Seizures in tuberous sclerosis complex: hitting the target

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Tuberous sclerosis complex is an autosomal dominant disorder of cellular proliferation and differentiation due to mutations in *TSC1* or *TSC2*. Although various types of tumours occur in patients with this disorder, arguably the most important clinical issue is neurological disease, consisting of high risk of seizures, autism spectrum and other behavioural disorders, and intellectual disability.[1] Two-thirds of infants with tuberous sclerosis complex develop epilepsy as infantile spasms or focal-onset disease.[2,3] Vigabatrin is an effective treatment for infants with tuberous sclerosis complex with infantile spasms, but eventually more than half of all affected individuals develop medically refractory epilepsy. The importance of seizure control in tuberous sclerosis complex has long been recognised, because refractory epilepsy is associated with a major risk of developmental and cognitive impairment.

Initially, positional cloning of the *TSC1* and *TSC2* genes in the 1990s provided little insight into the pathogenesis of disease. Eventually, studies in *Drosophila*, mice, and humans in the early 2000s converged to delineate a crucial and unique role of the protein products of these two genes in regulating the activation of mTORC1 by functioning as a GTPase-activating protein for RHEB (figure).[4,5] Fortuitously, rapamycin—an allosteric inhibitor of mTORC1—had been discovered, developed, and in clinical use as an immunosuppressant since the 1990s. This enabled rapid clinical translation of these discoveries into the use of rapamycin and its analogues (so-called rapalogs) for tumours associated with tuberous sclerosis complex, culminating in several positive randomised clinical trials.[6–8]

In tuberous sclerosis complex-related tumours there is complete loss of the TSC protein complex, which leads to high-level mTORC1 activation. However, the pathogenesis of brain disease in this disorder is more complex, with structural lesions known as cortical tubers, and haploinsufficiency effects leading to impaired neuronal migration, axon formation and connectivity, and synaptic plasticity. In mouse models,[9,10] complete loss of *TSC1* and *TSC2* from neuronal or glial cells leads to severe epilepsy and a fatal phenotype, and rapalogs are wonder drugs, completely preventing seizures and extending survival.

Improvement in seizure control and subjective improvement in behaviour and cognition were noted in trials of rapalogs for tuberous sclerosis complex-related tumours, which led to positive but uncontrolled trials of these agents for epilepsy in tuberous sclerosis complex.[11] These observations led to Jaqueline French and colleagues' randomised controlled trial in *The Lancet*,[12] which examines the safety and efficacy of the mTOR inhibitor everolimus (a rapalog) for treatment-resistant focal epilepsy due to tuberous sclerosis complex in patients aged 2–65 years. The investigators assessed the effects of two trough exposure concentrations of everolimus (3–7 ng/mL [low exposure] and 9–15 ng/mL [high exposure]), compared with placebo, on treatment-resistant focal-onset seizures during an 18 week treatment interval. The primary endpoint was response rate, defined as the proportion of patients achieving a ≥50% reduction in seizure frequency. Everolimus showed significant benefit: the response rate was 15·1% in the placebo group (95% CI 9·2–22·8; n=18), compared with 28·2% in the low-exposure group (20·3–37·3; n=33; p=0·0077) and 40·0% in the high-exposure group (31·5–49·0; n=52; p<0·0001). Similarly, the median percentage reduction in seizure frequency was 14·9% (95% CI 0·1–21·7) with placebo versus 29·3% with low-exposure everolimus (95% CI 18·8–41·9; p=0·0028) and 39·6% with high-exposure

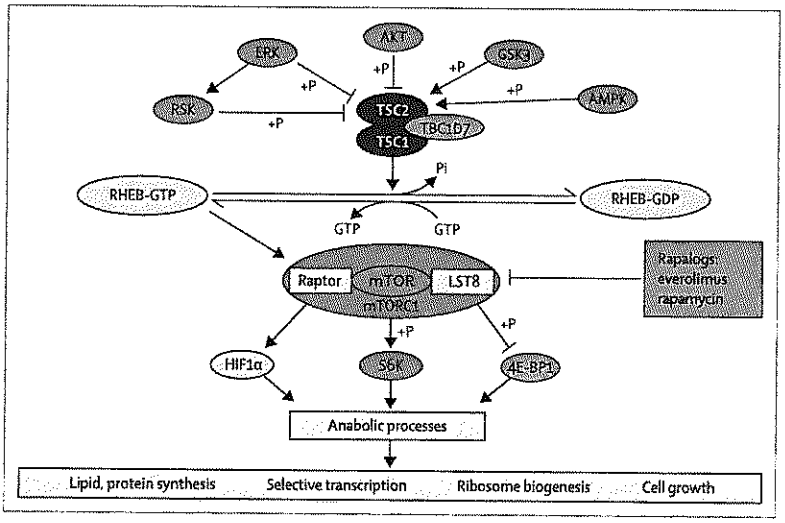

Figure: **The TSC protein complex and mTORC1 signalling**
In cells with loss of either TSC1 or TSC2, RHEB stays in a GTP-bound state and there is constitutive activation of mTORC1. Rapamycin and its analogues (so-called rapalogs) bind to mTORC1 through an interaction with FKBP12, to inhibit mTORC1 kinase activity, especially for S6K. TSC=tuberous sclerosis complex. mTORC1=mTOR complex 1.

everolimus (95% CI 35·0–48·7; p<0·0001). Serious adverse events were more common in patients given everolimus (14% each in the low-exposure and high-exposure groups *vs* 3% in the placebo group), although treatment discontinuation was rare (5%, 3%, and 2%, respectively).

These results can be viewed as a triumph of mechanism-based therapy for epilepsy in a population of patients with tuberous sclerosis complex with highly refractory epilepsy who had been treated with large numbers of anti-epileptic drugs (half of patients had received six or more drugs). However, a few concerns temper our enthusiasm. First, about 12% of patients in the high-exposure group had at least a 25% increase in seizure frequency, and only 4% became seizure free. Second, everolimus therapy is very expensive (US$15 000 for 4 weeks of therapy in the USA), meaning the cost was about $1000 per seizure eliminated. The possibility that everolimus might have other benefits in some patients with tuberous sclerosis complex (such as those with cognitive impairment, behavioural problems, or tumours) mitigates this concern to some extent. Third, the optimum everolimus dose or trough concentration is uncertain. Toxicity did not differ significantly between the high-exposure and low-exposure groups, and the response was clearly better in the high-exposure group. One question is whether even higher trough concentrations could lead to further benefit in some patients?

Many other questions are generated by this provocative trial. First, how exactly does mTORC1 inhibition by everolimus lead to seizure improvement in tuberous sclerosis complex? From mouse models, we know that rapalogs reverse many of the cellular and connectivity effects of mTORC1 activation,[9,10] but the precise mechanism of benefit at the anatomic or neurochemical level is unknown in patients with tuberous sclerosis complex and refractory epilepsy. Better understanding of this mechanism could pave the way toward further improvements in treatment of epilepsy in tuberous sclerosis complex.

Second, should rapalogs be given as first-line epilepsy treatment in patients with tuberous sclerosis complex, and could they be considered for presymptomatic treatment in some infants and children with this disease? Wu and colleagues[13] showed that serial electroencephalography in asymptomatic infants with tuberous sclerosis complex can identify signs of pre-epilepsy before seizure onset. Jozwiak and colleagues[14] provided preliminary evidence showing that treatment of this population of infants with vigabatrin before the onset of seizures improved long-term cognition and reduced the likelihood of subsequent refractory epilepsy. Could presymptomatic administration of an mTOR inhibitor reduce or prevent epilepsy development in tuberous sclerosis complex? Furthermore, could starting mTOR therapy at the time of diagnosis of tuberous sclerosis complex yield long-term benefit in the prevention of the manifest complications of the disease, beyond seizures? Additionally, what effects would life-long rapalog therapy have on growth, development, infection, and cancer risk, to name a few potential long-term complications, in children with tuberous sclerosis complex? Encouragingly, no major side-effects have been reported in children treated for longer than 5 years.[15] Despite these concerns, the outlook for tuberous sclerosis complex has improved greatly in the past decade based on the advent of molecularly targeted therapy. There is hope that improvement in treatments for all the manifestations of the disease will continue.

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ESR served on the Data Safety Monitoring Committee for everolimus trials done 3–4 years previously; Novartis compensated his medical school department for time spent doing this activity. ESR's centre considered becoming a clinical site for EXIST-3, but withdrew from consideration before patients were enrolled. DJK has received funding from Novartis for a clinical trial of everolimus in cancer and from AADi for clinical research support services for a trial of nab-rapamycin in patients with advanced malignant perivascular epithelioid cell tumours.

