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A Pilot Study of Sirolimus (Rapamycin, Rapammune®) in Subjects with Cowden Syndrome or other syndromes characterized by germline mutations in PTEN.

Abb. Title: Sirolimus in subjects with CS

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Drug Name: Sirolimus, commercial, (Rapamune®, Wyeth Pharmaceuticals; Philadelphia, PA)

PRÉCIS

Background:

- PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a tumor suppressor gene whose function is frequently lost through genetic and epigenetic mechanisms in cancer. Loss of PTEN increases activation of the PI3K/Akt/mTOR pathway, which increases cellular proliferation and survival.
- Germline mutations in PTEN are associated with a number of hamartomatous syndromes, of which Cowden Syndrome (CS) is the prototype. The set of syndromes that are defined by germline PTEN mutations has been labeled PTEN Hamartomatous Tumor Syndromes or PHTS.
- Patients with PHTS suffer increased morbidity and mortality. Benign tumors such as hamartomas occur in virtually every organ, most commonly in the skin and the gastrointestinal tract, which prompts frequent monitoring and resection and causes psychological and physical stressors on patients with this condition.
- CS patients develop thyroid, breast, and endometrial cancers at an earlier age than the general population, and have an overall increased incidence of these cancers compared to the general population. These patients have increased morbidity from heightened surveillance and diagnostic procedures.
- No medical therapies exist for PHTS patients.
- Because tumors from PHTS patients show increased activation of the PI3K/Akt/mTOR pathway, inhibitors of this pathway might have activity in patients with PHTS.
- Sirolimus (rapamycin) is a specific inhibitor of mTOR that is FDA-approved and is preferentially effective in cells with mutant PTEN.
- We hypothesize that sirolimus will have activity in patients with PHTS, as measured by biochemical techniques that will assess mTOR inhibition and clinical tests that will assess the growth and metabolism of benign and malignant tumors.

Objectives:

- The primary endpoint will be inhibition of the mTOR pathway in tissues obtained before and after therapy, as assessed using immunohistochemistry in benign as well as malignant tumors.
- Secondary endpoints will include inhibition of the mTOR pathway in PBMCs as assessed by immunoblotting, changes and duration of change in benign or malignant tumor size as assessed by CT, serial digital photography, digital dermoscopy, changes in tumor metabolism as assessed by PET, changes in lymphocyte counts, as well as changes in neuropsychological testing.

Eligibility:

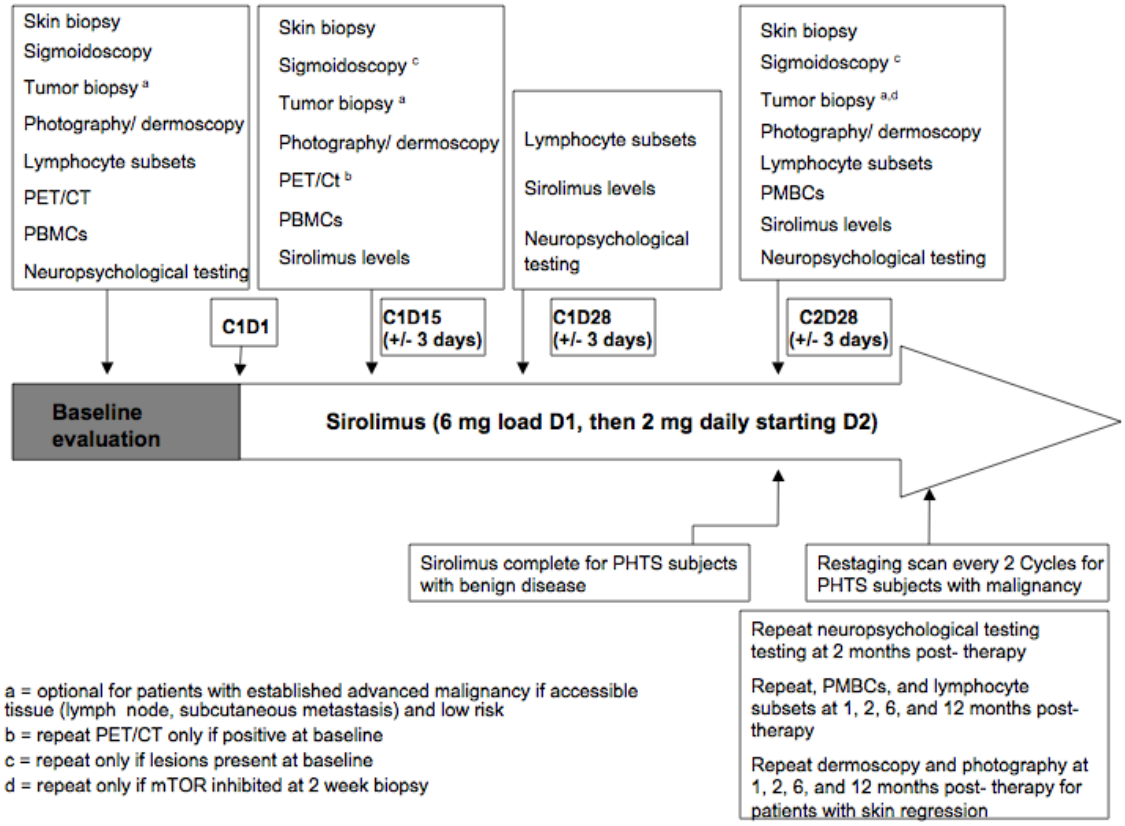
- Adult subjects with documented germline PTEN mutations who meet diagnostic criteria for Cowden Syndrome by international criteria.

Design:

- Subjects will undergo biopsy, imaging, photography, dermoscopy, and neuropsychological testing prior to and after a course of therapy with sirolimus to assess the efficacy of treatment.
- This pilot protocol will test sirolimus at an FDA-approved dose (6 mg PO loading dose/ 2 mg PO daily) in a group of twenty patients.

- Treatment will last for 56 days (plus 2-3 days to allow flexibility for scheduling of follow-up procedures) for PHTS subjects with benign hamartomatous tumors.
- For PHTS subjects with established malignancy, measurement of disease will be performed every other cycle and treatment will continue until disease progression or unacceptable toxicity.

Schema



Note: Effective with Amendment H, the 6- and 12-month post-therapy follow-up time points were discontinued.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary objective

1.1.1.1 To determine whether sirolimus, an mTOR inhibitor, will alter the activation status of components in the PTEN/Akt/mTOR pathway in benign and malignant tissues derived from PHTS subjects.

1.1.2 Secondary objectives

1.1.2.1 To explore inhibition of the mTOR pathway in PBMCs,

1.1.2.2 To explore inhibition of benign and malignant tumor growth and/or induction and duration of tumor regression using serial dermoscopy, photography, PET and CT scans

1.1.2.3 To evaluate for changes in lymphocyte counts,

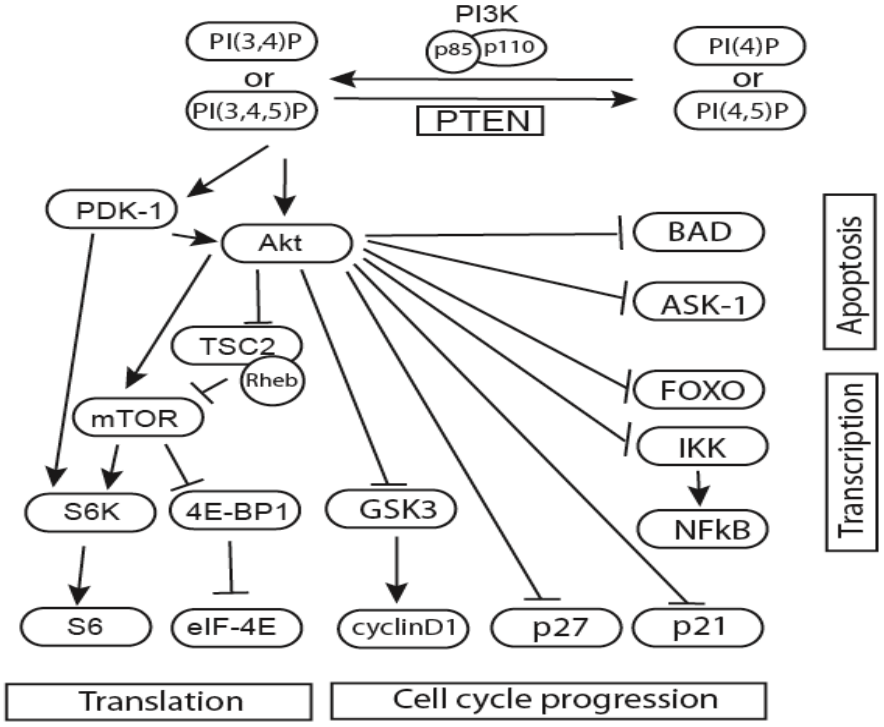
1.1.2.4 To evaluate possible changes in neuropsychological testing in subjects with PHTS after treatment with sirolimus.

1.2 BACKGROUND & RATIONALE

1.2.1 General

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a tumor suppressor gene located on chromosome 10q22–23. PTEN functions as a dual protein and lipid phosphatase, though its tumor suppressor activity is dependent upon its lipid phosphatase activity, which negatively regulates the PI3K/Akt/mTOR pathway (Figure 1). Loss of PTEN function occurs through genetic and epigenetic alterations and results in activation of this pathway.

Figure 1: The PTEN/Akt/mTOR Pathway. PTEN and PI3K act reciprocally to regulate the level of 3' phosphoinositides, which leads to activation of Akt. Akt then phosphorylates a number of substrates that control many important cellular processes. mTOR is a critical downstream component of Akt and propagates its signal to S6K, S6, and 4E-BP-1.



Uncontrolled activation of this pathway contributes to cellular transformation, as well as increased proliferation and/or survival of cancer cells. Somatic mutations or silencing of PTEN have been observed in many types of cancer, including glioblastoma multiforme, breast cancer, lung cancer, endometrial cancer, prostate cancer, renal cancer, melanoma, hepatocellular carcinoma, and microsatellite unstable colorectal cancers [1, 2].

PTEN hamartomatous tumor syndromes (PHTS) are a collection of syndromes characterized by germline mutations in PTEN, and include Cowden syndrome (CS), Lhermitte–Duclos disease (LD), Bannayan–Riley–Ruvalcaba syndrome (BRRS), Proteus syndrome (PS), and Proteus-like syndrome (PLS) (Table 1). Currently, the prevalence of detectable PTEN germline mutations in these syndromes is 80 percent for CS patients, 83 percent for LD patients, 60 percent for BRRS patients, 50 percent for PLS patients, and 20 percent for PS patients [3, 4]. Although not all patients with these syndromes have defined germline PTEN mutations, there may be other changes that occur at the gene or protein level that account for the remainder of PHTS, and the prevalence of non-mutational alterations in PTEN will undoubtedly increase with the availability of assays to detect such changes. Additionally, the genomic region that contains PTEN on chromosome 10q23 is a site of frequent loss of heterozygosity (LOH) in many human cancers [5]. Although early genetic studies did not find any genetic heterogeneity among BRRS and CS patients, subsequent studies have suggested rare locus heterogeneity that accounts for the majority of patients for whom genetic mutations were not initially described [6].

The nature of PTEN mutations may be specific for each syndrome in PHTS. For CS patients, two-thirds of the mutations have been mapped to exons 5, 7, and 8, with exon 5 (encoding the phosphatase core motif) accounting for 38 percent of the mutations [7]. Patients with adult-onset LD are likely to have PTEN mutations; however, PTEN mutations have not been described in childhood-onset LD [8, 9]. In BRRS, the PTEN mutation is allelic to CS, with patients having BRRS and CS at the same time manifesting with deletions or rearrangements involving PTEN [6, 10, 11]. The role of PTEN mutations in PS is less clear, because the defining characteristics of PS are controversial [12].

PHTS patients display greater morbidity and mortality rates than the general population, and commonly exhibit developmental delay and neurologic deficits. Most patients develop hamartomas in tissues derived from all embryonic germ cell layers by their late twenties, and CS and BRRS patients develop thyroid and breast cancers at an earlier age and with greater incidence than the general population [13, 14]. PS patients are at increased risk for premature death, as well as an increased rate of tumor occurrence [12]. These rare patients with germline PTEN loss have an increased risk of developing cancer compared with the general population, and provide a unique opportunity to study PTEN function in non-neoplastic and neoplastic tissues. Thorough analysis of tissues from these patients could identify new therapeutic targets, and could provide insight into the processes of benign growth and malignant transformation that is directly or indirectly related to loss of PTEN function.

1.2.2 PTEN biology

PTEN (also known as MMAC1 for mutated in multiple advanced cancers, or TEP1 for TGF-β regulated and epithelial cell-enriched phosphatase) acts as a tumor suppressor phosphatase gene by negatively regulating the PI3K/Akt/mTOR pathway. PTEN dephosphorylates the 3'-phosphoinositide products of PI3K, causing conversion of phosphatidylinositol (3,4,5) trisphosphate to phosphatidylinositol (4,5) bisphosphate and conversion of phosphatidylinositol (3,4) bisphosphate to phosphatidylinositol (4) phosphate. Reduction of 3´-phosphoinositides decreases activity of kinases downstream of PI3K such as phosphoinositide dependent kinase 1 (PDK-1), Akt, mTOR, and ribosomal protein s6 kinase 1 (S6K1). PTEN activity can also indirectly decrease phosphorylation of other substrates downstream of Akt such as p27, p21, GSK-3, Bad, ASK-1, as well as members of the forkhead transcription factor family (e.g., AFX, FKHR, FKHRL1). Thus, a loss or reduction in PTEN activity leads to increased phosphorylation of many key cellular proteins, which in turn can affect processes such as cell cycle progression, metabolism, migration, apoptosis, transcription, and translation.

Although PTEN's role as a tumor suppressor is dependent upon its lipid phosphatase activity, several studies have documented other activities for PTEN that are independent of its lipid phosphatase activity. For example, the protein phosphatase activity of PTEN can regulate cell survival pathways via utilization of the mitogen-activated protein kinase (MAPK) pathway [15, 16]. Moreover, Shen et al. recently showed that PTEN can promote chromosomal stability independent of its lipid phosphatase activity [17]. Targeted mutations of PTEN in its carboxy-terminal domain, but not its phosphatase domain, caused centromere breakage and chromosomal translocations [17]. Because mutations can occur throughout the PTEN gene in PHTS patients, it is possible that chromosomal instability is characteristic of tumors derived from PHTS patients that have mutations in the carboxy terminus.

PTEN function is commonly lost in human cancers through somatic mutations, gene silencing, or mismatch repair deficiency [2, 18, 19]. Somatic mutations have included missense, truncating, deletion, and promoter mutations, as well as mutations within the phosphatase core

motif. While several mechanisms can occur within one tumor type, there tends to be a predominance of one mechanism within a specific tumor. PTEN protein levels are regulated by its phosphorylation and ubiquitin-mediated proteasomal degradation [20-23]. Phosphorylation and subsequent caspase-mediated cleavage of PTEN in its C-terminus has been shown to reduce PTEN protein stability as well as its ability to interact with scaffolding proteins [21]. Recently, Wang et al. have identified HECT-domain protein NEDD4-1 as a ubiquitin ligase for PTEN, which can negatively regulate PTEN stability by catalyzing its polyubiquitination and subsequent degradation by the proteasome [24]. Interestingly, PTEN polyubiquitnation leads to cytoplasmic degradation, while monoubiquitination is essential for its import into the nucleus [23]. Whether ubiquitination of PTEN could be a mechanism of its inactivation in PHTS has not been explored.

The role of PTEN in tumorigenesis has been best demonstrated in mice that have been genetically engineered to lose one or both copies of PTEN. A crucial role for PTEN during embryogenesis was revealed when complete inactivation of both copies of the gene before embryonic day 13.5 resulted in embryonic death due to aberrant differentiation and patterning of the germ layers. Mutant embryos showed poorly organized ectodermal and mesodermal layers, and overgrowth of the cephalic and caudal regions [25, 26]. Monoallelic inactivation, however, results in PTEN heterozygous knockout mice that are capable of completing embryogenesis.

Many features observed in PTEN heterozygous knockout mice are similar to those seen in PHTS syndromes. PTEN+/- mice develop neoplasms in many organs, including the liver, prostate, endometrium, GI tract, thymus, and thyroid [27]. Specifically, several mice developed follicular or papillary thyroid adenocarcinoma, and 100% percent of the female PTEN+/- mice developed multifocal complex atypical hyperplasia of the endometrium, compared with a single focus of complex atypical hyperplasia in 8% of their wild-type littermates. In addition, clusters of intestinal polyps and large nodal masses representing significant lymph node hyperplasia were found in all PTEN+/- animals, but not in any of their wild-type littermates. A similar study by Luo and colleagues revealed intestinal polyps, epithelial neoplasia, as well as lymphocyte hyperplasia in PTEN+/- mice that was accompanied by Akt activation [28]. Using the opposite approach, several groups have demonstrated that reconstitution of wild-type PTEN into a mutant PTEN background decreases activation of the PI3K/Akt/mTOR pathway, alters cell cycle distribution, and/or induces apoptosis [29-33].

To bypass the issue of embryonic lethality, Trotman et al. generated a hypomorphic (i.e., loss of function) PTEN mutant in order to examine the 'dose-dependency' of PTEN in the prostate [34]. These mice express lower levels of PTEN than PTEN+/- mice, but do not quite constitute a PTEN null scenario. PTEN hypomorphic mutant mice develop extremely high levels of prostate hyperplasia and overt invasive prostate cancers. PTEN+/- mice also develop prostatic intraepithelial neoplasias, but these lesions occur in less than half of PTEN+/- mice after long latencies, and never progress to invasive prostate cancers, indicating that the relative expression levels of PTEN govern cancer progression in the prostate. Another group used conditional inactivation of PTEN in the thyroid gland to investigate the role of PTEN in thyroid function and disease, and found that these mice developed diffuse multinodular goiter and follicular thyroid adenomas with complete penetrance [35]. Although loss of PTEN did not result in any invasive thyroid cancers, it did significantly increase the thyroid proliferative index in all mice, raising the possibility that the hyperplastic follicular adenomas might eventually undergo full transformation.

Complex tumor phenotypes can occur in mice when PTEN loss is coupled to other genetic changes. For example, when mutant K-Ras was overexpressed concurrently with deletion of PTEN in the ovarian surface epithelium, invasive and metastatic endometrioid ovarian adenocarcinomas developed with full penetrance within 7 weeks [36]. Loss of INK4A/Arf combined with monoallelic loss of PTEN caused prostatic intraepithelial neoplastic lesions (PIN), endometrial

hyperplasia, and pheochromocytomas [37], while astrocytomas resulted from PTEN loss in conjunction with loss of tumor suppressor gene Rb in astrocytes [38]. Overexpression of Wnt-1, an oncogene, with simultaneous loss of PTEN resulted in earlier development of mammary gland ductal carcinomas compared to Wnt-1 overexpression in a PTEN wild-type background [39]. These studies demonstrate that the phenotypic manifestation of loss of PTEN varies with the context of other changes in tumor suppressor genes and/or oncogenes.

