Original article

Everolimus for epilepsy and autism spectrum disorder in tuberous sclerosis complex: EXIST-3 substudy in Japan

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Abstract

Background: Epilepsy and autism spectrum disorder (ASD) are the common neurological manifestations of tuberous sclerosis complex (TSC). EXIST-3 study has recently demonstrated that everolimus reduces seizures in patients with TSC and refractory epilepsy. Here we report the efficacy and safety of everolimus for treatment-refractory seizures in Japanese patients of EXIST-3, along with the exploratory analysis evaluating the everolimus effect on comorbid ASD symptoms in these patients.

Methods: Primary end point was change in seizure frequency from baseline defined as response rate ($\geq$50% reduction) and median percentage reduction in the seizure frequency. Pervasive Developmental Disorders Autism Society Japan Rating Scale (PARS) scores were assessed at baseline and at week-18 for ASD symptoms.

Results: Overall, 35 Japanese patients were randomized to everolimus low-exposure (LE; n = 10), everolimus high-exposure (HE; n = 14), or placebo (n = 11). The response rate was 30.0% and 28.6% versus 0% with the everolimus LE and HE versus placebo arm, respectively. Similarly, the median percentage reduction in seizure frequency was 6.88% and 38.06% versus −6.67%. Stomatitis was the most frequently reported adverse event (everolimus LE, 100%; HE, 78.6%; placebo, 9.1%). Four of 11 patients with ASD in the everolimus arms and 1 of 8 patients with ASD in the placebo arm showed $\geq$5 point decrease in PARS scores.

Conclusions: Adjunctive everolimus treatment improved seizure frequency with a tolerable safety relative to placebo among 35 Japanese patients with TSC-associated refractory seizures, consistent with the results of overall EXIST-3 study involving 366 patients. A favorable trend towards the improvement of ASD symptoms was observed.
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Keywords: Tuberous sclerosis complex; Epilepsy; Autism spectrum disorder; Everolimus; Refractory seizures; EXIST-3

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1. Introduction

Tuberous sclerosis complex (TSC) is a multisystem genetic disorder caused by mutations in either *TSC1* or *TSC2* gene, and involves multiple organs in the body, including the brain, heart, kidneys, lungs, eyes, and skin [1]. Epilepsy is the most common neurological symptom of TSC, affecting 72% to 85% of patients [1]. Early onset of epilepsy in TSC is associated with an increased risk of neurodevelopmental disorders, such as intellectual disability and autism spectrum disorder (ASD) [2]. ASD is very common in patients with TSC [3], with a prevalence rate of 17–63% [4]. Autism is more frequent in TSC children with early-onset seizures, and is occasionally noted in those without epilepsy. Previous studies suggested that the number and location of cortical tubers were associated with the development of ASD [3]. In patients with TSC, an abnormal brain circuitry has been implicated in both ASD and epilepsy [5].

In patients with TSC, epilepsy is often severe and intractable, with one-third of patients' seizures refractory to the current surgical and medical therapies [6]. As occurrence of seizures may increase the risk of ASD, early cessation of seizures may play a crucial role to mitigate the cognitive and behavioral difficulties associated with autism. The available antiepileptic drugs (AEDs) often fail to control TSC-associated seizures, thus increasing the need for an effective treatment option [7]. Furthermore, there is currently no specific psychopharmacotherapy for ASD in TSC patients, thus increasing the need for an effective treatment intervention [3,8].

Mutations of *TSC* gene leading to an abnormal signaling in the mammalian target of rapamycin (mTOR) pathway may serve a critical role in the pathogenesis of epilepsy and ASD in patients with TSC [9–11]. Between epilepsy and ASD, there are similarities and differences as to the alterations in *TSC* gene and mTOR pathway. Epileptic foci in brains with TSC are located in or around cortical tubers. Abnormal giant cells, a pathologic hallmark of cortical tubers, are caused by bi-allelic inactivation of *TSC1* or *TSC2* gene [12]. On the other hand, rodent models of TSC, *TSC1*^{+/-} and *TSC2*^{+/-} mice, have neither cortical tubers nor epileptic seizures, but do show ASD-like behaviors which are improved by treatment with rapamycin, an mTOR inhibitor [11]. Taken together, both epilepsy and ASD are caused by a *TSC* gene mutation. The presence of cortical tubers and bi-allelic inactivation (or second hit) is required for epilepsy, but not necessarily for ASD. ASD is attributed to haploinsufficiency, at least in part [11]. The preclinical evidence suggests that targeting the mTOR pathway could prevent the development of epilepsy and also ameliorate the symptoms of ASD [11,13,14]. Case reports and open-label studies showed the beneficial effects of mTOR inhibitors in patients with TSC-associated epilepsy [6,15,16]. Evidence is accumulating that mTOR inhibitors ameliorate epilepsy and the symptoms of TSC-associated neuropsychiatric disorders (TANDs) including ASD [17]. However, it was explored only in case series or reports. In addition, the correlation between seizure reduction and improvement in ASD symptoms is not clearly understood.

