ORIGINAL ARTICLE

Improvement of tuberous sclerosis complex (TSC) skin tumors during long-term treatment with oral sirolimus

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Background: Oral mechanistic target of rapamycin inhibitors have been shown to reduce visceral tumor volume in patients with tuberous sclerosis complex (TSC).

Objective: We sought to evaluate the cutaneous response to oral sirolimus in patients with TSC and an indication for systemic treatment, including long-term effects.

Methods: A retrospective analysis of 14 adult patients with TSC prescribed sirolimus to treat lymphangioleiomyomatosis was performed. Serial photographs of angiofibromas, shagreen patches, and ungual fibromas taken before, during, and after the treatment period were blinded, then assessed using the Physician Global Assessment of Clinical Condition (PGA). Microscopic and molecular studies were performed on skin tumors harvested before and during treatment.

Results: Sirolimus significantly improved angiofibromas (median treatment duration 12 months; median PGA score 4.5 [range 1.5-5]; Wilcoxon signed rank test, $P = .018$) and shagreen patches (median treatment duration 10 months; median PGA score 4.5 [range 3.5-5]; Wilcoxon signed rank test, $P = .039$), whereas ungual fibromas improved in some patients (median treatment duration 6.5 months; median PGA score 4.66 [range 2.75-5]; Wilcoxon signed rank test, $P = .109$). Clinical, immunohistochemical, or molecular evidence of resistance was not observed (range 5-64 months of treatment).

Limitations: This was a retrospective analysis limited to adult women with lymphangioleiomyomatosis.

Conclusion: Oral sirolimus is an effective long-term therapy for TSC skin tumors, particularly angiofibromas, in patients for whom systemic treatment is indicated. (J Am Acad Dermatol http://dx.doi.org/10.1016/j.jaad.2015.07.018.)

Key words: angiofibromas; lymphangioleiomyomatosis; mechanistic target of rapamycin inhibitor; shagreen patch; sirolimus; tuberous sclerosis complex; ungual fibroma.

Tuberous sclerosis complex (TSC) is an autosomal dominant neurocutaneous syndrome that leads to benign tumor formation in the brain, kidneys, lungs (ie, lymphangioleiomyomatosis [LAM]), and skin. It is caused by mutations in the *TSC1* or *TSC2* tumor suppressor genes, resulting in

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Conflicts of interest: None declared.

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hyperactivation of the mechanistic target of rapamycin (mTOR) signaling pathway and subsequent cell-cycle dysregulation.

Oral mTOR inhibitors, such as sirolimus (rapamycin) and everolimus, have been shown to reduce neurologic, lymphatic, pulmonary, and renal disease in patients with TSC.[1-12] However, attention has previously focused on internal disease and effects after 6 to 12 months of treatment.

Angiofibromas, shagreen patches, and ungual fibromas occur frequently in adult patients with TSC[13] and can be painful, disfiguring, emotionally distressful, or prone to bleeding. We sought to evaluate objectively the initial and long-term response of skin hamartomas to oral sirolimus, document the mucocutaneous side effects of treatment, and evaluate for resistance to ongoing treatment.

CAPSULE SUMMARY

- Oral sirolimus reduces internal tumors and lymphangioleiomyomatosis in patients with tuberous sclerosis complex.
- During oral sirolimus treatment for lymphangioleiomyomatosis, skin tumors improved in nearly all patients; there was no evidence for development of resistance during long-term therapy.
- Oral sirolimus instituted to treat internal disease improves tuberous sclerosis complex skin tumors.

METHODS

Patients

In all, 26 women with TSC and LAM, a TSC-associated lung disease with clinical manifestations that occur almost exclusively in women, were enrolled at the National Institutes of Health Clinical Center in Bethesda, MD. Fourteen patients were prescribed oral sirolimus to treat LAM. Sirolimus was started at 2 mg/d, and then titrated to achieve serum levels between 5 and 15 ng/mL in accordance to the MILES Trial.[3] The remaining 12 patients were not treated. Written informed consent was obtained according to protocols 00-H-0051, 95-H-0186, and/or 82-H-0032, which were approved by the National Heart, Lung, and Blood Institute Institutional Review Board.

