LAMP-1 Protocol

LAM Pilot study with imatinib mesylate

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Protocol

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Study Title: **LAM Pilot study with imatinib mesylate (LAMP-1)**

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1. INTRODUCTION

Lymphangioleiomyomatosis (LAM) is a rare and unusual cancer that involves the lungs either as part of Tuberous Sclerosis Complex (TSC) or in sporadic form[1]. The site of origin and the reason for almost exclusive female predominance remain unclear. Sporadic LAM has proven difficult to study because of the slow rate of tumor cell (LAM cell) growth with reliance on long duration treatment studies that have proven problematic in rare disease populations. Lung lesions are widely present at the time of diagnosis in all women. LAM cells line the lymphatics and spread diffusely through the lung exhibiting tiny nodules on computed tomography (CT)[2]. Lung destruction in part occurs from the secretion of matrix metalloproteinases[3] resulting in large cysts that are the clinical hallmark of this disease. Nevertheless, the rapid proliferation and growth of LAM is similar to that of other cancers, although the histologic appearance is benign[4]. The site of origin of the LAM cells remains unclear.

Vascular endothelial growth factor-D (VEGF-D) is generated by LAM cells. VEGF-D at high levels is found in no other cystic lung diseases in women and thus is a robust biomarker for LAM activity and response to treatment. In patients with LAM, sirolimus (rapamycin) was shown to stabilize lung function, reduce serum VEGF-D levels, and was associated with a reduction in symptoms and improvement in quality of life.

This pilot trial employs a dual agent design intended to generate safety and efficacy data sufficient to power and design a phase 3 study of imatinib mesylate vs placebo for Lymphangioleiomyomatosis (LAM). **The hypothesis is that imatinib mesylate will be equivalent to sirolimus (rapamycin) in short term efficacy and safety.** Durability of response will not be tested. Importantly, VEGF-D level will be used in this small clinical trial design using 20 participants.

Specific Aims

1) To determine if monotherapy imatinib mesylate suppresses serum VEGF-D in LAM compared to placebo.
2) To determine if sirolimus (rapamycin) withdrawal for 1 month is associated with serum VEGF-D elevation in patients on imatinib mesylate.
3) To determine the safety of imatinib mesylate in patients with LAM.

2. BACKGROUND

2.1 Literature and Previous Studies

Recently, two developments have changed the face of LAM clinical care. The first of these was the observation that patients with sporadic LAM often (but not always) have biallelic mutations in *TSC2* in LAM cells. Loss of TSC functionality leads to marked activation of the mammalian target of rapamycin (mTOR) complex 1, a master kinase that regulates cell growth. mTOR activation is seen in a number of diseases prompting clinical trials of sirolimus (also known as rapamycin). In LAM, these studies showed that inhibition of mTOR produced growth arrest in LAM cell cultures in many laboratories. After sirolimus was shown to shrink renal tumors called angioleiomyomas (AML) that are associated with both TSC and sporadic LAM[5], a trial targeting lung disease was designed and completed by the Rare Lung Disease Consortium of the NIH.

The Multicenter International Lymphangioleiomyomatosis Efficacy and Safety (MILES) study compared sirolimus vs placebo in a 1:1 randomized controlled, blinded study of individuals with impaired lung function as defined by a forced expiratory volume in 1 second (FEV1) < 70% predicted.

The randomization was intact for 1 year showing both efficacy and safety of the drug[6]. At the end of the year, the study design prompted cessation of sirolimus to determine the duration of the treatment response. Unfortunately, the majority of LAM affected women did not make it to the next scheduled 3 month visit because lung function rapidly deteriorated and consent was withdrawn.

The second development that has allowed the current grant to advance is the discovery that vascular endothelial growth factor-D (VEGF-D) is generated by LAM cells[7]. VEGF-D at high levels is found in no other cystic lung diseases in women and was added to the diagnostic criteria of the MILES study to facilitate study enrollment in the absence of lung biopsy. Importantly, VEGF-D was a robust biomarker for disease activity and response to sirolimus in the MILES study[8]. Historically, unlike all other cancers, disease activity depended on measures of lung function such as FEV1. CT scans have not been able yet to define an endpoint for therapy since micronodules are too small to measure and cyst volume is not known to correlate with disease activity. Hyperinflation, an important cause of symptoms[9], is difficult to measure serially. Hence, a biomarker for LAM allows a path forward for LAM clinical trials.

Studies in the laboratory of Dr. D'Armiento, co-investigator for this study, demonstrated that LAM and AML cells differentiate down several mesenchymal cell lineages, therefore she speculated that they possess an undifferentiated mesenchymal cell phenotype. One of the well-known characteristics of mesenchymal cells is the expression of the PDGF receptor. In order to confirm the fact that the LAM cell was of mesenchymal origin, she stained the cells for the PDGFR b receptor and demonstrated positive staining. Next, in order to test whether the PDGF pathway was activated in the LAM cells, she performed IHC and western blotting of both LAM and AML tissues. These cells were positive for phospho-PDGF receptor as shown in Figure 1.

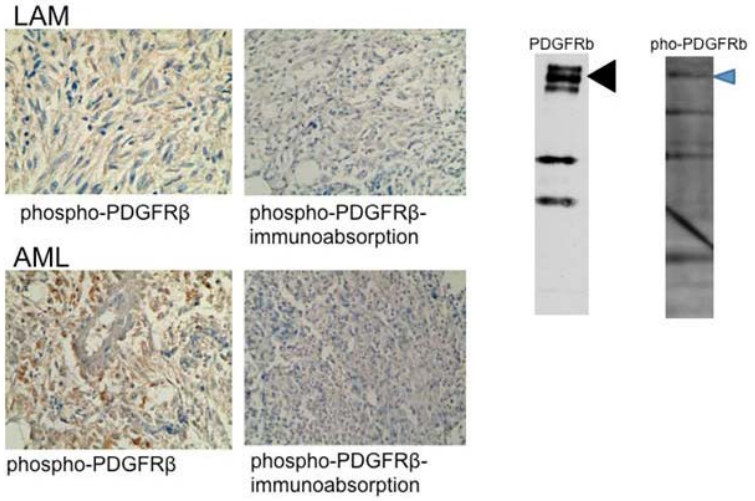

Figure 1. PDGFRb expression in LAM and AML. Immunohistochemistry of PDGFRb in LAM and AML tissue samples with a phosphor-PDGFRb antibody. Negative controls were performed after immunoabsorption of antibody. Western blot analysis on protein homogenates from AML tissue demonstrates expression of PDGFRb.

Imatinib mesylate (imatinib) is a tyrosine kinase inhibitor that functions by preventing the BCR-Abl tyrosine kinase enzyme from phosphorylating subsequent proteins and initiating the signaling cascade necessary for cancer development. Because the BCR-Abl tyrosine kinase enzyme exists only in cancer cells and not in healthy cells, imatinib is a targeted therapy that kills cancer cells through the initiation of apoptosis. In addition, imatinib blocks c-Kit and the platelet-derived growth factor receptor (PDGFR). As an inhibitor of PDGFR, imatinib mesylate has utility in other diseases. Imatinib has been reported to be an effective treatment for dermatologic diseases FIP1L1-PDGFR alpha+ mast cell disease, hypereosinophilic syndrome, and dermatofibrosarcoma protuberans[10] through this mechanism. After identifying the reactivity of the LAM cell with the PDGF antibodies Dr. D'Armiento demonstrated that

imatinib, which targets PDGF, could completely block the growth of the LAM/AML cells resulting in cell death (Figure 2, 3). Cells treated with Rapamycin, although growth inhibited, did not undergo cell death (Figure 3, [11]). These findings present Imatinib as a potential therapy in the treatment of Lymphangiomyomatosis.

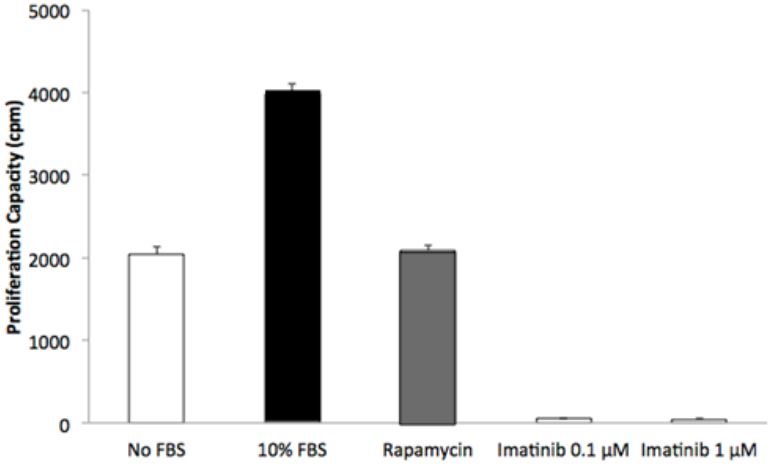

Figure 2. Primary human AML cells halt their growth after treatment with imatinib. Imatinib strongly inhibited the proliferation of primary AML cells at doses of imatinib that are achievable in the serum of humans.

