

Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (EXIST-1): a multicentre, randomised, placebo-controlled phase 3 trial



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Summary

Background Tuberous sclerosis complex is a genetic disorder leading to constitutive activation of mammalian target of rapamycin (mTOR) and growth of benign tumours in several organs. In the brain, growth of subependymal giant cell astrocytomas can cause life-threatening symptoms—eg, hydrocephalus, requiring surgery. In an open-label, phase 1/2 study, the mTOR inhibitor everolimus substantially and significantly reduced the volume of subependymal giant cell astrocytomas. We assessed the efficacy and safety of everolimus in patients with subependymal giant cell astrocytomas associated with tuberous sclerosis complex.

Methods In this double-blind, placebo-controlled, phase 3 trial, patients (aged 0–65 years) in 24 centres in Australia, Belgium, Canada, Germany, the UK, Italy, the Netherlands, Poland, Russian Federation, and the USA were randomly assigned, with an interactive internet-response system, in a 2:1 ratio to oral everolimus 4·5 mg/m² per day (titrated to achieve blood trough concentrations of 5–15 ng/mL) or placebo. Eligible patients had a definite diagnosis of tuberous sclerosis complex and at least one lesion with a diameter of 1 cm or greater, and either serial growth of a subependymal giant cell astrocytoma, a new lesion of 1 cm or greater, or new or worsening hydrocephalus. The primary endpoint was the proportion of patients with confirmed response—ie, reduction in target volume of 50% or greater relative to baseline in subependymal giant cell astrocytomas. Analysis was by intention to treat. This study is registered with ClinicalTrials.gov, number NCT00789828.

Findings 117 patients were randomly assigned to everolimus (n=78) or placebo (n=39). 27 (35%) patients in the everolimus group had at least 50% reduction in the volume of subependymal giant cell astrocytomas versus none in the placebo group (difference 35%, 95% CI 15–52; one-sided exact Cochran-Mantel-Haenszel test, $p<0\cdot0001$). Adverse events were mostly grade 1 or 2; no patients discontinued treatment because of adverse events. The most common adverse events were mouth ulceration (25 [32%] in the everolimus group vs two [5%] in the placebo group), stomatitis (24 [31%] vs eight [21%]), convulsion (18 [23%] vs ten [26%]), and pyrexia (17 [22%] vs six [15%]).

Interpretation These results support the use of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis. Additionally, everolimus might represent a disease-modifying treatment for other aspects of tuberous sclerosis.

Funding Novartis Pharmaceuticals.

Introduction

Tuberous sclerosis complex is estimated to affect more than 1 million people worldwide.¹ It is an autosomal dominant genetic disorder characterised by benign tumours (hamartomas) that arise in many organs, including the brain, kidneys, skin, eyes, lungs, heart, and liver.² The most common manifestations of tuberous sclerosis are neurological (eg, epilepsy, intellectual disability, and neurobehavioural and psychiatric problems, including autism spectrum disorder) followed by renal and pulmonary symptoms.^{1,3} Subependymal giant cell astrocytomas are slow-growing tumours, usually located near the foramen of Monro,¹ that develop in up to 20% of individuals with tuberous sclerosis.^{4–6} They are typically

asymptomatic until they reach a size sufficient to cause ventricular obstruction and hydrocephalus. Postoperative morbidity is substantial, although reports vary—about 20% of patients⁷ and up to 50%.^{6,8} Incomplete resection of subependymal giant cell astrocytomas leads to recurrence;^{1,9} in a retrospective analysis, recurrence or contralateral occurrence was reported in 34% of patients, with 13% requiring repeat operations.⁸

The tuberous sclerosis genes *TSC1* (hamartin) and *TSC2* (tuberin) encode proteins that form the hamartin-tuberin tumour suppressor complex, which restricts the activation of the mammalian target of rapamycin complex 1 (mTORC1), a protein kinase that regulates protein synthesis, and cell growth and proliferation, through Rheb

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(Ras homologue enriched in brain).¹⁰ Most patients with tuberous sclerosis have a mutation in either *TSC1* or *TSC2*,^{11–13} resulting in activation of mTORC1. This finding has led to the investigation of mTORC1 blockade as a treatment approach in tuberous sclerosis. The results of case reports and preliminary studies have shown that mTOR inhibition is associated with improvements in the manifestation of tuberous sclerosis including subependymal giant cell astrocytomas, angiomyolipomas (benign renal tumours), and facial angiofibromas.^{14–20} In an open-label study of 28 patients with evidence of serial growth of subependymal giant cell astrocytomas, the mTOR inhibitor everolimus (Afinitor, Novartis Pharma Stein AG, Stein, Switzerland) reduced the volume of subependymal giant cell astrocytomas, seizure frequency, and number of facial angiofibromas.²¹ We assessed the efficacy and safety of everolimus against placebo in patients with subependymal giant cell astrocytomas associated with tuberous sclerosis complex in the phase 3 EXAMining everolimus In a Study of Tuberous sclerosis complex (EXIST-1) trial.