Clinical response of skin lesions

A retrospective analysis of medical records, including dermatology consultation records and skin photography, was performed for each patient. Baseline presence of angiofibromas, shagreen patches, or ungual fibromas was documented. Incidence of mucocutaneous or systemic adverse events was also documented. Serial images taken before, during, and after the treatment period were scored by 2 blinded board-certified dermatologists (E. W. C. and T. N. D.) using the Physician Global Assessment of Clinical Condition (PGA).[14,15] According to this 7-point scale, unchanged lesions receive a score of 5. Improvement greater than or equal to 25% but less than 50% is scored as 4, greater than or equal to 50% to less than 75% is scored as 3, greater than or equal to 75% to less than 90% is scored as 2, greater than or equal to 90% to less than 100% is scored as 1, and 100% is 0. Worsening by greater than 25% is scored as 6.

Blind scoring was achieved by using a database of unlabeled skin photographs compiled by a third party without linkage to patient, treatment status, or date taken. One pair of photographs was created for each patient for right-sided facial angiofibromas, left-sided facial angiofibromas, individual shagreen patches, and closely spaced ungual fibromas. For treated patients, the pair consisted of 1 pretreatment photograph and 1 treatment photograph in random order. For non-treated patients (angiofibromas only, because of insufficient sample size for shagreen patches and ungual fibromas), the pair consisted of 2 photographs taken 1 to 3 years apart, also in random order. Other analyses compared the first and second treatment photograph, or 1 treatment photograph and 1 photograph after treatment cessation (for angiofibromas and shagreen patches only) also arranged in random order. For each pair of photographs, the reviewer was instructed to choose the photograph showing the most severely affected skin lesions and to treat this photograph as a baseline. Then, the second photograph was scored with respect to any change from the baseline photograph. If the reviewer appreciated a difference of less than 25% between the photographs, a score of 5 was assigned. In instances where the more recent photograph was chosen as the most severe by the reviewer, the third party would assign a score of 6 for the pair to denote disease progression. Scores from each pair of right- and left-sided angiofibromas, individual shagreen patches, and ungual fibromas from each reviewer were averaged to create an overall PGA for each type of skin lesion.

Statistical analysis

The Wilcoxon signed rank test was performed to evaluate clinical change in each lesion type before and during treatment, or between first and second treatment visit. The Mann-Whitney U test was

Abbreviations used:

LAM: lymphangioleiomyomatosis
mTOR: mechanistic target of rapamycin
PGA: Physician Global Assessment of Clinical Condition
pS6: phosphorylated ribosomal protein S6
TSC: tuberous sclerosis complex

performed to detect any difference between skin tumors from treated and untreated patients. The Mann-Whitney U test was also performed to evaluate the difference between treated lesions and lesions after treatment cessation.

Immunohistochemistry

Tissue sections from skin tumors obtained before drug initiation and during sirolimus treatment were incubated with anti-phospho-S6 ribosomal protein (Ser-235/236) (Cell Signaling Technology, Danvers, MA) or rabbit IgG (Santa Cruz Biotechnology Inc, Dallas, TX), and stained with ABC-alkaline phosphatase (Vectastain, Vector Laboratories, Inc, Burlingame, CA) by methods described in our previous work.[16]

Western blot analysis

Fibroblasts grown from normal-appearing skin and skin tumors obtained during the treatment period were plated on 100-mm dishes and were incubated in Dulbecco-modified Eagle medium with or without 10% fetal bovine serum, with or without 2 nmol/L sirolimus for 24 hours. Cells were lysed with protein extraction buffer, separated on 10% Mini-PROTEAN precast polyacrylamide gels (Bio-Rad, Hercules, CA), transferred to polyvinylidene fluoride membranes and immunoblotted with β-actin (Sigma-Aldrich, St Louis, MO), anti-TSC2 (D93F12), anti-phospho-S6 ribosomal protein (Ser-235/236), and anti-S6 ribosomal protein antibodies (Cell Signaling Technology) as described in previous methods.[17]

RESULTS

Patient characteristics

All 26 women met updated diagnostic criteria for TSC.[18,19] Fourteen patients were prescribed oral sirolimus at a median dose of 2 mg daily to treat LAM. Pretreatment (baseline) photographs were available for 11 patients. Pertinent patient characteristics are summarized in Table I.