The pivotal EXIST-3 study (EXamining everolimus In a Study of TSC) demonstrated that everolimus, as an adjunctive therapy, significantly improves the seizure frequency when compared to placebo in patients with treatment-refractory seizures associated with TSC [18]. Here, we report the efficacy and safety of everolimus for treatment-refractory seizures in the Japanese patients with TSC enrolled in the EXIST-3 study. This paper also presents the results from an exploratory analysis of EXIST-3, first of its kind in a randomized setting, assessing the efficacy of everolimus for the treatment of symptoms related to ASD in patients from the same population.

2. Methods

2.1. Patients

The EXIST-3 study included patients with clinically confirmed TSC [19] and treatment-refractory epilepsy receiving at least 1–3 AEDs at a stable dose for at least 12 weeks before randomization. Eligible patients had at least 16 partial-onset seizures during 8 weeks of baseline phase and with no continuous 21-day seizure-free period [18]. For the exploratory analysis, a subpopulation of the Japanese patients who had agreed to participate in the study were enrolled.

Patients were excluded if they had at least one of the following: SEGA requiring immediate surgical intervention, an episode of status epilepticus within 52 weeks prior to study, only nonmotor partial seizures without confirmation by ictal electroencephalography (EEG), untreated infantile spasms, seizures secondary to metabolic, toxic, infectious or psychogenic disorder, or drug abuse or current seizures related to an acute medical illness. Patients who received more than 3 AEDs, had changed their dosage during 4 weeks before screening or during the baseline phase, or had prior treatment with an mTOR inhibitor within 24 months of the study entry, were excluded.

2.2. Study design and treatment

The study design and methods used in the EXIST-3 study have been previously published [18]. This was a 3-arm, prospective, randomized, multicenter, double-blind, placebo-controlled, phase 3 study (NCT01713946). The study has 3 phases, a baseline phase for 8 weeks and a core phase of 18 weeks,

followed by an extension phase of ≥ 48 weeks. In the core phase, patients were randomized to receive placebo or everolimus titrated to a target trough concentration (C_{min}) of 3–7 ng/mL (everolimus low-exposure [LE] treatment) or to a target C_{min} of 9–15 ng/mL (everolimus high-exposure [HE] treatment) in addition to a stable regimen of 1–3 AEDs.

During the study, patients or their caregivers completed a seizure diary to record seizure type and frequencies. The seizure type was evaluated by the Epilepsy Study Consortium, and the seizures were categorized as focal seizures unless an electroencephalography (EEG) confirmed a generalized onset. The details of seizure classification, randomization, masking, and treatment procedure have been previously published [18].

2.3. Efficacy and safety assessments

The primary efficacy end point was change in seizure frequency from baseline in everolimus LE and everolimus HE arms compared with placebo during the 12-week maintenance period of the core phase expressed as response rate (the proportion of patients achieving 50% or greater reduction in seizure frequency), and median percentage reduction in the 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) [18].

The secondary end points included frequency of seizure-free days (100% reduction in the seizure frequency during the entire maintenance period), seizure-free rate, the proportion of patients achieving at least 25% reduction in the seizure frequency from baseline, categorical variable of 6 levels of reduction in the seizure frequency from baseline ($\leq -25\%$ [exacerbation]; $\geq -25\%$ to $<25\%$ [no change]; $\geq 25\%$ to $<50\%$; $\geq 50\%$ to $<75\%$; $\geq 75\%$ to $<100\%$; 100% [seizure freedom]), and safety reported as adverse events (AEs) assessed according to the National Cancer Institute Common Toxicity Criteria for Adverse Events version 4.03 [20].

The Pervasive Developmental Disorders Autism Society Japan Rating Scale (PARS) scores were used to evaluate the effect of everolimus on symptoms of ASD [21]. PARS scores were assessed at baseline, week 18, and at week 42 of the study duration. The diagnosis of ASD is suspected when the PARS scores are 9 points or more in the preschoolers, 13 points or more in the primary schoolers, and 20 points or more in the adolescents and adults. Symptoms of ASD were considered improved if the change in PARS scores from baseline to the end of core phase (week 18) was at least 5. Additional details on the PARS scale and assessment are provided in the supplementary material.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.braindev.2018.07.003.

2.4. Statistical analysis

The statistical methodology of the EXIST-3 study has been published previously [18]. The analysis of everolimus efficacy in the Japanese patients enrolled in the EXIST-3 study was preplanned. However, due to the small sample size, no statistical tests were applied and the data were presented using descriptive statistics. For the exploratory ASD study, all the Japanese patients who had at least 1 non-missing date of evaluation for PARS assessment were included. No planned statistical testing was applied.

