NHGRI IRB
Human Subjects Research
Protocol Template

Project title: Nonrandomized Open Label Pilot Study of Sirolimus Therapy for Segmental Overgrowth Caused by Somatic PI3K Activation

Draft/version number (October 04, 2018)

NHGRI Protocol Number (15-HG-0120)

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1. Precis:

The primary study objective is to determine the likely size of sirolimus treatment effect. The patient population will include male and female subjects, aged ≥ 3 years and ≤ 65 years of age with segmental overgrowth identified to have clinical and molecular findings of somatic *PIK3CA* gene mutation. The planned study size will be ten patients seen at the NIHCC. An additional 20 patients will be contributed by two other centers, who will be responsible for the conduct of the proposed research at their site, but the study procedures and dosing schedule will be identical to enable pooling of results for statistical analyses. The study design will be a nonrandomized, open label, phase II pilot study of sirolimus treatment. As patients have highly variable clinical presentations, and there are no established evidence-based methodologies for measuring serial changes in growth, the aim of this pilot study is to establish the optimal methodology for evaluating changes in growth to inform the design of a future randomized controlled trial, in addition to determining treatment effect size, and evaluating safety and toxicity of low dose sirolimus. Overall desired outcome will be reduced size of affected body part, and measures will include: reduction in affected tissue (fibroadipose or bone) size by clinical exam measurement and by radiological studies (MRI area measurements and/or DXA study measurements of fat).

2. Objectives and specific aims.

2.1 Primary Objectives

1. To measure the effect size of sirolimus therapy in reducing pathological overgrowth in *PIK3CA*-related overgrowth to enable statistical power calculations for a future randomized controlled trial (RCT). A stabilization or a reduction of the tissue volume at the target site of overgrowth will be considered as a success for a given patient.

   Specific Aim 1: To evaluate the use of measurements on physical examination of affected body part(s)

   Specific Aim 2: To evaluate use of quantitative MRI scan of affected and unaffected body part(s) to demonstrate negative change in fibrofatty, muscular, and/or bony overgrowth

   Specific Aim 3: To evaluate use of Dual-energy Xray Absorptiometry (DXA) for body composition to demonstrate reduction in fibrofatty overgrowth.

2.2 Secondary Objectives:

1. To establish if inter-patient comparison will be feasible in a future RCT

2. To evaluate alternative treatment outcomes for inclusion as primary or secondary end-points in a future RCT e.g. quality of life measures (both subjective and observational) in the pre- and post-treatment periods

3. To establish optimal sirolimus dosing algorithms for a future RCT

3. Brief Rationale and Background:

PIK3CA-Related Overgrowth Spectrum (PROS)

The PI3K-AKT-mTOR is a critical signaling pathway, which regulates cellular growth, proliferation and survival. Activating mutations in PIK3CA lead to increased activation of the AKT-mTORC1 axis, which in turn promotes excessive growth in affected tissue. Segmental overgrowth disorders are rare conditions characterized by abnormal growth, which is usually asymmetric and confined to discrete parts of the body. In the last two years, we have made significant progress towards developing a better clinical and molecular understanding of overgrowth disorders that overlap with Proteus Syndrome (PS) to some degree but that are generally quite poorly defined. In collaboration with researchers at Cambridge University in the UK, we delineated a second mosaic overgrowth disorder, which we originally termed fibroadipose overgrowth, which we determined to be caused by one of two activating mutations in the *PIK3CA* gene product, p.His1047Arg or p.His1047Leu [Lindhurst et al., 2012]. Other groups have found somatic variations in this gene in CLOVES syndrome [Kurek et al., 2012] and in asymmetric central nervous system overgrowth and dysplasia [Lee et al., 2012; Rivieré et al., 2012]. It is now clear that mutations in genes that activate this pathway can cause a wide range of overgrowth phenomena and our new challenge is to understand both the range of these phenotypes, their correlations with the nature and distribution of the mutations, and develop effective treatments. We have made significant progress towards this goal in the last year. A manuscript describing the natural histories and phenotypes of 35 patients with *PIK3CA* mutations was recently published AJMG [Keppler-Noreuil et al., 2014]. The PIK3CA-related overgrowth spectrum is wide, and depends upon the timing of the founder mutation in embryogenesis, and possibly on the exact mutation. Clinical presentation ranges from isolated enlargement of a digit, to extensive overgrowth of limbs, abdomen and in some cases the brain, and may be accompanied by vascular or lymphatic malformations. However, the natural history for PIK3CA overgrowth phenotypes is still not well characterized. In some patients, observed associated morbidity can be profound, with functional impairment, repeated surgical debulking of overgrown tissues and amputations, debilitating hemorrhages and thromboses, coupled with neurological sequelae and, in some cases, death

We hosted a workshop in September 2013 at the NIH in Bethesda, Maryland focusing on understanding the phenotypes of the various *PIK3CA*-related overgrowth syndromes, development of a set of clinical and research questions, and potential therapeutic approaches to the management of these disorders. We invited several research groups from around the country and the United Kingdom, along with representatives from patient-advocacy groups, to present their findings. Results were recently published summarizing diagnostic and testing eligibility criteria, differential diagnosis and evaluation of the *PIK3CA*-Related Overgrowth Spectrum (PROS) [Keppler-Noreuil et al., 2014].

Study Agent: Sirolimus

Mechanism of Action of Rapamycin and Indications
Sirolimus is an allosteric inhibitor of mTOR and acts by forming a complex with FK506 binding protein-12 (FKBP12), which then binds to a position adjacent to the kinase domain of mTOR [Sabers et al., 1995]. It is currently licensed for use in suppression of transplant rejection, and long-term safety has been established, with major adverse effects reported to be immunosuppression, pneumonitis, proteinuria, skin malignancy, dyslipidemia, and the development of insulin resistance.

Sirolimus has also been safely used in children. Falger et al. [2006] described the successful long-

term conversion to sirolimus of eight children (ages 4-15 years, median 12.8 years) with chronic allograft nephropathy (CAN) who were status-post renal transplant. All eight children tolerated sirolimus at a trough level of 15-20 ng/mL, and none had to stop the drug. Patients experienced increases in serum lipids within the first 3 months that seemed to dissipate by month 12 [Falger et al. 2006]. Sindhi and colleagues report that of thirty-nine children status-post liver or small intestine transplant who were switched to sirolimus, thirty (78%) tolerated the drug without incident [Sindhi et al., 2005]. In addition, over 50 children <13 years old status-post organ transplant have been safely treated with sirolimus at Cincinnati Children's Hospital and Children's Hospital of Philadelphia with the main toxicity being hyperlipidemia, mucositis, and poor wound healing [personal communication]. These, and other similar reports [Schachter et al., 2006] comprise a growing body of literature on the safety of sirolimus in children.

There are multiple trials for sirolimus treatment of tumors associated with Neurofibromatosis, type 1. There has been no clear consensus on the effect of sirolimus on linear growth, as its use has largely been confined to pediatric recipients of allografts with multi-factorial growth disruption. From animal studies, sirolimus treatment in juvenile rats lead to a reduction in length and tibia growth at two weeks, with no catch up growth after treatment cessation, however markers of chondrocyte proliferation and differentiation had improved by 4 weeks [Sanchez and He, 2009].

Off-label indications include topical treatment of facial angiofibromas [Bissler et al., 2008] systemic treatment for renal angiomyolipoma [Bissler et al., 2008] lymphangioleiomyomatosis. [Davies et al., 2008; Davies et al., 2011; McCormack et al., 2011] brain tumors associated with tuberous sclerosis [Franz et al., 2006; Krueger et al., 2010] and for chemotherapy of various malignancies (renal and hepatocellular cancer and mantle cell lymphomas) [Rao et al. 2004].

Pediatric Pharmacokinetics and Dosing of Sirolimus
In adults and children, sirolimus clearance has been reported to be associated with patient age. Dansirikul et al. [2006] reported sirolimus clearance was inversely related to age in 25 adult renal transplant patients. Similarly, Schachter et al., [2006] reported the sirolimus half-life to be shorter in children compared to adult data (10-24 hours vs. 49-70 hours, respectively) based on their analysis of 24 sirolimus pharmacokinetic profiles in 13 pediatric renal transplant patients [Schachter et al., 2004]. This study also reported that terminal half-life was significantly shorter in children ≤ 6 years of age (median 8.2 hours, range 4.4-10.6) compared to those > 6 years of age (median 12.6 hours, range 4.7-95.2) ($p < 0.05$). The authors attributed these findings to increased rates of drug metabolism in children. Scott et al. [2013] found that sirolimus clearance in their study population of children with NF1 when normalized to body weight (mean CL = 0.44 ± 0.15 L/kg) was much higher than in published adult data (mean CL = 0.21 ± 0.1 L/hr/kg). Clearance was most associated with body size parameters (BSA and total body weight) in children with NF1. When normalized for size, an age effect on clearance was observed in the youngest patients, most likely because of the maturational changes in drug absorption and metabolism [Scott et al., 2013]. Previous studies have also reported higher rates of sirolimus clearance in children, and the use of twice daily dosing was employed based on these previous findings [Schachter et al., 2004; Schubert et al., 2004]. After allometric scaling, a mean clearance of 24.2 L/hr/70kg was observed for children older than 4 years, which approaches adult values (approximately 28 L/hr) [Djebli et al., 2004; Le Meur et al., 2006; Zimmerman et al., 1997]

Imaging and Measurement of Fatty Overgrowth

Quantitative MRI scan for volumetric measurements of fatty overgrowth
Use of this imaging for measurement of connective tissue nevi (CCTN) and of bony overgrowth in Proteus syndrome has been used by our group with demonstrated feasibility and excellent

inter-rater reliability. In addition, other applications have included tumors of mixed tissue composition, like plexiform neurofibromas in NF1, which are frequently large, have a complex (non-spherical) shape, and have erratic growth pattern. In order to reproducibly quantify the size of these complex lesions and detect small changes in the size over time, previous investigators have used MR imaging of shortT1-InversionRecovery(STIR) MRimages [Solomon et al.2004]

DXA scan of body composition
Dual-energy X-ray absorptiometry (DXA) is a well-accepted technique for measuring body composition. Knowledge of measurement precision is critical for monitoring of changes in bone mineral content (BMC), and fat and lean masses. Our center and others have extensive experience in using DXA for body composition measurements and have found that total body precision for BMC, fat and lean mass had a 0.5%, 1.0%, and 0.5% coefficient of variation (CV), respectively. Regional body composition precision error was less than 2.5% CV for all regions except arms [Rothney et al., 2012; Carver et al., 2013; Agnihothri et al., 2014]

Rationale

PIK3CA-Related Overgrowth Spectrum (PROS) phenotypes are being characterized, and it is clear that there is a spectrum of phenotypes caused by somatic mutations in the *PIK3CA* gene, rather than a single phenotype. In some patients, observed associated morbidity can be profound, with functional impairment, debilitating hemorrhages and thromboses, coupled with neurological sequelae and, in some cases, death. At present, serial debulking surgery is the only available therapeutic option.

