Early Treatment with Vigabatrin Does Not Decrease Focal Seizures or Improve Cognition in Tuberous Sclerosis Complex: The PREVeNT Trial

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Objective: This study was undertaken to test the hypothesis that early vigabatrin treatment in tuberous sclerosis complex (TSC) infants improves neurocognitive outcome at 24 months of age.
Methods: A phase IIb multicenter randomized double-blind placebo-controlled trial was conducted of vigabatrin at first epileptiform electroencephalogram (EEG) versus vigabatrin at seizure onset in infants with TSC. Primary outcome was Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) cognitive assessment score at 24 months. Secondary outcomes were prevalence of drug-resistant epilepsy, additional developmental outcomes, and safety of vigabatrin.
Results: Of 84 infants enrolled, 12 were screen failures, 4 went straight to open label vigabatrin, and 12 were not randomized (normal EEG throughout). Fifty-six were randomized to early vigabatrin (n = 29) or placebo (n = 27). Nineteen of 27 in the placebo arm transitioned to open label vigabatrin, with a median delay of 44 days after randomization.

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Members of the PREVeNT Study Group who contributed to the completion of this trial are available as an online Supplementary file.

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Bayley-III cognitive composite scores at 24 months were similar for participants randomized to vigabatrin or placebo. Additionally, no significant differences were found between groups in overall epilepsy incidence and drug-resistant epilepsy at 24 months, time to first seizure after randomization, and secondary developmental outcomes. Incidence of infantile spasms was lower and time to spasms after randomization was later in the vigabatrin group. Adverse events were similar across groups.

Interpretation: Preventative treatment with vigabatrin based on EEG epileptiform activity prior to seizure onset does not improve neurocognitive outcome at 24 months in TSC children, nor does it delay onset or lower the incidence of focal seizures and drug-resistant epilepsy at 24 months. Preventative vigabatrin was associated with later time to onset and lower incidence of infantile spasms.

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Tuberous sclerosis complex (TSC) is an autosomal dominant multisystem genetic disorder affecting 1 in 6,000 live births[1] arising from deficiency in proteins encoded by the *TSC1* and *TSC2* genes, which form a protein complex that plays a critical role in the regulation of the serine–threonine kinase mechanistic target of rapamycin (mTOR).[2,3] Upward of 90% of affected individuals have central nervous system involvement, with epilepsy developing in up to 80 to 90% and nearly two thirds having drug-resistant epilepsy (DRE).[4] The majority develop epilepsy in the first year of life.[4,5] In addition, developmental delays, autism spectrum disorder, and psychiatric disorders are highly prevalent and demonstrate a strong association with early onset epilepsy and severity in this population.[5–8] Diagnosis of TSC is possible prior to the development of seizures due to advances in prenatal and early infancy testing.[9,10] In infants with TSC, epileptiform activity on electroencephalography (EEG) predicts the eventual development of epilepsy.[11] As a result, there is a window of opportunity to identify infants at high risk for seizures to initiate potential antiepileptogenic treatment prior to the onset of clinical seizures. The central hypothesis of this phase IIb trial was that preventative treatment with vigabatrin started at onset of interictal epileptiform activity on surveillance EEG will improve developmental outcomes at 24 months of age and lower the risk of developing refractory seizures.[11,12] Additionally, this study reevaluates the ability of epileptiform activity on routine surveillance EEG to predict impending epilepsy in asymptomatic infants with TSC.

Subjects and Methods

Study Design

A phase IIb multicentered, randomized, double-blind, placebo-controlled clinical trial was conducted at 13 TSC clinics across the United States (Fig 1). The study enrolled 84 TSC infants who were 6 months of age or younger and met the diagnostic criteria for TSC, with no history of seizures or evidence of subclinical electrographic seizures on EEG. Participants were excluded if they were born prematurely (<30 weeks of gestation), or received any antiseizure medication (ASM) or an mTOR inhibitor. Participants were ineligible if they were enrolled or planned to enroll in an experimental behavioral intervention study. The study was approved by a central institutional review board (IRB) and each participating site's IRB, and informed consent was obtained for each participant at the time of enrollment. The first trial participant was enrolled in December 2016, and the last enrolled in March 2020.

Study Evaluations

Study visit and EEG timing were based on the infant's chronological age; after enrollment, they entered the "watchful waiting" arm of the protocol, which included serial standardized EEG (International 10–20 system for EEG electrode placement or rarely a double distance montage for neonates if <6 weeks of age, 1-hour duration with wake and sleep, sampling rate of 2,000Hz, anterior posterior bipolar and reference montages available). EEGs were done every 6 weeks until 12 months of age followed by EEGs every 3 months until 24 months of age. Two central EEG readers board certified in clinical neurophysiology read the EEGs throughout the study, using a cloud-based EEG platform for near real-time review, which allowed for randomization at the time of the study visit. A high interrater agreement of $K = 0.8$ for interictal spikes and $K = 1.0$ for seizures was established prior to study launch in 14 practice EEGs across the neonatal and infantile age range. In the case of disagreement, a third reader would adjudicate.

Participants continued in the "watchful waiting" phase of the study until the emergence of an abnormal EEG with interictal epileptiform discharges and then were randomized to the blinded-treatment phase of the study. To improve accurate and timely identification of clinical seizures, caregivers reviewed a seizure recognition video at time of study enrollment. The educational video included information and videos on febrile seizures, infantile spams, focal seizures, drop/atonic seizures, myoclonic seizures, and generalized tonic–clonic seizures (https://www.youtube.com/watch?v=kOcwOm3dZko). Caregivers were encouraged to video record any suspicious events during the study for review by the clinician site investigator. Emergence of specific, predetermined EEG biomarkers (sharps waves, [poly-]spikes), but not seizures,[11] would prompt 1:1 randomization to vigabatrin or placebo. Both vigabatrin and placebo were dispensed in identical sachets and started at 50mg/kg/day for 3 days then continued at 100mg/kg/day in a blinded fashion. Only the lead statistician of the data coordinating center, independent medical monitor, and research pharmacists at each site were unblinded during this phase of the study.