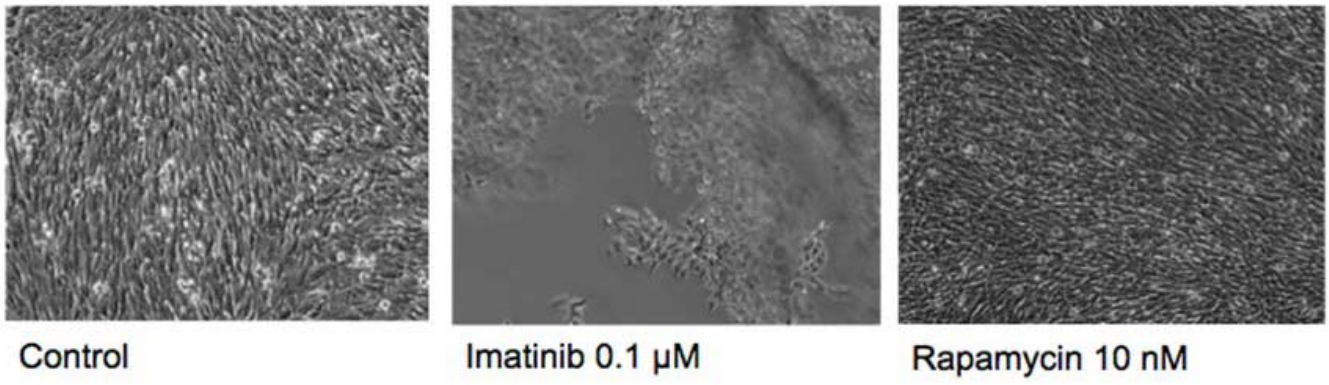

Figure 3. Cell death was achieved in the presence of fetal bovine serum (FBS) at 1µM and 0.1µM concentrations of imatinib. Previous studies have shown that sirolimus inhibits the cell growth but does not kill the cells as was seen in this study.[11]

2.2 Rationale for the Study

The proposed study design is a prospective, multicenter, randomized, stratified, double blind, placebo-controlled, proof of principal trial to examine the effect of imatinib mesylate on intrasubject VEGF-D, a biomarker associated with LAM disease activity. Since imatinib mesylate has not previously been used in LAM, a small pilot study is needed to gather safety data, confirm biomarker efficacy data, and determine if a clinical efficacy signal can be obtained in a short term study.

This pilot trial employs a dual agent design intended to generate safety and efficacy data sufficient to power and design a phase 3 study of imatinib mesylate vs placebo for LAM. Durability of response will not be tested. Importantly, VEGF-D level will be used in this small clinical trial design using 20 participants.

Since LAM is a rare disease a small trial is designed to assure enrollment is achievable.

This study will enable proper powering of subsequent studies. The authors believe that a proof of principal pilot study will be able to recruit 20 participants, the majority on rapamycin, for this study.

The overarching hypothesis that imatinib mesylate kills LAM cells, while sirolimus (rapamycin) provides growth suppression, suggests that removal of rapamycin may be necessary for the effects of imatinib mesylate to be seen. Therefore, a two stage pilot study has been designed with usual doses of imatinib mesylate used for other indolent tumors, in an attempt to confirm the hypothesis.

The findings of this pilot study are expected to enable powering of a larger clinical trial and advance knowledge of pathogenesis and treatment of LAM. All results will be shared at the end of the study with the LAM community.

2.3 Primary Hypothesis

The hypothesis is that imatinib mesylate will be equivalent to sirolimus (rapamycin) in short term efficacy and safety.

3. STUDY OBJECTIVES

3.1 Primary Aim

To determine whether imatinib mesylate will be equivalent to sirolimus in short term efficacy and safety in patients with LAM.

The primary efficacy analysis will be the log transformed change in intrasubject plasma VEGF-D before and 1 month after initiation of monotherapy imatinib mesylate.

3.2 Secondary Aims

1) To determine if monotherapy imatinib mesylate suppresses serum VEGF-D in LAM compared to placebo.

2) To determine if sirolimus (rapamycin) withdrawal for 1 month is associated with serum VEGF-D elevation in patients on imatinib mesylate.

3) To determine the safety of imatinib mesylate in patients with LAM.

3.2.1 To determine if a clinical efficacy signal can be obtained in a short term study.

3.2.2 To invite biobank participation.

With informed consent, the biosamples will be kept with study number identifiers to align with future clinical events. Individuals may participate in the study without agreeing to biobank participation.

3.2.3 Inform about impact of imatinib mesylate treatment on quality of life.

Quality of life questionnaire instruments are described in a later attachment as guided by the CDMRP Program will be administered.

4. RATIONALE

4.1 Study Population

Adult women with definite or probable LAM and impaired lung function will be invited to participate and screened by study inclusion and exclusion criteria below. Definite or probable LAM is established by the ERS LAM guidelines modified by addition of VEGF-D criteria as defined by the MILES Trail (see below). 20 women with LAM and impaired lung function will be enrolled. Many participants will be receiving sirolimus treatment at the time of enrollment; however, sirolimus naïve patients will not be excluded.

Participation of children is not allowed in this study because LAM does not cause clinical disease in this population.

Participation of women and minorities is encouraged, particularly since this disease only affects women. Sirolimus is pregnancy category C and imatinib is pregnancy category D. Pregnancy or intent to become pregnant or nurse during the 2 month study window and for 1 month afterwards is not allowed because of the safety risk of a category D medication. An adequate birth control method during these 3 months will be documented in all women of childbearing potential.

4.2 VEGF-D

VEGF-D at high levels is found in no other cystic lung disease and serves as a robust biomarker for LAM disease activity. Sirolimus (rapamycin) was demonstrated in the MILES study to be both safe and effective in the treatment of LAM. Importantly, VEGF-D was added to the diagnostic criteria of the MILES study. Hence, a biomarker for LAM allows a path forward for LAM clinical trials. This pilot study to determine whether imatinib will be equivalent to sirolimus in short term efficacy and safety in patients with LAM will similarly utilize the biomarker VEGF-D, measured at the University of Cincinnati laboratory. This test has been given a Humanitarian use device (HUD) designation for the disease LAM. Further information is at:
http://www.evaluategroup.com/Universal/View.aspx?type=Story&id=470067

4.3 Spirometry, Exercise (6MWT), and Dyspnea

Traditional methods to measure LAM disease activity have depended on measures of lung function such as FEV1 and FVC. Hyperinflation is an important cause of symptoms. Therefore, pre and post bronchodilator spirometry, plethysmographic lung volumes, and diffusion capacity are needed to accurately provide a physiologic assessment of lung function.

The ability to exercise is an important biomarker of lung disease and dyspnea. Exercise induced dyspnea remains a common symptom associated with the disease state. Exercise induced dyspnea correlates strongly to quality of life and exercise capacity correlates with mortality. Few studies of exercise capacity are available for LAM; however, the pathophysiology suggests that there is a high prevalence of hyperinflation and dynamic hyperinflation during exercise.

Although many specific exercises have been used as a biomarker for disease severity and progression, the distance walked in 6 minutes (6MWT) has the most data in COPD and is part of the LAM protocol in order to capture an exercise biomarker. Reproducibility is improved by a second 6MWT.

4.4 Laboratory Analysis

Samples of whole blood serum, DNA, blood mononuclear RNA, and urine will be obtained during this study. These samples serve the dual purpose of allowing a robust safety analysis of study timepoints and populating a LAM biobank of biosamples that are available to the larger LAM community (see Data Resource Sharing attachment). Biosamples will be stored both at Columbia University and MUSC and shared between laboratories with a sample and data sharing agreement in place with this study.

