A Randomized Phase 2 Study of Vincristine versus Sirolimus to treat High Risk Kaposiform Hemangioendothelioma (KHE).

Study Chair/Sponsor

Denise M. Adams, M.D.
300 Longwood Ave
Fegan 3 (VAC)
Boston, MA 02115
Phone: 617-355-5226
Pager: 5021
Fax: 617-730-0752
Email: denise.adams@childrens.harvard.edu

Coordinating Center:
Boston Children's Hospital
300 Longwood Avenue
Boston, MA 02115

Contact information:
Yvonne Sheldon BSN, RN
Project Management CTSU
Phone: 617-919-6299
Email: Yvonne.sheldon@childrens.harvard.edu

AGENT

Rapamune® (sirolimus)

Provided by:
Pfizer
235 East 42nd Street
New York, NY 10017

IND#105046

TABLE OF CONTENTS

1 ABSTRACT

Kaposiform hemangioendotheliomas (KHE) are extremely rare life threatening tumors that can be associated with Kasabach-Merritt Phenomenon consisting of profound thrombocytopenia and hypofibrinogenemia causing a significant risk of bleeding and an associated mortality rate as high as 20% to 30%. Despite the severity of potential complications, uniform guidelines are lacking both for the treatment and response to treatment of children and young adults with these tumors. KHE patients have been treated with a multitude of aggressive drug regimens without prospective evaluation of response or safety. Presently, vincristine is considered the standard of practice although there is not a lot of data to support this. We have treated a subset of these patients on study SIR-DA-0901 (FDA Grant# 5RO1FD003712-01). This study is a phase II trial assessing the efficacy and safety of sirolimus for the treatment of complicated vascular anomalies. Although the numbers are small, the response has been extremely promising with excellent tolerability. There is pre-clinical and clinical data supporting the essential regulatory function of the PI3 kinase/AKT/mTOR pathway in vascular growth and organization that suggests a therapeutic target for patients with complicated vascular anomalies. The overall goal of this trial is to objectively assess the efficacy of sirolimus compared to vincristine for the treatment of patients with high risk KHE.

2 OBJECTIVES (SCIENTIFIC AIMS)

2.1 Hypothesis:

Sirolimus treatment for children and young adults with Kaposiform hemangioendotheliomas will be more effective than vincristine, assessed by time to hematologic response in an induction period and provide equivalent safety parameters.

2.2 Primary Aims:

2.2.1 To determine the efficacy of oral sirolimus as compared to intravenous (IV) vincristine for an induction period of 2 courses in patients with high risk KHE. For this purpose, 50 patients with high risk KHE will be randomized to either receive oral sirolimus or IV vincristine for 2 courses. Both treatment groups will also be placed on a defined weaning dose of steroids over the induction period (initial 2 courses). Time to hematologic response will then be assessed at the end of 2 courses. Hematologic response will be defined as platelet count of > 100,000/μl x 2 consecutive lab measurements or an increase of 2 times the baseline for 2 consecutive lab measurements, whichever is greater, and a fibrinogen of > 150 mg/dl. (Baseline is initial laboratory evaluation without transfusions).

2.2.2 To determine the safety of oral sirolimus and IV vincristine in patients with high risk KHE. Toxicities will be assessed and recorded at specific intervals throughout therapy. Common Terminology Criteria for Adverse Events (CTCAE, version 4.0.3) will be used to categorize and grade all toxicities. Safety monitoring and stopping rules will be instituted to ensure that serious treatment-related toxicities are reported and study continuation re-evaluated.

2.3 Secondary Aims:

2.3.1 To determine the efficacy of these agents in the maintenance phase in patients with high risk KHE. Maintenance phase will last for 1 year and treatment change may occur for any patient that loses their response. (See research plan for details). Formal disease response will be assessed at end of maintenance courses 6 and 12 using radiographic, clinical and quality of life measures.

2.3.2 To determine the safety of these agents in the maintenance phase in patients with high risk KHE. Toxicities will be assessed and recorded at specific intervals throughout therapy. Common Terminology Criteria for Adverse Events (CTCAE, version 4.0.3) will be used to categorize and grade all toxicities with same monitoring.

2.3.3 To assess biomarker/genomic analysis on serum and tissue in children and adults with high risk KHE. Biomarkers in serum will be tested at baseline, end of induction, end of maintenance courses 6 and 12. Tissue availability will be more limited and will only be available at one time point.

3 BACKGROUND AND RATIONALE

3.1 Background and Significance:

Patients with Vascular Anomalies (VA) have a spectrum of diseases that can be broadly classified into vascular tumors and malformations (1, 2, 49) (Figure 1). Vascular tumors grow and spread through a proliferative process while vascular malformations enlarge through expansion of a developmental anomaly, with little cellular proliferative activity. Growth and/or expansion of VA cause clinical problems such as disfigurement, chronic pain, coagulopathies and bleeding, organ dysfunction and occasionally death. Individuals with VA often experience progressive clinical symptoms with worsening quality of life. Although limited treatment options are available, their efficacy has not been validated in prospective clinical trials and is usually based on case reports. Thus there are no validated standards of care for these patients (3).

Figure 1: Classification of Vascular Anomalies

ISSVA classification for vascular anomalies ©
(Approved at the 20th ISSVA Workshop, Melbourne, April 2014)

Overview table

Vascular anomalies				
Vascular tumors	**Vascular malformations**			
	Simple	**Combined °**	**of major named vessels**	**associated with other anomalies**
Benign Locally aggressive or borderline Malignant	Capillary malformations Lymphatic malformations Venous malformations Arteriovenous malformations* Arteriovenous fistula*	CVM, CLM LVM, CLVM CAVM* CLAVM* others	See details	See list

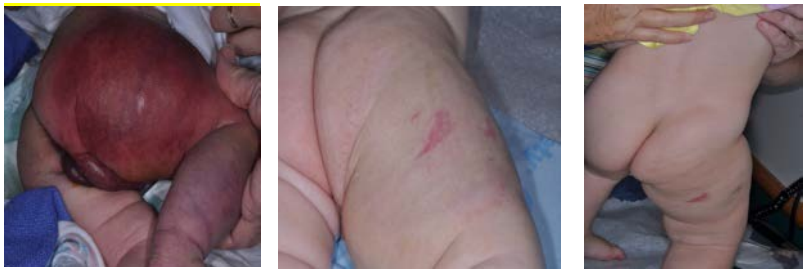
° **defined as two or more vascular malformations found in one lesion**
* **high-flow lesions**

Kaposiform hemangioendotheliomas (KHE) and tufted angiomas (TA) are rare tumors of vascular origin**.** There are no national registries documenting numbers of KHE patients but from extrapolating data at the 2 largest vascular anomaly centers in the U.S., the prevalence appears to be 0.91/100,000 children with an estimated total number of diagnosed cases in the US of 760 in 2011. These tumors pose difficult dilemmas for the practitioner particularly in regards to there being limited treatment options and a lack of validated biological markers. For example, KHE and TA can cause a coagulopathy known as Kasabach-Merritt Phenomenon (KMP) which has a mortality rate of 20 – 30% (4-7). A number of therapies have been reported via case reports but none have been uniformly effective. Steroids are often used as a first line therapy with varying results. (8, 9) Interferon has been used but has significant neurological side effects (10-12). Antifibrinolytic agents (e-aminocaproic acid or tranexamic acid) have been used with mixed responses, as have antiplatelet agents such as aspirin, or dipyridamole (13). Chemotherapy with agents such as cyclophosphamide, vincristine, and actinomycin has been used with variable responses as well (14-15). Other medical options that have been used include radiation therapy, compression therapy, and embolization therapy. We initially reported a case of refractory KHE with KMP whose hematologic parameters responded to sirolimus (16). Since that time, other cases have been reported that have improved outcome, at least in the short term (17).

We initially reported the use of sirolimus in the treatment of a 9 month old with refractory KHE with KMP on palliative treatment that had a hematologic recovery in 3 weeks to sirolimus and significant improvement in her clinical symptoms and quality of life (17). She began treatment in July 2007and has not suffered any long term effects. Since that time, other cases have been reported with excellent short term efficacy (18). Presently, 13 KHE patients are enrolled on SIR-DA-0901 (FDA Grant#

5RO1FD003712-04) (Figure 2 and Table 1). Twelve patients completed drug therapy and are in follow up. All twelve patients had a partial response to treatment (improvement of quality of life, organ function and radiologic imaging). Of the twelve patients who completed treatment, 8 were females and 5 were males. The average age at the start of treatment was 11 months with a range of 3 weeks to 3 years. Eleven patients presented at the time of diagnosis with KMP. Of the eleven, 3 had resolution of KMP prior to starting study but all were on steroid therapy and unable to wean treatment because of reoccurrence of KMP. When sirolimus was started all of these patients were weaned off steroids without reoccurrence of KMP. Of the 8 patients with KMP at study initiation, all had resolution of KMP with an average time to hematologic recovery of 3 weeks. One patient with multifocal KHE of the bone without KMP was taken off of study secondary to disease progression. This patient had been given a "wash-out period" (removal from previous therapy for 2 weeks prior to starting protocol). She was in severe pain from the beginning of the study, and also had multifocal bone disease which is extremely rare and perhaps has a different clinical spectrum than unifocal KHE. The "wash out" requirement was subsequently removed from the study. There were limited Grade III or IV toxicities in this group of patients, none of which required dose reduction or withdrawal from study. There have been no drug related infections and no obvious long term effects. Although we are presently evaluating this data, our preliminary conclusion is that sirolimus appears to be an effective and well-tolerated agent for high-risk KHE patients who otherwise have very limited options for treatment.

Figure 2. One patient's results with sirolimus.



a. Enrollment b. 6 months on study c. 12 months on study

Table 1. Summary of KHE patients on SIR-DA-0901

	Age at initiation of Sirolimus	Diagnosis	Location of Vascular Anomaly	Previous Treatment	Response time to KMP Resolution	Side Effects Grade III/IV Attributed to Sirolimus	Completed Study	Response
1	3 years	KHE with KMP initially Recovered at Start of Study	Retroperitoneal	Steroids, Vincristine, Actinomycin, Cytoxan	N/A	None	Yes	Yes
2	22 months	KHE with KMP initially Recovered at Start of Study	Multifocal Bone	Steroids, Interferon, Vincristine	N/A	N/A	Withdrew	Progressive Disease
3	9 weeks	KHE with KMP	Left Leg and Inguinal Area	Steroids	2 weeks	None	Yes	Yes
4	18 months	KHE with KMP	Chest, Pleura, Left arm	Steroids, Vincristine, Actinomycin, Cytoxan	2 weeks	None	Yes	Yes
5	2 months	KHE with KMP	Left leg	Steroids	3 weeks	None	Yes	Yes
6	5 weeks	KHE with KMP	Right Inguinal area, Buttocks, Back	Steroids	4 weeks	None	Yes	Yes
7	2 years	KHE	Right Mandible	Steroids	N/A	Grade III ANC	Yes	Yes
8	2 years	KHE with KMP	Left Forearm and Chest	Steroids, vincristine	3 weeks	Grade III ANC	Yes	Yes
9	4 weeks	KHE with KMP	Right Chest and Axilla	Steroids	1 week	None	Yes	Yes
10	5 months	KHE with KMP	Right Chest, Neck and Axilla	Steroids	3 weeks	None	Yes	Yes
11	13 months	KHE with KMP initially Recovered at Start of Study	Retroperitoneal	Steroids, Vincristine	N/A	Grade III/IV Neutrophils	Yes	Yes
12	3 months	KHE with KMP initially Recovered at Start of Study	Left Forearm	Steroids	N/A	Grade III Triglycerides	Yes	Yes
13	8 months	KHE with KMP	Left Leg	None	4 weeks	None	Yes	Yes

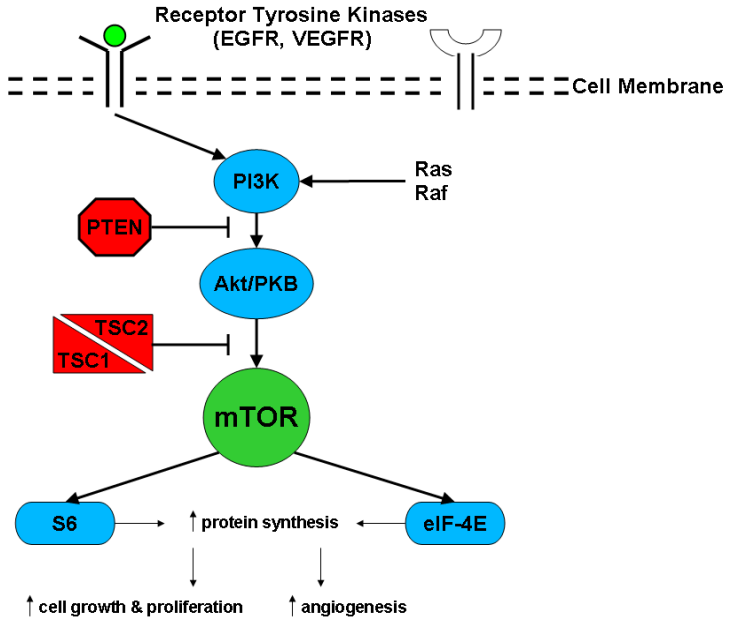
Sirolimus treatment has already made a tremendous difference in the morbidity and mortality/quality of life of these patients. However, because the numbers are small, the use of sirolimus needs to be

tested in a larger number of patients and compared to what is currently considered to be the standard of practice (vincristine). If our hypothesis is correct this drug will be proven to be more effective than vincristine and we will be one step closer to official FDA approval of sirolimus as a first line agent for the treatment of high-risk KHE.

3.2 PI3 Kinase/AKT/mTOR Signaling Pathway and Vascular Malformations

The PI3 kinase/AKT signaling pathway is critical to cell growth and survival and has been shown to govern normal vascular development, angiogenesis and even T cell differentiation (19). The mammalian target of Rapamycin (mTOR) integrates signals from the PI3 kinase/AKT pathway to coordinate cell growth and proliferation by regulating ribosomal biogenesis and protein synthesis (20) (Figure 4).

Figure 3: The mTOR pathway



Activation of AKT results in the phosphorylation of TSC proteins leading to mTOR activation with resultant cellular growth and proliferation. In addition, enhanced mTOR signaling has been shown to increase expression of the vascular endothelial growth factor (VEGF), a key regulator of angiogenesis and lymphangiogenesis (21-24). Disorders that lead to the inappropriate activation of the PI3 kinase/AKT/mTOR pathway would be predicted to result in tissue overgrowth with vascular anomalies. Indeed, there is preclinical and clinical data suggesting a critical role for this signaling pathway in vasculogenesis with disruptions that lead to hyperactivation of the pathway resulting in vascular pathology (25).

The over-expression of Akt/PKB (a key mediator of resistance to apoptosis in the PI3K pathway) in mice is known to cause cutaneous vascular malformations *in vivo* (26). Tie 2 receptor activating mutations have been associated with development of venous malformations through ligand-

independent AKT activation leading to endothelial survival (27). Several clinical studies have identified single gene mutations within the PI3 kinase/AKT pathway that are associated with vascular malformations and abnormalities of growth (28, 29). Tuberous sclerosis (TS) and lymphangioleiomyomatosis (LAM) are caused by inactivating mutations in the tuberous sclerosis complex tumor suppressor proteins TSC1 and TSC2 leading to increased activation of mTOR and unrestrained growth/lesions like hamartomas and various skin abnormalities (30). Loss of function mutations in the PTEN tumor suppressor gene, which inhibits the activation of Akt/PKB, are found in Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome, two disorders characterized by macrocephaly, vascular anomalies, excessive growth and hamartoma formation (31,32). These studies provide compelling evidence for a critical role for the PI3 kinase/AKT/mTOR pathway in the regulation of vascular growth and organization and suggest a therapeutic target for patients with these disorders.

3.3 Sirolimus and Inhibition of Growth

Sirolimus (Rapamycin) is a specific and potent inhibitor of mTOR. Inhibition is mediated through binding to the cellular protein receptor FKBP512. The exact mechanism of mTOR inhibition by the sirolimus-FKBP512 complex is not known, although it likely involves disruption of an effective signaling complex. Sirolimus has been used extensively as an immunomodulatory agent and in the treatment of patients with cancer. Single agent mTOR inhibition using a variety of mTOR inhibitors has resulted in objective radiographic responses in patients with low-grade astrocytomas (36), glioblastoma multiforme (37), renal cell carcinoma (33), mantle cell lymphoma (34), breast cancer (35), and sarcomas (36). Sirolimus has been shown to be effective and safe in both adult and pediatric populations. More recently, mTOR inhibition by sirolimus has been shown to be an effective strategy in the treatment of patients with hamartoma syndromes. Patients with TS (Tuberous Sclerosis) and LAM (lymphangioleiomyomatosis) showed a nearly 50% reduction in the size of angiomyolipomas and LAM lesions with sirolimus treatment. Upon withdrawal of the drug, however, there was some regrowth of the lesions but they did not to return to their pretreatment baseline (37). A recent case report of a patient with Proteus syndrome treated with sirolimus was also positive including some reversal of the manifestations of his tumors and malformations (38).

