



# Adjunctive everolimus for children and adolescents with treatment-refractory seizures associated with tuberous sclerosis complex: post-hoc analysis of the phase 3 EXIST-3 trial

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## Summary

**Background** Epilepsy occurs in 70–90% of patients with tuberous sclerosis complex. We aimed to assess the efficacy and safety of adjunctive everolimus for treatment-refractory seizures associated with tuberous sclerosis complex in paediatric patients enrolled in the EXIST-3 trial, a double-blind, placebo-controlled, randomised, phase 3 study.

**Methods** This post-hoc analysis focused on paediatric patients (age <18 years) in the EXIST-3 trial, which consisted of baseline (8 weeks), core (18 weeks), and extension phases (≥48 weeks) and was done at 99 centres in 25 countries worldwide. Briefly, patients with tuberous sclerosis complex-associated treatment-refractory seizures, who were receiving a stable dose of one to three antiepileptic drugs, were randomly assigned (1:1:1) to receive placebo, low-exposure everolimus (3–7 ng/mL), or high-exposure everolimus (9–15 ng/mL). Following the core phase, patients could enter the extension phase to receive everolimus at a targeted exposure range of 3–15 ng/mL up to 48 weeks after the last patient had completed the core phase. Efficacy endpoints were response rate (≥50% of reduction from baseline in average weekly seizure frequency) and median percentage reduction in seizure frequency during the 12-week maintenance period of the core phase, and at 12-week intervals throughout the extension phase. This study is registered with ClinicalTrials.gov, number NCT01713946.

**Findings** Between July 3, 2013, and May 29, 2015, 299 paediatric patients enrolled in the trial. In the younger subgroup (<6 years; n=104), 34 received placebo, 33 low-exposure everolimus, and 37 high-exposure everolimus; in the older subgroup (≥6 years to <18 years; n=195), 62 received placebo, 63 low-exposure everolimus, and 70 high-exposure everolimus. At the end of the core phase, response rate was higher in the treatment groups than placebo in both the younger subgroup (17·6% [6·8–34·5] for placebo vs 30·3% [95% CI 15·6–48·7; p=0·2245] for low-exposure everolimus vs 59·5% [42·1–75·2; p=0·0003] for high-exposure everolimus) and the older subgroup (12·9% [5·7–23·9] vs 27·0% [16·6–39·7; p=0·0491] vs 30·0% [19·6–42·1; p=0·0179]), as were median reduction in seizure frequency (12·3% [95% CI –10·1 to 24·8] vs 29·3% [95% CI 13·4 to 46·3; p=0·0474] vs 54·7% [43·5 to 73·1; p<0·0001] in younger patients; 13·5% [–3·0 to 26·8] vs 31·0% [16·1 to 42·9; p=0·0128] vs 34·8% [26·7 to 41·3; p=0·0006] in older patients). The efficacy persisted, with sustained seizure reduction after 1 year of treatment across both paediatric subgroups (response rate 48·9% [95% CI 38·1–59·8] for the younger subgroup vs 47·2% [39·3–55·2] for the older subgroup; median percentage reduction in seizure frequency 48·4% [95% CI 34·3–73·6] vs 48·0% [38·2–57·5]). At the cutoff date for the extension phase, grade 3 or 4 adverse events were reported in 45 (45%) younger patients (commonly pneumonia [n=16]) and 74 (38%) older patients (commonly pneumonia [n=8] and stomatitis [n=6]). Two deaths (pneumonia, which was suspected to be treatment-related, and sudden unexplained death due to epilepsy) were reported.

**Interpretation** Adjunctive everolimus resulted in sustained reductions in seizure frequency after 1 year and was well tolerated in paediatric patients with treatment-refractory seizures associated with tuberous sclerosis complex.

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## Introduction

Epilepsy is the most common presenting feature of tuberous sclerosis complex, affecting 70–90% of patients.<sup>1–4</sup> The onset of seizures occurs within the first year of life in roughly 60% of patients, and can even be observed in the neonatal period.<sup>1–5</sup> Adults with tuberous

sclerosis complex without a history of seizures continue to be at risk (12%) for developing seizures in later years.<sup>1</sup> Furthermore, patients with tuberous sclerosis complex have an 84–92% chance of having at least one seizure during their lifetime.<sup>6</sup> Early seizure onset is associated with an increased risk of refractory epilepsy, as well as

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## Research in context

### Evidence before this study

We searched PubMed for reports about clinical trials and case studies of patients with seizures associated with tuberous sclerosis complex using the primary search terms “tuberous sclerosis complex” or “tsc” and “epilepsy” or “seizure”, or “children” and “pediatric” and “paediatric”, published in English from database inception up to Sept 19, 2017. Our search identified exploratory clinical studies, case reports, and open-label trials, but did not identify any other placebo-controlled, randomised studies of everolimus, with the exception of EXIST-3. Evidence specific to children or paediatric patients with seizures associated with tuberous sclerosis complex is scarce, with only a few open-label studies with small sample sizes ( $n < 20$ ).

### Added value of this study

To our knowledge, this is the first phase 3 study to assess mTOR inhibitors for seizure reduction that included a large paediatric

population with a long follow-up period (up to 48 weeks). Our results show the benefit of adding everolimus as an adjunctive therapy for paediatric patients already taking one to three antiepileptic drugs for the treatment of tuberous sclerosis complex-associated treatment refractory seizures. Furthermore, our study provides important data for long-term use of everolimus, showing a sustained effect of seizure control over time with a manageable safety profile.

