A randomized controlled trial of everolimus for neurocognitive symptoms in PTEN hamartoma tumor syndrome

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Abstract

PTEN hamartoma tumor syndrome (PHTS) is a complex neurodevelopmental disorder characterized by mechanistic target of rapamycin (mTOR) overactivity. Limited data suggest that mTOR inhibitors may be therapeutic. No placebo-controlled studies have examined mTOR inhibition on cognition and behavior in humans with PHTS with/without autism. We conducted a 6-month phase II, randomized, double-blinded, placebo-controlled trial to examine the safety profile and efficacy of everolimus (4.5 mg/m^2) in individuals (5–45 years) with PHTS. We measured several cognitive and behavioral outcomes, and electroencephalography (EEG) biomarkers. The primary endpoint was a neurocognitive composite derived from Stanford Binet-5 (SB-5) nonverbal working memory score, SB-5 verbal working memory, Conners' Continuous Performance Test hit reaction time and Purdue Pegboard Test score. Forty-six participants underwent 1:1 randomization: $n = 24$ (everolimus) and $n = 22$ (placebo). Gastrointestinal adverse events were more common in the everolimus group ($P < 0.001$). Changes in the primary endpoint between groups from baseline to Month 6 were not apparent (Cohen's d $= -0.10$, $P = 0.518$). However, several measures were associated with modest effect sizes (≥ 0.2) in the direction of improvement, including measures of nonverbal IQ, verbal learning, autism symptoms, motor skills, adaptive behavior and global improvement. There was a significant difference in EEG central alpha power ($P = 0.049$) and central beta power ($P = 0.039$) 6 months after everolimus treatment. Everolimus is well tolerated in PHTS; adverse events were similar to previous reports. The primary efficacy endpoint did not reveal improvement. Several secondary efficacy endpoints moved in the direction of improvement. EEG measurements indicate target engagement following 6 months of daily oral everolimus. Trial Registration Information: ClinicalTrials.gov NCT02991807 Classification of Evidence: I.

Introduction

PTEN hamartoma tumor syndrome (PHTS) is a rare genetic disorder caused by germline pathogenic variant in *PTEN*, resulting in numerous neurodevelopmental and variable systemic features. Neurodevelopmental abnormalities include macrocephaly, intellectual disability and autism spectrum disorder (ASD) (1). *PTEN* is recognized as one of the most common predisposition genes for ASD (2). More specifically, PHTS is associated with variable impairment of frontal lobe systems, such as attention, impulsivity, reaction time, processing speed and motor coordination (3); however, other brain regions, such as the arcuate fasciculus, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus and uncinate fasciculus, may be affected (4). Systemic challenges encompass increased cancer risk, gastrointestinal (GI) polyps, vascular abnormalities and specific dermatological findings. Altogether, these symptoms can represent a significant source of distress for affected individuals and their caregivers (1).

The pathogenesis of PHTS is multifactorial, but one key and canonical component is upregulated mechanistic target of rapamycin (mTOR) signaling. In PHTS, loss of *PTEN* function leads to disinhibition of the PI3K (phosphatidylinositol-4,5-bisphosphate 3-kinase)-AKT–mTOR pathway (5), culminating in overactivation of mTOR signaling, implicated in cell growth, cell differentiation (6) and neuronal/synaptic development (7,8). mTOR overactivity leads to enhanced cell growth, underlying the increased risk for cancer and abnormal tissue growth seen in PHTS (9). Imbalance of mTOR activity also disrupts neural development, underlying the spectrum of synaptic and myelination abnormalities seen within the disorder (10).

Treatment of neurobehavioral symptoms in PHTS is supportive, but data from preclinical studies, case reports and pilot studies have suggested that mTOR inhibitors could be therapeutic. The mTOR inhibitor rapamycin rescued neuronal hypertrophy and autistic-like behavioral abnormalities in mice with deletion of *Pten* in postmitotic cortical and hippocampal neurons (11). In knockout mice with inactivated expression of *Pten* in cerebellar and dentate gyrus neurons, treatment with the mTOR inhibitor CCI-779 improved outcomes (seizures, death) and rescued/prevented cellular abnormalities (neuronal hypertrophy), particularly in the dentate gyrus (12). Small case reports have described variable impact of the mTOR inhibitor sirolimus on life-threatening somatic overgrowth (13), painful and functionally impairing vascular malformation (14) and thymus hyperplasia and lipomatosis (15) in individuals with PHTS. A single-arm interventional clinical trial on adults with PHTS (Cowden syndrome subtype), treated with 56 days of sirolimus, highlighted improvements in baseline symptoms, skin lesions, gastrointestinal polyps and cerebellar function (16). In a clinical trial assessing safety and response to sirolimus in individuals with complex vascular malformations, four out of four participants with PHTS who were still enrolled and could be evaluated by the end of the trial exhibited at least a partial response (17). No placebo-controlled studies have examined the safety and/or efficacy of mTOR inhibition on cognition and behavior in humans with PHTS.

To address this gap, we conducted a phase 2, double-blinded, placebo-controlled trial evaluating the safety and efficacy of everolimus, an mTOR inhibitor, for PHTS, targeting a variety of cognitive and behavioral endpoints. Although everolimus (a sirolimus analog) has received Food and Drug Administration (FDA) approval for specific indications in tuberous sclerosis complex (TSC) (18), another disorder of mTOR overactivity, its safety and efficacy remains untested in PHTS. In the current trial, we chose a wide array of cognitive and behavioral endpoints, as well as electroencephalography (EEG) measures, to account for the neurodevelopmental heterogeneity and complexity encompassed by PHTS.

Results
Enrollment

Out of the 55 participants who were enrolled, nine were ineligible for randomization after screening; specifically, $n = 4$ had nonverbal intelligence quotient (IQ) (NVIQ) < 50; $n = 2$ performed higher than one standard deviation below the mean on all three primary outcome measures (as defined in the Materials and Methods); $n = 1$ was unable to swallow pills; $n = 1$ withdrew consent and $n = 1$ was unable to adjust concomitant medication (due to plan to start new stimulant for treatment of severe attention deficit hyperactivity disorder). One of the two participants who performed higher than one standard deviation below the mean on all three primary outcome measures was errantly allowed to participate in the trial; we excluded this participant in our analysis. In total, 46 participants underwent 1:1 randomization and were included in our analysis: $n = 24$ in the treatment group and $n = 22$ in the placebo group following the intention-to-treat (ITT) principle (Fig. 1).