VEGF-D level will be measured at the beginning of the study, at one month and at the end of the study (56 days). For safety, all participants will also have laboratory monitoring of CBC, T4, TSH, Cholesterol, Sirolimus level and complete metabolic panel (CMP) at baseline, 28 days, and 56 days.

4.5 Biobank

With informed consent, a portion of biosamples will be kept with study number identifiers to align with future clinical events. Individuals may participate in the study without agreeing to biobank participation. These biosamples will allow proteomic and metabalomic analysis of a rare disease population with samples obtained on no LAM specific therapy, sirolimus (or other mTOR inhibitor) alone, or imatinib alone. These samples will be used and available to the LAM community to define the metabolic pathways affected by these drugs in intrasubject samples.

Because the LAM Foundation may in the future establish a biobank, the consent form will include an option of participant re-contact regarding the option moving biosamples to a LAM Foundation biobank, which may request identifying information. Participants in this study would be contacted prior to transfer of any biosample that includes identifying information and a new informed consent form for the LAM Foundation Biobank would be signed. Biosample requests from the LAM community will not receive identifying information to perform analysis on these biosamples.

4.6 Questionnaires

LAM is marked by premature disability, a shortened lifespan in most individuals, and frequent symptoms of disease. Disease specific quality of life, general quality of life, measures of exacerbation frequency, pre-existing lung function decline, and measures of dyspnea are collected by standardized and validated instruments. Quality of life questionnaire instruments are described in a later attachment as guided by the CDMRP program. Improved quality of life was determined among MILES study participants treated with sirolimus. Similarly, such measures will be collected in this imatinib pilot study.

Designed primarily as a safety study, the likelihood of achieving statistical differences between the groups in lung function or quality of life remains low; therefore, the questionnaires used in the study should be viewed as secondary and exploratory endpoints as explained in Attachment 1.
We will not be modifying any instrument for this study and will use the most recent validated version for each tool utilized. Recently, the St. Georges Respiratory Questionnaire (SGRQ) has been demonstrated to have longitudinal construct validity in LAM[2] Specifically, the SGRQ is a self-administered respiratory specific health care quality of life tool. When compared to FEV1, diffusion of the lung for carbon monoxide (DLCO), 6 minute walk test distance, and VEGF-D levels from the MILES cohort, the SGRQ change scores tracked for each of these four anchors. The strongest correlates were with FEV1. Plots of cumulative distribution functions further supported the longitudinal validity of the SGRQ in LAM.

The SGRQ is a 50 question examination that is available for use to academic investigators without charge. It is scored 1-100 with a clinically meaningful difference in obstructive lung diseases of 4 points change. Test to test reproducibility ranges from 0.795 to 0.9 for the American version that will be used for this study as a secondary variable. A copy of the questionnaire follows.

Exploratory endpoints for the study include mMRC dyspnea scale, the functional Assessment Inventory, and the EQ-5D score that were all used in the MILES study in a LAM population. None have been independently validated in LAM, yet in this pilot study, will be used to define if a treatment signal exists with these questionnaires. All have been used in more common respiratory diseases.

The modified Medical Research Council (mMRC) dyspnea scale is a four point scale that quantifies dyspnea and has correlations with mortality, quality of life in chronic obstructive pulmonary disease (COPD). The EQ-5D is a general Quality of Life scale that includes a visual analog scale to assess general wellbeing and may pick up non-respiratory toxicity of medications being studied. The

Functional Assessment Inventory is an instrument widely used to judge an individual's productive activity within 7 different domains. Copies of these 3 instruments are attached.

4.7 Facilities

University facilities at MUSC and Columbia University are available to see patients for research study inclusion. The pulmonary clinic in Rutledge Tower and the NEXUS center, and the clinical trials translational research center of the Clinical Translational Science Award (CTSA) at MUSC are available for this study. Personnel are available for biosample collection at the site of the CTSA biobank. The MUSC pulmonary clinical trials unit has 13 research coordinators working on studies at the present time. All are trained at time of hiring in bioethics and human subjects policy and procedures. All have current CITI certification through the University of Miami.

Biosamples for this study that will be collected include serum, plasma, DNA and blood mononuclear cell RNA collected in Paxgene tubes. Biosamples will be bar coded and stored at -80°C in a dedicated freezer running off an emergency powered circuit. Excess biosamples that are not used by the end of the study will be stored for the LAM community. Aliquots of samples will be made available as described in data sharing agreement form.

Pulmonary Division laboratories from both Universities have adequate freezer space, refrigerated centrifuges, and capability to isolate and store the biosamples described in the project.

5. STUDY DESIGN AND PROCEDURES

5.1 Overview

The proposed study design is a prospective, multicenter, randomized, stratified, double blind, placebo-controlled, proof of principal trial to examine the effect of imatinib mesylate on intrasubject VEGF-D, a biomarker associated with LAM disease activity.

The study is designed as a 3 visit study including baseline, 1 month, and 2 month visits. Since most patients with impaired lung function who would be eligible for this study are already on sirolimus, the proposed study would simultaneously address three issues: 1) provide a biomarker rich proof of principle study of imatinib mesylate in LAM, 2) determine if imatinib mesylate is safe when added to sirolimus, and 3) determine if imatinib mesylate controls vascular endothelial growth factor-D (VEGF-D) once sirolimus is removed. Small numbers of individuals are not on sirolimus; however, this proposal would not exclude them to define if there is a treatment response signal to imatinib mesylate alone. A stratified randomization strategy will differentially enroll participants on or off rapamycin at baseline.

At the conclusion of the study, participants will be returned to clinically available LAM therapy chosen with their treating physician. Imatinib will not be available through the investigators after participation in this study.

5.2 Patient Recruitment and Selection

10 eligible patients with LAM will be recruited and enrolled at each of Columbia University Medical Center and Medical University of South Carolina, for a total of 20 patients in the study. Both centers regularly follow LAM patients with sufficient patient numbers and characteristics to fulfill recruitment needs. Patients who are regularly cared for by the principal investigator and co-investigator will be reviewed for potential eligibility. In addition, a study advertisement will be placed in the LAM Foundation Newsletter and Website to inform individuals with LAM of this study. Screening procedures for established LAM patients and others are outlined below.

If a potential participant has received clinical LAM care at MUSC, authorized MUSC research personnel will review the potential participant's diagnosis, demographics, and lung function results. This information will be documented in Part 1 (page 2) of the Eligibility Screen. Participant identifiers are recorded on the cover sheet (page 1), in accordance with best practice.

Potential participants who appear eligible after Part 1 of the eligibility screen may be contacted and further screened by phone or in clinic. The appropriate telephone script or in-clinic script (see script documents) will be followed to introduce the research personnel, the research study and the option of engaging in the screening questions to determine eligibility. With the potential participant's verbal permission, questions on Part 2 (page 3) of the Eligibility Screen will be asked and responses recorded.

If someone is not a LAM patient at MUSC and is otherwise referred (e.g. self-referral from clinicaltrials.gov, advertisement response or referral from outside physician) and records do not accompany the request, the potential participant will be called and asked to voluntarily provide their records to the study team for preliminary review. When records are provided, the same procedures as above will be followed to screen the participant for interest and eligibility.

The Eligibility Screen and Scripts will allow the trained research coordinators to:

1) Verify in the records that the potential participant meets eligibility criteria (Eligibility Screen Part 1),

2) Verify that the individual is interested in participating in the LAMP-1 study (Telephone or In-Clinic Script).

3) Verify that the potential participant is preliminarily willing to perform study procedures and do not have contraindications (Eligibility Screen Part 2).

A participant who is interested and eligible after scripts and screening are complete will be asked if they wish to schedule their first study visit. The informed consent process will occur prior to study procedures at Visit 1.

The authorized investigator or coordinator engages in informed consent with the participant at the baseline visit and provides a written informed consent form for the participant to review. Following review of potential risks and benefits, the voluntary nature of participation, and ample opportunity to ask questions the consenting participant will provide signature on the informed consent document. At this point the participant is enrolled. The participant will be given a copy of the signed consent form to keep.

Participants will be given $200 travel money for each of the 3 study visits, for a cumulative payment of $600 per participant who completes the study.

5.2.1 Timeline

This timeline for this study is 1 years following after study drug is obtained. Grant funding from the Department of Defense has been approved (see attached budget). Imatinib mesylate (Gleevec®) for this study will be provided by Novartis. Placebo drug will be purchased. Enrollment will commence following IRB approval at both MUSC and Columbia University and subsequent approval by the human research protection office (HRPO) of the US Army Military Department. The anticipated enrollment schedule is detailed in the table below.

