

A Pilot Study of Sirolimus in Subjects with Cowden Syndrome or Other Syndromes Characterized by Germline Mutations in *PTEN*

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TRIAL INFORMATION

- **ClinicalTrials.gov Identifier:** NCT00971789
- **Sponsor(s):** National Cancer Institute
- **Principal Investigators:** Arun Rajan, Phillip A. Dennis
- **IRB Approved:** Yes

LESSONS LEARNED

- This is the first human interventional study in patients with Cowden syndrome that is driven by inactivation of germline *PTEN* gene.
- Single-agent sirolimus, a mTOR inhibitor, suppressed mTOR signaling in surrogate human tissues without significant toxicity.

ABSTRACT

Background. Cowden syndrome is characterized by inactivating germline *PTEN* mutations, which can lead to activation of the PI3K-Akt-mTOR pathway.

Methods. Adult subjects with germline *PTEN* mutation who met international diagnostic criteria for Cowden syndrome and who had Eastern Cooperative Oncology Group (ECOG) performance status 0–2 and adequate organ function were enrolled. Subjects were treated with a 56-day course of daily oral sirolimus. In addition to symptom assessment and physical examination, dermatologic, endoscopic, neurologic (cerebellar), and radiographic assessments were conducted. Inhibition of the mTOR pathway in benign skin and gastrointestinal (GI) lesion was assessed by immunohistochemistry.

Results. A total of 18 patients and 16 families were enrolled. *PTEN* mutations were located at exons 1–8. Regression of skin and GI lesions was observed by dermoscopy or endoscopy. Neurological evaluation showed improvement in cerebellar function score at 1 month. Immunohistochemistry (IHC) analysis in skin and GI benign lesions showed a decrease in the ratio of phosphorylated (p)S6 to total S6 in response to sirolimus. Ratios of pS6K to total S6 at days 14 and 56 were significantly lower than at baseline ($p = .0026$, $p = .00391$, respectively). A 56-day course of sirolimus was well tolerated.

Conclusion. A 56-day course of sirolimus was well tolerated in subjects with Cowden syndrome and was associated with

some evidence of improvement in symptoms, skin and GI lesions, cerebellar function, and decreased mTOR signaling. *The Oncologist* 2019;24:1510–e1265

DISCUSSION

Cowden syndrome is a rare, hereditary cancer syndrome that is characterized by germline *PTEN* mutation and development of malignant and benign tumors in various organs. There are several additional syndromes that are also driven by inactivated *PTEN* gene. There is no recommended systemic agent that can prevent cancer development in these syndromes. This is the first human study to investigate if sirolimus, a mTOR inhibitor that is clinically available as an immunosuppressive agent after organ transplantation, can modulate mTOR signaling in surrogate tissues in subjects with Cowden syndrome. Other endpoints included change in subjective symptoms, physical examination, dermatologic, endoscopic, neurologic (cerebellar), and radiographic assessments.

A total of 18 patients (16 families) with germline *PTEN* mutation were enrolled.

Overall, a 56-day course of sirolimus was well tolerated. Common toxicities (all grades >30%) are abnormalities in liver enzymes (39%), electrolytes (33%), and anemia (33%). With the exception of two individuals who developed grade 3 toxicities (hypophosphatemia and lymphopenia), all the

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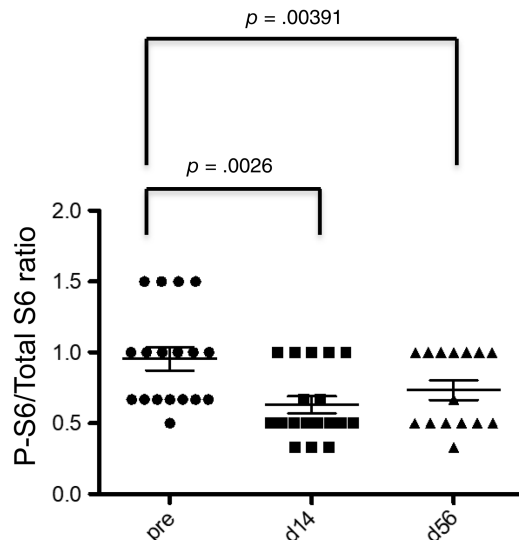


