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ORIGINAL ARTICLE

Everolimus for renal angiomyolipoma in patients with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis: extension of a randomized controlled trial

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ABSTRACT

Background. Mammalian target of rapamycin (mTOR) inhibitors are recommended as first-line treatment of renal angiomyolipoma associated with tuberous sclerosis complex (TSC) or sporadic lymphangioleiomyomatosis (sporadic LAM), but follow-up is limited. Longer term efficacy and tolerability data from a Phase 3, double-blind, placebo-controlled trial are presented.

Methods. Following favorable results from the primary analysis (data cutoff 30 June 2011) of the EXIST-2 trial, patients still receiving study treatment were allowed to enter an open-label extension. Everolimus was initiated at 10 mg once daily and titrated based on tolerability. The primary outcome was angiomyolipoma response rate (≥50% reduction from baseline in target lesion volumes). Safety was a secondary endpoint.

Results. As of the cutoff date (1 May 2013), 112 patients had received everolimus, and the response rate in 107 patients with angiomyolipoma (median duration of medication exposure of 28.9 months) was 54%. The proportion of patients achieving angiomyolipoma reductions of ≥30% and ≥50% increased over time, reaching 81.6% (62/76) and 64.5% (49/76), respectively, by Week 96. No everolimus-treated patients experienced renal bleeding. The long-term safety profile was consistent with previous reports; adverse events (AEs) were mostly Grade 1/2, and there were no new safety issues. The frequency of emerging AEs and severe AEs lessened over time.

Conclusions. Longer term everolimus treatment appeared safe and effective in patients with TSC- or sporadic LAM-associated renal angiomyolipoma not requiring surgical intervention. Continued reduction in angiomyolipoma volume was demonstrated, and there was no angiomyolipoma-related bleeding; AEs were predictable and generally manageable.

Trial Registration clinicaltrials.gov identifier. NCT00790400 (http://clinicaltrials.gov/ct2/show/NCT00790400).

Keywords: everolimus, mTOR inhibitors, renal angiomyolipoma, sporadic lymphangioleiomyomatosis, tuberous sclerosis complex

INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder affecting ~1.5 million people worldwide [1–3]. Diagnosed from birth through adulthood, TSC is characterized by growth of nonmalignant hamartomas in various organs throughout the body [4–6]. The majority of individuals with TSC have mutations in either the *TSC1* or *TSC2* genes [7, 8], and subsequent somatic mutation results in constitutive activation of mammalian target of rapamycin (mTOR), a critical regulator of cell growth and proliferation [9–11].

Radiological studies show that up to 80% of patients with TSC develop renal angiomyolipomas [8, 12–17], mesenchymal tumors arising from vascular pericytes [18] that are composed of dysplastic blood vessels, smooth muscle-like cells and adipose tissue [19]. The incidence of renal angiomyolipoma increases with age [15, 16], and most patients develop multiple bilateral lesions that pose a significant tumor burden on the kidneys [14]. Angiomyolipomas are slow-growing tumors but may lead to the formation of aneurysms, which can rupture, causing renal hemorrhage and shock [17, 20, 21]. Compromised renal function and end-stage renal disease have been reported [22–25], with renal disease being a leading cause of morbidity and mortality in patients with TSC [26].

Female patients with TSC may develop lymphangioleiomyomatosis (LAM) [27–30], which may result in progressive cystic destruction of the lung parenchyma with severe impairment of lung function [31]. LAM also occurs without TSC (i.e. sporadic LAM) [12, 32]. As with sporadic angiomyolipoma [33], somatic mosaicism of *TSC* gene mutations has been postulated as a mechanism for sporadic LAM [34–36].

mTOR inhibitors are recommended for first-line treatment of asymptomatic, growing renal angiomyolipoma associated with TSC [37], but long-term data are limited. Everolimus, an oral mTOR inhibitor, was investigated for the treatment of renal angiomyolipoma associated with TSC or sporadic LAM in EXIST-2, a double-blind, placebo-controlled, Phase 3 trial [38]. Results from the initial treatment phase demonstrated superiority of everolimus versus placebo; the response rate (proportion of patients with ≥50% reduction from baseline in the sum of volumes of target angiomyolipomas in the absence of progression) was 42% for everolimus and 0% for placebo [difference, 42%; 95% confidence interval (CI) 24–58%; $P < 0.0001$] [38]. Adverse events (AEs) were consistent with those previously reported for patients with TSC [38, 39].

Following initial positive results from the double-blind phase of the EXIST-2 trial, all patients still receiving treatment were permitted to participate in the extension phase and receive open-label everolimus. The longer term safety and efficacy results of everolimus in patients with TSC- or sporadic LAM-associated angiomyolipoma are presented here.

