Phase 2 Study of Sildenafil for the Treatment of Lymphatic Malformations

Study Protocol and Statistical Analysis Plan

NCT02335242

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Sildenafil for the treatment of lymphatic malformations

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Test Medication: Revatio®

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1. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS:

Abbreviation	Definition
AE	Adverse Event(s)
AF	Anchoring Fibrils
ALT	Alanine Transaminase
AST	Aspartate Transaminase
BSA	Body Surface Area
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CFR	Code of Federal Regulation
cGMP	Cyclic Guanosine Monophosphate
cm^2	Centimeters squared or square centimeters (area)
CPK	Creatine Phosphokinase
CRF	Case Report Form
CT	Computed Tomography
CYP3A	Cytochrome P_{450} 3A
DSMB	Data Safety Monitoring Board
EOS	End of Study
EOT	End of Treatment
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
Hgb	Hemoglobin
IB	Investigator's Brochure
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IND	Investigational New Drug
IRB	Institutional Review Board
IUD	Intrauterine Device
LDH	Lactate Dehydrogenase
LM	Lymphatic Malformation
N/A	Not Applicable
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hgb
MCHC	Mean Corpuscular Hgb Concentration
MRI	Magnetic Resonance Image
RBC	Red Blood Cells
RDW	RBC distribution width
SAE	Serious Adverse Event(s)
SMP	Safety Monitoring Plan
SOP	Standard Operating Procedures
UP	Unanticipated Problems
UPT	Urine Pregnancy Test
WBC	White Blood Cells

2. SYNOPSIS

Study Phase: 2

Financial Disclosure Information for U.S. FDA Submission to be Obtained? Yes

Test Medication:	Sildenafil 20 mg tablets, 2.5mg/ml suspension (Revatio®)
Control Medication:	Placebo tablets or suspension resembling Revatio®
Study Dosage / Usage:	Oral dosage of sildenafil. For pediatric patients 8 kg to 20 kg the suspension/tablets dose will be 10 mg/4ml three times a day (30 mg per day) and for patients > 20 kg the dose is 20 mg/8ml three times a day (60 mg a day). Subjects less than 8 kg will not be enrolled in the trial.
Route of Administration:	The medication will be given orally. The subjects who will receive the 10 mg dose will be given an oral suspension (using dose calculation by the study pharmacist) or half a tablet. Subjects will have the option of taking tablets or receiving an oral suspension of the medication in 2.5mg per mL.
Objective(s):	The objectives of this study are to assess the safety and effectiveness of sildenafil taken orally to improve or resolve lymphatic malformations.
Study Population:	Sixty Subjects (60) Subjects will be between the ages of 6 months to 10 years of age inclusive with lymphatic malformations (LM) that have a **minimum diameter of 3 centimeters** based upon MRI evaluation. Based on the patient numbers in pediatric dermatology clinics over the country, we expect that we will be able to screen 60 patients who will meet the inclusion criteria, and 55 patients will be enrolled in the study and begin medication. After 15 subjects are completed, an interim analysis will be performed to project effect size and estimate the total number of subjects required for the study. If after analysis of 15, 30 or 45 subjects we find that we are in the range of futility or efficacy, then we will notify the FDA as well as the DSMB, and begin to end the study by adding no additional subjects.
Structure:	Double-blind trial of placebo compared to sildenafil with an open label extension for subjects who received placebo. Two to one ratio of two sildenafil treated subjects who

receive active sildenafil to one placebo treated subject. During the twenty week evaluation visit, after completion of all evaluations, the randomization code will be broken and both the subject and investigator will know the results. The subjects who have randomized to placebo will have the option to begin a twenty week course of sildenafil. If the subjects who were on placebo elect to take active drug, the initiation visit can be completed at that time. The subject will then be followed with clinic visits at weeks 4, 12, 20 and 32 as indicated in the protocol. We will call the subjects at weeks 2, 8 and 16 to inquire about any questions that the subjects may have or any concerns. We will also request information on medication taken and their progress.

The subject's LMs will be categorized by the Pediatric Radiologist by the percent of the LM that is microcystic. Upon completion of the initial 15 subjects, the data will be evaluated in an interim analysis in order to determine if the percent of macrocystic component of the lesions suggests which subjects may have the best benefit. The initial 15 subjects will also be evaluated related to previous procedures, infections and other complications related to their lymphatic malformations. If specific characteristics of the LM are identified that suggests beneficial effect of the sildenafil, the protocol will be changed to enroll those subjects who are projected to have the best benefit. A new power analysis will be done to project the minimum numbers of subjects that should be enrolled further in the study.

Duration of Treatment: 20 weeks. Placebo subjects will complete 20 weeks of placebo and then have an option for 20 weeks of active sildenafil.

Duration of Assessment: The subjects will be evaluated for 20 weeks on therapy then followed for 12 weeks off therapy.

Open Label Extension: Placebo subjects will have an option to enter an open label treatment after they have completed 20 weeks of placebo.

Duration of treatment: Placebo 20 weeks
Washout Between Periods: none
Duration of Treatment: 20 weeks on active drug

After the completion of 20 weeks of sildenafil and 12 weeks of follow up, the subjects will have the option to be followed through an IRB approved long-term follow up trial not

covered in this IND application. That IRB trial would allow up to five years follow up on subjects and would only allow investigators to observe how the subject is doing long term, whichever treatment options the subject pursues in consultation with their regular doctor in that time. That planned follow up protocol will allow Stanford to contact the subjects several times a year in order obtain information about any therapy they may elect to follow, including sildenafil if their regular doctor prescribes it, and their response to any therapy.

Multi-Center: Yes

Number of Centers: Up to 4

Blinding:

Observer-Blind	Yes
Subject-Blind	Yes
Double-Blind	Yes

Randomization: Group Assignment Ratio: 2:1 randomization of two subjects who receive sildenafil to one subject who receives placebo.

Concurrent Control: No other treatments for lymphatic malformation will be allowed except antibiotics and/or surgical drainage if the lesions become infected. Treatment for recurrent infections in the lymphatic malformation will be allowed and documented during the trial. Concurrent medications will be documented. Subjects who require sclerosing agents or excisional surgery of their lymphatic malformation will be withdrawn from the trial.

Variable(s): PRIMARY: Change in lesion volume over twenty weeks of the test medication as evaluated by MRI examination. Comparison of placebo to sildenafil treated subjects.

SECONDARY: Change in lesion volume estimated using a soft tape measure to measure the length, width, and hemispheric measurement of each of the lymphatic malformations. (Berk, Berk et al. 2011) Completion of the form *Lymphatic Malformation Measurements.*

Subject's evaluation of the change in lesion clinical characteristics from baseline to 20 weeks by examining decreased discomfort, texture or thickness, distortion of normal anatomy or overall change obtained in the subject's

completion of *Subject's Assessment of Lymphatic Malformation Change* form.

Physician's evaluation of the lesion clinical characteristics on the day of the visit. This includes color, tenseness and texture, superficial component, deep component, and distortion of anatomical landmarks. This requires completion of the *Lymphatic Malformation Assessment* form for each LM site.

Physician's evaluation of the change in lesion clinical characteristics from baseline, such as change in texture or thickness, change in distortion of normal anatomy, and over change in lesions. This requires completion of the *Lymphatic Malformation Assessment of Change* form for each LM site.

Comparison of LM volume from MRI assessment to Body Surface Area (BSA) measurements before and after 20 weeks of sildenafil.

Blinded physician observer scored evaluation of clinical photographs taken prior to the initial dose of sildenafil or placebo and at the 20 week visit prior to the unblinding. Subjects who receive placebo and then enter the 20 week open label continuation will have additional photographs taken after 20 weeks of sildenafil.

Presence, frequency or absence of infection during trial.

Relationship of response to sildenafil to previous infections, sclerotherapy or surgery.

SAFETY: Adverse events and frequency of lesion infections.

Adverse Events: Both volunteered and elicited.

3. INTRODUCTION

3.1. BACKGROUND

Lymphatic malformations (LMs) are uncommon, congenital vascular anomalies that arise due to developmental dysplasia of the lymphatic network in utero.(Blei 2008) LMs are equally rare independent of sex or race.(Redondo, Aguado et al. 2011) About 65% of LMs are detected at delivery, 80% by 1 year of age, and 90% by 2 years of age

emphasizing their childhood expression.(Redondo, Aguado et al. 2011) The deeper ones may not be initially noticed but will become recognized as they increase in size or become infected. Although histologically benign, they are often locally invasive and affect the skin and soft tissues, as well as nerve, muscle or bone in some cases. Cystic hygromas are a specific type of congenital malformations of the lymphatic system that are commonly on the posterior neck. (Gallagher, Mahoney et al. 1999) They occur in about one in 1,775 live births (Howarth, Draper et al. 2005) to one in 2667 live births (Fisher, Partington et al. 1996) making them truly an orphan disease. The incidence may be higher as they can be associated with other fetal abnormalities resulting in spontaneous or induced abortions.(Fisher, Partington et al. 1996) In one series of 17 live born infants with cystic hygroma, 15 survived the first year of life yielding a survival rate of 88%.(Fisher, Partington et al. 1996) Lymphatic malformations often have considerable morbidity, associated with infection, bleeding and airway or vision obstruction as well as significant psychosocial concerns. (Greene, Perlyn et al. 2011) When present on the extremities, they often bleed with physical activity, may inhibit movement and ambulation, and are at times painful.

Clinical research in LMs has been largely neglected primarily due to the common misperception that only surgical and procedural interventions may help alleviate this disease. Unfortunately, despite the multiplicity of procedural therapies that exist, none are uniformly effective, all have the risk of significant adverse events, and no standard of care has been established.(Churchill, Otal et al. 2011; Greene, Perlyn et al. 2011) Sclerotherapy, surgical excision and laser ablation all require general anesthesia in the pediatric population, and even when initially effective, the LM may recur. In addition, many LMs are not resectable or able to be treated due to their infiltrative nature. Nonetheless, these lesions need to be treated due to risk of infection secondary to lymph stasis, acute enlargement as a reaction to infection or inflammation, disfigurement and pain. Unfortunately, active non-intervention is often the current recommendation for many patients with LMs due to the limitations of surgical and destructive modalities and lack of a disease-modifying medical therapy. Thus, many patients are forced to live with lesions which may inhibit movement and ambulation, can bleed and become infected, and are almost always of aesthetic and psychosocial concern.

A recent patient observation within our institution brought the possibility of using sildenafil to treat LMs to our attention. A female infant was born with a venous and lymphatic malformation affecting the entire right arm and upper chest with pleural, paravertebral, and para-aortic involvement. Examination findings included massive enlargement of the affected arm and chest wall. MRI study at 2 months of age confirmed that the affected areas were infiltrated with small, abnormal lymphatic spaces. Due to the nature and extent of the malformation, treatment with sclerotherapy, laser or surgery was not an option, and the patient was simply observed. She was diagnosed with pulmonary hypertension at 9 months of age after repeated admissions for respiratory distress, heart failure and failure to thrive. Sildenafil was started for the pulmonary hypertension, and the patient's mother noticed a decompression of the LM over the first 17 weeks of sildenafil. At 21 months of age, repeat MRI verified minimal

residual LM and continued severe pulmonary hypertension. Unfortunately, the patient was not able to be extubated after the study and she passed away due to cardiac and respiratory failure. The family refused an autopsy.