1.2.3 PTEN Hamartomatous Tumor Syndromes (PHTS)

Germline mutations in PTEN also occur, and are associated with a number of hamartomatous syndromes, of which Cowden Syndrome is the prototype. The collection of syndromes that are defined by germline PTEN mutations has been labeled PTEN Hamartomatous Tumor Syndromes or PHTS. PHTS includes Cowden Syndrome (CS), Lhermitte-Duclos disease (LD), Bannayan-Riley-Ruvalcaba Syndrome (BRRS), Proteus syndrome (PS), and Proteus-like syndrome (PLS), although not all patients with these syndromes have defined germline PTEN mutations. The prevalence of PTEN germline mutations in these syndromes is 80% for CS patients, 83% for LD patients, 60% for BRRS patients, 50% for PLS patients, and 20% for PS patients [10, 40].

1.2.4 Cowden Syndrome (CS)

CS was labeled as such after the family name of the first patient described with the disease by Lloyd and Dennis in 1963 [41]. CS was considered a rare entity with an estimated incidence of 1 in 1,000,000. However, once the susceptibility gene PTEN was identified, the estimated incidence of CS increased to 1 in 200,000. Nonetheless, this is likely still an underestimation, owing to a high degree of phenotypic variability that leads to under-diagnosis [42].

CS is an autosomal dominant multiple hamartomatous syndrome with age-related penetrance, characterized by trichilemmomas (hamartomas of the infundibulum of the hair follicle), mucocutaneous papillomatous papules, macrocephaly, and an increased risk of breast, thyroid, endometrial, and cerebellar tumors. Hamartomas are a histologically distinct subtype of benign tumors in which the cells maintain normal differentiation but are disorganized with respect to architecture. Hamartomas can be found in virtually any tissue. For example, hamartomas ranging between 1 mm to 6 mm in size were recently noted in the testes of males diagnosed with CS [43]. Ninety-nine percent of CS patients eventually develop these types of growths, and most patients will present with hamartomas by their early twenties.

Breast and thyroid cancers occur in two-thirds of cases of CS. The lifetime risk for breast cancer in women with CS is estimated to be as high as 50 percent, compared with 11 percent in the general population. CS patients typically manifest breast cancer in their forties with adenocarcinoma being the predominant histology [13, 14, 44]. Male breast cancer is also observed with increased incidence in CS. Lifetime risk of thyroid cancer in CS patients is as high as 10 percent, which is also significantly higher than the general population. There is a trend toward an earlier age of diagnosis with thyroid cancer, and the predominant histology is follicular carcinoma [7, 13].

Table 1 . International Cowden Consortium Diagnostic Criteria

Pathognomonic Criteria	Major Criteria	Minor criteria
Adult-Lhermitte-Duclos disease (LDD)	Breast carcinoma	Other thyroid lesions (adenoma or multinodular goiter)
Mucocutaneous lesions: Facial trichilemmomas Acral keratoses	Thyroid carcinoma (especially follicular)	Mental retardation (IQ $\leq$ 75)
	Macrocephaly (occipital frontal circumference $\geq 97^{th}$ percentile)	GI hamartomas

Papillomatous lesions	Endometrial carcinoma	Lipomas
		Fibrocystic disease of the breast
		Uterine Fibroids
		Fibromas
		GU tumors or malformations
Operational diagnosis		
Mucocutaneous lesions alone in conjunction with: - ≥6 facial papules (≥3 trichilemmomas) or - Cutaneous facial papules and oral mucosal papillomatosis or - Oral mucosal papillomatosis and acral keratoses or - ≥6 palmo-plantar keratoses Two or more major criteria One major criteria and three minor criteria Four minor criteria For individuals in a family in which one relative is diagnostic for CS: • A pathognomonic criterion • Any one major criteria with or without minor criteria • Two minor criteria • History of BRRS		

In 1995, the International Cowden Consortium, which represents a group of centers mainly in North America and Europe, established a set of operational diagnostic criteria from published data and expert opinions [45]. The International Cowden Consortium Criteria was revised in 2004 in light of increased knowledge of the molecular biology of CS (Table 1) [46].

1.2.5 Lhermitte-Duclos disease (LD)

LD, or dysplastic gangliocytoma of the cerebellum, was recently recognized as a phenotypic variance of CS and is now a major diagnostic criterion of CS. The clinical presentation of patients with LD includes ataxia, increased intracranial pressure, and seizures, all attributable to hamartomatous cerebellar tumors. Immunohistochemical analysis of tissues from patients with adult-onset LD has demonstrated activation of the PI3K/Akt/mTOR pathway. However, pathogenesis may be different in the childhood variant of LD, as the pathway was not constitutively active in these cases [47].

1.2.6 Bannayan-Riley-Ruvalcaba Syndrome (BRRS)

BRRS is an autosomal dominant condition that was initially reported by Riley and Smith, but was further refined by Bannayan, Zonana, and Ruvalcaba [48-50]. It is also known as Bannayan–Zonana syndrome (BZS), Ruvalcaba–Mhyre syndrome, and Riley–Smith syndrome. BRRS is characterized by macrocephaly, developmental delay, intestinal hamartomas, lipomatosis, hemangiomatosis, and can be distinguished from other PHTS by the presence of a speckled penis in male patients. Macrocephaly is the predominant feature. In addition, patients with BRRS usually have hypotonia, delayed psychomotor development, mental retardation, and seizures. Ocular abnormalities such as prominent Schwalbe lines and corneal nerves, lipid storage myopathy, and skeletal abnormalities such as joint hyperextensibility, pectus excavatum, and scoliosis are also commonly associated with patients with BRRS [51]. There is no clear increase of cancers in BRRS; however, any increase in incidence may be masked by the fact that BRRS patients have a limited

lifespan secondary to other comorbidities. PTEN loss in BRRS was delineated by Marsh *et al.* [4, 10, 52]. The clinical similarities between BRRS and CS have prompted the suggestion that both patient populations be managed similarly with regard to their cancer surveillance [7].

1.2.7 Proteus syndrome and Proteus-like syndrome

Proteus syndrome (PS) was originally described by Cohen and Hayden in 1979, and was given its name by Wiedemann, after the Greek sea god known for his ability to change his shape [53, 54]. PS is a rare disorder with about 100 cases reported in the medical literature. PS is a highly variable disorder that affects patients in a mosaic pattern and is characterized by congenital malformations, overgrowth of multiple tissues including connective tissue, fatty tissue, and epidermal nevi, and hyperostosis, a disproportionate asymmetric overgrowth of the skeleton. While several authors have reported that PS patients carry PTEN mutations [3, 12, 55], this remains controversial, as at least two-thirds of patients labeled as having PS did not meet established diagnostic Proteus criteria when assessed for enrollment in the NIH Proteus Research Study [12, 56]. In a study by Turner and colleagues, only 47.3 percent of 205 cases in the literature had 'bona fide' PS [57]. Thus far, 34 cases of 'indisputable' PS patients have been studied for PTEN mutations with negative results; nonetheless, a conclusive answer regarding PTEN mutations and PS awaits a better understanding of the molecular basis for this syndrome.

Zhou *et al.* were first to demonstrate that PLS was associated with PTEN mutations, and characterized PTEN mutations in six patients with PS-like manifestations, one of whom did not meet diagnostic criteria for PS and was designated as having PLS [3]. This patient with PLS was identified to have a germline PTEN R335X mutation in one allele. Additional biopsies from this patient that included a lipoma, an epidermoid nevus, and an arteriovenous malformation were all found to carry an R130X mutation on the second allele [58]. Both mutations have been described in other patients with CS and/or BRRS. When the other patients in this study were examined, five other patients had the same R335X mutation and a third had a M35T mutation. These studies suggest that PTEN is involved in PLS in some cases.

1.2.8 Clinical management of patients with PHTS

Patients with PHTS suffer increased morbidity and mortality. Developmental delay and neurologic deficits are commonly observed. The presence of hamartomas in multiple tissues, including the gastrointestinal tract, endometrium, thyroid, and skin is common and often results in significant anxiety and repetitive procedures and biopsies, which causes significant morbidity and decreases quality of life. Patients with PHTS often have benign hamartomatous lesions covering their entire gastrointestinal tract, which is frequently confused with familial adenomatous polyposis or other serious conditions. Although it has never been demonstrated that patients with PHTS are at increased risk for GI malignancy, in the care of their outside providers, PHTS patients are subject to frequent upper and lower endoscopies and numerous random biopsies, which is in excess of the screening guidelines advocated by the NCCN in 2007 (see table 2). Thus, depending on the awareness of medical professionals of the manifestations of this syndrome, PHTS patients may not receive optimal care.

1.2.9 Screening

While the phenotypic presentation of PHTS is variable, screening of asymptomatic patients could reduce their risk of developing malignancies, especially in CS patients. The likelihood that a CS patient will develop breast, thyroid, or endometrial cancer in his or her lifetime is 30–50%, [13, 59], 10%, and 5–10% [13, 46], respectively. Additionally, renal neoplasms, melanomas, and gangliocytomas of the cerebellum can occur in these patients. Given their susceptibility to these

potentially life-threatening malignancies, vigilant screening of these patients might prove beneficial in their long-term medical care. The current guidelines for screening and management are shown in table 2.

Table 2: Management guidelines for Cowden Syndrome advocated by NCCN

WOMEN	MEN AND WOMEN
• Training and education in breast self-exam and regular monthly BSE starting at age 18 y	• Annual comprehensive physical exam starting at age 18 y or 5 y younger than the youngest age of diagnosis of a component cancer in the family (whichever is younger), with particular attention to breast and thyroid exam
• Semiannual clinical breast exam starting at age 25 y or 5-10 y earlier than earliest known breast cancer in the family	• Annual urinalysis; consider annual urine cytology and renal ultrasound, if family history of renal cancer
• Annual mammography and breast MRI screening starting at age 30-35 y or 5-10 y earlier than earliest known breast cancer in the family (whichever is earlier)[1, 2]	• Baseline thyroid ultrasound at age 18 y, and consider annually thereafter
• Blind endometrial aspiration biopsies annually for premenopausal women starting at age 35-40, or 5 y before earliest diagnosis of endometrial cancer in the family, and annual endometrial ultrasound in postmenopausal women.	• Education regarding the signs and symptoms of cancer
• Discuss options for risk-reducing mastectomy on case-by-case basis and counsel regarding degree of protection, extent of cancer risk, and reconstruction options.	• Consider annual dermatologic exam
	RISK TO RELATIVES
	• Advise about possible inherited cancer risk to relatives and consideration of genetic consult and/or testing

[1]The appropriateness of imaging scheduling is still under study.
[2]High-quality breast MRI limitations include having: a need for a dedicated breast coil, the ability to perform biopsy under MRI guidances experienced radiologists in breast MRI, and regional availability.

1.2.10 Surgical management

Because studies of surgical prophylactic mastectomy in women who carry BRCA1 or BRCA2 mutations have shown substantial risk reduction in the incidence of subsequent breast cancers [60, 61], a recent review from the American Society of Clinical Oncology and the Society of Surgical Oncology has endorsed consideration of prophylactic mastectomies in women who have undergone genetic testing and have been found to express a mutated gene associated with high penetrance breast cancer [62]. The NCCN recommends discussing options for risk-reducing mastectomy on a case-by-case basis in women with Cowden Syndrome (see Table 2). However, these guidelines do not provide recommendations regarding prophylactic hysterectomies and/or thyroidectomies. The presence of symptomatic, suspicious tumors in these organs should be taken seriously, and for some PHTS patients such as postmenopausal women, the risk/benefit ratio may favor prophylactic hysterectomy.

1.2.11 Medical Management

There are currently no approved medical therapies for PHTS. Because PTEN mutations increase the activation of components of the Akt/mTOR pathway, therapeutic approaches to inhibit this pathway might be useful to treat or prevent tumorigenesis in PHTS patients. Our trial that employs an FDA-approved inhibitor of mTOR, sirolimus, could potentially identify effective therapy where none currently exists.

1.2.12 The potential use of mTOR inhibitors in PHTS patients

Although there are many inhibitors of the PI3K/Akt/mTOR pathway in development as cancer agents, the most promising and most clinically developed pathway inhibitors are those that inhibit mTOR. mTOR inhibitors are promising therapies for PHTS patients because loss of PTEN is a positive predictive factor for response to these drugs. Loss of one or both copies of PTEN removes the negative regulation on the PI3K/Akt/mTOR pathway and confers a survival advantage to affected cells [25, 32, 34, 63]. In a study using the mTOR inhibitor CCI-779, Shi *et al.* showed that PTEN-deficient human myeloma cells were over 1,000 times more sensitive to CCI-779 than myeloma cells expressing wild-type PTEN [64]. This relative sensitivity to CCI-779 conferred by inactive PTEN has also been observed in xenograft studies, where tumors that responded best to CCI-779 were those with inactive PTEN and/or activation of the Akt/mTOR pathway [65, 66]. Additionally, RAD001 was shown to be more effective as a single agent and as a radiation sensitizer in PTEN-deficient prostate cancer cells than in cells with wild-type PTEN [67]. Because a majority of PHTS patients bear identifiable PTEN mutations, the PI3K/Akt/mTOR pathway in these patients is no longer negatively regulated by PTEN. Therefore, these patients could be an ideal population for targeted pathway inhibition using mTOR inhibitors.

One inhibitor of mTOR, rapamycin, is FDA-approved to prevent rejection of renal transplants and temsirolimus, an analogue of rapamycin, was recently approved by the FDA to treat patients with renal cell carcinoma. Other mTOR inhibitors are being evaluated in different stages of clinical trials in oncology. Rapamycin (sirolimus, Rapamune®) is an oral anti-fungicide that was discovered in 1975 and is FDA-approved in combination with cyclosporine to prevent allograft rejection in renal transplant patients via inhibition of IL-2 induced T cell proliferation. It was shown over twenty years ago that rapamycin was able to inhibit the proliferation of cancer cells. In vitro, sirolimus inhibits mTOR and causes a cell cycle arrest in G1 and/or apoptosis, depending on the cell type. The response to sirolimus is also dose-dependent. Screening of NCI-60 cell line panel for sensitivity to sirolimus shows that at nanomolar doses cell cycle arrest is observed, but at micromolar doses cytotoxicity is achieved. Although sirolimus is more potent in cells with mutant PTEN, sirolimus is also effective in cells with wildtype PTEN but have activation of Akt and mTOR through other mechanisms. For example, sirolimus or its analogues are active in preclinical models of tumorigenesis. mTOR inhibitors can reverse Akt-dependent intraepithelial neoplasia in prostate cells and alveolar epithelial neoplasia in K-ras-driven model of lung cancer [68, 69]. Our group has shown that sirolimus, when given on a schedule that results in trough levels comparable to that of transplant patients, decreases tobacco carcinogen-induced lung tumorigenesis by 90% [70]. These studies indicate that inhibition of mTOR can reverse and/or delay tumorigenesis in many preclinical systems.

CCI-779 (cell cycle inhibitor 779, temsirolimus) and RAD001 (everolimus) are rapamycin analogues that have been designed exclusively as anti-cancer agents. Both have shown activity against many types of cancer in Phase I, II, and III clinical trials. Partial responses and stable disease have been observed in response to treatment with rapamycin analogues in NSCLC, breast, cervical, and uterine carcinomas, as well as sarcoma, mesothelioma, mantle cell lymphoma, neuroendocrine tumors, and glioblastomas (GBM) [71-80]. In a phase II study, CCI-779 produced an objective response rate of 7% in advanced RCC patients with a median time to tumor progression of 5.8 months and median survival of 15.0 months [81]. The results of this study prompted a Phase III trial, which was recently reported and showed that temsirolimus improved the overall survival of patients with metastatic renal cell carcinoma [82]. These results led to FDA approval of temsirolimus.

Biomarkers have been incorporated into some of these trials. For example, Galanis et al. demonstrated a statistically significant correlation between radiographic improvement in recurrent GBM patients and high levels of phosphorylated p70S6 kinase in baseline tumor samples [83]. Duran et al. showed that high levels of active mTOR (p-mTOR) predicted response to temsirolimus, and decreased levels of p-mTOR and p-S6 (and increased levels of p-Akt) with therapy correlated with an increased time to progression [80]. These studies indicate the potential utility of assessing p-mTOR, S6, and Akt before and after administration of an mTOR inhibitor.

The preclinical data and clinical results with rapamycin and its analogues show that overall these drugs are well tolerated and show signs of clinical efficacy in established cancers that correlates with mTOR inhibition. Rapamycin itself is already FDA-approved, and the extensive clinical experience with rapamycin has facilitated the development of CCI-779 and RAD001. These inhibitors of mTOR are different from other kinase inhibitors such as imatinib and sorafenib in that they are highly selective for their intended target, mTOR. Taken together, the FDA-approved status of rapamycin, its low cost in comparison to temsirolimus, and its specificity for mTOR that could increase the therapeutic index, make a strong rationale to test rapamycin in patients with PHTS. Moreover, the accessibility of tissues before and after rapamycin therapy in PHTS patients provides an opportunity to correlate changes in tumor growth and/or metabolism with changes in phosphorylation of pathway components such as Akt, S6K, S6 and 4E-BP1. This could provide a molecular basis for any observed effects on tumor growth.

1.2.13 Considerations in the use of mTOR inhibitors in PHTS patients

While it is possible that PHTS patients could benefit from drugs that inhibit the PI3K/Akt/mTOR pathway, this benefit might not be without cost. First and foremost, because these patients bear germline mutations in PTEN in every cell, greater toxicity than that observed in cancer patients treated with pathway inhibitors could be observed. This may not be the case, however, because anecdotal evidence with two PHTS patients treated with sirolimus indicates that toxicities were minimal (see below).

Two other considerations apply specifically to mTOR inhibitors. First, the use of mTOR inhibitors can lead to feedback activation of Akt, which could hypothetically negate any effect of mTOR inhibitors by promoting propagation of the Akt signal to other downstream substrates. Two different mechanisms have been described. mTOR can exist in two types of complexes, mTORC1 and mTORC2 complexes. Several preclinical studies have shown that mTOR inhibitors preferentially decrease the proportion mTORC1 complexes and increase the proportion of mTORC2 complexes, which are comprised of mTOR bound to rictor. mTORC2 complexes can directly phosphorylate at S473 and lead to its activation [84]. A separate mechanism for feedback activation has also been observed that involves IRS-1, which normally activates PI3K. S6K, a downstream substrate of mTOR, normally phosphorylates and inhibits IRS-1. Inhibition of mTOR by rapamycin decreases S6K activity, which indirectly activates IRS-1, prolongs the half-life of IRS-1, and activates PI3K [85]. Thus, the inhibition of mTOR could result in two modes of feedback activation of Akt, through direct activation of Akt by mTOR-rictor complexes, and through the indirect activation of IRS-1 by inhibition of S6K. Although either feedback mechanisms could possibly counter the efficacy of an mTOR inhibitor, there is no evidence that this occurs in humans, and the clinical relevance of these mechanisms is unclear. Nonetheless, this protocol will incorporate evaluation of these biomarkers to assess whether such a feedback mechanism occurs in tissues from PHTS patients.

Second, rapamycin has 'black-box' warnings regarding the possibility of developing serious infections, including opportunistic infections, as well as a possible association with the development

of lymphomas occurring in the setting of immune suppression. However, sirolimus has mainly been used for immune suppression post transplantation in combination with cyclosporine, a highly potent immunosuppressant. It has yet to be determined whether single-agent therapy with rapamycin or its analogues (which are metabolized to rapamycin in *vivo*), would produce the same effects on immune function. Preclinical studies in euthymic mice showed that CCI-779 did inhibit T cell activity, but this was reversible and returned to baseline within 24 hours of drug withdrawal. Importantly, several cycles of CCI-779 did not cumulatively impair T cell function, and an intermittent dosing schedule of CCI-779 curtailed immune suppression while preserving effects on tumor growth. Recently, a phase I study was conducted by Hidalgo and colleagues using an intermittent dosing schedule of daily CCI-779 for five days every two weeks [86]. In this study with 63 patients, CCI-779 was well tolerated, and there was no evidence of immune suppression. Because CCI-779 and RAD001 are metabolized to rapamycin, this data supports the possibility that rapamycin and its analogues may not cause profound immune suppression if used as single agents.