MATERIALS AND METHODS

Participants

A complete description of study participants, design and outcomes has been published [38]. In this study, eligible patients were aged ≥18 years with ≥1 renal angiomyolipoma lesion ≥3 cm in its longest diameter, as measured by computed tomography (CT) or magnetic resonance imaging (MRI), and had a diagnosis of TSC according to consensus criteria [40, 41] or sporadic LAM, as proved with biopsy or compatible chest CT scan. Patients with angiomyolipoma requiring surgery at the time of randomization were excluded, as were those with angiomyolipoma-related bleeding or embolization during the 6 months prior. Patients with LAM exhibiting severe impairment of pulmonary function were excluded.

Independent ethics committees and/or local ethics review boards approved the protocol. All patients provided written informed consent. An independent data monitoring committee performed safety reviews every 6 months, and a steering committee supervised study conduct.

Study design and treatment

This was a randomized, double-blind, placebo-controlled, multicenter, Phase 3 study of once-daily oral everolimus 10 mg versus placebo. Because the primary analysis, 6 months after the last participant was randomly assigned (data cutoff 30 June 2011), favored everolimus over placebo, the study was unblinded on 9 September 2011, and a preplanned open-label extension phase was launched. All patients still receiving double-blind study treatment or undergoing posttreatment evaluation could receive open-label everolimus. Patients initially randomized to everolimus continued to receive the same dose they were taking at the conclusion of the double-blind phase; those switching from placebo received everolimus 10 mg once daily. A starting dose of 10 mg was chosen as a means of providing adequate exposure to almost all patients based on dose proportionality in this adult age group. Dose modifications were to be determined clinically and were based solely on tolerability. Doses could be lowered to 5 mg/day or even to 5 mg/every other day. In the event that a patient required coadministration of a strong cytochrome P450 3A4 or P-glycoprotein inducer, everolimus dose could be increased in 5-mg increments up to twice the currently used daily dose based on tolerability. Data cutoff for a double-blind safety analysis were 14 October 2011, and the data cutoff for this longer term extension analysis were 1 May 2013.

Study outcomes

The primary efficacy outcome for this analysis was response rate, defined as the proportion of patients with renal angiomyolipoma with best overall confirmed response (confirmatory second scans performed no sooner than 8 weeks later), defined as ≥50% reduction from baseline in the sum of volumes of all target lesions (≤5 largest lesions ≥1 cm in longest diameter), in the absence of new lesions ≥1 cm, kidney volume increase of >20% from nadir, and angiomyolipoma-related bleeding grade ≥2 [as defined by the National Cancer Institute Common

Terminology Criteria for Adverse Events (NCI-CTCAE), version 3.0] [42]. Baseline was defined as the last available assessment on or before the start date of everolimus. Renal CT or MRI was performed at baseline, 12, 24 and 48 weeks, and annually thereafter; for each participant, the same kidney imaging modality was used throughout the study. Initial CT or MRI evaluations were performed locally, but a designation of response or progression was based on independent central radiologic review.

Additional end points included reduction in target lesion volume, time to progression (TTP), time to response, duration of response (DOR) and safety. TTP was the time from starting everolimus to first documented progression (i.e. ≥25% increase from nadir in angiomyolipoma volume or ≥20% increase from nadir in the volume of either kidney with a value greater than baseline, appearance of new angiomyolipoma ≥1 cm, or grade ≥2 angiomyolipoma-related bleeding). Time to response was the time to first documented angiomyolipoma response from the start of everolimus, and DOR represents the time from first response to first progression. TTP and DOR were censored at the last radiological assessment if progression was not observed before the cutoff date, the date the patient received further systemic medication or surgery, or patient death.

AEs were graded according to NCI-CTCAE criteria, version 3.0 [42]. Endocrine abnormalities were monitored via reproductive hormone levels [i.e. luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol and testosterone), menstrual status and relevant reproductive medical histories. Proportions of patients with severe renal impairment [glomerular filtration rate (GFR) <30 mL/min/1.73 m^2; chronic kidney disease stage IV/V] or with Grade 3/4 serum creatinine were determined, with GFR calculated using the Modification of Diet in Renal Disease formula. Proteinuria was assessed via standard urine dipstick test and abnormal values were reported as AEs based on the following urine protein results: 1+ (Grade 1), 2+ to 3+ (Grade 2), 4+ (Grade 3) and nephrotic syndrome (Grade 4) [42]. The AEs of hypercholesterolemia and hypertriglyceridemia were graded as follows: hypercholesterolemia—Grade 1, >upper limit of normal (ULN) (4.35 mmol/L for patients aged 3–19 years and 5.15 mmol/L for patients aged ≥20 years) to 7.75 mmol/L; Grade 2, >7.75 to 10.34 mmol/L; Grade 3, >10.34 = 12.92 mmol/L; Grade 4, >12.92 mmol/L; and hypertriglyceridemia—Grade 1, >ULN to 2.5× ULN; Grade 2, >2.5× ULN–5× ULN; Grade 3, >5× ULN–10× ULN; Grade 4, >10× ULN, where ULN = 2.24 mmol/L per central lab measurements.