Demographics and baseline clinical characteristics

The treatment and placebo groups showed no significant difference in age ($P = 0.592$), sex ($P = 0.331$), race ($P = 1.000$) or any of the primary growth parameters/vital signs (all $P > 0.244$) (Table 1). Eight participants in the placebo group and six participants in the treatment group had ASD ($P = 0.403$). The full-scale IQs (FSIQs) of the two groups were comparable ($P = 0.687$).

Safety of everolimus

Over 6 months of treatment, dropout rates were 9.1% for the placebo group and 12.5% for the everolimus group (Table 2). There were three dropout categories: dropout due to participant/parent request, dropout due to investigator request and dropout due to other requests. In the everolimus group, there were two participants who dropped out due to participant/parent request: one request was due to neutropenia and thrombocytopenia occurring before the Month 3 visit, and the other at Month 1 due to repeated instances of stomatitis. Included in the dropout due to other requests category was an investigator decision not to dose medication at baseline given safety concerns related to drug administration compliance. There was no significant difference in dropout rates among these three dropout categories ($P = 1.000$). The trial was completed during the COVID-19 pandemic, and all patients/families in the double-blind phase of the trial elected to continue their participation.

Ninety-four adverse events (AEs) occurred. Thirteen participants in the placebo group (59.0%) and 21 participants in the everolimus group (87.5%) reported having an AE. The number of participants who had an AE was significantly related to treatment arm ($P = 0.044$).

Among all participants, 60.9% reported at least one Grade 1 AE, and 34.8% reported at least one Grade 2 AE. All participants who experienced Grade 3 AEs were in the
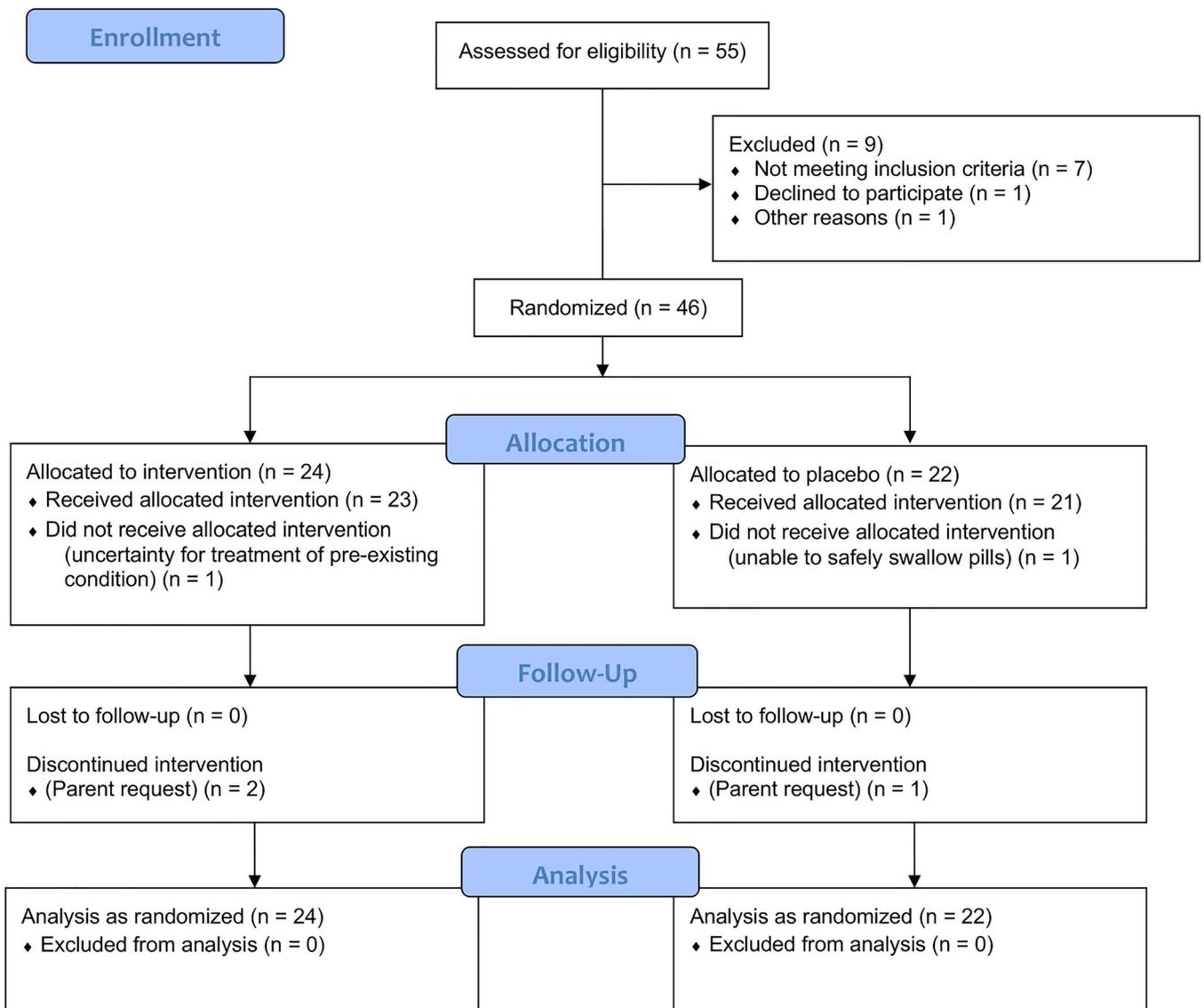
Figure 1. Consolidated standards of reporting trials (CONSORT) diagram for the trial.

everolimus treatment arm. None of the participants in the placebo or everolimus group reported Grade 4 or 5 AEs (Table 2).

