Everolimus for angiomyolipoma associated with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis (EXIST-2): a multicentre, randomised, double-blind, placebo-controlled trial


Summary

Background Angiomyolipomas are slow-growing tumours associated with constitutive activation of mammalian target of rapamycin (mTOR), and are common in patients with tuberous sclerosis complex and sporadic lymphangioleiomyomatosis. The insidious growth of these tumours predisposes patients to serious complications including retroperitoneal haemorrhage and impaired renal function. Everolimus, a rapamycin derivative, inhibits the mTOR pathway by acting on the mTOR complex 1. We compared the angiomyolipoma response rate on everolimus with placebo in patients with tuberous sclerosis or sporadic lymphangioliomyomatosis-associated angiomyolipoma.

Methods In this double-blind, placebo-controlled, phase 3 trial, patients aged 18 years or older with at least one angiomyolipoma 3 cm or larger in its longest diameter (defined by radiological assessment) and a definite diagnosis of tuberous sclerosis or sporadic lymphangioleiomyomatosis were randomly assigned, in a 2:1 fashion with the use of an interactive web response system, to receive oral everolimus 10 mg per day or placebo. The primary efficacy endpoint was the proportion of patients with confirmed angiomyolipoma response of at least a 50% reduction in total volume of target angiomyolipomas relative to baseline. This study is registered with ClinicalTrials.gov number NCT00790400.

Results 118 patients (median age 31·0 years; IQR 18·0–61·0) from 24 centres in 11 countries were randomly assigned to receive everolimus (n=79) or placebo (n=39). At the data cutoff, double-blind treatment was ongoing for 98 patients; 118 patients (median age 31·0 years; IQR 18·0–61·0) from 24 centres in 11 countries were randomly assigned to receive everolimus (n=79) or placebo (n=39). At the data cutoff, double-blind treatment was ongoing for 98 patients; two main reasons for discontinuation were disease progression (nine placebo patients) followed by adverse events (two everolimus patients; four placebo patients). The angiomyolipoma response rate was 42% (33 of 79 [95% CI 31–53%]) for everolimus and 0% (0 of 39 [0–9%]) for placebo (response rate difference 42% [24–58%]; one-sided Cochran-Mantel-Haenszel test p<0·0001). The most common adverse events in the everolimus and placebo groups were stomatitis (48% [38 of 79], 8% [3 of 39], respectively), nasopharyngitis (24% [19 of 79] and 31% [12 of 39]), and acne-like skin lesions (22% [17 of 79] and 5% [2 of 39]).

Interpretation Everolimus reduced angiomyolipoma volume with an acceptable safety profile, suggesting it could be a potential treatment for angiomyolipomas associated with tuberous sclerosis.

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Introduction Tuberous sclerosis complex is caused by decreased or absent expression of the genes TSC1 (hamartin) or TSC2 (tuberin), and is characterised by the growth of hamartomas in the kidney, brain, heart, liver, and skin.1,2 These hamartomas predispose patients to organ system dysfunction, including autism spectrum disorder, intellectual disability, and epilepsy.3 Angiomyolipomas are associated with loss of heterozygosity at the TSC1 or TSC2 gene locus, occur in roughly 80% of patients with tuberous sclerosis, and cause the largest proportion of adult deaths from the disease.4 As angiomyolipomas enlarge, aneurysm and haemorrhage risk increases.5 Enlarging renal angiomyolipomas can cause chronic kidney disease needing dialysis or eventual renal transplantation.6 Patients with angiomyolipomas often develop new lesions and recurrence of treated lesions.7 Lymphangioleiomyomatosis occurs in around 30% of women with tuberous sclerosis, and also rarely occurs in patients without tuberous sclerosis complex (sporadic lymphangioleiomyomatosis).8,9-11 Hamartin and tuberin form a complex with Tsc2B-Bub2-Cdc16 (TBC) 1 domain family, member 7 (TBC1D7), which is required for the proper regulation of Rheb and mamalian target of rapamycin complex 1 (mTORC1) by cellular growth conditions.12 The hamartin-tuberin complex inhibits mTORC1, and loss of the complex leads to constitutive mTORC1 activation, aberrant signalling, and tumour growth.13 The ability of sirolimus, an mTOR inhibitor, to improve manifestations of tuberous
sclerosis, including angiomyolipoma, subependymal giant-cell astrocytoma, and lymphangioleiomyomatosis, has been shown in a small number of studies that were reported after the initiation of our trial. Furthermore, angiomyolipomas are thought to arise from one progenitor cell called the perivascular epithelioid cell (PEC). The PEC gives rise to a family of neoplasms termed perivascular epithelioid cell neoplasms or PEComas. Since angiomyolipomas are an example of a PEComa, they have the potential to produce vascular collagen (ie, collagen type IV).