Sildenafil is well tolerated in both the adult and pediatric populations. It works by inhibiting phosphodiesterase isoform 5 (PDE5) and thus inhibits the breakdown of guanosine 3',5'-cyclic monophosphate (cGMP). PDE5 is found in high concentrations in vascular smooth muscle, as well as in the corpus cavernosum, platelets, and retina. (Shekerdemian, Ravn et al. 2002; Wang, Wu et al. 2003) It has also gained a second FDA approval for the treatment of patients with pulmonary arterial hypertension. (Pfizer 2007) Mortality associated with pulmonary hypertension is extremely high. Thus, despite the fact that sildenafil does not have an indication for the pediatric population in the United States of America, it has been used for pulmonary hypertension in the pediatric population with a very good results, an exceedingly benign side effect profile, and an excellent safety record. (Abrams, Schulze-Neick et al. 2000; Karatza, Bush et al. 2005; Barst, Ivy et al. 2012)

The possible mechanism for the success of sildenafil treatment in lymphatic malformations seems congruent with our current knowledge of LM pathogenesis and sildenafil's mechanisms of action. Although the pathogenesis of lymphatic malformations is not completely elucidated, it has been postulated that primitive lymphatic sacs separate from the normal network of developing lymph vessels during embryogenesis. A thickened coat of muscle fibers line these cisterns, and rhythmic contractions of the muscles increase intramural pressures leading to dilation.(Whimster 1976) On histopathology a muscular lymphatic channel is often present. Potential explanations for the therapeutic effects of sildenafil include simple smooth muscle relaxation, which may open the primary overactive muscular fiber leading to decreased intramural pressures and decompression of the malformation. Alternatively, simple smooth muscle relaxation could lead to secondary lymphatic channels opening, allowing decompression of the malformation. Both of these explanations intuitively seem very plausible. There are also many other possible explanations why sildenafil may work in lymphatic malformations including sildenafil's secondary effect on nitric oxide via its inhibition of cGMP as endothelial nitric oxide synthetase.

We initially started an IRB approved clinical trial giving the subjects 12 weeks of sildenafil therapy. We have currently enrolled 4 subjects in this trial. One subject enrolled, but because of study visit conflicts elected to not receive sildenafil. One subject had a suggestion of improvement at 8 weeks of treatment, but that subject had surgery on one of the lesions 8 weeks into the study and although the clinical lesion appeared improved the surgery decreased our ability to evaluate the results at the completion of the 12 week study. One subject completed the study with no other interventions. That subject had 25% to 50% improvement by the 12 week visit. The sildenafil was stopped as planned at 12 weeks and follow up at 16 weeks (4 weeks off sildenafil) documented expansion of the lesion. The parents of the first two children enrolled in the study requested continuation of the sildenafil and a prescription was given and follow up off the study was planned. Those children are now in an IRB

approved long term follow up study. The initial IRB approved protocol was changed to 20 weeks of sildenafil. The final subject completed the enrolment and has completed 12 weeks of sildenafil with 25% to 50% improvement.

One of the problems that we have recognized in this study is the inaccuracy of physical examination as the method to evaluate the response to therapy. The lesions seem to demonstrate improvement by first becoming soft when they were firm before. The skin develops a sagging redundant feel to palpation but the measurements of the volume of the lesions are slow to change because of the elasticity of the skin and slow retraction of redundant skin. In the proposed study, we will use MRI examinations to measure the volume change in the lesion over time as well as comparing subjects treated with sildenafil to subjects who initially receive placebo.

3.2. RATIONALE

Recently propranolol, a commonly used medication for hypertension, has been shown to have impressive efficacy in the treatment of infantile hemangiomas. (Leaute-Labreze, Dumas de la Roque et al. 2008) The new indication for this “old” drug was discovered when two children with infantile hemangiomas and heart problems (one with cardiac myopathy, the other with increased cardiac output) were placed on propranolol. Unexpectedly, their hemangiomas involuted quickly and dramatically after the initiation of the medication. To verify the effect of propranolol on hemangiomas, 9 other infants with infantile hemangiomas but no heart disease were then treated with propranolol, and all had rapid resolution of their infantile hemangiomas. (Leaute-Labreze, Dumas de la Roque et al. 2008) In addition, propranolol was well-tolerated and has a better side effect profile than oral corticosteroids, the traditional treatment for problematic hemangiomas. Additional studies have verified that hemangiomas respond quickly to propranolol, and the medication has been used successfully to treat not just infantile hemangiomas affecting the skin but of the airway and liver, as well as those complicated by ulceration.(Frieden and Drolet 2009; Sans, de la Roque et al. 2009; de Graaf, Breur et al. 2011; Fuchsmann, Quintal et al. 2011) After our own striking and serendipitous observation, we are hopeful to verify the effect of sildenafil on LMs. If successful, sildenafil has the potential to have just as large an impact in the treatment of LMs as propranolol has with hemangiomas. There is no effective nonsurgical treatment for this orphan disease.

3.3. OBJECTIVE

The objective in this study is to assess the safety and efficacy of sildenafil in reducing the volume and symptoms of lymphatic malformations when administered to subjects who have lymphatic malformations greater than 3 cm in diameter.

4. TEST MEDICATION

4.1. IDENTIFICATION

Test medication: Sildenafil (Revatio®) 20 mg tablets or suspension
Control Medication: Placebo or suspension resembling Revatio®

4.2. PACKAGING AND LABELING

The test medications will consist of Sildenafil 20 mg tablets (Revatio®) and Placebo tablets that resemble Revatio®. These medications will be shipped in bulk containers to the Lucile Packard Children's Hospital (LPCH) Pharmacy where they will be stored.

Each bottle will be labeled in accordance with Good Manufacturing Practice and will include the following information:

- Study Acronym Name
- Unique alpha-numeric identifier (Lot number)
- Storage requirement
- Investigational use and appropriate caution statements
- Sponsor identification
- Unique subject ID

The LPCH Pharmacy will dispense the Revatio® or Placebo in bottles labeled with the subjects identification information based upon the randomized code. The LPCH Pharmacist will compound an extemporaneously prepared oral suspension from Revatio® 20mg tablets or placebo tablets, depending on subject's preference. Pharmacy staff will know if the subject is receiving Placebo or Revatio®. Depending on subjects weight, the study pharmacist will formulate study drug into an oral suspension for 2.5mg per ml dosing volume.

Subjects. who weigh 8kg – 20kg will receive a 4-week supply 10mg/4ml TID, and the treatment will be compounded into a 480ml dosing volume, and then transferred to four bottles of 120 ml each.

Subjects weighing more than 20kg will receive a 4-week supply 20mg/8ml TID. The subject and the research physician will be blinded to Placebo or Revatio® until completion of the 20 week study visit. The code will also be broken in the situation of a Serious Adverse Event.

4.3. TEST MEDICATION ACCOUNTABILITY PROCEDURES

When a delivery of test medication is received at the investigational site, the Pharmacist, or a member of their staff specifically authorized and delegated by the Investigator, will check for accurate delivery and acknowledge receipt by signing (or initialing) and dating the required documentation and returning it as instructed. The

date, time, and condition of the package should also be documented at the time of receipt. A copy of this documentation will be retained for the Investigator file.

The LPCH Pharmacist will compound Revatio® 20 mg film-coated tablets or placebo. The pharmacy instructions for compounding are listed in section 17.1.

The packing list will indicate the expiry date for the material. Receipt and dispensing of the study treatment will be carefully recorded on appropriate accountability forms and made available for verification at each monitoring visit.

Used medication containers will be retained until checked by study monitor, after which they will be discarded at the site. Unused medication will be accounted for at the end of the trial and appropriately discarded at the site.

For study visits conducted at non-Stanford sites, medication will be prepared in the LPCH Pharmacy and shipped directly to the subject from Stanford for each visit.

A study monitor will perform an accounting of all test medication supplies, used or unused, at the study centers.

5. SUBJECTS

5.1. SUBJECT POPULATION

Subjects should be between the ages of six months and ten (10) years of age with the clinical diagnosis of lymphatic malformation that appears to be greater than 3 cm in diameter. Subjects will have a minimal weight of 8 kg to enter the trial. The diagnosis will be confirmed by an MRI examination performed within six (6) months of screening visit. The MRI will confirm that the lesion at least 3 cm in diameter and the lesion is either:

i. At least 50% of the lesion contains non-enhancing regions separated by septae, or
ii. No vessels in the lesion, or only a few small vessels that traverse the lesion but are not intrinsically a portion of the lesion, and no flow voids or only a few small flow voids that traverse the lesion but are not intrinsically a portion of the lesion.
iii. The volume of the total lesion will be calculated as well as the proportion of the lesion that is macrocystic will be calculated.

The enrollment goal will be forty subjects who have completed the twenty weeks of therapy followed by an MRI. We expect to enroll up to sixty (60) subjects.

The upper age limit is ten years old because as patients age they may have recurrent infections and scarring which may decrease our ability to evaluate the response of the lymphatic malformation to sildenafil. We will document previous therapies or interventions for the lymphatic malformation and previous infections in the lymphatic malformation.

5.2. INCLUSION CRITERIA

Subjects will be considered qualified for enrollment if they meet the following criteria:

1. Legally authorized representative of subjects willing and able to give consent. Assent will be obtained for subjects between the ages of 7 and 10 years old.

2. Subjects will need to be 6 months of age to 10 years of age at the time of entry into the study.

3. The minimum weight of a subject will need to be 8 kg at the time of enrollment.

4. Subjects will be required to have the clinical diagnosis of lymphatic malformation that appears to be over 3 cm in greatest diameter in order to be evaluated for entry. A review of a previous MRI examination may help confirm the entry criteria on subjects selected to come to Stanford for the MRI screening. The MRI must be performed within six (6) months of the screening visit if used as a baseline measure.

5. The lymphatic malformation should be causing enough disability for the subject requiring them to consider systemic therapy.

6. Females must not be pregnant or breast-feeding.

7. Female subjects of childbearing potential have negative urine-pregnancy test results.

8. A parent or legally authorized representative must be willing and able to ensure subject is present for all required study visits.

9. A parent or legally authorized representative must be able to follow instructions.

10. During the initial screening evaluation portion of the trial the subjects MRI examination will be required to confirm that the lymphatic malformation is present and is greater than 3 cm in diameter in order for the subjects to receive medication. MRI of less than six months is valid to use during screening with the discretion of the study radiologist.

11. Subjects will have no contraindications for the use of sildenafil.

12. Subjects will have a normal eye examination.

13. Subjects will have normal liver and kidney function.

14. Subjects will have no contraindication to MRI examinations such as metal implants, etc.

15. Subject must not be a smoker.

5.3. EXCLUSION CRITERIA

A participant will be ineligible if he/she meets any of the following criteria:

1. The participant has a medically unstable health status that may interfere with his/her ability to complete the study.

2. The participant presents with one or more of the following medical conditions: Hepatic impairment, severe renal impairment, lymphedema conditions such as Milroy disease, Meige lymphedema, Hennekam syndrome, Njolstad syndrome, Aagenaes syndrome, and Fabry disease, hypotension or at risk for hypotension, seizures or history of seizures, any significant cardiovascular risk factors and any condition which requires participants to use nitric oxide donors or nitrates in any form, underlying anatomic or vascular risk factor for developing non-arteritic anterior ischemic optic neuropathy (NAION) including low ocular cup to disc ratio, diabetes, hypertension, coronary artery disease, or hyperlipidemia Participants with Down syndrome, Turner syndrome and Noonan syndrome will be considered on a case-by-case basis.