PI3K regulates cellular growth and survival, and gain of function mutations in PIK3CA lead to increased activation of key growth signals, AKT and mTOR, which in turn promote excessive growth in affected tissue. Future targeted therapies may be possible with the identification of activated PI3K/AKT signaling, either through inhibition of PI3K, of AKT, or of downstream pathways such as mTORC1, using clinically available drugs. mTOR lies downstream from PI3K, and is a key determinant of cellular growth, and on this basis, mTOR inhibitors have been successfully used in allied conditions with excessive growth and hyperactivation of PI3K including; cancers [Rao et al., 2004] and mosaic loss of function of the PI3K negative regulator, PTEN. Rapamycin was reportedly beneficial in a child with type II segmental Cowden syndrome associated with PTEN deficiency [Marsh et al., 2008]. The progressive nature of overgrowth in PROS makes it a good target for pharmaceutical therapy because downregulation of the pathway may prevent the disease progression that is seen in many of the patients reported here.

Through our and our UK collaborators' preclinical trials using cells derived from patients with somatic *PI3KCA* mutations, we have demonstrated that exposure to an mTOR inhibitor (everolimus) reverses hyperactivation of AKT and mTOR in mutant cell lines. Target plasma concentration is based on cellular data; they found that approximately 0.0005ng/ml of everolimus (which is a derivative of sirolimus) completely reverses AKT activation and 0.005ng/ml reverses abnormal proliferation in affected cells. These experiments suggest that small concentrations of drug are required to be effective. We cannot be 100% sure of tissue concentrations from plasma concentrations, but data from sirolimus coated coronary stents suggests that plasma concentrations were roughly equivalent to concentrations obtained in coronary artery tissue i.e. a high degree of tissue penetrance occurs with the drug and, the volume of distribution is high for sirolimus which should help target it to overgrown fatty tissue. Our aim is therefore to have sirolimus levels as low as possible to avoid immunosuppression; therefore suggested a range of 2-6ng/ml to allow for inter-patient variability [Parker, unpublished 2013]. Further to this and through our UK collaborators clinical practice, one of our patients with severe overgrowth of her

legs caused by a mosaic PI3K mutation has been treated with sirolimus off-label on clinical grounds for one year. Preliminary results are encouraging with a reduction of 12% fat mass in her legs as estimated by DXA scanning, and near complete regression of a 7x11cm sacral epidermal nevus at one year. This has been achieved with a low dose of 8mcg/kg/24hrs (1mg once daily), avoiding significant side effects, and remaining below the therapeutic range for immunosuppression. Collectively these observations provide strong rationale for the use of mTOR inhibitors as potential therapies for PIK3CA related overgrowth.

Because of the many reports of the use and demonstrated safety and low risk of adverse side effects of Sirolimus in children less than 13 years of age at higher doses and trough levels than we propose to use, and the demonstrated morbidity and mortality of patients with somatic *PIK3CA* mutations, the potential benefit to risk ratio warrants study of the use of Sirolimus in treatment of these patients.

There are a number points that remain to be addressed:

- Emergence of off-label clinical therapy: Off-label sirolimus therapy is increasingly becoming available to patients, and is being sought by informed patient groups, and actively discussed in patient support group forums. This has given rise to concerns that recruitment to a placebo-controlled trial will be challenging with our current cohort sizes. Furthermore, through our own clinical experience and early unpublished reports of promising treatment responses, ethical reservations are now emerging about treating severely affected patients with placebo. This study design addresses this immediate problem, whilst enabling collation of standardized data for future trial planning, and will further give time to recruit sufficient numbers of patients who would be both appropriate and willing to be enrolled in a future placebo-controlled trial. These future placebo-controlled trials will likely be with alternative and different drugs than sirolimus that are targeted to act directly on PI3K in the PI3K/AKT pathway; therefore these placebo studies will be feasible in terms of patient recruitment. We believe that we will have no difficulty recruiting sufficient numbers of subjects for the described time period, and that we will have many additional patients for future therapy trials. Since September 2011 through passive recruitment at the NIH, we have enrolled 110 patients, therefore, 30 to 40 patients with this disorder per year. The majority of our patient referrals are from colleagues in genetics, and underestimate the total number of patients with *PIK3CA* mutations; those patients with isolated hemihyperplasia or macrodactyly, who may only be seen, for example, in Orthopedics may not have been referred for participation in this study to date. Therefore, the 30 patients from all three centers that will participate in the pilot study represent only about one-third of known patients to date. Based upon these referrals, and knowledge of other centers studying patients with PIK3CA-related overgrowth we estimate that this disorder is relatively common occurring in approximately 1 in 10,000 to 1 in 25,000 live births. For a future randomized trial, we also plan on actively recruiting patients, which will increase the number of patient referrals.
- Limited information of the natural history of the condition: In view of the rarity of these conditions and of their frequently poor clinical characterization in routine practice, knowledge of the natural history of the conditions is limited, being restricted to small case series with no prospective data on growth. In addition, although sirolimus is now being used in clinical practice there are no available data on effect size, and in particular whether sirolimus induces disease regression, merely slows growth, or is indeed effective at all. This study design will enable serial measurement of growth across all patients enrolled in the trial during the run-in period, and will further give an idea of effect size, and whether or not disease regression can be used as a primary end-point in a future trial.
- Marked clinical heterogeneity: Somatic *PIK3CA* mutations give rise to a diverse spectrum of clinical presentations ranging from an enlarged digit or limb, to more extensive overgrowth

of the body or brain. As a consequence, no two patients are phenotypically the same, and it is therefore unclear if primary end-points are cross-comparable between patients, or whether intra-patient comparisons will be required. This key difference is critical to determine, as the former would point towards a future randomized parallel study design, whereas the latter may necessitate a cross-over strategy.

- Sparse pharmacokinetic data: Although some pharmacokinetic data is available for sirolimus in both children and adults, many of these data were accrued in the context of renal failure, concurrent cyclosporin treatment and/or renal transplantation, with dosing aimed at achieving adequate plasma levels for immunosuppression. In contrast the target plasma concentration of this trial will be below or at the lower end of this range. Furthermore, renal and intestinal clearance of sirolimus in children is higher and has been shown to affect plasma concentrations; this may necessitate twice daily dosing in some patients, which would need to be incorporated into any study design. The proposed pilot study will thus generate pharmacokinetic data to refine the dosing algorithm for a larger scale trial.

We are proposing nonrandomized open label study with sirolimus treatment of patients with the PIK3CA-related overgrowth disorder. As patients have highly variable clinical presentations, and there are no established evidence based methodologies for measuring serial changes in growth, the aim of this pilot study is to determine treatment effect size, and to establish the optimal methodology for evaluating changes in growth to inform the design of a future randomized controlled trial.

4. Description of study design *(Brief description of what study design has been selected)*

The study design selected is a nonrandomized open-label pilot treatment study, which will involve treating ten patients with PIK3CA-related overgrowth with low dose sirolimus therapy at the NIH. The study design selected will involve a 6-month observational (non-treatment) period to measure serial changes in growth followed by a 6-month period with low dose sirolimus therapy. The primary aim of this study is to calculate an effect size for treatment with sirolimus, and further to inform the design of a future RCT with sirolimus and other small molecule inhibitors of the PI3K-Signaling pathway. The rationale for using this design will be to evaluate a variety of methods to measure changes in growth in patients with and without treatment to inform the optimal protocol for measuring primary outcome measures.

This study is being performed in association with a consortium involving three centers (NIHCC, Bethesda, USA, Cambridge UK, and Dijon, France). Each study center will function independently. Each center has been reviewed and approved by their respective IRBs with the UK and France sites having a designated Federalwide Assurance (FWA) number. Each site will have a designated PI, who will take responsibility for the conduct of the proposed research at that particular site, but the study procedures, including the methodologies for the primary outcome measures and sirolimus dosing schedule will be identical to enable pooling of results for statistical analyses under a data sharing agreement. Each of two additional centers will contribute 10 patients for a total of 30 patients, who will participate among three centers, and whose de-identified data will be analyzed. The toxicity information from the other sites will be part of the annual review.

The PIs and AIs from each of the separate centers, which include the statisticians and pharmacologists have met on a monthly basis since March, 2014 to develop the design of the study including the methodologies for the primary and secondary endpoints, along with the plan for sharing and analysis of the data. The data from the primary outcome measures, namely the MRI volumetric scans, and the DXA scans for whole body composition will be de-identified and

shared between the centers. The raw data from the MRI volumetric scans at the NIH will be sent to the UK Center for analysis with their software, SliceOmatic® (http://www.tomovision.com/products/sliceomatic.html. The DXA scan data from the UK and France will be fully anonymized or de-identified and sent in encrypted Excel sheets to the NIH for analysis by Dr. Kong Chen. Further, we will use the same quality of life measures, WHO-BREF- and PEDsQL for all three centers, which have been validated for English and French languages. The data from these measures will also be fully anonymized and sent in encrypted form to the NIH. Similarly, the results in identical format will be sent from the NIH to the UK and France centers and analyzed by the UK and French study teams. The data from these measures will be shared and analyzed at each center for comparison.

The NIH will also have additional secondary outcome measures, including additional quality of life measures recommended by the psychology staff at the NIH, as well as observational physical therapy and rehabilitation measures.