1.2.14 Defining immune effects of sirolimus as a single agent

We hypothesize that immune suppression will not be a significant safety issue in this trial, which is supported by many lines of evidence. First, immune suppression with sirolimus has mainly been reported with concurrent use of cyclosporine in transplant patients. Second, there are few reports in the literature of treatment related infection, opportunistic or otherwise, with either single agent sirolimus, or it analogs, CCI-779 or RAD-001 in phase I, II, and III trials of oncology patients. Third, we have preclinical data using a mouse model of lung tumorigenesis that demonstrates that the effects on the immune system are modest. A/J mice were treated with sirolimus (1.5 mg/kg/qod) for one, four and 12 weeks. The plasma concentration levels of sirolimus were stable throughout the study and ranged from 130-180 ng/ml, which is approximately tenfold the therapeutic trough concentrations achieved in humans with 2 mg per day dosing. Furthermore, the mice were treated for up to 12 weeks, which is approximately 10% of their total life span. (Note that the proposed study subjects without malignancy will only be treated for eight weeks.) At 12 weeks, sirolimus decreased CD4+ and CD8+ T cells by approximately 50% without affecting other lineages of immune cells. The CD4:CD8 ratio, however, remained constant at approximately 1.3, indicating there was not preferential depletion of one T cell subset. Induction of a Th2 phenotype, which has previously been associated with sirolimus [87], did not occur. Fourth, we retrospectively analyzed the lymphocyte count obtained from routine complete blood count differentials and sirolimus levels in Case 1 (Case 2 only had 3 data points). Case 1 had a modest decline in her absolute lymphocyte count which did correlate with drug levels. At no time during the ongoing 15 months of treatment with rapamycin have there been signs of immune suppression, nor has she suffered from serious infection, opportunistic or otherwise.

Based upon the above discussion, we do not believe that there will be sequelae of long-term immune suppression in PHTS patients with benign disease treated with sirolimus for 56 days (plus 2-3 days to allow flexibility for scheduling of follow-up procedures), nor do we believe that there will be significant toxicity related to immune suppression for PHTS subjects with malignancy who may be on study beyond 56 days. To reiterate, the blood levels achieved in the mouse model were approximately 10 fold the levels we will achieve in our study subjects, yet no immunosuppression was observed. The effects of sirolimus on the immune system in a non-transplant patient with or without a malignancy have not been well characterized. This study will contribute to our understanding of the effects of sirolimus on the immune system at doses capable of affecting mTOR inhibition. Given the known effects of sirolimus on cytokine driven T cell proliferation, we plan to follow lymphocyte subsets (CD4, CD8, CD20, NK) in our study subjects. If we observe a

decline in the CD4 count or percentage of CD4 cells that places our subjects at risk for opportunistic infections, appropriate prophylactic therapy will be instituted.

1.2.15 Summary of rationale

The development of medical therapies for PHTS would have advantages for PHTS patients and the general cancer community. For PHTS patients, it would provide medical therapy options for prevention of tumorogenesis and treatment of advanced malignancy where none currently exist. For the cancer community, initial use of agents that inhibit the PI3K/Akt/mTOR pathway in PHTS patients could credential drugs prior to testing in the general oncology community. The implications of using a credentialed inhibitor of the PTEN/PI3K/Akt/mTOR pathway in the general oncology population are important, because pathway activation is a common feature of cancer. Approximately 25-50% of sporadic primary tumors exhibit LOH in the chromosomal region of PTEN, and complete loss of PTEN has been found in several types of invasive and metastatic tumors. Activation of the PI3K/Akt/mTOR pathway can also occur independently of PTEN loss, and has been observed in more than 50% of human cancers, making it one of the most common molecular alterations in cancer. Although PHTS is a phenotypically variable, rare collection of syndromes, it has great relevance to human cancer because the study of these rare patients could provide insight into PTEN-driven tumorogenesis and facilitate drug development for millions of cancer patients.

1.2.16 Preliminary experience with sirolimus in PHTS patients

To determine if loss of PTEN in PHTS patients recapitulates preclinical studies and causes activation of the PI3K/Akt/mTOR pathway, we obtained multiple tissues from four PHTS patients after enrollment in a tissue procurement protocol, and assessed components in the pathway using immunohistochemistry or immunoblotting. The features of these patients are shown in table 3.

Analysis of peripheral blood mononuclear cells (PBMCs) from three PHTS patients using immunoblotting showed that phosphorylation of Akt, S6K, S6, and 4E-BP1 were increased compared to normal controls (Figure 2), indicating that loss of PTEN increases pathway activation in PBMCs.

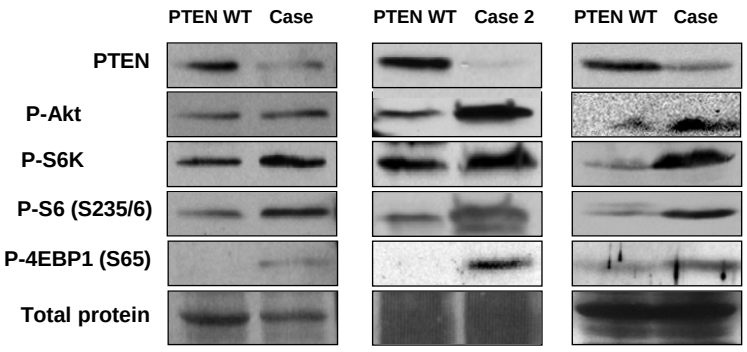


Figure 2. Loss of PTEN expression and increased activation of the Akt/mTOR pathway in PBMCs from PHTS patients. PBMCs from 3 PHTS patients and an unaffected control were extracted and immunoblotting was performed. The membrane was blotted with antibodies for total PTEN, and phosphorylated Akt (S473), S6K (T389), pS6 (S235/236), and 4EBP1 (S65). Fast green staining is shown for protein loading.

Table 3: Patient characteristics

	Age	Gender	Ethnicity	Syndrome	Malignant	PTEN mutation
Case 1	56	Female	Caucasian	CS	NSCLC, BC	R335X
Case 2	35	Female	Caucasian	CS, LDD	EC	L112P
Case 3	52	Female	Caucasian	CS, BRRS	SCC	Del (exons1-5)
Case 4	33	Female	Caucasian	CS	BC	R335X

CS=Cowden syndrome, LDD=Lhermitte-Duclos disease, BRRS=Bannayan-Riley-Ruvalcaba syndrome, NSCLC=non-small cell lung cancer, BC=breast cancer, EC=endometrial cancer, SCC=squamous cell carcinoma

Likewise, increased activation of Akt, mTOR, and S6 was also observed in benign and malignant tumors from multiple organs. Examples of staining for pathway activation using immunohistochemistry in GI polyps and trichilemmomas are shown in figure 3. Examples of staining in malignant tissues from each of 4 cases are shown in figure 4.

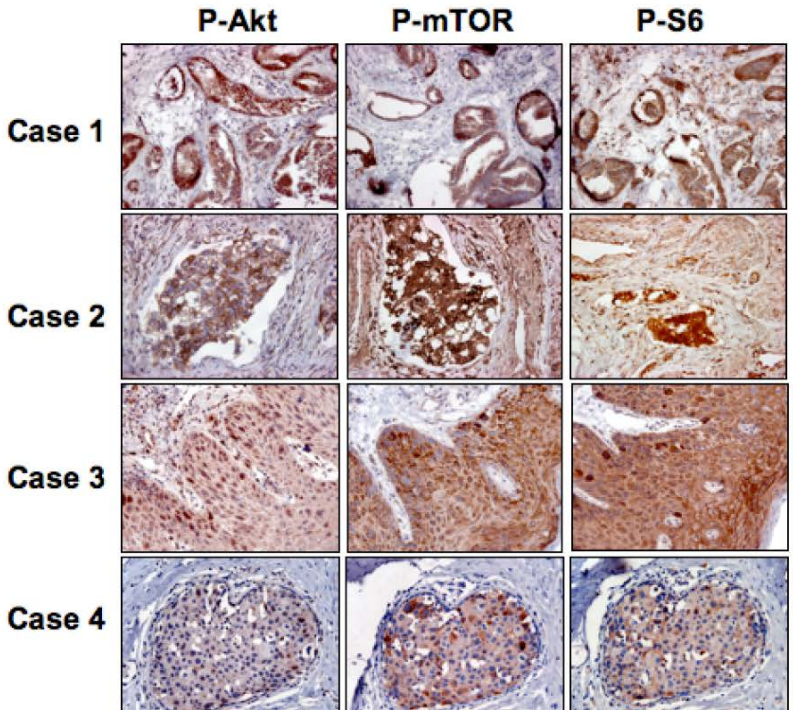


Figure 3. Increased activation of the Akt/mTOR pathway in benign tissues from PHTS patients. Stomach polyps from case 1 and trichilemmomas from case 2 were stained with antibodies for phosphorylated Akt (S473), mTOR (S2448), and pS6 (S235/236).

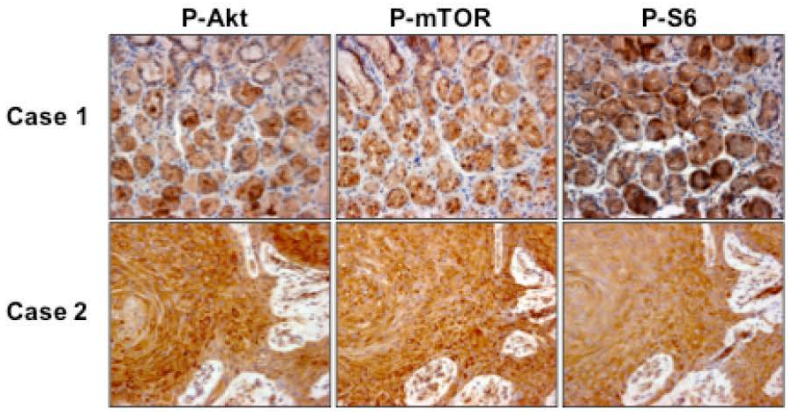


Figure 4. Increased activation of the Akt/mTOR pathway in malignant tissues from PHTS patients. Immunohistochemical analysis of the Akt/mTOR pathway in non-small cell lung cancer from case 1, endometrial carcinoma from case 2, squamous cell carcinoma of the tongue from case 3, and breast cancer from case 4.

These data are important for several reasons. First, they confirm that loss of PTEN leads to increased activation of the Akt/mTOR pathway in multiple tissues. Second, they show that immunoblotting and IHC are feasible to assess pathway activation in tissues from PHTS patients. Finally, these data strongly support the rationale to test drugs that target the Akt/mTOR patients in this rare patient population.

During the course of this biochemical analysis, two of the cases from which we derived tissues needed therapy for their cancers but declined options for standard chemotherapy. Case 2 had stage IV endometrial cancer with peritoneal carcinomatosis, declined standard chemotherapy, and sought treatment with sirolimus. This patient also had LD, which is characterized by dysplastic gangliocytomas of the cerebellum that cause ataxia, increased intracranial pressure, and seizures. After informed consent was obtained, the patient was started on 2 mg of sirolimus given orally every day (a standard dose for FDA approved usage). After 4 weeks of therapy, the patient was clinically improved, showed regression of skin hamartomas (data not shown), and exhibited decreased activation of the pathway in PBMCs (Figure 2). In addition, the patient showed a marked metabolic response in both benign and malignant tissues, as assessed by PET scan (Figure 5). This patient received sirolimus for 2 months, and then died 4 months later from acute cholecystitis (thought to be unrelated to sirolimus).

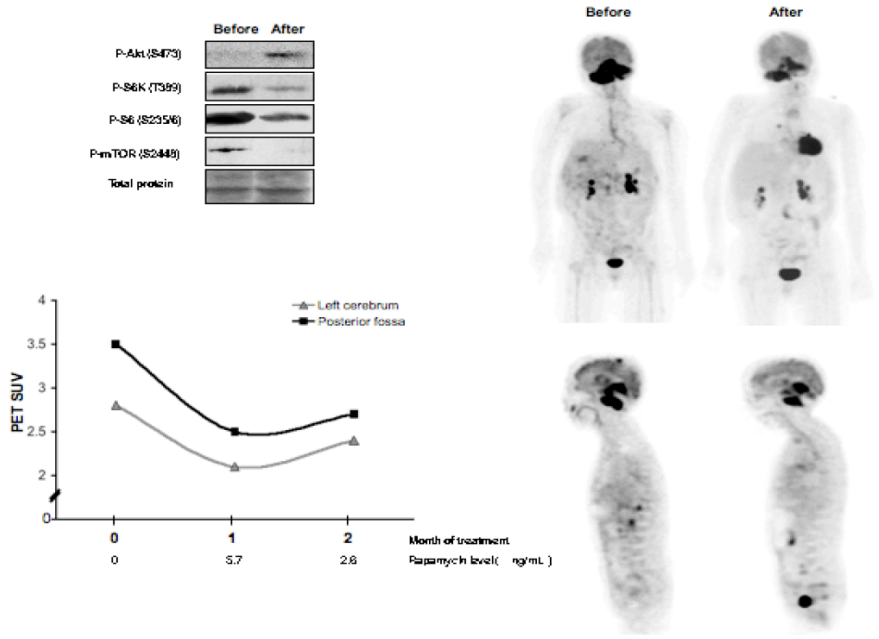


Figure 5: Response of Case 2 to sirolimus treatment. Activation of the Akt/mTOR pathway activity was decreased in PBMCs after sirolimus treatment (upper left panels). FDG-PET scans were performed before and 2 weeks after initiation of sirolimus treatment. Sirolimus reduced FDG uptake in presumed benign tissues such as cerebellum, esophagus, and bone marrow, as well as malignant tissues (peritoneum) (right panels). The metabolic activity was quantified in two brain areas, and correlated with rapamycin levels (lower left panels).

Case 1 has CS and stage IV NSCLC. After undergoing a therapeutic thoracentesis for increased dyspnea and chest pain, she declined standard chemotherapy and requested sirolimus. We obtained informed consent, and she was started on sirolimus 2 mg/d, but because the sirolimus levels progressively decreased and she remained asymptomatic, the dose was increased to 5 mg/d. She has clinically improved while on sirolimus, citing less cough, dyspnea, and nasal congestion. (She had a history of documented nasal polyps thought secondary to CS.) After 8 weeks of therapy, she was able to breathe through her nose for the first time in years, and noted a disappearance of facial lesions (Figure 6). Evaluation of other benign tissues showed tumor regression and/or decreased activation of mTOR. This patient also underwent FDG-PET scans, and similar to the results obtained in Case 2, sirolimus treatment was associated with a 30-50% decrease in metabolic activity in both benign and malignant tumors. Case 1 continues on single agent sirolimus, and after approximately 15 months of therapy, she continues to have stabilization of sites of NSCLC and has not required a repeat thoracentesis for the malignant pleural effusion.

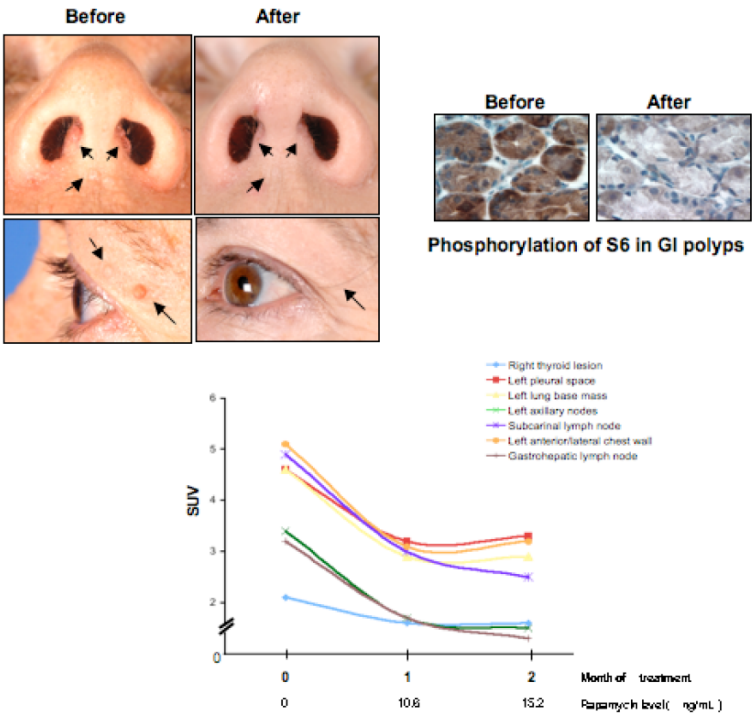


Figure 6: Response of Case 1 to sirolimus treatment. Regression of facial and intranasal trichilemmomas after treatment with sirolimus (upper left panels). Sirolimus also decreased phosphorylation of S6, a downstream substrate of mTOR, in a benign GI hamartomatous polyp (upper right panels). Sirolimus reduced FDG-PET metabolic activity in both presumed benign tissue (L axillary node, R thyroid lesion) and malignant tissue (L pleura, L lung base), which correlated with sirolimus level (lower panel).

1.2.17 Conclusions

Given the activation of the PI3K/Akt/mTOR pathway in tissues from PHTS patients, and the anecdotal biochemical and metabolic response of two PHTS patients to sirolimus, we hypothesize that sirolimus could have biological and clinical activity in PHTS patients. We propose to test sirolimus in a pilot trial in PHTS patients for monitoring of biochemical changes in accessible tissues, metabolic changes using PET imaging, and clinical changes using physical examination, photography, digital dermoscopy, and CT scans. This study will determine if sirolimus at the FDA approved dose can inhibit activation of downstream components of the mTOR pathway in affected (hamartomatous and malignant) tissues and unaffected (PBMCs) tissue. Biochemical changes will be correlated with levels of sirolimus as well as clinical outcomes, which will provide insight into the utility of sirolimus in this rare patient population. To our knowledge, this is the first clinical trial utilizing sirolimus in PHTS. A positive trial could provide the basis for future trials to assess the clinical efficacy of sirolimus, both for prevention of tumorogenesis and treatment of advanced malignancy, and could eventually lead to medical therapy for a rare population that currently has no medical options.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA:

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have documented germline PTEN mutation performed in a CLIA approved laboratory. [7, 88, 89].

2.1.1.2 Patients must meet clinical criteria for Cowden Syndrome. Cowden syndrome diagnostic criteria are listed in appendix 12.7.

2.1.1.3 Patients must have the capacity to provide informed consent and demonstrate willingness to comply with an oral regimen.

2.1.1.4 Patients must have at least 6 sites amenable to biopsy within the skin and/or GI tract and /or accessible malignant tumor (for patients with malignancy) and agree to the biopsy of these sites prior to and following sirolimus administration.

2.1.1.5 Patients do not need to have malignant tumors, but if they do, they must have relapsed or failed to respond to standard therapy, and the patient's current disease state must be one for which there is no known curative therapy. Patients who are diagnosed with cancer as a consequence of initial PET/CT scan will be managed according to the flow diagram illustrated in Figure 6.