1 de Vries PJ, Whittemore VH, Leclezio L, et al. Tuberous sclerosis associated neuropsychiatric disorders (TAND) and the TAND Checklist. *Pediatr Neurol* 2015; **52:** 25–35.
2 Chu-Shore CJ, Major P, Camposano S, Muzykewicz D, Thiele EA. The natural history of epilepsy in tuberous sclerosis complex. *Epilepsia* 2010; **51:** 1236–41.
3 Curatolo P, Moavero R, de Vries PJ. Neurological and neuropsychiatric aspects of tuberous sclerosis complex. *Lancet Neurol* 2015; **14:** 733–45.
4 Dibble CC, Manning BD. Signal integration by mTORC1 coordinates nutrient input with biosynthetic output. *Nat Cell Biol* 2013; **15:** 555–64.
5 Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012; **149:** 274–93.
6 McCormack FX, Inoue Y, Moss J, et al. Efficacy and safety of sirolimus in lymphangioleiomyomatosis. *N Engl J Med* 2011; **364:** 1595–606.
7 Bissler JJ, Kingswood JC, Radzikowska E, et al. Everolimus for angiomyolipoma associated with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis (EXIST-2): a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet* 2013; **381:** 817–24.

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8 Krueger DA, Care MM, Holland K, et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med* 2010; **363:** 1801–11.

9 Zeng LH, Xu L, Gutmann DH, Wong M. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Ann Neurol* 2008; **63:** 444–53.

10 Meikle L, Pollizzi K, Egnor A, et al. Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. *J Neurosci* 2008; **28:** 5422–32.

11 Krueger DA, Wilfong AA, Holland-Bouley K, et al. Everolimus treatment of refractory epilepsy in tuberous sclerosis complex. *Ann Neurol* 2013; **74:** 679–87.

12 French JA, Lawson JA, Yapici Z, et al. Adjunctive everolimus therapy for treatment-resistant focal-onset seizures 1 associated with tuberous sclerosis (EXIST-3): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet* 2016; published online Sept 6. http://dx.doi.org/10.1016/S0140-6736(16)31419-2.

13 Wu JY, Peters JM, Goyal M, et al. Clinical electroencephalographic biomarker for impending epilepsy in asymptomatic tuberous sclerosis complex infants. *Pediatr Neurol* 2016; **54:** 29–34.

14 Jozwiak S, Kotulska K, Domanska-Pakiela D, et al. Antiepileptic treatment before the onset of seizures reduces epilepsy severity and risk of mental retardation in infants with tuberous sclerosis complex. *Eur J Paediatr Neurol* 2011; **15:** 424–31.

15 Franz DN, Agricola K, Mays M, et al. Everolimus for subependymal giant cell astrocytoma: 5-year final analysis. *Ann Neurol* 2015; **78:** 929–38.

Adjunctive everolimus therapy for treatment-resistant focal-onset seizures associated with tuberous sclerosis (EXIST-3): a phase 3, randomised, double-blind, placebo-controlled study

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Summary

Background Everolimus, a mammalian target of rapamycin (mTOR) inhibitor, has been used for various benign tumours associated with tuberous sclerosis complex. We assessed the efficacy and safety of two trough exposure concentrations of everolimus, 3–7 ng/mL (low exposure) and 9–15 ng/mL (high exposure), compared with placebo as adjunctive therapy for treatment-resistant focal-onset seizures in tuberous sclerosis complex.

Methods In this phase 3, randomised, double-blind, placebo-controlled study, eligible patients aged 2–65 years with tuberous sclerosis complex and treatment-resistant seizures (≥16 in an 8-week baseline phase) receiving one to three concomitant antiepileptic drugs were recruited from 99 centres across 25 countries. Participants were randomly assigned (1:1:1), via permuted-block randomisation (block size of six) implemented by Interactive Response Technology software, to receive placebo, low-exposure everolimus, or high-exposure everolimus. Randomisation was stratified by age subgroup (<6 years, 6 to <12 years, 12 to <18 years, and ≥18 years). Patients, investigators, site personnel, and the sponsor's study team were masked to treatment allocation. The starting dose of everolimus depended on age, body-surface area, and concomitant use of cytochrome 3A4/P-glycoprotein inducers. Dose adjustments were done to attain target trough ranges during a 6-week titration period, and as needed during a 12-week maintenance period of core phase. Patients or their caregivers recorded events in a seizure diary throughout the study. The primary endpoint was change from baseline in the frequency of seizures during the maintenance period, defined as response rate (the proportion of patients achieving ≥50% reduction in seizure frequency) and median percentage reduction in seizure frequency, in all randomised patients. This study is registered with ClinicalTrials.gov, number NCT01713946.

Findings Between July 3, 2013, and May 29, 2015, 366 patients were enrolled and randomly assigned to placebo (n=119), low-exposure everolimus, (n=117), or high-exposure everolimus (n=130). The response rate was 15·1% with placebo (95% CI 9·2–22·8; 18 patients) compared with 28·2% for low-exposure everolimus (95% CI 20·3–37·3; 33 patients; p=0·0077) and 40·0% for high-exposure everolimus (95% CI 31·5–49·0; 52 patients; p<0·0001). The median percentage reduction in seizure frequency was 14·9% (95% CI 0·1–21·7) with placebo versus 29·3% with low-exposure everolimus (95% CI 18·8–41·9; p=0·0028) and 39·6% with high-exposure everolimus (95% CI 35·0–48·7; p<0·0001). Grade 3 or 4 adverse events occurred in 13 (11%) patients in the placebo group, 21 (18%) in the low-exposure group, and 31 (24%) in the high-exposure group. Serious adverse events were reported in three (3%) patients who received placebo, 16 (14%) who received low-exposure everolimus, and 18 (14%) who received high-exposure everolimus. Adverse events led to treatment discontinuation in two (2%) patients in the placebo group versus six (5%) in the low-exposure group and four (3%) in the high-exposure group.

Interpretation Adjunctive everolimus treatment significantly reduced seizure frequency with a tolerable safety profile compared with placebo in patients with tuberous sclerosis complex and treatment-resistant seizures.

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Introduction

Epilepsy is the most common neurological symptom of tuberous sclerosis complex, an autosomal dominant genetic disorder, and is reported in up to 85% of patients with the condition.[1,2] Nearly two-thirds of patients with tuberous sclerosis complex present with seizures in the first year of life, often as focal seizures or infantile spasms.[1,2] Early onset of epilepsy and particularly untreated early-onset epilepsy is associated with an increased risk of neurodevelopmental disabilities, including autism spectrum disorder and intellectual disability.[2] Seizures associated with tuberous sclerosis complex can be focal, multifocal, or generalised, and are typically difficult to control.[3] More than 60% of patients are resistant to standard therapies such as antiepileptic drugs, epilepsy surgery, ketogenic diet, and vagal nerve

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Research in context

Evidence before this study

Limited evidence is available on the optimal treatment of epilepsy in patients with tuberous sclerosis complex. We searched PubMed for English-language studies using the terms "tuberous sclerosis complex" or "tsc" and "epilepsy" or "seizure", published up to June 23, 2016. Our search identified exploratory clinical studies with small sample sizes and case reports demonstrating successful seizure control in some patients with various conventional antiepileptic drugs, but at least a third of patients are refractory to available medical and surgical therapies. Mutations in *TSC1* and *TSC2* genes responsible for the overactivation of mammallian target of rapamycin (mTOR) are the genetic causes of tuberous sclerosis complex. Pharmacological inhibitors of mTOR have demonstrated efficacy for the treatment of multiple features of tuberous sclerosis complex, including renal angiomyolipoma, subependymal giant-cell astrocytoma, and lymphangioleiomyomatosis. Early human case reports showed beneficial effects of mTOR inhibitors on seizures. Findings from a small prospective, single-group human clinical trial showed an improvement in seizure control in patients with tuberous sclerosis complex and refractory epilepsy. In two prospective human clinical trials, benefit could not be demonstrated when seizure frequency was analysed as a secondary outcome measure. No large randomised clinical trials are available.

Added value of this study

This phase 3 multicentre study provides the benefits and risks of adding everolimus as adjunctive therapy to between one and three antiepileptic drugs in patients with tuberous sclerosis complex who have shown a high burden of treatment-resistant seizures (at least 16 in an 8-week period before randomisation). Additionally, this study provides the first estimates of the optimal range of everolimus exposure suitable in patients with tuberous sclerosis complex and seizures.