Figure 1. Modulation of mTOR signaling in skin and gastrointestinal (GI) benign lesions. Skin and GI polyps were biopsied at baseline, day 15, and day 56 for mTOR analysis. Intensity of pS6 and S6 by immunohistochemistry was determined as 0, 1, 2, 3 using skin and GI surrogate tissues, and their ratio was plotted. Wilcoxon signed-rank test was used when comparing two matched samples.

remaining toxicities were grade 1 or 2, and none required dose modification. There was no pneumonitis in any of the participants.

Activity of sirolimus was evaluated by subjective and objective assessments. A majority (67%) of patients reported improvement in baseline symptoms. Dermatologic and endoscopic examinations showed improvement in skin (14/18, 77.8%) and GI polyps (2/14, 14.3%), respectively. Cerebellar function as assessed by the modified Scale for the Assessment and Rating of Ataxia (SARA) method showed a significant improvement in a total SARA score at one month ($n = 9$, $p = .034$, data not shown). Imaging studies for patients with measurable or positron emission tomography avid tumor were also assessed. Of the five patients with radiographically measurable disease, all showed stable disease by repeat computed tomography (CT) and magnetic resonance imaging (MRI) at day 56.

Biomarker analysis using biopsied skin or GI tissue was conducted. The ratio of pS6 to total S6 significantly decreased in response to sirolimus treatment at day 15 and day 56 (Fig. 1; $p = .0026$ and $p = .00391$, respectively). A median sirolimus trough level at day 28 was 6.7 ng/mL. There was no significant correlation between the trough level and grade 3 toxicities.

Given the tolerability, clinical availability, and target inhibition in surrogate tissue in this study, the use of sirolimus for the prevention of malignancy in patients with Cowden syndrome deserves further investigation. Duration and optimal dosing and scheduling of sirolimus for cancer prevention in this high-risk patient population need to be further defined.

TRIAL INFORMATION

Disease	Cowden syndrome as defined by International Cowden Consortium operational criteria, version 2000.
Stage of Disease/Treatment	Prevention
Prior Therapy	No designated number of regimens
Type of Study – 1	Phase I
Type of Study – 2	Pilot study
Primary Endpoint	Pharmacodynamic
Secondary Endpoint	Tolerability

Additional Details of Endpoints or Study Design

Patient eligibility: Eligible patients must have had a germline *PTEN* mutation that was confirmed at a Clinical Laboratory Improvement Amendments-approved laboratory, met International Cowden Consortium operational criteria for Cowden syndrome (Pilarski et al. *J Med Genet* 2004;41:323–326), been aged 18 years or older, and had at least six sites amenable to biopsy within the skin and/or gastrointestinal tract and/or an accessible malignant tumor (for patients with malignancy). Patients must have also had an expected survival of ≥ 3 months; an ECOG performance status of 0–2; and adequate organ functions as determined by absolute neutrophil count $\geq 1,500/\text{mL}$, platelets $\geq 100,000/\text{mL}$, total bilirubin $< 1.5 \times$ upper limit of institutional normal (ULN), aspartate transaminase (serum glutamic oxaloacetic transaminase) $< 2.5 \times$ ULN, alanine aminotransferase (serum glutamic pyruvic transaminase) $< 2.5 \times$ ULN, and serum creatinine $< 1.5 \times$ ULN. Patients must have recovered from any acute toxicity related to prior treatments, including surgery. Such toxicity should be grade 0–1 or must have returned to baseline. Patients were ineligible if they had interstitial lung disease, bleeding diathesis, or prior use of rapamycin or other mTOR inhibitor; if they were human immunodeficiency virus positive, pregnant, or lactating; or if they were taking drugs that modulated CYP3A4 and were unable to stop or replace.