2.1.1.6 Patients must have not received chemotherapy in the 28 days prior to enrollment.

2.1.1.7 Age ≥ 18 years of age.

2.1.1.8 ECOG performance score of ≤ 2.

2.1.1.9 An expected survival of ≥ 3 months.

2.1.1.10 Patients must consent to the use of effective barrier-based contraception during the course of treatment and for three months following discontinuation of treatment.

2.1.1.11 Patients must have normal organ and marrow function as defined below:

2.1.1.12 absolute neutrophil count ≥1,500/mL.

2.1.1.13 platelets ≥100,000/mL.

2.1.1.14 total bilirubin <1.5 X upper limit of institutional normal.

2.1.1.15 AST (SGOT) ≤2.5 X upper limit of institutional normal.

2.1.1.16 ALT (SGPT) ≤2.5 X upper limit of institutional normal.

2.1.1.17 Creatinine <1.5 X upper limit of institutional normal.

2.1.1.18 PHTS subjects with benign hamartomatous disease must have controlled fasting LDL and triglyceride levels as defined by NCEP ATP III guidelines. Please see Section 4.5 for further details.

2.1.1.19 Patients must have recovered from any acute toxicity related to prior treatments, including surgery. Toxicity should be ≤ grade 1 or returned to baseline.

2.1.1.20 If a patient withdraws consent within two weeks of starting study drug, he/she may request to re-enter study at the PI's discretion by re-signing consent and being re-registered through the CRO using the initial baseline studies. Sirolimus taken during the period on study (prior to withdrawal of consent) will not be considered as prior sirolimus therapy that otherwise would exclude enrolment.

2.1.2 Exclusion Criteria

2.1.2.1 Pregnant or lactating women, due to potentially harmful effects of sirolimus on the embryo or fetus or nursing child.

2.1.2.2 Any concurrent therapy with chemotherapeutic agents or biologic agents or radiation therapy.

2.1.2.3 Patients taking immuno-suppressive agents other than prescribed corticosteroids, which must not exceed the equivalent of 20 mg/d of prednisone

2.1.2.4 Patients that are on the following CYP3A4 inhibitors and cannot replace these medications with other equivalent medications for the period of the study: protease inhibitors, cyclosporine, fluconazole, itraconazole, ketoconazole, metoclopramide, felodipine, nifedipine, carbamazepine, Phenobarbital, grapefruit juice, and St. John's Wort.

2.1.2.5 Patients who have received live vaccines in the past 30 days.

2.1.2.6 Patients with human immunodeficiency virus (HIV) seropositivity, due to potential drug interactions between sirolimus and anti-retroviral medications, as well as the unknown effects of single agent sirolimus on the immune system in HIV patients.

2.1.2.7 Patients with interstitial lung disease or pneumonitis.

2.1.2.8 Patients with bleeding diathesis.

2.1.2.9 Patients with prior or active pneumocystis jirovecii (PJP) pneumonia.

2.1.2.10 Patients with prior use of rapamycin, a rapamycin analogue, or other mTOR inhibitor.

2.1.2.11 Patients who do not agree to have multiple repeated biopsies performed.

2.2 RESEARCH ELIGIBILITY EVALUATION

2.2.1 These items must be completed within 17 days of enrollment:

2.2.1.1 Complete history and physical examination: (including height, weight, vital signs, head circumference, facial trichilemmomas, acral keratoses, papillomatous lesions, mucosal lesions) with documentation of prior therapies for cancer and current medication history including over the counter medications, homeopathic remedies, vitamins, and alternative therapies.

2.2.1.2 Confirmation of Cowden Syndrome by International Cowden Consortium Diagnostic Criteria (see appendix 10.7) and documentation of PTEN germline mutation by sequence analysis from a CLIA approved laboratory will be assessed by the clinical team. Consultation with a dermatologist will be performed as needed to confirm pathognomonic criteria for Cowden Syndrome if this is unclear to the primary clinical team or in the patient's medical records.

2.2.1.3 Laboratory Evaluation and includes:

- Hematological Profile: CBC with differential and platelet count.
- Coagulation Profile: prothrombin time and activated partial thromboplastin time.
- Biochemical Profile: electrolytes, BUN, creatinine, fasting glucose, AST, ALT, bilirubin, calcium, phosphorous, albumin, magnesium, alkaline phosphatase, amylase and lipase.
- Fasting Lipid Profile: includes total cholesterol, HDL, LDL, and triglyceride.
- Serum or urine beta-hCG for female patients of childbearing age and anatomic ability.
- HIV 1&2 test

3 PATIENT REGISTRATION

3.1 ON-STUDY REGISTRATION

Authorized staff must register an eligible candidate with the NCI Central Registration Office (CRO) within 24 hours of signing the consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and faxed to 301-480-0757. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of registration will be forwarded electronically via e-mail. Please note, it is very important for all registrars to acquire encrypted e-mail from NIH Help Desk, since the verification of registration includes patient information. A recorder is available during non-working hours.

3.2 OFF-STUDY REGISTRATION

At the time of removal from study, CRO will also be notified when a patient is taken off-study. An off-study form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and faxed to 301-480-0757.

4 STUDY IMPLEMENTATION

4.1 STUDY DESIGN

Treatment will be administered on an outpatient basis except when admission is required for procedures or side effects of treatment. No other investigational agents or therapies other than those described below may be administered with the intent to treat the patient's syndrome (PHTS) or malignancy. Duration of treatment will be limited to a maximum of 2 cycles, each cycle equals 28 days, for patients with benign hamartomatous growths. For patients with malignant tumors, treatment will continue as long as patients have not met criteria to come off treatment as defined in Section 4.9.

To meet the statistical goals of this study, we hope to obtain 3 sets of 6 biopsies, (18 total- 6 biopsies at baseline, 2 weeks and 8 weeks) from each patient. As outlined in the statistical section 7, skin biopsies may be included in the analysis, but in at least 10 of 20 patients, up to 18 biopsies (3 sets of 6 biopsies) should be obtained by sigmoidoscopy as regression of skin lesions observed in Cases 1 and 2 (Section 1.2) may make analysis of pathway modulation after therapy impossible. In addition to physical examination, all patients will undergo baseline imaging with research PET/CT, and blood collection for research purposes. Patients will also undergo baseline digital photography, digital dermoscopy, and biopsy of skin lesions. Patients with advanced cancer will have optional tumor biopsy of accessible lymph nodes or subcutaneous metastasis at baseline. In addition, all patients will undergo baseline sigmoidoscopy. Given recent reports of cognitive and behavioral improvements in TSC2 deficient mice that are being treated with sirolimus, and the fact that neuropsychological testing is being incorporated into trials of sirolimus in other hamartomatous syndromes, neuropsychological testing will also be performed at baseline (see Section 4.6.6).

Patients with skin lesions only (and negative initial PET/CT) will undergo follow up digital photography, digital dermoscopy, and skin biopsy about 2 weeks (C1D14) and 8 weeks (C2D28), but will not undergo follow up sigmoidoscopy or PET/CT. Patients with lesions on baseline sigmoidoscopy will have follow up sigmoidoscopy digital photography, digital dermoscopy, and skin biopsy will be performed about 2 weeks (C1D14) and about 8 weeks (C2D28). PET/CT will be performed around 2 weeks only if baseline PET/CT identifies one or more lesions $\geq$ 1 cm or SUV

≥ 2. For patients with advanced cancer who had accessible tumor biopsied at baseline, follow up biopsy will be performed at 2 and 8 weeks. If there is no evidence of mTOR inhibition of tumor at 2 weeks, the 8 week biopsy will not be pursued. Repeat neuropsychological testing will be performed around 4 and 8 weeks on sirolimus.

Because patients with germline PTEN mutations can develop benign tumors in any organ, it is possible that scanning patients using PET/CT might reveal lesions that were previously unappreciated. Should any imaging study reveal a lesion suspicious of malignancy, appropriate additional imaging and biopsy will be performed. Furthermore, because of the high incidence of cancer in individuals with PHTS, it is anticipated that some study subjects will enroll with a diagnosis of malignancy. The algorithm in figure 6 describes the evaluation and disposition for subjects enrolling with a known cancer as well as those in whom cancer is diagnosed during the course of the trial.

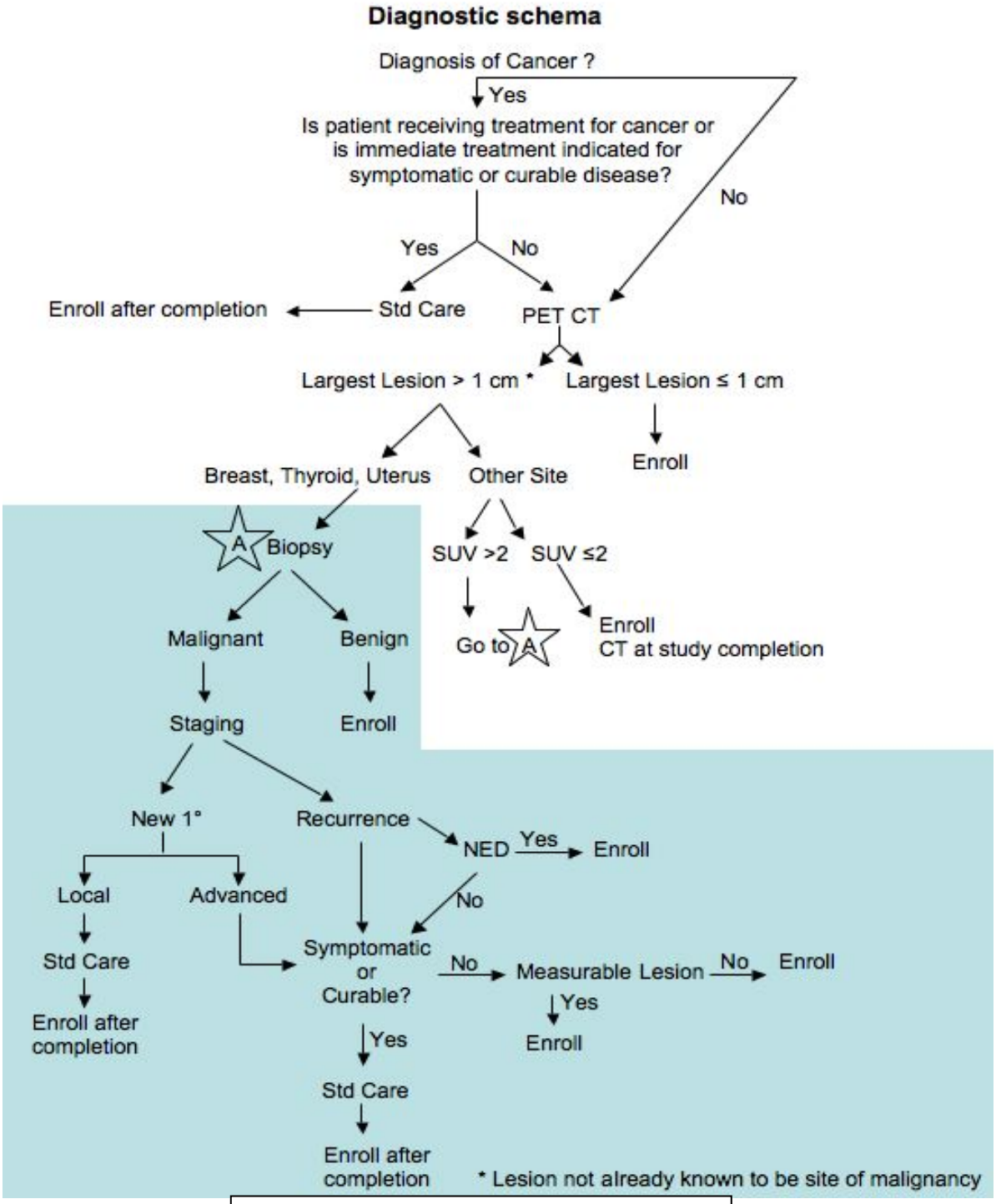


Figure 7. A diagnostic schema for PHTS patients

Clinically suspicious lesions for malignancy are outlined in figure 6. A distinction is made between organs that are known to undergo malignant transformation in PHTS patients, and lesions in organs that do not have a known predisposition for cancer. Biopsies in this setting will only be obtained for clinical purposes to rule out malignancy. If multiple lesions are present, the largest and

most metabolically active lesion will be biopsied. If there is no difference in size or metabolic rate in a number of lesions in the same organ, the lesion that can be biopsied with the least morbidity will be biopsied, and will be presumed to be representative of the lesions in that organ. The Clinical Pathology Department at the CRC will process and analyze biopsied specimens.

As outlined by the algorithm, PHTS subjects without evidence of malignancy at registration will be enrolled on the trial and receive sirolimus for a total of 56 days (plus 2-3 days to allow flexibility for scheduling of follow-up procedures). Those PHTS subjects with advanced and/or recurrent malignancy for which there is no curative therapy who have measurable disease will be eligible to continue on sirolimus beyond 56 days. At baseline, they will have appropriate imaging (such as CT, MRI or ultrasound) in order to fully assess disease status. These subjects with malignancy will have all studies assessing disease status performed every other cycle, approximately every 8 weeks until the patient meets off-treatment criteria as outlined in Section 4.9.1.

Given concern for immune suppression with sirolimus, stopping rules have been implemented to trigger cessation of the trial in the event of unacceptable rates of opportunistic infection as outlined in Section 4.3. To assess the duration of skin changes after treatment with sirolimus is completed, PHTS subjects with benign disease who had regression of benign skin lesions will have follow-up clinic visits, medical photography, digital dermoscopy, research blood and lymphocyte subsets and neuropsychological testing performed at the time points outlined in Section 4.4.3, 'Periodic Evaluations off Therapy'.

4.2 DRUG ADMINISTRATION

Sirolimus will be administered as a loading oral dose of 6 mg (day 1) followed by a daily oral dose of 2 mg (beginning day 2) for the remainder of the study. Sirolimus trough levels will be checked around C1D15 and day 1 of every cycle as well as any time that an unacceptable toxicity develops as outlined in Section 4.3.

Patients are to swallow the sirolimus tablets with approximately 250 ml of water each morning on an empty stomach. Tablets should not be taken with food. Patient compliance will be verified via pill count every 4 weeks from the beginning of the study. We will use the NCI CCR Pill Count Form found on the CCR intranet, Policies/SOP section (http://ccrintra.nci.nih.gov/).

4.3 TREATMENT MODIFICATIONS

Toxicities will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE Version 3.0) scale every 28 days.

The following toxicities will be defined as unacceptable and will cause a stop in treatment as per the rules below: grade 2 creatinine, grade 2 pneumonitis, or any other toxicities grade 3 or higher that are deemed possibly, probably or definitely related to the study drug. Toxicities unrelated to drug may not require dose adjustment.

If the toxicity develops within the first week of drug administration, patients will be removed from the study, sirolimus trough level will be checked, and patients will be replaced.

If the toxicity develops after week 1, patients will have their dose held and the trough level of sirolimus checked once a week until resolution of toxicity to $\leq$ grade 1. Once appropriately resolved, treatment will be restarted with 1 mg daily dosing for the remainder of the study. If resolution does not occur after three weeks, patients will be removed from the study and will be replaced. If toxicities recur on 1 mg daily dosing, sirolimus trough levels will be checked and the drug will be stopped until toxicities resolve to $\leq$ grade 1. Once resolved, drug will be restarted at 1

mg every other day. If toxicities do not resolve in three weeks, patients will not be restarted on treatment, and will not be replaced.

If a patient experiences a life-threatening toxicity or a grade 4 toxicity deemed possibly, probably or definitely related to the study drug, he/she will be removed from study.

In the event that a patient experiences multiple unacceptable toxicities, all toxicities must be resolved to ≤ grade 1 prior to re-initiation of therapy with dose reduction (if patient meets eligibility for dose reduction as outlined above).

If an unacceptable toxicity occurs after 4 weeks of therapy and requires discontinuation or holding of sirolimus, we would like to obtain one additional: skin examination with biopsies, sigmoidoscopy, medical photography, digital dermoscopy, and neuropsychological testing provided that these procedures/tests were otherwise planned, deemed medically safe, and can be performed within 2 weeks of stopping sirolimus.

4.4 PROTOCOL EVALUATION

4.4.1 On Study Evaluation

To be performed within 14 days prior to starting any medication.

4.4.1.1 History and Physical Examination including vital signs and head circumference

4.4.1.2 ECOG Performance status

4.4.1.3 Genetics evaluation by a practitioner trained in genetic counseling will be offered to the patient and his/her family members to ensure that the patient's and family's genetic counseling needs are met (see Section 5.7).

4.4.1.4 Clinical Digital Photography at the NIH clinical center.

4.4.1.5 Digital Dermoscopy at the dermatology clinic at the NIH clinical center.

4.4.1.6 Dermatologic evaluation and skin biopsy at the NIH clinical center.

4.4.1.7 Research PET/CT at the NIH clinical center.

4.4.1.8 EKG within prior 2 months.

4.4.1.9 Laboratory evaluation to include pregnancy test in women of childbearing potential, CBC with differential, electrolytes, BUN, creatinine, LDH, albumin, calcium, magnesium, phosphorus, SGOT, SGPT, T. Bilirubin, alkaline phosphatase, urinalysis, lymphocyte subsets (CD4, CD8, CD20, NK).

4.4.1.10 PT and aPTT

4.4.1.11 Sigmoidoscopy at the NIH Clinical Center.

4.4.1.12 Biopsy of suspicious tissue to rule out malignancy if clinically indicated (see section 4.1).

4.4.1.13 Research blood collection

4.4.1.14 Fasting Lipid panel

4.4.1.15 PHTS subjects with advanced malignancy and measurable disease will have disease status fully assessed at baseline by imaging (CT or MRI or Ultasound as clinically indicated).

4.4.1.16 Neuropsychological testing

4.4.1.17 Optional biopsy of accessible tumor (lymph node or subcutaneous metastasis) in patient with advanced malignancy

4.4.1.18 Clinical videography at the NIH Clinical Center

4.4.2 Periodic Evaluations during therapy

4.4.2.1 History and physical exam including vital signs and head circumference every 28 days

4.4.2.2 ECOG performance status every 28 days

4.4.2.3 Clinical tumor measurements, if clinically feasible, every 28 days

4.4.2.4 Clinical photography at NIH Clinical Center Medical Photography Department cycle 1 day 14 (+/- 3 days) and cycle 2 day 28 (+/- 3 days). For PHTS subjects with malignant tumors who continue on study beyond 2 cycles, additional clinical photography will be performed about every 2 cycles.

4.4.2.5 Sirolimus levels on cycle 1 day 14 and day 1 of every cycle thereafter or at the time of unacceptable toxicity.

4.4.2.6 Digital Dermoscopy will be performed cycle 1 day 14 (+/- 3 days) and cycle 2 day 28 (+/- 3 days). For PHTS subjects with malignant tumors who continue on study beyond 2 cycles, additional digital dermoscopy will be performed about every 2 cycles.

4.4.2.7 Dermatologic evaluation and skin biopsy at the NIH clinical center for patients with skin lesions at baseline cycle 1 day 14 (+/- 3 days) and cycle 2 day 28 (+/- 3 days).

4.4.2.8 Sigmoidoscopy for patients with lesions at baseline on cycle 1 day 14 (+/- 3 days) and cycle 2 day 28 (+/- 3 days).