Implications of all the available evidence

By comparison with previous studies, which were either case series, retrospective cohort studies, prospective studies of small sample size, or prospective clinical trials with seizure outcome assessed as secondary measure, the findings from EXIST-3 provide evidence that everolimus might be an effective treatment option as adjuvant therapy for children and adults with treatment-resistant epilepsy. EXIST-3 extends the use of everolimus for seizures in tuberous sclerosis complex, in addition to the benefit observed for other aspects of the disease (subependymal giant-cell astrocytoma, renal angiomyolipoma, and facial angiofibroma). The time to efficacy observed in the study suggests that everolimus, unlike conventional antiepileptic drugs, provides a unique mechanism of action through targeting of mTOR overactivation. This study's findings also demonstrate the short-term benefit of everolimus when added to best available antiepileptic drug therapy.

stimulation,[1,2] as opposed to only 30–40% of patients with epilepsy without tuberous sclerosis complex.[4]

To date, tuberous sclerosis complex is treated symptomatically with antiepileptic drugs that are not specific for the underlying cause. Targeting of disease-specific molecular signalling mechanisms that drive the development of seizures has been previously suggested in tuberous sclerosis complex or other epilepsy aetiologies,[5] but not implemented. Antiepileptic drugs render neurons less excitable, typically by interacting with transmembrane ion channels. They can be particularly effective for some specific seizure types or epilepsy syndromes (eg, carbamazepine for localisation related epilepsy, vigabatrin for infantile spasms, and clonazepam for Angelman's syndrome). However, antiepileptic drugs are not necessarily developed for or directed against a specific molecular pathomechanism.[5]

Aberrant mammalian target of rapamycin (mTOR) signalling results in hamartomas, and neuropsychiatric disorders and epilepsy associated with tuberous sclerosis complex.[2,3] Overactivation of mTOR leads to giant, dysplastic neurons, abnormal axonogenesis and dendrite formation, increased excitatory synaptic currents, reduced myelination, and disruption of the cortical laminar structure.[6–11] Dysregulated mTOR activity due to mutations in upstream pathway genes, including *STRADα*, *DEPDC5*, and *PI3K*, has also been implicated in epileptogenesis and seizures associated with cortical malformations.[12] At present, tuberous sclerosis complex is the best-characterised disease associated with mTOR pathway overactivation. Findings from preclinical studies[8,11] have shown that treatment with mTOR inhibitors could increase survival, prevent the development of new-onset seizures, and ameliorate existing epilepsy. Case reports and open-label studies suggest beneficial effects of everolimus in patients with epilepsy associated with tuberous sclerosis complex.[13–17] Everolimus is an mTOR inhibitor that has been approved for the treatment of subependymal giant-cell astrocytoma and renal angiomyolipoma in patients with tuberous sclerosis complex.[14,18] Another mTOR inhibitor, sirolimus, has been approved for the treatment of lymphangioleiomyomatosis in tuberous sclerosis complex.

We postulated that everolimus might improve seizures by targeting the specific molecular defect in patients with tuberous sclerosis complex and treatment-resistant focal epilepsy. Examining Everolimus in a Study of Tuberous Sclerosis Complex (EXIST-3) evaluated the efficacy and safety of two dosing regimens of adjunctive everolimus

compared with placebo in patients with tuberous sclerosis complex and treatment-resistant focal epilepsy.

Methods

Study design and participants

EXIST-3 is a three-arm, prospective, randomised, multicentre, double-blind, placebo-controlled, phase 3 study. It includes an initial 8-week baseline phase, followed by an 18-week core phase (reported here) and a 48-week extension phase (which will be reported on completion). Patients aged 2–65 years with a confirmed diagnosis of tuberous sclerosis complex and treatment-resistant epilepsy, with 16 or more seizures during the 8-week baseline phase (with no continuous 21-day seizure-free period) and receiving between one and three antiepileptic drugs at a stable dose for at least 12 weeks before randomisation were included. Patients were excluded if they had subependymal giant-cell astrocytomas requiring immediate surgical intervention, seizures secondary to drug abuse, psychogenic non-epileptic seizures, active infantile spasms, or an episode of status epilepticus within 1 year before study inclusion.

During the baseline phase, patients or their caregivers completed a seizure diary, recording seizure types and frequencies. In this population, many of whom have developmental delay, there are many seizure mimickers, including inattentive episodes, tics, and stereotyped behavioural events (known as stereotypies).[19] To ensure reliable and consistent classification of seizures across patients, seizures reported by patients and caregivers were entered into a seizure identification form, separated into probable seizures (>80% likelihood of being an epileptic seizure) and questionable seizures (50–80% likelihood) by the investigators. Only probable seizures were counted towards the primary outcome. Focal seizures with retained awareness (simple partial seizures) were considered questionable if they had no motor or observable component, unless they had electroencephalogram (EEG) confirmation. Stares without automatisms or other clear seizure-like manifestations were also counted as questionable. Independent reviewers (epileptologists from the Epilepsy Study Consortium, responsible for harmonising seizure classifications in the study) confirmed the seizure classification and designation as probable or questionable. Because multiple seizure types are present in tuberous sclerosis complex (a disease of focal pathology), all seizures were considered to be focal in onset unless an EEG confirmed a generalised onset. Seizures were assigned to one of six categories: focal motor with retained awareness; focal non-motor with impaired awareness (including atypical absence or bland focal with altered awareness); focal motor with impaired awareness; other focal motor seizures (including those often classified as of generalised origin—ie, focal to bilateral myoclonic, clonic, tonic, atonic); focal to bilateral tonic-clonic; and EEG-confirmed generalised onset seizures (not included in seizure count for primary analysis but included in an exploratory analysis).

All patients (or their legal representatives) provided written informed consent before entering the baseline phase. The study was done in accordance with the principles of Good Clinical Practice, the Declaration of Helsinki, and all local regulations. The study protocol (appendix) and all amendments were reviewed and approved by independent ethics committees or institutional review boards for each centre.

See Online for appendix

Randomisation and masking

At the end of the baseline phase, eligible patients entered the core phase and were randomly assigned (1:1:1), via permuted-block randomisation (block size of six) implemented by Interactive Response Technology (IRT) software, to receive placebo, everolimus titrated to a target trough concentration (C_{min}) of 3–7 ng/mL (low-exposure everolimus), or everlimus titrated to a target C_{min} of 9–15 ng/mL (high-exposure everolimus), in addition to a stable regimen of one to three antiepileptic drugs. Randomisation was stratified by age subgroup (<6 years, 6 to <12 years, 12 to <18 years, and ≥18 years). Dose adjustments to attain the target C_{min} were done during the first 6 weeks of the core phase, and as needed during the subsequent 12-week maintenance period.

Patients, investigators, site personnel, and the sponsor's study team were masked to treatment allocation, but allocation was not concealed from personnel in charge of drug supply, implementation of the randomisation list, and pharmacokinetic bioanalysis. The Data Safety Monitoring Board (DSMB) independent statistician and programmer were semi-blind to treatment allocation at the time of DSMB meetings. Study medication consisted of everolimus 2 mg pills and identical placebo pills, with both medication types being dispensed in yellow or blue blister packs. To maintain blinding, any patient in any treatment group could receive either or both colours of pills, and there were random dummy-dose titrations of placebo tablets in both the placebo group and the low-exposure group.

For the Epilepsy Study Consortium see http://epilepsyconsortium.org/

Procedures

We determined the starting dose of everolimus on the basis of patients' age, body-surface area, and concomitant use of cytochrome P450 3A4 (CYP3A4)/P-glycoprotein (PgP) inducers. For patients younger than 10 years, the starting dose of everolimus was 6 mg/m² per day for those not receiving CYP3A4/PgP inducers and 9 mg/m² per day for those receiving CYP3A4/PgP inducers; for patients aged 10–18 years, the equivalent doses were 5 mg/m² per day and 8 mg/m² per day, and for those older than 18 years were 3 mg/m² per day and 5 mg/m² per day, respectively. During the first 6 weeks of the core phase, up to three dose adjustments were allowed to reach the targeted everolimus trough range. Further dose adjustments were possible as needed during the 12-week

maintenance period of the core phase. Dose adjustments were 2 mg for patients not receiving concomitant CYP3A4 inducers and 4 mg for patients receiving CYP3A4 inducers. Dose increases and decreases were performed for patients with C_{min} values lower and higher than the target range, respectively.

Study treatment continued until the end of the core phase unless there was loss of seizure control, an episode of status epilepticus, interruption of one or more concomitant antiepileptic drugs for more than 7 days, intolerable toxic effects, or withdrawal of consent. Patients or their caregivers continued to record occurrence or absence of seizures on each day throughout the core phase. Patients were then offered to continue in the extension phase, in which all patients received everolimus titrated to achieve a C_{min} of 6–10 ng/mL (automated, IRT controlled) followed by non-automated, investigator-prescribed titrations to achieve a C_{min} of 3–15 ng/mL.

The Vineland II Adaptative Behavior Scale was completed at baseline, at completion of the core phase, then every 6 months thereafter. This scale was completed by the physician while interviewing and observing the patient (survey interview form), but could be completed by the parent or their caregiver if the patient was unable to provide the information required (parent or caregiver form).