3.4 Study Rationale

We propose a multi-center, phase II trial with participation from up to 12 sites. The study will consist of two phases. The first of these is an initial **induction phase** in which vincristine and steroids will be compared to sirolimus and steroids. Response in the induction phase will be assessed as **time to hematologic response**. At the end of induction phase, change of treatment can occur if there is failure to respond. Part 2 is a **maintenance phase** which will be 1 year in length. Continued safety and efficacy data will be collected during maintenance and there may be a treatment change at any time for patients who lose their response following induction. Failure will be defined as worsening of hematological parameters on two separate laboratory evaluations at the end of induction or at any time during maintenance, or if they meet the definition of progressive

disease following response assessments (or at the discretion of the PI). Formal response in maintenance will be evaluated by imaging studies, functional assessment, and quality of life as per study SIR-DA-0901. Present therapies are very limited and new therapies are desperately needed for this devastating disease. Based on our preliminary data, there is a very good rationale for sirolimus therapy in KHE patients and so a phase II trial is urgently needed to determine if this therapy is to become the new standard of care for KHE patients.

Our secondary aims will be addressing biomarker analysis. There are limited studies describing the biology of these tumors. Per study SIR-DA-0901 there is some preliminary data indicating the importance of VEGF-C and other upregulated markers in the mTOR pathway. This needs to be further investigated, especially in KHE patients. Furthermore there are no clear objective measurements to determine response data.

4 RECRUITMENT, PAYMENT, CONSENT, STUDY ENROLLMENT PROCEDURES

4.1 Recruitment

Patients will be recruited from the various clinics of the investigative sites. Outside referrals will be accepted. The study is open to all eligible patients (0-31 years of age) regardless of gender or ethnicity.

Upon coordinating center and institutional IRB approval or waiver, participating sites may post a summary of the study to their institutional web site including basic information such as study title, purpose of the study, protocol summary, basic eligibility criteria, study site location(s), and how to contact the study site for further information. This trial will also be posted on clinicaltrials.gov.

4.2 Obtaining Consent

Consent will be obtained by the PI or a sub-investigator on the trial. Informed consent may be obtained in person or may be obtained via telephone per institutional policies. Where deemed appropriate by the clinician and the child's parents or guardian, the child will also be included in all discussions about the trial. The informed consent process includes consultation with the parents or legal guardian (for subjects less than 18 years old) and the subject to discuss the investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies. The consent document will be provided in English. Non-English speaking participants will be consented using the institutional IRB approved process.

This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP) and the applicable regulatory requirements.

4.3 Protecting Pediatric Participants

To safeguard the participation of children in this study, Investigators will:

- Obtain written parent/legal guardian permission
- Obtain assent of children, as per institutional requirements
- Whenever possible and appropriate, purposefully solicit that the child and parent/legal guardian are still willing to participate (e.g., when new information regarding the risks/benefits is available)

4.4 Enrollment

Enrollment must take place within 30 days after obtaining written informed consent. If more than 30 days elapse, re-consent in writing must be obtained prior to enrollment.

After obtaining all required approvals and meeting the requirements outlined in the study's Manual of Operations, participating sites may enroll eligible subjects on this clinical trial. Procedures for subject enrollment outlined in the Manual of Operations must be followed.

Patients must be enrolled before treatment begins. All eligibility requirements will be reviewed with the Study PI or designee prior to enrollment.

Treatment must start within 7 calendar days after study enrollment. If more than 7 days elapse, the patient must be re-screened in order to enroll.

5 PATIENT ELIGIBILITY

5.1 Inclusion Criteria

5.1.1 Diagnosis: All patients must have one of the following vascular anomalies as determined by clinical, radiologic and histologic criteria (when possible). Biopsy strongly recommended (but not required) with suggested immunostains: CD34, PROX-1 or D240, Glut-1 and MIB-1.

- Kaposiform Hemangioendotheliomas
- Tufted Angioma

High Risk Stratification: In addition to the above diagnosis, all of the following criteria need to be met:

- Kasabach Merritt Phenomenon defined at a platelet counts less than 100,000/µl and/or fibrinogen level < 150 mg/dl at the time of diagnosis

5.1.2 Age: Patients must be 0 - 31 years of age at the time of study entry. Enrollment includes patients of both genders and all ethnic groups.

5.1.3 Organ function requirements:

Adequate liver function defined as:

- Total bilirubin ≤1.5 x ULN for age, and
- SGPT (ALT) ≤5 x ULN for age, and
- Serum albumin ≥2 g/dL.
- Fasting LDL cholesterol of <160 mg/dL
- Fasting triglyceride <400 mg/dl

Adequate Bone Marrow Function defined as:

- Peripheral absolute neutrophil count (ANC) ≥1000/μL
- Hemoglobin ≥8.0 g/dL (may receive RBC transfusions)
- No Platelet requirement

Adequate Renal Function Defined as:

- A serum creatinine based on age as follows:

Age (Years)	**Maximum Serum Creatinine (mg/dL)**
≤5	0.8
6 to ≤10	1.0
11 to ≤15	1.2
>15	1.5

- Urine protein to creatinine ratio (UPC) < 0.3 g/l

5.1.4 Performance Status: Karnofsky ≥ 50 (≥16 years of age) and Lansky ≥ 50 for patients <16 years of age (Appendix I).

5.1.5 Prior therapy

a. Patients who have undergone surgical resection or interventional radiology procedures for disease control are eligible if they meet all inclusion criteria after surgery/procedure
b. Surgery: At least 2 weeks since undergoing any major surgery
c. Radiation: ≥ 6 months from involved field radiation
d. Prior steroid and vincristine therapy is permitted. Patients may have received up to 2 doses of vincristine prior to randomization.

5.2 Exclusion Criteria

5.2.1 Concurrent severe and/or uncontrolled medical disease that could compromise participation in the study (e.g. uncontrolled diabetes, uncontrolled hypertension, severe infection, severe malnutrition, chronic liver or renal disease, active upper GI tract ulceration).

5.2.2 Patients who require medications that are strong inhibitors/inducers CYP3A4 enzyme activity, including anticonvulsants, (Appendix II) to control concurrent medical conditions are not eligible. Patients who discontinue use of prohibited medications with a one week washout prior to start of study treatment are eligible.

5.2.3 Known history of HIV seropositivity or known immunodeficiency. Testing is not required unless a condition is suspected.

5.2.4 Impairment of gastrointestinal function or gastrointestinal disease that may significantly alter the absorption of sirolimus (e.g. ulcerative disease, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome or small bowel resection). A gastric tube or nasogastric tube is allowed.

5.2.5 Females who are pregnant or breast feeding.

5.2.6 Males or females of reproductive potential may not participate unless they have agreed to use an effective contraceptive method during the period they are receiving the study drug and for 3 months thereafter. Abstinence is an acceptable method of birth control.

Females of childbearing potential will be given a pregnancy test within **7 days** prior to administration of study treatment and must have a negative urine or serum pregnancy test.

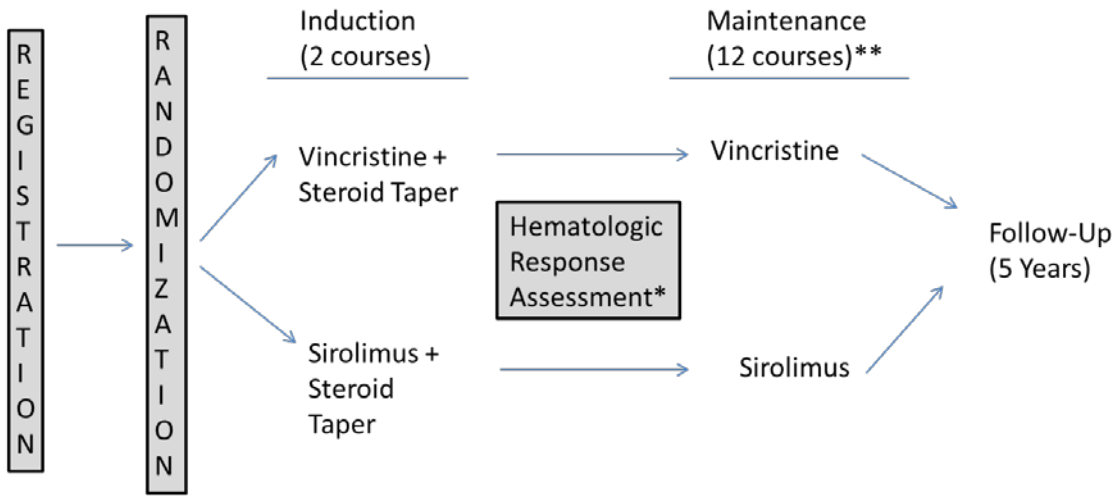
5.2.7 Patients who have received prior treatment with an mTOR inhibitor.

5.2.8 Patients unwilling or unable to comply with the protocol or who in the opinion of the investigator may not be able to comply with the safety monitoring requirements of the study.

5.2.9 Patients who currently have an uncontrolled infection, defined as receiving intravenous antibiotics.

6 TREATMENT PROGRAM

6.1 Study Schema



*Treatment change is permitted if failure to achieve Hematologic response.
** Formal Response assessment at end of maintenance courses 6 and 12.
Treatment change is permitted if PD or loss of response and any time of worsening hematologic parameters.

6.2 Research Design and Methods

A total of 50 patients will be enrolled over a 4 year period from up to 12participating institutions, targeting to yield a minimum of 45 evaluable patients by accommodating up to 10% non-evaluable rate, or 5 non-evaluable patients. Patients will be followed through an initial 14-course treatment period (2 courses of induction, 12 courses of maintenance), with 5-year follow up from time of enrollment for all patients who complete the study or have a response. Patients will be randomized to receive vincristine plus a weaning dose of steroids or sirolimus plus a weaning dose of steroids. Initial treatment combined with steroids is essential as many patients will be started on steroids at their primary institutions and there is speculation in our present study that this initial combination may have some synergistic benefit. This will provide consistency of treatment. After induction, patients will then continue on a 1 year maintenance phase. If patients fail to achieve hematologic response at the end of induction or lose their response during maintenance, a treatment change may occur. Formal response in maintenance will be evaluated by imaging studies, functional assessment, and quality of life as per study SIR-DA-0901. Failure at any time will be defined as worsening of hematological parameters on two separate laboratory evaluations.

The number of study patients was determined from projections of eligible patients seen by multidisciplinary vascular anomalies centers over the past three years. Study participation is limited to those institutions that have the required patient volumes, infrastructure and significant multidisciplinary expertise in investigator initiated clinical trials.

6.3 Randomization

The initial treatment assignment will be made using a response adaptive randomization algorithm: patients will be randomized in a 1:1 ratio (with equal probability) for the first 10 and in a skewed ratio for the rest to favor the better performing treatment arm by adjusting the treatment allocation ratio based on response data observed on previously accrued patients. The response data observed on previously enrolled patients will be available in a real time and continuously analyzed by the study statistician for treatment assignment of each newly enrolled patient. Clinical trial designs using such adaptive randomization algorithm are known to increase patient benefits over the course of trials without undermining the scientific rigor (39). We specifically will use the doubly adaptive biased coin design with a target allocation given by an urn model (40, 41).

Because of the time it takes to measure treatment response, we will modify the dynamic randomization in the following manner. Rather than using all previously enrolled patients response information to randomize the next patient, we will only base new randomization on the information from those previous patients who have had treatment response information available at the time of next randomization. Specifically, the probability of assigning a new patient to Sirolimus group is $\frac{1}{1+\frac{N_{m,A}}{N_{m,B}}\left(\frac{1-\hat{p}_{m,A}}{1-\hat{p}_{m,B}}\right)^2}$, where $N_{m,A}$ and $N_{m,B}$ are the number of numbers of patients who have been enrolled in Sirolimus and Vincristine respectively. $\hat{p}_{m,A}$ and $\hat{p}_{m,B}$ are respectively Sirolimus and Vincristine group observed response rates up to that time point.

6.4 INDUCTION

Arm 1: Vincristine + Steroid Taper
Arm 2: Sirolimus + Steroid Taper

6.4.1 **Steroid Taper –** Steroids may be started prior to study enrollment. All participants will continue to receive steroids during the Induction period. The steroid (prednisolone or equivalent) taper will begin following enrollment during 2 courses of the induction period along with either vincristine or sirolimus.

Steroid Taper Schedule

Table 2

Steroid Dose	Duration
2mg/kg/day divided BID	2 weeks
1.5 mg/kg/day divided BID	2 weeks
1.0 mg/kg/day divided BID	2 weeks
0.5 mg/kg/day QD	1 week
0.5 mg/kg/day QOD	Every other Day – 1 week

Stop	

6.5 Arm 1: Vincristine + Steroids (Induction)

6.5.1 **Administration** - Vincristine will be administered weekly for 2 courses (one course is 28 days) by central venous catheter (recommend PICC line) per institutional guidelines. Vincristine administration may be moved by one day per course. The vincristine dose used for this study is based upon weight with 10 kg as a cut-off.

Vincristine Dose

- 0.05 mg/kg/dose IV – for participants less than or equal to 10 kg
  OR
- 1.5 mg/m^2/dose IV – for participants greater than 10kg

6.6 Arm 2: Sirolimus + Steroids (Induction)

6.6.1 **Administration –** Sirolimus will be administered at a dose of 0.8 mg/m^2/dose for participants <6 months of age and 1mg/m^2/dose for participants ≥6 months of age twice a day on a continuous dosing schedule. One course will be 28 days, measured by a 24 hour clock, rather than calendar days, to accommodate instances when the first dose of a course is the evening dose. Previous studies support the role of therapeutic monitoring and dose adjustment, and the importance of twice daily dosing given the more rapid drug metabolism in pediatric patients (42). Sirolimus will be administered as an oral (liquid) preparation in this study.

6.6.2 **Pharmacokinetically Guided Dosing** – The target sirolimus trough level is 10-15ng/ml. Sirolimus dosing will be individually guided using real-time drug concentrations measurements in combination with a Bayesian population model-based target optimization approach (43)

The dosing of sirolimus will be guided by pre-dose concentration measurements (trough) collected prior to either the AM or PM sirolimus dose. Each participant will have sirolimus serum levels measured according to the timeline listed below. Analysis for sirolimus level will occur at each participating site, or at a local laboratory using a validated assay approved by the study Pharmacologist. Regardless of collection and analysis site, the study Pharmacologist (Sander Vinks, PharmD, PhD, FC at Cincinnati Children's Hospital Medical Center (CCHMC)) or designee will perform all modeling and make recommendations for dose adjustment. Trough levels will be taken as listed below:

Table 3.

Sirolimus	**Frequency of**	**Action**

	Trough levels	
Loading Dose	Anytime between Day 10-14*	Continue Weekly trough level monitoring until 2 consecutive trough levels are stable**.
Stable levels**	Each course ***	• If trough levels within target range – continue same sirolimus dose • If trough levels are outside of target range after consultation with study Pharmacologist – Dose Adjustment
Dose adjustment	7-14 days following dose adjustment	Continue Weekly monitoring until trough levels are stable**.
Toxicity	At time of toxicity	See section 8.1

* Children up to age 2 will have loading dose trough sirolimus levels drawn beginning at Week 1 (Days 6-9) or, at the discretion of the investigator. Trough levels may be drawn at 2 time points (12 hours apart) during Week 1 to determine an individualized PK (count business days only).

** Stable Level is defined as 2 consecutive trough levels that are within target range. After the pharmacologist verifies the trough is within target range, the pharmacologist recommendation for the second level should be received within 7 business days.

*** Trough levels are to be drawn at the beginning of each course (defined as first 5 days) if stable during the prior course (does not have to be repeated if trough level recommendation was obtained within 7 business days prior to start of next course).

If unable to achieve a target trough sirolimus levels within 4 weeks, patients may be asked to have a mini-PK collection of 2 blood draws, in addition to a trough level, in order to better estimate the participant's recommended dose. The mini-PK collection will be at: 1 hour and 3 hours after a sirolimus dose.

6.7 Hematologic Response Assessment –Induction (all participants)

During the induction phase, time to hematological recovery will be measured to assess response. Hematologic response will be defined as a platelet count >100,000/µl or an increase in platelet count of 2 times the baseline (whichever is greater) and fibrinogen of > 150mg/dl. Hematologic response must be documented within 7 days prior to completion of induction course 2 or earlier if failure is suspected. Platelet count and fibrinogen levels must meet these parameters on 2 consecutive lab measurements with a minimum of 1 day between evaluations. Baseline is initial laboratory evaluation without transfusions; lab results performed outside of enrolling site may qualify as baseline as long as supporting documentation is provided. Study treatment will be continued during the period of hematologic response assessment. Hematologic response can NOT be assessed within 24 hours of a platelet or cryoprecipitate infusion (Section 7.1).

6.8 Treatment Change (Induction)

Participants who fail to achieve hematologic response by the end of induction (2 courses of therapy) will change to the other treatment arm for the start of Maintenance. **Although participants are expected to finish their assigned induction period, a participant may switch treatment arm (change treatment) prior to the end of the induction period if it is in the best interest of the participant's safety and well-being and hematologic failure is documented. Such participant will be considered a treatment failure (a non-responder will be counted as failure and the participant will change to the other therapy, but will be counted in the intent to treat group).** Treatment change is permitted one time during the study. Maintenance should be initiated within one week following documentation of hematologic failure for patients who change treatment. Functional assessments (Appendix V) and Quality of Life Questionnaires will be repeated prior to start of new treatment arm. These will serve as the baseline for the subsequent assessments obtained during maintenance.

