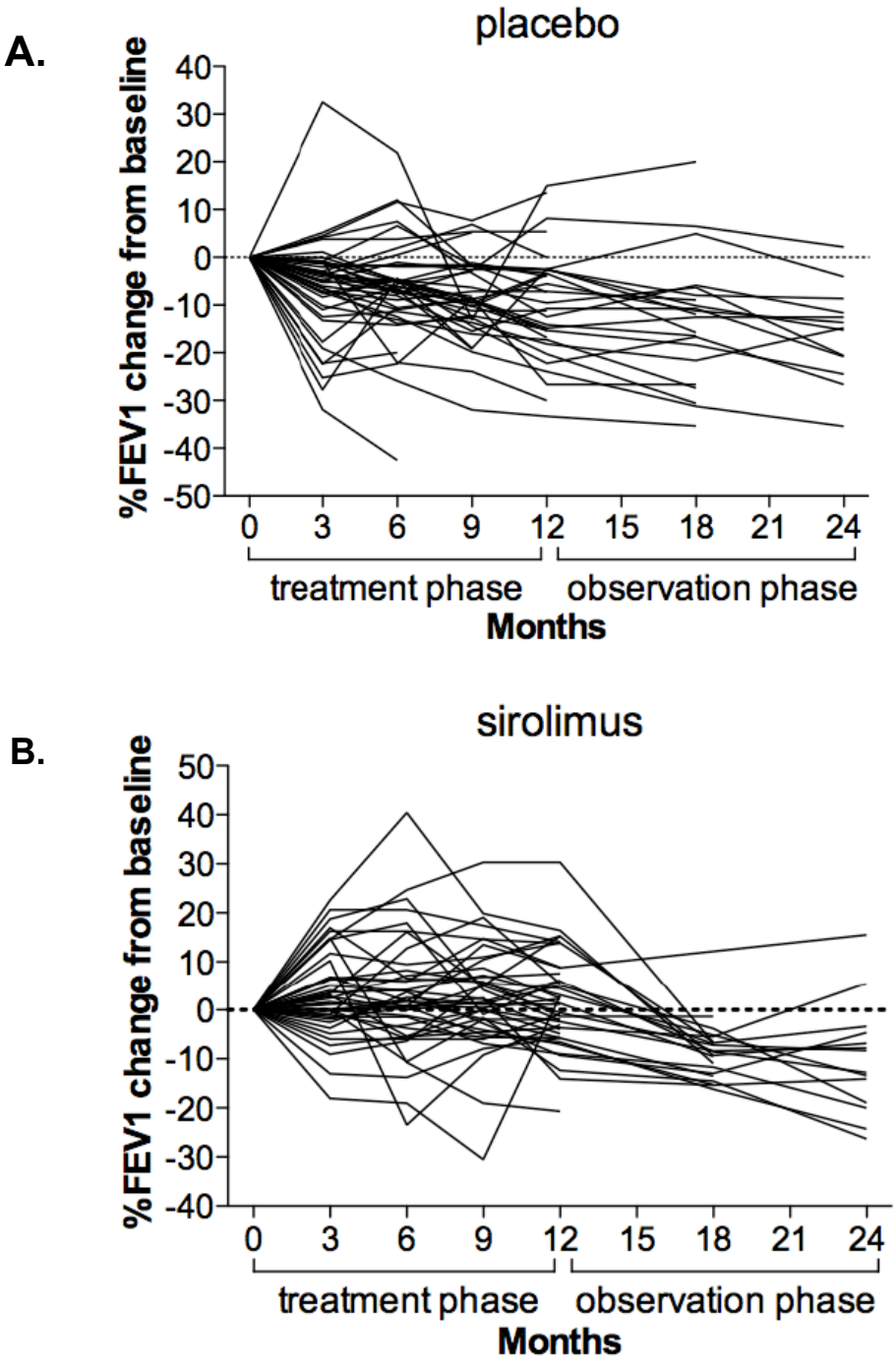
** The two major components of the Multiple Outcome Survey (MOS-SF 36 score), mental and physical component summary t-scores, are shown.