3. The participant has received at least one of the following medications contraindicated in association with sildenafil within 15 days of inclusion:

- Organic nitrates in any form, either regularly or intermittently -- Consistent with its known effects on the nitric oxide/cGMP pathway, sildenafil was shown to potentiate the hypotensive effects of nitrates.
- Ritonavir and other Potent CYP3A Inhibitors --- Concomitant use of REVATIO with ritonavir and other potent CYP3A inhibitors is not recommended.
- Alpha-blockers --- co-administering alpha-blockers with REVATIO because of additive blood pressure-lowering effects
- Amlodipine
- Cimetidine

4. The participant requires concomitant use of potent cytochrome P450 3A4 inhibitors (such as ketoconazole, itraconazole, erythromycin, saquinavir), or concomitant use of ritonavir. Also excluded are concomitant use of organic nitrates, alpha-blockers, amlodipine, or cimetidine.

5. The screening MRI cannot confirm that the lesion is a lymphatic malformation or the lymphatic malformation is less than 3 cm in its greatest diameter.

6. The patient has had extensive prior surgery or sclerotherapy to treat LM such that scarring may interfere with evaluation and treatment effect of sildenafil.

7. Patients who have had recurrent infection and significant scarring of the lesion secondary to infection to such an extent that the that scarring may interfere with evaluation and treatment effect of sildenafil

8. Participant is currently pregnant or considering becoming pregnant in the next 20 weeks.

9. The participant is known to have an allergy to sildenafil.

10. Ulcerated or currently infected LMs.

11. Diagnosis of the soft tissue tumor as LM is not clinically certain.

12. The participant is participating in another clinical study which may interfere.

13. The participant has a history of priapism or is diagnosed with sickle cell anemia or any other disorder which may predispose to priapism.

14. The Investigator may declare any subject ineligible for a valid medical reason.

15. Sirolimus should not be taken or applied within the last six months.

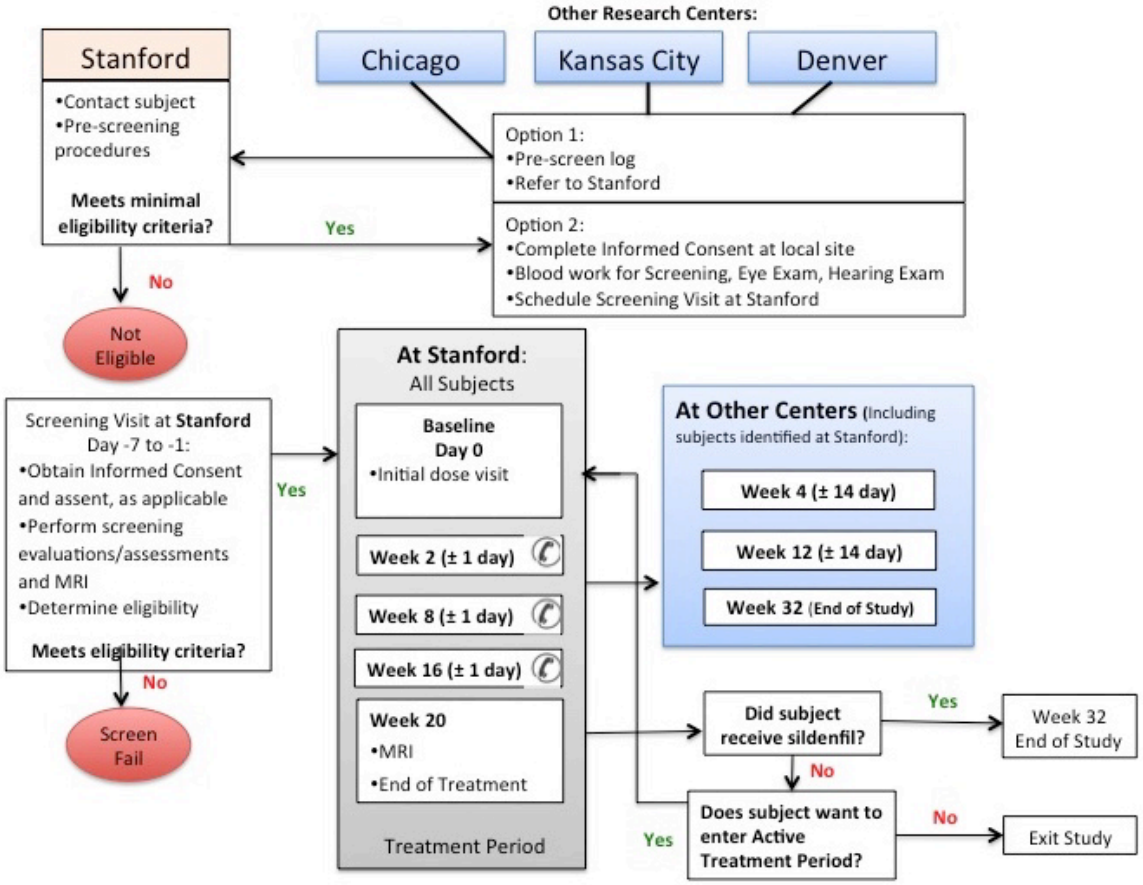
5.4. SCREENING LOG

All screened subjects initially considered for inclusion in this study will be documented. Each study site will maintain a Pre-Screening Log of identified potential subjects for the trial. Stanford will contact each potential subject for pre-screening procedures, detailed in section 7.1.1 on Page 18. If a subject is excluded from the study, the reasons for exclusion will be documented in the subject's source documents and noted on the Screening Log at Stanford and in the Pre-Screening logs at each site.

6. STUDY DESIGN

6.1. STUDY DESIGN

Figure 6.1-1: Study Design Schematic. Decision points are indicated in bold text. Treatment periods are indicated. Double lined boxes indicate visits that will occur at the subject's closest site, whether at Stanford or at a Cooperating Research Site (Denver, Chicago, Kansas City).



This will be a double blind placebo controlled trial with a ratio of 2 sildenafil subjects to 1 placebo subject in the Initial Treatment Period. Sildenafil or placebo will be given for 20 weeks.

60 research subjects will be enrolled.

There will be visits at Screening, Initial dose visit (includes a 2 hour observation period after initial dose), 4 weeks, 12 weeks, 20 weeks and 32 weeks visits. MRI examination will be part of the screening visit and will be repeated prior to the completion of medication at the 20 week visit. For subjects identified at Stanford, all visits will be conducted at Stanford University Medical Center. For subjects identified at other sites, all Screening, Initial Dose Visit, and Week 20 visits will be conducted at Stanford, including both MRIs. All other visits (weeks 4, 12, 32) will be conducted at the subject's local site. These subjects will be given the option to have the blood work for Screening done at their local site or at Stanford. They will also be given the option to have the eye exams and hearing exams at Screening and Week 20 done at their local site or at Stanford. Having these tests done locally at screening may make it easier to re-test if there is a change in vision or hearing while on sildenafil.

At the completion of 20 weeks of medication or placebo the subject will be evaluated by MRI, and will undergo an eye exam and a hearing exam. After the MRI, eye exam, and

hearing exam are completed, the subject will be evaluated by the investigator, and the subject will complete the self evaluation of response. The Investigator will complete the physician evaluation forms. After these evaluations are completed, the blind will be broken and the investigator and subject will know if the subject received placebo or sildenafil. The subject will also be informed of the of the MRI results of the screening MRI and the 20 week MRI once that report is available. Subjects who received placebo for the first 20 weeks will then have the option of starting 20 weeks on active drug.

A second method to evaluate the volume of the MRI will be done in a blinded manner by a pediatric radiologist, assisted by a pediatric radiology fellow. We will use segmentation to identify the surface area demonstrating LM involvement of multiple planes though the lesion. By calculating the surface area of multiple planes and knowing the distance between planes, the volume of the LM can be calculated. This is called segmentation analysis. The research radiologist and fellow will evaluate multiple MRIs in groups at the same time, unaware of the date or the subject, therefore blinded to treatment, placebo, or pretreatment. This evaluation will blinded in order to eliminate the bias of knowing who the subject is in the study. Although this is a research procedure, the results will be reported in the subjects Radiology Report in the Medical Record as an addendum. It will be signed by the research radiologist. This evaluation will be available several weeks after the subject completes the 20 weeks of sildenafil and/or placebo. The MRI will be read and the data recorded by the research radiologist without knowledge of the date the MRI was done. Once the data is collected and recorded, the research radiologist will learn name of the subject and the date of the MRI in order to attach the correct volume calculation to the correct MRI when placing the volume data in the Medical Record.

The volume data on the subject's LM(s) relative to the subject's BSA will then also be evaluated for any changes. Since all subjects will be children under 10 years old, using the BSA measurement may help to adjust our volume assessment to accommodate the amount that a subject has grown over the course of the course of treatment.

The process of screening will include an eye examination, hearing test, renal and liver function tests, and a MRI of the lesion. If the screening tests meet the inclusion and exclusion criteria the subject will be then be randomized to receive either placebo or sildenafil and entered into the trial. A hearing test can be difficult to accomplish if the child does not cooperate. If the hearing test is not successful at screening, we will repeat the test at subsequent visits until we obtain a hearing test result that is satisfactory to the pediatric audiologist. If the subject is referred from one of the cooperating research study sites, then we will give the subject the option to have the eye and hearing tests done at their closest cooperating research study site or at Stanford. In addition, we will attempt to obtain the hearing test at the cooperating research study site if the test is not successful during the screening at Stanford.

Initial dose of medication will be given in the dermatology clinic at Stanford before the two hour observation period. The subjects will then have follow-up visits at 4 weeks after beginning medication, 12 weeks after beginning medication and 20 weeks after

beginning medication. Subjects local to Stanford will be seen at Stanford for all visits. Subjects local to other cooperating study sites will be seen at Stanford for Screening, Initial Dose Visit, and Week 20, and at their local cooperating research study site for Week 4, Week 12, and Week 32. These subjects will also be given the option to have their Week 20 eye and hearing exams done at the cooperating research study site or at Stanford. Laboratory tests will be conducted at Stanford for baseline and follow-up. The Stanford site will contact the subject by phone at 2 weeks, 8 weeks and 16 weeks. Subjects who received placebo during the first 20 weeks of evaluation will have the option at the twenty-week visit of commencing use of the active drug for the next twenty weeks. If the subject who initially received placebo decides that they do not want to receive sildenafil for 20 weeks they will then complete the data collected at the 32 week visit in addition to the 20 week visit. The subject will not need to return for the 32 week visit. If the subject who initially received placebo selects to start the medication they will then be seen at the 4 week, 12 week and 20 week visit after commencing medication. Subjects local to other cooperating research study sites will attend the Week 4, Week 12, and Week 32 visits at their local site and Week 20 at Stanford. The medication will be stopped after the 20 week visit and the subject will be seen at Week 32, having been off medication for 12 weeks. The subject will have an additional MRI done at Stanford at the completion of the twenty weeks of active medication. Subjects on placebo who elect to receive the active drug will have three MRI examinations. Subjects on active medication will be requested to stop the medication in order to be followed over the next twelve weeks with no medication (week 32).

With the subject's permission, we will send a report to the subject's primary and specialty care physicians with the data that was obtained on the subject during the study, including the MRI LM research blinded segmentation results. Subjects will be returned to their primary care physician or specialty care physicians for follow-up at completion of the study. The subject can work with their local physician to decide to continue or not continue the sildenafil into the future independent of this research protocol and IND. As part of a separate IRB approved follow up protocol independent of this IND and after informed consent, we will request the subject to allow us to contact them several times a year for the next five years to ask how their lymphatic malformation lesion has progressed or changed and what additional therapies they have received.