2.5. Study oversight

This study was sponsored by Novartis Pharmaceuticals Cooperation and Novartis Pharma KK. All the study-related documents were approved by the institutional review board or ethics committee at each participating center. The study was conducted in accordance with the Good Clinical Practice principles, the Declaration of Helsinki, and all the local regulations. All the patients provided written informed consent prior to the initiation of any study-related procedure. Data were analyzed by the sponser's clinical and statistical teams and interpreted in collaboration with the study investigators. All authors were involved in development and approval of the manuscript and attested to the integrity of the data and adherence to the planned protocol and statistical analyses.

3. Results

3.1. Patients demographics

In the overall EXIST-3 study, a total of 366 patients with the treatment-refractory seizures associated with TSC were recruited from 99 centers in 25 countries [18]. Of these 366 patients, 35 were enrolled from the Japanese centers and randomized to the everolimus LE treatment arm (n = 10), everolimus HE treatment arm (n = 14), and placebo arm (n = 11) (Fig. 1). The overall median age of the Japanese patients was 8.76 years (range, 2.9–16.6 years) and all of them were below the age of 18 years (Table 1). The median age was 7.55 years (range, 3.9–13.8 years), 8.31 years (range, 2.9–16.6 years), and 10.12 years (range, 2.9–15.8 years) in the everolimus LE treatment arm, everolimus HE treatment arm, and placebo arm, respectively. One patient in the everolimus LE treatment arm discontinued the study
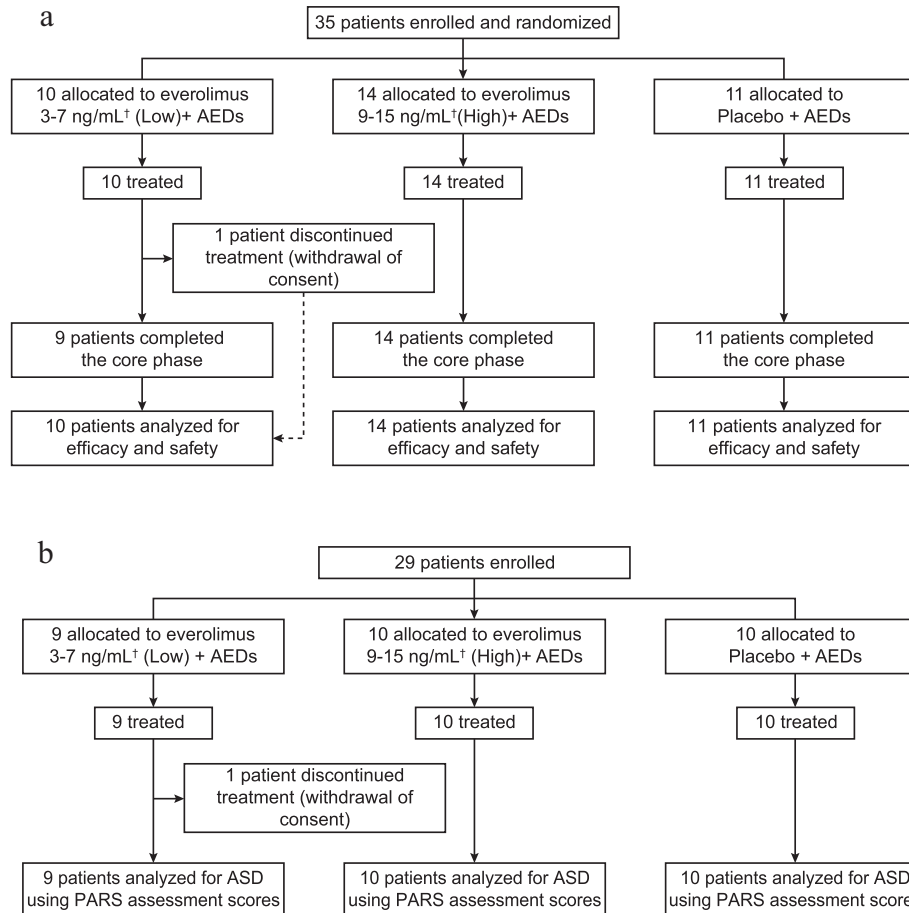
a

b

Fig. 1. Trial profile. (a) EXIST-3 – the Japanese subpopulation study. (b) EXIST-3 – the Japanese ASD exploratory study. AEDs, antiepileptic drugs; ASD, Autism spectrum disorder; PARS, Pervasive Developmental Disorders Autism Society Japan Rating Scale [†]Everolimus trough concentrations, everolimus 3–7 ng/mL represents low-exposure (LE) arm and everolimus 9–15 ng/mL represents high-exposure (HE) arm.

treatment during the maintenance period of core phase due to consent withdrawal.

3.2. Efficacy

The median seizure frequency per week at baseline was 9.09 (range, 3.3–56.1) and 13.69 (range, 2.9–71.8) in the everolimus LE and HE treatment arms, respectively, and 7.50 (range, 2.5–94.8) in the placebo arm. The median dose intensity observed in patients was 5.4 mg/m^2 per day (range, 4–9 mg/m^2) and 8.2 mg/m^2 per day (range, 5–14 mg/m^2) in the everolimus LE and HE treatment arms, respectively, and 7.1 mg/m^2 per day (range, 5–14 mg/m^2) in the placebo arm.