Study design and treatment plan: Following a loading dose of 6 mg, a 2-mg dose of sirolimus was administered orally for a period of 56 days for patients with only benign lesions. For those with malignant tumors or symptomatic benign tumors, treatment was allowed to continue until patients developed unacceptable toxicity or disease progression as assessed by CT or MRI obtained 56 days while on study. At baseline and at days 15 and 56, patients were assessed for subjective and objective findings by history, physical examination, dermatologic examination with digital dermoscopy, and neurologic examination. Cerebellar function was quantitated by modified SARA method (gait, stance, nose-finger, heel-shin slide tests, score 0–4; Schmitz-Hübsch et al. *Neurology* 2006;66:1717–1720). Patients with GI polyposis as assessed by baseline esophagogastroduodenal/colonoscopy underwent repeated endoscopy at days 15 and 56. Paired tissue samples at baseline and days 15 and 56 were obtained by biopsy from skin and GI lesions if available. Up to a total of six lesions were biopsied at any one time.

Pharmacokinetics and correlative studies: Serum trough levels of sirolimus were measured at baseline, day 15, and day 56 of the study. Immunohistochemistry of the biopsied materials were conducted for total S6, pS6, and pS6K and scored by traditional semiquantitative method (i.e., 0, 1+, 2+, 3+).

Statistics: This pilot study intended to enroll a total of up to 15 subjects, and it was considered desirable if at least 10 of these patients had six paired lesions amenable to biopsy for IHC in order to create a measure with reasonable statistical precision. The protocol was later amended to allow up to 20 patients. However, this study with a small sample size was not expected to provide any definitive conclusion but rather to generate hypotheses. The Wilcoxon signed-rank test was used when comparing two matched samples. A *p* value <.05 was considered as statistically significant.

Investigator's Analysis	Active and should be pursued further
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DRUG INFORMATION

Drug 1

Generic/Working Name	Sirolimus
Trade Name	Rapamune
Company Name	Wyeth Pharmaceuticals Inc., Philadelphia, PA
Drug Type	Small molecule
Drug Class	mTOR
Dose	2 milligrams (mg) per flat dose
Route	Oral (p.o.)
Schedule of Administration	Once daily

PATIENT CHARACTERISTICS

Number of Patients, Male	9
Number of Patients, Female	9
Stage	N/A
Age	Median (range): 42 (19–69)
Number of Prior Systemic Therapies	Median (range): N/A
Performance Status: ECOG	0 — 1 — 2 — 3 — Unknown — 18

Additional Patient Characteristics

	<i>n</i>
Number of patients/families	18/16
Organs involved	
Number range	3–6
Thyroid	15
GI polyps	13
Breast	8
CNS	
Total	18
Mental retardation	2
LDD	3
Macrocephaly	18
Skin	
Total	18
Acral keratosis	17
Trichilemmoma	11

Oral papules	17
Hemangioma	10
Lipoma	4
Kidney (RCC)	3
PET SUV + tumor	8
Family history	14
<i>PTEN</i> mutation	
Total	16
Location	
Exon 1–4	6
Exon 5	2
Exon 6	2
Intron 6	2
Exon 7	1
Intron 7	1
Exon 8	2
Type	
Missense	1
Stop codon	5
Ins/del + FS	7
Splice site	3

Abbreviations: CNS, central nervous system; del, deletion; FS, frameshift; GI, gastrointestinal; Ins, insertion; LDD, Lhermitte-Duclos disease; PET, positron emission tomography; RCC, renal cell carcinoma; SUV, standard uptake value.