Statistical analysis

All everolimus data from both the double-blind core phase and the open-label extension were combined; analyses were performed on all patients who received ≥1 dose of everolimus.

Baseline data and efficacy variables were summarized by descriptive statistics. Changes from baseline (actual and percentage) with exact 95% CI (obtained using the Clopper–Pearson method) were calculated for angiomyolipoma response. Kaplan–Meier curves and summary statistics (i.e. point estimates with 95% CI) were determined for TTP and DOR. Statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC, USA).

RESULTS

Baseline characteristics

A total of 118 patients with renal angiomyolipoma associated with TSC or sporadic LAM were enrolled from 24 centers in 11 countries between 28 April 2009 and 30 December 2010; seventy nine were randomized to everolimus and 39 to placebo in the double-blind phase. Overall, 112 patients received everolimus at any time during the study, including the 79 patients originally randomized to everolimus, and 33 patients who switched to open-label everolimus from placebo. Ninety-eight (87.5%) patients continued to receive everolimus and 14 (12.5%) had discontinued treatment at the cutoff date of 1 May 2013. The most common reason for everolimus discontinuation was AEs (including abnormal laboratory values), reported in nine patients (8%). Two patients withdrew due to administrative problems, one withdrew due to a protocol deviation and one withdrew consent. One patient (originally randomized to everolimus) with a history of intractable seizures died; the death was reported with the initial study report [38].

The median age of the 112 patients receiving everolimus was 32.2 years at baseline, and 65.2% were female (Table 1). The

Table 1. Patient demographics and disease characteristics

	Everolimus (N = 112)
Median age (range), years	32.2 (18.1–61.6)
Age, *n* (%)	
<30 years	49 (43.8)
≥30 years	63 (56.3)
Sex, *n* (%)	
Male	39 (34.8)
Female	73 (65.2)
Race, *n* (%)	
White	99 (88.4)
Asian	11 (9.8)
Other[a]	2 (1.8)
Diagnosis of TSC, *n* (%)	107 (95.5)
Diagnosis of sporadic LAM, *n* (%)	5 (4.5)
Diagnosis of LAM, *n* (%)	24 (21.4)
Target renal angiomyolipoma lesions (≥1 cm in longest diameter), *n* (%)	
0[b]	2 (1.8)
1–5	43 (38.4)
6–10	67 (59.8)
Longest diameter of the largest renal angiomyolipoma lesion, *n* (%)	
≥8 cm	33 (29.5)
≥4 cm and <8 cm	64 (57.1)
≥3 cm and <4 cm	7 (6.3)
<3 cm	6 (5.4)
Not applicable[c]	2 (1.8)
Sum of volumes of target renal angiomyolipoma lesions, cm^3 (n = 110)	
Mean (SD)	183.4 (251.3)
Median (range)	92.1 (2.8–1611.5)
Glomerular filtration rate, mL/min/1.73 m^2 (n = 98)	
Median (range)	85 (23–178)

LAM, lymphangioleiomyomatosis; TSC, tuberous sclerosis complex.
[a]A racial status of 'other' was applied to patients who were of mixed race.
[b]Lesions identified as not meeting target status were determined by central radiology, whereas eligibility criteria were based on the opinion of a local radiologist.
[c]Lesions marked as not applicable are those where there is not at least one target lesion.