Outcomes

The primary efficacy endpoint was change from baseline in seizure frequency for each of the two everolimus C_{min} ranges compared with placebo during the 12-week maintenance period of the core phase, expressed as response rate (reduction in seizure frequency) and median percentage reduction in seizure frequency. Seizure frequency corresponds to the ratio between the number of seizures and the number of days on which seizure information was known within the same period of time (baseline or maintenance phase).

The secondary endpoints included frequency of seizure-free days during the maintenance period, seizure-free rate (patients remaining seizure free during the entire maintenance period), the proportion of patients achieving at least a 25% reduction in seizure frequency from baseline, and exposure–response relationship analysis. A sensitivity analysis was done to assess the robustness of the primary and secondary efficacy endpoints by considering the assessment period as the entire core phase (we used the 12-week maintenance period in the primary analysis to comply with a European Medicines Agency guideline on clinical investigation of medicinal products in the treatment of epileptic disorders).[20] The study also assessed safety during the core phase. We assessed adverse events according to the National Cancer Institute Common Toxicity Criteria for Adverse Events version 4.03.[21]

Statistical analysis

EXIST-3 was planned as a phase 3 registration study in the absence of previous dose-finding studies in this indication of epilepsy associated with tuberous sclerosis complex. A literature review revealed that several recent studies of focal seizures and Lennox-Gastaut syndrome were powered to achieve seizure reductions of 16–22% greater than placebo. In these studies, median reduction in seizure frequency in placebo groups ranged from 11% to 18%.[22–25] In light of these studies, we chose a planned sample size of 345 patients (115 per group), to provide 90% power to detect a difference in response rate from 15% on placebo to 35% in each of the two everolimus groups, with each test at the 1·25% one-sided signficance level. At least 90% power was also expected for percentage reduction in seizure frequency, because response rate is less sensitive due to loss of information in dichotomising a continuous variable. Owing to a failure of the IRT system to titrate the everolimus dose during the first 5 months of the study, there was a concern about potential loss of power, particularly for the high-exposure everolimus group where most patients were expected to need at least one dose increase to reach the targeted trough range of 9–15 ng/mL. Without unblinding, we determined that a maximum of 18 patients in the high-exposure everolimus might have missed dose titrations. Therefore, to mitigate against a potential loss of power, we increased the planned sample size in the high-exposure everolimus group (in a blinded manner) by ten patients.

Descriptive statistics were used to summarise the baseline characteristics of the study population. The full analysis set was the primary efficacy population, comprising all randomly assigned patients. We compared response rate between each everolimus group versus the placebo group using Cochran-Mantel-Haenszel χ^2 tests stratified by age subgroup. For percentage reduction in seizure frequency, we used a rank ANCOVA model, with baseline seizure frequency as a covariate, and stratified by age subgroup. For each of the two primary variables (response rate and percentage reduction in seizure frequency), a Bonferroni-Holm procedure was used to ensure overall family-wise type I error rates of 2·5% (one sided), taking into account the comparison of each everolimus group with placebo; no multiplicity adjustment was made to take account of the two primary variables. The relationship between efficacy parameters and exposure to study drug was assessed using regression models (with logit function for response rate and linear function for seizure frequency). Safety analysis was done with the safety set of all patients who received at least one dose of study drug and had at least one post-baseline safety assessment.

We did statistical analyses with SAS (version 9.2). This trial is registered with ClinicalTrials.gov, number NCT01713946.

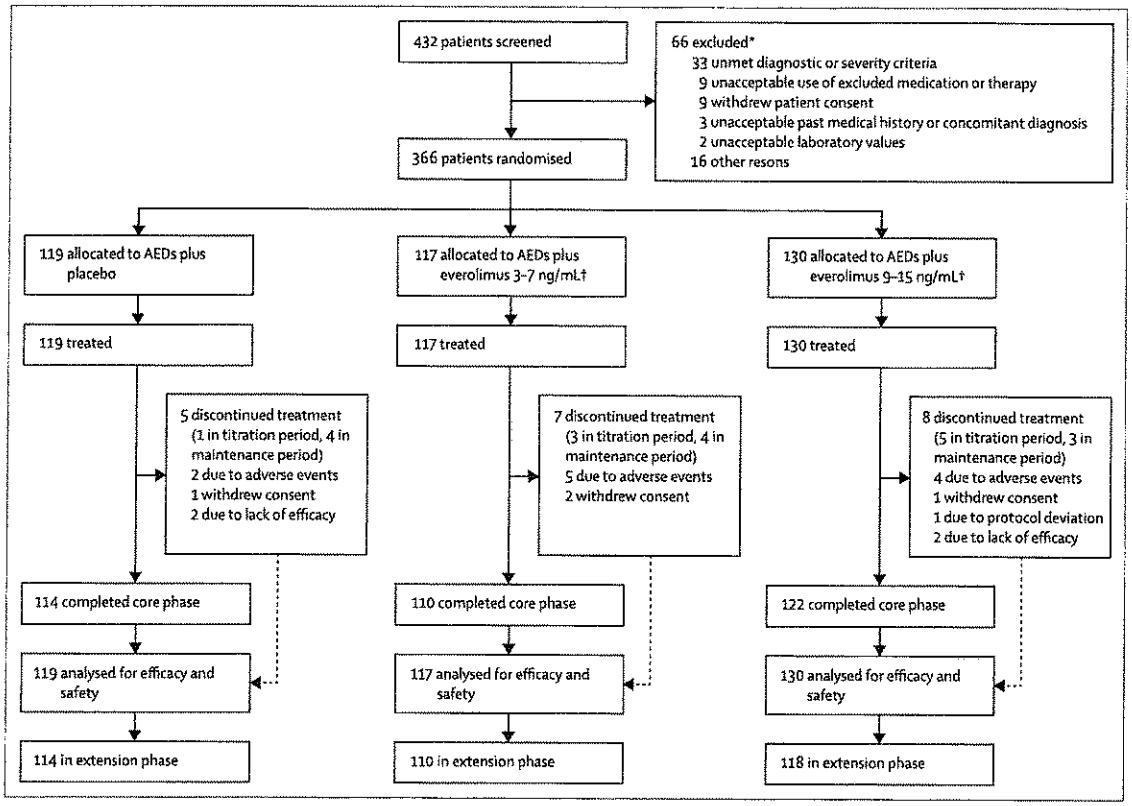

Figure 1: **Trial profile**
AEDs=antiepileptic drugs. *A patient could have had multiple reasons for screen failure. †Everolimus trough concentrations.

Role of the funding source

The study was designed by academic investigators and representatives of the funder, Novartis Pharmaceuticals Corporation. Data were collected electronically using data management systems of a contract research organisation designated by the funder and were analysed by the funder's statistical team. All authors had full access to the data for interpretation and analysis, were involved in development and approval of the report, and had the final responsibility for the decision to submit for publication. All authors vouch for the accuracy and completeness of the reported data, and attest that the study conformed to the protocol and statistical analysis plan.

Results

Between July 3, 2013, and May 29, 2015, 366 patients (190 men, 176 women) were enrolled from 99 centres in 25 countries worldwide and randomly assigned to receive placebo (n=119), low-exposure everolimus (n=117), or high-exposure everolimus (n=130; figure 1). The median age was 10·1 years (range 2·2–56·3) with 104 patients (28%) younger than 6 years and 67 (18%) patients aged 18 years or older (table 1). Of all patients enrolled, 178 (49%) had previously tried and not responded to six or more previous antiepileptic drugs. Antiepileptic drugs that failed most frequently before screening were levetiracetam in 245 (67%) patients, vigabatrin in 243 (66%) patients, and topiramate in 214 (58%) patients. At baseline, focal motor seizures with retained awareness were present in 71 patients (19%), focal non-motor seizures with impaired awareness in 165 (45%), focal motor seizures with impaired awareness in 95 (26%), other focal motor seizures in 149 (41%), focal to bilateral tonic-clonic seizures in 68 (19%), and EEG-confirmed generalised onset seizures in six (2%; table 2). The median seizure frequency per 28 days at baseline excluding EEG-confirmed generalised onset seizures was 42·0 (range 5·3–926·7) in the placebo group, 34·5 (5·5–771·5) in the low-exposure group, and 37·8 (1·0–873·5) in the high-exposure group.

