Official Title of the study: Resveratrol and Sirolimus in Lymphangioleiomyomatosis Trial

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MEDICAL IRB RESEARCH PROTOCOL

Protocol Synopsis

Title	Resveratrol and Sirolimus in LAM Trial
Short Title	The RESULT Trial
Rationale	Lymphangioleiomyomatosis (LAM) is a rare, progressive diffuse cystic lung disease characterized by the infiltration of lung parenchyma with abnormal smooth muscle cells, leading to progressive destruction of lung parenchyma. The origin of these smooth muscle cells is unknown, however, lung involvement happens as a result of metastatic spread of these smooth muscle cells via blood and lymphatics, thus characterizing LAM as a low-grade, destructive, metastasizing neoplasm. LAM is seen primarily in women with only a few reported cases in men. LAM can be seen in association with Tuberous Sclerosis Complex (TSC-LAM), or occur sporadically (S-LAM). Both TSC-LAM and S-LAM occur as a result of mutations in one of the two TSC genes: TSC1 or TSC2. Mutations in the TSC genes lead to constitutive activation of the mammalian target of rapamycin (mTOR) pathway, which drives cell proliferation and lymphangiogenesis. Sirolimus forms a complex with FKBP12, which binds to mTOR and blocks activation of downstream kinases, restoring homeostasis in cells with defective TSC function. Sirolimus has been shown to shrink tumors in animal models of TSC deficiency. The Multicenter International LAM Efficacy of Sirolimus (MILES) Trial was a double blind, randomized, parallel group trial of one year of treatment with sirolimus versus placebo followed by one year of observation. While patients treated with placebo lost approximately 10% of their lung volume in the treatment phase, patients treated with sirolimus exhibited stabilization of their lung function, and had an improvement in their functional performance and quality of life. During the observation year, lung function decline resumed in the sirolimus group and paralleled that of the placebo group. The results from the MILES trial formed the basis for a recent FDA approval for sirolimus as a treatment for LAM. However, the resumption of lung function decline following drug stoppage suggests that the function of sirolimus is suppressive rather than curative. In addition, while the drug was well tolerated for the trial duration, the long-term safety and efficacy of sirolimus in LAM remains unclear. These limitations of sirolimus highlight the need to explore novel treatment opportunities for patients with LAM. Resveratrol (*trans*-3, 5, 4′-trihydroxystilbene) is a naturally occurring polyphenol found in grapes, berries, and red wine. Multiple studies have shown resveratrol to

	have anticancer, anti-inflammatory, and anti-oxidant properties. Resveratrol has a complex mechanism of mTOR regulation. In addition, resveratrol inhibits the activity of Akt, AMPK and S6K1. Recent pre-clinical studies performed on TSC2 null cells as well as murine models have demonstrated that a combination of resveratrol and sirolimus leads to downregulation of autophagy and promotes apoptosis in TSC2 null cells, decreases the metastatic capability of uterine leiomyoma-derived smooth muscle cells, and causes a significant reduction in the size and growth of TSC2 deficient xenograft tumors. The unmet need for a remission inducing (cytotoxic) as opposed to a suppressive (cytostatic) treatment option for patients with LAM, combined with strong pre-clinical evidence demonstrating the efficacy of a combination of sirolimus and resveratrol form the basis for this dose-escalating, safety and efficacy clinical trial. Results from this trial will pave the way for pivotal phase III, randomized, controlled, efficacy studies of combined resveratrol and sirolimus in LAM.
Clinical Phase	II
IND Sponsor	Nishant Gupta, University of Cincinnati, Cincinnati OH
IND Number	131722
Principal Investigator	Nishant Gupta, University of Cincinnati, Cincinnati OH
Participating Site(s)	University of Cincinnati - Cincinnati, OH
Accrual Objective	20 evaluable subjects 25 target enrollment
Study Objectives	Phase II dose-escalating, open-label, safety and efficacy study to determine if there is a potential benefit of resveratrol in combination with sirolimus in patients with LAM. Based on the results of this trial, additional phase III clinical development trials may be designed.
Study Design	Phase II: Open label, intention to treat study
Study Duration	24 weeks treatment
Primary Objective	Assess the change in serum VEGF-D level after 24 weeks of treatment with a combination of resveratrol and sirolimus as compared to the VEGF-D level in patients on a stable dose of sirolimus alone.
Secondary Objectives	1. Assess the safety and adverse effect profile of combined resveratrol and sirolimus in adult patients with LAM.

	2. Determine the effect of treatment with a combination of resveratrol and sirolimus on changes in lung function and quality of life. 3. Determine the feasibility and reliability of performing regular home spirometry in patients with LAM.
Inclusion Criteria	Subjects enrolled in the trial must meet **all** of the following criteria. 1. Definitive diagnosis LAM based on the presence of characteristic cystic change on high-resolution computed tomography (HRCT) of the chest. The diagnosis must be confirmed by one of the following: A) Histopathological confirmation by biopsy (lung, abdominal mass, lymph node or kidney or cytology from thoracic or abdominal sources revealing HMB45+ staining of spindled/epithelioid cells) B) Compatible chest CT scan findings in the setting of tuberous sclerosis, angiomyolipomas (diagnosed by CT, MRI by the site radiologist or biopsy) or chylous pleural effusion (verified by tap) C) Chest CT scan findings compatible with LAM and a VEGF-D level ≥ 800pg/ml. 2. Age 18 years or greater. 3. Signed and dated informed consent 4. Currently on sirolimus for treatment of LAM for at least 20 weeks 5. Evidence of disease stabilization on sirolimus as demonstrated by two stable values of serum VEGF-D post initiation of sirolimus drawn at least 12 weeks apart from each other. For the purpose of this study, a variation in serum VEGF-D of less than or equal to 15% is considered stable.
Exclusion Criteria	Subjects who meet **ANY** of the following criteria are not eligible for enrollment as study participants: 1. Known allergy or hypersensitivity to Resveratrol 2. Inability to provide informed consent 3. Active enrollment in other clinical drug trials for LAM 4. Pregnant or plan to become pregnant in the next 6 months 5. Breast feeding 6. Inability to comply with pulmonary function tests or follow up visits 7. Inadequate contraception 8. Use of estrogen containing medications within the 30 days prior to randomization 9. History of organ transplant 10. Actively listed for lung transplantation 11. Inability to comply with study procedures or attend scheduled study visits 12. Any clinically significant medical disease (other than LAM) that is associated with an expected survival of less than 2 years, or likely to impact the ability of the patient to participate in the study in the opinion of the investigator, or impact the study efficacy or safety assessments.

Investigational Product	Resveratrol at escalating doses starting at 250mg per day to a maximum of 1000mg per day
Study Procedures	The primary objective of this trial is to evaluate the change in serum VEGF-D after 24 weeks of combined resveratrol and sirolimus as compared to the baseline serum VEGF-D value on sirolimus alone. Secondary objectives include an assessment of the safety and tolerability of resveratrol in combination with sirolimus in patients with LAM, as well as the effect of combined resveratrol and sirolimus on lung function and quality of life. The frequency, severity and duration of all adverse events, regardless of cause, will be tabulated by body system and by severity using the NCI Common Terminology Criteria for Adverse Events v4.0. Subjects will have a medical history, physical examination, and laboratory measurements of complete blood count, renal and liver function tests, serum VEGF-D, and sirolimus levels drawn at every in-person visit. Pre and post bronchodilator spirometry measurements will be performed at each visit. St. George's respiratory questionnaire (SGRQ), SD-SB, EuroQOL and ATAQ LAM will be used for quality of life assessment, and these questionnaires will be filled out at each in-person study visit. Patients enrolling in RESULT will be trained on proper technique for home spirometry using an advanced vertical turbine based device that records and wirelessly transmits full flow volumes loops. Once a week, patients will perform at least three spirometry maneuvers that pass ATS/ERS acceptability criteria and choose the best value as their weekly FEV1 and FVC. All spirometric data will be reviewed by an expert pulmonary physiologist, who will judge the spirometry data and loops for intra-test acceptability and reproducibility as specified in the ATS/ERS guidelines, as well as provide near real time electronic feedback to patients regarding their technique. We will correlate spirometry values and trends (absolute as well as rate of decline) obtained from weekly home spirometry with the office-based spirometry values, as well as serum VEGF-D, and quality of life assessment scores.
Data Analysis	The analysis will be based on an intention to treat design. All subjects that receive study drug will be included in the safety and efficacy analysis set. The primary analysis will occur after the last enrolled participant has completed the 24-week visit. All analyses will be performed using SAS version 9.4. A p-value less than 0.05 will be considered as statistically significant. Paired t-test will be performed to detect the difference of serum VEGF-D after 24 weeks of treatment with the serum VEGF-D prior to enrolment. The response variable VEGF-D will be measured repeatedly, at baseline and at weeks 8, 16, and 24. The change from baseline to the other study time points will indicate the treatment effect with resveratrol and sirolimus. Initially, by using the paired t-test we will test whether these effects are statistically significant. Multivariate linear regression models will be used to assess the relationship between serum VEGF-D change and the covariates including patient's clinical or demographic factors. A repeated measures ANOVA model and linear mixed models will be performed to compare the serum VEGF-D values at follow-up visits to the baseline taking into account the

	correlation between repeated measurements within the same patient. Subjects will be evaluable for safety if they have received at least one dose of the study drug. The frequency, severity and duration of all adverse events, regardless of cause, will be recorded on the case report forms. The frequency and severity of adverse events will be calculated by patient, with each patient counted once using the most severe grade experienced. The duration of adverse events will be calculated by the number of days each event persisted. Adverse events will be tabulated by body system and by severity using the NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.0, which can be obtained from the CTEP website: http://ctep.cancer.gov/reporting/ctc.html. Tables will be generated for all adverse events including those that are judged to be possibly, probably or definitely related to the drug. In order to assess the safety and adverse effect profile of resveratrol in LAM, we will assign a binary indicator variable to indicate a patient with or without adverse events. A multivariable logistic regression model will be used to assess of the effect of the covariates on the probability of having adverse events for patient with LAM. All interested factors will be adjusted in the logistic regression model. Model selection will be based on stepwise criterion when appropriate. Lung function parameters (FEV_1 and FVC) will be measured at the baseline visit, and at every in-person visit. DLCO will be measured at the baseline visit and the last study visit. Similar analysis plan as VEGF-D will be employed for the lung function parameters. We will use the following questionnaire-based assessments to determine the quality of life in this trial: St. George's Respiratory Questionnaire (SGRQ), EuroQOL scale, San Diego Shortness of Breath scale (SD-SOB), and A Tool to Assess Quality of Life in LAM (ATAQ-LAM). Written permission has been obtained to use all these questionnaires. The scores on each of these questionnaires will be calculated at baseline, and at every in-person visit. We will compare median values at baseline and 24 weeks with the Wilcoxon signed rank test for each of these scales. The spirometry values will be expressed as mean ± SD. All spirometry maneuvers will be checked for acceptability and reproducibility as specified in the ATS/ERS criteria by an expert pulmonary physiologist. The spirometry values obtained from maneuvers that do not satisfy the ATS/ERS criteria for acceptability will not be used in the primary analysis. If spirometric values meet the acceptability criteria but fail to satisfy the repeatability criteria, those values will be graded for quality, but will be included in the primary analysis. Overall compliance with weekly home spirometry will be recorded and reported as a percentage. Comparison of home spirometry and office-based spirometry values will be performed using the Bland-Altman method at baseline, and at each time frame that patients get an office-based spirometry (i.e. every 8 weeks). Rate of change of FEV1 (slope) over time will be calculated by using all available values (without imputations) in a linear mixed effects model after considering the correlation of the observations within each subject, and the time between measurements will be treated as a fixed effect. In

	order to analyze if the trends obtained from short-term follow up with frequent home spirometry correlate with overall disease trajectory, we will compare the rate of decline obtained after 8 weeks of home spirometry to the overall rate of decline obtained after 24 weeks of follow up. A similar analysis plan will be employed for serum VEGF-D, and other biomarkers. All statistical analyses for this study will be performed using SAS, version 9.4 (SAS Institute Cary NC).
Sample Size	The mean serum VEGF-D level at baseline was approximately 2,000pg/ml in both the MILES trial and the everolimus trial. In both studies, treatment with mTOR inhibitors resulted in an almost 50% reduction in the serum VEGF-D levels. Since patients in our study will be on sirolimus prior to enrolment, we estimate that the starting serum VEGF-D level in our study will be approximately 1,000pg/ml. The largest change between serial VEGF-D levels in the placebo group of MILES was 42%. Thus, VEGF-D responders in MILES trial were conservatively defined as patients in whom serum VEGF-D decreased to a value greater than 42% from its baseline. Accordingly, we made our power calculations based on the ability to show a greater than or equal to 42% change in serum VEGF-D levels. Assuming the correlation between the baseline and subsequent VEGF-D levels is 0.7, a sample size of 20 subjects will provide 80% power to detect a mean VEGF-D difference from baseline to post-treatment of 420pg/ml or higher with the level of significant of 0.05 and a standard deviation of 800 for the difference. Allowing for a 20% dropout rate, we expect to recruit 25 patients for our study. Sample size calculations were done using SAS version 9.4.

Table of Contents

1. Participating Institutions/Investigators Table (contact information)

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2. Glossary of Abbreviations

AE	Adverse event
AEDAMS	Adverse Event Data Management System
AMPK	5' AMP-activated protein kinase
ATAQ-LAM	A Tool to Assess Quality of Life in LAM
ATS	American Thoracic Society
AUC	Area under the curve
BD	Bronchodilators
CAPA	Corrective and Preventative Action
CBC	Complete blood count
CFR	Code of Federal Regulations
cGCP	Current Good Clinical Practice
Cmax	Peak plasma concentration
CMP	Comprehensive metabolic panel
CRF	Case report form
CT	Computerized tomography
CTCAE	Common Terminology Criteria for Adverse Events
CYP	Cytochrome P450
DLCO	Diffusion capacity for Carbon Monoxide
DSMB	Data Safety Monitoring Board
DSMP	Data Safety Monitoring Plan
ERS	European Respiratory Society
eCRF	Electronic Case Report Form
Evaluable Patients	Patients whose response to a treatment can be measured because enough information has been collected
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in first second

FVC	Forced vital capacity
FPI	Functional Performance Inventory
GEE	Generalized Estimating Equations
HIPAA	Health Insurance Portability and Accountability Act
HRCT	High-resolution computed tomography
ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
LAM	Lymphangioleiomyomatosis
MEF	Mouse epithelial fibroblasts
MILES	Multicenter International LAM Efficacy of Sirolimus
mTOR	Mammalian Target of Rapamycin
NCI	National Cancer Institute
NIH	National Institutes of Health
NSAES	Non Serious Adverse Events
OHRP	Office for Human Research Protection
PFT	Pulmonary function test
PI	Principal Investigator
SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction
SD-SOB	San Diego Shortness of Breath Scale
SGRQ	St. George's Respiratory Questionnaire
SUSAR	Suspected, Unexpected Adverse Reaction
TSC	Tuberous Sclerosis Complex
Tmax	Median time to achieve peak plasma concentration
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
VEGF-D	Vascular Endothelial Growth Factor - D

3. Background and Rationale

3.1 Background:

Lymphangioleiomyomatosis (LAM) is a rare, progressive diffuse cystic lung disease characterized by the infiltration of lung parenchyma with abnormal smooth muscle cells, leading to progressive destruction of lung parenchyma (1). The origin of these smooth muscle cells is unknown, however, lung involvement happens as a result of metastatic spread of these smooth muscle cells via blood and lymphatics, thus characterizing LAM as a low-grade, destructive, metastasizing neoplasm (2, 3). LAM is seen primarily in women with only a few reported cases in men (1, 4). LAM can be seen in association with Tuberous Sclerosis Complex (TSC-LAM), or occur sporadically (S-LAM). Both TSC-LAM and S-LAM occur as a result of mutations in one of the two TSC genes: TSC1 or TSC2 (5, 6). Mutations in the TSC genes lead to constitutive activation of the mammalian target of rapamycin (mTOR) pathway, which drives cell proliferation and lymphangiogenesis.

3.2 Role of Sirolimus in LAM

Sirolimus forms a complex with FKBP12, which binds to mTOR and blocks activation of downstream kinases, restoring homeostasis in cells with defective TSC function (7). Sirolimus has been shown to shrink tumors in animal models of TSC deficiency (8, 9). Based on these observations, mTOR inhibitors were studied as therapeutic agents in patients with LAM and TSC. Open label, phase 1-2 trials of sirolimus in patients with TSC, led to a reduction in the size of angiomyolipomas (10, 11). An improvement in lung function was also noted in a majority of patients on sirolimus (10). This formed the formed the rationale for conducting a larger clinical trial to evaluate the role of sirolimus in LAM.

The Multicenter International LAM Efficacy of Sirolimus (MILES) Trial was a double blind, randomized, parallel group trial of one year of treatment with sirolimus versus placebo followed by one year of observation (12). In the MILES trial, 89 patients with LAM and moderate lung impairment (defined as FEV_1 less than 70% predicted) were randomized to treatment with sirolimus or placebo for 12 months followed by 12 months of observation. The primary outcome for the MILES trial was the difference in the rate of change (slope) of FEV_1. While patients treated with placebo lost approximately 10% of their lung volume in the treatment phase, patients treated with sirolimus exhibited stabilization of their lung function, and had an improvement in their functional performance and quality of life.