We anticipate no problems in recruiting the ten patients from each center to complete this pilot study within one year. However, in the unlikely event that one of the centers is not able to accrue the 10 patients, we are confident that we have at least 30 patients ascertained and willing to participate in this study solely from the NIH. We believe that we will have no difficulty recruiting sufficient numbers of subjects for the described time period, and that we will have many additional patients for future therapy trials. Since September 2011 through passive recruitment at the NIH, we have enrolled 110 patients, therefore, 30 to 40 patients with this disorder per year. The majority of our patient referrals is from colleagues in genetics, and underestimate the total number of patients with *PIK3CA* mutations; those patients with isolated hemihyperplasia or macrodactyly, who may only be seen, for example, in Orthopedics may not have been referred for participation in this study to date. Therefore, the thirty patients from all three centers that will participate in the pilot study represent only about one-third of known patients to date. Based upon these referrals, and knowledge of other centers studying patients with PIK3CA-related overgrowth we estimate that this disorder is relatively common occurring in approximately 1 in 10,000 to 1 in 25,000 live births. For a future randomized trial, we also plan on actively recruiting patients, which will increase the number of patient referrals.

5. Description of procedures:

A summary chart of timing of procedures and evaluations from recruitment to completion, during the 13-month period of the study is found in Appendix A.

5.1. Medical information

In this study, we expect that we will recruit most of our patients from those participating in our ongoing natural history study (94-HG-0132). For prospective participants previously uninvolved with our research, we typically request materials such as photographs, imaging studies, and medical records so that we may make eligibility decisions. This information is typically retained for eligible participants who join the study or, in the case of unique items such as hard-copy imaging studies, returned to the participant or referring institution.

During the six-month period participants will be on sirolimus, they (or their parents in the case of minor children) will be asked to keep a daily diary detailing medication administration and any side effects noted (See Appendix P). These diaries will be turned into protocol staff on a weekly basis, either in person at the NIH or via fax or FedEx. See Section 9.1 for additional details.

5.2. Diagnostic studies

Upon admission to the NIH Clinical Center, a history and physical examination will be performed in addition to several laboratory tests, procedures, and consultations.

All patients enrolled in this protocol will also be enrolled in protocol 94-HG-0132: The phenotype and etiology of Proteus syndrome and related disorders. While a number of studies may be performed under that protocol, we will discuss here only the procedures we will employ specifically for and in this protocol. If studies for the two protocols coincide, they will not be duplicated (for example, if a patient was seen at the NIH on our natural history study 6 months prior to enrollment in this protocol, and one of our recommendations was to obtain a repeat abdominal ultrasound in 6 months, we will not perform two separate abdominal ultrasounds at the time of enrollment in this protocol).

After informed consent is obtained, participants will be asked to undergo a number of evaluations at the beginning of the 6-month run-in (observational period):

- General clinical examination with targeted confirmation of relevant aspects of the clinical history including information about prior medical problems, medications, measurement of height, weight, head circumference, blood pressure, and where relevant, sites of overgrowth [Appendices Y and Z]. A physical medicine and rehabilitation joint mobility and function evaluation will be done [Appendix BB]. At this time, we will assess for possible side effects of treatment, change in respiratory function, skin infections or changes suspicious for malignancy, and history of previous hospital admissions or surgical/radiological procedures. This will help rule out any contraindications to sirolimus therapy, and establish key investigations to monitor therapeutic response.
- Laboratory evaluations including: fasting blood samples Sodium, potassium, chloride, bicarbonate, calcium, phosphorus, magnesium, creatinine, glucose, blood urea nitrogen, albumin, total protein, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, uric acid, serum lipid panel, OGTT, insulin, C-peptide, HbA1C, Leptin, Adiponectin, Resistin, hepatitis and HIV serology, CBC, and sirolimus plasma levels
- Urine pregnancy test for reproductive-aged women before starting the drug
- Standardized quality of life and functional status questionnaires
- Physical measurement of affected and unaffected body parts
- DXA whole body composition scanning – will not be done if sedation is required (see below for details) and will only be done after a negative urine pregnancy test conducted within the preceding 24 hours.
- Volumetric MRI scan– may require the use of sedation (see below for details). [Appendix AA].

After the completion of the 6-month untreated period, participants will be asked to return to the NIH for a series of visits that will last between one and five days for commencement of treatment and subsequent monitoring. This initial visit will be for 1 month, during which time once a week evaluations will be completed. Participants will also be asked to undergo subsequent interim monitoring labs locally between the scheduled NIH visits at 3, and 6 months on treatment. The CBC with differential and sirolimus blood levels will be obtained every 1-4 weeks, after the initial sirolimus levels are within 10% of each other in the target range. If the ANC is below 1.5, then the CBC with differential will be checked at least every week until normalized. They will also be asked to complete a daily patient diary of sirolimus use and any potential side effects. They will be instructed on its completion and how to contact the PI and AIs if there are problems. See Appendix A for additional details.

Appendix B lists details about each monitoring procedure that does not involve radiation or sedation.

Pending approval from the NIH Radiation Safety Committee, in addition to the clinical/diagnostic studies outlined above in Appendices A and B, we will employ dual-energy x-ray absorptiometry (DXA) body composition testing (iDXA, GE Healthcare, Madison WI) at the beginning of the observation period (baseline), when the treatment period commences, after 3 months of treatment, and after 6 months of treatment. With this technique, one can determine total and regional body fat and lean soft tissue masses, bone mineral content and density. The use of DXA scan apparatus may cause some minimal discomfort in claustrophobic subjects and may cause some minimal back pain in a small minority of the individuals. We will only ask participants who can complete the DXA scan studies (which can take less than 10 minutes) without the use of sedation to undergo this procedure and will ask all female participants who are of reproductive age to undergo a urine pregnancy test prior to this study.

Participants in this study will not undergo any procedures involving research-indicated radiation as part of our natural history study (on which they will be co-enrolled) one year before enrolling and one year after completion of this protocol.
While we expect that participants at the lower age range for this protocol may be able to complete the DXA study without the use of sedation, sedation for the volumetric MRI may be required for very young participants (ages 3 to approximately 6 years). See Section 9.3 for additional details.

5.3. Biological Specimens

We will obtain blood and urine in this protocol for both clinical (e.g., to monitor glucose levels while on treatment) and research (to assess blood levels of sirolimus) purposes. We will not bank or retain biological specimens (i.e., create a cell line or store serum) under this protocol.

5.4. Approved Drugs Being Used for Research

Sirolimus is an FDA-approved for prophylaxis of organ rejection in patients > or = 13 years receiving renal transplants.

Sirolimus is being used off-label in this study. An IND is not required for clinical investigation of Sirolimus in this study because all the criteria under the FDA regulation 21 CFR312.2 (b), as follows (a response to each of these five criteria is in bold print):

1. The investigation is not intended to be reported to the FDA as a well-controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling for the drug. **True. This study is not intended nor designed. to extend the indication.**
2. If the drug that is undergoing investigation is lawfully marketed as a prescription drug product, the investigation is not intended to support a significant change in the advertising for the product. **True. This study uses an FDA-approved drug and there is no intent to change any advertising related to this drug.**
3. The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product. **True. The drug has been used in adults and pediatric patients at doses to produce higher blood levels (10 – 20 ng/mL) than targeted in this study. By targeting a lower blood level, we do not expect the risks to increase.**
4. The investigation is conducted in compliance with the requirements for institutional review set forth in 21 CFR part 56 (see also NIH SOP 7 “Requirements for the Ethical and Regulatory

Review of Research by NIH Institutional Review Boards (IRBs)"and SOP 8 "Procedures and Required Documentation for Submission and Initial Review of Protocols"), and with the requirements for informed consent set forth in 21 CFR part 50 (for NIH policy see also SOP 12 "Requirements for Informed Consent from Research Subjects"). **True.**

5. The investigation is conducted in compliance with the requirements regarding promotion of an investigational drug at 21 CFR 312.7. **True. There is no intent to represent in a promotional context that sirolimus is safe or effective for the treatment of overgrowth syndrome purposes or otherwise promote sirolimus for any purpose.**

Sirolimus is available as an oral solution and as tablets. Pfizer will provide funding for drug only support (for Sirolimus) for the participants in the study directly to the NIH, Pfizer is not a sponsor, nor does it have ownership of the data. We, at the NIH, are the Sponsor-Investigator for the study. However, since Pfizer is providing the drug, we will be responsible for reporting to Pfizer accurate and sufficient data on all reportable adverse events that occur within the study reporting period, in addition to reporting to the *PROS Sirolimus Study Safety Review Committee.*

Sirolimus Oral Solution

Each Sirolimus oral solution package, 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.

Sirolimus 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 for the bottles).

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). The syringe should be discarded after one use.

Sirolimus 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.

Sirolimus tablets

Sirolimus tablets are available in 0.5mg, 1mg, and 2mg strengths.

Sirolimus tablets should be stored at 20° to 25°C [USP Controlled Room Temperature] (68° to 77°F). Use cartons to protect blister cards and strips from light. Dispense in a tight, light-resistant container as defined in the USP.

The aim of sirolimus dosing will be to maintain sirolimus blood levels ranging between 2ng/ml and 6ng/ml thereby achieving detectable, but low concentrations of drug. This therapeutic range has been informed by pre-clinical studies in dermal fibroblasts which have established that very small doses of active drug are required to reverse aberrant signaling and slow growth in affected cells, with calculated IC_{50s} for AKT and p70S6 signaling of 0.7ng/ml. Furthermore, the UK index proband has been treated with sirolimus at a dose of 0.007mg/kg, and has experienced a 30% reduction in tissue mass, and the response to therapy has been sustained at 18 months with a mean plasma concentration of 3.5ng/ml (range 2-4ng/ml).

Dosing will vary between children and adults in view of the higher renal clearance and comparatively shorter $T_{1/2}$ in children. The upper limit of the starting dose for <18 years of age should be 1.5 mg total daily dose.