6.2. METHODS USED TO MINIMIZE BIAS

All subjects will have the option to receive 20 weeks of sildenafil in this open label extension study design. Subject assignment to sildenafil or placebo in the initial treatment period will be random.

7. STUDY PROCEDURES

For a summary of the required procedures by visit, refer to Table 17-1: Study Procedures by Treatment Day.

7.1. VISITS AND EXAMINATIONS

7.1.1. Prescreening communications

Authorization will be obtained by the Stanford Institutional Review Board (IRB) to screen subjects on the phone after they contact us. Subjects from the cooperating research sites will be referred to contact the Stanford study staff where they will be prescreened by Stanford study staff and all prescreening records kept at Stanford. The subjects will also obtain our contact information from ClinicalTrials.gov or through a local physician or community resource that is aware of our study. Potential subjects who contact the coordinator will undergo a telephone screening, using a script approved by the Stanford IRB, to verify that they meet the initial inclusion criteria for this trial. Potential subjects who meet the initial criteria will be mailed information about the study, including consent forms for their review. We will also send and ask them to sign and send back to us a release to obtain and examine their medical records in order to have more details about their current condition.

Once the prescreening communication documents that the patient is appropriate for the trial, the patient will be scheduled for a screening visit at the Stanford Dermatology Clinics. All screening visits may be conducted at Stanford, even for patients identified at non-Stanford cooperating research study sites. Patients identified at non-Stanford cooperating research study sites will be given the option to have the laboratory tests, ophthalmology evaluation and hearing test locally before coming to Stanford for the other screening tests. If they opt to do these locally, they will sign a consent form at the cooperating research study site prior to these exams. The visit at Stanford will be set up so that the subject can receive the screening evaluation, MRI, ophthalmology evaluation (if not done at the cooperating site), hearing test (if not done at the cooperating site), and laboratory tests (if not done at the cooperating site) during a two to three day visit to the Stanford site.

7.1.2. Visit 1 – Screening Visit [usually Day -7 to -1] at Stanford

NOTE: Any subject for whom an informed consent/assent has been obtained, but fails to meet the required entry criteria is considered to be a screen failure. All demographic information must be captured with the reason for screen failure specified.

1. Explain the purpose and nature of the study, and have the subject's parent or legally authorized representative, read, sign, and date the IRB-approved informed consent document. Even subjects who may have signed a consent form at the cooperating research study site prior to laboratory, eye and hearing exams will sign the Stanford consent form at this visit. The subject's assent to participate in the study will be obtained if the subject is between 7 and 10 years of age. The informed consent document must be signed and dated by the individual who consents the subject. A photocopy of the signed informed consent document will be provided to the parent or legally authorized representative. A copy of the informed consent will also be placed in the subject's medical record. The original copy will be maintained in the research files.

----- Do not proceed until consent has been obtained -----

2. Screen the subject against protocol inclusion and exclusion criteria (Sections 5.2, 5.3). Subjects not meeting the criteria are screen failures and must exit the study at this point.

3. Obtain demographic information and medical history, including information on all medications currently being taken at the time of signing the informed consent. Instruct the subject and/or caregiver of the excluded medications and give them a list. Document previous treatments for the lymphatic malformation and previous infections in the lymphatic malformation.

4. Review the subject's previously obtained MRI, X-rays or CT scans and other laboratory test results with the subject.

5. Perform a physical examination including heart and pulmonary auscultation, liver and spleen palpation, abdominal auscultation and palpation, and documentation of peripheral pulses. Examine the characteristics of their lymphatic malformation to see that it meets the criteria for the trial.

6. Obtain Vital Signs including height, weight, temperature, heart rate, blood pressure, respiratory rate, and oxygen saturation.

7. If the patient meets all the screening criteria then they will then be sent for an eye examination (if not done at the cooperating site), hearing test (if not done at the cooperating site), laboratory test evaluating a complete blood count (CBC) in addition to testing their liver and kidney function (if not done at the cooperating site) (see section 17.1), and an MRI. We will also obtain a pregnancy test on females who have achieved menarche.

7.1.3. Baseline Study [Day 0 (medication dispensed)] at Stanford

This will usually be the day after the screening.

1. Review inclusion and exclusion criteria with the subject, including the results of their laboratory data, eye examination, and MRI. If subject no longer meets criteria for participation, exit the subject from the study as a screen failure.

2. Obtain demographic information and medical history, including information on all medications currently being taken. Document concomitant medications. Instruct the subject and/or caregiver of the excluded medications and give them a list if they do not have their list that was previously given to them.

3. Perform a physical examination including heart and pulmonary auscultation, liver and spleen palpation, abdominal auscultation and palpation, and documentation of

peripheral pulses. Examine and describe the characteristics of their lymphatic malformation to see that it meets the criteria for the trial.

4. Obtain Vital Signs including height, weight, temperature, heart rate, blood pressure, respiratory rate, and oxygen saturation.

5. Clinical assessment of each lymphatic malformation including measuring the hemicircumference of the lesion by using a soft measuring tape placed on the skin over the area of the widest diameter of the lesion. The length, and width, will then be calculated as described in Figure 2 of Berk et al.(Berk, Berk et al. 2011) In order to document the site of the measurements a diagram will be drawn by hand or photographs will be taken of the methods. Each clinic visit will try to duplicate the measurements methods used in the initial visit.

6. Physician's evaluation of the lesion clinical characteristics on the day of the visit. This includes color, tenseness and texture, superficial component, deep component, and distortion of anatomical landmarks. This requires completion of the *Lymphatic Malformation Assessment* form for each LM site.

7. Site investigator will acquire digital photographs of each lymphatic malformation as described in Section 7.4.4, and summarized as follows:
    - Subject will be seated on a chair. If subject is an infant, then subject's parent or guardian will be seated on a chair with subject sitting on his/her knees or in his/her arms.
    - All photographs will include a color chart in the field of view, held in the same plane as the target lymphatic malformation.
    - Distance and angle of each photograph relative to subject will be documented at baseline and repeated for all subsequent photographs.
    - Photograph 1: front-on view of each lymphatic malformation.
    - Photograph 2: side-on view of each lymphatic malformation (to visualize thickness).

8. Review possible adverse events included in informed consent.

9. Medication journals will be given and instructions reviewed.

10. Study medication dose for 1 month will be calculated and obtained from the pharmacy. Subjects will receive an oral suspension of the study medication (see Section 18.2 for pharmacy instructions).

11. The first dose of the medication will be consumed in the clinic. The subject will remain within the clinic area and temperature, heart rate, blood pressure, respiratory rate, and oxygen saturation will be repeated every 30 minutes for the initial 2 hours after the initial dose.

12. Instruction sheet will be given to the subjects documenting contact information for the research physicians and the foods and medications to be avoided while on sildenafil.

13. If there are no safety issues identified during the 2 hours of observation, the participant or parent/guardian is dispensed enough study treatment until the next visit at Week 4 and the participant may leave the site. The subject and/or caregiver will again be instructed of the excluded medications and the list will again be reviewed. The subject will be advised to stop medication and notify the study team immediately if they notice a change in vision or hearing or if they feel dehydrated.

14. If a subject approves, we will send a copy of the dictated record for the study visit to their local primary care doctor and/or pediatric dermatologist and other specialty physicians that the subject requests. If the subject is to be followed at one of the local cooperating research sites, a copy to the record will be sent to the center. The MRI results, ophthalmology report, hearing test report and laboratory data will also be sent to the subject's local cooperating research site. We would like to be sure that the physicians in their home community are aware of the study and the subject's participation in the study.

7.1.4. Weeks 2, 8, and 16 [± 1 day]

Study staff at Stanford will call the subject on the phone and query subject and/or caregiver regarding any changes in general health and the use of concomitant medications. We will review previous and current concomitant medications. We will instruct the subject and/or caregiver of the excluded medications and answer any question that the subject may have.

7.1.5. Weeks 4 and 12 [± 14 days before or after scheduled visit] at subject's cooperating research site or at Stanford if the subject is local to Stanford.

1. If the subject is being seen at a cooperating research site the subject will need to review the purpose and nature of the study with the local investigator at the cooperating site. If not already done before local laboratory tests, eye and hearing exams prior to the screening visit at Stanford, the subject's parent or legally authorized representative must read, sign, and date the IRB-approved informed consent document for the local cooperating research site. The subject's assent to participate in the study will be obtained if the subject is between 7 and 10 years of age. The informed consent document must be signed and dated by the individual who consents the subject. A photocopy of the signed informed consent document will be provided to the parent or legally authorized representative. A copy of the informed consent will also be placed in the subject's medical record. A copy will be sent the Stanford. The original copy will be maintained in the research files. This consent will document the cooperation of the Stanford site and the local cooperating research site**.**

2. Query subject and/or caregiver regarding any changes in general health and the use of concomitant medications. Obtain demographic information and review interim medical history, including information on all medications currently being taken. Review previous concomitant medications and current concomitant medications. Instruct the subject and/or caregiver of the excluded medications and give them a list if they do not have their list with them.

3. If any adverse events are observed or reported, they must be recorded as instructed in Section 13, Adverse Events.

4. Review criteria for treatment discontinuation. If treatment must be permanently discontinued, proceed to Section 7.1.12 for Early Exit.

5. Perform a physical examination including heart and pulmonary auscultation, liver and spleen palpation, abdominal auscultation and palpation, and documentation of peripheral pulses. Examine and describe the characteristics of their lymphatic malformation.

6. Obtain Vital Signs including height, weight, temperature, heart rate, blood pressure, respiratory rate, and oxygen saturation.

7. Clinical assessment of each lymphatic malformation including measuring the hemicircumference of the lesion by using a soft measuring tape placed on the skin over the area of the widest diameter of the lesion. The length, and width, will then be calculated as describe in Figure 2 of Berk et al (2011). In order to document the site of the measurements a diagram will be drawn by hand or photographs will be taken of the methods. Each clinic visit will try to duplicate the measurements methods used in the initial visit.

8. Completion of subject's evaluation of the change in lesion clinical characteristics from baseline, such as decreased discomfort, texture or thickness, distortion of normal anatomy or overall change obtained in the subject's completion of *Subject's Assessment of Lymphatic Malformation Change* form.

9. Completion of the physician's evaluation of the lesion clinical characteristics on the day of the visit. This includes color, tenseness and texture, superficial component, deep component, and distortion of anatomical landmarks. This requires completion of the *Lymphatic Malformation Assessment* form for each LM site.

10. Physician's evaluation of the change in lesion clinical characteristics from baseline, such as change in texture or thickness, change in distortion of normal anatomy, and over change in lesions. This requires completion of the *Lymphatic Malformation Assessment of Change* form for each LM site.

11. Count study drug and perform compliance assessment; medication journal reviewed.

12. Study medication dose will be calculated based upon the weight of the subject during the visit. The Stanford site will be notified of the weight and dose. The Stanford physician will notify the pharmacy of the subject number and proposed dose. The Stanford pharmacy will dispense adequate study medication (sildenafil or placebo) to last until the next visit. The medication will be shipped directly to the subject's home.

13. Review possible adverse events included in informed consent.

14. New medication journals reviewed and instructions reviewed.

15. Arrange a MRI examination to be done before the 20 week visit at Stanford.

16. Instruction sheet will be given to the subjects documenting contact information for the research physicians and excluded medications and foods while on sildenafil if they do not have the instruction sheet previously given to them.

