Official Title of the Study: Trial of Sirolimus for Cognitive Impairment in Sturge-Weber Syndrome

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Principal Investigator: Dr. Anne M. Comi

Rare Diseases Clinical Research Network (RDCRN)

The Brain Vascular Malformations Clinical Research Network: Predictors of Clinical Course

Pilot Clinical Drug Trial of Sirolimus for Cognitive Impairments in Sturge-Weber syndrome

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1. Protocol Synopsis

Interventional Synopsis

Protocol Number:	BVMC6209
Protocol Title:	Pilot Clinical Drug Trial of Sirolimus for Cognitive Impairments in Sturge-Weber syndrome
Study Chair:	Dr. Anne Comi
Statistician:	Dr. Charles McCulloch
Consortium:	The Brain Vascular Malformations Clinical Research Network: Predictors of Clinical Course
Participating Sites:	Kennedy Krieger Institute, Johns Hopkins School of Medicine, Cincinnati Children's Hospital Medical Center
Activation Date:	June 22, 2016
Current Status:	Active
Sample Size:	10 (not including subjects screened or early withdrawals within first 2 months on study drug) Recruitment goal of five subjects from the Kennedy Krieger Institute and five subjects from Cincinnati Children's Hospital Medical Center. If Cincinnati Children's Medical Center (CCHMC) is unable to meet their recruitment goal, then Kennedy Krieger Institute (KKI) may enroll additional participants to meet the overall sample size of 10.
Target Enrollment Period:	October 1, 2016 – December 1, 2018
Study Design:	Interventional Pilot Treatment
Primary Study Objective:	1) To gain a preliminary understanding of the safety and 2) determine best outcomes to be used to assess the utility of sirolimus for the treatment of cognitive impairments in Sturge-Weber syndrome (feasibility of full-scale research design)
Study Population and Main Eligibility/ Exclusion Criteria:	Patients with Sturge-Weber syndrome brain involvement as defined on neuroimaging (n=10 subjects, male and female, ages 3 to 31 years) and the following: 1. Male or female patients ages 3 to 31 years of age. 2. Cognitive impairment as defined by the following: • SWS cognitive neuroscore of ≥ 1 3. Ability to participate in direct neuropsychological and developmental testing. 4. English as primary language. 5. Stable anti-epileptic drugs (no changes in medications except dose for >3 months). 6. Adequate renal function. GFR must be greater than 50 ml/min/m2 as determined by the Schwartz Formula for children and MDRD for adults: • http://www.nkdep.nih.gov/professionals/gfr_calculators/index.htm 7. If female and of child bearing potential, documentation of a negative pregnancy test prior to enrollment determined by a urine test is required. Sexually active pre-menopausal female patients (and female partners of male patients) must use adequate contraceptive measures, excluding estrogen containing contraceptives, while on the study drug. Abstinence will be considered an adequate contraceptive measure. 8. INR ≤1.5 (Anticoagulation is allowed if target INR ≤ 1.5 on a stable dose of warfarin or on a stable dose of LMW heparin for >2 weeks.) 9. Adequate liver function as shown by: • Serum bilirubin ≤ 1.5x ULN • ALT and AST ≤ 2.5x ULN

	10. Written informed consent according to local guidelines. Local guidelines for subject assent will also be followed. 11. Stable dose of medications affecting the cytochrome P 450 3A4 (CYP3A4) and p glycoprotein (P gp) systems for at least 3 months prior to consent.

Treatment	
Agent-	Rapamune® / Rapamycin® (sirolimus)
Dosage, schedule, route of administration-	Oral dose targeting a serum level dose range of 4-6 ng/mL given for a minimum of six months. Dose based on surface age, surface area, and mini-PK as outlined in the protocol. Subjects will receive no more than 2 mg/day given in two divided doses for a minimum of six months with the option to enter into the Extension Phase up to an additional year and a half.
Primary Outcome Measures:	1) Safety and Tolerability of sirolimus in patients with Sturge-Weber syndrome. Adverse Events will be determined at every visit and on every phone call using a case report with a list of side effects which can be seen with higher doses and serum levels than are expected with this study. Impact of the study drug upon seizure frequency, type, and duration will be assessed by patient/parent report using a seizure report form we have used in a prior study. Impact of study drug upon neurologic, ophthalmologic, endocrine, medical rehab, and dermatologic status will be assessed with case report forms, the physical exam, the Sturge-Weber syndrome neuroscore, the port-wine birthmark score, and other outcome measures described in the protocol. 2) Change over six months in cognitive functioning in Sturge-Weber syndrome is the primary outcome measure. This outcome will be assessed using a panel of testing selected based upon extensive experience in testing cognitive function in adults and children with SWS at the Kennedy Krieger Sturge-Weber Center. These will include the following measures from the National Institute of Health Toolbox (Gershon et al., 2010): **Attention/Executive Function – Flanker Inhibitory Control and Attention Test** (Eriksen & Eriksen, 1974) **Executive Functioning – Dimensional Change Card Sort Test** (Zelazo, Frye, & Rapus,1996) **Episodic Memory – Picture Sequence Memory Test** (Bauer et al., 2013; Dikmen et al., 2014) **Language – Oral Reading Recognition Test** **Language – Picture Vocabulary Test** (Dunn & Dunn, 1981) **Processing Speed – Pattern Comparison Processing Speed Test** (Carlozzi et al., 2014) **Working Memory – List Sorting Working Memory Test** (Weintraub et al., 2013) **Dexterity – 9-hole Pegboard Dexterity Test** (Wang, Bohannon, Kapellusch, Garg, & Gershon, 2015) **Strength – Grip Strength Test** (Hamilton, Balnave, & Adams, 1994)

	PROMIS measures self-report of emotional functioning (Cella et al., 2007) Each test in this panel is age normed. Therefore even though subjects will be of different ages, *the change in their performance as a percentage of the normed values* will be relevant and comparable from subject-to-subject. There are also published test-retest standard deviations from which to ascertain how much of a change in score is needed for a change to be regarded as significant.
Secondary Outcome Measures:	Secondary outcome measures include: change in quantitative EEG (power asymmetry), SWS clinical neuroscore, and birthmark score. Another secondary outcome measure will be an assessment of impact upon seizures. Parents/caregivers will report the following before starting study drug and at each visit: • Number of seizures (specifically motor seizures) - **This is the most reliable and important seizure outcome.** • Seizure duration • Seizure Type • Number of episodes of status epilepticus, defined as generalized convulsive seizure lasting longer than 10 minutes • Number of uses of rescue medication • Number of ER visits/ hospitalizations for seizures
Statistical Considerations (sample size and analysis plan):	Results of the above tests will be compared to age normed values. In addition, the difference in results between final test results and initial test results will be obtained for the cognitive functioning score of each subject. Mean and standard deviation of this difference for all 10 subjects will be calculated. These results will be used to determine whether individuals and the group as a whole demonstrated age normed changes in their cognitive functioning over the course of the six month trial. Neuropsychological testing will occur at both sites, KKI and CCHMC, and the de-identified results will be sent to KKI for analysis. There is no control group in this study of subjects who are not receiving study drug. The published test-retest standard deviations will be used to determine whether changes in testing results exceed expected test-retest changes in results. Expected seizure rate can vary greatly both between subjects and for an individual. Subjects must be on the same AEDs for the past three months to join the study. Subjects whose seizures increase to the point where it is necessary to add another seizure medication, start the ketogenic or Atkins diet, or go for surgery to control their seizures will be removed from the study. Quantitative EEG: An EEG will be done prior to starting study drug and at the end of the study while still on study drug. An asymmetry in power (roughly an integration of amplitude and frequency) in the affected side of the brain compared to the unaffected (or less affected) side of the brain, will be quantified as previously described (Hatfield et al., 2007; Ewen et al., 2009) for the quantitative EEG analysis. EEGs will occur at both sites, KKI and CCHMC, and the de-identified EEGs will be sent to KKI for analysis. The reason for this analysis is as a non-invasive biomarker of brain function which could be useful to develop as a marker of drug response. Quantitative EEG power analysis reflects an integration of the EEG activity amplitude and frequency. Therefore asymmetry of power on qEEG for these subjects reflects the increased slowing and decreased amplitude frequently seen in these patients interictally as their neurologic involvement progresses. Even patients with bilateral brain involvement have more involvement on one side than on the other side. Power asymmetry on qEEG has been shown to correlate with brain perfusion, brain atrophy, and SWS neuroscore, therefore, it has the potential as a biomarker responsive to