Methods

Patients

Eligible patients (aged 0–65 years) had a definite diagnosis of tuberous sclerosis complex according to consensus criteria,^{22,23} at least one target subependymal giant cell astrocytoma with the longest diameter 1 cm or greater as assessed with multiphase MRI, and one or more of the following when the results of an MRI done within 4 weeks of randomisation were compared with an earlier MRI: serial worsening (defined as an increase of at least 25% in volume of subependymal giant cell astrocytomas) based on the results of local imaging and radiographic assessment; presence of a new lesion 1 cm or greater in diameter; or new or worsening hydrocephalus (according to central radiological assessment of changes in ventricular configuration, periventricular oedema, and qualitative assessment of the dynamics of cerebrospinal fluid flow). Patients had to be medically stable and unlikely to require surgery for subependymal giant cell astrocytomas, with no critical hydrocephalus or imminent cerebral herniation.

The protocol was approved by an ethics committee at each centre, before the first patient was enrolled. The study was done in accordance with the principles of Good Clinical Practice, Declaration of Helsinki, and all local regulations. An independent data monitoring committee reviewed the safety every 6 months. All patients (or their legal representatives) provided written informed consent before enrolment.

Study design and treatment

The EXIST-1 double-blind, phase 3 trial was undertaken in ten countries (Australia, Belgium, Canada, Germany, UK, Italy, Netherlands, Poland, Russian Federation, and USA), in 24 centres. Patients were randomly assigned in a

2:1 ratio to everolimus or matching placebo, stratified according to the use of enzyme-inducing antiepileptic drugs. Everolimus was administered orally at a starting dose of 4.5 mg/m² body surface area per day and subsequently adjusted to attain blood trough concentrations of 5–15 ng/mL. In the event of treatment-related toxic effects, protocol-specified dose modifications were permitted. The starting dose was chosen to be just less than the maximum tolerated dose (5 mg/m² per day) in children with malignancies.²⁴ Patients were prohibited from using strong and moderate inhibitors of cytochrome P450 3A4 (CYP3A4) and P-glycoprotein (except antiepileptic drugs), strong inducers of CYP3A4 (except antiepileptic drugs), and concomitant use of anti-proliferative drugs (those who had previously used anti-proliferative agents were excluded from the study).

The trial consisted of a core phase from the start of the trial to the time when the last patient had been treated with everolimus or placebo for 6 months, and a planned extension phase in which all patients would be given the option of starting open-label everolimus if the results of the core phase favoured everolimus. The extension phase would continue until 4 years after the last patient was randomly assigned to treatment, ensuring follow-up of 4–5 years.

Randomisation and masking

An interactive internet-response system was used for random assignment of patients in a 2:1 ratio to everolimus and placebo and for management of their treatment to maintain allocation concealment. Patients were given masked study treatment (identical everolimus and placebo) unless discontinued as a result of unacceptable toxicity, withdrawal of consent, loss to follow-up, or progression of subependymal giant cell astrocytomas according to the results of independent, central radiological review. All study personnel were masked to treatment assignment. Dose adjustments for patients in the placebo group were recommended through the interactive internet-response system, in a randomised fashion, to maintain masking. Progression of subependymal giant cell astrocytomas was defined as an increase of 25% or more from the nadir volume at baseline; unequivocal worsening of non-target lesions of subependymal giant cell astrocytomas; the appearance of new lesions of 1 cm or more in diameter; or new or worsening hydrocephalus. Patients with progression of subependymal giant cell astrocytomas were unmasked to treatment, and those in the placebo group were offered open-label everolimus.

Efficacy and safety

The primary endpoint was the proportion of patients with confirmed tumour response, defined as a reduction in the total volume of all target subependymal giant cell astrocytomas of 50% or more relative to baseline, in the absence of worsening of non-target subependymal giant cell astrocytomas, new lesions of 1 cm or greater in

diameter, and new or worsening hydrocephalus. The initial tumour response required confirmation with an MRI scan 8–12 weeks later. Key secondary endpoints were absolute change from baseline to 24 weeks in seizure frequency per 24 h by use of a video electroencephalogram, time to progression of subependymal giant cell astrocytomas, and skin lesion response rate in patients with at least one skin lesion at baseline. Other secondary endpoints were angiomyolipoma response rate (defined as a reduction in the total volume of all target angiomyolipomas identified at baseline of 50% or more relative to baseline, with no new angiomyolipoma 1·0 cm or more in longest diameter, no increases in volume of kidney by more than 20% from nadir, and no angiomyolipoma-related bleeding of grade 2 or worse) in patients with one or more target angiomyolipomas, and time to, duration of, and correlation of response of subependymal giant cell astrocytomas with *TSC1* and *TSC2* gene mutation status.