No participants had clinical seizures prior to study enrollment, and any with electrographic seizures on the baseline EEG

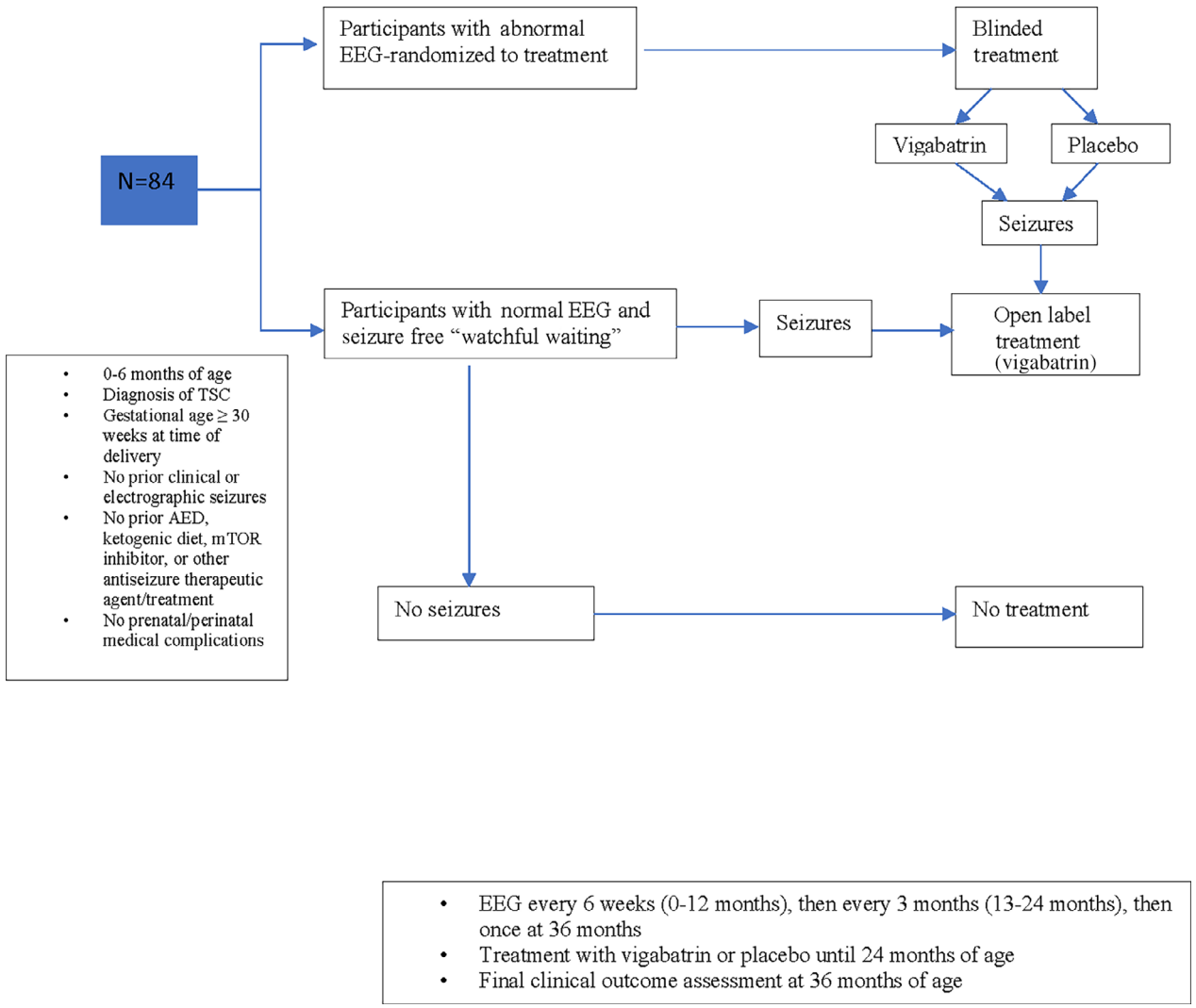

FIGURE 1: PREVeNT study design. AED = antiepileptic drug; EEG = electroencephalogram; mTOR = mechanistic target of rapamycin; TSC = tuberous sclerosis complex. [Color figure can be viewed at www.annalsofneurology.org]

were exited from the study. Participants who subsequently developed clinical and/or electrographic seizures during the "watchful waiting" period of the study but prior to developing epileptiform abnormalities on the EEG were not randomized. Instead, they immediately began treatment with open label vigabatrin (100mg/kg/day) with further optimization of ASM treatment if needed according to clinical judgment and established standards of care. In addition, participants with normal EEGs over the study duration and who experienced no clinical and/or electrographic seizures were never randomized to treatment. These additional groups continued in the study through completion at 36 months. These participants underwent identical assessments as those in the randomized groups for comparison.

Randomization occurred upon the identification of EEG abnormalities as described above during the "watchful waiting" period. Randomized infants who had their first seizure (clinical or electrographic) before 24 months followed a similar blinded 2-week transition phase to open label vigabatrin with automatic increase to a dosing target of 150mg/kg/day. Therapy could be further optimized thereafter if needed, according to the clinical judgment of the treating physician. Randomized infants who remained seizure-free at 24 months followed a similar blinded 2-week transition phase to open label vigabatrin before discontinuation of treatment.

All study participants followed a vigabatrin safety protocol consistent with the US Food and Drug Administration Risk Evaluation and Mitigation Strategy guidelines that included serial ophthalmologic examination assessment for potential vigabatrin-related changes.

