ORIGINAL ARTICLE

Sirolimus for Angiomyolipoma in Tuberous Sclerosis Complex or Lymphangioleiomyomatosis

John J. Bissler, M.D., Francis X. McCormack, M.D., Lisa R. Young, M.D., Jean M. Elwing, M.D., Gail Chuck, L.M.T., Jennifer M. Leonard, R.N., Vincent J. Schmithorst, Ph.D., Tal Laor, M.D., Alan S. Brody, M.D., Judy Bean, Ph.D., Shelia Salisbury, M.S., and David N. Franz, M.D.

ABSTRACT

BACKGROUND

Angiomyolipomas in patients with the tuberous sclerosis complex or sporadic lymphangioleiomyomatosis are associated with mutations in tuberous sclerosis genes resulting in constitutive activation of the mammalian target of rapamycin (mTOR). The drug sirolimus suppresses mTOR signaling.

METHODS

We conducted a 24-month, nonrandomized, open-label trial to determine whether sirolimus reduces the angiomyolipoma volume in patients with the tuberous sclerosis complex or sporadic lymphangioleiomyomatosis. Sirolimus was administered for the first 12 months only. Serial magnetic resonance imaging of angiomyolipomas and brain lesions, computed tomography of lung cysts, and pulmonary-function tests were performed.

RESULTS

Of the 25 patients enrolled, 20 completed the 12-month evaluation, and 18 completed the 24-month evaluation. The mean (±SD) angiomyolipoma volume at 12 months was 53.2±26.6% of the baseline value ($P<0.001$) and at 24 months was 85.9±28.5% of the baseline value ($P=0.005$). At 24 months, five patients had a persistent reduction in the angiomyolipoma volume of 30% or more. During the period of sirolimus therapy, among patients with lymphangioleiomyomatosis, the mean forced expiratory volume in 1 second (FEV_1) increased by 118±330 ml ($P=0.06$), the forced vital capacity (FVC) increased by 390±570 ml ($P<0.001$), and the residual volume decreased by 439±493 ml ($P=0.02$), as compared with baseline values. One year after sirolimus was discontinued, the FEV_1 was 62±411 ml above the baseline value, the FVC was 346±712 ml above the baseline value, and the residual volume was 333±570 ml below the baseline value; cerebral lesions were unchanged. Five patients had six serious adverse events while receiving sirolimus, including diarrhea, pyelonephritis, stomatitis, and respiratory infections.

CONCLUSIONS

Angiomyolipomas regressed somewhat during sirolimus therapy but tended to increase in volume after the therapy was stopped. Some patients with lymphangioleiomyomatosis had improvement in spirometric measurements and gas trapping that persisted after treatment. Suppression of mTOR signaling might constitute an ameliorative treatment in patients with the tuberous sclerosis complex or sporadic lymphangioleiomyomatosis. (ClinicalTrials.gov number, NCT00457808.)

From the Divisions of Nephrology and Hypertension (J.J.B.), Pulmonary Medicine (L.R.Y.), Neurology (G.C., J.M.L., D.N.F.), Radiology (V.J.S., T.L., A.S.B.), and Biostatistics (J.B., S.S.), Cincinnati Children's Hospital Medical Center; and the Division of Pulmonary and Critical Care, University of Cincinnati College of Medicine (F.X.M., L.R.Y., J.M.E.) — both in Cincinnati. Address reprint requests to Dr. Bissler at Cincinnati Children's Hospital Medical Center, MLC 7022, 3333 Burnet Ave., Cincinnati, OH 45229-3039, or at john.bissler@cchmc.org.

Drs. McCormack, Young, and Franz contributed equally to the article.

N Engl J Med 2008;358:140-51.