Brain MRI was done at months 3, 6, and 12 after initiation of the treatment and yearly thereafter until discontinuation of the patient from study. For patients with one or more angiomyolipoma of at least 1 cm in diameter at screening or baseline, MRI or CT of the kidney was done on the same schedule as the brain MRI. All scans were assessed by central radiological review. All patients completed a 24 h video electroencephalogram at baseline and 24 weeks (or end of treatment for those who discontinued) that was sent for independent central review. Skin lesions were assessed with the Physician's Global Assessment of Clinical Condition scale^{25,26} (a 7-point grading scale for evaluation of the overall extent of improvement or worsening of the patient's skin lesions compared with baseline) every 3 months. Blood was drawn every visit starting at week 2 for pharmacokinetic analysis. Laboratory assessments, including haematology and blood chemistry, were done every 2 weeks for the first 8 weeks, then at months 3, 4·5, and 6, and then every 3 months thereafter. DNA was isolated from whole blood at baseline for the purpose of sequencing *TSC1* and *TSC2* genes.

Adverse events were monitored continuously throughout the study with the Common Terminology Criteria for Adverse Events (version 3.0).²⁷ At each visit, patients or their carers were assessed for pulmonary symptoms consistent with interstitial pneumonitis, a known adverse effect of mTORC1 inhibition.

Statistical analysis

The planned sample size (n=99) was estimated with a simulation approach, giving the study 93% power to detect a 20% difference in response rates (assuming ≥20% with everolimus and 0 with placebo) of subependymal giant cell astrocytomas between treatment groups. The type 1 error was 2·5%.

Efficacy analyses included all patients (full analysis set) who were randomly assigned. Safety analyses

included all patients who were given at least one dose of study drug and had at least one post-baseline assessment. The per-protocol set was used for supportive analysis of the primary endpoint and consisted of all patients from the full-analysis set without any major protocol deviations who could be assessed for efficacy and had been treated for at least 50% of the days in the first 12 weeks since the first day of treatment. The everolimus and placebo groups were compared with a

	Everolimus group (n=78)	Placebo group (n=39)
Age (years; median, range)	9·5 (1·0–23·9)	7·1 (0·8–26·6)
Age (years)		
<3	13 (17%)	7 (18%)
3 to <18	55 (71%)	26 (67%)
≥18	10 (13%)	6 (15%)
Sex		
Male	49 (63%)	18 (46%)
Female	29 (37%)	21 (54%)
Ethnic origin		
White	73 (94%)	36 (92%)
Black	3 (4%)	1 (3%)
Other	2 (3%)	2 (5%)
Body surface area (m ² ; median, range)	1·07 (0·42–2·16)	0·96 (0·40–2·14)
Two or more main features of tuberous sclerosis complex	78 (100%)	39 (100%)
Use of enzyme-inducing antiepileptic drug	15 (19%)	7 (18%)
Presence of seizure on baseline electroencephalogram	27 (35%)	13 (33%)
One or more skin lesion	72 (92%)	38 (97%)
One or more angiomyolipoma	30 (38%)	14 (36%)
Hydrocephalus	8 (10%)	0
Previous treatment for subependymal giant cell astrocytomas	6 (8%)	2 (5%)
Drug	0	0
Surgery	6 (8%)	2 (5%)
Worsening subependymal giant cell astrocytomas confirmed by central review*	66 (85%)	34 (87%)
Serial growth	63 (81%)	32 (82%)
New lesion 1 cm or more in longest diameter	7 (9%)	5 (13%)
New or worsening hydrocephalus	5 (6%)	0
Number of target lesions of subependymal giant cell astrocytomas		
0	2 (3%)	0
1	40 (51%)	25 (64%)
2	34 (44%)	14 (36%)
3	1 (1%)	0
≥4	1 (1%)	0
Volume of subependymal giant cell astrocytomas (cm ³ ; median, range)	1·63 (0·18–25·15)	1·30 (0·32–9·75)
TSC mutation status†		
TSC1 and TSC2	1 (1%)	0
TSC1	10 (13%)	3 (8%)
TSC2	55 (71%)	29 (74%)
None	11 (14%)	7 (18%)

Data are number (%), unless otherwise indicated. *At least one criterion for worsening subependymal giant cell astrocytomas compared with prebaseline. †One patient in the everolimus group did not have mutation analysis.