Primary end points assessed development and epilepsy-related outcomes at 24 months. In addition, each study participant was followed until 36 months of age to assess for a single additional time point 12 months after completion of the study intervention phase.

Clinical psychologists remained blinded throughout the study to the participants' randomization arm, seizure control, and concomitant medication(s) while completing assessments. In-person assessments included the Bayley Scales of Infant and

Toddler Development, Third Edition (Bayley-III) and Vineland-II (Survey Interview Form), at 6, 12, 24, and 36 months of age. At the 6 months of age visit, the Bayley-III Social, Emotional, and Adaptive Behavior Parent questionnaire was completed in place of the Bayley-III cognitive assessment along with the Vineland-II. Modification to the developmental assessment protocol was necessitated during the COVID-19 pandemic to overcome restrictions to patient travel and/or the ability to conduct face-to-face evaluations at many sites for an extended period. These pandemic-related modifications included substitution of the Autism Diagnostic Observation Schedule (ADOS)-2 with the Brief Observation of Symptoms of Autism (BOSA) and Autism Diagnostic Interview-Revised.

A summary of the neurodevelopmental assessment was provided to the site investigators after each assessment. If the participant showed evidence of developmental delay, they were referred for early intervention services, often including physical, occupational, and speech therapy, and applied behavioral analysis, as indicated by the testing results.

Outcomes/Statistical Analysis

The sample size for this trial was derived from the ability to demonstrate a reduction or delay in seizures with a specified power to assess differences in cognitive scores on the Bayley-III. We utilized data from a prior study, which assessed the presence over time of an epileptiform EEG biomarker in TSC infants for our sample size calculation.[11] The prior study had 40 participants enrolled; 15 never developed an abnormal EEG or seizures, and 1 participant dropped out. Thus, we expected 37.5% not to develop epileptiform activity on the EEG and never to be randomized. Overall, we needed to recruit between 75 and 80 participants so that approximately 48 patients would develop an abnormal epileptiform EEG and be randomized with 24 to 25 participants per group.

In the placebo arm, we expected 22 of 24 (0.917) participants would eventually develop seizures. In the vigabatrin arm, if it mirrored the previous study, only 5 of 24 would have medically refractory seizures (0.208).[11] A Fisher exact test with a 0.05 2-sided significance level would have >95% power to detect the difference between the placebo proportion, p_1, of 0.917 and a vigabatrin proportion, p_2, of 0.208 when the sample size in each group was 24. If the vigabatrin treatment was 50% less effective than the prior data, 10 of 24 (0.417) of the vigabatrin arm would experience seizures, still providing >90% power to detect an antiseizure treatment effect.

The primary outcome measure, the Bayley-III cognitive composite standard scores, and the Vineland-II adaptive behavior composite standard scores were compared between the vigabatrin and placebo treatment groups at 12 and 24 months of age. A general linear model was used with repeated measures controlling for participant sex and whether the participant was randomized before or after 7 months of age. In these models, time was treated as a categorical variable and the covariance among cognitive scores was assumed to be unstructured. A contrast statement was used to test whether the difference in mean cognitive score differed between the two randomized groups, with a p value of 0.05 being considered significant for the primary outcome measure. A general linear multivariate model was used to simultaneously compare the mean Bayley-III language and motor composite standard scores, as well as the Vineland-II communication, daily living skills, socialization, and motor skills standard scores controlling for patient sex and whether the patient was randomized before or after 7 months of age. An unadjusted p value of 0.05 was considered significant for the Hotelling-Lawley Trace test for Bayley-III and Vineland-II standard scores at 12 and 24 months. We originally intended to include analysis of the autism risk between the randomized groups based on the ADOS scores at 24 and 36 months of age. However, protocol modifications were implemented for the autism assessments as COVID-19 precautions. This resulted in developmental evaluations with a mixture of ADOS and the BOSA across sites. The results of this analysis will be reported separately, along with the 24- to 36-month open label portion of the study.

The proportions of participants who developed seizures and DRE,[13] as well as the degree to which epilepsy was controlled, by 24 months of age in the vigabatrin and placebo groups were compared using a chi-squared test of independence or Fisher exact test. Time from randomization to first seizure of any type was compared between vigabatrin and placebo groups. A Cox proportional hazards model controlling for patient sex and whether a patient was randomized before or after 7 months of age was used. This decision was based on the results of the Wu et al publication.[11] In this study, the first epileptiform discharges appeared at the average age of 4.5 $\pm$ 4.0 (standard deviation) months, with a median age of 4.0 months. The age at onset of any seizure type with antecedent epileptiform activity averaged 7.5 months $\pm$ 4.4 months, with a median age of 6.0 months. As a result, 7 months was selected to control the randomization in an effort to balance those infants who developed infantile spasms and/or focal seizures early versus infants who would develop seizures later, possibly after 12 months of age.

Censoring occurred at the date of last study visit, and a p value of 0.05 with the Wald test was considered significant. Kaplan–Meier plots were generated to visually inspect the time to first seizure of any type, time to first focal seizure, and time to first infantile spasm from randomization.

The proportion of participants developing treatment associated adverse events (AEs) at any point during the study was compared between vigabatrin and placebo groups using Fisher exact test. Additionally, the time from enrollment to first adverse or serious AE was compared between vigabatrin and placebo groups using a log-rank test with censoring occurring at the date of last study visit.

The study was predicated on the EEG being sensitive and specific in identifying the biomarker predictive of future onset of seizures, and randomization occurred at the incidence of this biomarker. We assessed the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), similar to the analysis of the prior biomarker study used in planning the PREVeNT study design.[11]

Lastly, as many participants in both randomized groups received vigabatrin, we provided measurements of the time

between randomization and 24 months of age that participants received vigabatrin. The length of time during which randomized patients received identical treatments over the course of the study.