The tuberous sclerosis complex, a tumor-suppressor syndrome caused by mutations in the tuberin gene (*TSC2*) or the hamartin gene (*TSC1*), is characterized by hamartomas in organs including the brain, kidney, lung, skin, and heart.[1] Angiomyolipomas — tumors rich in fat, muscle, and blood vessels that can hemorrhage or infiltrate the kidney, leading to renal failure — develop in approximately 80% of patients.[2] Lymphangioleiomyomatosis, the major pulmonary manifestation in women with the tuberous sclerosis complex, is a progressive lung disease characterized by infiltration of smooth-muscle cells and formation of parenchymal cysts.[3] Sporadic lymphangioleiomyomatosis can develop in women without the tuberous sclerosis complex, owing to somatic mutations in tuberous sclerosis genes.[4] The cells comprising the lymphangioleiomyomatosis lesions and angiomyolipomas appear to arise from a common source.[5]

The hamartin–tuberin complex regulates the activity of the target of rapamycin complex 1, which lies downstream of cellular pathways controlling cell growth and proliferation. Abnormal signaling through the target of rapamycin complex 1 is involved in a number of tumor-suppressor syndromes[6-8] and cancers.[9] Sirolimus, an immunosuppressive agent approved by the Food and Drug Administration, forms a complex with FK binding protein 12 and inactivates the target of rapamycin complex 1, abrogating the signaling. Sirolimus corrects size defects in tuberin-deficient *Drosophila melanogaster* cells and induces the apoptosis of renal cystadenomas and hepatic hemangiomas in rodent models of the tuberous sclerosis complex.[10,11] In our phase 1–2, proof-of-concept study, we aimed to determine whether sirolimus has an effect on the volume of angiomyolipomas in patients with the tuberous sclerosis complex, sporadic lymphangioleiomyomatosis, or both.

METHODS

SELECTION AND ENROLLMENT OF PATIENTS

The consecutive enrollment of male and female patients, 18 to 65 years of age, began in May 2003 and continued until November 2004, when the target of 25 patients was reached. The voluntary provision of written informed consent, a confirmed diagnosis of the tuberous sclerosis complex[12] or sporadic lymphangioleiomyomatosis,[13,14] the use of contraception (for female patients), and the presence of at least one angiomyolipoma 1 cm or more in the largest dimension were required for participation. Exclusion criteria were the use of continuous supplemental oxygen; concurrent infection; surgery within 8 weeks before the start of sirolimus therapy; current or planned pregnancy; lactation; substantial hematologic, renal, hepatic, or metabolic abnormalities; and the use of an investigational drug within 30 days before entrance into the study. All female patients who could conceive were administered a pregnancy test before enrollment and at each visit.

STUDY DESIGN

The study was conducted at the General Clinical Research Center of the Cincinnati Children's Hospital Medical Center. Patients were recruited from the Tuberous Sclerosis Clinic and the patient registry of the LAM Foundation. The institutional review board approved the study protocol, and a data and safety monitoring board reviewed trial progress semiannually.

In our open-label, phase 1–2 trial, all patients received sirolimus for 1 year and were then followed for an additional year after the therapy was stopped. The primary end point was angiomyolipoma volume at 1 year, and secondary end points included angiomyolipoma volume at 2 years and spirometric measurements, lung volumes, diffusing capacity, results of the 6-minute walk test, and the percentage of the cyst volume at 1 and 2 years. Patients were seen at baseline; at week 2, 3, or 4; and at months 2, 4, 6, 9, 12, 18, and 24. The angiomyolipomas were imaged at all visits except the visit at week 2, 3, or 4 and the visit at month 9. Sirolimus dosing was based on serum target levels that would prevent rejection in patients who had renal transplants. The initial sirolimus dose was 0.25 mg per square meter of body-surface area. Sirolimus levels were measured at 2 weeks, and the dosage was adjusted to achieve a blood sirolimus level between 1 and 5 ng per milliliter. If the target angiomyolipoma lesions had not decreased by 10% of the baseline value in the longest coronal-plane dimension at the 2-month visit, the dose was increased to achieve a blood sirolimus level of 5 to 10 ng per milliliter. At the 4-month visit, if the threshold of a 10% reduction from the baseline value had not been reached,

the dose was increased to achieve a blood sirolimus level of 10 to 15 ng per milliliter. The dose chosen at the 4-month visit was continued through 12 months.

IMAGING

Cross-sectional brain and abdominal evaluations were performed by means of magnetic resonance imaging (MRI) with the use of a clinical 1.5-Tesla system (General Electric Medical Systems). Coronal and axial fast spin–echo T_2-weighted sequences were performed with and without fat suppression. At baseline, up to five lesions per patient were identified for volume measurement throughout the study. The volumes for all lesions from a given time point were averaged for use in analyses. Computed tomography (CT) of the lungs was performed during full inspiration and during full expiration, with the use of thin-section images. Brain MRI was performed with the use of an 8-channel phased-array head coil.