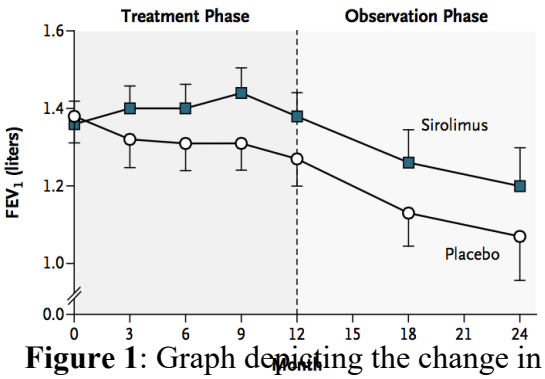


Figure 1: Graph depicting the change in FEV_1 in the MILES trial.

During the observation year, lung function decline resumed in the sirolimus group and paralleled that of the placebo group (Figure 1) (12).

The resumption of lung function decline following drug stoppage suggests that the function of sirolimus is suppressive rather than remission inducing. In addition, while the drug was well tolerated for the trial duration, the long-term safety and efficacy of sirolimus in LAM remains unclear. These limitations of sirolimus highlight the critical need to explore novel treatment opportunities for patients with LAM.

3.3 Serum Vascular Endothelial Growth Factor-D (VEGF-D) as a diagnostic, prognostic, and predictive biomarker of LAM

Serum vascular endothelial growth factor-D (VEGF-D), a ligand for the lymphatic growth-factor receptor VEGFR-3/Flt-4, is a major angiogenic growth factor that induces formation of lymphatics and promotes the spread of malignant tumor cells (13). VEGF-D has been noted to be present at increased levels in the serum of LAM patients (13-15). While the exact source of origin of VEGF-D in LAM is not well established, it is believed to be derived from the LAM cells and promotes the metastatic spread of LAM cells (15). The role of serum VEGF-D as a diagnostic biomarker for LAM is now well established. Among patients with cystic lung disease on chest imaging, elevated serum VEGF-D greater than 800pg/ml is almost 100% specific for the diagnosis of LAM (14). Serum VEGF-D measurement is commercially available in a CAP/CLIA laboratory at CCHMC.

In addition to its role as a diagnostic biomarker, serum VEGF-D is also a prognostic as well as a predictive biomarker. In the MILES trial, the baseline serum VEGF-D levels were similar in the placebo and treatment arms (12). Baseline serum VEGF-D levels correlated with multiple markers of disease severity, including pneumothorax, bronchodilator responsiveness and need for supplemental oxygen, and with physiologic parameters such as FVC ($p=0.002$), total lung capacity (TLC ($p<0.0001$)) and diffusion capacity for carbon monoxide ((DLCO) ($p<0.0001$)). Higher baseline serum VEGF-D levels were also associated with disease progression and treatment response. Each 1-unit increase in baseline log (VEGF-D) was associated with a baseline to 12-month change of -70 cc in the placebo group and +70cc in the sirolimus group ($p<0.001$). Placebo patients with baseline VEGF-D levels ≥600pg/ml were found to decline much faster than the placebo patients with VEGF-D <600pg/ml, and the ≥600pg/ml sirolimus group patients responded better than the sirolimus patients with VEGF-D <600pg/ml. While VEGF-D levels remained stable in the placebo arm, treatment with 12 months of sirolimus resulted in approximately 50% reduction in the mean VEGF-D levels. In the observation year, VEGF-D values remained stable in the placebo group, but increased again in the sirolimus group (Figure 2). Sirolimus induced reduction in serum VEGF-D was highly correlated with improvement in lung function. Improvement in baseline-to-12-month FEV1 occurred in 65% of VEGF-D responders (patients with a VEGF-D decrease ≥ 42% from its baseline value), as opposed to only 27% of VEGF-D non-responders ($p=0.04$) (Figure 3). These data indicate that VEGF-D is a clinically useful prognostic biomarker of disease progression and a predictive biomarker of treatment response (16). Similar findings have been replicated in another analysis (17). Similarly, reduction in serum

VEGF-D by approximately 50% was noticed after treatment with open-label everolimus in a recent trial (Figure 4) (18). These findings suggest that serum VEGF-D can serve as a biomarker to monitor the response to treatment in patients with LAM. Since LAM cells are the source of elevated VEGF-D, monitoring VEGF-D levels as treatment response can also serve as a surrogate for LAM cell burden and activity.

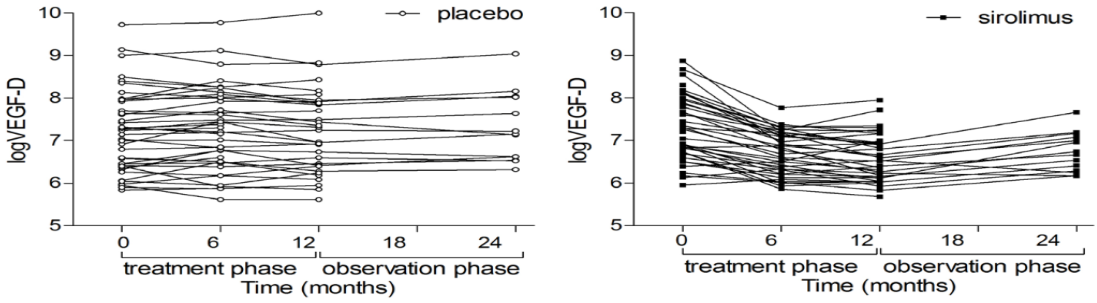


Figure 2: VEGF-D trends in the MILES trial. Serum VEGF-D stayed stable in the placebo group and decreased in the sirolimus group. VEGF-D levels increased again after stopping sirolimus.

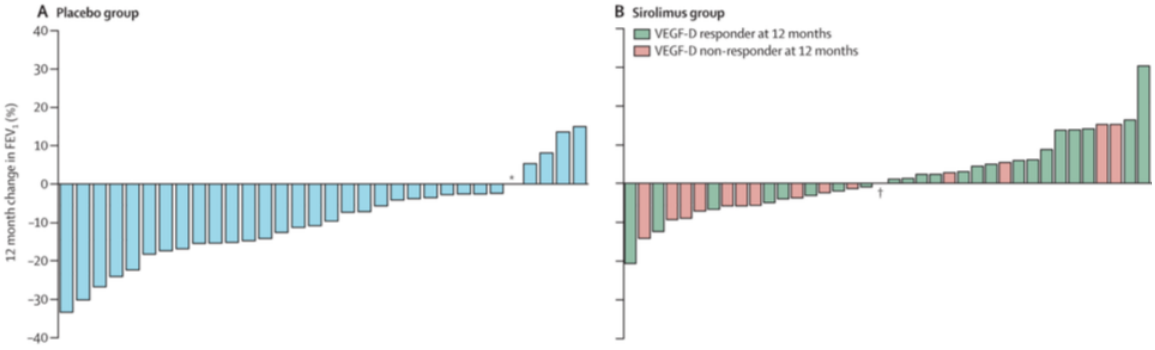


Figure 3: Baseline-to-12-month change in FEV1 in the placebo group (left), and sirolimus group (right) as stratified by VEGF-D responders and non-responders in the MILES trial.

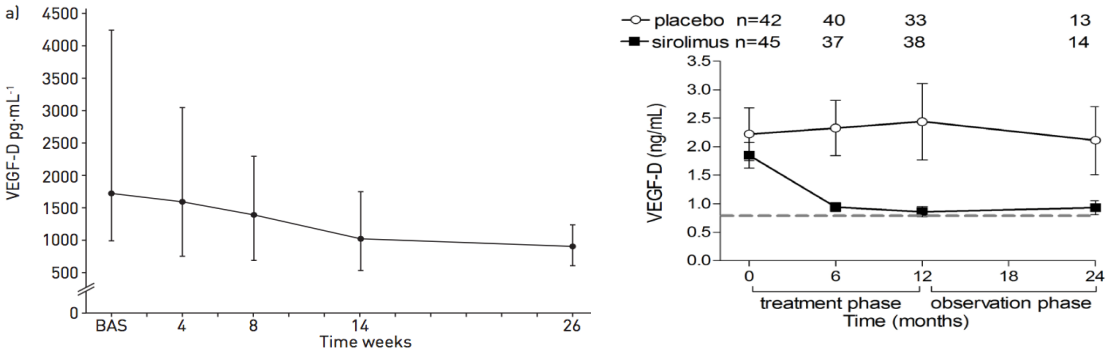


Figure 4: Graphs depicting the trend of serum VEGF-D in LAM patients following treatment with everolimus (left) and sirolimus (right). Serum VEGF-D declined to approximately 50% of its baseline value following treatment with mTOR inhibitors. The everolimus study (left) had frequent, early blood draws which revealed that most of the reduction in serum VEGF-D occurs in the first 8 weeks following treatment initiation, after which it plateaus.

3.4 Resveratrol and its role in treatment of LAM

Resveratrol (*trans*-3, 5, 4′-trihydroxystilbene) is a naturally occurring polyphenol found in grapes, berries, and red wine (19). Multiple studies have shown Resveratrol to have anticancer, anti-inflammatory, and anti-oxidant properties (19-23).

Resveratrol and mTOR regulation

Resveratrol has a complex mechanism of mTOR regulation. Resveratrol inhibits mTORC1 phosphorylation in a dose-dependent manner by inhibiting phosphorylation at S2448 residue (19, 24). Resveratrol has also been shown to have multiple effects on mTORC2 signaling (25). Resveratrol is believed to regulate mTOR activity through modulation of 5' AMP-activated protein kinase (AMPK), a key molecule for energy sensing that negatively regulates mTOR activity. Resveratrol induced phosphorylation of AMPK causes decreased phosphorylation of mTORC1 and its downstream targets (26). Resveratrol has also been shown to inhibit Akt phosphorylation in a dose-dependent and time-dependent manner, thus leading to mTORC1 inhibition (27). Lastly, Resveratrol has been shown to promote association between mTOR and DEPTOR, a negative regulator of mTORC1 and mTORC2 that inhibits mTOR kinase activity (28). The inhibition of PI3, Akt and mTOR activity has also been postulated to be responsible for the pro-apoptotic effects of Resveratrol (19).

Resveratrol and its role in regulating autophagy

Autophagy is an evolutionarily conserved process of recycling organelles or misfolded proteins that allows cells to maintain homeostasis under stress-induced conditions (29). Autophagy allows cancer cells to continue to survive by recycling intracellular components and persisting under low-nutrient or stress-induced conditions. The mTORC1 pathway is central to regulation of autophagy via its modulation of multiple autophagy-regulating factors such as Akt, AMPK, and S6K1. Resveratrol inhibits the activity of Akt, AMPK and S6K1, thus leading to autophagy down-regulation (19).

Sirolimus as a single agent may promote cell survival in LAM

Results from the MILES trial show that sirolimus as a single agent can lead to stabilization of lung function decline in patients with LAM (12). However, lung function decline resumed at a rate similar to the placebo group after stopping sirolimus, suggesting a suppressive rather than curative effect of sirolimus in LAM. The inhibition of mTOR activity with sirolimus releases the negative feedback signaling from S6K to Akt. The resultant increased Akt activity prevents cancer cell death by protecting them from apoptosis. Additionally, *TSC2–/–* LAM cells are dependent on autophagy for survival, and, since mTORC1 is a major regulator of autophagy, inhibition of this pathway with rapamycin induces autophagy, paradoxically leading to cell survival (30).

Combination of Resveratrol and Sirolimus treatment restores inhibition of Akt, while suppressing mTORC1 signaling

In a recent study, wild type and TSC2 null mouse embryonic fibroblasts (MEFs) were treated with both Resveratrol and Sirolimus (30). Sirolimus effectively suppressed mTORC1 signaling in both cell types, as measured by markers of S6K1 activity. Due to inhibition of the negative feedback loop, Sirolimus treatment increased Akt signaling, as measured by phosphorylation of Akt and its target PRAS40. Resveratrol treatment alone suppressed basal Akt signaling and slightly decreased S6K1 pathway activation. Interestingly, Resveratrol was able to prevent rapamycin-induced reactivation of Akt signaling as well as phosphorylation of its downstream substrate, PRAS40, while simultaneously effectively suppressing mTORC1/S6K1 signaling (Figure 5). Similar results showing inhibition of Akt signaling and mTORC1 inactivation by a combination of Resveratrol and Sirolimus were replicated in uterine leiomyoma-derived smooth muscle cells, human LAM-derived TSC2-null 621–101 cells, and normal human bronchial cells (Images not shown) (30).

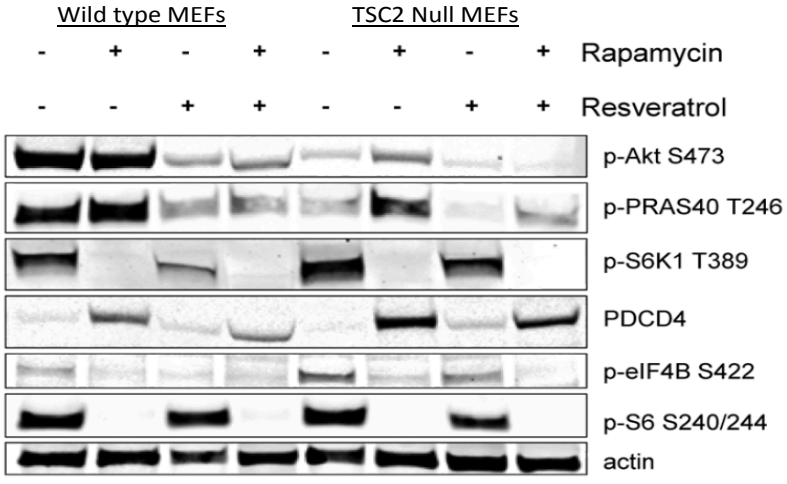


Figure 5: The combination of Resveratrol and Sirolimus inhibits PI3/Akt and mTOR signaling pathways.

Combination of sirolimus and resveratrol downregulates autophagy in TSC2-null cells

The effect of the combination of sirolimus and resveratrol on autophagy induction was studied in wild type and TSC2 null MEFs (30). Levels of p62/SQSTM1 protein inversely correlate with autophagy induction. While resveratrol alone did not have an effect on p62 levels, the combination treatment of sirolimus and resveratrol blocked autophagy induction in wild type and TSC2 null MEFs, and restored p62 levels back to baseline (Figure 6). Similar results showing a downregulation of autophagy were observed by treating human LAM-derived TSC2-null 621–101 cells with a combination of resveratrol and sirolimus (Images not shown) (30).

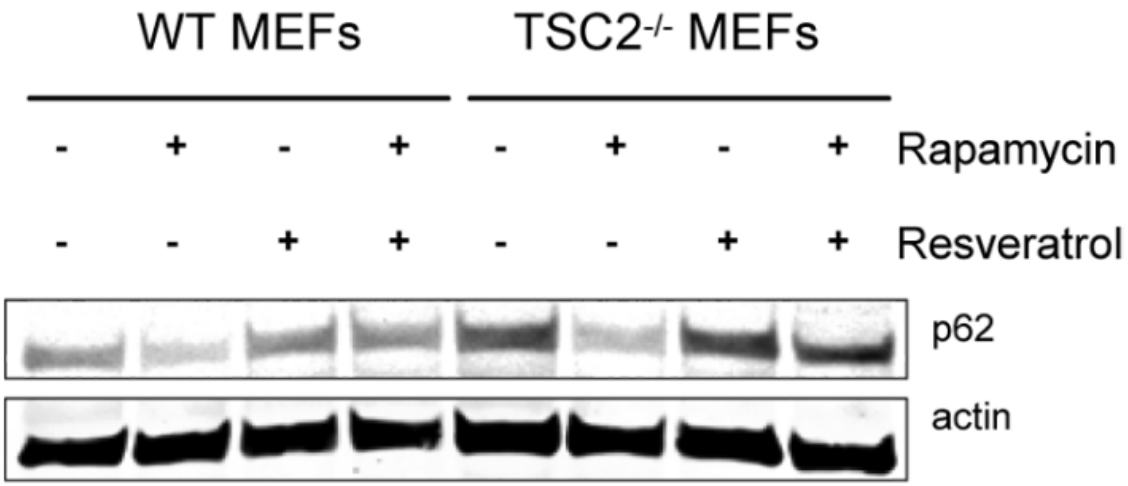


Figure 6: Resveratrol prevents sirolimus induced induction of autophagy. Wild type mouse epithelial fibroblasts (MEFs) and TSC2 null fibroblasts were treated with 20nM Sirolimus and 100μM Resveratrol for 24 hours. Cells were lysed and protein levels of p62 and actin were detected by immunoblot.

Apoptosis is induced in TSC2-null cells, but not in control cells

Wild type and TSC2 null MEFs were treated with sirolimus and/or resveratrol for 24h and assayed for apoptosis induction by examining cleavage of 2 markers, PARP and Caspase 3. Resveratrol treatment, alone or in combination with sirolimus, was able to strongly increase cleavage of PARP and Caspase 3 specifically in TSC2 null MEFs and not in wild type MEFs (Figure 7). These findings of increased apoptosis by resveratrol in TSC2 null cells were replicated in uterine leiomyoma-derived smooth muscle cells, human LAM-derived TSC2-null 621–101 cells (Images not shown) (30).