	Year 1				Year 2				Total
Target Enrollment Pilot Study (per quarter)	**Q 1**	**Q 2**	**Q 3**	**Q 4**	**Q 1**	**Q 2**	**Q 3**	**Q 4**	
MUSC	-	-	1	2	4	2	1	0	10
Columbia University	-	-	0	2	5	2	1	0	10
Target Enrollment (Cumulative)	**-**	**-**	**1**	**5**	**14**	**18**	**20**	**0**	**20**

5.3 Inclusion/Exclusion Criteria

5.3.1 Inclusion Criteria

Signed informed consent

- Age > 18 years* and < 65 years
- Definite or Probable LAM**
- FVC < 90% predicted or Postbronchodilator FEV1 <90% predicted
- Postbronchodilator FEV1 > 30% predicted
- DLCO > 20% predicted

5.3.2 Exclusion Criteria

- Current or planned pregnancy or lactation
- Unwillingness to discontinue sirolimus
- Change in the dose or use of sirolimus within the past month
- Inability to perform spirometry
- Other serious illness that would impact the outcome of the study
- Current lung transplant
- Current cigarette smoking
- Required use of ketoconazole, itraconazole, clarithromycin, or rifampin during the 2 months of the study.
- Unwillingness to avoid grapefruit juice or St. Johns Wort during the study.
- Planned surgery during the 2 months of the study..

*Children are excluded because LAM does not affect children

**Definite or Probable LAM is defined by the ERS LAM guidelines modified by addition of VEGF-D criteria as defined by the MILES Trail

Definite LAM:
1) Characteristic or compatible lung HRCT, and lung biopsy fitting the pathological criteria for LAM; or
2) Characteristic lung HRCT and any of the following: angiomyolipoma (kidney); thoracic or abdominal chylous effusion; lymphangioleiomyoma or lymph-node involved by LAM; and definite or probable TSC.

Probable LAM
1) Characteristic HRCT and compatible clinical history; or
2) Compatible HRCT and any of the following: angiomyolipoma (kidney); thoracic or abdominal chylous effusion; or VEGF-D >800 pg/ml at any time in the past.

5.4 Visit Schedule

The Baseline Visit will begin with signing the informed consent. A history and physical examination, post-bronchodilator spirometry, 6 minute walk test distance, and blood samples will be obtained. Questionnaires will be completed and the patient will be randomized to imatinib mesylate, at an oral dose of 400 mg once daily, or placebo stratified on whether sirolimus has been used in the last month. A sirolimus level and VEGF-D level will be measured.

A 1 week trough sirolimus level and telephone call will be obtained on all subjects entering the study on sirolimus therapy.

1 Month Visit- Post-bronchodilator spirometry, 6 minute walk test distance, and blood samples will be obtained. Questionnaires will be completed. Sirolimus will be discontinued if used at baseline.

2 Month Visit/ End of study- Post-bronchodilator spirometry, 6 minute walk test distance, and blood samples will be obtained. Questionnaires will be completed. A sirolimus level will be obtained to assure drug cessation. The participant is returned to LAM therapy of their choice.

Figure 1. Study Schedule, Stratification and Randomization

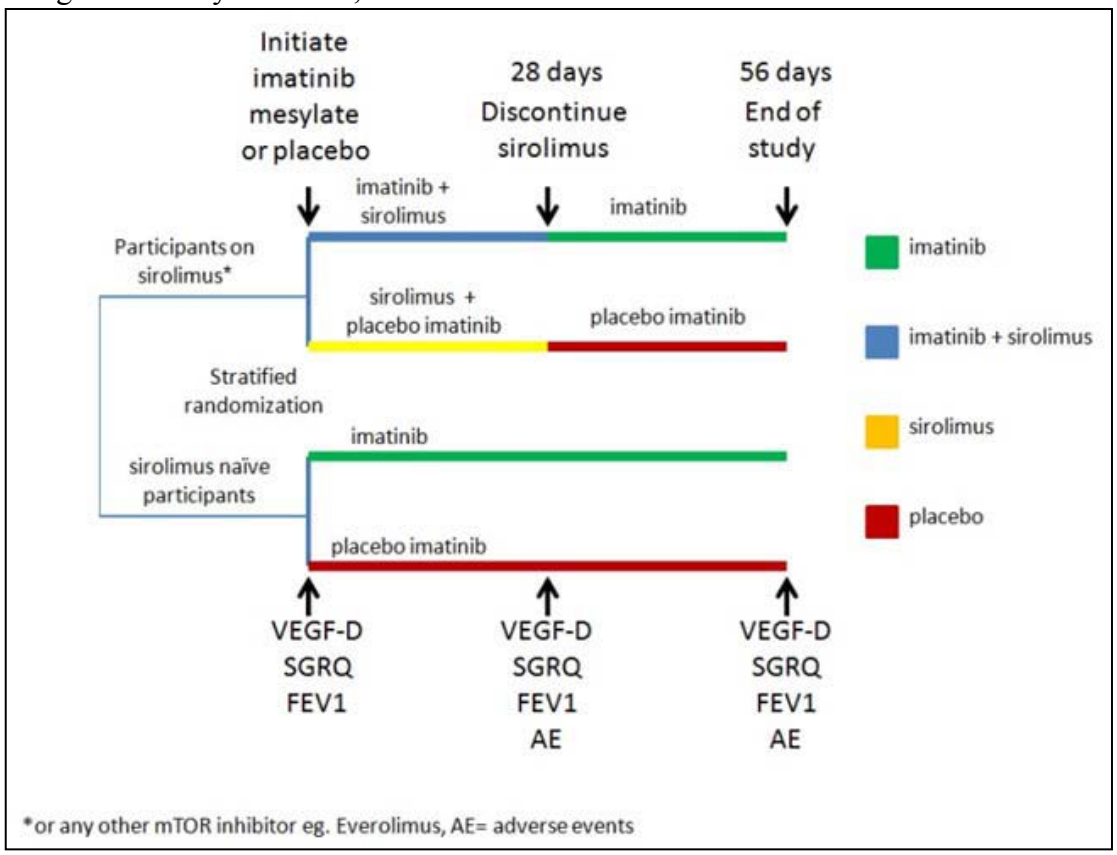

Table 1. Procedures by Visit

	Baseline (V1)	1 Month (V2)	2 Months (V3)
1.Informed Consent	X		
2.Pulmonary Function Testing	X	X	X
3.Six Minute Walk Test	X	X	X
4.Blood Sample Collection*	X	X	X
5.Urine Sample Collection*	X	X	X
6.Participant Questionnaires	X	X	X

*Table 2. Sample Collection and Usage

Collection	**Volume**	**Sample**	**Time(s)***	**Planned assays**
Blood each visit	30ml	Plasma	0, 1, 2	Cholesterol, CMP Sirolimus level, TSH, T4, VEGF-D level
	7 ml	Plasma	1 week in patients on sirolimus at baseline	Sirolimus level
	5ml	EDTA	0, 1, 2	CBC
	10ml	Paxgene DNA	0	DNA
	10ml	Paxgene RNA	0, 2	Blood mononuclear cell RNA
Urine each visit	10ml	Urine	0, 1, 2	Urinalysis, Pregnancy Test

*Time 0= Baseline, 1=1month, 2=2 months

5.5 Study Examinations
5.5.1 Blood and Urine Samples
These are collected at each study visit.

Safety: Universal and Occupational Safety and Health (OSHA) and all institutional-specific (such as bloodborne pathogen training) precautions and training requirements should be followed when processing blood and urine. For more detailed safety information see (http://www.osha.gov/SLTC/biologicalagents/index.html).

Urinalysis, pregnancy test, CBC, TSH, T4, Cholesterol, INR and CMP are performed on samples from each visit at the University Laboratories at MUSC and Columbia to look for adverse events and assure patient health and safety during the study. Trough Sirolimus level is performed on a sample from each visit to verify Sirolimus treated vs. naïve at baseline and that sirolimus was stopped in previous sirolimus receivers at the 1 month visit. This may require participants to take sirolimus at a time of the day that corresponds to anticipated study visit time. VEGF-D levels are performed at each visit to test efficacy of imatinib.

All tests except the VEGF-D will be performed in the clinical laboratory of the institution where the patient is seen (MUSC or Columbia University) and will be performed per standard protocol in these trained and certified laboratories. The costs of laboratory analysis are provided for in the study budget.