### Implications of all the available evidence

The findings from this paediatric subgroup analysis of a randomised trial add to the existing evidence of improving seizure control in this population by showing the safety and long-term efficacy of everolimus as an add-on therapy to antiepileptic drugs. Further exploration of everolimus is recommended as an earlier treatment for paediatric patients with tuberous sclerosis complex for the prevention of infantile spasms and refractory seizures.

neurodevelopmental and cognitive impairment.<sup>7</sup> In a retrospective chart review,<sup>8</sup> 38 (32%) of 120 adults and 21 (53%) of 40 children had treatment-refractory seizures. In children with a history of seizures, the prevalence of severe cognitive impairment ranges between 46% and 100%.<sup>9</sup>

The seizures in tuberous sclerosis complex are typically focal in onset and can precede, coexist with, or evolve into epileptic spasms.<sup>3</sup> The cause of seizures and the age at which the epileptiform activity manifests depend on the location of cortical tubers, the expression of which might be affected by the functional maturation of the cortex.<sup>5,10</sup> Epileptogenesis in tuberous sclerosis complex can result from dysregulation of the mammalian target of rapamycin (mTOR) pathway, leading to abnormal neuronal structure, increased cell growth and proliferation, reduced autophagy, and apoptosis. Epileptogenesis can also arise by an imbalance of decreased inhibition of  $\gamma$ -aminobutyric acid receptors in giant cells and dysplastic neurons, and increased excitation of glutamate receptors in dysplastic neurons.<sup>11,12</sup> Epilepsy secondary to tuberous sclerosis complex could also be a result of long-range neural network disorders with alterations in connectivity between the cortical and subcortical systems.<sup>11,12</sup>

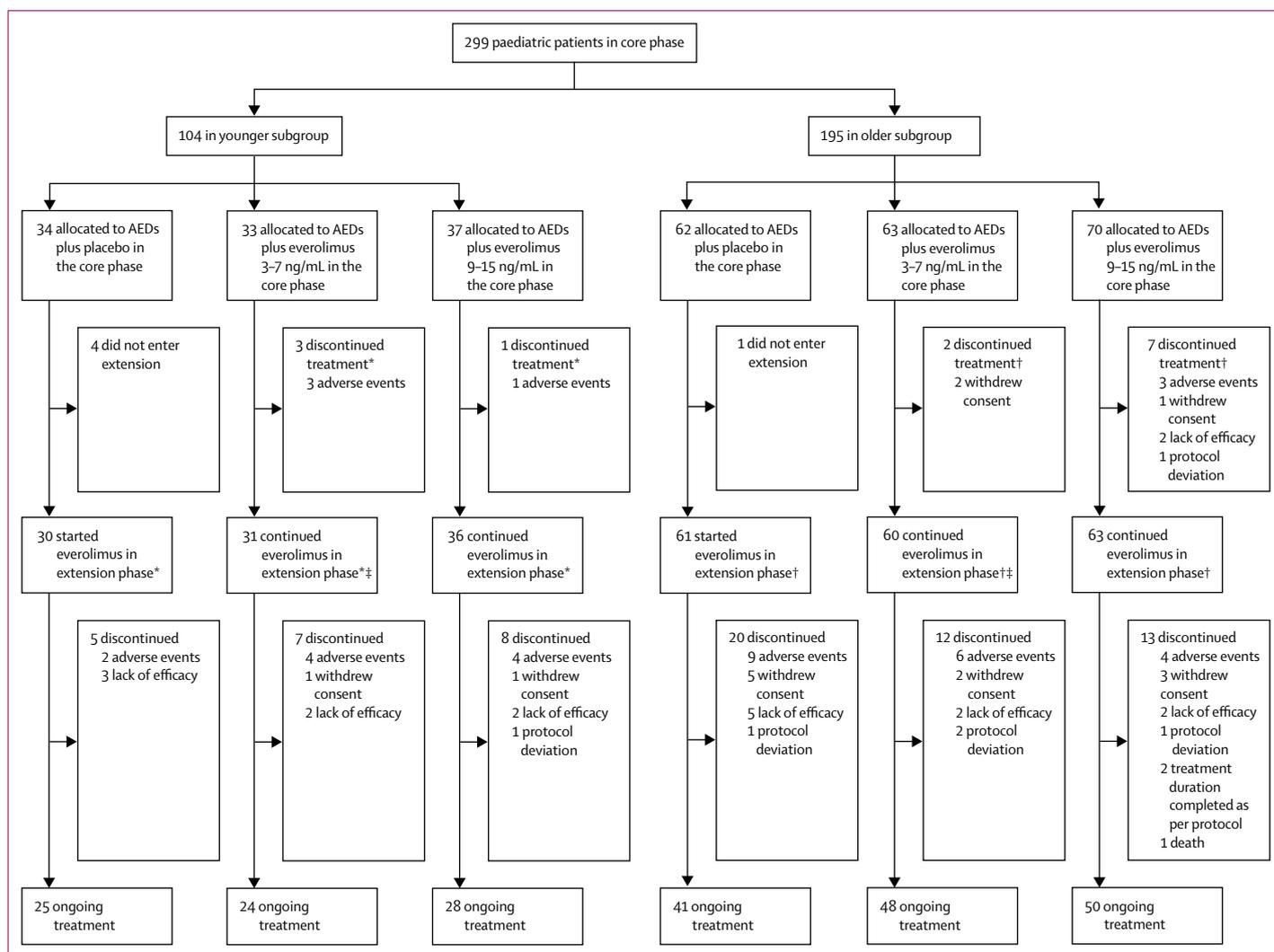
Everolimus, an mTOR inhibitor, is approved for the treatment of tuberous sclerosis complex-associated subependymal giant cell astrocytomas<sup>13</sup> and renal angiomyolipomas,<sup>14</sup> and has shown efficacy in reducing seizures associated with tuberous sclerosis complex.<sup>15</sup> Various preclinical and clinical studies<sup>12,13,16,17</sup> have reported the potential benefits of mTOR inhibition in tuberous sclerosis complex-associated epilepsy and neuropsychiatric symptoms. The phase 3, double-blind, placebo-controlled, EXAMining everolimus In a Study of

Tuberous sclerosis complex (EXIST-3) study<sup>18</sup> compared the efficacy and safety of two trough exposure ranges of everolimus with placebo in patients with treatment-refractory focal seizures associated with tuberous sclerosis complex. During the 12-week maintenance period of the core phase, the two targeted exposure ranges of everolimus (3–7 ng/mL [low exposure] and 9–15 ng/mL [high exposure]) showed greater reductions in seizure frequency (response rates of 28.2% [95% CI 20.3–37.3] for low exposure and 40.0% [31.5–49.0] for high exposure) compared with placebo (15.1% [9.2–22.8]). Based on the positive results of this trial, the European Commission approved everolimus as an adjunctive treatment for patients aged 2 years and older with refractory partial-onset seizures associated with tuberous sclerosis complex.<sup>19</sup> We aimed to do a post-hoc analysis of the paediatric subgroup enrolled in EXIST-3 (patients aged <18 years at the time of randomisation), using both the primary data in the core phase and the long-term data in the extension phase (at least 48 weeks after the last patient had completed the core phase). This analysis is crucial for the assessment of long-term efficacy of adjunctive everolimus in a treatment-refractory paediatric population with a high seizure burden and risk of cognitive impairment. Furthermore, we aimed to determine the long-term safety profile of everolimus, which is particularly important for the younger patients (aged <6 years).