	monitor sirolimus treatment, both from the standpoint of safety and efficacy in a future larger clinical trial. Seizure (motor seizures) number will be collected at each study visit and compared across visits. Seizure number will be recorded from the two months prior to joining the study at baseline (by history) and "since last visit" for all study visits. Seizure length and type, need for rescue medication, ER visit or hospitalization will also be recorded and compared across visits and at the end of the study (on drug) compared to the beginning of the study (prior to starting drug). There will be stopping rules for safety. Clinical neurologic severity scores (neuroscores) will be collected at each study visit, using a scale developed and validated by KKI. The neuroscore is comprised the frequency of seizures, extent of hemiparesis, assessment of visual field cut, and degree of cognitive functioning with a total score of 15 possible (Kelly, Hatfield, Lin, & Comi, 2005). Descriptive statistics for this measure will be reported in the form of median and range. Mann Whitney U Test will be used to assess whether a significant change in neuroscores has occurred after treatment compared to prior to treatment. Neuroscores will be collected at both sites, KKI and CCHMC, and the de-identified results will be sent to KKI for analysis. The Neurologic Quality of Life (Neuro QoL) will be collected prior to starting study drug and at each study visit. Descriptive statistics for this measure will be reported in the form of median and range. Mann Whitney U Test will be used to assess whether a significant change in quality of life has occurred after treatment compared to prior to treatment. NeuroQoL will be collected at both sites, KKI and CCHMC, and the de-identified results will be sent to KKI for analysis. The quantification of the facial birthmark score developed and validated at the KKI will occur at the beginning of the study, prior to starting study drug, and when the subjects reach the endpoint of the study at six months' time while still on study drug (Waelchli et al., 2014). If the subject elects to enter the Extension Phase, the facial birthmark will be rated at each Extension Phase visit. Descriptive statistics for this measure will be reported in the form of median and range. Mann Whitney U Test will be used to assess whether a significant change in the facial birthmark has occurred after treatment compared to prior to treatment. Facial birthmark scoring will occur at both sites, KKI and CCHMC, and the de-identified results will be sent to KKI for analysis. **Biomarker Development:** Spearman's rho will be used to assess for the strength, direction and significance of correlations between qEEG results and neuropsychological outcomes, NeuroQoL and the SWS Neuroscore. Significance will be taken at $p<0.05$. All of these outcomes will be used to determine whether sirolimus is 1) sufficiently well tolerated in this small group of subject and 2) what outcome measures and biomarkers demonstrate preliminary evidence of being responsive to sirolimus. This information will be used to determine the advisability of proceeding with a larger multicentered study and aid in the selection of outcome measures and biomarkers to be part of that follow-up randomized placebo-controlled trial.
Sponsors (federal, state, foundation and industry support):	National Institute of Health (NIH) Faneca 66 Foundation Pfizer (study drug only)

1.1 Overview

This study is a pilot project portion of an NIH Rare Disease Clinical Research Consortium focused on brain blood vessel malformations in three different rare diseases.

The focus of this specific project is on Sturge-Weber syndrome.
Sturge-Weber syndrome is a rare, congenital syndrome with vascular malformations of the brain, skin, and eye. The underlying cause of SWS is unknown and the extent of involvement varies greatly from patient to patient. Those with brain involvement present with cognitive impairments, seizures, stroke-like episodes, and neurologic deficits, usually in infancy. Those with eye involvement frequently develop vision-threatening glaucoma and retinal injury. Treatment has been largely symptomatic and hampered by delayed diagnosis and the rarity of the condition.

2. Specific Aims (Hypothesis and Objectives)

We aim to gain a preliminary understanding of safety of sirolimus in Sturge-Weber syndrome and the feasibility of a trial to determine the impact of sirolimus upon cognitive impairments in Sturge-Weber syndrome. The primary objectives are 1) to assess for adverse events in this population and 2) determine best outcomes and biomarkers to be used as a treatment trial of cognitive impairments in Sturge-Weber syndrome. Through this effort, we will determine the feasibility and design for a succeeding randomized placebo-controlled trial, if justified by this trial. At the end of this trial subjects who want to will have the opportunity to enter the Extension Phase of the trial and continue on the study drug.

3. Background

Children living with Sturge-Weber syndrome (SWS) urgently need more effective medications. Many children develop a range of cognitive impairments, including intellectual disability, attention deficit disorder, learning disabilities, and autism. Classic SWS consists of a vascular birthmark on the face (capillary malformation), abnormal blood vessels in the eye (choroid hemangioma), and a vascular malformation in the brain (leptomeningeal angioma). In conjunction with their cognitive impairments, patients develop seizures and strokes, brain atrophy, hemiparesis, and vision deficits. Typical treatment includes anticonvulsants and low-dose aspirin. Stimulants are frequently used to treat attention issues and have demonstrated to be effective and tolerated in some patients with SWS (Lance, Lanier, Zabel, & Comi, 2014). However, a recent review of a cohort of children at the Kennedy Krieger Institute treated with this approach reveals that cognitive impairments are common and only about 1/3 of patients have an IQ in the normal range (Kavanaugh et al., 2015). The PI3K/AKT/mTOR pathway, known for its essential regulatory functions in vascular growth and

organization, has previously been shown to be activated in capillary malformations associated with SWS (Shirazi et al., 2007). More recently, a sporadic R183Q mutation in GNAQ was identified at the Kennedy Krieger Institute (KKI) as the etiology of isolated capillary malformations and of SWS, further implicating this pathway, and thereby suggesting a potential therapeutic target (Shirley et al., 2013). The R183Q somatic mosaic mutation in GNAQ is predicted to hyperactivate the P13K/AKT/mTOR pathways.

Sirolimus has been used to treat individuals ages 0-31 years with complicated vascular anomalies as part of a Phase II clinical trial at Cincinnati Children's Hospital Medical Center (CCHMC). In that trial, sirolimus was safe even in the youngest cohort of patients, and pharmacokinetic studies were performed by Dr. Sander Vinks to elucidate appropriate dosing for patients based on age and goal drug level. While patients with Sturge-Weber syndrome were not eligible for that study, sirolimus has been tried for compassionate use in a teenage boy with Sturge-Weber syndrome, suffering from poorly controlled seizures with no significant toxicity (personal communication, D Franz) and in an infant with CLOVES syndrome and hemimegencephaly, including vascular leptomeningeal enhancement consistent with early angiomatous changes, for his poorly controlled seizures (personal communication, D Adams). In addition, a small clinical trial of 25 patients ages 2-18 has recently opened at Baylor using the mTOR inhibitor everolimus to treat refractory seizures in Sturge-Weber syndrome.