17. If a subject approves, the Stanford site or the local cooperating research site will send a copy of the dictated record for the study visit to their local primary care doctor and/or pediatric dermatologist and other specialty physicians that the subject requests. If the subject is to be followed at one of the local cooperating research sites, a copy to the record will be sent to the Stanford site. We would like to be sure that the physicians in the subject's home community are aware of the study and the subject's participation in the study.

7.1.6. Week 20 [± 21 days before or after scheduled visit] at Stanford

End of Treatment for subjects treated with sildenafil. Possible commencement of treatment for those subjects on placebo.

1. Query subject and/or caregiver regarding any changes in general health and the use of concomitant medications. Obtain demographic information and review interim medical history, including information on all medications currently being taken. Review previous concomitant medications and current concomitant medications. Instruct the subject and/or caregiver of the excluded medications and give them a list if they do not have their list with them.

2. If any adverse events are observed or reported, they must be recorded as instructed in Section 13, Adverse Events.

3. Ophthalmology evaluation, hearing exam, and blood draw performed. Subjects from cooperating research study sites will have the option to do these tests at their local site or at Stanford.

4. Perform a physical examination including heart and pulmonary auscultation, liver and spleen palpation, abdominal auscultation and palpation, and documentation of

peripheral pulses. Examine and describe the characteristics of their lymphatic malformation.

5. Obtain Vital Signs including height, weight, temperature, heart rate, blood pressure, respiratory rate, oxygen saturation.

6. Clinical assessment of each lymphatic malformation including measuring the hemicircumference of the lesion by using a soft measuring tape placed on the skin over the area of the widest diameter of the lesion. The length, and width, will then be calculated as describe in Figure 2 of Berk et al (2011). In order to document the site of the measurements a diagram will be drawn by hand or photographs will be taken of the methods. Each clinic visit will try to duplicate the measurements methods used in the initial visit.

7. Photographs of the Lymphatic malformation will be taken using standardized procedures described in Baseline Visit.

8. Completion of subject's evaluation of the change in lesion clinical characteristics from baseline, such as decreased discomfort, texture or thickness, distortion of normal anatomy or overall change obtained in the subject's completion of *Subject's Assessment of Lymphatic Malformation Change* form.

9. Completion of the physician's evaluation of the lesion clinical characteristics on the day of the visit. This includes color, tenseness and texture, superficial component, deep component, and distortion of anatomical landmarks. This requires completion of the *Lymphatic Malformation Assessment* form for each LM site.

10. Physician's evaluation of the change in lesion clinical characteristics from baseline, such as change in texture or thickness, change in distortion of normal anatomy, and over change in lesions. This requires completion of the *Lymphatic Malformation Assessment of Change* form for each LM site.

11. Collect study drug and perform compliance assessment; medication journal reviewed.

12. Obtain a pregnancy test if the subject is has achieved menarche.

13. The participant or parent/guardian and the investigator will complete the qualitative assessment of efficacy. The subject's evaluation of the response to the treatment will be reviewed. The results report of the MRI done at the twenty week visit will be shared with the subject and compared with the initial MRI reported results. After that discussion the subject will be told if they were on sildenafil or placebo. Subjects who received placebo will be given the option to start the sildenafil. Subjects who received active medication will be requested to stop the sildenafil for the next twelve weeks and follow up at the 32 week visit to examine the response of the lesion off of the sildenafil.

14. If subject received placebo and decides to receive sildenafil at this time, the study medication dose will be calculated and obtained from the pharmacy for adequate study medication to last until the next visit in 4 weeks. The 2 hour monitoring will be completed after the first dose as described in Baseline visit. For more detail, see Section 7.1.7. If the subject was on placebo and elects not to start sildenafil they will not need to return for the 32 week evaluation.

15. If the subject received placebo and decides to not receive sildenafil they will end the study at this time and complete additional information needed for the End of study 32 week visit described in 7.1.7. below.

16. If a subject approves, we will send a copy of the dictated record for the study visit to their local primary care doctor and /or pediatric dermatologist so that the physicians in their home community are aware of the study and the subject's participation in the study.

7.1.7. End of Study 32 weeks [± 2 Weeks] at subject's cooperating research site or at Stanford if the subject is local to Stanford for subjects who are on active sildenafil for the initial twenty weeks of the trial

1. Query subject and/or caregiver regarding any changes in general health and the use of concomitant medications. Obtain demographic information and review interim medical history, including information on all medications currently being taken. Review previous concomitant medications and current concomitant medications. Instruct the subject and/or caregiver of the excluded medications and give them a list if they do not have their list with them.

2. If any adverse events are observed or reported, they must be recorded as instructed in Section 13, Adverse Events.

3. Perform a physical examination including heart and pulmonary auscultation, liver and spleen palpation, abdominal auscultation and palpation, and documentation of peripheral pulses. Examine and describe the characteristics of their lymphatic malformation.

4. Obtain Vital Signs including height, weight, temperature, heart rate, blood pressure, respiratory rate, and oxygen saturation.

5. Clinical assessment of each lymphatic malformation including measuring the hemicircumference of the lesion by using a soft measuring tape placed on the skin over the area of the widest diameter of the lesion. The length, and width, will then be calculated as describe in Figure 2 of Berk et al (2011). In order to document the site of the measurements a diagram will be drawn by hand or photographs will be taken of the methods. Each clinic visit will try to duplicate the measurements methods used in the initial visit.

6. Completion of subject's evaluation of the change in lesion clinical characteristics from baseline, such as decreased discomfort, texture or thickness, distortion of normal anatomy or overall change obtained in the subject's completion of *Subject's Assessment of Lymphatic Malformation Change* form.

7. Completion of the physician's evaluation of the lesion clinical characteristics on the day of the visit. This includes color, tenseness and texture, superficial component, deep component, and distortion of anatomical landmarks. This requires completion of the *Lymphatic Malformation Assessment* form for each LM site.

8. Physician's evaluation of the change in lesion clinical characteristics from baseline, such as change in texture or thickness, change in distortion of normal anatomy, and over change in lesions. This requires completion of the *Lymphatic Malformation Assessment of Change* form for each LM site.

9. If a subject approves, we will send a copy of the dictated record for the study visit to their local primary care doctor and/or pediatric dermatologist and other specialty physicians that the subject requests. If the subject is to be followed at one of the local site cooperating research site, a copy to the record will be sent to Stanford. We would like to be sure that the physicians in their home community are aware of the study and the subject's participation in the study. By this visit the results of the segmentation analysis should be in the subjects Medical Record as an Addendum. Besides sharing this information with the subject, the information will be available for the subject's physicians.

10 After this visit the Stanford site will request the subject's parent or guardian's consent for a separate Stanford IRB-approved long term follow up protocol. If the subject is between 7 and 10 years old, we will request their assent. We will contact the subject on the phone if they are not at the Stanford site for the 32 week visit.

7.1.8. Baseline Active [Day 0 (medication dispensed)] at Stanford for subjects who are on placebo for the initial twenty weeks of the trial

For those subjects who were on placebo and request that they begin sildenafil, the first dose of the medication will be dispensed in the clinic, as described in the original baseline visit, Section 7.1.3. This visit will follow the same procedures described in Section 7.1.3.

7.1.9. Weeks 2 Active, 8 Active, and 16 Active [± 1 day]

These phone calls will follow the same procedures described in Section 7.1.4.

7.1.10. Weeks 4 Active and 12 Active [± 14 days before or after scheduled visit] at subject's cooperating research site for subjects who are on placebo for the initial twenty weeks of the trial

These visits will follow the same procedures described in Section 7.1.5.

7.1.11. Week 20 Active [End of Treatment, ± 21 days before or after scheduled visit] at Stanford for subjects who are on placebo for the initial twenty weeks of the trial

This visit will follow the same procedures described in Section 7.1.6, except that there is no unblinding and no option to start a treatment period of active sildenafil.

7.1.12. Week 32 Active [End of Study, ± 2 Weeks] at subject's cooperating research site for subjects who are on placebo for the initial twenty weeks of the trial

This visit will follow the same procedures described in Section 7.1.7.

7.1.13. Early Exit at subject's cooperating research site or at Stanford

In addition to the procedures required at the EOT visit:

1. The subject will be exited from the study.
2. Study medication will be collected. The subject may elect to not return to Stanford or the cooperating research site for this visit. In this case, we will have them mail the unused medication back to Stanford. We will give them pre-addressed shipping forms. If the subject does not return for this visit, we may also arrange to get the pregnancy test at a local hospital or local office.

7.1.14. Unscheduled Visits at subject's local site or at Stanford

Unscheduled exams may be conducted at the discretion of the Investigator with all obtained information recorded in the source documents. Subjects will be requested to have their local physician also send us any information on visits they may have with their local physician related to the lymphatic malformation or lymphatic malformation treatment.

7.1.15. Concomitant Medications

A concomitant medication is any drug or substance administered from the signing of the informed consent for the study through the last study visit Items 3 and 4 in Section 5.3 (Exclusion Criteria) list those medications that are not permitted during the study period. Investigational drugs or devices: The use of concomitant therapies must be documented in the subject's source documents.

7.2. DISCONTINUED SUBJECTS

Discontinued subjects are those who voluntarily discontinue participation, who are withdrawn for safety reasons or use of prohibited concomitant treatments, or who have missed a sufficient number of study visits, procedures or test medication doses as to be ineligible for further participation.

All subjects who discontinue the study prior to completing the regularly scheduled visits must complete the EOT and Exit Visit procedures described in Section 7.1.11. Any changes in medical health and/or the use of concomitant treatments must be captured. If any adverse events were observed since the previous visit, they must be recorded

(refer to Section 13, Adverse Events). Finally, if appropriate, the Investigator also should advise the subject's parent or legally authorized representative of subsequent therapy and/or procedures necessary for their medical condition.

In the case of subjects who persistently fail to return for scheduled visits, three attempts must be made to contact the subject's parent or legally authorized representative for the purpose of scheduling an Exit/EOS Visit. The third attempt should be via registered mail, return receipt requested.

7.3. SUBJECT PREGNANCY

Females of child-bearing potential (defined as post-menarche as documented in the medical history) are not excluded from the study, though we do not expect to enroll sexually active females since the upper age limit is 10 years old. However, if a subject becomes pregnant during the study, the investigators should be contacted immediately and the medication should be stopped. Pregnancy is not reportable as an adverse event; however, complications related to the pregnancy may be reportable as determined on a case-by-case basis. Pregnancy-related information will be collected until termination of the pregnancy. The subject who becomes pregnant will be removed from the trial.

7.3.1. Birth Control Measures

Females of childbearing potential (those who are post-menarche as documented in the medical history) may participate in the study if they meet all of the following conditions:

- they are not breast feeding;
- they have a negative urine or serum pregnancy test at screening;
- they agree to undertake a urine pregnancy test at the completion of week 20 of treatment or placebo
- they agree to undertake a urine pregnancy test upon exiting the study if they elect to withdraw prior to the week 20 pregnancy test;
- they do not intend to become pregnant during the study;
- they are using adequate birth control methods and they agree to continue using those methods for the duration of the twenty (20) weeks of treatment or placebo.

Adequate birth control methods are defined as: hormonal—topical, oral, implantable or injectable contraceptives; mechanical—spermicide in conjunction with a barrier such as a condom or diaphragm; IUD; or, surgical sterilization of partner. For non-sexually active females, abstinence may be regarded as an adequate method of birth control; however, if the subject becomes sexually active during the study, she must agree to use an adequate birth control method as described above for the remainder of the study. Since the oldest age at entry for this trial is 10 years of age we expect all females who have completed menarche to not be sexually active but we will still discuss this topic during the visits in a sensitive way with the parent or guardian so that the child will not be embarrassed during the visit. We will discuss this topic with the child in accordance with the wishes of the parent or guardian.