## Methods

### Study design and participants

EXIST-3 is a three-arm, prospective, randomised, multicentre, double-blind, placebo-controlled, phase 3 study consisting of baseline (8 weeks), core (6 weeks plus a 12-week maintenance period), and extension



**Figure 1: Trial profile**

Patients in the younger subgroup were younger than 6 years old, and those in the older subgroup were aged 6 years or older, and younger than 18 years. Age at randomisation was considered for the core phase (data cutoff date, Oct 2, 2015), whereas age at the start of everolimus was considered for the extension phase (data cutoff date, Sept 2, 2016). AEDs=antiepileptic drugs. \*n=101. †n=193. ‡One patient was included in the older subgroup at the time of the core phase, based on the age calculated from the exact date of birth. This date was then changed as per local requirement because only year of birth was allowed. Therefore, for the extension phase the date of birth for this patient was imputed and the patient was included in the younger subgroup.

(≥48 weeks) phases done at 99 centres in 25 countries worldwide. Patients aged 2–65 years with a confirmed diagnosis of tuberous sclerosis complex and at least 16 quantifiable partial-onset seizures reported in the baseline phase and who were receiving at least one to three antiepileptic drugs at a stable dose for at least 4 weeks before study entry were included. Patients with subependymal giant cell astrocytoma requiring immediate surgical intervention, seizures secondary to drug abuse, psychogenic non-epileptic seizures, patients younger than 2 years old with untreated infantile spasms, or an episode of status epilepticus within 1 year before study entry were excluded.<sup>18</sup> After the 12-week maintenance period of the core phase, patients were eligible to enter the prespecified extension phase, in

which they received everolimus up to 48 weeks after the last patient had completed the core phase, unless they had 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.

All participants (or their legal representatives) provided written informed consent according to local guidelines before enrolment. 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 the amendments were reviewed and approved by the institutional review boards or independent ethics committees of each participating centre.

See Online for appendix

	Core phase		Extension phase*	
	Younger subgroup (n=104)	Older subgroup (n=195)	Younger subgroup (n=101)	Older subgroup (n=193)
Age (years)	4.14 (2.2–5.9)	11.16 (6.1–18.0)	4.08 (2.2–5.9)	11.12 (6.1–18.0)
Sex				
Female	42 (40%)	98 (50%)	40 (40%)	97 (50%)
Male	62 (60%)	97 (50%)	61 (60%)	96 (50%)
Race				
Caucasian	66 (63%)	117 (60%)	64 (63%)	116 (60%)
Asian	26 (25%)	53 (27%)	25 (25%)	52 (27%)
Black	..	3 (2%)	..	3 (2%)
Native American	..	1 (1%)	..	1 (1%)
Pacific Islander	1 (1%)	..	1 (1%)	..
Other	11 (11%)	21 (11%)	11 (11%)	21 (11%)
Mutation analysis†				
TSC1	..	..	10 (10%)	26 (13%)
TSC2	..	..	63 (62%)	107 (55%)
Both	..	..	2 (2%)	5 (3%)
No mutation identified	..	..	13 (13%)	24 (12%)
No mutational status available	..	..	13 (13%)	31 (16%)
Number of concomitant antiepileptic drugs during the baseline phase				
1	12 (12%)	22 (11%)	12 (12%)	21 (11%)
2	41 (39%)	86 (44%)	39 (39%)	87 (45%)
3	50 (48%)	87 (45%)	49 (49%)	84 (44%)
4	1 (1%)	0	1 (1%)	1 (1%)

Data are median (range) or n (%). Patients in the younger subgroup were younger than 6 years old, and those in the older subgroup were aged 6 years or older, and younger than 18 years. \*Extension phase data are combined core phase and extension phase for patients randomly assigned to everolimus and extension phase only for patients randomly assigned to placebo. †Data collected at baseline and analysed at the cutoff date for the extension phase.

**Table 1: Baseline characteristics**

### Randomisation and masking

Eligible patients were randomly assigned (1:1:1; block size of six) to receive placebo, low-exposure everolimus, or high-exposure everolimus in addition to a stable regimen of one to three antiepileptic drugs during the core phase. Randomisation was stratified by age subgroup (<6 years, from 6 years to <12 years, from 12 years to <18 years, and ≥18 years). 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-masked to treatment allocation at the time of DSMB meetings. Placebo and everolimus pills were identical in appearance and patients could receive either the blue or yellow blister packets that they were dispensed in.<sup>18</sup> At entry into the extension phase, masking of the original randomisation group was maintained by adding the possibility of placebo tablet administration to patients initially randomised to either placebo or low-exposure everolimus groups.

### Procedures

For patients younger than 10 years old, the starting dose of everolimus was 9 mg/m<sup>2</sup> if the patients were receiving cytochrome 3A4 or P-glycoprotein inducers and 6 mg/m<sup>2</sup> if they were not receiving these drugs; for patients aged 10–18 years, the equivalent doses were 8 mg/m<sup>2</sup> or 5 mg/m<sup>2</sup>. Following the completion of the core phase, patients who entered the extension phase had an exposure range of 3–15 ng/mL until at least 48 weeks, regardless of drug exposure in the core phase. Once in the extension phase, patients were transitioned to an intermediate exposure range of 6–10 ng/mL, following which investigator-led transitions targeted an exposure range of 3–15 ng/mL. Tuberous sclerosis complex mutational analysis was done at baseline. For patients weighing 12–20 kg, mutational analysis was done at visit 4 during the core phase to reduce the blood sample collection at randomisation. For genetic testing, the whole blood samples were collected in EDTA (edetic acid) tubes and frozen at –70°C to –80°C immediately. DNA extraction was done by Quintiles Lab (Atlanta, GA, USA) and DNA sequencing by Novartis Next Generation Diagnostics group (Cambridge, MA, USA) with the PanCancer Next Generation Sequencing assay. Patients or caregivers recorded events in a seizure diary by date, with seizures lasting longer than 10 min being noted, throughout the study. Adverse events were assessed according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (version 4.03).