4.4.2.9 CBC with differential, Serum electrolytes and chemistries (Na+, K+, Cl-, CO2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT/SGPT, AST/SGOT, total bilirubin, LDH, amylase, and lipase), lymphocyte subsets (CD4, CD8, CD20, NK), PT and PTT every two weeks. For PHTS subjects with malignancy who continue on study beyond cycles 2, these blood tests will be performed every cycle after cycle 2.

4.4.2.10 Fasting lipid and triglyceride levels every two weeks for first 2 cycles and then every 4 weeks after cycle 2 (for patients eligible to remain on treatment beyond cycle 2).

4.4.2.11 Toxicity evaluation every cycle i.e. every 28 days.

4.4.2.12 Research blood collection cycle 1 day 14 (+/- 3 days) and cycle 2 day 28 (+/- 3 days). For PHTS subjects with malignant tumors who continue on study beyond 2 cycles, additional research blood will be collected about every 2 cycles.

4.4.2.13 Repeat research PET/CT at around 2 weeks for subjects with a positive baseline PET/CT.

4.4.2.14 PHTS subjects with advanced malignancy and measurable disease will have repeat disease measurements (as assessed by CT or MRI or Ultrasound as clinically indicated) every 2 cycles, approximately every 8 weeks.

4.4.2.15 Neuropsychological testing about every 4 weeks for 2 cycles.

4.4.2.16 Re-biopsy of accessible tumor (lymph node or subcutaneous metastasis) in patients with advanced malignancy who had biopsy at baseline cycle 1 day 14 (+/- 3 days) and cycle 2 day 28 (+/- 3 days) provided tumor still present. Cycle 2 day 28 biopsy will be performed only if there was evidence of mTOR inhibition at cycle 1 day 14 biopsy.

4.4.2.17 Clinical Videography at the NIH Clinical Center Cycle 1 Day 15 (+/- 3 days), Cycle 1 Day 28 (+/- 3 days), and Cycle 2 Day 28 (+/- 3 days).

4.4.3 Periodic Evaluations off Therapy

For PHTS patients with benign disease who had evidence of regression of skin lesions while on therapy (as determined by clinical judgment), repeat videography, digital dermoscopy and photography will be performed at 1 and 2 months after completion of therapy. In addition, all patients will have the following tests/evaluations performed at approximately 1 and 2 months after completion of therapy: clinic visit with history and physical, research blood collection and lymphocyte subsets. Neuropsychological testing will be repeated around 2 months post-therapy.

4.5 CONCURRENT THERAPIES

Given that one of the known side effects with sirolimus is hyperlipidemia, we may need to institute appropriate cholesterol lowering therapy in study subjects both prior to enrollment and while on study. We have set different criteria for instituting therapy depending on whether the PHTS subject has benign only disease or malignancy.

4.5.1 For PHTS subjects with benign hamartomatous disease

These subjects will be on trial for a total of 56 days (plus 2-3 days to allow flexibility for scheduling of follow-up procedures), so transient fluctuations in cholesterol are unlikely to have any long-term clinical significance. Nevertheless, we will adhere to the guidelines as outlined by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III). A version of the guidelines can be found on the internet at http://www.nhlbi.nih.gov/guidelines/cholesterol/atglance.pdf. Relevant portions of the NCEP ATPIII guidelines are included as Appendix Section 12.5.

At baseline, subjects will be stratified into high, intermediate, and low risk categories, as defined by NCEP ATP III guidelines. Major risk factors for coronary heart disease (other than LDL) are listed in 'Step 3' of the guidelines, and risk categories are listed in 'Step 5' of the guidelines (see Appendix Section 12.5). The following LDL thresholds will prompt referral back to the subjects' primary care provider for institution of appropriate medical therapy prior to enrollment into the trial: for low risk patients (0-1 risk factors) a fasting LDL >189 mg/dL, for intermediate risk patients (2+ risk factors) a threshold fasting LDL >159 mg/dL, and for high risk patients (coronary heart disease or chd equivalents) a threshold fasting LDL >129 mg/dL. Once the fasting LDL is below target values, subjects would be eligible for enrollment onto the trial.

If the fasting LDL levels rise to above threshold values while on study, patients will be started on HMG-CoA reductase inhibitors (statins) for the duration of the study. Only pravastatin or rosuvastatin will be utilized due to concern for cytochrome P450 interactions with sirolimus. Pravastatin is the preferred agent at the NIH clinical center. After 56 days (plus 2-3 days to allow flexibility for scheduling of follow-up procedures), when the study is complete, if subjects' fasting LDL remains above target, they will be referred back to their primary care physician for further management of hyperlipidemia.

A fasting triglyceride > 200mg/dL prior to enrollment will prompt referral back to the subjects' primary care provider for appropriate medical therapy and lifestyle modifications prior to study enrollment. Once the fasting triglyceride level is below 200mg/dL, subjects would be eligible to enroll on the protocol. If the fasting triglycerides rise to > 200mg/dL while on study, HMG-CoA reductase inhibitors will be initiated as as above. If the triglycerides rise to >499 mg/dL while on study, we will start gemfibrozil 600mg bid or clofibrate 1000mg bid or nicotinic acid. If the fasting triglycerides remain above threshold despite appropriate medical therapy and dietary modifications, we will continue the study agent (total of 56 days, plus 2-3 days to allow flexibility for scheduling of follow-up procedures) unless a grade III hypertriglyceridemia occured. After 56 days (plus 2-3 days to allow flexibility for scheduling of follow-up procedures) when the study is complete, if subjects' fasting triglyceride remains above target, they will be referred back to their primary care physician for further management of hypertrigyceridemia.

4.5.2 For PHTS subjects with malignancy

Given the fact that these subjects will be treated with sirolimus for advanced malignancy, we will tolerate higher fasting LDL and triglyceride levels irrespective of risk category before instituting medical therapy. All patients in this cohort will have a threshold of fasting LDL >189mg/dl or fasting hypertriglyceridemia of >200mg/dl, which will trigger initiation of HMG-CoA reductase inhibitors. Only pravastatin and rosuvastatin would be utilized to avoid any cytochrome P450 interactions with sirolimus. Pravastatin would be initiated at a dose of 40 mg orally once daily or rosuvastatin 20 mg orally once daily. Pravastatin is the preferred HMG-CoA reductase inhibitor as it is on formulary at the NIH Clinical Center. If the levels remain above the above-mentioned thresholds at the beginning of the next cycle, pravastatin will be doubled to 80 mg orally once daily or rosuvastatin will doubled to 40 mg once daily. If they remain above this threshold at the beginning of the following cycle, we will continue with the study until subjects meet criteria for grade III hypercholesterolemia. If triglycerides remain 200-499 after LDL goal is reached we will consider adding gemfibrozil 600 bid or clofibrate 1000 mg bid or nicotinic acid. For fasting triglyceride >499 mg/dl, we will start gemfibrozil 600 mg bid or clofibrate 1000mg bid or nicotinic acid. When triglycerides are below 500 mg/dl, we will then initiate LDL-lowering therapy (i.e HMG-CoA reductase inhibitors). If triglycerides remain above 500 mg/dl despite medical

management and dietary modifications, we will continue with the study medication until subjects meet criteria for grade III hypertriglyceridemia.

4.6 CORRELATIVE STUDIES

4.6.1 Measurement of pathway activation

4.6.1.1 Immunohistochemistry

Formalin-fixed, paraffin-embedded tumor biopsies from skin, and/or lower GI tract and/or malignant tissue will be assessed for activation of p-S6K, p-S6, p-4E-BP1, pS2448-mTOR, pS473-Akt, pIRS-1 levels using immunohistochemical techniques [90]. If tissue is scarce, we will prioritize measuring levels of p-S6, p-S6K and p-4E-BP1 as these are the downstream substrates of mTOR. Internal controls for IHC analysis will include slides made from cell blocks of lung cancer cell lines that have wide variation of pathway activation, which will assist assessment of staining intensity. Scoring of tumor biopsies will incorporate distribution and intensity of staining, and will be scored as being positive or negative as previously described [91].

4.6.1.2 Immunoblotting

Two speckled blue top tubes, 8ml each, will be collected from each patient on or about days 0 (prior to study drug administration), 14, and 56 to study inhibition of the pathway by sirolimus in peripheral blood mononuclear cells (PBMC) and to correlate pathway inhibition with levels of sirolimus. Subjects will have additional immunoblotting of PBMCs 1 and 2 months following cessation of sirolimus. Those subjects with PHTS and malignancy who will be continuing on the study beyond cycle 2 will have research blood drawn for PBMC analysis about every 2 cycles while on study. Levels of PTEN, and activation of mTOR, S6K1, 4E-BP1, S6, IRS-1 and Akt will be assessed by immunoblotting of PBMC lysates from each patient. Paired specimens will be run simultaneously and band intensity will be quantified via densitometry using NIH Image software. Levels of phospho-proteins will be normalized to corresponding levels of total proteins, and changes in phosphorylation during therapy will be compared to basal levels. This technique has been successfully employed in other protocols using rapamycin analogues [92].

4.6.2 Processing, Collection, and Storage of Tissue and Research Blood

The laboratory of Dr. William D. Figg, Pharm.D., will collect and store all specimens. The specimens will be labeled with a four digit number, the left two digits are the year of collection and the right two digits are the number of the patient enrolled on the protocol. A log- book that correlates the patient's four digit code with the actual name of the patient will be kept in the laboratory of Dr. William Figg. This information will be kept confidential. An abbreviation for the cycle and day the specimen was collected (e.g. D1, D2, D28) will be utilized.

Any residual samples will be stored permanently in the laboratory of Dr. William Figg. The IRB will be updated in the continuing review regarding the status of the specimen and any use beyond this protocol. We will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher.

Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if the risks of the new questions were covered in the consent document. If new risks are associated with the research (e.g. analysis of germ line genetic mutations), the principal investigator or the protocol chairperson must amend the protocol and obtain informed consent from all research subjects.

4.6.3 Digital Dermoscopy

Digital dermoscopy will be performed at baseline and around cycle 1 day 14 and cycle 2 day 28 while on therapy by Dermatology Branch personnel in the dermatology clinic of the NIH Clinical Center. For those PHTS subjects with malignancy who may be continuing on the trial, digital dermoscopy will be performed about every 2 cycles after cycle 2. For those patients with benign disease who by clinical judgment were thought to have regression of skin lesions while on sirolimus, dermoscopy will be performed at around 1 and 2 months off therapy to assess duration of response.

4.6.4 PET/CT scans

4.6.4.1 Technique

Patients will fast and avoid strenuous activity for 12 h before the PET/CT study. Baseline serum glucose will be obtained before the examination and must be below 130 mg/dl (7.2 mmol/l) before FDG injection. Diabetic patients will be managed on an individual basis in cooperation with the PET Department. FDG-PET studies will be performed 60 ± 10 min after intravenous injection of 15mCi. Three-dimensional acquisitions will be made using a dedicated PET-CT system (Discovery, GE Healthcare, Waukesha, WI) at the NIH PET department. The patient will void prior to imaging to reduce bladder activity. 15mCi of F-18 FDG will be utilized. All foci of elevated 18F-FDG uptake with SUV >2.0 will be considered suspicious for viable tumor. All repeat scans will be obtained on the same equipment using the same imaging conditions. Serial SUV activity as well as tumor diameter will be measured and recorded.

4.6.4.2 Analysis

For those subjects with a "postive" baseline PET/CT (defined as one or more lesions with FDG avidity $\geq$ 2 SUV or $\geq$ 1cm in size), repeat PET/CT scans will be performed around cycle 1 day 15 and will be compared to baseline research PET/CT from baseline. Serial PET/CT will assess the effects on metabolic activity in tumor tissues based on evidence that activation of the PI3K/Akt/mTOR pathway controls hexokinase activity [93], which controls retention of fluoro-deoxyglucose. We will attempt to correlate pathway inhibition with drug levels, toxicity, as well as changes in tumor metabolism and/or volume. PHTS subjects with benign hamartomatous disease will not undergo more than 2 research PET/CT's. Those PHTS subjects with malignancy who will continue on sirolimus will have follow-up PET/CT performed only as clinically indicated.

4.6.4.3 Risks

There is risk that is slightly greater than minimal from the administration of the fluorodeoxyglucose (FDG) as relates to the radiation exposure and development of cancer. Additionally, there is a rare risk of allergic reactions or vagal responses to injection of FDG. Prior to PET/CT scan, subjects will be required to sign a radiation safety consent form outlining the risks involved given the estimated cumulative radiation exposure they will receive by participating in the study.

4.6.5 Photography

Patients will undergo photography about Cycle 1 Day 1, Cycle 1 Day 15, and Cycle 2 Day 28 at the NIH patient photography studio. The images will be stored digitally in the clinical photography department, Building 10, Room B2C111. For those patients with benign disease who by clinical judgment were thought to have regression of skin lesions while on sirolimus, photography will be performed at around 1 and 2 months off therapy to assess duration of response.

4.6.6 Neuropsychological Testing

In mouse models, deletion of PTEN in the CNS results in neuronal hypertrophy and proliferation resulting in abnormal social interaction, providing a possible link with macrocephaly [94], and cognitive and developmental delay observed in PHTS, especially Lhermitte-Duclos and BRRS. PTEN mutations have also been reported in individuals with autism spectrum disorders (ASD), although a causal link between PTEN and ASD remains unclear [95]. Pre-clinical studies suggest that rapamycin administration to PTEN mutant mice inhibits macrocephaly by inhibiting neuronal hypertrophy. This is accompanied by a beneficial effect on the control of several abnormal behaviors [96]. This trial will investigate whether neuropsychological function will be modulated by a course of sirolimus in patients with PHTS.

Neuropsychological testing has been incorporated into trials of sirolimus in patients with tuberous sclerosis complex (TSC), another hamartomatous syndrome where mTOR is activated. TSC is associated with mental retardation, autism, and epilepsy. In studies of TSC 1 and 2 mutant mice, treatment with rapamycin markedly improves phenotype and mouse survival and at a cellular level reduces neuronal cell size and improves myelination [97, 98]. As part of an ongoing clinical trial of sirolimus in patients with TSC, neuropsychological testing is being performed and an interim analysis has found no evidence of clinically significant deterioration in memory or executive function [99].

Assessment of mood, attention, working memory, verbal and nonverbal memory, abstract reasoning and visuospatial abilities will be assessed with a battery of well normed neuropsychological tests. Patient results will be compared to normative values based on age. Neuropsychological tests will be completed in one session of approximately 2 hours duration, with ample time for explanation, breaks and debriefing included in that time estimate. The following tests will be administered:

WASI: four subtests assessing visuospatial abilities, word knowledge, abstract reasoning. Yields a Verbal IQ, Performance IQ and Full Scale IQ.
Beck Depression Inventory: assessment of mood
Hopkins Verbal Learning Test: verbal memory, immediate, delayed and recognition
Brief Visuospatial Memory Test-Revised: memory for visual information, immediate, delayed and recognition
Neuropsychological Assessment Battery: Attention Module: comprehensive assessment of attention and working memory
Trail Making Test: processing speed and working memory

Neuropsychological assessment will take place at baseline, at around 4 weeks of treatment and at the completion of treatment. In addition, a follow-up neuropsychological assessment will occur 2 months following cessation of treatment to assess for any changes in neuropsychological function once off therapy.

4.6.7 Clinical Videography

Patients will undergo clinical videography on Cycle 1 Day 15 (+/- 3 days), Cycle 1 Day 28 (+/- 3 days), and Cycle 2 Day 28 (+/- 3 days), plus periodic evaluations post-therapy, at the NIH Clinical Center. The purpose of the videography is to record the testing of cerebellar function with regard to movement, balance, and coordination. Patients will be consented according to NIH Medical Executive Committee policy. The images will be converted from tape to DVD by the clinical photography department, Building 10, Room B2C111, for storage.

4.7 SURGICAL GUIDELINES

4.7.1 Skin biopsies

4.7.1.1 Evaluation in the Dermatology clinic

The Department of Dermatology at the NIH Clinical Center will be performing up to six skin 3 mm tissue biopsies at a time. The patients will arrive at NIH Dermatology clinic. Consent will be obtained for each individual patient for pictures, tissue biopsies and skin exam. A full skin exam will be performed with an appropriate stand-by. Documentation of each individual skin lesion will be placed on the appropriate diagram. Up to six lesions will be identified for biopsy. The lesions will be chosen based on clinical relevance. Cosmetically sensitive areas such as the face will be avoided. The skin will be cleansed with an alcohol swab. An appropriate surgical drape will be applied. The 6 identified areas will be anesthetized under local anesthesia, lidocaine 1% with epinephrine. Once anesthetized, the 6 individual lesions will be removed with a 3 mm punch technique. Once hemostasis has been obtained, closure will be performed with 5.0 prolene. The biopsy site will be covered with petrolatum and a bandage. Wound care instructions will be provided. The patients will be advised to have their sutures removed in 7 days. The tissue obtained will be placed in formalin labeled bottles. A small portion of each lesion will be sent for routine histopathological examination and the remaining tissue will be stored in the laboratory of Dr. William Figg.

On or about C1 D14 and C2 D28, the patients will return to the dermatology clinic for up to 6 more tissue biopsies. The biopsies will be performed at sites in close proximity to the original biopsies. As per above, the same procedure will be performed.

Once all patients have been observed, all the diagrams and documentation of the skin lesions will be reviewed. The number, type and distribution of lesions will be accumulated. At the conclusion of the study, a report would be generated to record any changes in skin lesions after treatment with sirolimus. Once enrolled on the protocol, patients may refuse skin biopsy at any time and still be eligible to receive the study drug and all other correlative studies.

4.7.1.2 Contraindications

Contraindications to skin biopsy are an uncorrected bleeding disorder, tendency to keloid formation, a cutaneous infection at the site of the biopsy, and allergy to lidocaine.

4.7.1.3 Risks and Hazards

Potential complications of skin biopsy include pain, bleeding, infection, scar, and keloid formation.

4.7.2 Sigmoidoscopy

In flexible sigmoidoscopy, only the lower portion (rectum, sigmoid, and descending colon) of the large intestine closest to the anus is evaluated. Cleaning this part of colon is much simpler and entails the use of two enemas. Additionally, as this is a quick procedure, patients generally tolerate the procedure well with no need for sedation. This procedure usually takes 5 to 15 minutes. Flexible sigmoidoscopy will be performed by a trained gastroenterologist at the NIH clinical center. At the initial baseline sigmoidoscopy prior to sirolimus treatment, the total number of lesions in the lower portion of the large intestine will be divided into three, and one third will be biopsied at each sigmoidoscopy. The maximum number of lesions to be biopsied will be 18 (3 x 6), which is based on the statistical needs of the study. The gastroenterologist will biopsy the lesions and determine if the sample is adequate. The gastroenterologist will use jumbo forceps for these biopsies. These forceps have an open span of 8 mm, and biopsy superficial mucosa without interfering with muscularis propria or submucosa. The average biopsy specimen is 2 to 3 mm in diameter, with an estimated volume of 14 mm^3 and an estimated weight of 9.8 mg. Follow up flexible sigmoidoscopy and biopsy will occur around cycle 1 day 15 (provided there were lesions on initial sigmoidoscopy) and cycle 2 day 28. A portion of the biopsy specimen will be submitted for routine histopathology and the remainder will be placed in previously labeled cryovials (one biopsy per vial) and snap frozen in liquid nitrogen and stored in the laboratory of Dr. William Figg.