Table 1: Baseline demographic and clinical characteristics of patients

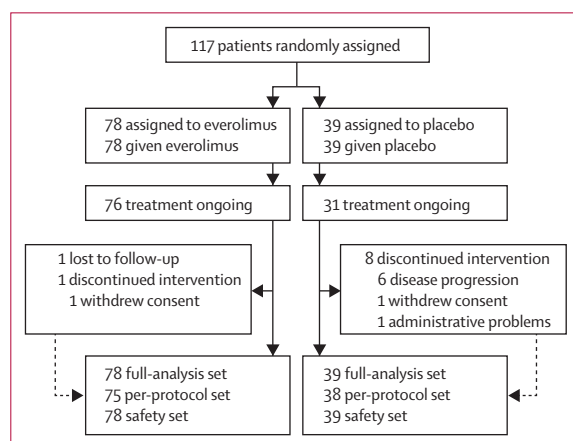


Figure 1: Trial profile

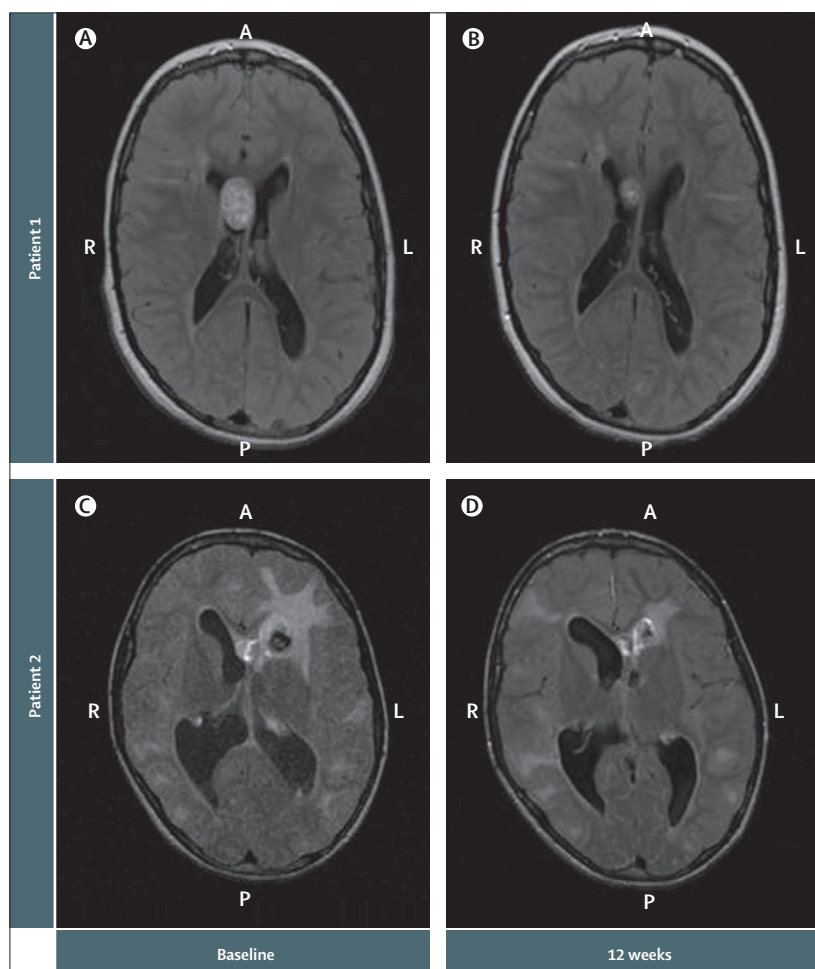


Figure 2: Contrast-enhanced axial fluid attenuated inversion recovery MRI of subependymal giant cell astrocytomas in two patients before (A, C) and after 12 weeks of everolimus (B, D)

Volume of subependymal giant cell astrocytomas was reduced in the two patients after 12 weeks of everolimus. Peritumoral oedema and white matter dysplasia were also reduced in patient 2, who had an unsuccessful resection for subependymal giant cell astrocytomas. Ventricular size was reduced overall in patient 2 despite an apparent increase in the right frontal horn due to resolution of the mass effect from the oedema. A=anterior. R=right. L=left. P=posterior.

one-sided exact Cochran-Mantel-Haenszel test for response rate of subependymal giant cell astrocytomas and skin lesions, a one-sided stratified log-rank test for the time to progression of the astrocytomas, and a one-sided test from rank ANOVA with baseline as covariate for seizure frequency.²⁸ All these tests were stratified according to the protocol (antiepileptic drug use vs no antiepileptic drug use) and done at the 2.5% level. Patients with unknown response of subependymal giant cell astrocytomas were judged non-responders for the analysis. For the key secondary endpoints, multiplicity was controlled through a predefined fixed-sequence testing procedure with a hierarchy of seizure frequency, time to progression of subependymal giant cell astrocytomas, and skin lesion response rate. Statistical analyses were done with SAS software (version 9.2). The data cutoff date for all analyses was 6 months after the last patient was randomly assigned to treatment.