Everolimus is a rapamycin derivative that inhibits the mTOR pathway by acting on mTORC1. A phase 1–2 clinical trial with everolimus was done in a small population of patients (n=38, median age 32 years, 28 were men) with tuberous sclerosis, lymphangioleiomyomatosis, or both (ClinicalTrials.gov identifier NCT00457964) at doses of 5 mg or 10 mg once daily, or 30 mg, 50 mg, or 70 mg once weekly. A mean reduction in the sum of the volumes of target angiomyolipoma lesions (defined as lesions with a maximum diameter of at least 1 cm) at 12 months of 47% was reported (p<0.0001). The response was similar between daily and weekly dosing arms. Adverse events were primarily characterised by mouth ulcers and infections, and were manageable and consistent with the known safety profile of everolimus in patients with tuberous sclerosis.

Here we report the first prospective, international, randomised, double-blind, placebo-controlled, phase 3 study to assess everolimus efficacy and safety in patients with angiomyolipoma associated with tuberous sclerosis or sporadic lymphangioleiomyomatosis. Additionally, the effect of everolimus on mediators of tumour vascularisation, vascular endothelial growth factor D (VEGF-D), and collagen type IV, are presented.

Methods

Patients

Eligible patients aged 18 years or older had at least one angiomyolipoma 3 cm or larger in its longest diameter, and a definite diagnosis of tuberous sclerosis per consensus criteria or sporadic lymphangioleiomyomatosis (biopsy-proven or chest CT scan). Patients were excluded if their angiomyolipoma required surgery at randomisation, or if they had angiomyolipoma-related bleeding or embolisation during the 6 months before randomisation. Patients with lymphangioleiomyomatosis were excluded if their carbon monoxide diffusion capacity (DLco) was 35% or less, oxygen saturation was below normal at rest, or oxygen saturation was 88% or less on a 6-min walking test with up to 6 L/min of oxygen.

All patients (or legal representatives if patients had developmental delays) provided written informed consent according to local guidelines before enrolment. Independent ethics committees, local ethics review boards, or both, approved the protocol, which was executed according to International Conference on Harmonisation Good Clinical Practice guidelines. An independent data monitoring committee did safety reviews every 6 months, and their responsibilities included minimising the exposure of patients to an unsafe therapy or dose, making recommendations for changes in trial processes where appropriate, advising on the need for dose adjustments because of safety issues, and endorsing continuation of the trial. A study steering committee supervised study conduct.

Study design and treatment

Patients were randomly assigned in a 2:1 fashion to receive either everolimus or placebo, stratified by enzyme-inducing antiepileptic drug use at randomisation and by the presence of sporadic lymphangioleiomyomatosis. Everolimus 10 mg per day was administered orally, with dose modifications allowed on the basis of safety findings. Concomitant use of strong inhibitors or inducers of cytochrome P450 3A4 or p-glycoprotein (PgP) was to be avoided during the study; use of antiproliferative agents other than study drug was prohibited.

Patients received blinded study treatment until angiomyolipoma progression, occurrence of unacceptable toxicity, or patient withdrawal for any other reason. Angiomyolipoma progression was defined as one or more of: increase from the nadir of 25% or more in angiomyolipoma volume (sum of volumes of all target angiomyolipomas identified at baseline) to greater than baseline; appearance of a new angiomyolipoma at least 1 cm in longest diameter; increase from nadir of 20% or more volume of either kidney to greater than baseline; or angiomyolipoma-related bleeding grade 2 or more as defined by the Common Terminology Criteria for Adverse Events, version 3.0. Patients with angiomyolipoma progression were unblinded, and placebo patients were offered open-label everolimus. The core phase of the trial, which lasted until the last randomised patient had been treated for 6 months, was analysed, and only data until the database lock on June 30, 2011 (before the start of the open-label phase), were considered in this analysis.