### Outcomes

This post-hoc analysis of the EXIST-3 study focuses on the efficacy and safety outcomes in paediatric patients following the treatment with low-exposure everolimus, high-exposure everolimus, or placebo assessed during the 12-week maintenance period of the core phase with the data cutoff date of Oct 2, 2015, and the extension phase with the data cutoff date of Sept 2, 2016. The efficacy endpoints included response rate (defined as percentage of patients with ≥50% reduction in average weekly seizure frequency) and the median percentage reduction in seizure frequency for the two everolimus exposure ranges compared with placebo, from baseline until the 12-week maintenance period of the core phase ended, and at 12-week intervals throughout the duration of everolimus treatment in the extension phase (for which some patients could have extended to >2 years). We report data for weeks 7–18 (8 weeks before start of everolimus treatment) and weeks 42–54 (corresponding to 1 year of everolimus exposure—ie, 18 weeks in the core phase plus 34 weeks in the extension phase for patients randomly assigned to everolimus treatment arms and 54 weeks in the extension phase for patients randomly assigned to placebo). Safety endpoints were frequency of adverse events, grade 3 or grade 4 adverse events, and deaths observed during the core and extension phases of the study.

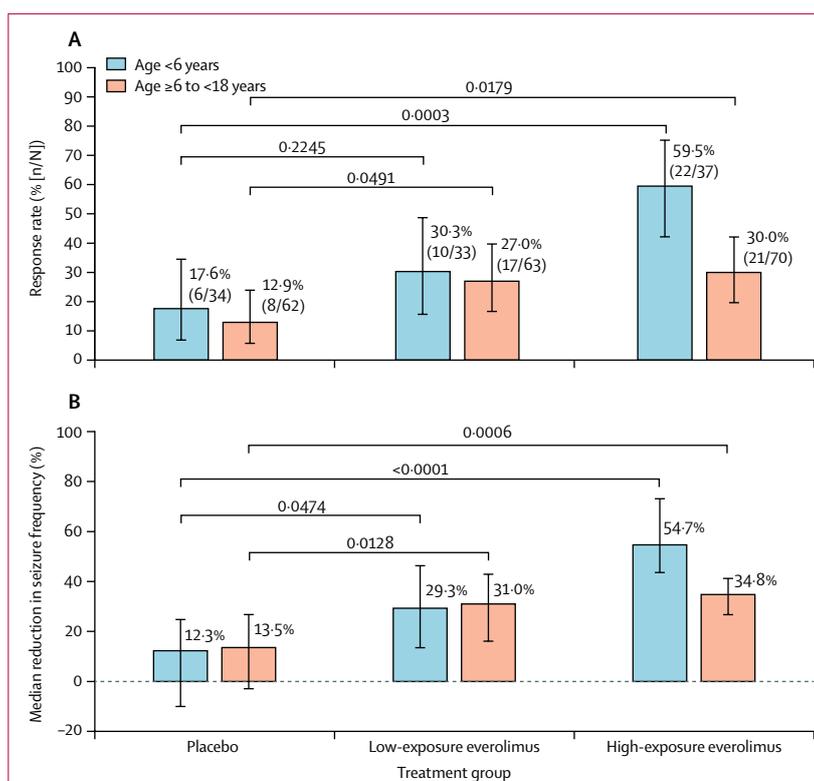
### Statistical analysis

The primary efficacy endpoints from the core phase were reported for all patients randomly assigned. In this post-hoc analysis, we included data from patients younger than 18 years (both children and adolescents) enrolled in EXIST-3 who received at least one dose of everolimus in either the core or extension phases of the study. For patients randomly assigned initially to one of the two everolimus treatment groups (low or high exposure), all the data from the core and extension phases of the study were included, and we considered baseline seizure frequency as that observed during the 8-week baseline phase before everolimus treatment. For patients randomly assigned to receive placebo initially, only the extension phase data were considered (ie, the time from initiation of everolimus), and we considered baseline seizure frequency as that observed in the last 8 weeks of the core phase.

The baseline data (the latest value on or before starting everolimus treatment) and efficacy variables were summarised by appropriate descriptive statistics (mean, SD, median, minimum, and maximum). We used descriptive statistics of percentage reduction from baseline in seizure frequency and response rate computed by time interval. Response rates with exact 95% CIs and the median percentage reduction from baseline for each treatment group, along with 95% bootstrap CIs (100 000 independent bootstrap replications were done), were calculated. A post-hoc comparison was performed. We compared response rate between each everolimus group and the placebo group with a  $\chi^2$  test and percentage reduction in seizure frequency by use of rank ANCOVA with baseline average weekly seizure frequency as a covariate. We made no adjustments for multiplicity. To analyse seizure outcomes in both age groups, the data are presented here as a comparison between the youngest age strata (younger subgroup; patients aged <6 years) and the other paediatric patients (older subgroup; aged from  $\geq 6$  years to <18 years). We did the statistical analysis using SAS software (version 9.2). The study is registered with ClinicalTrials.gov, number NCT01713946.

### Role of the funding source

The study was designed by an academic steering committee, including the sponsor (Novartis Pharmaceuticals Corporation). The data were collected electronically by 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 the development and approval of the report, and had the final responsibility for the decision to submit for publication. All authors vouched for the accuracy and completeness of the reported data, and attested that the study conformed to the protocol and statistical analysis plan.