Three serious AEs (SAEs) occurred in the everolimus group, and none occurred in the placebo group. The first SAE was potential suicidality (threat to cut oneself) reported by a school official which occurred during the screening phase before drug initiation. The school psychologist did not see a threat to the patient's safety; furthermore, the student may have made the comment after overhearing another student make the same comment. After complete psychiatric evaluation, the patient began participation in the trial (causality rated as 'definitely not related to study drug'). The second SAE consisted of an emergency room visit, before study drug administration, for abdominal pain in the setting of multiple renal lesions similar to prior episodes that occurred before enrollment in the trial (causality rated as 'definitely not related to study drug'). The third SAE consisted of hospitalization requiring intravenous fluid administration for dehydration in setting of possible enterovirus gastroenteritis (causality rated as 'possibly related to study drug'). In each case, treatment with study drug resumed after resolution of the SAE, and no side effects were reported. Among reported AEs, only three participants had AEs 'definitely related' to treatment; all were in the everolimus arm. Seventeen out of 24 participants (70.8%) in the everolimus group had AEs 'possibly or probably' related to treatment, in contrast to 7 out of 22 participants (31.8%) in the placebo group. There was only one participant with an AE rated as 'not recovered/not resolved'. This participant was in the placebo group and had ongoing dry skin (Grade 1 AE). The two most prevalent categories of AEs were skin/subcutaneous tissue complaints (21.7% of all participants) and gastrointestinal complaints (41.3% of all participants), including mucositis (Supplementary Material, Table S3). The prevalence of gastrointestinal AEs was higher in the everolimus group versus the placebo group ($P < 0.001$).

Table 1. Baseline demographics and clinical characteristics of the cohort

	Everolimus (n = 24)	Placebo (n = 22)	Total (n = 46)	P-value
Mean age in years (SD); range	16.5 (11.3), 5.0–44.0	14.7 (10.9), 5.7–44.0	15.6 (11.0), 5.0–44.0	0.592
Sex, n (%)				0.331[#]
Male	13 (54.2%)	15 (68.2%)	28 (60.9%)	
Female	11 (45.8%)	7 (31.8%)	18 (39.1%)	
Race, n (%)				1.000[†]
Black/African American	1 (4.2%)	1 (4.5%)	2 (4.3%)	
White	20 (83.3%)	18 (81.8%)	38 (82.6%)	
Other	1 (4.2%)	1 (4.5%)	2 (4.3%)	
Refused/Unknown	2 (8.3%)	2 (9.1%)	4 (8.7%)	
Growth parameters/vitals				
Mean weight in kg (SD), range	63.2 (43.6); 14.2–201.3	52.6 (24.5), 16.1–94.6	57.9 (35.4), 14.2–201.3	0.329
Weight z-score, Mean (SD), range	0.1 (1.2),−1.2 to 4.1	−0.1 (0.7),−1.2 to 1.0	0.0 (1.0),−1.2 to 4.1	0.329
Mean height cm (SD), range	155.9 (27.6), 92.0–192.0	150.7 (25.7), 106.0–188.3	153.3 (26.5), 92.0–192.0	0.520
Mean height z-score (SD), range	0.1 (1.0),−2.3 to 1.5	−0.1 (1.0),−1.8 to 1.3	0.0 (1.0),−2.3 to 1.5	0.520
Mean systolic BP in mmHg (SD), range, adults (age >18 years)	119.0 (14.4), 101.0–140.0	116.4 (10.0), 104.0–129.0	117.9 (12.3), 101.0–140.0	0.807[¥]
Systolic BP (mmHg), Mean (SD), range, children (age ≤ 18 years)	102.6 (6.3), 93.0–112.0	103.1 (11.3), 89.0–128.0	102.9 (9.3), 89.0–128.0	0.742[¥]
Diastolic BP (mmHg), Mean (SD), range, adults (age >18 years)	69.6 (12.7), 57.0–92.0	72.8 (8.6), 63.0–82.0	70.9 (10.8), 57.0–92.0	0.416[¥]
Diastolic BP (mmHg), Mean (SD), range, children (age≤ 18 years)	61.5 (7.2), 53.0–76.0	63.3 (9.6), 44.0–79.0	62.5 (8.5), 44.0–79.0	0.244[¥]
Body surface area (m^2), Mean (SD), range	1.6 (0.6), 0.6–3.0	1.5 (0.5), 0.7–2.1	1.5 (0.5), 0.6–3.0	0.431
Clinical features				
ASD, n (%)	6 (25.0%)	8 (36.3%)	14 (30.4%)	0.403[#]
Mean full scale IQ (SD), range	77.8 (20.3), 40.0–110.0	75.2 (23.5), 40.0–113.0	76.6 (21.6), 40.0–113.0	0.687

[#]P-values derived from Chi-square test. [†]P-values derived from Fisher's exact test. [¥]P-values derived from Mann–Whitney-U test. Unless otherwise indicated, P-values are derived from two-sample t-test.

Efficacy of everolimus on neurocognitive and behavioral endpoints

Longitudinal mixed effects modeling was used to estimate the efficacy of everolimus (Table 3). There were no statistically significance differences in baseline values between the everolimus and placebo groups on key cognitive measures. Regarding the change in the neurocognitive composite (as defined in the Materials and Methods) from baseline to Month 6 follow-up (end of the double-blind phase), there was no statistically significant difference between the everolimus and placebo groups, and effect size was negligible (Cohen's $d=-0.10$, $P = 0.518$). Similarly, none of the measures comprising the neurocognitive composite score showed a statistically significant difference between the two groups or clinically meaningful effect size (for all measures, $|d| < 0.2$).

Most of the secondary outcome measures (as defined in the Materials and Methods) also failed to show significant differences between the everolimus and placebo groups. However, several measures showed modest, but clinically meaningful effect sizes (all effect sizes≥0.2 are boldfaced in Table 3). In cognitive domains [Stanford Binet Intelligence Scales Fifth edition (SB-5) full scale IQ, SB-5 verbal IQ, and SB-5 NVIQ], NVIQ showed a modest, but favorable effect because of everolimus (Cohen's $d = 0.31$, $P = 0.294$). In the memory domain, the Wide Range Assessment of Memory and Learning-Second Edition (WRAML-2) verbal learning core subtest scaled score showed a greater improvement in the everolimus group compared with the placebo group (Cohen's $d = 0.27$, $P = 0.219$). Among autism symptoms, the Social Responsiveness Scale-Second Edition (SRS-2) total standard score (reverse coded; standard score generated from T-score) showed a statistically significant group difference (Cohen's $d = 0.34$, $P = 0.042$). Among behavioral and sensory processing measures, Child Behavior Checklist (CBCL) Total Problems standard score (reverse coded; standard score generated from T-scores) (Cohen's $d = 0.23$, $P = 0.276$) and Short Sensory Profile—Short Form (SSP) total score (Cohen's $d = 0.31$, $P = 0.296$) showed small, statistically insignificant effect sizes in the direction of more favorable change.