The trial is registered with ClinicalTrials.gov, number NCT00789828.

Role of the funding source

The study was designed by academic investigators and representatives of the sponsor Novartis Pharmaceuticals. The data were analysed by the sponsor (monitored and stored by PAREXEL, Waltham, MA, USA). All authors contributed to data interpretation and amendment of the report, 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 made the final decision about where to submit the paper for publication.

Results

Between Aug 20, 2009, and Sept 2, 2010, 117 patients who had subependymal giant cell astrocytomas associated with tuberous sclerosis were randomly assigned to the everolimus (n=78) or placebo group (n=39). Baseline demographics and clinical characteristics were well balanced between the treatment groups, but the everolimus group had a higher proportion of men than did the placebo group and had hydrocephalus (table 1). The median age of patients was 9.5 years (range 0.8–26.6). Skin lesions were present at baseline in 110 patients (94%) and eight (7%) had a history of surgery related to their subependymal giant cell astrocytomas (table 1). Worsening of tumours at baseline, as ascertained by the local investigator, was confirmed by central review in 100 (85%) patients; the frequencies of 17 individuals whose worsening subependymal giant cell astrocytomas were not confirmed by central review were balanced between the treatment groups (12 [15%] in the everolimus group and five in [13%] the placebo group). 84 (72%) patients had *TSC2* mutations (table 1).

The per-protocol set comprised 75 patients in the everolimus group and 38 in the placebo group. Two patients in the everolimus group could not be assessed

because they did not have any target subependymal giant cell astrocytomas identified at baseline central review, one patient in the everolimus group was excluded because of insufficient treatment exposure, and one placebo-treated patient was excluded for protocol deviation.

After a median follow-up of 9.7 months, 76 (97%) patients in the everolimus group and 31 (79%) in the placebo group were still undergoing double-blind treatment (figure 1). The most common reason for discontinuation was disease progression, which was reported exclusively in the placebo group (six [15%] patients); these patients had their treatment changed to open-label everolimus and their data for the double-blind analysis were censored at that point for the analysis of the double-blind period. The median duration of study treatment was 41.9 weeks (range 24.0–78.9) for individuals in the everolimus group and 36.1 weeks (13.9–79.7) for those in the placebo group. The median dose intensity of everolimus was 5.9 mg/m² per day (range 2.3–11.8).

In the full-analysis set, 27 (35%) of 78 patients in the everolimus group and none of 39 in the placebo group had a response in terms of a reduction in the total volume of all target subependymal giant cell astrocytomas of 50% or more relative to baseline (difference 35%; 95% CI 15–52; one-sided exact Cochran-Mantel-Haenszel test, $p < 0.0001$). The result obtained with the per-protocol analysis was similar—27 (36%) of 75 patients in the everolimus group versus none of 38 in the placebo group (36%, 17–53; $p < 0.0001$) had a tumour response. Reductions in volume of subependymal giant cell astrocytomas were detectable with MRI by 12 weeks (figure 2). By week 24, 31 (42%) of 74 patients in the everolimus group had a reduction in total tumour volume of at least 50% versus one (3%) of 34 in the placebo group, and 58 (78%) and five (15%), respectively, had a reduction of 30% or more.

Figure 3 shows that the treatment effect estimates of the tumour response ($\geq 50\%$ reduction relative to baseline) were in favour of everolimus, irrespective of the subgroup—antiepileptic drug use, sex, or age. Some subgroups had few patients, as shown by the wide 95% CIs for the estimates. At data cutoff, 107 patients (76 in everolimus group and 31 in placebo group) were still undergoing treatment; nine had discontinued and one in the everolimus group was lost to follow-up. No cases of progression of subependymal giant cell astrocytomas were seen in the everolimus group; as a result, the duration of tumour response was censored for all everolimus-treated responders. All responses of subependymal giant cell astrocytomas were ongoing at the data cutoff date, and the duration of response was from more than 63 days to more than 255 days. No responses were seen in the placebo group. Response to everolimus was noted irrespective of whether the *TSC* mutation was *TSC1* or *TSC2*, but the rate was lower in patients with a *TSC2* mutation—five (50%) of ten individuals with a mutation in *TSC1* compared with 16 (29%) of 55 with a *TSC2* mutation. None of the patients in the placebo group, irrespective of the mutation status (*TSC1* and *TSC2*, *TSC1*, or *TSC2*), had a tumour response. In everolimus-treated patients with no mutation identified, five (45%) of 11 had a response; none of the seven placebo-treated patients with no mutation identified had a response.