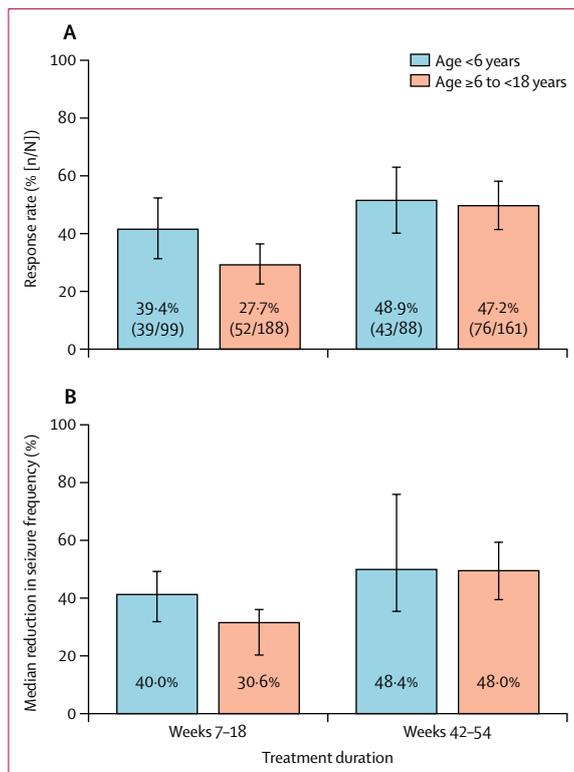
**Figure 2: Response rate (A) and reduction in seizure frequency (B) at the end of the core phase** (A) Error bars represent 95% CI obtained with Clopper-Pearson method. p values computed from  $\chi^2$  test. (B) Error bars represent 95% CI based on bootstrap percentiles. p values obtained from rank ANCOVA with baseline seizure frequency as covariate.

### Results

Between July 3, 2013, and May 29, 2015, 366 patients, of whom 299 (82%) were younger than 18 years, were initially enrolled in the EXIST-3 study (figure 1). Among the paediatric patients, 104 (35%) were younger than 6 years old (median age at start of the study 4.14 years, range 2.2–5.9) and 195 (65%) were aged 6–18 years (11.16 years, 6.1–18.0; table 1). Of the 104 patients in the younger subgroup, 34 were randomly assigned to receive placebo, 33 low-exposure everolimus, and 37 for high-exposure everolimus; seven discontinued treatment in the core phase (figure 1). Among 195 patients in the older subgroup, 62 were randomly assigned to receive placebo, 63 low-exposure everolimus, and 70 high-exposure everolimus; 11 discontinued treatment in the core phase (figure 1).

Overall, 294 (98%) of 299 paediatric patients received at least one dose of everolimus either in the core or extension phases of the study (figure 1). The median everolimus trough concentration ( $C_{\min}$ ) achieved in the younger patients at week 18 was 7.12 ng/mL (1.31–21.60; n=56), at 1 year was 6.50 ng/mL (1.61–18.20; n=57), and at 2 years was 7.43 ng/mL (1.42–19.20; n=25; data are not available for the older subgroup).

During the core phase, the median seizure frequency per week at baseline was 19.6 (range 2.5–95.1) in



**Figure 3: Response rate (A) and reduction in seizure frequency (B) from the start of treatment for all patients treated with everolimus**  
 (A) Error bars represent 95% CI obtained with Clopper-Pearson method.  
 (B) Error bars represent 95% CI based on bootstrap percentiles. Denominators include patients with a baseline value and a value at the assessment timepoint.

younger patients versus 10.8 (1.8–231.7) in older patients in the placebo group, 18.5 (1.4–138.1) versus 7.3 (2.1–192.9) in the low-exposure everolimus group, and 12.8 (1.9–110.3) versus 9.6 (0.3–218.4) in the high-exposure everolimus group. In both age groups, greater response rate and greater median reduction in seizure frequency was observed in the everolimus treatment arms than placebo. In the younger subgroup, response rates were 17.6% (6.8–34.5) for the placebo group, and 30.3% (95% CI 15.6–48.7;  $p=0.2245$  compared with placebo) for the low-exposure group and 59.5% (42.1–75.2;  $p=0.0003$ ) for the high-exposure group (figure 2A). The median reduction in seizure frequency was 12.3% (–10.1 to 24.8), 29.3% (95% CI 13.4 to 46.3;  $p=0.0474$ ), and 54.7% (43.5 to 73.1;  $p<0.0001$ ), for these groups, respectively (figure 2B). In the older subgroup, response rates were 12.9% (5.7–23.9) in the placebo group, 27.0% (95% CI 16.6–39.7;  $p=0.0491$ ) for the low-exposure group and 30.0% (19.6–42.1;  $p=0.0179$ ) for the high-exposure group (figure 2A). The median reduction in seizure frequency was 13.5% (–3.0 to 26.8), 31.0% (16.1 to 42.9;  $p=0.0128$ ) and 34.8% (26.7 to 41.3;  $p=0.0006$ ), respectively (figure 2B).

In the extension phase (following transition to everolimus for all the patients continuing on the trial),

the response rate at week 18 (corresponding to the 12-week window of weeks 7–18 after the start of everolimus treatment) was higher for the 99 younger patients at 39.4% (95% CI 29.7–49.7) than for the 188 older patients (27.7%; 21.4–34.6; figure 3A). These responses were sustained at 1 year (weeks 42–54), with response rates of 48.9% (38.1–59.8;  $n=88$ ) and 47.2% (39.3–55.2;  $n=161$ ), respectively (figure 3A). Similarly, the median percentage reduction in seizure frequency at week 18 was higher for the younger patients (40.0%; 30.8–47.7) than for the older patients (30.6%; 19.6–34.9). At 1 year, median reduction was increased to 48.4% (34.3–73.6) and 48.0% (38.2–57.5), respectively (figure 3B). A 25% or greater reduction in seizure frequency was sustained over time (appendix). The seizure outcomes improved over time in both everolimus treatment groups, with a greater benefit observed for the younger patients than for the older patients, up to 1 year of everolimus treatment (figure 4A and 4B). Furthermore, in patients who received high-exposure everolimus, response rates appeared higher in the younger subgroup than the older subgroup at the end of the core phase (63.9% [95% CI 46.2–79.2] vs 34.8% [23.5–47.6]; figure 4A), as was the median percentage reductions in the seizure frequency (57.3% [47.7–76.3] vs 35.2% [29.1–44.2]; figure 4B). However, after 1 year of everolimus treatment the differences between age groups was small (response rate 60.0% [42.1–76.1] vs 59.6% [45.8–72.4]; median percentage reductions in the seizure frequency 67.8% [42.0–92.2] vs 61.4% [48.0–66.1]).