††Scores on the EuroQol Visual Analogue Scale (VAS) are shown. VAS scores are based on self-ratings of health state on a 0 to 100 scale, with lower scores indicating worse functioning.

‡‡Scores on the Functional Performance Inventory are based on a scale of 1-4, with lower scores indicating lower health status

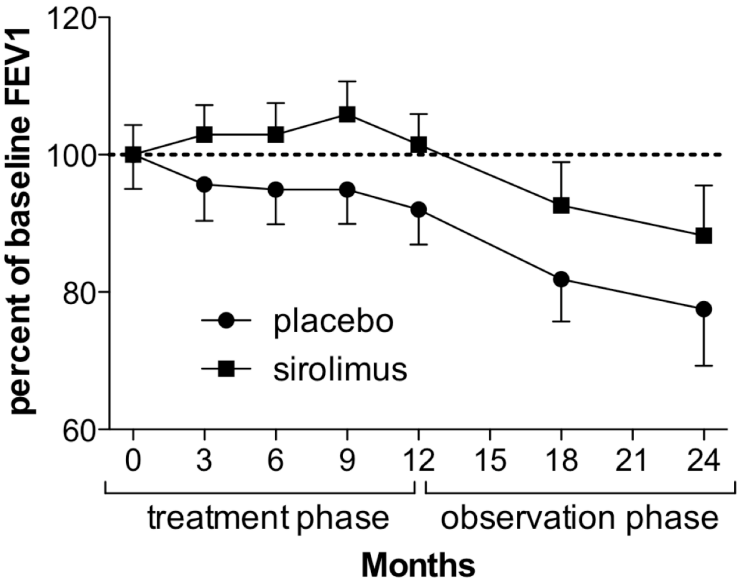
§§Scores on the General Well Being Questionnaire are based on a scale of 1-110, with lower scores indicating lower health status

SECTION 9. ADDITIONAL FEV1 ANALYSES AND FIGURES



Figs. A and B. Plot of the percent change in FEV1 from baseline for each individual in the placebo group (Panel A) and sirolimus group (Panel B) over the course of the 0-12 month treatment phase and 12-24 month observation phase of the trial.

C.



D.

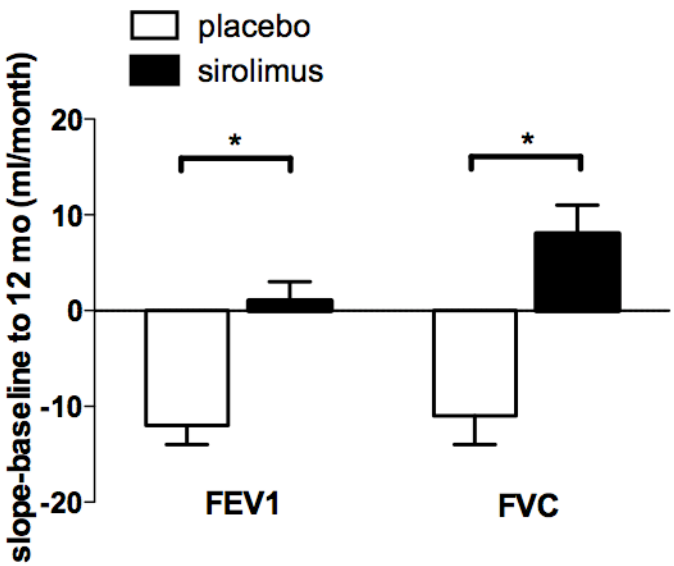


Fig. C. Plot of percent change in mean FEV1 from baseline in the placebo and sirolimus groups over the course of the 0-12 month treatment phase and 12-24 month observation phase of the trial. Data are mean (%) ± S.E.M.