At week 24, the median change from baseline in seizure frequency in 24 h with video electroencephalogram monitoring was 0 in the everolimus and placebo groups ($p = 0.004$). Because a large proportion of patients did not have any seizures at baseline 24 h electroencephalogram (table 1), we did a sensitivity analysis on the subset of individuals who had at least one seizure at baseline. Treatment groups were imbalanced—the placebo group

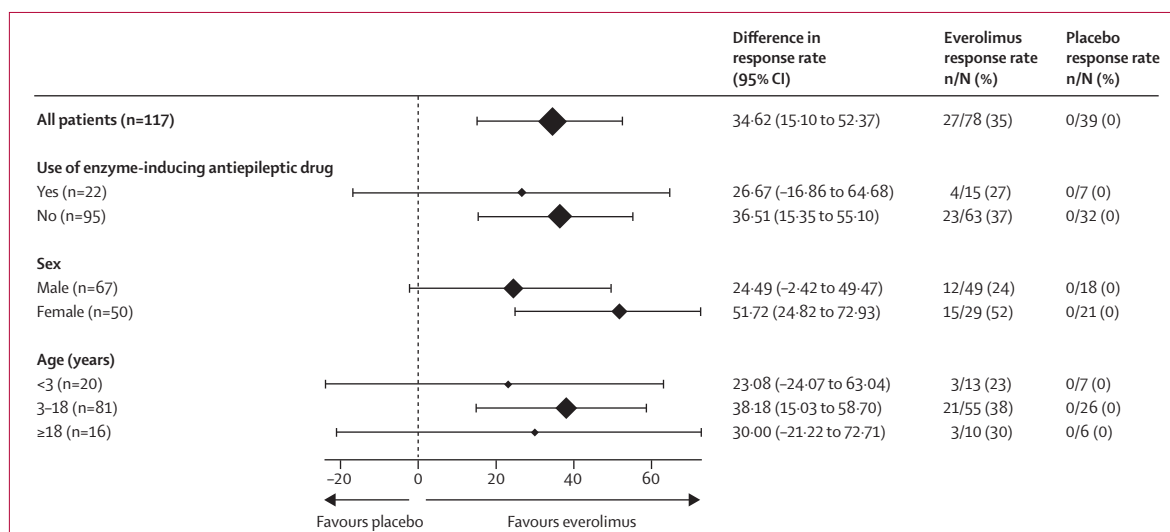


Figure 3: Forest plot of subependymal giant cell astrocytomas response rates in subgroups of patients

The area of each diamond is proportional to the number of patients in the subgroup. 95% CI were obtained from the exact unconditional confidence limits.

had a higher median baseline seizure frequency of 11.0 per 24 h (range 1.0 to 78.9) versus 5.9 per 24 h (1.0 to 42.6) in the everolimus group—the median change from baseline to week 24 was -2.9 per 24 h (95% CI -4.0 to -1.0) for the everolimus group and -4.1 per 24 h (-10.89 to 5.78) for the placebo group ($p=0.2988$).

As judged by central review, six patients, all in the placebo group, had progression of subependymal giant cell astrocytomas at the time of analysis. Median time to tumour progression was not reached in either treatment group, but the estimated progression-free rates at 6 months were 100% for everolimus and 86% for placebo ($p=0.0002$; figure 4).

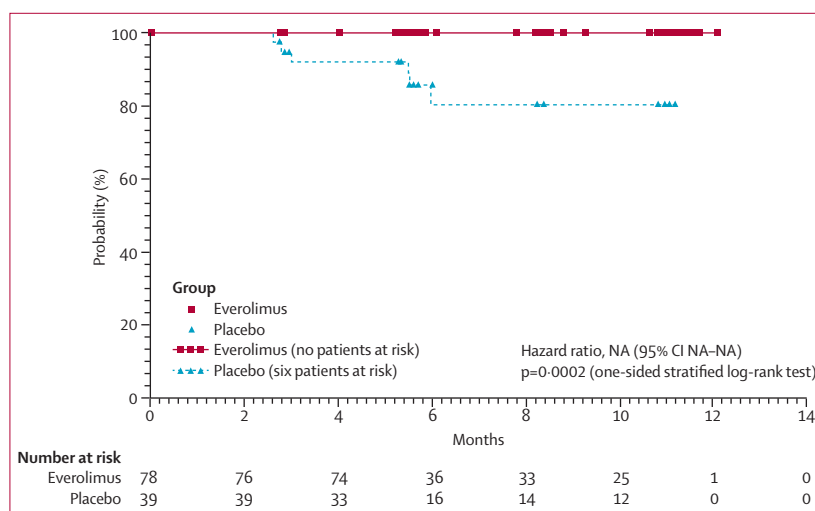


Figure 4: Kaplan-Meier plot of the estimated time to progression of subependymal giant cell astrocytomas. The hazard ratio could not be estimated because progression of the tumours occurred in the placebo group. NA=not applicable.