In motor functioning, the everolimus group showed noticeable improvement on the Purdue Pegboard task left hand standard score (generated from T-score), whereas the placebo group showed little change over time, leading to a statistically significant group difference (Cohen's $d = 0.40$, $P = 0.016$). There was also a modest advantage in the treatment group with performance on the Purdue Pegboard task with the dominant hand (Cohen's $d = 0.24$, $P = 0.264$) and non-dominant hand (Cohen's $d = 0.20$, $P = 0.396$). Figure 2 illustrates the significant results of two of these secondary measures (Purdue Pegboard task left hand standard score generated from T-score, SRS-2 total standard score reverse coded and generated from T-score).

Table 2. Summary of data pertaining to participant dropout and AEs

	Everolimus (n = 24)	Placebo (n = 22)	Total (n = 46)	P-value
Dropout, n (%)				
Total	3 (12.5%)	2 (9.1%)	5 (10.9%)	1.000
Due to participant/parent request	2 (8.3%)	1 (4.5%)	3 (6.5%)	1.000
Due to investigator request	0 (0.0%)	1 (4.5%)	1 (2.2%)	0.478
Due to other requests	1 (4.2%)	0 (0.0%)	1 (2.2%)	1.000
AEs overview, n (%)				
Any AE	21 (87.5%)	13 (59.1%)	34 (73.9%)	0.044
Non-SAEs	21 (87.5%)	13 (59.1%)	34 (73.9%)	0.044
SAEs	3 (12.5%)	0 (0%)	3 (6.5%)	0.235
AEs severity, n (%)				
Grade 1	18 (75%)	10 (45.5%)	28 (60.9%)	0.069
Grade 2	12 (50%)	4 (18.2%)	16 (34.8%)	0.032
Grade 3	4 (16.7%)	0 (0%)	4 (8.7%)	0.110
Grade 4	0 (0%)	0 (0%)	0 (0%)	—
Grade 5	0 (0%)	0 (0%)	0 (0%)	—
AEs relation to treatment, n (%)				
Definitely not related	5 (20.8%)	6 (27.3%)	11 (23.9%)	0.734
Probably not related	9 (37.5%)	4 (18.2%)	13 (28.3%)	0.197
Possibly or probably related	17 (70.8%)	7 (31.8%)	24 (52.2%)	0.017
Definitely related	3 (12.5%)	0 (0%)	3 (6.5%)	0.235
Patient recovery from AEs, n (%)				
Not recovered/not resolved	0 (0%)	1 (4.5%)	1 (2.2%)	0.478
Recovered/resolved with sequelae	1 (4.2%)	0 (0%)	1 (2.2%)	1.000
Recovered/resolved without sequelae	20 (83.3%)	13 (59.1%)	33 (71.7%)	0.103
Recovering/resolving	2 (8.3%)	0 (0%)	2 (4.3%)	0.490
AEs category, n (%)				
General	1 (4.2%)	1 (4.5%)	2 (4.3%)	1.000
Psychiatric	2 (8.3%)	1 (4.5%)	3 (6.5%)	1.000
Nervous system	2 (8.3%)	2 (9.1%)	4 (8.7%)	1.000
Ear and labyrinth	3 (12.5%)	0 (0%)	3 (6.5%)	0.235
Respiratory, thoracic and mediastinal	3 (12.5%)	4 (18.2%)	7 (15.2%)	0.694
Gastrointestinal	16 (66.7%)	3 (13.6%)	19 (41.3%)	<0.001
Renal and urinary	1 (4.2%)	0 (0%)	1 (2.2%)	1.000
Skin and subcutaneous tissue	7 (29.2%)	3 (13.6%)	10 (21.7%)	0.289
Blood and lymphatic system	2 (8.3%)	0 (0%)	2 (4.3%)	0.490
Musculoskeletal and connective tissue	1 (4.2%)	1 (4.5%)	2 (4.3%)	1.000
Infections and infestations	4 (16.7%)	2 (9.1%)	6 (13%)	0.667
Injury, poisoning and procedural complications	2 (8.3%)	1 (4.5%)	3 (6.5%)	1.000
Investigations	2 (8.3%)	3 (13.6%)	5 (10.9%)	0.659

P-values are on the basis of Fisher's exact test. *n* denotes number of participants, not number of events. Numbers of participants in each category (everolimus, placebo, total) do not subtract dropout, in line with ITT principle.

Adaptive functioning (Vineland Adaptive Behavior Scales-Third Edition (VABS-III) adaptive behavior composite standard score) showed an improvement in the treatment group compared with the placebo group but did not reach statistical difference (Cohen's $d = 0.32$, $P = 0.199$). In global improvement [Clinical Global Impressions-Improvement (CGI-I); reverse coded], a higher percentage of individuals demonstrated improvement in the everolimus (57.1%) arm versus the placebo arm (27.8%) at Month 6, leading to a considerable effect size (success rate difference = 29.3%, $P = 0.099$). The difference between the two groups in global improvement became statistically significant when adjusting for the following (data not shown in Table 3):

(1) baseline global severity [Clinical Global Impressions-Severity (CGI-S)] and FSIQ (success rate difference = 34.8%, $P = 0.042$); (2) CGI-S and verbal IQ (success rate difference = 35.9%, $P = 0.049$); and (3) CGI-I and NVIQ (success rate difference = 33.9%, $P = 0.040$). Finally, Table 4 shows the results of mixed effects modeling of some additional exploratory analyses for behavioral subscale measures. There was a trend for improvement in behavioral problems, particularly internalizing behaviors, in the everolimus group compared with placebo. There was also a small effect size in the direction of improvement in the everolimus group compared with placebo for some of the RBS-R subdomain scores.