Cutaneous responses to sirolimus therapy

Angiofibromas improved with treatment (Wilcoxon signed rank test, P = .018) (Table II). The clinical difference in angiofibromas between 9 treated patients and 12 untreated patients with TSC/LAM (mean age 39 years; median PGA score 4.88 [range 4.5-6]) was also significant (Mann-Whitney U test, P = .015). Shagreen patches improved with treatment (Wilcoxon signed rank test, P = .039). Ungual fibromas improved in some patients (median PGA score 4.66 [range 2.75-5]), although sample size was small (Wilcoxon signed rank test, P = .109). There were insufficient numbers of shagreen patches and ungual fibromas in the untreated group for comparison.

When individual patient responses were evaluated for those with a PGA score less than 5, angiofibromas improved in 7 of 9 (78%) patients, shagreen patches improved in 5 of 7 (71%) patients, and ungual fibromas improved in 3 of 5 (60%) patients. Representative improvement in each type of lesion before and during treatment is displayed in Fig 1.

Table I. Baseline characteristics of 14 patients with tuberous sclerosis complex/lymphangioleiomyomatosis prescribed oral sirolimus

Mean age (range)	37 (25-52) y
Sirolimus median daily dose (range)	2 (1-7) mg
Angiofibromas	9/11*
Prior ablative therapy	4/9
Shagreen patch	7/11*
Ungual fibroma	5/11*

*Eleven patients had pretreatment (baseline) skin photographs available for analysis of cutaneous responses to oral sirolimus. Of the 3 patients without baseline photographs, all had angiofibromas (1 with prior ablative therapy), 1 had a shagreen patch, and 1 had ungual fibromas.

Change in lesions after treatment cessation

Three patients with angiofibromas and shagreen patches were evaluated at a median of 14 months (range 6-48) after treatment cessation. Angiofibromas did not significantly worsen after cessation of treatment (median PGA score 4.75 [range 4.5-4.75]; Mann-Whitney U test, P = .633); in contrast, shagreen patches worsened after treatment cessation (median PGA score 5.25 [range 5-5.75]; P = .020).

Evaluation of treatment resistance

Clinical assessment. Six patients were evaluated 2 or more times during the treatment period (median treatment duration 26 months [range 18-64]). All patients remained stable or continued to improve between first and second treatment visits in angiofibromas (n = 6; median PGA score 4.75 [range

Table II. Change in tuberous sclerosis complex skin tumors during oral sirolimus treatment in 11 patients with tuberous sclerosis complex/lymphangioleiomyomatosis

Patients with baseline photographs (n = 11)	Median treatment duration, mo	Improvement (%)	Interquartile PGA score	Median PGA score (range)	Wilcoxon signed rank test
Angiofibromas (n = 9)	12	7/9 (78)	3.5-4.75	4.5 (1.5-5)	*P* = .018
Shagreen patch (n = 7)	10	5/7 (71)	4.25-5	4.5 (3.5-5)	*P* = .039
Ungual fibroma (n = 5)	6.5	3/5 (60)	4.5-5	4.66 (2.75-5)	*P* = .109

PGA, Physician Global Assessment of Clinical Condition.