Kidney CT or MRI (same modality used throughout the study for each patient) was done at baseline, 12, 24, and 48 weeks, and annually after start of study treatment and assessed with a blinded central radiology review. Skin lesions resulting from tuberous sclerosis complex include hypomelanotic macules, the shagreen patch, periangual or subungual fibromas, and facial angiofibromas, forehead plaques, or both, and were assessed every 12 weeks using the seven-point grading scale Physician’s Global Assessment of Clinical Condition (appendix). Adverse events were monitored throughout the study and graded according to the Common Terminology Criteria for Adverse Events v3.0 via patient-reported or caregiver-reported responses as well as investigator assessment. The primary efficacy endpoint was the proportion of patients with a confirmed angiomyolipoma response, defined as a reduction in angiomyolipoma volume (sum
of volumes of all target angiomyolipomas identified at baseline) of 50% or more relative to baseline and absence of angiomyolipoma progression. Initial response required confirmation by another scan. Key secondary endpoints were time to angiomyolipoma progression and skin lesion response rate. Additional endpoints included time to angiomyolipoma response, duration of angiomyolipoma response, duration of skin lesion response, pharmacokinetics of everolimus (including exposure and exposure-efficacy relation), plasma angiogenic molecules (including change from baseline and correlation with the angiomyolipoma volume), change from baseline in pulmonary function in lymphangioleiomyomatosis and sporadic lymphangioleiomyomatosis patients, and safety.

Other prespecified exploratory endpoints in the trial (which will be presented in another paper) included change in other lesions associated with tuberous sclerosis complex (namely subependymal giant-cell astrocytomas, tubers, and subependymal nodules), change from baseline in neuropsychological assessments and cognitive function, correlation between volume and longest diameter of angiomyolipomas, change from baseline in seizure severity, mutational analysis of TSC1 and TSC2 genes correlated with angiomyolipoma response rate and time to angiomyolipoma progression, relation between everolimus concentration and safety, and incidence of angiomyolipoma-related surgery.

At each visit, everolimus trough levels were measured in a central laboratory by high-performance liquid chromatography tandem mass spectrometry (WuXi App Tec, Shanghai, China). Plasma samples were collected predose at baseline and week 24 from all trial participants to measure VEGF-D (Quantikine, R&D Systems, Minneapolis, MN, USA) and collagen IV (Kamiya Biomedical, Seattle, WA, USA) levels by ELISA to assess their prognostic value for disease outcome or predictive value of treatment response. All samples were analysed in duplicate wells and mean concentrations were reported.

Statistical analysis

The planned sample size (N=99) provided 93% power to detect a 20% difference in angiomyolipoma response rates between treatments. Efficacy analyses were done on all randomised patients, and safety analyses were done on all patients who received at least one dose of study drug and had at least one post-baseline assessment. Patients not able to be assessed (either by drop out or other reasons) were considered as non-responders.

Treatment groups were compared by use of an exact stratified Cochran-Mantel-Haenszel test for angiomyolipoma and skin lesion response rates, and a one-sided stratified log-rank test for time to angiomyolipoma progression (all at the one-sided 2.5% significance level). Stratification was modified for statistical testing because only five patients had sporadic lymphangioleiomyomatosis. Therefore, before database lock and unblinding, we decided to group the sporadic lymphangioleiomyomatosis stratum with patients with tuberous sclerosis not using an enzyme-inducing antiepileptic drug; the modified stratification became use versus non-use of an enzyme-inducing antiepileptic drug. Multiplicity was controlled via a predefined fixed sequence testing procedure with a hierarchy of angiomyolipoma response rate, time to angiomyolipoma progression, and skin lesion response rate. The exposure-efficacy analysis was fitting a linear mixed model to log-transformed tumour size with log-baseline tumour size and log Cmin as covariates. The association of collagen IV and VEGF-D with angiomyolipoma volume was investigated by Spearman rank correlation (post-hoc analysis for the statistical test) to establish the relation between biomarker concentration and percentage change from baseline to week 24 in angiomyolipoma volume while adjusting for the baseline levels of the two variables. Statistical analyses were done with SAS software (SAS Institute).