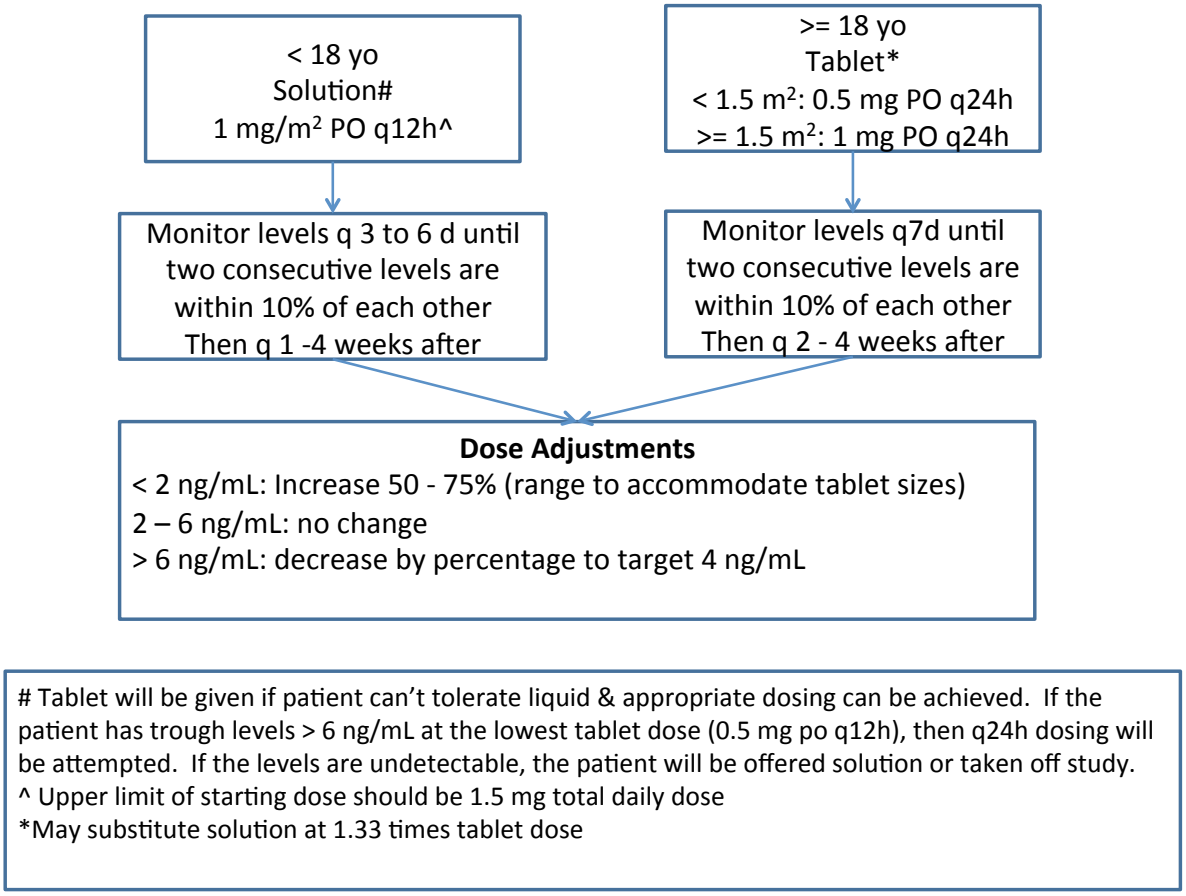
Participants will be advised to take sirolimus in the morning each day before breakfast and before evening meal in children on twice daily dosing.

Sirolimus blood levels will then be taken first thing in the morning, just prior to their usual dose of sirolimus to establish trough concentrations. If the sirolimus blood level is not in the expected range, it will be monitored every week until the desired therapeutic range (2-6 ng/ml) is achieved. Sirolimus blood levels will be monitored at all times where blood is taken and adjustments made as necessary, and a follow-up blood test one week after dose changes.

Dosing schedule takes into account the following assumptions based upon data on sirolimus in the medical literature:

1. Bioavailability of tablets = 0.17 and bioavailability of solution = 0.14
2. Apparent oral volume of distribution (Vd/F)= 12L/kg (so V= 2.04 L/kg)
3. Median weight =30 kg (from Scott et al., 2013)
4. Terminal elimination half life ($T^{1/2}$)= 12 hrs
5. Median BSA = 1.0 m^2 (from Scott et al., 2013)
6. Absorption rate constant (ka)= 2.77 hr^{-1} (from Scott et al., 2013)
7. Lower limit of detection = 2 ng/mL
8. Target of 2 – 6 ng/mL, with a desired level closer to 2ng/mL but still measurable

Depending upon the ability of the patient to take a tablet and if appropriate dosing can be achieved with the tablet, sirolimus will be given as a tablet or as an oral solution, as shown in the algorithm below.



Tablet will be given if patient can't tolerate liquid & appropriate dosing can be achieved. If the patient has trough levels > 6 ng/mL at the lowest tablet dose (0.5 mg po q12h), then q24h dosing will be attempted. If the levels are undetectable, the patient will be offered solution or taken off study.
^ Upper limit of starting dose should be 1.5 mg total daily dose
*May substitute solution at 1.33 times tablet dose

Concomitant medications

The interaction problems are mainly with STRONG inhibitors and inducers of CYP450 3A4. A comprehensive list can be found at:
http://medicine.iupui.edu/clinpharm/ddis/main-table/

Patients may not be currently receiving strong inhibitors of CYP3A4, and may not have received these medications within 1 week of entry.
These include:
• Macrolide Antibiotics: clarithromycin, telithromycin, erythromycin, troleandomycin.
• Gastrointestinal prokinetic agents: cisapride, metoclopramide.
• Antifungals: itraconazole, ketoconazole, fluconazole, voriconazole, clotrimazole
• Calcium channel blockers: verapamil, diltiazem, nicardipine
• Other drugs: rifampin, bromocriptine, cimetidine (tagamet), danazol, cyclosporine oral solution, lansoprazole (prevacid), calcium containing antacids.
• Grapefruit juice.

Patients must also avoid strong inducers of CYP3A4, and may not have received these medications within 1 week of entry. These include:
• Anticonvulsants: carbamazepine, phenobarbital, phenytoin
• Antibiotics: rifabutin, rifapentine.

• Herbal preparations: St. John's wort (*Hypericum perforatum, hypericine)*.

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 include:
• Carbamazepine (Tegretol)
• Felbamate (Felbtol)
• Phenobarbital
• Phenytoin (Dilantin)
• Primidone (Mysoline)
• Oxcarbazepine (Trileptal)

Participants will be advised report any new medications. The study team will consult with a pharmacist if any medication needs to be started. Where relevant, sirolimus levels should be measured if any of these additional drugs are commenced independently of the study.

1. Statins – monitor CK levels
2. Macrolides – may increase plasma concentrations of sirolimus
3. Azoles - may increase plasma concentrations of sirolimus
4. ACE/Angiotensin II inhibitors – risk of angiodema

Study medication accountability
Study drug accountability will be assessed and documented by NIHCC Pharmacy. Study tablets will be stored at room temperature and after use, the blister packs will be returned to pharmacy for drug accountability/adherence to be assessed. We will also account for liquid solution use; storage of the solution is described above.

Monitoring
Sirolimus blood levels will be performed using a commercially available assay from a CLIA-certified laboratory with a lower limit of detection of 2 ng/mL. A patient diary will be kept including timing and dose of Sirolimus, missed doses, side effects, and other medications taken.

5.5. Unapproved Drugs/Devices

Sirolimus, the drug used in this pilot treatment trial, is approved for another indication; however, it is not approved for use as treatment of the condition in this study, PIK3CA-Related Overgrowth Spectrum (PROS), and will be used off-label.

5.6. Specific results that will be given to participants or their health care providers

Results of clinical testing that we perform on any participant seen at the NIHCC (e.g., x-rays, cognitive testing) will be returned to the participants or their parents (in the case of minor children).

5.7. Describe questionnaires or other psychological instruments and estimate how long they will take to complete, and whether they address sensitive topics

All three centers will use a quality of life questionnaire, which will be performed before starting treatment and after 6 months of therapy. The WHO-QOL-BREF questionnaire will be used for adults [Appendix T]; this has been validated for use in chronic diseases [Skevington, Lotfy, & O'Connell, 2004]. For children, PedsQL™ questionnaire for children and parents report for children questionnaires will be used; these have also been validated in chronic diseases [Davis et al., 2006]. Both of these measures have been validated for the French language, in addition to English [Appendices U and V].

In addition, the participants and their parents at the NIH will be asked to complete the additional following measures of functioning, quality of life, and pain assessment at baseline and 6 months before treatment, then at 1, 3 and 6 months during the sirolimus treatment period (see Appendix A for frequency of administration of the questionnaires. Please see Appendices C through K, as listed below, for copies of these questionnaires. Parents (on behalf of pediatric participants ages 3-7 years) and adult participants will complete all appropriate/available measures. Pediatric patients aged 8 years old and older may directly complete some measures. The total time to complete all 4 measures for the parent will be approximately 15 minutes, while for the patients it will range from 20-30 minutes.

Quality of Life (IPI Scale)

Impact of Pediatric Illness Scale-Parent Proxy and Self-Report: The Impact of Pediatric Illness (IPI) Scale is a health-related quality of life (QOL) scale for children and adolescents and adults with CNS involvement. The IPI Scale assesses QOL in four domains: 1) Adaptive Behavior includes items on school attendance, social activities, and daily living skills, 2) Emotional Functioning includes items on anxiety, depression, and self-esteem, 3) Medical/Physical Status includes items on fatigue, pain, and physical functioning, and 4) Cognitive Problems includes items on attention, memory, and learning problems. There are two parent proxy report forms for primary caregivers of children from 3 to 5 years (Appendix C) and from 6 to 18 years of age (Appendix D). The self-report forms are for children from 10 - 18 years (Appendix E), and adults 18+ years (Appendix F). The parent and self-report forms have parallel content and the same number of items to allow direct comparison of parent and patient responses. The time period assessed by the scale is the past month, and higher scores indicate a poorer quality of life. A mean total score is derived from the 43 items of the scale, and mean scores are computed for the 4 domains. If a form has more than 3 missing responses then it is not considered valid. Data obtained in children with cancer indicates adequate internal consistency and good interrater reliability (Wolters, 2004; abstract).

Pain Intensity

The Numerical Rating Scale-11 is a segmented 11-point numeric scale that assesses pain intensity [Downie, 1978]. It has demonstrated reliability and validity in many studies, including clinical trials [Chang et al., 2014; Irving et al., 2011]. It consists of a horizontal line with 0 representing “no pain” at the right end of the line and 10 representing “worst pain you can imagine” at the left end. Respondents are asked to circle the one number from 0 to 10 that best describes the child’s “worst pain” during the past week. Participants ages 8 years and older will complete the self-report form (Appendix G) at each time point. It takes less than 1 minute to complete.