Results

Eighty-four participants were enrolled, with 12 meeting exclusion criteria postenrollment but prior to randomization; the most common reasons were evidence of clinical or electrographic seizures on their baseline EEG, and exposure to an mTOR inhibitor prenatally. Seventy-two continued in the study through 24 months of age (Fig 2). Participants were similar with respect to sex and age at randomization (Table 1). Of the 56 participants randomized, 1 participant withdrew at the parents' request. All the randomized participants completing the 24-month visit had at least one abnormal epileptiform EEG, of whom 39 (71%) went on to develop clinical or electrographic seizures (Table 2). Conversely, of the 16 individuals with normal EEGs, 1 withdrew before 24 months, leaving 15 participants, of whom 4 (27%) developed seizures by 24 months of age. As such, at age 24 months the EEG biomarker had a sensitivity of 0.826, (true positives (TP) randomized to placebo/TP + false negatives = 19/19 + 4 participants straight to open label = 19/23 = 0.826). The specificity was 0.611, given 11 true negatives (TN)/TN + false positives (7) = 11/18 = 0.611. Thus, the positive predictive value of the EEG biomarker was 0.731 (19/19 + 7 = 19/26), and the negative predictive value was 0.733 (11/11 + 4 = 11/15). Of those with seizures, 20 of 29 (69%) had been randomized to early treatment with vigabatrin versus 19 of 26 (73%) randomized to early treatment with placebo. All participants randomized to the vigabatrin treatment group started vigabatrin on the day of randomization (0 days) per the protocol. Participants in the placebo group were transitioned to open label vigabatrin in blinded fashion following the occurrence of a first clinical or electrographic seizure. In this latter group, seizure onset and vigabatrin treatment initiation was between 3 to 610 days (median = 44, interquartile range = 21–90) after randomization. As such, participants in both groups were receiving the vigabatrin treatment for a large portion of the randomized study period by the time they had reached the age of 24 months.

No differences were found between participants randomized to vigabatrin or placebo with respect to Bayley-III cognitive composite or Vineland-II adaptive behavior composite standard scores at 12 and 24 months of age. Similarly, no differences were found between either randomized group with respect to the additional Bayley-III and Vineland-II standard scores when jointly tested in multivariate analyses (Table 3). However, those randomized before 7 months of age had lower cognitive measures irrespective of treatment group.

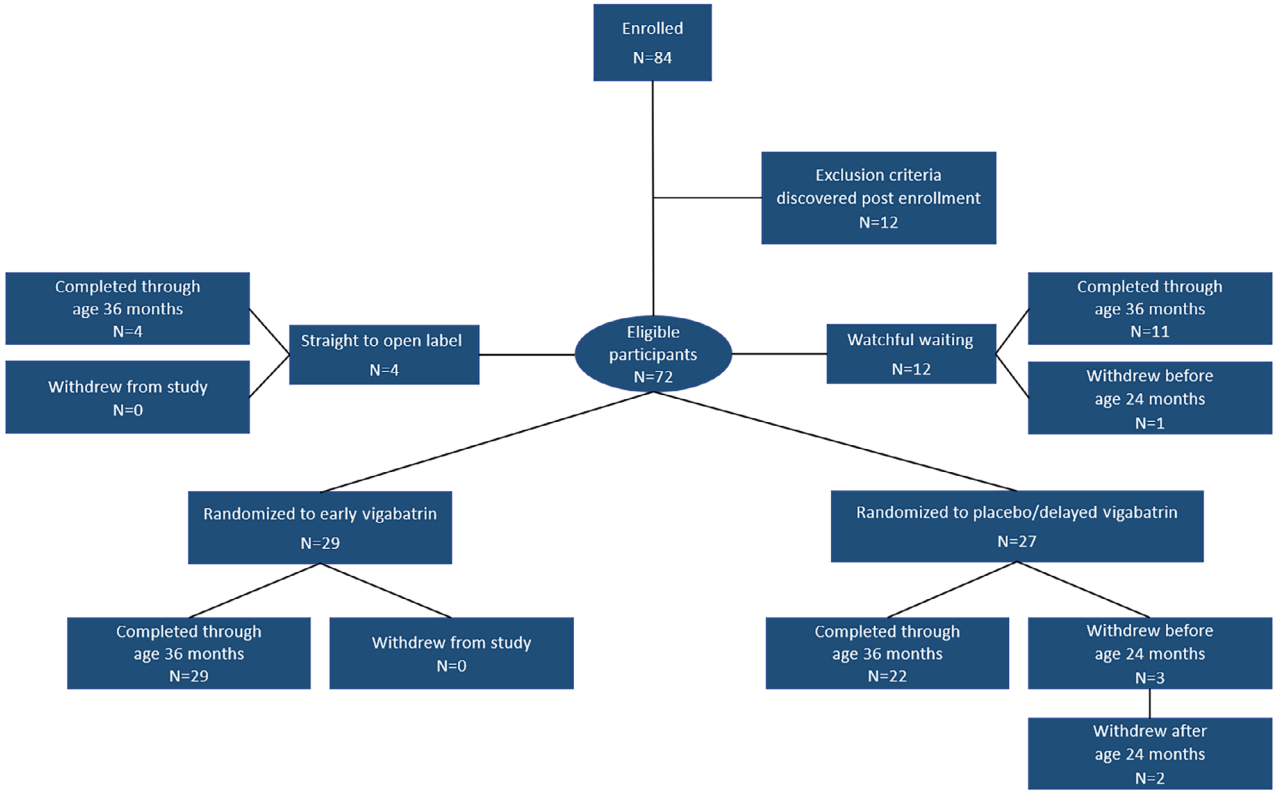

FIGURE 2: CONSORT diagram: PREVeNT participants across study arms. [Color figure can be viewed at www.annalsofneurology.org]