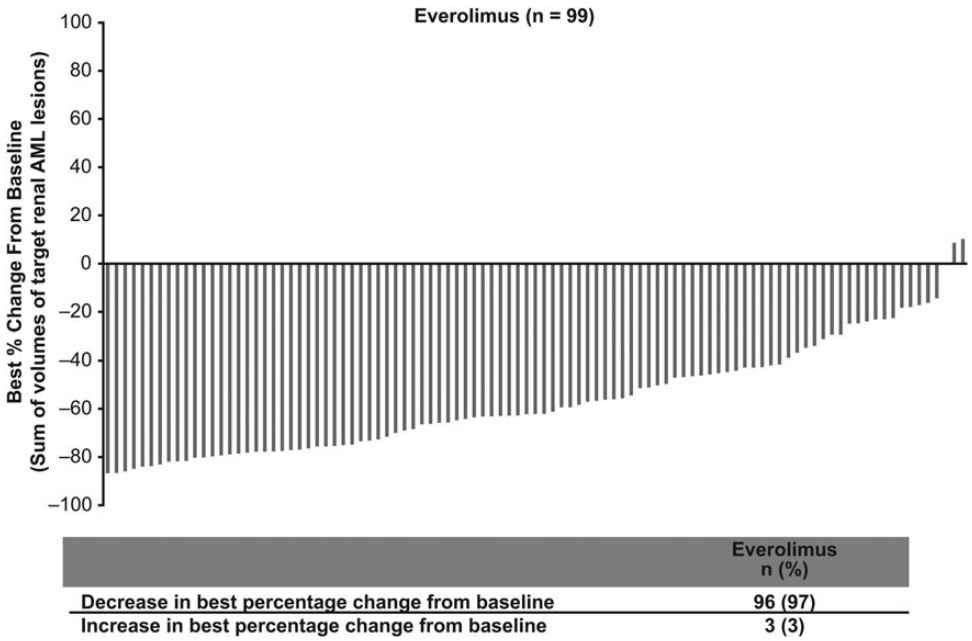

FIGURE 1: Best percentage change from baseline in renal angiomyolipoma volume. Excluded from the graph were patients for whom best percentage change in sum of volumes of target angiomyolipoma lesions was not available and patients with overall nonevaluable angiomyolipoma response.

majority of patients (59.8%) had ≥6 target lesions at baseline, and the median sum of volumes of target lesions was 92.1 cm^3. More than one-third of patients (37.5%) had undergone angiomyolipoma-related surgery before initiating everolimus, and the median GFR was 85 (range 23–178) mL/min/1.73 m^2.

Everolimus exposure

The median duration of everolimus exposure was 28.9 (range 0.5–46.2) months, and the median dose intensity was 8.91 (range 2.3–19.0) mg/day. Eighty patients (71.4%) required dose interruptions or reductions, with AEs being the most common reason (50.0 and 58.9% for dose reductions and dose interruptions, respectively).

Efficacy outcomes

Compared with an angiomyolipoma response rate of 42% (33/79 patients; 95% CI 31–53%) in everolimus-treated patients in the primary analysis (median treatment exposure 8.7 months) [38], the response rate increased to 54% (60/112 patients; 95% CI 44–63%); 38 patients (33.9%) had stable disease and 1 (0.9%) had disease progression as best overall response. Ninety-six of 99 evaluable patients (97.0%) experienced a reduction in the sum of volumes of target angiomyolipoma lesions relative to baseline per central radiology review (Figure 1). Of 13 (11.6%) nonevaluable patients, central review did not confirm the presence of target renal angiomyolipoma lesions in two patients; two patients had only one available baseline kidney scan and nine had ≥1 missing kidney volume measurement.

Response to everolimus increased over time (Figure 2). The proportion of patients who achieved ≥50% reduction from baseline in the sum of volumes of target lesions increased from 44.2% (46/104) after 12 weeks of treatment to 64.5% (49/76) at Week 96, and the proportion of patients with ≥30% reduction increased from 75.0% (78/104) after 12 weeks of treatment to 81.6% (62/76) after 96 weeks. Among the 60 patients achieving angiomyolipoma response at any time, the median time to response was 2.83 months and no progressions were observed. The duration between first response and the last radiological assessment in responders was 2.8–38.8 months.

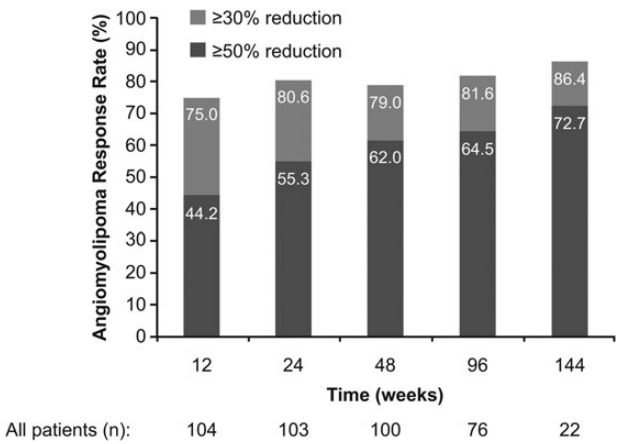

FIGURE 2: Effect of everolimus on renal angiomyolipoma volume over time.

Overall, the median time to angiomyolipoma progression was not reached, because 106/112 patients (94.6%) did not have angiomyolipoma progression (Figure 3). Estimated progression-free rates (95% CI) were 98.0% (92.1–99.5%) at 6 months, 95.7% (89.0–98.4%) at 12 months, 94.1% (86.1–97.5%) at 24 months and 89.4% (73.2–96.0%) at 36 months. Among six patients (5.4%) with angiomyolipoma progression