7.4. STUDY METHODS AND MEASUREMENTS

7.4.1. Demographic information

General demographic data include age, sex, race, and ethnicity. Location of the lymphatic malformation, age at diagnosis, confirmatory diagnostic tests (previous MRIs, biopsies, etc), family history of lymphatic malformation, and associated complications, e.g., infection, disability.

7.4.2. Vital signs

Vital sign measurements include measurement of temperature (oral or tympanic membrane), resting pulse rate, respiration rate, oxygen saturation, systolic and diastolic blood pressure; along with height and weight.

7.4.3. Lymphatic malformation assessment

The most precise method of lymphatic malformation evaluation will be the MRI examination. Clinical assessment of each lymphatic malformation includes measuring the hemicircumference of the lesion by using a soft measuring tape placed on the skin over the area of the widest diameter of the lesion. The investigators will be fully trained in the standardized methods to obtain these measurements. We have found in our preliminary subjects that the investigators vary in their measurement of length, width and hemispheric measurement unless they practice these measurements together in order to standardize their methods. The length, and width, will then be calculated as described in Figure 2 of Berk et al.(Berk, Berk et al. 2011) We will use linear mixed model to compare the changes in volume over time between and within subjects who receive placebo and those who receive sildenafil. In order to document the site of the measurements a diagram will be drawn by hand or photographs will be taken of the methods. Each clinic visit will try to duplicate the measurements methods used in the initial visit.

7.4.4. Photography methods

The digital photographs of each lymphatic malformation in this study will be acquired by the site investigators. The investigators will be fully trained in the standardized acquisition procedures. At least two digital photographs of the lymphatic malformation will be acquired for each subject at the baseline visit and the 20 week visit. Photographs may be taken at other visits but they will not be part of the final analysis. The first photograph will be taken with the image plane parallel to the lymphatic malformation (front-on view) and the second photograph will be taken with the image plane at a different angle to that of the first photograph (side-on view) so that the thickness of the lesion can be clearly visualized. The subject will be sitting on a chair while photographs are taken. If subject is an infant, subject's parent or guardian will be sitting on a chair with the infant sitting on his/her knees, if possible, or in his/her arms. In the field of view, all photographs will include a color chart for color and size calibration. Patient identification information, date of the photograph and therapy information will not be visible on the photograph. For each photograph acquisition, the color chart will be held in the same plane as the lymphatic malformation. The photographs will be acquired at a fixed distance from the patient at two different angles defined at baseline (one for Photograph 1 and one for Photograph 2).

The photographs will serve the following purposes:
1: To identify the lymphatic malformation or malformations if the subject has more than one malformation.
2: To qualitatively assess lymphatic malformation evolution between visits and to quantify the size and the color of the lymphatic malformation at baseline and Week 20.

7.4.5. Laboratory tests

The CBC and chemistry panel is detailed in Appendix 17. These are to be assessed at the Screening Visit and End of Treatment Visit (Week 20). All required lab tests must have been completed. At the discretion of the PI or designee, a topical lidocaine-based anesthetic (eg. Lidocaine 4% cream) may be used prior to clinical laboratory sample collection to decrease potential discomfort to the subject. A pregnancy test will be done at the end of treatment for females of childbearing potential.

8. DATA MANAGEMENT PLAN

Paper source documents will be prepared prior to study start. These source documents will be used in clinic to collect data for the study. Data will be collected at study visits, using the prepared data collection forms. All source documents will be retained in the subject's study binder. Source documents can include, but are not limited to:

- Signed informed consent documents
- Data collection forms (source documents)
- Laboratory reports
- MRI reports
- Dictations/summary reports

REDCap, an electronic data capture (EDC) system, will be utilized in this study as an electronic case report form (eCRF). REDCap is a HIPAA-secure database system available through Stanford at http://redcap.stanford.edu.This system can only be accessed by authorized users, and only users with permission to view identifiers will be able to view PHI. Users must sign into REDCap using their Stanford University ID and password. Additionally, users may only access the database from a Stanford IP address, or via a Virtual Private Network (VPN). Prior to study start, a database will be designed for this study that incorporates the data points collected in the source documentation. The Stanford site will receive a copy of the paper source documents from the local cooperating research sites after every visit of the subject at the local site. The Stanford site will place the information from the local site into REDCap.

After each study visit, data will be transcribed from paper source documents into the electronic data capture (EDC) REDCap system. Hard copies of source documentation will also be retained and stored in the subject binder. Photographs, laboratory reports, and dictations will be uploaded to REDCap.

Study staff will be responsible for "cleaning" the source document data and EDC prior to monitoring at each site. This includes but is not limited to: making sure that dates are

logical, reasons for concomitant medication have a corresponding adverse event, making sure that GCP is followed, etc.

At monitoring visits at Stanford, the study monitor will verify that the data entered into REDCap matches the data recorded in the source documentation. REDCap data will be printed and the monitor will verify that this information is the same as in the source documentation.

Data from REDCap will be exported to excel, SPSS, or SAS for analysis. When exporting data, PHI will not be included in the dataset.

9. DATA SAFETY MONITORING BOARD

A DSMB will be developed to monitor the study. The details of the DSMB are described in the Safety Monitoring Plan (SMP), in Item 10, section 2 of the IND application. The independent DSMB will assure that this trial is performed to the highest scientific and ethical standards. Prior to the start of the study, the DSMB will review the Clinical Protocol, Safety Monitoring Plan, Informed Consent forms, and proposed DSMB-specific reporting forms. Once the study has started, the DSMB will evaluate the scientific progress and safety of the trial, consider outside information that is relevant to the study, review proposed changes to the Clinical Protocol, ensure data integrity, ensure confidentiality of trial data and monitoring results, and make recommendations to the PI regarding the trial. The DSMB will meet prior to the start of the trial and then at least twice annually until 1 year after the last subject completes the last Week 32 or Week 32-A visit. Ad hoc or emergency meetings will occur as needed and the procedures for planning those meetings are described in detail in the Safety Monitoring Plan.

10. STATISTICAL DESIGN

10.1. EVALUABILITY

All subjects who receive study medication will be evaluable for the safety analysis. All subjects who receive study medication will be evaluable for the intent-to-treat analysis.

10.2. EFFICACY

All subjects who receive study medication will be evaluated for efficacy.

10.2.1. Primary Efficacy

Summary statistics will be determined for change in lesion volume by a radiologist blinded to the time of the MRI and whether the patent was on treatment or placebo. The initial MRI will be compared to the MRI after 20 week of sildenafil or placebo. If the subject on placebo elects to take sildenafil for 20 weeks the MRI used for comparison after 20 weeks of sildenafil will be the MRI taken after 20 weeks of placebo.

We will include in the interim analysis information about the characteristics of LMs. We will do a sub-analysis looking at specifics of location, lesion type (microcystic, macrocystic, or mixed and percent macrocystic), as well as previous procedures, infections or other interventions. If this process documents characteristics of lesions that do or do not respond to sildenafil, we will reconsider our study entry criteria and submit a protocol amendment for the IND and this grant, focusing more on specific characteristics of which type of lesion a subject should have in order to be enrolled in the trial.

10.2.2. Secondary Efficacy

The secondary efficacy variables will be change from baseline and at visits four weeks twelve weeks, twenty weeks and 32 weeks.

These will include the changes in the soft tape measurements of the lesion by comparing the measurements of length, width, and hemispheric measurement. The volume of the lymphatic malformation will be estimated based on these measurements.

Statistical analysis of the *Subject's Assessment of Lymphatic Malformation Change* form. This will give data on the subject's evaluation of the change in lesion clinical characteristics from baseline, such as decreased discomfort, texture or thickness, distortion of normal anatomy or overall change by correlating the data obtained in the subject's completion of *Subject's Assessment of Lymphatic Malformation Change* form.

Statistical analysis of the *Lymphatic Malformation Assessment* form for each LM site. This will give data on the physician's evaluation of the lesion clinical characteristics on the days of the visits. This includes color, tenseness and texture, superficial component, deep component, and distortion of anatomical landmarks.

Statistical analysis of the *Lymphatic Malformation Assessment of Change* form for each LM site. This will give data on the physician's evaluation of the change in lesion clinical characteristics from baseline, such as change in texture or thickness, change in distortion of normal anatomy, and over change in lesions.

Change in LM volume from MRI assessments in relation to BSA will also be carried out. REDCap calculates the BSA from height and weight measurements at each visit. Since growth can be non-linear, relating the LM volume to a subject's BSA may help to explain why a small change to the LM (by MRI volume analysis) is assessed as moderate by the investigator and parents of the subject (i.e., the LM may have stayed the same size but the child grew quickly, so the LM appears smaller by visual assessment).

The digital photographs of each lymphatic malformation in this study will be acquired by the site investigators. At least two digital photographs of the lymphatic malformation will be acquired for each patient at each scheduled study visit, a front-on view and a side-on view. See Section 7.4.4. The clinical photographs evaluated by blinded observers in a blinded manner by physicians not associate with this trial. The observers of the

photographs will compare the baseline photographs and score the additional visits as worse, no change, 1-25%, 26-50%, 51-75%, 76-100% improvement. We will also examine the potential value of 3D photography during the Baseline, 20 Week and 20-A Week visit.

Presence, frequency or absence of infection during trial will be documented.

10.2.3. Exploratory Efficacy
Not applicable.

10.3. SAFETY
All subjects who receive study medication will be evaluable for the safety analysis. Summary statistics will be provided with respect to patients requiring discontinuation from treatment and from study (any reason and for safety reasons). AE summaries will focus on treatment-emergent AE and will summarize subject AE incidences by severity, seriousness, and relationship to treatment. Vital signs, laboratory tests, and concomitant medication usage will be displayed as listings by subject.

11. SAMPLE SIZE JUSTIFICATION AND STATISTICAL ANALYSIS:
This is an exploratory, descriptive study for an orphan disease. The sample size is based on the prevalence of the disease and the availability of the subjects for the study. Based on our clinic numbers and expected numbers of subjects who will travel for this study, we expect 60 subjects to qualify for the study.

We will also perform analysis of responders vs. non-responders, taking multiple measurements for placebo patients into account, treating the patient as random effect, adjusting for age, sex and possibly other markers.

One of the potential trigger points to stop this study will be if milestones are reached that document statistically significant beneficial effect of sildenafil requiring no additional placebo treated subjects, or possibly no beneficial effect. We have reviewed the details of our study with our biostatistician. Because this is a very early study, we are unable to estimate the response to therapy and develop an effect size. For that reason we will do an interim analysis after completion of the first 15 subjects, 10 treated with sildenafil for the first 20 weeks and 5 treated with placebo for 20 weeks. We will evaluate the first 15 subjects for efficacy and futility and add to this analysis each additional 15 subjects as they complete the study. We should be able to use the interim analysis to project effect size and estimate the total number of subjects required for the study. If after analysis of 15, 30 or 45 subjects we find that we are in the range of futility or efficacy, then we will notify the FDA as well as the DSMB, and begin to end the study by adding no additional subjects.

We will include in the interim analysis information about the characteristics of LMs. We will do a sub-analysis looking at specifics of location, lesion type (microcystic,

macrocystic, or mixed and percent macrocystic), as well as previous procedures, infections or other interventions. If this process documents characteristics of lesions that do or do not respond to sildenafil, we will reconsider our study entry criteria and submit a protocol amendment, focusing on specific characteristics of which type lesion a subject should have in order to be enrolled in the trial.