Fig. D. Plot of the slope of FEV1 and FVC change in the placebo and sirolimus groups over the course of the 0-12 month treatment phase and 12-24 month observation phase of the trial. Data are mean (milliliters/month) ± S.E.M., *p<0.001

SECTION 10. ADDITIONAL FVC ANALYSES AND FIGURES

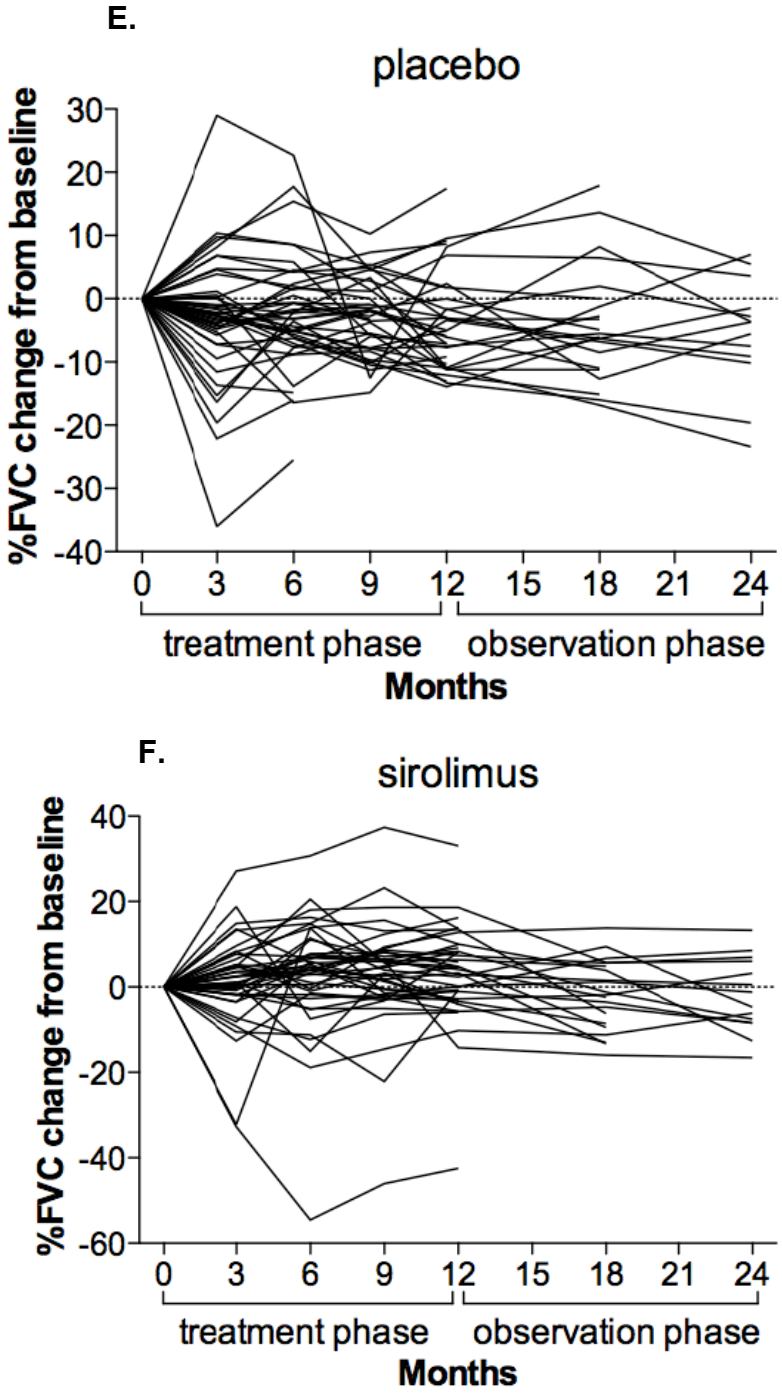


Fig. E and F . Plot of the percent change in FVC from baseline for each individual in the placebo (**E**) and sirolimus (**F**) group over the course of the 0-12 month treatment phase and 12-24 month observation phase of the trial