5.5.2 Pulmonary Function Testing and Exercise Tests
These are performed at each study visit.

Comprehensive lung function testing is obtained at each visit in this study. Baseline spirometry is obtained by following ATS guidelines. Post bronchodilator spirometry is obtained with an aggressive protocol of 4 puffs of albuterol and 4 puffs of ipratroprium to allow an optimal assessment. Lung volumes are obtained by plethysmography and diffusion capacity by the single breath method.

A 6MWT is performed at each of the 3 study visits. The 6MWT is performed without supplemental oxygen by methodology of the ATS Standards. Desaturation less than 80% has been associated with some adverse events and will be used to stop the test and measure the distance should that event occur. Oxygen saturation is determined throughout the entire test. A 10 point Borg dyspnea scale is obtained at rest and at the end of the 6MWT.

5.5.3 Questionnaires
Multiple questionnaires will be collected at each study visit as delineated in 4.6 above and seen in attachments to this study.

St. George's Respiratory Questionnaire (SGRQ)
This biomarker for COPD quantifies a patient reported outcome.

Modified Medical Research Council (MRC) Dyspnea Scale
Dyspnea is an important biomarker of disease severity and risk of death.

Functional Assessment Inventory
The FAI is widely used to judge an individual’s productive activity in 7 domains.

EQ-5D Health Questionnaire

Quality of Life scale that includes a visual analog scale to assess general wellbeing and may pick up non-respiratory toxicity of medications being studied.

5.5.3 Interim phone calls
A study coordinator will call each participant by phone at 1 week +/- 3 days and 2 weeks +/- 3 days after the baseline visit and again 2 weeks +/- 3 days after the 1 month visit. Phone calls will serve as an opportunity to touch base, inquire about any side effects or adverse events, encourage adherence and confirm the next visit. Completion and results of the phone call will be documented in REDCap.

5.6 Unblinding considerations
An unblinded study team (prescribing health care provider +/- coordinator) will be used to monitor sirolimus levels at 1 week and 1 month after baseline. Levels that increase by > 50% will lead to sirolimus dose reductions that will unblind the study participants. Such unblinding events will be reported to the research study monitor and be part of DSMB reporting.

6. ETHICAL CONSIDERATIONS
This study will be initiated only after a thorough review by the MUSC IRB, the Columbia University IRB, and the Human Research Protection Office (HRPO) of the US Army Military Department. A written informed consent will be obtained from every participant in which known side effects of imatinib are described. Participants may participate in this study and request that their biosamples be anonymized and/or not stored after the study analysis is complete.

An important consideration for this study is that sirolimus is not currently FDA approved for LAM. However, it is estimated that 90% of individuals with LAM and low lung function are using this drug and efforts are underway by Dr. Frank McCormack, principal investigator of the MILES trial and medical director of the LAM Foundation to obtain FDA approval via his IND for sirolimus.
This study is submitted because no study in LAM will advance in this rare disease population unless individuals on sirolimus are allowed to be enrolled since the disease is so rare. A study approved by the CDMRP in 2010 (the TRAIL trial) failed to achieve adequate enrollment because of this issue. Importantly, individuals enrolled in this study are on unchanging doses of sirolimus.

Subjects will be instructed at the beginning of the study to report to the investigator any adverse physical or mental changes they experience and they will be asked about adverse events at each visit, including those experienced at the baseline visit prior to, during, or immediately following treatment. All such adverse events reported by the subjects or observed by the investigators will be captured in the study forms. If the event is deemed to be Serious, the serious adverse events procedures will be followed and the IRB, DSMB, HRPO and Novartis notified.

An independent Data Safety Monitoring Board (DSMB) composed of 3 individuals (2 pulmonary specialists and one regulatory/statistical clinical trial specialist) will be established to provide independent benefit/risk oversight during the conduct of the study. The DSMB will: 1) Review the protocol and consent form prior to study initiation, 2) Evaluate Serious Adverse Events on an “as needed” basis and all adverse events on a quarterly basis, 3) Recommend discontinuation of the study in the event of the occurrence of Serious or Unexpected Adverse Events that are determined by the DSMB to pose a significant safety concern. The Chairperson of the DSMB will notify the HRPO and Dr. Strange who will in turn notify the FDA or other regulatory bodies of adverse safety outcome information sufficient to stop the study. This information will also be reported as part of required regulatory progress update reports Katherine Taylor, MS, will serve as independent research monitor in accordance with Department of Defense requirements. The research monitor will provide study oversight and will have the authority to take any action necessary, including stopping the research or

removing a subject, to protect the safety and well-being of human subjects until the IRB has a chance to assess the monitor's report.

Suchit Kumbhare is a trained study coordinator who will be in charge of creating and maintaining the randomization list at MUSC. M. Gwen Blanton is a trained study coordinator who will count and package study medication and placebo for both study sites, so that 10 sets of imatinib and 10 sets of placebo are prepared. Haitham Al Ashry, MD will oversee the counting, packaging and sorting as a double check mechanism. Dr. Al Ashry will ensure appropriate sorting of correct quantities of imatinb and placebo into two distinct groups. After this double check is complete, Gwen Blanton will label the two distinct and counted groups (imatinib and placebo) as A and B and Dr. Al Ashry will ensure that the labels have been accurately applied to the two distinct groups. Labels compliant with pharmaceutical standard of care will be applied. Five sets of A and 5 sets of B will be sent by FedEx to Columbia University for their 10 participants. At MUSC Suchit Kumbhare will randomize the participant without knowledge of whether A and B are drug or placebo. According to the randomization assignment, the blinded assessors, PI Charlie Strange, MD or Tatsiana Beiko, MD will dispense A or B to the participant. The label will contain space for the authorized blinded dispensing physician to write in the participant's name just prior to dispensing to the participant.

Columbia University will name a trained study team member to perform their randomization. Blinded assessor and co-investigator who will dispense medication at that site is Jeanine D'Armiento, MD. The Columbia IRB will review and approve their plan and designation.

Participation of children is not allowed in this study because LAM does not cause clinical disease in this population.

Participation of women and minorities is encouraged, particularly since this disease only affects women. Sirolimus is pregnancy category C and imatinib is pregnancy category D. Pregnancy or intent to become pregnant or nurse during the 2 month study window and for 1 month afterwards is not allowed because of the safety risk of a category D medication. An adequate birth control method during these 3 months will be documented in all women of childbearing potential.

6.1 Human Subjects' Protections and Risks

Risks of study participation and medications are described in detail in the informed consent, as are protections and the participant's right to not participate or withdraw at any time.

6.1.2 Pulmonary Function Tests

PFTs are a common medical procedure of generally low risk. Some participants may experience breathlessness, cough, fatigue, dizziness/lightheadedness (hyperventilation), all of which are brief and very rarely headache, syncope, musculoskeletal chest pain, rib fractures, or ear injury. An episode of stress incontinence (urine leakage) may be caused by the PFT maneuvers in susceptible individuals. A seated position has been specified to reduce risk related to dizziness or syncope. Transmission of airborne disease is rare and minimized or eliminated with single-use filters. Instructions for withholding bronchodilator medications prior to testing stress the continued use of rescue medication if needed. The use of albuterol or ipratropium generally relieves any symptoms related to the trough effect of long-acting bronchodilators.

- Albuterol has been reported to cause urticaria, angioedema, paradoxical bronchospasm, angina, arrhythmias, QT prolongation, hypertension, hypokalemia, seizures, tremor, nervousness, headache, tachycardia, muscle cramps, palpitations, insomnia, and dizziness.

- Ipratropium has been reported to cause cough, nausea, dry mouth, dizziness, headache, dyspnea, atrial fibrillation, tachycardia, paradoxical bronchospasm, laryngospasm, angioedema, anaphylaxis, hypersensitivity, and exacerbate narrow-angle closure glaucoma.

- The dose used in testing is twice the usual dose of albuterol (one dose every four hours) or ipratropium (one dose every six hours) used chronically. However, home management of exacerbations includes increasing the dose and/or frequency of bronchodilator therapy. Doses in patients hospitalized or visiting the Emergency Department for exacerbations may be ten times the usual dose. Repeat dosing after at least three hours, is unlikely to result in any additional side effects, if necessary for the scheduling of the computed tomography.