6.9 MAINTENANCE (12 courses)

Study treatment (all participants) will be continuous between the periods of Induction and Maintenance. For participants who change treatments prior to start of maintenance, study treatment should begin within one week after documentation of hematologic failure.

6.9.1 Arm 1: Vincristine

6.9.2 **Administration** – Participants randomized to Arm 1 and have achieved hematologic recovery as defined in Section 6.7 will continue to receive vincristine on the schedule outlined in Table 4. Participants randomized to Sirolimus for induction and who have failed to achieve hematologic recovery as defined in Section 6.7 will change treatment to Arm 1 and receive Vincristine as outlined in Table 4. Vincristine will be administered by central venous catheter (recommend PICC line) per institutional policy during Maintenance for a total of 12 courses (48 weeks).

Vincristine Dose

- 0.05 mg/kg/dose IV – for participants less than or equal to 10 kg
  OR
- 1.5 mg/m^2/dose IV – for participants greater than 10kg

Vincristine administration schedule:

Vincristine will be administered weekly for 2 courses (Maintenance Courses 1-2). After 2 courses vincristine will be administered every two weeks for 5 courses (Maintenance Courses 3-7). Treatment will then be every 3 weeks for 5 courses (Maintenance Courses 8-12). Doses held/ missed for toxicity will not be made up. Vincristine administration may be

moved by two days per course.

Table 4

Frequency	**Duration – 48 weeks**
Weekly	Maintenance Courses 1-2 (8 doses)
Every 2 weeks	Maintenance Courses 3-7 (10 doses)
Every 3 weeks	Maintenance Courses 8-12 (6 doses)

Frequency	Weekly (+/- 2 days)							
Maintenance Course	Course 1				Course 2			
Week	1	2	3	4	5	6	7	8
Vincristine	•	•	•	•	•	•	•	•

Frequency	Every 2 weeks (+/- 2 days)																			
Course	Course 3				Course 4				Course 5				Course 6				Course 7			
Week	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Vincristine		•		•		•		•		•		•		•		•		•		•

Frequency	Every 3 Weeks (+/- 2 days)																			
Course	Course 8				Course 9				Course 10				Course 11				Course 12			
Week	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
Vincristine			•			•			•			•			•			•		

6.9.3 **Monitoring** – Laboratory assessments and toxicity monitoring will occur as outlined in Section 9.

6.10 Arm 2: Sirolimus

6.10.1 **Administration –** Participants randomized to Arm 2 for Induction and who have achieved hematologic recovery as defined in Section 6.7 will continue to receive sirolimus. Participants currently receiving sirolimus will continue at their current dose unless the criteria for dose adjustment as described in Section 6.10.3 are met.

6.10.2 Sirolimus Administration for Treatment Change to Arm 2- Participants originally randomized to Arm 1 that fail to achieve hematologic recovery will then switch to Arm 2 and will be administered sirolimus at a dose of 0.8 mg/m^2/dose for participants <6 months of age and 1mg/m^2/dose for participants ≥6 months of age twice a day on a continuous dosing schedule. Sirolimus dosing will be monitored pharmacokinetically to achieve serum trough levels for sirolimus between 10-15ng/ml.

6.10.3 **Pharmacokinetically Guided Dosing** – The target sirolimus trough level is 10-15ng/ml. Sirolimus dosing will be individually guided using real-time drug concentrations measurements in combination with a Bayesian population model-based target optimization approach (43)

The dosing of sirolimus will be guided by pre-dose concentration measurements (trough). Each participant will have sirolimus serum levels measured according to the timeline listed below. Analysis for sirolimus level will occur at each participating site, or at a local laboratory using a validated assay approved by the study Pharmacologist. Regardless of collection and analysis site, the study Pharmacologist or designee will perform all modeling and make recommendations for dose adjustment. Trough levels will be taken as listed below:

Table 5

Sirolimus	**Frequency of Trough levels**	**Action**
Loading Dose	Anytime between Day 10-14*	Continue Weekly trough level monitoring until 2 consecutive trough levels are stable**.
Stable levels**	Each course ***	• If trough levels within target range – continue same sirolimus dose • If trough levels are outside of target range after consultation with study Pharmacologist – Dose Adjustment
Dose adjustment	7-14 days following dose adjustment	Continue Weekly monitoring until trough levels are stable**.
Toxicity	At time of toxicity	See section 8.1

* Children up to age 2 will have loading dose trough sirolimus levels drawn beginning at Week 1 (Days 6-9) or, at the discretion of the investigator. Trough levels may be drawn at 2 time points (12 hours apart) during Week 1 to determine an individualized PK.

** Stable Level is defined as 2 consecutive trough levels that are within target range. After the pharmacologist verifies the trough is within target range, the pharmacologist recommendation for the second level should be received within 7 business days.

*** Trough levels are to be drawn at the beginning of each course if stable during the prior course (does not have to be repeated if trough level recommendation was obtained within 7 days prior to start of next course).

If unable to achieve a target trough sirolimus levels within 4 weeks, patients may be

asked to have a mini-PK collection of 2 blood draws, in addition to a trough level, in order to better estimate the participant's recommended dose. The mini-PK collection will be at: 1 hour and 3 hours after a sirolimus dose.

6.11 Response Assessment – Maintenance (All Participants)

The optimal measure of disease response in patients with complex vascular anomalies has not been established. Current practice includes changes in physical exam, radiologic evaluations, laboratory assessments, and/or quality of life measures. These lesions are difficult to assess with any one method because of heterogeneity in growth patterns, diversity of associated clinical and laboratory abnormalities and a fluctuating clinical course dependent upon factors other than treatment, such as intercurrent infections, puberty, and trauma. It is currently unclear if there is a good correlation between lesion size, as determined radiologically and clinical changes or quality of life measures. For these reasons we have elected to assess **formal disease response** using three distinct methods:

1. Radiologic evaluation
2. Clinical measures of disease (cytopenias, coagulopathies, other clinically relevant radiologic evaluation beyond the lesion of interest such as chest X-rays), and/or parameters for grading functional impairment – (Functional Assessment - Appendix V)
3. Quality of life measures

Formal Responses will be assessed three times during the study: prior to initiation of study treatment (baseline), and at the end of courses 6 and 12 following the start of Maintenance. Disease assessments may be performed at any time during maintenance if clinically indicated. Participants with disease worsening as defined as "progressive disease" or "loss of response" based upon any one of the above methods will be permitted to change treatment. If worsened disease is based on laboratory parameters we suggest a repeat of laboratory evaluations to confirm. Refer to Section 11 for definitions of responses.

6.12 Treatment Change (During Maintenance)

Treatment change is permitted one time during the study. If participant has changed treatment at the start of maintenance and has 'progressive disease' or 'loss of response', off treatment criteria will have been met. If treatment change occurs during maintenance, treatment on the new arm will begin within 7 days of documentation of disease worsening. Functional Assessment and Quality of Life measures are to be repeated at the time of treatment change unless performed within the last 7 days.

6.13 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue for up to 14 courses (inclusive of Induction and Maintenance) or until one of the following criteria applies:

- Intolerable toxicity (leading to treatment interruption for more than 4 weeks)
- Refusal of further protocol therapy by patient and/or parent/guardian

- Physician determines it is in the best interest of the patient
- Progressive Disease or loss of response following change of treatment
- End of Protocol defined therapy
- Complete response to therapy*
  * If there is a Complete Response (CR) prior to completion of 12-24 weeks of therapy (3-6 courses), all study drugs will be stopped.
- Continuation of sirolimus or vincristine off study will be up to the patient's primary physician.

Participants who are off protocol therapy will be followed until they meet the criteria for Off Study (see below). Follow-up data will be required unless consent is withdrawn.

6.14 Duration of Follow-Up

All patients who complete the study or have a response (defined as stable disease or better), regardless of treatment arm, will continue to be followed for growth and complications of the vascular tumor and therapy-related toxicities for a total of 5 years from the date of study enrollment. Following completion of study therapy, patients will be followed at a minimum interval of every 6 months. Follow up visits may occur at participants home institutions or they may return to the study site for their routine follow up care. Participants should return to the study site on an annual basis during follow-up.

Patients who stop study treatment early for any reason and did not have a documented response to sirolimus or vincristine (defined as stable disease or better) will be followed for 30 days from the last dose of study treatment or until toxicities resolve to baseline or less than or equal to Grade 1.

6.15 Off Study Criteria

- Death
- Lost to follow-up
- Withdrawal of consent for any further data submission
- Complete Follow up as defined in Section 6.14
- Five years from protocol enrollment

7 GENERAL CONCOMITANT MEDICATION AND SUPPORTIVE CARE GUIDELINES

7.1 Supportive Care - Both Arms

Appropriate antibiotics, blood products, antiemetics and general supportive care are to be used as necessary.

- All blood products will be leukocyte depleted and irradiated to prevent graft-versus-host disease. **Platelet transfusions have been associated with an increase in pain and lesion size in patients with KHE/ KMP lesions. Platelet transfusions should be limited and only used if there is actual bleeding or prior to procedures.** Hematologic response can NOT be assessed within 24 hours of a platelet or cryoprecipitate infusion.
- Good oral hygiene and mouth care are encouraged, as mucositis is a common toxicity.
- Infection Prophylaxis will be administered according to treating center's institutional guidelines as immunosuppressed patients are susceptible to Pneumocystis carinii infection.
- GFs that support platelet or white cell number or function can only be administered for culture proven bacteremia, clinical sepsis, or invasive fungal infection with neutropenia.
- Constipation prophylaxis is highly recommended as constipation and abdominal cramps are common toxicities associated with vincristine.

7.2 Other therapy for vascular anomaly

Interventional procedures may be used to treat symptomatic lesions provided it does not interfere with radiographic evaluation. For any planned surgery, the treating investigator should consider holding sirolimus. Discussion with the study chair is required.

7.3 Concomitant Medications

7.3.1 Steroids - Steroid use in maintenance should be limited to physiologic replacement doses due to endocrine deficiencies, as a premedication for blood products and for short courses if medically indicated. This should be discussed with study chair.

7.3.2 Sirolimus is known to be a substrate for both cytochrome P-450 3A4 (CYP3A4) and p-glycoprotein (P-gp). Inducers of CYP3A4 and P-gp may decrease sirolimus concentrations whereas inhibitors of CYP3A4 and P-gp may increase sirolimus concentrations. Co-administration of Rapamune with strong inhibitors of CYP3A4 and/or P-gp (such as ketoconazole, voriconazole, itraconazole, erythromycin, telithromycin, or clarithromycin) or strong inducers of CYP3A4 and/or P-gp (such as rifampin or rifabutin) are not recommended. CYP3A4 – Known strong inducers and inhibitors of isoenzyme CYP3A4, as specified in Appendix II, must not be administered. If these treatments are administered, the patient will

be removed from protocol therapy. The listing of medications in Appendix II lists medications to avoid/ use caution with when taking sirolimus.

Enzyme inducing anticonvulsants: Patients may not be taking enzyme–inducing anticonvulsants, and may not have received these medications within 1 week of entry, as these patients may experience different drug disposition. These medications are listed in Appendix II.

7.3.3 Vaccinations- Patients receiving immunosuppressants, including sirolimus and vincristine, should not be administered live vaccines. All other vaccines are permitted and highly suggested.

8 DOSE MODIFICATIONS/DOSE DELAYS

Patients entered on the trial will be carefully monitored for the development of sirolimus-related toxicities and vincristine-related toxicities. This study will utilize the CTCAE of the National Cancer Institute for reporting of AEs. A copy of the current version of the CTCAE version 4.03 can be downloaded from CTEP: http://ctep.cancer.gov/reporting/ctc.html

8.1 Sirolimus Arm

Any patient experiencing a Grade 3 or 4 toxicity that is possibly, probably, or definitely related to sirolimus should have the drug held and a sirolimus trough level obtained as soon as possible. Other toxicities requiring dose adjustments will be defined based on categorization of toxicity and sirolimus trough levels.

8.1.1 Hematological Toxicity - If a patient experiences ≥Grade 3 neutropenia (ANC <750), anemia (Hgb <8), the sirolimus will be withheld. Patients should continue to be seen and have complete blood counts measured, in addition trough sirolimus levels will be obtained every week until recovery (≤Grade 1) is documented. See section 8.1.3 Table 6 for details on sirolimus trough target adjustments.

8.1.2 Non-Hematological Toxicity - During All Courses for toxicities attributable (possibly, probably, definitely) to sirolimus:

If a patient experiences a non-hematological toxicity as defined below, sirolimus will be withheld. Patients should continue to be seen and have appropriate labs/observations, in addition to trough sirolimus levels, obtained at least weekly until recovery (≤Grade 1) is documented. See section 8.1.3 Table 6 for details on sirolimus target adjustments.

Sirolimus-related (possibly, probably, or definitely) toxicities Requiring Dose Adjustments/Interruptions or other interventions

- Grade 3 or Grade 4 non-hematological toxicity **(*)**

- Grade ≥2 serum creatinine elevation
- Grade ≥2 allergic reaction
- Grade ≥2 hypertension
- Grade ≥1 non-hematologic toxicities related to sirolimus that are intolerable to the patient
- Any ≥ Grade 2 non-hematological toxicity that persists for ≥7 days without resolution (return to less than Grade 2 or baseline) and is considered sufficiently medically significant or sufficiently intolerable by patients that it requires treatment interruption and/or dose reduction (see Table 6).

(*) The only non-hematological toxicities that are *excluded* from requiring dose adjustments/interruptions or other interventions are the following:

- Grade 3 nausea and vomiting of less than 3 days duration
- Grade 3 transaminase elevations that return to levels that meet initial eligibility criteria within 7 days of study drug interruption and that do not recur upon study re-challenge with study drug
- Grade 3 GGT elevation
- Grade 3 lymphopenia

8.1.3 Dose Modification Algorithm for Toxicity - All recommendations for dose modifications will be made by the coordinating center after analysis of blood samples for sirolimus levels at local laboratory. New doses will be communicated to the treating institution by the CCHMC Pharmacologist or designee. The table below will be used to modify sirolimus dose for patients who develop toxicity.

All toxicities should recover to baseline or < Grade 2 prior to resuming sirolimus dosing.

Table 6

Sirolimus Trough Levels at time of Toxicity	**Dose Adjustment**	
	Toxicities attributable to sirolimus (possibly, probably, or related)	***Other explanation for toxicity (i.e., viral infection)***
>15ng/mL (target trough 10-15ng/mL)*	Resume at modified dose for target trough goal of 10-15ng/mL	Resume therapy at same dose (targeted trough level of 10-15ng/mL)
Between 10-15ng/mL	25% sirolimus dose reduction – resume at target trough goal of 7-10ng/mL	Resume therapy at same dose (targeted trough level 10-15ng/mL).
>10ng/mL (target trough 7-10ng/mL)*	Resume at modified dose for target trough goal of 7-10 ng/mL	Resume at same dose (targeted trough level 7-10ng/mL)
Between 7-10ng/mL	50% sirolimus dose reduction –	Resume at same dose (targeted

	resume at target trough goal of 5-7ng/mL	trough level 7-10ng/mL)
>7 (target trough 5-7ng/mL)*	Resume at modified dose for target rough goal of 5-7 ng/mL	Resume at same dose (targeted trough level 5-7ng/mL)
Between 5-7ng/mL	Remove from treatment	Remove from treatment
* If sirolimus trough level is above target range at time of toxicity; therapy may resume when trough level is within target range.		

8.1.4 Interventions for Hyperlipidemia/Hypertriglyceridemia

Hyperlipidemia has been reported as an AE in at least 10% of patients treated with Sirolimus. As per the recent American Heart Association Scientific Statement on Cardiovascular Risk Reduction in High Risk Pediatric Patients, Tier III (65) management of hyperlipidemia should occur for patients with a fasting LDL cholesterol > 160 mg/dL. Age appropriate dietary restrictions should be enforced. Consult with your pediatric dietary and/ or nutrition services.