Role of the funding source

Academic investigators and the sponsor designed the study, and the sponsor analysed the data (monitored and stored by PAREXEL). All authors had full access to the data and attest to the accuracy and completeness of the reported data and that the study conformed to the protocol and statistical analysis plan. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between May 8, 2009, and Dec 30, 2010, 118 patients across 24 centres in 11 countries were randomly assigned to receive everolimus (n=79) or placebo (n=39). At data cutoff (June 30, 2011), 83% (98 of 118) of patients were receiving double-blind study treatment, whereas 17% (20 of 118) had discontinued, mainly because of disease...
progression (placebo group only; figure 1). Median dose intensity was 10 mg per day for both treatment groups; mean dose intensity was 8·6 mg per day in the everolimus group and 9·6 mg per day in the placebo group. Median exposure duration was 38 weeks for everolimus and 34 weeks for placebo. Coadministration of strong and moderate cytochrome 3A inhibitors, PgP inhibitors, CYP 3A inducers, and PgP inducers was reported in 47 (59%) everolimus patients and 23 (59%) placebo patients.

Baseline demographic and disease characteristics were generally balanced between treatment groups; more everolimus patients had subependymal giant-cell astrocytomas (table 1). Overall, median age was 31 years (range 18–0–61–0), 78% (92 of 118) of patients had angiomyolipomas in both kidneys, and 29% (34 of 118) of patients had an angiomyolipoma at least 8 cm in longest diameter. Almost 40% (46 of 118) of patients had a previous intervention, including 19% (22 of 118) with nephrectomy.

Angiomyolipoma response rate was 42% (33 of 79 [95% CI 31–53%]) for everolimus compared with 0% (0 of 39 [0–9%]) for placebo (difference 42% [24–58%]; p<0·0001). Median time to angiomyolipoma response for everolimus was 2·9 months. The treatment effect was consistent across all subgroups evaluated, including modified strata, sex, age, and race (figure 2). At week 24, 55% (39 of 71) of everolimus patients had at least a 50% reduction from baseline in sum of volumes of target angiomyolipoma lesions compared with 0% (0 of 33) of placebo patients, and 80% (57 of 71) of everolimus patients had at least a 30% reduction compared with 3% (1 of 33) of placebo patients. Most everolimus patients had large decreases in best percentage change from baseline in sum of volumes of target angiomyolipoma lesions (figure 2, appendix). All angiomyolipoma responses were ongoing for between 10 and 85 weeks at the time of the data cutoff.

Everolimus was superior to placebo in time to angiomyolipoma progression (hazard ratio 0·08 [95% CI 0·02–0·37], p<0·0001; figure 3). Angiomyolipoma progressions were noted in three everolimus patients (4%) and eight placebo patients (21%). No patient achieving an angiomyolipoma response had progressed at the data cutoff date. Estimated progression-free rates (95% CI) for everolimus and placebo, respectively, were 98% (89–100%) and 83% (65–93%) at 6 months, and 92% (65–98%) and 25% (1–64%) at 12 months. Median time to angiomyolipoma progression was 11·4 months for placebo and was not reached for everolimus.

Skin lesions associated with tuberous sclerosis were present at baseline in 114 patients. Everolimus had a significantly higher skin lesion response rate than placebo (26% [20 of 77; 95% CI 17–37%] vs 0% [0 of 37; 95% CI 0–10%]; p=0·0002). At the data cutoff, skin lesion responses were ongoing in the 20 everolimus patients who had a skin lesion response (range 10–84 weeks).

Adverse events were consistent with the known everolimus safety profile. Stomatitis, nasopharyngitis, acne-like skin lesions, headache, cough, and hypercholesterolaemia were the most common adverse events with everolimus therapy (each reported in 20% of