Pain Interference

The Pain Interference Index is a 6-item parent and self-report questionnaire to measure pain interference using the past two weeks as a recall period. Respondents rate items on a 0 to 6 scale to indicate how much pain has interfered with various activities, and the total score is the mean of the 6 items. Preliminary reliability and validity are good [Wicksell et al., 2009]. The PII questionnaire has been adapted as a 6-item parent version that is formatted and scored the same way as the self-report PII, with total scores ranging from 0 to 6 (See Appendix H for both questionnaires). It will be administered at each time point to children ages 6 years and older and to parents of children ages 5 and older. Both the self-report and parent versions take about 2 minutes to complete.

Physical Functioning

The PROMIS Physical Function self-report measures for children include items assessing Upper Extremity function and Mobility (Appendix I). Each of these subscales contains 8 items formatted on a 1 – 5 Likert Scale, and are reliable and valid for children ages 8 to 17 [DeWitt et al., 2011].
For adults ages 18 and older, the PROMIS Physical Function 8-item short form will be used (Appendix J). Items are formatted on a 5-point Likert scale and the respondent indicates how much his or her health is limited in performing various activities (e.g., climbing stairs, lifting heavy objects, etc.) This scale has been found reliable and valid among the general population and chronically ill adults [Rose et al., 2014].
Parent report forms are available for the two PROMIS Physical Function subscales described above (Upper Extremity and Mobility; Appendix K). These also contain 8 items each and are completed by parents of children ages 5 to 17 years [Irwin et al., 2012]. For each of these PROMIS measures, item responses are summed, and total raw scores are converted to T-scores. Each scale typically takes about 2 minutes to complete.

5.8. Genetic counseling

Participants and families seen at the NIHCC, regardless of underlying diagnosis, will undergo genetic counseling with an Associate Investigator, who is also a certified genetic counselor, unless this has been accomplished on a prior visit to the NIHCC.

5.9. Description of criteria for withdrawal from study.

Study Withdrawal Criteria
The participant will be withdrawn from the study if any of the following apply:

- The participant elects to withdraw from the study
- Inability or failure to attend study visits
- Decision of *PROS Sirolimus Study Safety Review Committee*

Subjects may withdraw for any reason, at any time, according to federal regulations.

Unless we are specifically requested to destroy all research data collected by a subject who chooses to withdraw from this study, data collected from subjects who withdraw may be retained and analyzed if possible.

6. Description of Study Population:

6.1. Estimated number of participants, enrollment ceiling, and anticipated enrollment by year.

We are targeting the recruitment of ten to fifteen patients to the NIH through our study, Phenotype and Etiology of Proteus Syndrome and Related Overgrowth Disorders, Protocol # 94-HG-0132. The range provided reflects the possibility that some participants may withdraw and others may be found to be ineligible after enrollment. We anticipate that if we start the study in May, 2015 with two patients per month brought to the NIH, then the last two patients will complete the study in August, 2016. This study is being performed in association with a consortium involving three centers (NIHCC, Bethesda, USA, Cambridge UK, and Dijon, France). Each study center will function independently. Each center has been reviewed and approved by their respective IRBs with the UK and France sites having a designated Federalwide Assurance (FWA) number. Each site will have a designated PI, who will take responsibility for the conduct of the proposed research at that particular site, but the study procedures, including the methodologies for the primary outcome measures and sirolimus dosing schedule will be identical to enable pooling of results for

statistical analyses under a data sharing agreement. Each of two additional centers will contribute ten patients for a total of 30 patients, who will participate among three centers, and whose de-identified data will be analyzed. The toxicity information from the other sites will be part of the annual review.

6.2. Description and justification of clinical inclusion/exclusion criteria.

This study will include male or female participants with measurably progressive overgrowth.

Inclusion Criteria

- Age: ≥ 3 years to ≤ 65 years
- Male or Female
- Confirmed *PIK3CA* somatic mutation
- Measurably progressive overgrowth, in current progression or with clinical history of overgrowth progression
- Adequate Bone Marrow Function Defined as:
  - Peripheral absolute neutrophil count (ANC) ≥ 1500/μL, except for those participants with an absolute neutrophil count (ANC) of 1000-1500, caused by a benign condition associated with moderately decreased neutrophils known as Benign Ethnic Neutropenia (BEN), d those who have an ANC of 1000-1500 caused by a confirmed infection, which resolves with treatment of infection to ≥ 1500.
  - Platelet count ≥ 100,000/μL
  - Hemoglobin ≥ 10.0 gm/dL
- Adequate Renal Function Defined as:
  A serum creatinine based on age as follows:

Age (years)	**Maximum Serum Creatinine (mg/dl)**
≤ 5	0.8
5<age≤10	1.0
10<age≤15	1.2
>15	1.5

  OR a creatinine clearance or radioisotope GFR ≥ 70ml/min/1.73 m2
- Adequate Liver Function Defined As:
  - Bilirubin (sum of conjugated + unconjugated) ≤ 1.5 x upper limit of normal (ULN) for age, and
  - SGPT (ALT) ≤ 5 x upper limit of normal (ULN) for age, and
  - Serum albumin ≥ 2 g/dL.
- Fasting LDL Cholesterol:
- Patients must have a fasting LDL cholesterol of ≤ 160 mg/d
- All women of childbearing potential and all sexually active male patients must agree to use effective contraception
- Adolescent (15-17 year old) participants who are fluent in English and can thereby complete the pediatric self-report questionnaires and communicate well with the study team but whose parent(s) and/or legal guardian are primarily Spanish-speaking.

Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

- Age <3 years or >65 years
- Pregnant or breastfeeding
- Women and men of reproductive age without an effective method of contraception (during treatment and up to 12 weeks after sirolimus discontinuation)

- Hypersensitivity to sirolimus or any of the excipients
- Any current medical disorder or medication likely to impair ability to follow the study protocol safely and effectively
- Incapacity to give informed consent
- Sirolimus treatment in the prior 4 weeks
- If less than 3 months post-surgery
- Prior malignancy or ongoing investigations for malignancy
- Active skin infections requiring antibiotics or anti-viral medication
- HCV/HBV/HIV seropositivity
- Previous/ active MTB infection
- Pneumonitis
- Research radiation exposure within previous 12 months
- Adult participants or participants under the age of 15 years with insufficient English-language proficiency to complete informed consent and quality of life measures

We propose to restrict participation in this study to those with sufficient English language skills to complete the quality of life measures we will employ among all three study sites, as many of the specific quality of life measures we are employing at the NIH only are not available in other languages.

6.3. Location of study

The patients in the study will be evaluated at the NIH Clinical Center facilities, including the Pediatric Clinic or an Adult Clinic depending upon the age of the patient.

The patient may have interim blood draws done locally. We will work with participants to identify a local resource and expect that this will differ from patient to patient as some patients may be able to work directly with their doctors to have these labs drawn by a provider and others will need to visit a laboratory (i.e., LabCorp or Quest) independent from their local provider. We will provide participants with all supplies needed to package and ship specimens to us and we will cover all shipping charges. We will cover phlebotomy charges using the NIH Authorization for Payment for Medical Services Outside the Clinical Center form (NIH-2541).

6.4. Description of recruitment strategies

In the past, participants have been recruited primarily through knowledge of our study in the clinical genetics community and through the parent/ patient support groups in the US and UK. We will continue these efforts as the family support community for these disorders frequently asks us about developments in our research. We will notify the relevant family support groups (e.g. CLOVES Syndrome Community, CLOVES Syndrome Foundation, etc.) about the launch of this trial and will provide them the study website so that they may distribute this information to their memberships. Please see Appendix F for the text of the study website.

6.5. For existing sample/data sets, note whether samples were originally collected for research or clinical practice. If obtained for research, include a description of the original purpose of study and prior plans for sample storage. Was consent obtained that would be applicable to this study?

N/A

6.6. Description of any financial compensation. If participant withdraws early, describe whether compensation will be modified.

There is no financial compensation, other than support for travel expenses or our payment for shipping and reasonable handling expenses (e.g., blood drawing charges) for specimen collection.

7. Description of study statistical considerations and/or analytic plan:

Given the lack of standardized methodology for serial growth measurements and marked clinical heterogeneity, it is difficult to make formal statistical power calculations for this study. On the assumption that a treatment that achieves a success rate of less than 5% is unacceptable and that a success rate of around 20% is expected, it is necessary to include 27 patients with an alpha risk of 0.05 and a power of 80%. No single patient has ever been recorded to undergo spontaneous resolution or regression of disease.

Analysis of Endpoints

Primary outcomes:

The main objective of the pilot is to determine the best outcome measure to use in a subsequent RCT. In patients with established serial growth data, a measure of the relative percent excess tissue volume at the affected site will be taken at baseline and following 6 months sirolimus treatment to enable calculation of effect size. But rather than measuring simple differences in change, we propose to incorporate information from interim measurements using the following "broken stick" model for each outcome under consideration:

$$y_t = \beta_0 + \beta_1 t + \beta_2 (t - 24)_+ + \epsilon_t$$

Where

- y_t is the outcome at week t
- t is the follow-up time in weeks (baseline is time 0)
- β_0 represents the state of disease at baseline
- β_1 represents weekly progression during the untreated period
- β_2 represents the treatment effect, that is, the difference in weekly change between the treated period and the untreated period
- ϵ_t is a mean-zero normally distributed error term with variance σ_ϵ^2 and an AR(1) structure such that the correlation between adjacent error terms separated by k weeks is ρ^k.
- The "plus" variable $(u)_+$ has value u if $u > 0$, and 0 otherwise.

This model fits two line segments: the first during the untreated period, and, the second continuing where the first stopped (at week 24) , but with possibly a different slope, during the treated period. The treatment effect β_2 is the difference in slopes between the two line segments. This is in the spirit of calculating the difference between treated and untreated weekly changes, but allows consideration of the extra evidence offered during intermediate examinations of the patient. We suggest that the relative suitability of the various measures for a future trial can be assessed by comparing the ratios of their estimated expected values to their estimated standard deviations, or equivalently, by comparing their p-values (providing both effects are pointing in the right direction).

A complication is the irregularity of the measurement schedule: the temporal distance between adjacent time points may be a week, a month, and sometimes half a year. This precludes a standard AR(1) analysis with equally-spaced time points, but SAS PROC MIXED can handle this

using a spatial autocorrelation correlation structure. The broken stick model may be estimated in SAS PROC MIXED[1] using the following code fragment:

```
proc mixed data = dat noclprint;
        class person class week zero;
        model y = week weekplus / solution ddfm = kr;
        repeated classweek / subject = person type = sp(exp)(zero classweek);
        run;
```

In this code fragment, y is the outcome variable, week is numeric weeks, weekplus is the "plus" variable defined as in the "broken stick" model above, classweek is the same as week but is defined here as a class variable, and zero is a dummy class variable containing 0.