TABLE 1. Patient Demographics

Characteristic	Vigabatrin, n = 29	Placebo, n = 27	Straight to Open Label, n = 4	Watchful Waiting, n = 12
Sex, n (%)				
Male	13 (45%)	12 (44%)	2 (25%)	9 (75%)
Female	16 (55%)	15 (56%)	2 (25%)	3 (25%)
Genotyping, n (%)				
TSC1	1 (3%)	3 (11%)	0 (0%)	4 (33%)
TSC2	28 (97%)	18 (67%)	3 (75%)	6 (50%)
VUS or NMI	0 (0%)	4 (15%)	1 (25%)	2 (17%)
Not performed	0 (0%)	2 (7%)	0 (0%)	0 (0%)
Enrollment age, mo, mean (SD)	2.3 (1.4)	1.9 (1.3)	1.4 (0.6)	4.4 (2.1)
Randomization age cohort, n (%)				
Randomized before age 7 months	24 (83%)	19 (70%)	—	—
Randomized after age 7 months	5 (17%)	8 (30%)	—	—
Randomization age, mo, mean (SD)	5.1 (2.7)	5.1 (3.3)	—	—

Abbreviations: NMI = no mutation identified; SD = standard deviation; VUS = variant of unknown significance.

No differences were found between participants randomized to vigabatrin or placebo with respect to the proportion developing any type of seizures or DRE at 24 months (see Table 2). Similarly, no difference was found in time from randomization to first seizure of any type (Cox proportional hazard ratio = 0.593, $p = 0.1174$). Focal seizures were experienced by 38 (88%), and infantile spasms (21, 49%) were the most common seizure type reported. Time to first focal seizure from randomization was similar between treatment groups, whereas those randomized to vigabatrin had later onset and lower incidence of infantile spasms than those randomized to placebo (hazard ratio = 0.263 with 95% confidence interval = 0.097–0.710; Fig 3). The proportion of medically refractory spasms did not differ between treatment groups. These comparisons, however, were not specified a priori, and thus no statistical hypothesis testing was performed.

Rare AEs were reported in both groups. In total, 13 AEs were reported that were possibly related to vigabatrin, including 1 participant with ophthalmologic changes and 4 participants who had changes on brain magnetic resonance imaging (MRI). Only 1 participant, in the vigabatrin treatment group, stopped study drug after 12 months of age. This was a result of changes in the eye examination compared to the baseline examination. This participant was seizure-free and remained in the study, and the randomization was not broken. No difference between groups was found in the time to first AE from enrollment (log-rank $p = 0.0765$), and neither group appeared to be at higher risk for treatment-associated AEs than those on open label vigabatrin after experiencing a seizure.

Discussion

The PREVeNT Trial is the first phase IIb double-blind placebo-controlled multicenter trial of preventative vigabatrin in infants with TSC. The idea of preventative epilepsy therapy has been of great interest in the TSC community since the initial results were published by Jozwiak et al. and Cusmai et al in 2011,[14,15] and most recently the EPISTOP trial in 2021.[12] TSC infants are at high risk for developing DRE and epilepsy is the major identified risk factor for neurodevelopmental delay and autism spectrum disorder[4–8]; therefore, the primary outcome measure of PREVeNT was the Bayley-III cognitive assessment score at 24 months. The results of this trial showed no significant differences in vigabatrin versus

TABLE 2. Epileptic and Safety Outcomes by Study Arm

	Vigabatrin, n = 29	Placebo, n = 27	Straight to Open Label, n = 4	Watchful Waiting, n = 12	Nonmissing Vigabatrin vs Placebo, *p*
Seizures by age 24 months, n (%)					0.7375
Yes	20 (69%)	19 (70%)	4 (100%)	0 (0%)	
No	9 (31%)	7 (26%)	0 (0%)	11 (92%)	
Missing [withdrew]	0 (0%)	1 (4%)	0 (0%)	1 (8%)	
Drug-resistant epilepsy at 24 months, n (%)					**0.4653**
Yes	14 (48%)	14 (52%)	2 (50%)	0 (0%)	
No	15 (52%)	10 (37%)	2 (50%)	11 (92%)	
Missing [withdrew]	0 (0%)	3 (11%)	0 (0%)	1 (8%)	
Epilepsy control at 24 months, n (%)					**0.0807**[a]
Controlled	11 (38%)	5 (19%)	2 (50%)	0 (0%)	
Refractory	9 (28%)	12 (22%)	1 (25%)	0 (0%)	
Missing [withdrew]	0 (0%)	3 (11%)	0 (0%)	1 (8%)	
Patients experiencing adverse or serious adverse events, n (%)					**0.0626**[a]
None	27 (93%)	16 (56%)	2 (50%)	11 (92%)	
At least 1 event	2 (7%)	6 (26%)	2 (50%)	0 (0%)	
Missing [withdrew]	0 (0%)	2 (19%)	0 (0%)	1 (8%)	

[a]Fisher"s exact test used instead of χ^2 test of independence due to small cell counts.