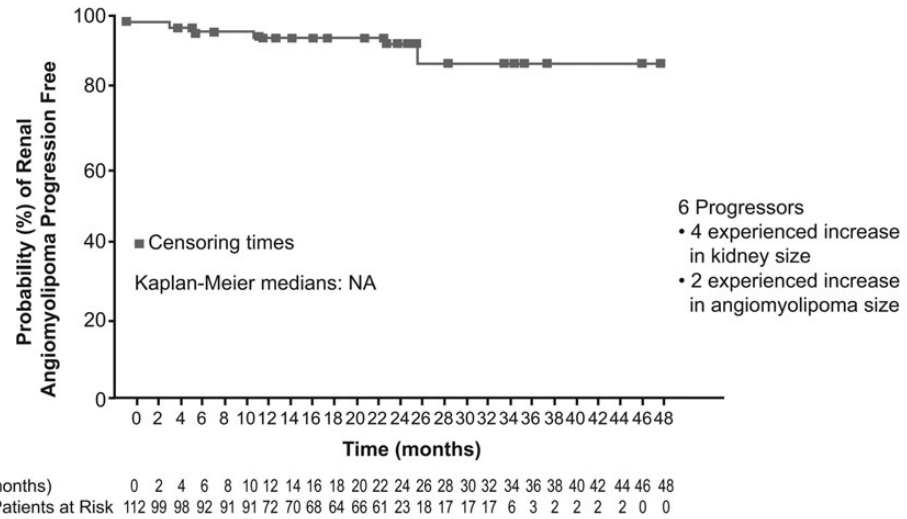

FIGURE 3: Time to renal angiomyolipoma progression.

at any time during the study, two had increased size of target lesions and four had increased kidney size. Four of the six patients with progression had intermittent dose reductions or temporary dose interruptions within 6 months prior to progression, whereas two of the six were still receiving everolimus 10 mg/day. Despite the progression, all six patients were ongoing at the data cutoff of 1 May 2013. At the last follow-up, two patients had increased angiomyolipoma volume after progression, three had decreased angiomyolipoma volume after progression and one did not have a follow-up assessment.

Safety outcomes

For the 112 patients in the longer term analysis who received everolimus, the most commonly reported AEs (>25% of patients) were nasopharyngitis, stomatitis, headache, acne, hypercholesterolemia, urinary tract infection (UTI) and aphthous stomatitis (Table 2). Most AEs were Grade 1/2 in severity. Overall, 42% of patients experienced Grade 3/4 AEs, 27% were suspected to be drug related; the most frequent Grade 3 AEs regardless of relationship to study drug were amenorrhea (4.2% of the 71 at-risk female patients, i.e. those aged 18–55 years) and decreased blood phosphorus (3.6%). Grade 4 AEs were blood uric acid increased (1.8%) and convulsion, hydrocephalus, hypertensive crisis, neutropenia, pancreatic carcinoma and rhabdomyolysis (each 0.9%). Overall, 97.3% of patients reported ≥1 AE considered everolimus related, including 26.8% with Grade 3/4 AEs. Approximately one-quarter of patients (28.6%) reported serious AEs (SAEs). Fourteen patients (12.5%) experienced SAEs suspected by the investigator to be everolimus related, including infections (3.6%) and gastrointestinal disorders (2.7%). Pneumonia was reported in 1.8% of patients, and ovarian cyst was reported in 2 of 73 female patients (2.7%).

After completion of the double-blind phase (safety analysis cutoff 14 October 2011), the most commonly reported AEs (>20%) were stomatitis, nasopharyngitis, hypercholesterolemia, acne, headache and cough in the everolimus group; and nasopharyngitis, headache and fatigue in the placebo group. Rates of new AEs while on treatment with everolimus decreased over time (Table 3). Most AEs reduced to incidences <10% in Year 2 and further declined in Year 3. Nasopharyngitis was the exception to this observation with an incidence of 32.1% in the

Table 2. Most common (≥10% of patients) adverse events by preferred term, regardless of relationship to everolimus

n (%)	Everolimus (*N* = 112)	
	All grades	Grade 3/4
Nasopharyngitis	48 (42.9)	0
Stomatitis	48 (42.9)	0
Headache	34 (30.4)	0
Acne	33 (29.5)	1 (0.9)
Hypercholesterolemia	33 (29.5)	1 (0.9)
Urinary tract infection	31 (27.7)	1 (0.9)
Aphthous stomatitis	29 (25.9)	1 (0.9)
Amenorrhea[a]	16 (22.5)	3 (4.2)
Cough	23 (20.5)	0
Diarrhea	22 (19.6)	0
Hypertension	21 (18.8)	1 (0.9)
Nausea	21 (18.8)	0
Fatigue	20 (17.9)	1 (0.9)
Edema peripheral	20 (17.9)	0
Vomiting	19 (17.0)	0
Back pain	18 (16.1)	0
Mouth ulceration	17 (15.2)	2 (1.8)
Proteinuria	17 (15.2)	1 (0.9)
Upper respiratory tract infection	16 (14.3)	0
Blood alkaline phosphatase increased	15 (13.4)	1 (0.9)
Hypophosphatemia	15 (13.4)	2 (1.8)
Hyperlipidemia	14 (12.5)	0
Leucopenia	14 (12.5)	0
Sinusitis	14 (12.5)	0
Abdominal pain	13 (11.6)	0
Activated partial thromboplastin time prolonged	12 (10.7)	0
Blood lactate dehydrogenase increased	12 (10.7)	0
Decreased appetite	12 (10.7)	1 (0.9)
Eczema	12 (10.7)	0
Pruritus	12 (10.7)	0

[a]Percentages calculated from the number of at-risk (aged 10–55 years) females (*n* = 71).