In the spirit of exploration, we will apply the "broken stick" model not only to absolute changes, but also to percent changes from baseline for each patient. In this case, the baseline value will be dropped and β_0 controlled to be 0.

Although power is not the main issue in this pilot study, Table 1 presents a simulation that demonstrates greater power using the "broken stick" method, in which $(\beta_0, \beta_1, \sigma_\epsilon)$ = (1, 0.1, 2), $\rho = (0.9,0.1)$ there were 30 patients, and measurements were taken at weeks (0, 24, 25, 26, 28, 32, 36, 40, 44, 48, 52). $\beta_1 = 0.1$ means that progression in the absence of treatment (during weeks 0-24) was 0.1 per week. Two cases were simulated: the null, in which $\beta_2 = 0$ (no treatment effect, so that progression continues unabated in the treated period), and an alternative in which $\beta_2 = -0.1$, that is, progression was completely stopped by treatment during weeks 24-52. In this example, as desired, all methods rejected the null hypothesis with probability about 0.05 when the null was true. Under the alternative, the broken stick enjoys a power advantage over Student's t and the non-parametric methods, with a greater power improvement when the weekly correlation is low, that is, when successive errors are more independent of each other.

Table 1: Simulated power of "broken stick" method at tw-tailed $\alpha = 0.05$ versus that of three other tests comparing treated to pre-treated changes. There were 10,000 iterations of each of the four scenarios (null, alt) X (p=0.9, 0.1).

	$\rho = 0.9$		$\rho = 0.1$	
	Null	Alt	Null	Alt
Broken stick	0.05	0.87	0.05	0.98
Sign	0.04	0.63	0.04	0.71
Signed Rank	0.05	0.81	0.05	0.88
Student's t	0.05	0.83	0.04	0.89

One difficulty involved in choosing the best of several outcomes is that the effect size of the best will likely be over-estimated. But note that, when applying our results to the design of a subsequent RCT, the design effect size chosen should ideally be predicated on what is clinically meaningful, rather than what was observed in a pilot study.

Although the aim of the study is to discover the best way to measure the treatment effect for a future RCT, we will nevertheless test the significance of the treatment in the current trial by the following stratagem: A predicted optimal method for assessing the outcome of each particular patient will be prospectively assigned to each patient at the time of enrollment. The candidate

[1] A similar but not identical GEE analysis may be carried out in SAS GENMOD using the WITHIN option on the REPEATED statement.

methods are as follows: physical examination measurements, volumetric MRI scan, and DXA scan for body composition. The optimal method may vary from one patient to the next, depending on the details of each patient's clinical status. On trial completion, significance of the treatment will be assessed by Wilcoxon signed rank test to compare the percent change of each patient in the treated period to the corresponding change in the observational period with respect to the purported optimal method. This percent change will be calculated for each patient by a simple OLS model through the origin, similar to the "broken stick" elaborated above (there are likely not enough data for a time series analysis in this case.) A secondary confirmatory analysis will score each patient as a success or a failure depending on whether the patient improved more or less during the treated period than the untreated period with respect to the purported optimal method. Significance may then be assessed by inquiring via the binomial distribution whether the probability of success exceeds 0.5. A third confirmatory analysis will involve calculating Wilcoxon signed rank tests for each method, and adjusting their p-values using Hochberg's method. The conclusions of this investigation must be interpreted taking into account the fact that the data do not come from an RCT.

Secondary outcomes:

Sirolimus dosing: Mean plasma levels achieved at steady state during the treated period, and the mean doses required to achieve the target plasma concentration for each age group will be calculated to characterize pharmacokinetics in this patient population and ultimately help identify optimal future dosing regimens.

Growth measurements: Means, variances, and correlations and graphical displays will be presented of change in the observation period, change in the treatment period, and difference between the changes, for each different methodology employed for measuring growth (DXA, MRI and circumferential measurements). These will also help to inform whether inter-patient comparison will be feasible in a future RCT. "Broken stick" models may be applied to these data as well.

8. Description of potential benefits of study:

8.1. Direct benefits to participants

The therapy may reduce or stop overgrowth, which is the cause of most of the patients' morbidity and need for surgical intervention may be reduced or eliminated.

8.2. Collateral benefit to participants

The potential direct benefit will be the reduction or reversal of overgrowth by the drug treatment. The indirect or collateral benefits of the study are substantial and include evaluation in a center where the physicians have had experience with *PIK3CA*-related overgrowth conditions, therapeutic interventions, and medical, genetic, and psychosocial counseling. Subjects may benefit from our diagnostic opinion as part of the eligibility determination.

8.3. Benefits to society

As mentioned in the Introduction, because sirolimus is an FDA-approved drug, it is currently being prescribed to patients with PIK3CA-related overgrowth without an opportunity to systematically collect safety and efficacy data. Expanding our understanding of how sirolimus may reduce

overgrowth in these patients in a controlled manner that allows for monitoring for side effects and other adverse events may benefit many patients with this disorder.

9. Description of likelihood and seriousness of harms and how safety will be maximized:

9.1. Therapeutic interventions

The greatest risks of this protocol are associated with the administration of Sirolimus. Sirolimus is approved for use as an immunosuppressive agent indicated for the prophylaxis of organ rejection in patients receiving renal transplants, where the drug dosage is approximately 4-10 times greater than the proposed dosing for this study, and the resultant target trough level (5-15 ng/ml) is 2-5 times greater than our proposed target level (2- 4 ng/ml). The most common (>30%) adverse reactions with this higher dosage and target trough level are: peripheral edema, hypertriglyceridemia, hypertension, hypercholesterolemia, creatinine increased, abdominal pain, diarrhea, headache, urinary tract infection, anemia, nausea arthralgia, pain and thrombocytopenia. These side effects are related to drug concentration and were improved with maintenance of sirolimus level between 10 to 20 ng/ml, which is approximately 5-10 times greater than the proposed level for this study; therefore, the risk of developing these side effects may be low.

Kasap et al. [2011] reviewed the side effects of Sirolimus use in pediatric renal transplantation. The reported adverse events were based upon 16 different studies with dosage ranging from 3 mg/m2 once daily to 2-10 mg/m2 divided BID. Reported adverse events included:

1) Myelosuppression: anemia, leukopenia, and thrombocytopenia were common adverse effects, which may lead to withdrawal of the drug. Twenty-five to 55% of pediatric RTR on sirolimus treatment with anemia have required erythropoietin therapy to sustain adequate erythrocyte counts. Leukopenia and thrombocytopenia are much less frequent adverse effects with an incidence of 4-7% and 3-7%, respectively. Thrombocytopenia in association with deteriorating renal function should alert the clinician to the possibility of TMA or HUS.
2) Hyperlipidemia: increases in triglycerides, LDL-cholesterol, and HDL-cholesterol levels are dependent on dose of sirolimus. Hyperlipidemia occurs in 40% of patients under sirolimus treatment, reaching maximum levels during the second or third post-treatment months. In pediatric renal transplant patients, it has been reported in a range of 10-61%. The treatment of choice is usually HMG-CoA reductase inhibitors (statins) and fibric acid derivatives (fibrates).
3) Nephrotoxicity (proteinuria). Sirolimus has traditionally been considered as a non-nephrotoxic drug. However, mean serum creatinine levels may increase after sirolimus introduction in those with underlying kidney disease (renal transplant, and glomerulonephritis patients). It may cause proteinuria in adults and children treated with sirolimus. Improvement of proteinuria after sirolimus withdrawl has suggested direct association of proteinuria with the drug.
4) Impaired wound healing: may be seen in the early phases of treatment (including lymphoceles, perigraft fluid collections in renal transplant patients).
5) Mouth ulcers/mucosal ulcers: is a well-described side effect seen in ~60% of patients, who are over immunosuppressed. Treatment has included administration of coated tablet forms instead of liquid forms, advance oral hygienic care, and the use of antiseptic rinses.
6) Liver function abnormalities: mild elevations of liver enzymes may be observed in patients receiving sirolimus therapy and usually respond to dose reduction or discontinuation.
7) Edema/angioedema: local edema of the legs, eyelids, face, upper extremities and the whole body as well as angioedema has been reported with sirolimus treatment. The mechanism of

edema has been associated with lymphatic obstruction rather than vascular obstruction. Angioedema is a self-limiting swelling in the deeper cutaneous and mucous membranes, and may be observed with an incidence of 15%. Neither edema nor angioedema has been reported in children.

8) Decreased testosterone levels: suppression of testosterone levels and increase in LH as long with disrupted spermatogenesis and an opposite increase in FSH has been reported in adult renal transplant patients. No study determining effect of sirolimus on testosterone levels in children or adolescents has been published.
9) Interstitial pneumonia: interstitial pneumonitis has been reported in both children and adults, and is associated with fever, dyspnea, fatigue, non-productive cough and occasional hemoptysis. X-ray and CT scan findings are bilateral patchy, ground-glass interstitial infiltrates in both lower lobes. These cases have been idiosyncratic with no identified infectious etiology. In some cases the interstitial lung disease has resolved after discontinuation of the drug or dose reduction of the sirolimus. The risk may be increased as the trough sirolimus concentration increases (this has been above the trough levels for immunosuppression).
10) Arthalgia: an occasional side effect of sirolimus, which is treated by supportive care and dose reduction.
11) Growth deficiency: has been reported in animal studies of rat pups, who had impaired longitudinal growth rate with a reduction of about 50% and distortion of the growth-plate structure. The growth velocity in children treated with sirolimus for a median of 10 years reported no change in 8 pediatric liver transplant recipients. In several other studies, there was no difference in growth. However, linear growth of pediatric patients on sirolimus should be monitored.

Our UK collaborators have treated a single adult patient with severe overgrowth of her legs caused by a mosaic *PI3KCA* mutation with low dose sirolimus off-label on clinical grounds for one year. This has been achieved with a low dose of 8mcg/kg/24hrs (1mg once daily), avoiding significant side effects.