Furthermore, sirolimus has been studied in multiple clinical trials for the treatment of cognitive impairments and seizures in tuberous sclerosis. In modeling this trial after the trial studying the impact of sirolimus upon cognition in tuberous sclerosis complex (TSC), we aim to determine the impact of sirolimus upon cognition in Sturge-Weber syndrome. The primary objective of this pilot study is to determine the tolerability of sirolimus as an adjunct treatment in children and adults with Sturge-Weber syndrome related cognitive impairments. Sirolimus is FDA-approved for organ rejection in kidney transplants for individuals ≥ 13 years old. Additionally, sirolimus is FDA-approved as a prescription medicine used to treat lymphangioleiomyomatosis (LAM), a rare progressive lung disease that affects predominantly women of childbearing age. Therefore, this study is a pilot trial of a clinically available medication for an indication (cognitive impairments in Sturge-Weber syndrome) which does not currently have FDA approval. The line of research represented by this particular pilot study is novel with respect to the specific disease state under study (SWS), but is modeled after earlier research utilizing the same compound in a different disease (TSC). Both diseases have constitutive activation of the mTOR pathway.

4. Study Design and Methods

4.1 Inclusion Criteria

Patients with Sturge-Weber syndrome brain involvement as defined on neuroimaging (n=10 subjects, male and female, ages 3 to 31 years) and the following:

1. Male or female patients ages 3 to 31 years of age.

2. Cognitive impairment as defined by the following:
    - SWS cognitive neuroscore of ≥ 1

3. Ability to participate in direct neuropsychological and developmental testing.

4. English as primary language.

5. Stable anti-epileptic drugs (no changes in medications except dose for >3 months).

6. Adequate renal function. GFR must be greater than 50 ml/min/m2 as determined by the Schwartz Formula for children and MDRD for adults: http://www.nkdep.nih.gov/professionals/gfr_calculators/index.htm

7. If female and of child bearing potential, documentation of a negative pregnancy test prior to enrollment determined by a urine test is required. Sexually active pre-menopausal female patients (and female partners of male patients) must use adequate contraceptive measures, excluding estrogen containing contraceptives, while on the study drug. Abstinence will be considered an adequate contraceptive measure.

8. INR ≤1.5 (Anticoagulation is allowed if target INR ≤ 1.5 on a stable dose of warfarin or on a stable dose of LMW heparin for >2 weeks.)

9. Adequate liver function as shown by:
    - Serum bilirubin ≤ 1.5x ULN
    - ALT and AST ≤ 2.5x ULN

10. Written informed consent according to local guidelines. Local guidelines for subject assent will also be followed.

11. Stable dose of medications affecting the cytochrome P-450 3A4 (CYP3A4) and p-glycoprotein (P-gp) systems for at least 3 months prior to consent.

4.2 Exclusion Criteria

1. Allergy to sirolimus or other rapamycin analogues.

2. Patients with seizures secondary to metabolic, toxic, infectious or psychogenic disorder, drug abuse or current seizures related to an acute medical illness.

3. Inability to keep follow-up appointments, maintain close contact with Principal Investigators, and/or complete all necessary studies to maintain safety.

4. Patients in need of immediate major surgical intervention.

5. Concurrent severe and/or uncontrolled medical disease, which could compromise participation in the pilot study (e.g. uncontrolled diabetes, uncontrolled hypertension, severe infection, severe malnutrition, chronic liver or renal disease, active upper GI tract ulceration, impaired or restrictive pulmonary function, pneumonitis or pulmonary infiltrates).

6. Chronic treatment with systemic steroids or another immunosuppressive agent. Patients with endocrine deficiencies are allowed to receive physiologic or stress doses of steroids if necessary. Inhaled steroids are allowed.

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

8. 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.

9. Patients with an active, bleeding diathesis.

10. Patients with uncontrolled hyperlipidemia: fasting serum cholesterol > 300 mg/dL
AND
fasting triglycerides > 2.5 x ULN.

11. Patients who have had a major surgery or significant traumatic injury within four weeks of study entry. Patients who have not recovered from the side effects of any major surgery (defined as requiring general anesthesia) or patients that may require major surgery during the course of the pilot study.

12. Patients with a prior history of organ transplant.

13. Patients who have received live attenuated vaccines within one week of start of sirolimus and during the pilot study.

14. Patients who have a history of malignancy.

15. Patients who are currently part of or have participated in any clinical investigation with an investigational drug within one month prior to enrollment.

16. Patients being treated with felbamate, unless treatment has been continuous for ≥ one year.

17. Patients currently receiving anticancer therapies or who have received anticancer

therapies within four weeks of study entry (including chemotherapy, radiation therapy, antibody based therapy, etc.).

4.3 Recruitment of Participants

Recruitment for the initial pilot will be from the Hunter Nelson Sturge-Weber Center database at the Kennedy Krieger Institute and from the Vascular Anomalies clinic at Cincinnati Children's Sturge-Weber syndrome database. Patients who are in research databases and have given the study team at the Kennedy Krieger Institute permission to contact them regarding research will be sent information regarding the trial and invited to contact the study team for more information if interested. Patients coming for clinical visits to either Dr. Comi or Dr. Hammill's clinics will be pre-screened for meeting inclusion criteria. A log will be kept of patients pre-screened for this trial and results of the pre-screening process.

Information about the pilot study will also be posted on the center websites, clinicaltrials.gov, the NIH Brain Vascular Malformation website, and offered for posting on the websites of relevant advocacy foundations. Participants will be either Dr. Comi's or Dr. Hammill's patients. If interested, individuals will contact Dr. Comi or Dr. Hammill from the website postings and will be seen first for a clinical visit. The investigational nature and objectives of this trial, the procedures, the treatments involved, the attendant risks, and discomforts, as well as potential alternative therapies, will be carefully explained to the patient or their parents or guardian if he/she is a child. A signed informed consent document will be obtained after the entirety of the pilot study is explained. 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. Assent will be obtained from all children able to provide assent according to our local IRB guidelines. Our consent contains an assent statement on the second-to-last page. The goal is to recruit up to ten participants total in this pilot study, with a recruitment goal of five subjects from the Kennedy Krieger Institute and five subjects from Cincinnati Children's Hospital Medical Center. If CCHMC is unable to meet their recruitment goal, then KKI may enroll additional participants to meet the overall sample size of 10.

4.5 Retention Strategies

When recruiting participants, the frequent visit schedule will be reviewed with the participants to ensure they are aware of the time commitment required to be in this study. All visits will be scheduled as far in advance as possible. Participants will receive reminders via email or phone before their appointments. All appointments surrounding holidays and travel will be scheduled far in advance.