The median duration of everolimus exposure in the core and extension phases combined was about 1.6 years (85.4 weeks; range 4–157) in the younger patients and 1.8 years (95.6 weeks; 2–165) in the older patients. The median dose intensity of everolimus was 8.35 mg/m<sup>2</sup> per day (range 2.5–27.8) for the younger patients and 6.97 mg/m<sup>2</sup> per day (1.7–18.8) for the older patients. During the core phase, pneumonia and bronchitis were reported at a higher incidence in the younger patients than in older patients (table 2). By contrast, stomatitis was more common in older than younger patients (table 2).

In both phases, the most common all-grade adverse events of any cause reported in 20% or more of patients in both age strata were pyrexia, diarrhoea, stomatitis, mouth ulceration, upper respiratory tract infection, nasopharyngitis, cough, and vomiting (table 2). Most adverse events were reported at similar frequencies among both patient subgroups; however, infections (including pneumonia and bronchitis) and diarrhoea seemed more common among the younger patients than among the older patients (table 2). At the time of data cutoff for the extension phase, grade 3 or 4 adverse events were reported in 45 (45%) of the 101 patients in the younger subgroup, the most common (reported in ≥5%) of which were pneumonia (16 [16%]), gastroenteritis (7 [7%]), and status epilepticus (5 [5%]) in the younger

patients (only those adverse events reported in  $\geq 20\%$  are shown in table 2). For the 193 patients in the older subgroup, grade 3 or 4 adverse events were reported in 74 patients (38%) with most reported in less than 1% of patients (table 2, appendix). Two deaths because of adverse events were reported. One patient (1%) died at the age of 3 years 10 months, after 17.3 months of treatment with everolimus, because of pneumonia which was suspected to be treatment-related (age at randomisation was 2 years). Another patient (1%) died at the age of 11 years 9 months, after 9.2 months of treatment with everolimus, because of sudden unexplained death due to epilepsy, which was not suspected to be treatment related (age at randomisation was 11 years). After the data cutoff date, one additional on-treatment death (1%) because of septic shock was reported at age 6 years 0 months after 25.5 months of treatment with everolimus (age at randomisation was 3 years).

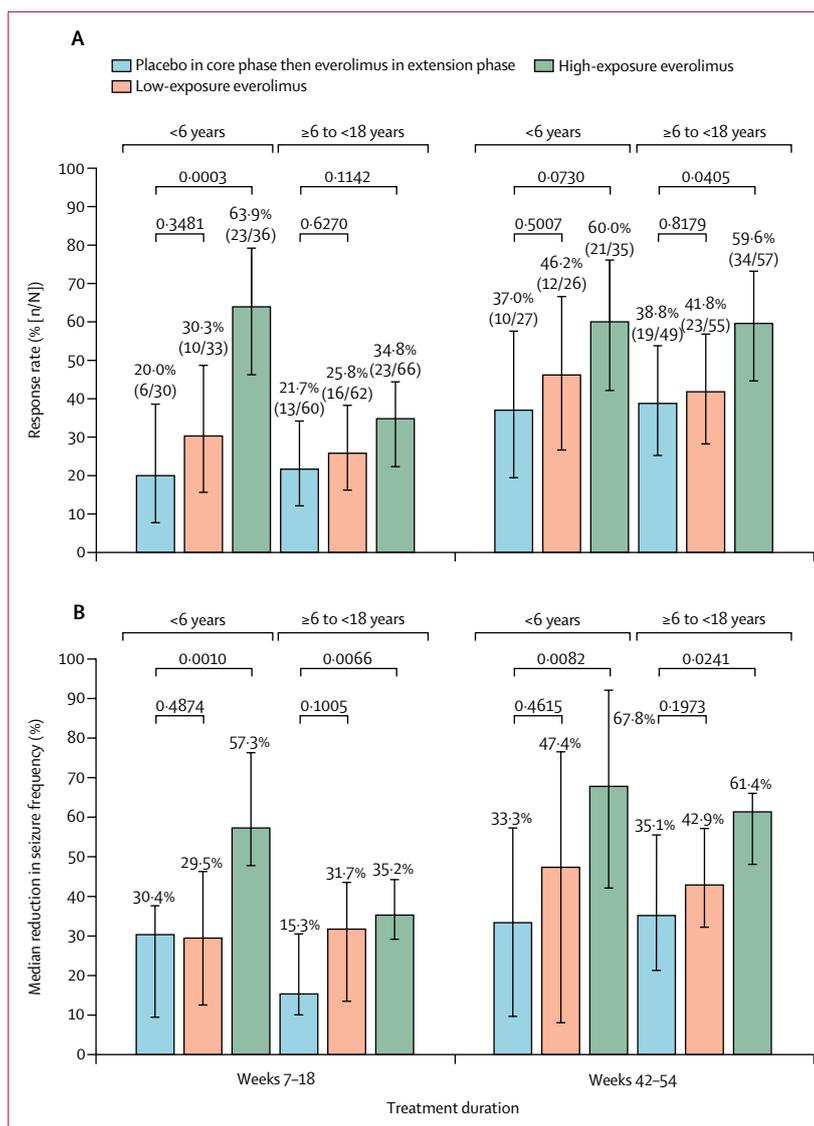
## Discussion

In this post-hoc analysis of the paediatric subpopulation of the EXIST-3 trial, we report long-term efficacy and safety data of adjunctive everolimus treatment for treatment-refractory seizures associated with tuberous sclerosis complex. Our results showed that everolimus produced a substantial and sustained reduction in seizure frequency compared with placebo in children and adolescents.

Treatment of epileptic seizures in tuberous sclerosis complex is notably difficult because most patients become treatment refractory. Furthermore, the onset of epilepsy most commonly occurs during infancy and early childhood, which can make treatment decisions challenging. The positive results from the EXIST-3 study<sup>18</sup> led to the approval of everolimus by the European Medicines Agency for the treatment of refractory seizures associated with tuberous sclerosis complex in patients aged 2 years and older. Thus, characterising the efficacy and safety profile of everolimus in children and adolescents is important to assess the feasibility of long-term treatment in this population with substantial disease burden.

The younger patients (<6 years) appeared to receive a greater benefit than the older patients ( $\geq 6$  years to <18 years), with up to 1 year of everolimus treatment (pharmacokinetic data to be reported in a separate publication). This outcome could be because of lower everolimus clearance or higher body surface area-normalised everolimus clearance in younger patients, as indicated by dose-normalised  $C_{min}$ , a surrogate marker of clearance (data not shown). Similarly, the seizure reduction improved with time among the older patients. However, these data should be interpreted with caution, taking patient discontinuations into account that resulted in fewer evaluable patients over time.