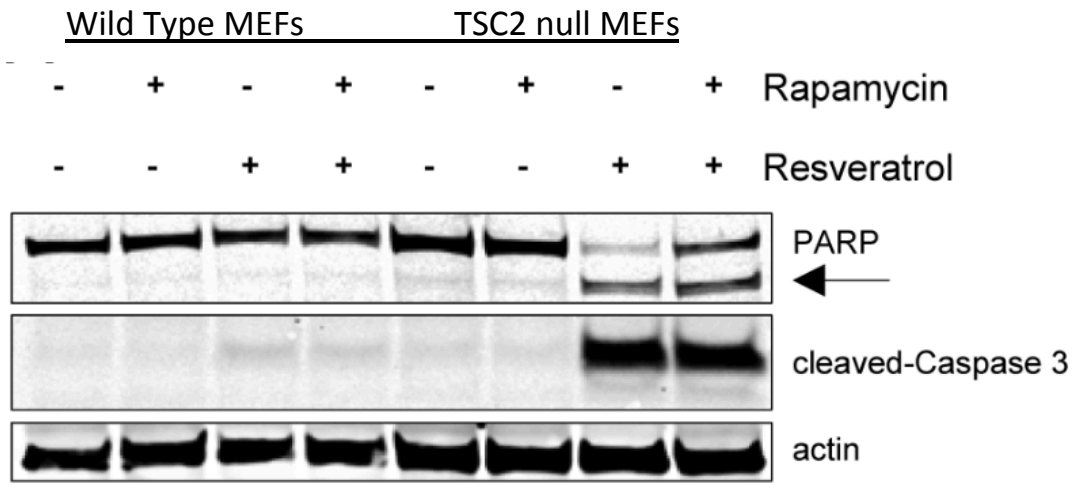


Figure 7: Resveratrol, alone or in combination with sirolimus, leads to apoptosis in TSC2 null mouse epithelial fibroblasts (MEFs) and not in wild type MEFs. Wild type and TSC2 null MEFs were treated with 20 nM rapamycin and/or 100 μM resveratrol for 24 h. Cells were lysed and probed for PARP, cleaved-Caspase 3, and actin. The arrow indicates cleaved PARP isoform.

Combination of sirolimus and resveratrol reduces the metastatic capacity of uterine leiomyoma-derived smooth muscle cells

TSC2 null uterine leiomyoma derived smooth muscle cells (ELT3 cells), metastasize to the lungs following tail vein injection into mice replicating an in vivo model for LAM (31). After 24 h treatment, mice treated with the combination therapy of resveratrol and sirolimus had significantly fewer lung metastases as assayed by photon flux compared with control mice or mice treated with either sirolimus or resveratrol alone (Figure 8) (30).

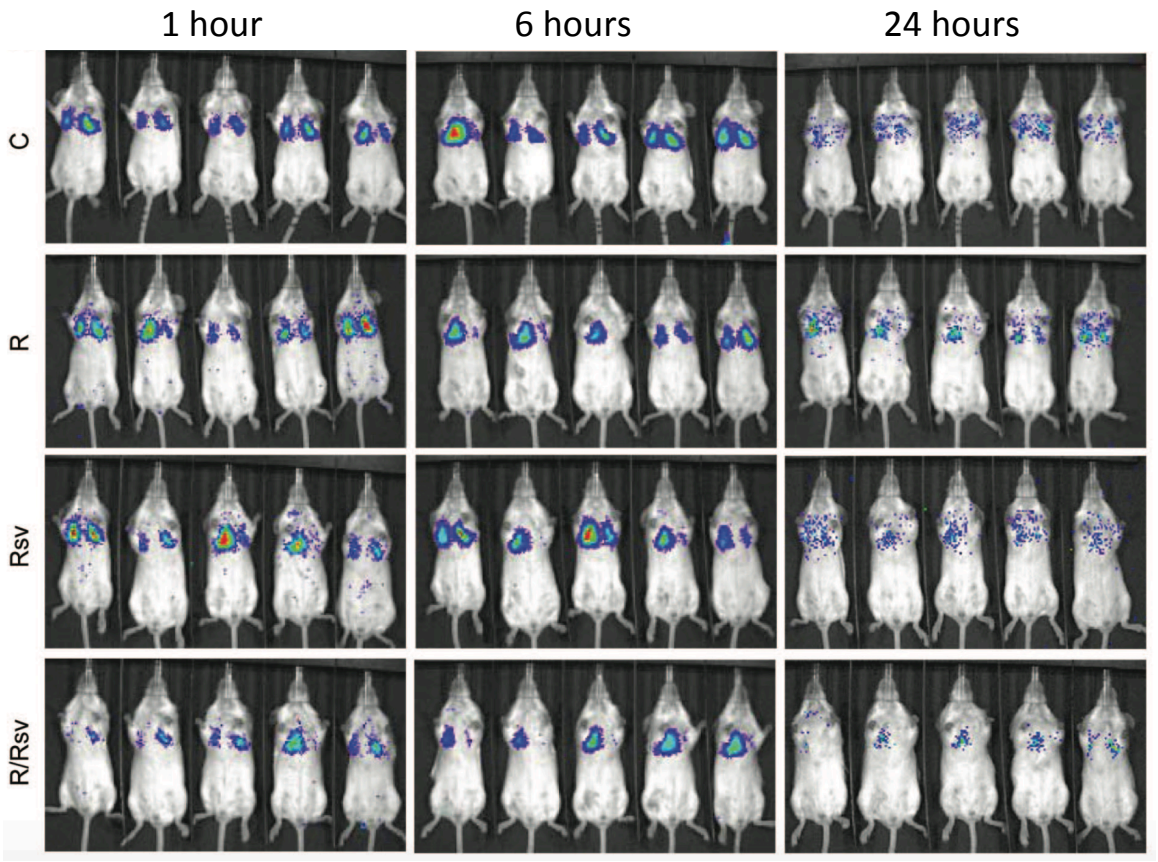


Figure 8: Combination of sirolimus and resveratrol strongly reduces the survival of Tsc2-null cells in vivo. Mice were treated with vehicle, sirolimus, resveratrol, or sirolimus plus resveratrol. ELT3-luciferase-expressing cells were inoculated intravenously. Representative bioluminescent images of lung colonization at 1, 6, and 24 hours post-cell injection are shown above depicting a significant decrease in pulmonary metastases following treatment with combination of resveratrol and sirolimus.

Combination treatment with resveratrol and sirolimus causes a significant reduction of TSC2 deficient xenograft tumor size and growth

Mice bearing ELT3-luciferase–expressing xenograft tumors were treated with sirolimus and resveratrol as single-agent treatments or in combination. There was no evidence of drug toxicity as evidenced by a lack of significant weight change among treatment groups. While sirolimus treatment alone showed a slight but significant reduction in tumor size in just 3 weeks of treatment, the combination of sirolimus and resveratrol caused a statistically significant reduction in tumor size as compared with sirolimus treatment alone by as early as Week 2 of treatment, and this reduction in tumor size was maintained at 4 weeks after treatment (Figure 9) (32).

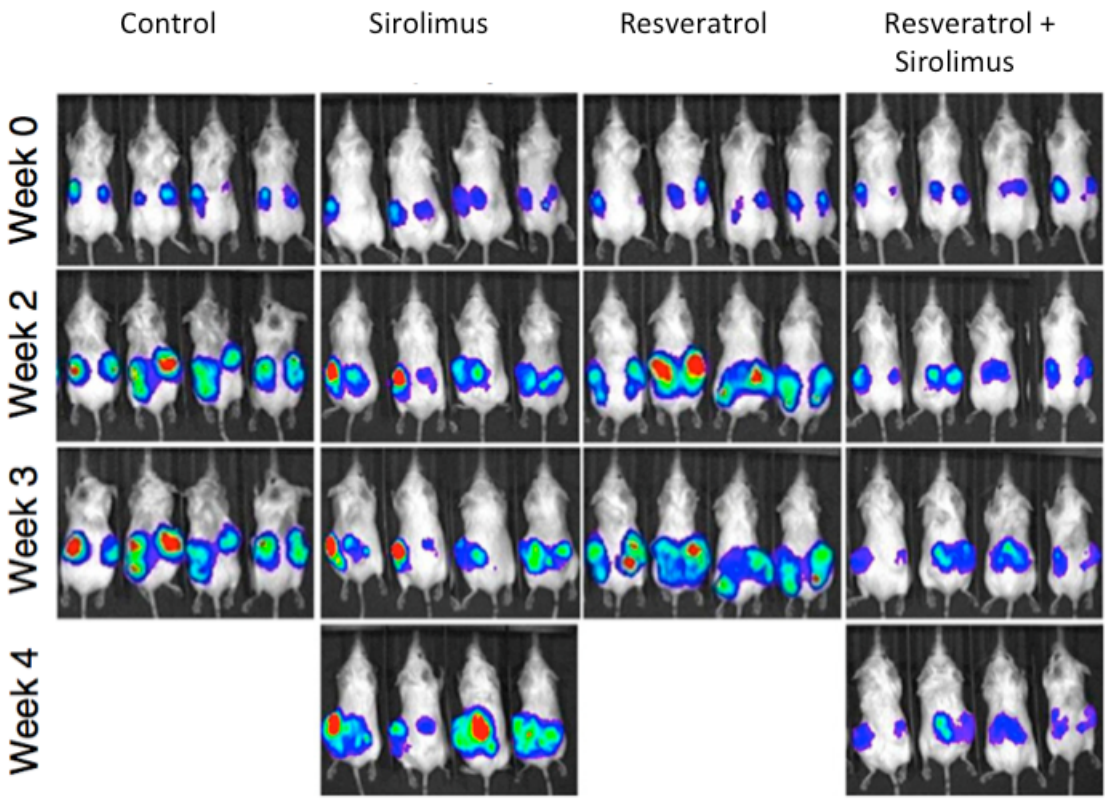


Figure 9: Combination treatment with sirolimus and resveratrol reduces tumor size and inhibits growth. Female CB17-scid mice were inoculated with ELT3-luciferase cells subcutaneously. Mice were treated with vehicle, sirolimus, resveratrol, or a combination of sirolimus and resveratrol for 4 weeks. Bioluminescent intensity in xenograft tumors was recorded and quantified weekly. Vehicle- and resveratrol-treated tumors progressed faster than the tumors in sirolimus or sirolimus–resveratrol groups. Combination of sirolimus and resveratrol caused a statistically significant reduction in tumor size as compared with sirolimus treatment alone.

The Combination of Sirolimus and Resveratrol Suppresses PI3K/Akt/ mTORC1 Activation in the TSC2 deficient xenograft tumor

Mice bearing ELT3-luciferase–expressing xenograft tumors were treated with sirolimus and resveratrol as single-agent treatments or in combination. The combination of sirolimus and resveratrol resulted in severe reduction of S6 phosphorylation and approximately 40% inhibition of 4EBP1 phosphorylation. Sirolimus alone resulted in mild reduction of S6 phosphorylation. Moreover, the combination of sirolimus and resveratrol caused a 45% inhibition of Akt phosphorylation at S473, with no effect on p-ERK signaling, thus indicating the specificity of this treatment in inhibiting Akt/mTORC1 signaling (32).

Combined Administration of Sirolimus and Resveratrol Reduces Proliferation and Induces Apoptosis within the TSC2 deficient xenograft tumor

Mice bearing ELT3-luciferase–expressing xenograft tumors were treated with sirolimus and resveratrol as single-agent treatments or in combination for 4 weeks. Sirolimus and resveratrol combination treatment caused a 2-fold up-regulation of cleaved caspase-3, a marker of apoptosis. In addition, analysis of TUNEL staining of tumor sections showed that combination treatment increased the levels of apoptotic TUNEL-positive cells by 3-fold, whereas single-agent treatment with either sirolimus or resveratrol had no effect on apoptosis (Figure 10). Taken together, these data indicate that a 4-week administration of the combination treatment of sirolimus and resveratrol inhibits mTORC1 signaling, inhibits Akt activation, and induces apoptosis (32).

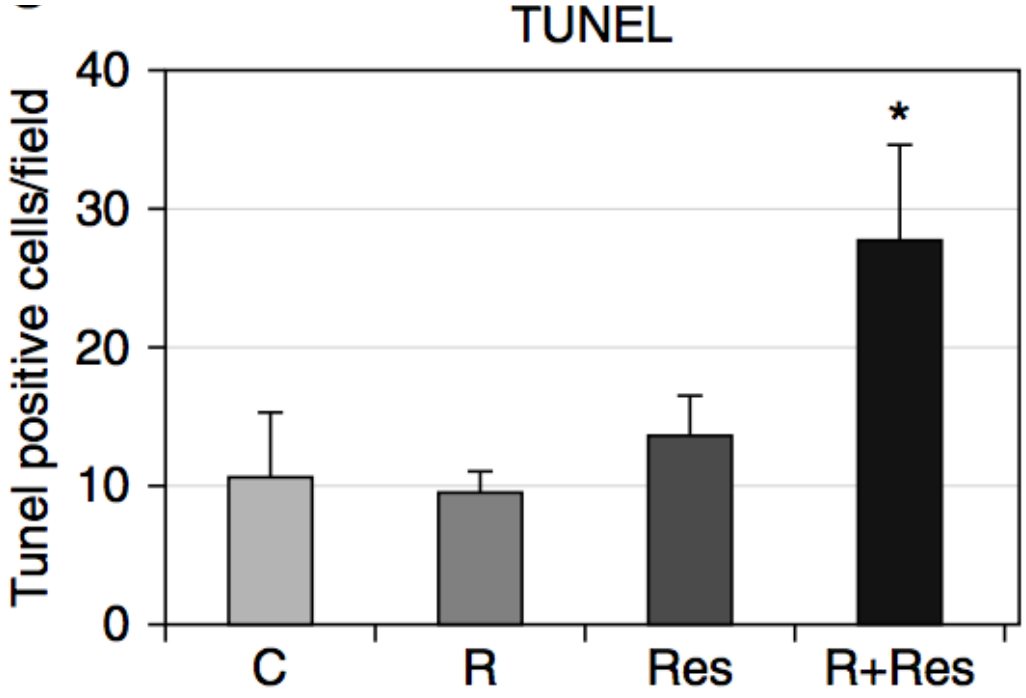


Figure 10: The combination of rapamycin and resveratrol induces apoptosis in xenograft tumors. TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labeling.

3.5 Role of home spirometry in pulmonary disorders

In a recent study, 50 patients with idiopathic pulmonary fibrosis (IPF) performed daily home spirometry for a median of 279 days. Office-based spirometry was performed 6- and 12-months after enrollment. In this study, over 80% of the patients adhered to the routine of performing daily spirometry, daily spirometry values showed excellent correlation with office-based spirometry (Figure 11), and the rate of decline in FVC obtained after 3 months of home spirometry was highly predictive of patient outcomes and subsequent mortality (HR: 1.04, CI: 1.02–1.06, p<0.001) (Figure 12) (33).

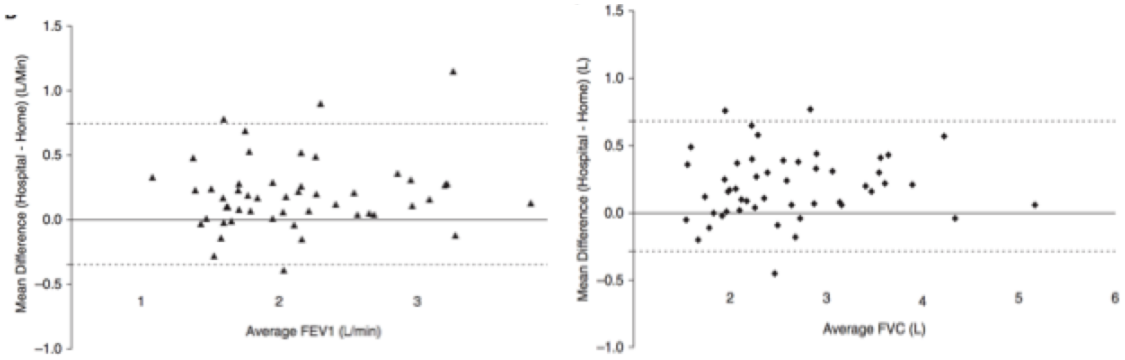


Figure 11: Bland-Altman plots demonstrating good agreement between office-based measurements of FEV1 (left) and FVC (right) with home-based measurements. There was a slight bias (0.20 liters) towards consistently higher readings obtained during office-based measurements as opposed to home-based measurements

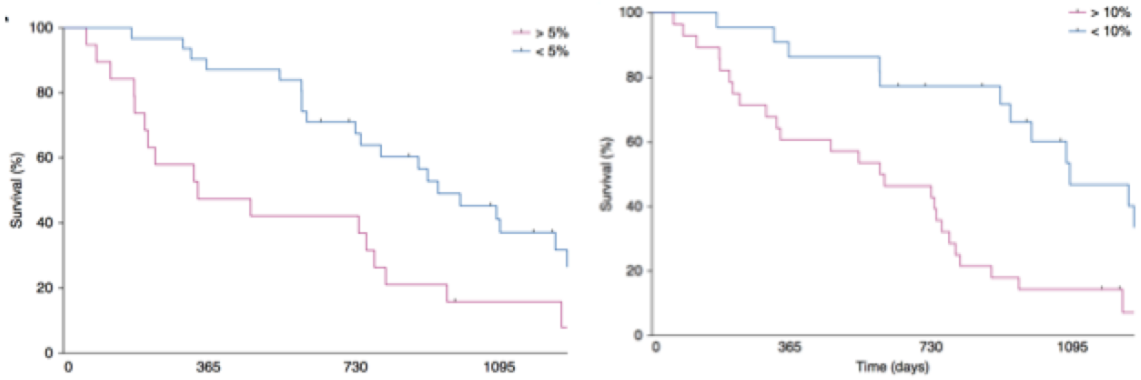


Figure 12: Kaplan-Meier curve demonstrating that the trends obtained from home-based FVC monitoring are predictive of the overall survival in patients with IPF. Left: Increased mortality in patients with FVC decrease more than 5% from baseline after 3 months of monitoring. Right: Increased mortality in patients with FVC decrease more than 10% from baseline after 12 months of monitoring.