12. ADVERSE EVENTS

12.1. GENERAL INFORMATION

Adverse event (AE): any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. AEs include any new events not present during the pre-intervention period or events that were present during the pre-intervention period, which have increased in severity.

All adverse events that occur during the course of the study will be recorded in the source documentation including the event's onset and resolution date (if the event has resolved), the frequency and severity of the event, a summary of any action taken as a result of the event (both in regard to any treatment given and to any change in dosing with the test medication), and an assessment of the event's relationship to the test medication.

12.2. NON-SERIOUS ADVERSE EVENTS

A nonserious adverse event is an adverse event that does not meet the requirements for a serious adverse event (see Section 13.3). Nonserious adverse events will be documented in study records and reported to the FDA in the annual IND report. All nonserious adverse events will be recorded in study documentation regardless of whether or not they are considered to be related to the test medication.

12.3. SERIOUS ADVERSE EVENTS

Life threatening AE: Any AE that places the subject, in the view of the investigator, 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.

Serious Adverse Event (SAE): Any adverse event that, in the view of the investigator, results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical

intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

12.4 REPORTING OF SERIOUS ADVERSE EVENTS

12.4.1. Reporting to FDA

For serious adverse events (SAE), a MedWatch Form FDA 3500A containing all available information must be sent to the FDA within 15 days of the Investigator's knowledge of the event; new information should be reported by telephone or fax as soon as it becomes available. Fatal or life-threatening SAEs will be reported to the FDA within 7 days of the Investigator's knowledge of the event. We will also inform Pfizer, the manufacturer of sildenafil (Revatio®), about the SAE. SAEs at any given site will be communicated to Stanford immediately by phone or email and reported to the FDA by Stanford on the timelines described above. All coordinating sites will be informed of SAEs promptly.

A MedWatch Form FDA 3500A must be completed for all serious adverse events and forwarded to the FDA within 15 days of the Investigator's knowledge of the event and to the Institutional Review Board/Independent Ethics Committee, according to their requirements. When new significant information is obtained as well as when the outcome of an event is known, the Investigator must inform FDA by telephone or fax. Fatal or life-threatening SAEs will be reported to the FDA within 7 days of the Investigator's knowledge of the event

12.4.2. Reporting to IRB

Unexpected deaths or life-threatening experiences related to the research must be reported to the IRB within 5 working days from the when the site's PI learns of the event. All SAEs (as defined in section 13.3) will be reported to the IRB within 10 working days from when the site's PI learns of the event.

Unanticipated problems involving risks to participants or others, or Internal Events that are unexpected and related to the research should be reported to the IRB within 10 working days from when the site's PI learns of the event or new information, or receives assessment from monitoring entity.

Additional reportable events include: unanticipated problems (UPs) involving risks to participants or others (including internal or external events, deaths, life-threatening experiences, injuries, breaches of confidentiality, or other problems) that occur any time during or after the research study.

Additional Reportable information:

- New information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency
- Protocol deviation. Protocol deviations/violations are to be reported only if:
  - Intended to eliminate apparent immediate hazard to a research participant, or
  - Harmful, or
  - Possible serious or continued noncompliance
- Complaint that is unresolved by the research team, or that indicates increased or unexpected risks
- Incarceration when, in the opinion of the PI, it is in the best interest of the participant to remain on the study

12.4.3. Reporting to DSMB

Reporting to the DSMB is described in detail in the Safety Monitoring Plan, under Item 10, section 2 of the IND application. In brief, SAEs will be reported as soon as possible to the DSMB Safety Officer, who will determine if it is necessary to convene the entire DSMB, and any other events that are potentially reportable to other regulatory agencies will also be reported to the DSMB.

12.5. ASSESSMENT OF ADVERSE EVENT SEVERITY, CAUSALITY, AND EXPECTEDNESS

Unexpected AE: Any adverse event, the specificity or severity of which is not consistent with the risk information described in the Investigator's Brochure.

Severity: Severity of the AE will be determined by the investigator. Severity may be mild, moderate, or severe:

- Mild – An event is mild if the subject is aware of, but can easily tolerate the sign or symptom
- Moderate – An event is moderate if the sign or symptom results in discomfort significant enough to cause interference with the subject's usual activities
- Severe – An event is severe if the sign or symptom is incapacitating and results in the subject's inability to work or engage in their usual activities

In addition, the Investigator will determine if the adverse event is may have been caused by the test product. The following circumstances would be considered to be possibly related:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome).
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture).
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation

or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

In source documentation, causality will be recorded using the following definitions:

- Not Related – An event is considered to be not related to the use of the test medication when the event is DEFINITELY UNRELATED or UNLIKELY to have any relationship to the use of the test medication
- Possibly Related – An event is considered to be possibly related to the use of the test medication when there is a REASONABLE POSSIBILITY that the test medication caused the adverse event

12.6. UNBLINDING OF TEST MEDICATION

If a subject develops an adverse reaction that requires withdrawal from the study we will document whether they are receiving placebo or active medication. The treatment assignment will also be unblinded during the twenty week visit after the MRI examination is completed.

12.7. FOLLOW-UP OF SUBJECTS WITH ADVERSE EVENTS

For subjects who are experiencing ongoing unresolved adverse events at the time of their study completion or early discontinuation from the study, the Investigator will schedule an appropriate follow-up visit in order to determine the outcome of the event.

13. INVESTIGATOR OBLIGATIONS

The Principal Investigator will comply with the commitments outlined in the Statement of Investigator (Form FDA 1572), with current Good Clinical Practice (GCP), and with all applicable regulatory requirements as outlined in Appendix 18.2 of this protocol. Additional information on Investigator Obligations is listed in Item 10, section 1 of the IND application.

14. INVESTIGATOR AND MONITOR RESPONSIBILITIES

The Investigator will designate a monitor to conduct the appropriate site visits monitoring at the appropriate intervals. The clinical investigation will be monitored to ensure that: the rights and well being of the subjects are protected; the reported data are accurate, complete, and verifiable from the source documents; and the study is conducted in compliance with the current approved protocol, with current GCP, and with applicable regulatory requirements.

Monitoring will be conducted periodically while the clinical study is ongoing. Monitoring methods may include site visits, telephone, written and fax correspondence. The study monitor will contact the site at appropriate intervals.

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16. STUDY PLAN

Table 16-1: Study Procedures by Treatment Day

Visit:	Screening *(Day -7 to +1)*	Baseline & Baseline-A[1] *(Day 0)*	Week 2 & Week 2-A[1] *(-/+ 1 Day)*	Week 4[6] & Week 4-A[1,6] *(-/+ 14 Days)*	Week 8 & Week 8-A[1] *(-/+ 1 Day)*	Week 12[6] & Week 12-A[1,6] *(-/+ 14 Days)*	Week 16 & Week 16-A[1] *(-/+ 1 Day)*	Week 20 & Week 20-A[1] *(-/+ 14 Days)*	End of Study[6]/ Early Exit[5,6] (Week 32) *(-/+ 14 Days)*
Procedures:									
Informed Consent/Assent	x								
Inclusion/Exclusion criteria	x								
Vital signs (height, weight, HR, BP, temp)	x	x		x		x		x	x
Complete history	x								
Interval history		x	x	x	x	x	x	x	x
Physical examination	x	x		x		x		x	x
Phone call			x		x		x		
Assessment of LM by MD	x	x		x		x		x	x
Assessment of LM by participant				x		x		x	x
LM measurements	x	x		x		x		x	x
LM photographs	x	x		x		x		x	x
Laboratory tests	x[7]							x	
Urine pregnancy test	x[3]							x[3]	
Ophthalmology exam	x[7]							x[7]	
Hearing exam	x[4,7]							x[7]	
Administer and dispense study medication		x		x		x			
Monitor vital signs every 30 mins for 2 hours		x							
Collect unused study medication				x		x		x	
Dispense or review medication diary		x		x		x		x	x
End of treatment								x	
MRI	x							x	
Review adverse events		x	x	x	x	x	x	x	x
Unblind treatment								x[2]	
End of study/ Exit form									x

[1] The "-A" suffix at end of Visit name indicates visits that are for the crossover Active Treatment Period, an option offered to any subject who received placebo during the Original Treatment Period.

[2] At Week 20, treatment will be unblinded and if subject received placebo then they have the option to enter the Active Treatment Period. Unblinding is performed only at the Week 20 Visit of the Original Treatment Period.

[3] If subject is female who has reached menarche.

[4] If hearing exam is not successful at screening, it will be repeated at subsequent visits until a satisfactory hearing test result is obtained.

[5] Subjects who exit early will not have a final MRI but we will attempt to obtain a Urine Pregnancy Test.

[6] Subjects local to a cooperating research site will attend Week 4, Week 12, and Week 32 visits at their cooperating site.

[7] Subjects local to a cooperating research site will be offered the option to have this procedure at their cooperating site or at Stanford.

17. APPENDICES

17.1. LABORATORY TESTS

Laboratory tests will be obtained upon entry (baseline) and end of treatment into the clinical trial. The exams will include:

- Complete Blood Count:
  - Hematocrit
  - Hemoglobin
  - White Blood Cell Count (WBC)
  - WBC differential
  - Red Blood Cell Count (RBC)
  - MCV (Mean Corpuscular Volume)
  - MCH (Mean Corpuscular Hgb)
  - MCHC (Mean Corpuscular Hgb Concentration)
  - RDW (RBC distribution width)
  - Stained Red Cell examination (Peripheral Smear)
  - Platelet Count

- Complete Metabolic Panel:
  - Albumin
  - Alkaline Phosphatase Total
  - ALT
  - AST
  - Urea Nitrogen
  - Calcium
  - Chloride
  - CO2 Content
  - Creatinine
  - Glucose
  - Globulin
  - Potassium
  - Sodium
  - Total Bilirubin
  - Total Protein
  - Anion Gap

- Urine Pregnancy if required (See Schedule of Events)

17.2 INSTRUCTIONS OF ORAL SUSPENSION FOR PHARMACIST

17.2.1 Instructions for Pharmacist (2 pages)

page 1:

INSTRUCTIONS FOR PHARMACIST FOR

COMPOUNDING REVATIO® 20MG TABLETS

Compounding of an extemporaneously prepared oral suspension from Revatio® 20 mg film-coated tablets (final concentration 10 mg/ml). Compounding of an oral suspension, by a pharmacist, following this procedure, will provide one patient with enough medicinal product for a: 28-day course of treatment (1 ml dosing volume, 10 mg dose) or a 14-day course of treatment (2 ml dosing volume, 20 mg dose).

Ingredients:

The compounding of the Revatio® oral suspension from Revatio® 20 mg tablets uses Ora-Sweet and Ora-Plus diluents.

Compounding instructions for a pharmacist:

1. Ensure Ora-Sweet and Ora-Plus have been equilibrated to room temperature.

2. Count 62 (sixty two) x 20 mg Revatio tablets.

3. Using a mortar and pestle, crush these 62 tablets, 2-10 tablets at a time, into a fine powder.

4. Measure out 30 ml Ora-Plus (cloudy white liquid), allowing time for any air bubbles to dissipate.

5. Add a portion (15-20 ml typical) of Ora-Plus from step 4 to the mortar and mix into a thick homogeneous paste. More Ora-Plus from step 4 may be added if necessary.