PRIMARY ASSESSMENT METHOD

Title	Response Assessment
Number of Patients Screened	18
Number of Patients Enrolled	18
Number of Patients Evaluable for Toxicity	18
Number of Patients Evaluated for Efficacy	18
Evaluation Method	Other (specify): Decrease in pS6-S6 ratio: significant
Outcome Notes	Suppression of mTOR signaling in surrogate skin and GI tissues were tested by pS6-S6 ratio in 18 and 15 patients at days 15 and 56, respectively (Figure 1).

ADVERSE EVENTS

All Cycles Name	NC/NA, %	Grade 1, %	Grade 2, %	Grade 3, %	Grade 4, %	Grade 5, %	All grades, %
Gastrointestinal - Abdominal pain	94	0	6	0	0	0	6
Phosphate, serum-low (hypophosphatemia)	88	6	0	6	0	0	12
Lymphopenia	88	6	0	6	0	0	12
Diarrhea	77	6	17	0	0	0	23
Albumin, serum-low (hypoalbuminemia)	88	6	6	0	0	0	12
Hemoglobin	55	39	6	0	0	0	45
Neutrophils/granulocytes (ANC/AGC)	94	0	6	0	0	0	6
Constipation	88	6	6	0	0	0	12
Fatigue (asthenia, lethargy, malaise)	72	22	6	0	0	0	28
Pain - headache	83	11	6	0	0	0	17

ALT, SGPT	67	33	0	0	0	0	33
AST, SGOT	89	11	0	0	0	0	11
Magnesium, serum-high (hypermagnesemia)	89	11	0	0	0	0	11
Magnesium, serum-low (hypomagnesemia)	89	11	0	0	0	0	11
Triglyceride, serum-high (hypertriglyceridemia)	78	22	0	0	0	0	22
Bilirubin (hyperbilirubinemia)	89	11	0	0	0	0	11
Calcium, serum-high (hypercalcemia)	94	6	0	0	0	0	6
Glucose, serum-high (hyperglycemia)	89	11	0	0	0	0	11
Cholesterol, serum-high (hypercholesteremia)	72	28	0	0	0	0	28
Glucose, serum-low (hypoglycemia)	94	6	0	0	0	0	6
Leukocytes (total WBCs)	94	6	0	0	0	0	6
Platelets	94	6	0	0	0	0	6
Dry skin	89	11	0	0	0	0	11
Mucositis/stomatitis (clinical exam)	89	11	0	0	0	0	11
Nausea	94	6	0	0	0	0	6
Insomnia	83	17	0	0	0	0	17
Rash: hand-foot skin reaction	89	11	0	0	0	0	11
Gastritis (including bile reflux gastritis)	94	6	0	0	0	0	6

Abbreviations: AGC, absolute granulocyte count; ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate transaminase; NC/NA, no change from baseline/no adverse event; SGPT, serum glutamic pyruvic transaminase; SGOT, serum glutamic oxaloacetic transaminase; WBC, white blood cell.

PHARMACOKINETICS/PHARMACODYNAMICS

Sirolimus trough level: median (range): 6.7 ng/mL (3.0–3.9)

ASSESSMENT, ANALYSIS, AND DISCUSSION

Completion	Study terminated before completion
Terminated Reason	Did not fully accrue. Original PI left institution
Investigator's Assessment	Active and should be pursued further

A number of oncogenic signaling pathways have been described as drivers of cell growth and survival in human cancers. The PI3K-Akt-mTOR pathway is activated in human malignancies, and several familial cancer syndromes with underlying genetic alterations are linked with this pathway. For instance, germline mutations in *PTEN*, *TSC2*, and *STK11*, which negatively regulate PI3K-Akt signaling, are linked with PTEN hamartoma tumor syndrome (PHTS), tuberous sclerosis syndrome, and Peutz-Jeghers syndrome, respectively [1].

PHTS encompasses multiple clinical syndromes with germline *PTEN* mutation. These include Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, Proteus syndrome, and Proteus-like syndrome, all of which are characterized by germline *PTEN* mutation [1, 2].