Johannson et al. were able to achieve a compliance rate of 90% with once-weekly home spirometry in 24 patients with IPF, and demonstrated that weekly home spirometry is both reliable and feasible in this patient population. Addition of weekly home spirometry resulted in enhanced precision, and was estimated to reduce the sample size needed to study the efficacy of a new intervention by four-fold (34). In another recent study, patients with cystic fibrosis randomized to twice-weekly home spirometry measurements had a statistically significant shorter time to detection of disease-related exacerbations as opposed to usual care. However, the adherence to home spirometry in this study was suboptimal with 50% of the population consistently performing once-weekly testing, and only 19% of the population adhering to the twice-weekly regimen (35). Home spirometry has been shown to facilitate early detection of bronchiolitis obliterans following lung transplantation as compared to office-based spirometry (36, 37).

3.6 Rationale for the proposed study

Treatment with sirolimus alone has a suppressive rather than remission inducing effect. In addition, mTORC1 inhibition by sirolimus leads to upregulation of autophagy and paradoxically increases LAM cell survival. As explained in Section 3.4, the combination of resveratrol and sirolimus is effective in blocking autophagy and inducing apoptosis of *TSC2*$^{-/-}$ cells, as well as reducing their survival *in vivo* with minimal effect on normal, TSC2-expressing cells. These pre-clinical results form the rationale for investigating the combination of resveratrol and sirolimus as a treatment option for patients with LAM and TSC. Since there is no clinical data on the adverse effect profile and effective dosing of resveratrol in patients with LAM, we plan to conduct a phase II, dose escalating, safety and efficacy study of resveratrol in combination with sirolimus with plans for a multi-center phase III study after successful completion of this project.

Home spirometry has the potential to shorten the length of monitoring time needed to determine the disease trajectory. Reliable estimates of lung function typically require multiple measurements (3 or more) obtained over a prolonged monitoring period, usually 12 months or more. We hypothesize that the trajectory of lung function decline and treatment response can be determined in a much shorter period of time using frequent home-based spirometric measurements. Our strategy of testing home spirometry in conjunction with RESULT will allow us to directly compare home spirometry to standard office-based spirometry in an ATS/ERS calibrated laboratory and with real time quality control for both home- and office-based pulmonary function studies. Our project will validate the use of home spirometry as a novel disease-monitoring tool for patients with LAM, and will help integrate home spirometry in clinical practice, and in future studies seeking to develop novel treatment approaches.

4. Study Objectives and Endpoints:

4.1 Primary Endpoint:

The primary objective of this study is to assess the change in serum vascular endothelial growth factor-D (VEGF-D) value after 24 weeks of treatment with a combination of resveratrol and sirolimus, as compared to the VEGF-D value on sirolimus alone.

4.2 Secondary Endpoints:

1. Safety and adverse effect profile of combined resveratrol and sirolimus in patients with LAM
2. Mean change in FEV_1 before and after treatment
3. Mean change in FVC before and after treatment
4. Difference in rate of change in FVC (slope) before and after treatment
5. Difference in rate of change in FEV_1 (slope) before and after treatment
6. Mean change in diffusion capacity of carbon monoxide (DLCO) before and after treatment
7. Difference in rate of change in DLCO (slope) before and after treatment
8. Baseline to 24-week change in the score on St. George's Respiratory Questionnaire (SGRQ)
9. Baseline to 24-week change in the score on ATAQ-LAM (A tool to assess quality of life in LAM)
10. Baseline to 24-week change in the score on SD-SOB score (San Diego Shortness of Breath Questionnaire)
11. Baseline to 24-week change in the score on EuroQOL scale
12. Overall compliance with weekly home spirometry.
13. Correlation between home spirometry values (absolute as well as trends) with office-based spirometry values and trends.

5. Study Design:

This is an open label, non-randomized, dose-escalation study conducted as a phase II safety and efficacy study. Patients will continue on their existing background therapy of sirolimus for treatment of LAM.

5.1 Identification and Screening:

Potential participants will be identified through the University of Cincinnati Medical Center LAM Clinic, which provides clinical care for patients with LAM. LAM patients may also learn of the study from the LAM Foundation web site or the study listing on ClinicalTrials.gov. Potential study participants will be screened for eligibility. Study staff expects to screen the required sample size of 25 patients over a 15-month time frame. Potential participants will be informed about the study, and following completion of the informed consent process, a screening evaluation will be performed. Informed consent will be obtained *before* any study procedures are performed. Informed consent for participation in this study may be obtained in-person or remotely via telephone.

5.2 Inclusion Criteria:

Subjects enrolled in the trial must meet **all** of the following criteria.

1. Definitive diagnosis LAM based on the presence of characteristic cystic change on high-resolution computed tomography (HRCT) of the chest. The diagnosis must be confirmed by one of the following:
   A) Histopathological confirmation by biopsy (lung, abdominal mass, lymph node or kidney or cytology from thoracic or abdominal sources revealing HMB45+ staining of spindled/epithelioid cells)
   B) Compatible chest CT scan findings in the setting of tuberous sclerosis, angiomyolipomas (diagnosed by CT, MRI by the site radiologist or biopsy) or chylous pleural effusion (verified by tap)
   C) Chest CT scan findings compatible with LAM and a VEGF-D level ≥ 800pg/ml.
2. Age 18 years or greater
3. Signed and dated informed consent
4. Currently on sirolimus for treatment of LAM for at least 20 weeks
5. Evidence of disease stabilization on sirolimus as demonstrated by two stable values of serum VEGF-D post initiation of sirolimus drawn at least 12 weeks apart from each other. For the purpose of this study, a variation in serum VEGF-D of less than or equal to 15% is considered stable.

5.3 Exclusion Criteria:

Subjects who meet **any** of the following criteria are not eligible for enrollment as study participants:

1. Known allergy or hypersensitivity to resveratrol
2. Inability to provide informed consent
3. Active enrollment in other clinical drug trials for LAM
4. Pregnant or plan to become pregnant in the next 6 months
5. Breast feeding
6. Inability to comply with pulmonary function tests or follow up visits
7. Inadequate contraception
8. Use of estrogen containing medications within the 30 days prior to randomization
9. History of organ transplant
10. Actively listed for lung transplantation
11. Inability to comply with study procedures or attend scheduled study visits
12. Any clinically significant medical disease (other than LAM) that is associated with an expected survival of less than 2 years, or likely to impact the ability of the patient to participate in the study in the opinion of the investigator, or impact the study efficacy or safety assessments.
13. Concomitant use of resveratrol with drugs with a narrow therapeutic range that are CYP3A, CYP1A2, CYP2D6, or CYP2C9 substrates (for example, theophylline, tizanidine, warfarin, phenytoin, alfentanil, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, cyclosporine, tacrolimus, thioridazine) will be prohibited from Day 1 until the end of study.

5.4 Recruitment:

Patients will be recruited from The University of Cincinnati Medical Center LAM Clinic, and referred by physicians and LAM support groups such as the LAM Foundation, and the Tuberous Sclerosis Alliance. IRB approved recruitment materials may be posted in public places such as LAM clinics, The LAM Foundation, the Tuberous Sclerosis Alliance, LAM patient and/or physician events, conferences and websites. The study will be posted on clinicaltrials.gov.

Potential study participants will be contacted by the study staff to determine interest in study participation. Contact may be made by mail, electronic mail, telephone or in person. Interested individuals will receive study information, including a cover letter and a consent form. Informed consent for study participation may be obtained in-person or over the phone.

5.5 Study Drug Treatment:

Patients who meet eligibility criteria, provide informed consent and are enrolled in the study will receive instructions regarding resveratrol dosage, route and administration. A 8-week supply of resveratrol 250mg daily will be provided at visit 1. Patients will be reminded about the side effects of resveratrol, and will be given a drug information sheet. Patients will be instructed to take the study medication at the same time every day. Patients will be carefully monitored monthly at subsequent visits for adverse effects

related to resveratrol. Common side effects of resveratrol include nausea, vomiting, diarrhea, flatulence, abdominal discomfort, and fatigue. Patients will be instructed to contact the study staff should they experience any of these side effects. Resveratrol doses may be held or reduced in the event of unacceptable side effects.

5.6 Treatment Regimen:

We have chosen an intra-patient dose escalation strategy because there is no previous data from human studies to demonstrate the safety of resveratrol in combination with sirolimus, in patients with LAM. Patients will begin resveratrol at a starting dose of 250mg daily, and increase the dose to 500mg daily after 8 weeks, followed by dose escalation to 1000mg daily in two divided doses (500 mg twice a day) at week 16 of the study. If no adverse effects are seen, patients should reach a dose of 1000mg per day at week 16 of the study, and then continue with this dose for the rest of the study duration. If patients do not tolerate a certain dose of resveratrol, the dose will be reduced to the last tolerated strength. Patients will remain on the last well-tolerated dose of resveratrol for the entire study duration. Patients will remain on their current dose of sirolimus without interruption, as clinically indicated. Patients who are unable to tolerate the lowest dose of resveratrol will be withdrawn from the study.

5.7 Home Spirometry

We will provide patients with a home spirometer (GoSpiro, Monitored Therapeutics Inc.) at the beginning of the study This device was chosen after a careful survey of 9 different home spirometers, based on its ability to assess acceptability and reproducibility of maneuvers and fully comply with the ATS/ERS standards of a spirometer (38). FEV1 and FVC will be obtained once weekly from patients agreeing to participate in the home spirometry sub study. Detailed instructions regarding the proper use of the home spirometer will be provided at the time of enrollment, along with access to detailed instruction manuals for future use. Once a week, patients will perform at least three spirometric measurements at approximately the same time, and record the best FEV1 and FVC values as their weekly results. Patients will also be asked to complete a brief survey to rate their experience with the home spirometry every week. These results, along with the associated flow-volume and volume-time loops will then be transmitted electronically to the study coordinator. The spirometric values as well as the loops will be reviewed in near real time along with direct feedback to the subject on the performance and trouble shooting of any issues by an expert pulmonary physiologist (Dr. Adam Cole and/or Dr. Nishant Gupta). The spirometry data and loops will be judged for their acceptability and reproducibility as specified in the ATS/ERS guidelines (38). A maneuver will have to satisfy both the satisfactory start as well as end of test criteria in order for it to be considered acceptable. Difference between the largest recorded FEV1/FVC and the next largest FEV1/FVC less than 150ml will determine satisfactory intra-test reproducibility. Inter-test reproducibility will be assessed by comparing the FEV1 values obtained during the first 4 weeks of home spirometry measurements, with a target between-test variation of less than 5%. Overall compliance to weekly home spirometry will be recorded with

success defined as greater than 80% compliance with weekly measurements throughout the 24-week study duration.

6. Study Procedures and Timelines

6.1 Study and Timeline Overview

Patients will be identified by the medical and research staff at the LAM clinic at the University of Cincinnati Medical Center. Study staff will screen up to 60 patients over 15 months with a goal of enrolling 25 participants. Patients who meet eligibility criteria, provide informed consent and are enrolled in the study will receive instructions regarding resveratrol dosage, route and administration. An 8-week supply of resveratrol will be provided at visit 1. Patients will begin resveratrol at a starting dose of 250mg daily, and increase the dose in two increments to a maximum dose of 1000mg daily. If patients do not tolerate a certain dose of resveratrol, the dose will be reduced to the last tolerated strength. Patients will remain on the last well-tolerated dose of resveratrol for the entire study duration. Patients who are unable to tolerate the lowest dose of resveratrol will be withdrawn from study.

The patients will be given a copy of the informed consent form. Patients will be given ample time to read and discuss the consent form with the study staff. The study staff will then answer any remaining questions asked by the patients. The patients will be offered the option of signing the consent form. Those who agree to participate will undergo the remainder of screening visit.

Prior to starting study drug, pre/post bronchodilator spirometry, diffusion capacity of carbon monoxide (DLCO), and serum VEGF-D will be obtained to establish baseline values prior to study drug initiation. The best values for FEV_1 and FVC obtained during the spirometry maneuvers will be used. Patients will be followed with serial spirometry and VEGF-D levels collected at the initial visit, followed by week 8, week 16 and week 24 visits. DLCO will also be obtained at the 24-week visit in addition to spirometry.

For patients who agree to the home spirometry study, we will teach them on the proper technique to perform, record and transmit home spirometry, and provide them with a home spirometer. The patients will perform their first test during the initial visit and we will use those values to compare the home spirometer values with the office-based spirometry values. After that patients will be instructed to perform home spirometry once a week as detailed in Section 5.7.

Section 6.2 provides a summary of the study timeline and procedures.

6.2 Schedule of Events:

Event	Screening Visit*	Initial Visit	Week 2	Week 4	Week 8	Week 12	Week 16	Week 18	Week 20	Week 24
Visit number	0 or Phone	1	Lab check	Phone	2	Phone	3	Lab check	Phone	4
Informed consent	X									
History and physical		X			X		X			X
CBC, CMP		X			X		X			X
Lipid panel		X			X					X
Serum VEGF-D	X	X			X		X			X
Sirolimus level		X	X***	X***	X	X***	X	X***	X***	X
Urine Pregnancy Test**		X			X		X			
Saved serum and plasma		X			X		X			X
Spirometry post BD		X			X		X			X
Diffusion capacity		X								X
Home spirometry dispensed		X ****								
Review concomitant meds		X		X	X	X	X		X	X
Review adverse events				X	X	X	X		X	X
SGRQ		X			X		X			X
ATAQ-LAM		X			X		X			X
EuroQOL		X			X		X			X
SD-SOB		X			X		X			X

Window for all visits after the initial visit: ±7 days, except Weeks 2 and 18 where the window is ±3 days

* Screening visit can be the initial visit if a patient meets all inclusion/exclusion criteria. Informed consent for study inclusion can be obtained remotely over the phone rather than in-person. VEGF-D levels to confirm eligibility for trial participation can be drawn remotely rather than an in-person visit.

** Only required for women of childbearing potential.

*** Can be drawn remotely. This is a trough level to be obtained 24 ± 4 hours after the last sirolimus dose.

**** FEV1 and FVC will be obtained once weekly from patients agreeing to participate in the home spirometry sub study. Detailed instructions regarding the proper use of the home spirometer will be provided at the time of enrollment, along with access to detailed instruction manuals for future use. Once a week, patients will perform at least three spirometric measurements at approximately the same time, and record the best FEV1 and FVC values as their weekly results. These results, along with the associated flow-volume and volume-time loops will then be transmitted electronically to the study coordinator.

ATAQ-LAM: A Tool to Assess Quality of Life in LAM, BD: Bronchodilators, CBC: complete blood count, CMP: comprehensive metabolic panel, SGRQ: St. George's Respiratory Questionnaire, SD-SOB: San Diego Shortness of Breath scale, VEGF-D: Vascular Endothelial Growth Factor-D.

6.3 Study Visits

Screening/Initial Visit

At the screening visit, the consent form will be presented to the potential patient. If the patient is competent to understand the risks, agrees to participate and provides informed consent and HIPAA release, the patient will undergo screening. Patients will also be informed about the home spirometry portion of this study, and given the option to opt-in or opt-out of the home spirometry evaluation. Patients may enroll in the clinical trial without agreeing to participate in the home spirometry portion. During this visit, we will review the inclusion/exclusion criteria, obtain a urine pregnancy test for women of childbearing potential (defined as any fertility status other than natural or surgical menopause or post hysterectomy), and check a serum VEGF-D level (if two previous stable values post initiation of sirolimus are not drawn). The screening visit and VEGF-D testing can be accomplished either remotely or in-person. If patients meet the study inclusion criteria, the screening visit can be their initial study visit. We anticipate that in most patients the screening visit will be the initial study visit, and the following additional steps will be completed during the visit:

- Review current medications.
- Review medical history and conduct a physical examination.
- Perform spirometry with appropriate safety precautions. All spirometry tests during all visits will be performed after supervised administration of bronchodilator in the pulmonary function laboratory.
- Perform diffusion capacity (DLCO) testing. DLCO will be performed in a supervised setting in the pulmonary function laboratory.
- Obtain clinical labs including: complete blood count (CBC), comprehensive metabolic panel (CMP), lipid panel, serum VEGF-D, sirolimus level, urine pregnancy test, and urinalysis.
- Quality of life, dyspnea and fatigue assessments
- Dispense resveratrol tablets for the next 8 week supply.