The response rate was 30.0% (95% confidence interval [CI], 6.7–65.2%) in the everolimus LE treatment arm, 28.6% (95% CI, 8.4–58.1%) in the everolimus HE treatment arm, compared with 0% (95% CI, 0.0–28.5%) in the placebo arm (Fig. 2a). The median percentage

reduction in the seizure frequency was 6.88% (95% CI, −79.26 to 55.99%) in the everolimus LE treatment arm and 38.06% (95% CI, 8.15–58.58%) in the everolimus HE treatment arm. On the other hand, the placebo arm showed a median reduction in the seizure frequency per week of −6.67% (95% CI, −31.03 to 29.99%), denoting the exacerbation of seizures (Fig. 2b).

The median change in frequency of the seizure-free days from baseline to core phase in the everolimus LE treatment arm, everolimus HE treatment arm, and placebo arm were 0.0 day (range, −8.7 to 20.8 days), 2.32 days (range, −4.4 to 13.3 days), and 0 day (range, −10.4 to 2.9 days), respectively. The seizure-free rate was 10.0% (95% CI, 0.3–44.5%) for the everolimus LE treatment arm, while the everolimus HE treatment and placebo arms showed 0% of seizure-free rate with 95% CI of 0 to 23.2% and 0 to 28.5%, respectively. The percentage of patients with ≥25% or more reduction in seizure frequency was 50.0% (95% CI, 18.7–81.3%), 57.1%

Table 1
Baseline characteristics by treatment group.

Demographic variable	Everolimus 3–7 ng/mL N = 10	Everolimus 9–15 ng/mL N = 14	Placebo N = 11	Overall N = 35
Median age, years (range)	7.55 (3.9–13.8)	8.31 (2.9–16.6)	10.12 (2.9–15.8)	8.76 (2.9–16.6)
Age category (years), n (%)				
<6	4 (40.0)	6 (42.9)	4 (36.4)	4 (40.0)
6 to <12	2 (20.0)	4 (28.6)	4 (36.4)	10 (28.6)
12 to <18	4 (40.0)	4 (28.6)	3 (27.3)	11 (31.4)
>18	0	0	0	0
Gender, n (%)				
Female	4 (40.0)	4 (28.6)	6 (54.5)	14 (40.0)
Male	6 (60.0)	10 (71.4)	5 (45.5)	21 (60.0)
Median seizure frequency at baseline (range)	9.09 (3.3–56.1)	13.69 (2.9–71.8)	7.50 (2.5–94.8)	–
Antiepileptic therapy during baseline phase				
Any background AEDs, n (%)	10 (100.0)	14 (100.0)	11 (100.0)	35 (100.0)
Number of AEDs in the regimen, n (%)				
1	0	2 (14.3)	2 (18.2)	4 (11.4)
2	5 (50.0)	5 (35.7)	5 (45.5)	15 (42.9)
3	5 (50.0)	7 (50.0)	4 (36.4)	16 (45.7)
>3	0	0	0	0
AEDs failed prior to study start*				
Number of AED's, n (%)				
<2	0	0	0	0
2	1 (10.0)	1 (7.1)	1 (9.1)	3 (8.6)
3	0	1 (7.1)	0	1 (2.9)
4	0	0	3 (27.3)	3 (8.6)
5	2 (20.0)	6 (42.9)	2 (18.2)	10 (28.6)
6	1 (10.0)	0	0	1 (2.9)
>6	6 (60.0)	6 (42.9)	5 (45.5)	17 (48.6)

AED, antiepileptic drug; HE, high-exposure; LE, low-exposure.
Everolimus 3–7 ng/mL represents LE arm and everolimus 9–15 ng/mL represents HE arm. *Prior to study start means before the screening visit.

(95% CI, 28.9–82.3%), and 27.3% (95% CI, 6.0–61.0%) in the everolimus LE treatment, everolimus HE treatment, and placebo arms, respectively (Fig. 2c).

3.3. Safety

All-grade AEs of any cause were reported in all the 24 patients (100%) treated with everolimus and 9 patients (81.8%) in the placebo arm. Grade 3 or 4 AEs occurred in 2 (20.0%) and 3 patients (21.4%) in the everolimus LE and HE treatment arms, respectively, and 2 patients (18.2%) in the placebo arm (Table 2). The most frequently reported AE was stomatitis, including aphthous ulcer, in the everolimus LE treatment arm (all grade, 100%; grade 3 or 4, 0%) and the everolimus HE treatment arm (all grade, 78.6%; grade 3 or 4, 14.3%). In contrast, the incidence of stomatitis was low (all grade, 9.1%; grade 3 or 4, 0%) in the placebo arm. Adverse events leading to dose adjustments or interruptions were reported in 3 (30.0%), 7 (50.0%) and 2 patients (18.2%) in the everolimus LE treatment, everolimus HE treatment, and placebo arms, respectively. No patient discontinued treatment due to AEs. No deaths were reported during the core phase.