346 (95%) patients completed the core phase. Five (4%) patients in the placebo group, seven (6%) in the low-exposure group, and eight (6%) in the high-exposure everolimus group discontinued treatment during the core phase. The most common reason for treatment discontinuation was adverse events in all treatment groups (placebo, two [2%] patients; low-exposure everolimus, six [5%] patients; and high-exposure everolimus, four [3%] patients). The median dose received by patients in the everolimus low-exposure group was 5·2 mg/m² per day (range 1·3–14·5) and in the

	Placebo (n=119)	Everolimus 3–7 ng/mL (n=117)	Everolimus 9–15 ng/mL (n=130)	All patients (n=366)
Age, years				
Median (range)	10·3 (2·2–52·0)	9·7 (2·2–56·3)	10·1 (2·3–50·5)	10·1 (2·2–56·3)
<6	34 (29%)	33 (28%)	37 (28%)	104 (28%)
6 to <12	37 (31%)	37 (32%)	39 (30%)	113 (31%)
12 to <18	25 (21%)	26 (22%)	31 (24%)	82 (22%)
≥18	23 (19%)	21 (18%)	23 (18%)	67 (18%)
Sex				
Female	58 (49%)	53 (45%)	65 (50%)	176 (48%)
Male	61 (51%)	64 (55%)	65 (50%)	190 (52%)
Race				
White	77 (65%)	76 (65%)	84 (65%)	237 (65%)
Black	1 (1%)	2 (2%)	1 (1%)	4 (1%)
Asian	27 (23%)	29 (25%)	31 (24%)	87 (24%)
Native American	0	0	1 (1%)	1 (<1%)
Pacific Islander	0	1 (1%)	0	1 (<1%)
Other	14 (12%)	9 (8%)	13 (10%)	36 (10%)
Body-surface area, m²				
Median (range)	1·10 (0·5–2·2)	1·09 (0·5–2·4)	1·09 (0·5–2·6)	1·10 (0·5–2·6)
Antiepileptic drugs failed before study start				
2	5 (4%)	4 (3%)	8 (6%)	17 (5%)
3	13 (11%)	15 (13%)	9 (7%)	37 (10%)
4	16 (13%)	22 (19%)	27 (21%)	65 (18%)
5	22 (18%)	22 (19%)	25 (19%)	69 (19%)
6	10 (8%)	10 (9%)	17 (13%)	37 (10%)
>6	53 (45%)	44 (38%)	44 (34%)	141 (39%)

Data are n (%), unless otherwise specified.

Table 1: **Demographic characteristics and medical history by treatment group**

high-exposure group was 7·5 mg/m² per day (1·4–24·4). The median C_{min} observed at the end of the core phase for patients randomly assigned to the low-exposure group was 5·1 ng/mL (1·4–25·3) and in the high-exposure everolimus group was 8·3 ng/mL (0·8–22·0).

The median seizure frequency per week at baseline was 10·5 (range 1·3–231·7) in the placebo group, 8·6 (1·4–192·9) in the low-exposure group, and 9·5 (0·3–218·4) in the high-exposure group, and at the end of core phase was 8·5 (0–217·7), 6·8 (0–193·5), and 4·9 (0–133·7), respectively. In the placebo group, 18 of 119 patients had a 50% or greater reduction in seizure frequency during the maintenance period compared with baseline, equivalent to a response rate of 15·1% (95% CI 9·2–22·8); the median percentage reduction in seizure frequency was 14·9% (95% CI 0·1–21·7). By comparison, everolimus was associated with a significantly greater response rate (33 of 117 patients in the low-exposure group, response rate 28·2% [95% CI 20·3–37·3], p=0·0077; and 52 of 130 patients in the high-exposure group, response rate 40·0% [31·5–49·0], p<0·0001) and a significantly greater median percentage reduction in seizure frequency (in the low-exposure group, 29·3% [95% CI 18·8–41·9], p=0·0028; and in the high-exposure group, 39·6% [35·0–48·7], p<0·0001; figure 2). The odds of achieving a 50% or greater reduction in seizure frequency was 2·2-times higher (95% CI 1·2–4·2) for low-exposure everolimus than placebo and 3·9-times higher (2·1–7·3) for high-exposure everolimus than for placebo. Results of the sensitivity analysis (using the full core phase instead of the 12-week maintenance period) were consistent with the primary analysis; 13 patients had a 50% or greater reduction in seizure frequency (response rate 10·9% [95% CI 5·9–18·0]) for placebo compared with 29 patients (24·8% [17·3–33·6]) for low-exposure everolimus and 42 patients (32·3% [24·4–41·1]) for high-exposure everolimus, and the median percentage reduction in seizure frequency was 10·7% (95% CI −1·6 to 17·6) for placebo compared with 18·4% (11·7–29·5) for low-exposure everolimus and 34·9% (28·5–41·1) for high-exposure everolimus.

A 25% or greater reduction in seizure frequency was observed in 45 patients (37·8% [95% CI 29·1–47·2]) in the placebo group, 61 patients (52·1% [42·7–61·5]) in the low-exposure everolimus group, and 91 patients (70·0% [61·3–77·7]) in the high-exposure everolimus group (figure 2). The seizure-free rate was 0·8% (95% CI 0–4·6; one patient) for the placebo group, 5·1% (1·9–10·8; six patients) for the low-exposure everolimus group, and 3·8% (1·3–8·7; five patients) for the high-exposure everolimus group. The median number of seizure-free days (per 28-day period) increased from baseline by 2·0 in the low-exposure group and 4·0 days in the high-exposure group, compared with 0·5 days in the placebo group. We noted seizure reduction with everolimus treatment among multiple seizure types (figure 2), and the seizure reduction findings were essentially unchanged when generalised onset seizures confirmed by EEG (reported in six patients) were included in the analysis (data not shown).

Logistic and linear regression models stratified by age subgroup and adjusted by baseline seizure frequency supported exposure (expressed as time-normalised [TN] C_{min}, denoting an estimated average of C_{min} over the maintenance period) as a strong predictor of response rate and seizure frequency in the maintenance period of the core phase; a doubling of TN C_{min} was associated with a statistically significant 2·2-times increase (95% CI 1·3–3·5; p=0·0017) in the odds for a response and a statistically significant 28·3% reduction (95% CI 11·7–41·8; p=0·0019) in seizure frequency.

In the placebo group, the median percentage reduction in seizure frequency peaked at week 10, and remained relatively stable throughout the rest of the core phase (figure 2). However, everolimus-treated patients reported an increasing benefit until the end of the core phase (week 18). A similar pattern of differentiation between patients in the placebo group and those in the everolimus

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groups was observed when the measure of efficacy was seizure freedom (figure 2). Quantifying these observations, a repeated measures analysis including both exposure and fixed time intervals of 2 weeks as predictors noted that a doubling of TN C_{min} was associated with a significant average reduction of 9·7% (95% CI 5·7–13·6; p<0·0001) in seizure frequency across the core phase. The time under treatment was also associated with a statistically significant 4·8% reduction (95% CI 3·2–6·3; p<0·0001) in seizure frequency for each period of 15 days more under treatment.

All patients assigned to placebo, low-exposure everolimus, or high-exposure everolimus received at least one dose of study drug and had at least one post-baseline assessment, and were therefore included in the safety anaylsis. The most common all-grade adverse events of any cause reported in more than 15% of patients in either treatment group during the core phase in the everolimus groups included stomatitis, diarrhoea, nasopharyngitis, pyrexia, and upper respiratory tract infection (table 3). Overall, 13 patients (11%) in the placebo group, 21 (18%) in the low-exposure everolimus group, and 31 (24%) in the high-exposure everolimus group experienced grade 3 or 4 adverse events. Stomatitis of grade 3 or 4 severity was reported in four patients (3%) in the low-exposure group and five patients (4%) in the high-exposure group. The other most frequent grade 3 or 4 adverse events (occurring in more than two patients) reported with everolimus included neutropenia (two [2%] patients in the low-exposure group and three [2%] patients in the high-exposure group), pneumonia (one [1%] patient and three [2%] patients, respectively), and irregular menstruation (three [2%] patients in the high-exposure group only). Serious adverse events were reported in three (3%) patients in the placebo group, 16 (14%) patients in the low-exposure group, and 18 (14%) patients in the high-exposure group. Adverse events leading to discontinuations were reported in two (2%) patients receiving placebo, six (5%) patients receiving low-exposure everolimus, and four (3%) patients receiving high-exposure everolimus, with stomatitis being the most common reason (two [2%] patients in the low-exposure group and two [2%] patients in the high-exposure group). Adverse events leading to a dose reduction or temporary interruption were reported in nine (8%), 28 (24%), and 46 (35%) patients in the placebo, low-exposure everolimus, and high-exposure everolimus groups, respectively. No deaths were reported during the core phase. Pharmacokinetic modelling assessing the exposure–safety relationship of everolimus showed that the changes in rates of stomatitis and infections observed upon doubling of exposure were not statistically significant (data not shown).