Blood chemistries, histories and physical examinations obtained on every NIHCC visit will help to detect any potential, unforeseen, or chronic toxicity of sirolimus. Sirolimus should be re-taken if vomiting occurs within 15 minutes of taking the dose, but not if vomiting occurs more than 15 minutes after taking sirolimus. Patients or their parents/guardians will keep a diary to document the intake of each dose of sirolimus and potential side effects (Appendix P). The patient diary will be reviewed with the patient's family at each required clinical study evaluation and participants will be asked to submit sirolimus diaries on a weekly basis when not at the NIH for a study visit. In addition, leftover study medication will be collected at each on study evaluation, with missed doses accounted for at this time. In addition, specific questioning about side effects will be performed on every admission by the PI and AIs.

Grading of toxicities associated with sirolimus therapy will be determined by the CTCAE criteria version 4.0 (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE4.03_2010-06-14_QuickReference_8.5X11.pdf).
Patients who develop greater than Grade 2 toxicities (i.e. Grade 3, 4, or 5 toxicities) that are probably or certainly related to sirolimus will have the sirolimus stopped and the patient will continue to be followed as per the protocol. Patients who develop Grade 2 toxicity that is probably or certainly related to sirolimus, will have sirolimus blood levels checked; if the level is in the 2-6 ng/ml (target level), sirolimus will be stopped, and the patient will be followed as per the protocol. If the level is >6 ng/ml, the investigators will stop the drug, but will monitor the patient for 2 weeks; if the toxicity resolves and the patient improves to baseline, then the sirolimus will be restarted at a lower dose, and levels will be checked at 4 days after restarting the sirolimus to reach target

level of 2-6 ng/ml. If the toxicity does not resolve, levels will be re-drawn; if the level continues >6 ng/ml, the drug will be held for 2 more weeks, and levels redrawn. With redraw of sirolimus level is in the 2-6 ng/ml range, and the toxicity resolves, the drug will be restarted at a lower dose.

To ensure safety, an interval analysis will be performed if and when 5 Serious Adverse Events have occurred or after 5 patients have completed their 6 month course of treatment.

If the patient is withdrawn from the study for any reason, every attempt will be made to obtain a final evaluation that will include a history and physical examination, AE evaluation and documentation and determination of the reasons for early termination, and follow up laboratory, radiological and other consults will be obtained.

Testing for HCV/HBV/HIV serology could give rise to a previously unrecognized diagnosis. If this scenario were to arise, additional counseling would be provided.

9.2. Diagnostic interventions

Please refer to list of diagnostic interventions in Appendix B. The primary procedure- associated risk of this study consists of phlebotomy requirements, while are approximately 15-18 ml per visit and will never exceed 50 ml in any 8-week period, including blood drawn for ancillary tests. Discomforts include venipunctures. Patients with these segmental overgrowth disorders will routinely be asked to consider skin biopsies to obtain tissue for research and genetics analyses. The likelihood of complications from tissue sampling (phlebotomy, skin biopsy) is low, and if these occur, they are unlikely to be other than minor (bruising, infection, small scar, etc). Topical numbing cream will be provided to those who request it before either blood draw or administration of injected anesthetic prior to skin biopsy. (Note that the consent form prompts subjects to request this if they wish.)

In this study, imaging studies including MRI scan for volumetrics, DXA scan for body composition, will be done to provide outcome measures for the sirolimus treatment. Some patients may not be able to complete both the MRI and DXA scans because of physical size limitations or inability to stay stationary (because of age) in the case of the DXA scan. Other imaging would be clinically indicated. Some patients may require sedation for the MRI scan.

9.3. Radiation

As outlined in Section 5.2, pending approval from the NIH Radiation Safety Committee, we will employ dual-energy x-ray absorptiometry (DXA) body composition testing at the beginning of the observation period, when the treatment period commences, after 3 months of treatment, and after 6 months of treatment.

Participants in this protocol may undergo other evaluations involving radiation in this protocol but these will only be done if they are clinically indicated. For example, we may order a chest x-ray for a patient who we are concerned may have signs of a pulmonary complication.

9.4. Sedation

Sedation is a major risk of participation in this study and we will make an effort to avoid it if possible. Currently, we would plan to utilize sedation for volumetric MRIs only as the DXA scans are less susceptible to motion artifact. Because all participants will be co-enrolled on our natural history study, which employs volumetric MRI, we will be aware, of which participants can

successfully undergo MRI without sedation and anticipate instances in which sedation is likely to be necessary. All sedation is done with formal consultation and involvement of the NIH CC anesthesia staff.

9.5. Psychological harms

This study requires multiple visits to the NIHCC and this could pose a significant burden to families and affected individuals. We have made every attempt to minimize the time spent at the NIHCC and have made provisions for participants to have as many monitoring labs as possible done close to home. As well, the potential efficacy of sirolimus is promising, but by no means well-established and some participants may be disappointed if their overgrowth does not resolve in the way that they had hoped. The unknown efficacy and tolerability of side effects of sirolimus for any individual patient is a major focus of both the informed consent and genetic counseling sessions in this study.

9.6. Risks to family relationships

N/A

9.7. Discrimination

All subjects who are candidates for the study must have a diagnosis of an overgrowth syndrome to be eligible, so we will rarely make a new diagnosis and therefore should not increase discrimination on this basis.

10. Description of how privacy and confidentiality of medical information/biological specimens will be maximized

10.1 Will participant identifiers be attached to data, or will samples/data be coded or unlinked?

Clinical data on patients who come to the NIH will be primarily stored in the NIH clinical center charts.. These will be labeled with names and NIH MRNs and are subject to the CC policies of confidentiality. Data collected from subjects by survey or questionnaires will be stored in the NHGRI clinical file and in secure electronic databases (i.e. LabMatrix). Any computer files will be password protected and the notebooks will be kept as described elsewhere in the protocol.

Data and consent forms will be stored by the PI or Lead AI. Consent forms must include identifiers and are stored in a locked office or other secure facility for that reason. Patient data forms and sheets will either be stored in locked files or in files in a locked room or in a password secured database or spreadsheet.

10.2 Description of any clinical/demographic information that will be included.

Research records for any participant seen at the NIHCC will include complete description of phenotype and molecular data. Age, sex, ethnicity, basic demographic data will be similarly stored.

Data will be kept in locked files or other secure files by the PI. Also, some data may be kept in password controlled files and/or databases.

10.3 How might this information make specific individuals or families identifiable?

As all of the subjects of this protocol have an extremely rare diagnosis and the manifestations of the disorder are often unique, each subject may be identifiable. This is an unavoidable aspect of rare disease research. It is our policy to consult with the families prior to publication of facial photographs that may be recognizable.

10.4 If research data will be coded, how will access to the "key" for the code be limited? Include description of security measures (e.g., *password-protected database, other*). List names or positions of persons with access to the "key" for the code.

As mentioned above in Section 10.1, participant data are kept in both paper and electronic forms in locked filing cabinets or password protected databases. The following individuals have access to stored physical and electronic patient data: Kim Keppler-Noreuil, Leslie Biesecker, Julie Sapp, Molly Crenshaw, Jennifer Johnston, Marjorie Lindhurst, and Rachna Patel. In addition, we have two slots dedicated to a lab technician and a clinical support technician who have access to the code. Physical or electronic access to identifiable patient data are not available to non-NIH investigators.

10.5 Will pedigrees be published? Include description of measures to minimize the chance of identifying specific families.

N/A

10.7 Will personally identifiable information be released to third parties?

It is our policy not to release any information to third parties without the consent of the subject or his/her parents if the subject is a child.

10.8 Under what circumstances will data/samples be shared with other researchers or deposited in various repositories, biobanks, and/or databases voluntarily or as mandated by NIH policies (e.g. dbGaP)?

We plan to share data between the three centers: NIH, University of Cambridge, and University of Dijon from each of our ten patients, which will be analyzed by all three centers since our methodologies for the primary and secondary endpoints are the same. The data to be shared will be from the primary outcome measures, namely the MRI volumetric scans, and the DXA scans for whole body composition with be de-identified and shared between the centers. The raw data from the MRI volumetric scans at the NIH will be sent to the UK Center for analysis with their software, SliceOmatic® (http://www.tomovision.com/products/sliceomatic.html. The DXA scan data from the UK and France will be fully de-identified and sent in encrypted Excel sheets to the NIH for analysis by Dr. Kong Chen. Further, we will use the same quality of life measures, WHO-BREF- and PEDsQL for all three centers, which have been validated for English and French languages. The data from these measures will also be fully de-identified and sent in encrypted form to the NIH. Similarly, the results in identical format will be sent from the NIH to the UK and France centers and analyzed by the UK and French study teams. The data from these measures will be shared and analyzed at each center for comparison.

10.9 Describe any additional features to protect confidentiality.

N/A

11. Assessment of Risk/Benefit Ratio

PIK3CA-related overgrowth disorders can be severe and progressive and can cause significant morbidity. Currently, only surgical approaches can be employed to manage the significant overgrowth experienced by many patients and amputation of digits and limbs is common. As such, we believe that the prospect of non-surgical approaches to the management of this disorder is worth consideration, even in the context of a protocol that delivers very minimal radiation dose and employs a pharmacologic intervention. This study has greater than minimal risk because of the use of research radiation (although the exposure is minimal), and the use of sirolimus; however, the potential benefit of treatment outweighs the minimal risk. Risks of Sirolimus therapy as described under section 9.1, occur at the higher doses and trough levels, which are required for immunosuppressive therapy to prevent rejection in the setting of kidney transplant (its approved indication). At the lower doses in this study, the risk of serious effects of sirolimus are less likely (as described in section 9). Furthermore, we will stop treatment with sirolimus if the patient experiences greater than Grade 2 toxicities that are probably or certainly related to sirolimus and the patient will continue to be followed as per the protocol. Patients who develop Grade 2 toxicity that is probably or certainly related to sirolimus (as described in section 12), will have sirolimus blood levels checked; if the level is in the 2-6 ng/ml (target level), sirolimus will be stopped, and the patient will be followed as per the protocol. If the level is >6 ng/ml, the investigators will stop the drug, but will monitor the patient for 2 weeks; if the toxicity resolves and the patient improves to baseline, then the sirolimus will be restarted at a lower dose, and levels will be checked at 4 days after restarting the sirolimus to reach target level of 2-6 ng/ml. If the toxicity does not resolve, levels will be re-drawn; if the level continues >6 ng/ml, the drug will be held for 2 more weeks, and levels redrawn. With redraw of sirolimus level is in the 2-6 ng/ml range, and the toxicity resolves, the drug will be restarted at a lower dose.