The median time-normalized C_{min}, which denotes an estimated average of C_{min} across the maintenance period of core phase, in everolimus LE and HE treatment arms was 4.62 ng/mL (range, 3.36–16.40 ng/mL) and 8.73 ng/mL (range, 3.61–15.40 ng/mL), respectively.

3.4. ASD exploratory study

Of the total 35 Japanese patients enrolled, 29 (12 females and 17 males) participated in the exploratory study (9 patients in the everolimus LE treatment arm and 10 patients each in everolimus HE treatment arm and placebo arm, respectively; (Fig. 1) The PARS assessment scores for all the 29 patients (irrespective of their ASD status) are presented (Table 3). At baseline, 20 patients were diagnosed with ASD, as per the PARS definition (supplementary material). The PARS analysis scores were assessed in 19 of 20 patients, who had completed the core phase.

In patients with ASD, 10 patients were reported with decrease in the PARS scores at the end of core phase (week 18; 3 in the placebo arm, 4 in the everolimus LE treatment arm, and 3 in the everolimus HE treatment arm) (Table 3). Of these, ≥5 point decrease in the total
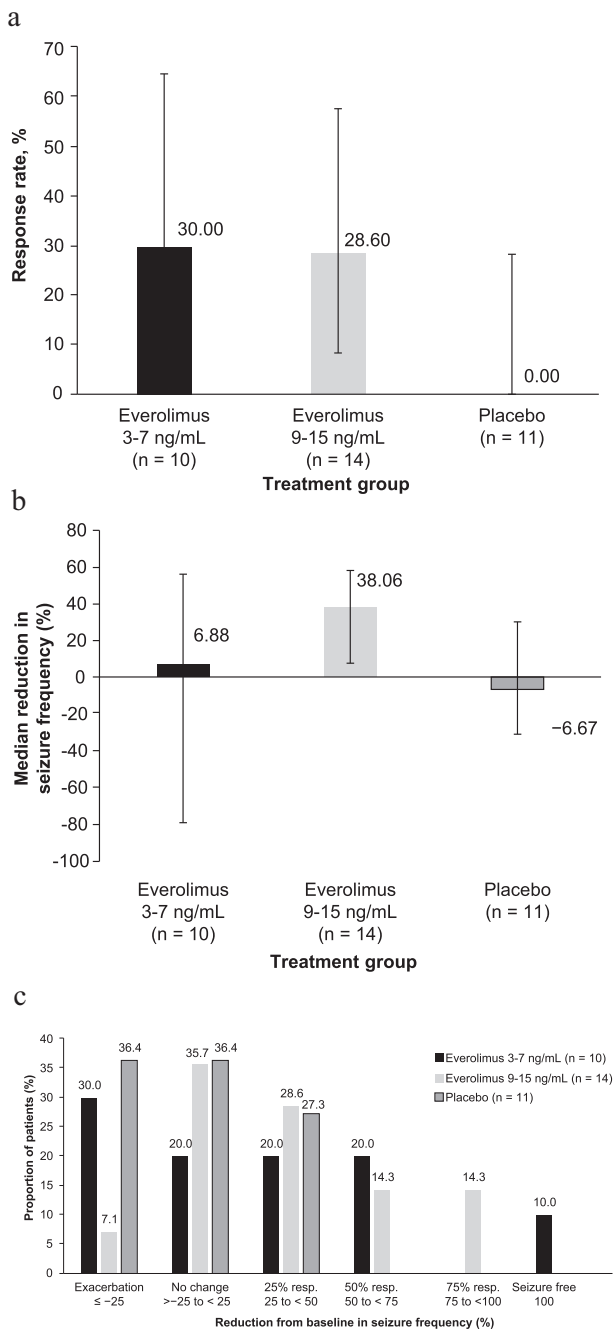
a

b

c

Fig. 2. Seizure outcomes. (a) Response rate by treatment group. Error bars denote 95% confidence intervals. (b) Median reduction in the seizure frequency by treatment group. Error bars denote 95% confidence intervals. (c) Distribution of reduction from baseline in the seizure frequency by treatment group. HE, high-exposure; LE, low-exposure; Everolimus 3–7 ng/mL represents LE arm and everolimus 9–15 ng/mL represents HE arm.

PARS score was reported in 5 patients along with the median percentage reduction in seizure frequency (2 in the everolimus LE treatment arm, 2 in the everolimus HE treatment arm, and 1 in the placebo arm). Overall no apparent correlation was noted between the change in PARS score and the median percentage reduction in

seizure frequency. However, in five patients with more than or equal to 5 change in PARS scores, a possible linear correlation seemed to be observed (Fig. 3). The representative everolimus-treated cases (Patients #2, #3 and #14) with a decrease of >5 points in PARS score are discussed below, which were reported across the age range of 4.0 to 13.5 years and also in both male (2 patients) and female patients (1 patient).