The procedures will be performed in the GI clinic at the NIH Clinical Center by a trained gastroenterologist. Patients will be counseled by the GI team as to risks, benefits, as well as preparatory regimens and post procedure activities. These risks may include: pain, risk of bleeding secondary to the sampling process, risk of infection secondary to introduction of the sterile sampling device(s), and risk of perforation. The consent process is separate from this protocol and will be obtained by the physician carrying out the sigmoidoscopy. Patients have the right to refuse the procedure at any time. Once enrolled on the trial, patients who refuse flexible sigmoidoscopy will be allowed to remain on the trial until s/he meets off study criteria as outlined in Section 4.9.2.

4.7.3 Biopsy of 'Other' Tissue

Biopsy of tissue other than skin and distal large intestine (by sigmoidoscopy) will only be performed for one of two reasons:

If PET/CT leads to the identification of lesions that are clinically suspicious for malignancy, then biopsy is required for clinical purposes to rule out cancer. The algorithm to determine suspicious lesions for malignancy is outlined in figure 6. Biopsies in this setting will only be obtained for clinical purposes to rule out malignancy and not for research purposes. If multiple lesions are present, the largest and most metabolically active lesion will be biopsied. If there is no difference in size or metabolic rate in a number of lesions in the same organ, the lesion that can be biopsied with the least morbidity will be biopsied, and will be presumed to be representative of the lesions in that organ. The Clinical Pathology Department at the CRC will process and analyze biopsied specimens. If PHTS patients with advanced, incurable malignancy have easily accessible tumor to biopsy (i.e. lymphadenopathy or subcutaneous metastasis), biopsy optional biopsy will be performed to allow comparison between benign and malignant tumors within the same patient. These patients may undergo optional tumor biopsy at baseline and around cycle 1 day 15 and cycle 2 day 28 (provided there was evidence of mTOR inhibition on cycle 1 day 15 biopsy) for research purposes. Biopsy will only be performed if deemed to be of low risk to the patient by the specialist performing the procedure and by the primary team. The use of imaging to facilitate biopsy will be determined by members of the Interventional Radiology team (or other specialist) and may include ultrasound or MRI. Local anesthesia will be administered to decrease pain associated with the procedure. If the PHTS subject with malignancy refuses a tumor biopsy, s/he will still remain on the study and

receive study medication, and all other correlative studies will be performed. Tissue samples will be submitted for routine histopathology and the remaining samples will be placed in previously labeled cryovials (one biopsy per vial) and snap frozen in liquid nitrogen and stored in the laboratory of Dr. William Figg.

4.8 RADIATION THERAPY GUIDELINES

There are no radiation therapy modalities administered in this protocol.

4.9 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

4.9.1 Off Treatment Criteria

4.9.1.1 Completion of 56 days (plus 2-3 days to allow flexibility for scheduling of follow-up procedures) of sirolimus for PHTS subjects with benign hamartomas.

4.9.1.2 Progression of disease (see Section 6.2.3) for PHTS subjects with malignancy as determined by CT scans (or alternate scans as clinically indicated) obtained every 2 cycles while on study.

4.9.1.3 Unacceptable toxicity as defined in Section 4.3

4.9.1.4 The patient may withdraw from the treatment at any time for any reason.

4.9.1.5 Medical or psychiatric illness, which in the investigator's judgment renders the patient incapable of further therapy.

4.9.1.6 Non-compliance with the oral regimen judged as missing >7 days' worth of sirolimus, unless this was secondary to resulting toxicities and coordinated with the principal investigator and/or study chairperson. All reasons for discontinuation of treatment must be documented in the medical record and the CRO must be notified.

4.9.2 Off Study Criteria

4.9.2.1 If a patient meets any of the criteria for 'Off Treatment Criteria' as outlined in Sections 4.9.1.2, 4.9.1.4, 4.9.1.5 or 4.9.1.6, s/he will also be taken off study.

4.9.2.2 If a patient develops an unacceptable toxicity requiring discontinuation of therapy, and met criteria to have one additional set of procedures/tests performed (see page 31, Section 4.3 'Treatment Modifications', last paragraph), the patient will be taken off study after these studies have been performed.

4.9.2.3 PHTS patients with benign hamartomas who completed 56 days (plus 2-3 days to allow flexibility for scheduling of follow-up procedures) of sirolimus will come off study after 2 months of follow-up

4.10 POST THERAPY EVALUATION (FOLLOW-UP)

Once off therapy, patients will receive the required supportive care for toxicities until stabilized or resolved. Patients are strongly encouraged to resume care with their previous medical provider. Patients with PHTS and benign hamartomas who by clinical judgment had evidence of regression of

skin lesions will have follow-up visits and testing as outlined in 'Periodic Evaluations off Therapy', Section 4.4.3.

5 SUPPORTIVE CARE

5.1 MEDICAL CARE

All medical, nutritional, and psychosocial care related to toxicities resulting from treatment will be provided until toxicities are stabilized or resolved.

5.2 HYPERCHOLESTEREMIA AND HYPERTRIGLYCERDEMIA

Subjects with PHTS and benign hamartomas with uncontrolled fasting LDL and triglyceride levels will be treated according to NCEP ATPIII guidelines prior to study enrollment. All subjects will be treated with cholesterol lowering agents should fasting LDL or triglyceride levels become elevated in the course of the study. Please see Section 4.5 for further details.

5.3 NUTRITIONAL AND PSYCHOSOCIAL TREATMENT

During cancer treatment and during treatment of rare conditions, it is sometimes difficult for patients to maintain good nutrition, either due to cachexia or due to depression. If it is deemed necessary, or it could benefit the patient, the patient will be referred for a nutritional consultation. Patients who are having emotional difficulty coping with their disease and/or their treatment will be referred to a social worker for evaluation and support.

5.4 PNEUMONITIS

Patients will be evaluated regularly and oxygen or further supportive care will be provided if patients develop symptomatic pneumonitis. Consultation with a pulmonary physician will be considered. Medication will be stopped for grade II pneumonitis as per Section 4.3.

5.5 IMMUNE SUPPRESSION

If CD4+ T cell counts decline to less than 200 cells/mm^3 or the percent CD4 cells falls to less than 20%, patients will be managed according to the HIV/AIDS opportunistic infection prophylaxis guidelines as recommended by the U.S. Public Health Service and the Infectious Diseases Society of America (http://www.aidsinfo.nih.gov/ContentFiles/OIpreventionGL.pdf). Periodic physical exams will be performed with attention to monitoring for the development of lymphoma. If suspicious adenopathy develops, appropriate imaging and diagnostic testing will be performed. Given concern for immune suppression with sirolimus, stopping rules have been implemented to trigger cessation of the trial in the event of unacceptable rates of opportunistic infection as outlined in Section 7.2.

5.6 RENAL TOXICITY

Patients will be supported with parenteral and oral fluids as necessary and will be monitored closely. Consultation with a nephrologist will be considered. Medication will be stopped for grade II creatinine as per Section 4.3.

5.7 GENETIC COUNSELING

Given that documented PTEN mutation is required for entry onto the protocol, we anticipate that the majority of patients who are screened for the trial will have received genetic counseling in the community prior to enrollment. For enrolled subjects as well as potential subjects undergoing screening for the protocol, a genetics assessment will be offered by a trained clinical geneticist. Such

an assessment may include an updated three generation pedigree analysis as well as assessment of a subjects understanding of PHTS and their level of communication regarding the syndrome to other family members. In addition, genetic counseling will be offered to family members of subjects. Any testing for germline PTEN mutation in potential protocol patients may be performed in the CLIA approved laboratory of Dr. Charis Eng of the Cleveland Clinic.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The protocol chairperson and/or the principal investigator of the protocol will be responsible for the collection, maintenance, security and quality control of all study data. Meetings chaired by the principal investigator and/or protocol chairperson will be held every 2 weeks to review the study data for quality, completeness, and interim analysis (see Section 7.2). Data will be captured on the NCI C3D database. Research records will be maintained to include but not limited to the following:

- Signed, dated consent form
- Completed eligibility checklist
- The sequence analysis of the PHTS mutation or deletion
- Source documents verifying eligibility criteria such as pathology reports
- Clinical photography images
- Recorded videography exams
- Dermoscopy images
- Adverse events assessment
- Interim monitoring test results
- Interim history and physical exam findings (i.e. new medications, change in history including smoking history, head circumference, palpable subcutaneous nodules)
- Response evaluations
- Correlative studies results
- Off study summary

6.2 RESPONSE CRITERIA

All subjects will be assessed for biochemical changes of the downstream substrates of the mTOR pathway in paired tissue biopsies and PBMCs as outlined in Section 7. Changes in number or size of hamartomas as assessed by PET/CT, dermoscopy, and/or digital photography will also be assessed. Changes in biochemical properties, number or size of benign tissues will not be subject to response criteria as there are no established criteria to define response for benign tissue. Such changes will undergo statistical analysis as outlined in Section 7.

For PHTS subjects with malignancy, response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [100]. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria. Note: Lesions are either measurable or non-measurable using the criteria provided below. The term "evaluable," in reference to measurability, will not be used because it does not provide additional meaning or accuracy.

6.2.1 Definitions of Disease State

6.2.1.1 Measurable disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm with conventional techniques (CT, MRI, x-ray) or as >10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

6.2.1.2 Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than four weeks before the beginning of the treatment.
Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. Tumor lesions that have been biopsied previously might or might not be considered measurable.

6.2.1.3 Non-measurable disease

All other lesions (or sites of disease), including small lesions (longest diameter <20 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

6.2.1.4 Target lesions

All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

6.2.1.5 Non-Target lesions

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as non-measurable lesions. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

6.2.1.6 Clinical lesions

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

6.2.2 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions

Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD

Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

6.2.3 Evaluation of non-target lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level

Incomplete Response/Stable Disease (SD): Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

Although a clear progression of "non-target" lesions only is exceptional, in such circumstances the opinion of the treating physician should prevail and the progression status should be confirmed at a later time by the principal investigator or the study chairperson.

6.2.4 Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR

PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration." Every effort should be made to document the objective progression, even after discontinuation of treatment.

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

6.2.5 Confirmatory Measurement/Duration of Response

6.2.5.1 Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed four weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry and at a minimum interval of eight weeks (see Section 6.2.1).

6.2.5.2 Duration of overall response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

6.2.5.3 Duration of Stable Disease

Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

6.2.6 Progression-Free Survival

Progression free survival will be determined from the date treatment began until radiographic evidence of progressive disease is noted.

6.2.7 Response Review

Patients with measurable disease will be assessed by standard criteria. The purpose of tumor measurements will be to assess benefit to the patients from treatment and to determine

appropriateness for continuing on study. For the purposes of this study, patients should be re-evaluated every cycle and undergo imaging studies every two cycles. Following documentation of an objective response, confirmatory scans will also be obtained four weeks after. The response will be evaluated by Dr Peter Choyke from the radiology department at the NIH Clinical Center.

6.3 TOXICITY CRITERIA

This study will utilize the CTCAEv3 for toxicity and adverse event reporting. A copy of the CTCAEv3 can be downloaded from the internet (**http://ctep.cancer.gov/forms/CTCAEv3.pdf**). All appropriate treatment areas should have access to a copy of the CTCAEv3.

7 STATISTICAL CONSIDERATIONS

7.1 PRIMARY STATISTICAL ANALYSIS

The primary endpoint of this trial will be to assess whether treatment with sirolimus in subjects with germline PTEN mutations will result in biochemical changes, namely inhibition of the mTOR pathway, as assessed using immunohistochemistry in benign lower gastrointestinal lesions and skin, as well as in malignant tumors. Skin biopsies may be included in the analysis, but the goal will be to try to obtain up to 6 'paired' biopsies at a given time point (up to 6 each at baseline, around 2 weeks, and around 8 weeks) from the lower GI tract (and accessible malignant tumor in patients with cancer) in 10 of 20 patients because of the possibility of disappearance of skin lesions following sirolimus treatment. A total of 6 lesions will be biopsied at any one time. For example, if 2 skin lesions are biopsied, than 4 lower GI hamartomas will be biopsied.

A biochemical change is defined as a decrease in the phosphorylation of S6K, S6, or 4E-BP1, as assessed via immunohistochemistry within the same patient, comparing baseline phosphorylation levels with levels at 2 and 8 weeks of sirolimus therapy. IHC will be quantified using a scoring system that utilizes intensity and distribution of staining as noted below [101]. Scoring of phosphorylation of pathway components will be based on distribution and intensity of staining. Distribution will be scored as 0 (0%), 1 (1% to 50%), and 2 (51% to 100%) to indicate the percentage of positive cells of interest in a single core. The intensity of the signal will be scored as 0 (no signal), 1 (weak), 2 (moderate), and 3 (strong). The distribution score and intensity score will then be summed into a total score (TS) of TS0, TS1, TS2, TS3, TS4, or TS5. Staining will be scored independently by two investigators who will be blinded to the clinical information, and will be supervised by two pathologists. Equivocal or borderline cases will be re-examined, and a consensus score will be reached by the observers. When assessing one variable for a given tumor, the observers will be blinded to the scores of the other variables and to outcome. A score of TS0-TS2 will be regarded as negative, whereas TS3-TS5 will be regarded as positive. Immunohistochemistry will be performed and interpreted in the NCI Laboratory of Pathology, under the direction of Dr. Stephen Hewitt, MD, PhD. Dr. Hewitt will be assisted by Dr. Marc Raffeld, M.D., also of the NCI Laboratory of Pathology, who is in the process of developing a CLIA approved test to measure activation of components of the Akt/mTOR pathway. Controls for this assay will be tissues and cell blocks known to be negative or positive for the pathway component of interest, as well as surrounding normal tissue when possible.

This pilot study will enroll a total of up to 15 subjects, and it would be desirable if at least 10 of these patients have 6 paired lesions amenable to biopsy for IHC, in order to create a measure with reasonable statistical precision. Prior to receiving sirolimus, biopsies will be obtained. For each patient, the average score for the up to 6 lesions will be obtained, and will represent the baseline

mean biochemical score. After approximately 2 and 8 weeks of sirolimus administration, biopsy of up to 6 matching lesions will be obtained and the average score will be calculated and will represent the mean biochemical score at 2 and 8 weeks, respectively. As a practical matter, if at baseline there are more than 18 total lesions to select (over 0, 2, and 8 weeks), 6 will be chosen at random to be used for the baseline evaluation and 6 matching lesions will be selected for evaluation at 2 weeks and 6 matching lesions for evaluation at 8 weeks. If there are fewer than 18 total potential lesions, the available number will be divided into three approximately equal sized groups for evaluation at each time point. (For example, if there are 12 available lesions at baseline, 4 will be biopsied on day 0, 4 will be biopsied at week 2 and 4 will be biopsied at week 8). The difference between the three mean values per patient will be determined by subtraction (that is 2 weeks – baseline and 8 weeks – baseline), and this will be evaluated as to whether it differs significantly from 0 by a paired t-test if normally distributed, or a Wilcoxon signed rank test if not normally distributed. The analysis will be done two ways: one including all patients enrolled and the second restricted to patients who have 18 total biopsied lesions—more lesions will yield a more reliable result. As this is a pilot study, both results will be used to help interpret the biochemical changes identified. The latter result may be more precise as the mean score will be determined based on more lesions. Under an assumption of normality, with at least 10 paired subjects, there will be at least 80% power to detect a difference equal to one standard deviation of each of the paired differences with a two-sided 0.05 alpha level t-test. There is no expectation of a biochemical change over a two and eight week period in the absence of treatment; thus a control group is not required to evaluate if any changes noted are meaningful in a pilot study. However, since regression to the mean is a possibility, even if unlikely, should a positive finding be realized in this pilot study, then any subsequent study should involve a randomized design employing a control arm. In the unlikely event that lesions disappear completely at 2 or 8 weeks, then analyses will be performed both including and excluding patients whose lesions disappear.

The lesions may be located on the skin, lower GI tract or accessible malignant tumor for patients with cancer. In our prior experience with Cases 1 and 2 (PHTS patients with malignancy treated with sirolimus- see Section 1.2), tissue analysis yielded consistent biochemical changes irrespective of anatomic site. Therefore, we anticipate that sirolimus will produce similar biochemical changes in the mTOR pathway in different tissue. To confirm this expectation, the study will allow all sites to be evaluated together, but will also evaluate changes within each anatomic site, provided sufficient data exist. The changes in biochemical and/or metabolic values between various sites of disease will also be compared in an exploratory manner due to limited numbers of subjects.

7.2 SECONDARY STATISTICAL ANALYSIS OF BIOLOGICAL DATA

Secondary endpoints of the study include: biochemical changes and duration of changes in the mTOR pathway as assessed by immunoblotting in PBMCs, changes in tumor size and duration of change as assessed by CT, digital photography, digital dermoscopy, changes in tumor metabolism as assessed by PET, changes in lymphocyte counts, and changes on a neurpsychological test battery.

The aforementioned biologic markers will be evaluated at baseline, around day 14 or day 28, as well as around day 56. PHTS subjects with benign disease who had evidence of regression of skin lesions will have additional clinical photography and digital dermoscopy, and all patients will have additional immunoblotting of PBMCs and lymphocyte subsets performed around months 1 and 2 off therapy to evaluate duration of changes. In addition, repeat neurpsychological testing will be performed around 2 months post-sirolimus treatment. Changes from baseline will be determined in either absolute or relative terms as appropriate, and evaluated for statistical significance, as well as to

determine if the changes or the actual values at a time point are associated with clinical response. Paired comparisons with baseline will be done using a paired t-test or Wilcoxon signed rank test as appropriate, and the changes will be compared between responders and non-responders using a two sample t-test (adequate numbers of subjects with normally distributed data in both groups) or a Wilcoxon rank sum test, if enough patients respond for this to be appropriate.

In all such cases, these secondary analyses will be considered exploratory and not formally adjusted for multiple comparisons. However, to ensure proper interpretation in the context of a potentially large number of explorations being performed, only p-values <0.01 will be interpretable as being associated with statistical significance.

Immunoblotting of PBMCs will be quantified using densitometry comparing levels of phosphorylated pathway components to total levels of expression and/or a standard (beta-actin). Pathway components to be checked include PTEN, p-AKT (S473), p-AKT (T308), p-mTOR (S2448), mTOR, p-S6k (T389), p-S6 (s235/6), p-4EBP1 (s65). Expression of other biomarkers may also be assessed in an exploratory fashion. Immunoblots will be analyzed as outlined in Section 4.6.1.2.
nMetabolic changes in the benign and/or malignant tissues from PHTS patients will be evaluated by use of PET scans employing quantitative SUV measurements. These evaluations will be considered secondary and exploratory since it is uncertain how to interpret the PET results in these patients. It has been noted that sirolimus may be associated with disappearance of benign lesions altogether. Changes in the number or size of hamartomas will be measured by PET/CT, dermoscopy or digital photography. Any evaluations of these secondary endpoints will be performed without formal adjustment for multiple comparisons, but any reported findings will be described in the context of the number and type of explorations performed.

Given concerns for immune suppression with sirolimus, we have instituted the following stopping rules. If within the first 8 patients, two patients develop opportunistic infection (as defined by the 1993 AIDS surveillance case definition, see Appendix Section12.6) while on sirolimus, the trial will be terminated for all subjects, as 2 out of 8 patients with an opportunistic infection could be a potentially excessive rate (the associated upper one sided 80% confidence interval boundary is 0.46, indicating a rate potentially consistent with as high as a 46% event rate).