Tumor volume is typically estimated with the use of orthogonal measurements, which assume that the masses are ellipsoid.[15] However, angiomyolipomas often have complex shapes, resulting from asymmetrical growth or the coalescence of multiple lesions.[2] MRI and CT are easily adapted for volumetric analysis.[16,17] Because volumetric techniques are superior to diameter-based approaches for measuring the size of tumors with complex shapes,[18] we used a standardized validated software program,[19] similar to software used for other renal-volume determinations,[20] for volumetric analyses of angiomyolipomas and cystic lung lesions. One investigator measured angiomyolipoma volumes. For validation, another investigator independently measured the lesion volumes by measuring the three orthogonal diameters.

PULMONARY FUNCTION AND 6-MINUTE WALK TESTING

Testing was performed according to guidelines of the American Thoracic Society.[21,22] Patients with lymphangioleiomyomatosis underwent complete pulmonary-function testing — including the measurement of spirometric variables, lung volumes, diffusing capacity, and the 6-minute walk distance — at baseline, 6 or 9 months, 12 months, and 24 months; simple spirometric measurements were obtained at other study visits. Assessment of reversible airflow obstruction was performed at baseline; if the results were positive, during subsequent visits, spirometric variables were measured after the administration of a bronchodilator.

LABORATORY STUDIES

To assess safety, at each visit we measured the levels of electrolytes, blood urea nitrogen, creatinine, glucose, hepatic enzymes, bilirubin, serum lipids, and sirolimus, and performed a complete blood count and urinalysis.

STATISTICAL ANALYSIS

We estimated that 25 patients would be needed for our study to have a statistical power of 95% to detect the difference between a 0% reduction in the mean angiomyolipoma volume from the baseline value (the null hypothesis) and a 30% reduction (the alternative hypothesis). A one-sided test was used, since angiomyolipomas do not spontaneously regress. An independent interim analysis was undertaken by the Cincinnati Children's Hospital Biostatistical Core, after 10 patients had completed 1 year of sirolimus therapy, to determine whether there was evidence of a reduction in lesion volume and of adequate safety.

Analyses were performed with the use of the Proc Mixed procedure with the Kenward–Roger correction (SAS software, version 9.1). To avoid bias due to missing data, least-square means (±SD) are reported. These are model-based estimates calculated from parameter estimates. Separate analyses were performed for the angiomyolipoma volumes alone, those expressed as percentages of the baseline value, and the percentages of the predicted values for the pulmonary-function outcomes, which are comparisons of the patients' pulmonary-function values with normative values derived from population studies.

For variables for which there was a significant difference between the means at baseline and follow-up, we also compared the least-square means at these time points. To estimate the slopes and determine whether the least-square means had changed significantly by 12 months, a random-coefficient model involving a spline function was applied. All tests reflected in Tables 1 and 2 were conducted a priori. P values of less than 0.05 were considered to indicate statistical significance. Reported P values were not adjusted for multiple testing.