- Provide a drug diary to document drug compliance. Patients will bring the drug diary at each subsequent visit
- If the patient has consented obtain a blood sample to be stored on-site for potential future use
- If the patient has consented, instruct them on the proper technique to perform home spirometry, provide them with a home unit, and obtain the first set of FEV1 and FVC values. Patients will also be taught on how to record and transmit electronically the home spirometry values on a weekly basis throughout the 24-week trial duration.

Lab Check (Week 2 ± 3 days)
During this study, the patient will have a sirolimus level checked for safety monitoring. This level can be drawn remotely.

Phone Visit (Week 4 ± 7 days)
During this visit a study coordinator will contact the patient and review the interval history in detail. A report of any adverse events will be assembled in detail, along with a review of their concurrent medications. Any concerns brought up during this phone call will be reviewed with the investigator. In addition, sirolimus level for safety monitoring will be drawn at this visit. The sirolimus level can be drawn remotely.

Visit 2 (Week 8 ± 7 days)
During this visit the following will be performed:

- Obtain a urine pregnancy test for women of childbearing potential
- Obtain urinalysis
- Review current medications
- Review the interval medical history, and perform a physical examination
- Perform spirometry after supervised administration of bronchodilator in the pulmonary function laboratory
- Quality of life, dyspnea and fatigue assessments
- Obtain blood for CBC, CMP, lipid panel, VEGF-D measurement and sirolimus level; if the patient has consented obtain an additional sample to be stored on-site for potential future use
- Dispense resveratrol tablets for the next 8 weeks
- Review drug diary and any reported adverse events
- Arrange for inter-visit phone call conduct

Phone Visit (Week 12 ± 7 days)
During this visit a study coordinator will contact the patient and review the interval history in detail. A report of any adverse events will be assembled in detail, along with a review of their concurrent medications. Any concerns brought up during this phone call will be reviewed with the investigator. In addition, sirolimus level for safety monitoring will be drawn at this visit. The sirolimus level can be drawn remotely.

Visit 3 (Week 16 ± 7 days)
During this visit, the following will be performed:

- Obtain a urine pregnancy test for women of childbearing potential
- Obtain urinalysis
- Review current medications
- Review the interval medical history, and perform a physical examination
- Perform spirometry after supervised administration of bronchodilator in the pulmonary function laboratory
- Quality of life, dyspnea and fatigue assessments
- Obtain blood for CBC, CMP, VEGF-D measurement and sirolimus level; if the patient has consented obtain an additional sample to be stored on-site for potential future use
- Dispense resveratrol tablets for the next 12 weeks
- Review drug diary and any reported adverse events

Lab Check (Week 18 ± 3 days)
During this study, the patient will have a sirolimus level checked for safety monitoring. This level can be drawn remotely.

Phone Visit (Week 20 ± 7 days)
During this visit a study coordinator will contact the patient and review the interval history in detail. A report of any adverse events will be assembled in detail, along with a review of their concurrent medications. Any concerns brought up during this phone call will be reviewed with the investigator. In addition, sirolimus level for safety monitoring will be drawn at this visit. The sirolimus level can be drawn remotely.

Visit 4 (Week 24 ± 7 days)
During this visit, the following will be performed:

- Obtain a urine pregnancy test for women of childbearing potential
- Obtain a urinalysis
- Review current medications
- Review the interval medical history, and perform a physical examination
- Perform spirometry after supervised administration of bronchodilator in the pulmonary function laboratory. LSEP
- Perform DLCO in the pulmonary function laboratory
- Quality of life, dyspnea and fatigue assessments
- Obtain blood for CBC, CMP, lipid panel, VEGF-D measurement and sirolimus level; if the patient has consented obtain an additional sample to be stored on-site for potential future use
- Review drug diary and any reported adverse events
- Advise women of childbearing potential that they should continue to use their method of birth control for at least 4 weeks after the study ends

- Remind patients they will be contacted via phone in 4 weeks as a follow up
- Remind patients to follow up with their regular physicians for routine care

If at visit 4, it is determined there has been clinical benefit, the patient and her regular physician will decide how to proceed after the conclusion of the clinical study.

End of study telephone call (Week 28 ± 7 days)
An end of study phone call will be conducted at 4 weeks ± 7 days after the last study visit. The purpose of the call is to follow up on any open or unresolved adverse events.

6.4 Enrollment

Enrollment in the study will continue until the enrollment target of 25 patients is reached. This research study will be explained in lay terms to each potential research participant. The potential participant will sign an informed consent form before undergoing any screening study procedures. Participants who are deemed eligible for the study (see sections 6.1 through 6.3) at each of the study sites will be enrolled and assigned a unique participant ID number.

6.5 Patient retention and drug compliance

Patient retention will be encouraged in several ways. Extensive contact information will be captured for each patient, at the time of study enrollment. This will include home, work, and cellular telephone numbers and that of an emergency contact or nearest family member. Prior to each study visit, the study staff will contact the patient to remind her about the upcoming study visit. A check for the reimbursement of expenses up to $500 will be issued after each study visit.

The study staff and physician will explain the importance of compliance with the study protocol at each patient contact. If a patient fails to comply with a study visit, the study staff will contact her by telephone. If the study staff is unsuccessful in reaching the patient, a follow up letter will be sent by overnight carrier, on two consecutive weeks, seeking follow-up.

Compliance with the protocol and drug administration will be strongly emphasized. Pill counts will be performed at visits and episodes of medication noncompliance will be recorded, including the reason for noncompliance. Patients will receive a calendar to document medication and any side effects experienced that day. If a patient wishes to withdrawal from the treatment phase of the study or has a serious adverse event (SAE) (whether related to study drugs or not), patient follow-up assessments will continue for safety monitoring and to avoid problems associated with missing data. The inclusion of such follow-up data will allow for analysis by intention-to-treat.

If a patient is withdrawn from study drug exposure for any reason, the patient will be strongly encouraged to continue with and complete the remainder of the study assessments, as scheduled.

6.6 Consent for future contact

During the consent process, patients will have the option to provide permission for future contact. This permission allows for patient contact information, including their full name, address and telephone number, to be retained. The permission for future contact allows for possible patient re-contact regarding future IRB-approved studies. If the patient denies permission, no future contact will be made.

6.7 Consent for continuing data review of medical records

During the consent process, patients will have the option to provide permission for future data review of their medical records. This permission allows for study staff to contact the patient (or the patient's caregiver(s)) to obtain medical records and other information for a period of up to two years after the patient has withdrawn from or completed the study. The purpose of this data review is to capture information including but not limited to clinical, radiographic, pathologic, laboratory and pulmonary function data, after the patient has exited the study. The rationale for future data review is that there may be long term effects of study treatment that are unknown to the investigators at this time.

6.8 Consent for storage of blood samples for future use

Patients will be asked to consent to the storage of leftover blood and serum samples for future use related to LAM. Patients can refuse without impacting their care or ability to participate in this study.

6.9 Consent for participation in the home spirometry substudy

Patients will be asked to consent for participation in the home spirometry evaluation, being done in conjunction with the clinical trial. Patients can refuse to participate in the home spirometry evaluation without impacting their care or ability to participate in this study.

7. Stopping rules

7.1 Participant withdrawal criteria

Participants may be terminated early from the study for the following reasons:

a. The participant elects to withdraw consent from all future study activities, including follow-up.

b. The participant is "lost to follow-up" (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed).

c. The participant dies.

d. The participant develops a medical condition or is started on new medication(s) not previously mentioned in the list of prohibited medications that, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality of the data obtained from the study.

e. The participant is listed for transplant and is required to stop sirolimus therapy.

f. In the opinion of the investigator, it is not in the participant's best medical interest to continue to participate in the study.

g. The participant meets any of the individual stopping rules as delineated in section 13.1.

7.2 Criteria for terminating the study

Up to 25 patients will be enrolled in this study. The maximum duration of patient participation is up to 28 weeks, including a follow-up phone contact 4 weeks after the last study visit. In the event of any death, or Grade IV Serious adverse event (SAE) attributable to the study agent and unexpected, enrollment will be suspended, pending Data Safety Monitoring Board (DSMB) review.

8. Study variables and measuring methods

8.1 Physical Exams:

Exams will be performed in a routine manner, consistent with the standard of care.

8.2 Pulmonary function testing

Pre and post bronchodilator spirometry and DLCO measurement will be performed in a standardized manner according to American Thoracic Society/European Respiratory Society criteria for acceptability and repeatability (38). The study site will attempt to have the same respiratory therapist administer testing to patients for consistency in the study. All PFTs obtained as part of the study will be performed post bronchodilator only, regardless of whether bronchodilator responsiveness is demonstrated. All spirometry data will be assessed for quality by spirometry standardization expert and study investigators, Dr. Francis X. McCormack, Dr. Adam Cole, and Dr. Nishant Gupta.

8.3 Questionnaires

St. George's Respiratory Questionnaire (SGRQ)

The SGRQ is a disease-specific instrument designed to measure impact on overall health, daily life, and perceived well-being. It has been developed to be used by patients with fixed or reversible airway obstruction. It has been shown to correlate well with physiologic parameters and to show changes with time in patients with LAM (39). The administration is performed by face to face or telephone interview; completion takes approximately 10 minutes. The questionnaire consists of 76 items divided into 3 domains: symptoms (frequency and severity); activity (activities that cause or are limited by breathlessness); and impacts (social functioning, psychological disturbances resulting from airways disease).

A Tool to Assess Quality of Life in LAM (ATAQ-LAM)

ATAQ-LAM is a disease-specific instrument designed to assess health related quality of life in patients with LAM. The questionnaire consists of 32 items divided into 4 domains: exertional dyspnea, cough, fatigue, and emotional well-being.

The EuroQOL visual analogue scale for quality of life

The EuroQOL visual-analogue scale measures self-reported ratings of health status. Scores range from 0 to100, with lower scores indicating worse functioning. Patients receiving sirolimus in the MILES trial had an increase in their scores on the EuroQOL scale as compared to the placebo group (12).

San Diego Shortness of Breath Questionnaire

The San Diego shortness of breath questionnaire measures the quality of life impairment secondary to dyspnea resulting from pulmonary disorders. It is a 24-item measure that assesses self-reported shortness of breath while performing a variety of activities of daily living (40).

8.4 Biomarker Analyses

VEGF-D biomarker analysis will be conducted on blood samples collected from each study patient at scheduled times. Assays will be performed by a central laboratory in Cincinnati. Serum VEGF-D has been shown to be elevated in patients with LAM and is a useful diagnostic, prognostic, as well as predictive biomarker (13, 14, 16).

8.5 Home Spirometry

FEV1 and FVC will be obtained once weekly from patients agreeing to participate in the home spirometry sub study. Detailed instructions regarding the proper use of the home spirometer will be provided at the time of enrollment, along with access to detailed instruction manuals for future use. Once a week, patients will perform at least three spirometric measurements at approximately the same time, and record the best FEV1 and FVC values as their weekly results. These results, along with the associated flow-volume and volume-time loops will then be transmitted electronically to the study coordinator. The spirometric values as well as the loops will be reviewed in near real time along with direct feedback to the subject on the performance and trouble shooting of any issues by an expert pulmonary physiologist (Dr. Adam Cole and/or Dr. Nishant Gupta).

9. Risks

9.1 Risks of the investigational product

9.1.1 Pharmacokinetics of resveratrol in humans

Absorption: In general, resveratrol is rapidly absorbed following oral administration. After oral administration, median time to achieve peak plasma concentration (Tmax) is approximately 30 minutes for low-dose resveratrol (41), and approximately 60-90 minutes for high-dose resveratrol (42, 43). Absorption of resveratrol is affected by food intake. Peak plasma concentration of resveratrol (Cmax) occurred 3 hours after a standard breakfast and 5 hours after consuming a high-fat meal (44). Co-administration of alcohol does not seem to affect resveratrol bioavailability (44).

Distribution: Because of its lipophilic nature, resveratrol has a high volume of distribution. Volumes of distribution ranging between 730 liters to 22000 liters have been described after ingesting resveratrol (44, 45).

Metabolism: Resveratrol undergoes extensive metabolism after human consumption. With small doses of resveratrol (up to 50mg per day), glucuronides are the main metabolites present in plasma (46). Monosulfates are the major metabolites when taking higher doses (> 250 mg per day) of resveratrol (47). It is possible that the resveratrol metabolites could act as a reserve for the parent molecule and, thus, contribute to resveratrol's pharmacological effects (48).

Elimination: The half-life of resveratrol varies depending upon the form of the drug. The half-life for micronized resveratrol is approximately 1 hour (49), whereas the half-life for nonmicronized resveratrol is approximately 8 hours (42). Resveratrol and its metabolites are mainly excreted through feces and urine (41). Fecal excretion is the major route of elimination for resveratrol and its metabolites accounting for greater than 70% of the excretion, with urinary elimination accounting for the remaining portions (41, 50).

9.1.2 Safety Profile of Resveratrol in Humans

In general, resveratrol is a well-tolerated drug with a robust safety profile. No adverse effects related to resveratrol were reported in clinical studies evaluating doses up to 1 gram of resveratrol per day (50-55). Resveratrol is well tolerated even at higher doses, however there is an increase in the gastrointestinal side effects at doses exceeding 1 gram per day. Howells et al. conducted a phase I study evaluating the safety profile of a micronized oral formulation of resveratrol in patients with liver metastases of colorectal cancer. Six patients received 5 grams per day of resveratrol for a total of 14 days. Resveratrol was well tolerated, in general, with the most common adverse effects being mild nausea and diarrhea. Other adverse effects included chills, lethargy, rash, peripheral neuropathy, skin irritation and vascular flushing, which resolved without sequelae (49). Using the same dose and formulation in 24 patients with multiple myeloma, Popat et al. noticed a higher rate of development of adverse effects. The most commonly reported adverse events were: nausea (79%), diarrhea (71%), vomiting (54%), fatigue (46%) and anemia (38%). 54% of patients reported grade 3 adverse events; most commonly 21% hematological (anemia and thrombocytopenia), 21% renal failure, 13% nausea and 13% infections (56).

In a phase I study, 10 healthy volunteers were given 4 different strengths of resveratrol (0.5 grams, 1 gram, 2.5 grams and 5 grams per day). Resveratrol was well tolerated at all doses, with no serious adverse effect noted. Minor adverse events were seen in 57% of the patients, and were self-limited resolving 2-4 days post administration (45). The same study was repeated again yielding similar results. There were no serious adverse events related to resveratrol. However, subjects taking the higher dose of resveratrol (2.5 grams and 5 grams per day) had an increased incidence of gastrointestinal symptoms, including nausea, flatulence, abdominal discomfort and diarrhea. Most of these events were mild (severity grade 1, NCI CTCAE v.4.0), although 4 participants on the 2.5 gram and 5 gram doses presented with nausea and/or diarrhea of moderate severity (grade 2). The gastrointestinal side effects commenced after 2-4 days of the intervention and occurred half to one hour after resveratrol ingestion (42). Other studies have reported similar findings with an increase in the incidence of gastrointestinal side effects at resveratrol doses higher than 1 gram per day, with most side effects being mild and spontaneously resolving after a brief drug holiday (41, 44, 47, 57, 58). Due to the increased incidence of gastrointestinal side effects related to resveratrol at high doses (42), and the increased incidence of renal failure noted in one study (56), we have decided to limit the highest dose in our study at 1 gram per day.

Adverse reaction	**Incidence (%)**
Gastrointestinal disorders	
Nausea	10 - 15
Vomiting	8 - 12
Diarrhea	20 – 30
Flatulence	5 – 10
Abdominal discomfort	5 - 10
Nervous system disorders	
Headache	< 1
Peripheral neuropathy	< 1
General disorders	
Fatigue	< 5
Chills	< 1
Musculoskeletal disorders	
Myalgias and muscle cramps	< 1
Skin and subcutaneous tissue disorders	
Rash	< 1
Skin discoloration/irritation	< 1
Acne	< 1
Flushing	< 1
Hematologic disorders	
Anemia	2 - 4
Thrombocytopenia	2 - 4
Renal disorders	
Acute renal failure	0 – 4%
Laboratory abnormalities	
Elevated bilirubin	< 1

Table 1: Adverse reactions associated with the use of resveratrol (41, 42, 44, 45, 47, 49, 56-58). Percentages for the incidence of a particular adverse event are estimated after combining the patient numbers in clinical studies evaluating high dose resveratrol.

9.2 Risks of study procedures

Questionnaires: Participants will complete questionnaires about their health and basic information about demographics will be obtained. These procedures represent no more than minimal risk. Care will be taken to ensure confidentiality. Research records will be held in locked cabinets or locked storage rooms when staff members are not in attendance. All transmission of data to the investigators' laboratory for analysis will contain only subject's coded identification. Subjects' names or any other personal identifying information will not be used. Publication of results will involve aggregate data only so that individual participants cannot be identified. On occasion, it may be necessary, for legal reasons, or for good clinical practice, for third parties such as the IRB, to review medical records that are identified by name, but this should be an uncommon occurrence, and every effort will be made by the investigators to provide confidentiality.