	Everolimus group (n=78)		Placebo group (n=39)	
	All grades	Grade 3 or 4	All grades	Grade 3 or 4
Any adverse event	75 (96%)	26 (33%)*	35 (90%)	9 (23%)†
Mouth ulceration	25 (32%)	1 (1%)‡	2 (5%)	0
Stomatitis	24 (31%)	6 (8%)‡	8 (21%)	1 (3%)‡
Convulsion	18 (23%)	4 (5%)‡	10 (26%)	2 (5%)‡
Pyrexia	17 (22%)	5 (6%)‡	6 (15%)	0
Nasopharyngitis	14 (18%)	0	9 (23%)	0
Vomiting	13 (17%)	1 (1%)‡	5 (13%)	0
Upper respiratory tract infection	12 (15%)	1 (1%)‡	7 (18%)	0
Fatigue	11 (14%)	0	1 (3%)	0
Cough	10 (13%)	0	4 (10%)	0
Diarrhoea	10 (13%)	0	2 (5%)	0
Rash	9 (12%)	0	2 (5%)	0
Bronchitis	8 (10%)	3 (4%)‡	4 (10%)	1 (3%)‡
Otitis media	8 (10%)	1 (1%)‡	2 (5%)	1 (3%)‡
Pharyngitis	8 (10%)	0	1 (3%)	0

Data are number (%). *All grade 3 except one grade 4 gastroenteritis. †All grade 3 except one grade 4 hyperuricaemia. ‡All grade 3.

Table 2: Adverse events of any cause in more than 10% of patients in the everolimus group

110 patients had at least one baseline skin lesion—30 (42%) of 72 patients in the everolimus group and four (11%) of 38 in the placebo group had a skin lesion response ($p=0.0004$). All skin lesion responses were incomplete.

44 patients had at least one renal angiomyolipoma at baseline (30 in everolimus group and 14 in placebo group); 16 (53%) of 30 patients in the everolimus group versus none of the 14 in the placebo group had an angiomyolipoma response.

The adverse event profile was consistent with the known safety profile of everolimus. Most adverse events were grade 1 or 2. The most common events were mouth ulceration, stomatitis, convulsion, and pyrexia (table 2). The most common grade 3 adverse events were stomatitis, pyrexia, and convulsion; grade 4 events were rare (table 2). Infections, mostly of the upper respiratory tract, were reported by 56 (72%) patients in the everolimus group and 26 (67%) in the placebo group. Other than one (1%) case of grade 1 herpes zoster in the everolimus group, no opportunistic infections were reported; one (1%) infection (gastroenteritis in the everolimus group) was classified as grade 4. One (1%) patient in the everolimus group had grade 2 interstitial pneumonitis after 197 days of treatment that resolved fully 8 weeks after reduction by one dose level.

38 (49%) patients in the everolimus group and four (10%) in the placebo group had adverse events requiring dose reduction or temporary interruption of treatment; most common were stomatitis (13 [17%] patients in everolimus group vs one [3%] patient in placebo group), mouth ulceration (six [8%] vs 0), pyrexia (five [6%] vs one [3%]), and pneumonia (four [5%] vs 0). No adverse events led to discontinuation from the study, and no patients died during the study.

In girls aged 13 years or older, three of eight in the everolimus group (aged 17 years, 19 years, and 19 years) and none of the five in the placebo group had secondary amenorrhoea lasting from 8 weeks to 14 months. Two cases resolved without intervention, and one resolved with progesterone.

Discussion

We noted a significant reduction in volume of subependymal giant cell astrocytomas associated with tuberous sclerosis complex in the everolimus group relative to the placebo group. Large astrocytomas are associated with increased morbidity and risk of hydrocephalus and potential death,²⁹ so stabilisation or even slight reductions in tumour volume translate into clinical benefit, and the reductions noted in this trial are judged clinically significant. This result in a placebo-controlled, double-blind trial, provides confirmation of the findings of previous small studies and case reports^{7,21,30,31} in which everolimus significantly reduced the tumour volume. The inclusion of a placebo group allowed the prospective comparison of efficacy and safety for the first time in this population. A placebo

group was judged necessary because no pharmacological treatments have been approved for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (panel).

Important to assess long term is whether continuous everolimus is necessary to maintain the reduction in the total volume of subependymal giant cell astrocytomas. Regrowth of subependymal giant cell astrocytomas after discontinuation of everolimus was reported in the earlier open-label phase 1/2 trial.²¹ The extension phase of our trial will provide data for long-term efficacy and safety that will help answer questions about the long-term effects of everolimus.

Analysis of change in seizure frequency was inconclusive because most patients had no seizures at baseline or at follow-up. Seizure frequency was evaluated as a secondary endpoint only and patients were selected for the trial on the basis of their need for intervention for progression of subependymal giant cell astrocytomas rather than presence of seizures.