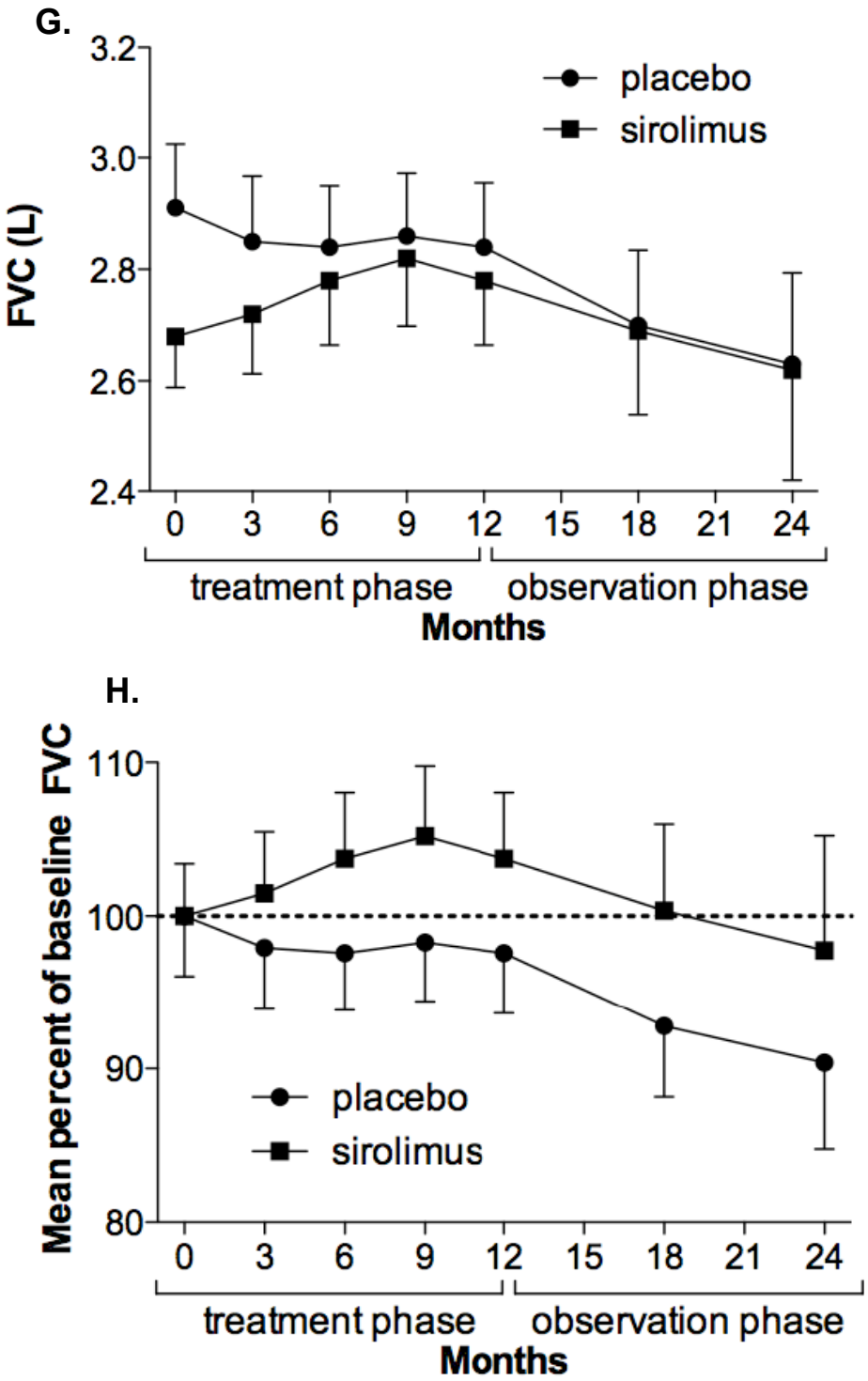


Fig.G. Plot of the mean FVC in the placebo and sirolimus groups over the course of the 0-12 month treatment phase and 12-24 month observation phase of the trial. Data are mean (%) ± S.E.M.

Fig. H. Plot of the percent change in FVC from baseline in the placebo and sirolimus groups over the course of the 0-12 month treatment phase and 12-24 month observation phase of the trial. Data are mean (%) ± S.E.M.