Table 7

Event	**Action**
Hyperlipidemia	
Fasting LDL >160 mg/dL and Patient ≥ 10 years old	• Diet (Nutritionist counseling – 30% of calories from fat, avoidance of trans-fats for 6 months) and exercise – Repeat Fasting LDL in 3 months. • If fasting LDL cholesterol is still >160 mg/dL, continue diet and exercise and initiate a triglyceride-lowering agent such as an HMG-CoA reductase inhibitor (pravastatin, atorvastatin, or fluvastatin). Patients should avoid drugs that inhibit or induce CYP3A4. Patients should be monitored clinically and through serum biochemistry for the development of rhabdomyolysis and other AEs as required in the product data sheets for HMG-CoA reductase inhibitors. Continue to follow lipid panel every 8-12 weeks and adjust statins as necessary. • If fasting LDL cannot be maintained ≤ 160mg/dL despite medical intervention, or the patient cannot tolerate medical intervention, then the sirolimus target goal should be reduced to a target goal of 7-10 ng/mL. • If after 3 months, fasting LDL cholesterol is still >160 mg/dL, then the sirolimus target goal should be reduced again to a target goal of 5-7 ng/mL. • If after 3 months at this new target goal fasting LDL cholesterol is still >160 mg/dL, then the patient must be removed from protocol therapy.
Fasting LDL >160mg/dL and Patient <10 years old	• Diet (Nutritionist Counseling <30% of calories from fat, avoidance of trans-fats for 6 months). Repeat Fasting LDL in 3 months. • If fasting LDL cholesterol is still >160mg/dL, continue diet and exercise and consider cholestyramine resin. • If fasting LDL cannot be maintained ≤ 160mg/dL despite medical intervention, or patient cannot tolerate medical intervention, then the sirolimus target goal should be reduced to a target goal of 7-10ng/mL. • If after 3 months, fasting LDL cholesterol is still 160 mg/dL, then sirolimus target goal should be reduced again to a target goal of 5-7ng/mL. • If after 3 additional months at this new target goal fasting LDL cholesterol is still >160 mg/dL, then the patient must be removed from protocol

	therapy.
Triglycerides	
150-699 mg/dL	• Nutritionist counseling for low simple-carbohydrate, low-fat diet. • If triglycerides remain 700mg/dL, sirolimus therapy may continue at current dose target.
≥ 700 – 1000 mg/dL	• Diet (nutritionist counseling) and exercise. Repeat lab work in 3 months. • If patient is ≥ 10 years old and fasting triglycerides still ≥ 700 mg/dL and HDL is low, the patient should continue diet and exercise and consider fibrate or niacin cholestyramine resin. o If after 3 months, the fasting triglycerides are still ≥ 700 mg/dL, then sirolimus target goal should be reduced to a target goal of 7-10 ng/mL. o If after 3 months the fasting triglycerides are still ≥ 700 mg/dL, then sirolimus target goal should be reduced again to a target goal of 5-7 ng/mL. o If after 3 months at this new target goal fasting triglycerides are still ≥ 700 mg/dL, then the patient must be removed from protocol therapy. • If patient is < 10 years old and fasting triglycerides are still ≥ 700 mg/dL or patient cannot tolerate medical intervention, then sirolimus target goal should be reduced to a target goal of 7-10 ng/mL. o If after 3 months the fasting triglycerides are still ≥ 700 mg/dL, then sirolimus target goal should be reduced again to a target goal of 5-7 ng/mL. o If after 3 months at this new target goal fasting triglycerides are still ≥ 700 mg/dL, then the patient must be removed from protocol therapy.

8.1.5 Toxicities Requiring Removal from Therapy – Sirolimus Arm only

If patients experience any of the following toxicities regardless of relationship to sirolimus or current trough level will be removed from protocol therapy.

Table 8

Toxicity	Criteria for Removal
Renal Function	If serum creatinine persistently increases (documented on at least 2 consecutive lab evaluations) to greater than 1.5X the baseline serum creatinine at trial entry, a creatinine clearance or GFR should be obtained. If the creatinine clearance or GFR is <70% of normal for age, regardless of attribution, the patient should be removed from protocol therapy.
Infection	Patients who develop a ≥ Grade 3 pneumocystis carinii pneumonia or systemic fungal infection, regardless of attribution, will be removed from protocol therapy.
Malignancy	Patients who develop lymphoma or other cancers, regardless of attribution, will be removed from protocol therapy.
Pneumonitis	Patients who develop ≥Grade 2 sirolimus-related pneumonitis (symptomatic but no intervention/oxygen therapy needed) will be removed from protocol therapy.
Hypertension	Patients who develop sirolimus-related ≥Grade 3 hypertension will be removed from protocol therapy.
Allergic Reaction	Patients who develop sirolimus-related >Grade 3 allergic reaction will be removed from protocol therapy.
Rash	Patients who develop sirolimus-related Grade 4 rash will be removed from protocol therapy.

Hyperlipidemia	Hyperlipidemia (with LDL cholesterol ≥160mg/dL) not responsive to diet, exercise, and medical intervention for patients after 2 dose reductions, regardless of attribution, will be removed from protocol therapy.

8.1.6 Other AE-related Criteria for Removal Therapy

- Patients who experience a sirolimus-related toxicity requiring a dose modification/interruption after two target level reductions will be removed from protocol therapy.
- Patients who experience a sirolimus-associated toxicity requiring a target level reduction/drug interruption whose toxicity does not recover within 4 weeks of stopping sirolimus will be removed from protocol therapy.

8.2 VINCRISTINE ARM

8.2.1 Vincristine Dose Modifications

Table 9

Toxicity	**Grade**	**Management/Dose Modification**
Neuropathic Pain	Grade 3 or greater	Hold dose. When symptoms subside, resume at 50% previous dose, then escalate to full dose as tolerated. Severe peripheral neuropathies, with or without a positive family history, might suggest the need for a molecular diagnosis evaluation to rule out hereditary neuropathy syndromes.
Vocal Cord paralysis	Any Grade	Hold Dose. When symptoms subside, resume at 50% previous dose, then escalate to full dose as tolerated.
Foot Drop, Paresis	Grade 3 or greater	Consider holding or decreasing dose. These toxicities are largely reversible but over months to years; physical therapy may be beneficial.
Jaw Pain	Any Grade	Treat with analgesics; do not modify vincristine dose.
Hyperbilirubinemia (Direct Bilirubin mg/dL)	<3.1 mg/dL	Full dose
	3.1-5.0 mg/dL	50% dose reduction
	5.1-6.0 mg/dL	75% dose reduction
	>6.0 mg/dL	Withhold dose and administer next scheduled dose if toxicity has resolved. Do not make up missed dose.
Constipation, Ileus, or Typhilits	Grade 3 or greater	Hold Dose. Institute aggressive regimen to treat constipation if present. When symptoms are less than or equal to Grade 2, resume at 50% dose and escalate to full dose as tolerated.
Extravasation	Discontinue the IV administration of the drug and institute appropriate measure to prevent further extravasation and damage according to institutional guidelines. Remaining dose may be introduced into another vein.	

For AEs not covered within section 8, it will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require that the subject to be removed from treatment. A subject may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the subject will be given appropriate care under medical supervision until symptoms cease or until the condition becomes stable.

9 REQUIRED OBSERVATIONS

9.1 Arm 1. Vincristine

STUDIES TO BE OBTAINED	Pre-Study[1]	Induction	Maintenance	Off treatment	Follow-up[5]
Informed Consent/Assent	X				
Eligibility Checklist	X				
Physical Assessments					
History	X	Weekly	X[2]	X	X
Physical Exam with vital signs (including T, P, RR, BP)	X	Weekly	X[8]	X	X
Height, weight, BSA	X	Weekly	X[8]	X	X
Performance Status (see protocol)	X	Weekly	X[2]	X	X
Concomitant Medications		Weekly	X[2]		
Laboratory Assessments[2]					
CBC, differential, platelets, fibrinogen, D-Dimer	X	X[2]	X[2]	X	X
Urinalysis[9]	X	X[9]	X[9]	X	X
Pregnancy test[3]	X	X[3]	X[3]		
Electrolytes including BUN, Creatinine, glucose, blood chemistry including albumin, total protein, SGOT, SGPT, bilirubin (total and direct)	X	X[2]	X[2]	X	X
Study Drug					
Vincristine administration (Vincristine administration may be moved by one day per course.)		Weekly	X[8]		
Diary Review		X[2]	X[2]	X[2]	
Response Assessments[4]					
Hematologic response[6]		X[6]			
Imaging analysis	X[1]		X[4]	X	
Health-related quality of life and pain assessments[4]	X		X[4]	X	
Functional Assessment (Appendix IV)[4]	X		X[4]	X	
Correlative Studies[7]					
Serum and tissue samples	X	X	X		

1. All studies to determine eligibility must be performed within 2 weeks prior to enrollment. Imaging may occur within 3 weeks of start of study treatment.
2. Physical Assessments and laboratory Assessments should be obtained at a minimum of the end of every course but may be repeated more frequently if clinically indicated. Participant Diary should be returned and reviewed at the end of every course.
3. For females of childbearing potential; must occur within 7 days prior to start of treatment. Pregnancy tests for females of childbearing potential will be repeated every 4 to 6 weeks.
4. Response assessments occur pre-study and at the end courses 6 and 12 in maintenance. Disease assessments may be performed at any time if clinically indicated. Quality of life and Functional assessments are to be repeated at time of treatment change. See section 11.

5. Follow-up assessments will be performed every 6 months or more frequently if clinically indicated. See Sections 6.14 and 9.6.
6. Hematologic response occurs within 7 days of completion of Induction course 2. This may occur sooner or if hematologic failure is suspected. Consecutive platelet count and fibrinogen levels must be drawn with a minimum of 1 day between evaluations. See Section 6.7. Hematologic response can NOT be assessed within 24 hours of a platelet or cryoprecipitate infusion. See Section 7.1

7. Optional archival tissue and Serum samples will be collected from consenting patients. See Section 9.6 for time points.
8. Physical exam occurs prior to administration of vincristine during maintenance. Vincristine will be administered weekly x 2 courses, q2weeks for 5 courses and q3 weeks for 5 courses. Missed doses will be discussed with Sponsor Investigator..
9. Urinalysis should be obtained every eight weeks.

Arm 2. Sirolimus

STUDIES TO BE OBTAINED	Pre-Study[1]	Induction	Maintenance	Off-treatment	Follow-up[5]
Informed Consent/Assent	X				
Eligibility Checklist	X				
Physical Assessments					
History	X	X[2]	X[2]	X	X
Physical Exam with vital signs (including T, P, RR, BP, SaO_2)	X	X[2]	X[2]	X	X
Height, weight, BSA	X	X[2]	X[2]	X	X
Performance Status (see protocol)	X	X[2]	X[2]	X	X
Concomitant Medications		X[2]	X[2]		
Laboratory Assessments					
CBC, differential, platelets, fibrinogen, D-Dimer	X	X[2]	X[2]	X	X
Urinalysis[10]	X	X[10]	X[10]	X	X
Pregnancy[3]	X	X[3]	X[3]		
Electrolytes including BUN, Creatinine, **& fasting glucose**, blood chemistry including albumin, total protein, alkaline phosphatase, uric acid, phosphorous & **fasting serum** lipid profile (triglycerides, total cholesterol, HDL and LDL), SGOT, SGPT, bilirubin(total and direct)	X	X[2]	X[2]	X	X
Study Drug Administration					
Sirolimus Administration (Oral)		**Continuous Dosing**			
Sirolimus Diary Review[11]		X[11]	X[11]	X	
Trough sirolimus levels		X[8]	X[9]		
Response Assessments[4]					
Hematologic response with fibrinogen and D-dimer[6]		X[6]			
Imaging analysis	X[1]		X[4]	X	
Functional Assessment (Appendix IV)	X		X[4]	X	
Health-related quality of life and pain assessments	X		X[4]	X	
Correlative Studies[7]					
Serum and Tissue samples	X	X	X		

1. All studies to determine eligibility must be performed within 2 weeks prior to enrollment unless otherwise indicated below. Radiological assessments may be done within 3 weeks prior to enrollment.

2. Physical Assessments and laboratory Assessments should be obtained at a minimum of the end of every course but may be repeated more frequently if clinically indicated.

3. For females of childbearing potential; must occur within 7 days prior to start of treatment. Pregnancy tests for females of childbearing potential will be repeated every 4 to 6 weeks.

4. Response assessments occur at baseline, end of maintenance courses 6 and 12. Quality of Life and pain assessment and functional assessments are to be repeated at treatment change. Disease assessments may be repeated if clinically indicated. See section 11.

5. Follow-up assessments will be performed every 6 months or more frequently if clinically indicated. See Sections 6.14 and 9.6.

6. Hematologic response occurs within 7 days of completion of Induction course 2. This may occur sooner if hematologic failure is

suspected. Consecutive platelet count and fibrinogen levels must be drawn with a minimum of 1 day between evaluations. See Section 6.7. Hematologic response can NOT be assessed within 24 hours of a platelet or cryoprecipitate infusion. See Section 7.1

7. Optional archival tissue and Serum samples will be collected from consenting patients. Sec Section 9.6 for time points.
8. The first trough level should not be measured until week 2 (Day 10-14) to allow for loading to occur and to approach steady state concentrations. (For patients less than 2 years of age there is allowance for additional levels in the first week; any questions should be discussed with the Sponsor Investigator). After any dose modification, subsequent sirolimus levels should be obtained 7-14 business days after the dose adjustment. If abnormal or dose is adjusted, sirolimus level must be repeated weekly until two consecutive trough levels are within target range (Stable levels). See Section 6.6.2 and Table 3.
9. For participants continuing on Maintenance, continue sirolimus trough levels at the end of every course if stable. For participants who switch to sirolimus arm at Maintenance, refer to Section 6.10.3, and Table 5 (Maintenance).
10. Urinalysis should be obtained every eight weeks.
11. Participant Diary should be returned and reviewed weekly.

9.2 Pre-study Assessments

All entry/eligibility studies must be completed within 2 weeks prior to start of treatment (unless otherwise specified). The baseline radiologic study documenting disease status is required within 3 weeks prior to start of study treatment. Urine or serum pregnancy test must be available within 7 day prior to start of study treatment.

9.3 Treatment Assessments

All assessments scheduled to be obtained during Induction and Maintenance must occur within (+/-) 1 day if scheduled to occur weekly. For assessments to be repeated at the end of courses, these must occur within (+/-) 4 days of scheduled day.

The following assessments will occur while participants are receiving study drug.

- Physical Assessments
  - Medical history
  - Physical exam with vital signs including temperature, pulse, respiratory rate, and blood pressure. Oxygen saturation will be recorded for patients randomized to the sirolimus arm only.
  - Height, Weight, BSA
  - Performance Status – Karnofsky performance status for participants ≥ 16 years of age or Lansky Performance scale for participants < 16 years of age
- Laboratory Assessments
  - CBC, Differential, Platelets, and fibrinogen and D-Dimer at assessment timepoints
  - Urinalysis
  - Pregnancy tests – serum or urine pregnancy tests may be performed if female is of childbearing potential. Pregnancy test should be within 7 days prior to start of treatment. Pregnancy tests will be repeated every 4 to 6 weeks for females of childbearing potential while receiving study treatment.
  - Chemistries
    - Arm 1 (Vincristine) – Electrolytes including BUN, creatinine, glucose, blood chemistry including albumin, total protein, SGOT, SGPT, bilirubin (total and direct).

        - Arm 2 (Sirolimus) - Electrolytes including BUN, creatinine, **fasting glucose**, blood chemistry including albumin, total protein, alkaline phosphatase, uric acid, phosphorous and **fasting serum** lipid profile (triglycerides, total cholesterol, HDL and LDL), SGOT, SGPT, and bilirubin (total and direct).
    - Any laboratory assessments may be performed more frequently if clinically indicated.
- Disease Assessments – Baseline, at the end of maintenance courses 6 and 12.
    - Hematologic response – Occurs within 7 days of completion of Induction course 2. This may occur sooner if hematologic failure is suspected to assess response including platelet count and fibrinogen levels. See Section 6.7 and 7.1 regarding platelet transfusions and cryoprecipitate infusions.
    - Imaging analysis at time of treatment change
    - Assessment of functional impairment (Appendix IV) – to be repeated at time of treatment change
    - Health-related quality of life and pain assessments – to be repeated at time of treatment change
    - Disease assessments may be repeated any time if clinically indicated.

9.4 Off treatment (completion of study drug assessments)

Assessments as described in Section 9.1 and 9.2 are to be performed at time of study drug discontinuation for any reason.

9.5 Follow up Assessments

All patients who complete the study or have a response (defined as stable disease or better), regardless of treatment arm, will continue to be followed for growth and complications of the vascular tumor and protocol therapy related toxicities for a total of 5 years from the date of study enrollment. Following completion of study therapy, patients will be followed at a minimum interval of every 6 months. Follow up visits may occur at participants home institutions or they may return to the study site at a minimum of every 6 months for their routine follow up care. Participants should return to the study site on an annual basis for a follow up visit. See section 6.14 for further information for follow-up criteria.

9.6 Correlative Studies (Biomarkers) - Optional Serum, plasma and Tissue (archival) collection

Correlative molecular biology studies will be conducted as part of the current trial. All patients should be approached for consent to all of these biology studies. Patients may participate in the treatment portion of this trial without consenting to the biology studies. Following completion of biomarker analysis at CCHMC, any remaining samples will be stored in the vascular tissue repository at CCHMC with participant consent.

Biomarker analysis

Biomarker analysis will be conducted on blood samples (serum and plasma) and tissue (when available) at baseline (blood and tissue), at the end of induction course 2, and at the end of maintenance courses 6 and 12 (following initiation of induction and maintenance). The change in levels of these vascular markers will be analyzed in LeCras' laboratory at CCHMC using ELISA. Analysis will include VEGF-A, -C, -D, Pleiotrophin, IGF-1, Endothelin-1, Thrombospondin and Angiopoietin-1, and-2 and future biomarkers of interest in this class of patients. Control serum and plasma from age and sex matched non-malformation patients may be obtained from left-over clinical samples or tissue repository. Tissue analysis (expression of the vascular markers) will include phosphorylated Akt, phosphorylated ERK-1/2, and mTOR and phosphor-S6 kinase, as well as biomarkers VEGF-A, -C, -D, Pleiotrophin, IGF-1, Endothelin-1, Thrombospondin and Angiopoietin-1, and-2 and future biomarkers of interest in this class of patients. There is limited information on biomarker analysis in this population of patients.