Table 3. Primary and secondary outcome estimates at baseline and Month 6 in the everolimus and placebo arms

	Everolimus (n = 24)		Placebo (n = 22)		Intent-to-treat effect on slope (Month 6 to baseline)	
Primary endpoints	Baseline	Month 6	Baseline	Month 6	Effect Size	P-value
Composite score	80.34	81.60	75.14	78.47	−0.10	0.518
SB-5 nonverbal working memory standard score	83.01	82.61	78.13	80.34	−0.14	0.391
SB-5 verbal working memory standard score	89.17	92.91	86.40	89.96	0.01	0.973
CPT-3 hit reaction time standard score (reverse coded; standard score generated from T-score)[a]	87.78	89.26	86.30	90.15	−0.12	0.592
Purdue Pegboard Test both hands standard score (generated from T-score)	62.83	67.12	55.92	61.49	−0.05	0.826
Secondary endpoints						
Cognition						
SB-5 full scale IQ	77.83	79.39	75.19	74.79	0.09	0.745
SB-5 verbal IQ	78.79	78.46	76.05	78.71	−0.13	0.632
SB-5 NVIQ	78.96	82.31	75.91	73.15	**0.31**	0.294
Memory						
WRAML-2 verbal learning core subtest scaled score	6.11	7.47	6.90	7.22	**0.27**	0.219
WRAML-2 verbal learning delayed recall scaled score	6.73	8.13	6.57	7.72	0.09	0.755
WRAML-2 verbal learning recognition scaled score	5.94	6.74	5.97	6.42	0.09	0.771
Executive functioning						
BRIEF-2 global executive composite standard score (reverse coded; standard score generated from T-score)	77.68	82.61	71.18	74.24	0.11	0.698
Autism symptoms						
SRS-2 total standard score (reverse coded; standard score generated from T-score)	73.12	78.91	67.52	66.90	**0.34**	0.042*
RBS-R total subscale score	16.44	14.42	23.35	19.96	0.11	0.767
Other behaviors						
CBCL total problems standard score (reverse coded, standard scores generated from T-scores)	85.90	90.10	80.54	81.53	**0.23**	0.276
SSP total score	137.46	143.08	128.25	126.52	**0.31**	0.296
Motor skills						
Purdue Pegboard Test right hand standard score (generated from T-score)	66.92	71.68	58.64	61.47	0.06	0.807
Purdue Pegboard Test left hand standard score (generated from T-score)	66.46	75.92	61.82	60.26	**0.40**	0.016*
Purdue Pegboard Test dominant hand standard score (generated from T-score)	67.92	75.60	58.63	59.25	**0.24**	0.264
Purdue Pegboard Test non-dominant hand standard score (generated from T-score)	69.37	75.58	61.89	62.36	**0.20**	0.396
DCDQ total score	37.51	40.01	36.29	37.64	0.08	0.652
Adaptive behavior						
VABS-III adaptive behavior composite standard score	76.96	81.56	74.41	74.71	**0.32**	0.199
Global severity & improvement						
Global severity (CGI-S) (reverse coded)	2.39	2.68	3.10	3.17	0.17	0.279
Global improvement (CGI-I)		57.1%		27.8%	**29.3%**	0.099

[a]$n < 10$ in the placebo group at Month 3 and Month 6. *Treatment effect is statistically significant ($\alpha = 0.05$, two-tailed). Some measures are reversely coded (CPT-3, SRS-2, BRIEF-2, CBCL, CGI-S). As a result, a higher score indicates better performance or less severe symptomology for all measures except for RBS-R total subscale score. Effect size > 0.2 or 20% (considered a small effect) is boldfaced. Except for RBS-R, positive effect size signifies improvement and negative effect size signifies worsening. SB-5 = Stanford Binet Fifth Edition (SB-5); CPT-3 = Conners' Continuous Performance Test-Third Edition; WRAML-2 = Wide Range Assessment of Memory and Learning-Second Edition; BRIEF-2 = Behavior Rating Inventory of Executive Function-Second Edition; SRS-2 = Social Responsiveness Scale-Second Edition; RBS-R = Repetitive Behavior Scale-Revised (RBS-R); CBCL = child behavior checklist; SSP = sensory profile questionnaire-short form; DCDQ = developmental coordination disorder questionnaire; VABS-III = Vineland Adaptive Behavior Scales-Third Edition; CGI-S = clinical global impressions-severity; CGI-I = clinical global impressions-improvement

Effect of everolimus on EEG

EEG power analysis (as defined in the Materials and Methods) results showed a significant difference in central alpha power ($P = 0.049$) and central beta power ($P = 0.039$) 6 months after everolimus treatment, with lower power measured in the treatment group (Fig. 3).
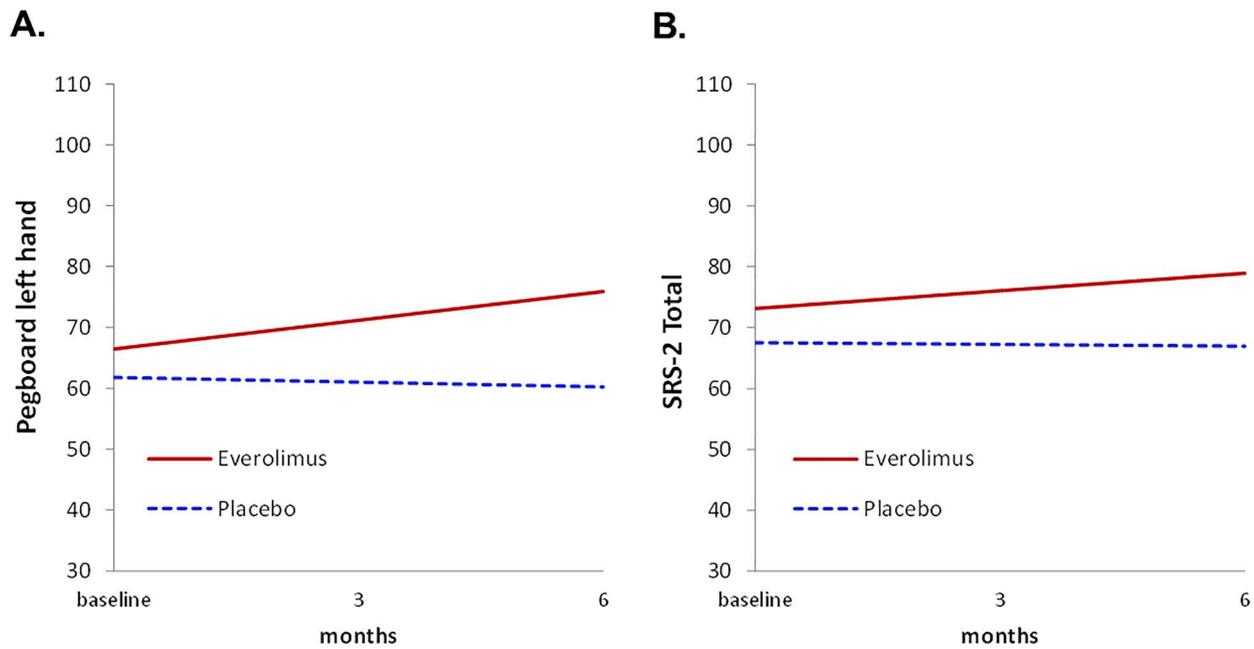
Figure 2. Estimated trajectories of Purdue Pegboard Test left hand standard score (**A**) and SRS-2 total standard score (**B**) over multiple timepoints for the everolimus and placebo groups. SRS-2 score is reverse coded; both standard scores are generated from T-scores. Larger scores are better. Outcomes are estimated on the basis of longitudinal mixed effects modeling allowing for random intercept.