The six-minute walk test is self-paced, but participants are encouraged to cover as much distance as they can in six-minutes. As with any walk, the participant may stumble or fall. It is expected that more severe participants will become short of breath and may need to stop to recover before six minutes have elapsed. The walk test will be stopped if the participant's SaO2 falls below 80%.

6.1.3 Imatinib side effects

The most common side effects of imatinib include nausea, diarrhea, headaches, leg cramps, fluid retention, visual disturbances, rash, weight gain, and reduced number of blood cells (any cell line can be affected). Imatinib is an immune suppressant and increases risks of common and uncommon infections.

Severe systolic congestive heart failure is an uncommon but recognized side effect of imatinib and mice treated with large doses of imatinib show toxic damage to myocardiocytes. Hypoxemia has been noted in one individual with LAM..

Since imatinib is mainly metabolised via the liver enzyme CYP3A4, substances such as ketoconazole, itraconazole, clarithromycin, and grapefruit juice can increase the plasma concentration of the drug. Conversely, CYP3A4 inducers like rifampin and St. John's Wort reduce the drug's activity. These medications will be prohibited during the study. A known interaction between imatinib and sirolimus in which sirolimus levels rise will be monitored during the study.

Imatinib also acts as an inhibitor of CYP3A4, 2C9 and 2D6, increasing the plasma concentrations of simvastatin, cyclosporin, pimozide, warfarin, and metoprolol. Imatinib also reduces plasma levels of levothyroxin via an unknown mechanism. INR, Cholesterol, T4, and TSH will be measured during the study for all individuals.

An imatinib dose reduction to 200 mg daily will be permitted should a participant experience persistent side effects.

6.1.4 Sirolimus side effects

The most common side effects of sirolimus are immune suppression, thrombocytopenia and impaired wound healing. For these reasons, individuals planning surgery (including oral surgery) during the study interval are excluded.

Lung toxicity is a serious complication associated with sirolimus therapy although it usually occurs shortly after beginning therapy. The mechanism of the interstitial pneumonitis is not clear. The toxicity does not appear to be dose dependent, but is more common in patients with underlying lung disease[13].

Sirolimus also acts on a related complex known as mTORC2. Disruption of mTORC2 produces the diabetes-like symptoms of decreased glucose tolerance and insensitivity to insulin also associated with sirolimus. As with all immunosuppressive medications, in theory sirolimus and imatinib may decrease the body's inherent anticancer activity. However, both of these medications have data that sirolimus can enhance the immune response to tumor targeting and promote tumor regression in clinical trials[14,15].

6.1.5 Vaccinations

Because both of these agents are immunosuppressants, live vaccinations are contraindicated because the microorganisms in the vaccine could multiply and infect the patient. Vaccines that are inactivated and toxoid vaccines do not hold this risk, but may not be effective during imatinib or sirolimus therapy.

6.2 Confidentiality

There is a risk to confidentiality. Source data at each center will be kept electronically under password protected systems. Questionnaires or paper documents will be kept in secure areas. Data transmission will be encrypted with a 128-bit VPN approach. The PFT software is designed to be HIPAA compliant for clinical use. Collection of DNA and RNA can lead to identification of patients.

All study personnel are certified in the ethical conduct of human biomedical and genetics research and HIPAA information security.

Longer descriptions of human subjects protections are provided in the consent form template.

6.3 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a study subject. This may include symptom(s), illness, clinically significant abnormal laboratory value or change in value, or worsening in a subject during a clinical study. Since one of the specific aims of the study is to establish the safety of imatinib in LAM affected individuals, it is the goal of the investigators to capture every AE.

6.3.1 Procedures for Adverse Events

Event, date of onset, severity, duration, and relationship to sirolimus or imatinb (if it can be determined) will be recorded on the study AE forms. All adverse events will be followed until they are adequately resolved or stabilized, or for 1 month following study completion, whichever comes first.

If an adverse event occurs during a study visit the study coordinator should first insure the participant receives any needed medical attention. If the study coordinator is notified by phone, the coordinator should confirm that the participant has received medical attention. The study coordinator should then notify the site Principal Investigator and complete the Adverse Event Form within seven days of learning of the event. Study coordinators will comply with local regulations and policies when notifying the institutional IRB.

The following general definitions for rating severity should be used for this study:
Mild: Awareness of signs or symptoms, but easily tolerated and transient; causing no loss of time from normal activities; symptoms would not require medication or a medical treatment; signs and symptoms are transient.
Moderate: Marked symptoms and discomfort severe enough to cause moderate interference with the subject’s usual activities. Symptomatic treatment is possible.
Severe: Incapacitating with inability to do work or usual activities; signs and symptoms may be of systemic nature or require medical intervention and/or treatment. Hospitalization may be required or prolonged.

The relationship of an AE or SAE to the underlying disease or to the procedure will be attributed using the following definitions:
Not Related: There is no evidence that the event has a relationship to the drug(s).
Possibly Related: The event has a timely relationship to the drug(s) used. However, a potential alternative etiology may be responsible for the adverse event.
Probably Related: The event has a timely relationship to the drug(s) used and the causative relationship can clearly be established. No potential alternative etiology is apparent.
The number of subjects and observation time in this pilot study is insufficient to develop a composite index or statistical plan for the evaluation of safety endpoints for this study. Therefore, all adverse events, their timing, severity, and attribution will be reported in study reports.

6.3.2 Serious Adverse Events

The FDA (2009) defines a serious adverse event as an adverse event that: results in the participant's death, is life-threatening, results in hospitalization (initial or prolonged), results in significant, persistent, or permanent change, impairment, damage, disruption, or disability in the participant's body function/structure, physical activities or quality of life, results in a congenital anomaly, or requires intervention to prevent permanent impairment or damage.

6.3.3 Procedures for Serious Adverse Events

There are no expected serious adverse events (SAE) in this study. In accordance with 21 CFR Parts 803 and 812, an SAE is defined as any untoward medical occurrence that: results in death, is lifethreatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect, or requires intervention to prevent permanent impairment or damage. All SAE will be reported to Dr. Strange and the Data Safety Monitoring Board Chair in 24 hours after knowledge of the occurrence.

If a serious adverse event (SAE) occurs during a study visit the study coordinator should first insure the participant receives any needed medical attention. If the study coordinator is notified by phone, the coordinator should confirm that the participant has received medical attention. The study coordinator should then notify the site Principal Investigator and complete the adverse event form. All SAE will be reported to Dr. Strange and the Data Safety Monitoring Board in 24 hours after knowledge of the occurrence. Study coordinators should comply with local regulations and policies when notifying the institutional IRB. The DSMB will review all SAEs and the Chairperson of the DSMB will notify the HRPO and principal investigator of adverse safety outcome information sufficient to stop the study.

The principal investigator has the obligation to report all serious adverse events to the FDA, IRB, and Novartis Pharmaceuticals Drug Safety and Epidemiology Department (DS&E). All events reported to the FDA by the investigator are to be filed utilizing the Form FDA 3500A (MedWatch Form), if applicable

To ensure patient safety, every SAE, regardless of suspected causality, occurring after the patient has provided informed consent and until at least 30 days after the patient has stopped study treatment must be reported to Novartis within 24 hours of learning of its occurrence. Information about all SAEs is collected and recorded on a Serious Adverse Event Report Form. The investigator must assess and record the relationship of each SAE to each specific study treatment (if there is more than one study treatment), complete the SAE Report Form in English, and send the completed, signed form along with the Novartis provided fax cover sheet to the Novartis Oncology Drug Safety and Epidemiology (DS&E) department by fax (fax: 877-778-9739) within 24 hours.

Any additional information for the SAE including complications, progression of the initial SAE, and recurrent episodes must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event.

Any SAEs experienced after a 30 day safety evaluation follow-up period should only be reported to Novartis if the investigator suspects a causal relationship to the study treatment.

Follow-up information is submitted in the same way as the original SAE Report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not, and whether the patient continued or withdrew from study participation.

If the SAE is not previously documented in the Investigator's Brochure or Package Insert (new occurrence) and is thought to be related to the Novartis study treatment, an oncology Novartis Drug Safety and Epidemiology (DS&E) department associate may urgently require further information from the investigator for Health Authority reporting. Novartis may need to issue an Investigator Notification (IN), to inform all investigators involved in any study with the same drug that this SAE has been reported. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

6.3.4 Pregnancy Procedures and Reporting

Any participant who becomes pregnant during the study will discontinue study medication and procedures for pregnancy follow-up and reporting will be followed:

To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to Novartis within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

Pregnancy should be reported by the investigator to the Novartis Oncology Drug Safety and Epidemiology Department (DS&E) by fax (fax: 877-778-9739). Pregnancy follow-up should include an assessment of the possible relationship of the pregnancy outcome to the study treatment. Any SAE experienced during pregnancy must be reported on the SAE Report Form.