9.6.1 Sample Collection

Tissue:

Archival tissue may be submitted to satisfy this correlative study. In the event tissue is available at another institution, the enrolling site should work with the institution to obtain the tissue samples, either snap-frozen or in paraffin block, or as prepared slides (10 unstained sections, if available. Sites should contact the coordinating center if fewer sections are available for submission). In the event no archival tissue is available and the patient has a cutaneous lesion, if consent is indicated on the study consent form, a 6 mm punch biopsy may be obtained to satisfy this correlative study.

Processing: For patients undergoing punch biopsy, the coordinating center should be contacted prior to the procedure to obtain further processing instructions.

Labeling: All tissue samples should be labeled with the subject's study ID number, initials, and the date of collection.

Shipping: See shipping instructions on the tissue specimen transmittal form.

9.6.2 Blood Biomarkers analysis:

Time point: Baseline, at end of induction and end of maintenance courses 6 and 12.

Sample collection: 1-6 ml of blood will be collected in a serum and plasma separator tubes

Processing:

- Specimens collected at CCHMC need to be received at LeCras' laboratory within 45 minutes of being drawn.
- Serum collection and processing: Collect 1-6 ml blood in serum separator tube (red top). Centrifuge at 1800xG for 15 minutes. Transfer serum to a 2-5 ml polypropylene cryogenic vial. Freeze and store at -80℃ until shipped.
- Plasma collection and processing: Collect 1-6 ml blood in a plasma separator tube (sodium citrate tube). Centrifuge at 1800xG for 15 minutes. Transfer plasma to a new tube and

repeat centrifugation 1800xG for 15 minutes. Transfer plasma to a 2-5ml polypropylene cryogenic vial. Freeze and store at -80℃ until shipped

- Due to blood volume limits, collect blood for clinical labs, then biomarker samples with serum as first priority then plasma.
- Polypropylene cryogenic vials will be provided to participating institutions.

Labeling: All samples should be labeled with the subject's study ID number, initials, the date of collection and time point (baseline, end of induction, and end of maintenance courses 6 and 12

Shipping: See shipping instructions on the serum and plasma specimen transmittal form. Frozen serum and plasma samples should be shipped frozen on dry ice overnight by FedEx or another overnight service. Please email tracking number and time/date/place of shipping to: HVMCresearch@cchmc.org.

Ship frozen serum/plasma samples in dry ice by FedEx or other overnight carrier; First Priority Overnight to:

Megan Metcalf
Cincinnati Children's Hospital Medical Center
3333 Burnet Ave.
MLC 7015
Cincinnati, OH 45229
Pager: (513) 736-3010

10 DRUG INFORMATION

10.1 Sirolimus (Investigational Brochure).

Sirolimus (Rapamune) is a macrocyclic lactone produced by *Streptomyces hygroscopicus*. In cells, sirolimus binds to the immunophilin, FK Binding Protein-12 (FKBP-12). The sirolimus: FKBP-12 complex binds to and inhibits the activation of the mammalian Target of Rapamycin (mTOR), a key regulatory kinase. Following administration of sirolimus oral solution, sirolimus is rapidly absorbed, with a mean time-to-peak concentration of approximately 1 hour (range 1-3 hours). The systemic availability of sirolimus was estimated to be approximately 14% after the administration of sirolimus oral solution. The mean bioavailability of sirolimus after administration of the tablet is about 27% higher relative to the oral solution. Sirolimus oral tablets are not bioequivalent to the oral solution; however, clinical equivalence has been demonstrated at the 2-mg dose level. Sirolimus is extensively metabolized by O-demethylation and/or hydroxylation to at least seven major metabolites. The parent drug contributes to more than 90% of the activity. The main route of elimination is through the feces (91%). The mean t 1/2 increased from 79 ± 12 hours in subjects with normal hepatic function to 113 ± 41 hours in patients with impaired hepatic function. Males have a 12% lower clearance of sirolimus than females after oral solution administration. No

differences were demonstrated between black and non-blacks. After administration of the oral solution and tablets with a high fat meal, the maximum concentration was reduced, and the time to maximum concentration was increased. The total exposure to drug (AUC) was also increased. Sirolimus is a substrate for both cytochrome P450 IIIA4 (CYP3A4) and P-glycoprotein (P-gp). Sirolimus is extensively metabolized by the CYP3A4 isozyme in the intestinal wall and liver and undergoes counter-transport from enterocytes of the small intestine into the gut lumen by the P-gp drug efflux pump. Sirolimus is potentially recycled between enterocytes and the gut lumen to allow continued metabolism by CYP3A4. Therefore, absorption and subsequent elimination of systemically absorbed sirolimus may be influenced by drugs that affect these proteins. Drugs that stimulate or inhibit p-450 enzymes will alter clearance of sirolimus and close attention to potential drug interactions is crucial.

10.1.1 Product Description:

For the purposes of this study, only sirolimus oral solution, containing 1 mg/mL sirolimus, will be used.
Sirolimus is a white to off-white powder and is insoluble in water, but freely soluble in benzyl alcohol, chloroform, acetone, and acetonitrile. The inactive ingredients in Rapamune® Oral Solution are Phosal 50 PG® (phosphatidylcholine, propylene glycol, mono- and di-glycerides, ethanol, soy fatty acids, and ascorbyl palmitate) and polysorbate 80. Rapamune Oral Solution contains 1.5% - 2.5% ethanol.

10.1.2 Solution Preparation and Storage:

Each Rapamune Oral Solution carton, NDC 0008-1030-06, contains one 2 oz (60 mL fill) amber glass bottle of sirolimus (concentration of 1 mg/mL), one oral syringe adapter for fitting into the neck of the bottle, sufficient disposable amber oral syringes and caps for daily dosing, and a carrying case.
Rapamune Oral Solution bottles should be stored protected from light and refrigerated at 2°C to 8°C (36°F to 46°F). Once the bottle is opened, the contents should be used within one month. If necessary, the patient may store the bottles at room temperatures up to 25°C (77°F) for a short period of time (e.g., not more than 15 days).
An amber syringe and cap are provided for dosing, and the product may be kept in the syringe for a maximum of 24 hours at room temperatures up to 25°C (77°F) or refrigerated at 2°C to 8°C (36°F to 46°F). After dilution, the preparation should be used immediately.
Rapamune Oral Solution provided in bottles may develop a slight haze when refrigerated. If such a haze occurs, allow the product to stand at room temperature and shake gently until the haze disappears. The presence of this haze does not affect the quality of the product.

10.1.3 Route of Administration:

Sirolimus may be taken either with or without food. Patients should be advised to take their sirolimus at the same time of the day and at the same time in relation to meals. Patients may not take sirolimus with grapefruit juice. It is recommended that the oral solution be added to at

least two ounces (60 mL) of water or orange juice immediately prior to consumption in order to hide the taste. This solution should be stirred vigorously and then consumed. The container may then be refilled with a minimum of 4 oz of additional water or orange juice, stirred vigorously, and immediately consumed. Special attention must be paid to avoiding foods and drugs that will affect CYP3A4. (See Appendix II)

10.1.4 Drug interactions

Sirolimus is known to be a substrate for both cytochrome P-450 3A4 (CYP3A4) and p-glycoprotein (P-gp). Inducers of CYP3A4 and P-gp may decrease sirolimus concentrations whereas inhibitors of CYP3A4 and P-gp may increase sirolimus concentrations.
Strong Inducers and Strong Inhibitors of CYP3A4 and P-gp - Avoid concomitant use of sirolimus with strong inducers (e.g., rifampin, rifabutin) and strong inhibitors (e.g., ketoconazole, voriconazole, itraconazole, erythromycin, telithromycin, clarithromycin) of CYP3A4 and P-gp. Alternative agents with lesser interaction potential with sirolimus should be considered.
Grapefruit Juice - Because grapefruit juice inhibits the CYP3A4-mediated metabolism of sirolimus, it must not be taken with or be used for dilution of Rapamune.
Inducers or Inhibitors of CYP3A4 and P-gp - Exercise caution when using sirolimus with drugs or agents that are modulators of CYP3A4 and P-gp. The dosage of Rapamune and/or the co-administered drug may need to be adjusted.
Refer to Appendix II for prohibited medications.
Immunosuppressants may affect response to vaccination. Therefore, during treatment with Rapamune, vaccination may be less effective. The use of live vaccines should be avoided; live vaccines may include, but are not limited to, the following: measles, mumps, rubella, oral polio, BCG, yellow fever, varicella, and TY21a typhoid.

10.1.5 Toxicity

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Headache (L), hypertension (L), nausea, immunosuppression (L), diarrhea, constipation, fever	Chest pain, insomnia, dysphagia, vomiting, dyspnea	Hypotension, asthma, increased cough, flu like syndrome, tachycardia, anorexia, sensitivity reactions

Prompt: Within 2-3 weeks, prior to the next course	Tremor (L), renal dysfunction, elevated creatinine/BUN, anemia, asthenia, pain (abdominal, back, pain), hyperlipidemia, hypercholesteremia, hypertriglyceridemia, hyperglycemia, peripheral edema, weight gain, arthralgia	Elevated LFTs, UTI, URIs, mild thrombocytopenia, leukopenia, hyper/hypokalemia (L), hypophosphatemia, rash, hives, pruritis, hyperuricemia, delayed wound healing, hypomagnesaemia (L)	Gastritis, esophagitis, flatulence, CNS abnormalities: (confusion (L), somnolence (L), depression (L), anxiety, anxiousness, paresthesias, emotional labiality, hypo/hypertonia, dizziness, neuropathy, hypesthesia, nervousness), infections (bacterial, fungal, viral–sepsis, cellulitis, herpes simplex & zoster, EBV, mycobacterial, sinusitis, pharyngitis, abscess, pneumonia, bronchitis, peritonitis), pleural effusions, pleural edema, hypoxia, thrombosis, thrombophlebitis, myalgia
Delayed: Any time later during therapy, excluding the above conditions	Acne		Skin ulcer, hirsutism (hypertrichosis) (L), gingival hyperplasia, abnormal vision, ear pain, cataracts, otitis, tinnitus, hemorrhage, ileus, chronic renal dysfunction, renal tubular necrosis, post-transplant diabetes mellitus (L), CHF, ascites, thrombocytopenic purpura (hemolytic-uremic syndrome), arthrosis, bone necrosis, osteoporosis
Late: Any time after completion of treatment			Lymphoproliferative disorders, skin malignancies
Unknown Frequency and Timing:	Sirolimus was embryo/feto-toxic in rats at dosages of 0.1 mg/kg and above (approximately 0.2 to 0.5 the clinical doses adjusted for body surface area). Embryo/feto toxicity was manifested as mortality and reduced fetal weights (with associated delays in skeletal ossification). Sirolimus is excreted in trace amounts in milk of lactating rats. It is not known whether sirolimus is excreted in human milk.		

(L) Toxicity may also occur later.

10.1.6 Therapeutic Drug Monitoring

Monitoring of sirolimus trough concentrations is recommended for all patients, especially in those patients likely to have altered drug metabolism, in patients ≥ 13 years who weigh less than 40 kg, in patients with hepatic impairment, when a change in the Rapamune dosage form is made, and during concurrent administration of strong CYP3A4 inducers and inhibitors. Therapeutic drug monitoring should not be the sole basis for adjusting Rapamune therapy. Careful attention should be made to clinical signs/symptoms, tissue biopsy findings, and laboratory parameters.

The above recommended 24-hour trough concentration ranges for sirolimus are based on chromatographic methods. Currently in clinical practice, sirolimus whole blood concentrations are being measured by both chromatographic and immunoassay methodologies. Because the measured sirolimus whole blood concentrations depend on the type of assay used, the concentrations obtained by these different methodologies are not interchangeable. Adjustments to the targeted range should be made according to the assay

utilized to determine sirolimus trough concentrations. Since results are assay and laboratory dependent, and the results may change over time, adjustments to the targeted therapeutic range must be made with a detailed knowledge of the site-specific assay used. Therefore, communication should be maintained with the laboratory performing the assay. A discussion of different assay methods is contained in Clinical Therapeutics, Volume 22, Supplement B, April 2000.

10.1.7 Supplier:

For this study, sirolimus (oral solution) will be supplied by Pfizer and is considered an "investigational" drug.

10.1.8 Return and Retention of Study Drug –

Study drug supplies may be destroyed on site per institutional policies. Documentation of destruction will be collected during on-site monitoring visits and provided to the coordinating center at the conclusion of the study.

10.2 Vincristine (See Package Insert for product information)

10.2.1 Supplier – commercial supplies are to be used for vincristine.

10.2.2 Source and Pharmacology

Vincristine is an alkaloid isolated from Vinca rosea Linn (periwinkle). It binds to tubulin, disrupting microtubules and inducing metaphase arrest. Its serum decay pattern is triphasic. The initial, middle, and terminal half-lives are 5 minutes, 2.3 hours, and 85 hours respectively; however, the range of the terminal half-life in humans is from 19 to 155 hours. The liver is the major excretory organ in humans and animals; about 80% of an injected dose of vincristine sulfate appears in the feces and 10% to 20% can be found in the urine. The P450 cytochrome involved with vincristine metabolism is CYP3A4. Within 15 to 30 minutes after injection, over 90% of the drug is distributed from the blood into tissue, where it remains tightly, but not irreversibly bound. It is excreted in the bile and feces. There is poor CSF penetration.

10.2.3 Administration

Vincristine is to be prepared and administered per institutional policies and the package insert recommendations. It is extremely important that the intravenous needle or catheter be properly positioned before any vincristine is injected. Leakage into surrounding tissue during intravenous administration of vincristine may cause considerable irritation. If extravasation occurs, the injection should be discontinued immediately and any remaining portion of the dose should then be introduced into another vein. Local injection of hyaluronidase and the application of moderate heat to the area of leakage will help disperse the drug and may minimize discomfort and the possibility of cellulitis.

The World Health Organization, the Institute of Safe Medicine Practices (United States) and the Safety and Quality Council (Australia) all support the use of minibag rather than syringe for the infusion of vincristine. The delivery of vincristine via either IV slow push or minibag is acceptable. Injection of vincristine sulfate should be accomplished as per institutional policy. Vincristine must be administered via an intact, free-flowing intravenous needle or catheter. Care should be taken that there is no leakage or swelling occurring during administration. The solution may be injected either directly into a vein or into the tubing of a running intravenous infusion. ***When dispensed the container or syringe containing vincristine must be enclosed in an overwrap bearing the statement*** "DO NOT REMOVE COVERING UNTIL MOMENT OF INJECTION. FATAL IF GIVEN INTRATHECALLY. FOR INTRAVENOUS USE ONLY."

10.2.4 ADVERSE REACTIONS

Prior to the use of this drug, patients and/or their parents/guardian should be advised of the possibility of untoward symptoms.

In general, adverse reactions are reversible and are related to dosage. The most common adverse reaction is hair loss; the most troublesome adverse reactions are neuromuscular in origin.

When single, weekly doses of the drug are employed, the adverse reactions of leukopenia, neuritic pain, and constipation occur but are usually of short duration (i.e., less than 7 days). When the dosage is reduced, these reactions may lessen or disappear. The severity of such reactions seems to increase when the calculated amount of drug is given in divided doses. Other adverse reactions, such as hair loss, sensory loss, paresthesia, difficulty in walking, slapping gait, loss of deep-tendon reflexes, and muscle wasting, may persist for at least as long as therapy is continued. Generalized sensorimotor dysfunction may become progressively more severe with continued treatment. Although most such symptoms usually disappear by about the sixth week after discontinuance of treatment, some neuromuscular difficulties may persist for prolonged periods in some patients. Regrowth of hair may occur while maintenance therapy continues.

10.3 Steroids (Prednisolone or equivalent) – See package insert for further information

10.3.1 Supplies – Commercial supplies of steroid are to be used for this study.

11 RESPONSE ASSESSMENTS

11.1 Evaluation of Disease Response:

Patients will be assessed at the baseline (prior to induction) and 6 and 12 courses after start of maintenance.

The optimal measure of disease response in patients with complex vascular anomalies has not been established. Current practice includes changes in physical exam, radiologic evaluations, laboratory assessments and/or quality of life measures. These lesions are difficult to assess with any one method because of heterogeneity in growth patterns, diversity of associated clinical and laboratory abnormalities and a fluctuating clinical course dependent upon factors other than treatment, such as intercurrent infections, puberty and trauma. It is currently unclear if there is a good correlation between lesion size, determined radiologically and clinical changes or quality of life measures. For these reasons we have elected to assess disease response using three distinct methods:

11.1.1 Response by Imaging:

For each imaging assessment time point (at baseline and end of maintenance courses 6 and 12), initial radiologic response will be reviewed by each site's radiologist. If the radiologist identifies an area of possible disease progression while evaluating the imaging, the imaging will be reviewed by the identified site study radiologist prior to dispensing drug.