In addition, we will be following lymphocyte counts as well as lymphocyte subsets and monitoring for changes with sirolimus therapy and sirolimus drug levels. If CD4+ T cell counts decline to less than 200 cells/mm^3 or the percent CD4 cells falls to less than 20%, institution of prophylaxis against pneumocystis pneumonia or other appropriate therapy will be instituted as recommended by the U.S. Public Health Service and the Infectious Disease society of America guidelines (http://www.aidsinfo.nih.gov/ContentFiles/OIpreventionGL.pdf).

Our group is in contact with over 100 families affected by this syndrome and could recruit up to 15 subjects who meet eligibility criteria within 24 months.

8 HUMAN SUBJECTS PROTECTIONS

8.1 RATIONALE FOR SUBJECT SELECTION

This study will be open to individual with PHTS, regardless of gender, ethnicity, or race provided that the aforementioned inclusion and exclusion criteria are met. For safety reasons, pregnant or lactating women, subjects with human immunodeficiency virus seropositivity, subjects with

interstitial lung disease and pneumonitis, subjects with pneumocystis jirovecci pneumonia, subjects with bleeding diathesis, and children are excluded from this study. This study will be recruited through internal referral, our local physician referral base, and through various cancer information hotlines (i.e., Clinical Studies Support Center, 1-800-4Cancer). Patients should realize that we are hopeful that they may gain benefit from this study, but there is little objective evidence to support our hypothesis at this time. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one ethnic group compared to another. Efforts will be made to extend accrual to each representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to ineffective treatments on the one hand and the need to explore racial/ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

8.2 INCLUSION OF WOMEN AND MINORITIES

Both men and women and members of all races and ethnic groups are eligible for this trial. Every effort will be made to recruit women and minorities in this study.

8.3 JUSTIFICATION FOR EXCLUSIONS

Due to lack of knowledge of the effects of sirolimus on the fetus or on infants, as well as the possibility of teratogenic effects, pregnant and nursing women will be excluded from this trial. Some animal studies have shown fetal malformations and fetal toxicity. Patients with unstable or serious medical or psychiatric conditions are excluded due to the possibility that the underlying condition may obscure the attribution of adverse events with respect to sirolimus.

8.4 PARTICIPATION OF CHILDREN

Patients under the age of 18 will be excluded from study because of the lack of data of the effect of sirolimus in this group. The effect of sirolimus in children may be investigated in studies that exclusively enroll children.

8.5 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The potential benefit to a patient who enters study is a reduction in the bulk or rate of growth of his/her tumor and/or hamartomas, which may or may not have a favorable impact on symptoms and/or survival. A patient with benign hamartomatous lesions will not be eligible to continue treatment beyond 56 days (plus 2-3 days to allow flexibility for scheduling of follow-up procedures), even if his/her lesions decrease in size or disappear. Given the relatively short duration of exposure to sirolimus on this study, patients with benign disease are unlikely to attain any potential long-term benefit that this drug may confer. This protocol will add to our scientific knowledge and increase the potential future application of sirolimus as a possible therapeutic or preventive agent for patients with PHTS. Potential risks include the possible occurrence of any of a range of side effects that are listed in the pharmaceutical section and the consent document. The procedure for protecting against or minimizing risks will be to medically evaluate patients on a regular basis as described earlier.

8.6 RISK/BENEFITS ANALYSIS

All care will be taken to minimize risks that may be incurred by tissue sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent. If patients suffer any physical injury as a result of the biopsies, immediate

medical treatment is available at NIH's Clinical Center in Bethesda, Maryland. Although no compensation is available, any injury will be fully evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

8.7 CONSENT AND ASSENT PROCESS AND DOCUMENTATION

An associate or principal investigator or the protocol chair on the trial will inform patients of the purpose, alternatives, treatment plan, research objectives and follow-up of this trial. The patient will be provided an IRB-approved consent for review and signature and his/her questions will be answered. After a decision is made to enroll into the study, a signature will be obtained from the patient at a subsequent visit. A copy of the signed informed consent will be placed in the patient's medical record and a copy will be placed in the research chart.
All patients must have a signed informed consent form and an on-study (confirmation of eligibility) form filled out and signed by a participating investigator before entering on study.

9 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

9.1 DEFINITIONS

9.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All AEs must be recorded on the AE case report form.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drug.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

9.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

9.1.3 Unexpected adverse reaction
An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

9.1.4 Serious
An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

9.1.5 Serious Adverse Event
An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

9.1.6 Disability
A substantial disruption of a person's ability to conduct normal life functions.

9.1.7 Life-threatening adverse drug experience
Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

9.1.8 Protocol Deviation (NIH Definition)
A protocol deviation is any change, divergence, or departure from the IRB approved research protocol

9.1.9 Non-complaince (NIH Definition)
The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

9.1.10 Unanticipated Problem
Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
  (b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**

- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

9.2 NCI-IRB REPORTING

9.2.1 NCI-IRB Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report to the NCI-IRB:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All serious non-compliance

Reports must be received by the NCI-IRB within 7 working days of PI awareness via iRIS.

These reports will be reviewed, entered into the protocol database, circulated and reviewed at the next scheduled IRB meeting, and then filed in the IRB protocol file. For confidentiality reasons, PI's should remove names, addresses and social security numbers from any supplemental information submitted with the AE/SAE form. Medical record numbers and/or study ID are the only identifiers that should be used.

9.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

For reporting of adverse events at time of continuing review, the NCI-IRB requires a summary report of adverse events that have occurred on the protocol since the previous continuing review and in aggregate. The method of presentation should provide the NCI-IRB with the information necessary to clearly identify risks to participants and to make a risk:benefit determination. The summary report is based on the following guidance: any unexpected severity and/or unexpected frequency of expected events needs to be reported and interpreted in relation to the risk:benefit of study participants in the narrative.

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:

- All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
- All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
- All Grade 5 events regardless of attribution;
- All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported.

NCI IRB Contact Information:
E-mail- nciirbadmin@mail.nih.gov
Mail- Building 82/Room 100, 9030 Old Georgetown Road, Bethesda, MD, 20814
FAX- 301-480-0106

9.3 DATA AND SAFETY MONITORING PLAN

All clinical research requires monitoring to ensure the quality data and human subjects protection. There are several types of DSM plans/programs that are necessary in clinical research. The following plan will be used for this study.

9.3.1 Principal Investigator/Research Team

All subjects will be monitored closely for adverse events during their participation in this study. This includes clinical as well as laboratory findings. Expected and unexpected events will be assessed according to CTCAEv3.0 criteria, recorded in the medical record and recorded in the C3D study data files. The list of known toxicities/ adverse events for each of the study agents can be found in Section 10.1.9.

The form for reporting to the FDA is the MedWatch 3500A Form which can be found at: http://www.fda.gov/medwatch/how.html.

Any finding that may affect the patient's willingness to continue in the study will be shared with them. Confidentiality will be maintained as much as possible, consistent with applicable regulations. Names of participants or identifying material will not be released without patient permission, except when such release is required by law. No patient's name or identifying information will be released in any publication or presentation. Records are maintained according to current legal requirements, and are made available for review according to the requirements of the Food and Drug Administration (FDA) or other authorized user, only under guidelines established by the Federal Privacy Act.

10 PHARMACEUTICAL AND INVESTIGATIONAL DEVICE INFORMATION

10.1 SIROLIMUS

10.1.1 Product Description
Other Names: Rapamycin, Rapammune®
10.1.2 Classification:
Immunosuppressive agent

10.1.3 Overview

Sirolimus (Rapamycin: Rapamune®: Wyeth-Ayerst, PA, USA), first isolated from the soil bacteria *Streptomyces hygoscopicus* in 1975 was approved by the FDA in 1999 as an immunosuppressive agent for use in preventing rejection in renal transplant recipients in combination with cyclosporine and corticosteroids.

10.1.4 Mechanism of Action

Sirolimus binds to the FK Binding Protein-12 (FKBP-12). This complex binds to and inhibits the activation of the mammalian regulatory kinase, Target Of Rapamycin (mTOR). This inhibition suppresses cytokine-driven T-cell proliferation, inhibiting the progression from the G1 to the S phase of the cell cycle.

10.1.5 Molecular Weight: 914.2

10.1.6 Storage Requirements

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

10.1.7 Stability

Sirolimus tablets are stable through the manufacturer's expiration date imprinted on the product container.

10.1.8 Administration

Sirolimus is to be administered orally consistently without food, at a dose and schedule defined by the protocol dose escalation schema. Study patients are not to ingest grapefruit juice while on sirolimus therapy.

10.1.9 Toxicity

Refer to FDA labeling for complete description of adverse events associated with Sirolimus.

- Body as a Whole: asthenia, fever, chills, malignancy, abnormal healing (including wound dehiscence and anastomotic disruption), bacterial infection, viral infection, anyphylactic/anaphylactoid reactions
- Cardiovascular: hypertension, tachycardia, congestive heart failure
- Digestive: diarrhea
- Hematologic and Lymphatic: lymphoproliferative disease, lymphoma, lymphadenopathy, anemia, leukopenia, thrombocytopenia, TTP
- Hepatic: hepatotoxicity, hepatic necrosis
- Metabolic: hypercholesterolemia, hyperlipidemia, hypokalemia, edema, weight gain, cushing's syndrome, diabetes mellitus
- Musculoskeletal: arthralgia, bone necrosis
- Nervous: insomnia, tremor, headache
- Respiratory: interstitial lung disease (pneumonitis, BOOP, pulmonary fibrosis)
- Skin: acne, rash, skin cancer
- Urinary: dysuria, urinary frequency

10.1.10 Pharmacodynamics

Rapamycin is a macrolide that acts as a signal transduction inhibitor of mTOR causing cell cycle arrest at G1 [102, 103]. It inhibits the serine-threonine kinase mammalian target of Rapamycin (mTOR) by forming a complex with the FK binding protein 12 (FKBP-12). mTOR is a member of the evolutionarily conserved phosphatidylinositide 3'-kinase (PI3K)-related family and it been shown to be involved in regulating many aspects of normal and neoplastic cell growth, including organization of the actin cytoskeleton, membrane trafficking, protein degradation, protein kinase C

(PKC) signaling, and transcription [104]. The rapamycin/FKBP-12 binding to mTOR leads to dephosphorylation and inactivation of the p70S6 kinase (s6k1) and 4E-BP1. In turn, this inhibits the translation and production of ribosomal components necessary for protein synthesis and thereby Akt activation and causes cell-cycle arrest in G1 phase [105]. This series of events effectively forms the rationale for the immunosuppressant role of Rapamycin in renal transplant patients since they effectively block IL-2 stimulation of T-cell proliferation.

10.1.11 Pharmacokinetics

Mean time to peak concentration (Tmax) of sirolimus following oral administration is 1 hour after a single dose in healthy volunteers and 2 hours after multiple daily doses in renal transplant recipients. The bioavailability of sirolimus after administration of the tablet is approximately 27% higher than that of the oral solution. Clinical equivalence of the two formulations has been demonstrated at the 2mg dose level. A high fat meal altered the bioavailability characteristics of sirolimus and therefore the drug should be taken consistently without food in this protocol. Sirolimus is extensively bound to plasma proteins including serum albumin (97%), α1-acid glycoprotein and lipoproteins. Sirolimus is a substrate for the cytochrome p450 IIIA4 (CYP3A4) and P-glycoprotein. Inhibitors of CYP3A4 may decrease the metabolism of sirolimus and increase sirolimus levels, while inducers of CYP3A4 may increase the metabolism of sirolimus and decrease sirolimus levels. The majority of sirolimus is excreted in the feces (91%) with only a minor amount (2.2% excreted in the urine). Mean whole blood sirolimus trough concentrations achieved steady state concentrations within 1 day after the start of dose administration following a loading dose of three times the maintenance dose.

10.1.12 Toxicology

There are no adequate studies in pregnant women therefore effective barrier-based contraception must be initiated before, during and for 12 weeks following discontinuation of therapy with sirolimus. In animal studies, sirolimus was embryo/fetal toxic resulting in mortality and reduced fetal weight but no teratogenecity. Sirolimus was used with Tacrolimus in liver transplant patients. In those patients, there has been an excess mortality and graft loss. In addition, there has been an increased incidence of hepatic artery thrombosis within 30 days post transplantation. In lung transplant patients, cases of bronchial anastomotic dehiscence has occurred leading to death.

10.1.13 Clinical Safety Data

The incidence of adverse reactions was determined utilizing renal transplant patients; therefore, it was determined in combination with cyclosporine and corticosteroids. Two randomized, double-blind, multicenter controlled trials were conducted. The first study included 558 renal transplant patients and the second 446 renal transplant patients. Patients in the sirolimus arm in both trials received Rapamune solution formulation orally in combination with cyclosporine and corticosteroids. A third study evaluated Rapamune tablet formulation in 228 renal transplant patients in combination with cyclosporine and corticosteroids. There was no difference in toxicity between the two formulations except for an increased incidence of acne with the oral liquid formulation and an increased incidence of tremor with the tablet formulation especially in black patients. Hyperlipidemia and hypercholesterolemia were found to be dose-related effects of sirolimus therapy. During clinical trials, renal transplant patients who began sirolimus with normal, fasting, total serum triglycerides developed hypertriglyceridemia after treatment with sirolimus 2 mg and 5 mg. Treatment of new onset hypercholesteremia was required in 42-52% of renal transplant patients on sirolimus compared to 16% in the placebo arm and 22% in the azathioprine arm. Patients treated with sirolimus tended to develop higher creatinine and lower glomerular filtration rate compared to the placebo and azathioprine arm. During clinical trials, hypophosphatemia

occurred in 15% to 23% of patients receiving sirolimus, while hypokalemia occurred in 11% to 21% of patients. A negative dose adjustment is recommended for patients with mild to moderate hepatic impairment since their steady state levels are affected by the hepatic functional level.

10.1.14 Drug interactions

Sirolimus is a substrate for P-glycoprotein and gut and liver CYP3A4. The co-administration of a potent inhibitor or inducer of CYP3A4 may respectively increase or decrease sirolimus AUC. Inhibitors of CYP3A4 include (but are not limited to): amprenavir, atazanavir, bromocriptine, cimetidine, clarithromycin, clotrimazole, cyclosporine, danazol, diltiazem, erythromycin, fluconazole, fosamprenavir, other HIV protease inhibitors, indinavir, itraconazole, ketoconazole, metoclopramide, nefazodone, nelfinavir, nicardipine, nifedipine, ritonavir, saquinavir, telithromycin, troleandomycin (TAO), verapamil, and voriconazole. Inducers of CYP3A4 include: nevirapine, rifampicin, rifampin, rifabutin, rifapentin, phenytoin, carbamazepine, phenobarbital and St. John's Wort.

10.1.14.1 The use of live vaccines should be avoided in patients receiving sirolimus.

10.1.14.2 Grapefruit juice inhibits CYP3A4. Patients should not ingest grapefruit juice while on sirolimus therapy.

10.1.15 Agent Ordering/Availability:

10.1.15.1 Agent Ordering

Sirolimus will be procured via commercial mechanisms.

10.1.15.2 Availability

Sirolimus is available commercially as 1 mg white triangular shaped tablets and 2 mg yellow to beige tablets, supplied in bottles of 100 tablets and cartons of 100 tablets (10 blister cards of 10 tablets each).

10.2 FLUORODEOXYGLUCOSE [F-18]

The FDG in this protocol will be purchased from an authorized commercial manufacturer of FDG for human use (under ANDA, as an approved drug). The formulations will be prepared on the day of use and injected within 9 hrs of formulation. FDG is supplied in saline as a sterile non pyrogenic clear solution. The product is in liquid form and will be administered intravenously using aseptic technique.

The FDG for human use is in a sterile isotonic phosphate buffer and is passed over a terminal Millipore filter (0.22μm). The total mass amount of FDG is approximately 9 microgram, a tracer dose. FDG obtained from commercial manufacturers will be used without further quality control, as it is done by the provider.

FDG dosimetry has been tabulated using methodology of the Medical Internal Radiation Dose (MIRD) Committee of the Society of Nuclear Medicine. The FDG radiation dosimetry will be submitted to the Radiation Safety Committee for approval. FDG is not expected to have any physiological or adverse side effects. It has been used extensively at the NIH, since about 1983, and no adverse effects have been documented. FDG will be administered under the supervision of Dr. Peter Herscovitch, an NIH authorized user of radioactivity for human use.

10.3 ROSUVASTATIN

Rosuvastatin must be obtained from commercial sources. Refer to the FDA-approved package insert accompanying the medication for information about the preparation, storage, and side effects of the drug. More common side effects (1-10%) include chest pain, hypertension, palpitation, peripheral edema, headache, anxiety, depression, dizziness, insomnia, neuralgia, pain, vertigo, rash, pharyngitis, abdominal pain, constipation, diarrhea nausea, vomiting, dyspepsia, gastroenteritis, elevated liver transaminases, anemia, bruising, myalgia, arthralgia, arthritis, back pain, hypertonia, paresthesia, weakness, bronchitis, cough, rhinitis, sinusitis, and flu-like syndrome. Potentially serious side effects would warrant discontinuation of treatment (e.g. persistent transaminase elevation, myopathy, markedly elevated CPK).

10.4 PRAVASTATIN

Pravastatin must be obtained from commercial sources. Refer to the FDA-approved package insert accompanying the medication for information about the preparation, storage, and side effects of the drug. More common side effects (1-10%) include chest pain, headache, fatigue, dizziness, rash, nausea/vomiting, diarrhea, heartburn, elevated liver transaminases, myalgia, cough, and flu-like syndrome. Potentially serious side effects would warrant discontinuation of treatment (e.g. persistent transaminase elevation, myopathy, markedly elevated CPK).

10.5 GEMFIBROZIL

Gemfibrozil must be obtained from commercial sources. Refer to the FDA-approved package insert accompanying the medication for information about the preparation, storage, and side effects of the drug. More common side effects (1-20%) include dyspepsia, fatigue, vertigo, headache, eczema, rash, abdominal pain, diarrhea, nausea, vomiting, constipation. Concomitant use with an HMG-CoA reductase inhibitor may lead to myopathy or rhabdomyolysis. Potentially serious side effects would warrant discontinuation of treatment (e.g. myopathy, markedly elevated CPK).

10.6 CLOFIBRATE

Clofibrate must be obtained from commercial sources. Refer to the FDA-approved package insert accompanying the medication for information about the preparation, storage, and side effects of the drug. Side effects include: chest pain, cardiac arrhythmias, phlebitis, urticaria, rash, pruritis, alopecia, toxic epidermal necrolysis, gallstones, nausea, vomiting, diarrhea, gastrointestinal upset, stomatitis, impotence, decreased libido, leucopenia, anemia, agranulocytosis, myalgia, flu-like symptoms, myopathy, fatigue, dizziness, headache, weight gain, elevated liver function tests, proteinuria. Potentially serious side effects would warrant discontinuation of treatment (e.g. persistent transaminase elevation, myopathy, markedly elevated CPK).

10.7 NIACIN

Niacin must be obtained from commercial sources. Refer to the FDA-approved package insert accompanying the medication for information about the preparation, storage, and side effects of the drug. Side effects include cardiac arrythmias, edema, flushing, hypotension, orthostasis, palpitation, tachycardia, chills, dizziness, headache, insomnia, migraine, acanthosis nigricans, rash, pruritis, urticaria, impaired glucose tolerance, gout, phosphorous levels decreased, uric acid level decreased, abdominal pain, dyspepsia, nausea, vomiting, decreased platelet count, increased liver enzymes, myopathy (with concurrent HMG-CoA reductase inhibitor), cystoid macular edema,

dyspnea, diaphoresis. Potentially serious side effects would warrant discontinuation of treatment (e.g. myopathy, markedly elevated CPK).