4.6 Schedule of Events

Table of Visits	**Screening**	**Baseline NP / EEG**	**Core Phase**			**Endpoint 6 months**	**Extension Phase**
Visit Number	1	2	3	4	5	6	7
Study Week	-2 ← ± 14 days →	0	2 ← ± 7 days →	6	14	26 ← ± 14 days →	38
Consent	X						
Physical Exam	X	X	X	X	X	X	X
Neuroscore	X	X	X	X	X	X	X
Neuro QoL	X	X	X	X	X	X	X
Incl/Excl Criteria	X	X	X	X	X	X	X
Past Medical History	X						
Diagnosis of SWS	X						
Seizure Hx	X	X	X	X	X	X	X
Previous AED Hx	X						
Current Med Hx	X	X	X	X	X	X	X
Picture Taken		X				X	X
Rescue Meds	X	X	X	X	X	X	X
EEG and NP Test	* ←	* →				X ± 7 days	
Labwork							
AED Blood Levels	X	X	X	X	X	X	X
CMP (renal, hepatic)	X	X	X	X	X	X	X
Lipid Profile	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X
Sirolimus Level		PK Study	X	X	X	X	X
Urinalysis	X	X	X	X	X	X	X

Pregnancy Test (if applicable)	X	X	X	X	X	X	X
Safety							
Adverse Events Check List		X	X	X	X	X	X
Safety Calls: Once Per Month		←	Monthly				→
Continue or Remove from Study?	X	X	X	X	X	X	X
Patient Reported Outcomes			X	X	X	X	X

** EEG and neuropsychological baseline testing to occur any time after consent is obtained and before the start of the study drug.
Please note that chemistries will include measurement of GGT.

5.0 Data and Safety Monitoring Plan

The Study Chair (Anne Comi, MD) has primary oversight responsibility of this clinical trial. Based on the determination by the NINDS Safety and Risk Assessment Committee, an NIH appointed Data Safety and Monitoring Board is not required. Dr. Comi, Dr. Hammill and the sub-investigators at each site will comprise the Safety Monitoring Committee. The Safety Monitoring Committee will review accrual, patterns and frequencies of all adverse events, and protocol compliance every six months. The Study Chair will provide Safety Monitoring Committee reports and meeting summaries to the NIH program officials regarding the continuation status of the protocol.

Dr. Comi and Dr. Hammill currently speak about weekly and will continue to do so. They will review data together every month for this small pilot trial by 1) Reviewing and analyzing the progress of the study; 2) Monitoring the safety of the study treatments and diagnostic procedures; 3) Ensuring data quality; 4) Recommending early stopping or continuation of the trial (if applicable); and 5) Reviewing recruitment and event rates. They will assess the proportion of enrolled versus projected enrollment and proportion of subjects who have completed the trial.

The research coordinator at each site will make safety calls and/or email/text contact with each participant bimonthly to ensure follow-up and assessment of any adverse events. Also, subjects will be asked to keep daily diaries of any side effects or concerns and asked to email or fax them in on a bimonthly basis. If any adverse events have occurred, the PIs will be notified immediately and corrective actions will take place. While patients with Sturge-Weber syndrome can have serious medical issues, we do not expect any serious adverse events (SAEs) directly attributable to the study drug.

Occurrence of an SAE directly attributable to the study drug will trigger a review to consider stopping the participant on the study within one month of the event. Based on a prior study, an adverse event rate of approximately 0.7 +/- events/subject/month is expected. An adverse event rate more than double this event rate will trigger an in-depth review to consider whether to consider continuing the participant on the study; an important consideration in this decision will be whether the adverse events are considered to be due to the study drug or not.

The trial PIs and clinical coordinators will review the study progress regularly. Adverse events will be reviewed to ensure the safety of the patients. Each site will have internal reviews carried out quarterly by clinical trials specialists to ensure that all protocol specifications are being followed and issues addressed promptly. Quarterly reports will be generated by KKI to assess completeness of data. There will be monthly phone conferences between KKI and CCHMC to address quality assurance (QA) issues.

Data provided must be treated with the 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.

Each subject enrolled will, from that point forward, be identified by a unique identifier (two codes: the local one that can be used by the registering site to obtain personal identifiers and a second code assigned by the DMCC). 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.

The study protocol will be reviewed and approved by the National Institutes of Health (NIH) before submission to individual center IRBs for approval. Participant enrollment may only begin with IRB approved protocol and consent forms.

5.1 Study Oversight

The Study Chair (Anne Comi, MD) has primary oversight responsibility of this clinical trial. Based on the determination by the NINDS Safety and Risk Assessment Committee, an NIH appointed Data Safety and Monitoring Board is not required. Dr. Comi, Dr. Hammill and the sub-investigators at each site will comprise the Safety Monitoring Committee. The Safety Monitoring Committee will review accrual, patterns and frequencies of all adverse events, and protocol compliance every six months. The Study Chair will provide Safety Monitoring Committee reports and meeting summaries to the NIH program officials regarding the continuation status of the protocol.

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

from the DMCC for site review. Adverse events will be reviewed monthly by the research team. A separate report detailing protocol compliance will also be available from the DMCC for site review on a monthly basis. The research team will then evaluate whether the protocol or informed consent document requires revision based on the reports.

This research study will be registered and results reported on clinicaltrials.gov.

5.2 Definitions and Standards

The Rare Diseases Clinical Research Network defines an adverse event as: "...an unfavorable and unintended sign, symptom or disease associated with a participant's participation in a Rare Diseases Clinical Research Network study."

Serious adverse events include those events that: "result in death; are life-threatening; require inpatient hospitalization or prolongation of existing hospitalization; create persistent or significant disability/incapacity, or a congenital anomaly/birth defects."

An unexpected adverse event is defined as any adverse experience...the specificity or severity of which is not consistent with the risks of information described in the protocol.

Expected adverse events are those that are identified in the research protocol as having been previously associated with or having the potential to arise as a consequence of participation in the study

All reported adverse events will be classified using the current version of the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 developed and maintained by CTEP at National Cancer Institute.

Prior to the use of this drug, patients and/or their parents/guardian will be advised of the possibility of untoward symptoms. These adverse reactions are not expected at the low doses being used in this pilot study.

5.3 Expected/Known Risks/Discomforts/Adverse Events Associated with Study Intervention and Procedures: Definition of Expected Adverse Events

The primary risk to the subjects from participation in this trial is from sirolimus. From prior studies, it is already known that in this dose range the risks of Serious Adverse Events due to sirolimus is very low, hence why this dose range was picked. The tables below have published adverse events for a much higher dose range. We do not expect any Serious Adverse Events. The occurrence of any Serious Adverse Event thought to be due to the study drug will trigger a review to consider a change in study protocol or stopping the trial. The risk of low-dose sirolimus, specifically in patients with Sturge-

Weber syndrome, is unknown, hence why this is the main purpose of this study. Risk of toxicity has been correlated with serum levels of sirolimus. At this low-dose range, the risk of toxicity is low. All participants will have a PK study in the beginning performed to guide dosing of the drug (aiming for the 4-6 ng/mL goal) and serum levels measured regularly to check compliance, and to minimize toxicity. Participants enrolled in this trial will be carefully monitored for the development of toxicities with guidelines for discontinuation of drug and stopping rules in place.

We will obtain institutional 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 (Dr. Comi). The treating physician at the clinical center will keep a list linking codes with patient identifying information in accordance with their institutional IRB guidelines. 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.

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

This trial will begin to assess the safety of sirolimus for Sturge-Weber syndrome. Outcomes (primary or secondary; see below) data which suggest potential benefit from the study drug and biomarker (see below) data suggesting a response to study drug will be used to guide the development of a larger, multi-centered, randomized, placebo-controlled trial. Finally, the participating institutions will develop a working collaboration that could provide the framework for future clinical trials in patients with this rare syndrome.