Table 3. Adverse events (preferred term) of any severity experienced by ≥15% of patients in any group by year of emergence

	Placebo	Everolimus			
	Double-blind[a,b] ($n = 39$)	Double-blind[a,c] ($n = 79$)	<12 months[d] ($n = 112$)	13–24 months[d] ($n = 101$)	25–36 months[d] ($n = 77$)
Stomatitis	3 (7.7)	39 (49.4)	46 (41.1)	9 (8.9)	2 (2.6)
Nasopharyngitis	14 (35.9)	22 (27.8)	36 (32.1)	19 (18.8)	14 (18.2)
Hypercholesterolemia	1 (2.6)	18 (22.8)	25 (22.3)	9 (8.9)	6 (7.8)
Acne	2 (5.1)	17 (21.5)	28 (25.0)	8 (7.9)	3 (3.9)
Headache	8 (20.5)	17 (21.5)	26 (23.2)	11 (10.9)	3 (3.9)
Cough	5 (12.8)	16 (20.3)	18 (16.1)	4 (4.0)	4 (5.2)
Aphthous stomatitis	4 (10.3)	15 (19.0)	21 (18.8)	14 (13.9)	6 (7.8)
Fatigue	8 (20.5)	14 (17.7)	19 (17.0)	2 (2.0)	2 (2.6)
Nausea	5 (12.8)	13 (16.5)	17 (15.2)	5 (5.0)	0
Mouth ulceration	2 (5.1)	13 (16.5)	17 (15.2)	3 (3.0)	2 (2.6)
Amenorrhea[e]	1 (3.8)	8 (15.4)	12 (16.9)	7 (10.8)	3 (6.0)
Urinary tract infection	6 (15.4)	12 (15.2)	16 (14.3)	14 (13.9)	6 (7.8)
Vomiting	2 (5.1)	12 (15.2)	15 (13.4)	7 (6.9)	1 (1.3)
Rhinitis	6 (15.4)	6 (7.6)	7 (6.3)	2 (2.0)	1 (1.3)
Flank pain	6 (15.4)	3 (3.8)	3 (2.7)	5 (5.0)	1 (1.3)
Diarrhea	2 (5.1)	11 (13.9)	17 (15.2)	6 (5.9)	3 (3.9)

[a]From 90-day safety update analysis of the double-blind phase.
[b]Median treatment duration, 10.3 months (45 weeks).
[c]Median treatment duration, 11.1 months (48 weeks).
[d]Based on the 1 May 2013, cutoff date. Median everolimus duration 28.9 months (126 weeks). Data occurring after 36 months of treatment not presented here due to the small number of patients at risk ($n = 18$). An adverse event is only counted in the time period in which it started.
[e]Number of females with at least 1 amenorrhea among the female patients only: 1/26 and 8/52 females, respectively, in the placebo and everolimus arm during the double-blind period; 12/71, 7/66 and 3/51 females aged 10–55 years in the first, second and third year, respectively, while on everolimus.

first 12 months, then 18.8 and 18.2% in the second and third years. Emergence of SAEs showed a similar pattern to AEs, with considerable reductions in incidence in Years 2 and 3 (7.9 and 7.8%, respectively) compared with Year 1 (19.6%). Most AEs could be managed successfully through dose reduction or interruption, and few patients withdrew from the study due to an AE.

Nine (8.0%) patients discontinued everolimus due to 12 AEs (two patients reported several AEs leading to discontinuation); these were angioedema, decreased blood phosphorous, bronchospasm, convulsion, diarrhea, hypersensitivity, localized edema, malaise, pancreatic carcinoma, proteinuria, rhabdomyolysis and skin toxicity (all in one patient each). AEs requiring dose adjustment/reduction were reported in 77 patients (68.8%), with the most common being stomatitis (10.7%), aphthous stomatitis (8.9%), upper respiratory tract infection (7.1%), diarrhea (6.3%), and mouth ulceration, sinusitis, and UTI (each 5.4%). Stomatitis, an identified risk of everolimus treatment, was also the AE that most required additional therapy (32.1%); however, most cases were easily managed and no patient discontinued treatment due to stomatitis.