| Table 1: Baseline patient demographic and disease characteristics |
|-------------------------|-----------------------------|
| **Everolimus** (n=79)   | **Placebo** (n=39)          |
| Age in years, median (range) | 32 (18–61) | 29 (18–58) |
| <30 years               | 35 (44) | 20 (51) |
| ≥30 years               | 44 (56) | 19 (49) |
| Sex                     |               |          |
| Men                     | 27 (34) | 13 (33) |
| Women                   | 52 (66) | 26 (67) |
| Race                    |               |          |
| White                   | 71 (90) | 34 (87) |
| Asian                   | 7 (9) | 4 (10) |
| Other                   | 1 (1) | 1 (3) |
| Diagnosis of tuberous sclerosis complex† | 77 (97) | 36 (92) |
| Diagnosis of sporadic lymphangiomyomatosis | 2 (3) | 3 (8) |
| Diagnosis of lymphangiomyomatosis | 22 (28) | 7 (18) |
| ≥1 skin lesion‡         | 77 (97) | 37 (95) |
| Presence of subependymal giant-cell astrocytoma| 43 (54) | 14 (36) |
| Previous angiomyolipoma therapy |         |          |
| Surgery/invasive procedure | 31 (39) | 15 (38) |
| Renal embolisation       | 19 (24) | 9 (23) |
| Nephrectomy              | 14 (18) | 8 (21) |
| Medication               | 0 | 0 |
| Longest diameter of largest angiomyolipoma lesion |               |          |
| ≥8 cm                   | 22 (28) | 12 (31) |
| ≥4 cm and <8 cm         | 45 (57) | 19 (49) |
| ≥3 cm and <4 cm         | 6 (8) | 4 (10) |
| <3 cm                   | 5 (6) | 2 (5) |
| Unknown§                | 0 | 1 (3) |
| Not applicable¶         | 1 (1) | 1 (3) |
| Bilateral angiomyolipoma | 65 (83) | 27 (71) |
| Number of target angiomyolipoma lesions |         |          |
| 0                       | 1 (1) | 1 (3) |
| 1–5                     | 32 (41) | 15 (38) |
| 6–10                    | 46 (58) | 23 (59) |
| Sum of volumes of target angiomyolipoma lesions |         |          |
| Number of patients with one or more target angiomyolipoma, n | 78 | 37 |
| Median (range), cm³     | 85 (9–1612) | 120 (3–4520) |

Data are n (%) unless otherwise stated. †All patients diagnosed with tuberous sclerosis complex had two or more major features. ‡Based on patients having skin lesion photos at baseline or assessment post-baseline, not based on the modified Gomez criteria. §Based on the major feature of subependymal giant-cell astrocytoma in the modified Gomez criteria being ticked yes. ¶Longest diameter of the largest angiomyolipoma lesion is unknown when at least one target lesion larger than 1 cm is confirmed but no precise diameter could be measured. ¶Lesions marked as not applicable are those where there is not at least one target lesion. ¶Lesions identified as not meeting target status were determined by central radiology whereas eligibility criteria were based on the local radiologist.
patients or more) and were primarily grade 1–2 (table 2). Infections (most frequently urinary tract and upper respiratory tract infections) occurred in 65% (51 of 79) of patients on everolimus and 72% (28 of 39) on placebo; there were no grade 4 infections. Adverse events leading to discontinuation occurred in 4% (three of everolimus patients and 10% (four of) placebo patients. In everolimus patients, these adverse events included grade 2 blood phosphorous decrease, one patient with concurrent grade 3 hypersensitivity, grade 3 angioedema, and grade 3 bronchospasm, and convulsion deemed not related to study drug, which resulted in death due to status epilepticus. The 28-year-old male patient had a medical history of intractable seizures, and the investigator did not consider the death treatment related. Dose reduction or interruption because of an adverse event occurred in 48% (38 of 79) of everolimus and 21% (eight of 39) of placebo patients.

One case of grade 2 non-infectious pneumonitis was reported in the everolimus group, which resolved within 14 days after dose reduction. Lung function in patients with lymphangioleiomyomatosis and sporadic lymphangioleiomyomatosis on everolimus showed slightly less deterioration during the study than did patients in the placebo group; median percentage change from baseline to week 24 for DLCO was –3% in the everolimus group and –8% in the placebo group, and for forced expiratory volume in 1 second was –1% for everolimus and –4% for placebo. Interpretation of this exploratory endpoint was limited because of the short duration of treatment exposure and the low number of patients.

The median values at baseline and week 24 for DLCO in the everolimus group were 6·56 and 6·00, respectively, and in the placebo group were 6·16 and 5·84, respectively. The median values at baseline and week 24 for forced expiratory volume in 1 second was –1% for everolimus and –4% for placebo. None of these patients had dose reductions or had to discontinue treatment.