Table 4. Estimates of mixed effects models corresponding to behavioral subscale scores at baseline and Month 6 in the everolimus and placebo arms

Subscale	Everolimus		Placebo		Intent-to-treat effect on slope (Month 6 to baseline)	
	Baseline	Month 6	Baseline	Month 6	Effect Size	P-value
CBCL						
Internalizing problems standard score (reverse coded, standard scores generated from T-scores)	88.76	95.91	81.57	80.75	**0.41**	0.109
Externalizing problems standard score (reverse coded, standard scores generated from T-scores)	95.52	99.88	90.17	90.80	**0.23**	0.390
RBS-R						
Stereotypic behavior subscale score	2.29	1.91	4.11	4.59	**−0.23**	0.277
Self-injurious behavior subscale score	1.35	0.98	1.83	2.17	**−0.41**	0.269
Compulsive behavior subscale score	2.44	2.39	4.12	5.04	**−0.25**	0.542
Ritualistic behavior subscale score	3.61	2.42	4.30	3.28	−0.07	0.887
Sameness behavior subscale score	5.30	4.37	6.58	4.57	**0.30**	0.421
Restricted interests subscale score	1.98	1.74	2.42	1.90	0.14	0.691
Total subscale score	16.44	14.42	23.35	19.96	0.11	0.767

CBCL = child behavior checklisr; subscales are reversely coded; therefore, a positive effect size signifies improvement. Repetitive Behavior Scale-Revised (RBS-R) subscales are not reversely scored; therefore, a negative effect size signifies improvement. Cohen's *d* is used for effect size for these measures. Effect size *d* > 0.2 (small effect size) is boldfaced.

Power in treatment and placebo groups did not differ significantly at baseline or Month 3 timepoints, and within-group power measures did not differ between baseline and Month 6 for any comparison.

Discussion

This study provides preliminary evidence that everolimus is safe for use in individuals with PHTS. The most common side effects of everolimus were GI-related, particularly stomatitis, similar to findings reported in other everolimus clinical trials in children and adolescents with TSC (19). Importantly, side effects of everolimus were non-life-threatening and non-permanent, with all the participants in the treatment arm reporting at least some degree of recovery from experienced side effects.

Although the study did not demonstrate everolimus-related improvement in the primary outcome measure, several secondary outcome measures showed changes in the direction of improvement with small effect size. The comprehensive nature of the neurocognitive battery was necessary given the pilot nature of this investigation and the limited number of validated outcome measures
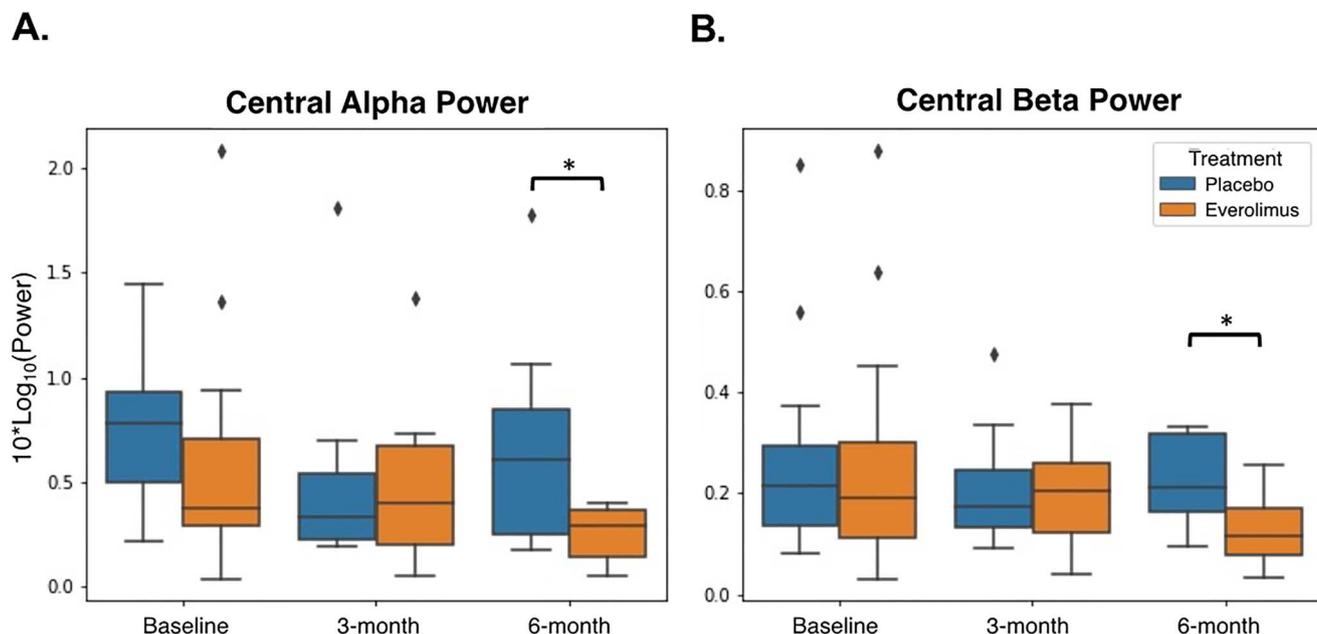
A.

B.

Figure 3. EEG central alpha (**A**) and beta power (**B**) over multiple timepoints for the everolimus and placebo groups. There were nine participants in the placebo group and seven participants in the everolimus group included in the 6-month EEG power analysis. *$P < 0.05$.

in this population. Furthermore, the study was not powered to detect modest to medium effect sizes in efficacy outcomes. Nonetheless, there were treatment-related improvements associated with small effect sizes pertaining to the domains of NVIQ, verbal memory, social symptoms, sensory processing, motor functioning, adaptive behavior and overall symptom severity. Particularly, meaningful clinical changes occurred in the area of social responsiveness (SRS-2), adaptive behavior (VABS-III) and global improvement (CGI-I). However, these findings should be considered preliminary, and future large trials are warranted to replicate these findings.