Blood draws: The risks of blood draws include bleeding, bruising, discomfort, fainting and lightheadedness infection or pain at the needle site. All research personnel drawing blood are trained in the procedure and are familiar with proper infection control protocol.

Pulmonary function tests: In general, PFTs involve minimum risks, which will be reviewed with the subject prior to performing the testing. In general, these tests are well tolerated but occasionally some people may experience temporary tiredness, mild chest tightening, and coughing. Short acting bronchodilators such as albuterol will be available and will be administered if needed. Subjects with LAM are routinely evaluated with breathing tests as a matter of clinical care.

9.3 Adequacy of protection against risks

Each participant in this study will be informed of the intent of the study and asked to sign an IRB approved informed consent form. The consent form will fully describe the procedures, risks, alternatives, and potential benefits of the study. Consent will be obtained from eligible participants by a physician investigator or by the study research coordinator. The participants will be given the opportunity to refuse to participate in the study, under the assurance that such refusal will in no way affect their regular treatment. All questions asked by study subject will be answered to the best of the knowledge of the investigator and the study staff. Time will be given for the patient to read the consent form and ask questions prior to performing any study procedures. A copy of the signed and dated consent form will be given to subject prior to leaving the clinic. There are no alternatives in this study other than not to participate. Potential risks and benefits will be thoroughly explained to the subjects participating and they are free to withdraw from these studies at any point in time prior to or during the course of these investigations.

All information, data, etc., collected as a result of these studies is considered confidential and will be released only as required by law. Information published as a result of this study will be protected completely preserving the anonymity of the participants. Per HIPAA guidelines, research records will be stored in a locked room identified specifically as a patient record room. All transmitting of data is done by coded names and does not identify any types of names (first or last). Results of any procedures or tests will also be de-identified so as to hide the true identity of patient's names or any other identifiers. On occasion, it may be necessary, for legal reasons, or for good clinical practice, for third parties such as the IRB, FDA, or DSMB members to review medical records that are identified by name. This however would be on special occasions and would not be a common occurrence. Every effort in all areas of care will be made to keep the patient's information as confidential as possible. As noted above, we will minimize risks to human subjects by creating and maintaining a standard of excellence such as we had with our previous clinical trials in our sites. Such standard includes active interactions between faculty and staff on a weekly formal basis, and as needed otherwise, careful attention to training of staff in procedures and their possible complications, and a philosophy of 'patient first' in all of our dealings. Specifically, we use only trained personnel to perform procedures. The plan to handle adverse events is described in detail in the section of data and safety monitoring plan.

9.4 Plans for assuring compliance with safety guidelines and regulations

All clinical staff will be required to participate in training offered through their respective Institutional Review Boards. Training is also required in Health Insurance Portability and Accountability Act (HIPAA) in order to have access to study subject records. International Conference of Harmonization (ICH) Good Clinical Practice Guidelines (GCPs) are utilized daily in the clinical research practice and are reviewed periodically. The clinical research teams will have twice a month conference calls to provide an opportunity for staff to review and discuss the activities of the study. All clinical and laboratory staff are required to take courses handling blood borne pathogens on a yearly basis as well as training in laboratory safety, handling of biohazards, storage and shipping of clinical and laboratory specimens including dangerous goods.

10. Benefits

10.1 Benefits of the investigational agent

As with other studies investigating novel treatment for LAM, there may or may not be any benefits for the subject participating in the study. However, participation in this study will provide additional data on the safety and effectiveness of resveratrol for the treatment of LAM. Some participants in this study may benefit from the general evaluation of their disease from a qualified specialist for the duration of their participation in the trial. The participants may benefit from the physical exams, pulmonary function tests, and other study procedures. The information learned from this research study may benefit other subjects with LAM in the future. Subjects who participate in the study will receive all study medications at no charge, will have all testing at no cost to them or their insurance carrier (and the results of those tests will be made available to their primary care physician).

10.2 Importance of the knowledge to be gained

In general, the knowledge gained from this study may benefit many subjects with LAM. The information obtained may improve the scientific community's understanding of the management of LAM. Specifically, the study will identify the safety and efficacy of the combination of resveratrol and sirolimus in LAM and thus may fundamentally alter the current clinical approach to patients with LAM.

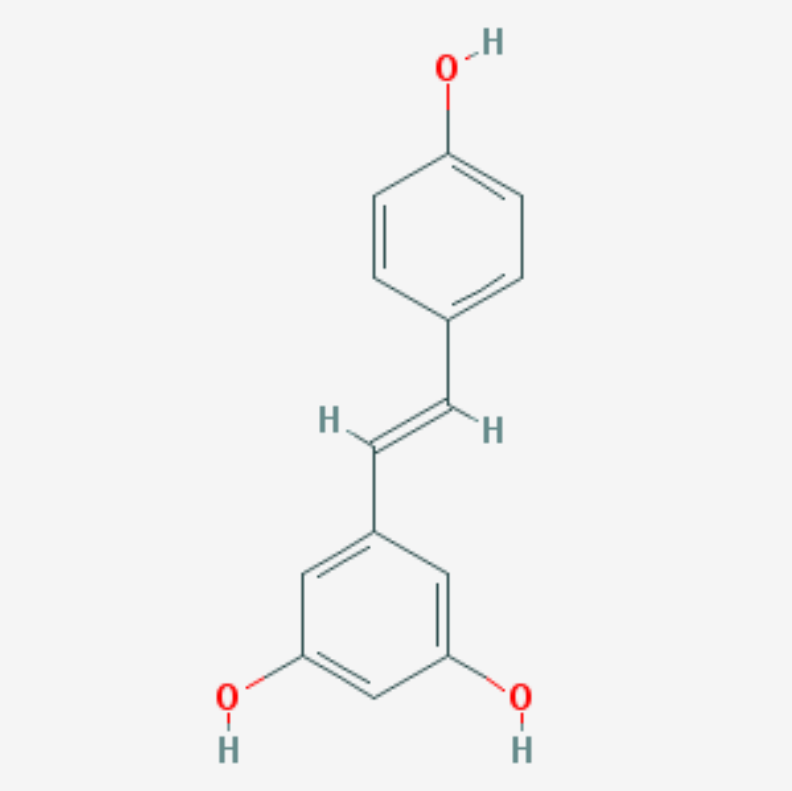
10.3 Benefits of Study Procedures

None.

11. Drug and Drug Procurement:

11.1 Resveratrol:

Resveratrol (3,5,4'-trihydroxystilbene) is a polyphenolic phytoalexin. It is a stilbenoid, a derivate of stilbene, and is produced in plants with the help of the enzyme stilbene synthase. It exists as two structural isomers: cis-(Z) and trans-(E). The molecular formula for resveratrol is C14H12O3 and its molecular weight is 228.24 g/mol. The structural formula of resveratrol is:

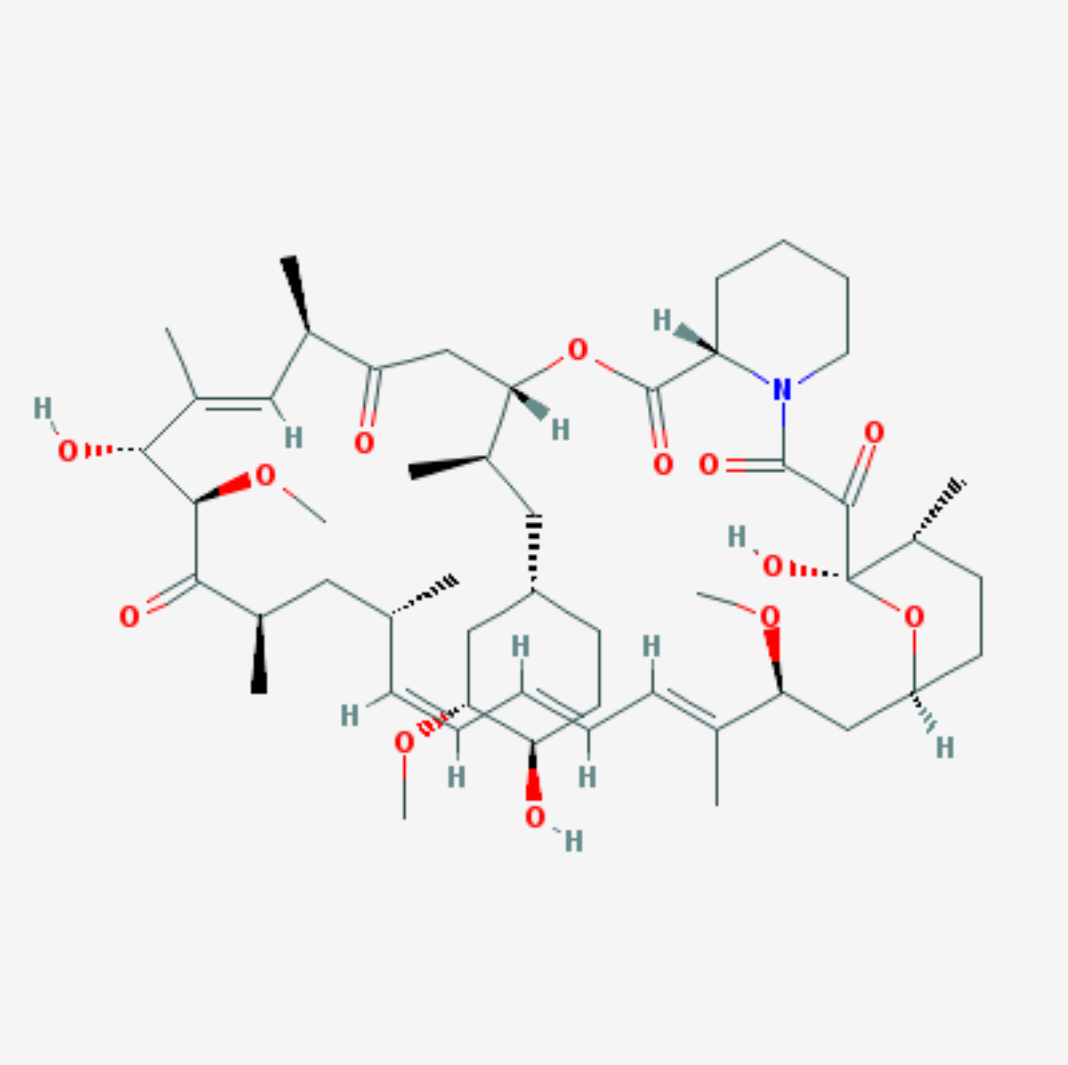


Resveratrol used in this study is manufactured by fermentation using genetically modified yeast in a process that results in *trans*-resveratrol with a purity of at least 98%. The product is free from DNA, proteins and cells from the production host. Resveratrol is available in the form of an off white powder that is highly lipophilic, and almost insoluble in water.

Resveratrol is available as white nº0 opaque capsules containing 125mg/capsule of resveratrol. The excipients present in the capsules are cellulose and magnesium stearate. Resveratrol capsules come in white plastic containers containing 200 capsules per container. Containers are to be stored at room temperature for a maximum of 2 years after encapsulation.

11.2 Sirolimus:

Sirolimus is an immunosuppressive agent. Sirolimus is a macrocyclic lactone produced by Streptomyces hygroscopicus. The chemical name of sirolimus (also known as rapamycin) is (3S,6R ,7E,9R ,10R,12R ,14S,15E ,17E,19E ,21S,23S ,26R,27R ,34aS)-9,10,12,13,14,21,22,23,24,25,26,27,32,33,34, 34a-hexadecahydro-9,27-dihydroxy-3-[(1R)-2-[(1S,3R,4R)-4-hydroxy-3-methoxycyclohexyl]-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3H-pyrido[2,1-c][1,4] oxaazacyclohentriacontine-1,5,11,28,29 (4H,6H ,31H)-pentone. Its molecular formula is C51H79NO13 and its molecular weight is 914.2. The structural formula of sirolimus is:



Sirolimus is a white to off-white powder and is insoluble in water, but freely soluble in benzyl alcohol, chloroform, acetone, and acetonitrile.

Sirolimus is available for administration as an oral solution containing 1 mg/mL sirolimus. Sirolimus is also available as a white, triangular-shaped tablet containing 1 mg sirolimus, and as a yellow to beige triangular-shaped tablet containing 2 mg sirolimus. The inactive ingredients in sirolimus Oral Solution are Phosal 50 PG® (phosphatidylcholine, propylene glycol, mono- and di-glycerides, ethanol, soy fatty acids, and ascorbyl palmitate) and polysorbate 80. Rapamune Oral Solution contains 1.5% - 2.5% ethanol.

The inactive ingredients in sirolimus tablets include sucrose, lactose, polyethylene glycol 8000, calcium sulfate, microcrystalline cellulose, pharmaceutical glaze, talc, titanium dioxide, magnesium stearate, povidone, poloxamer 188, polyethylene glycol 20,000, glyceryl monooleate, carnauba wax, and other ingredients. The 2 mg dosage strength also contains iron oxide yellow 10 and iron oxide brown 70.

Patients will remain on sirolimus and continue to obtain those agents in the manner already established. Sirolimus has approval from the FDA for the treatment of LAM.

11.3 Accountability of Investigational Product

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) the investigator will maintain adequate records of the disposition of the investigational product(s)/intervention material(s), including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any investigational product(s)/intervention material(s) accidentally or deliberately destroyed. Records for receipt, storage, use, and disposition will be maintained by the study site. A dispensing log will be kept current for each participant. This log will contain the identification of each participant and the date and quantity of investigational product(s)/intervention material(s) dispensed. All records regarding the disposition of the investigational product will be available for inspection by the DSMB, FDA, or IRB.

11.4 Assessment of compliance with the investigational product

Drug adherence will be assessed by pill counts. Pill counts will not be done in the presence of participants to mitigate “dumping” behavior, and therefore allow more accurate assessment of drug use. Adherence will be reinforced using standardized non-judgmental questioning and tailored behavioral strategies.

12. Modification or discontinuation of the investigational product

12.1 Modification of investigational product

The product will not be modified during the course of the study by the investigator, pharmacy or subject.

12.2 Premature discontinuation of investigational product

Refer to sections 12.3 and 13.1.

12.3 Medication adjustments

12.3.1 Dose Delays

For patients who are unable to tolerate the protocol-specified dosing schedule, dose adjustments are permitted in order to keep the patient on study drug. If administration of sirolimus must be interrupted because of unacceptable toxicity, drug dosing will be interrupted or modified according to rules described in Section 12.3.3. Sirolimus therapy may also be interrupted to accommodate surgical procedures or other invasive therapies at the discretion of treating clinicians. Major Events are non-treatment-related grade 3 and 4 pulmonary and non-pulmonary toxicities. Treatment should be delayed for major events if resveratrol may further complicate the non-treatment related event. If a major event requires a delay of treatment, treatment must be delayed until toxicity is resolved (≤ Grade 1 or ≤ Baseline). For treatment-related toxicities and major events, if toxicity is not resolved in ≤ 4 weeks, patient will be taken off the treatment that is most likely to be related to the toxicity. While this most likely will result in withdrawal from study drug exposure at the investigator's discretion patients may continue on single agent therapy, if toxicities are ≤ grade 2 and there is some evidence of clinical benefit to the patient.

Toxicity	**Dose reduce first**	**Dose reduce second**
Nausea	Resveratrol	Sirolimus
Diarrhea	Resveratrol	Sirolimus
Abdominal pain	Resveratrol	Sirolimus
Infections	Sirolimus	Resveratrol
Headache	Resveratrol	Sirolimus

Table 2: Approach to overlapping toxicities of sirolimus and resveratrol. The above table is a suggestion. Final decision on the order of dose reduction will be at the discretion of the investigator.

12.3.2 Resveratrol dose reduction

Any AE of ≥ Grade 3 and attributed as possibly, probably or definitely related to resveratrol will result in the dose being held until the AE has resolved to ≤ grade 1 or baseline, while sirolimus dosing may continue uninterrupted. If the AE resolves, reinstitution of treatment can occur but at a reduced dose as described in Table 3. If the AE recurs at the reduced dose, treatment will be held until the AE has resolved to ≤ grade 1 and when resolved treatment can be reinstituted at the next lower dose level. No more than 2 dose reductions are allowed during the maintenance cycles. Participants who are unable to tolerate the lowest dosage of resveratrol will be withdrawn from study drug exposure.

Toxicities that may be attributable to resveratrol include nausea, vomiting, abdominal pain, diarrhea, headache, and fatigue. If any of these AEs occur at grade ≤ 2, resveratrol may be continued and the AE managed with supportive care. For any AE with a grade ≥ 3, the rules outlined in Sections 14.2 – 14.10 apply for holding of dose, dose reduction, removal from study and reporting requirements.