Because males will not be enrolled in this study, there is no risk of pregnancy in partners of male participants.

6.4 Measures to Protect the Participant

All participants will have laboratory monitoring of CBC, T4, TSH, Cholesterol, INR, Sirolimus level and complete metabolic panel (CMP) at baseline, 28 days, and 56 days. Side effects may receive imatinib dose reduction to 200 mg daily if persistent. Study withdrawal for side effects is permitted. All side effects will be graded and followed. Side effects suggestive of an infection will prompt cessation of subsequent doses of rapamycin and imatinib mesylate/placebo for 1 week until such symptoms are improved. Any worsening of symptoms would prompt evaluation. Decline in FEV1 or FVC of 10% would prompt end of study, study unblinding, and transition to usual clinical care. A study

coordinator will call each participant to check on how they are doing 1 week +/- 3 days and 2 weeks +/- 3 days after the baseline visit and 2 weeks +/- 3 days after the 1 month study visits.

Study related injuries are addressed in the MUSC Statements of the informed consent form. In the event of a study related injury, the participant should go immediately to the emergency room of the Medical University Hospital, or in case of an emergency go to the nearest hospital, and tell the physician on call that they are in a research study. The doctor should call the study doctor to make arrangements for treatment. If the study sponsor does not pay for treatment, the Medical University Hospital and the physicians who render treatment will bill the participant's insurance. If the insurance company denies coverage or insurance is not available, the participant will be responsible for payment of all services rendered.

6.4.1 Withdrawal

A participant may withdraw from the study at any time for any reason. Study withdrawal for side effects is permitted. At the time of withdrawal the participant may 1) decline to provide any more data or specimens to the study but allow use of previously collected data and/or specimens, 2) request withdrawal of all his/her data from study databases and request that any stored samples be destroyed, or 3) withdraw some portion of the data collected (i.e., participants may withdraw specimens but not exam or questionnaire data or vice versa).

If a participant chooses to withdraw from the study, the research coordinator at the clinical site conducts an exit interview to determine the disposition of the participant's study data. The coordinator also provides the participant with any clinically relevant study results or establishes how the participant would like to be contacted if relevant results become available in the future. If the participant declines an exit interview, the consent form in place at the time of study withdrawal is used to determine the status of the participant's data.

Participants may be removed from the study if the clinical center PI determines that it is unsafe or unethical for the participant to continue in the study. Situations that might result in this kind of withdrawal include physical impairment such that the participant cannot complete the study protocol, institutionalization (e.g., long-term care facility, prison), or aggressive or antagonistic behavior towards clinical center staff. Any deterioration in symptoms or a 10% decline in either postbronchodilator FEV1 or FVC would end the study and participants can resume previous therapy.

7. STATISTICAL ANALYSIS

7.1 Sample Size

Since this is a pilot study, the estimate of sample size to determine a biologic signal in patients with LAM is extrapolated from other studies in which serial samples of VEGF-D has been measured. Since LAM is a rare disease a small trial is designed to assure enrollment is achievable. This study will enable proper powering of subsequent studies. The authors believe that a proof of principal pilot study will be able to recruit 20 participants, the majority on rapamycin, for this study. A larger study is not possible given the limitations of funding.

7.2 Statistics

Statistical analysis of the primary endpoint will compare the VEGF-D before and one month after imatinib montherapy by intrasubject testing. VEGF-D has been shown to be logarithmically expressed and log transformation of the data is planned. It is unlikely that data will be normal between the imatinib and placebo groups. Therefore, the intrasubject difference (log pg/ml) in VEGF-D between imatinib and placebo groups will be subjected to non-parametric analysis with Wilcoxen rank sum tests. All statistical tests will be 2-sided at a 0.05 level of significance.

Statistical analysis of secondary endpoints will be performed in identical manner using the % change in FEV1 and FVC for these analyses. Use of ml change disproportionately shows effects in this bronchodilator responsive disease for those with preserved lung function. All secondary endpoints will be reported in study manuscripts with adjustment for multiplicity testing. Data will be presented in spaghetti plots to allow an assessment for responder analysis.

Exploratory analyses are designed to not miss a biologic signal if present. The impact of any significant difference between groups will require additional study and will be presented as question generating and will not use adjustments for multiplicity testing.

7.3 Endpoints

7.3.1 The primary efficacy analysis will be the log transformed change in intrasubject plasma VEGF-D before and 1 month after initiation of monotherapy imatinib mesylate. Log transformation was needed in the MILES study because this serum concentration can often go quite high in some patients. VEGF-D will be obtained at baseline and at 28 and 56 days after initiation of imatinib. Note that this analysis will allow the use of sirolimus using and sirolimus naïve participants.

7.3.2 Secondary endpoints are obtained to test both efficacy and inform about quality of life. Quality of life questionnaire instruments are described in a later attachment as guided by the CDMRP program. Importantly, respiratory specific and general quality of life scores will be used in the study. Recently, the St Georges Respiratory Questionnaire (SGRQ) was retrospectively validated against four anchors in the MILES study[12] and will be used as a secondary efficacy endpoint in the combined analysis of both stratified randomization populations. To minimize multiplicative analysis, 3 endpoints are chosen for combined population secondary endpoints: Intrasubject Change in FEV1 (ml), Intrasubject Change in FVC (ml), and Intrasubject Change in SGRQ (points) between the timepoints of Baseline and 1 month after initiation of monotherapy imatinib/placebo. The last secondary endpoint compares a 2 month treatment with imatinib/placebo in sirolimus naive participants on intrasubject change in log VEGF-D (pg/ml).

7.3.3 Exploratory endpoints

The table below lists the exploratory endpoints that will be collected to define if there is a difference between the 4 populations in the study. All change variables are measured intrasubject.

Efficacy Endpoints	Difference in baseline and 1 month after initiation of monotherapy imatinib/placebo	Difference in baseline and 1 month in patients on sirolimus	Difference in baseline and 2 months in imatinb naive participants
Change in VEGF-D (log pg/ml)	**X (Primary)**	X (Exploratory)	**X (Secondary)**
Change in FVC (%)	**X (Secondary)**	X (Exploratory)	X (Exploratory)
Change in FEV1 (%)	**X (Secondary)**	X (Exploratory)	X (Exploratory)
Change in mMRC Dyspnea (score)	X (Exploratory)	X (Exploratory)	X (Exploratory)
Change in 6MWT distance (M)	X (Exploratory)	X (Exploratory)	X (Exploratory)
EQ-5D (score)	X (Exploratory)	X (Exploratory)	X (Exploratory)
Functional Assessment	X (Exploratory)	X (Exploratory)	X (Exploratory)

Inventory (score)			
SGRQ (score)	**X (Secondary)**	X (Exploratory)	X (Exploratory)

7.4 Study Limitations

Study limitations include that the exact mechanism by which imatinib kills LAM cells remains unknown. The Bcr-Abl pathway has many downstream pathways including the Ras/MapK pathway, which leads to increased proliferation due to increased growth factor-independent cell growth. It also affects the Src/Pax/Fak/Rac pathway that affects the cytoskeleton leading to increased cell motility and decreased adhesion. The PI/PI3K/AKT/BCL-2 pathway is also affected altering mitochondrial stability. The JAK/STAT pathway, responsible for cellular proliferation is also affected by imatinib. While further laboratory studies on LAM/AML cells with alternative agents and mechanisms will continue, the current safety profile for imatinib use in humans is sufficient to begin a clinical trial in LAM prior to definitive mechanism of its effect on LAM cells is known.

The study is small and of short duration. The time interval for LAM cells *in vivo* to grow once removed from the influence of sirolimus is unknown. The study design in this rare disease is based on experience from LAM patients that remains anecdotal. However, in balancing safety and the potential to see a signal of efficacy in a pilot study, the current design is advanced.

8. DATA HANDLING

8.1 Web Data Entry

Clinical data and research questionnaires will be captured through REDCap, an encrypted, password protected database developed for the purpose of clinical research. REDCap has been embraced by the CTSA program of the NIH. Data entry is performed via REDCap by trained study coordinators at each clinical site. An electronic copy of the REDCap submission is available at the end of the entry session and serves as the source document kept at the clinical site. Lung function data are entered by the clinical coordinators, but source documents are saved electronically according to Good Clinical Practice.