The adjunctive everolimus therapy also showed improvement in seizure reduction in paediatric patients



**Figure 4: Response rate (A) and reduction in seizure frequency (B) by age and treatment**

(A) Error bars represent 95% CI obtained with Clopper-Pearson method. p values computed from  $\chi^2$  test. (B) Error bars represent 95% CI based on bootstrap percentiles. p values obtained from rank ANCOVA with baseline seizure frequency as covariate. Denominators include patients with a baseline value and a value at the assessment timepoint.

who were initially randomly assigned to receive placebo and transitioned to everolimus in the extension phase, achieving additional reductions in seizure frequency beyond the placebo effect observed in the core phase. The reductions in seizure frequency observed in the paediatric subpopulation were greater than those reductions reported in the overall EXIST-3 trial population.<sup>18</sup> These results are also consistent with a previous smaller prospective study<sup>20,21</sup> that showed a clinically relevant reduction in seizure frequency in 12 (60%) of 20 patients with tuberous sclerosis complex treated with everolimus and with persistent efficacy in seizure control, with up to 4 years of continued treatment (13 [72%] of 18 patients had  $\geq 50\%$  of reduction in

	Core phase				Extension phase				
	Younger subgroup (n=104)		Older subgroup (n=195)		Younger subgroup (n=101)		Older subgroup (n=193)		
	All grades	Grade 3 or 4	All grades	Grade 3 or 4	All grades	Grade 3 or 4	All grades	Grade 3 or 4	
	Placebo (n=34)	Low-exposure everolimus (n=33)	High-exposure everolimus (n=37)	Placebo (n=62)	Low-exposure everolimus (n=63)	High-exposure everolimus (n=70)	Placebo (n=62)	Low-exposure everolimus (n=63)	High-exposure everolimus (n=70)
Pyrexia	5 (15%)	15 (45%)	14 (38%)	12 (19%)	20 (32%)	21 (30%)	1 (2%)	1 (2%)	0
Diarrhoea	9 (26%)	15 (45%)	10 (27%)	5 (8%)	15 (24%)	21 (30%)	0	0	1 (1%)
Stomatitis	9 (26%)	9 (27%)	14 (38%)	21 (34%)	22 (35%)	27 (39%)	0	3 (5%)	3 (4%)
Mouth ulceration	6 (18%)	10 (30%)	10 (27%)	8 (13%)	19 (30%)	17 (24%)	1 (2%)	1 (2%)	0
Upper respiratory tract infection	6 (18%)	9 (27%)	12 (32%)	7 (11%)	12 (19%)	13 (19%)	0	0	0
Nasopharyngitis	3 (9%)	7 (21%)	12 (32%)	9 (15%)	17 (27%)	15 (21%)	0	0	1 (1%)
Cough	3 (9%)	8 (24%)	7 (19%)	3 (5%)	12 (19%)	15 (21%)	0	0	0
Pneumonia	2 (6%)	7 (21%)	6 (16%)	1 (2%)	4 (6%)	2 (3%)	1 (2%)	1 (2%)	2 (3%)
Vomiting	3 (9%)	8 (24%)	7 (19%)	8 (13%)	13 (21%)	17 (24%)	0	0	0
Bronchitis	1 (3%)	5 (15%)	11 (30%)	1 (2%)	4 (6%)	2 (3%)	0	0	0

Data are n (%). Adverse events were reported in patients during the core (≥20% of patients in either of the everolimus treatment groups for both age groups) and extension phases (≥20% of patients in either of the age groups). Patients in the younger subgroup were younger than 6 years old, and those in the older subgroup were aged 6 years or older, and younger than 18 years. Extension phase data are both phases combined.

Table 2: Adverse events of any cause

seizures). In the paediatric population of the EXIST-3 trial, baseline seizure frequency was higher in younger patients than in older patients, indicating a severe seizure burden. Among the older patients, both the low-exposure and high-exposure everolimus treatment groups had lower baseline seizure frequencies than the placebo group. Reductions in seizure frequency with everolimus improved in children younger than 6 years with long-term follow-up. A similar observation was noted among children younger than 3 years (n=8) treated with everolimus for subependymal giant cell astrocytoma in tuberous sclerosis complex. Everolimus led to permanent cessation of seizures in one child with treatment-refractory epilepsy and at least a 50% of reduction in seizure frequency in two children.<sup>22</sup> In the EXIST-3 trial, seizure outcomes improved across all age groups including adults (≥18 years; appendix), with remarkably greater improvements in younger patients, possibly because of the higher everolimus exposure achieved in these patients.

To the best of our knowledge, this is the first reported analysis of a phase 3 study assessing mTOR inhibitors for seizure reduction in a large paediatric population for a long duration (nearly 2 years). Earlier trials<sup>21,23</sup> included the 4-year follow-up study of everolimus for 24 patients with tuberous sclerosis complex and refractory epilepsy (median age 8 years [range 2.0–21.3]) and a crossover trial assessing the effect of sirolimus on seizure reduction in 23 children aged between 3 months and 12 years, both of which had small sample sizes and open-label designs as major limitations.

For patients with treatment-refractory seizures, whether earlier intervention with everolimus would be similarly associated with improvements in cognitive and behavioural outcomes remains to be determined. The timing of seizure onset is considered a key predictor for neurodevelopmental outcomes and autism risk. A delay of 1 month in age at seizure onset corresponds to increased odds of no developmental delays by about 34% by 18 months of age and 61% by 24 months of age.<sup>24</sup> Additionally, seizure onset by 12 months of age was highly predictive of poor development outcomes (such as early learning, adaptive behaviour, and language) at later ages (18 months and 24 months), and this prediction was especially true for refractory seizures. Therefore, treatment within 6–12 months of age is recommended to improve seizure control, which could correspond to better developmental outcomes at 24 months in patients with infantile spasms.<sup>24</sup> In a retrospective study,<sup>25</sup> 44 infants presenting with seizures within the first 12 months of life were treated with vigabatrin. The long-term follow-up of these infants (≥3.5 years) showed that patients who received early treatment (within the first week of seizure onset) had higher rates of seizure freedom (65%) than those patients who were treated later (24% for ≥3 weeks after seizure onset; p<0.01) and that a smaller percentage of patients had intellectual disability

when treated early (61% vs 100%;  $p < 0.001$ ), suggesting that a shorter gap between seizure onset and start of treatment might improve outcomes.