Importance of Knowledge to be Gained:

The knowledge gained by this pilot study will help to determine potential for a new treatment for patients with SWS. Response criteria developed in this study could be utilized for future trials.

Serious toxicities are <u>not</u> expected with the low dose range being used for this pilot study. Any patient experiencing a Grade 3 or 4 toxicity that is possibly, probably, or definitely related to sirolimus will 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.

Hematological Toxicity - If a patient experiences ≥Grade 3 neutropenia (ANC <750), anemia (Hgb <8), the <u>sirolimus will be withheld and the subject will be removed from the study</u>. Patients will continue to be seen, treated as needed, and have complete blood counts measured. In addition, sirolimus trough levels will be obtained every week until recovery (≤Grade 1) is documented.

Non-Hematological Toxicity - For toxicities attributable (possibly, probably, definitely) to sirolimus:

If a patient experiences a non-hematological toxicity as defined below, sirolimus will be withheld and the subject will be removed from the pilot study. Patients should continue to be seen and have appropriate labs/observations. In addition sirolimus trough levels will be obtained at every visit until recovery (≤Grade 1) is documented.

Sirolimus-related (possibly, probably, or definitely) toxicities Requiring Dose Interruptions or other interventions and removal from study:

- 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 ≥ seven 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

(*) 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 three days duration
- Grade 3 transaminase elevations that return to levels that meet initial eligibility criteria within seven days of study drug interruption and that do not recur upon study re-challenge with study drug
- Grade 3 GGT elevation
- Grade 3 lymphopenia

Interventions for Hyperlipidemia/Hypertriglyceridemia

Hyperlipidemia has been reported as an AE in of patients treated with sirolimus; however, at the low dose being used in this study this is **not** expected to be seen. 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.

Table 1

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 six months) and exercise – Repeat Fasting LDL in three 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 after three 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 six months). Repeat Fasting LDL in three months. • If fasting LDL cholesterol is still >160mg/dL, continue diet and exercise and consider cholestyramine resin. • If after three 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 three 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 three 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 should be discontinued.

Toxicities Requiring Removal from Therapy:

Serious toxicities are not expected because a very low dose range is being used in this pilot study. If subjects experience any of the following toxicities regardless of

relationship to sirolimus or current trough level, they will be removed from protocol therapy.

Table 2

Toxicity	Criteria for Removal
Renal Function	If serum creatinine persistently increases (documented on at least two 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 will 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 ≥160 mg/dL) not responsive to diet, exercise, and medical intervention for patients after three months (see Table 1 above), regardless of attribution, will be removed from protocol therapy.

Drug Information:

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; therefore, close attention to potential drug interactions is crucial.

Product Description:

For the purposes of this pilot 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® (sirolimus) Oral Solution are Phosal 50 PG® (phosphatidylcholine, propylene glycol, mono- and di-glycerides, ethanol, soy fatty acids, and ascorbyl palmitate) and polysorbate 80. Rapamune Oral Solution contains 1.5% - 2.5% ethanol.

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). 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.

Route of Administration:

Sirolimus (Rapamune Oral Solution) may be taken either with or without food. Patients will be advised to take their sirolimus twice daily at the same two times of the day and at the same two times in relation to meals. Patients may not take sirolimus with grapefruit juice. It is recommended, but not required 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 should 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 or P-glycoprotein (P-gp; see below). Some anticonvulsants affect the CYP3A4 system and can be expected to decrease sirolimus; however, subjects will be allowed to be on these seizure medications. Both the levels of their seizure medications and the levels of the study drug will be monitored during the study. This is the approach taken with other similar clinical trials, such as in recent trials of mTOR inhibitors in subjects with Tuberous Sclerosis Complex. **Most subjects with Sturge-Weber syndrome will not be on the *other* drugs listed below that impact the CYP3A4 and P-gp systems;** however, if they are taking these medications then they should be on stable doses for at least three months prior to consent and if the dose of this medication needs to change then a sirolimus level will need to be checked within a week of this change.

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 sirolimus.
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 sirolimus and/or the co-administered drug may need to be adjusted.

Drugs that could increase sirolimus blood concentrations: Bromocriptine, cimetidine, cisapride, clotrimazole, danazol, diltiazem, fluconazole, HIV-protease inhibitors (e.g., ritonavir, indinavir), metoclopramide, nicardipine, troleandomycin, verapamil

Drugs and other agents that could decrease sirolimus concentrations: Carbamazepine, phenobarbital, phenytoin, rifapentine, St. John's Wort (*Hypericum perforatum*)

Drugs with concentrations that could increase when given with sirolimus: Verapamil

Vaccination - Immunosuppressants may affect response to the vaccination. Therefore, during treatment with sirolimus, 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.

Toxicity: **We do not expect any serious side effects with the low dose selected for this pilot study. The table below describes toxicities which have been reported for sirolimus over a wide dose range (up to 24 mg/m2 or higher).**

Table 3

	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, 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.

Laboratory tests and clinical procedures:

- Weight and vitals will be monitored at baseline and at each visit.
- As part of the current medical history assessed at each visit, patients will be screened for any change in their physical functioning.
- Neuroscores will be assigned at each visit by Dr. Comi or Dr. Hammill. Neurologic exam and general physical exam will also be done at each visit.

  Clinical neurologic severity scores (neuroscores) will be collected at each study visit, using a scale developed and validated by KKI. The neuroscore is comprised of the frequency of seizures, extent of hemiparesis, assessment of visual field cut, and degree of cognitive functioning with a total score of 15 possible (Kelly, Hatfield, Lin, & Comi, 2005). Descriptive statistics for this measure will be reported in the form of median and range. Mann Whitney U Test will be used to assess whether a significant change in neuroscores has occurred after treatment compared to prior to treatment. Neuroscores will be collected at both sites, KKI and CCHMC, and the de-identified results will be sent to KKI for analysis.
- Seizure severity will be assessed at each visit by Dr. Comi or Dr. Hammill.

  Parents/caregivers will report the following before starting study drug and at each visit to assess the impact of sirolimus upon seizures:
  - Number of seizures (specifically motor seizures) - **This is the most reliable and important seizure outcome.**
  - Seizure duration
  - Seizure Type
  - Number of episodes of status epilepticus, defined as generalized convulsive seizure lasting longer than 10 minutes
  - Number of uses of rescue medication
  - Number of ER visits/ hospitalizations for seizures
- Patients will be asked at each clinic visit or phone interaction about migraine frequency and severity and any change in neurologic symptoms.

- Frontal and profile photograph will be taken under standardized conditions with scoring of the port-wine birthmark for percent of face covered, thickness of birthmark, and darkness of birthmark color.

  The quantification of the facial birthmark score developed and validated at the KKI will occur at the beginning of the study, prior to starting study drug, and when the subjects reach the endpoint of the study at six months' time while still on the study drug (Waelchli et al., 2014). If the subject elects to enter the Extension Phase, the facial birthmark will be rated at each Extension Phase visit. Descriptive statistics for this measure will be reported in the form of median and range. Mann Whitney U Test will be used to assess whether a significant change in the facial birthmark has occurred after treatment compared to prior to treatment. Facial birthmark scoring will occur at both sites, KKI and CCHMC, and the de-identified results will be sent to KKI for analysis.