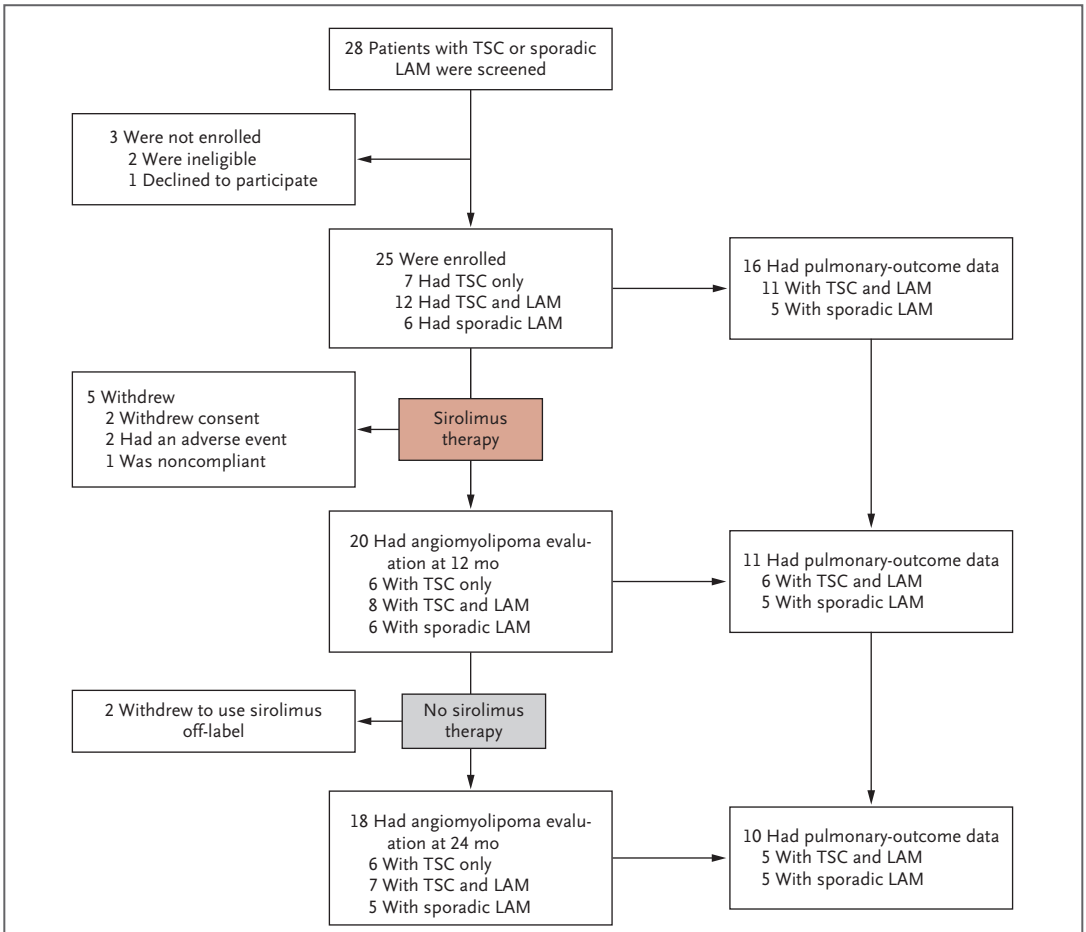

Figure 1. Overview of Screening, Enrollment, and Follow-up of the Patients with the Tuberous Sclerosis Complex (TSC) or Sporadic Lymphangioleiomyomatosis (LAM).

Angiomyolipoma volumes and pulmonary-function tests in patients with LAM were assessed during the period of sirolimus treatment, from baseline to 12 months, and during a post-treatment observation period, from 12 to 24 months. At baseline, 18 patients had LAM, but pulmonary data were uninterpretable in 2 patients because of chylothorax or pneumothorax. At the 12-month time point, one patient declined to undergo pulmonary-function tests, and four patients had withdrawn from the study. One patient withdrew during the second year.

RESULTS

CHARACTERISTICS OF THE PATIENTS

The 25 study patients consisted of 5 men and 2 women with the tuberous sclerosis complex only and 18 women with lymphangioleiomyomatosis, 12 of whom had the tuberous sclerosis complex with lymphangioleiomyomatosis and 6 of whom had sporadic lymphangioleiomyomatosis only. Five patients with the tuberous sclerosis complex (four who also had lymphangioleiomyomatosis) left the study during the first year: two withdrew consent, one had pyelonephritis and recurrent diarrhea, one had a unilateral renal hemorrhage, and one did not comply with the protocol (Fig. 1).

In one patient with sporadic lymphangioleiomyomatosis, the target serum sirolimus range of 1 to 5 ng per milliliter was maintained, but in all other patients the dose was increased to the highest range (10 to 15 ng per milliliter) on the basis of imaging results at 2 months and 4 months.