Infections, mostly Grade 1/2 in severity and involving the upper respiratory tract, were reported in 88.4% of patients. Grade 3 infections, reported in six patients (5.4%), were bronchopneumonia (two patients), erysipelas (one patient), and abscess, onychomycosis, pneumonia and UTI (one patient each). Although no event led to discontinuation, dose adjustment/interruption due to infection was required in 35 (31.3%) patients.

Renal events were reported in 19 (17.0%) patients. These events consisted of proteinuria (15.2%, $n = 17$ patients), increased blood creatinine (4.5%, $n = 5$ patients), and acute renal failure, chronic renal failure and renal impairment (each 0.9%, $n = 1$ patient each). The 17 patients with proteinuria reported a total of 34 episodes; as of the cutoff date, 27 episodes had resolved and 7 episodes had not. All but one episode of proteinuria were Grade 1/2 in severity. One patient, who had Stage IV chronic kidney disease at baseline, had Grade 3 proteinuria that was suspected to be related to everolimus; this resolved after the study drug was adjusted/temporarily interrupted. Grade 2 proteinuria episodes were treated with an angiotensin II receptor antagonist for two patients and with a renin inhibitor for one patient; the proteinuria resolved in two of the three cases. One event of Grade 2 proteinuria led to study discontinuation. No renal hemorrhages occurred in the extension phase; however, 1/39 placebo recipients (2.6%) had experienced severe bleeding with emergency nephrectomy.

The majority of patients had GFR ≥30 mL/min/1.73 m^2 (93.8%) or normal serum creatinine levels (86.6%) while receiving everolimus. Median GFR at baseline was 85 mL/min/1.73 m^2, and overall, GFR remained stable over time (median GFR at Week 120 was 84 mL/min/1.73 m^2). Severe renal impairment (GFR <30 mL/min/1.73 m^2) was observed in seven (6.3%) patients at least once postbaseline. All of these patients had compromised renal function (GFR <60 mL/min/1.73 m^2) prior to everolimus initiation. No patients had Grade 3/4 elevated serum creatinine, but 15 (13.4%) patients had Grade 1/2 elevations, which was temporary in seven patients. Eight patients experienced Grade 3 hypophosphatemia.

Amenorrhea occurred in 22.5% of 71 at-risk females and was mostly Grade 1/2 in severity. Events were transient and manageable, with only one patient requiring dose adjustment/interruption and no patients discontinuing treatment. Grade 3 amenorrhea was reported in three patients; all resolved spontaneously. There was no pattern of endocrine abnormalities detected based on periodic measurements of FSH, LH, testosterone and estradiol.

DISCUSSION

This longer term analysis of everolimus treatment demonstrated stability of everolimus effects over time in the treatment of TSC- or sporadic LAM-associated renal angiomyolipoma. This effect is especially important, because the majority of patients in the study had large, bilateral angiomyolipoma; 37.5% of patients had undergone angiomyolipoma-related surgery prior to the study; 2.7% had impaired renal function at baseline and 15.2% experienced proteinuria during the study, indicating a population at risk of long-term renal deterioration and hemorrhage. Although proteinuria occurred in some patients, there was no evidence of progression. The goal of treating TSC renal tumors is to prevent them from enlarging and thus avoiding the development of complications such as decreased renal function or hemorrhage.

In this study, a pronounced benefit was demonstrated with continued use of everolimus. The treatment effect was sustained over time, with an improvement in response rate from 42% in the primary analysis (median exposure 8.7 months) [38] to 54% (median exposure 28.9 months). Angiomyolipoma shrinkage continued over time and no hemorrhage occurred, in contrast to predicted outcomes for such a patient population [43]. None of the 60 responders experienced angiomyolipoma progression by the data cutoff date.

Of six patients with angiomyolipoma progression, four were receiving a reduced dose of everolimus and two were still treated with 10 mg/day before the progression. Previous analysis of the relationship between drug levels and treatment effect revealed a 10% reduction in tumor size for a 2-fold increase in C_{min} [38]; however, the measurement of drug levels did not continue into the extension phase of the study. Also, four patients had documented progression because of an increase in kidney size. For three of these patients, we observed a concordance between kidney increase and growth in angiomyolipoma target volumes even if the increase in target volume was dominated by a single lesion, explaining why the sum of volumes of all target lesions did not meet the definition for progression (≥25% increase from nadir in angiomyolipoma volume with a value greater than baseline). However, in one patient, the nonaffected kidney increased in size concurrent with an angiomyolipoma volume reduction from baseline of 85% in the affected kidney, illustrating the complexity of using kidney size as a surrogate measure for growth in poorly contoured angiomyolipoma lesions. Moreover, all six patients who reported progression were ongoing at the data cutoff of 1 May 2013, because investigators determined that continuation of treatment offered the patients substantial clinical benefit.