	Placebo (n=119)	Everolimus 3–7 ng/mL (n=117)	Everolimus 9–15 ng/mL (n=130)	All patients (n=366)
Seizure frequency	42·0 (5·3–926·7)	34·5 (5·5–771·5)	37·8 (1·0–873·5)	..
Antiepileptic therapy during baseline phase				
Number of AEDs in the regimen				
1	15 (13%)	7 (6%)	18 (14%)	40 (11%)
2	41 (34%)	55 (47%)	55 (42%)	151 (41%)
3	62 (52%)	55 (47%)	56 (43%)	173 (47%)
>3	1 (1%)	0	1 (1%)	2 (1%)
Vagal nerve stimulation	10 (8%)	13 (11%)	11 (8%)	34 (9%)
Ketogenic diet	4 (3%)	1 (1%)	2 (2%)	7 (2%)
Seizure types during baseline phase				
Focal motor with retained awareness	26 (22%)	20 (17%)	25 (19%)	71 (19%)
Focal non-motor with impaired awareness	47 (39%)	58 (50%)	60 (46%)	165 (45%)
Focal motor with impaired awareness	31 (26%)	32 (27%)	32 (25%)	95 (26%)
Other focal motor seizures	47 (39%)	51 (44%)	51 (39%)	149 (41%)
Focal to bilateral tonic-clonic	26 (22%)	20 (17%)	22 (17%)	68 (19%)
Generalised onset seizure (EEG confirmed)	2 (2%)	2 (2%)	2 (2%)	6 (2%)

Data are median (range) or n (%), unless otherwise specified. AEDs=antiepileptic drugs. EEG=electroencephalogram.

Table 2: **Antiepileptic therapy and seizure frequency during the baseline phase by treatment group**

Discussion

In this prospective, randomised phase 3 trial, everolimus produced a significant reduction in seizure frequency in patients with treatment-resistant epilepsy and tuberous sclerosis complex compared with placebo. At baseline, nearly half of patients had failed treatment with six or more previous antiepileptic drugs, and the median seizure frequency was 37·5 per 28 days, suggesting a heavily pretreated population with a severe seizure burden. Tuberous sclerosis complex is associated with various seizure types and epilepsy syndromes.[1] Previous studies of antiepileptic drugs have focused on either a specific syndrome (eg, Lennox-Gastaut syndrome) or a single seizure type (eg, primary generalised tonic-clonic seizure, atonic seizure).[26,27] EXIST-3 is, to our knowledge, the largest cause-specific epilepsy drug trial done so far; we enrolled patients with a known seizure cause, irrespective of ictal semiology or epilepsy syndrome, from 99 centres across 25 countries. Because everolimus targets the molecular basis of the underlying disease in patients with tuberous sclerosis complex, it could produce a clinically significant reduction in seizure frequency. Everolimus might also have activity in epilepsies associated with mTOR activation from other causes, such as *DEPDC5* or *STRAD* mutations.[5]

Few clinical trials have assessed the role of mTOR inhibitors for seizures associated with tuberous sclerosis complex.[15,28] Our study showed an improvement in seizure control with everolimus among multiple seizure types, suggesting that everolimus can treat a variety of seizure types in this population irrespective of epilepsy syndrome. The odds for response in patients treated with everolimus were 2·2-times (low-exposure group) and 3·9-times (high-exposure group) higher than with placebo. A previous uncontrolled study with everolimus

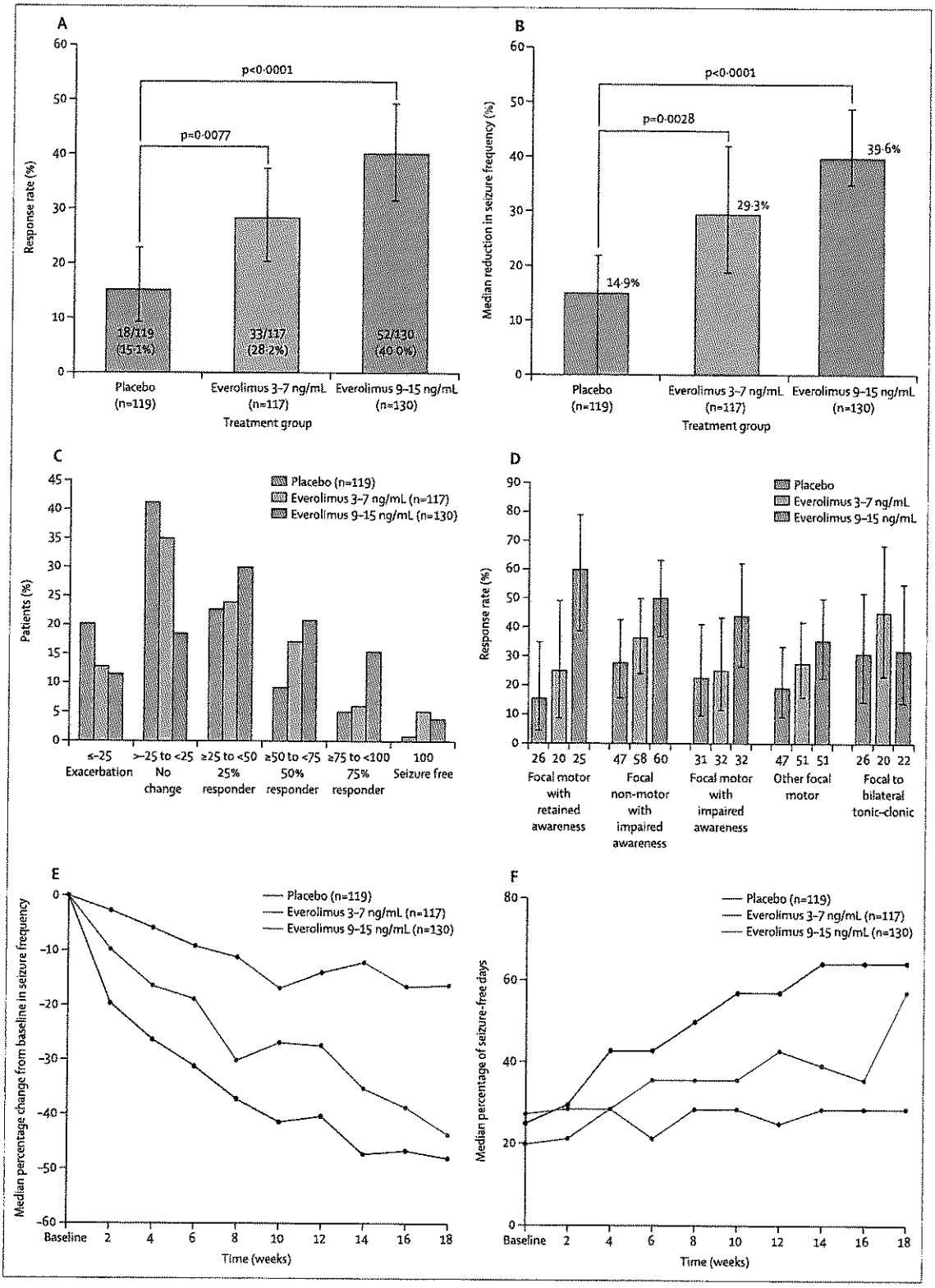

Figure 2: **Seizure outcomes**
(A) Response rate by treatment group. Bars represent 95% CIs. (B) Median percentage reduction in seizure frequency by treatment group. Bars represent 95% CIs. (C) Distribution of reduction from baseline in seizure frequency by treatment group. (D) Response rate among various seizure types. Numbers on the x axis denote the number of patients with at least one occurrence of the seizure type during the baseline phase; bars represent 95% CIs. (E) Median percentage change from baseline in seizure frequency. (F) Median percentage of seizure-free days.

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showed a 50% or greater reduction in seizures in 12 (60%) of 20 patients, with an overall median decrease in seizure frequency of 73% ($p<0·001$) and a median 70% decrease in cumulative seizure duration ($p=0·020$).[15] Results from the sensitivity analysis (adjusted for an 18-week follow-up period) in the EXIST-3 study were consistent with the primary analysis.

During the core phase, the safety profiles of the low-exposure everolimus and high-exposure everolimus groups were similar to each other. The decision to use the National Cancer Institute Common Toxicity Criteria for Adverse Events assessment scale to evaluate the safety profile of the study treatment was in accordance with our experience from previous studies of everolimus in tuberous sclerosis complex for the treatment of subependymal giant-cell astrocytoma and renal angiomyolipoma. In most epilepsy studies, adverse events are usually classified only into "mild", "moderate", and "severe". The number of severe adverse events and events leading to discontinuation in this study were consistent with findings from previous studies of antiepileptic drugs. For example, in a study of perampanel,[26] six (7·4%) of 81 patients experienced serious adverse events, and nine (11·1%) had adverse events leading to discontinuation; by comparison, we noted a somewhat higher frequency of severe adverse events (21%) with everolimus in this study, but a substantially lower frequency of adverse events leading to discontinuation (4%). The frequency and incidence of adverse events with everolimus reported here were consistent with the known safety profile of everolimus in tuberous sclerosis complex.[14,18,29] No new safety signals were identified. Importantly, the common side-effects of everolimus were generally non-overlapping with the typical side-effects of antiepileptic drugs such as sleepiness, dizziness, and fatigue, which might make it easier for patients to tolerate the medication in combination with other antiepileptic drugs, and could account for the relatively low dropout rate in the study.