Dose	**Reduce to**
250 mg daily	Hold or withdrawal from resveratrol exposure
500 mg daily	250 mg daily
1000 mg daily	500 mg daily

Table 3: Dose reduction schema for resveratrol

12.3.3 Sirolimus dose reduction

Known sirolimus toxicities will not be attributed to resveratrol but will only be attributed to sirolimus and may result in sirolimus dose modifications, at the discretion of the treating physician. Sirolimus trough levels will be checked at 2-, 4-, 8-, 12-, 16-, 18-, 20-, and 24- week intervals following study drug initiation. Sirolimus dose will be reduced if the serum trough level increases to a value greater than 10ng/ml. If sirolimus dose is reduced based on a high trough value, the trough level will be repeated 2 weeks after dose reduction. This process will be repeated until a sirolimus trough value less than 10ng/ml is achieved. The exact dose reduction scheme for sirolimus will be left at the discretion of the treating physician. Sirolimus toxicity monitoring labs will be performed as clinically indicated at the discretion of the physician. For severe/non-resolving toxicities, the treating physician may decide to stop sirolimus. Resveratrol may be continued as a single agent after sirolimus has been stopped. All reasonable efforts will be made to avoid sirolimus dose changes throughout the study duration, as long as it does not impact clinical patient care. A notification letter will be sent to the patient's primary pulmonologist about the patient's participation in the study and urging them to avoid sirolimus dose changes throughout the study duration, unless absolutely clinically indicated.

12.3.4 Concomitant medications

All concomitant medications including prescription and over-the-counter preparations taken during the course of the study will be documented on a CRF including start and stop dates, dose, and indication for use.

12.3.5 Prohibited medications

Participants will not be allowed to receive any concomitant medication(s) that may interfere in the interpretation of data or the subject's participation in the study in the Investigator's opinion.

Metabolism of sirolimus occurs in the liver and the small intestine by the CYP3A4 family of enzymes. Thus, concomitant administration of sirolimus with other CYP3A substrates or inducers can alter the oral bioavailability of sirolimus (59). Although not a strong modulator of CYP activity, resveratrol has been reported to inhibit the activity of CYP3A4 *in vitro* and *in vivo* (60). Thus, co-administration of sirolimus and resveratrol has the potential to increase the blood levels of sirolimus or lead to an increase in the side effects of sirolimus. We will periodically monitor the sirolimus level during the study, as well as monitor closely for any adverse effects related to the study medications.

Because of the potential interaction with other drugs involving CYP3A4 metabolic pathways, strong inhibitors and strong inducers of CYP3A4 will not be allowed to be used concomitantly with the study medication. A washout period of 14 days will be required after discontinuation of the below listed medications before beginning the study medication. A list of the prohibited medications is provided in Table 4 below:

List of prohibited medications for this study
Strong CYP inducers
Barbiturates
Carbamazepine
Phenytoin
Rifampin, Rifabutin
St. John's Wort
Strong CYP inhibitors
Ketoconazole
Voriconazole
Itraconazole
Nefazodone
Clarithromycin
Erythromycin
Telithromycin
Gemfibrozil
Grapefruit juice

Table 4: List of prohibited medications for this study

Concomitant use of resveratrol with drugs with a narrow therapeutic range that are CYP3A, CYP1A2, CYP2D6, or CYP2C9 substrates (for example, theophylline,

tizanidine, warfarin, phenytoin, alfentanil, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, cyclosporine, tacrolimus, thioridazine) will be prohibited from Day 1 until the end of study.

13. Procedures

13.1 Stopping rules

13.1.1 Study discontinuation

The DSMB, FDA, and IRB have the authority to stop or suspend this trial at any time. This study may be suspended or closed if:

- Early stopping rules have been met
- The study objectives have been met
- The study chair/investigators believe it is not safe for the study to continue
- The DSMB suspends or closes the trial
- The FDA suspends or closes the trial

13.1.2 Subject discontinuation

An intent-to-treat approach will be used. All data acquired prior to termination for the reasons outlined below will be included in the primary analysis unless patient withdraws consent. Every effort will be made to conduct a final study visit with the participant and participants will be followed clinically until, if applicable, all adverse events attributable to study drug resolve. Participants may be terminated early from the study for the following reasons:

- Withdrawal of consent
- Withdrawal by the participant
- Withdrawal by the investigator
- Intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease (e.g. mental status change, large pleural effusion etc.)

13.1.3 Study early stopping rules

Study enrollment will be suspended pending expedited review of all pertinent data by the IRB and the DSMB if any one of the following occurs:

- Death from any cause in any subject believed to be related to study drug
- Multiple medically similar serious adverse events related to study medication
- Events that, in the opinion of the Principal Investigator contraindicate further dosing of additional subjects

If one of the above-listed stopping rules are met, a prompt cumulative review of safety data and of the circumstances of the event(s) in question will be conducted to determine whether study entry should be resumed, whether the protocol will be modified, or whether the study will be discontinued permanently. Review and approval by the DSMB is required for resumption of the study in the event the study is interrupted because of one of the above-listed events.

13.1.4 Individual early stopping rules

A study participant will be terminated from the study if any of the following occurs during the study:

- Recurrent or persistent resveratrol-related toxicity unresponsive to treatment in spite of dose adjustments.
- Pregnancy or failure to comply with adequate contraception
- The participant refuses to continue in the study
- Continued non-compliance
- The investigator determines that the participant should be withdrawn from the study for reasons not listed above

A subject will be considered evaluable if they receive treatment for at least 8 weeks and the relevant information collected during this period. Subjects who are withdrawn after less than 8 weeks of treatment will be considered as pre-mature discontinuation, and will be replaced by another participant.

Every effort will be made to obtain endpoint evaluations for subjects who withdraw or who discontinue prematurely from the study. The same evaluations performed at final visit will be completed at the time of termination. If withdrawal is due to inability to tolerate investigational drug or other untoward event related to investigational drug, the withdrawal will be noted in data analysis as treatment failure.

13.2 Follow-up after early study termination

Participants who are prematurely withdrawn from study drug exposure due to adverse events will be followed to monitor safety for a minimum of 30 days or until resolution or stabilization of the disqualifying event or until the Principal Investigator determines that further follow-up is not needed.

Subjects considered “lost-to-follow-up” are those enrolled in the study, but, for whatever reason, refuse to return to complete the study per the protocol. Reasonable efforts will be made to contact any subject lost to follow-up during the course of the study in order to complete assessments and retrieve any outstanding data, study drugs or clinical supplies. The investigator must make at least three documented attempts to have the patient return for study withdrawal evaluations.

13.3 Participant replacement

Participants with early termination from this study will be replaced if they are deemed non-evaluable.

14. Data and Safety Monitoring Plan

The study protocol will be reviewed and approved by the DSMB and University of Cincinnati IRB prior to study initiation. Participant enrollment may only begin with IRB approved consent forms. This is an interventional exploratory phase II study.

14.1 Study Oversight

The Principal Investigator has primary oversight responsibility of this clinical trial. The DSMB has oversight responsibility of the Data Safety Monitoring Plan (DSMP) for this clinical trial. The DSMB will review accrual, patterns and frequencies of all adverse events, and protocol compliance every 6 months. The DSMB makes recommendations to the PI regarding the continuation status of the protocol.

The site Principal Investigator and their research team (co-Investigators, research nurses, clinical trial coordinators, and data managers) are responsible for identifying adverse events. Aggregate report - detailed by severity, attribution (expected or unexpected), and relationship to the study drug/study procedures – will be available for site review.

14.2 Adverse Events

This section defines the types of adverse events and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting and ICH E6: Guideline for Good Clinical Practice, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events version V 4.0. These criteria have been reviewed by the study investigators and have been determined appropriate for this study population.

14.3 Definitions

14.3.1 Adverse events

An adverse event (AE) is any occurrence or worsening of an undesirable or unintended sign, symptom, laboratory finding, or disease that is experienced during participation in the trial. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a medicinal (investigational) Study Agent(s) or a study procedure, whether or not related to the medicinal (investigational) Study Agent(s) or study procedure. Any medical condition that is present at the time that the subject is screened will be considered as baseline and not recorded as an AE. However, if the condition deteriorates or changes in severity at any time during the study it will be recorded and reported as an AE.

14.3.2 Suspected adverse reaction and adverse reaction

Suspected adverse reaction (SAR) means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, ‘reasonable possibility’ means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

An adverse reaction (AR) means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there are reasons to conclude that the drug caused the event.

14.3.3 Adverse events associated with study procedures

The following clinical situations will be considered to be outside of the normal range of findings and will be recorded as Adverse Events. These situations do not limit the principal investigator from reporting any other events, associated or not with these procedures, from being recorded and reported as AEs.

Blood draws

- Fainting/vasovagal events
- Bruising at puncture site greater than 2 cm in diameter
- Bleeding from puncture site lasting more than 5 minutes
- Swelling at puncture site larger than 2 cm

Pulmonary function testing

- Wheezing or bronchoconstriction requiring treatment with bronchodilators within 30 minutes from the procedure
- Coughing requiring treatment with bronchodilators within 30 minutes from the procedure

Adverse events related to study medication will be tabulated by body system and by severity using the NCI CTCAE v4.0.

14.3.4 Serious Adverse Event (SAE)

An AE or SAR is considered "serious" if it results in any of the following outcomes (21 CFR 312.32(a):

- Death: A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up.
- A life-threatening event: An adverse event or suspected adverse reaction is considered "life-threatening" if, in the opinion of the investigator, its occurrence places the subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.
- An inpatient hospitalization or prolongation of existing hospitalization.
- Persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, in the opinion of the investigator, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
- Congenital anomaly or birth defect.

Regardless of the relationship of the adverse event to the study, the event will be reported per Section 14.3.4 as an SAE if it meets any of the above definitions.

14.3.5 Expected adverse events

This section lists those adverse events regarded as expected for resveratrol:

Gastrointestinal: Nausea, vomiting, diarrhea, abdominal discomfort, flatulence
General: Fatigue
Hematological: Anemia, thrombocytopenia
Renal: Acute kidney injury
Liver: Increased serum bilirubin
Infectious: Increased risk of infections

14.3.6 Unexpected adverse event

An adverse event or suspected adverse reaction is considered "unexpected" when its nature, severity or frequency is not consistent with the information that is provided with the information in the protocol.

14.4 Collecting, recording and managing adverse events

14.4.1 Identifying adverse events

Any adverse event that occurs from the moment the subject has signed the consent form will be recorded and is reportable. Collection time for AEs and SAEs will begin after the screening visit when the consent form is signed and will continue until the end of the treatment.

Adverse events may be discovered through any of these methods:

- Observing the participant
- Questioning the participant, with standardized questions/procedures.
- Receiving an unsolicited complaint from the participant.
- An abnormal value or result from a clinical or laboratory evaluation can also indicate an adverse event when determined to be clinically significant by the Principal Investigator.

14.4.2 Recording AEs

Throughout the study all identified adverse events (serious and non-serious) will be recorded on all appropriate source document and adverse event case report forms regardless of their severity or relation to the study. A complete description of all adverse events will include event description, time of onset, investigator assessment of severity, relationship to study agent or procedures, time of resolution/stabilization of the event, expectedness, determination of whether the AE qualifies as a SAE, and action taken. A change in the severity of the AE will also be documented. Assessment of severity and relationship will be documented on the source documents or the on the CRF.

14.4.3 Recording SAEs

Serious adverse events will be recorded on the serious adverse event case report form and will include a narrative of the event signed and dated by the Principal Investigator.

Adverse event reporting timeline

- Within **24 hours** (of learning of the event), investigators must report any reportable Serious Adverse Event (SAE) that:
    - Is considered life-threatening/disabling or results in death of subject

    -OR-

    - Is unexpected/unanticipated
- Investigators must report all other reportable SAEs within **5 working days** (of learning of the event).
- All other (suspected) reportable AEs must be reported to the DSMB and the IRB within **20 working days** of the notification of the event or of the investigators becoming aware of the event.

14.5 Managing adverse events

The study investigator must apply his or her clinical judgment as to whether an AE is of sufficient severity to require that the participant immediately be removed from further receiving the study agent under the protocol. The investigator must institute any necessary medical therapy to protect a participant from any immediate dangers.

An adverse event will be followed until any of the following takes place: a) it is resolved, b) participant is stable, c) a minimum of 30 days after participant is terminated from the study and the Principal Investigator determines that follow-up is no longer needed.

If an abnormal value or result from a clinical or laboratory evaluation is determined to be an AE, then the evaluation that produced the value or result can be repeated until the value or result returns to normal, or the result can be explained, or the usual standard of care does not require further follow-up, and the participant's safety is not at risk.

Non-serious expected adverse events will be submitted to the DSMB in a timely fashion. The events will be presented in tabular form and given to the DSMB on a bi-annual basis. Study investigators are also required to fulfill all reporting requirements of their local institutions.

14.6 Grading and Attribution

14.6.1 Grading Criteria

In addition to determining whether an adverse event fulfills criteria for a serious adverse event or not, the severity of adverse events experienced by study participants will be graded according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events Version V4.0. This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events. All adverse events whether or not listed in the NCI-CTCAE will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual (A semi-colon indicates 'or' within the description of the grade):

Grade 1 = Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 = Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADLs (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc).

Grade 3 = Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADLs (bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).

Grade 4 = Life-threatening consequences; or urgent intervention indicated.

Grade 5 = Death related to AE.

14.7 Definition of Attribution

The attribution, of an adverse event to the study will be determined by the Principal Investigator or designated physician co/sub-investigator. The Principal Investigator or designee will record the determination of attribution on the appropriate adverse event or serious adverse event form. The attribution of an adverse event to the investigational drug or to a study procedure will be determined using the descriptors in the following table. For the purpose of this study, in addition to all study medications, the following procedures will be considered when determining attribution:

Attribution of adverse events

Code	Descriptor	Definition (guidelines)
UNRELATED CATEGORY		
1	Unrelated	The adverse event is clearly not related to study. The event is completely related to an etiology other than the study product or study intervention (the alternative etiology must be documented in the study subject's medical record)
2	Unlikely	The adverse event is doubtfully related to study and likely to be related to factors other than study product or study intervention.
RELATED CATEGORIES		
3	Possible	The adverse event may be related to study. There is an association between the event and the administration of study product and there is a plausible mechanism for the event to be related to the study product; there may be also an alternative etiology, such as characteristics of the subject's clinical status and/or underlying disease
4	Probable	The adverse event is likely related to study. There is (1) an association between the event and the administration of study product or study intervention, (2) a plausible mechanism for the event to be related to the study product, and (3) the event could not be reasonably explained by known characteristics of the subject's clinical status and or an alternative etiology is not apparent
5	Definite	The adverse event is clearly related to study. There is (1) an association between the event and the administration of the study product or study intervention, (2) a plausible mechanism for the event to be related to the related to the study product, and (3) causes other than the study product have been ruled out and/or the event re-appeared on re-exposure to the study product

(For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE website: http://ctep.cancer.gov/reporting/ctc.html) will be consulted.)

In a clinical trial, the study product\intervention will always be suspect when attributing an AE and the "unrelated" attribution will be used only when there is an undisputable or likely alternative explanation for the AE.

14.8 SAE Reporting Criteria and Procedures

The Principal Investigator will be notified by the study staff as soon as a study staff member becomes aware of the SAE. In the absence of the Principal Investigator, a physician sub-investigator will be notified.

The Principal Investigator determines causality (definitely not related, probably not related, possibly related, probably related, definitely related) of the adverse event. The DSMB Chair may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the adverse event. A back-up notification system will be instituted so that any delays in review by the Chair beyond a specified period of time are forwarded to a secondary reviewer.

14.8.1 Unexpected, non-serious adverse events

An unexpected, non-serious adverse event that is of Grade 3 severity or higher and study related will be recorded and reported under the serious adverse event reporting procedure (i.e. within 24 hours).

14.8.2 Notifying the FDA

The IND sponsor (The PI) is responsible for FDA safety submissions as follows: The IND Sponsor for this study will handle all regulatory communications with the Food and Drug Administration and will be responsible to update the IND to include this trial, file all regulatory related issues including any MEDWATCH reports which will be reviewed and approved by the PI and Steering Committee. The IND sponsor will be responsible for submission of yearly reports and final report for this study. All DSMB recommendations will be communicated to the IND sponsor in a timely fashion.

The following process for reporting a serious adverse event ensures compliance with the ICH guidelines, 21CFR §312.32.

- Expedited safety report to the FDA applies if the adverse event is classified as one of the following:
    - Serious and unexpected suspected adverse reaction (SUSAR) (Sections 14.3.4, 14.3.6, and 14.7) (i.e. serious, unexpected, and related)

      Or

    - Aggregate analysis of adverse events that suggest a causal relationship to the study medications

      Or

    - Any findings from clinical, epidemiological, pooled analysis of data pooled across multiple studies, published or unpublished scientific papers or any findings from animal or in vitro testing that would result in a safety-related change in the protocol, informed consent, investigator

brochure or other aspects of the overall conduct of the trial will be reported.

Expedited Safety Reports must be reported by the IND Sponsor to the FDA within 15 calendar days; fatal or immediately life-threatening serious, unexpected, suspected adverse reactions must be reported within 7 calendar days. SAEs that do not strictly fit the above criteria may be reported to the FDA in an expedited manner if the IND Sponsor chose to do so. Each 7-day report must be followed up by a 15-day report.

The following types of SAEs will be reported in the IND Annual Report:

- Serious, expected, suspected adverse reactions
- Serious but not a suspected adverse reaction

For standard reporting, the IND Sponsor or designee will file the IND Annual Report. All adverse events (not just those requiring 24 hour reporting) will be reported in the Annual IND Report.

14.8.3 Notifying the Data and Safety Monitoring Board

The PI will submit all safety reports on an ongoing basis to the DSMB. Individual or clusters of SAEs may be reported expeditiously to the DSMB upon determination.