MRI should be used as the radiologic study of choice. MRI will be performed using a 1.5 or 3.0 Tesla system with standard MRI protocols (Appendix III) to assess vascular anomaly response. In rare cases where the patient may have a contraindication to MRI, contrast-enhanced CT should be performed. Target lesions should be selected on the basis of their size and their suitability for accurate repeated measurements (either by imaging techniques or clinically). The same method of assessment and the same technique should be used to characterize the target lesion at baseline and during follow up. To improve reliability and consistency of response assessments all imaging will be centrally reviewed at BCH. Screening scan will be sent to BCH immediately for review to confirm eligibility. All scans will be sent by disc at the end of maintenance for assessment. If there is any question about radiologic disease response at any time during maintenance, the scans will be reviewed by the site radiologist and BCH study radiologist.

11.1.2 Quality of Life Assessments (QOL):

This study will evaluate the effects of disease and treatment with sirolimus on the health related quality of life (HRQOL) in children and young adults. QOL measurements in this population are complicated by the range of age of affected individuals (infants to young adults) and the lack of validated QOL scales for patients with vascular anomalies. However, there exist generic scales used to quantitate QOL in pediatric and adult patients with good reliability and validity for a variety of chronic diseases.

The Pediatric Quality of Life Inventory (PedsQL 4.0) will be used to assess the QOL of children from 3 to 18 years of age. (46) The infant PedsQL is a tool to assess QOL in patients under the age of 2 years. The PedsQL is a brief standardized multidimensional pediatric QOL scales consisting of both child self-report and parent proxy-report measures and includes

generic and disease specific modules. It consists of a 23-item core measure of global QOL that has four subscales: physical functioning, emotional functioning, social functioning, and school functioning. Based on the standard error of the mean, Varni has also defined minimum changes in total score as clinically meaningful (4.4 change in the child self-report and 4.5 change in the parent proxy report). Changes in total scores with maintenance therapy will be used to quantitate the effects of this drug on health related quality of life in the pediatric population. A brief description of the QOL scales being used in this protocol is attached in Appendix V.

The Functional Assessment of Chronic Illness Therapy (FACIT) system will be used to assess health-related QOL (47) in adult patients (19-31 years of age). Specifically, this study will utilize a 27-item generic core (FACT-G) that includes 4 core subscales - physical well-being, emotional well-being, social well-being, and functional well-being. The FACT-G is a brief, reliable, and valid QOL measure, which is sensitive to clinical change and has been used with patients with cancer and a variety of other chronic illnesses (48). Based on the standard error of the mean, the minimally clinically important difference in FACT-G scores is 3.99.

Quality of Life assessments will be used in determining disease response as outlined in section11.2. For Peds-QL, the patient's self-report will be used when appropriate. If the patient's self-report is not available or other circumstances such as developmental delays, mental retardation, psychosis, or other apparent psychiatric issue, that in the opinion of the investigator would impair the subject's ability to complete the scale accurately, then the proxy report will be used instead at the discretion of the investigator. Likewise, a proxy-report will be used over the patient's self-report as appropriate based on the developmental age of the patient. This determination will be made by the investigator at the baseline QOL time point and will remain consistent for all other QOL time points. In the event that there is neither a consistent parent nor proxy report, the QOL assessments will not be used as a measure of disease response.

Pain will be assessed as an exploratory outcome and is not part of the response criteria for this study. Minimal clinically meaningful values have not been established for these variables; however given the importance of pain from the patient's perspective, we will include its assessment. Pain will be measured using the FLACC pain questionnaire for infants and older subjects unable to complete their age appropriate pain questionnaire, the PedsQL Pediatric Pain Questionnaire (49) for children and the short-form McGill Pain Questionnaire (50) for adults.

11.1.3 Clinical criteria and functional impairment:

The degree of impairment of organ function caused by the vascular anomalies has been used historically to assess disease response to therapy. Currently, there are no validated scales, specific to vascular anomalies, to assess functional impairment. We therefore, have developed

a system of measurement which can be used across centers in a standardized manner (Appendix IV). This instrument has been adopted from the measures of organ function that have been validated in the quantification of AEs resulting from medical therapies or procedures (Common Terminology Criteria for AEs) and is currently in use In SIR-DA-0901 We will use these objective measures to quantify specific organ dysfunction resulting from vascular anomaly.

11.2 Disease Response

Response will be established by changes in at least 1 parameter at 6 and 12 courses after the initiation of maintenance using the following criteria:

Disease response – Complete Response (CR) and Partial Response (PR)

- **CR**
  - No evidence of disease on radiologic imaging and
  - No evidence of organ dysfunction due to disease and
  - Normalization of QOL criteria*
- **PR**
  - > 20 % reduction in volume by radiologic imaging, or
  - Improvement in target organ dysfunction by at least one grade, or
  - Improvement of proxy-report PedsQL by > 4.5; FACT-G by > 3.99*

Progressive disease

- > 20 % increase in target lesion volume by radiologic imaging, or
- Worsening in target organ dysfunction by at least one grade, or
- Worsening of Peds proxy-report PedsQL by > 4.5; FACT-G by > 3.99*

Stable disease: None of the above

Formal Responses will be assessed two times during maintenance at the end of courses 6 and 12 following start of Maintenance. Disease assessments may be performed at any time during maintenance if clinically indicated. Disease worsening as defined as “progressive disease” or “loss of response” based upon any one of the above methods will be permitted to cross-over. If worsened disease is based on laboratory parameters we suggest a repeat of laboratory evaluations to confirm.

* In the event there is an apparent discrepancy between the patient or parent proxy PedsQL score with either the investigator’s assessment of target organ dysfunction or response evident radiographically, the patient will remain on protocol therapy at the investigator’s discretion. In these particular instances, at the time of planned data analysis, the study statistician will determine if the patient should remain evaluable for overall response for the study.

Treatment will be continued until one of the criteria for removal from protocol therapy is met as outlined in section 6.13.

12 ADVERSE EVENT REPORTING REQUIREMENTS

12.1 Definitions

12.1.1 Adverse Event (AE)

An adverse event (AE) is any untoward medical occurrence (e.g., an abnormal laboratory finding, symptom, or disease temporally associated with the use of a drug) in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) need not be considered AEs and should not be recorded as an AE. Disease progression should not be recorded as an AE, unless it is attributable by the investigator to the study therapy.

12.1.2 Suspected Adverse Reaction (SAR)

A suspected adverse reaction (SAR) is any AE for which there is a *reasonable possibility* that the drug is the cause. *Reasonable possibility* means that there is evidence to suggest a causal relationship between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Causality assessment to a study drug is a medical judgment made in consideration of the following factors: temporal relationship of the AE to study drug exposure, known mechanism of action or side effect profile of study treatment, other recent or concomitant drug exposures, normal clinical course of the disease under investigation, and any other underlying or concurrent medical conditions. Other factors to consider in considering drug as the cause of the AE:

- Single occurrence of an uncommon event known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome)
- One or more occurrences of an event not commonly associated with drug exposure, but otherwise uncommon in the population (e.g., tendon rupture); often more than

once occurrence from one or multiple studies would be needed before the sponsor could determine that there is *reasonable possibility* that the drug caused the event.

- An aggregate analysis of specific events observed in a clinical trial that indicates the events occur more frequently in the drug treatment group than in a concurrent or historical control group

12.1.3 Unexpected AE or SAR

An AE or SAR is considered unexpected if the specificity or severity of it is not consistent with the applicable product information (e.g., package insert/summary of product characteristics for an approved product). Unexpected also refers to AEs or SARs that are mentioned in the package insert as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

12.1.3.1 Serious AE or SAR

An AE or SAR is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death;
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- Requires inpatient hospitalization (>24 hours) or prolongation of existing hospitalization;*
- Results in congenital anomaly/birth defect;
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. For reporting purposes, also consider the occurrences of pregnancy as an event which must be reported as an important medical event.

*Hospitalization for anticipated or protocol specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered serious adverse events.

12.2 Documentation and reporting of non-serious AEs or SARs

The severity of toxicities will be graded in accordance with the Common Terminology Criteria for AE (CTCAE) version 4.0.3. Grade 2 AEs deemed possible, probable or definitely attributed to study

medication and all Grade 3 and higher AEs, regardless of suspected causal relationship to study drug, will be recorded as AEs in the CRFs.

For non-serious AEs or SARs, documentation must begin from day 1 of study treatment and continue through the 30 day follow-up period after treatment is discontinued.

Laboratory results from non-pediatric institutions or laboratories often have significantly different lab reference ranges, not specific for age. In the event of a greater than ±10% difference in the reference range for a specific lab result, either BCH or other nationally recognized pediatric standards may be used to determine the grade of the laboratory value.

Collected information should be recorded in the Case Report Forms (CRF) for that patient. Please include a description of the event, its severity or toxicity grade, onset and resolved dates (if applicable), and the relationship to the study drug. Documentation should occur at least once a course.

Refer to the Manual of Operations for this clinical trial for more detail instructions regarding adverse event reporting.

12.3 Documentation of SAEs or Serious SARs

SAEs and Serious SARs will be recorded from the time of informed consent until 30 days after completion of study treatment.

12.3.1 SAE and Serious SAR reporting in the Follow Up Period

In the event that during the 5-year follow up period the Investigator identifies what is believed to be a new (unexpected) long-term risk or short-term risk (in the event the patient continued therapy on marketed off-label sirolimus) attributable to sirolimus, this SAE must be reported to the Sponsor and to the site's IRB consistent with any Unanticipated Problem reporting policy, as this would be a new risk directly related to any subject that might be participating in the research.

12.4 Participating Site Responsibilities:

12.4.1 SAE/Serious SAR Reporting

All SAEs and Serious SARs, regardless of attribution or expectedness, must be reported to BCH verbally to coordinating center and Denise Adams within 24 hours of site awareness and followed with a FDA MedWatch 3500a Form. For participating sites, this form must be submitted to BCH within 3 days of learning of the event.

MedWatch 3500a Reporting Guidelines: In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500a form:

- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome, if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow up information to a safety report should be submitted as soon as the relevant information is available. Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500a report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500a form
- Summarizing new information and sending by secure email or faxing it with a cover letter including subject identifiers (i.e. D.O.B., initial, subject number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The subject identifiers are important so that the new information is added to the correct initial report.)

12.4.2 IRB Reporting Requirements:

Participating sites are required to notify their local IRBs according to institutional reporting policies.

12.5 Sponsor Responsibilities to be performed by the Coordinating Center BCH:

12.5.1 FDA:

- Unexpected Fatal or Life-Threatening Suspected Adverse Reaction Reports
  Notify the FDA as soon as possible but no later than 7 calendar days after the sponsor's initial receipt of the information. Either the sponsor or the investigator can make the determination of life-threatening.

- Unexpected, Serious Suspected Adverse Reactions
  If an investigator or BCH deems that an event is both a serious SAR and unexpected, the FDA will be notified per 21 CFR 312.32(c) (1) (i), only if there is evidence to suggest a causal relationship between the drug and the adverse event. The serious SAR must be reported to the FDA as soon as possible but no later than 15 calendar days after the sponsor determines the information qualifies for reporting.

12.5.2 BCH IRB and~~:~~ BCH DSMB:

The sponsor will report any unexpected life threatening event or death (grade 4 or 5) adverse

event to the BCH IRB and BCH DSMB center program DSMB within 72 hours after the sponsor is made aware of the event.

12.5.3 Unexpected Grade 3 serious adverse events at least possibly attributable to the research will be reported to the BCH IRB per institutional policies.

12.5.4 Pfizer Reporting Requirements:

Notify Pfizer Drug Safety of all SAEs using the safety reporting form provided by Pfizer. Pfizer: Fax: (866) 997-8322

12.6 Reporting of Protocol Violations/Deviations and Unanticipated Problems

Any protocol violations, deviations, or unanticipated problems should be documented and reported according to the coordinating center's Manual of Operations. See the Manual for more detailed instructions.

In addition, each participating site should report protocol deviations/violations or unanticipated problems according to their site's policies, procedures and applicable regulations.

13 STATISTICAL ANALYSIS PLAN

13.1 Sample size and power calculation:

This is a two treatment arm randomized multi-center two-phase (induction/maintenance) clinical trial using the doubly adaptive biased coin design with a target allocation given by an urn model (40, 41) to continuously skew the randomization ratio to favor the better performing treatment arm. Patients will be randomized to receive either sirolimus or vincristine in the beginning of induction phase, and will be switched to the treatment they were not initially assigned to only if they fail to attain hematologic response (an increase of platelet count of >100,000 /µl or an increase in platelets of 2 times the baseline, whichever is greater, and a fibrinogen > 150 mg/dl; platelet and fibrinogen levels must meet these parameters on two consecutive lab measurements with a minimum of one day between evaluations). The primary efficacy endpoint is attainment of hematologic response by the end of the induction period.

We plan to enroll n=50 evaluable patients over a 4 year period from up to 8 participating institutions. In the study SIR-DA-0901 we observed the median time to hematologic response was 3 weeks and we anticipate 74%~90% response rate for the sirolimus treatment arm, whereas 20%~40% for the vincristine, depending on the underlying time to response mechanism and preliminary data. Based on a computer simulation study and assuming a response rate difference of 40% or greater, we anticipate the proposed sample of n=50 in total under the doubly adaptive biased coin design will provide 73%~90% power to detect a significant higher hematologic response rate of the sirolimus treatment arm. The simulation study also suggests that on average ≥20% more patients will be treated with the

more efficacious sirolimus treatment arm compared to the conventional fixed design.

In this adaptive design we will not conduct an interim analysis or stop the trial early. The adaptive design will treat more subjects with the sirolimus treatment arm by design in the case that the sirolimus treatment arm is more efficacious. We also do not expect any serious adverse events attributable to sirolimus. Therefore, futility may be the only legitimate reason to stop the trial early. However, the small total sample size limits the power of the interim analysis to test for the futility of sirolimus and splitting the type I error rate between the interim and the final analysis will lead to reducing the power of the final analysis without any substantial chance of early stopping for futility.

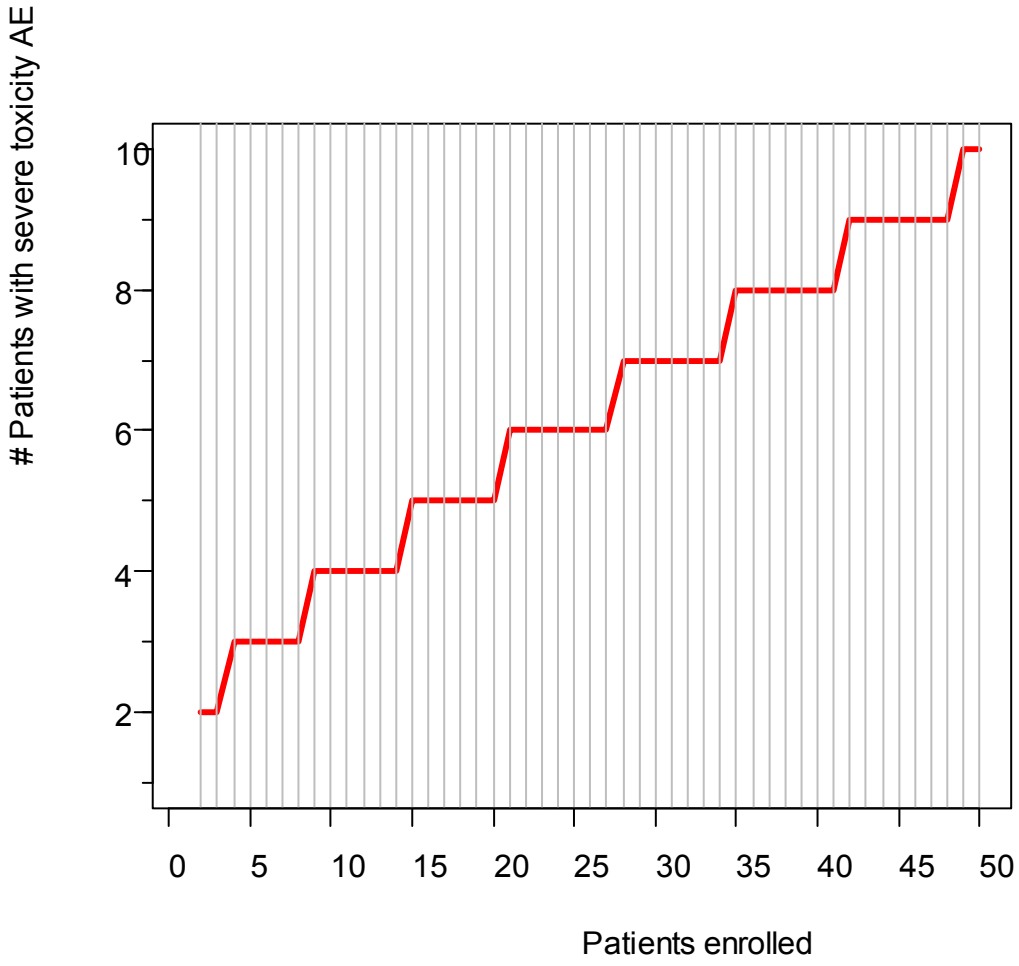
An early stopping rule will be invoked to prevent further accrual of patients onto the study in the event that Sirolimus or Vincristine is associated with a higher than acceptable rate of severe toxicity (10%) with severe toxicity (defined as Grade 4 infection, pneumocystis carinii pneumonia, Grade 2 pneumonitis, Grade 4 rash, Grade 4 hypertension, or Grade >2 allergic reaction) identified during the Induction period (the first 2 courses). We adopt a dynamic stopping rule based on cumulative number of patients enrolled into each treatment group. Enrollment to a treatment will be stopped, if the number of severe toxicity AE reaches a threshold defined in the table. This threshold is defined such that the 95% lower confidence bound of severe toxicity exceeds acceptable 10% rate, for either of the treatment groups. The stopping rule has high probability of stopping early if the probability of severe toxicity is moderately great (≥20%) and low probability of stopping if the underlying probability of severe toxicity is ≤10%.