Previously, everolimus was reported to reduce the volume of subependymal giant-cell astrocytoma[29] and renal angiomyolipoma,[18] which led to the approval of everolimus in these indications. Everolimus has also been reported to improve the appearence of skin lesions (facial angiofibroma) in these patients.[14] Yet results from the current study demonstrate the clinical benefits of everolimus for another manifestation of tuberous sclerosis complex, treatment-resistant seizures. Everolimus differs from other antiepileptic drugs by targeting the dysregulation of the mTOR cellular signalling pathway, providing an opportunity for therapeutic synergy. Everolimus initiated for one of its approved indications could also reduce seizures, and everolimus initiated for seizures could be expected to improve other manifestations of the disorder (eg, subependymal giant-cell astrocytoma, renal angiomyolipoma, facial angiofibroma). However, it should be noted that the exposure–response relationship differs among the different indications.

Linear mixed models supported a statistically and clinically significant association between a two-times increase in TN C_{min} and a 28·3% reduction in seizure frequency. In the studies of subependymal giant-cell astrocytoma and angiomyolipoma, a two-times C_{min} increase was associated with a non-clinically significant 10% (unpublished) and 13% statistically significant reduction in volumes,[30] respectively. This difference in exposure–response relationship might relate to higher interindividual variability of exposure and a threshold effect in the tumour-volume-based trials, or greater sensitivity of synaptic plasticity and neuronal hyperexcitability in this seizure study.

Several limitations in our study should be noted. Firstly, the results showed short-term benefit of everolimus when added to best-available antiepileptic drug therapy during the core phase. With a potential need for multiyear or lifelong therapy in patients with tuberous sclerosis complex, exploration of longer-term durability of efficacy and maintenance of safety and tolerability of everolimus is essential. With nearly 90% of patients continuing treatment in the extension phase, the EXIST-3 study could, in time, provide additional long-term efficacy and safety data relating to everolimus in patients with treatment-resistant seizures associated with tuberous sclerosis complex. Active surveillance and proactive management of adverse events are warranted in these patients, as in a phase 1/2 study where everolimus maintained seizure control for 4 years in 14 of 18 patients and had a tolerable safety profile.[31] Second, in this study we had intended to report the effect of everolimus on patient behaviour using the Vineland Adaptive Behavior Scale Survey; however, the substantial intellectual disability in the study population resulted in frequent failure of investigators to perform the survey at baseline and yielded profound flooring (ie, scores below which the test can no longer distinguish levels of behavioural attainment) in many surveys, limiting the interpretation of results. Finally, we noted less differentiation in exposure than anticipated between the two everolimus groups, because of lower-than-expected exposure in the high-exposure everolimus group (median C_{min} values at the end of the core phase were 5·1 ng/mL for the low-exposure everolimus group and 8·3 ng/mL for the high-exposure everolimus group). This lack of differentiation seems to have occurred because, in some patients, three titration steps of everolimus 2 mg or 4 mg might not have been sufficient to achieve the targeted trough range of 9–15 ng/mL (eg, patients with high everolimus clearance or high body-surface area). Despite overlap in actual C_{min} values among the everolimus treatment groups, the range of exposures acheived in the study population was adequately broad to permit a robust determination of the everolimus exposure–response relationship. We noted that doubling of exposure could more than double the likelihood of a response without a

	Placebo (n=119)		Everolimus 3–7 ng/mL (n=117)		Everolimus 9–15 ng/mL (n=130)	
	All grades	Grade 3 or 4	All grades	Grade 3 or 4	All grades	Grade 3 or 4
Any adverse event	92 (77%)	13 (11%)	108 (92%)	21 (18%)	123 (95%)	31 (24%)
Stomatitis*	11 (9%)	0	64 (55%)	4 (3%)	83 (64%)	5 (4%)
Diarrhoea	6 (5%)	0	20 (17%)	0	28 (22%)	0
Nasopharyngitis	19 (16%)	0	16 (14%)	0	21 (16%)	0
Upper respiratory tract infection	15 (13%)	1 (1%)	15 (13%)	0	20 (15%)	0
Pyrexia	6 (5%)	0	23 (20%)	0	18 (14%)	1 (1%)
Cough	4 (3%)	0	13 (11%)	0	13 (10%)	0
Rash	3 (3%)	0	7 (6%)	0	13 (10%)	0
Vomiting	11 (9%)	0	14 (12%)	0	13 (10%)	2 (2%)
Headache	6 (5%)	0	3 (3%)	0	11 (8%)	1 (1%)
Hypercholesterolaemia	1 (1%)	0	6 (5%)	0	9 (7%)	1 (1%)
Decreased appetite	7 (6%)	0	10 (9%)	1 (1%)	9 (7%)	1 (1%)
Acne	3 (3%)	0	3 (3%)	0	8 (6%)	0
Hypertriglyceridaemia	2 (2%)	0	6 (5%)	1 (1%)	8 (6%)	0
Pharyngitis	1 (1%)	0	6 (5%)	2 (2%)	8 (6%)	0
Ear infection	1 (1%)	0	2 (2%)	1 (1%)	7 (5%)	0
Epistaxis	1 (1%)	0	3 (3%)	0	7 (5%)	0
Influenza	4 (3%)	0	5 (4%)	1 (1%)	7 (5%)	0
Rhinorrhoea	1 (1%)	0	6 (5%)	0	4 (3%)	0

Data are n (%), unless otherwise specified. *Included all the related terms—mouth ulceration, aphthous ulcer, lip ulceration, tongue ulceration, mucosal inflammation, and gingival pain.

Table 3: **Adverse events of any cause reported in more than 5% of patients in either of the everolimus treatment groups**

statistically significant increase in reported adverse events such as stomatitis. This finding suggests that increased exposure of everolimus might be a reasonable option for patients who do not demonstrate a satisfactory reduction in seizure frequency.

Existing antiepileptic drugs are thought to reduce seizure frequency through a direct antiseizure effect on neuronal hyperexcitability via γ-aminobutyric acid (GABA)-ergic or glutamatergic mechanisms. Clinical effects of antiepileptic drugs are observed rapidly (seizure reduction is noted within days of initiating treatment) and remains stable through the duration of treatment.[32,33] The mechanism of action of everolimus and its clinical effects are more complex. Findings from animal models suggest that mTOR inhibitors, such as everolimus, have antiepileptogenic effects by altering signalling pathways and protein expression, and modifying downstream mechanisms involved in epileptogenesis. These mechanisms drive complex morphological changes to neuronal and glial cells that evolve over long periods of time in animal models.[12] Consistent with these observations, Krueger and colleagues[15] reported in a single-group study that everolimus reduced seizure frequency more in the later weeks than in earlier weeks of the 12-week study. This observation of a correlation between longer exposure and efficacy has been confirmed in this study, in which a clinically meaningful and statistically significant reduction in seizure frequency continued to improve throughout the core phase. Pharmacokinetic modelling demonstrated that every 15 days of treatment delivered an additional 4·8% reduction in seizure frequency. This observation contrasts with that expected for levetiracetam[33] and other antiepileptic drugs, which typically do not exhibit time-dependent amelioration of clinical benefit.

In conclusion, our findings demonstrate that everolimus treatment of mixed-type seizures in patients with tuberous sclerosis complex, despite the high baseline burden of seizures in these individuals, can lead to a clinically meaningful reduction in seizure frequency with a favourable benefit–risk ratio that improves with ongoing treatment. Everolimus, a disease-modifying drug targeting the underlying molecular pathology of tuberous sclerosis complex, represents a new treatment option for patients with treatment-resistant seizures associated with tuberous sclerosis complex. Further evaluation of mTOR inhibitors in patients with other diseases of treatment-resistant seizures and cortical malformations, resulting from PI3K-mTOR pathway mutations causing mTOR overactivation, could be warranted.

Contributors

JAF, JAL, PC, PJdV, DJD, NB, DP, and DNF designed the study. JAL, ZY, HI, TP, NB, MV, DP, and DNF contributed to patient accrual. JAL, ZY, PJdV, NB, MV, DP, and DNF managed the trial. JAL, ZY, HI, TP, MV, and DNF contributed to clinical care. JAL, ZY, HI, TP, DJD, MV, DP, and DNF collected the data. JAF, RN, PC, DJD, NB, MV, SP, and DNF analysed the data. JAF, JAL, TP, RN, PC, PJdV, DJD, NB, MV, SP, DP, and DNF interpreted the data. SP was the trial statistician. All authors contributed to drafting, revision, final review, and approval of the report.