14.8.4 Notifying the Institutional Review Board

The Principal Investigator will ensure the timely dissemination of SAE information, including expedited reports, to the IRB in accordance with IRB regulations and guidelines.

14.9 Reporting Pregnancy

A research subject will be terminated from the study due to pregnancy. The principal investigator will be informed immediately of any pregnancy and will report all pregnancies within 24 hours of becoming aware of the event to DSMB utilizing the SAE report form. This report is for tracking purposes only. All pregnancies that are identified during the study will be followed to conclusion and the outcome of each will be reported. The site principal investigator will discuss with the participant and/or the treating physician the known possible risks of the investigational product(s) on the fetus. Monitoring of the participant will continue until the conclusion of the pregnancy, and a follow-up SAE report form detailing the outcome of the pregnancy will be submitted to the DSMB.

14.10 Non-serious adverse events (NSAEs) reporting

14.10.1 Notifying the data and safety monitoring board

The DSMB will be notified of non-serious adverse events during their regularly scheduled meetings. The events will be presented to the Board in tabular format.

14.10.2 Notifying the FDA

The IND sponsor (the Study Chair) will file all adverse events per 21 CFR 312.32, whether expedited or part of the IND Annual Report. The IND sponsor will be responsible for compiling the IND Annual Report.

14.10.3 Notifying the Institutional Review Board

The Principal Investigator will ensure the timely dissemination of AE information to the IRB in accordance with applicable regulations and guidelines.

14.10.4 Unanticipated Problems

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Incidents or events that meet the OHRP criteria for unanticipated problems will be reported to the DSMB and IRB, in the following time frames:

1. Unanticipated problems that are serious adverse events should be reported to the IRB within 1 week of the investigator becoming aware of the event.

2. Any other unanticipated problem should be reported to the IRB according to the IRB's reporting policies.

14.11 Data Management

All study data will be collected via systems created by using RedCap (an online data management software) and will comply with all applicable guidelines regarding patient confidentiality and data integrity.

14.11.1 Registration

Registration of participants on this protocol will employ an interactive data system in which the clinical site (University of Cincinnati) will attest to the participant's eligibility as per protocol criteria and obtain appropriate informed consent. IRB approval for the protocol must be obtained before accrual can occur from the clinical site.

Each participant enrolled will be assigned a local identifier by the enrollment site. This

number can be a combination of the site identifier (location code) and a serial accession number. Only the registering site will have access to the linkage between this number and the personal identifier of the subject.

14.11.2 Data Entry

Data collection for this study will be accomplished with a combination of paper as well as online electronic case report forms. Using encrypted communication links, on-line forms will be developed that contain the requisite data fields.

14.11.3 Data Quality and Monitoring Measures

As much as possible data quality is assessed at the data entry point using intelligent on-line data entry via visual basic designed screen forms. Data element constraints, whether independent range and/or format limitations or 'relative' referential integrity limitations, can be enforced by all methods employed for data input. QA reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency.

The DSMB will review data quality on an ongoing basis. A risk-based monitoring plan will be developed to support the study. The purposes of study monitoring are to ensure the safety and welfare of study participants and to confirm the accuracy of study data.

An onsite monitoring visit will occur once during the conduct of the trial, with the option of making a second visit if determined by Principal Investigator to be needed, to address enrollment, or protocol deviation issues. The Monitoring Plan will detail the frequency and level of intensity of on-site monitoring visits. In general, the study will be monitored for all participants at a level of 100% of study data gathered for inclusion and exclusion criteria, informed consent procedures, adverse events and unanticipated problems. The study monitor(s) will collaborate with the Principal Investigator to conduct remedial site training to ensure compliance with GCP and all regulations.

During scheduled interim remote monitoring visits, the clinical monitors will verify that the protocol is being followed and that data are being collected according to protocol requirements. The clinical monitor(s) will review the Study Regulatory File to determine that all required documentation is being collected and that the IRB approval for the site is current. The monitor will then verify that each participant has signed the correct version of the informed consent document, and that this document is filed in the participant's source documents. At each visit, the monitor will perform an audit of the source documents in the subject's binder by checking them against the database. Adverse event documentation will be checked for completeness and accuracy. At the study closeout, the monitor(s) will confirm that all data have been reviewed, all source documents have been verified, and all required documents are present in the Study Regulatory File.

Protocol deviations and unanticipated problems will be reviewed by the Principal Investigator, and the regulatory and monitoring staff to determine the need for corrective and preventative action (CAPA). In the event that corrective and/or preventative action is

warranted, a root cause analysis will be performed and a CAPA will be completed as per institutional policy. The Principal Investigator is responsible for communicating the CAPA plan to appropriate staff (i.e. Site Investigator, study team) and obtaining appropriate signatures to address the issues at hand. The Principal Investigator will work with regulatory and monitoring staff to insure that the CAPA is followed and is effective in resolving the issue(s). Protocol deviations and unanticipated problems will be reported to the IRB as per institutional policy.

The table below describes the variables to be reviewed during monitoring visits.

Variable	**% of Records Reviewed**
Informed consents	100%
Eligibility criteria for all screened subjects	100%
Adverse events	100%
Protocol adherence	20% of active subjects
Verification of REDCap with source documents	20% of active subjects
Central study files-inclusion of all applicable documents	100%
Protocol deviations/violations	100%

14.12 Protocol Deviations

The practices below are consistent with Good Clinical Practice (GCP ICH E6) Sections:

- Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- Quality Assurance and Quality Control, section 5.1.1
- Noncompliance sections 5.20.1, and 5.20.2

14.12.1 Protocol deviation Definition

14.12.1.1 Protocol Deviation - Any change, divergence, or departure from the study design or procedures of a research protocol that affects the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data constitutes a protocol violation. Changes or alterations in the conduct of the trial which do not have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data are considered non-major protocol deviations. Site Principal Investigator is responsible for reporting protocol deviations to the IRB using the standard reporting form. As a result of deviations, corrective actions are to be developed

by the site and implemented promptly.

14.12.1.2 Major Protocol Deviation (Protocol Violation) **-** A protocol violation is a deviation from the IRB approved protocol that may affect the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data.

If the deviation meets any of the following criteria, it is considered a major protocol deviation. However, the lists of deviation examples that follow are not exhaustive.

- The deviation has harmed or posed a significant or substantive risk of harm to the research subject.

Examples:

1. A research subject received the wrong treatment or incorrect dose.
2. A research subject met withdrawal criteria during the study but was not withdrawn
3. A research subject received an excluded concomitant medication.

- The deviation compromises the scientific integrity of the data collected for the study.

Examples:

1. A research subject was enrolled but does not meet the protocol's eligibility criteria.
2. Changing the protocol without prior IRB approval, except for modification to ensure the safety of study participants.
3. Inadvertent loss of samples or data.

- The deviation is a willful or knowing breach of human subject protection regulations, policies, or procedures on the part of the investigator(s).

Examples:

1. Working under an expired professional license or certification.
2. Failure to follow federal and/or local regulations.
3. Repeated minor deviations.

- The deviation is inconsistent with the NIH Human Research Protection Program’s research, medical, and ethical principles.

Examples:

1. A breach of confidentiality.
2. Inadequate or improper informed consent procedure.

14.12.1.3 Non-Major Protocol Deviation - A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that has not been approved by the IRB and which DOES NOT have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

14.12.2 Reporting Protocol Deviations

Deviations from the protocol are not allowed. It is the responsibility of the study site to use continuous vigilance to identify and report any protocol deviations. Upon determination that a protocol deviation has occurred, the study staff will a) notify the Principal Investigator, b) notify Project Manager and c) complete the Protocol Deviation form. The Principal Investigator will complete and sign the Protocol Deviation form and submit it to the IRB, per IRB regulations. Major protocol deviations will be reported to the DSMB at the regular DSMB reporting interval. The IND sponsor (PI) will also be responsible for notifying the FDA.

15. Statistical considerations:

Sample size determination: As mentioned previously, the mean serum VEGF-D level prior to treatment was approximately 2,000pg/ml in both the MILES trial (12) as well as the everolimus trial (18). In both studies, treatment with mTOR inhibitors resulted in an almost 50% reduction in the serum VEGF-D levels (12, 18). Since patients in our study will be on sirolimus prior to enrolment, we estimate that the starting serum VEGF-D level in our study will be approximately 1,000pg/ml. The largest change between serial VEGF-D levels in the placebo group of MILES was 42%. Thus, VEGF-D responders in MILES trial were conservatively defined as patients in whom serum VEGF-D decreased to a value greater than 42% from its baseline (16). Accordingly, we made our power calculations based on the ability to show a greater than or equal to 42% change in serum VEGF-D levels. Assuming the correlation between the baseline and subsequent VEGF-D levels is 0.7, a sample size of 20 subjects will provide 80% power to detect a mean VEGF-D difference from baseline to post-treatment of 420pg/ml or higher with the level of significant of 0.05 and a standard deviation of 800 for the difference. Allowing for a 20% dropout rate, we expect to recruit 25 patients for our study. Sample size calculations were done using SAS version 9.4.

Data Analysis: The analysis will be based on an intention to treat design. All subjects that receive study drug will be included in the safety and efficacy analysis set. The primary analysis will occur after the last enrolled participant has completed the 24-week visit. All analyses will be performed using SAS version 9.4. A p-value less than 0.05 will be considered as statistically significant.

Specific Aim #1: To determine if combined resveratrol and sirolimus leads to reduction in serum VEGF-D compared to VEGF-D reduction on sirolimus alone. Paired t-test will be performed to detect the difference of serum VEGF-D after 24 weeks of treatment with the serum VEGF-D prior to enrolment. The response variable VEGF-D will be measured repeatedly, at baseline and at weeks 8, 16, and 24. The change from baseline to the other study time points will indicate the treatment effect with resveratrol and sirolimus. Initially, by using the paired t-test we will test whether these effects are statistically significant. Multivariate linear regression models will be used to assess the relationship between serum VEGF-D change and the covariates including patient's clinical or demographic factors. Linear mixed models will be performed to detect the correlation between serum VEGF-D and the covariates taking into account the correlation between repeated measurements within the same patient. A repeated measures ANOVA model and linear mixed models will be performed to compare the serum VEGF-D at follow-up visits to the baseline taking into account the correlation between repeated measurements within the same patient.

Specific Aim #2: To determine the safety and adverse effect profile of combined resveratrol and sirolimus in LAM. Subjects will be evaluable for safety if they have received at least one dose of the study drug. The frequency, severity and duration of all adverse events, regardless of cause, will be recorded on the case report forms. The frequency and severity of adverse events will be calculated by patient, with each patient counted once using the most severe grade experienced. The duration of adverse events

will be calculated by the number of days each event persisted. Adverse events will be tabulated by body system and by severity using the NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.0, which can be obtained from the CTEP website: http://ctep.cancer.gov/reporting/ctc.html. Tables will be generated for all adverse events including those that are judged to be possibly, probably or definitely related to the drug. In order to assess the safety and adverse effect profile of resveratrol in LAM, we will assign a binary indicator variable to indicate a patient with or without adverse events. A multivariable logistic regression model will be used to assess of the effect of the covariates on the probability of having adverse events for patient with LAM. All interested factors will be adjusted in the logistic regression model. Model selection will be based on stepwise criterion when appropriate.

Specific Aim #3: To determine the effect of combined resveratrol and sirolimus on lung function and quality of life parameters in patients with LAM. Lung function parameters (FEV1 and FVC) will be measured at the baseline visit, and at every in-person visit. DLCO will be measured at the baseline visit and the last study visit. Similar analysis plan as VEGF-D will be employed for the lung function parameters. We will use the following questionnaire-based assessments to determine the quality of life in this trial: St. George's Respiratory Questionnaire (SGRQ), EuroQOL scale, San Diego Shortness of Breath scale (SD-SOB), and A Tool to Assess Quality of Life in LAM (ATAQ-LAM). Written permission has been obtained to use all these questionnaires. The scores on each of these questionnaires will be calculated at baseline, and at every in-person visit. We will compare median values at baseline and 24 weeks with the Wilcoxon signed rank test for each of these scales.

Home spirometry evaluation: The spirometry values will be expressed as mean ± SD. All spirometry maneuvers will be checked for acceptability and reproducibility as specified in the ATS/ERS criteria (38) by an expert pulmonary physiologist. The spirometry values obtained from maneuvers that do not satisfy the ATS/ERS criteria for acceptability will not used in the primary analysis. If spirometric values meet the acceptability criteria but fail to satisfy the repeatability criteria, those values will be graded for quality, but will be used in the primary analysis. Overall compliance with weekly home spirometry will be recorded and reported as a percentage. Comparison of home spirometry and office-based spirometry values will be performed using the Bland-Altman method at baseline, and at each time frame that patients get an office-based spirometry (i.e. every 8 weeks). Rate of change of FEV1 (slope) over time will be calculated by using all available values (without imputations) in a linear mixed effects model after considering the correlation of the observations within each subject, and the time between measurements will be treated as a fixed effect. In order to analyze if the trends obtained from short-term follow up with frequent home spirometry correlate with overall disease trajectory, we will compare the rate of decline obtained after 8 weeks of home spirometry to the overall rate of decline obtained after 24 weeks of follow up. A similar analysis plan will be employed for serum VEGF-D, and other biomarkers. All statistical analyses for this study will be performed using SAS, version 9.4 (SAS Institute Cary NC).

16. Identification and access to source data:

16.1 Identifying source data

The site PI will keep accurate records to ensure that the conduct of the study is fully documented. Data derived from source documents will be transferred to protocol-specific CRFs. The results of all clinical and clinical laboratory evaluations will be maintained in the participant's medical records or hospital database and the data will be transferred to clinical CRFs, as applicable.

16.2 Updating source documentation

Documents describing the safety profile of an investigational product, such as the investigator's brochure, will be amended as needed by the investigational product manufacturer to ensure that the description of safety information adequately reflects any new clinical findings.

The site PI will provide the IRB with the most up-to-date versions of the above documents as soon as the PI becomes aware of any changes. For investigational product, the PI will confirm that there are no changes to the Investigator Brochure every 3 months.

16.3 Permitting access to source data

The investigational site participating in this study (University of Cincinnati) will maintain the highest degree of confidentiality for the clinical and research information obtained from the participants in this clinical trial. Medical and research records will be maintained at the study site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigator, the investigational site will permit the study monitor, authorized representatives of the IRB, the FDA, and health authorities to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other participant data may be copied (and all personally identifying information will be removed). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to identify individuals.

16.4 Quality control and quality assurance

The PI will keep accurate records to ensure that the conduct of the study is fully documented. The investigator will ensure that all CRFs and participant study files are legible and complete for every participant.

The PI, through the use of the DSMB, will be responsible for the regular review of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data and accuracy of source documentation verification.

When the CRFs are complete, they will be reviewed and signed by the PI. All discrepancies identified by the site monitor will be reviewed, and any resulting queries will be resolved with the site PIs and the CRFs will be amended as needed.

17. Ethical considerations and compliance with good clinical practice

17.1 Statement of Compliance

This study was designed to ensure the protection of subjects according to the ethical principles of the Declaration of Helsinki and amendments concerning medical research in human subjects. This clinical study will be conducted using current good clinical practice (cGCP), as delineated in ICH, Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance, and according to the criteria specified in this study protocol. Before study initiation, the protocol, the informed consent documents, and the CRFs will be reviewed and approved by the DSMB, IRB, FDA, as well as any other appropriate health authorities. Any amendments to the protocol or to the consent materials will also be approved by the appropriate bodies listed above prior to implementation.

17.2 Informed Consent

Each subject will be provided with oral and written information describing the nature and duration of the study. Written consent must be obtained prior to any study procedures. Prospective participant must be given ample opportunity to review the informed consent and inquire about the results of the study. All participants must read, sign, and date a consent form prior to study participation. Subject will enter the date of the consent. The original, signed consent form will be retained with the study center's records, and each subject will receive a copy. The informed consent form will provide information about the study to a prospective participant to allow for an informed decision about participation in the study. We anticipate all, or at least the vast majority, of the study participants to speak and understand English. In case a participant doesn't speak or understand English well, we will use the smart form and an interpreter to obtain informed consent.

The informed consent form will be revised and receive IRB approval if and when important new safety information is available, or whenever any new information becomes available that may affect participation in the trial.

A copy of the informed consent form will be given to a prospective participant for review. The Principal Investigator or an approved designee will discuss the consent with the prospective participant and answer questions. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. The site will document details of the informed consent process within the study records.

18. Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number and these numbers rather than names will be used during collection, storage, and reporting of participant information.

Data collection for this study will be accomplished with a combination of paper as well as online electronic case report forms. Using encrypted communication links, on-line forms will be developed that contain the requisite data fields. Data will also come from EPIC notes written as part of the research encounters, as well as field data collected using specific case report forms designed for this study. Field data will be entered into the eCRF in RedCap. Electronic data collected in the field will be protected from theft using whole disk encryption. Paper data will be stored in the locked secure office of study staff according to institution policies and procedures. During the study, data will be hosted at University of Cincinnati's secure Data Center. Upon completion of the study, private data will be destroyed by certified system administrators per UC policy.

19. Publications

Publication of any data from this study must be carried out in accordance with the clinical study agreements. The results of the study will be reported in clinicaltrials.gov per current NIH regulations.

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