The longer term safety profile of everolimus was consistent with that previously reported in this study [38] and in other studies of everolimus in TSC-associated clinical settings [39, 44, 45]. Consistent with current recommendations [37] in the treatment of asymptomatic growing angiomyolipomas larger than 3 cm, an mTOR inhibitor is preferable to the renal damage caused by angiomyolipoma progression [17, 20–26] or surgical and embolitic or ablative therapies [46, 47]. Although current consensus guidelines for the management of TSC caution that long-term safety studies are needed, this study confirms that AEs observed with prolonged use of everolimus are consistent with established risks and that no new safety issues manifest over time. The frequency of new AEs and SAEs was highest in the first year of treatment, and persistent or recurrent side effects were generally successfully handled with dose reductions. After 1 year on everolimus, nearly 60% of patients took 10 mg/day and 35% of patients took 5 mg/day, and these frequencies remained stable after 1 year. This appeared to be effective management, because the incidence of AEs reduced markedly over time and few patients (8%) discontinued because of AEs. This also supports the dosing rationale for this study. Whereas the EXIST-1 study in patients with TSC-associated SEGA [39] utilized a dosing strategy based on titrating to blood everolimus levels between 5 and 15 ng/mL, the EXIST-2 study initiated everolimus at 10 mg/day and titrated based on tolerability. The dosing strategy in EXIST-1 was necessary, because it was a predominantly pediatric population with a wide range of body sizes; however, there was much less body size variability in EXIST-2, because the patients were adults. Although blood levels are used to guide everolimus dosing in patients following renal transplant [48, 49], cell biology and drug levels in renal transplantation are vastly different than in the TSC population in EXIST-2. Unlike activated lymphocytes, such as those that could occur in renal transplant patients [50], angiomyolipomata cells are very slowly proliferating, and even fat-poor lesions contain significant intracellular lipid in which the drug could diffuse [18, 51]. Moreover, a poor relationship between angiomyolipoma tumor shrinkage and everolimus drug level has been noted previously [38].

Renal events were generally mild, but longer term follow-up of patients treated with everolimus indicates a larger decrease in renal function than at the earlier cutoff date [38]; however, given the longer duration of follow-up this could be expected. Also, it is important to note that there were no renal hemorrhages during the extension phase (only one patient discontinued due to proteinuria) and that the decrease in renal function was less than that reported earlier for placebo recipients [38]. Severe renal impairment occurred in a small number of patients, all of whom had decreased renal function prior to everolimus initiation; in the remainder of patients, GFR was stable or improved. Proteinuria is a known concern associated with mTORC1 inhibitors. Although the mechanism behind this is not completely understood, some evidence suggests that mTOR inhibition causes dysregulation of the autophagic pathway in podocytes [52]. It is especially important to monitor for proteinuria as it has been implicated in tubular cell epithelial dysfunction and tubulointerstitial injury, and may accelerate progression of kidney disease to end-stage renal failure [53]. In a large cohort registry study, heavy proteinuria (≥2+) increased the risk of progressing kidney disease to end-stage renal failure regardless of the level of estimated GFR [54]. In our study, proteinuria was not noted to be progressive and the majority of episodes resolved by the cutoff date.

Although the incidence of amenorrhea was also higher with longer term follow-up (22.5% of at-risk females versus 13.5% in the primary analysis [38]), events were transient and manageable, and all incidences of Grade 3 amenorrhea ($n = 3$) resolved.

Because ovarian cyst was reported in 2.7% of females, regular gynecologic follow-up is recommended.

The lack of a placebo arm in the extension phase of the study could be considered a limitation; however, given the magnitude of effect with everolimus in the primary analysis, maintenance of an untreated arm would have been unethical.

Everolimus is a safe and effective long-term therapeutic option for patients with TSC- or sporadic LAM-associated renal angiomyolipoma not requiring immediate surgical intervention. Continued reduction in angiomyolipoma size was observed over a median treatment period of 28.9 months, and AEs were predictable and manageable, with annual incidences decreasing over time. Ongoing follow-up of these patients will provide longer term data.

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CONFLICT OF INTEREST STATEMENT

J.J.B., J.C.K., E.R., M.F., E.B., M.S., P.J.dV and K.B. have served as consultants and/or participated in Advisory Boards for Novartis. J.J.B., J.C.K., B.A.Z., M.S., P.J.dV (donated to charity) and K.B. have received travel honoraria from Novartis. N.B., S.M., S.P. and S.S. are employees of Novartis. N.N. and S.B. have no conflicts to report. J.J.B., J.C.K., E.B. and K.B. have served as investigators on this study and received research grants (to their institutions) from Novartis. The authors attest to the originality of this manuscript, and note that the results presented in this paper have not been published previously in whole or in part, except in abstract format.

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