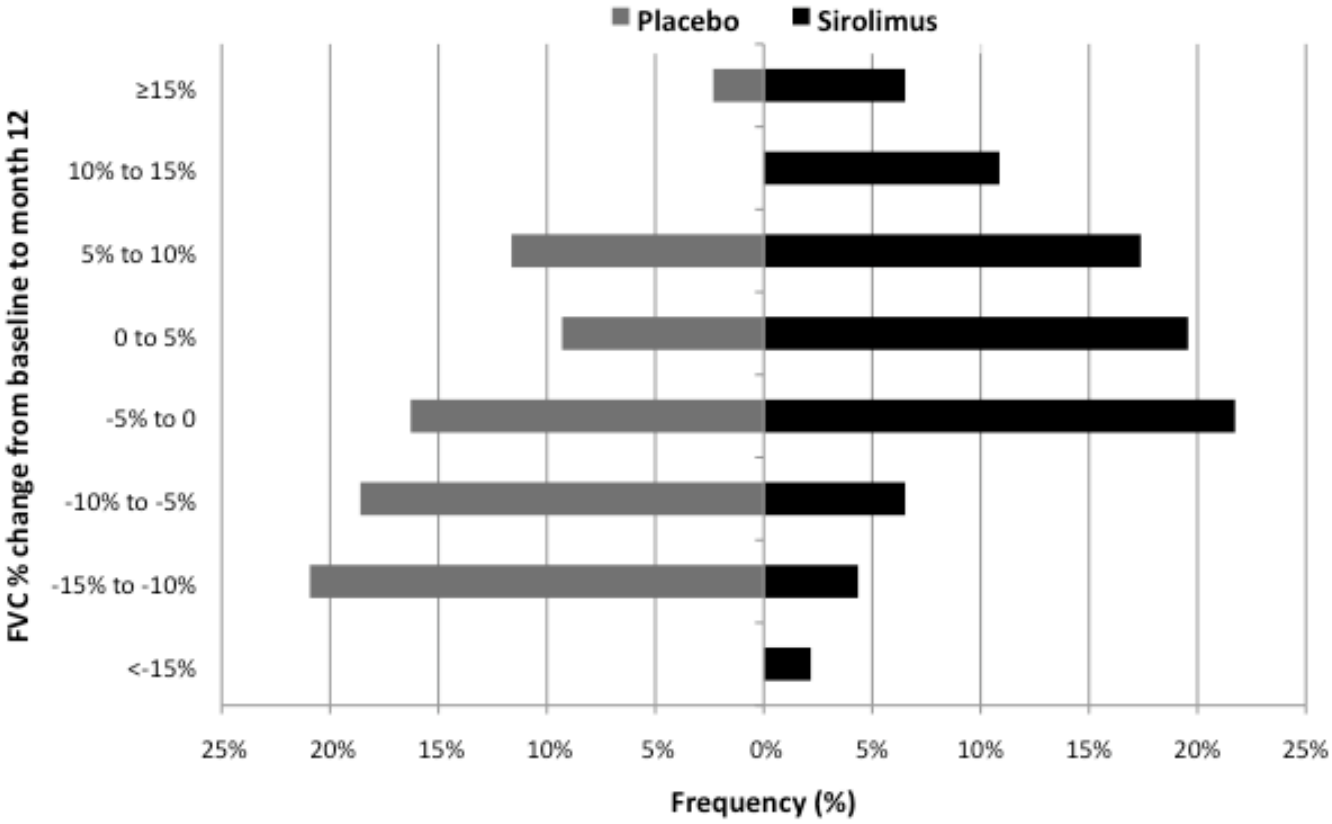


Fig. I. The percent change in FVC from baseline to month 12 according to treatment and absolute increments or decrements of 5% the baseline value. The percentage of patients that had any improvement in FVC was significantly greater in the sirolimus group. A significantly greater percentage of patients in the sirolimus group (54.3 percent) than in the placebo group (23.3 percent) ($p<0.001$). Conversely, a significantly greater percentage of those in the placebo group than in the sirolimus group (55.8 percent vs. 34.8 percent) had any worsening of the FVC.

SECTION 11. REFERENCES

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