Cowden syndrome is an autosomal-dominant, cancer susceptibility disorder in adulthood that is driven by germline mutation in a tumor suppressor, *PTEN*. *PTEN* is a dual phosphatase that dephosphorylates both protein and phospholipid substrates. Loss of *PTEN* function leads to upregulation of the

PI3K-Akt-mTOR pathway. Clinical features characteristic of Cowden syndrome include benign skin lesions, macrocephaly, intestinal polyposis, and malignancies in thyroid, breast, endometrium, intestine, and kidney [3]. Manifestations in skin range from acral keratosis to trichilemmomas to mucosal papillomatosis, whereas intestinal lesions include polyposis and malignancies.

In order to assist clinicians in the management of Cowden syndrome, the International Cowden Consortium established the first operational diagnostic criteria for PHTS in 1995, which was later revised in 2000 [4]. It was based on estimated risks of cancer and genotype-phenotype correlation in a total of 12 families with PHTS. Based on a study with over 3,000 cases, a new scoring system to assist clinicians in considering germline *PTEN* mutation tests was proposed in 2011 as well [5]. Most recently, a prospective study of 3,399 cases meeting the relaxed international diagnostic criteria with a subset of 368 cases with deleterious germline *PTEN* mutation was reported [3]. Increased

lifetime risk of cancer was found for carcinomas of breast, thyroid, endometrium, colorectum, kidney, and melanoma. This study recommended detailed cancer screening procedures for individuals with germline *PTEN* mutation.

Despite known cancer susceptibility and recommended cancer screening procedures, there has been no interventional or prevention study in humans with Cowden syndrome. Activation of Akt-mTOR signaling as a result of loss of *PTEN* can be theoretically counteracted by mTOR inhibitors in humans. Individuals with activated mTOR signaling likely benefit from mTOR inhibitors with tolerable toxicities. Here we present a pilot study intended to determine tolerability of sirolimus (rapamycin) and its modulation of mTOR signaling in human subjects with Cowden syndrome. This pilot study in patients with Cowden syndrome aimed to assess feasibility of treatment with sirolimus and modulation of mTOR signaling in accessible benign lesions (i.e., skin and gastrointestinal polyps). Administration of oral sirolimus 2 mg daily was tolerable and required no dose modification. Although pulmonary toxicity is anticipated for mTOR inhibitors, we observed no pneumonitis in this study. Although this study was exploratory, improvements in subjective and objective assessments such as cerebellar function seemed promising. mTOR signaling as determined by pS6-total S6 ratio was suppressed in response to sirolimus treatment.

Several human studies have also investigated if mTOR inhibitors can benefit patients with other nonmalignant conditions with activated Akt-mTOR signaling. For instance, McCormack et al. treated 89 patients with lymphangioleiomyomatosis (LAM) [6]. This condition is a progressive, cystic lung disease in young women, associated with activated mTOR as a result of defective tuberous sclerosis complex (*TSC*) gene. Loss of the *TSC* gene

results in constitutive activation of mTOR signaling, suggesting mTOR is a therapeutic target in LAM. This study demonstrated that a 12-month administration of sirolimus (2 mg daily) is tolerable and improved pulmonary function compared with placebo. Benign tumors regulated by mTOR signaling can also benefit from targeting mTOR. Everolimus, a rapamycin analogue, has activity in patients with defective *TSC* and subependymal giant cell astrocytoma [7]. These clinical findings suggest that a mTOR-driven benign condition with inactivation of various negative regulators such as *PTEN* may respond to mTOR targeted therapy.

This study is limited by its single arm, small sample size, and short exposure to the study drug. We acknowledge that confirmatory studies are required to define effectiveness in Cowden syndrome. Long-term administration of 2-mg daily sirolimus is reportedly tolerable in patients with LAM. We believe the current study provides meaningful information for studies planned in the future.

ACKNOWLEDGMENTS

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DISCLOSURES

Phillip A. Dennis: AstraZeneca (E, OI). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

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