Twenty patients underwent the 12-month evaluation. Two patients withdrew from the study after the 12-month visit to continue sirolimus therapy off-label because of self-perceived benefit, leaving 18 patients at the 24-month assess-

Table 1. Response of Angiomyolipoma Volume to Sirolimus Therapy.*

Value	Baseline (N=20)	12 Mo (N=20)	18 Mo (N=19)	24 Mo (N=18)
Least-square mean (95% CI) — ml	71.6±105.3 (24.9 to 118.2)	36.5±105.3 (–10.2 to 83.2)	64.8±106.1 (18.1 to 111.6)	74.9±108.0 (27.8 to 121.9)
Range — ml	1.0 to 389.0	0.7 to 169.9	0.7 to 357.2	1.1 to 462.4
Least-square mean (95% CI) — % of baseline value		53.2±26.6 (46.3 to 60.2)	76.8±27.5 (69.7 to 83.9)	85.9±28.5 (78.7 to 93.2)
P value for change from baseline value		P<0.001	P<0.001	P=0.005

* Plus–minus values are least-square means ±SD. The least-square means were calculated with the use of the Proc Mixed procedure (SAS software, version 9.1). Some patients had withdrawn from the study by the 18-month and 24-month follow-up visits. The two hepatic angiomyolipomas studied had the same pattern of response as that shown here for the renal angiomyolipomas.

ment of angiomyolipoma. Fourteen patients with lymphangioleiomyomatosis remained in the study for the second year, but chylothorax or pneumothorax at baseline precluded pulmonary-outcome assessments in two patients, and one patient who had the tuberous sclerosis complex with lymphangioleiomyomatosis declined the 12-month pulmonary-function tests.

Pulmonary end points after 1 year of receiving sirolimus were available for 11 patients with lymphangioleiomyomatosis (6 with the tuberous sclerosis complex with lymphangioleiomyomatosis and 5 with sporadic lymphangioleiomyomatosis). During the second year, 1 patient with lymphangioleiomyomatosis withdrew from the study to take the drug off-label; therefore, data for 10 patients with lymphangioleiomyomatosis were available for the 24-month analysis.

ANGIOMYOLIPOMA BURDEN

The targeted renal angiomyolipoma lesions were bilateral in 12 of the 20 patients (60%) and unilateral in 6 of the 20 patients (30%). Hepatic angiomyolipoma lesions were targeted in the remaining two patients (10%). The mean (±SD) angiomyolipoma volume at baseline was 71.6±105.3 ml (Table 1). After 12 months of therapy, the mean volume decreased to 53.2±26.6% of the baseline volume (P<0.001). At 12 months, 16 of the 20 patients for whom we had data for the first-year follow-up period (80%) had at least a 30% reduction in angiomyolipoma volume (Fig. 2). At 6 and 12 months after stopping sirolimus, the mean angiomyolipoma volume had increased to 76.8±27.5% of the baseline volume (P<0.001) and 85.9±28.5% of the baseline volume (P=0.005), respectively (Table 1 and Fig. 2). Angiomyolipomas in 5 of the 18 patients (28%) remained at least 30% smaller 1 year after therapy than they were at baseline. There was no correlation between statin use or lesion size at baseline and the response to sirolimus (see the Supplementary Appendix, available with the full text of this article at www.nejm.org). The response of a renal angiomyolipoma after 12 months of sirolimus therapy, visualized on MRI, is shown in Figure 3.

The angiomyolipoma volumes were divided into three categories: small (<6.5 ml), medium (6.5–85.0 ml), and large (>85.0 ml). The size assessments obtained with the use of volumetric techniques and those obtained by means of measuring the orthogonal diameters were correlated for each category. The intraclass correlation coefficients for the two methods ranged from 0.76 to 0.86 (mean, 0.81; P<0.001) across the visits. The statistical significance of the measurements obtained through either method was similar.

PULMONARY STUDIES

Pulmonary structural and functional data for 11 female patients with lymphangioleiomyomatosis are listed in Table 2. One patient was a current smoker, three were former smokers, and seven had never been smokers. At enrollment, spirometric measurements were normal in four patients, revealed moderate airflow obstruction (forced expiratory volume in 1 second [FEV_1], 50 to 70% of the predicted value) in three patients, and indicated severe airflow obstruction (FEV_1 <50% of the predicted value) in four patients. During sirolimus therapy, the mean FEV_1 increased from the baseline mean by 120±230 ml at 6 months (P=0.009) and by 118±330 ml at 12 months (P=0.06), with 5 of the 11 patients gaining 100 ml or more in volume during therapy (Fig. 4A and 4C). After 1 year of sirolimus therapy, the FEV_1 in

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.