Patient #2, a 5.8-year-old (age at baseline) male patient from the everolimus LE treatment arm reported PARS scores as 33, 26, and 27, at baseline, week 18, and week 42, respectively (change in PARS score: 7-points). At baseline, this patient showed repetitive behaviors like persistently lining up the bottles and stereotypic movement of his body. In addition, the patient reported mood changes following a change in situation or routine, and self-injurious behavior. After the treatment with everolimus, no such behavioral patterns were observed by the end of core phase (week 18) and also at week 42.

Patient #3, a 13.5-year-old (age at baseline) female from the everolimus LE treatment arm showed a 10-point decrease in the PARS score from 33 at baseline to 23 at week 18 and to 20 at week 42. After 5 months of treatment with everolimus, the patient was observed to have a reduction in irritability, an improved social interaction, and more cheerfulness compared to the baseline. An improvement in the communication skills was reported, as she responded better to her mother and was also able to express her feelings by improved speech.

Patient #14, a male aged 4 years (age at baseline) in the everolimus HE treatment arm (9–15 ng/mL) reported PARS scores as 19 at baseline and 11 at week 18 (change in PARS score: 8-points). At baseline, this patient showed unusual behavior patterns such as repetition of words from commercials, eating or swallowing nonfood items, unresponsiveness when called by name, inclination towards watching revolving things, moving the entire or part of the body repeatedly in the same pattern, and not maintaining the personal independence due to the disrupted lifestyle. At the end of core phase (week 18), these behaviors were not seen; however, the patient sometimes became involved in lining up toys and bottles, which was not observed at the baseline.

4. Discussion

The neurological manifestations including epilepsy, autism, and intellectual disability majorly account for the morbidity in patients with TSC [1,3]. The co-occurrence of epilepsy and neuropsychiatric disorders in TSC makes the treatment more challenging. In TSC, both the epilepsy and neuropsychiatric disorders are driven by the molecular etiology—mTOR overactivation [22]. The beneficial effects of the everolimus, an

Table 2
Adverse events of any cause reported in >20% of patients in either of the everolimus treatment groups.

	Everolimus 3–7 ng/mL N = 10		Everolimus 9–15 ng/mL N = 14		Placebo N = 11	
	All grades n (%)	Grade 3 or 4 n (%)	All grades n (%)	Grade 3 or 4 n (%)	All grades n (%)	Grade 3 or 4 n (%)
Any adverse event	10 (100.0)	2 (20.0)	14 (100.0)	3 (21.4)	9 (81.8)	2 (18.2)
Stomatitis*	10 (100.0)	0	11 (78.6)	2 (14.3)	1 (9.1)	0
Nasopharyngitis	5 (50.0)	0	5 (35.7)	0	3 (27.3)	0
Bronchitis	2 (20.0)	0	1 (7.1)	0	1 (9.1)	0
Diarrhea	2 (20.0)	0	1 (7.1)	0	0	0
Decreased appetite	2 (20.0)	1 (10.0)	0	0	0	0
Neutropenia	2 (20.0)	1 (10.0)	0	0	1 (9.1)	1 (9.1)

* Includes all the related terms—stomatitis and aphthous ulcer; HE, high-exposure; LE, low-exposure; Everolimus 3–7 ng/mL represents LE arm and everolimus 9–15 ng/mL represents HE arm.

mTOR inhibitor on multiple manifestations of TSC have been well researched and approved [18,23,24]. The recent positive results available from the primary analysis of EXIST-3 study showed that the adjunctive everolimus therapy led to a statistically significant reduction in seizure frequency compared to placebo in patients with treatment-refractory seizures associated with TSC [18]. This Japanese substudy of EXIST-3 is the first-ever study evaluating the effect of everolimus on ASD in patients with TSC associated with the treatment-refractory seizures in a randomized setting.

This Japanese sub-population of the EXIST-3 study involved a high-risk population with 48.6% of patients failed on >6 AEDs prior to the start of study treatment and with a median weekly seizure frequency at baseline of 9.09 (range, 3.3–56.1), 13.69 (range, 2.9–71.8) in the everolimus LE and HE treatment arms, respectively, and 7.50 (range, 2.5–94.8) in the placebo arm.