A graph is also given below to visually show threshold as a function of total number enrolled in a particular treatment group. If the cumulative number of aforementioned severe toxicity AE reaches or exceeds the red line, the enrollment to that treatment will be stopped. New enrollment to the study will only be into the other group at that point.

Number of patients enrolled to a particular treatment group	Stop enrollment If at least this many patients experienced severe toxicity AE cumulatively	Probability of stopping at this stage if the true toxicity rate is 10%	Probability of stopping at this stage if the true toxicity rate is 20%
2	2	0.01	0.04
3	2	0.028	0.104
4	3	0.004	0.027
5	3	0.009	0.058
6	3	0.016	0.099
7	3	0.026	0.148
8	3	0.038	0.203
9	4	0.008	0.086

10	4	0.013	0.121
11	4	0.019	0.161
12	4	0.026	0.205
13	4	0.034	0.253
14	4	0.044	0.302
15	5	0.013	0.164
16	5	0.017	0.202
17	5	0.022	0.242
18	5	0.028	0.284
19	5	0.035	0.327
20	5	0.043	0.37
21	6	0.014	0.231
22	6	0.018	0.267
23	6	0.023	0.305
24	6	0.028	0.344
25	6	0.033	0.383
26	6	0.04	0.423
27	6	0.047	0.461
28	7	0.018	0.322
29	7	0.022	0.357
30	7	0.026	0.393
31	7	0.031	0.429
32	7	0.036	0.465
33	7	0.042	0.5
34	7	0.048	0.534
35	8	0.02	0.401
36	8	0.024	0.434
37	8	0.027	0.467
38	8	0.032	0.5
39	8	0.037	0.532
40	8	0.042	0.563
41	8	0.048	0.593
42	9	0.021	0.469
43	9	0.024	0.5
44	9	0.028	0.53
45	9	0.032	0.559
46	9	0.036	0.588
47	9	0.041	0.616
48	9	0.046	0.642
49	10	0.022	0.528
50	10	0.025	0.556

Table 10. Threshold to stop enrollment if number of patients experienced pre-defined severe toxicity AE reaches or exceeds



13.2 Statistical analysis

We will summarize response and safety event rates by sample proportions and use exact binomial distributions to test for a significant difference in the event rates. We will also use the Kaplan-Meier estimator to estimate the distribution of time to hematologic response and use the log-rank test to test for a significant difference in the distributions. Secondary endpoints include efficacy and safety endpoints measured in the maintenance phase when some patients will switch treatment arms. As only non-responders or those who lose response will be only allowed to switch, this is not a cross-over trial and efficacy after switching will be separately measured and compared. Quality of life (QOL) measure data will be analyzed by observing changes in total scores. Different QOL test batteries will be used depending on the patient age and we will use standardized changes in total scores by the known standard deviation of the test batteries.

There will be two data analysis sets, one for per-protocol analysis and one for intent to treat analysis in order to account for any patients who stop treatment early.

Per Protocol Analysis;
The initial response in Induction analyses: Only patients who have completed the 2 courses of induction will be considered evaluable for response, for per-protocol analysis. The Maintenance phase analyses: Only patients who have completed 6 courses of therapy and finished the end of course 6 evaluations will be considered evaluable for response, for per-protocol analysis.

Intent-to-treat analysis data set:
All patients who received at least first 14 days of therapy (sirolimus or vincristine) will be included in the ITT data set. Those who terminated earlier than 2-course induction phase will be considered non-responders in ITT analysis. (The non-responder patients will change over to the other treatment and begin maintenance in that other treatment.)
Comparisons of early dropout rates (prior to 2 courses) will be compared between the two treatment arms.

13.3 Accrual

Infants to young adults up to 31 years of age with Kaposiform hemangioendothelioma or tufted angioma requiring systemic therapy will be eligible for this study. Children with vascular anomalies are usually otherwise healthy except for the presence of the vascular anomaly and any resulting organ dysfunction.

Anticipated number of patients to be enrolled

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or Unknown	Total
Female	1.6	2.5	4.2	2.5	14.2		25
Male	1.6	2.5	4.2	2.5	14.2		25
Total	3.2	5	8.4	5	28.4		50

13.4 Evaluable for Adverse Effects

Patients will be considered evaluable for adverse effects provided they have received at least the first 14 days of therapy (sirolimus or vincristine).

13.5 Evaluable for Response

Initial response in induction: Only patients who have completed the 2 courses of induction will be considered evaluable for response.
Maintenance: Only patients who have completed 6 courses of therapy and finished the end of course

6 evaluations will be considered evaluable for response.

14 HUMAN SUBJECTS PROTECTION & DATA SAFETY MONITORING PLAN

14.1 Sources of Material

If not contraindicated, tissue samples from the vascular tumor will be obtained before enrollment to ensure correct diagnosis. Blood tests will be obtained at study entry, at specific interval during treatment and at times required for the best care. In addition, results of blood work, evaluations including radiologic imaging, and adverse advents will be recorded at the treatment center and reported to BCH as coordinating center in a coded fashion. The treating physician at the clinical center will keep a list linking codes with patient identifying information in accordance with their institutional IRB guidelines. The Sponsor and BCH will not have access to identifying information from participating institutions.

14.2 Potential Risks

The primary risk to the subjects from participation in this trial is from sirolimus and/or vincristine toxicity. Risk of toxicity has been correlated with serum levels of sirolimus. All participants will have serum levels measured regularly to guide appropriate dosing and to minimize drug toxicity. Vincristine toxicity will be closely monitored and dose will be adjusted as needed. Participants enrolled on this trial will be carefully monitored for the development of toxicities, with guidelines for dose modifications, discontinuation of drug, and stopping rules in place.

14.3 Recruitment and Informed Consent

Patients may be recruited from the Dermatology, Oncology, Surgery, and Vascular Anomalies clinics at the participating institutions. The investigational nature and objectives of this trial, the procedures, and treatments involved and their attendant risks and discomforts and benefits, as well as potential alternative therapies will be carefully explained to the patient or the patient's parents or guardian if he/she is a child, and a signed informed consent document will be obtained. Consent will be obtained by the site PI or an associate investigator on the trial. Where deemed appropriate by the clinician and the child's parents or guardian, the child will also be included in all discussions about the trial. Requirement for documentation of assent will be determined by the local IRB.

14.4 IRB Approval and Required Documentation

This is a multi-institutional trial; BCH will require evidence of local IRB approval of the protocol prior to allowing for accrual of patients at that institution. This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP) and the applicable regulatory requirements.

14.5 Protection against Risks

We will obtain institution review board approval at all participating sites. Patient's risks of participating in research will be kept to a minimum with measures to protect confidentiality and planned interim analysis for safety monitoring. Confidentiality will be maximized by coding patient

information prior to transfer to the principle investigator. The treating physician at the clinical center will keep a list linking codes with patient identifying information in accordance with their institutional IRB guidelines. The principal investigators will not have access to identifying information. Best of care will be provided to patients in the event of toxicities associated with treatment. Parents will be provided appropriate contact number(s) for treating physicians at clinical sites in accordance with institutional IRB guidelines.

14.6 Potential Benefits of the Proposed Research to the Subject and Others

This is the first clinical trial to prospectively assess and compare the effectiveness and safety of two treatment options for patients with Kaposiform hemangioendotheliomas. There will be potential for a direct benefit for patients with KHE as this will determine the optimum therapy option for these patients. The instruments developed in this study will be beneficial for future trials for this population. Finally, the participating institutions will develop a working collaboration that could provide the framework for future clinical trials in patients with rare vascular diseases.

14.7 Importance of Knowledge to be Gained

The knowledge gained by this study will help to determine a standard treatment for patients with these rare tumors. Response criteria developed in this study could be utilized for future trials.

14.8 Data and Safety Monitoring Plan

The Pediatric DSMB at Boston Children's Hospital consists of individuals with recognized expertise in oncology, neurology, gastroenterology, conduct of clinical trials, and biostatistics. The DSMB meets a minimum of twice per year. Approximately 4 weeks before each meeting of the DSMB, the study chair or designee will be responsible for preparing study reports for review by the DSMB. The DSMB will provide recommendations to change the study or to continue the study unchanged.

The trial PI and clinical coordinator will review the study progress regularly. Patients entered on the trial and adverse events will be reviewed to ensure that the study is implemented as outlined in the protocol. Quarterly reports will be generated by BCH to assess completeness of data. There will be monthly phone conferences between BCH and the local Principal Investigators to address QA issues.

14.9 Study Monitoring and Auditing

This study will be monitored in accordance with the BCH Program Data Safety Monitoring Plan for Phase I, Pilot, and Phase II Studies. Monitoring and auditing procedures as outlined in BCH Program Data Safety Monitoring Plan will be followed to ensure that the entire study is conducted, documented, and reported in accordance with the IRB approved protocol, the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Guidelines, and applicable regulatory requirements of Boston Children's Hospital.

Verification of eligibility and appropriate documentation of informed consent will be performed for

all subjects enrolled into the study by BCH coordinating center. Monitoring of timeliness of Adverse Event and Serious Adverse Event reporting will be done as events are reported to the coordinating center. Case report forms for each subject enrolled into the study will be monitored for completeness and quality.

14.10 Privacy

Data provided must be treated in strictest confidence. No information provided from individual patient's records may be discussed with anyone other than those individuals mentioned in the collaborative research agreement. Data may not be released in any form except as provided in the agreement.

14.11 Confidentiality

Each subject enrolled will, from that point forward, be identified by a unique identifier (study subject number). This study subject number will also be used for any research specimens collected and shipped to analysts outside each study site. All records generated will be stored in a locked office area, only accessible to study personnel. Clinical information will be accessed, according to HIPAA requirements, by study personnel to complete study documents, as needed.

15 REFERENCES

1. Mulliken JB, Glowacki J. Hemangiomas and vascular malformations in infants and children: A classification base on endothelial characteristics. Plast Reconstr Surg. 1982;69(3):412-22.
2. Enjolras O, Mulliken JB. Vascular tumors and vascular malformations (new issues). Adv Dermatol.1997;13:375-423.
3. Adams DM, Wentzel MS. The role of the hematologist/oncologist in the care of patients with vascular anomalies. Pediatr Clin North Am 2008;55(2):339-55.
4. Kasabach H, Merritt K. Capillary hemangioma with extensive purpura: Report of a case. Am J Dis Child 1940; 59:1063-1070.
5. Sarkar M, Mulliken JB, Kozakewich HP, et al. Thrombocytopenic coagulopathy (Kasabach-Merritt phenomenon) is associated with Kaposiform hemangioendothelioma and not with common infantile hemangioma. Plast Reconstr Surg 1997;100(6):1377-86.
6. Enjolras O, M Wassef, Mazoyer E, et al. Infants with Kasabach-Merritt syndrome do not have "true" hemangiomas. J Pediatr 1997;130(4):631-40.
7. Gruman A, Liang MG, Mulliken JB et al. Kaposiform hemangioendothelioma without Kasabach-Merritt phenomenon. J Am Dermatol 2005;52(4):616-22.
8. Haisley-Royster, O Enjolras, Frieden IJ, et al. Kasabach-merritt phenomenon: A retrospective study of treatment with vincristine. J Pediatr Hematol Oncol 2002; 24(6):459-62.
9. Mulliken JB, Anupindi S, Ezekowitz RA, et al. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 13-2004: A newborn girl with a large cutaneous lesion, thrombocytopenia, and anemia. N Engl J Med 2004;350:1764-1775.
10. Barlow C F, Priebe CJ, et al. Spastic diplegia as a complication of interferon Alfa-2a treatment of hemangiomas of infancy. J Pediatr 1998; 132(3 Pt 1): 527-30.
11. Michaud AP, Bauman NM, Burke DK et al. Spastic diplegia and other motor disturbances in infants receiving interferon-alpha. Laryngoscope 2004;114(7):1231-6.
12. Harper L, Michel JL, Enjolras O, et al. Successful management of a retroperitoneal kaposiform hemangioendothelioma with Kasabach-Merritt Phenomenon using alpha-interferon. Eur J Pediatr Surg. 2006; 16(5):369-72
13. Morad AB, McClain KL, Ogden AK. The role of tranexamic acid in the treatment of giant hemangiomas in newborns. Am J Pediatr Hematol Oncol 1993;15:383-385.
14. Hauer J, Graubner U, Konstantopoulos N, et al. Effective treatment of kaposiform hemangioendotheliomas associated with Kasabach-Merritt phenomenon using four-drug regimen. Pediatr Blood Cancer 2007;49(6):852-54.
15. Blei F, Karp N, Rofsky N, et al. Successful multimodal therapy for kaposifrm hemangioendothelioma complicated by Kasabach-Merritt phenomenon: case report and review of the literature. Pediatr Hematol Oncol. 1998;15(4):295-305
16. Adams, DM, Dasgupta R. Elluru R, et al. Rapamycin used in the treatment of a kaposiform hemangioendothelioma with Kasabach Merritt Phenomenon. 17th International Workshop on Vascular Anomalies, Boston, June 2008.

17. Blatt J, Stavas J, Moats-Staats B, et al. Treatment of childhood kaposiform hemangioendothelioma with sirolimus. Pediatr Blood Cancer 2010;55:1396-1398.
18. Hamill A, Wentzel MS, Gupta A, et al. Sirolimus for the treatment of complicated vascular anomalies in children. Pediatr Blood Cancer. 2011 Dec 1;57(6):1018-24.
19. Vignot, S., S. Faivre, et al. (2005). "mTOR-targeted therapy of cancer with Rapamycin derivatives." Ann Oncol 16(4): 525-37.
20. Tee AR, Blenis J. mTOR, translational control and human disease. Seminar Cell Dev Biol 2005 ;6:29-37.
21. El-Hashemite N, Walker V, Zhang H, et al. Loss of Tsc1 or Tsc 2 induces vascular endothelial growth factor production through mammilian target of Rapamycin. Cancer Res 2003;63:5173-5177.
22. Lee DF, Hung MC. All roads lead to mTOR: Integrating inflammation and tumor angiogenesis. Cell Cycle. 2007 Dec;6(24):3011-4.
23. Jiang BH, Liu LZ. P13K/PTEN signaling in tumorigenesis and angiogenesis. Biochem Biophys Acta. 2008 Jan;1784(1):150-8.
24. Matsuo M, Yamada S, Koizumi K, et al. Tumor-derived fibroblast growth factor-2 exerts lymphangiogenic effects through Akt/mTOR/p70S6kinase pathway in rat lymphatic endothelial cells. Eur J Cancer. 2007. Jul;43(11):1748-54
25. Inoki K, Corradetti MN, Guan KL. Dysregulation of the TSC-mTOR pathway in human disease. Nat Genet. 2005 Jan;37(1):19-24.
26. Perry B, Banyard J, McLaughlin ER, et al. AKT1 overexpression in endothelial cells leads to cutaneous vascular malformations in vivo. Arch Dermatol. 2007. April;143(4):504-06.
27. Morris PN, Dunmore BJ, Tadros A, et al. Functional analysis of a mutant form of the receptor tyrosine kinase Tie2 causing venous malformations. J Mol Med 2005;83:58-63
28. Zhou X., Hampel H, Thiele H, et al.. Association of germline mutation in the PTEN tumour suppressor gene and Proteus and Proteus-like syndromes. Lancet. 2001 July;358(9277):210-211.
29. Lee CH, Inoki K, Guan KL. mTOR Pathway as a Target in Tissue Hypertrophy. Annu Rev Pharmacol Toxicol. 2007;47**:**443-467.
30. Inoki, K., M. N. Corradetti, et al. (2005). "Dysregulation of the TSC-mTOR pathway in human disease." Nat Genet 37(1): 19-24.
31. Franz DN, Leonard J, Tudor C et al. Rapamycin causes regression of astrocytomas in tuberous sclerosis complex.. Ann Neurol. 2006;59(3):490-8.
32. Galanis E, Buckner JC, Maurer MJ, et al. Phase II trial of temRapamycin (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. J Clin Oncol. 2005;23(23):5294-304.
33. Atkins MB, Hidalgo M, Stadler WM, et al. Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of Rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. J Clin Oncol. 2004; 22(5): 909-18.
34. Witzig TE, Geyer SM, Ghobrial I, et al. Phase II trial of single-agent temRapamycin (CCI-779) for relapsed mantle cell lymphoma. J Clin Oncol. 2005;23(23):5347-56.