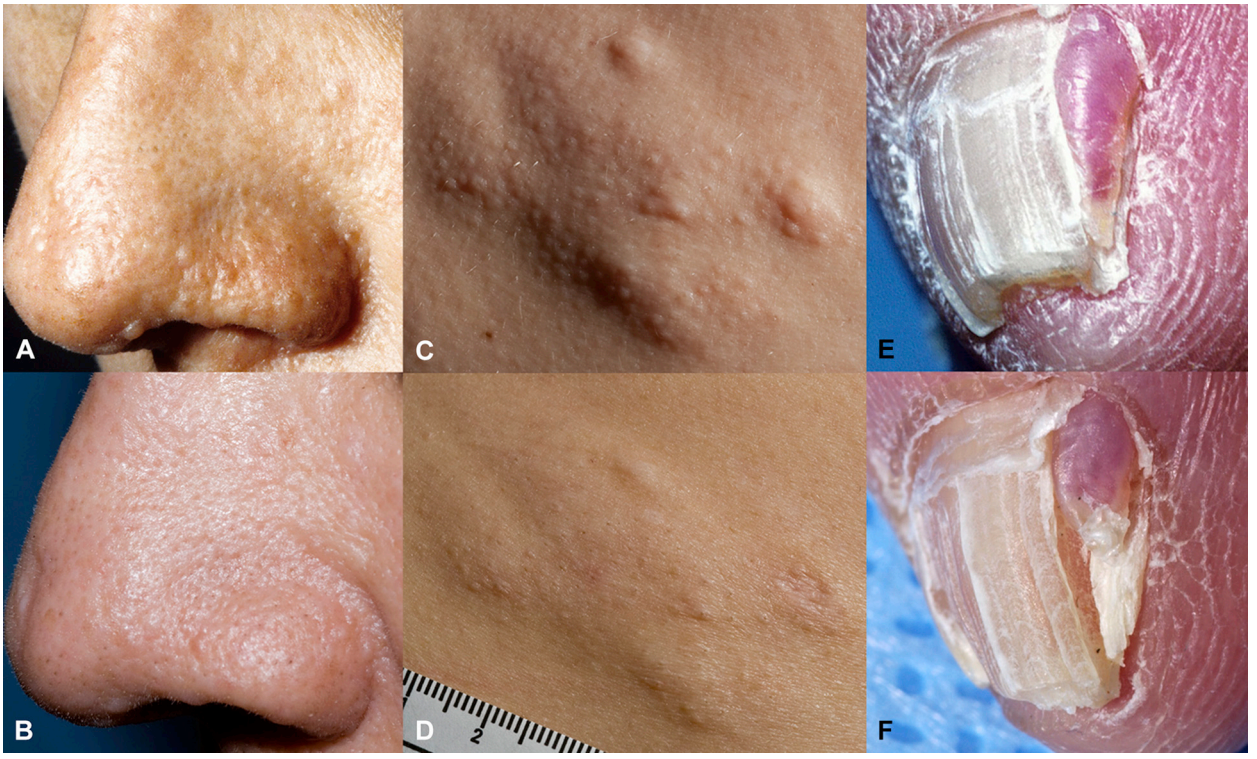

Fig 1. Tuberous sclerosis complex. Clinical improvement of angiofibromas, shagreen patch, and ungual fibroma in patients treated with oral sirolimus. **A**, Multiple skin-colored to pink papules on the nasal ala and alar crease on baseline assessment. **B**, After 1 month of oral sirolimus, papules are substantially diminished. **C**, Nodular plaque with follicular papules on baseline assessment. **D**, Flattening of plaque is noted after 10 months of oral sirolimus. **E**, Red, exophytic papule with hyperkeratotic tip on baseline examination. **F**, Reduction in size and erythema after 6 months of oral sirolimus.

2-5]; Wilcoxon signed rank test, *P* = .461), shagreen patches (n = 4; median PGA score 4.5 [range 2.5-4.5]; *P* = .102), and ungual fibromas (n = 2; median PGA score 4.5 [range 4-5]). Fourteen patients were evaluated 5 to 64 months after sirolimus initiation; during this range of treatment, there was no evidence of disease progression in any patient. Of these, 8 patients were taking sirolimus for more than 12 months.

Immunohistochemistry. Staining for phosphorylated ribosomal protein S6 (pS6), a marker of mTOR hyperactivation, was prominent in the tumor fibroblasts, epidermis, and adnexal structures in tissue sections from 4 skin tumors obtained from 2 patients before treatment initiation (Fig 2, *A*), confirming previously published results.[16] After 6 or 10 months of oral sirolimus treatment, an additional 4 skin tumors were harvested from the same 2 patients. Tissue sections from these tumors revealed markedly reduced staining (Fig 2, *B*).