Everolimus was associated with clinically meaningful increases in the time to progression of subependymal giant cell astrocytomas and skin lesion response rate compared with placebo. On the prespecified statistical analysis plan, formal significance could not be ascertained. However, if a Bonferroni approach, a more traditional means of controlling for multiplicity, had been used, the *p* values of 0.0002 for time to progression of subependymal giant cell astrocytomas and 0.0004 for best overall skin lesion response would have fallen to less than 0.025 and 0.0083, respectively, one-sided critical boundary. The benefit in time to progression of subependymal giant cell astrocytomas and skin lesion response rate is clinically relevant evidence of the efficacy of everolimus. Likewise, reduction or stabilisation of angiomyolipoma volume by everolimus is likely to have real clinical benefit by reducing the number of angiomyolipoma-related morbidities, such as risk of haemorrhage and chronic renal failure.

The safety profile of everolimus was consistent with that in the phase 1/2 study of everolimus in patients with tuberous sclerosis complex²¹ and the overall safety profile in the paediatric setting²⁴ with the exception of secondary amenorrhoea in three of eight girls aged 13 years and older. This adverse event might have been a consequence of mTOR inhibition because data suggest that mTOR might play a part in energy sensing and the control of puberty onset.³² A clear pattern was not noted in the three cases of amenorrhoea in our study in terms of risk factors, relation to study drug, or resolution patterns; however, continued vigilance is required. The EXIST-1 protocol was amended to add long-term assessment of potential effects of everolimus on growth, development, and sexual maturation in the paediatric population.

Although presence of a growing subependymal giant cell astrocytoma was the primary criterion for enrolment, everolimus resulted in clinically significant benefits not

Panel: Research in context

Systematic review

We searched PubMed for reports about clinical trials and case studies of patients with subependymal giant cell astrocytomas associated with tuberous sclerosis complex using the primary search terms “tuberous sclerosis complex,” “subependymal giant cell astrocytoma,” and “everolimus.” Our search, which was not limited by date, did not identify any other placebo-controlled, randomised studies of everolimus in this patient population.

Interpretation

The results of this study confirm the effectiveness of inhibition of mammalian target of rapamycin complex 1 (mTORC1) with everolimus for tuberous sclerosis complex. mTORC1 inhibition could benefit other disorders with overactivation of this pathway, such as *PTEN* (phosphatase and tensin homologue) mutations, Cowden's syndrome, and Birt-Hogg-Dube syndrome. Activation of autophagy by mTORC1 inhibitors is a potential treatment for other neurological disorders characterised by accumulation of pathogenic proteins such as Huntington's disease, Parkinson's disease, and collagen VI myopathies.

only with respect to reductions in volume of subependymal giant cell astrocytomas and delays in growth, but also reductions in comorbid skin lesions and kidney tumours. Other trials are in progress to investigate the effect of everolimus on intractable epilepsy and cognitive impairments associated with tuberous sclerosis complex. Our trial is now in the extension phase to assess whether the results can be safely maintained over a longer period. Our results support the use of everolimus for patients with subependymal giant cell astrocytomas associated with tuberous sclerosis. Furthermore, everolimus might represent a disease-modifying treatment for aspects of tuberous sclerosis other than subependymal giant cell astrocytomas.

Contributors

PJdV was a member of the study steering committee and reviewed the literature. DNF, PJdV, VHW, JPF, GS, DL, and TS designed the study. TS was the team leader for scientists and physicians to undertake the trial in accordance with good clinical practice. DNF did the research and oversaw the data gathering. SS, EMB, MF, RK, MHK, JRF, JYW, EAT, JPF, GS, and SJ gathered the data. DNF, SS, EMB, MHK, JYW, GS, HC, DL, and SJ did the data analysis. SS, EMB, RK, OW, JYW, PC, PJdV, VHW, GS, DL, and SJ did the data interpretation. JRF and HC reviewed the data. JPF managed the study. DNF, OW, PJdV, VHW, GS, DL, and SJ wrote the report. SS edited and contributed to the rewriting of the report and was the primary investigator at the study site. EB, MF, RK, MHK, JRF, PC, and EAT were involved in patient recruitment or enrolment.

Conflicts of interest

JPF, GS, HC, DL, and TS are employees of Novartis. DNF, SS, EMB, MF, MHK, JYW, PJdV, and SJ are consultants for Novartis (including advisory boards), and have received travel payments, research funding, or speaker honoraria from Novartis. DNF has received compensation from various attorneys for legal work reviewing medical malpractice cases and occasionally gives expert testimony. SS and JYW have received honoraria from Lundbeck Pharmaceuticals. JYW serves on a professional advisory board for and receives research support from the Tuberous Sclerosis Alliance. PJdV has been a coprincipal investigator on research studies partly funded by Novartis Oncology. The other authors declare that they have no conflicts of interest.

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