Note: Two patients withdrew prior to 24-month visit and before having seizures. Four patients withdrew prior to 24-month visit, with epilepsy resistance or control undeterminable. Three patients withdrew prior to 24-month visit and before having any treatment-related adverse events. Bold type indicates statistical significance.

placebo on the Bayley-III cognitive scales or Vineland-II adaptive behavioral standard scores (Fig 4). Our results are similar to those of the EPISTOP trial, which reported that early treatment with vigabatrin initiated at time of first abnormal EEG did not significantly differ from traditional treatment initiated at time of seizure onset with regard to neurodevelopmental outcome at 24 months of age.[12,16]

The PREVeNT study found that early vigabatrin treatment delayed the onset and reduced the overall prevalence of infantile spasms in TSC infants (see Fig 3C). However, the seizure prevention was not seen for other seizure types, including focal seizures (see Fig 3B), that are highly prevalent in this population.[4,11] PREVeNT, similarly to EPISTOP, reported a reduced incidence of infantile spasms up to 24 months of age.[12] The studies differ, however, in other epilepsy-related outcomes at 24 months. We found no difference in DRE at 24 months of age (48% in TSC infants randomized to early vigabatrin vs 52% in TSC infants randomized to placebo; see Table 2). In contrast, EPISTOP reported a reduction in DRE in the early vigabatrin group (28%) versus conventional treatment (64%).[12] Eligibility criteria, EEG monitoring, and follow-up procedures were similar between the two studies; however, key differences include the double-blind, placebo-controlled design of PREVeNT; in contrast, EPISTOP treatment was open label, with randomization or study site determining whether vigabatrin therapy was started at time of EEG abnormalities versus waiting until onset of clinical seizures. A centralized EEG review process determined randomization in PREVeNT versus local EEG review and randomization in EPISTOP. Additional investigation is needed to understand what was responsible for the differences in seizure outcomes between the studies.

TABLE 3. Mean (SD) of Neurocognitive Outcomes by Study Arm

Outcome	Early, n = 29	Placebo, n = 27	Straight to Open Label, n = 4	Watchful Waiting, n = 12
Bayley-III cognitive composite score				
12 months	89.8 (12.7)	87.3 (18.2)	96.3 (19.3)	102.5 (12.2)
24 months	80.9 (15.6)	83.9 (17.3)	85.0 (21.6)	97.3 (16.9)
Bayley-III sum language composite score				
12 months	82.4 (14.7)	79.9 (17.0)	85.5 (15.0)	89.7 (14.5)
24 months	71.4 (17.9)	78.0 (17.6)	80.5 (16.6)	90.5 (17.5)
Bayley-III sum motor composite score				
12 months	81.4 (11.8)	81.0 (17.7)	85.8 (3.8)	96.8 (17.4)
24 months	76.0 (17.2)	81.5 (17.0)	79.8 (13.0)	91.6 (12.5)
Vineland-II adaptive behavior composite score				
12 months	86.0 (9.8)	86.9 (13.1)	86.3 (7.0)	97.8 (14.2)
24 months	84.9 (11.9)	90.6 (14.3)	85.3 (10.7)	96.3 (5.8)
Vineland-II communication standard score				
12 months	86.9 (12.5)	88.7 (14.4)	86.8 (10.4)	92.3 (13.2)
24 months	83.6 (12.2)	89.6 (14.9)	89.5 (15.2)	96.1 (6.2)
Vineland-II daily living skills standard score				
12 months	88.2 (11.2)	89.6 (11.6)	87.5 (10.3)	98.3 (13.6)
24 months	87.3 (11.5)	91.9 (15.4)	90.5 (14.2)	99.2 (7.6)
Vineland-II socialization standard score				
12 months	93.9 (8.9)	92.7 (13.2)	92.5 (3.0)	99.8 (9.8)
24 months	88.3 (11.2)	94.7 (12.6)	87.3 (7.5)	94.7 (5.1)
Vineland-II motor skills standard score				
12 months	84.6 (14.7)	82.4 (16.0)	87.8 (11.4)	99.9 (18.5)
24 months	87.8 (13.7)	91.9 (12.9)	84.0 (4.9)	99.0 (7.5)

Note: For reference, Bayley-III composite and Vineland-II standard scores have mean of 100 and SD of 15 within the general population.
Abbreviations: Bayley-III = Bayley Scales of Infant and Toddler Development, Third Edition; SD = standard deviation.

The EEG biomarker in the PREVeNT study had similar ability as in a prior study, and was a moderately strong predictor of impending seizures,[11] with a PPV and NPV of >0.7. Seven participants in the placebo arm had epileptiform activity on 1 or more EEGs and did not go on to have seizures, and 4 participants developed clinical seizures with only normal EEGs. Thus, epileptiform activity on serial EEGs can anticipate impending epilepsy in most but not all infants with TSC and should precipitate discussion about the timing and potential benefit of ASM usage prior to the development of epilepsy. Development of guidelines for EEG surveillance that utilize the PREVeNT and EPISTOP results are needed and will be helpful for clinicians. Further analysis of the serial EEGs