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12 APPENDICES

12.1 NIH DERMATOLOGY EXAM NOTE

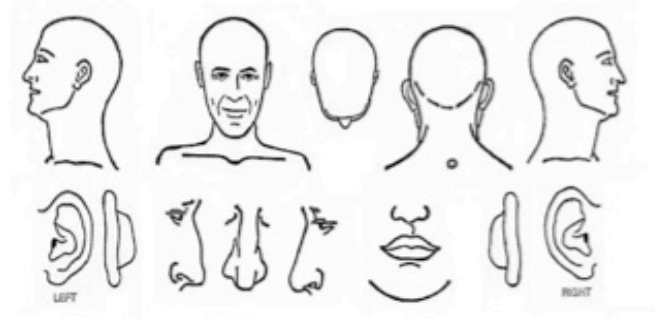
MEDICAL RECORD | **Outpatient Progress Notes**

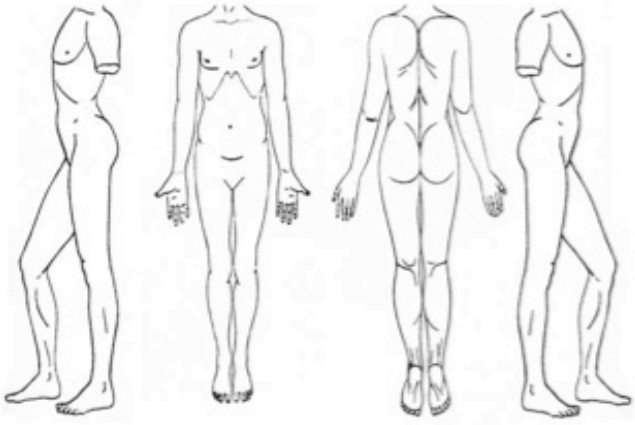
NATIONAL INSTITUTES OF HEALTH – DERMATOLOGY EXAM NOTE
Cowden Syndrome Protocol

Exam-_____0 week _____ 8 week

Skin Type: I II III IV V VI

	Normal	See Diagram		Normal	See Diagram		Normal	See Diagram
Conjunctiva & Lids	☐	☐	Scalp & Body Hair	☐	☐	Back	☐	☐
Lips, Teeth & Gums	☐	☐	Eccrine & Apocrine	☐	☐	RUE	☐	☐
Oropharynx	☐	☐	Perineum	☐	☐	LUE	☐	☐
Peripheral Vas Sys	☐	☐	Head	☐	☐	RLE	☐	☐
Axes	☐	☐	Neck	☐	☐	LLE	☐	☐
Lymph Nodes	☐	☐	Chest	☐	☐			
Digits & Nails	☐	☐	Abdomen	☐	☐			





Punch 3mm Sites
1. _____
2. _____
3. _____

☐ Site verified with patient. Time:_____
☐ Site labeled with surgical marking pen
☐ Consent Signed
☐ Area Cleansed / Prepped
☐ Anesthesia - 1% Lido with Epi
☐ Hemostasis with Drysol
☐ Closure:_____
☐ Bandage applied
☐ Wound care discused

Suture removal:
5 days / 7-10 days / 14 days

Pain upon leaving clinic
0 1 2 3 4 5 6 7 8 9 10

☐ **Patient Educated**

Patient Identification

Outpatient Progress Notes
NIH-532-10 (8-00)
P.A. 09-25-0099
File in Section 2: Progress Notes

12.2 ECOG PERFORMANCE STATUS

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

12.3 STUDY CALENDAR

Evaluation	C1 D1	C1 D15	C2 D1	C2 D15	C2 D28	Day 1 every cycle beyond C2[h]	Every 2 cycles beyond C2[h]	Off study/ treatment
Informed Consent	X[a]							
Medical History	X[a]							
Genetics assesment	X[a]							
Serum pregnancy Test (if applicable)	X[a]							
Inclusion/Exclusion Criteria	X[a]							
Physical exam (VS, HT, WT, head circumference)	X[a]		X		X	X		X
ECOG ps	X[a]		X		X	X		X
Adverse events			X		X	X		X
ECG	X[a]							
CBC	X[a]	X	X	X	X	X		
Lymphocyte Subsets	X[a]	X	X	X	X	X		X[j]
Biochemical Profile[c]	X[a]	X	X	X	X	X		
Coagulation Profile	X[a]	X	X	X	X	X		
Fasting Lipid Profile[d]	X[a]	X	X	X	X	X		
Urinalysis	X[a]	X	X	X	X	X		
Research Blood collection	X[a]	X			X		X	X[j]
Sirolimus Administration	X		X		X	X		
Research PET/CT	X[a]	X[f]						
Evaluation of malignant disease[h] (i.e. CT, MRI, US)	X[a]				X		X	X
Photography	X[a]	X			X		X	X[j]
Dermoscopy	X[a]	X			X		X	X[j]
Sirolimus level[b]		X	X		X	X		
Dermatology evaluation and Biopsy	X[a]	X			X			
Flexible Sigmoidoscopy	X[a]	X[e]			X[e.]			
Optional tumor biopsy [i]	X[a]	X			X [l]			
Neurocognitive Testing	X[a]		X		X			X[k]

[a]Must be performed within 14 days of start of cycle 1.
[b]Trough level, patient will not take the study drug until after level is drawn.
[c]Includes magnesium, sodium, potassium, chloride, bicarbonate, calcium, phosphorous, creatinine, BUN, fasting glucose, AST, ALT, total bilirubin, alkaline phosphatase, albumin, total protein, LDH, amylase, lipase.
[d] must be overnight fasting and includes triglycerides and cholesterol levels
[e] not repeated if no initial evidence of lower intestinal lesions
[f] not repeated if initial PET/CT shows no lesions $\geq$ 1cm or SUV $\geq$ 2
[h] applies only to PHTS subjects with malignancy who may be on study beyond two cycles
[i] only performed if accessible tumor in patients with malignancy
[j] evaluations/tests to be performed at months 1 and 2 post sirolimus therapy
[k] repeated around 2 months following cessation of sirolimus therapy
[l] not repeated if no evidence of mTOR inhibition at C1D15 biopsy

12.4 CYP3A4 INHIBITORS AND INDUCERS

CYP3A4 Inhibitors

Acetominophen	Diclofenac	Lomustine	Primaquine
Acetazolamide	Dihydroergotamine	Losartan	Progesterone
Amiodarone	Diltiazem	Lovastatin	Propofol
Amlodipine	Disulfiram	Mefloquine	Propoxyphene
Amprenavir	Docetaxel	Mestranol	Quinidine
Anastrozole	Doxorubicin	Methadone	Quinine
Aprepitant	Doxycycline	Methimazole	Quinupristin
Atazanavir	Drospirenone	Methoxsalen	Rabeprazole
Atorvastatin	Efavirenz	Methylprednisolone	Ranolazine
Azelastine	Enoxacin	Metronidazole	Risperidone
Azithromycin	Entacapone	Miconazole	Ritonavir
Betamethasone	Ergotamine	Midazolam	Saquinavir
Bortezomib	Erythromycin	Mifepristone	Selegiline
Bromocriptine	Ethinyl estradiol	Mirtazapine	Sertraline
Caffeine	Etoposide	Mitoxantrone	Sildenafil
Cerivastatin	Felodipine	Modafinil	Sirolimus
Chloramphenicol	Fentanyl	Nefazodone	Sulconazole
Chlorzoxazone	Fluconazole	Nelfinavir	Tacrolimus
Cimetidine	Fluoxetine	Nevirapine	Tamoxifen
Ciprofloxacin	Fluvastatin	Nicardipine	Telithromycin
Cisapride	Fluvoxamine	Nifedipine	Teniposide
Clarithromycin	Fosamprenavir	Nisoldipine	Testosterone
Clemastine	Glyburide	Nizatidine	Tetracycline
Clofazimine	Grapefruit juice (2)	Norfloxacin	Ticlopidine
Clotrimazole	Haloperidol	Olanzapine	Tranylcypromine
Clozapine	Hydralazine	Omeprazole	Trazodone
Cocaine	Ifosfamide	Orphenadrine	Troleandomycin
Conivaptan	Imatinib	Oxybutynin	Valproic acid
Cyclophosphamide	Indinavir	Paroxetine	Venlafaxine
Cyclosporine	Irbesartan	Pentamidine	Verapamil
Danazol	Isoniazid	Pergolide	Vinblastine
Dasatinib (1)	Isradipine	Phencyclidine	Vincristine
Delavirdine	Itraconazole	Pilocarpine	Vinorelbine
Desipramine	Ketoconazole	Pimozide	Voriconazole
Dexmedetomidine	Lansoprazole	Pravastatin	Zafirlukast
Diazepam	Lidocaine	Prednisolone	Ziprasidone

CYP3A4 Inducers

Aminoglutethimide	Nevirapine	Phenytoin	Rifapentine
Carbamazepine	Oxcarbazepine	Primidone	St. John's wort (3)
Fosphenytoin	Pentobarbital	Rifabutin	
Nafcillin	Phenobarbital	Rifampin	

When drugs classified as 'substrates' are co-administered with ***(Study Agent)***, there is the potential for higher concentrations of the 'substrate'. When ***(Study Agent)*** is co-administered with compounds classified as 'inhibitors', increased plasma concentrations of ***(Study Agent)*** is the potential outcome. The co-administration of 'inducers' would potentially lower plasma ***(Study Agent)*** concentrations.

Note: Adapted from Cytochrome P450 Enzymes: Substrates, Inhibitors, and Inducers. In: Lacy CF, Armstrong LL, Goldman MP, Lance LL eds. Drug Information Handbook 15TH ed. Hudson, OH; LexiComp Inc. 2007: 1899-1912.
Only major substrates and effective inducers are listed.
Additional information for drug interactions with cytochrome P450 isoenzymes can be found at http://medicine.iupui.edu/flockhart/.

(1) Investigator's Brochure: Dasatinib (BMS 354825). Bristol-Myers Squibb. October 2006.
(2) Malhotra *et al.* (2001). Clin Pharmacol Ther. 69:14-23.
(3) Mathijssen *et al.* (2002). J Natl Cancer Inst. 94:1247-1249.
Frye *et al.* (2004). Clin Pharmacol Ther. 76:323

12.5 NCEP ATPIII GUIDELINES

National Cholesterol Education Program

ATP III Guidelines At-A-Glance Quick Desk Reference

Step 1

Determine lipoprotein levels–obtain complete lipoprotein profile after 9- to 12-hour fast.

ATP III Classification of LDL, Total, and HDL Cholesterol (mg/dL)

LDL Cholesterol – Primary Target of Therapy	
<100	Optimal
100-129	Near optimal/above optimal
130-159	Borderline high
160-189	High
≥190	Very high
Total Cholesterol	
<200	Desirable
200-239	Borderline high
≥240	High
HDL Cholesterol	
<40	Low
≥60	High

Step 2

Identify presence of clinical atherosclerotic disease that confers high risk for coronary heart disease (CHD) events (CHD risk equivalent):

- Clinical CHD
- Symptomatic carotid artery disease
- Peripheral arterial disease
- Abdominal aortic aneurysm.

Step 3

Determine presence of major risk factors (other than LDL):

Major Risk Factors (Exclusive of LDL Cholesterol) That Modify LDL Goals

Cigarette smoking
Hypertension (BP ≥140/90 mmHg or on antihypertensive medication)
Low HDL cholesterol (<40 mg/dL)*
Family history of premature CHD (CHD in male first degree relative <55 years; CHD in female first degree relative <65 years)
Age (men ≥45 years; women ≥55 years)

** HDL cholesterol ≥60 mg/dL counts as a "negative" risk factor; its presence removes one risk factor from the total count.*

- Note: in ATP III, diabetes is regarded as a CHD risk equivalent.

High blood cholesterol

NATIONAL INSTITUTES OF HEALTH
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

Step 4

If 2+ risk factors (other than LDL) are present without CHD or CHD risk equivalent, assess 10-year (short-term) CHD risk (see Framingham tables).
Three levels of 10-year risk:

- >20% — CHD risk equivalent
- 10-20%
- <10%

Step 5

Determine risk category:

- Establish LDL goal of therapy
- Determine need for therapeutic lifestyle changes (TLC)
- Determine level for drug consideration

LDL Cholesterol Goals and Cutpoints for Therapeutic Lifestyle Changes (TLC) and Drug Therapy in Different Risk Categories.

Risk Category	LDL Goal	LDL Level at Which to Initiate Therapeutic Lifestyle Changes (TLC)	LDL Level at Which to Consider Drug Therapy
CHD or CHD Risk Equivalents (10-year risk >20%)	<100 mg/dL	≥100 mg/dL	≥130 mg/dL (100-129 mg/dL: drug optional)*
2+ Risk Factors (10-year risk ≤20%)	<130 mg/dL	≥130 mg/dL	10-year risk 10-20%: ≥130 mg/dL 10-year risk <10%: ≥160 mg/dL
0-1 Risk Factor†	<160 mg/dL	≥160 mg/dL	≥190 mg/dL (160-189 mg/dL: LDL-lowering drug optional)

* *Some authorities recommend use of LDL-lowering drugs in this category if an LDL cholesterol <100 mg/dL cannot be achieved by therapeutic lifestyle changes. Others prefer use of drugs that primarily modify triglycerides and HDL, e.g., nicotinic acid or fibrate. Clinical judgment also may call for deferring drug therapy in this subcategory.*

† *Almost all people with 0-1 risk factor have a 10-year risk <10%, thus 10-year risk assessment in people with 0-1 risk factor is not necessary.*

Step 6

Initiate therapeutic lifestyle changes (TLC) if LDL is above goal.

TLC Features

- TLC Diet:
  - Saturated fat <7% of calories, cholesterol <200 mg/day
  - Consider increased viscous (soluble) fiber (10-25 g/day) and plant stanols/sterols (2g/day) as therapeutic options to enhance LDL lowering
- Weight management
- Increased physical activity.

7

Step 7

Consider adding drug therapy if LDL exceeds levels shown in Step 5 table:

- Consider drug simultaneously with TLC for CHD and CHD equivalents
- Consider adding drug to TLC after 3 months for other risk categories.

Drugs Affecting Lipoprotein Metabolism

Drug Class	Agents and Daily Doses	Lipid/Lipoprotein Effects		Side Effects	Contraindications
HMG CoA reductase inhibitors (statins)	Lovastatin (20-80 mg) Pravastatin (20-40 mg) Simvastatin (20-80 mg) Fluvastatin (20-80 mg) Atorvastatin (10-80 mg) Cerivastatin (0.4-0.8 mg)	LDL HDL TG	↓18-55% ↑5-15% ↓7-30%	Myopathy Increased liver enzymes	Absolute: • Active or chronic liver disease Relative: • Concomitant use of certain drugs*
Bile acid sequestrants	Cholestyramine (4-16 g) Colestipol (5-20 g) Colesevelam (2.6-3.8 g)	LDL HDL TG	↓15-30% ↑3-5% No change or increase	Gastrointestinal distress Constipation Decreased absorption of other drugs	Absolute: • dysbeta-lipoproteinemia • TG >400 mg/dL Relative: • TG >200 mg/dL
Nicotinic acid	Immediate release (crystalline) nicotinic acid (1.5-3 gm), extended release nicotinic acid (Niaspan®) (1-2 g), sustained release nicotinic acid (1-2 g)	LDL HDL TG	↓5-25% ↑15-35% ↓20-50%	Flushing Hyperglycemia Hyperuricemia (or gout) Upper GI distress Hepatotoxicity	Absolute: • Chronic liver disease • Severe gout Relative: • Diabetes • Hyperuricemia • Peptic ulcer disease
Fibric acids	Gemfibrozil (600 mg BID) Fenofibrate (200 mg) Clofibrate (1000 mg BID)	LDL *(may be increased in patients with high TG)* HDL TG	↓5-20% ↑10-20% ↓20-50%	Dyspepsia Gallstones Myopathy	Absolute: • Severe renal disease • Severe hepatic disease

* *Cyclosporine, macrolide antibiotics, various anti-fungal agents, and cytochrome P-450 inhibitors (fibrates and niacin should be used with appropriate caution).*

12.6 CONDITIONS INCLUDED IN THE 1993 AIDS SURVEILLANCE CASE DEFINITION

- Candidiasis of bronchi, trachea, or lungs
- Candidiasis, esophageal
- Cervical cancer, invasive
- Coccidiomycosis, disseminated or extrapulmonary
- Cryptococcosis, extrapulmonary
- Cryptosporidiosis, chronic intestinal (greater than 1 month's duration)
- Cytomegalovirus disease (other than liver, spleen, or nodes)
- Cytomegalovirus retinitis (with loss of vision)
- Encephalopathy, HIV-related
- Herpes simplex: chronic ulcer(s) (greater than 1 month's duration); or bronchitis, pneumonitis, or esophagitis
- Histoplasmosis, disseminated or extrapulmonary
- Isosporiasis, chronic intestinal (greater than 1 month's duration)
- Kaposi's sarcoma
- Lymphoma, Burkitt's (or equivalent term)
- Lymphoma, immunoblastic (or equivalent term)
- Lymphoma, primary, of brain
- Mycobacterium avium complex or M. kansasii, disseminated or extrapulmonary
- Mycobacterium tuberculosis, any site (pulmonary or extrapulmonary)
- Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
- Pneumocystis carinii pneumonia
- Pneumonia, recurrent
- Progressive multifocal leukoencephalopathy
- Salmonella septicemia, recurrent
- Toxplasmosis of brain

Adapted from: http://www.cdc.gov/mmwr/preview/mmwrhtml/00018871.htm

12.7 COWDEN SYNDROME CRITERIA

COWDEN SYNDROME CRITERIA[a]

Pathognomonic criteria:
- Adult Lhermitte-Duclos disease (LDD) (cerebellar tumors)
- Mucocutaneous lesions
  - Trichilemmomas, facial
  - Acral keratoses
  - Papillomatous papules

Major criteria:
- Breast cancer
- Thyroid cancer, especially follicular thyroid carcinoma
- Macrocephaly (megalocephaly) (ie, ≥ 97th percentile)
- Endometrial cancer

Minor criteria:
- Other thyroid lesions (eg, adenoma, multinodular goiter)
- Mental retardation (ie, IQ ≤ 75)
- GI hamartomas
- Fibrocystic disease of the breast
- Lipomas
- Fibromas
- GU tumors (especially renal cell carcinoma)
- GU structural manifestations
- Uterine fibroids

Operational diagnosis in an individual, any single pathognomonic criterion, but:
- Mucocutaneous lesions alone if:
  - there are six or more facial papules, of which three or more must be trichilemmoma, or
  - cutaneous facial papules and oral mucosal papillomatosis, or
  - oral mucosal papillomatosis and acral keratoses, or
  - palmoplantar keratoses, six or more
- Two or more major criteria
  or
- One major and ≥ three minor criteria
  or
- ≥ four minor criteria

Operational diagnosis for individuals in a family where one relative is diagnostic for Cowden syndrome. The individual must also have one or more of the following:
- A pathognomonic criterion
- Any one major criteria with or without minor criteria
- Two minor criteria
- History of Bannayan-Riley-Ruvalcaba syndrome

[a] Adapted from Eng C. Will the real Cowden syndrome please stand up: revised diagnostic criteria. J Med Genet. 2000;37:828-830, with permission from the BMJ Publishing Group.