- hCG urine pregnancy test will be used for all female participants Tanner stage 2 (see Study Visit Table) and above. We will tell subjects that they must use adequate contraceptive measures, which may include abstinence, contraceptive implants, and condoms, but may not include estrogen containing contraceptives. We will confirm that this is the case each time they complete our clinic/telephone monitoring script. We will inform female subjects that they should not become pregnant or breastfeed a baby while on this pilot study or for 12 weeks after taking sirolimus. We will also inform male subjects that they should not get their partners pregnant while on this study or for 12 weeks after taking sirolimus. Anyone who becomes pregnant while on the pilot study will be removed immediately. All female subjects of childbearing potential will have a pregnancy test done at each study visit.

- A pharmacokinetic study will be done at the baseline visit. The subject will be started on a dose based on the recommended starting dose of mg/m2. The PK study results will be used to adjust their dose in order to get the patient to 4-6 ng/mL serum levels as quickly as possible. Subjects will be started on the study drug at study Visit 2. The PK results will be ready by study Visit 3 and the dose can therefore be adjusted. **There will be no dose escalation. The dose will only change as indicated by the PK study or if the concentration of sirolimus is outside of the 4-6 ng/mL goal. The maximum dose will be 2 mg/day.** If at the maximal dose, the sirolimus serum level is below the target level of 4-6 ng/ml, this result could impact the interpretation of the results if this subject has less of a response than other subjects. If it were to be the case that the majority of subjects are not able to reach this target range, then this trial will begin to evaluate safety and clinical trial measures for a lower than expected dose range of sirolimus as a treatment for cognitive impairments in Sturge-Weber syndrome. Furthermore, for each of the outcome measures we will plot the outcome versus serum level of the study drug. If there is a positive correlation between outcome and response, then this data would be used to select an optimal dose range to target in future trials.

The starting dose/m2 varies based on age and dosing strategy (often better tolerated when divided BID). Since our patients will be >3y, the starting dose should be 0.6mg/m2 q12h. We routinely do "miniPK" determinations using time 0, 1hour, and 3h post dose blood levels of sirolimus. Since with first dose the time 0 is 0, we will only draw the 1h and 3h levels. Dr. Sander Vinks at Cincinnati has an extensive background and multiple publications in this area and will do the PK analyses. While age-specific guidelines are usually appropriate to get to goal within 1 month, these patients may be on multiple anti-epileptics, some of which alter sirolimus metabolism. Most often, the metabolism is increased, meaning that levels decrease and patients who are "fully induced" for this particular CYP are likely to need 50% or more higher dosing than patients who are not on anti-epileptics. Subsequent levels will be trough levels done before their morning dose.

Based on prior experience, the risk of serious toxicity increases at serum levels great than 30 ng/dl. Blood samples will not be shipped between sites. Samples will be collected at each location and taken immediately to the respective lab for measurement of sirolimus levels. The two pathology labs (one at Hopkins and another at CCHMC) have communicated and confirmed they use comparable methods of measuring sirolimus levels.

The lab at Hopkins has confirmed they measure sirolimus using liquid chromatography-mass spectrometry (LC-MS). This analysis is performed after protein precipitation of the sample using an acetonitrile/methanol/zinc sulfate solution containing isotopically labeled sirolimus. The CLIA license number is 21D0680509 for the Hopkins lab. The assay range is 1.6-48.0 ng/mL with an LLOQ of the assay being 1.6 ng/mL and a between-batch variability percentage (%CV) of 6-9% for low, mid, and high QC.

The lab at CCHMC has confirmed sirolimus whole blood concentrations will be determined by a validated routine clinical assay accredited by the College of American Pathologists (CAP license #1667801) and with Clinical Laboratory Improvement Amendments certification (CLIA 88 license #36D0656333). The assay will be performed under GLP principles using LC-MS/MS on a Waters Quattro Micro API triple quadrupole mass spectrometer (Milford, MA) interfaced with Acquity UPLC instrument. The assay range is 0.5-100.0 ng/mL. The LLOQ of the assay is 1.0 ng/mL and within and between-batch variability (CV) is 12.8% and 14.0 %, respectively.

- Lipid panel, CBC, LFT, BUN, creatinine levels, electrolytes, urine protein, sirolimus levels, and levels of other AEDs will be measured at each scheduled visit after study drug is started.

- EEG will be done at baseline and after six months on study drug. Quantitative EEG analysis will be done with this EEG data. An EEG will be done prior to starting the

study drug and at the end of the study while still on the study drug. An asymmetry in power (roughly an integration of amplitude and frequency) in the affected side of the brain compared to the unaffected (or less affected) side of the brain, will be quantified as previously described (Hatfield et al., 2007; Ewen et al., 2009) for the quantitative EEG analysis. EEGs will occur at both sites, KKI and CCHMC, and the de-identified EEGs will be sent to KKI for analysis. The reason for this analysis is as a non-invasive biomarker of brain function which could be useful to develop as a marker of drug response. Quantitative EEG power analysis reflects an integration of the EEG activity amplitude and frequency. Therefore asymmetry of power on qEEG for these subjects reflects the increased slowing and decreased amplitude frequently seen in these patients interictally as their neurologic involvement progresses. Even patients with bilateral brain involvement have more involvement on one side than on the other side. Power asymmetry on qEEG has been shown to correlate with brain perfusion, brain atrophy, and SWS neuroscore, therefore, it has the potential as a biomarker responsive to monitor sirolimus treatment, both from the standpoint of safety and efficacy in a future larger clinical trial.

- Neuropsychological testing will be done at baseline and after six months on study drug.

  Change over six months in cognitive functioning in Sturge-Weber syndrome is the primary outcome measure. This outcome will be assessed using a panel of testing selected based upon extensive experience in testing cognitive function in adults and children with SWS at the Kennedy Krieger Sturge-Weber Center. These will include the following measures from the National Institute of Health Toolbox (Gershon et al., 2010):

  Attention/Executive Function – Flanker Inhibitory Control and Attention Test (Eriksen & Eriksen, 1974)

  Executive Functioning – Dimensional Change Card Sort Test (Zelazo, Frye, & Rapus,1996)

  Episodic Memory – Picture Sequence Memory Test (Bauer et al., 2013; Dikmen et al., 2014)

  Language – Oral Reading Recognition Test

  Language – Picture Vocabulary Test (Dunn & Dunn, 1981)

  Processing Speed – Pattern Comparison Processing Speed Test (Carlozzi et al., 2014)

  Working Memory – List Sorting Working Memory Test (Weintraub et al., 2013)

Dexterity – 9-hole Pegboard Dexterity Test (Wang, Bohannon, Kapellusch, Garg, & Gershon, 2015)

Strength – Grip Strength Test (Hamilton, Balnave, & Adams, 1994)

PROMIS measures self-report of emotional functioning (Cella et al., 2007)

Each test in this panel is age normed. Therefore, even though subjects will be of different ages, *the change in their performance as a percentage of the normed values* will be relevant and comparable from subject to subject. There are also published test-retest standard deviations from which to ascertain how much of a change in score is needed for a change to be regarded as significant.

- Neuro-QOL scale will be collected at each visit. The Neurologic Quality of Life (Neuro QoL) will be collected prior to starting study drug and at each study visit. Descriptive statistics for this measure will be reported in the form of median and range. Mann Whitney U Test will be used to assess whether a significant change in quality of life has occurred after treatment compared to prior to treatment. NeuroQoL will be collected at both sites, KKI and CCHMC, and the de-identified results will be sent to KKI for analysis.