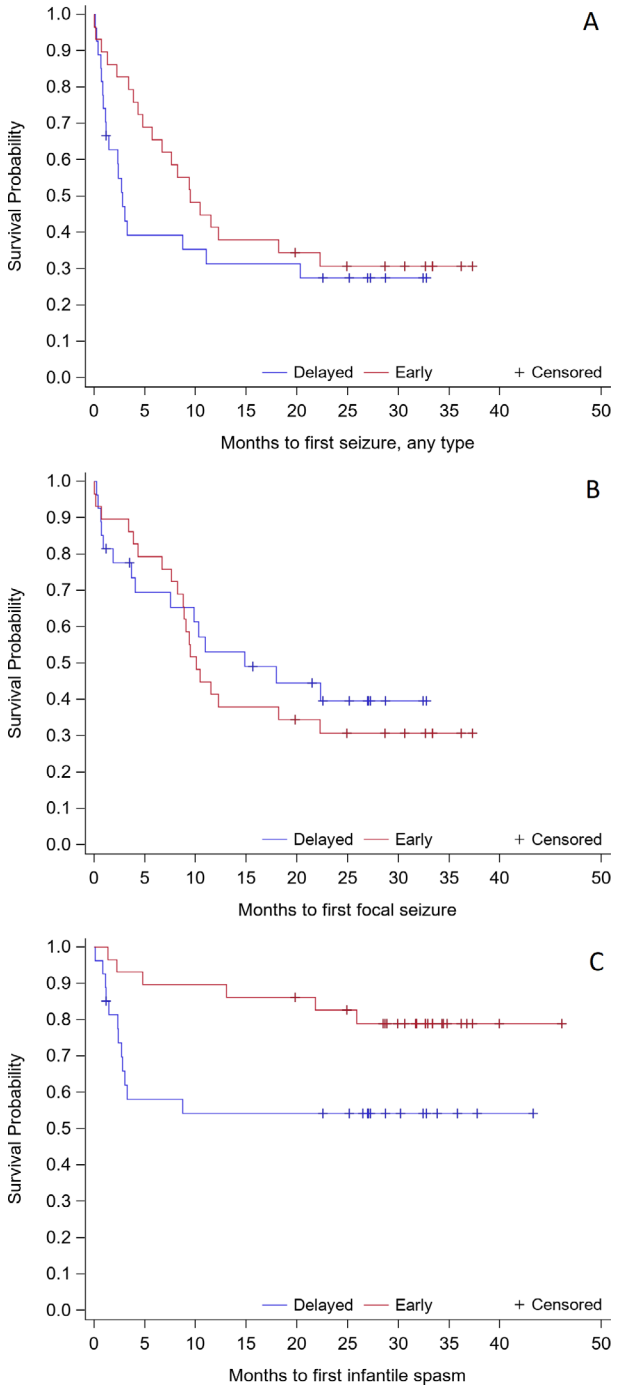

FIGURE 3: Time from randomization to first (A) any type of seizure, (B) focal seizure, (C) infantile spasms. [Color figure can be viewed at www.annalsofneurology.org]

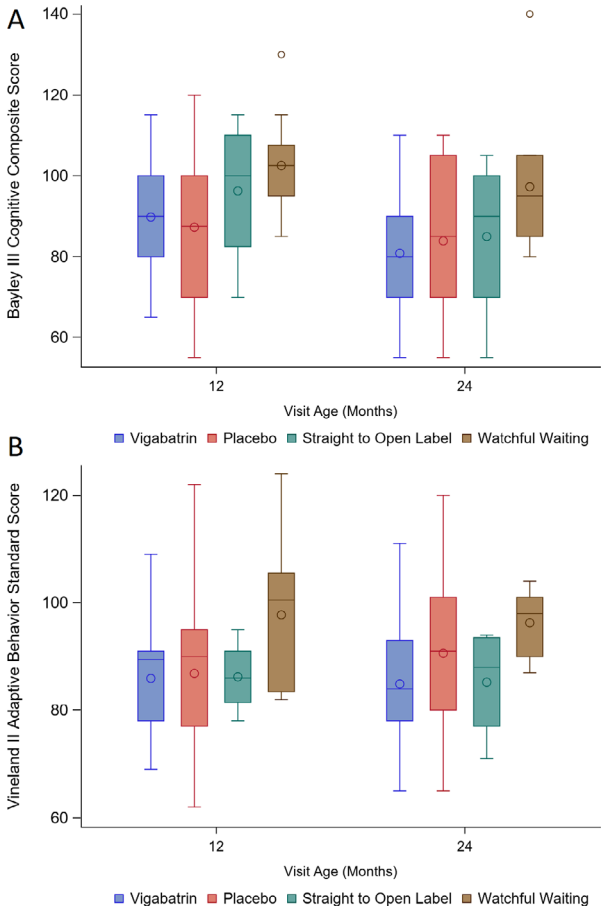

FIGURE 4: Developmental outcome scores for the (A) Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) cognitive composite standard scores and (B) Vineland-II standard scores. [Color figure can be viewed at www.annalsofneurology.org]

may identify features beyond interictal epileptiform activity that can predict epilepsy, specific seizure types, risk for DRE, or adverse neurodevelopmental outcomes.

Vigabatrin increases extracellular γ-aminobutyric acid (GABA)[17]; with minimal rationale that increased GABA would be procognitive, the proposed improvement in neurocognition was based on the efficacy of vigabatrin in preventing infantile spasms in TSC, as proposed in the studies by Jozwiak.[14,15] The presence of epileptiform activity and seizures earlier in development might have greater impact on neurocognitive outcomes as compared to later ages. Here, we show that the impact of vigabatrin as a preventative therapy was limited to infantile spasms, with no impact on the onset of focal seizure in this population. Furthermore, our data demonstrate that delay or prevention of infantile spasms had no measurable effect on cognitive outcomes at 2 years of life. This latter observation is contrary to past retrospective studies in TSC cohorts,[5,15] but consistent with more recent observations from the prospective EPISTOP[16] and TSC Autism Centers of Excellence Research Network[8] natural history study, both of which studied TSC infants. These results suggest that epilepsy prevention with vigabatrin including infantile spasms is not sufficient to prevent long-term neurocognitive delays. Future studies targeting epilepsy prevention via

TABLE 4. Neurocognitive Outcomes

Repeated Measure GLM Estimated Difference in Bayley-III Cognitive Composite Score Population Means between Early and Delayed Randomized Vigabatrin Groups		**Wald Test *p*-value**
12 months of age, n = 51	3.79 (Vigabatrin–placebo)	0.3389
24 months of age, n = 52	−0.66 (vigabatrin–placebo)	0.8681
Randomized at age <7 or >7 years	−13.42 (<7–>7)	0.0024
Male vs female	−11.74 (male–female)	0.0017
Repeated Measure GLM Estimated Difference in Vineland-II Adaptive Behavior Composite Score Population Means between Early and Delayed Randomized Vigabatrin Groups		**Wald test *p*-value**
12 months of age, n = 53	0.28 (vigabatrin–placebo)	0.9248
24 months of age, n = 53	−4.02 (vigabatrin–placebo)	0.2198
Male vs female	−5.74 (male–female)	0.0368
Randomized at age <7 or >7 years	−10.12 (<7–>7)	0.0023
Multivariate Test of Difference in Mean Bayley-III Composite Scores (language and motor) between Early and Delayed Randomized Vigabatrin Groups		**HLT *p*-value**
12 months, n = 49		0.7744
24 months, n = 51		0.5037
Multivariate Test of Difference in Mean Vineland-II Standard Scores (communication, daily living skills, socialization, and motor skills) between Early and Delayed Randomized Vigabatrin Groups		**HLT *p*-value**
12 months, n = 53		0.5949
24 months, n = 53		0.4888