Adverse events in this subgroup of paediatric patients are generally consistent with those previously reported for everolimus in patients with tuberous sclerosis complex.<sup>14,18,26</sup> Adverse events were predictable, primarily of grade 1 or 2 severity (mild or moderate), manageable with dose reductions or interruptions, and did not overlap with the typical side-effects of antiepileptic drugs. No new safety concerns were identified. Stomatitis and mouth ulceration, both known risks of everolimus treatment, were the most frequently reported adverse events, followed by infections. With the extended treatment, an increased incidence and severity of infections (primarily pneumonia) was observed in younger children, which is consistent with the known incidence pattern of pneumonia reported in the general population. The overall type, incidence, and severity of adverse events were comparable for adults ( $\geq 18$  years) and paediatric patients (appendix). However, infections were reported at a higher frequency in children than adults (appendix). Pneumonia is a major cause of morbidity and mortality worldwide in children under age 5 years in general,<sup>27,28</sup> whereas renal disease and sudden unexplained death due to epilepsy are considered the main causes of mortality in patients with tuberous sclerosis complex.<sup>29</sup> Furthermore, community-acquired infections, such as upper respiratory infections, have been estimated to occur 4–6 times per year in children and 2–4 times per year in young adults, and might not be related to everolimus exposure specifically, suggesting that continued surveillance is essential.<sup>21</sup> Two deaths that occurred during the reporting period in our study were because of pneumonia and sudden unexplained death due to epilepsy, which is recognised as a life-threatening event related to tuberous sclerosis complex.<sup>30</sup> Importantly, however, everolimus was well tolerated in the younger patients during the extended treatment duration (median 1.6 years), with only seven patients discontinuing because of adverse events during the extension phase.

Some of the limitations of the present analysis included an absence of data regarding the effect of concomitant antiepileptic drugs or change in antiepileptic drugs on everolimus efficacy, no control group in the extension phase of the study, and more patient withdrawals before later assessment timepoints. A further limitation was the imbalance in the baseline seizure frequency across the treatment groups and a wide range of everolimus exposure concentrations while transitioning from the core phase to extension phase of the study.

In conclusion, our findings showed an improvement in seizure control with adjunctive everolimus in paediatric patients, which adds to known improvements of other manifestations of tuberous sclerosis complex, including subependymal giant cell astrocytoma and

renal angiomyolipoma. Overall, the available evidence suggests that everolimus can affect the underlying mechanism of several tuberous sclerosis complex-associated clinical manifestations<sup>14,26</sup> by targeting the overexpression of mTOR, which is the molecular hallmark of tuberous sclerosis complex. Long-term treatment with everolimus might be required to maintain efficacy, and our findings show that everolimus is generally a well tolerated and effective therapeutic option for paediatric patients with tuberous sclerosis complex-associated seizures.

#### Contributors

PC, DNF, Pjdv, DJD, DP, and JAF designed the study. DNF, JAL, ZY, HI, TP, DJD, DP, and MV collected data. PC, DNF, RN, DJD, JF, MV, JAF, and AR analysed data. PC, DNF, JAL, TP, RN, Pjdv, DJD, JF, DP, MV, JAF, and AR interpreted data. DNF, JAL, ZY, HI, TP, DP, and MV were responsible for patient accrual. DNF, JAL, ZY, HI, Pjdv, JF, DP, and MV were responsible for trial management. DNF, JAL, ZY, HI, TP, and MV were responsible for clinical care. AR was the trial statistician. All authors drafted, revised, and reviewed and approved the final manuscript.

#### Declaration of interests

PC received consultation fees for advisory boards from Eisai, Shire, and Novartis, and received speaker honoraria and travel reimbursement from Novartis. DNF received speaker honoraria and travel reimbursements from Novartis; his employer, Cincinnati Children's Hospital, has received funds for consulting work and research grants from Novartis. JAL received consultation fees and research funding from Novartis. TP received consultation fees for advisory boards from Novartis and Desitin, and speaker honoraria from UCB Pharma and Desitin. RN received research funding from the European Union (Seventh Framework Programme), Shire, Zogenix, GW Pharma, and Eisai, and consultation fees from Eisai, Zogenix, and Novartis. Pjdv received research funding from Novartis and consultation fees from Novartis for being a member of Novartis steering committee, co-principal investigator, and working committee and scientific advisory board member of the Tuberous Sclerosis Registry to Increase Disease Awareness (TOSCA), sponsored by Novartis. DJD received research funding from The Epilepsy Study Consortium and US National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health. JF, AR, MV, and DP are employees of Novartis. JAF received research grants from Acorda, Alexza, Eisai, LCGH, Lundbeck, Pfizer, SK Life Sciences, Sunovion, Takeda, and UCB Inc, as well as grants from the Epilepsy Research Foundation, Epilepsy Study Consortium, Epilepsy Therapy Project, and NINDS. JAF is on the Scientific Advisory Board of Ovid, Sage Therapeutics, and Blackfynn, and is on the editorial board of *The Lancet Neurology*, *Neurology Today*, and *Epileptic Disorders*. JAF has received 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 Inc, Upsher-Smith, Zogenix, and Zynerba. JAF receives NYU salary support from the Epilepsy Foundation (for being a scientific officer) and for consulting work on behalf of the Epilepsy Study Consortium for Acorda, Adamas, Alexza, Anavex, Axcella Health, Biogen, BioPharm Solutions, Cerecor, Concert Pharmaceuticals, Engage, Eisai, GlaxoSmithKline, GW Pharma, Marinus, Nestle-Health Science, Neurelis, Novartis, Pfizer, Pfizer-Neusentis, Otsuka, Ovid, Sage Therapeutics, SK Life Sciences, Sunovion, Takeda, UCB Inc, Upsher Smith, Xenon Pharmaceuticals, Zogenix, and Zynerba. ZY and HI report no competing interests.

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