35. Chan S, Scheulen ME, Johnston S, et al. Phase II study of temRapamycin (CCI-779), a novel inhibitor of mTOR, in heavily pretreated patients with locally advanced or metastatic breast cancer. J Clin Oncol. 205;23(23):5314-22.
36. Schuetze SM, Baker LH, et al. (2006). Rapamycin reduced tumor-related morbidity and resulted in biochemical and radiographic response in patients with progressive sarcoma. ASCO. 2005; Atlanta, GA.
37. Bissler JJ, McCormack FX, Young LR, et al. Rapamycin for Angiomyolipoma in Tuberous Sclerosis Complex or Lymphangioleiomyomatosis. N Engl J Med. 2008;358**:** 140-151.
38. Marsh DJ, Trahair TN, Martin JL, et al. Rapamycin treatment for a child with germline PTEN mutation. Nat Clin Prac Oncol.2008;5(6):357-361.
39. Gallo et al. 2006, Adaptive designs in clinical drug development--an Executive Summary of the PhRMA Working Group, Journal of Biopharmaceutical Statistics, 2006, May;16(3):275-83. PMID:16724485.
40. Hu F, Zhang LX Asymptotic properties for doubly adaptive biased coin designs of multi-treatment clinical trials. Annals of Statistics, 2004, Vol. 32(1):268-301.
41. Jeffrey R Eisel and Michael B. Woodroofe. Central limit theorems for doubly adaptive biased coin designs, Annals of Statistics Volume 23, Number 1 (1995), 234,254.
42. Jelliffe RW, Schumitzky A, Bayard D, et al. Model-based, goal-oriented, individualised drug therapy. Linkage of population modeling, new 'multiple model' dosage design, bayesian feedback and individualised target goals. Clin Pharmacokinet.1998;34(1):57-77.
43. Marquet P. Clinical application of population pharmacokinetic methods developed for immunosuppressive drugs. Ther Drug Monit 2005;27(6) 727-32.
44. Varni JW, Seid M, Kurtin PS. PedsQL 4.0: reliability and validity of the Pediatric Quality of Life Inventory version 4.0 generic core scales in healthy and patient populations. Med Care 2001;39(8):800-12.
45. Cella D, Nowinski CJ. Measuring quality of life in chronic illness: the functional assessment of chronic illness therapy measurement system. Arch Phys Med Rehabil 2002;83(12 Suppl 2):S10-7.
46. Cella DF, Tulsky DS, Gray G et al. The Functional Assessment of Cancer Therapy scale: development and validation of the general measure. J Clin Oncol 1993;11(3):570-9.
47. Varni KW. The Varni/Thompson pediatric pain questionnaire. I. Chronic musculoskeletal pain in juvenile rheumatoid arthritis. Pain 1987;Jan. 28(1):27-38.
48. Melzack R. The short form McGill Pain Questionnaire. Pain 1987;Aug 30(2);191-197.
49. Wassef, M., Blei, F., **Adams, D.**, Alomari, A., Baselga, E., Berenstein, A., Burrows, P., Frieden, I. J., Garzon, M. C., Lopez-Gutierrez, J. C., Lord, D. J., Mitchel, S., Powell, J., Prendiville, J., Vikkula, M. Vascular Anomalies Classification: Recommendations From the International Society for the Study of Vascular Anomalies. Pediatrics. 2015 Jul 1; 136(1): e203-14. .

APPENDIX I: PERFORMANCE STATUS SCALES/SCORES

PERFORMANCE STATUS CRITERIA Karnofsky and Lansky performance scores are intended to be multiples of 10			
Karnofsky		**Lansky**	
Score	Description	Score	Description
100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

APPENDIX II: LIST OF MEDICATIONS TO AVOID/ USE CAUTION WHEN TAKING SIROLIMUS (RAPAMUNE)- THIS TABLE IS NOT ALL INCLUSIVE

- Avoid* and/or Use caution** when Using Sirolimus (Rapamune) and the following medications:
- (If these medications are medically necessary please consult a pharmacist prior to starting)
-

Azole Antifungals*: Ketoconazole, voriconazole, posaconazole, fluconazole, itraconazole, clotrimazole	**Antibiotics*:** Rifampin, Rifabutin, Erythromycin, telithromycin, clarithromycin, fusidic acid, troleandomycin**, rifapentine**	**Antiepileptics**:** carbamazepine, phenobarbital, phenytoin, fosphenytoin, oxcarbazepine, primidone**, stiripentol**
Protease inhibitors/antiretrovirals:** Ritonavir, indinavir, boceprevir, telaprevir, efavirenz, lopinavir, saquinavir, amprenavir, delavirdine	**Blood Pressure agents****: Diltiazem, verapamil, nicardipine, amiodarone	**Kinase Inhibitors*:** Crizotinib, tofacitinib, dabrafenib
Miscellaneous Medications*: Conivaptan, enzalutamide, mifepristone, nefazodone, natalizumab, pimecrolimus, tacrolimus, cyclosporine Use Caution**: Bromocriptine, Cimetidine, metoclopramide, danazol, dipyrone, leflunomide, lomatipide, mitotane,	**Food/herbal agents:** Grapefruit juice* Echinacea** St. John's Wort**	**Steroids** Dexamethasone, hydrocortisone, prednisone, prednisolone, methylprednisolone -make sure you are aware patient is on both due to immunosuppression. -no drug interaction

APPENDIX III: STANDARD VASCULAR MRI PROTOCOL

Trunk and extremities

Localizer
Cor T1
Ax 2D TOF (no sat bands)
Sag or Cor STIR
Ax T2 FSE with fat suppression
DWI
Ax T1 with fat suppression pre-contrast
Cor, Sag and Ax T1 with fat suppression post-contrast

Head and Neck

Localizer
Sag T1
Sag T2 FSE with fat suppression
Ax and Cor STIR
Ax 2D TOF (no sat bands)
DWI
Ax T1 with fat suppression pre-contrast
Cor and Ax T1 with fat suppression post-contrast

APPENDIX IV: PARAMETERS FOR GRADING FUNCTIONAL IMPAIRMENTS

Cardiac:

Grade 0	Normal
Grade 1	Mild asymptomatic diagnostic finding; no intervention required
Grade 2	Moderate asymptomatic diagnostic finding; no intervention required
Grade 3	Symptomatic, requiring intervention; responsive to therapy
Grade 4	Symptomatic, requiring intervention; not responsive to therapy; ventricular assist device or heart transplant indicated
Grade 5	Death

Skin:

Grade 0	Normal
Grade 1	Asymptomatic clinical changes of color, texture, warmth
Grade 2	Symptomatic skin lesions without breakdown
Grade 3	Skin breakdown with bleeding; pain; infection
Grade 4	Life-threatening consequences; major invasive intervention indicated
Grade 5	Death

Proptosis/enophthalmos:

Grade 0	Normal
Grade 1	Asymptomatic, intervention not indicated
Grade 2	Symptomatic and interfering with function, but not interfering with ADL
Grade 3	Symptomatic and interfering with ADL
Grade 4	Loss of vision
Grade 5	Not applicable

Obstruction of Visual Axis:

Grade 0	No obstruction of visual axis
Grade 1	Eyelid dysfunction Asymptomatic but not ADL; no astigmatism
Grade 2	Symptomatic; interfering with ADL; causing amblyopia or astigmatism
Grade 3	Surgical intervention indicated, amblyopia
Grade 4	Loss of vision
Grade 5	Not applicable

APPENDIX IV *(continued)*
PARAMETERS FOR GRADING FUNCTIONAL IMPAIRMENTS

Obstruction of airway:

Obstruction of: (select one or more)
– Bronchus
– Larynx
– Pharynx
– Trachea

Grade 0	No obstruction of airway
Grade 1	Asymptomatic obstruction on exam, endoscopy, or radiograph
Grade 2	Symptomatic (e.g., noisy airway breathing), but causing no respiratory distress; medical management indicated
Grade 3	Interfering with ADL; stridor or endoscopic intervention indicated (e.g., stent, laser, surgical removal)
Grade 4	Life-threatening airway compromise; tracheotomy or intubation indicated
Grade 5	Death

Hemorrhage/Bleeding From Coagulopathy of VM:

Grade 0	No hemorrhage/bleeding from coagulopathy of VM
Grade 1	Mild without transfusion
Grade 2	Moderate without transfusion
Grade 3	Transfusion indicated
Grade 4	Catastrophic bleeding, requiring major non-elective intervention
Grade 5	Death

Thrombotic Event from Coagulopathy of VM:

Grade 0	No thrombotic event from coagulopathy of VM
Grade 1	Phleboliths present on clinical or radiographic exam but asymptomatic
Grade 2	Phleboliths present on clinical or radiographic exam and symptomatic
Grade 3	Deep/Large Venous Thrombosis without Acute decompensation
Grade 4	Life threatening Venous Thrombosis with Acute decompensation requiring intensive intervention
Grade 5	Death

Neurologic/Compression by paraspinal/spinal VA:

Grade 0	No neurologic/compression by paraspinal/spinal VA
Grade 1	Asymptomatic radiologic findings only
Grade 2	Symptomatic, but not interfering with ADL
Grade 3	Symptomatic and interfering with ADL
Grade 4	Life-threatening; disabling; operative intervention indicated to prevent or treat CNS necrosis
Grade 5	Death

APPENDIX IV *(continued)*
PARAMETERS FOR GRADING FUNCTIONAL IMPAIRMENTS

Liver dysfunction:

Grade 0	Normal
Grade 1	Asymptomatic
Grade 2	Jaundice
Grade 3	Asterixis
Grade 4	Encephalopathy
Grade 5	Death

Abdominal compartment syndrome:

Grade 0	No abdominal compartment syndrome
Grade 1	Hepatomegaly
Grade 2	Mild hepatomegaly and abdominal distention
Grade 3	Moderate hepatomegaly and abdominal distention
Grade 4	Impairment of urine output or respiratory distress
Grade 5	Death

Bone involvement of VA:

Grade 0	No bone involvement of VA
Grade 1	Asymptomatic, radiologic findings only
Grade 2	Symptomatic, but not interfering with ADL
Grade 3	Altered ADL secondary to symptoms: pain, fracture
Grade 4	Complete loss of function
Grade 5	Death

Effusions caused by VA (pleural, cardiac):

Grade 0	No effusions caused by VA (pleural, cardiac)
Grade 1	Asymptomatic, radiologic findings only
Grade 2	Symptomatic, but not requiring immediate intervention
Grade 3	Requiring intervention, fluid removal,
Grade 4	Requiring ventilation or more aggressive surgical support
Grade 5	Death

Lymphedema caused by VA:

Grade 0	No lymphedema caused by VA
Grade 1	Pitting with application of pressure, edema improvement with elevation
Grade 2	Increased circumference, no longer pits with pressure
Grade 3	Worsened swelling with skin changes
Grade 4	Skin breakdown with or without infection

AVM Progression:

Grade 0	No AVM progression
Grade 1	Cutaneous blue stain, warm, shunting on Doppler
Grade 2	Enlargement, pulsation, thrill, bruit
Grade 3	Skin changes: ulceration, bleeding, pain
Grade 4	Cardiac failure
Grade 5	Death

APPENDIX IV *(continued)*
PARAMETERS FOR GRADING FUNCTIONAL IMPAIRMENTS

Grading System for Kaposiform Hemangioendotheliomas (KHE)/Tufted Angiomas (TA) and Kasabach Merritt Syndrome (KMP):

Grade 0	KHE/TA without KMP
Grade 1	KHE/TA with KMP without bleeding or blood product support
Grade 2	KHE/TA with KMP (thrombocytopenia, hypofibrinogenemia) with no active bleeding or DIC, but receiving blood product support
Grade 3	KHE/TA with KMP with DIC active bleeding
Grade 4 (mechanical	KHE/TA with KMP/DIC/bleeding requiring aggressive medical care ventilation, pressor support)
Grade 5	Death

Grading of Hematological Support of KHE/TA with KMP:

Grade 0	KHE/TA without KMP
Grade 1	No Blood product support
Grade 2	One of the following hematological supports: PRBC, platelets, fibrinogen (cryoprecipitate)
Grade 3	Two of the following hematological supports: PRBC, platelets, fibrinogen (cryoprecipitate)
Grade 4	All three of the following hematological supports: PRBC, platelets, fibrinogen (cryoprecipitate)
Grade 5	All three of the following hematological supports: PRBC, platelets, fibrinogen (cryoprecipitate), AND at least one other means of hematological support (including factor support)

Grading of Thrombocytopenia for KHE/TA with KMP:

Grade 0	KHE/TA with normal platelet count
Grade 1	plat > 40,000 above initial count
Grade 2	plat > 20,000 above initial count
Grade 3	Thrombocytopenia without bleeding
Grade 4	Thrombocytopenia with bleeding

Grading of Fibrinogen for KHE/TA with KMP:

Grade 0	KHE/TA with normal fibrinogen
Grade 1	Fibrinogen > 40 above initial count
Grade 2	Fibrinogen > 20 above initial count
Grade 3	Hypofibrinogenemia without bleeding
Grade 4	Hypofibrinogenemia with bleeding

ADL = Activities of Daily Life
KMP = Kasabach-Merritt Phenomenon
VA = Vascular Anomalies (vascular tumors and vascular malformations)
VM = Vascular Malformations

APPENDIX V: QOL INSTRUMENTS

Pediatric QOL Measures

Pediatric Quality of Life Inventory (PedsQL; Varni, 2001), 0-18 years: The PedsQL 4.0 Generic Core Scales are multidimensional child self-report and parent proxy-report scales to assess health-related quality of life (QOL) in children and adolescents ages 2 – 18 years. It is brief standardized pediatric QOL scale with good reliability and validity, which includes both generic and disease specific modules. It takes approximately 5 - 10 minutes to complete and consists of a 23-item core measure of global QOL that has four subscales: physical functioning, emotional functioning, social functioning, and school functioning. There are different forms for parents of children ages 0 – 18 years (infant 0 - 12 months; infant 13 - 24 months; toddler: 2 – 4; young child: 5 – 7; child: 8 – 12; adolescent: 13 – 18) and parallel self-report forms for ages 5 – 18 years (young child: 5 – 7; child: 8 – 12; adolescent: 13 – 18).

Adult QOL Measures

Functional Assessment of Chronic Illness Therapy (FACIT; Cella et al., 2002, 19-31 years): Adult self-report generic and disease-specific health-related QOL scales that consist of an assessment of general health status, the Functional Assessment of Cancer Therapy-General (FACT-G), and different subscales to assess disease specific issues of both cancer and non-cancer illnesses as well as specific symptoms. The 4 generic core subscales of the 27-item FACT-G are 1) physical well-being, 2) emotional well-being, 3) social well-being, and 4) functional well-being. The FACT-G is a brief, reliable, and valid QOL measure, which is sensitive to clinical change (Cella et al, 1993) and has been used with patients with cancer and a variety of other chronic illnesses (Cella et al, 2001). The response format is a 5-point likert scale. It will be administered to individuals 18 years and older and takes about 5 – 10 minutes to complete.

Pediatric Pain Measure

Peds QL Pediatric Pain Questionnaire (Varni et al., 1987): A pediatric pain scale designed so that parents and children from 5 – 18 years of age can independently assess the intensity of children's present pain and their worst pain in the past week. It consists of a visual analogue scale (VAS) with a happy face on one end of the line and a sad face on the other end. The child (or parent) is instructed to put a mark on the line that indicates how much pain the child is feeling. In addition, there is a drawing of a child and the child is asked to color where on the body the pain is. The VAS measure has been shown to be reliable and valid of pain perception in pediatrics. Higher scores indicate greater pain intensity. This scale takes approximately 5–10 minutes to complete.

Adult Pain Measure

The short-form McGill Pain - 2 Questionnaire (SF-MPQ-2) (Dworkin, RH, 2009): A revised self-report form of the original short form McGill Pain Questionnaire (SF-MPQ) that consists of

22 descriptors divided into 4 subscales. Three of these subscales consist of sensory descriptors and one consists of the original four SF-MPQ affective descriptors, as follows: (1) continuous pain descriptors (6 items): “throbbing pain”, “cramping pain”, “gnawing pain”, “aching pain”, “heavy pain”, and “tender”; (2) intermittent pain descriptors (6 items): ‘‘shooting pain”, ‘‘stabbing pain”, ‘‘sharp pain”, ‘‘splitting pain,” ‘‘electric-shock pain”, and ‘‘piercing”; (3)predominantly neuropathic pain descriptors (6 items): ‘‘hot-burning pain”, ‘‘cold-freezing pain”, ‘‘pain caused by light touch”, ‘‘itching”, ‘‘tingling or ‘pins and needles,’”, and ‘‘numbness”; and (4) affective descriptors (4 items): ‘‘tiring-exhausting”, ‘‘sickening”, ‘‘fearful”, and ‘‘punishing-cruel”

The FLACC will also be used for subjects unable to complete the age appropriate pain questionnaire.

APPENDIX VI INVESTIGATOR'S SIGNATURE

Study Title:	**A Randomized Phase 2 Study of Vincristine versus Sirolimus to treat High Risk Kaposiform Hemangioendothelioma (KHE)**
Study Number:	SIR-DA-1202
Protocol Version:	Version 6: 01/31/2018

I have read the protocol and agree that it contains all the details necessary for carrying out this study, and I will conduct the study as described herein.

Investigator's Signature: _____

Investigator (Printed name): _____

Date: _____

Institution: _____