Renal events, which were less common in the everolimus group than in the placebo group (5% [four of 79] vs 15% [six of 39]) and included proteinuria (everolimus 4% [three of 79] vs placebo 8% [3 of 39]), increased blood creatinine (everolimus 1% [one of 79] vs placebo 8% [three of 39]), and transient acute renal failure (everolimus 2–5% [two of 79] vs placebo 0% [0 of 39], all grade 1 or 2.

Mean everolimus trough levels ranged between 7·63 (SD 4·32) ng/mL (week 2) and 9·37 (SD 8·83) ng/mL at week 24 exhibiting large inter-individual variability (56–94%), with lower exposure in patients using enzyme-inducing antiepileptic drugs (5·10 [SD 3·02] vs 1·85 ng/mL) and was 11·4 months in the placebo treatment group, as of data cutoff. The hazard ratio and 95% CI were obtained from the Cox model, stratified by the modified stratification factor (use vs non-use of enzyme-inducing antiepileptic drug). The median percentage change from baseline or slight tumour growth.

Figure 2: Magnitude of treatment effect (A) Angiomyolipoma response rates by subgroup. A forest plot shows the effect of study treatment on the angiomyolipoma response rate across different subgroups (modified strata, sex, age, and race). The area of each diamond is proportional to the number of patients in a particular subgroup. The difference in response rates is everolimus minus placebo, and 95% CI were obtained from the exact unconditional confidence limits. Patients with an overall angiomyolipoma response as not evaluable were considered as non-responders. (B) Best percentage change from baseline in the sum of volumes of target angiomyolipoma lesions, per central radiology review. EIAED=enzyme-inducing antiepileptic drug. Patients were excluded if the overall angiomyolipoma response was not evaluable. Each bar represents one patient. *Subsequent scans for this patient did not demonstrate tumour shrinkage but instead revealed no change from baseline or slight tumour growth.

Figure 3: Kaplan-Meier plot showing time to angiomyolipoma progression, as assessed by central review The median time to angiomyolipoma progression was not reached for patients in the everolimus treatment arm and was 11·4 months in the placebo treatment group, as of data cutoff. The hazard ratio and 95% CI were obtained from the Cox model, stratified by the modified stratification factor (use vs non-use of enzyme-inducing antiepileptic drug). The p value was obtained from the one-sided log-rank test, stratified by use vs non-use of enzyme-inducing antiepileptic drug.
Abdominal pain 9 (11) 0 0 3 (8) 1 (3) 0
Nausea 13 (16) 0 0 5 (13) 0 0
Urinary tract infection 12 (15) 0 0 6 (15) 0 0
Vomiting 12 (15) 0 0 2 (5) 0 0
Anaemia 10 (13) 0 0 1 (3) 0 0
Arthralgia 10 (13) 0 0 2 (5) 0 0
Diarhoea 10 (13) 0 0 2 (5) 0 0
Abdominal pain 9 (11) 0 0 3 (8) 1 (3) 0
Blood lactate dehydrogenase increased 9 (11) 0 0 2 (5) 0 0
Hypophosphataemia 9 (11) 0 0 0 0 0
Eczema 8 (10) 0 0 3 (8) 0 0
Leucopenia 8 (10) 0 0 3 (8) 0 0
Oropharyngeal pain 8 (10) 0 0 4 (10) 0 0
Upper respiratory tract infection 8 (10) 0 0 2 (5) 0 0

Data are n (%). A patient with multiple occurrences of an adverse event is counted only once in that adverse event category. *Four grade 4 adverse events were reported in the everolimus group: two were laboratory abnormalities (blood uric acid increased and neutropenia), one was a convulsion, and one was a hypertensive crisis. One grade 4 adverse event occurred in the placebo group (volvulus).