12. Unanticipated Problems: Collection, monitoring, analysis and reporting of adverse events and protocol deviations

12.1. Describe all potential adverse events that can be anticipated and monitored for this protocol. If this is either a natural history or limited encounter protocol, explain this to the IRB and specify the occurrences that will be excluded from adverse event reporting.

The medical complications known to be associated with the PIK3CA-Related Overgrowth disorders will not be reported as adverse events. Anticipated adverse events that are known to be associated with sirolimus and that are Grade 1 or 2 will not be reported to the IRB unless two or more patients are affected from our total enrolled study group of ten patients. The common anticipated adverse events (reversible and usually not serious) usually are reported at greater than 20%. Those side effects that are likely (reversible and usually not serious) include: hyperlipidemia, hypercholesterolemia, anorexia, anemia, diarrhea, constipation, nausea, hypertension, stomatitis, rash, acne, arthralgias, peripheral edema, and mild to moderate headaches. Other examples of the expected adverse event include but are not limited to those events detailed in product labeling for sirolimus (See Appendix R) and in the protocol's risk section. The severity of organ and laboratory abnormalities is defined in the CTCAE(http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE4.03_2010-06 14_QuickReference_8.5X11.pdf). If the rate or severity of these events exceeds the rate or severity anticipated in the protocol or product labeling, the events will be classified and reported as though they are Unanticipated Problems.

12.2. Describe plan to monitor and report adverse events and protocol deviations, as outlined in SOP 16 (available at https://federation.nih.gov/ohsr/nih/index.php).

All adverse events (AEs) will be collected from the time of the study drug initiation to the 7-month follow up. All SAEs will be collected from the time informed consent is obtained to the 13-month visit. All AEs, whether observed by the Investigator, reported by the patient, from clinically significant laboratory findings, or other means, will be recorded in the patient's medical records and on the CRF, and reported. See Appendix Q for our Adverse Event Collection Form.

Adverse events will be monitored by phone calls or direct interviewing every week for the first two months of the study, monthly after that, and two weeks after the last study drug dose. Adverse events occurring at the NIH Clinical Center will be recorded in the medical records and in study documents. Nurses on the units and in outpatient setting will monitor excessive blood drawing by tabulating totals. In addition, patients will have easy access to the treatment team to report adverse events by phone or in person.

This monitoring period should provide adequate coverage for AEs related to our interventions. Adverse events will be recorded in clinical records and study documents. A laboratory abnormality should be reported as an adverse event if it requires an intervention. Interventions include, but are not limited to, discontinuation of treatment, dose reduction/delay, additional assessments, or concomitant treatment. In addition, any medically important laboratory abnormality may be reported as an adverse event at the discretion of the investigator. This could include a laboratory result for which there is no intervention, but the abnormal value suggests a disease or organ toxicity. For the purposes of this protocol all SAEs, and any Adverse Events that are of grade 3 or above will be reported to the trial safety office and will be further reported to the IRB, and the PROS Sirolimus Safety Review Committee, as may be appropriate and required. Adverse events, protocol deviations, unanticipated problems (UP), Unanticipated Adverse Device Effects (UADEs), serious adverse events, sponsor and serious, are defined as described in NIH HRPP SOP 16 ("Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations"). Unanticipated adverse events and serious protocol deviation will be reported in writing to the NHGRI Clinical Director (Dr. William Gahl) and to the chair of the NIHGRI IRB (or their designate) as soon as possible but not more than 7 days after the PI first learns of the event. Not serious unanticipated problems will be reported to the IRB and CD as soon as possible but not more than 14 days after the PI first learns of the event. Not serious protocol deviations will be reported to the IRB as soon as possible but not more than 14 days after the PI first learns of the event. Deaths will be reported to the Clinical Director within 7 days after the PI first learns of the event.

The PI will also report SAEs to the Pfizer as agreed upon in their Safety Reporting Reference manual. We will utilize the reporting forms requested by Pfizer (in Appendices W and X) in addition to the FDA Medwatch 3500A form (Appendix S).

This study will also have oversight by an NIH study monitor/ quality assurance (QA) auditors, Emily Huang and/or Lea Latham. She will review pilot clinical trial records every six months to whether we are complying with the protocol, including completion of consent forms, that each participant meets the eligibility criteria, and that safety tests were performed and properly evaluated.

12.3. Describe whether a Data Safety and Monitoring Board (DSMB) and/or any other additional monitoring measures will be used.

Data from this study will be reported to a standing PROS Sirolimus Study Safety Review Committee , which consists of the following members: Dr. Meryl Waldman, Dr. David Ng, Dr. Leslie Biesecker, and Dr. Kim Keppler-Noreuil. This committee will meet regularly every 3 months to review the patients' clinical progress during the 13 month study period. Additional meetings will be scheduled if an individual patient experiences a Grade 3 or higher adverse event. The PROS Sirolimus Study Safety Review Committee will tabulate and review the patient's symptoms and abnormal laboratory findings to determine course of action for clinical evaluation and whether the adverse event is probably or certainly related to sirolimus.

The PROS Sirolimus Study Safety Review Committee will decide whether to stop/pause the study, but two a priori pause rules will be in place. First, the study will be paused if any patient develops a Grade 4 adverse event in order to evaluate for an underlying or concurrent etiology. The pause will occur unless the events are of an accidental nature that could not reasonably be attributable to sirolimus. Second, if 5 serious adverse events occur, the study will be paused, the PROS Sirolimus Study Safety Review Committee will examine the data, and a determination will be made regarding whether to continue the study. A problem report will be generated and sent to the IRB for their consideration. A pause means that no new patients will be enrolled until the PROS Sirolimus Study Safety Review Committee lifts the pause; enrolled patients will discontinue sirolimus until the cause can be determined. In addition, individual patients who experience Grade 3 or higher adverse events that, after investigation, are probably or certainly related to sirolimus will be taken off the drug and followed as per the protocol. Patients who experience Grade 3 AEs may resume taking sirolimus if further investigation reveals that the AE was unrelated to sirolimus use. These decisions by the PROS Sirolimus Study Safety Review Committee and the PI will be reviewed with the IRB.

If a patient is terminated from the study for any reason, every attempt will be made to obtain a final evaluation that will include a history and physical examination, AE evaluation and documentation, and determination of the reasons for early termination, and follow-up laboratory testing and EKG.

13. Description of alternatives to participation

There are no approved drugs for this condition, and the alternative is not to participate in the study.

14. Description of Consent Process

14.1. Who will obtain consent *(PI, AIs)?*

The following individuals may obtain informed consent from participants and/or their guardians: Kim Keppler-Noreuil, Juile Sapp, Angela Wang, or Leslie Biesecker. Consent will be obtained in person at the NIHCC.

Patients who fulfill the inclusion criteria and express interest in participation will be provided with written information about the study (Appendix M) and the consent form prior to enrollment and consent. A member of the study team will discuss the trial in further detail by phone to confirm interest, eligibility and to answer questions.

The study will not include adults who are unable to consent for themselves, non-English-speaking adults or children under the age of 15 years whose guardians do not speak English, or vulnerable groups.

14.2. Setting where consent will be obtained

Consent will generally be obtained in the NIHCC as outlined above except when obtaining the consent of both parents/guardians of minor children. While we prefer to obtain consent from both parents or guardians of minor children, we will only require consent from one parent/guardian and only one parent/guardian will be required to accompany a child to NIHCC visits. When possible, we will obtain consent from the non-physically-present parent or guardian (when applicable) by telephone.

14.3. What information will be provided to participants?

Participants may access the information about the study posted on the web prior to enrollment (see Appendix L). Additionally, participants asked to consider undergoing research-indicated imaging procedures will receive the RSC pamphlet “An Introduction to Radiation for NIH Research Subjects” as part of the consent discussion.

As mentioned above, participants will be provided with both the consent form (Appendix N) and an information sheet describing the study (Appendix M) prior to their visit to the NIH to enroll.

14.4. Protections for participants who may be vulnerable to coercion or undue influences

Adults who are unable to provide informed consent are not eligible to participate in this protocol, as are pregnant women.

Children are eligible as described above. We believe that this protocol meets the 45CFR46 criteria for pediatric inclusion because the disease primarily affects children and the trial has been designed to meet the definition of greater than minimal risk but presenting the prospect of direct benefit to the individual subjects.

Minors will be asked to complete an adult research consent when they reach 18 years of age. If they do not complete the adult research consent form, they will be classified as a partial withdrawal. In the case of partial withdrawal, their specimens and data will be kept for future analyses, but they will not be re-contacted to request additional data or samples, or to recruit them into additional studies.

In cases where parents share joint legal custody for medical decision-making of a child (e.g., by a custody agreement or court order), it is NIH policy (OHSRP SOP 14D) that both parents must give their permission regardless of the risk level of the research. There are limited exceptions when one parent has since died, become incompetent, or is not reasonably available (e.g., in prison). Consent from the second parent can be obtained by telephone per NIH SOP 12.

Consent and assent discussions will follow standard guidelines for developmentally specific explanations of the study and the risks and benefits. The investigator and the family will determine if the child is capable of understanding a simple and clear explanation of the study. The parents will be asked for input based on the child's cognitive abilities and maturity. Parents should be familiar with this determination because most of the children eligible for the study will have been subjected to previous evaluations or procedures that required explanation. For these reasons we

believe that the parents will be familiar with the concept of assent and will be able to make an informed decision based on experience. The criteria for this decision are difficult to define in advance but should be straightforward to make in individual cases. In cases where the investigator believes the family decision is not reasonable the enrollment will be declined. The consent forms explain the concept of assent and record an agreement with the parents that assent is or is not appropriate, and if appropriate, was obtained. Please see Appendix O for the Assent Form.

14.5. Are there special circumstances regarding obtaining *consent?*

For children enrolled in this protocol, we will require informed consent from one parents/guardians consistent with HRPP procedures. As outlined above in Section 14.2, we do prefer to involve both parents/guardians in consent discussions, however, we will only require one to consent in person at NIH; we will provide the other parent with the form, and have a telephone informed consent discussion. Older adolescents (15-17-year-olds) whose parents speak primarily Spanish are eligible for participation. As such, we will have a certified Spanish translation of the consent document available so that Spanish-speaking parents or guardians may consent on their child's behalf.

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