6. Transfer the paste to an amber glass or high density polyethylene (HDPE) bottle (≥ 150 ml volume)

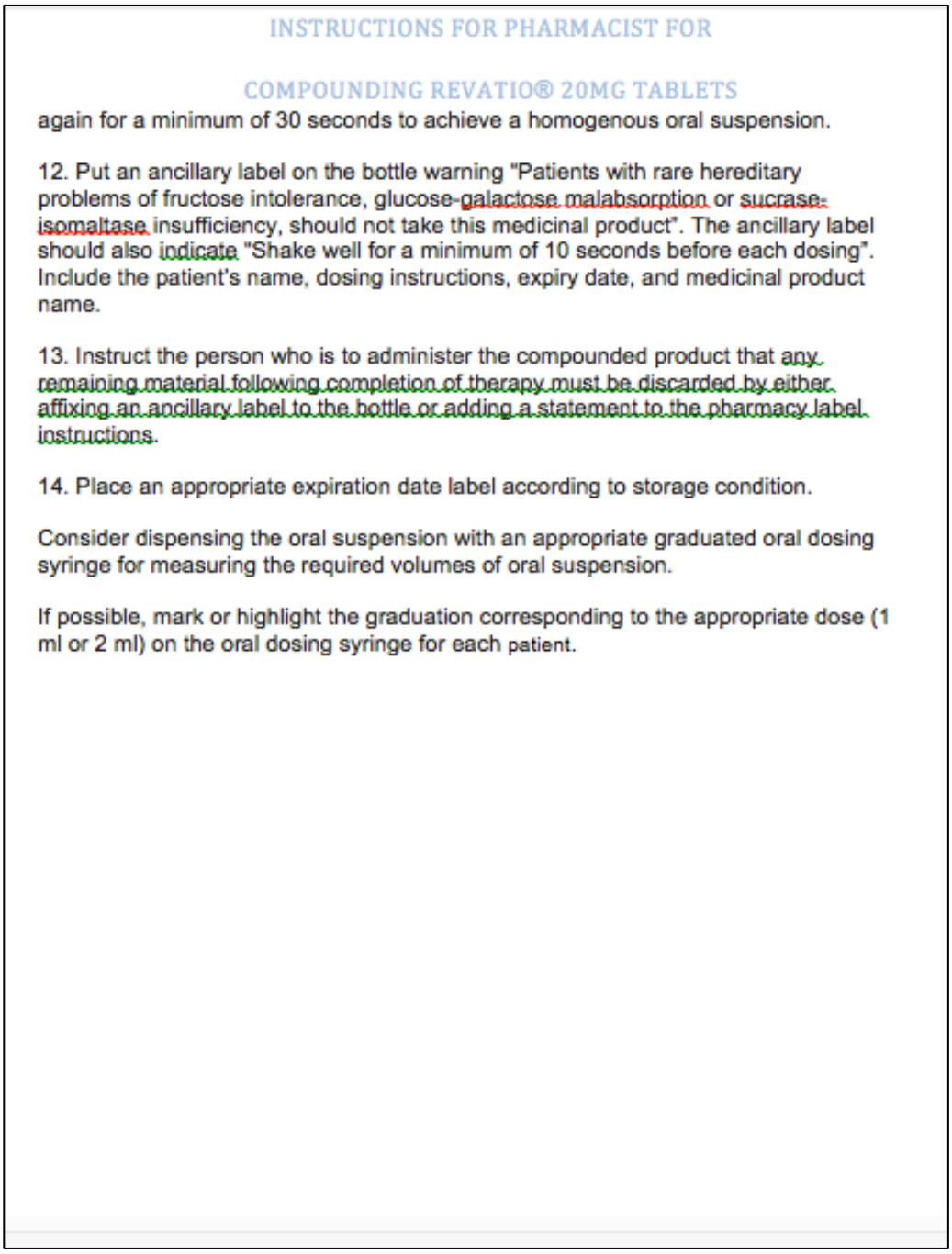
7. Rinse the mortar and pestle with the remaining Ora-Plus from step 4 and transfer rinses to the bottle to ensure complete transfer of the paste.

8. Measure 90 ml Ora-Sweet (clear pink liquid) allowing time for any air bubbles to dissipate.

9. Transfer approximately half of the volume of the Ora-Sweet from step 8 to the bottle containing the formulation prepared above.
10. Cap bottle and shake vigorously for a minimum of 30 seconds.

11. Transfer the remaining Ora-Sweet from step 8 to the bottle and shake vigorously

page 2:



INSTRUCTIONS FOR PHARMACIST FOR

COMPOUNDING REVATIO® 20MG TABLETS

again for a minimum of 30 seconds to achieve a homogenous oral suspension.

12. Put an ancillary label on the bottle warning "Patients with rare hereditary problems of fructose intolerance, glucose-galactose malabsorption or sucrase-isomaltase insufficiency, should not take this medicinal product". The ancillary label should also indicate "Shake well for a minimum of 10 seconds before each dosing". Include the patient's name, dosing instructions, expiry date, and medicinal product name.

13. Instruct the person who is to administer the compounded product that any remaining material following completion of therapy must be discarded by either affixing an ancillary label to the bottle or adding a statement to the pharmacy label instructions.

14. Place an appropriate expiration date label according to storage condition.

Consider dispensing the oral suspension with an appropriate graduated oral dosing syringe for measuring the required volumes of oral suspension.

If possible, mark or highlight the graduation corresponding to the appropriate dose (1 ml or 2 ml) on the oral dosing syringe for each patient.

17.2.2 Pharmacist Instructions for Oral Suspension of 10mg and 20mg

Subjects taking 20mg/8ml

LYMPHATIC MALFORMATION STUDY

Sildenafil 2.5mg/ml or Placebo suspension

COMPOUNDING FORMULA

		Lot#	*Exp Date*	*Rph1*	*RPh2*
Sildenafil 20mg or **Placebo**	105 tabs				
OraSweet	420ml				
OraPlus	420ml				

Use Study Sildenafil or Placebo tablet provided by Pfizer*

Date	Cpd by	Chk by	Exp Date	Appearance/Comments

Equipment: mortar and pestle, graduated cylinder, stirring rod

Preparation:

1-Count out 105 tablets of Sildenafil or Placebo.

2-Grind tablets in a mortar with a pestle, and triturate to a fine powder.

3-Mix 420ml of OraSweet with 420ml OraPlus. Use mixture as base solution.

4-Levigate with a small amount of base solution to form a uniform paste.

5-Add base solution in geometric proportions almost to 840ml, while mixing thoroughly.

6-Transfer contents of the mortar to a graduated cylinder.

7-Rinse the mortar and pestle with base solution and pour into graduated cylinder.

8-Add base solution to the graduated cylinder to achieve the total volume of 840ml.

9-Transfer contents of the graduated cylinder into bottles provided by the study.

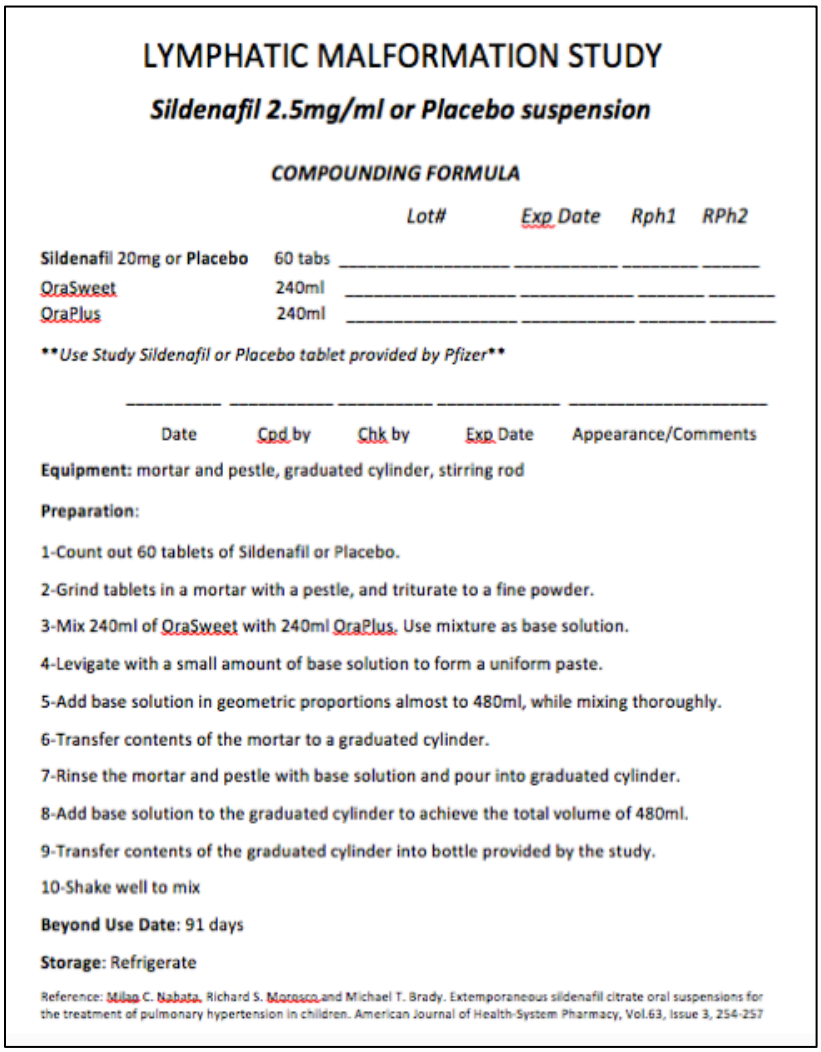
10-Shake well to mix

Beyond Use Date: 91 days

Storage: Refrigerate

Reference: Milap C. Nahata, Richard S. Morosco and Michael T. Brady. Extemporaneous sildenafil citrate oral suspensions for the treatment of pulmonary hypertension in children. American Journal of Health-System Pharmacy, Vol.63, Issue 3, 254-257

Subjects taking 10mg/4ml



LYMPHATIC MALFORMATION STUDY

Sildenafil 2.5mg/ml or Placebo suspension

COMPOUNDING FORMULA

		Lot#	*Exp Date*	*Rph1*	*RPh2*
Sildenafil 20mg or **Placebo**	60 tabs				
OraSweet	240ml				
OraPlus	240ml				

Use Study Sildenafil or Placebo tablet provided by Pfizer*

Date	Cpd by	Chk by	Exp Date	Appearance/Comments

Equipment: mortar and pestle, graduated cylinder, stirring rod

Preparation:

1-Count out 60 tablets of Sildenafil or Placebo.

2-Grind tablets in a mortar with a pestle, and triturate to a fine powder.

3-Mix 240ml of OraSweet with 240ml OraPlus. Use mixture as base solution.

4-Levigate with a small amount of base solution to form a uniform paste.

5-Add base solution in geometric proportions almost to 480ml, while mixing thoroughly.

6-Transfer contents of the mortar to a graduated cylinder.

7-Rinse the mortar and pestle with base solution and pour into graduated cylinder.

8-Add base solution to the graduated cylinder to achieve the total volume of 480ml.

9-Transfer contents of the graduated cylinder into bottle provided by the study.

10-Shake well to mix

Beyond Use Date: 91 days

Storage: Refrigerate

Reference: Milap C. Nahata, Richard S. Morosco and Michael T. Brady. Extemporaneous sildenafil citrate oral suspensions for the treatment of pulmonary hypertension in children. American Journal of Health-System Pharmacy, Vol.63, Issue 3, 254-257

18. DECLARATION OF HELSINKI

This clinical study will be performed in compliance with the ethical principles of the Declaration of Helsinki and Good Clinical Practice (GCP).