Table 2: Adverse events of any cause experienced by 10% or more patients in the everolimus treatment group, by grade

10·41 \(\text{[SD 9·47 ng/mL]}\). A large intersubject variability in response was also seen in the relation between percentage change from baseline in angiomyolipoma lesion volume and time-normalised minimum (trough) concentration \((C_{\text{trough}})\). An analysis fitting a linear mixed model to log-transformed tumour size with log-trialume tumour size and log \(C_{\text{trough}}\) as covariates indicated a 10% tumour size reduction from baseline for a 2-fold \(C_{\text{trough}}\) increase (95% CI −16 to −4%). There was no correlation between response-exposure and subgroups of angiomyolipoma volume at baseline (<100 cm³, 100–200 cm³, and >200 cm³).

Plasma VEGF-D and collagen IV levels decreased from baseline through week 24 only in the everolimus treatment group. Median VEGF-D concentration decreased 62% in everolimus-treated patients from 1307·0 to 503·0 compared with a 6% increase from 1762·5 to 1840·5 in placebo patients, and median collagen IV concentration decreased by 42% in everolimus patients from 216·7 to 130·4 compared with no change for placebo, indicating that decreases in VEGF-D and collagen IV concentrations were due to everolimus administration.

Plasma VEGF-D level correlated with angiomyolipoma lesion volume at baseline (appendix) and week 24 for the everolimus group (Spearman correlation coefficients 0·41 and 0·35, respectively, p=0·0191, baseline and p=0·0461 for week 24). Similarly, plasma collagen IV level correlated with angiomyolipoma lesion volume at baseline (appendix) in the everolimus arm (Spearman correlation coefficient 0·49, p=0·0001). Moreover, reductions in angiomyolipoma volume and VEGF-D levels were also correlated (Spearman partial correlation coefficient 0·43, p=0·0007) in the 62 everolimus patients for whom both VEGF-D concentrations and angiomyolipoma volume were determined at both baseline and week 24 (appendix).

Discussion
The results of this trial show the benefit of everolimus for treating angiomyolipomas associated with tuberous sclerosis complex. The best overall response rate was significantly higher for everolimus than for placebo and comparable with the response to embolisation. Everolimus had a homogeneous and consistent effect across all subgroups (modified strata, sex, age, or race). Furthermore, reductions in sum of target angiomyolipoma volumes were seen in around 95% of evaluable everolimus patients. Effectiveness of everolimus over placebo was also evident for the secondary endpoints, time to angiomyolipoma progression, and skin lesion response rate.

The key goal in patients with renal angiomyolipomas is prevention of bleeding and preservation of renal function, and in recent years, embolisation has been recommended to control active bleeding and to prevent future bleeds. Embolisation and surgical therapies can successfully treat solitary lesions and are often necessary interventions in emergency situations, but they are less useful for addressing coalescent renal angiomyolipomas. In this study, patients typically had large, bilateral, or multiple angiomyolipomas needing intervention, despite nearly 40% having already had an invasive procedure for angiomyolipoma (kidney embolisation in roughly 25% and nephrectomy in 19%), which highlights the limitations of current treatment approaches. There is an unmet clinical need for systemic treatments for angiomyolipoma to address the multifocal nature of renal involvement. Moreover, even focused invasive therapies carry risk to the surrounding normal parenchyma.

In our trial, few patients discontinued because of adverse events, most of which were grade 1 or 2 and reversible. Infections occurred with similar frequency and severity in both groups, most commonly upper respiratory infections. Although infection is an identified risk in patients treated with everolimus, our study recorded no increased risk of infection in this patient population. Pharmacodynamic findings suggest therapeutic drug monitoring might help to avoid overexposure and improve safety.

Amenorrhoea was reported in the study (everolimus 13% vs placebo 4%), and although the cause was unknown, disturbed hormone levels and ovarian cysts associated

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Panel: Research in context

Systematic review
We searched PubMed using the primary search terms of “tuberous sclerosis complex”, “angiomyolipoma”, “lymphangioleiomyomatosis”, and “everolimus”. Our search was not limited by date. The reference lists of publications identified in the search were also analysed for relevant articles. We identified reports of clinical trials and case studies of angiomyolipomas associated with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis, but did not identify any other placebo-controlled, randomised studies of everolimus in this patient population.