5.4 Reporting Timeline

- Within **24 hours** (of learning of the event), investigators must report any reportable Serious Adverse Event (SAE) that:
    1. Is considered life-threatening/disabling or results in death of subject
    -OR-
    2. Is Unexpected/Unanticipated
- Investigators must report all other reportable SAEs within **5 working days** (of learning of the event).
- All other (suspected) reportable AEs must be reported to the RDCRN within **14working days** of the notification of the event or of the site becoming aware of the event.

Local institutional reporting requirements to IRBs, any GCRC oversight committee and the FDA, if appropriate, remain the responsibility of the treating physician and the Study Chair.

5.5 RDCRN Adverse Event Data Management System (AEDAMS)

Upon entry of a serious adverse event, the DMCC created Adverse Event Data Management System (AEDAMS) will immediately notify the Study Chair, site PIs, the Medical Review Officer, and any additional agencies (if applicable- industry sponsor, CTEP, etc) of any reported adverse events via email.

Serious adverse events: The NIH appointed Medical Review Officer (MRO) determines causality (definitely not related, probably not related, possibly related, probably related, definitely related) of the adverse event. The MRO may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the adverse event. A back-up notification system is in place so that any delays in review by the MRO beyond a specified period of time are forwarded to a secondary reviewer. The Adverse Event Data Management System (AEDAMS) maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study.

Non-serious expected adverse events: Except those listed above as immediately reportable, non-serious expected adverse events that are reported to or observed by the investigator or a member of his/her research team will be submitted to the DMCC in a timely fashion (within 20 working days). The events will be presented in tabular form and given to the MRO on a bi-annual basis. Local site investigators are also required to fulfill all reporting requirements of their local institutions.

The DMCC will post aggregate reports of all reported adverse events for site investigators and IRBs.

5.6 Study Discontinuation (Interventional)

The NIH and local IRBs (at their local site) have the authority to stop or suspend this trial at any time. This study may be suspended or closed if:

- Early stopping rules have been met (defined below)
- Accrual has been met
- The study objectives have been met
- The Study Chair / Study Investigators believe it is not safe for the study to continue
- The NIH suspends or closes the trial
- The FDA suspends or closes the trial

5.7 Subject Discontinuation

All data acquired prior to termination for the reasons outlined below will be included in the primary analysis unless patient withdraws consent. Every effort will be made to conduct a final study visit with the participant and participants will be followed clinically until, if applicable, all adverse events resolve.

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

Early Stopping Rules: If a participant experiences a significant decline in his or her health or wellbeing, the PIs may remove the participant from the pilot study. This decision will be promptly made on a case-by-case basis with careful discussion between the PIs, the parents and/or patient, and in consultation with the IRB. In some cases, it can be more medically destabilizing to remove the subject from the study drug. However, in any case where a significant decline in health may be attributable to the study drug, or in any case where it is determined that it may not be in the subject's best interest to remain on the study drug, then they will be removed from the study. A significant decline in health or wellbeing could include a loss of seizure control and/or the occurrence of intolerable side effects. Loss of seizure control is defined as an increase in seizure frequency and/or severity or use of emergency medications that warrant the initiation of a new seizure medication or treatment such as, Ketogenic/Atkins diet, surgery, or VNS. If there has been a loss of seizure control, then the subject will be removed from the study drug at that time, he or she will complete the ending assessments, and then the subject will be removed from the trial. Other stopping criteria are listed in Tables 1 and 2 above. Other removal criteria include: death, lost to follow-up, withdrawal of consent for any further data submission, or inability to complete follow up two years from the protocol completion. **Early withdrawals within the first two months on the study drug will be replaced with a new study subject.**

5.8 Data Quality and Monitoring Measures

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

- Data Monitoring: The RDCRN DMCC identifies missing or unclear data and generates a data query to the consortium administrator contact.
- Data Delinquency Tracking: The Data Management and Coordinating Center will monitor data delinquency on an ongoing basis.

5.9 Quality Control: Study Related Procedures

1. Clinical coordinators at both sites will review the case report forms after completion by Dr. Comi or Dr. Hammill to resolve any issues.
2. Dr. Comi and Dr. Hammill will review the study progress regularly with monthly phone conferences. The two sites will have internal reviews performed regularly to ensure high quality of the study procedures and subsequent data.
3. The DMCC will perform an audit of the overall protocol including this pilot project.

6. Statistical Considerations

The proposed trial is a pilot drug trial. Based on the results, a sample size calculation will be done, using the cognitive outcome with the greatest response to the study drug, to plan a follow up randomized, placebo-controlled trial, should one be warranted. If no serious adverse events which are thought to probably or possibly be due to the study drug AND the cognitive data suggests a trend for possible benefit, then the results of this study will be used to plan a larger follow-up clinical trial.
Children and adults between 3 and 31 years of age with Sturge-Weber syndrome brain involvement and cognitive impairments will be eligible for this pilot study. A total of 10 patients will be enrolled at two centers including Cincinnati Children's Hospital Medical Center and the Kennedy Krieger Institute. This sample size was selected based on 1) availability of potential appropriate subjects at both centers and 2) upon the investigators experience with similar clinical trials.

Cognitive tests proposed have been previously validated in the literature and are normed to aged matched normal controls. These are published tests which are widely used. Therefore, responders in this trial will be subjects whose normed results improve on the task more than what is expected for the increase in age (6 months) +/- expected standard deviation.

Anticipated number of patients to be enrolled:

Table 4

	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	0	1	1	0	3		5
Male	1	0	0	1	3		5
Total	1	1	1	1	6		10

7. Data Management

Dr. Comi and Dr. Hammill currently speak about weekly and will continue to do so. They will review data together every month for this small pilot trial by 1) Reviewing and analyzing the progress of the study; 2) Monitoring the safety of the study treatments and diagnostic procedures; 3) Ensuring data quality; 4) Reviewing interim analyses and recommending early stopping or continuation of the trial (if applicable); and 5) Reviewing recruitment and event rates.

The trial PIs and clinical coordinators will review the study progress weekly. Patients entered on the trial and adverse events will be reviewed to ensure that the study is implemented as outlined in the protocol. Data will be collected on case report forms, which will be uploaded to the DMCC. All study data will be collected via systems created in collaboration with the RDCRN Data Management and Coordinating Center and will comply with all applicable guidelines regarding patient confidentiality and data integrity. Each site will have internal reviews carried out quarterly by clinical trials specialists to ensure that all protocol specifications are being followed and issues addressed promptly. Quarterly reports will be generated by KKI to assess completeness of data. There will be monthly phone conferences between KKI and CCHMC to address quality assurance (QA) issues. De-identified blood samples will be run at each site separately and the results will be sent from the KKI to CCHMC for PK testing. Dose adjustments for all patients in the study will be decided based on the PK testing performed at CCHMC.

The Principal Investigator at each site will review all data relating to safety and tolerability throughout the pilot study. Any subject experiencing significant side effects or medical concerns during the course of study treatment will be responded to appropriately as clinically warranted or as outlined in this protocol by Dr. Comi and Dr. Hammill. If the subject is not doing well clinically and the patient, parent, Dr. Comi or Dr. Hammill thinks that it is in the best interest of the subject to stop the study drug, then they will be removed from the pilot study. If the subject meets any of the early stopping requirements then they will be removed from the study. Internal monitoring of scientific data collection and quality of research will occur at both sites.