Abbreviations: Bayley-III = Bayley Scales of Infant and Toddler Development, Third Edition; GLM = generalized linear model; HLT = hotelling Lawley trace test.

mTOR inhibition, underlying epileptogenesis in TSC, and other developmental epilepsies are ongoing.[18–20] Currently, a clinical trial, TSC-STEPS, is underway in the United States and Australia, evaluating the benefit of early treatment with sirolimus, an mTOR inhibitor, before 6 months of age in TSC infants (clinicaltrials.gov/NCT05104983).

It is important to recognize that participants with seizures prior to or at the time of enrollment were excluded. All participants had close clinical follow-up and were referred to early intervention services if there were developmental concerns. The additional interventions and close clinical follow-up provided by the study would have been equally distributed due to the 1:1 randomization placebo-controlled design, and blinding of study participants and investigators.

PREVeNT study participants initially treated with placebo were quickly transitioned to open label vigabatrin at onset of seizures. Vigabatrin is a first-line therapy for young TSC patients at high risk for infantile spams and approved for the treatment of focal seizures in older patients.[1] Most participants randomized to placebo had onset of their seizures soon after randomization and moved to open label vigabatrin after a median of only 44 days. Thus, both randomized groups were relatively similar in terms of cumulative vigabatrin exposure over the study duration. It is possible that the vigabatrin exposure helped the cognitive outcomes of most of the randomized participants. Both groups scored in the low normal range at 12 and 24 months of age, similar to previously reported retrospective studies.[21,22] Perhaps timing of vigabatrin treatment initiation is less important than overall exposure to vigabatrin at these early ages in TSC infants.

Genotype and probably other clinical features influence seizure risk and cognitive and developmental

outcomes in TSC.[23] Epilepsy is more common and severe in individuals with *TSC2* versus those with *TSC1* mutations and variant of unknown significance/no mutation identified (VUS/NMI). By chance, *TSC1* and *TSC2* mutations and VUS/NMI were not evenly distributed across vigabatrin and placebo treatment groups (see Table 1). *TSC1* mutations and NMI/VUS were enriched in the placebo group versus the vigabatrin treatment group. The placebo group may have had milder underlying TSC disease burden, possibly impacting our ability to detect the effect of early vigabatrin treatment. Interestingly, participants randomized before 7 months of age had worse developmental outcomes irrespective of their treatment arm (Table 4). Earlier randomization due to an epileptiform EEG is consistent with risk for developing seizures at an earlier age[4,8,24] and is consistent with prior findings that earlier clinical seizure onset correlates with poor long-term cognitive and developmental outcome.[8] It is likely that multiple factors (genetic, molecular, electrophysiologic, and structural) contribute to seizure risk, DRE, and a higher risk for poor cognitive outcomes in early TSC. It will be important to incorporate this information into development of an epilepsy and neurocognitive risk stratification for treatment with vigabatrin and/or mTOR inhibition in TSC.

Finally, although there were no outcome differences in the primary end point, the Bayley-III, additional outcome data from the study need to be analyzed to understand the full impact of early treatment. We used norm-referenced standard scores as the primary outcome for assessing development, but these may mask changes in skills within and across individuals. The scores represent a child's performance and/or skills relative to age-matched peers but are less sensitive to changes in skills within an individual across time points. Further correlation with quantitative electrophysiological measures and structural MRI may also provide additional insight into specific subpopulations with more favorable response to early vigabatrin.

Conclusions

The PREVeNT clinical trial found that treating an initial epileptiform EEG prior to seizure onset with vigabatrin delayed and decreased the overall prevalence of infantile spasms in TSC infants. However, time to first seizure of any type was similar between placebo and vigabatrin groups, and preventative treatment with vigabatrin based on EEG epileptiform activity did not improve cognition (measured by the Bayley-III) or adaptive behaviors (measured by the Vineland-II) at 24 months of age. PREVeNT confirmed the utility of serial EEG during infancy in TSC to monitor for risk of developing seizures and identified a narrow window of opportunity for intervention between EEG changes and onset of clinical seizures. Vigabatrin therapy was overall well tolerated, and there were only a small number of vigabatrin-related AEs reported between 0 and 24 months of age.

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Author Contributions

F.B., E.M.B., J.M.P., B.E.P., S.O., G.C., and D.A.K. contributed to the conception and design of the study. M.D.F., R.R., E.M.B., J.M.P., B.E.P., T.O.M., S.O., K. S.T., S.C.R., W.M.M., M.K.K., H.A.N., K.W., D.A.N., M.W., J.L.K., K.P., G.C., and D.A.K. contributed to the acquisition and analysis of data. E.M.B., J.M.P., B.E.P., T.O.M., S.O., M.W., G.C., D.A.K., and M.S. contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest

Nothing to report.

Data Availability Statement

All data generated during the performance of the project will be fully disseminated to the research community through presentations at national/international meetings as well as publication of results and interpretation in peer-reviewed articles. Data collected via this trial (in deidentified format) will be available to interested researchers for secondary analyses after trial completion. In compliance with the NIH guidelines, a complete, cleaned, and deidentified dataset and any supporting documentation will be submitted to the NINDS Office of Clinical Research within 1 year of the primary

publication or within 18 months of the last study visit of the last subject, whichever occurs first.

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