Interpretation
This study confirms the effectiveness of mTORC1 inhibition with everolimus as a therapy for symptomatic angiomyolipomas associated with tuberous sclerosis complex. This finding opens an alternative avenue for treatment of patients who are not amenable to surgical intervention. As tuberous sclerosis complex is a life-long disorder that can affect multiple organ systems, the availability of a systemic treatment that can target different hamartous growths in the body simultaneously (eg, angiomyolipomas, lymphangioleiomyomatosis, and facial angiofibromas) is a positive development for future treatment of affected individuals. Inhibition of mTORC1 with everolimus might also prove useful in several other conditions that result from overactivation of the mTOR pathway, such as PTEN (phosphatase and tensin homologue) hamartoma tumour syndromes, Cowden’s syndrome, and Birt-Hogg-Dube syndrome.

with mTOR inhibitor therapy have been described. Amenorrhoea might not have been reported in other everolimus trials, since patients with renal cell carcinoma and neuroendocrine tumours are typically older adults. In a study of everolimus for the treatment of subependymal giant-cell astrocytomas associated with tuberous sclerosis, three of eight patients aged 17 or 19 years who were treated with everolimus had amenorrhoea. Although four of the seven cases of amenorrhoea in our study resolved without intervention, surveillance for this potential side-effect is warranted in women patients of child-bearing potential. Since this trial is the second to note amenorrhoea as an adverse event associated with everolimus treatment (panel), it is being considered as a potential risk and is being further investigated.

Patient MRIs can be challenging to obtain because of patient developmental status or implanted vagal nerve stimulators. Monitoring collagen IV and VEGF-D plasma levels might offer non-invasive methods to assess disease burden in untreated patients and help to monitor angiomyolipoma response to therapy. VEGF-D reduction by sirolimus has been reported in two tuberous sclerosis studies. Both studies showed VEGF-D reductions similar to that noted in our study, but neither had a control group to show unequivocally that the reductions were related to sirolimus. Sandra Dabora and colleagues also noted a correlation between VEGF-D concentration and lesion volume at baseline and week 52 in response to sirolimus, supported by our findings that change in VEGF-D correlated with disease burden and treatment response. Inclusion of collagen IV and VEGF-D assessments in future trials might yield new surrogate markers that could potentially limit the need for MRI in patients who need sedation or anaesthesia for such scans. These markers might also be useful for management of clinical response and potentially contribute to understanding angiomyolipoma pathobiology.

In summary, everolimus was more effective than placebo in angiomyolipoma response rate, time to angiomyolipoma progression, and skin lesion response rate. At week 24, over half the everolimus patients had at least a 50% reduction from baseline in target angiomyolipoma volume, whereas no placebo patients had volume reductions of 50% or more. The everolimus safety profile was consistent with previously reported mTORC1 inhibitor tolerability profiles. The mostly mild adverse events were generally managed while continuing everolimus treatment. The results of our study show that everolimus offers a potential pharmacological treatment option for patients with tuberous sclerosis and angiomyolipomas.

Contributors
JJB, JCK, PJdV, VHW, TS, GS, JL, and DL participated in the study design and discussions. JJB, JCK, ER, GS, and KB did the research. JJB, ER, GS, and KB oversaw data collection. JB, JCK, ER, BAZ, MF, EB, MS, SB, DC, TS, and KB collected the data. JJB, JCK, BAZ, SB, PJdV, DC, TS, GS, JL, DL, and KB participated in the data analysis and discussions. JJB, ER, BAZ, MF, PJdV, VHW, DC, TS, GS, JL, DL, and KB interpreted the data. JJB, JCK, PJdV, and KB did the literature review. JJB, JCK, PJdV, VHW, DC, GS, and KB wrote the manuscript. JJB, JCK, ER, MF, MS, SB, PJdV, GS, JL, DL, and KB edited and reviewed the manuscript. JJB, JCK, ER, EB, MS, NN, SB, and KB recruited and enrolled patients. All authors approved the final draft of the manuscript.

Conflicts of interest
DC, TS, GS, JL, and DL are employees of Novartis. DC, GS, and DL own Novartis stock. DL has Novartis stock options. JJB, JCK, ER, BAZ, MF, EB, MS, PJdV, and KB are Novartis consultants (including advisory boards), and have had travel paid, research funded, or speaker honoraria/speakers bureau by or for Novartis. MF is a member of speakers bureaus and advisory boards for Lundbeck and UCB. NN, SB, and VHW declare that they have no conflicts of interest.

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