7.1 Registration

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

The DMCC will use a system of coded identifiers to protect participant confidentiality and safety. Each participant enrolled will be assigned a local identifier by the enrollment site. This number can be a combination of the site identifier (location code) and a serial accession number. Only the registering site will have access to the linkage between this number and the personal identifier of the subject. When the participant is registered to participate in the study, using the DMCC provided web-based registration system: the system will assign a participant ID number. Thus each participant will have two codes: the local one that can be used by the registering site to obtain personal identifiers and a second code assigned by the DMCC. For all data transfers to the DMCC both numbers will be required to uniquely identify the subject. In this fashion, it is possible to protect against data keying errors, digit transposition or other mistakes when identifying a participant for data entry since the numbers should match to properly identify the participant.

7.2 Data Entry

Data collection for this study will be accomplished with online electronic case report forms created by the RDCRN DMCC. Using encrypted communication links, on-line forms will be developed that contain the requisite data fields. All data will be entered by each site into the DMCC online collection site which both study investigators (Comi and Hammill) will have access to along with their study coordinators.

7.3 Study Records Retention

Patient files will be kept until at least seven years after completion of the study.

7.4 Protocol Deviations (This section only applies to JHU/KKI)

The term "protocol deviation" is not defined by either the HHS human subjects regulations (45 CFR 46) or the FDA human subjects regulations (21 CFR 50). For JHM purposes, a protocol deviation is a minor or administrative departure (see definitions below) from the protocol procedures approved by the IRB that was made by the PI without prior IRB approval. Please note: Eligibility exceptions (or eligibility waivers granted by a sponsor) for enrollment of a specific individual who does not meet the inclusion/exclusion criteria in the IRB approved protocol are not deviations. Eligibility exceptions are considered changes in research that require IRB review and approval before a subject who does not meet the approved protocol inclusion/exclusion criteria may be enrolled.

A. **Protocol deviations that constitute unanticipated problems involving risks require prompt reporting to the JHM IRB**

A protocol deviation that constitutes an "unanticipated problem involving risks to subjects or to others" (see Policy No. 103.6(b) for the definition of an unanticipated problem) must be reported promptly to the IRB, as follows:

a. *Emergency deviations:* When a deviation occurs in an ***emergency situation***, such as when a departure from the protocol is required to protect the life or physical well-being of a participant. The sponsor and the reviewing IRB must be notified as soon as possible, but not later than 5 days after the ***emergency*** situation occurred (21 CFR 812.150(a)(4)). The PI must submit a report to the JHM IRB in eIRB under the Further Study Action activity, and use the Problem/Event Report, or for paper studies, use the Unanticipated Problem/Event Report Form (R.F.1)

b. *Major, non-emergent deviations without prior approval:* A planned deviation that is non-emergent and represents a major change in the protocol as approved by the IRB. The IRB must approve the request before the proposed change is implemented. The PI must submit

non-emergent deviations to the IRB for review in eIRB under the Further Study Action activity, and use the Change in Research activity; for paper studies, submit a Change in Research form. If a major, non-emergent deviation occurs without prior IRB approval the event is considered non-compliance. Non-compliance must be reported to the IRB promptly, in eIRB under the Further Study Action activity, and use the Problem/Event Report; for paper studies, use the Unanticipated Problem/Event Report Form (R.F.1). A PI's failure to report promptly any major, non-emergent deviation for which the PI did not obtain prior approval is itself an incident of non-compliance. Incidents of non-compliance will be managed in accordance with the Organization Policy on Investigator Non-Compliance Policy No. 103.7 .

B. Protocol deviations that are only minor or administrative

At JHM, minor or administrative protocol deviations are defined as those which do not "affect the scientific soundness of the research plan or the rights, safety, or welfare of human subjects." If a protocol deviation occurs which meets this definition, the deviation should be reported to the JHM IRB at the time the continuing review application is submitted. In eIRB and for paper studies, use the Protocol Deviation Summary Sheet (R.F. 4) to report these deviations. Examples of minor or administrative deviations could include: follow up visits that occurred outside the protocol required time frame because of the participant's schedule, or blood samples obtained at times close to but not precisely at the time points specified in the protocol.

8. Human Subjects

8.1 GCP Statement

This clinical trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with Good Clinical Practice and all applicable regulatory requirements.

8.2 Benefits

This study will gain a preliminary understanding of safety of sirolimus in Sturge-Weber syndrome and the feasibility of a trial to determine the impact of sirolimus upon cognitive impairments in Sturge-Weber syndrome. It will assess for adverse events in this population and determine best outcomes and biomarkers to be used as a treatment trial of cognitive impairments in Sturge-Weber syndrome.

8.3 Risks

The primary risk to the subjects from participation in this trial is from sirolimus. Risk of toxicity has been correlated with serum levels of sirolimus and at the low dose range being used in this study, the risk of toxicity is low. All participants will have serum levels

measured regularly to guide appropriate dosing and to minimize toxicity. Participants enrolled in this trial will be carefully monitored for the development of toxicities, with guidelines for discontinuation of drug and stopping rules in place. Please see section 5.3 for more detail and for protocol stopping rules.

We will obtain institutional review board approval at all participating sites. Patients' risks of participating in research will be kept to a minimum with measures to protect confidentiality and planned interim analysis for safety monitoring after 5 subjects have completed the study and continuous assessment of all serious adverse events at all times. Confidentiality will be maximized by coding patient information prior to transfer of data or samples between sites. The treating physician at the clinical center will keep a list linking codes with patient identifying information in accordance with their institutional IRB guidelines. 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. For additional risk information, please refer to section 5.3.

8.4 Recruitment

Patients will be recruited from the Sturge-Weber Center at the Kennedy Krieger Institute and from the Vascular Anomalies clinic at Cincinnati Children's Hospital Medical Center. See Section 4.3 for more details about subject recruitment. We expect to be able to recruit the subjects over a 6-12 month period of time at a rate of one subject per site per every two month time period.

8.5 Written Informed Consent

Written informed consent will be obtained from each participant before any study-specific procedures or assessments are done and after the aims, methods, anticipated benefits, and potential hazards are explained. The participant's willingness to participate in the study will be documented in writing in a consent form, which will be signed by the participant or legal guardian with the date of that signature indicated. The investigator will keep the original consent forms and signed copies will be given to the participants. It will also be explained to the participants that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. Written and/or oral information about the study in a language understandable by the participant will be given to all participants.

8.6 Process of Consent

The investigational nature and objectives of this trial, the procedures, the treatments involved, the attendant risks, discomforts, and potential benefits, as well as potential alternative therapies, will be carefully explained to the patient or their parents or guardian if he/she is a child. A signed informed consent document will be obtained after the entirety of the pilot study is explained. Consent will be obtained by the site PI or as

delegated by the PI and assigned on the delegation log for 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 and assent obtained where appropriate.

8.7 Certificate of Confidentiality

To help protect participant privacy, a Letter of Confidentiality has been obtained from the National Institutes of Health (NIH). With this Certificate, the researchers cannot be forced to disclose information that may identify a study participant, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify a participant, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of Federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

Even with the Certificate of Confidentiality, the investigators continue to have ethical obligations to report child abuse or neglect and to prevent an individual from carrying out any threats to do serious harm to themselves or others. If keeping information private would immediately put the study participant or someone else in danger, the investigators would release information to protect the participant or another person.

Department of Health and Human Services (DHHS) personnel may request identifying information for purposes of performing audits, carrying out investigations of DHHS grant recipients, or evaluating DHHS funded research projects.

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