In the Japanese subgroup, the adjunctive everolimus therapy demonstrated greater response rates in the everolimus LE (30.0%) and HE treatment arms (28.6%) than in the placebo arm (0%). This was consistent with the results from the overall study group of the EXIST-3 trial (placebo, 15.1%; everolimus LE treatment, 28.2%; everolimus HE treatment, 40.0%) [18]. The median percentage reduction in the seizure frequency was similar in the everolimus HE treatment arm of the Japanese subgroup (38.06%) and the overall study group (39.55%). On the other hand, a difference in the everolimus LE treatment arm and placebo arm was observed between the Japanese population (everolimus LE, 6.88% and placebo, −6.67%) and the overall study group (everolimus LE, 29.29% and placebo, 14.86%), respectively [18]. The reason for this difference is unclear, but could be attributed to differences in the background of the patients studied (e.g. higher number of AEDs as prior line of therapy in everolimus LE and placebo than everolimus HE in Japanese population, which suggested more treatment

resistant seizure) or the small sample size in the Japanese population. Similar to the overall study group, the Japanese population reported a favorable trend of a 25% or greater reduction in the seizure frequency in the everolimus arms (LE, 50% and HE, 57.1%) than the placebo arm (27.3%). The median time-normalized C_{min} observed in the Japanese population was also similar with the overall population [18].

The results from the ASD exploratory study showed a trend of improvement in the total PARS score with the everolimus treatment. Of the 19 patients with ASD at baseline, for whom the PARS scores were available at visit 11 (week 18), 4 of 11 patients in the everolimus arms showed an improvement of ≥5-point PARS score. In contrast, only 1 of 8 patients in the placebo arm showed a decrease of ≥5 points. A similar observation of improvement of autistic symptoms measured with the PARS was reported in a 27-year-old female patient treated with everolimus for renal angiomyolipomas [25].

The PARS scale was used in this study due to its comprehensiveness as a screening questionnaire for the diagnosis of ASD, wide usage in Japan, and reliability and validity demonstrated previously [26]. Originally designed to confirm the status of ASD in patients, PARS is also useful as a rating scale to evaluate the severity of a wide range of ASD symptoms [27]. The scores of the PARS scale correlate with the domain and total scores of the Autism Diagnostic Interview-Revised (ADI-R) and Autism Diagnostic Observational Schedule (ADOS) [21,28]. For Japanese examiners, it is much easier to acquire the skill of scoring for PARS than for ADI-R and ADOS [21]. In this study, the PARS score of ≥5-point decrease was considered to suggest a clinically meaningful improvement in ASD.

To the best of our knowledge, for the first time, this exploratory ASD study evaluated everolimus in a randomized setting for the treatment of ASD in patients with treatment-refractory seizures associated with

Table 3
Change of PARS scores from baseline phase to core phase in the Japanese patients.

Cohort	Patient number[#]	Age at baseline (years)	Gender	ASD status[†]	Baseline PARS scores	Visit 11 PARS score	Change	Seizure frequency 28 days, %		
								Baseline	Visit 11	% Reduction
Everolimus 3–7 ng/mL	1	12.4	Male	ASD	47	Discontinued	NA	35.2	40.67	−15.5
	2	**5.8**	**Male**	**ASD**	**33**	**26**	**−7**	**224.4**	**158.67**	**29.3**
	3	**13.5**	**Female**	**ASD**	**33**	**23**	**−10**	**65.06**	**28.03**	**56**
	4	3.9	Female	ASD	32	30	−2	37.5	57.69	−53.8
	5	5.8	Male	ASD	30	27	−3	24.84	73.67	−196.6
	6	7.1	Male	ASD	21	26	5	63	78.67	−24.9
	7	13.8	Female	Non-ASD	16	13	−3	223.5	128	42.7
	8	13.2	Male	Non-ASD	9	17	8	13.13	30.67	−133.7
	9	8.0	Female	Non-ASD	6	6	0	19.16	7.42	61.3
Everolimus 9–15 ng/mL	10	16.1	Male	ASD	44	51	7	191.8	337.77	−76.1
	11	3.2	Male	ASD	32	32	0	151.18	48.09	69.6
	12	**14.7**	**Female**	**ASD**	**26**	**21**	**−5**	**58.5**	**54.7**	**6.5**
	13	2.9	Male	ASD	20	24	4	95.28	56	41.2
	14	**4.0**	**Male**	**ASD**	**19**	**11**	**−8**	**65.33**	**36.1**	**44.8**
	15	3.9	Male	ASD	10	9	−1	51	50.4	1.2
	16	10.0	Female	Non-ASD	11	18	7	87.38	73.98	15.3
	17	14.8	Male	Non-ASD	10	8	−2	39.3	48.22	−22.7
	18	7.9	Male	Non-ASD	5	4	−1	287	159.33	44.5
	19	4.8	Female	Non-ASD	3	3	0	16.71	4.61	72.4
Placebo	20	9.5	Male	ASD	43	44	1	10	12.52	−25.2
	21	15.8	Female	ASD	31	28	−3	14.5	19	−31
	22	**4.9**	**Female**	**ASD**	**30**	**23**	**−7**	**166**	**141.33**	**14.9**
	23	4.4	Male	ASD	29	30	1	12.65	7.33	42
	24	10.3	Female	ASD	29	32	3	379.31	380	−0.2
	25	10.1	Male	ASD	24	25	0	58.5	76.75	−31.2
	26	2.9	Male	ASD	19	19	0	30	71	−136.7
	27	10.8	Male	ASD	19	18	−1	108.55	76	30
	28	14.4	Female	Non-ASD	8	8	0	42	25.67	38.9
	29	14.7	Female	Non-ASD	4	1	−3	19.42	23.39	−20.4