Declaration of interests

JAF reports salary support from NYU for consulting work on behalf of the Epilepsy Study Consortium for Acorda, Adamas, Alexza, Anavex, BioPharm Solutions, Cerecor, Concert Pharmaceuticals, Eisai, Georgia Regents University, GW Pharma, Marinus, Monteris, Nestle-Health Science, Neurelis, Novartis, Pfizer, Pfizer-Neusentis, Pronutria, Roivant, Sage, SciFluor, SK Life Sciences, Sunovion, Takeda, UCB Inc, Upsher Smith, Xenon Pharmaceuticals, Zogenix, and Zynerba. JAF reports personal fees for serving as Associate Editor of *Epilepsia*, received research grants from Acorda, Alexza, Eisai Medical Research, LCGH, Lundbeck, Pfizer, SK Life Sciences, Sunovion, UCB, Upsher-Smith, and Vertex, as well as grants from the Epilepsy Research Foundation, Epilepsy Study Consortium, Epilepsy Therapy Project, and NINDS, and is on the editorial board of *The Lancet Neurology*, *Neurology Today*, and *Epileptic Disorders*. JAF is a scientific officer for the Epilepsy Foundation for which NYU receives salary support. JAF reports travel reimbursement related to research, advisory meetings, or presentation of results at scientific meetings from the Epilepsy Study Consortium, the Epilepsy Foundation, Eisai, GW Pharma, Marinus, Nestle Life Sciences, Pfizer, Sage, SK Life Sciences, Takeda, UCB, Upsher-Smith, Zogenix, and Zynerba. JAL reports personal fees and research grants from Novartis. TP reports personal fees for advisory boards from Novartis and Desitin, and speaker honoraria from UCB Pharma and Desitin outside the submitted work. RN reports grants from EU (FP7), Shire, Zogenix, GW Pharma, and Eisai Medical Research, and personal fees from Eisai, Zogenix, and Novartis. PC reports personal fees for advisory boards from Eisai, Shire, Novartis, and speaker honoraria and travel reimbursement from Novartis. PJdV reports grants and personal fees from Novartis. DJD reports grants from The Epilepsy Study Consortium and NIH-NINDS. NB, MV, SP, and DP are employees of

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References

1 Chu-Shore CJ, Major P, Camposano S, Muzykewicz D, Thiele EA. The natural history of epilepsy in tuberous sclerosis complex. *Epilepsia* 2010; 51: 1236–41.

2 Curatolo P, Moavero R, de Vries PJ. Neurological and neuropsychiatric aspects of tuberous sclerosis complex. *Lancet Neurol* 2015; 14: 733–45.

3 Curatolo P. Mechanistic target of rapamycin (mTOR) in tuberous sclerosis complex-associated epilepsy. *Pediatr Neurol* 2015; 52: 281–89.

4 Kwan P, Schachter SC, Brodie MJ. Drug-resistant epilepsy. *N Engl J Med* 2011; 365: 919–26.

5 Wong M. Mammalian target of rapamycin (mTOR) inhibition as a potential antiepileptogenic therapy: From tuberous sclerosis to common acquired epilepsies. *Epilepsia* 2010; 51: 27–36.

6 Feliciano DM, Lin TV, Hartman NW, et al. A circuitry and biochemical basis for tuberous sclerosis symptoms: from epilepsy to neurocognitive deficits. *Int J Dev Neurosci* 2013; 31: 667–78.

7 McMahon J, Huang X, Yang J, et al. Impaired autophagy in neurons after disinhibition of mammalian target of rapamycin and its contribution to epileptogenesis. *J Neurosci* 2012; 32: 15704–14.

8 Meikle L, Pollizzi K, Egnor A, et al. Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. *J Neurosci* 2008; 28: 5422–32.

9 Talos DM, Kwiatkowski DJ, Cordero K, Black PM, Jensen FE. Cell-specific alterations of glutamate receptor expression in tuberous sclerosis complex cortical tubers. *Ann Neurol* 2008; 63: 454–65.

10 Weston MC, Chen H, Swann JW. Multiple roles for mammalian target of rapamycin signaling in both glutamatergic and GABAergic synaptic transmission. *J Neurosci* 2012; 32: 11441–52.

11 Zeng LH, Xu L, Gutmann DH, Wong M. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Ann Neurol* 2008; 63: 444–53.

12 Wong M, Roper SN. Genetic animal models of malformations of cortical development and epilepsy. *J Neurosci Methods* 2016; 260: 73–82.

13 Krueger DA, Care MM, Agricola K, Tudor C, Mays M, Franz DN. Everolimus long-term safety and efficacy in subependymal giant cell astrocytoma. *Neurology* 2013; 80: 574–80.

14 Krueger DA, Care MM, Holland K, et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med* 2010; 363: 1801–11.

15 Krueger DA, Wilfong AA, Holland-Bouley K, et al. Everolimus treatment of refractory epilepsy in tuberous sclerosis complex. *Ann Neurol* 2013; 74: 679–87.

16 Moavero R, Coniglio A, Garaci F, Curatolo P. Is mTOR inhibition a systemic treatment for tuberous sclerosis? *Ital J Pediatr* 2013; 39: 57.

17 Cardamone M, Flanagan D, Mowat D, Kennedy SE, Chopra M, Lawson JA. Mammalian target of rapamycin inhibitors for intractable epilepsy and subependymal giant cell astrocytomas in tuberous sclerosis complex. *J Pediatr* 2014; 164: 1195–200.

18 Bissler JJ, Kingswood JC, Radzikowska E, et al. Everolimus for angiomyolipoma associated with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis (EXIST-2): a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet* 2013; 381: 817–24.

19 Beach R, Reading R. The importance of acknowledging clinical uncertainty in the diagnosis of epilepsy and non-epileptic events. *Arch Dis Child* 2005; 90: 1219–22.

20 European Medicines Agency. Guideline on clinical investigation of medicinal products in the treatment of epileptic disorders. July 22, 2010. CHMP/EWP/566/98 Rev.2/Corr. http://wwwemaeuropaeu/docs/en_GB/document_library/Scientific_guideline/2010/01/WC500070043pdf (accessed July 8, 2016).

21 National Cancer Institute. Common terminology criteria for adverse events, (CTCAE), version 4.03. June 14, 2010. http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf (accessed April 15, 2016).

22 Ben-Menachem E, Biton V, Jatuzis D, Abou-Khalil B, Doty P, Rudd GD. Efficacy and safety of oral lacosamide as adjunctive therapy in adults with partial-onset seizures. *Epilepsia* 2007; 48: 1308–17.

23 Brodie MJ, Lerche H, Gil-Nagel A, et al. Efficacy and safety of adjunctive ezogabine (retigabine) in refractory partial epilepsy. *Neurology* 2010; 75: 1817–24.

24 French JA, Krauss GL, Biton V, et al. Adjunctive perampanel for refractory partial-onset seizures: randomized phase III study 304. *Neurology* 2012; 79: 589–96.

25 Glauser T, Kluger G, Sachdeo R, Krauss G, Perdomo C, Arroyo S. Rufinamide for generalized seizures associated with Lennox-Gastaut syndrome. *Neurology* 2008; 70: 1950–58.

26 French JA, Krauss GL, Wechsler RT, et al. Perampanel for tonic-clonic seizures in idiopathic generalized epilepsy: a randomized trial. *Neurology* 2015; 85: 950–57.

27 Perucca E, French J, Bialer M. Development of new antiepileptic drugs: challenges, incentives, and recent advances. *Lancet Neurol* 2007; 6: 793–804.

28 Wiegand G, May TW, Ostertag P, Boor R, Stephani U, Franz DN. Everolimus in tuberous sclerosis patients with intractable epilepsy: a treatment option? *Eur J Paediatr Neurol* 2013; 17: 631–38.

29 Franz DN, Belousova E, Sparagana S, et al. 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. *Lancet* 2013; 381: 125–32.

30 Budde K, Zonnenberg BA, Frost M, et al. Pharmacokinetics and pharmacodynamics of everolimus in patients with renal angiomyolipoma and tuberous sclerosis complex or lymphangioleiomyomatosis. *Br J Clin Pharmacol* 2016; 81: 958–70.

31 Krueger D, Wilfong A, Talley C, Agricola K, Franz DN. Everolimus shows sustained efficacy for refractory epilepsy in tuberous sclerosis complex (TSC). American Epilepsy Society; Dec 4–8, 2015; Philadelphia, PA, USA. 1251.

32 Meldrum BS, Rogawski MA. Molecular targets for antiepileptic drug development. *Neurotherapeutics* 2007; 4: 18–61.

33 French J, di Nicola S, Arrigo C. Fast and sustained efficacy of levetiracetam during titration and the first 3 months of treatment in refractory epilepsy. *Epilepsia* 2005; 46: 1304–07.