8.2 Data Sharing

All data collected in this study will be published in a peer reviewed medical journal to support access of imatinib mesylate to patients if it is effective in LAM. This study also allows for the collection of plasma, blood mononuclear cell RNA and DNA and urine from patients on sirolimus and on imatinib mesylate that will allow a large community of researchers to study these biosamples for differences in biologic activity of these two medications. When distributing these unique resources, the PI would include pertinent information on the nature, quality, or characterization of the materials. In addition, the PI will submit unique biological information, if samples are measured, to the appropriate data banks so that they can be made available to the broad scientific community.

At the end of the study the PI will share data with any interested party via a data-sharing agreement to impose a minimum of limitations on users. First publication rights and acknowledgement of original investigators in future secondary publications would be necessary for data access. Privacy and confidentiality standards will ensure data security at the recipient site, and prohibitions for manipulating data for the purposes of identifying subjects will be put in place.

8.2.1 Biobank

Biosamples will remain at MUSC, Columbia University, or the University of Cincinnati during the study until study parameters are complete. With informed consent, samples that are left after study completion will be kept in biorepositories at the respective institutions and will be available for sharing with qualified researchers. The informed consent form explains that samples may be transferred to qualified

researchers with coded participant information, but without use of protected health information (PHI). Biosamples will not be sent to researchers with attached PHI.

Biosamples will be available at the end of study for the larger number of LAM researchers to access the biosamples through a data and biosample sharing agreement with MUSC and/or Columbia University. Although the number of biosamples will be small, the ability to study active disease pathways between two medications used in humans is an attractive resource that would be advertised to the researcher community through the LAM Foundation and CDMRP resource listings. Shipping and laboratory costs would be paid by the requesting parties.

The biosamples will be collected under an informed consent that asks optional permission to re-contact the participant in order to link biosamples with future clinical events and introduce future LAM-related research opportunities. With effort ongoing to develop a LAM Registry through the University of Cincinnati, future research opportunities include the possibility of sample transfer if a larger biobank specific to LAM develops. If the LAM Foundation establishes such a biorepository, participants in this study who have given permission for biobanking of sample(s) and for recontact would be contacted prior to transfer of any biosample and consented with the new LAM biorepository consent form from the new site.

9. STUDY MONITORING

9.1 Data Fidelity and Quality Control

The clinical and biospecimen output data linked to a study ID will be archived and stored in the secure study database for a period of no less than 5 years after conclusion of the study. Data is password protected and secured by physical and firewall protected means.

9.2 Biospecimen Quality Control

Samples for study analysis related to clinical safety and efficacy are obtained, transported and processed through protocols of clinical laboratories at each institution. Samples for storage and biobank use, as expressly indicated in the participant's informed consent, are kept in dedicated repositories with all sample use logged. Quality control to define stability of all freezer temperatures and sample integrity is performed regularly.

9.3 Data Safety Monitoring Board

An independent Data Safety Monitoring Board (DSMB) composed of 3 individuals (2 pulmonary specialists and one regulatory/statistical clinical trial specialist) will be established to provide independent benefit/risk oversight during the conduct of the study. The DSMB will: 1) Review the protocol and consent form prior to study initiation, 2) Evaluate Serious Adverse Events on an "as needed" basis and all adverse events on a quarterly basis, 3) Recommend discontinuation of the study in the event of the occurrence of Serious or Unexpected Adverse Events that are determined by the DSMB to pose a significant safety concern. The Chairperson of the DSMB will notify the HRPO and Dr. Strange who will in turn notify the FDA or other regulatory bodies of adverse safety outcome information sufficient to stop the study. This information will also be reported as part of required regulatory progress update reports.

9.4 Research Monitor

A qualified research monitor, Katherine Taylor, is appointed in accordance with the Department of Defense (DoD) requirements. The research monitor will observe recruitment and enrollment procedures, the informed consent process and may oversee study visits and interactions. The research monitor will review monitoring plans and any unanticipated problem involving risks to subjects or others (UPIRTSO) reports. The research monitor will oversee data collection and analysis. The research monitor may

discuss the research protocol with the investigators, interview human subjects and consult with others outside of the study about the research. The research monitor will have the authority to stop the research in progress, remove individual human subjects from the research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report. The research monitor is responsible for promptly reporting their observations and findings to the IRB and HRPO.

10. STUDY ADMINISTRATION

The Principal Investigator, Charlie Strange, MD, oversees study design and implementation. Dr. Strange additionally oversees study administration and study personnel, including co-investigator, study coordinator (TBD) and other trained research personnel who will be involved in coordination and execution of this study. Under the auspices of Dr. Strange and institutional regulatory bodies trained study staff will coordinate meetings, conference calls, and web-conferences as needed; determine and delegate needs for administrative support (e.g. scheduling, minutes) for committees and subcommittees; ensure fidelity of all data management activities, including form design, regular reports, reimbursing, including contracts, identifying milestones for payment, and generating invoices and payments, maintaining directories and distribution lists, preparing reports, creating recruitment materials and assisting with recruitment, creating and updating the Manual of Operations, training and certification of study personnel, organizing in-person meetings, and coordinating and overseeing the publication process. Consultant funds (see attached budget) will be used for the formation of a Data Safety Monitoring Board, a research monitor and statistical analysis.

11. REFERENCES

1 Meraj R, Wikenheiser-Brokamp KA, Young LR, et al. Lymphangioleiomyomatosis: new concepts in pathogenesis, diagnosis, and treatment. Semin Respir Crit Care Med 2012; 33:486-497

2 Ogawa R, Miyagawa M, Ide K, et al. Exacerbation and remission of pulmonary micronodules with lymphangioleiomyomatosis around the time of childbirth. Jpn J Radiol 2013

3 Hayashi T, Fleming MV, Stetler-Stevenson WG, et al. Immunohistochemical study of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in pulmonary lymphangioleiomyomatosis (LAM). Hum Pathol 1997; 28:1071-1078

4 Kumasaka T, Seyama K, Mitani K, et al. Lymphangiogenesis-mediated shedding of LAM cell clusters as a mechanism for dissemination in lymphangioleiomyomatosis. Am J Surg Pathol 2005; 29:1356-1366

5 Bissler JJ, McCormack FX, Young LR, et al. Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangioleiomyomatosis. N Engl J Med 2008; 358:140-151

6 McCormack FX, Inoue Y, Moss J, et al. Efficacy and safety of sirolimus in lymphangioleiomyomatosis. N Engl J Med 2011; 364:1595-1606

7 Seyama K, Kumasaka T, Souma S, et al. Vascular endothelial growth factor-D is increased in serum of patients with lymphangioleiomyomatosis. Lymphat Res Biol 2006; 4:143-152

8 Young LR, Lee HS, Inoue Y, et al. Performance of serum VEGF-D as a biomarker of Lymphangioleiomyomatosis severity and treatment response: A prospective analysis from the MILES Trial. Lancet Resp Med 2013.

9 Baldi BG, Albuquerque AL, Pimenta SP, et al. Exercise performance and dynamic hyperinflation in lymphangioleiomyomatosis. Am J Respir Crit Care Med 2012; 186:341-348

10 Scheinfeld N. A comprehensive review of imatinib mesylate (Gleevec) for dermatological diseases. J Drugs Dermatol 2006; 5:117-122

11 Clements D, Markwick LJ, Puri N, et al. Role of the CXCR4/CXCL12 axis in lymphangioleiomyomatosis and angiomyolipoma. J Immunol 2010; 185:1812-1821

12 Swigris JJ, Lee HS, Cohen M, et al. St. George's Respiratory Questionnaire Has Longitudinal Construct Validity in Lymphangioleiomyomatosis. Chest 2013; 143:1671-1678

13 Errasti P, Izquierdo D, Martin P, et al. Pneumonitis associated with mammalian target of rapamycin inhibitors in renal transplant recipients: a single-center experience. Transplant Proc 2010; 42:3053-3054

14 Law BK. Rapamycin: an anti-cancer immunosuppressant? Crit Rev Oncol Hematol 2005; 56:47-60

15 Thanopoulou E, Judson I. The safety profile of imatinib in CML and GIST: long-term considerations. Arch Toxicol 2012; 86:1-12