ASD, autism spectrum disorder; PARS, Pervasive Developmental Disorders Autism Society Japan Rating Scale.
*Rows marked in bold represent patients with ≥5-point decrease in PARS scores.
[#] Patient number designated here refers only within the manuscript and is not related to the actual patient numbers assigned in the study.
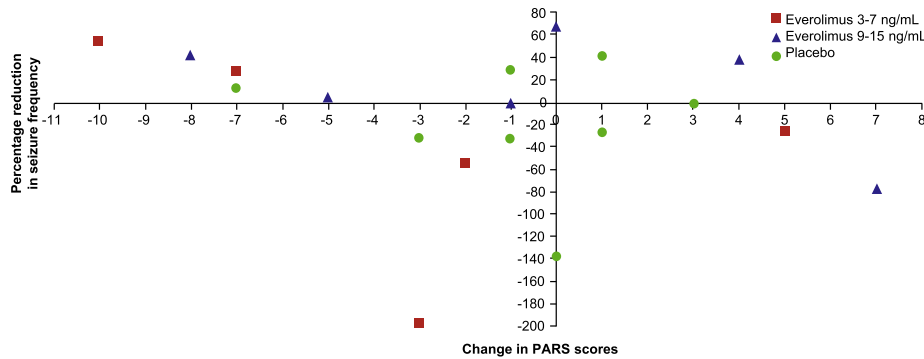[†] ASD status was determined based on the PARS scores at baseline.

Fig. 3. Correlation plot of change in PARS score with percentage reduction in seizure frequency. Figure illustrates the correlation between change in PARS scores (X axis) and percentage reduction in seizure frequency (Y axis). Although, no definite correlation between change in PARS scores and seizure reductions can be drawn for overall plot, a possible linear relationship was observed in the patients with ≥ 5 points change in PARS scores.

TSC. Earlier studies have shown that the treatment with mTOR inhibitors resulted in a considerable improvement in the ASD symptoms [17,29,30]. The treatment with everolimus for ASD was also studied in a series of 6 patients with TSC, of which, 4 showed improvement in their speech and verbal response with an increase in social behavior [17]. However, these results were restricted only to the case reports and open-label studies. Patients in this study showed improvement in the social communication deficits and in restricted and repetitive behaviors. These preliminary data suggest that everolimus can improve symptoms related to ASD in patients with TSC. Of interest, overall, there seems to be no clear relationship between the improvement in seizure frequency and PARS score. On the other hand, in 5 patients who achieved an improvement of ≥ 5-point PARS score, seizure frequency was decreased with possible linear correlation with reduction of PARS score. This observation may implicate that the pathogenetic differences (i.e., different association of second hit to TSC gene) and similarities (i.e. TSC gene haploinsufficiency) between epilepsy and ASD in TSC.

The safety evaluations showed that everolimus has an acceptable safety profile in the Japanese subgroup with no new safety concerns identified. No AEs led to the permanent treatment discontinuation in the core phase of the study. Although the overall incidence of AEs tended to occur with an increased frequency in the Japanese cohort, the incidence of grade 3 or 4 treatment-related AEs appeared to be similar with the overall population [18]. The AEs reported in this study were consistent with the known safety profile of everolimus in TSC, including a report for the Japanese patients with TSC-renal angiomyolipoma [23,24,31,32]. As reported in the past, a higher rate of the incidence of stomatitis was observed in the Japanese population, suggesting the need for appropriate monitoring and management [18,33]. The seizure frequency continued to decrease in a time-dependent manner; therefore, an appropriate AE management is essential to maximize the effect of everolimus. Limited is known on the effects of everolimus on growth and sexual maturation from EXIST-3, however, the long-term exposure to everolimus from EXIST-1 study (5-years follow up), indicate that growth and sexual maturation were not impacted by the use of everolimus [34]. The long-term data from the EXIST-3 study could provide more insights on the long-term efficacy and safety of everolimus and its effects on ASD symptoms in patients with treatment-resistant seizures associated with TSC. The limitations of this study included the small number of patients excluding precise statistical analyses, limited sensitivity of the PARS scale as an indicator to evaluate the ASD severity, and limited information on prior psychopharmacotherapy for ASD.

5. Conclusions

This analysis, along with that of the previous larger study, demonstrates that the adjunctive everolimus therapy is a valuable treatment option for the Japanese patients with the treatment-refractory seizures associated with TSC. The safety profile of everolimus in this subgroup was consistent with that of the overall population. The results from this study support the positive effect of everolimus on ASD in TSC.

Acknowledgments

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