Western blot analysis. Similar to treatment-naïve samples from our previous work,[20] *TSC2*-null skin tumor fibroblasts grown from 2 angiofibromas and 1 ungual fibroma obtained during in vivo

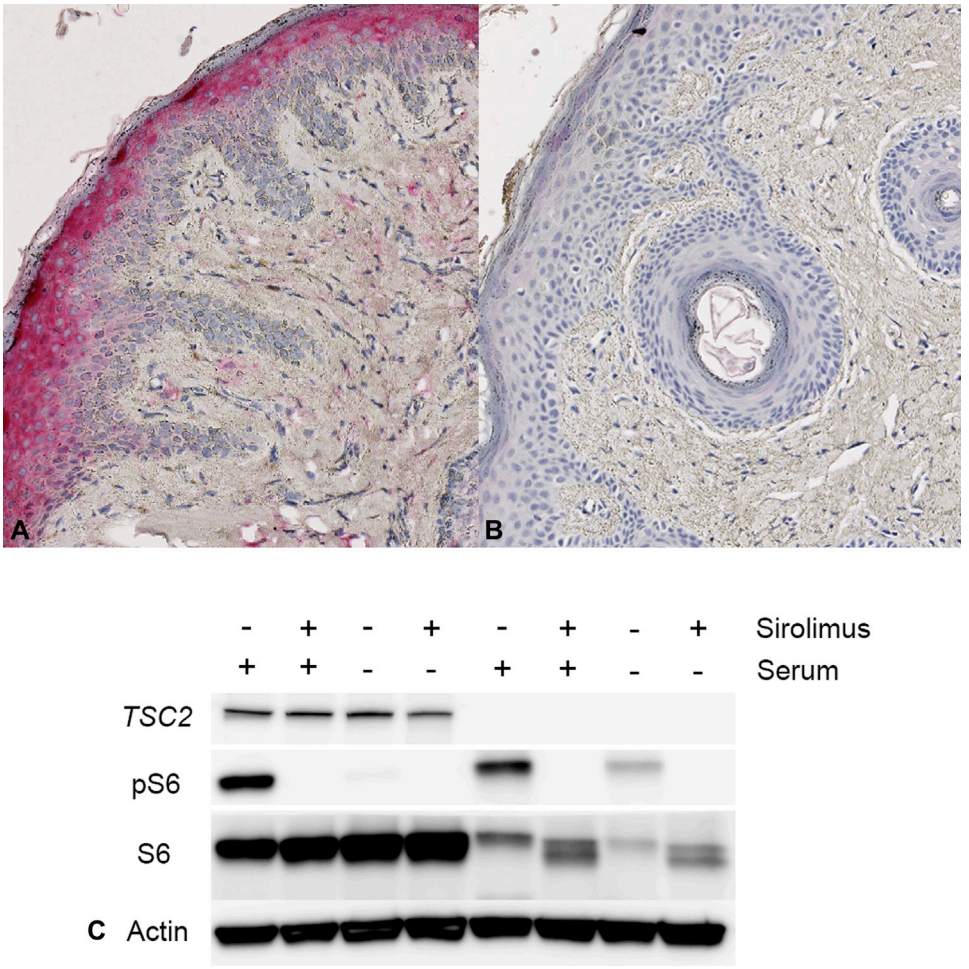

Fig 2. Tuberous sclerosis complex (*TSC*). Evaluation for resistance to oral sirolimus in skin tumors. **A**, Tissue section from treatment-naïve angiofibroma demonstrates increased staining for phosphorylated ribosomal protein S6 (pS6) in stromal fibroblast-like cells. **B**, pS6 Staining is decreased in an angiofibroma harvested after 10 months of treatment. **C**, Persistent pS6 expression in *TSC2*-null, serum-starved fibroblasts derived from an angiofibroma harvested after 5 months of treatment. Phosphorylation is blocked when cells are incubated in vitro with sirolimus.

sirolimus treatment (5, 10, or 15 months after treatment initiation) demonstrated constitutive expression of pS6 that was undetectable after treatment with sirolimus in vitro (Fig 2, *C*). Prior genetic analysis demonstrated biallelic *TSC2* mutations in 2 of these skin tumors (see samples identified as P3T1 and P22T in Tyburczy et al[17]).