Everolimus may impact electrophysiological measures in individuals with PHTS. The EEG findings in our study may reflect target engagement (i.e. drug is acting on the brain). Differences occurring specifically in central alpha and beta power at 6 months may reflect changes to the mu rhythm, which involves these frequency bands and this central location specifically. Mu rhythm changes may reflect alterations in the mirror neuron system, and may result in altered motor and imitation abilities (20). Our EEG paradigms were not set up to explicitly test this hypothesis; therefore, future studies would need to evaluate EEG during motor activity, imagery, and/or imitation for this purpose. Nonetheless, these findings are noteworthy, given that these changes are directly reflective of brain activity and may be more sensitive to an intervention than neuropsychological measures. Notably, although everolimus-induced changes in quantitative EEG measures such as alpha and beta power have not been previously described, there are data to suggest that everolimus can reduce seizure frequency and improve epileptiform activity on EEG in individuals with TSC, another mTOR pathway disorder; this effect is presumed to occur by addressing the neuroexcitability that

results from mTOR overactivation (21,22). Given a recent shift in the field toward using EEG-based biomarkers not only for epilepsy, but also for evaluating cognition (23), future studies are needed to evaluate the utility of EEG as a biomarker of cognitive outcomes in response to mTOR inhibitors (and other disease-modifying treatment).

The time to effect for everolimus on neurocognitive and electrophysiological measures in PHTS may be 3–6 months. Improvement in several of the secondary outcome measures occurred by 6 months but not necessarily by 3 months. In addition, changes in central alpha and beta power between the treatment and placebo arms became evident at the 6-month trial timepoint (not the 3-month trial timepoint), raising the question of whether buildup is required. Prior studies in the field have involved shorter study durations [such as 12 weeks for mavoglurant in fragile $\times$ syndrome (24)], which may be insufficient for determining treatment response in various cognitive domains. In contrast to the relatively short time needed for everolimus to cause an impact on somatic problems such as tumors, a longer duration may be necessary to assess an impact on neurocognition, which entails complex cognitive/memory/learning functioning. In other words, 3–6 months may be an optimal duration for a study to detect effects on cognition and behavior: a shorter study duration may not capture substantive changes in outcome measures, and a longer duration may incur challenges with respect to participant attrition.

The everolimus dose used in our trial was the same dose approved by the FDA and used in clinical practice in TSC. In a phase 3, randomized, double-blind, placebo-controlled study (EXIST-3), everolimus was effective as an adjunctive treatment for refractory focal epilepsy in TSC (25). A more recent study examined the safety and

efficacy of everolimus for neurocognition and behavior in children with TSC. In this double-blind, randomized, placebo-controlled, phase II study, participants diagnosed with TSC (age 6–21 years) received 6 months of daily treatment with 4.5 mg/m^2/day of oral everolimus ($n = 32$) or placebo ($n = 15$). Everolimus was overall well tolerated, but no significant improvement occurred on most neurocognitive and behavioral measures (19). Future studies are necessary to ascertain optimal dose for neurocognition in PHTS.

This study had three main limitations. First, a small number of patients participated in this study. However, recruitment is difficult for rare neurogenetic disorders [PHTS has an estimated prevalence of 1:200 000 (26)]. Second, a subset of participants was unable to complete all assessments, including EEG recording, largely because of the COVID-19 pandemic. Amidst the COVID-19 pandemic, participants completed instruments via remote assessment whenever possible, assuming the instrument did not require in person assessment. Reassuringly, there were only two outcome measures which had a sample size $n < 10$ at the Month 6 timepoint [Conners' Continuous Performance Test-Third Edition (CPT-3) hit reaction time and EEG central alpha and beta power], whereas the other measures had reasonable associated sample sizes in the analysis. Third, our enrollment numbers were insufficient to power subgroup analysis, such as participants with ASD.

In summary, our preliminary analysis suggests that everolimus is well tolerated in PHTS, and AEs were similar to reports in previous trials and clinical practice. Although the primary efficacy endpoint did not reveal improvement with everolimus, several secondary efficacy endpoints moved in the direction of improvement. EEG measurements indicate target engagement of everolimus in the doses used in this trial. These findings warrant further study in this population.

Materials and Methods
Participants and eligibility

Subject recruitment, enrollment and participation occurred at Stanford University, Cleveland Clinic and Boston Children's Hospital. Recruitment was a combination of (1) referrals from providers within specialized PTEN clinics (2) referrals from other specialists (including neurologists, developmental pediatricians, endocrinologists and vascular anomalies experts) following patients with PHTS, (3) self-referrals from members of the PHTS Foundation which advertised this study (4) referrals from SPARK (Simons Foundation Powering Autism Research). The Institutional Review Boards at all sites approved the study (ClinicalTrials.gov NCT02991807). All participants/guardians provided informed consent before study participation. Study occurred between 2017 and 2021.

Participants were eligible if they were English-speaking, 5–45 years of age, had a documented pathogenic variant

in *PTEN*, had a NVIQ ≥ 50 and performed lower than the age-adjusted population mean on one or more of three standardized measures assessing processing speed, working memory or fine motor skills. The CPT-3 hit reaction time standard score (reverse coded; standard score generated from T-score) assessed processing speed. Performance on the working memory subscale of the SB-5 assessed this domain. Performance with both hands on the Purdue Pegboard Test (standard score generated from T-score) assessed fine motor skills. Selection of these endpoints was on the basis of previous research indicating deficits in working memory, processing speed and fine motor abilities among individuals with PHTS and ASD (27). Inclusion/exclusion criteria are shown in Supplementary Material, Table S1.

Study design and procedure

The study was a phase II, double-blinded, randomized, placebo-controlled, multi-site trial. Eligible participants underwent 1:1 randomization for everolimus versus placebo, assigned via a data management center. Participants received 6 months of daily oral everolimus (target dose of 4.5 mg/m^2) or matching placebo.

The trial occurred in four phases: (1) pretreatment (screening) phase (2) 6-month blinded treatment phase (3) 6-month open label phase and (4) follow-up phase. During the screening phase, participants underwent vital signs assessment, medical history, physical/neurological examination, laboratory assessments, psychiatric screening with the Columbia Suicide Severity Rating Scale (C-SSRS) and neuropsychological evaluations with the SB-5, CPT-3 and the Purdue Pegboard Test to determine if they met eligibility criteria. During the blinded treatment phase, participants underwent assessments at baseline and Months 1–6. Participants underwent baseline evaluation within 6 weeks of screening visit and began the study drug within 7 days of baseline evaluation. Participants randomized to placebo who completed the blinded treatment phase could participate in the open label phase, consisting of the same assessments at the same discrete timepoints as those in the blinded treatment phase. Evaluation at Month 6 in the blinded treatment phase was the baseline evaluation in the open label phase. The follow-up phase consisted of a phone call 28 days after the last day of receiving study drug to ascertain the occurrence/resolution of AEs during this interval.

A Data and Safety Monitoring Board oversaw participant safety. The basis for AE monitoring was version 4.0 of the National Cancer Institute Common Terminology Criteria for Adverse Events (28). The basis for medication side effect monitoring was the Dosage Record and Treatment Emergent Symptom scale (29).

Outcome measures

The neuropsychological battery included standardized instruments and questionnaires (Supplementary Material, Table S2) assessing domains commonly impaired

and likely to be treatable in PHTS (30). From these instruments, we generated our primary outcome measure, a neurocognitive composite from a weighted average of performance on assessments of working memory (SB-5 nonverbal working memory standard score, SB-5 verbal working memory standard score), processing speed (CPT-3 hit reaction time standard score that is reverse coded and generated from T-score) and fine motor coordination (Purdue Pegboard Test both hands standard score that is generated from T-score). For ease of interpretation, we reverse-coded the following measures and transformed T scores into standard scores: CPT-3 hit reaction time; Behavior Rating Inventory of Executive Function-Second Edition (BRIEF-2) global executive composite; SRS-2 total score; CBCL internalizing, externalizing and total problems scores. We reverse-coded the CGI-S. We established reliability on the CGI-S and CGI-I (>90%) between the investigators before study initiation through the review of three case vignettes (30).

EEG

We collected resting-state EEG data at three timepoints (baseline, Month 3 and Month 6). EEG data acquisition systems at our three study sites were: Philips/EGI 128-channel HydroCel Geodesic Sensor Net (Boston Children's Hospital); Nihon Kohden 10–20 clinical EEG system (with additional 10–10 electrodes as tolerated) (Cleveland Clinic) and ANT Neuro 64-channel system (Stanford University). In total, placebo and treatment groups contributed 18 and 16 usable EEGs, respectively, at baseline; 11 and 8 EEGs, respectively, at Month 3; and 7 and 9 EEGs, respectively, at Month 6.

We processed EEG files consistently using the Batch EEG Automated Processing Platform (31). To compensate for different data acquisition electrode sets, we processed and analyzed the spatially distributed set of 18 channels in a 10–20 montage recorded at all three sites; we did not include Cz, a reference electrode unique to the Philips system. We filtered voltage signals with a 100 Hz low pass filter and a 1 Hz high pass filter, as well as a 60 Hz notch filter to remove any electrical power line contamination via CleanLine's multitaper approach (32). We down-sampled the data to 250 Hz to ensure consistency across sites and performance accuracy with artifact removal, conducted using the Harvard Automated Processing Pipeline for Electroencephalography (HAPPE) (33).

We segmented EEG data into contiguous 2-s windows in which no channel's amplitude exceeded 40 μV, the HAPPE default threshold after wavelet-thresholding and independent component analysis. We estimated EEG power using multi-taper spectral estimation with three tapers. We evaluated power in five frequency bands [delta (2–3.9 Hz), theta (4–7.9 Hz), alpha (8–12.9 Hz), beta (13–29.9 Hz), gamma (30–55 Hz)], and we averaged power in pairs of channels in occipital (O1, O2), central (C3, C4) and frontal (F3, F4) brain regions.

Statistical analysis

We analyzed safety of everolimus using the primary safety endpoint of dropout rate as well as several secondary safety endpoints (serious versus nonserious AEs; severity of AEs; relation of AEs to treatment; recovery rates from AEs; and categorization of AEs). We used Fisher's exact test to compare the incidence of these endpoints between the everolimus and placebo groups. We examined whether the everolimus group showed increased improvement from baseline to Month 6 follow-up, compared with the placebo group, on the basis of the primary efficacy endpoint (neurocognitive composite) as well as secondary efficacy endpoints (each component of the neurocognitive composite and other neurocognitive outcomes). Because this was a pilot trial with relatively small sample size, we did not perform statistical corrections for multiple testing.

For all outcome measures except CGI-I, we estimated the effect of everolimus with the ITT principle using standard linear mixed effects modeling (34,35) with repeated measurements collected at baseline, Month 3 and Month 6. We used maximum likelihood estimation so that all randomized cases with any available data could be included in the analyses in line with the ITT principle ($n = 24$ in the everolimus group; $n = 22$ in the placebo group). Given the modest sample size of our study, we used a robust standard error that is less sensitive to parametric assumptions. We assumed linear changes over time and allowed for variable individual baselines (random intercept). In line with accepted practices in modern longitudinal analysis, we assumed that missing data occurred randomly because of subject attrition or intermittent dropout unless observed otherwise (36). We calculated Cohen's *d* as the estimated group difference in the change divided by standard deviation at Month 6 pooled across the everolimus and placebo groups. For CGI-I, low sample sizes in several categories of the measure necessitated dichotomization of overall improvement (into improved or not); we used multivariate logistic regressions analysis instead of mixed effects modeling. Effect size was taken as success rate difference (in percentage).

For both mixed effects modeling and multivariate logistic regressions analysis, we used maximum likelihood estimation with robust standard errors, implemented in Mpus version 8.4 (37). We consistently used the nominal significance level ($\alpha = 0.05$, two-tailed) without adjusting for multiple testing, because we had one clear primary efficacy endpoint, a change in neurocognitive composite score from baseline to Month 6 and because most effects on secondary efficacy outcomes were insignificant with the nominal significance level. As a way of sensitivity analysis, we repeated all analyses conditional on the baseline FSIQ, which was a good predictor of missingness (attrition) in several cognitive outcomes. However, the results were not sensitive to the presence or absence of FSIQ as a covariate. Therefore, we reported the estimation

results from the analyses without using FSIQ as a covariate.

For EEG measures, we used *t*-tests to determine significant differences between placebo and treatment groups at each timepoint, as well as significant differences from baseline to Month 6 in each group.

The study protocol and statistical analysis plan were published (30).

Supplementary Material

Supplementary Material is available at *HMG* online.

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