Adverse events. Seven of 14 treated patients (50%) experienced mucocutaneous adverse events. Acne-like lesions affected 6 patients (43%). These were diffusely distributed on the face and scalp of 4 patients, whereas the scalp was primarily involved in 2 patients. Oral ulcers developed in 3 patients (21%), causing 1 patient (7%) to temporarily discontinue treatment. Nail fragility affected 1 patient. Systemic adverse events affected 3 patients. Pedal edema developed in 1 patient, elevated blood pressure and headache developed in 1 patient, and unspecified bowel discomfort developed in 1 patient, leading her to temporarily discontinue treatment.

DISCUSSION

Our blind assessment revealed that TSC skin tumors not only improve after treatment with systemic sirolimus, but also maintain improvement during at least 64 months of treatment. All but 1 treated patient (91%) demonstrated improvement in at least 1 skin lesion type, and no patients worsened during treatment. There was no evidence that resistance to sirolimus developed in skin tumors during treatment. Limitations of this study include small sample size, enrichment for adult women with

LAM, retrospective assessment of images, and between-subjects design for clinical comparison with nontreated angiofibromas.

In our cohort, angiofibromas appeared to respond more favorably to treatment than shagreen patches or ungual fibromas. Patients with angiofibromas demonstrated the greatest interquartile improvement of all 3 lesions types, with roughly 25% to 50% betterment. Moreover, angiofibromas improved in more than three-quarters of patients despite a history of ablative therapy in nearly one-half. Similarly, in a previous open-label trial of sirolimus for renal tumors that secondarily assessed skin lesions as "improved" or "unchanged," angiofibromas were found to improve in a greater percentage of patients than shagreen patches or ungual fibromas.[6] It is possible that the antiangiogenic capacity of sirolimus[21] is responsible for the apparent superior benefit of therapy on highly vascular TSC skin tumors.

Unexpectedly, angiofibromas did not seem to worsen after treatment cessation. This is in contrast to worsening of shagreen patches after treatment cessation in our cohort and progression of TSC-associated internal tumors in previous studies.[2,3,8] It has been observed that angiofibromas worsen during puberty.[22] likely because of increasing hormonal stimulation; thus, it is possible that decreased erythema or flattening of angiofibromas after treatment was maintained as a result of declining levels of growth-promoting hormones in this adult cohort.

In follow-up of patients up to 64 months after initiation of oral sirolimus, we did not observe any clinical evidence of treatment resistance. These findings were corroborated on the microscopic and molecular level. Immunohistochemical staining revealed that pS6 immunoreactivity in tumor fibroblast-like cells, the tumor-initiating cells,[16,20] is substantially decreased for at least 10 months after treatment initiation. The absence of pS6-positive stromal cells in the treated tumor is not a result of elimination of tumor cells, as cells with biallelic mutations in *TSC2* could be grown from treated lesions. Instead, tumor cells persisting in the tumor continue to show inhibition of mTOR with sirolimus both in vivo and in vitro. These results are consistent with the known cytostatic rather than cytotoxic effects of sirolimus and are in concordance with the persistence of tumor cells observed during sirolimus treatment in our xenograft model.[20] Although our results support the hypothesis that TSC-related cutaneous hamartomas remain susceptible to sirolimus years after initiation, development of resistance during prolonged mTOR inhibition has been reported in renal and thyroid cancer.[23,24]

Similar to previous clinical trials of oral mTOR inhibitors,[3,6,8,9] mucocutaneous adverse events of treatment were common, but generally tolerable. One-half of treated patients experienced at least 1 mucocutaneous adverse event, most frequently acne-like lesions and oral ulcers. In general, these were only treated if concerning to the patient, as the severity of the adverse events usually decreased with continued treatment. As systemic side effects developed in 3 patients, the authors do not recommend oral treatment for patients without concomitant internal disease requiring oral therapy. For patients with troublesome skin tumors only, topical sirolimus and more recently, topical everolimus, may be effective but are still under investigation.[25,26]

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